Genetic Diversity and Nutritional Content of Sorghum [*Sorghum bicolor* (L.) Moench] Accessions from Southern Africa

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Cover: An impression of sorghum grain diversity in Southern Africa
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Abstract

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop in semi-arid regions of the world especially in Africa, South Asia and China. It is an important food and fodder crop in the semi-arid tropics (SAT) of Africa, where it is used for making different kinds of food. In Southern Africa, it serves as a principal source of energy, protein, vitamins and mineral nutrients for the people in the region. The aim of this study was to characterise sorghum landrace accessions from Southern Africa in order to generate information that could help design appropriate breeding and conservation strategies in the region. Both agro-morphological and DNA markers were used to study the genetic diversity of accessions from five countries in the region. Nutritional diversity in terms of protein and mineral contents was also characterised.

A significant level of genetic variation was observed among 30 sorghum accessions from different agro-ecological regions in Botswana (70% among accessions and 30% within accessions), when genetic diversity was assessed using microsatellite (SSR) markers. The analysis of genetic diversity in 22 sorghum accessions from five countries (Botswana, Namibia, Swaziland, Zambia and Zimbabwe) of Southern Africa, revealed a significant variation in both agro-morphological traits and SSR markers. There were significant differences for protein and mineral content among 23 sorghum accessions from Southern Africa in terms of nutritional composition (protein and minerals). The patterns of genetic diversity and relationships observed in this research provide insights for genetic resource conservation and utilization of sorghum germplasm in Southern African. The protein and mineral content variation found among the sorghum accessions could also be exploited in sorghum improvement programs in the region.

Keywords: accessions, breeding, diversity, microsatellites, morphology, nutrition, sorghum

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Dedication

To my children: Bakang, Thuto and Thebe Moses Jr.

Jesus Christ is the same yesterday, today and forever. **Hebrews 13:8**

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Tiny Motlhaodi, Mulatu Geleta, Stephen Chite, Moneim Fatih, Rodomiro Ortiz, Tomas Bryngelsson (2014). Genetic diversity in ex-situ conserved sorghum accessions of Botswana as estimated by microsatellite markers. Australian Journal of Crop Science 8, 35-43.

II Tiny Motlhaodi, Mulatu Geleta, Stephen Chite, Moneim Fatih, Rodomiro Ortiz, Tomas Bryngelsson (2015). Genetic diversity in sorghum germplasm from Southern Africa as revealed by microsatellite markers and agro-morphological traits (in press).

III Tiny Motlhaodi, Tomas Bryngelsson, Stephen Chite, Moneim Fatih, Rodomiro Ortiz, Mulatu Geleta (2015). Nutritional diversity in sorghum accessions from Southern Africa as revealed by protein content and mineral composition (submitted).

The contribution of Tiny Motlhaodi to the papers included in this thesis was as follows:

I Planned, carried out all experimental work, analysed data and wrote the manuscript in cooperation with co-authors

II Planned, sourced germplasm, carried out field and laboratory experimental work, analysed data and wrote the manuscript in cooperation with co-authors

III Planned, carried out field experiments, evaluated and analysed data, wrote manuscript in cooperation with co-authors

Abbreviations

DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
NARS	National Agricultural Research Systems
NPGRCs	National Plant Genetic Resources Centres
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
SADC	Southern African Development Community
SPGRC	SADC Plant Genetic Resources Centre
SSR	Simple sequence repeats
UPGMA	Unweighted pair group method with arithmetic mean

1 Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) belongs to the genus Sorghum, tribe Andropogoneae, of the Poaceae family (Clayton & Renvoize, 1986). The species *S. bicolor* includes all cultivated sorghums as well as a group of semi wild and wild plants regarded as weeds (Mutegi *et al.*, 2011). Based on the morphological features of the inflorescence, grain and glumes, cultivated sorghum has been classified into five races: bicolor, caudatum, durra, guinea and kafir (Harlan & De Wet, 1972). Very high levels of diversity exist among and within the races (House, 1985).

The diversity in sorghum ensued mainly by practicing disruptive selection and isolation, recombination in the extremely varied habitats and movement of people carrying one or more cultivars of the species (Doggett *et al.*, 1970). A balance of farmer selection for cultivated traits and natural selection for wild characteristics has generated improved sorghum types, wild types and intermediate types (Doggett *et al.*, 1970). Sorghum is predominantly self-pollinating, with only 6% of the plants outcrossing. Hybrids are produced using a cytoplasmic male sterility system that prevents selfing (House, 1985).

1.1 Taxonomy of the genus Sorghum

Sorghum taxonomy has been variously described since Linnaeus's first description in 1753. Snowdon followed in 1936 (Snowden, 1936), whose work was tremendous and remains useful to scientists today, and then De Wet in 1970 also described the various groups of sorghum as well as their distribution.

Presently, 25 species of sorghum are recognized, and these are classified into five sections: Stiposorghum, Parasorghum, Eu-sorghum, Heterosorghum and Chaetosorghum. Under the section Eu-sorghum, three species are recognized: *S. halepense* (L.) Pers. occurring in India, *S. propinquum* (Kunth) Hitchc found in Southeast Asia and *S. bicolor* (L.) Moench, which originated in Africa (De Wet, 1978). *S. bicolor* (L.) Moench (2n=20), includes all annual and domesticated types found in Africa, India and Asia.

S. bicolor is divided into five basic races: bicolor, guinea, caudatum, kafir and durra as illustrated in figure 1. Harlan and De Wet (1972) described the races based on the morphological features of the inflorescence, grain and glumes. The

race bicolor has its grain elongated, with glumes clasping the grain, which may be completely covered or exposed. This race is mostly grown west of the Rift valley and also on a minor scale almost everywhere in Africa. Guinea is primarily West African with a secondary centre in Malawi and Tanzania. The grain is flattened dorso-ventrally, twisting at maturity 90 degrees between glumes that are nearly as long as or longer than the grain.

The caudatum grain is asymmetrical, with glumes half the length of the grain or less. This race is most abundant in east Nigeria, Sudan and Uganda. Kafir is mostly a race of east and Southern Africa. It has symmetrical grain, with glumes of variable length clasping the grain. Durra is dominant in Ethiopia and westward across the continent, covering the driest parts near the Sahara. Its grain is rounded and the glumes are very wide (House, 1985).

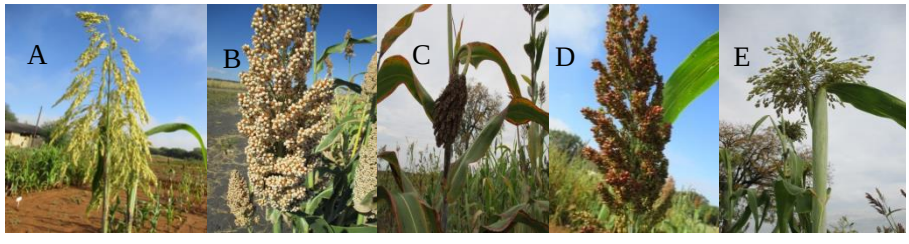

Figure 1. Morphological diversity in the sorghum accessions studied, illustrating the five sorghum races: A-Guinea; B-Caudatum; C-Durra; D-Kafir; E-Bicolor. (Photo: T. Motlhaodi)

1.2 Agro-ecology of sorghum

Sorghum is a short day plant but a wide genetic variation exists for its adaptation to a wide range of photoperiod and temperature conditions (Craufurd *et al.*, 1999). It requires a deep, well-drained fertile soil, fairly stable rainfall and a warm, frost-free period to grow well. A wide range of soil conditions can be tolerated, but growth on sandy soils is usually poor, unless heavy textured subsoil is present. A pH of between 5.5 and 8.5 is acceptable. Sorghum tolerates water logging better than maize.

Sorghum is a warm season crop, requiring high temperature for good germination and growth. Temperature ranges for germination are 7 to 10°C, but if there is sufficient moisture, germination occurs well at a soil temperature of 15°C or higher. After germination, temperatures of 27 to 30°C are required for optimum growth development. A temperature of as low as 21°C can however

have a dramatic effect on growth and yield (Vanderlip & Reeves, 1972). If temperatures are exceptionally high, grain yields can be reduced. Temperatures below zero can result in death of the plants, especially if plants are older than 3 weeks. Sorghum is mainly a rainfed crop of lowland, semi-arid areas of the tropics (Craufurd *et al.*, 1999). It requires an annual rainfall of 400 to800 mm, which should be well distributed over the cropping season (Ng'uni *et al.*, 2011).

1.3 Sorghum cultivation and utilization

Sorghum is usually cultivated as a field crop and has been, for centuries, one of the most important staple foods for millions of people in the semiarid tropics of Africa and Asia (Ali *et al.*, 2011). In most of these areas, sorghum remains the principal source of energy, proteins, vitamins and minerals. Presently, it is a staple food for more than 500 million people in more than 30 countries (Kumar *et al.*, 2011). This is probably because it can be cultivated in harsh environments where other crops such as maize and wheat cannot grow (Ali *et al.*, 2009). Cultivation in these areas is usually done without the application of fertilizers or other inputs (FAO, 1995). Sorghum can also tolerate cultivation on a wide range of soils and grows well on heavy vertisols commonly found in the tropics, where its tolerance to waterlogging is often required (Paterson *et al.*, 2009). It is equally suited to light sandy soils found in the dry areas. It can therefore produce grain on soils where many other crops would fail (FAO, 1995).

Sorghum has several uses. It is used for food, fodder and alcoholic beverages. Its stalks can also be used for fencing, firewood or for making brooms. The fibres can be used commercially to make wallboards and biodegradable packaging material (Delserone, 2007) and even solvents or dye can be extracted from the plant. A more recent use is as a source of ethanol and by-products from the ethanol production are also finding a place in the market (Delserone, 2007).

Variation in grain colour, shape and size as well as stalk thickness juiciness, sugar content and colour often influence the use of the crop. The white, large grains with corneous endosperm are usually preferred for human consumption. Nutritive value is increased if the endosperm is yellow with carotene and xanthophyll (Ng'uni *et al.*, 2012). The red varieties are preferred for making beer, especially in Africa where this sorghum-derived drink is very popular during traditional celebrations. The tall sweet varieties are usually used to make silage and hay for livestock feed. Those with succulent, sweet stalks and small heads and grains are preferred for chewing as with sugar cane. The white grained

varieties are usually soft and vulnerable to attack by birds during the dough stage of maturity, while the darker varieties are not (Ng'uni *et al.*, 2012).

Sorghum is used for human nutrition all over the world (Carter *et al.*, 1989). More than half of all sorghum produced in the world is used for human consumption. For subsistence farmers in arid, less developed regions of the world such as Africa, Central America and South Asia, it is the major food crop. The grain is used to make flour, porridge, couscous and molasses food supplement. In many parts of Africa, sorghum is used for making porridge, flat breads or the grains can be cooked as whole decorticated grain, more like rice. The nutritive value of sorghum based food is usually enhanced through its combination with locally grown edible oil crops such as sunflower, sesame and nigerseed in countries such as Ethiopia (Geleta *et al.*, 2002).

In the southern United States, sorghum syrup is used as a sweet condiment (like maple syrup) usually for biscuits, corn bread, pancakes, hot cereals or baked beans (Delserone, 2007). As an Arab cuisine, the milled grain is often cooked to make couscous, porridges, soups and cakes. In Central America, sorghum flour is sometimes used to make tortillas and especially in El Salvador where there is a shortage of corn. Sorghum can also be popped in the same manner as popcorn, although the popped kernels are smaller than popcorn. Sorghum has come into increasing use for homemade and commercial breads and cereals for gluten-free diets since 2000 (Delserone, 2007)

Sorghum can also be used for making alcoholic beverages. In China it is the most important ingredient for the production of distilled beverages such as *maotai* and *kaoliang.* In the United States sorghum can also be used as a main ingredient in production of gluten-free beer. This particular beer is aimed at those with celiac disease and its low carbohydrate content makes it popular among health-minded drinkers. African sorghum beer is a brownish pink beverage with a fruity, sour taste. The beer is not filtered so its appearance is cloudy and yeasty, and may contain bits of grain. This beer is a popular drink in Africa for traditional reasons (Van der Walt, 1956).

Sorghum is also considered to be a significant crop for animal feeds. Plants in the field can be used as pasture after harvesting the grain, where cattle and sheep can graze (Carter *et al.*, 1989). In most African countries this is a common practice and sometimes the stover can be cut and fed to livestock. Sorghum straw (stem fibre) can be made into very good wallboard for building houses. It can also be used to make biodegradable packaging; this kind of packaging does not accumulate static electricity so it is being used for packaging sensitive electronic equipment. In some countries the stems can be used for fencing, sweeping broom and for cooking fuel. For industrial purpose it is used for making ethanol and dye can be extracted from the plant to colour leather (Delserone, 2007). Sorghum

is also an important component in poultry feed and good progress has been made in the manufacturing of dog food, as well as pigeon and ostrich food (Delserone, 2007).

1.4 Nutritional status of sorghum

Sorghum is a principal source of energy, proteins, vitamins and minerals for people in the semi-arid tropics (Duodu *et al.*, 2003). It is a good energy source because it is about 70% starch. Proteins are the main constituents of sorghum after starch, making up to 12% dry weight of sorghum grain (Ng'uni *et al.*, 2012). The essential amino acid profile of sorghum protein differs (3-12% range) between varieties, soil and growing conditions (FAO, 1995). The digestibility of sorghum protein has also been found to vary between varieties, ranging from 30 to 70%. Sorghum's nutritional profile includes several minerals, though unevenly distributed and more concentrated in the germ and seed coat. Sorghum is a good source of the β-complex vitamins and some varieties contain B-carotene which can be converted to vitamin A by the human body. Some fat-soluble vitamins like D, E and K have also been detected, though not in sufficient amount (FAO, 1995).

1.5 World sorghum production

Cultivated sorghum is grown on about 42 million ha worldwide with an average production of 54 million t annually. About 90% of world sorghum is grown in developing countries, where it is a dietary staple food for more than 500 million people. It is estimated that 80% of the crop is produced by subsistence farmers, who often use local landraces that provide low but stable yields under marginal conditions; therefore it plays a vital role for farmers in dry areas where little else can grow (FAO, 2013). The five largest producers of sorghum in the world are the United States, India, Nigeria, Sudan and Ethiopia. USA usually leads total sorghum production and trade-off the crop due to very high yields, but India leads on acreage (FAO, 2013).

World sorghum production and area under cultivation have recorded mixed trends over the last five decades. Production expanded from 40 million tons at the beginning of the 1960s to 76 million t in the mid-1980s. However, by 1990 it had fallen to 58 million t. In Africa, production generally increased from 15 million t in the 1960s to 22 million t in 2010 (Fig. 2). The area under production worldwide also declined slightly during this period. The reduction in production was largely due to a decline in sorghum production in the USA and China.

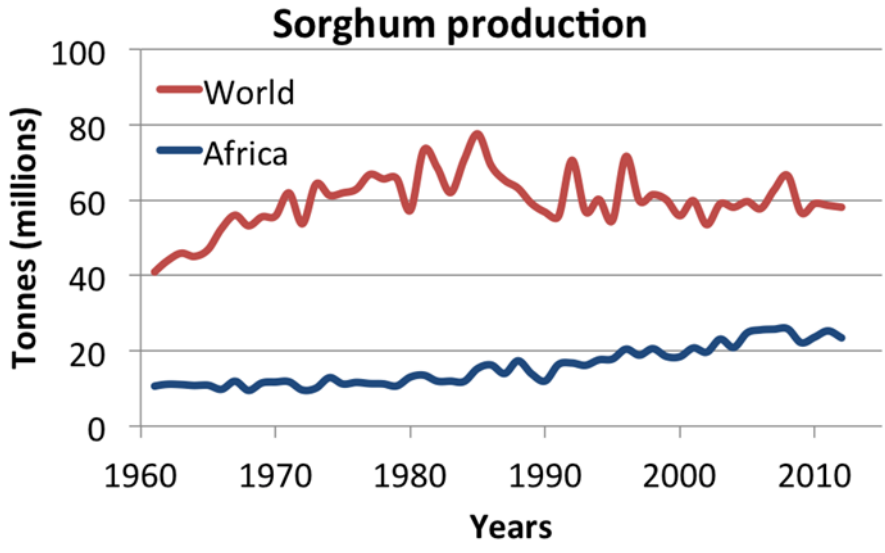

Figure 2. Sorghum production trend over the past five decades (FAO, 2013)

Production increased and in 2007/2008 it stood at 64.5 million tons worldwide. This increase could have been due to the increase in production area in countries like Brazil, Mali, Mexico, Niger, Sudan and Tanzania at the end of the 20th century (FAO, 2013).

In sub-Saharan Africa, the production data on sorghum can be considered as only the best estimates that are available as production data from small subsistence farms are difficult to obtain for countries. In many of the developing countries throughout the semi-arid tropics, inadequate infrastructure and lack of skilled manpower have contributed to the lack of information (FAO, 2013).

1.6 Breeding and conservation of sorghum

Breeding efforts on sorghum are largely conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Patancheru, India) and have been going on since this Institute was established in 1972. These efforts have been necessary because sorghum is threatened by both biotic and abiotic productivity-limiting constraints sorghum growing regions (Reddy *et al.*, 2004). The main aim of the breeding programs at ICRISAT has therefore been to improve the productivity of the crop. From the 1980s to the 2000s, there was a

gradual shift in their breeding strategy from initial wide adaptability to specific adaptations and trait-based breeding for threshold traits (Reddy *et al.*, 2004).

In Southern Africa, ICRISAT, together with regional governments established a Sorghum and Millet Improvement Programme (SMIP) in the early 1980s. Through this regional effort, enhanced germplasm was acquired from different sources and these were used with local collections to form the foundation of national breeding programmes (Chisi, 2010). Important breeding objectives for sorghum in the region have been towards increasing productivity of the crop and host plant resistance to pathogens and pests, and lately grain quality and drought adaptation (Chisi, 2010).

Breeding efforts are generally limited in Southern Africa (Mujaju, 2009), and sorghum is not an exception. Several sorghum cultivars have, however, been developed by local farmers throughout the region by systematic and gradual selection in their fields, both temporally and spatially. Some improved varieties have also been developed by breeders in the region, mostly by National Agricultural Research Systems (NARS) of different countries. The various NARS have directed their breeding research priorities towards increasing grain yield, excluding genetically low yielding landraces, developing resistant varieties to drought, the parasitic weed *Striga*, other pathogens and pests.

1.7 Genetic diversity and characterization

Genetic diversity within cultivated plant species is precious genetic resources that allows an increase in crop productivity and product quality as well as the development of varieties resistant to pests and pathogens (Geleta & Ortiz, 2013). Characterization of plant species using agro-morphological markers is the classical way of assessing genetic diversity for its use in plant breeding (Mujaju, 2011), especially in Southern Africa where resources for molecular characterization are limited. Doggett (1988) assets that in most crops, analyses of morphological traits that inherit according to Mendelian genetic principles were the earliest methods for estimating genetic diversity. The synthesis and categorization of morphological data into presumably genetic similarity groups is most useful when none is known about the population structure in a collection (Marshall & Brown, 1975). Different studies have been done in which phenotypic diversity index of morphological traits was used to measure genetic relationships in sorghum (Bucheyeki *et al.*, 2009; Habindavyi, 2009; Geleta *et al.*, 2005; Abdi *et al.*, 2002; Ayana & Bekele, 1999).

Characterization based only on morphological markers is usually not adequate to evaluate genetic diversity of plant genetic resources. This is because

they are highly influenced by environmental conditions, and different genes may contribute towards the expression of one morphological trait. Therefore, there is a need to complement them with molecular markers.

Molecular markers are basically nucleotide sequences corresponding to a physical position in a genome, and their polymorphisms between accessions allow the pattern of inheritance to be easily traced (Schulman, 2007). Their use for the genetic analysis and manipulation of important agronomic traits has become an increasingly useful tool in crop improvement and understanding of genetically complex quantitative traits. The availability of these markers for genetic diversity assessment is a quick way that breeders can use to select suitable genotypes for breeding (Lekgari & Dweikat, 2014). They have the potential to enhance the efficiency of plant breeding programs through a number of ways; DNA fingerprinting of elite genetic stocks, assessment of genetic diversity, increasing the efficiency of selection for difficult traits, and to make environment-neutral selection possible are some of them (Patil *et al.*, 2010). PCR-based markers are widely used in fingerprinting because of their high level of polymorphism (Warburton *et al.*, 2008) as well as their ease of detection (Sharon *et al.*, 1997).

Molecular tools, especially those employing DNA markers, have proven to be a robust and cost effective technology for the assessment of sorghum genetic diversity (Ng'uni *et al.*, 2011; Yang *et al.*, 1996; Deu *et al.*, 1994). Their use as a tool to assess relatedness in cultivated and between cultivated and wild sorghum have been successfully demonstrated (Ritter *et al.*, 2007; Menz *et al.*, 2004; Tao *et al.*, 1993).

Several types of molecular markers that are used for sorghum diversity assessments became available, and they vary in their complexity, reliability, as well as information generating capacity. The earliest DNA marker system, known as Restriction Fragment Length Polymorphism (RFLP), proved to be very useful, but their development and utilization is laborious, time consuming, expensive and not suitable for high-throughput automation. For these reasons, PCR-based markers such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Sequence Tagged Sites (STS), Single Nucleotide Polymorphism (SNPs) and their derivatives have become popular for molecular diversity research (Billot *et al.*, 2013). They provide useful information for breeders about genetic relationships between individuals (Mujaju, 2009). For sorghum, there have been a considerable number of studies designed to assess genetic diversity and phylogenetic relationships among sorghum cultivars. This research have used both isozyme and DNA based methods, for assessing cultivars from both developing and developed countries (Ramu *et al.*, 2013).

Out of the PCR based markers, simple sequence repeats (SSR) markers -also called microsatellites- quickly became the DNA markers of choice for plant and animal genomes because of the small quantity of genomic DNA required for their analysis and their suitability for automation and high-throughput platforms (Gutierrez *et al.*, 2005; Hearne *et al.*, 1992). SSRs are tandem repeats of di-, tri-, tetra-, penta- or hexa- nucleotide units in the DNA of plants and animals. They are abundantly distributed throughout the nuclear genomes of all studied plant species, which make them useful both for genetic mapping and for diversity studies (Ghebru *et al.*, 2002).

To date, a good number of microsatellite markers have been developed for sorghum that allows a very high rate of (and low cost) sorghum genotype assessment (Djè *et al.*, 2000; Smith *et al.*, 2000; Dean *et al.*, 1999). Several sorghum diversity studies involving SSR markers alone (Mutegi *et al.*, 2011; Ng'uni *et al.*, 2011; Thudi & Fakrudin, 2011; Ali *et al.*, 2008; Casa *et al.*, 2005; Ghebru *et al.*, 2002; Djè *et al.*, 2000) or in combination with other markers (Lekgari & Dweikat, 2014; Zhan *et al.*, 2012; Geleta *et al.*, 2006; Uptmoor *et al.*, 2003) have been undertaken. These studies have demonstrated that substantial genetic diversity exists between and among accessions both in the African gene banks as well as in the world sorghum collections, and that this diversity requires attention in terms of germplasm conservation. Poor correspondence between observed genetic structure and geographic origin is prevalent in most of the studies done in Africa (Mutegi *et al.*, 2011).

Lower genetic variation have been reported within than among accessions from recent SSR-based research involving sorghum accessions from Somalia (Manzelli *et al.*, 2007), from Zambia (Ng'uni *et al.*, 2011) and from Southern Africa (Ng'uni *et al.*, 2012). This observation is probably due to the predominantly selfing nature of the sorghum. Breeding systems of plant species are reported to have a significant impact on population variability with self-pollinating species being the least diverse and exhibiting higher between population than within population variation (Nybom & Bartish, 2000). The low levels of genetic variation among self-pollinated plant species is attributed to limited movement of genes through pollen, which also leads to greater differentiation among populations (Hamrick, 1983).

Ng'uni *et al.* (2012) reported clustering of sorghum accessions according to geographic origin of germplasm, when using SSR markers on sorghum accessions from Malawi, Tanzania and Zambia. However, Uptmoor *et al.* (2003) observed that accessions were not clustered according to their country of origin when using a combination of RFLP, RAPD and SSR markers on sorghum accessions from Botswana, Lesotho, South Africa, Zambia and Zimbabwe. Lack of clustering pattern of sorghum populations according to region or country of

origin could be attributed to out-crossing and seed movement across regions (Ayana *et al.*, 2000).

The various SSR studies have also demonstrated that these markers are highly polymorphic even among closely related sorghum cultivars, which demonstrates that they are highly informative (Uptmoor *et al.,* 2003).

2 Aim and objectives

The main aim of this thesis was to study the phenotypic and genotypic diversity of sorghum [*Sorghum bicolor* (L.) Moench] accessions from Southern Africa. The specific objectives were to:

1. Characterize genetic diversity among sorghum accessions from Southern Africa based on morphological traits
2. Assess patterns of genetic diversity revealed by microsatellite markers in order to identify genetic variation that is useful for sorghum genetic resources conservation and utilization in Southern Africa
3. Evaluate genetic relationships of sorghum accessions from different agro-ecological regions in Botswana
4. Determine the variation in protein and mineral contents among sorghum landrace accessions from Southern Africa
5. Identify locally adapted sorghum landraces that have high nutrients content for breeding and conservation programmes.

3 Materials and methods

3.1 Plant material

All sorghum germplasm used in this study were accessions provided by national gene banks of five countries in the Southern African Development Community (SADC) region.

Forty-seven *Sorghum bicolor* accessions were used for both the genetic diversity and nutritional diversity studies. Of these, thirty accessions obtained from the national gene bank of Botswana were used to analyse genetic diversity of sorghum accessions from Botswana (paper I). Twenty-two sorghum accessions from Botswana, Namibia, Swaziland, Zambia and Zimbabwe were used for the agro-morphological and SSR-based genetic diversity study (Paper II). Twenty-three accessions from Botswana, Namibia, Swaziland, Zambia and Zimbabwe were used for protein and mineral nutrient content analysis (Paper III).

3.2 Field experiments

The field experiment was conducted during the 2013-2014 growing season (December to July) at the Department of Agricultural Research at two research stations in Botswana, namely, Sebele (24°34'25"S and 25°58'00"E) and Pandamatenga (18°16'00"S and 25°39'00"E). The average minimum/maximum temperatures during the crop growing period at the two stations were 10/40°C and 12/42 °C, respectively, with an annual total rainfall of 281 and 558 mm. The soil types are sandy clay loam at Sebele and clay at Pandamatenga. The experimental design was randomised complete block design with two replicates. Each accession was planted in a separate plot, with rows of 5 m per accession. The distances were 1m between plots, 0.5m between rows and 0.25m between plants. No fertilizers were applied. Experimental measurement details are described in papers II and III.

3.3 Agro-morphological characterization

Agro-morphological data for 16 traits (10 qualitative and 6 quantitative) were recorded from 10 randomly chosen individual plants per accession in each replicate, based on the International Board for Plant Genetic Resources sorghum descriptors (ICRISAT, 1993). Ten qualitative traits recorded included waxy bloom, leaf midrib colour, stalk juiciness, juice flavour, awns, inflorescence compactness and shape, shattering, glume colour, grain covering and grain

colour. Quantitative characters recorded were plant height, days to flowering, inflorescence length, inflorescence width, 100 seed weight and grain yield. All agro-morphological traits used in the study are described in Paper II.

3.4 Nutritional analysis

3.4.1 Protein content determination

Sorghum kernels were ground to a fine powder with a Kinematica A10 Grinder (Switzerland). Sorghum flour samples were then freeze dried to constant weight and around 100 mg was weighed into tin capsules prior to total nitrogen analysis. An aliquot was burnt in an elemental analyzer (Vario max CN analyzer from Elementar) at 900°C. Passage of the produced gasses over special absorbent columns eliminated CO_2 and H_2O. Nitrogen content was measured by passing the remaining gasses through a column with a thermal conductivity detector at the end. Glutamic acid (C5H9NO4; C: 40.81% N: 9.52%) was used as standard reference. A protein factor of 6.25, equivalent to 0.16 g of nitrogen per gram of protein, was used to estimate protein content in sorghum, as recommended by Merril and Watt (1973).

3.4.2 Mineral content determination

Approximately 0.5 g of sorghum flour was packed in a Teflon capsule and placed in a microwave oven (MARS 5 from CEM with a regulated pressure and temperature, which were kept at 375 psi and 185°C, respectively). Each sample was digested in 10 ml solution (7 ml of concentrated nitric acid and 3 ml of water), which was then diluted with water up to 50 ml before analysis. The samples were analysed for mineral content at the Instrumental Chemistry Laboratory (Department of Biology, Lund University, Sweden) using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES; Perkin-Elmer, OPTIMA 8300). Atomic spectrometry standards from Perkin-Elmer, SPEX, Accu Standard and Merck were used for this analysis. The mineral content was recorded in microgram of mineral per gram of flour (µg/g).

3.5 DNA extraction

Seeds were planted in seedling trays and grown in the greenhouse at 25°C for approximately 10 days. Leaves (approximately 6 cm in length) were sampled into 2 ml Eppendorf tubes and frozen in liquid nitrogen, then freeze dried and maintained on silica gel at -80°C (Paper I) until they were milled using a Retsch MM400 shaker (Hann, Germany). For the second study (Paper II), leaf samples

for DNA analysis were collected from 10 plants grown in the field for approximately 2 weeks. The samples were kept on silica gel in small plastic bags to keep them dry until they were milled. DNA was separately extracted from leaves of 12 individual plants per accession using a modified cetyl trimethyl ammonium bromide (CTAB) protocol, as described in Bekele *et al.* (2007) for the first and second study.

3.6 SSR PCR reaction

For the genetic diversity analyses of Botswana sorghum accessions (Paper I), the screening of SSR primers for amplification, optimization of PCR conditions and detection of polymorphism lead to the selection of ten SSR primer pairs. Similarly, eleven primer pairs were selected for the diversity analysis of sorghum accessions from Southern Africa (Paper II). The selected primers and their amplification conditions were provided in Papers I and II.

The forward primers of each pair of selected primers were 5'-labeled with either HEX, 6-FAM, VIC or NED fluorescent dyes. The reverse primers were PIG-tailed with "GCTTCT" to avoid a non-template addition of a single nucleotide by Taq DNA polymerase to the PCR product, as previously described in Ballard *et al.* (2002). Reactions lacking DNA were included as negative controls. PCR reactions were prepared in 96-well thin wall PCR plates and amplifications were run in Gene Amp® PCR System 9700 (Applied Biosystems Inc., USA) at conditions optimized for each pair of primers. The PCR programs used are detailed in Papers I and II.

The PCR products were then multiplexed into panels. Different panels contained PCR products from different loci. PCR products labelled with the same fluorescent dye but multiplexed in the same panel had a size difference of at least 100 base pairs to avoid overlapping. Multiplexed PCR products were then analysed using ABI 3730 capillary DNA sequencer (Applied Biosystems) at University of Copenhagen, Denmark. The size standard ROX 58-352 was used as a molecular size marker.

3.7 Data scoring and analysis

3.7.1 Genetic diversity analysis

GeneMarker 2.4.0 (Softgenetics) was used for peak identification and fragment sizing. Allelic data for a particular locus was recorded as fragment size at a co-dominant locus and the genotype of each individual at each locus was recorded. When a PCR product was not obtained, data for the specific loci and samples were treated as missing values.

Genetic diversity parameters for each locus were estimated using POPGENE version 1.31 (Yeh *et al.*, 1999). Arlequin version 3.0 (Excoffier *et al.*, 2005) was used for the analysis of population genetic structure. For the analysis of molecular variance (AMOVA), sorghum accessions were grouped according to their ecological region, their race and the ethnicity of the local populations inhabiting the sites where the accessions were originally collected. Sorghum accessions were also grouped according to donor country. Cluster analysis and bootstrapping were performed with the FreeTree Freeware program (Pavlicek *et al.*, 1999) based on Nei's standard genetic distance (Nei & Li, 1979). TreeView 1.6.6 program (Page, 1996) was used to view the trees.

3.7.2 Agro-morphological data analysis

Analysis of variance (ANOVA) was performed on agro-morphological quantitative data using Minitab (version 17.0) statistical package. Details of analysis of both qualitative and quantitative data are provided in Paper II.

3.7.3 Mineral nutrient and protein content analyses

Minitab (version 17.0) was used for analysis of both protein and mineral nutrients. The data was subjected to analysis of variance (ANOVA). Tukey's test was carried out for pairwise comparisons of means. Pearson's correlation test was carried out to assess the association between pairs of nutrients.

4 Summary of results and discussions

4.1 Molecular genetic diversity in *Sorghum bicolor* (L.) Moench accessions (Papers I & II)

Characterization of genetic diversity in crop germplasm is essential for rational utilization and conservation of genetic resources (Geleta & Ortiz, 2013; Thudi & Fakrudin, 2011). The 10 microsatellite markers used in the genetic diversity study for Botswana sorghum accessions revealed significant genetic variation among accessions, indicating the importance of microsatellites for characterizing genetic diversity among closely related individuals (Ng'uni *et al.*, 2011; Uptmoor *et al.*, 2003). Genetic diversity analysis within the 30 sorghum accessions from Botswana revealed considerable amount of genetic diversity. The ten microsatellite loci used in the study revealed a total of 53 alleles, with 7 of the 30 accessions having accession specific rare alleles. The total number of alleles recorded in our study was lower than previously reported by other authors (Thudi & Fakrudin, 2011; Deu *et al.*, 2008; Folkertsma *et al.*, 2005; Ghebru *et al.*, 2002). These studies, however, assessed a higher number of accessions from different geographic areas. Comparable results to our study were obtained by Ng'uni *et al.* (2011).

A total of 11 SSR loci were used in the analysis of genetic diversity of sorghum accessions from five countries in Southern Africa. The loci were polymorphic and among them revealed a total of 70 alleles across all accessions, with 2 to 15 alleles per locus. This result suggests that a high genetic diversity exists in Southern African sorghum germplasm.

4.2 Genetic structure and relationships among sorghum accessions

Analysis of molecular variance (AMOVA) of the SSR data for 30 sorghum accessions from Botswana revealed significant differentiation among accessions ($P < 0.001$; Table 1). Genetic differentiation among accessions accounted for 70% of the total variation while the within accession variation accounted for the remaining 30%. AMOVA, however, revealed no significant variation among groups of sorghum accessions grouped according to agro-ecological zones, or ethnicity of the local populations of the collection sites of the accessions (Bakwena, Bakgatla, Barolong, Bangwato, Batawana and Bakalaka) and when the grouping was done according to races of the accessions (Bicolor, Durra, Guinea, Kafir). AMOVA from microsatellite data for the sorghum accessions from Southern Africa revealed significant differentiation among individual

plants, among accessions and among countries ($P < 0.001$; Table 1). Genetic differentiation among accessions accounted for 66.9% of the total variation while the within accession variation accounted for 23.6%. When accessions were grouped according to donor country, variation among groups accounted for 9.5%, and the remaining 5% variation was observed between individual plants within accessions.

The unweighted pair group method with arithmetic mean (UPGMA)-based cluster analysis did not group the sorghum accessions from Botswana according to racial classification, ethnicity or agro-ecological regions (Figure 3). A similar observation was made by Djè *et al.* (2000). They found a scattering of accessions belonging to the same race or geographical region when a matrix plot of individual sorghum accessions based on R_{ST} distances. Several other studies have reported weak differentiation among accessions according to geographic region (Uptmoor *et al.*, 2003; Ayana *et al.*, 2000). This observation could be attributed to the practice of seed exchange through traditional and commercial seed systems.

Farmers usually exchange seeds in order to access new cultivars with desirable traits. In Botswana, as also common in other sub-Saharan African countries, farmers exchange traditional crops like sorghum following collective socio-cultural and traditional activities that involve relationships between friends and relatives (Deu *et al.*, 2008). Ng'uni *et al.* (2011) however, reported that cluster analysis on Zambian sorghum accessions grouped them according to their geographic regions of origin, with 12.4% variation between regions. This finding was similar to that reported by Ghebru *et al.* (2002) for a collection of Eritrean and world sorghums, and this could be attributed to the fact that the accessions were sampled from a wider geographic region.

A pattern of genetic relationships where accessions from the same geographic region were genetically similar, as in Ng'uni *et al.* (2011), could be attributed to the existence of seed exchange patterns of such landraces between relatives or friends in the communities within that locality. A landrace, which constitute an accession, is the outcome of a continuous and dynamic development process involving maintenance and adaptation of germplasm to the environment and specific local needs by a community. Farmers often exchange seeds of landraces with other farmers within a locality (Ng'uni *et al.*, 2011)

Table 1. *SSR based analysis of molecular variance for 30 sorghum accessions from Botswana: without grouping the accessions (A), and by grouping the accessions according to agro-ecological zones (B), sorghum races (C) and ethnicity of the local population of the sampling site (D)*

Origin	Accessions	Source of variation	d.f.	Variance components	Percentage Variation
Botswana	(A) Ungrouped	AA	29	Va =1.69	69.7***
		WA	690	Vb = 0.73	30.3
		Total	719	2.40	
	(B) Ecological zones	AG	2	Va = 0.05	-1.2
		AAWG	27	Vb = 2.03	70.7***
		WA	690	Vc = 0.83	30.5
		Total	719	2.39	
	(C) Races	AG	3	Va =0.02	0.97
		AAWG	26	Vb = 1.66	68.8***
		WA	690	Vc = 0.73	30.2
		Total	719	2.41	
	(D) Ethnicity	AG	5	Va =0.06	2.53
		AAWG	24	Vb = 1.63	67.31***
		WA	690	Vc =0.73	30.16
Botswana, Namibia, Swaziland, Zambia, Zimbabwe	(A)ungrouped	AA	19	Vb =0.55	66.92***
		WA	180	Vc =0.147	17.98***
	(B)countries	AG	4	Va =0.07	9.56***
		AAWG	15	Vb =0.55	66.92***
		WA	180	Vc =0.147	17.98

AA = among accessions, WA = within accessions, AG = among groups, AAWG = among accessions within groups. *** indicates significant at 0.001

The Nei's standard genetic distance-based UPGMA cluster analysis for sorghum accessions from Botswana, Namibia, Swaziland, Zambia and Zimbabwe grouped the sorghum accessions into five groups (Figure 4). The dendrogram indicated the differentiation among the accessions, grouping those from different countries together. It is possible that some of these accessions, even though growing in different countries, could have been freely exchanged among farmers across borders of these countries

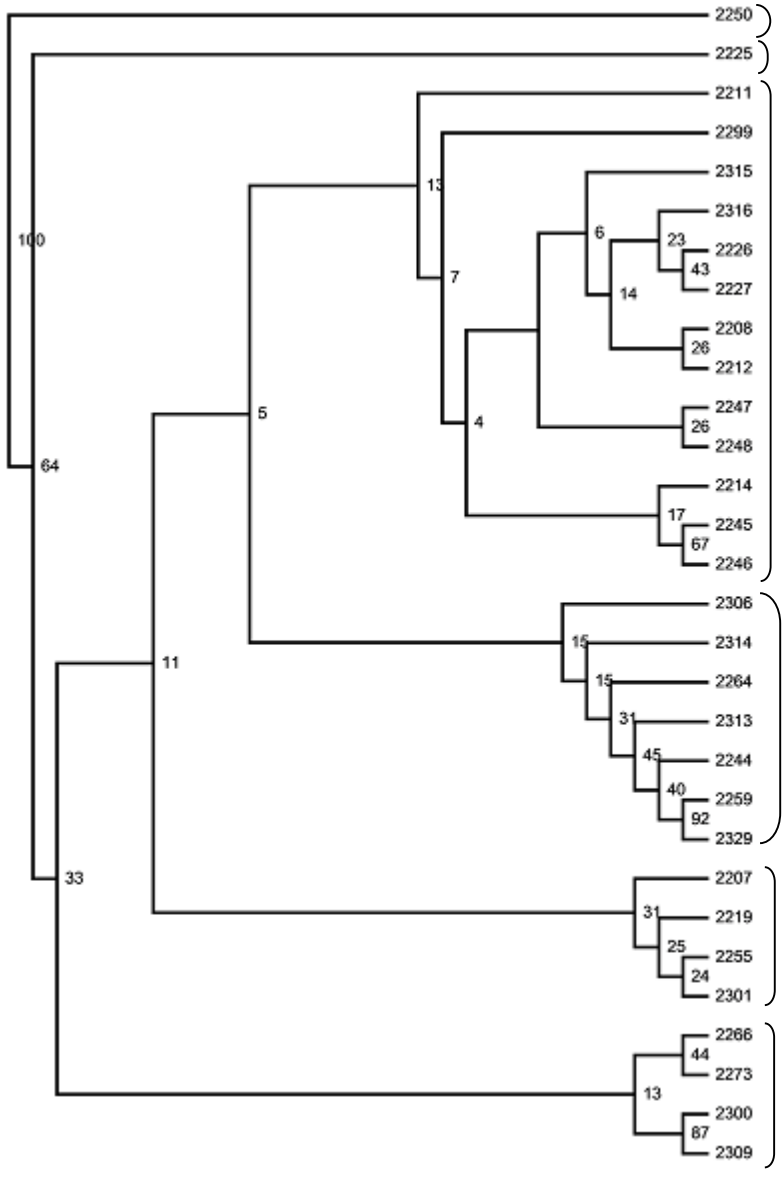

Figure 3. UPGMA dendrogram showing the clustering pattern of 30 sorghum accessions from Botswana based on Nei's standard genetic distance. Bootsrap values generated from 1000 resampling in the FreeTree program are shown between branches

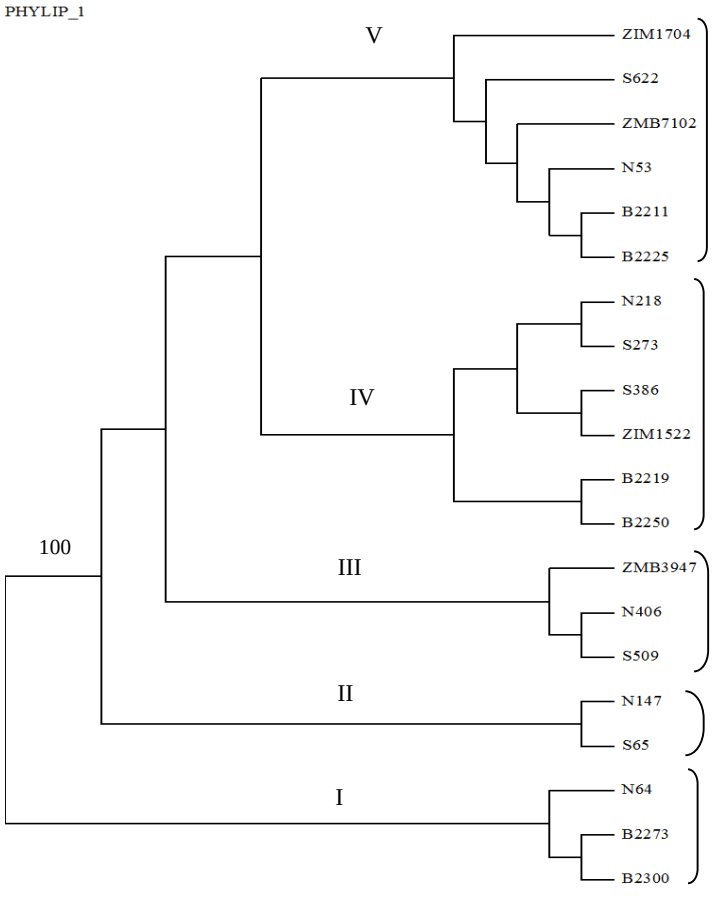

Figure 4. SSR based UPGMA dendrogram generated using Nei's standard genetic distance for 20 sorghum accessions. Bootsrap value generated from 1000 resampling using the FreeTree program is shown for the first branch. Letters before accession numbers represent the accession donor countries: B= Botswana, N= Namibia, S= Swaziland, ZMB= Zambia, ZIM= Zimbabwe.

4.3 Agro-morphological diversity in sorghum accessions

The variation among the 22 sorghum accessions studied was demonstrated by morphological traits. The high level of variation exhibited by both qualitative and quantitative traits indicates the potential of these accessions for sorghum breeding. The variation could be a valuable source for sorghum improvement programs in the five countries and the SADC region at large. Other studies

(Gerrano *et al.*, 2014; Geleta *et al.*, 2005) have also observed genetic variation among sorghum germplasm that could be useful for improvement programs. Traits such as 100 seed weight, inflorescence length and width, had positive effects on grain yield hence can easily be selected together when planning breeding programs.

4.3.1 Qualitative characters

A majority of the accessions (about 91%) had white leaf midrib colour and had non-juicy stalks. Only two accessions, B2219 from Botswana and ZMB3947 from Zambia, exhibited juicy stalks and dull green leaf midrib colour. A few (3) of the accessions were mostly bloomy, while the remaining 19 accessions had only slightly present to medium waxy bloom. Significant variation ($P < 0.05$) among accessions was noted for inflorescence compactness and shape.

Thirteen (59%) of the accessions had 75% grain covering, three accessions had 25% grain covering, 2 had 50% covering, 3 had glumes longer than grain and only one had its grains fully covered. Glume colour was skewed towards sienna (10 accessions), followed by black (5), then white (3), red (2), mahogany (1) and grey (1). Only five of the accessions had awns while the rest did not have awns. Twenty of the 22 recorded very low to low shattering, one was intermediate (B2273) and only one recorded high shattering (N406). Grain colour was skewed, with red dominating (13), 7 were whione (ZIM1317) had yellow grain and one (B2219) had buff coloured grain.

4.3.2 Quantitative characters

The ANOVA of the quantitative morphological traits revealed a significant variation for 5 of the 6 traits. Days to flowering among accessions was significantly different ($P < 0.05$). Accessions B2219, B2225 B2250, B2300 and ZIM1704 were early maturing, while late accessions included N53, N218, N406, ZIM1522, ZMB3947 and ZMB6986 were the latest.

Correlation coefficients of six pairs of the quantitative traits (Table 2) showed a significant positive correlation between yield and inflorescence length ($r = 0.611$); inflorescence width ($r = 0.897$), and 100 seed weight ($r = 0,620$). Correlation between inflorescence width and days to flowering was also significant ($r = 0.593$). Significant negative correlation was recorded between 100 seed weight and plant height ($r = -0.410$) as well as 100 seed weight and days to flowering ($r = -0.430$).

Table 2. *Correlation coefficients of quantitative morphological traits used to characterize sorghum accessions from Southern Africa.*

Traits	Height	DTF	Infl. length	Infl. width	100SW	Yield
Height	1.00	0.212	0.455*	0.164	-0.410*	0.334
DTF		1.00	-0.025	0.593*	-0.430*	0.356
Infl. length			1.00	0.254	-0.336	0.115
Infl. width				1.00	-0.170	0.897**
100 SW					1.00	0.620*
Yield						1.00

** indicates correlation is significant at 0.01 while * is for correlation is significant at 0.05 level

Based on morphological traits, accession B2219 from Botswana was the most distinct, and it grouped totally separately from the other accessions. This could be due to its sweet and juicy stalk, as well as its buff coloured grain. This accession is a non-grain or sweet type of sorghum belonging to the bicolor race and in Southern Africa its stalk is normally used for chewing whereas the grain is not palatable as food. It has been reported that bicolor types of sorghum have more primitive morphological characters (Harlan & De Wet, 1972) and are associated with a wide geographic distribution (Djè *et al.*, 2000)..

Three accessions N53, N218, B2250 had similar phenotypic traits and had the most compact and short inflorescence with curved peduncle typical of durra type sorghums, with the grain covered up to 75% by the glumes. They also had other phenotypic traits in common as they were among the few that had awns, were least shattering, had red grains and were of short stature. In the molecular analysis, these three accessions had three common alleles at three different loci, which were not found in the other accessions. These durra types of sorghum are not very common in Southern Africa and could have been introduced from other African locations. Two accessions had morphological traits typical of guinea types, 7 showed characteristics of caudatum and 7 displayed kafir type characteristics. Caudatum, guinea and kafir type sorghums are the most common in Southern Africa and evolved from other African sorghums (Doggett, 1988). With microsatellite analysis, these had the least number of rare alleles when compared to the other accessions. This is consistent with previous research showing an absence of rare alleles in southern equatorial accessions (Deu *et al.*, 2006), with kafir sorghums displaying little genetic differentiation (Doggett, 1988) and being mostly restricted to Southern Africa (Djè *et al.*, 2000).

4.4 Nutritional content variation in accessions

4.4.1 Nutrient variation in sorghum accessions

A significant variation was revealed among the sorghum accessions studied for grain protein and mineral nutrient content. Genetic factors played a major role for the variation between accessions, such as differences in the sorghum accessions' ability to absorb nutrients from the soil under prevailing environmental conditions. The accessions could also have different levels of requirements for these mineral elements, which could also be solely genetic. Hence, genetic background of sorghum genotypes is a very important factor determining nutrient contents. Appropriate cultivar choice for enhancing nutrient composition in sorghum genotypes is possible considering the reported variation.

Protein, Fe, Mg, Mn, Na and P were significantly different between the two sites while Ca, K and Zn were not. This suggests that environmental factors may have low effects the content for Ca, K and Zn. Hence, selection for breeding material can be done on different sites. For those nutrients with significant differences between sites, the effect of cultivation site should be considered when selecting cultivars for breeding. However, additional data from various sites (environments) should be analysed before a strong conclusion can be made regarding the effect of environment on these nutrients.

The significant variation obtained for grain protein and mineral content among accessions is encouraging for selecting potential accessions for genetic improvement. It has been emphasized that sorghum parents with more diversity among themselves are expected to exhibit a higher amount of heterotic expression and a broad spectrum of variability in segregating generations (Sabharwal *et al.*, 1995). Fe content reported in this study was lower than those reported by other authors (Ng'uni *et al.*, 2012; Shegro *et al.*, 2012; Kumar & Kumar, 2009), probably because of the different laboratory methods used in the different studies. However, protein and mineral nutrient content range reported in this study was similar to those reported by FAO (1995). Mn and P contents were similar to those obtained by Pontieri *et al.* (2014) and Shegro *et al.* (2012), who however reported higher contents of Ca, K, Mg and Fe.

4.4.2 Correlation among nutrients

Pearson's correlation coefficients among mineral elements and protein are given in Table 3. A significant positive correlation was obtained between different mineral nutrients. This has implications for the possibility to combine selection for correlated nutrients in a single agronomic background. However, it

has to be determined whether breeding for high concentration of one nutrient correlated to the other in such a manner increases the concentration of the other and vice-versa. Similar to the present study, significant positive correlation between Fe and Zn have previously been reported in sorghum (Ng'uni *et al.*, 2012; Shegro *et al.*, 2012; Kayodé *et al.*, 2006; Reddy *et al.*, 2005), wheat (Velu *et al.*, 2011) and in rice (Zhang *et al.*, 2004). Significant positive correlations were also observed between Ca and K, Fe and Mn and P as well as between Mg and P and P and Zn. Protein recorded a significant positive correlation with Mg and P.

Table 3. *Pearson's correlation coefficients showing pair-wise association among eight mineral elements and protein in sorghum accessions from five countries in Southern Africa.*

	Protein	**Ca**	**Fe**	**K**	**Mg**	**Mn**	**Na**	**P**
Ca	-0.092							
Fe	0.200	0.085						
K	-0.028	0.799***	0.129					
Mg	0.440*	0.203	0.327	0.227				
Mn	-0.132	0.163	0.508**	-0.101	0.682			
Na	-0.052	0.444*	0.278	0.255	0.160	-0.275		
P	0.512**	0.226	0.473*	0.449*	0.857***	0.640**	0.217	
Zn	0.275	0115	0.673**	0.045	0.655**	0.585**	0.043	0.524**

***significant at P = 0.001; **0.01; * 0.05.

4.4.3 Genotype x environment (G×E) interaction and heritability

Ca, Fe K, Mg, Mn and P recorded high G×E, ranging between 20 and 29%. Protein had the lowest G×E effect (2.6%). Na and Zn also recorded low values for G×E at 18% each (Table 4).

Table 4. *Relative variance explained by genotype (accession), environment (site), and genotype by environment (G×E) interaction on nutrient content of 23 sorghum accessions from five countries in Southern Africa*.

	Relative variance (%)								
Source	Protein	Ca	Fe	K	Mg	Mn	Na	P	Zn
Accession (genotype, G)	54.2	57.5	50,4	61.8	57.1	25.4	25.4	59.5	63.0
Site (environment, E)	1.9	0	14.9	0	9.8	37.8	10.1	11.0	1.0
G×E	2.6	20.1	21.5	25.1	21.2	28.9	17.7	24.9	17.9
Residual (Error)	17.7	21.4	12.0	9.0	10.2	7.7	41.8	4.4	16.6

Low G×E interaction and variation of genotypes for mineral nutrient concentration in the sorghum grain suggest that breeding for enhanced concentrations of the nutrients could be done across environments. The nutrient contents did not vary much across locations, hence their concentrations in the sorghum grains were stable. This was also reflected in quite high values for broad-sense heritability of grain nutrients (Table 5).

Broad-sense heritability (H^2) of the mineral elements and protein was estimated from the analysis of variance following Nyquist and Baker (1991). The formula used was $H^2 = V_G/V_P$ (Table 5).

Table 5. *Variance components and broad sense heritability for protein and mineral nutrient content in 23 sorghum accessions from Southern Africa.*

Variance component	**Nutrients**								
	Protein	Ca	Fe	K	Mg	Mn	Na	P	Zn
V_G	1.79	3307	64.4	248193	50045	48.7	20.3	308650	45
V_{GE}	0.86	1157	27.4	100837	18551	55.5	14.2	129247	12.8
V_R	0.29	616.8	7.7	18041	4460	7.4	16.7	11501	6.3
V_P	2.3	4039	80	303122	60435	78.2	31.6	376148	53
V_{GE}/V_G	0.48	0.35	0.43	0.41	0.37	1.14	0.70	0.42	0.28
H^2	0.78	0.82	0.80	0.82	0.83	0.62	0.64	0.82	0.85

H^2 = broad sense heritability, V_G = genotypic variance, V_{GE} = genotype x environment variance, V_R = residual variance, V_P = phenotypic variance

The ratio between the G×E variance components to the genotypic variance component gives an insight about the magnitude of the genotype-environment interaction (Gomez-Becerra *et al.*, 2010; Peterson *et al.*, 1986). In this study, low G×E interaction and low V_{GE}/V_G ratios indicate that there are no specific adaptation patterns for the accessions studied, so when breeding for higher nutrient content of these minerals in sorghum, any of the locations may be used for cultivation.

5 Conclusions and future prospects

5.1 Conclusions

Southern African sorghums contain significant and valuable genetic diversity as indicated by the observed number of alleles and the presence of rare and unique alleles in most of the accessions.

The pattern of genetic diversity revealed with both agro-morphological markers and microsatellites in Southern African sorghums may offer new opportunities to relate that diversity to the diversity structure for important agronomic traits such as grain nutrient quality and mineral content characteristics.

The patterns of genetic relationships observed in this study should provide more detailed insights for genetic resource conservation and utilization of sorghum germplasm in the SADC region.

The sorghum accessions from Botswana, Namibia, Swaziland, Zambia and Zimbabwe exhibited significant variation in grain protein, calcium, iron, magnesium, potassium and zinc contents. These results suggest that there is considerable variability for essential nutrients in the sorghum landrace accessions.

Correlation analysis of protein and mineral nutrients has indicated that it is possible for simultaneous improvement of different nutrient, assuming there is no penalty in the agronomic traits when combined with these minerals.

The study on nutritional contents variation has shown that identification of sorghum germplasm for breeding for improvement of mineral nutrients and protein is promising.

The observed low G×E interaction in this study indicate that there are no specific adaptation patterns for the accessions studied, so when breeding for higher nutrient contents, different sites can be used for cultivation.

5.2 Future prospects

Since the accessions studied actually constitute farmers' varieties, those that exhibited good levels of genetic diversity should be given priority in conservation strategies.

Some of the SSR loci used in this study may be significantly linked to important agronomic traits, so further characterization at both phenotypic and molecular levels is crucial. Quantitative trait loci (QTL) mapping that explain

the variation in the traits considered in the present study can contribute to more effective sorghum breeding and improvement.

In sorghum breeding, it is necessary to identify germplasm that breeders can use to improve not only yield, but essential nutrients needed for human nutrition. This is necessary to overcome malnutrition existing in world rural populations.

Accessions exhibiting a relatively high nutrient content in this study could be used to improve farmers' varieties with preferred agronomic traits such as early maturity, grain colour and yield.

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