Evaluation of advanced sorghum lines for use as possible parents in breeding for bio-ethanol production

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Dissertation submitted in fulfilment of the requirements for the degree of Master of Science in Plant Breeding

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Pietermaritzburg

South Africa

January 2017

Abstract

Production of bioethanol from renewable feedstocks is among the ways of reducing consumption of gasoline and environmental pollution. Renewable energy sources also ensure continuous energy supply. Efforts have been made to develop renewable feedstocks for production of fuels and chemicals and many crops have been investigated for this purpose. Grain sorghum is one of the most promising candidates because of many desirable characteristics it has, such as high starch content which is the major component for bioethanol production, drought tolerance, wide adaptability and short life cycle, cheap starting material, excellent nitrogen use efficiency, water logging tolerance and salinity resistance. The current study therefore, aimed at evaluating advanced sorghum lines and selected breeding lines for potential use in ethanol production under South African conditions and characterising sorghum breeding lines using agro-morphological traits under two planting dates.

The field evaluation was conducted using 45 advanced grain sorghum lines and five landrace/improved varieties. The breeding lines were planted using a 5 x 10 alpha lattice design with three replications at the Agricultural Research Council-Grain Crops Institute (ARC-GCI) experimental farm during 2015/2016 summer season. Data were recorded on 13 quantitative and four qualitative characters (agro-morphological characters) and three biochemical traits. Near Infrared Spectroscopy (NIR) was used in analysing starch, while protein content was determined through Bradford assay and *in vitro* pepsin method was used to determine protein digestibility. The analysis of variance for quantitative traits was highly significant for all traits implying that morphological traits differed among the advanced lines and varieties across the two planting dates. Most of the breeding lines were high yielding under the first planting date (10 December) with a mean grain yield of 3.6 t/ha, while under the second planting date (10 January) the mean grain yield was 3.2 t/ha. The majority of the breeding lines studied were early maturing as shown by the mean number of days to 50% flowering (71 days). The breeding lines also exhibited varying degrees of heritability estimates for the traits measured, where all traits showed high broad sense heritability (≥80%) and therefore would respond to selection. Correlation analysis indicated some important associations between the quantitative traits. Grain yield showed significant positive correlation with panicle weight, 1000 grain weight, number of panicles per plot, number of grain per panicle and plant height. The principal component analysis revealed that the three most important components contributed 33.21%, 16.76% and 14.38% to the total variation. The traits that contributed most to the variation were grain yield, plant height, panicle number per plot, 1000 grains weight and panicle

weight per plot. These results would be useful in a breeding programme for selecting sorghum breeding lines to improve production.

The ANOVA showed highly significant ($p \leq 0.001$) differences among the breeding lines for starch content, protein content and protein digestibility. The starch content varied from 63.28% to 71.29% across the two planting dates with a mean value of 67.51%. The protein content of the breeding lines ranged between 9.21% and 15.06% across the two planting dates with an overall mean of 12.24%. The protein digestibility ranged from 33.87% to 82.22% across the two planting dates with a mean value of 64.22%. A positive correlation was shown between starch content and grain yield, while the correlation between protein content and starch content was highly significantly negative. Therefore, there would be potential for selecting sorghum breeding lines for starch content, protein content and protein digestibility useful for bioethanol production. The breeding lines, 05 – Potch – 151, 15ELC F_6#2, 15ELC F_6#47, 15ELC F_6#43, and 15ELC F_6#45 showed high potential for bioethanol production, with high starch content, low protein content and high protein digestibility.

Declaration

I, Awadhi A. Mashombo, declare that:

1. The research reported in this dissertation, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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    a. their words have been re-written but the general information attributed to them has been referenced;
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5. This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the referencing sections.

Signed

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Awadhi A. Mashombo

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

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Dr. Julia Sibiya (Main-supervisor)

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Dr. Nemera Shargie (Co-supervisor)

Acknowledgements

Praise be to Almighty God, Lord of creation, The Compassionate, the Merciful, King of Judgement day! You alone we worship, and alone we pray for help.

I would like to convey my sincere gratitude, appreciation and thanks to various people and institutions that assisted and contributed to the successful completion of this study.

- First of all, I would like to express my special thanks and appreciation to my supervisors Dr. Julia Sibiya and Dr. Nemera Shargie for their close supervision, guidance, constructive criticism, support and hospitality during the whole period of my study.
- I would like to forward my gratitude to The Alliance for a Green Revolution in Africa (AGRA) for sponsoring my study at the University of KwaZulu-Natal, South Africa.
- Special thanks to the MSc Plant breeding administrators, Ms. Jayshree Singh and Ms Andile Mshengu for their devotion, positive and timely responses.
- I would like to acknowledge Dr. Amelework Assefa, Dr. Alina Mofokeng and Dr. Samson Tesfay for their technical support in laboratory work and advice.
- I remain very grateful to the Agricultural Research Council - Grain Crops Institute at Potchefstroom, South Africa for providing a platform for my research and hosting my internship and support in resources to successfully complete my research. Here it is appropriate to extend my special thanks to the research technicians' Mr. Paul Rantso, Ms Desiree Biya, and Ms Julia Sibiya for unreserved assistance in my research.
- Special thanks to my friends in the 2015 MSc. Plant breeding class, for their wonderful friendship and encouragement throughout the study.
- Last but not least, I would like to thank my family for the moral support, love and encouragement during my study.

Dedication

I would like to dedicate this work to:

My parents, Abdulrahmani Mashombo and Mwetabu Hassan, your love, wisdom and support has nourished my success.

My wife Ramla Rajabu and our son Abdulrahmani who endured the pain of separation and did not get much attention and love from me for the two years of this study.

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List of abbreviations

ANOVA	Analysis of variance
ARC-GCI	Agricultural Research Council – Grain Crops Institute
CTs	Condensed tannins
CV	Coefficient of variation
EV	Environment variance
FAOSTAT	Food and Agriculture Organization of the United Nations Statistical Databases
GA	Genetic advance
GAM	Genetic advance expressed as percentage of mean
GCV	Genotypic coefficient of variation
GV	Genotypic variance
H^2	Broad sense heritability
HTs	Hydrolysable tannins
NSP	Non-starch polysaccharides
PCA	Principal component analysis
PGV	Phenotypic coefficient of variation
PV	Phenotypic variance
R^2	Coefficient of determination
RFA	Renewable Fuels Association
USA	United States of America

CHAPTER 1

INTRODUCTION TO THE DISSERTATION

Sorghum (*Sorghum bicolor* (L.) Moench) is among the important cereal grain crops produced in the world. It is the fifth most important staple cereal food crop after wheat, rice, maize and barley based on the total grain production and area planted (FAOSTAT, 2016). In Africa, sorghum is ranked the second most important cereal after maize. It is the staple food crop for many people in semi-arid regions of the world, especially in Africa, where it is grown by many resource poor farmers (Kenga et al., 2004). The crop is a hardy, drought-tolerant and nutrient-efficient C4 grass, widely adapted throughout the world (Murray et al., 2008). In 2014 a total area of 44.2 million ha was cultivated worldwide (FAOSTAT, 2016). The area includes Africa, America, Asia, Europe and Oceania in the distribution shown in the map Figure 1.1.

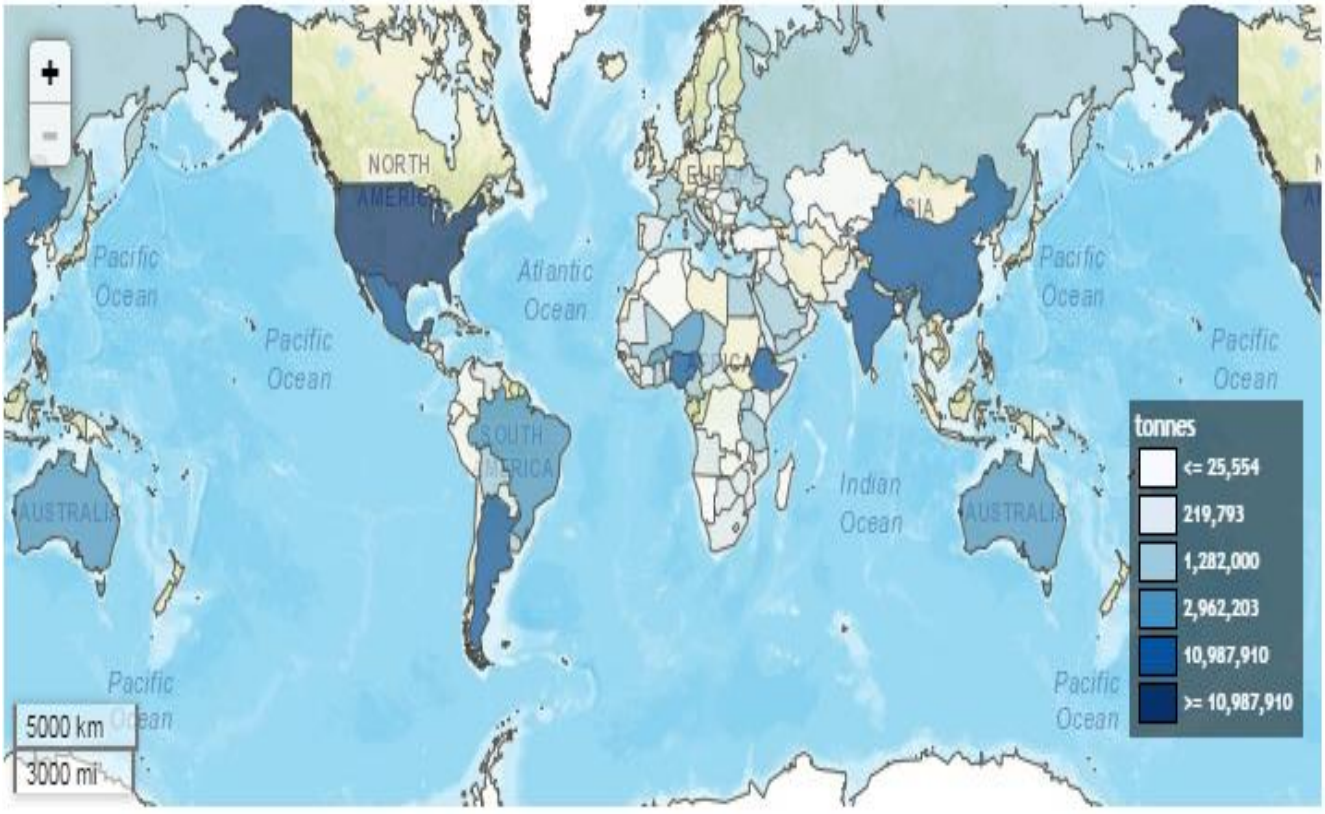

Figure 1.1 Global sorghum producing areas

(FAOSTAT, 2016)

The productivity between regions varies a lot due to the level of commercialisation and the corresponding adoption of new technologies. The average global production and yield of sorghum is shown in Table 1.1 below.

Table 1.1 World sorghum production by regions 2014

Region	Area cultivated (ha)	Yield (t/ha)	Total Production (t)	Production share by region (%)
Africa	29,017,018	1.10	28,985,167	42.70
America	6,861,651	4.28	26,635,394	39.20
Asia	7,403,629	1.43	9,591,470	14.10
Europe	389,556	3.88	1,371,640	2.00
Oceania	533,116	2.66	1,286,990	1.90

Source of Data: (FAOSTAT, 2016)

In Africa, sorghum production is mostly concentrated in areas where annual rainfall is less than 500 mm due to its drought tolerance. That being the case, most of the countries where sorghum is an important arable crop are dry and are areas at risk of desertification. These include the northern parts of Africa, dry parts of the west Africa and central African countries, the semi-arid parts of east Africa and the drier western parts of southern Africa (Botswana) (Taylor, 2003). In east Africa where overall rainfall is good, sorghum is also an important crop (Taylor, 2003). Total sorghum production in South Africa in 2014 was 150,920 tonnes and the average yield was 3.03 t/ha (FAOSTAT, 2016).

Sorghum has a diversity of uses, including human consumption and animal feed. Globally, over half of all sorghum is used for human consumption. Grain sorghum is used for flours, porridges and side dishes. It can also be used as a raw material for industry and can be processed into malted foods, beverages and beer (Kenga et al., 2004). In livestock, cattle and sheep are frequently fed on grain silage after harvest. Sorghum fibres are used in wallboard, fences, biodegradable packaging materials, and solvents, while dried stalks are used as a cooking fuel. A more recent use of sorghum is for the production of ethanol.

Sorghum is closely related to other potential biofuel crops such as sugarcane (*Saccharum officinarum* L.), the principal sugar feedstock, and maize (*Zea mays* L.), the most important starch feedstock (Murray et al., 2008). Researchers and ethanol producers have shown that

grain sorghum is a good feedstock for ethanol which can contribute in global ethanol requirement (Wu et al., 2007). The potential of grain sorghum for use in bioethanol production is due to its high starch content that is similar in composition to maize which has previously proved successful in bioethanol production (Rooney and Serna-Saldivar, 2000). Starch content in grain sorghum ranges between 60–77% and that of maize between 64–78% (Shelton and Lee, 2000). Therefore, sorghum grain just maize, would be appropriate for use in fermentation for the production of bioethanol. Grain sorghum is not only similar to maize in starch content composition, but also has advantage over maize on drought and heat tolerance, low fertiliser and pesticide input and high yield potential.

The potential of grain sorghum for production of bioethanol is due to the biochemical and physiological characteristics of sorghum plant. These characteristics include; high starch content, wide adaptability, and its use may be of particular benefit in areas where rainfall is limiting and maize does not grow well (Taylor et al., 2006). Sorghum has excellent nitrogen use efficiency (Bean et al., 2008), water lodging tolerance and salinity resistance (Nghiem et al., 2016). The ability of sorghum to withstand severe drought conditions and its high water usage efficiency makes it a good renewable feedstock suitable for cultivation in arid regions, such as the southern US and many areas in Africa and Asia (Nghiem et al., 2016). Under optimal conditions, however, sorghum has a grain yield potential equal to or greater than other cereal grains (Reddy et al., 2012).

Ethanol yield and efficiency of conversion is affected by several factors, including starch content of the grain, tannin content in the grain, protein digestibility and content. The higher the starch content in the grain the higher the yield of ethanol (Wang et al., 2008). Tannin content has a strong adverse effect on conversion efficiency and starch digestibility as these interact with proteins, metal ions, and polysaccharides (Wu et al., 2007). Protein content may be inversely proportional to starch content, thus ethanol production decreases as protein content increases. The relationship between protein content and theoretical percentage of ethanol yield indicated that protein content had no significant effect on ethanol yield (Zhan et al., 2003). Protein digestibility had a strong linear relationship with fermentation efficiency (Wu et al., 2007), thus conversion efficiency increased as protein digestibility increased.

1.1 Problem statement

Traditionally, sorghum is grown for grain as human food and fodder for animal feed. Besides its use as food, sorghum grain is also increasingly gaining importance for its potential use in bioethanol fuel production (Reddy et al., 2005). Sorghum grain has good potential as raw material for ethanol production. Researchers and ethanol producers have shown that grain sorghum is a good feedstock for ethanol and could make large contributions to a nation's fuel ethanol requirements (Farrell, 2006; Wu et al., 2006). Grain sorghum is a second major starch-rich raw material after maize for bio-ethanol production. Ethanol yield from sorghum grain is comparable to that from maize. Grain sorghum is not only similar to maize in composition of starch but also has an advantage over maize on drought and heat tolerance, low fertiliser and pesticide inputs. Most sorghum feedstock for bio-ethanol production is the normal non-tannin sorghum type.

According to the sorghum industry report, in South Africa the consumption of sorghum flour has doubled since 1997/1998 from 50,000 tons to more than 100,000 tons during 2010/11, while the malting market declined from 160,000 to less than 80,000 during the same period. There is a need, therefore, for developing high yielding cultivars to meet the growing needs for flour and improved quality as well as identifying dual-purpose types giving high yields of grain with acceptable quality for ethanol production.

1.2 Research objectives

The overall objective of this research was to evaluate advanced sorghum lines for potential use in ethanol production under South African conditions.

1.2.1 Specific objectives of the study

The specific objectives were:

I. To characterise sorghum breeding lines using agro-morphological traits under two growing environments (planting dates)
II. To evaluate and select sorghum lines based on starch content, protein content and protein digestibility.
III. To identify sorghum lines for direct bio-ethanol production and/or potential parents for breeding programme.

1.3 Research hypothesis

Based on the above objectives, the following hypotheses were tested in this study:

I. There is morphological variation among sorghum lines grown under two different environments
II. There are sorghum lines with high starch content, high protein digestibility and low protein content in the present germplasm
III. The present sorghum breeding lines possess lines with potential use for ethanol production that can serve as potential parents for breeding.

1.4 Outline of the dissertation

This dissertation is made up of four chapters as shown below:

Chapter 1: Introduction

Chapter 2: Literature Review

Chapter 3: Characterisation of sorghum breeding lines using agro-morphological traits under two environments

Chapter 4: Starch content, protein content, and protein digestibility analyses in grain sorghum

Chapter 5: General overview of the research findings

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CHAPTER 2

Literature Review

2.1 Introduction

This chapter reviews literature on topics that are important in the evaluation of advanced sorghum lines for bio-ethanol production. It is an important part of the research as it enables the recognition of the effort that has been put into sorghum breeding and to identify the research gap towards the development of sorghum cultivars for bio-ethanol production. The chapter critically reviews a) the origin and distribution of sorghum, b) the importance of sorghum, c) sorghum production constraints, d) morphological characterisation of grain sorghum, e) overview of bioethanol production, and f) grain quality influencing bioethanol yield in sorghum.

2.2 Sorghum origin, distribution and classification

Sorghum is among the important cereal grain crops produced in the world. It is the major cereal crop in semi-arid regions of the world, especially in Africa, where it is grown by many poor farmers (Kenga et al., 2004). It originated in north-eastern Africa (Ethiopia, Sudan and East Africa), about 5,000 years ago, where the largest diversity of both cultivated and wild species occurs (Sleper and Poehlman, 2006). Some researchers argue for multiple centres of origin for the crop. Theories of the origin and domestication of sorghum were based on archaeological evidence (Kimber, 2000). It was domesticated in Ethiopia and parts of Congo between 5,000 and 7,000 AD with secondary centres of origin in India, Sudan, and Nigeria.

Sorghum was distributed along trade and shipping routes throughout Africa, and through the Middle East to India at least 3,000 years ago. It was taken to India from eastern Africa during the first millennium BC (Acquaah, 2007). Along the way many distinct races evolved. This early distribution and introduction of the crop helped generate further genetic diversity in other continents, such as Asia. Based on morphological classification, all cultivated sorghums (*Sorghum bicolor* spp.) are grouped into five races: durra, kafir, guinea, bicolor, and caudatum. They differ in panicle morphology, grain size, and yield potential, among other characteristics (Acquaah, 2007; Harlan and De Wet, 1972). Based on potential agronomic uses, sorghum is classified into four groups; grain sorghums, sweet sorghums, grass sorghum and broom corn sorghum (Sleper and Poehlman, 2006).

2.3 Sorghum classification

The genus *Sorghum* Moench is characterized by spikelet borne in pairs: a bisexual and fertile sessile and spikelet and sterile or occasionally staminate flowered pedicillate spikelet. The genus is subdivided into five sections, the most important section being sorghum containing three species; *Sorghum bicolor* (Linn.) Moench, (2n=2x=20), the annual wild and domesticated sorghums, *Sorghum halepense* (Linn) pers., (2n=40-forage sorghum) a perennial, tetraploid, rhizomatous species, commonly known as Johnson grass in the United States and *Sorghum propinquum* (Kunth) Hitches., (2n=2x=20) a wild perennial, diploid, rhizomatous species with small hard seeds that is cross fertile with *S. bicolor* (Acquaah, 2007; Sleper and Poehlman, 2006).

2.4 Importance of Sorghum

Sorghum is a crop with great economic importance as the whole plant can be used in different ways. From ancient times, sorghum has been used for food, beverage, feed and building materials (Dicko et al., 2006; Reddy et al., 2007). The uses of sorghum differ from one place to another. In developed countries such as the United States and Australia, sorghum is grown essentially for animal feed. However, in Africa and Asia the grain is used both for human food and animal feed. It is estimated that more than 300 million people from developing countries rely on sorghum as a source of energy (Godwin and Gray, 2000). The grain is used for the production of traditional foods, for example: *ugali* (Tanzania), porridges such as *tô* (west Africa), *bogobe* (Botswana), *sankati* (southern Africa) and *ogi* (Nigeria); leavened breads such as *injera* (Ethiopia) and *kisra* (Sudan); unleavened breads such as *roti* (India), *chapatti* (south Asia) and *tortilla* (Latin America) and fermented beverages such as *umkhombothi* (South Africa) (Doggett, 1988). Additionally, the grains are used for making commercial beer and non-traditional products, such as animal fodder. After harvest, the grain sorghum stems can be used for fencing and building huts, while the roots are useful as fuel for cooking.

On commercial scale though, sweet sorghum and grain sorghum are used for production of biofuel and alcohol (Rooney et al., 2007; Woods, 2001; Zhao et al., 2009). The sweet sorghum juice from the stalk can be converted into sugar and syrup. The sugar is converted to biofuels, primarily used as a source of energy in transport industry. Bagasse, which is the remaining stalk after juice extraction, can be converted to heat and electricity through combustion and gasification (Claassen et al., 2004)

Statistics show that sorghum is one of the most important cereal crops, ranking fifth after wheat, rice, maize and barley (FAOSTAT, 2016). In terms of sorghum production, an area of 42 million ha with a total production of 61.5 million tonnes of grain was reported globally, of which 80% is produced in Africa and Asia (FAOSTAT, 2016). However, the potential for generating bioethanol from grain sorghum has not been quantified in most countries and environments in southern Africa.

2.5 Sorghum production constraints

The low sorghum yields in tropics and sub-tropics have been attributed to both abiotic production constraints (low and high extreme temperatures, poor soil fertility, drought) and biotic stresses such as *Striga* infestation, stem borers and shoot fly (Wortmann et al., 2006).

2.5.1 Abiotic production constraints

Low temperature cause poor pollen fertility, seed germination and retarded growth (Yu and Tuinstra, 2001). Drought affects the growth and development of sorghum plants, and the most damaging effects of this stress occurs from flowering through grain filling stage of crop growth (Harris et al., 2007). It has been reported by Reddy et al. (2007) that sorghum has a high yield potential, comparable to rice, wheat and maize especially under water limited conditions. In those areas where sorghum is commonly grown, yields of 3 - 4 t/ha have been obtained under non-stress conditions, dropping to 0.3 to 1.0 t/ha under stress. The traits associated with various drought aspects have been studied using different screening methods resulting in development of drought tolerant cultivars (Mutava et al., 2011).

Development of early maturing cultivars is advantageous in low rainfall regions as it allows the crop to escape damage during drought (Acquaah, 2007). Aluminium toxicity in the soil has also been shown to contribute to drought stress as it damages the root system (Magalhaes et al., 2007). High levels of soil aluminium reduce root development and predispose plants to drought injury. The affected plants can be vulnerable to mineral nutrient deficiencies. Soil management, including use of organic manure, reduces the effects of mineral deficit. Genotypes with aluminium tolerances have been identified for breeding (Acquaah, 2007). Lodging is also a serious problem in the tall sorghum introductions grown originally in the United States (Sleper and Poehlman, 2006). The problem of lodging has been improved by

breeding for short varieties and by development of cultivars with resistance to the root and stalk rots.

2.5.2 Biotic stresses

Striga spp. are notorious root parasitic weeds of cereals grown in most semi-arid and tropical regions such as sorghum, millet and maize. Striga infestation reduces photosynthesis in sorghum and generally causes yield losses of more than 50% (Lendzemo et al., 2007). These weeds are increasingly reported to be a threat to crop production particularly in the savannah regions of sub-Saharan Africa. It is difficult to control *Striga* by conventional management practices and the most effective control is the use of resistance cultivars.

Diseases such as grain mold, caused by a number of fungi including *Fusarium moniliforme* Sheld, *Curvularia lunata* etc., smut caused *Sphacelotheca spp* and leaf diseases e.g. leaf blight caused by *Exserohilum turcicum,* attack the crop (TeBeest et al., 2004). Various control measures are used to reduce the effect of diseases in sorghum so that farmers achieve optimum yields. These include use of resistant varieties and good agronomic practices.

Major insect pests of sorghum include greenbug, sorghum midge, stalk borers, and shoot fly. The insect pests cause significant grain yield losses, although the relative importance varies from one locality to another within and among the countries (Wortmann et al., 2006.). Host plant resistance has been important in controlling major insect pests in sorghum. Insect resistance in sorghum is commonly due to non-preference for insect feeding, or reduced reproductive capability of the insect (antibiosis) (Sleper and Poehlman, 2006). Bird damage, especially Quelea,, is among the major constraints in sorghum production in most areas. Bird scaring is a common method used by farmers to control birds. Without effective scaring, farmers will face significant yield losses. Breeders have developed some bird resistance varieties, though they are not 100% effective for some birds.

2.6 Genetic variability for grain yield in sorghum

Cultivar development is based on the exploitation of genetic variability in genotypes for the traits of interest. Genetic improvement for quantitative traits depends upon the nature and amount of variability present in the genetic stock and the extent to which the desirable traits

are heritable (Chavan et al., 2010). Durra (compact head) is the type of sorghum preferred by farmers in north-eastern coastal regions of Africa. It is preferred due to its high grain yield and quality (Abdi et al., 2002). It is characterized by compact head borne on recurved or goose-necked panicle that makes it unsuitable for mechanical harvest. Seeds are large and creamy yellow or white; stalks are slender, dry, and pithy and tiller freely (Sleper and Poehlman, 2006). Characteristics which bring variation for grain yield potential include: semi compact elliptic, compact elliptic, semi loose primary branches, very loose primary branches, very loose drooping primary branches, and half broomcorn head types in sorghum (Abdi et al., 2002; Doggett, 1988).

It was reported by (Doggett, 1988), that guinea sorghums are low yielding lowland sorghums compared to the durra types that are adapted to the high rainfall highlands. Variation in the grain yield and its components such as days to 50%flowering, days to maturity, panicle length, panicle width, plant height, number of primary branches per panicle, number of grains per panicle, test weight (g), harvest index and grain yield per panicle were reported by (Chavan et al., 2010) on the basis of genotypic and phenotypic variances. It has been demonstrated that the effectiveness of selection for any character depends not only on the extent of genetic variability but also on the extent to which it will be transferred from one generation to next (Makanda et al., 2009). Many African countries have rich collections of sorghum germplasm which are important because the more diverse the genetic base, the more distant the lines developed and consequently the higher the hybrid vigour that can be realised and maintained on crossing (Li and Li, 1998). In selection of sorghum lines for bio-ethanol production it is important to quantify genetic variability of germplasm.

2.7 Morphological characterisation of grain sorghum

In classical breeding, selection of cultivars is normally done using morphological traits (Warrick et al., 2002). Morphological characterisation of germplasm is indispensable for the utilisation of the available diversity in the crop improvement programme (Bucheyeki et al., 2008). Morphological characteristics are usually obtained in the field during crop growth and after harvesting the crop (Gaines et al., 1999). The individuals are discriminated based on physical characteristics for example plant height, maturity cycle, leaf area, panicle size, grain covering and colour. Characterisation of breeding lines developed at the ARC-GCI is needed to provide information on their present or potential future uses.

2.8 Overview of bioethanol production

Since the 1970s, the development of technology for production of fuels and industrial chemicals using renewable feedstocks has been dominant and currently shows no sign of slowing down (Nghiem et al., 2016). Bioethanol production from renewable feedstocks plays an important role in reducing both the environmental pollution and consumption of crude oil.

The term bioethanol can be defined as liquid biofuel produced from fermentation of sugar and starch components of plant by-products (Dias et al., 2009). Bioethanol is a promising alternative fuel because it is a renewable bio-based resource and it is oxygenated thereby provides the potential to reduce particulate emissions in compression–ignition engines (Balat et al., 2008). Previously, ethanol existed only in alcoholic drinks, but after some purification methods were established, ethanol utilization has highly expanded (Onuki, 2006). Bioethanol properties of higher octane number, higher flame speeds, wider flammability limits, and higher heats of vaporization than gasoline allow for a higher compression ratio, shorter burn time and leaner burn engine, which leads to a theoretical efficiency advantages over gasoline in an internal combustion engine (Balat et al., 2008).

The two countries with the largest ethanol production are the United States of America and Brazil contributing about 85% of global fuel production (Table 2.1) (RFA, 2016). During 2015, the annual ethanol production in the USA and Brazil were 14,806 million gallons (55.6 billion litres) and 7,093 million gallons (26.8 million litres), accounting for 57.6% and 27.6% of the total world production, respectively (RFA, 2016). In the USA, maize is the major feedstock for ethanol production, whereas in Brazil sugarcane is the leading crop for the same purpose (Nghiem et al., 2016).

Table 2.1 World fuel ethanol production by country or region (million gallons).

Country	2007	2008	2009	2010	2011	2012	2013	2014	2015
USA	6,521	9,303	10,938	13,298	13,248	13,300	13,300	14,300	14,806
Brazil	5,019	6,472	6,578	6,922	5,573	5,577	6,267	6,190	7,093
Europe	570	734	1,040	1,209	1,168	1,179	1,371	1,445	1,387
China	486	502	542	542	555	555	696	635	813
Canada	211	238	291	357	462	449	523	510	436
Rest of World	315	389	914	985	698	752	1,272	1,490	1,147
World	**13,123**	**17,644**	**20,303**	**23,311**	**22,404**	**21,812**	**23,429**	**24,570**	**25,682**

Data Source: RFA (2016). Analysis of public and private estimates

http://www.ethanolrfa.org/resources/industry/statistics/#1454098996479-8715d404-e546 (12/05/2016)

Substantial efforts have been made to develop feedstocks other than sugarcane and maize for production of fuels and chemicals in industries. Among many crops being investigated for this purpose, sorghum is one of the most promising candidate, principally in developing countries (Linoj et al., 2006). Grain sorghum has attracted strong interest in bioethanol production because of its many good characteristics including rapid growth (short life cycle), wide adaptability, excellent nitrogen usage efficiency, drought tolerance and waterlogging tolerance and salinity resistance (Nghiem et al., 2016). It is necessary to develop grain sorghum cultivars in South Africa which can be used in bioethanol production.

2.9 Chemical characteristics influencing bio-ethanol production from grain sorghum

In order to identify potential sorghum lines for bio-fuel production it is important to understand the key factors influencing ethanol production from grain sorghum. Ethanol yield and conversion efficiency are the two most important quality traits of cereal grains when they are used to produce fuel ethanol. Both ethanol yield and fermentation efficiency have been used to evaluate the performance of grain sorghum as a feedstock in ethanol production (Wu et al., 2007). Research shows that key factors affecting ethanol yield and ethanol fermentation efficiency of sorghum include starch content, starch digestibility, level of extractable proteins, protein and starch interaction, mash viscosity, amount of phenolic compounds, the ratio of

amylose to amylopectin, and formation of amylose-lipid complexes in the mash (Wang et al., 2008; Wu et al., 2007; Zhao et al., 2008).

2.9.1 Starch content

Starch is the major storage form of carbohydrate in sorghum. It is the main component of sorghum grain, followed by protein and non-starch polysaccharides (NSP) and fat. In most varieties, sorghum starches have 70 – 80% amylopectin and 20 – 30% amylose, waxy varieties have 85 – 100% amylopectin and 0 – 15% amylose (Rooney and Serna-Saldivar, 2000). Starch content is the major factor influencing ethanol production in cereals. The process of ethanol production first of all converts starch from grain into ethanol (Wang et al., 2008). Maize has been successful in ethanol production with its starch content of 64% - 78%. The USA, which is the largest bioethanol producing country, produced approximately 95% of the bioethanol from maize starch (Taylor et al., 2006). In general the higher the starch content in a grain, the higher the ethanol yield expected.

Sorghum is a starch-rich grain with similar composition to maize. It has the potential for being used in the production of bio-ethanol (Rooney and Serna-Saldivar, 2000). Many studies have shown that the starch content in most sorghum genotypes ranges between 60 – 77%. On average this starch difference should result in up to 15% calculated difference in ethanol yield per unit grain used. Variation in starch content is the result of several factors including growth environment, plant genetics, harvesting method and storage (Lacerenza et al., 2008). Wang et al. (2008) showed that not all starches in different sorghum varieties contribute equally to ethanol production. An analysis of sorghum varieties with similar starch percentages demonstrated that variations in ethanol yields could be as large as 7.4%. (Figure 2.1) (Wang et al., 2008).

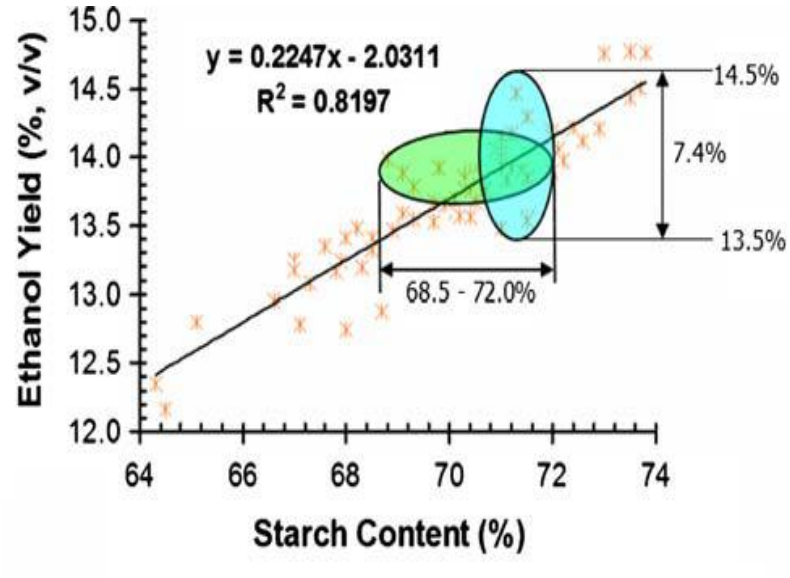

Figure 2.1 Relationship between ethanol yield and starch content of sorghum grain

Source (Wang et al., 2008)

Starch content in sorghum flour was a good predictor for ethanol yield (Lacerenza et al., 2008; Zhao et al., 2009). A study done by Wu et al. (2007) showed positive effects of starch content on ethanol yields. However, conversion efficiency to ethanol by fermentation did not correlate linearly with the starch contents of the sorghums (R^2 = 0.041). Therefore, starch content can be used to predict ethanol yield in grain sorghum but cannot be used to predict conversion efficiency of grain sorghum. This implies other factors than starch content affect the conversion process.

2.9.2 Tannin content

Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods (Chung et al., 1998). The name tannin originally was given to the plant extracts exhibiting astringency, without knowing their chemical structures (Okuda and Ito, 2011). The term tannin was first used in 1796 to indicate the chemical constituents of various plant extracts which were responsible for transforming fresh animal hides into leather (White, 1957). It was later defined by Bate-Smith and Swain (1962) as water soluble, polyphenolic compounds with molecular weights ranging from 500 to over 3,000. Serrano et al. (2009) defined tannins as a unique group of phenolic metabolites with molecular weights between 500 and 30,000. In plants, two main types of tannins can be distinguished; condensed tannins (CTs) and hydrolysable tannins (HTs). Condensed tannins consist of flavanol moieties, which are connected by C4-C8 linkages and sometimes C4-C6 links, they do not contain sugar

residues. Hydrolysable tannins have a sugar core to which gallic acids are bound through ester bonds (Nierop et al., 2005).

Sorghum is the only cereal that contains tannins. Tannins are associated with enhanced agronomic qualities such as reduced pre-harvest molding, enhanced resistance to pathogens and pests, lower bird depredation (in "bird-resistant" sorghums,) and lower pre-harvest germination (Bullard and York, 1996, Waniska, 2000). However, tannins are anti nutritional factors as they bind with proteins, precipitate them and make them unavailable during digestion. Therefore, the nutritive value of feeds containing tannins is consequently reduced. (Taylor, 2003).

Breeding efforts toward eliminating sorghum tannins have been done and the majority of sorghums currently produced are low in tannins. In the USA and Europe, 99% of the sorghum produced are tannin free (Awika and Rooney, 2004). However, in some countries, the use of high-tannin cultivars is economically advantageous,, especially under high bird predation (Kyarisiima et al., 2004). In southern Africa, small-scale farmers intercrop tannin and tannin-free sorghums in areas prone to high bird predation in order to reduce grain losses (Awika and Rooney, 2004).

There are some misperceptions about tannins in sorghum; that is, all sorghum contain tannins or that the presence of tannins is linked to seed colour (Boren and Waniska, 1992). Genotypes of sorghum having a pigmented testa layer, which is controlled by two complementary dominant genes designated B1 and B2 (B1_B2_), are the only sorghum types with tannins (Blakely et al., 1979). Sorghums are classified into three types based on the location and distribution of tannins. These are Type I (no pigmented testa layer and no tannins), Type II (tannins in pigmented testa), and Type III (tannins in pigmented testa and pericarp) (Waniska and Rooney, 2000). Therefore, not all the sorghums contain tannins. Only Type III includes the well-known “bird-resistant” sorghums or tannin sorghums.

Tannins are well known for their adverse effect on starch digestibility due to their ability to interact with proteins, polysaccharides and metal ions (Schofield et al., 2001). Tannin is the primary nutrient-limiting component in grain sorghum. High levels of condensed tannins can reduce starch and protein digestibility up to 10% (Leeson and Summers, 1997). In ethanol production the increase of the tannin content had a strong negative effect on the process efficiency (Wu et al., 2007). The study by Wu et al. (2007) found that the liquefaction of starch in tannin sorghums was more difficult and slower than in normal and waxy sorghums and

resulted in high-viscosity mash, slow starch-to-glucose conversion, and lower conversion efficiency. In particular, a study of nine sorghum samples showed that tannin contents had a strong adverse effect on conversion efficiency. The average efficiency of brown samples was 85.2 ±.31%, which was significantly ($P < 0.05$) lower than the averages of bronze (87.9 ± 1.01%), white (87.9 ± 1.36%), yellow (87.9 ± 0.88%), creamy (88.0 ± 1.28%), and red (88.2 ± 1.20%) samples. The difference in efficiencies among the other colour groups (except brown) was not significant ($P = 0.905$) (Wu et al., 2007). This further confirmed the adverse effects of tannin on conversion efficiency. Tannins could cause sorghum protein cross-linking during heating or cooking, prevent starch granules from absorbing water, and prevent enzymatic degradation (Duodu et al., 2003).

2.9.3 Protein content

Protein is the second major component in grain sorghum after starch. Traditionally, sorghum grain protein is classified based on its solubility in different solvents (Wong et al., 2009); that is, albumins (water-soluble), globulins (salt-soluble), kafirins (prolamins, aqueous alcohol-soluble), cross-linked kafirins (aqueous alcohol + reducing agent-soluble), cross-linked glutelins (detergent + reducing agent + alkaline pH-soluble) and unextracted structural protein residue (Afify et al., 2012). Albumins and globulins are primarily physiologically active proteins, while prolamins and glutelins are storage proteins. The prolamins in sorghum are called kafirin. A modern and more simplified classification design for sorghum proteins has been proposed that divides them into two groups, kafirins and non-kafirins. The later classification is based on the homogeneous nature and varied origin of the kafirin storage prolamins relative to the heterogeneous nature of the non-kafirin proteins (albumins, globulins and glutelins) that are involved in cellular functions (Hamaker and Bugusu, 2003).

The protein quality of cereals mainly depend upon their protein content, amino acid composition and amino acid availability (Sauer and Ozimek, 1986). Protein content in sorghum is variable. Generally protein content in sorghum lies between 6 to 18% depending on varieties and developmental condition. Protein content in sorghum is inversely proportional to starch content as in other cereal grains and thus show negative effect on ethanol yield (Zhan et al., 2003). In general, ethanol yields decreased as protein content increased, due to an inverse relationship between starch and protein content in a unit mass of grain. Ethanol fermentation efficiency could show as much as an 8% difference in sorghum varieties with similar protein content (Wang et al., 2008). The most probable reason for the adverse effects of protein on

ethanol fermentation could be the formation of web-like protein matrix by cross-linking of sorghum protein during mashing or cooking which prevents the starch granules in a mashed matrix from gelatinizing and limits accessibility to enzyme hydrolysis and consequently lowers the digestibility of sorghum starch (Duodu et al., 2003; Zhang and Hamaker, 1998).

2.9.4 Protein digestibility

Protein digestibility has been used as a quality indicator for human food and animal feeds, and protein with high digestibility has potentially a better nutritional value than those with low digestibility (Yan et al., 2011). Generally, the apparent digestibility of sorghum proteins is lower than that of other cereals (Axtell et al., 1981). Poor digestibility of sorghum proteins on cooking is a nutritional constraint to the use of sorghum as food. Digestibility may be used as an indicator of protein availability (Duodu et al., 2003).

Numerous factors contributes to the protein digestibility problems in sorghum. Duodu et al. (2003) divided these factors into two categories; i) exogenous factors which refer to factors that arise out of the interaction of sorghum proteins with non-protein components like polyphenols, non-starch polysaccharides, starch, phytates and lipids and ii) endogenous factors, which refer to factors that arise out of changes within the sorghum proteins themselves and do not involve interaction of the proteins with non-protein components.

Researchers for a long time have been investigating digestibility of protein as related to its use in foods and feeds. Recently, studies have been done on the effect of protein digestibility on ethanol fermentation efficiency (Wang et al., 2008; Wu et al., 2007; Zhao et al., 2009). Wang et al, (2008), using nine selected sorghum genotypes covering a broad range of ethanol fermentation efficiencies studied the effect of protein quality on ethanol fermentation efficiency. The results showed a strong linear relationship between protein digestibility and fermentation efficiency (R^2 = 0.91), implying that conversion efficiency increased as protein digestibility increased. The protein digestibility of waxy and normal sorghum were higher than those of high-tannin samples and the conversion efficiencies of waxy and normal samples were also higher than those of high-tannin samples (Wu et al., 2007). This show that the digestibility of protein relates to conversion efficiency. It is possible that sorghum samples with high protein digestibility provide more free-amino nitrogen for yeast growth during fermentation (Wang et al., 2008).

2.10 Conclusion

Based on the literature reviewed above, it can be concluded that grain sorghum is one of the most important renewable feedstock for bioethanol that can be used in semi-arid areas. The review showed that:

- Among the challenges with bioethanol production, is the availability of raw materials for the production. The availability of feedstocks for bioethanol can vary considerably from season to season and depends on geographic locations. Grain sorghum is the most promising feedstock considering its wide availability.
- Grain sorghum has a high starch content same as in maize which is essential for bioethanol production.
- The problem with some types of grain sorghum lines is the presence of tannins which has adverse effects on starch digestibility due to their ability to interact with proteins, polysaccharides and metal ions. Therefore, it is necessary to select lines with low tannins in breeding for bioethanol.
- There is no grain sorghum cultivar for bioethanol production in the market, hence it is necessary to develop such cultivar.

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CHAPTER 3

Assessment of variability in sorghum breeding lines using agro-morphological traits under two environments

Abstract

The main objective of this study was to characterise sorghum breeding lines using agro-morphological traits under two environments (planting dates). Fifty sorghum breeding lines from the Agricultural Research council - Grain Crops Institute (ARC-GIC), Potchefstroom were assessed for the diversity. Most of the breeding lines were high yielding under the first planting date (10 December, 2015) with a mean grain yield of 3.6 t/ha, while under the second planting date (10 January, 2016) the mean grain yield was 3.2 t/ha. The majority of the breeding lines were early maturing as shown by the mean number of days to 50% flowering (71 days). There were high phenotypic variance values for grain yield, panicle weight, number of grain per panicle and plant height. Genotypic variances for these characters were also high. A relatively high phenotypic coefficient of variation and genotypic coefficient of variation values (>20%) were obtained for plant height, number of panicles per plot, panicle weight, grain yield and number of grains per panicle. High broad sense heritability estimates (greater than 80%) were observed for all the characters. The highest genetic advance values were observed in number of grain per panicle, leaf area, plant height, and panicle number per plot. The estimated value of expected genetic advance expressed as percentage of the mean (GAM) at 5% proportion selected (selection intensity = 2.061) ranged from 2.29% to 148.0% across planting dates. Maximum GAM was recorded for leaf width (148.76%), grain yield (78.5%), panicle weight (69.13%) and panicle number per plot (61.62%). Grain yield showed significant positive correlation with panicle weight, 1000 grain weight, number of panicle per plot, number of grain per panicle and plant height. Three principal components accounted for 64.35% of the total variability observed. A dendrogram based on both qualitative and quantitative traits grouped the breeding lines into four clusters, but with different breeding line combinations. Five breeding lines; 15ELC F6#68, 15ELC F6#8, 15ELC F6#70, Maseka a swere and 15ELC F6#42 were ranked highest for grain yield. Overall, the study found considerable levels of genetic variability among sorghum breeding lines. The agro-morphological characterisation provides a useful measure of genetic diversity among sorghum breeding lines to identify potential parental material for future breeding programmes.

3.1 Introduction

The development of a broad genetic base of high yielding and stable sorghum cultivars requires a continuous supply of new germplasm as a source of desirable genes and gene complexes in crop breeding programmes (Noor et al., 2012). Characterisation and evaluation of existing germplasm are required for identifying potential germplasm for varietal improvement programmes (Dossou-Aminon et al., 2015; Elangovan et al., 2007). It involves distinctly identifying characteristics which are heritable, leading to a classification that will facilitate enhanced utilization of germplasm (Upadhyaya et al., 2008). Generally a well-characterised germplasm is needed for crop improvement programmes and strategic conservation of genetic resources (Amelework et al., 2016; Sergio and Gianni, 2005). Different methods have been used in crop genetics characterisation, including morphological, biochemical and molecular markers. The application of any type of marker in the evaluation of diversity among breeding lines will depend on the type of crop, technical expertise, laboratory equipment and cost, suitability for the specific study and the desired results (Chandra et al., 2001).

Morphological characterisation is the first, easiest and cheapest method of classifying germplasm, estimating diversity and registering a new cultivar (Rakshit et al., 2012). The classical approach of characterisation and evaluation of germplasms is based on variation of agronomic and morphological features (quantitative and qualitative characters) (Schut et al., 1997; Torkpo et al., 2006; Vega, 1993). Morphological characters are agronomic important characters measured directly from the population or from field specimens (Amelework et al., 2016; Gaines et al., 1999). The individuals are differentiated based on physical characteristics, for example, plant height, maturity cycle, leaf area, panicle, size, grain covering and colour (Van der Maesen et al., 1990). Morphological characterisation is influenced by the environment and is time consuming in general, but it can still be an important and practical means of making progress in sorghum germplasm evaluation (Geleta et al., 2005).

In morphological characterisation studies, both quantitative and qualitative characters are recorded including seedling vigour, days to 50% flowering (days), plant height (cm), leaf length and width (cm), panicle length (cm), panicle width (cm), number of basal tillers, glumes colour, grain colour, 1000-seed weight (g), panicle weight among others (Franco et al., 2001). Many studies have been done to evaluate patterns of sorghum genetic variation based on morphological characters (Agrama and Tuinstra, 2003). Agro-morphological characterisation

using quantitative and qualitative traits has been carried out to assess genetic diversity within and among the breeding lines of sorghum by several researchers (Abdi et al., 2002; Ayana and Bekele, 1998; Bucheyeki et al., 2008; Elangovan et al., 2007; Geleta et al., 2005; Noor et al., 2012)

The present study aimed at characterizing sorghum breeding lines advanced at ARC-GCI Potchefstroom for the purpose of evaluating the genetic variability of the germplasm of ARC-GCI. The relationship existing between the morphological traits was also determined and promising breeding lines with important agronomic traits like yield and maturity were identified for breeding purposes. Phenotypic data were recorded using days to 50% flowering, days to maturity, plant height, leaf length and leaf width, panicle length and panicle width in cm, panicle number per plot, panicle weight (kg/plot), grain yield (kg/plot), number of grains per panicle, 1000 grain weight in grams.

3.2 Material and methods

3.2.1 Germplasm

Forty-five advanced (F_7) sorghum breeding lines developed at the ARC-GCI and five landraces/improved varieties were used for the study and these are listed in Table 3.1.

Table 3.1: List of sorghum breeding lines used in this study

No	Name of breeding line	No	Name of breeding line
1	15ELC F_6#2	26	15ELC F_6#50
2	15ELC F_6#4	27	15ELC F_6#51
3	15ELC F_6#8	28	15ELC F_6#54
4	15ELC F_6#9	29	15ELC F_6#56
5	15ELC F_6#12	30	15ELC F_6#57
6	15ELC F_6#14	31	15ELC F_6#58
7	15ELC F_6#16	32	15ELC F_6#59
8	15ELC F_6#18	33	15ELC F_6#60
9	15ELC F_6#19	34	15ELC F_6#61
10	15ELC F_6#21	35	15ELC F_6#62
11	15ELC F_6#22	36	15ELC F_6#64
12	15ELC F_6#23	37	15ELC F_6#65
13	15ELC F_6#29	38	15ELC F_6#67
14	15ELC F_6#30	39	15ELC F_6#68
15	15ELC F_6#31	40	15ELC F_6#69
16	15ELC F_6#33	41	15ELC F_6#70

No	Name of breeding line	No	Name of breeding line
17	15ELC F_6#34	42	15ELC F_6#71
18	15ELC F_6#37	43	15ELC F_6#72
19	15ELC F_6#41	44	15ELC F_6#73
20	15ELC F_6#42	45	15ELC F_6#76
21	15ELC F_6#43	46	05Potch151
22	15ELC F_6#45	47	Framida
23	15ELC F_6#47	48	M48
24	15ELC F_6#48	49	Macia-SA
25	15ELC F_6#49	50	Maseka a swere

3.2.2 Experimental site

The field trials were conducted during 2015/16 summer season at two planting dates (10 December, 2015 and 10 January, 2016) at the Agricultural Research Council-Grain Crops Institute (ARC-GCI) experimental farm, in Potchefstroom. Potchefstroom is located at 26°74''S latitude; 27°8'E longitude and altitude of 1344 m above sea level and the average minimum and maximum temperature is 9.61°C and 25.48°C, respectively with an average annual total rainfall of 618.88 mm.

3.2.3 Field trial design and management

Experiments were laid out using a 10 x 5 alpha lattice design with three replications. The experimental materials were planted in two rows of 5 m long with inter-row spacing of 0.75 m and intra-row spacing of 0.15 m at two environments. The experiments were conducted under rain fed conditions with supplementary irrigation when needed. A compound fertiliser (3:2:1) NPK was applied as basal at planting at a rate of 100 kg/ha. A mixture of pre-emergence herbicides Dual Gold and Basagran® was applied to control weeds. This was augmented by hand weeding to keep the trial fields clean from weeds throughout the growing season. Insecticide KOMBAT® granule was applied to control stem borers in sorghum. All standard agronomic practices were followed as required. The harvested materials were threshed using a single head threshing machine

3.2.4 Data collection

Quantitative and qualitative characters of sorghum were measured based on sorghum descriptors (IBPGR, 1993).

Days to flowering: measured as the number of days from sowing to when 50% the plants in the plot started flowering.

Leaf length (cm): length of the 4th leaf measured from the flag leaf blade to the tip of the leaf. The mean of five plants randomly selected in each plot recorded.

Leaf width (cm): width of the 4th leaf measured at leaf length midpoint from the middle of the leaf. The mean of five plants randomly selected in each plot recorded.

NB: Leaf length and leaf width used to determine leaf area as by the formula described by (Khan et al. (2004)

Leaf area (cm^2) = was computed as leaf length (cm) × leaf width (cm) × 0.75 (correction factor).

Plant height (cm): measured from ground level to the tip of the panicle of the main stem. The mean of five randomly selected plants was recorded at maturity.

Days to maturity: measured from planting to the date at which 50% the plants in the plot reach physiological maturity.

Panicle exsertion: measured from the lower panicle branch to the tip of the panicle at maturity (cm) as 1= < 2 cm, slightly exserted; 2 = 2-10 cm, exserted; 3 = >10, well exserted; 4 = peduncle recurved.

Panicle compactness and shape: 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.

Panicle length (cm): measured from the base of panicle to the tip of the panicle at maturity. The mean of five randomly selected plants recorded.

Panicle width (cm): measured as width of panicle in natural position at the widest part. The mean of five randomly selected plants (panicles) recorded.

Panicle number per plot*:* recorded by counting the number of panicles harvested per plot. **Panicle weight (kg/plot):** weight of dry panicles per plot before threshing, then converted to t/ha).

Grain covering: amount of grain covered by glume at maturity. Descriptor used (1) 25% grain covered, (3) 50% grain covered (5) 75% grain covered (7) grain fully covered.

Grain colour: Colour codes given in parentheses besides descriptor states. (1) white (2) yellow (3) red (4) brown (5) buff.

Grain yield (kg/plot): was measured as grain weight per plot and then converted to t/ha.

Number of grains per panicle: was recorded by counting the number of grains per panicle of five representative panicles using a counting machine.

Thousand seed weight (g): was taken by weighing 1000 grains at 12% moisture content.

3.3 Statistical analysis

3.3.1 Analysis of variance

All the quantitative data generated for each plot were subjected to the analysis of variance (ANOVA). The GenStat Discovery 18th edition (Payne, 2009) was used for the ANOVA . Descriptive statistics (mean value, coefficient of variation (CV %), least significant difference at 5% level ($LSD_{0.05}$) and correlations (r) were used to compare levels of agronomic character variation between two environments used in this study. Multivariate analysis (principal component and cluster analyses) was carried out using Minitab 17 software (Minitab Inc. 2005).

3.3.2 Phenotypic and genotypic variability

Variability present in the population was estimated by simple measures, namely, mean, range, standard error, phenotypic and genotypic variances and coefficient of variation. Phenotypic and genotypic variances and coefficient of variation were calculated according to the method suggested by Singh and Chaudhary. (1985) as follows;

- Genotypic variance = $\sigma^2 g = \frac{MSt - \sigma^2 e}{r}$
  Where, MSt = Mean square of treatment

  $\sigma^2 e$ = environmental variance (error mean square) from ANOVA

  r = number of replications;
- Phenotypic variance = $\sigma^2 p = \sigma^2 g + \sigma^2 e$
- Phenotypic coefficient of variation (PCV) = $\frac{\sqrt{\sigma^2 p}}{\bar{x}}$ x 100

  Where $\bar{x}$ = sample mean

- Genotypic coefficient of variation (GCV) = $\frac{\sqrt{\sigma^2 g}}{\bar{x}}$ x 100

3.3.3 Estimate of heritability and genetic advance

Broad-sense heritability (H^2) for each variate was calculated based on the formula suggested by Hanson et al. (1956) as follows:

$$H^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where, H^2 = heritability in the broad sense

$\sigma^2 g$ = genotypic variance

$\sigma^2 p$ = phenotypic variance

Expected genetic advance (GA) was calculated according to Shukla et al.(2006) as:

GA = $Kxh^2x\sigma^2p$, and

Expected genetic advance percentage of mean was calculated as:

$\frac{(GA)}{\mu}$ X 100

Where, K is the standardized selection differential constant at 5% proportion selected (selection intensity = 2.06)

h^2 = broad sense heritability for the character selected,

$\sigma^2 p$ = phenotypic standard deviation

μ = grand mean

3.3.4 Correlation coefficients

Simple Pearson correlation coefficients were computed to examine the degree of association between two quantitative traits using IBM SPSS Statistics 24 software (SPSS Inc., 2006).

3.3.5 Principal component analysis

The principal component analysis (PCA) of the traits was employed to examine the percentage contribution of each trait to total genetic variation. A set of twelve quantitative traits were used

to group the breeding lines based on principal component using Minitab software version 17 (Minitab Inc. 2005).

3.3.6 Cluster analysis

Cluster analysis, based on Euclidean distances as similarity measures and the unweighted pair-group method with arithmetic averages (UPGMA), were used to analyse the genetic relationships among breeding lines using GenStat 18th edition (Payne, 2009).

3.4 Results

3.4.1 Summary comparison of phenotypic classes of qualitative traits

The frequency distribution of the breeding lines for the qualitative characters is presented in Table 3.2. The majority of the breeding lines (56%) had brown grain colour, followed by light brown (22%) and white (14%). Only 2% and 6% of the breeding lines studied were yellow and red seeded, respectively. The results showed that 60% of the grain were 50% covered by the glume and some of the breeding lines were ¼ covered (22%) and ¾ covered (18%) by glumes. Only two panicle compactness and shapes were observed; compactness dominated by semi-compact elliptic (60%) and compact elliptic shape (40%). Forty percent of the breeding lines were slightly exserted and others were well exserted (32%) and exserted (28%).

Table 3.2 Summary statistics frequency distribution of qualitative traits

Character	Variables and score	Frequency	Percentage (%)
Grain colour	Yellow (1)	1	2
	White (2)	7	14
	Red (3)	3	6
	L/brown (4)	11	22
	Brown (5)	28	56
Grain covering	75% Covering (3)	9	18
	50% Covering` (2)	30	60
	25% Covering (1)	11	22
Panicle compactness and shape	Compact elliptic (8)	20	40
	Semi-Compact elliptic(9)	30	60
Panicle exsertion	Well-exserted (1)	16	32
	Exserted (2)	14	28
	slightly exserted (3)	20	40

3.4.2 Analysis of variance for quantitative traits

Data were analysed for the two planting dates individually, and then combined and the results are presented in Table 3.3 – Table 3.6. The mean squares for the breeding lines were highly significant ($p \leq 0.01$) for all phenotypic traits, showing the high level of genetic diversity among them. Results indicated that most breeding lines flowered early to medium, with a mean of 71 days to 50% flowering and a range from 57 to 85 days. There was variation in flowering between two planting dates, the earliest flowering observed was 57 days in first planting date, while in the second planting date it was 61 days. Variations were observed for plant height among the breeding lines (Table 3.3). The tallest breeding line was 15ELC F_6#43 with 235 cm, followed by 15ELC F_6#48 with 221 cm. The shortest plant height was recorded for breeding line M48 which was 110 cm. The panicle length ranged between 25 cm for breeding line 15ELC F_6#21 to 33 cm for breeding line 15ELC F_6#23. The panicle width ranged from 5.93 cm in breeding line 15ELC F_6#58 to 9.34 cm in breeding line 15ELC F_6#14 with mean panicle width of 7.72 cm. There was also variation in yield among breeding lines in the two planting dates. The grain yield at the first planting date was relatively higher (3.60 t/ha) than that of second planting (3.20 t/ha). The trend was similar for the rest of the yield parameter traits such as number of panicle per plot, panicle weight, and number of grains per panicle. Only 1000 grain weight showed a slightly higher mean at the second planting date than at the first planting date. The combined mean for grain weight was relatively high at 28.4 g per 1000 grain, 2561 grains per panicle, 5.20 t/ha of panicle weight and 3.33 t/ha grain yield. Breeding line 15ELC F_6#68 had the highest mean of panicle weight and grain yield (8.12 t/ha and 5.64 t/ha, respectively). The grain weight of this breeding line was also high at 33.83 g per 1000 grains.

The coefficient of variation for most of the traits at each planting date (Table 3.4 and Table 3.5) had low to acceptable levels. In combined analysis the lowest CV was days to 50% maturity (0.7%) with LSD (0.05) of 1.4505 at 5% level indicating that flowering was less influenced by planting date. The highest CV was for panicle number per plot (12.6%) with LSD (0.05) of 11.073 at 5% probability level.

Table 3.3 Means of 13 quantitative characters for combined data

Entry	Name	GRY	PWT	GWT	NPP	NGP	PHT	DFL	DMT	PAL	PAW	LFL	LFW	LA
1	15ELC F6#2	2.81	3.38	25.75	49.50	2355	118.80	70	120.00	31.60	7.32	68.15	8.03	410.90
2	15ELC F6#4	3.36	3.79	27.85	50.67	2818	115.50	77	131.80	30.97	7.23	69.24	8.42	438.20
3	15ELC F6#8	5.07	5.36	36.65	42.50	2853	142.30	76	127.50	29.10	8.08	68.36	8.33	427.00
4	15ELC F6#9	3.65	4.93	32.32	48.17	2230	192.60	76	113.70	27.50	7.55	70.67	7.66	407.60
5	15ELC F6#12	3.00	3.47	28.63	41.17	3164	132.90	69	116.80	25.50	7.95	66.42	7.88	393.30
6	15ELC F6#14	2.03	2.38	27.15	31.00	2089	164.50	67	122.30	30.87	9.34	63.93	8.90	427.20
7	15ELC F6#16	2.32	2.90	28.97	27.50	2944	138.80	73	116.70	28.43	8.70	72.78	8.84	483.00
8	15ELC F6#18	1.75	2.23	24.09	28.00	1963	131.60	69	113.70	28.17	7.33	69.90	7.83	410.90
9	15ELC F6#19	1.43	1.70	23.78	23.17	1993	134.20	69	114.70	27.67	7.58	68.70	8.09	418.10
10	15ELC F6#21	2.72	2.78	25.22	36.67	2705	170.40	78	112.20	24.87	9.25	70.30	8.77	462.80
11	15ELC F6#22	2.24	2.60	25.20	34.83	2584	165.10	67	126.80	31.27	8.08	68.02	8.25	422.60
12	15ELC F6#23	4.31	4.51	30.15	57.50	2735	189.10	72	127.70	33.27	7.02	65.47	7.98	390.30
13	15ELC F6#29	2.71	3.55	25.87	40.00	2407	146.50	72	128.20	28.90	8.68	68.48	9.67	496.80
14	15ELC F6#30	1.89	2.77	26.38	34.67	2112	133.80	73	116.70	28.67	7.80	68.11	9.61	492.60
15	15ELC F6#31	3.12	3.48	28.25	39.50	2614	132.40	82	135.00	29.43	7.85	64.31	9.74	469.60
16	15ELC F6#33	2.79	3.22	27.90	54.33	1861	122.10	63	113.00	27.40	7.20	61.37	7.29	335.70
17	15ELC F6#34	2.45	3.43	25.72	43.83	3088	151.60	72	134.30	32.30	9.08	67.02	8.02	403.30
18	15ELC F6#37	2.28	3.24	28.52	48.50	1778	138.10	65	112.80	28.90	7.65	64.02	8.74	420.00
19	15ELC F6#41	3.93	4.73	34.18	72.67	2654	161.10	61	115.50	27.53	8.00	58.09	8.54	376.70
20	15ELC F6#42	4.51	5.34	30.65	69.83	2660	171.00	68	124.70	28.73	8.88	68.14	8.28	424.40
21	15ELC F6#43	4.43	4.66	31.93	66.67	2605	235.30	67	126.80	29.80	7.85	59.65	7.68	343.40
22	15ELC F6#45	4.35	4.70	29.30	67.00	2514	189.70	77	128.50	33.00	7.42	61.03	8.22	377.00
23	15ELC F6#47	4.27	4.33	29.62	67.33	2729	172.90	70	122.80	32.00	7.50	59.99	8.61	388.30
24	15ELC F6#48	3.40	3.16	28.68	49.92	2293	221.00	74	127.70	30.48	7.52	61.00	8.22	375.50
25	15ELC F6#49	4.27	4.77	30.58	66.33	2861	177.50	75	125.70	30.78	7.78	56.63	8.23	349.20
26	15ELC F6#50	3.08	3.80	28.53	59.17	2413	155.20	75	125.70	29.43	7.98	54.09	7.92	321.80

Entry	Name	GRY	PWT	GWT	NPP	NGP	PHT	DFL	DMT	PAL	PAW	LFL	LFW	LA
27	15ELC F6#51	3.68	4.16	26.78	69.17	2867	158.60	69	122.70	28.17	8.08	60.39	7.46	338.10
28	15ELC F6#54	3.05	3.75	22.35	58.50	2603	144.30	72	117.20	28.10	7.57	66.13	8.28	411.00
29	15ELC F6#56	4.25	4.76	30.53	64.83	3142	194.80	76	116.50	25.87	7.60	63.57	7.71	368.00
30	15ELC F6#57	3.53	4.53	25.75	63.83	2637	211.90	68	128.50	31.83	7.78	66.23	7.58	377.50
31	15ELC F6#58	2.15	3.31	23.22	70.17	2272	130.20	69	118.50	25.83	5.93	67.28	7.57	382.20
32	15ELC F6#59	4.48	4.87	32.17	79.83	2785	189.70	68	117.70	27.47	7.18	60.65	7.48	340.10
33	15ELC F6#60	2.61	3.37	26.15	55.67	2923	149.90	69	116.20	29.67	7.22	68.48	7.57	391.00
34	15ELC F6#61	3.47	4.58	30.23	59.17	2864	131.90	69	122.30	25.93	8.50	61.77	8.17	378.50
35	15ELC F6#62	3.21	3.67	27.65	59.83	2304	140.90	71	122.20	26.23	7.73	62.85	8.34	392.90
36	15ELC F6#64	4.36	4.80	29.43	72.67	2674	170.80	67	115.70	27.90	7.40	61.46	8.63	398.80
37	15ELC F6#65	3.15	3.34	27.10	39.17	2459	125.10	72	136.00	30.47	7.38	66.11	8.42	414.70
38	15ELC F6#67	3.39	3.85	27.88	50.17	2692	185.70	71	134.70	31.87	7.48	62.39	8.64	404.10
39	15ELC F6#68	5.64	6.08	33.83	69.50	3004	179.20	76	133.70	32.43	6.95	60.89	8.48	387.30
40	15ELC F6#69	4.24	4.64	30.32	56.50	3036	184.70	71	125.50	25.43	8.15	67.68	8.11	411.90
41	15ELC F6#70	4.97	5.71	32.53	64.00	3004	213.20	66	124.20	29.13	7.82	66.78	8.20	411.60
42	15ELC F6#71	3.01	2.88	27.95	34.83	2283	122.60	74	136.80	28.03	7.75	69.30	9.02	469.90
43	15ELC F6#72	2.55	2.92	25.18	61.33	2276	128.70	65	137.00	25.93	6.50	59.21	8.22	363.50
44	15ELC F6#73	3.24	3.53	27.68	57.50	2387	166.80	66	127.70	29.57	6.87	58.16	8.37	366.70
45	15ELC F6#76	2.64	3.39	25.68	64.33	1690	165.00	72	136.30	29.03	6.85	59.96	7.49	336.40
46	05-Potch-151	3.23	4.05	20.50	65.00	2794	125.30	71	119.50	32.93	7.38	69.21	7.36	381.90
47	Framida	3.60	4.07	31.78	71.17	2186	185.20	74	114.80	27.20	7.07	68.75	8.74	452.80
48	M48	3.97	4.41	29.08	66.83	2108	109.80	65	112.70	25.03	8.07	70.47	7.41	393.00
49	Macia-SA	4.16	4.90	31.38	49.67	3353	124.30	66	115.70	26.60	9.13	62.25	8.72	407.30
50	Maseka a swere	4.85	5.40	32.75	64.50	2668	165.60	72	135.50	31.07	7.00	57.65	8.03	347.20

GRY = Grain yield (t/ha), PWT = Panicle weight (t/ha), GWT = 1000 grain weight (g), NPP = No. panicle/plot, NGP = No. grain/panicle, PHT= Plant height (cm), DFL= Days to 50% flowering, DMT = Days to maturity, PAL = Panicle length (cm), PAW = Panicle width (cm), LFL = Leaf Length (cm), LFW = Leaf width (cm) LA = Leaf area (cm2)

Table 3.4 Means and mean squares from ANOVA of 13 quantitative characters for first planting date

Character	Range	Mean	MS	Error MS	L.s.d.(P=0.05)	C.V	SD
Days to 50% flowering	57-83	70	86.29**	0.96	1.59	1.40	5.38
Days to maturity	110-138	123	195.50**	0.81	1.46	0.70	8.05
Plant height (cm)	107-240	158	2835.06**	9.94	5.11	2.00	30.65
Leaf length (cm)	52-73	63.34	50.06**	2.51	2.57	2.50	4.25
Leaf width (cm)	6.50-10.50	7.90	1.40**	0.17	0.67	5.30	0.76
Leaf area (cm^2)	291.50-485.90	375.60	5745.60	583.40	39.14	6.40	43.76
Panicle length (cm)	24-36	29.33	19.49**	1.68	2.10	4.40	2.75
Panicle width (cm)	5-10	7.74	2.12**	0.19	0.69	5.60	0.91
No. of panicles/plot	17-101	56	1034.70**	44.02	10.75	11.60	19.38
Panicle weight (t/ha)	2.62-8.81	5.86	3.63**	0.11	0.53	7.50	1.13
Grain yield (t/ha)	1.40-5.81	3.60	1.95**	0.07	0.44	10.00	0.83
No. of grains per panicle	1151-3537	2606	550933**	31047	285.50	6.80	449.40
1000 grain weight(g)	20-40	27.72	37.08**	0.76	1.42	3.20	3.56

Table 3.5 Means and mean squares from ANOVA of 13 quantitative characters for second planting date

Character	Range	Mean	MS	Error MS	L.s.d.(P=0.05)	C.V	SD
Days of 50% flowering	61-84	71.673	73.99**	1.04	1.66	1.40	5.00
Days to maturity	112-136	123.76	167.35**	0.82	1.47	0.70	7.45
Plant height (cm)	107-241	158.23	2621.87**	7.55	4.45	1.70	29.45
Leaf length (cm)	48-82	65.92	113.15**	2.83	2.73	2.60	6.25
Leaf width (cm)	6.9-10.5	8.57	1.53**	0.09	0.48	3.40	0.75
Leaf area (cm^2)	289.90-546.90	424	9055.80	352.10	30.41	4.40	54.94
Panicle length (cm)	21.5-35	28.61	16.29**	1.63	2.07	4.50	2.53
Panicle width (cm)	5.00-10.50	7.70	1.69**	0.13	0.58	4.60	0.80
No. of panicles/plot	14-84	51.79	425.21**	49.46	11.39	13.6	13.23
Panicle weight (t/ha)	1.87-7.33	4.67	2.81**	0.06	0.40	7.20	0.79
Grain yield (t/ha)	1.19-5.45	3.20	1.78**	0.05	0.36	9.20	0.96
No. of grains/panicle	1328-4557	2516	723791**	101152	515.30	12.60	552.80
1000 grain weight (g)	20-37	29.08	36.45**	0.95	1.58	3.4	3.55

Table 3.6 Means and mean squares from ANOVA of 13 quantitative characters for combined data

Character	Range	Mean	MS	Error MS	L.s.d (P=0.05)	C.V	SD
Days to 50% flowering	61-82	71	1.08**	0.60	1.60	1.40	5.29
Days to maturity	110-138	123	358.19**	0.81	1.45	0.70	7.76
Plant height (cm)	107-241	158.16	5406.74**	8.73	4.76	1.90	30.00
Leaf length (cm)	48-81	64.63	2.00**	0.13	2.62	2.50	5.49
Leaf width (cm)	6.50-10.50	8.24	116.17**	2.65	0.58	4.40	0.83
Leaf area (cm^2)	321.80-496.80	3999	10369.70	468.60	34.85	5.40	41.57
Panicle length (cm)	22-36	28.97	31.77**	1.68	2.09	4.50	2.67
Panicle width (cm)	5 – 11	7.72	2.92**	0.16	0.65	5.20	0.86
No. of panicles/plot	14-101	54.37	1193.24**	47.29	11.07	12.60	16.77
Panicle weight (t/ha)	2.27-8.12	5.20	5.51**	0.08	0.47	7.40	1.14
Grain yield (t/ha)	1.43-5.64	3.33	3.05**	0.06	0.28	9.70	0.82
No. of grains /panicle	110-4557	2561	871829**	66387	414.90	10.10	505
1000 grain weight (g)	20-39	28.40	62.06**	0.85	1.49	3.30	3.62

3.4.3 Phenotypic and genotypic variability

Most of the characters had higher phenotypic and genotypic variance than environmental variance estimates across the two growing environments (Table 3.7). There were high phenotypic values, for number of grains per panicle (334867.67), plant height (1808.06 cm) and for number of panicle per plot (429.27). Genotypic variances for these characters were also as high as the phenotypic variances. The estimates of coefficient of variation for the combined data is presented in Table 3.8. It was observed that PCVs were higher in magnitude than GCVs in all characters across the two environments. The GCV ranged between 0.57% for days to 50% flowering and 74.7% for leaf width, while PCV ranged between 1.34% for leaf length and 77.27% for leaf width. Leaf width, grain yield, number of panicle per plot and panicle weight had high values of PCV and GCV (34.29 % - 77.27%). Plant height, number of grains per panicle, 1000 grain weight, panicle width and panicle length had moderate PCV and GCV values (10.93% – 20.23%), while days to 50% flowering, days to maturing and leaf length recorded low PCV and GCV values (0.57% - 8.85%).

Table 3.7 Estimates of components of variance across two planting dates

	GV	PV	EV	PVC	GVC
Days to 50% flowering	0.16	1.24	0.60	1.58	0.57
Days to maturity	119.13	119.94	0.81	8.88	8.85
Plant height (cm)	1799.34	1808.06	8.73	26.88	26.82
Leaf length (cm)	0.62	0.75	0.13	1.34	1.22
Leaf width (cm)	37.84	40.49	2.65	77.27	74.70
Panicle length (cm)	10.03	11.71	1.68	11.81	10.93
Panicle width (cm)	0.92	1.08	0.16	13.48	12.42
Panicle no/plot	381.98	429.27	47.29	38.11	35.95
Panicle weight (kg/plot)	1.81	1.89	0.08	35.29	34.50
Grain yield (kg/plot)	1.00	1.06	0.06	41.11	39.92
No. of grain per panicle	268480.67	334867.67	66387	22.60	20.23
1000 grain weight(g)	20.40	21.25	0.85	16.23	15.90

GV = Genotypic variance, PV = Phenotypic variance, EV = Environment variance, PVC = Phenotypic coefficient of variation GCV = Genotypic coefficient of variation

3.4.4 Heritability estimates and genetic advance

All agro-morphological characters showed high broad sense heritability estimates ranging from 80.18% for number of grain per panicle to 99.52% for plant height (Table 3.8). The genetic advance expressed as percentage of mean (GAM) ranged from 2.28% in leaf length to 148.76% in leaf width (Table 3.8).

Table 3.8. Estimates of heritability, genetic advance (GA) and genetic advance as percentage of mean (GAM) across two planting dates.

Character	H^2 (%)	GA	GAM (%)
Days of 50% flowering	97.28	12.15	17.19
Days to maturity	99.32	22.41	18.16
Plant height (cm)	99.52	87.17	55.11
Leaf length (cm)	82.74	1.47	2.28
Leaf width (cm)	93.46	12.25	148.76
Leaf area (cm^2)	87.57	110.74	27.69
Panicle length (cm)	85.68	6.04	20.85
Panicle width (cm)	84.87	1.82	23.56
Panicle no/plot	88.98	37.98	61.62
Panicle wt (kg/plot)	95.57	2.71	69.13
Grain yield (kg/plot)	94.29	1.99	78.50
Number of grains per panicle	80.18	955.75	37.31
1000 grain weight(g)	95.99	9.12	37.32

H^2 = Broad sense heritability, GA = Genetic advance GAM = Genetic advance as percentage of mean

3.4.5 Correlation coefficients

Table 3.9 shows the magnitude of correlations between the traits. There was a very high positive and significant correlation between some characters including grain yield and panicle weight, grain yield and 1000 grain weight, grain yield and number of panicles per plot, panicle weight and 1000 grain weight, panicle weight and number of panicle per plot. The number of grains per panicle showed a positive and significant correlation with grain yield, panicle weight, and 1000 grain weight. Plant height had a positive, significant correlation with grain yield, panicle weight, 1000 grain weight and number of panicles per plot. Leaf area showed a positive and significant correlation with leaf width, leaf length and panicle weight. There was positive and significant correlation between days to 50% flowering and days to maturity, panicle width and number of grains per panicle, leaf width and days to 50% flowering, leaf width and panicle width, leaf area and days to 50% flowering. High negative and significant correlations were recorded for number of panicle per plot with panicle weight, leaf length, and leaf width and leaf area.

Table 3.9 Correlation coefficients (n=50) between 13 morphologial characters in sorghum grown under two environments

	GRY	PWT	GWT	NPP	NGP	PHT	DFL	DMT	PAL	PAW	LFL	LFW	LA
GRY	1												
PWT	0.945**	1											
GWT	0.768**	0.730**	1										
NPP	0.651**	0.702**	0.327*	1									
NGP	0.533**	0.534**	0.334*	0.196	1								
PHT	0.492**	0.443**	0.402**	0.385**	0.203	1							
DFL	0.111	0.051	0.052	-0.206	0.170	0.085	1						
DMT	0.198	0.116	0.052	0.017	0.106	0.131	0.311*	1					
PAL	0.147	0.112	-0.048	0.006	0.082	0.255	0.205	0.498**	1				
PAW	-0.052	-0.03	0.102	-0.389**	0.311*	-0.017	0.017	-0.156	-0.12	1			
LFL	-0.341*	-0.297*	-0.290*	-0.517**	-0.027	-0.298*	0.169	-0.295*	-0.149	0.224	1		
LFW	-0.135	-0.187	0.087	-0.435**	-0.010	-0.141	0.300*	0.172	0.045	0.345*	0.112	1	
LA	-0.321*	-0.326*	-0.124	-0.635**	-0.029	-0.286*	0.315*	-0.08	-0.07	0.389**	0.729**	0.761**	1

*, ** Correlation is significant at 0.05 and 0.01 probability level, respectively

GRY = Grain yield, PWT = Panicle weight kg/plot, GWT = 1000 grain weight (g), NPP = No. panicle/plot, NGP = No. grain/panicle, PHT = Plant height (cm), DFL= Days to 50% flowering, DMT = Days to maturity, PAL = Panicle length (cm), PAW = Panicle width (cm), LFL = Leaf Length (cm), LFW = Leaf width (cm) LA = Leaf area (cm^2)

3.4.6 Principal components analysis (PCA)

Three principal components (PC1 to PC3) with eigenvalues of greater than 1 were extracted (Table 3.10), and these accounted for most of the variability observed and cumulatively explained about 64.35% of the total variation among the sorghum breeding lines studied. The first PC1 alone explained 33.21% of the total variation, followed by PC2 that accounted for 16.76% of the total variance and third PC3 contributed 14.38% of the total variation. PC1 was mainly due to variation in the grain yield (47.4%), plant height (46.6%), and panicle number per plot (38.1%), 1000 grain weight (36.9%) and panicle weight (31.1%). The second PC had high contributing factor loading from leaf width (48.9%), days to 50% flowering (46.7%), and panicle width (38.8%) and panicle number per plot (34.8%). The third PC had high contributing factor loading from days to maturity (54.3%), panicle length (52.1%) and panicle width (42.2%). The score plot of 50 sorghum breeding lines based on the first two principal components is presented in PCA biplot (Figure 3.1). The PCA biplot explains the existence of wide phenotypic diversity among sorghum breeding lines studied. The distribution of twelve agro-morphological traits in first two PCA is shown in the loading plot (Figure 3.2).

Table 3.10 Principal component analysis of quantitative characters among 50 sorghum breeding lines showing eigenvectors, eigenvalues and proportion of variation explained with the first three PC axes.

Variable	PC1	PC2	PC3
Date of 50% flowering	0.026	0.467	-0.178
Days to maturity	0.124	0.272	-0.543
Grain yield (kg/plot)	0.474	0.082	0.096
1000 grain weight(g)	0.369	0.144	0.237
Leaf length (cm)	-0.261	0.187	0.265
Leaf width (cm)	-0.117	0.489	0.008
Number of grain per panicle	0.26	0.295	0.241
Number of panicle per plot	0.381	-0.348	-0.03
Panicle length (cm)	0.106	0.214	-0.521
Panicle width (cm)	-0.069	0.388	0.422
Plant height (cm)	0.311	0.038	-0.085
Panicle wt (kg/plot)	0.466	0.035	0.148
Eigenvalue	*3.985*	*2.011*	*1.726*
% of total Variance	*33.21*	*16.76*	*14.38*
Cumulative % Variances	*33.21*	*49.97*	*64.35*

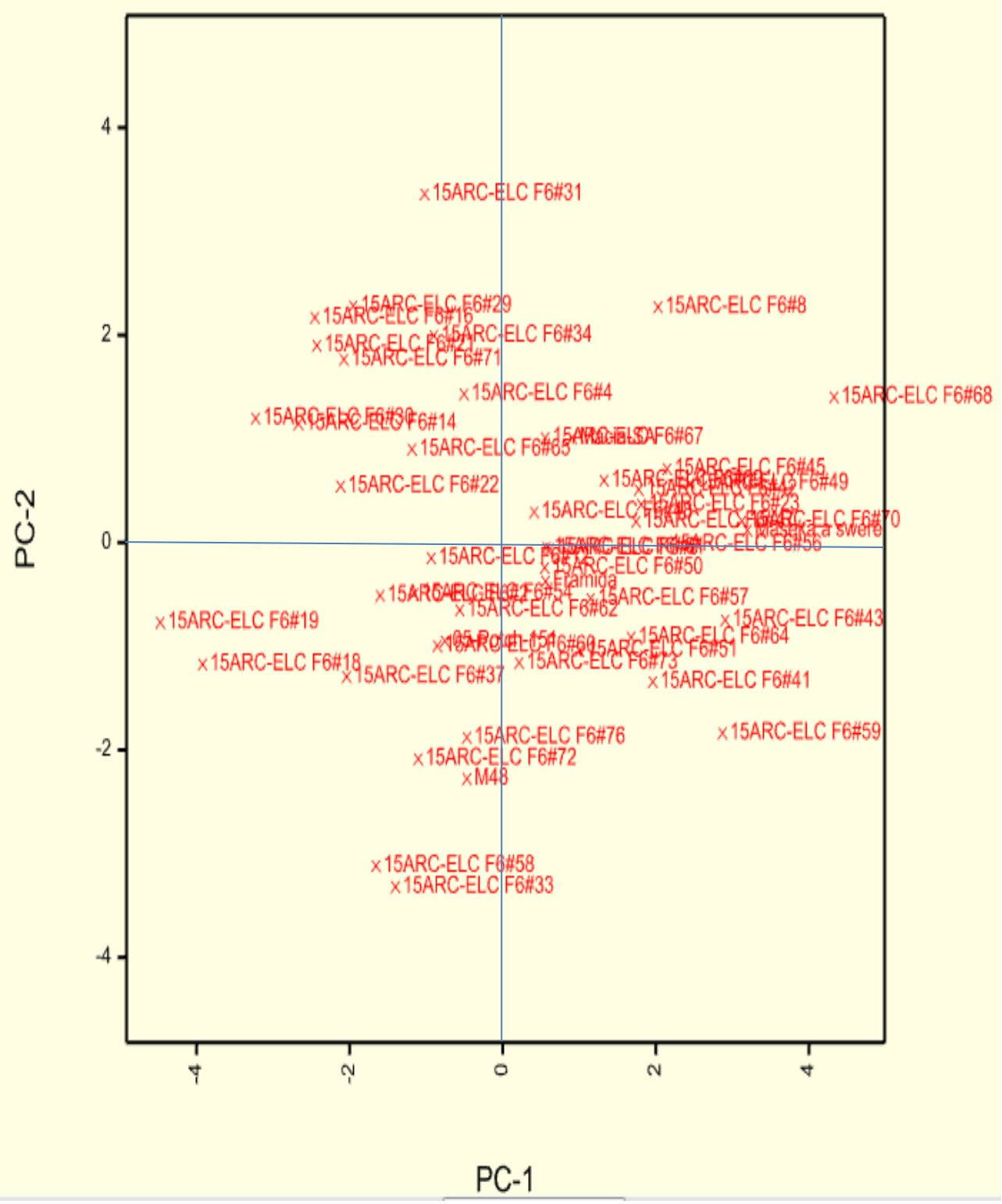

Figure 3.1 Principal component score plot of PC1 and PC2 describing the overall variation among sorghum breeding lines estimated using phenotypic character data

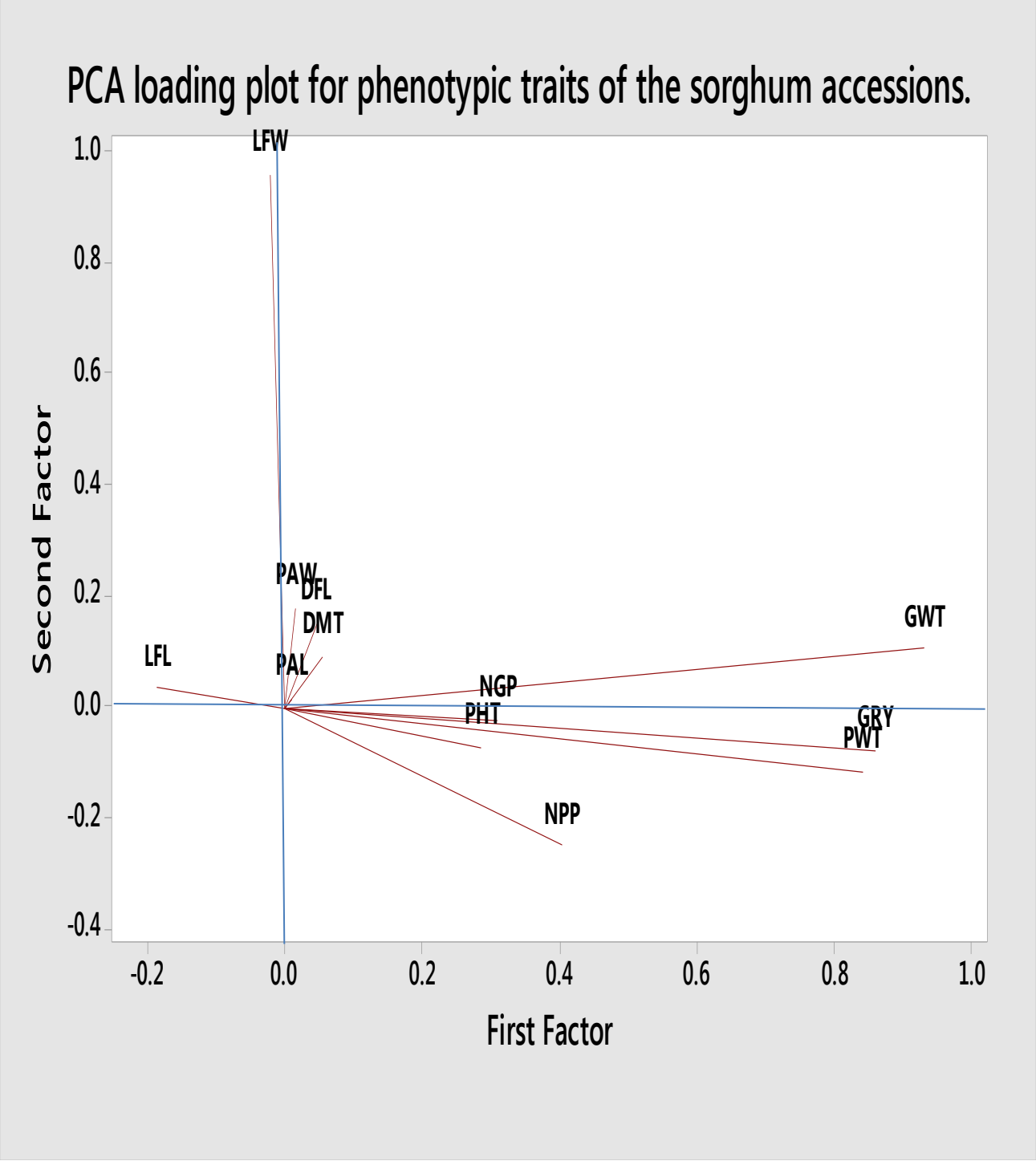

Figure 3.2 PCA loading plot for phenotypic traits of the sorghum breeding lines

3.4.7 Cluster analysis

The hierarchical clustering was performed on the Euclidean distance matrix utilizing complete linkage method by GenStat 18th edition (Payne, 2009) software to determine their phenotypic distances and relations among breeding lines. The quantitative traits dendrogram indicated differences among clusters of genotypes in the sorghum breeding lines (Figure 3.3). Four clusters were formed. The first cluster contained two breeding lines (15ELC F#72 and 15ELC F#76). The breeding lines in this cluster were characterised as late maturing. The second cluster consisted of 27 breeding lines characterised by narrow leaf width, long panicle length and narrow panicle width. Cluster III consisted of 12 breeding lines with long leaf length and large leaf area. Cluster IV consisted with 10 breeding lines characterised by early flowering and maturity, and short plant height.

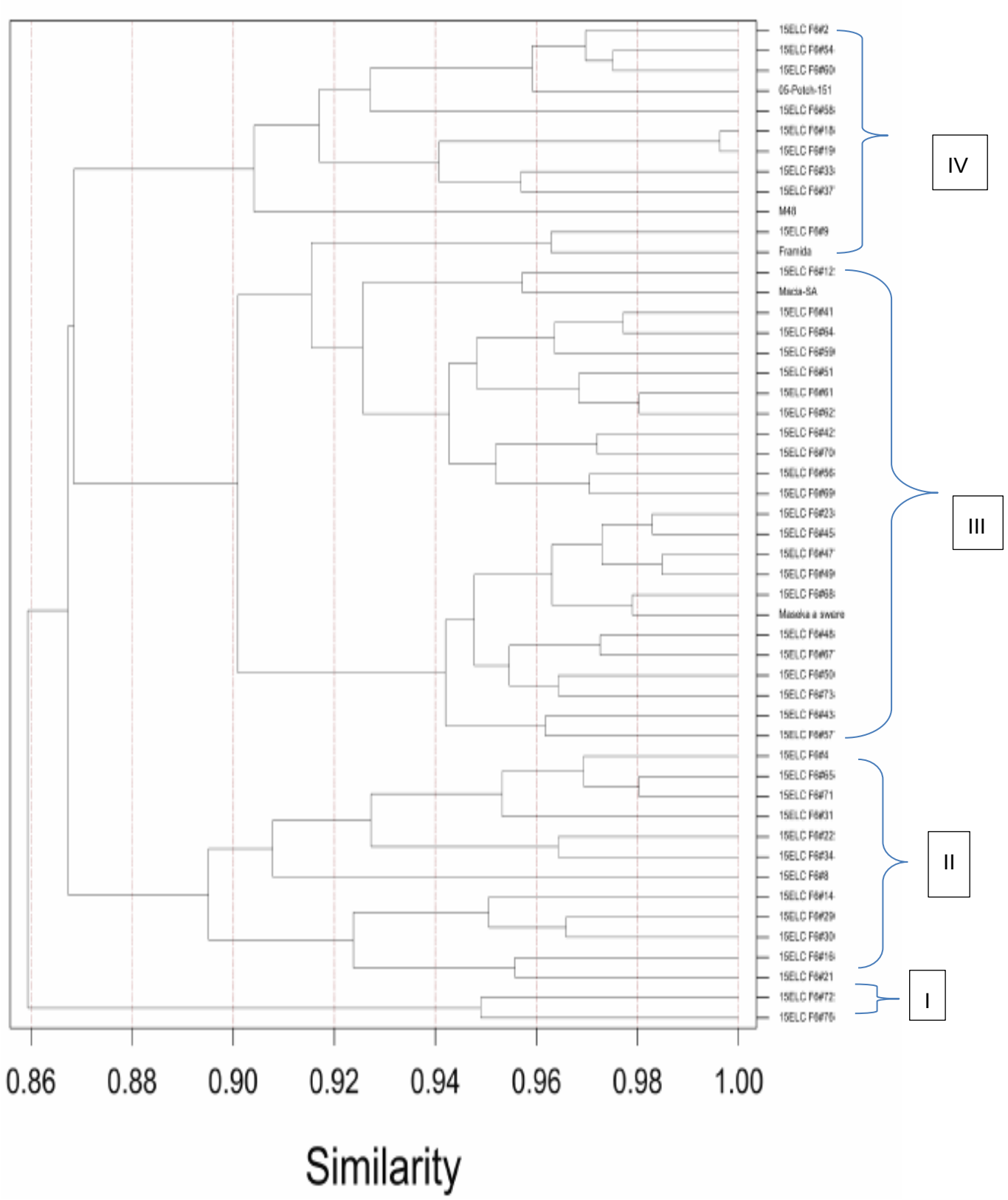

Figure 3.3 A dendogram of fifty sorghum breeding lines based on phenotypic traits

3.5 Discussion

Agro-morphological characterisation is one of the most important steps towards effective utilization of existing diversity in a crop species towards its genetic improvement and classification of the germplasm (Rakshit et al., 2012). Effective characterisation for agro-morphological traits is necessary to facilitate utilization of germplasm by breeders. This study provides details of genetic variability and functional correlations among 50 sorghum lines developed and advanced by the ARC (South Africa). The analysis was carried out using both qualitative and quantitative characters. The analysis of variance for the two planting dates and combined data showed highly significant differences for all traits. This implies that agronomic and morphological traits differed among the advanced sorghum lines and varieties at the two planting dates. The significant mean square values obtained for some of the characters indicated the effect the two planting dates had.

The study indicates that most of the breeding lines were high yielding as shown by the grand means of panicle weight (5.2 t/ha), grain yield (3.3 t/ha), number of grains per panicle (2561) and 1000 grain weight (28.4 g). The means of the grain yield parameters (panicle weight, grain yield per hectare, and number of grains per panicle and 1000 grain weight, panicle length and width) in the study were highest in the first planting date than at the second planting date. Variability obtained for grain yield, 1000 grain weight, and panicle weight were similar to earlier reports in sorghum (Elangovan et al., 2012). High grain yield is important in crop improvement programmes. The five top ranked breeding lines for grain yield were 15ELC F_6#68, 15ELC F_6#8, 15ELC F_6#70, Maseka a swere and 15ELC F_6#42. Therefore, breeding potential exists for these breeding lines as they showed good performance for yield and yield components under both environments. Days to 50% flowering ranged from 61 to 82 days. Days to 50% flowering is a useful criterion for determining the maturity range of the genotypes. Sorghum breeding lines can be grouped into three main categories, namely, early (less than 85 days), medium (86 - 105 days) and late (more than 105 days) (Kudadjie et al., 2007). Therefore, in this study all breeding lines were early maturing. Early maturing cultivars are advantageous in low rainfall regions as they allow the crop to escape damage from drought (Acquaah, 2007). The line 15ELC F_6#41 took 61 days to reach 50% flowering, followed by 15ELC F_6#33 (63 days) and 15ELC F_6#37, 15ELC F_6#72 and M48 (65 days). Breeding line 15ELC F_6#31 took 82 days to reach 50% flowering. Similar results showing variability for flowering dates was reported (Mahalakshmi and Bidinger, 2002; Noor et al., 2012).

There was considerable variation in plant height among breeding lines in this study. The maximum plant height was 235 cm for breeding line 15ELC F_6#43, followed by 15ELC F_6#48 (221 cm), and 15ELC F_6#70 (213 cm), while the minimum plant height was recorded for breeding line M48 (110 cm), 15ELC F_6#4 (116 cm) and 15ELC F_6#2 (119 cm). Similar results for plant height were observed in other studies (Nasim et al., 1993; Noor et al., 2012; Salah-ud-Din et al., 2002). Plant height is among the main yield components in sorghum and is directly proportional to yield (Noor et al., 2012). It is also an important agro-morphological trait that a breeder considers for lodging resistance. Tall cultivars are prone to lodging while short cultivars are mostly resistant to lodging. Short stature is also desirable for mechanized harvesting (Acquaah, 2012). The results revealed that most of the breeding lines were short to medium in height. Leaf length and leaf width ranged from 48 cm to 81 cm and 6.5 cm to 10.5 cm, respectively. Leaf length and leaf width was used to calculate leaf area which ranged from 321 cm^2 to 497 cm^2. Chaudhry et al. (1990) obtained similar results for the leaf area. Leaf area is an important yield component for fodder sorghum. Panicle length and panicle width showed considerable variability among breeding lines.

In both planting dates, the phenotypic and genotypic variance for all characters were higher than the environmental estimates. Genotypic variances for the characters were almost as high as phenotypic variances, indicating that the phenotype correlated well with the genotype thus selection based on phenotypic performance for these characters could be effective. According to Deshmukh et al. (1986) GCV and PCV values of roughly more than 20% are regarded as high, whereas values less than 10% are considered to be low and values ranging between 10 to 20% considered to be medium. High GCV and PCV were also observed for some characters such as plant height, leaf width, panicle number per plot, panicle weight, grain yield and number of grains per panicle. The finding is in agreement with the findings of Bello et al. (2007) and Williams et al. (1987) for cultivated sorghum cultivars of Adamawa State Nigeria. Similar results were also reported by Zaveri et al. (1989) for pearl millet. The means, ranges and coefficient of variation, namely the GCV and PCV across the planting dates in this study were highly variable. High GCV and PCV Similar results under different environments were reported by Ogunniyan and Olakojo (2014) for maize. This shows that the genotypes have a broad genetic bases as well as good potential to respond positively to selection.

High broad sense heritability estimates (greater than 80%) were observed for all characters in this study indicating the possibility of a positive response to selection. According to Singh (2001), high heritability estimate of a trait (≥ 80%) implies that selection for such traits could be fairly easy. This is due to a close correspondence between the genotypic and the

phenotypic expression, due to the relative small contribution of the environment to the phenotype. High heritability estimates obtained for most of the characters agreed with the findings of Mahajan et al. (2011), and Amare et al. (2015) for days to 50% flowering, days to maturity, plant height, panicle length and 1000 grain weight. Bello et al. (2007) reported similar results for most of the characters such as panicle length, days to 95% maturity, days to 50% flowering and plant height.

High heritability estimates with a low genotypic coefficient of variation may be a hindrance to selection and improvement of these traits (Amare et al., 2015). Genetic progress expected from selection increases with an increase in genotypic variance. Based on this benchmark, plant height, number of panicle per plot, panicle weight, grain yield and number of grains per panicle showed high heritability coupled with a high genotypic coefficient of variation across planting dates. This indicates that these characters may respond effectively to phenotypic selection. Amare et al. (2015) found a high heritability coupled with high genotypic coefficient of variation for traits such as plant height, grain yield, panicle yield, leaf area index, and harvesting index. Among these traits grain yield, panicle yield and plant height are in agreement with this study.

The highest genetic advance (GA) values were observed in number of grains per panicle, leaf area, and plant height. Singh (2001) explained that GA under selection refers to the improvement of characters in the genotypic value of the new population, compared with the base population under one cycle of selection at a given selection intensity. Estimated GA for grain yield was 2.65 t/ha and this implies that when we select the best 5% of high yielding genotypes as parents, the mean grain yield of progenies would be improved by 2.65 t/ha, that is, the mean genotypic value of the new population for grain yield will be improved from 3.33 to 5.98 t/ha. Based on the genetic advance to be expected panicle weight could increase from 5.2 to 8.8 t/ha.

The estimated value of expected genetic advance expressed as percentage of the mean (GAM) at 5% proportion selected ranged from 2.28% to 148% across planting dates. Maximum GAM was recorded for leaf width (148.76%), grain yield (78.5%), panicle weight (69.13%) and number of panicle per plot (61.62%). Amare et al. (2015) reported high GAM for leaf area index, plant height, grain yield, harvesting index and panicle yield per plant. Therefore, selection based on these traits with high GAM will result in improvement of performance of the varieties for these traits. High heritability estimates along with high genetic advance is usually

more helpful in predicting gain yield under selection than heritability estimates alone (Johnson et al., 1955). Therefore, from this study, traits with high heritability, coupled with the high expected genetic advance as percentage of the mean across locations, were leaf width, panicle weight, grain yield, and number of panicle per plot and plant height. These characters could be improved more easily than others due to their potential to respond positively to selection across the different locations.

The knowledge of the type of association among various characters in any breeding programme it helps in simultaneous selection for characters associated with desirable traits for improvement (Kumar et al., 2012). In the present study, correlation analysis indicated some important associations among the quantitative traits studied. Grain yield showed significant positive correlation with panicle weight, 1000 grain weight, number of panicles per plot, number of grains per panicle and plant height. El Naim et al. (2012) reported grain yield/ha had highly significant and positive correlation with number of grains per head and plant height. Number of grains per panicle had positive and highly significant correlation with grain yield. Similar results were observed by Tag El-Din et al. (2012). Grain yield had negative, significant correlation with leaf length and negative correlation with leaf width. This suggests selection for grain yield, panicle weight, 1000 grain weight, number of panicles per plot, number of grain per panicle and plant height can be carried out simultaneously, however, with an inverse selection pattern with leaf length and leaf width.

Breeding lines characterisation and clustering based on their morphological traits and genetic similarity assists in identification and selection of the best parents for hybridisation (Souza and Sorrells, 1991). The clustering demonstrated variation of breeding lines based on morphological traits that could be a valuable source for the sorghum improvement programmes. The study has shown that, diversity exists among the genotypes of the sorghum germplasm studied. The clustering pattern indicated the presence of variability among the grain sorghum breeding lines. Diversity of sorghum breeding lines was also reported by (Noor et al. (2012). The information obtained from this study is useful for the breeders in future sorghum breeding programmes for yield and yield related traits improvement.

The PCA was used as a data reduction tool to summarize the information from the data set to reduce causes of error and outliers on the results. The PCA is used to reveal the pattern of character variation among individual breeding lines in a population and allows the relationship between variables and observations to be studied, and recognizing the data structure (Chozin,

2007). The PCA is used to eliminate the redundancy in data set (Dossou-Aminon et al., 2015). In this study, three PCAs, having eigenvalues greater than 1, accounted for 64.35% of total variability among the sorghum breeding lines studied. The result suggested that grain yield, number of panicles per plot, plant height, 1000 grain weight, and panicle weight are important traits as they contributed maximum towards divergence of sorghum breeding lines. In the study reported by Sinha and Kumaravadivel. (2016), the first three PCAs with eigenvalues greater than 1 accounted for 73.2% of total variance. The PCA grouped the breeding lines into groups over the four quadrants based on the quantitative traits. The breeding lines remained scattered in all four quadrants. This shows large genetic variability for the traits studied. Some sorghum breeding lines overlapped in principal component axes showing that the traits have similarity.

3.6 Conclusion

The results from this study suggest that there is a considerable variation in the quantitative and qualitative morphological traits in the sorghum germplasm studied. The presence of morphological and genetic variations in agronomic traits of a crop would be of importance in determining the best method for yield improvement of that crop. A total of 50 sorghum breeding lines were evaluated for quantitative and qualitative traits to determine the extent of morphological diversity. The analysis of variance (ANOVA) revealed that there were statistically significant differences among the varieties for most of the traits across the two growing environments. The results showed that higher grain yield was obtained in the first planting date with mean grain yield of 3.6 t/ha, than in the second planting date, with mean grain yield of 3.2 t/ha. This shows that the sorghum breeding lines evaluated performed better when planted early in December than when planted in January at Potchefstroom. In both environments phenotypic and genotypic variance for most characters were higher than environment variance. This indicates that expression for most of the characters was genetic and can be exploited through breeding. High heritability coupled with high genotypic coefficient of variation was observed for traits such as plant height, number of panicle per plot, panicle weight, grain yield and number of grains per panicle. These traits also had high genetic advance values. Traits with high heritability and genetic advance should be given attention in order to bring an effective response of grain improvement of the concerned varieties. Correlation coefficient analysis revealed highly significant positive association between economic traits that can be used in the improvement of sorghum by breeders. Different breeding lines of sorghum exhibited the potential for selection of the desired characters. Morphological characterisation can still be a useful tool in breeding programmes.

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CHAPTER 4

Starch content, protein content, and protein digestibility analyses in grain sorghum

Abstract

The demand for ethanol derived from renewable feedstocks and fermentation as a substitute of gasoline has increased in recent years due to several reasons such as increase in the fossil fuel price, global energy crisis, and the environmental concerns over carbon emissions and international environmental agreements. Several crops have been investigated for bioethanol production. Grain sorghum is the second major starch-rich raw material (after maize) for bioethanol production. The aim of this study was to evaluate advanced grain sorghum breeding lines and elite varieties for their diversity in starch content, protein content and protein digestibility as, among others, the main factors influencing bioethanol yield from grain sorghum. Sorghum grains from 50 breeding lines used for morphological characterisation (Chapter 3) were analysed for starch content using near- infrared (NIR) spectroscopy, and for protein content using Bradford protein assay procedure. The *in vitro* protein digestibility was also determined. The analysis of variance showed highly significant differences in sorghum breeding lines for starch content, protein content and protein digestibility. The starch content for the breeding lines varied from 63.28% to 71.29% across the two planting dates with a mean value of 67.51%. The protein content ranged between 9.21% and 15.06% across the two planting dates with an overall mean of 12.24%. The protein digestibility ranged from 33.87% to 82.22% over the two planting dates with a mean value of 64.21%. There were positive correlations between starch content and grain yield, while protein content was negatively correlated with starch content. The breeding lines were grouped according to grain colour, with the light brown grain types having a high starch and low protein content. In general, there was great variability in starch content, protein content and digestibility across the two planting dates. The presence of genetic diversity among breeding lines studied is essential for quality improvement as there would be a potential of selection of sorghum breeding lines based on starch content, protein content and protein digestibility. The breeding lines that showed desirable characteristics for bioethanol production with high starch content, low protein content, and high protein digestibility were 05-Potch-151, 15ELC F_6#2, 15ELC F_6#47, and 15ELC F_6#43 and 15ELC F_6#45.

4.1 Introduction

Sorghum is an important staple food crop in Africa and Asia, and is a major feed grain in developed countries. During recent years, sustainable alternative bioethanol feedstocks are being sought, particularly in regions of low water availability. One viable solution is bioethanol from sorghum grain. Grain sorghum is among the major important cereal crops grown in South Africa with the potential use for bioethanol production. Despite its potential, grain sorghum has been underutilized as a renewable feedstock for bioenergy. The possibility of grain sorghum being used in bioethanol production is due to its high starch content, which is similar in composition to maize, a crop that has been successfully used for bioethanol production (Rooney and Serna-Saldivar, 2000). Thus sorghum grain would be appropriate for use in fermentation for bioethanol (Nghiem et al., 2016).

There are different varieties of sorghum grains that can be used for bioethanol purposes. The variations between sorghum varieties affect the amount of ethanol production and conversion efficiency. It is approximated that the yield of ethanol from sorghum grain is comparable to that of maize grain (Murray et al., 2008). In the past, more attention was given to studying the factors influencing ethanol yield from maize than sorghum. The potential of grain sorghum for use as a feedstock in commercial ethanol production has also attracted interest from several research groups (Nghiem et al., 2016).

Researchers have investigated the digestibility of sorghum starch and protein in relation to feed or food uses (Rooney and Pflugfelder, 1986; Streeter et al., 1990; Zhang and Hamaker, 1998). In order to identify potential sorghum lines for bioethanol production, it is important to understand the key factors influencing ethanol production from grain sorghums. Ethanol yield and conversion efficiency are the two most important quality traits of cereal grains when they are used to produce fuel. Both ethanol yield and fermentation efficiency have been used to evaluate the performance of grain sorghum as a feedstock in ethanol production (Wu et al., 2007). Research shows that key factors affecting ethanol yield and ethanol fermentation efficiency of sorghum include starch content, protein digestibility, level of extractable proteins, protein and starch interaction, mash viscosity, the amount of phenolic compounds, the ratio of amylose to amylopectin, and the formation of amylose-lipid complexes in the mash (Wu et al., 2007; Wang et al., 2008; Zhao et al., 2009).

Starch is the major component followed by protein in grain sorghum. Starch content in sorghum flour was a good predictor for ethanol yield (Lacerenza et al., 2008; Zhao et al.,

2009). Sorghum genotypes with high starch content are good candidates for bioethanol production. Starch is the major biochemical indicator for bioethanol production in cereals. Grain sorghum is known to be less digestible than maize and is the reason why it has limited use for bioethanol purposes (Duodu et al., 2003).

Most plant breeding programmes have been focusing on increasing yields of sorghum grain but little attention has been paid to the biochemical quality of the grain. Therefore, the objective of this study was to evaluate the variations in starch content, protein content and protein digestibility of grain sorghum breeding lines as the key factors influencing bioethanol production.

4.2 Materials and methods

4.2.1 Germplasm material

Grain samples of 50 sorghum breeding lines were harvested from field trials grown under two planting dates during 2015/16 summer season at the ARC-GCI experimental Farm, in Potchefstroom, South Africa (26°74''S; 27°8'E). The list of the sorghum breeding lines used for the analyses are presented in Table 4.1. The samples were manually cleaned by removing foreign materials and plant debris and then ground to flour with a Udy cyclone sample mill.

Table 4.1: List of sorghum breeding lines used in the study

Entry	Name	Entry	Name
1	15ELC F_6#2	26	15ELC F_6#50
2	15ELC F_6#4	27	15ELC F_6#51
3	15ELC F_6#8	28	15ELC F_6#54
4	15ELC F_6#9	29	15ELC F_6#56
5	15ELC F_6#12	30	15ELC F_6#57
6	15ELC F_6#14	31	15ELC F_6#58
7	15ELC F_6#16	32	15ELC F_6#59
8	15ELC F_6#18	33	15ELC F_6#60
9	15ELC F_6#19	34	15ELC F_6#61
10	15ELC F_6#21	35	15ELC F_6#62
11	15ELC F_6#22	36	15ELC F_6#64
12	15ELC F_6#23	37	15ELC F_6#65
13	15ELC F_6#29	38	15ELC F_6#67
14	15ELC F_6#30	39	15ELC F_6#68
15	15ELC F_6#31	40	15ELC F_6#69
16	15ELC F_6#33	41	15ELC F_6#70
17	15ELC F_6#34	42	15ELC F_6#71

Entry	Name	Entry	Name
18	15ELC F_6#37	43	15ELC F_6#72
19	15ELC F_6#41	44	15ELC F_6#73
20	15ELC F_6#42	45	15ELC F_6#76
21	15ELC F_6#43	46	05-Potch-151
22	15ELC F_6#45	47	Framida
23	15ELC F_6#47	48	M48
24	15ELC F_6#48	49	Macia-SA
25	15ELC F_6#49	50	Maseka a swere

4.2.2 Determination of starch content

Starch content was analysed using Near-Infrared (NIR) spectroscopy. A FOSS NIR machine, NIR Systems Composite Monochomator 6500, (FOSS NIR Systems Inc., 7703 Montpelier Rd, Laurel, MD, USA) was used at ARC - CGI Potchefstroom Grain quality laboratory. Sorghum grains of each sample from the two planting dates were placed in a sample cup that was used for scanning of the whole seeds for analysis of starch content. The whole grains were scanned, then put into envelopes and shaken for 5 seconds before re-scanning. The grains were scanned in triplicates.

4.2.3 Protein content analysis

Total soluble protein extraction

Total soluble proteins were extracted according to the methodology of Kanellis and Kalaitzis (1992) with slight modification: freeze-dried, milled mesocarp tissue (1.0 g DM) was extracted in 5mL 50mM Tris–HCl buffer (pH 7.4) containing 0.2M NaCl, 20mM MgSO4, 1mM EDTA, 5mM -mercaptoethanol, 0.5mM PMSF, 10mM leupeptin, and 10% (v/v) glycerol. The samples were then homogenised using the ultrasonic cell disrupter to extract free and membrane-bound proteins. Subsequently, the mixture was allowed to stand on ice for 15 min and centrifuged at 20,000 × g for 20 min. The supernatant was used for enzyme assays after being filtered through Miracloth®.

Quantification of total protein content

Protein concentration was measured using the Bradford micro assay (Bradford, 1976) after diluting Bradford dye reagent concentrate with distilled water at a ratio of 1:4. A 1 mL of diluted dye was added to a plastic cuvette and mixed with protein extract. The samples were then incubated for 5 min at room temperature, followed by spectrophotometer reading at 595 nm. The concentration of proteins was determined by comparing spectrophotometry results with the standard curve of bovine serum albumin (R^2 = 0.98) (Figure 4.1).

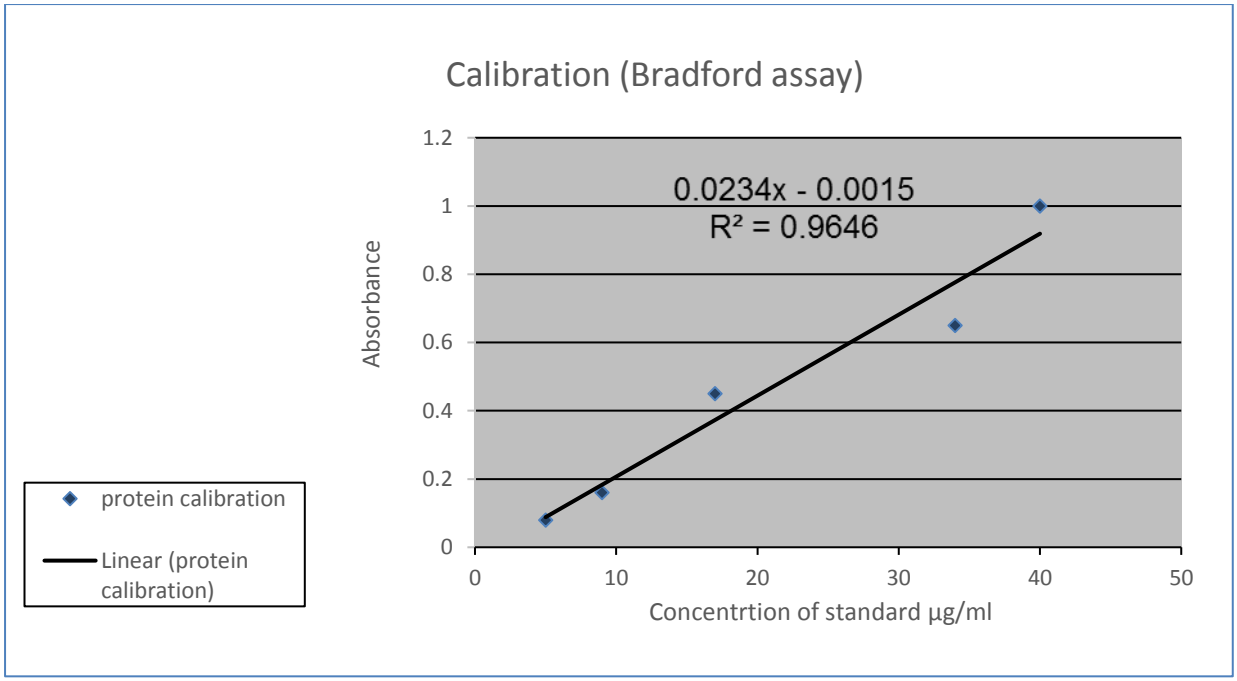

Figure 4.1 Standard curve for Bradford assay

4.2.4 Determination of protein digestibility

In vitro pepsin protein digestibility was determined using the pepsin method as described by Mertz et al. (1984) with some modification: the pH 2.0 citrate buffer containing pepsin (105 mg pepsin/100 ml buffer) was pre-heated to 37°C. A 200 mg sample was weighed into a 50 ml plastic centrifuge tube and 35 ml of the pH 2.0 citrate buffer containing pepsin added to the samples and suspended by swirling. Tubes were incubated for 2 hours at 37°C in a shaking water bath and mixed every 15 min. The reaction was stopped by the addition of 2 ml of 2M sodium hydroxide. After terminating the enzyme reaction with sodium hydroxide, samples were vortexed and centrifuged at 4500 rpm for 10 min to form a firm pellet and the clear supernatant was pipetted off using a pasteur pipette. The pellet was washed once with 35 ml distilled water, centrifuged and the clear supernatant pipetted off. Residues were dried in the centrifuge tubes. The dried material was carefully crushed and completely scraped out of the centrifuge tube, weighed and the protein content determined.

Calculation of results

% Protein digestibility = (X - Y /X) x 100

X = mean total protein content

Y = mean residual protein

4.3 Statistical data analysis

Data obtained was subjected to analysis of variance (ANOVA) using GenStat software, 17th edition (Payne, 2009). Pearson's correlation test was carried out to assess the significance of degree of association between total starch, protein content, protein digestibility, grain size, grain colour and 1000 grain weight using GenStat software, 17th edition (Payne, 2009).

4.4 Results

The ANOVA revealed highly significant (P≤ 0.001) differences among the sorghum breeding lines for content, protein content and protein digestibility across the two planting dates suggesting a high degree of variability among the parameters.

4.4.1 Starch content

Results of starch content of the 50 sorghum breeding lines across the two planting dates are presented in Table 4.2. The starch content of sorghum breeding lines at first planting date ranged from 65.07% to 73.72%, with a mean of 69.27%. There was distinct variation among the sorghum breeding lines where 05Potch151 (73.72%), 15ELC F_6#47 (72.34%), 15ELC F_6#69 (72.03) and 15ELC F_6#43 (71.93%) had the highest starch contents. The breeding lines 15ELC F_6#22 (66.09%), 15ELC F_6#73 (66%), Macia-SA (65.77%), and Framida (65.44%) had the lowest starch contents.

At the second planting date, the breeding lines exhibited starch content ranging from 61.13% to 68.9% with a mean of 65.75% (Table 4.2). There was a marked variation among sorghum breeding lines where 05Potch151 (68.9%), 15ELC F_6#22 (68.86%), M48 (67.58%) and 15ELC F_6#12 (67.42%) had the highest starch contents. The breeding lines 15ELC F_6#73 (61.13%), 15ELC F_6#67 (61.37%) 15ELC F_6#70 (62.65%), 15ELC F_6#56 (62.98%) and Framida (63.12%) had lowest starch contents.

The combined ANOVA showed a high degree of variability among the sorghum breeding lines for starch content. The overall mean ranged between 63.28% and 71.29% with the grand mean of 67.51%.The breeding lines with highest values of starch content in the combined environment were 05Potch151 (71.29%), 15ELC F_6#2 (70.28%), 15ELC F_6#47 (69.8%), and 15ELC F_6#43 (69.64%). The lowest starch content was observed in the breeding lines Framida (63.28%), 15ELC F_6#58 (64.03%), 15ELC F_6#22 (64.27%), 15ELC F_6#21 (64.37%) and Macia-SA (64.45%).

Table 4.2 Grain starch content (%) of 50 sorghum breeding lines grown at two planting dates, in the 2015/16 season at Potchefstroom, South Africa

Entry No.	Name of breeding line	First planting	Second planting	Overall mean
1	15ELC F_6#2	71.65	68.90	70.28
2	15ELC F_6#4	71.52	66.37	68.95
3	15ELC F_6#8	68.49	64.44	66.46
4	15ELC F_6#9	68.08	65.78	66.93
5	15ELC F_6#12	68.31	64.40	66.36
6	15ELC F_6#14	68.85	67.35	68.10
7	15ELC F_6#16	70.23	67.02	68.63
8	15ELC F_6#18	69.08	63.67	66.38
9	15ELC F_6#19	68.41	65.97	67.19
10	15ELC F_6#21	67.17	61.37	64.27
11	15ELC F_6#22	66.09	62.65	64.37
12	15ELC F_6#23	69.28	66.07	67.67
13	15ELC F_6#29	69.55	64.77	67.16
14	15ELC F_6#30	70.91	63.64	67.28
15	15ELC F_6#31	66.35	64.52	65.44
16	15ELC F_6#33	68.99	66.57	67.78
17	15ELC F_6#34	68.47	65.65	67.06
18	15ELC F_6#37	66.64	64.89	65.76
19	15ELC F_6#41	68.79	65.87	67.33
20	15ELC F_6#42	71.60	67.33	69.47
21	15ELC F_6#43	71.93	67.35	69.64
22	15ELC F_6#45	71.50	66.94	69.22
23	15ELC F_6#47	72.34	67.26	69.80
24	15ELC F_6#48	70.78	66.48	68.63
25	15ELC F_6#49	69.49	67.38	68.44
26	15ELC F_6#50	68.66	66.18	67.42
27	15ELC F_6#51	67.12	65.20	66.16
28	15ELC F_6#54	70.03	64.81	67.42
29	15ELC F_6#56	68.70	65.82	67.26
30	15ELC F_6#57	71.45	65.89	68.67
31	15ELC F_6#58	65.07	62.98	64.03
32	15ELC F_6#59	68.31	67.19	67.75
33	15ELC F_6#60	69.92	66.26	68.09
34	15ELC F_6#61	69.58	67.58	68.58
35	15ELC F_6#62	69.90	66.75	68.32
36	15ELC F_6#64	68.55	66.52	67.53
37	15ELC F_6#65	70.93	66.82	68.88
38	15ELC F_6#67	71.21	67.42	69.32

Entry No.	Name of breeding line	First planting	Second planting	Overall mean
39	15ELC F_6#68	69.46	65.97	67.71
40	15ELC F_6#69	72.03	66.53	69.28
41	15ELC F_6#70	71.01	65.92	68.47
42	15ELC F_6#71	69.37	66.64	68.01
43	15ELC F_6#72	67.48	63.66	65.57
44	15ELC F_6#73	66.00	63.93	64.97
45	15ELC F_6#76	69.69	67.01	68.35
46	05Potch151	73.72	68.86	71.29
47	Framida	65.44	61.13	63.28
48	M48	68.52	65.83	67.18
49	Macia-SA	65.77	63.12	64.45
50	Maseka a swere	70.89	66.79	68.84
	Mean	69.27	65.75	67.51
	Max	73.72	68.9	71.29
	Min	65.07	61.13	63.28
	L.s.d.(P=0.05)	1.34	2.90	2.25
	CV%	1.20	2.70	2.10

4.4.2 Protein content

Results of the protein contents of 50 sorghum breeding lines across two planting dates, first planting and second planting are presented in Table 4.3. The protein content of sorghum breeding lines at first planting ranged from 8.04% to 14.33% with a mean of 11.81%. Under this environment the breeding lines with the highest value of protein content were 15ELC F_6#22 (14.33%), 15ELC F_6#31 (14.11%), 15ELC F_6#33 (14.09%), 15ELC F_6#37 (14.06%) and 15ELC F_6#19 (13.77%) and the lowest values of protein content were observed for 15ELC F_6#67 (8.04%), 05Potch151 (8.50%), 15ELC F_6#69 (8.56%), 15ELC F_6#14 (8.65%) and 15ELC F_6#47 (9.05%).

At the second planting, protein content ranged between 9.91% and 15.78%. The breeding lines with highest values for protein content under this condition were 15ELC F_6#22 (15.78%), Framida (14.93%), 15ELC F_6#18 (14.72%), 15ELC F_6#72 (14.52%) and 15ELC F_6#54 (14.84%), while breeding lines with the lowest protein content value were 05Potch151 (9.91%), 15ELC F_6#47 (9.93%), 15ELC F_6#67 (10.31%), 15ELC F_6#14 (10.57%) and 15ELC F_6#62 (10.68%).

Overall, there was a high degree of variability among the sorghum breeding lines when tested at both first planting and second planting dates. In the combined analysis, the protein content

ranged from 9.21% to 15.06% with an overall mean of 12.24%. The breeding lines which showed high protein content across planting dates were 15ELC F_6#22 (15.06%), 15ELC F_6#18 (14.15%), 15ELC F_6#72 (14.08%), 15ELC F_6#19 (13.95%) and 15ELC F_6#54 (13.92%), and breeding lines with lowest protein contents were 05-Potch-151 (9.21%), 15ELC F_6#67 (9.36%), 15ELC F_6#47 (9.49%), 15ELC F_6#14 (9.61%), 15ELC F_6#69 (10.21%).

Table 4.3 Grain protein content (%) of 50 sorghum breeding lines from two planting dates in the 2015/16 season at Potchefstroom, South Africa

Entry No.	Name of breeding line	First planting	Second planting	Overall mean
1	15ELC F_6#2	10.71	12.33	11.52
2	15ELC F_6#4	10.29	13.06	11.68
3	15ELC F_6#8	11.06	13.71	12.38
4	15ELC F_6#9	12.56	11.31	11.93
5	15ELC F_6#12	11.89	13.14	12.52
6	15ELC F_6#14	8.65	10.57	9.61
7	15ELC F_6#16	10.62	11.57	11.09
8	15ELC F_6#18	13.58	14.72	14.15
9	15ELC F_6#19	13.77	14.14	13.95
10	15ELC F_6#21	11.26	13.73	12.49
11	15ELC F_6#22	14.33	15.78	15.06
12	15ELC F_6#23	13.26	13.23	13.25
13	15ELC F_6#29	11.80	14.21	13.01
14	15ELC F_6#30	12.98	13.80	13.39
15	15ELC F_6#31	14.11	13.55	13.83
16	15ELC F_6#33	14.09	13.50	13.80
17	15ELC F_6#34	12.41	13.62	13.01
18	15ELC F_6#37	14.06	13.42	13.74
19	15ELC F_6#41	12.52	12.18	12.35
20	15ELC F_6#42	10.77	11.09	10.93
21	15ELC F_6#43	10.29	12.67	11.48
22	15ELC F_6#45	10.32	10.68	10.50
23	15ELC F_6#47	9.05	9.93	9.49
24	15ELC F_6#48	12.33	10.75	11.54
25	15ELC F_6#49	11.82	11.22	11.52
26	15ELC F_6#50	12.14	11.48	11.81
27	15ELC F_6#51	13.15	13.50	13.32
28	15ELC F_6#54	13.37	14.48	13.92
29	15ELC F_6#56	11.79	13.03	12.41
30	15ELC F_6#57	9.81	11.73	10.77
31	15ELC F_6#58	12.32	13.15	12.73
32	15ELC F_6#59	12.82	12.55	12.69
33	15ELC F_6#60	12.81	10.88	11.85
34	15ELC F_6#61	10.55	10.73	10.64
35	15ELC F_6#62	10.50	10.31	10.40
36	15ELC F_6#64	11.12	12.48	11.80
37	15ELC F_6#65	13.44	14.41	13.92
38	15ELC F_6#67	8.04	10.68	9.36
39	15ELC F_6#68	12.55	14.15	13.35
40	15ELC F_6#69	8.56	11.86	10.21
41	15ELC F_6#70	12.09	12.52	12.31
42	15ELC F_6#71	13.36	12.97	13.16

Entry No.	Name of breeding line	First planting	Second planting	Overall mean
43	15ELC F_6#72	13.65	14.52	14.08
44	15ELC F_6#73	12.24	13.08	12.66
45	15ELC F_6#76	12.26	12.10	12.18
46	05Potch151	8.50	9.91	9.21
47	Framida	10.82	14.93	12.88
48	M48	12.15	13.45	12.80
49	Macia-SA	12.17	13.62	12.89
50	Maseka a swere	11.69	12.77	12.23
	Mean	11.81	12.67	12.24
	Max	14.33	15.78	15.06
	Min	8.04	9.91	9.21
	L.s.d.(P=0.05)	1.10	1.41	1.28
	CV%	5.70	6.90	6.50

4.4.3 Protein digestibility

Results of the protein digestibility analysis of the 50 sorghum breeding lines across the two planting dates are presented in Table 4.4. The protein digestibility at the first planting date ranged from 36.15% to 84.28% with a mean of 65.57%. Breeding lines with the highest protein digestibility percentage were 15ELC F_6#43 (84.28%), 05Potch151 (80.97%), 15ELC F_6#47 (80.61%), 15ELC F_6#2 (80.48%), and 15ELC F_6#42 (76.38%), while breeding lines15ELC F_6#62 (36.15%), 15ELC F_6#73 (37.78%), Framida (46.08%), 15ELC F_6#58 (53.02%), and 15ELC F_6#21 (54.31%) recorded the lowest protein digestibility.

At the second planting date, protein digestibility ranged between 31.59% and 80.17%. The breeding lines with highest protein digestibility values were 80.17%, 79.43%, 78.95%, 78.51%, and 78.48% for 15ELC F_6#43, 15ELC F_6#47, 05Potch151 15ELC F_6#45, and 15ELC F_6#2, respectively. On the other hand, the lowest protein digestibility values were recorded in breeding lines 15ELC F_6#62 (31.59%), 15ELC F_6#73 (38.66%), Framida (42.68%), 15ELC F_6#51 (48.59%), and 15ELC F_6#58 (49.60%).

Overall, there was a higher degree of variability among the sorghum breeding lines when tested at first planting than second planting (Table 4.4). The protein digestibility ranged between 33.87% and 82.22% across the environments with the grand mean of 64.22%. The breeding lines that showed high protein digestibility across the two planting dates were 15ELC F_6#43, 15ELC F_6#47, 05Potch151, 15ELC F_6#2, and 15ELC F_6#45, with values of 82.22%, 80.02%, 79.96%, 79.48%, and 76.91%, respectively.

Table 4.4 Protein digestibility(%) of 50 grain sorghum breeding lines grown under two planting dates in the 2015/16 season at Potchefstroom, South Africa

Entry	Name of breeding line	First planting date	Second planting date	Overall
1	15ELC F_6#2	80.48	78.48	79.48
2	15ELC F_6#4	75.36	75.69	75.53
3	15ELC F_6#8	65.30	60.45	62.87
4	15ELC F_6#9	67.07	61.11	64.09
5	15ELC F_6#12	59.29	53.13	56.21
6	15ELC F_6#14	67.52	64.52	66.02
7	15ELC F_6#16	74.52	71.74	73.13
8	15ELC F_6#18	60.19	56.38	58.28
9	15ELC F_6#19	65.61	61.97	63.79
10	15ELC F_6#21	54.31	54.82	54.56
11	15ELC F_6#22	60.32	55.11	57.72
12	15ELC F_6#23	68.05	62.31	65.18
13	15ELC F_6#29	62.52	60.31	61.41
14	15ELC F_6#30	76.30	76.00	76.15
15	15ELC F_6#31	62.50	58.57	60.54
16	15ELC F_6#33	64.76	62.61	63.68
17	15ELC F_6#34	66.61	64.32	65.47
18	15ELC F_6#37	56.18	53.47	54.82
19	15ELC F_6#41	74.48	69.88	72.18
20	15ELC F_6#42	76.38	77.09	76.74
21	15ELC F_6#43	84.28	80.17	82.22
22	15ELC F_6#45	75.31	78.51	76.91
23	15ELC F_6#47	80.61	79.43	80.02
24	15ELC F_6#48	74.43	69.02	71.72
25	15ELC F_6#49	73.08	69.00	71.04
26	15ELC F_6#50	57.58	56.53	57.05
27	15ELC F_6#51	50.43	48.59	49.51
28	15ELC F_6#54	65.61	62.29	63.95
29	15ELC F_6#56	73.86	71.10	72.48
30	15ELC F_6#57	73.51	70.20	71.85
31	15ELC F_6#58	53.02	49.60	51.31
32	15ELC F_6#59	68.56	65.72	67.14
33	15ELC F_6#60	65.92	63.48	64.70
34	15ELC F_6#61	56.11	53.29	54.70
35	15ELC F_6#62	36.15	31.59	33.87
36	15ELC F_6#64	60.31	56.77	58.54
37	15ELC F_6#65	71.46	66.19	68.83
38	15ELC F_6#67	65.96	63.36	64.66
39	15ELC F_6#68	69.25	62.21	65.73
40	15ELC F_6#69	74.91	74.07	74.49
41	15ELC F_6#70	56.42	51.04	53.73
42	15ELC F_6#71	61.13	58.80	59.97

Entry	Name of breeding line	First planting date	Second planting date	Overall
43	15ELC F_6#72	55.69	51.42	53.56
44	15ELC F_6#73	37.78	38.66	38.22
45	15ELC F_6#76	62.54	60.16	61.35
46	05Potch151	80.97	78.95	79.96
47	Framida	46.08	42.68	44.38
48	M48	71.07	74.73	72.90
49	Macia-SA	64.56	61.87	63.21
50	Maseka a swere	74.15	75.72	74.94
	Mean	65.57	62.86	64.21
	Max	84.28	80.17	82.22
	Min	36.15	31.59	33.87
	L.s.d.(P=0.05)	2.18	2.88	2.53
	CV%	2.10	2.80	2.40

4.4.4 Relationship between grain colour, starch content, protein content and protein digestibility

The relationships observed between the grain colour and the starch content, protein content and protein digestibility in sorghum grain are presented in Figure 4.2 – Figure 4.4. The sorghum breeding lines with highest mean starch contents were from the group with light brown grain colour while the red coloured breeding lines, on the other hand, showed the lowest starch content (Figure 4.2). The breeding lines group with the highest protein contents were those with the red grain colour, while the light brown breeding lines had the lowest protein content (Figure 4.3). The highest protein digestibility was recorded in breeding lines with yellow colour, while the lowest were the red colour (Figure 4.4).

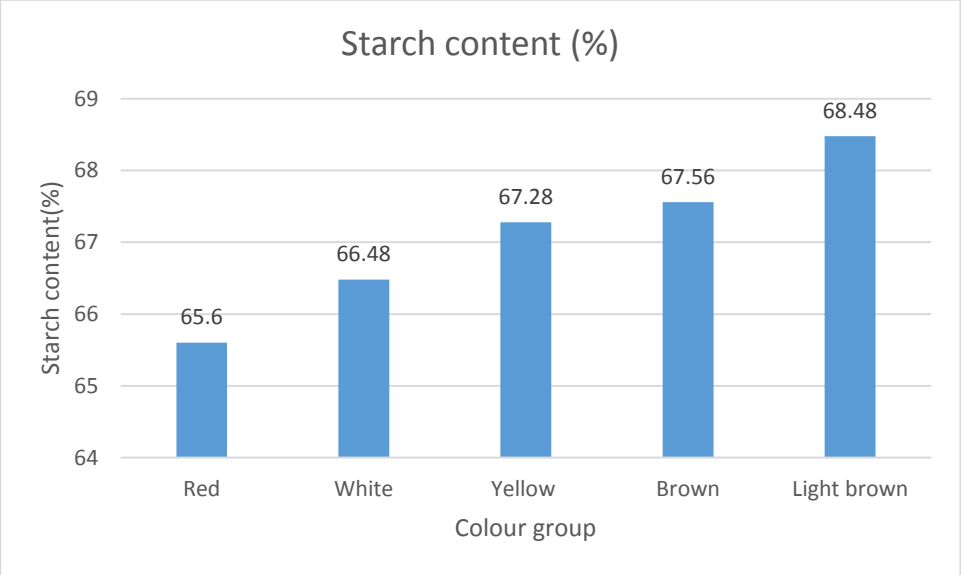

Figure 4.2 Average starch content in grain sorghum according to grain colour groups

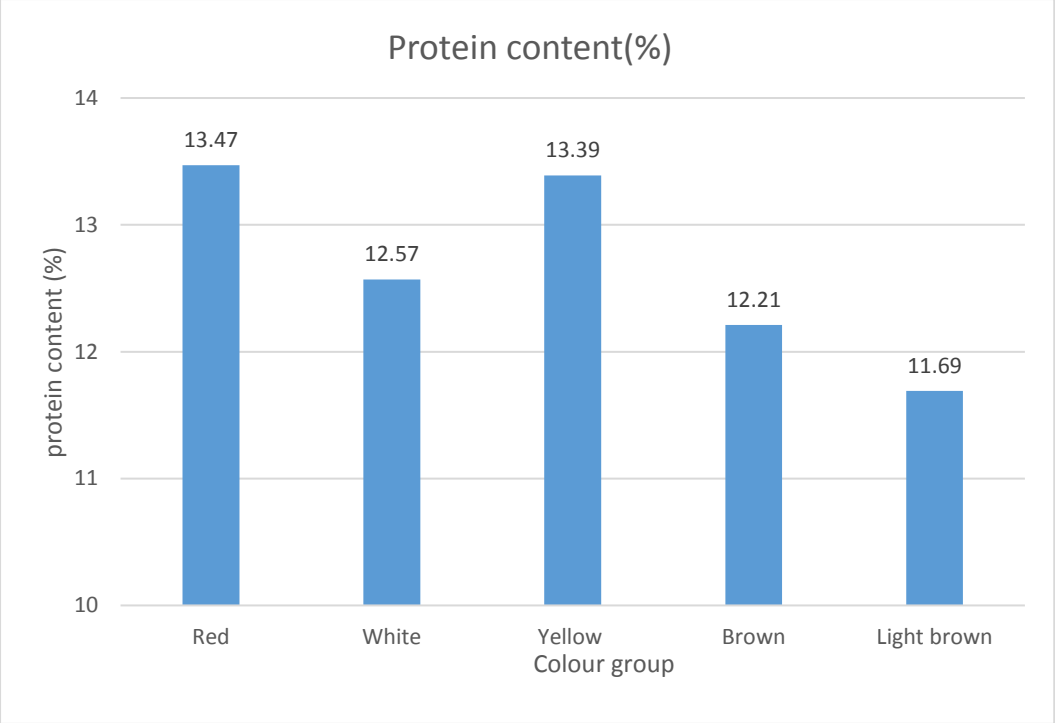

Figure 4.3 Average protein content according to colour groups

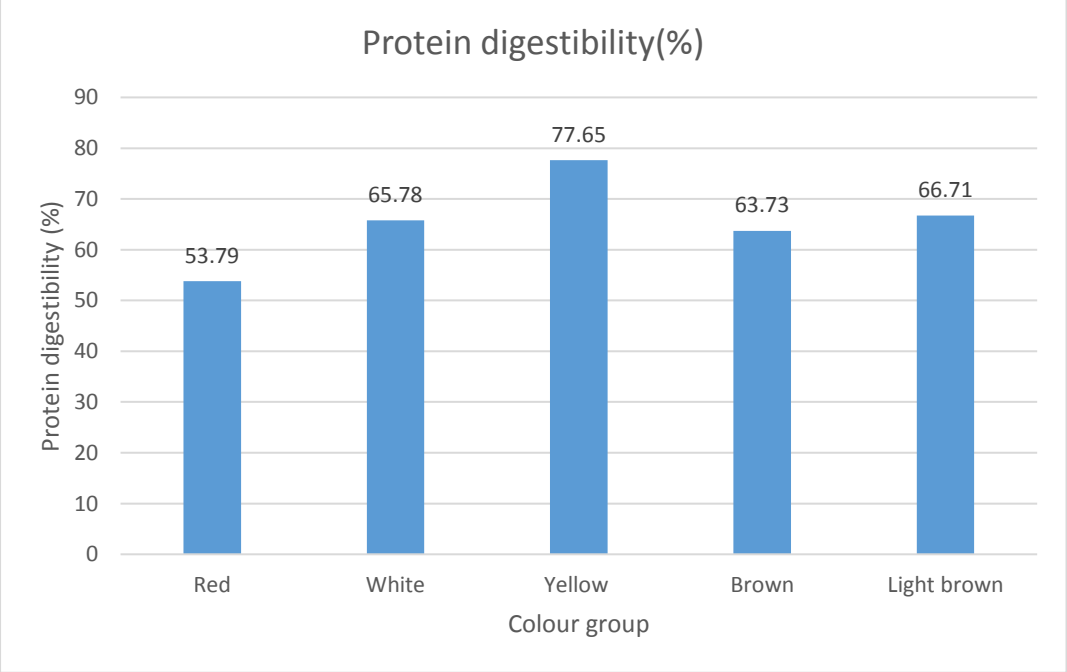

Figure 4.4 Average protein digestibility in grain sorghum according to grain colour groups

4.5 Correlation coefficient

Table 4.5 shows the phenotypic correlation coefficients between protein content, starch content protein digestibility and grain yield. There was a highly significant, negative correlation between starch content and protein content. Starch content was highly significant and positively correlated with protein digestibility. Protein content was significant but negatively correlated with protein digestibility. There was a non-significant correlation between grain yield with protein digestibility, starch and protein content.

Table 4.5 Phenotypic correlation coefficients showing pair-wise association among starch content, protein content, protein digestibility and grain yield.

Trait	Starch content	Protein content	Protein digestibility	Grain yield
Starch content	-			
Protein content	-0.65**	-		
Protein digestibility	0.66**	-0.34*	-	
Grain yield	0.19	-0.19	0.14	-

In this study, the breeding lines which performed better across the two planting dates were selected for direct ethanol production and/or in the sorghum breeding programme for further improvement for ethanol production (Table 4.6). The line selected were stable in both locations for all traits.

Table 4.6 Potential sorghum breeding lines that can be used for improvement of bioethanol production

Name of Breeding line	Starch (%)	Protein (%)	Protein digestibility (%)	Grain yield (t/ha)
05-Potch-151	71.29	9.21	79.96	3.23
15ELC F_6#47	69.8	9.49	80.02	4.27
15ELC F_6#69	69.28	10.21	74.49	4.24
15ELC F_6#45	69.22	10.5	76.91	4.35
15ELC F_6#42	69.47	10.93	76.74	4.51

4.6 Discussion

The study indicated the presence of substantial variability among the sorghum breeding lines based on their biochemical qualities (starch content, protein content and protein digestibility). The variation was observed among the breeding lines at each planting date and across planting dates. This suggested that the biochemical quality of sorghum grain was influenced by the time of planting. The overall starch content ranged between 63.28% and 71.29% across the two environments. The starch content in this study was in the range obtained by other researchers (Salinas et al., 2006; Wu et al., 2007; Wang et al., 2008). In most studies, starch content in the sorghum genotypes ranged between 57 and 74% of grain dry weight (Geleta et al., 2005; Ragaee et al., 2006; Boudries et al., 2009). Yan et al. (2011) also reported genetic variation in waxy grain sorghum for bioethanol production. In previous reports, the total starch content ranged from 65.4% to 76.3%. Many studies have revealed that ethanol yield is highly correlated with total starch content. The ethanol production process basically converts starch from grain sorghum into ethanol, hence the higher the starch content in sorghum grains, the higher the ethanol yield produced (Wang et al., 2008). Therefore, total starch content of grain sorghum can be a predictor of ethanol yield, a higher starch content means high ethanol yield, better processing efficiency and reduced amount of residues after fermentation (Wu et al., 2008). In this study, there was considerable variability in starch content between samples from the two planting dates. Relatively high grain starch content was observed from the first planting (mean of 69.3%).

Significant differences ($p \leq 0.01$) were found for the protein content among the breeding lines over the two planting dates. The combined protein content varied from 9.21% to 15.06%. This was in the range reported by Beta et al. (1995), Geleta et al. (2005) and Wu et al. (2007), but higher than that reported by Subramanian and Jambunathan (1984). Awadelkareem et al. (2009) reported protein grain content ranging from 5.44% to 12.90%. Ragaee et al. (2006) found protein content ranging from 8.07% to 19.80%. It was further reported by Ng'uni et al. (2012) that protein content of southern African (Malawi, Tanzania, and Zambia) sorghum cultivars varied from 9.7% to 16.3%. Ethanol yield decreases with an increase in protein content. This is because of the inverse relationship between starch and protein contents in a unit mass of grain. Zhan et al. (2003) reported that protein content in sorghum is inversely proportional to starch content as in other cereal grains and hence show a negative effect on ethanol yield.

In this study, protein content showed variability between the first and second planting dates. Overall, a high protein content was observed in the second planting date. The protein content under both planting dates was inversely related with starch content. The results obtained in

this study agree with the previous studies as most breeding lines with a high starch content suitable for bioethanol production had low protein content.

Protein digestibility varied significantly among breeding lines across the two planting dates. Some breeding lines had relatively higher protein digestibility, comparable to those of previous studies on protein digestibility of uncooked sorghum grains. The highest protein digestibility in this study was 82.22%, with a mean of 64.22%. The result was similar in range with the study by Wu et al. (2007), Oria et al. (1995a), and Oria et al. (1995b). However, the values in this study were lower than the findings by Axtell et al. (1981), Hamaker et al. (1987) and Rom et al. (1992). Some breeding lines were characterised by low digestibility, below 50%. The low digestibility of sorghum proteins is presumably due to the high protein cross-linking (Afify et al., 2012). The variability of protein digestibility among genotypes was also observed by several researchers (Oria et al., 1995b; Mokrane et al., 2010). Samples from the first planting date performed better than samples from the second planting date in terms of protein digestibility.

Conversion efficiency increases as protein digestibility increases. Therefore, breeding lines with high protein digestibility could be the suitable candidates for ethanol production. Good quality proteins are those that are readily digestible and contain essential free amino acids for yeast growth during fermentation (Wang et al., 2008). Previous studies have shown that there is a positive correlation between protein digestibility and ethanol yield in sorghum genotypes studied (Zhan et al., 2006; Wu et al., 2007; Zhao et al., 2008).

The quality of sorghum grain is affected by several factors like climate, soil type, genotype and fertilization among others and these can affect the nutrient composition (Ebadi et al., 2005). In the breeding lines studied several factors could account for variation of starch, protein digestibility and protein content. The main factors that possibly contributed to the variation are genetic differences in the breeding lines, variation in growing time, and the interaction between genotype and the environment. Genetic influence (genotypes) results in variation of breeding lines growing in the same location at the same time. This large genotypic variability for grain starch content, protein digestibility and protein content among advanced sorghum breeding lines provides an opportunity for selecting potential breeding lines and genetic improvement for bioethanol production.

In this study, grain colour showed significant differences among the breeding lines in starch content, protein content, and protein digestibility. It was observed that the starch content was relatively high in light brown coloured breeding lines, followed by brown coloured breeding lines across the two planting dates. Protein content was low in light brown and brown coloured

breeding lines. There was no significant difference in starch content and protein content based on grain size. A study by Ng'uni et al. (2012) indicated no significant differences in protein, total starch, Fe, and Zn contents among breeding lines based on grain colour among some southern African sorghum breeding lines. The results of this study suggest that grain colour should be considered in improvement of starch and protein content for bioethanol production.

The Pearson correlation coefficients revealed significant relationships between starch content, protein content and protein digestibility in this study. The strong negative correlation (r = -0.65**) observed between starch content and protein content, suggests that the improvement of grain starch content will have an inverse effect on protein content. This relationship is crucial for bioethanol production as high starch content and low protein contents are the desired traits.

4.7 Conclusion

The sorghum breeding lines used in this study exhibited a considerable level of variability for starch content, protein content and protein digestibility. The level of starch content and protein content was in the range reported by previous researchers. The differences in performance among the breeding lines due to the planting time was observed. In general, sorghum breeding lines planted early performed better, i.e. had a high grain yield, a high starch content, low protein content and high protein digestibility, compared to the breeding lines planted four weeks later. For Potchefstroom and similar areas, it is recommended to plant sorghum earlier in the season than late planting to produce good quality grain for ethanol production.

The presence of genetic variation among sorghum breeding lines studied is important for future genetic improvement in sorghum for bioethanol production. The consideration should be given to sorghum breeding lines that show relatively high genetic variability. In this study, high performing breeding lines were identified, and these should be further studied for other factors influencing bioethanol production such as tannin content and understanding the effect of starch properties and protein structures and function on ethanol production before testing for stability in different agro-climatic regions. High starch content, low protein content and high protein digestibility were recorded at the first and second planting dates for breeding lines 05-Potch-151, 15ELC F_6#67, 15ELC F_6#47, 15ELC F_6#69, 15ELC F_6#45 and 15ELC F_6#42. Hence, these breeding lines can be recommended for further investigation and use as parental lines in breeding programmes for bioethanol yield improvement. Furthermore, breeding lines with high digestibility potentially have better nutritional value than those with low digestibility, and these breeding lines can be good for human food and animal feed.

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CHAPTER 5

General overview of the research findings

5.1 Introduction and objectives of the study

Sorghum (*Sorghum bicolor (L.)* Moench) is among the important cereal grain crops produced in the world. The development of a broad genetic base, high yielding and stable sorghum cultivars requires a continuous supply of new germplasm as a source of desirable genes and gene complexes in crop breeding programmes. Therefore, characterisation and evaluation of existing germplasm is required for identifying potential germplasm for varietal improvement programmes. Sorghum is closely related to other potential biofuel crops such as sugarcane (*Saccharum officinarum* L.), the principal sugar feedstock, and maize (*Zea mays* L.), the most important starch feedstock. This chapter outlines the findings of the study conducted at the ARC-GCI advanced sorghum lines. The objectives, summary of the research findings, implication of the research findings and recommendations are highlighted.

The specific objectives were to:

i. Characterise sorghum breeding lines using agro-morphological traits under two environments (planting dates).
ii. Evaluate sorghum breeding lines based on starch content, protein content and protein digestibility and identify sorghum lines for direct bioethanol production and potential parents for the breeding programme.

5.2 Summary of research findings

Characterisation of sorghum breeding lines using agro-morphological traits under two environments (planting dates)

- The ANOVA revealed highly significant differences among the breeding lines for most of the traits across two planting dates.
- The mean performance results of traits studied across two planting dates showed that sorghum breeding lines performed better at the first planting date than at the second planting date.
- High heritability estimates and genetic advance coupled with high genotypic coefficient of variation were observed for traits such as plant height, number of panicle per plot, panicle weight, and grain yield and number kernels per panicle.

- Correlation coefficient analysis revealed a highly significant positive association between economic traits that can be used in the improvement of traits through breeding in sorghum. These relationships can be exploited in indirect selection for grain yield improvement.
- Three principal components contributed 33.21%, 16.76%, and 14.38% of the total variation. The traits that contributed most to the variation were grain yield, plant height, panicle number per plot, 1000 grain weight and panicle weight.
- Five breeding lines; 15ELC F_6#68, 15ELC F_6#8, 15ELC F_6#70, Maseka a swere and 15ELC F_6#42 were ranked top for grain yield.

Evaluation of sorghum lines based on starch content, protein content and protein digestibility and identification of sorghum lines for bioethanol production and use as potential parents for breeding programme

- Generally the sorghum breeding lines studied had a great variability in their starch, protein content and protein digestibility
- The starch content of the breeding lines varied from 63.28% to 71.29% across the two planting dates with mean value of 67.51%. The breeding lines with high starch content under both planting dates were; 05 Potch 151 (71.29%), 15ELC F_6#2 (70.28%), 15ELC F_6#47 (69.8%), and 15ELC F_6#43 (69.64%).
- The protein content of sorghum breeding lines ranged from 9.21% to 15.06% with an overall mean of 12.24%. The five breeding lines with low protein content values were 05-Potch-151 (9.21%), 15ELC F_6#67 (9.36%), 15ELC F_6#47 (9.49%), 15ELC F_6#14 (9.61%), 15ELC F_6#69 (10.21%).
- The protein digestibility ranged between 33.87% and 82.22% across the two planting dates with a mean of 64.22%. The breeding lines that showed high protein digestibility across two planting dates were 15ELC F_6#43, 15ELC F_6#47, 05-Potch-151, 15ELC F_6#2 and 15ELC F_6#45 at 82.22%, 80.02%, 79.96%, 79.48% and 76.91%, respectively.
- Starch content and protein digestibility showed a positive correlation, while protein content had a negative correlation with starch content and protein digestibility. Such relationship is important in the selection of materials for bioethanol production. Indirect selection can be exploited by selecting materials with low protein contents to improve starch content and protein digestibility.

The breeding lines with high starch content, low protein content, and high protein digestibility that showed high potential for bioethanol production and/or for use as parents

in breeding for ethanol yield improvement were; 05 -Potch151, 15ELC F_6#2, 15ELC F_6#47 and 15ELC F_6#43 and 15ELC F_6#45.

5.3 General implications and the way forward

The following implications and future directions were identified:

- Coefficients of variation and ranges for the agro-morphological characters of the sorghum breeding lines showed that significant variation exists for all the characters. Selection can be made among these traits for further improvement.
- High heritability and high genetic advance for most traits indicated the presence of additive genes in the traits and suggested reliable sorghum improvement through selection of the traits.
- Mean performance results of traits for both morphological traits and biochemical traits studied across two planting dates showed that sorghum breeding lines performed better at the first planting than at the second planting date.
- Relationships existed among biochemical traits (starch content, protein content and protein digestibility) suggest indirect selection can be exploited by selecting materials with low protein contents to improve starch content and protein digestibility.

5.4 Conclusion and recommendations

The main objective of the study was to evaluate advanced sorghum lines for potential use in bioethanol production under South African conditions. The results revealed that among the advanced sorghum lines used for the study most of them possess the desirable agronomic and biochemical characteristics for the purpose of ethanol production. It is recommended that further evaluations be conducted on other factors influencing bioethanol production from grain sorghum and the actual bioethanol production process such as fermentation and conversion efficiency to quantify ethanol yield from these breeding lines.

Appendix 1: Weather data of Potchefstroom 2015/2016 planting season

	Temperature °C			Rainfall (mm)			
Month	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Total
November 2015	0.00	22.35	30.45	36.44	23.12	1.22	36.58
December 2015	0.00	19.3	33.44	38.48	28.81	2.09	64.70
January 2016	0.00	24.89	30.34	39.58	20.95	3.16	94.74
February 2016	0.00	26.67	31.59	34.63	27.7	2.65	76.96
March 2016	0.00	24.38	28.7	32.85	21.15	1.94	60.20
April 2016	0.00	50.29	26.46	31.44	17.27	2.57	76.96
May 2016	0.00	21.59	22.08	26.63	13.39	1.37	42.42