CHARACTERISATION OF LANDRACES OF DRUMSTICK (*Moringa oleifera* Lam.)

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

Master of Science in Horticulture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2004

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DECLARATION

I hereby declare that this thesis entitled "Characterisation of landraces of drumstick (*Moringa oleifera* Lam.)" 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.

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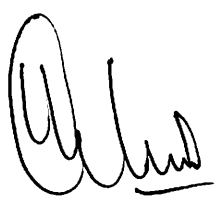
CERTIFICATE

Certified that this thesis entitled "Characterisation of landraces of drumstick (*Moringa oleifera* Lam.)" is a record of research work done independently by Ms. Resmi.D.S 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.



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Dedicated to

My Family

ACKNOWLEDGEMENT

"Do your duty dedicating all works to me in a spiritual frame of mind, free from desire, attachment and mental grief"

The above lines have always inspired me in times of need. The successful completion of the work is due to His grace and blessing. My pranams to our Supreme Benefactor.

Dr.V.A.Celine, Associate Professor, Department of Olericulture and Chairman of the Advisory Committee with her valuable guidance and suggestions has contributed much to my work. I express my heartfelt gratitude and indebtedness to her for her proper guidance and always lending a patient ear to my queries.

The same is also to be said of my Advisory Committee member, Dr. L. Rajamony who has always taken a special interest in my work and queries and ensured that my work progressed in the right direction.

Dr. I. Sreelathakumary has provided ample inspiration and guidance for successful completion of my work as Advisory Committee member.

And last but certainly not the least of my Advisory Committee members, Dr.K.B.Soni with her expertise in molecular biology has always been there to provide guidance and direction to my work.

My heartfelt thanks and gratitude to all my Advisory Committee members for sharing their time and knowledge with me.

I owe my thanks to Dr. Abdul Vahab, who has always encouraged me in my research.

Dr.V.K.Girija, Department of Plant Pathology, with her expertise in pathogen identification has played a pivotal role in completion of my research.

Dr. N. Anitha, Department of Entomology, has provided valuable suggestions and recommendations on pest control and identification of major pests. My sincere gratitude and thanks to her for enlightening me on areas I needed guidance on.

I am indebted to all the staff of the Department of Plant Physiology who permitted me to use their lab facilities.

My friends, Resmi and Suresh have always provided me with the right mix of moral support and encouragement in times of self-doubt. Also special mentions is to be made of my friends Krishnapriya, Neema, Juliya, Sheena, Palani, Manuel, and all my friends of the '96 batch who have contributed in their own unique ways. My heart felt thanks to Pradeep, Satheesh and Anitha chechi for being with me throughout my research . I would like to thank Simi chechi, Robi, Manju chechi and Geetha chechi for their advices and help during my research.

My thanks to Achan, Amma and Sandy for standing by me through the whole course of study.

Also my sincere gratitude to my Amma, Sooraj and Kavitha for their prayers and moral support.

Special thanks to my husband, Sajai for his unconditional help in preparing the thesis and for supporting me throughout my studies.

And finally a special thanks to my son, Ramu for his much enjoyed but most unexpected interruptions to my work.

Resmi D. S.

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LIST OF ABBREVIATIONS

%	per cent
µl	microlitre
µM	micromolar
A.O.A.C	Association of Official Agricultural Chemists
cm	Centimetre
et al.	And others
FIB	Farm Information Bureau
Fig.	Figure
g	Gram
h	Hour
i.e.,	That is
I.U	International Unit
KAU	Kerala Agricultural University
min	minutes
ml	millilitre
ng	Nanogram
No.	Number
^{o}C	Degree Celsius
PCR	Polymerase chain reaction
PVP	Poly vinyl pyrrollidone
RAPD	Random Amplifying Polymorphic DNA
rpm	Revolutions per minute
s	seconds
SCAR	Sequence Characterised Amplified Region
SDS	Sodium dodecyl sulphate
SSR	Symbol sequence repeats
UPGMA	Unweighted Pair Group Method with the Arithmetic Means
viz.	Namely

Introduction

1. INTRODUCTION

Drumstick (*Moringa oleifera* Lam.), is a multipurpose tree vegetable belonging to the family Moringaceae. The tree is native to the Sub Himalayan regions of North India and is also known by other names as 'Horse Radish Tree', 'West Indian Ben', and 'Never Die'. In India, it is grown all over for its highly nutritious pods, leaves, and flowers. They are rich sources of proteins, vitamins and minerals (Rajkumar *et al*., 1973). Drumstick leaves contain protein 6.7 g, vitamin A 11, 300 I. U., vitamin C 220 mg, Fe 7 mg and Ca 440 mg per 100 g. The pods of drumstick play an important role in South Indian cuisine and are much valued for their distinctly inviting flavour. Moreover, the pods are processed to various products such as dehydrated powder, drumstick oil, drumstick pickle, canned drumstick etc. and are exported.

Ancient Indian literature makes mention about drumstick as a miracle plant due to its widespread applications in agriculture, medicine and industry. Almost all parts of the tree like root, bark, stem, leaf, flower, and pods have medicinal values. The two alkaloids, namely moringine and moringinine present in the tree, are being responsible for many of the medicinal properties of the tree (Peter, 1979). The seeds contain 38-40 % ben oil which is used as a lubricant and also in cosmetics. The press cake remaining after oil extraction is used as manure (Prabhakar *et al*., 2003). The seeds are widely used for water purification (Manickam and Ghosh, 1986). The bark fibre is used for making mats, paper and cordage (Verma *et al*., 1976).

Though drumstick is not cultivated as a commercial crop in Kerala, it is grown in most of the homesteads. The total area is estimated as 19632 ha with a production of 20825 t (F. I. B. 2003).

In spite of its nutritional, economic and medicinal importance, the crop still remains under exploited. Kerala, being a state with tremendous variability, it

is high time to collect and characterise the available genetic wealth of drumstick for further utilization in crop improvement programmes.

Conventionally, characterisation is done based on morphological and agronomic traits. But these methods are susceptible to environmental conditions. The emergence of molecular marker techniques aids to characterise the genetic differences at the molecular level and hence provide a more accurate characterisation. The most commonly used molecular technique; the Random Amplified Polymorphic DNA (RAPD) is quick, reliable and widely applicable. RAPD markers have been used for finger printing genomes (Welsh and Mc Clelland, 1990), population biology studies (Astley, 1992), mapping of genetic traits, identification of genome specific markers, gene flow studies (Graham *et al*., 1997), heterozygosity studies (Lanham, 1996) and other uses (Williams *et al*., 1990). Several authors have applied the RAPD technique to investigate genetic variability and found the technique very efficient and reliable (Brown *et al.*, 1993).

Considering the importance of the above facts, the present study was conducted with the following objectives:

- To collect and characterise the available landraces of drumstick in the southern regions of Kerala using morphological characters and RAPD markers.
- To identify superior genotypes.
- To establish and maintain the identified genotypes as a part of germplasm conservation and management.

Review of Literature

2. REVIEW OF LITERATURE

Drumstick (*Moringa oleifera* Lam.) is a popular "Tree vegetable" grown widely in India. Though much variability is noted in this crop, very little effort has been carried out to characterise the available genetic material. The present study was done to analyse the extent of genetic variability in this crop, so as to utilize them for crop improvement.

This review attempts to survey at length the morphological and molecular characterisation studies done in drumstick and other perennial horticultural crops and to classify them under appropriate headings as the literature on drumstick especially molecular characterisation is very meagre.

2.1 TAXONOMIC STUDIES

Drumstick belongs to the family Moringaceae and genus *Moringa*. Verdcourt (1985) developed a taxonomic key for the genus *Moringa* and classified it into 14 species. Edwards *et al*. (2000) stated that the taxonomic position of the family Moringaceae is not yet clear and although it has some features similar to those of Brassicaceae and Capparidaceae, the seed structure does not agree with either of the above families. According to Olson (2002) *Moringaceae* consists of 13 species of trees and shrubs and the inter-generic studies within Caricaceae -Moringaceae clade showed that Caricaceae and Moringaceae are sister taxa. The 13 species are *M. drouhardii, M. hildebrantii, M. ovalifolia, M. stenopetala, M. concanenesis, M. oleifera, M. peregrina, M. arborea, M. borziana, M. longituba, M. pygmaea, M. rivae, M. ruspoliana.*

2.2. VARIABILITY STUDIES

2.2.1 Vegetative Characters

The *Moringa* types were broadly classified into two groups based on the branching habit of the tree and also into three groups based on the pigmentation of

the floral parts and fruits (Muthusamy, 1954; Seemanthini, 1964; Irulappan, 1991). Mohideen and Shanmugavelu (1982) found that the tree height ranged from 2.2-7.8 m and the number of branches varied from 2-23.

Sadasakthi (1997) studied the extent of genetic variability in annual drumstick and observed that the tree height variation was high among the hybrids (246.6 - 445.8 cm). The number of primary branches ranged from 5.2-13.4 in parents and in hybrids it ranged from 6.8-14.6. Olson and Carlquist (2001) studied the anatomical correlations with life form diversity, ecology and systematics in *Moringa* species and identified 4 habits, *i.e.* bottle trees, sarcorhizal trees, slender trees and tuberous shrubs. Vijayakumar *et al.* (2002) reported that maximum tree height was noticed in March sowing (4.66m) followed by January sowing (4.36m) and the least height was seen in September sowing (2.84m) in annual drumstick. The number of primary branches was recorded highest in March sowing (27.7) followed by January sowing (26.4) and the lowest level in September sowing (20.1).

2.2.2 Leaf Characters

Olson (2002) observed that seedlings of most species of *Moringa* have palmate leaves. The three Asian species, *M. concanensis* Nimmo., *M. oleifera* Lam., and *M. peregrina* Forssk. have pinnate seedling leaves. The early leaves of some species of *Moringa* are entire and bear traces of palmate venation. The two *Moringa* species, *M. drouhardii* and *M. hildebrantii* have irregular leaf margins while other *Moringa* species have entire margins.

2.2.3 Flowering Habit and Flower Characters

Drumstick is considered as an evergreen tree in India. Flowering in drumstick varies depending on environmental or genetic factors. Generally two flowering seasons are observed in South India, whereas only one flowering has been noticed under North Indian conditions.

Muthusamy (1954) reported two main flowering seasons *viz.,* March- April and July- September under Coimbatore conditions. Ramachandran *et al*. (1980) reported that 'Chemmurunga' flowered throughout the year with heavy yield in South India. In Bangalore, flowering was observed in two seasons *ie.* March - May and July - September (Devar *et al.*, 1981). Mohideen and Shanmugavelu (1983) reported that annual drumstick had precocious flowering and bearing tendencies. Jyothi *et al.* (1990) reported that in Visakhapatanam, drumstick flowered twice a year, February to May and September to November. Annual drumstick had early flowering habit commencing from August-September with a peak period of blossoming during December -January in Thrissur. Even though it flowered early, fruit set occurred only in December-January blooms (Babu and Rajan, 1996). Veeraragavathatham *et al.* (1998) evaluated the local drumstick plants available in Tamil Nadu and identified two annual types, KM 1 and PKM 1 based on the flowering habit. Studies conducted at College of Horticulture, Vellanikkara revealed that most of the perennial drumstick clones flowered late in the season (KAU, 1999). Mathew (2002) studied the floral biology of 60 drumstick plants at Vellayani and two flowering peaks, July-August and February- March were observed.

There was only one flowering during February-March in Lucknow and Punjab (Singh, 1962; Nair and Singh, 1974). Indira and Peter (1988) reported that flowering and fruiting occurred in February under North Indian conditions. Pushpangathan *et al*. (1996) reported that in N-E part of India, drumstick flowered during February and gave mature fruits during March - April.

Ochse (1977) observed that in West Indies there were drumstick types which rarely flower and were principally cultivated for their foliage. Duke (1978) reported that drumstick thrived well in tropical and subtropical climate with free and continous flowering and fruiting.

Suthanthirapandian *et al.* (1989) recorded the number of flowers per panicle in annual moringa and it ranged from 19-126. Sadasakthi (1997) reported

that the number of flowers per panicle ranged from 75.9- 99.87 in parents and in hybrids it ranged from 82.37-100.10 in annual drumstick in a study involving parents and 24 hybrids.

2.2.4 Fruit Characters

Mc Cann (1966) observed a variety with nearly cylindrical wavy fruits and was considered superior for economic purposes. Jaffna is an introduced variety from Sri Lanka seen in some parts of Madras state, which bears long pods of 2-3 feet with soft flesh of good taste. Chavakacheri Moringa, also a Jaffna type bears fruits as long as 90-120 cm and Chemmuringa produces red tipped fruits. Another variety known as Palmurungai with thick pulp, bitter taste and another called Punamurungai are also noted. Kodikalmurungai is another variety in Tamil Nadu, which produces very short pods. Kadumurungai is a wild type producing small inferior quality pods (Muthusamy, 1954; Seemanthini, 1964; Sundararaj *et al*., 1970; Gopalakrishnan, 1978; Ramachandran *et al*., 1980).

Mohideen and Shanmugavelu (1982) reported that the length and girth of moringa fruits ranged from 21.5-42.2 cm and 4.3-7.0 cm respectively. Palanisamy *et al.* (1985) recorded the fruit length, fruit fresh weight and number of seeds per fruit in annual moringa. The maximum mean fruit length was 42.8 cm and the highest fresh weight recorded was 103.72 g. The seed number per fruit remained significantly unaltered throughout the maturation period of the fruits. Suthanthirapandian *et al.* (1989) found that the fruit weight exhibited wide variation (29-231.5 g) and the number of seeds per fruit showed a variation of 4.3-24.7. Suthanthirapandian *et al.* (1992) evaluated twenty seed moringa types and studied the variation in fruit length, fruit weight and the seed number per fruit. Pushpangathan *et al.* (1996) reported that moringa fruits were about 60-100cm long, green and splitted longitudinally on ripening. Sadasakthi (1997) reported that fruit weight, fruit length and fruit girth showed variation in parents and hybrids in annual moringa and the hybrids exceeded the parents in the above three values. The number of seeds per fruit ranged from 16.23 to 24.67 in parents and in hybrids it varied from 17.53-22.53.

2.2.5 Yield Characters

At the College of Horticulture, Vellanikkara, a large variability has been reported in the germplasm pool consisting of 122 accessions. Seven accessions were selected with the mean yield of more than 10 kg/tree/year. The maximum yield was recorded as 26.23 kg/ tree in MO 70 (Gopalakrishnan, 1978; Peter, 1979). Suthanthirapandian *et al.* (1989) reported that the yield by number of fruits per plant varied from 1- 155 in annual moringa. Sherkar (1993) reported that an individual moringa tree can yield 50-70 kg of pods in one year under favourable conditions. Sadasakthi (1997) reported that number of fruits per tree ranged from 75.67 to 307.00 among lines and 55.33 to 213.33 in testers and in hybrids it ranged from 135 to 342.67. The fruit yield also showed variation among the parents and hybrids, and hybrids showed better results when compared to parents in annual moringa.

2.2.6 Quality Characters

2.2.6.1 Vitamin A

Das (1965) reported that 100 g of the fresh leaves of drumstick contain 110 µg of ß- carotene and 13 µg of alpha carotene. Ramachandran *et al.* (1980) reported the vitamin A content in leaves and fruits of drumstick as 11, 300 I.U and 184 I.U per 100 g of the edible portion respectively. Sadasakthi (1997) reported that the carotene content of the fruits in hybrids ranged from 119.97 µg to 142.13 µg per 100 g of edible portion in annual drumstick. Rai *et al.* (2004) studied the carotene content in drumstick, agathi, spinach and reported that drumstick contains 6780 µg of carotene.

2.2.6.2 Ascorbic Acid

Dogra *et al.* (1975) reported that both leaves and fruit juice of moringa to be rich in vitamin C content and it varied from 126.41 to 132.17 mg/100 g pulp. Verma *et al.* (1976) determined the vitamin C contents of fresh leaves and pods of

M. oleifera and *M. concanensis* at tender and mature stages. The vitamin C content in tender stage of the pods of different clones of *M. oleifera* varied from 75-131 mg /100g and in mature stage it varied from 93-143 mg /100 g, whereas in those of *M. concanensis* it varied from 75-106 mg /100 g and 103-130 mg /100 g respectively. Ramachandran *et al.* (1980) reported the ascorbic acid content in leaves and fruits of moringa as 220 mg and 120 mg per 100g of the edible portion respectively. The mean ascorbic acid value ranged from 114.57 mg to 133.77 mg per 100 g in fruits among the parents and hybrids showed a range of 123 mg -146.77 mg per 100 g (Sadasakthi, 1997). Sato *et al.* (2003) evaluated the ascorbic acid in young shoots and leaves of *Moringa* spp. and it showed a range of 158-323 mg per 100 g of fresh weight. Prabhakar *et al.* (2003) has reported a vitamin C content of 120 mg for every 100 g fresh pod weight in drumstick. Rai *et al.* (2004) reported that drumstick leaves has a high vitamin C content of 220 mg in comparison with other leafy vegetables.

2.2.6.3 Organoleptic Evaluation

Dogra *et al.* (1975) reported extensive variation in the taste of pods of *M. oleifera* and *M. concanensis* which varied from very sweet to bitter. An organoleptic test of PKM 1 done recently at ICRISAT-Niamey found the variety to be superior in taste compared to local varieties and to a relative from East Africa called *M. stenopetala* (ICRISAT, 2004)

2.2.7 Incidence of Pests and Diseases

Seemanthini (1964) and Ramachandran *et al.* (1980) reported a root rot caused by *Diplodia* sp. in drumstick. Mandokhot *et al.* (1994) reported *Fusarium pallidoroseum* as the causal agent of twig canker in drumstick.

The hairy caterpillar, *Eupterote mollifera* Wlk, causes defoliation in drumstick (Ayyar, 1940; Muthusamy, 1954). Two caterpillar pests *viz.*, *Eupterote mollifera* and *Noorda blitealis* are serious pests of drumstick and another pest *Noorda moringae* infest unopened flower buds, causing them to drop

and cause 100% damage (Seemanthini, 1964). Sivagami and David (1968) reported the following insect pests in drumstick: caterpillars of *Tetragonia siva, Metanastria hyrtaca and Heliothes armigera* ; an aphid, *Aphis craccivora* ; a scale insect , *Ceroplastodes cajani* ; and a fruit fly, *Gitonia* sp.

2.3 MOLECULAR MARKERS

A molecular marker may be defined as a DNA sequence used for chromosome mapping as it can be located at a specific site in the chromosome. Molecular markers have been shown to be useful for diversity assessment in a number of plant species (Waugh and Powell, 1992). The specific sequence of DNA which is unique for an individual is known as molecular genetic marker. On the other hand, isozymes being the product of gene expression are designated as biochemical markers. The use of marker genes in perennial plants will have the most practical value, because breeding and genetic studies in these species are so difficult and time consuming using conventional techniques (Gloria and Richard, 1992). Various types of molecular markers are utilized to evaluate DNA polymorphism and are genetically classified as PCR based markers and hybridization based markers (Joshi *et al.*, 1999).

2.3.1 PCR Based Marker Techniques

These are fingerprinting techniques that use an *in vitro* enzymatic reaction to specifically amplify a multiplicity of target sites in one or more nucleic acid molecules (Michelli and Bova, 1996; Anolles and Trigiano, 1997).

Among the PCR based marker techniques the important ones are Random Amplified Polymorphic DNA, Amplified Fragment Length Polymorphism, Microsatellites and Sequence Characterized Amplified Region.

2.3.1.1 Random Amplified Polymorphic DNA (RAPD)

RAPDs are DNA fragments amplified by the Polymerase Chain Reaction (PCR) using short synthetic primers of random sequence. These oligonucleotides

serve as both forward and reverse primer and usually are able to amplify fragments from 3-10 genomic sites simultaneously. Amplified fragments are separated by gel-electrophoresis and polymorphisms are detected as the presence or absence of bands of particular size. These polymorphisms are considered to be primarily due to variation in the primer annealing sites. RAPDs have been used for many purposes, ranging from studies at the individual level (e.g. genetic identity) to studies involving closely related species. Due to their very high genomic abundance, RAPDs have also been applied in gene mapping studies (Williams *et al.*, 1990).

They are also used in fingerprinting genomes (Welsh and Mc Clelland, 1990), population biology studies (Astley, 1992), linkage mapping (Williams *et al*., 1993), genetic diversity estimation (Graham *et al.*, 1994, Lanham *et al*., 1995), heterozygosity studies (Lanham, 1996), gene flow studies (Graham *et al*., 1997).

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

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

i. RAPD and linkage maps

A detailed genetic map has been constructed from a single intraspecific cross of *Lactuca sativa* which comprises of 319 loci. Thirteen major, four minor linkage groups and several unlinked markers are identified for this genome. RAPD markers show similar distributions throughout the genome and were much

easier to identify (Kesseli *et al.*, 1994). Caporali *et al.* (1996) constructed nine linkage groups integrating 23 RFLP and two RAPD markers with some isoenzyme markers previously detected in *Asparagus officinalis*.

RAPD assay has been used by several groups as an efficient tool for identification of markers linked to agronomical traits which are introgressed during the development of near isogenic lines. Traits of interest studied include jointless pedicel in tomato (Wing *et al*., 1994), spotted wilt virus resistance in tomato (Chaque *et al*., 1996), anthracnose resistance in mango (Subramanian *et al.*, 1996), scab resistance in apple (Hong *et al.*, 1997) etc.

The genetic linkage map have been created in sweet cherry (Stockinger *et al*., 1996), citrus (Christophani *et al*., 1999) and litchi (Liu and Mei, 2003) using RAPD.

ii. RAPD and taxonomic studies

RAPD markers have been widely used for taxonomic and related studies. Demeke *et al.* (1992) investigated the potential use of RAPDs for taxonomic studies using *Brassica, Sinapsis* and *Raphanus* taxa. Analysis of the RAPD bands revealed the relationship between diploid and amphidiploid *Brassica* taxa. Results show that the *Raphanus sativus* and *Sinapsis alba* were distinct from the *Brassica* taxa.

iii. RAPD and somaclones

RAPD markers were found to be useful for confirmation of genetic fidelity in micro propagated plants (Gupta *et al*., 1996). Hollingsworth *et al.* (1999) showed that RAPD technology can be used to detect somatic variation occurring during long-term culture of asparagus embryogenic calli maintained on PGR medium. Raimondi *et al.* (2001) carried out RAPD and cytogenetic analyses to detect somaclonal variation in somatic embryo-derived plants from two elite genotypes of *Asparagus officinalis*. Results showed that molecular markers are

complimentary approaches that allow a correct assessment of somaclonal variation in asparagus.

RAPD markers were used for genetic analysis in somaclones of mangosteen (*Garcinia mangostana* L.). The results showed that RAPD markers can be used to rapidly point out genetic similarities or dissimilarities in micropropagation systems (Chato, 2000)

iv. RAPD and hybrids

RAPD technique has been used for the identification of hybrids and their parent determination as well. Wang *et al.* (1994) proposed RAPD fingerprinting as a convenient tool for the identification, protection and parentage determination of plant hybrids.

Interspecific hybrid plants of *Carica papaya* and *C. cauliflora* were created through hybridization followed by embryo rescue. RAPD markers were developed to mark the genetic hybrids and all 120 hybrid plants analysed were confirmed genetic hybrids (Magdalita *et al.*, 1998). DNA fingerprints and phylogenetic relationships were established for 10 *Carica papaya* genotypes based on RAPD markers. Seven parents from the Solo and Formosa groups and three hybrids from the cross between these two groups were used. Some genotypes could be fingerprinted by the presence or absence alone of some bands, but in general the DNA fingerprint could only be obtained by a set of RAPD markers. The phylogenetic analyses showed greater similarity among the parents of each group and the intermediary position of the hybrids, as expected (Angela *et al*., 2004).

Evaluation of coffee hybrids and their respective parents were carried out with RAPD markers by Fontes *et al.* (2002). The results indicated that RAPDs were efficient in evaluating the genetic diversity in *Coffea arabica* and in certifying the hybrid condition of the genetic materials obtained by artificial crosses.

v. RAPD for identification of somatic hybrids

RAPD analysis was done to verify the hybridity of 20 citrus intergeneric, interspecific and intertribal somatic hybrids regenerated from protoplast fusion (Deng *et al.*, 2000)

vi . RAPD in sex determination

Attempts to identify the sex of dioecious species at an early stage have remained almost unsuccessful. In recent years, by using molecular marker tools, efforts are being taken to identify sex at an early stage (Mulcahy *et al.*, 1993). Early identification of sex in papaya (Somsri *et al*., 1998), and nutmeg (Shibu *et al.*, 2000) is helpful for growers.

A PCR based Seedling Sex Diagnostic Assay (SSDA) specially designed for early sexing of papaya seedlings was reported by Parasnis *et al*. (2000). They developed a male specific sequence characterised amplified region (SCAR) marker in papaya by cloning a male specific RAPD fragment and designing longer primers. RAPD markers were used to differentiate between the sexual forms of three commercial papaya cultivars belonging to the solo group (Lemos *et al.*, 2002). Deputy *et al*. (2002) have developed RAPD products that determine sex in papaya. The sexing technique was used to correctly predict hermaphrodite papaya plants in a population of seedlings with an overall accuracy of 99.2 %.

vii. RAPD in cultivar identification and estimation of genetic variability

Identification of plant cultivars is important for practical breeding purposes and for related areas like plant proprietary rights protection. Assessment of genetic diversity among cultivars and their wild relatives has recently attracted increased attention in order to cope up with the reduction of diversity resulting from the breeding process. Cultivar identification and estimation of genetic variability had now been achieved with RAPD technique in a large number of horticultural crops.

Scientists at AVRDC analyzed the genetic diversity in 27 accessions of drumstick. A total of 75 primers out of 98 tested could be scored as unambiguously polymorphic bands (76.5 %). A total of 524 unambiguously polymorphic bands were generated using 75 primers. The drumstick accessions tested were divided into four clusters and all the *M. oleifera* were included in cluster 1. Genetic diversity recognized among *M. oleifera* accessions in cluster 1-b and cluster 1-c showed a reflection of geographical isolation (AVRDC, 2003).

Van and Madeira (1998) used RAPD markers to evaluate the genetic relationship between three biotypes of water spinach (*Ipomoea aquatica* Forsk.). Forty eight decamer primers were screened, eighteen of these were informative and yielded 188 resolvable bands, of which 58 (31%) were polymorphic. Five primers produced unique DNA fingerprints useful for the identification of biotypes. Hwang *et al.* (2002) investigated the genetic variation and intra-specific relationships between 35 cultivars of *Lactuca sativa* using external morphology and RAPD by random decamers and simple sequence repeat primers. 37 primers were amplified and 230 bands showed polymorphism.

RAPD technique was used for the analysis of relationship among 10 cultivars of papaya (Stiles *et al*., 1993). 11 primers amplified 102 distinct fragments and the results confirm that RAPD is a reliable and sensitive technique for genome analysis.

Schnell and Knight (1993) studied the phylogenetic relationships among nine *Mangifera* species using RAPDs and ten selected primers produced 109 usable bands. RAPD generated clusters did not always agree with the taxonomic classification based on phenotypic traits. When the two subsections of the genus were analyzed separately, the classification more closely agreed with the traditional taxonomic analysis. The use of RAPD markers for germplasm classification and clonal identification in mango was reported by Schnell *et al.* (1995). Lopez-Valenzuela *et al*. (1997) grouped mango cultivars into four categories according to their geographical origin based on RAPD data.

Assessment of genetic relatedness among mango cultivars of India were done using RAPD markers by Ravishankar *et al*. (2000). 18 cultivars were subjected to RAPD analysis using 30 arbitrary primers, out of which 27 amplified mango genomic DNA. This shows a clear segregation of mango cultivars mainly depending on their geographical location. 50 mango cultivars were analysed using ten primers. A dendrogram based on Jaccard's coefficient of similarity indicated a moderate degree of genetic diversity among the cultivars (Kumar *et al*., 2001). RAPD analysis was carried out in 29 mango cultivars comprising popular landraces and advanced cultivars. PCR amplification with 24 primers generated 314 bands and the differences among the regions were significant and the study revealed that 94.7 % of the genetic diversity in mango existed within regions (Karihaloo *et al*., 2003). De Souza and Lima (2004) studied the genetic variability in forty mango genotypes using RAPD markers. Thirteen primers were selected for the RAPD reactions and each genotype was characterised by its banding pattern.

RAPD markers were used to determine the genetic diversity within and between twenty elite lines of cashew (*Anacardium occidentale* L.) and RAPD polymorphisms were obtained among the geographically diverse lines (Mneney *et al*., 2001). RAPD markers were used to determine the genetic diversity among ninety cashew accessions. A dendrogram confirmed that the diversity of Indian cashew collections can be considered to be moderate to high (Dhanraj *et al*., 2002). DNA fingerprinting of Indian cashew varieties were done using RAPD and ISSR techniques (Archak *et al.*, 2003). Molecular profiles of 24 selections and 11 hybrids were developed using a combination of five RAPD and four ISSR primers pre-selected for maximum discrimination and repeatability. Difference in the average similarity coefficients between selections and hybrids was low indicating the need and scope for identification of more parental lines in enhancing the effectiveness of hybridization programme. Samal *et al.* (2004) assessed the genetic relationship of twenty varieties of cashew by using RAPD markers. A total of 80 distinct DNA fragments were amplified by using 11

selected random 10-mer primers. Analysis of genetic relationships in cashew using RAPD banding data may be useful for plant improvement, in descriptions of new variety and also assessing variety purity in plant certification programmes.

Prakash *et al.* (2002) studied 41 genotypes of guava using RAPD technique. The results revealed maximum genetic distance of 54 per cent among *P. guajava* and *P. quadrangularis,* while minimum distance was only 11 per cent between SWY-1 and GR-1 Navalur collections.

RAPD markers were used to analyse 15 citrus genotypes and genetic similarities obtained with 12 random primers indicated a minimum similarity of 0.81 among the mandarins. The four genotypes of sweet oranges could not be differentiated using RAPD markers, indicating maximum similarity (Bastianel *et al*., 2001). Corazza-Nunes *et al.* (2002) studied the genetic variability of 38 grape fruit (*Citrus paradisi* Macf.) and 3 pummelos (*Citrus maxima* (Burm.) Merr.) using RAPD and SSR analyses. About 49 per cent of RAPD were polymorphic and 4.6 alleles per SSR loci were identified. The majority of grape fruit accessions showed a narrow genetic base suggesting that observed polymorphism might be associated with somatic mutations, which were not detected by molecular markers. To evaluate the genetic similarity and inter relationship among 31 acid citrus species, RAPD markers were used (Abkenar and Isshiki, 2003). Out of 60 primers screened, 27 were selected which produced 108 markers and 76 were polymorphic. The result shows that RAPDs allowed distinction of very close cultivars.

RAPD markers were used for fingerprinting genotypes within and between nine accessions of Annona cultivars (Ronning *et al.*, 1995). Brown *et al*. (2003) estimated the genetic variability among nine accessions of *Annona muricata.* Seventeen fragments were obtained of which 14 were polymorphic and the phenogram identified two groups.

RAPD was used to detect polymorphism among 7 different cultivars of date palm. Out of 140 primers, 42 detected polymorphism. Out of 213 amplified fragments, 132 (61.97%) were polymorphic. Cluster analysis revealed two clusters (Askari *et al.*, 2003). RAPD was used to compare genetic material from four female date palms and four unknown male trees. The genetic similarity between four females range from 87.5-98.9 per cent. Banding profiles suggest that both males 3 and 4 are genetically related to the four female cultivars (Soliman *et al.*, 2003). Al-Khalifah and Askari (2003) assessed the genetic diversity among 13 different cultivars of date palm (*Phoenix dactylifera* L.) using RAPD. The selected primers revealed polymorphism and the genotypes were distinguishable by their unique banding pattern. The variation detected among closely related genotypes indicates the efficiency of RAPD markers over the morphological and isozyme markers.

RAPD markers have been used to characterise cocoa clones representing the three main cultivated sub populations *viz.* Criollo, Forestero and Trinitario (Wilde *et al*., 1992). The use of single primers of arbitrary nucleotide sequence resulted in the selective amplification of DNA fragments, which were unique to the individual cocoa clones studied. Lashermes *et al*. (1996) employed RAPD markers to analyse the genetic diversity among cultivated and sub spontaneous accessions of *Coffea arabica.* The narrow genetic bases of commercial cultivars were confirmed by their study. On the other hand, relatively large genetic diversity was observed within the germplasm collection. A study was done to assess the genetic variability among *Coffea arabica* cultivars using RAPD markers and this proved to be a useful tool for the genetic characterization of coffee genotypes (Sera *et al*., 2003).

Duran *et al*. (1997) analysed 48 coconut types belonging to East African tall types by different DNA marker techniques including RAPD analysis. Results detected DNA polymorphism among the genotypes and allowed identification of single genotypes by individual specific fingerprints. Varghese *et al.* (1997) evaluated the applicability of RAPD markers in cultivated rubber tree, *Hevea*,

using 43 primers in a set of 24 clones selected from different Asian countries. Out of the total 220 fragments amplified, 111 were polymorphic. The statistical analysis indicated the absence of a distinct geographical group. An analysis of 175 accessions of Brazilian oil palm (*Elaeis oleifera*) collection was carried out using RAPD markers. Results show that Brazilian oil palm accessions have moderate level of genetic diversity compared to African oil palm (Moretzsohn *et al*., 2002).

2.3.1.2 Amplified Fragment Length Polymorphism (AFLP)

AFLP is based on PCR amplification of restriction fragments generated by specific restriction enzymes and oligo nucleotide adaptors of few nucleotide bases (Vos *et al*., 1995). This technique has been used in tagging of agronomic traits (Cervera *et al*., 1996), genetic mapping (Ellis *et al*., 1997a; Forster *et al*., 1997), genetic diversity experiments (Ellis *et al*., 1997b), and for the study of mating system in plant populations (Muluvi *et al*., 2004).

Genetic analysis of seven populations of *Moringa oleifera* was performed using amplified fragment length (AFLP) markers. The four pairs of AFLP primers generated a total of 236 amplification products of which 157 (66.5%) were polymorphic between or within populations. Analysis of molecular variance (AMOVA) revealed significant differences between regions and populations (Muluvi *et al.*, 1999). An AFLP data set comprising 95 accessions from 20 species of *Lactuca* and related genera were generated and results show that all species are conspecific (Koopman *et al*., 2001). Dolezalova *et al*. (2003) studied the relationships among morphological characters, isozyme polymorphism and DNA variability in 51 accessions of *Lactuca* species. The results from AFLP analysis and the relative DNA content measurement corresponded well with recent taxonomic classification of the genus *Lactuca.*

The phylogenetic relationships among *Mangifera* species were analysed by comparing 217 AFLP markers and AFLP technique was confirmed to be useful for phylogenetic analysis (Eiadthong *et al.*, 2000). AFLP information was used

for identification of 16 mango cultivars and 7 rootstocks for construction of genetic map (Kashkush *et al*., 2001).

Kim *et al.* (2002) established the genetic relationships among papaya cultivars using AFLP markers. 71 accessions were analysed and 186 markers were generated. The results show that self pollinated hermaphrodite cultivars were as variable as open pollinated dioecious cultivars. Droogenbroeck *et al.* (2002) used AFLP technique to assess the genetic relationships among the cultivated papaya and related species. 496 polymorphic bands were generated with five primer combinations. The groupings of accessions of each species corresponded largely with their taxonomic classification and were found to be consistent with other studies related to RAPD data.

AFLP markers were used to fingerprint and to examine genetic diversity among twelve genotypes of the gooseberry (*Ribes grossularia* subgenus *Grossularia*). AFLP generated unique profiles for each genotype (Lanham and Brennan, 1999).

PCR-based DNA profiling of coconut palms was conducted using AFLPs. A total of 322 amplification products were generated from the 42 genotypes with eight pairs of primers. Overall most variation was detected in the tall rather than the intermediate and dwarf forms. Information from this study will facilitate the management of coconut germplasm and optimise the choice of genetically divergent parents for crossing (Perera *et al.*, 1998). 11 accessions of *Coffea arabica* were evaluated by AFLP and 107 markers were used to calculate the genetic distances. The results enabled a discussion of the genetic diversity reductions that occurred during dissemination of *Coffea arabica* from its primary centre of diversity (Anthony *et al*., 2002). Steiger *et al.* (2002) carried out AFLP analysis of 61 accessions of *Coffea arabica* cultivars and 74 informative markers were generated. The variation among Arabica cultivars was similar to variation within cultivars and no cultivar specific DNA marker was detected.

2.3.1.3 Microsatellite

Microsatellite consists of randomly arranged di-tri-tetra nucleotide repeats, which are hyper variable and ubiquitously distributed throughout eukaryotic genomes. Microsatellite DNA markers, which can be directly amplified by PCR, have been developed using the unique sequences that flank microsatellite (Weber and May, 1989).

Perera *et al*. (2000) used microsatellite DNA markers to investigate the level of genetic diversity and population genetic structure of coconut (*Cocos nucifera* L.). Using eight SSRs, it was possible to uniquely discriminate 116 of 130 individuals. These results provide important information for conservation and breeding purposes.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled 'Characterisation of landraces of drumstick (*Moringa oleifera* Lam.) was carried out at the Department of Olericulture and Department of Plant Biotechnology, College of Agriculture, Vellayani during the year 2002-2004.

3.1 SURVEY AND COLLECTION OF LANDRACES

A survey was carried out in different agro-climatic situations for collecting the available landraces of drumstick. Special emphasis was given to include morphologically distinct, high yielding and regular bearing types from southern regions of Kerala. Morphological data on growth and yield aspects were recorded. Information on the history, local names, reaction towards pests and diseases were also collected. The cuttings of the various accessions were collected by conducting field visit. The collection was done from May 2003 to January 2004. The details of the accessions are presented in Table 1.

3.2 MAINTENANCE OF THE ACCESSIONS

The limb cuttings of the 28 accessions collected during the survey were planted and maintained at the experimental field of the Department of Olericulture, College of Agriculture, Vellayani for further studies. All the plants were maintained with uniform management practices. These plants served as the source material for further characterisation.

3.3 MORPHOLOGICAL CHARACTERISATION OF LANDRACES

3.3.1 Observations Recorded

The biometrical observations were recorded both from the located plants and from the plants maintained at the experimental field by conducting frequent field visits.

Table 1 List of drumstick accessions used for the study

Accession number	Location	Age	Other features
MO 1	Puthusserry, Palakkad	15	Medium-sized tree with large sized fruits
MO 2	Paravattom, Thrissur	10	Medium-Sized tree with small fruits
MO 3	Kalathode, Thrissur	12	Medium-sized tree with profuse branching & fruiting
MO 4	Poonkulam, Trivandrum	10	Medium-sized tree with profuse bearing
MO 5	Vellayani, Trivandrum	12	Medium sized tree with regular bearing
MO 6	Vellayani, Trivandrum	15	Medium-Sized tree with regular bearing
MO 7	Nagercoil	10	Large sized tree with very long fruits
MO 8	Vellayani, Trivandrum	12	Large sized tree with medium sized fruits
MO 9	Vellayani, Trivandrum	15	Medium sized tree with medium sized fruits
MO 10	Vellayani, Trivandrum	15	Large tree with profuse bearing medium sized fruits,susceptible to fruit rot
MO 11	Kanjikuzhy, Kottayam	17	Medium sized tree with profuse bearing
MO 12	Thrissur	14	Medium sized tree with medium sized fruits
MO 13	Statue, Trivandrum	10	Medium tree with profuse flowering and fruiting, susceptible to fruit rot
MO 14	Kallada, Kollam	14	Medium sized tree with profuse bearing

Table 1 List of drumstick accessions used for the study

MO 15	Coimbatore	10	PKM-1 released from TNAU
MO 16	Ettumanoor, Kottayam	16	Small tree with profuse bearing
MO 17	Edapazhanji, Trivandrum	18	Medium Sized Tree with profuse flowering and fruiting
MO 18	Vazhuthacaud, Trivandrum	12	Large tree with heavy bearing
MO 19	Vallakadavu, Trivandrum	15	Medium Sized Tree with profuse flowering and fruiting
MO 20	Vallakadavu, Trivandrum	10	Medium sized tree with small pointed fruits
MO 21	Manacaud, Trivandrum	12	Large tree with profuse flowering and fruiting, leaflets were larger in size
MO 22	Manacaud, Trivandrum	14	Large tree with long, fleshy fruits, tree seen drooping due to the weight of the fruit
MO 23	East Fort, Trivandrum	17	Medium sized tree producing small fruits with seeds bulging
MO 24	Santhinagar, Trivandrum	20	Large tree with flowering and fruiting throughout the year
MO 25	Pulimoodu, Trivandrum	15	Medium sized tree with regular bearing
MO 26	Vazhuthacaud, Trivandrum	20	Medium sized tree with flowering and fruiting
MO 27	Vattiyoorkavu, Trivandrum	20	Medium sized tree with average sized fruits
MO 28	Parottukonam, Trivandrum	10	Large sized tree with very long and fleshy fruits

3.3.1.1 Plant Characters

1. Age of the Tree

The age of the tree was recorded by personal enquiry with the farmer.

2. Height of the Tree

The tree height was recorded from the ground level to the topmost leaf laid and presented in metre.

3. Number of Main Branches

Number of branches arising from the main stem were counted.

4. Length and Breadth of the Leaf

Fully mature leaf was selected for making the above observation. The length was measured as the distance from the base of the petiole to the leaf tip and expressed in cm. The breadth of the same leaf, used for recording the length was taken at the region of maximum width.

5. Colour of Tender Stem

The colour of tender stem at the region of emerging leaves was noted.

3.3.1.2 Flowering Characters

1. Time of Flowering

The flowering pattern of the accessions was observed and recorded for a period of 12 months.

2. Number of Flowering Peaks per Year

The number of flowering peaks in a period of 12 months was observed and the intensity of flowering at peak stage was noted.

3. Number of Flowers per Inflorescence

Five inflorescences were selected randomly from each tree during summer flowering and number of flowers per inflorescence were counted and average worked out.

4. Flower Colour

Colour of the flowers in the inflorescence selected for the above observation was noted.

3.3.1.3 Fruit Characters

1. Fruit Length and Girth (cm)

Five mature fruits of each accessions were collected after the summer flowering. The length was measured as the distance from pedicel attachment of the fruit to the apex using twine and scale, mean taken and expressed in cm.

Girth of the fruits was measured at the region of maximum width using twine and scale, mean taken and expressed in cm.

2. Fruit Colour

The colour of the fruits selected for making the above observation was noted.

3. Number of Fruits

The total of all fruits produced by the tree was calculated accounting the weekly harvest data.

4. Average Fruit Weight (g)

Weight of the fruits selected for recording length and girth was noted, average taken and expressed in g.

5. Yield (kg)

Weight of all fruits harvested from the selected trees was noted.

6. Number of Seeds per Fruit

The number of seeds in five fruits were counted, average taken and recorded.

3.3.1.4 Nutritional Quality Characters

The nutritional quality characters *viz*., vitamin A and vitamin C of the fruits and leaves of 28 accessions were analysed.

1. Vitamin A

β-carotene content of fruits and leaves was estimated by following the method of Srivastava and Kumar (1998). Carotene values expressed in µg / 100 g were divided by 0.6 to get the vitamin A content in IU (A.O.A.C, 1984).

Reagents:

Acetone

Anhydrous sodium sulphate

Petroleum ether

Procedure: Five grams of fresh fruit was crushed in 15 ml acetone and added a few crystals of anhydrous sodium sulphate. The supernatant was decanted into a beaker. The process was repeated twice and transferred the combined supernatant to a separatory funnel. 15 ml petroleum ether was added and mixed thoroughly. The lower layer was discarded and the upper layer was collected in a 100 ml volumetric flask, made upto 100 ml with petroleum ether and recorded optical density (OD) at 452 nm using petroleum ether as blank.

$$\beta\text{-carotene } (\mu g/100\ g) = \frac{OD\ (452\ nm) \times 13.9 \times 10^4 \times 100}{\text{Weight of sample} \times 560 \times 1000}$$

$$\text{Vitamin A (I.U.)} = \frac{\beta\text{-carotene } (\mu g/100\ g)}{0.6}$$

The same procedure was followed for the analysis of vitamin A content in leaf also.

2. Vitamin C

Vitamin C content of leaves and fruits was estimated by 2,6-dichlorophenol indophenol dye method (Sadasivam and Manickam, 1992).

Reagents : 4 per cent oxalic acid

Ascorbic acid (standard)

2,6-dichlorophenol indophenol dye

Procedure : One gram of fresh fruit was extracted in an acid medium (4 per cent oxalic acid) and titrated against 2,6-diclorophenol imidacloprid dye to a pink colour which persisted at least 5 s.

Vitamin C content in mg/100 g

$$= \frac{\text{Titre x dye factor x volume made up x 100}}{\text{Aliquot of extract taken x weight of sample}}$$

The vitamin C content of the leaves was calculated following the same procedure as above.

3. Organoleptic Evaluation

The organoleptic quality and acceptability traits were done in fruits and leaves using a scoring method proposed by Jijiamma (1989). The major quality attributes included in the score were colour, doneness, bitterness, odour and taste (Appendix I).

Each of the above mentioned quality was assessed by a five point rating scale.

The fruits were washed thoroughly in water and cut into pieces. 100 g cut fruits were boiled in 150 ml water and 1 g salt for 10 minutes. The prepared sample was used for organoleptic quality scoring.

The leaves were washed in water and mixed with grated coconut and salt and cooked for 10 minutes. The sample was used for organoleptic quality scoring.

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

3.3.1.5 Scoring for pests and diseases

1. Incidence of Fruit Rot

All the selected trees were maintained and occurrence of fruit rot was recorded.

2. Incidence of Hairy Caterpillar, *Eupterote mollifera*

All the selected trees were closely monitored for the incidence of hairy caterpillar.

3. Incidence of Moringa Bud Borer, *Noorda moringae*

The accessions were observed for the incidence of moringa bud borer and the extent of damage was calculated. The percentage of damage was calculated in all the accessions by counting the number of leaves with damage and total number of leaves in a branch.

3.3.2 Data Analysis

The data on various morphological characters were utilized for preparing mean tables and graphical representations. Relative performance of the accessions in terms of various growth, flowering and yield attributes was assessed through graphical representation either by categorizing them or by calculating the percentage to the highest values. Ranking was done on all the 28 drumstick accessions based on important morphological and quality characters as proposed by Rajamony *et al*. (1994).

3.4 MOLECULAR CHARACTERISATION OF LANDRACES

3.4.1 Isolation of Genomic DNA

DNA was isolated from 25 accessions of drumstick which were found to be morphologically distinct. For the isolation of genomic DNA, the young, tender leaves were used. Isolation was done as proposed by Murray and Thompson (1980) with slight modifications.

Tender leaf tissues were collected and washed with distilled water. The leaves were dried using a tissue paper and they were chopped coarsely. About 5 gm of chopped leaf sample was transferred to a cool dry mortar and ground to a fine powder by using liquid nitrogen. The dry powder was dispersed in extraction buffer (0.7 N NaCl, 1 % CTAB, 50Mm Tris HCL, 10 Mm EDTA and 1% β-mercaptoethanol). Enough extraction buffer was added, so as to disperse the clumps easily. For this, 1.0 ml/30-100 mg dry weight powder was required. The mixture was incubated for 30 min at 60°C with gentle mixing. It was then subjected to centrifugation at 15,000 rpm for 10 minutes. The clear supernatant was taken and the remaining extraneous matter was discarded.100 µl of phenol chloroform isoamyl alcohol was added to the supernatant and centrifuged for 10 min at 10,000 rpm. The supernatant was again collected and 200 µl of chloroform isoamyl alcohol (24 %) was added and centrifuged at 10,000 rpm for 10 minutes. Supernatant was collected and the above step was repeated. Again the supernatant was collected and 100 µl of 3 M sodium acetate and 200 µl of isopropanol was added. The mixture was kept at -80° C for 30 minutes and again centrifuged at 10,000 rpm for 10 minutes, supernatant was removed and the pellet was given 70 % alcohol wash twice. The supernatant was removed and the pellet was kept for drying. Later the pellet was suspended in 100 µl TE buffer and dissolved.

3.4.2 Quantification of DNA

Reliable quantification of DNA concentration is important for many applications in molecular biology. DNA quantification was carried out with the help of UV-Vis spectrophotometer (Spectronic Genesys 5). The spectrophotometer was calibrated at 260 nm and 280 nm wavelength using TE buffer (10Mm Tris HCl, pH 8.0,1 Mm EDTA, pH 8.0) in which DNA was dissolved. The optical density (OD) of the DNA sample dissolved in the buffer was recorded at both 260 nm and 280 nm.

Since an OD of 1.0 at 260 nm represent 50 µg/ml of DNA the quantity of DNA in the sample was estimated by the following formula.

Amount of DNA ($\mu g\ ml^{-1}$) = A_{260} x 50 x dilution factor

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

The quality of DNA could be guided from the ratio of the OD values recorded at 260 nm and 280 nm. A ratio between 1.8 and 2.0 indicates good quality DNA.

3.4.3 Agarose Gel Electrophoresis

The agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit. The concentration of agarose used was 0.7 per cent for visualizing the genomic DNA. A concentration of 1.2 per cent was used for PCR products. A voltage level of 50 V was used.

The required amount of agarose was weighed out and added to 1 x TAE buffer. Agarose was dissolved by boiling. After cooling to about 50°C, ethidium bromide was added to a final concentration of 0.5 $\mu g\ ml^{-1}$. The mixture was poured immediately to a preset template with appropriate comb. After solidification, the comb and scaling tapes were removed and the gel was mounted in an electrophoresis tank filled with 1 x TAE (0.04 M Tris acetate, 0.01M EDTA, pH 8.0) running buffer. The gel was completely covered on the surface by the buffer. The DNA sample was

mixed with required volume of gel loading buffer (6.0 x loading dye containing 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 with required volume of gel loading buffer. Electrophoresis was performed at 50 V until the tracking dye reached ¾th of the length of the gel. The gel was visualized using a transilluminator (Appligene Oncor, France).

3.4.4 Random Amplified Polymorphic DNA Analysis

RAPD analysis was performed following the method recommended by Williams *et al*. (1990) with required modifications.

Thirty five arbitrarily designed decamer primers supplied by Operon Inc. USA were used.

Genomic DNA (20 ng) was amplified *in vitro* in a 25 µl of reaction mixture containing 2.5 µl of 10 x buffer (10 mM Tris Hcl pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl and 0.01 per cent gelatin), 5 picomoles of primer, 200 µM each of dNTPs and 0.6 units of Taq DNA polymerase (Invitrogen, USA). Amplifications were carried out in a Programmable Thermal Controller (MJ Research, Inc.) set for the following programme: An initial denaturation at 94°C for 1 min followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 34°C for 1 min 30 S and extension at 72°C for 2 min and a final step of extension at 72ºC for 5 min. Finally the products of amplification were cooled to 4°C until attended. A negative control, containing water instead of template was included in each reaction set.

Amplified products along with DNA molecular weight marker were separated by 1.2 per cent agarose gel electrophoresis, stained with ethidium bromide and visualized on a UV transilluminator. The number of bands were recorded and those primers which produced maximum number of bands were used to amplify the DNA of all twenty five drumstick

clones. The photographs of the amplification profile were taken using a gel documentation system.

3.4.5 Data analysis

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

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

Where,

a: number of bands present in both the clones in a pair.

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

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

Based on the similarity co-efficient, the distance between the clones was computed with the help of the software package NTSYS (Version 2.02i). Using these values of distances between accessions, a dendrogram was constructed by UPGMA method. Association between the various accessions was found out from the dendrogram.

Results

4. RESULTS

A study on the characterisation of landraces of drumstick (*Moringa oleifera* Lam.) was conducted at the Department of Olericulture and Department of Plant Biotechnology, College of Agriculture, Vellayani. The data on biometrical characters were collected from twenty eight diverse accessions of drumstick during a calendar year starting from June 2003 to May 2004. The results of the study are presented here under in the following subheadings:

4.1 Vegetative characters

4.2 Flowering characters

4.3 Fruit characters

4.4 Quality characters

4.5 Pest and disease incidence

4.6 Data analysis

4.7 Molecular characterisation

4.1 VEGETATIVE CHARACTERS

The data collected on vegetative characters are presented in Table 2.

4.1.1 Tree Height

The height of the selected trees ranged from 5.0 m to 12.5 m with a mean of 7.9 m. The height of the trees was analysed by categorising them into five groups as furnished below and the relative height is illustrated in Fig.1. Out of the twenty eight accessions studied, MO 22 alone recorded a height more than 12 m.

Table 2 Vegetative characters of drumstick accessions in the study

Accession number	Height (m)	No of main branches	Leaf			Colour of tender stem
			Length (cm)	Width (cm)	Weight (g)	
MO 1	5.5	4	40.22	24.40	4.77	Light green
MO 2	7.3	7	31.40	19.40	3.21	Light green
MO 3	5.8	8	32.20	19.80	4.65	Light green
MO 4	5.5	4	46.20	22.60	5.31	Light green
MO 5	6.0	2	39.60	20.20	3.78	Green
MO 6	8.3	3	50.80	21.20	4.30	Light green
MO 7	9.0	6	50.40	37.80	6.77	Light green
MO 8	10.0	3	37.40	24.60	5.23	Light green
MO 9	7.5	5	52.20	45.20	4.32	Light green
MO 10	10.0	3	41.60	31.30	4.65	Light green
MO 11	6.5	6	49.20	38.10	5.15	Light green
MO 12	5.5	4	37.80	28.60	6.11	Light green
MO 13	7.0	3	45.20	41.40	5.45	Deep purple
MO 14	5.5	2	38.20	30.20	4.82	Light green
MO 15	5.0	4	45.40	39.10	6.52	Light green
MO 16	5.5	4	44.20	30.80	6.31	Light green
MO 17	6.0	5	36.50	29.66	4.75	Purplish green
MO 18	10.0	5	45.60	36.70	5.36	Purplish green
MO 19	6.5	4	39.30	31.20	6.24	Light green
MO 20	9.0	2	55.20	48.20	4.16	Light green
MO 21	12.0	4	43.60	38.40	5.86	Light green
MO 22	12.5	1	42.60	40.10	5.48	Deep purple
MO 23	10.0	2	49.40	37.20	4.28	Light green
MO 24	12.0	3	56.80	48.90	4.19	Light green
MO 25	8.5	4	47.40	40.20	5.42	Light green
MO 26	6.0	3	57.80	46.80	6.17	Light green
MO 27	10.0	5	54.60	47.20	5.53	Light green
MO 28	8.0	3	37.40	26.90	5.95	Purplish green
Mean	7.9	3.9	44.58	33.79	5.17	

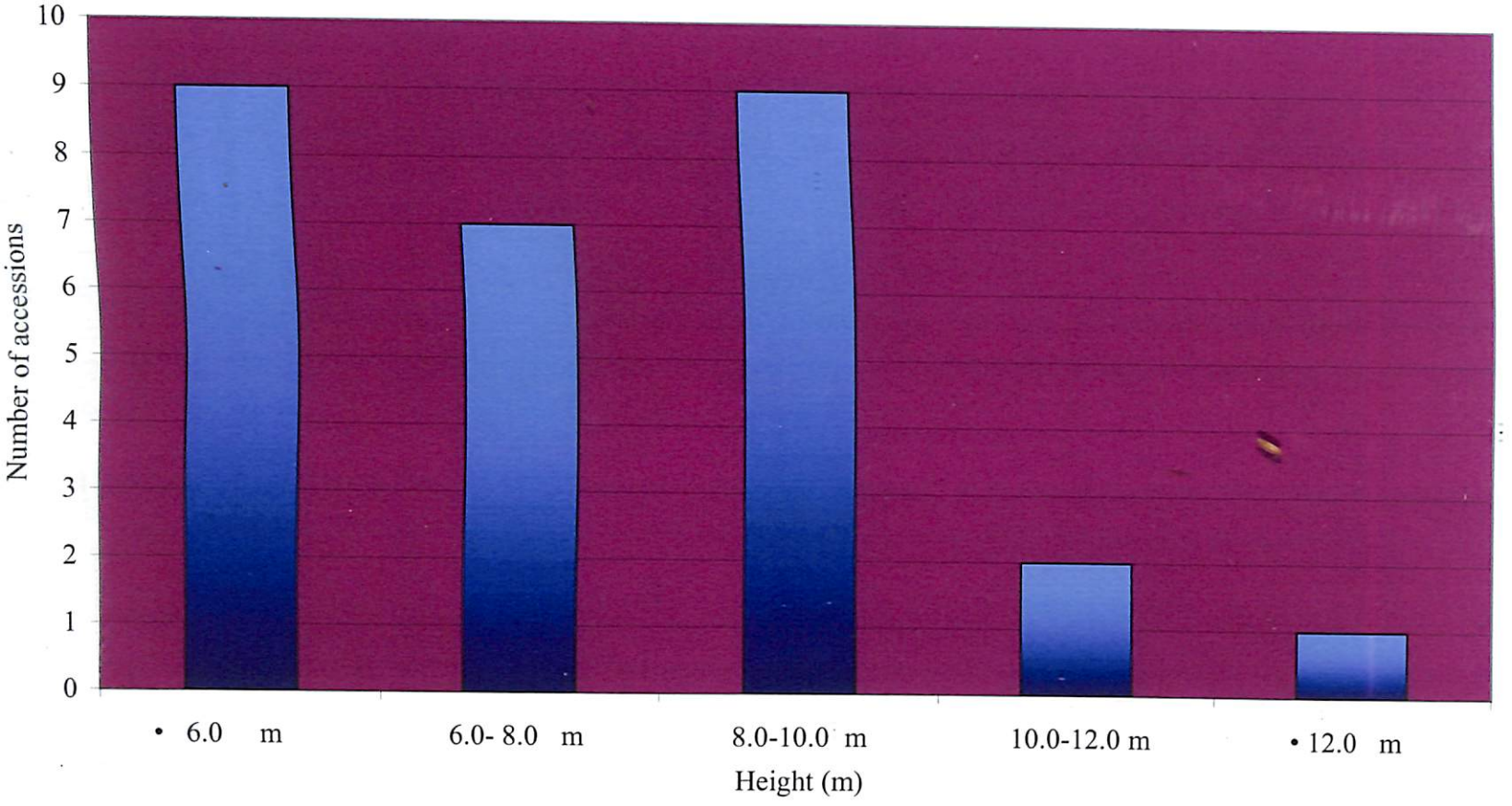


Fig.1 Classification of drumstick accessions based on height

Category	Number of accessions
≤ 6.0 m	9
6.0- 8.0 m	7
8.0-10.0 m	9
10.0-12.0 m	2
≥ 12.0 m	1

4.1.2 Number of Main Branches

The number of main branches varied from 1-8 with a mean value of 3.9. The maximum number of main branches was recorded in the accession MO 3 and the minimum number in MO 22.

4.1.3 Leaf Parameters

The leaf length showed a range of 57.8 cm (MO 26) to 31.4 cm (MO 2) with a mean of 44.58 cm (Plate 1). But the maximum leaf width was in accession MO 20 (48.2 cm) and the minimum in MO 2 (19.4 cm). Leaf width recorded a mean of 33.79 cm. The leaf weight ranged from 3.21 g (MO 2) to 6.77 g (MO 7) with a mean of 5.17 g. The relative length, breadth and weight of leaves were estimated by keeping the highest values in each category as cent percent and the same was expressed in Fig.2.

4.1.4 Colour of Tender Stem

The variation in the colour of tender stem was observed among the accessions. It ranged from light green to purple (Plate 2). Accessions MO 13

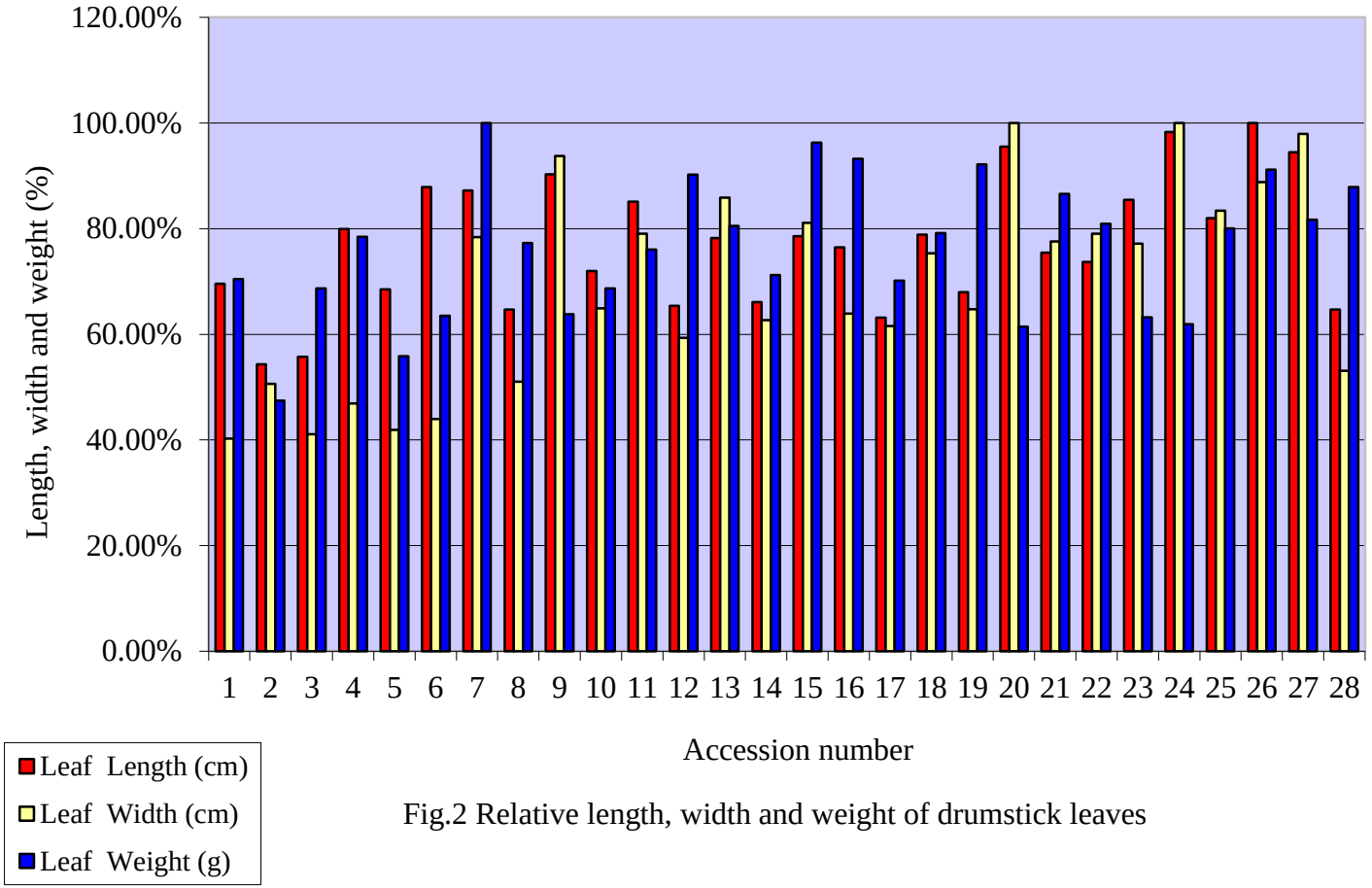


Fig.2 Relative length, width and weight of drumstick leaves

Plate 1
Variation in Leaf Length

Plate 2
Range in Stem Pigmentation

and MO 22 had deep purple colour. MO 17, MO 18 and MO 28 had purplish green stem and others had light green tender stem.

4.2 FLOWERING CHARACTERS

The data on flowering characters are presented in Table 3. Flowering was recorded through out the year except in the months of November and December.

4.2.1 Flowering Peaks per Year

All the accessions showed variation in the peak flowering time in a year. The accessions MO 13, MO 24, MO 26 showed three flowering peaks in a year whereas MO 16 recorded only one peak flowering. All other accessions exhibited two peaks flowering in a year. The flowering pattern of the drumstick accessions for one calendar year from June 2003 to May 2004 is presented in Fig. 3.

4.2.2 Number of Flowers per Inflorescence

The number of flowers per inflorescence varied from 65.23 (MO 28) to 25.4 (MO 22) with a mean of 49.9. The relative percentage of flowers per inflorescence was calculated by categorizing the accessions into five groups based on number of flowers as shown below and is illustrated in Fig.4.

Category	Number of accessions
25-35	3
35-45	7
45-55	8
55-65	8
65-75	2

Table 3 Flowering characters of the drumstick accessions in the study

Accession number	Flowering peaks/year	Time of flowering			Flowers/ inflorescence	Flower colour	Flower shedding
		I	II	III			
MO 1	2	Feb-Mar	-	July-Aug	55.68	C	+
MO 2	2	Mar-Apr	-	July-Aug	52.8	C	+
MO 3	2	Mar-Apr	-	July-Aug	64.44	C	+
MO 4	2	Feb-Apr	-	Aug-Sep	36.6	C	+
MO 5	2	Mar-Apr	-	July-Aug	54.6	C	+
MO 6	2	Mar-Apr	-	July-Aug	52.6	C	+
MO 7	2	Feb-Mar	-	July-Sept	61.2	C	+
MO 8	2	Mar-Apr	-	Aug-Sep	60.2	C	+
MO 9	2	Feb-Mar	-	Aug-Sep	50.4	C	+
MO 10	2	Feb-May	-	Sep	63.75	C	+
MO 11	2	Mar-Apr	-	July-Aug	53.2	C	+
MO 12	2	Mar-Apr	-	July-Aug	52.8	C	+
MO 13	3	Feb	May	Aug	37.2	C	+
MO 14	2	Feb-Mar	-	Jul-Aug	33.8	C	+
MO 15	2	Feb-Mar	-	Jul-Aug	56.7	C	+
MO 16	1	Mar-May	-	-	44.6	C	+
MO 17	2	Mar-May	-	Aug-Sep	45.6	C	+
MO 18	2	Apr-May	-	Sep	43.2	C	+
MO 19	2	Feb-May	-	Aug	34.8	C	+
MO 20	2	Feb-May	-	Aug-Sep	43.4	C	+
MO 21	2	Feb-May	-	Jul-Aug	51.6	C	+
MO 22	2	Feb-May	-	Aug-Sep	25.4	C	+
MO 23	2	Feb-Mar	-	Jul-Aug	60.2	C	+
MO 24	3	Feb	May	Sep	45.2	C	+
MO 25	2	Apr-May	-	Aug-Sep	48.8	C	+
MO 26	3	Feb	May	Sep	62.8	C	+
MO 27	2	Feb-Mar	-	Aug-Sep	39.65	C	+
MO 28	2	Feb-Mar	-	Jul-Aug	65.23	Y	+
Mean	2				49.9		

+ denotes present
C = Cream
Y = Yellow

Fig. 3 Flowering Pattern of the drumstick accessions for one calendar year

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
MO 1	-	+	+	-	-	-	+	+	-	-	-	-
MO 2	-	-	+	+	-	-	+	+	-	-	-	-
MO 3	-	-	+	+	-	-	+	+	-	-	-	-
MO 4	-	+	-	+	-	-	-	+	+	-	-	-
MO 5	-	-	+	+	-	-	+	+	-	-	-	-
MO 6	-	-	+	+	-	-	+	+	-	-	-	-
MO 7	-	+	+	-	-	-	+	-	+	-	-	-
MO 8	-	-	+	+	-	-	-	+	+	-	-	-
MO 9	-	+	+	-	-	-	-	+	+	-	-	-
MO 10	-	+	-	-	+	-	-	-	+	-	-	-
MO 11	-	-	+	+	-	-	+	+	-	-	-	-
MO 12	-	-	+	+	-	-	+	+	-	-	-	-
MO 13	-	+	-	-	+	-	-	+	-	-	-	-
MO 14	-	+	+	-	-	-	+	+	-	-	-	-
MO 15	-	+	+	-	-	-	+	+	-	-	-	-
MO 16	-	-	+	-	+	-	-	-	-	-	-	-
MO 17	-	-	+	-	+	-	-	+	+	-	-	-
MO 18	-	-	-	+	+	-	-	-	+	-	-	-
MO 19	-	+	-	-	+	-	-	+	-	-	-	-
MO 20	-	+	-	-	+	-	-	+	+	-	-	-
MO 21	-	+	-	-	+	-	+	+	-	-	-	-
MO 22	-	+	-	-	+	-	-	+	+	-	-	-
MO 23	-	+	+	-	-	-	+	+	-	-	-	-
MO 24	-	+	-	-	+	-	-	-	+	-	-	-
MO 25	-	-	-	+	+	-	-	+	+	-	-	-
MO 26	-	+	-	-	+	-	-	-	+	-	-	-
MO 27	-	+	+	-	-	-	-	+	+	-	-	-
MO 28	-	+	+	-	-	-	+	-	-	-	-	-

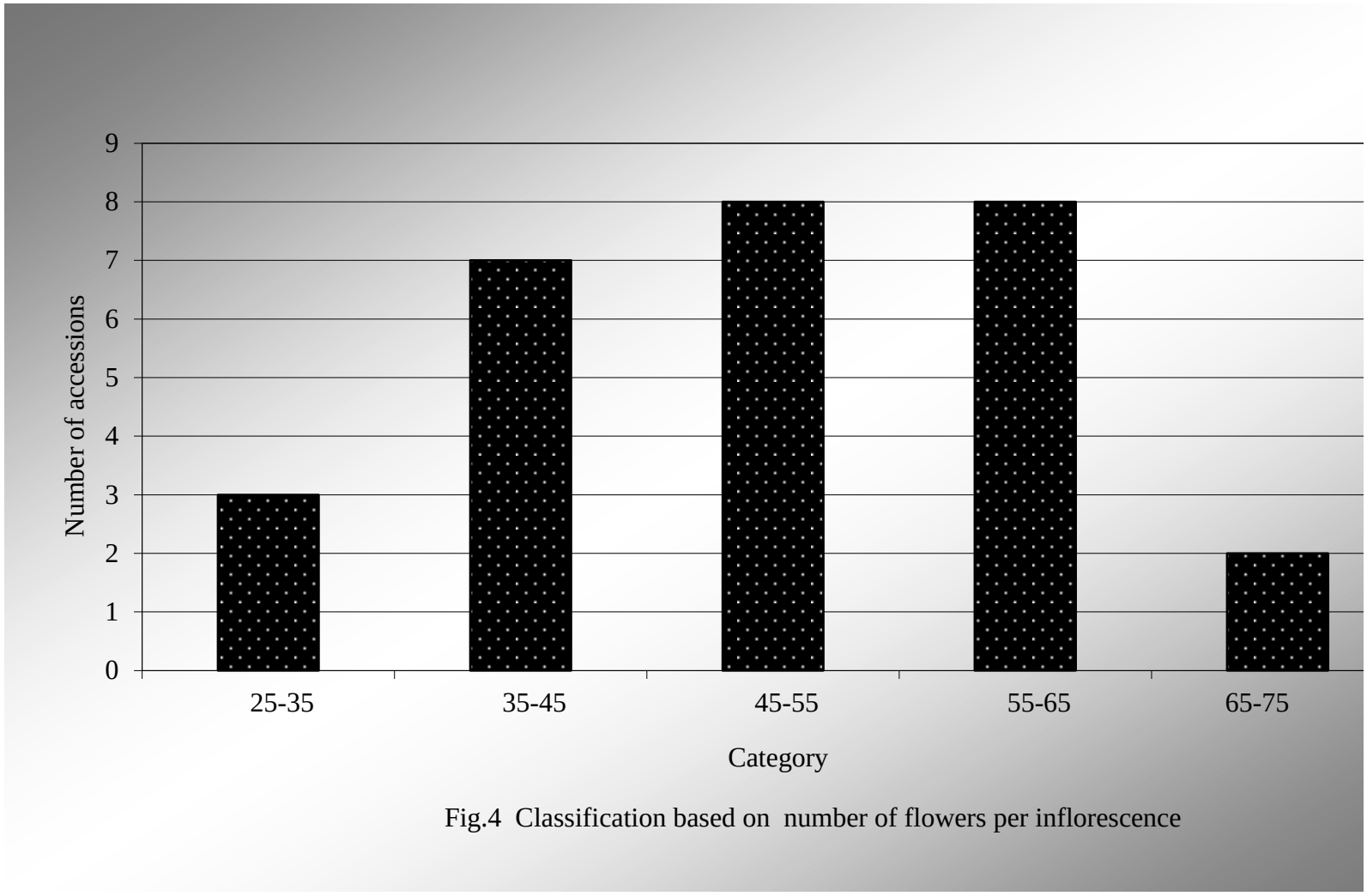


Fig.4 Classification based on number of flowers per inflorescence

4.2.3 Flower Colour

The variation in flower colour was very less. All the accessions produced cream flowers except accession MO 28 which produced yellow flowers.

4.2.4 Flower Shedding

All the accessions exhibited flower shedding and it was noted that shedding was more in those accessions which produced more number of flowers.

4.3 FRUIT CHARACTERS

The data on fruit characters and yield are presented in Table 4.

4.3.1 Fruit Length

The fruit length ranged from 32.3 cm - 100.02 cm. The mean fruit length was found to be 51.64 cm. The longest fruit was recorded in accession MO 28 and the shortest fruit in MO 20.

Category	Number of accessions
≤ 40 cm	7
40.0-50	14
55-70	2
70-90	1
≥ 90	4

The fruits were grouped into five categories based on length as shown above and the fruit length of twenty eight accessions are represented in Fig.5.

Table 4 Fruit characters of drumstick accessions in the study

Accession number	Fruit		Fruits Plant^{-1}	Fruit weight (g)	Yield Plant^{-1}(kg)	No.of seeds/fruit	Colour	Fruit shedding
	Length (cm)	Girth (cm)						
MO 1	92.80	8.24	352	212.52	69.77	23.6	G	-
MO 2	32.56	4.50	205	91.25	20.56	24.4	G	-
MO 3	64.66	4.23	206	55.86	24.92	18.6	G	-
MO 4	73.80	4.66	315	115.35	39.50	23.8	G	-
MO 5	32.75	4.23	523	25.30	12.25	19.6	G	-
MO 6	38.84	6.12	270	41.32	11.15	20.8	G	-
MO 7	55.26	4.25	252	196.30	49.47	25.2	G	-
MO 8	32.74	4.22	245	36.50	8.94	19.4	G	-
MO 9	43.58	5.52	247	33.50	8.30	20.6	G	-
MO 10	38.76	5.18	460	63.55	46.64	17.4	G	-
MO 11	42.60	6.86	286	56.22	19.56	22.8	G	-
MO 12	50.62	5.16	508	65.78	48.59	16.4	G	-
MO 13	39.56	5.22	242	48.97	17.41	21.2	G	-
MO 14	36.50	5.45	223	36.54	27.09	16.4	G	-
MO 15	72.45	6.43	265	120.00	31.80	25.2	G	-
MO 16	47.94	6.52	174	53.21	17.83	22.8	G	-
MO 17	36.84	5.54	288	48.72	20.20	18.2	G	-
MO 18	40.84	5.14	373	62.45	31.83	20.2	GG	-
MO 19	44.80	5.26	294	35.35	10.50	19.8	G	-
MO 20	32.30	4.42	276	27.89	9.04	19.6	GG	-
MO 21	51.34	5.94	375	26.70	9.50	14.6	G	-
MO 22	96.02	8.20	196	148.10	29.02	25.2	G	-
MO 23	48.64	5.34	534	30.08	16.35	19.8	G	-
MO 24	46.88	5.96	612	54.57	37.40	20.8	GG	-
MO 25	54.82	6.36	177	90.87	14.30	16.2	G	-
MO 26	54.54	6.32	565	63.43	43.14	18.8	GG	-
MO 27	43.58	5.36	363	41.35	20.40	16.2	G	-
MO 28	100.02	8.36	310	227.30	70.46	25.6	G	-
Mean	51.64	5.68	326	75.32	27.35	20.47		

G = Green
GG = Greyish Green
- denotes absent

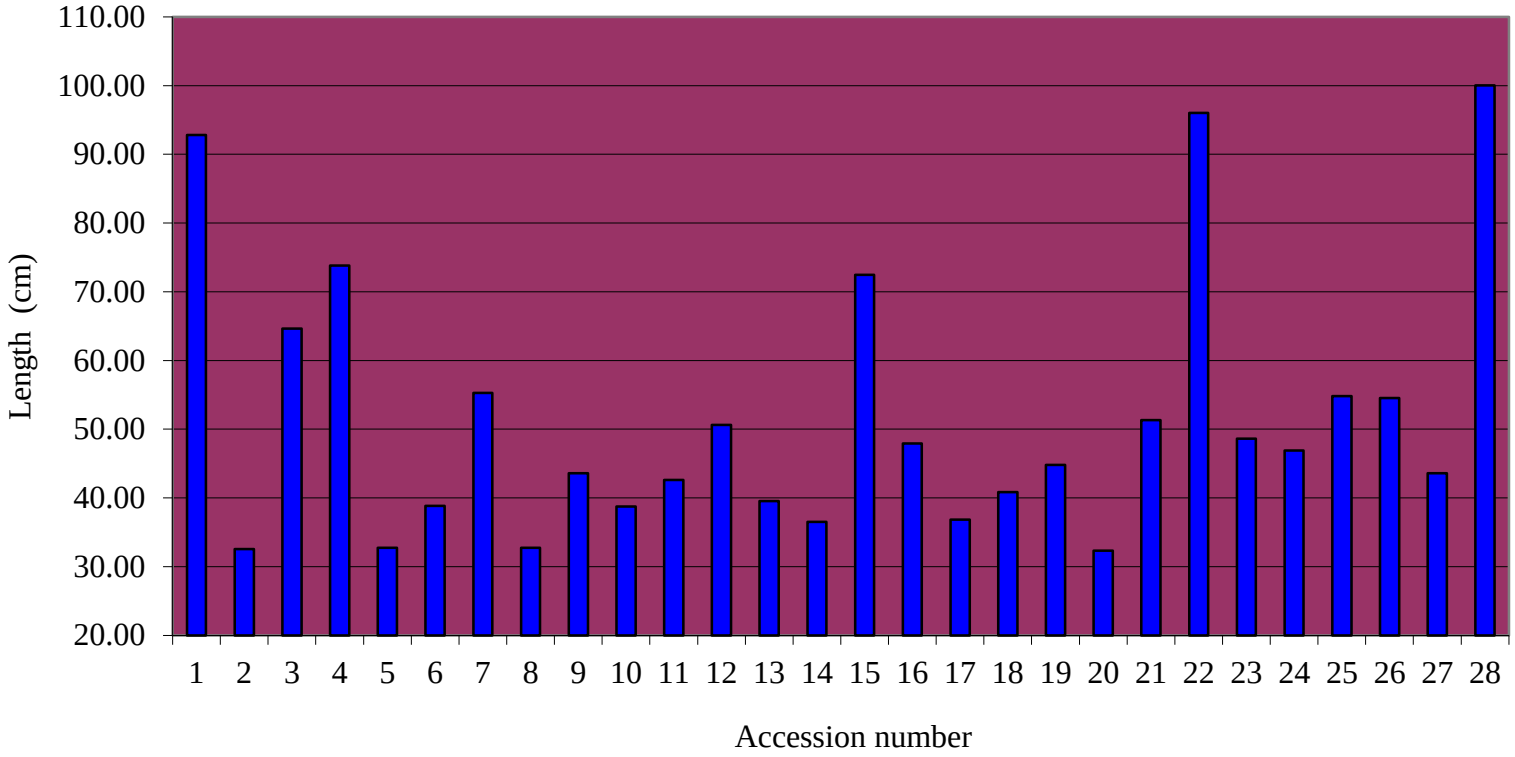


Fig.5 Fruit length of drumstick accessions

4.3.2 Fruit Girth

The fruit girth ranged from 4.22 cm (MO 8) to 8.36 cm (MO 28) with a mean girth of 5.68 cm.

Category	Number of accessions
≤ 5 cm	4
5.0-6.0	13
6.0-7.0	7
7.0-8.0	2
≥8.0	2

The girth of fruits were analysed by categorizing the accessions into five groups based on the girth as furnished above and the fruit girth is illustrated in Fig. 6. Plate 3 shows the variation for fruit size and colour.

4.3.3 Number of Fruits per Plant

The fruit number ranged from 174 (MO 16) to 612 (MO 24) with a mean value of 326. The relative numbers of the fruits in the drumstick accessions were studied by categorizing the accessions into five groups as shown and the number of fruits is illustrated in Fig.7.

Category	No: of accessions
≤ 200	3
200-300	13
300-400	6
400-500	1
≥ 500	5

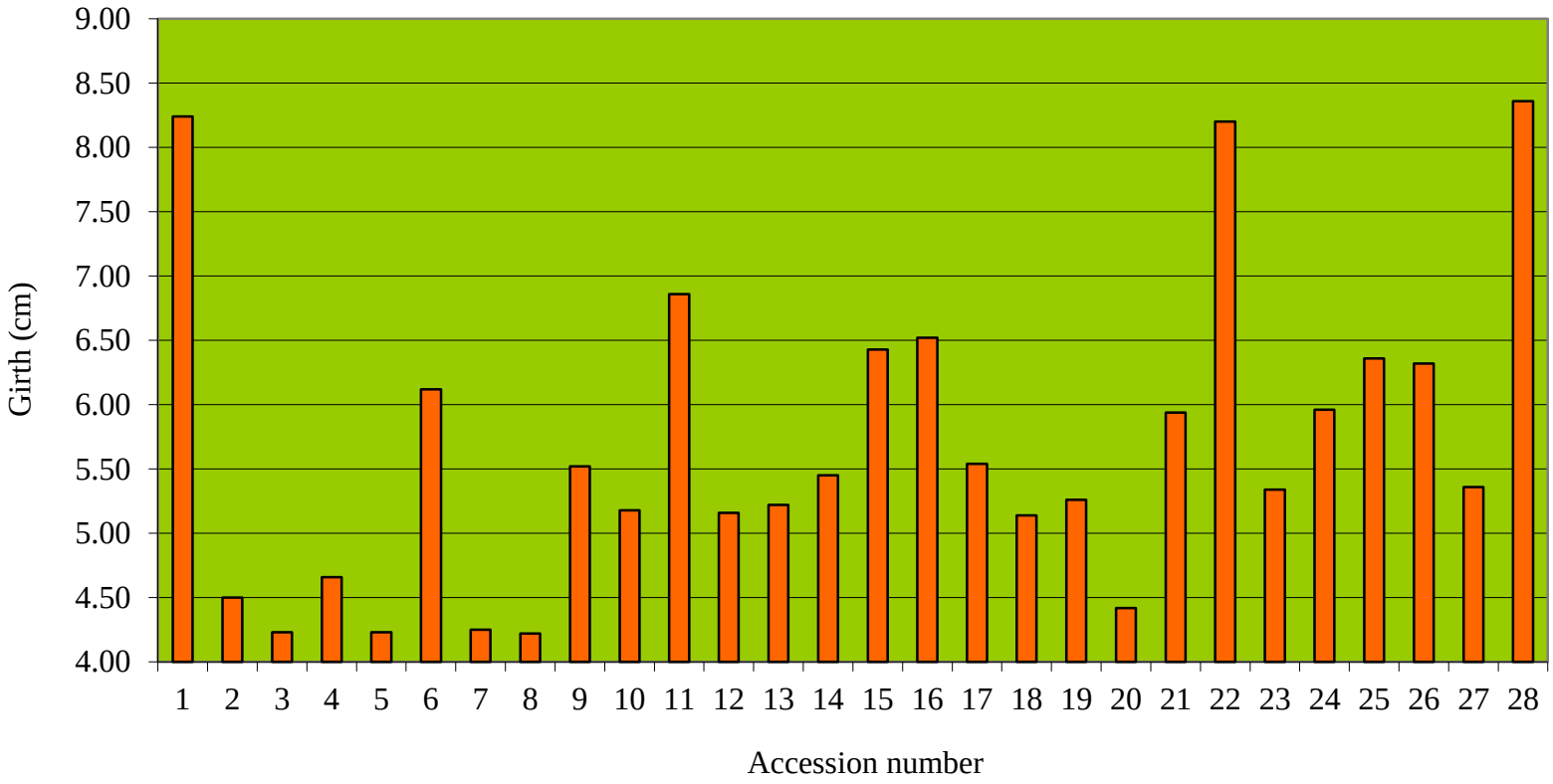


Fig.6 Fruit girth of drumstick accessions

Plate 3. Variation in fruit morphology on drumstick accessions - A representative sample

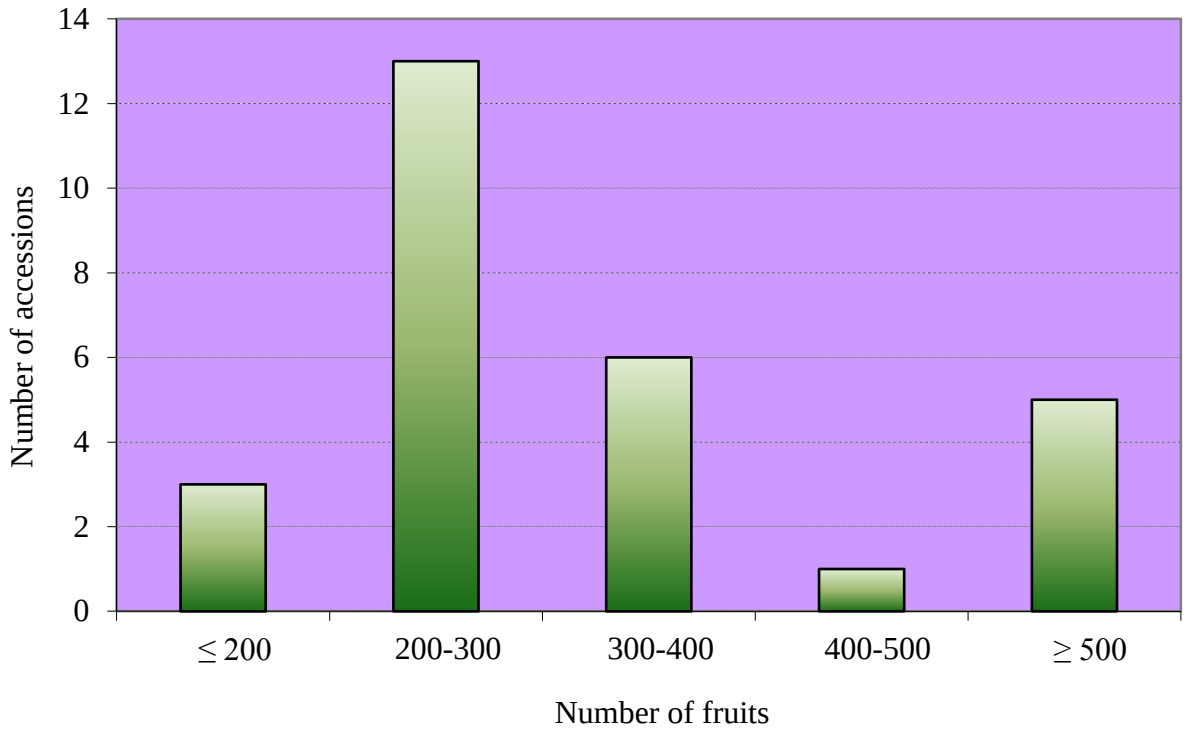


Fig.7 Classification based on number of drumstick fruits

4.3.4 Fruit Weight

The fruit weight ranged from 25.3 g (MO 5) to 227.3 g (MO 28) with a mean value of 75.32. The weight of the fruits are expressed in Fig.8.

4.3.6 Total Fruit Yield

The total fruit yield ranged from 8.94 kg (MO 8) to 70.46 kg (MO 28). The total yields of the accessions is illustrated in Fig. 9.

4.3.6 Number of Seeds per Fruit

The number of seeds per fruit was recorded highest in MO 28 (25.6), followed by MO 7 (25.2). The minimum number of seeds per fruit was recorded in MO 21 (14.6).

4.3.7 Fruit Colour

Accessions MO 24, MO 20, MO 18, MO 23 produced grayish green fruits, while all others produced fruits green in colour.

4.3.8 Fruit Shedding

None of the accessions studied showed fruit shedding at any stages of fruit development.

4.4 QUALITY CHARACTERS

The quality attributes considered in the present study were Vitamin A, Vitamin C and organoleptic qualities of fruit and leaf. The vitamin content of the leaves and fruits of drumstick are presented in the Table 5.

4.4.1 Vitamin A

The vitamin A content in leaf was lowest in MO 1 (8107.86) and highest in MO 26 (13,215.79 I.U.). The vitamin A content in fruit showed a range of 94.59 (MO 25) to 184.74 I.U (MO 10). The mean vitamin A content in leaf and fruit was 10839.38 I.U. and 141.24 I.U. respectively.

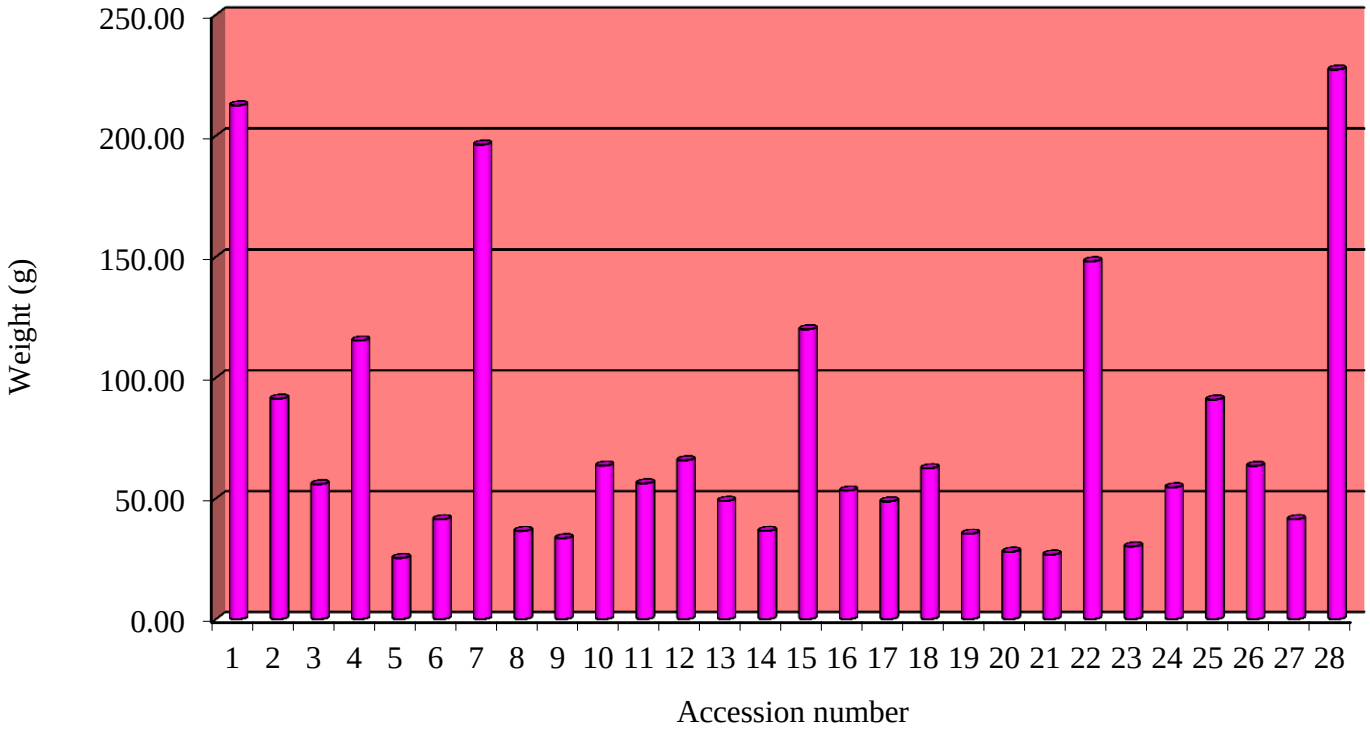


Fig.8 Weight of drumstick accessions

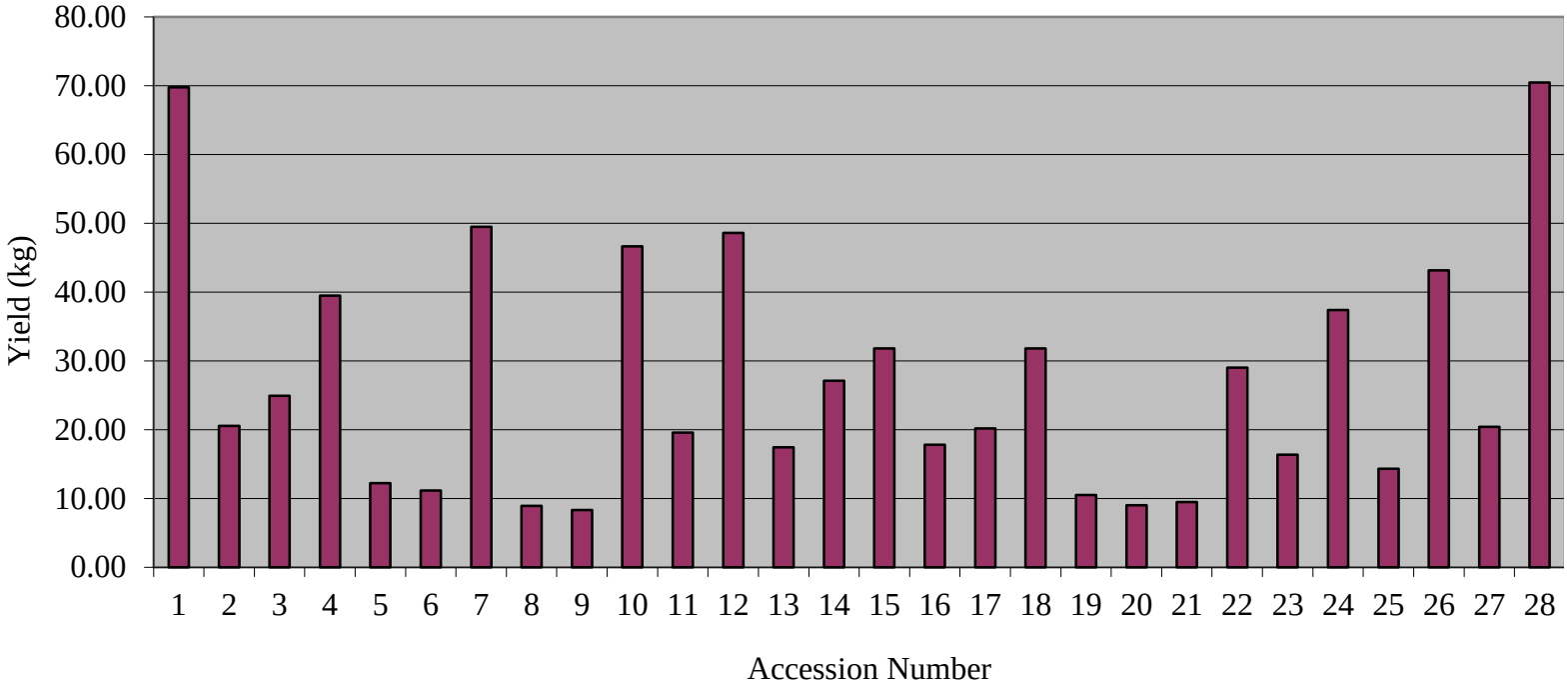


Fig.9 Yield of drumstick accessions

Table 5 Vitamin A and C content of drumstick leaves and fruits

Accession number	Vitamin A (I.U)		Vitamin C (mg/100g)	
	Leaf	Fruit	Leaf	Fruit
MO 1	8107.86	128.370	214.56	120.80
MO 2	9459.15	118.914	187.21	100.35
MO 3	11486.53	135.130	202.08	87.50
MO 4	11079.99	162.150	182.91	122.93
MO 5	8203.45	99.560	175.21	91.25
MO 6	12160.87	108.100	200.55	112.50
MO 7	11180.77	101.340	225.22	127.08
MO 8	9053.78	175.660	231.25	124.32
MO 9	8513.10	123.509	206.25	125.50
MO 10	12195.87	184.740	220.56	119.66
MO 11	11119.83	121.617	220.50	90.32
MO 12	11123.89	148.640	190.23	122.70
MO 13	11215.79	183.730	215.32	124.35
MO 14	11486.05	179.720	210.22	116.45
MO 15	10540.14	182.420	211.11	110.63
MO 16	10269.88	118.914	206.32	96.57
MO 17	10675.27	105.401	214.65	120.33
MO 18	12837.35	116.220	225.56	129.16
MO 19	10107.61	183.730	193.75	108.37
MO 20	11702.25	179.720	222.25	106.25
MO 21	8986.14	182.450	214.53	118.77
MO 22	12567.09	102.690	176.35	110.40
MO 23	10540.14	122.960	200.32	117.75
MO 24	11621.18	148.640	216.55	124.66
MO 25	11283.35	94.590	225.00	118.33
MO 26	13215.79	162.150	210.15	122.26
MO 27	11283.35	108.104	220.85	125.45
MO 28	11486.05	175.660	212.50	119.76
Mean	10839.38	141.244	208.28	114.80

4.4.2 Vitamin C

The vitamin C content in leaf and fruit was recorded highest in MO 18 (225.56 mg / 100 g in leaf and 129.16 mg / 100 g in fruit). The lowest vitamin C content in leaf was noted in MO 5 (175.21 mg / 100 g) and MO 3 recorded the lowest value in fruit (87.50 mg / 100 g).

4.4.3 Organoleptic Evaluation

The quality attributes considered under organoleptic evaluation were colour, doneness, tenderness, odour and taste. The accessions showed significant differences for all these attributes. The leaves of the accession MO 13 (21.4) was organoleptically superior to others based on the score and the lowest score was obtained for MO 14 (13.3). The fruit of accession MO 10 (23.3) was organoleptically superior to others based on the score and the lowest score was obtained for fruits of MO 25 (14.22). Organoleptic score of the leaves and fruits are given in Table 6.

4.5 INCIDENCE OF PESTS AND DISEASES

4.5.1 Diseases

Though much serious diseases were not observed in drumstick, a severe attack of Fusarium rot was noted during the course of study. The fruits and twigs of accessions MO 10, and MO 13 were severely infected. The symptoms appeared as water soaked sunken spots, later affecting the entire surface of the fruits and twigs. In advanced stages of infection, internal rotting of the fruit occurred (Plate 4). Finally the fruit shrivels and becomes unconsumable. The causal organism of the disease was identified as *Fusarium pallidoroseum* (Cooke) Sacc. (Plate 5).

4.5.2 Insect Pests

Hairy caterpillar (*Eupterote mollifera* W.) and bud borer (*Noordae moringae* Wlk.) were the major pests observed in the field.

Table 6 Organoleptic score of the leaves and fruits of drumstick accessions in the study

Accession number	Organoleptic score	
	Leaves	Fruits
MO 1	16.20	17.50
MO 2	19.50	21.40
MO 3	14.30	-
MO 4	13.95	22.50
MO 5	16.75	15.50
MO 6	15.30	20.32
MO 7	15.50	15.60
MO 8	18.60	16.50
MO 9	19.20	-
MO 10	20.33	23.30
MO 11	19.40	-
MO 12	20.50	-
MO 13	21.40	17.40
MO 14	13.30	19.25
MO 15	19.66	-
MO 16	21.10	-
MO 17	18.70	-
MO 18	15.40	15.30
MO 19	14.50	19.40
MO 20	18.25	20.00
MO 21	15.25	16.35
MO 22	20.12	20.20
MO 23	16.48	10.55
MO 24	19.50	18.45
MO 25	17.80	14.22
MO 26	16.60	22.26
MO 27	18.25	17.75
MO 28	20.90	22.55

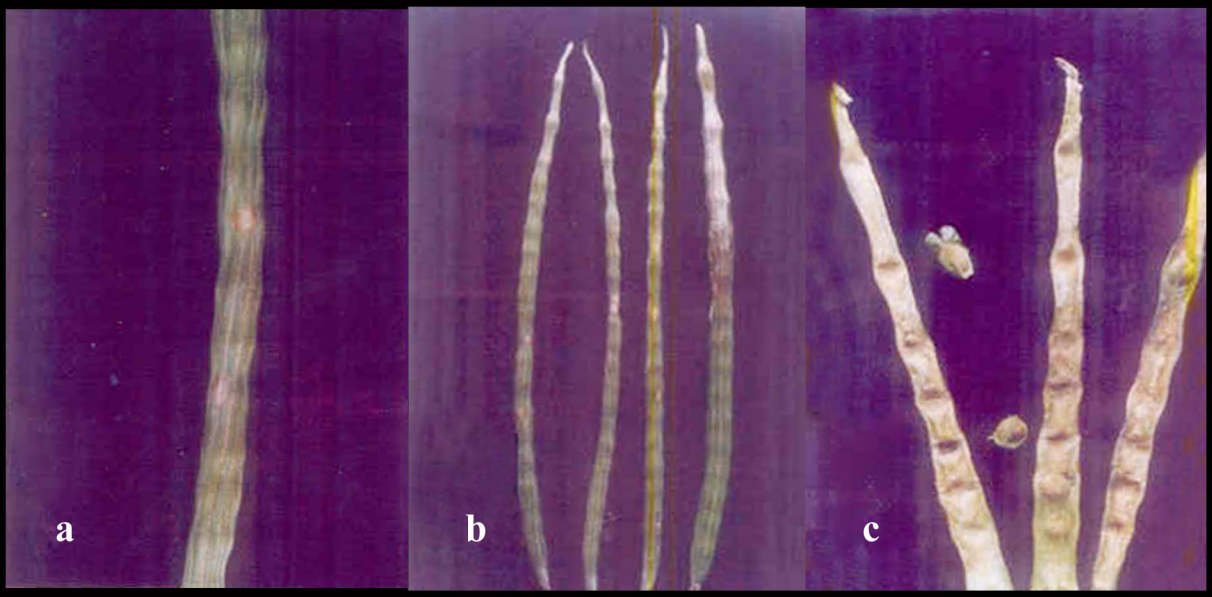


Plate 4

(a) Well developed spots (b) Advanced stage of infection (c) Internal rotting

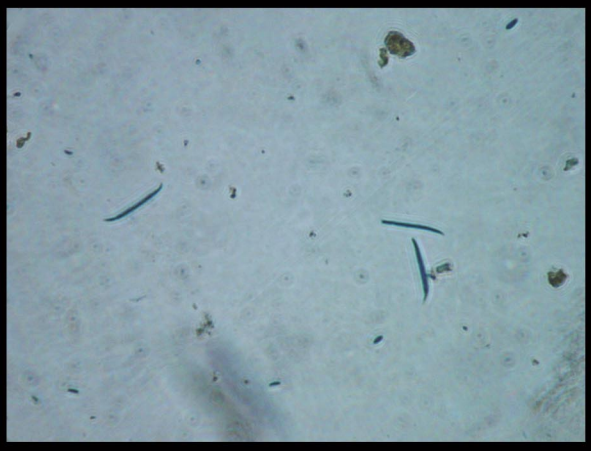


Plate 5

Conidia of *Fusarium pallidoroseum*

All the 28 accessions were observed for the incidence of bud borer (*Noordae moringae* Wlk.). The percentage of damage in leaf was calculated. The caterpillar feeds extensively on the leaves and the tree becomes devoid of leaflets. The severity of attack varied in the accessions and 100 % attack was recorded in accession MO 4 (Plate 6).

In the present study, all the accessions were observed for the incidence of hairy caterpillar, *Eupterote mollifera* W. The attack was noted during the months of November-December and all the accessions were equally susceptible. The eggs masses and larvae were found on all accessions. The caterpillar feeds extensively on the leaves and scrapes the bark (Plate 7).

4.6. DATA ANALYSIS

Based on the important morphological and quality characters, ranking was done for the twenty eight drumstick accessions as per Rajamony *et al.* (1994) (Table 7). Accessions with the least score was considered superior among the 28 accessions studied. Accession 26 was ranked first with a score of 88, followed by accession MO 28 with a score of 102.

4.7 MOLECULAR CHARACTERISATION

In this study, 25 accessions of drumstick from diverse geographical locations were analysed using RAPD markers to assess the extent of genetic diversity. The three accessions, MO 5, MO 8, and MO 19 were not included in the molecular analysis since they were found inferior compared to others. So they were excluded from RAPD analysis.

4.7.1 DNA Isolation

DNA isolation was done in morphologically different twenty five accessions following the procedure of Murray and Thompson (1980) with slight modifications.

Plate 6.
Attack of Bud borer (*Noordae moringae*)

a) Whole tree **b) Twig**

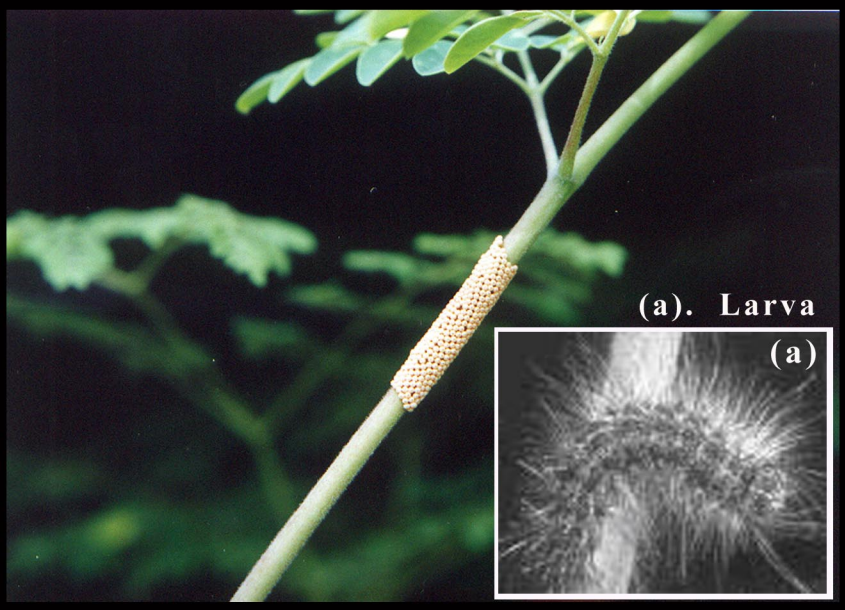


Plate 7.
Twig with the Egg mass of Hairy caterpiller (*Eupterote mollifera*)

Table 7 Overall ranking of drumstick accessions for important morphological and quality characters

Accession number	Leaf			Flowers/ inflorescence	Fruit			Yield (kg plant^{-1})	Vitamin A (I.U)		Vitamin C (mg/100 g)		Cumulative
	Length (cm)	Width (cm)	Weight (g)		Length (cm)	Girth (cm)	weight (g)		Leaf	Fruit	Leaf	Fruit	Rank
MO 1	19	28	18	9	3	2	2	2	28	15	12	11	149
MO 2	28	23	28	12	27	23	7	14	23	19	25	24	253
MO 3	27	27	20	3	6	26	14	13	8	14	20	28	206
MO 4	11	24	14	25	4	22	6	7	17	10	28	8	176
MO 5	20	26	27	10	25	26	28	22	27	27	27	26	291
MO 6	6	25	23	14	21	9	20	23	5	23	21	19	209
MO 7	7	11	1	1	7	25	3	3	14	26	3	2	103
MO 8	24	22	15	6	26	28	22	27	24	8	1	7	210
MO 9	5	4	22	16	16	13	24	28	26	16	19	3	192
MO 10	18	15	20	4	22	19	10	5	4	1	7	14	139
MO 11	9	9	16	11	18	4	13	17	16	18	8	27	166
MO 12	23	20	6	12	11	20	9	4	15	12	24	9	165
MO 13	14	6	11	24	20	18	17	19	13	2	10	6	160
MO 14	22	18	17	26	24	14	21	12	9	6	16	18	203

Table 7 Overall ranking of drumstick accessions for important morphological and quality characters

MO 15	13	8	2	8	5	6	5	10	19	5	15	20	116
MO 16	15	17	3	18	13	5	16	18	21	19	18	25	188
MO 17	26	19	19	22	23	12	18	16	18	24	11	12	220
MO 18	12	14	13	21	19	21	12	9	2	21	2	1	147
MO 19	21	16	4	26	15	17	23	24	22	2	23	22	215
MO 20	3	1	26	20	28	24	26	26	6	6	5	23	194
MO 21	16	12	8	15	10	11	27	25	25	4	13	15	181
MO 22	17	9	10	28	2	3	4	11	3	25	26	21	159
MO 23	8	13	24	6	12	16	25	20	19	17	22	17	199
MO 24	2	1	25	19	14	10	15	8	7	12	9	5	127
MO 25	10	7	12	17	8	7	8	21	11	28	4	16	149
MO 26	1	5	5	5	9	8	11	6	1	10	17	10	88
MO 27	4	3	9	23	16	15	19	15	11	22	6	4	147
MO 28	24	21	7	2	1	1	1	1	9	8	14	13	102

Emerging leaves collected fresh were found to be best with respect to DNA yield and purity. Addition of 1% PVP and ß- mercaptoethanol to the extraction buffer along with other reagents reduced the browning of the pellet. The DNA yield of the accessions ranged from 51µg/ml to 4140 µg/ml. The purity of DNA (A260/A280) ranged from 1.5 to 1.97 (Table 8).

4.7.2 Gel Electrophoresis

Gel electrophoresis was done to assess the quality of DNA isolated by using 0.7% agarose. For RAPD analysis, 1.2 % agarose was used. The gel electrophoresis was done at a voltage of 75 V. 1 X TAE buffer (0.04 M Tris acetate, 0.01M EDTA, pH 8.0) was used for carrying out the electrophoresis. DNA was observed as a single crisp band showing unsheared DNA.

4.7.3 Polymerase Chain Reaction

Polymerase chain reaction was done in twenty five accessions of drumstick. The 25µl of the reaction mixture contained 2.5µl of 10 x buffer (10mM Tris HCL pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl and 0.01 per cent gelatin), 5 picomoles of primer, 200 µM each of dNTPs, 0.6 units of Taq DNA polymerase and 20 ng of DNA. The programme consisted of an initial denaturation at 94°C for 1 min followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 34°C for 1 min 30 S and extension at 72°C for 2 min, and a final step of extension at 72ºC for 5 min. Finally the products of amplification were cooled to 4°C after the reaction.

35 primers were screened for their efficiency using the DNA isolated from MO 1 as the representative sample. Out of the 35 primers screened, nineteen primers namely, OPA-1, OPA-3, OPA-4, OPA-7, OPA-10, OPA-11, OPA-13, OPA-17, OPA-19, OPA-20, OPB-3, OPB-4, OPB-6, OPB-7, OPB-8, OPB-10, OPB-11, OPB-14 and OPB-15 yielded amplification products with

Table 8 Quality and Quantity of DNA isolated from drumstick accessions

Sl.no	Accession number	A_{260} nm	A_{280} nm	A_{260} /A_{280}	DNA yield (µg/ml)
1	MO 1	0.030	0.019	1.58	900.00
2	MO 2	0.091	0.059	1.54	2730.00
3	MO 3	0.017	0.009	1.89	510.00
4	MO 4	0.020	0.012	1.67	600.00
5	MO 6	0.023	0.014	1.64	690.00
6	MO 7	0.045	0.027	1.67	1350.00
7	MO 9	0.007	0.004	1.75	210.00
8	MO 10	0.138	0.070	1.97	4140.00
9	MO 11	0.059	0.033	1.79	1770.00
10	MO 12	0.010	0.006	1.67	300.00
11	MO 13	0.017	0.009	1.89	51.00
12	MO 14	0.038	0.021	1.81	1140.00
13	MO 15	0.017	0.009	1.89	510.00
14	MO 16	0.035	0.020	1.75	1050.00
15	MO 17	0.013	0.008	1.63	390.00
16	MO 18	0.016	0.009	1.78	480.00
17	MO 19	0.006	0.004	1.50	180.00
18	MO 21	0.035	0.022	1.59	1050.00
19	MO 22	0.009	0.005	1.80	270.00
20	MO 23	0.006	0.004	1.50	180.00
21	MO 24	0.014	0.008	1.75	420.00
22	MO 25	0.020	0.012	1.67	600.00
23	MO 26	0.016	0.009	1.78	480.00
24	MO 27	0.014	0.009	1.56	420.00
25	MO 28	0.010	0.018	1.80	300.00

the DNA. The total number of bands, number of faint bands, and number of intense bands produced by all the primers used for screening are presented in Table 9.

The primers produced 34 bands, of which 58 % (20 bands) were polymorphic. The highest numbers of RAPDs were produced by the primers OPA-10, OPA-13 and OPB-7. Of these primers, OPA-10 produced the highest numbers of three intense bands and two faint bands. The primers OPA-13 and OPB-7 produced two intense bands and two faint bands each.

The three primers namely OPA-10, OPB-7, OPA-13 were used for amplifying the DNA from the twenty five accessions. The three primers used in this analysis (OPA-10, OPA-13 and OPB-7) yielded 18 scorable bands with an average of 6 bands per primer. The nucleotide sequence of these three primers and no: of informative markers given by each primer are presented in Table 10.

Primer OPA-10 could amplify the DNA sample from all the accessions. There were seven scorable bands. Two bands were monomorphic for all the accessions. Accessions MO 1, MO 4, MO 6, MO 7, MO 11, MO 12, MO 15, MO 18, MO 22, MO 23, and MO 25 gave a total of five bands each. Four bands each were produced by accessions MO 2, MO 3, MO 6, MO 14, MO 17, MO 16, MO 28, MO 26, MO 24, and MO 27. The accessions MO 9, MO 10, MO 13 and MO 24 gave three bands each (Plate 8).

Primer OPB-7 produced seven scorable bands when used for amplification. Four bands each were produced by the accessions MO 1, MO 4, MO 6, MO 15, and MO 27. Only two faint bands were given by MO 3. MO 16 gave one intense band and two faint bands. There was no amplification in MO 28. All the other accessions gave three bands each. Two bands were monomorphic for all the accessions (Plate 9).

Table 9 Primer associated banding patterns with the DNA of MO1 using 35 primers

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

Table 10 Nucleotide sequence of primers and total number of informative RAPD markers amplified with them

Primer	Sequence	Number of informative RAPD markers
OPA-10	GTGATCGCAG	7
OPA-13	CAGCACCCAC	4
OPB-7	GGTGACGCAG	7

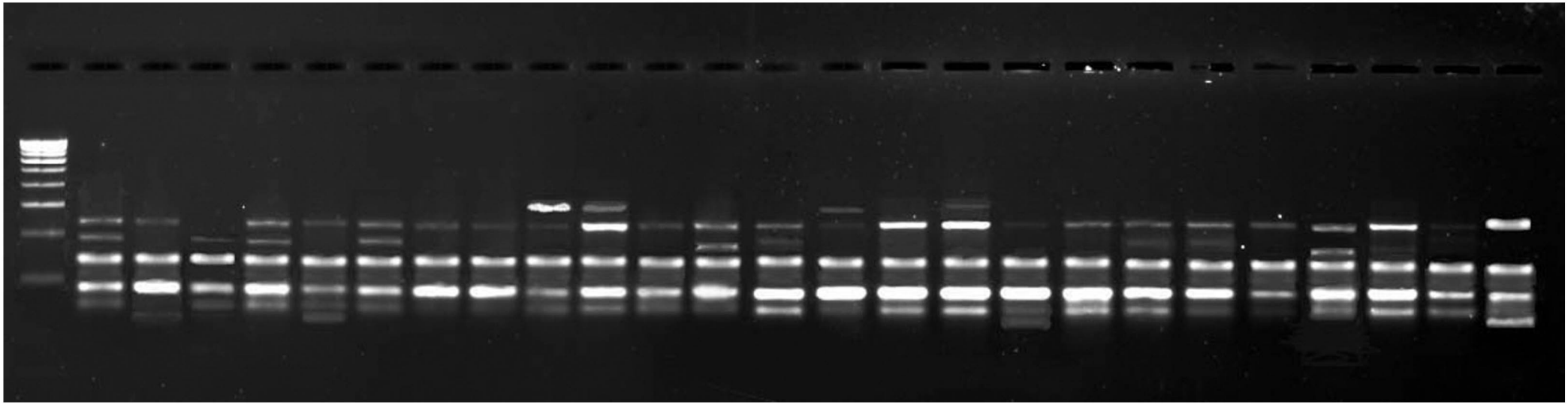
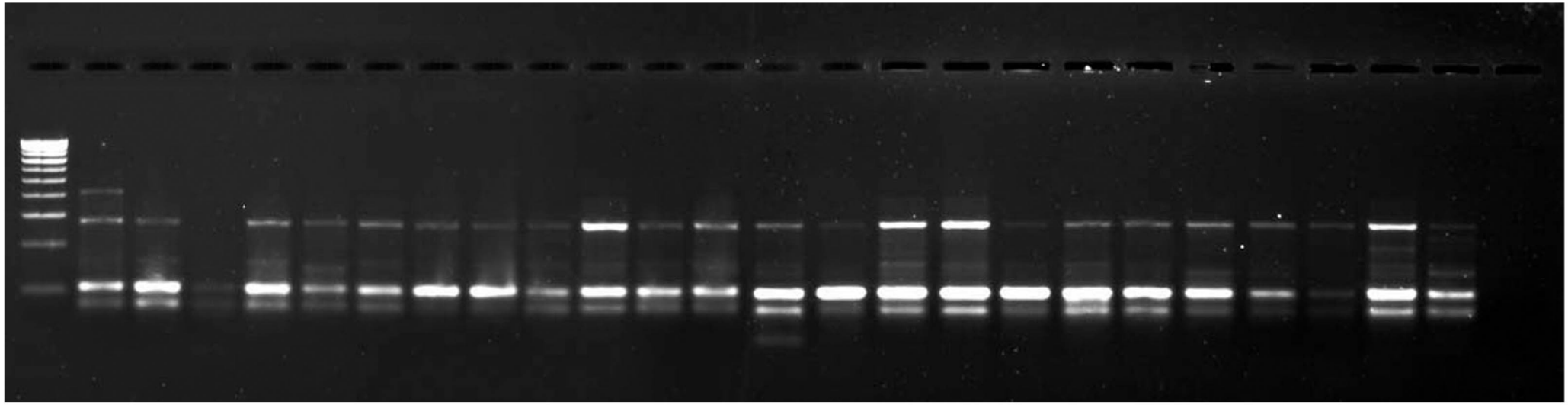


Plate 8

Amplification profile of the DNA of 25 drumstick accessions using primer OPA 10



1 2 3 4 6 7 9 10 11 12 13 14 15 16 17 18 19 21 22 23 24 25 26 27 28

Plate 9

Amplification Profile of the DNA of 25 drumstick accessions using primer OPB 7

Primer OPA-13 produced four scorable bands when used for amplification. Two bands were monomorphic for all the accessions except for accessions MO 11, MO 16, MO 18. Accession MO 27 produced a faint band. Accession MO 1 gave four bands and accession MO 28 produced three bands each (Plate 10). The PCR reaction was repeated at least twice in order to check the reproducibility.

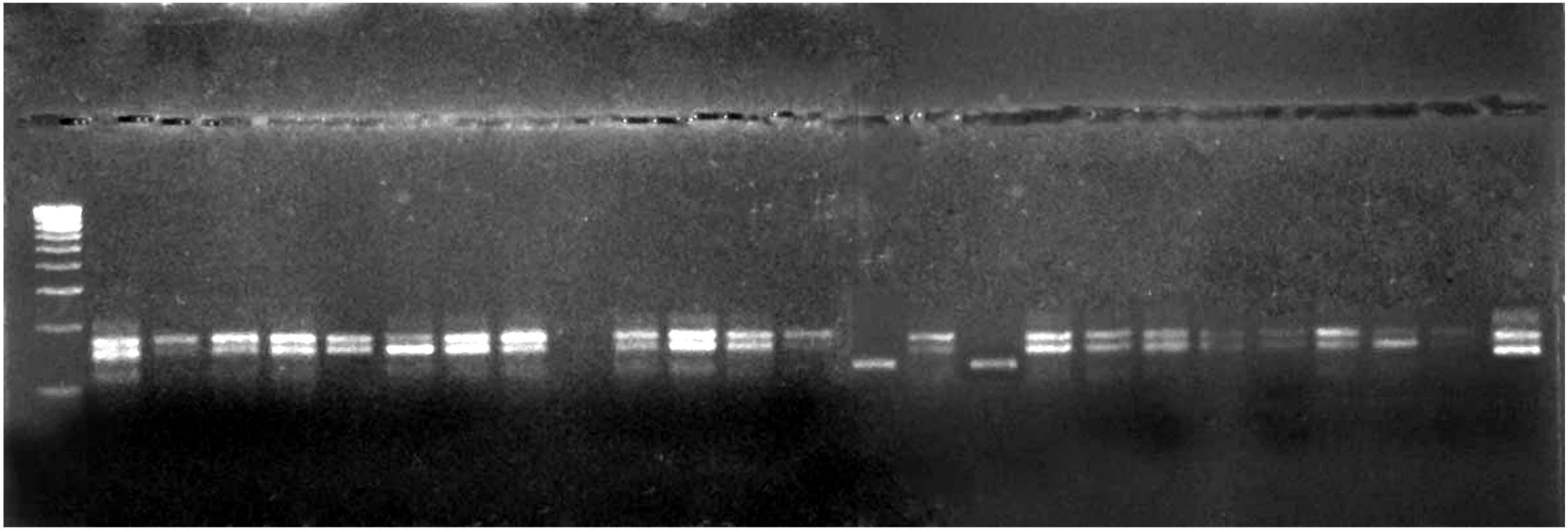
Amplification profiles of the DNA of the drumstick accessions using primers OPA-10, OPB-7 and OPA-13 are presented in Fig. 10, 11 and 12 respectively. Data obtained from all these three primers were used for statistical analysis.

4.7.4 Data Analysis

RAPD marker data were subjected to cluster analysis using NTSYS program to estimate the similarity indices and genetic relatedness among the accessions. The reproducible bands were scored for their presence (+) or absence (-) for all the accessions studied. A genetic similarity matrix was constructed using the Jaccard's coefficient method (Table 11). The pair wise coefficient values varied between 0.28 and 1.00. The least similarity coefficient values were that of accession MO 2 with MO 6 (0.28). The highest value for similarity index was obtained for accessions MO 12 with MO 14, MO 11 with MO 16 and MO 7 with MO 20 (1.00).

A dendrogram was generated by UPGMA cluster analysis based on similarity coefficient values (Fig. 13). Cluster analysis revealed that at about 65 % similarity index, the accessions grouped into five clusters.

Accessions MO 1, MO 9, MO 10, MO 13, MO 7, MO 20, MO 21, MO 4, MO 12, MO 14, MO 17, MO 25, MO 26 and MO 27 grouped together to form the largest cluster. Accessions MO 7 and MO 20 belonging to distant locations were having similarity index value of 1.00. Accessions



1 2 3 4 6 7 9 10 11 12 13 14 15 16 17 18 20 21 22 23 24 25 26 27 28

Plate1 0

Amplification profile of the DNA of 25 drumstick accessions using primer OPA 13

Fig.10 Representation of amplification profile of DNA using primer OPA-10

1	2	3	4	6	7	9	10	11	12	13	14	15	16	17	18	19	21	22	23	24	25	26	27	28
-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+
+	-	+	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	-	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-
-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+

Fig.11 Representation of amplification profile of DNA using primer OPB-7

1	2	3	4	6	7	9	10	11	12	13	14	15	16	17	18	19	21	22	23	24	25	26	27	28
+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
-	+	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	+	+	-
+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
+	+	-	+	+	+	-	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	-	-
-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

Fig.12 Representation of amplification profile of DNA using primer OPA-13

1	2	3	4	6	7	9	10	11	12	13	14	15	16	17	18	19	21	22	23	24	25	26	27	28
+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+
+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+
+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+

Table 11. Similarity matrix of 25 drumstick accessions based on the Jaccard's similarity index

	1	2	3	4	6	7	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25	26	27	28
1	1.00																								
2	0.44	1.00																							
3	0.62	0.66	1.00																						
4	0.66	0.71	0.71	1.00																					
6	0.33	0.28	0.50	0.37	1.00																				
7	0.77	0.62	0.62	0.87	0.33	1.00																			
9	0.87	0.50	0.71	0.75	0.37	0.87	1.00																		
10	0.77	0.44	0.62	0.66	0.50	0.77	0.87	1.00																	
11	0.37	0.33	0.60	0.42	0.75	0.37	0.42	0.37	1.00																
12	0.60	0.62	0.62	0.87	0.50	0.77	0.66	0.77	0.37	1.00															
13	0.77	0.44	0.62	0.66	0.33	0.77	0.87	0.77	0.37	0.60	1.00														
14	0.60	0.62	0.62	0.87	0.50	0.77	0.66	0.77	0.37	1.00	0.60	1.00													
15	0.50	0.50	0.71	0.55	0.57	0.50	0.55	0.66	0.42	0.66	0.50	0.66	1.00												
16	0.37	0.33	0.60	0.42	0.75	0.37	0.42	0.37	1.00	0.37	0.37	0.37	0.42	1.00											
17	0.50	0.71	0.71	0.75	0.57	0.66	0.55	0.66	0.42	0.87	0.50	0.87	0.75	0.42	1.00										
18	0.30	0.42	0.42	0.50	0.80	0.44	0.33	0.44	0.60	0.62	0.30	0.62	0.50	0.60	0.71	1.00									
20	0.77	0.62	0.62	0.87	0.33	1.00	0.87	0.77	0.37	0.77	0.77	0.77	0.50	0.37	0.66	0.44	1.00								
21	0.70	0.55	0.55	0.77	0.44	0.88	0.77	0.88	0.33	0.88	0.70	0.88	0.60	0.33	0.77	0.55	0.88	1.00							
22	0.75	0.57	0.83	0.62	0.42	0.75	0.85	0.75	0.50	0.55	0.75	0.55	0.62	0.50	0.62	0.37	0.75	0.66	1.00						
23	0.55	0.57	0.83	0.62	0.66	0.55	0.62	0.75	0.50	0.75	0.55	0.75	0.85	0.50	0.85	0.57	0.55	0.66	0.71	1.00					
24	0.50	0.50	0.71	0.55	0.37	0.50	0.55	0.50	0.42	0.50	0.50	0.50	0.75	0.42	0.55	0.33	0.50	0.45	0.62	0.62	1.00				
25	0 75	0.57	0.83	0.85	0.42	0.75	0.85	0.75	0.50	0.75	0.75	0.75	0.62	0.50	0.62	0.37	0.75	0.66	0.71	0.71	0.62	1.00			
26	0.54	0.55	0.55	0.77	0.44	0.70	0.60	0.70	0.33	0.88	0.54	0.88	0.60	0.33	0.77	0.55	0.70	0.80	0.50	0.66	0.60	0.66	1.00		
27	0.54	0.55	0.55	0.77	0.44	0.70	0.60	0.70	0.33	0.88	0.70	0.88	0.60	0.33	0.77	0.55	0.70	0.80	0.50	0.66	0.45	0.66	0.80	1.00	
28	0.37	0.60	0.60	0.42	0.40	0.37	0.42	0.37	0.50	0.37	0.37	0.37	0.42	0.50	0.42	0.33	0.37	0.33	0.50	0.50	0.42	0.50	0.33	0.33	1.00

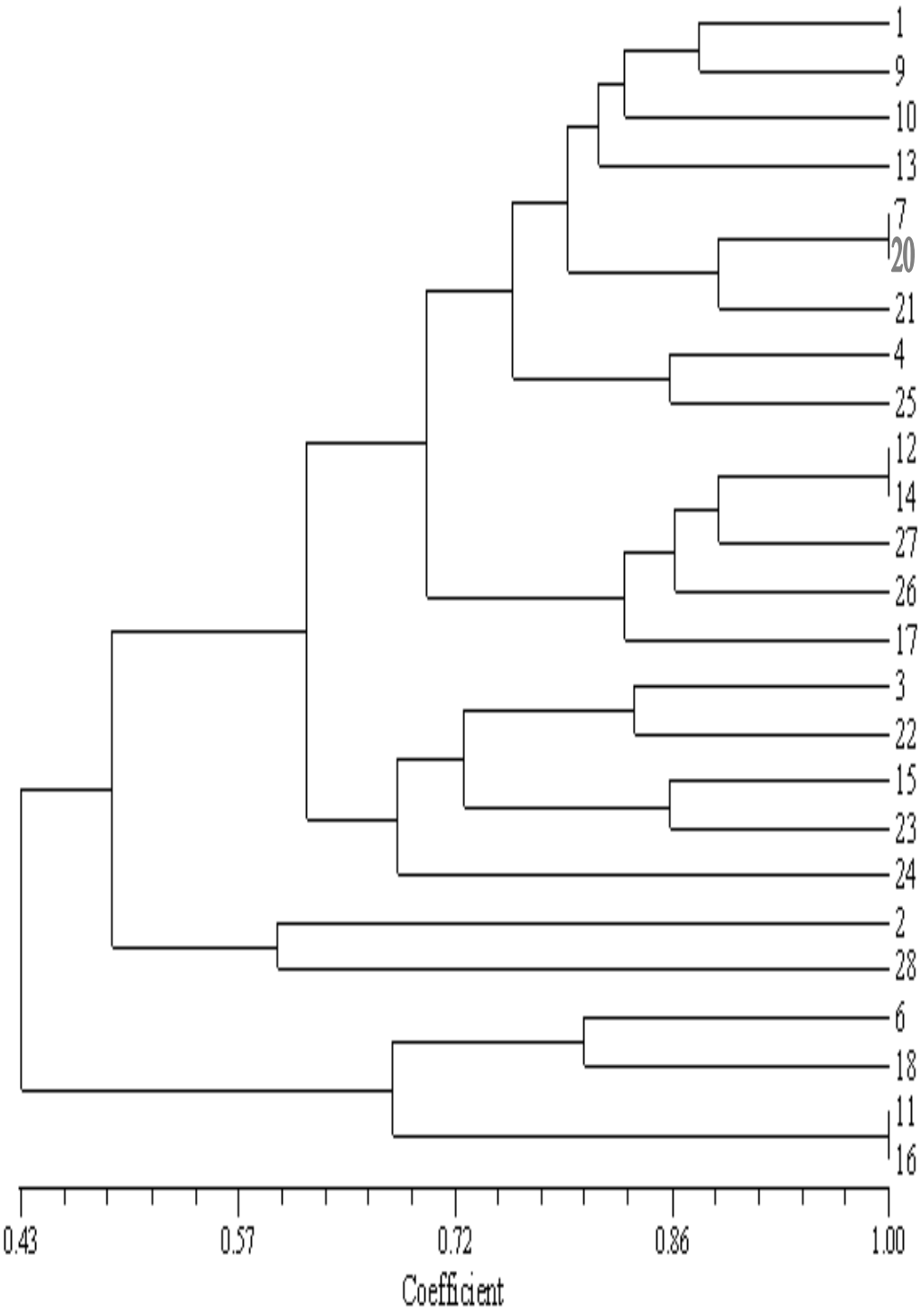


Fig. 13 Dendrogram obtained from RAPD analysis based on similarity coefficient values

MO 12 and MO 14 were having a similarity index value of 1.00, but they belonged to different geographical locations.

Accessions MO 3, MO 22, MO 15, MO 23 and MO 24 grouped together to form cluster II. Accessions MO 22, MO 23, MO 24 belonged to same location, but they were not morphologically similar.

Accession MO 28 and accession MO 2 formed two separate clusters each. Accession MO 28 was already distinct as superior as morphological ranking. Accessions MO 6, MO 18, MO 11, and MO 16 together formed cluster V. Accessions MO 11 and MO 16 which showed a similarity index of 1.00 belonged to adjacent locations.

Based on this cluster analysis also, accession MO 28 was found to be quite distinct and these can be used for their desirable characteristics in further breeding programmes. The narrow genetic base revealed in the present study emphasizes the need to exploit larger germplasm collections having more diverse morpho-agronomic traits in cultivar improvement programmes.

Discussion

5. DISCUSSION

Drumstick (*Moringa oleifera* Lam.) is a highly nutritious and popular tropical tree vegetable grown in South India. It is well known for its multipurpose attributes, wide adaptability and ease of establishment. Its leaves, pods and flowers are packed with nutrients important to both humans and animals. Although detailed studies on the distribution of genetic variability are limited, considerable variation has been reported in natural populations in India (Ramachandran *et al.*, 1980) indicating considerable potential for improvement. In spite of the wide variability, the drumstick landraces in this region has not been systematically characterised so far.

As the initial step in any crop improvement programmes, knowledge of the variability present in the available genetic material is very essential. Such information would facilitate crop improvement programmes and the conservation and exploitation of drumstick genetic resources. Morphological and agronomic traits usually form the basis for characterising the available material. However, it fails to provide reliable information at genetic level. The use of molecular markers along with morphological and agronomic traits is of relevance in this context. Molecular markers like Random Amplified Polymorphic DNA (RAPD) markers are widely used to characterise plant genetic resources. Compared to other techniques, RAPD is fast, easy and cheap. Also it is free from environmental influences.

Twenty eight diverse accessions of drumstick were collected from different places to study and assess the extent of variability present in these regions. The information on biometric and quality characters was recorded. Molecular characterisation using RAPD markers were also done and the genotypes were grouped to clusters based on similarity coefficient values. Distinct accessions were identified by morphological and molecular characterisation and the results are discussed below:

5.1 MORPHOLOGICAL CHARACTERISATION

Morphological characterisation provides information to aid the identification of accessions and to recognise important traits related to economic yield and potential constraints to yield.

5.1.1 Vegetative Characters

The vegetative characters were recorded both from the identified plants and from the plants maintained at the experimental field as mentioned in the chapter 3.

In the present study, tree height showed a range of 5 m to 12.5 m. The variability noted may be due to environmental factors, as trees tend to grow tall where resources are abundant, stresses are minor, and competition for light is less. The present study showed that trees under shaded condition remained stunted and were low in productivity. The height to which trees can grow and the biophysical determinants of maximum height are poorly understood. Shorter trees are preferred generally, as they are less expensive to prune, thin, and harvest. But the study reveals that the taller trees are more productive compared to shorter ones.

The number of main branches showed a range of 1 - 8. Accession MO 3 showed profuse branching even at a very young stage. Earlier Mohideen and Shanmugavelu (1982) have reported that the number of branches varied from 2-23. The variation seen may be due to the pruning done after each harvest.

The leaf geometry depends on factors such as light availability and it is found that as trees grow taller and older, the increasing leaf water stress due to gravity and path length resistance may ultimately limit leaf expansion. In this study, the leaf length ranged from 31.4 cm to 57.8 cm and leaf width showed a range of 19.4 cm to 48.2 cm. It was found that trees growing in shade were having poor leaf development and those trees in open favourable conditions had luxuriant vegetative growth. Studies conducted at AVRDC have shown that there is variability in leaf size and shape of leaflets (AVRDC, 2003).

5.1.2 Flowering Characters

Flowering in plants is dependant on climate factors as well as genetic factors. It can be continous, seasonal or erratic. Continuity of flowering can be due to constancy of environmental conditions or insensivity to environmental fluctuations.

The present study revealed that drumstick produces flowers in all the months except in the months of November and December. The results fully agree with the findings of Mathew (2002) and Mathew and Rajamony (2004). The equitable climate prevailing in this region may be attributed for this. The accessions MO 13, MO 24 and MO 26 showed three flowering peaks in a year whereas MO 16 recorded only one peak flowering. Variability in flowering time was reported by several workers in many locations (Duke, 1978; Ramachandran *et al*., 1980; Pushpangathan *et al*., 1996). However, Muthusamy (1954) reported two main flowering seasons *viz.,* March-April and July- September under Coimbatore conditions. There was only one flowering during February-March in Lucknow and Punjab (Singh, 1962; Nair and Singh, 1974).

The non-flowering noted in this study during November- December may be due to comparatively colder climate during these months and also the trees may be having a dormancy phase. It was seen that most accessions produced flowers during March and no or very less flowers were produced during June. This may be due to the sunny and rainy weather during these months.

Sadasakthi (1997) reported that the number of flowers per panicle ranged from 75.9- 99.87 in parents and in hybrids it ranged from 82.37-100.10 in annual drumstick in a study involving parents and 24 hybrids. In the present study, the number of flowers in an inflorescence varied from 25.4 (MO 22) to 65.23 (MO 28). The lesser number of flowers noted shows that all the accessions exhibited flower shedding.

Flower colour of all the accessions was cream except for accession MO 28 (Yellow). The variable floral architecture and colour determine the mating

systems in plant populations and also it plays a major role in attracting insects like bees and ants which are the main pollinators in drumstick. Flower shedding was seen in all the accessions. This may be due to moisture stress or other physiological reasons.

5.1.3 Fruit Characters

Fruit length is an economically important character as it decides the handling, packing and transportation of the fruits. The accessions showed variation in fruit characters as they were selected from different geographical locations. From the results, it was inferred that accession MO 28 produced the longest fruits (100.02 cm) and shortest fruits were recorded in MO 20 (32.3 cm). The fruit girth ranged from 4.22 cm (MO 8) to 8.36 cm (MO 28). These results are in agreement with the findings of Suthanthirapandian *et al.* (1992) and Pushpangathan *et al.* (1996).

The number of fruits per tree is the most important character in deciding the yield potentiality and economic value of the crop. The results showed a range from 174 (MO 16) to 612 (MO 24) fruits per tree. It was seen that lengthier fruits were produced lesser in number. Fruits of moderate length were produced more in number.

The average fruit weight showed a range of 25.3 (MO 5) to 227.3 g (MO 28) and the total fruit yield ranged from to 8.94 kg (MO 8) to 70.46 kg (MO 28). Similar results have been reported by Suthanthirapandian *et al.* (1989). The wide variation in yield may be due to the diverse geographical and ecological conditions or it may be due to genetic factors.

Kernal oil of drumstick seeds is used as a lubricant in precision equipments. This shows the economic and industrial importance of drumstick seeds. Additionally, the oil cake has been used as water purifier in industry. Therefore, industry prefers fruits with more seeds while culinary preference was directed to selection of fruits with less seeds. The number of seeds per fruit in the present

study ranged from 14.6 (MO 21) to 25.6 (MO 28). It was noted that larger sized fruits had more number of seeds.

5.1.4 Quality Characters

Drumstick is perhaps one of the richest sources of nutrients and vitamins, according to leading nutritional experts. It has been estimated that one glassful of fresh Moringa leaves contains the daily requirement of vitamin A for up to ten people.

Vitamin A, which acts as a shield against eye diseases, skin diseases, heart ailments, diarrhoea, and many other diseases is present in high quantities in drumstick fruits and leaves. The results showed that vitamin A content in leaf ranged from 8107.86 (MO 1) to 13,215.79 I.U (MO 26). The maximum vitamin A content in fruit was noted in MO 10 (184.74 I.U.) and lowest in MO 25 (94.59 I. U.). Similar results have been reported by Ramachandran *et al.* (1980).

Vitamin C, which fights illnesses including colds and flu are present in high amounts in leaves and fruits of drumstick. The vitamin C content in leaf and fruit was highest recorded in MO 18 (225.56 mg / 100 g). The lowest vitamin C content in leaf was noted in MO 5 (175.21 mg / 100 g) and MO 3 recorded the lowest value in fruit (87.50 mg / 100 g). The results are in agreement with the findings of Prabhakar *et al.* (2003) and Rai *et al.* (2004). Sato *et al.* (2003) evaluated the ascorbic acid in shoots and leaves of *Moringa* species and it showed a range of 158-323 mg per 100 g of fresh weight.

Organoleptic evaluation was done to assess the variation in consumer acceptance of pods and leaves. The quality attributes considered under organoleptic evaluation were colour, doneness, tenderness, odour and taste. Variability was noticed among the accessions studied and the slight bitterness noted in the leaves of accessions MO 14 and MO 25 are with high ascorbic acid content as reported by Dogra *et al.* (1975).

5.1.5 Incidence of Pests and Diseases

Among the insect pests, bud borer (*Noordae blitealis* Wlk.) and hairy caterpillar, *Eupterote mollifera* W. were noted during the present study. All the accessions showed incidence of bud borer. The pest was seasonal and was noted in January and the infestation varied from 13.3 - 100 per cent. The accessions in which infestation were lesser are to be selected. All the accessions were susceptible to the attack of hairy caterpillar. These pests have already been reported in drumstick by Seemanthini (1964) and Sivagami and David (1968).

Fruit rot caused by *Fusarium pallidoroseum* (Cooke) Sacc. was reported for the first time in Trivandrum district. The fruits were severely damaged by the attack and accessions MO 10 and MO 13 were highly susceptible to the disease during March-April. Mandokhot *et al.* (1994) had reported *Fusarium pallidoroseum* as the causal agent of twig canker in drumstick. Accessions which were resistant to the attack are more preferred for further selection in breeding programmes. Accessions MO 1, MO 2, MO 9, MO 24 and MO 28 were found to be totally free of the disease.

5.2. MOLECULAR CHARACTERISATION

The molecular characterisation was done in 25 accessions which were morphologically different among the 28 accessions studied.

5.2.1 DNA Isolation

As the biochemical composition of plant tissues and species varies considerably, it is difficult to use the same protocol for DNA isolation for different plant species. Even closely related species may require different DNA isolation procedures (Weising *et al.*, 1995). But the method proposed by Murray and Thompson (1980) with slight modifications was found to yield DNA of good quality and quantity. Young emerging leaves were used for DNA isolation and they gave suitable quantity of DNA. The quantity and quality of isolated DNA depends on the source of tissue as well as efficient disruption of plant cell wall (Babu, 2000). Mondal *et al*. (2000) suggested that tender leaves contain actively

dividing cells with lesser intensity of extra nuclear material like proteins, carbohydrates and other metabolites that interfere with the isolation of nucleic acids which in turn improve the quality of DNA. Tender leaves also facilitate easy cell disruption for DNA extraction.

The DNA yield of accessions ranged from 51µg ml^{-1} to 4140 µg ml^{-1}. The ratio A_{260}/A_{280} ranged from 1.5 to 1.8. This may be due to the interference of various compounds in the plant tissue during the experiment.

5.2.2 Agarose Gel Electrophoresis and RAPD Analysis

Agarose gel electrophoresis was used for analyzing the genomic DNA isolated from drumstick accessions as well as for RAPD products.

0.7 per cent agarose gel was used for visualizing genomic DNA and 1.2 per cent for RAPD analysis while Mulcahy *et al.* (1993) had used 0.9 per cent agarose for genomic DNA. Lim *et al.* (1999) used 0.8 per cent for the same purpose. Shibu *et al*. (2000) observed the separation of amplified products through 1.5 per cent agarose gel while Mondal *et al*. (2000) found that 1.4 per cent agarose the best. Low concentration of 1.2 per cent gel was found best by Padmesh *et al*. (1999). Voltage level of 75 V was found most suited for RAPD analysis. Galderisi *et al*. (1999) reported that amplification of the PCR products was obtained at a voltage of 100-120 V.

5.2.3 Polymerase Chain Reaction

To identify promising primers for RAPD analysis, 35 decamer primers (Operon Inc., USA) were screened using the DNA of MO 1. Five primers out of 35 primers yielded amplification products. The total number of bands ranged from 1-5. The primers which did not yield any amplification indicate that there was no sequence complimentary to the sequence of these primers in the DNA of MO 1.

A total of 34 RAPDs were generated of which 20 bands were polymorphic.

In the present study, three primers were identified based on the number of polymorphic bands observed. Prasannalatha *et al.* (1999) has suggested that individual primers have the capability to amplify the less conserved and highly repeated regions of the genomic DNA.

For further study, the three primers namely, OPA-10, OPA-13 and OPB-7 were used for amplifying the DNA of 25 accessions. PCR was repeated twice to check the reproducibility. Wolf *et al*. (1999) observed that the problems encountered with the reproducibility of RAPD patterns could be avoided by choosing primers with a dinucleotide sequence and high G-C content.

Works done at AVRDC in drumstick had used 75 primers out of 98 tested and 524 polymorphic bands were generated, which shows that by using a specific primer an average of seven bands could be obtained per primer (AVRDC, 2003). In the present study, an average of six bands per primer was obtained. However, Bhat and Jarret (1995) suggested that the number of polymorphism may be more important than the number of primers selected for the generation of stable phenogram. They also suggested that the number of polymorphisms required to generate a stable phonetic analysis would vary with the plant material under investigation and the sequences that are amplified.

The three primers used in this analysis (OPA-10, OPA-13 and OPB-7) yielded 18 scorable bands with an average of 6 bands per primer. Primer OPA-10 produced seven scorable bands when used for amplification. All the accessions could produce amplification with this primer. Primer OPB-7 produced seven scorable bands when used for amplification. Primer OPA-13 produced four scorable bands when used for amplification. The highest number of bands was given by the primer OPA-10.

5.2.4 Statistical Analysis

RAPD marker data were subjected to cluster analysis using NTSYS program to estimate the similarity indices and genetic relatedness among the accessions. The pair wise coefficient values varied between 0.28 and 1.00. Cluster

analysis revealed that at about 65 per cent genetic similarity, the accessions grouped into five clusters.

Accessions MO 1, MO 9, MO 10, MO 13, MO 7, MO 20, MO 21, MO 4, MO 12, MO 14, MO 17, MO 25, MO 26 and MO 27 grouped together to form the largest cluster. Accessions MO 7 and MO 20 from distant locations were having similarity index value of 1.00. Similarly accessions MO 12 and MO 14 which belonged to distant locations showed a similarity index value of 1.00.

Accessions MO 3, MO 22, MO 15, MO 23, and MO 24 grouped together to form cluster II. Accession MO 28 and accession MO 2 formed two separate clusters each. Accessions MO 6, MO 18, MO 11, and MO 16 together formed cluster V. Accessions 11 and 16 were from adjacent locations. Based on this cluster analysis also, accession MO 28 was found to be quite distinct and it can be used for desirable characteristics in further breeding programmes.

It was noted that the cluster formation was not fully in agreement with the geographical location. Also the accessions used in this study had narrow genetic variation and the numbers of primers were not sufficient for accurate results.

Not much literature is available about the morphological and agronomic characterisation of drumstick genotypes. So proper comparison of the results of this study with those of earlier studies is not possible. The polymorphism obtained in the present study will be useful for future studies on analysis of drumstick landraces. Knowledge about the extent of genetic variability present in drumstick landraces will be useful for developing new varieties with desirable characteristics and to establish a core collection as a part of germplasm conservation and management.

Summary

6. SUMMARY

The present study entitled "Characterisation of landraces of drumstick (*Moringa oleifera* Lam.) was conducted during the period 2002-2004. The objectives of this study was to analyse the extent of genetic variability using morphological characters and Random Amplified Polymorphic DNA (RAPD) markers and to identify superior genotypes in terms of yield, quality and reaction towards pest and disease incidence. The study included 28 diverse accessions of drumstick collected mainly from the southern part of Kerala. The biometrical observations were recorded from the located trees by conducting field visits and the materials are conserved at the Department of Olericulture for future studies. The results of the morphological and molecular characterisation are summarised hereunder:

Variability for morphological characters were present in all the 28 accessions studied. The tallest tree recorded was accession MO 22 (12.5 m) and the shortest was MO 15 (5.0 m). The number of branches varied from 1 (MO 22) to 8 (MO 3). The mean values for leaf length, width and weight were 44.58 cm, 33.79 cm and 5.17 g respectively. The variation in the colour of tender stem ranged from light green to deep purple. The accessions MO 13, MO 24, and MO 26 showed three flowering peaks in a year whereas MO 16 recorded only one peak flowering. The number of flowers per inflorescence varied from 65.23 (MO 28) to 25.4 (MO 22) with a mean of 49.9. The fruit length ranged from 32.3 cm (MO 20) to 100.02 cm (MO 28). The fruit girth varied from 4.16 cm (MO 5) to 8.36 cm (MO 28). Fruits per plant showed a range from 174 (MO 16) to 612 (MO 24). The maximum fruit weight recorded was in MO 28 (227.3 g). The total fruit yield varied from 8.94 kg (MO 8) to 70.46 kg (MO 28). The number of seeds per fruit was recorded highest in MO 28 (25.6) and the minimum number of seeds per fruit was recorded in MO 21 (14.6).

The quality attributes considered were Vitamin A, Vitamin C and organoleptic qualities of fruit and leaf. The vitamin A content in leaf was lowest in MO 1 (8107.86) and highest in MO 26 (13,215.79 I.U.). The vitamin A content in fruit showed a range of 94.59 (MO 25) to 184.74.18 I.U (MO 10). MO 18 recorded highest vitamin C content in leaf and fruit (225.56 mg / 100 g in leaf and 129.16 mg / 100 g in fruit). MO 5 recorded lowest vitamin C content in leaf (175.21 mg / 100 g) and MO 3 recorded the lowest value in fruit (87.50 mg / 100 g). The leaves of the accession MO 13 (21.4) was organoleptically superior to others and the lowest score was obtained for MO 14 (13.3). The fruit of accession MO 10 (23.3) was organoleptically superior to others based on the score and the lowest score was obtained for fruits of MO 25 (14.22).

Though serious diseases are not reported in drumstick, a fruit rot was reported for the first time in Trivandrum district during the course of study. The causal organism was identified as *Fusarium pallidoroseum*. The fruits and the twigs were severely damaged in accessions MO 10 and MO 13. Bud borer (*Noordae blitealis* Wlk.) and hairy caterpillar (*Eupterote mollifera* W.) were the major pests observed in the field. The severity of attack by bud borer varied in the accessions and 100 % attack was seen in accession MO 4 during March-April. Incidence of hairy caterpillar was noted during the months of November-December and all the accessions were equally susceptible.

Based on the morphological data, overall ranking for important characters was done for the 28 accessions. Accession MO 26 and accession MO 28 were found superior to others as per the ranking.

As part of the molecular characterisation, DNA was isolated from 25 accessions which were morphologically superior in terms of yield and quality characters. The DNA yield of the accessions ranged from 51µg/ml to 4140 µg/ml. The purity of DNA (A260/A280) ranged from 1.5 to 1.97. Out of 35 primers, 19 yielded amplification products. 5 primers showed polymorphism among the accessions and finally 3 primers were selected (OPA 10, OPA 13 and OPB 7) for RAPD analysis. These primers gave 18 scorable bands with an average of 6 bands

per primer. Dendrogram was generated by UPGMA cluster analysis. At about 65 per cent similarity index, the accessions formed five clusters. Cluster I had all accessions from Trivandrum except accessions MO 1, MO 7, MO 12 and MO 14. Accessions MO 22, 23 and 24 from adjacent locations had grouped in cluster II, but the accessions MO 3 and MO 15 from distant locations had also grouped in this. Accessions MO 2 and MO 28 formed separate clusters each. Accessions MO 11 and MO 16 from adjacent location had grouped together in cluster V. Cluster formation was not fully in agreement with geographical locations. Accessions MO 26 which was distinct in morphological data analysis had grouped in cluster I with thirteen other accessions. Accession MO 28 which was ranked second as per morphological analysis formed a separate cluster confirming its distinctness.

The study has revealed considerable variability in the landraces of drumstick which can be used for evolving new types with better yield and quality attributes. As this a preliminary study on this crop, detailed studies including more number of trees from wider geographic area has to be done for more precise and accurate results. As per this study accessions MO 26 and MO 28 can be used for their desirable characters in future breeding programmes.

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*Original not seen

CHARACTERISATION OF LANDRACES OF DRUMSTICK (*Moringa oleifera* Lam.)

RESMI, D.S.

Abstract of the thesis submitted in partial fulfillment of the requirement for the degree of

Master of Science in Horticulture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2004

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ABSTRACT

The present investigation on "Characterisation of landraces of drumstick (*Moringa oleifera* Lam.)" was carried out at the Department of Olericulture and Department of Plant Biotechnology, College of Agriculture, Vellayani during 2002-2004. The experiment was carried out using 28 diverse accessions of drumstick collected from different agro climatic situations particularly from southern parts of Kerala. The objective of the study was to assess the extent of genetic variability in the landraces of drumstick using morphological characters and Random Amplified Polymorphic DNA (RAPD) markers.

A survey was conducted to identify the morphologically distinct drumstick trees in the different regions of Kerala. Biometrical observations were recorded from the selected plants by conducting field visits. Morphological data was collected on vegetative characters, flowering characters, fruit characters, quality characters and reaction towards pest and disease incidence. Planting materials of each accession were also collected from the identified plants and planted them at the Department of Olericulture for further investigation and conservation.

All the accessions showed variation for the morphological characters studied. Height varied from 5.0-12.5 m. The leaf length, width and weight showed variability with a mean value of 44.58 cm, 33.79 cm and 5.17 g respectively.

Accessions MO 13, MO 24, MO 26 recorded 3 flowering peaks per year whereas all the remaining accessions showed two peak flowering except MO 16 which showed only one peak per year. The number of flowers per inflorescence varied from 65.23 (MO 28) to 25.4 (MO 22). The fruits per plant varied from 174 (MO 16) – 612 (MO 24) and the fruit yield ranged from 8.94 kg (MO 8) to 70.46 kg (MO 28).

The range of values for the quality characters were 8107.86 (MO 1) to 13,215.79 I. U. (MO 26) for vitamin A in leaf, 94.59 (MO 25) to 184.74 (MO 10) for vitamin A in fruit, 175.21 (MO 5) to 225.56 mg / 100 g (MO 18) for vitamin C in leaf and 87.5 (MO 3) to 129.16 mg / 100 g (MO 18) for vitamin C in fruit. The leaves of MO 13 and fruits of MO 10 were organoleptically superior compared to others studied.

A severe attack of fruit rot was noted in accession MO10 and MO 13. The fungus was identified as *Fusarium pallidoroseum* (Cooke) Sacc. All the accessions were observed for the incidence of bud borer and hairy caterpillar and all of them were equally susceptible. Based on important morphological characters, overall ranking was done in all the accessions and accession MO 26 and MO 28 were superior compared to others.

Molecular characterisation of 25 accessions of drumstick which were identified distinct as per morphological characterisation was done. Out of 35 primers tested, 19 yielded amplification products. Five primers showed polymorphism among the accessions and finally three primers were selected (OPA 10, OPA 13 and OPB 7) for RAPD analysis. These primers gave 18 scorable bands with an average of 6 bands per primer.

Dendrogram was generated by UPGMA cluster analysis. At about 65 per cent similarity index, the accessions formed five clusters. Accessions MO 1, MO 9, MO 10, MO 13, MO 7, MO 20, MO 21, MO 4, MO 12, MO 14, MO 17, MO 25, MO 26 and MO 27 grouped together to form the largest cluster. Accessions MO 7 and MO 20 which showed a similarity index value of 1.00 belonged to different locations. Accessions MO 12 and MO 14 were having a similarity index value of 1.00, but they also belonged to different geographical locations. Accessions MO 3, MO 22, MO 15, MO 23 and MO 24 grouped together to form cluster II. Accessions MO 22, MO 23, MO 24 belonged to same location, but they were not morphologically similar. Accession MO 28 and accession MO 2 formed each separate cluster respectively. Accession MO 28 was

already distinct as superior as morphological ranking. Accessions MO 6, MO 18, MO 11 and MO 16 together formed cluster V. Accessions MO 11 and MO 16 were from adjacent locations and showed a similarity index of 1.00.

Cluster formation was not fully in agreement with geographical locations. Morphological studies had shown that MO 26 and MO 28 were superior when compared to others. Molecular studies also confirms the distinctness of MO 28 and it can be used in further breeding programmes.

The present study using morphological characters and RAPD markers have shown considerable variability in the available landraces. However, accessions with broader genetic base have to be studied with more number of primers for getting accurate results.

Appendices

APPENDIX-1

Score card for the organoleptic evaluation of cooked drumstick leaves and fruits

Quality attributes	Subdivisions of Attributes	Score of each attribute	1	2	3	4
Appearance(Colour)	Natural Colour well preserved	5				
	Colour fairly preserved	4				
	Moderately preserved	3				
	Slightly discoloured	2				
	Highly discoloured	1				
Doneness	Well Cooked	5				
	Fairly Cooked	4				
	Just Cooked	3				
	Slightly Cooked	2				
	Slightly overcooked	1				
Tenderness	Very Soft	5				
	Soft	4				
	Fairly Soft	3				
	Fibrous	2				
	Very Fibrous	1				
Odour	Very Pleasant	5				
	Fairly Pleasant	4				
	No Odour	3				
	Fairly Unpleasant	2				
	Unpleasant	1				
Taste	Very good	5				
	Good	4				
	Bland	3				
	Bad	2				
	Very bad	1				