Diversity Analysis of South African Sorghum Genotypes Using Agronomic Traits, SSR Markers and Protein Content and Amino Acid Composition

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A thesis submitted in fulfilment of the academic requirements for the degree of
Doctor of Philosophy (PhD) in Plant Breeding

African Centre for Crop Improvement (ACCI)

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University of KwaZulu-Natal

Pietermaritzburg

South Africa

June 2015

Thesis Abstract

Sorghum is an important food security crop and ranks fifth after wheat, rice, maize, and barley in total area of production globally. In South Africa, sorghum is mainly grown for food and livestock feed both by small-holder and large-scale farmers. Diverse sorghum genotypes are grown in South Africa, which have not been fully characterized using agronomic, molecular or protein markers for breeding or strategic conservation. There is also little knowledge of farmers' views and perception of the constraints affecting sorghum production, and their trait preferences, information that is needed to direct sorghum breeding programmes. The objectives of the study were to: (i) determine farmers production constraints and preferences of sorghum varieties in the Limpopo Province in South Africa; (ii) assess the level of genetic diversity present among South African sorghum genotypes using agro-morphological traits; (iii) compare random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers and high resolution melt (HRM) analyses to determine genetic variation among selected sorghum genotypes; (iv) assess the genetic diversity present among South African sorghum genotypes using genetic distances as measured by SSR markers; and (v) determine genetic diversity of selected South African sorghum genotypes grown in two different agro-ecologies, especially for protein content and amino acid composition, and to select candidate lines for subsequent breeding and conservation.

A participatory rural appraisal (PRA) study was conducted in two selected districts of Limpopo province to determine sorghum production constraints and variety preference involving 311 respondent farmers. The PRA data was collected using semi-structured questionnaires, focus group discussion, matrix ranking and transect walks. The main constraints affecting sorghum production were bird damage (53.08 %), storage pests (weevils) (50.05%), parasitic weeds (35.00%), drought (35.25%) and postharvest diseases (30.75%). Good taste, high yields, resistance to bird damage, insect pests (weevils), and diseases, early maturity and drought tolerance were farmers-preferred traits of sorghum varieties in the study areas.

Ninety eight diverse South African sorghum genotypes were characterized using agro-morphological traits. Principal component analysis revealed that the three most important components contributed 38.9%, 30.96% and 18.13% to the total variation. The traits that

contributed most to the variation were plant height, seed weight and panicle weight. A dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean, and this grouped the genotypes into three major clusters. The grouping of the genotypes was independent of the source or place of origin. The genotypes MP 4277, EC 2934, KZ 5097, FS 4909, and LP 4303 were phenotypically identified as the most diverse. The best lines with quantitative and qualitative attributes were MP 4276, NW 5430, 05-Potch-167 and EC 3217 across the locations.

Eight selected sorghum genotypes were used to compare high resolution melt (HRM) analysis with random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) analyses. DNA was extracted using the CTAB extraction method. The template DNA was amplified, using three RAPD and SSR primers for each sample. Both markers revealed variation among the sorghum genotypes, with a moderate correlation between the RAPD and SSR results. The genotypes were further subjected to high resolution melt (HRM) analysis, which showed considerable variation between the genotypes. There was a high level of correspondence between the clustering of genotypes when using SSR markers or HRM analysis.

One hundred and three sorghum genotypes collected from various South African provinces by the Department of Plant Genetic Resources, the African Centre for Crop Improvement (ACCI) and the Agricultural Research Council-Grain Crops Institute (ARC-GCI) were genotyped using 30 polymorphic SSR markers. The SSR analysis revealed extensive variation among the sorghum genotypes. The genotypes Macia-SA and AS4 had the lowest dissimilarity index, whereas POTCH-115 and MP 2048 showed the highest dissimilarity index. The size and number of alleles ranged from 90 to 294, and 2 to 15, respectively. The polymorphic information content (PIC) varied between 0.02 and 0.84. The heterozygosity data points ranged between 0.02 and 0.85, with the genetic distances ranging between 0 and 8.4.

Fifty nine selected sorghum genotypes were grown at two locations, Makhathini and Ukulinga Research Farm, Pietermaritzburg. These were analysed for crude protein content using near-infrared spectroscopy (NIR). The genotypes that had a high protein content at both locations were AS4 (15.07%), Maseka-a-swere (15.13%), AS19 (15.22%), Macia-SA (15.31%), AS16 M1 (15.57%) and Mammopane (16.18%). Nineteen sorghum genotypes with high crude protein content were selected and analysed for their amino acid composition. The genotypes with high lysine content were KZ 5246 (2.27%), AS17 (2.25%), Manthate

(2.16%) and LP 1481 (2.11%). Lines identified with high leucine levels were LP 1948 (14.3%), FS 4905 (14.3%), MP 4154 (14.26%) and LP 1481 (14.25%). The genotype AS16cyc was the best candidate for high phenylalanine content (5.99%).

Overall, the study found considerable levels of genetic variability among South African sorghum germplasm using agro-morphological, SSR markers and protein content and amino acid levels. The selected lines should be useful for future breeding programmes. Knowledge of the genetic diversity can be used to direct efforts to conserve the diversity of sorghum germplasm present in South Africa.

Declaration

I, Maletsema Alina Mofokeng, hereby declare that:

1. This document is a token of my own work and has not previously, in its entirety or in part, been submitted to any other institution for any academic award.

2. All sources of information used in this literature review have been duly acknowledged.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other scientists. Where other written sources have been quoted, then:

    i) Their words have been rewritten but the general information attributed to them has been referenced.

    II) Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

4. This thesis does not contain text, graphics or tables copied and pasted from the internet unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Maletsema Alina Mofokeng (Candidate)

Signature:……  ……..

As the candidate's supervisors, we agree to the submission of this thesis:

Supervisor: Prof Hussein Shimelis	**Co-supervisor: Prof Mark Laing**
Signature:……..	Signature: ……
Date:……..	Date:……..…

Dedication

To God Almighty

Praises be unto the Lord for steadfast love, His grace and mercy.

For strength when pursuing my study

To my parents

Koos and Fransina Mofokeng, these are the fruits of your daughter's hard work.

You are my rock, and pillars of strength.

Thank you for your love.

Publications from this thesis

1. Comparison between random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers with high resolution melt analyses in genetic variation analysis among selected sorghum genotypes. African Journal of Biotechnology 2012:11(102):16697-16707.
2. A genetic diversity analysis of South African sorghum genotypes using SSR markers. South African Journal of Plant and Soil 2014. 31(3):145-152.

Acknowledgements

My sincere gratitude and appreciation goes to the following people and institutes:

- My major supervisor, Prof Hussein Shimelis, and co-supervisor, Prof Mark Laing, for guidance, support, patience and ensuring the success and completion of this study.
- The Alliance for a Green Revolution in Africa (AGRA) for sponsoring my study in African Centre for Crop Improvement (ACCI), University of KwaZulu-Natal.
- The Generation Challenge Programme (GCP) for funding through the genotyping support services.
- The Professional Development Programme (PDP) of the Agricultural Research Council (ARC) for funding, training and giving me a platform and opportunity to become a professional scientist.
- The National Research Foundation (NRF) for funding my first year of PhD study.
- The ACCI administrators, Lesley Brown, Rowelda Donnelly, Jayshree Singh and Mr Ian Doidge for administrative and field work support.
- The Agricultural Research Council is appreciated for research support.
- Incotec (Pty) Ltd for assistance with the SSR data analysis.
- The Limpopo Department of Agriculture and various stakeholders are appreciated for assistance and support with participatory rural appraisal.
- My parents, brothers and a sister: thank you for your love, prayers, sacrifices, support, understanding and encouragement during tough and good times, even when spending a long time away from home. May the Lord continue to shower us with His blessings.

Above all, praises unto the Lord, God Almighty.

Table of Contents

Introduction to the thesis

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal crops grown worldwide. It ranks fifth after wheat, maize, rice and barley (Dogget, 1988; Bryden et al., 2009; FAO, 2011). According to Vijayakumar et al. (2014) sorghum produced worldwide is 64.20 million tonnes with a cultivated area of 41 million hectares. Of this grain, about 26 million tonnes are produced in Africa. The four leading sorghum producers in Africa are Nigeria, Ethiopia, Burkina Faso and Niger. About 74% of sorghum in Africa is used for food (Acquaah, 2012). In 2012 South Africa produced 150 000 tonnes of sorghum grains on a harvested area of 60 000 hectares (FAO, 2013). According to the Department of Agriculture, Forestry and Fisheries (2010) the Free State Province is the main sorghum producer with approximately 53% of the production area, followed by Mpumalanga (30%), Limpopo (9%), North West (6%), and Gauteng (2%) Provinces. Although the production varies widely among provinces, sorghum remains an important food constituent in the diet of rural households in the country (Taylor, 2003).

In South Africa sorghum is mostly grown in marginal areas, serving as a source of calories for many people. It ranks the third after maize and wheat. Historically, it has only been a subsistence food crop. However, it is increasingly becoming the foundation for successful food and beverage industries, in addition to its other uses (Taylor and Dewar, 2000). It also serves as the best alternative to barley for beer brewing in the competitive environment of multinational brewing enterprises. In addition, sorghum crop residues and greens are good sources of animal feed and fodder (Rooney and Waniska, 2001; Chakauya et al., 2006). Apart from its contribution to food security, sorghum is broadly adapted and can be grown in a wide range of environments. One of its strongest traits is its great adaptability to tropical and subtropical areas of the world where water availability and soil conditions are marginal for other grain crops such as maize (ICRISAT, 2009). It can be produced in a wide variety of soil types, but yields are typically highest in deep, well drained soils with good fertility. It also appears to have a high capacity for osmotic adjustment to stress to maintain turgor pressure in cells (Nguyen et al., 1997), while some sorghum varieties possess “stay green” genes that enable them to continue to photosynthesize post-flowering, during drought (Malala, 2010). Because of these abilities to survive in harsh conditions it is a vital crop for household food security for many rural communities farming in marginal agro-ecologies, such as in the dry regions of South Africa, Botswana and Namibia.

According to de Man (1999), the grains of various cereal crops possess different levels of protein. These include maize (9-10%), rice (6-9%), barley (10%), wheat (8-14%) and rye

(12%) among others. Sorghum has a protein level ranging from 7.3-15.6% (Hulse et al., 1980), which is higher than most other cereals consumed by humans (Ahmed et al., 1996). However, sorghum's protein content is lower when compared to leguminous crops. The negative aspect is that sorghum protein is highly indigestible, with limited bio-availability (around 2%), leading to malnutrition where people depend upon sorghum for protein intake. South Africa is one of the 36 countries most highly challenged with its public health nutrition state, according to the World Health Organisation. Furthermore, it is reported that the state of children and adult's nutrition health has deteriorated in South Africa (Steyn et al., 2006). To ensure food and nutrition security, households need sufficient and nutritious food (Faber et al., 2011). This can be achieved through breeding and growing crops with high levels of digestible protein.

Proteins are an essential component of the diet needed for the survival of humans and animals. About 63% of the world's protein intake is from grains or grain products (Hoveland, 1980). The protein's basic function in nutrition is to supply adequate amounts of essential amino acids. The quality of a protein, or its nutritive value, depends on its amino acid content and on their bioavailability after digestion, absorption, and minimal obligatory rates of oxidation. In countries where cereals are staple foods, protein malnutrition is a widespread problem, often associated with one or two deficient amino acids. Sorghum cultivars with improved lysine have been reported (Singh and Axtell, 1973), but most cultivars are deficient in essential amino acids (especially lysine, methionine, cysteine and tryptophan). Information on protein content and amino acid levels among sorghum landraces is important for growers and breeders. The protein content is influenced by environment and the cultivars used (Hoveland, 1980; Rharrabti et al., 2001; Almodares et al., 2009). Hence, it is essential to assess the levels of protein and the essential amino acids present in sorghum varieties grown locally by farmers. Varieties with increased protein and amino acid levels can be considered for selection in breeding programmes aiming to improve nutritional quality of sorghum.

Many farmers grow local sorghum cultivars with low-inputs for consumption, of which their potential as sources of food constituents such as proteins has not yet been fully researched. Protein quality is a measure of levels of amino acids present in a cultivar (Waggle and Deyoe, 1966; Audilakshmi and Aruna, 2005) but information on these levels in South African landraces is still scanty. Wenzel et al. (2001) reported variation in grain quality among South African sorghum landraces. However, these landraces with high grain quality exhibited low yield potential. Their protein and amino acid levels varied depending on cultivars used, and in different environments. Hence, it is important to assess the level of proteins and their constituents in diverse genotypes for improvement.

Proper characterization and evaluation of germplasm is an important component for effective management of genetic resources and their utilization in breeding programmes (Frankel, 1989). Accurate identification of genotypes is very useful throughout the process of breeding, starting from initial parent selection to the final utilization of cultivars. Agro-morphological descriptors have been used traditionally to distinguish one accession from another (Smith and Smith, 1992). Sorghum is a very diverse crop when using morphological descriptors, with cultivated sorghums exhibiting great phenotypic variability (Aruna and Audilakshmi, 2008). Hence it is vital to monitor genetic diversity because the availability of the resources determines the current and potential future sustainability of agricultural productivity (Huang et al., 2007).

Exploitation of diversity at the genotypic level requires an efficient system such as molecular marker technology. The use of molecular marker technology has been attempted in various crops for management and improvement. The estimation of plant genetic resource diversity has become simpler and more reliable with the advent of DNA-based molecular markers. This is because these molecular markers are discrete, co-dominant or dominant, and free from epistatic gene action (Tanksley et al., 1989; McIntyre et al., 2001). In contrast to morphological or biochemical marker techniques, DNA-based methods are independent of environmental factors and give rise to a high level of polymorphism (Karp et al., 1997). The DNA-based fingerprinting techniques are important tools for genetic identification, and in determination of variation in plant breeding, and for germplasm management (McGregor et al., 2000; Simioniuc et al., 2002).

Many molecular marker technologies have been developed and applied for studying patterns of genetic diversity in sorghum germplasm collections and in breeding programs (Gupta and Varshney, 2002). The most commonly used marker techniques in sorghum for diversity studies include the following: random amplified polymorphic DNA (RAPD) (Prakash et al., 2006; Shivjee and Khanna, 2010; Abdel-Fatah et al., 2013), amplified fragment length

polymorphism (AFLP) (Wu et al., 2006; Pecina-Quintero et al., 2012; Shegro et al., 2013), single nucleotide polymorphism (SNP) (Murray et al., 2009), microarrays (Huang, 2011), diversity array technology (DArT) (Mace et al., 2008; Mace et al., 2009) and simple sequence repeats (SSRs)/microsatellites (Dje et al., 2000; Gupta and Varshney, 2002; Adguna and Bekele, 2013; Beyene et al., 2014). The SSR markers are co-dominant, discrete and highly informative (Karp et al., 1997).

A method referred to as high resolution melt (HRM) analysis was invented in 2003, and has been used to detect DNA variations in plants and animal species (Wittwer et al., 2003). This technique is a closed tube, and post-PCR technique that differentiates amplicon products in the presence of a saturating fluorescent dye on the basis of melting profiles (Naidoo et al., 2013). The melting profiles are evaluated by normalized fluorescence curves, derivative plots, or difference plots to detect variation (Vaugn and Elenitoba-Johnson, 2004). It is helpful for distinguishing genotypes or accessions in combination with other molecular marker methods. The HRM technique is fast, accurate, efficient, cost-effective, repeatable, and has high throughput. It has been used in various crops including maize (Abakemal et al., 2012; Naidoo et al., 2013), and barley (Lehmensiek et al., 2008) among other crops.

Several landraces of sorghum are widely grown by small-scale farmers across different provinces in South Africa. Despite their low yielding potential, these landraces are preferred by the smallholder farmers because of their broad adaptation, tolerance to abiotic and biotic stresses, and suitable quality and agronomic attributes (DAFF, 2010). These sorghum landraces constitute an important source of genetic material for future breeding programmes. Hence, it is necessary to carry out efficient characterization of sorghum landrace collections from various provinces within the country. This will help streamline sorghum breeding for improved yield and protein quality. Identification of suitable sorghum genotypes and development of improved cultivars which are more suited to the marginal areas would help in food security and alleviation of malnutrition (Slabbert and Dorfling, 2001).

Farmers' knowledge and preference on the use of the local cultivars, characterization and improvement of the sorghum landraces with special reference to yield, protein content and amino acid is important for selections and breeding. Accurate documentation of the constraints affecting sorghum production that are encountered by farmers is also essential in establishing the breeding goals in future sorghum breeding programmes. This can be achieved through farmer-scientist partnership and information sharing.

The overall objective of this study was to determine the genetic diversity present among South African sorghum germplasm using agro-morphological traits, SSR markers, and crude protein content and amino acid composition. Furthermore, the study aimed to explore farmers' perceptions of the constraints affecting sorghum production, and their varietal trait selection criteria, in a study conducted in selected districts of Limpopo Province.

Research Objectives

The specific objectives of the study were:

i. To determine farmers's perceptions of the constraints affecting sorghum production, and their criteria for choice of sorghum varieties in the Limpopo Province in South Africa.
ii. To assess the level of genetic diversity present among South African sorghum genotypes using agro-morphological traits.
iii. To compare the capacity of RAPD and SSR markers and high resolution melt (HRM) analysis, to determine the genetic variation present among selected sorghum genotypes.
iv. To assess the genetic diversity present among South African sorghum genotypes using genetic distances, as measured by SSR markers.
v. To determine the genetic diversity of selected South African sorghum genotypes grown in two diverse environments, especially for their protein content and amino acid composition, in order to select candidate lines for future breeding and conservation.

Research Hypotheses

i. Farmers are aware of sorghum production constraints and have preferences for specific traits in the sorghum varieties that they choose to grow.

ii. There is distinctive genetic variation in agro-morphological traits among the South African sorghum genotypes.

iii. SSR markers are more effective than RAPD markers for genotype analyses in sorghum.

iv. HRM analysis is a technique that can match the efficacy of SSR markers for genotypic studies in sorghum.

v. There is genetic divergence in South African sorghum genotypes when tested using high resolution melt analysis in combination with RAPD and SSR markers.

vi. There is considerable genetic diversity among the South African sorghum genotypes when evaluated using 30 SSR markers.

vii. There exists genetic diversity for crude protein content and amino acid composition among South African sorghum genotypes.

Thesis Outline

Table 01 shows the thesis outline. The thesis is written in the form of discrete research chapters, each following the format of a stand-alone research paper (whether or not the chapter has already been published). This is the dominant thesis format adopted by the University of KwaZulu-Natal. As such there is some unavoidable repetition of references and some introductory information between chapters.

Table 01. Thesis outline.

Chapters	Objective
-	Introduction to Thesis
Chapter 1	Literature review
Chapter 2	Appraisal of farmers' sorghum production constraints and variety preferences in the Limpopo Province, South Africa
Chapter 3	Assessment of genetic relatedness among South African sorghum genotypes using agro-morphological traits
Chapter 4	Comparison between random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers with high resolution melt analyses in genetic variation analysis among selected sorghum genotypes
Chapter 5	Genetic diversity analysis of South African sorghum genotypes using SSR markers
Chapter 6	Genetic diversity of selected South African sorghum genotypes for protein and amino acid composition
Chapter 7	Thesis overview

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CHAPTER 1

Literature review

1.1 Introduction

This section presents a literature review with the purpose of supporting and enhancing the value of the anticipated research study. The review examines genetic variation and characterization using agro-morphological traits, DNA-based markers, grain nutritional quality and farmer's perceptions, and constraints of sorghum production.

1.2 Sorghum origin and domestication

It has been estimated that sorghum (*Sorghum bicolor* (L.) Moench) was originally domesticated between 5,000 and 7,000 years ago, probably in northern Africa (Murdock, 1959; Ehret, 1988; Harlan, 1989). Distribution of sorghum followed, and has been associated with human migration, trade, and shipping routes from Africa, to the Middle East to India, 3,000 years ago (Kimber, 2000). Some authors have even suggested that the origin of sorghum was in India (Haaland, 1995; Meadow, 1996). Other researchers have proposed that the origin and domestication of sorghum was in China (Kimber, 2000). Sorghum has been found in Africa, Asia, Australia, and some parts of America (Harlan and Wet., 1972; Dogget, 1988; Acquaah, 2007). Numerous studies have demonstrated that sorghum is a very diverse crop, with cultivated sorghums exhibiting great phenotypic variability.

1.3 Sorghum genetics and classification

Several reports indicate that sorghum (2n=2x=20) can be classified into two groups, the wild and the cultivated sorghums (Dogget, 1988; Smith and Frederiksen., 2000; Ayana et al., 2002). The wild sorghums include *Sorghum halepense* (L) Pers, S. *propinquum* (Kunth) Hitchc, *S. bicolor* sub-species *drummondii* and *S. bicolor* subspecies *verticilliflorum*. The cultivated germplasm has been classified into five major races, *bicolor*, *caudatum*, *durra*, *guinea* and *kafir,* and 10 intermediate races based on panicle morphology. According to Harlan and De Wet (1972) the *bicolor* race is characterised by loose panicle with grains covered by large closed glumes, and is mostly distributed in Asia and Africa. The *caudatum* race is characterised by asymmetric grain, flattened on the ventral surface and convex on the dorsal surface. The panicle morphological structure varies with shape. This race is

mainly found in Central and East Africa. The *durra* sorghums have very compact panicles with curved penducles, and tiny glumes that are attached to globular grain. This race is mostly grown in East Africa, the Middle East and India. The *guinea* sorghums are tall with loose panicles, spikelets with open glumes enclosing an elliptical grain and are photoperiod sensitive. These sorghums are found in West Africa. The *kafir* varieties are small sorghums with relatively compact and cylindrical panicles consisting of symmetrical grain flattened on the ventral surface and convex on the dorsal. This race is grown mostly in eastern and southern Africa (Mann et al., 1983). Morphologically intermediate races have been reported in Africa (Tesso et al., 2008; Mutegi et al., 2009). Due to the vast diversity in cultivated sorghum, breeders are interested in exploiting this diversity, to develop improved varieties using various breeding methods.

1.4 Constraints to sorghum production

There are various biotic and abiotic stresses that affect the production and productivity of sorghum in various parts of the world. The biotic stresses include diseases, insect pests, birds, and parasitic weeds. The most prevalent diseases are ergot, grain mould, various smuts, root and stalk rots, and leaf diseases such as rust, zonate leaf spot, mildews, anthracnose and leaf blight (Dogget, 1988; McLaren and Smit, 1996). The major insect pests include stalk borers, maize and sorghum aphids, panicle feeding bugs, beetles, bollworms, wireworms, cutworms, weevils, shootfly, sorghum midge, and armoured cricket (Dogget, 1988; van den Berg and Drinkwater, 1997). The most commonly known parasitic weeds include *Striga* spp., commonly known as witchweed. Whereas weeds affects sorghum produce and plant growth development, *Striga* spp. feed on the sorghum roots, extracting water, minerals and photosynthetic assimilates (Press and Steward, 1987; Robert, 2011). Researchers have developed sorghum varieties with resistance to *Striga* spp. (Haussmann et al., 2000), and have developed an Integrated *Striga* Management (ISM) tool for control of *Striga* spp. (Teshome, 2013). Most of these diseases and insect pests are controlled by chemicals, natural enemies, cultural practices and resistant cultivars. In order to reduce bird damage, high tannin cultivars have been grown in some parts of Africa, although this grain has a bitter taste. This approach was mainly used by enterprises growing sorghum for large scale beer brewing. For direct human consumption, non-bitter varieties have been preferred. Hence, some commercial farmers and researchers try to manage bird damage using bird repellents such as methiocarb (Dogget, 1988) and gas-driven bird scarers. This still remains a challenge for small scale farmers, showing the need for development, access and

availability of bird resistant varieties that can be utilized by large- and smallholder farmers and communities in marginal areas of the world.

The most prevalent abiotic stress in sorghum is drought, which is experienced in many parts of the world. Plants become stunted and their growth and development is retarded (Moussa and Abdel-Aziz, 2008; Younesi and Moradi, 2009). Drought has been reported to affect all growth stages of sorghum, including the pre- and post-flowering stages. Many studies have been conducted in breeding for sorghum cultivars for enhanced levels of drought tolerance, including the identification of component traits affecting tolerance to drought (Khanna-Chopra and Sinha, 1988; Blum, 2011; Assefa, 2012).

Malnutrition is one of the factors contributing to food and nutritional insecurity of mankind. Malnutrition related ailments affect communities globally, but especially in sub-Saharan Africa (FAO, 2010).Traditionally, crop improvement studies have focussed on addressing environmental stresses, insect pests and diseases. Breeding for improved nutrition has been neglected in the past, for many crops, including sorghum. More recently, plant breeders have started to breed for improved nutritional quality traits such as enhanced levels of Fe and Zn, vitamins, starch, amino acids and proteins (Ashok et al., 2010; Sanjana et al., 2010). The breeding strategies include biofortification, mutation breeding and genetic engineering (Monyo et al., 1988; Oria et al., 2000; Tesso et al., 2006; Prasad, 2010).

1.5 Analysis of genetic diversity

Assessment of genetic diversity is of paramount importance for current and future breeding programmes (Mohammadi and Prasanna, 2003). This is because it is essential for sourcing and pyramiding of genes that may be considered valuable, and that can be used in various breeding programmes such as in nutritional quality breeding. Many local germplasm/landraces has been lost, or are likely to disappear due to modern agricultural practices, environmental factors, and genetic erosion (Teshome, 2001). Genetic diversity is valuable for selections of varieties that may serve as future parents for hybrids and other new varieties (Geleta and Labuschagne, 2005). Knowledge of the genetic and nutritional diversity of the local genetic material and their interrelationship is an invaluable aid for plant breeders making decisions about which crosses to make, and for germplasm conservation. Genetic diversity among accessions provides opportunities for improvement of agronomic and nutritional quality traits in crops (Huang, 2004). It aids plant breeders to characterize and classify accessions into heterotic groups (Menz et al., 2004) and it also affects the potential genetic gain via selection (Kotal et al., 2010).

1.6 Sorghum characterisation and evaluation

Characterisation and evaluation of germplasm are a prerequisite for utilization of the available diversity in cultivar improvement. The germplasm can be characterised morphologically and genotypically, as well as by using nutritional quality traits.

1.6.1 Morphological characterization of sorghum

Morphological or phenotypic descriptors are used to distinguish one accession from another. The characterisation of genotypes gives descriptive information of their traits, and aid in understanding the similarities and differences among genotypes (IBPGR and ICRISAT, 1993). Phenotypic characterisation is usually based on both qualitative and quantitative morphological characters (Upadhyaya *et al*., 2010). According to Geleta and Labuschagne (2005) the qualitative traits include leaf midrib colour, grain colour, glume colour, endosperm texture, pericarp colour, leaf trichomes, awns, testa colour, pericarp thickness, and panicle compactness. Quantitative traits are also useful for determination of genetic diversity among genotypes. Typical quantitative traits include plant height, maturity, leaf area, leaf width, leaf length, number of leaves, panicle length, grain yield per plant, grain size, 1000 grain weight, grain number per panicle, panicle width, number of primary branches per panicle, and panicle weight (Punitha *et al*., 2010). Abdi *et al*. (2002) reported patterns of variation in sorghum for qualitative traits in Ethiopia. Rao *et al*. (1996) observed great morphological and agronomical diversity in sorghum germplasm in India. Ganesamurthy *et al*. (2010) observed great phenotypic variability among sorghum landraces collected in Tamil Nadu. Mehmood et al. (2008), Reddy et al. (2012), and Rakshit et al. (2012) studied the genetic variation within and among sorghum landraces using agro-morphological traits. The researchers identified great genetic diversity among the landraces. Furthermore, Ngugi and Maswili (2010) characterised sorghum genotypes from Kenya using phenotypic descriptors. Although there are reports on morphological diversity in other regions, there appear to have been few such studies reported in South Africa. Thus, morphological diversity analysis is required because most of the landraces grown by smallholder farmers in South Africa have not been characterised. Morphological characterisation is useful for breeding programmes, identification of duplicates, establishment of patterns of genetic variation, and establishment of relationships of agronomic traits for direct and indirect selection.

Although agronomic and morphological characterization provides useful information to breeders, the challenge is that the phenotypic characters are influenced by environmental factors (Smith and Smith, 1992; Cadee, 2000). The alternative is to undertake genotypic characterisation, using molecular marker systems because these are not subjected to

environmental influences, they do not require fixed plant developmental stages and they have the potential to give results rapidly from both seed and seedlings. On the other hand, most scientists prefer to use both morphological descriptors and molecular markers to characterize sorghum varieties (Geleta et al., 2006; Shehzad et al., 2009; Abdel-Fatah et al., 2013).

1.6.2 Molecular marker technology

Accurate estimates of genetic diversity levels among and within crop plant species are increasingly important for crop improvement. This is because they can assist to reduce population bottlenecks, threats of genetic losses, and also for the determination of variation to be found in local landraces at country, regional and local levels. This requires an efficient DNA marker technology. Among the marker techniques used for DNA analysis are simple sequence repeats (SSR) (sometimes referred to as microsatellites) and random amplified polymorphic DNA (RAPD) analysis. Both have been widely employed for the evaluation of genetic variation among crop genotypes.

1.6.2.1 Random amplified polymorphic DNA (RAPD)

RAPD is a fast, simple and reliable fingerprinting technique used for various applications in plant breeding (Williams et al, 1990). It is a useful tool for genetic diversity analysis of plant populations. This technique can be used for phylogenetic studies (Agrahama and Tuinstra, 2003), genetic diversity analysis, cultivar identification, quantitative traits, marker assisted selection and to estimate the extent of genetic variation in sorghum (Menkir et al., 1997; Chowdari et al., 1998a,b; Ayana et al., 2002; Dahlberg et al., 2002). Shivjee and Khanna (2010) used RAPDs to evaluate the level of genetic diversity in selected sorghum varieties. Agrahama and Tuinstra (2003) used both RAPDs and SSRs to study phylogeny and genetic relationships among sorghum genotypes. Arya et al (2006) evaluated levels of genetic diversity among Indian sorghum germplasm. The advantage of RAPD is that DNA probes and prior sequence information for the specific design of primers are not required, (Williams et al 1990). According to Kumar and Gurusubramanian (2011) the technique is PCR based and involves no blotting or hybridization. It involves low costs per unit assay when compared to other DNA marker systems. It requires only small amounts of DNA, produces high numbers of DNA fragments and can be automated. Arbitrary primers can be easily purchased. The disadvantages of this method are that the technique is not reproducible, is a dominant marker, and is species specific. It has challenges with co-migration, and mismatches between the primer and the template, which may result in the total absence of PCR product, or a decreased amount of the PCR product.

1.6.2.2 Simple sequence repeats (SSR) (microsatellites)

Simple sequence repeats consist of short tandem repeated nucleotide motifs flanked by conserved sequences in the loci (Tautz, 1989). The SSRs are multi-allelic and generally more informative than most of the marker techniques, and are based on heterozygosity values (Powell et al., 1996). SSR markers are easily maintained and shared among laboratories (Maughan et al., 1995). They have high reproducibility, co-dominance, low cost, and abundance in the plant genome. The SSRs serve as an ideal marker system for genetic analysis. Hence, this technique has been used widely in genetic diversity studies of various crops including sorghum.

The SSR reveals a large number of polymorphisms. Hence, they can be used to study plant species in which previous methods have found little or no variation (Echt et al., 1998). They can give high throughput and are co-dominant markers, therefore, homozygotes and heterozygotes can easily be distinguished. The technique is inexpensive once the primers have been developed. It is repeatable, easily automated, requires small quantities of DNA, and gel runs can be multiplexed. It is not influenced by environmental conditions. However, the development can be long and expensive because of the requirement for complex electrophoresis methods such as polyacrylamide gels, and sequencing. Co-migrating fragments may not always be homologous. According to Ciofi et al. (1998) slippage of the polymerase enzyme may yield products that differ with 1-5 repeat units. This can be a significant problem when analyzing mono- and di-nucleotide repeats.

SSRs have become the molecular markers of choice for a wide range of applications. The applications include genetic mapping and genome analysis (Chen et al., 1997; Li et al., 2000), gene and quantitative trait locus analysis (Blair and McCough, 1997) and in marker assisted breeding (Ayres et al., 1997; Weising et al., 1998). In sorghum, microsatellites are highly polymorphic (Brown et al., 1996; Taramino et al., 1997; Bhattramakki et al., 2000; Kong et al., 2000) except those located in coding regions that are relatively conservative (Schloss et al., 2002). Furthermore, Wu and Huang (2007) used SSR for mapping sorghum genome in comparison with the existing genetic linkage maps. Furthermore, SSRs have been used in genetic diversity studies among elite sorghum inbred lines (Smith and Frederiksen, 2000; Menz et al., 2004; Shehzad et al., 2014), among germplasm collections from different geographic locations (Yang et al., 1996; Dje et al., 2000), and in the assessments of the population genetic structure and relatedness within or among landraces (Dje et al., 1999; Uptmoor et al., 2003; Folkertsma et al., 2005; Bhosale et al., 2011). Smith

et al. (2010) used SSR analysis to determine the level of genetic diversity in sorghum hybrids widely grown in the USA. El-Way et al. (2008) used SSR analysis to assess genetic diversity in nine sorghum lines from different regions. Wide genetic diversity has been reported in many studies of sorghum diversity (Han et al., 2011; Ngugi and Onyango, 2012; Rajput et al., 2012; Beyene et al., 2014; Kimani et al., 2014; Tesfamichael et al., 2014). In addition, SSRs can be used in conjunction with other molecular techniques (Geleta and Labuschagne, 2005).

The use of the SSR technique has been reported for other crops such as barley (Wang et al., 2010), rice (Yao et al., 2015), wheat (Sutapa et al., 2014), cassava (Njoku et al., 2013), maize (Shiri et al., 2014), cotton (Zhao et al., 2015) and cowpea (Adetiloye et al., 2013). Among cereal crops, rice and maize have been more intensively characterized for DNA markers (Romero et al., 2009) than sorghum. In South Africa, some of the sorghum germplasm has been characterized but not all collections of landraces grown by small scale farmers in rural areas have been characterized

1.6.3 High resolution melt (HRM) analysis

The HRM analysis is a post-PCR technique that has been developed relatively recently for use in genotyping nuclear genes in plants and animals (De Koeyer et al., 2010). It has been identified as a simple, rapid, consistent and powerful technique for genotyping, and detection of polymorphism and mutations (De Leeneer et al., 2008; Muleo et al., 2009). It can further differentiate homozygotes by a shift in melting temperature (T_m) and heterozygotes by a change of shape of melting curves of the samples analyzed (Wittwer et al., 2003; Reed and Wittwer, 2004). The method involves a standard PCR reaction and the use of a fluorescent dye, whereby the primers bind the double stranded DNA in the presence of the dye. Following the PCR, the fluorescence is captured while the PCR products are melting. The melting profiles are determined by GC content, length, and the homozygosity and /or heterozygosity of the samples under investigation (Reed et al., 2007). The reactions are analyzed by normalized fluorescence curves, derivative plots, or difference plots to discover variants (Vaugn and Elenitoba-Johnson, 2004). HRM can provide an excellent specificity and sensitivity with high throughput.

HRM analysis has been used in various crops, including sorghum. Mofokeng et al. (2012) reported the presence of genetic diversity among selected sorghum genotypes when comparing RAPDs and SSRs with HRM analysis. Abakemal et al. (2012) reported genetic variation within and between quality protein maize inbred lines when using RAPDs, and SSRs in combination with HRM analysis. HRM analysis in combination with SSR has been

used to fingerprint hybrids and their parental lines in rice (Zhu et al., 2013). The researchers concluded that HRM analysis should be given more priority due to its high accuracy, low-cost, speed and efficiency. The technique has the potential to analyze for genetic diversity. It can help to speed up selections in plant breeding programmes due to its convenience, time saving, and cost reduction of post-PCR processing, especially relative to gel electrophoresis and sequencing (Zhu et al., 2013). In addition, SSR-HRM can be used for variety identification in grape and olive cultivars (Mackay et al., 2008), common bean (Ganopoulos et al., 2012), and sweet cherry (Ganopoulos et al., 2011).

HRM has also been used in other studies, including the detection of SNPs in crops such as maize (for a low phytic acid gene) (Naidoo et al., 2013), potato (De Koeyer et al., 2010), almond (Wu et al., 2009), white lupin (Croxford et al., 2008), barley (Lehmensiek et al., 2008), perennial rye grass (Studer et al., 2009), tomato (van Deynze et al., 2007) and pepper (Park et al., 2009).

1.7 Sorghum nutritional quality

Nutritional quality is an inherited trait of a crop. It may vary as a result of environmental changes. Most plant breeding research on sorghum has focused on other factors such as biotic and abiotic stresses and agronomic characters such as stem height. In the past nutritional aspects such as vitamins, mineral content and proteins have received little attention. Proteins are essential to human beings. They contribute to tissue building, which is dependent on amino acids, which are sometimes referred to as the “building blocks of life” (Hoveland, 1980; Azevedo et al., 2006; Lea and Azevedo, 2006). Amino acids are derived from digested proteins, sourced from plants and animals. Whilst there are many amino acids, only eight are essential amino acids that have to be supplied in the diet of human beings. These include: leucine, phenylalanine, threonine, tryptophan, isoleucine, lysine, methionine and valine (Ferreira et al., 2005; Ferreira et al., 2006) The synthesis of amino acids, and their role in the nitrogen pathway has been subjected to much research (Ayongwa et al., 2006; Kingston-Smith et al., 2006; Tcherkez and Farquhar, 2006). Nutritional quality considerations in the breeding of crops usually include protein levels and the amino acid profiles (Helm et al., 2004; Pompeu et al., 2006). Globally, about 65% of proteins consumed by humans are supplied by plants, with 47% coming from cereals. In underdeveloped countries, plant proteins are the major source of protein for most people, and in many instances they may be the only source of protein most of the time (Millward, 1999).

1.7.1 Protein content and amino acid composition in sorghum

Protein is one of the important nutritional attribute of sorghum quality. It is located in the endosperm, germ, and pericarp, which are composed of about 80%, 30% and 16% protein, respectively (Taylor and Schussler, 1986). The average protein content varies from 7.3-15.6% (Hulse et al., 1980). The major proteins found in sorghum are kafirins or prolamins, account for approximately 80% of the total grain protein (Taylor et al., 1984). The remainder are glutelins. These protein fractions are located primarily within the protein bodies and protein matrix of the endosperm. The prolamins are characterised by their low contents of essential amino acids, particularly lysine which accounts for only 0.2% of the total amino acids in sorghum kafirin, less than 2% in the endosperm and less than 3% in the whole grain. The levels of protein can be increased significantly by generous nitrogen fertilization (Warsi and Wright, 1973).

The grain germ is rich in albumins, and globulins, while the endosperm contains kafirins and glutenins. The albumins, globulins, and glutenins fractions are rich in lysine and other essential amino acids. Cultivars exhibiting improved protein quality usually contain more of these, with a corresponding lower proportion of kafirins. These cultivars have been selected and bred to contain a larger germ-to-endosperm ratio, and contain high levels of albumins, globulins, and glutenins (Mohan and Axtell, 1975). The poor nutritional quality of the kafirins is compounded by the fact that they are difficult to digest and that their digestibility decreases on cooking (Duodu et al., 2003).

Researchers have attempted to improve the nutritional quality of sorghum, based on the identification of high lysine mutants (Mertz et al., 1964; Nelson et al., 1965). Two mutants were identified in sorghum, with the *hl* gene in an Ethiopian line (Singh and Axtell, 1973) and the P721 opaque gene, which was induced with the chemical mutagen diethylsulphate (Axtell *et al*., 1979). Studies show that these lines contain ‘‘low prolamin’’ levels, whereby the proportion of kafirin is reduced by about 50%, with compensatory increases in other more lysine-rich proteins and free amino acids. The lysine content is enhanced by about 40-60%. The limiting factor with these lines is the association of high lysine with deleterious effects on seed weight and yield. Oria *et al*. (2000) reported the identification of a novel line with high protein digestibility from a cross involving the high lysine *P721* opaque mutant. The Africa Biofortified Sorghum (ABS) project developed transgenic sorghum line with increased lysine content (50%), ABS#1 (ABS, 2009). In the ABS project they also developed another line, ABS#2, with improved grain digestibility, whereby the bioavailability of essential amino acids such as lysine, threonine, and tryptophan are increased, together with beta-carotene.

Further lines with enhanced bioavailability of Fe and Zn were to be developed in this project (Zhao, 2007). However, due to regulatory issues, these GMO sorghum lines have not been released to the public.

1.8 Genetic diversity of protein content and amino acids composition

Malnutrition is one of the challenges facing Africans. It was recorded that in the 1980's and 1990's in sub-Saharan Africa, the rate of mortality due to protein malnutrition ranged between 25-35% on average (Rutherford and Mahanjane, 1985; Gernaat et al., 1998). In the current decade, sub-Saharan Africa is faced with the highest level of diseases associated with malnutrition in the world, as a fraction of the population, and this is increasing, whereas it is decreasing for the rest of the world (FAO, 2008). South Africa is also faced with the challenge of malnutrition, especially in the rural, tribal areas. Hence it is important to assess the available genetic pool of sorghum for nutritional quality traits such as their protein and amino acid digestibility, profile and content. Assessment of the nutritional profile of plant genotypes is essential to reduce malnutrition by breeding for improved content and composition of minerals, proteins and vitamins in crops such as sorghum (Welch and Graham, 2004; Feil et al., 2005). Knowledge of genetic and nutritional diversity can impact on conservation of sorghum genetic resources and the breeding of improved varieties (Simionuc et al., 2002).

Genetic variation in protein, mineral composition, total starch and its components was also observed among Ethiopian sorghum landraces (Shegro et al., 2012). Shegro et al. (2013) again reported nutritional diversity among a total of thirty one sorghum landraces from Western Ethiopia. Nguni et al. (2012) reported genetic and nutritional diversity among the sorghum accessions from Malawi, Tanzania, and Zambia. The authors assessed grain iron and zinc, total protein, and starch contents among the accessions, and used ten SSR markers to estimate genetic diversity. Considerable variation in minerals (Fe and Zn) was observed among cultivars, breeding lines and selected sorghum accessions (Hariprasanna et al., 2014). The results show that there is hope for enrichment of micronutrients in sorghum in order to combat malnutrition.

Mokrane et al. (2010) found differences among Algerian sorghum genotypes in protein and amino acid concentrations. Variation in amino acid profile and storage protein content was also reported among the commercial sorghum, MASSA 03, and nine ICRISAT high-lysine genotypes form India (Vendiamatti et al,. 2008). Furthermore, when white sorghum hybrids

from Foggia in Italy and Kansas, USA were evaluated for chemical composition, protein and lipid content, and total amino acid, the protein content was higher in Foggia, southern Italy, than in Kansas, USA, although the grain quality was comparable between sorghums grown in this two regions (Pontieri et al., 2010). Therefore, sorghum can serve as source of essential amino acids and also potential parents with high amino acid concentrations can be selected for further breeding improvement.

1.8.1 Methods of protein and amino acid composition analysis

The amino acid composition of sorghum refers to the levels of various amino acids present in the protein fraction of grain. The amino acid profiles can be assessed by various methods such as chromatography procedures including gel filtration, ion exchange chromatography, preparative IEF, and hydrophobic interaction chromatography. However, near-infrared spectroscopy (NIR) is the most effective and non-destructive technique for analysis of quality traits such as protein and amino acid levels relative to the other techniques (Brauteseth, 2009).

1.8.1.1 Near-infrared spectroscopy

The use of near infrared spectroscopy (NIR) in the analysis of amino acid compositions has been reported in various studies (Olesen et al., 2011). Near-infrared spectroscopy is a technique that was first developed in the 1950's (Barton *et al*., 2002). It was reported in the early 1960 as a non-destructive method that can be utilized in various ways. This technique functions with wavelengths between 750-2600 nm in which overtones and combinations of vibrations of numerous functional groups (-OH, -CH, -NH, -SH, etc) can be excited and detected. Hence, it can give information about structural and physical characteristics of biological compounds (Alexandrakis *et al*., 2008). It is fast, cheap, accurate, and can identify multiple chemical components in sample composite matrices. It offers an uncomplicated sample presentation. Hence, it is useful for speeding up selections in breeding programmes aiming to increase quality traits and decrease toxins.

The NIR has been used for analysis of many traits in various crops (Fontaine *et al*., 2001). Hacisalihoglu *et al*. (2010) NIR to measure the levels starch, protein, and seed dry matter in common bean. The starch and protein parameters have been estimated by NIR in potatoes. Starch content was 90% while total protein content was 62% (Haase, 2006). Schultz *et al*. (2005) analyzed carotenoids in plants using the NIR. Pedro and Ferreira (2005) reported various levels of total and insoluble solids, lycopene and β-carotene in tomato fruit. The NIR technique was also reported to be useful in the analyses of starch, ash, cellulose, total nitrogen, and total sugars in the roots and tubers such as cassava, taro, yams and

sweetpotato (Lebot *et al*., 2009). The NIR technique can be used in the analysis of *trans* fatty acids of various ground cereal products (Kim and Kays, 2009). Amino acid composition can be determined in soybean using NIR (Kovalenko *et al*., 2006). In wheat, the NIR has been calibrated for amino acids and showed useful for explaining variation of about 70-98% (Fontaine *et al*., 2002). Delwiche *et al*. (2011) identified waxy starch in wheat using NIR analysis (high levels of amylopectin relative to amylose).

Several studies have shown the efficiency of NIR analyses when applied to sorghum. Figueiredo *et al*. (2006) used NIR to measure amylose, protein, and lipid contents, endosperm texture, and hardness in cultivated sorghum core collections in whole and ground grain. Hicks *et al.* (2002) compared whole and ground grain NIRS calibrations of sorghum genotypes and hybrids for starch, lipid, and protein content, together with protein digestibility, in two sites. NIR analysis has been reported to provide accurate and efficient measurements in the analysis of protein for nutritional value and in the labelling of seeds of a number of cereal cultivars (YoungYi et al., 2010). Fontaine *et al*. (2002) used NIR to evaluate protein content and amino acid composition of milled sorghum grains. The protein and starch levels were also determined in sorghum lines and single hybrids for nutritional value and the protein, which ranged from 9.43-17.7% (Pepó et al., 2011). Roberts et al. (2011) analyzed sweet sorghum bagasse for gross calorific value, *in vitro* true digestibility and crude protein using NIR. The correlation coefficients were above 0.9 for all analyses. Although this technique has potential for the analysis of quality traits, there is still limited information on the application of NIR in sorghum landraces for breeding purposes, in particular, in terms of developed, open source calibration models, which is essential if plant breeders are to make use of the technology.

1.8.1.2 High performance liquid chromatography

High performance liquid chromatography (HLPC) is one of the methods used for analysis of chemical constituents of plants, including proteins and amino acids. The technology is widely used because of its reliability, the high level of reproducibility, and the low detection limits that HPLC offers (Breithaupt, 2004). However, this method requires a long process of sample preparation, and extraction of the desired pigments, which may be attached to other fractions in the plants that can mask the authentic content (Schulz *et al*., 2005).The use of HPLC has been reported in the analysis of sorghum (Taylor *et al*., 2007). Loerger *et al*. (2007) studied the variation in proteins of vitreous and flowery sorghum endosperm using HPLC methods. Mokrane *et al*. (2009) characterized the primary sorghum proteins, kafirins, using a combination of SDS-PAGE, SE-HPLC, and RP-HPLC, as found in various sorghum genotypes and concluded that sorghum could be an excellent source of amino acids and

protein, if the problems of amino acid profile, and digestibility of proteins, could be solved. El Nourf *et al* (1998) classified sorghum kafirins in relation to cross linking behaviour. Dykesa *et al*. (2011) reported other quality traits in lemon-yellow sorghum genotypes grown in two locations. Although several studies have reported the use of HLPC for analysis of protein and amino acid composition, the HPLC method can be time consuming and expensive when large collections require analysis for quality traits in plant breeding programmes.

1.9 Participatory rural appraisal: farmer production constraints and variety preference

Participatory rural appraisal (PRA) is one of the effective tools for attempting to solve farmer's local concerns through involvement of community members, understanding their situation and learning from them, usually using an interdisciplinary team of researchers (Blaney and Thibault, 2003). The PRA involves engaging with community members/farmers, both individually and in groups, and with sector representation, whereby the views and concerns of farmers and concerned parties are expressed and recorded. The presentation and analysis of information of the PRA is done in a relaxed manner in contrast to other methods such as rapid rural appraisal (RRA) (Chambers, 1992). It emphasizes local knowledge, empowerment and sustainability of natural resource, agricultural, health, social or other issues (Chambers, 1997). It has been used mainly by NGO's and government officials in developing countries in the past 25 years (Cornwall *et al*., 2001). It can also be a first step in participatory plant breeding, which tends to encourage widespread and significant adoption of new varieties (Cecarralli *et al*., 2003).

The PRA technique maybe applied in various sectors, including the agricultural sector. It has been used to determine the challenges farmers face in the development and production of crops of interest and also the preferences of traits of interest they may have. Bucheyeki *et al*. (2011) reported that the major rice production constraints in Tanzania included the lack of improved varieties, the susceptibility of landraces to diseases, the unavailability of seeds, frequent drought, and high input prices. In Ghana, most important sorghum production constraints have been reported to be poor soils, erratic rainfall and pest infestation of the grain during storage (Kudadjie *et al*., 2004). The PRA technique has also been used to solicit farmers' views on crops such as banana, where farmers preferred varieties that produced heavy bunches, with resistance to pests and diseases, tolerance to drought, and which were fast maturing, with good quality traits (Bareke *et al*., 2009). In root crops such as sweet potato farmers preferred sweet taste, high yield, early maturity good storability, high dry mass, less fibrous, leaves that make good vegetable stews, and resistance to pests and

diseases (Chiona, 2009). In cassava, the preferences of farmers were for high yield, pest and disease tolerance, sweetness, high dry matter content, and cultivars that cooked well and were marketable and which could be stored for a long time without rotting (Mtunda, 2009). In cereals such as maize, farmers preferred affordable seeds, high yield, early maturity, and low input costs (Sibiya, 2009), whereas with rice, traits such as large grain size, good aroma, early maturity and high yield were the preferred by farmers (Singh et al., 2004; Lamo, 2009). Odendo *et al*. (2002) solicited farmers' views on the selection of maize varieties that they planted, and reported that earliness and high yield were the most important traits to farmers. More information on other cereal crops through participation of farmers is still required to ensure that when new varieties are developed, widely adopted by farmers because they meet the selection criteria of the farmers (Sperling *et al*., 1993).

Sorghum is of regional importance as a staple food in South Africa. The sorghum growers in the Limpopo province are both small and large scale farmers. The province is considered to be the centre of sorghum genetic diversity in South Africa. To enhance sorghum breeding it is necessary to understand farmer's objectives and requirements (Kudadjie *et al*., 2004). Farmer's and end user's involvement in research usually enhance the chances of adoption of new varieties (Danial, 2003). This is because they can specify the type of sorghum material they prefer, also have their own selection criteria (vom Brocke et al., 2010). Local varieties are an important source of germplasm in plant breeding programmes due to presence of traits preferred by farmers and because of their local adaptation (Danial *et al*., 2007). For instance, farmers in the Lake Zone of Tanzania prefer sorghum varieties on the basis of color and taste of sorghum ugali (Mafuru et al., 2007). Makanda (2009) conducted participatory rural appraisal (PRA) in Zimbabwe and South Africa on their preferences for sweet stem sorghum. He determined that farmers preferred high yielding and early to intermediate sorghum varieties with improved stem sugar content. Mekbib (2007) used a PRA to classify and characterise sorghum genotypes. Rana *et al*. (2000) reported farmer desired characteristics including high yield, quality of both grain and fodder, bold and lustrous grains, and resistance to biotic and abiotic stresses. Nkongolo *et al.* (2008) documented farmers' indigenous knowledge of the major traits of sorghum landraces. They found that farmers had selected landraces that were superior to existing varieties. Farmers in Ethiopia preferred sorghum varieties that are drought tolerant, resistant to *Striga*, and which produced good quality grain (Gebretsadik et al., 2014). However, there have been no studies based on the use of the PRA method to evaluate sorghum production constraints, and the trait preferences in sorghum varieties preferred by farmers, millers, breeders, and end-users in South Africa.

1.10 Summary

Sorghum is grown in various environments around the world, often in dry environments where other crops do not yield reliably. Many genotypes of this crop are grown by farmers to meet a variety of purposes, in particular as a staple food, and for brewing traditional beer. Most genotypes grown today by small-scale farmers in South Africa are landraces. These are important as germplasm for conservation, and as a source of genes for the improvement of traits of interest in sorghum breeding programmes. Hence the landraces need to be characterised using phenotypic and genotypic methods including analyses for their nutritional quality. Understanding the agronomic and quality traits preferred by farmers, end user's, and other stakeholders is essential for the establishment of appropriate breeding goals for new sorghum varieties, that meet stakeholders needs, and therefore result in the adoption of new sorghum varieties. This can be achieved with the use of participatory rural appraisal.

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CHAPTER 2

Appraisal of farmers' sorghum production constraints and variety preferences in the Limpopo Province, South Africa

2.1 Abstract

Participation of farmers in crop breeding programs is vital for selection of new varieties and for wider and enhanced adoption and use of newly developed cultivars. The objective of this study was to determine sorghum production constraints, farmers' preferred traits and ideal sorghum varieties under smallholder farming systems in the Limpopo Province of South Africa. A participatory rural appraisal (PRA) was conducted during the period 2013-2014, involving 311 farmers in two districts and four municipalities known for their sorghum production. The PRA tools used were semi-structured questionnaires, group discussions, key-informants, matrix ranking and transect walks. Both primary and secondary data were collected. Results indicated that the most important production constraints to sorghum production in the study areas were bird damage (53.1 %), storage pests (weevils) (50.1%), parasitic weeds (35.0%), drought (35.3%) and postharvest diseases (30.8%). Respondent farmers indicated that their ideal varieties should have good taste, high yields, resistance to bird damage, insect pests (weevils), and diseases, early maturity and drought tolerance. Incorporating farmer's preferred traits in sorghum breeding programs may enhance adoption and use of improved cultivars in the study areas.

Keywords, farmers' preferred traits, Limpopo Province, participatory rural appraisal, sorghum

2.2 Introduction

Sorghum ranks second after maize in sub-Saharan Africa in terms of production and it provides food for millions of people (Gerda and Christopher, 2007). In South Africa, sorghum remains one of the most important grain crops grown for food and feed. It grows in various provinces including Mpumalanga, North West, Northern Cape, Eastern Cape, KwaZulu-Natal, and the Free State. Sorghum is being commercially produced in the Free State Province (DAFF, 2010). Genetically diverse sorghum landraces and varieties are grown by small- to large-scale farmers in Limpopo Province. Sorghum breeders need to understand farmer's production constraints, trait preferences and varietal requirements (Kudadjie et al., 2004). Farmers' and end users' involvement in variety development can enhance the levels of adoption of new varieties (Danial, 2003). This is because users can specify the type of sorghum material they prefer. Information on preferences and attributes of the type of sorghum variety required can be acquired through the involvement of farmers and other stakeholders in the early stages of plant breeding research, and the use of participatory rural appraisal.

Participatory rural appraisal (PRA) is one of the sociological tools used to understand local farmer's concerns through involvement of community members, understanding their situation and learning from them using interdisciplinary teams of researchers (Blaney and Thibault, 2003). The PRA process involves groups of community members/farmers, and various sectors whereby their views and concerns are systematically expressed and recorded. It emphasizes local knowledge, empowerment and sustainability of natural resources, agricultural, health, social or other issues (Chambers, 1997). It has been used mainly by government and non-governmental organizations (NGOs) scientists in developing countries in the past decades (Cornwall et al., 2001). It can also be a first step in participatory plant breeding, which favors a wide/ spread adoption of new varieties (Cecarralli et al., 2003).

Information obtained through PRA studies help plant breeders to define their breeding goals and objectives. Local varieties are important in plant breeding programmes because they carry selected traits preferred by farmers (Danial et al., 2007). Farmers in the Lake Zone of Tanzania preferred sorghum varieties on the basis of the color and taste of 'ugali', a porridge prepared from sorghum (Mafuru et al., 2007). Rana et al. (2000) reported farmer desired characteristics to be high yield, quality of both grain and fodder, bold and lustrous grains, and tolerance to biotic and abiotic stresses. Mekbib (2007) used PRA for classification and characterization of sorghum in Ethiopia. Nkongolo et al. (2008) studied farmers' preferences

in sorghum landraces and reported that farmer characterization of sorghum varieties had allowed selection of superior landraces from which to develop modern varieties. Makanda (2009) conducted PRA studies in Zimbabwe and South Africa on sweet stemmed sorghum. He found that farmers preferred high yielding and early to intermediate sorghum varieties with high stem sugar content. There is no recent information on sorghum production constraints and varieties preferred by farmers, millers, and end-users in South Africa using the PRA research methodology. The objective of this study was to determine sorghum production constraints, farmers' preferred traits and the profile of ideal sorghum varieties under smallholders farming systems in Limpopo Province of South Africa.

2.3 Materials and methods

2.3.1 Description of the study sites

The PRA was conducted during 2013 and 2014 in two districts: Sekhukhune and Waterberg of Limpopo Province, South Africa (Figure 2.1). The Sekhukhune District is divided into five municipalities but only three municipalities were sampled for the PRA study, based on their scale of sorghum production. The Municipalities sampled were Fetakgomo, Makhuduthamaga and Tubatse Municipalities. The Sekhukhune District covers an area of 1,326,437 ha, the major part being rural small-scale farms (Aird and Archer, 2004). It is characterized by a mean rainfall of 600-800 mm in the south, and 500-600 mm in the north, extending from November to March. The temperatures can reach up to 38^{o}C in summer and range from 7-28^{o}C in winter. Due to limited number of sorghum farmers, only Lephalale Municipality was selected from Waterberg district for the PRA study. The areas are characterized by hot and semi-arid, subtropical climate with temperatures ranging from 18 to 38.2^{o}C and a mean rainfall of 600-650 mm mostly falling during November to February, the major season of sorghum production.

Figure 2.1. Map of Limpopo Province in South Africa, showing the study areas indicated in solid stars. (Source: Magwede et al., 2014).

2.3.2 Sampling method

A purposive sampling method was used to select districts, municipalities, villages and farmers.

The Lephalale municipality of the Waterberg District, and the Makhuduthamaga, Fetakgomo, Greater Tubatse municipalities within the Sekhukhune District were selected for the study. Three municipality sections: North, South and Central in the Lephalale municipality, and six villages: three (Mashite, Manganeng and Mothibeng) from Makhuduthamaga, two (Malekaskraal and Lerajane) from Fetakgomo, and one (Ga-Matokomane) from Greater Tubatse were selected for the study (Table 2.1).

Table 2.1 summarizes the geographical descriptions of the districts and municipalities selected for the study. Greater Tubatse had the highest population number (335 676 inhabitants) and area coverage (4602 Km^2) when compared to other municipalities. Makhuduthamaga had a population of 274 358 and an area of 2097 Km^2, whereas Lephalale occupied 66.94 Km^2 with a population of 17639. Fetakgomo was the least populated municipality (93 795 inhabitants) with an area of 1105 Km^2 and elevation of 1209 m above sea level. Makhuduthamaga and Fetakgomo municipalities represent the Lowveld areas, whereas Lephalale and Greater Tubatse are situated in the Highveld.

Table 2.1. Geographical descriptions of the study areas.

District	Municipality	Population	Area (Km^2)	Elevation (m)	Latitude	Longitude
Waterberg	Lephalale	17639	66.94	820	23°40'S	27°45'E
Sekhukhune	Makhuduthamaga	274 358	2097	1179	24°45'S	29°45'E
	Fetakgomo	93 795	1105	1209	24°41'67"S	29°91'67"E
	Greater Tubatse	335 676	4602	680	24°40'S	30°20'E

Source: Statistics South Africa (Census, 2011)

A total of 311 male and female sorghum growers were sampled across the study areas (Table 2.2). The villages were selected in collaboration with the extension officers and municipal managers of the Department of Agriculture, Forestry and Fisheries in various districts.

Table 2.2. Districts, municipalities, villages and the number of male and female sorghum farmers sampled for the study.

District	Municipality	Section/village	Gender		No. of participants
			Female	Male	
Waterberg	Lephalale	North	15	45	60
		South	16	11	27
		Central	8	18	26
Sekhukhune	Makhuduthamaga	Mashite	49	30	79
		Manganeng	45	9	54
		Mothibeng	17	7	24
	Fetakgomo	Malekaskraal	14	7	21
		Lerajane	9	0	9
	Greater Tubatse	Ga-Matokomane	1	10	11
Total			169	142	311

2.3.3 Data collection and analysis

The research team consisted of the principal investigator, extension officers, some local managers, and the chiefs of the villages. The PRA tools used for data collection included; semi-structured questionnaires (Appendix), group discussions, matrix ranking, key informants, and transect walks in the study areas. The farmers were interviewed using a

questionnaire summarised in the Appendix. The data collected were analysed using SPSS version 22.0 computer package (SPSS, 2013).

2.4 Results and discussion

Description of households

The total numbers of respondents were 113 in Waterberg and 198 in Sekhukhune. Overall, 169 female and 142 male farmers participated in the study (Table 2.2). For some variables, the results of which are discussed below, the number of farmers interviewed varied from 166 to 311. Fewer farmers were unavailable for interviews than initially planned. The male farmers were generally more articulate than the females during group discussion. In the rural areas males, chiefs, and leaders are normally considered as heads of the households and villages. Therefore, this group are given more platforms and preferences in decision making and heading the households.

The contingency chi-square analysis revealed highly significant differences for education levels across municipalities. Most respondents (46.3%) did not have basic primary school education (Table 2.3). Twenty six percent of the participants attended primary school, 18.3% been to secondary school and 0.32% had studied at a tertiary level out of a total of 311 participants in both districts. Most sorghum growers did not have a good level of education, which may impact on sorghum production and the adoption of new technology. Generally, experience and education levels are expected to influence knowledge and the farming enterprises undertaken in rural areas. There is a need for a better education and farmers training to sustain the production status of the crop in these farming systems. Affiliation with farming cooperatives, farmer support groups and working with extension officers may enhance the farmers' knowledge of production of sorghum (Nkgodi, 2005).

Table 2.3. Education level of the respondent farmers across the four sampled municipalities in Limpopo Province

Municipality	Education level				Total
	none	primary	secondary	tertiary	
Lephalale	56	25	15	0	96
Makhuduthamaga	68	48	21	1	138
Fetakgomo	12	3	17	0	32
Tubatse	8	5	4	0	17
Total	144	81	57	1	283
Pearson X^2 value, df=9 =29.557					
Asymp. Sig. =0.001					

The chi-square analysis revealed highly significant differences for education and gender balances across municipalities. The ages of participants ranged from 21 to above 70 years old (Table 2.4). Respondent farmers were largely (81) represented within age category of 51-60 years, followed by respondents aged 61-70 (66), 41-50, 31-40 and above 70. The youth category (21-30) was the least numerous of respondent farmers. In general, the study found a low level of involvement of the youth in agricultural activities. Adults and elderly were the dominant sorghum growers across the municipalities (Table 2.4). The present findings agree with the report of Mmbengeni and Mokoka (2002) who reported a low level of involvement of the youth in agriculture in the Limpopo Province.

Table 2.4. Cross tabulation of age group of respondent farmers' across four municipalities in Limpopo Province.

Municipality	Age (years)						Total
	21-30	31-40	41-50	51-60	61-70	>70	
Lephalale	7	20	33	21	16	6	103
Makhuduthamaga	1	3	21	50	41	24	140
Fetakgomo	8	5	7	6	4	4	34
Tubatse	0	1	2	4	5	4	16
Total	16	29	63	81	66	38	293
Pearson X^2 value, df =15=76.598							
Asymp. Sig.=0.000							

Major crops grown and area of production

There were highly significant differences among the farm sizes recorded in the municipalities (Table 2.5). The farm sizes ranged from 1 to > 10 hectares (ha). Most respondents had farm sizes of 1-2 ha (95) and 3-4 ha (57) followed by 5-6 ha and 7-8 ha. The numbers of farmers owning farms of 9-10 ha and >10 ha were very small both at 2.1% (4). This shows that the municipalities are dominated by smallholder farmers growing sorghum for subsistence purposes. Makhuduthamaga Municipality had the largest number (95) of farmers owning small farms of 1-2 ha, followed by Lephalale (19) and Tubatse (11). In general, farmers in the Fetakgomo Municipality had bigger farms of ranging between 1 and 6 ha. Farmers in Fetakgomo and Tubatse Municipalities owned smaller farms than those in the Lephalale and Makhuduthamaga Municipalities. The study of Diale (2011) found that the size of the farm seemed to have an effect on the adoption of hybrids. Heisey et al. (1998) found that large scale farmers are more likely to adopt new technologies such as hybrid technology than small landholders. The study results were in agreement with those of Bekuretsion (2005) who reported that the average land holding size in the South and Anseba regions was 0.25 to 4 hectares while in Gash Barka it ranged from 1.3 to 40 hectares.

Table 2.5. Farm sizes of respondent farmers across four municipalities in Limpopo Province

	Farm size (ha)						Total
Municipality	1-2	3-4	5-6	7-8	9-10	>10	
Lephalale	19	16	7	1	0	0	43
Makhuduthamaga	57	27	4	9	1	3	101
Fetakgomo	8	9	8	1	2	1	29
Tubatse	11	5	1	0	1	0	18
Total	95	57	20	11	4	4	191
Pearson X^2 value, df=15=31.198 Asymp. Sig.=0.008							

The total area planted by sorghum showed a significant difference between municipalities (Table 2.6). Most farmers (69.9%) cultivated sorghum in 1-2 ha of land in all municipalities except in Fetakgomo municipality where most farmers grow sorghum using 3-4 and 5-6 ha of land (Table 2.6). In Tubatse, most farmers planted sorghum with farm areas measuring 1-2 ha. Most farmers in Makhuduthamaga Municipality cultivated sorghum on 1-2 ha of land followed by 3-4 ha and very few using 5-6 ha of cultivated lands. The results for the farm sizes and area under sorghum cultivation concur with those of Shargie (2015) who found that sorghum in the Limpopo Province is mainly produced by smallholder farmers. These

farmers are faced with the challenge of improving their production and productivity due to small landholding and limited access to modern production technology.

Table 2.6. Areas allocated to sorghum production by respondent farmers in four selected municipalities of Limpopo Province

	Area allocated to sorghum production (ha)				
Municipality	1-2	3-4	5-6	9-10	Total
Lephalale	40	3	0	0	43
Makhuduthamaga	69	29	3	0	101
Fetakgomo	7	10	9	1	27
Tubatse	14	0	1	0	15
Total	130	42	13	1	186
Pearson X^2 value, df=9=62.2260 Asymp. Sig.=0.000					

In two of the three municipalities sorghum was the most important crop and second after maize in Fetakgomo. Respondent farmers planted sorghum with other grains, legume and vegetable crops in the study areas (Table 2.7). Other crops being grown included millet (*Pennisetum glaucum* (L.) R. Br.), maize (*Zea mays* L.), watermelon [*Citrullus lanatus* (Thunb.)], pumpkin (*Cucurbita pepo* L.), beans (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* [L.] Walp.), sugarcane (*Sacharum officinarum* L.), bambara groundnut [*Vigna subterranean* (L.) Verdc.], sunflower (*Helianthus annuus* L.), groundnut (*Arachis hypogaea* L.), sweetpotato [*Ipomoea batatas* (L.) Lam.] and various vegetables. The list of common crops grown across the villages corresponds with a report of WOMIWU Rural Development (2005).

Table 2.7. List and order of importance of major crops grown by respondent famers in four municipalities of Limpopo Province.

Municipality and crops grown			
Lephalale	Makhuduthamaga	Fetakgomo	Tubatse
Sorghum	Sorghum	Maize	Sorghum
Pearl Millet	Maize	Sorghum	Pearl Millet
Bambara groundnut	Dry bean	Cowpea	Watermelon
Dry bean	Pumpkin	Pumpkin	Pumpkin
Watermelon	Melon	Vegetables	Dry beans
Maize	Sugar cane	Sugarcane	Maize
Sunflower	Watermelon	Groundnut	
Melon	Pearl Millet	Watermelon	
	Groundnut		
	Cowpea		

Uses of sorghum

There were significant differences among the uses of sorghum across municipalities ($P \leq 0.001$). Sorghum was mainly used for food (home consumption) followed by brewing, feed and fodder, and for industrial purposes (Table 2.8). In Lephalale, the respondents did not indicate any use of sorghum for industrial purposes. The various foods commonly prepared from sorghum included stiff porridge, soft porridge, "Ting" (a fermented porridge) and beer (Table 2.9). Stiff porridge was the most preferred by farmers as a staple food followed by soft porridge, and beer. "Ting" was the least preferred and was only eaten in the Fetakgomo and Lephalale Municipalities.

Table 2.8. Various uses of sorghum reported by respondent famers in four municipalities of Limpopo Province.

	Sorghum uses				
Municipality	Food	Feed and fodder	Beer	Industrial use	Total
Lephalale	25	10	1	0	36
Makhuduthamaga	116	8	17	3	141
Fetakgomo	19	1	10	1	30
Tubatse	13	2	1	2	16
Total	173	21	29	6	229
Pearson X^2 value, df=9=37.505 Asymp. Sig.= 0.000					

Table 2.9. Number of respondent farmers who prepares food types or drink from sorghum in four municipalities of Limpopo Province.

	Food types				
Municipality	Soft porridge	Stiff porridge	Ting	Beer	Total
Lephalale	12	7	1	17	37
Makhuduthamaga	56	26	0	4	86
Fetakgomo	17	6	3	2	28
Tubatse	6	4	0	5	15
Total	91	43	4	28	166
Pearson X^2 value, df=9=47.962 Asymp. Sig. = 0.000					

Sorghum as a cash crop

Most farmers (71.1%) indicated that they did not sell their sorghum harvests (Table 2.10). They only sold a small portion to neighbouring farmers and villages when cash was required for various social needs by the family. Sorghum was mainly grown for home consumption and the rest was kept as seed for the next growing cycle. When sorghum produced was in excess it was sold to other farmers. Sorghum was sold in the form of threshed seeds or panicles/heads in the studied municipalities (Table 2.11). It is evident that in Lephalale Municipality sorghum grain was being sold in larger quantities than the other areas.

Table 2.10. Proportion of farmers producing sorghum for sale in four municipalities in the Limpopo Province

	Do you sell sorghum		
Municipality	No	Yes	Total
Lephalale	22	15	37
Makhuduthamaga	66	19	85
Fetakgomo	16	8	24
Tubatse	9	4	13
Total	113	46	159
Pearson X^2 value, df=3=4.462			
Asymp. Sig. = 0.216			

Table 2.11. Various forms of sorghum traded by smallholder farmers across four municipalities in the Limpopo Province

	Which part is sold			
Municipality	Do not sell	Heads	Seeds	Total
Lephalale	0	14	1	15
Makhuduthamaga	15	13	0	28
Fetakgomo	0	6	0	6
Tubatse	0	4	0	4
Total	15	37	1	53
Pearson X^2 value, df=6=20.613				
Asymp. Sig. = 0.002				

Productivity of sorghum under smallholder farming systems

The yields of sorghum in the study areas were expressed in number of bags (50 Kg) harvested per plot. Table 2.12 shows the number of sorghum varieties grown in the four municipalities, together with their mean yields. The mean yields varied from 1.05 to 7.7

bags ha^{-1}. The variety 'Macia' was the highest yielder (7.7 bags) followed by 'Sefubetswane' (3.38 bags). The mean yield of variety 'Mapinkana' was 2.6 bags and 'Maseka-a-swere' was 2.15 bags. 'Khunamang' yielded 1.75 bags, 'Manthate' (1.23 bags) and a red variety ('Mahubedu') yielded 1.05. The overall low yields can be attributed to the ongoing use of relatively low yielding landrace varieties, grown on fragmented, small piece of lands of 1-2 ha. Moreover, farmers typically use low quality seeds kept under poor storage conditions (Ashiono et al. 2005).

Table 2.12. Sorghum varieties grown and mean yields per growing season reported by respondent famers in four municipalities of Limpopo Province.

Variety	Municipality and mean yield (bags)					
	Lephalale	Makhuduthamaga	Fetakgomo	Tubatse	Mean[1]	Rank
Macia	8.60	5.80	6.00	10.40	7.70[a]	1
Sefubetswane	2.50	3.00	4.20	3.80	3.38[b]	2
Mapinkana	1.20	2.00	4.00	3.20	2.60[bc]	3
Maseka-a-swere	2.00	3.00	2.20	1.40	2.15[bc]	3
Khunamang	0.50	2.00	1.50	3.00	1.75[c]	4
Manthate	0.60	0.90	2.10	1.30	1.23[c]	4
Mahubedu	2.00	0.80	1.00	0.40	1.05[c]	4
Mean	2.49	2.50	3.00	3.36	2.84	
P-value	<.001					
LSD (0.05)	1.55					
CV (%)	43.80					

[1]Means followed by the same letter do not differ significantly according to Fischer's least significant difference test (P≤ 0.05)

Farmers' preferences of sorghum varieties

There were highly significant differences between the sorghum varieties preferred by the farmers of the study areas (Table 2.13). Farmers preferred the improved variety Macia as their number one choice followed by their local variety Sefubetswane. The least preferred local variety was Khunamang. Overall, this study indicated that farmers in the study sites would prefer to grow improved sorghum varieties although they will still continue to plant their local varieties for their adaptation to harsh growing conditions and quality attributes for the preparation of various food products. Like most farmers in sub-Saharan Africa, farmers in the study areas keep seeds from the previous harvest for planting in the next season (Taylor, 2003). Sorghum in the study areas has become a successful foundation crop for food and beer or local industries. The levels of knowledge, age, labour, land holding, and resource availability are determinants of the farmers' choice of varieties and enterprise mix. For instance, Abebe et al. (2005) reported that generally farmers in Ethiopia have their own way of selecting a variety for their localities. In some cases the farmers' preferences coincide

with the breeders' selection criteria. It is, therefore, important to determine farmers preferred traits in crop varieties or include the farmers in a variety selection process during breeding. This enhances the potential for adoption of the varieties in the respective communities where the varieties are released.

Table 2.13. Names of sorghum varieties grown and percentage of farmers growing these in four municipalities of Limpopo Province.

	Municipality and % preference						
Variety	Lephalale	Makhuduthamaga	Fetakgomo	Tubatse	Type	Mean[1]	Rank
Macia	32.00	41.00	45.00	56.00	Improved	43.50[d]	1
Sefubetswana	38.00	24.00	34.00	21.00	Local	29.25[c]	2
Mapinkana	15.90	18.00	13.00	0.60	Local	11.88[b]	3
Maseka-a-swere	7.00	12.00	10.00	6.30	Local	8.83[ab]	4
Manthate	11.00	6.00	3.00	15.00	Local	8.75[ab]	4
Mahubedu	9.00	4.00	9.00	8.00	Local	7.50[ab]	4
Khunamang	4.00	3.00	1.00	10.20	Local	4.55[a]	5
Mean	16.70	15.43	16.43	16.73		16.32	
P-value	<.001						
LSD (0.05)	8.50						
CV (%)	38.90						

[1]Means followed by the same letter do not differ significantly according to Fischer's least significant difference test (P≤ 0.05)

Farmers' perception of traits of an ideal sorghum variety

There were significant differences among the traits that the farmers preferred across municipalities (Table 2.14). The most important farmer preferred traits were high yield and good taste. This was followed by early maturity, insect and disease resistance, heat and drought tolerance, and the ability to grow with low production inputs. Farmers were asked if soil acidity could be a problem in their fields by explaining to them the symptoms of this constraint. Farmers did not appear to experience problems with acidic soils. The results concur with the study by Nkgodi (2005) where farmers in Sekhukhune preferred sorghum varieties with characteristics of early maturity, drought tolerance and porridge making quality and good taste, but not yield. Tesfamichael et al. (2013) indicated that the farmers chose the sorghum varieties for seeding based on panicle and seed size, grain color and maturity dates. The panicles for seeds were selected at physiological maturity. Furthermore, the farmers preferred white and red grains for injera and porridge while those with brown grains

were used to prepare alcoholic beverages. Sibiya (2009) indicated that the farmers in KwaZulu-Natal in South Africa preferred their ideal maize varieties to be high yielding, have a good taste, inexpensive seed and should require minimum inputs.

Table 2.14. Traits of sorghum varieties preferred by farmers in four municipalities of Limpopo Province

	Municipality and trait preference (%)					
Preferred Traits	Lephalale	Makhuduthamaga	Fetakgomo	Tubatse	Mean[1]	Rank
High yield	52.00	43.00	61.30	56.00	53.075[a]	1
Good Taste	56.00	44.00	51.00	49.20	50.05[a]	1
Early Maturity	41.00	37.00	34.00	28.00	35.0[b]	2
Insect resistance	36.00	43.00	36.00	26.00	35.25[b]	2
Disease resistance	32.00	28.00	25.00	38.00	30.75[b]	2
Drought tolerance	23.20	25.00	26.70	24.00	24.73[c]	3
Heat tolerance	24.30	23.10	25.00	24.00	24.1[c]	3
Low input for growing	25.60	22.10	23.20	24.90	23.95[c]	3
All purpose type	18.10	16.30	18.00	17.00	17.35[d]	4
Tolerant to acid soils	5.60	6.10	4.50	4.20	5.1[e]	5
Mean	31.38	28.76	30.47	29.13	29.95	
P-value	<.001					
LSD (0.05)	6.04					
CV (%)	15.7					

[1]Means followed by the same letter do not differ significantly according to Fischer's least significant difference test (P≤ 0.05).

Respondent farmers rated good taste as a highly preferred trait found in the varieties Mapinkana, Manthate, Khunamang, Sefubetswane and a red seeded variety (Mahubedu) (Table 2.15). Early maturity was the most preferred trait found in Maseka-a-swere and Sefubetswana. High yield was regarded as the most preferred trait found in Macia with its resistance to diseases and insect pests. The results concur with the studies of Marsalis et al. (2010) who found that drought and heat tolerance of sorghum combined with the re-growth ability after drought makes sorghum an ideal candidate for silage systems in drier areas. Sorghum is known to be a resilient crop well adapted to climatic change (Reddy et al., 2011). Mativavarira et al. (2011) reported that farmers in Zimbabwe considered grain yield of sorghum varieties as most important trait. The farmers in the present study areas indicated

that they needed better sorghum varieties to increase production and to reduce pre- and post-harvest losses.

Table 2.15. Various traits of sorghum varieties listed and preferred by farmers across four municipalities of Limpopo Province.

Trait	Variety						
	Mapinkana	Maseka-a-swere	Manthate	Macia	Khunamang	Sefubetswana	Mahubedu
Early maturity	44	68	23	38	14	56	34
Good taste	66	44	48	25	49	63	68
High yield	13	10	15	65	23	20	18
Resistance to pests and diseases	8	6		45	5	36	3
Other	14	12	5	-	-	28	-

Major constraints to sorghum production

The major challenges to sorghum production as perceived by farmers are indicated in Table 2.16. The stress factors varied across municipalities. The production constraints are ranked in order of importance (Table 2.16). Bird damage and weevils were rated as the most prevalent and serious problems in farmers' fields and during storage, respectively. Parasitic weeds (*Striga)*, drought, and storage rot were the next most important constraints followed by stem borer, rust, anthracnose and heat stress. Downy mildew, northern leaf blight, and soil fertility were considered the least important stress factors. Chikuta et al. (2014) reported that low yield, limited availability of improved sorghum varieties, poor access to improved seed, inconsistent grain market, and pests and diseases were the most important constraints faced farmers in sorghum production in Zambia. Mwadalu and Mwangi (2013) and Habindavyi (2009) reported that the quelea birds, sometimes referred to as "pest birds" were one of the production constraint of sorghum in farmers' fields in Kenya and Burundi, respectively. These birds flock in large numbers and can cause devastating grain losses of small grained cereals. Makanda (2009) reported the major constraints in sorghum production in Zimbabwe were drought, poor soil fertility, diseases, pests, seed availability, markets, and labour shortages. Drought stress has been identified as one of the major constraints to most rain-fed crop production throughout the world (Ludlow et al., 1994; Haussmann et al., 1999; Borrell et al., 2000). Among other factors and interventions, problems of drought and low productivity can be addressed through breeding for higher yields under drought stress conditions. In Kenya, addressing poor soil fertility through credit schemes was identified as key role which improved maize production six-fold (Achieng et al., 2001).

Table 2.16. Important biotic and abiotic constraints to sorghum production indicated by farmers across four municipalities of Limpopo Province

Biotic/abiotic constraint	Municipality and farmers (%)				Mean[1]	Rank
	Lephalale	Makhuduthamaga	Fetakgomo	Tubatse		
Birds	52.00	43.00	61.30	56.00	53.08a	1
Weevils	56.00	44.00	51.00	49.20	50.05a	1
Parasitic weeds	41.00	37.00	34.00	28.00	35.00b	2
Drought	36.00	43.00	36.00	26.00	35.25b	2
Storage rots	32.00	28.00	25.00	38.00	30.75b	2
Stem borer	23.20	25.00	26.70	24.00	24.73c	3
Rust	24.30	23.10	25.00	24.00	24.10c	3
Anthracnose	25.60	22.10	23.20	24.90	23.95c	3
Heat	18.10	16.30	18.00	17.00	17.35d	4
Downy mildew	5.60	6.10	4.50	4.20	5.10e	5
Northen leaf blight	4.80	5.30	5.00	3.90	4.75e	5
Phaeosphaeria leaf spot	2.50	3.10	2.30	3.80	2.93e	5
Soil fertility	0.60	1.20	1.10	0.80	0.93e	5
Mean	24.75	22.86	24.08	23.06	23.69	
P-value	<.001					
LSD (0.05)	5.25					
CV (%)	17.4					

[1]Means followed by the same letter do not differ significantly according to Fischer's least significant difference test (P≤ 0.05).

2.5 Conclusions

Participatory plant breeding is important to bridge the knowledge gap between breeders and farmers. It provides an accurate picture of farmers' production systems and seed management. The most important production constraints to sorghum production in the study areas were bird damage, storage pests (weevils) parasitic weeds, drought and postharvest diseases, respectively. Respondent farmers indicated that their ideal varieties should have high yields and good taste, early maturity, resistance to insect pests (weevils), and diseases, plus drought and heat tolerance. Incorporating farmer's preferred traits in sorghum breeding programs may enhance adoption of improved cultivars in the study areas.

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CHAPTER 3

Assessment of genetic relatedness among South African sorghum genotypes using agro-morphological traits

3.1 Abstract

Sorghum is an important cereal crop providing food, feed and bioenergy worldwide. Knowledge of genetic diversity among sorghum genotypes is essential for current and future breeding. The objective of this study was to assess the level of genetic diversity present among South African sorghum genotypes using agro-morphological traits. Ninety eight sorghum accessions collected from the Department of Agriculture Forestry and Fisheries, African Centre for Crop Improvement and Agricultural Research Council-Grain Crops Institute were phenotyped at two sites: Makhathini flats in KwaZulu-Natal and Burgershall in Mpumalanga during 2012. Experiments were laid out using an alpha lattice design with three replications. Data on eight quantitative and six qualitative traits were collected and subjected to principal component (PC), hierarchal cluster, and multivariate analyses and a dendrogram constructed using the Unweighted Pair Group Method with Arithmetic Mean. The principal component analysis revealed three important PCs that contributed to the total variation of 88.9% observed among genotypes across locations. PC1, PC2 and PC3 contributed to 46.69, 30.74, and 11.45% of the total variation. A dendrogram revealed three main clusters of genotypes. The grouping of the sorghum genotypes was not based on source of collection. The most diverse accessions identified were MP 4277, EC 2934, KZ 5097, FS 4909, and LP 4303 which are useful for breeding.

Keywords: Agro-morphology, genetic diversity, *Sorghum bicolor,* South Africa

3.2 Introduction

Genetic variation in crop plants is essential for crop improvement. Maintenance of genetic diversity within and among crop species is crucial for sustainable agriculture, especially under low-input production conditions of marginal environments (Worede, 1993). It is estimated that by 2050 the global population will rise by 8.9 billion due to population growth (FAO, 2006). Hence it is vital to boost agricultural production and productivity to ensure food security to the growing population. Availability of diverse and resilient genetic resources determines the current and future plant breeding and sustainability of agricultural productivity (Huang et al., 2007; Van de Wouw et al., 2010). A wide range of genetic diversity provides security for farmers against pests, diseases, environmental and other random stresses.

Morphological or phenotypic descriptors are useful to characterize crop genotypes (Ngugi and Maswili, 2010). Morphological traits provide descriptive information of ideotypes and aid in understanding the similarities and differences between genotypes (IBPGR and ICRISAT, 1993). Unlike DNA markers, morphological markers are influenced by the environment. Various studies used phenotypic traits to effectively distinguish sorghum genotypes (Abdi et al., 2002; Geleta and Labuschagne, 2005).

Sorghum is the most important cereal crop worldwide after maize, wheat, rice and barley (FAO, 2011). It ranks second after maize in sub-Saharan Africa in terms of production, providing food for millions of people (Gerda and Christopher, 2007). It is classified into two groups: wild and the cultivated types (Smith and Frederiksen, 2000; Ayana et al., 2002). The wild sorghum species include *Sorghum halepense*, S. *propinquum*, *S. bicolor* subspecies d*rummond*ii and *S. bicolor* subspecies *verticilliflorum*. The cultivated sorghum has been classified into five major races: *bicolor*, *caudatum*, *durra*, *guinea* and *kafir,* and 10 intermediate races based on panicle and spikelet characteristics (Dogget, 1988; Assar et al., 2005).

In South Africa both wild and domesticated sorghum species are present. Mann et al. (1983) reported that the South African sorghum race 'Kafir' might have arisen from introgression between domesticated and wild sorghum. In South Africa sorghum is cultivated by small- and large-scale commercial farmers in various provinces including Mpumalanga, North West, Northern Cape, Eastern Cape, KwaZulu-Natal, and the Free State. The Free State Province is the main commercial sorghum production area (DAFF, 2010). Sorghum is a

foundation crop in the food and beverage industries in the country (Taylor, 2000; Taylor, 2003). In addition, sorghum crop residues are a good sources of animal feed and fodder (Rooney and Waniska, 2001; Chakauya et al., 2006).

Phenotypic characterization and evaluation of sorghum genotypes are dependent on records of qualitative or quantitative morphological traits (Upadhyaya et al., 2010). Geleta et al. (2005) used qualitative traits including leaf midrib colour, grain colour, glume colour, endosperm texture, awns and panicle compactness. Quantitative traits include plant height, maturity period, leaf area, leaf width, leaf length, number of leaves, panicle length, grain yield per plant, grain size, 1000 grain weight, grain number per panicle, panicle width, number of primary branches per panicle, and panicle weight (Punitha et al., 2010). However, some of the aforementioned traits may have limited agronomic importance for growers. Previous studies demonstrated that sorghum is genetically diverse with cultivated sorghums exhibiting great phenotypic variability (Rao et al., 1996; Aruna and Audilakshmi, 2008). A wide range of genetic diversity was observed among sorghum landraces in Cameroon (Barnaud et al., 2007). Ganesamurthy et al. (2010) observed a great phenotypic variability among sorghum landraces collected in Tamil Nadu. In Kenya, Muraya et al. (2011) reported a wide range of morphological and structural diversity among the wild sorghums. Gerrano (2011) reported a wide range of morphological diversity among 22 sorghum genotypes from Ethiopia and South Africa using qualitative and quantitative descriptors. Among the 11 South African sorghum genotypes studied by Gerrano (2011) only two were landraces. Morphological diversity analysis of South African sorghum germplasm is required using a relatively greater number of samples, representing the diverse sorghum growing provinces. This will allow gathering adequate information on the phenotypic performance and relatedness among the sorghum germplasm originating from South Africa for effective use of the sorghum genetic resources and future conservation strategies. The objective of the study was to assess the level of genetic diversity present among South African sorghum genotypes using agro-morphological traits.

3.3 Materials and methods

3.3.1 Plant material

A total of 98 South African sorghum genotypes were used for the study. The genotypes were obtained from the Department of Agriculture Forestry and Fisheries (DAFF)-Plant Genetic Resources, the Agricultural Research Council-Grain Crops Research Institute (ARC-GCRI), and the African Centre for Crop Improvement (ACCI) (Table 3.1). The genotypes from DAFF

were originally collected from the following provinces: KwaZulu-Natal, Limpopo, Free State, Eastern Cape, North West and Mpumalanga.

3.3.2 Description of study sites and experimental design

Experiments were conducted at two localities: Makhathini Flats and Burgershall. The experiment at Makhathini Flats was established at the research sub-station of the ARC situated in KwaZulu-Natal Province at 27º 24' S and 32º 11' 48"E with an altitude of 72 meter above sea level (masl) dominated by the Hutton soil. The second site was Burgershall (25° 06' S 31° 05' E) which is the research sub-station of ARC situated in Mpumalanga Province. The site has an elevation of 697 masl and receives a total annual rainfall of above 950 mm with mean minimum and maximum temperatures of 18°C and 40°C in summer and 8ºC to 25ºC in winter, respectively. This site also has a Hutton soil. The experiment was laid out in an alpha lattice design replicated three times.

3.3.4 Data collection

Data was collected according to the Standard Key Descriptor Lists for Characterizations for sorghum (IBPGR/ICRISAT, 1984).

Quantitative data:

The quantitative characters measured were: plant height (PTH) measured from the ground to the tip of the panicle at maturity and expressed in cm; Panicle length (PAL) measured from the lower panicle branch to the tip of the panicle at maturity (cm); Panicle width (PAW) measured as width of panicle in natural position at the widest part (cm); grain weight (TSW) measured by weighing 1000 grains at 12% moisture content; seed yield per panicle (SWT) measured as weight of grain per panicle (g); Weight of head (panicle) (PWT) was recorded before threshing. Rachis number (RCN) measured as number of rachis per panicle. Panicle exsertion (PEX) measured as length of peduncle from flag leaf to the base of inflorescence (cm) as 1= < 2 cm, Slightly exserted; 2 = 2-10 cm, Exserted; 3 = >10, Well exserted; 4 = Peduncle recurved.

Qualitative data:

Grain color was recorded using the Munsell Color Chart for Soil Scientists as 1 = White, 2 = Reddish white, 3 = Brown, 4 = Reddish yellow, 5 = Strong brown, 6 = blackish brown, 7 = dark red, 8 = dark reddish brown, and 9 = yellow, 10 = black, 11 = blackish strong brown, 12 = yellow red, 13 = yellowish brown, 14 = greyish brown, 15 = reddish brown, 16 = light brown, 17 = reddish black, 18 = light grey, 19 = red. Glume colour labelled as 1 = White, 2 =

Reddish white, 3 = Brown, 4 = Reddish yellow, 5 = Strong brown, 6 = blackish brown, 7 = dark red, 8 = dark reddish brown, and 9 = yellow, 10 = black, 11 = blackish strong brown, 12 = yellow red, 13 = yellowish brown, 14 = greyish brown, 15 = reddish brown, 16 = light brown, 17 = reddish black, 18 = light grey, 19 = red, 20 = dark grey, 21 = reddish dark grey, 22 = dusky red, 23 = dark brown, 24 = light green, 25 = grey, and 26 = dark yellow. Data were collected from the inner rows. Midrib color measured as 1 = Colorless, 2 = Pale green, 3 = Green, 4 = Purple, and 5 = White.

Inflorescence shape and compactness were recorded as 1 = Very lax; 2 = Very loose erect primary branches; 3 = Very loose drooping primary branches; 4 = Loose erect primary branches; 5 = Loose drooping primary branches; 6 = Semi-loose erect primary branches; 7 = Semi-loose drooping primary branches; 8 = Semi-compact elliptic; 9 = Compact elliptic; 10 = Compact oval; 11 = Half broom corn; 12 = Broom corn. Glum covering recorded as the amount of grain covered glume as 1 = 25%; 3 = 50%; 5 = 75%; 7 = 100% or grain fully covered; and 9 = Glumes longer than grain. Awns were recorded as either present or absent at maturity.

3.3.5 Data analysis

Data was subjected to correlations and Principal Component Analysis (Jackson, 1991) of the Multivariate analysis program in Genstat (GenStat, 2011). The quantitative data was analysed in GenStat 14.1 (GenStat, 2011) using the general linear model procedure. The dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean in the Agglomerative Hierarchical clustering in XLSTAT. The qualitative data was analysed using the Kruskal-Wallis test procedure.

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Table 3.1 List of sorghum accessions used in the study

Serial number	Accession name	Place of collection/ Origin	Serial number	Accession name	Place of collection/ origin
1	5405	North-West	54	5281	KwaZulu-Natal
2	5333	North-West	55	4547	KwaZulu-Natal
3	5464	North-West	56	5097	KwaZulu-Natal
4	5436	North-West	57	5245	KwaZulu-Natal
5	5454	North-West	58	4952	Free State
6	5430	North-West	59	4909	Free State
7	5393	North-West	60	4905	Free State
8	5337	North-West	61	4891	Free State
9	2167	Eastern Cape	62	Motlerane	ARC
10	3416	Eastern Cape	63	Mammopane	ARC
11	3414	Eastern Cape	64	Macia-SA	ARC
12	3262	Eastern Cape	65	M153	ARC
13	3319	Eastern Cape	66	05 Potch-151	ARC
14	2922	Eastern Cape	67	Maseka-a-swere	ARC
15	3364	Eastern Cape	68	Mammolokwane	ARC
16	3403	Eastern Cape	69	05- Potch-138	ARC
17	2975	Eastern Cape	70	M48	ARC
18	3184	Eastern Cape	71	05 Potch-115	ARC
19	2934	Eastern Cape	72	05 Potch-167	ARC
20	2985	Eastern Cape	73	Manthate	ARC
21	3217	Eastern Cape	74	AS 82	ACCI
22	4276	Mpumalanga	75	AS13	ACCI
23	5476	Mpumalanga	76	AS 1	ACCI
24	4265	Mpumalanga	78	AS 4	ACCI
25	4052	Mpumalanga	79	AS 18	ACCI
26	2055	Mpumalanga	80	AS 19	ACCI
27	5518	Mpumalanga	81	AS 17	ACCI
28	4259	Mpumalanga	82	AS 16	ACCI
29	5502	Mpumalanga	83	AS 11	ACCI
30	5541	Mpumalanga	84	AS 6	ACCI
31	1990	Mpumalanga	85	AS 1 M_2 Ctrl	ACCI
32	4277	Mpumalanga	86	AS 16 M2 Ctrl	ACCI
33	2048	Mpumalanga	87	AS 21	ACCI
34	4161	Mpumalanga	88	AS 8	ACCI
35	1450	Limpopo	89	4942	Free state
36	4312	Limpopo	90	4403	Limpopo
37	1948	Limpopo	91	3281	Eastern Cape
38	1390	Limpopo	92	3439	Eastern Cape
39	5258	KwaZulu-Natal	93	3281	Eastern Cape
40	AS66	ACCI	94	5542	Mpumalanga
41	1413	Limpopo	95	1417	Limpopo
42	1394	Limpopo	96	AS1ems	ACCI
43	1481	Limpopo	97	4442	Limpopo
44	4303	Limpopo	98	4052	Mpumalanga
45	5088	KwaZulu-Natal			
46	5287	KwaZulu-Natal			
47	5233	KwaZulu-Natal			
48	5237	KwaZulu-Natal			
49	4722	KwaZulu-Natal			
50	5246	KwaZulu-Natal			
51	4606	KwaZulu-Natal			
52	4531	KwaZulu-Natal			
53	5274	KwaZulu-Natal			

3.4 Results

3.4.1 Meteorological data of the study sites over an eight year period (2005-2012)

The meteorological data of the study sites including relative humidity, temperature, rainfall, solar radiation and total relative evapo-transpiration over an eight year period are presented in Tables 3.2 and 3.3. The present studies were conducted during 2012.

At Makhathini (Table 3.2), the mean minimum temperatures ranged from 16.82 to 18.00^{o}C with the mean of 17.31^{o}C and a mean maximum temperatures varied between 28.51 to 29.86^{o}C with a mean maximum temperature of 29.13^{o}C. The mean minimum relative humidity ranged between 40.86 to 44.68% with a mean of 42.59% and the mean maximum relative humidity varied between 53.98 to 90.62% with a mean of 85.40%. The mean total rainfall varied between 267.21 to 813.40 mm with a mean of 541.38 mm. In 2006 the mean total rainfall was high (813.40 mm) followed by the years 2010 at 684.28 mm and 2012 at 611.12 mm. The lowest rainfall was recorded in 2008 at 332.87 mm. The solar radiation varied between 12.06 and 17.52 MJ/m^2 with a mean of 14.53 MJ/m^2. The highest solar radiation was recorded in 2006 at 17.52 MJ/m^2 followed by the year 2011 at 15.04 MJ/m^2. The lowest radiation was observed in 2009 at 12.06 MJ/m^2. The total relative evapo-transpiration ranged from 71.21 to 109.07 mm with a mean of 93.57 mm. The highest total relative evapo-transpiration of 109.07 mm was recorded in 2006 followed by 101.50 mm in 2011 and 99.30 mm in 2010. The lowest total relative evapo-transpiration was observed in 2012. There was minimal variation among mean minimum and maximum temperatures, mean minimum relative humidity, and solar radiation (Table 3.2).

Table 3.2 Meteorological data of Makhathini from 2005 to 2012.

Year	Mean Min temp (°C)	Mean Max Temp (°C)	Mean Min Rel. Humidity (%)	Mean Max. Rel. humidity (%)	Mean Total Rainfall (mm)	Solar radiation (MJ/m^2)	Total relative evapo-transpiration (mm)
2005	18.00	29.86	44.65	53.98	477.60	14.34	93.22
2006	17.42	29.17	41.46	88.10	813.40	17.52	109.07
2007	17.07	29.18	41.67	90.29	547.90	14.44	93.21
2008	17.22	29.17	41.73	89.83	332.87	13.72	91.77
2009	17.16	28.51	44.68	90.62	267.21	12.06	71.21
2010	17.70	29.24	43.42	90.22	684.28	14.63	99.30
2011	17.07	28.93	42.23	90.29	596.65	15.04	101.50
2012	16.82	28.96	40.86	89.85	611.12	14.51	89.29
Min	16.82	28.51	40.86	53.98	267.21	12.06	71.21
Max	18.00	29.86	44.68	90.62	813.40	17.52	109.07
Mean	17.31	29.13	42.59	85.40	541.38	14.53	93.57
Variance	0.15	0.14	2.18	161.75	32155.68	2.29	122.54
Stdev	0.38	0.38	1.48	12.72	179.32	1.51	11.07

Mean Min temp = Mean minimum temperature, Mean Max Temp = mean maximum temperature, Mean Min Rel. Humidity = Mean minimum relative humidity, Mean Max. Rel. humidity = Mean maximum relative humidity.

At Burgershall (Table 3.3), the mean minimum temperatures ranged from 13.27 to 14.90°C with the mean of 14.24°C and the mean maximum temperatures varied between 25.68 and 26.78°C with the mean maximum temperature of 26.22°C. The minimum and maximum temperatures were almost similar over the eight years. The total rainfall varied between 692.90 and 1330.88 mm with the mean of 908.39 mm. The highest maximum rainfall of 1330.88 mm was recorded in 2011 followed by rainfall of about 1296.50 mm in 2006. The highest minimum rainfall of 14.90 mm was also experienced in 2005. The mean minimum relative humidity ranged from 40.95 to 54.79% with a mean of 46.82%. The mean maximum relative humidity ranged between 92.03 to 97.30 % with a mean of 94.00%. The highest mean relative minimum and maximum humidities of 54.79% and 97.30% were recorded in 2006, respectively. The solar radiation varied between 15.87 and 19.13 MJ/m^2 with a mean of 17.29 MJ/m^2. The highest radiation was experienced in 2007. The total relative evapo-transpiration ranged between 84.89 and 108.63 mm with a mean of 97.78 mm. The highest evapo-transpiration was recorded in 2007 followed by 2005 at the evapo-transpiration of 103.42 mm.

Immense variation was observed among the mean maximum humidity, mean total rainfall and total relative evapo-transpiration over the eight year period at Makhathini.

Moreover, great variation was observed among total rainfall, and total relative evapo-transpiration. A slight variance was observed for mean minimum relative humidity. Furthermore, there was minimal variance for mean minimum and maximum temperatures, mean maximum relative humidity and solar radiation among the years.

When comparing and contrasting the meteorological data of the two locations, the mean total rainfall in Burgershall (908.39 mm) was higher than in Makhathini (541.38 mm). The mean minimum and maximum temperatures of the Makhathini (17.31 and 29.13^{o}C) were higher than the Burgershall temperatures (14.24 and 26.22^{o}C). The Burgershall had high minimum and maximum humidity (46.82 and 94.00%), solar radiation (17.29 MJ/m^2) and total relative evapo-transpiration (97.78 mm) than the Makhathini with 42.59 and 85.40% minimum and maximum relative humidity, 14.53 MJ/m^2 solar radiation and 93.57 mm total relative evapo-transpiration (Tables 3.2 and 3.3). The two locations had mean minimum and maximum temperatures, solar radiation and total relative evapo-transpiration in a decreasing trend over the eight years.

Table 3.3. Meteorological data of Burgershall from 2005 to 2012.

Year	Mean min temp (^{o}C)	Mean max temp. (^{o}C)	Total rainfall (mm)	Mean min relative humidity (%)	Mean max relative humidity (%)	Solar radiation (MJ/m^2)	Total relative evapo-transpiration (mm)
2005	14.90	26.78	692.90	50.11	97.06	17.96	103.42
2006	14.38	25.69	1296.50	54.79	97.30	18.37	101.33
2007	14.09	26.72	744.50	48.49	96.40	19.13	108.63
2008	14.34	26.57	703.50	42.64	92.37	17.68	102.34
2009	14.32	25.68	755.50	46.20	92.32	16.87	94.02
2010	14.70	26.15	914.30	46.29	92.05	15.90	93.33
2011	13.91	25.82	1330.88	45.08	92.43	16.53	94.28
2012	13.27	26.34	829.06	40.95	92.03	15.87	84.89
Min	13.27	25.68	692.90	40.95	92.03	15.87	84.89
Max	14.90	26.78	1330.88	54.79	97.30	19.13	108.63
Mean	14.24	26.22	908.39	46.82	94.00	17.29	97.78
Variance	0.25	0.21	67729.50	18.97	5.95	1.41	56.47
STDEV	0.50	0.45	260.25	4.35	2.44	1.19	7.51

Mean Min temp = mean minimum temperature, Mean Max Temp = mean maximum temperature, Mean Min Rel. Humidity = Mean minimum relative humidity, Mean Max. Rel. humidity = Mean maximum relative humidity.

3.4.2 Quantitative responses of sorghum genotypes tested at two locations

3.4.2.1 Mean response of sorghum genotypes in two locations

The data of the eight quantitative traits was analysed using linear mixed models. The results are indicated in Table 3.4. Highly significant (P≤ 0.001) differences were observed for all traits measured in both locations except for panicle length at Makhathini which was significant at P≤ 0.05.

Plant height

There were highly significant differences (P < 0.001) among the sorghum genotypes for plant height in Makhathini experiment (Table 3.4). Plant height ranged from 18.30 to 259.30 cm. The genotypes that showed high plant height were AS 8, KZ 5246, LP 4403, AS 16, EC 3439, KZ 4606, EC 3319, KZ 5233, KZ 4547, Maseka-a-swere and KZ 5258. The lowest were AS 21, MP 4276, EC 2985, AS1 M_2 Ctrl, AS 18, AS 19, and 05-Potch-151, AS 17, LP 1417, and LP 1450. In Burgershall the plant height ranged from 67.89 cm to 160.90 cm. The genotypes that had the tallest plants were AS 82 and NW 5436 followed by AS 6, EC 2167, EC 3416, NW 5436, MP 5542 and LP 4303.The genotypes that had short plants were Motlerane, and LP 4442.

Panicle width

There were highly significant differences among the genotypes based on panicle width (Table 3.4). The panicle with ranged from 3.33 to 17.67 cm for Makhathini experiment. The genotypes with the broad panicles were FS 4891, MP 4276, KZ 4606, EC 3364, and Motlerane. The genotypes with the narrow panicles were AS 1 M_2 Ctrl, 05-Potch-138, NW 5405, AS 17 and AS 8. In Burgershall, the panicle width varied between 2.83 and 15.63 cm. The NW 5405, LP 1450, AS 1 M_2 Ctrl, 05-Poth-167 and EC 2985 genotypes had broad panicles and Mammolokwane, NW 5430, EC 3403, and Maseka-a-swere had narrow panicles.

Panicle length

There were significant differences observed among the genotypes based on panicle length (Table 3.4). The length varied between 6.67 and 27.67 cm for Makhathini trial. The genotypes that had long panicles were KZ 4722, LP 1413, 05-Potch-167, LP 4403, MP 5518, KZ 5258. The genotypes with short panicles were AS 21, LP 1417, Mammolokwane, AS 17 and AS 19. In Burgershall, the panicle length varied between 9.86 cm and 45.60 cm.

The EC 3217, LP 4312, 05-Potch-115, and MP 2048 had long panicles. LP 4442, MP 5542, and EC 3403 exhibited short panicles.

Number of rachis

Highly significant differences were recorded among the sorghum genotypes based on rachis number (Table 3.4). The rachis number ranged from 11.00 to 55.00. The genotypes that showed high number of rachis were Mammolokwane, LP 4312, KZ 5245, MP 4161, Manthate, KZ 5287 and Mammopane. The genotypes with low number of rachis were LP 1417, MP 2055, MP 4052, MP 5476, EC 3217, MP 5542, MP 4259 and 05-Potch-151. The number of rachis ranged from 21.33 to 74.00 in Burgershall. LP 4312 showed high number of rachis followed by EC 2167, EC 2922, NW 5333, AS 82, NW 5464 and AS 19. The genotypes MP 2055, Mammolokwane, KZ 4606, LP 1390 and Motlerane had low number of rachis.

Panicle exsertion

There were highly significant differences among the sorghum genotypes based on panicle exsertion (Table 3.4). The panicle exsertion varied between 6.00 and 68.33 cm. The genotypes KZ 4722, KZ 5281, LP 1417, MP 4052, and KZ 5233 were exserted. The LP 1394, MP 2048, EC 3281, FS 4909, LP 4312, EC 3319, KZ 4606, LP 4403, and KZ 4531 had maximum values and were well exerted together with the remainder. In Burgershall, the panicle exsertion varied between 2.69 to 40.33. Only Motlerane was exserted. The genotypes LP 1394, EC 3184, KZ 5237, LP 4403, KZ 5274, M153, AS 8, and AS 1_{ems} had maximum values and together with the remainder were well exserted.

Panicle weight

There were highly significant differences among the sorghum genotypes based on panicle weight (Table 3.4). The panicle weight ranged from 6.27 to 135.67 g. The genotypes that exhibited high panicle weight were NW 5430, NW 5393, Mammolokwane, LP 1450, 05-Potch-138 and LP 4442. The genotypes that had low panicle weights were EC 3281, EC 3416, LP 4403, AS 17, AS 6, and AS 66. In Burgershall, the panicle weight ranged from 5.67 g to 84.67 g. The genotype EC 2975 had a high panicle weight followed by 05-Potch-138, LP 1948, NW 5464 and LP 4403. The genotypes that exhibited low panicle weight were LP 1450, NW 5430, and Motlerane.

Thousand seed weight

There were highly significant differences recorded among the genotypes based on thousand seed weight (Table 3.4). The seed weight varied between 0.81 g and 13.50 g. The

genotypes that had a high thousand seed weight were MP 4277 followed by FS 4909, NW 5393, 05-Potch-138, and LP 1417. The lowest were MP 4052, EC 2985, NW 5436 and EC 3416. In Burgershall the thousand seed weight varied between 1.33 g and 48.00 g. The EC 3281 followed by FS 4942, LP 1450, EC 3281, EC 3184 and MP 4161 had high thousand seed weight whereas Macia-SA and AS 82 were low.

Grain yield

There were significant differences among the genotypes based on grain yield (Table 3.4). The grain yield varied between 1.00 and 180.40 g for Makhathini trial. The genotypes 05-Potch-151, LP 1413, KZ 5245 and AS 1_{ems} showed high grain yield, and NW 5436, 05-Potch-167 and AS 82 had low grain yield. In Burgershall, the grain yield varied between 30.30 and 217.30 g. The highest grain yields were observed in MP 4265, NW 5464, LP 4303, 05-Potch-167 and AS 17. The genotypes that had low grain yield were EC 2985, MP 4052, EC 3319, NW 5430 and KZ 5258.

3.4.2.2 Combined analysis of variance of eight morphological traits across two locations

Table 3.5 summarizes the mean squares of sites, genotypes and genotype by site interaction from a combined analysis of variance. The two sites showed significant differences ($P \leq 0.001$) in all of the quantitative traits measured. Highly significant differences ($P \leq 0.001$) were also observed among the genotypes on panicle length, rachis number, thousand seed weight and weight per panicle. Furthermore, genotype effects were significantly different ($P \leq 0.05$) for panicle weight. Grain yield, plant height, panicle width, and panicle exsertion were non-significant. On the other hand, highly significant differences were observed on the genotype by site interaction on panicle length, rachis number, and thousand seed weight. The significant differences in genotype by environment could be attributed to the different reaction of the genotypes to sites or due to differences between the sites.

3.4.2.3 Mean response of ninety eight sorghum genotypes planted across two sites

There were highly significant differences ($P \leq 0.001$) observed for variety and the interaction of variety within the site in all traits measured. For site, plant height, panicle exsertion, panicle width, panicle weight, rachis number and thousand seed weight were highly significant. Panicle length and seed yield were non-significant.

Plant height

There were highly significant differences (P≤ 0.001) observed for plant height across the sites (Table 3.6). The mean plant height ranged from 47.5 cm to 174.7 cm. The genotypes that exhibited tall plants were AS 8, AS 16, KZ 5246, LP 4403 and EC 3439. The genotypes AS21, AS 18, AS 1ems, 05.Potch-151 and LP 4442 and MP 4276 had shortest plants.

Panicle exsertion

The panicle exsertion varied between 7.74 cm and 48.23 cm (Table 3.6). Only Motlerane was exserted (7.74 cm). The other genotypes were well exserted (above 10 cm).

Panicle width

Panicle width ranged from 3.44 cm to 15.83 cm among the genotypes (Table 3.6). Genotypes LP 4312, 05.Potch-167, AS $1M_2$ Ctrl and MP 4276 showed broad panicles while genotypes FS 4905, MP 5542, MP 5476, NW 5337 and NW 5436 had narrow panicles.

Panicle weight

Panicle weight ranged from 8.08 g to 92.02 g (Table 3.6). The genotypes that had higher panicle weight were 05-Potch-138, Mammolokwane, AS 18, NW 5430 and NW 5393. The genotypes with smaller panicle weight were LP 4312, AS 8, EC 3281, AS 6, MP 5541, AS 11 and EC 3439.

Thousand seed weight

Thousand seed weight ranged between 2 g and 43 g (Table 3.6). The genotypes EC 3281, EC 2934, KZ 4547, KZ 5097 and LP 4312 had the highest thousand seed weight and AS 6, MP 2048 and AS 82 had low seed weights.

Table 3.4. Mean of eight quantitative traits of 98 sorghum genotypes evaluated at Makhathini and Burgershall

Var	Makhathini								Burgershall							
	PHT	PAL	PAW	PEX	RCN	PWT	SWP	TSW	PHT	PAL	PAW	PEX	RCN	PWT	SWT	TSW
1	126.7	11.67	7	12	22.33	24.01	65.7	3.67	126.97	16	15.63	31.67	37	44.1	149.3	26.33
2	183	13	15	19.33	34	37.81	114.7	3.67	111.48	17.07	5.6	21.67	58	46.17	78.2	28
3	127	17.83	15	33	25.5	55.01	72.5	4	83.23	21.67	4.93	33.33	38.33	65.1	61.3	38
4	156	22.67	15.67	15	25.33	23.01	1.50	1	98.89	15.8	6.2	10.67	57.33	47.83	190.7	28
5	117	17.33	17	68.33	33	42.67	64	5	148.11	15	3.73	25	26	15	66.7	34.67
6	110.4	15.67	16	10.33	30	129.67	78.3	5	72.17	19	3.9	27	38	13.9	75	33
7	133.7	14.67	14.93	34.4	23.33	135.67	112.7	6.34	78.78	13.07	2.96	35	36	7.63	44.9	41.33
8	184.7	17	15.67	35.75	18	95.01	114	5	94.61	17	3.4	32.67	35.5	22.25	70	38
9	167.7	15	13.67	16.67	26.67	77.67	83.3	3	102.8	14.8	3.88	24.17	41.17	36.33	141.6	31.33
10	181.7	18.33	16	31.33	34	10.27	84.7	1	68.01	17.87	4.53	27.33	67.33	40.67	116.5	28
11	120	14	13.33	31.67	27	60.01	128.7	4.67	132.93	17	5.05	27.67	35.33	30.3	121.7	38
12	155.7	10	14	12	23.33	83.34	93	4.67	133.59	14.53	3.93	33.33	24.67	27	127.3	28
13	227	19.33	15.33	11.33	30.33	63.67	152	2.34	79.29	17.2	5.33	33	35	11.17	79.3	28
14	182	15.33	16	15.67	21	31.34	41.7	2	99.55	16.65	3.4	36	32.33	30.51	136.5	35
15	140	18.33	17.33	12.33	19.67	77.34	143.3	3	85.76	18.73	3.13	36.67	24.67	18.67	43	31.33
16	111.3	12.67	16.33	30	30	67.01	36.3	6	87.07	13.99	4.23	36	58.33	37.83	74.6	28
17	154	18.67	15.67	25.67	24.33	24.34	69.5	3	87.41	15.36	3.67	29.67	33.5	50.17	80.8	36.33
18	131	18	17	35.33	33	50.26	23.5	2	81.1	10.84	3	33	30	59.07	75.3	33
19	146.3	15.33	15.33	33.67	20	23.01	72.3	5.23	81.1	18	3.6	28.67	37.5	84.67	102.8	38
20	30	14.67	16.33	36.33	28	33.34	26.3	1	84.77	16.57	5.1	40	47	43	132.7	43
21	148.6	16.33	15.67	31.33	14.67	44.01	173.5	3.67	100.22	19.33	6.4	25	54	23.17	135.1	28
22	24.7	15	17.67	16.67	22.33	20.67	62	3.34	122.45	15.78	12.7	35.67	24.67	27.17	30.3	28
23	84.7	17.33	13	28.67	14	92.51	31.5	3	105.98	45.6	3.82	34.67	30.33	13.43	119.7	31.33
24	155.1	14.67	17	16.33	26.67	77.01	141.3	4.34	95.06	19.4	5.13	24.33	24.67	15.67	78.8	34.67
25	158.3	12.67	15.67	10.67	14	17.01	127	5.34	81.63	19.8	5.8	34.33	41.33	43.5	162.7	31.33
26	123.3	11.67	15	13	12.67	15.17	122	3.34	77.75	15.33	5.6	33.67	42.33	58.83	217.3	31.33
27	134.3	23.33	16.33	15.33	34	39.67	120.3	4.34	125.26	16.46	3.4	30	27.5	10.27	39.2	32
28	164.5	16.67	15.33	9.33	14.67	45.34	122.7	5.67	101.49	17.13	3.93	31.33	21.33	32.5	53.3	34.67
29	118.3	13.67	13.33	17.33	30	79.34	153	4.67	104.77	12.2	3.33	20	35	24.83	85.3	31.33
30	163.3	13.67	15	15.33	26	14.01	141	3	83.79	15.6	5.33	23.67	43.67	23.25	102.8	38
31	181.7	15.33	15.67	15.67	26	80.67	154	4.34	80.69	14.4	4.33	24.33	37	15.5	57.2	38.67
32	173	15	15	13.33	24.5	15.01	162.5	13.5	72.77	13.6	4.6	34	35.5	38.25	86	33
33	147.7	12	14	32.67	28.5	87.34	76	5	91.94	16.23	4.94	30.35	37.62	30.16	102.8	34.13
34	126.7	14.67	15.33	12.33	43	20.67	47	3.67	99	23.77	6.37	22	45.33	32.97	177.3	41.1
35	46	16.67	16.67	8.33	33.17	123.14	128	4	104.75	13.13	4.1	37	34	11.25	156	43
36	178	21	14.33	34.33	44	14.67	161.7	4.34	100.51	16.82	15.35	25	42.33	5.67	87.8	44.67
37	53	11	15	31.67	27	59.67	155	4.67	96.19	24	6.4	27	74	34	78.9	28
38	105.6	13.33	15.33	12.33	34.67	49.67	127	5.34	94.32	14	4.67	25	27	66.5	108	31.33

39	192	23.33	15.67	33.33	35	35.34	41.7	5.34	74.38	16.42	4.56	35.67	22.33	25.83	87	39.67
40	111.2	14.67	16.33	19.33	37.33	13.34	136.8	2	86.38	14.73	3.33	33.67	31	43.5	46.8	31.33
41	140.3	26	13.67	32.33	31.67	42.67	180.3	6.67	79.5	18.13	5.6	25	50	37	83.3	28
42	115.7	16	15	35.33	19.67	93.67	78	4.67	107.76	15.6	4.33	27.67	49	17.67	94	34.67
43	48	12.33	16	10.67	24.33	77.67	167.7	5	112.71	11.47	3.09	40.33	25	14.83	74.6	40.33
44	70	17.67	16.33	30.33	32.67	23.34	32.7	3.34	130.41	15.73	4.13	20	41	17	183.9	31.33
45	111.7	22.33	15	32.67	37.67	90.01	92	4.34	118.61	14.53	4.93	34.33	43.67	32.5	167	41.33
46	66	16.67	16.33	6	40.33	47.67	147.7	5.34	127.35	21.67	4.87	35.67	41.33	44.83	49.1	34.67
47	210.5	14.67	14	8.67	32.33	25.34	149.3	3.34	79.04	17.93	6.53	34.33	33	34.67	119.3	31.33
48	132	12.67	15.33	32	38.33	40.34	41.7	4	118.39	15.07	6.67	39.33	40.5	15.33	130	28
49	146.1	27.67	12.5	34	29.67	32.01	55	4.67	98.54	16.06	5.66	34	36.33	14.17	157.3	31.33
50	251.7	14.67	15	22	26	82.34	166.7	3.34	83.11	16.2	5.07	31.33	52.5	27.5	73.3	28
51	229.3	16.67	17.33	34.33	32	66.67	66.7	6	90.15	13.73	3.53	23	22.33	27.17	98.2	28
52	151.7	15.33	14	15.33	25.67	52.01	40.7	2.34	99.77	14.95	4.37	23.67	33.33	44.33	100.3	31.33
53	107.7	16	15.33	21.33	17	27.67	48.3	5	76.09	17.6	4.67	39	25	18.1	121	31.33
54	144.3	12.67	16.33	14.33	31.33	59.67	117	5.34	110.93	16.2	4.33	20	41.67	25.17	162.8	38
55	207.2	13.67	17	32	31.67	23.34	42.5	5.31	86.64	16.8	6.2	36	35.67	13.2	79.3	38
56	138.3	16	15.33	35	32.67	50.67	148.8	5.46	117.01	14.27	4	27	47.67	27.83	147.3	38
57	151	12.33	16.33	11.33	43.33	91.34	180	5.34	115.95	14.8	3.73	35.67	37	35.47	61.7	34.67
58	155.4	22	16.67	18.67	21.67	78.67	154.7	5.34	96.57	17.13	3.63	35.67	24.67	39.53	73.3	41.33
59	142	14.67	15	26.67	22.33	66.67	32.7	6.34	114.43	16.55	3.38	36	39	22.8	99.5	31.33
60	89.3	18.33	14.67	24.33	26.83	75.01	130.2	6	115.73	17.26	3.32	32.67	30.33	24.67	73.2	34.67
61	63	14	17.67	14.33	22.33	45.01	35.7	3.67	106.92	15.93	4.73	34	41.33	37.17	92	34.67
62	72.3	17.67	17.33	13.67	23.33	79.34	89.3	5	67.89	17.8	5.13	2.69	23	8.67	68.7	28.15
63	160	15.67	15	13.67	39.33	46.34	42.3	2.34	100.01	13.41	3.83	26.33	45.33	13	51	31.33
64	159.3	15	14.33	30	21.67	44.67	130.3	4.34	74.42	15.27	4.2	25.67	36.33	29.5	90	1.33
65	136.7	18	15.67	33	23	54.34	149.3	4.34	86.78	15.2	3.47	39	31.33	25	71.7	38
66	37	21.33	14.67	35.33	14.67	57.67	180.4	4.34	80.5	12.01	3.1	36.67	35	12	71.4	38
67	204.3	20	14.67	32.67	27.33	29.34	68.7	5.34	115.53	11.68	3	30	38.67	45.5	143.7	28
68	141.3	7.72	14	16.67	55	126.01	136	4.67	80.13	11.72	2.83	30	21.67	33.83	79.7	34.67
69	130.5	14	4	8.67	15.67	119.01	85.7	6.34	83.47	15.67	3.68	30	36.17	67.25	137	36.33
70	177.9	17	15.67	19.33	28.67	64.67	84.4	4.67	103.91	13.97	4.11	30	26.67	58.33	138.9	28
71	72	15.33	13.67	14.33	30.33	28.34	163.7	3	117.88	24	5.6	30.33	42.5	27.33	109.3	41.33
72	80	23.67	15.67	22.33	30.67	23.34	111	2	73.45	15.76	13.45	36.67	53.33	27.75	176.3	31.33
73	87.8	13	15.67	35.33	41	37.51	121	5	128.66	14.09	4.25	32.67	37.62	14	71.1	28
74	110.5	14.67	15.67	13.67	23.67	39.01	12.3	2.67	160.9	19.9	5.23	35	57.33	14.42	168	18.9
75	115	15.67	14	13.67	25.33	28.67	117.7	5.67	83.67	13.44	3.38	30.67	34.67	25.33	89.8	34.67
76	175	14.67	13.67	14.33	29.67	84.51	159	4.67	78.93	15.73	4.2	33	31	30.33	83.7	31.33
77	79.7	14.67	15.33	17	22.67	77.51	50.7	5.34	121.6	15.15	3.88	27.67	35.33	6.17	129.7	33.26
78	115.6	15	14.33	11	33.67	78.67	138.7	3	79.07	13.39	4.54	36	34.17	28.83	90.2	33.15
79	35.3	10	14.67	27	15.67	99.01	50.3	5.67	80.75	14.1	4	33	30	58.43	132	38
80	36.7	9.33	16.33	33.33	33	57.01	74.7	5.34	128.39	16.12	5.57	34.67	56	35.25	93.5	41.33
81	43.7	8	8.33	23.33	30.67	11.51	113.8	2	95.86	12.67	4.4	25.33	37	45.5	176	38

82	247	21	16.67	26	38.33	66.67	131	4.67	96.19	17.53	3.87	35	26	30.17	85.7	33.26
83	52.2	11	15.33	35.33	34	16.34	59.2	3.34	108.79	16.37	5.53	33	36.33	17.75	78.7	32.5
84	166	16.33	14.67	14.67	38.67	12.01	102.3	2	131.4	20.5	6.83	31.67	37.62	20	102.8	34.13
85	34.7	16.67	3.33	31.33	31	59.01	73	3.67	91.43	14.07	13.93	30	50	36.83	174.7	33
86	181.3	15.67	16	17	33	34.67	39	2.34	110.03	15.03	3.57	24.67	53.67	11.5	154.3	31.33
87	18.3	6.67	16.33	18	28	20.17	74.7	4	90.21	11.88	3.93	20	42.67	56	120	37.59
88	259.3	12.33	10.67	23.67	33	14.01	135.3	3.67	98.13	15.2	4.87	38.67	24.67	14.5	60	31.33
89	137	16	15.67	15.67	33.33	41.84	55	3.67	118.3	17.93	4.5	35.33	33.33	33.83	151	44.67
90	249.7	23.33	17	25	25	10.67	135	4.34	99.18	18.87	5.1	39	47.67	63.33	78.3	38
91	92.4	15.03	16.67	32.67	27.93	6.27	38.5	4.23	89.62	14.27	4.23	32.33	35.33	22.67	90	43.26
92	236.3	13	13.67	16.67	36.33	16.01	23	3.34	110.4	15.93	4.43	20	35.67	20.67	72.7	34.67
93	58.7	16.67	14.67	8	16	53.67	156.3	3	113.97	18.12	5	33	31.33	16.5	83.3	48
94	170.7	16.67	13.67	15.67	14.67	30.34	165.7	3.31	131.17	10.73	4.47	23.67	35.33	59.17	99.7	34.67
95	44.7	7	15.67	17	11	63.34	123	6.34	110.23	17.47	4.87	20	32.33	16.6	90	28.15
96	53.7	12.33	15.33	34.33	23.67	56.01	180	4.34	75.19	14.67	3.33	38.33	41.33	12.17	91.8	41.33
97	56.7	14.67	13.33	12.67	31	113.67	82.7	5	69.48	9.86	3.35	30	31	22.67	83.1	33
98	78.2	14.45	14.33	27.33	29.69	79.82	108.6	0.81	94.41	13.33	4.47	30	35.33	16.67	65.3	41.33
CV (%)	18.7	19.9	14.2	19.5	30.3	27.8	20.3	37.5	11.2	37.3	36.2	8.6	14.5	39.7	18.5	15.8
SE	24.2	3.12	2.12	4.37	8.47	14.59	20.15	1.58	11.109	6.06	1.79	2.61	5.47	11.98	18.97	5.39
LSD (5%)	43.58	5.61	3.82	7.88	15.24	26.27	36.28	2.85	17.891	9.76	2.88	4.2	8.82	19.29	30.55	8.69
R^2	0.89	0.7	0.64	0.89	0.58	0.87	0.9	0.65	0.82	0.41	0.73	0.9	0.84	0.74	0.87	0.57
F pr.	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.043	<.001	<.001	<.001	<.001	<.001	<.001

CV = coefficient of variation; SE = Standard error; LSD = Least significance Difference; R^2 = R square value; F pr. = F probability; Var = variety; PTH = plant height, PAL = panicle length, PAW = panicle width, PEX = panicle exsertion, RCN = rachis number, PWT = panicle weight, SWP = grain weight per panicle, TSW = thousand seed weight

Table 3.5 Combined analysis of variance of eight quantitative traits in two locations, Makhathini and Burgershall..

Source of variation	DF	PTH	PAL	PAW	PEX	RCN	PWT	SWP	TSW
Site	1	819.01**	15.43**	807.07**	366.65**	248.24**	316.54**	247.28**	6585.49**
Genotype	97	109.59	281.43**	111.4	119.55	283.41**	139.54*	123.75	171.26**
Gen*Site	95	96.99	177.13**	97.45	117.79	206.83**	116.01	103.01	158.78**
Residual		2786	12.33	12.97	60.17	73.97	692.2	2082	20.99

DF = Degrees of freedom, Gen*Site = Genotype and site interaction, PTH = plant height; PAL = panicle length, PAW = panicle width; PEX = panicle exsertion; RCN = rachis number; PWT = panicle weight; SWP = grain weight per panicle, and TSW = thousand seed weight.

Table 3.6. Means and sources of variance of eight quantitative traits of 98 sorghum genotypes evaluated across two sites.

Variety (Accession)	Plant Height (cm)	Panicle length (cm)	Panicle width (cm)	Panicle exsetion (cm)	Rachis No	Panicle weight (g)	Grain yield (g)	Thousand Seed weight (g
1	130.6	14.43	15.83	32.12	30.1	34.05	100.8	14.84
2	151.9	15.45	10.34	26.65	45.66	42.29	94.3	15.66
3	114.3	20.35	10.29	29.22	32.14	59.93	64.4	20.76
4	156.7	19.66	6.8	17.15	40.94	35.71	91.7	14.31
5	97.6	16.63	8.64	19.98	29.8	29.28	64.3	19.62
6	101.3	17.8	9.64	23.35	33.52	72.66	72.8	18.8
7	120.8	14.34	8.5	25.33	29.15	72.54	75.5	23.58
8	150.5	17.47	6.55	28.35	26.21	59.46	88.5	21.26
9	157	15.37	8.63	29.47	33.39	57.81	108.1	16.96
10	160.7	18.56	9.21	20.48	50.72	25.87	99.3	14.31
11	106.3	15.9	9.6	20.64	30.86	45.83	121.1	21.09
12	133.8	12.65	8.44	25.07	23.35	55.41	106.1	16.16
13	163.2	18.74	9.86	34.37	32.2	38.24	112.4	14.98
14	138.1	16.46	8.91	23.69	27.24	31.8	86.9	18.26
15	118	19.38	9.29	27.43	22.39	48.09	92.5	16.96
16	100.5	14.24	10.45	26.3	44.07	52.6	53.9	16.84
17	121.9	17.86	9.06	21.1	29.11	37.26	73.6	19.43
18	109.1	15.41	8.64	24.04	32.49	55.04	50.5	17.28
19	128	17.67	9.01	31.38	29.02	54.63		24.83
20	78.8	16.5	10.17	35.32	38.09	38.31	78.5	21.7
21	124.6	17.39	11.36	28.41	34.14	31.63	153.8	15.66
22	59.7	16.3	13.91	28.27	25.04	22.29	43.9	15.49
23	81.5	31.76	5.04	22.93	22.98	51.58	72.8	16.96
24	113.6	17.26	10.1	24.59	26.59	43.58	107.5	19.28

25	140.6	16.39	9.66	21.01	28.2	28.09	143.2	18.15
26	112.5	13.73	10.17	27.09	27.99	36.17	168.6	17.13
27	116.4	19.61	8.07	22.12	31.04	23.71	78.9	17.97
28	119.8	16.53	8.57	22.39	18.22	36.29	86	19.96
29	94.1	12.82	9.54	25.38	33.11	50.14	116.5	17.81
30	119.4	13.47	9.72	19.44	34.27	17.66		20.25
31	123.4	13.63	10.45	19.71	30.99	45.83	102.4	21.25
32	131.2	13.71	8.86	33.53	28.57	25.17	123.8	23.11
33	-	-	-	-	-	-	-	5
34	116.3	18.44	11.4	19.13	43.39	25.09	108.5	22.12
35	71	13.58	10.23	34.86	32.92	65.64	139.5	23.22
36	134.1	18.39	15.75	29.74	42.05	8.08	122.4	24.21
37	70.5	16.97	10.95	22.71	49.09	44.76	114.6	16.16
38	89.4	12.95	10.61	18.68	30.88	56.25	114.9	18.15
39	136.9	19.24	10.83	24.86	28.94	28.11	59.4	22.25
40	92.7	13.54	9.56	32.61	33.75	27.43	89.6	16.46
41	121.2	21.42	10.85	20.25	40.6	39.5	131.3	17.18
42	113.4	15.66	10.82	48.23	34.34	57.24	87.7	19.45
43	65.2	11.54	8.55	35.89	24.79	47.82	128	22.41
44	100.2	16.28	10.06	25.62	37.47	22.01	112.4	17.13
45	116	18.09	9.8	31.79	41.07	62.22	133.6	22.57
46	97.5	18.88	11.38	24.46	41.08	47.3	104.3	19.79
47	144.9	15.87	11.1	21.73	33.35	31.83	140	17.13
48	126	13.52	11	26.08	39.84	28.78	89.8	15.83
49	123.2	21.53	11.17	19.58	33.39	24.02	110	17.81
50	170.1	15.25	10.2	21.45	39.85	56	124.5	15.49
51	161.9	14.94	10.38	28.74	27.79	47.41	85.9	16.84
52	125.9	14.79	9.96	29.16	29.96	50.14	75.7	16.62
53	93.2	16.53	9.39	26.85	21.58	24.39	88.7	17.98

54	130.2	14.18	9.55	13.76	37.38	43.36	142.9	21.43
55	147.1	14.87	11.04	34.3	34.14	20.24	66.3	24.74
56	127.7	14.71	9.66	29.95	40.76	41.11	153.5	24.56
57	136.7	13.81	9.54	35.21	41.09	64.6	122.7	19.79
58	129.2	19.75	10.05	34.84	24.22	60.16	115.3	23.08
59	129	15.61	9.2	36.16	31.38	46.73	68.1	18.65
60	103.3	17.87	3.44	32.36	29.19	51.96	105	20.13
61	84.2	15.31	10.11	32.54	30.53	39.66	62.1	18.94
62	70.8	17.83	11.23	7.74	24.66	41.86	74.8	14.06
63	129.3	14.88	8.83	28.72	41.11	28.26	45.4	16.62
64	118	15.4	10.24	21	27.5	36.33	111.2	17.64
65	115.5	16.7	9.9	24.53	28.37	39.5	109.7	20.93
66	58	17.02	9.63	27.68	23.52	33.44	125.6	20.93
67	163.7	15.95	10.67	31.59	34.18	37.21	104	16.5
68	110.8	9.94	9.29	27.88	38.67	78.18	105.6	19.45
69	108.8	14.98	10.61	27.51	25.73	92.02	109.9	21.12
70	142	15.74	10.48	29.8	27	60.02	108.3	16.16
71	97.1	20.06	10.32	26.94	37.38	26.96	135	21.89
72	78.7	20.08	15.4	26.16	41.33	24.37	92.7	16.46
73	109.7	14.06	10.86	22.24	-	25.28	96	16.33
74	136.4	18	10.33	25.89	38.57	26.87	89.7	6.45
75	100.6	15.04	8.58	23.5	28.09	26.22	106	19.96
76	127.3	15.64	10.23	21.52	30.02	55.63	120.6	17.81
77	102.6	15.27	10.46	22.85	28.42	40.72	89.9	16.22
78	98.8	14.65	9.55	23.36	33.8	53.18	114.2	14.82
79	57.5	12.61	8.66	25.03	21.12	77.3	90.6	21.6
80	85	13.28	11.46	24.05	42.49	46.71	86.9	23.08
81	65.1	10.26	9.39	26.96	33.71	29.91	146.9	19.74
82	172.1	19.16	9.76	25.67	31.88	48.37	109	15.82

83	75.8	13.61	10.29	28.1	35.06	18.48	71.3	17.71
84	144.1	18.34	10.44	22.42	-	17.43	-	2
85	61.3	14.97	13.95	22.73	39.89	47.95	122.8	18.12
86	139	14.99	8.97	29.53	42.99	24.64	97.8	16.62
87	47.5	8.91	9.66	27.2	35.03	39.59	99.1	17.32
88	174.7	13.39	10.78	25.67	28.53	15.03	99.4	17.3
89	121.1	16.78	8.88	33.07	33.73	40.59	108.4	23.87
90	167.8	20.75	10.24	38.01	35.98	38.49	109.3	20.93
91	86.2	14.43	10.45	35.63	31.12	15.98	65.6	43
92	166.8	14.11	9.74	24.36	35.79	19.87	49.6	18.78
93	79.6	17.03	9.36	23.64	23.36	36.65	122.6	25.18
94	144.2	13.35	4.42	16.99	24.66	46.26	135.4	21.95
95	70.7	11.87	10.96	14.99	21.32	41.54	108.9	14.87
96	57.8	13.23	8.83	28.42	32.48	36.26	140.8	22.57
97	58	11.47	9.86	20.88	30.94	68.27	85.4	18.8
98	81.7	13.38	10.06	32.69	32.96	48.92	86.7	20.88
Variety	201**	50**	19**	196**	245**	1430**	3782**	40.24**
Site	131379**	43.24	14299**	9516**	13966**	72196**	1387.7	127628.63**
Variety x Site	5405**	41**	14**	228**	229**	2087**	2241**	40.33**
Mean	114.2	15.9	9.9	26.4	32.6	41.3	101.1	18.77
CV (%)	16.4	30.27	19.46	14.02	21.79	32.35	19.25	20.73
SE	18.72	4.82	1.93	3.7	7.13	13.46	19.48	3.941
R^2	0.9	0.53	0.93	0.91	0.77	0.87	0.89	0.959
LSD (5%)	31.5	5.898	2.363	4.527	8.736	16.46	23.86	4.608

CV = coefficient of variation, SE = standard error, R^2 = R square value, LSD = least significance difference and Rachis No = rachis number.

3.4.2.3 Correlation analysis among phenotypic traits

Grain yield and related traits were analysed using pair-wise rank correlations coefficients. The results and association of the quantitative traits were reported based on the significance levels of 5% ($P< 0.05$) and 1% ($P< 0.01$), respectively (Table 3.7). Plant height significantly and positively correlated with weight per panicle, and negatively with thousand seed weight. It was further positively and significantly correlated with panicle length and seed yield, and negative and significantly correlated with panicle exsertion. Panicle length was significant and negatively associated with panicle width, and panicle weight. It was also significant and positively correlated to rachis number. Panicle width had significant and negative correlation with panicle exsertion, and significant and positively correlated with grain yield. Panicle width was also highly significant and positively correlated with panicle weight, and negative for rachis number and thousand seed yield. Panicle exsertion was significantly correlated with rachis number, and thousand seed yield. It was further, negatively and significantly correlated with panicle weight and seed yield. Rachis number positively correlated with thousand seed weight and a negative correlation was observed for panicle weight. It was further positively and significantly associated with thousand seed weight. Grain weight was significant and positively correlated with panicle weight. Thousand seed weight was positively correlated to panicle weight and was highly significant.

Table 3.7.Pairwise correlation coefficients of eight quantitative traits of 98 sorghum genotypes evaluated at two locations.

	PTH	PAL	PAW	PEX	RCN	SWP	TSW	PWT
PTH	1							
PAL	0.1213*	1						
PAW	0.0481	-0.0827*	1					
PEX	-0.1034*	0.0267	-0.1095*	1				
RCN	0.0012	0.0594*	-0.167**	0.1445**	1			
SWP	0.103*	0.0034	0.1204*	-0.0651*	0.1126*	1		
TSW	-0.3063**	0.0288	-0.3752**	0.4221**	0.36**	0.0391	1	
PWT	0.0481	-0.0827*	1.0000**	-0.1095*	-0.167**	0.1204*	-0.3752**	1

* P = 0.05; ** P ≤ 0.01; PTH = plant height, PAL = panicle length, PAW = panicle width, PEX = panicle exsertion, RCN = rachis number, SWP = seed yield, TSW = thousand seed weight, PWT = panicle weight

3.4.3 Principal component analysis

The quantitative data of the two locations, Makhathini and Burgershall were subjected to principal component analysis (PCA) in a Multivariate analysis. The principal component analysis revealed three most important PC's each contributing 38.9%, 30.96% and 18.13% (Table 3.8). This shows great variation among the genotypic traits under investigation. The factor loadings from the principal component analysis ranged from -0.10884 to 0.76384 for PC1, -0.55215 to 0.81235 for PC2, and -0.68285 to 0.2985 for PC3. Plant height, grain yield and panicle weight were the traits that contributed most to the variation in the first PC. Seed weight and plant height were the traits that contributed most to the variation in the second PC, and panicle weight, plant height, and thousand seed weight were the most contributors to the variation observed in the third PC.

Table 3.8. A factor loading of the eight morphological traits in 98 sorghum genotypes evaluated across two locations showing most important PCs.

	Factor loading		
Trait	PC1	PC2	PC3
Plant height	0.76	-0.55	0.3
Panice length	0.01	-0.01	0.02
Panicle width	0.04	-0.03	-0.08
Panicle Exsertion	-0.03	0.01	0.06
Rachis number	0	0.04	0.11
Panicle weight	0.14	0.02	-0.68
Seed yield	0.57	0.81	0.07
Thousand seed weight	-0.11	0.11	0.25
% variation	38.91	30.96	18.13
Latent Roots	2644	2103	1232
Cumulative variation	38.91	69.87	88

3.4.3 Cluster analysis

The quantitative and qualitative traits were analysed using Agglomerative Hierarchical clustering to construct a dendrogram (Figure 3.1). Three major clusters were formed among the sorghum accessions. Cluster I was composed of thirty one genotypes and it was further sub-divided into two sub-clusters, Ia and Ib. The Sub-cluster Ia was composed of twenty two sorghum accessions, and sub-cluster Ib consisted of nine sorghum accessions. In sub-cluster Ia, entries KZ 5097 (56) and EC 2985 (20) were similar but distantly related with the other accessions in the same sub- cluster. In sub-cluster Ib, the genotypes KZ 5274 (53) and NW 5464 (3) were similar and also distantly related with the rest of the accessions in the same cluster.

Cluster II was composed of thirty three accessions which were classified into two sub-clusters, IIc and IIb. The sub-clusters formed two sub-groups in each sub-cluster. In sub-cluster IIc, the following genotypes: LP 1481 (43), Manthate (73), AS16 (82), MP 2048 (33), KZ 4722 (49), and FS4909 (59) were genetically similar but distantly related with other accessions in the same sub-cluster.

In sub-cluster IId, entries formed three sub-sub clusters. MP 4259 (28) and MP 5502 (29) were distant from the other genotypes but closely related with AS 82 (74), FS 4891 (61), and FS 4942 (89). EC 3217 (21) and KZ 4722 (49) were closely related by allocated far apart from the group of MP 5476 (23), LP 1948 (37), AS 18 (79) and FS 4952 (58).

Cluster III consisted of thirty four accessions and was categorized into sub-cluster IIIe and IIIf. Sub-cluster IIIe was further divided into two groups where KZ 5258 (39), EC 2975 (17), and LP 4303 (44) KZ 5245 (57) were similar and formed a group, but distantly related with the other accessions in the second group in the same sub-cluster. The accessions in the sub-cluster IIIf grouped themselves into two groups, where LP 1450 (35), EC 3414 (11), AS66 (40), MP 4276 (22), and EC 2934 (19) were closely related. The genotype MP 4052 (98) was different from the other accessions in the second group consisting of accessions AS1 M_2 Ctrl, NW 5337 (8), AS1 (76), EC 3403 (16), and M48 (70). The most diverse sorghum accessions identified were MP 4277 (32), EC 2934 (19), KZ 5097 (56), FS 4909 (59), and LP 4303 (44).

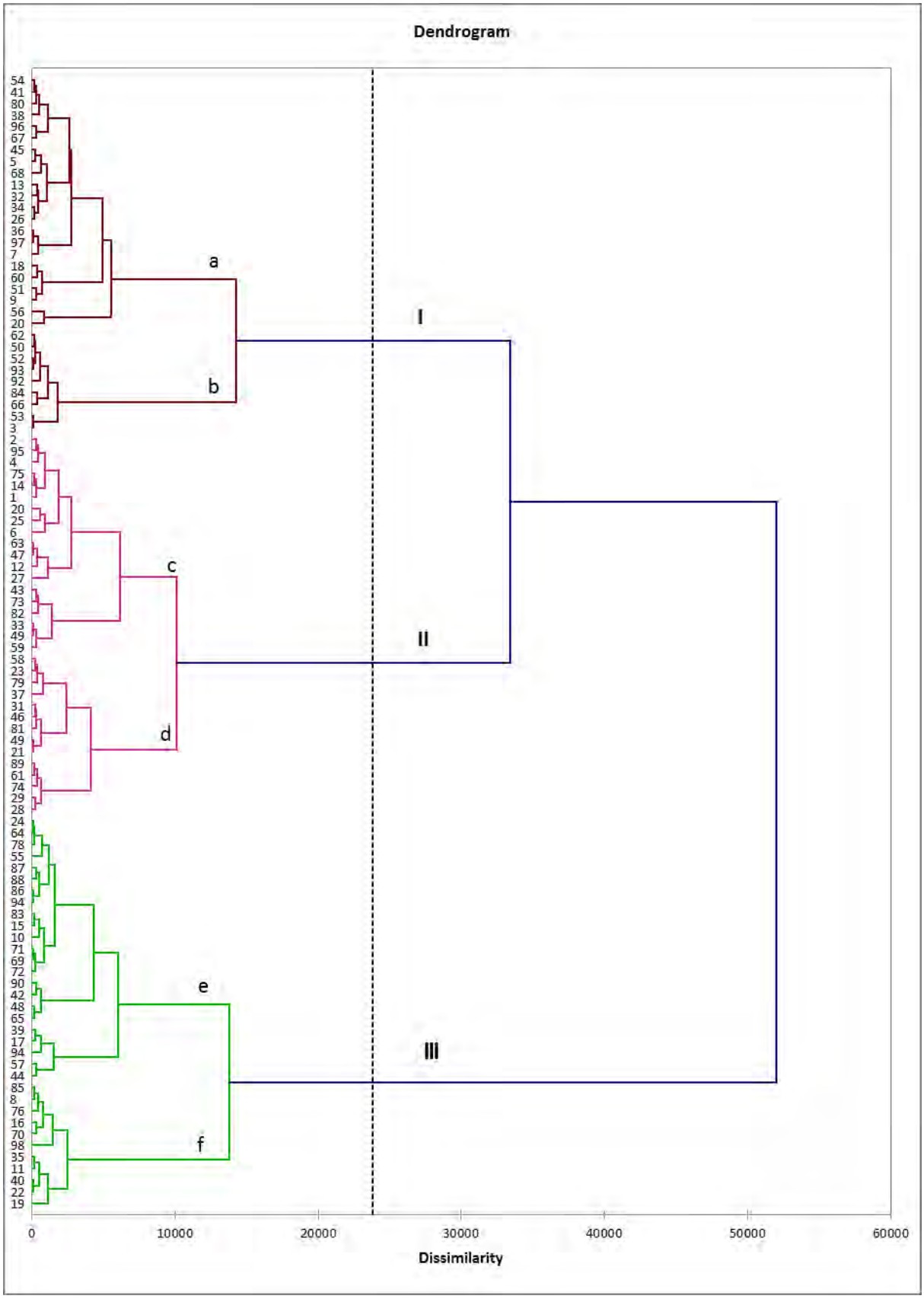

Figure 3.1. A dendrogram of ninety eight sorghum lines based on morphological traits using Agglomerative Hierarchical clustering.

3.4.4 Response of sorghum genotypes to six qualitative traits

3.4.4.1 Spearman's rank correlation analysis of six qualitative traits

Six agronomically important qualitative traits were analysed using Spearman's rank correlation coefficients (Table 3.8). The qualitative traits were grain and midrib colors, inflorescence shape and compactness, glume covering, glume color and either presence or absence of awns. The glume coverage was significant and positively correlated with the awns. The glume color was significant at P <0.001 and positively correlated with the midrib color. The midrib color was further correlated negatively with seed color and was highly significant. The glume coverage was significantly and negatively correlated with seed color. The head shape and head compactness was highly significant and negatively correlated with the midrib color. The correlations of the other traits were non-significant when correlated with one another.

Table 3.9. Spearman's correlation coefficients of six qualitative traits of 98 sorghum genotypes evaluated at two locations.

	Awns	Glume color	Glume coverage	Head shape	Midrib color	Seed color
Awns	1					
Glume color	-0.012	1				
Glume coverage	0.004	-0.059	1			
Head shape	-0.002	0.172**	0.002	1		
Midrib color	0.049	-0.017	-0.005	-0.177**	1	
Seed color	0.102*	0.029	-0.101*	-0.012	-0.073	1

*= significant at P ≤ 0.05, ** = highly significant at P ≤ 0.001

3.4.4.2 Kruskal-Wallis analysis

The six qualitative traits were analysed using Kruskal Wallis test procedure with one way analysis of variance. The qualitative traits analysed are shown in Table 3.9. There were significant differences (P < 0.001) observed among the sorghum genotypes based on midrib color (Table 3.10). All other qualitative traits measured were non-significant at both P< 0.05 and P < 0.00 significance levels.

Table 3.9. Kruskal-Wallis analysis, and significant tests of six qualitative traits in sorghum.

Trait	X^2
Awns	0.110
Glume color	0.947
Glume coverage	0.471
Head shape and compactness	0.496
Midrib color	< 0.001
Grain color	0.968

3.5 Discussion

Principal component analysis

Principal Component analysis revealed the traits that contributed the most to the variation observed among the sorghum lines. The traits included plant height, rachis number, and panicle weight. The yield related traits can be useful in selection for the purpose of yield improvement. Yield is a complex trait which has many components contributing to its totality. Improving some of the yield components contributes to the yield improvement. It is important for researchers to consider the association among the pairs of the yield related traits; hence correlation studies are essential for assessing the association between them. In this study, there were significant correlations observed among the yield related traits. The genotypes with the traits that are highly significant and have low coefficient of variation can be used as a selection criteria for use of genetic material for handling and improvement.

Analysis of quantitative and qualitative traits

Information on significant correlation among the characters is important for initiation of breeding programmes as it gives a chance for selection of desirable genotypes with desirable traits concurrently. Various studies reported correlations among yield and yield components in their crop of interest. In sorghum, Tesso et al. (2011) reported significant positive correlation between leaf traits and yield components excluding the thousand seed weight and panicle length. In their study, the authors indicated that yield components were positively correlated with each other excluding panicle length and thousand seed weight. Nabin et al. (2013) also observed significant correlations among the fodder yield per plant with thousand grain weight and plant height. In other studies, grain yield has been reported to have significant positive correlations with harvest index, ear head dry matter, total dry

matter, head length, and width, leaf area index, and duration (Kadam et al., 2001). In many studies grain yield per panicle was also reported to have a positive significant correlation with plant height, panicle length, panicle weight, and number of seeds per panicle (Prasuna et al., 2012). Grain yield was also reported elsewhere that it was found to be positively correlated with plant height, panicle length and number of seeds per head (Chavan et al., 2011; Mahajan et al., 2011).

El-Din et al. (2012) reported a positive and highly significant correlation between number of grain/head and grain yield and positive significant correlation between panicle length and grain yield. Moreover, non-significant negative correlation was observed between panicle width and grain yield/panicle. Jankovic et al. (2012) also reported presence of very strong to almost complete, statistically very significant positive correlations among the morphological productive indicators per species. Plant height, leaf length, leaf width, stem diameter, and other quality traits were significantly and positively correlated with fodder yield per plant (Prakash et al., 2010). In sorghum, grain yield was significantly and positively correlated with panicle weight and fodder yield per plant. It further had a positive correlation among days to flowering, plant height, leaf number and panicle width (Mallinath et al., 2004). Ramesh et al. (2012) reported positive and highly significant association between yield and other quality components at two sites.

There was enormous variation among the quantitative and qualitative traits measured in the study. The variation in qualitative traits was also observed and reported in Ethiopian and South African sorghum accessions by Gerrano et al. (2014). Updhyaya et al. (2010) reported variation on the qualitative and quantitative traits and identified specific traits as new sources in sorghum germplasm. The traits included early flowering, short plant height, medium panicle exsertion and medium sized seeds. Shegro et al. (2013) further reported highly significant differences among the quantitative traits in sorghum accessions under study and the qualitative diversity index values ranged from 31% for panicle shape and compactness to 84% of the glume color. Lekgari and Dweikat (2014) reported genetic diversity based on the plant height, days to anthesis and moisture content in sweet sorghum. Knowledge of patterns of diversity of genetic material is of great importance and is key component in crop improvement and breeding (Warburton et al., 2008).

Analysis of genetic diversity (clustering and genetic distance)

Studies on analysis of genetic diversity using qualitative and quantitative phenotypic traits in sorghum were reported (Ayana and Bekele, 2002; Abdi et al., 2002; Agrama and Tuinstra, 2003; Geleta and Labuschagne, 2005; Torkpo et al., 2006; Bucheyeki et al., 2009;

Ganesamurthy et al., 2010; Ngugi and Maswili, 2010; Abdel-Fatah et al., 2013). Shegro et al. (2013) further reported genetic diversity among the sorghum lines from Ethiopia using morphological descriptors. In addition, Suliman and Abdelbagi (2010) further reported the presence of genetic diversity among the sorghum accessions for Sudan.

Characterization of the accessions gives an overview of the traits and helps to understand similarities and differences among the accessions under investigation (IBPGR and ICRISAT, 1993). Based on the dendrogram, some accessions among the clusters and the sub-clusters were distantly related. The closely related accessions are those that are grouped within the same subgroup and share the same ancestral history. The DAFF collections were found in almost all of the clusters and were mixed in terms of the provincial collection. The grouping of accessions was not based on the source of collection, hence were mixed. This shows the presence of genetic diversity among the accession within and among the South African provinces. It may also be due to gene flow from the neighboring areas/provinces and sharing of seeds by farmers amongst themselves (Manzelli et al., 2005). Moreover, farmers share seeds and name the same accessions differently in various areas or regions (Chakauya et al., 2006). Farmer's practices may also influence the handling and conservation of the genetic material on their fields.

The results in this study concur with the study of Uptmoor et al. (2003) where the authors reported genetic diversity among the sorghum accessions, and the clustering of accessions was not based on the place of origin or source. The presence of vast diversity among the genotypes in this study was clearly shown by the distant relationships among the genotypes. The diverse genotypes could be useful for selections in plant breeding programmes and for further genetic improvement (Upadhyaya et al., 2010). They can also serve as potential parents for hybridization and for development of hybrids in line x tester analysis (Ngugi and Maswili, 2010; Ngugi and Onyango, 2012; Shehzad and Okuno, 2014). The presence of genetic diversity in the genetic pool allows breeders to make selections of the distantly related genotypes based on the phenotypic traits of interest, more especially the traits that may be appealing to researchers, farmers and end-users. Furthermore, for evaluation of inheritance of some of the specific traits of interest, and enhancing genetic gain (Abdel-Fatah et al., 2013). Morphological traits are excellent indicators of presence of genetic variability among the genotypes under investigation, local differentiation and conservation, and can be employed to categorize morphological diversity (Grenieret et al., 2001).

3.6 Conclusions

Phenotypic evaluation of germplasm can be useful for characterisation, conservation and maintenance of genetic resource. The study revealed three most important principal components among the sorghum accessions evaluated in the two locations. Traits that were highly significant, and had positive correlation were observed among the yield related traits and could be selected for strategic improvement in breeding programmes. There was morphological diversity present among the sorghum accessions studied and the following lines, MP 4277, EC 2934, KZ5097, FS4909, and LP 4303, were identified as the most diverse. The phenotypic diversity based on quantitative and qualitative traits is imperative for germplasm conservation, classification and identification, and for strategic selection and isolation of novel genes based on specific traits. The best lines with quantitative and qualitative traits were MP 4276, NW 5430, 05-Potch-167 and EC 3217 across locations. They have intermediate plant height, well exserted, broad panicles, intermediate panicle weight, high weight per panicle and medium seed size (Intermediate thousand seed weight). The geotypes can be selected for further breeding based on the morphological traits.

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CHAPTER 4

Comparison between random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers with high resolution melt analyses in genetic variation analysis among selected sorghum genotypes

4.1 Abstract

Understanding the genetic diversity of germplasm is essential in plant breeding programmes and germplasm management. Molecular markers are efficient and effective tools widely used for assessing genetic diversity among crop genotypes. Recently, high resolution melt analysis (HRM) has been reported for detecting genetic variability. However, there is limited information on the use of HRM in conjunction with other molecular marker techniques for assessing genetic variation in sorghum. This study was conducted to compare RAPD and SSR markers with HRM analyses to determine genetic variation among selected sorghum genotypes. Eight diverse sorghum accessions obtained from the plant genetic resources, Department of Agriculture, Forestry and Fisheries/South Africa were subjected for both analyses. DNA was extracted from fresh leaves of the eight accessions and amplified using three RAPD and three SSR primers. The HRM analysis was performed and temperature normalised melting curves and difference plots were created and results compared. Both the molecular markers, the SSR and RAPD and HRM revealed variations among the accessions. The HRM melting profiles fairly well correlated with results from the RAPD and SSR analysis. The clustering of sorghum accessions using SSR marker highly corresponded with the HRM analysis. Therefore, the HRM can be a useful tool in genetic diversity and classification of sorghum genotypes without post-PCR analysis or processing.

Keywords: Genetic diversity, HRM, high resolution melt analysis, RAPD, SSR, sorghum

4.2 Introduction

Sorghum [*Sorghum bicolor* L. (Moench)] is one of the most important food security crops worldwide after wheat, rice, maize, and barley (FAO, 2011). It is grown by small and large scale farmers for food and livestock feed including environments considered to be marginal for other cereal crops such as maize and wheat. In spite of its economic potential, there has been limited research and extension in sorghum compared to other cereal crops such as maize and wheat in South Africa (Wenzel et al., 2001). Characterisation of sorghum germplasm is an important aspect in plant breeding programs to find new sources of genetic variation. Exploitation of diversity at the genotypic level requires an efficient system such as molecular marker technology (Iqbal et al., 2010). This technology allows estimation of genetic resource diversity more efficiently and reliably than phenotypic markers which are subject to genotype by environment interaction (Staub et al., 1997). The use of molecular markers aid the conventional breeding in many aspects including selection of parents for hybridization through genotypic diversity analysis studies (Jain and Kharkwal, 2004).

The DNA-based fingerprinting techniques are important tools for genetic variation studies in plant breeding and germplasm management and gene identification (McGregor et al., 2000; Simionic et al., 2002). Various marker techniques have been used for analysis of genetic diversity in sorghum including randomised amplified polymorphism (RAPD) (Prakash et al., 2008; Iqbal et al., 2010; Shivjee and Khanna, 2010) and simple sequence repeat (SSR) (Shehaz et al., 2009; Rajput et al., 2012; Reddy et al., 2012). These marker techniques are discrete, co-dominant or dominant, and free from epistatic gene action (Tanksley et al., 1989). In contrast to morphological or biochemical marker techniques, DNA-based methods are independent of environmental factors and results to a high level of polymorphism (Karp et al., 1997).

The high resolution melt (HRM) analysis is helpful for discriminating genotypes in combination with other molecular marker techniques. This technique has been reported in various studies and is advantageous due to lack of post-PCR sample processing and/or separation (Montgomery et al., 2007). The HRM can be used for genotyping in various ways including the use of amplicon melt with temperature controls (Seipp et al., 2007), unlabelled probes with the 3'-end to prevent extension by *Taq* polymerase (Zhou et al., 2004), and fluorescently labeled primers. The most common approach is the use of fluorescently labeled primers whereby the primers binds the genomic DNA in the presence of a dye. The

fluorescence is captured while samples are melting, following the PCR. The melting profiles are determined by GC content, length and the homozygosity and/or heterozygosity status of the sample genotypes (Reed et al., 2007). The melting profiles can be evaluated by normalized fluorescence curves, derivative plots, or difference plots to detect variation (Vaugn and Elenitoba-Johnson, 2004). This takes a very short time which is more beneficial to marker assisted selection in plant breeding programmes. The HRM has been reported in various studies including maize (Naidoo, 2010), potato (De Koeyer et al., 2010), almond (Wu et al., 2009), apple (Chagne et al., 2008), white lupin (Croxford et al., 2008), and perennial rye grass (Studer et al., 2009). The objective of the study was to compare RAPD and SSR marker and high resolution melt (HRM) analyses to determine genetic variation among selected sorghum genotypes.

4.3 Materials and methods

Plant materials

Eight sorghum genotypes were used for this study supplied by the plant genetic resources unit of the Department of Agriculture, Forestry and Fisheries (South Africa). Lines were coded as KZ87 (KZ 5287), EC67 (EC 2167), NW93 (NW 5393), LP48 (LP 1948), FS52 (FS 4952), EC3217 (EC 3217), FS489 (FS 4891) and MP65 (MP 4265) and originally collected from KwaZulu-Natal, Eastern Cape, North West, Limpopo, Free State, and Mpumalanga provinces as prefixed in the codes. The sorghum genotypes were planted in the African Centre for Crop Improvement (ACCI) tunnel at the University of KwaZulu-Natal. Two sorghum seeds of each accession were planted in 40 cm diameter plastic pots filled with seedling mix in five replicates. Each line was planted in 5 pots. Cultural practices were applied as necessary and water supplied as drip irrigation.

DNA extraction and quantification

The DNA was extracted using the CTAB extraction method (Kang et al., 1998; Saghai-Maroof et al., 1984). Young fresh leaves of each sorghum genotype were sampled via a lid of 1.5 ml microfuge tubes where a disc of leaf material was punched out into a tube. Four hundred µl of 2% CTAB extraction buffer [2% (w/v) CTAB 100 mM of Tris-HCl (pH 8.0), 1.4 M of NaCl, 20 mM of EDTA (pH 8.0) and 1% (w/v) PVP] was added to each tube. Samples were treated with a bead beater. The leaf material was then incubated in a waterbath at 66^{o}C for an hour. Four hundred µl of chloroform:isoamyl alcohol (24:1 v/v) was added and then centrifuged at 4^{o}C for 10 minutes at 12 000 g. The top aqueous part was transferred into fresh 1.5 ml microfuge tubes and 0.62 volumes of ice-cold isopropanol was added,

mixed gently and incubated at room temperature to precipitate the DNA. The precipitated product was centrifuged for 5 minutes at 12 000 g and the supernatant were removed. The DNA pellet was washed by adding 1 ml 70% (v/v) ethanol followed by centrifugation at 12000 g. The supernatant was discarded and following a quick spin the pellet was aspirated and then air dried at room temperature overnight. The DNA was suspended in 25 µl TE buffer. The genomic DNA concentrations were quantified using a NanoDrop spectrophotometer ND-1000 (NanoDrop Technologies, Wilmington, Delaware, USA) with absorbance 260/280 nm wavelengths. The DNA was diluted to a working concentration of 25 ng/µl TE buffer.

PCR and HRM conditions

The HRM analysis followed previous studies of Wu et al. (2008). The stock solution of the template DNA for each sample was diluted to a final concentration of 25 ng/µl in 0.1× TE buffer. A total of 15 µl per reaction mixture was prepared for PCR amplification. The reaction mixture contained 4.5 *µ*l water, 7.5 *µ*l KAPA (2×) Universal (KAPA SYBR$^{(R)}$ FAST qPCR Kit) master mix, 1.5 *µ*l of 500 nM RAPD primers (Table 4.1) and the 1.5 *µ*l of 25 ng/*µ*l template DNA. Negative and positive controls were included in each PCR setup to ensure non-contamination of the reagents. The reaction mixtures were amplified using a Rotor-Gene 6000 real-time rotary analyser. The machine was also used for HRM analysis. The PCR was performed as follows: an initial step of 95^{o}C 2 minutes followed by 40 cycles of 95^{o}C 10 seconds, 37^{o}C 20 seconds, 72^{o}C for 20 seconds. The HRM was performed after the PCR analysis with the ramp temperature of 70^{o}C-90^{o}C with each step rising with 0.2^{o}C, the pre-melt conditioning for 90 seconds on the initial step, and 2 seconds equilibration after each step. The melting curves were created and the Rotor-Gene software was used to distinguish sorghum accessions by normalisation and difference plots.

A total of 15 µl reaction mixture of each accession was prepared as described for the previous method but using the forwards and reverse SSR primers at 200 nM (Table 4.1). The PCR setup was 95^{o}C for 2 minutes for the initial denaturation temperature, followed by 95^{o}C for 10 seconds, 72^{o}C for 20 seconds for 40 cycles. The HRM was performed at ramp 75-85 degrees with each step rising with 0.1 degrees, and the pre-melt conditioning for 90 seconds on the initial step, and 2 seconds interval after each step. The melting curves were normalised and the Rotor-Gene software was used to distinguish sorghum accessions by difference plots.

Table 4.1. List of SSR and RAPD primers used for the experiment.

Marker	Primer		Sequence
SSR	GWKZN43	Xtxp335	Forward: 5′ TATTTCCTCTTGAAAGAATCAGGG 3′
	GWKZN44	Xtxp335	Reverse: 5′ TATTCATCGAGCAAAAGGCA 3′
	GWKZN45	Xtxp258	Forward: 5′ CACCAAGTGTCGCGAACTGAA 3′
	GWKZN46	Xtxp258	Reverse: 5′ GCTTAGTGTGAGCGCTGACCAG 3′
	GWKZN47	Xtxp145	Reverse: 5′ GTTCCTCCTGCCATTACT 3′
	GWKZN48	Xtxp145	Reverse: 5′ CTTCCGCACATCCAC 3′
RAPD		OPA-12	5′ TCGGCGATAG 3′
		OPA-16	5′ AGCCAGCGAA 3′
		OPA-18	5′ AGGTCACCGT 3′

Gel electrophoresis, data collection and analysis

About 2 µl of the amplified PCR products, 3 µl of water and 1 µl of loading buffer were mixed and loaded onto an agarose gel. A low molecular weight DNA ladder (6x) [1× Gel loading dye, blue: 2.5% Ficoll-400, 11 mM EDTA, 3.3 mM Tris-HCL (pH 8.0 at 25^{o}C), 0.017% SDS, and 0.015% bromophenol blue] was also included in the reaction. For RAPD analysis, the samples were electrophoresed on a two and half percent (w/v) agarose gel in a 1× TBE buffer for 1 hour 40 minutes with the current of 150 voltage. For the SSR analysis, the samples were electrophoresed on a 2% (w/v) agarose gel in a 1× TBE buffer for 1 and half hours with the current of 150 voltage. Bands were visualized by staining the gel in an ethidium bromide 0.5 µg/ml for 25 minutes and destained in water. The destained gel was then photographed under UV light. Bands were scored as either present (1) or absent (0). Dendrograms were constructed using a Dice Coefficient analysis (Dice, 1984) in NTSYS v2.1 software (Numerical Taxonomy and Multivariate Analysis) Computer Programme.

4.4 Results

RAPD analysis

Agarose gel analysis of RAPD marker

Polymorphisms were observed in all primer sets used for RAPD marker analysis. The fragment sizes ranged from 200 bp to more than 700 bp for the RAPDs (Figure 4.1). A high molecular weight DNA ladder could have been used for estimating the fragment sizes. Different banding patterns were observed among the genotypes. OPA 12 did not amplify very well and the bands associated with OPA 18 were difficult to score although some levels of polymorphism were observed, hence were not considered. The OPA 18 primer seemed to amplify the same region in all genotypes except for the MP65 accession. However, problems with data scoring and the reproducibility in amplification of RAPD markers have been reported (Jones et al., 1998).

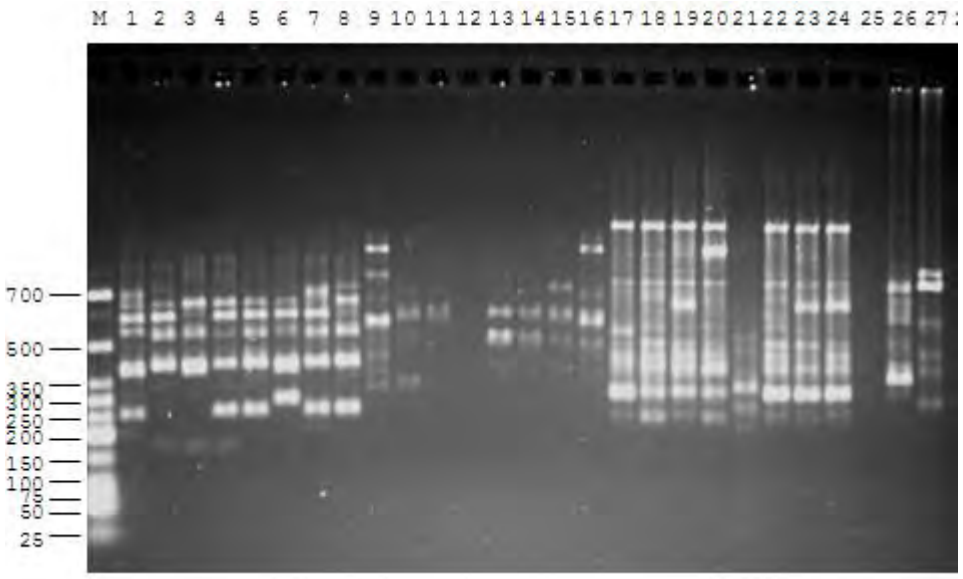

Figure 4.1. Agarose gel electrophoregrams of three RAPD primers (OPA-12, OPA-16, and OPA-18 from left to right); where: M = molecular weight ladder, 1= KZ87, 2= FS52, 3=NW93, 4= EC67, 5=MP65, 6= LP48, 7= FS489, 8= EC3217, and the same lines were replicated in that order from 9-16 and 17-24 across the remaining two markers. Number 25 represents the no temperate control; 26-27 represent the control (human DNA).

The numbers of bands scored are shown in Table 4.3. When comparing the RAPD and the SSR, the RAPD marker system revealed more polymorphic bands than the SSR. The number of polymorphic bands for RAPD was up to 12 whereas for SSR marker was 5.

High Resolution Melt analysis for RAPD marker

The melting profiles were created and normalised for three RAPD primers (Figure 4.2). The temperature normalised melting curves displayed the change in the fluorescence to 100% with the temperature of 76^{o}C and 0% fluorescence at about 86^{o}C. Variation was observed in

all the three RAPD primers. OPA12 showed differences among the sorghum genotypes studied.

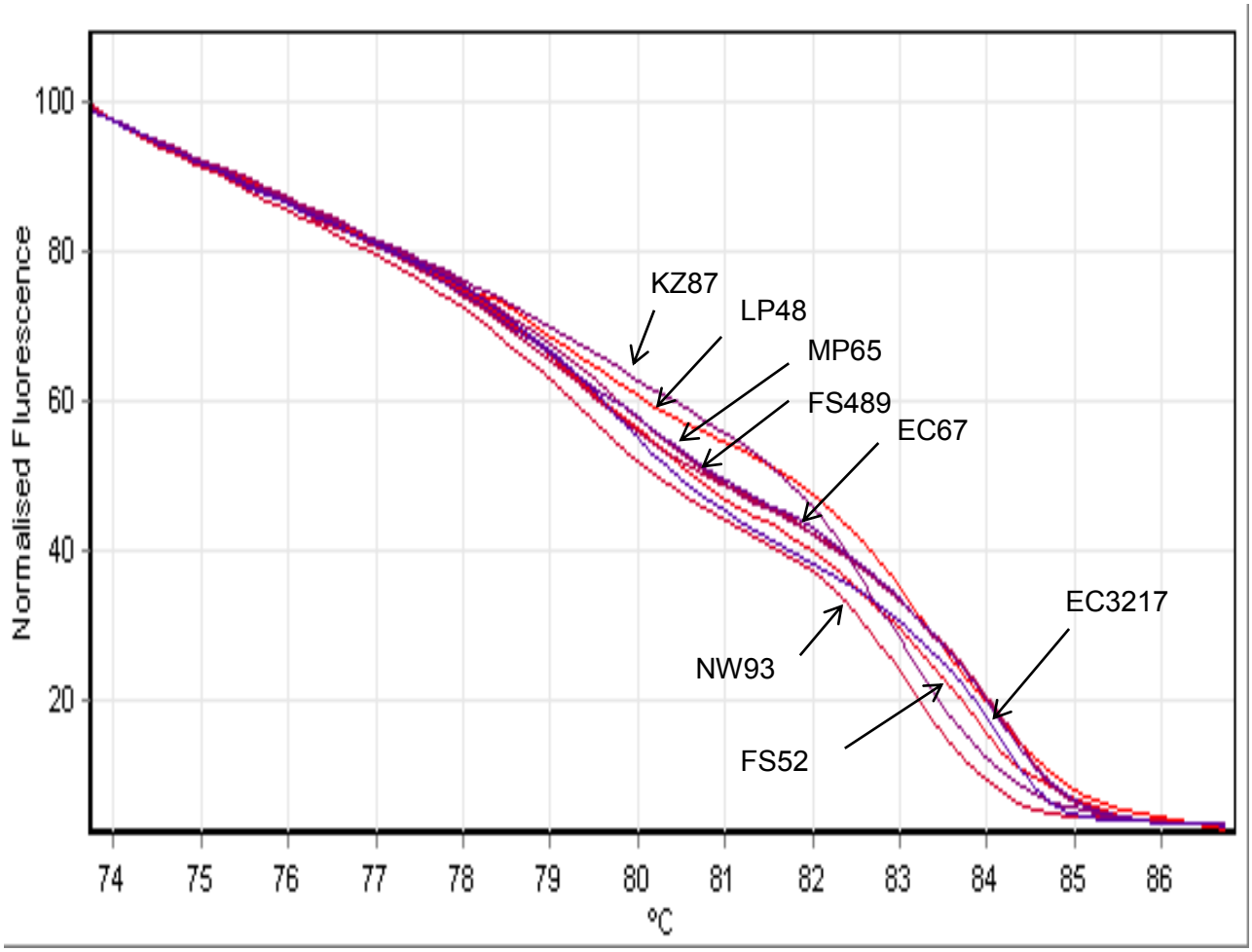

Figure 4.2. The temperature normalised HRM melting curves of sorghum genotypes using RAPD OPA 12 primer.

Genotypes EC67, FS489 and EC3217 had similar melting curves. The NW93 had a different curve compared to other genotypes followed by LP48. This could be due to early maturity, and short stature of the accession itself. Genotypes FS52 and EC3217 had similar normalised melting profiles. Also NW93 and LP48 had similar normalised melting profiles. The genotypes that had similar melting profiles are closely related. These genotypes with unique profiles from others can be selected and used in breeding programmes as sources of valuable genes. Although some variations were observed among the melting curves, the curves created were difficult to interpret for OPA 16 and OPA 18 RAPD primers.

Similar results were observed in the difference plots. Genotype MP65 was used as a baseline with a threshold confidence level set at 90% in a difference plot analysis. The

results are shown in Table 4.2. Genotypes EC67, and FS489 were very closely related with the baseline entry, MP65. NW93 was not related to MP65 and the rest of the genotypes. LP48 distantly related with MP65 and other accessions. Although different from the baseline and other genotypes, FS52, EC3217 and KZ87 appeared to be closely related.

Table 4.2. Similarity of sorghum genotypes from difference plot of HRM analysis.

Genotype	KZ87	FS52	NW93	EC67	MP65	LP48	FS489	EC3217
Confidence %	66.56	80.41	19.98	93.07	100	41.31	97.09	77.01

Analysis using the SSR

Gel electrophoresis

Polymorphisms were observed among sorghum genotypes studied using three SSR primer pairs. The sizes of the SSR fragments ranged from 150 bp to 300 bp (Figure 4.3). The Xtxp 258 primer showed monomorphic banding pattern with only EC67 showing a small difference in fragment size.

Figure 4.3. Agarose gel electrophoregrams of three SSR primers (Xtxp335, Xtxp145, and Xtxp258- from left to right); where: M = molecular weight ladder, 1= KZ87, 2= FS52, 3= NW93, 4= EC67, 5= MP65, 6= LP48, 7= FS489, 8= EC3217, and the same lines were replicated in that order from 10-17 and 20-27 across the remaining two markers. Numbers 9, 18, and 27 represent the no template control.

Table 4.3. Number of bands scored for five primers showing polymorphism.

Marker/primer		Number of bands scored for each sample							
		KZ87	FS52	NW93	EC67	MP65	LP48	FS489	EC3217
SSR	Xtxp335	1	1	2	2	2	2	1	1
	Xtxp258	1	1	0	1	1	1	0	1
RAPD	*OPA12*	6	4	4	5	5	5	5	6
	OPA16	3	1	1	0	2	2	3	4
	OPA18	4	5	5	4	1	4	5	4

Polymorphic bands were scored for both SSR and RAPD marker analyses. The numbers of bands scored are shown in Table 4.3. The RAPD marker system revealed more polymorphic bands than the SSR. The number of polymorphic bands for RAPD was up to 12 whereas for SSR marker analysis was 5. Xtxp 145 did not form any bands suggesting its non-polymorphism to the template DNA.

High resolution Melt analysis for SSR marker

The normalised temperature melting profiles were created in the HRM analysis. Differences were observed among normalised melting profiles in all three SSR primer pairs. In this study, only data on one SSR primer, Xtxp 335 is interpreted. The Xtxp 335 primer showed clear variation among the genotypes tested (Figure 4.4). KZ87 showed a different melting curve from the entire set of sorghum accessions. Genotypes FS52, FS489 and EC3217 had very similar melting pattern. Similar melting profiles were also observed for genotypes EC67 with MP65, and NW93 with LP48. These genotypes melting profiles were different from the EC3217, FS52 and FS489.

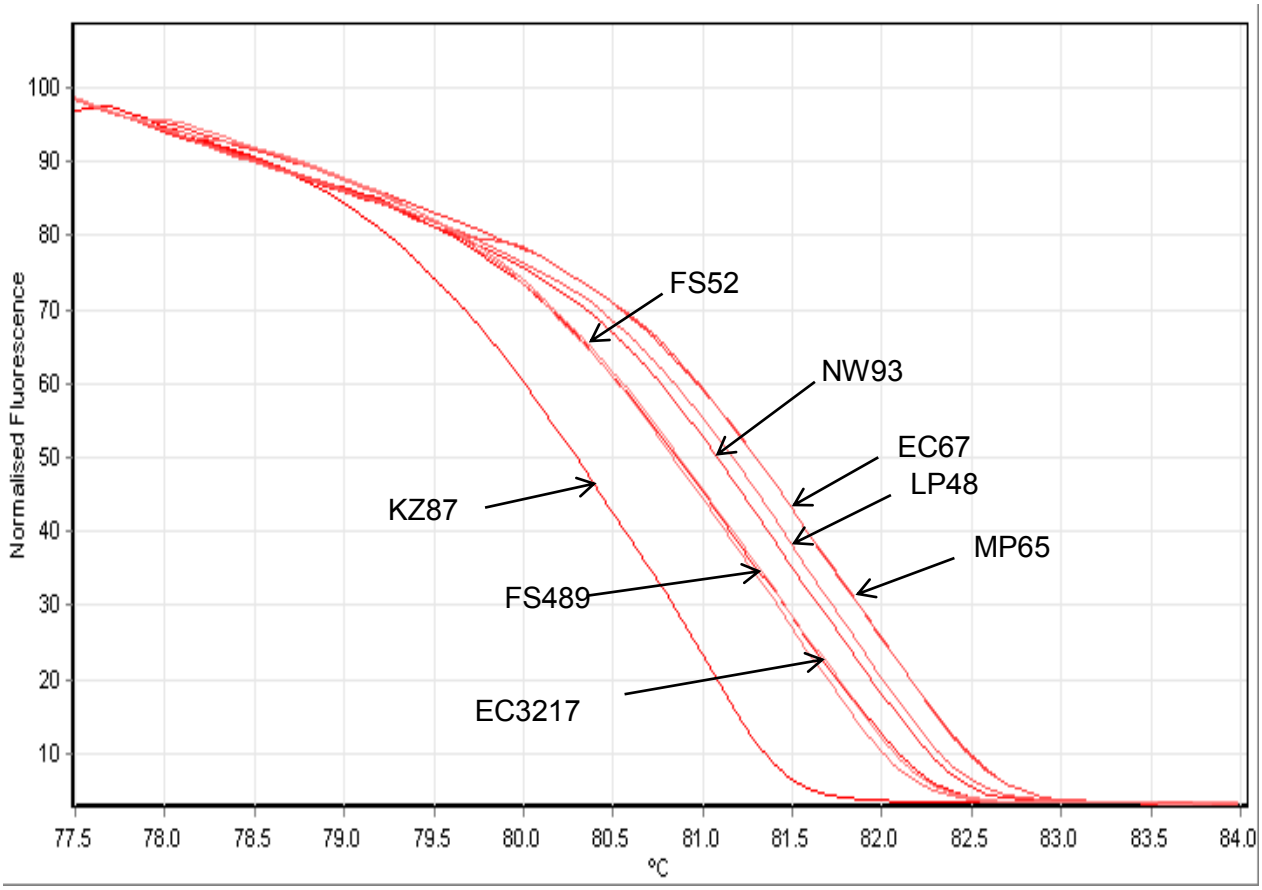

Figure 4.4. The temperature normalised HRM curve analysis of sorghum genotypes using SSR primer, Xtxp335.

The genotype FS489 was used as a reference genotype in the difference plot analysis with the threshold confidence level set at 90%. The results are presented in Table 4.4 and are similar to the normalised melting curves (Figure 4.4). Accessions FS52, and EC3217 were closely related to the reference genotype, FS489. KZ87 followed by MP65, EC67, LP48 and NW93 accessions were distantly related to all other sorghum accessions. These genotypes can be grouped together and the FS52 and EC3217 can also fall into the same group with the baseline genotype, FS489.

Table 4.4. Similarity of sorghum genotypes from difference plot of HRM analysis using Xtxp 335 primer.

Genotype	KZ87	FS52	NW93	EC67	MP65	LP48	FS489	EC3217
Confidence %	0.21	94.79	33.24	2.03	1.9	14.62	100	94.64

Melting profiles were also created for the two SSR primer pairs, Xtxp 258 and Xtxp 145. Differences were observed among the sorghum genotypes when using Xtxp 258 and Xtxp

145 primer sets although most seemed to have similar melting profiles (Figure 4.5). This suggests that either the marker had been monomorphic for that particular locus and hence incapable of discriminating the melt peaks or may not be possible to see the polymorphism at the given resolution.

Clustering of eight accessions by RAPD and SSR analysis

RAPD

The RAPD markers grouped the sorghum genotypes into two clusters, cluster I and cluster II (Figure 4.5). The first cluster (cluster I) was divided into two groups where KZ87, FS489, and EC3217 were similar with genetic similarity value of 1.00, and EC67 and MP65 were also 100% similar. The second cluster (cluster II) grouped FS52, NW953 and LP48 together. FS52 and NW93 were the same and LP48 varied. LP48 could be a useful candidate as a parent in breeding programmes.

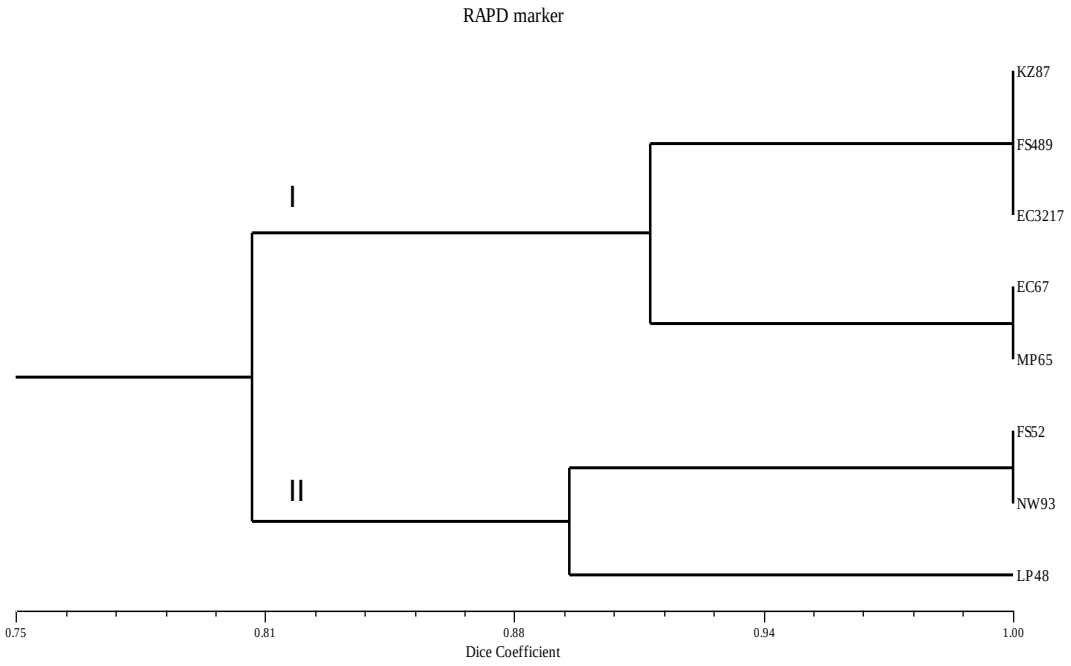

Figure 4.5. Dendrogram constructed based on RAPD marker showing genetic distance and cluster groups among eight sorghum accessions.

Table 4.5. Dice similarity coefficient for RAPD analysis on eight sorghum accessions.

Genotype	KZ87	FS52	NW93	EC67	MP65	LP48	FS489	EC3217
KZ87								
FS52	0.8000	1.0000						
NW93	0.8000	1.0000	1.0000					
EC67	0.9091	0.8889	0.8889	1.0000				
MP65	0.9091	0.8889	0.8889	1.0000	1.0000			
LP48	0.7273	0.8889	0.8889	0.8000	0.8000	1.0000		
FS489	1.0000	0.8000	0.8000	0.9091	0.9091	0.7273	1.0000	
EC3217	1.0000	0.8000	0.8000	0.9091	0.9091	0.7273	1.0000	1.0000

The RAPD analysis showed a matrix with the genetic distances ranging from 0.72 to 1.00 among genotypes under study (Table 4.5). Most of the genotypes are closely related.

SSR

Variation was observed among sorghum genotypes when using SSR markers. Dice similarity matrix was used to cluster accessions using the UPGMA algorithm. The resulting dendrogram revealed two clusters (Figure 4.6). The first cluster grouped FS489 together with FS52 and EC3217 which were closely related. The second cluster was divided into two sub-clusters; where LP48, MP65 and EC67 were the same with a genetic distance of 1.0 and were similar to NW93. Although clustered together, KZ87 was distantly related to NW96, EC67, MP65 and LP48.

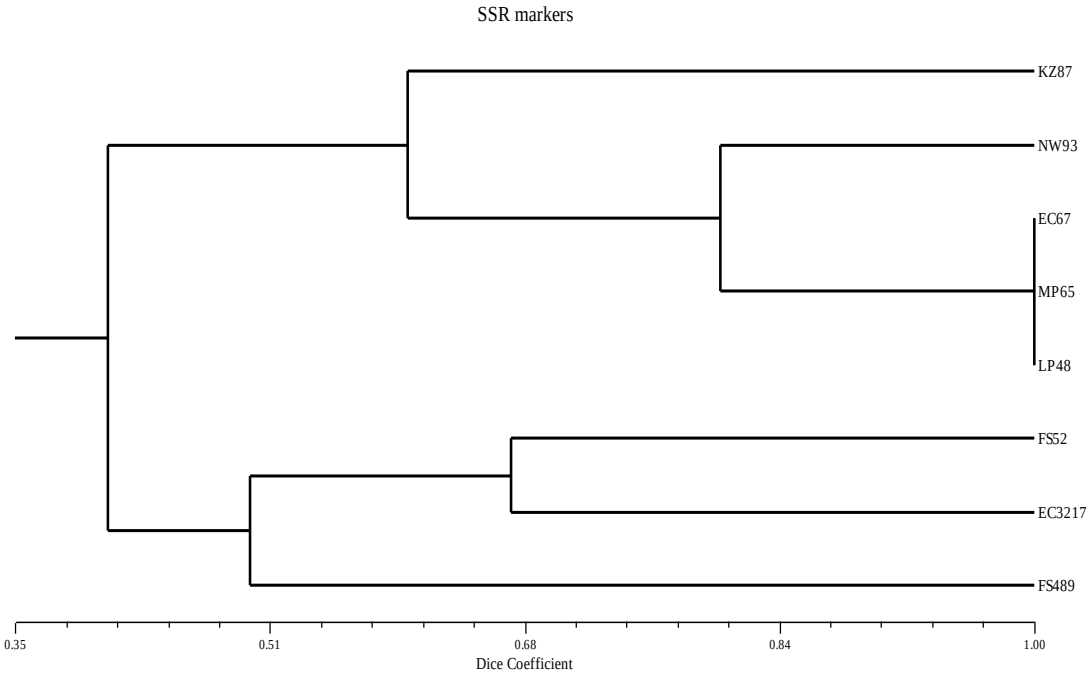

Figure 4.6. Dendrogram constructed based on SSR data, showing genetic distance and cluster groups among eight sorghum accessions.

Table 4.6. Dice similarity coeffiicient for SSR analysis on eight sorghum accessions.

Genotype	KZ87	FS52	NW93	EC67	MP65	LP48	FS489	EC3217
KZ87	1.0000							
FS52	0.3333	1.0000						
NW93	0.4000	0.0000	1.0000					
EC67	0.6667	0.3333	0.8000	1.0000				
MP65	0.6667	0.3333	0.8000	1.0000	1.0000			
LP48	0.6667	0.3333	0.8000	1.0000	1.0000	1.0000		
FS489	0.3333	0.3333	0.4000	0.3333	0.3333	0.3333	1.0000	
EC3217	0.6667	0.6667	0.4000	0.6667	0.6667	0.6667	0.6667	1.0000

The SSR analysis showed genetic similarity values ranging from 0.33 to 1.00 among the genotypes tested (Table 4.6).

4.4 Discussion

Gel electrophoresis

Variation was observed among the genotypes studied when using SSR and RAPD analyses. In SSR analysis, monomorphic banding pattern observed implied that the primer amplified the same region in the genome. Further, an alternative gel such as acrylamide could be useful due to its good resolution power compared to agarose. The heterozygotes were observed among the genotypes which concurred with the ability of this technique to distinguish among homozygosity and heterozygosity status of the genotypes. In RAPD analysis, a large number of bands were revealed due to the random priming nature and potential confounding effects associated with co-migration with other markers (Tessier et al., 1999). Several studies compared various molecular marker systems in sorghum and the SSR was highly correlated with the morphological markers in contrast to AFLP (Geleta and Labuschagne, 2005). Panwar et al. (2010) compared the efficiency and effectiveness of RAPD and the SSR markers in finger millet. Similar studies for comparison of marker systems were also reported by Agrahama and Tuinstra (2003).

High resolution melt analysis

The HRM analysis showed existence of genetic variation among the sorghum genotypes when using SSR and RAPD primers. The melting profiles and difference plots grouped genotypes for SSR and RAPD similarly. The genotypes denoted in various groups can be selected for crosses as potential parents to undertake selection. Similar studies were performed in sweet cherry where HRM was used together with SSR to distinguish sweet cherry cultivars (Ganopoulos et al., 2011). Olive cultivar genotyping was performed using HRM and SSR (Muleo et al., 2009). Mackay et al. (2008) reported microsatellite high resolution melt analysis as useful tool for variety identification and verification in olive and grapevine plants. The use of HRM in conjunction with RAPD has been reported in parasitological studies thus far (Tulsiani et al. 2010). However, in other studies, the use of the HRM analysis was reported effective and efficient when used with other molecular markers (De Koeyer et al., 2010; Naidoo, 2010). For instance, Hofinger et al. (2009) reported the accuracy and sensitivity of the HRM analysis for predicting wide range of nucleotide polymorphisms in barley.

Clustering of the genotypes

In the RAPD analysis, the genetic distances ranged from 0.72 to 1.00. The results concur with Grenier et al. (2000) who reported diversity range of 0.71 to 0.93. The RAPD marker analysis showed some distinction among the genotypes and most of them were very similar. Ayana et al. (2000) also reported weak differentiation of Ethiopian and Eritrean sorghum accessions. The results of the RAPD clustering appear to correspond fairly with the melting profiles of the HRM analysis (Figure 4.2). On the other hand, the SSR analysis showed genetic similarity values ranging from 0.33 to 1.00. When comparing SSR and RAPD analysis, similarity matrices constructed based on shared allele analysis revealed the lowest average genetic similarity between genotypes when estimated using SSR markers (0.33) and was higher among entries when determined using RAPD markers (0.72). These results indicated that RAPD markers provide less resolving power than SSR markers.

The accessions that are distantly related can be selected and used for crossings as parents for use in a breeding programme, estimation of genetic advance as well as further improvement of sorghum cultivars. The results appear to show a wide diversity among the sorghum genotypes. Dje *et al*. (2000) estimated a wide genetic diversity when analysing 25 sorghum landraces derived from a restricted area of North Western Morocco with three SSR markers.

4.5 Conclusions

The SSR and RAPD markers as well as HRM analysis revealed genetic variation among the sorghum genotypes. The HRM was useful in detecting variation among the sorghum genotypes using melting profiles and corresponded well with the SSR clustering of sorghum genotypes. Hence, the HRM can be useful for genetic diversity analysis and assigning sorghum genotypes into heterotic groupings without post-PCR analysis or processing. It can be useful in accelerating selections in plant breeding programmes as is timely, efficient and effective and can be extended with other molecular marker techniques.

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CHAPTER 5

A genetic diversity analysis of South African sorghum genotypes using SSR markers

5.1 Abstract

Diverse landraces of sorghum are widely grown by small-holder farmers in South Africa. The objective of the study was to assess the genetic diversity present in the South African sorghum genotypes using genetic distances as measured by SSR markers. In total 103 diverse landraces and breeding lines were genotyped using 30 SSR primers. A wide genetic diversity was observed with the allele sizes ranging from 90 to 294 bp. The numbers of alleles ranged from 2 to 15 with an average of 6.4 per locus. The polymorphic information content ranged from 0.0192 to 0.8351 (average of 0.5031) with heterozygosity values of 0.0194 to 0.8524 (average 0.5483). The Euclidian genetic distances varied from 0 to 8.4 with average of 5.67. Genotypes Macia-SA and AS4 had the lowest dissimilarity index, whereas 05-POTCH-115 and MP2048 exhibited the highest value. The study established the existence of considerable genetic diversity among South African sorghum germplasm. This may enable breeders to exploit the potential of transgressive segregation and for strategic conservation.

Keywords: genetic diversity, landrace, *Sorghum bicolor,* South Africa, SSR markers

5.2 Introduction

Genetic diversity analysis of sorghum (*Sorghum bicolo*r (L.) Moench; 2*n* = 2x = 20) germplasm is fundamental for breeding and conservation strategies. Genetic advancement during selection depends on the availability of genotypes possessing favourable alleles for desired traits, which relies on the available genetic diversity. Genetic diversity analysis can be carried out using phenotypic or molecular markers. DNA based molecular markers are more efficient to analyse a greater number of genotypes (Reif et al., 2003). Furthermore, molecular markers detect the presence of favourable alleles among germplasm and allow estimation of genetic diversity more reliably and efficiently than phenotypic markers, which are subject to genotype by environment interaction. Summarily, the molecular marker technology aid conventional breeding in various aspects such as to 1) assess genetic diversity and establish heterotic patterns, 2) screen for useful single gene traits, 3) accelerate backcross breeding programs via selection of gene(s) of interest and 4) identify and protect commercial cultivars through fingerprinting (Xiao et al., 1996).

Several DNA based marker systems have been successfully used for assessing genetic diversity in sorghum (Nguni et al., 2011). Simple sequence repeats are reportedly the marker of choice for diversity analysis because of their ability to produce informative multiallelic loci and greater genotypic differentiations. SSRs are highly polymorphic (Anas and Yoshida, 2004) and provide wider genome coverage than amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers. Moreover, compared to single nucleotide polymorphisms (SNPs), SSR markers with moderate density are more informative than SNPs for assessment of genetic diversity in crops (Yang et al., 2011).

The sorghum genome sequence project identified 71 000 SSRs in the genome (Paterson et al., 2009). The availability of this large number of SSR markers provides a more cost-effective and rapid method for DNA profiling (Smith et al., 1997).

Sorghum is one of the most important crops worldwide after wheat, rice, maize, and barley, providing food, fodder and bio-energy feedstock (FAO, 2006). In sub-Saharan Africa sorghum ranks second after maize for preference and importance, and it remains a critical food security crop for the livelihoods of more than 100 million people. Sorghum grows in low-rainfall, arid to semi-arid environments considered to be marginal for other cereal crops such as maize and wheat. It has exceptional tolerance to drought, high temperature stresses and low soil fertility making it the crop of choice by millions of farmers in marginal agro-ecologies.

In South Africa sorghum is grown by both small- and large-scale commercial farmers. Further, sorghum has increasingly become a foundation crop in the food and beverage industries (Wenzel et al., 2001; Taylor, 2003). Several landraces of sorghum are widely grown by small-scale farmers across different provinces in the country. Despite low yielding potential, the landraces are preferred by the small-scale farmers because of broad adaptation, tolerance to abiotic and biotic stresses, and suitable quality and agronomic attributes (DAFF, 2010). In South Africa both wild and domesticated sorghum species and their hybrids are present. Mann et al. (1983) reported that the South African sorghum race 'Kafir' might have arisen from introgression between domesticated and wild sorghum. Sorghum productivity in small-scale producing regions of South Africa could be enhanced through effective breeding using locally adapted and well-characterised germplasm.

Previous genetic diversity studies using the SSR markers from east and southern Africa (Nkongolo and Nsapato, 2003) demonstrated the prevalence of wide genetic variation in sorghum collections. Uptmoor et al. (2003) indicated the existence of high genetic diversity among Southern African sorghum accessions. The authors used 46 sorghum accessions and subjected them to SSR, RAPD and AFLP markers. Of the accessions 23 were landrace collections from four Southern Africa Development Cooperation countries including Botswana, Lesotho, Malawi, and South Africa. Only five of the landraces were sampled from the RSA each representing the North West, Mpumalanga, Gauteng, KwaZulu-Natal, or Limpopo provinces. The remaining 23 accessions in this study were modern breeding lines acquired from Lesotho, Zimbabwe, Zambia, South Africa and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The previous reports did not fully cover the landraces collected from various provinces in South Africa. An exhaustive assessment of the genetic diversity present in the South African sorghum germplasm is required using relatively greater number of samples, representing the diverse sorghum growing provinces with suitable and sufficient number of SSR markers. This may provide adequate information on the genetic relationship among the sorghum germplasm originating from South Africa for effective use of national sorghum genetic resources and future conservation strategies. Thus, the objective of the study was to assess the genetic diversity present among 103 South African sorghum genotypes using genetic distances as measured by 30 selected SSR markers.

5.3 Materials and methods

5.3.1 Plant materials and study sites

The study used 103 sorghum genotypes obtained from the Department of Agriculture, Forestry and Fisheries (DAFF), the African Centre for Crop Improvement (ACCI) and Agricultural Research Council-Grain Crops Institute (ARC-GCI) in South Africa (Table 5.1). Sixty nine genotypes from the DAFF were landraces collected from North West, Eastern Cape, Mpumalanga, Limpopo, KwaZulu-Natal, and Free State Provinces in South Africa. Twelve genotypes were obtained from the ARC-GCI and twenty two were from the ACCI. For DNA sampling, the genotypes were planted in the field under the alpha lattice design replicated twice at Ukulinga (29°37′S, 30°22 E), the Research Farm of the University of KwaZulu-Natal. Recommended cultural practices were followed to grow healthy and vigorous sorghum plants.

Table 5.1. List of sorghum genotypes used in the study[a].

No.	Genotype		Source/origin	No.	Genotype		Source/origin
	Code	name/pedigree			Code	name/pedigree	
1	SA-1	5405	North-West	66	SA-66	4952	Free State
2	SA-2	5451	North-West	67	SA-67	4909	Free State
3	SA-3	5333	North-West	68	SA-68	4905	Free State
4	SA-4	5464	North-West	69	SA-69	4891	Free State
5	SA-5	5436	North-West	70	SA-70	Motlerane	ARC-GCI
6	SA-6	5454	North-West	71	SA-71	Mammopane	ARC-GCI
7	SA-7	5430	North-West	72	SA-72	Macia-SA	ARC-GCI
8	SA-8	5393	North-West	73	SA-73	M153	ARC-GCI
9	SA-9	5337	North-West	74	SA-74	05 Potch-151	ARC-GCI
10	SA-10	2167	Eastern Cape	75	SA-75	Maseka-a-swere	ARC-GCI
11	SA-11	3416	Eastern Cape	76	SA-76	Mammolokwane	ARC-GCI
12	SA-12	3414	Eastern Cape	77	AR-2	05 Potch-138	ARC-GCI
13	SA-13	3262	Eastern Cape	78	AR-3	M48	ARC-GCI
14	SA-14	3319	Eastern Cape	79	AR-4	05 Potch-115	ARC-GCI
15	SA-15	2922	Eastern Cape	80	AR-5	05 Potch-167	ARC-GCI
16	SA-16	3364	Eastern Cape	81	AR-6	Manthate	ARC-GCI
17	SA-17	3403	Eastern Cape	82	AR-7	AS 82	ACCI
18	SA-18	2975	Eastern Cape	83	AR-8	AS13	ACCI
19	SA-19	3184	Eastern Cape	84	AR-9	AS 1	ACCI
20	SA-20	2934	Eastern Cape	85	AR-10	AS 4	ACCI
21	SA-21	2985	Eastern Cape	86	AR-11	AS 18	ACCI
22	SA-22	3217	Eastern Cape	87	AR-12	AS 19	ACCI
23	SA-23	4154	Mpumalanga	88	AC-1	AS 17	ACCI
24	SA-24	4276	Mpumalanga	89	AC-2	AS 16	ACCI
25	SA-25	5476	Mpumalanga	90	AC-3	AS 16 Cycl	ACCI
26	SA-26	4265	Mpumalanga	91	AC-4	AS 11	ACCI
27	SA-27	4052	Mpumalanga	92	AC-5	AS 6	ACCI
28	SA-28	2055	Mpumalanga	93	AC-6	AS 1 M2 Ctrl	ACCI
29	SA-29	5518	Mpumalanga	94	AC-7	AS 16 M2 Ctrl	ACCI
30	SA-30	4259	Mpumalanga	95	AC-8	AS 21	ACCI
31	SA-31	5502	Mpumalanga	96	AC-9	AS 8	ACCI
32	SA-32	5541	Mpumalanga	97	AC-10	AS 2	ACCI
33	SA-33	1990	Mpumalanga	98	AC-11	N13 Striga resistant	ACCI
34	SA-34	4277	Mpumalanga	99	AC-12	#32 2384443	ACCI
35	SA-35	2048	Mpumalanga	100	AC-13	#14 235929	ACCI
36	SA-36	4161	Mpumalanga	101	AC-14	SRN39 FRAMIDA	ACCI
37	SA-37	1455	Limpopo	102	AC-16	HORMAT	ACCI
38	SA-38	1450	Limpopo	103	AC-18	#21237289	ACCI
39	SA-39	4312	Limpopo				
40	SA-40	1948	Limpopo				
41	SA-41	1390	Limpopo				
42	SA-42	4441	Limpopo				
43	SA-43	3132	Limpopo				
44	SA-44	4442	Limpopo				
45	SA-45	1413	Limpopo				
46	SA-46	1394	Limpopo				
47	SA-47	1481	Limpopo				
48	SA-48	1473	Limpopo				
49	SA-49	4303	Limpopo				
50	SA-50	4311	Limpopo				
51	SA-51	5088	KwaZulu-Natal				
52	SA-52	5287	KwaZulu-Natal				
53	SA-53	5233	KwaZulu-Natal				
54	SA-54	5258	KwaZulu-Natal				
55	SA-55	AS66	ACCI				
56	SA-56	5237	KwaZulu-Natal				
57	SA-57	4722	KwaZulu-Natal				
58	SA-58	5246	KwaZulu-Natal				
59	SA-59	4606	KwaZulu-Natal				
60	SA-60	4531	KwaZulu-Natal				
61	SA-61	5274	KwaZulu-Natal				
62	SA-62	5281	KwaZulu-Natal				
63	SA-63	4547	KwaZulu-Natal				
64	SA-64	5097	KwaZulu-Natal				
65	SA-65	5245	KwaZulu-Natal				

[a] North West, Eastern Cape, Mpumalanga, Limpopo, KwaZulu-Natal, and Free State are administrative provinces in South Africa; ARC-GCI, Agricultural Research Council-Grain Crops Institute; ACCI-African Centre for Crop Improvement.

5.3.2 DNA extraction, purification and quantification

Young fresh leaves were harvested from ten plants of each genotype four weeks after planting. The leaf samples were bulked per genotype and placed in a 1.5 ml eppendorf tubes containing silica gel (Rogstad, 2003). Samples were sent to the Biosciences east and central Africa (BecA) of the International Livestock Research Institute (ILRI), Kenya for SSR analysis. Accordingly, the DNA was extracted using a solvent extraction method and the quality of the extracted DNA was evaluated on a 1% agarose gel.

5.3.3 PCR and SSR analysis

PCR reactions were performed using GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). The DNA concentrations of sorghum samples were diluted to a final concentration of 50 ng/µl TE buffer. Thirty SSR primers provided by the Generation Challenge Program-Genotyping Support Service were used for analysis of reactions (Table 5.2). The markers used in this study were selected from the SSR kit (http://sat.cirad.fr/sat/sorghum_SSR_kit) from all the linkage groups of sorghum (Table 5.2). Genotyping was conducted at BecA-ILRI/Kenya using ABI-3730 genetic analyser (Applied Biosystems).

Data analysis

Data were captured using the Genscan®software (Applied Biosystems) and the resulting fragments were analyzed and the alleles scored using the GeneMapper®software version 4.1 (Applied Biosystems). PCR was done for all the 30 primers (Table 5.2). A dissimilarity matrix was generated using DARwin 5.0 software (Perrier and Jacquemoud-Collect, 2006). The data matrices of the genetic distances were used to create the dendrogram using the unweighted pair group method with arithmetic mean (UPGMA) algorithym. The assay efficiency index referred to as polymorphism information content (PIC) was calculated using the following: PIC = 1-∑fi, where fi is the frequency of the ith allele (Smith *et al*. 1997).

Table 5.2. Descriptions of thirty SSR markers used in the study.

No.	Marker	Chromosome	Motif	Forward primer	Reverse primer	Annealing Tm	Min allele	Max allele
1	gpsb067	8	(GT)10	TAGTCCATACACCTTTCA	TCTCTCACACACATTCTTC	49	160	190
2	gpsb123	8	(CA)7+(GA)5	ATAGATGTTGACGAAGCA	GTGGTATGGGACTGGA	50	284	304
3	Isep0107	3	(TGG)4	GCCGTAACAGAGAAGGATGG	TTTCCGCTACCTCAAAAACC	59	199	206
4	Isep0310	2	(CCAAT)4	TGCCTTGTGCCTTGTTTATCT	GGATCGATGCCTATCTCGTC	60	159	214
5	mSbCIR223	2	(AC)6	CGTTCCAATGACTTTTCTTC	GCCAATGTGGTGTGATAAAT	55	104	124
6	mSbCIR240	8	(TG)9	GTTCTTGGCCCTACTGAAT	TCACCTGTAACCCTGTCTTC	55	104	180
7	mSbCIR246	5	(CA)7+(GA)5	TTTTGTTGCACTTTTGAGC	GATGATAGCGACCACAAATC	55	86	114
8	mSbCIR248	10	(GT)7	GTTGGTCAGTGGTGGATAAA	ACTCCCATGTGCTGAATCT	56	81	121
9	mSbCIR262	7	(CATG)3	GCACCAAAATCAGCGTCT	CCATTTACCCGTGGATTAGT	57	208	446
10	mSbCIR276	3	(AC)9	CCCCAATCTAACTATTTGGT	GAGGCTGAGATGCTCTGT	53	222	252
11	mSbCIR283	7	(CT)8(GT)8	TCCCTTCTGAGCTTGTAAAT	CAAGTCACTACCAAATGCAC	54	111	157
12	mSbCIR286	1	(AC)9	GCTTCTATACTCCCCTCCAC	TTTATGGTAGGATGCTCTGC	55	110	150
13	mSbCIR300	5	(GT)9	TTGAGAGCGGCGAGGTAA	AAAAGCCCAAGTCTCAGTGCTA	61	74	118
14	mSbCIR306	1	(GT)7	ATACTCTCGTACTCGGCTCA	GCCACTCTTTACTTTTCTTCTG	55	118	126
15	mSbCIR329	10	(AC)8	GCAGAACATCACTCAAAGAA	TACCTAAGGCAGGGATTG	54	73	121
16	SbAGB02	5	(AG)35	CTCTGATATGTCGTTGTGCT	ATAGAGAGGATAGCTTATAGCTCA	55	96	160
17	Xcup02	6	(GCA)6	GACGCAGCTTTGCTCCTATC	GTCCAACCAACCCACGTATC	54	189	207
18	Xcup53	1	(TTTA)5	GCAGGAGTATAGGCAGAGGC	CGACATGACAAGCTCAAACG	54	182	198
19	Xgap206	6	(AC)13+(AG)20	ATTCATCATCCTCATCCTCGTAGAA	AAAAACCAACCCGACCCACTC	55	100	162
20	Xgap72	9	(AG)16	TGCCACCACTCTGGAAAAGGCTA	CTGAGGACTGCCCCAAATGTAGG	55	175	211
21	Xgap84	2	(AG)14	CGCTCTCGGGATGAATGA	TAACGGACCACTAACAAATGATT	55	171	227
22	Xtxp012	4	(CT)22	AGATCTGGCGGCAACG	AGTCACCCATCGATCATC	55	143	213
23	Xtxp015	10	(TC)16	CACAAACACTAGTGCCTTATC	CATAGACACCTAGGCCATC	55	199	235
24	Xtxp021	4	(AG)18	GAGCTGCCATAGATTTGGTCG	ACCTCGTCCCACCTTTGTTG	60	163	223
25	Xtxp057	9	(GT)21	GGAACTTTTGACGGGTAGTGC	CGATCGTGATGTCCCAATC	55	213	285
26	Xtxp136	10	(GCA)5	GCGAATAGCATCTTACAACA	ACTGATCATTGGCAGGAC	55	240	246
27	Xtxp141	7	(GA)23	TGTATGGCCTAGCTTATCT	CAACAAGCCAACCTAAA	55	127	175
28	Xtxp265	9	(GAA)19	GTCTACAGGCGTGCAAATAAAA	TTACCATGCTACCCCTAAAAGTGG	55	168	246
29	Xtxp320	1	(AAG)20	TAAACTAGACCATATACTGCCATGA TAA	GTGCAAATAAGGGCTAGAGTGTT	54	251	329
30	Xtxp321	8	(GT)4+(AT)6+(CT)21	TAACCCAAGCCTGAGCATAAGA	CCCATTCACACATGAGACGAG	55	182	240

5.4 Results and discussion

5.4.1 Polymorphism and allelic diversity of SSR markers

The number of alleles and size ranges, heterozygosity, and PIC values are summarised in Table 5.3. The SSR markers generated a total of 306 putative alleles (different fragment sizes) among the 103 sorghum genotypes. Most of the markers generated 2-7 alleles but nine markers generated 9-15 alleles each. The current analysis found heterozygosity values ranging from 0.02 to 0.85 with an average of 0.55.Marker Xisep0310 had the lowest, and Xtxp320 the highest, range of heterozygosity. The PIC values ranged from 0.02 for the marker Xisep0310 to 0.84 for Xtxp320 with an average of 0.50.

Table 5.3. Genetic information generated by thirty SSR markers on 103 sorghum genotypes.

Marker	Allele number	Allele size ranges in base pairs	Heterozygosity	PIC value
gpsb123	5	284.79 - 293.88	0.64	0.58
gpsb067	4	172.53 - 183.07	0.31	0.29
mSbCIR246	4	93.33 - 100.00	0.27	0.25
mSbCIR262	5	214.26 - 224.37	0.59	0.54
Xtxp321	14	187.85 - 223.94	0.77	0.74
Xtxp320	10	253.92 - 283.17	0.85	0.84
Xtxp141	9	143.56 - 165.99	0.82	0.80
Xtxp136	3	236.48 - 239.58	0.60	0.53
Xtxp057	10	234.38 - 263.28	0.70	0.67
Xtxp021	10	159.46 - 196.19	0.60	0.58
Xtxp015	5	175.50 - 203.45	0.52	0.48
Xtxp012	15	162.28 - 210.23	0.75	0.72
Xisep0310	2	175.75 - 203.45	0.02	0.02
Xgap265	10	174.75 - 218.61	0.80	0.78
Xgap84	7	185.01 - 202.98	0.74	0.70
Xgap72	7	182.11 - 192.39	0.54	0.51
Xcup53	4	182.57 - 199.11	0.08	0.07
Xcup02	4	191.41 - 199.92	0.43	0.36
mSbCIR329	4	108.27 - 114.26	0.54	0.44
mSbCIR306	3	119.92 - 124.17	0.52	0.40
mSbCIR300	3	102.16 - 108.60	0.41	0.35
mSbCIR286	7	103.94 - 125.15	0.67	0.62
SbAGB02	11	97.87 - 157.55	0.50	0.48
mSbCIR283	6	115.47 - 145.52	0.50	0.45
mSbCIR276	3	229.26 - 233.20	0.54	0.45
mSbCIR240	6	104.95 - 104.95	0.34	0.32
mSbCIR248	3	90.09 - 100.78	0.65	0.58
mSbCIR223	3	104.55 - 113.09	0.57	0.49
Xgap206	13	105.40 - 147.45	0.74	0.71
Isep107	2	220.82 - 227.15	0.44	0.34
Average	6.4	163.755 - 183.516	0.55	0.50

PIC = Polymorphic information content

5.4.2 Genetic distance and dissimilarity analysis

The Euclidean dissimilarity matrix estimates as measures of genetic distances ranged from 0 to 8.4 with an average value of 5.67 (data not shown). This shows a wide diversity among the sorghum genotypes investigated. Among the 103 genotypes studied, SA-72 and AR-10 had the lowest dissimilarity index (0.00) whereas AR-4 and SA-35 exhibited the highest dissimilarity (8.4). SA-55 (AS66), SA-72 (Macia-SA) and SA-73 (M15) were distantly related to most genotypes tested. AS66 was sourced from ACCI and the other two are breeding

lines from ARC-GCI. Among the landraces, SA-28, SA-42, and SA-59 were the most dissimilar among the tested genotypes. These landraces were sourced from Mpumalanga, Limpopo and KwaZulu-Natal Provinces, respectively. The breeding lines such as AR-2, AR-3, AR-4, AC-1 AC-6, AC-18 and SA-73, displayed high genetic dissimilarity in spite of continued selection of such genotypes that would result in narrow genetic diversity and low allelic richness when compared to the tested landraces (data not shown).

5.4.3 Cluster analysis

The Euclidean dissimilarity matrix was used to cluster genotypes using the Unweighted Pair Group Method with Arithmetic mean algorithm (UPGMA). The resulting dendrogram revealed two major distinct clusters of sorghum genotypes, cluster I with 0.25, and cluster II with 0.48 Euclidian distances (Figure 5.1). The genotypes in cluster I comprised 35 genotypes that formed subgroups Ia and Ib. The genotypes were breeding lines obtained from the ARC-GCI and ACCI except for SA-61, SA-57, SA-37, SA-13 and SA-5. Genotypes in subgroup Ia (SA-61 and AC-9) were distantly related, whereas AC-12 and AC-16 were distinct from the remainder of the genotypes.

Cluster II was composed of two subgroups (IIa and IIb), which consisted of 68 sorghum genotypes mainly landrace collections from the DAFF and 5 breeding lines. Among the the breeding lines, four breeding lines (AR-6, SA-70, SA-71 and SA-75) from the ARC-GCI and line AC-4 from the ACCI were grouped in this cluster. The subgroup IIa consisted of 31 genotypes and subgroup IIb of 37 genotypes SA-47, SA-63, SA-60, SA-40, SA-10, SA-30, SA-31, SA-23, SA-41 and SA-48 were distantly related to the other genotypes. Interestingly, the genotypes in cluster II subgroup IIa were closely related with the widely cultivated South African landraces AR-6 (Manthate), SA-70 (Motlerane), SA-71 (Mammopane) and SA-75 (Maseka-a-swere) sourced from the ARC-GCI.

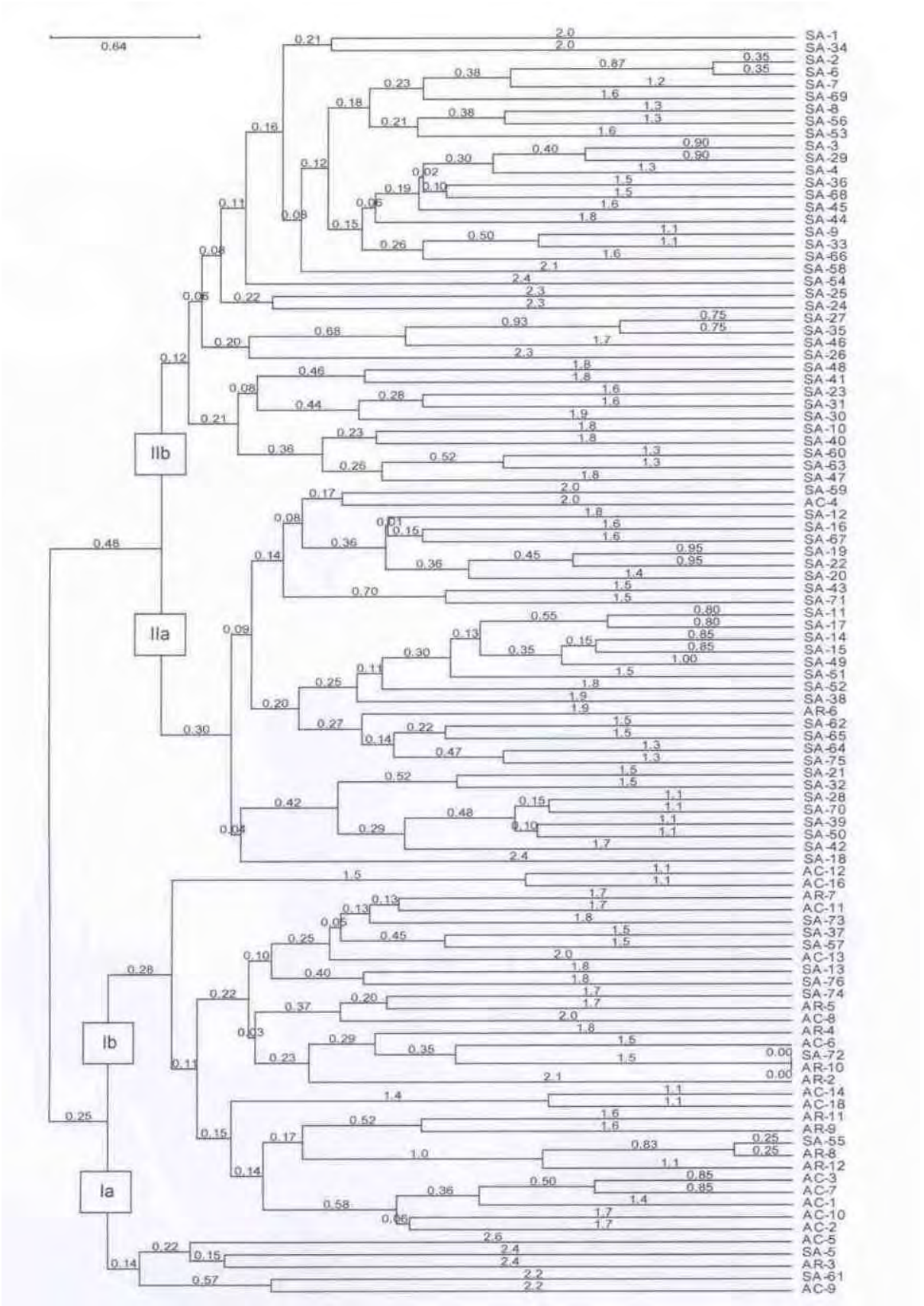

Figure 5.1. Dendrogram revealing genetic relationships among 103 sorghum genotypes from South Africa based on SSR analysis. Euclidian's similarity coefficients and UPGMA clustering. Ia, Ib, IIa, and IIb are sub-groups within the clusters.

5.5 Discussion

In the current study, the average number of alleles was 6.4 per locus, which was similar to 6.5 alleles per locus reported by Reedy et al. (2010) but higher than 4.9 alleles per locus reported by Pei et al. (2010). In sorghum genetic diversity studies a greater number of alleles (27) were reported by Muraya et al. (2011) and 7.6 by Wang et al. (2009). Greater number of alleles generated by SSR markers suggests allelic richness, a useful indicator of worthiness for subsequent selection and conservation strategies. The average heterozygosity observed in this study was fairly similar to that previously reported by Uptmoor et al. (2003) at 0.60 in Southern Africa, who used 25 SSR markers and compared 46 sorghum accessions of diverse geographical collections. Low levels of heterozygosity (0.04) were recorded among Zambian sorghum genotypes (Nguni et al. 2011) and a comparatively high value (0.8) was reported by Thudi and Frakrudin (2011) among rabi sorghum genotypes. The higher value of allelic diversity of SSR loci found in this study was probably associated with the wide ranged of genetic diversity represented in the germplasm of South African sorghum tested. Increased levels of heterozygosity indicate significantly greater proportion of genetic diversity, which will enhance selection response in breeding programs. The high level of heterozygosity observed among the genotypes signified the fact that genotypes used in this study were collected from a wide range of geographic areas with different levels of selection pressure. Increased allelic number in the present study is probably attributed to significant genetic variation among the sampled sorghum gene pool. Farmers maintain a large number of landraces on a single plot to cope with the diverse environmental conditions, resulting in a continuous exchange of genes through pollen flow (Manzelli et al., 2007; Baurnard et al., 2008). In addition, farmers exchange seeds through gifts and via markets to renew old seed stocks or to acquire new varieties. Consequently, there may be a continuous exchange of genes among the genotypes.

The SSR markers revealed marked genetic diversity among the sorghum genotypes. The PIC values ranged from 0.02 for the marker Xisep0310 to 0.84 for Xtxp320 with an average of 0.50. These estimates were fairly similar to the mean PIC ranges obtained in other sorghum genetic studies by Ali et al. (2008) and Geleta et al. (2006). In sorghum genetic diversity studies, Thudi and Fakrudin (2011) reported a greater mean PIC value than the present estimates. Assar et al. (2005) found PIC values ranging from 0.46 to 0.87 among sorghum genotypes evaluated using 16 SSR markers. High PIC values suggest their informative potential to detect differences among the sorghum lines.

Reedy et al. (2010) reported genetic dissimilarity estimates ranging from 0.384 to 0.728 with an average dissimilarity value of 0.54 among 14 sorghum accessions studied. Kumar and Khanna (2009) reported genetic similarity values ranging from 0.261 to 0.762 among 10 diverse cultivated sorghum genotypes. The genetic dissimilarity estimates (genetic distances) assist in selection for parental lines and creation of a segregating population in order to maintain genetic diversity in crop breeding programmes. Genetic estimates are useful when assigning genotypes to heterotic groups in hybrid development from different intergroup crosses (Xiao et al., 1996). Dje et al. (2000) estimated a wide genetic diversity when analysing 25 sorghum landraces assembled from a limited area of north-western Morocco using SSR markers. SSR markers also revealed wide genetic diversity among sorghum collections in Zambia. The wide genetic diversity is important in breeding programmes for selections and inclusion of landrace/genotypes with genes of novelty (Nguni et al. 2011).

Some of the genotypes were closely related with the widely cultivated South African landraces. This may suggest that the genotypes are genetically related as in the other four widely cultivated landraces obtained from the ARC-GCI. Farmers practice mass selection, i.e. healthy and large panicles are selected based on appearance, not considering the genetic purity every year, and this seed is used in the following planting season. According to Harlan (1975), such practices exert a particular balance of selection pressure and allow for genetic variability within populations.

In general, the genotypes used in the study did not appear in the same cluster based on the source or area of collection. This suggests wide genetic variation among the germplasm. According to Barnaud et al. (2007) farmers' practices and historical factors affect patterns of genetic diversity. Despite the gene flow, farmers' practices are key to maintenance of genetic diversity of landraces with related agronomic traits and agroecologies. For instance, Tukeswa et al. (2000) reported specific selection pressure towards the brewing trait in sorghum selected by farmers. Similar results were reported in Somalia where farmers selected and preserved landraces on the basis of the phenotypic and agronomic traits (Manzelli et al., 2007). Ganesamurthy et al. (2010) reported absence of relationship between the geographic and the genetic diversity, and concluded that the geographic location could be used as a diversity index for selection.

Nguni et al. (2011) reported that some of the sorghum genotypes from the same area of collection were grouped in different clusters, although most of them were grouped according to the area of collection. Several studies were conducted based on geographical location, either at country, regional or agroclimatic level (Nkongolo and Nsapato, 2003). The results of the present study concur with the report by Uptmoor et al. (2003) who also found the clustering of genotypes studied did not agree based on the diverse origins of the sorghum genotypes. Breeders use different methods in selecting the best parents for making crosses and for assigning lines to a particular genetic group. These methods include (1) phenotypic performance for specific traits, (2) pedigree relationships, (3) adaptability and yield stability, (4) top crosses and (5) diallel crosses. Genetic distance estimates from molecular markers have been reported as very useful tools in selecting the best parent combination for new pedigree and for assigning lines into genetic groups (Reif et al., 2003; Ali et al., 2008; Paterson et al., 2009). With complementary phenotypic traits, the genotypes showing distant genetic relationship across clusters and subclusters should be strategically selected as parental lines for subsequent crosses for genetic recombination and to improve genetic advancement in sorghum breeding programs. An understanding of genetic diversity among inbred lines can be particularly useful in planning crosses, in assigning lines to specific heterotic groups and for precise identification of plant material for strategic conservation (Deu et al., 2006; Geleta et al., 2006; Perumal et al., 2007).

The SSR markers used in the study were sampled from 10 linkage groups of the sorghum genome (Dean et al., 1999), which collectively had moderate to high PIC values that provided information to uniquely identify most of the genotypes profiled in the study. All 30 SSR markers were polymorphic, confirming that each marker would be effective and valuable for genetic analysis. The degree of precision of molecular markers in estimating genetic relatedness between genotypes is strongly dependent on the type and number of markers that are used and their genome coverage (Menz et al., 2004; Geleta et al., 2006; Perumal et al., 2007). The use of SSRs for analysis of population genetic diversity in sorghum could reduce the limitations in identifying polymoprhisms and result in more complete genomic coverage (Perumal et al., 2007).

Given the high level of gene flow among genotypes and high level of variation, farmers' traditional agricultural production system has played a vital role in maintaining and directing genetic diversity and evolution. Case et al. (2005) studied genetic diversity analysis of sorghum using 98 SSR markers and found that local landraces captured 86% of the total variation found in wild species. Similarly, Deu et al. (2006) pointed out the role of farmers in the management and preservation of genetic diversity over time. The high genetic variability

among landraces provide enough genetic plasticity to adapt to the diverse environmental conditions in the tropical areas (Manzelli et al., 2007), and allow circumvention of crop failure by reducing vulnerability to environmental stresses._

5.6 Conclusions

The study examined the genetic diversity present in 103 sorghum genotypes collected from six administrative provinces and two breeding programs in South Africa using 30 polymorphic SSR markers. The SSR markers revealed wide genetic diversity among the sorghum genotypes studied. The analyses formed two major distinct clusters without allocating genotypes based on the source or origin. The results showed clear separation between the breeding lines and landrace collections from diverse provinces in South Africa. Further sub-grouping showed close genetic relationship of genotypes SA-28, SA-42, SA-50, SA-39, SA-28, SA-32, SA-21, SA-42, SA-62, SA-65, SA-64, SA-38, SA-52, SA-51 SA-50, SA-39, SA-43, SA-20, SA-22, SA-19, SA67, SA-16, SA-12, AC-4 and SA-59 with the widely cultivated landraces Motlerane, Manthate, Maseka-a-swere, and Mammopane in South Africa. The distantly related sorghum genotypes, such as SA-23, SA-28, SA-42, SA-44, SA-57 and SA-59, can be useful in introducing genes of novelty into sorghum breeding programmes. Given that the collection provinces are diverse with heterogeneous agroecologies, the selected lines with high genetic diversity could serve as important sources of novel alleles for breeding and genetic conservation. Furthermore, phenotypic evaluations are needed to select suitable agronomic traits associated with the genetic markers for breeding and conservation strategies.

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CHAPTER 6

Genetic diversity among selected South African sorghum genotypes for protein content and amino acid composition

6.1 Abstract

Malnutrition is a challenge in developing and underdeveloped countries of the world. Sorghum is an important food security crop in sub-Saharan Africa providing food and feed. However, sorghum has inadequate and variable protein content and levels of essential amino acids required for balanced human and animal diets. The objectives of this study were to determine the genetic diversity present among selected South African sorghum genotypes for protein and amino acid content and to select candidate lines for breeding or direct production. Fifty nine selected South African sorghum genotypes grown at two localities (Makhathini and Ukulinga) were analysed for crude protein content using near-infrared spectroscopy (NIR). Nineteen genotypes with high crude protein content from each location were selected and analysed for amino acid profiles using protein hydrolysates. The crude protein content of the genotypes varied from 7.69 to 16.18% across the two sites with a mean of 13.07%. The genotypes that had high crude protein content at both sites were Mammopane (16.18%), AS16 M1 (15.57%), Macia-SA (15.31%), AS19 (15.22%), Maseka-a-swere (15.13%), and AS4 (15.07%). The genotypes identified with superior leucine content were LP 1948 at 14.3%, FS 4905 (14.3%), MP 4154 (14.26%) and LP 1481 (14.25%). High lysine content was detected in the genotypes KZ 5246 at 2.27%, AS17 (2.25%), Manthate (2.16%) and LP 1481 (2.11%). The genotype AS16cyc was the best candidate for high phenylananine content at 5.99%. Manthate and Maseka-a-swere were best candidates for high protein values and good amino acid compositions. Overall, the studied lines had great variability in their protein and amino acid profiles. Low levels of cysteine, lysine, methionine, and histidine were measured. The presence of genetic diversity is essential for quality improvement to achieve balanced protein and amino acid levels in sorghum.

Keywords: amino acids, genetic diversity, protein content, sorghum

6.2 Introduction

Food security and malnutrition are major challenges in the world today (FAO, 2010). In South Africa there are great disparities among communities. It is estimated that 14 million people are food insecure and 1.5 million children suffer from malnutrition in South Africa (HSRC, 2004). However, in South Africa there is a coexistence of both under- and over-nutrition across all age groups (Steyn et al., 2006).

Proteins are essential component of the diet needed for humans. About 63% of the world protein consumption is from grains or grain products (Hoveland, 1980). The protein's basic function in nutrition is to supply adequate amounts of required amino acids. These proteins are composed of numerous amino acids of which eight are essential for the human diet. In food plants the protein quality is a measure of the amino acid levels present in a given genotype (Waggle and Deyoe, 1966; Arun et al., 2009). The protein quality or its nutritive value depends on its amino acid content and on the physiological availability of specific amino acids after digestion, absorption and oxidation. Sorghum, the most important food security crop in sub-Saharan Africa, has poor protein digestibility and inadequate levels of some of the essential amino acids such as lysine compared to other cereals (FAO, 1995).

In countries where cereals are staple foods, protein malnutrition is a widespread problem. The low levels of some critical amino acids in African cereals contribute to hunger and malnutrition reported in sub-Saharan Africa (FAO, 2010). Furthermore, one of the challenges of sorghum production under small-scale farming system in South Africa is a lack of varieties that produce stable yields which have adequate protein and amino acid contents. Hence, it is essential to characterize sorghum collections from various provinces within South Africa. Characterization and identification of suitable sorghum genotypes and development of improved cultivars that are more suited to the marginal areas would help in food security and alleviation of malnutrition (Slabbert et al., 2001). Efforts have been made to improve levels of amino acids such as lysine in sorghum via mutation breeding (Singh and Axtell, 1973; Axtell et al., 1979). Monyo *et al*. (1988) reported a hybrid with improved lysine and yield derived from a genotype designated as P-721 Opaque. Other studies have also reported the high lysine sorghum mutants (Oria *et al*., 2000). Genetic engineering has been attempted to improve sorghum protein and amino acid levels (Zhao *et al*., 2002). According to Hoveland (1980) the protein content is influenced by environment and the cultivars used. Sorghum selections with improved lysine have been reported (Singh and Axtell, 1973) but most

released cultivars are still deficient in essential amino acids. Information on protein content and amino acid levels among sorghum landraces are important for growers, millers, end-users and breeders. However, sorghum cultivars grown by subsistence farmers are low yielders and their protein content and amino acid levels are unknown. Hence, it is essential to assess the levels of protein and the essential amino acids present in sorghum cultivars grown by farmers. Cultivars with superior levels of protein and amino acid levels could be used in breeding programmes aimed at improving the nutritional quality of sorghum.

Various methods have been employed to assess levels of proteins and amino acids in crops (Workman and Burns, 2001; Coetzee, 2003). Near-infrared spectroscopy (NIR) is one of the methods used by researchers to assess various quality traits. NIR can be quick, affordable and accurate. It is a non-destructive method for analysing quality traits including protein and amino acids, among others (Brauteseth, 2009). NIR has been used in various studies for determination of protein and other nutritional quality traits (YoungYi et al., 2010; Olesen et al., 2011). Hence, it is an important tool for use in characterization and making selections in plant breeding programmes.

In other studies, the protein fraction in cereal crops like sorghum was characterized by size exclusion, reverse phase HPLC and SDS–PAGE (Mokrane et al., 2009) and via *in vitro* protein digestibility of the extracted proteins (Mokrane et al., 2006). The methods used for the analysis of amino acids include ion exchange chromatography (Adeyeye, 2010), capillary electrophoresis (Waldhier et al., 2009), anion-exchange chromatography with integrated pulsed amperometric (IPA) detection equipped with a gold electrode (Rombouts et al., 2009) and high performance liquid chromatography (HLPC) (Ilisz et al., 2008), among others. Liquid chromatography-mass spectrometry (LC-MS) is the most widely used analytical technique for amino acids in food sources. The technique is effective and efficient for analysis of amino acids in food crops. It is fast with high throughput and provides precision and accuracy without requiring antibodies for the quantification of peptides. It also allows structurally and chemically similar peptides and proteins to be differentiated (Ewles et al., 2010; Ewles and Goodwin, 2011; Nowatzke et al., 2011). Developments in chromatographic methodology have reduced sample and reagent requirements and improved identification, resolution, and sensitivity of amino acid analyses of food samples (Peace and Gilani, 2005). The objectives of this study were to determine the genetic diversity present among selected South African sorghum genotypes, in particular to assess their protein and amino acid composition and to select candidate lines for breeding or direct production.

6.3 Materials and methods

6.3.1 Plant materials and growing environments

Fifty nine sorghum genotypes were selected and grown at Ukulinga Research Farm (29.67'S and 30.14"E, 812 m.a.s.l) of the University of KwaZulu-Natal, and the Makhathini Research Station (27º 24' S and 32º 11' 48"E, 697 m.a.s.l) of the Agricultural Research Council. A list of the sorghum genotypes used in the study is presented in Table 6.1. The studies were conducted in the 2011/2012 growing season and March to August 2012.

6.3.2 Analysis of crude protein

Crude protein content was analysed using Near-Infrared Spectroscopy (NIR) (VISION, 2008) using a FOSS NIR machine, NIRSystems Composite Monochomator 6500, (FOSS NIRSystems Inc., 7703 Montpelier Rd, Laurel, MD, USA) at the Department of Plant Pathology, University of KwaZulu-Natal. About 10 g of sorghum grains of each sample from the two locations, i.e., Makhathini and Ukulinga, were placed in a sample cup that was used for scanning of the whole seeds for analysis of crude protein. The whole grains were scanned, then put into envelopes and were shaken for 5 seconds before re-scanning. The grains were scanned in triplicates. The sorghum genotypes analysed for crude protein are indicated in Table 6.1

6.3.3 Analysis of amino acids

Nineteen sorghum genotypes that showed high protein content were selected for analysis of amino acids. The amino acids were analysed at the Central Analytic Facility, University of Stellenbosch, South Africa. The sorghum samples were first hydrolysed according to the AOAC (2003) method. About 0.1 g of samples were weighed using vibrator apparatus. A 6 ml of 6N HCl and 15% phenol were added into the sample inside the hydrolysis tubes.

Table 6.1. A list of sorghum genotypes used in the study.

Serial Number	Genotype	Source/place of collection	Serial Number	Genotype	Source/place of collection
1	Mammopane	ARC	31	FS4891	Free State
2	NW5436	North West	32	KZ5246	KwaZulu-Natal
3	EC3414	Eastern Cape	33	LP1390	Limpopo
4	EC3217	Eastern Cape	34	KZ5233	KwaZulu-Natal
5	AS16 cyc	ACCI	35	KZ5245	KwaZulu-Natal
6	05-POTCH-115	ARC	36	EC3416	Eastern Cape
7	EC3319	Eastern Cape	37	NW5454	North West
8	LP4442	Limpopo	38	05-Potch-151	ARC
9	MP4265	Mpumalanga	39	MP4277	Mpumalanga
10	EC3364	Eastern Cape	40	NW5393	North West
11	EC3403	Eastern Cape	41	MP1990	Mpumalanga
12	AS11	ACCI	42	Maseka-a-swere	ARC
13	AS21	ACCI	43	Macia-SA	ARC
14	Mamolokwane	ARC	44	MP4259	Mpumalanga
15	KZ5287	KwaZulu-Natal	45	Manthate	ARC
16	M153	ARC	46	LP1413	Limpopo
17	LP4303	Limpopo	47	EC2985	Eastern Cape
18	EC3184	Eastern Cape	48	FS4905	Free State
19	MP4276	Mpumalanga	49	MP4154	Mpumalanga
20	AS16 M1	ACCI	50	LP1481	Limpopo
21	AS2	ACCI	51	05-Potch-167	ARC
22	AS16 M2	ACCI	52	MP2048	Mpumalanga
23	AS4	ACCI	53	KZ5088	KwaZulu-Natal
24	KZ5281	KwaZulu-Natal	54	NW5337	North West
25	MOTLERANE	ARC	55	NW5333	North West
26	LP1948	Limpopo	56	AS17	ACCI
27	AS19	ACCI	57	FS4909	Free State
28	AS1	ACCI	58	KZ5237	KwaZulu-Natal
29	KZ5258	KwaZulu-Natal	59	LP1473	Limpopo
30	NW5430	North West			

The hydrolysis tubes made of glass were sealed following the standard procedure for sample vacuum hydrolysis according to the manufacturer's instructions, Thermo Scientific. The hydrolysis tubes were placed inside glass beakers and put in an oven at a temperature of 110^{o}C. After 24 hours, these were removed from the oven and allowed to cool to room temperature. The vials were transferred into two 2ml Eppendorf tubes and the remainder of each sample was discarded. One eppi was used for analysis of amino acids in the Liquid

Chromatography Mass Spectroscope. The other eppi was stored at -20^{o}C. The eppi samples were subjected to the Water AccQ Tag Ultra Derivitization Kit (Waters Corporation, MA, USA). A 10 µl of undiluted sample was added to the Waters AccQ Tag Kit constituents and placed in a heating block at a temperature of 55^{o}C for ten minutes. The column was an AccQ Tag C18, 1.7 µm, 2.1 x 100 mm, and sample injection was of 1 µl with the ESI + source. The solvents, Eluent A2 contained 100 ml Eluent A concentrate and 900 ml water and Eluent B was supplied in the AccQ Tag Kit. The samples were run with the capillary voltage of 3.5 kilo volts (kV) and core voltage of 15 volts (V) at 120^{o}C. The desolvation temperature, desolvation gas and core gas were 350^{o}C, 350Lh^{-1} and 50Lh^{-1}, respectively. The list of amino acids analysed is shown in Table 6.2.

Table 6.2. Full names of the amino acids and abbreviations.

Amino acid	Abbreviation	Amino acid	Abbreviation
Histidine	His	Lysine	Lys
Serine	Ser	Tyrosine	Tyr
Arginine	Arg	Methionine	Met
Glycine	Gly	Valine	Val
Aspartic acid	Asp	Isoleucine	Ile
Glutamic acid	Glu	Leucine	Leu
Threonine	Thr	Phenylalanine	Phe
Alanine	Ala		
Proline	Pro		
Cysteine	Cys		

6.3.4 Data analysis

The spectral data of the scanned sorghum samples were entered into VISION software (VISION, 2008). The data was further analysed using Unscrambler software version 3.0 (Esbesen, 1994). The model used for protein predictions was adapted from Brauteseth (2009) for sorghum protein. The protein content and amino acid profiles of the genotypes were compared using the analysis of variance of GenStat 14th edition computer package (Payne et al., 2011).

6.4 Results

6.4.1 Protein content

Results of crude protein content of the 59 sorghum genotypes across the two sites, Makhathini and Ukulinga are presented in Table 6.3. The protein content of sorghum

lines at Makhathini ranged from 5.50 to 16.95% with a mean of 12.78% (Table 6.3). There was marked variation among the sorghum genotypes where MP4259 (16.18%), Manthate (16.47%), Mammopane (16.5%), Macia-SA (16.65%) and MP4154 (16.95%). had the highest crude protein content. The genotypes KZ 5233 (5.55%), EC3416 (8.84%) and MP4265 (8.92%) had the lowest crude protein contents.

At Ukulinga, the sorghum genotypes exhibited crude protein ranging from 8.92 to 16.81% with a mean of 13.37% (Table 6.3). Genotypes that had high protein content were 05-POTCH-115, AS1, AS16 M1 at 16.06%, 16.15% and 16.81%, respectively. Genotypes LP1390, MP4259, KZ5233 had the lowest crude protein content of 8.92%, 9.75% and 9.83%, respectively.

Overall, there was higher degree of variability among the sorghum genotypes when tested at Makhathini than Ukulinga (Table 6.3). The crude protein content ranged from 7.69 to 16.18% averaged across the two sites with a grand mean of 13.07%. The genotypes that showed high protein across the two sites were AS4, followed by Maseka-a-swere, AS19, Macia-SA, AS16 M1 and Mammopane at 15.07%, 15.13%, 15.22%, 15.31%, 15.57%, and 16.18%, respectively. The lowest crude protein contents were noted in the genotype KZ 5233 LP 1390 at 7.69% and 9.65%, respectively.

Table 6.3. Protein content (%) of 59 sorghum genotypes when grown at Makhathini and Ukulinga, 2011/2012.

Number	Genotype	Makhathini	Ukulinga	Overall mean
1	Mammopane	16.5	15.85	16.18
2	NW5436	11.28	15.44	13.36
3	EC3414	11.79	11.66	11.73
4	EC3217	14.73	14.12	14.43
5	AS16 cyc	15.15	13.01	14.08
6	05-POTCH-115	12.27	16.06	14.17
7	EC3319	12.29	12.83	12.56
8	LP4442	12.54	12.7	12.62
9	MP4265	8.92	11.81	10.37
10	EC3364	11.99	12.52	12.26
11	EC3403	12.65	13.13	12.89
12	AS11	12.52	14.96	13.74
13	AS21	14.47	12.31	13.39
14	Mamolokwane	12.53	14.28	13.41
15	KZ5287	9.54	12.14	10.84
16	M153	12.72	15.96	14.34
17	LP4303	11.03	13.4	12.22
18	EC3184	11.46	12.44	11.95
19	MP4276	12.43	13.19	12.81
20	AS16 M1	14.32	16.81	15.57
21	AS2	12.25	15.01	13.63
22	AS16 M2	12.73	15.33	14.03
23	AS4	14.24	15.9	15.07
24	KZ5281	11.56	13.49	12.53
25	MOTLERANE	12.88	15.29	14.09
26	LP1948	13.74	11.14	12.44
27	AS19	14.68	15.75	15.22
28	AS1	12.07	16.15	14.11
29	KZ5258	13.53	12.79	13.16
30	NW5430	12.12	14.36	13.24
31	FS4891	12.55	12.6	12.58
32	KZ5246	10.65	11.15	10.90
33	LP1390	9.38	8.92	9.15
34	KZ5233	5.55	9.83	7.69
35	KZ5245	12.5	13.15	12.83
36	EC3416	8.84	14.58	11.71
37	NW5454	12.34	13.03	12.69
38	05-Potch-151	11.58	14.79	13.19
39	MP4277	11.81	12.74	12.28
40	NW5393	10.88	10.8	10.84
41	MP1990	11.73	13.91	12.82
42	Maseka-a-swere	15.88	14.38	15.13

43	Macia-SA	16.65	13.97	15.31
44	MP4259	16.18	9.75	12.97
45	Manthate	16.47	13.19	14.83
46	LP1413	14.94	14.8	14.87
47	EC2985	15.56	14.42	14.99
48	FS4905	13.05	12.07	12.56
49	MP4154	16.95	11.62	14.29
50	LP1481	15.83	12.67	14.25
51	05-Potch-167	12.53	11.7	12.12
52	MP2048	15.8	13.48	14.64
53	KZ5088	13.7	11.36	12.53
54	NW5337	13.73	12.42	13.08
55	NW5333	10.09	10.26	10.18
56	AS17	13.24	13.33	13.29
57	FS4909	11.82	14.96	13.39
58	KZ5237	13.1	14.53	13.82
59	LP1473	9.49	14.77	12.13
	Min	5.55	8.92	7.69
	Max	16.95	16.81	16.18
	Mean	12.78	13.37	13.07
	Variance	4.89	3.14	2.51
	SD	2.21	1.77	1.58
	SE	0.31	0.27	
	F-probability	< 0.001	< 0.001	

6.4.2 Amino acid composition of sorghum genotypes at Makhatini

The selected 19 genotypes were grown and their seed samples were profiled for 17 amino acids (Table 6.2). All genotypes were assessed for amino acid composition except for Macia-SA due to financial constraint. The levels of amino acids were expressed as percent of the total protein (Tables 6.4).

Percent amino acids showed significant differences among tested genotypes. Levels of all amino acids in different cultivars were highly significantly different at $P < 0.001$ (Table 6.4). The essential amino acids include: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine. Histidine content ranged between 1.81 and 2.32% with a mean of 2.10%. The genotypes: AS17, MP 2048 and MP 4276 had high histidine content at 2.32, 2.26 and 2.26%, respectively. Low histidine values were recorded in the genotypes 05-Potch-115, 05-Potch-167 and AS16cyc at 1.97, 1.91 and 1.81%, respectively. Lysine ranged from 1.09 to 2.17% with a mean of 1.80%. The genotypes that had high lysine percent were Manthate, 05-Potch-115, FS 4905, LP 1413 and EC 2985 at 2.17, 2.09, 2.04,

2.02 and 2.02%, in that order. The lowest was in the genotype MP 4276 at 1.09%. Threonine ranged from 2.26 to 3.24% with a mean of 3.00%. The genotypes that had a high threonine percent were 05-Potch-115, KZ 5246, Maseka-a-swere and LP 1481 at 3.23, 3.19, 3.15 and 3.12%, respectively. The lowest was recorded in the genotype AS16cyc at 2.26%. Methionine levels ranged from 1.40% to 4.28% with a mean of 2.10%. The genotype that had the highest methionine percent was EC 2985 at 15.85% and the lowest was MP 4276 at 1.40%. Valine ranged from 4.28 to 5.33% with a mean of 5.00%. The genotypes that had high valine percent were MP 2048, LP 1948, AS11, KZ 5246 and AS17 with 5.33%, 5.24%, 5.23%, 5.19% and 5.17%, while the lowest was noted in the genotype FS 4905 at 4.28%. Isoleucine ranged from 3.26 to 4.17% with a mean of 3.90%. The genotypes that had high isoleucine percent were 05-Potch-115, LP 1481, LP 1413 and MP 4259 with 4.17%, 4.13%, 4.11% and 4.09%, in that order. The lowest isoleucine content was recorded in the genotype LP 1948 at 3.26%. Leucine ranged from 13.40% to 14.47% with a mean of 14.10%. The genotypes that had high leucine percent were AS16cyc, Maseka-a-swere, 05-Potch-167 and 05-Potch-115 at 14.14%, 14.45%, 14.28% and 14.17%. The leucine content was the lowest in LP 4303, AS17 and AS11 at 13.6%, 13.54% and 13.4%, respectively. Phenylalanine ranged from 5.10% to 6.86% with a mean of 5.40%. The genotype Maseka-a-swere had the highest phenylalanine content at 6.86% and the lowest was noted in the genotype MP 4276 with 5.1%.

6.4.3 Amino acid composition of sorghum genotypes at Ukulinga

Amino acid compositions of the 19 sorghum genotypes when evaluated at Ukulinga are presented in Tables 6.5. The ANOVA displayed highly significant differences ($P < 0.001$) for the seventeen amino acids. Only the major amino acids are discussed below.

Percent amino acids of the total protein showed significant differences among the tested genotypes (Table 6.6). All amino acids were highly significant at $P < 0.001$ (Table 6.6). Histidine showed variation ranging between 1.78 to 2.28% with a mean percent of 2.06. LP 1413, MP 2048, MP 4259, MP 4276 and EC 2985 expressed high histidine levels of 2.28%, 2.25%, 2.23%, 2.23% and 2.21%, respectively. 05-Potch-167 had low histidine of 1.78%. The threonine composition showed differences ranging between 2.79% and 3.26% with an average percent of 3.05%. The genotypes 05-Potch-115 and LP 1948 had the highest threonine content of 3.26% and 3.24%, respectively. Manthate expressed the lowest content of 2.79%. The lysine levels ranged from 1.71 to 2.5% with the mean of 2.05%. The LP 1481 had the highest lysine content of 2.5% and MP 4154 had low content of 1.71%. The methionine values ranged from 1.7% to 2.33% with an average of 2.06%. The genotypes 05-

Potch-115, MP 4154, AS16cyc, LP 4303 and LP 1948 expressed high levels of 2.33%, 2.33%, 2.32%, 2.29% and 2.27%, respectively. The lowest content was observed in MP 2048 at 1.7%.

The valine content varied between 4.89% and 5.28% with an average of 5.03%. The genotypes that showed high values were Macia-SA, Manthate, AS17, LP 1481 and LP 1413 of 5.27%, 5.19%, 5.19%, 5.17% and 5.15%, respectively. The lowest values were observed in LP 4303 and MP 2048 both at 4.89%. The isoleucine content among the genotypes varied from 3.63% to 4.06% with an average of 3.83%. The genotypes that had high isoleucine levels were EC 2985, 05-Potch-167 and MP 4276 at 4.06%, 4.06% and 4.01%, respectively. The lowest levels were noted in genotypes MP 4259 at 3.64% and 05-Potch-115 at 3.63%. The leucine levels ranged from 13.04% to 14.29% with the average of 13.79%. The genotypes that had high leucine were LP 1413 and 05-Potch-115 at 14.28% and 14.25%, respectively. The genotype AS17 had the lowest leucine level of 13.04%. The phenylalanine levels varied between 4.82% and 5.7% with the average of 5.17%. Manthate had the highest phenylalanine content of about 5.7% and MP 4259 had the lowest content of 4.82%. The arginine values varied between 3.11% and 4.19% with an average of 3.62%. The genotypes 05-Potch-115 and FS 4905 both expressed increased arginine level of 4.19% each. Macia-SA had the lowest level of 3.11%. The tyrosine values ranged from 3.84% to 4.53% with an average of 4.13%. The highest tyrosine level was noted in Manthate having 4.53% and the lowest in EC 2985 having tyrosine content of 3.84%.

Table 6.4. Amino acids composition (%) of 18 sorghum genotypes grown at Makhathini, 2011/2012.

	Amino acids																
Genotype	His	Ser	Arg	Gly	Asp	Glu	Thr	Ala	Pro	Cys	Lys	Tyr	Met	Val	ILe	Leu	Phe
AS11	2.23	4.74	3.78	3.18	6.28	21.84	3.03	9.29	8.45	0.33	1.66	4.48	2.09	5.00	3.87	14.42	5.32
AS16cyc	1.81	3.20	2.47	5.16	5.03	18.01	2.26	7.24	15.15	0.00	1.09	5.75	4.28	4.28	3.26	14.14	6.86
AS17	2.32	4.85	4.23	3.36	7.01	21.00	3.23	8.73	7.92	0.30	2.17	4.42	2.03	5.09	3.76	14.18	5.39
EC 2985	2.07	4.89	3.52	3.41	6.44	22.01	3.08	8.87	8.48	0.32	1.68	4.36	2.35	4.85	3.92	14.11	5.64
FS 4905	2.18	4.80	4.03	3.75	6.63	21.36	3.08	8.76	7.85	0.25	1.87	4.65	2.73	4.98	3.80	13.87	5.42
KZ 5246	2.10	4.65	3.75	3.64	6.75	21.33	3.19	8.93	8.11	0.22	2.04	4.47	2.31	5.33	4.01	13.91	5.26
LP 1413	2.14	4.79	3.67	3.17	7.59	22.20	2.93	9.56	7.70	0.20	2.02	4.14	1.70	5.06	3.84	14.14	5.15
LP1481	2.11	4.79	3.60	3.39	7.14	22.60	3.03	9.52	7.73	0.19	2.09	3.98	1.85	5.17	4.09	13.60	5.13
LP1948	2.00	4.71	3.06	3.02	6.91	22.72	2.91	9.63	8.03	0.14	1.85	4.10	1.72	5.10	4.17	14.47	5.44
LP 4303	2.00	4.67	3.59	3.19	7.39	22.42	2.91	9.21	7.89	0.21	2.02	4.22	1.68	5.24	3.99	14.24	5.15
MP 2048	2.26	5.19	3.54	3.32	6.74	22.18	2.93	9.22	7.97	0.20	1.89	4.27	1.81	5.09	4.11	13.95	5.34
MP 4154	2.08	4.77	3.20	3.02	6.61	22.93	2.91	9.53	8.25	0.20	1.72	4.08	1.79	4.95	4.13	14.44	5.40
MP 4259	2.23	4.90	3.33	3.57	6.86	22.89	3.12	9.17	8.20	0.22	1.87	4.05	2.23	4.98	3.74	13.54	5.10
MP 4276	2.26	4.85	3.28	3.37	6.46	23.01	3.15	9.25	8.11	0.23	1.73	4.05	2.09	4.98	3.89	13.92	5.35
Manthate	2.23	4.57	3.80	3.82	7.27	22.48	3.00	8.96	7.62	0.17	1.98	4.15	2.42	5.19	3.70	13.40	5.26
Maseka-a-swere	2.08	4.69	3.43	3.04	7.01	22.76	2.99	9.58	8.10	0.23	1.67	3.97	1.56	5.23	3.92	14.45	5.29
05-Potch-115	1.97	4.82	3.24	3.03	7.81	22.65	3.01	9.56	8.10	0.25	1.87	4.09	1.52	4.88	3.79	14.17	5.23
05-Potch-167	1.91	5.01	3.27	3.23	7.31	22.80	3.00	9.59	8.09	0.28	1.88	3.94	1.40	4.85	3.88	14.28	5.26
Min	1.81	3.20	2.47	3.02	5.03	18.01	2.26	7.24	7.62	0.00	1.09	3.94	1.40	4.28	3.26	13.40	5.10
Max	2.32	5.19	4.23	5.16	7.81	23.01	3.23	9.63	15.15	0.33	2.17	5.75	4.28	5.33	4.17	14.47	6.86
Mean	2.10	4.70	3.50	3.40	6.80	22.10	3.00	9.10	8.40	0.20	1.80	4.30	2.10	5.00	3.90	14.10	5.40
F-probability	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SE	0.03	0.10	0.09	0.12	0.14	0.28	0.05	0.13	0.40	0.02	0.06	0.10	0.15	0.05	0.05	0.08	0.09
Variance	0.02	0.16	0.16	0.25	0.37	1.38	0.04	0.32	2.87	0.01	0.06	0.17	0.42	0.05	0.04	0.10	0.15

Table 6.5. Amino acid composition (%) of 18 sorghum types grown at Ukulinga 2011/2012.

Genotype	Amino acids																
	His	Ser	Arg	Gly	Asp	Glu	Thr	Ala	Pro	Cys	Lys	Tyr	Met	Val	ILe	Leu	Phe
AS11	2.03	4.94	3.39	3.31	6.85	22.66	3.14	9.15	7.94	0.32	1.85	4.37	2.19	4.90	3.74	14.18	5.06
AS16cyc	1.89	4.80	3.52	3.55	7.42	22.26	3.07	8.92	7.70	0.26	2.17	4.26	2.32	4.94	3.76	14.03	5.12
AS17	1.94	5.14	3.65	3.81	7.38	22.10	3.26	8.93	7.61	0.25	2.33	4.07	2.20	4.98	3.70	13.72	4.94
EC2985	2.21	5.29	3.66	3.72	6.29	21.36	3.17	8.62	8.29	0.29	1.71	4.53	2.33	4.89	3.86	14.07	5.70
FS4905	2.05	4.75	3.71	3.30	7.06	22.05	3.06	9.25	7.88	0.24	1.95	4.27	1.88	5.00	3.94	14.28	5.35
KZ5246	2.15	4.77	3.74	3.82	7.72	21.90	3.24	9.14	7.59	0.22	2.50	4.03	2.13	5.17	3.82	13.24	4.82
LP1413	2.28	4.55	3.84	3.10	6.72	22.02	2.99	9.24	8.26	0.20	1.89	4.40	1.80	5.19	4.06	14.02	5.44
LP1481	1.99	4.68	3.48	3.29	7.24	23.33	2.90	9.46	7.65	0.16	2.13	3.91	1.95	5.10	3.92	13.69	5.13
LP1948	2.04	4.76	3.52	3.47	7.47	22.96	2.96	9.45	7.68	0.13	2.34	3.84	1.72	5.19	4.01	13.49	4.97
LP4303	2.05	4.66	3.58	3.68	7.04	22.80	2.86	8.96	7.96	0.16	2.12	3.98	2.29	5.09	3.82	13.81	5.15
MP2048	2.25	4.70	3.61	3.53	6.74	22.91	2.79	9.27	7.88	0.24	1.77	4.24	2.27	5.08	3.75	13.69	5.27
MP4154	1.94	5.21	3.11	3.20	7.16	23.17	2.87	9.58	7.80	0.16	1.75	4.07	1.70	4.91	3.95	14.16	5.28
MP4259	2.23	4.93	3.82	3.38	6.76	21.82	3.17	9.14	7.90	0.21	2.04	4.20	2.02	5.15	4.06	13.89	5.27
MP4276	2.23	4.75	4.19	3.76	7.12	22.46	3.18	8.92	7.57	0.23	2.35	4.03	2.12	5.04	3.79	13.13	5.11
Manthate	2.15	4.67	4.15	3.96	7.15	22.19	3.16	8.93	7.55	0.16	2.34	4.07	2.33	5.27	3.72	13.16	5.05
Maseka-a-swere	2.04	4.64	3.48	3.15	7.19	23.56	2.95	9.45	7.74	0.30	1.77	4.13	1.96	4.95	3.64	14.06	4.98
Macia-SA	1.96	5.08	3.86	3.88	8.05	22.44	3.02	9.31	7.75	0.21	2.23	3.87	1.87	4.94	3.63	13.04	4.87
05-Potch-115	1.86	4.69	3.19	3.44	7.41	23.03	3.08	9.02	8.01	0.17	1.85	4.11	1.93	4.95	3.80	14.07	5.40
05-Potch-167	1.78	4.89	3.19	3.44	7.13	22.95	3.03	9.37	7.92	0.23	1.77	4.00	2.04	4.89	3.75	14.25	5.38
Min	1.78	4.55	3.11	3.1	6.29	21.36	2.79	8.62	7.55	0.13	1.71	3.84	1.7	4.89	3.63	13.04	4.82
Max	2.28	5.29	4.19	3.96	8.05	23.56	3.26	9.58	8.29	0.32	2.5	4.53	2.33	5.27	4.06	14.28	5.7
Mean	2.06	4.84	3.62	3.52	7.15	22.52	3.05	9.16	7.83	0.22	2.05	4.13	2.06	5.03	3.83	13.79	5.17
F-probability	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
SE	0.03	0.05	0.07	0.06	0.10	0.14	0.03	0.06	0.05	0.01	0.06	0.04	0.05	0.03	0.03	0.09	0.05
Variance	0.02	0.05	0.10	0.08	0.21	0.64	0.02	0.08	0.05	0.00	0.07	0.04	0.05	0.02	0.02	0.18	0.06

6.4.4 Effect of location on amino acid composition

Percentage amino acids showed significant differences among the tested sorghum genotypes. The amino acids showed significant differences (Table 6.9). The phenylananine, lysine and leucine were significant at $P \leq 0.05$. The phenylalanine content ranged from 5.04 to 5.99 percent of the total with the mean percent of 0.23. AS16cyc had high phenylananine of about 5.99% and KZ 5246 had the lowest level of 5.04%. The lysine content ranged from 1.63 to 2.27% of the total with a mean of 1.94%. The genotypes that had high lysine were KZ 5246, AS17, Manthate and LP 1481 at 2.27%, 2.25%, 2.16% and 2.11% contents, respectively. The lowest lysine was recorded in AS16cyc at 1.63%. The leucine values ranged from 13.28% to 14.30% of the total with the mean of 13.94%. The genotypes that showed high leucine content were LP 1948, FS 4905, MP 4154 and LP 1481 at 14.3%, 14.3%, 14.26%, and 14.25%, respectively. Macia-SA showed the lowest level of leucine at 13.28%.

Among the amino acid profiles assessed, cysteine was the most deficient. It was in the range of 0.13 to 0.33% and a mean of 0.22%, followed by lysine varying between 1.63 to 2.27% with a mean of 1.94%, methionine varied between 1.67 to 3.30% with the mean of 2.07%, and histidine further varied between 1.78 and 2.26% with the mean of 2.08% (Table 6.9). The most abundant amino acids were glutamic acid ranging between 20.14 to 23.66% with an average acid of 22.35%, followed by leucine ranging between 13.28% and 14.30% with the mean of 13.94%, alanine varying between 8.08 and 9.56% with the mean of 9.16%; and lastly, the proline content with a range of 7.59% to 11.43% and a mean of 8.12%.

Generally, the genotypes LP 1948 (14.3%), FS 4905 (14.3%), MP 4154 (14.26%) and LP 1481 (14.25%) were the best on leucine content across the two locations. KZ 5246 (2.27%), AS17 (2.25%), Manthate (2.16%) and LP 1481 (2.11%) were the best genotypes for lysine and AS16cyc (5.99%) was the best candidate genotype for phenylananine content across the two locations. Hence, these genotypes can be selected for further quality improvement in sorghum breeding programmes.

The genotype Maseka-a-swere, had high protein content (15.13%), and high valine (5.23%) and leucine (14.45%) at Makhathini. At Ukulinga, Maseka-a-swere had high glutamic acid (23.56%) and cysteine (0.30%) and on average (across two locations) it showed high glutamic acid (23.16%), alanine (9.52%), and leucine (14.26%). Manthate had a high protein content of 14.83% across the two locations, while arginine of 3.80% and glucine of 3.82% at Makhathini. At Ukulinga, Manthate showed high arginine of 4.15%, glycine of 3.96%, lysine of 2.34%, methionine of 2.33% and valine of 5.27%. Across the locations, Manthate

exhibited arginine of 3.98%, glycine of 3.89%, lysine of 2.16%, and valine of 5.23%. These two genotypes have high protein content and amino acid levels useful for breeding and/or conservation.

Table 6.6. Mean amino acid composition (%) among 19 sorghum genotypes when grown at Makhathini and Ukulinga 2011/2012.

Genotype	Amino acid																
	Hist	Ser	Arg	Gly	Asp	Glu	Thr	Ala	Pro	Cys	Lys	Tyr	Met	Val	Ile	Leu	Phe
AS11	2.13	4.84	3.59	3.25	6.57	22.25	3.09	9.22	8.20	0.33	1.76	4.43	2.14	4.95	3.81	14.30	5.19
AS16cyc	1.85	4.00	3.00	4.36	6.23	20.14	2.67	8.08	11.43	0.13	1.63	5.01	3.30	4.61	3.51	14.09	5.99
AS17	2.13	5.00	3.94	3.59	7.20	21.55	3.25	8.83	7.77	0.28	2.25	4.25	2.12	5.04	3.73	13.95	5.17
EC 2985	2.14	5.09	3.59	3.57	6.37	21.69	3.13	8.75	8.39	0.31	1.70	4.45	2.34	4.87	3.89	14.09	5.67
FS 4905	2.12	4.78	3.87	3.53	6.85	21.71	3.07	9.01	7.87	0.25	1.91	4.46	2.31	4.99	3.87	14.08	5.39
KZ 5246	2.13	4.71	3.75	3.73	7.24	21.62	3.22	9.04	7.85	0.22	2.27	4.25	2.22	5.25	3.92	13.58	5.04
LP 1413	2.21	4.67	3.76	3.14	7.16	22.11	2.96	9.40	7.98	0.20	1.96	4.27	1.75	5.13	3.95	14.08	5.30
LP1481	2.05	4.74	3.54	3.34	7.19	22.97	2.97	9.49	7.69	0.18	2.11	3.95	1.90	5.14	4.01	13.65	5.13
LP1948	2.02	4.74	3.29	3.25	7.19	22.84	2.94	9.54	7.86	0.14	2.10	3.97	1.72	5.15	4.09	13.98	5.21
LP 4303	2.03	4.67	3.59	3.44	7.22	22.61	2.89	9.09	7.93	0.19	2.07	4.10	1.99	5.17	3.91	14.03	5.15
MP 2048	2.26	4.95	3.58	3.43	6.74	22.55	2.86	9.25	7.93	0.22	1.83	4.26	2.04	5.09	3.93	13.82	5.31
MP 4154	2.01	4.99	3.16	3.11	6.89	23.05	2.89	9.56	8.03	0.18	1.74	4.08	1.75	4.93	4.04	14.30	5.34
MP 4259	2.23	4.92	3.58	3.48	6.81	22.36	3.15	9.16	8.05	0.22	1.96	4.13	2.13	5.07	3.90	13.72	5.19
MP 4276	2.25	4.80	3.74	3.57	6.79	22.74	3.17	9.09	7.84	0.23	2.04	4.04	2.11	5.01	3.84	13.53	5.23
Manthate	2.19	4.62	3.98	3.89	7.21	22.34	3.08	8.95	7.59	0.17	2.16	4.11	2.38	5.23	3.71	13.28	5.16
Maseka-a-swere	2.06	4.67	3.46	3.10	7.10	23.16	2.97	9.52	7.92	0.27	1.72	4.05	1.76	5.09	3.78	14.26	5.14
05-Potch-115	1.97	4.95	3.55	3.46	7.93	22.55	3.02	9.44	7.93	0.23	2.05	3.98	1.70	4.91	3.71	13.61	5.05
05-Potch-167	1.89	4.85	3.23	3.34	7.36	22.92	3.04	9.31	8.05	0.23	1.87	4.03	1.67	4.90	3.84	14.18	5.33
Macia-SA	1.78	4.89	3.19	3.44	7.13	22.95	3.03	9.37	7.92	0.23	1.77	4.00	2.04	4.89	3.75	14.25	5.38
Min	1.78	4.00	3.00	3.10	6.23	20.14	2.67	8.08	7.59	0.13	1.63	3.95	1.67	4.61	3.51	13.28	5.04
Max	2.26	5.09	3.98	4.36	7.93	23.16	3.25	9.56	11.43	0.33	2.27	5.01	3.30	5.25	4.09	14.30	5.99
Mean	2.08	4.78	3.55	3.47	7.01	22.32	3.02	9.16	8.12	0.22	1.94	4.20	2.07	5.02	3.85	13.94	5.28
Variance	0.02	0.19	0.19	0.27	0.45	1.26	0.04	0.23	2.99	0.01	0.09	0.15	0.34	0.04	0.05	0.17	0.19
STDEV	0.13	0.44	0.43	0.51	0.67	1.12	0.20	0.48	1.73	0.08	0.30	0.39	0.59	0.20	0.22	0.41	0.44
SE Mean	0.03	0.10	0.10	0.12	0.16	0.26	0.05	0.11	0.41	0.02	0.07	0.09	0.14	0.05	0.05	0.10	0.10
F-probability	0.216	0.273	0.157	0.452	0.069	0.118	0.213	0.946	0.152	0.926	0.006	0.112	0.828	0.565	0.342	0.005	0.043

6 .5 Discussion

Assessing local sorghum genotypes for protein and amino acids is essential for exploiting the existing potential residing in the local landraces for improved human nutrition. There was variation present among the sorghum genotypes based on the crude protein and amino acid profiles. Shegro et al. (2012) also found genetic variation among the sorghum landraces when analysing protein and other mineral elements. In their report the protein content varied between 8.08 and 15.26%. Nguni et al. (2012) further reported grain protein content ranging between 9.7 and 16.3% in Southern African sorghum genotypes. In the present study the crude protein content varied between 7.69% and 16.18%, which is similar to the crude protein reported by Shegro et al. (2012) and Nguni et al. (2012). Pepo et al. (2011) reported protein levels ranging between 9.43-17.7% among sorghum cultivars and single hybrids. Perdesen and Kofoid (2003) reported crude protein ranging from 106 to 128 g/kg with a mean of 117 g/kg for sorghum lines without testa and 107 to 124 g/kg protein with testa containing sorghum lines when assessing the sorghum conversion lines for protein content. Mokrane et al. (2010) reported protein content of about 16% in various Algerian sorghum cultivars. Douglas et al. (1990) found crude protein levels in sorghum lines to be higher than maize, ranging from 8.8 to 15.0%. Crude protein in the range of 6% to 16% has been reported by other researchers (Youssef, 1998; Afripro, 2003).

Protein content and amino acid compositions are highly variable due to differences in genotypes, environments and genotype x environment interaction. The protein content of barley was reported to be 11.5% and fractionated as B, C, D and *γ* hordeins. The B and C fractions accounted for 70% to 80% and 10% to 12%, respectively, of the total hordein, while the D and *γ* fractions were minor components (Jun-cong et al., 2005). Jaradat (1991) studied grain protein variability among populations of wild barley in Jordan. The author reported protein content ranging from 106.3 to 239.1 g kg-1, and thousand kernel weight from 21.17 to 31.8 mg. Previous studies have attempted to determine the content, composition and structure of the grain proteins in wheat (Shewry et al., 2002; Bradová and Štočková, 2010; Özbek et al., 2011). Mature wheat grains contain 8-20% protein. The major storage proteins are gliadins and glutenins, which interact in the presence of water to form gluten, the protein complex responsible for the visco-elastic properties that make durum wheat superior for pasta making (Peña et al., 1994; Wieser, 2000). Genes conferring high grain protein have been identified in wild tetraploid wheats (Brevis et al., 2010) or in mutant barley lines (Roesler and Rao, 2000).

The protein content a crop is influenced by the production environment. The levels of protein can be increased significantly by generous nitrogen fertilization (Warsi and Wright, 1973). Environmental factors such as location, chemical fertilizers, plant population and chemical treatments influence the protein content and amino acid patterns (Salunkhe et al., 1977). In this study, the protein content varied across locations and genotypes. The genotypes that exhibited high protein content in this study have potential to be selected for breeding, conservation or direct production at the target agro-ecology. The genotypes with high protein were also exhibiting intermediate plant height, medium sized panicle width, intermediate panicle weight, medium seed size and were well exserted. More studies are needed in different agro-ecologies to select genotypes with stable protein expression.

There were significant differences among the sorghum genotypes based on the amino acid composition. The amino acid levels were different for lysine, isoleucine and phenylananine across the two locations. Lysine and methionine were in low levels than other amino acids. Glutamic acid, leucine, alanine and proline were found in high levels. These results concur with the reports of other researchers who found low levels of lysine and methionine (Azevedo et al., 1997; Amjad et al., 2003). Ebadi et al. (2005) also found low levels of lysine and methionine in high tannin sorghums. High levels of proline and glutamic acid were also recorded (Landry et al., 2005; Landry and Delhaye, 2007). Hicks et al. (2002) reported genetic variation among the sorghum inbred lines and hybrids for crude protein and other quality traits. Mokrane et al. (2010) found different levels of amino acids analysed in Algerian sorghum cultivars. The amino acid profiles had amino acid score of 1.0-2.6 of the human protein requirement. Moreover, the amino acids contents ranged from 0.9 to 2.6 g/100g except for lysine, methionine, and cysteine. Genetic variation was also observed among high lysine sorghum genotypes from India and MASSA 03 based on protein and amino acids. There were high lysine and threonine soluble concentrations observed among the sorghum genotypes which could serve as potential food sources due to a better balanced amino acid profile.

A large part of the differences observed in amino acid profiles was due to genetic effects. Hence, breeding for enhanced amino acid profiles is feasible. The best sorghum genotypes can be used as parents to develop superior cultivars and/or hybrids with improved protein and amino acids.

6.6 Conclusions

The sorghum genotypes showed a wide variation of crude protein and amino acid profiles. High crude protein content recorded at Makhathini and Ukulinga were in the genotypes Mammopane (16.18%), AS16 M1 (15.57%), Macia-SA (15.31%), AS19 (15.22%), Maseka-a-swere (15.13%) and AS4 (15.07%). Hence, these lines can be recommended for further quality improvement in sorghum breeding or direct production. The candidate genotypes with superior levels of leucine were LP 1948 (14.3%), FS 4905 (14.3%), MP 4154 (14.26%) ad LP 1481 (14.25%). The genotype KZ 5246 (2.27%), AS17 (2.25%), Manthate (2.16%) and LP 1481 (2.11%) were the best genotypes for high lysine, whereas AS16cyc (5.99%) was the best candidate for phenylalanine. Manthate and Maseka-a-swere were best candidates for high protein and good amino acid composition. The presence of genetic diversity among the sorghum genotypes studied is imperative for further genetic improvement in sorghum breeding programmes as well as for improved human nutrition value.

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CHAPTER 7

Thesis overview

Sorghum is one of the most important cereal crops grown globally. Understanding the genetic diversity and its interaction with the environment is of paramount importance in developing cultivars considering farmer's preferred traits. The objectives of the study were to: (i) determine farmers production constraints and preferences of sorghum varieties in the Limpopo Province in South Africa, (ii) assess the level of genetic diversity present among South African sorghum genotypes using agro-morphological traits, (iii) compare RAPD and SSR marker and high resolution melt (HRM) analyses to determine genetic variation among selected sorghum genotypes, (iv) assess the genetic diversity present among South African sorghum genotypes using genetic distances as measured by SSR markers, and (v) determine genetic diversity of selected South African sorghum genotypes grown at two diverse environments for protein and amino acid composition and select candidate lines for breeding and conservation.

Major findings

i) Appraisal of farmers' sorghum production constraints and variety preferences in the Limpopo Province, South Africa

- The constraints to sorghum production were bird damage, storage pests (weevils), parasitic weeds, drought and postharvest diseases.
- The farmers' preference traits of an ideal/ideotype sorghum variety were good taste, high yields, resistance to bird damage, insect pests (weevils) and diseases, early maturity and drought tolerance.
- Farmers grew sorghum predominantly for home consumption
- Macia was the most preferred sorghum variety and the highest yielder

ii) Assessment of genetic relatedness among South African sorghum genotypes using agro-morphological traits

- Three principal components contributed to 38.9%, 30.96% and 18.13% to the total variation. The traits that contributed most to the variation were plant height, grain weight and panicle weight.
- The dendrogram grouped the genotypes into three major clusters. The grouping of the genotypes was not based on a source or place of origin.

- The genotypes MP 4277, EC 2934, KZ5097, FS4909, and LP 4303, were identified as the most diverse lines.
- The best lines with quantitative and qualitative attributes were MP 4276, NW 5430, 05-Potch-167 and EC 3217 across the locations.

iii) Comparison between random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers with high resolution melt analyses in genetic variation analysis among selected sorghum genotypes.

- Both SSR and RAPD markers and HRM revealed variations among the sorghum accessions.
- The HRM melting profiles correlated well with the outcomes from RAPD and SSR analyses.
- The clustering of sorghum accessions using SSR marker highly corresponded with the HRM analysis.

iv) Genetic diversity analysis of sorghum genotypes using SSR markers in South Africa.

- The analyses formed two major distinct clusters without allocating genotypes based on the source or origin.
- The results showed clear separation between the breeding lines and landrace collections from various provinces in South Africa
- The Euclidian genetic distances varied from 0 to 8.4 with an average of 5.67.
- Genotypes Macia-SA and AS4 had the lowest dissimilarity index, and 05-POTCH-115 and MP2048 had the highest value.
- A wide genetic diversity was observed with the allele sizes ranging from 90 to 294 bp. The numbers of alleles ranged from 2 to 15 with an average of 6.4 per locus.
- The polymorphic information content ranged from 0.0192 to 0.8351 (average of 0.5031) with heterozygosity values of 0.0194 to 0.8524 (average 0.5483).

v) Genetic diversity among selected South African sorghum genotypes for protein content and amino acid composition.

- Generally, the sorghum lines had great variability in their protein and amino acid profiles.

- The crude protein content of the genotypes varied from 7.69 to 16.18% across the two sites with a mean of 13.07%.
- The genotypes that had high crude protein content at both sites were Mammopane (16.18%), AS16 M1 (15.57%), Macia-SA (15.31%), AS19 (15.22%), Maseka-a-swere (15.13%), and AS4 (15.07%).
- The genotypes identified with superior leucine content were LP 1948 at 14.3%, FS 4905 (14.3%), MP 4154 (14.26%) and LP 1481 (14.25%).
- High lysine content was detected in the genotypes KZ 5246 at 2.27%, AS17 (2.25%), Manthate (2.16%) and LP 1481 (2.11%).
- The genotype AS16cyc was the best candidate for high phenylananine content at 5.99%.
- Low levels of cysteine, lysine, methionine, and histidine were observed.

Overall, the study established the existence of considerable genetic diversity among South African sorghum germplasm phenotypically, genotypically and when using nutritional quality traits. The lines identified with superior performance can be selected and used for further quality breeding to achieve balanced protein diet and amino acid levels. They can also be utilised for strategic conservation, genetic enhancement and development of breeding populations.

Appendix

A copy of a questionnaire used for conducting participatory rural appraisal in two districts of the Limpopo province.

A. **General information** **Date**_____

District_____ Municipality_____

Village/sublocation_____

Name of respondent_____ M ☐ F ☐

Age (range)_____

Number of Household_____

Education level: Tick the highest level: None_____ Primary_____ Secondary _____ College _____ University _____

B. Sorghum farming systems

1. What is the approximate size of your farm?
2. On how many hectares do you plant sorghum?
3. Do you plant any improved sorghum varieties
    i. Yes
    ii. No
4. If yes, what improved varieties do you plant- Names?

    _____
    _____
    _____
5. Why do you prefer the improved varieties?

    _____
    _____
    _____

6. What other local sorghum cultivars do you plant- give names?

    _____
    _____
    _____
7. Why do you prefer the local varieties?

    _____
    _____
    _____

8. What is your average sorghum yield approximately (bag= 50 kg)?

_____
_____

9. What are the main uses of the sorghum you grow?
    i. Home consumption
    ii. Animal feed
    iii. Brewing
    iv. Other _____
10. How many bags do you require for the family consumption every year?
    i. Less than 5 bags
    ii. 6 to 10 bags
    iii. More than 10 bags

a) What type of food preparation you make from sorghum?_____

_____

b) Do you sell your sorghum? Which part? _____

_____

C. Constraints to sorghum production

11. What are the sorghum production constraints that you face in order of importance?(the constraints maybe ranked in order to see their importance)

Sorghum production constraints				
1	High cost of inputs	Fertilizers	Tick appropriately	Rank
		Seed		
		Labor		
2	Storage pests	Weevils		
		Larger grain borer		
		Moths		
3	Field pests	Stem borer		
		Greenbugs		
		Sorghum midge		
		Stem and stalk borers		
		Shootfly		
4	Diseases	Downy mildew		
		Anthracnose		
		Phoma Leaf Spot		
		Zonate Leaf Spot		
		Bacterial leaf spot		
		Bacterial streak		
		Northern Leaf Blight		
		Rust		

		Common, head and long smut		
		Ergot		
		Stem rots		
		Maize dwarf mosaic		
		Sooty stripe		
		Storage rots		
	Parasitic weeds	Striga		
	Other	Birds		
5	Abiotic	Drought		
		Soil fertility and acid soil		
		Heat		
6	Policies	Low market prices		

D. Farmers' variety preference

12. Which sorghum varieties do you grow in your area? List them

_____
_____
_____

13. List factors you consider when selecting sorghum varieties and rank them

Factor	Reason	Rank
High Yield		
Resistance to disease/pest		
Tolerance to drought/heat		
Resistance to storage pests		
Resistance to birds		
Maturity period		
Grain colour		
Grain size		
Head size		
Head shape		
Taste		
Plant height		
Biomass		
Other		

Taste: 1= Sweet, 2= Non-sweet, 3= Bitter Colour: 1 White, 2= Tan, 3= Brown, 4= Red
Head shape: 1= Compact, 2= Semi-compact, 3= Loose

14. What other crops do you grow in your area?

_____
_____

15. Rank them in order of importance

Crops	Rank

16. In your opinion, what should be the focus of the current sorghum research efforts in order of priority?

_____
_____
_____

17. In your opinion, what would be an ideal sorghum variety?

_____

_____

_____