MORPHOLOGICAL AND GENETIC CHARACTERISATION OF MANGO (*MANGIFERA INDICA* L.) VARIETIES IN MOZAMBIQUE

by

Cecilia Ruth Bila Mussane

Submitted in accordance with the requirements for the Magister Scientiae Agriculturae Degree in the Department of Plant Sciences (Plant Breeding), in the Faculty of Natural and Agricultural Sciences at the University of the Free State

University of the Free State

Bloemfontein

South Africa

May 2010

Supervisor: Dr. A. van Biljon

Co-supervisor: Prof. L. Herselman

DECLARATION

"I declare that the thesis hereby submitted by me for the degree of Magister Scientiae Agriculturae at the University of the Free State is my own independent work and has not previously been submitted by me to another University/Faculty.

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DEDICAÇÃO

Esta pequena contribuicao para a fruticultura em Moçambique dedico para os meus filhos Neyde e Siaka, pelo longo tempo que estiveram longe de mim.

ACKNOWLEDGEMENTS

I am grateful to all organisations, institutions and individuals that supported me during my studies, there where so many and it is not possible to mention all of them, but I address my sincere gratitude to all who contributed to the success of this study. The list below contains just a few of the many contributors.

- Southern African Development Community/Implementation and coordination of Agricultural Research and Training in the SADC Region (SADC/ICART) programme hosted at the University of the Free State, South Africa, for the financial support that enabled me to accomplish my Baccalaureus Scientiae Agriculturae, Baccalaureus Scientiae Honours and Magister Scientiae Agriculturae degrees.
- The Government of Mozambique, especially the Agricultural Research Institute of Mozambique (IIAM), for allowing me to proceed with my studies.
- Dr. Angie van Biljon for excellent supervision, technical advice, encouragement and huge patience that touched my heart.
- Prof. Liezel Herselman for the valuable input and expertise that she provided, including molecular work at the laboratory of Plant Breeding until the end of document submission that will be unforgettable indeed for the rest of my life.
- Dr. Elizma Koen for her useful input, and encouragement during the first year of the research.
- I wish to thank all staff of the Plant Breeding department, Prof. M.T. Labuschagne for her leadership and guidance, Mrs. Sadie Geldenhuys for her valuable help in various issues with kindness during my studies.
- Dr. L. Schwalbach, for his appreciated coordination and administrative effort that made the University of the Free State, SADC/ICART a success. He was committed to find financial solutions in difficulty times.
- My husband Cuna, my children Neyde and Siaka, my mother, my brothers and all my relatives and friends for their encouragement, understanding and patience.
- My fellow postgraduate students and colleagues in the SADC/ICART programme at the University of the Free State, South Africa for their cooperation and friendship.

- My colleagues from the fruit crop programme at Umbeluzi Research Station, Mario Faniquico and Albino Langa. Their support was fundamental during the field work.
- My fellow students and colleagues, Tyson, Katleho, Godwin, Fred, Scott, Oscar, Davies, and Abe for their cooperation and assistance.
- Above all, I thank God almighty and omnipotent because he is my guider and without him I can’t do anything.

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List of abbreviations

ACC	1-aminocyclopropane-1-carboxylate
AFLP	Amplified fragment length polymorphism
AFS	Adherence of fibre to fruit skin
ARC	Agricultural Research Council
ASF	Adherence of fibre to fruit skin
ASP	Adherence of fruit skin to pulp
ATC	Alpha tocopherol equivalent
ATP	Adenosine triphosphate
bp	Base pair
BSA	Bovine serum albumin
cDNA	Complementary DNA
CEW	Crown east-west
CITEM	Centre for International Trade Expositions and Missions
CNS	Crown north-south
CoA	Cofactor A
CSH	Crown shape
CTAB	Hexadecyltrimethylammonium bromide
DFI	Density of flowers
DFS	Depth of fruit stalk cavity
DLF	Density of lenticels
DNA	Deoxyribose nucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithriotreitol
EDTA	Ethylenediaminetetraacetate
EMBRAPA	Empresa Brasileira de Pesquisa Agropecuária

FAT	Fruit attractiveness
FBC	Fruit background colour
FBI	Fruit bearing intensity
FBT	Fruit beak type
FDE	Foliage density
FIA	Intensity of anthocyanin
FLP	Fibre length in the pulp
FMP	Fruit maturation period
FNP	Fruit neck prominence
FRD	Fruit diameter
FRL	Fruit length
FRW	Fruit weight
FSH	Fruit shape
FSS	Pulp texture of ripe fruit
FSW	Fruit waxyness
HGT	Tree height
IAG	Inflorescence axis growth
IFC	Inflorescence colour
IFL	Inflorescence length
IFP	Inflorescence position
IFS	Inflorescence shape
IFW	Inflorescence width
IPGRI	International Plant Genetic Resources Institute
ISSR	Inter-sequence repeat microsatellites
KCl	Potassium chloride
LAS	Leaf apex shape
LAT	Leaf attitude
LBLL	Leaf blade length
LBLS	Leaf blade shape
LBLW	Leaf blade width

LFR	Lead fragrance
LMA	Leaf margin
LSF	Length of stone fibre
LSP	Length of stamens
$MgCl_2$	Magnesium chloride
min	Minute
MSH	Maternal half-sib
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NDI	Nature of disc
NSS	Number of stamens
PAGE	Polyacrylamide gel electrophoresis
PAR	Pulp aroma
PCA	Principal Co-ordinate analysis
PCF	Pulp colour of ripe fruit
PCR	Polymerase chain reaction
PIR	Pubescence of inflorescence raquis
PJU	Pulp juiceness
PLB	Presence of leaf bracts
PLE	Petiole length
PSV	Pattern of veins stone
PTF	Presence of turpentine flavour
PTR	Pulp texture of ripe fruit
QFP	Quantity of fibre in the pulp
QFS	Quantity of fibre on stone
QLO	Quantity of fibre in the pulp, latex oozing by penduncle
RAPD	Random amplified polymorphic DNA
RBT	Ratio Bix/titratable acidity
RFLP	Restriction fragment length polymorphism

rpm	Revolutions per minute
SAM	S-adenosyl methionine
SCF	Skin colour of ripe fruit
SDL	Seed length
SDS	Seed shape
SDW	Seed width
SDWG	Seed weight
SFA	Shape of fruit apex
spp	Specie
SSR	Simple sequence repeat
STL	Stone length
STT	Stone thickness
STW	Stone width
STWG	Stone weight
TA	Titratible acidity
TBE	Tris-Boric acid-EDTA
TE	Tris-EDTA
TEB	Type of embryony
TGH	Tree growth habit
Tris-Cl	Tris (hydroxymethyl) aminomethane
TRK	Tree circumference
TSF	Texture of stone fibre
UPGMA	Unweighted pair group method of arithmetic averages
USA	United States of America
USDA	United States Department of Agriculture
VST	Veins on stone

SI UNITS

°C	Degrees Celsius
µ	Micron
µg	Microgram
µl	Microlitre
cm	Centimetre
ft	Feet
g	Gram
h	Hour
ha	Hectare
IU	Amount of a substance based on measured biological activity or effect
kcal	Kilocalories
kg	Kilogram
kj	Kilojoule
km	Kilometre
m	Metre
M	Molar
meg-RE	Micrograms of retinol equivalent
mg	Milligram
ml	Millilitre
mm	Millimetre
mM	Millimolar
MT	Million ton
N	Normal
ng	Nanogram
pH	Measure of acidity/basicity
pmol	Picomolar
t	Ton
U	Unit
V	Volt
v/v	Volume per volume
W	Watt
w/v	Weight per volume

CHAPTER 1
GENERAL INTRODUCTION

In the 1960's little was known about mangoes outside the tropics and there was virtually no international trade involving fresh fruit (Litz, 1997). Today mangoes are the fifth most important fruit crop, following citrus, banana, grape and apple. In 2002 the world export market for fresh and processed mango fruits had a value of US$ 396 700 000 (FAO, 2002). In 2008 mangoes comprised nearly 40% of the global tropical fruit harvest that was estimated at over 82.7 million ton (MT) (FAO, 2009; FAO, 2010). The increase in mango production worldwide can be attributed mainly to the green revolution, which through the use of Mendelian inheritance principles of crop breeding has brought additional supply of staple food as well as horticultural crops to developing countries. Furthermore, this rise in productivity is also due to optimisation of agronomical and horticultural field practices and better control of pests and diseases. Mango because of its long juvenile period and heterogeneity, has taken advantages of these technologies. This is reflected in an extension of new planting areas, planting of regular bearing cultivars, control of flowering, irrigation management, fertilisation and use of agrochemicals (Mukherjee, 1997). Genetic markers are recognised as one technique that increased the advance in mango improvement as well as the other classifiable methods such as harmonised open pollination and clonal selection (Iyer and Dinesh, 1997).

Mozambique has a favourable climate that enables the commercial production of mango trees. An average of about 11 000 mango trees represent the most abundant fruit tree throughout the country. The Zambezia province has 25.4%, followed by Manica with 16.2% and Sofala province with 11.6% (INE-MADER, 1999/2000). Despite the many fruit trees in the Zambezia province, the Manica province has a more suitable subtropical climate that is ideal for mango cultivation (World Bank, 2006).

In Mozambique mango trees are extensively cultivated and are commonly planted in a scattered manner in fruit-gardens and orchards. It is also found on small properties, where trees with fruit not appreciated by the external markets, characterised by the presence of

fibre, a turpentine smell, small sized or overweight fruit and inadequate fruit colour are often used by the local people (Ferrao, 1999). Fruit producers in Mozambique have received South African plantlets and “Tommy Atkins”, “Kent”, “Keitt” and “Heidi” appear to be the most popular cultivars. The acceptance of these varieties is associated with their eating quality (World Bank, 2006). Research on fruit growing in Mozambique is in the preliminary stage. More work is needed with respect to germplasm collection throughout the country, as well as the characterisation and evaluation of the germplasm for future improvement activities (Ferrao, 1999). The phenomenon of allopolyploidy, out breeding, and the different agro-climatic conditions in mango growing areas, has resulted in a high level of genetic diversity in mangoes. There furthermore exists confusion in the nomenclature of mangoes due to different local names for the same variety. Characterisation of germplasm is thus important for better use of genetic resources (Ravishankar et al., 2004). Unique and important problems are found in mango production, like pre- and post-harvest anthracnose, irregular bearing, short shelf life and internal breakdown of the fruit. The causes of these problems are genetic and according to Litz (2004) they can be solved through conventional breeding.

Mozambique has a tropical climate with summer rainfall that is conducive to the production of tropical crops. Mozambique therefore has a large local market for mangoes. There is a possibility of substituting imports by increasing production (World Bank, 2006) and because of climatic conditions Mozambique has a great opportunity to access international markets through the early mango season, starting in November**.** To extend the period of availability of the product throughout the year, fruit developing later can be an advantage for local markets in the country (Ferrao, 1999).

The study will include morphological and molecular characterisation of 30 mango varieties from Mozambique. Morphological characterisation is traditionally the most common method used and many different crops have been studied (González et al., 2002) such as mango (Ascenso et al., 1981; Illoh and Olorode, 1991; Jintanawong et al., 1992; Subedi et al., 2009), banana (Gibert et al., 2009), citrus (Domingues, 1999), cashew nut (Chipojola et al., 2009) and tropical trees (Hargreaves, 2006).

Molecular characterisation encompasses modern methods that complement morphological descriptors and has become quite popular, each with its own advantages and disadvantages (Lavi et al., 1993). Studies in *Mangifera indica* L. have been conducted using different molecular markers. Techniques used include random amplified polymorphic DNA (RAPD) (Karihaloo et al., 2003; Schnell et al., 2004), restriction fragment length polymorphism (RFLP) (Eiadthong et al., 1992; Chunwongse et al., 2000; Ravishankar et al., 2004), amplified fragment length polymorphism (AFLP) (Eiadthong and Yonemori, 2000; Hautea et al., 2001; Kashkush et al., 2001; Teo et al., 2002; Yamanaka et al., 2006), microsatellites or simple sequence repeats (SSRs) (Eiadthong et al., 1999; Duval et al., 2005; Honsho and Nishiyama, 2005; Schnell et al., 2005) and inter-SSRs (González et al., 2002; Pandit et al., 2007; Xian-Mei and Cheng-Xiang, 2007).

The main aims of this study were to describe and evaluate the main plant and fruit characteristics of 30 local varieties from Mozambique. The specific objectives were to:

a. Determine the genetic relationship and diversity among 30 mango varieties using morphological characterisation, including assess the chemical characteristics by determining the brix and acid content in the fruit and make varietal recommendations regarding the suitability of different varieties for fresh consumption, local consumption, exportation or fruit processing.

b. Determine the genetic relationship and diversity between 30 mango varieties using AFLP molecular characterisation.

c. Compare dendrograms produced from morphological and AFLP molecular markers.

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CHAPTER 2

LITERATURE REVIEW

2.1 ORIGIN, DISTRIBUTION AND TAXONOMY OF MANGO

According to history, the emperor Akbar, who reigned in northern India, from 1556 to 1605, planted an orchard of a hundred thousand mango trees. Because of the phyto-geographical distribution of related species, the fossil records and the presence of plenty of wild and cultivated varieties in India, it was stated that the region of mango origin was most likely Indo-Burma. From here mangoes were probably exported to other countries and continents (Singh, 1968; Kostermans and Bompard, 1993). Today the production areas for mango fruits can be grouped into different groups viz. Florida (USA), Mexico, Central America, West Indies (Caribbean islands), South America, Africa/Arabian Peninsula, Indian subcontinent and Indochina (China/Indonesia/Pacific) (Anonymous, 2008).

Mangoes are a member of the Anacardiaceae family that comprises 73 genera, fitted in the order Sapindales. This order belongs to the sub-class Rosidae from the class Magnoliopsida and division Magnoliophyta (Bompard and Schnell, 1997; Anonymous, 2008). The genus *Mangifera* to which mangoes belong consists of 69 species and is classified into two sub-genera with several sections based on morphological characters. Among the species, *M. indica* is the most important, although there are other species that also produce edible fruits such as *M. altissima* Blanco, *M. lagenifera* Griff., *M. macrocarpa* Blume, *M. odorata* Griff. and *M. sylvatica* Roxb. (Bompard, 1993).

2.1.1 HISTORY AND CURRENT DISTRIBUTION OF MANGO IN AFRICA

Mango trees were reported in Somalia as early as 1331 (Griesbach, 2003). Ivory and slave traders brought seed into Kenya beginning in the fourteenth century and even today Kenya exports mature mangoes to France and Germany and mature and immature mangoes to the United Kingdom, the latter for chutney-making (Anonymous, 2008).

In the sixteenth century the Portuguese disseminated mangoes from Goa to East and West Africa and Brazil, because of the trade with spices and other vegetables (Mukherjee, 1953). Cultivation started in the Caribbean islands in Barbados in 1742 and Jamaica in 1782. Fruit was transported from the Philippines to Mexico in colonial times. Egypt produces 110 000 tons of mangoes annually and exports reasonable amounts to 20 countries in the Near East and Europe. Mango culture in Sudan occupies about 10 000 ha producing a total of 60 000 ton per year (Anonymous, 2008). There is no documentation of the introduction of mangoes into South Africa. However, a plantation was established in Kwazulu-Natal in 1860. Today the South African market, in all probability, has achieved about 60 000 tons annually and fresh mangoes are exported to Europe (Human, 2008).

2.2 IMPORTANCE OF MANGO WORLDWIDE

2.2.1 ECONOMY

The world´s total mango production has increased over the years, from about 24.4 MT in 1999 (FAO, 2000) to 33.8 MT in 2008 (FAO, 2009). The major producers are Asia with about 74%, followed by Latin America and the Caribbean with 16%, Africa with 10% and less than 1% for Europe and Oceania (Galan Sauco, 2004; FAO, 2009). Importation of processed mango such as canned mangoes, mango flavoured beverages and processed mango pulp has also increased in the last few years (de Almeida et al., 2000). The major importers are France, Great Britain, Netherlands, Germany, Belgium, Italy, Denmark and United States of America (Pimentel et al., 2000; Human, 2008), while Mexico, Philippines, Pakistan, India, Thailand, India and South Africa are the major mango exporting countries (de Almeida et al., 2000). In 1998 the total value of mango exportation was about US$ 375.5 million and the total exported volume was 510 thousand ton, compared with a production of 23-28 MT. This implies that only a small quantity of production was exported and consequently there is a possibility to increase the export market. The main characteristics of international mango markets are that the price is established at the import market. The consumer profit is also an important

variable that determines mango demand and it is important that consumers are given information about alternative forms of consumption (de Almeida et al., 2000).

2.2.2 Nutritional value

Mango is renowned for combating nutritional disorders (Griesbach, 2003). Each part of the plant has a number of functions: The fruit can heal many diseases such as beriberi, bronchial diseases, kidney stones, insomnia, brain fatigue, mental depression and heartburn. It is a good laxative, depurative, digestive and diuretic and is advised for nervous people (Arcos, 1999). Unripe fruit can be used against exhaustion and heat stroke and a half ripe fruit mixed with salt and honey is indicated to cure gastro-intestinal disorders. The leaves can be prepared as an infusion and help for tooth ache, weak teeth, throat infections and elimate pyorrhoea. A bark infusion can be a remedy for mouth infections in children (Bally, 2006). A gum, tannin and a yellow dye can furthermore be obtained from the trees (Narasimha Char et al., 1979).

Mango fruit contains a large fraction of the human's daily needed essential minerals and vitamins. The calorific value of mango is generally derived from the sugars and is as high as that of grapes and even higher than that of apples, pears or peaches. The protein content is usually a little higher than that of other fruits, except avocado. Mangoes are also a good source of thiamine and niacin and contain some calcium and iron as seen in Table 2.1. Mango fruits are an excellent source of vitamin A and C, potassium and beta-carotene. It is also high in fibre, but low in calories (approximately 110 calories per average sized mango), fat (only 1.0 g) and sodium. Research results indicate that dietary fibre may help prevent certain types of cancer and can reduce blood cholesterol levels and that one medium mango fruit can contain up to 40% of the daily fibre requirement (Griesbach, 2003). Fresh mangoes are processed and preserved into a wide range of products including pulps, juices, frozen slices, dried slices, pulp (fruit leather), chutneys, jam, pickles, canned in syrup, and sliced in brine (Bally et al., 2009).

Table 2.1 Nutritional value per 100 g fresh mango pulp

Constituent	Per 100 g fresh pulp	Constituent	Per 100 g fresh pulp
Water	81.79 g	**Aminoacids**	
Energy	65 kcal (272 kJ)	Tryptophan	0.008 g
Protein	0.51 g	Threonine	0.019 g
Fats	0.27 g	Isoleucine	0.018 g
Carbohydrates	17.00 g	Leucine	0.031 g
Total dietary fiber	1.80 g	Lysine	0.041 g
Ash	0.50 g	Methionine	0.005 g
		Phenylalamine	0.017 g
Minerals		Tyrosine	0.010 g
Calcium	10.00 mg	Valine	0.026 g
Iron	0.13 mg	Arginine	0.019 g
Magnesium	9.00 mg	Histidine	0.012 g
Phosphorus	11.00 mg	Alanine	0.051 g
Potassium	156.00 mg	Aspartic acid	0.042 g
Sodium	2 .00 mg	Glutamic acid	0.060 g
Zinc	0.04 mg	Glycine	0.021 g
Copper	0.11 mg	Proline	0.018 g
Manganese	0.027 mg	Serine	0.022 g
Selenium	0.60 mg		
Vitamin			
Vitamin C (Total ascorbic acid)	27.20 mg		
Thiamine	0.056 mg		
Riboflavin	0.57 mg		
Niacin	0.58 mg		
Pantothenic acid	0.16 mg		
Vitamin B6	0.16 mg		
Total folate	14.00 meg_RE		
Vitamin A IU	3894.00 IU		
Vitamin A RE	389.00 mg_RE		
Vitamin E	1.12 mg_ATE		
Tocopherols, alpha	1.12 mg		
Lipids			
Total saturated fatty acids	0.07 g		
Total monounsaturated fatty acids	0.10 g		
Total polyunsaturated fatty acids	0.05 g		
Cholesterol	0.00 mg		

Source: USDA nutrient standard reference (release 14 July 2001)
meg_RE = micrograms of retinol equivalent, IU = amount of a substance based on measured biological activity or effect, ATE = alpha tocopherol equivalent (Vitamin E activity)

Adapted from Bally (2006).

2.3 CULTIVAR CHARACTERISATION

In mangoes pre- and post-harvest anthracnose, irregular bearing, short shelf life and internal breakdown are the most important production constraints. The causes of these problems are genetic and to solve this, genetic diversity is needed (Litz, 2004). According to Krishna and Singh (2007) the phenomena of allopolyploidy, out crosses and the different agro-climatic conditions in the mango growing areas has proportionate a high level of genetic diversity in mangoes. Confusion however exists in mango nomenclature due to different local names for the same varieties, thus making characterisation of germplasm important for better use of all genetic resources available. The International Plant Genetic Resources Institute (IPGRI) has developed a descriptor list to assist with the identification of cultivars. The list contains passport data for identifying the accession and information recorded by collectors; characterisation data, include recorded characters, marked as being highly heritable, that can be easily seen in the field and expressed in all environments; and evaluation data to help assess abiotic and biotic stress susceptibility (IPGRI, 2006).

2.3.1 MORPHOLOGICAL CHARACTERISATION

The application of morphological markers is the simplest of the formal, standardised and repeatable methods of evaluating crop genetic diversity. Some of the most important advantages of using morphological characterisation are that published descriptor lists are readily obtainable for most major crop species, it can be carried out *in situ*, is relatively low-cost and easy to perform. Morphological characterisation is the first step that should be done before more profound biochemical or molecular studies are carried out (Hoogendijk and Williams, 2001).

Various studies with different tropical trees have utilized morphological characterisation, including *M. indica*. Differentiation between cooking and dessert bananas was done based on morphological, physical and chemical characteristics of 23 unripe cultivated varieties of Colombian *Musaceae* (Gibert et al., 2009). Morphological characterisation of Mandarin fruits from the *Citrus* germplasm active bank of Centro de Citricultura Sylvio Moreira/IAC was done using 38 fruit morphological description characters and large

phenotypic variation in most of the analysed characters was observed (Domingues, 1999). Morphological characterisation of cashew (*Anacardium occidentale* L.) in four populations in Malawi (Chipojola et al., 2009) detected that variation between accessions could be attributed to genetic history, eco-geographic origin and selection for desired agronomic traits by farmers. Another study was done for identification of tropical trees through vegetative morphology and the existence of marked differences, even discontinuities of distributions of characters between those families included in the study was detected (Hargreaves, 2006). A preliminary selection of 19 mango accessions and cultivars from a collection at the Umbeluzi Research Station in Mozambique was done. The study focused on colour, size, shape, weight and volume of fruit, number of embryos per seed, peel thickness, adherence, flavour, texture, fibre content, juice, soluble solids, sugars, acidity, pH and ratio of soluble solids to acidity. As a result, the five most desirable varieties were selected (Ascenso et al., 1981).

2.3.1.1 MANGO TREE DESCRIPTION

The main objective of variety characterisation is to obtain a better understanding of the principal characteristics of the different parts of the plant. Successful mango varieties are chosen for essential agronomic traits such as taste, colour and weight, shape of the fruits as well as tree height, leaves and inflorescences rather than yield (Chadra and Pal, 1986).

Tree

Mango trees have different types of canopies, according to the propagation type, density, type of variety and eco-geographical conditions. Some varieties, such as “Latra”, are considered to have a creeper-growth habit because of its spreading nature. The biggest mango tree in the world is found in India and has a spreading crown of 36.6 x 45.7 m (Singh, 1968). When trees are propagated by seed they develop a sympodially branched appearance according to the Scarron’s model, while grafted trees tend to be shorter. The tree height can reach 8-35 m, depending on cultivar, climate, soil type and rootstock (Human, 2008).

Root

The root system of mango trees is composed of a taproot about 6-8 m deep, superficial feeder-roots and fibrous anchor roots. Sometimes feeder-roots can develop above the water table and fibrous roots may extend away from the drip line. This effective root system can reach 7.5 m to the lateral side and 1.2 m depth in 18 years or older plants in well drained soil (Anonymous, 2008). The volume of feeder roots of mango varies during the annual cycle, with the majority of root development occurring during the wet periods of the year and declining during the dry periods. Root growth is periodical, slowing or stopping throughout major canopy growth periods (Bally, 2006).

Leaf

Characteristic leaf shapes include entire, leathery, short, pointed and oblong to lanceolate leaves. The length is about 450 mm. Differences are due to varietal variation, climate, cultural practises and growth stages. Young leaves from different varieties can present different colours. This can vary from copper-red to purplish in colour. At maturity the leaf colour changes to dark green and usually smells like turpentine (Fivaz, 2008).

Inflorescence and flowers

The mango inflorescence is primarily terminal on a panicle (Bally, 2006). Singh (1968) found that the inflorescence is most commonly pubescent, although at times it is glabrous. Inflorescence colour ranges from yellow to light green with crimson patches or with crimson flushes on branches. The number of panicles per plant ranges from 600-6 000 and the number of flowers per panicle varies from 200-4 000. The majority of flowers open between 9-11 am and the receptivity of the stigma occur about 72 h after anthesis (Genu and Pinto, 2002). The greenish-white or pinkish flowers are borne in inflorescences usually located on current or previous year's growth. Male flowers usually outnumber the bisexual or perfect flowers (Griesbach, 2003). The hermaphroditic flowers have a shiny, green, globous, superior ovary with an anatropic ovule and a style with a single lobe. The male and hermaphroditic flowers normally appear on the apical end of the inflorescences and are long pedicellate. The calyx and corolla have five pubescence sepals and five white, pink or purplish petals, followed by five yellowish nectar glands, a

single fertile stamen and a number of non-fertile stamens of different sizes, known as staminodes (Fivaz, 2008).

Pollen and pollination

According to Singh (1968) mango trees have limited fruit production, since only 35% of all flowers are pollinated and only 0.01% is transformed into fruits. Mango pollen has an oblong shape when dried and spherical when hydrated and each anther can produce between 250-650 grains of pollen. Pollen viability is more prominent immediately after anther opening. High temperatures are favourable for pollen viability and low temperatures cause abnormal pollen production (Neto and Cunha, 2000). In hermaphroditic flowers the pistil and stamen have the same length, allowing insects to transfer pollen from the anther to stigma. According to Genu and Pinto (2002) the reduced number of fertilised flowers is provoked by the small number of perfect flowers that have been pollinated and due to the large number of male flowers.

Fruit

Mango fruit of the different cultivars varies in shape, size, appearance and internal characteristics. The fruit is a fleshly drupe, varying in size from 2.5-30 cm long, may be kidney-shaped, ovate or round and weigh from approximately 200 g to over 2 000 g. The leathery skin is waxy and smooth and when ripe entirely pale green or yellow marked with red, depending on the cultivar (Griesbach, 2003)

2.3.2 GENETIC CHARACTERISATION

Genetic characterisation is important to provide scientists, producers and all interested people, with information about certain properties of some cultivars. There are different genetic methods to describe cultivars, such as cytology and molecular markers. These methods combined with morphological characterisation are essential to understand genetic diversity of mango.

2.3.2.1 CYTOLOGY

The number of chromosomes of *M. indica*, *M. sylvatica* and *M. caloneura* Kurz is 2n=40. The somatic chromosomes have lengths from 0.4-2.0 μ. There are eleven chromosome types that vary for at least one chromosome between different cultivars. There are a large number of somatic chromosomes with high numbers of nucleolar chromosomes, resulting in regular pairing and disjunction of chromosomes during meiosis, absence of any multivalent formation and good fertility that can be linked to the polyploid nature of mango (Mukherjee, 1950; 1963). Singh (1968) reported studies that were undertaken regarding morphological characterisation of leaves, flowers, fruits etc., and indicated a gradual variation of parameters between two limits, e.g., green-yellow to yellow fruit colour or very thin and very thick leaves. The appearance of different degrees of variation indicate mango's polyploid and hybrid nature. In production of new varieties it is important to consider natural hybridisation because of high compatibility between varieties, resulting from large morphological similarity of chromosomes (Mukherjee, 1963; Bompard, 1993; Iyer and Degani, 1997). The phenomenon of allopolyploidy is believed to have originated from amphidiploidy, because differentiation of many varieties occurred primarily through gene mutations, selection and preservation of some of them through grafting (Mukherjee, 1953; Mathews and Litz, 1992; Yonemory et al., 2002). In recent times, two spontaneous tetraploid mango seedlings were identified. A tetraploid "Gomera-1" from Canary Island (Galan Sauco et al., 2001) and another one from Katrine in Australia and both are used for rootstock breeding purposes (Bally et al., 2009).

2.3.2.2 GENETIC DIVERSITY

Of more than 1 000 known varieties of mango, only 350 are of commercial importance. The original wild mangoes had small fruits with little, fibrous flesh and it is believed that natural hybridisation occurred between *M. indica* and *M. sylvatica* in South Asia. Selection for better quality has been carried out for 4 000-6 000 years and vegetative propagation for 400 years (Morton, 1987). There are three main groups of mango cultivars: a) Most improved tropical cultivars with fibreless fruit and no turpentine flavour; b) Improved subtropical cultivars, with attractive, good quality fruit, but with unsatisfactory yield and less resistance to disease and c) Unimproved cultivars with high

fibre content, external green colour, turpentine flavour and poor shelf life, e.g. “Peach” and “Sabre” (Human, 2008). There are many mango varieties produced in different countries around of the world. Some are listed in Table 2.2.

Table 2.2 Most important mango cultivars in major producing countries.

Continent	Country	Cultivars
Africa	Cote d'voire	Amelie, Kent
	Egypt	Alphonso, Bullock's Heart, Hindi Be Sennara, Langra, Mabrouk
	Kenya	Boubo, Ngowe, Batawi
	Mali	Amelie, Kent
	South Africa	Fascell, Haden, Keitt, Kent, Sensation, Tommy Atkins, Zill
Asia	Bangladesh	Aswina, Fazli, Gopal Bhog, Himsagar, Khirapati, Langra
	India	Alphonso, Banganapalli, Bombay, Bombay Green, Chausa, Dashehari, Fazli, Fernandian, Himsagar, Kesar, Kishen Bhog, Langra, Mallika, Mankurad, Mulgoa, Neelum, Pairi, Samar Behisht, Suvarnarekha, Totapuri, Vanraj, Zardalu
	Indonesia	Arumanis, Dodol, Gedong, Golek, Madu, Manalagi
	Israel	Haden, Tommy Atkins, Keitt
	Malaysia	Arumanis, Kuala Selangor 2, Golek, Apple Rumani, Malgoa
	Myanmar	Aug Din, Ma Chit Su, Sein Ta Lone, Shwe Hin Tha
	Pakistan	Anwar Ratol, Began Pali, Chausa, Dashehari, Gulab Khas, Lang siroli, Sindhri, Suvarnarekha, Zafran
	Philippines	Carabao, Manila Super, Pico
	Thailand	Nam Doc Mai, Ngar Charn, Ok Rong, Keow Savoey, Pimsen mum
Australia		Kensigton Pride
North America	Costa Rica	Haden, Irwin, Keitt, Mora, Tommy Atkins
	Guatemala	Haden, Kent, Tommy Atkins
	Haiti	Francine, Madame Francis
	Mexico	Haden, Irwin, Kent, Manila, Palmer, Sensation Tommy Atkins, Van Dyke
	USA	Keitt, Kent, Tommy Atkins
South America	Brazil	Bourbon, Carlota, Coracao, Epada, Itamaraca, Maco, Magoada, Rosa, Tommy Atkins
	Ecuador	Haden, Keitt, Kent, Tommy Atkins
	Peru	Haden, Keitt, Kent, Tommy Atkins
	Venezuela	Haden, Keitt, Kent, Tommy Atkins

Source: Mukherjee (1997)

2.3.2.3 Molecular markers

Molecular markers have the advantages of being abundant, phenotypically neutral, show absence of epistasis and are not influenced by the developmental stage or tissue of the plant or environmental conditions (Mohapatra, 2007). Many molecular markers are nowadays utilised for numerous purposes, e.g., characterisation of germplasm, varietal identification and clonal fidelity testing, assessment of genetic diversity, validation of genetic relationships and marker-assisted selection (Hoogendijk and Williams, 2001). Different classes of DNA markers, each with its own advantages and disadvantages, are available.

Restriction fragment length polymorphism (RFLP)

RFLPs were developed by Botstein et al. (1980). RFLP uses restriction enzymes that cut the DNA molecule at specific sites, called restriction sites, resulting in different fragments of variable lengths. After separation by electrophoresis, fragments are transferred to nitrocellulose or nylon filters through Southern blotting followed by hybridisation with radioactively labelled DNA probes and visualisation using photographic film (Varshney et al., 2004).

A study by Eiadthong et al. (1999a) on 13 *Mangifera* species classified the species into two groups based on eight informative mutation sites detected by four endonuclease enzymes. The monomorphic group of 11 *Mangifera* species formed a cluster with *A. occidentale*. This study used a combination of two types of molecular markers, RFLP and AFLP.

Random amplified polymorphic DNA (RAPD)

In RAPD analysis, the sequence of the fragment to be amplified is unknown. Primers are drawn with random sequences of about 10 bp and the technique is used in organisms where the DNA sequence is unknown (Williams et al., 1990). RAPD analysis have the advantages of being neutrally selective, do not use radio-isotopes, can use DNA of low quality and primers are more accessible than that of the RFLP technique. However, disadvantages include a limited detection of polymorphisms, a low resolution profile that

may result in low bands and detection of only the dominating allelomorphs. It was found that RAPD, due to low annealing temperatures, are less reproducible than other techniques (Williams et al., 1990; Kapteyn and Simon, 2002).

Schnell and Knight (1993) used nine *Mangifera* species to determine genetic relationships using RAPDs. The classification of species based on RAPD data was different compared to classification based on phenotypic characteristics. This was the first study involving molecular markers in *Mangifera.*

Twenty-five mango accessions were analysed using RAPDs for the identification of cultivars and validation of genetic relationships in *M. indica* (Schnell et al., 1995). Eighty random decamer primers were used and 28 of these gave polymorphisms. This study included a maternal half-sib (MSH) family. RAPD data were used to create simple matching coefficients that were analysed phenetically and by means of Principal Co-ordinate Analyses (PCA). The randomly selected accessions were scattered with no apparent pattern while the MSH clustered together in both the phenetic dendrogram and the PCA.

The RAPD technique was used to investigate two species of bush mango [*Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. and *I. wombolu* Vermoesen] from Central/West Africa. Significant genetic integrity was detected and no confirmation of hybridisation was seen. Results of the study indicated two different species, despite morphological similarity and previous misidentification as being the same species. This study also confirmed that the RAPD technique can be applied in species with diminutive genetic diversity information available (Lowe et al., 2000). Forty genotypes from the Brazilian Research Institute (EMBRAPA) were analysed using 13 primers that produced 176 reproducible RAPD markers. Of the 176 markers, 116 were polymorphic, detecting 65.9% polymorphism. The authors concluded that RAPD analysis showed efficient differences to determine genotype polymorphism in mango germplasm (de Sousa and Costa Lima, 2004).

RAPD analysis furthermore was used for the identification of molecular markers linked to differential flowering behaviour of mangoes in Andaman and Nicobar Islands

(Damodaran et al., 2007). The study reported that specific bands in the range 200-300 bp, amplified by the primers OPX9, OPX10, OPF4 and OPC2, were found only in multiple-flowering open-pollinated clones of Neelam and Banganapali, while the same was absent in single-flowering clones and varieties.

Amplified fragment length polymorphism (AFLP)

AFLP is a polymerase chain reaction (PCR)-based method, similar to RAPD analysis and can be performed on genomes of any crop and complexity. It is a universal and multi-locus marker and applies PCR amplification of restriction fragments from total double-digested genomic DNA, under highly stringent conditions. AFLP analysis utilises six (*Eco*RI, *Pst*I, *Hin*dIII) and four-base (*Mse*l or *Taq*I) cutters for template preparation. Following digestion, adapters are added to the restricted DNA to create primer annealing sites. The initial PCR step uses primers with a single selective nucleotide and reduces the whole complexity of the combination up to 16-fold, allowing the target sequence to become the predominant species. Products from the first PCR are used as templates for a second amplification that uses three selective nucleotides on the 3'-end of each primer. Other enzyme systems substitute six-base for eight-base cutting enzymes, such as *Sse*8387I or its isochizomer *Sda*I or *Sbf*I (Mohle and Schwarz, 2004). The AFLP technique results in predominant amplification of those restriction fragments that have a rare cutter sequence on one end and a frequent cutter sequence on the other end. The basis for using two restriction enzymes is the following:

a) The frequent cutter will produce small DNA fragments that will amplify well and are in the best size range for separation on denaturing gels (sequence gels).

b) The number of fragments to be amplified is decreased by using the rare cutter and this limits the number of selective nucleotides desired for selective amplification.

c) The use of two restriction enzymes makes it possible to label one strand of the double stranded PCR products that prevents the incidence of doublets on the gels due to different mobility of the two strands of the amplified fragment.

d) Using two different restriction enzymes gives the most agility in tuning the number of fragments to be amplified.

e) A large number of different fingerprints can be created by the diverse combinations of a small number of primers (Vos et al., 1995).

A study using 31 F_1 progenies from crosses between "Alphonso" and "Palmer" led to the construction of maps for each cultivar that were useful for analysing correlations of traits like fruit size, shape and colour (Phumichai et al., 2000). AFLP analysis was demonstrated to be useful for identification of mango cultivars and rootstocks (Kashkush et al., 2001). The authors reported genetic relationships and diversity within *Mangifera* species, with no differences between morphological and molecular data in this study. Hence AFLP analysis can be considered an applicable and effective tool in taxonomic analysis (Phumichai et al., 2000). A study was done to clarify the effectiveness of AFLP markers for the identification of accessions in four *Mangifera* species that are important in Malaysia and to explore the genetic relationship and diversity among these *Mangifera* species for the basic knowledge of *Mangifera* breeding. They concluded that AFLP is robust, useful and an appropriate tool for identifying *Mangifera* species and for detecting genetic relationships between the four species tested (Yamanaka et al., 2006).

Simple sequence repeat (SSR)

According to Holton (2001), microsatellites or SSRs are simple sequence repeats of about 1-6 nucleotides. The advantages are that they are dispersed and plentiful in all genomes, with elevated levels of polymorphism compared to other molecular markers. As a disadvantage SSR analysis is an expensive and time-consuming process mainly when the creation of a library is needed. For many crops, to construct a high resolution linkage map, using only SSR markers is expensive, but it is usually more reasonable to combine SSR and AFLP analysis. Other advantages of SSR include co-dominant inheritance, analytical simplicity and its transferability (Weber, 1990; He et al., 2003).

In Thailand a study was done to identify mango cultivars and evaluate their genetic variation using SSR anchored primers. Results indicated that two Thai mango cultivars were found to be far distant of the genetic relationship from the other cultivars. Seven

cultivars were in the same group as two Florida cultivars, one Philippine cultivar and one Indonesian cultivar. Four other Thai cultivars were divided into two groups: each group enclosed Indian cultivars. The analysis did not present evident distinction between the polyembryonic and monoembryonic seed races (Eiadthong et al., 1999b).

Viruel et al. (2004) reported on the development of a set of 16 SSRs for mango using two genomic libraries enriched with CT repeats from DNA extracted from "Tommy Atkins". The analysis of 28 mango genotypes using these 16 SSRs showed three main groups using both cluster analysis and PCA indicating similar distribution of the genotypes. Cultivars were grouped according to their geographical origin and pedigree history and the two main types of mangoes (monoembryonic and polyembryonic) were clearly differentiated.

Honsho et al. (2004) isolated and characterised new SSRs in mango to identify 36 cultivars from different places, namely Thailand, Australia, USA and Taiwan. An AC genomic library was created using the mango variety "Irwin". SSR alleles indicated high frequencies and tended to be shared by Thailand cultivars, whereas rare alleles were found in cultivars from others regions. This could have been due to the similar genetic background in 29 of the 36 cultivars from Thailand.

SSR analysis shows great potential for mango improvement and can be performed for variety identification, validation of parentages, estimation of genetic variation in existing populations and characterisation of rootstocks (Brettell et al., 2002).

2.4 MANGO BREEDING

2.4.1 BREEDING OBJECTIVES

Mango breeding programmes have gained significant progress in the past with regard to release of new hybrid varieties from many centres. Many countries have initiated mango breeding programmes with well defined objectives (Iyer and Dinesh, 1997). Breeding programmes have to be carried out with consideration of the breeding objectives. In mangoes, breeders should distinguish between objectives for either the rootstock or the crown. Rootstock objectives focus on the introduction of disease and stress resistant

varieties and selection of genotypes that mainly possesses high production of small fruits (250-300 g), seeds that can be removed easily from the endocarp, with good germination percentages and that are polyembryonic, young plants with good development and high grafting index. Resistance to pests and diseases is determined by inoculation of plantlets at nursery stage through irrigation water containing dissolved fungi or by direct inoculation of the plantlets (Genu and Pinto, 2002).

Breeding for improved crown resistance focuses mainly on diseases and pests that normally appear in different countries such as fruit flies, anthracnose, powdery mildew, as well as for malformation and physiological disorders. The first step is always the introduction and selection of resistant varieties. Secondly, the use of hybridisation and selection must be considered. For selection, two types of pest and disease occurrence must be taken into account. Firstly infections that normally appear during the production cycle of the plant and secondly infections that require specific conditions and do not occur regularly. For the last type of infection, inoculation with pathogens must be done during the nursery stage and differentiation can be seen after 90 days (Genu and Pinto, 2002).

2.4.2 METHODS OF MANGO BREEDING

There are very few commercially important hybrids that have resulted from conventional breeding (Usman et al., 2001). However there are several methods available for breeding mangoes that can be adopted by breeders. The selected method depends essentially on financial resources, qualified people, physical space and time accessible and the characteristics of available cultivars.

Recurrent selection is one of the methods mostly utilised in the past. It was used to generate “Haden” in 1910 in Florida. The method is based on the selection of plants in a certain area, harvesting of fruits and evaluation of the offspring resulting from cross pollinated crosses. The parental lines of selected offspring are then carefully selected, planted and evaluated. Because mango is a cross pollinated crop, only the female parental line is known to work with this method, unless controlled crosses are done. The application of molecular markers during recurrent selection has been demonstrated to be

useful, as it is possible to select the desirable genotypes at an early stage, not having to wait for many years, considering that mango trees are perennial and takes a long time to reach maturity (Genu and Pinto, 2002).

Selected hybrids can easily be vegetatively propagated and a wide range of genetic variation exists in mangoes thus making mango breeding an attractive method to improve the crop. Three methods could be considered in asexually propagated crops, namely hybridisation, clonal selection and mutation breeding. According to Singh and Sturrock (1969) mango is a heterozygous plant. Therefore they suggested that hybridisation is viable for mango breeding. Hybridisation followed by clonal selection can be applied to create genetic variability and transfer specific characters in asexually propagated crops (Agrawal, 1998). Hybridisation success depends on the selection of parents. This requires that the breeding value of parents have to be calculated from the performance of hybrid progeny. After pollination all flowers must be removed from the panicle except the pollinated flowers. If the desirable characteristics are present in the progeny, it indicates good general combining ability. The parents will then be used for wide crosses and propagated through vegetative means. In some cases undesirable characteristics found in the F_1 progeny can be removed through backcrosses (Singh, 1968).

Mango development is based on hybridisation through open pollination followed by evaluation of the progeny for desirable morphological traits. Each individual in the progeny comprises a unique new genotype. Selections made from these individuals are then vegetatively propagated for evaluation. This clonal material should be established in multiplication trials and evaluated for disease and pest resistance, yield and quality of production. That is genetically uniform, generated by a single individual and vegetatively propagated. It is recommended to establish multiplication trials to select superior clones. This method has got some limitations such as a low multiplication ratio and it requires large variability in the population. In some cases, intra-clonal selection can occur, where variations are observed in clones resulting from the original variety. The use of molecular markers can determine if new clones are different from the original cultivar and they are effective in reducing the time required using conventional breeding alone (Agrawal, 1998); this is referred to as marker assisted selection (MAS)

Mutations are also used to create variability in mango trees (Singh and Sturrock, 1969). They found that physical and chemical treatments, such as applying X-rays or other mutagenic agents before grafting or budding, can generate different varieties. Mutations can also occur spontaneously, as verified in cultivars like “Hirasonia”. According to Agrawal (1998) the dosage of mutagens depends on the type of material to be treated and the availability of the mutagen.

According to Genu and Pinto (2002) polycrosses and selection between half-sib populations are commonly used as a method for breeding mangoes. It requires planting various genotypes in the same space, in such a way that crosses may occur between all of them. This method is particularly useful for mangoes, because of the small size of the flowers that makes hand pollination difficult. Additional advantages are that the method does not require skilled labour and a large number of different genotypes are obtained. The method is based on the following:

- Selection of parental lines based on desirable characteristics and flowering time that must be similar to increase the efficiency of crosses.
- Establishment of field trials using the Latin square design.
- Selection of progenies based on phenotypic characteristics, followed by clonal evaluation.

2.4.3 BREEDING ACHIEVEMENTS

Significant progress has been reported in many research centres with regard to release of new mango hybrid varieties. The following varieties were released from the Indian Research Institute (Iyer and Dinesh, 1997):

> “Arka Neekiram” - Regular bearing with medium sized fruits, free from spongy tissue, good pulp colour, excellent skin colour and the tree is semi-vigorous and consequently suitable for close planting.
>
> “Sindhu” - Medium sized fruit (215 g), high pulp to stone ratio (26:1) and very thin (30 mm) and small stone (6.7 g).

"Jawahar" - Attractive shape, high pulp content, fibreless and precocious in bearing.

The following varieties were selected from the Volcani Research Centre (Lavi et al., 1997a; 1997b):

"Naomi" - Smooth skin with red pigmentation weighing about 450 g and a mid season bearer.

"Shelly" - A late season cultivar having good taste, red orange skin, good shelf life and weighing 400-500 g.

"Tango" - An early season cultivar, with very attractive appearance, good taste and weighing 300-400 g.

A breeding programme in Brazil has released the following varieties (Pinto et al., 2000):

"Alfa" - slow growth, semi-dwarf and highest and most regular yield.

"Roxa" - Medium to very firm pulp, few fibres to fibreless, excellent taste because of higher Brix acidity ratio and highest and most regular yield.

The Agricultural Research Councils´ Institute for Tropical and Sub-tropical Crops (ARC-ITSC) released three new cultivars namely, "Heidi", "Joa" and "Chené". "Joa" shows a high tolerance of bacterial black spot (de Villiers and Joubert, 2008).

2.4.4 PROBLEMS ASSOCIATED WITH MANGO BREEDING

The long juvenile period of mango trees, from seed until maturity and from one generation to the next, results in several years before variety release. Another breeding constraint is the time required to achieve economical production, which is about 20 years, and it is only possible to calculate the average yield at that time (Singh, 1968).

The problem of low fruit set is a common phenomenon in crosses obtained from mango breeding programmes. Identifying the genotype of the progeny, because of high heterozygosity of the crop, can be troublesome, due to the polyembryonic nature of some cultivars and the large space necessary to establish field plantation, as well as the

maintenance of hybrid populations in the field during this lengthy period (Genu and Pinto, 2002). Incompatibility between some species is reported (Ram et al., 1976). Low temperatures (≤ 10°C at night) cause pollen grain with low viability (≤ than 50%). The optimum temperature for normal meioses is 15-33°C (70-85% viability) (Iyer and Degani, 1997).

2.4.5 BREEDING FOR PESTS AND DISEASES

Mango trees may be infested with several pests and diseases caused by fungi and bacteria. Reports indicate that there are 492 species of harmful insects, including grasshoppers, mealy bug, inflorescence midge, fruit fly, scale insect, shoot borer, leaf webber and stone weevil. Apart from these pests there are 17 species of mites and 26 species of nematodes that can attack mango trees. Some of these pests cause injuries during flowering and fruiting, resulting in high production losses. Other pests like aphids and termites not mentioned here, are considered to be minor pests on mango (Anonymous, 2008). In this section only anthracnose, powdery mildew, fruit flies and weevil will be discussed, since samples used for this study were collected in an orchard infested by these pests and diseases.

2.4.5.1 MANGO SEED WEEVIL

The mango seed weevil is an important pest in nurseries and orchards. Adults become reproductively active during the flowering stage and the female insects lay their eggs on developing fruit. The insect *Sternochetus mangiferae* (Fabricius) damages the flesh of ripe fruit when adults emerge from the seed because the eggs are laid near the fruit surface (Hansen et al., 1989; Johnson, 1989). After hatching, the larvae tunnel through the pulp to the young developing seed (Hansen et al., 1989) where they excavate cavities into the seed and pupate. After one week adults are formed, but about one or two months later the adults emerge from the seed (Balock and Kozuma, 1964). Control of this pest through field sanitation is required but is labour-intensive. Chemical control can be done through some insecticides such as organophosphate or fenthion that reduce infestation to ≤ 17% (Pena and Mohyuddin, 1997). Resistant cultivars should be seedless selections,

cultivars that form seed early or cultivars that produce fruit out of season (Balock and Kozuma, 1964).

2.4.5.2 FRUIT FLIES

Fruit flies belong to the genera *Anastrepha* (eight species), *Bactrocera* (30 species), *Ceratitis* (seven species), *Dirioxa* (two species) and *Toxotrypana* (one species). *Bactrocera* species are pests of major importance, especially in the eastern hemisphere. The female fruit flies belonging to this species introduce their eggs underneath the skin of the ripe fruit and after hatching, the larvae burrow deeper into the fruit. They contaminate the fruit with frass and provide access for fungi and bacteria that can cause secondary infections. Fully grown larvae drop to the ground and enter the soil where they pupate. Humid weather is considered to be favourable for *Bactrocera* fruit flies and *Bactrocera* populations decrease during dry periods. Chemical control involves Malathion for three months (Pena and Mohyuddin, 1997).

2.4.5.3 ANTHRACNOSE

According to Arauz (2000), anthracnose is a post-harvest disease that occurs frequently on mangoes in humid growing areas and up to 100% fruit loss can occur in wet or very humid conditions. It is caused by the fungi, *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk, *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. and/or *C. acutatum* Simmonds. The disease, presents various symptoms on different parts of the plant.

On the leaves it shows irregular shaped black necrotic spots on both sides of the mango leaves often causing the young leaves to curl. The damage coalesces and forms irregular lesions (0.3-1.0 cm) along the leaf, giving it a ‘shot-hole’ appearance). Anthracnose also infects the panicle. It is also called blossom blight, infesting either the inflorescence or the stalk of the individual flowers. The stalks, when infected, present dark grey to black lesions and the flowers are dry and brown to black. Fruit symptoms include rounded brown to black lesions, without a definite border on the fruit surface. The lesions appear in different sizes and some coalesce, forming large areas in the fruit superficies. Normally the lesions occur in the peel, but in severe infestations it can affect the pulp (Dodd et al., 1997).

It was found that bagging fruit about 56 days before harvest reduced anthracnose symptoms, when bagging was done for periods longer than 56 days, no differences were observed, compared to 56 days. Fruit mass, colour, total soluble solids, acidity and eating quality were not affected by bagging (Hoffman et al., 1997).

Another method that is applied to reduce attacks of microorganisms like anthracnose on ripe mangoes is the hot water bath treatment. Mango fruits in a typical pack line, alongside other processes such as washing and pre-sorting of fruits, are submitted to water temperatures of 50°C (de Villiers and Joubert, 2008). This method is also used to extend the shelf life of mangoes, mainly for exportation to USA (Kim et al., 2007).

2.4.5.4 POWDERY MILDEW

Powdery mildew is caused by *Oidium mangiferae* Berth. a fungus which only attacks mango (Prakash and Srivistava, 1987). The disease can cause losses up to 80-90% and is most severe during cool, dry conditions. Conidia are wind disseminated, germinating at temperatures ranging from 9-32°C (Schoeman et al., 1995). Symptoms become visible on shoots of the inflorescences, leaves and fruits. It causes abortion of the small fruits, and the affected panicles may set few or no fruit. On the leaves, a white, powdery coating appears either on the underside, both sides or top side of the leaves, depending on the cultivar that is attacked (Ploetz and Prakash, 1997). “Zill” and “Kent” are susceptible while “Tommy Atkins” and “Sensation” are more resistant (Ploetz et al., 1994). Powdery mildew can be controlled with sulphur and other newer fungicides that should be applied at the onset of symptom development in order to be effective (Ploetz and Prakash, 1997).

2.5 POST- HARVEST PROBLEMS AND UTILISATION

2.5.1 POST-HARVEST PHYSIOLOGY

Mango is a perishable fruit that needs 3-9 days to mature, complicating delivery of this product to distant markets. Some techniques are available for controlling pests and diseases to protect fruit against damage during transportation and packing. However, methods for improved storage still need to be developed and improved. It is during the storage stage that the quality of mango fruit decreases, showing poor colour and changes

of flavour. Studies about the processes involved in the ripening process of mango fruit would be useful (Gomez-Lim, 1997). A study to analyse the chemical and biochemical changes taking place during freezing and cold storage of four Spanish mango varieties was undertaken. Results indicated titratable acidity decreased with an increase in pH during the freezing period. Ascorbic acid, β-carotene, peroxidase and polyphenoloxidase activities decreased during frozen storage (Marin et al., 1992).

2.5.2 FRESH CONSUMPTION

According to Genu and Pinto (2002) the majority of the world's mango production is consumed as fresh fruit. In Brazil and Mozambique many attempts to industrialise mango was not very successful due to lack of uniform raw material in large volume, the seasonality of the crop and short period of harvesting that limits the constant supply of raw material as well as the lack of infrastructure for proper transport, ripening and storage of fresh fruit before processing.

Some of the major problems associated with mango fruits are perishability and short shelf life. Increasing the shelf life of mango can be considered as the main concern in post-harvest production, according to Jaskani et al. (2008). An increase in shelf life can be obtained in two ways:

a) Stopping ethylene activity, by atmosphere control or use of ethylene absorbents such as potassium permanganate or activated charcoal/vanadium oxide.
b) Using genetic transformation of mangoes using the mango ACC (1-aminocyclopropane-1-carboxylate) synthase and ACC oxidase genes in order to decrease the susceptibility of over ripening fruits to anthracnose infection.

When harvested at full ripeness fruit does not store well and if harvested green the maturation process is not suitable. Mangoes are furthermore susceptible to low temperatures that cause damage of fruit flesh in storehouses. In order to reduce the post-harvest damage the challenge is to control ethylene production and activity. It is therefore necessary to stop the expression of genes encoding ethylene biosynthetic enzymes using transformation with the respective antisense genes and decreasing the level of the

ethylene precursor ACC by deamination or hydrolysis. Ethylene biosynthesis is mediated by three major enzymes, S-adenosyl methionine (SAM) synthase, ACC synthase and ACC oxidase (Litz, 2004). Two ACC synthase and ACC oxidase cDNA clones were identified from a mango cDNA library and were utilised in genetic transformation antisense experiments of mango to control ethylene production (Cruz-Hernandez and Litz, 1997).

2.5.3 PROCESSING

According to Genu and Pinto (2002), in the majority of tropical developing countries, the natural abundance of fresh mango fruits frequently brings an excess in local demand. Unfortunately, the excess fresh fruit is not always completely made use of and only a small quantity of fruit is processed. Mango fruit used for processing should have firm texture, high yield, medium size, high sugar content, bright colour, good flavour and aroma after ripening, low acid content, no fibre and low tannin content and these varieties normally show resistance to some pests and diseases and are used as rootstocks (Mannepun and Yunchaland, 2004). There are several mango fruit processing methods using different types of equipment such as peeling and slicing machines, high capacity pulp pasteurisers, continuous pulp filling units, adoption of forced circulation evaporators and scraped surface evaporators for concentration, aseptic filling systems and essence recovery systems. It is estimated that 35-55% of fruit mass is wasted, that can be recovered to produce biogas. India is the country with the highest volume of processed products, constituting approximately 1% of the total global production (Nanjundaswamy, 1997).

The world trade for processed mango juice was valued at about US$ 9.9 million and for mango pulp US$ 5.8 million in 2000. The products have been exported to the Middle East, Europe and North America. The demand for mango pulp and mango concentrate is increasing due to its exotic flavour and is utilised in the beverage industry, dairy industry and in baby food formulations (Galan Sauco, 2004). Uses of green and ripe mangoes are listed in Table 2.3.

Table 2.3 Uses of green and ripe mango

Green mango	Ripe mango
Salted pickles, sour pickle, sweet pickle before and after fermentation, salted pickle before and after fermentation, juice, mango powder, mango sauce, chutney, jelly, slices in syrup, jam, mango pie and mango tart.	Dried sweet mango, nectar, puree and canned mango slices or pieces in syrup, toffee, mango leather and mango bar. Jelly, jam, powder for ingredients of bakery products, mango ice-cream, pies, tarts, wine etc

Nanjundaswamy (1991)

Mango pickles: There are two types of pickles namely salt and oil pickles. Oil pickles are prepared with mustard or ginger oil. Mango fruit is sliced, cured and drained and mixed with spices and oil before being bottled and sealed. Varieties with a high acid percentage (5-6%) are the best.

Frozen processed mango: The best variety to use for this process is Alphonso. The method involves freezing mangoes at -30°C in 40° Brix sugar syrup containing citric acid and ascorbic acid, followed by storage at -18°C. It is necessary to do a pre-soaking with 2% calcium chloride to maintain the firmness of the slices and it was found that this also helps to preserve the flavour. According to Marin et al. (1992) it is possible to store frozen mango slices for four months using this method. Mango slices are placed at -40°C until the centre of slices reach -24°C. The frozen slices are vacuum-packed in plastic-bags with 250 g of fruit. The final stage is to freeze the mangoes at -18°C. Recently, a new technique has been developed for use with minimal processed products (Quick frozen fresh). The main characteristics of these products are similar to the fresh products, but with longer shelf life and more practical storage (Genu and Pinto, 2002).

Mango beverages: Beverages include juice, mango nectar and mango ready-to-serve beverages. These beverages are obtained by mixing pulp, citric acid (0.3%), water, and sugar (15° Brix). The processing methods of these differ only for the proportion of pulp required, that varies between 35%, 20% and 10 % pulp respectively (Nanjundaswamy, 1997).

Dehydrated products: These types of products are becoming popular worldwide. Through-flow air-drying was the most efficient process and slices steeped in 40° Brix sugar syrup resulted into a good dried product. The osmotic dehydration process used sugar syrup (70° Brix) as the osmotic agent that is applied to sliced mangoes dipped into a sulphite solution, drained and finally dried in air or using a vacuum drier (Nanjundaswamy, 1997).

Mango powder: Various methods have been utilized to produce powder that can be used as beverage base or as flavouring agent. The production costs of producing mango powder are high due the required technology. The pulp should be placed in a foam drying mat and whipped. Through a foam inducer the pulp forms heat stable low density foam. The foam is spread on a wire mesh tray or continuous belt in a thin layer and dried with hot air.

Mango concentrate: Mango concentrate can be produced through treatment of pulp with pectolytic enzyme and the serum should be separated from the fibre pulp residue. The serum and fibre are then remixed and homogenised to obtain 40° Brix concentrate. Or by a forced circulation evaporator under vacuum up to 30° Brix (Nanjundaswamy, 1997).

Although mango fruit play an important role worldwide, it still presents a small average yield per ha and the processed products create away to avoid losses of fresh fruit. The major production comes from Philippines with 11.9 t/ha, followed by Mexico with 10.2 t/ha and the third position is occupied by Pakistan with 10.2 t/ha. The low index rate of technology adoption by farmers is the main cause of low yield (Pimentel et al., 2000). However, new technologies are still needed and though some work has been done in mango improvement mango genetic resources remain under collected and poorly represented in herbarium collections (Bompard, 1995). The current study was undertaken to improve understanding of mango diversity lead to the improvement of varieties, simultaneously increasing yield and improving quality of fruits, especially in Mozambique.

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CHAPTER 3
MORPHOLOGICAL CHARACTERISATION

3.1 INTRODUCTION

Historically mango genotypes have been characterised using morphological markers (Singh, 1969). Morphological markers have the advantage of being simple to identify and do not need specialised labour. Although molecular characterisation is increasingly being used, morphological characterisation continues to be a useful component that enhances the power of molecular methods. Morphological characterisation can be used as an important tool since published descriptors lists are readily available for most major crop species (Hoogendijk and Williams, 2001). Morphological characterisations criteria are thus important and some main characteristics that can be used as references in mango breeding programmes or in marketing are needed.

The most common commercial mango varieties are “Tommy Atkins”, “Haden”, “Keitt” and “Kent”, and these are also often used as references for export fruits. For exportation, mangoes must be red or deep yellow in colour with a reddish blush, based on consumer preferences in the USA and Europe, respectively (Anonymous, 2008). Other desirable characteristics found in mango fruits are regular bearing, good fruit shape and flesh quality, weight of 300-400 g per fruit, extended production period and extended storage and shelf life. Trees must be compact, tolerant of adverse soil conditions and resistant to pest and disease (Iyer and Dinesh, 1997).

Mangoes in general have good flavour, taste, aroma and texture and most varieties are consumed fresh. The perishable nature of mangoes and its short harvest season severely limits its use for fresh consumption and exportation (Marin et al., 1992). However, there are many processed mango products on the world market, such as canned mango slices in syrup, mango juice and nectar, mango jam and chutney, dehydrated mango etc. (Mannepun and Yunchakad, 2004). It is calculated that only 0.22% of mangoes produced in the world are used for processing (Nanjundaswamy, 1997). Varieties for processing should have fruit of 200 g, good yield of mango pulp and a pH of $\leq$ 4.3 since it improve resistances to pathogen and microorganism development (Martins, 2006).

Several studies have been carried out using morphological characterisation to identify and evaluate mango varieties. Subedi et al. (2009) reported on the identification of some mango cultivars that have different positive characteristics such as ability to grow in dry and marginal areas or at high altitude, absence of fibre in pulp, high fruit setting rate and off-season fruiting. A preliminary mango evaluation study of 19 mango accessions and cultivars resulted in the selection of the five most desirable varieties at a research station in Mozambique (Ascenso et al., 1981). Numerous taxonomic studies of mango varieties in Nigeria were done using 64 morphological markers, including exomorphic and endomorphic as well as vegetative and reproductive characters. Conclusions indicated separation of varieties into two or four groups and PCA indicated the importance of combining qualitative and quantitative data with some vegetative characters and a large number of reproductive characters (Illoh and Olorode, 1991). Cultivars from Thailand were described using IPGRI descriptors and the authors concluded that the heritable characters were useful to characterise all cultivars, since leaf and fruit parameters were used together to identify cultivars (Jintanawong et al., 1992).

The general objective of this study was to describe and evaluate the main characteristics of 30 varieties at the Umbeluzi research station in Mozambique. The specific objectives was to determine the genetic relationship and diversity among 30 mango varieties using morphological characterisation, assess the chemical characteristics by determining the Brix and acid content in the fruit and make recommendations regarding the suitability of different varieties for fresh consumption, local consumption, exportation or fruit processing.

3.2 MATERIALS AND METHODS

3.2.1 PLANT MATERIAL

An orchard of mango trees including 110 varieties was established from 1964 to 1972 by Portuguese settlers at the Umbeluzi research station located in the Boane district situated 25 km from Maputo, the capital of Mozambique. The varieties planted came from different provinces or districts inside and outside the Mozambican borders. In Table 3.1 varieties used in this study are listed with their respective origins.

Table 3.1 Varieties used in this study

Nr.	Varieties	Collected	Origin	Code	Nr.	Varieties	Collected	Origin	Code
1	Malcorada	Nampula	India	1	**16**	Xavier	Maputo	Local	3
2	Polenta	Gondola	Local	2	**17**	Boane	Boane	Local	3
3	Umbeluzi	Umbeluzi	Local	3	**18**	Q.Aurora	Umbeluzi	Local	3
4	Monsserate	Maputo	Local	3	**19**	Zoologia	Umbeluzi	Local	3
5	Matola 2	Matola	Local	3	**20**	Kent	Nelspruit	Florida	4
6	Malcurada	Maputo	India	3	**21**	Haden	Nelspruit	Florida	4
7	Nametil 1A	PAMongovolas	Local	1	**22**	Xinavane	Xinavane	Local	3
8	Sabre	Nelspruit	S E Asia	4	**23**	Sensation	NI	Florida	NI
9	Afonsa Pairy	Maputo	India	3	**24**	Ruby	Angola	Florida	5
10	Lima 2	Nampula	Local	1	**25**	Peach	Nelspruit	S E Asia	4
11	Papaia	Matola	Local	3	**26**	Melo	Marracuene	Local	3
12	Anzo	Mossuril	Local	1	**27**	Lourenco	Manica	Local	2
13	Keitt	Angola	Florida	5	**28**	Matola 3	Matola	Local	3
14	Fernandinha	Nampula	India	1	**29**	Matola 4	Matola	Local	3
15	PA Mocuba	Mocuba	Local	1	**30**	Matola 6	Matola	Local	3

Code:

1 = North of Mozambique, 2 = Central of Mozambique, 3 = South of Mozambique, 4 = Nelspruit-South Africa, 5 = Angola, NI = No information; PA= Posto Agrario, Q.Aurora = Quinta Aurora, SE Asia = South East Asia.

The study included 30 of the 110 varieties (Table 3.1) as this offered a starting point for characterisation and evaluation of the entire collection planted at the research station. The 30 varieties were randomly chosen based on their proximity in the orchard at time of sampling and the presence of young and healthy leaves for DNA analysis (Chapter 4).

3.2.2 MORPHOLOGICAL CHARACTERISATION

The IPGRI descriptors for mangoes were used as a guide to collect data of the 30 selected varieties. These descriptors were used to assist with the identification of varieties and record characteristics marked as either highly heritable, such as fruit taste and type of embryony, abiotic and biotic stress susceptibility, maturity period, fruit availability period, etc.

Most of the characterisation of the varieties was done in the field where the trees were planted. Collected data included the following descriptors for the different parts of the plants: tree, leaf, inflorescence, fruit descriptors, stone and seed, and chemical characteristics of the fruit. The collected data was classified into two types, quantitative and qualitative data, as indicated in Appendix 1. For the extent of data collection, 10 leaves, 10 inflorescences and 20 fruits were used as established by the IPGRI method.

3.2.3 CHEMICAL CHARACTERISTICS OF MANGO FRUITS

3.2.3.1 BRIX DETERMINATION

Ten fruits from each of the 30 varieties were collected and transported to the Agricultural Research Council (ARC) - Nelspruit laboratory for Brix and acidity analyses. The Brix value was determined by squashing the fruits on the lens of an ATAGO hand held refractometer. Calibration of the instrument was done using distilled water. Brix was measured one day after harvest although some samples were only measured one week later because the fruit was not mature enough and other varieties were rotten because of pests and diseases. Therefore only 28 varieties were used to determine Brix value.

3.2.3.2 ACID DETERMINATION

Acid was determined for the same 28 varieties used in the Brix determination and this was also done at ARC-Nelspruit. The method was based on using an acid base titration with sodium hydroxide (NaOH). Juice (10 ml) from each of the ten fruits per variety was pipetted into a conical flask and four drops of phenolphthalene indicator solution was added. This was titrated with 0.1562 N NaOH from a burette until a colour change from clear to pink was noted. The amount of NaOH was recorded and divided by 10 to obtain the acid %.

3.2.3.3 RATIO BRIX/ACIDITY DETERMINATION

The ratio was obtained by dividing the Brix value by titratable acidity (TA) for each of the ten fruits per variety.

3.2.4 STATISTICAL ANALYSES

The qualitative morphological data was scored as 0's and 1's and converted manually into a binary matrix. The average of the quantitative data was standardised using the following formula (Microsoft Office Excel, 2007):

$Z = X-\mu/\sigma$

X = Value to standardise

μ = Aritmetic mean

σ = Standard deviation of distribution

Two dendrograms, one for quantitative data and other for qualitative data, were constructed using NTSYSpc software, version 2.2 (Rohlf, 1993). For correlations Agrobase (2000) software were used. PCA and PCA bi-plot were done using NCSS (2004) statistical software and Microsoft Office Excel (2007). For quantitative data pairwise genetic distances were expressed as the complement of the Euclidean distances (Kaufman and Rouseeuw, 1990) while Dice's coefficient (Dice, 1945) was used for qualitative data. Cluster analyses were performed using the unweighted pair group method of arithmetic averages (UPGMA) (Sokal and Michener, 1958). Dendrograms were created using the SAHN programme of NTSYSpc. The robustness of the

dendrograms was tested by estimating the cophenetic correlation values using the COPH and MXCOMP programmes.

3.3 RESULTS AND DISCUSSION

3.3.1 MORPHOLOGICAL CHARACTERISATION

3.3.1.1 QUANTITATIVE ANALYSES

According to the data obtained in the study, as summarised in Table 3.2, “Matola 6” had the biggest fruit length (21.70 cm), fruit diameter (37.22 cm), fruit weight (1 089.29 g), stone length (10.07 cm), stone width (6.28 cm), stone thickness (1.82 cm) and stone weight (69.00 g) amongst the tested varieties. Values for seed length, seed width and seed weight could not be determined for “Matola 6” and a few other varieties because of rotten seed. The lowest fruit length (12.50 cm) was recorded for “Malcurada”. “Umbeluzi” had the lowest fruit diameter (16.53 cm), stone width (3.46 cm), seed width (2.51 cm) and seed weight (10.78 g). “Ruby” had the lowest stone thickness (0.31 cm) while the lowest stone weight was found in “Matola 2” and “Keitt” (18.50 g). “Nametil 1A” had the smallest stone and seed length (5.55 and 4.83 cm respectively) and “Lourenco” had the lowest fruit weight (116.67 g). Mango fruit can have a weight range that varies from as little as a few grams up to 1 kg (Human, 2008) and fruit lengths can vary from 2.5–30.0 cm in different varieties (Morton, 1987).

“Keitt” had the greatest leaf blade width (7.31 cm), “Xavier” the greatest leaf blade length (27.58 cm) and petiole length (4.23 cm), while “Matola 4” had the lowest leaf blade length (13.49 cm) and “Anzo” the lowest leaf blade width (3.48 cm). For petiole length, the lowest value was found for “Kent” (1.55 cm). Mango leaves can measure up to 30 cm in length and 13 cm in width (Griesbach, 2003). “Haden” had the highest rachis pubescence value of 2.00 while “Matola 3” had the lowest value (0.09).

Table 3.2 Quantitaive characteristics of 30 mango varieties

Varieties	FRL cm	FRD cm	FRW g	STL cm	STW cm	STT cm	STWG g	SDL cm	SDW cm	SDWG g	LBLL cm	LBLW cm	PLE cm	IFL cm	IFW cm	PIR	HGT m	TRK m	CNS m	CEW m	RBT %
Malcorada	13.39	19.49	173.53	6.87	3.83	0.54	23.67	5.83	2.97	21.00	22.40	4.95	3.15	23.45	12.70	1.00	5.75	1.53	5.05	4.55	67.50
Polenta	15.08	30.56	516.67	6.73	4.87	0.37	33.00	5.40	3.55	14.00	22.50	4.53	2.72	22.25	9.95	1.70	7.37	1.66	5.85	6.15	21.55
Umbeluzi	14.03	16.53	144.12	8.81	3.46	0.75	20.94	5.89	2.51	10.78	21.16	3.99	3.05	16.50	10.50	0.11	5.88	1.15	6.30	6.60	63.64
Monsserate	14.17	22.19	453.85	7.47	4.05	0.55	25.87	5.71	3.01	20.50	22.02	4.49	2.97	20.73	11.05	0.94	8.18	1.79	6.75	8.75	26.50
Matola 2	19.40	27.15	575.00	9.67	4.57	0.57	18.50	-	-	-	18.69	3.85	2.44	29.61	20.67	0.67	6.30	1.79	6.35	6.80	75.79
Malcurada	12.50	20.52	204.17	6.36	4.24	0.52	27.25	5.50	3.20	16.00	20.44	4.48	3.46	28.10	13.15	1.50	6.30	2.49	7.05	-	14.71
Nametil 1A	15.39	25.02	330.00	5.55	3.98	0.45	23.40	4.83	3.30	15.50	23.71	5.19	3.34	23.10	15.20	1.10	6.63	1.94	7.00	6.45	76.50
Sabre	13.75	16.58	150.00	8.17	3.60	0.59	24.33	6.60	2.80	14.20	20.58	5.01	3.63	20.50	15.10	0.50	5.15	1.44	6.00	5.30	-
Afonsa Pairy	13.54	22.38	269.23	6.09	4.19	0.59	21.67	5.50	3.10	11.00	22.40	5.05	2.65	26.60	14.70	1.80	7.78	2.49	7.60	7.55	20.86
Lima 2	18.63	26.70	462.50	9.33	5.80	0.60	47.67	7.00	3.87	24.33	16.15	3.68	2.02	27.80	21.30	1.50	6.13	1.73	6.40	6.35	17.50
Papaia	13.17	21.15	150.00	6.40	4.20	0.60	24.00	5.50	3.00	12.00	14.30	4.37	2.49	19.50	13.50	1.20	5.19	0.45	4.55	4.40	25.21
Anzo	16.75	22.59	490.00	9.18	4.70	0.58	37.20	6.50	3.50	-	14.01	3.48	3.17	25.10	21.40	1.00	5.44	1.57	6.20	5.80	30.86
Keitt	16.06	24.19	468.75	8.17	4.50	0.40	18.50	-	-	-	20.11	7.31	2.79	47.65	27.15	0.40	7.68	0.99	5.45	6.15	30.00
Fernandinha	13.69	21.16	166.67	7.04	4.09	0.59	27.18	6.48	3.17	16.00	17.94	3.71	3.38	25.14	14.59	1.55	6.09	1.50	5.85	5.35	54.62
PA Mocuba	14.73	21.59	323.08	7.40	4.80	0.51	34.33	6.30	3.63	25.00	20.36	4.57	2.68	34.05	27.55	0.60	4.90	1.22	6.65	-	8.62
Xavier	14.31	22.04	268.75	6.91	4.23	0.39	25.17	5.54	2.88	12.50	27.58	6.08	4.23	33.35	18.20	1.80	6.60	0.70	7.45	6.20	30.98
Boane	17.68	26.49	754.55	8.99	5.66	0.56	57.00	6.53	4.40	29.50	17.06	4.20	3.07	19.50	13.90	0.60	5.10	1.50	4.30	11.70	16.89
Quinta Aurora	18.14	27.03	546.15	8.96	5.14	0.42	43.75	6.90	4.00	29.50	23.71	4.28	2.61	33.39	30.72	1.78	11.98	2.52	13.95	13.85	43.14
Zoologia	15.91	23.93	312.50	7.13	4.47	0.48	25.67	6.47	3.58	20.50	14.63	3.71	2.74	35.13	21.50	0.60	7.14	2.55	7.65	7.74	73.50
Kent	19.41	29.05	656.25	8.70	5.02	0.40	38.20	6.57	4.22	30.00	14.47	4.19	1.55	36.00	16.35	0.90	6.38	0.69	5.50	4.75	48.28
Haden	18.56	29.04	512.50	8.94	5.26	0.42	46.20	7.00	3.90	30.33	19.45	4.56	2.41	26.90	11.45	2.00	5.59	1.61	5.25	4.85	40.86
Xinavane	16.35	26.53	470.00	5.95	4.55	0.58	22.00	4.85	3.25	15.00	19.88	4.37	2.80	29.15	17.10	0.60	5.88	1.67	7.05	6.70	53.70
Sensation	15.31	23.60	300.00	6.60	4.30	0.58	19.20	6.00	3.50	-	17.48	4.19	2.85	36.55	18.15	1.40	10.38	1.23	5.60	3.00	85.00
Ruby	13.80	20.32	260.00	8.29	3.93	0.31	22.57	6.50	3.33	-	17.06	4.99	2.48	24.35	10.30	1.90	6.13	1.82	6.25	7.18	40.86
Peach	13.92	22.15	276.92	6.59	4.28	0.50	25.33	5.40	3.17	13.83	21.61	4.79	3.73	30.56	26.06	1.00	5.74	0.55	5.90	5.95	47.08
Melo	18.77	28.32	815.38	8.63	5.38	0.58	48.25	-	-	-	21.25	3.83	2.13	24.35	16.40	1.10	8.23	2.04	9.65	8.60	22.73
Lourenco	15.21	24.30	116.67	6.16	4.36	0.50	26.00	5.00	3.60	18.00	19.68	4.64	3.33	23.45	14.10	1.10	4.90	1.29	4.80	9.15	29.14
Matola 3	16.97	26.42	433.33	7.72	5.28	0.49	43.55	6.49	3.79	25.33	14.38	4.04	2.95	35.32	22.05	0.09	5.30	1.04	5.60	5.55	-
Matola 4	16.35	24.82	403.85	8.93	4.80	0.51	39.25	5.73	3.87	19.50	13.49	3.49	2.16	27.85	25.40	0.78	5.30	1.77	6.40	7.80	39.74
Matola 6	21.70	37.22	1089.29	10.07	6.28	1.82	69.00	-	-	-	17.78	3.72	2.26	26.89	21.83	1.44	4.90	0.96	6.25	6.20	13.43

FRL = fruit length, FRD = fruit diameter, FRW = fruit weight, STL = stone length, STW = stone width, STT = stone thickness, STWG = stone weight, SDL = seed length, SDW = seed width, SDWG = seed weight, LBLL = leaf blade length, LBLW = leaf blade width, PLE = petiole length, IFL = inflorescence length, IFW = inflorescence width, PIR = pubescence of inflo-rescence raquis, HGT = tree height, TRK = trunk circumference, CNS = crown north-south, CEW = crown east-west, RBT = ratio Brix/titratable acidity, " - " = missing value

Data indicated that “Quinta Aurora” had the highest inflorescence width average of 30.72 cm followed by “PA Mocuba” and “Keitt” with 27.55 and 27.15 cm, respectively. The lowest value was obtained for “Polenta” (9.95 cm). “Keitt’s” inflorescence was the longest with a value of 47.65 cm while “Sensation” and “Kent” measured 36.55 and 36.00 cm, respectively (Table 3.2). According to Campbell (1992) “Keitt” is a popular and heavy producer. This factor can be attributed to the dimensions of the inflorescence and consequently the number of flowers potentially fertile. The lowest inflorescence length was obtained for “Umbeluzi” (16.50 cm).

Mango trees can achieve heights of 3-10 m and even 30-38 m (Morton, 1987; Bally, 2006). “Quinta Aurora” was the largest three among all tested varieties with a crown north-south measurement of 13.95 m and a crown-east west measurement of 13.85 m and a height of 11.98 m. These tree dimensions make “Quinta Aurora” a productive variety, since other studies on perennial species found correlation between fruit production and diameter of the canopy (Glendinning, 1966; Sing Dhaliwal, 1968; Garcia and Nicolella, 1985). The smallest varieties were “PA Mocuba”, “Lourenco” and “Matola 6” all with a tree height of 4.9 m. “Zoologia” had the biggest trunk circumference (2.55 m) while the lowest value was obtained for “Papaia” (0.45 m) (Table 3.2).

The Brix/acidity ratio is a balance between sugars and acids and is an indication of the palatability of the juice (Echeveria, 1990). The highest Brix/acidity ratios were in “Sensation” (85%), “Nametil 1A” (76.5%) and “Matola 2” (75.79%). The lowest values were detected for “PA Mocuba”, “Matola 6” and “Malcurada” with 8.62%, 13.43% and 14.71%, respectively (Table 3.2).

Fruits for exportation, as discussed in section 3.1, should weigh approximately 300-400 g and have good flesh quality. Therefore varieties that can be selected are “Nametil 1A”, “Zoologia” and “Sensation” because they not only satisfy the weight requirement but also have a good Brix/acidity ratio. “Matola 2”, although possessing a good Brix/acidity ratio has excessive weight. However all commercial varieties exceeded the required ideal weight indicating that “Matola 2” should be acceptable. “Umbeluzi” and “Malcorada”, despite having good Brix/acidity ratios (63.64 and 67.50) cannot be selected because of

low fruit weight (173.53 and 144.12 g). These varieties should possibility be useful in processing products like juice, dehydrated pulp etc.
“Matola 6”, “Matola 4”, “Matola 3”, “Peach”, “Boane”, “Anzo”, “Papaia”, “Sabre” and “Malcorada” are dwarf varieties. Dwarfness enables high density planting, ease of harvesting and when associated with other characteristics referred to in section 3.3.1.2 can be selected for rootstocks.

3.3.1.2 QUALITATIVE ANALYSES

Data obtained for 50 of the qualitative morphological descriptors are listed in Table 3.3. Characteristics that did not show variation are not included in the table, e.g., curvature of secondary veins, colour of fully developed leaf, presence of leaf pubescence and type of flower; all descriptors are listed in Appendix 1.

The majority of varieties presented an oblong crown shape (56.7%) and 50% of the trees had a spreading and 43.3% an erect growth habit. It was reported by Morton (1987) that the canopy can be rounded, erect, oval or slender.

In general, the leaves were lanceolate (70%), with a semi-erect leaf attitude (90%), the leaf apex shape was mainly acute (70%), with an entire leaf margin (96.7%) and leaves had a mild fragrance (56.7%) and intermediate foliage density (56.7%). All leaves presented absence of curvature of secondary veins, were dark green in colour and fully developed leaves lacked pubescence. Bally et al. (2009) reported that mango leaves are variable in shape and size, frequently oblong with tips from rounded to acuminate and young leaves varied from brown to purple and old leaves were dark green in colour. Leaf shapes were entire, leathery, short, pointed and oblong to lanceolate. The length was about 450 mm. Differences found are due to varietal variation, climate, cultural practises and growth stages. At maturity leaves usually smell like turpentine (Fivaz, 2008).

Results for inflorescence indicated that the majority had a terminal position (60%), horizontal inflorescence axis growth habit (53.3%) and a pyramidal inflorescence shape (76.6%).

Table 3.3 Qualitative morphological characteristics of 30 tested mango varieties

Varieties	CSH	TGH	FDE	LBLS	LAT	LAS	LMA	LFR	IFP	IAG	IFS	PLB
Malcorada	Oblong	Erect	Intermed.	Elliptic	S. erect	Acute	Entire	Strong	Axilary	S.erect	Pyramidal	Present
Polenta	Oblong	Erect	Sparse	Lanceolate	S. erect	Acute	Wave	Strong	Terminal	S.erect	Pyramidal	Absent
Umbeluzi	S.circular	Drooping	Dense	Lanceolate	S. erect	Acuminate	Entire	Mild	Axilary	S.erect	Conical	Present
Monsserate	Oblong	Spreding	Dense	Lanceolate	S. erect	Acuminate	Entire	Strong	Terminal	Horizontal	Pyramidal	Present
Matola 2	Broadly p.	Spreding	Dense	Lanceolate	S. erect	Acute	Entire	Mild	Terminal	Drooping	Pyramidal	Present
Malcurada	Broadly p.	Spreading	Dense	Lanceolate	S. erect	Acuminate	Entire	Mild	Axilary	Horizontal	Pyramidal	Present
Nametil 1A	Broadly p.	Erect	Intermed.	Lanceolate	S. erect	Acute	Entire	Mild	Terminal	S.erect	Pyramidal	Present
Sabre	Broadly p.	Spreading	Intermed.	Lanceolate	S. erect	Acuminate	Entire	Mild	Axilary	Horizontal	Conical	Present
Afonsa Pairy	Broadly p.	Spreading	Intermed.	Lanceolate	S. erect	Acuminate	Entire	Mild	Terminal	Horizontal	Pyramidal	Present
Lima 2	Broadly p.	Erect	Dense	Lanceolate	S. erect	Acute	Entire	Mild	Axilary	S.erect	Broadly p.	Present
Papaia	Broadly p.	Erect	Sparse	Lanceolate	S. erect	Acute	Entire	Mild	Terminal	Horizontal	Pyramidal	Absent
Anzo	Spherical	Spreading	Dense	Elliptic	S. erect	Acute	Entire	Strong	Terminal	S.erect	Pyramidal.	Present
Keitt	Oblong	Erect	Sparse	Elliptic	S. erect	Acute	Entire	Mild	Terminal	-	Broadly p.	Absent
Fernandinha	Oblong	Erect	Dense	Lanceolate	S. erect	Acute	Entire	Mild	Axilary	S.erect	Pyramidal	Present
PA Mocuba	Broadly p.	Erect	Dense	Oblong	S. erect	Acute	Entire	Strong	Terminal	S.erect	Pyramidal	Present
Xavier	Oblong	Spreading	Dense	Ovate	S. erect	Acuminate	Entire	Mild	Terminal	S.erect	Pyramidal	Present
Boane	Oblong	Erect	Intermed.	Lanceolate	S. erect	Acute	Entire	Mild	Axilary	Horizontal	Pyramidal	Present
Quinta Aurora	Oblong	Drooping	Intermed.	Lanceolate	Horizontal	Acuminate	Entire	Absent	Terminal	Horizontal	Broadly p.	Absent
Zoologia	Oblong	Erect	Dense	Oblong	S. erect	Acute	Entire	Mild	Axilary	S.erect	Pyramidal	Absent
Kent	Oblong	Erect	Intermed.	Lanceolate	S. erect	Acute	Entire	Mild	Terminal	Horizontal	Broadly p.	Present
Haden	Oblong	Spreading	Intermed.	Lanceolate	Horizontal	Acute	Entire	Mild	Terminal	S.erect	Pyramidal	Present
Xinavane	Oblong	Spreading	Intermed.	Ovate	S. erect	Acute	Entire	Strong	Terminal	Horizontal	Pyramidal	Present
Sensation	Oblong	Spreading	Intermed.	Lanceolate	S. erect	Acute	Entire	Strong	Terminal	Horizontal	Broadly p.	-
Ruby	Oblong	Erect	Intermed.	Lanceolate	S. erect	Acute	Entire	Mild	Terminal	Horizontal	Pyramidal	Present
Peach	Broadly p.	Spreading	Intermed.	Lanceolate	S. erect	Acute	Entire	Strong	Terminal	Horizontal	Pyramidal	Present
Melo	Oblong	Spreading	Intermed.	Lanceolate	Horizontal	Acute	Entire	Absent	Terminal	S.erect	Pyramidal	Present
Lourenco	Broadly p.	Spreading	Intermed.	Lanceolate	S. erect	Acute	Entire	Mild	Axilary	Horizontal	Pyramidal	Absent
Matola 3	Oblong	Spreading	Intermed.	Elliptic	S. erect	Acuminate	Entire	Strong	Axilary	Horizontal	Pyramidal	Absent
Matola 4	Oblong	Erect	Intermed.	Ovate	S. erect	Acute	Entire	Strong	Axilary	Horizontal	Pyramidal	Absent
Matola 6	Broadly p.	Spreading	Intermed.	Lanceolate	S. erect	Acuminate	Entire	Strong	Axilary	Horizontal	Pyramidal	Present

CSH = crown shape, TGH = tree growth habit, FDE = foliage density, LBLS = leaf blade shape, LAT = leaf attitude, LAS = leaf apex shape, LMA = leaf margin, LFR = leaf fragrance, IFP = inflorescence position, IAG = inflorescence axis growth, IFS = inflorescence shape, PLB = presence of leaf bracts, Broadly p.= broadly piramydal, Intermed.= intermediate, S.erect = semi-erect, S.circular = semi-circular, "-" = missing values.

Table 3.3 Continued

Varieties	DFI	IFC	LSP	NDI	NSS	FIA	FBI	FSH	SFA	FSS	FBC	FAT
Malcorada	Dense	Yellow	Equal	Solen br.	1 fertile	Medium	High	Roundish	-	Smooth	Yellow	Average
Polenta	Medium	Yellow	Equal	Narrow	2-3 fert.	Low	Medium	Roundish	-	Smooth	-	Poor
Umbeluzi	Sparse	Green.	Equal	Solen br.	-	Medium	Medium	Oblong	Acute	Smooth	Orange	Poor
Monsserate	Medium	L. green	Equal	Narrow	1 fertile	Low	High	Oblong	Obtuse	Smooth	Green	Poor
Matola 2	Medium	Pink	Longer	Narrow	1 fertile	Low	Medium	Oblong	-	Rough	Green	Average
Malcurada	Dense	Green.	Longer	Solen br.	1 fertile	Low	High	Roundish	Obtuse	Smooth	Green	Poor
Nametil 1A	Dense	Yellow	Equal	Solen br.	1 fertile	Low	High	Roundish	-	Smooth	Green	Average
Sabre	Sparse	Green.	Equal	Solen br.	1fertile	Low	Medium	Oblong	Acute	Smooth	Yellow	Average
Afonsa Pairy	Medium	Yellow	-	-	-	-	Medium	Roundish	Obtuse	Smooth	Green	Poor
Lima 2	Dense	Yellow	Equal	Solen br.	1 fertile	Medium	Medium	Elliptic	Obtuse	Smooth	Yellow	Average
Papaia	Medium	Yellow	Longer	Solen br.	1 fertile	Low	High	Roundish	Obtuse	Smooth	Green	Average
Anzo	Dense	Yellow	Equal	Solen br.	1 fertile	Medium	High	Oblong	Acute	Smooth	Green	Good
Keitt	Medium	Yellow	Equal	Solen br.	1 fertile	Low	High	Roundish	Obtuse	Smooth	Yellow	Average
Fernandinha	Dense	Yellow	Equal	Solen br.	1 fertile	High	High	Obovoide	Obtuse	Rough	Green	Average
PA Mocuba	Medium	Greenw.	Longer	Narrow	2-3 fert.	High	Medium	Obovoide	-	Smooth	Green	Average
Xavier	Medium	Yellow	Longer	Solen br.	1 fertile	Low	High	Roundish	-	Rough	Green	Average
Boane	Medium	Yell. green	Equal	Solen br.	1 fertile	High	High	Oblong	Obtuse	Smooth	Green	Poor
Quinta Aurora	Medium	Yell. green	Equal	Narrow	1 fertile	Low	Medium	Oblong	Obtuse	Smooth	Green	Poor
Zoologia	Sparse	Yell. green	Equal	Solen br.	1 fertile	-	Medium	Roundish	Obtuse	Smooth	Yellow	Average
Kent	Dense	Pink	Equal	Solen br.	1 fertile	-	High	Obovoide	Obtuse	Smooth	Yellow	Good
Haden	Medium	Pink	Equal	Solen br.	1 fertile	Low	High	Roundish	Obtuse	Smooth	Yellow	Good
Xinavane	Sparse	L. green	Equal	Narrow	2-3 fert.	Low	Medium	Roundish	-	Smooth	Green	Average
Sensation	Medium	Pink	Equal	Solen br.	1 fertile	Low	High	Obovoide	Acute	Rough	Green	Average
Ruby	Medium	L. orange	Longer	Narrow	1 fertile	-	High	Oblong	Obtuse	Smooth	Yellow	Good
Peach	Medium	Pink	Longer	Narrow	1 fertile	Low	High	Roundish	Obtuse	Smooth	Orange	Average
Melo	Medium	L.green	Longer	Solen br.	-	-	Medium	Oblong	Obtuse	Smooth	Green	Poor
Lourenco	Sparse	Green.	Equal	Solen br.	5 fert	Medium	Medium	Roundish	Round	Smooth	Yellow	Average
Matola 3	Medium	Green.	Equal	Solen br.	1 fertile	Low	Medium	Roundish	-	Smooth	Yellow	Good
Matola 4	Sparse	Yell. green	Longer	Narrow	1 fertile	Low	Medium	Oblong	-	Rough	Green	Poor
Matola 6	Dense	L.green	Shorter	Solen br.	1 fertile	Low	Low	Oblong	-	Rough	Green	Average

DFI = density of flowers, IFC = inflorescence colour, LSP = length of stamens, NDI = nature of disc, NSS = number of stamens, FIA = intensity of anthocyanin, FBI = fruit bearing intensity, FSH = fruit shape, SFA = shape of fruit apex, FSS = fruit skin surface texture, FBC = fruit background colour, FAT = fruit attractiveness, Green. = greenish, Yell. = yellowish, L. = light, Fert. = fertile, br. = broader, "-" = missing values.

Table 3.3 Continued

Varieties	DLF	DFS	FNP	FBT	FST	FSW	SCF	PCF	PTR	ASP	QLO	QFP	PJU
Malcorada	Sparse	Shallow	Pro.	Percep.	Shallow	No waxy	Yellow	Y. orange	Intermed.	Intermed.	Low	Low	Sligtly juicy
Polenta	Sparse	Absent	Absent	Percep.	Absent	No waxy	Yellow	Orange	Soft	Strong	Medium	Intermed.	Very juicy
Umbeluzi	Sparse	Absent	Pro.	Mammifor.	Deep	No waxy	Greenish	Y. orange	Soft	Strong	High	Intermed.	Sligtly juicy
Monsserate	Dense	Shallow	Sligtly pro.	Pointed	Shallow	No waxy	Green	Yellow	Soft	Strong	Medium	High	Juicy
Matola 2	Sparse	Medium	Absent	Pro.	Absent	No waxy	Yellow	Y. orange	Soft	Strong	Low	Low	Very juicy
Malcurada	Sparse	Shallow	Absent	Percep.	Absent	No waxy	Green	Golden y.	Soft	Weak	High	High	Juicy
Nametil 1A	Medium	Medium	Absent	Percep.	Absent	No waxy	Greenish y.	Golden y.	Soft	Weak	Low	Low	Very juicy
Sabre	Sparse	Absent	Pro.	Mammifor.	Absent	No waxy	Greenish y.	Golden y.	Soft	Weak	High	Intermed.	Sligtly juicy
Afonsa Pairy	Sparse	Shallow	Absent	Pointed	Absent	No waxy	Greenish y.	Yellow	Soft	-	Medium	Low	Juicy
Lima 2	Sparse	Shallow	Absent	Percep.	Absent	No waxy	Yellow	-	Firm	-	High	Intermed.	-
Papaia	Sparse	Shallow	Sligtly pro.	Percep.	Absent	No waxy	Green	Golden y.	Soft	Intermed.	Low	Intermed.	Juicy
Anzo	Sparse	Absent	Pro.	Pointed	Absent	No waxy	Green p. p	Golden y.	Soft	Weak	Absent	Intermed.	Juicy
Keitt	Sparse	Shallow	Very pro.	Percep.	Absent	No waxy	Yellow	Y. orange	-	-	Medium	Low	-
Fernandinha	Sparse	Shallow	Absent	Percep.	Absent	No waxy	Green r. b.	Light y.	Intermed.	Weak	Medium	Low	Juicy
PA Mocuba	Sparse	Shallow	Sligtly pro.	Percep.	Absent	No waxy	Greenish y.	Orange	Soft	Weak	Medium	Intermed.	Juicy
Xavier	Medium	Shallow	Sligtly pro.	Percep.	Absent	No waxy	Green	Golden y.	Soft	Weak	Medium	Low	Juicy
Boane	Sparse	Absent	Absent	Pointed	Shallow	No waxy	Green	Light y.	Firm	Weak	High	High	Sligtly juicy
Quinta Aurora	Sparse	Absent	Sligtly pro.	Pro.	Shallow	No waxy	Green	Light y.	Firm	Weak	Medium	High	Very juicy
Zoologia	Medium	Shallow	Sligtly pro.	Percep.	Absent	No waxy	Green r. b.	Orange	Soft	Strong	Low	High	Juicy
Kent	Dense	Shallow	Very pro.	Percep.	-	No waxy	Yellow	Orange	Intermed.	Intermed.	Low	Low	Very juicy
Haden	Medium	Shallow	Very pro.	Percep.	Absent	No waxy	Green r. b.	Golden y.	Intermed.	Strong	Absent	Low	Very juicy
Xinavane	Dense	Medium	Absent	Percep.	Absent	No waxy	Greenish y.	Orange	Intermed.	Strong	Medium	Intermed.	Juicy
Sensation	Sparse	Absent	Absent	Pointed	Absent	No waxy	Greenish y.	Yellow	Intermed.	Intermed.	Medium	High	Sligtly juicy
Ruby	Sparse	Absent	Very pro.	Pointed	Absent	No waxy	Green r. b.	Light y.	Intermed.	Weak	Medium	Intermed.	Sligtly juicy
Peach	Sparse	Medium	Very pro.	Percep.	Absent	No waxy	Yellow	Y. orange	Firm	Weak	Low	High	Sligtly juicy
Melo	Sparse	Shallow	Sligtly pro.	Percep.	Shallow	No waxy	-	Orange	Soft	Intermed.	Low	High	Sligtly juicy
Lourenco	Medium	Medium	Absent	Percep.	Absent	No waxy	Greenish y.	Orange	Soft	Weak	Absent	Intermed.	Juicy
Matola 3	Sparse	Medium	-	Percep.	Shallow	No waxy	Yellow	Golden y.	Soft	Strong	Low	High	Very juicy
Matola 4	Sparse	Absent	Pro.	Pointed	Deep	No waxy	Green	Golden y.	Soft	Intermed.	Low	Intermed.	Juicy
Matola 6	Sparse	-	Sligtly pro.	Percep.	Shallow	Waxy	Yellow	Y. orange	Firm	Intermed.	Medium	High	Very juicy

DLF = density of lenticels, DFS = depth of fruit stalk cavity, FNP = fruit neck prominence, FBT = fruit beak type, FST = fruit sinus type, FSW = fruit waxyness, SCF = skin colour of ripe fruit, PCF = pulp colour of ripe fruit, PTR = pulp texture of ripe fruit, ASP = Adherence of fruit skin to pulp, QLO = latex oozing by peduncle, QFP = quantity of fibre in the pulp, PJU = pulp juiceness, Intermed. = Intermediate, Mammifor. = mammiforme, Percep. = perceptible, Pro = prominente, b. = brown, p.p. = purple patch, r. = red, Y. = yellow, y. = yellowish, "-" = missing values

Table 3.3 Continued

Varieties	AFS	FLP	PAR	PTF	VST	PSV	QFS	LST	AFS	TSF	SDS	TEB	FMP
Malcorada	Low	Short	Intermed.	Intermed.	Surface	Forked	Low	Short	Weak	Soft	Reniform	Mono	Medium
Polenta	Medium	Short	Mild	Mild	Surface	Forked	Low	Short	Weak	Soft	Oblong	Mono	Medium
Umbeluzi	Medium	Short	-	Absent	Depressed	Parallel	Intermed.	Long	Intermed.	Coarse	Oblong	Poly	Early
Monsserate	Low	Long	Strong	Intermed.	Depressed	Forked	Low	Short	Strong	Coarse	Reniform	Mono	Late
Matola 2	High	Medium	Intermed.	Strong	Surface	Forked	Low	Medium	Weak	Coarse	-	-	Early
Malcurada	Medium	Medium	Strong	Mild	Depressed	Parallel	Intermed.	Medium	Intermed.	Coarse	Reniform	Mono	Medium
Nametil 1A	Medium	Medium	Mild	Mild	Surface	Parallel	Intermed.	Medium	Weak	-	Oblong	Poly	Medium
Sabre	Medium	Medium	Mild	Intermed.	Surface	Forked	Low	Short	Intermed.	Soft	Ellipsoid	Poly	Early
Afonsa Pairy	Medium	Short	Intermed.	Intermed.	Depressed	Forked	Low	Short	Intermed.	Soft	Reniform	Mono	Early
Lima 2	-	Short	Strong	Strong	Elevated	Forked	Intermed.	Long	Intermed.	Soft	Reniform	Mono	Early
Papaia	Medium	Short	-	Strong	Surface	Parallel	Low	Short	Strong	Soft	Reniform	Mono	Medium
Anzo	Medium	Long	Intermed.	Strong	Depressed	Forked	Low	Long	Weak	-	Reniform	Mono	Medium
Keitt	Medium	-	Mild	Absent	Surface	Forked	Intermed.	Short	Weak	Soft	Reniform	Mono	Medium
Fernandinha	High	Short	Mild	Mild	Depressed	Parallel	Intermed.	Short	Weak	Coarse	Ellipsoid	Mono	Medium
PA Mocuba	Medium	Medium	Intermed.	Mild	Depressed	Forked	Intermed.	Medium	Intermed.	Soft	Reniform	Poly	Early
Xavier	Medium	Short	Intermed.	Strong	Depressed	Parallel	Low	Medium	Strong	Soft	-	Mono	Medium
Boane	Medium	Long	Intermed.	Strong	Elevated	Parallel	Intermed.	Long	Intermed.	Soft	Reniform	Mono	Medium
Q. Aurora	Medium	Long	Strong	Intermed.	Elevated	Forked	Intermed.	Short	Weak	Soft	Oblong	Mono	Medium
Zoologia	High	Medium	Strong	Mild	Depressed	Forked	Low	Medium	Intermed.	Coarse	Oblong	Mono	Early
Kent	High	Short	Strong	Intermed.	Elevated	Forked	Low	Short	Weak	Soft	Oblong	Mono	Medium
Haden	Low	Short	Mild	Absent	Surface	Forked	Low	Short	Weak	Soft	Oblong	Mono	Medium
Xinavane	Low	Short	Strong	Strong	Surface	Forked	Low	Short	Weak	Soft	Reniform	Mono	Medium
Sensation	High	Long	Intermed.	Mild	Elevated	Forked	Low	Short	Weak	Soft	-	-	Medium
Ruby	High	Medium	Intermed.	Mild	Depressed	Parallel	Low	Short	Weak	Soft	Reniform	Mono	Medium
Peach	Medium	Long	Intermed.	Absent	-	-	High	Long	Strong	Coarse	Reniform	Poly	Late
Melo	High	Medium	Intermed.	Intermed.	Elevated	Forked	Low	Short	Intermed.	Soft	Reniform	Mono	Medium
Lourenco	Medium	Long	-	Strong	Surface	Forked	Low	Long	Intermed.	Coarse	Oblong	Poly	Early
Matola 3	High	Short	Mild	Mild	Depressed	Parallel	Intermed.	Long	Strong	Coarse	Reniform	Poly	Early
Matola 4	High	Medium	Strong	Intermed.	Elevated	Parallel	Intermed.	Medium	Intermed.	Soft	Reniform	Mono	Early
Matola 6	Medium	Medium	Mild	Mild	Depressed	Parallel	High	Medium	Strong	Coarse	Oblong	Mono	-

AFS = adherence of fibre to fruit skin, FLP = fibre length in the pulp, PAR = pulp aroma, PTF = presence of turpentine flavour, VST = veins on stone, PSV = pattern of veins stone, QFS = quantity of fibre on stone, LSF = length of stone fibre, AFS = adherence of fibre to fruit skin, TSF = texture of stone fibre, SDS = seed shape, TEB = type of embryony, FMP = fruit maturation period, Intermed. = intermediate"-" = missing values

Seventy percent of inflorescence had pubescence rachis, medium density of flowers (53.3%) and 33.3% were yellow and 43% had a green or greenish colour. The intensity of anthocyanins was 10% high, 16.7% medium and 56.7% low. The lengths of stamens were mostly equal (63%), the disc was solen broader (66.7%) and one fertile stamen (76.6%) was seen for most varieties but all showed pentamerous flowers. The mango inflorescence is primarily terminal and flowers are usually small (Bally, 2006).

Fruits shape were more roundish (46.7%) and oblong (36.7%) although some were obovoid (13%) or elliptic (3.3%). The bearing intensity varied between high (50%), medium (46.7%) and the rest had a low intensity. The majority of fruit apices had an obtuse shape (50%). Average fruit attractiveness (53.3%), green fruit background colour (56.7%), and smooth fruit skin texture (80%), sparse density of lenticels (73.3%) and shallow fruit stalk cavity (46.7%) were observed. Thirty six percent of the varieties showed an absent fruit neck prominence while 43.4% showed a slightly prominent or prominent fruit neck. A perceptible fruit beak type (63.3%), absent fruit sinus (66.7%), no waxy fruit skin (96.7%) and yellow fruit skin colour (30%) were observed for the majority of varieties. The ripe fruit had mainly a golden yellow (30%), orange (23.3%) or yellow orange (20%) pulp colour, the others were yellow or light yellow. Most of the varieties (56%) showed a soft pulp texture and the adherence of fruit skin to pulp was mainly weak (40%). The pulp juiciness was 26.7% very juicy, 40% juicy and 26.7% slightly juicy. The quantity of latex oozing at the peduncle was mainly medium (40%) or low (33.3%). The fibre quantity in the pulp was 36.7% intermediate while 33.3% were high and 30% low. The adherence of fibre to fruit skin were found to be medium (53.3%), fibre length in the pulp were short (40%) and 33.3% of the fruit showed a mild presence of turpentine.

Mango fruit is classified as a drupe and can be highly versatile in shape, colour, taste and texture according to the variety. Fruits shapes vary from round to ovate to oblong and long with lateral compression. Fruits mainly have a dark green background that become lighter green to yellow as they ripen. Some fruit develop a red background at fruit set that persists until the fruit ripen. Pulp normally has a sweet and slightly turpentine flavour and when it ripens the colour changes from yellow to orange with a smooth to fibrous texture

(Bally, 2006).Results also indicated the following major characteristics to be present: depressed (40%), surface (33%) or elevated (23.3%) veins on the stone, a forked pattern of veins on stone (60%), low fibre on stone (56.7%), and short fibres on the stone (50%). Fibre adherence to the stone is mainly weak (43.3%). Most fibres show a soft texture (60%). The most common seed shape was reniform (53.3%) and 70% of the varieties had monoembryonic seed, most (56.7%) of the fruit had a medium maturation period (Table 3.3). Some mango cultivars have polyembryonic seed and those types originated in the tropics. The embryos arise from nucellar tissue and generally inhibit the development of the zygotic embryo. In contrast cultivars most developed in the subtropics, are monoembryonic and contain a single zygotic embryo (Human, 2008).

Based on these results it will be difficult to choose specific varieties for different purposes, since no single variety has all the recommended characteristics mentioned in section 3.1 for exportation and in Chapter 2 for processing. Accordingly, based on some important characteristics it is possible to say that “Matola 3”, “Ruby” and “Anzo” have good fruit attractiveness that is good for exportation. “Matola 3”, “Matola 4”, “Lourenco”, “PA Mocuba”, “Lima 2”, “Afonsa Pairy”, “Matola 2” and “Umbeluzi” presented an early fruit maturation period that is important for Mozambique because of international market opportunities. Consumers mainly prefer a rounded fruit shape making “Matola 3”, “Nametil 1A” and “Malcorada” candidates that can be selected in breeding programmes. Other varieties listed in the Table 3.3 with rounded shapes were not included because of unfavourable eating quality. There are no huge distinctions between export and domestic markets, except for the former, pulp texture should be firm for transport over long distances. For processing products varieties with high juice content are more suitable such as “Matola 6”, “Matola 3”, “Quinta Aurora”, “Nametil 1A” and “Polenta”.

3.3.1.3 COMMERCIAL VARIETIES

Characteristics listed for commercial varieties in Table 3.4 represent a comparison between some results obtained in this study and those reported by Knight (1997). The methodologies used were similar as both studies used the IPGRI descriptors as

Table 3.4 Results from morphological characterisation of commercial varieties obtained in this study compared with Knight (1997)

	Afonsa Pairy		Fernandinha		Haden		Keitt		Kent		Sensation	
	Study	**Knight**	**Study**	**Knight**	**Study**	**Knight**	**Study**	**Knight**	**Study**	**Knight**	**Study**	**Knight**
Tree size	Vigorous	Mod. vig.	Mod. vig.	Mod. vig.	Mod. vig.	Mod. vig.	Mod. vig.	Mod. vig.	Mod. vig.	Vigorous	Mod. vig.	Vigorous
Crown spread	B. pyram.	B. round	Oblong	D. round	Oblong	L. spread.	Oblong	Upright o.	Oblong	D. upright	Oblong	Mod. sy.
Fruit colour	Golden orange	Yellow	Green/red blush	Yellow/ red blush	Yellow/ red blush	Bright yellow red blush	Yellow/ red blush	Yellow red blush	Green yellow	Yellow red blush	Green red blush	Yellow red blush
Fruit shape	Roundish	Ovate-oblique	Obovoide	Ovate-oblique	Roundish	Oval-rounded	Oblong	Oval rounded base	Obovoide	Oval rounded base	Obovoide	Oval rounded base
Fruit length	13.54 cm	6 cm	13.69 cm	12.2 cm	18.56 cm	10.5-14 cm	16.06 cm	13-15 cm	19.41 cm	9-9.5 cm	15.31cm	9-11.5 cm
Fruit weight	269.23 g	225-325 g	166.67 g	450 g	512.25 g	510-680 g	468.75 g	510-2000 g	656.25 g	600-750 g	300 g	280-340 g
Pulp texture	Soft	Firm-soft	Intermed.	Thick	Intermed.	Firm	-	Firm	Intermed.	Firm	Intermed.	Firm
Pulp fibre	Low	Low	Low	No fibre	Low	Abund. fibre	Low	Low	Low	Low	High	Absent
Pulp colour	Yellow	Yellow	Light yellow	Bright Yellow	Golden Yellow	Deep yellow	Yellow orange	Lemon orange	Orange	Yellow/ orange	Yellow	Deep Yellow
Pulp aroma	-	Character.	Mild	Piquant	Strong	Pleasant	Mild	Pleasant	Intermed.	Pleasant	Intermed.	pleasant
Type Embry.	Mono	Mono	Mono	Mono	Mono	Mono	Mono	Mono	Mono	Mono	Mono	Mono
Pulp juicy	Juicy	-	Slightly j.	Mod.-very	Very j.	Juicy	-	Juicy	Very juicy	Juicy	Slighly j.	Medium j.
Maturation p.	Early	Late-mid	Midseason	Late	Midseason	Early	Midseason	Late	Midseason	Late	Midseason	Mid-late
Skin type	-	Thin	Easily s.	Adherent	Adherent	Adherent	-	Adherent	Intermed.	Adherent	Intermed.	Easily s.

Abund. = abundant, B. = broadly, Character. = characteristic, D. = dense, Embry. = embryony, Intermed. = intermediate, j. = Juicy, L. = large,open, Mod = moderately, o. = open, P. = period, Pyram. = pyramidal, s. = separated, Spread. = spreading, sy. = Symmetrical, Vig. = vigorous.

guidelines. However, the nomenclature used in literature in some cases was different from that used in this study because different versions of the IPGRI descriptors were used. In this study the 2006 version and in Knight (1997) the 1989 version was used. It was found that crown shape, fruit shape and pulp aroma had different descriptions for the characteristics though it probably means the same e.g. for:

a) Crown: broadly pyramidal vs. broadly rounded dense;

b) Fruit shape: roundish vs. oval rounded

c) Aroma: pleasant aroma vs. intermediate; piquant aroma vs. strong aroma

Results showed some similarities such as fruit weight (excluding “Fernandinha” and “Keitt”), pulp colour, tree size (except for “Afonsa Pairy”, “Kent” and “Sensation”) and type of embryony (Table 3.4). The introduction of cultivars from one agroclimatic region to another has indicated that for certain characteristics some genotypes were stable while others were highly influenced by the environment (Iyer and Dinesh, 1997). Fruit length values for all varieties in this study were higher than stated in literature. There were also variations in results for fruit skin colour: “Afonsa Pairy”, “Fernandinha”, “Kent” and “Sensation” were found to be green in colour, but Campbell (1992) and Knight and Schnell (1994) stated the fruit skin colour to be yellow with red blush. Skin colouration of mature fruit in part results from anthocyanins that develop when tissues are exposed to light. Research is necessary to set up physiological parameters from which pruning and orchard strategies can be developed (Proctor and Creasey, 1971; Schaffer et al., 1992). The mango fruit collection at the Umbeluzi research station has not received the same maintenance and care (see 3.3.4).

3.3.2 CORRELATIONS

Results obtained for correlations using Spearman’s coefficient showed a positive and highly significant correlation between fruit length and various traits such as fruit diameter, fruit weight, stone length, stone width, stone weight, seed length, seed width and seed weight (Table 3.5). This table only contains highly significant and significant correlations. All correlations can be seen in Appendix 2.Fruit length presented a negative.

Table 3.5 Correlation matrix using Spearman's coefficient

Char.1	Char.2	Values	Char.1	Char.2	Values	Char.1	Char.2	Values	Char.1	Char.2	Values	Char.1	Char.2	Values
FRL	**FRD**	0.7776**	**FRD**	**STWG**	0.5364**	**STL**	**SDW**	0.4574*		**LBLW**	-0.3858*	**LBLL**	**LBLW**	0.5869**
	FRW	0.8161**		**SDW**	0.7786**		**SDWG**	0.5773**		**RBT**	-0.3922*		**PLE**	0.5772**
	STL	0.7078**		**SDWG**	0.5622**	**STW**	**STWG**	0.8494**	**SDL**	**SDW**	0.481**		**CNS**	0.3839*
	STW	0.7584**		**PLE**	- 0.4622*		**SDL**	0.4725*		**SDWG**	0.6653**	**HGT**	**CNS**	0.7098**
	STWG	0.6105**	**FRW**	**STL**	0.5715**		**SDW**	0.8821**		**IFW**	0.3836*	**Brix**	**STW**	-0.3964*
	SDL	0.6082**		**STW**	0.7523**		**PLE**	-0.4258*	**SDW**	**SDWG**	0.8346**		**STWG**	-0.3941*
	SDW	0.7663**		**STWG**	0.6268**		**IFW**	0.4152*		**LBLL**	-0.4166*		**HGT**	0.4895**
	SDWG	0.7201**		**SDL**	0.4353*		**SDWG**	0.7259**		**PLE**	-0.4982**		**TA**	-0.4013*
	PLE	-0.4585*		**SDW**	0.7517**	**STT**	**LBLW**	-0.462**		**IFW**	0.3757*		**RBT**	0.6133**
	IFW	0.4417*		**SDWG**	-0.6843		**PIR**	-0.3933*	**SDWG**	**PLE**	-0.462*			
FRD	**FRW**	0.7918**	**STL**	**STW**	0.4681*	**STWG**	**SDL**	0.5568**	**IFL**	**STT**	-0.4225*			
	STW	0.7857**		**STWG**	0.555**		**SDW**	0.7943**		**IFW**	0.722**			
	STT	-0.3855*		**SDL**	0.83**		**SDWG**	0.7567**	**IFW**	**CNS**	0.4955**			

CNS = Crown north-south, FRL = fruit length, FRD = fruit diameter, FRW = fruit weigth, HGT = height, IFL = inflorescence length, IFW = inflorescence width, LBLL = leaf blade length, LBLW = leaf blade width, PIR = pubescence of inflorescence rachis, PLE = petiole length, RBT = ratio Brix/TA, SDL = seed length,SDW = seed width, SDWG = seed weight, STL = stone length, STT = stone thickness, STW = stone width, STWG = stone weight, TA = titratable acidity, char. = characteristic

**$P \leq 0.01$ = higly significant, *$P \leq 0.05$ = significant

correlation with petiole length and a significant correlation with inflorescence width. According to Gupta et al. (1996) a highly significant correlation between fruit weight and fruit length and fruit length and diameter of mango fruits do exist as seen in this study as well.

Fruit diameter was highly significantly correlated with fruit weight, stone width, stone weight, seed width and seed weight. There was a significant but a negative correlation between fruit diameter and stone thickness as well as petiole length. Fruit weight was highly significantly correlated with stone length, stone width, stone weight and seed width and significantly correlated with seed length but negatively correlated with seed weight.

Stone length was significantly correlated with seed width and stone width and a highly significantly correlated with stone weight, seed length and seed weight (Table 3.5).

Stone width was highly significantly correlated with stone weight, seed width and seed weight but negatively correlated with petiole length and significantly correlated with inflorescence width and seed length.

Stone thickness was found to be negatively correlated with leaf blade width and pubescence of inflorescence rachis.

Stone weight was highly significantly correlated with seed length, seed width and seed weight however it was negatively correlated with leaf blade width and Brix/TA ratio. A study performed by Wright et al. (2007) reported that seed size was correlated with plant height, but these results could not be confirmed in this study.

Seed length was highly significantly correlated with seed width and weight and significantly correlated with inflorescence width.

Seed width was highly significantly correlated with seed weight and was negative correlated with petiole length. The results show a significant correlation between seed width and inflorescence width and negative correlation with leaf blade length. Seed weight was also found to be negatively correlated with petiole length. Seed weight was also found to be negatively correlated with petiole length. Inflorescence length was

negatively correlated with stone thickness and highly significantly correlated with inflorescence width. Tree height and inflorescence width were highly significantly correlated with crown north-south, probably because the north-south side of the trees are more exposed to sunlight during the day. The crown east-west side are normally covered by the shade of other trees. According to a study by de Azevedo et al. (1998) the genetic and phenotypic correlations among plant height and crown north-south and crown east-west were positive and significantly in cashew trees, a fruit tree from the same family as mango trees. Leaf blade length was highly significantly correlated with leaf blade width and petiole length and significantly correlated with crown north-south. Other research found that leaf size was positively correlated with fruit size (Wright et al., 2007).

In Table 3.5 Brix was highly significantly correlated with tree height and Brix/TA ratio. Brix was significantly negatively correlated with stone width, stone weight, and TA. In other tropical fruit trees, specifically in citrus, there was no correlation between Brix and acid content (Widodo et al., 1996).

3.3.3 PRINCIPAL COMPONENT ANALYSIS

The central idea of PCA is to decrease the dimensionality of a data set consisting of a large number of interrelated variables. While PCA does not take into account covariance and correlations, it concentrates on variances. The variation can be explained by the components with e.g. 60% of variation and the correlations between principal component and original variables less than 0.25 can also be discarded (Jolliffe, 1986). The eigenvectors were obtained through a linear function $\alpha_i x$ of the elements of x having maximum variance, where α_i is a vector of P constants. PCA of the quantitative data of this study showed that a reduced number of characteristics could be used efficiently to discriminate between varieties. PCA grouped the 23 characteristics into 23 components which accounted for 100% of the variability existing among the mango varieties. Most of the variation was explained by the first seven PCs with a cumulative eigenvalue of 80.60% and these seven were selected since it presented eigenvalues higher than one. PC1 contributed 30.01% to the total variation, PC2 12.17% and PC3 contributed 11.07% and the percentage was reduced successively until PC7 with a contribution of 5.42% (Table 3.6) to the total variation. Fruit weight contributed 36% to the variation, fruit

Table 3.6 Principal component analyses (PCA) of the different characteristics evaluated in the 30 mango varieties studied

	Eigenvectors						
Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**
HGT	-0.012	0.47	0.09	0.23	0.15	0.10	-0.13
TRK	-0.13	-0.11	0.17	-0.32	0.03	0.40	0.04
CNS	-0.08	0.42	0.13	0.30	0.11	0.04	-0.16
CEW	-0.012	0.22	0.06	0.34	-0.02	-0.28	0.42
FRL	-0.35	0.01	-0.10	0.06	0.07	0.04	0.02
FRD	-0.32	0.01	0.06	0.01	0.02	0.25	0.27
FRW	-0.36	0.02	0.03	-0.02	0.04	0.03	0.05
STL	-0.28	-0.06	-0.13	0.08	0.02	-0.25	-0.55
STW	-0.34	-0.03	-0.01	0.07	-0.18	0.16	0.06
STT	-0.21	-0.23	0.14	-0.18	0.21	0.08	-0.10
STWG	-0.31	-0.14	0.05	0.14	-0.15	0.12	-0.17
SDL	-0.25	0.13	-0.00	-0.28	0.32	-0.28	-0.08
SDW	-0.26	0.12	-0.01	-0.34	0.29	-0.25	0.01
SDWG	-0.04	0.14	-0.27	-0.08	-0.50	-0.22	-0.31
LBLL	0.17	0.31	0.34	-0.13	0.15	0.01	-0.03
LBLW	0.14	0.29	0.05	-0.43	-0.10	-0.03	0.01
PLE	0.25	0.05	0.06	-0.18	0.02	0.05	-0.17
IFL	-0.08	0.35	-0.28	-0.27	-0.17	0.27	0.06
IFW	-0.15	0.30	-0.24	-0.10	-0.21	0.08	0.08
TA	-0.06	-0.04	0.20	-0.14	-0.41	0.02	-0.00
Brix	0.09	0.07	-0.39	0.11	0.18	0.41	-0.01
RBT	0.10	-0.05	-0.42	0.04	0.33	0.18	-0.21
PIR	-0.014	0.09	0.44	0.13	-0.08	0.30	-0.42
Eigenvalues	6.90	2.80	2.55	1.96	1.67	1.42	1.25
Individual %	30.01	12.17	11.07	8.51	7.25	6.16	5.42
Cumulative %	30.01	42.18	53.25	61.77	69.01	75.17	80.60

HGT = height, TRK = trunk diameter, CNS = crown north-south, CEW = crown east-west, FRL = fruit length, FRD = fruit diameter, FRW = fruit weight, STL = tone length, STW = stone weight, STT = stone thickness, STWG = stone weight, SDL = stone length, SDW = seed width, SDWG = seed weight, LBLL = leaf blade length, LBLW = leaf blade width, PLE = petiole length, IFL = inflorescence length, IFW = inflorescence width, TA = titratable acidity,
RBT = Brix/titratable ratio, PIR = pubescence of inflorescence raquis,

Underlined values are the traits contributing most to the PC.

length 35% and stone width 34% in PC1. The variables that contributed the most to PC2 were tree height (47%), crown north-south (42%) and inflorescence length (35%). For PC3 pubescence of inflorescence rachis (44%), Brix/TA ratio (42%) and Brix (39%) were the highest contributing characteristics. For PC4 the characteristics with the most

influence were leaf blade width (43%), crown east-west (34%) and seed width (34%). Seed weight, titratable acidity and Brix/TA ratio contributed 50%, 41% and 33% to PC5 respectively and for PC6 Brix (41%) and trunk diameter showed a value of 40%. Petiole length was the unique characteristic accounted for PC7 with 17%. Eigenvalues expressed as individual percentage means the distribution of the source data among each eingenvector (Stasoft, 2010).

3.3.3.1 PCA BI-PLOT

PCA biplot is used to order the genotypes and to visualise genetic relationships among varieties. The principle is based on the representation of the relationships according to presence or absence of markers and differs from other methods that use a hierarchical structure to group individuals (Laurentin, 2009).

The results showed that “Quinta Aurora”, “Keitt”, “Matola 6”, “Boane”, “Papaia” and “Xavier” were the most diverse varieties (Figure 3.1). The most similar varieties were “Peach” and “Monsserate” followed by “Lima 2” and “Kent”, “Haden” and “Matola 4” as well as “Lourenco” and “Fernandinha”. Other varieties scattered on the left side are characterised by high fruit weight namely, “Quinta Aurora”, “Keitt”, “Melo”, “Matola 2”, “Matola 6”, “Boane”, “Lima 2”, “Kent”, “Haden”, “Anzo”, “Matola 4”, “Matola 3” and “PA Mocuba”. On the right side are varieties with low fruit weight such as “Zoologia”, “Xinavane”, “Sensation”, “Afonsa Pairy”, “Nametil 1A”, “Peach, Papaia”, “Lourenco”, “Fernandinha”, “Ruby”, “Malcurada”, “Malcorada”, “Sabre” and “Xavier”. “Monsserate”, “Polenta” and “Xinavane” are outliers.

The characteristics that contribute to clustering of the varieties by each quadrant are depicted in Figure 3.2. In quadrant I characteristics such as height, crown north-south, inflorescence length, inflorescence width, crown east-west, seed weight, pubescence of inflorescence rachis, seed length, seed width and seed length are found and contribute to cluster quadrant I Figure 3.1 and these results confirm what was found in section 3.3.1.

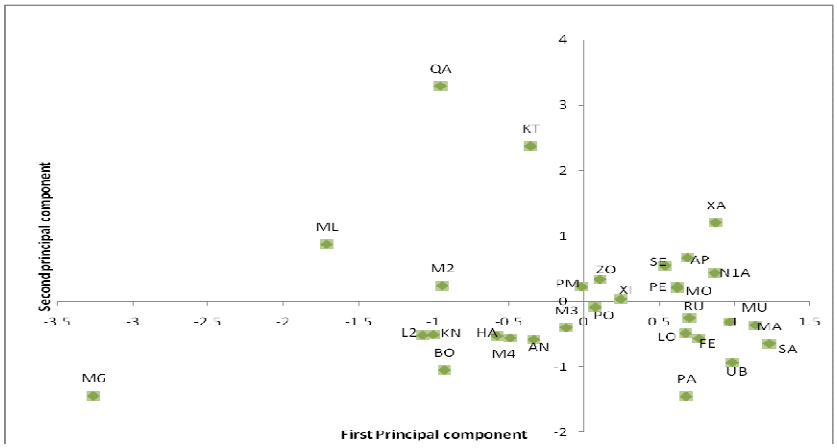

Figure 3.1: PCA bi-plot scatter gram showing the relative positions of 30 mango varieties. QA = Quinta Aurora, KT = Keitt, ML = Melo, M2 = Matola 2, M6 = Matola 6, BO = Boane, L2 = Lima 2, KN= Kent, HA= Haden, M4 = Matola 4, AN = Anzo, M3 = Matola 3, PO = Polenta, RU = Ruby, MU = Malcurada, MA = Malcorada, SA = Sabre, LO = Lourenco, FE = Fernandinha, UB = Umbeluzi, PA = Papaia, XI = Xinavane, PM = PA Mocuba, MO = Monsserate, ZO= Zoologia, SE = Sensation, AP = Afonsa Pairy, N1A = Nametil 1A, XA = Xavier

Quadrant II (Figure 3.2) showed that leaf blade length, leaf blade width, petiole length and Brix contributed most to quadrant II Figure 3.1 (“Zoologia”, “Xinavane”, “Polenta”, “Xavier”, “Sensation”, “Afonsa” “Pairy”, “Nametil 1A”, “Peach” and “Monsserate”). “Xavier” accordingly section 3.3.1 had the highest leaf blade length; Quadrant III present characteristics of stone width, stone weight, stone length, stone thickness, trunk and titratable acidity and also are found fruit length, fruit weight, fruit diameter positioned in the middle line of the first component axis (meaning that the characteristics had influence for both sides) determined for clustering “Haden”, “Matola 4”, “Anzo”, “Lima 2”, “Kent”, “Boane” and “Matola 6”, which are the varieties with highest fruit weight. In the fourth quadrant only Brix/TA ratio value determined the clustering of “Fernandinha”, “Lourenco”, “Ruby”, “Malcurada”, “Malcorada”, “Sabre”, “Umbeluzi” and “Papaia”. “Malcurada” presented the lowest Brix/TA ratio value (14.75) among the low fruit weight varieties (section 3.3.1). The most similar characteristics indicate that there are correlations between them as we see in quadrant III that most of the characteristics are correlated as was discussed in Section 3.3.2.

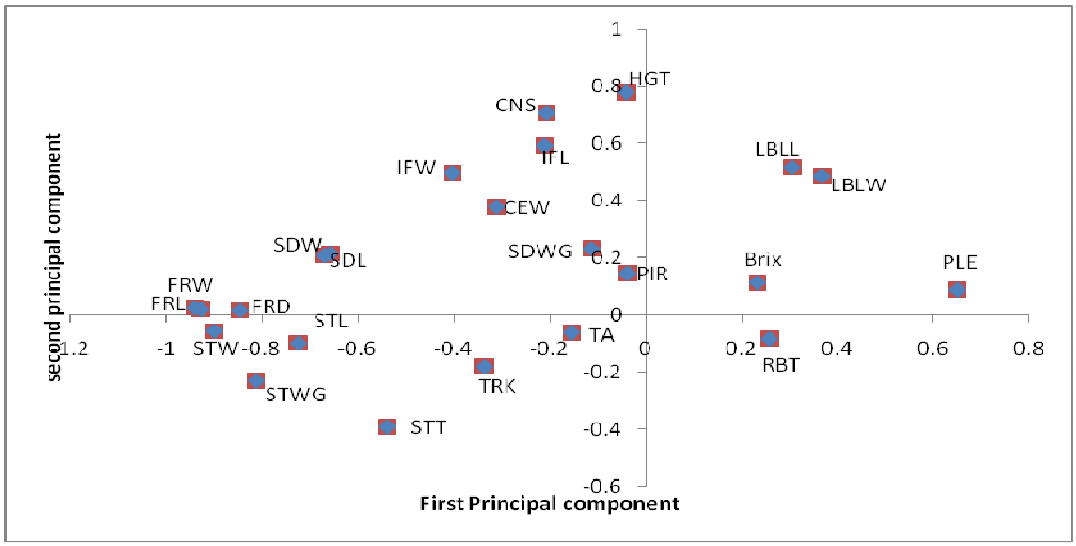

Figure 3.2. PCA bi-plot clustering 23 quantitative morphological characteristics. HGT = height, TRK = trunk, CNS = crown north south, CEW = crown east west, FRL = fruit length, FRD = fruit diameter, FRW = fruit weight, STL = stone length, STW = stone width, STT = stone thickness, STWG = stone weight, SDL = seed length, SDW = seed width, SDWG = seed weight, LBLL = leaf blade length, LBLW = leaf blade width, PLE = petiole length, IFL = inflorescence length, IFW = inflorescence width, TA = titratable acidity, RBT = Brix/titratable acidity ratio, PIR = pubescence of inflorescence rachis.

3.3.4 CHEMICAL CHARACTERISTICS

Due to the presence of rotten fruit in same of the samples (“Sabre” and “Lima 2”), analysis was done using the remaining 28 varieties.

The °Brix is depicted in Figure 3.3A and ranged from 9.4 for”Matola 6” to 17.0 for “Sensation”. Brix values for “Malcorada” and “Monsserate” were 16.2 and 15.9, respectively and on the other end of the scale values of 10.0 and 10.2 were found for “Melo” and “Lourenco” respectively.

“Matola 2” had the lowest TA of 0.19, followed by “Sensation” (0.20) and “Zoologia” (0.20). “PA Mocuba” (1.30), “Malcurada” (0.85), “Matola 6” (0.70) and “Afonsa Pairy” (0.70) were varieties with the highest TA values (Figure 3.3B).

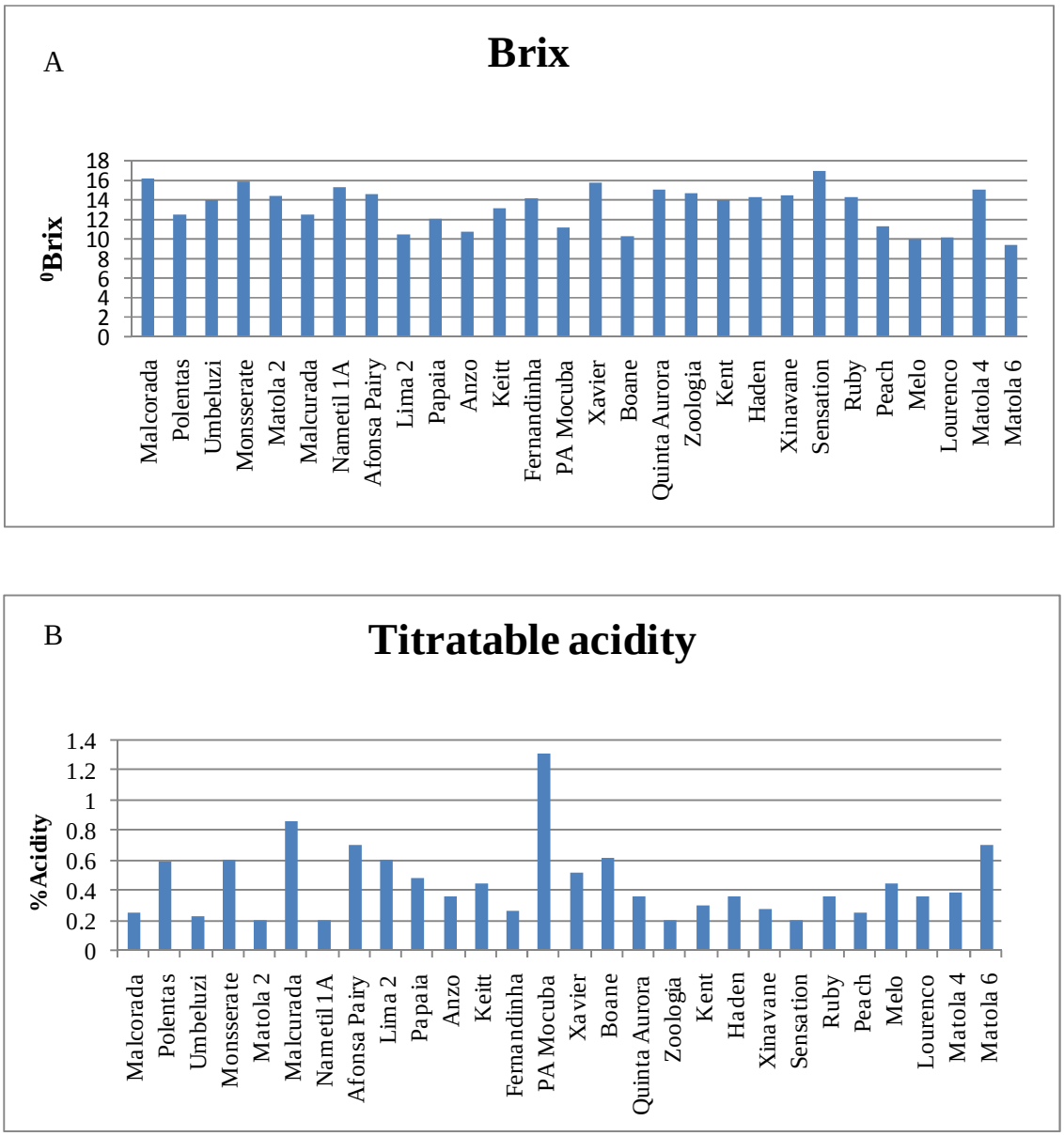

Figure 3.3 Brix (A) and titratable acidity (B) values for 28 mango varieties

The Brix value measured in this study was lower than that found in literature except of "Sensation" and the TA values were all higher compared to literature (Table 3.7).

Table 3.7 Brix and titrabale acidity obtained in this study compared with literature

Varieties	Brix		TA	
	Study	Literature	Study	Literature
Afonsa Pairy	14.6	19.0*	0.7	0.41*
Keitt	13.2	18.4*, 21.07***	0.44	0.22, , 0.45***
Kent	14.0	21.0*, 17.2**, 21.93***	0.29	0.11*, 0.50**, 0.44***
Haden	14.3	18.9*, 16.0**	0.35	0.12*, 0.11**
Sensation	17.0	15.7*	0.2	0.15*

Source: *Litz, 1997; **Lakshminarayana, 1975; ***Uddin et al., 2006.

Differences between results obtained in the current study and literature might be due to a lack of nutrients in the soil at the research station. Trees are maintained under poor conditions with hardly any fertilisation, irrigation and hardly any pest and disease control as well as no pruning and weed control. The author believes that better management of the orchard will improve the quality of the fruits. A field trial conducted by Symal and Mishra (1989) concluded that an increase in application of NPK fertiliser resulted in an increase in the total soluble solids (Brix) and a lower total fruit acidity. Furthermore, the application of urea and super phosphate alone or simultaneously increased the acidity and total soluble solids (Singh, 1975).

3.3.5 CLUSTERING OF 30 MANGO VARIETIES BASED ON QUANTITATIVE DATA

A total of 690 data points were used for analysis of quantitative data and the total variation between the 30 varieties based on the Euclidean dissimilarity coefficient ranged from 2.45 to 10.65. Excluding “Matola 6” and “Quinta Aurora” the other varieties were 6.81% dissimilar. The varieties clustered into five main groups namely Group I, II, III, IV and V (Figure 3.4). The cophenetic coefficient was 0.84 suggesting the presence of a good fit between the dendrogram and the genetic similarity matrices. Group III, IV and V contained one variety each namely, “Keitt”, “Quinta Aurora” and “Matola 6”, respectively. The variety that was the most different from the rest was “Matola 6”, with the highest fruit weight and the second lowest Brix/TA ratio (Table 3.4). “Quinta Aurora” and “Keitt” also presented high fruit weight (≥ 300 g). “Quinta Aurora” vs. “Matola 6” showed the highest dissimilarity of 10.6% followed by “Keitt” vs. “Quinta Aurora” with 9.05%, “Kent” vs. “Keitt” with 6.81% and “Xavier” vs. “Matola 2” with 6.50%.

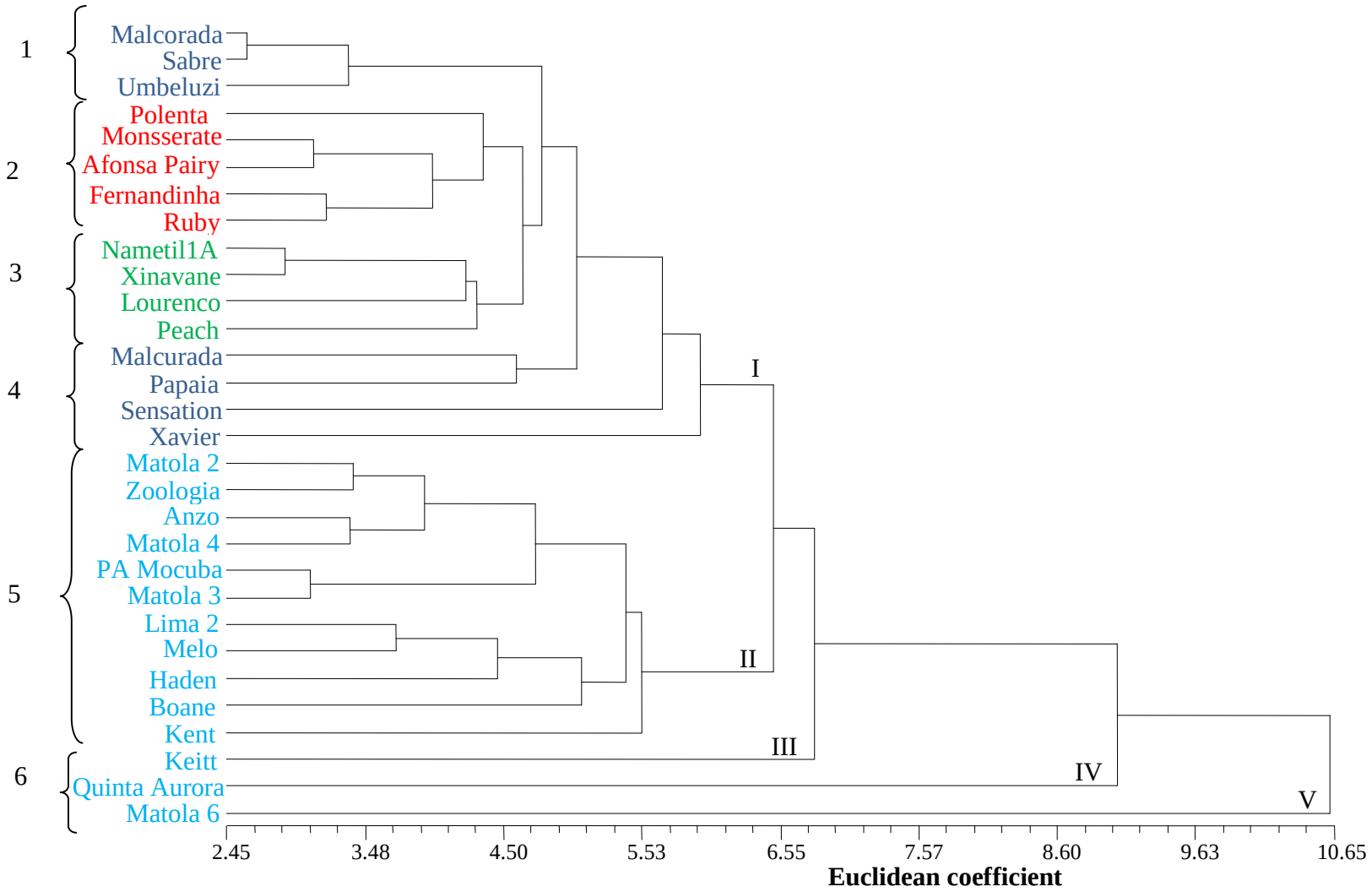

Figure 3.4 Dendrogram of clustering of 30 mango varieties based on quantitative traits using UPGMA clustering and the Euclidean coefficient. Dark blue: low fruit weight, green: low TA, light blue: high fruit weight, red: high TA,

Group II (subgroup 5) contained 11 varieties with high fruit weight. This subgroup contained two polyembryonic varieties, “Matola 3” and “PA Mocuba” that clustered together. The rest of the varieties were all monoembryonic.

Group I contained 16 varieties divided into four subgroups. Subgroups 1 and 4 present the same characteristic of low fruit weight. “Sabre” and “Umbeluzi” are polyembryonic varieties and clustered together in subgroup 1 while polyembryonic varieties “Lourenco” and “Peach” clustered together in subgroup 3. Subgroup 2 is characterised by varieties with high TA values (> 0.4) and included “Polenta”, “Monsserate”, “Afonsa Pairy”, “Fernandinha” and “Ruby”. Subgroup 3 contained “Nametil 1A”, “Xinavane”, “Lourenco” and “Peach” with low TA values (≤ 0.35).

“Malcorada” and “Sabre” were the most similar varieties with a dissimilarity coefficient of 2.60. These varieties had similar characteristics regarding low fruit weight (173.53 g and 150.00 g), and fruit length (13.39 cm and 13.75 cm). The second most similar varieties were “Nametil 1A” and “Xinavane” with similar seed width (3.30 cm and 3.25 cm) and seed weight (15.50 cm and 15.00 cm) and had a dissimilarity of 2.88. The third most similar varieties with a dissimilarity coefficient of 3.07 were “PA Mocuba” and “Matola 3” because of similarity observed in stone thickness (0.51 cm and 0.49 cm), trunk diameter (1.22 m and 1.04 m) and petiole length (2.68 cm and 2.95 cm). “Monsserate” and “Afonsa Pairy” varieties were the fourth most similar with a coefficient of 3.19 and had similar values for stone width (4.05 cm and 4.19 cm) and stone thickness (0.55 cm and 0.59 cm). In addition, in general it seemed that fruit weight contributed significantly to the clustering in Figure 3.4. Comparing the PCA bi-plot and dendrogram there are large similarities such as the most different varieties namely “Matola 6”, “Quinta Aurora” and “Keitt”. The clusters in both analyses were grouped according to low and high fruit weight.

The present study is the first one using local varieties and it is thus not possible to compare the clustering with previous studies but based on the dendrogram there are some relevant aspects that can be considered for the breeding programme in Mozambique. “Malcorada” has a good Brix/acidity ration, roundish fruit shape, average attractiveness but low weight and could be a good parent combined with varieties with high fruit weight

like “Matola 3” that also possesses good attractiveness, early maturation and good taste. Since these two varieties clustered in different groups, they represent a high potential to generate large genetic diversity. “Matola 3” and “Nametil 1A” should also be considered as parents since “Nametil 1A” showed good desired characteristics for the exportation market such as a good Brix/acidity ratio, but with high tree height while “Matola 3” is a dwarf variety. “Lourenco” was the shortest (116.67 g) variety but with a low Brix/acidity (29.14) and should be a good parent when crossed with “Kent” with a high tree height (656.25 g) and good fruit quality.

3.3.6 CLUSTERING OF 30 MANGO VARIETIES BASED ON QUALITATIVE DATA

For the qualitative analysis, 1740 data points were used to create the dendrogram (Figure 3.5). The cophenetic coefficient was 0.63 suggesting the presence of a poor fit between the dendrogram and the genetic similarity matrices. The most similar varieties were “Papaia” and “Xavier” with a Dice similarity coefficient of 0.71 and the following characteristics were similar: Semi-erect leaf attitude, mild leaf fragrance, terminal inflorescence position, pyramidal inflorescence shape, medium density of flowers, yellow inflorescence colour, solen broader nature of disc, one fertile stamen, low intensity anthocyanin, high fruit bearing intensity, roundish fruit shape, average fruit attractiveness, green fruit ground colour, shallow depth of fruit stalk cavity, slightly prominent fruit neck, perceptible fruit beak type, absent fruit sinus type, green skin colour of ripe fruit, golden yellow pulp colour of ripe fruit, soft pulp texture of ripe fruit, medium adherence of fibre to fruit, short fibre length in the pulp, juicy fruit, strong presence of turpentine taste, low quantity of fibre on the stone, strong adherence of fibre on stone, soft texture of stone fibre and medium maturation period.

The second most similar varieties were “Malcorada” and “Keitt” with a Dice similarity coefficient of 0.709. The similarities between these varieties were obvious when morphological traits such as oblong fruit shape, erect tree growth habit, elliptic leaf blade shape, semi-erect leaf attitude, acute leaf apex shape, yellow inflorescence colour, equal length stamen, one fertile stamen, high fruit bearing intensity, roundish fruit shape, average fruit attractiveness, yellow fruit ground colour, red fruit blush, smooth fruit skin surface texture, sparse density of lenticels, perceptible fruit beak type, yellow skin of ripe

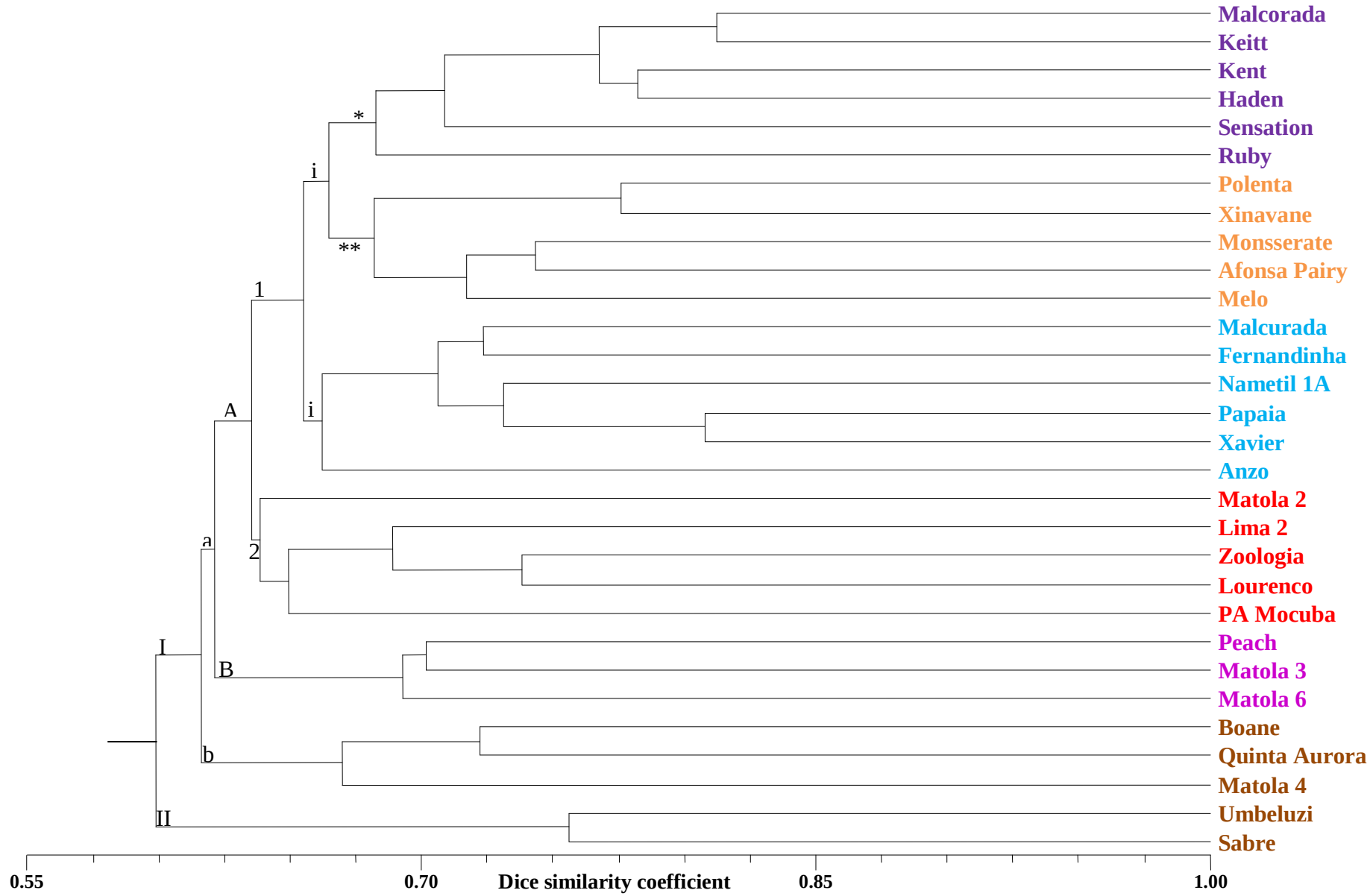

Figure 3.5 Dendrogram showing the clustering of 30 mango varieties based on qualitative traits using UPGMA clustering and the Dice similarity coefficient. Purple: good eating (commercial varieties), orange: low fibre, light blue: green fruit ground colour, red: early maturing, pink: high fibre, brown: oblong fruit shape

fruit, yellow orange pulp colour of ripe fruit, low quantity fibre in the pulp, surface veins on stone, forked pattern of veins on stone, short length of stone fibre, weak adherence of fibre on stone, soft texture of stone fibre and medium fruit

maturation period were scanned. “Keitt” and “Malcorada” are varieties with good fruit storage characteristics (Table 3.3). These varieties kept for nine days after harvesting, while other varieties only had 3-7 days storage life before the fruit was spoiled. Since mangoes are denominated as climactic fruit and ripen quickly after harvest in addition to disease problems and general perishable nature of the fruit (Mitra and Baldwin, 2001), good storage life is very important in a breeding programme.

“Matola 6” vs. “Umbeluzi” were the two most different varieties with a similarity of 0.397 followed by “PA Mocuba” and “Peach” with 0.420 and “Matola 4” and “Polenta” with a coefficient of similarity of 0.440.

This dendrogram resulted in two main clusters I and II. Cluster II contained only two varieties while cluster I contained the other 28 varieties. All varieties in main group II and subgroups b (brown in Figure 3.5) presented the same characteristics including oblong fruit shape, one fertile stamen and sparse density of lenticels. Unfortunately this elongated fruit shape (oblong shape) makes it difficult for mechanical picking and packing systems (Bally et al., 2009).

The varieties in subcluster B (pink) had the following similar traits: spreading tree growth habit, lanceolate leaf blade shape, strong leaf fragrance, horizontal inflorescence axis growth habit, pyramidal inflorescence shape, one fertile stamen, sparse density of lenticels, abruptly sloping of fruit ventral, perceptible fruit beak type, yellow skin colour of ripe fruit, high quantity of fibre in the pulp, high adherence of fibre to stone and coarse texture of stone fibre. The presence of fibre in pulp is not a desirable quality characteristic to select for in any breeding programme and according to Mannepun and Yunchakad (2004) fruit for processing should have firm texture, high yield, medium size, high sugar content, bright colour, good flavour and aroma after ripening, low acid content, no fibre and low tannin content, however “Peach” are often use as rootstock.

The cluster coloured in red, subgroup 2, contained varieties with acute leaf apex shapes, medium fruit bearing intensity, average fruit attractiveness, absent fruit sinus type, forked pattern veins on stone and early maturation period. “Lourenco” and “PA Mocuba” are found in this group and are polyembryonic varieties.Varieties “Malcurada”, “Fernandinha”, “Nametil 1A”, “Papaia”, “Xavier” and “Anzo” clustered together in subgroup ii (blue) and showed similarities such as semi-erect leaf attitude, pyramidal inflorescence shape, solen broader nature of disc, high fruit bearing intensity, green fruit ground colour and absent fruit sinus type. The fifth group in yellow/light brown containing “Polenta”, “Xinavane”, “Monsserate”, “Afonsa Pairy” and “Melo” (subgroup i **) presented identical characteristic for terminal inflorescence position, pyramidal inflorescence shape, forked pattern of veins, low quantity of fibre in stone and short length of fibre in stone.

“Keitt”, “Kent”, “Haden”, “Sensation” and “Ruby” are all commercial varieties and clustered together in subgroup i* (dark purple) and presented acute leaf apex shape, presence of leaf bracts, one fertile stamen, high fruit bearing intensity, short length of stone fibre, weak adherence of fibre to stone, soft texture of stone fibre and medium fruit maturation period. “Malcorada” clustered with the commercial varieties due to good eating qualities.

The clustering indicated that varieties possessing a characteristic of early maturation are potentially good parents since Mozambique can take advantages of this in international markets. Subgroup 2 (red) contained all the varieties with this characteristic. However, some varieties such as “Lourenco” and “PA Mocuba” can be used as rootstock since that are polyembryonic varieties. “Zoologia” and “Keitt” clustered in different groups thus with a potential to generate large genetic diversity considering that “Zoologia” contains the desired characteristic of early maturation period but with high fibre content while “Keitt” contains low fibre and is a medium to late variety. “Lima 2” from subgroup 2 has undesired characteristics of elliptic fruit shape and strong presence of turpentine taste but crossed with “Keitt” or “Kent”, with absent to intermediate presence of turpentine and roundish shape, but with medium period of maturation, can be useful in a breeding programme. “Matola 2” with early maturation but with oblong fruit shape can be

improved through association with “Haden” and “Keitt” that contains a roundish shape and medium maturation season.

The six polyembryonic varieties clustered together in pairs of two in different groups throughout the dendrogram. “Umbeluzi” and “Sabre” grouped together in cluster II, “Matola 3” and “Peach” in subcluster B and “Lourenco” and “PA Mocuba” in subcluster 2. Varieties such as “Lourenco” and “PA Mocuba” could be used as rootstocks, considering the type of embryony and dwarfness but further evaluation is needed to obtain more information about their suitability as rootstocks. According to Samson (1986), a good rootstock must be uniform, grow vigorously, have tolerance to soil-borne diseases and induce regular bearing. In Mozambique “Peach” and “Sabre” are used as rootstock. However, the use of more diversified rootstock varieties, better adapted to the region, should be considered because the rootstock variety affects yield, tree size, fruit appearance and maturity periods (de Villiers and Joubert, 2008).

3.3.7 Clustering of 30 mango varieties based on both quantitative and qualitative data

A dendrogram was constructed using both quantitative and qualitative data with a total of 2430 data points. The dendrogram was based on Euclidean distances and four main clusters I, II, III and IV was observed (Figure 3.6). The cophenetic coefficient was 0.83 suggesting the presence of a good fit between the dendrogram and the genetic similarity matrices. Group III and IV contained one variety each, namely “Quinta Aurora” and “Matola 6” respectively. Results indicated that these two varieties were morphologically the most different from the other varieties mainly based on high fruit weight. The total variation between all varieties ranged from 6.50 to 13.20. If these two varieties were excluded the total variation between the remaining 28 varieties was 9.85.

Cluster II was characterised by varieties with high fruit weight (light blue) and three commercial varieties clustered together in the subgroup iv of cluster II (“Haden”, “Kent” and “Keitt”). “Matola 4”, “Matola 3” and “Matola 2” from the same collection region (Matola) also clustered together in subgroup C in cluster II.

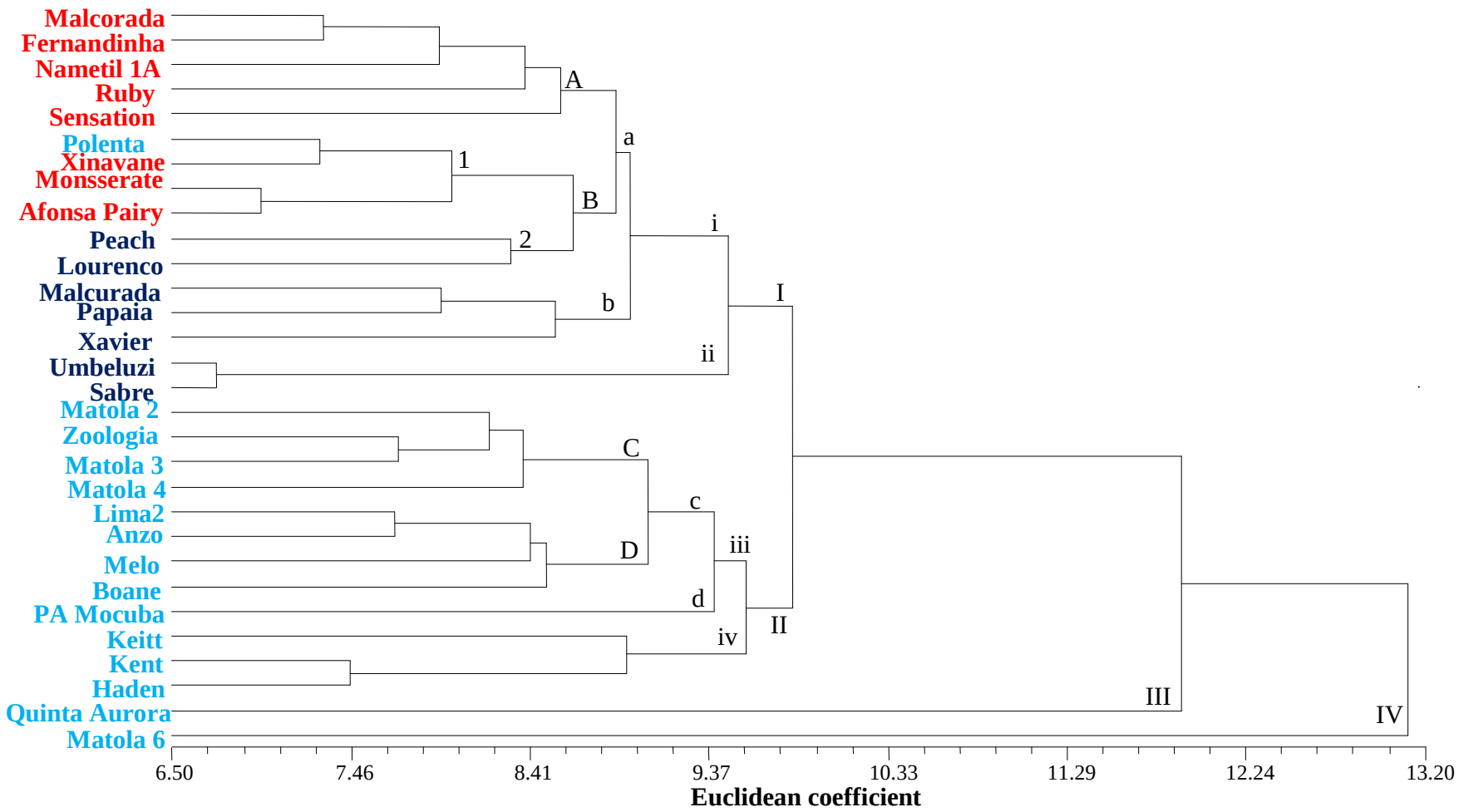

Figure 3.6 Dendrogram showing the clustering of 30 mango varieties based on both quantitative and qualitative data using UPGMA clustering and the Euclidean dissimilarity coefficient. Dark blue: low fruit weight, light blue: high fruit weight, red: high Brix content

Cluster I was subdivided into two subclusters i and ii. Subgroup ii contained the polyembryonic varieties “Umbeluzi” and “Sabre”. Subgroup i was further subdivided into two subgroups (a and b) and subgroup a had two subgroups A and B. All varieties in subgroup A had high Brix content (above 14). Varieties in subgroup B1 also had high Brix content except for “Polenta”. “Polenta” showed some similarities with “Xinavane” and “Monsserate” because of high fruit weight. Subgroup B2 contained two polyembryonic varieties “Peach” and “Lourenco”. All seven varieties with low fruit weight (Table 3.8) were included in cluster I coloured in dark blue.

The most similar varieties were “Umbeluzi” and “Sabre” with a Euclidean dissimilarity coefficient of 6.73. These two varieties had similar fruit weights (173.53 g and 150.00 g) and an oblong fruit shape as main characteristics.

“Monsserate” and “Afonsa Pairy” was the second most similar varieties and their similarities were already mentioned in the quantitative and qualitative data sets. These varieties clustered together in all three dendrograms and their coefficient of dissimilarity for this dendrogram was 6.97. “Polenta” and “Xinavane” clustered together because of the following characteristics: similar values for seed width (3.55 and 3.25 cm), leaf blade width (4.53 and 4.37 cm), trunk circumference (1.66 and 1.67 cm), medium fruit bearing intensity, roundish fruit shape, orange pulp colour of ripe fruit and intermediate quantity of fibre in stone as the main characteristics and appear as the third most similar varieties with a coefficient of dissimilarity of 7.28.

“Malcorada” and “Fernandinha” were the fourth most identical varieties (dissimilarity of 7.30) and characteristics that contributed to their similarity were similar values for fruit length (13.39 and 13.69 cm), the same high fruit bearing intensity, average fruit attractiveness, red fruit blush, low quantity of fibre in the pulp and being slightly juicy as the main characteristics.

“Matola 6” clustered as the most different variety compared to all other 29 varieties. It had a dissimilarity of 14.66 with “Umbeluzi”, 14.57 with “Malcorada” and 14.55 with “Sabre”. The fourth most different varieties were “Matola 6” and “Sensation” with a dissimilarity coefficient of 14.28. The main reason for this was probably because of the

high fruit weight of “Matola 6” that by far exceeded the weight of the other varieties and according to correlation analysis, fruit weight is correlated with other mango fruit characteristics including stone length, stone width, stone weight, seed length, seed width and seed weight (Section 3.3.5). Many other different qualitative traits (Table 3.5) were also seen for these varieties.

“Matola 6” presents an opportunity in breeding for processing because the high fruit weight is a good characteristic for mango pulp. The Brix content for this variety is very low (9.40). Since “Sensation” had the highest Brix value of 17.00, combining these two varieties could produce a variety with acceptable characteristics to produce mango pulp. “Matola 6”, “Quinta Aurora” and “Polenta” are very juicy varieties thus with the potential to be good parents in breeding programmes for juice production when crossing them with varieties with high Brix values from subgroups coloured in red.

3.4. CONCLUSIONS

It is difficult to combine the best characteristics in one variety. Even commercial varieties do not have all desired characteristics. There were similarities and some deviations between characteristic found in the literature and from results of this study. Future studies should generate more data over years on these varieties and include a genotype x environment study to obtain more reliable results.

Based on their morphological characteristics varieties can be grouped according to their potential to be a source of material for the following breeding objectives:
Exportation: “Nametil 1A” is a sweet variety, good size, roundish fruit shape, fibreless, mild turpentine, polyembryonic seed and early harvesting period. “Malcorada” is a sweet variety, fibreless, roundish fruit shape, yellow fruit colour with red blush, yellow orange pulp colour, good storage and late harvesting period. “Zoologia” is a sweet variety, good fruit size, roundish fruit shape, yellow fruit colour, orange pulp colour, mild turpentine taste and early harvesting period. “Anzo” has good attractiveness, good size, good quality and red blush, high bearing intensity, good storage and early harvesting period. “Matola 3” is a good tasting variety, roundish shape, dwarf, good attractiveness, mild turpentine taste, early period of maturation and good size.

Processing**:** Based on good yield for pulp, good fruit size and juicy pulp the following varieties are promising for processing: **"**Matola 6", "Quinta Aurora", "Matola 2", "Monsserate", "Afonsa Pairy", "Ruby", "Xinavane", "Polenta" and "Melo". Varieties with intermediate Brix/acidity ratio and good fruit size are "Fernandinha" and "Umbeluzi". "Umbeluzi" specifically has a good flavour with absence of turpentine taste.

Local market: It is important to extend the availability of mango fruit throughout the year, using late varieties of good quality. The promising varieties are the following: "Malcorada", "Umbeluzi", "Monsserate" and "Xinavane", "Keitt", "Kent" and "Sensation".

The highest level of variation (60% similarity) between the 30 tested mango varieties was detected using qualitative data, followed by the combined qualitative and quantitative data (86.8% similarity). The lowest level of variation was detected using quantitative data (89.4%) but this might be due to the fact that only 690 data points were used during the quantitative analysis compared to 1740 and 2430 respectively for qualitative data and combined quantitative and qualitative data. The quantitative and qualitative dendrograms showed some similarities between clustering of varieties e.g. Monsserate always clustered with "Afonsa Pairy", "Keitt" with "Kent", "Umbeluzi" with "Sabre", "Matola2" with "Zoologia" as well as "Papaia" with "Xavier".

The combined data analysis was more accurate to differentiate between varieties and the quantitative data, although having fewer data points, contributed more towards clustering of varieties in main groups mainly based on fruit weight, TA and Brix. This was confirmed by PCA. However, the qualitative analysis had an influence on clustering of varieties in the small subgroups such as Papaia and Xavier, Fernandinha and Nametil 1A, Ruby and Sensation, Polenta and Xinavane and Keitt, Kent and Haden. Prior studies on diversity have demonstrated the importance of quantitative traits outside the centre of diversity and qualitative traits within the centre of diversity (Tolbert et al., 1979; Witcombe and Gilani, 1979). According to PCA 18, characteristics from 23 were considered efficient to determine variation. The morphological characterisation of the 30 mango varieties suggested the availability of a broad genetic base in the mango varieties of Mozambique that could be exploited for future mango selection and breeding in Mozambique.

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CHAPTER 4

MOLECULAR CHARACTERISATION

4.1 INTRODUCTION

Plant genetic resources are among the most valuable properties available to man. Characterisation of these resources is important not only for identification of different species but also to determine genetic relatedness among them. Information thereby generated can be used successfully in breeding programmes worldwide. This is also important in the present context of intellectual property rights and trade agreements (Anand, 2007). Molecular markers are useful for identifying cultivars and landraces and for studying genetic similarities among them (Duneman, 1994). According to Bally et al. (2009), although there are some constraints in conventional mango breeding programmes, molecular markers open up new opportunities to produce new cultivars with improved characteristics that will improve productivity, fruit quality and the economic competitiveness of the mango industry. Molecular markers have the advantage of being highly heritable, obtainable at a high number and frequency and display enough polymorphism in closely related genotypes (Stuber et al., 1999; Archak et al., 2003; Weising et al., 2005). Morphological markers on the other hand have limited application in breeding as they are few in number as well as dependent on the season and developmental stage of the plant (Krishna and Singh, 2007) and are influenced by the environment.

The most frequently used molecular markers include RAPDs, RFLPs, AFLPs and more recently microsatellites (SSRs) and inter-sequence repeat microsatellites (ISSR) (Wunsch and Hormaza, 2002). Most techniques were used for identification on the one hand and improvement of conventional breeding on the other (Jeffreys et al., 1985). Applications include individual identification of cultivars or rootstocks for various horticultural purposes such as breeder's rights, identification of pollen donors and determination of genetic relatedness (Hillel et al., 1989; Lavi et al., 1991). Improvements of breeding projects include detection of genetic linkage between agriculturally important traits and

DNA fragments (Plotsky et al., 1990) and a significant decrease in the number of backcross generations needed for gene introgression (Hillel et al., 1989).

AFLP analysis is a genotyping technique that produces informative and highly reproducible fragments (Vos et al., 1995). AFLP markers have been used to study the genetic diversity of various fruit species including apricot (Hagen et al., 2002; Geuna et al., 2003;), citrus (Krueger and Roose, 2003), date (El-Assar et al., 2005), mango (Kashkush et al., 2001), olive (Montemurro et al., 2005), peach (Aranzana and Carbor, 2003), European plum (Goulao et al., 2001) and sweet cherry (Zhou et al., 2002).

Based on informativeness and robustness, the use of AFLPs and SSRs have been favoured for determining the genetic relationships and dissemination paths in some plant species (de Rick et al., 2001; Rivera-Ocasio et al., 2002). AFLP markers are reliable, high throughput, cost effective, have wide genome coverage (Hansen et al., 1999; Prashanth et al., 2002) and do not need sequence information.

The objective of this study was to determine the genetic relationship and diversity among 30 Mozambican mango varieties using AFLP molecular characterisation.

4.2 MATERIALS AND METHODS

Fresh leaves of 30 local varieties from Mozambique listed in Table 4.1 were collected from the research station at Umbeluzi, with three leaf samples per variety. The collection established at Umbeluzi consists of 102 varieties from different regions in the country including eight varieties from Nelspruit, South Africa. This study includes only 30 varieties, providing a starting point for characterisation and evaluation of the entire collection. Varieties were randomly chosen based on their proximity in the orchard and the presence of young and healthy leaves on the trees. The last mentioned being an important factor, because it was observed that some varieties during certain periods of the year did not present leaf buds.

Table 4.1 List of mango varieties used for AFLP analysis

Nr.	Entry	Collected	Code	Origin	Nr.	Entry	Collected	Code	Origin
1	Malcorada	Nampula	1	India	16	Kent	Nelspruit	4	Florida
2	Fernandinha	Nampula	1	India	17	Haden	Nelspruit	4	Florida
3	Xavier	Maputo	3	Local	18	Sensation	NI	NI	Florida
4	Malcurada	Maputo	3	India	19	Ruby	Nelspruit	4	Florida
5	Afonsa Pairy	Maputo	3	India	20	Matola 2	Matola	3	Local
6	Lima 2	Nampula	1	Local	21	Nametil 1[a]	PA Mongovolas	1	Local
7	Anzo	Mossuril	1	Local	22	Boane	Boane	3	Local
8	Papaia	Papaia	3	Local	23	Quinta Aurora	Umbeluzi	3	Local
9	Melo	Marracuene	3	Local	24	Zoologia	Umbeluzi	3	Local
10	PA Mocuba	Mocuba	1	Local	25	Polenta	Gondola	2	Local
11	Matola 3	Matola	3	Local	26	Peach	Nelspruit	4	Southeast Asia
12	1 E	Mocuba	1	Local	27	Xinavane	Xinavane	3	Local
13	Matola 4	Matola	3	Local	28	Umbeluzi	Umbeluzi	3	Local
14	Matola 6	Matola	3	Local	29	Correia	Mossuril	1	Local
15	Keitt	Angola	5	Florida	30	Lourenco	Manica	2	Local

Code: 1 = northern Mozambique, 2 = center zone of Mozambique, 3 = southern Mozambique, 4 = South Africa, 5 = Angola, NI = no information, PA = Posto agrario

4.2.1 DNA EXTRACTION

DNA extraction and all subsequent steps for molecular characterisation were done at the Plant Breeding molecular biology laboratory at the University of the Free State, Bloemfontein in South Africa.

The collected leaf samples were washed with 70% (v/v) alcohol, freeze-dried for five days and ground to a fine powder using Qiagen's TissueLyser. This was followed by DNA extraction using the CTAB (hexadecyltrimethylammonium bromide) method (Saghai-Maroof et al., 1984). DNA was extracted by mixing approximately 50 mg ground leaf tissue and 750 µl extraction buffer [100 mM Tris-HCl (tris hydroxymethyl aminomethane) pH 8.0, 20 mM EDTA (ethylenediaminetetraacetate) pH 8.0, 1.4 M NaCl, 2% (w/v) CTAB, 0.2% (v/v) β-mercaptoethanol and 200 mM urea]. The mixture was incubated at 65°C for one hour. After incubation, 500 µl chloroform: isoamylalcohol [24:1 (v/v)] was added. DNA was precipitated from the aqueous phase with 500 µl isopropanol at room temperature for 20 min. The phases were separated by centrifugation for 10 min at 12 000 rpm. The precipitate was washed at room temperature for 20 min with 500 µl 70% (v/v) ethanol and centrifuged at 12 000 rpm for 5 min. The pellet was air-dried and resuspended in 200 µl TE buffer (10 mM Tris-Cl pH 8.0 and 1 mM EDTA pH 8.0) and incubated at 37°C. DNA was further purified by extracting the DNA from the aqueous phase using 0.75 M ammonium acetate and an equal volume chloroform: isoamylalcohol [24:1 (v/v)] and centrifugation at 12 000 rpm for 5 min. DNA was precipitated by adding 500 µl ice-cold 100% ethanol and incubated at 4°C overnight. After centrifugation at 12 000 rpm for 15 min, the pelleted DNA was washed twice with 70% (v/v) ethanol through centrifugation at 12 000 rpm for 10 min. The resulting pellet was air-dried and resuspended in 50 µl TE buffer containing 100 µg/ml RNase, incubated at 37°C for two hours and stored at -20°C till further use.

4.2.2 AFLP ANALYSIS

AFLP analysis was done according to Vos et al. (1995) as modified by Herselman (2003). It was performed using *Eco*RI and *Mse*I as well as *Sbf*I enzymes, adapters and primer combinations. Since problems were experienced in the laboratory with the

*Eco*RI/*Mse*I AFLP protocol and not enough data could be obtained using this system, an alternative enzyme combination, *Sbf*I/*Mse*I was also used. Primers and adapters were synthesised by Integrated DNA Technologies Inc. USA. Oligonucleotides used for adapters were polyacrylamide gel electrophoresis (PAGE) purified. Adapters were prepared by mixing equal amounts of both strands, heating at 65°C for 10 min and leaving the mixture to cool down to room temperature.

4.2.2.1 DOUBLE DIGESTION AND LIGATION OF GENOMIC DNA

Genomic DNA (± 1 µg) was digested at 37°C for five hours using 1x *Mse*I-buffer [50 mM NaCl, 10 mM Tris-Cl, 10 mM $MgCl_2$ and 1 mM dithriotreitol (DTT) pH 7.9] and 5 U *Mse*I (New England Biolabs). Thereafter it was further digested using 5 U *Eco*RI (Roche Diagnostics) and NaCl to a final concentration of 100 mM and incubated overnight at 37°C. Adapter ligation of digested DNA was performed by adding a solution containing 50 pmol of *Mse*I-adapter, 5 pmol *Eco*RI-adapter (Table 4.2), 1 U T4 DNA Ligase, 0.4 mM adenosine triphosphate (ATP) and 1x T4 DNA ligase buffer (66 mM Tris-Cl pH 7.6, 6.6 mM $MgCl_2$, 10 mM DTT and 66 µM ATP) followed by overnight incubation at 16°C.

For digestion and ligation using *Sbf*I the process was similar except for the following: ± 1 µg DNA was digested at 37°C for five hours using 1x NEB buffer 4 (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate and 1 mM DTT pH 7.9), 100 µg/ml bovine serum albumin (BSA) and 5 U *Mse*I. After *Mse*I digestion, DNA was further digested overnight at 37°C using 5 U *Sbf*I. The adapter-ligation step was the same as for *Eco*RI except that the *Eco*RI adapter was replaced with a *Sbf*I adapter.

Table 4.2 *Eco*RI-, *Sbf*I- and *Mse*I-adapter and primer sequences used in AFLP analysis

Enzyme	Type	Sequence (5'-3')
*Eco*RI	Adapter-F	CTCGTAGACTGCGTACC
	Adapter-R	AATTGGTACGCAGTCTAC
*Sbf*I	Adapter-F	CTCGTAGACTGCGTACATGCA
	Adapter-R	TGTACGCAGTCTAC
*Mse*I	Adapter-F	GACGATGAGTCCTGAG
	Adapter-R	TACTCAGGACTCAT
*Eco*RI	Primer+0	GACTGCGTACCAATTC
	Primer+3	GACTGCGTACCAATTCNNN
		NNN = AAC
*Sbf*I	Primer+0	AGACTGCGTACATGCAGG
	Primer+3	GACTGCGTACATGCAGGNN
		NN = CC, GA, TA
*Mse*I	Primer+0	GATGAGTCCTGAGTAA
	Primer+3	GATGAGTCCTGAGTAANNN
		NNN = CAG, CAT, CTA, CTT, CGG

4.2.2.2 PRE-SELECTIVE AMPLIFICATION REACTIONS

Pre-selective amplification reactions were done in 50 µl reaction mixtures containing 5 µl template DNA (undiluted digestion-ligation mixture), 1 x GoTaq® Flexi buffer [10 mM Tris-Cl pH 9.0, 50 mM KCl and 0.1% (v/v) Triton X-100], 2 mM $MgCl_2$, 0.02 U GoTaq® Flexi DNA polymerase (Promega, Madison, USA), 200 µM of each deoxynucleotide triphosphate (dNTP) and 30 ng of each pre-selective primer *Eco*RI- or *Sbf*I- and *Mse*I-primer+0 (Table 4.2). Fragments were amplified using the following programme: 5 min at 94°C followed by 30 cycles of 30 s at 94°C, 60 s at 56°C and 60 s at 72°C and a final elongation step of 10 min at 72°C (Herselman, 2003). All PCR reactions were performed using a DYAD^{TM} (DNA Engine) Peltier thermal cycler. Quality and quantity of pre-selective reactions were determined by electrophoresis in 1.5% (w/v)

agarose gels, at 60 V for 45 min. Pre-selective amplification products were diluted accordingly (1:10 to 1:30) before selective amplification.

4.2.2.3 SELECTIVE AMPLIFICATION REACTIONS

Selective amplification reactions were performed in a final volume of 20 µl, containing 5 µl diluted pre-selective product, 2 mM $MgCl_2$, 200 µM of each dNTP, 100 µg/ml BSA, 30 ng *Mse*I-primer, 30 ng *Eco*RI- or *Sbf*I-primer (Table 4.2), 1 x GoTaq® Flexi buffer and 0.75 U GoTaq® Flexi DNA polymerase. The following cycling programme was used for selective amplification: one cycle of denaturation at 94°C for 5 min followed by one cycle of 30 s at 94°C, 30 s at 65°C and 60 s at 72°C. The annealing temperature was lowered by 1°C per cycle during the next eight cycles after which 25 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 60 s were performed, followed by one last elongation of five min at 72°C. *Eco*RI, *Sbf*I and *Mse*I primers were coded starting with E, S and M respectively followed by the selective nucleotides of each primer. AFLP analysis was done using two *Eco*RI/*Mse*I (E-AAC/M-CAT and E-AAC/M-CTA) and five *Sbf*I/*Mse*I primer combinations (S-CC/M-CAT, S-CC/M-CGG, S-GA/M-CGG, S-TA/M-CAT and S-TA/M-CGG). Primers were randomly selected.

4.2.2.4 POLYACRYLAMIDE GEL ELECTROPHORESIS AND SILVER STAINING

Preceding loading, amplification products were mixed with an equivalent volume of formamide loading buffer [98% (v/v) de-ionised formamide, 10 mM EDTA pH 8.0, 0.05% (w/v) bromophenol blue and 0.05% (w/v) xylene cyanol] and denatured at 95°C for 5 min. The mixture was immediately placed on ice. Aliquots of 5 µl of each sample were separated on 5% (w/v) denaturing polyacrylamide gels [19:1 acrylamide:bis-acrylamide, 7 M urea and 1 x TBE buffer (89 mM Tris-Cl, 89 mM boric acid and 20 mM EDTA)] at a constant power of 80 W for approximately two hours.

The silver staining process for DNA visualisation of the denaturing acrylamide gels was done using the Silver Sequence™ DNA Sequencing System of Promega. Gels were fixed in 10% (v/v) acetic acid for 30 min and rinsed three times in de-ionized water, first for 10 min and 5 min each the last two washes. Gels were stained in a solution of 0.1% (w/v)

silver nitrate and 0.056% (v/v) formaldehyde for 30 min and rinsed in de-ionized water for 10 s before being immersed in a cold (4-10°C) developer [3% (w/v) sodium carbonate, 0.056% (v/v) formaldehyde and 2 µg/ml sodium thiosulphate]. Gels were shaken manually in the developer until the DNA fragments became visible. The 10% acetic acid was used to stop the developing process and shaking continued for a further 2-3 min. Gels were rinsed in de-ionised water again. Gels were left to air-dry overnight and photographs were taken by exposing photographic paper placed under the gel to dim light for approximately 20 s. This produced a negative image of the same size and scale as the gel.

4.2.3 DATA ANALYSIS

AFLP fragments were scored manually using binary unit characteristics for presence (1) or absence (0) across the 30 varieties for the seven primer combinations utilised. The binary data matrix was used to calculate the genetic similarity matrix using Dice similarity coefficient (Dice, 1945). The similarity matrix was subjected to UPGMA (Sneath and Sokal, 1973) clustering in the SAHN programme parameter and utilised to construct the dendrogram using the TREE programme of NTSYS-pc software, version 2.1 (Rohlf, 1993). The goodness of fit of clustering data matrices was calculated using COPH and MXCOMP programmes in NTSYSpc.

4.3 RESULTS AND DISCUSSION

A total of 207 data points were produced by the seven AFLP primer combinations. The cophenetic coefficient was 0.785 suggesting the presence of a poor fit between the dendrogram and the genetic similarity matrices. According to Table 4.3, primer combination S-TA/M-CGG generated the highest number of fragments (39) followed by E-AAC/M-CAG (37). A mean of 30 fragments were produced per primer combination. S-CC/M-CGG yielded the highest percentage of polymorphic fragments (87.5%) followed by S-GA/M-CGG (78.8%) and E-AAC/M-CAG (78.4%). Although E-AAC/M-CTA produced the lowest percentage of polymorphic fragments, the other *Eco*RI/*Mse*I-primer combination (E-AAC/M-CAG) produced the fourth highest percentage polymorphic fragments. The two AFLP systems, *Eco*RI/*Mse*I and *Sbf*I/*Mse*I, thus

produced similar results and both systems can be used for diversity analysis in mango. Since *Sbf*I is usually used for more complex genomes (recognising eight base pairs and thus reducing the number of amplified fragments) it was expected that a lower number of fragments would be amplified compared to other studies on mango using *Eco*RI as rare cutter.

Table 4.3 Information generated using seven AFLP primer combinations

Primer combination	Total scorable fragments	Polymorphic fragments	% Polymorphism
E-AAC/M-CTA	29	14	48.3
E-AAC/M-CAG	37	29	78.4
S-GA/M-CGG	33	26	78.8
S-TA/M-CAT	20	16	80
S-TA/M-CGG	39	30	76.9
S-CC/M-CAT	25	19	76
S-CC/M-CGG	24	21	87.5
Total	207	155	74.9
Average	30	22	-

Yamanaka et al. (2006) reported on a study using AFLP to analyse 35 mango accessions using eight primer combinations that produced a total of 518 fragments and 96.3% of them (499) were polymorphic. *Eco*RI-ACT/*Mse*I-CTA generated the highest percentage of polymorphisms (100%) and *Eco*RI-ACT/*Mse*I-CAT yielded the most fragments (84). A total level of variation of 53% similarity was detected among different species of *Mangifera* that was higher compared to this study that mainly used *Sbf*I as rare cutter and only one *M. indica* species was analysed.

A study done on genetic diversity and relationship among 112 mango plants from different states in Mexico using AFLPs, indicated high genetic similarity with heterozygosity values ranging from 0.38-0.68, and the amplified products were 308 with 87.3% polymorphism (Galvez-Lopez, et al., 2009). These results were similar to those in this study where the varieties were from different places and a high genetic similarity between varieties was found. High genetic diversity is frequent when comparing different species (Xin-hua et al., 2007) in a study with 36 mango cultivars and related species using chloroplast inter-simple sequence repeat markers with eight primers that generate

77.2% polymorphism. Genetic diversity of 25 mango genotypes from the same species *M. indica* was assessed using RAPDs and 45 primers (Rajwana et. al., 2008). The results showed high genetic similarity between the studied genotypes with total variation of 89%, which is higher than that found in this study (72.45%). Values of high genetic similarity were found by Schnell et al. (1995) and he concluded that varieties from Florida are not genetically distant from the varieties from India because most of them i.e., "Haden" and "Kent", have originated from a Mulgoba seedling originally from India.

The dendrogram (Figure 4.1) showed two main clusters, I and II. Cluster II, consisted of one single variety, "Lourenco" that was genetically the most different from all other varieties with a similarity of 72.45% to the rest of the varieties in this study. This variety is unique in the sampled list as it is the only variety from the Manica province, located in the centre of Mozambique. Cluster I contained 29 varieties clearly divided into two main subgroups A and B, with most of the varieties in subgroup A. However "Zoologia" was an outlier in subgroup A and is morphologically closer to varieties in subgroup B.

AFLP analysis detected relatively low levels of variation within the tested mango varieties. If "Lourenco" is excluded, the other 29 varieties were 80% similar and this value is closer to that found by Rajawana et al. (2008) and mentioned above. The most similar varieties based on genotypic data were "Matola 4" and "Matola 6", with a similarity of 93.95%. These two varieties have the same origin and have intermediate adherence of fruit skin to pulp, medium fibre length in the pulp, strong leaf fragrance, axilary inflorescence position and a horizontal inflorescence growth habit, pyramidal inflorescence shape, one fertile stamen, low intensity of anthocyanin, intermediate foliage density, semi-erect leaf attitude, parallel pattern of vein stone, medium length of stone fibre, monoembrionic seed, medium fruit bearing intensity, oblong fruit shape, green fruit ground colour, rough fruit skin texture and sparse density of lenticels. These two varieties showed similar values for quantitative data such as leaf blade width (3.49 and 3.72 cm), petiole length (2.16 and 2.26 cm), inflorescence length (27.85 and 26.89 cm), tree height (5.3 and 4.9 m) and crown north-south (6.4 and 6.25 m).

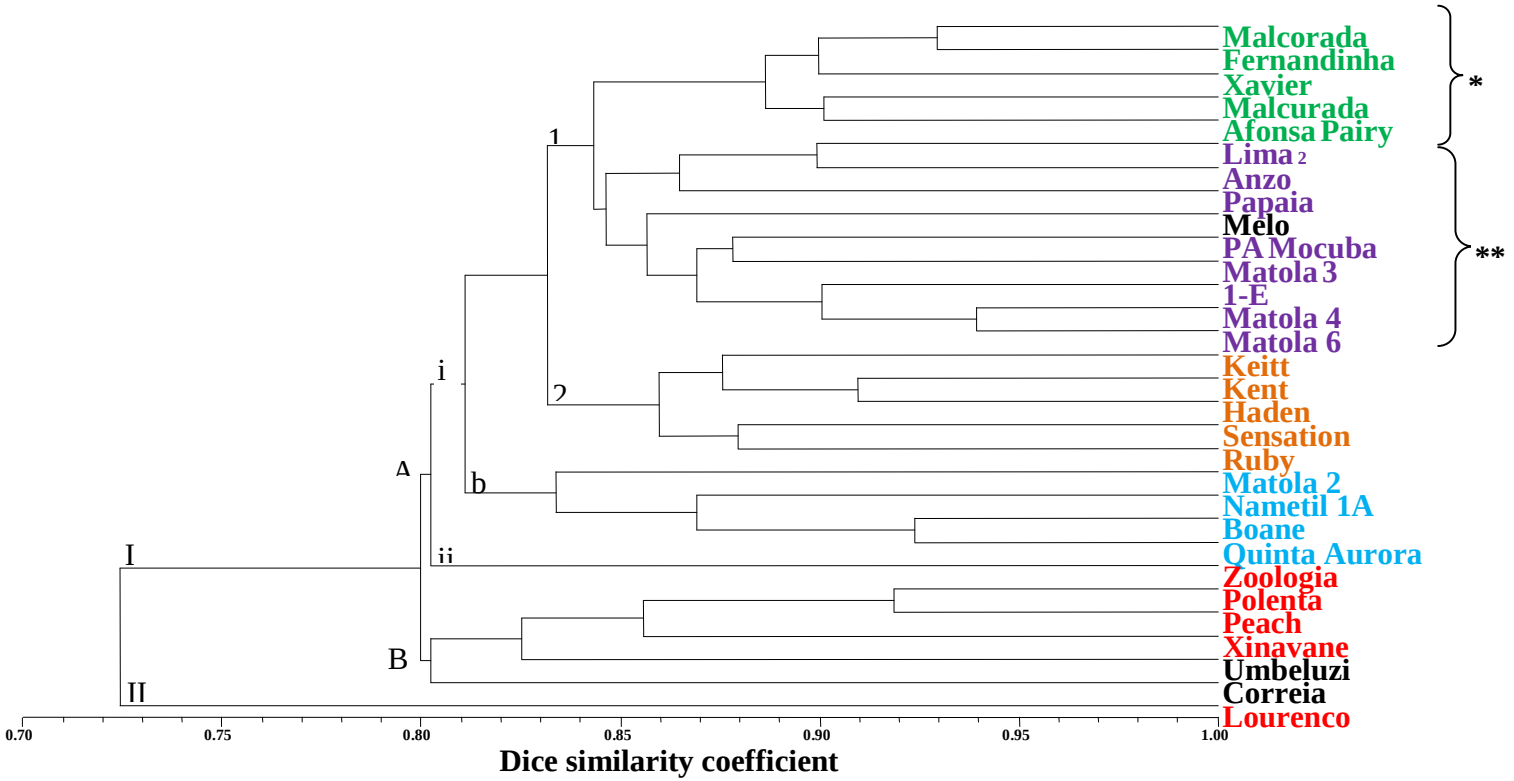

Figure 4.1 **Clustering of 30 mango varieties based on AFLP data analysis using UPGMA clustering using the Dice similarity coefficient. Green = low fruit weight, purple = Dwarf, low Brix, brown = commercial varieties, blue = high fruit weight, red = roundish fruit shape, black = outliers.**

“Malcorada” and “Fernandinha” were the second most similar varieties with a similarity coefficient of 92.95% and this similarity can be attributed principally to the similar values for fruit weight (173.53 and 166.67 cm). Furthermore these two varieties have oblong crown shape, erect tree growth habit, semi-erect leaf attitude, acute leaf apex shape, entire leaf margins, yellow inflorescence colour, equal length of stamens, solen broader nature of the disc, one fertile stamen, high fruit bearing intensity, average fruit attractiveness, red fruit blush, sparse density of lenticels, shallow depth stalk cavity, perceptible fruit beak type, short length fibre, weak adherence of fibre to stone, monoembryonic seed, medium fruit maturation period, no waxy fruit skin, intermediate pulp texture of ripe fruit, low quantity of fibre in pulp, short fibre length in the pulp and are slightly juicy. Both these varieties originated from India.

“Quinta Aurora” and “Boane” are from southern Mozambique and were the third most similar varieties with a similarity of 92.37%. This similarity is justified by similar values for stone length (8.99 and 8.96 cm), seed width (4.40 and 4.00 cm), seed weight (29.50 cm) and leaf blade width (4.20 and 4.28 cm). These two varieties present an oblong crown shape, intermediate foliage density, lanceolate leaf blade shape, horizontal inflorescence axis growth habit, medium density of flowers, equal length of stamen, solen broader nature of disc, one fertile stamen, oblong fruit shape, obtuse shape of fruit apex, poor fruit attractiveness, green fruit ground colour, smooth fruit skin surface texture, elevated veins on stone, intermediate quantity fibre in stone, soft texture of stone fibre, monoembryonic seed, medium maturation period, shallow fruit sinus, no waxy fruit skin, green skin colour of ripe fruit, light yellow pulp of ripe fruit, firm pulp texture of ripe fruit, weak adherence of fruit skin to pulp, high quantity of fibre in pulp, medium adherence of fibre to fruit skin and long fibre length in the pulp.

“Polenta” and “Peach” were the fourth most similar varieties with a similarity coefficient of 91.89% with identical characteristics such as the same seed length value (5.40 cm), lanceolate leaf blade shape, acute leaf apex shape, strong leaf fragrance, terminal inflorescence position, pyramidal inflorescence shape, medium density of flowers, narrow nature of disc, roundish fruit shape, sparse density of lenticels, perceptible fruit beak type, absent fruit sinus, no waxy fruits, yellow skin colour of ripe fruit, yellow orange to orange pulp colours of ripe fruit and medium adherence of fibre to fruit skin

Varieties in subgroup IB (red coloured) had the characteristic of roundish fruit shape except for “Umbeluzi” and “Correia”. Four of the varieties have yellow orange and orange pulp fruit colour (“Polenta”, “Peach”, “Xinavane” and “Umbeluzi”). “Peach” and “Umbeluzi” are polyembryonic varieties and also the most similar varieties in this subgroup. “Zoologia”, clustering in subgroup IA also present orange pulp fruit and is morphologically more similar to varieties in subgroup B than A.

Subgroup b (light blue coloured) is characterised by high fruit weight, up to 300 g and contained four varieties, “Matola 2”, “Nametil 1A”, “Boane” and “Quinta Aurora”. “Keitt”, “Kent”, “Haden” and “Sensation” are commercial varieties and clustered together in subgroup 2 (light brown coloured) with Ruby. All these varieties have a high Brix content of ≥ 13. Subgroup 1** (purple) contained nine varieties, “Lima 2”, “Anzo”, “Papaia”, “Melo”, “PA Mocuba”, “Matola 3”, “1E”, “Matola 4” and “Matola 6”. Most varieties in this group have a low Brix content ≤ 13 value and the tree height is equal or less than 6 m and can be considered as dwarf varieties. “Melo” is an outliner in this group.

Subgroup 1* (green coloured) contained varieties characterised by low fruit weight. “Malcorada” and “Malcurada” clustered in this subgroup and these two varieties probably came from a variety from India named “Mankurad” or are closely related. There is considerable confusion regarding cultivar nomenclature since similar cultivars grown in different areas are known by different names (Lakshminarayana, 1980; Campbell, 1992). “Fernandinha” and “Afonsa Pairy” both originated in India and have the same growth habit and yellow fruit (Knight, 1997).

Mangoes are subjected to natural or induced pollination, segregation as well as genetic recombination, all playing a major role in genetic diversification (Galvez-Lopez et al., 2009). Schnell et al. (2005) reported highly related cultivars from Florida based on SSR data. The constant selection and clonal propagation may have reduced genetic diversity between the analysed germplasm. Most varieties at Umbeluzi Research Station were vegetatively propagated, thus it is probably one of the causes for the low genetic variations found. To increase genetic diversity within these tested varieties, it is necessary to cross varieties genetically most different and “Lourenco” can be a good parent

combined with all varieties in the dendrogram, due to good characteristics such as dwarfness and resistance to anthracnose.

4.4 CONCLUSIONS

AFLP analysis is a functional, powerful and suitable tool for identification of variation within and between of *Mangifera* species and for detecting genetic diversity and relationships among the 30 mango varieties grown in Mozambique. The total level of variation detected in the study was not high, although AFLPs could uniquely distinguish between the different varieties.

The clustering patterns of mango varieties in this study showed some relationship between genetic distance and origin of some varieties such as “Malcorada”, “Malcurada”, “Fernandinha” and “Afonsa Pairy” from “India”, “Matola 6”, “Matola 4” and “Matola 3” from the Matola district and all commercial varieties having Florida as origin. Furthermore, some polyembryonic varieties grouped together, namely “Peach”, “Umbeluzi” and “Lourenco” and “Matola 3” and “PA Mocuba”. The polyembryonic varieties have the same origin, i.e., Thailand and Myanmar (Bompard and Schnell, 1997). Lourenco was genetically the most distant from other varieties and its resistance to anthracnose makes it a good parent in a breeding programme.

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CHAPTER 5

COMPARISON BETWEEN MORPHOLOGICAL AND MOLECULAR CHARACTERISATION

5.1 INTRODUCTION

Mango is one of the most popular fruit crops of tropical and subtropical zones worldwide, particularly in Asia. The status and importance can easily be realised by the fact that it is often mentioned as “the king” of fruits in the tropical world (Singh, 1996). Mango originated in the Indo-Burma region and in SE Asia, there are two ecogeographic races: Tropical (SE Asia) and subtropical (Indo-Burma). Paintings of mango fruits, flowers and leaves are still found in Buddist and Hindu temples throughout India (CITEM, 1985). Mango has been cultivated for more than 4 000 years (Morton, 1987). The common mango (*M. indica* L.) and closely related genera (*Mangifera* spp.) belong to the family Anarcardiaceae that consists of dicotyledonous trees and shrubs (Bally et al., 2009). Domestication in the Indian region led to the development of monoembryonic varieties while domestication in Indochina (SE Asia), Thailand and Myanmar regions gave rise to polyembryonic varieties (Bompard and Schnell, 1997). Many of the commercial varieties grown in the world today, such as Haden, Keitt and Kent, originated from a secondary centre of diversity in Florida during the twentieth century (Campbell, 1992; Knight and Schnell 1994).

Mango is effectively cultivated under conditions that vary from very hot, humid, to cool and dry, to very hot arid conditions and can survive in areas with an annual rainfall of ≤ 300 mm and temperatures as high as 45°C (de Villiers and Joubert, 2008). The optimal rainfall volumes for mangoes are about 400-600 mm and the crop can tolerate drought (rainfall < 40 mm) for up to eight months under certain circumstances (Bally, 2006). Other optimal conditions needed include: temperatures of about 20-26°C, altitude of 0-12 000 m and pH value of the soil between 5.5 and 7.5 and deep soils of at least 3 m (Griesbach, 2003).

While relative few varieties are considered good for commercial trade and suited for national and international markets, there are many other local minor varieties with remarkable characteristics that form a vast genetic resource available to the mango breeder. Locally selected and wild varieties are often well adapted to local environmental conditions and offer a useful source of locally adapted genes (Bally et al., 2009). Genetic diversity can be revealed by a number of methods, including morphological data, agronomic performance and biochemical and molecular characterisation (Mohammadi and Prasanna, 2003).

Morphological characterisation cannot be seen as an older method that was replaced by molecular markers, but as a useful tool that complements the new techniques (Campos et al., 2005). Molecular markers have the advantages of being abundant, having phenotypic neutrality, absence of epistasis and are not influenced by developmental stage, tissue and environmental expression (Mohapatra, 2007).

Genetic diversity using a combination of techniques, such as morphological and molecular markers have been conducted in potato (Fisher et al., 2008), *Echinochloa* spp. and rice (Ruiz-Santaella et al., 2006), banana (Mohamed, 2007), beans (Duran et al., 2005), globe artichoke (Crino et al., 2008) and wheat (Cox and Murphy, 1990; Vieira et al., 2007).

The objective of this study was to compare dendrograms produced using morphological characteristics and AFLP molecular markers

5.2 MATERIALS AND METHODS

The methods described in Chapters 3 and 4 are also valid for this chapter, since this is a comparison between morphological and molecular characteristics. The combined morphological and AFLP dendrogram was constructed based on qualitative morphological and AFLP data. The binary data were combined and introduced into NTSYSpc software referred to in previous chapters and a dendrogram was produced using tree similarity using the Dice similarity coefficient and UPGMA clustering method. For comparison between dendrograms the number of varieties was reduced to 28 in order

to obtain an uniform method of comparison, since some varieties used for morphological characterisation were not used during AFLP analysis due to the absence of budding leaves when samples were taken for AFLP analysis. “Sabre” and “Monsserate” were included in morphological analysis but not for AFLP analysis while “1-E” and “Correia” were included in AFLP analysis but not in morphological characterisation. New dendrograms were thus constructed without these four varieties.

5.3. RESULTS AND DISCUSSION

5.3.1. COMPARISON OF MORPHOLOGICAL QUALITATIVE AND AFLP DENDROGRAMS

Dendrograms generated revealed that some varieties clustered together for both analyses (Figures 5.1 and 5.2). The commercial varieties clustered together in the morphological (subgroup *, Figure 5.1) and AFLP (subgroup 2, Figure 5.2) dendrograms. This clustering confirmed previous reports and indicates that these varieties share a close genetic relatedness. According to Campbell (1992) and Knight and Schnell (1994) “Haden”, “Keitt” and “Kent” have the same parent of origin, the seedling “Mulgoba”. Bally et al. (2009) reported that the success of these varieties is due to their adaptability to different environments worldwide while maintaining their fruit quality and regular bearing characteristics. “Boane” and “Quinta Aurora” clustered together in both the morphological (subcluster b) and AFLP (subcluster b) dendrograms as well as “Polentas” and “Xinavane” in the morphological (subcluster 2) and AFLP (subcluster ii) dendrograms. “Matola 6” and “Matola 3” clustered together in subgroup B of the morphological and in subcluster ** of the AFLP dendrogram. However, some discrepancies were found between the two dendrograms. “Polenta” and “Xinavane” are clustered with “Peach” and “Umbeluzi” in the same subcluster (ii) in the AFLP dendrogram but in Figure 5.1 “Polenta” and “Xinavane” clustered in subgroup 2 with “Afonsa Pairy” and “Melo”. “Peach” clustered with “Matola 6”, “Matola 3” and “Umbeluzi 3” in subgroup ii of the morphological dendrogram. “Matola 6”, “Matola 4” and “Matola 3” with same origin and “PA Mocuba” clustered together in the AFLP (subgroup **) dendrogram. “PA Mocuba”clustered with Matola 2,

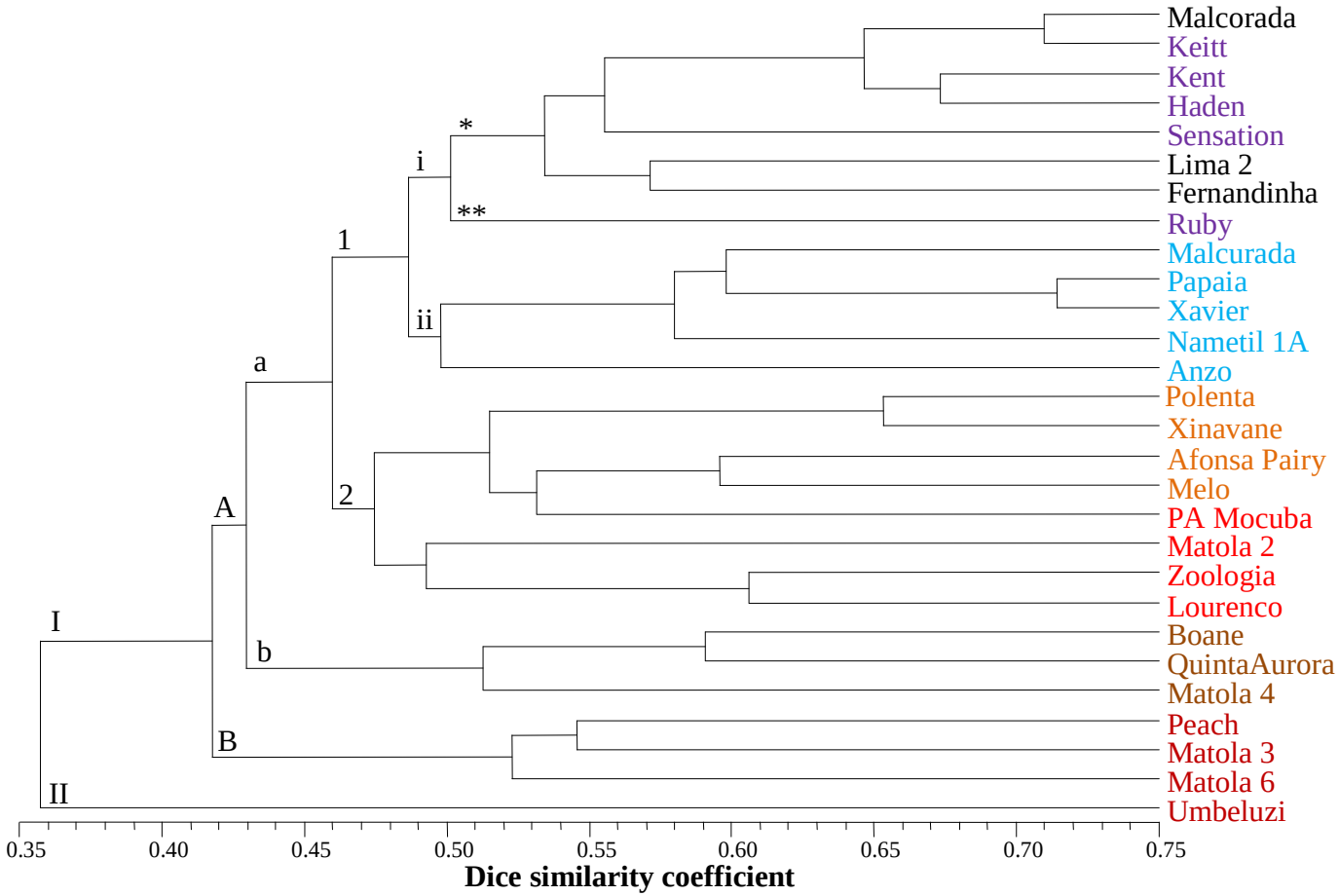

Figure 5.1 Clustering of 28 mango varieties based on qualitative traits using UPGMA clustering and the Euclidean coefficient. Purple = commercial varieties, black = outliers, blue = green fruit colour, brown = low fibre content, red = early maturation, dark-brown = oblong fruit shape, pink = high fibre content.

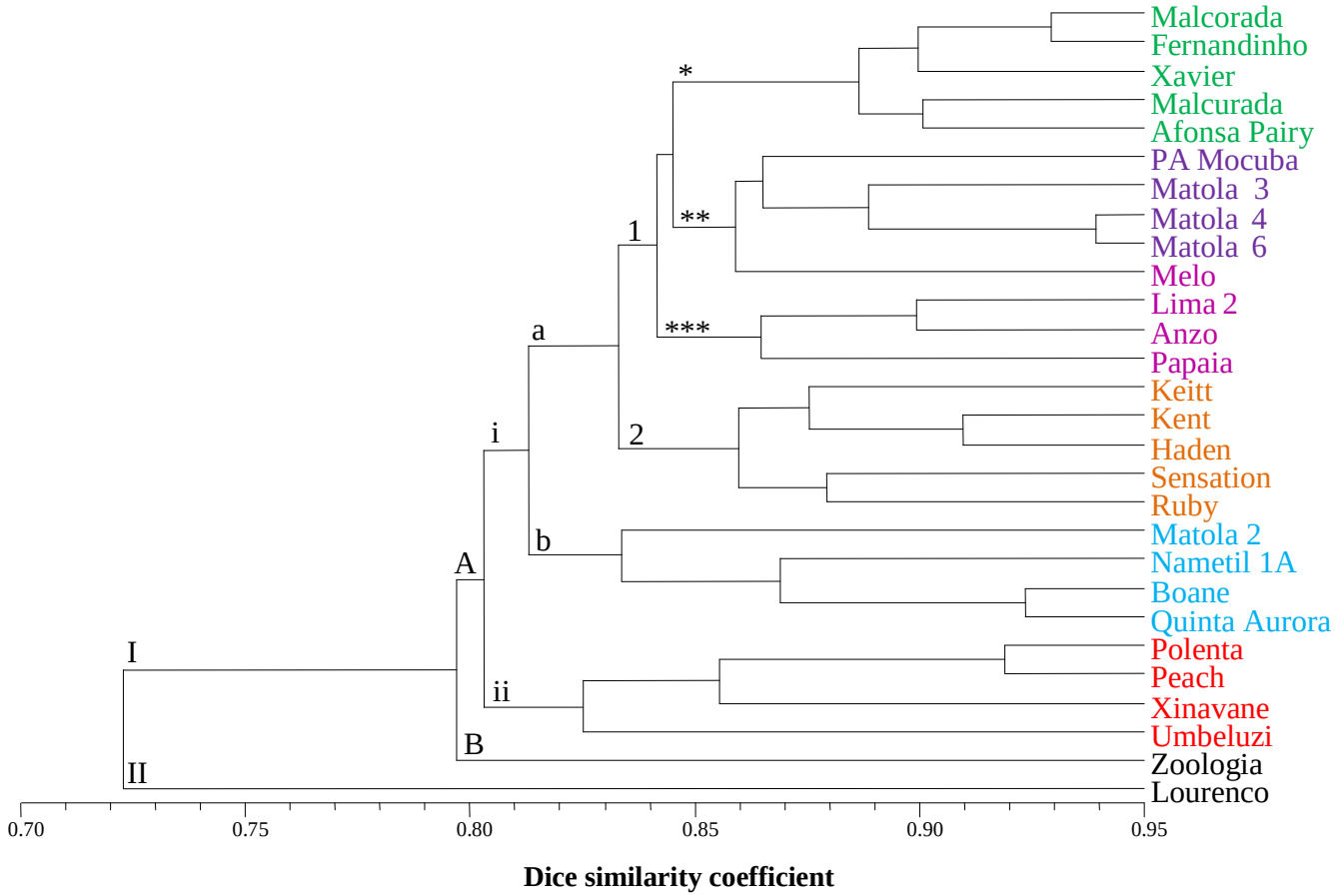

Figure 5.2 Clustering of 28 mango varieties based on AFLP analysis and UPGMA clustering using the Dice similarity coefficient. Green = low fruit weight, purple = dwarfeness, pink = low Brix, brown = commercial varieties, blue = high fruit weight, red = roundish shape black = outlier.

“Zoologia” and “Lourenco” during morphological analysis. “Lourenco” is the unique variety in group II and “Zoologia” in subgroup B of the AFLP dendrogram. “Afonsa Pairy” clustered with “Malcurada”, “Xavier”, “Fernandinha” and “Malcorada” in subcluster * of the AFLP dendrogram but during morphological analysis clustered in subgroup 2 with “Polenta”, “Xinavane”, “Melo” and “PA Mocuba”. “Malcorada”, “Lima 2” and “Fernandinha” are outliers between the commercial varieties in subgroup * of the morphological dendrogram but were separated during AFLP analysis and did not cluster with commercial varieties. “Boane” and “Quinta Aurora” clustered with “Nametil 1A” and “Matola 2” during AFLP analyis while they clustered together with “Matola 4” during morphological analysis. “Lima 2”, “Anzo” and “Papaia” were in the subgroup *** in the AFLP dendrogram but are dispersed throughout the morphological dendrogram.

AFLP provided a more accurate clustering of varieties because of better efficiency with a cophenetic coefficient of 0.78 compared to a cophenetic coefficient of 0.67 for the morphological dendrogram. “Malcorada” clustered with commercial varieties during morphological qualitative analysis, but during AFLP analysis it clustered with other more similar varieties from the same origin. Dissimilarities observed between these two dendrograms are because AFLP analysis targets the entire genome (including all characteristics, quantitative and qualitative). This was confirmed by the formation of definite subgroups in the AFLP dendrogram based on high fruit weight (light blue), low fruit weight (green) and the low Brix content (pink). Although dwarfness is a distinguishing qualitative trait it was not evident as a separate subgroup in the morphological qualitative dendrogram but varieties with this characteristic were found in the same subgroup (purple) in the AFLP dendrogram, excluding “Matola 4”, indicating that this is a highly heritable trait. The total variation for morphological analysis was 35.29% similarity while AFLP analysis presented 69.43% similarity between varieties. These results suggest that morphological analysis detected higher levels of variation than molecular analysis thus the morphological dissimilarities detected between varieties were not necessarily the product of genotypic dissimilarity. A study done in citrus which detected differences between dendrograms from morphological data with 40% of similarity and AFLP with 50% similarity suggested that morphological and molecular differences are apparently independent, due to different selection and evolutionary factors

(Koehler-Santos et al., 2003). Use of morphological characterisation for the differentiation of citrus species frequently lacks the efficiency needed to identify individual genotypes since most such characters can be influenced by environmental factors and also depend on the developmental stage of the plant (Campos et al., 2005).

In the current study, 23 quantitative, and 58 qualitative morphological traits and seven AFLP markers were used to investigate the level of genetic diversity within 28 mango varieties using morphological and AFLP characterisation respectively. The AFLP dendrogram was based on 207 data points, whereas the morphological qualitative dendrogram had 1624 data points. Results obtained agree with Zacarias (2008) who detected similar patterns of grouping using morphological and AFLP analysis in cassava. Morphological traits are not the best way to evaluate genetic distance since the degree of divergence between genotypes at the phenotypic level is not necessarily correlated with a similar degree of genetic difference (Hamrick and Godt 1989). The differences mentioned above could be derived from the fact that phenotypic markers such as flower and fruits are intricate and multigenic characteristics which leads to subjective evaluation. Molecular characterisation is more efficient in the generation of an impartial picture of diversity than agronomic traits. However, the agronomic characteristics are still important in germplasm management and determination of molecular diversity and should be included (Campos et al., 2005).

5.3.2 COMBINED MORPHOLOGICAL AND AFLP DENDROGRAM

Figure 5.3 shows the combined morphological and AFLP dendrogram created using 1820 data points, revealing a total variation of 63% and with a cophenetic coefficient of 0.81 suggesting the presence of a good fit between the dendrogram and the similarity matrices. The dendrogram presented two main groups, I and II. Group II contained two polyembryonic varieties, “Umbeluzi” and “Lourenco”. Group I comprised of 26 varieties subdivided into different subgroups. Subgroup 6 included all four “Matola” varieties from the same origin, Matola. The main similar characteristics found between these four varieties are sparse density of lenticels, pyramidal inflorescence shape, one fertile stamen, very juicy (“Matola 4” is only juicy) and early maturation period. “Matola 3” and

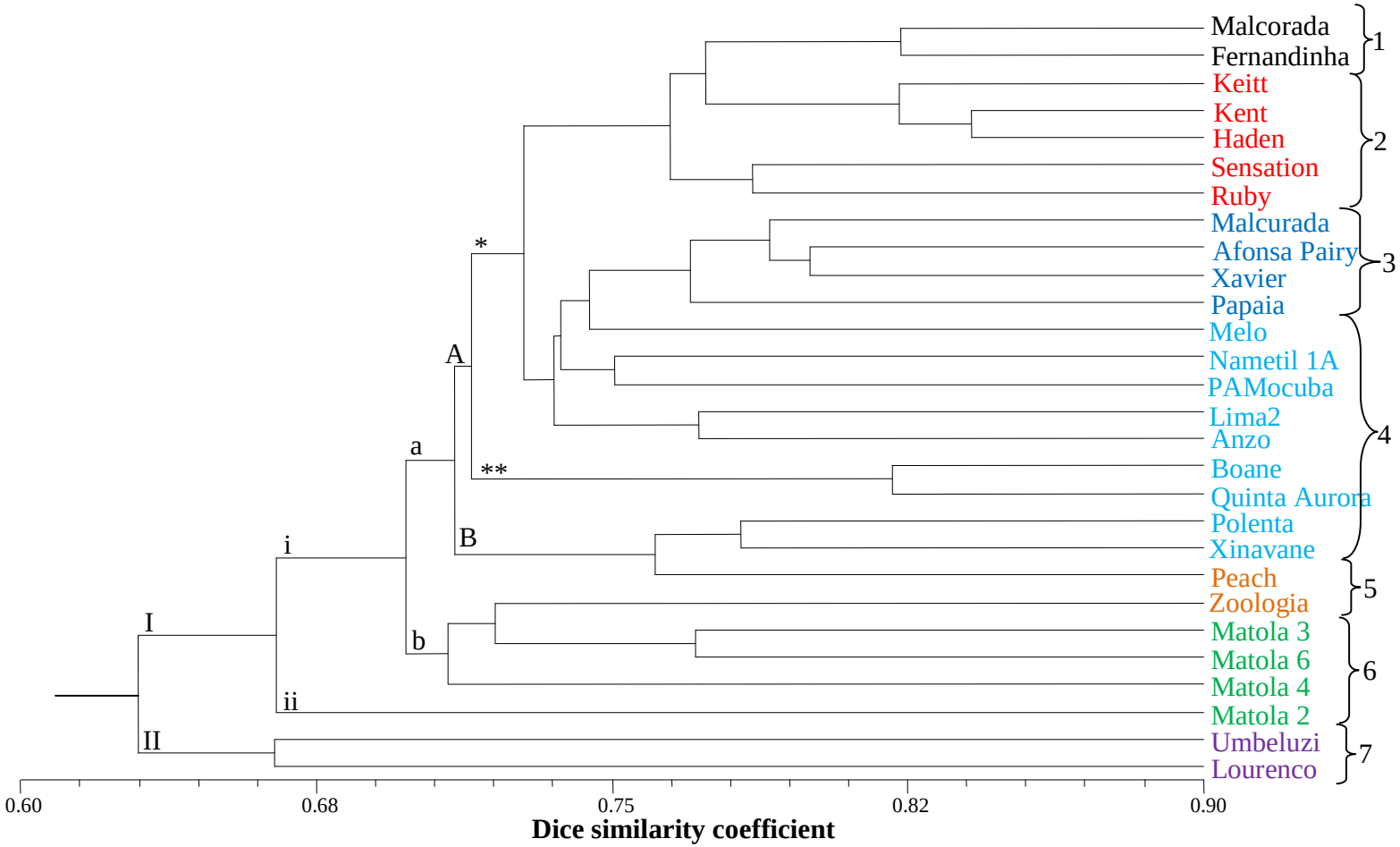

Figure 5.3 Dendrogram showing the clustering of 28 mango varieties based on both morphological and AFLP characterisation using UPGMA clustering and the Dice similarity coefficient. Black = low fruit weight and low content fibre, red = commercial varieties, dark blue = low fruit weight, light blue = high fruit weight, brown = high content of fibre, green = very juicy and early maturation and purple = polyembrionic

“Matola 6” was the most similar within this subgroup and the similarities are based on fruit sloping abruptly, perceptible beak type, mild presence of turpentine, strong adherence of fibre to stone, mild pulp aroma and solen broader nature of disc. “Matola 2” was the most different of the “Matola” varieties and it is isolated in subgroup ii. “Peach” and “Zoologia” although not clustering together, are closer to each other and shared a 69 % similarity (subgroup 5), The characteristics found to be similar between these two varieties are mainly quantity of fibre in the pulp. Except for “Peach” and “Lourenco”, all varieties in subgroups 5, 6 and 7 come from zone 3 (Table 3.1). Subgroup 4 are composed of varieties with high fruit weight, and most of the varieties originate from zone 1 (Table 3.1) namely “Melo”, “Nametil 1A”, “PA Mocuba”, “Lima 2”, “Anzo”, “Boane”, “Quinta Aurora”, “Polenta” and “Xinavane”. “PA Mocuba” and “Nametil 1A” are polyembryonic varieties. Varieties from zone 1 (Table 3.1) clustered together in subgroup 4, such as “Anzo”, “Lima 2”, “PA Mocuba” and “Nametil 1A”. “Malcurada”, “Afonsa Pairy”, “Xavier” and “Papaia” are varieties with low fruit weight and all came from zone 3 (Table 3.1) and are found in subgroup 3. “Malcurada” and “Afonsa Pairy” according to literature, originated in India. The commercial varieties clustered together in subgroup 2 and included the most similar varieties, “Kent” and “Haden” with a Dice similarity coefficient of 0.84. The similarity between “Kent” and “Haden” was because they are the juiciest varieties among all commercial varieties. The third most similar varieties, “Keitt” and “Kent” were also found in this subgroup and the main similar characteristic is yellow orange to orange pulp colour of ripe fruit. “Malcorada” and “Fernandinha”, both from India, clustered in subgroup 1. These two varieties are the second most similar (82%) and the identical characteristics between these varieties were already discussed in Chapter 3.

The most different varieties were “Umbeluzi” and “Lourenco” (66% similarity) and the main differences were absence of turpentine taste, high quantity of latex and oblong fruit shape in “Umbeluzi” and the absence of fruit sinus, absence of latex and roundish fruit shape for “Lourenco”. The second most different varieties were “Peach” and “Zoologia” (69% similaritiy). The differences found are for juicy fruit pulp, early maturation and monoembryonic seed for “Zoologia” and slightly juicy fruit pulp, late maturation and polyembryonic seed for “Peach”. “Anzo” and “Boane” were 71% similar because “Anzo” has a spherical crown shape, elliptic leaf blade

shape, purple patches on skin of ripe fruit, intermediate quantity of fibre in the pulp, soft texture of ripe fruit and medium intensity of anthocyanin. “Boane” is characterised by having an oblong crown shape, lanceolate leaf blade shape, high quantity of fibre in the pulp, green colour of ripe fruit, firm texture of ripe fruit and high intensity of anthocyanin.

The combined dendrogram was more similar to the AFLP than the morphological dendrogram. The morphological qualitative dendrogram detected the highest level of variation (35.29% similarity) of the three dendrograms, followed by the AFLP and combined morphological and dendrogram, based on the clusters formed by “Malcorada” and “Fernandinho” as well as “Malcurada” and “Afonsa Pairy”. Furthermore, three of the “Matola” varieties clustered together in the same sugroup ** in the AFLP dendrogram (excluding “Matola 2”) and clustered together in the combined dendrogram as well. Despite this, the morphological data also contributed towards clustering in the combined analysis, e.g. clusters such as “Polenta” and “Xinavane”, “Matola 3” and “Matola 6” and “Papaia” versus “Xavier”. The combined dendrogram showed clear clustering based on region of origin of the varieties as well as morphological characters such as fruit weight, fibre content, commercial varieties, juiciness, maturation and embryony.

Clustering indicated that all “Matola” varieties could be good parents combined with the commercial varieties since the last mentioned ones are medium to late maturation and the former are early maturation varieties. “Zoologia” has good eating quality but has a high fibre content therefore crosses between this variety and “Malcorada” and “Fernandinha” can improve quality while maintaining high levels of genetic diversity.

5.4. CONCLUSIONS

AFLP analysis was more precise in clustering varieties based on known morphological characteristics, mainly due to better efficiency. However, some similarities in clustering of varieties based on morphological as well in AFLP data were observed. For example all commercial varieties, “Boane” and “Quinta Aurora”, “Polentas” and “Xinavane” as well as “Matola 6” and “Matola 3” always clustered together in the same subgroups irrespective of the technique used. This finding indicated that morphological and AFLP complement each other in evaluation of

genetic diversity in crops. The total variation detected using morphological analysis was 35.29% while AFLP presented 69.43% similarity between varieties. Molecular markers were more reliable to detect genetic differences among mango varieties. The combined morphological and AFLP analysis was more accurate with more data points compared to morphological and AFLP dendrograms.

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CHAPTER 6

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The current study is the first including not only morphological but also genetic characterisation of 30 of 110 mango varieties of the collection at the Umbeluzi research station in Mozambique.

Morphological characterisation indicated high levels of diversity in mango varieties growing in Mozambique. All desired characteristics were not found in one unique variety, although some varieties showed potentially good characteristics for international markets including good Brix/acidity ratio, low fibre content, good fruit weight, dwarfness, fruit shape and maturation period for different seasons. For processing products, varieties with characteristics for each purpose were identified. Some varieties presented high fruit juice content, for juice processing, high fruit weight for mango pulp and high acid content for mango pickles. Since domestic markets require mango fruit throughout the year, the study identified different varieties with different maturation periods. More work is needed to prolong the availability of mango fruit throughout the year through breeding programmes. Results indicated that it should be possible to obtain improved varieties to fulfil the market opportunity that Mozambique has in October and November and to improve fruit quality.

The morphological dendrogram based on quantitative data clustered varieties based on primary characteristics such as high fruit weight, low acidity, high acidity and low fruit weight. The morphological dendrogram based on qualitative data divided the varieties into six groups based on the following main characteristics: high Brix/acidity ratio, green fruit colour, low quantity in fibre, early maturation, oblong fruit shape and high quantity of fibre.

The lowest level of variation was detected using quantitative data (89.4% similarity) and qualitative data presented the highest (60% similarity). The quantitative and

qualitative dendrograms showed some similarities between clustering of varieties e.g. “Monsserate” always clustered with “Afonsa Pairy”, “Keitt” with “Kent”, “Umbeluzi” with “Sabre”, “Matola 2” with “Zoologia” as well as “Papaia” with “Xavier”. The polyembryonic varieties clustered together throughout the two dendrograms. Both dendrograms revealed useful information for future breeding programmes in Mozambique, especially with regard to the possibility to obtain high levels of genetic diversity between early maturation and commercial varieties as well as to improve fruit quality of local varieties. In the combined quantitative and qualitative dendrogram, fruit weight and Brix content contributed more towards clustering of varieties into three subgroups. A breeding programme for fruit quality improvement versus processing can be considered.

Genetic characterisation gave reliable results and the AFLP technique proved to be powerful for identification of mango varieties. The origin of varieties played a significant role in clustering and led to clustering of varieties from India, commercial varieties from Florida and polyembryonic varieties from Thailand. The other main characteristics observed for each cluster were low fruit weight, dwarfness, low Brix content, high Brix/acidity ratio, high fruit weight and oblong fruit shape. Lourenco was the most dissimilar variety, being a potential good parent in breeding programmes because of its dwarf characteristic. Future studies should include analysis of anthracnose resistance.

The combined AFLP and morphological dendrogram provided the most accurate results, since it was based on more data points and clustered varieties not only from outside the country but also from different places in the country, south and north. The combined dendrogram was more similar to the AFLP dendrogram than the morphological dendrogram and breeding programmes for maturation period as well fruit quality improvement were emphasised by this dendrogram. Genetic analysis detected lower levels of variation than morphological analysis, implying that more germplasm conservation efforts should be done in the country.

PCA indicated that fruit weight was the variable contributing most towards variation and this confirmed the pattern of clustering in the morphological quantitative dendrogram, combined quantitative and qualitative as well as AFLP and combined AFLP and morphological dendrogram where varieties were mainly divided into

clusters based on fruit weight. Other characteristics obtained in PCA that determined clustering in the dendrograms were tree height, Brix/TA ratio, TA and Brix.

Similar results between this study and other studies concerning the total genetic variation of the AFLP dendrogram, total variation obtained in morphological combined quantitative and qualitative dendrogram and clustering of commercial varieties were found. The current study provides breeders with knowledge regarding the extent of mango genetic diversity in the Umbeluzi collection as well as a means to do selections for better parents to use in crossing schemes. Mango producers in Mozambique are thus provided with options to obtain varieties for different purposes, such as international markets, processing and the domestic market.

It is recommended that future studies should include more varieties from the collection at Umbeluzi. Studies should also focus on morphological data, taking into account pest and disease resistance as well as genetic and cytological analysis. Future studies should also include genotype x environment interaction analysis over years. It is also recommended to introduce new varieties from different countries in order to develop a better breeding programme.

SUMMARY

Mango (*Mangifera indica* L.) belongs to the Anacardiaceae family. Consumption is increasing worldwide due its their nutritional value that is rich in vitamins A and C, potassium, β-carotene, fibre as well as proteins. Mangoes are economically important for Mozambique because of suitable climatic conditions and international market opportunities. This study compared morphological and AFLP characterisation of 30 mango varieties from the Umbeluzi research station, including some commercial varieties: “Keitt”, “Kent”, “Haden”, “Sensation” and “Ruby”. Morphological characterisation using IPGRI descriptors were divided into quantitative (23 characteristics) and qualitative (58 characteristics) data. The total variation was lower using quantitative (89.4% similarity) than qualitative data (60% similarity). The combined quantitative and qualitative dendrogram had 63% similarity and clustered varieties in three subgroups based on high fruit weight, high Brix content and low fruit weight. AFLP characterisation used seven primer combinations, generating 207 data points and detecting 74.9% polymorphism with an average of 30 fragments per primer combination. The total variation was low (72.49% similarity) and the dendrogram showed two main groups subdivided in five subgroups based on low fruit weight, dwarfness and low Brix content, high fruit weight and roundish fruit shape. The combined morphological and AFLP dendrogram was more accurate in clustering varieties based on geographic origin within the country and from outside. The dendrograms presented in the study will help breeders to select parents for crosses. PCA results confirmed the pattern of clustering of varieties based on fruit weight and Brix/acidity ratio. It is recommended to extend the study to include, the entire collection at Umbeluzi and to include morphological data, considering pest and diseases resistance characterisation, as well as genetic and cytological analysis. A study of genotype x environment interactions done over years should be undertaken in future.

Key words: AFLP, Brix, commercial varieties, IPGRI descriptors, PCA, quantitative data, qualitative data. titratible acidity.

OPSOMMING

Veselperske (*Mangifera indica* L.) behoort aan die familie Anacardiaceae. Verbruik het wêreldwyd toegeneem as gevolg van die voedingswaarde; veselperskes is ryk aan Vitamien A en C, kalsium, β-karoteen, vesel en proteïene. Die gewas is vir Mosambiek ekonomies belangrik omdat die land geskikte klimaatstoestande vir die gewas het en daar internasionale markgeleenthede bestaan. Die studie vergelyk morfologiese en AFLP analises van 30 variëteite afkomstig van die Umbeluzi navorsingsstasie. Kommersiële variëteite: “Keitt”, “Kent”, “Haden”, “Sensation” en “Ruby” was ook ingesluit by die studie. Vir die beskrywing van morfologiese eienskappe is IPGRI beskrywers gebruik en die eienskappe is verdeel in kwantitatiewe (23 eienskappe) en kwalitatiewe (58 eienskappe) data. Die totale variasie vir die kwantitatiewe data (89.4%) was meer ooreenstemmend as variasie vir die kwalitatiewe data (60%). Die gekombineerde kwalitatiewe en kwantitatiewe dendrogram het 63% ooreenstemmendheid gehad en die variëteite groepeer in drie subgroepe gebaseer op hoë vrugmassa, hoë Brix inhoud en lae vrugmassa. AFLP merkers met sewe priemstuk-kombinasies is gebruik om data punte te genereer en 74.9% polymorfismes is bepaal met 'n gemiddeld van 30 fragmente per priemstuk-kombinasie. Die totale variasie was laag (72.49%) en die dendrogram toon twee hoof groepe wat in vyf subgroepe verdeel is op grond van lae vrugmassa, dwergagtigheid en lae Brix waarde, hoë vrugmassa en ronde vrugvorm. Die gekombineerde morfologiese en AFLP dendrogram was meer akkuraat in groepering van variëteite gebaseer op geografiese oorsprong binne en buite die land. Die dendrogramme in die studie sal telers help om die geskikste ouers vir kruisings te kies. PCA resultate bevestig die groeperings patroon gebaseer op vrugmassa en Brix/suur verhouding. Dit word aanbeveel dat die studie uitgebou word om al die inskrywings in die Umbeluzi versameling te ondersoek en dat morfologiese data vir peste en siekte weerstand as ook sitologie daarby ingesluit word. 'n Studie oor jare om die genotipe x omgewing interaksie te bepaal moet in die toekoms onderneem word.

Sleutel woorde: AFLP, Brix, IPGRI beskrywers, kommersiële variëteite kwalitatiewe data, kwantitatiewe data, PCA, titreerbare suur.

Appendix 1

Table A.1 Tree descriptors

Type	Code	Description
Quantitative		
	HGT	Tree height
	TRK	Trunk circumference
	CNS	Crown diameter north-south
	CEW	Crown diameter east-west
Qualitative		
	CSH	Crown shape
	TGH	Tree growth habit
	FDE	Foliage density

Table A.2 Leaf descriptors

Type	Code	Description
Quantitative		
	LBLL	Leaf blade length
	LBLW	Leaf blade width
	PLE	Petiole length
Qualitative		
	LBLS	Leaf blade shape
	CSV	Curvature of secondary veins
	LAS	Leaf apex shape
	LBS	Leaf base shape
	LMA	Leaf margin
	LPU	Leaf pubescence
	CDL	Colour of fully developed leaf
	LFR	Leaf fragrance
	LAT	Leaf attitude

Table A.3 Flower/inflorescence descriptors

Type	Code	Description
Quantitative		
	IFL	Inflorescence length
	IFW	Inflorescence width
	PIR	Pubescence of inflorescence rachis
Qualitative		
	IFP	Inflorescence position
	IAG	Inflorescence axis growth habit
	IFS	Inflorescence shape
	PLB	Presence of leaf bracts
	DFI	Density of flowers in inflorescence
	FTY	Type of flower
	IFC	Inflorescence colour
	LSP	Length of the stamen in relation to the pistil
	NDI	Nature of the disc
	NSS	Number of stamens/staminoides
	FIA	Intensity anthocyanin

Table A.4 Fruit descriptors

Type	Code	Description
Quantitative		
	FRL	Fruit length
	FRD	Fruit diameter
	FRW	Fruit weight
Qualitative		
	FBI	Fruit bearing intensity
	FSH	Fruit shape
	SFA	Shape of fruit apex
	FAT	Fruit attractiveness
	FBC	Fruit background colour
	FSS	Fruit skin surface texture
	DLF	Density of lenticels on fruit skin
	DFS	Depth of fruit stalk cavity
	FNP	Fruit neck prominence
	FBT	Fruit beak type
	FST	Fruit sinus type
	FSW	Fruit skin waxyness
	SCF	Skin colour of ripe fruit
	PCF	Pulp colour of ripe fruit
	PTR	Pulp texture of ripe fruit
	ASP	Adherence of fruit skin to pulp
	QLO	Quantity of latex oozing from peduncle
	QFP	Quantity of fibre in pulp
	AFS	Adherence of fibre to fruit skin
	FLP	Fibre length in the pulp
	PAR	Pulp aroma
	PTF	Presence of turpentine flavour
	FMP	Fruit maturity period
	PJU	Pulp juiciness

Table A.5 Stone and seed descriptors

Type	Code	Description
Quantitative		
	STL	Stone length
	STW	Stone width
	STT	Stone thickness
	STWG	Stone weight
	SDL	Seed length
	SDW	Seed width
	SDWG	Seed weight
Qualitative		
	VST	Veins on stone
	PSV	Pattern of stone venation
	QFS	Quantity of fibre on stone
	AFS	Adherence of fibre to stone
	TSF	Texture of stone fibre
	SDS	Seed shape
	TEB	Type of embryony

Table A.6 Chemical characteristics of fruits

Type	Code	Description
Quantitative		
	Brix	Brix
	TAC	Titratable acidity
	RBT	Ratio Brix/Titratable acidity

Appendix 2

Table A.7 Correlation matrix

	SL	SW	ST	SWG	SDL
SW	0.4681				
	0.0120*				
ST	0.1086	-0.2125			
	0.5823	0.2777			
SWG	0.555	0.8494	-0.0803		
	0.0022**	0.0000**	0.6847		
SDL	0.83	0.4725	0.0283	0.5568	
	0.0000**	0.0111*	0.8865	0.0021**	
SDW	0.4574	0.8821	-0.3225	0.7943	0.481
	0.0144*	0.0000**	0.0942	0.0000**	0.0096**
SDWG	0.5773	0.7259	-0.2276	0.7567	0.6653
	0.0013**	0.0000**	0.244	0.0000**	0.0001**
LL	-0.2956	-0.3239	-0.166	-0.2864	-0.2788
	0.1267	0.0926	0.3987	0.1395	0.1508
LW	-0.2861	-0.2742	-0.462	-0.3858	-0.2099
	0.14	0.1579	0.0133*	0.0426*	0.2837
PL	-0.3431	-0.4258	0.0855	-0.2645	-0.2531
	0.0739	0.0239*	0.6653	0.1737	0.1937
IW	0.3459	0.4152	-0.0943	0.2454	0.3836
	0.0714	0.0280*	0.633	0.2081	0.0439*
PU	-0.2331	0.0192	-0.3933	0.0115	-0.1029
	0.2326	0.9229	0.0384*	0.9536	0.6023
HGT	0.0916	0.0631	-0.1158	-0.0421	0.2382
	0.643	0.7497	0.5572	0.8315	0.2222
TRUNK	-0.0683	-0.0092	-0.2146	-0.0256	-0.075
	0.7298	0.963	0.2727	0.8973	0.7044
CNS	0.1827	0.1065	-0.1161	0.1228	0.2428
	0.3521	0.5896	0.5564	0.5336	0.2132
TA	-0.0675	0.2312	-0.0291	0.2479	0.0228
	0.7329	0.2364	0.883	0.2033	0.9081
RBRIX	-0.0209	-0.3264	0.1247	-0.3922	-0.0109
	0.9158	0.09	0.5273	0.0390*	0.9562

Table: Continued

	IL	Brix	FL	FD	FW
Brix	0.1477 0.4531				
FL	0.3571 0.0621	-0.0943 0.633			
FD	0.3126 0.1053	-0.0844 0.6694	0.7776 0.0000**		
FW	0.2305 0.2379	-0.1019 0.606	0.8161 0.0000**	0.7918 0.0000**	
SL	0.1117 0.5716	-0.136 0.49	0.7078 0.0000**	0.2543 0.1916	0.5715 0.0015**
SW	0.3349 0.0815	-0.3964 0.0368*	0.7584 0.0000**	0.7857 0.0000**	0.7523 0.0000**
ST	-0.4225 0.0251*	-0.1071 0.5875	-0.0906 0.6466	-0.3855 0.0428*	-0.2037 0.2986
SWG	0.0058 0.9766	-0.3941 0.0380*	0.6105 0.0006**	0.5364 0.0033**	0.6268 0.0004**
SDL	0.2844 0.1424	-0.0739 0.7085	0.6082 0.0006**	0.2142 0.2738	0.4353 0.0206*
SDW	0.3243 0.0922	-0.2868 0.1389	0.7663 0.0000**	0.7786 0.0000**	0.7517 0.0000**
SDWG	0.257 0.1867	-0.0936 0.6356	0.7201 0.0000**	0.5622 0.0018**	0.6843 0.0001**
LL	0.014 0.9438	0.2787 0.1509	-0.2673 0.169	-0.1432 0.4674	-0.1913 0.3294
LW	0.3656 0.0557	0.1364 0.4888	-0.2771 0.1534	-0.1625 0.4086	-0.1698 0.3877
PL	-0.0543 0.7838	-0.0037 0.9851	-0.4585 0.0141*	-0.4622 0.0133*	-0.4475 0.0169*
IW	0.722 0.0000**	-0.1037 0.5994	0.4417 0.0186*	0.2313 0.2364	0.2736 0.1589
PU	-0.1621 0.4098	0.1361 0.4897	-0.1867 0.3416	0.1275 0.5178	-0.107 0.5878
HGT	0.3543 0.0643	0.4895 0.0082**	0.2134 0.2757	0.2526 0.1948	0.246 0.207
TRUNK	0.1873 0.3398	-0.0102 0.9589	0.0226 0.9089	0.0486 0.8062	0.0133 0.9466
CNS	0.2521 0.1956	0.2967 0.1252	0.2228 0.2544	0.1381 0.4834	0.1666 0.3969
TA	0.0221 0.9113	-0. 4013 0.0343*	-0.198 0.3125	-0.0761 0.7002	0.0237 0.9048
Rbrix	0.1204 0.5417	0.6133 0.0005	0.1558 0.4285	0.002 0.992	-0.0801 0.6852

Table : Continued

	SDW	SDWG	LL	LW	PL
SDWG	0.8346				
	0.0000**				
LL	-0.4166	-0.3016			
	0.0275*	0.1188			
LW	-0.2982	-0.2478	0.5869		
	0.1233	0.2036	0.0010**		
PL	-0.4982	-0.462	0.5772	0.3561	
	0.0070**	0.0133*	0.0013**	0.0629	
IW	0.3757	0.2954	-0.0324	0.0467	0.0011
	0.0488*	0.127	0.8701	0.8135	0.9956
PU	0.0076	-0.0501	0.249	0.0709	-0.0677
	0.9693	0.8002	0.2013	0.7198	0.7321
HGT	0.1363	0.171	0.3241	0.0977	-0.0649
	0.4891	0.3843	0.0924	0.6208	0.7428
TRUNK	-0.04	-0.082	0.1004	0.2022	0.1078
	0.84	0.6784	0.6112	0.3021	0.5849
CNS	0.1105	0.1634	0.3839	-0.0429	0.0321
	0.5757	0.4062	0.0437*	0.8284	0.8713
TA	0.094	0.1002	0.1347	0.1267	-0.0145
	0.6343	0.6119	0.4943	0.5207	0.9415
RBRIX	-0.1447	-0.0714	0.0073	-0.1555	0.018
	0.4624	0.7181	0.9705	0.4295	0.9274

Table: Continued

	TA
RBRIX	-0.7922

Table : Continued

	SDW	SDWG	LL	LW	PL
SDWG	0.8346				
	0.0000**				
LL	-0.4166	-0.3016			
	0.0275*	0.1188			
LW	-0.2982	-0.2478	0.5869		
	0.1233	0.2036	0.0010**		
PL	-0.4982	-0.462	0.5772	0.3561	
	0.0070**	0.0133*	0.0013**	0.0629	
IW	0.3757	0.2954	-0.0324	0.0467	0.0011
	0.0488*	0.127	0.8701	0.8135	0.9956
PU	0.0076	-0.0501	0.249	0.0709	-0.0677
	0.9693	0.8002	0.2013	0.7198	0.7321
HGT	0.1363	0.171	0.3241	0.0977	-0.0649
	0.4891	0.3843	0.0924	0.6208	0.7428
TRUNK	-0.04	-0.082	0.1004	0.2022	0.1078
	0.84	0.6784	0.6112	0.3021	0.5849
CNS	0.1105	0.1634	0.3839	-0.0429	0.0321
	0.5757	0.4062	0.0437*	0.8284	0.8713
TA	0.094	0.1002	0.1347	0.1267	-0.0145
	0.6343	0.6119	0.4943	0.5207	0.9415

Table: Continued

	IW	PU	HGT	TRUNK	CNS
PU	-0.3155				
	0.1019				
HGT	0.3054	0.2676			
	0.114	0.1687			
TRUNK	0.1023	0.1049	-0.0581		
	0.6045	0.5954	0.7691		
CNS	0.4955	0.1908	0.7098	-0.0491	
	0.0073**	0.3308	0.0000**	0.8039	
TA	0.0854	0.076	-0.1427	-0.0818	0.0247
	0.6655	0.7008	0.4687	0.6791	0.9007
RBRIX	0.0278	-0.1639	0.2843	-0.0323	0.0928
	0.8884	0.4047	0.1426	0.8703	0.6384