CHARACTERIZATION AND *IN VITRO* MULTIPLICATION OF BITTERGOURD (*Momordica charantia* L.) GENOTYPES

RESMI J.

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

Doctor of Philosophy in Horticulture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2009

Department of Olericulture
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM 695522

Dedicated
to
my family

DECLARATION

I hereby declare that this thesis entitled "**Characterization and *in vitro* multiplication of bittergourd (*Momordica charantia* L.) genotypes**" 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,
12-11-2009.

RESMI J.
(2005-22-103)

CERTIFICATE

Certified that this thesis entitled "**Characterization and *in vitro* multiplication of bittergourd (*Momordica charantia* L.) genotypes**" is a record of research work done independently by Mrs. Resmi. J. (2005-22-103) 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.

Sreelatha
12-11-09

Vellayani,
12-11-2009.

Dr. I. Sreelathakumary
(Chairperson, Advisory Committee)
Professor,
Department of Olericulture,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

APPROVED BY

Chairperson:

Dr. I. SREELATHAKUMARY
Professor,
Department of Olericulture,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Members:

Dr. M. ABDUL VAHAB**,**
Professor and Head,
Department of Olericulture,
College of Agriculture, Vellayani,
Thiruvananthapuram – 695522.

.

Dr. K. RAJMOHAN
Professor and Head,
Department of Plant Biotechnology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695522.

Dr. C.R. SUDHARMAIDEVI
Professor,
Department of Soil Science and Agricultural Chemistry,
College of Agriculture, Vellayani,
Thiruvananthapuram – 695522.

Dr. ROY STEPHEN
Associate Professor,
Department of Plant Physiology,
College of Agriculture, Vellayani,
Thiruvananthapuram – 695522.

External Examiner:

Dr. V. PONNUSWAMI
Dean (Horticulture),
Horticultural College and Research Institute,
Tamil Nadu Agricultural University,
Periyakulam-625604

ACKNOWLEDGEMENT

"By the grace of God I am What I am"

I am deeply thankful to God for the numberless blessings he showered upon my life, I bow my head before the mighty power for his eternal love and grace for rendering help through various hands in completing the work successfully.

It is my proud privilege to have worked under the guidance of Dr. I. Sreelathakumary, Chairperson of the Advisory Committee, Professor, Department of Olericulture. I feel immense pleasure in extending my heartfelt gratitude and sincere thanks for her kind treatment, ever-willing help, valuable guidance, expertise, patience and critical scrutiny of the manuscript, constructive suggestions and motivation throughout the course of this research work and in the preparation of the thesis. I owe much to her for the moral support and affection which helped me to complete this endeavor successfully.

From deep within my heart, I owe my indebtedness to Dr. M. Abdul Vahab, Professor and Head, Department of Olericulture, for his genuine interest, periodic suggestions, constructive perusal of manuscript and whole hearted help.

My heartfelt gratitude to Dr. K. Rajmohan, Professor and Head, Department of Plant Biotechnology for his valuable comments, timely help and encouragement throughout the study.

I owe my immense thanks to Dr. C.R. Sudharmaidevi, Professor, Department of Soil Science and Agricultural Chemistry, for her personal attention, kind concern and also helping me to conduct the biochemical analysis.

I convey my deep sense of gratitude to Dr. Roy Stephen, Associate Professor, Department of Plant Physiology, for sincere help rendered during the course of investigation, critical scrutiny of the manuscript and valuable suggestions.

I am particularly grateful to Dr. V. A. Celine, Professor, Department of Olericulture, for her valuable suggestions and wholehearted approach throughout the course.

I convey my deep sense of gratitude to Dr. R.V. Manju, Dr. Vasantha Kumar, Dr. B.R. Reghunath and Dr. B. Sony for the sincere help rendered during the course of investigation.

I express my indebtedness to Mr. C.E. Ajithkumar, Senior Computer Programmer, Department of Extension in executing the experimental data.

I sincerely acknowledge each and every non-teaching staff and labourer of the Department of Olericulture, for their whole hearted co-operation and sincere efforts for the successful completion of my research work.

I am grateful to National Bureau of Plant Genetic Resources, Regional Centre, Thrissur for their help for providing seeds of bittergourd genotypes for conducting the study.

I feel special pleasure in expressing my whole hearted thanks to my dearest classmates Dr. Beena Thomas, Dr. Gauri Priya, Dr. Nirmalatha, Dr. Reshmi C.R., Sajitha J.P., Dr. Jithesh and Sreeram for their affection, moral support, selfless help and pleasant company.

I must thank specially Ajitha, Arpitha, Asha, Akhila, Neenu, Shershah, Sony, Thankamany, Udaya and Vineetha for their noble help and kind concern throughout the course of research.

I acknowledge KSCSTE for providing Research Fellowship and Kerala Agricultural University for providing the facility to conduct thesis work.

My loving mother Mrs. N. Ratnaveni (now late) initiated me into the scientific lore. My hearty prayers for the departed soul.

Lastly I thank my husband, Dr. Jijo. S., for his constant moral support, encouragement and co-operation; our son Harikiran, for never being demanding; in-laws and other family members for their commendable sacrifice, mental support, prayers and for bearing the inconveniences caused during the study.

Resmi

RESMI J.

CONTENTS

LIST OF TABLES

LIST OF TABLES CONTINUED

LIST OF TABLES CONTINUED

LIST OF FIGURES

LIST OF FIGURES CONTINUED

LIST OF PLATES

LIST OF PLATES CONTINUED

LIST OF APPENDICES

Sl. No.	Title	Appendix No.
1.	Score card for the organoleptic evaluation of cooked bittergourd fruits	I
2.	Composition of MS basal media tried for *in vitro* culture of bittergourd genotype MC 4	II

LIST OF ABBREVIATIONS

%	–	Per cent
°C	–	Degree Celsius
°E	–	Degree East
µg	–	Micro gram
µl	–	Micro litre
µM	–	Micro molar
°N	–	Degree North
BAP	–	Benzyl amino purine
bp	–	Base pair
CD	–	Critical difference
cm	–	Centimetre
d.f.	–	Degrees of freedom
DNA	–	Deoxy ribonucleic acid
dNTPs	–	deoxyribonucleotide triphosphates
2,4-D	–	2,4-dichloro phenoxy acetic acid
2,4,5-T	–	2,4,5-dichloro phenoxy acetic acid
EDTA	–	Ethylene diamino tetra acetic acid disodium salt
et al.	–	And others
Fig.	–	Figure
g	–	gram
GA_3	–	Gibberellic acid
GA	–	Genetic advance
GCV	–	Genotypic coefficient of variation
IAA	–	Indole-3-acetic acid
IBA	–	Indole-3-butyric acid
i.e.	–	That is
kg	–	Kilogram
Kn	–	Kinetin
mM	–	Millimolar
N	–	Normality
NAA		α-Naphthalene acetic acid
NBPGR	–	National Bureau of Plant Genetic Resources
Ng	–	Nanogram
nm	–	Nanometer
No.	–	Number
NS	–	Non significant
PCR	–	Polymerase chain reaction
PCV	–	Phenotypic coefficient of variation
pH	–	Per Hydrogen
pM	–	Pico mole
SE	–	Standard error
spp.	–	Species
Tris HCl	–	Tris (hydroxy methyl) aminomethane hydrochloride
viz.	–	Namely

1. INTRODUCTION

Momordica is a genus of herbaceous annual or perennial climbers with 45 species native to tropical Asia and Africa. The name for the genus is derived from the Latin word 'mordicus', meaning bitten; it refers to the fact that the jagged edges of the seed look like bite marks. About six species are cultivated for their fruits used as vegetable, the most common being *Momordica charantia* Linn.

M. charantia is a fast growing, trailing or climbing cucurbit with thin stem and tendrils that grow naturally on the edge and in gaps of low land tropical forests (Burkill, 1985). It is known by different names such as Bittergourd, Bitter melon, Balsam pear, Bitter cucumber or African cucumber in English, Karela in Hindi, Gujarathi and Punjabi, Karla in Marathi, Pagal in Tamil and Pavakka in Malayalam.

M. charantia is a pantropical species. According to Zeven and Zhukovsky (1975) the origin of bittergourd remains unknown. It is probably a native of Africa (Williams and Ng, 1976) or Indo-Burma (Garrison, 1977). However the regions of eastern India and southern China are suggested as possible centres of domestication (Seshadri, 1993). Wild *M. charantia* var. *abbreviata* Ser., a native of Asia, may be the progenitor to the domesticate (Miniraj et al., 1993).

Bittergourd is grown for its bitter tender fruits. Among the cucurbits, it is considered as a prized vegetable because of its high nutritive values with respect to vitamins (especially ascorbic acid) and minerals particularly iron, calcium and phosphorous. Small fruited variety is richer in nutrients than long fruited variety. The small fruits contain 2.0% protein, 1.0% fat, 2.8% carbohydrates, 9.8 mg iron, 210 IU of vitamin A and 88 mg vitamin C per 100 g edible portion (Saimbhi, 1993; Dey et al., 2005). Big and long fruit contains 1.6% protein, 0.2% fat, 4.2% carbohydrates, 2.2 mg iron, 210 IU of vitamin A and 88-92 mg vitamin C

per 100 g edible portion. White-fruited Indian varieties are, in fact, relatively high in polypeptides, phenolics and polyphenolic compounds, which are natural antioxidants; thus are alternatives to replace synthetic antioxidants to enhance food quality (Horax et al., 2005, Krawinkel and Keding, 2006). Fruit contains as many as 14 carotenoids (five at the immature, 6 in the mature-green and 14 in the ripe stages) and cryptoxanthin (the principal chloroplast and chromoplast based pigment) in ripe fruit (Behera et al., 2007a).

Bittergourd is one of the important vegetable crops of India which cover 0.3 lakh ha with a total production of 3.5 lakh tones (IIVR, 2005). The cultivation of the crop is very lucrative especially in South India from where green fruits are exported to Gulf and European countries, which holds a major share of the total export of fresh vegetables (Rajan and Markose, 2005). Dehydrated fruit is gaining attention in market. The market preference for colour, shape, size and bitterness also vary with locations. White coloured varieties are less bitter in taste and preferred in South India. The fruits are dried, boiled and stuffed for ready use while preserved by sundrying or pickled for use in the off-season. The annual import of bittergourd in UK comes to 1000 tonnes. The area and production are fast increasing and some of the leading states in the country are Uttar Pradesh, Orissa, Maharashtra, Andhra Pradesh, Tamil Nadu and Kerala. The cultivation of bittergourd in Kerala is limited to an area of 2129 ha (FIB, 2008).

Bittergourd has excellent medicinal virtues. The roots, vines, leaves, flowers and seeds of bittergourd are used in medicinal preparations (Morton, 1967). Its medicinal value is probably due to the presence of a cocktail of chemical compounds such as momordicin and polypeptides. Presence of health promoting substances such as vicine and charantin has been reported in bittergourd (Dutta et al., 1981). Two novel phytochemicals in bittergourd have been demonstrated of which one have

clinical properties of insulin and the other one have the ability to inhibit an enzyme named guanylate cyclase, which is linked to pathogenesis and replication of not only psoriasis, but leukemia and cancer as well (Takemoto, 1982). The bitter principle momordicin lowers blood glucose content in humans. Its juice consumption is also very useful for diabetic patient due to its potent oxygen free radical scavenging activity of the fruit juice (Sreejayan and Rao, 1991). The plant is gaining a lot of attention due to its anti-HIV (Lee-Huang et al., 1995), anti-diabetic (Raman and Lau, 1996) and anti-tumor activity (Xue et al., 1998). Bittergourd fruits contain anticarcinogens or chemo preventive agent (Etoh et al., 2002; Yasui et al., 2005; McCue et al., 2005). Thus bittergourd has been widely studied for its utilization as a traditional phytomedicine, with more recent attention focused on its as a hypoglycemic agent (Chen and Li, 2005; Li, 2008).

A wide range of variability with respect to fruit shape, size, colour, surface texture and bitterness has been reported in bittergourd. Evaluation of genotypes for phenotypic characters based on morphological variation, supplemented with DNA characterization, helps in documentation and deployment of the available genetic variability. Study of genetic polymorphism provides scientific basis for the utilization of germplasm resources efficiently in crop improvement. Though a range of plant characters are currently available for distinguishing closely related individuals, their sensitivity to environment and scanty genome coverage hinders their further usage in breeding. DNA based molecular markers are in abundance and clearly allow the comparison of genetic material avoiding any environmental influence on gene expression. Random amplified polymorphic DNA (RAPD), a PCR based DNA marker technology, offers advantages in speed, technical simplicity, random coverage of genome and relatively higher level of polymorphism (Newburry and Lloyd, 1993).

Bittergourd being a highly cross pollinated and seed propagated crop, maintenance of desirable quality and morphological aspects are very difficult. *In vitro* conservation of plantlets could hence be an alternative method for overcoming these constraints. Standardization of a protocol for production and maintenance of novel types of bittergourd is an imperative need.

Taking into consideration of all these aspects, the present study was undertaken with the following objectives:

1. To genetically catalogue the available *M. charantia* genotypes.
2. To identify superior genotypes based on yield, quality, pest and disease resistance.
3. To estimate genetic variability, components of variation and divergence in bittergourd.
4. To document the biochemical traits of bittergourd germplasm.
5. To characterize and assess the extent of variability in available germplasm of bittergourd by RAPD based DNA marker.
6. To standardize the *in vitro* propagation techniques for rapid multiplication of elite bittergourd genotypes.

2. REVIEW OF LITERATURE

Bittergourd is an important cucurbitaceous vegetable cultivated for its immature fruits. The diverse morphological characters of *M. charantia* in India provide a relatively broad phenotypic species variation (Robinson and Decker-Walters, 1999; Behera et al., 2006). Though it is extensively grown in India, authentic reports on characterization and *in vitro* standardization of the crop is meagre. Hence an attempt has been made to review the available literature on various aspects in some important cucurbitaceous crops and presented under the following subheads.

2.1 Morphological characterization

2.2 Biochemical characterization

2.3 Molecular characterization

2.4 Standardization of *in vitro* techniques

2.1 MORPHOLOGICAL CHARACTERIZATION

2.1.1 Genetic Cataloguing

Genetic cataloguing helps to easily describe the morphological features of a genotype and thus helps exchange of information about new accessions. Characterization of the germplasm using standard descriptors is essential for the documentation and conservation.

India is endowed with large amount of genetic diversity in bittergourd based on morphological characters (growth habit, maturity and various fruit characters including shape, size, colour and surface texture). Two types of cultivars have been grown in northern India and nine types in southern India. Today, India cultivate primarily two varieties of

M. charantia (i) var. *charantia*, which produces large fusiform fruits and (ii) var. *muricata* (wild) with small and round fruits (Chakravarty, 1990).

A wide range of morphological variability was noted for fruit shape, size and colour in bittergourd (Ramachandran, 1978; Vahab, 1989).

Dey et al. (2006) reported that the bittergourd germplasm of 38 indigenous genotypes including commercially released varieties of India and 2 promising gynoecious lines showed a very large morphological variation with respect to fruit shape, size and colour.

2.1.2 Variability

The information on varietal suitability and the extent of genetic variability will be useful for the crop improvement. Many workers have reported considerable variability in bittergourd genotypes.

2.1.2.1 Plant Characters

In bottlegourd, Kumar et al. (2007) observed days to germination ranging from 7.23 to 11.01, with a mean of 8.91 while Ram et al. (2007) reported a range of 6.3 to 14.7 with a mean of 10.7.

High phenotypic and genotypic variance was reported for vine length in bittergourd (Choudhary, 1987). In bittergourd, Yadav et al. (2004) and Ram et al. (2006a) reported wide range of variability for vine length.

In bittergourd, Yadav et al. (2008) reported a range of 4.50 - 10.00 cm for internodal length.

Yadav et al. (2004) noticed high variability for number of primary and secondary branches per plant in bittergourd.

2.1.2.2 Flowering Characters

Ramachandran and Gopalakrishnan (1979) observed wide varietal variation for days to first male and female flower production in bittergourd. Limited variability was reported in ashgourd (George, 1981) and bittergourd (Mangal et al., 1981; Choudhary, 1987). Rao and Rao (2008) obtained a range of 41.27 to 60.33 days for first male flower and 48.87 to 64.20 days for first female flower in ridgegourd.

High PCV and GCV were recorded for days to first male and female flower production in bittergourd (Rajput et al., 1996; Devmore and Dhonukshe, 2007).

Prasad and Singh (1989) noticed limited variability for node to first male and female flower in ridgegourd. High genotypic coefficient of variation was recorded for the character in snapmelon (Jeeva and Pappiah, 2002) and pointedgourd (Dora et al., 2003).

In case of sex ratio, high variability was noticed in bittergourd (Shrivastava and Srivastava, 1976; Singh et al., 1977; Thakur et al., 1994).

2.1.2.3 Fruit Characters

Wide genetic diversity among the cultivars for yield and yield related characters were reported in bittergourd (Lawande and Patil, 1991; Bhave et al., 2003).

In ridgegourd, Reddy and Rao (1984) observed high phenotypic and genotypic coefficient of variation for fruit character. A high genetic variation for days to first fruit harvest was observed by Radhika (1999) in snakegourd. In ashgourd, days to first fruit harvest exhibited lowest PCV and GCV (Resmi, 2004).

Changlin (1998) obtained a range of 12.15 to 33.21 cm for fruit length in ashgourd. High GCV and PCV were observed for fruit length in snakegourd (Mathew and Khader, 1999).

For fruit girth, high genetic variation was observed by Iswaraprasad (2000) in bittergourd.

In bittergourd low genetic variance was observed for fruits per plant by Babu et al. (1986), whereas Vahab (1989) and Katiyar *et al.* (1996) observed high values.

Wide variability in fruit weight was noticed in bittergourd (Jaiswal et al., 1990). High GCV and PCV were observed for average fruit weight in ridgegourd (Varalakshmi et al., 1995) and bottlegourd (Narayan et al., 1996).

A high genetic variation for fruit yield was observed in bittergourd (Indiresh, 1982; Reddy et al., 1995; Katiyar et al., 1996). High PCV and GCV were reported for the character by Lovely (2001) in ashgourd and Dora et al. (2003) in pointedgourd.

2.1.2.4 Seed Characters

Ashok (2000) found wide variation in seed characters in snakegourd.

Lowest GCV was reported for seeds per fruit in bittergourd by Ramachandran and Gopalakrishnan (1979). In snakegourd, Varghese (1991) noticed high genetic variability for the character.

Mathew (1999) reported wide range of variation for 100-seed weight in bottlegourd. High phenotypic and genotypic coefficients of variation were observed for the character by Narayanankutty et al. (2006) in snakegourd.

2.1.2.5 Pest and Disease Incidence

Wide range of variation was reported for mosaic disease resistance in ashgourd. The vulnerability index for mosaic incidence ranged from 27.50 to 75.00 (Resmi, 2004).

2.1.3 Heritability and Genetic Advance

Heritability along with genetic advance will be helpful in assessing the reliability of a character for selection. Rajeswari and Natarajan (2002) in bittergourd reported high values of heritability and genetic advance for most of the characters.

In bottlegourd, Ram et al. (2007) observed high heritability and genetic advance for days to germination, while Kumar et al. (2007) noticed low values.

Parkash et al. (2000) reported low heritability and genetic advance for vine length in ashgourd. High heritability coupled with high genetic advance was observed for vine length in snapmelon (Reddy et al., 2005).

Menon (1998) noticed high values of heritability and genetic advance for primary branches in ashgourd. Prabha et al. (2007) recorded low heritability and high genetic advance for the character in ridgegourd.

In bittergourd, Mangal et al. (1981) observed low heritability and genetic advance for days to first female flower. High heritability and low genetic advance was noted for days to first male and female flower in ashgourd (Lovely, 2001) and ivygourd (Varghese, 2003).

High heritability and low genetic advance for node to first female flower was reported by Prasad and Singh (1989) in ridgegourd. High values of heritability and genetic advance was noticed for node to first male and female flower in snapmelon (Jeeva and Pappiah, 2002).

High heritability and moderate to high genetic advance for sex ratio was found in bittergourd (Babu et al., 1986).

Iswaraprasad (2000) reported high heritability and low genetic advance for days to fruit harvest in bittergourd.

High heritability coupled with high genetic advance for fruit girth was noticed in spongegourd (Prasad et al., 1984). Similar estimates for fruit length were reported by Varalakshmi et al. (1995) in ridgegourd and Mathew (1999) in bottlegourd.

Sarkar et al. (1990) found out in pointedgourd that fruit girth had high heritability and low genetic advance while Ananthan et al. (2005) reported similar result for fruit length in ridgegourd.

Number of fruits per plant registered moderate heritability and low genetic advance in bittergourd (Babu et al., 1986), while high values of heritability and genetic advance was reported in bittergourd (Choudhary et al., 1991).

High heritability and high genetic advance for average fruit weight was noticed in bittergourd (Vahab, 1989) and ridgegourd (Prabha et al., 2007).

Fruit yield per plant was observed to have high heritability along with low genetic advance in snakegourd (Varghese and Rajan, 1993), while high estimates of the character was reported in ashgourd (Parkash et al., 2000) and chow-chow (Sanwal et al., 2008).

High values of heritability and genetic advance was reported for seeds per fruit in spongegourd (Singh et al., 2000) and snakegourd (Narayanankutty et al., 2006)

In the case of 100-seed weight, high heritability coupled with high genetic advance was noted in ashgourd (Lovely, 2001) and bottlegourd (Kumar et al., 2007).

2.1.4 Correlation Studies

The knowledge of correlation between yield and component characters and among component characters themselves is essential for a rational and directed improvement in yield.

In bottlegourd, a positive correlation was observed between yield and days to germination (Ram et al. 2007). Kumar et al. (2007) reported positive correlation between days to germination and days to first fruit harvest.

A high positive correlation was observed between vine length and fruit yield in ashgourd (George, 1981) and pointedgourd (Singh et al., 1986), whereas Varalakshmi and Reddy (1994) reported that in ridgegourd yield was negatively correlated with vine length.

Fruit yield was observed to have a positive correlation with internodal length in ashgourd (Menon, 1998) and ivygourd (Sarnaik et al., 1999).

A positive correlation was observed between yield and number of primary branches in bottlegourd (Murali et al., 1986). In pointedgourd, Sarkar et al. (1999) reported that number of secondary branches was positively correlated with yield.

Narayan et al. (1996) revealed in bottlegourd that days to first male flower was negatively correlated with fruit yield. Days to first female flower was found to have a positive correlation with yield in ridgegourd (Anitha, 1998), whereas a negative correlation was observed in ivygourd (Varghese, 2003; Suresh, 2004).

A negative association of fruit yield with node to first flower was reported in ridgegourd (Rao et al., 2000), whereas a positive correlation was found in snapmelon (Pandey et al., 2003).

Negative association was observed with days and node to first female flower in ridgegourd (Rao et al., 2000). In ashgourd, Lovely (2001) reported a negative correlation for days to first female flower and fruits per plant. Ram et al. (2006b) found that days to germination and node to first female flower were negatively correlated in bittergourd.

Murali et al. (1986) reported negative correlation of sex ratio with fruit yield in bottlegourd. In ashgourd, Resmi and Sreelathakumary (2006) observed that sex ratio was positively correlated with fruit yield.

Murali et al. (1986) noticed that sex ratio and number of fruits per vine is negatively correlated in bottlegourd. Ashok and Rajan (2004) found that sex ratio was positively correlated with 100-seed weight in snakegourd.

In bittergourd, Parhi et al. (1995) observed a positive correlation between fruit yield and days to first fruit harvest, while in sweetgourd, Sanwal et al. (2007) noticed a negative correlation between these two characters.

In *Luffa hermaphrodita*, days to first fruit harvest was positively and significantly correlated with vine length and average fruit weight (Ram et al., 2006a).

A positive correlation between yield and fruit length was found in parwal (Singh et al., 1987) and ridgegourd (Varalakshmi and Reddy, 1994), whereas positive association between yield and fruit girth was reported in ashgourd (Parkash et al., 2000) and bittergourd (Bhave et al., 2003).

In parwal, Singh et al. (1987) reported positive correlation of fruit length with fruit girth and average fruit weight. Parhi et al. (1995) recorded positive correlation of fruit girth with average fruit weight in bittergourd.

Number of fruits produced was found to have a positive association with yield in bittergourd (Indiresh, 1982; Lawande and Patil, 1989**)** and in bottlegourd (Ram et al., 2006b).

A high negative correlation between fruits per plant and fruit yield were reported by Lovely (2001) and Resmi (2004) in ashgourd. Number of fruits per plant was positively correlated with vine length in chow-chow (Singh, 2004).

Fruit yield showed a negative association with average fruit weight in watermelon by Singh and Singh (1988), whereas a positive correlation was reported in snakegourd (Narayanankutty et al., 2006) and *Luffa* (Ram et al., 2006a).

Fruit weight showed negative and non-significant correlation with number of fruit per plant in chow-chow (Singh, 2004). In bottlegourd, Ram et al. (2006b) reported that fruit weight was correlated with number of seeds per fruit and 100-seed weight.

Prasad and Singh (1990) in pointedgourd and Menon (1998) in ashgourd noticed positive association between number of seeds per kilogram of flesh and fruit yield, while Hazra et al. (2003) in pointedgourd reported a negative correlation.

In snakegourd, Ashok and Rajan (2004) reported that seeds per fruit had significant and negative correlation with days to first male flowering, and days to first fruit harvest, while Narayanankutty et al. (2006) recorded that seeds per fruit had significant and positive correlation with days to first male and female flowering, fruit girth and fruit weight. In bottlegourd, Kumar et al. (2007) obtained a positive and significant correlation between yield and 100-seed weight.

Rakhi (2001) reported in melon that virus disease incidence was negatively correlated with vine length, sex ratio and average fruit weight.

In ashgourd, Resmi and Sreelathakumary (2006) observed high positive correlation for fruit length with mosaic incidence.

2.1.5 Path Coefficient Analysis

Path coefficient analysis provides better index for selection than mere correlation coefficient by separating correlation coefficients of yield and its components into direct and indirect effects.

In bittergourd, Ramachandran (1978) reported high positive effects of fruit weight, fruits per plant and vine length had on yield. Number of primary branches and fruit length had negative direct effect on fruit yield.

Average fruit weight and vine length exhibited maximum direct effect on fruit yield per vine in ashgourd (George, 1981), whereas node at which female flower appeared and fruit length had positive direct effect on yield in ridgegourd (Varalakshmi and Reddy, 1994).

Paranjape and Rajput (1995) found that vine length, number of branches, fruits per plant and seed number indirectly contributed to yield in bittergourd.

In bittergourd, days to opening of first male and female flower, fruit girth and number of seeds per fruit had maximum positive direct effect on yield, whereas number of primary branches and fruit length had weak positive direct effect on yield (Parhi et al., 1995).

Rajput et al. (1995) found that days to first female flower appearance and days to fruit harvest exhibited direct negative effect on yield in bittergourd. Narayan et al. (1996) revealed appreciable amount of positive direct effect of days to first fruit harvest, average weight of edible fruit, number of fruits per plant and days to first female flower opening on yield in bottlegourd.

Menon (1998) observed that in ashgourd, average fruit weight exhibited the highest positive direct effect on fruit yield followed by fruits per plant, female flowers per plant, vine length, internodal length and number of seeds per fruit.

Path analysis in ivy gourd revealed that fruits per plant had the highest positive direct effect on yield (Joseph, 1999), while in pointed gourd, fruit weight and fruit diameter had maximum direct effects on yield (Sarkar et al., 1999). In bottlegourd, fruit length had its highest positive direct effect on yield followed by fruit girth (Mathew, 1999).

Parkash et al. (2000) in ashgourd and Rao et al. (2000) in ridgegourd reported that fruits per plant and average fruit weight had high direct effect on yield.

Lovely (2001) found that in ashgourd days to first female flower, fruits per plant, fruit length and girth had positive direct effects, while seeds per fruit and vine length had negative direct effects.

Path analysis in bittergourd revealed that fruit length, average fruit weight, number of seeds per fruit had direct and positive effect on fruit yield, whereas fruit girth had positive and indirect effect (Bhave et al., 2003).

The principal direct or indirect contributors to fruit yield were fruit length, fruits per plant and fruit weight as reported in pointedgourd (Hazra et al., 2003), snapmelon (Pandey et al., 2003) and ashgourd (Resmi, 2004).

Singh (2004) reported that in chow-chow, number of fruits per plant, days to germination and vine length had negative direct effect on yield.

In snakegourd, fruit weight and fruits per plant have the maximum positive direct effects on yield and the indirect contribution of other

characters was mainly through days to first harvest, seeds per fruits and 100-seed weight (Narayanankutty et al., 2006).

Ram et al. (2007) reported that highest positive direct effect was exerted by vine length followed by fruit weight and days to first flowering in bottlegourd. Sanwal et al. (2007) revealed that the characters days to maturity and fruit diameter has negative direct effect on yield per plant in sweetgourd.

2.1.6 Selection Index

Selection index involves discriminant function analysis, which is meant for isolating superior landraces based on the phenotypic and genotypic correlations.

Parhi et al. (1993) prepared a selection index for a collection of 13 bittergourd genotypes based on major components of yield namely, 100-seed weight, number of seeds per fruit and yield per plant.

Fruit length and fruit girth are important characters that should be taken for selection in ashgourd improvement programme (Lovely, 2001).

Varghese (2003) reported in ivygourd that the characters, number of fruits per plant and average fruit weight can be used as a criteria for selection of genotypes.

Resmi (2004) formulated selection index with better yield, fruit quality, earliness in male and female flowering, narrow sex ratio and mosaic resistance for 25 ashgourd landraces.

Ram et al. (2006b) reported that the characters like fruit weight along with number of fruits needs to be given more emphasis in selecting high yielding genotypes in bittergourd.

2.1.7 Genetic Divergence

The multivariate analysis using Mahalanobis's D^2 statistic, which measures the forces of differentiation at intracluster and intercluster level is a valuable tool in obtaining quantitative estimates of divergence. Anand and Murthy (1968) have emphasized the merit of D^2 statistics for genetic grouping of germplasm.

Genetic divergence was studied for eight quantitative characters in a collection of 25 cultivars of bittergourd by Ramachandran et al. (1981). They were grouped into ten clusters and yield per plant, fruits per plant and fruit size were the important factors contributing towards divergence.

Kadam and Kale (1985) studied genetic divergence for fourteen quantitative characters in a collection of thirty cultivars in ridgegourd. The cultivars were grouped into 20 clusters and fruit number per vine and yield per vine were noticed to be important factors contributing towards divergence.

Vahab and Gopalakrishnan (1993) grouped 50 genotypes of bittergourd into five clusters based on D^2 values. The pattern of clustering indicated that there was no association between ecogeographic distribution of genotypes and genetic divergence as the same group consisted of genotypes from diverse locations and the genotypes of same source fell into different groups also.

Parhi et al. (1993) grouped 13 genotypes of bittergourd into six clusters considering 14 quantitative characters in which fruit yield, number of seeds per fruit and 100-seed weight made maximum contribution to total divergence.

Narayanankutty et al. (2004) conducted genetic divergence studies in snakegourd and grouped 36 genotypes into six clusters. The analysis of cluster mean values indicated considerable differences for the thirteen characters studied.

Kutty and Dharmatti (2005) assessed genetic divergence among 40 genotypes of bittergourd of different ecogeographic origin. The total genotypes were grouped into 10 clusters on similarities of D^2 values. The character productive length of vine contributed maximum to divergence.

Maharana et al. (2006) studied genetic divergence in ivygourd and grouped 16 genotypes into five clusters with one to nine genotypes each. Canonical analysis confirms the clustering pattern. The intracluster average D^2 value ranged from 395.99 between cluster I and III to 133.09 between cluster III and IV.

Genetic divergence using Mahalanobis's D^2 statistics was studied for 15 quantitative characters among the 15 bittergourd genotypes (Devmore et al., 2007). The genotypes were grouped into eight clusters irrespective of geographic divergence, indicating no parallelism between genetic diversity and geographic divergence in this crop. The cluster mean indicated that days to first female flower, nodal position for first female flower, 100 seed weight, branches per vine, fruits per vine, days to first fruit harvest, fruit weight, fruit length, fruit girth and fruit yield were important characters towards maximum genetic divergence.

Dey et al. (2007) conducted genetic divergence studies in thirty eight bittergourd genotypes including two promising gynoecious lines for 17 characters. These genotypes were grouped into 6 clusters irrespective of geographic divergence indicating no parallelism between geographic and genetic diversity.

Genetic divergence studies by Resmi and Sreelathakumary (2008) resulted in clustering of 25 landraces of ashgourd into seven gene constellations based on Mahalanobis's D^2 statistic.

2.1.8 Reaction Towards Pests and Diseases

2.1.8.1 Reaction Towards Mosaic Virus

In India, mosaic diseases are common on almost all the cucurbit vegetables. There are different strains like cucumber mosaic virus, watermelon mosaic virus, pumpkin yellow vein mosaic virus and kakri mosaic virus.

A variety of mosaic symptoms occur on different members of the Cucurbitaceae (Singh, 1992). The mosaic disease symptoms consisted of distinct pattern of irregular dark green and light green patches on the leaf lamina, raised blisters on the leaf lamina, reduced leaf size, shortened and retarded growth.

The yield loss due to virus infection in pumpkin was 100 per cent when the plants were inoculated at seedling stage (Jayasree, 1984). Nandakumar (1999) reported that the incidence of mosaic in bittergourd adversely affected not only the yield but also the quality of the bittergourd fruits.

Source of Resistance

In bittergourd, Purushothaman (1994) reported that all the varieties tested were highly susceptible to the virus except Arka Harit, which was seen least susceptible. Early infection by the virus significantly affected the growth of plant and yield.

Out of 86 genotypes of bittergourd screened against Bittergourd Distortion Mosaic Virus (BDMV), Arunachalam (2002) observed that nine genotypes from northern and central parts of Kerala were found to be resistant.

2.1.8.2 Reaction Towards Fruit Fly

Bactrocera cucurbitae Coq., commonly known as fruit fly or melon fly, is highly polyphagous and its preferred hosts are bittergourd, musk melon, snap melon and snakegourd (Srivastava and Butani, 1998). It is known to be found at certain heights in a canopy of host gourds when these are grown on a pandal (Jiji et al., 2005; Sisodiya et al., 2005) and also typically to roost in hedges and other taller vegetation around the perimeter of fields and thus to be more common in field edges than centres.

The fruitfly causes more than 50 per cent loss in the yield of bittergourd fruits (Narayanan and Batra, 1960; Fischer and Butch, 1989).

Source of Resistance

Source of resistance have been found in bittergourd (Robinson and Decker-Walters, 1999). High silica content of the fruit rinds has also been associated with fruitfly resistance.

In host resistance studies of fruitfly, 66 accessions were screened by Padmanabhan (1989) and classified into highly susceptible, moderately susceptible and moderately resistant. Moderately resistant lines had relatively short duration than susceptible ones.

Bittergourd varieties ‘Green Rough’, ‘Green Smooth’, ‘White Rough’ and ‘White Smooth’ showed resistance to fruitfly. In India, it was found that the more prickly variety ‘Phule BG 4’ was comparatively resistant to fruitfly (Peter, 1998). A variety namely Kalyanpur Baramasi was also found tolerant to fruit fly (Rai et al., 2005).

Satpathy et al. (2005) assessed ten different varieties of bittergourd for differential infestation by fruit fly in field. They reported that levels of

infestation varied between 21 and 29 per cent and did not significantly differ. This implies that among the varieties tested, no significant level of resistance was available.

2.1.9 Quality Characters

2.1.9.1 Keeping Quality

The perishable nature of the bittergourd accounts to 20-30 per cent of spoilage. In bittergourd, Veenakumari et al. (1994) reported that a shelf life of 2.3 days under normal storage.

Developing fruits of *M. charantia*, harvested at horticultural maturity can be stored up to 14 days in humidified air at different temperatures. Fruit quality was best maintained if bitter melon was stored at 10 and 12.5°C. Fruits at 15°C continued to develop, showing undesirable changes including seed development, loss of green color, and fruit splitting. Immature fruit maintained postharvest quality better than fruit harvested at the fully developed green stage (Zong et al., 1995).

2.1.9.2 Organoleptic Quality

Suresh (2004) conducted organoleptic evaluation of 25 ivygourd accessions by considering the quality attributes like colour, doneness, bitterness, odour and taste. The accessions did not differ significantly for all these attributes. The accessions CG 14 (20.14) followed by CG 23 (18.85) and CG 17 (18.4) were organically superior to others based on the score. The accessions CG 8, CG 5, CG 21 and CG 22 which were highly bitter did not have any score.

Organoleptic quality of 4 ivygourd genotypes was evaluated using score card. In observed vegetative maturity there was no significant variation in the overall acceptability in between genotypes, but when individual criterias were considered significant difference was observed

only with respect to texture in CG 81 and CG 82 with a maximum score of 3.80 (Renjumol, 2006).

2.2 BIOCHEMICAL CHARACTERIZATION

2.2.1 Beta Carotene

Carotenoids derive their name from the main representative of their group, β-carotene, the orange pigment first isolated from carrots by Wackenroder in 1831. β-carotene is the most wide spread provitamin A, it being present in almost all vegetables. Gross (1991) reported 2.3 µg/g fresh weight β-carotene in bittergourd.

In *M. cochinchinensis*, total carotenoid concentrations were 497 µg/g fruit material with a beta-carotene concentration of 83 µg/g (Thuy et al., 2006).

Reddy et al. (2007) reported a range of 43.07 to 1860.33 µg/100 g total carotenoids in snapmelon. The genotype DBSM 3 showed maximum value for total carotenoids.

2.2.2 Ascorbic acid or vitamin C

Ascorbic acid or vitamin C is a sugar acid. It acts as an antioxidant and protects the cell membrane from the toxic action of powerful oxidizing agents.

Vahab (1989) reported that vitamin C content varied from 45.5 to 122.38 mg/100 g in 50 genotypes of bittergourd. MC 82, the medicinal bittergourd had maximum vitamin C content.

The ascorbic acid content in bittergourd fruit declined with increasing age (Karla et al., 1998).

In bittergourd, Sankaran et al. (2002) reported that ascorbic acid content seemed to decrease with maturity and larger fruits had lower ascorbic acid content. Vijayaraghavan et al. (2006) also reported that there is decreasing trend in ascorbic acid content with the advancement of age and the fruits harvested at 15 days after anthesis showed high content.

Yadav et al. (2008) found that the vitamin C content of bittergourd genotypes ranged from 44.67 to 120.00 mg/100 g and the maximum was observed in the genotype IC-85648A.

2.3.3 Iron

Iron plays important roles in cellular processes such as the synthesis of DNA, RNA and proteins; electron transport; cellular respiration; cell proliferation and differentiation; and regulation of gene expression.

In bittergourd, Tee et al. (1997) reported an iron content of 6.3 mg/100g while, Kumar and Sagar (2003) found that a 100g edible portion contain 1.8 mg of iron.

Renjumol (2006) found that the iron content of ivygourd genotypes ranged from 0.52 to 0.97 mg/100 g and the maximum was observed in the genotype CG-81.

2.3.4 Chlorophyll

The chlorophylls are the essential components for photosynthesis and occur in chloroplasts as green pigments in all photosynthetic plant tissues. Chlorophylls a and b occur in higher plants.

In bittergourd, Veenakumari et al. (1994) found that chlorophyll a and b contents are 0.026 mg/g and 0.009 mg/g respectively.

Mili et al. (2007) reported that the chlorophyll content in spine gourd was increased linearly upto 12 DAF in round type and 13 DAF in

long type and thereafter it decreased linearly in both the types. The degradation of chlorophyll in vegetables was mainly due to the activity of chlorophyllase enzyme which converted chlorophyll to pheophytin that produced the olive green colour.

2.3.5 Bitterness

All parts of the bittergourd plant are bitter in taste except the ripe aril covering the seed. The bitter principals are designated as momordicins, which are oxygenated tetracyclic triterpenoids with a cucurbitane skeleton that have been identified by Yasuda et al. (1984). Momordicins are repellent to many insects in the same way that cucurbitacins are (Tailamy, 1985). However, unlike cucurbitacins which strongly attract cucumber beetles, momordicins are still very repellent to these pests of Cucurbitaceae plants (Chandravadana, 1987). Very few studies have attempted to separate and quantify momordicins (Chandravadana and Chander, 1990; Zhu et al., 1990; Yasui et al., 1998).

Fatope et al. (1990) found lower concentrations 0.04% of momordicin I and 0.19% of momordicin II in dry plant of *M. charantia* collected in Nigeria.

Devadas and Ramadas (1994) evaluated triterpenoid content of fruits of 12 morphologically and geographically diverse bittergourd. The highest amounts were observed in P7 (MC 41) and P12 (White Long) and the lowest in P3 (MC 13-Coimbatore Local) and P5 (Arka Harit).

Mathew (2005) conducted factor analysis of bitterness in *C. melo* var. *conomon* and reported that cucurbitacin content decreased with fruit maturity. Results of thin layer chromatographic studies of cucurbitacin extract confirmed higher concentration of cucurbitacin in the placanta followed by flesh and rind.

Momordicin concentrations in leaves and stems of *M. charantia* were found to vary considerably between the 54 collecting sites. Results range from 1.47 to 37.5 mg of total momordicins per g of dry plant or 0.15 to 3.75% (w/dry w) with a mean of 12.68 mg/g (Beloin et al., 2005).

Chen et al. (2008) isolated five cucurbitacins namely kuguacins A–E, together with three known analogues including momordicin I from the roots of *M. charantia*.

2.3 MOLECULAR CHARACTERIZATION

The advent of molecular markers has revolutionized phylogenetic studies and characterization of germplasm. DNA markers offer precise means to measure genetic diversity and affinity among germplasm collections than the morphological and biochemical markers due to their environmental insensitivity and abundance in genome. Random Amplified Polymorphic DNA (RAPD) is a multiplex marker system that conventionally uses single-primer PCR to amplify random DNA fragments. RAPD analysis is now being routinely used in identification of duplicate collections in the germplasm. RAPD marker profiles were highly consistent and useful in establishing the cultivar identity.

2.3.1 RAPD for detection of genetic variability

RAPD analysis was used to determine the levels of genetic diversity among 22 accessions of bittergourd. Six of 47 screened primers gave reproducible RAPD patterns. One hundred and nine reproducible RAPD bands were generated across the investigated species. The dendrogram divided 22 accessions of bittergourd into 2 groups that were correlated to diameter size of the fruits (Pala, 2001).

Suresh (2004) investigated genetic diversity among 25 ivygourd genotypes using RAPD. A total of 41 RAPDs (average 1.46 bands per primer) were generated by 17 primers of which 39 were polymorphic.

Sureja et al. (2006) while analysing genetic diversity among nine parental lines of ashgourd using RAPD marker observed that five primers (19.2 per cent of primers used) showed high percentage (>50 per cent) of polymorphic bands and three primers (11.5 per cent of primers) showed low percentage (<15 per cent) polymorphic bands.

Morimoto et al. (2006) investigated genetic diversity of Kenyan landraces of the white-flowered gourd (*Lagenaria siceraria*), which exhibits tremendous morphological variation. RAPD analyses were performed on 53 landraces of the cultivated species *L. siceraria* and 42 accessions of three wild species (40 *L. sphaerica*, 1 *L. abyssinica* and 1 *L. breviflora*). A total of 432 polymorphic bands were detected using 54 primers. The four species were clearly differentiated from one another.

Molecular characterization of promising genotypes in bottlegourd including a novel segmented leaf type through RAPD was reported by Ram et al. (2006c). The results also demonstrate that the primers are genotype specific and obviously distinct type of germplasm based on a particular morphological variation conditioned by qualitative inheritance may not necessarily show wide diversity based on RAPD.

Dey et al. (2006) carried out genetic diversity studies in 38 bittergourd genotypes using RAPD markers. Among 116 random decamer primers screened, 29 were polymorphic and informative enough to analyse these genotypes. The clustering pattern based on yield related traits and molecular variation was different.

Resmi et al. (2007) used RAPD markers to characterize genetic variability and relationships among 25 landraces of ashgourd at molecular level. Landraces with morphologically distinct smooth and waxy textured fruits grouped into two major clusters.

Verma et al. (2007) analysed genetic diversity of ashgourd inbred lines based on RAPD. A total of 282 reproducible bands were produced by using 42 RAPD primers, out of these amplified fragments, 130 (46.1 per cent) were found to be polymorphic and the rest were monomorphic.

RAPD based grouping analysis revealed that Indian snapmelon was rich in genetic variation and region and sub-region approach should be followed across India for acquisition of additional melon landraces. Accessions of var. *agrestis* and var. *momordica* clustered together and there was a separate cluster of the accessions of var. *reticulatus* (Dhillon et al., 2007).

Rahaman et al. (2007) characterized 81 sweetgourd germplasm using RAPD as molecular markers. The UPGMA dendrogram demonstrated 8 clusters. Clustering of the germplasm based on morphological characters did not match with the clusters obtained from RAPD analysis. In case of RAPD analysis, no relationship was noticed between the genetic diversity and geographical distribution of the sweet gourd germplasm.

Genetic relationship and variation of 29 accessions of teasle gourd (*M. dioica* Roxb.) and 1 accession of *M. cochinchinensis* Spreng. (wild relatives of teasle gourd) were examined by 44 RAPD primers. In the phenetic dendrogram, *M. cochinchinensis* was out grouped as single accession, while others showing relatively weak grouping formed four

groups. Clustering pattern did not demonstrate any relationship between geographical origin and genetic diversity (Rasul et al., 2007).

A genetic analysis of 38 diverse Indian bittergourd accessions of *M. charantia* var. *charantia* (domesticated) and var. *muricata* (wild, free-living) was performed using 29 RAPD primers. RAPD yielded 208 amplicons of which 76 (36.5 per cent) were polymorphic providing an average of 2.6 amplicons per primer. The accessions examined were genetically distinct, and their differences provided for the development of strategies for genetic analyses and crop improvement (Behera et al., 2007b).

Rahman et al. (2007) evaluated genetic diversity of bittergourd accessions collected from different parts of Bangladesh based on RAPD markers. The UPGMA dendrogram based on genetic distance suggested 2 main clustering of 20 selected bitter gourd germplasm – 2 accessions forming cluster I, and the rest 18 accessions forming cluster II, where 16 accessions were grouped together in sub-cluster I, while 2 accessions formed sub-cluster II.

Behera et al. (2008) conducted diversity assessment using different DNA marker systems (RAPD, ISSR and AFLP) in detecting genetic diversity of bittergourd. The studies using RAPD and ISSR markers were not able to uniquely discriminate all the bittergourd accessions examined, whereas AFLP analysis was discriminatory and allowed for a more complete dissection of unique differences among accessions of bitter gourd within and between collection sites.

2.4 STANDARDIZATION OF *IN VITRO* TECHNIQUES

Tissue culture has opened a new vista and has proved to be an effective technique for faster multiplication of plants. Tissue culture

techniques indicate the scope and potential in exploiting the regenerating behaviour and cell totipotency of plants.

In vitro multiplication of elite clones will be an attractive and alternative approach to the conventional vegetative propagation methods. Morel (1960) first showed the potential of clonal propagation using *in vitro* culture techniques. Tissue culture technique provides a rapid and reliable system for production of a large number of genetically uniform disease-free plantlets. These plants are reported to grow faster and mature earlier than seed propagated plants (Vasil and Vasil, 1980).

According to Murashige (1974) there are three possible routes available for *in vitro* propagule multiplication *viz.,* (a) enhanced release of axillary buds (b) production of adventitious shoots through organogenesis and (c) somatic embryogenesis. In shoot tip culture, genetic uniformity is favoured. Callus mediated somatic organogenesis is not recommended for clonal propagation, but is ideal for recovery of useful variant lines. Somatic embryogenesis though limited to a few plant species is the most rapid mode of plant regeneration (Evans et al., 1981).

In recent year *in vitro* procedures are used to some degree in almost every major agronomic, vegetable and fibre crops. The success of such technology requires an efficient protocol for plant regeneration from isolated organs, tissues and cells. The plants belonging to the family Cucurbitaceae provide a major portion of vegetable and they need to be investigated for maximum utilization. Plantlet formation has already been reported in *Cucurbita pepo* (Jelaska, 1974), cucumber (Chee, 1990; Gambley and Dodd, 1990), watermelon (Dong and Jia, 1991) and squash (Rahman et al., 1993). *Momordica charantia* Linn. is an economically important crop among vegetable species and probably the most expensive summer vegetable in India. The attempt of *in vitro*

propagation of bittergourd was demonstrated by Islam et al. (1994) and Sultana and Bari Miah (2003). There have not been many studies conducted in the *in vitro* propagation of bittergourd. Hence the review highlights the research on the various techniques of *in vitro* propagation of cucurbits with special emphasis on enhanced release of axillary buds and callus mediated (indirect) somatic organogenesis.

2.4.1 Enhanced Release of Axillary Buds

Micropropagation by axillary bud proliferation has proved to be the most reliable method for large scale production of many crop plants (Satyakala et al., 1995). Axillary and apical shoots contain quiescent or active meristems depending on the physiological state of the plant. For shoot tip culture, relatively large explants (sometimes up to 1 cm in length), consisting of the shoot apical meristem and a number of leaf primordia and small unexpanded leaves at different stages of development are needed. The dominance of the apical meristem is removed and development of precocious axillary shoots is stimulated by incorporation of growth regulators (usually cytokinin) in the growth medium. This results in a highly branched shoot system (Jha and Ghosh, 2007). These clusters can further be subdivided into smaller clumps of shoots, which will form similar clusters when sub cultured on fresh medium. About 5-10 multiplication rates can be achieved on a regular four to eight week propagation cycle and can thus lead to rapid clonal propagation levels in the range of 0.10 to 3.00 $\times 10^6$.

There are many reports on *in vitro* regeneration of economically important members of the Cucurbitaceae. The regeneration techniques used by different researchers for cucurbits vary greatly with respect to types of explant used and types and concentrations of growth regulators applied even where the goals of the investigations were very similar

(Nadolska-Orczyk and Malepszy, 1984; Moreno et al., 1985; Trulson and Shahin 1986; Kathal et al., 1988; Niedz et al. 1989). Literature on cucurbits indicates a low rate of regeneration and survival of plants with abnormalities such as premature flowering (Gambley and Dodd, 1990). Regeneration from cotyledon, sections of hypocotyls and apical buds with varying regeneration frequency has been reported by Gambley and Dodd (1991). Huda and Sikdar (2006) developed standard protocol for obtaining healthy disease free bitter gourd plants by *in vitro* production through apical meristem culture.

2.4.1.1 Factors Affecting Enhanced Release of Axillary Buds

Morphogenesis and proliferation rate of culture depend on the key factors that include explant, culture medium, plant growth substances, media supplements added, mode of culture and culture conditions.

a. Explant

The proper choice of explants is of cardinal importance and makes an absolute difference between success and failure in inducing regeneration *in vitro*. The response varies according to the type, stage and physiological age of the explants. The commercial application of *in vitro* techniques in cucurbitaceous taxa has been well demonstrated and the regeneration of plants has been reported from excised cotyledons (Halder and Gadgil, 1982a; Gambley and Dodd, 1990; Singh et al., 1990), leaf explants (Mishra and Bhatnagar, 1995) and nodal segments (Debnath et al., 2000; Uddin, 2000).*Gambley and Dodd (1991) observed that cucumber* explants including at least part of the cotyledon, a short section of hypocotyl, and the apical bud, are capable of producing multiple axillary buds from the seedling apex and adventitious shoots from the hypocotyl base when

cultured on medium supplemented with kinetin. Removal of the apical bud triples the number of shoots produced from the apex of explants with two intact cotyledons but does not affect shoot production from explants with some or all of their cotyledons removed.

The micropropagation in squash has been reported by Cassells and Bajaj (1991) through shoot apices. Sheo et al. (1991) reported micropropagation using shoot tips or axillary buds with MS medium in pumpkin. Burza and Malepszy (1995) described rapid and efficient plant regeneration from leaf explants of *Cucumis sativus* and *C. anguria*.

Spestidis et al. (1996) described micropropagation using axillary buds in watermelon while, Kumar et al. (2002) reported the use of apical and axillary bud explants in *Coccinia grandis* and *Trichosanthes dioica*.

In squash (*Cucurbita pepo*), Ananthakrishnan et al. (2003) prepared the explants with half a cotyledon and a hypocotyl segment (including removal of apical bud) stimulate shoot regeneration.

Sultana and Bari Miah (2003) investigated in *M. charantia* that the nodal segments comparatively produced large number of multiple shoots than shoot tips when cultured on different BA and NAA hormonal combinations. Sikdar et al. (2003) also observed similar results and they reported that performance of the nodal explants for multiple shoot regeneration in bitter gourd was better than shoot tip.

Sarowar et al. (2003) developed an efficient *in vitro* micropropagation protocol was for direct shoot growth of *Cucurbita* hybrid variety using shoot–tips of 5-day-old explants.

In muskmelon (Keng and Hoong, 2005) and cucumber (Matakiadis and Kintzios, 2005), nodal segments proved to be good explants for micro

propagation. Sikdar et al. (2005) established a regeneration system of bittergourd using immature cotyledonary node.

Saha and Kazumi (2007) used cotyledonary node of 7 days old *in vitro* germinated seeds in bottle gourd while, Vasudevan et al. (2007) used embryonal axis explants from 2-day old *in vitro* germinated seeds in cucumber to induce multiple shoot production,.

In pointedgourd, Malek et al. (2007) indicated that the nodal explants were more suitable for shoot regeneration and multiplication and also maximum shoot elongation compared to those of the shoot tip explants.

Lee et al. (1995) investigated *in vitro* propagation of various muskmelons cultured from nodal explants. They found node from the upper part of the stem shooted well, shooting efficiency decreased with nodes taken from the lower part of the stem. These findings are reverse in case of results obtained for Malek et al. (2007) in pointedgourd.

b. Culture Medium

The plant cell culture medium is complex and contains nutritional components, which are essential for the growth and development of the cultured tissue. The optimal medium formulation for a particular application depends on the plant species used and the purpose of cell, tissue or organ culture resorted to (Wang and Charles, 1990).

The principal components of most plant tissue culture media are inorganic nutrients (macronutrients and micronutrients), carbon source (s), organic supplements, growth regulators and a gelling agent. Media components satisfy one or more functions in the *in vitro* growth of plants.

A number of media have been devised for specific tissues and organs. The earliest and widely used were White's (1943) and

Heller's (1953) basal media. Since 1960's, most researchers have been using MS (Murashige and Skoog, 1962), B5 (Gamborg et al., 1968) and SH (Schenk and Hildebrant, 1972) media. As MS medium is characterized by high concentration of mineral salts, some workers found it beneficial to reduce its strength (Griffis et al., 1981).

MS medium as the ideal medium for *in vitro* propagation of many cucurbits was reported by various workers (Jelaska, 1974; Barnes et al., 1978; Halder and Gadgil, 1982a; Niedz et al., 1989; Chee, 1990; Gambley and Dodd, 1990; Dong and Jia, 1991; Rahman et al., 1991, 1993; Nabi et al, 2002; Sarowar et al., 2003; Keng and Hoong, 2005; Malek et al., 2007; Saha and Kazumi, 2007; Vasudevan et al., 2007; Li et al., 2008).

Sultana et al. (2005) reported that *in vitro* axillary shoot multiplication of bittergourd could be established in MS medium. Sikdar et al. (2005) cultured immature cotyledonary nodes of bittergourd plants on MS medium. Huda and Sikdar (2006) noticed that MS medium supplemented with different concentrations of growth regulators was ideal for culturing apical meristems of bittergourd *in vitro*.

c. Plant Growth Regulators

Plant growth regulators (PGRs) are the critical media components in determining the developmental pathway of the plant cells. The PGRs used most commonly are plant hormones and their synthetic analogues (Hota, 2007).

The growth and morphogenesis *in vitro* are regulated by the interaction and balance between the growth regulators supplied in the medium and the growth substances produced endogenously by the cultured

cell. Commonly used growth regulators in tissue culture include auxins, cytokinins, gibberellins and abscissins (Razdan, 2003).

Murashige (1974) reported cytokinin as ideal growth regulator for the axillary shoot proliferation by overcoming the apical dominance. BAP has been the most effective cytokinin for meristem, shoot tip and bud cultures followed by kinetin. The role of exogenous auxin in culture proliferation medium is to nullify the suppresive effect of high cytokinin concentrations on axillary shoot elongation, there by resorting normal shoot growth (Lundergan and Janick, 1980). For axillary bud prolifeation, exogenous auxin was not always needed. Although exogenous auxins do not induce axillary shoot proliferation, culture growth has been improved by their presence (Wang and Hu, 1980). Naturally occuring auxins like IAA, IBA and syntheitc forms like NAA; 2,4-D; 2,4,5-T are regularly used in tissue culture experiments. Gibberellins are involved in regulating cell elongation and GA_3 is the most common gibberellin used in tissue culture media.

The *in vitro* studies in crop plants show that there is a differential response of various explants to different concentrations of auxins and cytokinins. High cytokinin to auxin ratio generally induced multiple shoot formation while high auxin to cytokinin ratio usually induced rhyzogenesis (George and Sherrington, 1984).

In general, herbaceous plants are highly responsive to BAP treatments and most of the cultures produce robust well formed shoots suitable for further shoot proliferation (Debergh and Zimmerman, 1991). The ability of BAP to induce axillary branching is well documented (George, 1993).

In watermelon, Ahad et al. (1994) observed that the MS medium with BA (0.5-1 mg l^{-1}) and IAA (0.1 mg l^{-1}) was suitable for adventitious

shoot regeneration and with addition of 100 mg l^{-1} casein hydrolysate improved shoot regeneration efficiency. Hoque et al. (1995) found that a combination of 1.5 mg l^{-1} BA and 0.1 mg l^{-1} NAA was more suitable for adventitious multiple shoot formation in teasle gourd.

In pointed gourd, Hoque et al. (1998) achieved shoot formation in seedling explants when cultured on MS medium supplemented with 4.44 µM BA while, higher number of shoots per explant (5.78), number of nodes/shoot (6.11) and longest shoot (3.57 cm) were observed on the medium containing 8.88 µM BA by Kumar et al. (2003).

Sarowar et al. (2003) conducted i*n vitro* micropropagation of *Cucurbita* cultivar and reported that the best condition for shoot growth was with 3 mg l^{-1} 6-benzyladenine (BA) in MS medium. The shooting frequency was 84% and five shoots were obtained from each explant after 30 days of culture.

Sultana and Bari Miah (2003) reported that in bittergourd, 2.0 mg l^{-1} BA + 0.2 mg l^{-1} NAA was optimal for the multiple shoot regeneration from nodal segments *in vitro*. In this combination, the frequency of multiple shoot regeneration response was 95% and the average number of shoots per explant was 8.20±0.26.

Sultana et al. (2004) reported that MS medium supplemented with 1.0 mg l^{-1} BA + 0.2 mg l^{-1} NAA was found to be the best medium for the production of multiple shoots in watermelon.

MS medium supplemented with 2.0 mg l^{-1} BAP + 0.1 mg l^{-1} IAA + 2.0 mg l^{-1} GA_3 was the most effective medium for shoot proliferation of bittergourd (Sikdar et al., 2005).

NAA, an auxin was not necessary for shoot proliferation of musk melon (Keng and Hoong, 2005). In their experiment, nodal segments that

were cultured on MS medium supplemented with 8.0 mg l^{-1} BA without NAA produced the most multiple shoots with an average of 17.9 shoots per nodal segment.

Huda and Sikdar (2006) observed that the growth of meristem was maximum when they were cultured on MS medium supplemented with 0.05 mg l^{-1} Kn + 0.1 mg l^{-1} GA_3. Best formulation for shoot initiation with elongation was 1.0 mg l^{-1} BAP + 0.1 mg l^{-1} IBA + 0.3 mg l^{-1} GA_3.

In pointedgourd, Malek et al. (2007) reported that highest percent of shoot regeneration was 93.86 when nodal explants were cultured on MS + 2.0 mg l^{-1} BAP. The maximum number of shoots (4.00) per explant was observed in MS + 2.0 mg l^{-1} BAP + 0.3 mg l^{-1} NAA from nodal segment.

Saha and Kazumi (2007) conducted *in vitro* micropropagation of bottle gourd and found that the highest number of shoots (7 shoots/explant) was obtained when cultured in MS medium supplemented with 2 mg l^{-1} BA. Kinetin showed the highest percentage of shoot regeneration from bud, ranging from 92.9 to 1000, but induced less buds as well as less shoots, 4.25 shoots/explants.

d. Carbon Source

Plant cells and tissues in culture medium lack autotrophic ability and therefore need external carbon for energy. The addition of an external carbon source to the medium enhances proliferation of cells and regeneration of green shoots. Sucrose is the most preferred carbon source in plant tissue culture. The concentration of sucrose varied from 20-30 g l^{-1}. Sucrose also acts as an osmoticum that can stimulate and regulate morphogenesis (Brown et al., 1979; Wethrell, 1984; George and Sherrington, 1984).

In bittergourd, Sultana et al. (2005) reported that the media having 30 g l^{-1} sucrose showed highest percentage of explants responded to shoot proliferation and that was 100 per cent. This sucrose concentration also showed the optimum result of total number of shoot per culture, number of usable shoots per culture and average length of shoots. At 40 and 50 g l^{-1} sucrose concentrations, the shoot size was bigger but its number decreased and root growth was inhibited.

Application of 3.00 per cent sucrose as best for *in vitro* propagation was also reported in cucumber (Ahmad and Anis, 2005), musk melon (Keng and Hoong, 2005) and bottlegourd (Saha and Kuzami, 2007).

e. Hexitols

Myo-inositol is a hexitol. Most tissue culture media have this compound. It serves various purposes in sugar transport, carbohydrate metabolism, membrane structure and cell wall formation etc.

f. Natural Complexes

Natural complexes are mostly undefined substances. Inclusion of natural complexes is not essential or may not be critical but is often beneficial. Coconut milk or liquid endosperm of *Cocos nucifera* is used in many tissue culture works since 1940.

g. Solidifying Agent

Gelling or solidifying agents are commonly used for preparing semisolid and solid tissue culture media. They support the tissues growing in static conditions. Agar is the most commonly used gelling agent in plant tissue culture work. A change in agar concentration affects the nutrients in it as well as the overall nutrient concentration in the

experiment (Skirvin 1981; Debergh, 1983). Whether explants grow better on agar or other supporting agents depend on the tissue and the species (Razdan, 2003).

In bittergourd, Sultana et al. (2005) observed that highest response of shoot proliferation was in MS medium having 7 g l^{-1} of agar and the frequency was 100 per cent. But, Sikdar et al. (2005) applied agar 6.00 g l^{-1} in MS medium for the micropropagation in bittergourd. Agar 6.00 g l^{-1} was best for solidifying the media, for the purpose of *in vitro* production through apical meristem (Huda and Sikdar, 2006).

h. Activated Charcoal

The addition of activated charcoal (AC) to culture media is reported to stimulate growth and differentiation in carrot, ivy and tomato. Paradoxically, its effect on soybean has proved inhibitory. Inhibition of growth is attributed to the absorption of phytohormones to AC whereas stimulation could be due to any one of the factors, namely, adsorption of inhibitory compounds to AC and darkening of the medium. It also helps to reduce toxicity by removing toxic compounds (e.g., phenols) produced during the culture and permits unhindered cell growth (Mohamed-Yasseen et al., 1995).

i. Culture Conditions

Murashige (1977) reported that light intensity, quality and duration affect the growth of *in vitro* grown cultures. According to him, the optimum light intensity for shoot formation in a large number of herbaceous species is around 1000 lux.

Sultana et al. (2005) reported that bittergourd cultures could be kept at 26±1°C under 16 hour photoperiod with a photon flux density of about 70 µ mol m^{-2} s^{-1}.

Ahmad and Anis (2005) maintained cultures of cucumber at 25± 2°C under a 16 hour photoperiod provided by cool white fluorescent tubes with light intensity of 2000 Lux. Keng and Hoong (2005) reported that muskmelon cultures could be kept at 25± 2°C under continuous cool white fluorescent lights with a photon flux density of 2500-3000 Lux.

Huda and Sikdar (2006) incubated apical meristem cultures of bittergourd at 25±2°C under 16 hour photoperiod.

In pointedgourd, Malek et al. (2007) placed the cultures in the temperature set on 26±1°C with a light intensity of 2000-3000 Lux from fluorescent tubular lamps. The maintained photoperiod was 16 hours light and 8 hours dark (16 L/8 D) and relative humidity of 60-70%.

Ananthakrishnan et al. (2007) noticed that squash cultures could be placed under 25±1°C, 16 hour photoperiod, 30–40 μ mol m^{-2} s^{-1} cool white fluorescent light.

2.4.2 Callus Mediated (Indirect) Somatic Organogenesis

Indirect somatic organogenesis is not adventive and requires the redetermination of the differentiated cells leading to callus formation. Separate shoot and root initials are characteristically formed in callus cultures (George and Sherrington, 1984). Exogenously supplied phytohormones not only control the process but are required for organogenesis to occur. Levels of plant growth regulators in the culture medium, particularly high auxin and low cytokinins often lead to callus formation. Callus culture is often performed in the dark as light can encourage differentiation of the callus. Disadvantage of callus culture is that the propagules may not be true to type and a callus phase may result in genotypic and phenotypic abnormalities.

The organogenic route of regeneration from callus culture was reported in *M. charantia* (Halder and Gadgil, 1982b). Dong and

Jia (1991) had successfully produced *in vitro* plantlets of watermelon using callus derived from cotyledon. Sultana et al. (2004) produced *in vitro* plantlets of watermelon from the callus culture of leaf segments.

An adverse effect of prolonged *in vitro* culture is reported (Jureti and Jelaska, 1991; Ficcadenti and Rotino, 1995). Thomas and Sreejesh (2004) in ashgourd and Pal et al. (2007) in summer squash observed that friable and creamy calli derived from hypocotyl can be used to initiate organogenic calli and shoot-bud induction with subsequent regeneration.

2,4-D is among the most widely used auxins for *in vitro* callus induction in a wide range of plant species. In summer squash, Leljak-Levanic et al. (2004) and Pal et al. (2007) achieved successful induction of potentially organogenic callus from hypocotyl and cotyledons using 2,4-D.

Kim et al. (1988) reported that cotyledonary explants from cucumber formed callus tissue on MS medium supplemented with 0.5 µM 2,4-D and 5 µM BA.

Srivastava et al. (1989) obtained organogenic calli in watermelon using a combination of BAP and NAA. In cucumber, Selvaraj et al. (2007) produced green, compact nodular organogenic callus in MS medium containing NAA (2.69 µM) and BA (4.44 µM).

Molvig and Rose (1994) suggested that GA_3 along with a cytokinin stimulates the development of shoot primordia induced by 2,4-D. A similar effect of this combination was noted by Ananthakrishnan et al. (2003) and Pal et al. (2007) in *C. pepo*.

Moreno et al. (1985) observed in melon that, in response to 1.5 mg l^{-1} IAA and 6.0 mg l^{-1} Kn, more than 90% of the calli produced well-developed shoots. In cucumber, Kim et al. (1988) achieved

shoot development from callus on using MS medium supplemented with 0.5 µM NAA and 5 µM BA.

In cucumber, Selvaraj et al. (2007) produced adventitious shoots from the organogenic callus when it was transferred to MS medium supplemented with NAA (1.34 µM), BA (8.88 µM), zeatin (0.91 µM) and l-glutamine (136.85 µM) with shoot induction frequency of 75.6%.

2.4.3 *In vitro* rooting

Rooting of *in vitro* produced plantlets is a very important part of plant tissue culture. For successful micro propagation, healthy, strong and functional root system is required. Rooting response variation may be affected by different conditions of the shoots used for root induction, variations in the medium used for multiplication before root induction, the number of subcultures before root induction and the culture period on multiplication medium before transfer to root induction medium. The differences in rooting response may be a result of genotype or cultural conditions.

In most of the cucurbits the root induction was achieved on either basal MS medium alone or with very low level of auxin (Mythili and Thomas, 1999). Ananthakrishnan et al. (2003) in *C. pepo*, Lee et al. (2003) in *C. maxima,* Keng and Hoong (2005) in muskmelon and Li et al. (2008) in cucumber reported that all the *in vitro* shoots produced root when transferred to MS medium without any plant growth regulator.

NAA singly was found to be effective in the induction of root at the base of regenerated shoots in different plants like *M. charantia* (Islam et al., 1994) and *Trichosanthes dioica* (Uddin 2000; Debnath et al., 2000). Sultana and Bari Miah (2003) in bittergourd observed that the best root development was obtained in the rooting medium containing half strength MS medium with 0.5 mg l^{-1} NAA.

Rooting of isolated *in vitro* raised microshoots of cucumber was readily achieved with NAA (1.0 µM) in half MS (Ahmad and Anis, 2005).

IBA is a widely used plant growth regulator for root induction in cucurbits (Abrie and Staden, 2001; Sarowar et al., 2003; Thomas and Sreejesh, 2004; Krug et al., 2005). Efficiency of IAA in root induction was observed in bottlegourd (Saha and Kazumi, 2007).

In pointedgourd, Kumar et al. (2003) reported that among the media tested for root induction a very low concentration of IBA (0.49 µM) produced significantly higher number of roots per shoot. Higher concentrations of IBA as well as a very low level of NAA (0.27 µM) favoured formation of malformed and thick roots, which were not suitable for pot transfer. Sarowar et al. (2003) in *Cucurbita*, Pal et al. (2007) in summer squash and Vasudevan et al. (2007) in cucumber found that the elongated shoots rooted on MS medium with IBA.

For root formation the MS semisolid medium fortified with 0.1 mg l^{-1} NAA and 0.5 mg l^{-1} IBA was found to be the best formulation for bittergourd (Huda and Sikdar, 2006).

In cantoloupe, Nagesha et al. (2007) reported that MS basal medium with 1.2 mg l^{-1} BAP had the distinguishing feature of producing roots simultaneously with the shoots from the explants. This overcame the need of a separate rooting media.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study entitled "Characterization and *in vitro* multiplication of bittergourd (*Momordica charantia* L.) genotypes" was carried out at the Department of Olericulture, utilizying the facilities of the Departments of Plant Biotechnology and Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during 2005-2008. For morphological characterization, the experimental field (Plate 1) was laid at an area located at 8.5°N latitude, 76.9°E longitude at an altitude of 29.0 m above MSL. The site has a lateritic red loam soil with a pH of 5.2. The area enjoys a humid tropical climate.

The study consisted of the following experiments.

3.1 Morphological characterization

3.1.1 Genetic cataloguing of bittergourd

3.1.2 Genetic variability and divergence

3.2 Biochemical characterization

3.3 Molecular characterization

3.4 Standardization of *in vitro* techniques of bittergourd

3.1 Morphological characterization

3.1.1 Genetic Cataloguing of Bittergourd

The basic material for the study included 33 genotypes of bittergourd collected from different agroclimatic regions of the country through survey and correspondence. The details of the genotypes and their sources are presented in Table 1. The descriptor developed by IBPGR (1983) for cucurbits was used for cataloguing (Table 2).

Plate 1. General views of experimental field and different genotypes of *M. charantia* with its fruits

1

2

3

Plate 1. Continued

MC 1

MC 2

MC 3

MC 4

MC 5

MC 6

MC 7

MC 8

MC 9

Plate 1. Continued

MC 10

MC 11

MC 12

MC 13

MC 14

MC 15

MC 16

MC 17

MC 18

Plate 1. Continued

MC 19

MC 20

MC 21

MC 22

MC 23

MC 24

MC 25

MC 26

MC 27

Plate 1. Continued

MC 28

MC 29

MC 30

MC 31

MC 32

MC 33

Table 1. Particulars of genotypes of *Momordica charantia* used in the study and their sources

Sl. No.	Accession No.	Source
1	MC 1	Thiruvalla, Pathanamthitta, Kerala
2	MC 2	CO-1, Tamil Nadu Agricultural University, Coimbatore
3	MC 3	IC 68314, National Bureau of Plant Genetic Resources , Thrissur
4	MC 4	Preethi, Kerala Agricultural University, Thrissur
5	MC 5	Kalpetta, Wayanad, Kerala
6	MC 6	Pusa Do Mausami, Indian Agricultural Research Institute, New Delhi
7	MC 7	Kuzhipalam, Thiruvananthapuram, Kerala
8	MC 8	IC 85632, National Bureau of Plant Genetic Resources , Thrissur
9	MC 9	Anchal, Kollam, Kerala
10	MC 10	MDU-1, Tamil Nadu Agricultural University, Madurai
11	MC 11	Arka Harit, Indian Institute of Horticultural Research, Bangalore
12	MC 12	Konkan Tara, Konkan Krishi Vidyapeeth, Dapoli
13	MC 13	IC 85650, National Bureau of Plant Genetic Resources , Thrissur
14	MC 14	IC 85603, National Bureau of Plant Genetic Resources , Thrissur
15	MC 15	Priya, Kerala Agricultural University, Thrissur
16	MC 16	Haripad, Alappuzha, Kerala
17	MC 17	IC 85627, National Bureau of Plant Genetic Resources , Thrissur
18	MC 18	IC 50523, National Bureau of Plant Genetic Resources , Thrissur
19	MC 19	Kattakada, Thiruvananthapuram, Kerala
20	MC 20	Priyanka, Kerala Agricultural University, Thiruvalla
21	MC 21	Vellathuval, Idukki, Kerala
22	MC 22	Chathamangalam, Kozhikode, Kerala
23	MC 23	IC 113878, National Bureau of Plant Genetic Resources , Thrissur
24	MC 24	IC 85636, National Bureau of Plant Genetic Resources , Thrissur
25	MC 25	IC 470569, National Bureau of Plant Genetic Resources , Thrissur
26	MC 26	Thripunithara, Ernakulam, Kerala
27	MC 27	Charuplasseri, Palakkad, Kerala
28	MC 28	Kadakkal, Thiruvananthapuram, Kerala
29	MC 29	IC 68326, National Bureau of Plant Genetic Resources , Thrissur
30	MC 30	Chennai, Tamil Nadu
31	MC 31	IC 85642, National Bureau of Plant Genetic Resources , Thrissur
32	MC 32	IC 85612, National Bureau of Plant Genetic Resources , Thrissur
33	MC 33	Pala, Kottayam, Kerala

Table 2. Genetic cataloguing of *Momordica charantia*

1. Vegetative characters
Growth habit – Less viny/Moderately viny/Highly viny
Tendril – Present/Absent
Density of foliage hairs per branch – Few/Moderate/Dense
Leaf size – Small/Medium/Large
Leaf shape – Ovate/Pedate/Reniform
Leaf lobes – No lobes/Shallowly lobed/Deeply lobed
Leaf dorsal surface pubescence – Soft hairy/Bristle like
2. Flowers and fruits
Flower size – Very small/Small/Medium/Large/Very large
Fruit size – Very small/Small/Medium/Large/Extra large
Fruit form – Round/Oval/Cylindrical/Globular/Elliptical/Elongate/ Elongate flattened/Elongate bottlelike
Skin texture –Smooth/ Rough/ Waxy/ With spines
Skin colour – White/Green/Light green/Dark Green/Others
Fruitshape at stemend – Deeprounded/ Rounded/ Flattened/ Taperpoint
Fruitshape at blossomend - Deeprounded/ Rounded/ Flattened/ Taperpoint
Peduncle length – Short/Medium/Long
Peduncle detachment from fruit – Easily/Difficult
3. Seeds
Seed quantity per fruit – Very few (<5)/ Few (5-15)/ Intermediate (15-20)/ Many (20-30)/ Very many (>30)
Seed size – Small/Medium/Large/Very large
Seed coat colour – Whitish yellow/Yellow/Brown
Seed surface lushe – Dull/Glossy
Seed separation from placenta – Easily/Medium/Difficult

3.1.2 Genetic Variability and Divergence

Thirty three genotypes of bittergourd were grown during May 2006 to September 2006, to identify superior genotypes with yield, quality and reaction towards the incidence of pests and diseases.

Statistical details were as furnished below:

Design	: RBD
Replications	: 2
Treatments	: 33 genotypes
Spacing	: 2.0 × 2.0 m
Number of plants per plot	: 8 (4 pits; 2 plants per pit)

The cultural and management practices were adopted according to package of practices recommendations of Kerala Agricultural University (KAU, 2007).

3.1.2.1 Observations

Four plants per genotypes per replication were selected for taking observations and the mean worked out for each replication as per standard procedures. Four fruits per genotypes per replication were selected for taking observations of fruit characters.

3.1.2.1.1 Plant characters

a. Days to seedling emergence

Number of days taken from sowing to emergence of seedling.

b. Vine length (cm)

Measured from the collar region to the tip of the main vine using the measuring tape after pulling out the vine at the time of last harvest.

c. Internodal length (cm)

Distance between 10^{th} and 11^{th} nodes of the vine.

d. Number of primary branches

The number of primary branches per plant counted at the full maturity of the plant.

e. Number of secondary branches

The number of secondary branches per plant counted at the full maturity of the plant.

3.1.2.1.2 Flowering characters

a. Days to first male flower

The number of days from sowing of seeds to the opening of the first male flower.

b. Node to first male flower

Node of the first male flower counting from the first true leaf.

c. Days to first female flower

The number of days taken from sowing to the bloom of the first female flower.

d. Node to first female flower

Number of nodes from the base of the plant to the node where the first female flower appeared.

e. Sex ratio

Number of male and female flowers were counted starting from the commencement of flowering till its completion and expressed as male to female ratio.

$$\text{Sex ratio} = \frac{\text{Number of male flowers}}{\text{Number of female flowers}}$$

3.1.2.1.3 Fruit characters

a. Days to first fruit harvest

Number of days taken from sowing to the harvest of the first formed fruit.

b. Fruit length (cm)

The length of the fruit measured from the stalk end to the blossom end.

c. Fruit girth (cm)

The girth at the middle of the same fruit used for the length measurement.

d. Fruits per plant

The total number of fruits produced on a single plant observed.

e. Average fruit weight (g)

Weight of four fruits from each genotype from each replication were taken and average worked out.

f. Yield per plant (kg)

Weight of whole fruits from each plant of the genotypes.

g. Seeds per fruit

One well-ripened fruit from each plant was selected at random and seeds with the mucilage were extracted carefully. It was washed, cleaned and dried under shade for three to four days and number of seeds were counted.

h. One hundred seed weight (g)

The dry weight of randomly selected 100 seeds were taken.

3.1.2.1.4 Quality characters

1. Keeping quality (days)

The harvested fruits were kept under ordinary room conditions to study their shelf life and the number of days up to which the fruits remained fresh for consumption without loss of colour and firmness as suggested by Veenakumari et al. (1994). For recording colour change of fruits during storage the following score chart was used.

Characteristic of fruit	Score
Green colour, fresh and firm without any symptoms of shrinkage	0
Green colour with shriveled appearance	1
Slight yellowing starting from the tip	2
50 per cent yellowing	3
While fruits turning yellow, rind remained firm	4
Whole fruit yellow, soft and decayed	5

2. Organoleptic analysis

The organoleptic quality and acceptability traits were done using a scoring method proposed by Jijiamma (1989). The major quality attributes included in the score were colour, doneness, flavour, taste and bitterness (Appendix 1). Each of the mentioned quality was assessed by a four point rating scale.

The fruits were thoroughly washed in water and cut into pieces. 100 g cut fruits were boiled in 100 ml water and 1 g salt for 7 minutes. The prepared sample was used for organoleptic quality scoring.

The panel members were selected from a group of healthy adults in the age group of 25 to 35. They were requested to taste each sample and score it. Each quality was assessed by the panel member after tasting the same sample several times if needed.

3.1.2.1.5 Scoring for pests and diseases

The incidence of major pests and diseases was recorded under natural field conditions.

1. Incidence of fruit fly

Characterization of fruit fly incidence was done as suggested by Nath (1966).

The incidence of fruit fly on fruits was assessed by calculating percentage of infested fruits over total number of healthy fruits at different pickings (Plate 2). Pest rating was done as per the following scale

% of fruit infestation	Score
0-20	1
21-40	2
41-60	3
61-80	4
81-100	5

2. Scoring for mosaic virus incidence

The rating scale given by Rajamony et al. (1990) in melon was used for scoring with minor modifications. This was done according to the characteristic symptoms of the individual plant (Table 3 and Plates 3 to 6). The scoring was done 60-75 days after sowing.

Table 3. Scoring for mosaic virus incidence

Rating scale	Symptom	Category
0	No symptom	Highly resistant
1	Very light mottling of green colour	Resistant
2	Mottling of leaves with light and dark green colour	Moderately resistant
3	Blisters and raised surface on the leaves	Moderately susceptible
4	Distortion of leaves	Susceptible
5	Stunting of the plants with negligible or no flowering	Highly susceptible

The individual plant score was utilized to work out the 'Severity Index' or 'Vulnerability Index' (V.I.), so as to measure the resistance. The vulnerability index was calculated using an equation adopted by Silbernagel and Jafri (1974) for measuring resistance in snap bean (*Phaseolus vulgaris*) to beet curly top virus and modified later by Bos (1982).

$$\text{Vulnerability Index (V.I.)} = \frac{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5)}{n_t\,(n_c-1)} \times 100$$

where,

$n_0, n_1, \ldots, n_5$ = number of plants in category 0,1,…,5 respectively

n_t = total number of plants

n_c = total number of categories = 6

The Vulnerability Index was used to classify the genotypes into different categories.

Sl. No.	Vulnerability Index	Category
1	0-20	Highly resistant
2	21-40	Resistant
3	41-60	Moderately resistant
4	61-80	Susceptible
5	81-100	Highly susceptible

Plate 2. Symptoms and fruit fly infestation on different bittergourd genotypes

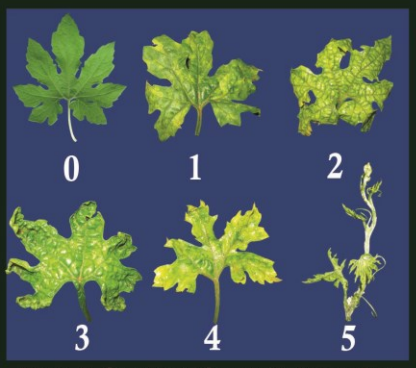


Plate 3. Scoring of mosaic intensity on the leaves of *M. charantia*

Plate 4. MC 33 - A genotype found highly resistant in the field

Plate 5. MC 27 - A genotype found moderately resistant in the field

Plate 6. MC 17 - A genotype found highly susceptible in the field

3.1.2.1.6 Statistical analysis

1. Analysis of variance (ANOVA) and covariance (ANCOVA) for Randomized Block Design (RBD) in respect of the various characters was done (Panse and Sukhatme, 1985).

2. Mean : The mean of the character X_i ($\overline{X}_i$) was worked out.

3. Variability components (phenotypic and genotypic) for different characters was estimated as suggested by Kempthorne (1977).

(a) The variance and covariance components were calculated as per the following formulae :

For the character X_i,

$$\text{Environmental variance, } \sigma_{ei}^2 = \text{MSE}$$

$$\text{Genotypic variance, } \sigma_{gi}^2 = \frac{\text{MST - MSE}}{r}$$

$$\text{Phenotypic variance, } \sigma_{pi}^2 = \sigma_{gi}^2 + \sigma_{ei}^2$$

where, MST and MSE are respectively, the mean sum of squares for treatment and error from ANOVA and 'r', the number of replications.

For two characters X_i and X_j,

$$\text{Environmental covariance, } \sigma_{eij} = \text{MSPE}$$

$$\text{Genotypic covariance, } \sigma_{gij} = \frac{\text{MSPT - MSPE}}{R}$$

$$\text{Phenotypic covariance, } \sigma_{pij} = \sigma_{gij} + \sigma_{eij}$$

where, MSPT and MSPE are respectively, the mean sum of products between the i^{th} and j^{th} characters for genotypes and environment respectively from Analysis of Covariance (ANCOVA).

(b) Coefficient of variation

Variability that existed in the population for various characters were apportioned using the estimates of coefficient of variation (Singh and Choudhary, 1985).

For the character X_i,

$$\text{Phenotypic coefficient of variation, PCV} = \frac{\sigma_{pi}}{\overline{X_i}} \times 100$$

$$\text{Genotypic coefficient of variation, GCV} = \frac{\sigma_{gi}}{\overline{X_i}} \times 100$$

$$\text{Environmental coefficient of variation, ECV} = \frac{\sigma_{ei}}{\overline{X_i}} \times 100$$

where, σ_{pi}, σ_{gi} and σ_{ei} are respectively the phenotypic, genotypic and environmental standard deviations with respect to each character.

4. Heritability

Hanson et al. (1956) proposed the mathematical relationship of variance estimates on computation of heritability, which is usually expressed as a percentage.

$$\text{Heritability (broad sense), } H^2 = \frac{\sigma_{gi}^2}{\sigma_{pi}^2} \times 100$$

The range of heritability was categorized as suggested by Robinson et al. (1949) namely, low (0 – 30 per cent), moderate (31 – 60 per cent) and high (61 per cent and above).

5. Genetic advance

Genetic advance as percentage over mean was calculated as per the formula given by Lush (1949) and Johnson et al. (1955).

$$\text{Genetic advance, GA} = \frac{kH^2\,\sigma_{pi}}{\overline{X_i}} \times 100$$

where, H^2 - heritability in broad sense.

σ_{pi} - phenotypic standard deviation

k - selection differential which is 2.06 in case of 5 per cent selection in large samples (Miller et al., 1958; Allard, 1960).

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

6. Correlation analysis

Phenotypic, genotypic and environmental correlation coefficients were worked out according to the procedure suggested by Singh and Choudhary (1985).

7. Path analysis

The direct and indirect effects of yield contributing factors were estimated through path analysis technique (Wright, 1954; Dewey and Lu, 1959).

8. Mahalanobis's D^2 analysis

Genetic divergence was studied based on eleven characters taken together using Mahalanobis's D^2 statistic as described by Rao (1952). The genotypes were clustered by Tochers method.

9. Selection index

The selection index developed by Smith (1936) using the discriminant function of Fisher (1936) was used to discriminate the genotypes based on eleven characters. The selection index is described by the function

$$I = b_1 X_1 + b_2 X_2 + \ldots b_k X_k$$

The function $H = a_1G_1 + a_2G_2 + \ldots a_kG_k$ describes the merit of a plant, where $X_1, X_2,\ldots, X_k$ are the phenotypic values and $G_1, G_2,\ldots, G_k$ are the genotypic values of the plant with respect to the characters $X_1, X_2,\ldots, X_k$. H denotes the genetic worth of the plant. The economic worth assigned to each character is assumed to be equal to unity i.e., $a_1, a_2,\ldots, a_k = 1$. The regression coefficients $b_1, b_2, \ldots, b_k$ are estimated in such a way that the correlation between H and I is maximum. The procedure will reduce to an equation of the form $b = P^{-1}Ga$, where P and G are the phenotypic and genotypic variance-covariance matrices respectively. Based on the 'b' estimates and the mean values for the eleven characters with respect to each genotypes, scores were calculated and the genotypes were ranked.

3.2 Biochemical characterization

The nutritional quality characters like beta-carotene, vitamin C and iron of 33 genotypes were analysed. The presence of photosynthetic pigments *viz.*, chlorophyll a, chlorophyll b and total chlorophyll in fruits were estimated. Grouping the genotypes into bitter and non-bitter categories was done by determining the bitterness value.

1. Beta-carotene

β-carotene content of fruits was estimated by following the method of Srivastava and Kumar (1998).

2. Ascorbic acid content (Vitamin C)

Ascorbic acid content of fruits was estimated by 2,6-dichlorophenol indophenol dye method (Sadasivam and Manickam, 2002).

3. Iron

Fruit samples were taken from each plot from the middle harvest, dried and powdered. Iron content of fruit samples was estimated by Atomic Absorption Spectrophotometer after wet digestion of the sample using di-acid mixture as suggested by Perkin-Elmer Corporation (1982).

4. Photosynthetic pigments (Chlorophyll, mg/g tissue)

The photosynthetic pigments *viz.*, chlorophyll a, chlorophyll b and total chlorophyll were estimated in fruit samples of all the thirty three genotypes by following the method of Arnon (1949).

5. Bitterness

The bitter properties of fruits were determined by comparing the threshold bitter concentration of an extract of the fruit material with that of a dilute solution of quinine hydrochloride R. The bitterness value was expressed in units equivalent to the bitterness of a solution containing 1 g of quinine hydrochloride R in 2000 ml (WHO, 2002).

3.3 Molecular characterization

3.3.1 Materials

Molecular characterization of thirty three genotypes of bittergourd was carried out using RAPD markers. Young leaf samples from each genotype was collected and used for DNA extraction.

3.3.2 Methods

1. Isolation of genomic DNA

The method of isolation of genomic DNA followed was modified from that of Murray and Thompson (1980). Briefly 0.5 g of leaf material was first washed in running tap water and later in distilled water two or

three times after chopping the leaves coarsely. After wiping off the water using tissue paper, the chopped leaves were pulverized in liquid nitrogen in a pre-cooled mortar by rapid grinding to a fine powder. Dry powder of plant material was transferred to a 2.0 ml centrifuge tube and enough extraction buffer (0.7 N NaCl, 2% CTAB, 50 mM Tris HCl (pH 8.0), 10 mM EDTA) was added to it so that clumps can easily be dispersed but the solution remains somewhat viscous. For this, 1.0 ml per 30-100 mg dry weight of powder was required. 200-300 µl PVP and 50-100 µl β-mercaptoethanol was also added to the centrifuge tube and was incubated in waterbath at 60°C for 45 minutes with occasional gentle shaking. The mixture was then subjected to centrifugation at 5000 rpm for 5 minutes. The clear supernatant was taken and the remaining extraneous matter was discarded. After that one-third volume of Phenol : Chloroform: Isoamyl alcohol (25:24:1) solution was added to the centrifuge tube, the two phases were mixed gently and centrifuged at 5000 rpm for 5 minutes at 4.0°C. Then the supernatant was collected and to this one-third volume of Chloroform : Isoamyl alcohol (24:1) solution was added and centrifuged as in the previous step after thorough mixing. After collecting the upper phase, again the Chloroform : Isoamyl alcohol (24:1) extraction was repeated until the interphase disappeared. After that to the supernatant, one-tenth volume of 3.0 M Sodium acetate followed by double volume of chilled absolute isopropyl alcohol were added. It was stored at -20°C overnight to precipitate the DNA. The mixture was then centrifuged at 5000 rpm for 4 minutes at 4°C to pellet the DNA. The supernatant was discarded and the pellet was washed in 50 per cent ethanol. Then it was centrifuged at 5000 rpm for 4 minutes at 4°C. The supernatant was again discarded and the pellet was air dried for 20 minutes. Then the pellet was dissolved in 0.5 ml of 1x Tris EDTA buffer (10 mM Tris HCl, 1 mM EDTA, pH 8) and stored at -20°C.

All the materials used in the preparation and storage of reagents including reagent bottles, conical flasks, centrifuge tubes, spatula, glassrodes and tips of micropipettes were washed with Labolin solution and rinsed with distilled water and autoclaved.

2. Quantification of DNA

The quantification of DNA is necessary before it is subjected to amplification by PCR. DNA quantification was carried out with the help of UV-Vis Spectrophotometer (Spectronic Genesys 5).

The buffer in which the DNA was already dissolved was taken in a cuvette to calibrate the Spectrophotometer at 260 and 280 nm wavelength. The optical density (O.D.) of the samples dissolved in the buffer was recorded at both 260 and 280 nm.

The quantity of DNA in the sample was estimated by employing the following formula :

$$\text{Amount of DNA } (\mu g\ \mu l^{-1}) = \frac{A_{260} \times 50 \times \text{dilution factor}}{1000}$$

where, A_{260} – absorbance at 260 nm

The quality of DNA could be judged from the ratio of the O.D. values recorded at 260 and 280 nm. The A_{260} / A_{280} ratio between 1.8 and 2.0 indicates good quality of DNA, where A_{280} is the absorbance at 280 nm.

3. Agarose gel electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit supplied by the Bangalore Genei. The required amount of agarose was weighed out (0.7 per cent for visualizing the genomic DNA and 1.2 per cent for visualizing the amplified products) and melted in 1x TAE buffer (0.04 mM Tris acetate, 0.001 mM EDTA, pH 8) by boiling. After cooling to about 50°C, ethidium bromide was added to a

final concentration of 0.5 µg ml^{-1}. The mixture was then poured to a preset template with appropriate comb. After solidification, the comb and the sealing tapes were removed and the gel was mounted in an electrophoresis tank filled with 1x TAE buffer, so that the gel was fully immersed in the buffer. The DNA sample was mixed with the required volume of gel loading buffer (6x loading dye *viz.* 40 per cent sucrose, 0.25 per cent bromophenol blue). Each well was loaded with 20 µl of sample. One of the wells was loaded with 5.0 µl of molecular weight marker along the required volume of gel loading buffer. Electrophoresis was performed at 75 volts until the loading dye reached $3/4^{th}$ of the length of the gel. The gel was visualized using a gel documentation system (BIO RAD, USA).

4. Random Amplified Polymorphic DNA (RAPD) analysis

DNA amplification was done using 80 arbitrarily designed decamer primers (Operon Inc., CA, USA) adopting the procedure of Staub et al. (2000) with required modifications.

Polymerase chain reactions of genomic DNA were performed in 25 µl containing 2.5 µl 10x PCR buffer, 1 µl $MgCl_2$, 2 µl each of dNTPs, 10 pM primer, 1 unit of Taq DNA polymerase (Invitrogen, USA) and 40 ng genomic DNA. Amplification was performed in a Programmable Thermal Controller (PTC-100, MJ Research Inc.) for an initial denaturation at 94°C for 5 minutes, followed by 44 cycles of denaturation at 94°C for 15 seconds and annealing at 35°C for 15 seconds. An extension at 72°C for 75 seconds was included after the last cycle. Finally the products of amplification were cooled to 4°C. A negative control containing sterile water instead of template was included in each reaction set.

The PCR product was size fractioned on a 1.2 per cent agarose gel prepared in 1x TAE buffer and stained with ethidium bromide.

DNA fragments were visualized and photographed using a gel documentation system (BIO RAD, USA). The RAPD bands were represented as '+' for presence and '-' for absence and recorded. The PCR was repeated twice in order to confirm the reproducibility. The amplified products of five primers alone which could produce amplification for most of the clones were used for further analysis.

5. Data analysis

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

$$Sj = a / (a + b + c)$$

where, a : number of bands present in both the genotypes in a pair

b : number of bands present in the first genotype but not in the second one

c : number of bands present in the second genotype but not in the first

Based on the similarity coefficient, the distance between the genotypes was computed with the help of the software package NTSYS PC (Version 2.02i). Using these values of distances between genotypes, a dendrogram was constructed by UPGMA (Unweighted pair group method with arithmetic average). Association between the various genotypes was found out from the dendrogram.

3.4 Standardization of *in vitro* techniques of bittergourd

The materials and methods used for the standardization of *in vitro* propagation of bittergourd *via* enhanced release of axillary buds and callus mediated somatic organogenesis (indirect) are presented in this section.

3.4.1 Genotype

The genotype MC 4 was used for *in vitro* standardization.

3.4.2 Explants

Very young fresh shoot tips, nodal segments, internodes and leaves are used as explants for the *in vitro* culture. Explants were taken from tender and actively growing vines in the experimental field of bittergourd.

3.4.3 Collection and preparation

The explants were washed thoroughly in tap water, containing a few drops of the wetting agent, Labolene for 30 minutes. Explants are then washed several times in running tap water for five minutes and then in glass distilled water. They were kept in open beakers with proper aeration until surface sterilization and inoculation were carried out.

3.4.4 Surface sterilization

Surface sterilization of the explants was carried out inside a laminar air flow (Klenzaids, Model 1104) just before inoculation. The explants were transferred to a sterile beaker and treated with mercuric chloride (0.08 per cent) for 2 minutes, with intermittent shaking. The solution was drained and the explants were washed 4-5 times with sterile distilled water. The explants were transferred carefully to sterile blotting paper over sterile petri plate to remove excess water.

3.4.5 Inoculation and incubation

All the inoculation operations were carried out in a laminar air flow chamber. The vessels and tools (beakers, petri plates, blades, forceps etc.) required for inoculation were washed thoroughly, rinsed with glass distilled water, covered air tight with aluminium foil and autoclaved at 121°C temperature and 1.06 kg cm^{-2} pressure for 45 minutes. They were

further flame sterilized just before inoculation using a spirit lamp inside the laminar air flow chamber.

To inoculate the explants on culture medium, the cotton plugs of the culture vessels were removed and the rim was flamed. The explants were inoculated into the medium using sterile forceps. The mouth of the culture vessels were flamed again and cotton plugs were replaced.

The cultures were then transferred to the culture room provided with light or darkness at 26 $\pm$ 1°C. Sub culturing was carried out as and when the plants attained required growth.

3.4.6 Culture media

Standard procedures (Murashige and Skoog, 1962) were followed for the preparation of the plant tissue culture media. The details of the MS media composition are given in Appendix II. Specific quantities of the stock solutions were pipetted out into a 1000 ml beaker. Sucrose and inositol were added fresh. After making up the volume up to 1000 ml using glass distilled water, the pH of the medium was adjusted to 5.7 using 0.1 N NaOH/HCl with the aid of an electronic pH meter (Global Electronic – Model DPH 500).

For the preparation of solid media, the solution was then heated by placing the beaker on a heating mantle. Agar was added to the solution as and when it started boiling and thoroughly stirred for uniform mixing, till agar melted. Activated charcoal when used in the medium was added at this stage and stirred well for uniform distribution. Then the medium was dispensed to the pre-sterilized culture vessels at the rate of 15 ml in the case of test tubes and 40 ml in the case of Erlenmeyer flasks. The mouth of the culture vessels were plugged with sterilized cotton, covered with aluminium foil or paper, labeled and autoclaved at 121°C temperature and 1.06 kg cm^{-2} pressure for 20 minutes. After autoclaving, the culture media

were taken out and allowed to cool and solidify at room temperature and stored in culture room until used. Before inoculation, the sterilized media were observed for three days for any sign of contamination.

3.4.7 Enhanced release of axillary buds

The surface sterilized shoot tips were inoculated on basic MS medium and transferred to culture room under light for culture establishment. After one week, the aseptic explants in the establishment medium showing complete greening and shoot initiation were transferred MS medium containing the different growth regulators and supplements (Table 4) suggested by Sultana and Bari Miah (2003) and Sikdar et al. (2005).

Table 4. Treatments tried to study the effect of MS medium containing the different growth regulators and supplements on shoot proliferation in bittergourd

Treatment No.	Plant growth regulators (mg l^{-1})	Inositol (mg l^{-1})	Agar concentration (g l^{-1})	Sucrose concentration (g l^{-1})
MSP_1	BA 2.00 + NAA 0.20	100	7.00	30.00
MSP_2	BA 2.00 + IAA 0.10 + GA_3 2.00	100	6.00	30.00

3.4.8 Somatic organogenesis

The cleansed leaf, shoot tip, nodal and internodal explants were inoculated on MS medium with various types and combinations of plant growth regulators were tried as treatments for inducing various types of morphogenetic responses. Induced calli were subcultured into fresh media after a 12-18 day interval for developing an organogenic nature. Watery, spongy, very compact, brown and dead portions of calli were discarded during every subculture. Friable, nodular calli were assumed potentially organogenic and were selected for maintenance and regeneration. After the second subculture, the callus tissue was transferred on to MS medium supplemented with BA (1.00 and 2.00 mg l^{-1}) along with different concentrations of activated charcoal for adventitious shoot initiation and proliferation.

Each treatment was replicated six times. Observations were recorded on the number of cultures initiating callus from the explants. Callus Index (CI) was computed by multiplying percent cultures initiating callus with growth score (G), which was assessed by visual rating (poor=1, medium=2, good=3, and profuse=4). The mean score was expressed as growth score (G). Data on days to callus induction, percent callus showing regeneration, days to regeneration, adventitious shoot length (cm), number of leaves per shoot and length and breadth of leaf (cm) were recorded after four weeks of culture. Hormone free medium was treated as control.

3.4.8.1 Plant Growth Regulators for Callus Induction and Proliferation

The effects of plant growth regulators like cytokinins *viz.,* BA alone and in combination with auxins like NAA, IBA and 2,4-D were assessed for callus induction and proliferation (Table 5).

Table 5. Treatments tried to study the effect of plant growth regulators on callus induction and proliferation in bittergourd

(Medium –MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1})

Treatment No.	Plant growth regulators (mg l^{-1})
MOP_1	BA 0.05
MOP_2	BA 0.05 + IAA 1.00
MOP_3	BA 0.05 + IAA 2.00
MOP_4	BA 0.05 + IAA 3.00
MOP_5	BA 0.05 + IAA 4.00
MOP_6	BA 0.05 + IAA 5.00
MOP_7	BA 0.05 + IAA 4.00+ 2,4-D 1.00
MOP_8	BA 0.05 + IAA 4.00+ 2,4-D 2.00
MOP_9	BA 0.05 + IAA 4.00+ 2,4-D 3.00
MOP_{10}	BA 0.05 + IAA 5.00+ 2,4-D 1.00
MOP_{11}	BA 0.05 + IAA 5.00+ 2,4-D 2.00
MOP_{12}	BA 1.00
MOP_{13}	BA 0.05 + IBA 1.00
MOP_{14}	BA 0.05 + IBA 2.00
MOP_{15}	BA 0.05 + IBA 3.00
MOP_{16}	BA 0.05 + IBA 4.00
MOP_{17}	BA 0.05 + IBA 5.00
MOP_{18}	BA 0.05 + IBA 4.00+ 2,4-D 1.00
MOP_{19}	BA 0.05 + IBA 4.00+ 2,4-D 2.00
MOP_{20}	BA 0.05 + IBA 4.00+ 2,4-D 3.00
MOP_{21}	BA 0.05 + IBA 5.00+ 2,4-D 1.00
MOP_{22}	BA 0.05 + IBA 5.00+ 2,4-D 2.00
MOP_{23}	BA 0.05 + NAA 1.00
MOP_{24}	BA 0.05 + NAA 2.00
MOP_{25}	BA 0.05 + NAA 3.00
MOP_{26}	BA 0.05 + NAA 2.00+ 2,4-D 1.00
MOP_{27}	BA 0.05 + NAA 2.00+ 2,4-D 2.00
MOP_{28}	BA 0.05 + NAA 2.00+ 2,4-D 3.00
MOP_{29}	BA 0.05 + NAA 3.00+ 2,4-D 1.00
MOP_{30}	BA 0.05 + NAA 3.00+ 2,4-D 2.00

3.4.8.2 Sucrose for Callus Induction and Proliferation

Sucrose at different levels (20.00, 30.00 and 40.00 g l^{-1}) were tried to assess the effect on callus induction (Table 6).

Table 6. Treatments tried to study the effect of sucrose on *in vitro* callus induction in bittergourd

(Medium – MS + inositol 100 mg l^{-1} + agar 6.30 g l^{-1} + IBA 4.00 mg l^{-1} + BA 0.50 mg l^{-1} + 2,4- D 2.00 mg l^{-1})

Treatment No.	Sucrose (g l^{-1})
MOS_1	20.00
MOS_2	30.00
MOS_3	40.00

3.4.8.3 Agar for Callus Induction and Proliferation

Different levels of agar were tried to study the effect on callus induction (Table 7).

Table 7. Treatments tried to study the effect of agar on *in vitro* callus induction in bittergourd

(Medium – MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + IBA 4.00 mg l^{-1} + BA 0.50 mg l^{-1} + 2,4- D 2.00 mg l^{-1})

Treatment No.	Agar (g l^{-1})
MOA_1	5.30
MOA_2	6.30
MOA_3	7.30

3.4.8.4 Plant Growth Regulator and Activated Charcoal on Callus Regeneration and Adventitious Shoot Production

Different levels of activated charcoal along with plant growth regulator (BA) were tried to study the effect on callus regeneration and adventitious shoot production (Table 8).

Table 8. Treatments tried to study the effect of growth regulator and activated charcoal on *in vitro* callus regeneration and adventitious shoot production in bittergourd

(Medium –MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1})

Treatment No.	BA (mg l^{-1})	Activated charcoal (%)
$MOAC_1$	1	0.50
$MOAC_2$	2	0.50
$MOAC_3$	1	1.00
$MOAC_4$	2	1.00

3.4.8.5 Culture Conditions on Callus Regeneration and Adventitious Shoot Production

The cultures were kept in light or darkness in order to study the effect of light on callus regeneration (Table 9). Light was provided using cool white fluorescent tubes. Light intensity was measured using Lutron LX-101 lux meter. Darkness was provided by keeping the cultures in a temperature controlled dark room (26 ± 1°C).

Table 9. Treatments tried to study the effect of light on *in vitro* callus regeneration in bittergourd
(Medium – MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1} + activated charcoal 0.50 g l^{-1} + 0.50 BA 1.00 mg l^{-1})

Treatment No.	Light intensity (lux)
MOL_0	0
MOL_1	1000

3.4.9 *In vitro* rooting

Trails on *in vitro* rooting were carried out by transferring *in vitro* shoots of bittergourd to a rooting medium. For root induction, 3-5 cm long regenerated shoots were excised and transferred to MS medium containing varying concentrations of auxin IBA. The treatment details are given in Table 10. Observations on number of cultures initiating roots (percentage), number of days for root initiation, number of roots per culture, length of roots (cm) and nature of roots were recorded after four weeks of culture.

Table 10. Treatments tried to study the effect of plant growth regulators on *in vitro* rooting in bittergourd
(Medium –MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1})

Treatment No.	Plant growth regulators (mg l^{-1})
R_1	IBA 0.50
R_2	IBA 1.00
R_3	IBA 2.00

3.4.10. Statistical analysis

Completely randomized design (CRD) as per Panse and Sukhatme (1985) was followed for statistical analysis of the data wherever necessary.

RESULTS

4. RESULTS

Experimental data recorded during the course of investigation were subjected to statistical analysis and the results are presented under the following heads.

4.1 Morphological characterization

4.1.1 Genetic cataloguing

4.1.2 Genetic variability and divergence

4.2 Biochemical characterization

4.3 Molecular characterization (RAPD)

4.4 Standardization of *in vitro* techniques

4.1 Morphological characterization

4.1.1 GENETIC CATALOGUING

Thirty-three genotypes of *M. charantia* were genetically catalogued for vegetative, flower, fruit and seed characters as per IBPGR (1983) descriptor list (Tables 11 to 13 and Plates 7 to 9).

Most of the genotypes fall in moderate to high viny growth habit and medium to long internodal length. Tendrils are present in all the thirty three genotypes. Leaf size varied from small to large with ovate, pedate or reniform shape. Most of the genotypes had shallowly lobed leaves but exceptions of deeply lobed cases were also found. All the genotypes had soft hairy leaf dorsal surface pubescence and moderate density of foliage hairs per branch.

Variability was more pronounced for flower and fruit characters. Flower and fruit size ranged from small to very large. Fruit form was either round, oval, globular, cylindrical, elliptical, elongate or dumbell. Skin texture was either smooth, rough or with spines. Wide variation was noticed in skin colour ranging from white to dark green, majority having green skin coloured fruits. Fruit shape at stem end and blossom end ranged

Table 11. Vegetative characters in *M. charantia* genotypes

Accession No.	Growth habit	Internodal length	Leaf size	Leaf shape	Leaf lobes
MC 1	Less viny	Medium	Small	Ovate	Shallowly lobed
MC 2	Moderately viny	Medium	Medium	Ovate	Shallowly lobed
MC 3	Moderately viny	Long	Small	Ovate	Deeply lobed
MC 4	Highly viny	Long	Large	Ovate	Deeply lobed
MC 5	Moderately viny	Long	Medium	Pedate	Deeply lobed
MC 6	Moderately viny	Medium	Small	Reniform	Shallowly lobed
MC 7	Moderately viny	Long	Medium	Ovate	Deeply lobed
MC 8	Moderately viny	Long	Large	Ovate	Shallowly lobed
MC 9	Moderately viny	Long	Medium	Pedate	Deeply lobed
MC 10	Moderately viny	Medium	Medium	Ovate	Shallowly lobed
MC 11	Less viny	Long	Medium	Pedate	Deeply lobed
MC 12	Moderately viny	Medium	Medium	Ovate	Deeply lobed
MC 13	Moderately viny	Medium	Medium	Pedate	Deeply lobed
MC 14	Highly viny	Medium	Medium	Ovate	Shallowly lobed
MC 15	Highly viny	Long	Medium	Ovate	Shallowly lobed
MC 16	Moderately viny	Medium	Medium	Ovate	Shallowly lobed
MC 17	Less viny	Medium	Small	Reniform	Shallowly lobed
MC 18	Less viny	Medium	Medium	Ovate	Shallowly lobed
MC 19	Moderately viny	Long	Medium	Ovate	Deeply lobed
MC 20	Highly viny	Medium	Medium	Pedate	Shallowly lobed
MC 21	Less viny	Medium	Small	Ovate	Shallowly lobed
MC 22	Moderately viny	Medium	Medium	Ovate	Shallowly lobed
MC 23	Moderately viny	Short	Small	Ovate	Shallowly lobed
MC 24	Highly viny	Medium	Medium	Ovate	Deeply lobed
MC 25	Less viny	Medium	Medium	Ovate	Shallowly lobed
MC 26	Highly viny	Medium	Small	Reniform	Shallowly lobed
MC 27	Highly viny	Medium	Large	Ovate	Deeply lobed
MC 28	Moderately viny	Medium	Large	Ovate	Deeply lobed
MC 29	Moderately viny	Short	Medium	Ovate	Shallowly lobed
MC 30	Less viny	Medium	Small	Pedate	Shallowly lobed
MC 31	Moderately viny	Medium	Small	Pedate	Shallowly lobed
MC 32	Less viny	Short	Medium	Ovate	Shallowly lobed
MC 33	Less viny	Short	Medium	Ovate	Deeply lobed

Table 12. Flower and fruit characters in *M. charantia* genotypes

Accession No.	Size		Fruit form	Skin		Fruit shape at		Peduncle	
	Flower	Fruit		Texture	Colour	Stem end	Blossom end	Length	Detachment from fruit
MC 1	Medium	Medium	Round	With spines	Light green	Rounded	Flattened	Medium	Easily
MC 2	Large	Large	Oval	Rough	Green	Flattened	Rounded	Long	Easily
MC 3	Medium	Medium	Globular	Rough	Green	Rounded	Taper point	Short	Difficult
MC 4	Large	Large	Round	With spines	Light green	Rounded	Flattened	Medium	Difficult
MC 5	Medium	Small	Cylindrical	With spines	White	Taper point	Taper point	Medium	Easily
MC 6	Small	Large	Dumbbell	Smooth	Green	Taper point	Flattened	Long	Easily
MC 7	Small	Large	Elongate flattened	With spines	Green	Flattened	Taper point	Long	Easily
MC 8	Medium	Large	Oblong elliptical	With spines	Light green	Rounded	Flattened	Short	Easily
MC 9	Medium	Medium	Oval	With spines	Light green	Rounded	Flattened	Long	Difficult
MC 10	Medium	Extra large	Elongate flattened	Smooth	Green	Taper point	Rounded	Medium	Difficult
MC 11	Small	Small	Oval	Smooth	Dark green	Rounded	Deep rounded	Short	Easily
MC 12	Small	Large	Oblong elliptical	With spines	Green	Rounded	Taper point	Medium	Difficult
MC 13	Small	Medium	Oblong elliptical	With spines	Green	Flattened	Taper point	Short	Easily
MC 14	Small	Medium	Oblong elliptical	With spines	Green	Flattened	Taper point	Short	Difficult
MC 15	Medium	Extra large	Elongate	With spines	Dark green	Taper point	Taper point	Long	Easily
MC 16	Medium	Medium	Elongate	With spines	Green	Rounded	Taper point	Medium	Easily
MC 17	Medium	Small	Cylindrical	With spines	Green	Taper point	Taper point	Medium	Difficult
MC 18	Small	Large	Elongate	Smooth	Green	Rounded	Taper point	Short	Difficult
MC 19	Medium	Medium	Elliptical	With spines	Green	Rounded	Flattened	Short	Difficult
MC 20	Large	Extra large	Elongate	With spines	Light green	Rounded	Taper point	Medium	Difficult
MC 21	Medium	Large	Elongate	With spines	Light green	Flattened	Deep rounded	Medium	Easily
MC 22	Medium	Large	Oblong elliptical	With spines	Light green	Flattened	Rounded	Short	Easily
MC 23	Small	Medium	Oblong elliptical	Smooth	White	Taper point	Taper point	Long	Easily
MC 24	Medium	Large	Elongate	With spines	Green	Taper point	Rounded	Long	Difficult
MC 25	Small	Very small	Oval	With spines	Dark green	Taper point	Taper point	Short	Easily
MC 26	Large	Large	Elongate	With spines	Light green	Taper point	Rounded	Long	Easily
MC 27	Small	Large	Oblong elliptical	With spines	Light green	Rounded	Taper point	Medium	Easily
MC 28	Small	Medium	Oblong elliptical	With spines	Green	Rounded	Taper point	Short	Difficult
MC 29	Small	Medium	Oblong elliptical	With spines	Dark green	Rounded	Flattened	Short	Easily
MC 30	Small	Medium	Oblong elliptical	With spines	Green	Rounded	Taper point	Medium	Difficult
MC 31	Medium	Medium	Oblong elliptical	With spines	Green	Taper point	Taper point	Short	Easily
MC 32	Small	Medium	Oblong blocky	Rough	Light green	Rounded	Taper point	Short	Easily
MC 33	Medium	Very small	Round	Smooth	Green	Rounded	Taper point	Long	Easily

Table 13. Seed characters in *M. charantia* genotypes

Accession No.	Seed quantity per fruit	Seed size	Seed coat colour	Seed surface lushe	Seed separation from placenta
MC 1	Intermediate	Large	Whitish yellow	Glossy	Difficult
MC 2	Many	Medium	Whitish brown	Dull	Difficult
MC 3	Intermediate	Large	Whitish brown	Dull	Medium
MC 4	Intermediate	Large	Brown	Dull	Easily
MC 5	Intermediate	Medium	Brown	Glossy	Medium
MC 6	Many	Medium	Yellow	Dull	Easily
MC 7	Intermediate	Small	Yellow	Dull	Difficult
MC 8	Intermediate	Medium	White	Glossy	Easily
MC 9	Few	Very large	Dark brown	Dull	Medium
MC 10	Intermediate	Very large	Brown	Dull	Easily
MC 11	Few	Medium	Brown	Dull	Difficult
MC 12	Intermediate	Large	Yellow	Glossy	Easily
MC 13	Intermediate	Medium	White	Glossy	Difficult
MC 14	Few	Large	Brown	Dull	Medium
MC 15	Many	Medium	Brown	Dull	Difficult
MC 16	Few	Small	Brown	Dull	Medium
MC 17	Few	Medium	Brown	Dull	Medium
MC 18	Many	Medium	Brown	Dull	Difficult
MC 19	Intermediate	Large	Brown	Glossy	Easily
MC 20	Very many	Very large	Brown	Dull	Difficult
MC 21	Few	Medium	Brown	Dull	Medium
MC 22	Many	Large	White	Glossy	Medium
MC 23	Few	Large	Brown	Dull	Difficult
MC 24	Very many	Large	Whitish yellow	Glossy	Easily
MC 25	Few	Medium	Brown	Dull	Difficult
MC 26	Many	Large	White	Glossy	Easily
MC 27	Many	Medium	Brown	Dull	Medium
MC 28	Many	Medium	Whitish brown	Dull	Difficult
MC 29	Few	Very large	Yellow	Glossy	Medium
MC 30	Many	Medium	White	Glossy	Easily
MC 31	Intermediate	Medium	Yellow	Dull	Difficult
MC 32	Intermediate	Large	Brown	Dull	Difficult
MC 33	Very few	Small	Brown	Dull	Medium

Plate 7. Variability in leaf characters of *M. charantia*

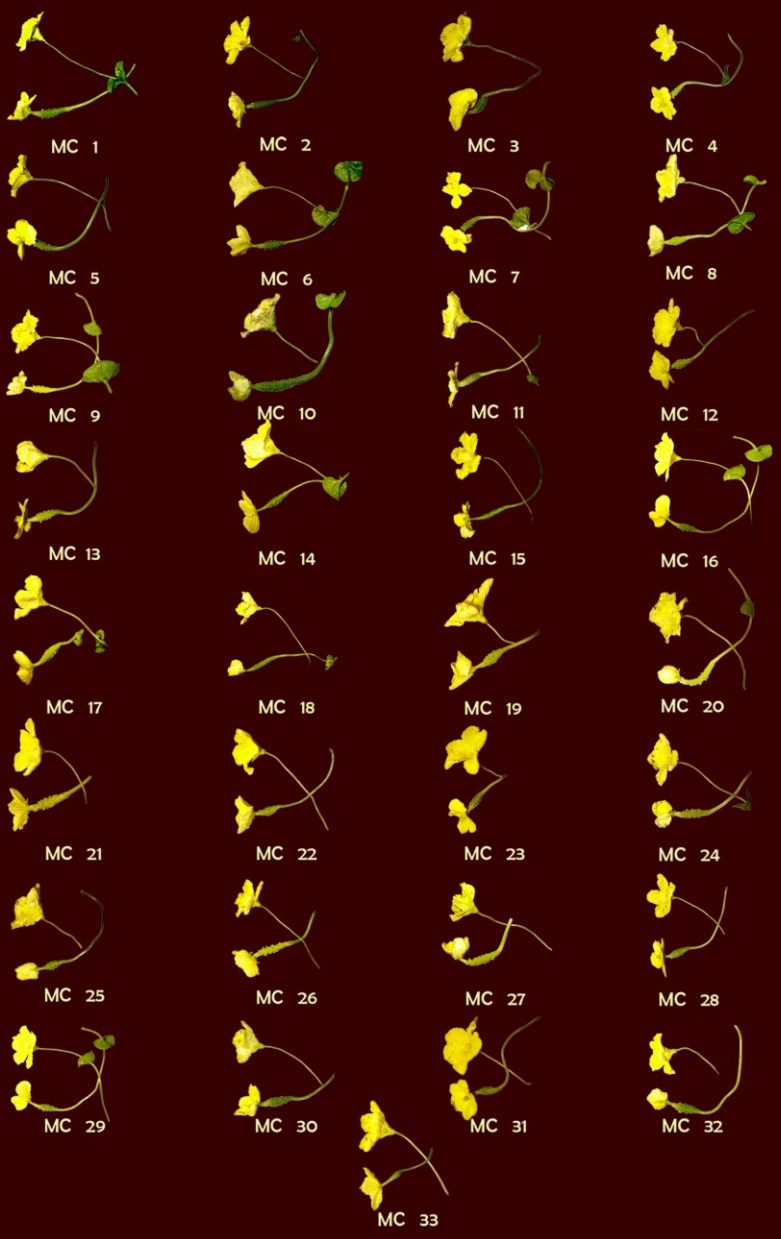


Plate 8. Variability in flower characters of *M. charantia*

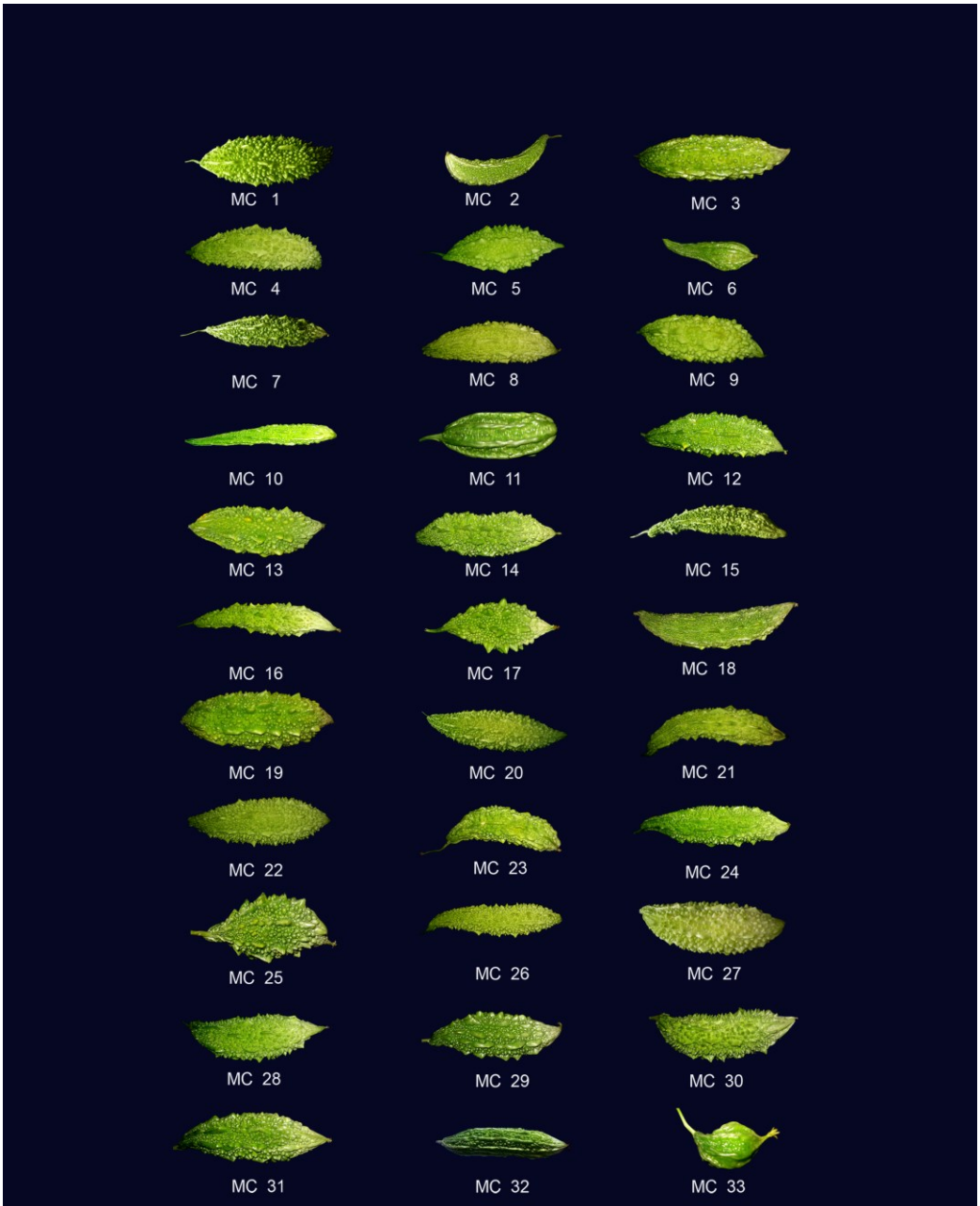


Plate 9. Variability in fruit characters of *M. charantia*

from deep round to taper point. Peduncle length ranged from short to long. Peduncle detachment from fruit was easy in most of the genotypes.

Seed quantity per fruit ranged from very few to many with small to very large seed size. Most of the genotypes had brown seed colour, while a few of them possessed yellow, white and dark brown colour as well. Glossy to dull seed surface lushe was noticed. Seed separation from placenta was difficult in most of the genotypes.

4.1.2 GENETIC VARIABILITY AND DIVERGENCE

4.1.2.1 Mean Performance

Analysis of variance showed significant differences among the genotypes for all the characters studied indicating inherent genetic variability in the materials taken up for the study except fruit fly infestation (%). The genotypes with high mean value can directly be used for adaptation or as parents in hybridization. The mean values of 33 genotypes for different characters are presented in Table 14.

Days to seedling emergence

Days to seedling emergence exhibited a range of 6.00 to 12.75. MC 9 was the earliest to emerge (6.00), while MC 26 was the latest (12.75).

Vine length

There was significant difference among the genotypes for vine length. It ranged from 103.75 - 620.00 cm with an overall mean of 329.45 cm. MC 1 was the longest with a length of 620.00 cm, which was on par with MC 10 (572.5 cm). The genotype MC 33 (103.75 cm) was the shortest, which was on par with MC 17 (103.75 cm), MC 8 (155.00 cm) and MC 28 (166.25 cm).

Table 14. Mean value of biometric characters in *M. charantia*

Treatments	Days to seedling emergence	Vine length (cm)	Internodal length (cm)	No. of primary branches	No. of secondary branches	Days to first male flower	Days to first female flower	Node to first male flower	Node to first female flower	Sex ratio
MC 1	8.25	620.00	3.55	18.00	26.25	41.25	46.25	14.50	12.25	23.14
MC 2	8.25	360.00	2.13	16.75	35.75	38.75	43.75	16.75	16.25	17.69
MC 3	6.50	303.75	4.50	12.25	26.75	35.75	40.75	7.00	12.75	23.18
MC 4	6.75	290.00	5.27	8.75	26.50	37.75	39.50	12.25	19.25	16.68
MC 5	10.25	452.50	4.48	14.00	17.25	41.75	42.00	17.75	19.00	21.79
MC 6	9.25	378.75	2.23	14.75	19.75	37.75	41.00	12.25	17.00	24.68
MC 7	9.25	463.75	3.70	11.00	16.75	36.50	40.00	10.75	13.75	22.38
MC 8	11.50	155.00	3.45	13.25	18.50	35.75	37.50	16.75	17.75	21.41
MC 9	6.00	295.00	4.60	7.75	15.25	41.75	43.50	16.25	18.50	18.81
MC 10	11.75	572.50	3.28	21.00	26.50	51.00	54.50	17.75	20.00	17.19
MC 11	6.75	182.50	3.55	8.50	21.50	36.50	40.25	11.00	15.50	20.11
MC 12	9.50	334.75	2.85	20.50	39.50	39.00	43.50	16.25	22.75	15.94
MC 13	9.00	438.75	2.93	21.50	42.50	42.00	45.75	14.00	21.25	25.74
MC 14	8.00	251.25	2.28	9.75	16.00	44.75	45.25	12.75	14.75	29.13
MC 15	7.75	500.00	3.70	13.25	24.25	36.25	39.50	12.25	16.00	15.46
MC 16	10.50	335.00	2.30	16.25	27.00	44.50	36.00	20.75	24.25	25.12
MC 17	9.50	103.75	2.23	9.50	15.50	41.50	44.00	19.50	22.50	21.97
MC 18	9.00	408.75	2.80	21.00	43.75	41.00	45.00	16.25	24.50	21.62
MC 19	9.75	362.50	3.75	14.25	20.25	41.75	48.00	22.00	28.00	20.90
MC 20	7.75	468.75	5.58	13.25	19.50	44.25	51.00	16.50	23.25	17.17
MC 21	9.25	212.50	2.55	21.00	33.00	36.50	42.50	15.00	19.50	21.69
MC 22	8.00	438.75	2.50	27.50	49.00	38.50	43.50	10.75	13.50	16.43
MC 23	9.00	295.00	1.60	12.25	20.75	44.50	55.70	21.00	29.25	25.99
MC 24	10.00	437.50	3.38	29.25	56.75	46.75	57.25	20.25	25.50	23.66
MC 25	11.50	338.75	2.45	17.00	27.50	40.50	43.50	20.75	22.00	16.49
MC 26	12.75	326.25	3.03	24.25	47.00	39.50	45.25	10.25	13.50	17.85
MC 27	8.25	346.25	2.90	20.75	32.00	40.25	41.25	13.25	13.25	18.71
MC 28	8.50	166.25	2.15	11.25	21.00	41.00	47.25	16.00	18.50	17.01
MC 29	7.25	195.00	1.83	20.00	30.25	31.50	36.25	11.50	14.50	19.92
MC 30	11.75	216.25	2.53	24.00	38.25	39.00	41.50	18.25	26.25	19.78
MC 31	10.00	197.50	3.65	20.50	31.00	37.00	45.50	19.50	22.00	20.73
MC 32	8.50	321.00	1.78	29.50	48.50	40.75	42.75	10.75	12.75	21.63
MC 33	8.00	103.75	1.25	9.00	18.50	41.00	52.75	18.50	20.50	21.28
Mean	9.03	329.45	3.05	16.71	28.86	40.19	44.30	15.42	19.09	20.64
F ratio	5.68**	32.79**	9.37**	7.29**	11.59**	5.87 **	6.56**	6.88**	6.54**	17.44**
CD	1.98	1.42	1 0.98	6.57	9.57	4.47	6.15	4.19	5.38	2.31

* Significant at 5 % ** Significant at 1%

Table 14. Continued

Treatments	Days to first fruit harvest	Fruit length (cm)	Fruit girth (cm)	Fruits per plant	Average fruit weight (g)	Yield per plant (kg)	Seeds per fruit	100-seed weight (g)	Fruit fly infestation (%)	Mosaic incidence (V.I.)
MC 1	55.50	25.02	17.07	14.25	166.67	1.78	16.00	18.95	11.73	58.00
MC 2	58.50	23.62	15.54	31.75	236.34	4.89	22.50	17.45	4.22	40.50
MC 3	64.25	20.17	19.95	10.50	207.15	0.99	21.00	16.15	6.68	42.50
MC 4	68.00	29.18	20.08	21.00	219.05	3.09	20.00	23.75	5.77	35.00
MC 5	61.00	17.65	18.85	13.50	123.30	1.14	17.00	18.00	4.74	38.50
MC 6	51.00	17.94	11.99	23.75	109.09	2.77	24.00	20.60	2.78	47.50
MC 7	53.00	19.40	15.80	11.50	161.83	1.26	19.00	13.30	4.86	61.00
MC 8	48.00	17.80	16.37	13.75	155.50	1.36	12.50	16.65	4.56	39.00
MC 9	49.00	17.40	16.35	17.50	141.80	1.57	9.50	19.55	4.52	46.00
MC 10	56.50	33.66	8.48	34.25	183.05	4.41	16.00	25.10	4.57	38.00
MC 11	64.00	10.83	11.00	8.75	51.88	0.67	19.00	16.85	6.25	60.50
MC 12	46.90	21.52	19.55	14.50	210.70	3.08	21.00	22.05	3.14	35.00
MC 13	46.50	21.00	19.60	14.75	168.67	1.55	14.50	16.45	6.56	33.00
MC 14	52.00	17.30	15.55	13.75	128.66	1.64	13.50	18.90	4.58	39.00
MC 15	46.50	27.04	10.35	22.50	159.72	2.81	28.50	16.85	8.89	60.00
MC 16	47.00	12.95	14.75	11.75	182.50	1.32	11.50	16.25	3.16	36.50
MC 17	53.50	13.40	13.63	14.00	47.84	1.59	14.50	12.10	5.13	65.00
MC 18	55.34	20.89	14.46	9.00	140.21	0.61	25.00	23.45	8.35	37.00
MC 19	55.50	16.45	16.30	26.25	99.70	1.63	18.00	22.40	2.77	41.50
MC 20	59.55	38.83	25.53	14.75	578.75	5.89	33.00	21.60	8.75	41.00
MC 21	45.50	24.50	13.40	22.75	131.25	1.71	15.00	17.60	7.59	20.50
MC 22	50.30	27.03	19.98	21.75	262.83	4.21	23.00	17.45	5.42	19.50
MC 23	58.00	11.77	15.30	12.50	120.42	1.40	13.00	23.40	5.19	17.00
MC 24	64.50	23.40	13.83	11.75	180.30	1.84	38.00	16.95	7.22	14.00
MC 25	47.50	11.38	14.08	30.25	42.75	1.09	11.00	17.50	2.49	45.00
MC 26	46.50	28.88	14.88	22.25	234.50	4.52	25.00	22.40	4.43	16.00
MC 27	51.00	23.29	20.59	26.25	235.74	4.18	26.50	20.40	5.39	20.50
MC 28	54.00	19.10	14.70	13.25	152.08	1.36	20.50	8.70	5.31	54.00
MC 29	60.50	18.65	18.03	23.00	102.41	1.47	12.00	18.90	4.65	16.00
MC 30	52.00	16.05	14.72	16.25	95.38	0.99	15.50	17.50	8.22	62.50
MC 31	46.00	15.99	14.31	29.75	104.00	1.65	16.00	15.40	5.77	15.50
MC 32	43.50	21.50	16.44	23.75	130.25	1.01	15.50	25.90	3.01	13.00
MC 33	61.50	4.91	6.96	16.50	4.26	0.06	3.00	7.15	1.58	10.00
Mean	53.71	20.26	15.71	18.54	159.65	2.11	18.48	18.35	5.49	36.91
F ratio	6.90**	11.71 **	6.11**	6.35**	52.86**	24.63**	34.36**	10.02**	1.49	9.01**
CD	7.37	5.79	4.33	8.01	38.32	0.82	3.45	3.85	5.21	15.76

* Significant at 5 % ** Significant at 1%

Internodal length

Internodal length varied from 1.25 to 5.58 cm. The genotypes on an average had 3.05 cm internodal length. Maximum internodal length was observed in MC 20 (5.58 cm), which was on par with MC 4 (5.27 cm). Minimum internodal length was found in MC 33 (1.25 cm), which was on par with MC 23 (1.60 cm), MC 32 (1.78 cm), MC 29 (1.83 cm), MC 2 (2.13 cm) and MC 28 (2.15 cm).

Number of primary branches

Among the genotypes, the number of primary branches varied from 7.75 to 29.50 with a general mean of 16.71. Maximum number of 29.50 was found in MC 32 and minimum of 7.75 in MC 9.

Number of secondary branches

Mean number of secondary branches varied from 15.25 in MC 9 to 56.75 in MC 24 with a general mean of 28.86.

Days to first male flower

Days to first male flower exhibited a range of 31.50 to 51.00. MC 29 was the earliest to flower (31.50), which was on par with MC 3 (35.75) and MC 8 (35.75). MC 10 (51.00) was the latest, which was on par with MC 24 (46.75).

Days to first female flower

Among the genotypes, days to first female flower ranged from 36.00 in MC 16 to 57.25 in MC 24 with an overall mean of 44.30.

Node to first male flower

Node to first male flower ranged from 7.00 in MC 3 to 22.00 in MC 19 with a general mean of 15.42.

Node to first female flower

Range in node to first female flower among the genotypes was from 12.25 in MC 1 to 29.25 in MC 23.

Sex ratio

Sex ratio had a range from 15.46 in MC 15 to 29.13 in MC 14 with an overall mean of 20.64.

Days to fruit harvest

Days to fruit harvest exhibited a range of 43.50 to 68.00. MC 32 was the earliest to harvest (43.50) while MC 4 (68.00) was the latest.

Fruit length

A wide range of variation was noticed for fruit length. Maximum fruit length was observed in MC 20 (38.83 cm), which was on par with MC 10 (33.66 cm) and minimum in MC 33 (4.91 cm).

Fruit girth

Girth of fruits varied significantly among the genotypes from 6.96 to 25.53 cm. Maximum fruit girth was recorded in MC 20 (25.53 cm). Genotype MC 33 (6.96 cm) had the minimum fruit girth.

Fruits per plant

Fruit number varied considerably from 8.75 in MC 11 to 34.25 in MC 10 with an overall mean of 18.54.

Average fruit weight

Range in average fruit weight among the genotypes was from 4.26 to 578.75 g, highest in MC 20 (578.75 g) and lowest in MC 33 (4.26 g).

Yield per plant

A wide range of variation was observed for yield per plant from 0.06 to 5.89 kg. MC 20 had the highest yield (5.89 kg), which was significantly different from all other genotypes. The lowest yield was obtained from MC 33 (0.06 kg).

Seeds per fruit

Seeds per fruit observed a range from 3.00 in MC 33 to 38.00 in MC 24 with an overall mean of 18.48.

100-seed weight

Range in 100-seed weight among the genotypes was from 7.15 g in MC 33 to 25.90 g in MC 32.

Fruit fly infestation

Among the genotypes, the fruit fly infestation (%) ranged from 1.58 to 11.73. The genotypes on an average had 5.49 fruits infested.

Mosaic incidence

Mosaic was the only disease observed at later stage of crop growth and hence scoring based on visual observations was done for mosaic. The vulnerability index for mosaic incidence ranged from 3.50 to 25.50. Maximum mosaic incidence was observed in MC 17 (65.00), whereas MC 33 (10.00) was the least affected. The reaction of genotypes towards mosaic incidence indicated that ten genotypes were highly resistant, ten genotypes were resistant, ten genotypes were moderately resistant and remaining three were susceptible to the disease (Table 15).

Table 15. Reaction of 33 genotypes of *M. charantia* towards mosaic virus under field conditions

Category	Highly resistant	Resistant	Moderately resistant	Susceptible	Highly susceptible
Vulnerability index	0-20	21-40	41-60	61-80	81-100
Treatments	MC 21, MC 22, MC 23, MC 24, MC 26, MC 27, MC 29, MC 31, MC 32, MC 33	MC 2, MC 4, MC 5, MC 8, MC 10, MC 12, MC 13, MC 14, MC 16, MC 18	MC 1, MC 3, MC 6, MC 9, MC 11, MC 15, MC 19, MC 20, MC 25, MC 28	MC 7, MC 17, MC 30	Nil

4.1.2.2 Genetic Parameters

The population mean, range, phenotypic, genotypic and environmental variances, phenotypic and genotypic coefficients of variation are given in Table 16.

High phenotypic and genotypic variances were observed for several characters including average fruit weight and vine length. Wide variation was observed in phenotypic and genotypic variances among the characters. A close association between phenotypic and genotypic variances was noticed for yield per plant, average fruit weight and seeds per fruit. For most of the characters, genotypic variance makes up the major portion of the phenotypic variance, with very little effect of environment.

Phenotypic and genotypic coefficients of variation (PCV and GCV respectively) observed were high for most of the characters (Fig. 1). Yield per plant had the highest PCV (68.61) and GCV (65.87) followed by average fruit weight (61.06 and 59.91 respectively). The lowest PCV and GCV were exhibited by days to first male flower (10.11 and 8.52 respectively).

4.1.2.3 Heritability and Genetic Advance

Heritability and genetic advance for different characters are presented in Table 17 (Fig. 2).

High heritability coupled with low genetic advance was observed for most of the characters, except fruit fly infestation and mosaic incidence.

Heritability estimates were high for most of the characters studied *viz.,* average fruit weight (96.29), seeds per fruit (94.34), vine length (94.08) and yield per plant (92.19). Fruit fly infestation recorded a low heritability estimate (19.67).

Table 16. Range, mean, phenotypic, genotypic and environmental variances, phenotypic and genotypic coefficients of variation for different characters in *M. charantia*

Sl. No.	Character	Range	Mean ± SEm	σp^2	σg^2	σe^2	PCV (%)	GCV (%)
1	Days to seedling emergence	6.00 - 12.75	9.03 ± 0.69	3.14	2.20	0.94	19.63	16.43
2	Vine length (cm)	103.75 - 620.00	329.45 ± 22.37	16913.32	15912.32	1001.00	39.47	38.29
3	Internodal length (cm)	1.25 - 5.58	3.05 ± 0.34	1.19	0.96	0.23	35.70	32.08
4	Number of primary branches	7.75 - 29.50	16.71 ± 2.28	42.99	32.61	10.38	39.23	34.17
5	Number of secondary branches	15.25 - 56.75	28.86 ± 3.32	138.79	116.77	22.03	40.82	37.44
6	Days to first male flower	31.50 – 51.00	40.19 ± 1.55	16.52	11.71	4.81	10.11	8.52
7	Days to first female flower	36.00 - 57.25	44.30 ± 2.13	34.41	25.31	9.09	13.21	11.33
8	Node to first male flower	7.00 - 22.00	15.42 ± 1.45	16.69	12.46	4.23	26.49	22.88
9	Node to first female flower	12.25 - 29.25	19.09 ± 5.38	26.20	19.26	6.95	26.81	22.98
10	Sex ratio	15.46 - 29.13	20.64 ± 0.79	11.79	10.52	1.28	16.64	15.71
11	Days to first fruit harvest	43.50 - 68.00	53.71 ± 2.56	51.63	38.57	13.06	13.57	11.72
12	Fruit length (cm)	4.91 - 38.83	20.26 ± 2.01	51.26	43.19	8.07	35.35	32.44
13	Fruit girth (cm)	6.96 - 25.53	15.71 ± 1.50	16.03	11.52	4.51	25.49	21.61
14	Fruits per plant	8.75 - 34.25	18.54 ± 2.78	56.58	41.18	15.40	40.58	34.62
15	Average fruit weight (g)	4.26 - 578.75	159.65 ± 13.28	9502.45	9149.60	352.85	61.06	59.91
16	Yield per plant (kg)	0.06 - 5.89	2.11 ± 0.29	2.09	1.93	0.16	68.61	65.87
17	Seeds per fruit	3.00 - 38.00	18.48 ± 1.19	50.43	47.58	2.85	38.42	37.32
18	100-Seed weight (g)	7.15 - 25.90	18.35 ± 1.33	19.58	16.03	3.55	24.11	21.81
19	Fruit fly infestation (%)	1.58 - 11.73	5.49 ± 1.81	8.13	1.60	6.53	51.91	23.02
20	Mosaic incidence (Vulnerability Index)	3.50 - 25.50	36.91 ± 5.46	34.01	20.63	13.38	53.68	41.80

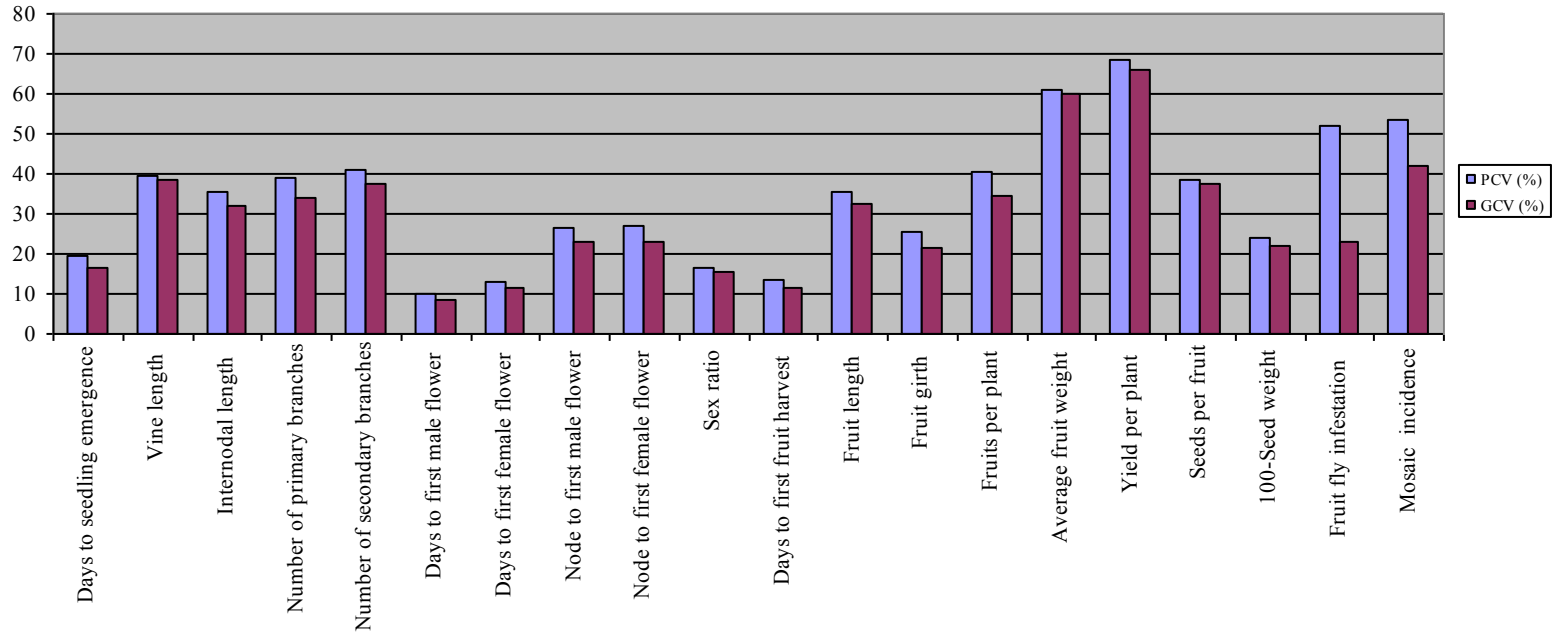


Fig. 1. Phenotypic and genotypic coefficients of variation for twenty characters in *M. charantia*

Table 17. Heritability and genetic advance for different characters in *M. charantia*

Sl. No.	Characters	Heritability (%)	Genetic advance (%)
1	Days to seedling emergence	70.04	2.56
2	Vine length	94.08	252.05
3	Internodal length	80.71	1.81
4	Number of primary branches	75.86	10.25
5	Number of secondary branches	84.13	20.42
6	Days to first male flower	70.90	5.94
7	Days to first female flower	73.56	8.89
8	Node to first male flower	74.63	6.28
9	Node to first female flower	73.49	7.75
10	Sex ratio	89.15	6.31
11	Days to first fruit harvest	74.69	11.06
12	Fruit length	84.26	12.43
13	Fruit girth	71.87	5.93
14	Fruits per plant	72.78	11.28
15	Average fruit weight	96.29	193.35
16	Yield per plant	92.19	2.74
17	Seeds per fruit	94.34	13.80
18	100-Seed weight	81.85	7.46
19	Fruit fly infestation	19.67	7.47
20	Mosaic incidence	60.65	7.29

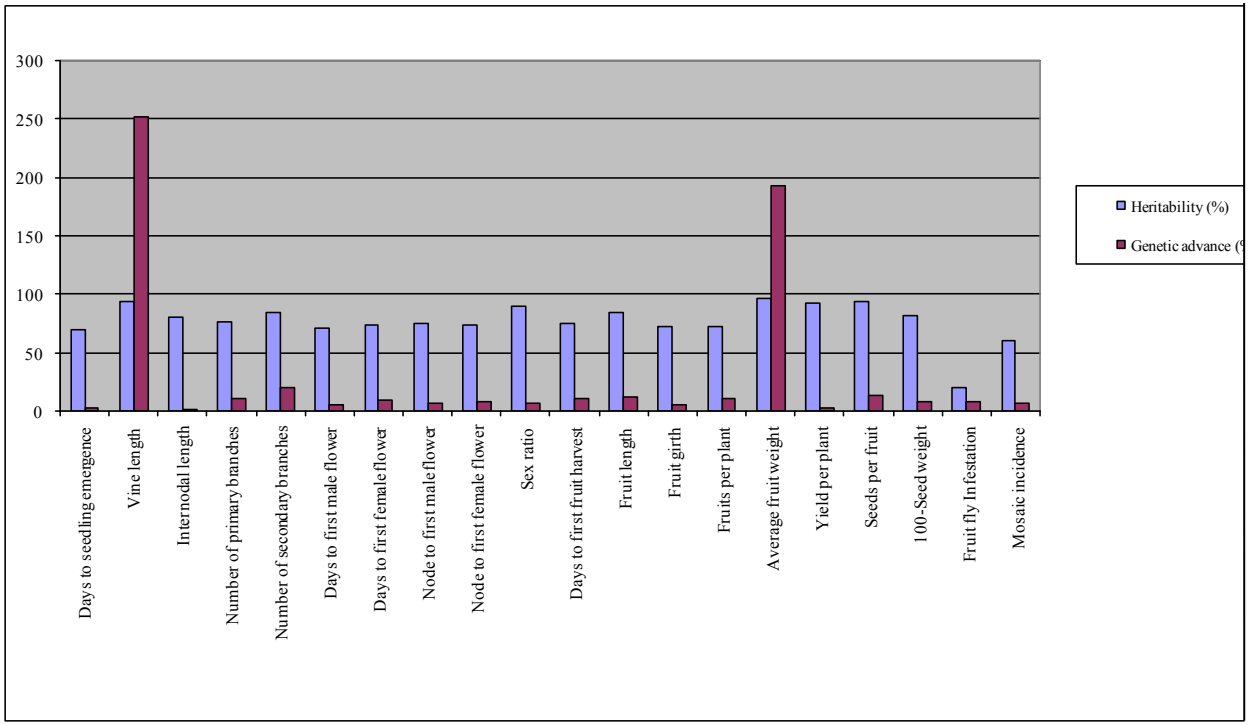


Fig. 2. Heritability and genetic advance for twenty characters in *M. charantia*

Genetic advance was highest for vine length (252.05), followed by average fruit weight (193.35) while, lowest for internodal length (1.81) and days to seedling emergence (2.56). High heritability combined with high genetic advance was observed for vine length and average fruit weight. Moderate heritability with low genetic advance was noted for mosaic incidence. Fruit fly infestation showed low heritability with low genetic advance.

4.1.2.4 Correlation Analysis

The phenotypic, genotypic and environmental correlation coefficients were estimated for 20 characters (Tables 18, 19 and 20). In general, the correlation parameters indicated high magnitude of genotypic correlation coefficients than the phenotypic ones for the characters studied.

(A) Phenotypic correlation

(i) Correlation between yield and other characters

Yield per plant recorded high positive significant correlation with vine length (0.4104), fruit length (0.7275), fruit girth (0.3381), fruits per plant (0.4693), average fruit weight (0.7412), seeds per fruit (0.5370) and 100-seed weight (0.3767). Node to first male and female flower (-0.1716 and -0.1771 respectively) and sex ratio (-0.4802) was negatively correlated with yield.

(ii) Correlation among the yield component characters

Days to seedling emergence was found to be uncorrelated with most of the yield component characters except number of primary branches (0.3419), node to first male and female flower (0.3914 and 0.3145 respectively). Days to first fruit harvest (-0.3344) showed significant negative correlation with days to seedling emergence.

Table 18. Phenotypic correlation coefficients among yield and its components in *M. charantia*

Characters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20
Days to seedling emergence (X1)	1.0000																			
Vine length (X2)	0.0664	1.0000																		
Internodal length (X3)	-0.2082	0.3399**	1.0000																	
Number of primary branches (X4)	0.3419**	0.2935*	-0.2122	1.0000																
Number of secondary branches (X5)	0.1897	0.2148	-0.1777	0.8866**	1.0000															
Days to first male flower (X6)	0.2244	0.3373**	0.0229	0.0863	0.0286	1.0000														
Days to first female flower (X7)	0.2349	0.2425*	0.0166	0.2150	0.1685	0.6621**	1.0000													
Node to first male flower (X8)	0.3914**	-0.0864	-0.1456	-0.0037	-0.1274	0.4812**	0.3748**	1.0000												
Node to first female flower (X9)	0.3145**	-0.1063	-0.0736	0.0004	-0.0188	0.3874**	0.4505**	0.7973**	1.0000											
Sex ratio (X10)	-0.0239	-0.0744	-0.2363	-0.0861	-0.1213	0.1972	0.0791	0.0522	0.0922	1.0000										
Days to first fruit harvest (X11)	-0.3344**	0.1551	0.4019**	-0.1870	-0.0933	0.0357	0.1641	-0.1187	0.0018	0.0062	1.0000									
Fruit length (X12)	-0.0252	0.5672**	0.4289**	0.3273**	0.2958*	0.1538	0.2068	-0.2673*	-0.2008	-0.3857**	0.2157	1.0000								
Fruit girth (X13)	-0.2086	0.1609	0.3897**	0.1216	0.1229	-0.1112	-0.0334	-0.1745	-0.0623	-0.0828	0.2644*	0.4264**	1.0000							
Fruits per plant (X14)	0.2123	0.1136	-0.0643	0.2373	0.1006	-0.0441	0.0567	0.0241	-0.1372	-0.4071**	-0.2354	0.1989	-0.0955	1.0000						
Average fruit weight (X15)	-0.1206	0.4433**	0.4651**	0.1449	0.1625	0.1577	0.1571	-0.1747	-0.0738	-0.2836*	0.2196	0.7837**	0.6499**	-0.0287	1.0000					
Yield per plant (X16)	0.0377	0.4104**	0.2607*	0.1876	0.1657	0.1528	0.1844	-0.1716	-0.1771	-0.4802**	0.0919	0.7275**	0.3381**	0.4693**	0.7412**	1.0000				
Seeds per fruit (X17)	-0.0283	0.4420**	0.3360**	0.3037*	0.3838**	0.0687	0.2422*	-0.1867	-0.0467	-0.2410*	0.3836**	0.6176**	0.2950*	-0.0428	0.5982**	0.5370**	1.0000			
100-seed weight (X18)	0.0573	0.4081**	0.1881	0.2749*	0.2551*	0.2144	0.2162	-0.1042	0.0456	-0.0806	0.1632	0.4380**	0.2235	0.2571*	0.3036*	0.3767**	0.2360	1.0000		
Fruit fly infestation (X19)	-0.0988	0.2563*	0.2944**	0.1416	0.1823	0.0622	0.2255	-0.1602	-0.0621	-0.0187	0.1895	0.3729**	0.0669	-0.1148	0.2714*	0.1342	0.2949*	0.1129	1.0000	
Mosaic incidence (X20)	0.0054	0.0179	0.1287	-0.3280**	-0.3863**	-0.0653	0.0538	-0.0305	-0.0753	-0.0389	0.1525	-0.1254	-0.2124	-0.0925	-0.1867	-0.0899	-0.0181	-0.2353	0.2384*	1.0000

Table 19. Genotypic correlation coefficients among yield and its components in *M. charantia*

Characters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20
Days to seedling emergence (X1)	1.0000																			
Vine length (X2)	0.0730	1.0000																		
Internodal length (X3)	-0.2208	0.3948	1.0000																	
Number of primary branches (X4)	0.5163	0.3170	-0.3205	1.0000																
Number of secondary branches (X5)	0.2728	0.2243	-0.2408	0.9185	1.0000															
Days to first male flower (X6)	0.3617	0.4167	-0.0533	0.1115	0.0245	1.0000														
Days to first female flower (X7)	0.3817	0.2967	-0.0465	0.3723	0.2918	0.7219	1.0000													
Node to first male flower (X8)	0.5250	-0.1147	-0.1551	-0.0758	-0.1600	0.5617	0.5553	1.0000												
Node to first female flower (X9)	0.3894	-0.1241	0.0036	-0.0199	0.0039	0.4978	0.6545	0.8897	1.0000											
Sex ratio (X10)	0.0334	-0.0863	-0.2742	-0.1064	-0.1633	0.2199	0.0599	0.0854	0.1319	1.0000										
Days to first fruit harvest (X11)	-0.3510	0.1443	0.5582	-0.2703	-0.0942	-0.0274	0.1729	-0.1281	0.0443	-0.0095	1.0000									
Fruit length (X12)	-0.0137	0.6418	0.5665	0.3548	0.3418	0.1545	0.2321	0.4192	-0.2942	-0.4561	0.2225	1.0000								
Fruit girth (X13)	-0.3296	0.2067	0.5101	0.0818	0.1680	-0.1631	-0.1402	-0.2962	-0.1388	-0.1250	0.3705	0.4065	1.0000							
Fruits per plant (X14)	0.2346	0.1467	-0.1971	0.3842	0.1854	0.0230	0.0020	0.1777	-0.0934	-0.4934	-0.2642	0.3370	-0.1800	1.0000						
Average fruit weight (X15)	-0.1497	0.4558	0.5591	0.1500	0.1744	0.1939	0.1783	-0.2367	-0.1058	-0.3072	0.2448	0.8226	0.7295	0.0123	1.0000					
Yield per plant (X16)	0.0573	0.4323	0.2660	0.2533	0.2291	0.2031	0.1870	-0.1387	-0.1711	-0.5167	0.1074	0.8580	0.4356	0.4596	0.8032	1.0000				
Seeds per fruit (X17)	-0.0506	0.4747	0.4225	0.3575	0.4552	0.0671	0.2872	-0.2503	-0.0417	-0.2733	0.4152	0.6588	0.3239	-0.0316	0.6229	0.5782	1.0000			
100-seed weight (X18)	0.1267	0.4666	0.2666	0.4382	0.3327	0.2337	0.2903	-0.1003	0.0842	-0.0839	0.1620	0.5445	0.3739	0.3192	0.3449	0.4027	0.2685	1.0000		
Fruit fly infestation (X19)	-0.1716	0.1892	0.0377	0.2244	0.0834	0.1540	0.1873	-0.1307	-0.0181	-0.1335	0.1097	0.0305	0.1427	-0.1517	0.2818	0.2606	0.1348	0.1607	1.0000	
Mosaic incidence (X20)	-0.0790	-0.0537	0.1282	-0.2312	-0.1893	-0.0472	-0.0047	0.0046	-0.0210	-0.1085	0.2381	-0.1717	-0.3302	-0.2724	-0.2565	-0.1876	-0.0082	-0.2702	0.3598	1.0000

Table 20. Environmental correlation coefficients among yield and its components in *M. charantia*

Characters	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20
Days to seedling emergence (X1)	1.0000																			
Vine length (X2)	0.0536	1.0000																		
Internodal length (X3)	-0.1754	-0.0381	1.0000																	
Number of primary branches (X4)	-0.1281	0.2150	0.1788	1.0000																
Number of secondary branches (X5)	-0.0905	0.1570	0.1186	0.7811	1.0000															
Days to first male flower (X6)	-0.1030	-0.0230	0.2667	0.0169	0.0453	1.0000														
Days to first female flower (X7)	-0.1389	-0.0347	0.2321	-0.2499	-0.2981	0.5073	1.0000													
Node to first male flower (X8)	0.0429	0.0794	-0.1141	0.2157	-0.0033	0.2674	-0.1417	1.0000												
Node to first female flower (X9)	0.1245	-0.0242	-0.3378	0.0602	-0.1065	0.1010	-0.1162	0.5337	1.0000											
Sex ratio (X10)	-0.2794	0.0577	-0.0253	0.0090	0.1536	0.1258	0.1807	-0.1052	-0.0858	1.0000										
Days to first fruit harvest (X11)	-0.2925	0.2791	-0.1427	0.0668	-0.0929	0.2049	0.1391	-0.0909	-0.1197	0.0840	1.0000									
Fruit length (X12)	-0.0672	-0.0433	-0.2193	0.2239	0.0509	0.1609	0.1182	0.3257	0.1503	0.0738	0.1962	1.0000								
Fruit girth (X13)	0.0870	-0.0705	0.0052	0.2349	-0.0366	0.0181	0.2514	0.1589	0.1412	0.0987	-0.0266	0.5233	1.0000							
Fruits per plant (X14)	0.1572	-0.0613	0.3790	-0.1879	-0.2139	-0.2154	0.2060	-0.4066	-0.2568	-0.0563	-0.1545	-0.3139	0.1253	1.0000						
Average fruit weight (X15)	0.0219	0.2014	-0.3289	0.1764	0.0719	-0.0240	0.0714	0.2678	0.1537	0.0152	0.1232	0.5600	0.4211	-0.3872	1.0000					
Yield per plant (X16)	-0.0546	0.1133	0.2546	-0.1765	-0.3245	-0.0759	0.2117	-0.4016	-0.2521	-0.1275	0.0194	-0.2595	-0.1118	0.6369	-0.2876	1.0000				
Seeds per fruit (X17)	0.0988	-0.0906	-0.3131	0.0110	-0.2295	0.1080	0.0241	0.1944	-0.0976	0.1232	0.2930	0.3196	0.2242	-0.1336	0.0982	-0.0336	1.0000			
100-seed weight (X18)	-0.1656	-0.0133	-0.1528	-0.3362	-0.1235	0.1585	-0.0413	-0.1203	-0.0901	-0.0636	0.1707	-0.0840	-0.2800	0.0480	-0.0306	0.2261	0.0003	1.0000		
Fruit fly infestation (X19)	-0.0716	0.0311	0.0013	0.1246	0.4156	0.0096	-0.0780	-0.2440	-0.1196	0.1262	-0.1831	-0.1313	0.0279	0.1199	0.1056	0.2261	-0.1018	0.1269	1.0000	
Mosaic incidence (X20)	0.1657	0.3835	0.1415	0.1050	0.1387	-0.1015	0.1765	-0.1063	-0.1899	0.1980	-0.0247	-0.0108	0.0168	0.2701	0.0774	0.2875	-0.0798	-0.1679	0.2030	1.0000

Vine length was positively correlated with internodal length (0.3399), days to first male flower (0.3373), fruit length (0.5672), average fruit weight (0.4433), seeds per fruit (0.4420) and 100-seed weight (0.4081).

Internodal length had positive correlation with days to first fruit harvest (0.4019), fruit length (0.4289), fruit girth (0.3897), average fruit weight (0.4651), seeds per fruit (0.3360) and fruit fly infestation (0.2944).

A positive correlation was observed between number of primary branches and number of secondary branches (0.8866), while negative correlation with mosaic incidence (-0.3280).

Number of secondary branches observed positive correlation with seeds per fruit (0.3838) and negative correlation with mosaic incidence (-0.3863).

Days to first male flower recorded high positive correlation with days to first female flower (0.6621) and node to first male and female flower (0.4812 and 0.3874 respectively). Days to first female flower also observed high positive correlation with node to first male and female flower (0.3748 and 0.4505 respectively).

Node to first male flower exhibited positive correlation with node to first female flower (0.7973) and negative correlation with fruit length (-0.2673).

Sex ratio recorded negative correlation with most of the characters, the highest being with fruits per plant (-0.4071), followed by fruit length (-0.3857) and average fruit weight (-0.2836).

Fruit girth (0.2644) and seeds per fruit (0.3836) showed positive correlation with days to first fruit harvest.

Fruit length observed high positive correlation with fruit girth (0.4264), average fruit weight (0.7837) seeds per fruit (0.6176),

100-seed weight (0.4380) and fruit fly infestation (0.3729). On the other hand, fruit girth had positive correlation with average fruit weight (0.6499) and seeds per fruit (0.2950).

Fruits per plant recorded positive correlation with 100-seed weight (0.2571). Average fruit weight exhibited high positive correlation with seeds per fruits (0.5982) and 100-seed weight (0.3036). Fruit weight showed negative and non-significant correlation with number of fruits per plant (-0.0287).

Seeds per fruit was positively correlated with fruit fly infestation (0.2949). Mosaic incidence showed significant positive correlation with fruit fly infestation (0.2384).

(B) Genotypic correlation

(i) Correlation between yield and other characters

High positive correlation was observed between yield per plant and vine length (0.4323), fruit length (0.8580), fruit girth (0.4356), fruits per plant (0.4596), average fruit weight (0.8032), seeds per fruit (0.5782) and 100-seed weight (0.4027), whereas node to first male and female flower (-0.1387 and -0.1711 respectively) and sex ratio (-0.5167) exhibited a high negative correlation.

(ii) Correlation among the yield component characters

Days to seedling emergence had high positive correlation with number of primary branches (0.5163) and node to first male flower (0.5250), whereas days to first fruit harvest (-0.3510) and fruit girth (-0.3296) was negatively correlated.

Vine length showed high positive correlation with days to first male flower (0.4167), fruit length (0.6418), average fruit weight (0.4558), seeds per fruit (0.4747) and 100-seed weight (0.4666).

Internodal length observed positive correlation with days to first fruit harvest (0.5582), fruit length (0.5665), fruit girth (0.5101), average fruit weight (0.5591) and seeds per fruit (0.4225), while sex ratio (-0.2742) showed negative correlation.

Number of primary branches exhibited positive correlation with number of secondary branches (0.9185), days to first female flower (0.3723), fruits per plant (0.3842) and 100-seed weight (0.4382). Number of secondary branches had negative correlation with node to first male flower (-0.1600) and sex ratio (-0.1633).

Days to first male flower observed high positive correlation with days to first female flower (0.7219), node to first male flower (0.5617), node to first female flower (0.4978) and sex ratio (0.2199). Days to first female flower showed positive correlation with node to first male flower (0.5553) and node to first female flower (0.6545).

Node to first male flower had positive correlation with node to first female flower (0.8897), sex ratio (0.0854) and fruit length (0.4192), while negatively correlated with fruit girth (-0.2962), average fruit weight (-0.2367) and seeds per fruit (-0.2503).

Fruit length (-0.2942), fruit girth (-0.1388) and average fruit weight (-0.1058) were negatively correlated with node to first female flower.

Sex ratio recorded negative correlation with most of the characters, the highest being with fruits per plant (-0.4934), followed by fruit length (-0.4561), average fruit weight (-0.3072), seeds per fruit (-0.2733) and fruit girth (-0.1250).

Days to first fruit harvest showed positive correlation with fruit length (0.2225), fruit girth (0.3705) and seeds per fruit (0.4152), while fruits per plant had negative correlation (-0.2642).

Fruit length had high positive correlation with fruit girth (0.4065), fruits per plant (0.3370), average fruit weight (0.8226), seeds per fruit (0.6588) and 100-seed weight (0.5445), while negative correlation with mosaic incidence (-0.1717). Fruit girth had high positive correlation with average fruit weight (0.7295), seeds per fruit (0.3239) and 100-seed weight (0.3739), while negatively correlated with fruits per plant (-0.1800) and mosaic incidence (-0.3302).

Fruits per plant observed positive correlation with 100-seed weight (0.3192) while, negative correlation with fruit fly infestation (-0.1517) and mosaic incidence (-0.2724). Average fruit weight exhibited high positive correlation with seeds per fruits (0.6229) and 100-seed weight (0.3449).

Seeds per fruit was positively correlated with 100-seed weight (0.2685) and fruit fly infestation (0.1348). 100-seed weight showed negative correlation with mosaic incidence (-0.2702).

Fruit fly infestation exhibited positive correlation with mosaic incidence (0.3598).

(C) Environmental correlation

Environmental correlation coefficients were found to be negligible among yield and its component characters, except for the correlation between fruits per plant and yield per plant (0.6369).

4.1.2.5 Path Analysis

In path analysis, the genotypic correlation coefficients among yield and its component characters were partitioned into direct and indirect contribution of each character to fruit yield (Table 21). Days to first female flower, node to first female flower, sex ratio, fruit length, fruit girth, fruits per plant, average fruit weight, seeds per fruit, fruit fly

Table 21. Direct and indirect effect of selected yield components on fruit yield in *M. charantia*

Character	Days to first female flower	Node to first female flower	Sex ratio	Fruit length (cm)	Fruit girth (cm)	Fruits per plant	Average fruit weight (g)	Seeds per fruit	Fruit fly infestation	Mosaic incidence	Correlation with yield
Days to first female flower	**0.1465**	-0.1322	-0.0007	-0.0744	0.0088	0.0011	0.1749	0.0399	0.0236	-0.0006	0.1844
Node to first female flower	0.0959	**-0.2020**	-0.0015	0.0943	0.0087	-0.0536	-0.1038	-0.0058	-0.0006	-0.0027	-0.1711
Sex ratio	0.0088	-0.0266	**-0.0116**	0.1463	0.0078	-0.2834	-0.3014	-0.0380	-0.0046	-0.0140	-0.5167
Fruit length (cm)	0.0340	0.0594	0.0053	**-0.3207**	-0.0255	0.1936	0.8071	0.0915	0.0354	-0.0221	0.8580
Fruit girth (cm)	-0.0205	0.0280	0.0015	-0.1304	**-0.0627**	-0.1034	0.7157	0.0450	0.0049	-0.0425	0.4356
Fruits per plant	0.0003	0.0189	0.0057	-0.1081	0.0113	**0.5744**	0.0121	-0.0044	-0.0155	-0.0351	0.4596
Average fruit weight (g)	0.0261	0.0214	0.0036	-0.2638	-0.0457	0.0071	**0.9811**	0.0865	0.0200	-0.0330	0.8032
Seeds per fruit	0.0421	0.0084	0.0032	-0.2113	-0.0203	-0.0182	0.6111	**0.1389**	0.0252	-0.0011	0.5782
Fruit fly infestation	0.1007	0.0037	0.0016	-0.3305	-0.0089	-0.2595	0.5708	0.1021	**0.0343**	0.0463	0.2606
Mosaic incidence	-0.0007	0.0042	0.0013	0.0551	0.0207	-0.1565	-0.2517	-0.0011	0.0124	**0.1287**	-0.1876

Residue = 0.3425715
Direct effects- diagonal elements
Indirect effects- off diagonal elements

infestation and mosaic incidence were selected for path coefficient analysis.

Average fruit weight exhibited the highest positive direct effect on fruit yield (0.9811), followed by fruits per plant (0.5744). The direct effects of days to first female flower, seeds per fruit and mosaic incidence were negligible, whereas fruit length, fruit girth and node to first female flower exerted small negative direct effect on yield.

Indirect effects through average fruit weight was consistently high signifying the importance of this character. Thus in the case of days to first female flower (0.1749), positive correlation with yield was mainly due to its positive indirect effects through average fruit weight. At the same time fruit length, fruit girth and seeds per fruit made high positive correlation with yield due to their positive indirect effects through average fruit weight (0.8071, 0.7157 and 0.6111 respectively). Sex ratio exhibited high negative correlation with yield due to negative indirect effect through average fruit weight (-0.3014) and fruits per plant (-0.2834). Similarly, node to first female flower had negative correlation with yield due to negative indirect effect through average fruit weight (-0.1038) and fruits per plant (-0.0536). Fruit fly infestation showed comparatively high positive genotypic correlation with yield per plant, its indirect effect was relatively high and positive through average fruit weight (0.5708). In the case of mosaic incidence, the negative correlation was mainly built by the indirect negative effect through average fruit weight (-0.2517) and fruits per plant (-0.1565).

4.1.2.6 Selection Index

A discriminant function analysis was carried out for isolating superior genotypes. Selection index involving characters *viz.,* days to seedling emergence (X_1), vine length (X_2), internodal length (X_3), number of primary branches (X_4), number of secondary branches (X_5), days to first male flower (X_6), days to first female flower (X_7), node to

first male flower (X_8), node to first female flower (X_9), sex ratio (X_{10}), days to first fruit harvest (X_{11}), fruit length (X_{12}), fruit girth (X_{13}), fruits per plant (X_{14}), average fruit weight (X_{15}), yield per plant (X_{16}), seeds per fruit (X_{17}), 100-seed weight (X_{18}), fruit fly infestation (X_{19})and mosaic incidence (X_{20}) were selected for the analysis.

The selection index worked out was as follows :

$$I = 2.80366\ X_1 + 0.9297854\ X_2 + 13.17556\ X_3 - 0.3482765\ X_4 - 0.08907298\ X_5 + 2.054818\ X_6 - 3.167724\ X_7 + 2.133132\ X_8 + 1.726119\ X_9 - 0.2468289\ X_{10} - 0.9902639\ X_{11} - 0.4898278\ X_{12} + 0.8298175\ X_{13} + 0.5986971\ X_{14} + 0.7364406\ X_{15} + 10.9873\ X_{16} + 3.522038\ X_{17} + 1.487722\ X_{18} + 2.278038\ X_{19} - 3.183979\ X_{20}$$

The scores obtained for the genotypes based on the selection index were given in Table 22.

Based on selection index, MC 20 (2575.39) ranked first, followed by MC 10 (2010.98), MC 26 (1909.59), MC 22 (1840.43) and MC 27 (1837.84) (Plates 10, 11, 12, 13 and 14). The minimum scores were obtained for MC 33 (535.66) and MC 17 (627.37).

4.1.2.7 Mahalanobis's D^2 Analysis

Following Mahalanobis's D^2 statistic, the 33 genotypes of *M. charantia* were subjected to cluster analysis, based on twenty characters *viz.,* days to seedling emergence, vine length, internodal length, number of primary branches, number of secondary branches, days to first male flower, days to first female flower, node to first male flower, node to first female flower, sex ratio, days to first fruit harvest, fruit length, fruit girth, fruits per plant, average fruit weight, yield per plant, seeds per fruit, 100-seed weight, fruit fly infestation and mosaic incidence.

The 33 genotypes fell under five clusters. The clustering pattern is furnished in Table 23. Cluster I was the largest with 11 genotypes,

Table 22. Selection indices on the genotypes of *M. charantia* arranged in descending order

Rank	Treatments	Selection index
1	MC 20	2575.39
2	MC 10	2010.98
3	MC 26	1909.59
4	MC 22	1840.43
5	MC 27	1837.84
6	MC 12	1755.82
7	MC 2	1684.30
8	MC 24	1656.74
9	MC 13	1625.75
10	MC 9	1602.57
11	MC 18	1572.87
12	MC 7	1562.19
13	MC 5	1550.52
14	MC 15	1544.84
15	MC 4	1513.77
16	MC 6	1415.24
17	MC 3	1397.47
18	MC 19	1392.56
19	MC 16	1345.16
20	MC 32	1318.76
21	MC 23	1282.39
22	MC 1	1249.73
23	MC 14	1157.17
24	MC 25	1139.97
25	MC 21	1115.82
26	MC 31	1007.21
27	MC 30	1081.39
28	MC 28	1046.91
29	MC 8	970.60
30	MC 29	910.96
31	MC 11	812.76
32	MC 17	627.37
33	MC 33	535.66

T

Plate 10. MC 20 - A genotype ranked first based on selection index

Plate 11. MC 10 - A genotype ranked second based on selection index

Plate 12. MC 26 - A genotype ranked third based on selection index

Plate 13. MC 22 - A genotype ranked fourth based on selection index

Plate 14. MC 27 - A genotype ranked fifth based on selection index

followed by cluster III and V with 10 genotypes each. Cluster II and IV had one genotype each.

The average inter and intra cluster distances are presented in Table 24. The intracluster distance was highest for cluster I (1197.78), followed by clusters III and V (1149.66 and 903.03 respectively). The highest intercluster distance was observed for clusters III and IV (2515.57), followed by clusters II and III (2088.12) and clusters I and IV (1856.82). The genetic distance (D) between clusters I, III and V were largest with cluster IV. The minimum intercluster distance was observed between clusters I and V (1022.33) indicating a close relationship among the genotypes included. The data in this table clearly showed that the genotypes usually did not cluster according to their geographical distribution.

The cluster means of 33 genotypes (Table 25) showed that the mean values of the clusters varied in magnitude for all the twenty characters. Cluster I consisted of genotypes with medium sized fruits with shortest internode, male and female flowers at lower nodes, earliness in fruit harvest and highest mosaic resistance. Cluster II (MC 20) had earliness in seedling germination, longest internode, lowest sex ratio as well as highest fruit length, fruit girth, average fruit weight, yield per plant and seeds per fruit. Cluster III comprised of genotypes with smallest fruits, shorter vine length and less number of branches with lower fruit yield. Cluster IV (MC 10) consisted of genotype with medium sized fruits with longest vine length, highest number of primary and secondary branches, fruits per plant and 100-seed weight along with lowest fruit fly infestation. Cluster V comprised of small sized fruits with lowest fruit yield**.** The best cluster with rest to yield and other component characters represented by cluster II followed by IV.

Table 23. Clustering pattern of thirty three genotypes of *M. charantia*

Cluster No.	Number of accessions	Treatments
I	11	MC 1, MC 2, MC 4, MC 12, MC 15, MC 21, MC 22, MC 26, MC 27, MC 29, MC 32
II	1	MC 20
III	10	MC 3, MC 6, MC 7, MC 8, MC 9, MC 11, MC 14, MC 17, MC 28, MC 33
IV	1	MC 10
V	10	MC 5, MC 13, MC 16, MC 18, MC 19, MC 23, MC 24, MC 25, MC 30, MC 31

Table 24. Average inter and intracluster distances in the thirty three genotypes of *M. charantia*

Cluster	I	II	III	IV	V
I	1197.78	1570.86	1566.15	1856.82	1022.33
II		0.00	2088.12	1545.21	1595.39
III			1149.66	2515.57	1167.00
IV				0.00	1822.31
V					903.03

Diagonal elements- intracluster values

Off diagonal elements- intercluster values

Table 25. Cluster means of twenty biometric characters in *M. charantia*

Cluster	Days to seedling emergence	Vine length (cm)	Internodal length (cm)	Number of primary branches	Number of secondary branches	Days to first male flower	Days to first female flower	Node to first male flower	Node to first female flower	Sex ratio
I	8.59	358.59	2.92	20.02	35.64	38.18	42.18	13.05	15.77	18.65
II	7.75	468.75	5.58	13.25	19.50	44.25	51.00	16.50	23.25	17.17
III	8.33	240.38	2.99	10.70	18.95	39.23	43.23	14.08	17.15	21.99
IV	11.75	572.50	3.28	21.00	26.50	51.00	54.50	17.75	20.00	17.19
V	10.08	348.25	2.99	19.00	32.50	41.88	30.38	19.05	24.20	22.18

Table 25. Continued

Cluster	Days to first fruit harvest	Fruit length (cm)	Fruit girth (cm)	Fruits per plant	Average fruit weight (g)	Yield per plant (kg)	Seeds per fruit	100-seed weight (g)	Fruit fly infestation	Mosaic incidence
I	52.06	24.56	16.90	22.16	189.95	2.97	20.45	20.15	5.84	21.68
II	59.55	38.83	25.53	14.75	578.75	5.89	33.00	21.60	8.75	41.00
III	55.03	15.82	14.23	14.33	116.01	1.33	15.65	15.00	4.62	46.45
IV	56.50	33.66	8.48	34.25	183.05	4.41	16.00	25.10	4.57	38.00
V	53.33	16.75	15.62	17.58	125.72	1.32	17.95	18.73	5.45	34.05

4.1.2.8 Quality Characters

i. Keeping quality (days)

Keeping quality of fruits were studied based on shelf life and spoilage during storage as measured by rotting percentage and weight loss. The mean values of 33 genotypes for different characters are furnished in Table 26.

Maximum shelf life of 8.0 days was recorded by MC 25, followed by MC 12 and MC 30 with 6.5 days simultaneously.

The increased physiological loss in fruit weight was observed with the advancement of storage period. The percentage loss in weight on zero to two days after storage was lowest in MC 25 (1.30 %), while highest in MC 17 (12.35 %). Meanwhile, minimum percentage loss in weight on two to four days after storage was observed in MC 15 (1.65 %) and maximum in MC 33 (13.90 %).

Among the genotypes, rotting of fruits within four days was lowest in MC 25 (3.00 %), while highest in MC 17 (95.00 %). Within six days, 100 per cent rotting of fruits was observed in MC 17 and MC 21, while minimum rotting of six per cent was found in MC 25.

With regard to general appearance of bittergourd fruits, no change in colour for 2 days was seen in fruits obtained from the genotypes MC 25 and MC 12. Slowest change in colour of fruits for 2 days was found in MC 22, MC 1, MC 31, MC 3 and MC 2. Accordingly, change of colour was slower in MC 25 and MC 12 for four days. Colour change was faster in MC 17 and MC 33 with a score of 2.5 within two days and 4.5 within four days.

ii. Organoleptic analysis

The organoleptic quality of 33 genotypes were evaluated with respect to appearance, doneness, flavor, taste and bitterness by a four point rating scale (Table 27 and Plate 15). The genotypes differ significantly for all these attributes.

Table 26. Mean performance of bittergourd genotypes in terms of keeping quality

Treatments	Shelf life (Days)	Weight loss during storage (%)		Rotting of fruits (%)		Score of colour change	
		0-2 days	2-4 days	4 days	6 days	2 days	4 days
MC 1	2.5	5.85	5.50	42.50	78.50	0.5	3.5
MC 2	2.5	4.90	6.55	48.00	63.50	0.5	4.0
MC 3	5.5	3.00	2.35	9.50	17.00	0.5	1.5
MC 4	4.0	4.55	5.35	14.50	43.50	1.5	2.0
MC 5	2.5	7.85	6.10	62.50	85.50	1.0	2.5
MC 6	2.0	4.95	6.50	73.00	88.50	1.5	3.5
MC 7	2.0	9.20	7.15	76.50	99.00	2.0	4.0
MC 8	2.5	7.40	6.00	73.50	95.00	1.0	3.5
MC 9	4.0	3.90	4.60	17.00	39.50	1.0	2.5
MC 10	5.0	2.70	3.35	16.00	24.00	1.0	1.5
MC 11	3.0	3.65	4.50	57.00	72.50	1.5	2.0
MC 12	6.5	2.40	3.20	16.50	20.50	0.0	1.0
MC 13	2.5	5.70	4.45	79.00	92.50	1.5	3.0
MC 14	3.0	7.00	5.85	84.00	95.50	1.0	2.5
MC 15	5.0	2.80	1.65	11.00	15.50	1.0	2.0
MC 16	3.5	4.70	4.50	13.00	20.00	1.5	2.0
MC 17	1.5	12.35	10.75	95.00	100.00	2.5	4.5
MC 18	3.0	6.95	6.05	83.00	94.00	1.5	3.5
MC 19	4.0	5.75	6.55	11.00	17.50	1.0	1.5
MC 20	3.5	5.55	4.55	9.50	12.00	1.5	2.5
MC 21	2.0	9.15	7.85	92.00	100.00	1.0	3.5
MC 22	4.5	5.90	4.20	18.00	23.50	0.5	1.5
MC 23	3.0	5.40	5.55	24.00	35.00	1.5	2.0
MC 24	2.0	4.50	3.30	80.50	97.50	2.0	3.0
MC 25	8.0	1.30	2.40	3.00	6.00	0.0	1.0
MC 26	4.5	3.45	3.20	13.00	17.00	1.5	2.0
MC 27	3.5	5.15	5.60	3.50	31.00	1.5	2.0
MC 28	5.5	2.35	2.75	18.50	21.00	1.5	1.5
MC 29	4.5	2.00	2.35	16.00	37.00	1.5	2.0
MC 30	6.5	1.65	2.00	10.50	16.50	1.0	1.5
MC 31	3.5	7.10	8.75	13.00	35.50	0.5	1.5
MC 32	3.0	7.20	6.20	28.50	58.00	1.5	3.5
MC 33	2.0	10.75	13.90	49.50	97.50	2.5	4.5
CD (0.05)	1.27	1.88	1.63	13.01	12.36	1.50	1.62

Table 27. Organoleptic evaluation of bittergourd genotypes

Treatments	Appearance	Doneness	Flavour	Taste	Bitterness
MC 1	2.89	2.96	2.67	2.67	2.44
MC 2	2.33	3.11	2.33	2.11	2.11
MC 3	2.89	2.56	2.78	2.78	2.44
MC 4	2.11	2.89	2.56	2.56	2.67
MC 5	2.67	2.33	2.22	2.33	2.44
MC 6	2.89	1.89	2.00	2.22	2.78
MC 7	1.89	2.89	2.22	2.22	2.44
MC 8	2.11	3.22	2.67	2.44	3.33
MC 9	2.11	1.89	2.22	2.33	2.11
MC 10	2.56	3.11	2.56	2.33	1.78
MC 11	2.67	3.11	2.44	2.56	2.67
MC 12	2.56	2.33	2.22	2.22	1.89
MC 13	2.22	3.00	2.00	2.22	2.00
MC 14	2.22	2.11	2.56	2.78	3.11
MC 15	2.78	2.44	2.56	2.67	2.78
MC 16	2.22	2.78	2.33	2.00	2.00
MC 17	2.44	3.00	2.67	2.67	2.22
MC 18	2.56	3.00	2.78	2.67	3.00
MC 19	3.33	2.44	2.33	2.67	2.00
MC 20	2.56	3.00	2.78	3.11	2.44
MC 21	2.00	2.67	2.44	2.44	2.22
MC 22	2.00	3.00	2.89	3.11	3.11
MC 23	2.44	2.67	2.33	2.00	1.89
MC 24	2.67	2.00	2.22	1.89	2.56
MC 25	2.22	2.11	2.67	1.78	1.22
MC 26	2.22	2.00	2.78	2.33	1.89
MC 27	2.44	3.33	2.78	2.67	2.67
MC 28	2.67	2.56	2.56	2.22	2.44
MC 29	2.22	3.00	2.22	3.00	2.11
MC 30	2.00	2.11	2.56	2.44	1.89
MC 31	3.44	3.00	2.11	2.89	2.22
MC 32	2.56	2.33	2.44	2.11	2.89
MC 33	2.67	2.11	2.00	2.00	1.89

Plate 15. Organoleptically superior genotypes

MC 20 - Very good taste

MC 22 - Pleasant flavour and very good taste

MC 27 - Highly acceptable doneness

MC 31 - Well preserved natural colour

Among the bittergourd genotypes, MC 31 well preserved natural colour (appearance/colour) with a highest score of 3.44, while the moderately discoloured appearance was recorded by MC 7 (1.89). Highly acceptable doneness was showed by MC 27 (3.33) and slightly acceptable doneness point of 1.89 scored by MC 6 and MC 9. MC 22 had pleasant flavor with highest score of 2.89, while MC 6, MC 13 and MC 33 had moderately flavor with a lowest score of 2.00. Very good taste was offered by MC 20 and MC 22 with a maximum score of 3.11, while MC 25 (1.78) had very poor taste. MC 8 (score 3.33) had lowest bitterness while, MC 25 was highly bitter with a very high score of 1.22.

4.2 Biochemical characterization

Biochemical characterization based on beta-carotene, vitamin C, iron, chlorophyll and bitterness value was carried out and the mean values of 33 genotypes for different characters are presented in Table 28 and Plate 16.

Beta Carotene

The range and overall mean of β-carotene was 52.58 to 138.96 µg/100 g and 100.63 µg/100 g respectively. Maximum β-carotene content of 138.96 µg/100 g was found in MC 5 and minimum of 52.58 µg/100 g in MC 8 (Fig. 3).

Ascorbic acid

Ascorbic acid value ranged from 62.54 to 124.29 mg/100 g with a general mean of 83.95 mg/100 g. The highest ascorbic acid content was recorded in MC 26 (124.29 mg/100 g) and lowest in MC 29 (62.54 mg/100 g) (Fig. 4).

Iron

Range in iron content among the genotypes was from 2.38 to 6.88 mg/100 g, highest in MC 25 (6.88 mg/100 g) and lowest in MC 7 (2.38 mg/100 g) (Fig. 5). The genotypes on an average had 4.33 mg/100 g iron content.

Table 28. Mean performance of bittergourd genotypes in terms of biochemical characters

Treatments	β-carotene (µg/100 g)	Ascorbic acid (mg/100 g)	Iron (mg/100 g)	Chlorophyll content (mg/g)			Bitterness (units/g)
				Chlorophyll a	Chlorophyll b	Total chlorophyll	
MC 1	95.41	85.23	4.25	0.02250	0.00235	0.02595	1782.50
MC 2	102.97	74.11	4.50	0.02200	0.00045	0.01945	3900.00
MC 3	82.11	69.92	3.88	0.03475	0.00825	0.02710	1915.00
MC 4	70.15	98.54	4.50	0.00970	0.00405	0.01380	1227.50
MC 5	138.96	68.98	4.38	0.00700	0.00365	0.01175	1320.00
MC 6	110.51	83.36	3.88	0.06145	0.02775	0.09150	2340.00
MC 7	72.97	84.74	2.38	0.05690	0.01330	0.04365	5200.00
MC 8	52.58	113.03	4.63	0.00780	0.00200	0.00970	1227.50
MC 9	63.07	92.53	3.75	0.00190	0.00410	0.00710	1300.00
MC 10	115.71	70.95	4.63	0.06445	0.01135	0.02530	4333.00
MC 11	84.70	73.99	5.25	0.05205	0.00520	0.04340	1950.00
MC 12	86.33	72.38	4.75	0.01240	0.00870	0.02385	4333.00
MC 13	114.30	82.56	3.63	0.03555	0.01100	0.02640	1950.00
MC 14	131.84	110.34	3.63	0.03300	0.00765	0.02790	2042.50
MC 15	119.35	93.10	5.13	0.07600	0.00185	0.08235	3033.00
MC 16	91.66	74.52	4.50	0.01450	0.00270	0.01175	2340.00
MC 17	83.79	84.27	3.38	0.02745	0.00065	0.02835	2042.50
MC 18	95.09	83.89	4.13	0.04075	0.02365	0.03435	1617.50
MC 19	103.48	73.55	5.50	0.06235	0.03075	0.09405	2080.00
MC 20	63.15	84.85	3.38	0.00110	0.00085	0.00305	1320.00
MC 21	109.14	68.42	5.50	0.01065	0.00175	0.00557	1782.50
MC 22	119.95	123.88	4.38	0.02135	0.00205	0.01375	1617.50
MC 23	101.47	68.42	4.25	0.05385	0.01565	0.03870	1877.50
MC 24	126.07	75.37	4.38	0.04250	0.01100	0.03205	3466.00
MC 25	123.84	63.49	6.88	0.54365	0.02320	0.12000	10400.00
MC 26	107.15	124.29	3.13	0.01525	0.00815	0.02670	1782.50
MC 27	130.55	113.37	3.88	0.00245	0.00190	0.00300	1320.00
MC 28	96.49	76.78	5.50	0.02805	0.00740	0.01750	4333.00
MC 29	97.38	62.54	6.38	0.01180	0.00200	0.05750	6933.00
MC 30	122.68	77.87	4.50	0.04105	0.00725	0.03230	3640.00
MC 31	126.08	91.48	3.50	0.08810	0.01900	0.07095	2608.00
MC 32	120.86	86.73	3.38	0.00811	0.00435	0.01185	3342.50
MC 33	60.97	63.04	3.38	0.08800	0.00655	0.07790	7800.00
Mean	100.63	83.95	4.33	0.04839	0.00850	0.03669	2974.22
CD (0.05)	12.95	9.60	1.66	0.22	0.24	0.23	2640.39

Plate 16. Biochemically superior genotypes

MC 26 - Maximum ascorbic acid content

MC 25 - Maximum iron content and shelf life, highest bitterness

MC 5 - Maximum beta carotene content

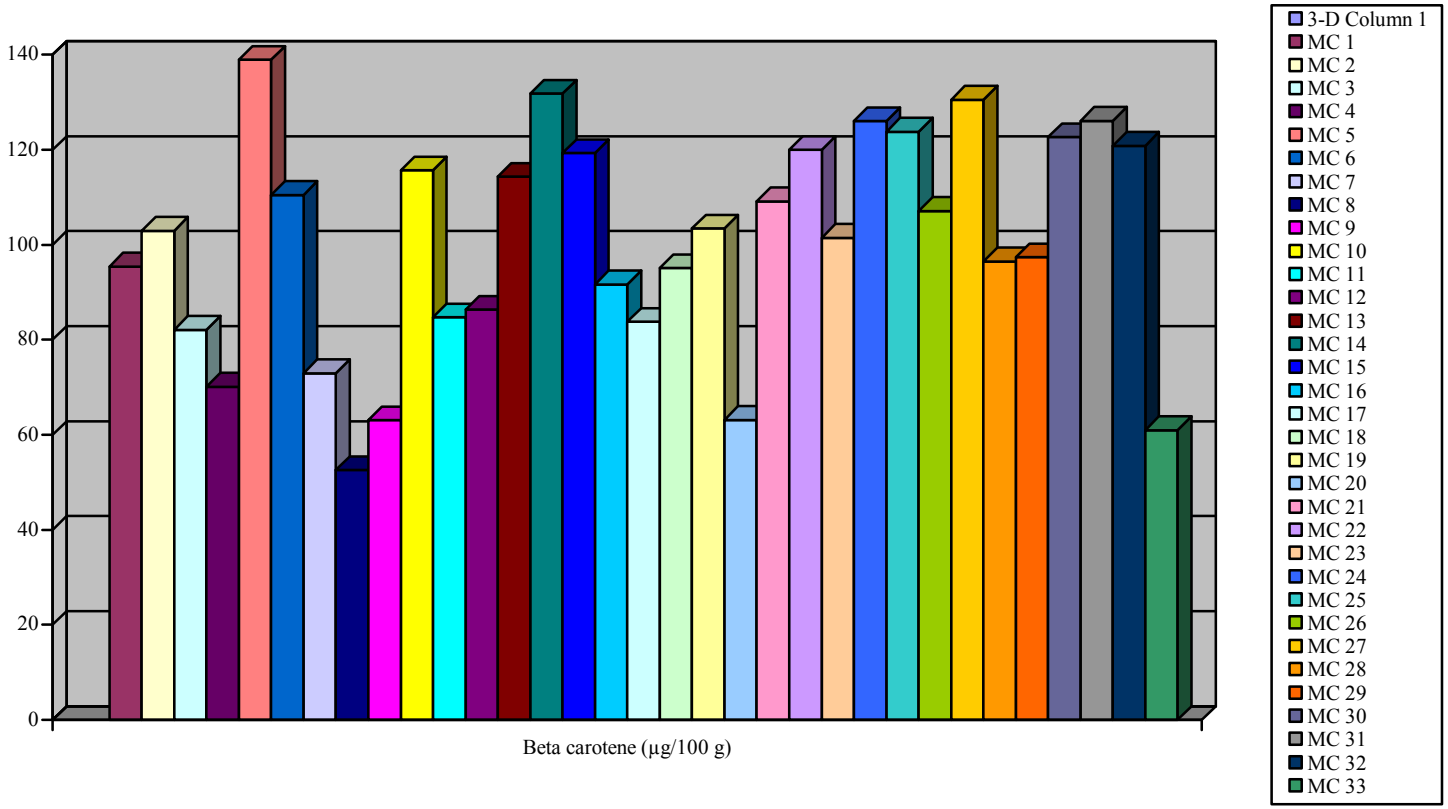


Fig. 3. Beta carotene content in 33 *M. charantia* genotypes

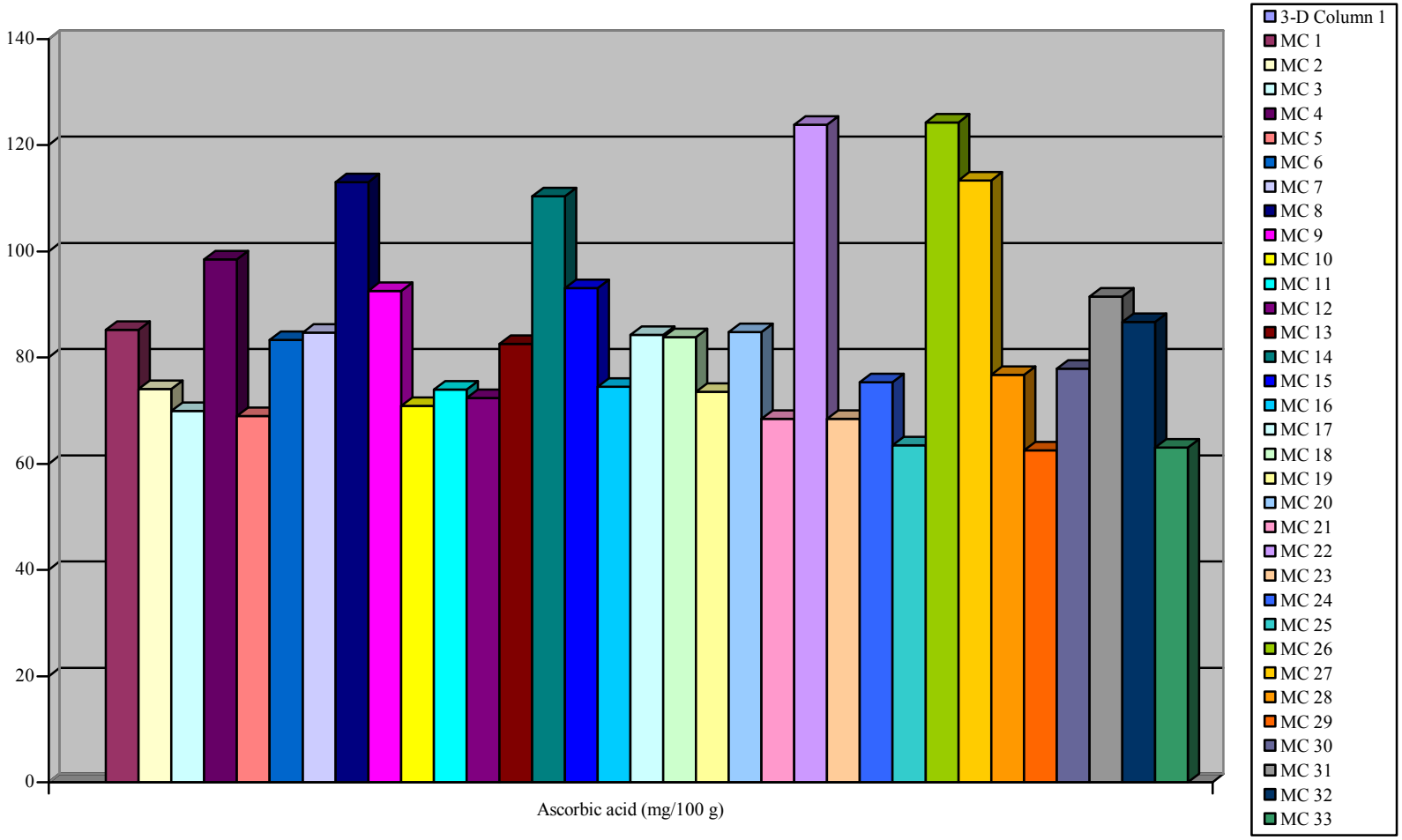


Fig. 4. Ascorbic acid content in 33 *M. charantia* genotypes

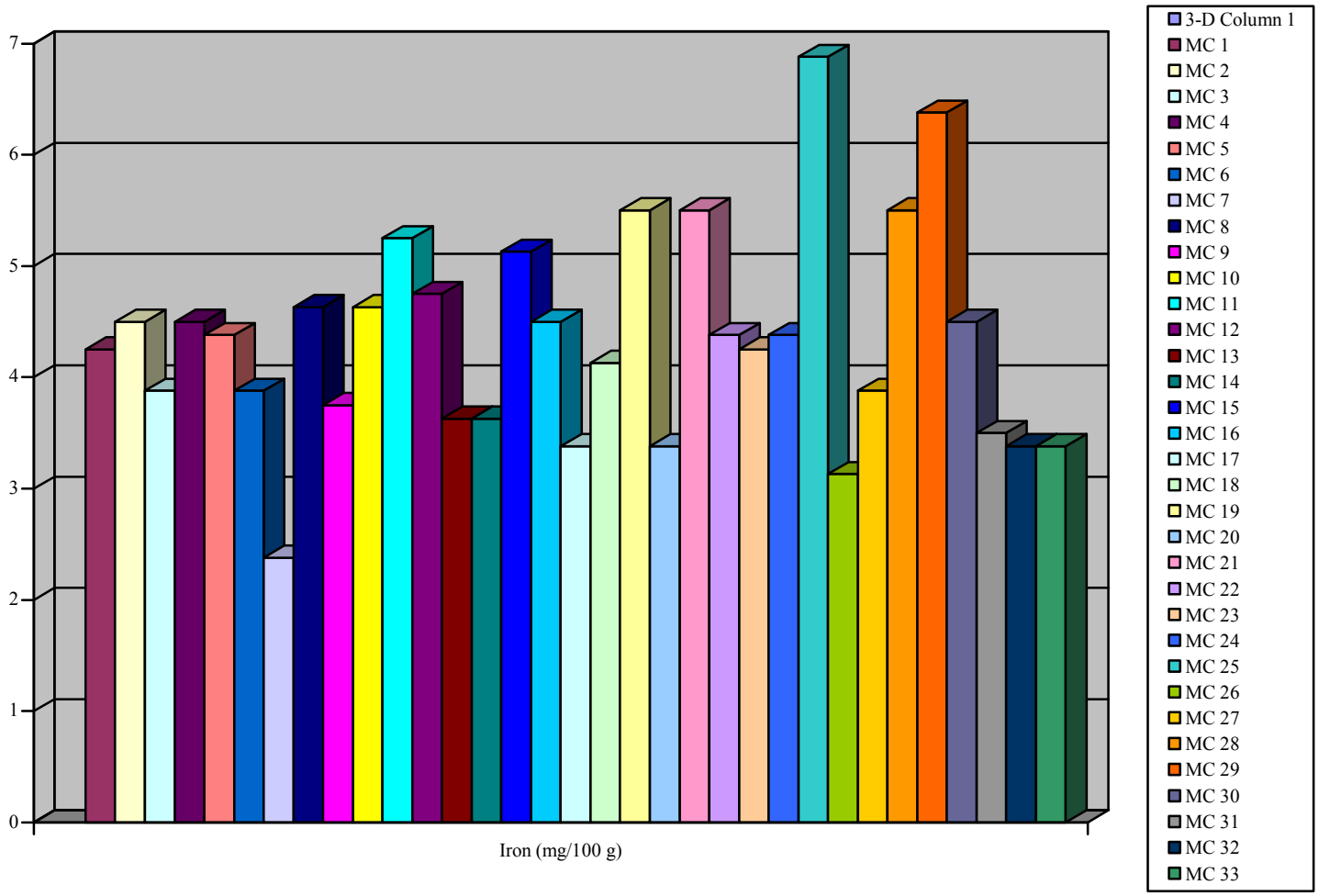


Fig. 5. Iron content in 33 *M. charantia* genotypes

Chlorophyll

Chlorophyll a content varied from 0.00110 to 0.54365 mg/g and 0.04839 mg/g was the general mean. Chlorophyll b content ranged from 0.00045 to 0.03075 mg/g and 0.00850 mg/g was the general mean. The range and overall mean of total chlorophyll was 0.003 to 0.12 mg/g and 0.03669 mg/g respectively (Fig. 6-8).

Bitterness

Bitterness value ranged from 1227.50 to 10400 units/g with a mean value of 2974.22 units/g. The highest bitterness value of 10400 units/g was observed in MC 25 and the lowest in MC 8 (1227.50 units/g) (Fig. 9).

4.3 Molecular characterization (RAPD)

RAPD (Random amplified polymorphic DNA) analysis was used to characterize genetic variability and relationships among thirty three genotypes of *M. charantia* from diverse ecosystems.

4.3.1 Isolation of Genomic DNA

Etiolated 15-20 days old seedlings were used to extract genomic DNA from various genotypes of *M. charantia* following the protocol modified from that of Murray and Thompson (1980).

The DNA yield for thirty three genotypes of *M. charantia* ranged from 1.05 to 3.96. The purity of DNA (A_{260} / A_{280} ratio) (Table 29) ranged from 1.40 to 2.09 $\mu g\ \mu l^{-1}$.

4.3.2 Testing the Quality of DNA

For RAPD profile analysis, the DNA should be free of RNA and protein. Moreover, it needs intact, unsheared DNA sample of sufficient quantity. To access the quality, all the genomic DNA samples were run on 0.8 per cent agarose gel and the gel was stained with ethidium bromide

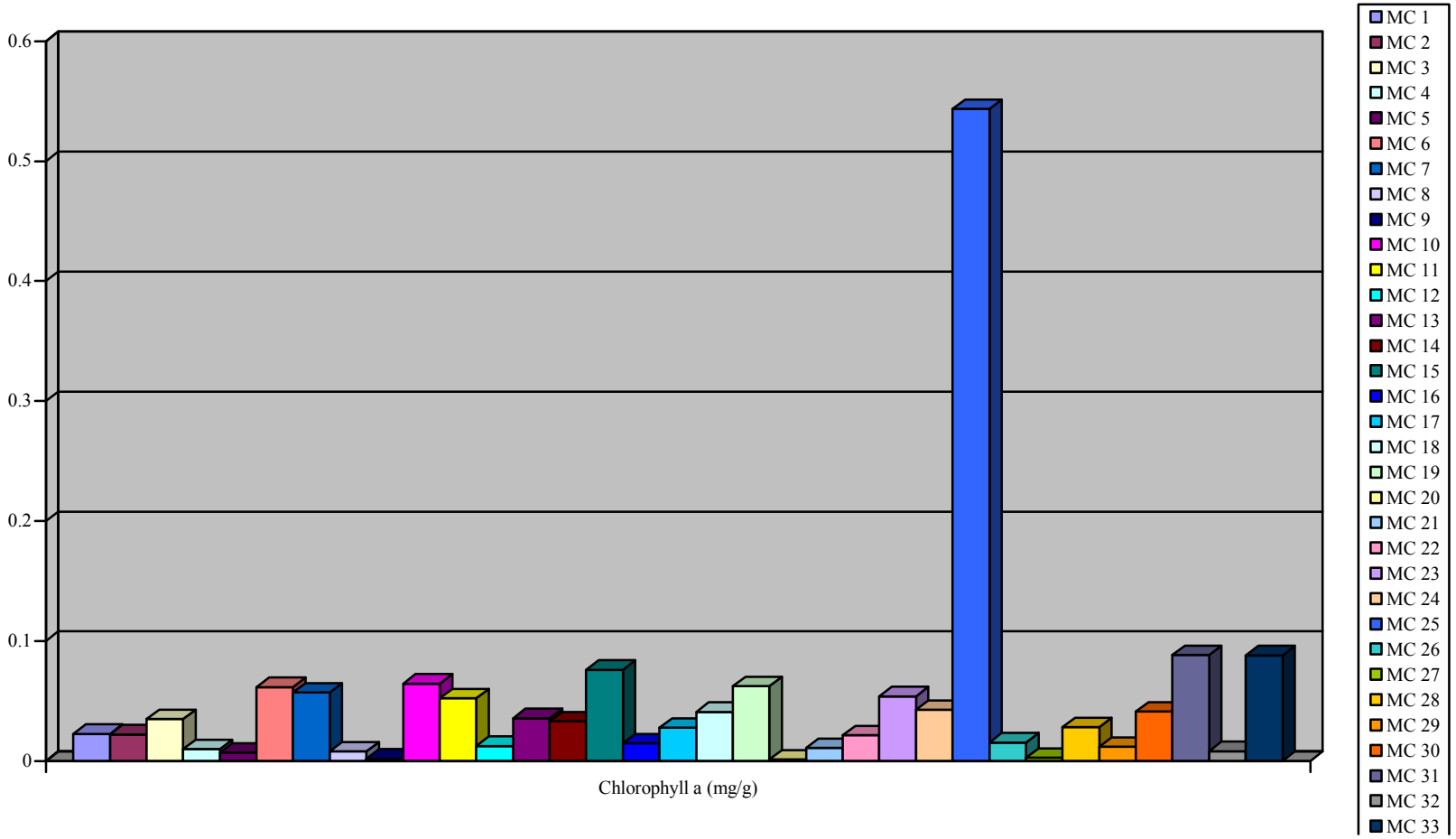


Fig. 6. Chlorophyll a content in 33 *M. charantia* genotypes

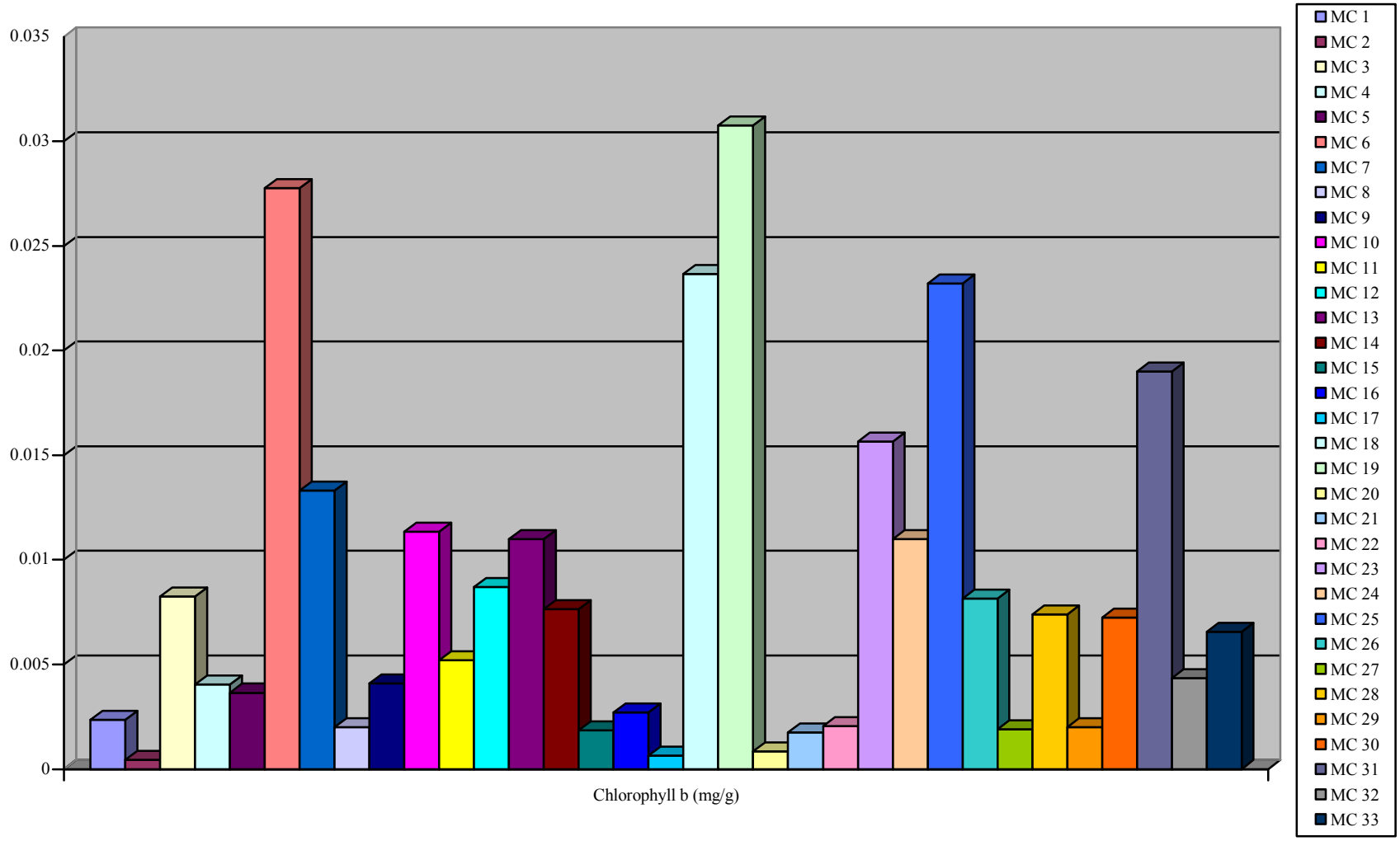


Fig. 7. Chlorophyll b content in 33 *M. charantia* genotypes

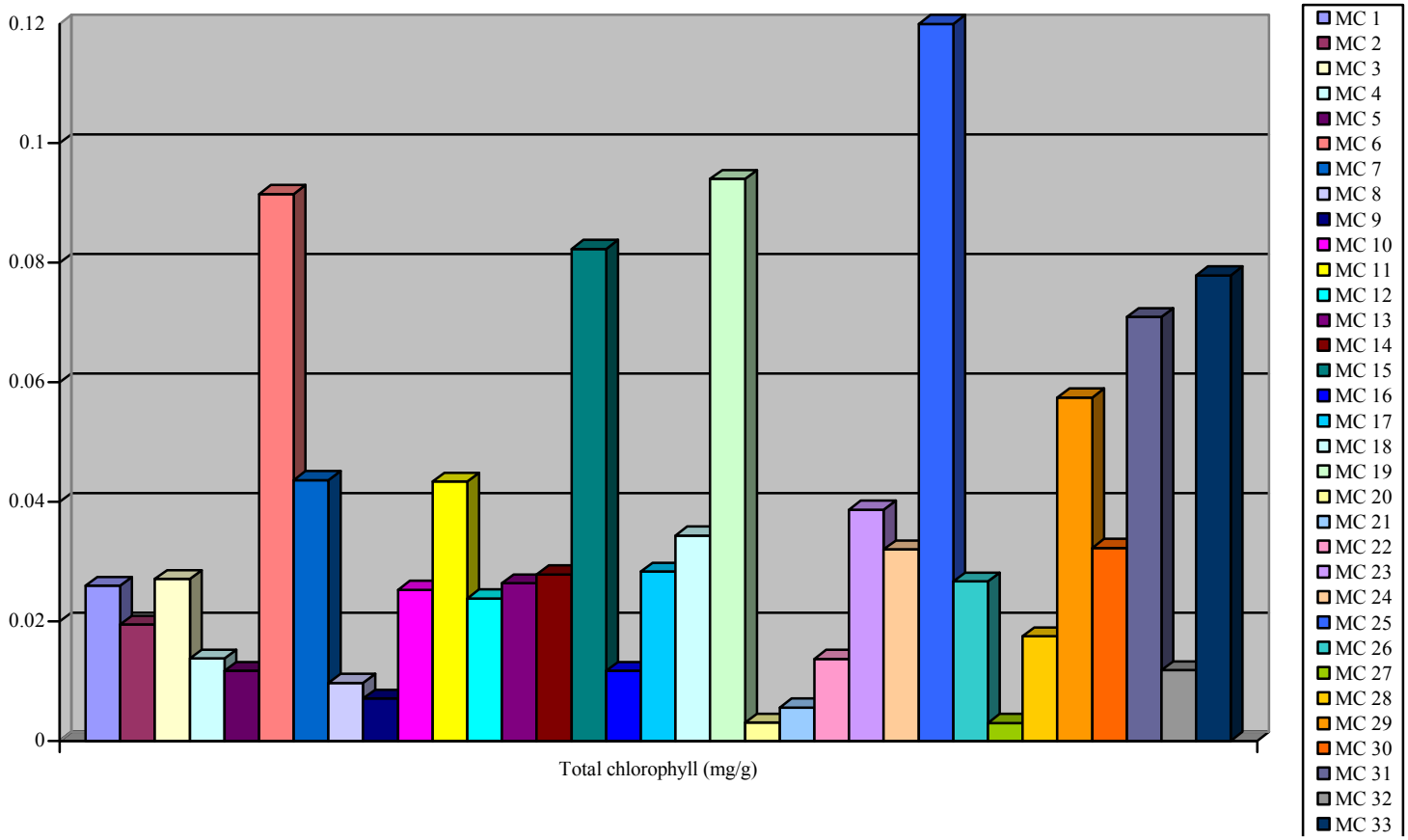


Fig. 8. Total chlorophyll content in 33 *M. charantia* genotypes

*Table 29***.** Quantitative and qualitative characters of DNA isolated from genotypes of *M. charantia* using modified Murray and Thompson method

Sl. No.	Treatments	260 nm	280 nm	Ratio $\left[\frac{260}{280}\right]$	DNA yield (µg µl-1)
1	MC 1	0.093	0.052	1.79	2.79
2	MC 2	0.058	0.034	1.71	1.74
3	MC 3	0.081	0.045	1.75	2.43
4	MC 4	0.084	0.043	1.63	2.52
5	MC 5	0.070	0.037	1.81	2.10
6	MC 6	0.077	0.039	1.54	2.31
7	MC 7	0.115	0.060	1.79	3.45
8	MC 8	0.076	0.041	1.40	2.28
9	MC 9	0.092	0.049	1.92	2.76
10	MC 10	0.085	0.049	1.83	2.55
11	MC 11	0.087	0.044	1.61	2.61
12	MC 12	0.096	0.055	1.46	2.88
13	MC 13	0.089	0.047	1.70	2.67
14	MC 14	0. 070	0.044	1.87	2.10
15	MC 15	0. 114	0.066	1.69	3.42
16	MC 16	0.087	0.046	1.89	2.61
17	MC 17	0.042	0.022	1.91	1.26
18	MC 18	0.055	0.027	2.04	1.65
19	MC 19	0.039	0.022	1.77	1.17
20	MC 20	0.080	0.045	1.77	2.40
21	MC 21	0.100	0.055	1.82	3.00
22	MC 22	0.062	0.033	1.88	1.86
23	MC 23	0.132	0.069	1.91	3.96
24	MC 24	0.067	0.038	1.73	2.01
25	MC 25	0.063	0.036	1.75	1.89
26	MC 26	0.068	0.036	1.89	2.04
27	MC 27	0.035	0.019	1.84	1.05
28	MC 28	0.067	0.037	1.81	2.01
29	MC 29	0.088	0.045	1.96	2.64
30	MC 30	0.055	0.028	1.96	1.65
31	MC 31	0.048	0.023	2.09	1.44
32	MC 32	0.039	0.022	1.77	1.17
33	MC 33	0.079	0.043	1.84	2.37

and bands appeared in the gel were visualized, using gel documentation system.

4.3.3 Polymerase Chain Reaction (PCR)

Polymerase chain reaction, standardized for the amplification of the DNA from *Cucumis melo* L. (Staub *et al*., 2000) was used for thirty three genotypes of *M. charantia*. Eighty decamer primers of series A, B, E, J and UBC were screened for their efficiency using the DNA isolated from genotype MC 12 as the representative sample. Out of the 80 decamer primers, fifty six yielded amplification products. The total number of bands, number of intense bands and number of faint bands produced by the primers are given in Table 30.

A total of 158 RAPDs (average 1.98 bands per primer) were generated by the 56 primers, of which 86.08 per cent were polymorphic (136 bands) and twenty two were monomorphic. Eight primers showed high level of polymorphism. The maximum number of RAPDs (9 bands) were produced by primer 0PA-02 and 0PA-18, followed by UBC-05 (8 bands), OPE-14 (7 bands), OPB-01 (6 bands), OPB-12 (6 bands), UBC-03 (6 bands) and OPB-06 (5 bands). For further PCR amplification, these eight primers were selected based on their performance in DNA amplification and production of highest number of bands as well as intense bands (Table 31). Also the selected primers were consistent and heritable when checked for their reproducibility. Hence these were used for DNA amplification of 33 genotypes of *M. charantia*. Data obtained from the eight primers that give reproducible bands were used for statistical analysis.

The RAPD profile generated by three selected primers *viz.,* 0PA-02, 0PA-18, OPB-01, OPB-06, OPB-12, OPE-14, UBC-03 and UBC-05 were shown in Plates 17 to 24 and Figures 10-17**.** A total of 56 scorable bands (average of 7.00 bands per primer) were generated of which 23 were

Table 30. Primer associated banding patterns in DNA sample of genotype MC 12

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

Table 30. Continued

Sl. No.	Primers	Total number of bands	Number of intense bands	Number of faint bands
41	OPE-01	0	0	0
42	OPE-02	1	1	0
43	OPE-03	0	0	0
44	OPE-04	0	0	0
45	OPE-05	2	0	2
46	OPE-06	0	0	0
47	OPE-07	1	1	0
48	OPE-08	1	1	0
49	OPE-09	2	1	1
50	OPE-10	2	1	1
51	OPE-11	1	1	0
52	OPE-12	0	0	0
53	OPE-13	2	0	2
54	OPE-14	4	2	1
55	OPE-15	2	1	1
56	OPE-16	0	0	0
57	OPE-17	2	1	1
58	OPE-18	0	0	0
59	OPE-19	0	0	0
60	OPE-20	2	2	0
61	OPJ-01	1	0	1
62	OPJ-02	2	0	2
63	OPJ-03	0	0	0
64	OPJ-04	3	2	1
65	OPJ-05	3	1	2
66	OPJ-06	1	0	1
67	OPJ-07	2	1	1
68	OPJ-08	2	1	1
69	OPJ-09	0	0	0
70	OPJ-10	0	0	0
71	UBC-01	3	1	2
72	UBC-02	0	0	0
73	UBC-03	6	4	2
74	UBC-04	0	0	0
75	UBC-05	8	6	2
76	UBC-06	3	0	3
77	UBC-07	2	0	2
78	UBC-08	3	1	2
79	UBC-09	0	0	0
80	UBC-10	2	1	1

Table 31. Nucleotide sequences of primers and total number of informative RAPD markers amplified with them in the genotypes of *M. charantia* used in this study

Sl. No.	Primer	Sequence (5'-3' direction)	Number of informative RAPD markers
1	0PA-02	TGCCGAGCTG	9
2	0PA-18	AGGTGATCGT	9
3	OPB-01	GTTTCGCTCC	6
4	OPB-06	TGCTCTGCCC	5
5	OPB-12	CCTTGATGCA	6
6	OPE-14	TGCGGCTGAG	7
7	UBC-03	CCTGGGCCCC	6
8	UBC-05	CCCGCCTCCC	8

Fig. 10. Representation of amplification profile of the DNA of thirty three genotypes of *M. charantia* using the OPA-02

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	-	+	+	+	+	+	+	+	-
-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	+	+	-	+	-	+	+	-	+	+	+	+	-
-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
+	+	+	-	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-
+	+	+	-	+	-	+	-	+	-	+	-	+	+	+	-	-	-	-	+	-	+	-	+	-	+	+	+	-	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+

Fig. 11. Representation of amplification profile of the DNA of thirty three genotypes of *M. charantia* using the OPA-18

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	-	-	-	-	-	-	+	+	+	-	-	+	-	-	+	+	-	-	-	-	-	+	+	-	+	+	+	+	+	+
-	+	-	+	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Plate 17. Amplification profiles of the DNA of thirty three genotypes of *M. charantia* using the primer OPA-02

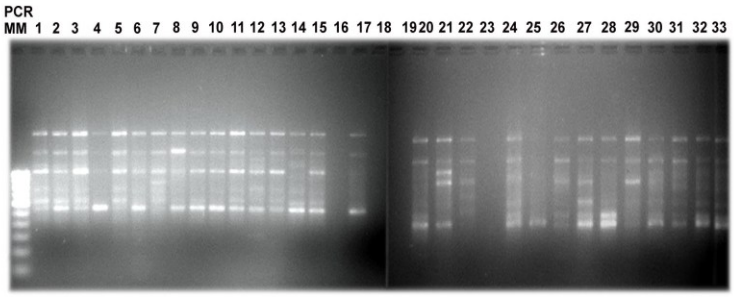


Plate 18. Amplification profiles of the DNA of thirty three genotypes of *M. charantia* using the primer OPA-18

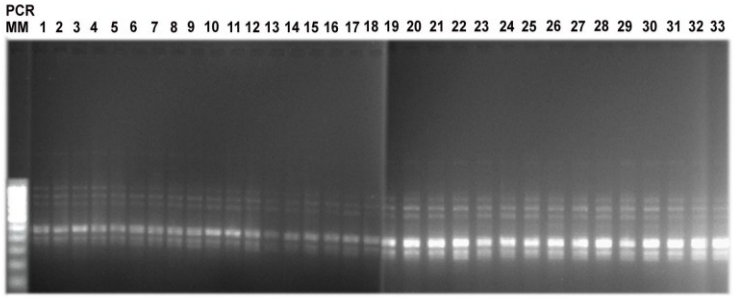


Fig. 12. Representation of amplification profile of the DNA of thirty three genotypes of *M. charantia* using the OPB-01

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	-
-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-

Fig. 13. Representation of amplification profile of the DNA of thirty three genotypes of *M. charantia* using the OPB-06

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-
-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	+	-	+	-	-	-	+	+	-
+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
+	+	+	+	-	-	-	+	-	+	+	+	-	+	-	-	-	-	-	-	+	-	+	-	-	+	-	-	+	-	+	-	-
-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-

Fig. 14. Representation of amplification profile of the DNA of thirty three genotypes of *M. charantia* using the OPB-12

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+	-	-	-
+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+
-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	+	-	+	-	-	+	+
+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Plate 19. Amplification profiles of the DNA of thirty three genotypes of *M. charantia* using the primer OPB-01

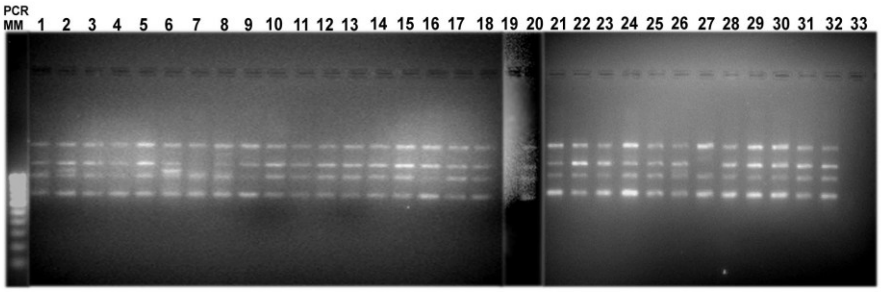


Plate 20. Amplification profiles of the DNA of thirty three genotypes of *M. charantia* using the primer OPB-06

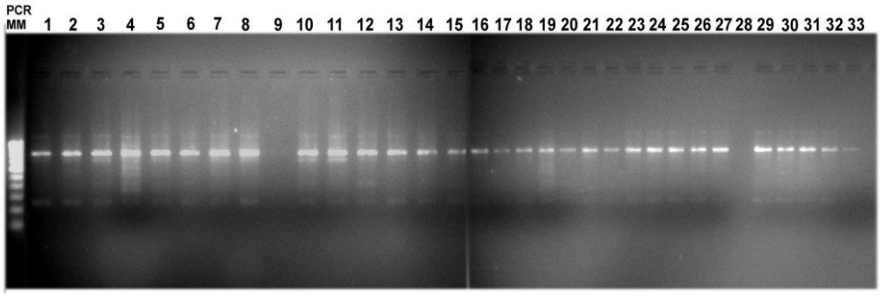


Plate 21. Amplification profiles of the DNA of thirty three genotypes of *M. charantia* using the primer OPB-12

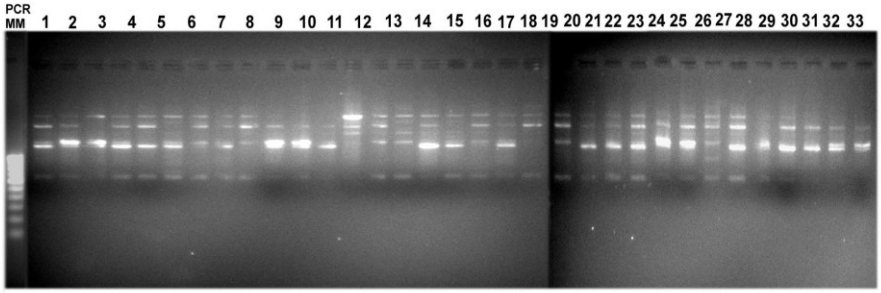


Fig. 15. Representation of amplification profile of the DNA of thirty three genotypes of *M. charantia* using the OPE-14

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-
-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-
-	+	-	+	-	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
-	-	-	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-
-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-

Fig. 16. Representation of amplification profile of the DNA of thirty three genotypes of *M. charantia* using the UBC-03

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
-	+	-	-	-	+	+	+	+	-	+	-	-	-	-	+	+	+	+	-	+	-	-	+	-	-	+	-	-	+	-	-	-
+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
-	+	-	-	+	+	-	-	-	-	+	-	-	+	-	+	+	-	-	+	+	-	-	-	-	-	+	-	+	+	-	-	-
-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Fig. 17. Representation of amplification profile of the DNA of thirty three genotypes of *M. charantia* using the UBC-05

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	-	+	-	-	+	-
+	+	-	+	+	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Plate 22. Amplification profiles of the DNA of thirty three genotypes of *M. charantia* using the primer OPE-14

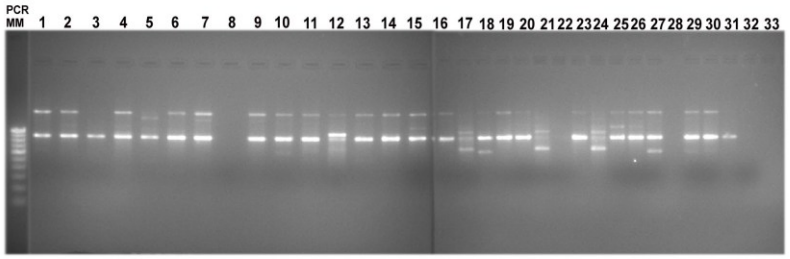


Plate 23. Amplification profiles of the DNA of thirty three genotypes of *M. charantia* using the primer UBC-03

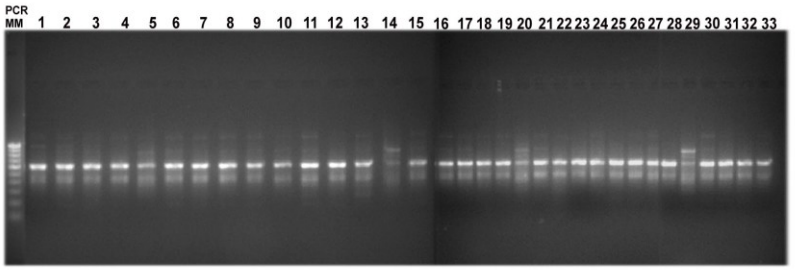
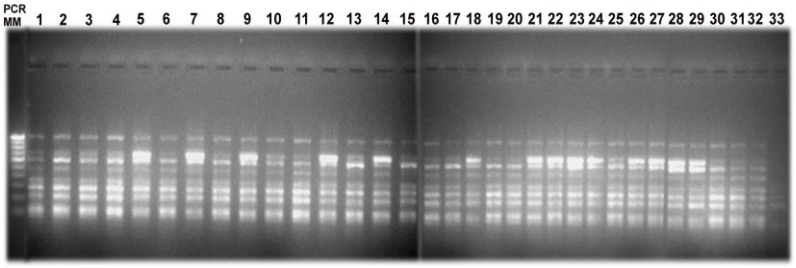


Plate 24. Amplification profiles of the DNA of thirty three genotypes of *M. charantia* using the primer UBC-05



monomorphic and rest, 33 were polymorphic (58.93 %). The number of bands ranged from 1 to 9 with an average of 1.25 per primer.

The primer OPA-02 was unique as it could distinguish maximum polymorphism among the genotypes tested. The highest number of scorable bands was given by OPA-18 of which seven of the bands produced were monomorphic. The primer UBC-05 produced eight scorable bands of which six bands were monomorphic for all the genotypes. The primer UBC-03, which produced a total of six scorable bands had two bands as monomorphic. Among the OPB group, primer OPB-01 had three monomorphic bands, while OPB-06 and OPB-12 had 2 and 1 monomorphic bands respectively. The primer OPE-14 had only one band as monomorphic while the rest, 6 bands were highly polymorphic.

4.3.4 Data Analysis

The banding pattern from RAPD analysis for each primer was scored by visual observation. Reproducible bands were scored for their presence (+) or absence (-) for all the genotypes of *M. charantia* studied. From this RAPD marker data, Jaccard's similarity coefficient values were calculated for each pair-wise comparison between genotypes and a similarity coefficient matrix was constructed (Table 32). This matrix was subjected to UPGMA to generate a dendrogram for the thirty three genotypes (Fig.18). All computing were carried out using NTSYS-pc software.

Overall similarity indices ranged from 0.65 to 0.90. Cluster analysis revealed that at about 0.65 similarity coefficient, the thirty three genotypes of *M. charantia* grouped into two clusters. The genotype with smallest fruit M 33 got differentiated from the rest of the bittergourd germplasm at 0.65 similarity coefficient.

Table 32. Similarity matrix for the thirty three genotypes of *M. charantia* generated using RAPD primers

x	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8	MC9	MC10	MC11	MC12	MC13	MC14	MC15	MC16	MC17	MC18	MC19	MC20	MC21	MC22	MC23	MC24	MC25	MC26	MC27	MC28	MC29	MC30	MC31	MC32	MC33
MC 1	1.00																																
MC 2	0.64	1.00																															
MC 3	0.70	0.76	1.00																														
MC 4	0.71	0.73	0.72	1.00																													
MC 5	0.69	0.75	0.78	0.79	1.00																												
MC 6	0.67	0.72	0.72	0.77	0.83	1.00																											
MC 7	0.73	0.71	0.82	0.83	0.85	0.79	1.00																										
MC 8	0.68	0.70	0.78	1.00	0.76	0.78	0.80	1.00																									
MC 9	0.65	0.75	0.74	0.83	0.81	0.79	0.85	0.85	1.00																								
MC 10	0.65	0.76	0.80	0.85	0.79	0.76	0.75	0.78	0.75	1.00																							
MC 11	0.69	0.78	0.78	0.79	0.77	0.84	0.81	0.80	0.81	0.83	1.00																						
MC 12	0.67	0.76	0.85	0.81	0.83	0.76	0.75	0.78	0.75	0.80	0.83	1.00																					
MC 13	0.68	0.76	0.80	0.72	0.87	0.76	0.74	0.73	0.78	0.80	0.74	0.76	1.00																				
MC 14	0.68	0.77	0.80	0.77	0.88	0.77	0.75	0.75	0.75	0.77	0.75	0.85	0.80	1.00																			
MC 15	0.60	0.86	0.82	0.74	0.76	0.73	0.72	0.71	0.80	0.78	0.76	0.73	0.82	0.74	1.00																		
MC 16	0.67	0.66	0.65	0.74	0.72	0.73	0.83	0.80	0.80	0.69	0.76	0.69	0.73	0.70	0.75	1.00																	
MC 17	0.68	0.70	0.69	0.79	0.76	0.82	0.80	0.80	0.76	0.74	0.72	0.70	0.73	0.75	0.75	0.80	1.00																
MC 18	0.67	0.76	0.72	0.77	0.79	0.80	0.75	0.78	0.87	0.80	0.79	0.76	0.83	0.77	0.82	0.78	0.78	1.00															
MC 19	0.68	0.70	0.69	0.75	0.72	0.78	0.72	0.76	0.76	0.78	0.76	0.74	0.73	0.71	0.80	0.84	0.76	0.82	1.00														
MC 20	0.70	0.63	0.65	0.70	0.72	0.65	0.72	0.71	0.76	0.65	0.72	0.65	0.73	0.70	0.70	0.79	0.67	0.69	0.71	1.00													
MC 21	0.68	0.72	0.76	0.81	0.83	0.80	0.83	0.82	0.79	0.76	0.79	0.85	0.76	0.81	0.69	0.73	0.74	0.76	0.74	0.69	1.00												
MC 22	0.87	0.69	0.72	0.81	0.79	0.76	0.83	0.78	0.83	0.76	0.83	0.72	0.76	0.73	0.73	0.78	0.74	0.72	0.74	0.82	0.72	1.00											
MC 23	0.68	0.69	0.76	0.85	0.80	0.73	0.75	0.86	0.80	0.77	0.80	0.77	0.76	0.82	0.78	0.78	0.70	0.77	0.75	0.78	0.77	0.82	1.00										
MC 24	0.69	0.77	0.72	0.77	0.88	0.77	0.83	0.75	0.83	0.69	0.75	0.77	0.80	0.77	0.88	0.74	0.75	0.77	0.75	0.70	0.81	0.77	0.78	1.00									
MC 25	0.66	0.65	0.68	0.78	0.71	0.69	0.71	0.75	0.80	0.73	0.75	0.77	0.68	0.69	0.78	0.78	0.83	0.82	0.75	0.70	0.69	0.73	0.78	0.69	1.00								
MC 26	0.66	0.82	0.79	0.80	0.77	0.75	0.73	0.81	0.81	0.79	0.81	0.79	0.79	0.76	0.85	0.72	0.69	0.79	0.77	0.68	0.87	0.75	0.80	0.80	0.76	1.00							
MC 27	0.72	0.77	0.69	0.70	0.76	0.70	0.80	0.75	0.85	0.70	0.72	0.70	0.81	0.74	0.67	0.71	0.75	0.73	0.68	0.67	0.73	0.73	0.66	0.74	0.63	0.72	1.00						
MC 28	0.69	0.73	0.72	0.73	0.75	0.65	0.75	0.75	0.75	0.73	0.75	0.73	0.72	0.73	0.78	0.74	0.71	0.73	0.75	0.82	0.73	0.77	0.82	0.77	0.73	0.80	0.67	1.00					
MC 29	0.61	0.70	0.78	0.79	0.76	0.78	0.68	0.76	0.76	0.87	0.80	0.82	0.73	0.75	0.79	0.67	0.72	0.82	0.76	0.63	0.74	0.70	0.75	0.67	0.75	0.77	0.68	0.71	1.00				
MC 30	0.65	0.78	0.83	0.71	0.80	0.78	0.76	0.80	0.80	0.78	0.80	0.78	0.78	0.79	0.75	0.71	0.76	0.82	0.76	0.71	0.78	0.78	0.79	0.79	0.70	0.75	0.77	0.75	0.76	1.00			
MC 31	0.67	0.76	0.80	0.81	0.75	0.68	0.79	0.78	0.79	0.80	0.87	0.85	0.72	0.77	0.73	0.69	0.70	0.72	1.00	0.73	0.76	0.85	0.82	0.73	0.77	0.79	0.73	0.81	0.78	0.82	1.00		
MC 32	0.71	0.77	0.80	0.77	0.79	0.69	0.79	0.75	0.79	0.77	0.88	0.85	0.76	0.77	0.74	0.74	0.71	0.73	0.71	0.74	0.73	0.85	0.73	0.73	0.78	0.76	0.78	0.73	0.75	0.79	0.90	1.00	
MC 33	0.58	0.64	0.66	0.68	0.65	0.60	0.65	0.65	0.73	0.67	0.73	0.67	0.62	0.64	0.72	0.68	0.65	0.75	0.69	0.68	0.60	0.67	0.71	0.64	0.81	0.70	0.61	0.68	0.69	0.69	0.71	0.72	1.00

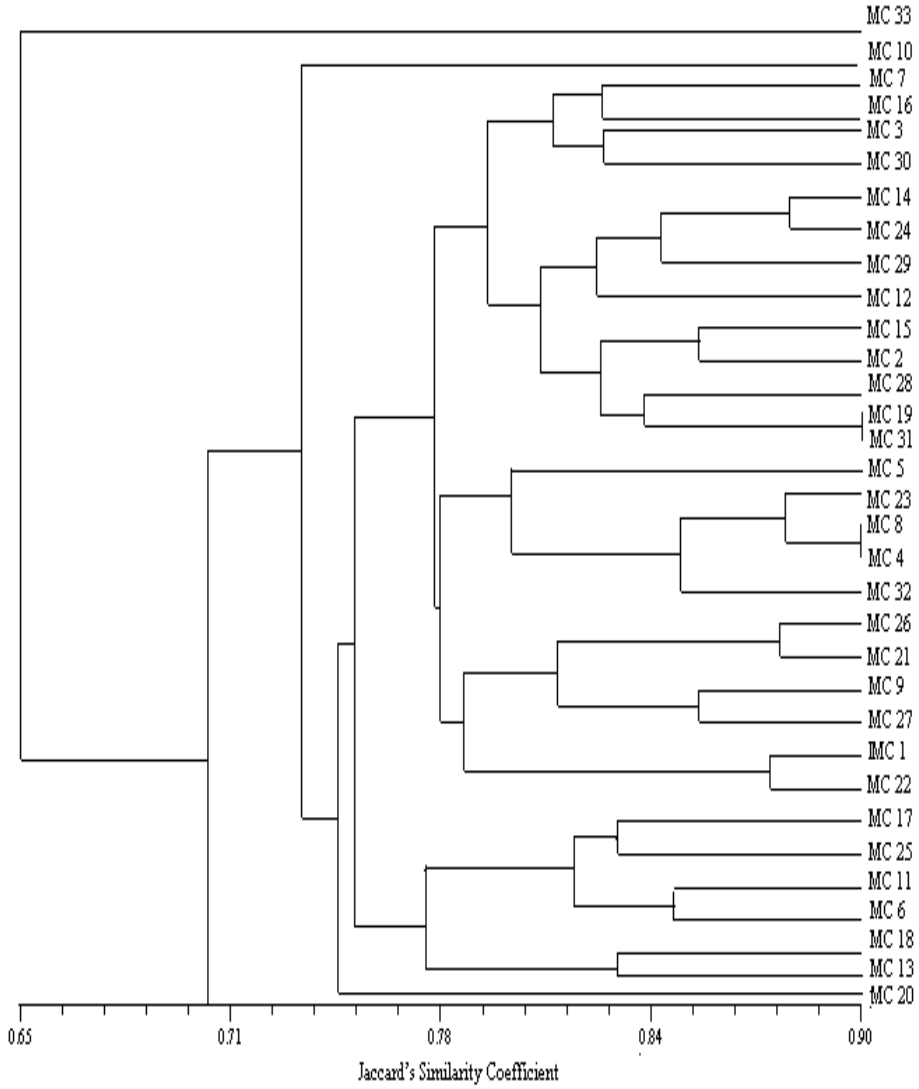


Fig. 18. Dendrogram for thirty three genotypes of *M. charantia* based on data from RAPD primers

At 0.70 similarity coefficient, the long fruited and mutant genotype MC 10 got distinct from the rest of 32 bittergourd genotypes. The genotype with highest average fruit weight, MC 20 stood out from the rest of the group at 73.1 per cent similarity. Genotypes with low average fruit weight stood out from the rest of the group at 75.2 per cent similarity. These genotypes with low fruit weight again grouped into 2 subclusters at 77.65 per cent similarity. The first subcluster was further divided into two subgroups with two members each at 81.9 per cent similarity. MC 17 showed 83.1 per cent similarity with MC 25 while MC 6 showed 84.6 per cent similarity with MC 11. The second subcluster consisted of two members MC 13 and MC 18 which showed 83.1 per cent genetic similarity.

Genotypes with medium fruit size could be split into two subclusters of 13 and 11 members respectively at 77.8 per cent similarity. This grouping was in concordance with their fruit skin colour.

At 79.2 per cent similarity, first subcluster of genotypes with medium fruit size was again grouped into two subgroups with 4 and 9 members respectively. The members had green skin colour on fruits with exceptions of MC 15 and MC 29 having dark green skin colour falling in this subcluster.

The first subgroup with four members (MC 3, MC 7, MC 16 and MC 30) further divided into two at 82.5 per cent similarity. MC 7 showed 83.7 per cent similarity with MC 16. Similarly, MC 3 showed 83.7 per cent similarity with MC 30.

At 80.7 per cent similarity, second subgroup comprising of genotypes with same skin texture was further grouped into two with 4 and 5 members respectively. MC 14, MC 24 and MC 29 formed one group while MC 12 remained distinct at 0.83 similarity coefficient. At 85.5 per cent similarity, the member with dark green skin colour MC 29 got distinct from MC 14 and MC 24. MC 14 showed 88.2 per cent

similarity with MC 24. Similarly, the second division of this subgroup with five members MC 2, MC 15, MC 19, MC 28 and MC 31 was again divided into two at 82.8 per cent similarity. Among these, MC 2 and MC 15 fall in one group while MC 19, MC 28 and MC 31 formed another group. At 0.86 similarity coefficient, MC 15 which had fruits with dark green skin colour got distinct from MC 2. Similarly, MC 28 stands singly at 0.84 per cent similarity coefficient while MC 19 and MC 31 showed 100 per cent similarity.

The second subcluster of eleven genotypes with medium fruit size was again grouped into two subgroups with 5 and 6 members respectively at 0.78 similarity coefficient. Most of the members of this subcluster had light green skin colour, but exceptions of white skin colour was also found.

At 81.3 per cent similarity, the first subgroup with 5 members was again divided into two with MC 4, MC 8 and MC 23 falling in one subgroup and MC 5 stand singly. At 85.6 per cent similarity, MC 23 got distinct from MC 4 and MC 8. MC 4 showed 100 per cent similarity with MC 8.

Among the second subgroup, genotypes with light green skin coloured fruits (MC 1, MC 9, MC 21, MC 22, MC 26 and MC 27) was again grouped into 2 with four and two members respectively at 78.6 per cent similarity. MC 21 showed 87.9 per cent similarity with MC 26 while MC 9 showed 85.2 per cent similarity with MC 27. Rest of the genotypes in second subgroup, MC 1 and MC 22 showed 87.3 per cent similarity.

In this study, RAPD marker analysis has revealed and grouped the *M. charantia* genotypes according to their genetic relationships reliably. The clusters based on RAPD analysis using eight primers depict genetic variation among the genotypes of *M. charantia*. The 33 genotypes of *M. charantia* that were studied formed 8 clusters in the UPGMA cluster analysis. Quite distinct among these were two cluster formed

at 0. 65 similarity coefficient which clearly separates genotypes based on average fruit weight and fruit skin colour.

4.4 Standardization of *in vitro* techniques

4.4.1 Enhanced Release of Axillary Buds

Shoot tip explants were used for multiple shoot production following the protocol of Sultana and Bari Miah (2003) and Sikdar et al. (2005). Complete greening and shoot initiation was shown by aseptic explants in the establishment medium. Development and multiplication of axillary buds from shoot tip explants was also observed in the specified media (Plate 25). But multiplication and elongation of axillary buds on the same media could not be obtained even after one month.

4.4.2 Somatic Organogenesis

Callus mediated (indirect) somatic organogenesis was induced in bittergourd using leaf, nodal, internodal and shoot tip explants. The explants were cultured on a Murashige and Skoog (MS) basal nutrient medium containing plant growth regulators (cytokinin or auxin- cytokinin) with various combinations and concentrations for the study of callus induction and proliferation. Efficient plant regeneration *via* organogenesis was also established. The results of the study are presented as follows.

4.4.2.1 Effect of plant growth regulators on callus induction and proliferation

Thirty treatments with different combinations of plant growth regulators (BA, IAA, IBA, NAA and 2,4-D) were tried to assess its effect on callusing and proliferation from leaf, nodal, internodal and shoot tip explants (Table 33-36 and Plates 26-29, 31-33).

The results revealed that the lowest minimum number of days for callusing in leaf (12.67), nodal (12.00), internodal (13.33) and shoot tip (11) explants was observed in the treatment MOP_{19} followed by MOP_{27}

Table 33. The effect of plant growth regulators on leaf explants for callus induction and proliferation in bittergourd
(Medium – MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1})

Treatment No.	Days to callusing	Callusing percentage (%)	Growth score	Callus index
MOP_1	16.50	66.67	1.16	77.34
MOP_2	-	0.00	0.00	0.00
MOP_3	-	0.00	0.00	0.00
MOP_4	18.00	33.33	1.00	33.33
MOP_5	16.33	50.00	1.33	66.50
MOP_6	20.00	16.67	1.16	19.34
MOP_7	16.33	33.33	1.50	49.99
MOP_8	14.83	83.33	2.00	166.66
MOP_9	16.50	16.67	1.33	22.17
MOP_{10}	-	0.00	0.00	0.00
MOP_{11}	-	0.00	0.00	0.00
MOP_{12}	-	0.00	0.00	0.00
MOP_{13}	-	0.00	0.00	0.00
MOP_{14}	15.00	33.33	1.00	33.33
MOP_{15}	16.00	66.67	1.33	88.67
MOP_{16}	13.33	100.00	2.00	200.00
MOP_{17}	16.00	100.00	1.33	133.00
MOP_{18}	14.00	66.67	2.83	188.68
MOP_{19}	12.67	100.00	3.83	383.00
MOP_{20}	15.00	100.00	2.17	217.00
MOP_{21}	16.67	16.67	1.50	25.01
MOP_{22}	-	0.00	0.00	0.00
MOP_{23}	18.00	50.00	2.00	100.00
MOP_{24}	16.00	100.00	3.17	317.00
MOP_{25}	16.17	33.33	1.17	38.99
MOP_{26}	14.33	66.67	2.50	166.68
MOP_{27}	13.00	100.00	3.67	367.00
MOP_{28}	17.17	100.00	3.50	350.00
MOP_{29}	18.83	50.00	1.67	83.50
MOP_{30}	22.00	16.67	1.00	16.67
Control	-	0.00	0.00	0.00

*Treatment combinations are given in Table 5.
The data represents mean value of the six replications.

Table 34. The effect of plant growth regulators on nodal explants for callus induction and proliferation in bittergourd
Medium – MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1})

*Treatment No.	Days to callusing	Callusing percentage (%)	Growth score	Callus index
MOP_1	15.00	83.33	1.40	116.66
MOP_2	-	0.00	0.00	0.00
MOP_3	15.67	50.00	1.00	50.00
MOP_4	16.00	50.00	1.30	66.66
MOP_5	14.67	83.33	1.80	149.99
MOP_6	18.00	33.33	1.50	49.99
MOP_7	14.00	33.33	1.00	33.33
MOP_8	13.50	50.00	1.67	83.33
MOP_9	15.50	50.00	1.00	50.00
MOP_{10}	19.67	50.00	2.00	100.00
MOP_{11}	-	0.00	0.00	0.00
MOP_{12}	-	0.00	0.00	0.00
MOP_{13}	16.00	33.33	1.00	33.33
MOP_{14}	15.83	33.33	1.00	33.33
MOP_{15}	15.50	83.33	2.60	216.66
MOP_{16}	13.50	100.00	2.67	267.00
MOP_{17}	14.00	100.00	2.50	250.00
MOP_{18}	13.50	100.00	3.00	300.00
MOP_{19}	12.00	100.00	4.00	400.00
MOP_{20}	15.00	100.00	2.33	230.00
MOP_{21}	15.67	50.00	2.00	100.00
MOP_{22}	-	0.00	0.00	0.00
MOP_{23}	17.00	33.33	2.00	66.66
MOP_{24}	13.50	66.67	2.75	183.34
MOP_{25}	19.00	16.67	1.00	16.67
MOP_{26}	15.00	83.33	2.80	233.32
MOP_{27}	13.00	100.00	4.00	400.00
MOP_{28}	14.00	100.00	2.83	283.00
MOP_{29}	18.67	83.33	1.80	149.99
MOP_{30}	20.00	16.67	2.00	33.34
Control	-	0.00	0.00	0.00

*Treatment combinations are given in Table 5.
The data represents mean value of the six replications.

Table 35. The effect of plant growth regulators on internodal explants for callus induction and proliferation in bittergourd
(Medium – MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1})

*Treatment No.	Days to callusing	Callusing percentage (%)	Growth score	Callus index
MOP_1	18.33	100.00	2.66	266.00
MOP_2	20.00	16.67	1.33	22.17
MOP_3	18.00	16.67	1.50	25.01
MOP_4	18.00	33.33	1.83	60.99
MOP_5	17.33	50.00	2.33	116.50
MOP_6	19.17	33.33	1.33	44.33
MOP_7	15.33	100.00	2.66	266.00
MOP_8	14.83	100.00	3.00	300.00
MOP_9	17.83	33.33	2.17	72.33
MOP_{10}	19.00	33.33	2.00	66.66
MOP_{11}	-	0.00	0.00	0.00
MOP_{12}	-	0.00	0.00	0.00
MOP_{13}	17.00	33.33	1.17	38.99
MOP_{14}	15.17	33.33	2.00	66.66
MOP_{15}	15.00	50.00	2.83	141.50
MOP_{16}	14.50	100.00	3.00	300.00
MOP_{17}	16.50	83.33	3.00	249.99
MOP_{18}	18.50	83.33	3.33	277.49
MOP_{19}	13.33	100.00	4.00	400.00
MOP_{20}	18.33	83.33	2.67	222.49
MOP_{21}	20.17	66.67	2.00	133.34
MOP_{22}	20.83	66.67	1.17	78.00
MOP_{23}	16.33	33.33	2.67	88.99
MOP_{24}	16.00	100.00	3.00	300.00
MOP_{25}	17.67	100.00	2.83	283.00
MOP_{26}	15.83	66.67	3.00	200.01
MOP_{27}	14.17	100.00	3.67	367.00
MOP_{28}	15.50	100.00	3.00	300.00
MOP_{29}	18.83	83.33	1.67	139.16
MOP_{30}	23.50	33.33	1.00	33.33
Control	-	0.00	0.00	0.00

*Treatment combinations are given in Table 5.
The data represents mean value of the six replications.

Table 36. The effect of plant growth regulators on shoot tip explants for callus induction and proliferation in bittergourd
(Medium – MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1})

*Treatment No.	Days to callusing	Callusing percentage (%)	Growth score	Callus index
MOP_1	17.67	100.00	2.00	200.00
MOP_2	19.00	16.67	2.00	33.34
MOP_3	19.00	16.67	1.00	16.67
MOP_4	16.83	50.00	1.33	66.50
MOP_5	15.00	83.33	1.83	152.49
MOP_6	16.33	83.33	1.33	110.83
MOP_7	15.00	66.67	3.33	222.01
MOP_8	13.67	100.00	2.67	267.00
MOP_9	14.33	83.33	1.83	152.49
MOP_{10}	16.83	83.33	2.00	166.66
MOP_{11}	-	0.00	0.00	0.00
MOP_{12}	-	0.00	0.00	0.00
MOP_{13}	14.00	33.33	1.17	38.99
MOP_{14}	13.83	33.33	2.00	66.66
MOP_{15}	13.33	66.67	2.17	144.67
MOP_{16}	13.00	100.00	2.67	267.00
MOP_{17}	15.00	100.00	2.00	200.00
MOP_{18}	12.00	100.00	3.17	317.00
MOP_{19}	11.00	100.00	4.00	400.00
MOP_{20}	13.00	100.00	2.83	283.00
MOP_{21}	15.00	83.33	1.83	152.49
MOP_{22}	16.50	66.67	1.50	100.01
MOP_{23}	14.17	66.67	1.83	122.01
MOP_{24}	12.67	83.33	2.00	166.66
MOP_{25}	19.00	33.33	1.33	44.33
MOP_{26}	12.50	66.67	3.00	200.01
MOP_{27}	11.33	100.00	4.00	400.00
MOP_{28}	13.00	100.00	3.16	316.00
MOP_{29}	16.50	50.00	3.00	150.00
MOP_{30}	19.33	50.00	2.33	116.50
Control	-	0.00	0.00	0.00

*Treatment combinations are given in Table 5.
The data represents mean value of the six replications.

Plate 25. The effect of MS medium containing the different growth regulators and supplements on shoot tip explants for shoot proliferation in bittergourd

Medium – MS + BA 2.00 mg l^{-1} + NAA 0.20 mg l^{-1}

A. Complete greening and shoot initiation after one week of culture

B. Development and multiplication of axillary buds after four weeks of culture

Medium – MS + BA 2.00 mg l^{-1}+ IAA 0.10 mg l^{-1}+ GA_3 2.00 mg l^{-1}

C. Complete greening after 5 days of culture

D. Shoot initiation after two weeks of culture

E. Development and multiplication of axillary buds after four weeks of culture

Plate 26. Effect of plant growth regulators on different explants for callus induction in bittergourd (BA)
Medium – MS + BA 0.05 mg l^{-1}

Leaf explant

A. Callus initiation within two weeks

B. Callus proliferation within 3-4 weeks

Nodal explant

C. Callus initiation within two weeks

D. Callus proliferation within 3 weeks

Internodal explant

E. Callus initiation within two weeks

F. Callus proliferation within 3-4 weeks

Shoot tip explant

G. Callus initiation within two weeks

H. Callus proliferation within 3 weeks

Plate 25. The effect of MS medium containing the different growth regulators and supplements on shoot tip explants for shoot proliferation in bittergourd

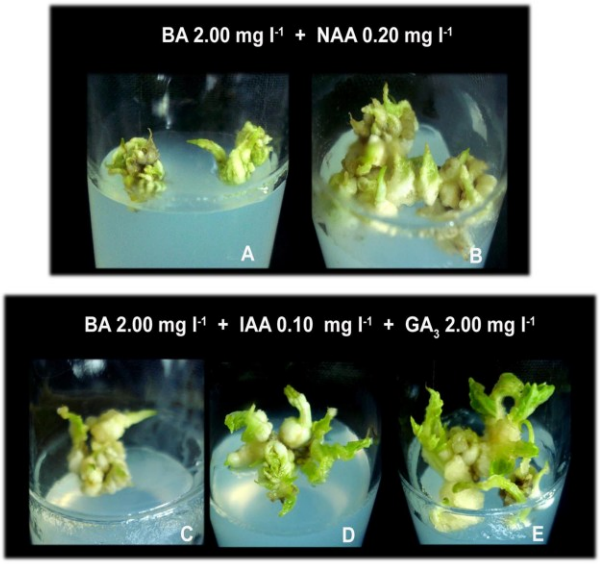


Plate 26. The effect of plant growth regulators on different explants for callus induction in bittergourd (BA)

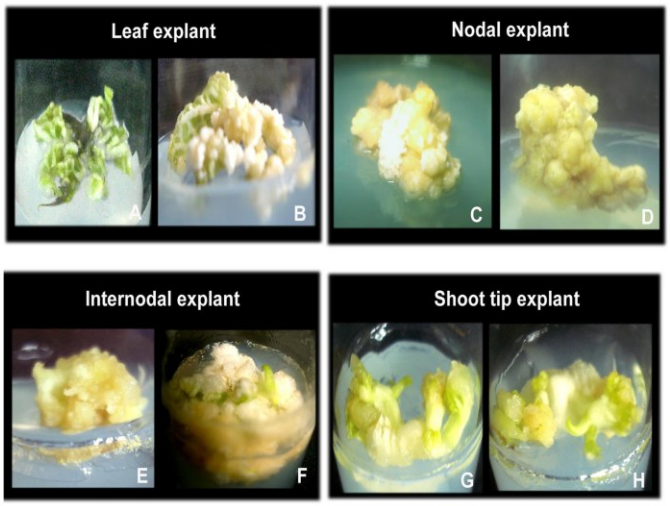


Plate 27. Effect of plant growth regulators on different explants for callus induction in bittergourd (BA + IAA) Medium – MS + BA 0.05 mg l^{-1} + IAA 4.00 mg l^{-1}

Leaf explant

I. Callus initiation within two weeks

J. Callus proliferation within 3-4 weeks

Nodal explant

K. Callus initiation within two weeks

L. Callus proliferation within 3 weeks

Internodal explant

M. Callus initiation within two weeks

N. Callus proliferation within 3-4 weeks.

Shoot tip explant

O. Callus initiation within two weeks

P. Callus proliferation within 3 weeks

Plate 28. Effect of plant growth regulators on different explants for callus induction in bittergourd (BA + IBA) Medium – MS + BA 0.05 mg l^{-1} + IBA 4.00 mg l^{-1}

Leaf explant

A. Callus initiation within two weeks

B. Callus proliferation within 3-4 weeks

Nodal explant

C. Callus initiation within two weeks

D. Callus proliferation within 3 weeks

Internodal explant

E. Callus initiation within two weeks

F. Callus proliferation within 3-4 weeks

Shoot tip explant

G. Callus initiation within two weeks

H & I. Callus proliferation within 3 weeks

Plate 27. The effect of plant growth regulators on different explants for callus induction in bittergourd (BA + IAA)

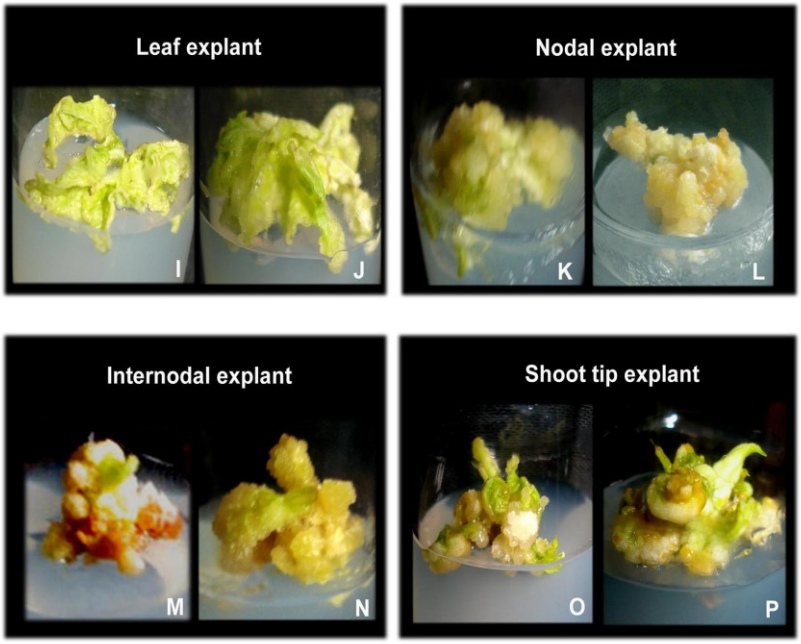
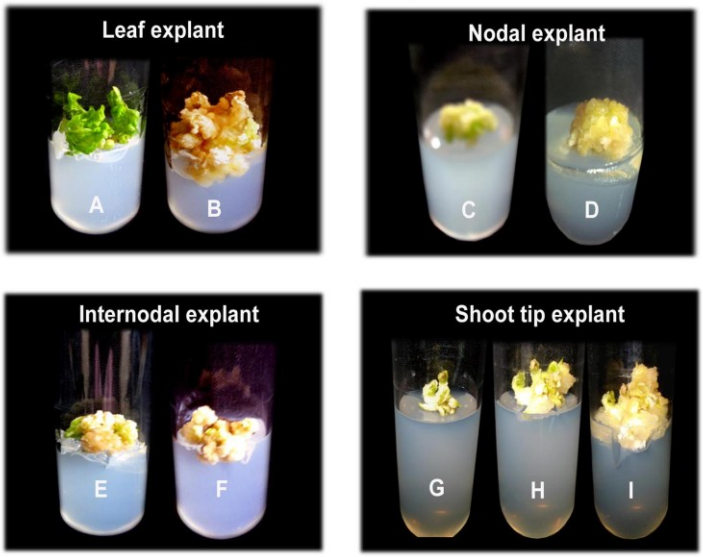


Plate 28. The effect of plant growth regulators on different explants for callus induction in bittergourd (BA + IBA)



with 13.00 days each for leaf and nodal explants while, 14.17 and11.33 days for internodal and shoot tip explants respectively.

The per cent of explant cultures initiating callus varied widely (0.00 to 100.00). Cent percent leaf cultures in the treatments MOP_{16}, MOP_{17}, MOP_{19}, MOP_{20}, MOP_{24}, MOP_{27} and MOP_{28} initiated callus. In case of nodal cultures, 100 per cent callusing was obtained in MOP_{16}, MOP_{17}, MOP_{18}, MOP_{19}, MOP_{20}, MOP_{27} and MOP_{28}. Treatments MOP_{1}, MOP_{7}, MOP_{8}, MOP_{16}, MOP_{19}, MOP_{24}, MOP_{25}, MOP_{27} and MOP_{28} initiated cent percent callusing in internodal explants. 100 per cent callusing have been induced from shoot tip explants by the treatments MOP_{1}, MOP_{8}, MOP_{16}, MOP_{17}, MOP_{18}, MOP_{19}, MOP_{20}, MOP_{27} and MOP_{28}. The treatments MOP_{11}, MOP_{12} and control failed to initiate callus in all explants.

Highest growth score (4.00) was recorded by the treatments MOP_{19} and MOP_{27} in nodal and shoot tip explants. But MOP_{19} and MOP_{27} registered 3.83 and 3.67 growth score in leaf explants, while in internodal explants the growth score was 4.00 and 3.67 respectively by the above treatments. Lowest growth score (1.00) was recorded by the treatments MOP_{3} in shoot tip; MOP_{30} in internodal; MOP_{4}, MOP_{14}, MOP_{30} in leaf and MOP_{3}, MOP_{7}, MOP_{9}, MOP_{13}, MOP_{14} and MOP_{25} in nodal explants.

Medium containing callus induction treatment MOP_{19} recorded the highest callus index (400.00) in node, internode and shoot tip explants followed by 383.00 in leaf explants. Treatment MOP_{27} also registered highest callus index (400.00) in node and shoot tip explants, while an index of 367.00 each in internode and leaf explants. Maximum callus proliferation occurred when induced calli was transferred in the same medium at an interval of 12-18 days.

Out of the thirty treatment combinations tried, MOP_{29} as well as MOP_{30} led to profuse callusing from shoot tip explants and also exhibited rhizogenesis. The roots formed were thick and spongy (Plate 30).

Plate 29. Effect of plant growth regulators on different explants for callus induction in bittergourd (BA + NAA)

Medium – MS + BA 0.05 mg l^{-1} + NAA 4.00 mg l^{-1}

Leaf explant

J. Callus initiation within two weeks

K. Callus proliferation within 3-4 weeks

Nodal explant

L. Callus initiation within two weeks

M. Callus proliferation within 3 weeks

Internodal explant

N. Callus initiation within two weeks

O. Callus proliferation within 3-4 weeks

Shoot tip explant

P. Callus initiation within two weeks

Q. Callus proliferation within 3 weeks

Plate 30. Effect of plant growth regulators on shoot tip explants leading to callusing and rhizogenesis (BA + NAA + 2,4-D)

Medium – MS + BA 0.05 mg l^{-1} + NAA 3.00 mg l^{-1} + 2,4-D 1.00 mg l^{-1}

Plate 29. Effect of plant growth regulators on different explants for callus induction in bittergourd (BA + NAA)

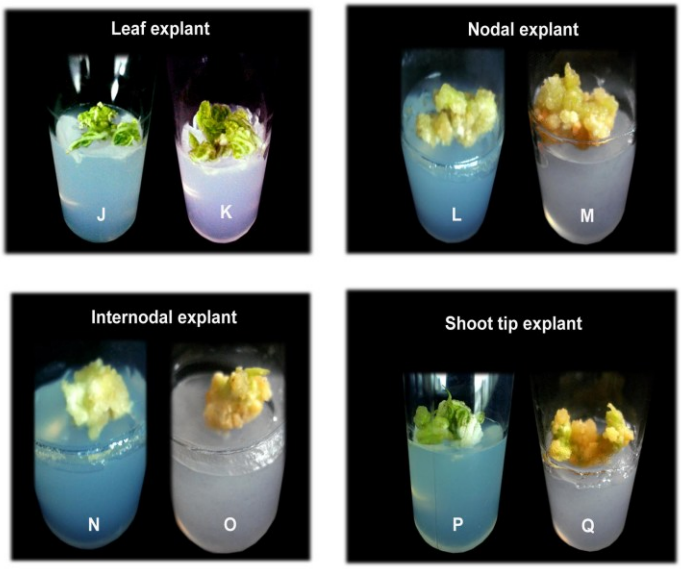


Plate 30. Effect of plant growth regulators on shoot tip explants leading to callusing and rhizogenesis (BA + NAA)

Plate 31. Effect of plant growth regulators on different explants for callus induction in bittergourd (BA + IAA + 2,4-D)

Medium – MS + BA 0.05 mg l^{-1} + IAA 4.00 mg l^{-1} + 2,4-D 2.00 mg l^{-1}

Leaf explant

A. Callus initiation within two weeks

B. Callusing after three weeks

C. Callusing after six weeks

D. Callusing after eight weeks

Nodal explant

E. Callus initiation within two weeks

F. Callusing after three weeks

G. Callusing after six weeks

H. Callusing after eight weeks

Internodal explant

I. Callus initiation within two weeks

J. Callusing after three weeks

K.Callusing after six weeks

L. Callusing after eight weeks

Shoot tip explant

M. Callus initiation within two weeks

N. Callusing after three weeks

O. Callusing after six weeks

P. Callusing after eight weeks

Plate 31. The effect of plant growth regulators on different explants for callus induction in bittergourd (BA + IAA + 2,4-D)

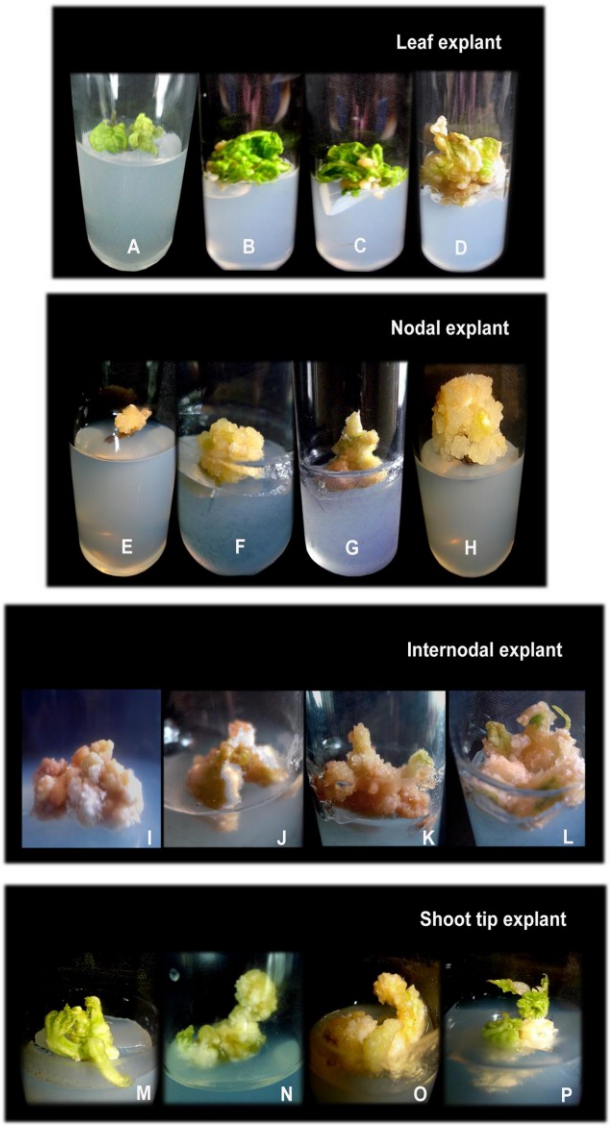


Plate 32. Effect of plant growth regulators on different explants for callus induction in bittergourd (BA + IBA + 2,4-D)

Medium – MS + BA 0.05 mg l^{-1} + IBA 4.00 mg l^{-1} + 2,4-D 2.00 mg l^{-1}

Leaf explant

A. Callus initiation within two weeks

B. Callusing after three weeks

C. Callusing after six weeks

D. Callusing after eight weeks

Nodal explant

A. Callus initiation within two weeks

B. Callusing after three weeks

C. Callusing after six weeks

D. Callusing after eight weeks

Internodal explant

A. Callus initiation within two weeks

B. Callusing after three weeks

C.Callusing after six weeks

D. Callusing after eight weeks

Shoot tip explant

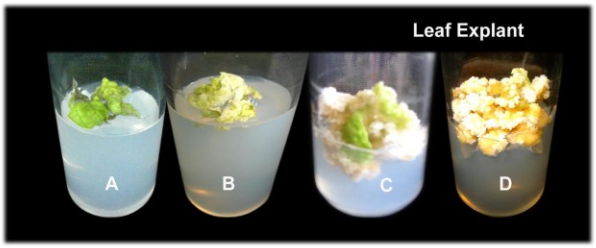
A. Callus initiation within two weeks

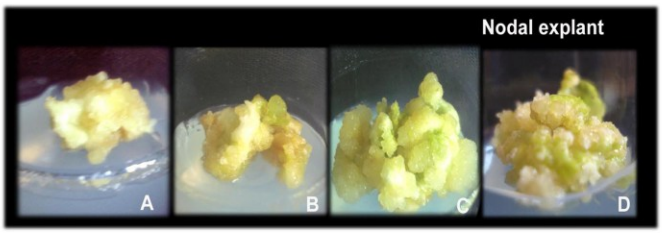
B. Callusing after three weeks

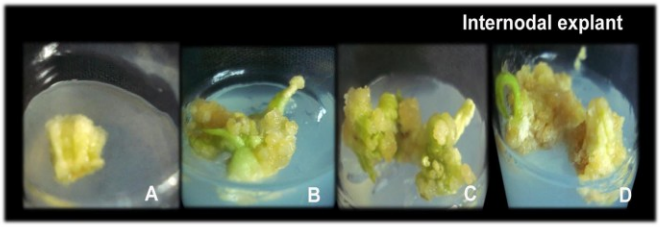
C. Callusing after six weeks

D. Callusing after eight weeks

Plate 32. The effect of plant growth regulators on different explants for callus induction in bittergourd (BA + IBA + 2,4-D)







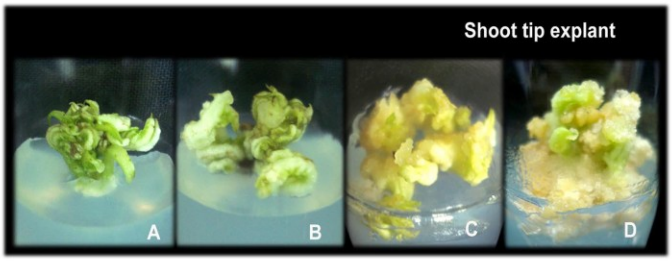


Plate 33. Effect of plant growth regulators on different explants for callus induction in bittergourd (BA + NAA + 2,4-D)

Medium – MS + BA 0.05 mg l^{-1} + NAA 4.00 mg l^{-1} + 2,4-D 2.00 mg l^{-1}

Leaf explant

A. Callus initiation within two weeks

B. Callusing after three weeks

C. Callusing after six weeks

D. Callusing after eight weeks

Nodal explant

E. Callus initiation within two weeks

F. Callusing after three weeks

G. Callusing after six weeks

H. Callusing after eight weeks

Internodal explant

I. Callus initiation within two weeks

J. Callusing after three weeks

K.Callusing after six weeks

L. Callusing after eight weeks

Shoot tip explant

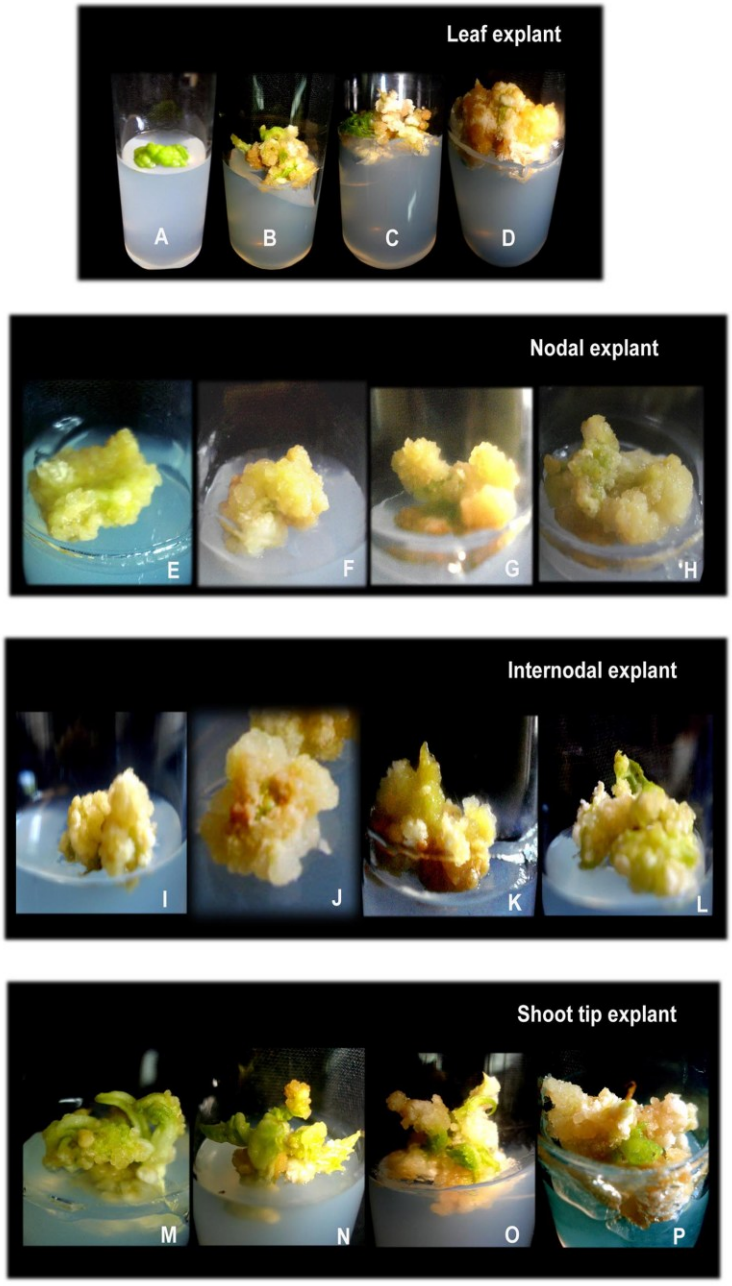
M. Callus initiation within two weeks

N. Callusing after three weeks

O. Callusing after six weeks

P. Callusing after eight weeks

Plate 33. Effect of plant growth regulators on different explants for callus induction in bittergourd (BA + NAA + 2,4-D)



4.4.2.2 Effect of sucrose on callus induction and proliferation

Data pertaining to the results of the trail conducted to study on the effect of three different levels of sucrose (20.00, 30.00 and 40.00 g l^{-1}) on callus induction and proliferation is presented in Table 37.

Regarding the minimum days required for callusing, the treatment MOS_2 (30.00 g l^{-1}) recorded lowest values for all the explants. As far as callusing percentage is concerned, cent percent callusing was found in leaf and shoot tip explants for all the three levels, while variation was found in nodal and internodal explants among the three sucrose levels.

In the case of growth score, maximum score of 4.00 was obtained in MOS_2 in nodal, internodal and shoot tip explants. Among the three different levels of sucrose tried, sucrose at 20.00 g l^{-1} (MOS_1) was found to be inferior to all others and produced least growth score for all the explants.

With respect to callus index also, the treatment MOS_2 was superior to other treatments by producing highest index of 400.00 in nodal, intermodal and shoot tip explants (Plate 34).

4.4.2.3 Effect of agar on callus induction and proliferation

Data recorded on the effect of three different levels of agar (5.30, 6.30 and 7.30 g l^{-1}) on callus induction and proliferation is presented in Table 38.

Among the three treatments, days for callusing was earliest for all the explants in MOA_2. The treatment MOA_1 took lesser days for callusing than MOA_3 for all the explants.

No variation was noticed among the treatments for callusing percentage. Cent percent callusing was obtained in all the explants.

Table 37. The effect of sucrose on *in vitro* callus induction and proliferation in bittergourd
(Medium – MS + inositol 100 mg l^{-1} + agar 6.30 g l^{-1} + IBA 4.00 mg l^{-1} + BA 0.50 mg l^{-1} + 2,4- D 2.00 mg l^{-1})

Type of explant	*Treatment No.	Days to callusing	Callusing percentage (%)	Growth score	Callus index
Leaf	MOS_1	15.17	100.00	1.67	167.00
	MOS_2	12.83	100.00	3.17	317.00
	MOS_3	13.50	100.00	2.33	233.00
Node	MOS_1	16.00	100.00	1.83	183.00
	MOS_2	13.17	100.00	4.00	400.00
	MOS_3	17.50	83.33	3.00	249.99
Internode	MOS_1	18.10	50.00	1.00	50.00
	MOS_2	14.16	100.00	4.00	400.00
	MOS_3	19.50	66.67	2.16	144.01
Shoot tip	MOS_1	13.00	100.00	1.83	183.00
	MOS_2	11.50	100.00	4.00	400.00
	MOS_3	15.17	100.00	3.50	350.00

*Treatment combinations are given in Table 6.
The data represents mean value of the six replications.

Table 38. The effect of agar on *in vitro* callus induction and proliferation in bittergourd
(Medium – MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} +IBA4.00 mg l^{-1} + BA 0.50 mg l^{-1} + 2,4- D 2.00 mg l^{-1})

Type of explant	*Treatment No.	Days to callusing	Callusing percentage (%)	Growth score	Callus index
Leaf	MOA_1	14.66	100.00	3.00	300.00
	MOA_2	13.17	100.00	3.33	333.00
	MOA_3	16.17	100.00	1.50	150.00
Node	MOA_1	16.83	100.00	2.17	217.00
	MOA_2	14.50	100.00	4.00	400.00
	MOA_3	18.50	100.00	1.33	133.00
Internode	MOA_1	17.33	100.00	2.67	267.00
	MOA_2	15.66	100.00	4.00	400.00
	MOA_3	21.83	100.00	1.33	133.00
Shoot tip	MOA_1	15.33	100.00	3.17	317.00
	MOA_2	13.00	100.00	4.00	400.00
	MOA_3	16.67	100.00	2.00	200.00

*Treatment combinations are given in Table 7.
The data represents mean value of the six replications.

Plate 34. Effect of sucrose on different explants for callus induction in bittergourd

Medium – MS + BA 0.05 mg l^{-1} + IBA 4.00 mg l^{-1} + 2,4-D 2.00 mg l^{-1}

Leaf explant

A. Sucrose 20.00 g l^{-1}

B. Sucrose 30.00 g l^{-1}

C. Sucrose 40.00 g l^{-1}

Nodal explant

D. Sucrose 20.00 g l^{-1}

E. Sucrose 30.00 g l^{-1}

F. Sucrose 40.00 g l^{-1}

Internodal explant

G. Sucrose 20.00 g l^{-1}

H. Sucrose 30.00 g l^{-1}

I. Sucrose 40.00 g l^{-1}

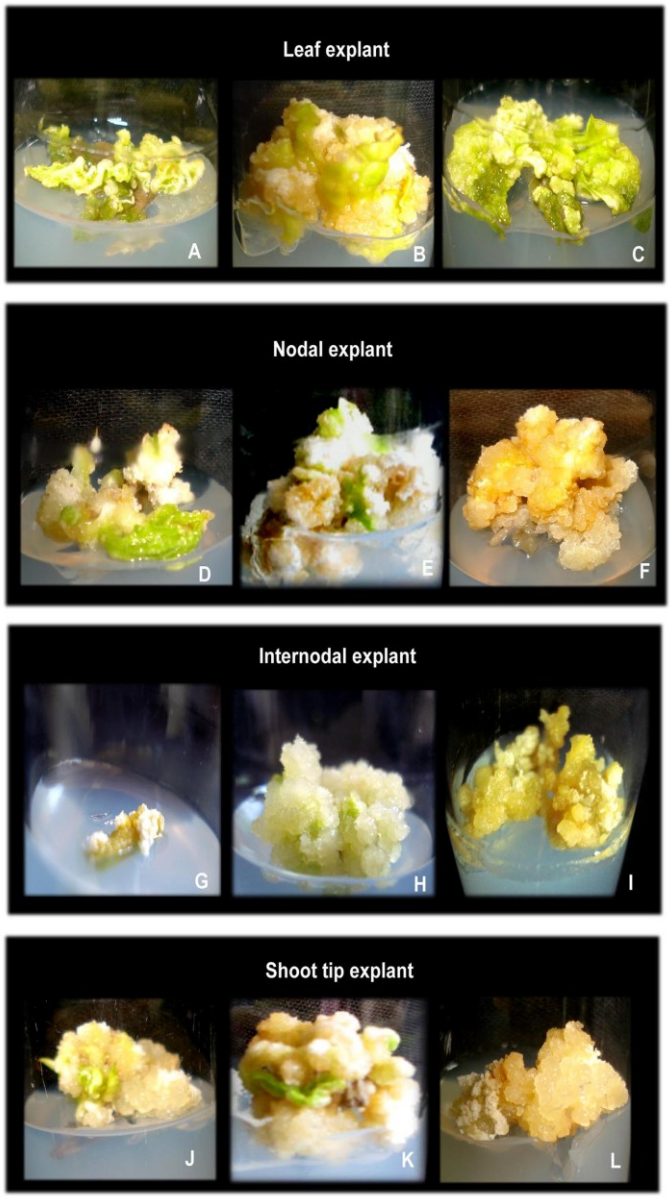
Shoot tip explant

J. Sucrose 20.00 g l^{-1}

K. Sucrose 30.00 g l^{-1}

L. Sucrose 40.00 g l^{-1}

Plate 34. Effect of sucrose on different explants for callus induction in bittergourd



Wide variation was noticed with regard to callus growth score among the explants for the three levels of agar tried. Highest callus growth score of 4.00 was obtained in MOA_2 for nodal, internodal and shoot tip explants. Higher levels of agar exhibited inhibitory action on callus growth.

Regarding the callus index also, the treatment MOA_2 registered the maximum value of 400.00 for nodal, internodal and shoot tip explants (Plate 35). The treatment MOA_2 was found to be inferior to all others and produced least callus index values for all the explants.

4.4.2.4 Effect of plant growth regulator (BA) and activated charcoal on different explant callus for regeneration and adventitious shoot production

The calli obtained in the initiation media were further transferred to four treatments with various combinations and concentrations of cytokinin (BA) and activated charcoal in the culture medium.

The results revealed that callus regeneration and adventitious shoot production was noticed only in shoot tip derived callus (Plate 36). Nodal (Plate 37) and internodal (Plate 38) derived callus resulted in regeneration and shoot initiation or greening, but shoot elongation could not be obtained. Callus regeneration could not be initiated in leaf derived callus even after one month.

The effect of plant growth regulator (BA) and activated charcoal on shoot tip derived callus for regeneration and adventitious shoot production are given in Table 39. The per cent of shoot tip calli cultures developing into adventitious shoot varied widely from 16.67 to 100.00. Cent percent cultures differentiated into shoots in the treatment $MOAC_1$, whereas $MOAC_2$ and $MOAC_3$ led to shoot regeneration to the tune of 66.67 and 50.00 per cent respectively. The control, which had no plant growth

Plate 35. Effect of agar on different explants for callus induction in bittergourd

Medium – MS + BA 0.05 mg l^{-1} + IBA 4.00 mg l^{-1} + 2,4-D 2.00 mg l^{-1}

Leaf explant

A. Agar 5.30 g l^{-1}

B. Agar 6.30 g l^{-1}

C. Agar 7.30 g l^{-1}

Nodal explant

D. Agar 5.30 g l^{-1}

E. Agar 6.30 g l^{-1}

F. Agar 7.30 g l^{-1}

Internodal explant

G. Agar 5.30 g l^{-1}

H. Agar 6.30 g l^{-1}

I. Agar 7.30 g l^{-1}

Shoot tip explant

J. Agar 5.30 g l^{-1}

K. Agar 6.30 g l^{-1}

L. Agar 7.30 g l^{-1}

Plate 35. Effect of agar on different explants for callus induction in bittergourd

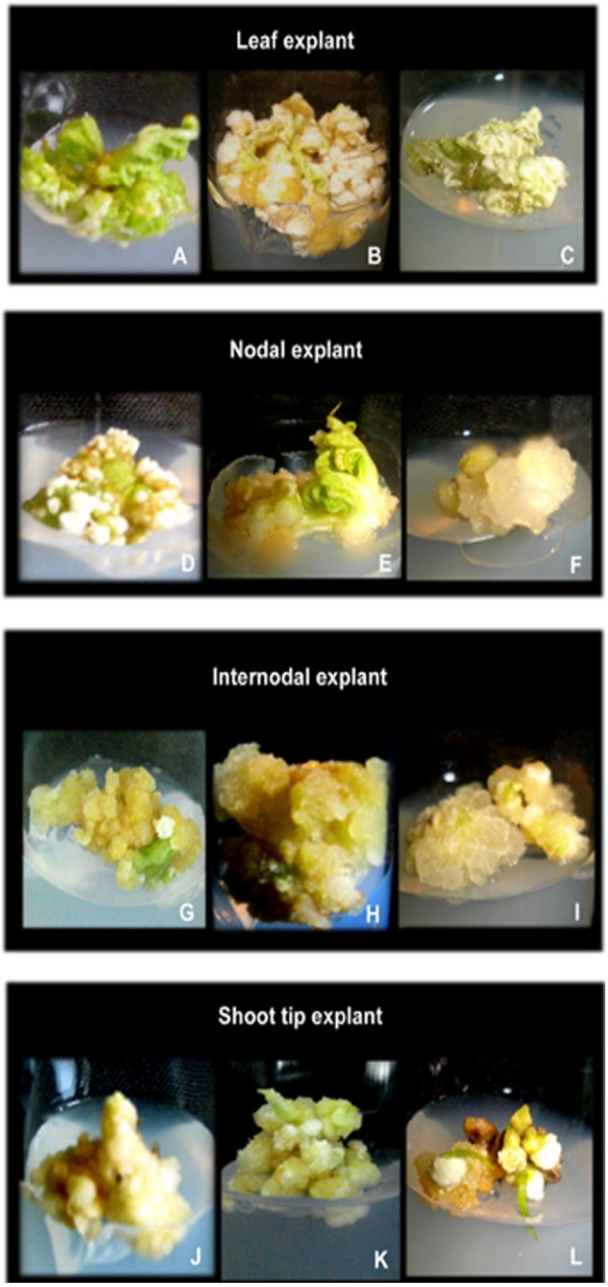


Table 39. Effect of plant growth regulator (BA) and activated charcoal on shoot tip callus for regeneration and adventitious shoot production in bittergourd

(Medium – MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1})

*Treatment No.	Regeneration percentage (%)	Days to regeneration	Adventitious shoot length (cm)	No. of leaves per shoot	Length of leaf (cm)	Breadth of leaf (cm)
$MOAC_1$	100.00	18.17	8.28	11.50	1.75	1.75
$MOAC_2$	66.67	23.33	6.90	4.50	4.33	2.92
$MOAC_3$	50.00	24.83	2.92	2.00	0.45	0.30
$MOAC_4$	16.67	31.33	1.27	1.00	5.15	4.10
Control	0.00	0.00	0.00	0.00	0.00	0.00
F		45.581**	7.485**	2.445**	2.094**	2.589**
SE		0.922	0.393	0.303	0.151	0.101
CD (0.05)		2.664	1.136	0.893	0.447	0.298

*Treatment combinations are given in Table 8.

**Significant at 1% level

The data represents mean value of the six replications.

Plate 36. The effect of growth regulator and activated charcoal on shoot tip callus for regeneration and adventitious shoot production in bittergourd

Medium – MS + 0.50 BA 1.00 mg l^{-1} + activated charcoal 0.50 g l^{-1}

A. Callus regeneration

B. Shoot initiation or greening

C. Adventitious shoot production

D & E. Shoot elongation

F. Fully grown shoot formation

Medium – MS + 0.50 BA 2.00 mg l^{-1} + activated charcoal 0.50 g l^{-1}

A. Callus regeneration

B. Shoot initiation or greening

C, D & E. Adventitious shoot production

Medium – MS + 0.50 BA 1.00 mg l^{-1} + activated charcoal 1.00 g l^{-1}

A & B. Callus regeneration

C. Shoot initiation or greening

D & E. Adventitious shoot production

Medium – MS + 0.50 BA 2.00 mg l^{-1} + activated charcoal 1.00 g l^{-1}

A. Callus regeneration

B. Shoot initiation or greening

C, D & E. Adventitious shoot production

Plate 36. The effect of growth regulators and activated charcoal on shoot tip callus for regeneration and adventitious shoot production in bittergourd

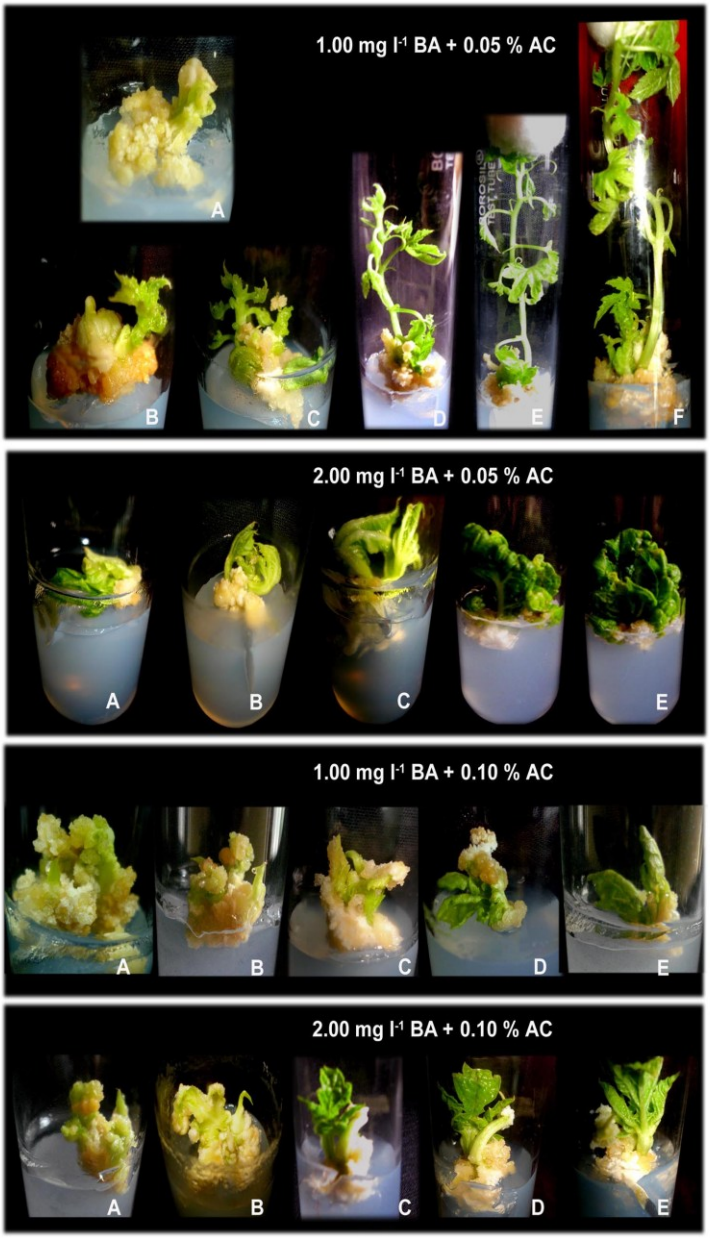


Plate 37. The effect of growth regulator and activated charcoal on nodal callus for regeneration and adventitious shoot production in bittergourd

Medium – MS + 0.50 BA 1.00 mg l^{-1} + activated charcoal 0.50 g l^{-1}

A & B. Callus regeneration

C, D & E. Shoot initiation or greening

Medium – MS + 0.50 BA 2.00 mg l^{-1} + activated charcoal 0.50 g l^{-1}

A. Callus regeneration

B & C. Shoot initiation or greening

Medium – MS + 0.50 BA 1.00 mg l^{-1} + activated charcoal 1.00 g l^{-1}

A. Callus regeneration

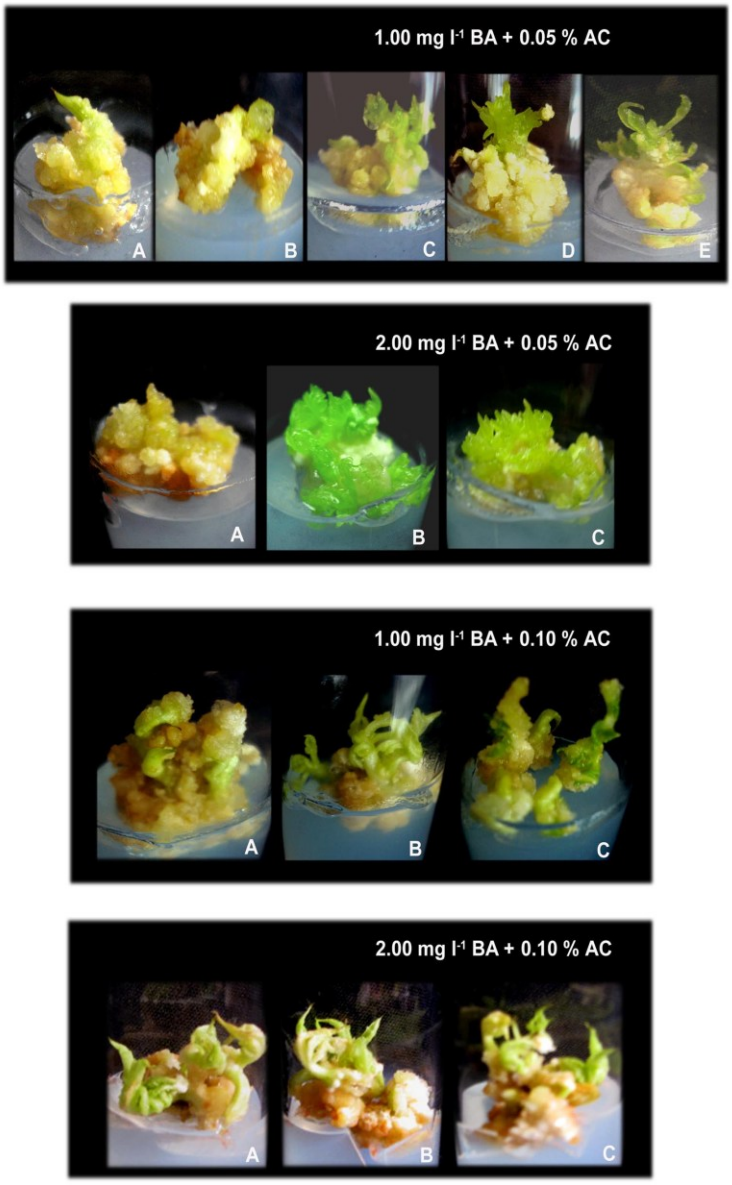
B & C. Shoot initiation or greening

Medium – MS + 0.50 BA 2.00 mg l^{-1} + activated charcoal 1.00 g l^{-1}

A. Callus regeneration

B & C. Shoot initiation or greening

Plate 37. The effect of growth regulators and activated charcoal on nodal callus for regeneration and adventitious shoot production in bittergourd



regulator and AC failed to develop shoots. Only one shoot was produced from shoot tip calli for all the treatments.

Callus regeneration from shoot tip calli was observed in 18.17 days in the treatment $MOAC_1$. Also, the callus regeneration resulted in 23.33 days in the treatment $MOAC_2$ and was closely followed by the treatment $MOAC_3$ (24.83 days).

With regard to length of the adventitious shoot produced from shoot tip derived callus, $MOAC_1$ recorded the highest value (8.28 cm). The lowest value (1.27cm) was recorded by the treatment $MOAC_4$. Number of leaves per shoot was also highest (11.50) in the treatment $MOAC_1$ and least (1.00) in the treatment $MOAC_4$. Maximum leaf length (5.15 cm) and leaf breadth (4.10 cm) was observed in the treatment $MOAC_4$, while minimum values for above two characters, 0.45 cm and 0.30 cm respectively was obtained from the treatment $MOAC_3$.

4.4.2.5 Culture conditions on callus regeneration and adventitious shoot production

Light had significant influence on the callus regeneration and adventitious shoot production. Cultures under lighted condition went to callus regeneration, while darker conditions lead to profuse callusing (Plate 39). It was further observed that the absence of light (MOL_0) affected callus regeneration also. Regenerated plants started callusing when kept under continuous darkness.

Cultures under lighted condition produced on cent percent callusing and took an average 19.17 days for regeneration (Table 40). With regard to length of the adventitious shoot produced from shoot tip derived callus, MOL_1 recorded the highest value (7.45 cm). Number of leaves per shoot was also highest (9.50) in the treatment MOL_1 Maximum leaf length (0.90 cm) and leaf breadth (0.950 cm) was observed in the treatment MOL_1.

Plate 38. The effect of growth regulator and activated charcoal on internodal callus for regeneration and adventitious shoot production in bittergourd

Medium – MS + 0.50 BA 1.00 mg l^{-1} + activated charcoal 1.00 g l^{-1}

A. Callus regeneration

B & C. Shoot initiation or greening

Medium –MS + 0.50 BA 2.00 mg l^{-1} + activated charcoal 1.00 g l^{-1}

A & B. Callus regeneration

C. Shoot initiation or greening

Plate 39. The effect of light on *in vitro* callus regeneration in bittergourd

Medium –MS + 0.50 BA 1.00 mg l^{-1} + activated charcoal 0.50 g l^{-1}

A. Regenerated plants started callusing in continuous darkness (0 lux)

B. Callus regeneration in light (1000 lux)

Plate 40. The effect of plant growth regulators on *in vitro* rooting in bittergourd

A. Medium – MS + IBA 0.50 mg l^{-1}

B. Medium – MS + IBA 1.00 mg l^{-1}

C. Medium – MS + IBA 2.00 mg l^{-1}

Plate 38. The effect of growth regulator and activated charcoal on internodal callus for regeneration and adventitious shoot production in bittergourd

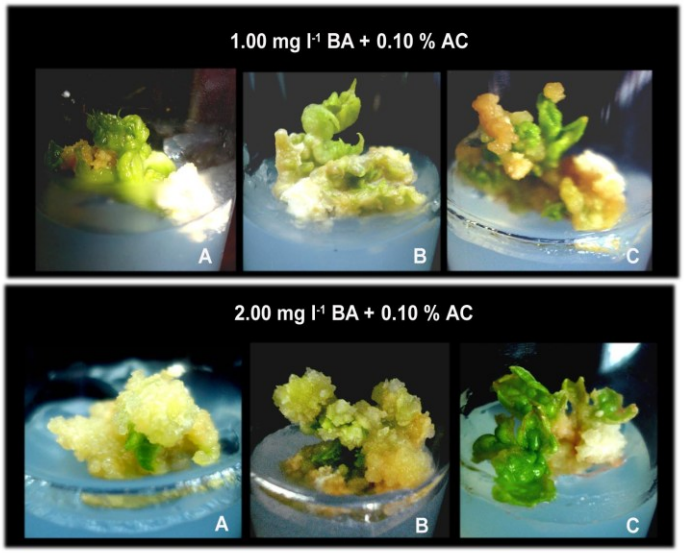


Plate 39. The effect of light on *in vitro* callus regeneration in bittergourd

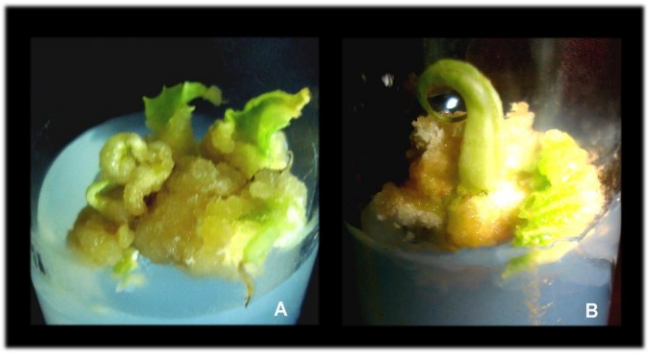
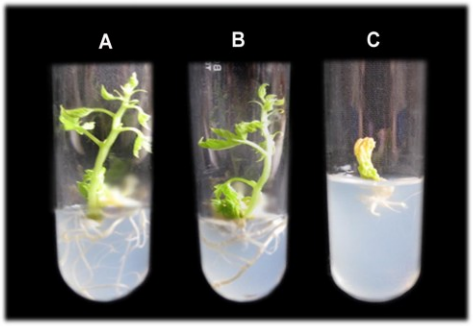


Plate 40. The effect of plant growth regulators on *in vitro* rooting in bittergourd



4.4.3 *In vitro* rooting

Well developed shoots (3-5 cm long) were excised and transferred to rooting media with varying levels of auxin IBA (0.05, 1.00 and 2.00 mg l^{-1}) were tried to study its effect on *in vitro* rooting. With regard to all aspects of *in vitro* rooting, significant difference was noticed among the different treatments (Table 41).

The percentage of rooting varied from 16.67 to 66.66. The treatment R_2 recorded the highest per cent (66.66) of rooting.

Wide variation was noticed in the case of number of days taken for root initiation (10.33 to 22.33 days). Earliest rooting (10.33 days) was observed in R_1 (IBA 0.50 mg l^{-1}) followed by R_2 (IBA 1.00 mg l^{-1}).

With respect to number of roots formed and length of the roots, the treatment R_2 was found to be superior by recording a maximum of 10.83 roots and 6.83 cm root length (Plate 40). The treatment R_3 produced the shortest roots (1.20 cm).

Only 33.33 per cent of the shoots rooted in the control (MS) which resulted in single elongated roots (1.78cm). The exhibited significantly delayed root initiation also (22.33 days).

The roots formed in all the treatments were white in colour and without root hairs. The treatment R_2 was significantly superior to all others and produced medium thicker and long roots.

Table 40. Effect of light on shoot tip callus for regeneration and adventitious shoot production in bittergourd
(Medium –MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1} + activated charcoal 0.50 g l^{-1} + 0.50 BA 1.00 mg l^{-1})

*Treatment No.	Regeneration percentage (%)	Days to regeneration	Adventitious shoot length (cm)	No. of leaves per shoot	Length of leaf (cm)	Breadth of leaf (cm)
MOL_0	-	-	-	-	-	-
MOL_1	100.00	19.17	7.45	9.50	0.90	0.95

*Treatment combinations are given in Table 9.
The data represents mean value of the six replications

Table 41. Effect of plant growth regulator on *in vitro* rooting in bittergourd
(Medium –MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1})

*Treatment No.	Rooting response (%)	Minimum days for root initiation	Total number of roots	Length of the longest root (cm)	Remarks
R_1	50.00	10.33	6.67	5.50	Medium thick, long roots
R_2	66.66	11.33	10.83	6.83	Medium thick, long roots
R_3	16.67	18.17	5.17	1.20	Thick, short roots
Control	33.33	22.33	1.00	1.78	Thin, long root

*Treatment combinations are given in Table 10.
The data represents mean value of the six replications.

DISCUSSION

5. DISCUSSION

Bitter gourd or balsam pear (*Momordica charantia* L.) belonging to the family Cucurbitaceae is a popular vegetable in India, having considerable nutritional, economic and medicinal importance. It is a very rich source of calcium, phosphorous, iron, protein, vitamin A and C to make the food wholesome and healthy. Drinking fresh bittergourd juice is recommended by naturopaths. The bitter principle in bittergourd is momordicin (non-toxic alkaloid), which prevents the spoilage of cooked vegetable and keeps fit for consumption even for two to three days. It plays an important role in the agro-economy of our country and costlier compared to other vegetables available in the season. In spite of its economic importance as a common vegetable, no serious attempt has been made so far to improve the quality of fruits.

M. charantia is grown all over the country in tropical and temperate climates. India is endowed with the wealth of bittergourd germplasm consisting of both wild and cultivated forms. Being a monoecious and highly cross-pollinated crop, large variation is observed in fruit and vegetative characters. Variation is also observed in respect of extent of bitterness. However information on nature and magnitude of genetic diversity is meagre in this crop. It is desirable to evaluate and characterize the genetic resources of bittergourd for sustainable utilization. The characterization can be achieved through morphological, biochemical or molecular markers. Identification of superior genotypes among the existing germplasm becomes imperative for promoting production per unit area of bittergourd.

A large number of varieties have been developed in bittergourd through direct selection from indigenous germplasm. Moreover, in India, several local landraces or local cultivars have established themselves based on selection by local growers in the past. Though wide variability

exists between original indigenous varieties and cultivated types but at present mostly mixed types are available among common cultivated types. These varieties are similar to each other morphologically. Recently, use of molecular markers has assumed great significance in germplasm characterization and assessment of genetic diversity. Universal acceptance of PCR- based markers has accelerated the use of molecular markers, more specifically DNA based markers, that are valid in assessing genetic diversity as these are genetically stable and detectable during all stages. Hence to identify and characterize the genotypes of bittergourd, molecular markers have to be used.

The genetic improvement of any crop aims at increasing the production potential and quality by altering the genetic make up of the existing varieties. For rational approach to improve the yield, it is essential to have the knowledge of variability, heritability, genetic advance and association between characters. In India, genetic improvement of bittergourd has been accomplished primarily by conservative breeding strategies aimed at satisfying the consumers' preferences for fruit size, shape, colour and bitterness. Keeping in view of the genetic variability existing in bittergourd in India, a research work should be done to explore its variability and select elite materials for developing high-yielding and quality rich varieties. The rich diversity in bittergourd has many value added characters and will bring fruits in its improvement.

Bittergourd is conventionally propagated by seeds. Since this crop is highly cross-pollinated, variation in seedling types is anticipated. Desirable quality and morphological aspects are very difficult to be maintained. In this context, adoption of *in vitro* technique became an imperative approach as it produces planting materials 'true to type'. Very recently, a few scientists have tried to obtain differentiation of shoot-buds and plantlets from *in vitro* cultured various explants of some cucurbits. *In vitro* regeneration of bittergourd cultivars grown under Indian

conditions is limited and hence an investigation should be undertaken to explore the possibility of developing a suitable protocol for *in vitro* multiplication of bittergourd.

Hence a study was undertaken to collect, catalogue and characterize the available genotypes of *M. charantia* at morphological, biochemical and molecular level. Morphological and biochemical characterization helps to assess the magnitude of genetic variability for identifying superior genotypes based on yield, quality, earliness and pest and disease resistance. Molecular characterization gives a comprehensive picture on diversity and relatedness of available genotypes. The present study was also undertaken to develop a rapid micro-propagation protocol for plant regeneration of elite bittergourd genotypes through *in vitro* culture of different explants.

5.1 MORPHOLOGICAL CHARACTERIZATION

5.1.1 Genetic cataloguing

Thirty three genotypes of *M. charantia* upon cataloguing showed distinct variations among each other with respect to vegetative, flower, fruit and seed characters. Most of the genotypes fall in moderate to high viny growth habit and short to long internodal length. Variability was more pronounced for flower and fruit characters. Flower and fruit size ranged from small to very large. Fruit colour and fruit shape showed wide variations among the genotypes. Seed quantity per fruit ranged from very few to many with small to very large seed size. There is a report on high variability for morphological characters in bittergourd (Dey et al., 2006). The rich variability among bittergourd genotypes have many value-added characters and will bring fruits in its improvement. If they are considered while breeding, no doubt bittergourd will be a promising vegetable.

5.1.2 Genetic variability and divergence

Bittergourd exhibits tremendous variability in vegetative and fruit characters, keeping quality and reaction towards pest and diseases. The extent of genetic variability indicates the amenability of a given character for its improvement. Hence knowledge on the nature and magnitude of genetic variation contributing to gain under selection is essential.

In the present investigation, analysis of variance revealed considerable genetic diversity among elite genotypes of *M. charantia* for aggregate effect of most of the characters studied namely, days to seedling emergence, vine length, internodal length, number of primary branches, number of secondary branches, days to first male flower, node to first male flower, days to first female flower, node to first female flower, sex ratio, days to first fruit harvest, fruit length, fruit girth, fruits per plant, average fruit weight, yield per plant, seeds per fruit, 100-seed weight and mosaic incidence indicating sufficient diversity among the genotypes. Such variation indicated the scope for improving the population for these characters as reported earlier by Vahab (1989), Jaiswal et al. (1990), Katiyar et al. (1996), Iswaraprasad (2000), Bhave et al. (2003), Devmore and Dhonukshe (2007) and Yadav et al. (2008) in bittergourd.

In respect of vegetative characters, ample variability was observed as evident from the wide range obtained for days to seedling emergence, vine length and internodal length. Among the genotypes evaluated, MC 9 was the earliest to emerge (6.00), MC 1 was the most vigorous registering the highest values for vine length and MC 20 for internodal length. Ram et al. (2007) observed wide range of variability for days to germination in bottlegourd. The variation in vine length might be due to the specific genetic constitution, inherent character and vigour of different genotypes. Considerable variability was reported by Ram et al. (2006a) for vine length and Yadav et al. (2008) for internodal length in bittergourd.

Number of primary branches and secondary branches recorded a high range of variation as reported by Yadav et al. (2004) in bittergourd.

Days to first male and female flower plays an important role in deciding the earliness or lateness of the crop. The early and late female flower appearance helps in occurrence of early or late flush of the crop. Days to first male and female flower showed wide range of variation among the genotypes. Similar results were also reported by Rao and Rao (2008) in ridgegourd. Node to first male and female flower also recorded wide range of variability. The variation in the character days to first male and female flowering might be due to intermodal length, number of nodes and vigour of the crop.

Sex ratio in the present study ranged from 15.46 to 29.13. Considerable variation for the character was also reported by Thakur et al. (1994) in bittergourd.

Days to first fruit harvest recorded narrow range of variation. Most of the genotypes are harvested within 55 days.

Among the genotypes, maximum fruit weight was observed in MC 20. Other genotypes with better fruit weight were MC 22, MC 2, MC 27 and MC 26. Both fruit length and fruit girth contributed to better fruit weight in high yielders. In the present study, fruit length ranged from 4.91 to 38.83 cm. Similarly, fruit girth also varied from 6.96 to 25.53 cm, suggesting ample variability and scope for improvement for fruit size in *M. charantia*.

Fruits per plant and yield per plant exhibited high variability as reported by Katiyar *et al.* (1996) in bittergourd. The variation in number of fruits per plant might be due to fruit set percentage, sex ratio and vine length. Among the genotypes evaluated, fruits per plant was maximum in MC 10 (MDU-1, TNAU). Other genotypes with better fruit number were MC 2 (CO-1, TNAU) and MC 25 (IC 470569, NBPGR, Thrissur).

All these genotypes have green fruit skin colour. MC 27, MC 32, MC 21, MC 26 and MC 22 are genotypes with light green skin colour that had better fruit number.

Significant variation in yield per plant might be attributed to fruit set percentage, sex-ratio, fruit length, number of fruits per plant, fruit weight and fruit girth. This finding was supported by Shrivastava and Srivastava, 1976; Singh et al., 1977; Indiresh, 1982; Reddy et al., 1995; Katiyar et al., 1996 and Yadav et al., 2004 in bittergourd.

Among the genotypes MC 20 (Priyanka, KAU) is the highest yielder followed by MC 26 (Thripunithara, Ernakulam), MC 10 (MDU-1, TNAU), MC 22 (Chathamangalam, Kozhikode) and MC 27 (Charuplasseri, Palakkad). The high yield in MC 20 may be attributed to the high fruit length, fruit girth and average fruit weight. MC 26 was characterized by low sex ratio and earliness in male and female flowering and harvest. The genotype MC 10 got highest number of fruits per plant along with higher fruit length, average fruit weight and 100-seed weight. The genotypes MC 22 and MC 27, apart from being better for most of the fruit characters like fruit length, fruit girth, average fruit weight, seed number and 100-seed weight, they also registered high values for resistance against fruit fly and mosaic disease resulting in better yield. This confirms the fact that fruit yield is a complex trait and is the ultimate expression of many component characters.

Seeds per fruit and 100-seed weight exhibited a wide range of variation from 3.00 to 38.00 and 7.15 g to 25.90 g respectively. Similar results were reported for seeds per fruit by Varghese (1991) in snake gourd and for 100-seed weight by Mathew (1999) in bottlegourd. Varieties with high fruit seed weight and fruit seed number are preferred not only to increase crop production, but also to meet the needs of the seed industry and farmers.

One of the major obstacles in the cultivation of bittergourd is the attack of pest like fruitfly, *Bactrocera cucurbitae*. Fruit fly infestation percentage was less than 20 per cent for all the genotypes.

Mosaic disease is a major constraint in cucurbit cultivation in Kerala. Significant differences were observed among the bittergourd genotypes for mosaic incidence, which clearly indicated that the level of resistance or susceptibility to the disease varied with the genotype. Out of the 33 genotypes evaluated, ten genotypes were highly resistant, ten genotypes were resistant, ten genotypes were moderately resistant and remaining three were susceptible to the disease. Screening for mosaic resistance was also done by Arunachalam (2002) in bittergourd.

The absolute variability in different characters cannot be the criteria for deciding as to which character is showing the highest degree of variability. The estimates of genotypic and phenotypic coefficients of variation provided suitable parameter for comparison of characters regarding the extent of genetic variation. In the present investigation, the GCV was very near to PCV for most of the characters indicating that these characters are least influenced by the environment and are under the control of genotype itself. In such a situation, selection can be effective on the basis of the phenotype alone with equal probability of success. High values of GCV are an indication of high genetic variability among the genotypes and the scope for improvement of these characters through simple selection. High coefficients of variation (phenotypic [PCV] and genotypic [GCV]) were observed for yield per plant and average fruit weight indicating broad genetic base for these traits. Similar results were also reported in bittergourd (Jaiswal et al., 1990), ridgegourd (Varalakshmi et al., 1995), bottlegourd (Narayan et al., 1996), ashgourd (Lovely, 2001) and pointedgourd (Dora et al., 2003). The high PCV and GCV observed for these characters are evident from their high variability, which in turn offers good scope for selection.

5.1.2.1 Heritability and Genetic Advance

Heritability provides information on heritable portion of variation. The high values of heritability estimates in broad sense indicate that substantial improvement can be made using standard selection procedures. Heritable variation can be found out with greater degree of accuracy when heritability (h, broad sense) is taken into consideration along with genetic advance (GA). Hence, both heritability and genetic advance were determined to get a clear picture of the scope of improvement in various characters through selection.

High values of heritability were observed for most of the characters studied. The High values of heritability estimates in broad sense indicate that substantial improvement can be made using standard selection procedures. Higher magnitude of heritability (>60 %) was recorded for average fruit weight, seeds per fruit, vine length and yield per plant. Similar findings were also reported in bittergourd (Vahab, 1989) for average fruit weight, in ashgourd (Parkash et al., 2000) for yield per plant, in snapmelon (Reddy et al., 2005) for vine length and in snakegourd (Narayanankutty et al., 2006) for seeds per fruit. High heritability estimates indicate the presence of large number of fixable additive factors and hence the phenotype could provide fairly reliable measure of genotype. Therefore, these traits can be improved by selection exercised on the basis of phenotypic performance.

High heritability estimates does not necessarily mean a high genetic advance for a particular character. The present investigation revealed high heritability coupled with low genetic advance for several biometric characters including yield per plant, fruits per plant, fruit girth and fruit length. High heritability coupled with low genetic advance attributable to non-additive gene action and hence hybridization followed by selection may be used for improvement of these traits. Sarkar et al. (1990) in

pointedgourd and Ananthan et al. (2005) in ridgegourd also observed high heritability and low genetic advance for several yield characters.

High heritability coupled with high genetic advance was noticed for vine length and average fruit weight. The high heritability combined with high genetic advance could be treated as an indication of additive gene action and the consequent high expected genetic advance from selection for these characters. Simple selection therefore could be effective for the improvement of these traits. Similar results were reported in snapmelon (Reddy et al., 2005) for vine length and in ridgegourd (Prabha et al., 2007) for average fruit weight.

Moderate heritability with low genetic advance was observed for mosaic incidence. It may be inferred that these characters were conditioned by non-additive gene action and presence of high genotypic and environmental interaction.

On the basis of the present study, it can be concluded that simultaneous selection based on multiple characters having high estimates of heritability associated with greater genetic advance may be useful for the improvement of this crop. Also, high variability and heritability along with genetic advance expressed by these traits indicated that the potential genotypes could be tested in multi-locational trials and selected as donors for these characters or used as parent in hybridization programme.

5.1.2.2 Correlation Studies

The correlation coefficient analysis measures the mutual relationship between various characters and it determines the component traits on which selection can be relied upon the effect of improvement.

In the present study, the correlation parameters in general indicated high magnitude of genotypic correlation coefficients than the phenotypic ones for characters studied, thereby establishing inherent relationship

among the characters. Both at phenotypic and genotypic levels, the characters *viz.,* vine length, fruit length, fruit girth, fruits per plant, average fruit weight, seeds per fruit and 100-seed weight showed strong positive association with yield per plant indicating that these characters are the most important yield components and that effective improvement in yield can be achieved selection based on these characters in bittergourd. The yield was increased when there was an increase in number of fruits per vine and fruit weight. Node to first male and female flower and sex ratio were negatively correlated with yield.

The very high positive association of fruit length and average fruit weight with yield indicated that these characters were the primary yield attributes in bittergourd. Similar reports were there in bittergourd (Mangal et al., 1981), ridgegourd (Varalakshmi and Reddy, 1994), snakegourd (Narayanankutty et al., 2006) and *Luffa* (Ram et al., 2006).

Fruit length had high positive correlation with fruit girth, fruits per plant, average fruit weight, seeds per fruit and 100-seed weight. Fruit length was positively correlated with fruit weight, indicated that the fruit length and fruit weight could be improved simultaneously. Earlier, Singh et al. (1987) explained positive correlation of fruit length with fruit girth and average fruit weight in parwal. Average fruit weight also exhibited high positive correlation with seeds per fruits and 100-seed weight. Similar report was given by Ram et al. (2006b) in bottlegourd for average fruit weight and number of seeds per fruit.

Days to seedling emergence showed significant negative correlation with days to first fruit harvest. Vine length was positively correlated with internodal length and number of primary and secondary branches.

The negative correlation of node to first male and female flower observed in the present investigation corroborates with the results of Rao et al. (2000) in ridgegourd. Strong positive correlation of node to first male flower with node to first female flower was found.

Sex ratio exhibited strong negative correlation with fruit yield and fruits per plant. Similar report was made in bottlegourd by Murali et al. (1986). This suggested that a direct relationship existed between number of female flowers and number of fruits set and total fruit yield.

Days to first fruit harvest showed positive correlation with fruit girth and seeds per fruit. Positive correlation of fruit girth with fruit yield, average fruit weight and seeds per fruit was in agreement with the findings of Parhi et al. (1995) and Bhave et al. (2003) in bittergourd.

The present investigation revealed that number of fruits was positively correlated with yield. Lawande and Patil (1989) in bittergourd and Ram et al. (2006b) in bottlegourd reported strong positive correlation between fruits per plant and fruit yield. Fruits per plant also recorded positive correlation with 100-seed weight.

Fruit weight showed negative and non-significant correlation with number of fruits per plant, this indicated that increase in number of fruits per plant was associated with reductions in fruit number. Earlier, Singh (2004) explained the negative correlation between fruit number and fruit weight in chow-chow and reported that genotypic yield limitation necessitated competition between the two divergent forces and resulted in negative correlation. This makes a clear indication that increase in number of fruits per vine would indirectly affect the total yield which is more dependent on fruit weight. Therefore, aiming at selecting genotypes with more number of fruits of very small size may not be successful. The best alternative would be to select genotypes with fairly good number of fruits of medium size.

Seeds per fruit was positively correlated with fruit fly infestation. Mosaic incidence showed significant positive correlation with fruit fly infestation.

5.1.2.3 Path Coefficient Analysis

Path coefficient analysis furnishes a method for separating out the direct and indirect effects so as to measure the relative importance of each component characters. As evidenced from correlation studies, path coefficient analysis also signifies the importance of character average fruit weight, which exhibited the highest direct and indirect effect. Fruits per plant showed the next highest positive effect on yield. These confirmed the role of above characters in determining the fruit yield and therefore, are valuable in constructing the selection criteria. Similar results were also reported in bittergourd (Bhave et al., 2003), pointedgourd (Hazra et al., 2003), snapmelon (Pandey et al., 2003), ashgourd (Resmi, 2004), bottlegourd (Ram et al., 2007) and sweetgourd (Sanwal et al., 2007).

The direct effects of days to first female flower, seeds per fruit and mosaic incidence were negligible, whereas fruit length, fruit girth and node to first female flower exerted small negative direct effect on yield.

The direct effects of fruit length, fruit girth and seeds per fruit were negligible, but their indirect effect through average fruit weight were consistently high indicating that indirect selection for average fruit weight can increase yield. Negative correlation of sex ratio and node to first female flower with yield was due to high negative indirect effect through average fruit weight and fruits per plant. In bittergourd, Ramachandran (1978) reported that fruit length had negative direct effect on yield while, Bhave et al. (2003) observed that fruit girth had positive and indirect effect on yield. Narayanankutty et al. (2006) found that seeds per fruit indirectly contributed to yield in snakegourd. It can be concluded that traits like fruit length, fruit girth, number of fruits per plant, average fruit weight and seeds per fruit could be considered as most important yield contributing characters since they have expressed strong direct and indirect effect towards yield and also showed substantial positive correlation with yield. It appears that it would be rewarded to lay stress on

the above mentioned traits for selection programme based on the phenotypic performance for improvement in bittergourd.

5.1.2.4 Selection Index

Selection of genotypes based on a suitable index is highly efficient for any crop improvement programme. Identification of superior genotypes of *M. charantia* based on discriminant function analysis was done by Ram *et al*. (2006b) in bittergourd. A model involving the same set of eleven characters which was used for path coefficient analysis was selected for ranking the genotypes. On ranking the scores obtained, the genotype MC 20 (Priyanka, KAU) ranked first, followed by MC 10 (MDU-1, TNAU), MC 26 (Thripunithara, Ernakulam), MC 22 (Chathamangalam, Kozhikode) and MC 27 (Charuplasseri, Palakkad). These genotypes with better yield, fruit quality, earliness in male and female flowering, narrow sex ratio, fruit fly and mosaic resistance may be recommended as elite types after refinement and multilocational testing.

5.1.2.5 Mahalanobis's D^2 Analysis

Mahalanobis's D^2 statistics is a powerful tool for determining the degree of divergence between populations and relative contribution of different components to the total divergence in isolation of suitable parents. Genetic divergence was assessed by Lovely (2001), Resmi and Sreelathakumary (2008) in ashgourd; Narayanankutty *et al.* (2004) in snakegourd and Devmore et al. (2007), Dey *et al.* (2007) in bittergourd.

In the present study, based on Mahalanobis's D^2 statistic, the 33 genotypes were grouped into five gene constellations. The maximum number of genotypes (11) were included in Cluster I, followed by cluster III and V with 10 genotypes. Cluster II and IV had one genotype each. The pattern of clustering almost followed the ranking obtained from selection index. The commercially cultivated varieties like CO-1, Preethi,

Konkan Tara and Priya grouped under cluster I, while Pusa Do Mousami and Arka Harit group into cluster III. It may be inferred from this result that almost all the commercially cultivated genotypes of our country may have originated from closely related sources. Other commercially released cultivars like Priyanka and MDU-1 grouped singly into cluster II and IV respectively, which indicate that these genotypes are quite distinct from rest of the germplasm.

Considering the cluster means for various characters studied, clusters II and IV were superior for most of the biometric characters, whereas clusters III was generally poor. Cluster I and V were found to be intermediate. It is also evident that except cluster III and V (represented by small fruited genotypes), all the clusters showed higher yield potential than cluster I, which was represented by most of the commercially cultivated varieties.

Based on the results, Mahalanobis's D^2 was found to be useful tool in grouping the genotypes phenotypically. It may be concluded that in bittergourd, there is vast scope to develop new varieties with more yield potential and other attributes of economic importance by using this elite germplasm collection. For crop improvement programmes, intercrossing among genotypes with outstanding mean performance for these characters would be effective. To develop early varieties with more yield, selection from cluster I will be effective as it showed higher yield with early maturity. It is clear that for getting maximum yield with largest and heaviest fruits from early crop, cluster II would be a good option. To breed good varieties from small fruited group, selection from cluster V will be highly useful and to breed long, slender fruited varieties having some demand in specific region of our country, selection from cluster IV will be useful.

In general, the pattern of distribution of genotypes from different regions into different clusters was random. Similar observations were also

reported by Vahab and Gopalakrishnan (1993), Devmore et al. (2007) and Dey et al. (2007) in bittergourd. One of the possible reasons may be the fact that it is very difficult to establish the actual location of origin of a genotype. The free and frequent exchange of genetic material among the farmers and breeders in the country makes it very difficult to maintain the real identity of the genotype. The absence of relationship between genetic diversity and geographical distance indicates that forces other than geographical origin such as exchange of genetic stock, genetic drift, spontaneous variation, natural and artificial selection are responsible for genetic diversity. Another possibility may be that estimates of diversity based on the characters used in the present investigation might not have been sufficient to account for the variability caused by some other traits of physiological or biochemical nature which might have been important in depicting the total genetic diversity in the population. Therefore, selection of genotypes for hybridization should be based on genetic diversity other than geographic divergence.

The present investigation on morphological characterization of 33 *M. charantia* genotypes showed wide variation for most of the characters. High heritability coupled with high genetic advance was observed for biometric characters namely, vine length and average fruit weight indicates the scope for effective selection. Correlation and path coefficient analysis revealed that average fruit weight and fruit number are the primary yield components. The genotypes MC 20 (Priyanka, KAU) ranked first, followed by MC 10 (MDU-1, TNAU), MC 26 (Thripunithara, Ernakulam), MC 22 (Chathamangalam, Kozhikode) and MC 27 (Charuplasseri, Palakkad) were found to be promising with regard to yield, fruit quality, earliness in male and female flowering, narrow sex ratio, fruit fly and mosaic resistance. It is noteworthy that the genotypes under evaluation were diverse and had great potential for further improvement in quality fruit yields.

5.1.2.6 Quality Characters

i. Keeping quality (days)

Bittergourd is valued for its nutritive and medicinal properties and is a good source of minerals. Because of these unique properties there is always a consumer preference for bittergourd among the cucurbits. However, the highly perishable nature of fresh fruits resulting in produce loss has been a handicap in bittergourd production. Hence, selection of genotypes with maximum shelf life by minimizing physiological loss in weight and maintaining the product acceptability upto a considerable period is necessary. Maximum shelf life of 8.0 days was recorded by MC 25, followed by MC 12 and MC 30 with 6.5 days simultaneously. In bittergourd, Veenakumari et al. (1994) reported that a shelf life of 2.3 days under normal storage.

The physiological loss of weight expressed in terms of percentage increased progressively on par with storage. MC 25 and MC 15 had lower percentage loss in weight in four days after storage, while higher in MC 17 and MC 33. The results also showed that lowest rotting percentage occurred in fruits obtained from genotype MC 25. Among the genotypes, change in colour was slower in MC 25 and MC 12 upto 4 days.

ii. Organoleptic analysis

Organoleptic evaluation was done to assess the variation in consumer acceptance for quality attributes. Organoleptic quality of 33 bittergourd genotypes was evaluated by considering the quality attributes like appearance / colour, doneness, flavour, taste and bitterness score card. The genotypes differ significantly for all these attributes.

Among the bittergourd genotypes, MC 31 well preserved natural colour (Appearance/colour) , MC 27 showed highly acceptable doneness, MC 22 had pleasant flavour, MC 20 and MC 22 offered very good taste, MC 8 had lowest bitterness while, MC 25 was highly bitter.

In general, fruits with light green skin colour had overall acceptability and were superior to others based on the score. For canning, the genotypes MC 20 and MC 22 were found to the best, mainly due to their superior flavor and taste. For the purpose of dehydration, MC 31 was recommended, because they re-constituted to their original appearance reasonably well.

5.2 BIOCHEMICAL CHARACTERIZATION

Bittergourd fruits are highly nutritious (Gopalan et al., 1982). Bittergourds are very low in calories but dense with precious nutrients. In terms of nutritive value, bittergourd ranks first among cucurbits, the most important nutritional contribution being vitamins and minerals, especially iron, phosphorous and ascorbic acid. Nutritional value of green, small fruited types of bittergourd is surprisingly high for a cucurbit fruit. It has an ANV of 4.10, due to high iron and ascorbic acid content.

A variety of bittergourd with a higher content of beta carotene, ascorbic acid and iron would be of great use. Results of an association analysis indicated that breeding for an increase in total soluble solids would improve the content of ascorbic acid while maintaining a reasonably high protein content but that improving the iron content would demand a separate breeding programme (Ramachandran and Gopalakrishnan, 1980).

Variability in the important biochemical constituents like beta carotene, ascorbic acid, iron, chlorophyll and bitterness in bittergourd has been reported by several workers (Tee et al., 1997; Kumar and Sagar, 2003; Yadav et al., 2008). The present study also reveals wide variation in nutritive value among the genotypes.

Bittergourd is an excellent source of carotenoids such beta-carotene which is a precursor of vitamin A. Beta carotene is an antioxidant and known to be a potent booster for the immune system, as well as an

important nutrient for good eye health. In the present investigation, the β-carotene content was found to range from 52.58 µg/100 g in MC 5 to 138.96 µg/100 g in MC 8 respectively.

Vitamin C is ubiquitous (Chatterjee, 2009). It is found in almost all plants and animal kingdoms, where its precise role to prevent or cure scurvy is well understood. Also ascorbate could prevent or cure heart disease, stroke, cancer and infections. Humans, in contrast to many animals, are incapable of synthesizing this vitamin and are totally dependent on its dietary intake for their survival. The immature fruits of bittergourd are a good source of ascorbic acid. The results showed that the maximum ascorbic acid content of 124.29 mg/100 g was seen in MC 26 and minimum of 62.54 mg/100 g in MC 29 which is in accordance with the findings of Vahab (1989) in bittergourd.

Bittergourd is believed to have a high iron content, which increases its value as a preventive medicine for many health conditions. In the present study, iron content was highest in MC 25 (6.88 mg/100 g) and lowest in MC 7 (2.38 mg/100 g). In bittergourd, Tee et al. (1997) evaluated iron content and reported as 6.3 mg/100g while, Kumar and Sagar (2003) found that a 100g edible portion contain 1.8 mg of iron.

The medium sized fruits were associated with higher β-carotene while, small sized fruits found to have higher iron content and large fruited genotypes contained higher ascorbic acid content. A distinct difference in the content of vitamins and minerals is observed in different genotypes. However, correlation between colour of fruits and their iron content is found in some of the genotypes.

Bittergourd is known for its unique bitter taste. Bitterness of fruit is due to the presence of an alkaloid, momordicin. Immature fruits are less bitter and have a tangy flavour that adds spice to oriental cuisine.

Much variability is noted in the content of chlorophyll and bitterness factors. It's also notable that there is some correlation between chlorophyll content and bitterness value. Genotypes with higher the chlorophyll content, greater will be the bitterness value. The results of organoleptic evaluation of the quality attribute 'bitterness' is also in concordance with this fact.

5.3 MOLECULAR CHARACTERIZATION

The diverse visual characters (*i.e.,* sex expression, growth habit, maturity, fruit shape, size, color and surface texture) of bitter gourd in India provide for relatively broad diverse plant species. However, the genetic diversity of bitter gourd at the DNA level is not known. Obtaining estimates of genetic diversity is extremely important to plant improvement since it increases the efficiency and effectiveness of selection of improved cultivars. The traditional approach of identifying parents for breeding programmes by their morphological differences is constrained by several factors such as the environmental plasticity of the traits under consideration, and the limited number of phenotypic markers available. Characterization of germplasm based on horticultural traits needs complementation with molecular markers as they can contribute greatly to the utilization of genetic diversity through descriptive information of structure of genotypes, analyses of relatedness, the study of identity and location of diversity. In addition, characterization data in combination with evaluation data provide most representative core subset collection. DNA based genetic diversity assessment will help to eliminate duplications in the collection, making the process cost effective. The molecular markers are independent of environment, available in unlimited numbers, and can be assessed in a comparatively short period of time.

Random amplified polymorphic DNA (RAPD) analysis provides a quick and reliable method for resolving genetic relationships. RAPD analysis has been successfully employed to analyse genetic

diversity in ivygourd (Suresh, 2004), bottlegourd (Ram *et al.,* 2006), ashgourd (Verma *et al.*, 2007; Resmi et al., 2007), teasle gourd (Rasul *et al*., 2007), sweetgourd (Rahaman *et al.*, 2007) and bittergourd (Rahman et al., 2007; Behera *et al.,* 2008).

In the present study an attempt was made to determine the extent of genetic diversity in 33 genotypes of *M. charantia* of diverse geographic origin based on RAPD markers, making use of arbitrary primers to amplify random DNA sequence in the genome.

Isolation of genomic DNA of bittergourd was done using modified Murray and Thompson (1980) method. Tissues from young tender leaves were found to yield good quality DNA.

The DNA yield for thirty three genotypes of *M. charantia* ranged from 1.05 to 3.96. The purity of DNA (A_{260} / A_{280} ratio) ranged from 1.40 to 2.09 $\mu g\ \mu l^{-1}$.

To identify the promising primers for RAPD analysis, eighty decamer primers of kit A, B, E, J and UBC were screened using the DNA of genotype MC 12. The procedure standardized by Staub *et al*. (2000) in *Cucumis melo* germplasm was tried for amplification. Fifty six primers, out of the eighty decamer primers yielded amplification products indicating presence of sequence complementary to these primers in the DNA of MC 12 genotype. A total of 158 RAPDs (average 1.98 bands per primer) were generated by the 56 primers, of which 86.08 per cent were polymorphic (136 bands) and twenty two were monomorphic. Eight primers showed high level of polymorphism. This could be explained by the capability of individual primers to amplify the less conserved and highly repeated regions of the genomic DNA. There is high possibility for the amplified fragments to contain repeated sequences.

For further amplification of DNA from thirty three bittergourd germplasm, the eight promising primers were identified for RAPD

analysis based on performance in DNA amplification, production of highest number of polymorphic bands as well as intense bands and reproducibility. They were 0PA-02, 0PA-18, OPB-01, OPB-06, OPB-12, OPE-14, UBC-03 and UBC-05. Bhat and Jarret (1995) suggested that the number of polymorphisms might be more important than the number of primers for the generation of stable phenogram and it would vary with plant material under investigation and the sequences that are amplified. In bittergourd, Pala (2001) identified six RAPD primers to show genetic relationship among the genotypes while Behera *et al*. (2007) used twenty nine RAPD primers for genetic diversity studies.

A total of 56 scorable bands (average of 7.00 bands per primer) were generated by the selected eight primers of which 23 were monomorphic and rest, 33 were polymorphic (58.93 per cent). The number of bands ranged from 1 to 9 with an average of 1.25 per primer.

The primer OPA-02 was unique as it could distinguish maximum polymorphism among the genotypes tested. The highest number of scorable bands was given by OPA-18 of which seven of the bands produced were monomorphic. The primer UBC-05 produced eight scorable bands of which six bands were monomorphic for all the genotypes. The primer UBC-03, which produced a total of six scorable bands had two bands as monomorphic. Among the OPB group, primer OPB-01 had three monomorphic bands, while OPB-06 and OPB-12 had 2 and 1 monomorphic bands respectively. The primer OPE-14 had only one band as monomorphic while the rest, 6 bands were highly polymorphic.

The estimation of Jaccard's similarity coefficients and construction of dendrogram by using UPGMA revealed the presence and extent of genetic similarities among the thirty three genotypes of *M. charantia* examined. The overall similarity coefficients ranged from 0.65 to 0.90. Cluster analysis revealed that at about 0.65 similarity coefficient, the

thirty three genotypes of *M. charantia* grouped into two clusters. The genotype with smallest fruit M 33 got differentiated from the rest of the bittergourd germplasm at 0.65 similarity coefficient. This substantiates the moderately broad distribution of genetic variability, which can be attributed to the broad genetic base in their origin.

At 0.70 similarity coefficient, the long fruited and mutant genotype MC 10 got distinct from the rest of 32 bittergourd genotypes. The genotype with highest average fruit weight, MC 20 stood out from the rest of the group at 73.1 per cent similarity. Genotypes with low average fruit weight grouped into 2 subclusters at 77.65 per cent similarity.

Genotypes with medium fruit size could be split into two subclusters of 13 and 11 members respectively at 77.8 per cent similarity. This grouping was in concordance with their fruit skin colour. The members had green skin colour on fruits with exceptions of MC 15 and MC 29 having dark green skin colour falling in one subcluster. Most of the members of second subcluster had light green skin colour, but exceptions of white skin colour was also found.

At 81.3 per cent similarity, the first subgroup with 5 members was again divided into two. MC 4 showed 100 per cent similarity with MC 8. At 80.7 per cent similarity, second subgroup comprising of genotypes with same skin texture was further grouped into two with 4 and 5 members respectively. Also MC 19 and MC 31 showed 100 per cent similarity. The results indicated that most of the collected genotypes examined were genetically distinct, and these differences can be provided for the development of strategies for genetic analysis and crop improvement in this species.

Thus the study revealed that RAPD technique can be suggested as an objective and viable alternative or supplement to ampelography for bittergourd identification. The clusters based on RAPD analysis using eight primers depict wide genetic variation among the bittergourd

genotypes and provided varietal profiles. It can easily differentiate *M. charantia* genotypes, even the closely related ones. Polymorphism obtained in the present study will be further useful in fingerprinting and in determining genetic diversity among bittergourd genotypes. For future studies on analysis of bittergourd genotypes, wider genetic base and greater number of RAPD primers are to be included for accurate results. Finally, the results support the idea that RAPD technique being relatively simpler, quicker, inexpensive, non-radioactive, versatile and universal can detect sufficient polymorphisms for germplasm characterization and genetic distance studies. This marker system has the ability to amplify DNA from dispersed polymorphic loci and has its power to detect small genetic differences.

5.4 STANDARDIZATION OF *IN VITRO* TECHNIQUES

Generally, bittergourd is propagated by seed. Being a cross pollinated crop, the seedling progeny is highly heterogenus. Hence the maintenance of desirable quality and morphological aspects are very difficult in bittergourd. By considering its economic importance, effort should be made to utilize biotechnology for further improvement of this valuable crop. Hence standardization of a protocol for production and maintenance of novel types of bittergourd has been attempted.

The commercial application of *in vitro* techniques in cucurbitaceous taxa has been well demonstrated and the regeneration of plants has been reported for *Cucurbita pepo* (Jelaska, 1974), cucumber (Chee, 1990; Gambley and Dodd, 1990), melon (Moreno et al., 1985), watermelon (Dong and Jia, 1991) and squash (Rahman et al., 1993) and bittergourd (Islam et al., 1994; Sultana and Bari Miah, 2003; Huda and Sikdar, 2006).

5.4.1 Somatic Organogenesis

Callus mediated (indirect) somatic organogenesis is characterized by redetermination of the differentiated cells leading to callus formation and subsequent dedifferentiation to form separate shoots and

root initials from it. However, the key technology for regeneration that starts from tissues or callus cultures, i.e. the induction of organogenesis, is still insufficient in bittergourd. Somatic organogenesis through callus pathway is achieved in several cucurbits like *M. charantia* (Halder and Gadgil, 1982b), watermelon (Dong and Jia, 1991; Sultana et al., 2004), ashgourd (Thomas and Sreejesh, 2004) and summer squash (Pal et al., 2007).

In vitro induction of organogenesis depends on the endogenous concentration of plant growth regulator, their distribution in the cultured tissue and interaction with exogenously supplied growth regulator. Growth promoting substances were found to induce rapid cell division. The present results indicated that explants dedifferentiated and initiated calli at the cut surfaces within 11 to 15 days of inoculation and proliferated within 3 weeks of culture. Combination of BA and 2,4-D along with IBA/NAA/IAA derived positive response for all the explants.

The induction of potentially organogenic callus successfully from all the explants was obtained either on the medium supplemented with IBA (4.00 mg l^{-1}) + BA (0.50 mg l^{-1}) + 2,4- D (2.00 mg l^{-1}) or NAA (2.00 mg l^{-1}) + BA (0.50 mg l^{-1}) + 2,4- D (2.00 mg l^{-1}). Kim et al. (1988) has come out with the results that combination of 2,4-D and BA could help to induce callus from cotyledonary explants of cucumber. Moreover, Srivastava et al. (1989) obtained organogenic calli using a combination of BAP and NAA. The use of 2,4-D alone were reported previously in summer squash by Leljak-Levanic et al. (2004) and Pal et al. (2007).

On the other hand, BAP along with IAA, IBA or NAA initiates callus initiation. But the callus index and growth score was poor. The use of high doses of NAA or IBA in combination with BA and 2,4-D had negative effects on organogenesis. Furthermore, these combinations had a marked effect on the quality of callus, especially on those from leaf.

There was, however, no callus formation from all the explants cultured on media fortified with higher concentration of BA.

Better proliferation of callus (high callus index of 350.00 and more) was obtained in the same initiation media. BA as a cytokinin might be responsible for rapid cell division and proliferation.

Both the color and texture of the callus derived from the four explants varied. Calli derived from shoot tips, internodes and nodes were mostly friable and creamy in color with very few brownish exceptions. Leaf derived calli were mostly watery and pale brown and had less potential for further organogenesis.

Callus morphology varied with different growth regulators used in the culture medium. Media with IBA or NAA in combination with BA and 2,4-D developed creamy white friable fast growing callus. Yellowish green slow growing compact calli which turned slightly brownish on further subculturing was induced on medium with IAA in combination with BA and 2,4-D. Callus morphology was also in agreement with Thomas and Sreejesh (2004) as in *B. hispida* and with Pal et al. (2007) as in summer squash. Friable and creamy calli derived from shoot tip, nodal and internodal explants can be used to initiate organogenic calli and shoot-bud induction. Subsequent regeneration of bittergourd plant was obtained in shoot tip derived calli only. Calli induced from shoot tip explants were larger in size than those from other explants, and when transferred to a basal medium at low levels of PGRs (in particular BA) promoted shoot organogenesis and elongation.

Sucrose is the most utilizable carbon form and energy source as well as an osmoregulatory factor (Brown et al., 1979; Wethrell, 1984; George and Sherrington, 1984). In the present investigation, effect of various concentrations of sucrose (20.00 g l^{-1}, 30.00 g l^{-1} and 40.00 g l^{-1}) on callus initiation and proliferation was studied. It was observed that

different concentrations of sucrose affected callus initiation and proliferation in bittergourd variously. Among the three different levels of sucrose tried, sucrose at 30.00 g l^{-1} was found to be superior to all others not only by initiating callus at minimum days, higher callusing percentage but also produced maximum growth score and callus index for all the explants. The present findings also indicate that the sucrose not only acts as a carbon cum energy source in the medium but also acts as an osmoticum and different concentrations of it acts as one of the controlling factors for the induction and growth of callus.

Agar at optimum concentration creates a favourable osmotic potential for the uptake of nutrients. Three different levels of agar (5.30 g l^{-1}, 6.30 g l^{-1} and 7.30 g l^{-1}) were tried to standardize the optimum agar concentration for callus initiation and proliferation. The treatment with 6.30 g l^{-1} of agar recorded the earlier callus initiation, cent percent callusing, maximum growth score and callus index. This may be due to the easy availability of nutrients like Ca, Mg, K and Mn in the soft gel medium at lower agar concentration. The treatment with 7.30 g l^{-1} of agar was inferior with respect to all the parameters. It recorded delayed callus initiation also. This could be due to restricted diffusion of macro nutrients or reduced availability of organic matter or water. It is evident from the present investigation and those of other (Skirvin, 1981; Debergh, 1983) that concentrations of the agar in the media can affect callus growth. Therefore, the level of agar in the medium should be such that it minimizes the water loss and allows the good diffusion of nutrient elements.

The present investigation has demonstrated that plant growth regulators, sucrose and agar on the medium play an important role on *in vitro* callus growth and proliferation of bittergourd. It showed that MS medium containing sufficient growth regulators in combination with 30.00 g l^{-1} sucrose and 6.30 g l^{-1} agar level proved to be more effective for callus initiation and proliferation.

The compact callus tissue was sectioned and transferred on to MS medium fortified with cytokinin (BA) and activated charcoal in various combinations for shoot initiation and elongation. Generally, shoot initiation was observed 2-3 weeks after inoculation on regeneration medium. Green adventitious shoot buds developed on the compact masses was subcultured on the same media at 2 to 3 weeks interval for shoot elongation. Subculturing of regenerated shoots with minimal basal callus into same medium exhibited further shoot elongation.

Of all the explants tested, highest overall callus regeneration and shoot elongation was noted from shoot tip explants. Leaf calli were unresponsive even after 8 weeks of culturing for regeneration. Shoot elongation was not observed in nodal and internodal derived calli, instead only short green shoot primordia were visible.

Good (100 per cent) shoot organogenesis was observed using BA in combination activated charcoal from shoot tip derived calli. It is suggested that activated charcoal along with a cytokinin stimulates the development of shoot primordia. On the other hand, BA along with a low concentration of activated charcoal initiates shoot elongation also. MS medium fortified with 0.05 % AC + 1.00 mg l^{-1} BA recorded earlier regeneration, highest regeneration percentage, lengthy adventitious shoot and more number of leaves under light conditions. Higher levels of BA and AC in the medium reduced the growth of the shoots *i.e.*, reduced shoot length, lesser number of leaves and also the leaves produced were greener than those produced in other treatments. The synergistic effect of cytokinin and AC may be the reason for earlier regeneration. Stimulation for callus regeneration could be due to adsorption of inhibitory compounds to AC also. Activated charcoal added to the medium might have eliminated the residual effect of 2,4-D and IBA or NAA by adsorption and thus promoting the activities of BA for shoot initiation.

No substantial difference in regeneration rates was found when shoot tip derived calli were taken from the first 2 subcultures. It was observed that from the third subculture and onwards regeneration rates showed marked differences. An adverse effect of prolonged *in vitro* culture is reported (Jureti and Jelaska, 1991; Ficcadenti and Rotino, 1995). Although the callus maintains its regeneration capacity for a longer period, prolonged subculturing may lead to a higher frequency of mutants, especially in higher concentrations of BA.

The nodal and internodal derived calli were able to regenerate shoots only from older callus when compared with shoot tip derived callus. The incubation period of callus induced from nodal and internodal explants appears to be critical for induction of organogenesis. Longer incubation periods (9 weeks) lead to shoot organogenesis in these calli. The selected callus (having regeneration potential) was multiplied on callusing medium (BA 0.05 mg l^{-1} + IBA 4.00 mg l^{-1} + 2,4-D 2.00 mg l^{-1}) and maintained a high regeneration rate from 1-5 subcultures on this medium.

After 1-2 weeks culture of nodal derived morphologically distinct organogenic calli on regeneration medium, green spots and development of several shoots were observed gradually from a single callus mass. The distinct shoot-like structures could be distinguished by the presence of green, opaque, and compact leaves. There was, however, no shoot elongation from these shoot like structures cultured on media fortified with BA (1.00 or 2.00 mg l^{-1}) with 0.05 per cent AC. On the other hand, higher levels of cytokinin (BA) and AC leads to shoot initiation and differentiation in nodal and internodal derived callus. Among various combinations, 1.00 mg l^{-1} BAP with 0.10 per cent AC was most effective. Moreover, 2.00 mg l^{-1} BAP plus 0.10 per cent AC was also impressive.

Light had significant influence on callus regeneration and shoot elongation. Light inhibit callusing while dark condition induced better callusing. According to Murashige (1977), the optimum light intensity

for shoot formation in a large number of herbaceous species is around 1000 lux. Profuse callusing by darkness is due to the etiolation effect. Etiolated tissues may be less lignified than the light grown tissues, which facilitate easy de-differentiation. Also, the regenerated shoots and leaves appeared pale and chlorotic when kept under dark conditions. This may be attributed to the lack of chlorophyll development required for photosynthesis. Moreover, continuous darkness initiated callusing of regenerated plants.

5.4.2 *In vitro* rooting

Adventitious shoots developed in cultures in the presence of cytokinin especially BA lack roots. Easy root formation is very important for establishment of *in vitro* regenerated plantlets. Media having a low concentration of salts have proven satisfactory for rooting of micropropagated shoots. Roots are mostly induced in the presence of a suitable auxin (IBA, IAA or NAA) in the medium (Mythili and Thomas, 1999).

For rooting, the well developed elongated microshoot (3-5 cm) is excised and transferred to the rooting medium. In the present study, different levels of auxin IBA along with the control (full MS) were tried for *in vitro* rooting of bittergourd shoots. Roots were observed as early as 2 weeks after placing on the rooting medium. Delayed rooting response, even after 3 weeks in root induction medium observed in higher concentration of IBA can be attributed to the persisting effect of BA.

Rooting occurred in all concentrations but with different rooting percentages, and the optimal response was observed on MS + IBA (1.00 mg l^{-1}) in terms of earlier root initiation, better rooting percentage (66.66%), average number of roots (10.83) with mean root length of 6.83 cm. IBA has been reported to give better results compared to other auxins for *in vitro* rhizogenesis in cucurbits (Abrie and Staden, 2001; Sarowar et al., 2003; Thomas and Sreejesh, 2004; Krug et al., 2005).

MS supplemented with 0.50 mg l^{-1} IBA induced early root initiation. Shoot exposed to higher concentrations of IBA (2.00 mg l^{-1}) even though produced thick, short roots, the shoot became necrotic, lost its leaves and the shoot tips died gradually. With regard to number of roots in control (MS medium without any plant growth regulator), single root formation was observed. Ananthakrishnan et al. (2003) in *C. pepo*, Lee et al. (2003) in *C. maxima,* Keng and Hoong (2005) in muskmelon and Li et al. (2008) in cucumber reported that all the *in vitro* shoots produced root when transferred to MS medium without any plant growth regulator.

By characterizing all the thirty three genotypes of *Momordica charantia* using morphological (selection index and D^2 analysis) and molecular (RAPD marker analysis) methods revealed that morphologically distinct and superior lines were genetically differentiable. The clustering pattern based on yield related traits and molecular variation was different. Also, the diversity based on yield related traits and molecular analysis was not in consonance with ecological distribution. In the present study, standardization of *in vitro* multiplication of bittergourd through callus mediated (indirect) somatic organogenesis using shoot tip explant was successful.

Development of high-yielding bittergourd with early fruiting, more number of fruits and nutritionally rich fruits will have an advantage for higher returns to the farmers. The superior genotypes in terms of light green fruits *viz.,* MC 26, MC 22 and MC 27 identified in the present study need special attention in terms of multilocational testing. Among the genotypes bearing dark green fruits, MC 25 is rich in biochemical and keeping quality characters.

SUMMARY

6. SUMMARY

The present investigation on "Characterization and *in vitro* multiplication of bittergourd (*Momordica charantia* L.) genotypes" was carried out at the Department of Olericulture, the Department of Plant Biotechnology and the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during 2005-2008.

The study envisaged genetic cataloguing of the available germplasm in *Momordica charantia*, assessment of genetic variability, divergence, association among the characters including direct and indirect effects of various characters on yield, formulation of a selection index for identifying suitable lines based on yield, quality, pest and disease resistance, biochemical characterization for quality traits, molecular characterization using RAPD analysis and standardization of *in vitro* techniques.

The experimental material consisted of 33 genotypes of bittergourd collected from different agroclimatic regions of the country. The genotypes were genetically catalogued based on the descriptor list for cucurbits (IBPGR, 1983). The results revealed distinct variations among the genotypes with respect to vegetative, flower, fruit and seed characters.

Significant differences were observed among thirty-three genotypes of *M. charantia* for all the characters except fruit fly infestation (%) studied namely, days to seedling emergence, vine length, internodal length, number of primary branches, number of secondary branches, days to first male flower, days to first female flower, node to first male flower, node to first female flower, sex ratio, days to first fruit harvest, fruit length, fruit girth, fruits per plant, average fruit weight, yield per plant, seeds per fruit, 100-seed weight and mosaic incidence indicating sufficient diversity among the genotypes.

Among the genotypes MC 20 (Priyanka, KAU) is the highest yielder followed by MC 26 (Thripunithara, Ernakulam), MC 10 (MDU-1, TNAU), MC 22 (Chathamangalam, Kozhikode) and MC 27 (Charuplasseri, Palakkad). The high yield in MC 20 may be attributed to the high fruit length, fruit girth and average fruit weight. MC 26 was characterized by low sex ratio and earliness in male and female flowering and harvest. The genotype MC 10 got highest number of fruits per plant along with higher fruit length, average fruit weight and 100-seed weight. The genotypes MC 22 and MC 27, apart from being better for most of the fruit characters like fruit length, fruit girth, average fruit weight, seed number and 100-seed weight, they also registered high values for resistance against fruit fly and mosaic disease resulting in better yield. Fruit fly infestation percentage was less than 20 per cent for all the genotypes. The reaction of thirty three genotypes of *M. charantia* towards mosaic incidence indicated that ten genotypes were highly resistant, ten genotypes were resistant, ten genotypes were moderately resistant and remaining three were susceptible to the disease. High coefficients of variation (phenotypic [PCV] and genotypic [GCV]) were observed for yield per plant and average fruit weight. The lowest PCV and GCV were exhibited by days to first male flower.

High heritability coupled with low genetic advance was observed for most of the characters, except fruit fly infestation and mosaic incidence. High heritability coupled with high genetic advance was observed for vine length and average fruit weight indicating scope for improvement of these characters through selection.

Correlation studies at both phenotypic and genotypic levels revealed that the characters like vine length, fruit length, fruit girth, fruits per plant, average fruit weight, seeds per fruit and 100-seed weight were positively correlated with yield per plant. Node to first male and female flower and sex ratio were negatively correlated with yield.

Path coefficient analysis based on direct and indirect effects of different characters on fruit yield indicated that it would be rewarded to lay stress on average fruit weight and number of fruits per plant for selection programme based on the phenotypic performance for improvement in bittergourd.

Based on the selection index scores obtained, the genotype MC 20 (Priyanka, KAU) ranked first, followed by MC 10 (MDU-1, TNAU), MC 26 (Thripunithara, Ernakulam), MC 22 (Chathamangalam, Kozhikode) and MC 27 (Charuplasseri, Palakkad). They were found to be promising based on their superiority in yield, fruit quality, earliness in male and female flowering, narrow sex ratio and fruit fly infestation and mosaic resistance. Hence these genotypes can be utilized to incorporate particular traits for tailoring high yielding bittergourd hybrids/selections.

The 33 genotypes of *Momordica charantia* were grouped into five gene constellations based on Mahalanobis's D^2 statistic. Cluster I was the largest which contained 11 genotypes, followed by cluster III and V with 10 genotypes. Cluster II and IV had one genotype each. With regard to cluster means, clusters II and IV performed better for most of the biometric characters taken. The maximum intercluster distance was observed for clusters III and IV (2515.57), followed by clusters II and III (2088.12) and clusters I and IV (1856.82). The intracluster distance was highest for cluster I (1197.78).

A study was conducted to assess the keeping quality of all the bittergourd genotypes based on maximum shelf life and minimal physiological loss in weight. Maximum shelf life of 8.0 days was recorded by MC 25, followed by MC 12 and MC 30 with 6.5 days simultaneously. MC 25 and MC 15 had lower percentage loss in weight in four days after storage, while higher in MC 17 and MC 33. The results also showed that lowest rotting percentage occurred in fruits obtained from genotype

MC 25. Among the genotypes, change in colour was slower in MC 25 and MC 12 upto 4 days.

The organoleptic quality of 33 bittergourd genotypes was evaluated by considering the quality attributes like appearance / colour, doneness, flavor, taste and bitterness score card. The genotypes differ significantly for all these attributes. In general, fruits with light green skin colour had overall acceptability and were superior to others based on the score.

The biochemical characterization of bittergourd germplasm was done by estimating parameters like β-carotene, ascorbic acid, iron, chlorophyll and bitterness. MC 5 had the maximum β-carotene content of 138.96 µg/100 g while MC 8 had the minimum content of 52.58 µg/100 g. ascorbic acid was highest in MC 31 (124.29 mg/100 g) and lowest in MC 29 (62.54 mg/100 g). Iron content varied from 2.38 in MC 7 to 6.88 mg/100 g in MC 24. Chlorophyll a and b content varied from 0.00110 to 0.54365 mg/g and 0.00045 to 0.03075 mg/g respectively. The range of total chlorophyll was 0.003 to 0.12 mg/g. Bitterness value was minimum in MC 8 (1227.50 units/g) and maximum in MC 25 (10400 units/g). It's also notable that there is some correlation between chlorophyll content and bitterness value. Genotypes with higher the chlorophyll content, greater will be the bitterness value. A distinct difference in the content of vitamins and minerals is observed in different genotypes. However, correlation between colour of fruits and their iron content is found in some of the genotypes.

RAPD (Random amplified polymorphic DNA) analysis was used to characterize genetic variability and relationships among thirty-three genotypes of *M. charantia* of diverse geographic origin at molecular level. Tissues from young tender leaves were found to yield good quality DNA. The DNA yield for thirty-three bittergourd genotypes ranged from 1.05 to 3.96. The purity of DNA (A_{260} / A_{280} ratio) ranged from 1.40 to 2.09 µg μl^{-1}. Each sample was subjected to RAPD analysis. Out of the

80 decamer primers, fifty six primers yielded amplification products. A total of 158 RAPDs (average 1.98 bands per primer) were generated by the 56 primers, of which 86.08 per cent were polymorphic (136 bands) and twenty two were monomorphic. Eight primers showed high level of polymorphism and were informative enough to analyse these genotypes. 0PA-02, 0PA-18, OPB-01, OPB-06, OPB-12, OPE-14, UBC-03 and UBC-05 were identified for RAPD analysis based on their performance in DNA amplification, reproducibility and production of highest number of polymorphic bands as well as intense bands.

The primer OPA-02 was unique as it could distinguish maximum polymorphism among the genotypes tested. The highest number of scorable bands was given by OPA-18 of which seven of the bands produced were monomorphic. The primer UBC-05 produced eight scorable bands of which six bands were monomorphic for all the genotypes. The primer UBC-03, which produced a total of six scorable bands had two bands as monomorphic. Among the OPB group, primer OPB-01 had three monomorphic bands, while OPB-06 and OPB-12 had 2 and 1 monomorphic bands respectively. The primer OPE-14 had only one band as monomorphic while the rest, 6 bands were highly polymorphic. The selected primers yielded 56 scorable bands (average of 7.00 bands per primer) of which 23 were monomorphic and rest, 33 were polymorphic (58.93 per cent).

The estimation of Jaccard's similarity coefficients and construction of dendrogram by using UPGMA revealed the presence and extent of genetic similarities among the thirty-three genotypes of *M. charantia* examined. The overall similarity coefficients ranged from 0.65 to 0.90. Cluster analysis revealed that at about 0.65 similarity coefficient, the thirty three genotypes of *M. charantia* grouped into two clusters. Quite distinct among these were two cluster formed at 0. 65 similarity coefficient which clearly separates genotypes based on average fruit weight and fruit skin colour.

By characterizing all the thirty three genotypes of *Momordica charantia* using morphological (selection index and D^2 analysis) and molecular (RAPD marker analysis) methods revealed that morphologically distinct and superior lines were genetically differentiable. The clustering pattern based on yield related traits and molecular variation was different. Also, the diversity based on yield related traits and molecular analysis was not in consonance with ecological distribution.

The standardization of an *in vitro* protocol for production and maintenance of novel types of bittergourd through callus mediated (indirect) somatic organogenesis has been attempted. The induction of potentially organogenic callus and better proliferation of callus successfully from all the explants (shoot tip, node, internode and leaf) was obtained either on the MS medium supplemented with IBA (4.00 mg l^{-1}) + BA (0.50 mg l^{-1}) + 2,4- D (2.00 mg l^{-1}) or NAA (2.00 mg l^{-1}) + BA (0.50 mg l^{-1}) + 2,4- D (2.00 mg l^{-1}) in combination with 30.00 g l^{-1} sucrose and 6.30 g l^{-1} agar level. The present results indicated that explants dedifferentiated and initiated calli at the cut surfaces within 11 to 15 days of inoculation and proliferated within 3 weeks of culture.

The shoot tip explants were more responsive among all the explants tried in terms of callus induction and subsequent plant regeneration. Adventitious shoots were produced from the organogenic shoot tip derived callus when it was transferred to MS medium fortified with 0.05 % AC + 1.00 mg l^{-1} BA with earlier regeneration, highest regeneration percentage, lengthy adventitious shoot and more number of leaves under light conditions. The use of high doses of BA and AC in the medium had negative effects on organogenesis. Shoot elongation was not observed in nodal and internodal derived calli, instead only short green shoot primordia were visible. Efficient rooting was achieved on MS fortified with IBA (1.00 mg l^{-1}).

REFERENCES

REFERENCES

Abrie, A.L. and Staden, J. 2001. Development of regeneration protocols for selected cucurbit cultivars. Pl. Growth Regul., 35: 263–267.

Ahad, A.R., Islam, M.H., Kuzzaman, K. and Joarder, O.I. 1994. Plant regeneration from immature and mature axis of watermelon. Pl. Tiss. Cult., 2: 39-44.

Ahmad, N. and Anis, M. 2005. *In vitro* mass propagation of *Cucumis sativus* L. from nodal segments. Turk. J. Bot., 29: 237-240.

Allard, R.W. 1960. *Principles of Plant Breeding*. John Wiley and Sons Inc., New York, 468 p.

Anand, I.J. and Murthy, B.R. 1968. Genetic divergence and hybrid performance in Linseed. Indian J. Genet. Pl. Breed., 28: 178-185.

Ananthakrishnan, G., Xia, X., Amutha, S., Singer, S., Muruganantham, M., Yablonsky, S., Fischer, E. and Gaba, V. 2007. Ultrasonic treatment stimulates multiple shoot regeneration and explant enlargement in recalcitrant squash cotyledon explants *in vitro*. Pl. Cell Rep., 26:267–276.

Ananthakrishnan, G., Xia, X., Elman, C., Singer, S., Paris, H.S., Galon, A. and Gaba, V. 2003. Shoot production in squash (*Cucurbita pepo*) by *in vitro* organogenesis. Pl. Cell Rep., 21:739–746.

Ananthan, M., Balakrishnamoorthy, G. and Natarajan, S. 2005. Genetic variability in ridgegourd (*Luffa acutangula* (Roxb.) L.). S. Indian Hort., 53: 326-328.

Anitha, C.A. 1998. Variability in ridgegourd (*Luffa acutangula* (Roxb.) L.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 89 p.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplast. Pl. Physiol., 24: 1-5.

Arunachalam, P. 2002. Breeding for resistance to distortion mosaic virus in bittergourd. Ph.D. thesis, Kerala Agricultural University, Thrissur, 132 p.

Ashok, P. 2000. Character association of seeds on plant morphology in snakegourd (*Trichosanthes anguina* L.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 121 p.

Ashok, P. and Rajan, S. 2004. Character association of seeds on plant morphology in snakegourd (*Trichosanthes anguina* L.). The Andhra Agric. J., 51: 52-58.

Babu, B.S., Reddy, E.N. and Rao, M.R. 1986. Inheritance of certain quantitative and qualitative characters in bittergourd (*Momordica charantia* L.). S. Indian Hort., 34: 380-386.

Barnes, L. R., Cochran, F. D., Mott, R. L. and Henderson, W. R. 1978. Potential uses of micropropagation for cucurbits. Cucurbit Genet. Coop. Rep., 1:21.

Behera, T.K., Singh, A.K. and Staub, J.E. 2007b. A comparative analysis of genetic diversity in bittergourd (*Momordica charantia* L.) genotypes using RAPD and ISSR markers. Abstr. Am. Soc. hort. Sci. 104th Annual Conference, 42(4):915.

Behera, T.K., Dey, S.S. and Sirohi, P.S. 2006. 'DBGy-201' and 'DBGy-202': two gynoecious lines in bittergourd (*Momordica charantia* L.) isolated from indigenous source. Indian J. Genet. Pl. Breed., 66: 61–62.

Behera, T.K., Staub, J.E., Philipp, B. and Simon, W. 2007a. Bittergourd and human health. Medicinal Aromatic Pl. Sci. Biotech., 22: 224-226.

Behera, T.K., Ambika B.G., Singh, A.K. and Staub, J.E. 2008. Relative efficiency of DNA markers (RAPD, ISSR and AFLP) in detecting genetic diversity of bitter gourd (*Momordica charantia* L.). J. Sci. Fd. Agric., 88: 733-737.

Beloin, N., Gbeassor, M., Akpagana, K., Hudson, J., Soussa K., Koumaglo, K. and Arnason, J.T. 2005. Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. J. Ethnopharmacol., 96:49-55.

Bhave, S.G., Bendale, V.M., Pethe, U.B., Berde, S.A. and Mehta, J.L. 2003. Correlation and path analysis in segregating generations of bittergourd. J. Soils Crops, 13: 33-40.

Bhat, K.V. and Jarret, R.L. 1995. Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. Genet. Resour. Crop Evol., 42: 107-118.

Bos, L. 1982. Crop losses caused by viruses. Adv. Virus Res., 2: 31-57.

Brown, D.C.M., Leung, D.W.M. and Thrope, T.A. 1979. Osmotic requirements for shoot formation in tobacco cellus. Physiol. Pl., 46: 36-41.

Burkill, H. M. 1985. *The useful plants of West Tropical Africa*. Second edition. Vol. 1. Royal Botanic Gardens, Kew, 322 p.

Burza, W. and Malepszy, S. 1995. Direct plant regeneration from leaf explants in cucumber (*Cucumis sativus* L.) is free of stable genetic variation. Pl. Breed., 114: 341-345.

Cassells, A.C. and Bajaj, Y.P.S. 1991. High-tech micropropagation. In: Bajaj, Y.P.S. (ed.). *Biotechnology in Agriculture and Forestry* Springer-Verlag Press, New York pp. 286-306

Chakravarty, H.L., 1990. Cucurbits of India and their role in the development of vegetable crops. In: Bates, D.M., Robinson, R.W. and Jeffrey, C. (eds.). *Biology and Utilization of Cucurbitaceae.* Cornell University Press, Ithaca, New York, pp. 325–334.

Chandravadana, M. V. 1987. Identification of triterpenoid feeding deterrent of red pumpkin beetles (*Aulacophora foveicollis*) from *Momordica charantia*. J. Chem. Eco., 13: 1689-1 694.

Chandravadana, M. V. and Chander, S. 1990. Subcellular distribution of Momordicine II in *Momordica charantia* L. Indian J. Exp. Biol., 28: 185-186.

Changlin, W. 1998. *Waxgourd Varietal Trial*. AVRDC-ARC Research Report, Taiwan, 202 p.

Chatterjee, J.B. 2009. The history of vitamin C research in India. J. Biosci. 34: 185-194

Chee, P.P. 1990. High frequency of somatic embryogenesis and recovery of fertile cucumber plants. Hort Sci., 25: 792-793.

Chen, J., Tian, R., Qiu, M., Lu, L., Zheng, Y. and Zhang, Z. 2008. Trinorcucurbitane and cucurbitane triterpenoids from the roots of *Momordica charantia*. Phytochemistry, 69 : 1043–1048.

Chen, Q. and Li, T.S.C. 2005. Bitter melon reduces fat accumulation in rats. Br. J. Nurt. 93: 747-754

Choudhary, S.M. 1987. Studies on heterosis, combining ability and correlation in bittergourd (*Momordica charantia* L). Ph. D. thesis, Mahatma Phule Agricultural University, Rahuri, 136 p.

Choudhary, S.M., Kale, P.N. and Desai, U.T. 1991. Variability studies and scope of improvement for fruit yield in bittergourd. J. Maharashtra agric. Univ., 16: 15-17.

Debergh, P.C. 1983. Effects of agar brand and concentration on tissue culture medium. Physio. Pl. 59: 270-276

Debergh, P. C. and Zimmerman, R. H. 1991. *Micropropagation technology and application*. Kluer Academic Publishers, Netherlands, 189 p.

Debnath, R. K., Roy, S. K., Ahmed, G. and Hossain, M. 2000. Micropropagation of patal (*Trichosanthes dioica* Roxb) from nodal segment and shoot tip. Pl. Tiss. Cult., 10: 125-130.

Devadas, V.S. and Ramadas, S. 1994. Genetic analysis of bitter principles in *Momordica charantia* L. Cucurbit Genet. Coop. Rep., 17: 129-131.

Devmore, J.P. and Dhonukshe, B.L. 2007. Genotypic variability in floral biological attributes of bittergourd (*Momordica charantia* L.). Veg. Sci., 34: 82-83.

Devmore, J.P., Dhonukshe, B.L., Apte, U.B. and Jadhav, B.B. 2007. Genetic divergence in bittergourd (*Momordica charantia* L.). South Indian Hort. 55: 20-23

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

Dey, S. S., Behera, T. K., Pal, A. and Munshi, A. D. 2005. Correlation and path coefficient analysis in bittergourd (*Momordica charantia* L.). Veg. Sci., 32: 173–176.

Dey, S.S., Behera, T.K., Munshi, A.D. and Sirohi, P.S. 2007. Studies on genetic divergence in bittergourd (*Momordica charantia* L.). Indian J. Hort., 64: 53-57.

Dey, S.S., Singh, A.K., Chandel, D. and Behera, T.K. 2006. Genetic diversity of bittergourd (*Momordica charantia* L.) genotypes revealed by RAPD markers and agronomic traits. Scientia Horticulturae, 109: 21–28.

Dhillon, N., Ranjana, R., Singh, K., Eduardo, I., Monforte, A., Pitrat, M. and Singh, P. 2007. Diversity among landraces of Indian snapmelon (*Cucumis melo* var. *momordica*). Genet. Resour. Crop Evol., 54: 1267-1283.

Dong, J. and Jia, S. 1991. High efficiency plant regeneration from cotyledons of watermelon (*Citrullus vulgaris* Schrad). Pl. Cell Rep., 9: 559-562.

Dora, D.K., Behera, T.K., Acharya, G.C., Mohapatra, P. and Mishra, B. 2003. Genetic variability and character association in pointedgourd (*Trichosanthes dioica* Roxb.). Indian J. Hort., 60: 163-166.

Dutta, P.K., Chakravarty, A.K. and Chowdhary, U.S. 1981. Vicine, a favism-inducing toxin from *Momordica charantia* L. seeds. Indian J. Chem. 20: 669–671.

Etoh, S., Keiko, I., Toshiyuki, T., Kohji, Y., Kazuki, S. and Masuko, K. 2002. Bittergourd induces apoptosis in cancer cell lines. Nippon-Shokuhin-Kagaku-Kogaku-Kaishi 49: 250-256

Evans, D.A., Sharp, W.R. and Flick, C.E. 1981. Growth and behaviour of cell cultures: Embryogenesis and organogenesis. In: Thorpe, T.A. (ed.). *Plant Tissue Culture - Methods and Applications in Agriculture*, Academic Press, New York, pp. 45–113.

Fatope, M. O., Takeda, Y., Yamashita, H., Okabe, H. and Yamauchi, T. 1990. New cucurbitane triterpenoids from *Momordica charantia*. J. Nat. Prod., 53: 1491 -1497.

FIB, 2008. *Farm Guide 2008*. Farm Information Bureau, Government of Kerala.

Ficcadenti, N. and Rotino, G.L. 1995. Genotype and medium affect shoot regeneration of melon. Pl. Cell Tiss. Org. Cult. 40: 293

Fischer, C.P. and Butch, P.E. 1989. Pest status: Temperate Europe and West Asia. World Crop Pest, 3: 37-50.

*Fisher, R.H. 1936. The use of multiple measurements in taxonomic problems. Ann. Eugen., 7: 179-188.

Gambley, R.L. and Dodd, W.A. 1990. An *in vitro* technique for the production of de novo multiple shoots in cotyledon explants of cucumber (*Cucumis sativus* L.). Pl. Cell Tiss. Org. Cult., 20: 177-183.

Gambley, R.L. and Dodd, W.A. 1991. The influence of cotyledon in axillary and adventitious shoot production from cotyledonary nodes of *Cucumis sativus* L. (cucumber). J. Exp. Bot., 42: 1131-1135.

Gamborg, O.L., Miller, R.A. and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res., 50: 151-158.

Garrison, W. 1977. The world crops plant germplasm and endangered resources. The Bull. Atomic Scientists, 33: 9-16.

George, E.F. 1993. Plant tissue culture techniques. In: George, E.F. (ed.). *Plant Propagation by Tissue Culture*. Part 1 The technology. Second edition. Exegetics Ltd. Edinghon, U. K., p. 42.

George, E.F. and Sherrington, P.D. 1984. *Plant Propagation by Tissue Culture*. Eastern Press Reading, Berks, 171 p.

George, T.E. 1981. Biometrical studies in ashgourd (*Benincasa hispida* (Thunb.) Cogn.). M.Sc.(Hort.) thesis, Kerala Agricultural University, Thrissur,78 p.

Gopalan, C., Ramashastri, B.V. and Balasubramanian, S. C. 1982. *Nutritive value of Indian food.* I.C.M.R, Hydrabad, 328 p.

Griffis, J.L., Sheehan, T.J. and Dennis, B. 1981. Excised floral tissues of coconut palm (*Cocos nucifera* L.) cultured *in vitro*. HortScience, 16: 460-463.

Gross, J. 1991. *Pigments in vegetables: Chlorophylls and Carotenoids*. Van Nostrand Reinhold, New York, 298 p.

Halder, T. and Gadgil, V.V. 1982a. Morphogenesis in some species of the family cucurbitaceae. In: Proc. Symp. on Tissue Culture of Economically Important Plants. (Ed. Rao, A.N.), Singapore: Natl. Univ., pp 98-103

Halder, T. and Gadgil, V.V. 1982b. Shoot bud differentiation in long term callus cultures of *Momordica* and *Cucumis*. Indian J. Expt. Biol., 20: 780-782.

Hanson, C.H., Robinson, H.F. and Comstock, R.E. 1956. Biometrical studies on yield in segregating population of Korean lespedesa. Agron. J., 48: 267-272.

Hazra, P., Ghosh, R. and Nath, S. 2003. Identification of important yield components in pointedgourd (*Trichosanthes dioica* Roxb.). Crop Res. 25: 244-252

Heller, R. 1953. *In vitro* release of axillary shoots from apical dominance in monocotyledonous plantlets. Ann. Bot., 40: 1323-1325.

Hoque, A., Bhomik, M.E. and Khalequzzaman, M. 1998. *In vitro* culture of pointed gourd. Thai J. Agric. Sci., 31: 369.

Hoque, A., Islam, R. and Joarder, O.I. 1995. *In vitro* plantlets differentiation in kakrol (*Momordica dioica* Roxb.) in teasle gourd. Pl. Tiss. Cult., 5: 119-124.

Horax, R., Hettiarachchy, N. and Islam, S. 2005. Total phenolic contents, phenolic acid constituents in 4 varieties of bitter melon (*Momordica charantia*) and antioxidant activities of their extracts. J. Fd. Sci., 70: 275–280.

Hota, D. 2007. *Modern Biotechnology in Plant Breeding*. Gene Tech Books, New Delhi, 262 p.

Huda, A. K. M. N. and Sikdar, B. 2006. *In vitro* plant production through apical meristem culture of bittergourd (*Momordica charantia* L.). Pl. Tiss. Cult. Biotech., 16: 31-36.

IBPGR. 1983. *Genetic resources of Cucurbitaceae*. International Board for Plant Genetic Resources, Rome, 71 p.

Indiresh, B.T. 1982. Studies on genotypic and phenotypic variability in bittergourd (*Momordica charantia* L.). Thesis Abstr., University of Agricultural Science, Bangalore, 8: 52

Islam, R., Sarkar, P.K., Naderuzzaman, A.T.M. and Joarder, O.I. 1994. *In vitro* regeneration of plants from cotyledons of *Momordica charantia* L. Pl. Tiss. Cult., 4: 105-109.

Iswaraprasad, C.M. 2000. Combining ability and heterosis in bittergourd (*Momordica charantia* L.). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 107 p.

*Jaccard, P. 1908. Nouvelles rescherches suv la distribution florale. Bull. Soc. Vandoise des Sciences Neturelles, 44: 223-270.

Jaiswal, R.C., Kumar, S., Raghav, M. and Singh, D.K. 1990. Variation in quality traits of bittergourd (*Momordica charantia L.*) cultivars. Veg. Sci., 17: 186-190.

Jayasree, P.K. 1984. Yellow vein mosaic disease of pumpkin in Kerala. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 77 p.

Jeeva, S. and Pappiah, C.M. 2002. Variability studies in snapmelon. S. Indian Hort., 50: 238-240.

Jelaska, S. 1974. Embryogenesis and organogenesis in pumpkin explants. Physiol. Pl., 31: 157-161.

Jha, T.B. and Ghosh, B. 2007. *Plant Tissue Culture - Basic and Applied*. Universities Press, Hyderabad 206 p.

Jiji, T., Stonehouse, J.M., Senthilkumar, Mumford, J.D. and Verghese, A. 2005. Heights of food bait trap attraction of melon fruit flies in bittergourd in Southern India. Int. J. trop Insect Sci., 25: 1-4.

Jijiamma, N.C. 1989. Nutritional profile of amaranthus as influenced by post harvest handling. MSc. (FS & N) thesis, Kerala Agricultural University, Thrissur, 110 p.

*Johnson, H.W., Robinson, H.F. and Comstock, R.E. 1955. Estimation of genetic and environmental variability in soybeans. Agron. J., 47: 314-318.

Joseph, S. 1999. Evaluation of diploids and polyploids of ivygourd (*Coccinia grandis* (L.) Voigt). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 98 p.

Jureti, B. and Jelaska, S. 1991. Plant development in long-term embryogenic callus lines of *Cucurbita pepo*. Pl. Cell Rep. 9: 623-626.

Kadam, P.Y. and Kale, P.N. 1985. Genetic divergence in ridgegourd (*Luffa acutangula* L. Roxb.). Veg. Sci., 12: 97-104.

Karla, C.L., Berry, S.K. and Kulkarni, S.G. 1998. The bittergourd (*Momordica charantia* L.) - A unique vegetable. Indian Fd. Packer, 42: 35-43.

Kathal, R., Bhatnagar, S.P. and Bhojwani, S.S. 1988. Regeneration of plants from leaf explants of *Cucumis melo* cv. Pusa Sharbati. Pl. Cell Rep., 7: 449–451.

Katiyar, R.S., Mishra, A. and Prasad, A. 1996. Genetics of bittergourd (*Momordica charantia* L.). Indian J. agric. Sci., 66: 551-552.

KAU. 2007. Package of Practices Recommendations – Crops. Directorate of Extension, Kerala Agricultural University, Thrissur, 334 p.

*Kempthorne, O. 1977. *An Introduction to Genetic Statistics*. Jagmander Book Agency, New Delhi, 221 p.

Keng, C.L. and Hoong, L.K. 2005. *In vitro* plantlets regeneration from nodal segements of muskmelon (*Cucumis melo* L.). Biotechnology, 4: 354-357.

Kim, S., Chang, J. Cha, H. C. and Lee, K. 1988. Callus growth and plant regeneration in diverse cultivars of cucumber (*Cucumis sativus* L). Pl. Cell Tiss. Org. Cult., 12: 67-74.

Krawinkel, M.B. and Keding, G.B. 2006. Bitter gourd (*Momordica charantia*): a dietary approach to hyperglycemia. Nutr. Rev., 64: 331–337.

Krug, M.G.Z., Stipp, L.C.L., Rodrignez, A.P.M. and Mendez, B.M.J. 2005. *In vitro* organogenesis in watermelon cotyledons. Pesq. agropec. bras., Brasília, 40: 861-865.

Kumar, S., Singh, M., Ram, D. and Banerjee, M.K. 2002. Biotechnology in vegetables. Abstr. In: International Conference on Vegetables, PNASF, 11-14 November, 2002, Bangalore pp. 61-65

Kumar, S., Singh, M., Singh, A.K., Srivastava, K. and Banerjee, M.K. 2003. *In vitro* propagation of pointed gourd (*Trichosanthes dioica* Roxb.). Cucurbit Genet. Coop. Rep., 26:74-75.

Kumar, S., Singh, R. and Pal, A.K. 2007. Genetic variability, heritability, genetic advance, correlation coefficient and path analysis in bottlegourd. Indian J. Hort., 64: 163-168.

Kumar, S.P. and Sagar, V.R. 2003. Bittergourd not bad at all. Processed Fd. Ind., 6: 23-25.

Kutty, M.S. and Dharmatti, P.R. 2005. Genetic divergence in bittergourd. Karnataka J. Agric. Sci., 18: 740-743.

Lawande, K.E. and Patil, A.V. 1989. Correlation studies in bittergourd. J. Maharashtra agric. Univ., 14: 77-79.

Lawande, K.E. and Patil, A.V. 1991. Studies on gene action in bittergourd (*Momordica charantia* L.). Veg. Sci., 18: 192-199.

Lee, Y.K., Chung, W.I. and Ezura, H. 2003. Efficient plant regeneration *via* organogenesis in winter squash (*Cucurbita maxima* Duch.). Pl. Sci., 164: 413–418.

Lee, W. S., H. Y., Lee, H. J., Kwon, H. S., Hwang, H. A. and Hong, S. Y. 1995. *In vitro* production of plantlets from node culture of oriental melon and muskmelon (*Cucumis melo* L.). J. Korean Soc. hort. Sci., 36: 199-210.

Lee-Huang, S., Huang P.L. and Chen, H.C. 1995. Anti- HIV and anti-tumor activities of recombinant MAP 30 from bitter melon. Gene 161: 151-156.

Leljak-Levanic, D., Bauer, N., Mihaljevic, S. and Jelaska, S. 2004. Somatic embryogenesis in pumpkin (*Cucurbita pepo* L.): control of somatic embryo development by nitrogen compounds. J. Pl. Physiol., 161: 229-36.

Li, J.X., Li, J.W. and Ge, G.M. 2008. *In vitro* culture and plantlet regeneration from cotyledon of *Cucumis sativus* L. J. Changjiang Vegetables, 1: 44-46.

Li, T.S.C. 2008. *Vegetables and Fruits – Nutritional and Therapeutic Values*. CRC Press, New York, 286 p.

Lovely, B. 2001. Evaluation of genetic divergence in ashgourd. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 76 p.

Lundergan, C.A. and Janick, J. 1980. Regulation of apple shoot proliferation and growth *in vitro*. Hort. Res., 20: 19-24.

*Lush, J.L. 1949. *Animal Breeding Plans*. Iowa State University Press, Annes, 473 p.

Maharana, T., Mandal, P., Sahoo, G.S. and Mahapatra, B. 2006. Multivariate analysis of genetic divergence in Kunduru (*Coccinia grandis* (L.) (Voigt). Abstracts. First International Conference on Indigeydnous Vegetables and Legumes, 12 - 15 December 2006, Hyderabad, India.70 p.

Malek, M. A., Bari Miah, A., Al-Amin, M., Khanam, D. and Khatun, M. 2007. *In vitro* regeneration in pointed gourd. Bangladesh J. agric. Res., 32: 461-471.

Mangal, J.L., Dixit, J., Pandita, M.L. and Sidhu, A.S. 1981. Genetic variability and correlation studies in bittergourd (*Momordica charantia* L.). Indian J. Hort., 38: 94-99.

Matakiadis, T. and Kintzios, S. 2005. The effect of ATP on cucumber regeneration from nodal explants: association with α-tocopherol, H_2O_2 and size of culture vessel. Pl. Growth Regul., 45: 127-137.

Mathew, A. 1999. Genetic variability in bottlegourd (*Lagenaria siceraria* (Mol.) Standl.) in relation to yield and yield attributes. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 65 p.

Mathew, A. 2005. Factor analysis of bitterness in *C. melo* var. *conomon*. Mak. Ph.D. (Hort.) thesis, Kerala Agricultural University, Thrissur, 121 p.

Mathew, S.S. and Khader, K.M.A. 1999. Genetic studies in snakegourd (*Trichosanthes anguina* L.). J. trop. Agric., 37: 71-72.

McCue, P., Kwon, Y. and Shetty, K. 2005. Anti-amylase, anti-glucosidase and anti-angiotensin I – converting enzyme potential of selected foods. J. Fd. Biochem., 29: 278-294.

Menon, M.P. 1998. Cataloguing and identification of promising ashgourd ecotypes in relation to season and maturity. M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 105 p.

Mili, J.P., Deka, B.C. and Saikia, L. 2007. Standardization of maturity indices of spine gourd. Acta Hort., 752: 477-480.

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

Miniraj, N., Prasanna, K.P. and Peter, K.V. 1993. Bittergourd (*Momordica charantia L.*.). In: Bergh, B.O. and Kalloo, G. (eds.). *Genetic Improvement of Vegetable Crops*. Pergamon Press, New York, pp. 239-246

Mishra, A.K. and Bhatnagar, S.P. 1995. Direct shoot regeneration from the leaf explant of cucumber (*Cucumis sativus* L.). Phytomorphology, 45: 47-55.

Mohamed-Yasseen, Y., Barringer, S., Schloupt, R.M. and Splittstoesser, W.E. 1995. Activated charcoal in tissue culture: An overview. Pl. Growth Regul. Soc. Am., 23: 206-213.

Molvig, L. and Rose, R.J. 1994. A regeneration protocol for *Spinacia oleracea* using gibberellic acid. Aust. J. Bot., 42: 763 - 769.

Morel, G. 1960. Producing virus free Cymbidiums. Am. Orchid. Soc. Bull. 29: 495-497

Moreno, V., Garcia-Sogo, M., Granell, B., Garcia-Sogo, B. and Roig, L.A. 1985. Plant regeneration from calli of melon (*Cucumis melo* L. cv. Amarillo Oro). Pl. Cell, Tiss. Org. Cult., 5: 139–146.

Morimoto, Y., Maundu, P., Kawase, M., Fujimaki, H. and Morishima, H. 2006. RAPD polymorphism of the white-flowered gourd (*Lagenaria siceraria* Standl.) landraces and its wild relatives in Kenya. Genet. Resour. Crop Evol., 53: 963-974.

Morton, J.F. 1967. The balsam pear – an edible medicinal and toxic plant. Eco. Bot., 212: 57-68.

Murali, B., Babu, K.H. and Reddy, V.P. 1986. Correlation studies in bottlegourd (*Lagenaria siceraria* (Mol.) Standl.). S. Indian Hort., 34: 338-340.

Murashige, T. 1974. Plant propagation through tissue cultures. Ann. Rev. Pl. Physiol., 25: 135-166.

Murashige, T. 1977. Clonal propagation of horticultural crops through tissue culture. Scient. Hort., 9: 238-243

Murashige, T. and Skoog, S. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Pl., 15:473-497.

Murray, M. and Thompson, W. 1980. The isolation of high molecular weight plant DNA. Nucl. Acids Res., 8: 4321-4325.

Mythili, J.B. and P. Thomas. 1999. Micropropagation of *Trichosanthes dioica* Roxb. Sci. Hortic., 79: 87.

Nabi, S.A., Rashid, M.M., Al-Amin, M. and Rasul, M.G. 2002. Organogenesis in teasle gourd (*Momordica dioica* Roxb.). Pl. Tiss. Cult., 12: 173-180.

Nadolska-Orczyk, A. and Malepszy, S. 1984. Cucumber plant regeneration from leaf explants-selected characteristics. Bulletin Pol. Acad. Sci., 32:423-428.

Nagesha, N., Devaiah, B.N., Ramesh, B.N. and Gowda, R.P.H. 2007. *In vitro* micropropagation and transformation of cantoloupe (*Cucumis melo* L.) with GUS gene through *Agrobacterium* mediated transformation. In: Kesavachandra, R. et al. (eds.). Recent Trends in Horticultural Biotechnology New India Publishing Agency, New Delhi, pp. 753-759

Nandakumar, C. 1999. Monitoring and management of the pest complex of bittergourd (*Momordica charantia* L.). Ph.D. thesis, Kerala Agricultural University, Thrissur, 210 p.

Narayan, R., Singh, S.P., Sharma, D.K. and Rastogi, K.B. 1996. Genetic variability and selection parameters in bottlegourd. Indian J. Hort., 53: 53-58.

Narayanan, E.S. and Batra, H.N. 1960. *Fruitflies and their control*. ICAR Monograph, New Delhi, 68p.

Narayanankutty, C., Sunanda, C.K. and Jaikumaran, U. 2004. Genetic divergence in snakegourd (*Trichosanthes anguina* L.). Veg. Sci., 31: 175-176.

Narayanankutty, C., Sunanda, C.K. and Jaikumaran, U. 2006. Genetic variability and character association analysis in snakegourd. Indian J. Hort., 63: 402-406.

Nath, P. 1966. Varietal resistance of gourds to the fruit fly. Indian J. Hort., 23: 69-78.

Newburry, H.J. and Lloyd, B.V.F. 1993. The use of RAPD for assessing variation in plants. Pl. Growth Regul., 12: 43-51.

Niedz, R.P., Smith, S.S., Dunbar, K.B., Stephens, C.T. and Murakishi, H.H. 1989. Factors influencing shoot regeneration from cotyledonary explants of *Cucumis melo*. Pl. Cell Tiss.Org Cult., 18: 313–319.

Padmanabhan, V. 1989. Host resistance studies in bittergourd to the infestation by the fruitfly (*Dacus cucurbutae* Coq.). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 80 p.

Pal, S. P., Alam, I., Anisuzzaman, M., Sarkar, K.K., Sharmin, S. and Alam, A.M.F. 2007. Indirect organogenesis in summer squash (*Cucurbita pepo* L.). Turk. J. Agric., 31: 63-70.

Pala, K. 2001. Intraspecific variation of bitter gourd *Momordica charantia* Linn. by RAPD analysis. M.Sc. (Ag.) thesis, Chulalongkorn University, Thailand, 97 p.

Pandey, S., Rai, M., Ram, D., Singh, B. and Chaubey, P.K. 2003. Component analysis in snapmelon (*Cucumis melo* var. *momordica*). Veg. Sci., 30: 64-67.

Panse, V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers*. Indian Council of Agricultural Research, New Delhi, 347 p.

Paranjape, S.P. and Rajput, J.C. 1995. Association of various characters in bittergourd and direct and indirect effects on yield. J. Maharashtra agric. Univ., 20: 193-195.

Parhi, G., Mishra, H.N. and Mishra, R.S. 1995. Correlation and path-coefficient studies in bittergourd. Indian J. Hort., 52: 132-136.

Parhi, G., Mishra, H.N. and Tripathy, P. 1993. Genetic divergence in bittergourd (*Momordica charantia* L.). S. Indian Hort., 41: 344-349.

Parkash, C., Singh, K.P. and Kalloo, G. 2000. Variability analysis and cause and effect relationship in ashgourd (*Benincasa hispida* (Thunb.) Cogn.). Indian J. Pl. Genet. Resour., 13: 298-301.

Perkin-Elmer Corporation. 1982. *Analytical Methods for Atomic Absorption Spectrophotometry*. Perkin-Elmer Corporation, Norwalk, Connecticut, U.S.A., 176 p.

Peter, K.V. 1998. *Genetics and breeding of vegetables*. Indian Council of Agricultural Research, New Delhi, 333 p.

Prabha, J.R., Padmalatha, T., Sankar, R. and Rao, V.S. 2007. Variability, heritability and genetic advance in ridgegourd (*Luffa acutangula* (Roxb.) L.). The Andhra Agric. J., 54: 204-106.

Prasad, A., Singh, P.D. and Nalini, K.M. 1984. Heritability and genetic advance in *Luffa cylindrica* L. Prog. Hort., 16: 312-315.

Prasad, V.S.R.K. and Singh, D.P. 1989. Studies on heritability, genetic advance and correlation in ridgegourd (*Luffa acutangula* Roxb.). Indian J. Hort., 46: 390-394.

Prasad, V.S.R.K. and Singh, D.P. 1990. Studies on morphological and agronomical components of pointedgourd (*Trichosanthes dioica* Roxb.). Indian J. Hort., 47: 337-340.

Purushothaman, V. 1994. Investigation on mosaic disease of bittergourd. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 110 p.

Radhika, V.S. 1999. Estimation of combining ability and heterosis in snakegourd (*Trichosanthes anguina* L.). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 117 p.

Rahaman, E.H.M.S., Rabbani, M.G. and E.J. Garvey. 2007. Molecular Characterization of sweet gourd using RAPD. Book of Abstract- Conference on Promotion of Biotechnology in Bangladesh- National and International Perspectives. April 6-8, 2007, p. 105

Rahman, M.A., Rabbani, M.G., Ahammed, G.J., Akhter, J. and Garvey, E.J. 2007. Genetic diversity of bittergourd germplasm of Bangladesh. Book of Abstract- Conference on Promotion of Biotechnology in Bangladesh- National and International Perspectives. April 6-8, 2007, p. 100

Rahman, S.M., Hossain, M., Islam, R. and Joarder, O.I. 1993. Plant regeneration from internode segments of *Cucurbita maxima* Duch.× *Cucurbita moschata* Duch. Curr. Sci., 65: 562-564.

Rahman, S.M., Hossain, M., Joarder, O.I., and Islam, R. 1991. Plant regeneration from cotyledons of an interspecific hybrid of *Cucurbita*. Pl. Tiss. Cult., 1: 91-95.

Rai, M., Kumar, S., Pandey S., Singh, M., Ram, D. and Prasanna, H.C. 2005. Promising solanaceous and cucurbitaceous vegetables. Indian Hort., 49: 32-35.

Rajamony, L., More, T.A., Seshadri, V.S. and Varma, A. 1990. Reaction of muskmelon collection to cucumber green mottle mosaic virus. J. Phytopath., 129: 237-244.

Rajan, S. and Markose, B.L. 2005. Bittergourd (Momordica charantia L.). In. Thamburaj, S. and Singh, N. (eds.). *Vegetables, Tuber crops and Spices*. ICAR, New Delhi, pp. 279-285

Rajeswari, K.S. and Natarajan, S. 2002. Genetics and inheritance of yield and its components in bittergourd (*Momordica charantia* L.). S. Indian Hort., 50: 82-90.

Rajput, J.C., Paranjape, S.P. and Jamadagni, B.M. 1995. Correlation and path analysis studies for fruit yield in bittergourd. J. Maharashtra agric. Univ., 20: 377-379.

Rajput, J.C., Paranjape, S.P. and Jamdagni, B.M. 1996. Variability, heritability and scope for improvement for yield components in bittergourd (*Momordica charantia* L.). Ann. Agric. Res., 17: 90-93.

Rakhi, R. 2001. Collection and characterization of landraces of culinary melon (*Cucumis melo*) in Kerala. M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 83 p.

Ram, D., Rai, M., Rai, N., Yadav, D.S., Pandey, S., Lal, V., Singh, S. and Singh, S. 2007. Characterization and evaluation of winter fruited bottlegourd (*Lagenaria siceraria* (Mol.) Standl.). Acta Hort., 752: 231-237.

Ram, D., Rai, M., Singh, H.K., Verma, A., Pandey, S. and Kumar, A. 2006b. Cause and effect analysis of yield in off-season bittergourd (*Momordica charantia* L.). Veg. Sci., 33: 63-64.

Ram, D., Rai, M., Verma, A. and Singh, Y. 2006a. Genetic variability and character association analysis in *Luffa* sp. Indian J. Hort., 63: 294-297.

Ram, H.H., Sharma, K. and Jaiswal, H.R. 2006c. Molecular characterization of promising genotypes in bottlegourd including a novel segmented leaf type through RAPD. Veg. Sci., 33: 1-4.

Ramachandran, C. 1978. Genetic variability, correlation studies and path coefficient analysis in bittergourd (*Momordica charantia* L.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 131 p.

Ramachandran, C. and Gopalakrishnan, P.K. 1979. Correlation and regression studies in bittergourd (*Momordica charantia* L). Agric. Sci., 49: 850-854.

Ramachandran, C. and Gopalakrishnan, P.K. 1980. Variability studies for biochemical traits in bittergourd. Agris. Res. J. Kerala 18: 27

Ramachandran, C., Gopalakrishnan, P.K. and Peter, K.V. 1981. Genetic divergence in bittergourd. Veg. Sci., 7: 100-104.

Raman, A. and Lau, C. 1996. Anti-diabetic properties and phytochemistry of *Momordica charantia* L. (Cucurbitaceae). Phytomedicine 2: 349-362

Rao, B.N. and Rao, P.V. 2008. Sex reversion in ridgegourd (*Luffa acutangula* (Roxb.) L.). Int. J. trop. Agric., 26: 181-184.

Rao, B.N., Rao, P.V. and Reddy, I.P. 2000. Character association and path-coefficient studies in ridgegourd (*Luffa acutangula* (Roxb.) L.). The Andhra Agric. J., 47: 103-107.

*Rao, C.R. 1952. *Advanced Statistical Methods in Biometric Research*. John Wiley and Sons Inc., New York, 390 p.

Rasul, M.G., Hiramatsu, M. and Okubo, H. 2007. Genetic relatedness (diversity) and cultivar identification by randomly amplified polymorphic DNA (RAPD) markers in teasle gourd (*Momordica dioica* Roxb.). Scientia Horticulturae, 111: 271-279.

Razdan, M.K. 2003. *Introduction to Plant Tissue Culture.* Second edition. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 375p.

Reddy, A.N.K., Munshi, A.D. and Behara, T.K. 2007. Diversity in snapmelon genotypes. Indian Hort., 52: 16-17.

Reddy, A.N.K., Munshi, A.D., Behara, T.K. and Kaur, C. 2005. Studies on genetic and biochemical parameters of introduced and indigenous germplasm in snapmelon (*Cucumis melo* L. var. *momordica* Duth.). Indian J. Pl. Genet. Resour., 18: 91-93.

Reddy, B.S., Thammaiah, N., Patil, R.V. and Nandihalli, B.S. 1995. Studies on the performance of bittergourd genotypes. Adv. Agri. Res. India 4: 103-108.

Reddy, K.S.S. and Rao, M.R. 1984. Studies on heritability, genetic advance and character association in ridgegourd. S. Indian Hort., 32: 97-100.

Renjumol, P.V. 2006. Nutritional evaluation and acceptability of ivygourd genotypes (*Coccinia indica* (L.) Voigt.). M.Sc. (FS & N) thesis, Kerala Agricultural University, Thrissur, 86 p.

Resmi, J. 2004. Characterization of landraces of ashgourd (*Benincasa hispida* (Thunb.) Cogn.). M.Sc. (Ag.). thesis, Kerala Agricultural University, Thrissur, 100 p.

Resmi, J. and Sreelathakumary, I. 2006. Variability, heritability and correlation studies in ashgourd (*Benincasa hispida* (Thunb.) Cogn.). Extended Abstract, XVIII Kerala Science Congress, 29-31 January 2006, CESS, Akkulam, Thiruvananthapuram, pp. 138-140

Resmi, J. and Sreelathakumary, I. 2008. Divergence studies in ashgourd (*Benincasa hispida* (Thunb.) Cogn.). Extended Abstract, 20th Kerala Science Congress, 28-31 January 2008, Nalanchira, Thiruvananthapuram, pp. 174-177

Resmi, J. Sreelathakumary, I. and Rajmohan, K. 2007. Detection of genetic variability in ashgourd (*Benincasa hispida* (Thunb.) Cogn.) using RAPD markers. In. Keshavachandran, R., Nazeem, P.A., Girija, D., John, P.S. and Peter, K.V. (eds.). *Recent Trends in Horticultural Biotechnology*. New India Publishing Agency, New Delhi, pp. 571-577

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

Robinson, R.W. and Decker-Walters, D.S., 1999. *Cucurbits*. CAB International, Wallingford, Oxford, UK, 226. p.

Sadasivam, S. and Manickam, A. 2002. *Biochemical Methods*. Second edition. New Age International (P) Ltd., New Delhi, 256 p.

Saha, S. and Kazumi, H. 2007. In vitro micropropagation of bottle gourd (Lagenaria siceraria; Cucurbitaceae): prospective rootstocks for the grafting of watermelon and other cucurbits. Acta Hort., 731:151-158.

Saimbhi, M.S. 1993. Agro-techniques for cucurbits. In: Kalloo, G. and Chadha, K.L. (eds.). *Advances in Horticulture*: 5. Vegetable Crops, Part I Malhotra Publishing House, New Delhi, pp. 401-432

Sankaran, M., Thangarajan, T. and Veeraraghavathatham, D. 2002. Studies on physico-chemical changes associated with maturity of bittergourd (*Momordica charantia* Linn) cv. Co 1. S. Indain Hort., 50: 554-560.

Sanwal, S.K., Yadav, R.K., Rai, N., Yadav, D.S. and Singh, P.K. 2007. Genetic diversity and interrelation analysis in sweetgourd (*Momordica cochinchinensis*) genotypes of northeast India. Veg. Sci., 34: 64-66.

Sanwal, S.K., Yadav, R.K., Singh, P.K. and Rai, N. 2008. Variability and genetic diversity studies in indigenous chow-chow genotypes of northeast India. Indian J. Hort., 65: 167-170.

Sarkar, S.K., Maity, T.K. and Som, M.G. 1999. Correlation and path coefficient studies in pointedgourd (*Trichosanthes dioica* Roxb.). Indian J. Hort., 56: 252-255.

Sarkar, S.K., Maity, T.K., Roy, K. and Som, M.G. 1990. Studies on genetic variability in pointedgourd (*Trichosanthes dioica* Roxb.). Exp. Genet., 6: 68-73.

Sarnaik, D.A., Verma, S.K. and Sharma, G.L. 1999. Character association in ivygourd (*Coccinia grandis*). A. agric. Res., 20: 436-438.

Sarowar, S., Oh, H.Y., Hyung, B.W., Harn, C.H., Yang, S.K., Ok, S.H. and Shin, J.S. 2003. *In vitro* micropropagation of a *Cucurbita* interspecific hybrid cultivar – a root stock plant. Pl. Cell Tiss. Org. Cult., 75: 179-182.

Satpathy, S., Rai, S., Shivalingaswamy, T.M., Stonehouse, J.M., Mumford, J.D. and Verghese, A. 2005. Resistance and susceptibility of bittergourd varieties to melon fly in eastern Uttar Pradesh. Pest Mgmt. hort. Ecosystems, 11: 162.

Satyakala, G., Rao, M.M. and Lakshmisita, G. 1995. *In vitro* micropropagation of scented geranium. Curr. Sci., 68: 762-765.

Schenk, R.V. and Hildebrant, A.C. 1972. Techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot., 50: 199-204.

Selvaraj, N., Vasudevan, A., Manickavasagam, M., Kasthurirengan, S. and Ganapathi, A. 2007. High frequency shoot regeneration from cotyledon explants of cucumber *via* organogenesis. Scientia Horticulturae, 112: 2-8.

Seshadri, V.S. 1993. Cucurbits. In. Bose, T.K., Som, M.G. and Kabir, J. (eds.). *Vegetable Crops*. Second edition. Naya Prokash, Calcutta, pp. 40-124

Sheo,Y.K., Paek, K.Y., Hwang, J.K. and Cho, Y.H. 1991. Micropropagation using shoot tips in pumpkin. J. Korean. Soc. Hort. Sci., 32: 137-145.

Shrivastava, V.K. and Srivastava, L.C. 1976. Genetic parameter, correlation and path coefficient analysis in bittergourd (*Momordica charantia* L.). Indian J. Hort., 33: 66-70.

Sikdar, B., Shafiullah, M., Chowdhury,A.R., Sharmin, N., Nahar, S. and Joarder, O.I. 2005. *Agrobacterium*-mediated *GUS* Expression in bittergourd (*Momordica charantia* L.). Biotechnology, 4: 149-152.

Sikdar, B., Sharmin, N. and Chowdhury, A. R. 2003. Direct *in vitro* regeneration of bittergourd (*Momordica charantia* L.). Bangladesh J. Genet. Biotechnol., 4: 67-70.

*Silbernagel, M.J. and Jafri, A.M. 1974. Temperature effects on curly top resistance in *Phaseolus vulgaris*. Phytopathology 64: 825-827

Skirvin, R.M. 1981. Fruit Crops. In: Conger, B.V. (ed.). *Cloning Agricultural Plants via In Vitro Techniques*. CRC Press, Boca Raton, pp. 51-139

Singh, D.K., Singh, R.D. and Singh, M. 2000. Genetic variability in spongegourd (*Luffa aegyptiaca* L.). New Agriculturist, 11: 51-54.

Singh, H.N., Srivastava, J.P. and Prasad, R. 1977. Genetic variability and correlation studies in bittergourd. Indian J. Agric. Sci., 47: 406-407.

Singh, M.N., Kathal, R. and Bhatnagar, S.P. 1990. Regeneration of plants from hypocotyl and cotyledon cultures of *Cucumis melo* cv Pusa Maduras. Phytomorphology, 40: 401-405.

Singh, N.K. and Singh, R.K. 1988. Correlation and path coefficient analysis in watermelon (*Citrullus lanatus* Thunb. Mansf.). Veg. Sci., 15: 95-100.

Singh, R.K. 2004. Correlation and path coefficient studies in chow-chow (*Sechium edule*). Indian J. Hort., 61: 154-157.

Singh, R.K. and Choudhary, B.D. 1985. *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publishers, New Delhi, 300 p.

Singh, R.R., Mishra, G.M. and Jha, R.N. 1987. Inter relationship between yield and its components in parwal. S. Indian Hort., 35: 245-246.

Singh, R.S. 1992. *Diseases of Vegetable Crops*. Second edition. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 362 p.

Singh, V.P., Singh, K. and Jaiswal, R.C. 1986. Geneitc variability and correlation studies in pointedgourd (*Trichosanthes dioica* Roxb.). Narendra Deva J. agric. Res., 1: 120-124.

Sisodiya, D.B., Jhala, R.C., Stonehouse, J.M., Verghese, A. and Mumford, J.D. 2005. Horizontal and vertical distribution of melon fly in gourd fields in central Gujarat. Pest Mgmt. hort. Ecosystems, 11: 163.

Smith, F.H. 1936. A discriminant function for plant selection. Ann. Eugen. 7: 240-250

Spestidis, N., Sapountzakia, G. and Tsaftaris, A.S. 1996. Micropropagation of the melon hybrid 'Galia'. Cucurbit Genet. Coop. Rep., 19: 63-65.

Sreejayan, M. and Rao, M. A. 1991. Oxygen-free radical scavenging activity of *Momordica charantia* fruits. Fitoterpeda, 62: 344-346.

Srivastava, D.R., Andrianov,V.M. and Piruzian, E.S. 1989. Tissue culture and plant regeneration of watermelon (*Citrullus vulgaris* Schrad. cv. Melitopolski). Pl.Cell Rep., 8: 300-302.

Srivastava, K.P. and Butani, D.K. 1998. *Pest Management in Vegetables*. Research Periodicals and Book Publishing House, New Delhi, 294p.

Srivastava, R.P. and Kumar, S. 1998. *Fruit and Vegetable Preservation-Principles and Practices*. Second Edition. International Book Distributing Co., Lucknow, 444 p.

Staub, J.E., Danin-Poleg, Y., Fazio, G., Horejsi, T., Reis, N. and Katzir, N. 2000. Comparative analysis of cultivated melon groups (*Cucumis melo* L.) using random amplified polymorphic DNA and simple sequence repeats markers. Euphytica, 115: 225-241.

Sultana, R.S. and Bari Miah, A. 2003. *In vitro* propagation of Karalla (*Momordica charantia* Linn.). J. Biol. Sci., 3:1132-1139.

Sultana, R.S., Bari Miah, A., Rahman, M.M., Rahman, M.H. and Mollah, M.U. 2005. Aseptic multiplication and maintenance of bittergourd (*Momordica charantia* Linn.) as affected by sucrose, agar and pH. J. Biol. Sci., 5: 781-785.

Sultana, R.S., Bari Miah, A., Rahman, M.M., Rahman, N.A., Siddique, N.A. and Khatum, N. 2004. *In vitro* rapid regeneration of plantlets from leaf explants of watermelon (*Citrullus lanatus* Thumb.). Biotechnology, 3: 131-135.

Sureja, A.K., Sirohi, P.S., Behera, T.K. and Mohapatra, T. 2006. Molecular diversity, its relationship with hybrid performance and heterosis in ashgourd (*Benincasa hispida*). J. Hort. Sci. Biotech., 81: 33-38.

Suresh, S. 2004. Molecular characterization of ivygourd (*Coccinia grandis* (L.) Voigt). M.Sc. (Ag.). thesis, Kerala Agricultural University, Thrissur, 92 p.

Tailamy, D. W. 1985. Squash beetle feeding behavior: an adaptation against induced cucurbit defenses. Ecology, 66: 1574-1579.

Takemoto, D.J. 1982. Guanylate cyclase activity in human leukamic and normal lymphocytes, enzyme inhibition and cytotoxic toxic activity of plant extracts. Enzyme, 27: 179-188.

Tee, E.S., Ismail, M.N., Nasir, M.A. and Khatijah, I. 1997. *Nutrient composition of Malaysian Foods*. 4th Edition. Malaysian Food Composition Database Programme, Institute for Medical Research, Kuala Lumpur, 367 p.

Thakur, J.C., Khatra, A.S. and Brar, K.S. 1994. Genetic variability and heritability for quantitative traits and fruit fly infestation in bittergourd. Punjab agric. Univ. J. Res., 31: 161-166.

Thomas, T.D. and Sreejesh, K.R. 2004. Callus induction and plant regeneration from cotyledonary explants of ash gourd (*Benincasa hispida* L.). Sci. Hortic., 100: 359-367.

Thuy, L., Franke, V., Custer, A.A., and Murphy, L.J. 2006. *Momordica cochinchinensis* spreng. fruit carotenoids reevaluated. J. Fd. Composition Analysis, 19: 664-668.

Trulson, A.J. and Shahin, E.A. 1986. *In vitro* plant regeneration in the genus *Cucumis*. Pl. Sci., 47: 35–43.

Uddin, S. 2000. *In vitro* propagation of pointed (*Trichosanthes dioica* Roxb.). M. Sc. thesis. BSMRAU, Salna Gazipur 92 p.

Vahab, M.A. and Gopalakrishnan, P.K. 1993. Genetic divergence in bittergourd (*Momordica charantia* L.). S. Indian Hort., 41: 232-234.

Vahab, M.A. 1989. Homeostatic analysis of components of genetic variance and inheritance of fruit colour, fruit shape and bitterness in bittergourd (*Momordica charantia* L.). Ph.D. thesis, Kerala Agricultural University, Thrissur, 148 p.

Varalakshmi, B. and Reddy, Y.N. 1994. Correlation and path analysis studies in ridgegourd. Indian J. Hort., 51: 85-89.

Varalakshmi, B., Rao, P.V. and Reddy, Y.N. 1995. Genetic variability and heritability in ridgegourd (*Luffa acutangula*). Indian J. agric. Sci., 65: 608-610.

Varghese, P. and Rajan, S. 1993. Genetic variability and heritability studies in snakegourd (*Trichosanthes anguina* L.). J. trop. Agric., 31: 13-17.

Varghese, P. 1991. Heterosis in snakegourd (*Trichosanthes anguina* L.). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 106 p.

Varghese, S. 2003. Genetic analysis in ivygourd (*Coccinia grandis* (L.) Voigt.). Ph.D. thesis, Kerala Agricultural University, Thrissur, 112 p.

Vasil, V. and Vasil, I.K. 1980. Isolation and culture of cereal protoplasts. II. Embryogenesis and plantlet formation from protoplasts of *Pennisetum americanum*. Theor. appl. Genet., 56: 97-99.

Vasudevan, A., Selvaraj, N., Ganapathi, A., Choi, C.W., Manickavasagam, M. and Kasthurirengan, S. 2007. Direct plant regeneration from cucumber embryonal axis. Biologia Plantarum, 51: 521-524.

Veenakumari, D., Raju, V.K. and Devdas, V.S. 1994. Yield, quality and shelflife of bittergourd (*Momordica charantia* L.). J. trop. Agric., 32: 126-128.

Verma, V.K., Behera, T.K., Munshi, A.D., Swarup, K.P. and Mohapatra, T. 2007. Genetic diversity of ashgourd [*Benincasa hispida* (Thunb.) Cogn.] inbred lines based on RAPD and ISSR markers and their hybrid performance. Scientia Horticulturae, 113: 231–237.

Vijayaraghavan, V., Natarajan, S., Pugalendhi, L. and Anand, M. 2006. Influence of maturity stage on physico-chemical characters of bittergourd. National seminar on ASTH, Annamalai University, March 20-21, 2006, 197 p.

Wang, P.J. and Charles, A. 1990. *Biotechnology in Agriculture*. Springer-Verlag Press, New York, 350p.

Wang, P.J. and Hu, C.Y. 1980. Regeneration of virus free plants through *in vitro* culture. HortScience, 12: 437-445.

Wethrell, D.F. 1984. Enhanced adventive embryogenesis resulting from plasmolysis of cultured wild carrrot cells. Pl. Cell Tiss. Org. Cult., 3: 221-227.

White, P.R. 1943. Nutrient deficiency studies and improved inorganic nutrient for cultivation of excised tomato root. Growth, 7: 53-65.

WHO. 2002. *Quality Control Methods for Medicinal Plant Materials*. A. I. T. B.S Publishers and Distributors, New Delhi, 115 p.

Williams, J.T. and Ng, N.0. 1976. Variation within *Momordica charantia* L., the bittergourd (Cucurbitaceae). Ann. Bogorienses, 6: 111-123.

*Wright, S. 1954. The interpretation of multivariate systems. In: Kempthorne, O., Bancroft, T.A., Gowen, J.W. and Lush, J.L. (eds.). *Statistics and Mathematics in Biology*, State University Press, Iowa, pp. 11-13

Xue, Y., Song, S.H., Chen, Y., Rubatzky, V.E., Hang, C. and Peron, J.Y. 1998. Possible anti-tumor promoting properties of bittergourd and some Chinese vegetables. Acta Hort.467: 55-64.

Yadav, M., Choudhary, R., Chandra, A., and Mehta, A.K. 2004. Genetic variability of different genotypes of bittergourd (*Momordica charantia* L.). The Allahabad Farmer, 2: 70-76.

Yadav, M., Singh, D.B., Choudhary, R. and Singh, D. 2008. Genetic variability in bittergourd (*Momordica charantia* L.). J. Hort. Sci., 3: 35-38.

Yasuda, M., Iwamoto, M., Okabe, H. and Yamauchi, T. 1984. Structures of Momordicines I, II, and III, the bitter principle in the leaves and vines of *Momordica charantia* L. Chem. Pharm. Bull., 32.2044-2047.

Yasui, H., Kato, A. and Yazawa, M. 1998. Antifeedants to armyworms, *Spodoptera litura* and *Pseudaletia separata*, from bittergourd leaves, *Momordica charantia.* J. Chem. Eco., 24: 803-813.

Yasui, Y., Hosokawa, M., Sahara, T., Suzuki, R., Ohgiya, S. and Kohno, H. 2005. Bittergourd seed fatty acid rich in 9c, 11t, 13t-conjugated linolenic acid induces apoptosis and up-regulates the GADD45, p53 and PPARγ in human colon cancer Caco-2 cells. Essential Fatty Acids 73: 113–119.

Zeven, A.C. and Zhukovsky, P.M. 1975. *Dictionary of cultivated plants and their centres of diversity*, Wageningen, 575 p.

Zhu, Z.J., Zhong, Z.C., Luo, Z.Y. and Xiao, Z.Y. 1990. Studies on the active constituents of *Momordica charantia* L. Acta Pharm. Sinica, 25: 898–903.

Zong, R., Morris, L. and Cantwell, M. 1995. Postharvest physiology and quality of bitter melon (*Momordica charantia* L.). Postharvest Biol. Tech., 6: 65-72.

http://www.iivr.org.in/2005

*Original not seen

APPENDICES

APPENDIX-1

Score card for the organoleptic evaluation of cooked bittergourd fruits

Sl. No.	Quality attributes	Subdivisions of attributes	Score of each attribute	Treatments			
				1	2	3	4
1.	Appearance/ Colour	Natural colour well preserved	4				
		Slightly discoloured	3				
		Moderately discoloured	2				
		Highly discoloured	1				
2.	Doneness	Highly acceptable	4				
		Moderately acceptable	3				
		Slightly acceptable	2				
		Least acceptable	1				
3.	Flavour	Very pleasant	4				
		Pleasant	3				
		Moderately pleasant	2				
		Unpleasant	1				
4.	Taste	Very good	4				
		Good	3				
		Fair	2				
		Poor	1				
5.	Bitterness	No bitterness	4				
		Slight bitterness	3				
		Moderate bitterness	2				
		High bitterness	1				

APPENDIX-1I

Composition of MS basal media tried for *in vitro* culture of bittergourd genotype MC 4

Nutrients	Quantity (mg l^{-1})
Macronutrients	
NH_4NO_3	1650.00
$(NH_4)_2SO_4$	-
$NH_4H_2PO_4$	-
KNO_3	1900.00
KH_2PO_4	170.00
$MgSO_4.7H_2O$	370.00
$Ca(NO_3)4H_2O$	-
$NaH_2PO_4.H_2O$	-
$CaCl_2.\ 2H_2O$	440.00
Micronutrients	
H_3BO_3	6.20
$MnSO_4.4H_2O$	22.30
$ZnSO_4.7H_2O$	8.60
KI	0.83
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	27.85
$FeSO_4.7H_2O$	37.25
Vitamins	
Thiamine, HCl	0.10
Pyridoxine, HCl	0.50
Nicotinic acid	0.50
Aminoacid	2.00
Glycine	-
Others	
Inositol	100.00
Sucrose*	30.00
Agar*	6.30

*g l^{-1}

ABSTRACT

CHARACTERIZATION AND *IN VITRO* MULTIPLICATION OF BITTERGOURD (*Momordica charantia* L.) GENOTYPES

RESMI J.

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

2009

Department of Olericulture
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM 695522

ABSTRACT

The research project "Characterization and *in vitro* multiplication of bittergourd (*Momordica charantia* L.) genotypes" was carried out at the Department of Olericulture, the Department of Plant Biotechnology and the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani during 2005-2008. The objective of the study was to catalogue the genotypes based on the IBPGR descriptor for cucurbits, to estimate the genetic parameters for different traits in the germplasm for identifying superior lines based on yield, quality, pest and disease resistance, to document the biochemical traits, to characterize the genotypes using molecular techniques (RAPD analysis) and to standardize the *in vitro* techniques for bittergourd.

Thirty three genotypes of *Momordica charantia* collected from various sources upon cataloguing pointed out wide variation for several morphological characters. Analysis of variance revealed significant differences among the genotypes for all the characters except fruit fly infestation (%) studied namely, days to seedling emergence, vine length, internodal length, number of primary branches, number of secondary branches, days to first male flower, days to first female flower, node to first male flower, node to first female flower, sex ratio, days to first fruit harvest, fruit length, fruit girth, fruits per plant, average fruit weight, yield per plant, seeds per fruit, 100-seed weight and mosaic incidence.

Among the genotypes, MC 20 (Priyanka, KAU) recorded the maximum yield (5.89 kg), average fruit weight (578.75 g), fruit length (38.83 cm) and fruit girth (25.53 cm). MC 1 was the longest in vine length (620.00 cm). MC 33 (1.25 cm) was shortest in internodal length along with least vulnerability index for mosaic (10.00). MC 10 was characterized by maximum fruits per plant (34.25). MC 16 was the earliest to flower (36.00 days for female flowering) while, MC 32 was the earliest to harvest (43.50). High phenotypic and genotypic coefficients of variation were observed for yield per plant and average fruit weight.

High heritability coupled with low genetic advance was observed for most of the characters. High heritability coupled with high genetic advance was observed for vine length and average fruit weight.

Correlation studies and path coefficient analysis revealed that average fruit weight and number of fruits per plant are the primary yield components as evidenced from its high positive correlation as well as direct and indirect effects on yield.

In the discriminant function analysis, the genotype MC 20 (Priyanka, KAU) ranked first, followed by MC 10 (MDU-1, TNAU), MC 26 (Thripunithara, Ernakulam), MC 22 (Chathamangalam, Kozhikode) and MC 27 (Charuplasseri, Palakkad). They were found to be promising based on their superiority in yield, fruit quality, earliness in male and female flowering, narrow sex ratio, fruit fly infestation and mosaic resistance and hence they may be utilized for further crop improvement.

Based on the analysis for genetic divergence, the 33 genotypes of *M. charantia* were grouped into five clusters, with the highest intercluster distance observed between clusters III and IV.

The keeping quality studies indicated that maximum shelf life, lower percentage loss in weight, lowest rotting percentage and slower change in colour was obtained from fruits of the genotype MC 25.

The organoleptic quality evaluation revealed that all the genotypes differ significantly for attributes like appearance / colour, doneness, flavor, taste and bitterness. In general, fruits with light green skin colour had overall acceptability and were superior to others based on the score.

The biochemical characterization revealed that the medium sized fruits were associated with higher β-carotene while, small sized fruits found to have higher iron content and large fruited genotypes contained higher ascorbic acid content. Genotypes with higher the chlorophyll content, greater will be the bitterness value. A distinct difference in the content of vitamins and minerals is observed in different genotypes. However, correlation between colour of fruits and their iron content is found in some of the genotypes.

The DNA isolated from the 33 genotypes of *M. charantia* were subjected to RAPD analysis. Out of the 80 decamer primers, fifty six yielded amplification products. A total of 158 RAPDs (average 1.98 bands per primer) were generated by the 56 primers, of which 86.08 per cent were polymorphic (136 bands) and twenty two were monomorphic. Eight primers *viz.*, 0PA-02, 0PA-18, OPB-01, OPB-06, OPB-12, OPE-14, UBC-03 and UBC-05 showed high level of polymorphism and were informative enough to analyse the genotypes The selected primers yielded 56 scorable bands (average of 7.00 bands per primer) of which 23 were monomorphic and rest, 33 were polymorphic (58.93 %). The overall Jaccard's similarity coefficients ranged from 0.65 to 0.90. Cluster analysis revealed that at about 0.65 similarity coefficient, the thirty three genotypes of *M. charantia* grouped into two clusters which clearly separates genotypes based on average fruit weight and fruit skin colour.

By characterizing all the thirty three genotypes of *Momordica charantia* using morphological (selection index and D^2 analysis) and molecular (RAPD marker analysis) methods revealed that morphologically distinct and superior lines were genetically differentiable. The clustering pattern based on yield related traits and molecular variation was different. Also, the diversity based on yield related traits and molecular analysis was not in consonance with ecological distribution.

The induction of potentially organogenic callus and better proliferation of callus successfully from all the explants was obtained either on the MS medium supplemented with IBA (4.00 mg l^{-1}) + BA (0.50 mg l^{-1}) + 2,4- D (2.00 mg l^{-1}) or NAA (2.00 mg l^{-1}) + BA (0.50 mg l^{-1}) + 2,4- D (2.00 mg l^{-1}) in combination with 30.00 g l^{-1} sucrose and 6.30 g l^{-1} agar level. The shoot tip explants were more responsive among all the explants tried in terms of callus induction and subsequent plant regeneration. Shoot regeneration response from shoot tip derived callus was best on MS medium fortified with 0.05 % AC + 1.00 mg l^{-1} BA with earlier regeneration, highest regeneration percentage, lengthy adventitious shoot and more number of leaves under light conditions.