EVALUATION OF SELECTED SWEET SORGHUM (*Sorghum bicolor* L. Moench) GENPTYPES FOR INDUSTRIAL ETHANOL PRODUCTION

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EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

DECLARATION

I hereby declare that this is my original work and has not been submitted in part or in whole for an award in any institution.

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RECOMMENDATION

This thesis has been submitted with our approval as supervisors according to Egerton University regulations

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DEDICATION

I dedicate this work to my lovely parents and my siblings for their moral, emotional and financial support.

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ABSTRACT

Agrochemical industries are faced with inadequate supply of molasses that is the main raw material for ethanol production creating a need to develop an alternative feedstock. The use of sweet sorghum will alleviate the problem. Three sweet sorghum (*Sorghum bicolor* L. Moench) genotypes, EUSS10, EUSS11, and EUSS17 were tested against five controls, SS21, SS17, SS14, ACFC003/12 and SS04 to evaluate their ethanol yield potential, stability, and adaptability in Busia, Siaya and Kisumu Counties of Kenya. The genotypes were from ICRISAT. A Randomized Complete Block Design (RCBD) experiment with three replications was carried out at Sinyanya and Masumbi in Siaya County and Mundika in Busia County during the first season and Nyahera and Sagam in Kisumu County and Mundika during the second growing season in 2014. Seeds were planted at the onset of rainy season in each location in plots measuring 2.5 m by 4 m each consisting of four rows of sorghum. Days to 50% heading and plant height was taken in the field. Plant height was recorded for 3 plants per plot and two central rows harvested for juice extraction. Juice was fermented and distilled to obtain ethanol. Juice samples were taken for Brix and sugar quality analysis. All data on agronomic traits and yields were subjected to analysis of variance using SAS version 9.1 and means separated using Least Significant Differences. Adaptability and stability analysis was carried out using Genstat version 15.1. The results showed high variability on all measured attributes among genotypes except juice extractability and bagasse moisture. Genotypes by environment interactions were also high for cane and juice yield. Overall, genotypic correlations showed a linear positive correlation of ethanol yield with plant height, juice volume and cane yield. The genotypes that performed above the environmental mean for cane yield were: EUSS10, ACFC003/12, SS14, SS17 and EUSS11 and juice yield were SS14, EUSS11, EUSS10, and ACFC003/12. For ethanol yield, the best performing genotypes were EUSS10, ACFC003/12, SS14 and SS04. The stable genotype that performed well across environments for cane, juice and ethanol yield was SS14. Therefore, SS14 is recommended for cultivation in all tested environments. EUSS10 and SS14 were adapted to lower midland (LM)1, LM2, and LM3. Genotypes EUS11, ACFC003/12, and EUSS17 are suitable for cultivation in LM1 and LM3 agro-ecological zones (AEZ). EUSS10 and SS14 would be highly recommended for rainfed conditions at LM1, LM2, and LM3 AEZs.

Keywords: Sweet sorghum, Genotypes, Ethanol

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LIST OF ABBREVIATIONS AND ACRONYMS

AEC	Average environment coordinates
AEZ	Agro-ecological zone
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
ASALs	Arid and semi-arid lands
ASL	Above sea level
ASV	AMMI stability value
DNS	Dinitrosalicyclic acid
E1O	Biofuel made of 10% ethanol and 90% petrol
EPZ	Export processing zone
GEI	Genotype-environment interaction
GGE	Genotype main effect plus genotype x environment interaction
GHG	Greenhouse gases
IPCA	Interaction principal component axis
LM	Lower midland
LSD	Least significant difference
NADH	Nicotinamide dinucleotide
NRS	Non-reducing sugars
PCA	Principal component analysis
RCBD	Randomized complete block design
RS	Reducing sugars
TS	Total sugars
UDP	Uridine diphosphate
ICRISAT	International crops research institute for the semi-arid tropics
KEPHIS	Kenya Plant Health Inspectorate service
KALRO	Kenya Agricultural and Livestock Research Organization

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Sweet sorghum (*Sorghum bicolor* L. Moench) belongs to the kingdom Plantae, family Poaceae and genus Sorghum (Mazumdar *et al*., 2012). It is grown for grain as it is one of the drought resistant crops which can be grown under minimal inputs and can be harvested within a span of four months. The sorghum originated from North-eastern Africa near Egyptian-Sudanese border and the crop spread to other parts of Africa, India, United states, China and several regions around the world. Currently the US is the largest producer of the grain sorghum in the world followed by other countries including India, China, Mexico and Nigeria, Australia and Argentina (Lovis, 2003). Sorghum is staple food grain for millions of people in semi-arid areas of the world especially in Africa and India (Shiferaw *et al*., 2013). Therefore, sorghum has enabled utilization of low rain-fed areas where maize and sugarcane are not viable for food production. The introduction of sweet sorghum will further double the benefits as the grain can be used for food whereas stem is sold to distilleries for ethanol production.

Energy crisis as reflected by rising price of fossil fuels is a major challenge faced by several countries (Basavaraj *et al*., 2013). Besides, global climate change and the emission of greenhouse gases resulted from increased consumption of fossil fuels (Li *et al*., 2013). Use of ethanol as biofuel in vehicles have environmental benefits as it reduces vehicular pollution and greenhouse gas emissions (Subramanian *et al*., 2005; Cao *et al*., 2014). Biofuels are an alternative source of renewable energy derived from plant biomass and organic wastes. The potential plant biomass for production of biofuels is sugarcane, maize, sorghum, wheat, sugarbeet, cassava, barley and sweet potatoes (Donghai and Xiaorong, 2010; Manea *et al*., 2010; Souza *et al*., 2013). Among the main energy crops, sweet sorghum stands out as a very promising cheap and renewable feedstock, resulting in many studies by different researchers worldwide (Agung *et al*., 2013; Souza *et al*., 2013) as it produces sugars which can be converted to alcohol that can be used as energy source (Rao *et al*., 2012).

The market for renewable energy is growing due to increased desire worldwide to reduce reliance on conventional energy and reduce carbon emissions. Bio-energy covers a range of technologies including electricity from waste combustion, biomass electricity and transportation energy with its feedstock including energy crops, waste wood, woodchip, manure, agricultural waste and sewage sludge (Grandview research, 2014). Biomass fuels

especially wood fuel is the largest primary energy in Kenya accounting for over 68% of total primary energy consumption particularly for rural households and cottage industries (Muchiri, 2008).

Modern bioenergy technology such as ethanol blended with petrol for transportation was tried in the 1980s but was discontinued after world oil prices declined, however, Ministry of Energy in Kenya pledged to introduce ethanol blending Kenya in 2012. The initiative, however, has never materialized due to inadequate raw material for ethanol production. Kenya launched new policy through National Sugar Board to enhance bio-energy sector by requiring new sugar mills seeking operating licences to include ethanol and electricity production in their operations (Muok *et al*., 2008; Karekezi and Kimani, 2010). Therefore, the development of liquid biofuels such as ethanol and biodiesel will significantly lower oil imports. The introduction of alternative energy crops such as sweet sorghum to supplement molasses for ethanol production will significantly raise total ethanol production enabling a revival of bioenergy sector in the country.

Ethanol producing industries in Kenya experienced a shortage and unreliability of molasses that is a primary feedstock due to decline in sugarcane harvest and set up of more distilleries by sugar millers (Spectre International, 2015). This necessitates the development of alternative and supplementary feedstock for which sorghum is a potential target crop. The use of sweet sorghum as raw material for ethanol production will result in improvement of livelihoods and poverty alleviation through economic empowerment. It will also reduce reliance on petroleum imports when internal supply of ethanol is adequate to be used as bio-fuel. *Sorghum bicolor* possesses a significant amount of genetic diversity for traits of agronomic importance (Ritter *et al*., 2007). Knowledge of genetic diversity is of great importance (Warburton *et al*., 2008) and is a key component in crop improvement and plant breeding. The diversity of selected eight sorghum genotypes is not well understood and comprehensive knowledge of diversity and genetic relationship among varieties will aid in crop improvement strategies.

The raw materials used in the production of ethanol via fermentation are classified into three main types which are sugars, starches, and cellulose materials. Sugars from sugarcane, sugar beets, sweet sorghum, molasses, and fruits can be converted into ethanol directly. Starches from corn, cassava, potatoes, and root crops are first hydrolyzed to fermentable sugars by the action of thermally stable α-amylase and amyloglucosidase or raw granule hydrolyzing α-amylase enzymes from the malt. Cellulose from wood, agricultural residues, waste liquor from pulp, and paper mill are converted into sugars, by the action of

mineral acids (Manea *et al*., 2010). Dilute sulphuric acid pre-treatment at 140 ℃ for 30 minutes is effective in removing most of the hemicelluloses, pectin, and proteins from sorghum biomasses for fermentation (Donghai and Xiaorong, 2010). Lack of knowledge of inherent genotypic characteristics of potential sweet sorghum in different environmental conditions is a major hindrance in the production of sweet sorghum. The present study aimed at identifying best cultivars across different agro-ecological zones that can be used for bio-ethanol production.

1.2 Statement of the problem

The main feedstock used by bio-energy industries in Kenya for bio-ethanol production is molasses, a by-product from sugarcane processing. Bio-energy companies are faced with an inadequate supply of molasses due to competing demand by livestock sector and sugar companies that are now converting it to ethanol. Therefore, there is a need for alternative raw material for bioenergy industries to augment molasses. Sweet sorghum stem juice can easily be fermented just like molasses; however, there has to be a suitable sweet sorghum variety with a constant supply of cane throughout the year. Sweet sorghum can be grown for ethanol production. However, sweet sorghum genotypes vary in cane stalk yield, juice volume and ethanol yield with each genotype responding differently to changes in climatic factors across different environments. Unfortunately, the production and stability of these genotypes in the different agro-ecological zone of the country is not known. Therefore, there was a need to evaluate the performance of genotypes across locations to recommend best genotypes for adoption by farmers.

1.3 Objectives

1.3.1 General objective

The general objective of this study was to evaluate the potential of selected sweet sorghum genotypes for industrial ethanol production and contribute to its increased production for industrial use.

1.3.2 Specific objectives

1. To determine cane yield, juice volume and ethanol yield of selected sweet sorghum genotypes across selected agro-ecological zones.
2. To determine interaction between genotype and environment on stalk yield, juice volume and ethanol yield.

3. To determine amount of fermentable sugars in sweet sorghum juice extract.

1.4 Hypotheses

1. There is no difference in cane yield, juice volume and ethanol yield of selected sweet sorghum genotypes across selected agro-ecological zones.
2. There is no significant interaction between genotype and environment on stalk yield, juice quality and ethanol yield.
3. There is no difference in amount of fermentable sugars in sweet sorghum juice extract among genotypes.

1.5 Justification for the study

Several studies have shown that sweet sorghum has great potential as a feedstock for bio-ethanol production yet there are no known sweet sorghum genotypes recommended for this purpose in Kenya. Different environments have biotic and abiotic factors that affect the performance of sorghum genotypes differently resulting in varied yields. Therefore, identification of sorghum cultivars with superior qualities for ethanol yields will help alleviate the problem. The use of sorghum by bio-energy industries will commercialize the crop and its cultivation will ensure a continuous supply of feedstock. Alternative raw material for ethanol production will result in increased volume and stabilised market prices. Furthermore, Sweet sorghum will have higher commercial value, which will translate into increased income and thus improved livelihood for households growing this crop. The main consumers of ethanol are alcoholic beverages and chemical industries that make industrial products such as paints, solvents, perfume, and disinfectants in medicine. Bio-ethanol used as a replacement or blended with the gasoline can reduce petroleum imports and offer a solution to energy supply by meeting growing demand for energy for transportation. Furthermore, bio-ethanol is a biodegradable environment-friendly energy source. Sweet sorghum has the potential for increasing incomes in Kenya considering the potential economic opportunities accrued when ethanol is exported.

CHAPTER TWO
LITERATURE REVIEW

2.1 Taxonomy of sweet sorghum

Sweet sorghum (*Sorghum bicolor* L. Moench) belongs to genus sorghum in the family of Gramineae (Zhan *et al*., 2012). In 1974, Moench established genus sorghum and brought all the sorghum together under the name *Sorghum bicolor* (Teshome *et al*., 1997). *Sorghum bicolor* is further broken down into three subspecies: *Sorghum bicolor bicolor*, *Sorghum bicolor drummondii* and *Sorghum bicolor verticilliflorum*. *S. bicolor bicolor* is represented by agronomic types such as grain sorghum, sweet sorghum, sudangrass and broomcorn (Dahlberg *et al*., 2011). Grain sorghum is mainly used as principal food and raw material for alcoholic beverages. Broomcorn and sweet sorghum are used as raw materials for making broom and sweetener syrup respectively while grass sorghum is grown for green feed and forage use. The subspecies bicolor has been partitioned into five races namely; *bicolor*, *guinea*, *caudatum*, *kafir* and *dura* (Harlan and De Wet, 1972).

2.2 Biology of sorghum

Sorghum has a chromosome number 2n=20, a nuclear DNA content of 1.6 picogram and a genome size of 735 Mbs (Motlhaodi *et al*., 2014). The complete genome of sorghum was made available to public use in 2008 which enable the sorghum geneticist to understand complex traits at the sequence level (Ramu *et al*., 2013). Cultivated sorghum can be divided into three main categories based on end product utilization: grain sorghum for starch, sweet sorghum for sugar, forage and energy sorghum for biomass. Sweet sorghum is one of the many types of cultivated sorghum due to its high sugar content in the stem. Sweet sorghum is a very efficient source of bio-energy compared with sugar cane (*Saccharum officianarum* L.) and corn (*Zea mays* L.) as it uses C4 photosynthetic pathway to produce sucrose which can be directly fermented (Ali *et al*., 2008). Sweet sorghum is characterized by low grain yields, but high biomass production. It is tall and contains juicy stalks with 10-25% sugars (Wang *et al*., 2009). Though categorized, there are virtually no biological or taxonomic boundaries among these cultivated forms and they all belong to the same species: *Sorghum bicolor* (Ritter *et al*., 2007). The sweet sorghum can grow taller with ticker stem as shown in figure 2.1.

Figure 2. 1: Sorghum plants in Masumbi, Siaya County

2.3 Kenya agro-ecological conditions and suitability for sorghum production

The climatic requirements for sweet sorghum production are soil temperature of 15 ^{o}C for seed germination, soil with clay percentage of between 10 and 30% and pH between 5.5 and 8.5 and annual rainfall range of 300 to 750 mm (AFF, 2010; Ouma *et al*., 2013). Sorghum being a C4 tropical grass and drought-resistant is adapted to the latitudes ranging from 40°N to 40°S of the equator and can be cultivated in the rain-fed areas of semi-arid tropics. The crop can be grown successfully on clay, clay loam or sandy loam soils which can tolerate salinity and alkalinity to a large extent and is considered a natural replacement for less water-efficient crops (Nahar, 2011; Basavaraj *et al*., 2013).

About 83% of Kenya's land surface is classified as arid and semi-arid lands (ASALs) characterized by low rainfall (100-900 mm per annum) which is not suitable for sustainable rain-fed agriculture (Njeru *et al*., 2013). These ASALs could, therefore, be utilized for cultivation of drought-resistant crops like sorghum to meet demand for brewing industries that has recently risen in East Africa (Ringo *et al*., 2015). In Kenya, sorghum is grown principally in marginal areas of formerly Eastern, Nyanza and Coast provinces (Muui *et al*.,

2013) characterized by drought, water logging, saline-alkaline, infertile soils and high temperatures (E. P. Z., 2005). Nyanza province is the leading producer of sorghum in Kenya, estimated to produce 52% of country's total production followed by western at 23%. Sorghum production in Kenya has steadily increased from 150,127 tonnes in the year 2005 to 159,877 tonnes in 2011 (Kilambya *et al*., 2013). The production is expected to rise even higher as new sorghum cultivars and hybrids are released. Sorghum grain is utilized in preparing food like *ugali*, porridge, and alcoholic beverages whereas stalks are used as fodders and fencing material (Okuthe *et al*., 2013). There is the need to improve overall income from sorghum through improving productivity and increasing value by locating and exploiting alternative uses such as bio-ethanol production.

2.4 Sweet sorghum production in Kenya

The study conducted in western and coastal regions of Kenya showed that sweet sorghum is the most suitable bio-ethanol feedstock at 185,822 km^2 of the country surface area followed by cassava and sugarcane at 66,092 km^2 and 12,591 km^2 respectively (Olweny *et al*., 2013). Their study showed that production of bio-ethanol from sweet sorghum is the most profitable with a gross margin of over sixty-seven thousand Kenya shillings per hectare. Technology for producing ethanol using sweet sorghum already exist in the country (Ndegwa *et al*., 2011) therefore, sweet sorghum should be exploited to supplement use of molasses for ethanol production. Sweet sorghum and sugarcane are similar in gross structure and chemical composition and cost component of their use for bio-ethanol production is significantly reduced by a consistent and reliable year-round supply of feedstock (Kim and Day, 2011). Furthermore, genetic diversity study of sorghum by Kimani *et al*. (2014) found that Kenya is among countries with highest genetic diversity and rare alleles which could be used to improve yields through a breeding programme. Food security in Kenya is a major concern that can be addressed by growing sorghum in semi-arid areas where maize performs poorly (Mwadalu and Mwangi, 2013).

2.5 Significance of the sweet sorghum crop

Investigations have demonstrated that the concentrate juice of sweet sorghum is a valuable food product which could be used in confectionery, bakery, milk and canned food processing, for making alcohol, citric acid, alcoholic and non-alcoholic beverages and for bee feeding. The sweet sorghum juice can be used for drinks, made of fruits and jellies, without diminishing their organoleptic and physical-chemical characteristics (Elena, 2007). Sweet

sorghum can be used for the production of paper or electricity through combustion of total biomass and stillage from sweet sorghum has a higher biological value than bagasse from sugarcane when used as fodder for animals as it is rich in micronutrients and minerals (Cao *et al*., 2014).

Use of sweet sorghum overcomes many of the shortcomings of other energy crops because only stalks are used to produce ethanol while the grain is saved for food or livestock feed. The juice from its stalk can be used for making syrup, molasses or ethanol. The bagasse and green foliage are excellent fodder for the animal; moreover, it can be used as an organic fertilizer (Braconnier *et al*., 2011; Nahar, 2011).

2.6 Attributes of sweet sorghum important for ethanol production

Sugar content and profile in sweet sorghum juice of different cultivars can be different (Prasad *et al*., 2007; Imam and Capareda, 2011). Fermentable sugars in sweet sorghum are mainly sucrose, glucose, and fructose (Widianto *et al*., 2010). The mature stems of sweet sorghum contain about 73% moisture and 27% solids, consisting mainly carbohydrates. About 13% of solids consist of sucrose, glucose, and fructose (Ceclan *et al*., 2012).

The advantages of sweet sorghum as sugar crop include short growth period and can be harvested 1 to 3 times a year compared to growth period of sugarcane which last between 8 to 24 months and requires 4.5 to 6.0 t/ha of the cane for sowing. Besides, it is not easy to sow with a machine. Sweet sorghum can easily be sown using a machine with seed quantity of 4.5-7.5 kg/ha; (Massoud and El-razek., 2011; Mazumdar *et al*., 2012; Ratnavathi *et al*., 2012). Sweet sorghum produces five times more fresh stem weight (830 g) and dry stem mass (164 g) than grain sorghum (150 g and 27 g respectively); Sweet sorghum produces a higher volume of juice (366 ml) and higher yield of sugars (42 g) per stem than grain sorghum (70 ml and 4 g respectively) (Wang *et al*., 2012).

2.7 Ethanol production through yeast fermentation

Sweet sorghum has the potential to become a multipurpose feedstock for large-scale ethanol production from stem juice, cellulose from stalks, and starch from grain. Ethanol is a clear, colourless, flammable, oxygenated hydrocarbon with the chemical formula C_2H_5OH (Udhayaraja *et al*., 2012), has melting point of -114 °C, boiling point of 78.4 °C and has a density of 789 g/l at 20 °C (Gnansounou and Dauriat, 2005).

Although many microbes have been used in ethanol production, yeast, *Saccharomyces cerevisiae* is primarily used in industry, using starch and sugars from plants as the starting material for the process. The most common carbon sources utilized by the microbes are agricultural products which can easily be processed to create the simple sugars needed for fermentation (Widianto *et al*., 2010). Ethanol fermentation by *S. cerevisiae* is primarily done via the standard glycolysis pathway. In the process, a single molecule of glucose is oxidized to two molecules of pyruvate. Anaerobic conditions are required so that molecular oxygen is not available for use as an electron acceptor, and instead pyruvate must be used as the terminal electron acceptor. This fermentative process involves the decarboxylation of pyruvate to form CO_2 and acetaldehyde and the subsequent reduction of acetaldehyde to produce ethanol.

Yeast ferments glucose through Embden-Meyerhof pathway to pyruvate which is decarboxylated to acetaldehyde and carbon dioxide, a reaction catalysed by pyruvate decarboxylase. Acetaldehyde is then reduced to ethanol by alcohol dehydrogenase enzyme using NADH. Alcohol fermentation by the bacterium, *Zymomonas mobilis* through Entner-Doudoroff pathway is advantageous over yeast fermentation as it produces a single molecule of ATP thus inhibiting high biomass that could have led to wastage of nutrients, therefore, more carbon is channelled to fermentation products (Muller, 2003). Engineered *Escherichia coli* are used for ethanol production as it has the ability to ferment a wide spectrum of sugars. Conversion of cellulose to ethanol can be achieved by using various organisms such as *Clostridium thermocellum*, *Neurospora crassa* and *Zygosaccharomyces rouxii* (Lin and Tanaka, 2006).

2.8 Uses of ethanol

Absolute and 95% ethanols are good solvents and are used in many industrial products such as paints, perfumes and tinctures. Solutions of ethanol (70-85%) are used as disinfectants in medicine. Bio-ethanol, however, captures the alcoholic beverages market and a small share of the vehicle fuels market. Ethanol intended for non-food uses is made unfit for human consumption by addition of small amounts of toxic or unpleasant substances such as methanol or gasoline (Gnansounou and Dauriat, 2005). Bio-ethanol is a valid and concrete alternative to petrol and can be used pure (E100), or in blending, generally in the proportion of 10% bio-ethanol and 90 % petrol (E10), or 85% bio-ethanol and 15 % petrol (E85) (Ratnavathi *et al*., 2012). Thus to obtain self-sufficiency in terms of ethanol production, it is essential to diversify the cropping pattern and introduce crops like sweet sorghum.

High prices of oil are reported to have negative impacts on the economic balance of oil importing countries especially the poorest ones (Braconnier *et al.*, 2011). Geopolitical tensions in some oil producing regions and the uncertainties surrounding the future availability of fossil fuels have created a strong interest for bio-fuels. Therefore, it is suggested to plant sweet sorghum for biofuel (bio-ethanol) production in hot and dry countries to solve problems such as increasing the octane of gasoline and to reduce greenhouse gases (GHG) and gasoline imports (Almodares and Hadi, 2009). The European Union mandated 10% of all transport fuels to come from biofuels by the year 2020 and the Kenyan government is trying to revive the ethanol-blending programme (Ndegwa *et al*., 2011). The crop residues including bagasse and leaves can be used for thermal and electrical energy production (Ceclan *et al*., 2012).

2.9 Nutrient management to increase yield of sweet sorghum

Crop management and cultivar choice strategies are important to attain higher stalk and juice yield in sweet sorghum. Both cane and juice yield increase with increasing rates of nitrogen application. Feedstock yield in sweet sorghum can be improved by various nitrogen levels, spacing and plant growth regulators (Kumar *et al*., 2008; Wortmann *et al*., 2010). A similar study by Mosali *et al*. (2010) showed a positive response to fertilizer rates. Management in terms of nitrogen input is critical to increasing the green stalk yield which is linearly and positively related to juice yield. Maximum millable cane yield and optimum Brix value in sweet sorghum could be achieved by adopting 120 kg N ha^{-1}, 7.2 plants m^{-2} population (intra-row spacing of 30 cm with 45 cm inter-row spacing) (Djanaguiraman and Ramesh, 2013).

2.10 Genetic diversity

Genetic diversity refers to variety in genes in a given species (Horden *et al*., 1993) whereas species diversity refers to a variety of species within a population (Prescott-Allen and Prescott-Allen, 2013). *Sorghum bicolor* contains both cultivated and wild races possess a significant amount of genetic diversity for traits of agronomic importance (Hart *et al*., 2001). Genotypic differences have been reported for Brix and plant height in a panel of 125 sorghum genotypes (Murray *et al*., 2009), sugar content and juice yield among United States sorghum collections (Makanda *et al*., 2009).

Sweet sorghum has high sugar rich stalks like sugar cane, however, sorghum genotypes vary in terms of field Brix, juice Brix, and juice volume, total soluble sugars,

reducing sugars, juice purity and biomass (Mohammed *et al*., 2011; Ratnavathi *et al*., 2012; Elangovan *et al*., 2014). Effect of cultivar have been reported to be significant on plant height, stem diameter, the number of tiller per plant, stem fresh weight and ethanol yield (Soleyman *et al*., 2013). Variability has also been recorded in sweet sorghum for grain yield from 1.5 to 7.5 t ha^{-1}, ranging from 7.2% to 15% for sucrose, for cane yield from 24 to 120 t ha^{-1} and for biomass yield from 36 to 140 t ha^{-1} (Manea *et al*., 2010). This necessitated evaluation of genotypes to identify ones with outstanding performance for ethanol production.

Similarly, both grain and sweet sorghum lines exhibit phenotypic variations though they originated from same species *Sorghum bicolor* L. The two lines show a difference in their trancriptomes with considerable numbers of variety-specifically or differentially expressed genes (Jiang *et al*., 2013). Using complementary DNA microarray chips, Calvino *et al*. (2008) identified 154 differentially expressed genes between grain and sweet sorghum lines. It is important to identify superior genotypes for ethanol production in terms of percent juice Brix, juice volume, total soluble sugars and biomass.

2.11 Key enzymes involved in sucrose metabolism

In both sweet sorghum and sugarcane, photoassimilates are first used for growth and development during early vegetative stages. Afterwards, when internodes have elongated, stems become storage organs, where most of the accumulated carbon are stored as sucrose. Sweet sorghum (*Sorghum bicolor*) and sugarcane (*Saccharum officinarum*) accumulate a high level of sucrose in their stems (Bihmidine *et al*., 2013). The variation in the pattern of sucrose accumulation is due to the interplay of activities of key enzymes of sucrose metabolism (Zhu *et al*., 1997). The key enzymes of sucrose metabolism include sucrose phosphate synthase, sucrose synthase, sucrose phosphate phosphatase and invertase (Sturm and Tang, 1999). Invertase catalyses irreversible breakdown of sucrose into glucose and fructose whereas sucrose synthase cleaves sucrose to fructose and UDP-glucose in a reversible reaction. Sucrose phosphate synthase and sucrose phosphate phosphatase are jointly responsible for the irreversible synthesis of sucrose from UDP-glucose and fructose-6-phosphate (Lunn and Macrae, 2003). In sugarcane stem, sucrose accumulation has been linked to differential RNA or protein expression or localization of enzymes involved in sucrose metabolism (Schafer *et al*., 2004; Grof *et al*., 2006). Positive linear relationship between sucrose phosphate synthase activity and sucrose accumulation has been reported in sugarcane (Botha and Black, 2000; Grof *et al*., 2007).

There are two types of invertases; cell wall-bound and soluble acid invertase having sucrolytic activities and results in an increase in invert sugars (glucose and fructose) due to the breakdown of sucrose. The maturity-linked increase in activity of cell wall-bound invertase has been reported in sugarcane to have a direct bearing on sucrose levels in the internodes (Lontom *et al*., 2008). There is a decrease in activity of sucrose metabolizing enzymes, particularly acid invertase as the stem elongation nears completion in sweet sorghum (Lingle, 1987). The individual or combined activities of these enzymes can influence sucrose levels in stems of sorghum. Since both sugarcane and sweet sorghum accumulate sucrose in their stems it is expected they use similar gene products in sucrose metabolism. Studies of expression patterns of the key enzymes involved in sucrose metabolism in the stem of sweet sorghum is necessary to explain variation among genotypes in terms of sucrose levels. This aids in selecting sweet sorghum varieties with highest content of sugar in the stem.

Distilleries and sugarcane-based industries are showing increasing interest using sweet sorghum as an alternative to making up for the possible deficit of energy resources (Reddy *et al*., 2005). Successful development of new cultivars for ethanol production depends largely on the availability of source germplasm with desirable traits such as biotic and abiotic tolerance. Furthermore, effective genetic enhancement of traits like Brix, height, and fermentable sugars depends on the level of genetic diversity available in crop species. Therefore, knowledge of the genetic relationship among eighth genotypes is essential for developing appropriate strategies for breeding and germplasm management.

Sugar yield is a quantitative trait affecting ethanol yield, which is as a result of various traits affected by environment contributing together during crop growth. It is, therefore, desirable to study the association between yield and yield attributing traits across agro-ecological zones to facilitate effective selection for simultaneous improvement of one or more yield influencing components. This study can indicate which genotypes are suitable for conservation, agronomic evaluation and for breeding strategies to improve sweet sorghum productivity for ethanol production in the country.

CHAPTER THREE

INFLUENCE OF GENOTYPIC VARIABLES OF SWEET SORGHUM (*Sorghum bicolor* L. Moench) ON CANE, JUICE AND ETHANOL YIELD

Abstract

Sweet sorghum (*Sorghum bicolor* L. Moench) contains fermentable sugars in the stem that can be converted to ethanol. The current study aimed at evaluating the performance of three sweet sorghum genotypes with five checks and contributes towards availing suitable sweet sorghum for industrial ethanol production. Field studies were carried out in Kenya at varied locations in a randomized complete block design with three replications. Sorghum was harvested at dough stage of grain development and evaluated for several stem juice production traits including plant height, cane yield, juice volume, degrees Brix, total, reducing, and non-reducing sugars, and ethanol yield via juice fermentation. Analyses of variance using SAS version 9.1 showed a significant effect of genotype for morphological characters and ethanol yield. Genotype EUSS10 produced the greatest cane (27.4 t/ha) and juice yield (7806.7 l/ha) whereas ACFC003/12 recorded the greatest ethanol yield (423.1 l/ha). At all sites, EUSS10 was taller and late to 50% heading whereas SS04 had higher Brix and total sugar concentration. The greatest grain yield and non-reducing sugar concentration was produced by SS17 and SS21, respectively. Results of this study show that though Brix and total sugars are desirable for ethanol yield, cane yield, and juice volume of sweet sorghum determines the ultimate volume of ethanol produced. Genotypes EUSS10, EUSS11, and EUSS17 performed better in terms of juice, cane, and ethanol yields, therefore, the three varieties have a high potential for bio-ethanol production.

Keywords: Sweet sorghum, Genotypes, Stalk juice, Ethanol yield

3.1 Introduction

Sweet sorghum **(***Sorghum bicolor* L. Moench**)** is an energy crop that produce large quantities of stem juice with readily fermentable sugars that can be converted to ethanol through fermentation. It is a C4 species with high photosynthetic capacity and drought tolerance and therefore, can be cultivated in most temperate and tropical climates (Dalvi *et al*., 2013). Juice composition affects the amount of ethanol produced (Widianto *et al*., 2010) and composition is affected by genotype, environment and crop harvesting time (Almodares and Hadi, 2009). Sweet sorghum fermentable sugars in the juice are comparable to that of

sugarcane and can be fermented directly into ethanol with an efficiency of more than 90% (Wu *et al*., 2010). Sweet sorghum biomass is renewable and can be used for transportation fuel, electricity and chemical production (Ceclan and Pop, 2012). It stands out as the most promising source of raw material for energy and industry among several bio-energy crops (Gosse, 1996). It has rapid growth, higher biomass yield, and wider adaptability than other crops (Pavli *et al*., 2013) and it is a renewable, cheap and widely available resource (Thanapimmetha *et al*., 2011).

It is projected that world energy demands will continue to expand by 45% between 2008 to 2030, forcing countries to develop alternative fuel sources such as the use of gasoline blended with ethanol for automobile fuel as in India and Brazil (Ratnavathi *et al*., 2012). Bio-ethanol fuels produced from agricultural raw materials are considered clean fuels for automobiles and are an alternative to fossil fuels (Imam and Capareda, 2012). Favourable traits of sorghum bioethanol are: less sulphur content in ethanol, a high octane rating and automobile friendly as up to 25% of the ethanol-petrol mixture can be used without engine modification (Rao *et al*., 2010). Sweet sorghum fulfils requirements for energy crop proposed by Matsuoka *et al*. (2014) including being a perennial plant, have well-developed agronomic practise, the feedstock is easily and reliably transformed into useful forms of energy and has a favourable cost of production and delivery. Sweet sorghum accumulates more sugars in their stems than other sorghum types as it matures. It consists approximately 75% cane, 10% leaves, 5% grain and 10% roots when mature (Grassi *et al*., 2002).

Sweet sorghum cane juice is mainly comprised of three fermentable sugars; sucrose (70%), glucose (20%) and fructose (10%) which vary depending on variety and environment (Prasad *et al*., 2007). A high sucrose level at maturity is attributed to low activity of soluble acid invertase and high activity of sucrose synthase in the stem (Tarpley *et al*., 1994). After flowering, the sucrose content increases while invert sugar decreases (Almodares *et al*., 2010). Total sugars comprise reducing sugars and non-reducing contained in the stem juice. The total reducing sugars in sweet sorghum is the sum of glucose and fructose contained in stem juice and is used as one of the quality parameters by sugar and ethanol industries (Parrella *et al*., 2016). Sugars have significant bearing on ethanol yield therefore high sugar yielding genotypes need to be selected (Prasad *et al*., 2013).

To obtain maximum ethanol yield, sweet sorghum genotypes could be selected for height, Brix, total sugars, non-reducing sugars, reducing sugars, biomass, cane yield, and juice yield as these characters have a positive relationship with ethanol yield (Rani and Umakanth, 2012). Under favourable conditions, sweet sorghum can produce 7682 litres of

ethanol per hectare (Murray *et al*., 2009). In central Greece, the cultivar 'Keller' produced high dry biomass and ethanol yield ranging 21.0-33.6 Mg/ha and 5120-8390 l/ha, respectively (Sakellariou-Makrantonaki *et al*., 2007). However, in Kenya sweet sorghum has not received much attention and it has not been cultivated commercially on a large scale. The objective of this study was to identify superior sweet sorghum cultivars for ethanol production by evaluating their productivity in different regions of Kenya.

3.2 Materials and methods

3.2.1 Site description

Sweet sorghum field experiments were conducted in Kisumu, Siaya and Busia Counties of Kenya (Fig. 3.1). The specific sites were Sinyanya (00^{o} 06' 68.5'' S; 034^{o} 08' 66.0'' E, at 1168 m ASL), Masumbi (00^{o} 01' 73.0'' N; 034^{o} 21' 87.4'' E, at 1370 m ASL) both in Siaya county, Mundika (00^{o} 24' 56.6'' S; 034^{o} 07' 93.1'' E, at 1222 m ASL) in Busia, Nyahera (00^{o} 0.02' 52.78'' S, 034^{o} 39' 03.59'' E, at 1387 m ASL) and Sagam (00^{o} 03' 20.86'' N, 034^{o} 32' 31.06'' E, at 1216 m ASL) both in Kisumu County.

3.2.2 Experimental design

The control genotypes SS04, SS21, SS17and SS14 was sourced from Kenya seed company, a commercial seed merchant while ACFC003/12 came from Kenya Agricultural and Livestock Research Organization (KALRO). The controls were at advanced stage of sweet sorghum variety release by Kenya Plant Health Inspectorate service (KEPHIS) and fitted as standard checks. An initial study was conducted using 25 sorghum lines from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) to determine their potential for ethanol yield. The three best-performing genotypes EUSS10, EUSS11 and EUSS17 were selected for evaluation against the five controls in different environments.

Eight sweet sorghum genotypes were grown in a randomized complete block design (RCBD). The genotypes were: EUSS10, EUSS11, and EUSS17 as candidates with the controls being ACFC003/21, SS04, SS14, SS21, and SS17. Seed sowing was done at the onset of the rains at a seed rate of 8 kg/ha. Sowing took place on 18th March in Sinyanya and Masumbi and 19th March 2014 in Mundika during the first season. Sowing in the second season was done in September 2014 for both Mundika and Sagam while Nyahera was planted on 24th September 2014. Genotypes were sown in 0.60 m rows in plots measuring 4×2.5 m in a randomized complete block design with three replications. Each plot consisted of four rows of sorghum and the blocks were separated by a 1.5 m alley. Triple superphosphate fertilizer

was applied uniformly to all plots at a rate of 17.2 kg/ha at planting. Weeds were controlled manually using hoes three weeks after seedling emergence and sorghum was thinned to a spacing of 0.10 m within-row and top dressed with calcium ammonium nitrate (25% N) at the rate of 20 kg N/ha. Birds guarding was initiated as soon as seeds formed in the panicles to prevent damage to sweet sorghum grains.

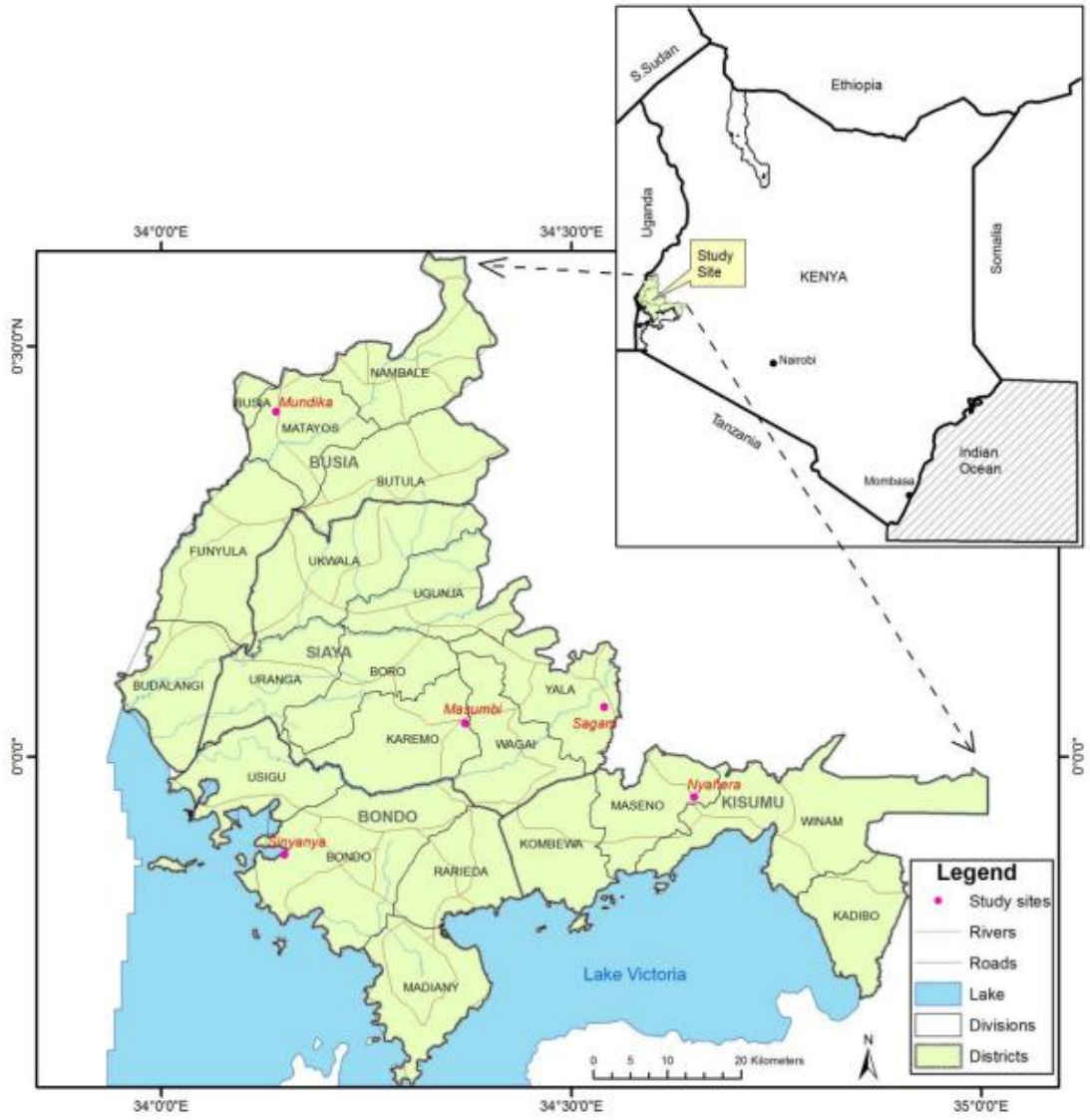

Figure 3. 1: Map of Study area (Source: Kenya survey)

3.2.3 Field Data Collection

Emergence was observed in all plots two weeks after planting and stand counts were conducted at 3-4 leaf stage or later. Days to 50% heading was determined by calculating the number of days from sowing to when 50% of the sorghum heads in each plot had produced

grains. Sorghum genotypes were monitored until they attained the hard dough stage at which, plant height was recorded and crops harvested. Three randomly selected plants from each cultivar in all replicates were used for recording plant height. Plant height was measured from base of stem to tip of panicle and data averaged across three plants.

Harvesting was done approximately 16 weeks after sowing in the three sites: Masumbi, Mundika and Sinyanya for the first season, and approximately 14 weeks at Sagam and Mundika and 13 weeks at Nyahera for the second season. Plants from the middle two rows of each plot were cut to a stubble height of 0.05 m, leaves were stripped off by hand, and panicles removed using secateurs. Panicles were sun dried, threshed and winnowed manually. The grain was weighed and yields in tonnes/ha was calculated. The fresh weight of harvested stalks was determined and stalks were then transported to the laboratory for juice extraction. Juice was extracted with a one roller crusher (FuanLiyuan, China, type YC 80B-4) and strained through a sieve into a juice container. The volume of juice was recorded and degrees Brix (%) was measured with a hand refractometer (RHB0-90ATC, Fujian, China). After juice extraction, wet bagasse weight was recorded immediately. The bagasse moisture content was determined through modified method of Anwar (2010) where the wet bagasse was kept in microwave at 65 ^{o}C for three days to get constant dry weight. Juice extractability and bagasse moisture was calculated as follows:

$$Juice\ extractability\ (\%) = \frac{weight\ of\ juice\ (kg)}{fresh\ cane\ weight\ (kg)} \times 100 \quad \text{..... 3.1}$$

$$Bagasse\ moisture\ (\%) = \frac{wet\ bagasse\ wt.(kg) - dry\ bagasse\ wt.(kg)}{dry\ bagasse\ wt.(kg)} \times 100 \quad \text{..... 3.2}$$

Figure 3.2: Mature sweet sorghum plants in the field, Masumbi, Siaya county (a) and harvested stalks (b)

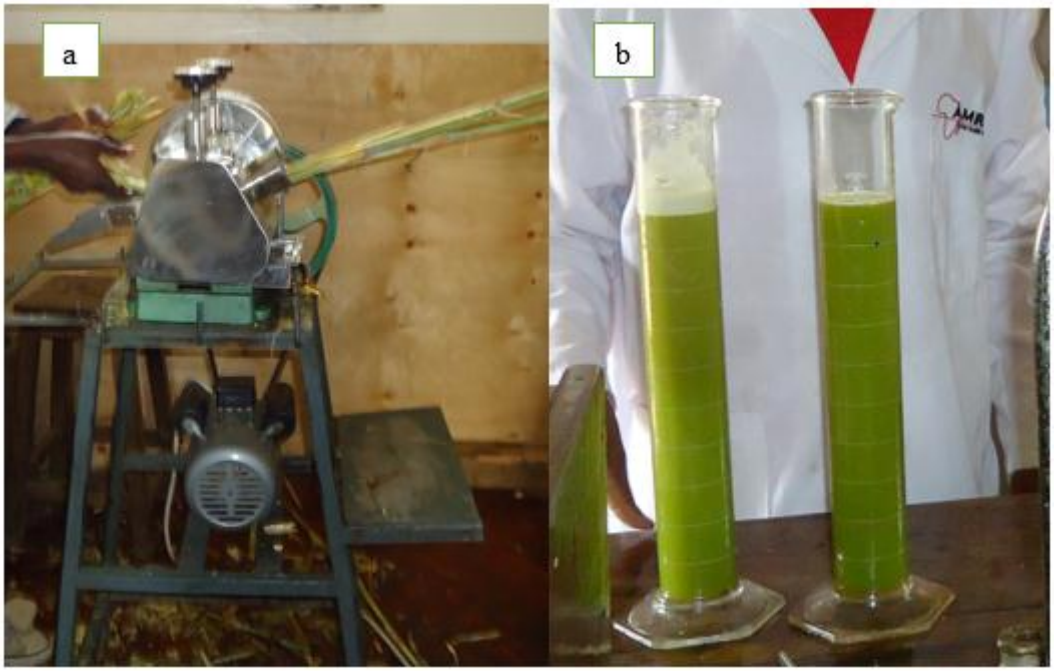

Figure 3.3: Juice extraction (a) and stem juice being measured (b)

Figure 3. 4: Juice fermentation and, b) ethanol obtained after distillation

3.2.4 Ethanol analysis

A 100 ml aliquot of extracted juice from each plot was fermented at 35 ^{o}C for four days using *Saccharomyces cerevisiae* (1.5%) and then distilled to obtain ethanol. The active

dry brewer's yeast (Angel Yeast Co., Ltd., china) was added directly to sample juice bottles, mixed then sealed and left to ferment. The fermented juice was transferred to rotary evaporator (Buchi Rotavapor R-205) and run for 30 minutes at 78 ^{0}C. Ethanol yield was determined by volume. A refractometer (RFM 3330, Bellinghant Stanley limited) was used to determine the concentration of ethanol in the distillate. The refractive index of distillate was compared with a standard curve created from absolute ethanol diluted with distilled water to create concentrations of 0, 5, 10, 15, 20, 25 and 30% ethanol. Ethanol was then expressed as mL of ethanol per litre of fermenting sweet sorghum juice. Ethanol yield (l/ha) was estimated from juice yield per hectare of each genotype as follows:

$$Ethanol\ yield\ (l/ha) = \frac{L\ ethanol}{L\ juice} \times litres\ of\ juice\ per\ hectare \quad \text{..... 3.3}$$

3.3 Data Analysis

Data on cane yield, juice volume, Brix, percent juice extractability, plant height, days to 50% heading, grain yield, and ethanol yield were subjected to analysis of variance (ANOVA) and treatment means were compared using Least Significant Difference (LSD) at a significance level of P<0.05. ANOVA was conducted with SAS software version 9.1 with genotype and environment as fixed effects and replication as random. Data as presented for the genotypes was pooled across locations. The model for the ANOVA is represented in equation (4) below.

$$Y_{ijk} = \mu + E_i + G_j + GE_{ij} + B_k + E_{ijk} \quad \text{..... 3.4}$$

Where; $Y_{ijk,}$ observation of j^{th} genotype in i^{th} environment and in k^{th} block; μ, overall mean; E_i, i^{th} environment effect (i = 1, 2, 3, 4 and 5); $G_{j:}$ j^{th} genotype effect (j =1, 2, 3…, 8); GE, interaction effect of j^{th} genotype in i^{th} environment; B_{kj}, effect of k^{th} block in i^{th} environment; E_{ijk}, random error component.

3.4 Results and discussion

3.4.1 Days to 50% heading, plant height and green cane yield

Days to 50% heading, plant height, and cane yield varied by genotype. The time difference between early and late maturing was about 2 weeks. Genotype SS21 was early maturing while EUSS10 was late to mature taking 67 and 82 days, respectively to reach 50% heading (Table 3.1). Genotype EUSS10 took a similar number of days to reach 50% heading

with the control SS14, both taking about 4 more days than SS04, EUSS11, EUSS17, and ACFC003/12. EUSS11 and EUSS17 took 3 and 10 more days than the controls SS17 and SS21, respectively to reach 50% heading. These results are in agreement with findings of Shivani and Sreelakshmi (2014) where days to 50% flowering were found to range from 51 to 79 days.

Table 3.1: Days to 50% heading, plant height and cane yield among eight sweet sorghum genotypes (n=18)

Genotype	Days to 50 % heading	Plant height (m)	Cane yield (t/ha)
SS04	76.4 ± 5.2^{bc}	1.80 ± 0.41^{b}	21.1 ± 9.7^{b}
SS14	81.5 ± 6.1^{a}	1.78 ± 0.39^{b}	22.0 ± 7.8^{b}
SS21	67.4 ± 5.7^{d}	1.53 ± 0.41^{c}	16.1 ± 8.4^{c}
SS17	74.6 ± 5.6^{c}	1.78 ± 0.40^{b}	20.1 ± 9.0^{bc}
EUSS17	77.3 ± 3.5^{b}	1.78 ± 0.38^{b}	20.2 ± 9.8^{bc}
EUSS10	82.1 ± 6.0^{a}	1.95 ± 0.47^{a}	27.4 ± 14.9^{a}
EUSS11	77.8 ± 3.8^{b}	1.70 ± 0.43^{b}	23.5 ± 9.2^{ab}
ACFC003/12	75.8 ± 6.8^{bc}	1.78 ± 0.48^{b}	23.5 ± 9.3^{ab}
$LSD_{0.05}$	2.7	1.2	4.85

Means are presented as mean ± standard deviation

Means followed by the same letter in a column are not significantly different at 5% LSD

Genotype EUSS11 and EUSS17 plant height was similar to that of the controls, but approximately 0.17 m greater than that of SS21. EUSS10 plant height was greater than all other genotypes and 0.42 m taller than SS21. Moreover, EUSS10 plant height was about 0.15 m taller than SS04, SS14, SS17, and ACFC003/12. In terms of cane yield, EUSS10, EUSS11 and Control ACFC003/12 produced about 18.7% more cane biomass than SS21. Genotype EUSS17 cane yield was similar to other controls and produced 10.9% more cane biomass than SS21. Cane yield is known to be significantly positively correlated with stem diameter and plant height (Audilakshmi *et al*., 2010) and was so with plant height in this study. Plant height and cane yield have been reported to significantly differ among sorghum cultivars ranging from 191 to 268 cm and from 54 to 69 t/ha, respectively (Almodares *et al*., 2008; Prasad *et al*., 2013). Late maturing genotypes tend to accumulate high biomass (Ouma and

Akuja, 2013) which explains high cane yield produced by EUSS10 which took longer to mature. Similar to sugarcane, an important yield component in sweet sorghum is plant height which determines harvestable stalk. The longer the stalk, the more likely that genotype will provide greater cane yield thus it is not surprising that EUSS10 gave highest yield while SS21 gave the least.

3.4.2 Juice yield, extractability, bagasse moisture and grain yield

There were no differences among sorghum genotypes for percent extractability and bagasse moisture, which averaged 42.9 and 38.8%, respectively (Table 3.2). The juice yield was influenced by sorghum genotypes. EUSS10 had the highest juice volume of 7806.7 l/ha than all other genotypes while SS21 produced the least juice volume (3098.6 l/ha). All other genotypes were similar with juice volume ranging from 4635 to 5835 l/ha.

Table 3.2: Sweet sorghum genotype effect on juice yield, extractability, bagasse moisture and grain yield (n=18)

Genotype	Juice yield (l/ha)	Extractability (%)	Bagasse moisture content (%)	Grain yield (t/ha)
SS04	5116±2529^{b}	41.6±6.4^{a}	38.6±12.8^{a}	2.07±1.95^{ab}
SS14	5835±2255^{b}	43.6±5.8^{a}	37.1±7.3^{a}	1.56±1.41^{bc}
SS21	3098±2114^{c}	41.4±14.3^{a}	38.9±7.2^{a}	1.36±0.92^{bc}
SS17	4635±3107^{b}	43.5±7.0^{a}	37.0±6.1^{a}	2.60±1.71^{a}
EUSS17	5018±2759^{b}	42.9±7.2^{a}	37.0±7.1^{a}	2.51±1.73^{a}
EUSS10	7807±2690^{a}	44.8±13.5^{a}	38.3±9.2^{a}	1.31±0.90^{c}
EUSS11	5794±4345^{b}	45.7±9.9^{a}	35.9±8.3^{a}	2.40±0.35^{a}
ACFC003/12	5455±2138^{b}	39.8±10.2^{a}	39.7±7.9^{a}	2.42±0.70^{a}
$LSD_{0.05}$	1488	6.0	4.6	0.75

Means are presented as mean ± standard deviation

Means followed by the same letter in a column are not significantly different at 5% LSD

Grain yield was badly damaged by birds in Sinyanya and was not harvested. Grain yield was affected by genotype at all experimental sites. These results are in harmony with findings of Abdalla and Gamar (2011) and Showemimo (2007) who found a significant

difference between sorghum lines for grain yield in Sudan. Interestingly, EUSS10 produced the lowest grain yield (1.3 t/ha) which was similar to controls SS14 and SS21 and about 22.5 % lower than other genotypes. From this study, it is evident that considerable juice was retained in bagasse and this seems to be influenced by genotype.

3.4.3 Juice Brix, sugars and ethanol yield

EUSS11 Brix was similar to that of all control genotypes while EUSS17 Brix was similar to all controls except that of SS04 (Table 3.3). The Brix for SS04 was 1.4% greater than that of EUSS17. Meanwhile, EUSS10 Brix was less than that of all genotypes. Genotype SS04 recorded greatest percent total sugar (11.1%) and it was similar to that of SS21 and EUSS17. EUSS10 had the least total sugar that was approximately 2 percentage points less than the three control genotypes. Reducing sugar did not differ between genotypes and ranged from 1.4 to 1.9%. Lowest non-reducing sugar (sucrose) was recorded by EUSS10 and it was similar to only that of SS14 and about 1.5% lower than other genotypes. Genotype ACFC003/12 and EUSS10 produced the greatest ethanol yields (423 and 420 l/ha, respectively), but yields were similar to those of SS04, SS14, EUSS17, and EUSS11. Ethanol yields of EUSS10 and ACFC003/12 were about 83% greater than that of the least yielding genotype SS21 and about 37% greater than that of SS17.

Table 3.3 : Brix, total sugar, reducing sugar, non-reducing sugar and ethanol yield among eight sweet sorghum genotypes (n=18)

Genotype	Brix (%)	Total sugars (%)	Reducing sugar (%)	Non-reducing sugar (%)	Ethanol yield (l/ha)
SS04	17 ± 2^{a}	11.1 ± 1.8^{a}	1.86 ± 0.68^{a}	8.2 ± 1.8^{a}	349 ± 182^{ab}
SS14	16 ± 3^{ab}	9.9 ± 1.8^{c}	1.87 ± 0.41^{a}	7.4 ± 1.5^{ab}	376 ± 185^{ab}
SS21	16 ± 3^{ab}	10.8 ± 2.2^{ab}	1.93 ± 0.61^{a}	8.6 ± 2.3^{a}	230 ± 181^{c}
SS17	15 ± 3^{b}	10.2 ± 1.2^{bc}	1.86 ± 0.67^{a}	8.2 ± 0.7^{a}	306 ± 242^{bc}
EUSS17	15 ± 3^{b}	10.3 ± 1.4^{abc}	1.85 ± 0.60^{a}	7.9 ± 1.3^{a}	359 ± 285^{ab}
EUSS10	12 ± 3^{c}	8.2 ± 1.5^{d}	1.43 ± 0.53^{a}	6.3 ± 1.0^{b}	420 ± 270^{a}
EUSS11	16 ± 3^{ab}	10.0 ± 1.5^{bc}	1.85 ± 0.46^{a}	7.9 ± 2.0^{a}	413 ± 266^{ab}
ACFC003/12	17 ± 2^{ab}	9.7 ± 1.8^{c}	1.54 ± 0.48^{a}	7.8 ± 2.4^{a}	423 ± 196^{a}
$LSD_{0.05}$	1.3	0.87	0.52	1.32	113

Means are presented as mean ± standard deviation

Means followed by the same letter in a column are not significantly different at 5% LSD

Genotypic differences for juice volume, Brix and ethanol yield have also been reported (Reddy *et al*., 2011; Reddy *et al*., 2013; Soleyman *et al*., 2013; Elangovan *et al*., 2014; Reddy *et al*., 2014). Low ethanol yields recorded by control SS21 was due to its short height and early maturity hence accumulating low biomass and producing low cane and juice yield across the sites. Cane yield (19.6-34.2 t/ha) and juice extractability (48.5-54.7%) obtained from six sweet sorghum varieties by El-Geddawy *et al* (2014) is comparable to results from our study. However, they recorded higher Brix (17.5-21.8%) and plant height (223.4-411.3 cm).

3.4.4 Correlation among stem traits

Ethanol yield, cane yield, juice yield, plant height and grain yield were all positively correlated at $P<0.001$ (Table 3.4). This result is consistent with the findings of Wang *et al*. (2012). Juice yield was positively correlated with days to 50% heading at $P<0.01$, whereas Brix was positively correlated ($P<0.001$) with total sugar and non-reducing sugar. A negative relationship was observed between extractability and non-reducing at $P<0.05$; plant height and total sugar at $P<0.001$ and reducing sugar and non-reducing sugar at $P<0.05$. Fermentable sugars form a major component of total soluble solids in sweet sorghum stem juice thus a positive linear correlation between Brix and total sugars is expected. Sucrose (non-reducing sugar) is the predominant stalk sugar in sweet sorghum and is converted to reducing sugars (glucose and fructose) by invertase, thus a negative relationship is likely to be observed between reducing and non-reducing sugar. In this study, the greatest ethanol yielding genotypes also had the greatest cane yield, juice yield and plant height. Thus, tall sorghum genotypes producing high cane yield should be selected for planting to enhance juice yield and consequently high ethanol production.

Genotype EUSS10 produced the lowest Brix values and total sugar yield. However, it yielded the greatest cane and juice volume, and produced ethanol yields that were similar to ACFC003/12. This result highlights the interplay of sugar concentration and juice yield and indicates clearly that sugar yield per hectare (juice yield x sugar concentration) is more indicative of high ethanol yields than sugar concentration alone. In this case, low sugar concentration in EUSS10 was compensated by higher yields of cane and cane juice resulting in a higher sugar yield for ethanol fermentation. Brix values indicate total soluble solids in

juice extract and are positively correlated with sugars, and ethanol yield. According to Erickson *et al*. (2011), a low Brix value is generally associated with greater fresh biomass production. In their study, they found a negative correlation between Brix values in juice and fresh biomass yield of sweet sorghum genotypes grown in the year 2009 and 2010. Genotype EUSS10 had highest cane yield and lowest Brix concurring with their findings.

Genotypes that had a high cane and juice yield, and plant height produced high ethanol yield. These traits together with days to 50% heading were found to be positively correlated with ethanol yield as reported by Prasad *et al*. (2013) and Rani and Umakanth (2012). Genotypes that took more time to mature accumulated more biomass, which translated to high juice and consequently high ethanol yield. This is similar to Houx and Fritschi (2013) who also found that the late maturing 'M 81E' genotype had the lowest Brix, but greatest juice yield that resulted in high sugar yields and subsequent ethanol yields that were among the greatest of 12 genotypes evaluated. However, Sweet sorghum should be harvested before stem sugars are converted to starch and stored in grain. The present study showed relatively low performance in cane yield, explaining low juice and ethanol volume recorded. More effort needs to devise methods for improving yields to enable Kenya to compete globally in terms of ethanol production.

Table 3.4: Correlation among stem traits and grain yield of sweet sorghum

	Cy	Jy	Ey	Brix	Ext	Plt hgt	Dh	Gy	TS	RS	NRS
Cy	-	0.910***	0.823***	-0.133ns	0.030ns	0.687***	0.089ns	0.368***	-0.205ns	-0.260ns	-0.015ns
Jy		-	0.843***	-0.161ns	-0.001ns	0.636***	0.229**	0.379***	-0.204ns	-0.193ns	-0.085ns
Ey			-	-0.025ns	0.001ns	0.505***	0.019ns	0.322***	-0.168*	-0.141ns	-0.017ns
Brix				-	0.132ns	-0.485***	0.022ns	0.109ns	0.564***	0.038ns	0.455***
Ext					-	-0.105ns	0.137ns	0.129ns	-0.090ns	0.148ns	-0.242*
Plt hgt						-	-0.058ns	0.117ns	-0.337***	-0.226ns	-0.116ns
Dh							-	0.187*	-0.012ns	-0.153ns	-0.140ns
Gy								-	-0.044ns	0.094ns	-0.133ns
TS									-	0.037ns	0.947***
RS										-	-0.284*
NRS											-

Key; Cy-Cane yield (t/ha), Jy-Juice yield (l/ha), Ey-Ethanol yield (l/ha), Brix (%), Ext-Extractability (%), Plt hgt-Plant height (m), Dh-Days to 50% heading, Gy-Grain yield (t/ha), TS-Total sugar (%), RS-Reducing sugar (%), NRS-Non-reducing sugar (%)

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CHAPTER FOUR
ADAPTABILITY AND STABILITY OF SELECTED SWEET SORGHUM GENOTYPES ACROSS ENVIRONMENTS

Abstract

The genotype and environment interaction manipulates the selection criteria of sorghum (*Sorghum bicolor*) genotypes. Eight sweet sorghum genotypes were evaluated at five different locations in two growing seasons of 2014. The aim was to explore the adaptation and identify best genotypes for bio-ethanol production in Kenya. The experiments were conducted in a randomized complete block design replicated three times. Sorghum canes were harvested at hard dough stage of grain development and passed through rollers to obtain juice that was then fermented to obtain ethanol. Cane, juice and ethanol yield was analyzed using the additive main effect and multiplication interaction model (AMMI) and genotype plus genotype by environment (GGE) biplot. The combined analysis of variance of cane and juice yield of sorghum genotypes showed that sweet sorghum genotypes were significantly (P<0.05) affected by environments (E), genotypes (G), and genotype by environment interaction (GEI). GGE biplot showed high yielding genotypes EUSS10, ACFC003/12, SS14 and EUSS11 for cane yield; EUSS10, EUSS11 and SS14 for juice yield and EUSS10, SS04, SS14 and ACFC003/12 for ethanol yield. Genotype SS14 and SS17 were low yielding and showed general adaptability for both cane and juice yield whereas SS14 was widely adapted for ethanol yield. The mean yield value of genotypes averaged over environments indicated that EUSS10 had highest cane yield (26.9 t/ha) and juice yield (7807 l/ha) while ACFC003/12 had highest ethanol yield (423 l/ha). Genotype EUSS10 exhibited specific adaptation to favorable environments and is the promising genotype of superior performance for ethanol production.

Keywords: Sweet sorghum, GEI, Cane yield, Ethanol yield

4.1 Introduction

Sweet sorghum is gaining popularity for ethanol production due to its high sugar level in their stem juice. It is widely grown for food, feed and fuel in semi-arid tropics of Asia, Africa, America and Australia (Kumar *et al*., 2011) due to its drought tolerance. Drought is regarded as important abiotic stress causing yield instability and food insecurity (Abdalla and Gamar, 2011). Drought can be mitigated through irrigation as one of the available options, however, developing countries find it challenging due to huge capital investment. The introduction of drought-tolerant crops such as sorghum in ASALs remains the most desirable alternative. Sweet sorghum accumulates high amount of fermentable sugars in the stem desirable for ethanol production. Uses of sweet sorghum include brewing for both industrial and local products, baking and home consumption as food. Sorghum is a multipurpose crop which can be adopted in semi-arid parts of the country to help in the eradication of poverty through the supply of grain for food and sale of the stem to distilleries for ethanol production.

Studies of adaptability and stability provide information about the behavior of each genotype under different environmental conditions. The phenotypic performance of each genotype is influenced by abiotic and biotic factors, some genotypes may perform well in one environment but fail in several others (Fentie *et al*., 2013). These factors include rainfall, temperature, soil fertility, light, pests and diseases which vary across locations and significantly influence yield ability of crop varieties. These factors make it difficult to establish the superiority of cultivar across diverse environments (Aslam *et al*., 1993). A major drawback in the selection of genotypes with high yielding capacity in different environments is genotype by environment interaction. New genotypes must be stable for yields and should be stable across environments or suited to target regions (Mendes *et al*., 2012).Yield is controlled by the complex polygenic system and strongly varies depending on environmental conditions (Panayotov and Dimova, 2014). Stability analysis is an important step in developing cultivars for a wide range of environments or for a specific location (Joshi, 2004). Genotype by environment interaction has to be studied for yields, which are cane, juice and ethanol in our case as they are considered the most important economic traits (Moussa *et al*., 2011).

Genotype × Environment interaction complicates breeding, testing and selection of superior genotypes (Romagosa *et al*., 2013). Additive main effects and multiplicative interactions (AMMI) analysis is used to determine stability of genotypes across locations using

the principal component axis (PCA) scores and AMMI stability values (ASV) while genotype plus genotype by environment (GGE) analysis is effective method which is based on principal component analysis to fully explore multi-environment trials (Hagos and Abay, 2013). Average environment coordinates (EAC) of GGE biplot separates entries with below-average means from those with above-average means (Reddy *et al*., 2014). Stability of various crops have been studied by applying AMMI and GGE biplots successfully in Soybean (*Glycine max* L. Meril) (Ikeogu and Nwofia, 2013), sweet potatoes (*Impomea batatas*) (Moussa *et al*., 2011), pepper (*Capsicum annuum*) (Panayotov and Dimova, 2014), finger millet (*Eleusina coracana*) (Lule *et al*., 2014), wheat (*Triticum aestivum*) (Ayalneh *et al*., 2013), grain sorghum (Patil *et al*., 2007) and rice (*Oryza sativa*) (Islam *et al*., 2014). GGE and AMMI analysis were applied to determine stability and adaptability of eight sorghum genotypes grown in five different ecological zones.

4.2 Materials and Methods

4.2.1 Site description

Sweet sorghum field experiments were carried out in Kisumu, Siaya and Busia Counties of Kenya. The specific sites were Sinyanya (00^{o} 06' 68.5" S; 034^{o} 08' 66.0" E) at 1168 m above sea level (ASL), Masumbi (00^{o} 01' 73.0" N; 034^{o} 21' 87.4" E) at 1370 m ASL both in Siaya County, Mundika (00^{o} 24' 56.6" S; 034^{o} 07' 93.1" E) at 1222 m ASL in Busia, Nyahera (00^{o} 0.02' 52.78" S, 034^{o} 39' 03.59" E) at 1387 m ASL and Sagam (00^{o} 03' 20.86" N, 034^{o} 32' 31.06" E) at 1216 m ASL both in Kisumu County. Sinyanya receives an annual rainfall of between 900 and 1000 mm and the mean annual temperature range of 22.3 to 22.7 ^{o}C. The climate in Masumbi is mainly humid type, with bi-nomial rainfall pattern. The mean annual rainfall and temperature in the area are 1500-1900 mm and 20.9-21.8 ^{o}C, respectively. Average annual rainfall and temperature of Mundika range 1450-1650 mm and 21.4-22.3 ^{o}C, respectively. Nyahera receives low annual rainfall range: 1220 to 1390 mm and high mean annual temperature (22.0-22.7 ^{o}C) as compared to Sagam. Sagam receives bi-nomial rainfall as to other sites with high rainfall experienced during the 1st season (February-July) and low 2nd rainy season between August and December. The average annual rainfall in Sagam is 1450-1650 mm with a mean annual temperature range of 21.2 to 22.8 ^{o}C (Jaetzold *et al*., 2009).

4.2.2 Experimental design

Eight sweet sorghum genotypes were grown in a randomized complete block design (RCBD). The genotypes were: EUSS10, EUSS11 and EUSS17 as candidates with the controls being ACFC003/21, SS04, SS14, SS21 and SS17. Sowing was done on 18th March in Sinyanya and Masumbi and 19th March 2014 in Mundika for first season. Sowing in the second season was done on 13th September 2014 for both Mundika and Sagam while Nyahera was planted on 24th September 2014. Genotypes were sown in plots measuring 4×2.5 m in a randomized complete block design with three replications. Each plot consisted of four rows of sorghum at a spacing of 60 cm by drill and the blocks were separated by 1.5 m path. Triple superphosphate fertilizer was applied uniformly to all plots at a rate of 17.2 kg/ha during sowing. Control of weeds was done manually using hoes, three weeks after seedling emergence and sorghum were thinned to a spacing of 10 cm within the row then top dressed with calcium ammonium nitrate (25% N) at the rate of 20 kg N/ha. Birds guarding was effected soon after the panicles formed to prevent grains damage.

4.2.3 Data collection

Emergence was observed in all plots two weeks after planting and stand counts determined for all sorghum experimental units. Days to 50% heading was determined by calculating the number of days from sowing to when 50% of the sorghum panicles in each plot emerged. Sorghum was monitored untill when various genotypes had attained hard dough stage of grain when plant height was determined and panicles harvested. Three randomly selected plants from each cultivar in all replicates were used for recording plant height. Plant height was measured from base of the stem to tip of panicle and data averaged across three plants.

Harvesting took place on 9th July 2014 at the three sites: Masumbi, Mundika, and Sinyanya for the first season while Sagam and Mundika were harvested on 12th December 2014 and Nyahera on 29th December 2014 for the second season. Eight different cultivars were harvested at hard dough stage of grain by taking plants in two inner rows of each plot. The leaves were stripped off by hand from harvested stalk and panicles removed using secateurs. Sorghum panicles from harvested area were sun dried to 12 % moisture, threshed and winnowed manually. The grains were weighed and data was used to calculate yield in tonnes per hectare. The harvested stalks were weighed with a weighing balance to get fresh cane weight then transported

to the laboratory for juice extraction. Juice from the stalk was extracted in one roller crusher (Fuan Liyuan, China, type YC 80B-4) and strained through a sieve into a juice container. The volume of juice was measured and recorded and Brix (%) was taken using hand refractometer. After juice extraction wet bagasse weight was taken immediately then dried in an oven at 65 ^{o}C for three days to get dry weight. The data obtained was used to calculate percent juice extractability and bagasse moisture as follows:

$$Juice\ extractability\ (\%) = \frac{weight\ of\ juice\ (kg)}{fresh\ cane\ weight\ (kg)} \times 100 \quad \text{4.1}$$

$$Bagasse\ moisture\ (\%) = \frac{wet\ bagasse\ wt.(kg) - dry\ bagasse\ wt.(kg)}{dry\ bagasse\ wt.(kg)} \times 100 \quad \text{4.2}$$

4.2.4 Ethanol analysis

Juice was sampled from each plot taking 100 ml for fermentation. Yeast (1.5%), *Saccharomyces cerevisiae* was added to juice and fermentation process carried out at 35 ^{o}C for four days then distilled to obtain ethanol whose volume was determined. Refractometer (RFM 3330 code 25-330, Bellinghant Stanley limited) was used to determine the concentration of ethanol. The refractive index of distillate was taken then compared with that of a standard curve. Absolute ethanol was mixed with distilled water to give the concentrations of 0, 5, 10, 15, 20, 25 and 30% ethanol whose refractive index was taken to obtain standard curve showing the relationship between the refractive index and percent of ethanol in the distillate.

4.2.5 Statistical analysis

Statistical computations were carried out using Genstat software version 15.1 (VSN International limited, 2012) for AMMI and GGE biplot analysis. The graphic representation of genotypes and environments by AMMI analysis results from a model of main additive effects and multiplicative interaction (Gebremedhin *et al*., 2014). This model is expressed mathematically by;

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger} \quad \text{4.3}$$

Where; Y_{ger}: mean yield of genotype *g* in the environment *e* for replication *r*;

μ: grand mean;

α_g: deviation of the genotype *g* from the grand mean;

β_e: deviation of the environment *e* from the grand mean;

λ_n: singular value for the interaction principal component axis (IPCA) *n*;

N: the number of PCA axis retained in the model;

γ_{gn}: the PCA score of a genotype for PCA axis *n*;

δ_{en}: the environmental PCA score for PCA axis *n*;

ρ_{ge}: AMMI residual and ε_{ge}: the error term when the experiment is replicated.

AMMI stability value (ASV) was calculated by:

$$ASV = \sqrt{\left[\frac{SS_{IPCA_1}}{SS_{IPCA_2}}\right](IPCA1\ score)]^2 + (PCA2\ score)^2} \quad \text{4.4}$$

AMMI analysis was used to determine the stability of genotypes across locations using principal component axis (PCA) scores and ASV. Genotypes having least ASV were considered as widely adapted genotypes. Similarly, IPCA2 score close to zero reveal more stable, while large values indicated more responsive and less stable genotypes. GGE biplot analysis was used to visualize the relationship between testers and entries and to determine 'which won where' portion. GGE biplot also reveals stability of genotypes, genotypes located near the biplot origin are considered as widely adapted genotypes while genotypes located far as specifically adapted.

4.3 Results and discussions

4.3.1 Effect of environment on maturity, height and biomass of selected sweet sorghum

The effects of genotype were significant on days to 50% heading, with SS21 being early maturing genotype across environments (Table 4.1). Genotypes SS14, EUSS10 and EUSS11 took long to mature across environments. The time difference between early and late maturing genotypes was more than two weeks across environments except in Masumbi and Mundika (Season II). Generally, genotypes matured early during the first season compared to the second season. From the study, it was observed that the least number of days to reach 50% heading was about eight and a half weeks (61 days).

Plant height differed among sweet sorghum genotypes and across locations. The tallest and shortest plant height was recorded by genotypes in Sagam and Sinyanya, respectively (Table 4.2). Genotype SS21 was ranked among the shorter genotypes during the first and the second season while SS04, EUSS10, and ACFC003/12 grew taller consistently across environments. In Nyahera, the result indicates plant height was similar for all genotypes ranging from 1.62 to 1.81 m except for ACFC003/12, which recorded maximum plant height of 1.89 m. A similar trend was observed in Masumbi whereby all genotypes were similar in height except SS21 that recorded 0.60 m shorter than EUSS10.

Table 4.1: Influence of environment on maturity (days to 50% heading) of sweet sorghum genotypes

Genotypes	**Environments**					
	Masumbi (LM1)	Mundika (LM2)	Sinyanya (LM3)	Mundika (LM2)	Nyahera (LM3)	Sagam (LM1)
	← Season one (March-July) →			← Season two (Sept-Dec) →		
	← Number of days to 50% heading →					
SS04	79±3^{abc}	80±0^{b}	80±4^{ab}	69±6^{bcd}	73±4^{ab}	76±3^{abcd}
SS14	87±4^{a}	85±0^{a}	85±0^{ab}	76±4^{a}	74±4^{ab}	82±7^{ab}
SS21	73±10^{c}	72±1^{c}	66±0^{c}	66±4^{d}	61±1^{c}	66±6^{d}
SS17	76±2^{bc}	79±3^{b}	80±4^{ab}	67±4^{cd}	71±4^{b}	73±5^{bcd}
EUSS17	80±4^{abc}	80±1^{b}	78±0^{b}	72±1^{abc}	75±2^{ab}	78±4^{abc}
EUSS10	84±3^{ab}	86±2^{a}	86±1^{a}	74±2^{ab}	77±1^{a}	85±9^{a}
EUSS11	80±2^{abc}	81±1^{b}	80±4^{ab}	73±1^{abc}	75±2^{ab}	77±2^{abc}
ACFC003/12	80±5^{abc}	79±2^{b}	82±10^{ab}	71±3^{abcd}	73±4^{ab}	70±7^{cd}
$LSD_{0.05}$	8.5	2.8	8.0	5.6	4.2	10.5

Means are presented as mean ± standard deviation

Means followed by the same letter do not differ in a column at 5% LSD

Genotypes varied with environments for cane yield (Table 4.3). EUSS10 gave higher yields across environments both in season one and two except in Nyahera. However, the performance was similar to EUSS11 and ACFC003/12 across environments except in Mundika during the first season. Among the genotypes, SS21 gave relatively very low yield across

environments during the first season. Though there was varying genotypic performance across environments, Sagam environment seems to have favoured better performance of genotypes. EUSS10 shows that it is more suited for lower midland zone 1 and 2 (Figure 4.1 and 4.2) whose rainfall and temperature ranged between 804-846 mm and 20.3-29.0 ^{o}C respectively during the study period. The significant difference between sweet sorghum genotypes with regard to height and cane yield indicates that genotypes vary genetically. Genotypes that took long to mature grew taller and recorded high cane yield showing a positive relationship between plant height and cane yield. These morphological characters together with cane diameter and number of internodes per stalk have been reported to affect final yield in sugarcane (Singh and Singh, 1954; Panhwar *et al*., 2003). Hence, tall sweet sorghum genotypes should be selected to maximize cane yield.

Table 4.2: Effect of environment on plant height of eight sweet sorghum genotypes

Genotypes	**Environments**					
	Masumbi (LM1)	Mundika (LM2)	Sinyanya (LM3)	Mundika (LM2)	Nyahera (LM3)	Sagam (LM1)
	← Season 1 (March-July) →			← Season 2 (Sept-Dec) →		
	← Plant height (m) →					
SS04	1.8±0.3^{ab}	2.2±0.5^{ab}	1.1±0.1^{a}	2.0±0.6^{abc}	1.8±0.4^{ab}	2.0±0.2^{a}
SS14	1.8±0.2^{ab}	1.9±0.1^{c}	1.1±0.2^{a}	2.1±0.2^{ab}	1.6±0.2^{b}	2.2±0.5^{a}
SS21	1.4±0.1^{c}	2.0±0.6^{c}	0.9±0.3^{a}	1.8±0.2^{bc}	1.8±0.9^{ab}	2.0±0.3^{a}
SS17	1.9±0.2^{ab}	1.4±0.2^{d}	1.1±0.2^{a}	1.8±0.1^{c}	1.7±0.2^{ab}	2.2±0.2^{a}
EUSS17	1.8±0.2^{ab}	2.1±0.1^{bc}	1.1±0.1^{a}	2.0±0.1^{abc}	1.7±0.1^{b}	2.1±0.2^{a}
EUSS10	2.1±0.3^{a}	2.3±0.0^{a}	1.1±0.0^{a}	2.2±0.2^{a}	1.8±0.0^{ab}	2.3±0.3^{a}
EUSS11	1.7±0.2^{bc}	1.9±0.1^{c}	0.9±0.1^{a}	1.8±0.0^{bc}	1.7±0.1^{b}	2.2±0.3^{a}
ACFC003/12	1.7±0.2^{abc}	2.0±0.1^{bc}	0.8±0.2^{a}	2.1±0.1^{abc}	1.9±0.2^{a}	2.1±0.1^{a}
$LSD_{0.05}$	3.62	1.84	3.44	3.20	2.13	4.04

Means are presented as mean ± standard deviation

Means followed by the same letter do not differ in a column at 5% LSD

Table 4.3: Cane yield (t/ha) of eight sweet sorghum genotypes across environments

Genotypes	Environments					
	Masumbi (LM1)	Mundika (LM2)	Sinyanya (LM3)	Mundika (LM2)	Nyahera (LM3)	Sagam (LM1)
	← Season 1 (March-July) →			← Season 2 (Sept-Dec) →		
	← Cane yield (t/ha) →					
SS04	26.4±11.9^{ab}	28.5±3.2^{ab}	13.9±2.4^{ab}	16.0±6.0^{b}	22.2±10.7^{a}	19.4±15.8^{b}
SS14	24.3±7.9^{ab}	20.8±3.6^{cd}	16.7±0.0^{a}	20.8±7.2^{b}	16.6±7.5^{ab}	32.6±7.9^{ab}
SS21	9.7±1.2^{b}	13.2±3.2^{e}	8.3 4±2^{b}	16.7±8.3^{b}	20.1±4.3^{a}	28.6±7.9^{ab}
SS17	27.8±7.9^{ab}	15.3±6.4^{de}	13.9±4.8^{ab}	16.6±4.1^{b}	16.0±3.2^{ab}	33.3±7.2^{ab}
EUSS17	25.0±8.3^{ab}	18.1±3.2^{cde}	16.7±0.0^{a}	16.6±11.0^{b}	10.1±4.2^{b}	33.3±11.0^{ab}
EUSS10	31.9±21.1^{a}	30.8±1.2^{a}	12.5±4.2^{ab}	30.0±6.4^{a}	11.8±1.2^{b}	44.4±12.7^{a}
EUSS11	18.8±2.1^{ab}	22.9±4.2^{bc}	13.9±2.4^{ab}	23.6±6.4^{ab}	21.5±6.4^{a}	40.3±2.4^{a}
ACFC003/12	23.6±6.7^{ab}	24.3±6.4^{bc}	11.1±4.5^{ab}	25.7±6.7^{ab}	21.5±11.0^{a}	34.7±6.4^{ab}
$LSD_{0.05}$	19.0	7.6	6.0	10.7	7.7	17.5

Means are presented as mean ± standard deviation

Means followed by the same letter do not differ in a column at 5% LSD

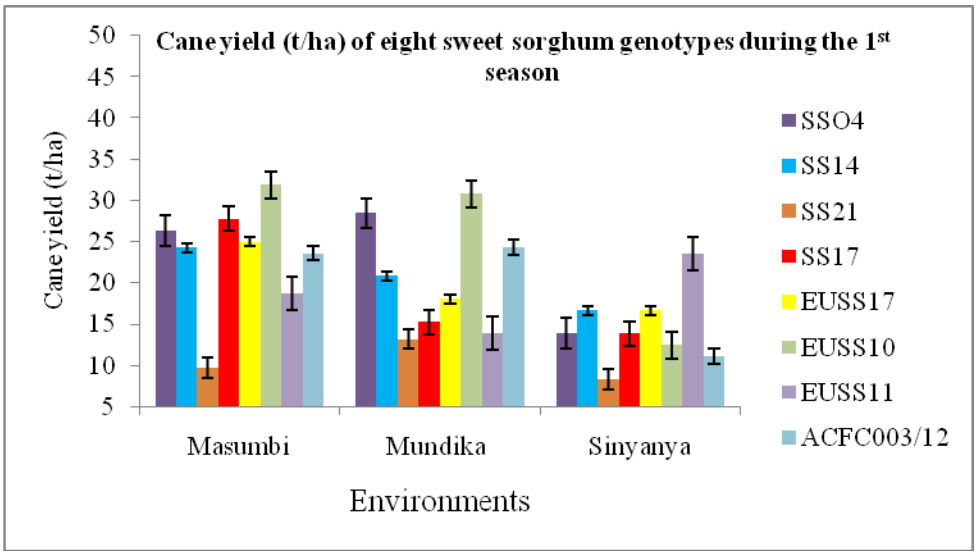

Figure 4.1: Influence of environment on cane yield (t/ha) of sweet sorghum genotypes in first season

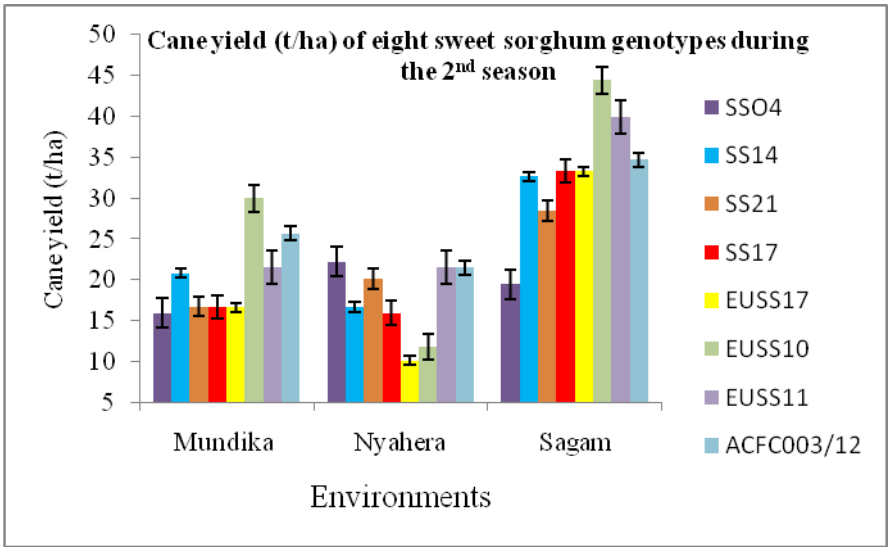

Figure 4.2: Influence of environment on cane yield (t/ha) of sweet sorghum genotypes in second season

In most developing countries, the socio-economic needs of rapidly increasing populations are the main driving forces in the allocation of land resources to various kinds of uses, with food production as the primary land use. Heavy population pressure and the related increased competition from different types of land users have emphasized the need for more effective land-use planning and management. Rational and sustainable land use is an issue of great concern to governments and land users interested in preserving the land resources for the benefit of present and future populations. FAO has developed and successfully applied the agro-ecological zones (AEZ) to address issues including linking land-use outputs with other development goals in such areas as food production, food self-sufficiency, cash crop requirements, issues of soil fertility constraints, soil erosion risks and land degradation (FAO, 1996). Zoning help in recommending the use of different types of land in different locations by ascertaining potential yields that vary among locations, years and seasons. AEZ defines zones on the basis of combinations of soil, landform and climatic characteristics.

Each zone has a similar combination of constraints and potentials for land use, and serves as a focus for the targeting of recommendations designed to improve the existing land-use situation, either through increasing production or by limiting land degradation. The thermal zones in Kenya describe the temperature regime available for crop growth during the growing

period. It is usually defined based on ranges of mean temperature that is associated with altitude. The thermal zone 1 in Kenya records mean daily temperature and altitude below 25 ^{o}C and 800 m asl, respectively. The mean daily temperature range and altitude range is 22.2-25.0 ^{o}C and 800-1200 m asl, respectively for Zone 2 and 20.0-22.5 ^{o}C and 1200-1550 m asl, respectively for Zone 3 (FAO, 1996). The leading crop in lower midland zone 1 and 2 is sugarcane whereas LM3 has cotton as the best performing crop. Upper midland zone 1-3 and lower midland zone 1-3 are regarded as sorghum zones (Jatzold and Kutsch, 1982).

Kenyan climate is tropical with the temperature varying widely from cool high altitude around central highland regions to high temperatures at the coast. Kenya experiences two distinct wet periods; the short rains occur during the second season between October to December and the long rains in March to May (season 1) (McSweeney *et al*., 2011). The onset, duration and intensity of these rainfalls also vary considerably from year to year.

4.3.2 Influence of environment on juice volume, extractability and bagasse moisture

Juice volume differed significantly among sweet sorghum genotypes; there were high and low performers (Table 4.4). Among the genotypes, EUSS10 gave consistently high yields across environments. All genotypes recorded highest juice volume in Sagam except SS04. Though genotypes performed differently across environments, LM1 agro-ecological zones (Masumbi and Sagam) favored better performance. In Sinyanya, SS14 had about seven times more juice volume than SS21. Similarly, EUSS10 recorded juice volume about five times more than SS21 in Mundika during the first season. Among the genotypes, SS21 and EUSS17 gave relatively low juice volume in Masumbi and Nyahera, respectively. Genotypes responded differently to the varied environments during season one and two (Figure 4.3 and 4.4). The genotypes performed better in Sagam (LM1) due to high total rainfall experienced during the growth period.

Table 4.4: Juice volume (l/ha) of eight sweet sorghum genotypes across environments

Genotypes	Environments					
	Masumbi (LM1)	Mundika (LM2)	Sinyanya (LM3)	Mundika (LM2)	Nyahera (LM3)	Sagam (LM1)
	← Season 1(March-July) →			← Season 2 (Sept-Dec) →		
	← Juice yield (l/ha) →					
SS04	8044±3217^{ab}	6304±335^{bc}	3567±1525^{ab}	4225±1653^{bc}	4518±2873^{a}	4011±2910^{c}
SS14	7014±1675^{ab}	5090±923^{cd}	5061±1006^{a}	5588±1995^{bc}	3648±1995^{ab}	8611±2882^{abc}
SS21	1938±116^{b}	2850±1352^{e}	761±569^{c}	4281±3218^{bc}	4000±831^{ab}	4763±2646^{c}
SS17	7086±3993^{ab}	3472±1860de	3310±2207^{ab}	2188±742^{c}	3311±659^{ab}	8442±3130^{abc}
EUSS17	7057±2795^{ab}	3906±189de	4617±577^{ab}	4063±2934^{bc}	2332±1034^{b}	8135±3430^{abc}
EUSS10	9615±5261^{a}	9051±996^{a}	3861±890^{ab}	10111±2889^{a}	3056±265^{ab}	11146±2906^{a}
EUSS11	4867±1008^{ab}	4848±1358^{cde}	3411±1488^{ab}	6344±1121^{b}	4646±1784^{a}	10647±1751^{ab}
ACFC003/12	5990±1135^{ab}	7363±1544^{ab}	2406±1787^{bc}	5674±1939^{bc}	5028±2628^{a}	6269±418^{bc}
$LSD_{0.05}$	6346	2141	2307	3587	1975	4731

Means are presented as mean ± standard deviation

Means followed by the same letter do not differ in a column at 5% LSD

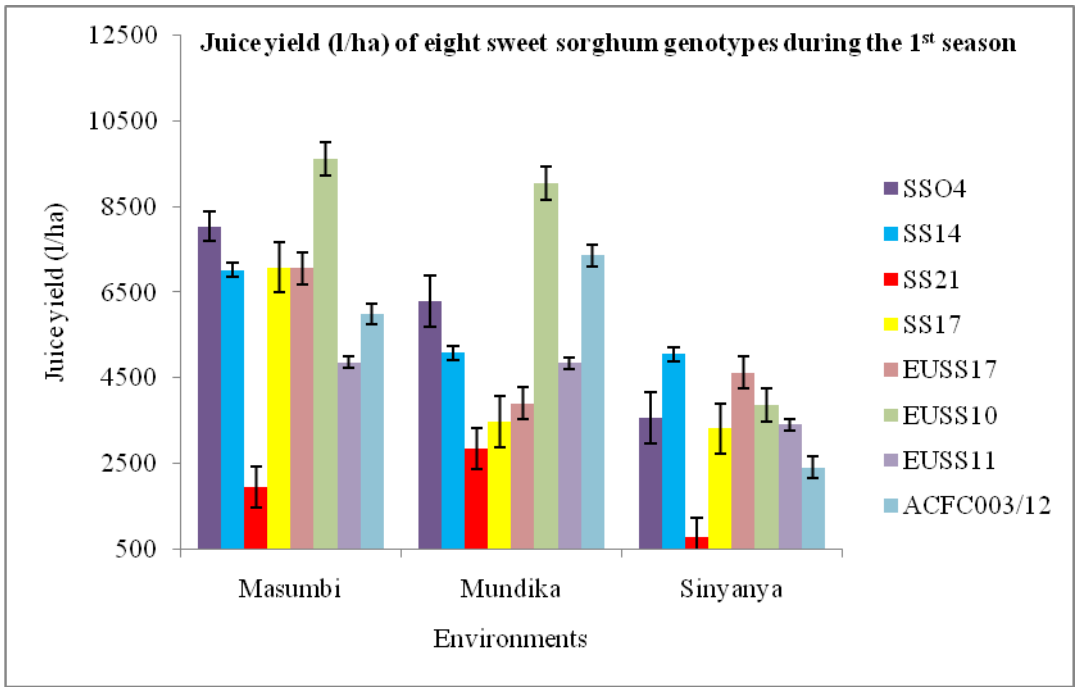

Figure 4.3: Influence of environment on juice yield (l/ha) of sweet sorghum genotypes during the first season

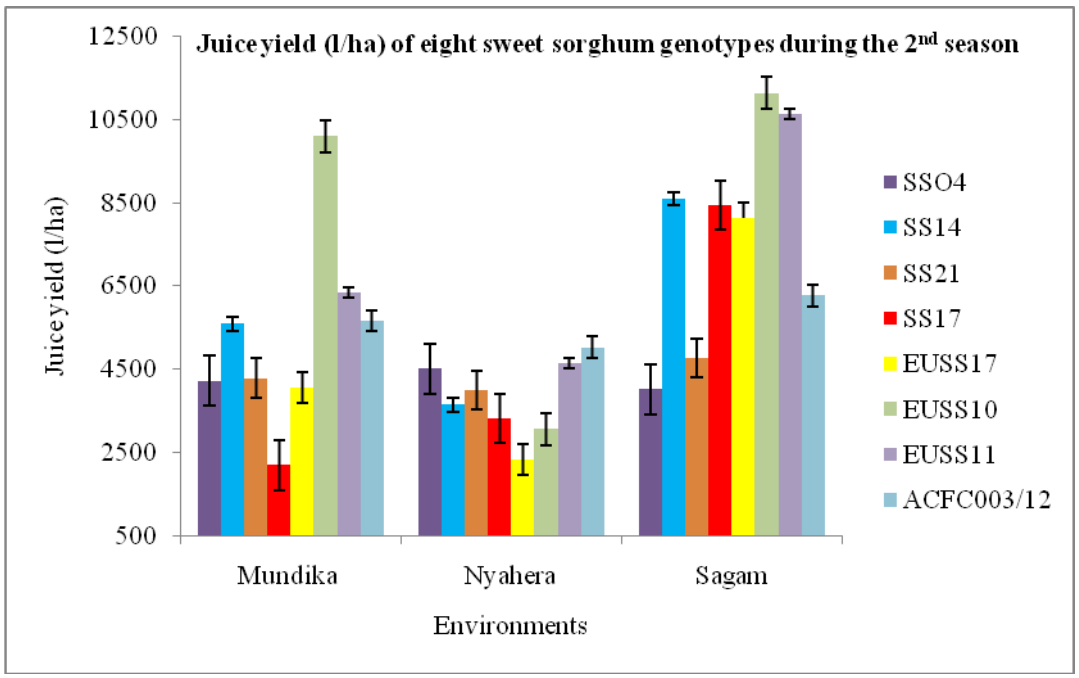

Figure 4.4: Influence of environment on juice yield (l/ha) of sweet sorghum genotypes during the second season

Generally, genotype and environment did not affect juice extractability (Table 4.5). Juice extractability did not differ among sweet sorghum genotypes in Mundika (Season I) and Nyahera averaging 38 and 39%, respectively. Interestingly, SS21 had the highest and the lowest juice extractability in Sinyanya and Sagam respectively.

Table 4.5: Juice extractability (%) of eight sweet sorghum genotypes across environments

Genotypes	**Environments**					
	Masumbi (LM1)	Mundika (LM2)	Sinyanya (LM3)	Mundika (LM2)	Nyahera (LM3)	Sagam (LM1)
	← Season 1(March-July) →			← Season 2 (Sept-Dec) →		
	← Juice extractability (%) →					
SS04	44.9±1.3^{b}	38.4±4.6^{a}	43.9±4.8^{b}	38.2±5.1^{b}	38.6±4.0^{a}	45.3±13.2^{bc}
SS14	46.3±1.0^{b}	44.8±3.8^{a}	41.5±14.0^{b}	46.7±1.0^{ab}	39.1±3.1^{a}	43.2±1.5^{abc}
SS21	44.3±1.9^{b}	30.8±12.8^{a}	66.6±14.7^{a}	38.1±1.7^{b}	34.7±2.5^{a}	33.8±6.4^{c}
SS17	44.0±3.3^{b}	38.7±4.0^{a}	48.4±8.7^{ab}	48.8±11.9^{a}	39.2±5.5^{a}	41.9±1.0^{abc}
EUSS17	43.0±2.1^{b}	44.7±13.5^{a}	42.2±8.9^{b}	43.2±0.8^{ab}	38.6±4.0^{a}	45.8±0.6^{abc}
EUSS10	48.0±8.2^{ab}	43.4±2.9^{a}	39.0±14.0^{b}	42.6±2.1^{ab}	39.9±1.6^{a}	56.0±15.7^{a}
EUSS11	58.3±16.0^{a}	38.2±4.6^{a}	42.6±5.3^{b}	42.2±0.7^{ab}	42.9±28.5^{a}	50.2±6.4^{ab}
ACFC003/12	45.3±2.9^{b}	27.7±17.7^{a}	43.3±12.8^{b}	42.6±1.8^{ab}	38.7±7.5^{a}	41.3±6.0^{bc}
$LSD_{0.05}$	11.4	17.9	18.9	10.4	18.7	14.3

Means are presented as mean ± standard deviation

Means followed by the same letter do not differ in a column at 5% LSD

There was no significant difference among sweet sorghum genotypes in Nyahera in terms of percent bagasse moisture averaging 42% (Table 4.6). Though there were similar percent bagasse moisture among genotypes in each location, SS21 lost the highest amount of juice through the bagasse in Masumbi and Sagam. Bagasse moisture for ACFC003/12 was 16 and 15% higher than SS14 and SS21 in Mundika season I and Sinyanya respectively. It is evident that more juice was lost in the bagasse accounting for more than 30% of harvested cane weight. Since the genotypes were subjected to the same machinery, the difference in extractability could be attributed to different abilities of genotypes to retain moisture during extraction process. Stage

of harvesting also affects juice volume and extractability. Channappagoudar *et al*. (2007) compared the juice extractability at three stages; flowering, grain filling and physiological maturity and found high yielding genotypes recorded higher percent juice extractability at physiological maturity.

Table 4.6: Bagasse moisture (%) lost by eight sweet sorghum genotypes across environments

Genotypes	**Environments**					
	Masumbi (LM1)	Mundika (LM2)	Sinyanya (LM3)	Mundika (LM2)	Nyahera (LM3)	Sagam (LM1)
	← Season 1(March-July) →			← Season 2 (Sept-Dec) →		
	← Bagasse moisture (%) →					
SS04	33.6±0.9^{ab}	41.5±10.8^{ab}	30.3±14.9^{ab}	43.6±3.6^{a}	41.3±6.0^{a}	40.4±3.9^{ab}
SS14	35.0±0.6^{ab}	30.2±4.2^{b}	34.9±6.0^{ab}	39.2±1.3^{ab}	42.0±0.9^{a}	41.8±1.1^{ab}
SS21	38.3±7.7^{a}	41.8±3.3^{ab}	20.9±9.9^{b}	42.3±5.6^{ab}	44.8±5.2^{a}	45.4±6.8^{a}
SS17	36.5±1.3^{a}	37.5±3.0^{ab}	33.3±10.0^{ab}	34.6±2.9^{b}	38.9±7.3^{a}	41.3±10.2^{b}
EUSS17	33.9±9.3^{ab}	33.1±4.5^{ab}	31.7±2.9^{ab}	41.6±2.1^{ab}	42.1±2.3^{a}	39.2±1.7^{ab}
EUSS10	34.3±1.2^{ab}	38.0±1.8^{ab}	34.7±10.2^{ab}	43.5±7.9^{a}	47.5±5.8^{a}	31.76.3^{b}
EUSS11	31.5±2.9^{b}	38.8±0.3^{ab}	31.3±10.7^{ab}	40.7±1.5^{ab}	38.0±18.6^{a}	35.0±4.8^{ab}
ACFC003/12	34.3±4.8^{ab}	45.8±13.1^{a}	35.5±9.4^{a}	40.6±0.5^{ab}	42.5±1.5^{a}	39.6±2.1^{ab}
$LSD_{0.05}$	6.3	13.0	12.8	8.0	17.9	12.8

Means are presented as mean ± standard deviation

Means followed by the same letter do not differ in a column at 5% LSD

4.3.3 Influence of environment on Brix, ethanol and grain yield

Genotypes EUSS11 recorded consistent high Brix values across environments with the controls SS04, SS14 and ACFC003/12 except in Sagam (Table 4.7). Among the genotypes, EUSS10 had the least total soluble solids (Brix) across environments. All genotypes recorded similar Brix in Mundika during the second season ranging from 13 to 15.7%. Though SS21 performed poorly in terms of morphological characters, it was the best for Brix in Mundika during season I and II and in Sagam. A high Brix shown by genotypes in Sinyanya is attributed to higher temperatures experienced at that site (Figure 4.7). Johnson and Seebaluck (2013)

reported that sugarcane requires higher solar radiation during initial growth stage and during ripening in order to accumulate more sucrose at ripening.

Table 4.7: Brix (%) of sweet sorghum genotypes across environments

Genotypes	**Environments**					
	Masumbi (LM1)	Mundika (LM2)	Sinyanya (LM3)	Mundika (LM2)	Nyahera (LM3)	Sagam (LM1)
	← Season 1(March-July) →			← Season 2 (Sept-Dec) →		
	← Brix (%) →					
SS04	18.3±0.6^{a}	18.0±1.0^{a}	19.0±2.0^{a}	15.0±1.0^{a}	16.6±1.2^{ab}	13.7±2.1^{abc}
SS14	16.7±0.6^{ab}	14.8±1.6^{abc}	21.0±1.0^{a}	13.0±2.0^{a}	17.0±0.0^{ab}	13.3±1.2^{abc}
SS21	14.0±0.0^{cd}	18.3±0.6^{a}	15.7±2.3^{bc}	15.7±3.2^{a}	12.3±0.6^{c}	17.0±1.0^{a}
SS17	16.3±0.6^{b}	11.7±3.8^{c}	18.3±2.3^{ab}	15.0±3.0^{a}	15.3±0.6^{b}	15.3±0.6^{ab}
EUSS17	15.7±0.6^{bc}	13.0±4.6^{c}	18.7±2.3^{ab}	15.0±1.7^{a}	15.0±1.0^{b}	15.0±1.0^{ab}
EUSS10	12.3±2.3^{d}	13.3±0.6^{bc}	15.0±1.0^{c}	12.0±3.5^{a}	9.3±1.5^{d}	10.7±3.1^{c}
EUSS11	17.0±1.0^{ab}	15.3±3.8^{abc}	18.7±0.6^{ab}	15.0±0.0^{a}	17.7±6.6^{a}	13.0±4.4^{bc}
ACFC003/12	17.0±1.0^{ab}	14.8±1.6^{abc}	18.3±1.2^{ab}	15.3±1.2^{a}	16.3±2.5^{ab}	17.0±1.0^{a}
$LSD_{0.05}$	1.9	4.7	3.0	4.1	2.3	3.9

Means are presented as mean ± standard deviation

Means followed by the same letter do not differ in a column at 5% LSD

Genotypes varied within environments for ethanol yield (Table 4.8). Genotypes performed similarly during the second season with SS17, EUSS10 and SS04 recording the lowest volume of ethanol per hectare in Mundika, Nyahera and Sagam respectively. In Nyahera, EUSS10 and EUSS17 had ethanol yield that was lower than that produced by other genotypes by about 14%. During season I, the performance of the two controls SS21 (in Masumbi and Sinyanya) and SS17 (in Mundika) was relatively poor. The maximum ethanol yield among the genotypes across environments was obtained from EUSS11 (838 l/ha) in Sagam. The performance of EUSS17, EUSS10, and EUSS11 were comparable to the best controls SS04, SS14, and ACFC003/12 in most of the tested environments. Genotypes performed better in LM1

agro-ecological zones (Masumbi and Sagam) compared to LM3 (Sinyanya and Nyahera) for ethanol yield (Figure 4.5 and 4.6).

Table 4.8: Ethanol yield (l/ha) of sweet sorghum genotypes across environments

Genotypes	**Environments**					
	Masumbi (LM1)	Mundika (LM2)	Sinyanya (LM3)	Mundika (LM2)	Nyahera (LM3)	Sagam (LM1)
	← Season 1(March-July) →			← Season 2 (Sept-Dec) →		
	← Ethanol yield (l/ha) →					
SS04	539±216^{ab}	247±57^{bc}	276±116^{abc}	359±87^{ab}	350±235^{a}	325±278^{b}
SS14	470±112^{ab}	170±89^{c}	371±68^{a}	402±51^{ab}	212±176^{ab}	629±133^{ab}
SS21	130±8^{c}	130±53^{c}	117±14^{d}	352±187^{ab}	200±40^{ab}	451±303^{ab}
SS17	475±268^{ab}	113±67^{c}	174±92^{cd}	205±108^{b}	194±126^{ab}	677±142^{ab}
EUSS17	473±187^{ab}	120±69^{c}	317±27^{ab}	369±369^{ab}	148±79^{b}	722±369^{ab}
EUSS10	644±486^{a}	417±65^{a}	177±11^{cd}	569±73^{a}	138±24^{b}	573±157^{ab}
EUSS11	326±68^{ab}	155±78^{c}	244±127^{abcd}	574±43^{a}	337±85^{ab}	838±308^{a}
ACFC003/12	401±76^{ab}	336±157^{ab}	224±66^{bcd}	500±141^{a}	377±263^{a}	699±63^{ab}
$LSD_{0.05}$	425	152	138	283	229	430

Means are presented as mean ± standard deviation

Means followed by the same letter do not differ in a column at 5% LSD

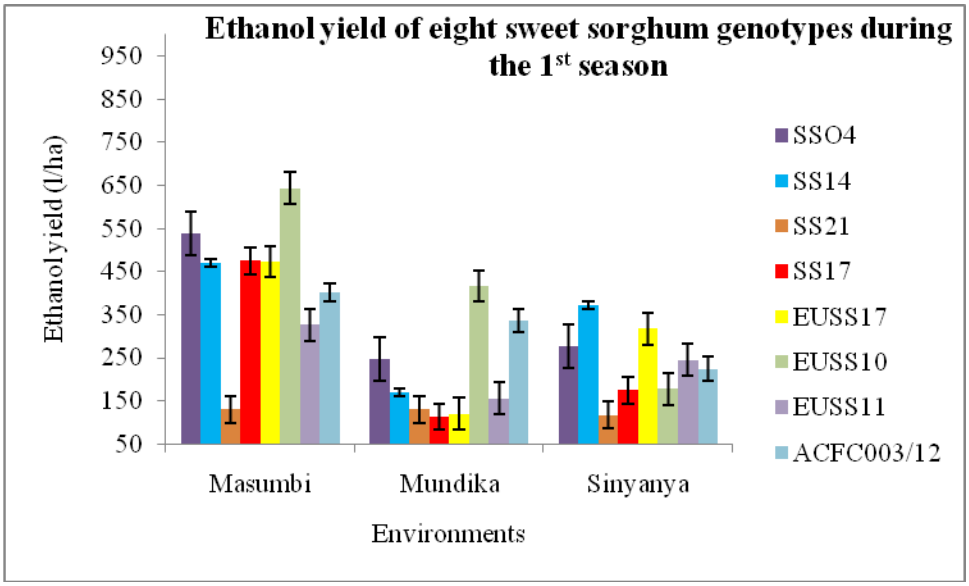

Figure 4.5: Influence of environment on ethanol yield (l/ha) of sweet sorghum genotypes during the first season

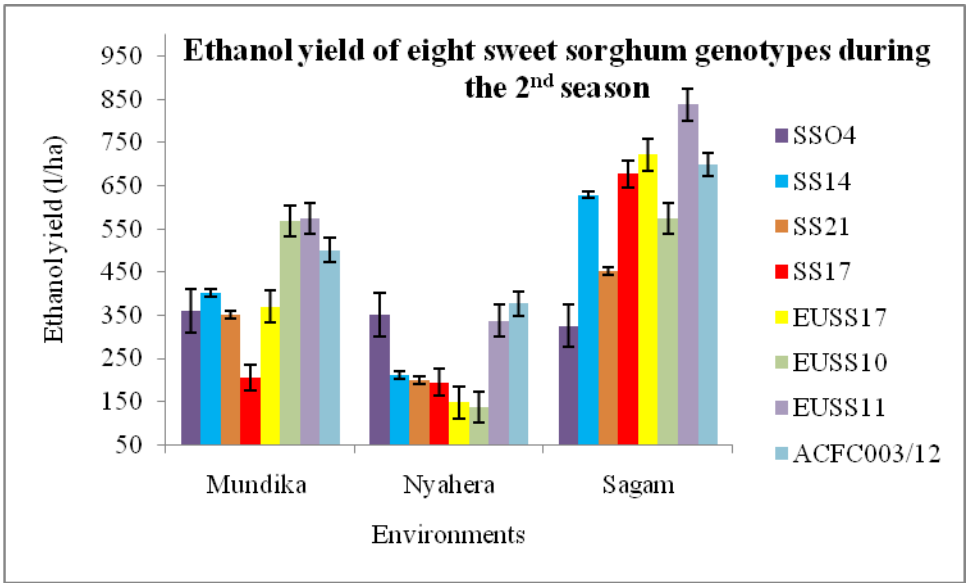

Figure 4.6: Influence of environment on ethanol yield (l/ha) of sweet sorghum genotypes during the second season

In all sites, grain yield was affected by genotype (Table 4.9). Genotypes produced maximum grain yield in Masumbi with SS17 and EUSS17 recording the highest. Grain yield in Mundika Season I and II fell within a narrow range and averaged 1.8 and 1.1 t/ha, respectively. EUSS10 was among the least performing genotypes for grain yield across environments. Tall

genotypes had low Brix values conforming to the findings of Ahmed and Obeid (2012) who found a negative association between juice Brix and stalk height. This complicates the selection of tall genotypes to improve on cane yield since tall genotypes might have poor juice quality. Selection can be balanced by selecting genotypes with high juice Brix and cane yield to improve ethanol yield per hectare. More breeding programs are needed to improve sucrose and hence Brix for taller genotypes.

Table 4.9: Grain yield (t/ha) of eight sweet sorghum genotypes across environments

Genotypes	**Environments**				
	Masumbi (LM1)	Mundika (LM2)	Mundika (LM2)	Nyahera (LM3)	Sagam (LM1)
	← Season 1(March-July) →		← Season 2 (Sept-Dec) →		
	← Grain yield (t/ha) →				
SS04	4.96±2.34^{ab}	2.55±1.00^{ab}	0.23±0.15^{b}	1.31±1.03^{bc}	1.52±0.25^{bc}
SS14	2.96±1.49^{bc}	0.52±0.49^{b}	0.10±0.07^{b}	0.36±0.25^{d}	2.84±0.84^{ab}
SS21	1.91±1.10^{c}	0.70±0.50^{b}	0.71±0.35^{a}	2.42±0.02^{a}	1.06±0.88^{c}
SS17	7.25±2.14^{a}	2.19±2.01^{ab}	0.72±0.27^{a}	1.41±0.41^{b}	1.42±0.68^{bc}
EUSS17	7.01±2.15^{a}	1.85±1.51^{ab}	0.25±0.13^{b}	0.56±0.56^{cd}	2.91±0.64^{ab}
EUSS10	2.94±1.50^{bc}	0.86±0.33^{b}	0.24±0.04^{b}	0.22±0.11^{d}	2.29±1.08^{bc}
EUSS11	4.38±0.96^{abc}	2.10±0.99^{ab}	0.22±0.23^{b}	0.95±0.71^{bcd}	4.36±0.83^{a}
ACFC003/12	2.28±0.78^{abc}	3.73±0.26^{a}	0.38±0.26^{ab}	1.46±1.10^{b}	2.23±1.67^{bc}
$LSD_{0.05}$	2.99	1.95	0.35	0.82	1.74

Means are presented as mean ± standard deviation

Means followed by the same letter do not differ in a column at 5% LSD

All experimental environments fell within the same agro-ecological zone, lower midland (LM); the difference in yield was due to difference in sub agro-ecological zones as depicted in Table 4.10. In general, the soil in these areas was sandy clay loam, slightly acidic (pH =4.4-6.0) and was poor in nitrogen and phosphorous. Sinyanya and Nyahera both in lower midland zones were characterized by high mean maximum temperature (Fig. 4.7) and lower precipitation (Fig. 4.8). The test locations vary in latitude, rainfall, soil types and temperature. The three

environments with high yielding potential, Masumbi, Mundika, and Sagam are characterized by high bimodial rainfall patterns as compared to lowest yielding environments Sinyanya and Nyahera. LM1 and LM2 agro-ecological zones can be utilized for commercial production of sweet sorghum. Genotypes showed satisfactory yields in the most favorable environments (LM1) such as Sagam and Masumbi. The reason being the ability of genotypes to respond advantageously to better environmental conditions in LM1 compared to LM3 agro-ecological zones. High temperatures and low precipitation are some of contributing factors to poor performance in LM3 AEZ. The slightly better performance of genotypes in Mundika during season one compared to season two could be due to the difference in rainfall during early growth stages of sorghum plants. SS14 was seemingly not fluctuating in yields across environments and it was possible to infer superior yields in unfavourable environments compared to other genotypes.

Table 4.10: The influence of environment on soil components

County	site	**AEZ	pH	Soil type	Nitrogen (%)	Phosphorous (ppm)
Siaya	Masumbi	*LM1	4.4	Clay loam	0.11	9.75
Siaya	Sinyanya	LM3	5.4	Sandy clay loam	0.17	8.8
Busia	Mundika I	LM2	4.4	Sandy clay loam	0.09	6.4
Busia	Mundika II	LM2	4.4	Sandy clay loam	0.09	6.4
Kisumu	Sagam	LM1	5.8	Sandy clay loam	0.12	8.5
Kisumu	Nyahera	LM3	6.0	Sandy clay loam	0.15	5.5

*Lower Midland Zone **Agro-ecological Zone

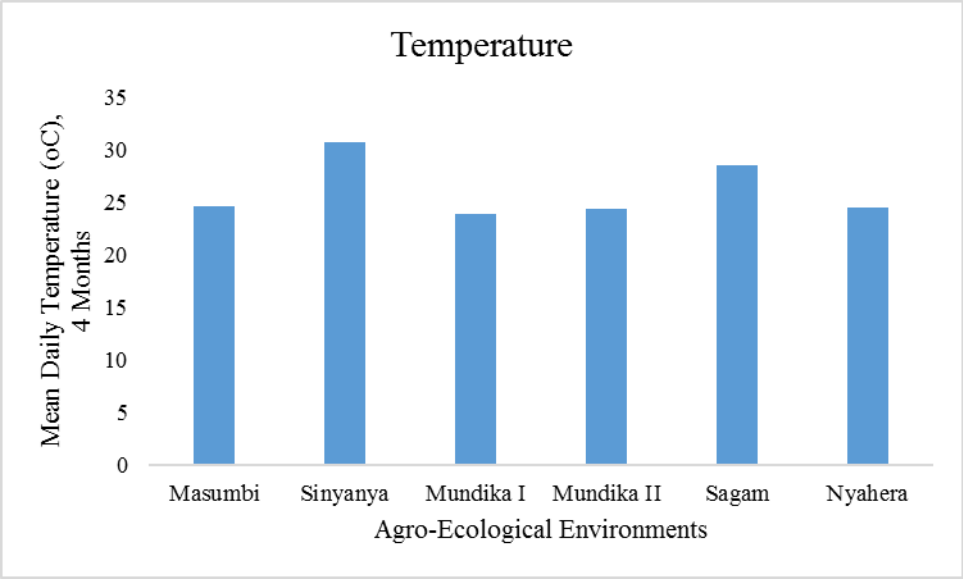

Figure 4.7: Mean daily temperature during sorghum growing season

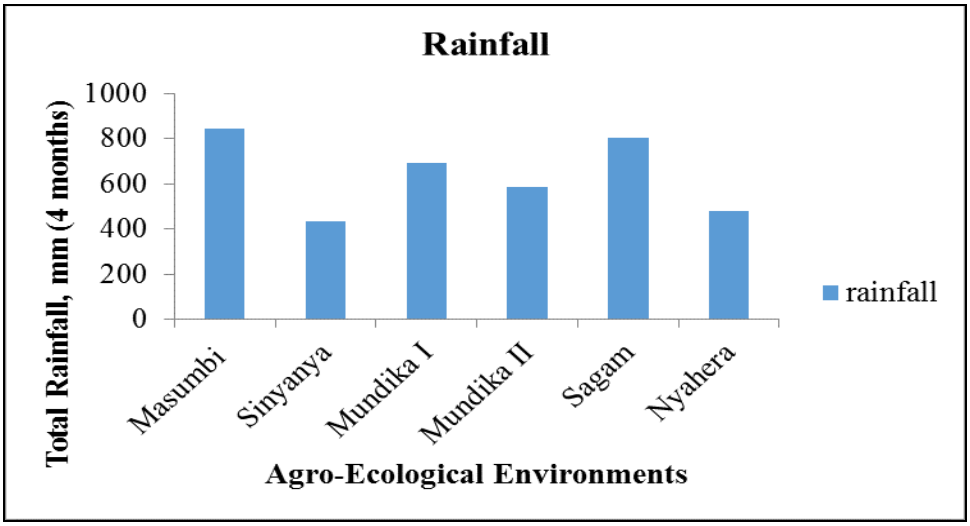

Figure 4.8: Cumulative rainfall during sorghum growing period

4.3.4 AMMI stability values analysis

The combined analysis of variance of cane and juice yield of sorghum genotypes showed that sweet sorghum genotypes were affected by environments (E), genotypes (G), and genotype by environment interaction (GEI) (Table 4.11). However, assessment of genotype by environment (GxE) interaction on ethanol yield stability indicated that GxE was not present for ethanol yield indicating that genotypes did not respond differently to varying environmental conditions. However, some genotypes had higher yields than others indicating genotype

identification to specific environments. G, E, and GEI effects accounted for 8.6, 36.9 and 19.4% respectively for cane yield total sum of squares; 18.8, 24.3 and 22.2% respectively for juice yield total sum of squares and 7.1, 38.4 and 18.5% respectively for ethanol yield total sum of squares (Table 4.11). It is important to note that environment contributed largely to variation in yields.

A large sum of squares shows that environments were diverse, influencing yields differently which was in harmony with the findings of Reddy *et al*. (2014) in sweet sorghum production. Traits such as green biomass, plant height, stem diameter, juice extractability and stem sugar content are major contributors of sweet sorghum's economic importance for bio-fuel production (Almodares *et al*., 2008; Murray *et al*., 2008). However, variability exists in morphological characters of sweet sorghum among genotypes and across locations. Identification of adaptable, stable and high yielding genotypes under different environmental conditions prior to release have been reported by Lule *et al*. (2014) to be the first and foremost steps for plant breeding. Environment expresses most of the total yield variation while genotype and genotype by environment interactions are less effective (Mortazavian *et al*., 2014). The soil's constituents such as moisture content, mineral availability and pH that is an integral part of environment cause large annual variation in yield performance of a crop. GEI can be reduced by identifying genotypes that are most stable (Eberhart and Russel, 1966).

Table 4.11: Additive Main effects and Multiplicative Interaction analysis of variance for cane, juice and ethanol yield of the genotypes across environments

Source of variation	D.f	Cane yield			Juice yield			Ethanol yield		
		SS	MS	Explained (%)	SS	MS	Explained (%)	SS	MS	Explained (%)
Total	143	14691	14691	-	1306609520	9137130	-	7724869	54020	-
Treatments	47	9507	9507***	64.7	827867592	17614204	63.4	4944624	105205***	64.0
Genotypes	7	1257	1257**	8.6	220070892	31438699***	16.8	548971	78424*	7.1
Environments	5	5423	5423***	36.9	317801649	63560330***	24.3	2963587	592717***	38.4
Block	12	957	957	-	70966844	5913904	-	313774	26148	-
Interaction	35	2826	2826*	19.4	289995052	8285573*	22.2	1432066	40916ns	18.5
IPCA1	11	1253	1253*	56.9	107425316	9765938*	54.1	638384	58035*	62.0
IPCA2	9	950	950*	43.1	91016710	10112968*	45.9	391863	43540ns	38.0
Residuals	15	623	623	-	91553026	6103535	-	401819	26788	-
Error	84	4228	50.3	-	407775083	4854465	-	2466471	29363	-

*P<0.05, **P<0.01, ***P<0.001, ns non-significant, d.f = degrees of freedom, SS=sum of square and MS=mean square.

The first Interaction Principal Component (IPCA 1) and the second (IPCA 2) accounted for 56.9 and 43.1% respectively of the cane's IPCA mean squares. The IPCA1 accounted for 54.1 and 62% of juice and ethanol yield interaction sum of squares respectively while IPCA2 accounted for 45.9 and 38% (Table 4.11). The first two principal component axis were significant thus best explain interaction sum of squares and were used in cane and juice yield analysis. However, AMMI model 1 can be used when only one principal component axis is significant to explain the interaction between genotype and environment (Gebremedhin *et al*., 2014) as for ethanol yield in our case.

Environments and genotypes with least ASV scores are considered the most stable. Accordingly, genotypes SS14, SS17 and ACFC003/12 had a general adaptation for cane yield while SS14 was most stable for juice and ethanol yield (Table 4.12). However, SS04 was most unstable for cane, juice and ethanol yield. Similarly, environments were classified using ASV as stable for cane yield (Sinyanya, Mundika season 1 and II), juice yield (Masumbi, Mundika season I and Sinyanya) and ethanol yield (Mundika season II). Nyahera and Sagam were least stable for cane yield while Sagam was unstable for both juice and ethanol yield (Table 4.13).

Furthermore, the IPCA2 scores of genotypes in AMMI analysis indicate stability of genotypes across locations; high IPCA2 score (either negative or positive) are unstable while those with low scores are most stable (Hagos and Abay, 2013). Table 4.12 revealed that genotypes ACFC003/12, SS17, and SS14 for cane yield; SS14, SS04 and EUSS11 for juice yield and EUSS10, EUSS11 and SS14 for ethanol yield were most stable genotypes as they had low IPCA2 scores. The most unstable genotypes were SS04 and SS21 for cane yield, SS17 and EUSS10 for juice yield and SS21, SS17 and EUSS17 for ethanol yield. Stable genotypes follow genes that affect the trait in question and their expression relative to the environment being similar to average cultivar while unstable genotypes have genes that are challenged differently by a different environment (Ngeve and Bouwkamp, 1993). Table 4.13 further revealed that Masumbi had the highest IPCA2 score for both cane and ethanol while Mundika season II had highest IPCA2 score for juice yield hence they were the most interactive environments. Sinyanya, Nyahera and Mundika season II were the least interactive for cane, juice and ethanol yield respectively.

Table 4.12: The first two interaction principal component axis (IPCA) scores and AMMI stability values (ASV) for genotypes

Genotype	Cane yield				Juice yield				Ethanol yield			
	Mean (t/ha)	PCA1	PCA2	ASV	Mean (l/ha)	PCA1	PCA2	ASV	Mean (l/ha)	PCA1	PCA2	ASV
SS04	21	-3.10	2.07	4.58	5112	-50.24	-18.57	62.1383	350	-13.18	3.76	21.80
SS14	22	-0.06	0.32	0.33	5835	11.11	-13.30	18.68	376	0.19	-4.64	4.65
SS21	16	-1.17	-2.56	2.99	3099	-17.65	27.03	34.12	230	2.97	10.12	11.22
SS17	20	0.30	0.70	0.80	4635	14.65	-38.73	42.43	306	1.74	-9.28	9.70
EUSS17	20	0.87	1.07	1.57	5018	17.68	-29.10	35.81	358	3.82	-9.02	10.96
EUSS10	27	2.90	1.02	3.97	7807	25.14	34.07	45.17	420	-10.19	-1.04	16.64
EUSS11	23	0.39	-1.94	2.01	5794	30.20	18.18	40.01	413	12.31	3.69	20.40
ACFC003/12	24	-0.13	-0.68	0.17	5455	-30.89	20.44	41.79	423	2.343	6.41	7.46

Table 4. 13: AMMI stability values of cane, juice and ethanol yield for 8 sweet sorghum genotypes evaluated in different environments

Environments	Cane yield				Juice yield				Ethanol yield			
	Mean (t/ha)	PCA1	PCA2	ASV	Mean (l/ha)	PCA1	PCA2	ASV	mean (l/ha)	PCA1	PCA2	ASV
Masumbi	23	0.49	2.96	3.03	6451	-6.83	-36.33	37.21	432	-11.81	-11.14	22.24
Mundika I	22	-0.58	1.19	1.41	5361	-26.19	22.27	38.09	211	-7.78	5.53	13.83
Mundika II	21	0.96	-0.98	1.60	5309	6.01	48.53	49.04	416	1.66	1.66	3.18
Nyahera	18	-3.04	-1.87	4.42	3817	-36.46	0.45	43.04	245	1.54	8.64	8.99
Sagam	33	3.00	-1.72	4.32	7753	62.33	1.63	73.58	614	16.00	-7.11	27.02
Sinyanya	14	-0.84	0.42	1.18	3374	1.14	-36.53	36.56	238	0.39	-4.01	4.06

4.3.5 Genotype plus genotype by environment (GGE) biplot analysis

GGE biplots refer to biplot that displays the G and GE of a genotype-by-environment data. The biplot analysis presents a holistic picture of multi-environment variety trials. Genotypes or environments located on the right-hand side of the midpoint of the axis (IPCA1) have higher yields than those on the left-hand side (Asio *et al*., 2005). In this study, genotypes EUSS10, ACFC003/12, SS14 and EUSS11 for cane yield (Fig. 4.9); EUSS10, EUSS11 and SS14 for juice yield (Fig. 4.10) and EUSS10, SS04, SS14 and ACFC003/12 for ethanol yield (Fig. 4.11) were generally high yielding as they were placed on right-hand side of midpoint of IPC1 axis (representing grand mean). Similarly, Mundika season I and II, Sagam and Masumbi were considered best performers for cane yield (Fig. 4.9), while all sites except Nyahera produced high juice yield (Fig. 4.10). However, all sites performed above average in terms of ethanol yield (Fig. 4.11).

The polygon view of GGE biplot for cane yield (Fig. 4.9) indicates the best genotypes(s) for each environment(s). The genotypes EUSS10, ACFC003/12 and SS14 were found to be promising in Masumbi, Sagam, and Mundika season I and II (LM1 and LM2). EUSS17 and SS04 were better adapted to Nyahera (LM3) which is low performing site. The genotypes located on the vertex of a polygon are the ones that gave the highest yield for the environment that fall within that quadrant. The vertex genotypes were EUSS17, SS04, SS21, EUSS10 and EUSS11 for cane yield. Genotype EUSS10 recorded the highest cane in Masumbi and Mundika during season I and II. EUSS11 gave the highest cane in Sagam while both SS04 and EUSS17 were best-performing genotypes in Nyahera and Sinyanya. The polygon reflects that SS21 is poor cane yielding not suitable for neither of the environments. The genotypes located on the vertex of a polygon are best or poorest genotypes in some or all environments except left-bottom quadrant (Hagos and Abay, 2013).

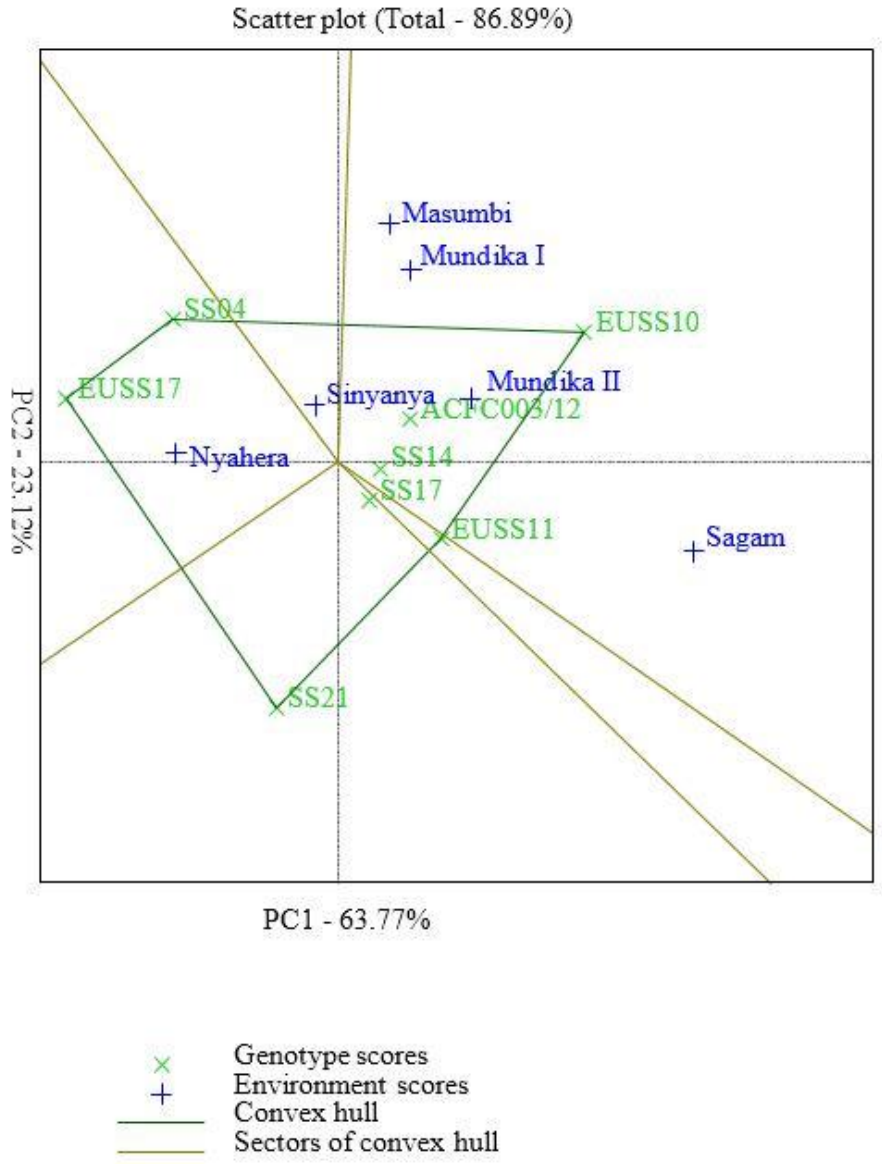

Figure 4.9: The which-won-where view of GGE biplot for cane yield

The GGE biplot for juice yield (Fig. 4.10) indicates that SS14 and EUSS10 are suitable for cultivation in LM1, LM2, and LM3 as represented by Mundika during season I and II, Masumbi, Sinyanya and Sagam while ACFC003/12 and SS04 were better adapted to Nyahera (LM3). EUSS11 recorded the highest juice volume in Sagam, Sinyanya, and Mundika during season II while SS04 recorded the highest in Nyahera. EUSS10 was the best performer in Masumbi and Mundika season I. Genotypes SS21, SS17 and EUSS17 felled into sectors where there were no locations. These genotypes are poorly adapted to all environments that were tested. Locations in one sector having best-performing genotype can be considered as mega-environments for that genotype (Gebre and Mohammed, 2015). These

results are in conformity with the findings of Reddy *et al*. (2014) who observed high yielding and stable genotypes for cane and juice yield.

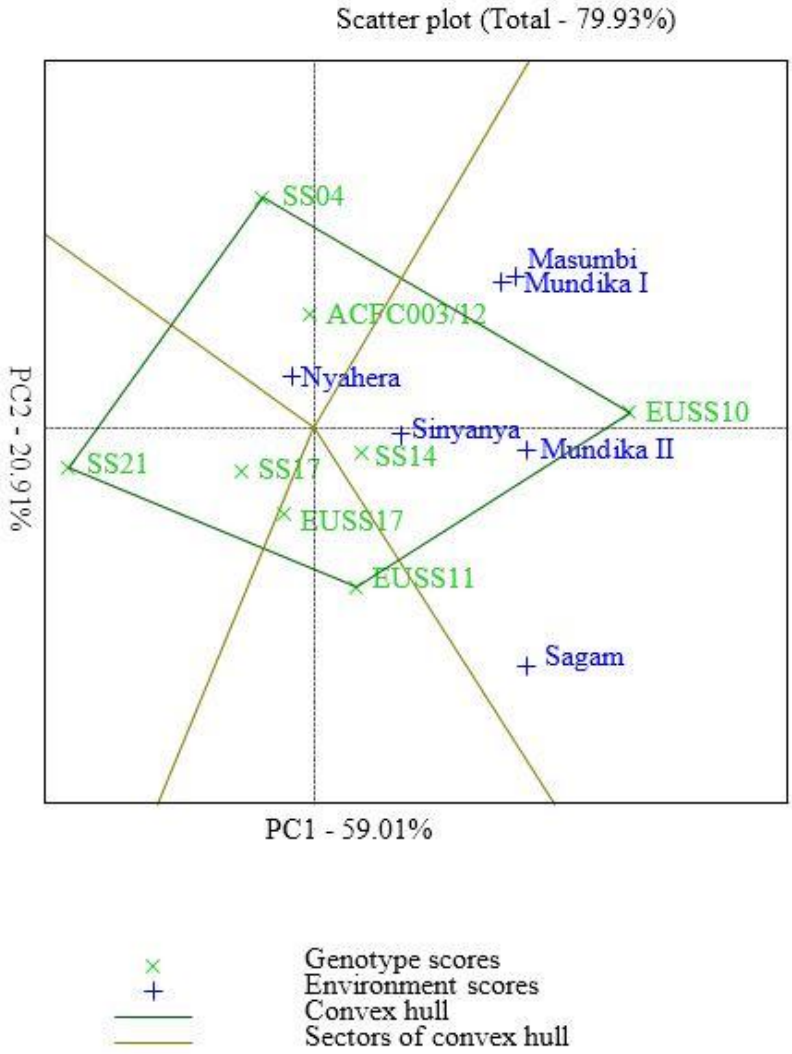

Figure 4.10: The which-won-where view of GGE biplot for juice yield

Biplots were divided into four sectors in figure 4.11; genotypes which fall in same sector as with environment are said to be adapted to those locations. In the present study, genotypes EUSS10 and SS14 were adapted to Masumbi, Mundika season I and II and Sinyanya (LM1, LM2 and LM3). EUS11, ACFC003/12 and EUSS17 were suitable for cultivation in Nyahera and Sagam (LM1 and LM3). Furthermore, figure 4.11 displays 'which won where' feature of biplots. EUSS11 had the highest ethanol yield in Sagam. Genotypes SS04 and EUSS10 were the winning genotypes in Masumbi and Mundika during the 1st season for ethanol yield. SS21 and SS17 were poor performers for ethanol yield and were not suitable for tested environments.

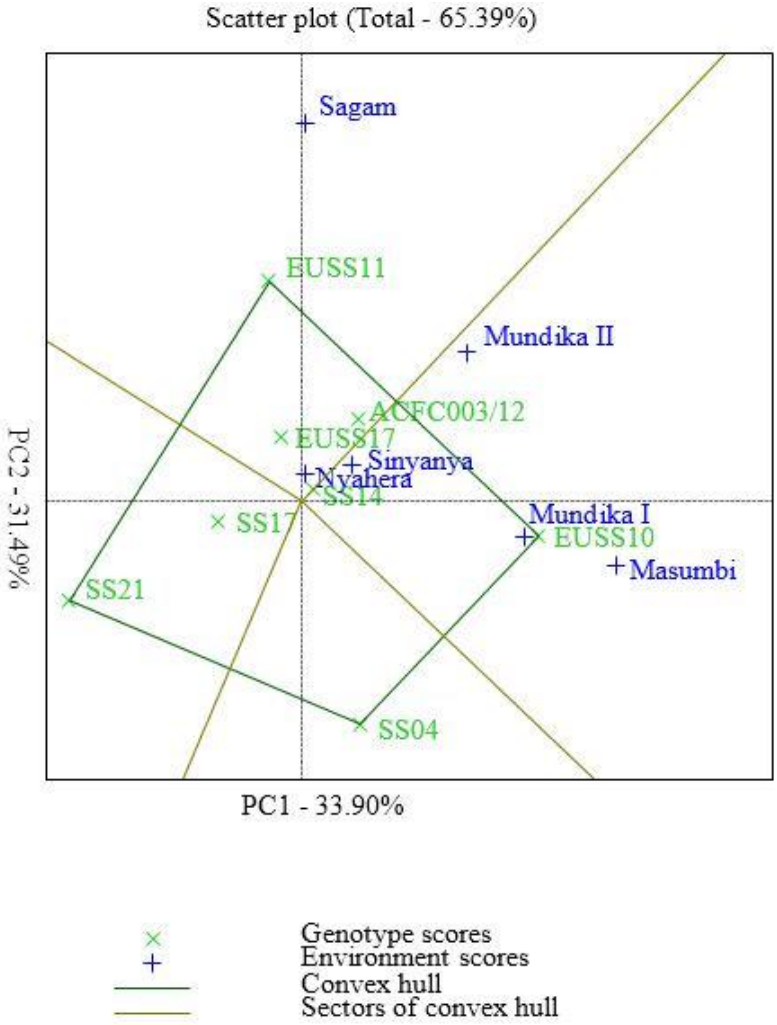

Figure 4.11: The which-won-where view of GGE biplot for ethanol yield

Furthermore, figures 4.9, 4.10 and 4.11 shows the stability and adaptability of genotypes in terms of cane, juice and ethanol yield respectively. Genotypes SS14 and SS17 for cane yield had the shortest vector from origin whereas SS14 for both juice and ethanol yield were close to the origin. Moreover, SS14 genotype had IPCA1>0 and is therefore regarded as stable and high yielding. Genotype EUSS10 had the highest IPCA1 score and located close to IPCA2 axis for both juice and ethanol yield, indicating it is high yielding genotype but specifically adapted. Dynamic as opposed to static stability is preferred by breeders and agronomist in order to have genotypes which can produce more yields when optimal agronomic inputs and favorable environmental conditions are provided (Djurovic *et al*., 2014). Therefore, SS14 can be chosen for wider adaptability and EUSS10 for favorable environments. Genotype ACFC003/12 had medium stability for cane and ethanol yield across environments.

Figures 4.12, 4.13 and 4.14 gives vector view of GGE biplot in which environments are connected with biplot origin via lines. It also shows the relationship among genotypes. This view of biplot aids in the understanding of interrelationship among environments. The cosine of the angle between the vectors of two environments approximates the correlation coefficient between them. Environments with a small angle between them are highly positively correlated, and they provide similar information on genotypes. Present investigations showed that Masumbi and Mundika for cane, juice and ethanol yield (Fig. 4.12, 4.13 and 4.14) and Nyahera and Sinyanya for ethanol yield (Fig. 4.14) were considered to be similar as they had small angle between them. In contrast, genotypes EUSS10 and SS21, SS04 and EUSS11 were located in opposing quadrants for cane, juice and ethanol yields; therefore, the angles between them were larger and are considered dissimilar genotypes. Similarly, Nyahera and Sagam were dissimilar for both cane and juice yield. Sinyanya and Nyahera lay closest to the origin, therefore, contributed the least to GEI for cane, juice and ethanol yield while Sagam made the highest contribution. From this study, it is evident that low-performing genotypes are stable and have wider adaptability whereas high performing genotypes are less stable.

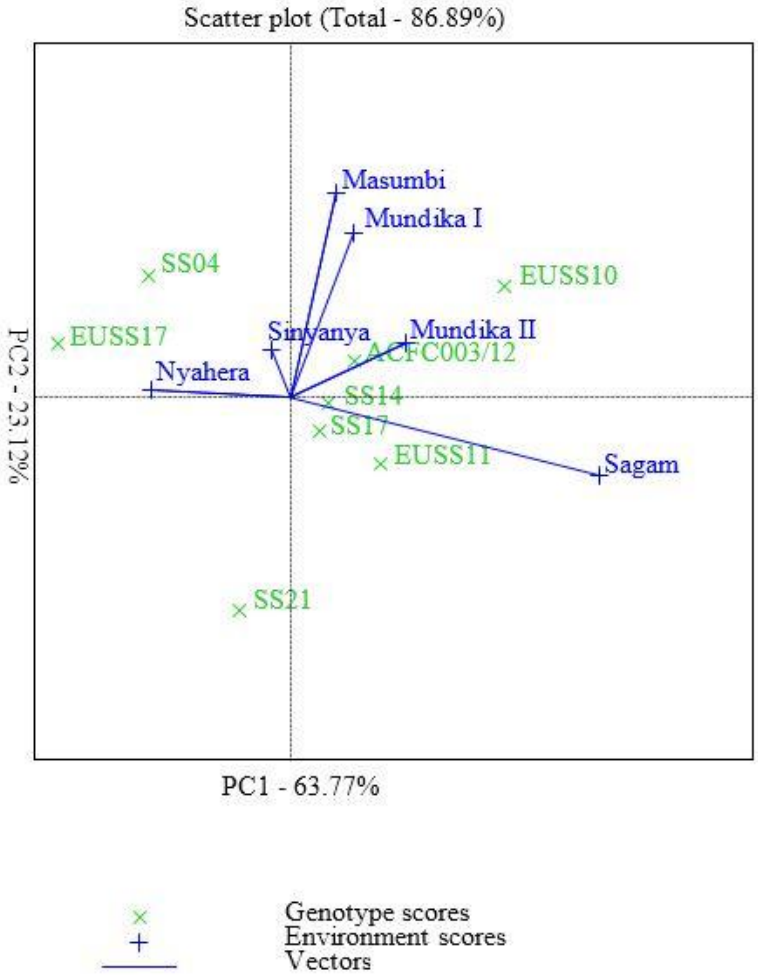

Figure 4.12: The biplot showing relationship between testers and mega environments for cane yield

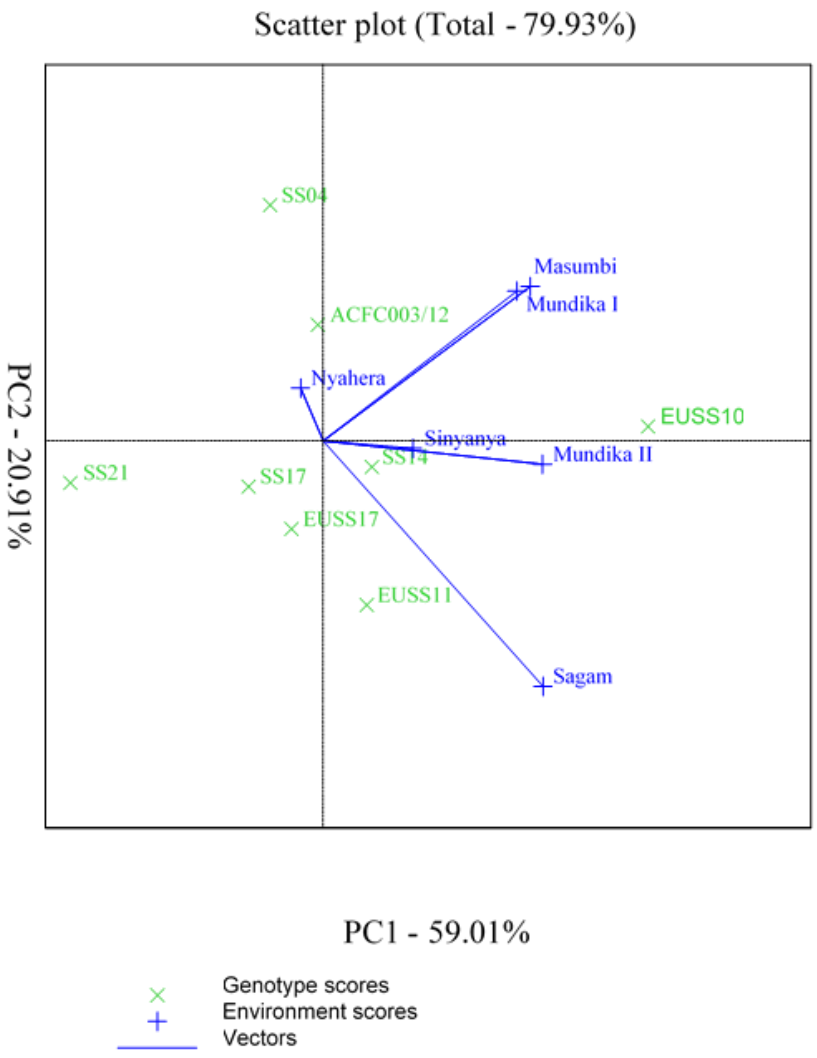

Figure 4.13: The biplot showing relationship between testers and mega environments for juice yield

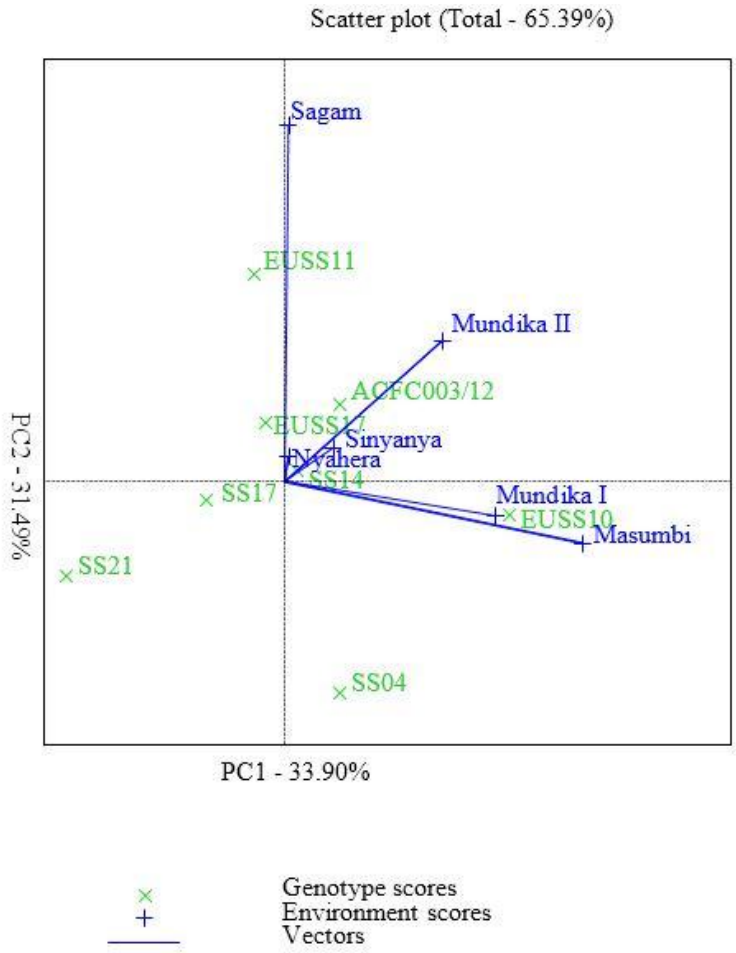

Figure 4.14: The biplot showing relationship between testers and mega environments for ethanol yield

Cane yield was found to be highly correlated with plant height. A study by Abubakar and Bubuche, (2013) in Nigeria found out that genotype by environment interaction had a significant influence on sorghum plant height. Differences in plant height can result in changes in cane yield across environments; therefore, genotypes adapted to specific locations have to be selected. Biomass yield and plant height have been found to be major contributors to economic yields in sweet sorghum (Bahadure *et al*., 2014). Furthermore, ANOVA revealed there was a significant effect due to genotype by environment interaction. This indicates that genotypes performed differently at each site which is expected due to differences in soil composition, rainfall, and temperature. Ideal cultivars and environments are those having large PC1 scores (high mean yield) and small PC2 scores (high stability) (Frashadfar *et al*., 2012). Based on this Mundika season I and Masumbi were found to be ideal environments whereas SS14 was ideal genotype for ethanol production. Genotype EUSS10 was the

winning genotype for ethanol yield in Masumbi, Mundika both in season one and two and in Sinyanya, therefore, suitable for those sites.

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CHAPTER FIVE
SUGAR PROFILE OF JUICE EXTRACT IN SELECTED SWEET SORGHUM GENOTYPES

Abstract

Sweet sorghum (*Sorghum bicolor* L. Moench) is a high sugar yielding crop. Owing to its early maturity, adaptability to low rain-fed conditions and high sugar content in its stem, it is regarded as best alternative feedstock for ethanol production. The aim of the study was to determine the amount of total and reducing sugars among selected eight sweet sorghum genotypes grown at diverse locations. Stalk juice extract of sweet sorghum was collected for each genotype across experimental sites and kept at 4 ^{0}C before laboratory analysis. Total soluble sugars were determined by phenol-sulphuric acid method whereas reducing sugars was determined by Dinitrosalycyclic acid method. Non-reducing sugars were estimated by subtracting reducing sugars from total sugars. Analysis of variance was performed using SAS software and means separated by least significance difference at $P<0.05$. There was no significant difference among sweet sorghum genotypes for reducing sugars in Nyahera and Sagam locations. However, significant differences were observed for reducing sugar in Sagam and total sugars in all sites among genotypes. On average, Sagam, Mundika season II and Nyahera recorded 1.7, 1.9 and 1.7% reducing sugars respectively. The highest amount of total sugars was recorded by SS04 (11.8%), SS21 (13.6%) and SS14 (12.3%) in Masumbi, Mundika and Sinyanya respectively during the first season. The least was recorded by ACFC003/12 (9%) in Mundika and EUSS10, both in Masumbi (7.1%) and Sinyanya (9.6%). During the 2nd growing season, the highest amount of sugar was recorded by SS21 in both Mundika (11.8%) and Sagam (10.8) and EUSS11 (11.8%) in Nyahera. Non-reducing sugars averaged 7.8, 7.4 and 8.1% in Nyahera, Sagam and Mundika respectively. Significant positive correlation was observed between total sugars and Brix and total sugars and ethanol for Sinyanya. From this study, it is evident that eight sweet sorghum genotypes have a relatively high amount of total sugars though there was no significant link between sugars and ethanol yield.

Keywords: Total sugars, Reducing sugars, Non-reducing sugars, Genotypes

5.1 Introduction

Sorghum (*Sorghum bicolor* L. Moench) is major cereal crop mainly cultivated in arid and semi-arid regions of the world (Smith and Frederiksen, 2000). Sweet sorghum is distinguished from grain sorghum variety as it accumulates high concentration of soluble sugars in stem juice. Stem sugar content is an important trait in sweet sorghum that contributes to ethanol yield from fermented stem juice. However, there are variations in sugar content among the sweet sorghum genotypes. These sugars are stored intracellularly within large vacuoles of stem parenchyma cells (Tarpley and Vietor, 2007). Sucrose storage tissues exhibit low metabolic activities upon maturation of stem (Tarpley *et al*., 1996) thus stored sucrose accumulates. Research conducted by Poloewetse (2012) found that amount of sucrose decreases from top internode to basal internode of matured sweet sorghum whereas sucrose levels along the mature stem of sugarcane are constant. Sugars are translocated from leaves and are stored in relatively more vacuolated stem thus a higher level of sugars in the stem (Bhatia and Singh, 2001). Production of bioethanol from cane crops depends on sucrose levels in their stems (Moreira, 2000, Chohnan *et al*., 2011) and a high amount of sucrose is an important attribute of sweet sorghum for production of ethanol used as biofuel (Waclawovsky *et al*., 2010; Calvino and Messing, 2012). Therefore, it was necessary to determine the amount of sugar among selected sweet sorghum genotypes to assess its association with ethanol yield.

Sweet sorghum stalks have higher sugar content compared with other sorghum types and can be used to produce both first and second generation bioethanol. First generation ethanol is obtained from hydrolysis of starch in the grains whereas stem provides soluble sugars in juice when extracted. Second generation ethanol can be obtained from all lignocellulosic materials such as bagasse, however, technology is still not yet available for efficient ethanol production from such biomass. Sweet sorghum juice pH is between 4 and 5.5 that are optimal for yeast growth and ethanol production (Narendranath and Power, 2005). In addition, juice contains trace essential elements for microbial growth and ethanol production (Laopaiboon *et al*., 2009). Sugars in sweet sorghum stem require less energy as they are readily fermentable compared to starch in the grain which has to be hydrolyzed to simple sugars before conversion to ethanol. Furthermore, sweet sorghum has a potential of producing ratoon crop after harvest which can further provide more material for ethanol production. These special attributes make sweet sorghum juice suitable raw material for ethanol production. To enhance food security in Kenya, sweet sorghum can be used for the

production of grain for food and ethanol from stems thus results in the development of rural zones that are marginal for sugarcane and maize.

Sugarcane juice is used for commercial production of table sugar while sweet sorghum is not. Sweet sorghum stem juice has relatively high aconitic acid and starch that inhibit crystallization of sugar and therefore developing new technologies to reduce inhibitors can lead to commercial production of sugar from sweet sorghum (Bamber, 1980). At the moment, most of the sugar in sorghum is geared towards production of ethanol used as biofuel either pure or blended with petrol.

High ethanol production in sweet sorghum is attributed to green cane yield, Brix, juice stalk extractability, the content of reducing and non-reducing sugars and grain yield (Iraddi *et al*., 2014). However, the concentration of soluble sugars in sorghum varies widely depending upon the variety. Several attempts have been tried to improve sugar levels in sweet sorghum stems. Investigation on the effect of nitrogen fertilization and plant density on productivity and quality of sweet sorghum by Mahmoud *et al*. (2013) found that Brix and reducing sugars increased significantly when they increased nitrogen rate up to 120 kg per feed. Removal of heads before grain formation has been reported to increase Brix and sugar in sweet sorghum stalks (Broadhead, 1973; Erickson *et al*., 2011). Greater sugar accumulation in the stem associated with sterility and top removal has been attributed to changes in patterns of assimilate partitioning with stem becoming the predominant alternative sink (Lin and Lin, 1994).

Sweet sorghum accessions that accumulate more starch tend to accumulate less sucrose in stem tissues. Furthermore, high starch levels in seeds tend to have a negative effect on sucrose accumulation in stems, accessions which accumulate high amount of sucrose tends to accumulate less starch in their seeds (Poloewetse, 2012). This observation may be due to the competition of triose phosphate, a product of photosynthesis which can either be diverted for sucrose or starch biosynthesis. Grain filling has been reported to be supported by photosynthesis and remobilization of stored carbohydrates in the stem (Yang and Zhang, 2006). Starch synthesis is promoted when sucrose synthesis is restricted and in many plant species, leaf starch serves as a transient sink to accommodate excess photosynthate that cannot be converted to sucrose and exported (Paul and Foyer, 2001). High photosynthetic activity occurs during high light intensity and CO_2 levels and triosephosphate have to be converted either to starch or sucrose to prevent feedback inhibition of photosynthesis. Another approach to increasing sugar levels is breeding to increase the size of stem; both thickness and height which results in higher juice volume and an increase in sucrose

concentration (Patrick *et al*., 2013). Similarly, sucrose content in stem can be increased by reducing sink strength of grain thereby reducing competition for photoassimilates through generation of sweet sorghum varieties with larger stems and reduced panicle size (Bihmidine *et al*., 2013).

Several studies have been carried out to characterize the interaction pattern of sugar accumulation and its components between sweet sorghum genotypes and the environment (Makanda *et al*., 2012; Elangovan *et al*., 2014). The results confirmed the presence of a significant interaction between the genotype and the environment as a consequence of the differential response of the genotypes to environmental changes. In all of the cases, the variation that resulted from the environment had the largest contribution to the total observed variation in the sugar contents (De Vries *et al*., 2010). In sweet sorghums, the sugar content and the stem juice yields are quantitative traits with polygenic inheritance, highly affected by the environment (Zou *et al*., 2011). There is great variation in the sugar content in the stems of sorghum varieties, the Brix in the juice extracted from the stem of 200 cultivars ranged from 8.0% to 19.1% (Zhao *et al*., 2008). This variation is even more complex because not all of the genotypes responded to environmental changes in the same way (Elangovan *et al*., 2014).

Among other agronomical traits, sugar content in sweet sorghum stalks affects ethanol yields. The ability of sorghum genotypes to produce more ethanol depends on the amount of fermentable sugars. However, there is no information in literature revealing the sugar profile of sweet sorghum under study and how it influence ethanol. Therefore, the objective of present study was to investigate the amount of fermentable sugars of sweet sorghum genotypes grown at diverse geographical locations. The use of sweet sorghum to supplement molasses will lead to increased ethanol which can be exported to earn country foreign exchange.

5.2 Materials and methods

5. 2. 1 Obtaining cane juice

Sorghum stalks were harvested at hard dough stage of grain from two inner rows and crushed to obtain juice after heads and leaves were removed. Juices for each genotype were sampled from all the experimental sites and filtered through a sieve to remove chaff then stored at 4^0C in fridge for sugar analysis. Extracted juice was analyzed for Brix using portable refractometer, total sugar by phenol-sulphuric acid method (Dubois *et al.,* 1956) and reducing sugar by Dinitrosalycyclic acid method (Miller, 1959) as detailed below.

5. 2. 2 Determination of total soluble sugars

Total soluble sugar content was determined by modified phenol-sulphuric acid method (Dubois *et al.,* 1956). A standard curve was prepared to quantify total sugar content in the stem juice. Phenol, 5 % was prepared by dissolving 50 g of reagent grade phenol in water and diluted to one liter. Reagent grade sulphuric acid (96%) was prepared by delivering 960 ml of absolute concentrated sulphuric acid into a beaker having 40 ml of distilled water.

Stock glucose solution (0.1 %) was prepared by dissolving 100 mg of glucose in 100 ml of distilled water. Working standard solution (100 µg/ml) was then prepared by diluting 10 ml of stock solution to 100 ml with distilled water. A set of standard glucose solutions of strengths 0, 20, 40, 60. 80 and 100 µg/ml was then prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard and respectively adding into each tube 1.0, 0.8, 0.6, 0.4, 0.2 and 0 ml of distilled water so that the volume in each tube was made up to 1 ml. The 1 ml distilled water ('0%' concentration) served as blank.

The sample solution (1 ml) collected from each plot was diluted with distilled water. Diluted sample (0.1 ml) was then pipette into a separate test tube and volume in each tube made to 1 ml with distilled water. To each test tube contents, 1 ml of 5 % phenol was added followed by addition of 5 ml of concentrated sulphuric acid (96 %) with concurrent stirring to mix. After 10 minutes the contents in tubes were shaken and kept at room temperature for 20 minutes. The optical density of each tube was then read in a spectrophotometer at a wavelength of 490 nm. A standard curve of glucose was prepared by plotting a graph of absorbance against glucose concentration. The amount of sugar in the sample was then determined by reference to a standard curve. Absorbance corresponds to 0.1 ml of test = *x* mg of glucose. Percent total carbohydrate present is equal to amount of total sugar (grams) in 100 ml of sample solution and is given by:

$$Total\ carbohydrate\ (\%) = \frac{x}{0.1}\ x\ 100\ mg\ of\ glucose\ x\ dilution\ factor \quad \ldots\ldots\ldots\ldots\ 5.1$$

5. 2. 3 Determination of total reducing sugars

Total reducing sugars was determined by modified dinitrosalicyclic acid (DNS) method (Miller, 1959). The DNS reagent was formed by dissolving 10 g of Dinitrosalicyclic acid, 2 g crystalline phenol, 0.5 g sodium sulphite and 10 g sodium hydroxide in one litre of distilled water. The stock glucose (0.1 %) solution was prepared by dissolving 100 mg of glucose in 100 ml of distilled water. Working standard solution (100 µg/ml) was then prepared by diluting 10 ml of stock solution to 100 ml with distilled water. A set of standard

glucose solutions of strengths 0, 20, 40, 60. 80 and 100 µg/ml were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of working standard and respectively adding into each tube 1.0, 0.8, 0.6, 0.4, 0.2 and 0 ml of distilled water so that the volume in each tube was made up to 1 ml. DNS reagent (1 ml) was added to each test tube of the standard solutions, mixed and then heated at 90 ^{o}C for 5 minutes. A 1 ml of 40% Rochelle salt solution was added to each tube contents and allowed to cool before taking absorbance at 510 nm. Spectrophotometer readings were used to prepare standard curve for estimating the amount of reducing sugars in the juice samples.

Sample juices (1 ml) were diluted with distilled water before the development of colour. Diluted sample juice (0.5 ml) was put in separate test tubes and volume equalized to 3 ml with distilled water, followed by addition of 3 ml DNS reagent. The mixture was then heated in boiling water at 90 ^{o}C for 5 minutes. It was removed from the water bath and 1 ml of 40% Rochelle salt solution (potassium sodium tartrate) added when the contents of tubes were still warm. The mixture was allowed to cool to ambient temperature and absorbance read at 510 nm.

A standard curve of glucose was prepared by plotting a graph of absorbance against glucose concentration. The amount of reducing sugar in the sample was then determined by reference to a standard curve. Absorbance corresponds to 0.5 ml of test= *x* mg of glucose. The amount of reducing sugars in milligrams in 0.5 ml of sample is represented by *x*. Percent total reducing sugar present is equal to amount of reducing sugar (in grams) in 100 ml of sample solution and is given by:

$$Total\ reducing\ sugars\ (\%) = \frac{x}{0.5}\ x\ 100\ mg\ of\ glucose\ x\ dilution\ factor \quad \text{.....5.2}$$

The amount of non-reducing sugar was obtained by subtracting reducing sugar from total sugar.

5. 2. 4 Statistical analyses

Statistical analyses were performed using SAS program version 9.1. ANOVA was done and the means were compared according to least significant difference test. Linear regression analyses were done by MS Excel.

5.3 Results and discussion

5. 3. 1 Influence of environment on sugar content among sweet sorghum genotypes

Total soluble sugars were determined for all genotypes across experimental sites in both seasons; however, reducing sugars was determined during the second growing season. There were no differences among genotypes in Nyahera and Sagam in terms of percent reducing sugars averaging 1.7% in both sites (Table 5.1). Genotypes SS14, SS21, SS17 and EUSS17 recorded significantly higher total reducing sugars than ACFC003/12 in Mundika during season II. Highest and lowest reducing sugar was recorded by SS21 and ACFC003/12 respectively in Mundika season II. Similar results were found by Shinde *et al*. (2013) for reducing sugars ranging 0.8-3.5% though they reported much diversity for non-reducing sugar among 46 sweet sorghum genotypes. Almodares *et al*. (2010) obtained highest invert sugars (3.9%) on the application of 100 kg/ha of urea during planting and 200 kg/ha urea at 4 leaf stage of sweet sorghum plants. Reducing sugars majorly comprise of glucose and fructose in sweet sorghum juice and are first converted to ethanol before sucrose by yeast during the fermentation process. Sucrose, glucose and fructose were found to be the only fermentable sugars at maturity stage of twelve cultivars of sweet sorghum in a study by Oldham *et al*. (2003). They reported total soluble sugars in juice to vary from 7.9 to 17.6%. From the study reducing sugars did not significantly affect ethanol yield of sweet sorghum genotypes.

Table 5.1: Reducing sugars among sweet sorghum genotypes during September-December season, 2014

Genotype	Reducing sugars (%)		
	Environments		
	Mundika (LM2)	Nyahera (LM3)	Sagam (LM1)
SS04	1.68±0.74[ab]	1.92±0.61[a]	1.99±0.92[a]
SS14	2.20±0.07[a]	1.47±0.27[a]	1.92±0.44[a]
SS21	2.28±0.17[a]	2.31±0.36[a]	1.20±0.34[a]
SS17	2.24±0.29[a]	1.45±1.09[a]	1.88±0.25[a]
EUSS17	2.24±0.72[a]	1.79±0.19[a]	1.51±0.69[a]
EUSS10	1.56±0.75[ab]	1.59±0.52[a]	1.14±0.32[a]
EUSS11	1.90±0.61[ab]	1.60±0.29[a]	2.05±0.47[a]
ACFC003/12	1.18±0.04[b]	1.79±0.39[a]	1.66±0.69[a]
LSD	0.87	0.99	1.00

Means are presented as mean ± standard deviation

Means followed by the same letter in a column are not significantly different at 5% LSD

Total soluble sugars were affected by genotypes in each location during the first season (Table 5.2). The control SS04 recording higher total sugars across environments whereas EUSS10 performed poorly. Genotypes had higher total sugars in Sinyanya except SS04 and SS21. EUSS10 recorded 4.7 and 2.7% lower total sugars compared to SS04 and SS14 in Masumbi and Sinyanya respectively, the other genotypes were similar. EUSS17 was among the superior sugar yielding genotypes in Mundika for total sugar with SS21 recording the highest. High sugar levels obtained from Sinyanya was due to desiccation of stalks resulting in more concentrated juice. Massacci *et al*. (1996) reported that water stress in sweet sorghums leads to a reduction in the internode elongation as a result of a decrease in the enzyme activities responsible for sugar degradation, which in turn stimulates stem sucrose accumulation. This could explain the high sucrose recorded by genotypes in Sinyanya site that was characterized by low amount of rainfall during the growth period. Alhajturki *et al.* (2012) suggested that the accumulation of sugar in sweet sorghum under water stress was an osmotic adjustment mechanism that allowed the plant to maintain water absorption for higher

rates of photosynthesis and plant growth. The sugar content in stem juice was affected by the variety of sweet sorghum as reported by Almodares *et al*. (1994) and Reddy *et al*. (2005).

Table 5.2: Total sugars among eight sweet sorghum genotypes during March-July season, 2014

Genotypes	**Total sugars (%)**		
	← Environments →		
	Masumbi (LM1)	Mundika (LM2)	Sinyanya (LM3)
SS04	11.82±1.32^{a}	13.05±1.23^{a}	11.71±0.80^{ab}
SS14	9.74±2.21^{ab}	9.39±1.08^{c}	12.34±1.83^{a}
SS21	8.88±0.96^{bc}	13.55±1.26^{a}	10.79±1.34^{ab}
SS17	9.76±1.45^{ab}	9.82±0.70^{bc}	11.42±2.08^{ab}
EUSS17	9.16±0.44^{bc}	11.67±0.67^{ab}	11.49±1.91^{ab}
EUSS10	7.09±0.80^{c}	9.61±1.51^{c}	9.63±0.89^{b}
EUSS11	10.12±1.57^{ab}	8.98±0.77^{c}	11.41±0.74^{ab}
ACFC003/12	10.15±1.90^{ab}	8.93±1.24^{c}	10.93±1.06^{ab}
$LSD_{0.05}$	2.55	2.04	2.47

Means are presented as mean ± standard deviation

Means followed by the same letter in a column are not significantly different at 5% LSD

The total sugars and non-reducing sugars for genotypes in Nyahera and Sagam were similar (Table 5.3). SS17 was consistently performing well across environments for total and non-reducing sugars. However, the performance of ACFC003/12 was similar with SS17 except in Sagam where it had the least percent sugars. Though EUSS10 and EUSS11 were low performing genotypes in Mundika for both total and non-reducing sugars, they had similar sugars with the controls SS17 and SS14. EUSS17 recorded similar sugars with all controls in Mundika and Sagam. In Nyahera, EUSS11 had the highest total sugars that were not different from other controls except SS21. From this study, it is evident that EUSS10 recorded generally lowest total sugars compared to other genotypes though it had the highest cane, juice and ethanol yields. The total soluble sugar (14.8-19.4%), sucrose (10.2-17.8%) and reducing sugars (4.5-5.5%) recorded by El-Geddawy *et al*. (2014) in six sweet sorghum varieties is relatively higher than from current study.

Table 5.3: Influence of environment on total and non- reducing sugars among sweet sorghum genotypes during September to December season 2014

Genotype	Environments					
	←Mundika (LM2)→		←Nyahera (LM3)→		←Sagam (LM1)→	
	Total sugars (%)	Non-reducing sugars (%)	Total sugars (%)	Non-reducing sugars (%)	Total sugars (%)	Non-reducing sugars (%)
SS04	11.1±1.8^{a}	9.4±1.8^{ab}	10.2±1.9^{ab}	8.2±2.2^{ab}	8.8±0.8^{bc}	6.8±0.2^{bc}
SS14	8.6±1.0^{b}	6.4±1.1^{c}	10.1±0.6^{ab}	8.7±8.9^{ab}	9.0±1.7^{bc}	7.1±1.8^{bc}
SS21	11.8±2.8^{a}	9.5±3.0^{ab}	8.9±1.0^{bc}	6.6±1.0^{bc}	10.8±1.5^{a}	9.6±1.3^{a}
SS17	10.3±0.7^{ab}	8.1±0.4^{abc}	9.8±0.5^{ab}	8.3±0.7^{ab}	10.2±1.0^{ab}	8.3±1.1^{ab}
EUSS17	10.2±1.1^{ab}	8.0±0.8^{abc}	9.2±0.4^{bc}	7.4±0.6^{bc}	10.0±1.6^{ab}	8.4±2.3^{ab}
EUSS10	8.7±0.9^{b}	7.2±0.3^{bc}	7.1±0.8^{c}	5.5±0.9^{c}	7.3±1.2^{c}	6.2±1.2^{c}
EUSS11	8.7±0.6^{b}	6.8±1.0^{c}	11.8±1.3^{a}	10.2±1.6^{a}	8.9±0.6^{bc}	6.8±0.3^{bc}
ACFC003/12	11.0±1.3^{a}	9.8±1.3^{a}	9.7±1.2^{ab}	8.0±2.3^{abc}	7.3±1.2^{c}	5.7±1.6^{c}
LSD	2.2±5	2.4	2.3	2.5	1.8	2.0

Means are presented as mean ± standard deviation

Means followed by the same letter do not differ in a column at 5% LSD

Sucrose has been reported to be dominant to glucose and fructose in sweet sorghum stem juice (Amaducii *et al*., 2004; Han *et al*., 2012). Non-reducing sugars can be regarded as sucrose in our case and highest values were recorded by EUSS11 (10.2%) in Nyahera and SS21 both in Sagam (9.6%) and Mundika season II (9.5%). From this study, total sugar and non-reducing sugar percentage were found to vary depending on sweet sorghum genotype and it was lower than that recorded by Soleyman *et al*. (2013). Almodares and Sepahi (1996) found sucrose to vary ranging from 6 to 14.4% among 36 sweet sorghum cultivars.

Sweet sorghum is normally harvested from hard dough to physiological maturity because the highest total sugar and sucrose content and the lowest invert sugar can be obtained (Almodares *et al*., 2010). The results showed that sugars differed among sorghum genotypes, those genotypes that recorded greatest total sugars had highest sucrose. It shows that the sucrose content has an important contribution in total sugar content. There was diversity among sweet sorghum for non-reducing and total sugars. The figures obtained for

some genotypes regarding sugar percentage were similar to those obtained by Sami *et al*. (2013) for thirty genotypes.

Sugar production in sweet sorghum is affected by the environment (Bernal *et al*., 2014). The results indicated that stem sugar content was controlled by the genetic constitution of the genotype with the environment contributing to their expression. The most variation seen in stem sugar content among sweet sorghum genotypes across environments indicates that the sugars traits are under quantitative polygenic inheritance. Murray *et al*. (2008) indicated that the sugar concentration in sweet sorghum is affected by many genes and by gene-environment interactions. The environments that resulted in a greater cane yield among the genotypes reduced the stem sugar concentration.

5. 3. 2 Correlation analysis for sugar and stem juice traits

The correlation between total sugar and Brix was significant for Masumbi (r^2=0.96) and Sinyanya (r^2=0.93) at P<0.001 (Fig. 5.1 and 5.2), Nyahera (r^2= 0.92, P<0.01) and Mundika season II (r^2=0.75, P<0.05). Brix is a measure of total dissolved solids including fermentable sugars (fructose, glucose and sucrose), starch, organic acids and soluble minerals and is often used to estimate sugar (Lingle *et al*., 2012). Therefore, when total soluble solids levels are high in juice it results in higher refractive index thus high Brix values is recorded and vice versa. Fermentable sugars form the major component of total soluble solids in sweet sorghums stem juice thus a positive linear correlation between Brix and total sugars. Positive correlation between sugar yield and Brix has also been reported in other studies (Liu *et al*., 2008; Pfeiffer *et al*., 2010). Brix and sugars increase with maturity with Brix attaining its peak when grain reaches hard dough stage (Gutjahr *et al*., 2013). The increase in total sugars is attributed largely to increase in sucrose and to less extent increase in glucose and fructose between 90 and 115 days after planting (Lingle *et al*., 2012). Sweet sorghum stores carbohydrates as sugars in stalks (Rains *et al*., 1990) and they tend to accumulate sucrose rapidly during grain filling period and juice quality may improve or deteriorate slowly after hard dough stage but will deteriorate rapidly after the seed is fully ripe (Inman-Bamber, 1980). At dough stage, sweet sorghum can, therefore, be harvested to provide grain for food and stems for juice extraction before fermentable sugars start to decrease.

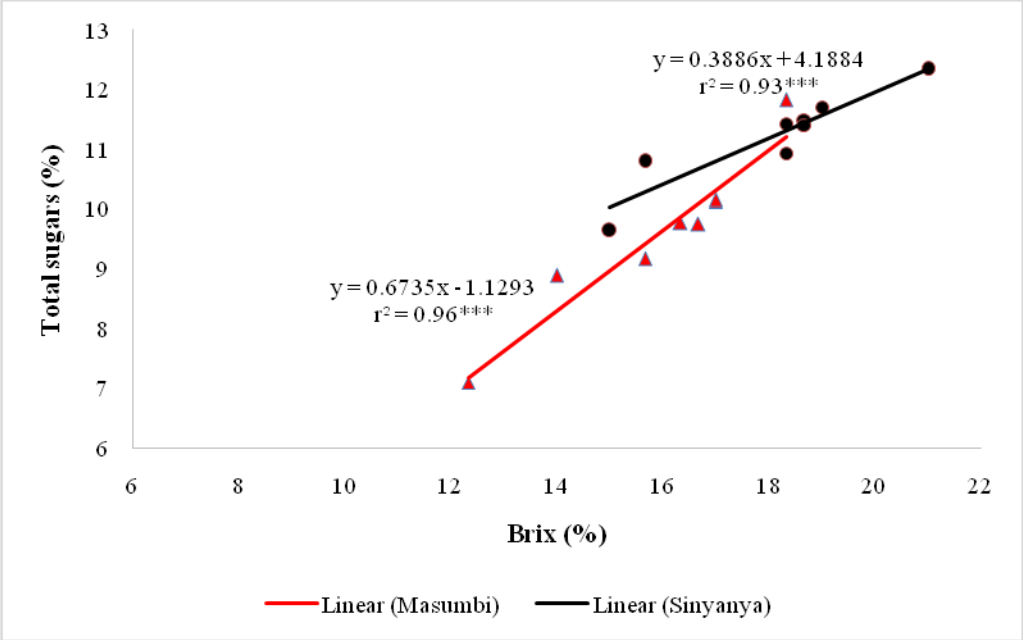

Figure 5.1: Correlation between total sugars and Brix during season I

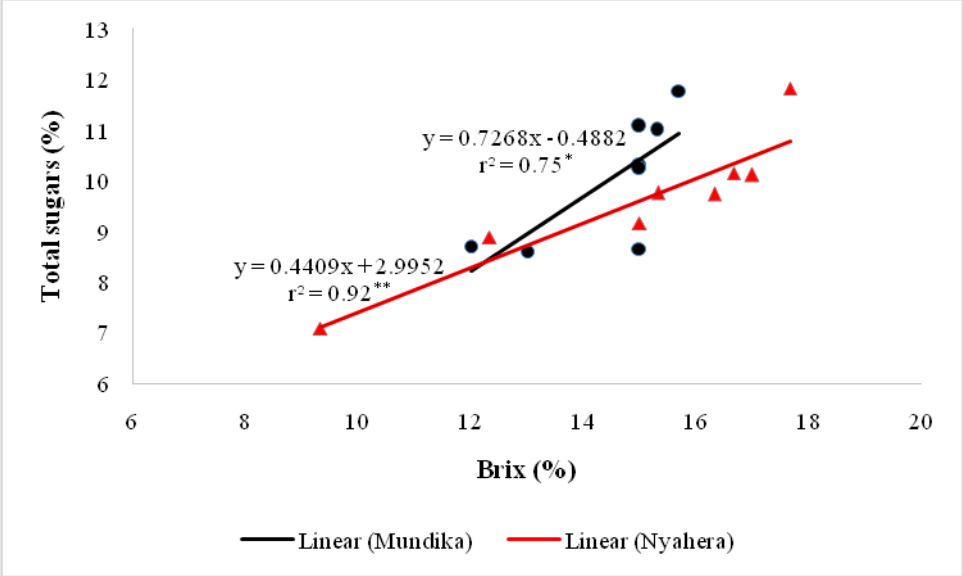

Figure 5.2: Correlation between total sugars and Brix during season II

Linear positive correlation was observed between non-reducing sugars and Brix in Nyahera with r^2=0.92 (n=8, *P*<0.01, (Fig. 5.3) and total sugars and non-reducing sugars for Mundika season II (r^2=0.95), Nyahera (r^2=0.98) and Sagam (r^2=0.96) at *P*<0.001 (Fig.5.4). Similar results have been reported by Tsuchihashi and Goto (2004) and Davila-Gomez (2011). Brix is, therefore, a good estimate of sugar in sweet sorghum stem juice. In a separate study by Wang *et al*. (2012), a significant correlation between sucrose weight and total sugar

yield was observed in sweet sorghum. This is expected since sucrose is a major component of total sugar. Sucrose is a predominant stalk sugar contributing largely to total fermentable sugars and is approximated that sucrose, fructose and glucose are 54, 20 and 26% respectively in mature sweet sorghum stem (Smith and Buxton, 1993). Sucrose can be converted to glucose and fructose by invertase enzyme thus a negative relationship is likely to be observed between reducing and non-reducing sugar. Sucrose is a major product of photosynthesis and is a major carbohydrate form used as energy source for growth. The enzymes controlling sucrose levels in sweet sorghum include sucrose synthase and soluble acid invertase. Sucrose synthase catalyzes reversible interconversion of sucrose and Uracil diphosphate (UDP) to UDP-glucose and fructose (Scafer *et al*., 2004). Soluble acid invertase (SAI) lowers sucrose levels in stem juice of sweet sorghum therefore low levels of SAI results in high sucrose levels (Zhu *et al*., 2000). The interconversion by sucrose synthase can either increase sucrose by lowering glucose and fructose and vice versa thus the negative correlation between reducing and non-reducing sugars as reported by Yang *et al*. (2013).

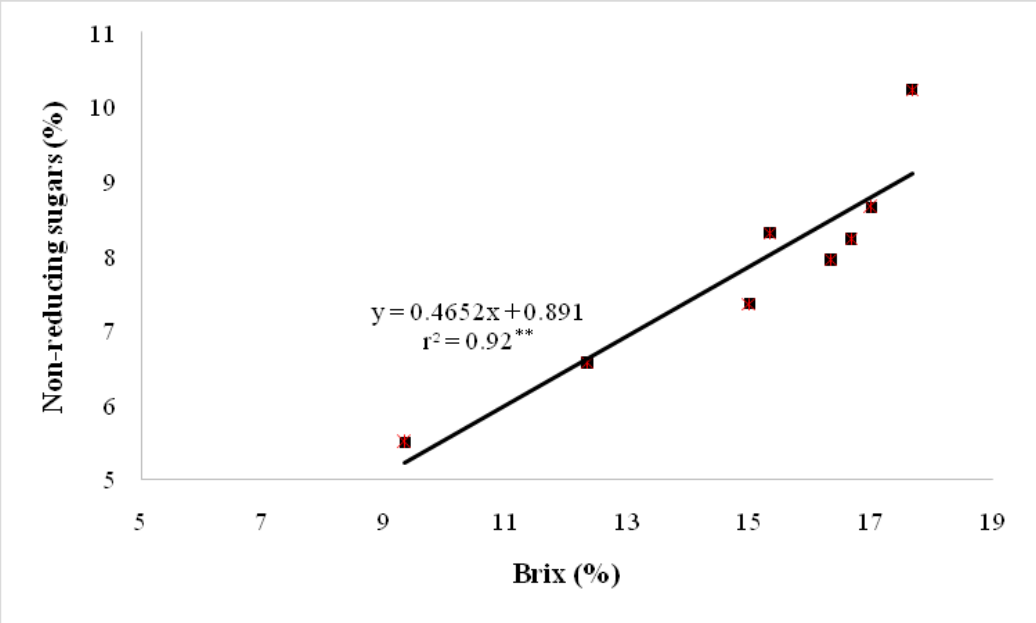

Figure 5.3: Correlation between non-reducing sugars and Brix in Nyahera

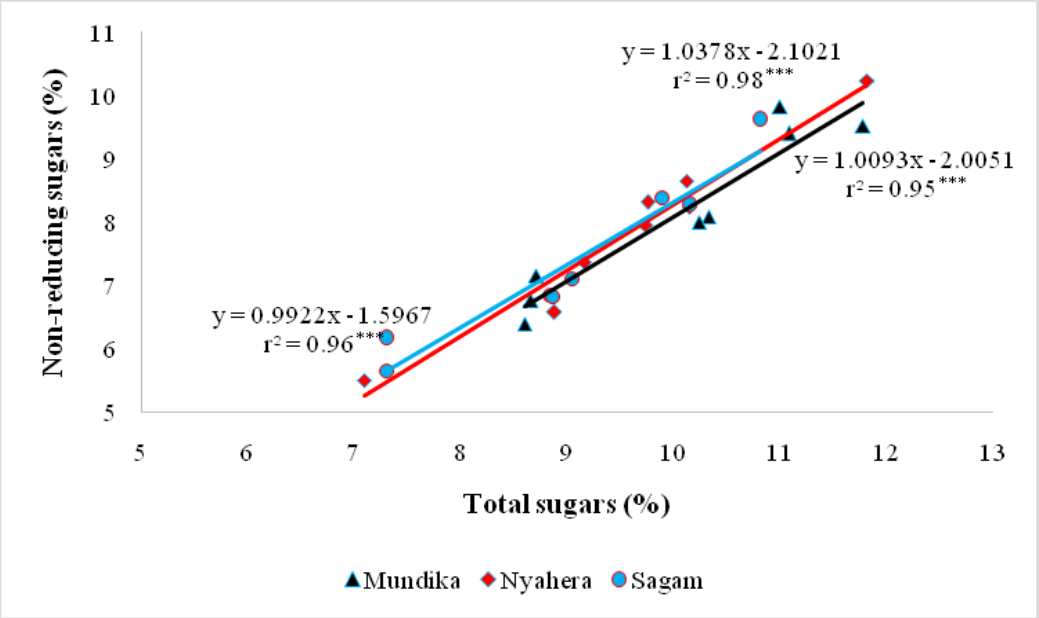

Figure 5.4: Correlation between non-reducing sugars and total sugars during season II

Similarly, total sugars were significantly correlated with ethanol yield in Sinyanya at $P<0.05$ ($r^2=0.71$, Fig. 5.5). Previous studies have reported a significant positive correlation between ethanol and sugar concentration of stalk juice (Murray *et al*., 2008; Zou *et al*., 2011). From this study genotypes which yielded highest ethanol volume recorded highest, cane yield, juice volume and plant height though they had low Brix and total sugars. Low sugar content in high ethanol producing genotypes for instance EUSS10 was compensated by higher yields of cane and finally juice increase for fermentation.

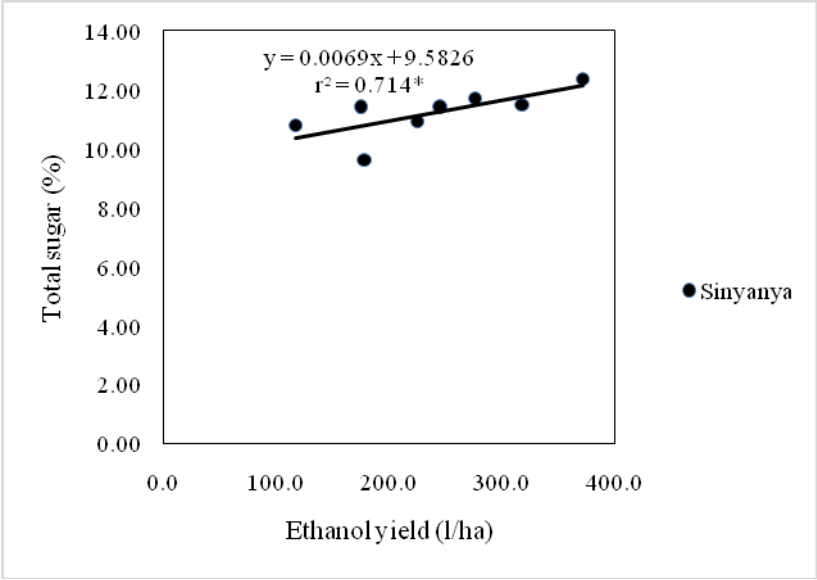

Figure 5.5: Correlation between total sugar and ethanol yield in Sinyanya

The reducing sugars are monosaccharides that can be oxidized in presence of oxidizing agents in alkaline solution because they have free carbonyl and ketone group (Robyt, 2012). In this study, reducing sugars considered as glucose and fructose was not affected by the genotypes. These sugars did not show any association with ethanol meaning that sucrose can be the target sugars for improvement since it showed positive correlation with ethanol in Sinyanya. Despite the fact that the sugars were variable for each genotype, it was possible to observe relatively high sugar content trend for genotypes SS21, SS17, SS04, ACFC003/12 and EUSS11 across the environments.

Previous studies have shown that plant height and the number of internodes were positively correlated with sugar concentration of cane juice (Ganesh *et al*., 1995), suggesting that genetic improvement of these two traits could improve the total biomass and sugar content (Murray *et al*., 2008; Zou *et al*., 2011). However, this study indicated independence of sugar and biomass traits that should be confirmed with further experimentation. Gutjhar *et al*. (2013) reported that a high variability in stem biomass did not allow the finding of a correlation between stem sugar content and juice sugar concentration in sorghum genotypes. The sugar yield was more dependent on the stem weight and the juice volume rather than on the sugar concentration, which was high in most of the genotypes. The principal drivers for biomass accumulation, the genetic constitution and the environment were responsible for stem sugar storage.

There were significant differences among sweet sorghum genotypes for total and non-reducing sugars which is attributable to genetic differences between eight genotypes. The presence of relatively high sugar levels in stem indicates that sweet sorghum genotypes have the capacity to be used as feedstock for ethanol production. Genotype EUSS10 had generally low sugar levels though it produced high cane, juice and ethanol yield, therefore, improvement of sucrose levels through breeding will significantly raise its ethanol yield. The sugar content and Brix from genotypes under study are far much lower than those recorded in other studies; therefore, these traits have to be improved.

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CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i) LM1 agro-ecological zone has a high potential for sweet sorghum production with appreciable ethanol yield. Major contributors of ethanol yield are cane yield, juice volume, and plant height.

ii) The genotypes SS14 and SS21 had high stable yield performance though they recorded low performance. EUSS10 though it had low sugar and least stable was highest performing genotype and thus could be considered as the most suitable genotype for ethanol production. Stable sweet sorghum genotypes could be considered suitable for broad adaptability while development of specific genotypes for specific regions of production would utilize to advantage genotypes with narrow "specific" adaptability.

iii) This study demonstrates that although Brix (%) and total sugar concentration are desirable traits in sorghum stalk juice, juice volume is the main determinant for ethanol yield.

iv) Environmental effects, as well as, GEI had the strongest effect on yield of sweet sorghum genotypes. The significant genotype by environment interaction for cane and juice yield observed from analysis of variance in this study shows that sweet sorghum genotypes respond differently when grown in different environmental condition.

v) The best performing genotypes were EUSS10, ACFC003/12 and SS04 while average performers were; EUSS11, EUSS17, and SS14. The genotypes SS21 and SS17 were poor performers for ethanol yield located outside limits of any environments. It is evident that performance of sweet sorghum is attributed to both genetic make-up and environment.

6.2 Recommendations

i) Since tested genotype EUSS10 exhibited high yielding superiority over check varieties across various locations, it can be used to produce ethanol in the country; therefore, I recommend this genotype be released and to be incorporated in a breeding program for developing better genotypes.

ii) To widen sweet sorghum for food and ethanol production, more multi-location trials should be conducted in all sorghum production zones. The high yielding genotypes

with superior agronomic performance, EUSS10, SSO4, ACFC003/12, EUSS11, and EUSS17 should be tested extensively in on-farm trials and promoted for adoption and commercialization in Kenya.

iii) Special attention should also be paid to developing tall sweet sorghum hybrid varieties with high sugar content in their stem to enhance ethanol productivity per unit area of land.

iv) For ethanol yield SS14 may be recommended as broadly adapted and stable to the LM1, LM2 and LM3 of the environments tested. EUSS10 is adapted to the favorable environment and is recommended for cultivation in such environments where it is likely to express its maximum yield potential.

v) LM1 (Masumbi and Sagam) is the best environment for sweet sorghum genotypes for ethanol production.

vi) There is a need to understand expression pattern of key enzymes involved in carbon partitioning of photosynthesis assimilates between storage tissues of sucrose in stems and starch storage in grains sinks. This can help understanding trade-offs between sugar accumulation in the stem and starch deposition in the seeds thus will aid in the manipulation of genes geared towards an increase in stem sucrose levels at the expense of starch storage in grain.

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