BIODIVERSITY IN PLANT, GRAIN AND NUTRITIONAL CHARACTERISTICS OF SORGHUM [*Sorghum bicolor* (L.) Moench] ACCESSIONS FROM ETHIOPIA AND SOUTH AFRICA

By

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DEDICATION

This piece of work is dedicated to my father Shegro Gerrano, and my mother Edessie Beyso who sent me to school and supported me from the financial hardship that they were experiencing on a subsistence farm, for my better future. I also dedicate this thesis to my lovely and wonderful wife Tinebeb Nega and our child Saron who endured the pain of separation and did not get much attention and love from me for the three years of this study.

DECLARATION

“I declare that the thesis hereby submitted by me for the Philosophiae Doctorate Degree in Agriculture at the University of the Free State is my own independent work and has not previously been submitted by me to another University/Faculty.

I further more cede copyright of the thesis in favour of the University of the Free State.”

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Abe Shegro Gerrano Date

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Nothing shall be impossible for God!

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ABBREVIATIONS AND SYMBOLS

%	percent
µg	microgram
µl	microlitre
^{o}C	degree celsius
A	Absorbance
AFLP	amplified fragment length polymorphism
ALP	amplicon length polymorphism
Am:Ap	amylose to amylopectin ratio
ANOVA	analysis of variance
ARC-GCI	Agricultural Research Council-Grain Crops Institute
AsARC	Asossa Agricultural Research Centre
ATP	adenosine triphosphate
bp	base pair
Ca	calcium
cm	centimetre
CSA	Central Statistical Authority
COPH	co-phenetic
CTAB	hexadecyltrimethylammonium bromide
CV	coefficient of variation
cv	cultivar
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DNase	deoxyribonuclease
DTT	dithiotreitol
EDTA	ethyl diamine tetra acetic acid
EIAR	Ethiopian Institute of Agricultural Research
ESIP	Ethiopian Sorghum Improvement Project
et al	'et alii/alia' (and others)
FAO	Food and Agricultural Organization
Fe	iron
g	gram
GD	genetic distance
GOPOD	glucose oxidase peroxidase 4-aminoantipyrine
GS	genetic similarity
H'	phenotypic diversity index
H_2O	water
HNO_3	nitric acid
h	hour
IAR	Institute of Agricultural Research
IBC	Institute of Biodiversity Conservation
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
INTSORMIL-CRSP	International Sorghum and Millet Collaborative Research Support
IPGRI	International Plant Genetic Resources Institute
K	potassium
LSD	least significant difference

m	meter
M	molar
MARC	Melkassa Agricultural Research Centre
MAS	marker assisted selection
masl	meter above sea level
max	maximum
mg	miligram
mg kg^{-1}	miligram per kilogram
Mg	magnesium
min	minute
ml	milliliter
Mn	manganese
Mol	mole
MOPS	Morpholinopropanesulfonic acid
MoARD	Ministry of Agriculture and Rural Development
N	nitrogen
Na	sodium
NaOH	sodium hydroxide
NCSS	number cruncher statistical system
nm	nano meter
P	posphorus
PC	principal component
PCA	principal component analysis
PCoA	principle coordinate analysis
PCR	polymerase chain reaction
PIC	polymorphic information content
pmol	pico mol
ppm	part per million
PU	Purdue University
QTL	quantitative trait loci
RAPD	random amplified polymorphic DNA
RCBP	rural capacity building project
RFLP	restriction fragment length polymorphism
rpm	revolutions per minute
SA	South Africa
SAHN	sequencial agglomerative hierarchial nested
SCAR	sequence characterized amplified region
SDS	sodium dodecyl sulphate
SPLAT	single polymorphic amplification test
SSR	simple sequence repeat
STS	sequence tagged sites
UPGMA	unweighted pair group method using arithmetic averages
USDA	United States Department of Agriculture
UPOV	International union for the protection of new varieties of plants
UV	ultraviolet
v/v	volume by volume
W	watt
w/v	weight by volume

w/w	fresh weight basis
WHO	World Health Organization
Zn	zinc
Λ	lambda

CHAPTER 1

GENERAL INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench), a tropical plant belonging to the Poaceae family, is one of the most important cereal crops in the world (Anglani, 1998). More than 35% of sorghum is utilised as a food grain and the balance is used primarily for animal feed, alcohol production and industrial products (FAO, 1995; Awika and Rooney, 2004; Dicko et al. 2006; Mehmood et al., 2008). In terms of cereal grains production, sorghum ranks fifth in cereal crop after wheat, rice, maize and barley (Smith and Frederiksen, 2000; FAO, 2005). In sub Saharan Africa sorghum is the second most important cereal crop after maize (*Zea mays* L*)* (Zidenga, 2004) and the second preferred cereal after tef (*Eragrostis tef* (Zucc.) Trotter) for preparing '*injera*', which is the staple food in Ethiopia and Eritrea (Gebrekidan and Gebrehiwot, 1982; Doggett and Prasada Rao, 1995; Ayana, 2001).

Doggett (1988) suggested that sorghum was domesticated and originated in the north-east quadrant of Africa, most likely in the Ethiopian-Sudan border regions. The presence of wild and cultivated sorghums in Ethiopia reveales that Ethiopia is the primary centre of origin and centre of diversity (Mekibeb, 2009). Given the diversity of sorghum, studying genetic diversity (Ayana, 2001) and biochemical composition of sorghum germplasm from Ethiopia is very important for several reasons.

Ethiopia, the primary centre of origin for sorghum, where the crop was domesticated (Vavilov, 1951) and diversified (Harlan, 1969; Rosenow and Dalhberg, 2000) is characterised by a diversity of climate, physiography, soils, vegetation, farming systems and socio-economic conditions (Ayana, 2001). The presence of a highly variable agro-ecology presents a possibility due to a favourable combination of circumstances and a challenge for germplasm conservationists and plant breeders (Ayana, 2001). According to Gebrekidan (1973; 1981), in Ethiopia sorghum is extremely diverse throughout the growing areas, which contain pockets of isolation with an extremely broad and valuable genetic base for potential breeding and improvement in the country and the world at large. Since its domestication, the crop has been under intensive human selection for traits of interest by farmers and this led to being existence of extremely diversified local landraces. Diversity in Ethiopian sorghum is based on maturity, adaptation to different soils and fertility levels, moisture regimes, panicle types, seed colour, seed size, disease and insect resistance and grain quality. The presence of such a highly variable genetic

pool with diverse agro-ecology adaptations poses a enormous challenge as well as opportunity for improvement of the crop (Doggett, 1988).

Sorghum requires less moisture than other cereal crops and is more tolerant to drought-prone and of poorly drained soil, making production easier in most agro-ecological zones subject to limited rainfall areas which are unfavourable for most cereals (Maunder, 2002). Sorghum is an important food crop in Ethiopia where it is widely grown in the high lands, low lands and semi-arid regions of the country (Abdi et al., 2002), especially in moisture stressed parts where other crops can least survives. According to the Central Statistics Authority of Ethiopia (CSA, 2008), sorghum ranks third after maize and tef in total production, after maize in yield per hectare and after tef and maize in area harvested.

Being an indigenous crop, a large amount of variability exists in the country. As a result, a large number of sorghum germplasm have been collected by the Ethiopian Sorghum Improvement Project (ESIP) and the Institute of Biodiversity Conservation (IBC). Many of these accessions have not been evaluated in the country using morphological, biochemical and DNA molecular markers.

The chemical composition in food crops can vary considerably between regions within a country as well as between countries. Such divergences might be due to variation in genotype, temperature, rainfall and access to water, use of fertilizer, and nutrient content of the soil (Greenfield and Southgate, 1992). The physical seed characteristics and variation in nutritional composition due to production in diverse environments, and processing methods used, affects the quality of sorghum. Genetic improvement of sorghum can improve food quality in arid and semi-arid regions where sorghum is predominantly growing and is a key food crop. Improvement of sorghum productivity in developing countries depends on the development and availability of new technologies. Sorghum breeding programmes have offered a wide range of new varieties with interest of traits that improved production and productivity (FAO and ICRISAT, 1996). Sorghum breeders developed and released several improved genotypes every year that contained desirable traits over a wide range of environmental conditions. Screening and selection of improved varieties for specific local food and industrial requirements from this great biodiversity is of utmost importance for food security and alleviation of poverty (Anglani, 1998; Akintayo and Sedgo, 2001; Dicko et al., 2006). In the past, studies have been devoted to assessing patterns of sorghum genetic variation based on morphology or pedigree. However, this approach has its limitations. Complex quantitatively inherited traits are difficult to trace based solely on morphology. For this reason, DNA-based

methods have been employed in studies of sorghum genetic diversity and in genetic improvement of the crop (Zidenga, 2004). DNA markers have provided breeders with new tools to understand and more efficiently select for complex traits in breeding programmes (Akinbo et al., 2007; 2008).

Diversity studies have been carried out in the Ethiopian/Eritrea area, which, like most areas, is threatened by loss of landraces due to introduction and development of improved varieties. Ethiopian sorghum germplasm is noted worldwide as a source of useful genes such as high lysine content (Singh and Axtell, 1973), cold tolerance (Singh, 1985), good grain quality, and disease and insect resistance (Kebede, 1991). Evaluating genetic diversity of germplasm can assist to distniguish accessions with the greatest novelty which thus, is most desirable for incorporation into crop improvement programmes. Genetic distance estimates determined by phenotypic and molecular markers help identify suitable germplasm for incorporation into future plant breeding programmes. Hence, assessment of genetic diversity in sorghum germplasm and determination of sorghum phenotypic and biochemical activities would help to know the breeding potential of the accessions in Ethiopia and South Africa.

Since Ethiopia is one of the Vavilovian centres of genetic diversity and origin for many cultivated and wild plants (Vavilov, 1951; Harlan, 1969; Mengesha 1975), the Institute of Biodiversity Conservation was established to collect and conserve the invaluable plant genetic resources in the country. Since its establishment, the institute has collected and maintained 9 824 sorghum germplasm accessions. Most of the accessions are landraces, which have acquired under diverse agro-ecological conditions and complex farming systems.

Regardless of the economic use of sorghum; the important position of Ethiopia in terms of its domestication and diversity; the fact of existing of a large number of landraces in the Ethiopian national gene bank as well as under subsistence agriculture; and the wide consideration of these landraces have as sources of useful genes for sorghum improvement, a limited number of studies have been done on the genetic diversity (Gebrekidan, 1973; Gebrekidan and Kebede, 1977; Teshome et al., 1977 and Ayana, 2001) and physicochemical and morphological characterisation of sorghum germplasm from Ethiopia. There are many introductions and several local collections that need to be characterised before they can be utilised effectively and efficiently in sorghum breeding programmes.

The overall objective of this study was to analyze and describe the magnitude of genetic and biochemical diversity in Ethiopian sorghum accessions for the benefit of future breeding programmes. As this study was carried out in South Africa in collaboration with the sorghum breeding programme of the Grain Crops Institute in Potchefstroom, it was decided to include the 11 most important sorghum genotypes of the South African programme for comparison. The specific objectives of this study therefore were to:

1) Assess the genetic diversity of sorghum accessions from Ethiopia and South Africa using amplified fragment length polymorphism (AFLP) marker technique and classify accessions in different groups based on their genetic distances.
2) Estimate the level of morphological variability and genetic distances among sorghum germplasm accessions from Ethiopia and South Africa.
3) Compare the relative advantages of both morphological descriptors and AFLP markers for their usefulness in discriminating accessions.
4) Assess the variation of biochemical composition of grain of the sorghum germplasm accessions.
5) Identify specific accession(s) with valuable traits that can be used in future sorghum breeding programmes.

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CHAPTER 2

LITERATURE REVIEW

2.1 Morphological traits in sorghum diversity study

Diverse taxonomic characteristics have been used to separate and assess patterns of phenotypic diversity in the relationships of species and germplasm collections of crops (Perry and MacIntosh, 1991; Rabbani et al., 1998). A great extent of variability exists in quantitative and qualitative traits among sorghum local landraces, such as maturity, yield, plant height, plant pigmentation, midrib colour, panicle length and width, panicle compactness and shape, glume colour, grain colour, size and weight and disease reaction (House, 1985; Mukuru, 1993).

Traditionally, characterisation and evaluation of genetic diversity in crop species is based on variation in quantitative characters and qualitative characters (Vega, 1993; Schut et al., 1997), this might be due the morpho-agronomic traits does not need any advanced equipment or complex experiments. They are simple, rapid and inexpensive to score and measure. Phenotypic estimates are used to present the degree of genetic relationship and difference between lines; it is presumed that similarity in phenotype characteristics reflects genetic similarity of genotypes (Cox et al., 1985). The application of agro-morphological traits has been used as a powerful tool in the classification and grouping of lines, to study taxonomic status, identification, determination of genetic variation and correlation of characters with agronomic potential (Millan and Cubero, 1995; Van Beuningen and Busch, 1997). Before the advent of DNA-technology, genetic diversity analysis was only studied using morphological and physiological descriptors (Liu and Furnier, 1993; Neinhuis et al., 1995). Characterisation and studying evolutionary relationships of crop species involves the cultivation of sub-samples and their subsequent morphological and agronomic description (Vega, 1993). Therefore, it is paramount important to know and comprehend the nature of the interaction and relationships between genetic, physiological, morphological and physico-chemical characters, in order to employ intensive selection criteria effectively and efficienlty.

Morphological markers are important in the study of genetic diversity and relationships in plant breeding programmes (Cox and Murphy, 1990; Van Beuningen and Busch, 1997) because (1) the existing data based on the germplasm collection or breeding stock can often be used for genetic analysis; (2) statistical procedures for morphological trait

analysis are readily available; (3) morphological information is essential in understanding the ideotype performance relationships; (4) explanations of heterosis may be enhanced if morphological measures of distances are included as an independent variable. However, use of morphological traits for the study of genetic diversity and relationship has been criticized since the study of genetic relationship among germplasm using morphological characteristics is time consuming and costly process (Cooke, 1984). Furthermore, the genetic control of morphological characters is complex, involving epistatic interactions (Smith and Smith, 1989). Morphological markers are recessive and only expressed in the homozygous condition. Most elite cultivated and breeding line does not grow vigorously with observable morphological markers, a large number of which have deleterious effects on agronomic traits (Smith, 1986). Morphological traits are usually subject to genotype x environment interaction effects (Kumar, 1999) that results a limited number of stable characters. Thus, morphological appearance cannot adequately describe genotypes without extensive trials (Lin and Binns, 1994) and, therefore, valid comparisons are only possible for descriptions taken at the same location during the same season (Smith and Smith, 1989). On the other hand, discrete morphological traits are the basis for description of identity, distinctness and uniformity of cultivars in plant variety protection and registration under the guidelines of the International Union for the protection of new varieties of plants (UPOV, 1980). Geleta et al. (2006) also indicated that although morpho-agronomical characterisation is influenced by the environment and is time consuming in general, among other disadvantages in relation to Amplified Fragment Length Polymorphisms (AFLPs) and Single Sequence Repeats (SSRs), it can still be an important and practical means of making progress in germplasm evaluation by conservationists and breeders. Furthermore, morphological traits are almost entirely used for crop diversity analysis in countries like Ethiopia where economy and trained manpower are the limiting factors to establish modern technologies for crop diversity analysis.

In sorghum, studying genetic diversity include concepts of Mendelian hereditary analysis of discrete morphological traits (Doggett, 1988) and statistical analysis of quantitative agro-morphological traits together with eco-geographic information (de Wet et al., 1976; Murty et al., 1976, Ayana, 2001). Using *ex situ* and conserved sorghum germplasm accessions from Ethiopia and Eretria, Ayana and Bekele (1998) reported that high and comparable levels of phenotypic variation exist between the regions of origin.

2.2 Genetic diversity

Genetic diversity refers to the variation of heritable characteristics present among alleles of genes in different individuals of populations of species that serves as an important role in evolution by allowing a species to adapt to a new environment (IPGRI, 1993; Weir, 1996; Kremer et al., 1998). The ultimate source of genetic diversity is gene mutation, it is a permanent change in the DNA sequence, molded and shaped by selection, recombination, gene flow, genetic drift, and migration in heterogeneous environments in space and time (Hartl and Clark, 1997). Natural selection chooses the best fit among and within a population; there can be no adaptive evolution without genetic variation (Ayana, 2001). Genetic diversity is an essential raw material for evolution, which enables populations of the crop species to survive, adapt new circumstances, and evolve to produce new genetic variants, where some of them may become the most fit variants that meet long-term changes in the environment (Hedrick, 2000, Ayana, 2001).

Likewise, genetic diversity is vital in plant breeding for developing new and high yielding varieties and protecting the productivity of such varieties by integrating genes/traits for disease and insect pest resistance as well as tolerance to abiotic stresses (Allard, 1999) to address ever-increasing food requirement. So, the level of genetic diversity determines the evolutionary potential of a species and the rate of gain from human selection in breeder's materials. Therefore, a major focus of research in genetics has been to determine the amount of genetic variation in both natural and domestic populations and describing the possible mechanisms maintaining such variability in meeting new environmental challenges (Weir, 1996, Ayana, 2001).

Plant genetic resources, the part of biodiversity, comprehends cultivated varieties in current use and newly developed, obsolete cultivars, primitive varieties (landraces), wild and weedy species, near relatives of cultivated varieties; and special stocks including elite and current breeders lines and mutants and are useful resources in the biological basis for food security (Wasswa, 2001). Genetic resources have evolved as a product of domestication, intensification, diversification, and improvement through selection by farmers for different purposes. The local landraces and newly developed improved cultivars provide raw materials for crop improvement worldwide, for present and future generations (Rai, 2002). Therefore, it is pivotal importance to conserve the diversity of crop species.

Genetic diversity can be expressed, through a large number of associations of genes which exist in individuals of a single species and are shown as characters that differ among cultivated varieties of the same plant species in growth pattern, resistance to disease and pests, tolerance to environmental conditions and productivity (Frankel and Brown, 1984). Genetic diversity is an important factor in breeding procedures that is aimed at improving crop varieties for desirable traits. It is crucial factor against climatic stress and pests. Genetic diversity provides more importantly a reasonabe yield and resistant to adverse environmental conditions that elucidate farmers to grow several crop varieties in their field (McNaught, 1988).

Genetic diversity can be measured using different approaches within and between populations as the number of organisms differing from others and the relationships among individuals of their relative frequency at genus, species, population, individual, genome locus and DNA base sequence levels (Kresovich and McFreson, 1992; Gaston, 1998; Kumar, 1999). Although, the process of assessment needs to be interactive and dynamic, due to evolutionary changes (Gaston, 1998). Genetic divergence acts as a vital role in the successful breeding programmes. Genetically diverse parents produce high heterotic effects and yield desirable segregates. Thus, quantitative assessment of genetic diversity is significantly important to determine the extent of genetic differences between and within crop species (Adugna, 2002).

Genetic variability within a taxon is of great importance for plant geneticists, breeders, physiologists, taxonomists and biosystematists (Prince et al., 1992). Diversity within a given plant population is a product of biotic factors, physical environment, artificial selection and plant characters such as size, mating system, mutation, migration and dispersal (Frankel et al., 1995) and the influence of man through domestication ans selection (Allard, 1988).

The genetic diversity in the germplasm of a breeding programme affects the potential genetic gain through selection. Estimates of genetic diversity using new molecular tools, especially molecular markers have proven to be a useful way to delineate existing heterotic groups, identify new heterotic groups and assign inbreds of unknown genetic origin to established heterotic groups (Dubreuil et al., 1996; Hongtrakul et al., 1997; Saghai-Maroof et al., 1997; Pejic et al., 1998; Casa et al., 2002).

Ethiopia is a centre for genetic diversity for many domesticated crop plant species such as sorghum, barley, tef, chickpea and coffee, largely represented in the country by local

landraces and wild types that are exceptionally adapted to adverse environmental conditions, genetically diverse forms. Much of this crop diversity is found in small fields of small scale farmers, have played a great role in the creation, maintenance and effiiecient utilisation of resources (Worede et al., 2000).

In a country like Ethiopia, which is characterised by highly varied agro-ecological and diverse growing conditions, the existence of genetic diversity is significantly important for the maintenance, conservation and enhancement of production and productivity in agricultural crops. Such diversity provides security for the farmer against biotic and abiotic stresses. Genetic diversity grants farmers to exploit highly varied micro-environments differing in characteristics such as soil, water, temperature, altitude, slope, and fertility. Genetic diversity between and within species is especially significant to Ethiopia as it represents an important genetic resource to the subsistence farming communities at regional and country level (Worede et al., 2000).

An intensive study of genetic diversity in sorghum local landraces based on race, latitude of origin, photoperiod-sensitivity, grain and nutritional quality, agro-morphological traits and DNA markers, has provided an evidence that sorghum has appreciable genetic variation that has been poorly used in terms of crop improvement programme (Wu et al., 2004; Abu Assar et al., 2005; Deu et al., 2006; Kayode et al., 2006; Dillon et al., 2007).

Previously, genetic improvement of sorghum has been achieved using conventional plant breeding scheme. However, genetic diversity and the advent of molecular marker technologies offer great potential to add to the genetic improvement in sorghum breeding programmes. In recent years, SSRs and AFLPs have been used effectively in marker-assisted breeding of different crops and are often considered the molecular markers of choice. With respect to efficient breeding, the conservation and effective use of genetic resources is paramount important, since different farmers' varieties provides greater genetic variability and furnish useful genes that are especially useful in resistance breeding and quality traits (Tanksley and McCouch, 1997). However, the success of genetic conservation and breeding programmes depend on understanding the distribution of genetic diversity and evolutionary relationships present in the gene pool (Zhang et al., 2000). Hence, the assessment of the genetic diversity and evolutionary relationships between and within local crop species could provide their high potential use and ensure rapid adoption of the improved germplasm by growers (Van Leur and Gebre, 2003).

In general, knowledge of genetic diversity and evolutionary relationships among individual germplasm within a species or among different species and its potential merit would be beneficial to crop improvement programme (Lee, 1996). Evaluation and characterisation of genetic diversity levels among germplasm provides the estimates of genetic variation among segregating progeny for pure line development (Manjarreze-Sandoval et al., 1997) and the degree of heterosis in the progeny of certain parental combinations (Cox and Murphy, 1990; Barbosa-Neto et al., 1996).

2.3 Genetic distance

Genetic distances are measures of the average genetic divergence between two equences, species or between populations within a species or taxa (Souza and Sorrells, 1991). The distance in gene frequency between the parent genotypes is important because the higher the difference in gene frequency, the higher the amount of heterosis which indicated that a more distant genetic relationship and vis-versa for smaller genetic distance (Carrera et al., 1996). Genetic distances among progeny confirm their origin and the genetic relationships between them and their parents (Carrera et al., 1996). Efficient identification and selection of the desirable genotypes largely depends on a comprehensive understanding of the genetic relatedness and variation present within the crop and its closely related wild species (Muench et al., 1991; Kresovich and McFreson, 1992; Kearsey, 1993). Information concerning genetic relatedness is crucial, for it indicates the rate of adaptive evolution and the extent of response in crop improvement (Vega, 1993). Furthermore, it is essential as a guideline in the choice of parents for breeding programmes (McNaught, 1988; Loarce et al., 1996), to detect the genetic duplicates in germplasm collections and implementing an effective genetic conservation programme (Frankel and Brown, 1984; Muench et al., 1991).

Analysis of the extent and distribution of genetic variation in a crop are essential in understanding the evolutionary relationships between accessions and to sample genetic resources in a more systematic fashion for breeding and conservation purposes (Ejeta et al., 1999). Menkir et al. (1997) suggested that molecular markers, in particular genetic distance estimates determined by molecular markers, are suitable to assess genetic diversity and to identify diverse sources in crop germplasm collections. Genetic distance is the extent of gene differences between cultivars, as measured by allele frequencies at a sample of loci (Nei, 1987). Genetic similarity is the converse of genetic distances, i.e., the extent of gene similarities among cultivars. The measure of distance or similarity

among cultivars is the covariance of allele frequencies summed for all characters (Smith, 1984).

Several genetic distance measures have been used to quantify genetic relationships among cultivars or germplasm accessions. Each variable of molecular bands such as DNA-based marker bands are considered as a locus so that every locus has two alleles. Banding profiles of each accession can be scored as present (1) or absent (0). Therefore, two approaches are used to derive phylogenetic relationships from DNA fingerprinting data. The first widely used approach involves the cluster analysis of pairwise genetic distances for the construction of dendrograms. Pair wise genetic distances are calculated from input data containing present (1) or absent (0) values for all DNA markers. One of the most commonly used genetic distance formulae is Euclidean distance, which is the square root of the sum of squares of the distances between the multidimensional space values of the distances for any two cultivars (Kaufman and Rouseeuw, 1990) and it can be put as:

$$GD = \sqrt{\sum_{i=1} \frac{(Xi - Yi)2}{N}}$$

Where, GD is the genetic distance between individual X and individual Y; i=1 to N; N is the total number of bands, and Xi and Yi are i^{th} band scores (1 or 0) for individual Xs and Ys. The process is repeated for all possible pair wise groupings of individuals and the pair wise distance values tabled in a pair wise distance matrix. Genetic distance has also been calculated from several genetic similarity indices (GS) that can be calculated using either: D=1-S or D=-In (S). One useful similarity index is that of Nei and Li (1979): GD=1-[2Nxy/Nx+Ny]. Here 2Nxy is the number of shared bands, and the Nx and Ny are the number of bands observed in individual X and individual Y, respectively. Other similarity indices such as Jaccard's (Rohlf, 1993) and Gower's similarity coefficients (Gower, 1971) have been used in calculating genetic distance (Barrett and Kidwell, 1998).

The pattern of genetic relationships between and within accessions can be shown by multivariate analyses. Clustering analysis is a useful statistical tool for studying the relationships among closely related accessions. In cluster analysis, accessions are arranged in hierarchy by agglomerative algorithm according to the structure of a complex pairwise genetic proximity measure. The hierarchies emerging from the cluster analysis

are highly dependent on the proximity measures and clustering algorithm used (Kaufman and Rouseeuw, 1990).

2.4 Molecular markers in sorghum diversity studies

Molecular genetic markers are defined as differences at the genotype level that can be used to answer and explain questions of genetics (Lokko et al., 2005). To be useful as a genetic marker, the marker locus has to show experimentally detectable variation among individuals (Sørensen et al., 2008).

Variation in nucleotide sequence is exploited to assess the genetic diversity and relationships in sorghum germplasm. Molecular marker-assisted selection (MAS) involves selection of plants carrying genomic regions that are associated with favourable trait of interest. With the development and availability of an array of molecular markers and dense molecular genetic maps in crop plants, MAS has become possible for traits governed by both major genes and by quantitative trait loci (QTL) (Singh and Lohithaswa, 2006).

Molecular markers have provided a powerful approach to analyse genetic diversity and evolutionary relationships among and within germplasm accessions in many crop species. Molecular markers are useful DNA techniques that complement morphological and physiological characterisation of cultivars since they are found in the whole genome, independent of plant tissue, influence of environmental and management practices and allow cultivar identification (Manifesto et al., 2001; Altintas et al., 2008). Molecular characterisation of cultivars is also useful to evaluate potential genetic erosion due to the extensive selection, biotic and abiotic factors resulting in a reduction of genetic diversity.

The use of DNA-based markers for the genetic analysis and manipulation of important agronomic traits has become an increasingly useful tool in plant breeding. DNA markers have the potential to enhance the operation of a plant breeding programme in a number of ways, ranging from fingerprinting of elite genetic stocks, assessment of genetic diversity, increasing the efficiency of selection for difficult traits, to making environment-neutral selection possible. However, their greatest potential appears to be in accelerating the rate of gain from selection for desirable genotypes and in the manipulation of QTL that condition complex economic traits. DNA markers also permit plant breeders to correctly map or place the various interacting genes that condition complex agronomic traits (Ejeta et al., 1999). DNA markers are used to evaluate the genetic variation in gene

banks as well as to identify phylogenetic and molecular structure of crops and their associated wild species. Molecular markers assisted genetic analysis provides a means to locate and select genes controlling important agronomic, pest resistance, stress tolerance, and food quality traits (Singh and Lohithaswa, 2006). Markers are identifiable DNA sequences found at specific locations of the genome and transmitted by the standard laws of inheritance from one generation to the next. In contrast to morphological markers, which are based on visible traits, and biochemical markers, which are based on proteins produced by genes, molecular markers rely on a DNA assay. Molecular markers have been used to identify and characterize QTL associated with several different traits in sorghum including plant height and maturity (Pereira and Lee, 1995), characters related to plant domestication (Patterson et al., 1995), diseases resistance (Gowda et al., 1995), and drought tolerance (Tuinstra et al., 1996, 1997, 1998).

Compared to morphological and biochemical characteristics, the DNA genome provides a significantly more powerful source of genetic polymorphism (Beckmann and Soller, 1986). They allow direct comparison of genetic diversity to be made at the DNA level, have the potential to identify a large number of polymorphic loci with whole coverage of an entire genome, are phenotypically neutral, allow scoring of plants at any developmental stage and are not modified by environment and management practices (Tanksley et al., 1989; Messmer et al., 1993, Prabhu et al., 1997). They also render to detect the exact genetic constitution of an individual plant in a segregating population (Phillip et al., 1994). DNA markers are now widely used in constructing genetic maps, QTL mapping, and diversity analysis and as tool for marker assisted selection in breeding programmes.

Molecular markers have the advantage of improving the effectiveness of conventional breeding through the selection of desirable characteristics based on the presence of molecular markers, which are linked to the particular trait in question (Lee, 1996). Molecular markers are discrete and non-deleterious and are unaffected by environmental conditions and free of epistatic interaction (Tanksley et al., 1989; Mclntyre et al., 2001). Molecular marker technology can greatly improve the efficiency and effectiveness of sorghum breeding programmes by helping to select genes for traits of interest that are otherwise difficult to measure or that require particular conditions for their expression. Molecular markers are laboratory based tests in which the presence or absence of bands on a gel is used to indicate the presence or absence of a favourable version of a gene for a particular trait (Jordan, 2006). DNA markers provide a possibility due to a

favourable combination of circumstances to detect, monitor and manipulate genetic variation more precisely compared to morphological and biochemical markers (Yamamoto et al., 1994).

2.4.1 Concept of polymorphism

Polymorphism refers to different forms of the same basic structure. In the context of a population, these differences in DNA sequences are called polymorphisms; they may occur in coding regions (exons) or noncoding regions of genes. It occurs when two or more clearly different phenotypes exist in the same population of a species. Polymorphism is common in nature; it is related to biodiversity, genetic variation and adaptation. If modifications of a gene exist at a specific locus in a population, the locus is polymorphic. At the molecular level, polymorphism ranges from a single nucleotide base change to the number of tandem repeats in a repetitive DNA sequence. The changes may be neutral, with no detectable phenotypic effect, or they may result in the production of different forms of the same enzyme (isozymes) active under different environmental conditions, such as pH or temperature. If a specific recognition base sequence is present, the restriction enzyme recognising that site will cleave the DNA molecule and result in fragments of specific base pair lengths. If the site is absent, a different length DNA fragment will be produced (Kirby, 1992). Assessment of genetic relationships using molecular markers provides polymorphism information about a germplasm pool, which is useful for developing, mapping and breeding populations or lines (Beer et al., 1997). Polymorphism information is also useful for selecting and identifying parents to be used in future breeding programme.

2.4.2 DNA fingerprinting techniques

The DNA markers are considered to be the most suitable means for estimating genetic diversity analysis because of their abundant polymorphism and the fact that they are independent of environment conditions (Gepts, 1993). Variation in nucleotide sequence has been exploited to assess the genetic diversity and relationships in sorghum germplasm. DNA markers such as restriction fragment length polymorphisms (RFLPs), PCR-based DNA markers such as sequence characterised amplified regions (SCARs), randomn amplified polymorphic DNAs (RAPDs), SSRs, sequence tagged sites (STSs), single polymorphic amplification test (SPLAT), AFLPs, amplicon length polymorphisms (ALPs) and others have been used to assess and characterise genetic variability in sorghum genetic resources (Menkir et al.,1997; Dean et al., 1999; Ayana et al., 2000a; b;

Thimmaraju et al., 2000; Geleta, 2003. RFLPs (Helentjaris et al., 1986; Hulbert et al., 1990; Chittenden et al., 1994; Pereira et al., 1994; Xu et al., 1994) and PCR-based approaches such as RAPDs (Williams et al., 1990; Tao et al., 1998), SSRs (Taramino et al., 1997) or microsatellites and AFLPs (Zabeau and Vos, 1993; Vos et al., 1995; Boivin et al., 1999) have been used successfully to assess genetic relationships in sorghum.

2.4.2.1 Restriction fragment length polymorphism (RFLP)

Among the various DNA molecular markers, RFLP was the first to be used in human genomic mapping (Botstein et al., 1980). They were the first to suggest that large numbers of genetic markers might be found by studying differences in the heredity material of the DNA molecule itself. At later stage RFLP was adopted for plant genomic mapping (Weber and Helentjaris, 1989). Restriction enzymes are highly specific “molecular shears” which cleave the DNA at particular sequences (restriction sites). If two individuals differ by as little as a single nucleotide in the restriction site, the restriction enzyme will cut the DNA of one but not the other, generating restriction fragments of different lengths which can then be separated (in an electrical field) and visualized by specific binding of radioactive probe (Andrew et al., 1991).

RFLPs are co-dominant markers that are abundant in all organisms, stable and unlimited in number (Kochert, 1994). The RFLP technique has been successfully employed for identification and characterisation of cultivars (Gebhardt et al., 1989a; Gorg et al., 1992), phylogenetic studies (Debener et al., 1990), parental tracing (Hosaka, 1986), genetic map construction (Gebhardt et al., 1989b; Gebhardt et al., 1991), and genetic relationship and diversity studies (Miller and Tanksley, 1990).

RFLP analysis consisted of DNA isolation from a suitable set of plants followed by digestion of the DNA with a specific restriction endonucluase. The DNA fragments generated in such a way are then separated by agarose-gel electrophoresis and transferred to a nitrocellulose or nylon filter by Southern blotting. Subsequently, nucleic acid hybridisation is done with radioactively labelled cloned probes. RFLPs are then scored by direct comparison of banding patterns (Kochert, 1994; Morell et al., 1995).

RFLP is limited by the relatively large amount of DNA required for restriction digestion, Southern blotting and hybridisation plus the requirement of radioactive isotopes and autoradiography which makes this technique relatively slow, laborious and expensive (Kochert, 1994).

2.4.2.2 Random amplified polymorphic DNA (RAPD)

Williams et al. (1990) and Welsh and McClelland (1990) were the first to use DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequences for plant, humans and animals. RAPD markers are generated by PCR amplification of random genomic DNA segments with single synthetic decamer primers of arbitrary sequence (Williams et al., 1990; Jacobson and Hedrén, 2007). Since the process uses PCR primers (10 bp) the products are easily separated by standard electrophoretic techniques and visualised by ultraviolet (UV) illumination of ethidium bromide stained gels (Williams et al., 1990; Thottappilly et al., 2000). The amplification product will vary in size according to the distance between primer homology within the target DNA, a polymorphism will result. Generally, in this technique, no primer sequence information is required to design the primers involved in the PCR reaction; hence the term “random” in the naming (Thottappilly et al., 2000).

Provided that the RAPD primer sequences are arbitrarily chosen, the genome is expected to be sampled randomly. Most RAPD fragments are inherited as dominant markers, i.e., they are either present or absent. A fragment is seen in the homozygous (AA) as well as in the heterozygous (Aa) situation, and only the absence of the fragment reveals the underlying genotype (aa) (Weising et al., 2005). RAPD markers have been used to estimate genetic diversity in several crops, including sorghum (Yang et al., 1996; Menkir et al., 1997; Ayana et al., 2000b). However, the need to repeat each PCR reaction multiple times and the inability to obtain identical banding patterns in different laboratories have limited the use of the RAPD technique (Bai et al., 1999).

2.4.2.3 Simple sequence repeats (SSRs)

Simple sequence repeats (SSR), also called microsatellites, are among the most variable types of tandemly repeated DNA. The fragment polymorphism relates to total sequence length, as determined by the number of repeat units, and the heterozygote for different fragments in diploid genomes can be identified (Mc Gregor et al., 2000). The sequences are regions of short, tandemly repeated DNA motifs and consist of two to eight bp repeat units with an overall length in the order of tens of bp, which are found dispersed throughout the genomes (Tautz and Rentz, 1984).

Microsatellites are important genetic markers in identification and characterisation of plant species. They are co-dominant markers and are a PCR-based technique,

amenable to automation and thus permit analysis of large populations/lines in a short period of time. Microsatellites are highly polymorphic and evenly spread throughout a genome (Areshchenkova and Ganal, 1999). Microsatellite markers are multi allelic and detect a much higher level of DNA polymorphism than any other known marker system (Rafalski and Tingey, 1993). The products generated have been found to be highly reproducible and cost effective (Jones et al., 1997) and the polymorphisms can easily be detected both by southern hybridisation and by PCR (Arens et al., 1995).

2.4.2.4 Amplified fragment length polymorphism (AFLP)

AFLP is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA (Vos et al., 1995). The genomic DNA is digested with two restriction enzymes, usually a rare cutter and a frequent cutter. Double stranded oligonuceotides, known as adapters, are ligated to the ends of the genomic DNA at the specific restriction sites. Adaptors have a nucleotide overhang known as a ‘sticky end’ complementary to that of the restriction site. Separate adaptors are needed for each of the different restriction enzymes. The ligated DNA is then used as template for PCR reactions. The primers are specific to the combination of the adaptor sequence and restricted site sequence (Huang et al., 2007).

In this technique, specific double-strand DNA adapters are ligated to the DNA restriction fragments, so that the sequence of the adapters and the adjacent restriction sites serve as primer-binding sites. The primers are designed to contain the sequences that are complementary to those of adapters and the restriction sites, along with one to three selective bases added at their 3’ ends. The use of selective bases allows amplification of only a subset of the restriction fragments, which still generates a large number of bands facilitating the detection of polymorphism (Vos et al., 1995).

The AFLP approach is powerful molecular marker since it requires no prior sequence information of the target genome, and it is applicable to a variety of crop species. Moreover, it is easily standardised and readily automated for high-throughput application. AFLP technology offers the fastest, most reproducible, and most cost-effective way to generate high-density genetic maps for marker-assisted breeding of desirable traits of interest. It is also the ideal tool for determining varietal identity and assessing trueness to type (Perkin-Elmer, 1996).
AFLP is a molecular marker technique for fingerprinting DNA of any origin and has several advantages over other DNA fingerprinting techniques. Some of its advantages

are the possibility to detect small sequence variations using small quantities of genomic DNA (0.05 to 0.5 µg), the ability to reveal many polymorphic loci per assay and the simultaneous analysis of numerous germplasm accessions. The markers are reliable and reproducible between laboratories and are relatively easy and inexpensive to generate. Unlimited number of markers can be generated by varying the restriction enzymes, and the nature and the number of selective nucleotides (Blears et al., 1998).

This DNA fingerprinting technique, detects mostly dominant markers based on the PCR amplification of the genomic restriction fragments (Kiem et al., 1995). AFLP markers combine the best characteristics of the other DNA markers while avoiding their disadvantages. Unlike RFLPs, AFLP technology is PCR based, requires only minimal amounts of starting DNA, and is readily automatable and, unlike RAPD, AFLP markers have proven to be robust, reliable and reproducible, unlike SSRs, AFLP analysis requires no prior sequence knowledge of the target genome and, therefore has no up to front characterisation costs.

Although some concerns have been raised about the clustering of AFLP markers (Kiem et al., 1995) advantages of the AFLP procedure include the inherent simplicity associated with markers, based on the polymorphism detected per primer pair. With a small number of primer pairs, it is possible to assess with adequate precision and reasonable cost the parental genetic contribution to subsequent progeny generations. AFLP markers may also allow breeders to follow changes, which result from selection, genetic drift, mutation, seed mixtures and hybridisation (Van Toai et al., 1997).

The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. The technique involves three steps: (1) restriction of the DNA and ligation of oligonucleotide adaptors, (2) selective amplification of sets of restriction fragments, and (3) gel analysis of the amplified fragments. PCR amplification of restriction fragments is achieved by using the adapter and restriction site sequence as target sites for primer annealing. The selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. Using this method, sets of restriction fragments may be visualised by gel elecrophoresis without knowledge of nucleotide sequence. The method allows the specific co-amplification of high numbers of restriction fragments. The number of fragments that can be analyzed simultaneously depends on the resolution of the detection system. Typically 50-100 restriction fragments are amplified and detected on denaturing polyacrylamide gels. The

AFLP technique provides a novel and powerful DNA fingerprinting technique for DNA of any origin or complexity (Vos et al., 1995). High reproducibility, rapid generation and high frequency of identifiable polymorphisms make AFLP analysis an attractive fingerprinting technique for identifying polymorphisms and for determining linkages by analysing individuals from a segregating population (Tamiru et al., 2007). AFLP marker generates high levels of polymorphic fragments in a single gel; it has been used increasingly for mapping and genetic diversity analysis (Hongtrakul et al., 1997; Fregene et al., 2000; Lima et al., 2002; Ubi et al., 2003; Altintas, 2008).

2.5 Utilization of the grain

Sorghum is one of the major cereal crops consumed in Ethiopia and the total consumption of sorghum closely follows the global pattern of output, since most of it is consumed in the countries where it is grown (FAO, 1995). It is one of the main staples for the world's poorest and most food insecure people. Sorghum is important in many African countries for human consumption and animal feed (Vogel and Graham, 1979) and more than 95% of total food use of sorghum occurs in developing countries (FAO, 1995).

The vegetative portions of plant are important sources of fuel for cooking and the stems of the wild varieties are used to make baskets or fish traps (Singh and Lohithaswa, 2006). The plant stem and foliage are used for green chop, hay, silage, and pasture. In some areas, the stem is used for hut making. The grain plays a dominant role in the traditional beer brewing, at household and industrial levels (House et al., 2000). Grain sorghum is used to make products such as potable alcohol, malt, beer, liquids, gruels, starch, adhesives, core binders for metal casting, ore refining, and grits as packaging materials. Grains are a rich and cheap source of starch and have applications in the food, pharmaceutical, textile, and paper industries. Malt drinks and malt cocoa-based weaning food and baby food industries are popular in Nigeria (Chandel and Paroda, 2000). In Africa, sorghum is fermented not only to make beer but porridge, *injera* (fermented bread) and other products, in a process that makes available the much-needed proteins it contains. Traditional foods made from sorghum include unfermented and fermented breads, porridges, couscous, and boiled rice resembling foods and snacks, as well as, alcoholic beverages. Sorghum blended with wheat flour is used to produce baked products, including yeast-leavened pan, hearth and flat breads, cakes, muffins, cookies, biscuits, and flour tortillas (Badi et al., 1990). Most production is consumed by the households producing the crop, and only a small proportion of harvests

enter the commercial market. Since many sorghum producing areas still experience periodic food deficits, production must be increased in order to improve household food security (FAO and ICRISAT, 1996).

Sorghum therefore presumes significant importance in the economics of several countries in Africa and Asia, largely inhabited by resource-poor farmers. Although, a broad range of traditional, novel, and industrially important products are produced from sorghum, the quality products, such as ethanol and extraction breakfast foods, needs to be improved by utilizing the variability present in the crop (Chandel and Paroda, 2000).

2.6 Food quality

The quality of grain sorghum is determined by visual quality, nutritional quality (including whole grain, protein and starch digestibility; nutrient bio-availability), and anti-nutritional factors such as tannins, processing characteristics, cooking quality and consumer acceptability (Hulse et al., 1980). Grains of most cereal species, such as wheat, maize and sorghum, provides food and are important economical commodities, contain inadequate amount of some essential amino acids, particularly lysine, threonine, tryptophan and methionine. Wide range of variability has been observed in the essential amino acid composition of sorghum protein, because the crop is grown under diverse agro-climatic conditions which affect the nutritional composition of the grain (FAO, 1995).

The mature sorghum kernel (dry weight) is composed of the embryo (10%), the pericarp (8%) and the endosperm (80%). The relative proportions may vary with genetic background, environment and degree of maturity. The embryo is rich in protein, lipids, minerals and vitamin B; thus removal of the outer pericarp increases the protein and reduces the cellulose, lipid and mineral content of the grain. Sorghum is an important source of minerals that are located in the pericarp, aleurone layer and germ. Sorghum is a good source of K and an adequate source of Mg, Fe, Zn, and Cu (Smith and Frederiksen, 2000). Grain sorghum is also rich in Ca and P (Hulse et al., 1980). The mineral composition of sorghum grain is highly variable. More than genetic factors, environmental conditions prevailing in the growing region affect the mineral content of this food grain (FAO, 1995).

Sorghum is a good source of vitamins, especially the B vitamins (thiamin, riboflavin, pyridoxine), and the liposoluble vitamins A, D, E and K (Dicko et al., 2006). Among the B-group vitamins, concentrations of thiamin, riboflavin and niacin in sorghum were

comparable to those in maize. Wide variations have been observed in the values reported, particularly for niacin (Hulse et al., 1980). Ethiopian high-lysine sorghum varieties were also high in niacin; values per 100 g were 10.5 mg in IS11167 and 11.5 mg in IS11758, against 2.9 to 4.9 mg in normal sorghum (Pant, 1975).

Sorghum product quality is determined by endosperm texture and endosperm type which are important characteristics of the grain (Pushpamma and Vogel, 1982). Endosperm type refers to either a horny or floury endosperm (Dewar et al., 1993), while endosperm texture is the proportion of horny to floury (soft) endosperm (Cagampang and Kirleis, 1984).

Seed hardness, weight, and size are important parameters for assessment of sorghum grain quality (House, 1985). In addition to average values for hardness, weight and size, uniformity of these characters is important in sorghum grain quality assessment because of its impact on processing. Grain size, shape, lustre and colour are the important grain quality traits that contribute to consumer preferences and acceptability. Grain size is more importantly influences both market and yield. Grain size has a positive correlation with grain yield (Potdukhe et al., 1994; Senthil and Palanisamy, 1995; Sankarapandian et al., 1996; Asthana et al., 1997; Muppidathi et al., 1999; Audilakshmi and Aruna, 2005), crude protein content (Hicks et al., 2002) and the stay-green trait in sorghum (Borrell et al., 2003). Furthermore, grain size, grain shape and lustre are also important quality characters that determine the market price of sorghum grain. Sorghum varieties with round and/or large grain have higher hulling yield (FAO, 1994; Audilakshmi and Aruna, 2005). Understanding the significatn importance of gene effects is beneficial in choosing the breeding programmes and selection procedures to develop new sorghum cultivars with enhanced grain size, grain colour, round grain shape and a high degree of grain lustre (Audilakshmi and Aruna, 2005).

2.6.1 Chemical composition of sorghum grain

2.6.1.1 Carbohydrates

Carbohydrates are sugars and starches, which provide energy for humans and animals, and cellulose which make up many plant structures. The quality and quantity of carbohydrates present in sorghum are significantly important quality parameters that can influence consumer acceptance of the end product (Pushpamma and Vogel, 1982).

Starch is the most abundant chemical component, while soluble sugars and crude fibre are low (Waniska and Rooney, 2002).

2.6.1.2 Starch

The primary carbohydrate, starch, is the most abundant chemical component in sorghum and makes up about 60 to 80% of the normal, non-waxy, kernels. It is deposited as granules in the endosperm cells, being the main constituent of the endosperm. Inspite of the botanical source, starch is structurally composed of two high molecular weight homopolysaccharides known as amylose, a straight chain and amylopectin, a branched-chain polymer of glucose which are held together by hydrogen bonds and are arranged radically in spherical granules (Rooney and Pflugfelder 1986).

Amylose content in sorghum grain is genotype dependent. Waxy sorghums contain very low levels of amylose (level < 1%) and the content of amylose in normal sorghums ranges from 10 to 17% (w/w, fresh weight basis), constituting approximately 20-30% of starch. With balance in both being amylopectin there is no significant difference between red and white sorghum grains in their starch contents (Dicko et al. 2006). They also found that screening of starch content in 50 sorghum varieties before and after germination showed that there is an inter-varietal difference of content in these compounds.

Amylose is composed of essentially homogenous linear units of α-(1→4)-D-glucopyranose, which can form helicoidal structures in solution (Manners, 1974; Jarvis and Walker, 1993; Dicko et al. 2006). The interior of the helix is hydrophobic, allowing amylose to form a complex with free fatty acids and iodine (Fennema, 1985). There is a significant inter-varietal difference of content of amylose among sorghum varieties (Beta and Corke, 2001; Dicko et al., 2006).

Amylopectin is comprised of short chains of α-(1→ 4)-D-glucopyranose (majority 10-20 units in sorghum starch) branched to α-(1→ 6)-D-glucopyranoses to form a highly branched structure (Blennow et al., 2001). Amylopectin content is variety dependent and ranges from 45 to 54% (w/w, fresh weight basis) (Dicko et al., 2006).

Contents of amylose and amylopectin are important characteristics of starch for the selection of sorghum genotypes (Dicko et al., 2006). Since amylose has a higher gelatinisation temperature than amylopectin (Whistler et al., 1984), sorghum with low

amylose content could be targeted for industrial brewing (Dicko et al. 2006). Amylose is more susceptible to retrogradation than amylopectin and waxy sorghum is less viscous than normal sorghum (FAO, 1995; Dicko et al. 2006). Low amylose-containing sorghum varieties are preferred for extrusion-cooking since they give better functional characteristics of the extrudates, such as enzyme susceptibility and solubility (Gomez et al., 1988; Dicko et al. 2006). Dicko et al. (2006) suggests that sorghum varieties with low amylose content may be recommended for infant porridge preparation. Hydrated amylose forms a helix that can interact with iodide to form a blue or purple colour. Amylopectin interacts with iodide to form a brown colour. In its properties, sorghum starch resembles maize starch and the two can be used interchangeably in many industrial and feed applications. When boiled with water, the starch forms on opaque paste of medium viscosity. On cooling, this paste sets to a rigid, non-reversible gel. The gelatinisation temperature ranges from 68°C to 80°C (Sweat at al., 1984; National Research Council, 1996).

2.6.1.3 Soluble sugars

According to Murty et al. (1985), soluble sugar content of the caryopsis changes during development but the maximum can be 5.2%. At maturity, the average soluble sugar content ranges from 0.8 to 4.2% with sucrose being 75% of the sugars (Subramanian et al., 1980; Jambunathan et al. 1984). Mature caryopsis contains 2.2 to 3.8% soluble sugars, 0.9 to 2.5% free reducing sugars, and 1.3 to 1.4% non reducing sugars (Bhatia et al., 1972). Glucose ranges from 0.6 to 1.8% and fructose from 0.3 to 0.7%. High lysine and sugary cultivars contain more soluble sugars than normal sorghums (Subramanian et al., 1980; Murty et al. 1985). Subramanian et al. (1980) reported that the high-lysine sorghum lines IS11167 and IS11758 from Ethiopia comprised of the highest percentages of total soluble sugars (5.2 and 4.4%, respectively). During germination, sugars accumulate in the endosperm after the second day with maximum concentration occurring after eight days (Newton et al., 1980; Waniska and Rooney, 2000). The major soluble carbohydrate in the caryopsis changes from sucrose to glucose and fructose after two days. The monosaccharides are located in the endosperm and the scutellum, after two days. However, sucrose is localised in the scutellum and is the highest on the fourth day after germination (Waniska and Rooney, 2000). Rooney and Pflugfelder (1986) reported that the soluble sugar content, free glucose and maltose increase during germination.

2.6.1.4 Protein

Sorghum protein is significanlty important for the human diet in many countries in the world, thought, it is less digestible when wet cooked, due to exogenous and endogenous factors, since it is substantially adapted to adverse agro-climatic conditions that are not favourable to most other cereals (Cecil, 1992; Gomez, 1993).

The protein quality of sorghum is associated with the distribution of protein fractions in the grains which importantly attributed in terms of consumer acceptability (Pushpamma and Vogel, 1982), and nutritional composition (Serna-Saldivar and Rooney, 1995). Sorghum is mainly utilised in developing countries where cereals are a staple food crops. This might cause unbalanced diet, since sorghum and most other cereal grains, when examined for albumin, glutelin and globulin proteins are limiting essential amino acids, particularly lysine, tryptophan and threonine.

The average protein content of sorghum is usually variable ranged from 11 to 12 % (Dendy, 1995). Lasztity (1996) reported that the protein content varies from 6 to 25%. The protein content and its amino acid composition in sorghum varies due to genotype and environmental conditions at which the crop is grown (water availability, soil fertility, temperatures and environmental conditions during grain development) (Taylor and Schussler, 1986; Frey, 1997) that affect the grain composition. Sorghum proteins are located in the endosperm (80%), germ (16%), and pericarp (3%) (Taylor and Schussler, 1986). Kafirins, or prolamins, and glutelins comprise the major protein fractions in sorghum. These fractions are located primarily within the protein bodies and protein matrix of the endosperm. Nitrogen fertilization significantly increases grain yield, kafirin accumulation and protein content (Warsi and Wright, 1973). Protein quality is critically important in developing countries where the human diet consists mainly of cereals grains.

In several cereal grains, including sorghum, an inverse correlation has been observed between grain yield and protein content (Frey, 1997). Moreover, the protein content of the grain is significantly and inversely correlated with its weight and starch content (FAO, 1995). Likewise, the ash content and protein content of the sorghum grain are positively correlated with each other (Subramanian and Jambunathan, 1982). Grain protein and its amino acid composition in sorghum differ with the environmental conditions (Deosthale et al., 1972). Wide variability has been observed in the essential amino acid composition of sorghum protein (Hulse et al., 1980; Jambunathan et al., 1984).

As in other cereals grains, lysine is the first limiting essential amino acid in sorghum grain. After screening more than 9 000 accessions in the world germplasm collection, Singh and Axtell (1973) identified two sorghum lines of Ethiopian origin, IS 11758 and IS 11167 that had exceptionally high lysine at relatively high levels of protein. The average lysine content of the whole kernel of IS 11758 was 3.13 g per 100 g protein and the total protein content of the kernel was 17.2%. IS 11167 contained 3.33 g lysine per 100 g protein and 15.7% protein. Normal sorghum grown under similar conditions contained 12% protein and 2.1 g lysine per 100 g protein. Both lines were also high in oil percentage. Inheritance studies suggested that the increased amount of lysine in each line was controlled by a single recessive gene that could be easily transferred by plant breeding procedures (Singh and Axtell, 1973).

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CHAPTER 3

ASSESSMENT OF GENETIC DIVERSITY IN SORGHUM USING PHENOTYPIC MARKERS

Abstract

Sorghum is an important food crop in moisture stressed regions of the world. It is a staple crop for millions in Africa and Asia. In Ethiopia, it is a multipurpose crop used for food, fuel, housing materials, fencing and livestock feed. Data on genetic diversity levels among sorghum accessions will increase the efficiency of the sorghum improvement programme. Field experiments were conducted at the ARC Grains Crops Institute in Potchefstroom South Africa, in 2009 and 2010, to estimate the level of phenotypic diversity among 22 sorghum accessions. The experiment was laid out in a randomised complete block design with three replications. Nine qualitative and 20 quantitative morphological traits were recorded. Analysis of variance for the quantitative traits revealed that differences among accessions were highly significant for all traits. This indicated that morphological traits differed in amount of variation between sorghum accessions studied. Qualitative traits diversity index values varied from 31% (panicle compactness and shape) to 84% (glume colour). The pair-wise genetic distances based on phenotypic traits showed varying genetic distances. The principal component analysis showed that the first five principal components (PC) contributed 87% of variability among the accessions. Leaf number, days to 50% flowering, number of internodes, plant height and panicle width contributed mainly to PC1 and leaf width, leaf area, grain yield, leaf sheath length, internode length and panicle weight to PC2. Cluster analysis of the phenotypic traits resulted in four distinct groups of accessions with genetic distances ranging from 0.40 to 1.59. Therefore, the phenotypic markers provide a useful measure of genetic distances among sorghum accessions to identify potential donors or parental material for future breeding efforts.

3.1 Introduction

Sorghum is one of the world's most important cereals, with over 500 million people in the hot dry tropics dependent on it. It is grown for different purposes such as grain, forage, syrup and sugar and industrial uses of the stems and fibers. Sorghum is very hardy and can be grown in areas where other crops cannot be grown successfully (Dumitru, 2008;

Evan, 2009**)**. It is the second most important cereal in terms of both area harvested and annual production in Africa. According to the FAO (2008), Africa contributed over 60% to the total land area dedicated to cultivation of sorghum. It is especially important for food security in arid and semi-arid regions of the world (Evan, 2009).

Sorghum is widely grown in different agro-ecological zones in Ethiopia. It is the most crucial cereal crop in the lowland areas because of its biotic and abiotic stress tolerance (Kebede, 1991; Abdi et al. 2002). Sorghum displays significant genetic variability in different regions of Africa. This genetic diversity is subjected to serious genetic losses due to introduction of new varieties and habitat destruction (Brown, 1983; Hawkes, 1983; Abdi et al., 2002; Shewayrga et al., 2006) that could result in the loss of useful and desirable traits. Reduced diversity may eliminate options to use unexploited resources for food production, industry and medicine.

Morphological characterisation has been carried out to assess genetic diversity within and among the accessions by previous researchers (Harlan and de Wet, 1972; de Wet et al., 1976; de Wet, 1978; Abdi et al., 2002). Many studies have been conducted to assess the patterns of genetic diversity in landraces of different crops using different methods. Examples include studies on tef (Bekele, 1996); wheat (Bekele, 1984; Negassa, 1986; Tesfaye et al., 1991; Bechere et al., 1996), barley (Tolbert et al., 1979; Bekele, 1983; Negassa, 1985; Demissie and Bjørnstad, 1996), and sorghum (Modern et al., 1988; 1989; 1990; Aldrich et al., 1992; Ayana and Bekele, 1998; 1999; 2000; Dje et al., 1998; Abdi et al., 2002; Geleta, 1997; 2003). Since sorghum is grown throughout Ethiopia, which genetically varies extensively, characterisation and evaluation of germplasm are pre-requisites for the utilisation of the available diversity in the crop improvement programme. Hence, it is very important to assess genetic diversity based on quantitative and qualitative traits and identifying promising accessions for different traits that could be utilised in breeding programmes.

3.2 Materials and methods

3.2.1 Experimental material and study site

The experimental material comprised of 22 sorghum accessions obtained from the IBC, ESIP at MARC/Ethiopia and the ARC-Grain Crops Institute, South Africa (Table 3.1). The landrace accessions (Table 4.1) obtained from the IBC, Ethiopia performed poorly and failed to set seed under field conditions. Therefore, morphological characterisation was

only carried out for those accessions that performed well under field conditions. The study was conducted on the research farm of the ARC-GCI located at Potchefstroom (26°74''S; 27°8'E) during the 2009 and 2010 growing seasons. Potchefstroom is located at an altitude of 1344 m above sea level and the average minimum and maximum temperature is 9.61˚C and 25.48˚C respectively with an average annual total rainfall of 618.88 mm (Appendix 1). The type of soil was sandy clay loam.

Table 3.1 List of sorghum accessions, with their collection site and status

No.	Accession*	Locality/Origin	Status
1	216737	Itang	Landrace
2	216743	Abobo	Landrace
3	Birmash	MARC	Variety
4	Gambella 1107	MARC	Variety
5	IS9302	ICRISAT	Introduction
6	Baji	MARC	Variety
7	97MW6129	MARC	Advanced cv.
8	97MW6127	MARC	Advanced cv.
9	NO253	ICRISAT	Introduction
10	PI308453	Purdue University	Introduction
11	97MW6113	MARC	Advanced cv.
12	Macia-SA	South Africa	Variety
13	M48	South Africa	Variety
14	M141	South Arica	Breeding line
15	M81	South Africa	Breeding line
16	M105	South Africa	Breeding line
17	M26	South Africa	Breeding line
18	M101	South Africa	Breeding line
19	M163	South Africa	Breeding line
20	Masekaswere	South Africa	Landrace
21	Mamolokwane	South Africa	Landrace
22	M153	South Africa	Variety

ICRISAT = International Crops Research Institute for the Semi-Arid Tropics, MARC=Melkassa Agricultural Research Center, *Accession number for the landraces is from the IBC and the naming of varieties and introductions from the EIAR and ARC-GCI, South Africa

3.2.2 Methods

The accessions were sown in a plot size of 5 x 0.9 m with three replications in a randomised complete block design for two consecutive seasons. Each entry was planted in two rows, keeping plant to plant distances of 25 cm and the distances between blocks 1.5 m. Uniform crop management practices were applied to all entries in the trial as recommended for the area. For every accession, five randomly selected individual plants were used for recording data for quantitative traits, except days to 50% flowering and number of tillers, which were recorded on plot basis (Table 3.2). For the qualitative characters (Table 3.3), the most frequent character state was recorded. Seed colour, glume colour, and leaf mid rib colour were examined and scored using the Munsell colour film (1990). For both the quantitative and qualitative characters, data was recorded based on sorghum descriptors (IBPGR/ICRISAT, 1993). For leaf characteristics measurement, a procedure developed by Stickler et al. (1961) was used.

3.2.3 Statistical analysis

3.2.3.1 Quantitative traits

Using the data of the 22 sorghum accessions, ANOVA was carried out for the 20 quantitative traits separately for the 2009 and 2010 cropping seasons. Data were combined over seasons after doing the homogeneity test of variances as suggested by Gomez and Gomez (1984). Combined ANOVA was done over the 2009 and 2010 cropping seasons for the quantitative traits (Agrobase, 2005). Means for each trait were separated by the least significant difference (LSD) at ($p \leq 0.01$). Phenotypic correlation coefficients were computed to examine the degree of association among the quantitative traits (Table 3.2). Multivariate analysis was employed using the appropriate procedure of the Number Cruncher Statistical System (NCSS, 2004). Principal component analysis (PCA) was used as a data reduction tool to summarise the information from phenotypic data so that the influence of noise and outliers on the clustering results is reduced. Means of each quantitative character were standardised prior to PCA as suggested by Ruiz et al. (1997) to avoid the effect due to difference in scale. Standardisation is achieved by subtracting from each observation the mean value of the character and subsequently dividing it by its respective standard deviation (Ruiz et al., 1997; Upadhyaya et al., 2002).

Table 3.2 List of quantitative characters recorded in the study

Character	Code	Description
Days to 50% flowering (count)	DF	Number of days from emergence to when 50% of plants have started flowering in a plot
Leaf number (count)	LN	Count of total number of leaves per plant (main stalk)
Leaf length (cm)	LL	Length of the third leaf from the flag leaf
Leaf width (cm)	LW	Width of the third leaf from the flag leaf
Leaf area (cm^2)	LA	Area of the third leaf from the flag leaf, computed as (leaf length x leaf width x 0.747) suggested by Stickler et al. (1961)
Stalk diameter (cm)	SD	Diameter measured on the third internode from the ground surface
Internode length (cm)	IL	Length of the third internode counted from the ground surface
Number of internodes (count)	NI	Count of total internodes per plant
Number of tillers (count)	NT	Average number of basal tillers at maturity without counting the main stem
Leaf sheath length (cm)	LSL	Length measured on leaf sheath found on the third internode from the ground
Plant height (cm)	PH	Height of the main stalk from the ground to the tip of the main panicle
Length of peduncle exertion (cm)	LPE	Length between the base of flag leaf and the base of the panicle
Panicle length (cm)	PL	Length of the panicle from its base to tip
Panicle width (cm)	PW	Width of panicle in natural position at the widest part
Panicle weight (g)	PWt	Average weight of the five harvested whole panicles
Number of primary branches per panicle (count)	NPBP	Number of branches arising directly from the rachis of the panicle
Grain yield per panicle (g)	GYP	Weight of grain per panicle (average of five plants or panicles in the plot)
1000-seed weight (g)	TSW	Weight of 1000 seed counts at 12% moisture content
Threshing percent (%)	TP	The ratio of grain weight per panicle to the head weight of the same multiplied by 100
Grain number per panicle (count)	GNP	The ratio of grain weight per panicle to the average 1000 seed weight per panicle of the same multiplied by 1000

Table 3.3 List of qualitative characters recorded in the study along with their codes and descriptions

Qualitative characters	Code	Description
Leaf mid rib colour	LMC	White (1), Dull green (2), Yellow (3)
Plant colour	PC	Pigmented (1) and Tan (2)
Panicle compactness and shape	PCS	Semi-loose erect primary branches (6), Semi-loose dropping primary branches (7), semi-compact elliptic (8)
Glume colour	GLC	White (1), Brown (3), Red (4), Black (6)
Grain covering	GCOV	25% grain covered (1), 50% grain covered (3), 75% grain covered (5)
Grain colour	GCOL	White (1), Yellow (2), Red (3), Brown (4)
Grain size	GSI	Small (1), Bold (2), Medium (3)
Grain shape	GSH	Round (1), Eliptical (2), Flat(3)
Grain luster	GLU	Lusterous (1), Non-lusterous (2)

This resulted in standardised values for each character with average zero and standard deviation of one or less. A dendrogram was generated, depicting relationships among the genotypes using NCSS (2004). The measure of dissimilarity was Euclidean distance and the hierarchical agglomerative clustering method using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) was used to examine the resemblance and grouping of genotypes. Euclidean or straight-line measure of distance was used for estimating genetic distance (GD) among accessions (Mohammadi and Prasanna, 2003). The matrix of average GD between two individuals i and j, having observations on phenotypic characters (p) denoted by x_1, x_2, ..., x_p and y_1, y_2, ..., y_p for i and j, respectively, was calculated using Euclidean distance, where:

$$GD\ (i,j) = [(x1-y1)^2 + (x2-y2)^2 + \ldots (xp-yp)^2]^{1/2}$$

3.2.3.2 Qualitative traits

The Shannon-Weaver diversity index (H') was computed using the phenotypic frequencies to assess the phenotypic diversity for each character for all accessions. This index as described by Perry and Mclntosh (1991) is given as:

$$H' = 1 - \sum_{i=1}^{n} P_i \log_e Pi$$

Where, Pi is the proportion of accessions in the i^{th} class of an n-class character and n is the number of phenotypic classes of traits. Each H' value was divided by its maximum value ($\log_e n$) and normalised in ordered to keep the values between 0 and 1. By pooling various characters across the accessions, the additive properties of H' were used to evaluate diversity of characters between the accessions. The average diversity index ($\overline{H}'$) over n traits was estimated as, $\overline{H}' = \sum \overline{H}' / n$.

3.3 Results and discussion

3.3.1 Quantitative traits

3.3.1.1 Univariate statistics

The mean squares for the accessions as shown in Table 3.4, were highly significant ($p \leq 0.01$) for all phenotypic traits revealing a high level of genetic diversity among them. Of all accessions evaluated, 97MW6129 showed the highest values for number of days to 50% flowering, leaf number, number of internodes, plant height and number of primary braches per panicle. Tall plants easily logde, however, they are very important in areas where people are using them as fire wood, construction of houses, fences and for animal feed. Moreover, selection for tallness was achieved for this accession when yield was not the priority. This variety may produce more dry weight. This accession showed longer flowering but exhibit lower grain yield per plant and moderately light kernels. This might be due to limitations of available resources that influence leaf area leading to a reduction in grain yield and kernel size, that have a great impact on end-use quality and kernel size. This result agrees with what Botha (2002) found. The mean number of primary branches per panicle, a yield component, was also high for accessions IS9302 and M81. The highest number of leaves per plant and number of internodes per plant was also recorded for accessions 97MW6127 and PI308453. Doggett (1988) and Ayana and Bekele (2000) found the longer the duration of the induction of floral primordia the more leaves are formed which causes plants to grow taller and these are more likely to be tall.

Table 3.4 Means, mean squares and least significant difference for the 20 quantitative characters† averaged over two years

No	Accessions	DF	LN	LL	LW	LA	SD	IL	NI	NT	LSL	PH	LPE	PL	PW	PWt	NPBP	GYP	TSW	TP	GNPP
1	216737	86.00	14.00	78.98	11.08	652.32	2.62	15.76	13.00	39.00	23.11	233.90	6.48	22.13	8.78	115.30	54.00	92.15	36.00	79.73	2561.00
2	216743	82.00	13.00	86.62	11.24	727.10	2.56	13.38	12.00	53.00	23.77	198.85	6.23	24.68	7.28	103.50	58.00	80.73	34.00	78.36	2384.00
3	Birmash	82.00	14.00	76.30	10.32	585.85	2.74	10.75	13.00	48.00	20.38	205.07	8.30	34.23	6.63	111.90	64.00	79.87	23.33	71.78	3369.00
4	Gambella1107	76.00	12.00	69.31	10.15	533.00	2.29	9.38	8.00	26.00	17.98	130.75	4.23	27.73	8.08	90.98	60.00	66.08	21.33	72.39	3044.00
5	IS9302	83.00	14.00	74.21	10.83	599.62	2.80	10.11	13.00	37.00	19.53	197.90	8.83	35.56	6.65	112.80	72.00	85.10	24.67	75.53	3427.00
6	Baji	86.00	14.00	77.81	10.50	609.97	2.58	10.86	11.00	39.00	20.10	167.90	4.50	33.15	6.30	105.10	66.00	70.40	25.17	67.74	2751.00
7	97MW6129	100.00	17.00	75.46	9.76	548.83	2.76	11.95	15.00	52.00	20.68	238.10	9.28	34.13	6.48	82.65	72.00	61.00	20.00	73.75	3050.00
8	97MW6127	96.00	17.00	74.93	9.70	544.80	2.78	10.95	15.00	60.00	19.27	230.45	6.12	33.97	6.87	81.60	70.00	58.65	20.33	71.90	2940.00
9	NO253	83.00	15.00	70.79	10.49	554.97	2.68	8.65	13.00	39.00	20.48	193.62	8.35	34.40	6.35	94.20	62.00	71.60	25.33	76.13	2906.00
10	PI308453	95.00	17.00	75.35	9.55	533.55	2.76	9.87	15.00	60.00	19.03	214.60	7.75	33.03	6.33	78.00	64.00	51.80	21.00	66.30	2475.00
11	97MW6113	88.00	15.00	76.52	10.81	617.90	2.86	8.23	13.00	48.00	19.63	199.62	9.44	32.58	6.00	111.20	56.00	81.90	21.50	72.50	3677.00
12	Macia-SA	75.00	12.00	72.06	10.76	580.05	2.70	8.07	9.00	18.00	15.68	129.10	6.25	25.22	9.43	97.95	66.00	75.45	17.00	75.93	4376.00
13	M48	69.00	11.00	72.11	9.42	509.15	2.52	5.27	8.00	41.00	13.92	115.85	9.13	26.13	7.48	75.90	46.00	59.35	24.00	77.55	2455.00
14	M141	76.00	13.00	72.66	10.25	558.37	2.61	7.18	11.00	33.00	17.03	147.43	6.93	29.72	9.32	117.60	54.00	97.55	22.33	83.18	4395.00
15	M81	79.00	12.00	84.95	11.38	722.95	2.58	9.00	9.00	39.00	18.22	155.77	9.22	26.93	9.53	125.40	73.00	95.35	23.33	75.94	4101.00
16	M105	84.00	15.00	77.80	12.18	703.78	2.43	12.93	13.00	35.00	23.10	217.05	4.50	19.62	9.27	135.90	53.00	113.90	29.67	83.78	3833.00
17	M26	74.00	11.00	76.70	9.92	569.92	2.39	8.98	9.00	40.00	16.02	157.08	9.83	28.70	9.37	121.80	51.00	92.85	24.33	75.33	3764.00
18	M101	76.00	12.00	74.01	9.84	547.32	2.57	5.38	8.00	33.00	15.90	113.10	8.43	26.97	7.43	70.30	70.00	51.70	17.33	72.42	2943.00
19	M163	81.00	12.00	81.15	10.77	656.85	2.38	6.47	9.00	57.00	17.16	139.20	16.55	22.38	7.22	75.20	53.00	64.03	29.00	88.63	2234.00
20	Masekaswere	82.00	14.00	65.23	10.39	506.60	2.40	8.93	11.00	32.00	20.95	182.35	11.53	32.20	6.53	89.65	60.00	69.50	29.33	78.21	2394.00
21	Mamolokwane	80.00	15.00	69.50	9.87	515.35	2.10	12.64	13.00	70.00	20.72	234.70	10.30	30.33	7.02	81.95	54.00	66.65	34.17	83.93	1970.00
22	M153	82.00	14.00	85.16	10.47	667.80	2.53	9.63	13.00	40.00	20.26	172.10	6.12	17.95	7.65	91.03	54.00	78.14	32.33	87.59	2433.00
	Mean Squares	34**	1.1**	41**	0.51**	3811**	0.1**	7.2**	2.5**	490**	4.9**	688**	11**	7.2**	1.3**	652**	51**	433**	33**	193**	407562**
	LSD	1.68	0.84	2.32	0.40	29.22	0.22	0.72	1.04	2.02	1.29	7.62	0.75	1.38	0.49	2.45	1.88	2.89	1.96	3.37	295.67
	CV%	1.77	5.29	2.68	3.41	4.29	7.63	6.49	7.82	4.12	5.85	3.68	8.15	4.21	5.75	2.17	2.71	3.34	6.76	3.83	8.40

†Character abbreviations as given in Table 3.2, **p≤0.01

M48 was the earliest maturing accession. Furthermore, accession M48 revealed lower mean values for leaf number, internode length, number of internodes, leaf sheath length and plant height than the other accessions. This accession could be selected for earliness as well as had an opportunity to accumulate genes for short height in areas where moisture stress is prevalent. Moreover, early maturing can also be useful as drought escape mechanism and is suited to drought enviromental conditions. Therefore, this trait is very important in crop improvement programmes. Gebrekidan (1981) reported early flowering and short plant height sorghum types are suitable for lowland areas with limited amount of rainfall and short growing season in Ethiopia. Ayana and Bekele (2000) found similar results in Eritrean sorghum accessions.

Among all accessions, accession 216737 had significantly higher internode length and thousand seed weight than other entries, while accession M101 had significantly lower number of internodes, plant height, panicle weight, thousand seed weight and grain yield per panicle than the other entries. This accession could be also selected for shortness genes. A wide range in thousand seed weight was observed, from 17 g for accession Macia-SA to 36 g for accession 216737. These values were higher than what Kwolek et al. (1986); Ayana and Bekele (2000); Bello et al. (2007) and Chozin (2007) found but similar to what Shakoor (1999) and Yetneberk et al. (2005) found. This accession had large seed size. This finding agrees with those of Kirleis and Crosby (1982) who reported that kernel size has a major influence on thousand seed weight. Moreover, these findings are in line with what Aboubacar et al. (1999) found. Kwolek et al. (1986), Kebede and Menkir (1987) and Ayana and Bekele (2000) reported that large seed size in sorghum is associated with increased germination percentage, improved stand establishment and increased grain yield. Lothrop et al. (1985) and Ayana and Bekele (2000) reported the possibility of improving seed size in sorghum by mass selection.

Both M141 (4395) and Macia-SA (4376) had a significantly higher value for grain number per panicle over the other accessions. The highest panicle weight and grain yield per panicle were observed in accession M105 and the lowest in accession M101.The mean values recorded for the grain yield per panicle in the present study were superior to what Shakoor (1999) and Bello et al. (2007) found. The variability present in the grain yield can be used to separate grain sorghum and fodder sorghum. Higher yield is crucial in crop improvement programmes but it is highly influenced by many contributing traits both in positive and negative directions. Therefore, selection indirectly for improved yield is desirable than direct selection for yield due to its low heritability nature. Mamolokwane had the highest tillering capacity of all accessions, which would be a valuable source of

high tillering traits/genes for the sorghum breeding programme, since it is an important parameter in determining yield. However, this accession showed comparatively lower numbers of grain and the lowest grain yield per panicle compared to the other accessions.This might be due to non fertile tillers and death of tillers. By contrast, a low tillering potential of accession Macia-SA was observed, which could be used in the adverse environmental conditions such as water limited lowland regions of Ethiopia and South Africa.

Accessions from Ethiopia, ICRISAT and PU were characterised by taller plants and later flowering than accessions from South Africa. These accessions mature after the surcease of the rain in lowland areas where the rainfall is high like the north west, western and south west regions of Ethiopia and hence avoid grain damage. Similarly, Morgan and Finlayson (2000) reported that late maturing plants are generally taller than early flowering sorghum plants. On the contrary, the accessions from South Africa were characterised by short plants and early flowering, suggesting the possibility of obtaining genes for early flowering and short plants from these accessions. The accessions possessed high levels of variability for important agronomic traits of primary interest in sorghum breeding programmes, such as days to 50% flowering, plant height, number of tillers, and panicle characteristics. These accessions must be considered as sources of important genes/traits that plant breeders need to exploit and utilise.

In general, the studied sorghum accessions showed significant variation in phenotypic characters, indicating that the accessions had high genetic diversity which should allow development of new genotypes of desired traits through selection and crossing programmes. This is in agreement with previous studies (Kebede, 1991; Ayana and Bekele, 2000; Geleta, 2003; Bello et al., 2007; Chozin, 2007; Bucheyekei, 2009). It would be useful to include some of the tested South African material in the Ethiopian breeding programme to make crosses to improve selected traits, and the same can be done for the South African breeding programme, including Ethiopian material to broaden the genetic base. The Ethiopian material performed very well under South African conditions (comparable to the South African material), and the South African material should therefore also grow well in Ethiopia. This could be to the mutual benefit of sorghum breeding in the two countries.

3.3.1.2 Bivariate statistics

Plant height was significantly and positively correlated with days to 50% flowering, number of leaves per plant, number of internodes, and leaf sheath length (Table 3.5). The positive correlation between plant height and days to 50% flowering was observed in this study, which was also reported in previous studies (Zongo et al., 1993; Ayana, 2001; Kebede et al. 2001) and are genetically controlled and heritable traits that can be transferred into desired genotypes. Positive association between plant height and number of internodes was found. This agrees with what Kumar and Singhania (1984) found. Leaf area was significantly and positively correlated with leaf length and width. Ayana and Bekele (2000) reported that this was due to the functional relationship of leaf area = leaf length × leaf width × 0.75. Leaf number was significantly correlated with days to 50% flowering, internode length, number of internodes, leaf sheath length, plant height, number of tillers, panicle length but negatively correlated with panicle width. Ayana and Bekele (2000) found significant and positive correlation of leaf number with plant height and days to 50% flowering. Quinby (1967) and Hesketh et al. (1969) also reported significant positive correlations between these characters. Hesketh et al. (1969) and Ayana and Bekele (2000) reported that these characters determine the competitive ability of a genotype when grown with other varieties or species.

Length of leaf sheath was positively and significantly correlated with plant height, days to 50% flowering, number of leaves, leaf width, leaf area, internode length and number of internodes. Stalk diameter and leaf sheath length are used as indicators for lodging resistance (Esechie et al., 1977; Ayana and Bekele, 2000) and were positively correlated with height. Stalk diameter was not significantly correlated with plant height in this study, and almost all correlation coefficients involving panicle exertion were negatively correlated.

Table 3.5 Correlation coefficient matrix for 20 phenotypic characters†

No.	Char.	DF	LN	LL	LW	LA	SD	IL	NI	LSL	PH	NT	PL	PW	PWt	LPE	TP	NPBP	TSW	GYP
1	DF	1.00																		
2	LN	0.91**	1.00																	
3	LL	0.14	-0.09	1.00																
4	LW	-0.05	-0.11	0.47**	1.00															
5	LA	0.03	-0.14	0.87**	0.84**	1.00														
6	SD	0.50*	0.40	0.17	0.00	0.07	1.00													
7	IL	0.50*	0.50*	0.26	0.37	0.35	-0.02	1.00												
8	NI	0.84**	0.94**	0.08	0.01	0.03	0.45*	0.62**	1.00											
9	LSL	0.54**	0.58**	0.27	0.51**	0.44**	0.04	0.85**	0.68**	1.00										
10	PH	0.77**	0.84**	0.08	0.13	0.09	0.23	0.81	0.92**	0.79**	1.00									
11	NT	0.52**	0.53**	0.22	-0.29	-0.03	-0.04	0.30	0.55**	0.28	0.59**	1.00								
12	PL	0.39	0.44**	-0.49*	-0.46*	-0.57**	0.42*	-0.06	0.35	-0.03	0.29	0.23	1.00							
13	PW	-0.52**	-.55**	0.20	0.38	0.34	-0.29	0.00**	-0.47*	-0.24	-0.35	-0.50**	-0.60**	1.00						
14	PWt	-0.12	-0.13	0.31	0.71**	0.56**	0.11	0.37	0.05	0.32	0.17	-0.34	-0.12	0.52**	1.00					
15	LPE	-0.08	-0.16	-0.07	-0.16	-0.12	-0.18	-0.39	-0.17	-0.28	-0.10	0.37	0.10	-0.23	-0.33	1.00				
16	TP	-0.33	-0.24	0.22	0.36	0.35	-0.51	0.02	-0.11	0.12	-0.06	0.03	-0.67**	0.37	0.08	0.32	1.00			
17	NPBP	0.44**	0.33	0.00	-0.04	-0.03	0.51*	0.05	0.20	0.05	0.14	-0.06	0.52**	-0.24	-0.08	-0.14	-0.57**	1.00		
18	TSW	-0.04	0.02	0.32	0.41	0.43**	-0.48	0.58**	0.20	0.64**	0.38	0.29	-0.46*	0.03	0.19	0.09	0.63**	-0.53**	1.00	
19	GYP	-0.21	-0.19	0.33	0.76	0.61**	-0.04	0.34	0.01	0.32	0.13	-0.33	-0.34	0.63**	0.95**	-0.24	0.38	-0.26	0.34	1.00
20	GNP	-0.20	-0.21	0.00	0.33	0.17	0.35	-0.19	-0.18	-0.28	-0.23	-0.55	0.06	0.58	0.66	-0.26	-0.13	0.23	-0.54**	0.59**

*p≤ 0.05, **p≤ 0.01, † Character abbreviations as given in Table 3.2

Ayana (2001) also reported negative correlations for peduncle exertion. Grain yield per panicle revealed strong and positive association with leaf area, panicle width and weight. Grain number per panicle was directly and positively correlated to grain yield per panicle, but negatively correlated with thousand seed weight. This negative association might be due to seasonal variation during seed filling and maturation as well as trade off more, smaller seed or fewer larger seed. The positive associations indicate that selecting for grain number per panicle would have a positive effect on panicle related traits particularly providing higher yields. A negative significant correlation was observed among some traits which could be utilised in breeding for negative correlated traits and indicated an inherent association of these traits. High correlation between and among characters may show that the characters share some common genetic and geographical information (Thorpe, 1976; Bekele, 1984; Ayana and Bekele, 2000) as well as pleiotropic and linkge of genes governing the traits. Ayana (2001) reported that correlations among characters are of interest to plant breeders because they help in the identification of easily measured characters that could be used as indicators of more important (but more complex to score) characters. They are also useful in selection of desirable traits (Amurrio et al., 1993; Ayana and Bekele, 2000). Chozin (2007) reported that evaluation of the pattern of variation and genetic relationship among breeding material can facilitate precise identification of genetic divergence and reliable classification of specific heterotic groups, which particularly useful in planning crosses. Grenier et al. (2004) found morphological diversity of sorghum accessions in Sudan and reported the importance of germplasm collection and efficient utilisation of genetic resources.

3.3.1.3 Principal component analysis

The Principal component analysis (PCA) was used as a data reduction tool to summarize the information from the data set so that the influence of noise and outliers on the results is reduced. PCA also decreases the number of descriptors responsible for the highest percentage of total variance of the experimental data. It allows the relationship between variables and observations to be studied, as well as recognizing the data structure. Falcinelli et al. (1988) and Chozin (2007) showed multivariate analyses to be a valid system to deal with germplasm collections and evaluation. Similarly, Dasgupta and Das (1984) and Chozin (2007) considered multivariate analysis best for choosing parents for hybridization. PCA is used to reveal the pattern of character variation among individual accession in a population (Chozin, 2007).

The PCA grouped the 20 phenotypic characters into 20 components, which accounted for the entire (100%) variability among the studied accessions. As Chatfied and Collins (1980) stated, components with an eigenvalue of less than 1 should be eliminated so that fewer components are dealt with. Furthermore, Hair et al. (1998) suggested that eigenvalues greater than one are considered significant and component loadings greater than ±0.3 were considered to be meaningful. Hence, from this study, only the first five eigenvectors which had eigenvalues greater than one and cumulatively explained about 86.53% of the total variation among the accessions was discussed (Table 3.6). The first principal component (PC) alone explained 29.60% of the total variation, mainly due to variation in the leaf number, days to 50% flowering, number of internodes, plant height and panicle width. Characters which contributed more to the second PC accounted for 27.02% of the total variation and was dominated by traits such as leaf width, grain yield per panicle, leaf sheath length, internode length and panicle weight. The third PC with 16.78% of the variation was composed of grain number per panicle, stalk diameter, number of primary branches per panicle, thousand seed weight and threshing percent. Grain number per panicle showed the most variation among the characters in this PC with a high positive loading. Stalk diameter and thousand seed weight had the same weight for this component but in opposite directions. The fourth PC with 7.64% of variance comprised leaf length and leaf area with positive loadings. The eigenvectors of PC5 showed large negative loadings for the length of peduncle exertion which is responsible for 5.50% of total variation. The first and the second PCs explained the most variation among the accessions, revealing a high degree of association among the characters studied.

The existence of wider phenotypic diversity among sorghum accessions studied was further explained by the PCA biplot (Figure 3.1). The PCA biplots provide an overview of the similarities and differences between the quantitative traits of the different accessions and of the interrelationships between the measured variables. The biplot demarcated the accessions with characteristics explained by the first two dimensions. The PCA grouped the accessions into groups over the four quadrants based on the quantitative traits (Figures 3.1, 3.2). The accessions remained scattered in all four quadrants, showing large genetic variability for the traits studied. Accessions which overlapped in the principal component axes had similar relationships in the traits.

Table 3.6 Principal component analysis of 20 quantitative characters in 22 sorghum accessions showing eigenvectors, eigenvalues, individual and cumulative percentage of variation explained by the first five PC axes

Characters	Eigenvectors				
	PC1	PC2	PC3	PC4	PC5
Days to 50% flowering	0.37	0.06	0.08	0.14	-0.00
Leaf number	0.38	0.05	0.04	-0.08	-0.06
Leaf length	-0.00	0.26	-0.00	0.57	0.14
Leaf width	-0.10	0.35	0.11	0.10	-0.05
Leaf area	-0.08	0.35	0.05	0.41	0.07
Stalk diameter	0.17	-0.03	0.36	0.30	-0.09
Internode length	0.21	0.31	-0.02	-0.22	0.22
Number of internodes	0.36	0.14	0.03	-0.06	-0.13
Leaf sheath length	0.23	0.31	-0.05	-0.12	0.13
Plant height	0.34	0.20	-0.02	-0.16	-0.14
Number of tillers	0.26	0.01	-0.27	0.20	-0.23
Panicle length	0.24	-0.21	0.20	-0.15	-0.28
Panicle width	-0.30	0.16	0.12	-0.12	0.02
Panicle weight	-0.10	0.30	0.28	-0.17	-0.23
Length of peduncle exertion	-0.02	-0.12	-0.26	0.27	-0.64
Threshing percent	-0.16	0.19	-0.32	0.00	-0.27
Number of primary braches per panicle	0.16	-0.09	0.32	0.23	0.15
Thousand seed weight	0.02	0.29	-0.36	-0.11	0.03
Grain yield per panicle	-0.15	0.33	0.17	-0.17	-0.28
Grain number per panicle	-0.16	0.05	0.45	-0.06	-0.29
Eigen value	5.92	5.40	3.36	1.53	1.10
Individual %	29.60	27.02	16.78	7.64	5.50
Cumulative %	29.60	56.61	73.39	81.04	86.53

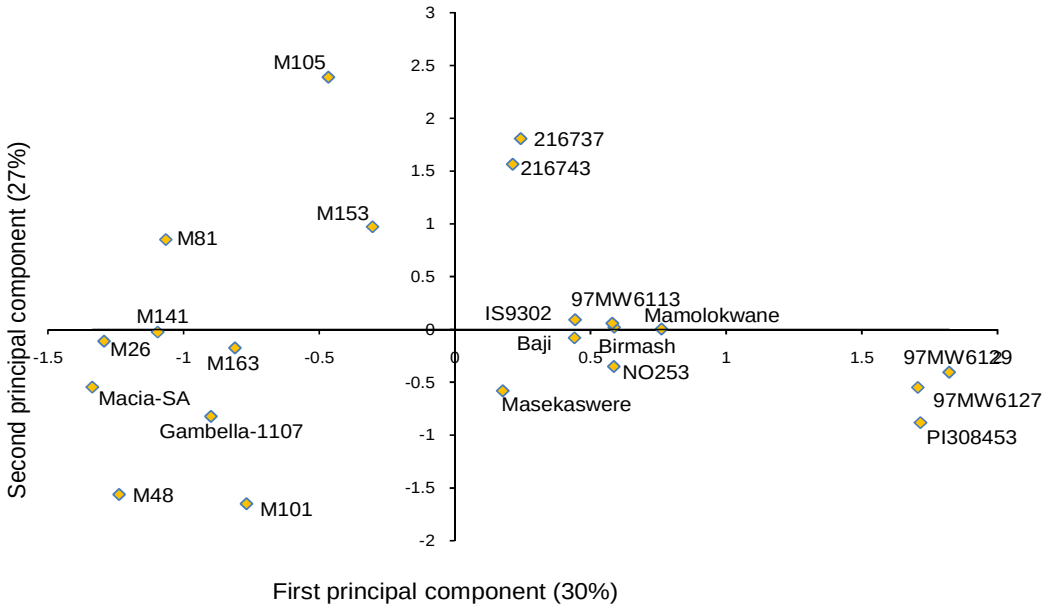

Figure 3.1 Principal component score plot of PC1 and PC2 describing the overall variation among sorghum accessions estimated using phenotypic character data

Both the loading biplot (Figure 3.2) and the correlation matrix (Table 3.5) showed that leaf sheath length, internode length, plant height, number of internodes, days to 50% flowering, number of tillers and leaf number were close to each other. These variables were positively correlated. There was significant negative correlation between days to 50% flowering and panicle width. Panicle width was also negatively correlated with leaf number, number of internodes, number of tillers and panicle length while was positively correlated with internode length. No correlation was observed between length of peduncle exertion and other quantitative traits.

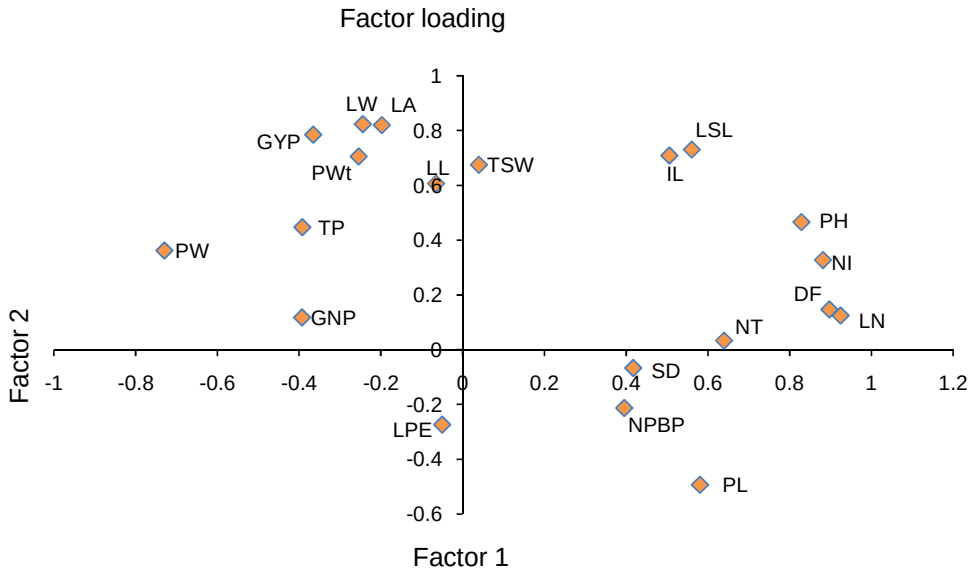

Figure 3.2 PCA loading plot for phenotypic traits of the sorghum accessions. Character abbreviations as given in Table 3.2

3.3.1.4 Genetic distance and cluster analysis

Estimates of genetic distances matrix based on phenotypic characters for all pair-wise combinations of (22 × 21)/2 = 231 for the 22 sorghum accessions are presented in Table 3.7. Genetic distances from 0.38 to 1.59 were observed in the pair-wise combinations, indicating that the accessions were diverse for the phenotypic characters measured. The minimum genetic distance of 0.38 was recorded between accessions 97MW6127 and 97MW6129. On the other hand, the highest genetic distance of 1.59 was recorded between accession M163 (South African) and the rest of the accessions, indicating that there was a high genetic diversity between the accessions. What is interesting is that the genetic distances between the South African material and the Ethiopian material (1.44) compared to distances within South African material (1.41, data not shown), were very similar. Within the Ethiopian material there were 13 genetic distance values of lower than 0.8 indicating some relatedness within the material, with an average distance of 1.16. In the South African material only one value was lower than 0.90. One would have expected that genetic distances from material from countries as far apart as these two countries should have been bigger than genetic diversity within South African material.

Table 3.7 Estimates of genetic distance based on phenotypic characters for all pair-wise comparisons of 22 sorghum accessions

No.	Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	216737	1.00																				
2	216743	0.72	1.00																			
3	Birmash	1.55	1.55	1.00																		
4	Gambella-1107	1.55	1.55	1.48	1.00																	
5	IS9302	1.55	1.55	0.43	1.48	1.00																
6	Baji	1.55	1.55	0.72	1.48	0.72	1.00															
7	97MW6129	1.55	1.55	1.06	1.48	1.06	1.06	1.00														
8	97MW6127	1.55	1.55	1.06	1.48	1.06	1.06	0.38	1.00													
9	NO253	1.55	1.55	0.54	1.48	0.54	0.72	1.06	1.06	1.00												
10	PI308453	1.55	1.55	1.06	1.48	1.06	1.06	0.49	0.49	1.06	1.00											
11	97MW6113	1.55	1.55	0.6	1.48	0.6	0.72	1.06	1.06	0.6	1.06	1.00										
12	Macia-SA	1.55	1.55	1.48	1.24	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.00									
13	M48	1.55	1.55	1.48	0.94	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.24	1.00								
14	M141	1.55	1.55	1.48	1.24	1.48	1.48	1.48	1.48	1.48	1.48	1.48	0.91	1.24	1.00							
15	M81	1.55	1.55	1.48	1.24	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.09	1.24	1.09	1.00						
16	M105	1.09	1.09	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.00					
17	M26	1.55	1.55	1.48	1.24	1.48	1.48	1.48	1.48	1.48	1.48	1.48	0.91	1.24	0.69	1.09	1.55	1.00				
18	M101	1.55	1.55	1.48	0.83	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.24	0.94	1.24	1.24	1.55	1.24	1.00			
19	M163	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.59	1.00		
20	Masekaswere	1.55	1.55	0.97	1.48	0.97	0.97	1.06	1.06	0.97	1.06	0.97	1.48	1.48	1.48	1.48	1.55	1.48	1.48	1.59	1.00	
21	Mamolokwane	1.55	1.55	1.40	1.48	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.48	1.48	1.48	1.48	1.55	1.48	1.48	1.59	1.4	1.00
22	M153	0.89	0.89	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.09	1.55	1.55	1.59	1.55	1.55

The original material from which the cultivars and advanced lines were developed, may have come from the same source. This whole data set confirms that sufficient genetic diversity is present for the measured characteristics, although there is some relatedness in the Ehtiopian material. Material with the biggest genetic distances between them can be included in crossing blocks of breeding programmes in both countries.

Cluster (segmentation) analysis for phenotypic traits shows a clear demarcation between sorghum accessions (Figure 3.3). Furthermore, Table 3.8 showed differences among clusters by summarsing cluster means for the 20 quantitative traits. Based on these traits, the accessions were grouped into different clusters. The dendrogram divided the accessions into three main clusters and a singleton. The first main cluster was produced at a genetic distance of 1.10 and included the accessions 216737, 216743, M153 and M105. Acession M105 was separated within the cluster indicating that it had some differences in the traits. Cluster I characterised by the longest and widest leaves, highest leaf area, the longest internodes, thickest stalks and longest leaf sheath, lowest panicle length and peducle exertion, heaviest panicle and thousand seed as well as grain yield.

The second main cluster was also formed at a genetic distance of 1.40 and comprised of five accessions from Ethiopia, two from South Africa, one from Perdue University and two from ICRISAT. Mamolokwane was separated as a singleton accession from the rest in this cluster. This cluster consisted of ten accessions with the longest days to 50% flowering, highest number of leaves, shortest leaves, narrowest leaves width, smallest leaf area, the highest number of interodes, medium leaf sheath length, tallest plant, longest panicle, narrowest panicle, average panicle weight, lowest threshing percent and highest number of primary branches per panicle.

The third cluster consisted of seven accessions which all originated from South Africa except Gambella-1107 that was from Ethiopia. This indicated that this accession had genetic similarity with the rest of the accessions within the cluster. This cluster grouped the accessions with early flowering, the smallest number of leaves, the smallest number of internodes, shortest leaf sheath and plant height, smallest number of tillers, widest panicle, lowest thousand seed weight and highest grain number.

Cluster IV contained only one accession, M163 and formed at a genetic distance of about 1.58 and was from South Africa. This cluster consisted of an accession with average days to 50% flowering, medium leaf width, shortest internode and small stalk

diameter, highest number of tillers, lightest panicle weight, longest penducle, highest treshing percent, lowest number of primary branches, grain yield and grain number.

Accession M163 was not included in any of the clusters and grouped as a singleton and stood individually as a separate cluster, and this indicates that it was phenotypically dissimilar from the other accessions. Accessions M105, Mamolokwane and M81 were separated within the clusters and hence they are phenotypically dissimilar from the others based on the 20 phenotypic traits recorded. Accession M163 revealed the highest genetic dissimilarity coefficient value of 1.59 and appeared as the most divergent accession. This indicated that the accessions included in this study could be valuable sources of genetic variability in the sorghum improvement programmes. Therefore, the present study showed that the phenotypic traits could classify the accessions according to geographic origin to some extent, by using cluster analysis.

Characterisation of accessions and clustering them on the basis of their morphological traits and genetic similarity will help in identification and selection of the best parents for hybridisation (Souza and Sorrells, 1991). Therefore, the grouping of accessions by multivariate methods of analysis based on their similarity in the present study would be valuable for sorghum breeders in that the most important accessions in the population may be selected from different clusters for improvement programmes.

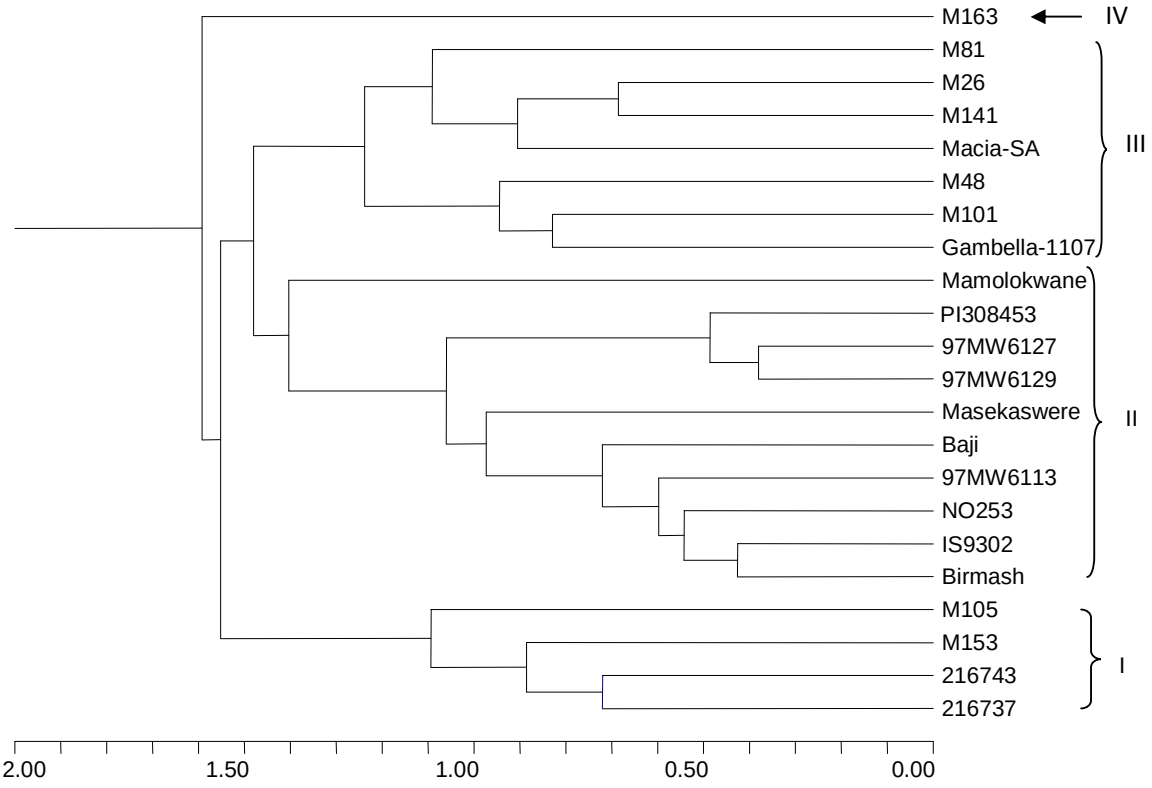

Figure 3.3 Dendrogram of 22 sorghum accessions revealed by UPGMA cluster analysis based on phenotypic data

Table 3.8 The summary of cluster means of 20 quantitative traits for the sorghum accessions based on data set

	Cluster means				
Characters	I	II	III	IV	Mean
Days to 50% flowering	83.50	87.50	75.00	81.00	81.75
Leaf number	14.00	15.20	11.86	12.00	13.27
Leaf length	82.14	73.61	74.54	81.15	77.86
Leaf width	11.24	10.22	10.25	10.77	10.62
Leaf area	687.75	561.74	574.39	656.85	620.18
Internod length	12.93	10.29	7.61	6.47	9.33
Stalk diameter	2.54	2.41	2.52	2.38	2.46
Number of internods	12.75	13.20	8.86	9.00	10.95
Leaf sheath length	22.56	20.01	16.39	17.16	19.03
Plant height	205.48	206.43	135.58	139.20	171.67
Number of tillers	41.75	48.50	32.86	57.00	45.03
Panicle length	21.01	33.36	27.34	22.38	26.02
Panicle width	8.25	6.52	8.67	7.22	7.67
Panicle weight	111.43	94.91	99.99	75.20	95.38
Length of peduncle exertion	5.83	8.44	7.72	16.55	9.64
Threshing percent	82.37	73.78	76.12	88.63	80.23
Number of primary branches per panicle	54.75	64.00	60.00	53.00	57.94
Thousand seed weight	33.00	24.48	21.38	29.00	26.97
Grain yield per panicle	91.23	69.65	76.90	64.03	75.45
Grain number per panicle	2802.75	2895.90	3582.57	2234.00	2878.81

3.3.2 Qualitative characters

Estimates of phenotypic diversity [Shannon Weaver diversity index as described by Perry and Mclnntosh (1991)] for individual qualitative traits over all accessions are shown in Table 3.9. Individual traits showed a different pattern of variation among accessions. Estimates of phenotypic diversity indices (H') for individual traits varied from 0.31 for panicle compactness and shape to 0.84 for glume colour with an overall mean phenotypic diversity index of 0.59. Panicle compactness and shape, plant colour and grain luster were monomorphic, while glume colour, grain colour and grain size were polymorphic and leaf mid rib colour and grain covering were relatively polymorphic. The diversity values for the characters showed a wide variability among them. Thus, the diversity among accessions varied depending on the characters. According to Brown and Weir (1983) this index is used in genetic resource studies as a convenient measure of both allelic richness and allelic evenness when using genetic data, but because of the log transformation it is not readily interpretable in genetic terms. A low H' indicates

extremely unbalanced frequency classes for an individual trait and lack of genetic diversity.

Table 3.9 Estimates of diversity indices for qualitative traits from different localities among sorghum accessions

Qualitative traits	Diversity index (H')
Leaf mid rib color	0.61
Plant color	0.46
Glume color	0.84
Panicle compactness and shape	0.31
Grain size	0.71
Grain shape	0.52
Grain luster	0.48
Grain covering	0.60
Grain color	0.79
Average diversity index	0.59

3.4 Conclusions

A total of 22 sorghum accessions were evaluated for 20 quantitative and nine qualitative traits to determine the extent of phenotypic diversity. The ANOVA identified the relative importance of each of the quantitative traits. The traits substantially contributed to differentiating the accessions studied. Moreover, the simple correlations between each pair of quantitative characters recorded, clearly depicted the close association between some traits. Selection of secondary highly associated characteristics can be used to improve important primary characteristics. Subjecting the 20 quantitative traits to multivariate analysis provided supporting results to univariate and bivariate analysis. High genetic distances were observed among some accessions, although differences within South African material, and between South African and Ethiopian material were much the same. It showed a possibility of the varieties to form heterotic groups for improving these phenotypic traits in breeding programmes of both South Arica and Ethiopia. Genetic distance is an important parameter in selecting parents for breeding. Cluster analysis grouped the accessions into three main clusters and a singleton. All the accessions were distinctly separated from each other and the accessions with similar morphological characters grouped together. Phenotypic variation for nine qualitative

(categorical) traits was estimated using the Shannon-Weaver diversity index. High and comparable levels of phenotypic diversity were found among the accessions. Based on the observed variation both for quantitative and qualitative traits, it could be concluded that studying the phenotypic diversity among sorghum accessions is important to identify the genetic potential of parental lines and increase the efficiency of the sorghum breeding programmes. Thus all the accessions are phenotypically variable and phenotypic markers can be used to distinguish accessions.

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CHAPTER 4

ASSESSMENT OF GENETIC DIVERSITY IN SORGHUM ACCESSIONS USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) ANALYSIS

Abstract

This study was conducted to determine the genetic relationships of 46 sorghum accessions collected from the north-western, western and central parts of Ethiopia as well as South Africa. Thirty of the accessions were landraces from Ethiopia. They were evaluated for genetic diversity using six AFLP primer combinations. Dice similarity coefficients were calculated and a dendrogram constructed following the UPGMA method of cluster analysis. A total of 186 fragments were amplified of which 78 (43.10%) were polymorphic among accessions. The number of polymorphic fragments amplified per primer combination varied from 9 to 21. Genetic polymorphism present among sorghum accessions was low, as evidenced by the high level of similarity in the AFLP marker profiles of different sorghum accessions. Pair-wise genetic similarity coefficients ranged from 0.87 to 0.99**,** with an average of 0.92. This indicated low levels of genetic diversity among tested sorghum accessions. The landraces were genetically very similar, while the differences between landraces and the Ethiopian cultivars and the South African material were somewhat higher. Genetic similarity within the South African pure breeding lines and within the Ethiopian cultivars was very high. Almost all accessions clustered according to geographical origin. Results of this study indicated that the landraces were related, and were probably exchanged between farmers in the collection regions, although no duplications were found in the material. The similarity between the Ethiopian and South African material was also high, indicating that there must have been a common source of material somewhere in the history of the breeding programmes.

4.1 Introduction

The eastern African region (Abyssinia), , is described as one of the centres of diversity (Vavilov, 1926; Ghebru et al., 2002; Zidenga, 2004) and a possible area of domestication for sorghum (Doggett, 1965). Worede (1988) reported on the existence of high levels of genetic variability of crops such as sorghum, wheat and teff in Abyssinia. Recent studies using molecular markers have also confirmed that the central and north-eastern regions of Africa were the principal areas of sorghum domestication (Deu et al., 1994).

Assessment of sorghum genetic diversity and the evolutionary relationship among and within accessions are crucial for sorghum improvement programmes (Dean et al., 1999; Abu Assar et al., 2005) and high levels of variation were reported from Ethiopia (Ayana and Bekele, 1998; 1999; 2000). Since Ethiopia is within the geographical range where sorghum has originated, the greatest genetic diversity for cultivated and wild sorghum was found there (Doggett, 1988). Grenier et al. (2004) found that diversity of sorghum appears to be correlated with duration of domestication and adaptation zones.

DNA markers have been applied to assess and characterise genetic variation within and among crop species and can help in generating new sources of genetic variability by adding new characters of importance and superior recombinants in crop species through MAS (Menz et al., 2004; Todorovska et al., 2005; Kumar et al., 2008). Genetic erosion resulted in loss of biodiversity which has emphasised the importance of characterising germplasm accessions, including locally adapted landraces and other plant materials for genetic resource conservation (FAO 1998; Todorovska et al., 2005). Hence, consideration must be given to evaluate genetic resources such as landraces as well as breeding material at DNA level for evaluation, maintenance and improvement of genetic diversity (Todorovska et al., 2005).

Conservation of genetic diversity within a species and among its populations is very important to be taken into consideration to achieve genetic gain towards targeted goals (Gray, 1996). Conventional plant breeding is time consuming and highly dependent on environmental conditions. The application of molecular markers is crucial and more efficient for selection in breeding programmes as well as to assess genetic diversity amongst since these markers are not influenced by the environment (Fufa et al., 2005; Geleta et al., 2006; Shehzad et al., 2009). Molecular markers play a vital role in the determination of genetic relationships and different kinds of markers have been used in many studies of sorghum (Ritter et al., 2007; Mehmood et al., 2008; Shehzad et al.,

2009) for example RFLPs (Tao et al., 1993; Deu et al., 1994; Ahnert et al., 1996), RAPDs (Tao et al., 1993; Ayana et al., 2000b; Uptmoor et al., 2003; Jeya Prakash et al., 2006; Mehmood et al., 2008; Iqbal et al., 2010), SSRs (Smith et al., 2000; Ghebru et al., 2002; Uptmoor et al., 2003; Anas and Yoshida, 2004; Menz et al., 2004; Casa et al., 2005; Folkertsma et al., 2005; Abu Assar et al., 2009; Bucheyeki et al., 2009) and AFLPs (Uptmoor et al., 2003; Menz et al., 2004; Ritter et al., 2007). These marker systems have been used succesfully to characterise and estimate the genetic diversity in sorghum germplasm. Therefore, the present study was designed to assess the biodiversity of sorghum accessions from the north-western, western and central parts of Ethiopia as well as from South Africa, using AFLP analysis.

4.2 Materials and methods

The sorghum accessions used were obtained from the IBC/Ethiopia and were collected from the north-western, western and central parts of Ethiopia. Eleven accessions from South Africa were included for comparison purposes (Table 4.1).

4.2.1 DNA isolation

The leaf material was harvested from three, three week-old plants of each sorghum accession. Young harvested leaves were freeze-dried and then ground to a fine powder using a Qiagen TissueLyser. The total genomic DNA was isolated from the tissuelysed material using the CTAB (hexadecyltrimethylammonium bromide) method (Saghai-Maroof et al., 1984). A volume of 750 µl CTAB buffer [100 mM tris hydroxymethyl aminomethane, pH 8.0); 20 mM EDTA (ethylene-diaminetetra acetate), pH 8.0; 1.4 M NaCl; 2% (w/v) CTAB; 0.2% (v/v) β-Mercaptho-ethanol] was added to approximately 250 µl fine leaf powder in a 1.5 ml microfuge tube and incubated in a water bath at 65^{o}C for 1 h. The suspension was extracted with 500 µl chloroform: isoamylalcohol [24:1 (v/v)] and the phases separated by centrifugation at 12000 *g* for 3 min. DNA was precipitated from the aqueous phase with 0.66 volumes isopropanol at room temperature for 20 min and centrifuged at 12000 *g* for 10 min.

Table 4.1 List of sorghum accessions, collection sites and the status of accessions used for AFLP analysis

No.	Acc. No./Name*	Major agro-ecology	Region	Zone	District	Adaptation zone	Status
1	69029	North-west	BGR	Metekel	Dibate	Lowland	Landrace
2	69030	North-west	BGR	Metekel	Dibate	Lowland	Landrace
3	69032	North-west	BGR	Metekel	Dangur	Lowland	Landrace
4	69128	West	BGR	Assosa	Assosa	Lowland	Landrace
5	69147	West	BGR	Metekel	Wenbera	Intermediate	Landrace
6	69164	West	BGR	Metekel	Wenbera	Lowland	Landrace
7	69165	West	BGR	Metekel	Dangur	Intermediate	Landrace
8	69538	West	Oromya	Illubabor	Yayu	Intermediate	Landrace
9	216737	West	Gambella	Zone 1	Itang	Lowland	Landrace
10	216743	West	Gambella	Zone 2	Abobo	Lowland	Landrace
11	223525	West	Oromiya	W.Wollega	Ghimbi	Lowland	Landrace
12	223543	West	Oromiya	Illubabor	Bure	Intermediate	Landrace
13	223548	West	Oromiya	Illubabor	Yayu	Lowland	Landrace
14	223551	West	Oromiya	Illubabor	Yayu	Lowland	Landrace
15	223552	West	Oromiya	Illubabor	Dedesa	Intermediate	Landrace
16	223554	West	Oromiya	Illubabor	Dedesa	Intermediate	Landrace
17	223555	West	Oromiya	Illubabor	Bedele	Intermediate	Landrace
18	223558	West	Oromiya	Jimma	Kerssa	Intermediate	Landrace
19	228736	Central	Oromiya	West Showa	Ambo	Lowland	Landrace
20	228739	Central	Oromiya	West Showa	Ambo	Lowland	Landrace
21	228740	Central	Oromiya	West Showa	Ambo	Lowland	Landrace
22	228741	Central	Oromiya	West Showa	Ambo	Lowland	Landrace
23	228919	West	Oromiya	Illubabor	Bure	Intermediate	Landrace
24	229831	Noth-west	BGRS	Metekel	Mandura	Lowland	Landrace
25	229834	Noth-west	BGRS	Metekel	Mandura	Lowland	Landrace
26	229835	Noth-west	BGRS	Metekel	Mandura	Lowland	Landrace
27	229838	Noth-west	BGRS	Metekel	Dibate	Lowland	Landrace
28	237762	Central	Oromiya	West Showa	Bako Tibe	Intermediate	Landrace
29	237763	Central	Oromiya	West Showa	Bako Tibe	Intermediate	Landrace
30	237779	West	Oromiya	West Wellega	Ghimbi	Lowland	Landrace
31	Geremw	Central	Oromiya	East Showa	Adama	Intermediate	Cultivar
32	97MW6129	Central	Oromiya	East Showa	Adama	Intermediate	Cultivar
33	97MW6127	Central	Oromiya	East Showa	Adama	Intermediate	Cultivar
34	NO253	N/a	ICRISAT	ICRISAT	N/a	Intermediate	Cultivar
35	PI308453	N/a	PU	PU	N/a	Intermediate	Cultivar
36	97MW6113	Central	Oromiya	East Showa	Adama	Intermediate	Cultivar
37	Macia-SA	North-west	North-west	North-west	Potchefstroom	Dry	Cultivar
38	M48	North-west	North-west	North-west	Potchefstroom	Dry	Cultivar
39	M141	North-west	North-west	North-west	Potchefstroom	Dry	Breeding line
40	M81	North-west	North-west	North-west	Potchefstroom	Dry	Breeding line
41	M105	North-west	North-west	North-west	Potchefstroom	Dry	Cultivar
42	M101	North-west	North-west	North-west	Potchefstroom	Dry	Breeding line
43	M163	North-west	North-west	North-west	Potchefstroom	Dry	Breeding line
44	Masekaswere	North-west	North-west	Limpopo	Potchefstroom	Dry	Landrace
45	Mamolokwane	North-west	North-west	Limpopo	Sekhukhune	Dry	Landrace
46	M153	North-west	North-west	North west	Sekhukhune	Dry	Cultivar

Acc.= name of accessions, *accession numbers/name as obtained from the Institute of Biodiversity, Ethiopia, and the ARC-GCI, South Africa, BGRS=Benishangul-Gumuz Regional State, MARC=Melkassa Agricultural Research Center, ICRISAT= International Crops Research Institute for the Semi-Arid Tropics, PU=Purdue University, N/a=Not available,

The precipitate was washed at room temperature with 500 µl 70% (v/v) ethanol for 20 min followed by centrifugation at 12000 *g* for 5 min. The pellet was air-dried for 1 h and resuspended in TE buffer (10 mM tris hydroxymethyl aminomethane, pH 8.0; 1 mM EDTA, pH 8.0). Resuspended DNA was precipitated with 0.75 M ammonium acetate and an equal volume chloroform: isoamylalcohol [24:1 (v/v)].

DNA was precipitated from the aqueous layer with two volumes of ice-cold absolute ethanol. After an overnight incubation at -20^{o}C, DNA was recovered by centrifugation at 12000 *g* for 15 min and washed twice with ice-cold 70% (v/v) ethanol for 5 min. The pellet was air-dried and resuspended in TE buffer and treated with 0.1 µg µl^{-1} DNase-free RNase for 2 h at 37^{o}C. DNA quantity and quality were estimated using a UV spectrophotometer by measuring absorbances at A_{260} and A_{280}. DNA samples were diluted to a working solution of 200 ng µl^{-1}.

4.2.2 AFLP analysis

AFLP analysis was performed using six primer pair combinations (Table 4.2). *Mse*I-primers were screened in combination with *Eco*RI-primers (*Eco*RI and *Mse*I primers were given names beginning with E and M, respectively. The code following E or M refers to the three selective nucleotides at the 3'-end of the primer. This coding system was used throughout). Primers and adapters were synthesized by Integrated DNA Technologies Inc. (Coralville, USA). Oligonucleotides used for adapters were PAGE (polyacrylamide gel electrophoresis) purified. Adapters were prepared by adding equimolar amounts of both strands, heating for 10 min to 65^{o}C in a water bath and then leaving the mixture to cool down to room temperature. AFLP analysis were performed as described by Vos et al. (1995) and modified by Herselman (2003).

Table 4.2 *Eco*RI and *Mse*I adapter, primer+1 and primer+3 sequences used in AFLP analysis

Enzyme	Type	Sequence (5'-3')
*Eco*RI	Adapter-F	CTCGTAGACTGCGTACC
	Adapter-R	AATTGGACGCAGTCTAC
*Mse*I	Adapter-F	GACGATGAGTCCTGAG
	Adapter-R	TACTCAGGACTCAT
*Eco*RI	Primer+1	GACTGCGTACCAATTCA
	Primer+3	GACTGCGTACCAATTCACA
*Mse*I	Primer+1	GATGAGTCCTGAGTAAC
	Primer+3	GATGAGTCCTGAGTAACNN
		CNN=CAC, CAG, CTA, CTC, CTG, CTT

4.2.2.1 Restriction digestion and ligation

Genomic DNA (±1.0 µg) was digested using 4 U of *Mse*I (New England Prolabs) and 1x *Mse*I-buffer [50 mM NaCl; 10 mM tris hydroxymethyl aminomethane, pH 7.9; 10 mM $MgCl_2$; 0.1 mM DTT (dithiotreitol)] in a final volume of 50 µl for 5 h at 37^{o}C. Following *Mse*I digestion, DNA was further digested overnight at 37^{o}C with 5U *Eco*RI and NaCl to a final concentration of 100 mM. Adapter ligation of the digested DNA was obtained by adding a solution containing 50 pmol *Mse*I-adapter, 5 pmol *Eco*RI-adapter, 1 U T4 DNA Ligase (USB Corporation), 0.4 mM ATP (adenosinetriphosphate) and 1x T4 DNA ligase buffer (66 mM tris hydroxymethyl aminomethane, pH 7.6; 6.6 mM $MgCl_2$; 10 mM DTT; 66 mM ATP) followed by overnight incubation at 16^{o}C.

4.2.2.2 Pre-amplification reactions

Pre-amplification reactions were carried out in 50 µl reaction mixtures containing 5 µl template DNA (restriction/ligation mixture), 30 ng of each pre-amplification primer (*Eco*RI- and *Mse*I-primer+1) (Table 4.2), 1 x Promega *Taq* polymerase buffer (10mM tris hydroxymethyl aminomethane, pH 9.0; 50 mM KCl; 0.1% (v/v) Trition x-100), 2 mM $MgCl_2$, 200 µM of each dNTP and 1 U *Taq* DNA polymerase (Promega, Madison, WI, USA). Amplifications were performed using the following cycling programme: 5 min at 94^{o}C, 30 cycles of 30 s at 94^{o}C, 60 s at 56^{o}C and 60 s at 72^{o}C and a final elongation of 10 min at 72^{o}C. Quality and quantity of pre-amplification reactions were determined by

electrophoresis in 1.5% (w/v) agarose gels and diluted accordingly (1:5 to 1:15 times) prior to selective amplification.

4.2.2.3 Selective amplification

Selective amplification reactions were performed in a total volume of 20 µl reaction containing 5 µl of diluted pre-amplification product, 1x Promega *Taq* polymerase buffer, 2 mM $MgCl_2$, 200 µM of each dNTP, 100 µg ml^{-1} bovine serum albumin, 30 ng *Mse*I-primer+3, 30 ng *Eco*RI-primer+3 and 0.75 U Promega *Taq* DNA polymerase. The selective amplification cycling programme consisted of: one cycle of denaturation at 94^{o}C for 5 min followed by one cycle of 30 s at 94^{o}C, 30 s at 65^{o}C and 60 s at 72^{o}C. The annealing temperature was reduced by 1^{o}C per cycle during the next eight cycles after which 25 cycles were performed at 94^{o}C for 30 s, 56^{o}C for 30 s and 72^{o}C for 60 s followed by one last elongation of 5 min at 72^{o}C. AFLP products were separated in denaturing polyacrylamide gels and DNA fragments visualised using silver staining.

4.2.3 Gel electrophoresis

Polymerase chain reaction (PCR) products were mixed with 20 µl formamide dye [98% (v/v) de-ionized formamide; 10 mM EDTA, pH 8.0; 0.05% (w/v) bromophenol blue; 0.05% (w/v) xylene cyanol] and denatured by incubation for 5 min at 95^{o}C. Mixtures were immediately placed on ice prior to loading. The PCR products (2.5 µl) were separated on 5% (w/v) denaturing polyacrylamide gels [19:1 acrylamide:bis-acrylamide; 7 M urea; 1x TBE buffer (89 mM Tris-borate; 2.0 mM EDTA)]. Electrophoresis was performed at constant power of 80 W for approximately 2 h.

4.2.4 Silver staining for DNA visualisation

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 5 s before being immersed in a cold (4 to 10^{o}C) developing solution [3% (w/v) sodium carbonate; 0.056% (v/v) formaldehyde and 0.002 mg ml^{-1} thiosulphate] solution. Gels were shaken manually in the developer until DNA fragments became visible. The

10% acetic acid was used to stop the developing process and shaking continued for a further 2 to 3 min. The gel was rinsed in de-ionised water and left upright to dry overnight at room temperature. A photograph of the gel was taken by exposing the photographic paper (Ilford multigrade IV RC de Luxe) directly under the gel to dim light for 20 s. This produced a negative image of the same size as of the gel.

4.2.5 Data analysis

A binary matrix of specific AFLP fragments as present (1) or absent (0) was generated for each accession. Only reliable (between 300 and 700 bp) and repeatable bands (at least three repetitions) were considered. Pairwise genetic distances were expressed as the complement of the Dice coefficient (Dice, 1945). Cluster analyses were performed using UPGMA (unweighted pair-group method using arithmetic averages; Sokal and Michener, 1958) analysis. Statistical analyses were performed using NTSYS-pc version 2.21c (Exeter Software, NY, USA). Dendrograms were created using the SAHN (Sequencial Agglomerative Hierarchical Nested) programme of NTSYS and goodness of fit of clustering to data matrixes was calculated using COPH and MXCOMP programmes and correlated with the original distance matrices in order to test for the association between the cluster in the dendrogram and the Dice matrix. Principal co-ordinate analysis (PCoA) employed the DCENTER and EIGEN procedures of NTSYS-pc.

AFLP data were evaluated using Shannon Weaver diversity index (H') and polymorphic information content (PIC). Shannon Weaver diversity index was calculated over all loci as described by Perry and Mclntosh (1991) see Chapter 3, section 3.2.3.2. The PIC for each primer combination was calculated, to know its capablility of making distinctions, assess the quality of markers and to compare the effectiveness of each enzyme primer combination in rendering genetic information (Lanteri et al., 2004). PIC was calculated according to Riek et al. (2001) for the dominant marker as follows: $PIC = 1- [f^2 + (1- f)^2]$ where f is the frequency of the marker in the data set. PIC values were averaged to provide PIC value for a primer-pair.

4.3 Results and discussion

4.3.1 Genetic information of AFLP markers

A total of 186 fragments were amplified using six AFLP primer combinations. The number of scorable fragments amplified by each AFLP primer combination varied from 22 for E-ACA/M-CAG to 36 for E-ACA/M-CTT with an average value of 31 per primer combination (Table 4.3). That was lower than the average value of 39.3 obtained by Uptmoor et al. (2003) using 28 AFLP primer combinations.

Table 4.3. Genetic information generated by six AFLP primer combinations using 46 sorghum accessions

Primer	TNF	NPF	MF	P (%)	PIC	H'
E-ACA/M-CAG	22	15	7	68.18	0.256	0.209
E-ACA/M-CTC	35	21	14	60.00	0.222	0.206
E-ACA/ M-CTT	36	12	24	33.33	0.139	0.203
E-ACA/ M-CTG	34	10	24	29.41	0.221	0.251
E-ACA/ M-CTA	29	9	20	31.03	0.123	0.146
E-ACA/M-CAC	30	11	19	36.67	0.106	0.123
Total	186	78	108			
Average	31	13	18	43.10	0.178	0.190

TNF=total number of fragments; NPF=number of polymorphic fragments; MF=monomorphic fragments;
P (%) = percentage polymorphism; PIC=polymorphic information content; H'=Shannon Weaver diversity index

A total of 78 polymorphic fragments were scored between the different sorghum accessions with the number of polymorphic fragments for each primer pair ranging from nine (31.03%) for E-ACA/M-CTA to 21 (60%) for E-ACA/M-CTC with an average value of 13 polymorphic fragments per primer combination (43.10%). A total of 108 (18%) monomorphic fragments were also detected. The E-ACA/M-CAG primer combination amplified the lowest bands (22) but at a higher rate of polymorphism (68.18%). The level of polymorphism (%) was calculated as the ratio of the number of polymorphic amplified fragments to the total number of detected fragments. This formula as described by Shevchuk et al. (2009) is given as $P=n_p/(n_p+n_{np}) \times 100\%$, where n_p is the number of polymorphic PCR fragments and n_{np} is the non-polymorphic PCR fragments.

The smaller number of fragments per primer combination detected in this study compared to previous studies may be due to the smaller number of primers used. Ritter et al. (2007) reported an average value of 17 polymorphic fragments using 16 AFLP primer combinations on 95 sorghum lines that was higher than the values reported in this

study. Furthermore, of the 598 scored fragments, the 277 (46%) polymorphic fragments detected that was almost similar to the value obtained in the present study. Perumal et al. (2007) reported 30.53% polymorphic fragments using 16 AFLP primer combination using 46 converted sorghum lines which was lower than the value obtained in the present study. Uptmoor et al. (2003) also reported 61.80% polymorphic fragments among 46 South African sorghum accessions using AFLP markers and Geleta (2003) reported 85% using 45 sorghum accessions collected from the eastern parts of Ethiopia using eight primer combinations. Ayana et al. (2000a) detected 69% polymorphic fragments among 93 individuals representing 11 wild sorghum populations in Ethiopia using RAPD.

In the present study, primer combination E-ACA/M-CAG was highly discriminative compared to other enzyme primer combinations. The PIC and Shannon diversity index values for each primer combinations ranged from 0.106 to 0.256 with over all average of 0.178 and 0.123 to 0.251 with an over all mean of 0.190, respectively (Table 4.3). Primer enzyme combinations E-ACA/M-CAG revealed the highest PIC value which indicated its usefulness in differentiating individuals and demonstrated high information content compared to other combinations. The number and frequency of the fragments affected the PIC values of the informativeness of the markers. The highest H' value of 0.251 was recorded for primer combination E-ACA/M-CTG. Primer combinations E-ACA/M-CAC showed the lowest values for PIC and H'. In the present study, the low levels of H' values indicated that there was low genetic diversity detected among the accessions tested. Genetic polymorphism present among sorghum accessions was low, as evidenced by the high level of similarity in the AFLP marker profiles of different sorghum accessions. The narrow genetic diversity detected in the present study may contribute the use of some sorghum accessions as parents in the breeding programme to produce desirable and interesting qualitative traits and their adaptability to various agro-ecological conditions in both Ethiopia and South Africa.

4.3.2 AFLP genetic distance similarity and cluster analysis

Estimates of genetic similarity matrices based on the AFLP molecular marker data for all pairwise combinations of the 46 sorghum accessions are presented in Table 4.4. The genetic similarity varied from 0.87 to 0.99. The high levels of genetic similarity indicated that accessions were related and the variation was limited.

Table 4.4 Genetic similarity among 46 sorghum accessions generated using six AFLP primer combinations based on Dice's similarity coefficient

No.	Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
1	69029	1.00																																												
2	69030	0.99	1.00																																												
3	69032	0.98	0.98	1.00																																											
4	69128	0.94	0.94	0.95	1.00																																									
5	69147	0.91	0.91	0.92	0.89	1.00																																								
6	69164	0.93	0.93	0.94	0.92	0.94	1.00																																							
7	69165	0.93	0.93	0.94	0.92	0.91	0.94	1.00																																						
8	69538	0.97	0.97	0.98	0.95	0.91	0.92	0.93	1.00																																					
9	216737	0.87	0.87	0.88	0.85	0.92	0.90	0.90	0.88	1.00																																				
10	216743	0.90	0.91	0.92	0.89	0.95	0.92	0.91	0.92	0.94	1.00																																			
11	223525	0.90	0.91	0.91	0.90	0.92	0.90	0.90	0.93	0.91	0.91	1.00																																		
12	223543	0.89	0.90	0.90	0.91	0.93	0.91	0.91	0.92	0.91	0.93	0.94	1.00																																	
13	223548	0.90	0.90	0.92	0.89	0.97	0.94	0.92	0.93	0.92	0.96	0.93	0.95	1.00																																
14	223551	0.93	0.92	0.93	0.90	0.87	0.89	0.89	0.94	0.90	0.87	0.94	0.89	0.89	1.00																															
15	223552	0.98	0.97	0.97	0.93	0.92	0.94	0.93	0.97	0.89	0.91	0.91	0.89	0.91	0.94	1.00																														
16	223554	0.97	0.96	0.96	0.93	0.91	0.93	0.92	0.96	0.88	0.90	0.91	0.89	0.91	0.94	0.99	1.00																													
17	223555	0.97	0.97	0.96	0.94	0.92	0.93	0.92	0.96	0.88	0.91	0.92	0.90	0.91	0.93	0.99	0.99	1.00																												
18	223558	0.93	0.93	0.93	0.92	0.93	0.93	0.93	0.95	0.92	0.92	0.94	0.93	0.94	0.91	0.95	0.94	0.94	1.00																											
19	228736	0.91	0.92	0.92	0.90	0.93	0.91	0.90	0.92	0.93	0.93	0.91	0.95	0.93	0.89	0.91	0.90	0.91	0.93	1.00																										
20	228739	0.97	0.96	0.96	0.93	0.90	0.92	0.92	0.97	0.87	0.89	0.93	0.91	0.91	0.96	0.97	0.97	0.96	0.94	0.91	1.00																									
21	228740	0.97	0.96	0.97	0.95	0.92	0.92	0.93	0.98	0.90	0.91	0.93	0.93	0.93	0.98	0.97	0.98	0.98	0.95	0.92	0.99	1.00																								
22	228741	0.96	0.94	0.95	0.92	0.90	0.91	0.91	0.97	0.88	0.90	0.92	0.92	0.92	0.97	0.96	0.96	0.95	0.93	0.91	0.99	0.99	1.00																								
23	228919	0.96	0.95	0.95	0.93	0.90	0.91	0.91	0.96	0.86	0.89	0.92	0.91	0.91	0.95	0.96	0.96	0.96	0.93	0.92	0.98	0.98	0.97	1.00																							
24	229831	0.89	0.89	0.90	0.88	0.94	0.92	0.91	0.90	0.92	0.94	0.90	0.95	0.95	0.87	0.89	0.89	0.90	0.92	0.93	0.89	0.92	0.89	0.89	1.00																						
25	229834	0.95	0.93	0.94	0.92	0.89	0.91	0.93	0.93	0.88	0.88	0.92	0.90	0.89	0.93	0.93	0.93	0.93	0.92	0.91	0.95	0.97	0.94	0.95	0.89	1.00																					
26	229835	0.95	0.94	0.94	0.92	0.92	0.93	0.94	0.95	0.88	0.91	0.90	0.92	0.93	0.91	0.94	0.94	0.94	0.93	0.93	0.95	0.95	0.94	0.94	0.92	0.95	1.00																				
27	229838	0.92	0.92	0.92	0.92	0.94	0.96	0.94	0.93	0.90	0.92	0.91	0.92	0.93	0.88	0.93	0.92	0.93	0.94	0.92	0.91	0.93	0.91	0.91	0.92	0.92	0.94	1.00																		
28	237762	0.88	0.89	0.89	0.87	0.93	0.89	0.90	0.91	0.92	0.92	0.93	0.94	0.95	0.88	0.89	0.89	0.90	0.94	0.93	0.91	0.92	0.91	0.91	0.94	0.91	0.90	0.92	1.00																
29	237763	0.94	0.93	0.94	0.90	0.88	0.89	0.89	0.96	0.87	0.88	0.92	0.91	0.89	0.97	0.94	0.94	0.93	0.91	0.90	0.97	0.99	0.98	0.95	0.89	0.94	0.92	0.89	0.91	1.00															
30	237779	0.88	0.90	0.90	0.88	0.95	0.92	0.91	0.91	0.91	0.95	0.94	0.94	0.96	0.88	0.90	0.90	0.91	0.94	0.92	0.90	0.91	0.90	0.90	0.94	0.89	0.91	0.92	0.95	0.89	1.00														
31	Geremw	0.91	0.91	0.91	0.90	0.92	0.93	0.92	0.93	0.90	0.93	0.90	0.90	0.93	0.88	0.91	0.91	0.93	0.91	0.89	0.90	0.92	0.89	0.90	0.92	0.90	0.92	0.92	0.89	0.88	0.93	1.00													
32	97MW6129	0.89	0.88	0.89	0.88	0.90	0.89	0.91	0.90	0.91	0.92	0.89	0.92	0.91	0.90	0.89	0.89	0.89	0.90	0.92	0.90	0.93	0.90	0.91	0.92	0.93	0.92	0.90	0.92	0.90	0.90	0.91	1.00												
33	97MW6127	0.89	0.88	0.89	0.88	0.89	0.88	0.92	0.90	0.91	0.90	0.90	0.90	0.90	0.88	0.89	0.89	0.89	0.89	0.91	0.89	0.92	0.89	0.91	0.90	0.93	0.90	0.90	0.92	0.92	0.88	0.90	0.98	1.00											
34	NO253	0.89	0.88	0.88	0.86	0.87	0.88	0.89	0.90	0.89	0.89	0.89	0.90	0.88	0.88	0.89	0.89	0.89	0.88	0.91	0.88	0.91	0.89	0.90	0.91	0.92	0.90	0.89	0.90	0.91	0.87	0.91	0.96	0.95	1.00										
35	PI308453	0.91	0.91	0.90	0.89	0.92	0.92	0.92	0.91	0.93	0.94	0.90	0.91	0.92	0.89	0.92	0.92	0.92	0.92	0.92	0.90	0.93	0.89	0.91	0.92	0.92	0.93	0.92	0.91	0.89	0.92	0.94	0.97	0.96	0.94	1.00									
36	97MW6113	0.92	0.92	0.92	0.89	0.92	0.93	0.92	0.92	0.93	0.94	0.91	0.92	0.92	0.89	0.93	0.93	0.93	0.94	0.92	0.91	0.93	0.90	0.90	0.94	0.91	0.93	0.93	0.90	0.90	0.93	0.94	0.94	0.93	0.95	0.97	1.00								
37	Macia-SA	0.91	0.91	0.92	0.89	0.94	0.92	0.91	0.92	0.95	0.97	0.89	0.93	0.94	0.88	0.92	0.92	0.93	0.93	0.94	0.90	0.93	0.90	0.89	0.95	0.89	0.92	0.92	0.91	0.89	0.94	0.93	0.93	0.92	0.91	0.95	0.96	1.00							
38	M48	0.89	0.91	0.90	0.88	0.91	0.91	0.92	0.92	0.91	0.91	0.91	0.91	0.90	0.88	0.91	0.91	0.91	0.92	0.90	0.90	0.93	0.89	0.90	0.93	0.91	0.91	0.92	0.91	0.89	0.91	0.93	0.93	0.93	0.92	0.95	0.96	0.93	1.00						
39	M141	0.89	0.89	0.90	0.87	0.90	0.90	0.90	0.91	0.92	0.92	0.88	0.92	0.91	0.88	0.90	0.89	0.90	0.90	0.92	0.88	0.91	0.88	0.89	0.92	0.90	0.90	0.90	0.91	0.89	0.90	0.91	0.95	0.93	0.93	0.94	0.94	0.94	0.92	1.00					
40	M81	0.89	0.89	0.90	0.88	0.93	0.91	0.91	0.90	0.91	0.92	0.88	0.92	0.92	0.87	0.90	0.89	0.90	0.91	0.91	0.89	0.91	0.88	0.89	0.93	0.90	0.91	0.90	0.91	0.88	0.92	0.91	0.93	0.92	0.90	0.93	0.92	0.95	0.92	0.95	1.00				
41	M105	0.88	0.87	0.89	0.87	0.91	0.90	0.90	0.90	0.93	0.93	0.88	0.93	0.92	0.88	0.89	0.88	0.88	0.90	0.91	0.89	0.91	0.88	0.88	0.92	0.89	0.90	0.89	0.90	0.89	0.91	0.90	0.93	0.92	0.90	0.93	0.93	0.94	0.91	0.96	0.97	1.00			
42	M101	0.89	0.88	0.90	0.87	0.88	0.88	0.87	0.92	0.91	0.89	0.88	0.92	0.89	0.91	0.90	0.89	0.89	0.89	0.94	0.91	0.94	0.92	0.89	0.90	0.89	0.90	0.87	0.88	0.93	0.88	0.87	0.90	0.88	0.90	0.90	0.91	0.92	0.89	0.92	0.91	0.93	1.00		
43	M163	0.91	0.91	0.90	0.89	0.89	0.90	0.92	0.93	0.91	0.91	0.89	0.91	0.89	0.90	0.92	0.91	0.93	0.92	0.92	0.91	0.94	0.92	0.92	0.92	0.91	0.92	0.91	0.91	0.92	0.89	0.91	0.93	0.93	0.92	0.94	0.94	0.94	0.94	0.93	0.92	0.92	0.93	1.00		
44	Masekaswere	0.88	0.88	0.88	0.86	0.88	0.90	0.90	0.90	0.91	0.90	0.89	0.91	0.89	0.88	0.89	0.89	0.89	0.91	0.91	0.88	0.91	0.88	0.88	0.91	0.90	0.89	0.90	0.90	0.90	0.90	0.91	0.93	0.92	0.94	0.93	0.94	0.91	0.92	0.95	0.92	0.92	0.93	0.93	1.00	
45	Mamolokwane	0.91	0.90	0.91	0.89	0.92	0.92	0.90	0.92	0.89	0.93	0.91	0.90	0.92	0.88	0.91	0.91	0.91	0.91	0.91	0.90	0.92	0.90	0.92	0.91	0.92	0.91	0.90	0.90	0.89	0.92	0.94	0.94	0.93	0.93	0.95	0.94	0.93	0.92	0.94	0.93	0.92	0.91	0.92	0.93	1.00	
46	M153	0.89	0.88	0.90	0.89	0.90	0.89	0.90	0.91	0.91	0.92	0.88	0.92	0.91	0.89	0.90	0.90	0.90	0.90	0.92	0.89	0.91	0.90	0.90	0.90	0.89	0.90	0.89	0.90	0.89	0.90	0.90	0.94	0.93	0.92	0.94	0.92	0.94	0.91	0.94	0.94	0.95	0.93	0.93	0.92	0.94	1.00

The genetic similarity within the 30 landraces was very high, with very few values lower than 0.90. Landrace 9 (Table 4.4) was the only one that was less similar (<0.90) than nine other landraces, but all values were still higher than 0.80. This indicated that the landraces were genetically very similar, and were related to each other. Farmers may have exchanged landraces in the collection areas and crosses could have been made within the material, by design, or by cross pollination. However, AFLP analysis detected no duplications (100% similarity) within the tested accessions. Quite a number of landraces showed genetic similarity of <0.90 in pairwise comparisons with the Ethiopian sorghum cultivars as well as South African breeding material, showing more dissimilarity than within landraces. The genetic similarity between the Ethiopian cultivars and the landraces, and the South African material and the landraces was much the same. As with the phenotypic characterisation, it would seem that the South African material and the Ethiopian material may have come from a common source somewhere in their breeding history. It is possible that some material kept at, for example, ICRISAT may have been collected from Ethiopia in the past, as Ethiopia is the source of origin of sorghum. The South African breeding programme could have obtained their breeding material from a source like ICRISAT, as sorghum is not indigenous to South Africa. Similarity within the Ethiopian cultivars and within the South African breeding material was very high, indicating a very small genetic base. This should be addressed in the future by introducing unrelated material to both programmes.

Menz et al. (2004) reported genetic similarity ranging from 0.81 to 0.91 for 50 sorghum inbred lines using AFLP marker analysis. The average genetic similarity coefficient for the pairwise combinations was 0.92. Uptmoor et al. (2003) reported mean genetic similarities among 46 sorghum accessions of 0.88 based on AFLP analysis. Similarly, Geleta et al. (2006) reported that a genetic distance coefficient of 0.62 among 45 sorghum accessions using eight AFLP primer combinations. Furthermore, Abu Assar et al. (2005) found an average genetic similarity value of 0.30 among 96 sorghum genotypes which was much lower than the values reported in this study.

The dendrogram produced four distinct clusters (Figure 4.1). Cluster I comprised of 13 accessions at a genetic similarity of 0.932. All accessions in this cluster were cultivars except accessions Mamolokwane and Masekaswere. These accessions were the two landrace accessions from South Africa and were separated from the subgroups as singletons.

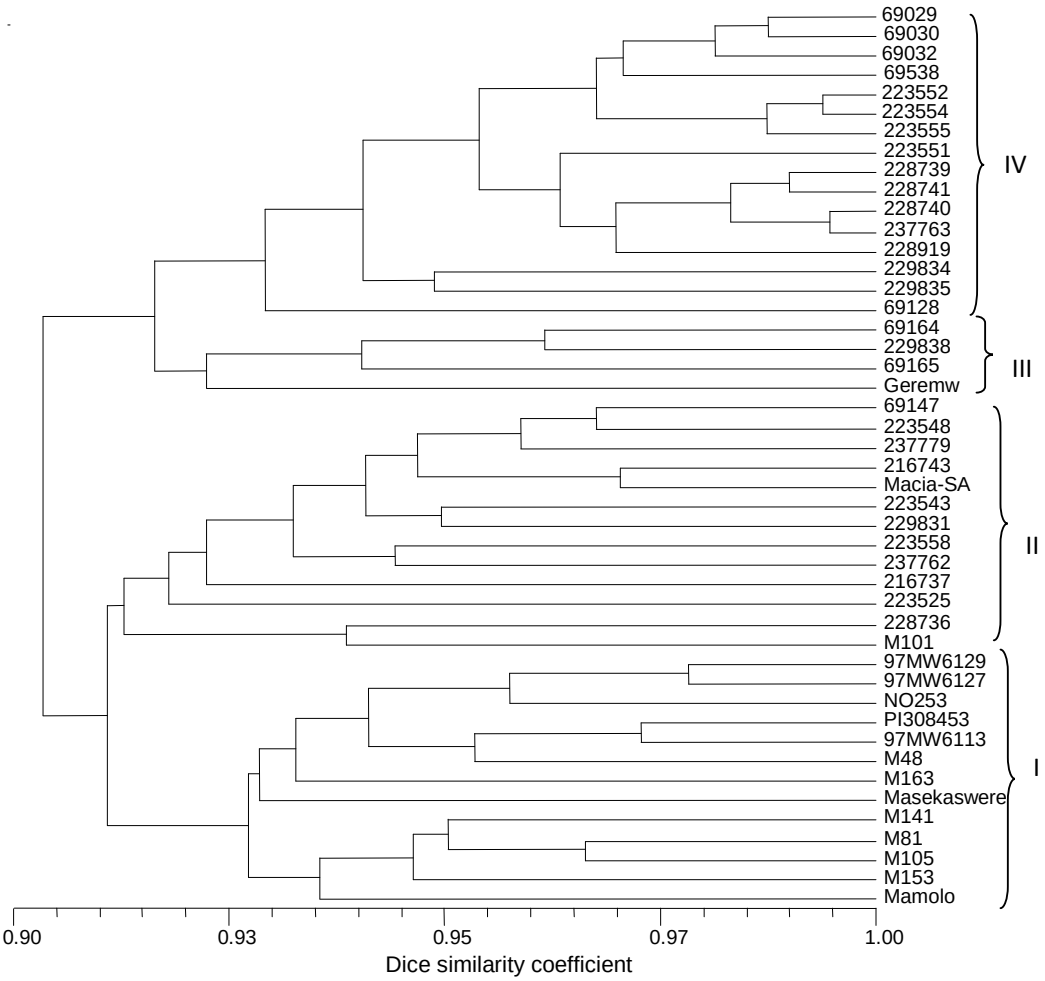

Figure 4.1 Dendrogram revealing genetic relationships among 46 sorghum accessions from Ethiopia and South Africa based on AFLP analysis, Dice similarity coefficients and UPGMA clustering, Mamolo=Mamolokwane

In this main cluster (I), accessions were grouped mainly according to geographical origin. All accessions from South Africa, except Macia-SA and M101 clustered together in Cluster I. Clustering of the Ethiopan accession with the South African ones might be due to the same reasons as hypothesised before, that they have a common source of ancestry. The only Ethiopian accession that did not cluster in cluster I were Geremw, Macia-SA and M101 (South Africa). For example, accessions 97MW6129, 97MW6127 and 97MW6113 were advanced lines from Ethiopia and NO253 and PI308453 were introductions from ICRISAT and Purdue University to Ethiopia. The rest of the accessions in this cluster were from South Africa. Accessions in cluster I were breeding lines that had undergone higher levels of selection as breeders only select for a limited number of traits. Selection thus might have narrowed down genetic diversity in the breeding lines, thus clustering together despite their geographical origin. The two exotic

germplasm accessions, NO253 and PI308453 showed the relationship between them and with accessions from Ethiopia and South Africa cultivars. Moreover, AFLP marker detected that they were distinctly related in the cluster though separated into different sub-groups within a cluster. Therefore, identification of intra-specific accessions is crucial factor for the success of introgression breeding in sorghum. Similarly, Uptmoor et al. (2003) detected genetic relatedness and diversity within 46 sorghum accessions grown in Southern Africa and revealed a clear separation between landraces and breeding varieties using AFLP, RAPD and SSR markers. The clustering of accessions based on their genetic similarity in this study would help in selection of genetically diverse parental lines to get superior recombinants for future sorghum breeding programmes (Jeya Prakash et al., 2006).

Similarly, cluster II consisted of 13 accessions at a genetic distance of 0.912. This cluster was subdivided into two subgroups at a genetic similarity of 0.914 and contained accessions M101 (South Africa) and 228736 (Ethiopia) as one of the subgroups. Accessions M101 and Macia-SA were from South Africa and clustered together in cluster II with 11 landrace accessions of Ethiopia. This must be due to some genetic relationships with the rest of the accessions in the cluster. All 11 landrace accessions in cluster II were collected from the north-western and western agro-ecologies of Ethiopia, although they were from different localities (Table 4.1). Accessions 237762 and 228736 were collected from central Ethiopia in the west Showa zone and grouped in this cluster based on regional backgrounds.This might be due to gene flow, and seed exchange among farmers within and between regions, thus causing clustering. Bucheyeki et al. (2009) reported the clustering of 40 sorghum landraces using SSR-based analysis in Tanzania based on their area of collection sites and pedigree relationship and showed variation and diversity of the landraces. According to Barnaud et al. (2008), Bucheyeki et al. (2009) and Muray et al. (2010) the gene flow plays a large role in structuring the genetic variability within and among sorghum populations. Manzelli et al. (2007) similarly reported continuous exchanges of genes between sorghum population results the genetic diversity.

Cluster III comprised of four accessions at a genetic distance similarity of 0.926. This group contained landrace accessions from the north-western parts of Ethiopia that were collected from the Metekel zone of the Benishangul-Gumuz region, except for cultivar Geremw, which was obtained from Melkassa Agricultural Research Center (MARC) of east Showa zone of Oromiya region in Adama district and was an outlier in this cluster.

Cluster IV contained 16 accessions which was a mixture of accessions collected from the north-western and western as well as central parts of Ethiopia. Accession 69128 clustered separately from the other accessions in the cluster and was collected from the Assosa zone of the Benishangul-Gumuz region border to Sudan.

Accessions Geremw, 223525, Masekaswere, Mamolokwane, M163, M153 and 69128 clustered separately from the rest of the accessions in their group, but that only makes them genetically distinct in that particular group, not with all accessions tested. The presence of this difference within the cluster could have benefits in breeding programmes and selection of parental lines. Accessions 228740 and 237763 were the most similar of all accessions evaluated. Both these accessions were from the west Showa zones of the Oromya region (with a similarity coefficient of 0.994). The two collection sites, Ambo and Bako Tibe, were close in distance to each other in the west Showa zone. Similarly, accessions 223552 and 223554 were the second most similar accessions, both collected from Dedesa in the Illubabor zone.

Accessions 228739, 228741, 228740 and 237763 that clustered closely together in cluster IV, were collected from the Ambo and Bako Tibe districts of the west Showa zone in the central parts of Ethiopia. These accessions clustered closely together with accessions 223551 and 228919 that were collected from west of Ethiopia from the Illubabor zone (Yayu and Bure districts). Many accessions from the same region of origin and those closely situated regions clustered together. Regions in close geographical proximities, example Benishangul-Gumuz Region and Oromya regions, clustered together (cluster IV). Similarly, Vittal et al. (2010) reported that SSR markers grouped 23 sorghum genotypes from the United State of America based on their geographical origin. Moreover, Shehzad et al. (2009) also found that 320 sorghum accessions from Ukraine were distinctly classified according to geographic distribution. In line with this, Abu Assar et al. (2005) reported that 96 sorghum genotypes from Sudan grouped together based on their geographical origin as well as adaptation zones. Folkertsma et al. (2005) also reported on the variation among Guinea-race sorghum landraces based on eco-geographical regions using SSR markers.

4.3.3 Principal co-ordinate analysis using AFLP markers

The PCoA biplot (Figure 4.2) clustered accessions similarly to the dendrogram based on their genetic similarity (Figure 4.1) with some differences. Accessions grouping together in cluster I of the dendrogram (Figure 4.1) also grouped together in the biplot. Similarly,

those accessions grouping together in clusters II, III, and IV also grouped together in the biplot with some exceptions. Accessions Geremw, 69165, 229838 and 69164 clustered together in the dendrogram, but in the biplot cultivar Geremw was separated from the group and clustered together with other commercial cultivars. This might be due to the pedigree relationships with the rest of the accessions in cluster I of the dendrogram. Accessions 229834 and 229835 clustered together with accessions 69164, 69165 and 229838, all collected from the Metekel zone in Ethiopia. Accessions Macia-SA, 223525 and 223558 clustered together in cluster II of the dendrogram (Figure 4.1) but with the PCoA they appeared to be far apart from the group and fell in different groups. Macia-SA grouped together with the cultivars, whereas, accessions 223525 and 223558, (both) collected from the Ghimbi and Keresa districts of the Oromya region clustered together and both these districts were in close proximity to each other. Therefore, the PCoA separated accessions better than the dendrogram based on the genetic similarity analysis and geographical location. PCoA provided a better diversity structure than the dendrogram since PCoA used three dimentions, compared to one dimension for the dendrogram.

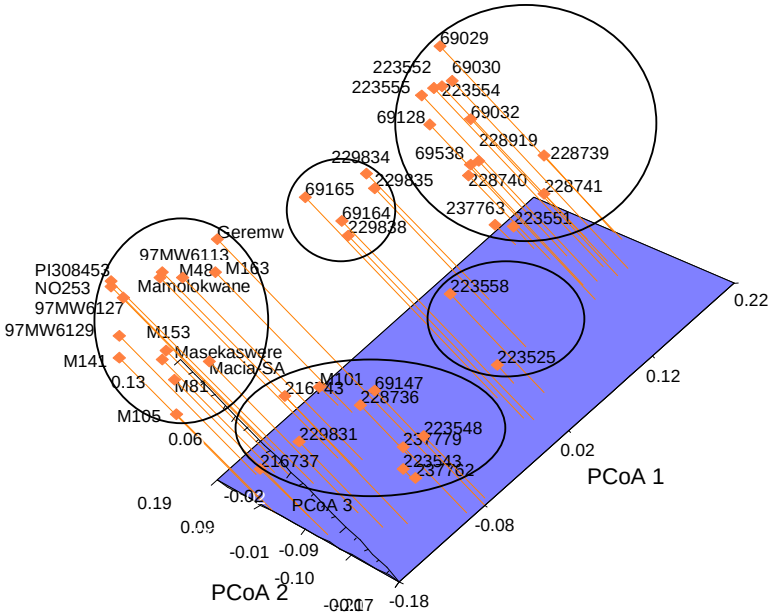

Figure 4.2 Principal co-ordinate analysis biplot for genetic characterisation of 46 sorghum accessions using AFLP analysis

4.4 Conclusions

Understanding the diversity of sorghum germplasm collections is important for effective exploitation of their genetic potential as well as for selection of landraces and other genotypes as breeding lines, maintenance and for conservation. The AFLP marker analysis has successfully provided a precise estimation of genetic diversity among tested sorghum accessions. The similarity shown with the DNA markers was much higher than reported in the previous chapter where morphological markers were used, although the landraces were excluded in that chapter. DNA markers cover the whole genome while morphological markers cover only selected traits in breeding programmes and DNA markers are therefore more reliable. One reason for the low levels of genetic diversity among Ethiopian landraces might be due to the reduced population size/small number of samples collected from different regions and most landraces were from three regions in Ethiopia, which did not represent the whole of Ethiopia or Africa. The fact remains, however, that genetic similarity for the other material (cultivars from Ehtiopia, and cultivars, breeding lines and two landraces from South Africa) was equally high, also showing low levels of genetic diversity.

AFLP marker data identified and clustered accessions mainly according to their collection sites. The PCoA provided a similar structure but with some differences to that of the dendrogram's clustering patterns, suggesting the effectiveness of PCoA analysis in genetic diversity analysis. In future, more primer combinations should be included for genetic analysis in sorghum germplasm. Therefore, further investigations could involve a larger number of samples and more primers with a wider range of collection sites for accessions within regions including other regions from the northern, southern and eastern parts of the country to obtain a clear picture of genetic diversity in Ethiopia using different DNA-based molecular markers analysis.

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CHAPTER 5

COMPARISON OF GENETIC DIVERSITY ASSESSMENT IN SORGHUM ACCESSIONS USING QUALITATIVE MORPHOLOGICAL AND AFLP MARKERS

Abstract

The comparison of different methods for estimating genetic diversity could provide useful information to increase the efficiency in plant breeding and conservation programmes. During this study a total of nine qualitative morphological traits and six AFLP primer combinations were used to assess the genetic diversity among 17 sorghum accessions, seven from Ethiopia and 10 from South Africa. The mean morphological genetic similarity (0.49 with a range of 0.00-0.89) was lower in comparison to similarity computed using AFLP markers (0.93 with a range 0.88-0.98) and combined morphological and AFLP markers (0.90 with a range of 0.82-0.97). Genetic similarity measured by AFLP markers was similar within the Ethiopian and South African material, as well as between South African and Ethiopian material. The morphological similarity was much higher in the Ethiopian material than South African material, indicating that the genotypes were related. The PCoA biplots grouped accessions in a similar way to that of dendrograms. Genetic diversity was limited. The morphological and combined morphological and AFLP dendrograms separated accession 216737 as genetically distinct from the rest of accessions. Similarly, combined morphological and AFLP dendrogram separated accession M101 from the rest of the accessions.

5.1 Introduction

Information on genetic diversity has been successfully used for efficient germplasm management and utilisation (Frankel, 1989; Blakeney, 2002), genetic fingerprinting and genotype selection (FAO, 1998; Engles et al., 2002) and has played an important role in achieving success in crop improvement. Genetic evaluation and morphological characterisation of genotypes gives basic information of the characters and assists in understanding the similarities and differences among genotypes (IBPGR and ICRISAT, 1993; Bucheyeki et al., 2009). Morphological descriptors, biochemical and molecular markers are the different methods that are used for measuring genetic diversity among crop species (Geleta and Labuschagne, 2005; Mehmood et al., 2008). Morphological

traits are commonly used to analyze genetic diversity (Weining and Langridge, 1991; Teshome, 1997; Abdi et al., 2002; Geleta and Labuschagne, 2005), since they provide a simple way of measuring genetic diversity while studying genotype performance under normal growing conditions, but are influenced by environmental factors (Tuinstra et al., 1996; van Beuningen and Busch, 1997; Fufa et al., 2005). Regardless of these restrictions, they have been used for genetic diversity assessment and genotype development (Fufa et al., 2005).

Geleta and Labuschagne (2005) underlined the importance of using molecular markers as an additional tool for varietal description, as the genetic control of morphological traits are mostly polygenic and their expression depends on environmental factors. Molecular markers have proved to be invaluable for understanding the genetic make-up of agricultural crops. They differ from morphological traits in that molecular markers usually occur in greater numbers, they can be distinguished without relying on the complete development of the plant and their expression is not altered by the environment.

Knowledge of genetic distance and/or similarity not only generates a better understanding of germplasm organisation and efficiency during genotypic sampling, but also has implications on the choice of parents for crosses and gene introgression from exotic germplasm. It can also be used to recommend cultivars for a given region (Vieira et al., 2007). Thus, studying genetic diversity of accessions is indispensable for identification of the genetic similarity and differences among the available genetic resources.

DNA markers are not influenced by environmental conditions like that of morphological traits, therefore, the use of molecular markers for variety fingerprinting and identification of crop plants for breeding programmes is of paramount importance (Jeya Prakash et al., 2006; Tabbasam et al., 2006; Mehmood et al., 2008; Abu Assar et al. 2009). Therefore, using both morphological and molecular characterisation of crop species can provide complementary advantages in selection of unique gene (s) trait (s) of interest.

Genetic diversity studies using a combination of techniques, such as morphological and molecular markers (example, RAPD and AFLP), have been conducted in cotton (Bie et al., 2001; Wu et al., 2001; Lukonge, 2005), wheat (Cox and Murphy, 1990; Vieira et al., 2007) and oilseed (Riaz et al., 2003). Combined agro-morphological, SSR and AFLP markers have also been used to assess genetic diversity in sorghum (Geleta, 2003) and maize (Beyene et al., 2005) and generated useful information on genetic diversity that is

useful for breeders. Both morphological and molecular analyses are informative tools for estimation of genetic distances (Vieira et al., 2007). Hence, the objective of this study was to compare the use of both morphological and AFLP markers to assess biodiversity in sorghum accessions.

5.2 Materials and methods

5.2.1 Experimental material

The sorghum accessions in Chapter 3 (Table 3.1) were used. The landrace accessions (Chapter 4, Table 4.1) from IBC/Ethiopia were not included in the comparison of morphological and AFLP marker data because morphological traits recording was not complete on them as they were photoperiod-sensitive. Of the 22 accessions used in the morphological characterisation, only 17 were included in the AFLP analysis due to problems associated with DNA purity and concentration. Only these 17 accessions that were evaluated both morphologically and DNA markers were included in the comparison of the results. The first seven were from Ethiopia, and the last 10 from South Africa.

5.2.2 Morphological traits

The data collection procedure for nine morphological (qualitative) traits (Table 3.3) as described in Chapter 3 (section 3.2.2) was used. The morphological traits were coded as present (1) or absent (0) to compare them with AFLP marker data.

5.2.3 AFLP markers

AFLP analysis was performed as described in Chapter 4 (sections 4.2.1-4.2.5). A total of 186 AFLP fragments were scored as present (1) or absent (0) and used for comparative analysis with morphological data.

5.2.4 Data analysis

The morphological data was subjected to analysis using NTSYS-pc version 2.21c (Exeter Software, NY, USA). Similarity matrices were compiled for all pairs of accessions using Dice similarity coefficients (Dice, 1945), using SIMQUAL (similarity of qualitative data). Cluster analysis was done using UPGMA analysis (Sokal and Michener, 1958) and dendrograms were constructed using the SAHN programme. Cophenetic analysis

was done as described in Chapter 4 (section 4.2.5). Similar analysis was done on AFLP as well as combined AFLP and morphological data for comparison. Principal co-ordinate analysis (PCoA) was done to show differentiation of accessions as described in Chapter 4 (section 4.2.5).

5.3 Results and discussion

5.3.1 Genetic similarity based on morphological and AFLP data

The genetic similarity for morphological data ranged from 0.00 to 0.89 (Table 5.1 below diagonal), indicating that some accessions were morphologically very similar (0.89) while others were totally different (0.00). The average genetic distance for all pairwise comparisons (N = 136) was 0.49. A 0.89 similarity coefficient was obtained between accessions 97MW6113 and 216743, 97MW6129, NO253 and PI308453, as well as between PI308453 and 97MW6129 and Masekaswere and 216743. Morphologically, these accessions were clustered together based on similarities in longest days to 50% flowering, highest number of leaves, shortest leaves, narrowest leaves width, smallest leaf area, the highest number of internodes, medium leaf sheath length, tallest plant, longest panicle, narrowest panicle, average panicle weight, lowest threshing percent, and highest number of primary branches per panicle. Furthermore, a high level of genetic similarity of 0.78 was observed for some accessions. The genetic similarity between M141 and 216737 was zero. These two accessions shared no similar morphological traits and were not related at all. These accessions were from South Africa and Ethiopia, respectively. The genetic similarity within the Ethiopian material (0.678) was much higher than within South African material (0.43, data not shown). The genetic similarity between South African and Ethiopian material was similar to that within South African material (0.46).

Table 5.1 Genetic distances for morphological (**below diagonal**) and AFLP (above diagonal) data based on Dice similarity coefficients for 17 sorghum accessions

No.	Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	216737	1.00	0.94	0.91	0.91	0.89	0.93	0.93	0.95	0.91	0.92	0.91	0.93	0.91	0.91	0.91	0.89	0.91
2	216743	**0.44**	1.00	0.92	0.90	0.89	0.94	0.94	0.97	0.91	0.92	0.92	0.93	0.89	0.91	0.90	0.93	0.92
3	97MW6129	**0.44**	**0.78**	1.00	0.98	0.96	0.97	0.94	0.93	0.93	0.95	0.93	0.93	0.90	0.93	0.93	0.94	0.94
4	97MW6127	**0.33**	**0.78**	**0.78**	1.00	0.95	0.96	0.93	0.92	0.93	0.93	0.92	0.92	0.88	0.93	0.92	0.93	0.93
5	NO253	**0.22**	**0.78**	**0.78**	**0.67**	1.00	0.94	0.95	0.91	0.92	0.93	0.90	0.90	0.90	0.92	0.94	0.93	0.92
6	PI308453	**0.33**	**0.78**	**0.89**	**0.78**	**0.78**	1.00	0.97	0.95	0.95	0.94	0.93	0.93	0.90	0.94	0.93	0.95	0.94
7	97MW6113	**0.33**	**0.89**	**0.89**	**0.78**	**0.89**	**0.89**	1.00	0.96	0.96	0.94	0.92	0.93	0.91	0.94	0.94	0.94	0.92
8	Macia-SA	**0.33**	**0.33**	**0.44**	**0.44**	**0.22**	**0.33**	**0.33**	1.00	0.93	0.94	0.95	0.94	0.92	0.94	0.91	0.93	0.94
9	M48	**0.44**	**0.44**	**0.44**	**0.33**	**0.44**	**0.44**	**0.56**	**0.11**	1.00	0.92	0.92	0.91	0.89	0.94	0.92	0.92	0.91
10	M141	**0.00**	**0.33**	**0.33**	**0.22**	**0.44**	**0.33**	**0.44**	**0.44**	**0.33**	1.00	0.95	0.96	0.92	0.93	0.95	0.94	0.94
11	M81	**0.33**	**0.22**	**0.44**	**0.22**	**0.22**	**0.33**	**0.33**	**0.78**	**0.22**	**0.67**	1.00	0.97	0.91	0.92	0.92	0.93	0.94
12	M105	**0.33**	**0.44**	**0.44**	**0.33**	**0.22**	**0.33**	**0.33**	**0.67**	**0.22**	**0.67**	**0.78**	1.00	0.93	0.92	0.92	0.92	0.95
13	M101	**0.44**	**0.33**	**0.44**	**0.44**	**0.22**	**0.33**	**0.33**	**0.78**	**0.22**	**0.22**	**0.56**	**0.44**	1.00	0.93	0.93	0.91	0.93
14	M163	**0.33**	**0.67**	**0.67**	**0.56**	**0.67**	**0.67**	**0.78**	**0.22**	**0.67**	**0.33**	**0.22**	**0.22**	**0.44**	1.00	0.93	0.92	0.93
15	Masekaswere	**0.33**	**0.89**	**0.67**	**0.67**	**0.67**	**0.67**	**0.78**	**0.44**	**0.44**	**0.44**	**0.33**	**0.56**	**0.44**	**0.56**	1.00	0.93	0.92
16	Mamolokwane	**0.44**	**0.67**	**0.56**	**0.67**	**0.44**	**0.56**	**0.56**	**0.33**	**0.44**	**0.33**	**0.33**	**0.56**	**0.33**	**0.33**	**0.78**	1.00	0.94
17	M153	**0.56**	**0.78**	**0.67**	**0.67**	**0.56**	**0.78**	**0.67**	**0.44**	**0.22**	**0.11**	**0.33**	**0.33**	**0.44**	**0.44**	**0.67**	**0.56**	1.00

The genetic similarity estimates for all pairwise combinations of the 17 accessions using AFLP molecular marker data are presented in Table 5.1 (above diagonal). The genetic similarity coefficients for AFLP marker data ranged from 0.88 to 0.98. The average genetic similarity for all pairwise comparisons was 0.93. The highest genetic similarity coefficient (0.98) was observed between accessions 97MW6129 and 97MW6127. This indicated that these two accessions were closely related and one cannot expect to make much genetic gain if they are chosen as parents for crossing. Both of these accessions were from MARC, Ethiopia. The lowest genetic similarity (0.88) was obtained between accessions M101 and 97MW6127. M101 was from South Africa while 97MW6127 was obtained from MARC and they were the most dissimilar accessions based on AFLP data in this study. Agrama and Tuinstra (2003) found the genetic similarity values of 0.437 for SSR and 0.612 for RAPDs on 22 sorghum genotypes that were lower than the values obtained in this study. Selection of the parents, based on genetic distance information, could provide a basis for choosing parents for the crossing programme (Zhong-hu, 1991; Benesi 2005). Accessions PI308453 and 97MW6129 that had a similarity value of 0.89 based on morphological data were 0.97 similar based on AFLP data. The most similar accessions based on AFLP data were 97MW6129 and 97MW6127 (both from MARC in east Showa), and accessions 216743 and 97MW6129, 97MW6127, NO253, PI308453 as well as 97MW6129 and 97MW6127, NO253; Macia-SA and M81, M101 and M81 and M105, as well as Masekaswere and Mamolokwane had a 0.78 similarity based on morphological data (second highest morphological value). Accessions revealing the second highest similarity value (0.97) based on AFLP data were 97MW6113 and PI308453; Macia-SA and 216743; and M105 and M81. M141 and 216737 that shared no similarity based on morphological data (0.00) had a genetic similarity of 0.91 based on AFLP data. The most dissimilar accessions based on AFLP data, M101 and 97MW6127 (0.88) had a genetic similarity of 0.44 based on morphological data. The genetic similarity was much the same within the Ethiopian and South African material, as well as between the two groups of material (0.94, 0.93 and 0.93 respectively, data not shown).

5.3.2 Morphological cluster analysis

The goodness of fit for the cluster analysis was confirmed by the cophenetic coefficient of r = 0.85. This signified that the generated clusters accurately represented distances between accessions as determined by the similarity coefficients. The percentage similarity between accessions ranged from 33 to 89% (Figure 5.1). The resulting phenetic dendrogram revealed three main clusters (I, II and III, Figure 5.1) at a genetic distance of 0.33. Cluster I contained five accessions, subdivided into two sub-groups,

M101 in one subgroup and M141, M105, M81 and Macia-SA in the second at a genetic similarity of 0.50. Accession M141 was separated from this group due to unique traits such as the semi-loose drooping primary branches, panicle type and black glume colour and related to M105, M81 and Macia-SA. Accession M101 had pigmented plant colour and flat grain shape which caused this accession to cluster separately from other accessions within the cluster. All accessions in cluster I were from South Africa and were characterised by 50% grain covering, small to medium grain size with white grain colour and yellow leaf mid rib colour. Furthermore, the cluster showed tan type of plant, semi-loose drooping primary branches, semi-compact elliptic panicle and non-lustrous as well as elliptical grain shape. Accessions M141, M81 and M105 were introductions from ICRISAT/Zimbabwe to South Africa. The closest accessions in this cluster were M81 and Macia-SA with a genetic similarity coefficient at 0.78. These accessions were clustered together based mainly on collection sites and pedigree relationship. Likewise, Bucheyekei et al. (2009), Dean et al. (1999) and Ghebru et al. (2002) detected clustering of sorghum accessions based on their collection site and pedigree relationship.

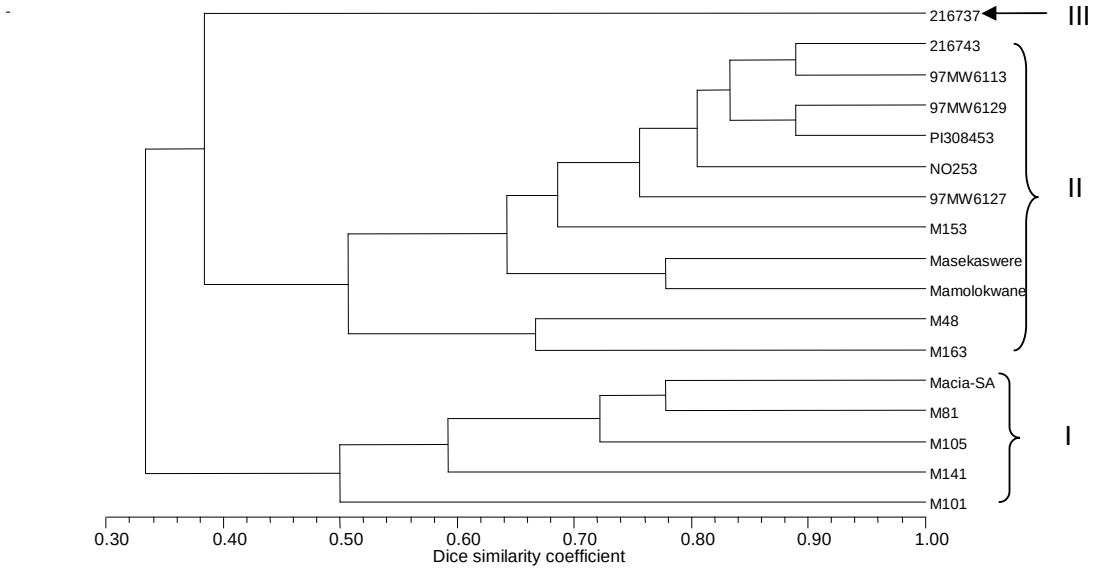

Figure 5.1 Phenetic dendrogram generated using morphological data of 17 sorghum accessions depicting their relationships based on UPGMA clustering from pairwise comparisons employing Dice genetic similarity coefficient

Cluster II contained the majority of accessions (Figure 5.1) and of those 11 accessions four were from Ethiopia, one from PU, one from ICRISAT and five from South Africa with varied morphological characters. Two accessions, M163 and M48 clustered separately at a genetic distance of 0.51 from the rest of the accessions and had a genetic similarity coefficient of 0.67 and were grouped based on red grain colour. All accessions in this

cluster were characterised by round grain shape, dull green leaf mid-rib colour, with pigmented type of plant colour, lustrous with elliptic shape of the grain, yellow grain colour, and semi-compact elliptic type of panicle, bold seed size and 25% grain covering. Two other accessions, Mamolokwane and Masekaswere clustered separately from the remaining seven accessions in cluster II, at a genetic similarity coefficient of 0.78. These accessions are landraces from South Africa and were collected in the Sekhukhune district of the Limpopo province. Among accessions grouped in cluster II, 216743 and 97MW6113 as well as 97MW6129 and PI308453 were the most similar accessions at a genetic similarity coefficient of 0.89, indicating a higher morphological similarity.

Cluster III contained only accession 216737 that was linked with the other accessions at a genetic distance of 0.39 and was the most distant from the rest of the accessions. This accession is a landrace from Ethiopia and it was characterised by white leaf mid rib colour, red glume colour, round grain shape, non lustrous grain and yellow grain colour.

The morphological dendrogram demonstrated variation of accessions based on morphological traits that could be a valuable source for the sorghum improvement programmes in the two geographical regions, Ethiopia and South Africa. Similarly, Geleta and Labuschagne (2005) found the existence of morphological variation among sorghum accessions collected from eastern parts of Ethiopia using 10 morphological traits and concluded that the variation among the sorghum germplasm implies the need for the genetic resource collection and maintenance. Teshome et al. (1997) evaluated 117 sorghum accessions from North Shewa and South Welo regions of Ethiopia based on 14 morphological traits and reported extensive variation of the accessions. Grenier et al. (2004) observed the morphological diversity among sorghum accessions as well as a high level of diversity within region and was distributed with geographical origin using 2 017 Sudanese sorghum landraces. Barro-Kondombo et al. (2010) also found a high level of morphological and genetic variability in sorghum varieties from Burkina Faso.

5.3.3 Principal co-ordinate analysis based on morphological analysis

The PCoA biplot is shown in Figure 5.2. The first eight principal components with eigenvalues greater than 1 accounted for 89.20% of total variation. However, the first three components accounted for 56.73% of variation and effectively grouped the 17 sorghum accessions. PCoA clustered accessions similarly to the morphological dendrogram with some differences. As observed in Cluster I of the dendrogram (Figure 5.1), accessions with similar morphology also grouped together using PCoA (Figure 5.2), however, accession M141 and M101 were positioned far apart from the group. Similarly, accessions that grouped together in cluster II using cluster analysis also grouped together using PCoA except accessions M48 and M163 that were separated individually from the cluster. Accession 216737 was separated as in the dendrogram. This indicated that PCoA can separate accessions more efficiently than that of cluster analysis based on genetic similarity analysis.

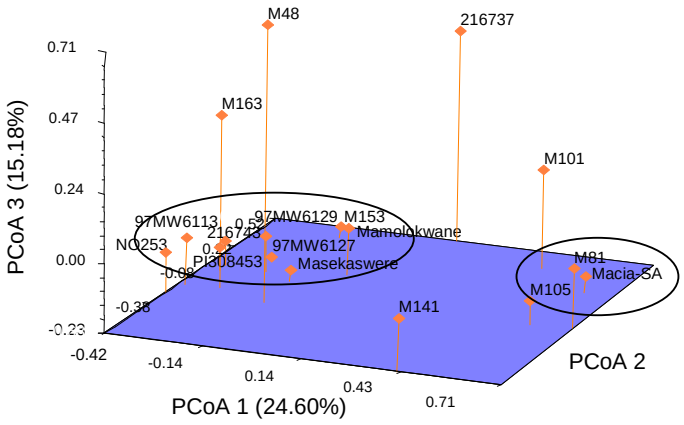

Figure 5.2 Principal co-ordinate analysis biplot for characterisation of 17 sorghum accessions using morphological markers employing NTSYS-pc

5.3.4 Cluster analysis based on AFLP markers

The dendrogram generated based on Dice genetic similarity coefficient using UPGMA cluster analysis and AFLP marker data revealed four main clusters split into two main

clusters at a genetic similarity coefficient of 0.927 (Figure 5.3). The four main clusters were split into two main clusters at a genetic similarity of 0.916. The cophenetic correlation coefficient computed for the goodness of fit of the cluster analysis was 0.68, which indicated a poor fit of the dissimilarity and cophenetic matrices.

Cluster I consisted of two accessions, Masekaswere and M101, at a genetic distance of 0.934 which were both from South Africa. Cluster II comprised of five accessions, all from South Africa at a genetic similarity coefficient of 0.936. Accession Mamolokwane was clustered separately in this cluster and was the only landrace in the cluster. Cluster III contained seven accessions, three from Ethiopia, one from ICRISAT and one from PU and two from South Africa. M163 clustered separately from the rest of the accessions in this cluster with a 0.933 genetic dissimilarity coefficient and was from South Africa, indicating a unique accession. M48 was also from South Africa and genetically related to PI308453 and 97MW6113 with a 0.952 genetic dissimilarity coefficients, indicating that it might share some genetic information with accessions of Ethiopia and PU. Cluster IV comprised of three accessions, accession 216737 being dissimilar at a genetic distance of 0.944 in the group. Accessions 216737 and 216743 were collected from the same region, Gambella, Ethiopia and could have shared the same genetic background with Macia-SA which originated from South Africa. Agrama and Tuinstra (2003) reported the clustering of sorghum genotypes based on their geographical origins. Thus selection of accessions as parental material based on their genetic distances and clustering could increase the genetic diversity among accessions. Sabharwal et al. (1995) and Chozin (2007) reported that sorghum parents with more diversity among themselves are expected to show a higher amount of variability. Abu Assar et al. (2009) found that the 40 genotypes studied in Sudan were clustered based on morphological and/or pedigree relationship using 16 SSRs primers.

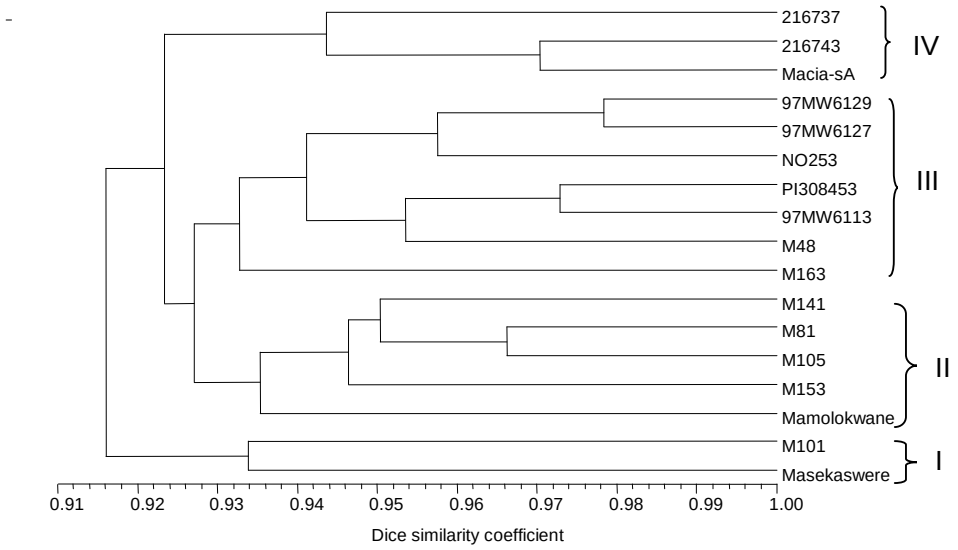

Figure 5.3 Dendrogram generated based on the AFLP data using UPGMA cluster analysis of Dice genetic similarity coefficients

5.3.5 Principal co-ordinate analysis based on AFLP data

The PCoA biplot based on AFLP data (Figure 5.4) revealed similar clustering as in the dendrogram (Figure 5.3) using Dice genetic distance. However, accessions Masekaswere and M101 were positioned far apart in the biplot, but clustered together in the dendrogram (Figure 5.3). Accessions 216737, 216743 and Macia-SA grouped together using both cluster analysis and the PCoA. However, during cluster analysis, 216737 clustered separately from 216743 and Macia-SA while PCoA analysis indicated a closer association of 216737 with 216743 than Macia-SA. This might be because both accessions were collected from the same region in Ethiopia. The PCoA separated accessions into two groups that were clustered together in cluster II using the dendrogram. Mamolokwane and M141 were separated from accessions M153, M81 and M105 using PCoA analysis. Similarly, it also separated accessions that were clustered together in cluster III into three groups. The first group comprised of accessions 97MW6129 and 97MW6127. The second group consisted of accessions Masekaswere, PI308453, M163, 97MW6113 and M48, while NO253 was separate.

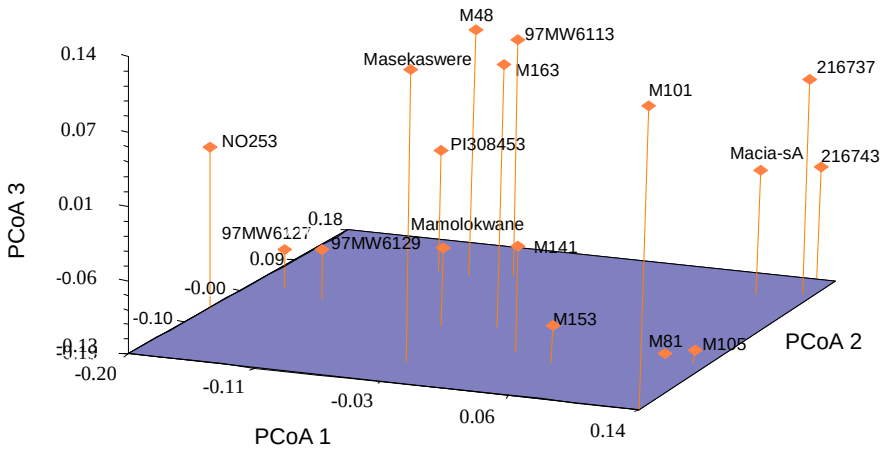

Figure 5.4 Principal co-ordinate analysis biplot for characterisation of 17 sorghum accessions using AFLP markers employing NTSYS-pc

5.3.6 Comparison of morphological and AFLP dendrograms

Comparison of the morphological and AFLP dendrograms showed that some accessions clustered together for both analyses (Figure 5.1 and 5.3). Accessions M163, M48, 97MW6113, PI308453, NO253, 97MW6127, 97MW6129 and 216743 clustered together in both the morphological and AFLP dendrograms (clusters II, III and IV). Accessions that clustered differently in these clusters, were M153, Masekaswere and Mamolokwane that clustered with above mentioned accessions based on morphological data, but clustered separately from these accessions in another main group using AFLP data. Macia-SA also clustered differently based on morphological and AFLP data. M141, M105 and M81 clustered together using both morphological and AFLP data. In the morphological dendrogram, accessions M48 and M163 were separated from the cluster II at a genetic similarity of 0.51, while in the AFLP dendrogram accession M163 was separated from the rest of the accessions in cluster III at a genetic similarity of 0.933. This showed that accession M163 was both morphologically and genetically different from the rest of the accessions in the cluster. Accessions PI308453 and 97MW6129 as well as 97MW6113 and 216743 were the most similar based on the morphological data. However, AFLP

data positioned them in different sub-clusters although they were still in the same main cluster (III). Accession 216737 was the most distinct accession based on morphological clustering and grouped totally separately. However, it clustered together with accessions 216743 and Macia-SA in the AFLP dendrogram. Furthermore, landrace accessions Mamolokwane and Masekaswere grouped together in cluster II of the morphological dendrogram but in the AFLP dendrogram they were placed in different cluster groups. Accessions M81, M105 and M141 grouped together in both the morphological and AFLP dendrograms. AFLP molecular analysis is a good technique to discriminate and group closely related sorghum accessions and also to describe the origin and pedigree relationship among them. Similarly, Abu Assar et al. (2005) found that 96 sorghum genotypes were grouped together based on their geographic and pedigree relationships.

The genetic similarity ranged from 0.00 to 0.89 and 0.88 to 0.98 for morphological and AFLP analysis, respectively. The genetic similarity for morphological data was generated by nine data points while AFLP analysis was based on 186 data points. Morphological data is based on the expression of a few genes that have been selected for, while AFLP analysis covers the entire genome and targets both expressed, but mainly unexpressed genes that were not selected for. Ritter et al. (2007) found a genetic dissimilarity value of 0.66 using AFLP data on 95 sorghum accessions that was lower than the values found in this study.

5.3.7 Genetic similarity based on combined morphological and AFLP data

The genetic similarity for combined morphological and AFLP data for all pairs varied from 0.82 to 0.97, with an average value of 0.90 (Table 5.2). The highest similarity coefficient was observed between accessions PI308453 (PU) and 97MW6129 (Ethiopia) and between PI308453 (PU) and 97MW6113 (Ethiopia). Accessions NO253 (ICRISAT) and 216737 (Ethiopia) revealed the lowest genetic similarity coefficient (0.82).

Table 5.2 Combined morphological and AFLP genetic distance based on Dice similarity coefficients for 17 characterised sorghum accessions employing NTSYS-pc

No.	Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	216737	1.00															
2	216743	0.91	1.00														
3	97MW6129	0.88	0.91	1.00													
4	97MW6127	0.86	0.89	0.96	1.00												
5	NO253	0.82	0.89	0.95	0.93	1.00											
6	PI308453	0.89	0.93	0.97	0.95	0.93	1.00										
7	97MW6113	0.89	0.94	0.94	0.92	0.94	0.97	1.00									
8	Macia-SA	0.90	0.93	0.91	0.89	0.86	0.92	0.92	1.00								
9	M48	0.88	0.89	0.90	0.89	0.89	0.92	0.93	0.88	1.00							
10	M141	0.85	0.89	0.91	0.88	0.90	0.91	0.91	0.91	0.89	1.00						
11	M81	0.87	0.88	0.90	0.87	0.85	0.89	0.89	0.94	0.88	0.93	1.00					
12	M105	0.89	0.90	0.90	0.88	0.85	0.89	0.89	0.92	0.87	0.94	0.96	1.00				
13	M101	0.87	0.86	0.88	0.85	0.84	0.86	0.87	0.91	0.85	0.88	0.89	0.90	1.00			
14	M163	0.87	0.90	0.92	0.91	0.90	0.92	0.93	0.90	0.93	0.90	0.88	0.88	0.90	1.00		
15	Masekaswere	0.87	0.90	0.91	0.90	0.92	0.91	0.93	0.88	0.89	0.92	0.88	0.90	0.90	0.91	1.00	
16	Mamolokwane	0.86	0.91	0.92	0.91	0.89	0.93	0.92	0.90	0.90	0.91	0.90	0.90	0.87	0.89	0.93	1.00
17	M153	0.88	0.91	0.93	0.91	0.90	0.93	0.91	0.91	0.87	0.90	0.91	0.92	0.90	0.90	0.90	0.92

5.3.8 Combined morphological and AFLP cluster analysis

Four main clusters were generated for the combined morphological and AFLP dendrogram with a total similarity value of 0.871 (Figure 5.5). Cluster I comprised of only a single accession, M101, a breeding line from South Africa at a genetic distance of 0.878. Cluster II contained the majority of the accessions, similar to that of the morphological dendrogram (Figure 5.1), at a genetic distance of 0.902. The accessions were collected from different places at the MARC, ICRISAT, PU and ARC-GCI/South Africa. Mamolokwane and Masekaswere, landrace accessions from South Africa grouped together within this cluster, while they were separated in the AFLP dendrogram (Figure 5.3), but grouped together in the morphological dendrogram (Figure 5.1). M153 was close to these accessions using AFLP and combined data. AFLP and combined dendrograms also grouped accessions 97MW6129 and 97MW6127 together both from MARC based on pedigree relationships as well as geographical location. Similarly, accessions M48 and M163 clustered together using morphological and combined dendrograms. Cluster III contained five accessions, with all of them from South Africa except for 216743 which was from the Gambella region in Ethiopia. Accessions 216743 clustered together with Macia-SA, but separately from the other accessions in the cluster

at a genetic distance similarity of 0.909. Accessions 216743 and Macia-SA had a genetic distance similarity of 0.935 and also clustered together in the AFLP dendrogram. Cluster IV revealed only one distinct accession, 216737, similar to that of the morphological dendrogram. However, it grouped together with 216743 and Macia-SA in the AFLP dendrogram.

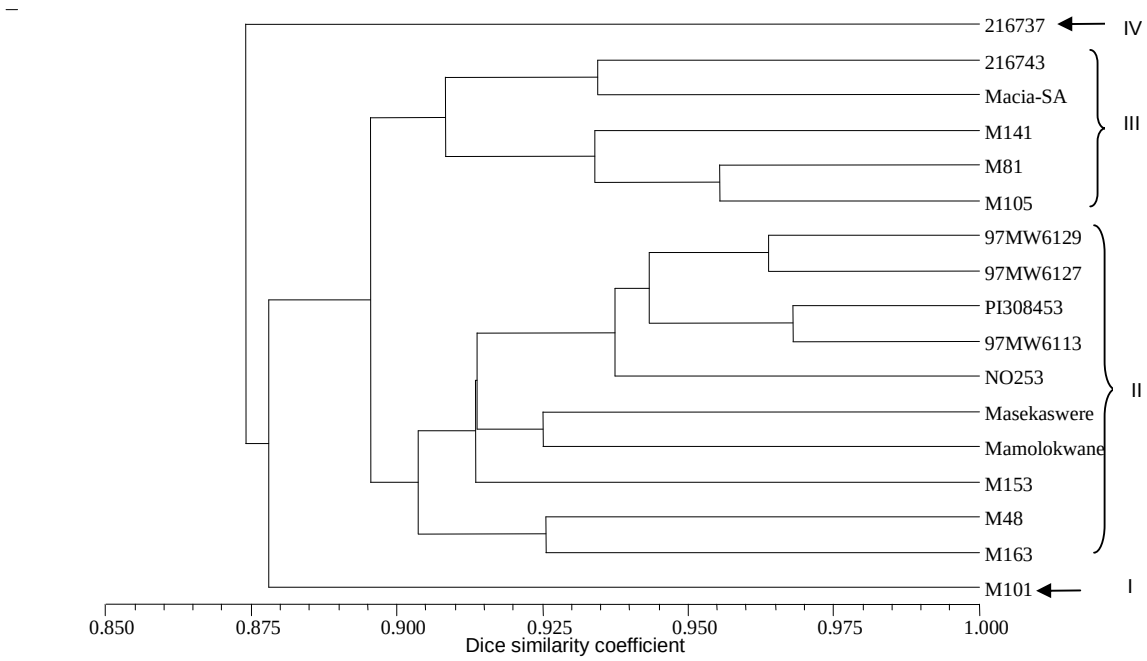

Figure 5.5 Combined AFLP and morphological data of 17 sorghum accessions using Dice similarity coefficient employing NTSYS-pc

Based on the dendrograms, the results indicated some relationship among accessions that might be due to human selection for the unique traits existing between accessions (Camussi et al., 1985; Ahnert et al., 1996; Dillon et al., 2005). The genetic diversity analysis using association of AFLP and morphological data is more powerful because it covers both the phenotypes as well as the polymorphisms on DNA level. Thus, the AFLP and combined analysis is more effective than using morphological data alone. Conventional breeding methods in combination with molecular markers have been reported in development of elite productive varieties in sorghum (Geleta, 2003). Similarly, Abu Assar et al. (2005) reported that combining the molecular information and morphological traits enhanced the incorporation of desirable genes into well-adapted cultivars and landraces.

5.3.9 Principal co-ordinate analysis based on combined morphology and AFLP data

The PCoA for combined analysis is presented in Figure 5.6. The PCoA separated M101 from the rest of accessions as in the combined dendrogram (Figure 5.5). This suggests that there was a significant contribution of AFLP data to the construction of the combined dendrogram. PCoA separated accession NO253 from the rest of accessions in cluster II. Similarly, PCoA separated accessions M141, M163 and Masekaswere from their respective groups in the dendrogram and clustered them together. Accession 216737 clustered as a single accession in the dendrogram but PCoA clustered it with accessions Macia-SA, M105 and M81. PCoA separated accession 216743 from its cluster in the dendrogram and positioned it as a single accession. The separation of the accessions such as M101 was confirmed and apparent with cluster analysis (Figure 5.5). This accession was from South Africa. Therefore, selection of such distinct accession as parent lines for the future sorghum improvement programmes is of paramount importance to gain genetic potential.

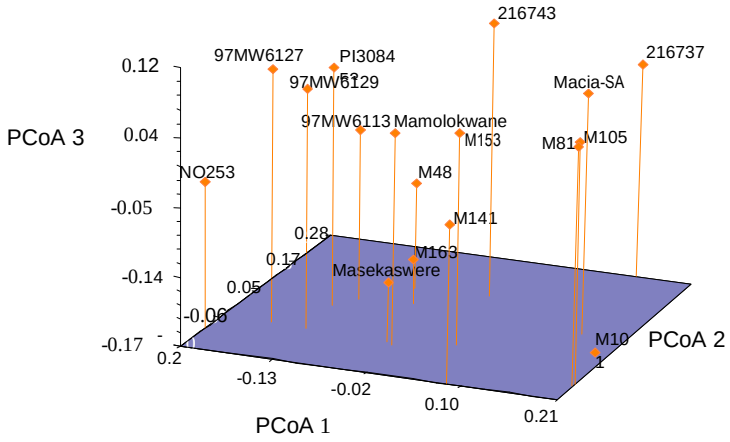

Figure 5.6 Principal co-ordinate analysis biplot for 17 characterised sorghum accessions using combined morphological and AFLP markers with the aid of NTSYS-pc

5.4 Conclusions

The morphological traits used were able to distinguish between accessions and the AFLP markers complemented the data obtained to separate the most similar accessions. Genetic similarity was lower in the morphological data, but was very high in the AFLP data. The combination of the two types of characterisation showed slightly lower similarity than with AFLP data alone. Combining of both morphological and molecular markers obtained more precise information about the accessions. Breeders and curators of collections will probably feel more comfortable working with both morphological and AFLP data, and combining the data for both breeding and conservation purposes. Once again it was clear that there was very little genetic diversity in the tested data set, although the material came from two different breeding programmes. Both breeding programmes will benefit from introducing unrelated material to broaden the genetic base of the breeding material.

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CHAPTER 6

GENETIC VARIABILITY AMONG SORGHUM ACCESSIONS FOR SEED STARCH AND STALK TOTAL SUGAR CONTENT

Abstract

Sorghum is a staple food grain in many semi-arid and tropical areas of the world, notably in sub-Saharan Africa because of its adaptation to harsh environments. Among important biochemical components for sorghum to processes are levels of starch (amylose and amylopectin) and total sugar contents. The objective of this study was to determine the genetic variation for total starch in the seed, its components and total sugar in the stalks of the sorghum accessions from Ethiopia and South Africa. Samples of 22 sorghum accessions were evaluated. Accessions showed a significant variation in total starch (31.01 to 64.88%), amylose (14.05 to 18.91%), amylose/amylopectin ratio (0.31 to 0.73) and total stalk sugar content (9.36 to 16.84%). The variation found among the sorghum accessions shows that improved total starch and starch components and stalk sugar contents can be achieved through crossing selected genotypes.

6.1 Introduction

For millions of people in the developing countries, mainly in Africa, sorghum is an important staple food. This crop sustains the lives of many poor people in rural areas. Improvements in production, availability, storage, utilisation and consumption of sorghum will significantly contribute to these communities as source of food security and nutrition (FAO, 1995).

The world sorghum economy consists of a traditional, subsistence, smallholder farming sector mainly in Africa and Asia where most production is consumed directly as food and a modern, mechanised, high-input, large-scale sector, mainly in the developed countries and in Latin America where output is used largely as animal feed. The sorghum economy is linked with its contribution to food security and efficient use of water in drought-prone regions in much of the developed world (Léder, 2004). As a food crop, sorghum can provide good nutrition in the human diet (Mudjisihino and Damardjati, 1987). More than 35% of sorghum is grown directly for human consumption; the rest is used primarily for animal feed, alcohol production and industrial products (FAO, 1995;

Awika and Rooney, 2004; Dicko et al. 2006; Mehmood et al., 2008). This crop can also be grown either alone or in mixture with legume fodders for nutritious and palatable fodder production. It can tolerate drought and hot weather and can be successfully grown on all types of soils except water logged and saline soils. Several improved sorghum varieties adapted to semi-arid and tropic environments are released every year by breeders. Selection of varieties meeting specific local food and industrial requirements is very important (Anglani, 1998).

Cereal grains contain macronutrients (carbohydrate, protein and fat) required by humans for growth and maintenance (David, 2007). Starch is the predominant storage carbohydrate in plants and the most important source of carbohydrate in the human diet where it is an important source of energy (Bednar et al., 2000). In sorghum grain, starch is the major proximate component (63 to 74%) and the major energy supplier in sorghum grain (Perez-Maldonado and Rodriguez, 2007). Starch granules consist of a linear polysaccharide called amylose (20-30% of starch) and a highly branched polysaccharide called amylopectin (70-80% of starch). The two starch components have different properties and are not suitable for the same applications (Zobel, 1988). The total starch and the relative proportion of amylose and amylopectin vary considerably within plant species, plant organs, and depends on organ development and growth conditions. Sorghum starch granules are surrounded by a protein matrix that can limit access of enzymes (Oria et al., 2000; Benmoussa et al., 2006). Sorghum has resistant starch, which makes it of interest for obese and diabetic people. In addition, sorghum may be an alternative food for people who are allergic to gluten. Typical levels of amylose and amylopectin in cereal starches are 25-28% and 72-75% respectively, although, for starches of some botanical sources, high amylose (up to 70% amylose) and waxy (<1% amylose) genotypes also exist (Colonna and Buléon, 1992).

Sorghum varieties containing 10 to 25% sugar in their stalks near the time of grain maturity that can be used to produce sorghum sugar or syrup (Hunter and Anderson, 1997). This importantly adds another benefit for farmers for the stalks, and genotypes with both good yield and sugary stalks can be selected by breeders for specific dual type cultivars. Hence, the objective of the study was to determine the starch contents, and its components in seed, as well as total sugar in stalks of sorghum accessions.

6.2 Materials and methods

6.2.1 Plant material

The sorghum accessions used for phenotypic characterization (Chapter 3, Table 3.1) were used for determination of total starch and its components as well as stalk sugar content for two consecutive seasons.

6.2.2 Starch extraction

Starch content was determined using a Total Starch Assay procedure [Amyloglucosidase/α-Amylase Method (Megazyme International Ireland Ltd, Bray, Ireland)]. Sorghum seeds were cleaned manually and ground using an IKA Analysis A10 Grinder. One hundred mg of each sorghum flour sample was weighed into test tubes. The samples were wetted with 0.2 ml aqueous ethanol (80% v/v) to aid dispersion and mixed on a vortex mixer. Dimethyl sulphoxide (DMSO, 2 ml) was added to the samples immediately after which they were vigorously stirred on a vortex mixer. The samples were incubated in a boiling water bath at 95˚C for 5 min. Three ml of thermostable α-amylase in 3-morpholinopropanesulfonic acid (MOPS) buffer was added to the samples and vigorously mixed using a vortex mixer. The samples were then incubated in a boiling water bath at 95˚C for 6 min while stirring after 2 and 4 min using a vortex mixer. Four ml sodium acetate buffer and 0.1 ml amyloglucosidase were added to each tube and vortexed. The samples were incubated in a water bath at 50˚C for 30 min followed by the addition of 0.1 ml distilled water. The samples were mixed thoroughly and centrifuged at 3000 rpm for 10 min. One ml aliquot from each tube was transferred into new tubes, this was done in duplicates. Nine ml distilled water was added to duplicates and the tubes were shaken. Duplicate aliquots of 0.1 ml of diluted solution were transferred to the bottom of new glass test tubes and 3.0 ml glucose oxidase peroxidase 4-aminoantipyrine (GOPOD) reagent was added to each tube (including the glucose controls and reagent blanks) and incubated at 50˚C for 20 min. The absorbance was read against the reagent blank at 510 nm for each sample, including the glucose control. The percentage total starch (dry weight basis) was calculated as follows:

$$\text{Starch} = [\Delta A \times F \times 1000 \times 1/1000 \times 100/W \times 162/180]$$
$$= \Delta A \times F/W \times 90$$

Where:

ΔA = Absorbance (reaction) read against the reagent blank

F = 100 (µg of glucose)/Absorbance of 100 µg glucose
1000 = Volume correction (0.1 ml taken from 100 ml)
1/1000 = Conversion from micrograms to milligrams
100/W = Factor to express “starch” as a percentage of flour weight
W = The weight in milligrams (“as is” basis) of the flour analysed
162/180 = Adjustment from free glucose to anhydro glucose (as occurs in starch)

6.2.3 Amylose/amylopectin content determination

Amylose content was extracted and estimated by the iodine binding method (Cruz and Khush, 2000). One hundred mg of sorghum flour sample was weighed. The samples were wetted with addition of 1 ml of 95% (v/v) ethanol followed by 9 ml of 1 M NaOH to aid dispersion and stirred using a vortex mixer. The samples were placed in a boiling water bath for 15 min and stirred using a vortex mixer every 5 min. The samples were cooled at room temperature for 1 h and then centrifuged at 3000 rpm for 5 min. Duplicate 0.1 ml aliquots of the solution were transferred into clean test tubes and 0.1 ml of 1 M acetic acid was added to each test tube followed by addition of 0.2 ml iodine solution and 9.6 ml distilled water. The contents were vortexed and left to stand for 20 min. The absorbance was read against the reagent blank at 620 nm for each sample. The amylose percentage was calculated using the formula:

Amylose% = {[Concentration (mg/ml) × 1000] / Mass of the sample (mg)} × 100

Amylopectin was calculated from as 100 – amylose %.

6.2.4 Sugar content determination

The total sugar content in stalks at physiological maturity was estimated as the Brix% using a hand held refractometer. The refractometer was calibrated with distilled water, and the sugar content was measured. The samples were taken from the third internode from the base of the plant for uniformity of sampling. This portion of the stalk seemed to be the sweetest throughout the growing season and after the plants reached the physiological maturity stage. The sap was squeezed and extracted from the cut stalks with pliers and placed on a hand held refractometer, after which readings were taken.

6.2.5 Statistical analysis

The data for the starch and its components was subjected to ANOVA using Agrobase (2005) for the 2009 and 2010 cropping seasons. A combined ANOVA over two years was also done for each parameter measured.

6.3 Results and discussion

The ANOVA revealed that there were highly significant (P≤ 0.01) differences (Table 6.1) among the accessions, suggesting that there was a high degree of variability among them for all measured parameters. Variation was also observed in the mean values for the same parameters for the accessions grown in the 2009 and 2010 seasons. The accessions from the 2009 cropping season had an almost 10% lower total starch content than in 2010. Amylopectin and amylose:amylopectin ratio were similar for the two seasons. Stalk sugar content was slightly higher in 2010. The lower starch values in 2009 might have been due to a much lower rainfall in 2009 compared to 2010 (Appendix I). Temperatures in the first three months after planting were 1-2^{o}C warmer in 2009 than in 2010, but the following three months (February to April) were 1-2^{o}C cooler than in 2010. This could have influenced the measured values. Ellies et al. (1998) reported that starch content is influenced by the genetic and environmental conditions.

Highly significant variation was seen for all variables in the combined ANOVA (Table 6.2). Total starch content ranged from 44.39 to 68.05% in the present study. Among all accessions, the highest value was recorded in accession 97MW6113 (from Ehtiopia); while the lowest was found in Birmash (also from Ethiopia). Intermediate starch content was obtained in accessions NO253 and PI308453. Wester et al. (1992) reported similar starch content for 48 commercial grain sorghum hybrids.

Table 6.1 Means, mean squares, least significant differences and coefficient of variation for total starch, and its components[a] and stalk sugar in sorghum accessions for the 2009 and 2010 cropping seasons

		Year 2009					Year 2010				
No.	Accessions	Starch	Am	Amp	Am:Amp[b]	Total sugar	Starch	Am	Amp	Am:Amp[b]	Total sugar
1	216737	47.86	14.53	83.47	0.17	16.33	57.64	13.57	86.43	0.16	17.02
2	216743	66.52	13.05	86.95	0.15	13.7	57.53	15.61	84.39	0.19	13.42
3	Birmash	31.01	14.9	85.1	0.18	13.85	57.77	16.02	83.98	0.19	9.68
4	Gambella-1107	55.02	16.31	83.69	0.19	3.69	62.96	16	84	0.19	11.33
5	IS9302	49.34	18.1	81.9	0.22	10.34	55.86	18.8	81.2	0.23	14.33
6	Baji	54.32	18.1	81.9	0.22	16.19	47.31	18.23	81.77	0.22	15.57
7	97MW6129	51.94	19.91	80.09	0.25	11.23	59.22	16.82	83.18	0.2	14.61
8	97MW6127	36.78	18.8	81.2	0.23	11.53	53.96	15.82	84.18	0.19	14.79
9	NO253	49.95	12.98	87.02	0.15	14.35	60.25	18.02	81.98	0.22	11.04
10	PI308453	49.96	15.34	84.66	0.18	14.6	59.9	17.53	82.47	0.21	8.27
11	97MW6113	61.54	15.19	84.81	0.18	16.85	74.57	18.22	81.78	0.22	10.4
12	Macia-SA	60.86	17.53	82.47	0.21	12.59	57.86	15.23	84.77	0.18	14.36
13	M48	64.88	17.7	82.3	0.22	6.17	62.77	17.07	82.93	0.21	12.56
14	M141	56.34	17.69	82.31	0.21	14.62	56.8	15.23	84.77	0.18	19.07
15	M81	34.23	16.59	83.41	0.2	7.99	64.44	15.37	84.63	0.18	12.83
16	M105	39.78	18.09	81.91	0.22	13.94	63.33	14.71	85.29	0.17	13.52
17	M26	52.88	20.47	79.53	0.26	15.93	54.83	15.77	84.23	0.19	14.25
18	M101	47.14	17.53	82.47	0.21	16.64	63.9	20.29	79.71	0.25	16.57
19	M163	51.75	13.31	86.69	0.15	10.55	59.43	16.34	83.66	0.19	15.15
20	Masekaswere	37.6	17.85	82.15	0.22	18.58	57.21	16.69	83.31	0.2	14.95
21	Mamolokwane	35.56	17.97	82.03	0.22	15.65	63.99	14.6	85.4	0.17	14.37
22	M153	54.47	14.12	85.88	0.16	13.67	64.39	15.58	84.42	0.18	20.78
	Mean squares	303.377**	14.081**	14.081**	0.003**	40.324**	84.391**	7.486**	7.486**	0.002**	25.411**
	LSD	3.07	1.21	1.21	0.01	1.45	3.43	1.5	1.5	0.02	1.25
	CV%	3.76	4.42	0.88	5.64	6.72	3.48	5.56	1.09	7.07	5.44
	Mean	49.53	16.64	83.36	0.2	13.13	59.81	16.43	83.57	0.2	14.04

**P≤ 0.01; [a]Mean percent values of total starch and its components in kernels of diverse sorghum accessions Am=Amylose; Amp=Aylopectin; [b]Am:Amp=Amylose to amylopectin ratio

Table 6.2 Means, mean squares, least significant difference and coefficient of variation for the starch and its components averaged over two years

No.	Accessions	Starch	Amylose	Amylopectin	Am:Amp[b]	Total sugar
1	216737	52.75	14.05	85.95	0.16	16.68
2	216743	62.02	14.33	85.67	0.17	13.56
3	Birmash	44.39	15.46	84.54	0.18	11.76
4	Gambella-1107	58.99	16.16	83.85	0.19	7.51
5	IS9302	52.60	18.45	81.55	0.23	12.33
6	Baji	50.81	18.16	81.84	0.22	15.88
7	97MW6129	55.58	18.36	81.64	0.23	12.92
8	97MW6127	45.37	17.31	82.69	0.21	13.16
9	NO253	55.10	15.50	84.50	0.19	12.70
10	PI308453	54.93	16.43	83.57	0.20	11.44
11	97MW6113	68.05	16.71	83.29	0.20	13.63
12	Macia-SA	59.36	16.38	83.62	0.19	13.48
13	M48	63.82	17.38	82.62	0.21	9.36
14	M141	56.57	16.46	83.54	0.20	16.84
15	M81	49.33	15.98	84.02	0.19	10.41
16	M105	51.55	16.40	83.60	0.20	13.73
17	M26	53.86	18.12	81.88	0.22	15.09
18	M101	55.52	18.91	81.09	0.23	16.60
19	M163	55.59	14.83	85.17	0.17	12.85
20	Masekaswere	47.40	17.27	82.73	0.21	16.76
21	Mamolokwane	49.77	16.28	83.72	0.20	15.01
22	M153	59.43	14.85	85.15	0.17	17.22
	Mean squares	182.851**	10.329**	10.329**	0.002**	26.156**
	LSD	2.26	0.95	0.95	0.01	0.94
	CV%	3.61	5.02	0.99	6.38	6.07
	Mean	54.67	16.54	83.47	0.20	13.59

**p≤ 0.01; [b]Am:Amp= amylose/amylopectin ratio

Amylose content is important for food processing in the industry and for quality. From the accessions studied, the highest amylose content was recorded in accession M101 (18.91%, from South Africa); while the lowest was found in accessions 216737 (14.05%) and 216743 (14.33%), both from Ethiopia. These values were lower than those reported by the FAO (1995), Beta et al. (2000), Beta and Corke (2001), Geleta (2003), Léder (2004), Geleta et al. (2005), Salinas et al. (2006), Chanapamokkhot and Thongngam (2007) and Boudries et al. (2009) but within the range of what McDonouch et al. (1998) reported (2.0 to 28%). Benmoussa et al. (2006) found that the amylose content ranged between 19.2 to 22.4%. Further more, Beta and Corke (2001) reported amylose content ranging from 20.9 to 30.2%, while Salinas et al. (2006) reported the amylose content

from 25.28 to 28.26%. Grains with low amylose contents (1-2%) are termed “waxy”, and are associated with homogenous recessive genes (*wxwxwx*). Non-waxy grains are those with normal levels of amylose (23-28%). According to Ring et al. (1982), accessions grouped as heterowaxy contain lower amylose content than non-waxy starches (24 to 30%). Heterowaxy grains have lower amylose contents than normal grains (20%) but display many of the physical attributes of non-waxy grains (McDonough et al. 1998). Both genetic and environmental factors affect the amylose content of sorghum (Ring et al., 1982). Wang et al. (2008) reported that waxy and heterowaxy sorghum varieties have higher ethanol yields than non-waxy varieties, at the same starch level.

The amylose content has been reported to vary with the botanical source of the starch and is affected by the climatic and soil conditions during grain development (Yano et al., 1985; Boundries et al. 2009). It was also reported that environmental and genetic factors determine amylose levels in sorghum (Ring et al., 1982; Taylor et al., 1997). Beta and Corke (2001) reported that sorghum genotype and growing environment significantly affect amylose content. Amylose content of rice was also reported to be affected by both environment and genotype (Juliano et al., 1965; Paule, 1977). It is possible to increase the amylose content of seed through crossing, as large variation is observed in grain sorghum. In this study there was a 4.86% difference between the highest and lowest amylopectin values, which leaves some options for selection and recombination to increase amylopectin, if needed. The values for Ethiopian and South African material were very similar (16.45 and 16.62%, data not shown).

Amylose/amylopectin ratio is an important characteristic of starch that governs much of starch functionality. The ratio of amylose to amylopectin varied from 0.16 to 0.23. The highest ratio was observed in accessions IS9302, 97MW6127 and M101. The values obtained were lower than those reported by Arora and Luthra (1972) and Boudries et al. (2009).

The studies by Arora and Luthra (1972), Sabramanian et al. (1987), FAO (1995), Ali et al. (2008) and Audilakshmi et al. (2010) were conducted earlier to estimate total stalk sugars in various sorghum accessions. In the current study the content of total stalk sugar ranged from 7.51 to 17.22% among the accessions. The highest total sugar content of all accessions measured was in M153 (17.22%) and M141 (16.84); while the lowest total sugar content cultivar was Gambella-1107. Accessions M153 and M141 may have good genetic potential for future use as dual purpose sorghum types for sugar and grain production. Sweet sorghum fodders may contain up to 21% total sugars (Coleman,

1970). Hunter and Anderson (1997) reported that sorghum varieties which contained 10 to 25% sugar in their stalks near the time of grain maturity can be used to produce sorghum sugar. Therefore, selection and breeding accessions of higher grain yield as well as higher sugar content is important in future sorghum breeding. Rajvanshi and Nimbkar (2008) suggested that the production of grain and sugar from the same plant are very important attributes since food and fuel can be produced simultaneously from the same piece of land. The sugar content of the South African material was on average 1.43% higher than that of the Ethiopia material, but the potential is there to select high sugar yielding cultivars from both sets of material.

These values were higher than the values reported by Ragaee et al. (2006) and Boudries et al. (2009) but lower than values reported by Owuama (1997), Lee et al. (2002), Geleta (2003), Osman (2004), Salinas et al. (2006) and Sang et al. (2008). Wang et al. (2008) found that starch content in sorghum genotypes ranged between 64 and 74% of grain dry weight. The average values for Ethiopian and South African material were very similar (54.6 and 54.75, data not shown).

6.4 Conclusions

The results revealed very high variation for total starch among the sorghum accessions (23.7% difference between the highest and lowest), which will certainly allow for selection for specific applications of cultivars, for example for bio-fuel. The range of amylose content was smaller, but some variation was seen. There was quite a large variation in the stalk sugar content (almost 10% difference between the highest and the lowest), which suggest very good potential of selection for dual purpose cultivars, where seed and sugar can be produced from the same plants.

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CHAPTER 7

VARIATION OF MINERAL AND PROTEIN CONTENTS OF SORGHUM ACCESSIONS

Abstract

Among the factors affecting grain nutritional quality, mineral and protein content are important, but little attention has been given to determining these concentrations in grain sorghum improvement programmes. The concentration of the mineral elements and protein content in sorghum vary due to genotypic and environmental influences and genotype by environment interactions. The objective of this study was to determine the contents of eight mineral elements (Ca, Fe, K, Mn, Na, P, Zn and Mg) and protein in sorghum accessions for two consecutive seasons. Varietal and seasonal differences were observed in all the determined parameters. The average values combined over years for accessions showed a wide variation in protein (7.20 to 11.00%), Ca (121.60-279.85 mg kg^{-1}), Fe (22.59-37.65 mg kg^{-1}), K (1492.33-2575.00 mg kg^{-1}), Mn (11.28-18.54 mg kg^{-1}), Na (14.13-39.52 mg kg^{-1}), P (2050.83-3452.83 mg kg^{-1}), Zn (20.89-33.42 mg kg^{-1}), and Mg (977.50-1390.33 mg kg^{-1}) concentrations. A significant degree of association was observed between Ca and P; Zn and Fe, Mn and P and between protein and Mn, P and Zn. The principal component analysis showed that Fe, Mn, P, Zn and protein contributed largely to clustering of the accessions in the PC1; Ca, P and Mg to PC2 and Ca, K and Na to PC3. The presence of a considerable degree of compositional variability of mineral and protein contents among tested accessions suggests that sorghum can be a valuable source of genes for improved mineral and protein content in sorghum.

7.1 Introduction

In Africa, India and China, grain sorghum ranks third among cereals for human consumption (El Khalifa and El Tinay, 2002). Sorghum has an advantage of being drought tolerant and many subsistence farmers in these regions cultivate it as a staple food crop for consumption (Murty and Kumar, 1995). Sorghum contains protein (6-25%), ash (1.2-1.8%), oil (3.4-3.5%), fiber (2.3-2.7%) and carbohydrate (71.4-80.7%) with dry matter ranging from 89.2 to 95.3% depending on the type of cultivar (Lasztity, 1996; Samia et al., 2005; Idris et al., 2007). Further, they reported that sorghum flour contained 11.0-13.0, 285-310 and 4.0-5.50 mg 100 g^{-1} Ca, P and Fe, respectively. Sorghum is

reported to be a good source of more than 20 minerals (BSTID-NRC, 1996; Dicko et al., 2006) and is also rich in P, K, Fe, and Zn (Glew et al., 1997; Anglani, 1998; Dicko et al. 2006).

Furthermore, it is a staple crop for people living across water-stressed regions in Ethiopia and acts as a source of energy, protein, vitamins and minerals (Klopfenstein and Hoseney, 1995). Breeding of cereal crops with increased protein and micronutrient concentration requires genetic variation in the trait among germplasm (Morgounov et al., 2007). Selection of varieties for a higher concentration of mineral elements and crossing them with those revealing lower concentrations of mineral elements through evaluation help to improve human health (Gorz et al. 1987). Previous work with several plant species suggested that mineral concentration was under genetic control and that considerable progress was possible by selecting for either high or low mineral accumulation (Gorsline et al., 1964; Kleese et al., 1968; Hill and Jung, 1975; Gorz et al. 1987).

Intensive plant breeding programmes have increased yields of sorghum grain but little attention has been paid to the nutritional quality of the grain. Therefore, the objective of this study was to study the variation in mineral elements and protein contents of grain of sorghum accessions and determine association between them, if any, over two consecutive seasons.

7.2 Materials and methods

7.2.1 Plant material

The sorghum accessions used for phenotypic characterisation (Chapter 3, Table 3.1) were used for quantification of mineral elements and crude protein content.

7.2.2 Mineral analyses

Sorghum kernels were ground to a fine powder with an IKA Analysis A10 Grinder. Two gram flour samples were weighed, placed into porcelain crucibles and the dry ashing procedures were used for determination of Ca, Mg, Mn, Zn, Fe, Na, K and P in the Soil Laboratory, Department of Soil, Crop and Climate Sciences at the University of the Free State. Samples were placed in a muffle furnace at 550˚C for 3 h after which samples were acid-digested by addition of 1 ml of 55% (v/v) HNO_3 after cooling. The acid was

evaporated to dry from the samples using a sand bath and oven dried in the muffle furnace again. Ten ml of 1:2 v/v 55% HNO_3 solution was added to the samples to moisten them and placed in the sand bath for 5-10 min to warm it up. The samples were stirred in porcelain crucibles using glass rods and transferred into 100 ml volumetric flasks. The samples were shaken immediately and allowed to dissolve overnight to extract the minerals. The samples were then transferred into glass test tubes and diluted with distilled water 100 times. Mineral concentrations were then determined by an Atomic Absorption Spectrophotometer (SpectrAA 300).

7.2.3 Protein content determination

Flour sample (250 mg) was weighed, oven dried over night at 95˚C and protein content (N×6.25) was determined by the combustion method (Leco ®model, FP-528, St. Joseph, Ml) in the Nutritional Laboratory, Department of Animal, Wild and Grassland Sciences, University of the Free State.

7.2.4 Statistical data analysis

Agrobase Generation II software (Agrobase, 2005) was used for ANOVA. Bivariate Pearson's correlation coefficient test was applied to assess the significance of degree of association in protein content and concentrations among mineral elements using NCSS (2004). Standardisation of data was carried out as described in Chapter 3, Section 3.2.3.1. Principal component analysis (PCA) was used to visualise the pattern of variation of the data.

7.3 Results and discussion

7.3.1 Mineral and protein content

Significant differences (P≤ 0.01) among accessions were found for the concentration of mineral elements and protein content (Tables 7.1 and 7.2) for the 2009 and 2010 seasons. Accessions Macia-SA (279.85 mg kg^{-1}) and 97MW6127 (218.70 mg $kg^{-}1$) were significantly higher in Ca content compared to all other accessions (Table 7.2). These were higher than values reported by Mohammed et al. (2010).

Table 7.1 Means, mean squares, least significant differences and coefficient of variation for mineral elements and protein content in sorghum accessions during 2009 and 2010 seasons

		Mineral elements (mg kg^{-1} dried basis)																	
		Year 2009									Year 2010								
No.	Accession	Ca	Fe	K	Mn	Na	P	Zn	Mg	Protein	Ca	Fe	K	Mn	Na	P	Zn	Mg	Protein %
	216737	277.50	55.13	1262.50	17.50	30.83	2944.50	22.00	1237.50	10.75	67.53	20.17	2752.00	14.33	48.23	3456	44.83	1250	10.33
2	216743	237.50	46.00	1300.00	13.75	17.75	2861.00	18.25	1159.38	10.44	67.27	16.50	2720.70	15.10	15.27	3246	35.07	1300	8.90
3	Birmash	240.00	34.50	1325.00	11.33	24.75	2557.75	18.75	1368.77	9.71	49.13	17.80	2450.30	11.23	16.00	2855	33.57	1300	6.50
4	Gambella-1107	308.33	38.00	1637.50	15.33	24.25	3357.00	19.17	1631.17	11.76	78.37	18.50	2902.00	20.17	19.37	3542	44.77	1149	10.24
5	IS9302	248.75	45.50	1643.77	19.75	34.00	2565.47	21.25	1368.77	8.92	51.47	17.17	2407.00	12.83	33.87	3262	30.83	1261	8.14
6	Baji	252.50	34.83	1412.50	14.17	21.13	2454.04	17.67	1237.50	10.04	60.87	19.83	2635.00	15.17	43.20	2824	35.23	1334	8.72
7	97MW6129	223.75	31.50	1487.50	19.38	17.00	2293.38	17.75	1150.00	9.24	79.10	18.10	3085.00	15.00	62.03	3535	39.57	1243	8.66
8	97MW6127	371.25	36.75	1037.50	12.75	14.50	3529.25	17.75	1062.50	9.58	66.13	18.50	2473.00	15.10	42.17	3376	33.00	892	9.22
9	NO253	200.00	41.25	950.00	14.00	19.00	2429.50	15.63	1168.77	9.51	44.57	15.57	2034.70	12.17	16.77	3449	24.37	1001	9.14
10	PI308453	229.38	34.50	1181.25	11.33	19.00	2560.50	18.25	946.88	9.39	83.83	18.43	3254.00	16.33	16.50	3575	34.17	1360	8.23
11	97MW6113	268.00	35.67	1337.50	16.63	38.75	2129.15	15.83	1046.88	7.86	53.00	14.60	2342.00	11.17	14.40	2883	32.73	1135	6.53
12	Macia-SA	477.04	42.63	1625.00	13.38	21.75	3831.01	16.17	1159.17	11.99	82.67	13.73	2265.00	15.43	38.75	3075	34.83	854	8.62
13	M48	250.00	36.33	1440.63	14.38	16.38	2858.75	15.75	1212.50	10.43	56.50	15.77	2799.00	12.33	34.63	3266	34.40	1347	8.79
14	M141	266.25	28.75	1734.38	11.92	23.13	2367.57	14.33	1118.77	10.45	82.90	16.43	3415.70	13.77	46.60	3073	29.43	1366	7.68
15	M81	247.50	36.75	1231.25	22.75	22.25	2825.31	23.25	1137.50	11.54	75.43	19.17	2315.00	14.33	14.65	3433	35.50	1066	8.74
16	M105	218.75	34.25	1567.98	14.00	14.00	2144.25	12.00	1121.88	10.13	72.73	15.90	2635.00	14.10	14.27	3349	33.00	1268	9.20
17	M26	296.67	35.25	1548.44	17.58	20.50	2258.82	13.63	1340.42	10.22	57.23	13.50	2752.00	14.77	46.87	3193	37.00	1349	9.65
18	M101	327.50	43.50	2146.88	15.17	23.50	3555.19	20.00	1259.38	10.09	86.23	14.07	2322.70	14.43	44.20	3264	27.53	1149	9.29
19	M163	255.00	45.75	1229.69	12.50	58.63	2627.63	23.00	1156.27	11.59	76.23	18.33	2948.70	14.17	12.50	3235	31.77	1168	7.80
20	Masekaswere	267.50	42.38	1000.00	14.88	20.17	2469.82	19.50	1118.77	9.43	47.57	15.73	2751.00	11.50	32.77	3213	34.73	1327	7.31
21	Mamolokwane	195.00	33.00	900.00	16.88	15.25	2565.75	19.00	1131.17	9.40	48.20	19.43	2642.70	13.50	14.00	3775	38.70	1250	8.74
22	M153	296.00	44.13	1457.82	11.75	22.50	2042.58	12.75	928.89	9.45	77.37	14.07	3366.00	13.60	16.43	3226	29.03	1052	8.07
	Mean squares	114100**	113**	254496**	27**	296**	722891**	28.96**	68640**	3.034**	565**	13**	399021**	11.56**	701**	175331**	71**	67219**	2.99**
	LSD	24.72	4.01	39.99	1.53	4.22	38.12	1.29	17.77	1.28	2.11	1.61	40.87	1.16	1.94	4.47	4.36	150.30	0.83
	CV%	5.55	6.27	1.75	6.18	10.86	0.86	4.43	0.91	7.71	1.93	5.79	0.92	5.03	4.04	0.08	7.72	7.60	5.93
	Mean	270.37	38.92	1384.41	15.05	23.59	2692.19	17.80	1184.67	10.09	66.56	16.88	2694	14.12	29.25	3272.70	34.28	1201	8.57

**$p \leq 0.01$

Table 7.2 Means, mean squares, least significant differences and coefficient of variation for mineral elements and protein content in sorghum accessions over two cropping seasons

No.	Accession	Minerals (mg kg^{-1} dried basis)								
		Ca	Fe	K	Mn	Na	P	Zn	Mg	Protein%
1	216737	172.52	37.65	2007.33	15.92	39.53	3200.33	33.42	1243.83	10.54
2	216743	152.38	31.25	2010.33	14.43	16.51	3003.33	26.66	1229.67	9.67
3	Birmash	144.57	26.15	1887.67	11.28	20.38	2706.50	26.16	1334.50	8.11
4	Gambella-1107	193.35	28.25	2269.83	17.75	21.81	3449.50	31.97	1390.33	11.00
5	IS9302	150.12	31.33	2025.50	16.29	33.93	2913.67	26.04	1315.00	8.53
6	Baji	156.68	27.33	2023.83	14.67	32.16	2639.17	26.45	1285.83	9.38
7	97MW6129	151.43	24.80	2286.33	17.19	39.52	2913.50	28.66	1196.33	8.95
8	97MW6127	218.70	27.63	1755.33	13.93	28.33	3452.50	25.38	977.50	9.40
9	NO253	122.28	28.41	1492.33	13.08	17.88	2939.17	20.00	1085.17	9.32
10	PI308453	156.62	26.47	2217.67	13.83	17.75	3067.83	26.21	1153.45	8.81
11	97MW6113	160.50	25.13	1839.83	13.90	26.58	2505.83	24.28	1091.00	7.20
12	Macia-SA	279.85	28.18	1945.00	14.41	30.25	3452.83	25.50	1006.50	10.31
13	M48	153.25	26.05	2120.17	13.36	25.51	3062.50	25.08	1279.83	9.61
14	M141	174.58	22.59	2575.00	12.84	34.86	2720.17	21.88	1242.50	9.06
15	M81	161.47	27.96	1773.17	18.54	18.45	3129.00	29.38	1101.83	10.14
16	M105	145.75	25.08	2101.50	14.05	14.13	2746.50	22.50	1195.00	9.67
17	M26	176.95	24.38	2150.33	16.28	33.68	2725.83	25.31	1344.67	9.94
18	M101	206.87	28.78	2234.83	14.80	33.85	3409.50	23.77	1204.33	9.69
19	M163	165.62	32.04	2089.33	13.33	35.56	2931.50	27.38	1162.00	9.70
20	Masekaswere	157.53	29.06	1875.50	13.19	26.47	2841.50	27.12	1223.00	8.37
21	Mamolokwane	121.60	26.22	1771.33	15.19	14.63	3170.33	28.85	1190.67	9.07
22	M153	183.68	29.10	2412.00	2.68	19.47	2634.33	20.89	990.45	8.76
	Mean squares	4993**	65**	293957**	19**	591**	404625**	37**	57147**	1.65**
	LSD	12.22	2.13	28.19	0.94	2.29	18.89	2.24	74.56	0.75
	CV%	6.32	6.66	1.20	5.67	7.55	0.55	7.50	5.44	7.05
	Mean	168.47	27.90	2039.28	14.58	26.42	2982.52	26.04	1192.88	9.33

**p≤ 0.01

Among accessions, over the two seasons the highest concentration of Fe was recorded in accession 216737 (37.65 mg kg^{-1}); while the lowest was 22.59 mg kg^{-1} and found in accession M141. The Fe concentration was much higher in the first season. A reason for this could be that the rainfall and the yield were much higher in the second season, which diluted the concentration of some minerals, such as Fe, Ca and Zn. This is due to the amount of rainfall variation in the growing seasons (403 mm) 2009 and (867.66 mm) 2010 (Appendix I). The values in this study were lower than the findings of Miller and Boswell (1976); Lèder (2004); Waters and Pedersen (2009) and Mohammed et al. (2010) but were higher than the values reported by Asha et al. (2005). The present study indicated that accessions

Gambella-1107, 97MW6127, NO253, Macia-SA and M81 were similar in their Fe content. This indicated that they had some common characters in the uptake and biological process of this mineral element. Similarly substantial differences in K concentration was also observed ranging from 1492.33 to 2575 mg kg^{-1} with the highest being in accession M141 (2575 mg kg^{-1}) and lowest in accession NO253 compared to other accessions. The values were higher than those reported earlier (Lèder, 2004; Ragaee et al., 2006).

Mn content was very similar for the two seasons (Table 7.1). Over the two seasons (Table 7.2) Mn content varied from 11.28-18.54 (mg kg^{-1}). It was highest in accession M81 and lowest in accession Birmash. These values are lower than those reported by Waters and Pedersen (2009) but higher than those reported by Lèder (2004). Accessions 216743, 97MW6127, PI308453, 97MW6113, Macia-SA and M105 were similar in Mn content (Table 7.2). Accessions Birmash and M105 showed similar values for Mn and accessions IS9302 and M105 showed similar values for Na content (Table 7.1) compared to other accessions and would be selected as stable accessions. The Na concentration varied from 14.13 to 39.53 mg kg^{-1}. The highest concentration was found in 216737 and 97MW6129 compared to other accessions. These values were higher than values reported by Lèder (2004) but lower than those reported by Badi (2004) and Awadelkareem et al. (2009). The concentration of P varied from 2505.83 mg kg^{-1} in accession 97MW6113 to 3453 mg kg^{-1} in accessions Macia-SA and 97MW6127. The value obtained was higher than what has been reported by Mohammed et al. (2010).

The concentration of Zn over the two seasons (Table 7.2) ranged from 20.00 to 33.42 (mg kg^{-1}). The Zn content of accessions 216737 and Gambella-1107 was significantly higher than all other accessions. The Zn content of accession NO253 (20.00 mg kg^{-1}) was significantly lower than that of the other accessions, while accessions Birmash, IS9302, Baji, PI308453 and Macia-SA had similar Zn contents (26.00 mg kg^{-1}). These values were lower than those reported by Waters and Pedersen (2009) and Mohammed et al. (2010) but higher than those previously reported by Miller and Boswell (1976); Lèder (2004). Among all accessions, the highest concentration of Mg was recorded in the accession Gambella-1107 (1390.33 mg kg^{-1}); and the lowest in 97MW6127 (977.50 mg kg^{-1}). The values obtained were found to be higher than those reported by Miller and Boswell (1976) and Mohammed et al. (2010).

Protein content varied from 7.20 to 11.00%. The highest protein content was recorded in accession Gambella-1107 (11.00%); while the lowest was in 97MW6113 (7.20%) combined

over two years (Table 7.2). It was in the range reported by Subramanian and Jambunathan (1984) but lower than the values reported by Ragaee et al. (2006), Mohammed et al. (2010), Waters and Pedersen (2009) and Mokrane et al. (2010). Subramanian et al. (1990) reported grain protein content ranging from 6.80 to 19.60%. Ebadi et al. (2005) and Awadelkareem et al. (2009) also found protein content from 5.44 to 12.90% in sorghum grains. Similarly, Neucere and Sumrell (1980), Awadelkareem (2002) and Awadelkareem et al. (2009) found 10.00 to 14.00% protein content. The genotypes as well as the environment influence the protein content (Deosthale et al., 1972; Benzian et al., 1983; Ebadi et al., 2005).

Compared to all other accessions, accessions 216737, 97MW6127 and NO253 showed the most stable protein content over the two cropping seasons (Table 7.1). This trait is significantly affected by environmental factors that affect grain filling. This could explain the big difference in mean protein values for 2009 (10.09%) and 2010 (8.57%). This indicated that the protein content of sorghum grain grown under limited soil moisture is higher than that of sorghum grain produced with plentiful soil moisture (Table 7.1; Appendix I). The protein quality of sorghum depends on their protein content. Thus, genetic variation in protein content is important for sorghum improvement.

The accession, growing season and soil factors as well as the relationship of these factors highly influenced the variation in mineral composition and protein content of sorghum accessions. Of the mineral elements studied, P, K, Mg and Ca were the most abundant and small amounts of Fe, Na, Mn and Zn were present. Khalil et al. (1984) also reported that the highest concentrations of minerals present in sorghum grain are K, P, Ca and Mg. Fe values were lower than that reported by Lèder (2004) but values for Ca, K, Na, Mn, Zn and Mg were higher in the present study.

The highest correlations were between Ca with P; Mn with Zn and protein (Table 7.3). Ca was highly significantly positively correlated with P. Significant positive correlations between Zn and Fe, Mn, and P were observed. There was also highly significantly positive correlation between protein and Mn, P and Zn. Waters and Pedersen (2009) also reported positive correlation between sorghum grain protein and Zn and P. The mineral elements are important in the nutrition of humans and animals as well as their metabolic activities and inter-relationships that regulate other factors which are needed for living organisms like enzymes, anti-oxidants and vitamins. Hence, it is important to determine the mineral content of sorghum accessions for the future breeding programmes in Ethiopia and South Africa. Genotype, location and other weather conditions could influence the concentration levels of

the mineral elements in the crop plant. The variation found among the sorghum accessions combined over years shows that breeding for high concentration of mineral elements and protein content in the sorghum can be obtained by selecting appropriate genotypes for crosses. From the results of this study, the following accessions could be selected and incorporated into a sorghum improvement programme in two regions of Africa (Ethiopia and South Africa): accession Macia-SA (Ca and P), accession 216737 (Fe, Na and Zn), accession M141 (K), accession M81 (Mn), accession 97MW6129 (Na), accession 97MW6127 (P) and accession Gambella-1107 (Zn, Mg and protein). On average, Ca and K content was significantly higher in South African material and Mn was significantly higher in Ethiopian material (data not shown) which shows that there was a translocation difference of these minerals for the two sets of material. The content of the other minerals were very similar. The protein content of the two sets of material differed with only 0.3%. This is probably due to a lack of selection for protein in both breeding programmes, but the fact that some accessions had protein content of more that 10% indicates that there is selection potential.

Table 7.3 Phenotypic correlation among mineral elements and protein contents of 22 sorghum accessions

	Minerals (mg kg^{-1} dried basis)							
Variables	Ca	Fe	K	Mn	Na	P	Zn	Mg
Fe	0.09	1						
K	0.21	-0.20	1					
Mn	0.08	0.14	0.03	1				
Na	0.34	0.21	0.33	0.19	1			
P	0.53**	0.32	-0.14	0.38	0.03	1		
Zn	0.02	0.45*	-0.06	0.62**	0.22	0.42*	1	
Mg	-0.36	-0.03	0.28	0.22	0.18	0.38	0.38	1
Protein%	0.39	0.34	0.12	0.51**	0.09	0.41**	0.41**	0.14

**P≤ 0.01, *P≤ 0.05

7.3.2 Principal component analysis

Principal component analysis grouped the nine traits into nine components which accounted for the entire (100%) variability but there were only three principal components which had eigenvalues greater than one and cumulatively accounted for 68.72% of the total variation among the accessions (Table 7.4). Chatfield and Collins (1980) and Hair et al. (1998)

suggested that eigenvalues greater than one are considered significant and component loadings greater than ±0.30 were considered to be meaningful. Consequently, only the first three principal component axes were retained in this study and traits with loading greater than ±0.30 were viewed to represent the corresponding principal component axis.

Table 7.4 Eigenvalues, total variance and variable eigenvectors for nine principal components that describe the variation of nine measured variables in 22 sorghum accessions

PC	Eigenvalue	Total variance		Eigenvectors (loadings) for								
		Indiv. %	Cuml. %	Ca	Fe	K	Mn	Na	P	Zn	Mg	Protein%
1	2.9655	32.95	32.95	-0.27	-0.31	-0.04	-0.41	-0.21	-0.44	-0.44	-0.10	-0.47
2	1.7047	18.94	51.89	0.45	0.07	-0.28	-0.22	-0.18	0.34	-0.28	-0.66	0.07
3	1.5141	16.82	68.72	-0.47	0.25	-0.64	0.11	-0.48	0.07	0.25	0.00	-0.03
4	0.9865	10.96	79.68	-0.01	0.65	-0.17	-0.28	0.56	-0.20	0.11	-0.10	-0.31
5	0.6594	7.33	87.00	0.07	-0.43	-0.33	0.55	0.36	-0.09	0.15	-0.30	-0.38
6	0.4390	4.88	91.88	0.22	-0.34	-0.41	-0.44	0.18	0.37	0.11	0.51	-0.14
7	0.3637	4.04	95.92	-0.14	-0.00	-0.40	0.14	0.33	-0.18	-0.59	0.15	0.54
8	0.2107	2.34	98.27	0.58	0.00	-0.21	-0.02	-0.25	-0.67	0.26	0.12	0.18
9	0.1560	1.73	100.00	-0.32	-0.34	0.01	-0.41	0.22	-0.12	0.46	-0.40	0.44

Indiv. %=individual percent, Cuml. %=cumulative percent

The proportion of the total variance explained by each principal component is additive, with each new component contributing less than the preceding one to the explained variance. The first principal component which alone explained 32.95% of the total variability among the accessions was mainly due to variations in Fe, Mn, P, Zn and protein with high negative loading (Table 7.4). The sign of the loading indicated the direction of the relationship between the components and the variables (Johnson, 1998). The second principal component which accounted for 18.94% of the total variation was predominantly a function of Ca, P and Mg with positive and negative loading, respectively. Accessions with high PCA2 scores, therefore, would have high Ca, P and Mg values. The third principal component with 16.82% variance separated the accessions on Ca, K and Na all with negative loadings. The PC1 and PC2 explained most of the variation among the accessions, depicting a high degree of association among the protein and mineral elements. The eigenvectors of the PC1 revealed large negative loadings for all variables and a few positive loading for the variables in PC2. A plot of the first principal component axis (PCA 1) against the second principal component axis (PCA 2) (Figure 7.1) revealed that Macia-SA, 97MW6127, NO253, M153, 216737 and Gambella-1107 were most distant from the major group which was concentrated around zero.

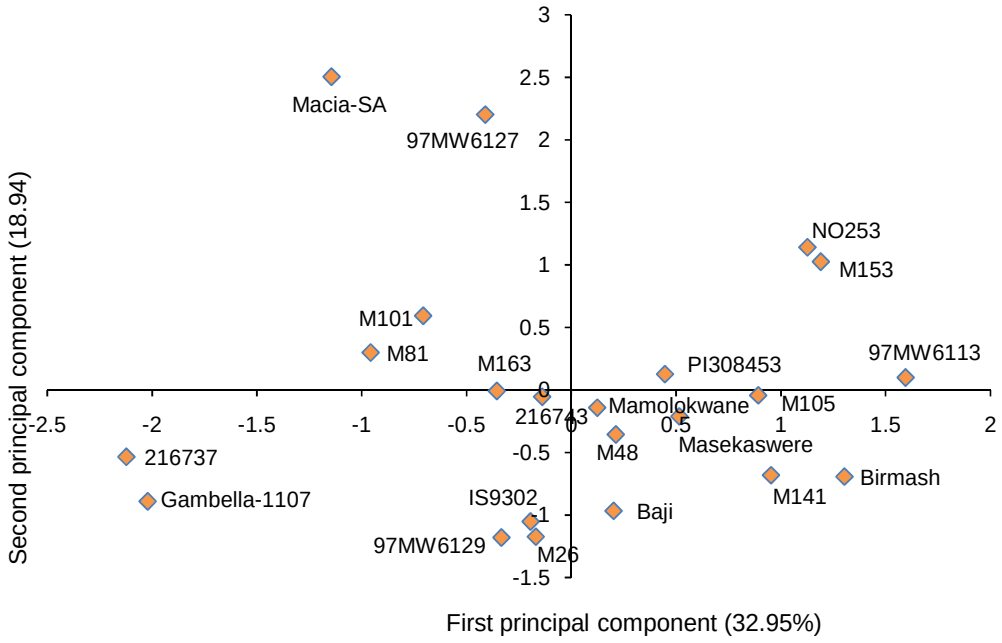

Figure 7.1 Configuration of the sorghum accessions under principal component axis 1 and 2

7.4 Conclusions

There was considerable variation among the accessions for mineral and protein content. It is conceivable that these differences are genetic and may have value as bio-chemical markers in sorghum breeding. Accessions with high concentration of the most important mineral elements and protein content are potential genetic sources for the future development of improved lines. With the exception of Ca, K and Mn, the mineral and protein contents of the material from Ethiopia and South Africa were similar. Principal component analysis elucidated three principal component axes that explained most of the variability among the accessions.

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CHAPTER 8

DIVERSITY IN STARCH, MINERAL AND PROTEIN COMPOSITION OF SORGHUM LANDRACE ACCESSIONS FROM ETHIOPIA

Abstract

Cereals, especially sorghum, are an important staple food crop in parts of Africa, Asia, and Latin America. In Ethiopia, sorghum is grown for food and cash income by subsistence farmers. The objective of this study was to determine the extent of genetic variation in protein, mineral composition, total starch and its components of sorghum landraces from Ethiopia. Sorghum whole grains were analyzed for crude protein, total starch and its components and mineral profile (Ca, Mg, K, P, Fe, Mn, Zn and Na). The analysis of variance showed significant differences among the sorghum landraces in nutritional composition. There was highly significant correlation between P and Mg; and between Zn and P and protein which indicated that some interaction existed in absorption and translocation between minerals and protein content. The PCA revealed that the first four principal components (PCs) contributed 71.77% of the variability among the landraces. Zn, Mn, Mg, P and protein contributed more to PC1, while Fe, Na and Ca contributed to PC2. Therefore, the differences found among the sorghum landrace accessions in chemical characteristics shows that high nutritional values in the sorghum can be achieved through screening and selection.

8.1 Introduction

Grain sorghum is a drought tolerant cereal that is produced worldwide. It is the fifth most important crop after wheat, rice, maize, and barley (Bryden et al., 2009). Sorghum shows a higher tolerance to drought, that makes it a crop of preference in the tropical and subtropical areas (Hulse et al., 1980; Gualtieri and Rappaccini, 1990). However, a large number of different landraces, which are adapted to biotic and abiotic stress factors, are still cultivated by growers. Unfortunately, the low productivity of varieties on the marginal areas causes food shortage in main sorghum growing areas that requires extensive breeding programmes (Haussmann et al., 2000) that pay a significant role in the local economy (Shumba, 1994; Beta and Corke, 2001). It was reported that compared to maize, sorghum breeding has been neglected in recent decades and furthermore, the availability of high-yielding maize varieties

has led to the displacement of sorghum. However, in general, maize is less adapted to drought-prone conditions and this result often lower yield stability (Wenzel et al., 2001). With respect to efficient and effective breeding, the conservation of genetic resources is important, since sorghum landraces may bear advantageous genes that are especially useful in resistance breeding and in terms of quality traits (Tanksley and McCouch, 1997). Knowledge of genetic diversity has an important impact on the improvement of crop productivity as well as on the conservation of genetic resources (Dean et al., 1999; Simioniuc et al., 2002).

In developing countries, sorghum can play an important role in achieving food security at the household level (Dendy, 1995) and generates income. In Ethiopia, for the traditional farmer, every part of the sorghum plant is utilised - the grain for food; the leaves for feed; the sweet stalk for chewing; the dry stalk for construction and fuel.

Sorghum supply important minerals, vitamins, protein and other micronutrients (Fe, Zn, and Ca) essential for optimal health and development. Cereals also have the potential for health enhancement and that their consumption can lower the risk of diet-related diseases (David, 2007).

Determination of nutritional composition in sorghum is paramount important for improving malnutrition due to mineral elements, protein and vitmans in food stuff (Peters et al., 2003; Welch and Graham, 2004; Feil et al., 2005). Feil et al. (2005) reported that breeding for higher concentration of minerals in food crops is an alternative for improving the health of humans suffering from the consequences of mineral deficiency. Adequate intakes of mineral elements are important not just for ensuring optimal health but also growth and development (Salgueiro et al., 2002; Untoro et al., 2005; Chan et al. 2007). Cereals and legumes are rich in minerals but the bioavailability of these minerals is sometimes inhibited by the presence of anti-nutritional factors such as phytate, and polyphenols (Valencia et al., 1999; Idris et al. 2007). An adequate mineral absorption is important especially for infants, children, elderly and ill people (Bergman et al., 1999).

Identifying and manipulating the appropriate varieties can improve yield and quality of sorghum varieties. The chemical composition of sorghum landraces has not yet been adequately studied to meet the needs of growers, consumers and traders of sorghum for both food and industrial uses in Ethiopia. Therefore, exploration of available genetic variation in landraces for chemical composition requires the selection of sorghum accessions before

inclusion in sorghum breeding programmes. Selection of sorghum accessions for improved mineral elements, total starch and protein content is dependent on knowledge of the extent of genetic variation expressed in a given environment. Hence, the objective of the present investigation was to examine the variability among sorghum landrace accessions from Ethiopia for mineral, protein and starch composition.

8.2 Materials and methods

8.2.1 Plant material

All 31 sorghum landrace accessions used for this study were received from the IBC/Ethiopia as described in Chapter 4, Table 4.1.

8.2.2 Mineral and protein content determination

The procedure for determination of mineral elements and protein content following the procedures as described in Chapter 7 under Sections 7.2.2 and 7.2.3.

8.2.3 Starch extraction

The procedure for the extraction of starch and its components as well as total sugar content of the stalks is as described in Chapter 6, Sections 6.2.2, 6.2.3 and 6.2.4.

8.2.4 Statistical data analysis

Data obtained was subjected to analysis of variance (ANOVA) (Agrobase, 2005). Pearson's correlation test was carried out to assess the significance of degree of association in protein content, total starch and concentrations among mineral elements. PCA was used to investigate the sources of variation (NCSS, 2004).

8.3 Results and discussion

The ANOVA revealed that there were highly significant differences ($P \leq 0.01$) among the sorghum landrace accessions for minerals, protein, total starch and its components (Table 8.1) indicating the presence of genetic differences among them. The total starch content for the landraces ranged from 32.11 to 57.09% and it was highest for accession 228741

(57.09%), followed by accession 229833 (56.38%). This value was lower than the values reported by other authors (Geleta, 2003; Ragaee et al., 2006; Salinas et al., 2006; Boudries et al., 2009). The lowest starch contents were recorded in accessions 229832 (32.11%) and 69147 (33.72%).

For amylose the highest value was 18.59% followed by 17.86% and were recorded in accessions 228739 and 69128, respectively. The lowest amylose content was 3.51% and found in accession 223525. According to Ring et al. (1982) these accessions are classified as waxy types (contains less than 5% amylose), while most of the accessions in this study can be grouped as heterowaxy ones (contains a lower amylose content than non-waxy starches (24 to 30%). This indicated that there is a significant variation in the amylose content among these accessions. Similar results were reported by Benmoussa et al. (2006) and Dicko et al. (2006a) but the values in this study were lower than those reported by other authors (Beta et al., 2000; Beta and Corke, 2001; Geleta et al., 2005; Salinas et al., 2006; Chanapamokkhot and Thongngam, 2007; Sang et al., 2008; Boudries et al., 2009). The amylose content has been reported to vary with the botanical source of the starch and is affected by the climatic and soil conditions during grain development (Yano et al., 1985). It was also reported that genetic factors determine amylose levels in sorghum (Ring et al., 1982; Taylor et al., 1997). Amylose content of rice is similarly affected by environment and heredity (Juliano et al., 1965; Paule, 1977; Beta and Corke, 2001). In sugary sorghum the amylose content of the starch is about 5 to 15% higher than in normal sorghum (Singh and Axtell, 1973). The highest amount of amylopectin (96.50 and 92.90%) was recorded in accessions 223525 and 223558 respectively. The lowest was 81.40% and found in the accession 228739. These values were higher than that reported by Boudries et al. (2009). The amylose to amylopectin ratio also varied from 0.04 to 0.23. The highest ratios were 0.23 and 0.22 and were obtained in accessions 228739 and 69128, respectively. The lowest was 0.04 and found in accession 223525.

Table 8.1 Mean, mean squares, least significant differences and coefficient of variation for minerals, protein, total starch and its components in 31 sorghum germplasm landraces

		Starch as is and its components					Minerals (mg kg^{-1} dried basis)								
No.	Acc.*	Starch	Amylose	Amylopectin	Am:Ap[a]	Sugar	Ca	Fe	K	Mn	Na	P	Zn	Mg	% Protein
1	69029	53.18	14.50	85.50	0.17	12.55	237.33	56.50	2218.77	12.67	54.38	2855.88	24.67	1000.00	10.90
2	69030	46.52	8.03	92.00	0.09	10.30	371.00	56.00	1439.10	13.75	18.75	2845.79	24.75	837.50	11.10
3	69032	50.07	12.09	87.90	0.14	11.46	368.88	41.17	2350.00	11.00	25.25	3198.94	22.33	1050.00	10.65
4	69127	41.50	15.46	84.50	0.18	6.37	447.50	74.25	2568.75	17.67	18.00	3339.36	32.00	1331.30	12.41
5	69128	48.66	17.86	82.10	0.22	8.85	403.75	86.50	2225.00	16.17	18.00	3263.25	24.83	1450.00	10.83
6	69147	33.72	12.64	87.40	0.14	10.90	365.00	48.00	1150.00	9.50	34.50	1498.00	13.50	750.00	10.38
7	69164	53.60	15.81	84.20	0.19	8.99	295.00	52.00	1900.00	13.00	25.00	2822.00	24.50	1150.00	11.05
8	69165	41.47	12.68	87.30	0.15	10.60	235.00	86.17	1566.67	14.83	26.50	3050.88	20.67	1246.70	9.47
9	69538	49.73	11.51	88.50	0.13	8.76	337.50	56.00	2425.00	16.67	19.83	3539.50	34.67	1331.30	12.32
10	223525	53.93	3.51	96.50	0.04	10.01	442.50	107.50	1259.40	20.67	19.00	3140.58	21.75	1231.30	14.52
11	223543	38.44	11.15	88.90	0.13	12.68	265.00	54.75	1937.50	13.50	34.25	2244.00	24.00	831.27	9.78
12	223548	48.61	9.21	90.80	0.10	14.09	340.00	127.50	1350.00	15.00	33.50	2308.00	28.50	950.00	10.30
13	223551	53.88	9.08	90.90	0.10	11.43	330.88	53.79	2337.50	12.00	18.33	2858.25	18.58	1000.00	10.52
14	223552	43.50	12.43	87.60	0.14	9.13	325.00	62.00	1837.50	16.83	25.00	2657.00	22.00	1300.00	9.63
15	223554	45.13	12.16	87.80	0.14	12.18	325.00	73.50	1437.50	20.83	20.00	3263.50	18.50	1179.30	9.25
16	223555	48.77	9.62	90.40	0.11	9.67	382.50	65.67	1759.40	22.50	29.61	3774.13	34.00	1431.30	15.26
17	223558	52.86	7.08	92.90	0.08	5.25	287.50	60.63	2256.27	23.83	22.25	3787.25	33.25	1506.30	14.13
18	228736	39.46	12.22	87.80	0.14	12.30	295.00	78.50	1825.00	13.00	20.00	3153.50	24.75	1231.30	9.58
19	228739	45.43	18.59	81.40	0.23	9.84	270.00	57.75	1540.67	12.00	20.00	3185.75	27.00	1231.30	10.73
20	228740	46.50	14.63	85.40	0.17	9.95	230.00	52.25	1262.50	20.17	15.00	2337.00	20.67	1230.00	10.95
21	228741	57.09	16.55	83.50	0.20	8.82	242.50	49.08	1837.50	15.50	28.38	3429.13	24.33	1184.70	10.66
22	228919	43.05	8.26	91.80	0.09	6.77	422.50	53.38	1731.25	17.83	16.00	3041.50	29.50	1219.00	10.52
23	229831	40.16	12.69	87.30	0.15	14.93	320.00	43.17	2112.50	15.00	18.65	2424.50	17.67	1131.30	8.78
24	229832	32.11	10.44	89.60	0.12	13.59	287.50	64.17	2437.50	15.50	18.75	2641.00	29.00	1100.00	10.77
25	229833	56.38	13.34	86.70	0.15	6.98	257.50	53.13	1753.13	12.50	26.50	2348.50	20.00	1140.70	11.28
26	229834	38.29	11.07	88.90	0.12	9.77	330.00	43.33	1762.50	15.83	11.50	3157.50	22.67	1300.00	10.60
27	229835	45.20	17.24	82.80	0.21	9.60	227.50	66.50	1600.00	17.17	23.75	2741.75	28.00	1050.00	10.72
28	229838	38.86	11.29	88.70	0.13	9.97	250.00	50.25	1625.00	16.67	18.00	3529.00	24.50	1412.70	11.13
29	237762	53.73	12.62	87.40	0.14	13.19	280.00	45.36	1715.63	17.75	16.00	1967.75	17.00	973.43	9.56
30	237763	38.21	13.00	87.00	0.15	8.61	207.50	43.50	1743.75	15.33	19.75	3225.00	26.17	1381.30	9.76
31	237779	46.00	14.83	85.20	0.17	7.14	270.00	41.50	1725.00	15.83	17.75	2068.50	17.50	1008.33	8.08
	Mean squares	133.06**	32.82**	32.895**	0.005**	16.43**	12948.66**	1139.47**	433403.43**	33.36**	200.21**	891754.50**	82.28**	108149.48**	7.30**
	LSD	2.19	1.10	1.11	0.01	1.40	20.49	3.66	45.46	1.01	4.30	169.65	1.05	11.37	1.19
	CV%	2.92	5.51	0.78	6.24	8.48	4.03	3.65	1.52	3.93	11.49	3.59	2.65	0.60	6.77
	Mean	45.94	12.31	87.70	0.14	10.15	311.27	61.41	1828.72	15.82	22.97	2893.44	24.23	1168.79	10.83

**p≤ 0.01

[b]Am:Ap=amylose/amylopectin ratio

*Accession number for the landraces is from the Institute of Biodiversity Conservation

The values obtained were lower than those reported by Arora and Luthra (1972) and Boudries et al. (2009). The total sugar content for the stalks varied between 5.25 to 14.93%. The chance of deriving sweet sorghum may be possible from the high brix accessions. The highest total sugar content was 14.93% and found in accession 229831 followed by accession 223548 (14.09%). The lowest was 5.25% and recorded in accession 223558. These values were lower than those reported by Sabramanian et al. (1987) and higher than those reported by Arora and Luthra (1972). Few of these accessions may have good potential for further use as sweet sorghum and grain sorghum lines and may be used in sorghum improvement programmes for incorporation of this trait into breeding lines. Coleman (1970) reported that sweet sorghum fodders may contain up to 21% total sugars. Determination of starch and starch components such as amylose, amylopectin and total sugar content is crucial for the selection of the accessions for the food type needed (Dicko et al., 2006b). Amylose has a higher gelatinisation temperature than amylopectin (Whistler et al., 1984; Dicko et al., 2006b), sorghum with low amylose content could be used in industry (Dicko et al., 2006b). Low amylose-containing sorghum accessions could give better characteristics of the extrudates (Gomez et al., 1988, Dicko et al., 2006b). Sorghum varieties with low amylose content may be recommended for infant porridges (Dicko et al., 2006b). Hibberd et al. (1982) reported the higher starch digestibility in low-amylose, waxy sorghum than the normal sorghum.

The highest Ca concentration of 477.50 mg kg^{-1} followed by 442.50 mg kg^{-1} was found in accessions 69127 and 223525, while the concentration in accession 237763 (207.50 mg kg^{-1}) was the lowest. The Ca content was significantly higher than results reported by Awadelkareem et al. (2009) and Samia et al. (2005). Ca is important mineral element used for human consumption, especially for bone development and strength. The highest concentration of Fe was 127.50 mg kg^{-1} and was found in accession 223548 followed by 223525 with a concentration of 107.50 mg kg^{-1}. The lowest concentration was found in accessions 69032 and 237779. Deosthale and Belvady (1978) reported Fe content ranging from 4.70 to 14.05 mg/100 g in sorghum cultivars. The highest K concentration was 2568.75 mg kg^{-1} followed by 2437.50 mg kg^{-1} in accessions 69127 and 229832 respectively. The values obtained were lower than those recorded by Badi (2004). The highest concentration of Mn (about 50% higher than the other accessions) was found in accessions 223558 and 223555 and the lowest in accessions 69147, 69032, 223551, and 228739. The findings indicated the existence of significant variation in the mineral elements between the accessions. For Na the highest concentration was 54.38 mg kg^{-1} and found in accession 69029. The lowest Na concentration was 11.50 mg

kg^{-1} and found in accession 229834. This result agrees with that of Awadelkareem et al. (2009) who reported that Na content of two sorghum cultivars ranged from 5.83 to 6.18 mg/100 g. This was lower than the results of Badi (2004) who reported that the Na content of two sorghum cultivars ranged from 6.3 to 7.0 mg/100g.

The highest P concentration of 3787.25 mg kg^{-1} was found in accession 223558 followed by accessions 223555 and 69538. The lowest concentration of 1498 mg kg^{-1} was recorded in accession 69147 which was lower than the 407 and 396 mg/100g reported by Khalil et al. (1984) and 388 to 756 mg/100 g by Deosthale and Belvady (1978). P is an essential mineral in human nutrition and plays an important role in the structure and function of the human body. The highest concentration of Zn was 34.67 mg kg^{-1} followed by 34 mg kg^{-1} in accessions 69538 and 223555 respectively. Zn plays an important role in human nutrition for growth and development as well as in the proper functioning of the immune system. Zn was reported to be an essential mineral adversely affected by phytate (Urga et al., 1997). The highest concentration of Mg was 1506.17 mg kg^{-1} in accession 223558; while the lowest concentration of 750 mg kg^{-1} was found in accession 69147. Liu et al. (2003) found significant differences among rice cultivars in the absorption and translocation of Fe, Mn, Zn, Cu, and Mg which were also dependent upon the growth stages. Such variability in the content of mineral elements for the same species may be related to genetic origin, geographical source and the levels of the fertility of the soil. Generally, the concentration of mineral elements of the landrace accessions revealed the presence of higher levels of K, Ca, Mg and P than those of Fe, Mn, Na and Zn. Na was present at an intermediate level while Zn and Mn were present in very low amounts with Mn being the lowest. Sorghum is an important source of Fe, Ca and Zn in diets of Ethiopians, especially for people living under poor socio-economic conditions. Therefore, selection of potential accessions for these mineral elements is important for the future breeding programme.

Several factors may account for the variation of mineral contents in the accessions studied. This may include the genotype, mineral concentration in the soil as well as translocation rates of the elements by the accessions from the soil. Sorghum, like other crops, depends on the soil for mineral elements needed for structural and catalytic functions. Variation could be due to differences in the accessions' ability to absorb the mineral elements from the soil. Another possible reason is that the 31 accessions may differ in their level of requirements for these mineral elements. For all minerals there were significant ($P \leq 0.01$) differences between the accessions, and it is assumed that a large part of this is due to genetic effects. This suggests that breeding progress can be

expected with selection. Some accessions were superior. Depending on the breeding objective, the germplasm accessions can be genetically recombined to serve as parents for developing new superior sorghum varieties for higher mineral concentration. Sabharwal et al. (1995) emphasised that sorghum parents with more diversity among themselves are expected to exhibit a higher amount heterotic expression and a broad spectrum of variability in segregating generations.

Protein content varied between 8.08 and 15.26% and the range was similar to those reported by other authors (FAO, 1995; Beta et al., 1995; Lasztity, 1996; Dicko et al. 2006b). Among the accessions, protein content was highest for accession 223555 (15.26%), followed by 223525 (14.52%). The lowest protein content was found in accession 237779 (8.08%), followed by 229831(8.78%). Wide variability in the composition of the grain, including its protein content, was reported (Hulse et al., 1980; Geleta, 2003). Similarly, Douglas et al. (1990) reported that most sorghum cultivars have a higher crude protein content than maize (8.8-15%). This large genotypic variability for grain protein content of the Ethiopian sorghum landraces is encouraging for selecting potential accessions and genetic improvement. Proteins are important components of human diet required for growth and development of the body, maintenance and repairing of worn out tissues and for production of enzymes and hormones required for many body processes.

Very high correlation coefficients ($r = 0.80$, $p \leq 0.000$ and $r = 0.75$, $p \leq 0.000$) were found between P and Mg and between protein and Zn, respectively (Table 8.2). This indicates that some interactions existed between minerals and protein content. Therefore, it remains to be determined whether breeding for high concentration of Mg inevitably increases the concentration of P and vice-versa and similar situation is expected for Zn and protein content. Shah and Mehta (1958) reported that the correlation between Mg and P is due to the functions ascribed to Mg in plant nutrition namely that of a carrier of the P used by the plant. Similarly, Khan et al. (2008) found positive correlation between Mg and protein on date varieties in Pakistan. Flynn et al. (1987) reported that cultivars with higher Zn concentration in the grain also had higher levels of protein and of lysine and methionine in protein. In particular, the finding that the total sugar content was inversely significantly correlated with total protein content might have implications for plant breeding programmes in relation to human nutrition and animal feed.

Table 8.2 Phenotypic correlation coefficients showing pair-wise association among eight mineral elements, protein, starch and sugar composition in sorghum

	Minerals (mg kg $^{-1}$)									
Variable	Zn	Fe	Mn	Ca	Mg	K	Na	P	Protein	Starch
Fe	0.38*	1								
Mn	0.38*	0.03	1							
Ca	0.26	0.32	0.19	1						
Mg	0.53**	-0.20	0.51**	0.04	1					
K	0.16	-.025	-0.11	0.19	0.10	1				
Na	0.35*	0.59**	-0.04	0.08	-0.12	-0.07	1			
P	0.64**	0.00	0.37	0.19	0.80**	0.20	0.08	1		
Protein	0.75**	0.16	0.53**	0.27	0.59**	0.20	0.32	0.64**	1	
Starch	-0.10	0.05	0.10	0.00	-0.18	-0.05	0.41**	-0.04	0.30	1
Sugar	-0.32	0.34	-0.30	0.07	-0.53	-0.01	0.04	-0.38*	-0.39*	-0.31

*P≤ 0.05, **P≤ 0.01

The PCA showed that the first four PCs were important and explained 71.77% of the total variation among the accessions (Table 8.3). PC1 had an eigenvalue of 3.82 and accounted for 34.76% of the variation. This represents an equivalent of five variables and indicated that Zn, Mn, Mg, P and protein were important contributing variables. Accessions with high PC1 scores, therefore, would have high levels of these mineral elements and protein contents. PC2 had an eigenvalue of 1.56, contributing 14.16% of the variation and had Fe concentration as the main contributing factor compared to Na, Ca and protein content. PC3 had eigenvalues of 1.38, indicating that Mn, K and Na were contributing 12.51% variation. The PC4 with 10.33 of the variance was composed of Ca, total starch and sugar content, while K concentration contributed a lesser amount. Protein concentration was important in at least two PCs, indicating its relative importance to variation among the accessions. The PC1 and PC2 explained most of the variation among the accessions.

Table 8.3 Principal components (PCs) analysis of protein, total starch, sugar content and eight mineral elements in 31 sorghum accessions showing eigenvectors, eigenvalues and their percentage contribution to the total variations explained with the first four principal component axes

	Eigenvectors			
Variables	PC 1	PC 2	PC 3	PC 4
Zinc	0.39	0.07	-0.23	0.25
Iron	0.12	0.61	0.25	0.16
Manganese	0.35	0.04	0.34	-0.22
Calcium	0.18	0.30	0.24	0.39
Magnesium	0.42	-0.25	0.10	-0.02
Potassium	0.17	-0.23	-0.53	0.44
Sodium	-0.14	0.43	-0.54	-0.09
Phosphorus	0.43	-0.09	-0.13	0.09
Protein	0.39	0.30	-0.08	-0.10
Starch	0.14	0.25	-0.32	-0.59
Sugar content	-0.30	0.26	0.01	0.36
Eigenvalue	3.82	1.56	1.38	1.14
Individual%	34.76	14.16	12.51	10.33
Cumulative%	34.76	48.92	61.43	71.77

The biplot demarcated the accessions with characteristics explained by the first two dimensions. A breeder, in consequence, can easily visualise the distances between the accessions and decide on the best varieties to be selected, based on several variables, compressed in the two major principal components and analysed simultaneously. Accessions close to each other in the scores plot are similar; accessions located near the origin are distinctive accessions and those far from the origin are extremes. This is because the principal component has been constructed with the data centred by subtracting the average of each variable. The PCA analysis grouped the accessions into groups over the four quadrants based on the concentrations of mineral elements, protein and total starch content (Figures 8.1). The accessions remained scattered in all four quadrants, showing large genetic variability in composition. The accessions in the top left quadrant were closely related in Na and total sugar contents. The right top quadrant consisted of the accessions with related contents of Ca, Fe, Mn, Zn, protein and total starch. The right bottom quadrant comprised the accessions associated with the P, K and Mg on the first principal component. The distance between the locations of any two accessions on the score plot is directly proportional to the degree of difference/similarity

between them in terms of the mineral and protein contents. Figures 8.2 therefore, revealed that accessions 69147 (#6), 223525 (#10), 223548 (#12), 223555 (#16), 223558 (#17), 237763 (#30) and 237779 (#31) were the most distant/diverging from the major group which in the principal component axes was concentrated on zero depicting some similarity in terms of the nutritional values. However, accessions 223548 (#12) showed a similar relationship in the second principal component axis with accession 237763 (#30). Furthermore, accessions 223558 (#17) and 223555 (#16) revealed a close relationship in the second principal component axis. Accessions which overlapped in the principal component axes had similar relationships in the concentration of the mineral elements, starch, sugar and protein content. The loading plot indicates the similarities/correlations and differences between the chemical compositions. The elements with small loadings located near the origin have little influence on data structure; where as the elements, starch, sugar and protein with high loadings represent the greatest influence on data structure on the clustering and separation of sorghum accessions.

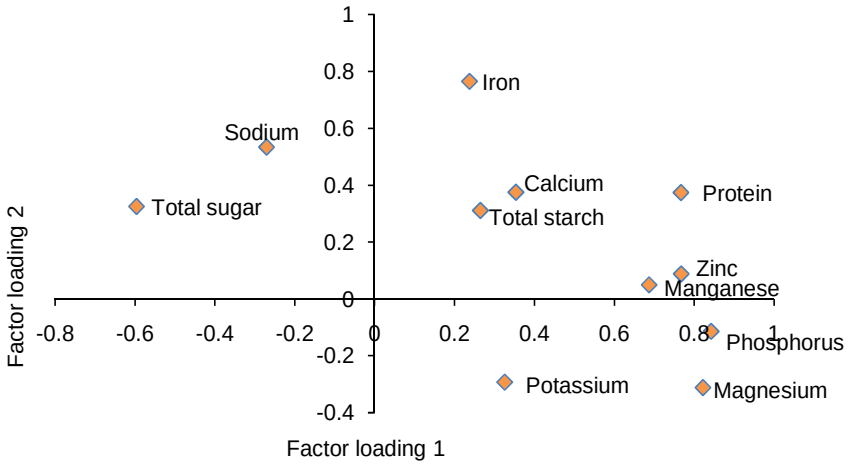

Figure 8.1 PCA loading plot of PC1 and PC2 describing the variation among the different mineral elements and protein content determined from the 31 sorghum landrace accessions

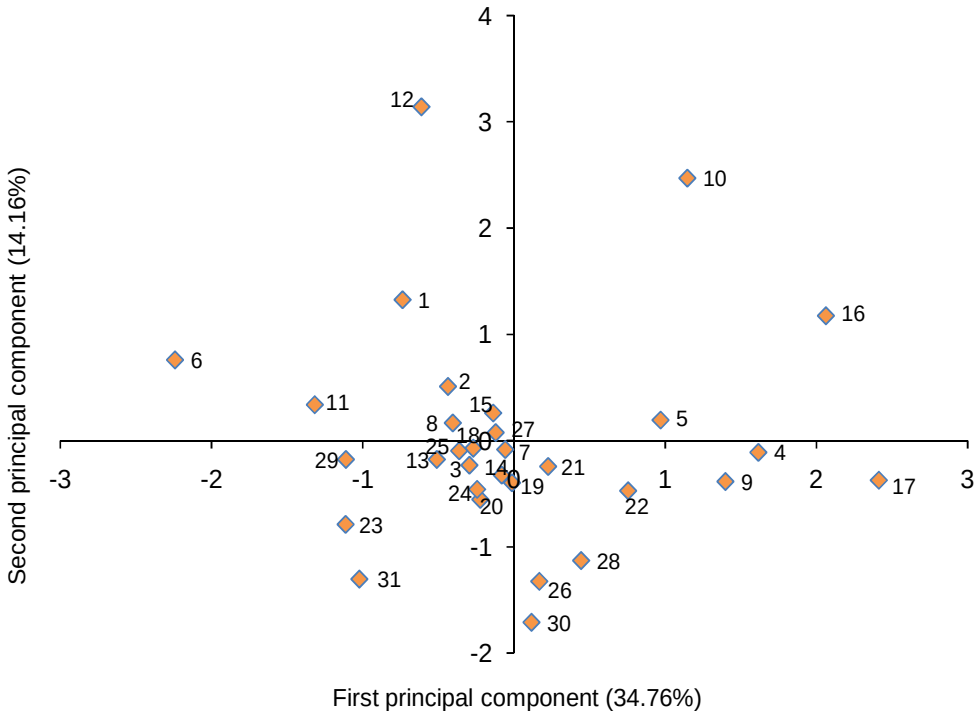

Figure 8.2 PCA score plot of PC1 and PC2 describing the overall variation among nutritional compositions from the 31 sorghum landrace accessions; Accession number as given in Table 8.1

8.4 Conclusions

Production of sorghum will remain high and expand in the future. Breeding of sorghum with careful concern for its quality will provide useful grain for the new or modified technological processes that will emerge. In sorghum breeding, it is necessary to identify germplasm that breeders can use to improve not only yield, but also mineral concentration, total starch and sugar content. Diversity in nutritional composition was observed among the sorghum landrace accessions studied. There was significant variation in mineral composition and concentration among the sorghum landrace accessions. High values were seen in accessions 228741(total starch), 228739 (amylose and amylose to amylopectin ratio), 223525 (amylopectin, Fe), 229831 (total sugar), 69127 (Ca, and K), 223558 (Mn, P and Mg), (Na), and 223555 (Zn and protein). This was a single set of data, and therefore only gives an indication of the variation available. These landraces should be tested in more than one environment and season to really identify individuals which can be included in future breeding efforts.

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CHAPTER 9

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Information on the levels and patterns of genetic diversity is valuable for efficient management of germplasm and for effective utilisation of materials in breeding programmes to meet the ever-changing needs of growers and consumers in the face of changing and unpredictable environmental challenges. A prerequisite for the genetic improvement programme of sorghum is knowledge of the extent of genetic variation present between accessions and genetic distances between them, and between sorghum and closely related species with which hybrids could be produced. This could be achieved through characterisation of germplasm using morphological, biochemical and DNA markers. Sorghum accessions were obtained from IBC/Ethiopia, Melkassa Agricultural Research Centre/EIAR, and ARC-GCI/South Africa for characterisation based on phenotypic and AFLP markers, a combination of both markers as well as evaluation of starch, minerals and protein content diversity. Nine categorical morphological and 20 quantitative traits measured proved to be a useful tool in determining the genetic variations as well as the relationships among accessions as differences were observed between the accessions for the traits studied. Estimates of genetic distances matrix based on phenotypic characters for all pair-wise combinations of the 22 sorghum accessions were examined. As a result a genetic distance ranging from 0.38 to 1.59 was computed, indicating phenotypic diversity among accession. Qualitatively inherited morphological characteristics could be used to characterise accessions for collection and maintenance of germplasm and for parental selection through heterotic groups to improve sorghum. In addition, cluster analysis and PCA based on phenotypic markers separated and grouped different accessions according to their genetic distances/similarity. Since morphological traits do not cover the entire genome, this has to be confirmed on DNA level. Furthermore, conventional breeding supplemented with Marker Assisted Selection of designed traits hastens progeny selection for increased yield, disease and pest resistance as well as nutritional quality. Grain yield per panicle revealed high and positive association with leaf width, leaf area, panicle width and weight. Grain number per panicle was also positively and significantly correlated with number of tillers, panicle width, panicle weight, thousand seed weight and grain yield per panicle. The positive associations indicate that selecting for more tillers and other positively associated panicle related traits would have a positive effect on grain number per panicle. Negative significant correlations were observed among some traits

which could be utilised in breeding for negatively correlated traits. AFLP analysis showed genetic distance values ranging from 0.88 to 0.98 for AFLP and 0.00 to 0.89 for morphological traits. The morphological characterisation therefore picked up much more variation, but it was based on a relatively small number of selected characteristics. AFLP results indicated a narrow genetic base as shown by high genetic similarities. The South African and Ethiopian materials were genetically very similar, indicating a common ancestral source of the breeding materials used in the two breeding programmes. AFLP is a promising marker system especially for sorghum accessions, which are closely related with low levels of polymorphism and thus high levels of genetic uniformity. It is recommended that a combination of both morphological and DNA characterisation can be used for selection of breeding material and for genetic conservation. It is also recommended that both the South African and the Ethiopian sorghum breeding programme include new genetically unrelated genotypes in their breeding programmes in order to broaden the genetic base of the material.

Sorghum is an important source of mineral elements that are located in the pericarp, aleurone layer, and germ. Sorghum is a good source of K and P, and an adequate source of Mg, Fe, Zn, Ca, and Na. P was the mineral element found in the largest concentrations in the tested material. In the present study, those accessions which revealed moderate to high concentrations could be selected and incorporated into breeding programmes. Protein content varied due to environmental conditions and genotypes. Adequate content of protein is critically important in developing countries where human diets consist mainly of cereals. Therefore, sorghum accessions containing high amounts of protein could be incorporated in the future sorghum breeding programmes.

In conclusion, the present study has explained the relevance of employing phenotypic markers together with molecular markers to determine genetic distances and evolutionary relationships for sorghum as well as mineral, starch, stalk sugar and protein content diversity to identify genetically valuable potential accession/s for future sorghum improvement. Distinct sorghum accessions that have been identified with a combination of phenotypic and AFLP markers as well as those accessions revealing good biochemical traits need to be included in breeding programmes. Moreover, the results from the present study could contribute to *in situ* and *ex situ* conservation and effective utilisation of sorghum material.

SUMMARY

Keywords: *AFLP, Characterisation, Genetic diversity, Germplasm, Nutritional composition, phenotypic diversity, Sorghum.*

This study was conducted with the objectives of assessing the genetic diversity of sorghum accessions that were obtained from different geographical locations in Ethiopia, as well as South Africa, using phenotypic traits, AFLP markers, minerals, starch and protein.

Twenty phenotypic traits were subjected to ANOVA and highly significant differences were observed for all traits among accessions. Some important characteristics were found to be significantly correlated, which could allow for selection of related secondary characteristics to improve primary characteristics. Cluster analysis grouped accessions into five clusters based on similarity. These results would be useful in a breeding programme for selecting sorghum accessions to improve production.

Nine qualitative morphological traits were also analysed using the Shannon Weaver diversity index ($H^{'}$). The results showed that the average $H^{'}$ was 0.59. The highest polymorphism was recorded for the glume colour (0.84), while the lowest was recorded in panicle compactness and shape (0.31). This indicated that there was wide variability among accessions studied. The cluster analysis for the qualitative traits also identified accessions based on their similarity and differences based on their genetic distances. Hence, visual selection and measurements of the sorghum attributes in field trials could be used to select the desirable traits and improving yield and stability of the accessions in breeding programmes.

AFLP analysis showed high genetic similarity of Ethiopian landraces, as well as breeding material from the Ethiopian and South African sorghum breeding programmes, even though the phenotypical characterisation showed high variability. This indicated the possibility that South African and Ethiopian breeding material may have a common ancestry. The morphological, AFLP and combined morphological and AFLP cluster analysis clearly distinguished between all accessions, even though they were genetically similar.

Sorghum kernels were used to determine the mineral, starch and protein composition of the sorghum accessions. The ANOVA of the traits revealed highly significant variation among the accessions studied. Furthermore, PCA grouped accessions according to their similarity/differences in the four quadrants which confirmed that there was variation among them for these nutritional traits. Therefore, there would be potential for selecting accessions for specific mineral and protein content for human consumption, and specific starch and amylose content for industrial use. The sugar content of the stalks also indicated the potential to develop dual sorghum cultivars where both the seed and sugar can be produced from the same plants.

OPSOMMING

Hierdie studie is uitgevoer met die doel om genetiese diversiteit in sorghum inskrywings van verskillende geografiese gebiede in Ethiopië en van Suid Afrika te bepaal, met behulp van fenotipiese eienskappe, AFLP merkers, minerale, stysel, amilose en amilopektien, proteïen en stamsuiker inhoud.

Twintig fenotipiese eienskappe is met variansie analise ontleed, en hoogs betekenisvolle verskille is tussen al die inskrywings vir die gemeette eienskappe gekry. Sommige belangrike eienskappe was betekenisvol gekorreleer wat die seleksie van sekondêre eienskappe sal toelaat vir die verbetering van primêre eienskappe. Groeperingsanalise het vyf groepe gevorm gebasseer op fenotipiese ooreenkomste. Hierdie resultate sal nuttig wees in teelprogramme om sorghum inskrywings te selekteer wat gebruik kan word vir verbeterde produksie.

Nege kwalitatiewe morfologiese eienskappe is ook geanaliseer met die Shannon Weaver diversiteitsindeks (H'). Die resultate het ‘n gemiddelde H' van 0.59 aangetoon. Die hoogste polimorfisme is vir kafkleur aangetoon (0.84), terwyl die laagste vir panikel kompaktheid en vorm was (0.31). Dit het die variabiliteit tussen die inskrywings beklemtoon. Die groeperingsanalise vir die kwalitatiewe eienskappe het ook die inskrywings volgens ooreenkomste gegroepeer en volgens genetiese afstande. Dus kan visuele seleksie van eienskappe in die veld wel gebruik word om die beste ouers te selekteer vir die verbetering van opbrengs en stabiliteit van inskrywings in teelprogramme.

AFLP analise het groot genetiese ooreenkomste in die Ethiopiese landrasse uitgewys, sowel as ooreenkomste in die teelmateriaal van die Ethiopiese en die Suid Afrikaanse sorghum teelprogramme, al het die fenotipiese karakterisering baie meer variasie getoon. Dit wil voorkom asof die inskrywings van die Suid Afrikaanse en die Ethiopiese teelprogram in die verlede ‘n gemeenskaplike bron van teelmateriaal gehad het. Die morfologiese, AFLP en gekombineerde groepering van die twee kon al die inskrywings van mekaar onderskei, al was die genetiese ooreenkomste in die material baie groot.

Sorghum saad is gebuik om mineraal, stysel en proteïeninhoud diversiteit van die inskrywings te evalueer. Die variansie analise het hoogs betekenisvolle verskille tussen inskrywings vir al die eienskappe getoon. Hoofkomponent analise het die inskrywings in vier kwadrante verdeel volgens die gemeette eienskappe, wat groot genetiese diversiteit

aangetoon het. Daar is dus potensiaal om ouers te selekteer vir die verbetering van mineraal en proteïeninhoud vir menslike gebruik, en vir spesifieke styseleienskappe vir industriële gebruik. Die variasie vir suikerinhoud van stamme het gewys dat dubbeldoel sorghum cultivars ontwikkel kan word waar saad en suiker van dieselfde plante af geproduseer kan word.

Appendix I

Weather data for the growing seasons

Year (2008/2009)	Elements	Maximum temperature (^{o}C)	Minimum temperature (^{o}C)	Average temperature (^{o}C)	Total rainfall (mm)
	November	29.07	15.54	22.31	89.41
	December	31.17	17.38	24.28	73.92
	January	29.68	17.65	23.67	16.24
	February	27.37	16.24	21.81	20.83
	March	26.79	13.51	20.15	73.9
	April	26.11	9.98	18.05	41.91
	May	21.87	6.41	14.14	58.17
	June	19.21	3.95	11.58	28.96
	Total				403.34
Year (2009/2010)	November	27.26	13.50	20.38	87.88
	December	30.58	16.31	23.45	204.98
	January	27.26	17.09	22.18	242.57
	February	29.16	16.16	22.66	86.36
	March	28.30	15.15	21.73	143.00
	April	24.47	12.16	18.32	77.47
	May	23.23	6.78	15.01	25.4
	June	19.94	-0.26	9.84	0.00
	Total				867.66

Source: ARC-GCRI, Potchefstrom, South Africa (2008-2010).