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Assessment of forage quality Among the sudangrasses, sweet and grain sorghum inbred lines at different cutting time points

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ASSESSMENT OF FORAGE QUALITY AMONG THE SUDANGRASSES, SWEET AND GRAIN SORGHUM INBRED LINES AT DIFFERENT CUTTING TIME POINTS

A Thesis Submitted to

The Master of Biotechnology Program

In partial fulfillment of the Requirements for

The Degree of Master of Science in Biotechnology

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Dept. Chair/Director Date Dean Date

DEDICATION

I dedicate this work to my dearest and loving mother, Hajjat Aisha Ndagire Mulagguusi. Your bountiful love has always given me the courage to fly at greater heights. Thank you, mum, for believing in me.

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Abstract

Sorghum bicolor (L.) Moench is the world's fifth mostly cultivated cereal after wheat, corn, barley, and oats. Although originated in Ethiopia, the United States is the leading producer and exporter of grain sorghum worldwide. In Africa, it is the second most widely grown crop after corn and mainly cultivated in the arid and semi-arid regions of the continent. Its hardiness to environmental stress and low costs of production has made it a more viable forage crop for animal consumption in marginal agricultural regions. In this study, twelve sorghum varieties were evaluated for their forage quality based on their agro-morphological traits and cell wall composition. Results of the agro-morphological trait analysis showed that black-seeded Sudangrass had the lowest dry weight compared to the sweet sorghum cultivars (Sugar Drip, Rex and Ramada) and this was significant at 90 days after sowing (DAS). This was reflected on its low *in vitro* digestibility and thus its low forage quality. In addition, the Sudan grasses exhibited a significant decrease in their fresh and dry weights, stalk diameter, leaf width and leaf number with advancing plant maturity. This correlated with their forage quality thus the best cutting time point for the Sudan grasses was at 75 DAS. Results of fiber fraction, nutritive analysis and *in vitro* digestibility indicated that Sugar Drip had the highest forage quality as evident from its low lignin content, high Relative Feed Value and highest Net Energy of Lactation at and this was significant at 90 DAS. This was followed by Rex, Ramada, MN1054, white-seeded Sudangrass, GK Aron and black-seeded Sudangrass. Grain sorghum cultivars were harvested at grain maturity and results of *in vitro* digestibility of their cell wall components were slightly comparable to sweet sorghum. However, Sohag was significantly superior to LG35 in terms of its RFV and *in vitro* digestible dry matter. Single nucleotide polymorphisms (SNPs) in one of the lignin biosynthesis genes; caffeic acid 3-O-methyltransferase (*COMT*) were evaluated for their effect on forage quality. The detected SNPs is expected to affect protein function. No correlation was noted between the *COMT* SNPs and lignin content and accumulation in the studied cultivars. Likewise, the detected SNPs did not have any effect of forage quality.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ADL: Acid Detergent Lignin

ADF: Acid Detergent Fiber

BMR: Brown midrib mutants

CAD: Cinnamyl Alcohol Dehydrogenase

CEL: Cellulose

COMT: Caffeic acid 3-*O*-Methyltransferase

CP: Crude Protein

DCPI: Digestible Crude Protein Intake

DOMI: Digestible Organic Matter Intake

DAS: Days after sowing

DNA: Deoxyribose nucleic acid

FAO: Food and Agriculture Organization

Gp: Gas production

GPSF: Gas Production Structural Fraction

GPNSF: Gas Production Non-Structural Fraction

HEM: Hemicellulose

INDDM: *In vitro* Digestible Dry Matter

INDOM: *In vitro* Digestible Organic Matter

IPCC: Intergovernmental Panel on Climate Change

LIG: Lignin

ME: Metabolic Energy

MP: Microbial protein

NCBI: National Institute of Biotechnology Information

NEl: Net Energy of Lactation

NDF: Neutral Detergent Fiber

PCR: Polymerase Chain Reaction

RFQ: Relative Forage Quality

RFV: Relative Feed Value

SCFA: Short Chain Fatty Acids

SNPs: Single Nucleotide Polymorphism

TDN: Total Digestible Nutrients

TSS: Total soluble sugars

4-CL: 4-Caumorate CoA: Ligase

LIST OF TABLES

LIST OF FIGURES

Chapter 1

1.1 Introduction

One of the greatest challenges facing livestock production today is climate change. This is attributed to the decline in the quality and quantity of animal feed and forage crops due to a plethora of factors such as increasing temperatures and water scarcity. Therefore, there is a dire need to search for alternative feed and forage crops that can withstand devastating environmental conditions (Rojas-Downing et al., 2017). Sorghum is currently the most viable forage crop due to its tolerance to various environmental stress factors such as high temperatures, drought, pests and diseases but most importantly, its low energy input compared to other warm-season forage crops like corn (Getachew et al., 2016).

Crop-livestock integration is currently one of the sustainable mixed cropping systems that involves intercropping certain crops with forages within the same production unit area (Borghi et al., 2013; Sani et al., 2011). A previous study indicated that intercropping grain sorghum [*Sorghum bicolor* (L.) Moench] with other perennial forages such as palisade grass [*Brachiaria brizantha* (Hochst. Ex A. Rich) Stapf] and Guinea grass (*Panicum maximum* Jacq.) under fertilizer supplementation resulted in increased grain yield that reached 6238 and 6127 kg/ha respectively. Furthermore, the cost benefit analysis of intercropping sorghum indicated that higher revenues were obtained when grain sorghum was intercropped with guinea grass than sorghum alone (Borghi et al., 2013).

In dairy cattle, grains can be an efficient source of starch, which provides energy to the animal hence enhancing milk production (Santos et al., 1997). In the past, sorghum grain was considered inferior to corn in terms of its nutritive value due to decreased digestibility. However, there is no correlation between milk production and decreased digestibility upon

feeding animals on dry rolled or ground sorghum grains (Mitziner et al., 1994). It is worth noting that energy production efficiency in the rumen from starch depends on the processing method of sorghum grain. Dry-rolling and steam-flaking of sorghum grains have been evaluated for their effect on ruminal starch degradability and animal performance. Studies showed that the latter enhances milk production and protein yield compared to dry-rolling (Theurer, 1986; Theurer et al., 1995).

The vegetative part of sorghum is also a suitable source of energy for ruminants. Sudangrasses and the sorghum-sudangrass hybrids are examples of tall sorghum cultivars used as forage. These cultivars are characterized by production of several tillers, thin stalks and can be ratooned. The latter is of good economic importance because it reduces labor costs with regard to field preparation and sowing. However, the nutritive value of ratoons is lower compared to the main stalk (Vinutha et al., 2017). Furthermore, with rising concerns of climate change, forage sorghum is more desirable than corn for animal consumption due to its hardiness against unfavorable environmental conditions (Getachew et al., 2016; Mut et al., 2017). Nevertheless, for maximum productivity, forage sorghum requires balanced nitrogen fertilization to enhance its yield and quality as well as suppressing the effects of drought on plant growth (Mut, et al., 2017). In addition, supplemental irrigation has been shown to improve forage quality of sorghum (Carmi et al., 2006; Jahansouz et al., 2014).

Nonetheless, the suitability of sorghum for animal consumption is assessed on several forage quality parameters such as:

Soluble fraction

Carbohydrates:

Sorghum contains several carbohydrates notably; Sucrose, fructose, glucose, stachyose and raffinose. In sorghum grains, sucrose is the most abundant carbohydrate present (Watson & Hirata, 1960). Furthermore, previous studies on sweet and forage sorghum have shown that their stalks are rich in soluble carbohydrates hence making them suitable for silage production (Tjandraatmadja et al., 1991; Borges et al., 1999; Fazaeli et al., 2006). Feeding dairy lactating cows on sorghum silage has been reported to result in similar milk yield production as compared to both corn and alfalfa silage (Grant et al., 1995; Lundeen, 2000).

Crude protein:

Crude protein (CP) is one of the most important soluble fractions of forage sorghum. CP is the amount of total nitrogen present in the forage crop. For good quality forage, it is recommended that CP should be >7.0% (Milford & Minson, 1966). The CP content of sorghum mostly depends on the nitrogen content of the soil. Previous studies have showed that nitrogen applications in forage sorghum increases the CP content of the forages thus increasing the palatability and digestibility of the forage (Sher et al., 2016; Almodares et al., 2009).

Insoluble fiber fraction

Neutral detergent fiber (NDF):

NDF refers to the total amount of cellulose, hemicellulose and lignin found in the cell wall of plants (Van Soest et al., 1991). The amount of NDF and forage intake are inversely proportional. Thus, the higher the NDF, the lower the forage intake and vice versa.

The fiber fraction content of forages depends on plant genotype (Di Marco et al., 2009). A previous study on NDF content between grain and sweet sorghum cultivars (Behling Neto et al., 2017) indicated that the latter contain lower NDF compared to the grain sorghum cultivars.

Acid Detergent Fiber (ADF) and Acid detergent lignin (ADL):

ADF is a measure of cellulose and lignin present in a plant cell wall (Van Soest, 1963). The amount of ADF and energy are inversely proportional. Thus, the higher the ADF, the lower the energy from the feed.

ADL on the other hand refers to the measure of lignin and ash present in a forage sample. The higher the ADL, the lower the digestibility of the forage. This is because lignified plant tissues are not easily accessible by digestive enzymes for cell wall hydrolysis and breakdown.

Other forage quality parameters

Digestible Dry Matter (DDM):

DDM refers to the proportion of the forage that is digestible. DDM is influenced by plant age. The younger the plant, the higher the DDM. This is because young plant tissues have a low lignin content compared to old plant tissues. Furthermore, the dry matter intake of the forage is influenced by lignin content. Forages with highly lignified tissues have a low dry matter intake since the indigestible portion creates a fill feeling effect in the animal hence resulting in low forage consumption (Moore & Jung, 2001).

Relative Feed Value (RFV) and Total Digestible Nutrients (TDN):

RFV is an index that estimates the Digestible Dry Matter and Dry Matter Intake based on the laboratory analyses for ADF and NDF, respectively. Both livestock producers and hay farmers have for a long time used the RFV to price hay and determine the economic value of forage (Moore & Undersander, 2002). The higher the RFV of forages, the higher the palatability and digestibility (Jahansouz et al., 2014). The recommended RFV for good quality forage is > 151 (Horrocks & Vallentine, 1999).

TDN on the other hand refers to the sum of the proteins, lipids, sugars and digestible fiber present the diet or feedstuff. It is calculated based on the ADF. For beef cattle rations that are primarily forage, TDN is a useful forage quality parameter to be put under consideration.

TDN is believed to have been the first measure used by animal nutritionists to determine the amount of available energy present in animal feeds. Although Net energy (NE) is currently used as a measure of the available energy required for growth, maintenance and lactation, TDN could also be used to calculate the NE (NRC, 2001).

Relative Forage Quality (RFQ):

RFQ is currently a more preferable quality index used in feedstuffs compared to RFV and TDN. This is because the RFV takes into account the DDM, which is neither a conventional measure of available energy requirements nor feed energy concentration (Moore & Undersander, 2002). Likewise, RFQ combines both Dry matter intake and TDN for determining the voluntary intake of available energy upon feeding animals with a forage as a single source of protein and energy (Moore & Undersander, 2002; Salama & Zeid, 2016).

Digestible Organic Matter (DOM):

The proportion of the organic matter in the feed that is apparently digested in the total digestive tract of the ruminant is referred to as Digestible Organic Matter. DOM is positively correlated to the Metabolizable Energy (ME): the amount of energy utilized by the ruminant (Hamid et al., 2007). The higher the DOM, the higher the digestibility and thus high ME.

Rationale:

Although there are different varieties of annual and perennial warm-season grasses used for forage, sorghum is the most preferred of all due to its low production inputs and tolerance to several environmental constraints. Animal husbandry is a widely practiced economic activity in certain regions of Africa, but this industry is currently threatened by poor quality traditional forage sorghum cultivars. Hence, there is need to identify pure sorghum lines that are of good forage quality.

Objective:

The main objective of this research project was to assess the forage quality of the Sudangrasses, sweet and grain sorghum inbred lines at different cutting time points expressed as Days after Sowing (DAS).

To achieve the above objective, twelve sorghum-inbred lines were cultivated at the Centre for Applied Research on the Environment and Sustainability (CARES) and harvested at different cutting time-points. This was followed by fiber fraction, nutritive composition analysis and *in vitro* digestibility to elucidate their forage quality. DNA extraction and *COMT* sequence PCR amplification and analysis were also conducted to detect for SNPs that were expected to affect forage quality.

Chapter 2: Literature Review

2.1 Origin and description of Sorghum

Sorghum [*Sorghum bicolor* (L.) Moench] includes the cultivated sorghum cultivars that are widely grown across the world. Its origin is traced all the way back from Abyssinia, present day Ethiopia more than 5,000 years ago. The genus *Sorghum* has been classified into five sections; *Heterosorghum* (2n = 40), *Striposorghum* (2n = 10), *Chaetosorghum* (2n = 40), *Parasorghum* (2n = 10) and *Eusorghum* (2n = 20, 40). The latter is believed to be the source of modern cultivated sorghum, and it was further subdivided into two; *Arundinacea* and *Halepensia*. The latter comprises of sorghums that fall under the rhizomatous taxa, which are widely distributed across the Mediterranean, extending to the South-east Asia, India and to the Pacific Islands. The former comprises of 48 taxa, which contain 13 wild species, 28 cultivated species, and 7 hybrids that are a result of crosses between the cultivated sorghum and their wild relatives (De Wet & Harlan, 1971). It was from the work of de Wet and Huckabay (1967), that the 48 taxa were all grouped into a single race; *bicolor*. Other races such as *durra*, *caudatatum*, *guinea* and *kafir* have been previously reported by other groups (Elangovan et al., 2014). The race *durra* is believed to have been dominantly cultivated in Anatolia (present day modern Turkey and Armenian highland) and the Middle East including India and Ethiopia whereas *guinea* was widely grown in West Africa. Race *kafir* was however predominantly cultivated in the southwestern part of the Democratic Republic of Congo though also grown in Nigeria. For the *bicolor*, some cultivars are predominantly African although a lot of diversity of this race is mostly seen in Asia (de Wet & Huckabay, 1967).

Sorghum bicolor (L.) Moench is an annual, warm-season crop, whose growth and development are favored by long days and high temperatures, though susceptible to low ambient temperatures. It is highly tolerant to drought, thus its successful wide cultivation in the arid and semi-arid regions of the world (Rooney & Saldivar, 2003).

Botanically, sorghum is classified under family Gramineae, subfamily Panicoideae and belongs to the Andropogeneae tribe. It is a highly diverse plant, and according to its economic value, it can be regarded as grain sorghum, sweet sorghum, forage sorghum, broomcorn (*Sorghum vulgare* var. technicum) or Sudangrass (*Sorghum sudanese*) (Lukow & Mcvetty, 2004).

Morphologically, sorghum is much more like corn in terms of its general appearance and both share a similar pattern of carbon dioxide fixation (C4 plant). However, its height ranges from 5-18 feet and the plant consist of an imperfect complete flower in which self-pollination takes place. Sorghum has panicles that are either compact or open depending on the variety and its seeds are smaller than those of millet (Rooney & Saldivar, 2003). **Fig. 2.1** shows grain sorghum, Sudan grass and sweet sorghum cultivars at CARES.

2.2 Global production of grain sorghum

Concerning the global production of cereals, Sorghum is the fifth most widely cultivated crop in the world. Nigeria, Sudan and Ethiopia are the largest producers of grain sorghum on the African continent. **Table 2.1** shows the top 10 grain producing countries in Africa. India and China are the largest grain sorghum producers in Asia (Rooney & Saldivar, 2003; Lukow & Mcvetty, 2004). The United States is currently the global giant producer and

exporter of grain sorghum. Sorghum was introduced in the United States as a promising animal feed during the 18th century, and currently, it is the preferred biofuel feedstock to corn and sugarcane (Rosentrater & Evers, 2018).

The global total grain production of sorghum by 2017 stood at approximately 57 metric tons under a cultivation area of approximately 40 million hectares. (FAOSTAT, 2017). A shown in **Fig. 2.2**, the Americas and Africa are the largest producers of grain sorghum. By country, the United States, Nigeria and India are the largest producers of grain sorghum around the globe (**Fig. 2.3**).

2.3 Uses of Sorghum.

2.3.1 Sorghum as a food crop

In Sub-Saharan Africa, grain sorghum is the second most widely grown cereal crop after corn and is a staple food to millions of families in Africa. Its flour is gluten-free thus a very good source of energy and protein for people with gluten allergies. Sorghum flour can be used to make a variety of food products such as porridge, bread, local brew and confectionaries (Arendt & Zannini, 2013). Juice extracted from the sweet sorghum varieties (syrup-type sweet sorghum and saccharine-type sweet sorghum) is used in the manufacture of syrup and sugar for human consumption (Almodares & Hadi, 2009).

2.3.2 Sorghum as an animal feed

One of the greatest challenges facing livestock production sectors today is climate change. In the United States particularly, a search for alternative forages and silage crops tolerant to abiotic stress factors is of a great priority for agricultural sustainability. *Sorghum bicolor* (L.) Moench is a more viable forage and silage crop compared to corn due to its hardiness to unfavorable environmental conditions and excellent phenotypic traits that renders it a suitable crop for animal feed. However, traditional forage sorghum cultivars accumulate high levels of lignin; a complex phenolic polymer that is deposited in the secondary cell wall of plants (Pillonel et al., 1991). The quality of forage crops is dependent on their lignin content and composition. This is because lignin impedes the enzymatic breakdown of the plant cell wall to release sugars and other nutrients required by ruminants to produce more milk and put on more body mass. In addition, lignin itself is an anti-nutritional compound because it is completely not digested.

Brown midrib mutants of sorghum (BMR) have attracted the scientific community for their high forage quality (i.e. low lignin content and absence of prussic acid) thus a more viable alternative of animal feed and forage to the traditional sorghum varieties. BMR phenotypes are a result of mutations in one of the genes involved in lignin biosynthesis. Particularly in Sorghum (*Sorghum bicolor* (L.) Moench), there are 4 BMR loci known and these are; *bmr*-12, *bmr*-18, *bmr*-6 and *bmr*-2. The latter has been linked to a decrease in enzymatic activity of 4-Coumarate: CoA Ligase (4-CL) (Saballos et al., 2012) whereas two allelic forms (*bmr*-12 and *bmr*-18) as well as *bmr-6* have been linked to a decrease in enzymatic activity of Caffeic acid 3-*O*-Methyltranferase (COMT) and Cinnamyl Alcohol Dehydrogenase (CAD) respectively (Oliver et al., 2005). BMR mutants are characterized by a brown midrib

phenotype of their leaves from which their name is derived (**Figure. 2.4. A**) (Getachew et al., 2016).

The effect of these brown midrib mutants (BMR) on animal performance has been extensively studied. Aydin and his colleagues (1999), reported an increased fiber digestion and milk production in lactating dairy cows fed on BMR sorghum compared to the wild type (Aydin et al., 1999).

Forage sorghum and the Sudangrasses on the other hand, have for long been cultivated for animal consumption. Their multi-cut nature and lower costs of production have rendered them suitable candidates for animal grazing. However, these sorghum cultivars accumulate prussic acid in their leaves during abiotic stress and at a young age, which is toxic to animals. They also build up high nitrate concentrations under heavy nitrogen applications; therefore, not safe for grazing due to potential nitrate poisoning (Patel et al., 2013). Therefore, it is imperative to note that animals should not be allowed to graze in sorghum fields after a hail storm and over nitrogen fertilization should be avoided to protect them from prussic acid and nitrate poisoning.

Several sorghum hybrids have been evaluated for their forage quality and suitability for animal consumption. A previous study by Pires and others (2017), indicated several Sorghum-Sudangrass hybrids having a high protein content (> 7%) and *in vitro* dry matter digestibility, thus suggesting their high nutritional value and suitability for animal consumption.

Another highly important sorghum variety used as forage is sweet sorghum. Due to their high sugar content, these varieties have been utilized as forage thus improving the milk yield of lactating animals (Adewakun et al., 1989).

Grains of sorghum cultivars are also an important animal feed in poultry (Fernandes et al., 2013). Moreover, grains from certain sorghum varieties are a cheaper alternative source of energy and protein to corn required for body maintenance and profitability of broiler chicken (Dowling et al., 2002). Likewise, in Asia, sorghum Stover is used as a dry fodder for livestock during the dry seasons (Reddy et al., 2010).

2.3.3 Sorghum as a biofuel feedstock

The detrimental effects of fossil fuels on the environment and the recently increasing fuel prices have urged the search for safer and cheaper alternatives whose impact on the environment is negligible. Biofuel production is thus rendered a better alternative to fossil fuels and huge investments have been incurred to broaden research into this form of energy.

By 2010, bioethanol production had grown by 13.8% and hence accounting for 0.5% of the world's energy market (Calviño & Messing, 2012). Currently, the global road transport fuel supply from biofuels stands at 3% and is expected to increase to 9% by 2050. The United States and Brazil are the world's leading producers of bioethanol from corn and sugarcane, respectively. However, both crops are in the food chain hence negatively affecting the food price (Calviño & Messing, 2012).

Sweet sorghum varieties (*Sorghum bicolor* (L.) Moench) are currently the preferred biofuel feedstock to corn and sugarcane due to their low costs of production, high content of

soluble sugars, high lignocellulosic biomass accumulation, short life cycle (120 – 140 days) and high tolerance to abiotic stress (Ratnavathi et al., 2011). In addition, the potential of BMR forage sorghum for biofuel production has been extensively explored. A previous study (Dien et al., 2009) reported enhanced carbohydrate conversions and increased bioethanol yields for forage sorghum lines that exhibited low lignin contents. It was noted that cellulose conversion to ethanol was highest with the double mutants and brown midrib mutants (*bmr*-6 and *bmr*-12) at 43%, 22% and 21% respectively compared to the wild type (Dien et al., 2009).

Furthermore, grain sorghum Stover is currently a viable alternative to corn in the bioenergy industry. A recent study (Sekhon et al., 2016) compared the composition of non-structural carbohydrates (free glucose, sucrose, and starch) between corn and sorghum Stover internodes at physiological maturity. Results indicated similar trends in composition of non-structural carbohydrates and hence a promising source of bioethanol production. It should be noted that biofuels from sorghum are superior to those from sugarcane due to their high-octane content and less sulfur hence environmentally friendly (Rooney et al., 2007; Sharma et al., 2009; Mathur et al., 2017).

Although sweet sorghum varieties are mainly used in the production of bioethanol, they can also play an important role in the production of biodiesel. A previous study indicated the potential of sweet sorghum juice to be a better carbon source than glucose for *Chlorella protothecoids* used in the production of biodiesel (Gao et al., 2009).

2.3.4 Other uses of the crop

Despite various uses of sorghum in the food and feed industry, stalks of the traditional tall varieties in Africa are used as a fuel to prepare meals as well as in the construction of houses; whereas, broomcorn varieties are used to make brooms. Sorghum fibers are used in the manufacture of several products including; biodegradable packaging materials, solvents and dyes used in the coloration of leather (Getachew et al., 2016).

Just like sugarcane and corn, sorghum is a C4 plant hence capable of efficiently fixing carbon dioxide into its bundle sheath cells in conditions of high ambient oxygen concentration. This is due to the presence of RuBisco enzyme that has a high affinity for carbon dioxide (Monson et al., 1984).

In the bioenergy industry, sorghum is the most viable alternative model system of C4 plants due to its small genome of approximately 730 Mb (Paterson et al., 2009). Its small genome has hence generated more information on very complex genomes of other grasses such as sugarcane, corn, switchgrass, and miscanthus. In addition, molecular markers such as quantitative trait loci (QTLs) can be used to easily trace for desirable traits in sorghum hybrids developed from crosses between sweet and grain sorghum varieties (Calviño & Messing, 2012).

2.4 Production constraints of sorghum

Mostly in Sub-Saharan Africa, sorghum production is constrained by birds because the grain varieties widely cultivated in this region lack polyphenols on seed undercoats that would irritate birds. In addition, most farmers in Africa depend on subsistence agriculture, thus less attracted to its commercial production. They consider it as a low profitable cash crop. Another major constraint of sorghum production in developing countries is lack of capital. The rising labor costs of sorghum production such as land preparation, fertilizer and pesticide applications as well as grain harvest and processing have discouraged farmers into such a business venture. Likewise, African governments have not prioritized sorghum production as they have done with other cereals like corn and rice. There are no adequate government policies that would favor the commercial production of sorghum. Therefore, the usefulness of sorghum has been underestimated by most African governments (FAO, 1994).

Parasitic plants such as *Striga hermonthica* (Del.) Benth are one of the major obstacles affecting grain sorghum production in Africa. This weed attacks sorghum roots and withdraws nutrients from the plant hence causing wilting and death. Due to the capability of its seeds to overwinter under infested soils, most farmers in Africa abandon their fields in search for *Striga* free lands (Zeyaur et al., 2006). Grain molds such as *Fusarium moniliforme* and *Curvularia lunata* are among the major production constraints of grain sorghum in the tropics. These attacks and colonize young developing kernels therefore, reducing grain yield and quality (Williams & McDonald, 1983).

In East and Southern Africa, stem-borers are a major nuisance in regions where corn and sorghum are cultivated. Their impact on crop development could lead to 88% losses, thus

the need for integrated pest management and control to reduce their devastating effects on corn and sorghum production (Midingoyi et al., 2016).

Global climate change is Africa's greatest threat to her food availability and security. The Intergovernmental Panel on Climate Change (IPCC) reported that Africa would experience a 3-4^{0}C temperature increase in the forthcoming century ((Harvest, 2007). The expected long dry spells that are soon hitting the continent will greatly affect the quality of sorghum with regard to its biomass despite its tolerance to drought. A previous study on the effect of drought on sorghum stem biomass (Perrier et al., 2017) indicated that exposure of sorghum to water deficit resulted in a decreased accumulation of stem biomass thus lowering its forage quality with regard to its stem dry weight.

Nevertheless, elevated CO_2 concentrations in the atmosphere greatly affect the chemical composition of plants (Turunen et al., 2009). A previous study on the effect of increasing concentrations of atmospheric CO_2 on different C3 and C4 grasses (Wand et al., 1999) showed a decrease in crude protein content with increasing CO_2 hence lowering their forage quality. Furthermore, elevated CO_2 levels have been reported to increase lignin content of plants (AbdElgawad et al., 2014). Despite lignin's structural and protective significance in plants, its high accumulation in various economically important crops is undesirable.

2.5 Lignin biosynthesis

Lignin is a secondary metabolite: complex cross-linked phenolic polymer usually deposited in the secondary cell wall of plants (Pillonel et al., 1991). Its abundance on earth follows that of cellulose hence making up 30% of the earth's organic carbon (Bonawitz & Chappel, 2010; Holtman et al., 2003: Boerjan et al., 2003). Moreover, the dry weight of lignocellulosic biomass comprises of 15-40% lignin thus creating a major concern for their use in feed and biofuel production (Cline & Smith, 2017). Lignin biosynthesis starts from a precursor molecule phenylalanine, an amino acid that undergoes enzymatic modifications to form various products and intermediates leading to the formation of monolignols; 4-hydroxy cinnamyl alcohol, coniferyl alcohol and sinapyl alcohol (**Fig. 1.5**). The metabolic grid of lignin biosynthesis comprises of a variety of enzymes involved in the hydroxylation and *O*-methylation processes to generate monolignols. One of the key enzymes required for the downstream biosynthesis of monolignols is Caffeic acid 3-*O*-methyltransferase (COMT). COMT is an S-adenosyl methionine-dependent methyltransferase required for the methylation of 5-hydroxyconiferyl alcohol and 5-hydroxyconiferyl aldehyde to generate S unit precursors; sinapyl alcohol and sinapyl aldehyde respectively. (Jung et al., 2012). In sorghum, only one locus encodes a functional COMT (SbCOMT) whose crystal structure and enzymatic kinetics have been elucidated (Green et al., 2014). Genetic manipulation of *COMT* in sorghum is underway for improved forage quality.

In this study, analysis of *COMT* SNPs in 12 sorghum-inbred lines was conducted to elucidate their effect on lignin accumulation and forage quality.

Fig. 2.1: Grain sorghum, Sudangrass and Sweet sorghum cultivars at CARES. Note that black-seeded Sudangrass is taller and its panicles are open and loose whereas grain sorghum cultivar at the border is shorter with larger leaves compared to the black-seeded Sudangrass. The sweet sorghum cultivar, Rex is next to the black-seeded Sudangrass and is of an intermediate height.

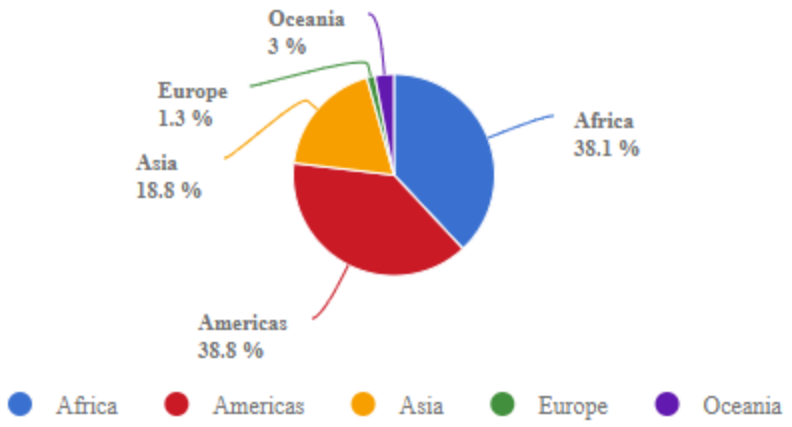


Fig. 2.2: Global grain sorghum production (2017). Image retrieved from http://www.fao.org/faostat/en/#data/QC/visualize.

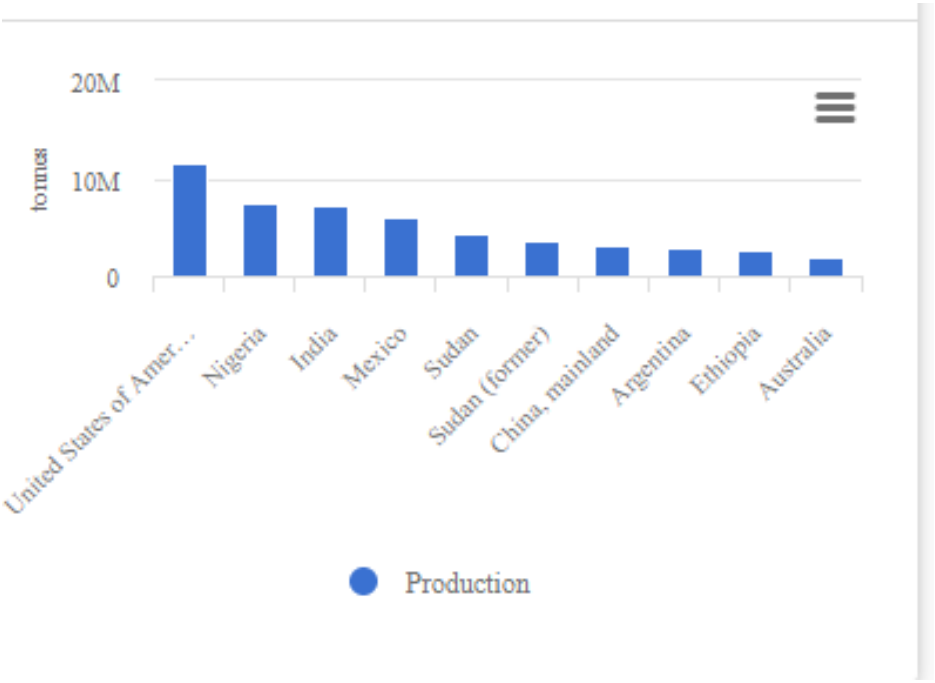


Fig. 2.3: Top 10 grain sorghum producing countries (2017). Image retrieved from http://www.fao.org/faostat/en/#data/QC/visualize.

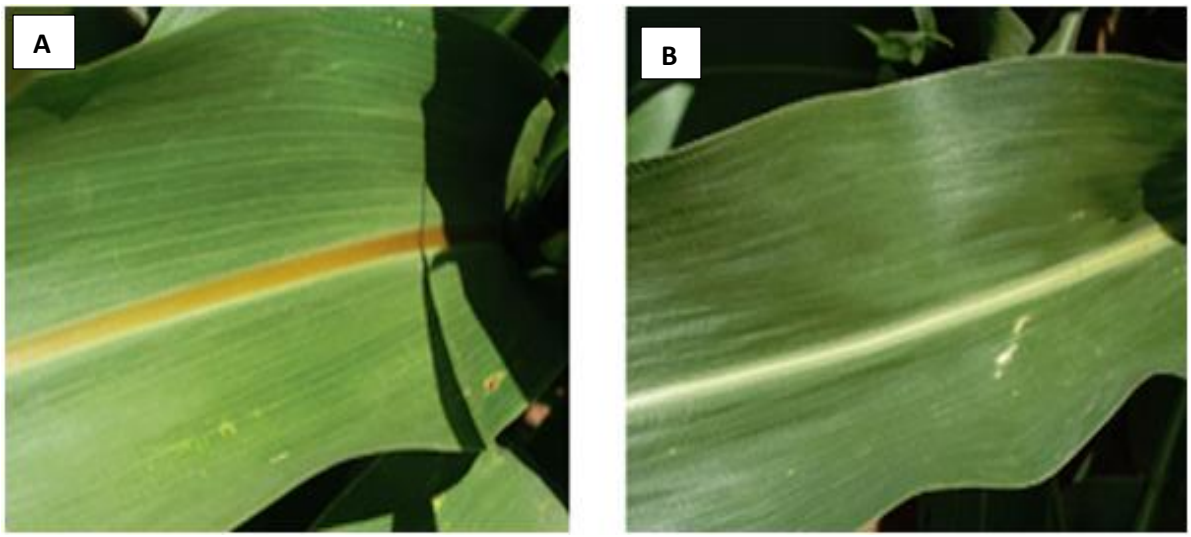


Fig. 2.4: Leaf midribs of two sorghum varieties. (**A)**: brown mutant and (**B)**: traditional sorghum plants. Image retrieved from https://aces.nmsu.edu/pubs/_a/A332/welcome.html.

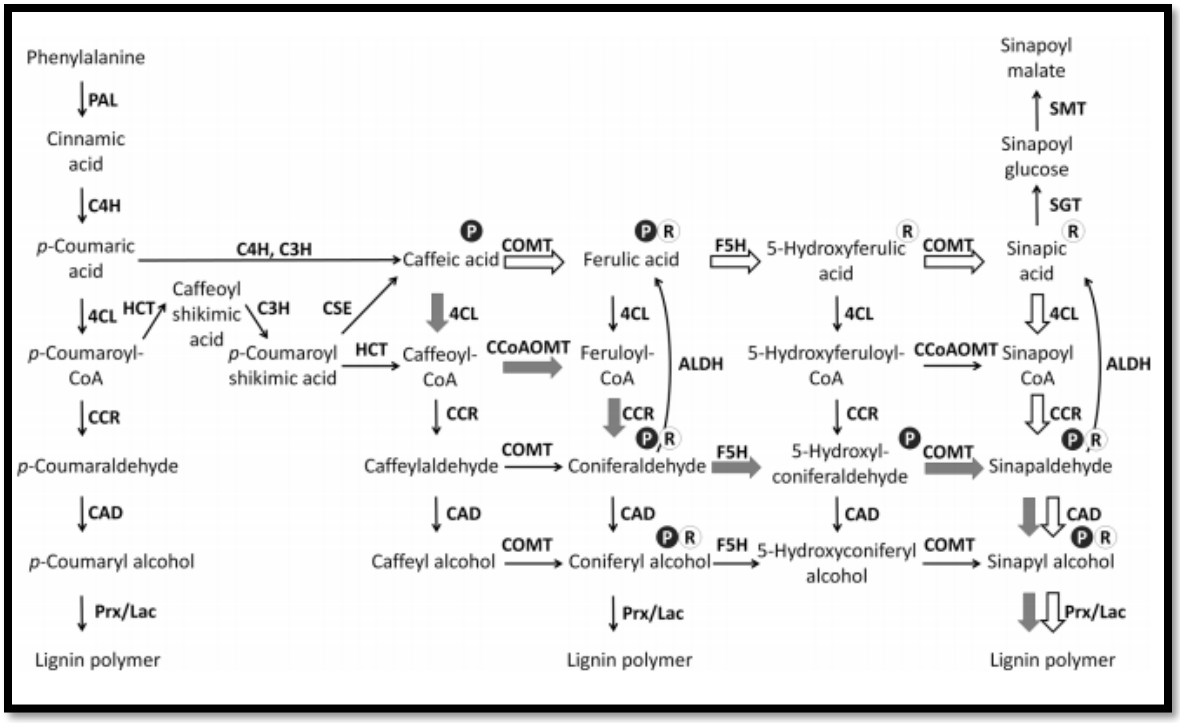


Image retrieved from (Shigeto et al., 2017).

Fig. 2.5: Proposed pathway for lignin biosynthesis. The sinapyl alcohol biosynthesis in *Arabidopsis* and poplar is indicated by gray arrows. White arrows show the pathway through sinapic acid. Monolignol intermediates detected in developing xylem of *Populus alba* and *Robinia pseudoacacia* are indicated by circled ***P*** and ***R*** respectively. **PAL** phenylalanine ammonia-lyase, **C4H** cinnamate 4-hydroxylase, **C3H** p-coumarate 3-hydroxylase, **COM**T caffeic acid O-methyltransferase, **CCoAOMT** caffeoyl-CoA O-methyltransferase, **F5H** ferulate 5-hydroxylase, **4CL** 4-coumarate:CoA ligase, **CSE** caffeoyl shikimate esterase, **HCT** p-hydroxycinnamoyl-CoA:quinate/shikimate p-hydroxycinnamoyltransferase, **ALDH** aldehyde dehydrogenase, **CCR** cinnamoyl-CoA reductase, **CAD** cinnamyl alcohol dehydrogenase, **SMT** sinapoylglucose:malate sinapoyltransferase, **C4H** cinnamate 4-hydroxylase, **SGT** sinapate glucosyltransferase, **Prx** class III peroxidase, **Lac** Laccase.

Table 2.1: Top 10 grain sorghum producing countries in Africa.

Country	Area harvested (ha)	Production (tons)	Average production per ha in tons
Nigeria	5,820,000	6,939,000	1.193
Ethiopia	1,840,018	4,815,595	2.617
Sudan	5,411,500	3,743,000	0.692
Niger	3,820,696	1,945136	0.509
Mali	1,585,986	1,393826	0.879
Burkina Faso	1,667,193	1,365898	0.819
Cameroon	852,456	1,351,966	1.586
Chad	1,147470	946,295	0.825
Tanzania	782,717	796,570	0.825
Egypt	147,961	727,648	4.918

FAOSTAT Data, 2017

Chapter 3. Methodology

3.1 Plant materials and experimental design

Seeds of sorghum inbred lines were obtained from the Agricultural Genetic Engineering Research Institute (AGERI) Giza, Egypt. The field experiment was then conducted at the Centre for Applied Research on the Environment and Sustainability (CARES) at the American University in Cairo, New Cairo – Egypt (30°01'11.7"N 31°29'59.8"E). The study was conducted to investigate the forage quality of inbred lines of the Sudangrasses, sweet and grain sorghum cultivars at different cutting time points (60, 75, 90 DAS and at grain maturity). The experiment was conducted in a completely randomized design (CRD) with two replicates.

3.2 Cultivation practice

Sowing was performed only in one experimental season. Plants were hand-sown in 4 rows, 75 and 50 cm inter and intra-row spacing respectively based on a planting density of 26,667 plants/ha. Black-seeded Sudangrass was planted at the boarders of the plot. Weeding was done by hand and plants were drip irrigated according to the crop requirements. Insect control was performed by spraying with Lambda-cyhalothrin insecticide when necessary.

3.3 Measurements and sampling procedure

At each cutting time point, cutting and sampling procedures were done within the borders of the plot. Five plants from each variety (Sudangrasses and sweet sorghum) were randomly selected and plant heights were measured using a meter rule, stalk diameters were measured from the second internode from the bottom -up of the plant using a Vernier caliper, leaf widths of the 3rd bottom leaf were measured using a ruler. Juice from sweet sorghum varieties was extracted from the second internode bottom-up of the stalk and Brix measurements were taken using a hand-held refractometer.

Each of the five plants from each variety were divided in stalks, leaves and panicles. Stalks were cut into smaller pieces of approximately 5 cm. All plant fractions were weighed to obtain the fresh weights and then oven dried to a constant weight at 70^{0}C for 3 days to obtain the dry weights. Juice yield was calculated by subtracting the stalk dry weight from the stalk fresh weight and expressed as g/plant. Sugar yield was calculated as a product of brix and juice yield.

3.4 Fiber fraction(s), nutritive value analysis and *in vitro* digestibility

Fiber fraction(s) analysis is an important technique for determining the forage quality of different plants and it considers certain important parameters including; Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergent lignin (ADL). In this study, fiber fraction and nutritive value analysis [Crude protein (CP)] for all the cultivars were performed at the Regional Center for Food and Feed, Giza, Egypt. Briefly, whole plant samples were ground using a Wiley mill and the fine powder passed through a 1 mm screen.

Samples were analyzed in triplicates. Total nitrogen content of the samples was determined by Kjeldahl technique followed by determination of concentrations of crude protein (CP) according to the Association of Official Analytical Chemists 2016 (AOAC no.984.13 and no. 968.06 respectively). Likewise, Neutral detergent fiber (NDF, AOAC no. 2002.04), Acid detergent fiber (ADF, AOAC no. 973.18) and Acid detergent lignin (ADL, AOAC no. 973.18) were sequentially determined by semiautomatic ANKOM^{220} Fiber Analyzer (ANKOM Technology, Macedon, NY, USA). Cellulose (ADF-ADL), Hemicellulose (NDF-ADF) and Lignin were calculated from the organic matter of the detergent fiber fractions. Relative Feed Value (RFV) and Total Digestible Nutrients (TDN) were calculated as described by Atis et al., 2012 and Jahansouz et al., 2014 respectively.

Assessment of the quality of animal feeds has for a long time been performed by *in vitro* digestibility, gas production technique. This technique involves the use of Rumen fluid extracted from animals as an inoculum to mimic the in vivo fermentation of feed thus, allowing a proper estimation of the nutritive composition and fermentation kinetics of ruminant feeds through gas production. Merits of this technique over the in vivo fermentation in the determination of nutritive value of feedstuffs include; it is cheaper, faster, less labor intensive, suitable for both small quantities of feed and large-scale evaluation of ruminant feeds (Getachew et al., 1998). In this study, the gas production technique was performed according to (Menke & Steingass, 1988) at the Regional center for food and feed, Agricultural Research Center, Giza.

Briefly, ammonium free rumen fluid was collected in equal proportions from two animal donors (sheep) before their morning feed and put into thermo flasks. The rumen fluid was later filtered through a 1 mm sieve and the obtained filtrate was incubated at 39 ^{0}C. Rumen

liquor and buffer solution were mixed together in the ratio of 1:2 (v/v) and all laboratory procedures of handling rumen liquor were conducted under a continuous flow of carbon dioxide gas. 200 mg test samples were fed into 100 ml capacity graduated plastic syringes and the lubricated pistons were inserted onto the syringes. 30 ml of rumen liquor (inoculum) were introduced into the plastic syringes via silicon tubes at the tips of the syringes and these were subjected to incubation (± 39 ^{0}C). Gas production was measured at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 h. This experiment was conducted in triplicates.

In-vitro digestible dry matter (INDDM) and in-vitro digestible organic matter (INDOM) were determined as described by Menke & Steingass, (1998), whereas microbial protein was calculated from the equation: MP (g/kg DOM) = 120 X DOM/100 as described by Czerkawski, (1986). Gas production structure fraction (GPSF), gas production non-structural fraction (GPNSF), *in-vitro* digestibility crude protein intake (INVDCPI), *in-vitro* digestibility organic matter intake (INVDOMI), Metabolic Energy (ME), Net Energy of lactation (NEl) and Short chain fatty acids (SCFA) for all varieties were determined using the following formulas as described by Van Gelder et al., 2005:

GPSF (ml/g DM) = (GP3h-5.5) x 0.99-3 Equation 1

GPNSF (ml/g DM) = (1.02 x (GP24h-5.5) – (GP3h-5.5) + 2) Equation 2

INV-DCPI (g/day) = (-203.242 + (14.797 x GP24 + 6.249 x GP48h) Equation 3

INV-DOMI (g/day) = (-1763.07 + 42.5 x GP24h) + 13.52 x GP48h) Equation 4

ME(MJ/kg DM)= 2.20 + 0.1357 x GP + 0.0057 x CP + 0.0002859 x CP x CP Equation 5

NEl(MJ/kg DM)=0.54 + 0.0959 x GP + 0.0038 x CP + 0.0001733 x CP x CP Equation 6

SCFA (mmol/ml gas) = (0.0239 x GP + 0.0601) Equation 7

3.5 DNA extraction and quantification

To acquire a representative population of each sorghum variety, young leaves were sampled from 3-4 two weeks old seedlings for DNA extraction and this was referred to as batch 1. Then, leaves were again sampled from two different plants within each variety at 90 DAS for DNA extraction and these were referred to as batch 2 and 3 respectively.

DNA from all batches was isolated using GeneJet Plant Genomic DNA Purification Mini kit (Cat No. K0791) according to the manufacturer's protocol. All centrifugation was performed using a mini spin plus tabletop microcentrifuge. Briefly, 100 mg of leaf tissue was placed into liquid nitrogen and grinded thoroughly to a fine powder using a mortar and pestle. The fine powder was then placed in an eppendorf tube containing 350 µl of Lysis buffer A. 50, 20 µl of Lysis buffer B and RNase respectively were quickly added into the eppendorf tube, and the mixture was incubated for 10 min at 65^0C in a shaking incubator. This was followed by addition of 130 µl of precipitation solution and the eppendorf was inverted 2-3 times to mix up the contents after which it was placed on ice for 5 min. The mixture was centrifuged for 5 min at 20,000 x g. 550 µl of the supernatant were carefully collected and transferred to a clean microcentrifuge tube. This was followed by addition of 400 µl of Plant gDNA Binding Solution and 400 µl of 96% ethanol, mixing was performed. 700 µl of the prepared mixture was transferred to the spin column and centrifugation was performed for 1 min at 6,000 x g. 500 µl of wash buffer I were added to the spin column and centrifuge for 1 min at 8,000 x g. The flow-through was discarded. 500 µl of wash buffer II were added and centrifuge for 3 min at 20,000 x g using a mini spin plus table

centrifuge and the flow-through was discarded. Genomic DNA was eluted by addition of 100 µl of the Elution buffer to the center of the column membrane, followed by incubation for 5 min at room temperature and centrifuge for 1 min at 8,000 x g. A second elution was performed as previously described and the purified DNA was stored at 4^0C for downstream applications.

Assessment of DNA quality and quantity was performed using a SpectroStar Nanodrop (BMG LABTECH) followed by running the DNA on 1% (w/v) agarose gel stained with 3 µl ethidium bromide. Visualization of DNA bands (**Fig. S2**) was done by using Gel Doc EZ System (Bio-Rad, USA).

3.6 Primer design and PCR amplification of *COMT*.

A reference sequence (>NC_012876.2:4721553-4724381) for *COMT* was obtained from the National Center of Biotechnology Information (NCBI) and multiple primers were designed to amplify the coding sequence of *COMT* (**Table 1.1**).

Extracted genomic DNA of all varieties was diluted to 15 ng / µl with nuclease free water and used as a template to amplify the exons of *COMT*. PCR was performed using COSMO DNA Polymerase Enzyme kit (Cat No. W1020201) according to the manufacturer's protocol. Briefly, genomic DNA was first denatured for 3 min at 95^0C before setting up the PCR reaction. For 25 µl reaction volume, 20 µl of COSMO 5x RED buffer (pH 9) were pipetted into an eppendorf tube followed by addition of 2 µl of COSMO Taq DNA polymerase. 10 pmol of the first set of forward and reverse primers were added into the mixture to form a master mix. The master mix was vortexed and 13 µl of the freshly

prepared master mix were pipetted into a 50 µl PCR reaction tube. This was followed by the addition of 15 ng / µl denatured genomic DNA and the volume was completed to 25 µl by addition of 10 µl of PCR water. PCR conditions for the amplification of exon 1 were: Initial denaturation: 94^0C for 3 min; denaturation: 94^0C for 30 s; annealing: 55^0C for 15 s; extension: 72^0C for 30 s; final extension: 72^0C for 10 min; 35 cycles. Conditions for the nested PCR of exon 1 were the same as those of the first pair of primers for exon 1 except the annealing conditions (60^0C for 15 s). PCR conditions for the amplification of exon 2 were: Initial denaturation: 94^0C for 3 min; denaturation: 94^0C for 30 s; annealing: 67^0C for 15 s; extension: 72^0C for 30 s; final extension: 72^0C for 10 min; 35 cycles. Conditions for the nested PCR of exon 2 were the same as those of the first pair of primers for exon 2 except the annealing conditions (58^0C for 15 s). The PCR amplicons were run on 1% agarose gel and visualized using Gel Doc EZ System (Bio-Rad, USA).

3.7 PCR purification, DNA sequencing and analysis

For both exon 1 and exon 2, PCR products from all the 3 batches were pooled and purification was performed using QIAGEN MinElute PCR Purification Kit (Cat. No. 28004) according to the manufacturer's protocol. All centrifuge steps were carried out using a mini spin plus tabletop microcentrifuge. Accordingly, 5 volumes of PB buffer were added to 1 volume of the PCR reaction and mixed together by shaking. To bind the DNA, the mixture was then pipetted into a MinElute column placed in a 2 ml collection tube and centrifugation was performed for 1 min at 10,000 x g. The flow-through was discarded and 750 µl of buffer PE were added into the MinElute column and centrifuged for 1 min at 10,000 x g. The flow-through was discarded and centrifugation was repeated for 1 min at

10,000 x g. To elute the purified DNA, 10 µl of Buffer EB (10 mM Tris-Cl, pH 8.5) was carefully added to the center of the membrane and the column was left to stand at room temperature for 1 min. Centrifugation was then performed for 1 min at 10,000 x g. The purified PCR products were stored at -20^{0}C. Cycle sequencing of the purified PCR products using the nested primers was performed at Macrogen Inc., South Korea.

Sequences were merged using a sequence merger online tool (http://hvdr.bioinf.wits.ac.za/fmt/) and multiple alignments were performed using MAFFT version 7 (Katoh et al., 2017). Phylogenic analysis was conducted using MEGA and DnaSP v6 software.

3.8 Statistical analysis

Data collected was analyzed using IBM SPSS statistical software (version 22). Analysis of variance (ANOVA) was conducted to detect for significant difference in forage quality parameters among the studied cultivars at one or more cutting time points and this was followed by Duncan multiple range test ($p \leq .05$). A t-test was performed to detect for significant differences in forage quality parameters between two cultivars at a single cutting time point.

Table 3.1: Multiple primers used in the amplification of *COMT*.

First primer set	**Sequence**
Forward primer (Exon 1)	ATGGGGTCGACGGCGGA
Reverse primer (Exon 1)	CCATGAGGACCTTGTCCTGGT
Forward primer (Exon 2)	TACTACCTGAAGGACGCGGTGCT
Reverse primer (Exon 2)	TTACTTGATGAACTCGATGGCCCA

Nested primer set	**Sequence**
Forward primer (Exon 1)	GTCGACGGCGGAGGACGT
Reverse primer (Exon 1)	CTCTCCATGAGGACCTTGTCCT
Forward primer (Exon 2)	TGAAGGACGCGGTGCTTGA
Reverse primer (Exon 2)	CTTGATGAACTCGATGGCCCA

Summary of the overview of the workflow

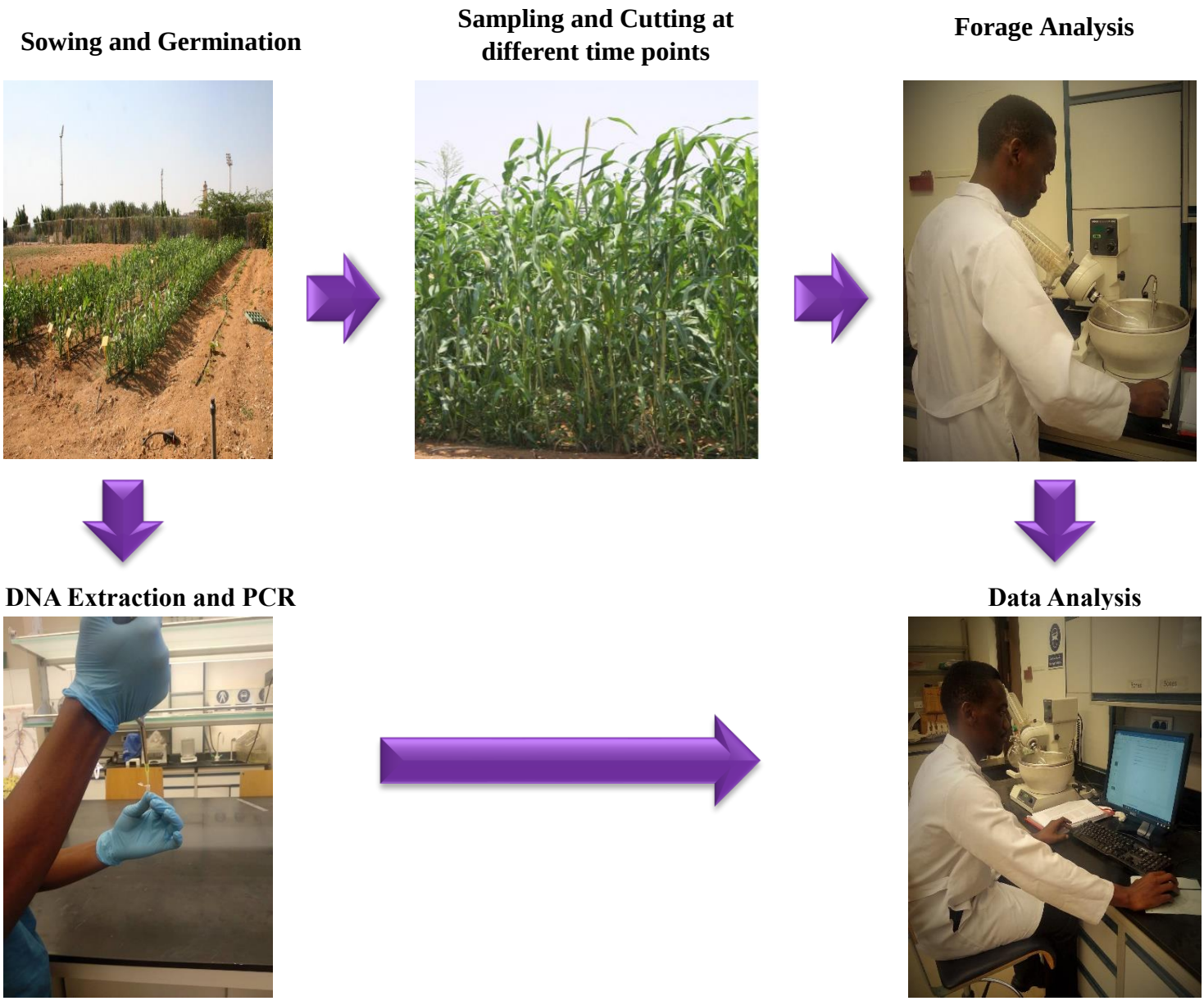


Fig. 3.1: Summary of the overview of the workflow

Chapter 4. Results.

4.1 Agro-morphological traits analysis.

4.1.1 Assessment of the average plant heights among the Sudan grasses and sweet sorghum varieties at 60, 75 and 90 DAS.

The mean of plant heights of the Sudangrasses and sweet sorghum cultivars were assessed at 60, 75 and 90 DAS (**Fig. 4.1**). No significant difference in plant heights was noted between the Sudangrasses at 60 DAS.

Data collected at 75 DAS indicated that Sugar Drip significantly had the lowest average plant height (327.26 cm) compared to black-seeded Sudangrass (392.40 cm) and MN1054 (376.50 cm) ($p \leq .05$). No significant difference in the average plant height was noted between Sugar Drip and white-seeded Sudangrass.

Results of this study showed that at 90 DAS, MN1054 significantly had the highest average plant height (370.20 cm) compared to Ramada (312.40 cm), Rex (307.00 cm) and Sugar Drip (303.60 cm) ($p \leq .05$). Likewise, Sugar Drip significantly had the lowest average plant heights compared to the Sudangrasses and MN1054 ($p \leq .05$).

The effect of the two-way interaction between plant ages at cutting and the Sudangrasses (varieties) is presented in **Table S1**. Both plant ages at cutting and variety were statistically significant at the .05 significance level. The main effect for plant ages at cutting yielded an F ratio of $F(2, 24) = 18.79$, $p < .001$, indicating a significant difference in the average plant heights at 60, 75 and 90 DAS. The main effect for variety yielded an F ratio of $F(1, 24) = 9.21$, $p = .006$, indicating a significant difference in the average plant height between the Sudangrasses. In addition, the interaction effect (Plant ages at cutting x Variety) was

significant F (2, 24) = 3.85, p = .036. For sweet sorghum varieties (MN1054 and Sugar Drip), no significant difference was noted in the mean of their plant heights at 75 and 90 DAS, condition; t (18) = .84, p = .411 (**Table S2**).

Overall, results of this study indicate an increase and decrease in the average plant heights of the Sudangrasses at 75 and 90 DAS respectively compared to the sweet sorghum cultivars.

4.1.2 Assessment of the average leaf number among the Sudan grasses and sweet sorghum varieties at 60, 75 and 90 DAS.

Results of the leaf number assessment among the studied sorghum varieties (**Fig. 4.2**) showed no significant difference in the mean leaf number between the Sudangrasses at 60, 75 and 90 DAS.

At 75 DAS, Sugar Drip and MN1054 significantly had the highest mean leaf number (15.40 and 14.40) compared to the Sudangrasses ($p \leq .05$). However, black-seeded Sudangrass significantly had the lowest mean leaf number (10.80) compared to MN1054 and Sugar Drip ($p \leq .05$) except for white-seeded Sudangrass (11.40).

Data collected at 90 DAS showed no significant difference in the mean leaf number among Ramada, Sugar Drip, MN1054 and Rex. However, GK Aron significantly had the least mean leaf number (9.00) compared to Rex (12.80), MN1054 (13.80), Sugar Drip (14.20) and Ramada (14.80) ($p \leq .05$) except for the Sudangrasses.

The effect of the two-way interaction between plant ages at cutting and the Sudangrasses (varieties) is presented in **Table S3**. Both plant ages at cutting and variety were statistically significant at the .05 significance level. The main effect for plant ages at cutting yielded an F ratio of $F(2, 24) = 5.30$, $p = .012$, indicating a significant difference in the mean of leaf number at 60 and 75 DAS and at 90 and 75 DAS. The main effect for variety yielded an F ratio of $F(1, 24) = 7.12$, $p = .013$, indicating a significant difference in the mean of leaf number between the Sudangrasses at the cutting time points. No significant difference was noted on the interaction effect (Plant ages at cutting x Variety), $F(2, 24) = 0.86$, $p = .437$.

Likewise, no significant difference in the mean of leaf numbers was noted between MN1054 and Sugar Drip at 75 and 90 DAS, condition; $t(18) = 1.445$, $p = .166$ (**Table S4)**.

Generally, results of this study indicated that there was an increase and decrease in mean leaf number of the Sudangrasses at 75 and 90 DAS respectively compared to the sweet sorghum cultivars. Furthermore, all the sweet sorghum varieties had a higer leaf number compared to the Sudangrasses except for GK Aron.

4.1.3 Assessment of the average leaf width among the Sudan grasses and sweet sorghum varieties at 60, 75 and 90 DAS.

The mean leaf width of the studied forage and sweet sorghum varieties was assessed at different cutting time points (60, 75 and 90 DAS) (**Fig. 4.3**). At 60 DAS, no significant difference was noted between the Sudangrasses.

At 75 DAS, Sugar Drip and black-seeded Sudangrass significantly had the highest and lowest mean of leaf width (9.48 cm and 5.80 cm respectively) compared to MN1054 (7.76 cm) and Sudan white grass grass (7.24 cm) ($p \leq .05$).

At 90 DAS, results indicated that Sugar Drip and Ramada significantly had the highest mean of leaf widths (9.04 cm and 9.48 cm respectively) compared to other varieties and ($p \leq .05$). However, no significant difference in mean of leaf width was noted between the Sudangrasses and GK Aron. Furthermore, no significant difference was noted between Rex and MN1054 at this time point.

The effect of the two-way interaction between plant ages at cutting and the Sudangrasses (varieties) is presented in **Table S5**. Both plant ages at cutting and variety were statistically significant at the .05 significance level. The main effect for plant ages at cutting yielded an F ratio of $F(2, 24) = 9.99$, $p = .001$, indicating a significant difference at 60 and 75 DAS and at 90 and 75 DAS. The main effect for variety yielded an F ratio of $F(1, 24) = 8.45$, $p = .008$, indicating a significant difference in the mean of leaf widths between the Sudangrasses at the cutting time points. In addition, the interaction effect (Plant ages at cutting x Variety) was significant $F(2, 24) = 5.45$, $p = .011$.

No significant difference in the mean of leaf widths of MN1054 and Sugardrip was noted at 75 and 90 DAS, condition; t(18) = 0.785, p = .442 (**Table S6**).

Overall, results of this study indicate an increase and decrease in leaf width between the Sudangrasses at 75 and 90 DAS respectively comapred to the sweet sorghum cultivars.

4.1.4 Assessment of the average stalk diameter among the Sudan grasses and sweet sorghum varieties at 60, 75 and 90 DAS.

Plant stalk diameters of both Sudangrasses and sweet sorghum cultivars in our study were assessed at 60, 75 and 90 DAS (**Fig. 4.4**). No significance difference was noted in the average stalk diameter between the Sudangrasses at 60 DAS.

However, at 75 DAS, black-seeded Sudangrass significantly had the least average stalk diameter (18.32 mm) compared to white-seeded Sudangrass (26.26 mm), MN1054 (23.80 mm) and Sugar Drip (26.62 mm) ($p \leq .05$).

Likewise, black-seeded Sudangrass significantly had the least average stalk diameter (15.89 mm) compared to other varieties ($p \leq .05$) at 90 DAS, except white-seeded Sudangrass. Among the sweet sorghum varieties, GK Aron significantly had the least average stalk diameter (20.08 mm) at a cut off $p \leq .05$. However, Ramada significantly had a larger average stalk diameter (27.10 mm) compared to MN1054 (22.56 mm) and GK Aron (20.08 mm) ($p \leq .05$).

The effect of the two-way interaction between plant ages and the Sudangrasses (varieties) is presented in **Table S7**. Both plant age at cutting and variety were statistically significant

at the .05 significance level. The main effect for plant age at cutting yielded an F ratio of $F(2, 24) = 27.76$, $p < .001$, indicating a significant difference in the average stalk diameter at 60 and 75 DAS and at 90 and 75 DAS. The main effect for variety yielded an F ratio of $F(1, 24) = 18.58$, $p < .001$, indicating a significant difference in the average stalk diameter between the Sudangrasses at the sampled time points. The interaction effect (Plant ages at cutting x Variety) was significant $F(2, 24) = 10.34$, $p = .001$.

No significant difference in the average of stalk diameters of MN1054 and Sugar Drip was noted at 75 and 90 DAS, condition; $t(18) = 1.56$, $p = .136$ (**Table S8**).

Generally, results of this study show an increase and decrease in the mean of the stalk diameters between the Sudan grasses at 75 and 90 DAS respectively compared to the sweet sorghum cultivars.

4.1.5 Sugar yield and quality traits.

Across the 5 sweet sorghum cultivars (**Table 4.2**), brix ranged from 10% to 20% with a mean of 13.8. At 90 DAS, Sugar Drip had the highest brix (20%) whereas GK Aron had the lowest brix (10%). The juice yield (g/plant) ranged from 313.09 to 754.44 with a mean of 444.73. Ramada had the highest juice yield followed by Sugar Drip, Rex, MN1054 and GK Aron respectively. The mean sugar yield (g/plant) ranged from 31.31 to 90.53 with a mean of 60.72. Ramada had the highest sugar yield followed by Sugar Drip, Rex, MN1054 and GK Aron respectively.

4.1.6 Assessment of total plant biomass for the Sudangrasses and sweet sorghum cultivars at 60, 75 and 90 DAS.

The mean total fresh weights of the Sudangrasses and sweet sorghum cultivars were assessed at 60, 75 and 90 DAS (**Fig. 4.5**). Although the white-seeded Sudangrass had a higher average total fresh weight than the black-seeded Sudangrass at 60 DAS, both varieties showed no significant difference.

Results obtained at 75 DAS showed that the black-seeded Sudangrass significantly had the lowest average total fresh weight (405.57 g/plant) compared to other varieties ($p \leq .05$). No significant difference in the average total fresh weight was noted among the white-seeded Sudangrass, MN1054 and Sugar Drip.

Data collected at 90 DAS indicated that the black-seeded Sudangrass and Ramada significantly had the lowest and highest average total fresh weight (294.48 g/plant and 1148.57 g/plant respectively) compared to other varieties ($p \leq .05$).

The effect of the two-way interaction between plant ages at cutting and the Sudangrasses (varieties) is presented in **Table S9**. Both DAS and variety were statistically significant at the .05 significance level. The main effect for plant ages at cutting yielded an F ratio of $F(2, 24) = 10.56$, $p = .001$, indicating a significant difference in the average total fresh weights at 60 and 75 DAS and at 90 and 75 DAS. The main effect for variety yielded an F ratio of $F(1, 24) = 13.04$, $p = .001$, indicating a significant difference in the average total fresh weights between the Sudangrasses. However, the interaction effect (Plant ages at cutting x Variety) was not significant $F(2, 24) = 1.93$, $p = .167$.

For the sweet sorghum varieties (MN1054 and Sugar Drip), no significant difference was noted between 75 and 90 DAS, conditions; t (18) = 1.13, p = .274 (**Table S10**).

Overall, results of this study indicate a significant increase and decrease in the average total fresh weights of the Sudangrasses at 75 and 90 DAS respectively compared to the sweet sorghum cultivars.

Similarly, the mean total dry weights of the studied sorghum varieties were assessed at 60, 75, 90 DAS (**Fig. 4.6**). No significant difference in the average total dry weights was noted between the Sudangrasses at 60 DAS.

At 75 DAS, no significant difference was noted between the white-seeded Sudangrass and sweet sorghum cultivars. However, the black-seeded Sudangrass significantly had a lower average total dry weight (132.56 g/plant) compared to the white-seeded Sudangrass (186.54 g/plant) ($P \leq .05$).

Results obtained at 90 DAS showed that Ramada significantly had the highest average total dry weight (261.41 g/plant) compared to GK Aron, MN1054 and the Sudangrasses ($p \leq .05$). However, the black-seeded Sudangrass significantly had the lowest average total dry weight (109.37 g) compared to MN1054, Sugar Drip, Rex and Ramada ($p \leq .05$). No significant difference in total dry weight was noted among Ramada, Rex and Sugar Drip.

The effect of the two-way interaction between plant ages at cutting and the Sudangrasses (varieties) is presented in **Table S11**. Both plant ages at cutting and variety were statistically significant at the .05 significance level. The main effect for plant ages at cutting yielded an F ratio of F (2, 24) = 25.29, $p < .001$, indicating a significant difference in the average total dry weights at 60 and 75 DAS and 90 and 75 DAS. The main effect for variety

yielded an F ratio of F (1, 24) = 8.99, $p < .001$, indicating a significant difference in the average total dry weights between Sudangrasses. However, the interaction effect (plant ages at cutting x Variety) was not significant F (2, 24) = 2.27, p = .125.

For sweet sorghum cultivars (MN1054 and Sugar Drip), no significant difference was noted between 75 and 90 DAS, conditions; t (18) = .965, p = .35 (**Table S12**).

Overall, results of this study indicated a significant increase and decrease in the mean of total dry weights of the Sudan grasses at 75 and 90 DAS respectively compared to the sweet sorghum cultivars.

4.1.7 Correlation analysis.

A Pearson product-moment correlation coefficient was computed to analyze the relationship between Sugar yield and agro-morphological traits of sweet sorghum cultivars at 90 DAS. Results of this study showed there was a strong positive correlation between sugar yield and juice yield, r = .837, n = 25, $p < .0001$ (**Fig. 4.7. A**). Likewise, a moderate positive correlation between sugar yield and Brix was noted, r = .408, n = 25, $p < .0403$ (**Fig. 4.7. B**). In addition, a strong positive correlation between sugar yield and stalk dry weight, r = .845, n = 25, $p < .0001$ (**Fig. 4.7. C**), sugar yield and stalk fresh weight, r = .752, n= 25, $p < .0001$ (**Fig. 4.7. D**), sugar yield and stalk diameter, r = .083, n =25, $p < .0001$ (**Fig. 4.7. F**) was noted. No correlation was noted between sugar yield and plant heights, r = -.299, n = 25, $p < .0001$ (**Fig. 4.7. E**).

4.1.8 Assessment of grain sorghum biomass at grain maturity.

Assessment of the mean total fresh weights for grain sorghum varieties was conducted at grain maturity (**Fig. 4.8. A**). No significant difference was noted among all the varieties.

Data collected for average total dry weights at grain maturity indicated a significant difference in the mean between Sohag104 (155.60 g/plant) and TX430 (97.96 g/plant) at a cut off $p \leq .05$ (**Fig. 3.8. B**). Both of these varieties, however, did not show any significant difference in the mean of their total dry weights with other grain sorghum varieties.

4.1.9 On set of flowering at which 50% anthesis was observed in different sorghum varieties.

The Sudangrasses and Ramada had the earliest and latest anthesis. Within the sweet sorghum cultivars, GK Aron had the earliest anthesis. **Table 4.2** shows plant ages expressed as DAS at which 50% anthesis was observed with the Sudangrasses exhibiting the earliest anthesis between 45 and 48 DAS. This was followed by sweet sorghum and grain sorghum cultivars whose anthesis was observed between 57 and 83 DAS respectively.

4.1.10 Inflorescence-panicles of different sorghum varieties 90 DAS and a comparison of root structure between black-seeded Sudangrass and GK Aron.

The structure of inflorescence-panicles was of keen interest in our study. It was noted that grain sorghum varieties had compact panicles with larger seeds. Sweet sorghum varieties

had slightly open-compact panicles but with smaller immature seeds compared to the grain sorghum varieties. However, forage sorghum varieties had open panicles with much smaller mature seeds compared to the sweet sorghum varieties (**Fig. 4.9**).

The root system of black-seeded Sudangrass and GK Aron were compared 90 DAS. Both varieties showed a well-established identical fibrous and prop root system. No rhizomes were identified in both varieties (**Fig. 4.10**).

4.2 Molecular Analysis.

4.2.1 Multiple alignment of Exon 1 and 2, phylogenetic relationships and detection of Single Nucleotide Polymorphisms (SNPs).

PCR amplification for exon 1 and 2 of *COMT* was performed (**Fig. 4.11**) and the amplicons sequenced. With indels included, there were a total of 308 and 446 positions in the final alignment for both exon 1 and exon 2 (**Fig. 4.12. A** and **B**) respectively. Exon 1 has a total of 17 sites (5.52%) with alignment gaps and 252 (81.81%) monomorphic sites. Furthermore, 39 (12.66%) are polymorphic, 9 (2.92%) are parsimony informative and 30 (9.74%) are singletons. These singleton variable sites are located on Sudan white grass, LG 35 and Rex.

For exon 2, there are 5 sites (1.12%) with alignment gaps and 417 (93.5%) are monomorphic. Furthermore, 24 (5.38%) are polymorphic, 4 (0.9%) are parsimony informative and 9 (2.02%) are singleton variable. Notably, these singleton variable sites are also located Sudan white grass, LG 35 and Rex.

Phylogenetic analysis for exon 1 showed two clades with a relatively high bootstrapping of 54% (**Fig. 4.12. C**). The first clade was divided into two subclades in which sequences of black-seeded Sudangrass, GK Aron and Dwarf clustered together in the first subclade with those of MN1054, Sohag and rex clustering together in the second subclade. TX430 formed the second clade of the tree. However, the white-seeded Sudangrass and LG 35 are anticipated to be ancestral sequences since they are outgroups. Exon 2 has three clades with a relatively high bootstrapping above 60% (**Fig. 4.12. D**). The first clade consists of two subclades. Sequences of the white-seeded Sudangrass and Sugar Drip are clustered within the first subclade whereas those of Rex, Dwarf, Ramada and GK Aron are clustered together in the second subclade. The second clade consists of two subclades. The first subclade consists of a sequence of LG 35 whilst the second subclade consists of sequences of MN1054 and TX430 clustering together. The third clade consists of two subclades. The first subclade consists of black-seeded Sudangrass sequence whilst the second subclade consists of Sohag and Sohag104 sequences clustering together.

4.3 Forage analysis.

4.3.1 Fiber fraction, nutritive value and in *vitro* digestibility for the Sudan grasses and sweet sorghum varieties at 60, 75 and 90 DAS.

The mean lignin content of Sudangrasses and sweet sorghum varieties was determined at different cutting time points (**Fig. 4.13. A**). No significant difference was noted between the Sudangrasses at 60 and 75 DAS. However, Sugar Drip had the lowest lignin content (7.60%) at 75 DAS compared to MN1054 (11.28%) and this was significantly different.

Data collected at 90 DAS indicated that Sugar Drip had the lowest lignin content (5.11%) compared to other varieties and this was significantly different. Overall, there was a significant decrease in lignin content with advancing plant maturity except for the black-seeded Sudangrass.

The relative feed value (RFV) of the Sudangrasses and sweet sorghum cultivars was determined at different cutting time points (**Fig. 4.13. B**). Results of this study show that no significant difference was noted between the Sudangrasses at 60, 75 and 90 DAS. Sugar Drip however had a higher RFV (82.45) than the black-seeded Sudangrass (58.79) at 75 DAS and this was significantly different ($p < .05$). At 90 DAS, Sugar Drip and Rex had the highest RFV (106.58 and 101.95 respectively) compared to the Sudangrasses, MN1054 and GK Aron and this was significantly different ($p < .05$). Generally, our data shows that RFV significantly increased with advancing plant maturity depending on the variety. The RFV of the white-seeded Sudangrass and Sugar Drip increased (53.14% and 22.64% respectively) with advancing plant maturity except for the black-seeded Sudangrass and MN1054.

The effect of plant age on digestible crude protein intake (DCPI) was elucidated (**Fig. 4.13. C**). Results of this study indicated no significant difference between the Sudangrasses at 60, 75 and 90 DAS. However, Sugar Drip had a higher DCPI (352.42 g/day) compared to the black-seeded Sudangrass (201.72 g/day) at 75 DAS and this was significantly different ($p < .05$). Data obtained at 90 DAS indicate that Sugar Drip and Rex had the highest DCPI (414.42 g/day and 366.44 g/day) compared to the Sudangrasses and GK Aron and this was significantly different ($p < .05$). Nevertheless, DCPI of Rex was not significantly different to that of MN1054 and Ramada. Generally, depending on the plant variety, DCPI increased

or decreased with advancing plant maturity. For the white-seeded Sudangrass, the DCPI significantly increased from 60 to 75 DAS (14.66%) but later declined (36.58%) at 90 DAS. Furthermore, the DCPI of the black-seeded Sudangrass significantly decreased with advancing plant maturity. The same trend was observed with MN1054 (47.86%) at 90 DAS. Nonetheless, there was a 14.96% increase in DCPI of Sugar Drip at 90 DAS.

The Net Energy of Lactation (NEl) was assessed among the Sudangrasses and sweet sorghum cultivars at different cutting time points (**Fig. 4.13. D**). Results of this study indicate no significant difference in NEl between the Sudangrasses and sweet sorghum cultivars at 60 and 75 DAS. However, results obtained at 90 DAS indicate that Sugar Drip and Rex had the highest NEl (4.65 MJ/kg DM and 4.01 MJ/kg DM respectively) compared to the black-seeded Sudangrass and GK Aron and this was significantly different ($p < .05$). Nevertheless, the NEl for Rex was not significantly different to that of the white-seeded Sudangrass, MN1054 and Ramada. Overall, results of this study indicate a decrease and increase in NEl with advancing plant maturity depending on the variety ($p < .05$). The NEl of the black-seeded Sudangrass significantly decreased from 65 to 90 DAS (22.55%). Sugar Drip however, showed an increase in the NEl from 75 to 90 DAS (25.8%). No significant change was noted with MN1054.

4.3.2 Fiber fraction, nutritive value and *in vitro* digestibility of the grain sorghum varieties at grain maturity.

Fiber fraction analysis on the grain sorghum varieties was conducted at grain maturity. No significant difference was noted in most of the forage quality parameters except for the Relative feed value (RFV) and Acid detergent lignin (ADL) (**Table 4.3**). Nevertheless, Sohag had a higher RFV (104.02) compared to LG35 (66.24) and this was significantly different ($p < .05$) except for Sohag104 and Dwarf (**Fig. 4.14. A**). Furthermore, results of the *in vitro* digestibility indicate a no significant difference in all the forage quality parameters analyzed except the *in vitro* digestible dry matter (INDDM) (**Table 4.4**). Sohag had a higher INDDM (57.59%) compared to LG35 (50.44%) and this was significantly different ($p < .05$) except for Sohag104 and Dwarf (**Fig. 4.14. B**). overall, results of this study show that LG35 significantly had the least forage quality compared to other grain sorghum varieties.

Assessment of the average plant heights among the Sudangrasses and sweet sorghum cultivars at 60, 75 and 90 DAS.

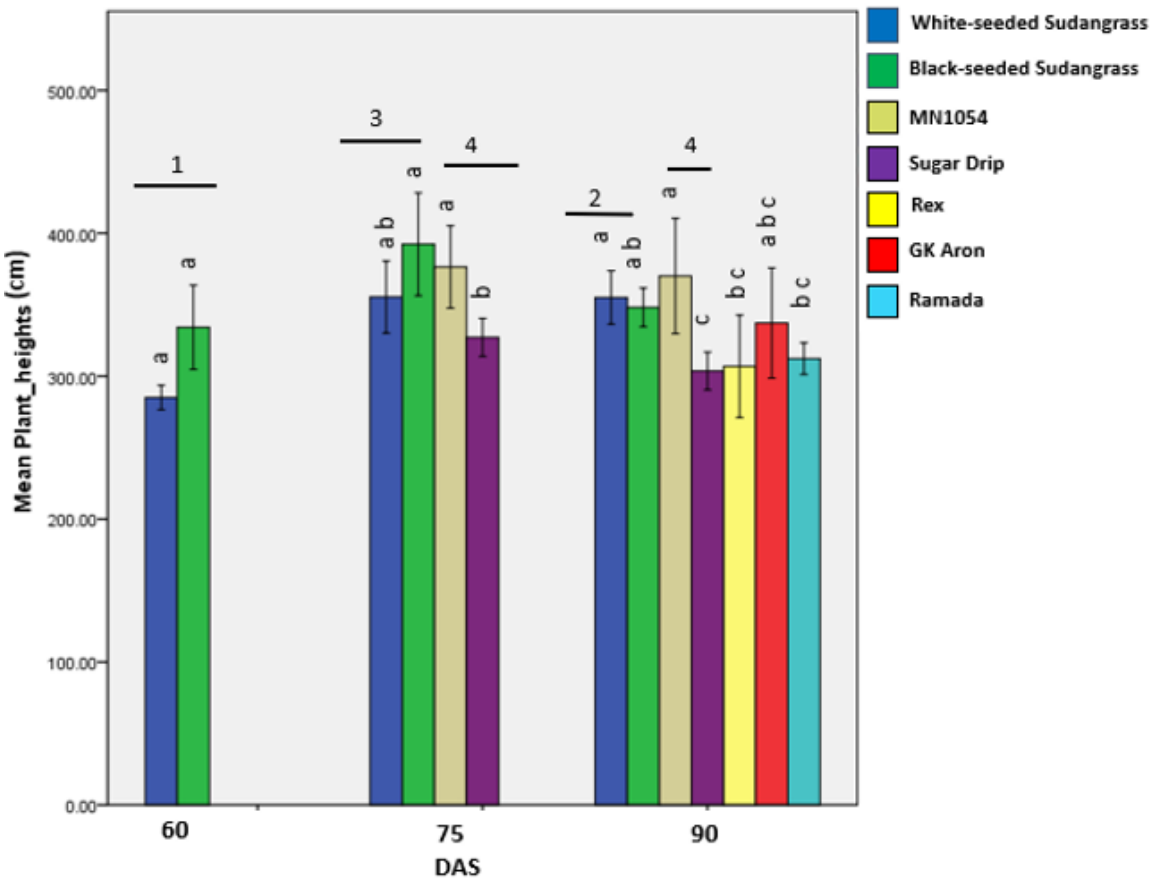


Fig. 4.1: Average plant heights of the Sudangrasses and sweet sorghum cultivars recorded at 60, 75 and 90 DAS. Error bars represent the standard deviation. Data expressed as mean ± SD (n = 5). Bar columns at the same time point having different letters at the top indicate a significant difference at $p \leq .05$. Horizontal bars at the top of the bar columns having different numbers indicate a significant difference at $p \leq .05$.

Assessment of the average leaf number among the Sudangrasses and sweet sorghum cultivars at 60, 75 and 90 DAS.

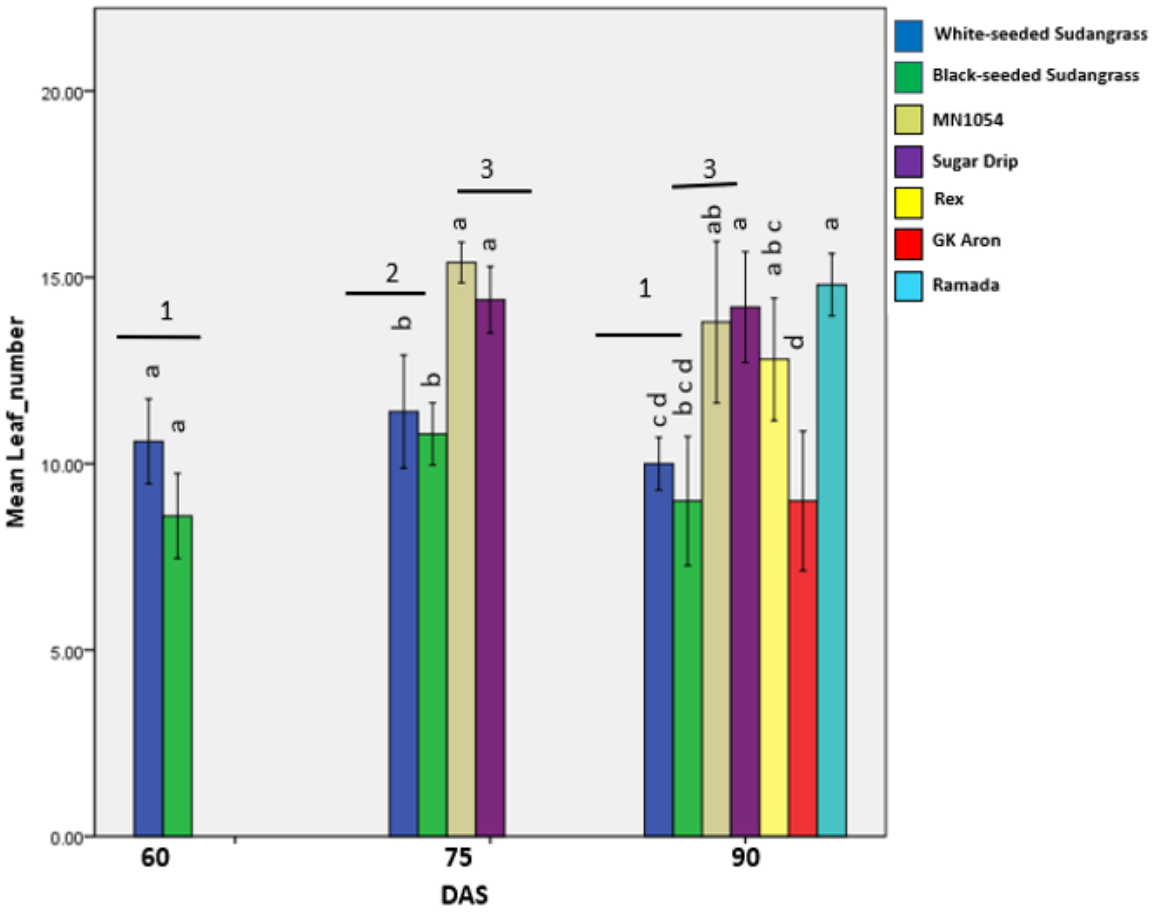


Fig. 4.2: Mean leaf number of the Sudangrasses and sweet sorghum cultivars recorded at 60, 75 and 90 DAS. Error bars represent the standard deviation. Data expressed as mean ± SD (n = 5). Bar columns at the same time point having different letters at the top indicate a significant difference at $p \leq .05$. Horizontal bars at the top of the bar columns having different numbers indicate a significant difference at $p \leq .05$.

Assessment of the average leaf width among the Sudangrasses and sweet sorghum cultivars at 60, 75 and 90 DAS.

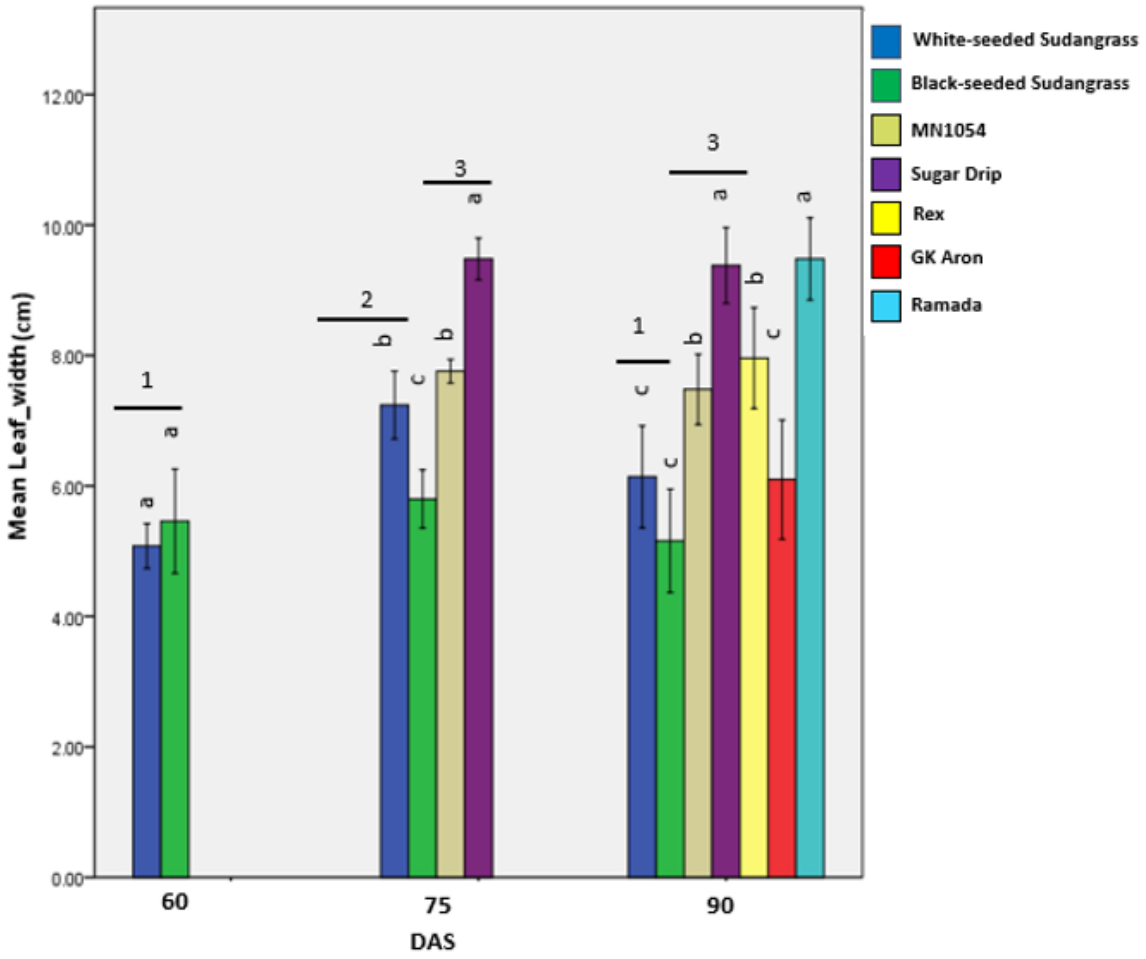


Fig. 4.3: Mean leaf widths of the Sudangrasses and sweet sorghum cultivars recorded at 60, 75 and 90 DAS. Error bars represent the standard deviation. Data expressed as mean ± SD (n = 5). Bar columns at the same time point having different letters at the top indicate a significant difference at $p \leq .05$. Horizontal bars at the top of the bar columns having different numbers indicate a significant difference at $p \leq .05$.

Assessment of the average stalk diameter among the Sudangrasses and sweet sorghum cultivars at 60, 75 and 90 DAS.

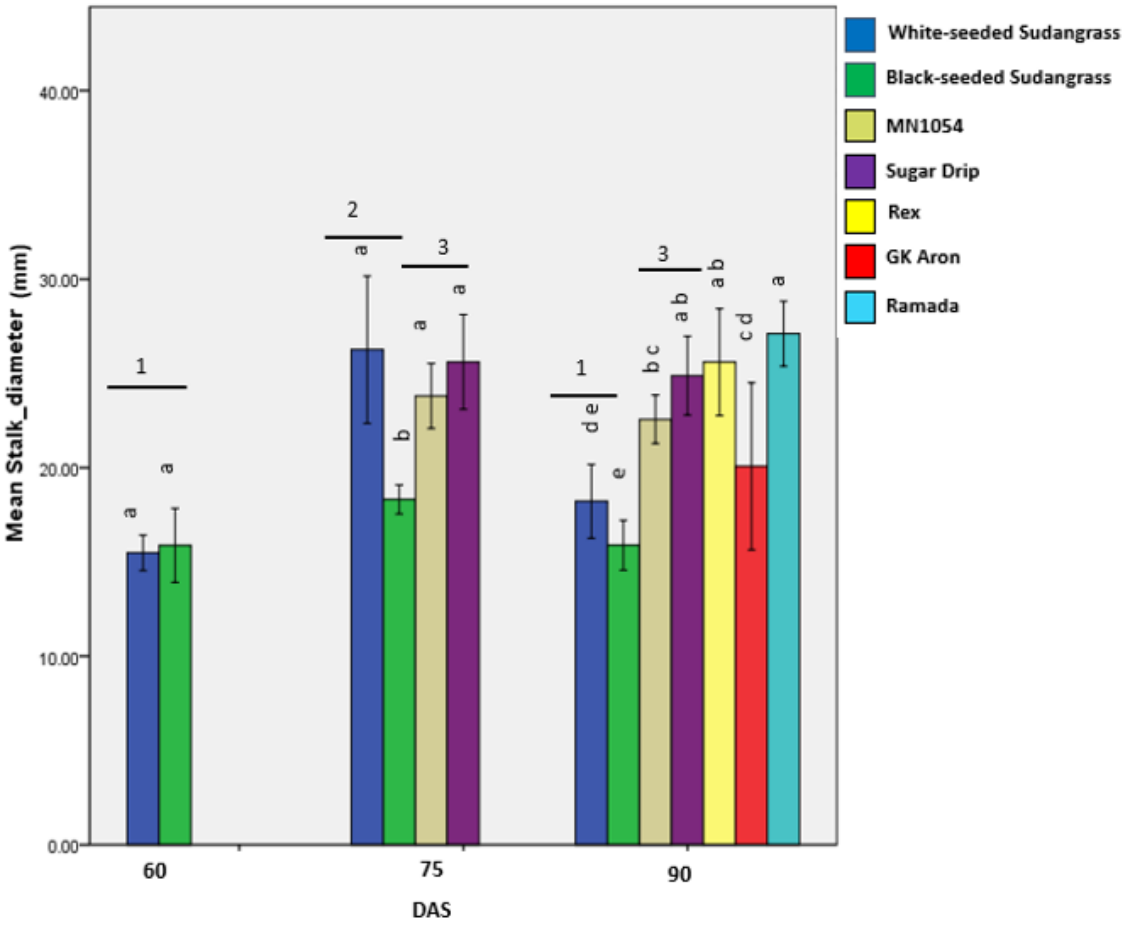


Fig. 4.4: Average stalk diameters of the Sudangrasses and sweet sorghum cultivars recorded at 60, 75and 90 DAS. Error bars represent the standard deviation. Data expressed as mean ± SD (n = 5). Bar columns at the same time point having different letters at the top indicate a significant difference at $p \leq .05$. Horizontal bars at the top of the bar columns having different numbers indicate a significant difference at $p \leq .05$.

Assessment of mean total fresh weights for the Sudangrasses and sweet sorghum cultivars at 60, 75 and 90 DAS

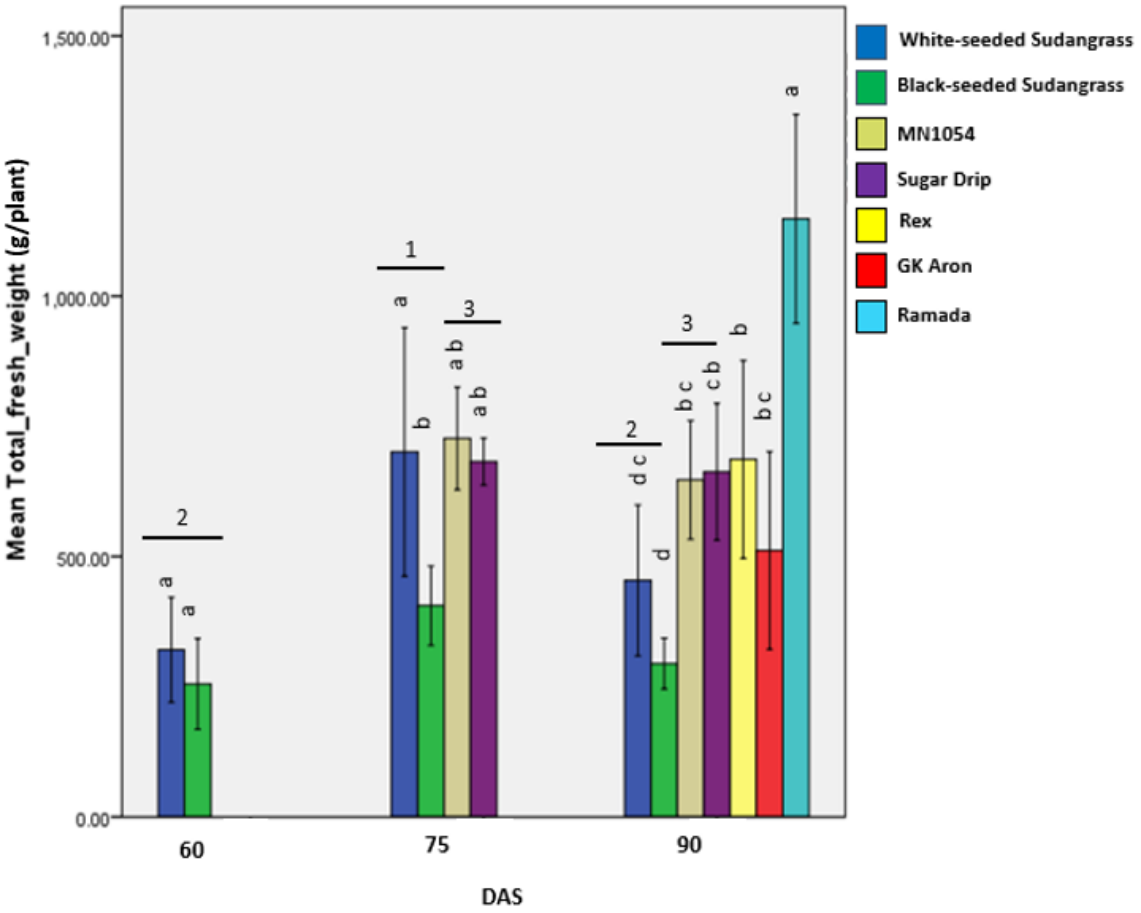


Fig. 4.5: Average total fresh weights of the Sudangrasses and sweet sorghum cultivars at 60, 75 and 90 DAS. Error bars represent the standard deviation. Data expressed as mean ± SD (n = 5). Bar columns at the same time point having different letters at the top indicate a significant difference at $p \leq .05$. Horizontal bars at the top of the bar columns having different numbers indicate a significant difference at $p \leq .05$.

Assessment of mean total dry weights for the Sudangrasses and sweet sorghum cultivars at 60, 75 and 90 DAS

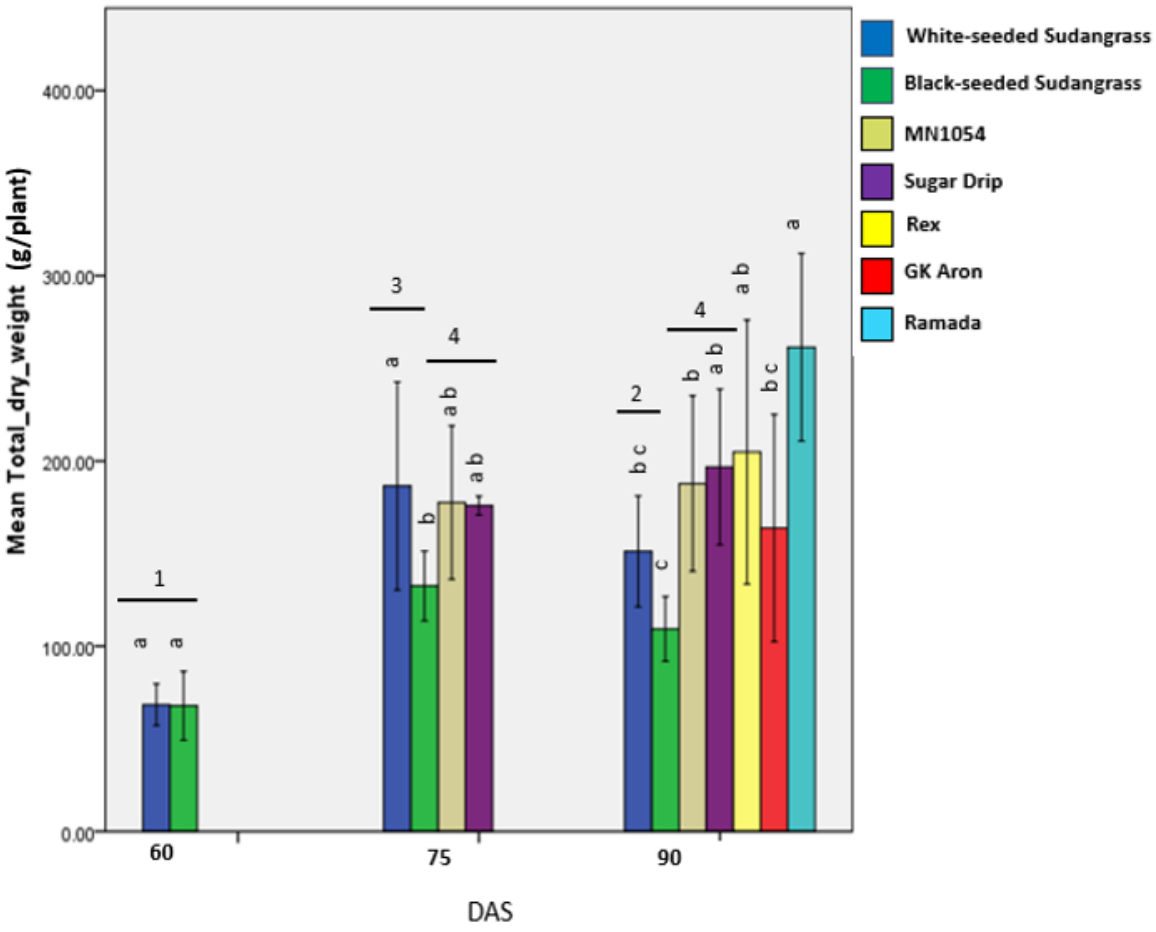
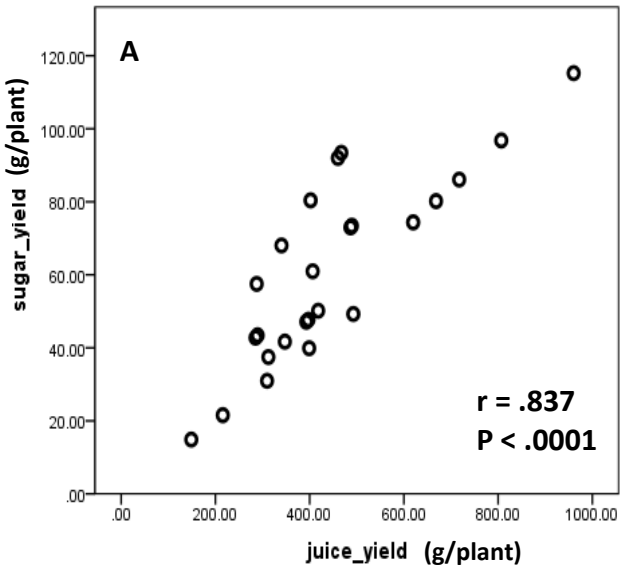
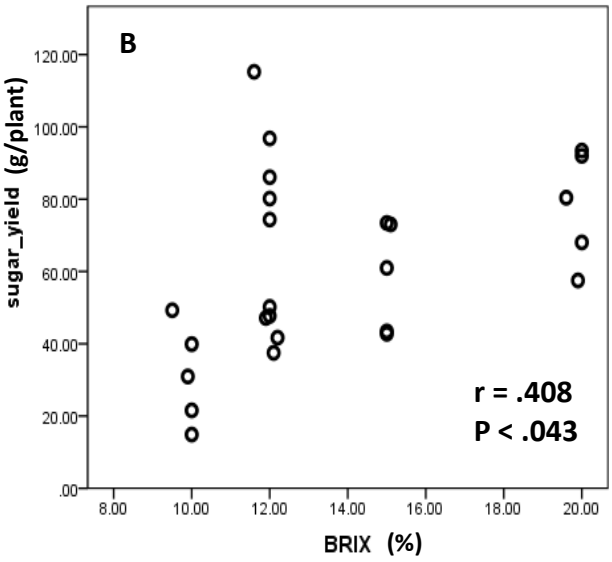
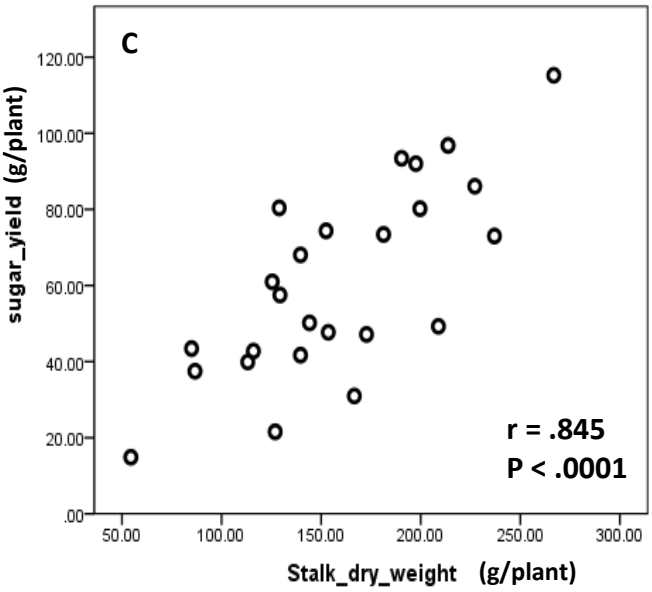


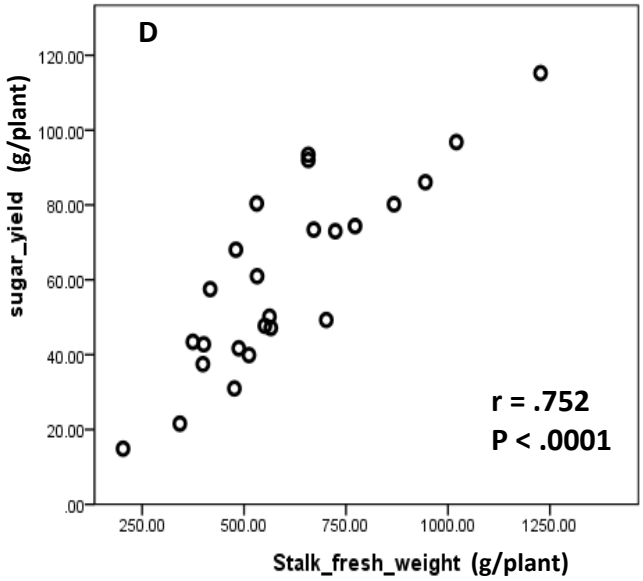
Fig. 4.6: Average total dry weights of the Sudangrasses and sweet sorghum cultivars recorded at 60, 75 and 90 DAS. Error bars represent the standard deviation. Data expressed as mean ± SD (n = 5). Bar columns at the same time point having different letters at the top indicate a significant difference at $p \leq .05$. Horizontal bars at the top of the bar columns having different numbers indicate a significant difference at $p \leq .05$.

Correlation analysis









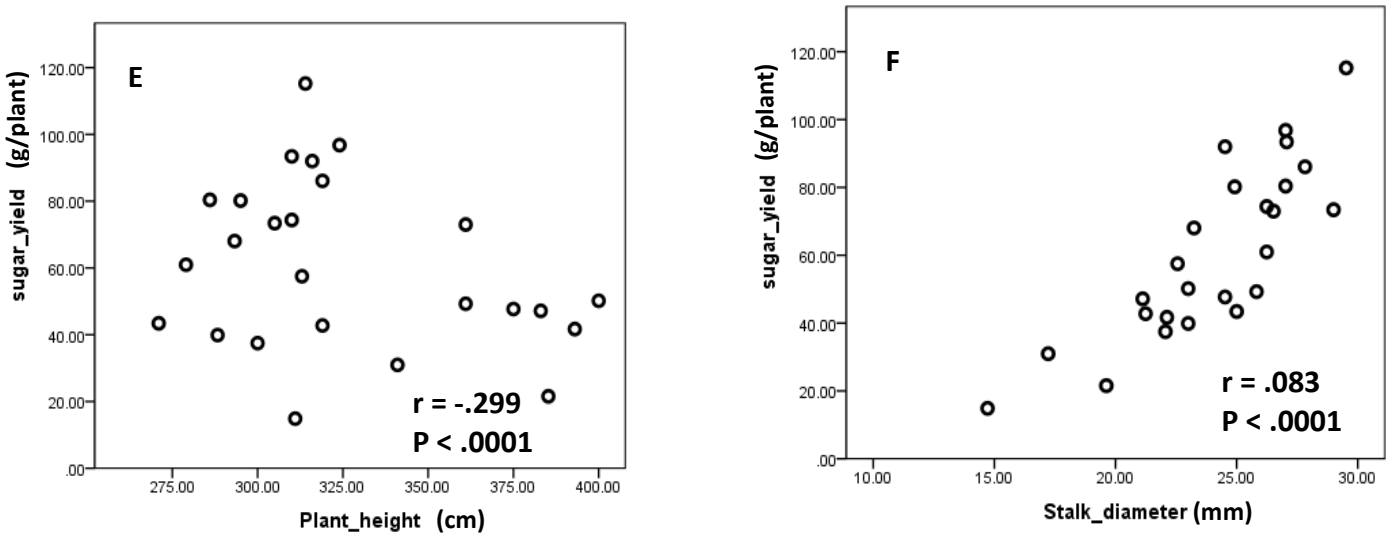


Fig. 4.7: Correlation analysis between sugar yield and agro-morphological traits 90 DAS; **(A)**: sugar yield and Juice yield; **(B)**: sugar yield and Brix; **(C)**: sugar yield and stalk dry weight; **(D)**: sugar yield and stalk fresh weight; **(E)**: sugar yield and plant height; **(F)**: Sugar yield and stalk diameter.

Assessment of the average total fresh and dry weights for grain sorghum varieties at grain maturity.

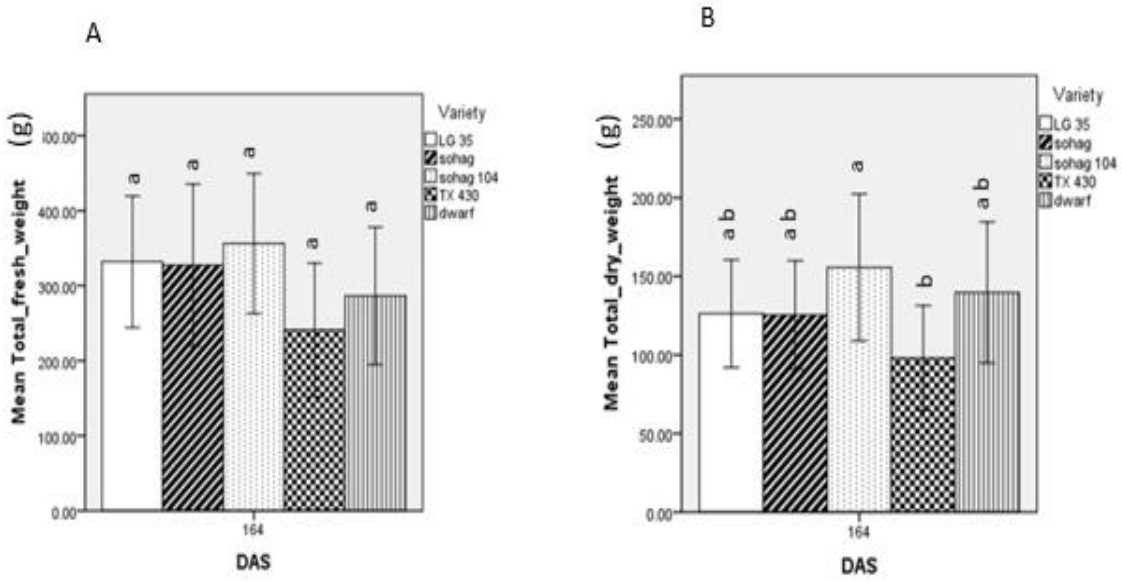
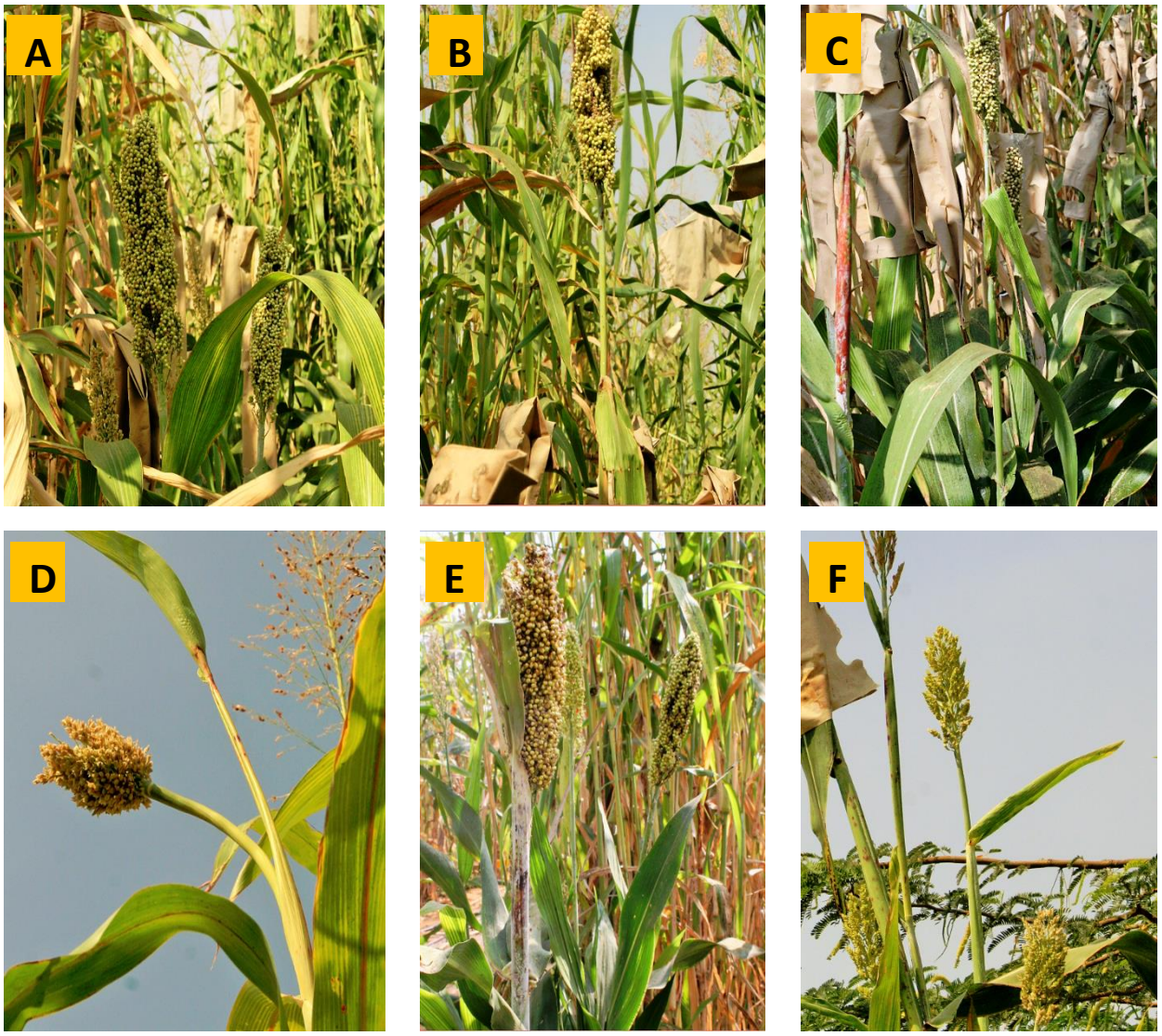


Fig. 4.8: Average biomass of grain sorghum cultivars at grain maturity; (**A)**: mean total fresh weights; (**B)**: mean total dry weights of grain sorghum cultivars. Error bars represent the standard deviation. Data expressed as mean ± SD (n = 5). Bar columns having different letters at the top indicate a significant difference at $p \leq .05$.

Inflorescence-panicles of different sorghum varieties 90 DAS.



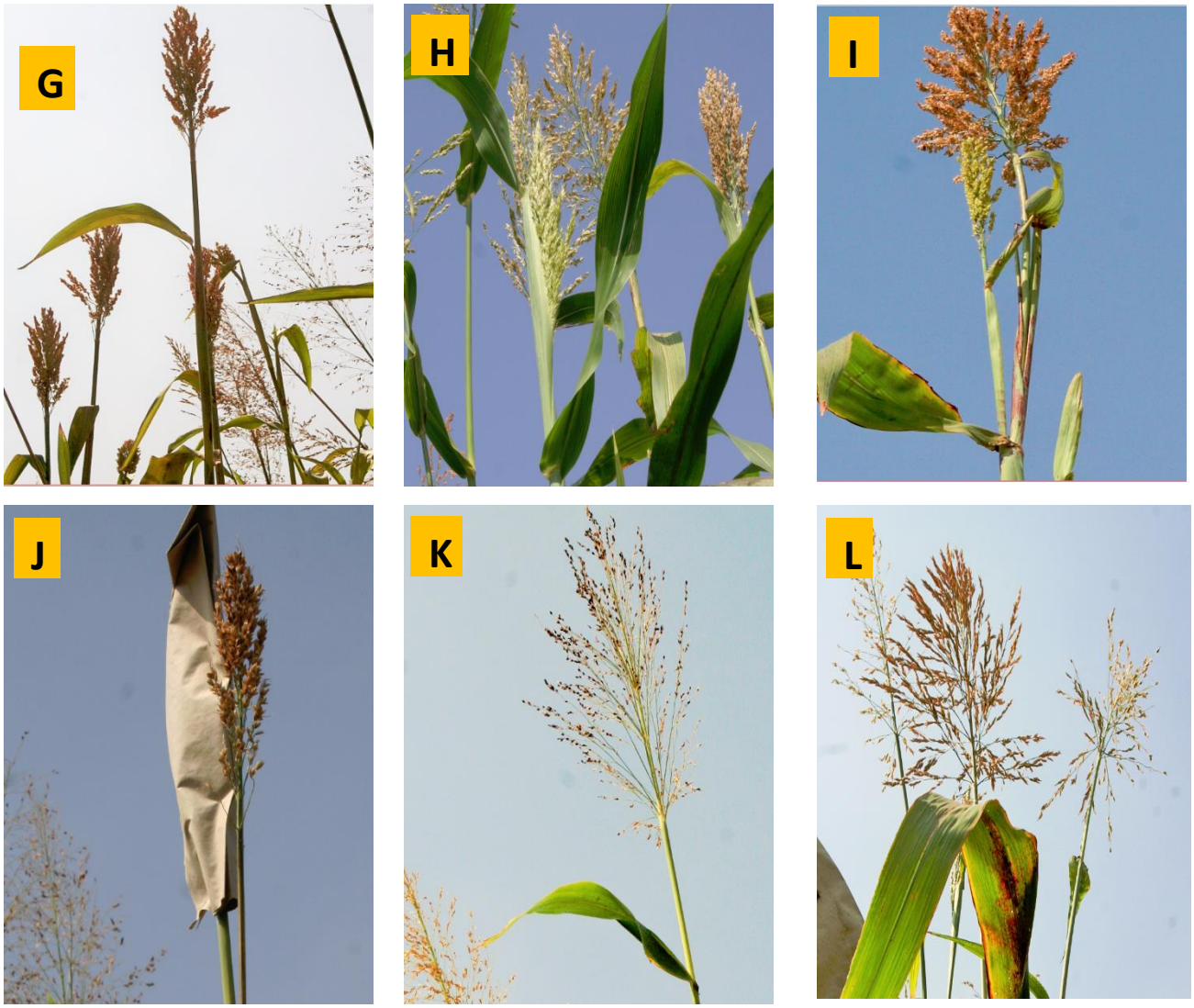


Fig. 4.9: Inflorescence-panicles of different sorghum varieties 90 DAS. Compact panicles of (**A)**: Sohag104; (**B)**: Sohag; (**C)**: Dwarf; (**D)**: LG 35 and (**E)**: TX430. Slightly compact panicles of (**F**) Ramada; Slightly open panicles of; (**G)**: Sugar Drip; (**H)**: GK Aron; (**I)**: MN1054; (**J):** Rex. Open panicles of (**K):** Black-seeded Sudangrass and (**L**): white-seeded Sudangrass.

Comparison of the root system between black-seeded Sudangrass and GK Aron

Fig. 4.10: Fibrous and prop root system of two different sorghum varieties. (**A)**: black-seeded Sudangrass and (**B)**: GK Aron. In both cultivars, the fibrous and prop root systems are well developed and established. No emergence of rhizomes was observed from any of the cultivars.

Multiple alignment of Exon 1 and 2, phylogenetic relationships and detection of Single Nucleotide Polymorphisms (SNPs).

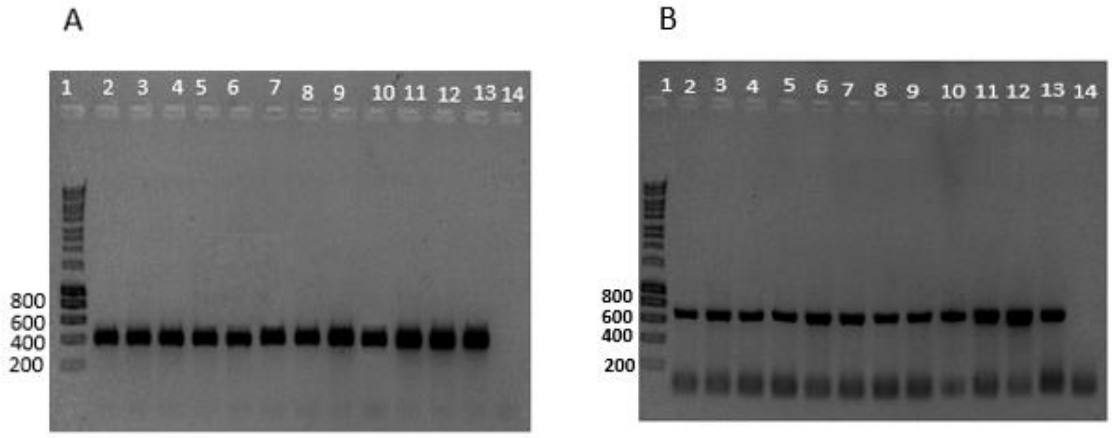
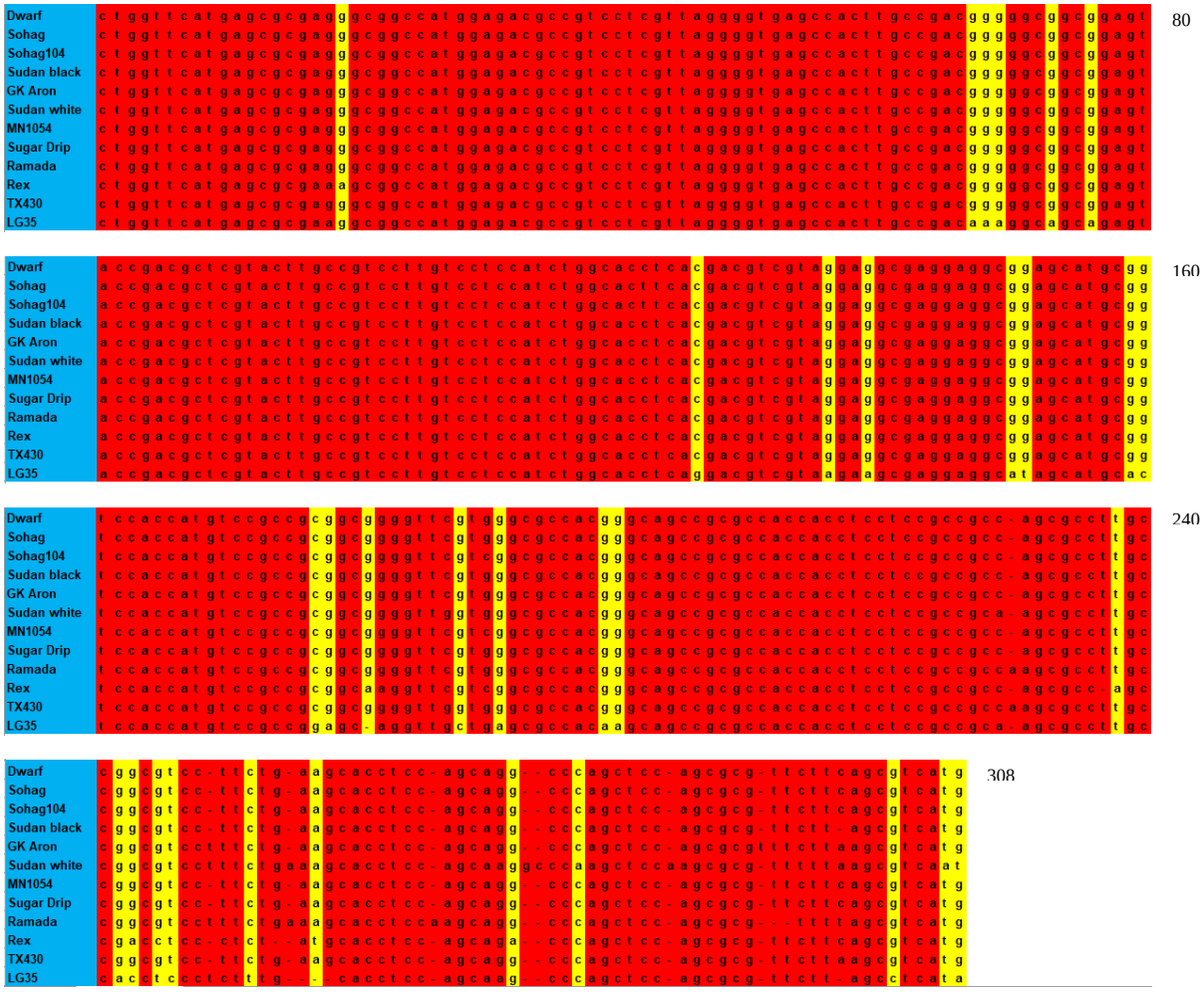


Fig. 4.11: Image of 1% Agarose gel for PCR amplicons of; (**A)**: exon one and (**B)**: exon two. Lanes **1-14**: 1 Kb Hyper DNA ladder; white-seeded Sudangrass; black-seeded Sudangrass; MN1054; Sugar Drip; Rex; GK Aron; Ramada; TX430; LG 35; Sohag; Sohag104; Dwarf; Negative control, no DNA added, respectively.

Multiple sequence alignment for exons 1 and 2

A



B

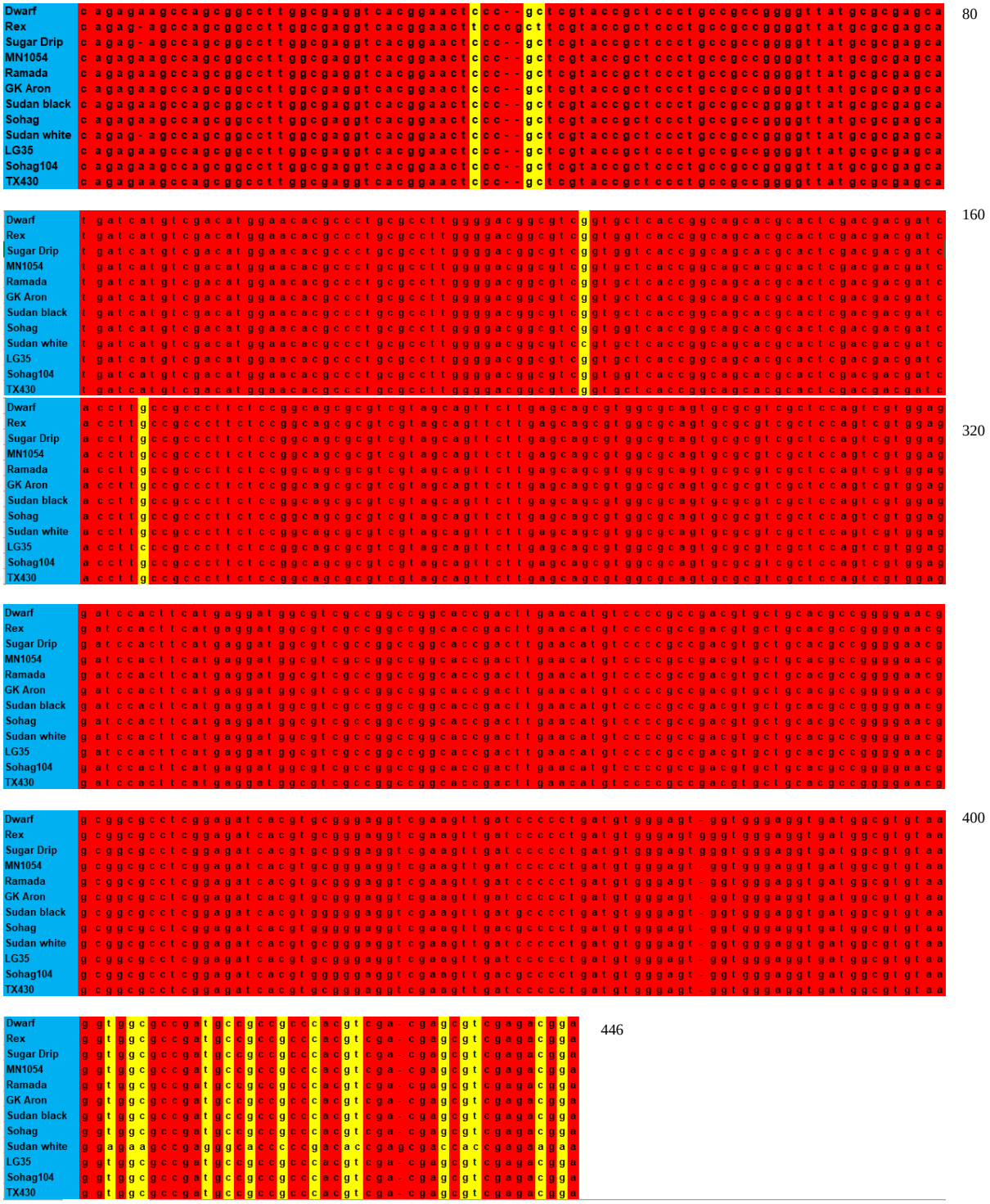


Fig. 4.12: Multiple sequence alignment for; (**A**): Exon 1 and (**B**): exon 2. Sequences of 12 sorghum cultivars are aligned and the yellow columns indicate regions of singleton variables (SNPs). Rex, LG35 and white-seeded Sudangrass have SNPs in the second exon of COMT.

Phylogenic tree of exon 1

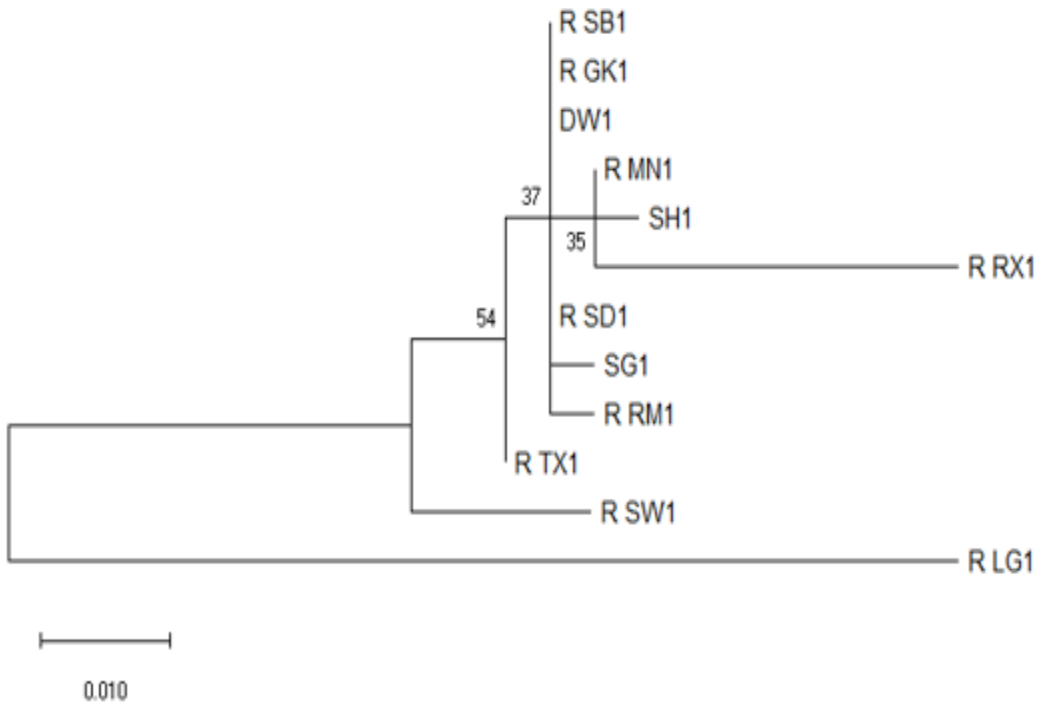


Fig. 4.12. C: A phylogenetic tree of exon 1. Evolutionally history was inferred using the maximum Likelihood method and Kimura 2-parameter model. The tree is scaled, and the branch lengths are measured in the number of substitutions per site. All gaps and missing data were eliminated, and the final dataset contained 291 positions. The tree with a highest log likelihood = -639.55 is shown. The varieties analyzed were; **SW1**: white-seeded Sudangrass; **SB1**: black-seeded Sudangrass; **MN1**: MN1054; **SD1**: Sugar Drip; **RX1**: Rex; **GK1**: GK Aron; **RM1**: Ramada; **TX1**: TX430; **LG1**: LG 35; **SH1**: Sohag; **SG1**: Sohag104; **DW1**: Dwarf respectively.

Phylogenetic tree of exon 2

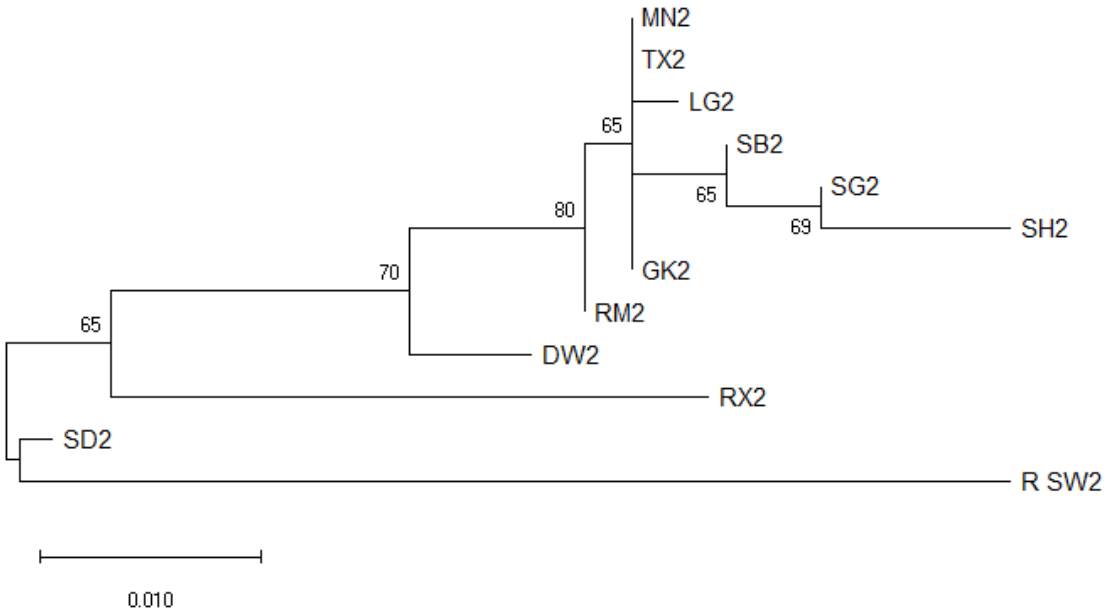


Fig. 4.12. D: A phylogenetic tree of exon 2. Evolutionally history was inferred using the maximum Likelihood method and Kimura 2-parameter model. The tree is scaled, and the branch lengths are measured in the number of substitutions per site. All gaps and missing data were eliminated, and the final dataset contained 441 positions. The tree with a highest log likelihood = -997.04 is shown. The varieties analyzed were; **SW2**: white-seeded Sudangrass; **SB2**: black-seeded Sudangrass; **MN2**: MN1054; **SD2**: Sugar Drip; **RX2**: Rex; **GK2**: GK Aron; **RM2**: Ramada; **TX2**: TX430; **LG2**: LG 35; **SH2**: Sohag; **SG2**: Sohag104; **DW2**: Dwarf respectively.

Fiber fraction, nutritive value and in *vitro* digestibility for the Sudangrasses and sweet sorghum cultivars at 60, 75 and 90 DAS.

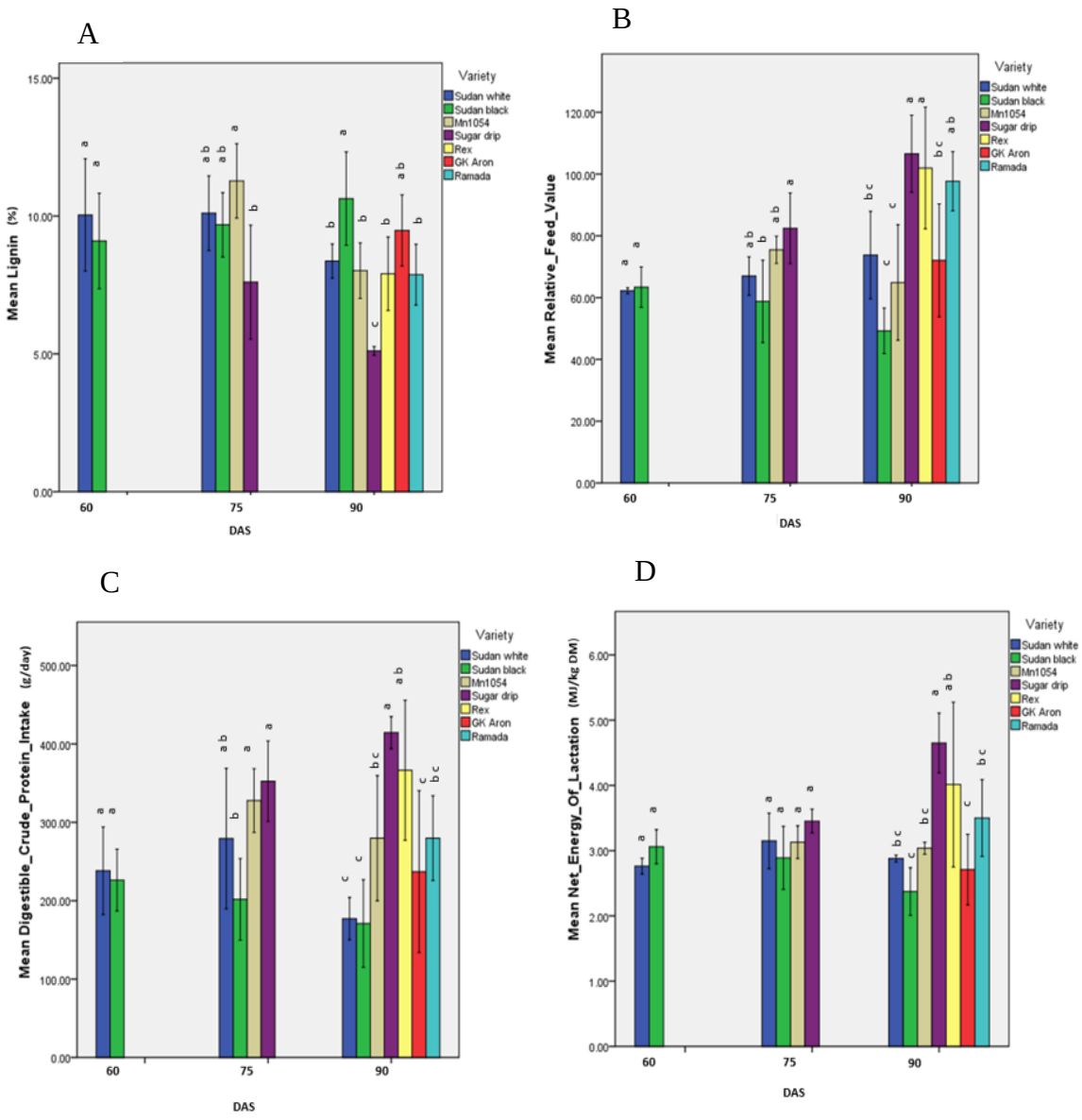


Fig. 4.13: Fiber fraction, nutritive value and *in vitro* digestibility for Sudangrasses and sweet sorghum cultivars at 60, 75 and 90 DAS; (**A)**: mean lignin content; (**B)**: mean relative feed value; (**C)**: mean digestible crude protein; (**D)**: mean net energy of lactation for the Sudan grasses and sweet sorghum varieties determined at 60, 75 and 90 DAS. Error bars represent the standard deviation. Bar columns at the same time point having different letters at the top indicate a significant difference at p <. 05.

Nutritive value and *in vitro* digestibility of the grain sorghum cultivars at grain maturity.

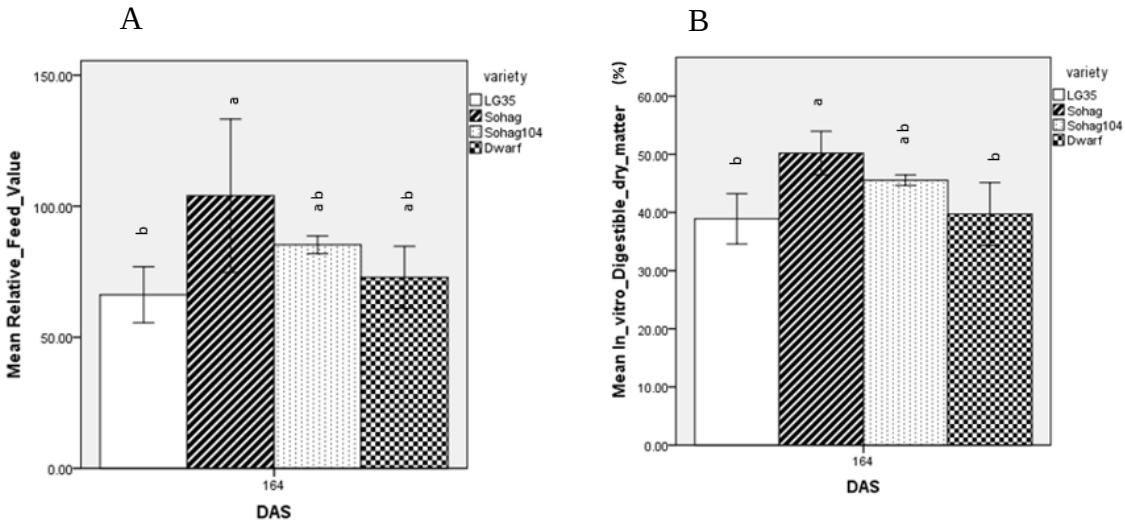


Fig. 4.14: Nutritive value and *in vitro* digestibility for grain sorghum cultivars at grain maturity; (**A)**: mean relative feed value; (**B)**: mean in vitro digestible dry matter of the grain sorghum varieties at grain maturity. Error bars represent the standard deviation. Bar columns having different letters at the top indicate a significant difference at $p < .05$.

Table 4.1: On set of flowering at which 50% anthesis was observed in different sorghum varieties.

Grain Sorghum Cultivars	**DAS**	**Sweet Sorghum Cultivars**	**DAS**	**Sudangrasses**	**DAS**
TX430	59	**MN1054**	65	**White-seeded Sudangrass**	48
LG35	65	**Sugar Drip**	64	**Black-seeded Sudangrass**	45
Sohag	65	**Rex**	62		
Sohag104	65	**GK Aron**	57		
Dwarf	65	**Ramada**	83		

Table 4.2: Stalk fresh and dry weights, juice yield, sugar yield and BRIX of sweet sorghum cultivars at 75 and 90 DAS.

DAS	Variety	Stalk fresh weight (g)	Stalk dry weight (g)	Juice yield (g/plant)	Sugar yield (g/plant)	Brix (%)
75	**MN1054**	592.57	132.2	460.37	50.64	11
	Sugar Drip	557.20	134.11	423.09	46.54	11
	Mean	574.89	133	441.73	80.27	11
90	**MN1054**	513.03	139.37	373.67	44.84	12
	Sugar Drip	548.55	157.17	391.38	78.28	20
	Rex	540.38	148.94	391.06	58.66	15
	GK Aron	447.10	134.01	313.09	31.31	10
	Ramada	966.38	211.95	754.44	90.53	12
	Mean	603.09	158.29	444.73	60.72	13.8

Table 4.3: Results of fiber fraction and nutritive value analysis for the Sudan grasses and sweet sorghum cultivars at 60, 75 and 90 DAS.

DAS	variety	NDF (%)	ADF (%)	ADL (%)	RFV	TDN (g/kg)	HEM (%)	CEL (%)	LIG (%)	CP (%)	DCPI (g/day)
60	**Sudan white**	55.21a	42.38a	10.45a	62.20a	46.64a	12.82a	31.92a	10.04a	5.50a	238.27a
	Sudan black	58.39a	43.48a	9.77a	63.40a	45.21a	14.91a	33.71a	9.09a	4.60a	226.37a
75	**Sudan white**	56.52a	42.07ab	10.69ab	66.98ab	47.03b	14.44a	31.38ab	10.10ab	4.77a	279.25ab
	Sudan black	58.58a	44.19a	10.45ab	58.79b	44.29b	14.38a	33.73a	9.68ab	3.80ab	201.72b
	MN1054	54.46a	39.20b	11.96a	75.48ab	50.74ab	15.26a	27.24bc	11.28a	4.53ab	327.76a
	Sugar Drip	48.83b	34.33c	8.27b	82.45a	55.57a	14.49a	26.05c	7.60b	3.33b	352.42a
90	**Sudan white**	52.16b	39.49b	9.00b	73.79bc	50.36b	12.67b	30.49a	8.37b	3.17b	177.11c
	Sudan black	60.40a	43.50a	11.44a	49.27c	45.19c	16.89a	32.06a	10.63a	2.27c	170.91c
	MN1054	52.62b	38.18b	8.76b	64.89c	52.07b	14.44ab	29.41a	8.02b	3.43b	279.78bc
	Sugar Drip	43.71c	30.56c	5.53c	106.58a	61.90a	13.15ab	25.04b	5.11c	3.43b	414.42a
	Rex	45.94bc	31.72c	8.25b	101.95a	60.40a	14.21ab	23.46b	7.91b	3.13b	366.44ab
	GK Aron	52.47b	40.08ab	9.93ab	72.04bc	49.60bc	12.38b	30.15a	9.48ab	3.40b	237.09c
	Ramada	43.94c	33.39c	8.35b	97.68ab	58.23a	10.54b	25.04b	7.87b	4.27a	279.78bc

Means within a column followed by the same letters at each cutting time point were not significantly different according to the Duncan multiple range test ($p > .05$). NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; RFV: Relative feed value; TDN: Total digestible nutrients; HEM: hemicellulose; CEL: cellulose; LIG: lignin; CP: crude protein; DCPI: digestible crude protein intake. DAS: days after sowing

Table 4.4: Results of in vitro digestibility for the Sudangrasses and sweet sorghum cultivars at 60, 75 and 90 DAS.

DAS	Variety	GP (ml/200 mg DM)	GPSF (ml/g Dm)	GPNSP (ml/g Dm)	INDDM (%)	INDOM (%)	DOMI (g/day)	ME (MJ/kg DM)	NEl (MJ/kg DM)	SCFA (mmol/ml gas)	Mp (g/kg DOM)
60	**Sudan white**	22.92a	4.73a	51.25a	36.87a	50.82a	1802.23a	5.35a	2.76a	0.61a	60.99a
	Sudan black	26.06a	4.34a	48.22a	39.96a	53.17a	1737.92a	5.77a	3.06a	0.68a	63.80a
75	**Sudan white**	27.00a	21.54a	48.44a	40.62a	53.86a	2079.91ab	5.89a	3.15a	0.71a	64.63a
	Sudan black	24.35a	3.60b	45.82a	36.89a	51.88a	1608.03b	5.53a	2.89a	0.64a	62.26a
	MN1054	26.77a	10.16b	51.10a	44.23a	53.69a	2061.89ab	5.86a	3.13a	0.69a	64.43a
	Sugar Drip	30.91a	19.98a	46.97a	43.28a	56.25a	2250.72a	6.32a	3.45a	0.78a	67.50a
90	**Sudan white**	24.25bc	5.74a	45.36a	41.01abc	51.81bc	1555.76b	5.51bc	2.88bc	0.64bc	62.17bc
	Sudan black	19.01c	5.45a	49.22a	32.02c	47.90c	1404.45b	4.79C	2.37c	0.52c	57.48c
	MN1054	25.88bc	14.86a	51.11a	36.93bc	53.03bc	2019.79ab	5.73bc	3.04bc	0.68bc	63.63bc
	Sugar Drip	42.70a	16.55a	45.49a	49.78a	65.59a	2548.13a	8.02a	4.65a	1.08a	78.70a
	Rex	36.08ab	16.32a	48.05a	50.04a	60.64ab	2388.03a	7.12ab	4.01ab	0.92ab	72.77ab
	GK Aron	22.49c	11.05a	44.37a	39.77abc	50.49c	1640.21b	5.28c	2.71c	0.59c	60.59c
	Ramada	30.70bc	8.96a	43.47a	46.06ab	56.63bc	1901.79ab	6.39bc	3.50bc	0.79bc	67.95bc

Means within a column followed by the same letters at each cutting time point were not significantly different according to the Duncan multiple range test ($p > .05$). GP: gas production; GPSF: gas production structure fraction; GPNSF: gas production non-structure fraction; INDDM: In vitro-digestible dry matter; INDOM: In vitro-digestible organic matter; DOMI; digestible organic matter intake; ME: metabolic energy; NEl: net energy of lactation; SCFA: short chain fatty acids; Mp: microbial protein; DAS: days after sowing.

Table 4.5: Results of fiber fraction and nutritive value analysis of grain sorghum cultivars at grain maturity.

Variety	NDF (%)	ADF (%)	ADL (%)	RFV	TDN (g/kg)	HEM (%)	CELL (%)	LIG (%)	CP (%)	DCPI (g/day)
LG35	54.91a	37.76a	8.55a	66.24b	52.58a	17.13a	29.22a	7.65a	2.93a	219.68a
Sohag	47.60a	30.49a	7.03b	104.02a	61.99a	17.11a	23.45a	6.45a	3.47a	369.49a
Sohag104	49.66a	33.32a	7.76ab	85.32ab	58.34a	16.32a	25.55a	7.20a	3.60a	320.18a
Dwarf	50.84a	33.99a	8.60a	72.88ab	57.46a	16.84a	25.39a	7.11a	3.33a	263.24a

Means within a column followed by the same letters were not significantly different according to the Duncan multiple range test ($p > .05$). NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; RFV: Relative feed value; TDN: Total digestible nutrients; HEM: hemicellulose; CEL: cellulose; LIG: lignin; CP: crude protein; DCPI: digestible crude protein intake.

Table 4.6: Results of *in vitro* digestibility of grain sorghum cultivars at grain maturity.

Variety	GP (ml/200 mg DM)	GPSF (ml/g Dm)	GPNSP (ml/g Dm)	INDDM (%)	INDOM (%)	DOMI (g/day)	ME (MJ/kg DM)	NEl (MJ/kg DM)	SCFA (mmol/ml gas)	Mp (g/kg DOM)
LG35	22.51a	8.88a	46.44a	38.91b	50.44a	1898.32a	5.26a	2.70a	0.60a	60.52a
Sohag	31.99a	15.73a	59.93a	50.20a	57.59a	2952.72a	6.56a	3.62a	0.82a	69.10a
Sohag104	28.97a	8.71a	59.19a	45.55ab	55.34a	2575.07a	6.16a	3.34a	0.75a	66.40a
Dwarf	24.32a	9.46a	52.16a	39.70b	51.87a	2220.11a	5.52a	2.89a	0.64a	62.24a

Means within a column followed by the same letters were not significantly different according to the Duncan multiple range test ($p > .05$). GP: gas production; GPSF: gas production structure fraction; GPNSF: gas production non-structure fraction; INDDM: In vitro-digestible dry matter; INDOM: In vitro-digestible organic matter; DOMI; digestible organic matter intake; ME: metabolic energy; NEl: net energy of lactation; SCFA: short chain fatty acids; Mp: microbial protein.

Chapter 5. Discussion

5.1 Agro-morphological traits

5.1.1: Total plant biomass, Average plants heights, leaf number, leaf width and stalk diameter.

In this study, the agro-morphological traits of the Sudangrasses and two sweet sorghum cultivars (MN1054 and Sugar Drip) were assessed at different cutting time points. Results indicated a significant decline in the agro-morphological traits of the Sudangrasses with advancing plant maturity with the highest plant heights, stalk diameter, leaf width and leaf number recorded at 75 DAS. This was in correlation with their forage quality. We recommend that the best cutting time point for the Sudangrasses is 75 DAS. This is because at 90 DAS, the Sudangrasses were reaching senescence, a physiological state characterized by deterioration of plant tissues and death. Senescence affects yield of forage sorghum and corn in terms of their biomass and grain production (Gregersen et al., 2013). It is of great importance therefore, to harvest sorghum before reaching senescence in order to obtain high yields and good quality forage.

5.1.2 Lodging

MN1054 is one of the African landraces that has been widely used in the breeding programs of sweet sorghum for biofuel production (Murray et al., 2008). In our study, MN1054 exhibited lodging post 75 DAS and our results indicated a decrease in its forage quality at 90 DAS. This could be attributed to several factors such as differential gene expression

upon root lodging and stalk cannibalization. A previous study on root lodging and its effect on the nutritive composition of sorghum stalks by Mizuno and his colleagues (2018), revealed that an increase in expression of sucrose or starch degradation genes occurs upon lodging hence resulting in a decrease of carbohydrate concentration in stalks (Mizuno et al., 2018). In addition, MN1054 slightly had heavier panicles compared to other sweet sorghum cultivars. This could have led to stalk cannibalization; a process by which stalk nutrients are withdrawn and translocated to the head panicles to fill the seeds. This was in accord with a previous study on corn (Hladik, 2012). Ramada also exhibited root lodging post 75 DAS. Since we did not sample Ramada for nutritive composition analysis and *in vitro* digestibility at 75 DAS, we could not determine whether its forage quality had declined by 90 DAS.

5.1.3. BRIX

Sugar Drip, Rex and Ramada are commercially cultivated sweet sorghum cultivars in the United States due to their high sugar content. In this study, Sugar Drip and Rex had the highest Brix (**Table 4.2**); a measurement of total soluble sugars most especially sucrose. Our results contradict to those of Ali and his colleagues (2007), in which Ramada had the highest Brix (17.93), followed by Rex (17.27) and Sugar Drip (16.73) (Ali et al., 2007). This could be due to several factors such as weather conditions, time of planting, planting density and soil fertility among others. Nevertheless, it was noted that Brix of Sugar Drip, and MN1054 increased with advancing plant maturity. Our results are in accord to those of Zhao and his colleagues (2011), who reported a 66.59% significant increase in total soluble sugar content (TSSC) of five sorghum cultivars (Italy, Zaoshu, Chutian-2, Lvneng-

3 and M-81E) from 0-40 days after anthesis in 2006 and a 49.10% increase in TSSC from 0-40 days after anthesis in 2007. With Sugar Drip and Rex having the highest Brix in this study, this would make them good candidates for silage production. This is because the quality of good silage production highly depends on the sugar content of the silage crop (McDonald, 1981). Sugars neutralize the acidic conditions in the silage to prevent its spoilage.

Correlation analysis showed that juice yield and sugar content are positively correlated (**Fig. 4.8. A**). A high juice producing cultivar has a high sugar content regardless of its brix. We therefore suggest that in plant breeding programs, selection of sweet sorghum cultivars should base on those that have a high juice yield rather than high brix. Indeed, Makanda and his colleagues (2009), suggested that genotypes with lower brix but high juice yield were preferable stalk sugar accumulators compared to their counterparts.

5.2 Forage analysis

5.2.1. NDF, ADF and ADL

The digestibility of forages is dependent on several forage quality parameters such NDF, ADF, ADL and as such, the higher the lignin content, the lower the digestibility (Traxler et al., 1998). Generally, the Sudangrasses had a higher fiber fraction composition compared to the sweet sorghum and grain sorghum cultivars. This could be attributed to early panicle development of the Sudangrasses, which led to the transition of most soluble sugars to the panicles thus increasing the insoluble fibre content of their stalks.

5.2.2 Relative Feed Value

RFV is one of the important parameters used in elucidating the forage quality and digestibility of animal feeds. RFV of grasses, legumes and their mixture has been previously categorized (Rohweder et al., 1978). Although Sugar Drip and Rex significantly have the highest RFV compared to the Sudangrasses, MN1054 and GK Aron, they do not fall under the same range as per quality standards of Hay Market Task Force of American Grassland and Forage Council. Likewise, their RFV was below the recommended range of > 151 (Horrocks & Vallentine, 1999). Our results are in accord with a previous study by Jahansouz and others (2014), in which the sorghum cultivars studied had a low RFV below the acceptable range and this was attributed to deficit irrigation. We suggest that a similar effect could have happened in our study. In addition, other factors such as planting date could have affected the RFV of the sorghum varieties in this study. Sorghum is a short-day plant that quickly flowers during short days hence resulting into low vegetative growth and low plant biomass (Wolabu & Tadege, 2016). In this study, sorghum was planted in early July during a period in which days were getting shorter. This led to early anthesis (**Table 4.1**) thus transitioning most of the nutrients for panicle development and grain filling. Overall, the RFV increased with advancing plant maturity.

5.2.3 Crude protein

Crude protein content (CP) of whole plant was assessed in all sorghum cultivars in this study (**Table 4.3 and Table 4.5**). It was noted that the CP of all cultivars did not exceed 5.50% thus below the recommended range (7%) for animal feeds (Milford & Minson,

1966). This could be attributed to several factors such as climatic conditions, planting date, agronomical practices and cutting time. However, Ramada significantly had the highest CP (4.27%) compared to other cultivars and this was probably due to its larger leaves and high leaf number. Leaves contain higher CP than stalks (Atis et al., 2012).

5.2.4 *In vitro* digestible organic matter and microbial protein

A correlation between the *in vitro* digestible organic matter (INDOM) and microbial protein (Mp) was noted. Sugar Drip and Rex had the highest INDOM and this correlated with their high Mp (**Table 4.4**). DOM provides the necessary energy required for the synthesis of microbial proteins that support the growth and survival of rumen microbes (Andrade-Montemayor et al., 2009). Indeed, amino acids obtained from Mp are absorbed by ruminal microbes to carry out their metabolic functions thus enhancing animal performance with regard to milk production of lactating dairy cows (Clark et al., 1992). Therefore, Sugar Drip and Rex would better influence the activities of the microbial community in the rumen than would the black-seeded Sudangrass due to its low DOM and Mp.

5.2.5 Gas production

Gas production of methane, carbon dioxide and Short chain fatty acids (SCFA) such as propionate, acetate and butyrate are the end products of carbohydrate fermentation in the rumen by microbes. The amount of gas produced (Gp) depends on the composition of SCFA (Calabro et al., 2001) and on the content of water-soluble carbohydrates (WSC)

(Neto et al., 2017). Data from our study indicated that Sugar Drip, Rex and Ramada significantly had the highest SCFA and thus their high Gp at 90 DAS (**Table 4.4)** compared to other cultivars**.** Results of this study are in accord to those obtained by Neto and others (2017), in which sweet sorghum varieties BRS 506 and CMSXS 647 had a higher Gp than the grain sorghum varieties and this was attributed to their high-water soluble carbohydrates (WSC). In our study however, a grain sorghum cultivar, Sohag, exhibited a high gas production of 31.99 ml/200 mg DM (**Table 4.6**) which was in the same range as that of Sugar Drip (42.70 ml/200 mg DM), Rex (36.08 ml/200 mg DM) and Ramada (30.70 mg/200 mg DM) (**Table 4.4**). Therefore, its quality could produce a similar outcome as the three sweet sorghum cultivars with regard to dry matter intake and improved animal performance.

6.0 Conclusion

Sugar Drip, Rex and Ramada have the highest forage quality followed by whited-seeded Sudangrass, GK Aron, MN1054 and black-seeded Sudangrass. For the Sudangrasses, the most suitable cutting date was 75 DAS since their forage quality and agro-morphological traits declined by 90 DAS. For sweet sorghum cultivars, the most suitable cutting date was 90 DAS except for MN1054. Its forage quality declined with advancing plant maturity. For grain sorghum cultivars, their forage quality at grain maturity is comparable except for Sohag and LG35 cultivars. At the molecular level, no correlation was noted between COMT SNPs and forage quality.

6.1 Future perspectives

Another field experiment is required under different conditions.

Manipulation of COMT through genetic engineering will have an effect on lignin content and composition, which we hope, will further improve on the forage quality of cultivars in our study. Furthermore, there is need to improve on other forage quality parameters of the cultivars either through genetic engineering or traditional plant breeding. This will increase on the palatability of the forages hence improving animal performance in regard to dry matter intake and milk production.

There is need of confirming the obtained results of in vitro digestibility by performing in vivo feeding trials.

It is also important to elucidate the suitability of these cultivars for biofuel production through conducting further studies such as acid pre-treatments, fermentation and subsequent determination of ethanol yields.

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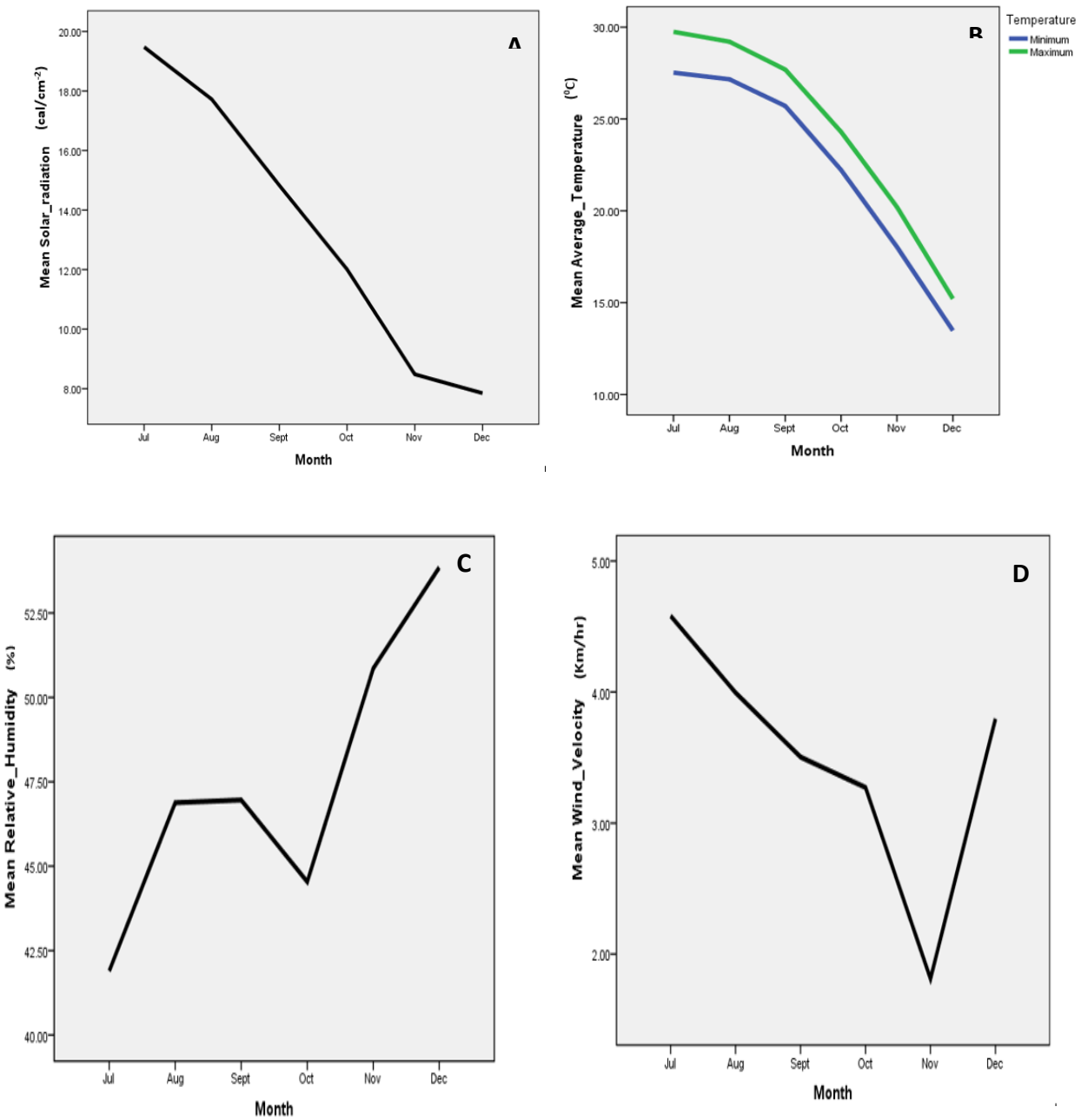
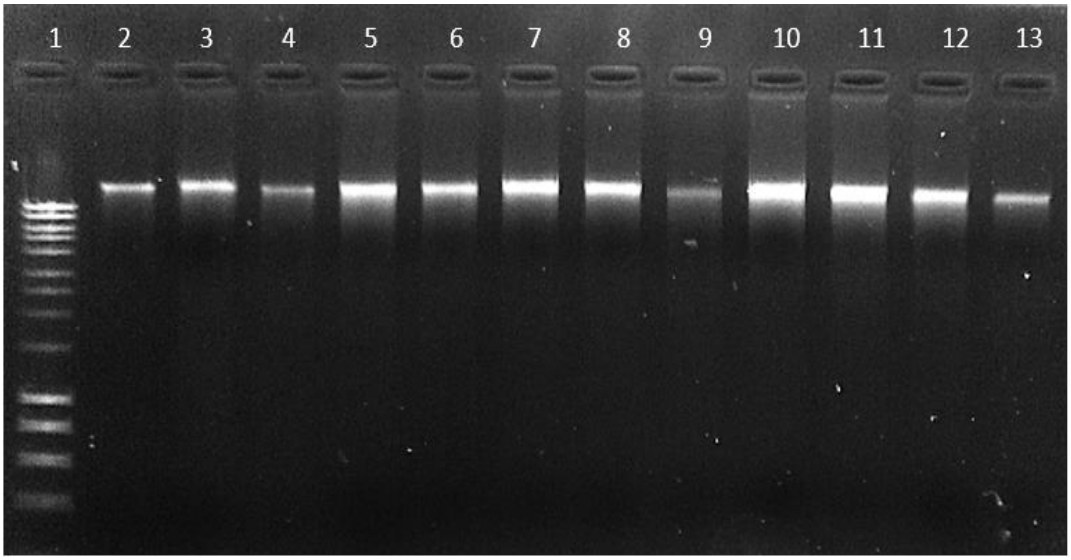
Appendix

Average solar radiation, minimum and maximum temperature, relative humidity and wind velocity recorded during the field experiment at CARES.



Supplementary Fig. S1: Image of; **A**: mean solar radiation; **B**: average temperature; **C**: mean relative humidity; **D**: mean wind velocity recorded during the filed study at CARES.



Supplementary Fig. S2: Image of 1% Agarose gel DNA samples for the different sorghum varieties. **1**: Thermo Scientific ™ GeneRuler™ 1kb DNA Ladder; **2**: Dwarf; **3**: black-seeded Sudangrass; **4**: LG 35; **5**: Sohag; **6**: TX430; **7**: Mn 1054; **8**: white-seeded Sudangrass; **9**: GK Aron; **10**: Sugar Drip; **11**: Rex; **12**: Ramada; **13**: Sohag104 respectively.

Supplementary Table S1: Two-way ANOVA on the effect of plant age at cutting (DAS) and variety on plant heights of the Sudan grasses

Source	df	F	Sig.
Plant age at cutting	2	18.878	.000
Variety	1	9.207	.006
Plant age at cutting X Variety	2	3.846	.036
Error	24		

Supplementary Table S2: t-test for plant heights of sweet sorghum cultivars (Mn1054 and Sugar Drip)

	Levene's test for equality of variances				
	F	Sig.	t	df	Sig.(2-tailed)
Plant heights					
Equal variances assumed	3.673	.071	.842	18	.411
Equal variances not assumed			.842	16.611	.412

Supplementary Table S3: Two-way ANOVA for the effect of plant age at cutting (DAS) and variety on leaf number for the Sudan grasses

Source	df	F	Sig.
Plant age at cutting	2	5.297	.012
Variety	1	7.121	.013
Plant age at cutting X Variety	2	.857	.437
Error	24		

Supplementary Table S4: t-test for leaf number of sweet sorghum cultivars (Mn1054 and Sugar Drip)

	Levene's test for equality of variances				
	F	Sig.	t	df	Sig.(2-tailed)
Leaf number					
Equal variances assumed	2.123	.162	1.445	18	.166
Equal variances not assumed			1.445	13.182	.172

Supplementary Table S5: Two-way ANOVA for the effect of plant age at cutting (DAS) and variety on leaf widths for the Sudan grasses

Source	df	F	Sig.
Plant age at cutting	2	9.999	.001
Variety	1	8.445	.008
Plant age at cutting X Variety	2	5.452	.011
Error	24		

Supplementary Table S6: t-test for leaf width of sweet sorghum cultivars (Mn1054 and Sugar Drip)

	Levene's test for equality of variances				
	F	Sig.	t	df	Sig.(2-tailed)
Leaf width					
Equal variances assumed	.294	.594	.785	18	.442
Equal variances not assumed			.785	18.000	.442

Supplementary Table S7: Two-way ANOVA for the effect of plant age at cutting (DAS) and variety on stalk diameter for the Sudan grasses

Source	df	F	Sig.
Plant age at cutting	2	27.763	.000
Variety	1	18.576	.000
Plant age at cutting X Variety	2	10.340	.001
Error	24		

Supplementary Table S8: t-test for stalk diameter of sweet sorghum cultivars (Mn1054 and Sugar Drip)

	Levene's test for equality of variances				
	F	Sig.	t	df	Sig.(2-tailed)
Stalk diameter					
Equal variances assumed	.148	.705	1.562	18	.136
Equal variances not assumed			1.562	17.881	.136

Supplementary Table S9: Two-way ANOVA on the effect of plant age at cutting (DAS) and variety on total fresh weight of the Sudan grasses

Source	df	F	Sig.
Plant age at cutting	2	10.559	.001
Variety	1	13.035	.001
Plant age at cutting X Variety	2	1.930	.167
Error	24		

Supplementary Table S10: T-test for total fresh weight of sweet sorghum cultivars (Mn1054 and Sugar Drip)

	Levene's test for equality of variances				
	F	Sig.	t	df	Sig.(2-tailed)
Total fresh weight					
Equal variances assumed	2.022	.172	1.129	18	.274
Equal variances not assumed			1.129	15.486	.276

Supplementary Table S11: Two-way ANOVA on the effect plant age at cutting (DAS) and variety on total dry weight of the Sudan grasses

Source	df	F	Sig.
Plant age at cutting	2	25.291	.000
Variety	1	8.990	.006
Plant age at cutting X Variety	2	2.273	.125
Error	24		

Supplementary Table S12: T-test for total dry weight of sweet sorghum cultivars (Mn1054 and Sugar Drip)

	Levene's test for equality of variances				
	F	Sig.	t	df	Sig.(2-tailed)
Total dry weight					
Equal variances assumed	2.319	.145	-.965	18	.347
Equal variances not assumed			-.965	15.529	.349

Supplementary Table S13: One-way ANOVA for the plant heights of the Sudan grasses and sweet sorghum cultivars at 75 DAS

Plant heights

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11912.694	3	3970.898	5.413	.009
Within Groups	11737.549	16	733.597		
Total	23650.243	19			

Supplementary Table S14: One-way ANOVA for the plant heights of the Sudan grasses and Sweet sorghum varieties at 90 DAS

Plant heights

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20444.287	6	3407.381	4.553	.002
Within Groups	20955.223	28	748.401		
Total	41399.510	34			

Supplementary Table S15: One-way ANOVA for the leaf number of the Sudan grasses and sweet sorghum varieties at 75 DAS

Leaf number

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	75.600	3	25.200	24.585	.000
Within Groups	16.400	16	1.025		
Total	92.000	19			

Supplementary Table S16: One-way ANOVA for the leaf number of the Sudan grasses and sweet sorghum varieties at 90 DAS

Leaf number

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	153.600	6	25.600	4.716	.002
Within Groups	152.000	28	5.429		
Total	305.600	34			

Supplementary Table S17: One-way ANOVA for the leaf widths of the Sudan grasses and sweet sorghum varieties at 75 DAS

Leaf width

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	34.630	3	11.543	76.573	.000
Within Groups	2.412	16	.151		
Total	37.042	19			

Supplementary Table S18: One-way ANOVA for the leaf widths of the Sudan grasses and sweet sorghum varieties at 90 DAS

Leaf width

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	78.119	6	13.020	24.956	.000
Within Groups	14.608	28	.522		
Total	92.727	34			

Supplementary Table S19: One-way ANOVA for the stalk diameters of the Sudan grasses and Sweet sorghum varieties at 75 DAS

Stalk diameter

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	220.339	3	73.446	13.377	.000
Within Groups	87.849	16	5.491		
Total	308.188	19			

Supplementary Table S20: One-way ANOVA for the stalk diameters of the Sudan grasses and sweet sorghum varieties at 90 DAS

Stalk diameter

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	514.828	6	85.805	14.199	.000
Within Groups	169.202	28	6.043		
Total	684.030	34			