COMBINING ABILITIES AND HETEROSIS FOR ETHANOL RELATED TRAITS IN SWEET SORGHUM (*Sorghum bicolor* L.)

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A thesis submitted to the Graduate School in partial fulfillment for the requirement for the degree of Doctor of Philosophy in Plant Breeding of Egerton University

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been presented to any other university for any degree or other award.

Signature …… Date ……

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RECOMMENDATION

This thesis has been submitted with our approval as University Supervisors.

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DEDICATION

This thesis is dedicated to my family.

ABSTRACT

Sweet sorghum [*Sorghum bicolor* (L). Moench] is important for production of fodder and ethanol worldwide. Objectives of this study were to determine: (*i*) the most suitable harvesting stage for sweet sorghum for which ethanol production is a maximum, (*ii*) the combining abilities for ethanol related traits and (*iii*) the best sweet sorghum hybrids for production of industrial alcohol. Experiments were conducted in Nakuru (0° 23′S, 35° 35′E), Homa Bay (0.35°13′07″S, 34°07′44″), and Kisumu (0°04′06″S, 34°49′03″E), counties in Randomized Complete Block Design (RCBD). They involved harvesting of sorghum at various stages for analysis of sugar traits to determine the harvesting stage for sweet sorghum in Western and Rift Valley regions in Kenya for which ethanol production is optimum. Hybrids were made by crosses in a line by tester mating design and the products were evaluated across three locations. Fourteen lines were used as females while four lines were used as testers. Genotype, Genotype × Environment (GGE) biplot and Principal Component Analyses (PCA) were used to select the best performing and most stable hybrids. Results indicated that harvesting sweet sorghum at 104 to 117 days after planting is appropriate for production of kernels and ethanol. Line EUSS10 exhibited the highest ethanol (1062.78 L ha^{-1}) from juice volume of 22976.9 L ha^{-1} while Line EUSS11 produced 985.26 L $ha^{-1}$with a brix of 16.21. All the traits such as height, girth, cane yield, brix, juice volume and ethanol yield showed both negative and positive heterosis. Hybrids exhibited positive heterosis ranging from 36.63% to 101.17% and 22.87% to 113.77% for juice and ethanol volume, respectively. Line IS25547 and tester EUSS10 had the highest General Combining Ability (GCA) for ethanol production while BM39 × EUSS10 had the highest Specific Combining Ability (SCA). GCA effects accounted for a larger portion of the treatment sum of squares than SCA effects suggesting that additive gene effects are more pronounced than non additive gene effects in the inheritance of sweet sorghum traits such as ethanol and juice volume. GGE biplot analysis distinguished the best performing hybrids from the rest and PCA revealed that juice volume was one of the most significan components in ethanol production. This study suggests that hybrids IS9203 × EUSS10, GS001 × EUSS10 and NYANGEZI × SS04 can be developed for use by farmers due to their good performance and high stability across the tested environments with long rainy season in the low lands seen to be the best for sweet sorghum production.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABS	African Biotechnology Sorghum project
CGIAR	Consultative Group for International Agricultural Research
CSIR	Council for Science and Industrial Research
FAO	Food and Agriculture Organization of the United Nations
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
INTSORMIL-CRSP	International Sorghum and Millet Collaborative Research Support Program
KALRO	Kenya Agricultural and Livestock Research Organization
NARS	National Agricultural Research System
SAFGRAD	Semi-Arid Food Grain Research and Development
SMIP	Sorghum and Millet Improvement Program
SPAAR	Special Program for African Agricultural Research
SRI	Sugar Research Institute
USAID	United States Agency for International Development
WCA	West and Central Africa

CHAPTER ONE
INTRODUCTION

1.1 Background of the study

The demand for biofuel is on the increase globally with many countries especially the developed countries working towards the reduction of the use of fossil fuels due to environmental concerns (Almodares and Hadi, 2009). The first generations biofuels include bioethanol, biodiesel and straight vegetable oil which are used in domestic and industrial energy supply (Sorda *et al*., 2010). Bioethanol has been produced from sugar beet (*Beta vulgaris*) (Dodic *et al*., 2009), sugarcane (*Saccharum officinarum*) (Luo *et al*., 2009 b), sweet sorghum (*Sorghum bicolor*) (Almodares and Hadi, 2009), maize (*Zea mays*) (Torres *et al*., 2015), wheat (*Triticum aestivum*) (Talebnia *et al*., 2010) and cassava (*Manihot esculenta*) (Nguyen *et al*., 2007). Biodiesel has been obtained from soybean (*Glycine max*) (Santos *et al*., 2009), oil palm (*Elaeis guineensis*) (Sumathi *et al*., 2008), coconut (*Cocos nucifera*) (Nakpong and Wootthikanokkhan, 2010), peanut (*Arachis hypogaea*) (Kaya *et al*., 2009), rapeseed (*Brassica napus*) (Yoo *et al*., 2010), mustard (*Brassica juncea*) (Hasib *et al*., 2011), camelina (*Camelina sativa*) (Frohlich and Rice, 2005), safflower (*Carthamus tinctorius*) (Meka and Tripathi, 2007), sunflower (*Helianthus annuus*) (Guan *et al*., 2009) and jatropha (*Jatropha curcus*) (Tiwari *et al*., 2007). In Africa, the growing of high potential energy crops has been compromised by low adoption of agricultural technology and harsh environmental conditions (Webersik and Wilson, 2009). Development emphasis in this region is to ensure improved productivity for industrial crops and growing sweet sorghum for ethanol production is an opportunity which should be explored since it is a multi-purpose crop with great yield potential and can be used as an alternative feedstock for ethanol production (Ali *et al*., 2007; Rooney *et al*., 2007).

In many parts of Africa, poverty is concentrated in rural areas and a high proportion of the population (25%) living in arid and semi-arid lands (ASALs) can benefit from development of new cultivars which is necessary to enhance technological changes (Sanders *et al*., 1996). The major emphasis of technological change in the ASALs over the last three decades has been new cultivars (Ahmed *et al*., 2000). The area under sorghum (*Sorghum bicolor* L. Moench) in Africa has nearly doubled in the recent past but yields have not increased (Olembo *et al*., 2010). As a result of this phenomenon, various National Agricultural Research Systems (NARS), International Crops Research Institute for Semi-Arid Tropics (ICRISAT) and International

Sorghum and Millet Collaborative Research Program (INTSORMIL CRSP), have directed their breeding research towards the yield reducing factors such as genetically unexploited low yielding landraces, drought, striga (*Striga hermonthica*), pests and diseases (ICRISAT, 2014). Sorghum has been evaluated for physical and functional properties of the grains with very little effort on improving their nutritional values (Atokple, 2010). However, there is an emphasis geared towards enhancing industrial properties of sorghum such as the production of ethanol. Despite lagging behind many other commodity-based research programmes, such as maize and cotton (*Gossypium hirsutum*), sorghum research in sub-Saharan Africa has been successful in diffusing a large number of new cultivars onto farmers' fields for food but not ethanol production (Olembo *et al.,* 2010).

Sorghum being drought tolerant among cereals with ability to withstand high temperature and floods is important for food security in Africa (Taylor, 2009). Sorghum performs well in areas whose annual rainfall range from 500 to 700 mm per year (Taylor, 2009). Most of the countries in Africa where sorghum is a significant arable crop are generally dry and at risk of desertification. In fact, the rain in sub-tropical Africa is intermittent and characterized by brief periods of very high precipitation with subsequent dry spell (Taylor, 2009). There is an increasing demand for sorghum mainly in brewing industry to replace barley yet the amount produced by farmers is too low to satisfy demand in Kenyan (Kilambya and Witwer, 2013). The crop is predominantly self pollinated and development of new varieties is a natural option for crop improvement and makes it amenable for use in population improvement and hybrid development by exploitation of heterosis (Khawanja, 2014).

Content of sugar in the stem is one of the most important traits of sweet sorghum (Liu *et al*., 2013). However, there are large variations in sugar contents of stem among sweet sorghum varieties. Content of sugar in the sorghum cane can be assessed by checking brix which is a measure of sugar and soluble starch in plant sap based on light refraction (Ready *et al*., 2005). Sucrose is the predominant sugar in the sorghum cane and varies with plant maturity and is lowest when plant is at the boot stage but highest at the soft dough stage (Lingle, 1987). Sucrose contents at different internodal region of sorghum stem has up-down tendency with the internodal number increasing from top to base (Subramanian *et al*., 1987). The upper most internodes represent strong 'utilization sinks' until final development of the peduncle during anthesis. However, at the physiological maturity stage, it is not clear how sugar is accumulated in different internodes (Hoffmann-Thoma *et al.,* 1996).

1.2 Statement of the problem

Demand for energy has created new food security problems with biofuels and animal feeds competing for food crops. There is need to balance those crops that produce food and fuel. Greenhouse gases and climate change have complicated the matter with man trying to venture into new avenues for continued supply of energy. Production of food crops such as maize and industrial crops such as sugarcane have been complicated by the expansive ASALs in Kenya which form almost 80% of the total land area. The few agrochemical industries in Kenya engaged in ethanol production compete for molasses, a by-product in sugar production which is also in very high demand by animal feed industries, breweries and sugar industries which now engage in product diversification using molasses. Development of sorghum hybrids in Kenya has been stagnant for decades with the current varieties in the market having been released more than three decades ago. With increasing demand on industry based crops, there is need to develop elite sweet sorghum genotypes with higher ethanol yield to support energy sector in Kenya.

1.3. General objective

The main objective of this study was to develop commercial sweet sorghum hybrid (s) for high ethanol yield and grain yield in Kenya.

1.4 Specific objectives

a) To determine the most suitable harvesting stage for sweet sorghum for optimum production of ethanol.
b) To determine heterosis and the combining ability for brix, cane yield, juice yield and ethanol production in sweet sorghum.
c) To identify elite sweet sorghum hybrids for ethanol production.

1.5 Hypotheses

a) There is no suitable harvesting stage for sweet sorghum for production of ethanol.
b) There is neither heterosis nor combining ability for brix, cane yield, juice yield and ethanol production in sweet sorghum.
c) There is no significant difference in performance of sweet sorghum hybrids.

1.6 Justification of the study

Food and energy security along with environmental protection are a prerequisite for economic development. Poverty reduction is best approached through rural households.

Improvement of an ASAL friendly crop such as sweet sorghum will increase household income for many rural poor households who can grow food and be linked to new biofuel markets or industries for ethanol production. Ethanol is blended with fossil fuels in varying percentages and used in automobiles without modification of the engines. Its production from the juice of sweet sorghum stalks is more cost effective as compared to other feed stocks (Reddy *et al*., 2013). The inherent tolerance of sorghum to marginal lands and environmental conditions, its versatility as a food, fuel and fodder and its ability to produce high yields, makes it difficult to ignore. Biofuel is an alternative to the growing demand for energy and its ability to negate climate change (Atabani *et al*., 2012). In this regard, the need for alternative source of ethanol for industrial use and as a biofuel cannot be overemphasized. Sorghum is known to survive drought and grow well under low input conditions thus increasing productivity in otherwise low production areas. There is an emerging demand for sweet sorghum whose production is low in Kenya. The challenge for plant breeders is to develop sweet sorghum varieties that will produce higher ethanol yield to supplement sugar cane whose supply is less than the industrial demand. Hybridization has been the most important method of crop improvement in history and development of hybrid seeds will enhance the production of sweet sorghum to meet industrial needs in Kenya.

1.7 Scope and limitations of the study

This study encompassed production of sweet sorghum hybrids with increased ethanol production potential. The croses were done in a line × tester design in which the male lines were crossed with the female lines. Only the F_1 population and the parents were considered in the study. This study did not include molecular characterization of the ethanol related traits. The hybrids were not subjected to selfing to obtain F_2 population which would have reflected segregation of the alleles that contain ethanol related traits.

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CHAPTER TWO
LITERATURE REVIEW

2.1 Origin and classification of sorghum

Sorghum [*Sorghum bicolor* (L) Moench] is a dual purpose, self-pollinated diploid (2n = 20), annual, C_4 grass with a high photosynthetic efficiency (Khawanja, 2014). It is in the family *Poaceae* and has a small genome size of 730 Mbp that is 25% the size of maize or sugarcane and is fully sequenced making it an attractive model for functional genomics among the C_4 plants (Ordonio *et al*., 2016). Sorghum was domesticated in 3000 BC and could have arisen from *Sorghum verticillillorum* which easily crosses with sorghum (De Wet *et al*., 1970). Early domestication of sorghum was associated with changing the small-seeded, shattering open panicles to larger, non-shattering seeds and more compact panicles (Dhillon *et al*., 2006). Sorghum bicolor has three sub-species, namely *arundinaceum, bicolor* and *drummondii*. The cultivated sorghum varieties are all from *bicolor*, which has five local races; *bicolor, caudatum durra*, *guinea* and *kafir* (Harlan, 1972). Commercial cultivars of sorghum are categorized according to the purpose and these are for grain, forage, fiber, broom, sweet and biomass sorghum depending on their agronomic importance (ICRISAT, 2014). Although sorghum is a self pollinated crop, some florets are protogynous resulting into cross pollination hence classified as often cross-pollinated (Sing, 1995).

2.2 Genetics, botany and growth development of sorghum

The genus *Sorghum* comprises a high genetic diversity hence there is potential for crop improvement and increased productivity (Uptmoor *et al*., 2003; Assar *et al*., 2005). Diversity of color and shape shows the enormous amount of genetic variation in *Sorghum* species. Cultivated sorghum can be divided into three main categories based on end product utilization, thus: grain sorghum for grain production, sweet sorghum for sugar and grain production and biomass sorghum for biomass production. Although phenotypic differences exist between grain and sweet sorghum with the latter having sugar rich stems, taller plant, higher biomas but less grain production (Victor and Miller, 1990; Rooney *et al*., 2007), how it differs genetically from grain sorghum is not well understood (Murray *et al*., 2008). There are virtually no biological or taxonomical barriers or boundaries among these cultivated forms for hybridization and they all belong to the same species. Plant breeders have focused on improving sorghum to serve as food, feed and fuel (Tarpley and Victor, 2007; Vermerris *et al*., 2007; Laopaiboon *et al*., 2007) depending on the local needs. This involves improving

characters such as yield performance and stability, resistance to pests and pathogens, grain and stem qualities among others.

Important genes have been maped indicating their roles and approximate locations in the sorghum genome (Cheng *et al*., 2010). On chromosome number 1; *Tb1, Sh, Ma, Y,* genes which are responsible for grain color, maturity, grain shattering, stem tillering respectively have been located (Graham, 1916; Karper and Quinby, 1945; Karper and Quinby, 1947; Webster *et al*., 1965). On chromosome number 2; Rf_2, B_2, *Z, Ma*, responsible for fertility, grain testa, grain mesocarp, and maturity have also been detected (Vinall and Cron, 1921; Ayyagar, 1934; Miller and Picket, 1964; Rooney and Aydin, 1999). Chromosome number 3 has *Pl, R, Alt, ms, A* which represents genes responsible for disease resistance, grain colour, abiotic stress tolerance, male sterility and presence of awns respectively (Graham, 1916; Sieglinger *et al*., 1934; Webster 1965; Duncan, 1988; Reddy *et al.,*1992). Number 4 and 5 has *opr* and *bmr* respectively (Schertz and Stephens, 1966; Poter *et al*., 1978). Genes related to coleoptiles color, such as Rs_1, Rs_2 and those responsible for the leaf traits such as *bmr6, bmr12* have also been mapped (Mace *et al*., 2009). The gene for lodging resistance (*Dw1)* has also been discovered (Yamaguchi *et al*., 2016). Cloning of C4 specific gene *Ppc* which encodes phosphoenolpyruvate carboxylase, and PEP case in sorghum has been done and transferred to transgenic rice (Zang *et al*., 2003). While studying response to biotic and abiotic stresses, Kadier *et al*. (2017) conducted a genome wide investigation using 145 non-redundant NAC genes and identified NAC genes which are distributed among the 10 sorghum chromosomes and are responsible for coping with the stresses in the sorghum genome. Intergeneric hybrids of sugarcane and sorghum have been used to show differential expression of genes related to sugar accumulation such as SPS, SuSy and SAI have been mapped and shows the possibility of improving sucrose content in such hybrids (Ramalashmi *et al*., 2014).

Breeders have tapped genes for insect and disease resistance (Gowda *et al*., 1995), drought tolerance (Tsago *et al*., 2013), photosensitivity adaptation to climatic conditions (Obilana, 1985), duration of growth period, response to low nitrogen level (Miri and Rana, 2012), sugar accumulation in the stem for syrup and ethanol (Zheng *et al*., 2011), high grain yield for food (Qazi *et al.*, 2012) and high biomass yields for use as feedstock for animal or second generation biofuels (Srinivasa *et al*., 2012). Sorghum is propagated by seed and sweet sorghum cultivars produce sugar in the stalk and starch in the grains making it a multipurpose crop. It has a high water and nutrient use efficiency, its bagasse has high biological value when

used as forage and good combustion when used for cogeneration and finally it has a wide adaptability to the environment (Khawanja *et al.*, 2014).

Sorghum seeds germinate after three to four days especially under warm environment (20 ^{o}C or above). A period of up to 10 days, is required in colder soils between 13 ^{o}C and 20 ^{o}C (Khawanja *et al*., 2014). The plant remains in a vegetative phase for about 30 to 40 days, during which all leaves are formed. After this period, growth occurs by cell elongation. The floral initial forms 30 to 40 days after germination. Floral initiation marks the end of the vegetative growth due to meristematic activity. Anthesis starts from the proximal end of the panicle towards dystal end at a rate of 2-5 cm day^{-1} and completes within 7 to 10 days, pollen grains are only viable for a short period and stigma remains receptive for 8-16 hours (Ayyangar and Rao, 1931). The grand period of growth in sorghum follows the formation of a floral bud and consists largely of cell enlargement (Reddy and Sanjana, 2003). The crop has a growth cycle of about four months and its production can be easily mechanized (Khawanja *et al*., 2014).

2.3 Importance of sorghum

Sorghum is the fifth most important cereal crop in the world after rice (*Oryza sativa*), barley (*Hordeum vulgare L*.), wheat (*Triticum aestivum*) and maize (*Zea mays*) (Taylor, 2009). It is very essential to diets of the poor people in the arid and semi-arid lands (ASALs) where droughts cause frequent crop failures (FAO and ICRISAT, 1996). It is grown on 40 million hectares (ha) in 105 countries of Africa, Asia, Oceania and North and South America. Major producers of sorghum globally include USA, India, Mexico, Nigeria, Sudan and Ethiopia. Other sorghum producing countries include Australia, Brazil, Argentina, China, Burkina Faso, Mali, Egypt, Niger, Tanzania, Chad and Cameroon. Grain is mostly used as food in Asia and Africa and as feed in America. Sorghum stover is an increasingly important source of dry season fodder for livestock in Asia (ICRISAT, 2014).

In USA, sorghum is one of the most preferred fodder crops with high biomass accumulation and high calories value (Almodares *et al*., 2011). Therefore, forage sorghum is a major contributor in the development of livestock industry in USA (McDermott *et al.*, 2011). However, sorghum has not been utilized for forage in developing countries because the most common variety is the grain sorghum which does not have good forage properties (Rooney *et al.*, 2007). Sweet sorghum has been known to have high sugar content in the stem and used both as a source of ethanol and forage (Rooney, 2007; Yuan, 2008).

Grain sorghum is food for millions of people living in the arid and semi-arid tropics (Sing and Sing, 1992; Sharma and Ortiz, 2000). It is a food security crop with good storage properties and various communities have diversified the use ranging from *ugali*, porridge, *githeri* and even as a beverage (Leder, 2004). Sorghum is rich in carbohydrates and proteins but lacks preference due to the high presence of phenolic compounds in the grains resulting to high tannin concentration in the seed coat (Jansman, 1993). Although tannins are an adaptive mechanism to protect the grains from damage caused by birds (Husle *et al.,* 1980). Sorghum forms an alternative crop for farmers living in the arid and semi arid lands giving them an opportunity to harvest from the otherwise non productive land with minimal input. It is an indigenous crop to Africa and is a pro-poor multipurpose crop providing food, feed, fiber and fuel across a range of agro-ecosystems (Kilambya and Witwer, 2013).

In Kenya, sorghum is used as human food, where it is a staple food for millions of people (MoA, 2012). It is still largely grown as a subsistence food crop in most African countries (Kilambya and Witwer, 2013). It is also used as animal feed and industrial raw material. Industrially, the grain is used to manufacture wax, starch, syrup, alcohol, dextrose, agar, edible oils and gluten feed. As food, the grain is used in making fermented and non fermented porridge, bread, and other traditional dishes (MoA, 2012). Sorghum is grown by small-scale, resource-poor farmers and is mainly used for home consumption in Kenya. As the only indigenous cereal species, it is produced throughout much of the country, even in areas with low agricultural potential (Kilambya and Witwer, 2013).

Some sorghum grain is also processed into flour by commercial mills and sold in urban markets. However, presence of anti-nutritional factors such as phytate and tannins (Selle *et al.*, 2010) restricts its use as food source. Sorghum tannins are condensed type while hydrolysable tannins do not occur in sorghum (Nyachoti *et al.*, 1997). Multiple phenolic hydroxyl groups of tannins may form stable complexes with protein, metal ions and other micromolecules like polysaccharides (Kondo *et al*., 2007). Stable complexes of tannins with proteins may lead to coagulation or precipitation of protein molecules (Mahmood *et al.,* 2014). In many cases, sorghum flour is used to enrich cassava flour before it is packaged and sold to consumers (Chemonics, 2010). However, more recently developed cultivars can produce grain yields similar to that of good grain sorghum hybrids. Forage sorghums are typically taller, leafier, and later maturing than grain sorghum hybrids (Camara *et al*., 2006).

Sweet sorghum is a natural variant of common grain sorghum with high stem sugar content and often considered a smart crop because it can produce both food and fuel (Rao *et al*., 2009). As a C_4 crop with a high level of directly fermentable stem sugars and the ability to produce high biomass under adverse conditions, sweet sorghum is considered an ideal biofuel crop for the first and second generation bioethanol production, particularly having the advantages of exploitation of marginal land and avoiding competition for land for food crops. However, the genetic basis for these remarkable traits of sweet sorghum is poorly understood (Reddy and Sajana, 2003). The conversion of sugar to ethanol requires less energy as compared to starch in which more energy is used for depolymerization. In the USA, sweet sorghum has demonstrated potential to produce up to 6000 L ha^{-1} of ethanol (Smith and Buxton, 1993). However, estimated ethanol yields were on average 33% more with grain of corn and grain sorghum compared with sugar of sweet sorghum for seven rainfed site-years in Nebraska U.S.A. (Wortmann *et al.*, 2010). Utilization of sweet sorghum as an energy crop is hindered by seasonal availability, the need to transport and store much mass and storability of the sweet sorghum canes. However, sweet sorghum is more accessible to poor farmers because of its low cost of cultivation and its ability to grow in areas that receive a minimum of 700 mm annual rainfall. It also has a higher net energy balance, (3.63) compared to grain sorghum (1.50) and maize (1.53) (Wortmann *et al*., 2008). Even though the ethanol yield per unit weight of feedstock is lower for sweet sorghum compared to sugarcane, the much lower production costs and water requirement for this crop more than compensates for the difference and hence, returns a competitive cost advantage in the production of ethanol (Rao *et al*., 2004). It produces three valuable products: food, fuel and feed, raising small holder incomes by about 23% in central India while probably reducing net greenhouse gas emissions (Rao *et al*., 2009).

2.4 Production of sorghum hybrids

Hybridization occurs as a result of crossing plants of different inbreds or species resulting to new offspring which may have improved performance due to heterosis (Duvick, 2005). This concept has been used in crop improvement for many decades and on various crops. Heterosis is caused by dominance and or over dominance gene actions (Birchler *et al.*, 2010; Kaeppler, 2012) and is important in improvement of crops. Sorghum crop is about 6% cross pollinated meaning that it is mostly self pollinated making hybrid seed production challenging due to complex procedures in emasculation (Sing, 1995). However, with the discovery of male sterility among some varieties of sorghum, hybrid production has been made highly feasible.

Application of male sterility in hybrid production involves identification of the male sterile line (*A* line), male fertile line (*B* line) and the restorer line (*R* line) (Wang *et al*., 2006). It has been utilized in hybrid production in several crops (Sing, 1995). In maize, Texas male sterile cytoplasm was used to develop hybrids (Deway *et al*., 1996); in *Petunia* (Young and Hanson, 1987), in sunflower (*Helianthus annus*) (Monenger *et al.*, 1994) and in common beans (*Phaseolus vulgaris)* (Abad *et al*., 1995) and this technique has also been used on sorghum originating from India, Africa and America (Sane *et al.*, 1996).

Globally, USA is leading in sorghum production for the animal feeds (Stewart *et al*., 2005) while India is leading in grain sorghum production (Almoderas and Hadi, 2009). There has been a declining trend in the production of sorghum in Kenya (Connelly, 1994; Thornton, 2010). Many sorghum hybrids have been developed to meet various needs such as fodder, ethanol and grains (Edgerton, 2009). Several seed companies have released various sorghum hybrids for use in other countries (Tripp and Rohrbach, 2001). USA is known to be leading in utilization of hybrid seeds and is currently the leading in production (Steduto *et al*., 2012). In India, hybrid sorghum productivity was 6.2 t ha^{-1} (Patil, 2007). In both Nigeria and Niger, hybrid sorghum performed better that the local varieties (House *et al*., 1997). In Sudan, hybrid sorghum yielded more than the local varieties by producing between 50 and 85% under field conditions and between 300 and 400% under irrigation (Ejeta, 1986).

2.5 Utilization of sorghum hybrids

Attitude of farmers towards sorghum has greatly affected its production (Muui *et al*., 2013). Many farmers only grow sorghum for household consumption due to the fact that grain sorghum has been characterized with low production, low prices and inadequate market (Bennett and Anex, 2009). There are no hybrid sorghum seeds in the market due to the fact that seed industries are not willing to invest in the production of sorghum seeds with the market base as the rural poor communities. Crop development challenges include the fact that sorghum is a self pollinated crop and that the development of hybrid seeds will depend on the successful utilization of male sterile lines with those that have the ability to restore fertility. The diversity of sorghum makes it have very many uses such as animal feeds, human food and industrial sorghum which can be used in baking, brewing and ethanol processing industries (Berenji and Dahlberg, 2004) creating great opportunities for hybrid seed utilization. The demand for sorghum is growing but its potential utilization is hindered by continued low production

(Muendo *et al*., 2004). The brewing and baking industries are in need of sorghum to help diversify their products (Kilambya and Witwer 2013). This situation makes it necessary for Kenya to import sorghum from neighboring countries despite its high production potential (Muendo *et al.,* 2004).

Health consciousness and blood sugar related complications demand the consumption of high fiber and low calorie foods (World Health Organization, 2016). This has boosted the demand for sorghum and other related grains which have been associated with low income earners. The availability of technology and skill for the development of sorghum hybrid seeds by the Kenya Agriculture and Livestock Research Organization (KALRO) as well as universities offers good opportunity for seed improvement.

2.6 Sorghum production in Kenya

Sorghum is one of the most important cereal crops in Kenya (Taylor, 2009). It is highly adapted to a wide range of climatic conditions including marginal lands due to the fact that it is a C4 crop hence can be grown without competition with the other weather sensitive crops (Khawanja *et al.,* 2014). It is one among the few resilient crops that can adapt well to future climate change conditions, particularly the increasing drought, soil salinity and high temperatures (ICRISAT, 2014). The crop has high water use efficiency making it suitable to soil moisture deficit environment (Khawanja *et al*., 2014). It can grow at an altitude range of 900 m to 2500 m above the sea level, temperatures of 12 to 37 ^{o}C and an optimum rainfall of 550 to 800 mm (Chemonics, 2010; Srinivasa *et al*., 2013). Sorghum does well in a wide range of soils such as alvisols and vertisols and can tolerate pH of 5.5 to 8.5 (Du Plessis, 2008). Kenya has a wide range of climatic and edaphic factors that can favor sorghum growing. Although Kenya has high production potential of the crop, it has remained under utilized (Muui *et al.,* 2013). Most sorghum production is concentrated in Kenya's southwestern and south-central districts namely within the Eastern, Nyanza, Western and Rift Valley regions, which accounted for about 43, 41, 9 and 7 percent respectively, of Kenya's total sorghum production in 2011. Collectively, these regions produce 99 percent of the country's sorghum (MoA, 2012).

Agricultural productivity of Kenya rests on the quality of improved seeds (Denning *et al*. 2009). Correct selection of seeds also comes with incentives for better management practices. Use of improved varieties makes the subsistence farmers grow to commercial production due to increased productivity (Altieri *et al*., 2012). Studies indicate that 78% of total

seeds for the top 7 grains grown in Kenya are from the informal sector and not the seed industries (Tegemeo Institute, 2004). The seed industries in Kenya have been known to be slow in the production of seeds that target the rural poor for fear of failing to get returns on their investment (Nyoro and Ariga, 2004). The rural poor farmers are known to depend on their saved seeds and have low capacity to purchase the improved seeds (Lipton and Longhurst, 2010). This makes the seed companies unwilling to produce seeds of crops such as sorghum.

Although research by scholars around the world have revealed that sorghum hybrids can be better performing in various environmental conditions including drought, soil infertility, acidity and aluminum toxicity, production of the sorghum hybrid seeds has not been done by the seed companies (Chapman *et al.*, 2000). This is likely because of the fear that the companies would not realize the return on their investment due to low purchase of the products. This vicious situation has led to continuous low productivity of sorghum in Kenya. However, the realization of sorghum as an industrial crop has created a new increased demand for sorghum making it an important crop that will demand improved seeds to meet its market requirement (Vermeris *et al,* 2007).

Hybrids have the potential of increasing sorghum productivity by more than twice the potential under the same conditions as the non hybrids (Rooney *et al*., 2007). Utilization of hybrid sorghum seeds significantly increases yields (House *et al*., 1997). Bantilan *et al*. (2004) quantified the increase in production potential of sorghum due to hybrid utilization to be between 20 to 60%. Hybridization has the potential of increasing both the grain and the plant biomass accumulation in sorghum (Zeng *et al*., 2011). Scientists have to tailor their studies to the market needs of the sorghum crop (Pingali and Traxler, 2002).

Adoption of new agricultural technologies has been a subject of study for decades in developing countries (Evenson and Gollin, 2003). It is paradoxical that new technologies come up every day but only few of them end up with the farmer due to various challenges (Hamel and Prahalad, 2013). It has been observed that access and utilization of hybrid seeds can improve food security in Kenya but agricultural industry is still dominated by seeds from the informal sector (Tegemeo Institute, 2004).

Sorghum production in Kenya is inadequate to sustain the ever increasing population (FAOSTAT, 2014). In eastern Kenya both the local and improved varieties are being grown (Timu *et al.*, 2014). Out of 17 seed companies operating in Kenya 7 are involved in production of sorghum from open polinated varieties. Pannar Company produces hybrid sorghum that are

only sold in other countries like South Africa (Chepng'etich *et al*., 2015). Pioneer and Monsanto produce hybrid sorghum seeds though are currently not available in the Kenyan market. There is poor adoption of new technologies in Kenya especially in sorghum farming. To improve the production trend, it is essential to ensure the use of hybrid seeds as well as the adoption of new technologies (Chepng'etich *et al*., 2015).

2.7 Sorghum varieties in Kenya

Several varieties of sorghum are currently grown in Kenya. The improved varieties such as *Seredo, Serena, Gadam, Sila* and *KARI Mtama* are available with many seed stockists all over the country (Timu *et al.,* 2014). The local varieties are currently rare and can only be found with a few farmers in the rural areas. In the western part of Kenya there is *Gopari, Kisudi, Andiwo, Ochuti* while in Eastern Kenya farmers grow *Kiambere, Gatururu* and *Muceru.*

2.8 Factors influencing sorghum production in Kenya

Eighty percent of Kenya land area is favorable for growing of sorghum due to favourable environmental conditions (Place *et al*., 2006). Sorghum can do well in the high lands, midlands and lowlands (Muui *et al*., 2013). Many parts of the country is dominated by both alvisols and vertisols which can favor the growth of sorghum (Muui *et al*., 2013). It is more tolerant to alkaline salts and can do well in a pH ranging from 5.5 to 8.5. Sorghum has the ability to withstand short periods of water logging and can do well in soils with high clay content. The optimal production of sorghum can be attained at between 10 and 30% clay content (Lewandowski *et al*., 2003).

Sorghum productivity can be supported by a broad range of precipitation between 300 to 2500 mm per year (Place *et al*., 2006). Kenya receives rainfall amounting to 2500 mm per year depending on the area with about 250 mm rainfall on very dry environments making it possible for sorghum production in most places. Sorghum can survive drought due to its drought tolerance mechanisms including folding of leaves, smaller leaf surface area limiting transpiration and rapid stomatal closure. This makes sorghum a suitable crop for the marginal lands in Kenya (Muui *et al*., 2013).

Biotic factors including diseases and insects cause economic loss to sorghum growing farmers. The most common disease is headsmut caused by fungi (*Sorosporium reilianum*). Although it is not clear whether shoot flies (*Atherigona soccata)* cause substancial loses in Kenya, 50% loss had been recorded in India (Jotwani, 1982). Planting of resistant varieties is the most prefered management option for shoot fly in sorghum (Sharma *et al*., 2005). Aphid

(*Sipha flava*), maize stalk borer (*Busseola fusca*) and ball warm are potential threats to the production of sorghum but can be managed by use of integrated pest management practices. Birds are among the most destructive sorghum pests and can cause up to 100% loss depending on the season and the variety (Hiron *et al*., 2014). They can be managed by avoiding offseason planting and bird scare for small holder farmers.

Among economic factors, high poverty levels have strong influence in sorghum production which mostlty targeted household consumption (Herrero *et al.*, 2010). This has resulted to low market for sorghum especially at the local level and futher discouraged its production. The emergence of industrial utilization of sorghum products has created new market demands for the products (Perlack *et al*., 2005). Currently, there is increased demand for sorghum for brewing, baking, and ethanol industries (Taylor *et al*., 2006). In order to realize the full potential of sorghum, it is necessary to develop sorghum hybrids which can meet the rising market demand.

2.9 Breeding of sorghum for economicaly important traits

Sorghum breeding started as early as 1931 in West Africa in a research station established in 1921 at Bambey, Senegal (Atokple, 2010). Over the last two decades improved sorghum technologies for West and Central Africa have been developed mainly by ICRISAT, one of the 15 CGIAR centres, with significant inputs from its national partners (Olembo *et al*., 2010). In Asia, currently available sweet sorghum varieties are more photoperiod-sensitive than available hybrids, and hybrids are earlier maturing and have significant heterosis (30 to 40%) for cane, juice and sugar yields (Reddy *et al*., 2013).

In East and Southern Africa (ESA), results for 2007 and 2008 indicated that most of the sweet sorghum lines that are locally developed had higher brix percent (15−17%) than the Indian check NTJ 2 with 11.6%. Some 20 hybrids also exhibited brix values between 15% and 21% and the average stalk yield for hybrids were 16 t ha^{-1} with a range of 9.5 to 25.5 t ha^{-1}). Stalk yields (10.52 t ha^{-1}) of ESA-developed lines were lower than that of materials received from ICRISAT which was 12.9 t ha^{-1} (Reddy *et al*., 2013).

A breeding program specifically aimed to increase the sweet stalk yield potential while optimizing both grain and giving higher returns to the farmer has been initiated. Genotype by environment (G × E) interactions are significant for sweet sorghum productivity-related traits. It has been noted that the genotypes that perform well in the rainy season are not necessarily the top performers in the post rainy season and *vice versa*. In ESA, the evaluation of sweet stalk

sorghum varieties across three locations in Mozambique and four environments in Kenya indicate a significant G × E for sugar content, stalk yield, grain weight and biomass yield (Reddy *et al.,* 2013).

2.10 Accumulation of sucrose in plants

Accumulation of sugar in the stems of plants is a complex biochemical mechanism that accumulates and controls sugar in the stalk (Jackson, 2005; Inman-Bamber *et al.,* 2008). It is hypothesized that this process is directed by sink strength which is the competitive ability of a sink organ to import photoassimilates and depends on both physical size and physiological activity capabilities (Marcelis, 1996). In the USA, an elite sweet sorghum genotype, *Keller* had been developed through breeding and has high performance in a wide range of environmental conditions (Sami *et al*., 2013). Relationship between the source (plant leaves) and the sink (stem) regulates accumulation of sugar and is responsible for the different sugar levels in the stems of sugarcane varieties (Chandra *et al*., 2012).

Sugar accumulation in the stem occurs against the concentration gradient, a situation which demands the use of energy (Batta and Sing, 1996). Accumulation of sucrose in the stem of sugarcane is controlled by genotype, environment and the stage of development (Chandra *et al.,* 2012). There are also specific enzymes whose presence plays critical roles in accumulation of sucrose. Sucrose Synthase (SuSy), Sucrose-Phosphate-Synthase (SPS) and invertase found in cell wall, neutral and soluble acids play very critical roles in sucrose accumulation. Sucrose accumulation is regulated by both the action of SuSy and SPS. SPS is present in the leaves of the plant and controls the flux of carbon into sucrose making it the major exporter of photosynthate from the source to the sink tissues (Jang and Sheen, 1994; Huber and Huber, 1996). SPS has been detected in wheat (Castleden *et al.,* 2004) and in sugarcane (Grof *et al*., 2006). Although much work has been done towards the understanding of sucrose accumulation in the stems, precise understanding of the biochemistry and genetics of sucrose accumulation is still at large (Chandra *et al*., 2015).

In sweet sorghum, sucrose accumulates in the large pith parenchyma cell vacuoles and driven to the apoplast from the leaves by the phloem vessels. This is then taken up by the pith cells and sequestered in the vacuoles by minimum hydrolysis (Tarpley and Vietor, 2007). Compartmentalization of the structural carbohydrates has brought about variaties that can be used for biofules and bio-power as well as forage like in the case of brown midrib sorghum which has been developed for easy digestibility hence used in forage for enhanced

saccarification and fermentation efficiency (Dien *et al*., 2009; Jung *et al*., 2012). During the vegetative growth, there is low sucrose level in the stems of sweet sorghum while a significant increase is seen at floral initiation with maximum levels at grain physiological maturity (McKinley *et al*., 2016). There is high invertase activity during vegetative phase while a considerable reduction during the post anthesis phase. On the other hand, there is low activity of sucrose synthase during the vegetative phase and high activity during post anthesis phase (Farooq *et al*., 2011).

2.11 Estimation of heterosis in plant breeding

In plant breeding, progeny of diverse varieties of species or crosses between species exhibit greater biomass, speed of development and fertility than both parents due to heterosis. Heterotic effect of the hybrid is the mean difference from the mid parent (Falconer and Mackay, 1996). It can be estimated from mean deviation from better parent, check and mid-parent value (Lamkey and Edwards, 1998). It is also defined as the difference between the hybrid and the mean of the two parents (Falconer and Mackay, 1998). This can be expressed as:

$$H_{F_1} = \mu F_1 - \frac{\mu P_1 + \mu P_2}{2} \text{ (Lamkey and Edwards, 1998)} \quad (2.1)$$

Considering the allele frequencies for a diallelic locus in populations as 1 and 2 represented by p and $p + \partial p$ respectively, the expression (2.1) explains heterosis. Assuming that the genotypes in P_1 and P_2 are in Hardy Weinberg equilibrium proportions and that the lines are completely inbred, the means (μ) will be given as:

$$\mu P_1 = (2p - 1)a + 2p(1 - p)d \quad (2.2)$$

$$\mu P_2 = \mu P_1 + 2(\partial p)a - 2(\partial p)^2 d \quad (2.3)$$

The probability of getting an A_1A_2 locus is the probability of receiving an A_1 from P_1 and A_2 from P_2 (p[1-(p+∂p)]) or an A_2 from P_1 and A_1 from P_2 ([1-p][p+∂p]). The mean of F_1 can be expressed in terms of P as:

$$\mu F_1 = \mu P_1 + (\partial p)a \quad (2.4)$$

This gives the contribution of heterosis in this locus as:

$$H_{F_1} = \mu F_1 - \frac{\mu P_1 - \mu P_2}{2} = (\partial p)^2 d \quad (2.5)$$

For this locus to show heterosis (H>0), a difference in allele frequencies ($\partial p \neq 0$) and dominance is required (d > 0). This can be summarized as:

$$H_{F_1} = \sum_{i=1}^{n} (\partial P_i)^2 d_i \text{(Lamkey and Edwards, 1998).} \qquad (2.6)$$

This suggests that heterosis depends on dominance and that without dominance there will be no inbreeding depression and there will be no heterosis. In quantitative genetics, the allelic frequencies can be used to estimate heterosis like in the case of maize (Lamkey and Edwards, 1998). Panmitic-midparent heterosis also called midparent heterosis is the difference between the mean of the F_1 hybrid and the mean of the two random-mating parental populations (panmictic-midparent value). Better parent or F_2-midparent heterosis is the difference between the mean of the F_2 generation (derived by random mating the F_1) and the panmictic-midparent value. Finally, Heterosis of the F_1- population selfed is defined as the difference between the mean of the F_1-population selfed and panmictic-midparent value. With the assumption that there are two alleles per locus, the means for generations can be given by the expression:

$$\bar{F}_{1(f)} = (1 - f)(\bar{F}_2 + 2\Delta^2 d) + fa(\bar{p}_1 - \bar{p}_2) \qquad (2.7)$$

$$\bar{F}_{2(f)} = (1 - f)(\bar{F}_2) + fa(\bar{p}_1 - \bar{p}_2) \qquad (2.8)$$

$$\bar{P}_{1(f)} = (1 - f)(\bar{F}_2 + 2\,\Delta \propto d - 2\Delta^2 d) + fa(\bar{p}_1 - \bar{p}_2 + 2\,\Delta) \qquad (2.9)$$

$$\bar{P}_{2(f)} = (1 - f)(\bar{F}_2 - 2\,\Delta \propto d - 2\Delta^2 d) + fa(\bar{p}_1 - \bar{p}_2 - 2\,\Delta) \qquad (2.10)$$

(Source: Lamkey and Edwards, 1998).

Where f = inbreeding coefficient of a generation

P_i= frequency of the ith allele in population 1

P_i'= frequency of the ith allele in population 2

$\bar{p}_i = \frac{p_i + p_i'}{2}$ = the average allele frequency in the cross of population 1 and 2

$\partial_i = \frac{p_i - p_i'}{2}$ = one half the difference in allele frequency between populations.

In case of only two alleles then;

$\partial_i = -\partial_2 = \Delta$ and d = the deviation from the homozygote midparent;

a = half of the difference between homozygotes;

$\propto = a + d(\bar{p}_2 - \bar{p}_1)$ = average effect of an allele substitution

$\bar{F}_{2(f)} = a(\bar{p}_1 - \bar{p}_2) + 2\bar{p}_1\bar{p}_2 d$ which is the mean for F_2 generation.

These heterosis values can be represented as: Panmictic-midparent heterosis or midparent heterosis = $4\Delta^2 d$ where Δ is the difference in allele frequency between populations and d is the deviation of the heterozygote from homozygote mid parent. Panmictic-midparent F_2 heterosis or better parent heterosis = $2\Delta^2 d$. Panmictic-midparent F_1-selfed heterosis or standard heterosis = $3\Delta^2 d$-$p_1 p_2 d$, where p_1 is frequency of i^{th} allele in parent 1 and parent 2 is the frequency of the i^{th} allele in parent 2.

Although genetic divergence (difference in allelic frequency) and dominance are necessary for there to be heterosis, they are not sufficient in the case of multiple alleles. Multiple alleles segregating in a population the lack of heterosis cannot be used to infer a lack of genetic divergence between the parental populations (Cress, 1996; Lamkey and Edwards, 1998). Heterosis in sorghum was first observed by Corner and Karper (1927), but commercial exploitation was not possible until the discovery of cytoplasmic genetic male sterility system by Stephens and Holland in 1954. Sorghum exhibits both negative and positive heterosis and could be exploited for sorghum improvement (Umakanth *et al*., 2012). In USA, the sorghum production has trippled since the adoption of hybrid sorghum cultivars and exploitation of hybrid vigor in conjunction with intensive management practices (Rani *et al*., 2013). Positive heterosis in F_1 hybrids is higher in early maturity, high stripped stalk yield, percent cane juice extracted and grain yield which is suitable for dual purpose (Pothisoong and Jastil, 2014). Heterosis has been exploited in; wheat through classical breeding approaches (Bailey *et al.*, 1980) and molecular approaches (Li *et al*., 2014), maize (Springer and Stupar, 2007) and Sorghum (Singhania, 1980; Makanda *et al*., 2009).

2.12 Estimation of combining ability

Combining ability is defined as the ability to transfer the desired properties of appropriated lines entered into hybrid combinations to hybrid offspring (Hayes and Immer, 1942). They are of two types; general combining ability (GCA) and specific combining ability (SCA). GCA is the average performance of a line in a hybrid combination and SCA is the better or poorer performance than expected of a given hybrid combination (Sprague and Tatum, 1942). Properties under the influence of general combining ability are affected by additive gene action, while properties under the influence of specific combining ability are affected by non-additive gene action or dominant and/or epistatic gene action (Falconer, 1975).

Falconer (1975) established that the difference in GCA stems from additive variance (V_A) and additive by additive interaction due to different environmental conditions, while the

difference in SCA is attributed to non-additive genetic variance. General or specific combining power can be estimated via various methods, the most common of which is diallel analysis (Griffling, 1956). Line × tester (multiple sequence) analysis is the modified version of the top-cross method proposed by Kemphtorne (1957) and is used as a suitable method for hybrid variety breeding programs especially where cytoplasmic sterile and restorer lines are included as parents (Stephens and Holland, 1954). While investigating gene action in sesame (*Sesamum indicum*), Anyanga *et al*. (2016), identified genotypes which were good general combiners for height, capsule branches and length of capsules and one hybrid which had a significant SCA which was recommended for hybrid production.

Combining ability estimates were used to investigate the adaptation of maize to acidic soils (Welcker *et al*., 2005). Tadesse *et al.* (2008) estimated the combining abilities of introduced sorghum parental lines for major morpho-agronomic traits and found that GCA was significant for height, grain yield and panicle length while SCA effects were not significant. Combining ability for quantitative characters in sunflower was estimated using half diallel mating design to predict their usefulness in hybrid development (Machikowa *et al*., 2011). While conducting studies on trees, Wu *et al*. (2004) observed that a positive correlation of SCA and breeding values are sufficient to conduct selection for a breeding population. On the contrary, the effect of reciprocal recurrent selection on relative efficiency of genetic value was assessed using SCA and GCA in maize breeding population in which efficient process of selection was observed to be based on GCA effects (Makumbi *et al*., 2011). Identification of potential parental lines and a combination of hybrids in breeding programs have been done using GCA and SCA in maize hybrids in which consequences of tester of choice was seen to be significantly reduced (Lariepe *et al*., 2017).

Specific and general combining abilities have been determined for growth traits in fish breeding in which the growth traits of blunt snout beam (*Megalobrama amblycephala*) were analysed in a diallel cross and the general combining ability was detected to be higher for dam than for the sire (Luo *et al*., 2014). In chickpea (*Cicer arietinum*), GCA and SCA were conducted in order to understand the gene action governing biomass and harvest index, the selection of parents was seen to be best determined by *per se* performance, the combining abilities and heterosis (Hegde *et al.*, 2007). SCA was observed to be better than GCA in the prediction of hybrid combinations for high mineral content in cabbage (*Brassica oleracea*) head (Singh *et al.*, 2012). The ratio of GCA to SCA has been used to indicate the predominance

of non additive genes in mulberry (*Morus spp*.) (Vijayan *et al*., 1997). In resistance of potato (*Solanum tuberosum*) to early blight (*Alternaria solani)*, combining ability analysis showed that both additive and non-additive gene actions were important (Gopal, 1998). While looking at *per se* performance, combining ability and heterosis in sugarcane, Verma and Singh, (2004), found out that there was no relationship between *per se* performance, heterosis and combining ability and suggested that both the *per se* performance and combining ability be considered for selection in determining hybrid combinations. On the contrary, SCA was seen to be more important than GCA in grain performance of rice (*Oryza sativa*) when an analysis was conducted using North Carolina II and North Carolina III breeding designs (Zhou *et al.*, 2017).

In sorghum, Sharma *et al*. (2007) observed significant GCA and SCA for feeding score, number of nodes and overall resistance to spotted stem borer (*Chilo partellus*) and suggested that the traits were associated with resistance to spotted stem borer and was believed to be influenced by additive gene action. Variance component estimates of SCA were observed to be greater than that of GCA for total biomass, juice extraction and grain yield of sorghum indicating the non-additive control of genetic variation while the GCA variance was higher than the SCA variance for fresh stalk yield, juice yield, brix content, total sugar yield and computed bioethanol yields indicating additive gene action (Umakanth *et al*., 2012).

2.13 Heritability of important traits in sorghum

Heritability refers to the ratio of genetic variance to the phenotypic variance (Sing, 1995). It denotes the proportion of phenotypic variance that is due to the genotype hence heritable (Sing, 1995). It measures the fraction of phenotype variability that can be attributed to genetic variation. It is usually denoted by h^2 or H representing narrow sense and broad sense heritability respectively:

Narrow sense heritability $$h^2 = \frac{\sigma^2_A}{\sigma^2 g + \sigma^2 ge + \sigma^2 e} \quad (2.11)$$

$$h^2 = \frac{\sigma^2 A}{\sigma^2 p} \quad (2.12)$$

Broad sense heritability $$H = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 ge + \sigma^2 e} \quad (2.13)$$

$$H = \frac{\sigma^2 g}{\sigma^2 p} \quad (2.14)$$

Where σ^2_A is variance due to additive effect σ^2g is genetic variance and σ^2p is phenotypic variance (Sing, 1995). Broad sense heritability (*H*) values can also be calculated according to Kempthorne (1957). Narrow sense heritability (h^2) values can be calculated according to Falconer (1975), (Tan, 2010). Bakers ratio refers to the ratio of narrow sense heritability to broad sense heritability with the formular derived from narrow sense heritability and broad sense heritability formula proposed by Baker (1978):

$$\text{Baker's ratio} = (2\,\sigma^2 g\,ca)\,/(2\sigma^2 gca+\sigma^2 sca) \qquad (2.14)$$

It was used to show how much the parents contributed to the offspring in a study of sesame (*Sesamum indicum*) (Ayanga *et al.,* 2016).

$$H = (G / F) \text{ and } h^2 = (A / F)$$

Where G-variance due to genotype effect, F-variance due to phenotype and A-variance due to additive effect.

2.14 Male sterility in plants

In male sterile plants, the male parts of the flower are not functional while the female parts are functional (Chase, 2007). It occurs in nature sporadically due to mutation and manifests in three forms thus: genetic, cytoplasmic and cytoplasmic-genetic (Chase, 2007). Genetic male sterility is mostly governed by single recessive gene though dominant genes have also been known like in the case of safflower (*Carthamus tinctorius* L*).* Cytoplasmic Male Sterility (CMS) is usually caused by the cytoplasm and can be easily transferred to a given strain using it as a recurrent parent. It has been utilized in hybrid production in several crops (Sing, 1995). In maize, Texas male sterile cytoplasm was used to make hybrids (Dewey *et al*., 1996); in *Petunia* (Young and Hanson, 1987), in sunflower (*Helianthus annus*) (Moneger *et al*., 1994) and in *Phaseolus* (Abad *et al*., 1995), several hybrids have been produced. Cytoplasmic male sterility is determined by the cytoplasm. In sorghum, this has been observed in many varieties originating from India, Africa and America (Sane *et al*., 1996). Cytoplasmic-Genetic male sterility is where nuclear gene restores fertility when male sterile line is known (Sing, 1995).

Manifestations of male sterility are diverse and can range from complete absence of male organs, failure to develop normal sporogenous tissues (no meiosis), abortion of pollen at any step of its development, absence of stamen dehiscence or the inability of mature pollen to germinate on receptive stigma (Buda and Pelletier, 2000). Cytoplasmic Male Sterility is a maternally inherited trait that is often associated with mitochondrial genes (Chase and Babay-Laughnan, 2004; Hanson and Bentolila, 2004). It can also arise spontaneously in breeding lines as a result of wide crosses or interspecific exchange of nuclear and cytoplasmic genes, or following mutagenesis (Hanson and Bentolila, 2004).

There are a number of different types of CMS systems with distinct genetic features, both within and among different species, but key features that appear to be shared across different types are that CMS is associated with chimeric mitochondrial Open Reading Frames (ORFs) and fertility restoration is often associated with genes encoding pentatricopeptide repeat (PPR) proteins (Chase and Laughnan, 2004; Hanson and Bentolila, 2004). Wang (2006) described details of the molecular basis of CMS and fertility restoration in the CMS-Boro II system in rice, which is likely to have implications for CMS systems in general.

2.15 Sources and uses of ethanol

Ethanol, chemically known as ethyl alcohol (CH_3CH_2OH), is a colourless hydrocarbon with molecular weight of 46.07 g mol^{-1}. It is miscible with ethyl ether, acetone and chloroform and highly soluble in benzene (Wyman and Hinman, 2015). It has a density of 0.78 g cm^{-3}, a boiling point of 78.2^{o}C, a melting point of -117^{o}C and heat combustion of 1336.8 KJ mol at 25^{o}C. Ethanol can either be obtained from petrochemical or fossil feedstock or from sugar or starch from crops (Balat, 2005). The former is referred to as synthetic ethanol while the latter is referred to as bioethanol. There are two main types of ethanol that are used as fuel; hydrous and anhydrous; hydrous alcohol contains 4% water and can only be used in vehicles that are specifically designed for it. On the other hand, anhydrous alcohol has almost no water and can be blended with gasoline and used in ordinary vehicles (Wyman and Hinman, 2015). Blends ranging from 10% ethanol and 90% gasoline (referred to as E10) to 85% ethanol and 15% gasoline have been used in various parts of the world (MOE/GTZ, 2008). Ethanol is used as a solvent and a preservative in pharmaceutical industry as well as an active ingredient in alcoholic beverages (MOE, 2009). It is also used as an oxygenous biomass fuel considered as a predominant alternative to *Methyl tert-butyl ether* (MTBE) used for oxygenating fuel for vehicles to reduce air pollution. MTBE is toxic and carcinogenic (Belpoggi *et al*., 1995) whose

use should be avoided. Ethanol is the preferred alternative due to its biodegradable, low toxicity, persistence and regenerative characteristics (Cassada *et al*., 2000). The United States gasoline supply, for example, is promoting ethanol blend fuels hence increasing the importance of ethanol as more health issues related to air quality are diagnosed (Almodares *et al.*, 2009). Ethanol can be produced from high energy crops such as sweet sorghum, naturally occurring feedstock like sugarcane, cassava, wheat and maize of which analysts have confirmed sweet sorghum as the most suitable feedstock for ethanol production through the process of fermentation (Ndegwa *et al*., 2011). Ethanol can be derived from the alcoholic fermentation of simple sugars or starch (first generation bioethanol) or polysaccharides (second generation bioethanol) under anaerobic conditions (Khawanja *et al*., 2014).

2.15.1 First generation bioethanol production

The simple sugars accumulated in the stems of sweet sorghum can be fermented to produce ethanol (Almodares and Hadi, 2009). Fermentation involves the action of yeast (*Saccharomyces cerevisiae*) on sugars from the sweet sorghum's pith juice (Kundiyana, 2006). As sugars are consumed by yeast, they are converted to ethanol and carbon dioxide. Since yeasts are living organisms, it is important to provide an environment that will support their growth and allow them to complete the conversion process. There are various types of yeasts that can be used for fermentation such as *Pichia stipitis*, *Pachysolen tannophilus*, *Candida shehatae*, *Candida tropicalis, Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* (Jeffries and Jin, 2004). There are also others like *Candida* spp. which grow as parasites on both plants and animals. *Saccharomyces cerevisiae* was chosen due to its efficiency in conversion of sugars to alcohol and its availability (da Cunha-Pereira *et al*., 2011). There are various important critical steps for creating a suitable environment for ethanol fermentation. It is important to ensure the medium is sterile to remove other microorganisms such as bacteria and fungi that can compete for the same substrate with yeast (Smith *et al*., 1987). This is done using antiseptic solutions. Fermentation requires anaerobic environment hence oxygen should be sealed from entering the container. Optimum temperature of 37 ^{o}C is recommended with a near neutral pH of between 5.5 and 7.5 (Belpoggi *et al*., 1995).

2.15.2 Second generation bioethanol production

The second generation bioethanol refers to the production of ethanol produced from ligno-cellulosic biomas like woody crops, agricultural residues or solid biowaste like sorghum

bagasse but the main disadvantage of the second generation bioethanol production is the enomous costs involved (Khawaja *et al*., 2014). The degradation of lignin can generate toxic substances hence the second generation bioethanol production is not preferred (Jacobsen and Wyman, 2000).

2.15.3 Fermentation efficiency and ethanol yields

Theoretical yield of ethanol was determined to be highest for starch, glucose or fructose, sucrose and lowest in maize grain (Smith and Buxton, 1993). They estimated that 5% of the sugar is used to produce microbial growth and non ethanol products. Sweet sorghum juice can be converted to alcohol either by fermenting the juice or by fermenting the chopped stalks in a solid state process (Rein, 1984). The U.S. Department of energy estimated potential sweet sorghum ethanol yield to be 5590 L ha^{-1} (U.S. Department of energy, 1979). In Iowa State, sweet sorghum cultivars, yields between 3850 to 4410 L ha^{-1} of ethanol production assuming 95% extraction rate (Hunter, 1994). Tew *et al.* (2008) found out that up to 6,060 L ha^{-1} could be obtained from sweet sorghum hybrids. The varietal differences indicate potential improvement of productivity through genetic improvement (Wortmann *et al.*, 2010). The cost of producing ethanol from sweet sorghum was found to be greater than that of maize in California U.S.A. because of high harvest cost (Geng *et al*., 1989) but yield of hexose was greater for sweet sorghum and cost of ethanol production from the juice was lower and closer to that of sugarcane (Rahmani and Hodges, 2006).

2.16 Estimation of genetic variance

Knowledge of genetic variances enables the plant breeder to understand how the genes of interest are transferred from parents to offsprings. In quantitative genetics, there are various methods of estimating genetic variances. The two main methods are use of genetic models and application of mating designs. Genetic model has Additive (A) effects, Dominance (D) effects and (A × A) epistatic effects. These effects can also be refered to as ADAA model (Cockerhan, 1980). It can also be used in the estimation of G × E effect. This method requires at least two generations such as F_1 and F_2 for proper analysis (Zhu, 1998). The mixed linear model for this method is given as:

Parents:

$$Y_{hijk(P)} = \mu+E_h+2A_i+D_{ij}+4AA_{ii}+2AE_{hi}+DE_{hii}+4AAh_{ij}+B_{k(h)}+\varepsilon_{hijk} \qquad (2.15)$$

$$F_1 \quad Y_{hijk(F1)} = \mu+E_h+(A_i\ A_i\ )+D_{ij}+AA_{ii}+(AA_{jj}+2AA_{ij})+(AE_{hi} +AE_{hj})+DE_{hii}+(AAEh_{ij}+\ AAEh_{jj}\ )+2AAEh_{ij}+B_{k(h)}+\varepsilon_{hijk} \quad (2.16)$$

$$F_2 \quad Y_{hijk(F2)} = \mu+E_h+(A_i\ A_j\ )+(\tfrac{1}{4}D_{ii}+\tfrac{1}{4}D_{jj}+\tfrac{1}{2}D_{ij})+(AA_{ii}+AA_{jj}+2AA_{ij})+(AE_{hi} +AE_{hj})+(\tfrac{1}{4}DE_{hii}+\tfrac{1}{4}DE_{hij}\ )+\tfrac{1}{2}DE_{hij}+\ (AEEh_{ij}+\ AAEh_{jj})+2AAEh_{ij} +B_{k(h)}+\varepsilon_{hijk} \quad (2.17)$$

$$F_3 \quad Y_{hijk(F3)} = \mu+E_h+(A_i\ A_j\ )+(\tfrac{3}{8}D_{ii}+\tfrac{3}{8}D_{jj}+\tfrac{1}{4}D_{ij}\ )+(AA_{ii}+AA_{jj}+2AA_{ij})+(AE_{hi} +AE_{hj})+(\tfrac{3}{8}DE_{hii}+\tfrac{3}{8}DE_{hjj}\ )+\ \tfrac{1}{4}DE_{hij}+\ (AEEh_{ii}+\ AAEh_{jj})+2AAEh_{ij} +B_{k(h)}+\varepsilon_{hijk} \quad (2.18)$$

Where μ is the population mean, which is a fixed effect, E_h is environment effect which can either be random or fixed, A_i or A_j is additive effect from parent *i* or *j;* D_{ii}, D_{jj} or D_{ij} is the dominance effect; AA_{ii}, AA_{jj} or AA_{ij} is the additive × additive epistatic effect; AE_{hi} or AE_{hj} is the additive × environment interaction effect; DE_{hii}, DE_{hjj} or DE_{hij} is the dominance × environment interaction effect; AAE_{hii}, AAE_{hjj} or AAE_{hij} is the additive × additive × environment interaction effect; $B_{k(h)}$ is the block effect and ε_{hijk} is the random error. The above equations can be expressed in terms of vectors and matrices such that:

$$Y=\mathbf{1}\mu+\mathbf{X}_E\mathbf{b}_E+\mathbf{U}_A\mathbf{e}_A+\mathbf{U}_D\mathbf{E}_D+\mathbf{U}_{AA}\mathbf{e}_{AA}+\mathbf{U}_{AE}\mathbf{e}_{AE}+\mathbf{U}_{DE}\mathbf{e}_{DE}+\mathbf{U}_{AAE}\mathbf{e}_{AAE}+\mathbf{U}_B\mathbf{e}_B+e_e \quad (2.19)$$
$$=\mathbf{Xb}+\textstyle\sum_{u=1}^{\infty} U_u e_u$$

The assumption made in this model is that E is a fixed model, where μ is the population mean, 1 is the vector with all elements 1; **e**A is the vector for additive effects, $\mathbf{e}_A \approx N(0,\sigma^2_A\mathbf{I})$; U_A is the incidence matrix for dominance effects$\mathbf{e}_D \approx N(0,\sigma^2_D\mathbf{I})$; U_D is the incidence matrix for additive × matrix for additive and additive effects, e_{AA} is the vector for additive × additive effects, $e_{AA} \approx N(0,\sigma^2_{DE}\mathbf{I})$; $\mathbf{U}_{DE}$ is the incidence matrix for dominance × environment effects, e_{AAE} is the vector for additive × additive × environment effects,$\mathbf{e}_{AA} \approx N(0,\sigma^2_{DE}\mathbf{I})$ e_{AAE} is the incidence matrix for AAE effects; e_B is the vector for block effects $e_B \approx N(0, \sigma^2_B\mathbf{I})$; U_B is the

incidence matrix for block effects; e_e is the vector for random errors $e_e \approx N(0,\sigma^2_e\mathbf{I})$ (Wu *et al*., 2006). This method of estimating genetic variances is limited in the sense that the breeder must have data for atleast two generations. Mating designs have been developed to estimate genetic variances even when only one generation is available.

Estimation of genetic variances can also be done by estimating variances due to phenotypes and environment so as to predict the variances due to genetics of the organism (Bernardo and Yu, 2007). Estimation of phenotypic and genotypic coefficients of variation were suggested by Burton and De Vabe (1953):

$$\text{Environmental variance } (\sigma^2 e) = MSe$$

$$\text{Genotypic Variance } (\sigma^2 g) = \left[\frac{MSg - MSe}{r}\right]$$

$$\text{Phenotypic Variance } (\sigma^2 p) = \sigma^2 g + \sigma^2 e$$

$$\text{Phenotypic coefficient of variation (PVC)} = \frac{\sigma p}{x} \times 100$$

$$\text{Genotypic coefficient of variation (GVC)} = \frac{\sigma g}{x} \times 100$$

$$\text{Where } x = \text{grand mean of a character}$$

Covariance analysis has been used in maize breeding to compare the effects of using genome wide selection over marker assisted recurrent selection (Bernardo and Yu, 2007).

Studies in genetic variability have been conducted (Basu, 1981; Soltani *et al.,* 1998; Ahnert *et al.,* 2000). The extent of variability is measured by genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) which provides information about relative amount of variation in different characters. According to Roychowdhury and Tah, (2011), the extent of variability is measured by GCV and PCV which provide information about relative amount of variation in different traits studied. In sweet sorghum, high heritability estimates for days to maturity and height of the plant has been observed (Totok 1997; Aba *et al.,* 2001; Ahmed *et al.,* 2011) but very low h^2 for weight of the panicle which suggest that it would not respond to selection. In sweet sorghum, Sami (2013) observed high GCV for content of sucrose followed by grain yield and height of the plant. Sweet sorghum yield of sucrose, juice, cane, juice extraction, days to 50% flowering (DTF), sucrose per cent, juice volume, juice weight, millable cane weight, fresh cane weight, stay green trait, stem girth and plant height have high heritability and can be selected for to enhance ethanol production (Singh *et al*., 2012; Rani and Umakanth, 2012). Increased selection pressure on stalk yield, juice yield

and brix, can lead to realization of elite sweet sorghum germplasm with superb ethanol production (Sami *et al*., 2013).

2.17 Mating designs for plant breeding experiments

Mating design refers to the procedure of producing progenies in plant breeding. Plant breeders and geneticists theoretically and practically use different forms of mating designs and arrangements for targeted purpose. There are various factors that determine mating designs and arrangements used by breeders and geneticists to generate improved plants (Khan *et al*., 2009). They include; the type of pollination, the type of crossing to be used, the type of pollen dissemination, the presence of a male-sterility system, the purpose of the project and the size of the population required among others (Sharma, 2006).

The choice of a mating design for estimating genetic variances should be dictated by objectives of the study, time, space, cost and other biological limitations. Thus, several studies (Kearsey and Pooni, 1996; Griffing, 1956; Hallauer *et al*., 2010; Acquaah, 2012) described and contrasted different mating designs. In all mating designs, the individuals are taken randomly and crossed to produce progenies which are related to each other as half-sibs or full-sibs. A form of multivariate analysis or the analysis of variance can be adopted to estimate the components of variances (Nduwumuremyi *et al*., 2013).

2.17.1 Bi-parental mating

According to Marther, (1982) bi-parental mating design is the simplest and also known as paired crossing design where a large number of plants (n) are selected at random and crossed in pairs to produce $\frac{1}{2}$n full-sib families (Comstock and Robinson, 1948; Griffing, 1956). It has been used successfully in rice breeding (Manickavelu *et al*., 2006). Their progenies are tested and the observed variation partitioned by straight forward analysis of variance into between and within families (Hill *et al*., 1998). The analysis of variances is usually within families and between families. The covariance of full sibs σ^2b is such that:

$$\sigma^2 b = \text{CovFS} = \tfrac{1}{2}V_A + \tfrac{1}{4}V_D + V_{EC} = 1/r\ (MS_1 - MS_2) \qquad (2.20)$$

$$\sigma^2 w = [\sigma^2_G - \text{CovFS}] + \sigma^2_{EW} = \tfrac{1}{2}V_A + \tfrac{3}{4}V_{D+} V_{EW} = MS_2 \qquad (2.21)$$

MS_2 is the environmental source of variation for variance within the crosses.

Assuming dominance effects are zero then:

$\sigma^2 b = \frac{1}{2}V_A$

$\sigma^2 w = \frac{1}{2}V_A + \frac{3}{4}V_{EW}$ (Acquaah, 2012).

Although this design is simple and easily executed, it is unable to yield sufficient information required to estimate all the parameters in a study and can estimate only V_A, V_D, V_{EC} and V_{EW}. This is because the progenies are either full sibs or unrelated (Sharma, 2006).

2.17.2 Polycross mating design

Polycross mating design involves intermating a group of cultivars by natural crossing in isolated blocks. The term polycross was coined by Tysdal, Kiesselbach and Westover in 1942, to indicate progeny from seed of a line that was subject to outcrossing with other selected lines growing in the same nursery (Hill *et al*., 1998). It is most suited to species that are obligate cross-pollinaters, for example, forage grasses and legumes, sugarcane, sweet potato (*Impomea batatas*), and those that can be vegetatively propagated like cassava (*Manihot esculenta*) (Acquaah, 2012). It is critical that the entries be equally represented and randomly arranged in the crossing block (Falconer and Mackay, 1996). Morgan (1988), suggested that Latin square experimental design should be used as the most appropriate design to ensure all entries have equal chance of random mating with each other in the polycross nursery (Morgan, 1988).

The polycross design has an advantage of producing synthetic cultivars, recombining selected genotypes in the recurrent selection procedure and evaluating the general combining ability of the parent genotypes (Falconer and Mackay, 1996; Sleper and Poehlman, 2006; Sharma, 2006). Polycross design can provide an opportunity for estimation of GCA, however, since the parents are of different origin and the crop is sensitive to environmental changes, the performance of the parental lines and their progenies such as flowering is likely to be affected (Morgan, 1988). In addition, the differences in performance of progeny clones could arise from variations in heritability of trait measured (Gorz and Haskins, 1971). In polycross, the progenies from individual plants which are tested are half-sib families and the covariance within the family can be given by:

$$\mathrm{Cov(HS)} = (1 + F)/4(\sigma^2{}_A)$$

Where *F* is the inbreeding coefficient of genotypes being tested.

Polycross design is one of the designs which is used in the application of halfsib mating which is useful in breeding cross pollinated spacies (Nguyen and Sleper, 1983). It has also largely been used in understanding the genetics of trees such as red spruce in which paternity and

parentage analysis were conducted to determine the sib relationships among the trees in an orchard (Doerksen and Herbinger, 2008).

2.17.3 Top cross mating design

Top cross also called inbred variety cross refers to a mating between a selection, line, clone and a common pollen parent which may be a variety, inbred line or single cross (Nduwumuremyi *et al*., 2013). The selected plants are crossed with a common tester (s) of known performance, generally in open pollination. The design was proposed by Jenkins and Brunsen in 1932 for testing inbred lines of maize in cross-bred combinations and later renamed topcross by Tysdal and Grandall in 1948 (Hill *et al*., 1998). The tester parent should have well known genetic background; either narrow- or broad-based testers (Aly *et al*., 2011). It is mainly used to increase chances of obtaining desirable gene (s) from exotic or poorly combining materials.

In making top crosses, only single cross F_1s are utilized because they are uniform. The top cross F_1s will be segregating and it is impossible to identify superior plants at crossing; therefore, they are not used. The F_1s are selected for desirable agronomic characteristics or for desirable parentage. Top cross has been widely used for preliminary evaluation of combining ability of new inbred lines (Mosa, 2010). The possible numbers of crosses are *n* x 1, given *n* number of inbreds with the progenies yielding only GCA information, not SCA (Sleper and Poehlman, 2006). It is a simple and efficient system of screening inbred lines for combining ability before pairing inbreds in single-cross yield trials. This design can provide preliminary rapid screening of genetic stocks as it involves the lowest crossing load and simple statistical analysis (Mosa, 2010). In topcross, the progenies from individual plants are tested, these progenies are half-sib families. The covariance within the families is

$$\text{Cov (HS)} = (1+F)/4(\sigma^2_A)$$

where *F* is the inbreeding coefficient of genotypes being tested.

The model for experiment conducted in one environment is:

$$y_{ij} = \mu + p_i + b_j + \varepsilon_{ij} \qquad (2.22)$$

Where μ is the overall mean, p_i variance due to progenies, b_j is variance due to blocks, ε_{ij} is the error term.

The variance component σ^2_p is an estimate of $(1+F)/4(\sigma^2_A)$, calculated from $\sigma^2_p = V(m_1)+V(m_2)$, when the parents are non-inbred, F = zero (Wricke and Weber, 1986). However, top crosses require 5 heads per cross; this number is necessary because these crosses will segregate in the next F_1 generation and at least 80 plants to facilitate the selection of desirable plants in the F_1. The design has two shortfalls thus a single tester variety may not offer wide genetic background for testing the inbred stocks and the numbers of crosses become large if the test inbredlines are many. This design was used in increasing oil content of two synthetic maize varieties (Rosulj *et al.*, 2002).

2.17.4 North Carolina mating designs

After using diallel mating design for a long time, North Carolina designs were developed. North Carolina mating design is labor intensive. Three designs were developed and have been numbered Noth Carolina I, II and III (Comstock and Robinson, 1948). The first North Carolina design is popular due to its usefulness in both theoretical and practical plant breeding applications (Sharma, 2006). It is commonly used to estimate additive and dominance variances as well as for the evaluation of full- and half-sib recurrent selection. The sources of variation are due to males, females and within the plots (Comstock and Robinson, 1948; Griffing, 1956). The disadvantage of this design is that it requires sufficient seed for replicated evaluation trials, and hence is not of practical application in breeding species that are not capable of producing large amounts of seed. NC I design can be used for both self and cross-pollinated species. Being a nested design, each member of a group of parents which have been designated as males is mated to a different group of parents. In this design, the offsprings can be either full-sibs or half-sibs. Individuals that share a common father are refered to as half sib family group and a set of families with both parents in common constitutes a full-sib family (Kearsey and Pooni, 1996; Hallauer *et al.*, 2010). The model for experiment conducted in one environment is:

$$Y_{ijk} = \mu + m_i + f_{ij} + r_k + e_{ijk} \qquad \text{(Source: Sharma, 2006} \qquad (2.23)$$

where μ is the mean, m_i is the effect of the i^{th} male, f_{ij} is the effect of the j^{th} female mated to the i^{th} male, and e is the experimental error (Hallauer *et al.*, 2010). The design is commonly used

to estimate additive and dominance variances (Acquaah, 2012). It has also been used in maize (Hung *et al*., 2012), sorghum (Makanda *et al.*, 2009) and wheat (Cox and Murphy, 1990) improvement.

The North Carolina II mating design is a factorial design. In this design, each member of a group of parents used as males is mated to each member of another group of parents used as females. According to Kearsey and Pooni (1996), the design is useful in evaluating inbred lines for combining ability. This design is better when the plants under study have multiple flowers which can be used repeatedly as both male and female. Blocking is used in this design to allow all mating involving a single group of males to a single group of females to be kept intact as a unit (Comstock and Robinson, 1948). The design is essentially a two-way ANOVA in which the variation may be partitioned into difference between males (m) and females (f) and their interaction (Nduwumuremyi *et al*., 2013).

North Carolina III mating design which is also called the triple testcross was developed to estimate additive and dominance variance (Acquaah, 2012). It is capable of testing non-allelic (epistatic) interactions, which the other designs are not able to estimate. It is called triple test cross because a third tester is included in the design. This is done by including a random sample of F_2 plants which is then backcrossed to the two inbred lines from which the F_2 was descended. (Hill *et al*, 1998). North Carolina III is the best among the North Carolina desigs and is known to be the most powerful. The modifications by Kearsey and Jinks which included the third tester made the design have capacity to measure aspects that the other two could not measure (Kearsey and Pooni, 1996). The F_2 population is usually the reference population for the North Carolina III mating design (Hallauer *et al*., 2010).

In order to conduct analysis in this design, it is usually divided into two components in which the first component tests for epistasis while the second component assesses the significance and provides estimates of the additive and dominance components of variation. Although the triple test cross is the most powerful of the available mating designs, it is rarely used by plant breeders because it is more demanding than necessary (Hill *et al*., 1998). The NC designs have been used in development of hybrids in wheat (Cox and Murphy, 1990), maize (Williams, 2006) and sorghum, (Eberhart *et al.*, 1967; Makanda, *et al*., 2009).

2.17.5 Diallel mating design

Complete diallel mating design allows the parents to be crossed in all possible combinations including selfs and reciprocals (Schlegel, 2010). This kind of mating scheme is

required to achieve Hardy–Weinberg equilibrium in a population (Sharma, 2006). The diallel is the most used and abused of all mating designs in obtaining various genetic information (Hallauer *et al*., 2010). Much of its abuse could probably be due to the presence of two models for diallel analysis; random and fixed models (Griffing, 1956). A random model involves parents that are random members of a random mating population. It is useful for estimating GCA and SCA variances. In contrast, when parents are considered fixed effects, the aim is to measure the GCA effect for each parent and the SCA effect for each pair of parents. It is also widely used for developing breeding populations for recurrent selection (Sharma, 2006). Johnson and King (1998) suggested that diallel mating designs can be used to provide the maximum opportunity to manage co-ancestry in breeding population and maximize selection differential. However, in practice, a diallel with selfs and reciprocals is neither practical nor useful. This is because selfing does not contribute to the recombination of genes between parents. Furthermore, recombination is achieved by crossing in one direction making reciprocals unnecessary (Sharma, 2006). Nursery arrangements for the application vary depending either complete or partial diallel design and four methods under the diallel mating design have been so far described. The number of progenies generated from each method are different, the number of progeny families (pf) for methods 1 to 4 are: pf = n2, pf = 1/2n (n + 1), pf = n (n − 1), and pf = 1/2 n (n − 1), respectively (Sharma, 2006).

The method I or full diallel design consists of parents, one set of F_1's and reciprocal F_1's. The system gives n2 genotypes (Griffing, 1956). The mathematical models for combining ability analysis for the fixed and random effects are given by; fixed effect model or model I is given by:

$$Y_{ij}=\mu+g_i+g_j+s_{ij}+r_{ij}+1/bc\sum_k\sum_l\varepsilon_{ijkl} \qquad (2.24)$$

where; μ is the population mean, g_i, g_j is the general combining ability effect for the i^{th} and j^{th} parents, s_{ij} is the specific combining ability effect of the cross between the i^{th} and j^{th} parents such that $s_{ij}= s_{ji}$, r_{ij} is the reciprocal effect involving the reciprocal crosses between the i^{th} and j^{th} parents such that $r_{ij}= r_{ji}$ and, ε_{ijkl} is the experimental error due to environmental effect associated with the $ijkl^{th}$, which is assumed to be uncorrelated and normally distributed with zero mean and variance, V_E. Random effect model or model II:

$$Y_{ij}=\mu+g_i+g_j+s_{ij}+r_{ij}+1/bc\sum_k(bv) + 1/bc\sum_k\sum_l\varepsilon_{ijkl} \qquad (2.25)$$

The other three models have also been elaborated by Griffing, (1956). Diallel mating design was used in introgression of genes from upland cotton to Pima cotton (Zhang, Percy and McCarty, 2014). Half diallel mating design was used in determining GCA and SCA of sesame (*Sesamum indicum L.*) (Ahmed and Adam, 2014). With the aid of molecular markers, the design was used to correct pedigree errors in breeding populations (Munoz *et al*., 2014).

2.17. 6 Line × Tester mating design

Line x tester design is basically an extension of topcross design in the sense that instead of one tester as used in topcross, more than one is used. It was first proposed by Kempthorne in 1957 and cited by Sharma (2006). It was used successfully in analysis for grain yield and yield related traits in bread wheat (*Triticum aestivum L.*) (Fellai *et al.*, 2013). The statistical model is:

$$Y_{ijkl}=\mu+ a_l+b_{kl}+ V_{ij}+(av)_{ijl}+ E_{ijkl} \quad (2.26)$$

where Y_{ijkl} = observed value from each experimental unit; μ = population mean; a_l = location effect; b_{kl} = block or replication effect within each location; V_{ij} = F_1 hybrid effect = $g_i + g_j + s_{ij}$. However, $V_{ij} = g_i + g_j + s_{ij}$, where gi = general combining ability (GCA) for the i^{th} parental line; gj = GCA effect of j^{th} tester; sij = specific combining ability (SCA) for the ij^{th} F_1 hybrid and $(av)_{ijl}$ = interaction effect between i^{th} F_1 hybrid and i^{th} location; and ε_{ijkl} = residual effect (Fellai *et al*., 2013). The significance of mean square for line × testers provides a direct test of significance of dominance variance, σ^2_D while significance of σ^2_A is provided by significance of lines and testers mean squares (Nduwumuremyi *et al*., 2013). Line by tester design has been used in maize (Hallauer *et al*., 2010) wheat (Fellai *et al.*, 2013) and sorghum (Kenga *et al*., 2004).

2.18 Linear regression

Linear regression shows the relationship between independent and dependent variables (Gomez and Gomez, 1984). The simple linear regression analysis deals with the estimation and tests of significance concerning two parameters α and β in the equation:

$$Y = \alpha + \beta X \quad (2.27)$$

The data required for the application of the simple linear regression analysis are the *n* pairs (with $n > 2$) of *Y* and *X* values. Here, consideration of the relationship between cane yield,

juice yield, brix, and how each is related to ethanol production was made. Hypothesis $\alpha = \alpha_0$ was tested and t_α calculated according to Gomez and Gomez (1984) as follows:

$$t_\alpha = \frac{\alpha - \alpha_0}{\sqrt{s^2_{y.x}\left[\frac{1}{n} + \frac{\bar{X}^2}{\sum_X 2}\right]}} \quad (2.28)$$

The computed t_α value was compared to the tabular **t** value with n = 2 degrees of freedom and at a prescribed level of significance.

Multiple linear regression explains the relationship between one continuous dependent variable and two or more independent variables. It incorporates a large number of predictors and the analyses are best conducted using matrices. It helps in understanding how dependent variable changes with the change in the independent variable, identifying the strength of the effect of independent variables on dependent variables and very useful in predicting future trends and values. For example, every value of the independent variable *x* is associated with value of dependent variable *y*. The population regression line for variable *p* explanatory variables x_1, x_2,..., x_p is given by:

$\mu y = \beta_0+\beta_1 x_1+\beta_2 x_2+\ldots+\beta_p x_p$ (Montgomery and Runger, 2010) (2.29)

2.19 Correlation analyses

This refers to a measure of degree of association between two variables. Coefficient of correlation *r* can be expressed with a value ranging from -1 to 1 according to Montgomery and Runger, (2010) as:

$$r = \frac{\sum_{i-1}^{n} y_i(x_i - \bar{x})}{[\sum_{i-1}^{n} y_i(x_i-\bar{x})^2 \sum_{i-1}^{n} y_i(y_i-\bar{y})]^{1/2}} \quad (2.30)$$

$$= \frac{S_{xy}}{(S_{xx}SS_T)^{1/2}} \quad (2.31)$$

$$\widehat{\beta_1} = (\frac{SS_T}{S_{xx}})^{1/2} \quad (2.32)$$

Where r is the correlation coefficient and SS_T is the total sum of squares

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CHAPTER THREE

EFFECTS OF HARVESTING STAGE OF SWEET SORGHUM (*Sorghum bicolor* L.) GENOTYPES ON ETHANOL RELATED TRAITS IN KENYA

3.1 Abstract

Harvesting stage of sweet sorghum [*Sorghum bicolor* (L.) Moench] cane is important for sugar production of industrial alcohol. Four sweet sorghum genotypes were evaluated to determine the effects of harvesting stage on sweet sorghum traits in a randomized complete block design in four different environments. To determine sorghum harvest growth stage for bioethanol production, sorghum canes were harvested at intervals of seven days after anthesis. The genotypes were evaluated at different development stages from flowering time to physiological maturity to ascertain development stage with maximum production of bioethanol. The canes were crushed and extracted juice fermented to produce ethanol. Chlorophyll content were measured at various stages. Panicles were harvested at each of the stages and kernels dried and weighed at 14% moisture content. Chlorophyll, grain weight, absolute ethanol volume, juice volume, cane yield and brix, showed significant ($p \leq 0.001$) differences for genotypes, locations and the stages of harvesting. Harvesting sweet sorghum after 28 and 35 days after anthesis (104 to 117 days after planting) would be appropriate for production of kernels and ethanol in western Kenya while 157 to 200 days will be suitable for areas with climate similar to that of Egerton University. Genotypes evaluated at Kibos and Kendu-Bay showed higher ethanol production potential than those evaluated at Egerton site. EUSS10 had the highest ethanol potential (1062.78 Lha^{-1}) due to excellent juice volume (22976.9 L ha^{-1}) and EUSS11 (985.26 L ha^{-1}) due to its high brix (16.21).

Key Words: *Bioethanol, Brix, Cane yield, Sweet Sorghum , Volume of Juice*

3.2 Introduction

Sweet Sorghum (*Sorghum bicolor* L.) is an indigenous diploid (2n = 2*x* = 20), C4 crop to Africa and is a multipurpose crop providing food, feed, fiber and fuel across a range of agro-ecosystems (Kilambya and Witwer, 2013). In Kenya, sweet sorghum has the potential to improve the food security situation by providing food from kernel and feed as well as supply of cane in sugarcane industries for ethanol production (Naylor *et al*., 2007). Sorghum is well adapted to environmental conditions ranging from tropical to temperate conditions within 40 ^{o}N and 40 ^{o}S of the equator (Dogget, 1988). The potential of sorghum for production of

industial alcohol is enormous because the kernel and stalk can be harvested for both fodder and fuel production (Woods, 2001).

The stage of harvesting sorghum for biofuel is important for maximum juice extraction and ethanol processing (Zegada-Lizarazu and Monti, 2012). Stage of harvesting sweet sorghum cane for ethanol production is important to farmers as well as food, fodder and biofuel industries (Zegada-Lizarazu and Monti, 2012). Studies have been done on sweet sorghum sugar traits but those concerned with the harvesting stage of sweet sorghum relevant to its food, fodder and fuel utilities are minimal (Reddy *et al*., 2005; Antonopoulou *et al*., 2008; Ritter *et al*., 2008; Murray *et al*., 2008; Wang *et al*., 2009). In Indonesia, the optimum sorghum harvesting stage for industrial alcohol was determined to be hard dough stage (Tsuchihashi and Goto, 2004) but the effect of various stages on properties of sucrose was not considered. Biofuel industry is constantly competing with food supply globally because biofuel plant sources are the same that supply food to human population (Timilsina *et al*., 2012). In USA, 13.5 million tonnes of grain starch are used annually for production of biofuels (Simon *et al.*, 2008). This has elicited arguments concerning the competition between using food products as biofuels and using them to enhance food security in developing countries (Rathman *et al*., 2010).

The need to grow obligate cash crops such as sugarcane competes unfavorably with food production due to stiff competition on the arable land hence complicating the food security situation especially in developing countries (Bonin and Lal, 2012). There is need to balance between the growing of cash crops and food crops or else countries remain at the brink of famine (Paarlberg and Paarlberg, 2013). The knowledge of the harvesting stage of sweet sorghum can help breeders to further develop improved varieties and for biofuel farmers to know when to harvest their sorghum crop (Yuan *et al*., 2013). In the USA, 6000 Lha^{-1} of ethanol has been produced from sweet sorghum cane but this production is low compared to the quantity obtained from sugarcane (Smith and Buxton, 1993). However, even though the ethanol yield per unit weight of sweet sorghum cane feedstock is lower compared to that of sugarcane, the low production costs and water requirement for this crop compensates for the difference and returns a competitive cost advantage in the production of ethanol (Rao *et al*., 2004).

Sugar accumulation in the stems of sweet sorghum is a function of metabolism and transport processes in the plant (Patrick *et al*., 2013). The physiological size and activities of

sink organ influence the competitive ability to import photoassimilates (Marcelis, 1996). Therefore, it is necessary to increase sucrose content in large stems. A sweet sorghum cultivar, *Keller* had been developed and has high performance in a wide range of environmental conditions in the USA (Sami *et al*., 2013). Currently, there are efforts globally to promote the production of biofuel from sweet sorghum cane (Sokan-Adeaga and Ana, 2015). In Australia, sweet sorghum is grown in South Eastern Queensland and canes are supplied to the biofuel industry to produce industrial alcohol. In the USA, sweet sorghum is used for ethanol and fodder production (Zarco-Tejada *et al.,* 2005; Ratnavathi *et al*., 2015).

Carbohydrates in sorghum are mainly synthesized in the leaves and translocated to demand sites which include panicles and stem (Bihmidine *et al*., 2015). The main pigment in the leaves that enable sorghum to synthesize carbohydrates is chlorophyll in the presense of light energy (Slewinski, 2012). Chlorophyll content indicates the photosynthetic activity of the plant and can be used to predict maturity and harvesting time of sweet sorghum cane. High chlorophyll content in the leaves is related to high photosynthetic activity and *vice versa* and detection of chlorophyll content through remote sensing can be used to predict appropriate harvesting time of sorghum for production of alcohol (Megio *et al*., 2010). However, low concentration of chlorophyll content in the leaves is an indicator of nitrogen deficiency and may indicate false maturity in the crop (Zarco *et al*., 2005). To avoid false detection, the precision of agricultural practices can be increased by remote sensing (Haboudane *et al*., 2002. It is necessary to set a baseline and investigate soil nutrient conditions before using this approach (Shepherd and Walsh, 2007). The objective of this study was to determine the effect of harvesting stage of sweet sorghum on yield of the cane, volume of juice, ethanol and brix of sweet sorghum at different harvesting stages.

3.3 Materials and Methods

3.3.1 Genotypes

Three sweet sorghum lines EUSS10, EUSS11 and EUSS17 and cultivar, SS04 were used in this study. SS04 is a cultivated sweet sorghum variety of medium height with cream coloured kernels. EUSS11 and EUSS17 have cream kernels and also have sweet stalks. The other three are under development by Egerton University and have not been released as cultivars.

3.3.2 Experimental site and environmental conditions

This study was carried out at Egerton University (0° 23′32″S and 35° 35′12″E), Kendu-Bay (0.35°13′07″S, 34°07′44″) and KALRO Kibos (0°04′06″S, 34°49′03″E). These were considered as environments in which first and second seasons in Kibos were considered as different environments. Egerton University Njoro Campus is located at an altitude of 2265 m above the sea level (a.s.l). The site is classified as Lower Highland 2 to 5 (LH2 – LH5) agro ecological zones and has a sub humid modified tropical climate (Sombroek *et al*., 1982). The annual average precipitation of the area is 908 mm with 60% reliability. Mean temperatures ranges between 16°C and 17°C while soils are classified as Mollic Andosols. KALRO Kibos Research Station has an altitude of 1173 m a.s.l, about 8 km East of Kisumu City. This area experiences mean precipitation of 1323 mm per year with the conventional onset of long rains usually in March. The short rains usually commence in August with a drop in September, reaching the peak of about 374.4 mm in December. Generally, high precipitation is experienced during the long rainy season with the average maximum temperature of this location is 30°C with a minimum of 15.5°C, while the soils are heavy black cotton (Hansen *et al*., 2011). Kendu-Bay lies at an altitude of 1132 m a.s.l, about 5 km east of Kendu-Bay Township. The area experiences an average rainfall of about 1200 mm per annum with the onset of long rains in March while the short rains commence in September. The average temperature in this area is 27 °C with a minimum of 17 °C. The soil in this area is predominantly sandy and drains very easily.

3.4 Experimental procedure

Four sweet sorghum genotypes were planted at the four aforementioned sites. The fields were disc ploughed and harrowed twice to minimize weed growth and achieve a fine tilth suitable for planting sorghum. The experiment was conducted in a Randomized Complete Block Design (RCBD) with four replications. Within the replicates, sorghum genotypes were planted at seeding rate of 10 kg ha^{-1} in plots measuring (3 × 5) m. Sorghum was sown at a spacing of (60 × 15) cm. At planting time, fertilizer application rate was 30 kg ha^{-1} of P, 10 kg ha^{-1} of K and 40 kg ha^{-1} N with NPK in the ratio 20:10:10 respectively. Fourteen days after emergence, plants were thinned to one plant per hole. Six weeks after planting, the crop was top dressed at the rate of 40 kg ha^{-1} N using Calcium Ammonium Nitrate (CAN). Infestation of shoot fly (*Atherigona soccota*) on young seedlings was controlled by spraying a systematic

insecticide, Bulldock® (*beta-cyfluthrin* 25 gl^{-1}) at 25 g ha^{-1} at intervals of 14 days for one month. Within the experimental plots, weed growth was managed by mechanical weeding. Weeding and inter cultivation operations was done twice between 5-leaf and booting stage. Between booting stage and the end of anthesis, a second dose of Bulldock® was applied at 25 g ha^{-1} to control sorghum midge (*Contarinia sorghicola*). After heading, sorghum panicles were covered with paper bags to protect them from bird damage. Sorghum canes were harvested by cutting plants at the base, leaf sheath stripped and panicles clipped. This was done at different developmental stages from the onset of flowering to maturity (Table 3.1).

Table 3.1 Duration after anthesis, harvest stages and description of crop appearance.

Duration after anthesis (days)	Harvest Stages	Description of the crops during various stages of harvesting
7	I	Plants are at 50% flowering; panicles have no pollen
14	II	Plants have all flowered and pollen shed
21	III	Pollination complete and grain filling begins; the milk stage
28	IV	Grain filling complete and kernel begin to harden; soft dough stage
35	V	Grains almost mature; the hard dough stage
42	V	The crops are at physiological maturity; the grains begin to dry

3.5 Data collection

Mean heights of the plants were determined by measuring from the base level to the panicle while height to flag leaf was determined by measuring plants from the base to the flag leaf collar. Mean girth was determined by measuring circumference at the fifth internode using Vanier calipers. From each entry, a sample of five plants per plot from the middle row were harvested by cutting the plant at the base and stripped, at an interval of seven days for six weeks. Cane and juice yield was estimated by cutting sorghum at the base from the middle two rows of plot and stripped off leaf sheath. Harvesting commenced at anthesis at an interval of 7 days for 42 days.

Juice was extracted from canes using a three roller crusher (Fuan Liyuan, China, type YC 80B-4) once and strained through a 1 mm pore sieve to remove large particles. The wet

weight of bagasse was determined immediately using Ashton Meyer's digital balance. Thereafter, brix in the juice samples were estimated using refractometer. Juice extraction (%) was computed by dividing weight of fresh juice by weight of fresh cane and multiplying by 100. Juice yield was computed by multiplying average juice weight from 5 plants by plants per hectare. Juice was fermented by adding approximately 1.5 g yeast (*Saccharomyces cerevisiae*) to each 100 ml of sample juice and incubated at 35ºC for 4 days. The fermented samples were then transferred into conical flask connected to a Liebig condenser and distilled. Ethanol concentration was determined by measuring the refractive indices on a hand held refractometer (Model: Standard line Alla made in France). The concentrations were based on a standard curve drawn by measuring the refractive indices of absolute ethanol solutions (0, 5, 10, 15, 20, 25 and 30%) in distilled water.

Chlorophyll level in the leaves was determined from the 50% flowering stage and at all stages of harvesting, using SPAD 502 chlorophyll meter. The chlorophyll level was determined from the leaf on the 5^{th} node from the ground and at the mid section of the leaf for consistency. The 5^{th} leaf also happens to have the largest surface area and remains attached to the plant till maturity. Five plants were randomly selected per plot and harvested by cutting at the base which is the middle of the first internode. For each of the harvested plants, the panicles were removed and air dried (25 ºC) for 21 days. Thereafter, cane yield, excluding the leaves was determined from the stalks. The average grain yield in mass was determined for every stage of harvesting for each genotype after drying them to approximately 14% moisture content.

3.6 Data analyses

Further investigation was done on the brix to understand its rate of accumulation. The first derivatives of the function were taken to determine the rate of accumulation of brix using the formula:

$$f' = \frac{dy}{dx} = \frac{(\Delta y_1 - \Delta y_2)}{(\Delta x_1 - \Delta x_2)} \qquad (3.1)$$

Data were analyzed using Proc GLM of SAS (Statistical Analysis System) Version 9.1 with location, replication and genotype considered as fixed factors while interactions between genotype and stage, stage and location and interactions between stage, genotype and location considered as random factors. During the analysis, the hypothesis on location was tested using location × stage as the error term, hypothesis on genotypes was tested using genotype × stage as the error term, hypothesis on stages of harvesting was tested using genotype × stage as the

error term and finally, the hypothesis on interaction between location × genotype, genotype × location × stage was used as the error term. For every trait under investigation, error variances and means were computed for genotypes and locations. They were compared and declared significant or not at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$. Analyses of data for traits were done using the following statistical model:

$$Y_{ijkl} = \mu + L_i + R_{ij} + G_k + S_l + GL_{ik} + GS_{kl} + SL_{il} + SGL_{ikl} + \varepsilon_{ijkl} \qquad (3.2)$$

where Y_{ijkl} is observation, μ is the overall mean, L_i is the effect due to location, R_{ji} is effect due to j^{th} replicate in the i^{th} location, G_k is the effect due to k^{th} genotype, GL_{ik} is the effect due to i^{th} location and k^{th} genotype, S_l is the effect due to the stage of harvesting, SL_{il} is due to the interaction between stage of harvesting and location GS_{kl} is due to interaction between stage of l^{th} stage of harvesting and k^{th} genotype, SGL_{ikl} is the effect due to interaction between i^{th} location, k^{th} genotype and l^{th} stage of harvesting and ε_{ijkl} is the error term as outlined by Gomez and Gomez (1984). SAS code used was as indicated in appendix IV.

3.7 Results and Discussion

3.7.1 Environmental conditions

The growing period commenced on 14th April 2016 to July 2016 for first season in Kibos (0°04′06″S, 34°49′03″E). At the time of planting on 14th April, the seedbed was saturated with moisture after receiving an average of 20.6 mm of rainfall. April was the month with the highest rainfall in the growing season with moderate rainfall experienced in May (8.2 mm) and June (1.7 mm) (Figure 3.1). Temperatures of 23.1°C in April, May 22.6°C and June at 16°C were experienced during the growing period. During this period low temperatures (15°C) and rainfall (1 mm) were experienced (Figure 3.1). The second season in Kibos was from September to December 2016. Rainfall during the second season was generally lower and with higher mean temperatures. At Kendu-Bay (0.35°13′07″S, 34°07′44″) the growing period commenced September 16th to January 2017.

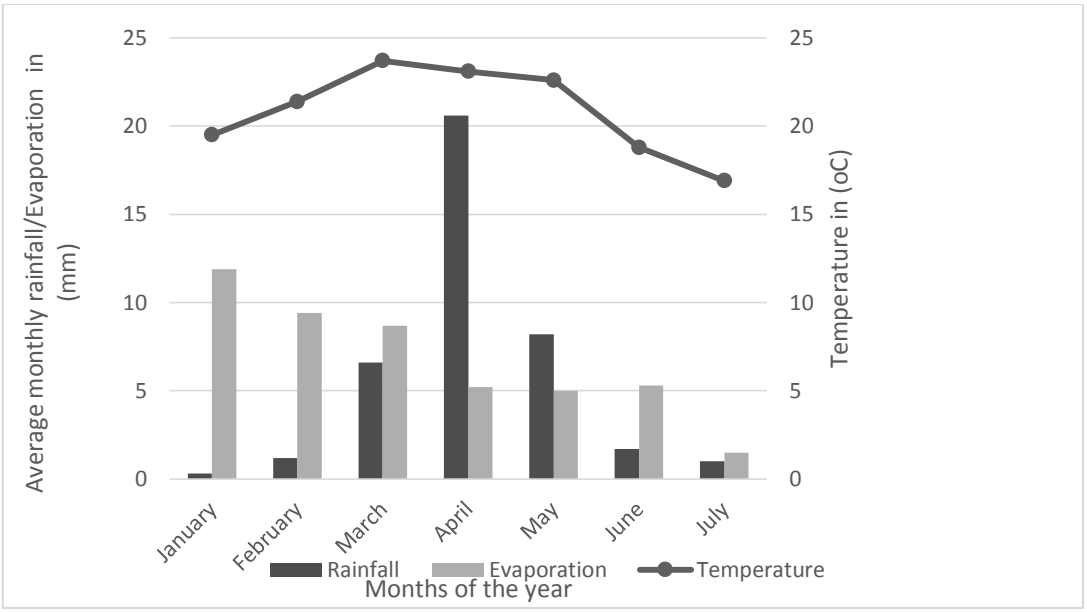

Figure 3.1 Weather data for the period January to July 2016 from SRI meteorological station about 200 m from the experimental field (NB: This is monthly average and to get the total monthly rainfall or evaporation you multiply by 30 or 31 depending on the days of the month).

3.7.2 Analyses of variance of sweet sorghum traits

There were significant ($p \leq 0.001$) effects due to genotype for height, girth, juice volume, brix, ethanol content and chlorophyll (Table 3.2).

Table 3.2 Expected Mean squares and mean squares of four sweet sorghum genotypes evaluated at different harvesting stages in four environments in 2016 for agronomic traits.

Sources of Variation	df	Expected Mean Squares	Height	Girth	Chlorophyll	Days to flowering
Environment	3	$\sigma^2_E+4\sigma^2_{GLS}+16\sigma^2_{LS}+24\sigma^2_{GL\,c}+24\sigma^2_{RL}+96\sigma^2_L$	657.05	31.08	0.80	44943.92***
Replicate (Location)	12	$\sigma^2_E+24\sigma^2_{R(L)}$	549.95	1.27	2.05	0.13
Location × Stage	15	$\sigma^2_E+4\sigma^2_{GLS}+16\sigma^2_{LS}$	111.79**	17.78***	6.07	5.50
Genotype	3	$\sigma^2_E+4\sigma^2_{GLS}+16\sigma^2_{GS}+24\sigma^2_{GL}+\sigma^2_G$	4934.80**	198.28***	1504.18***	7207.21***
Stage	5	$\sigma^2_E+4\sigma^2_{GLS}+16\sigma^2_{LS}+\sigma^2_S+16\sigma^2_{GS}$	103.45	49.60***	7443.85***	8.82
Genotype × Environment	9	$\sigma^2_E+4\sigma^2_{GLS}+24\sigma^2_{GL}$	578.77***	1.17	0.21	433.57***
Genotype × stage	15	$\sigma^2_E+4\sigma^2_{GLS}+16\sigma^2_{GS}$	41.89	25.36***	732.60***	9.14
Genotype × Environment × Stage	45	$\sigma^2_E+4\sigma^2_{GLS}$	30.33	1.61*	6.28	10.53***
Error	276	σ^2_E	61.46	1.01	8.56	0.14
CV%			4.57	5.42	6.88	0.45
R^2			0.65	0.86	0.95	0.99

df-degrees of freedom,

*,**, *** significantly different at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, respectively.

Test H = Location, E = Location × Stage; Test H=Genotype, E= Genotype × Satge; Test H= Stage, E= Genotype × Stage; Test H= Genotype × Location, E= Genotype × Location × Stage

Continued on the next page

Table 3.2 *Continue*

Sources of Variation	df	Expected Mean Squares	Cane yield (ton ha^{-1})	Volume of juice (l ha^{-1})	Brix	Absolute ethanol (l ha^{-1})	Grain Weight (tons ha^{-1})
Environment	3	$\sigma^2_E+4\sigma^2_{GLS}+16\sigma^2_{LS}+24\sigma^2_{GL}+24\sigma^2_{RL}+96\sigma^2_L$	662.43	9708.29	31.44	8075611.10	14722.00
Replicate (Environment)	12	$\sigma^2_E+24\sigma^2_{RL}$	9.90	208.06	1.31**	132343.80	68673.00
Environment × Stage	15	$\sigma^2_E+4\sigma^2_{GLS}+16\sigma^2_{LS}$	165.59***	4535.36***	9.31***	4456531.00***	19175.00***
Genotype	3	$\sigma^2_E+4\sigma^2_{GLS}+16\sigma^2_{GS}+24\sigma^2_{GL}+\sigma^2_G$	4042.87***	115329.89***	953.41***	53188319.40***	26662551.00***
Stage	5	$\sigma^2_E+4\sigma^2_{GLS}+16\sigma^2_{GS}+16\sigma^2_{LS}+\sigma^2_S$	320.55	4705.67***	618.16***	13997372.80***	1183648746.00***
Genotype × Environment	9	$\sigma^2_E+4\sigma^2_{GLS}+24\sigma^2_{GL}$	133.07***	1795.57*	6.94***	1442274.30*	3286.00
Genotype × stage	15	$\sigma^2_E+4\sigma^2_{GLS}+16\sigma^2_{GS}$	231.46***	6075.12***	15.40***	2982527.40***	2724333.00
Genotype × Environment × Stage	45	$\sigma^2_E+4\sigma^2_{GLS}$	29.34***	743.93***	1.18***	610428.60***	5095.00
Error	276	σ^2_E	6.59	112.03	0.53	164456.60	34981.00
CV%			6.38	7.36	5.19	9.37	5.31
R^2			0.93	0.95	0.97	0.89	0.99

Significant ($p \leq 0.001$) effects due to stage and genotype × stage interactions were detected for girth, juice volume, brix, ethanol and content of chlorophyll. No significant effects due to genotype × stage of harvesting interactions were observed for grain weight.

Significant ($p \leq 0.001$) difference due to location was observed for days to flowering. For cane yield, girth, height, brix, volume of juice, chlorophyll content, weight of kernel and ethanol volume, location effects were not significant ($p \leq 0.05$). The effect due to interactions between location and stage was significant ($p \leq 0.001$) for height, girth, cane yield, volume of juice, brix, volume of ethanol and kernel weight. All the other traits did not show any significant difference. Location × Genotype effects were significant ($p \leq 0.001$) for height, days to flowering, cane yield and brix. Volume of juice and volume of ethanol were significant ($p \leq 0.05$) while all the others did not show any significant difference. The effect due to interactions between genotype × location × stage was significant ($p \leq 0.001$) for days to flowering, cane yield, volume of the juice, brix, volume of ethanol (Table 3.2).

There was significant variation between genotypes for days to flowering. EUSS17 took the least (57 days) to flower followed by SS04 (69 days) and EUSS11 (73 days) but it took 82 days for EUSS10 to attain anthesis stage. Line EUSS10 attained a height of 182 cm, followed by EUSS17 (179 cm) and EUSS11 (175 cm). Cultivar SS04 attained a height of 188 cm. However, harvesting stage did not influence height for the sorghum lines tested. There was a clear distintion in the appearance of the grains with the ones obtained more shriveled and lighter than the ones in the later stages (Figure 3.2). The harvest after 42 days had the most suitable kernels (Figure 3.2).

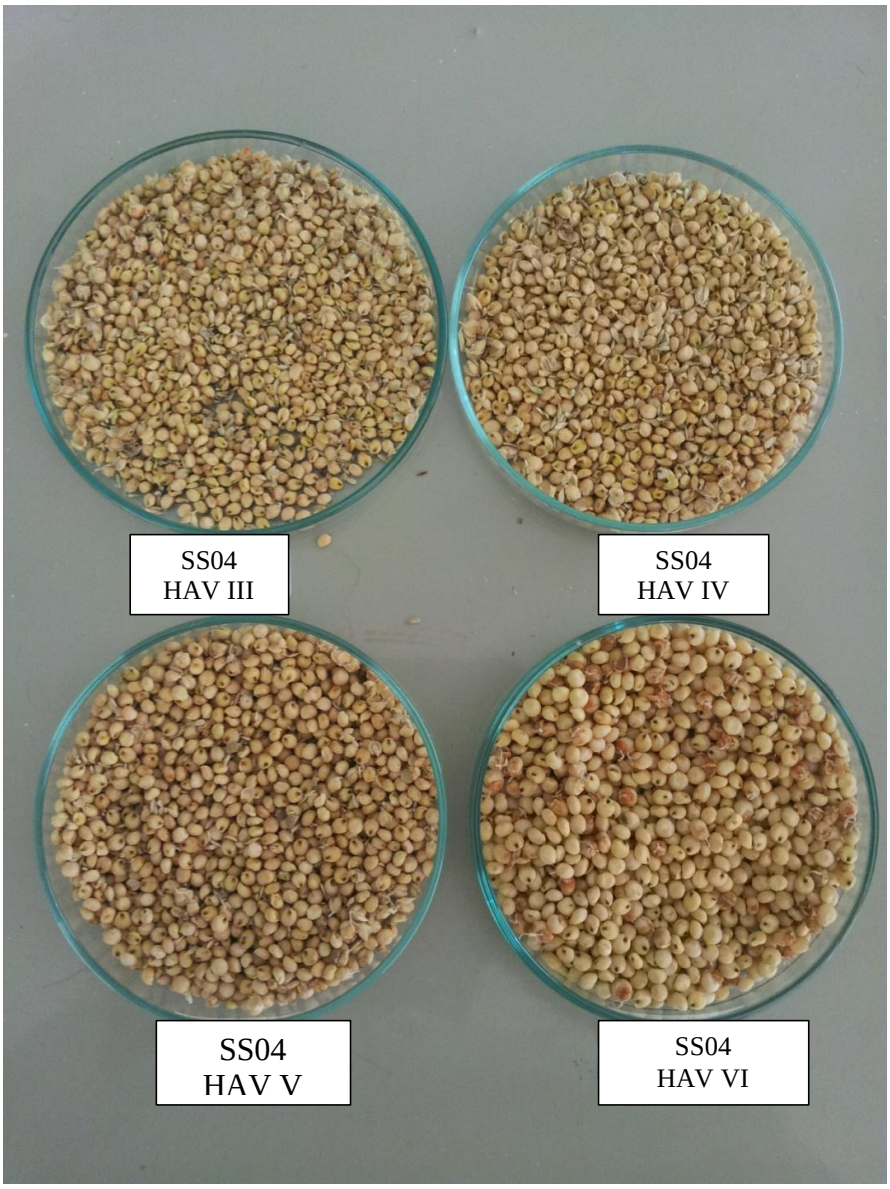

Figure 3.2 Kernels of sweet sorghum (*Sorghum bicolor* L.) variety SS04 obtained at various stages.

NB: HAV III -Harvest at stage 3 , HAV IV –Harvest at stage 4, HAV V-Harvest at stage 5, HAV VI-Harvest at stage 6.

Table 3.3 Mean values of some agronomic traits and ethanol related traits from sweet sorghum genotypes evaluated and harvested at different development stages in four environments in Kenya in 2016.

Genotype	Height (cm)	Cane yield (tons ha^{-1})	Girth (mm)	Volume of juice (L ha^{-1})	Brix (%)	Absolute ethanol (L ha^{-1})	Chlorophyll level	Grain Weight (ton ha^{-1})	Days to flowering
EUSS10	188.00 a	50.85 a	20.79 a	22976.9 a	9.16 d	1062.78a	46.54 a	4277.06 a	93.21 a
EUSS11	182.25 b	45.39 b	18.44 b	20600.8 b	16.21 a	985.26 b	44.13 b	3428.52 b	81.87 b
EUSS17	179.00 bc	35.76 c	18.11 b	14808.8 c	15.29 b	961.96 b	40.42 c	3308.06 c	72.67 d
SS04	174.00 c	35.73 c	17.64 b	13546.6 d	13.91 c	805.96 c	38.06 d	3046.30 d	78.48 c
Lsd 0.05	1.99	4.68	1.54	317.6	1.2	40.29	1.55	94.33	0.93
Days after anthesis									
7	177.39 c	43.45 a	20.24 a	17314.2 dc	8.56 e	609.37 c	57.04 a	0.00 e	81.25 a
14	177.95 ab	44.84 a	18.31 c	18322.9 b	12.56 d	803.07 b	50.97 b	0.00 e	81.89 a
21	179.69 ab	44.18 a	19.28 b	19220.8 a	13.81 c	1073.06 a	45.14 c	1170.10 d	80.97 a
28	179.78 a	41.17 b	16.80 d	18100.4 b	14.18 c	1078.63 a	40.43 d	2295.99 c	81.79 a
35	180.30 a	39.40 bc	18.79 b	17821.0 bc	15.56 b	1089.08 a	30.75 e	7262.43 b	81.67 a
42	180.42 a	38.55 c	18.86 bc	17120.4 d	17.18 a	1070.72 a	29.39 e	10362.89 a	81.84 a
Lsd 0.05	2.44	5.73	1.89	537.5	1.47	49.35	1.14	115.54	1.13
Environments									
Egerton	176.44 a	41.91a	18.99a	15650.8 ab	14.29 b	846.45 b	42.52 a	3522.20 a	114.00 a
Kibos 1	180.89 a	40.57b	18.74a	15548.9 b	13.51 c	906.25 a	42.47 a	3515.24 a	70.34 c
Kibos 2	177.69 a	36.36c	17.75b	13588.3 b	13.65 c	789.67 c	42.45 a	3514.86 a	72.02 b
Kendu-Bay	181.99 a	41.89a	18.89a	15881.8 a	14.74 a	929.72 a	42.47 a	3541.26 a	69.92 d
Lsd 0.05	7.37	0.98	0.35	479.02	0.36	35.22	0.45	53.14	0.11

Means designated by the same letter within columns are not significantly different at $p \leq 0.05$

Among the four genotypes, there was significant difference ($p \leq 0.05$) in the total cane yield per hectare between EUSS10 and EUSS11 but there was no significant difference among EUSS11, EUSS17 and EUSS17 and SS04 (Table 3.2). Sorghum line EUSS10 gave the highest cane yield of 57 t ha $^{-1}$ when harvested 21 days after anthesis. Both EUSS17 and SS04 produced similar yields when harvested 21, 28, 35, and 42 days after anthesis (Figure 3.5). Although harvesting after 7, 14 and 21 days after anthesis were not significantly different for cane yield, there were significant difference ($p \leq 0.05$) in cane yield for harvests conducted after 28 and 42 days after anthesis (Table 3.2). For cane yield, Egerton and Kendu-Bay site had the highest followed by Kibos 1 (season 1) and Kibos 2 (season 2).

In this study, concentration of chlorophyll decreased from the highest in the first stage of harvesting to the lowest in the last stage of harvesting (Figure 3.3). The highest level of chlorophyll was observed at anthesis which was 57, 69, 73 and 82 days after sowing for EUSS17, SS04, EUSS11 and EUSS10, respectively. In general, there was a steady reduction of chlorophyll content as sorghum matured. Among the four sorghum genotypes, EUSS10 had the highest chlorophyll content. From analysis of linear regression on the level of chlorophyll in the leaves, it was evident that SS04 had the highest rate of decrease of chlorophyll at -8.93. This was followed by EUSS11 and EUSS17 at -8.20 and -5.82, respectively. The lowest rate of reduction of chlorophyll concentration was observed on sorghum line EUSS10 at -2.22 as well as the lowest y-intercept. Comparison of the chlorophyll content revealed that there was no significant difference in the chlorophyll levels in the four locations. It is interesting to note that sweet sorghum performed competitively well at Egerton which is more than 2000 m a.s.l and Kisumu which is about 1000 m a.s.l.

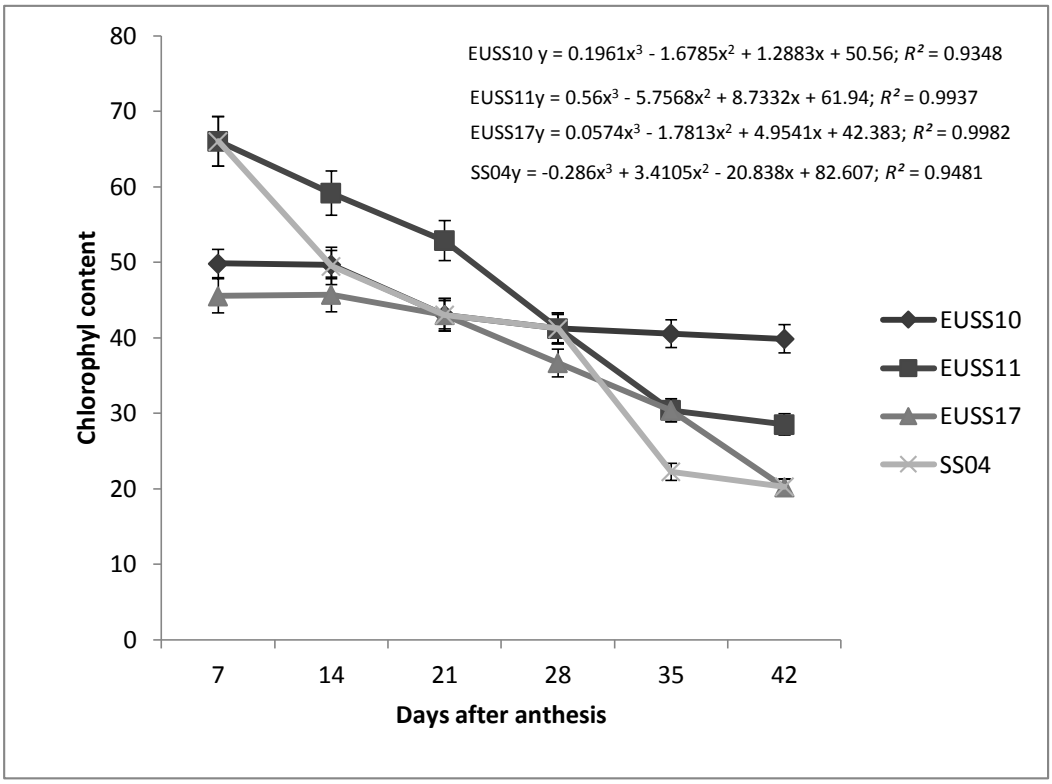

Figure 3.3 Chlorophyll content at different harvesting stages of four sweet sorghum genotypes evaluated in four environments.

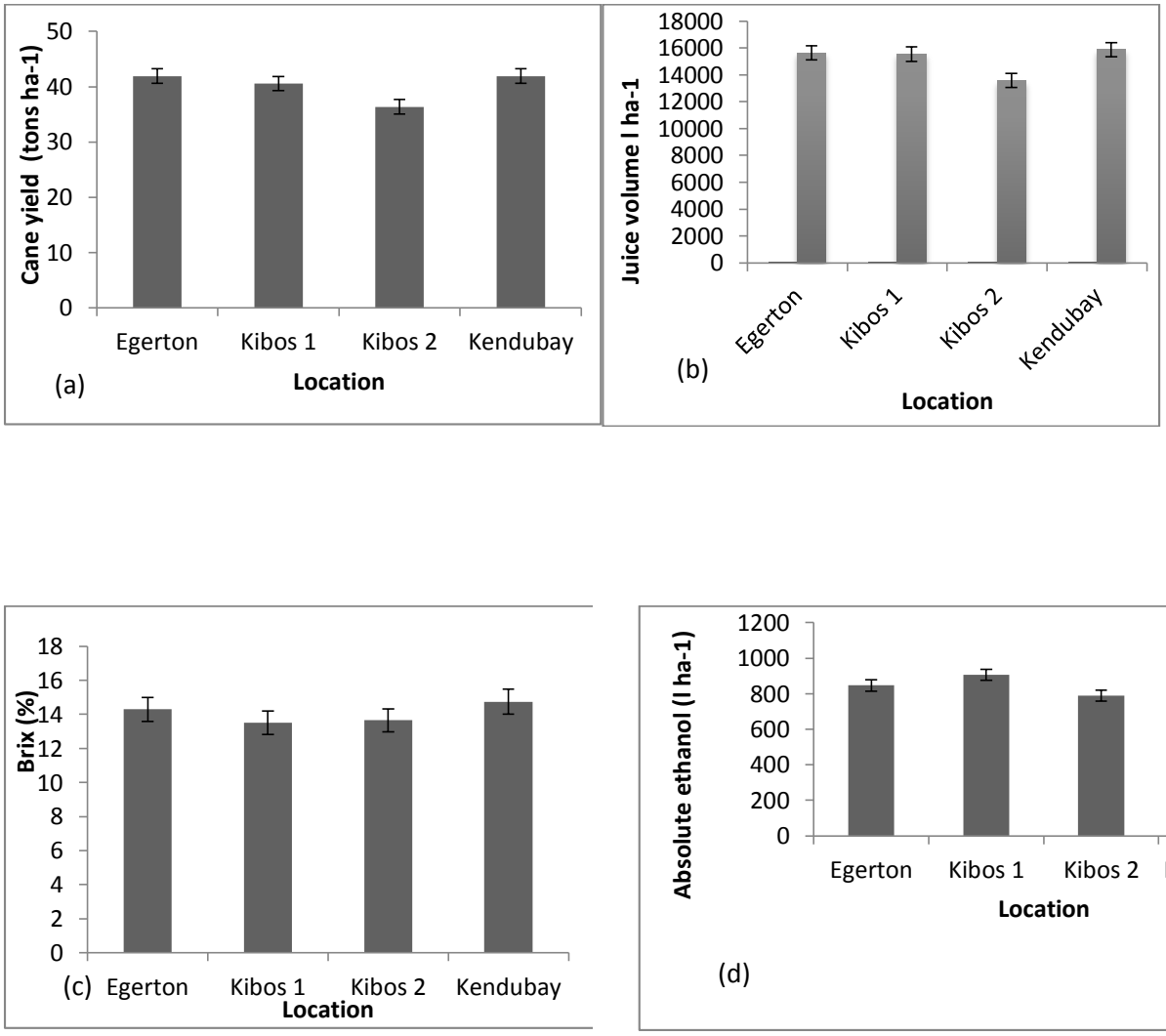

Figure 3.4 (a) Cane yield, (b) Juice volume, (c) Brix, (d) Ethanol volume of sweet sorghum in four different environments

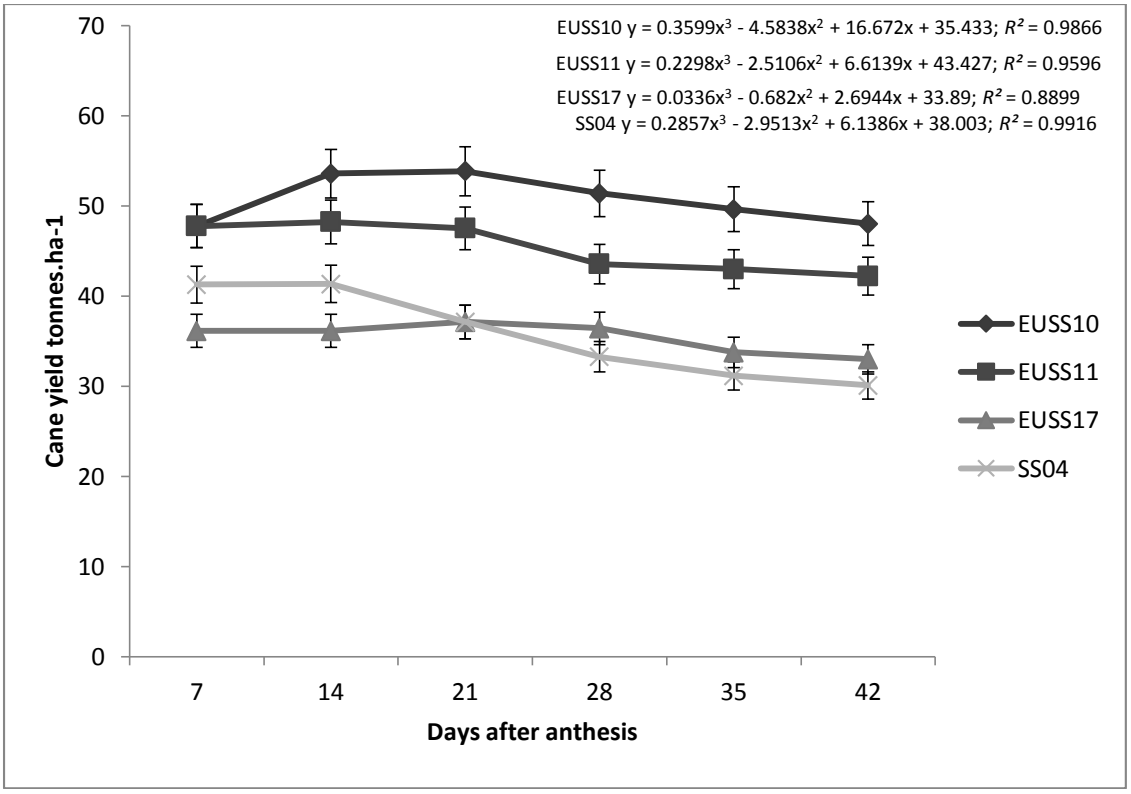

Figure 3.5 Effect of harvesting stage on cane yield of four sweet sorghum genotypes evaluated in four environments.

There was significant mean difference ($p \leq 0.001$) in the brix for all genotypes evaluated (Table 3.2) and for all the stages of harvesting except for harvests done after 21 and 28 days after anthesis (Table 3.3). The genotypes, the stages of harvesting and their interaction showed significant variation in brix accumulation (Table 3.2). The important traits related to ethanol production were seen to be highest at Kendu Bay (Figure 3.4). Cane yield was highest (42.0 t ha^{-1}) in Kendu Bay followed by Egerton (41.5 t ha^{-1}), it also had high (15,888 L ha^{-1}) volume of juice followed by the first season in Kibos (15,548 L ha^{-1}) (Figure 3.4 b). Among the four different environments, Kendu-Bay had the highest (14.74) brix followed by Egerton (14.29) and Kibos (13.65) (Figure 3.4 c). There was significant ($p \leq 0.001$) difference in brix in the four locations as well as the interaction between genotypes and locations and genotype by location and by stages of harvesting (Table 3.2). Materials evaluated at Kendu-Bay had the highest brix followed by Egerton (Figure 3.4 c). There was no significant difference between the brix levels observed during the first and second season in Kibos. Brix is a good indicator of the amount of sucrose in the stalk for ethanol production. EUSS11 and EUSS17 had the highest brix at all the harvesting stages (Figure 3.6). EUSS10 had low brix and the increase

was slower and almost stagnant after 35 and 42 days after anthesis. Furthermore, the highest rate of brix accumulation was exhibited for genotype EUSS11 which was at the rate of 1.9 after every 7 days. This was followed by SS04, EUSS17 and EUSS10 at 1.53, 1.5 and 0.98, respectively (Figue 3.7).

There was rapid decrease in brix in line EUSS11, EUSS17 and SS04 to 21 days after anthesis followed by a steady increase up to 42 days after anthesis. Further analysis of rate of accumulation of brix was done by performing regression and determining equation of the curves, finding the first derivatives of the equations and plotting the functions to obtain parabolic curves. For example, for EUSS10, $y = 0.11818x^3-1.4137x^2+5.6825x+1.5$ was differentiated to $y = 0.35454x^2-2.8274x+5.6825$ and EUSS11, $y = 0.3634x^3-3.999x^2+14.709x-1.3333$ differentiated to $y = 1.0902x^2-7998x+14.709$ and the products used to estimate the rates of accumulation of brix. Rate of accumulation of brix of EUSS11 was decreasing at the rate of 0.4% per day. For the same genotype, the rate increased by 0.2% per day. Accumulation of brix steadily decreased for SS04 at the rate 0.47% for the first 21 days, slowed down for 7 days to minimum then increased steadily for 14 days at the rate of 0.21%. Genotype EUSS10 showed very gradual decrease for all the stages of harvesting (Figure 3.7). For EUSS11, EUSS17 and SS04, the parabolas indicated that the rates of brix accumulation dropped from anthesis to 21 days after anthesis then started to increase at increasing rate from 28 days to 42 days after anthesis. A detailed comparison of brix (Figure 3.10), volume of juice (Figure 3.11) and volume of ethanol (Figure 3.12) have been illustrated for all the four environments.

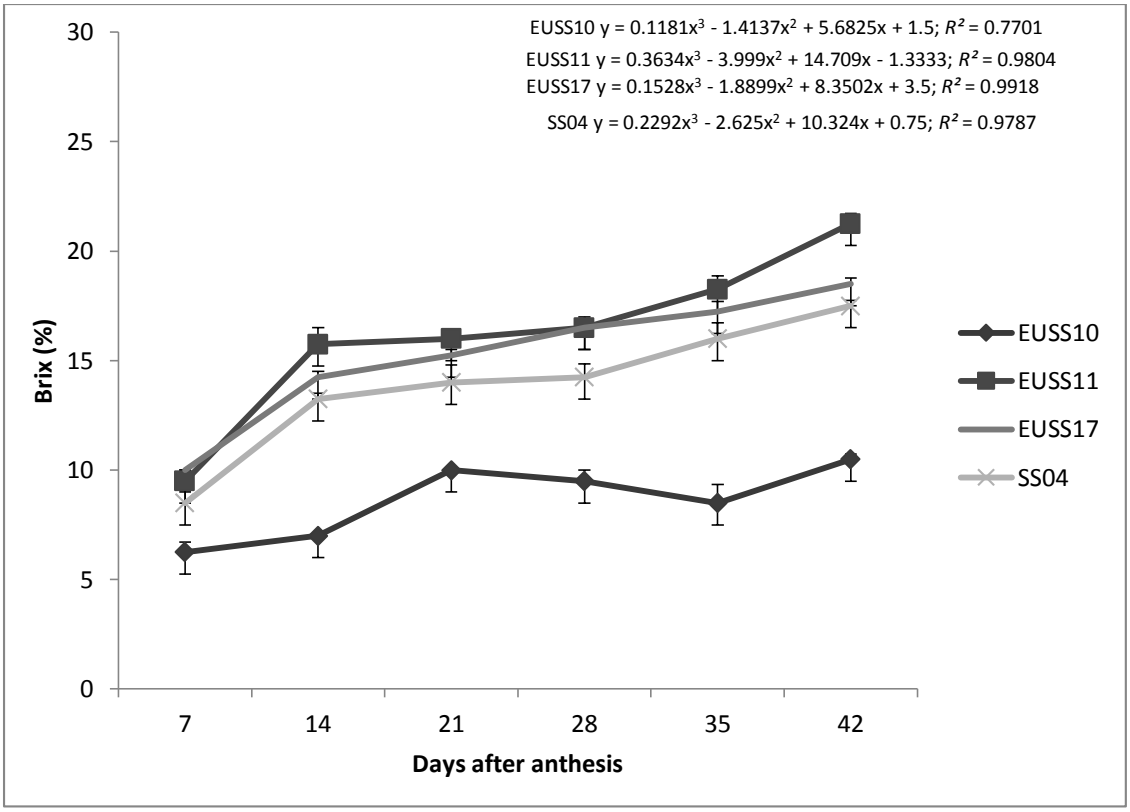

Figure 3.6 Effect of harvesting stage on brix (%) of four sweet sorghum genotypes evaluated in four environments.

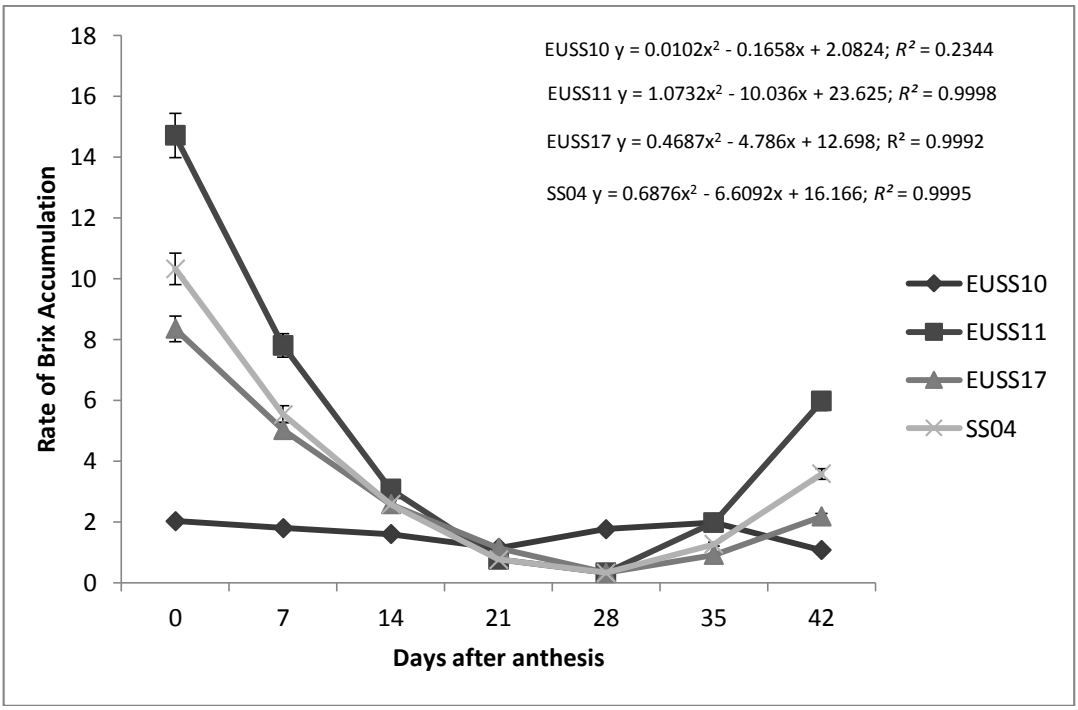

Figure 3.7 Rate of accumulation of brix against the stages of harvesting among the four sweet sorghum genotypes observed in four envriornments in Kenya.

There was significant ($p \leq 0.001$) difference in juice volume among all the genotypes (Table 3.2). Juice volume increased with maturity until 21 days after flowering then declined (Figure 3.8). Juice volume also showed significant difference in the means at anthesis, after 7, 14, and 21 days after anthesis. There was a significant difference between 28, 35 and 42 days after anthesis (Table 3.3). There was no significant ($p \leq 0.001$) difference in juice volume in the environments except for Kendu-Bay. There was also no significant difference in the interaction between stages of harvesting and the locations. However, there was a significant ($p \leq 0.001$) difference for the interaction between location and genotype. At the 21st and 28th day after flowering, most of the genotypes were at their peak in juice production. For Egerton University location, the peak juice production was observed in the 40th day after flowering. The highest juice volume was observed on line EUSS10 35 days after flowering and this was in Kendu-Bay.

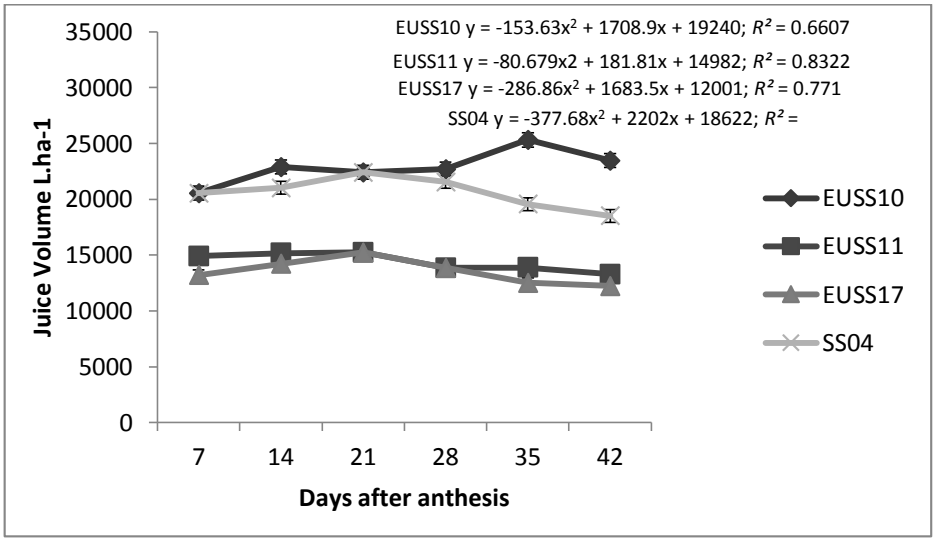

Figure 3.8 Effect of harvesting stage on juice yield of four sweet sorghum genotypes evaluated in four environments in Kenya.

There was significant ($p \leq 0.001$) difference in the absolute ethanol volume for genotype EUSS10, SS04, and EUSS11 (Table 3.2). However, there was no significant ($p \leq 0.001$) difference observed between EUSS11 and EUSS17 (Table 3.3). Mean difference in absolute ethanol volume at anthesis (609.37 L ha^{-1}), 14 days (803.07 L ha^{-1}) and 21 days (1073.06 L ha^{-1}) were observed but there was no significant difference after 28 days (1078.63 L ha^{-1}), 35 days (1089.08 L ha^{-1}) and 42 days after anthesis (1070.72 L ha^{-1}) (Table 3.3). Volume of ethanol obtained from all genotypes increased rapidly from anthesis to 21 days after then a decrease from 21 days to 42 days after anthesis. However, the decrease of ethanol

observed in EUSS10 commenced after 35 days but the volume was highest between 21 and 28 days after anthesis except for EUSS10 which had the highest volume after 35 days after anthesis (Figure 3.9). Among the sorghum genotypes, the mean volume of ethanol was (1,062, 985, 961 and 805) L ha^{-1} for EUSS10, EUSS11, EUSS17 and SS04 respectively (Table 3.3). Crops grown at Kendu-Bay had the highest volume of ethanol followed by those that were grown at Egerton (Figure 3.9). There was significant difference in absolute ethanol volume in the four environments and the interaction between the environment, stage and genotypes. Kendu-Bay was seen to be the highest in production of ethanol followed by the first season in Kibos (Figure 3.4).

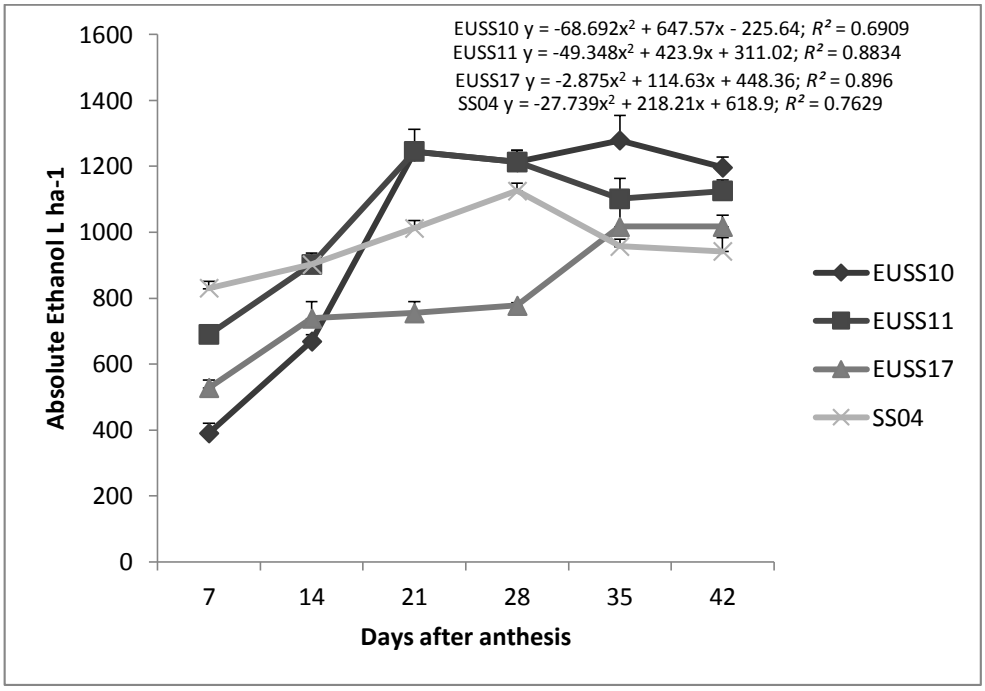

Figure 3.9 Effect of harvesting stage on Ethanol yield of four sweet sorghum genotypes evaluated in four environments in Kenya.

In this study, grain yield was investigated at the various stages of harvesting. Interaction between genotype × stage was not significant. It was observed that ESS10 had higher kernel means (1337.95, 2923.27, 8913.43 and 12936.79) t ha^{-1} for 21, 28,35 and 42 days after anthesis respectively, than the rest of the genotypes (Figure 3.11). There was significant difference in the grain yield among the genotypes and the stages of harvesting (Table 3.2). At anthesis and 14 days after anthesis, there was no grain yield for all the genotypes. Significant grain yield was realized after 28, 35 and 42 days after anthesis (Figure 3.13). EUSS10 emerged the most productive variety in terms of grain yield. There was significant difference in grain production in the four different environments.

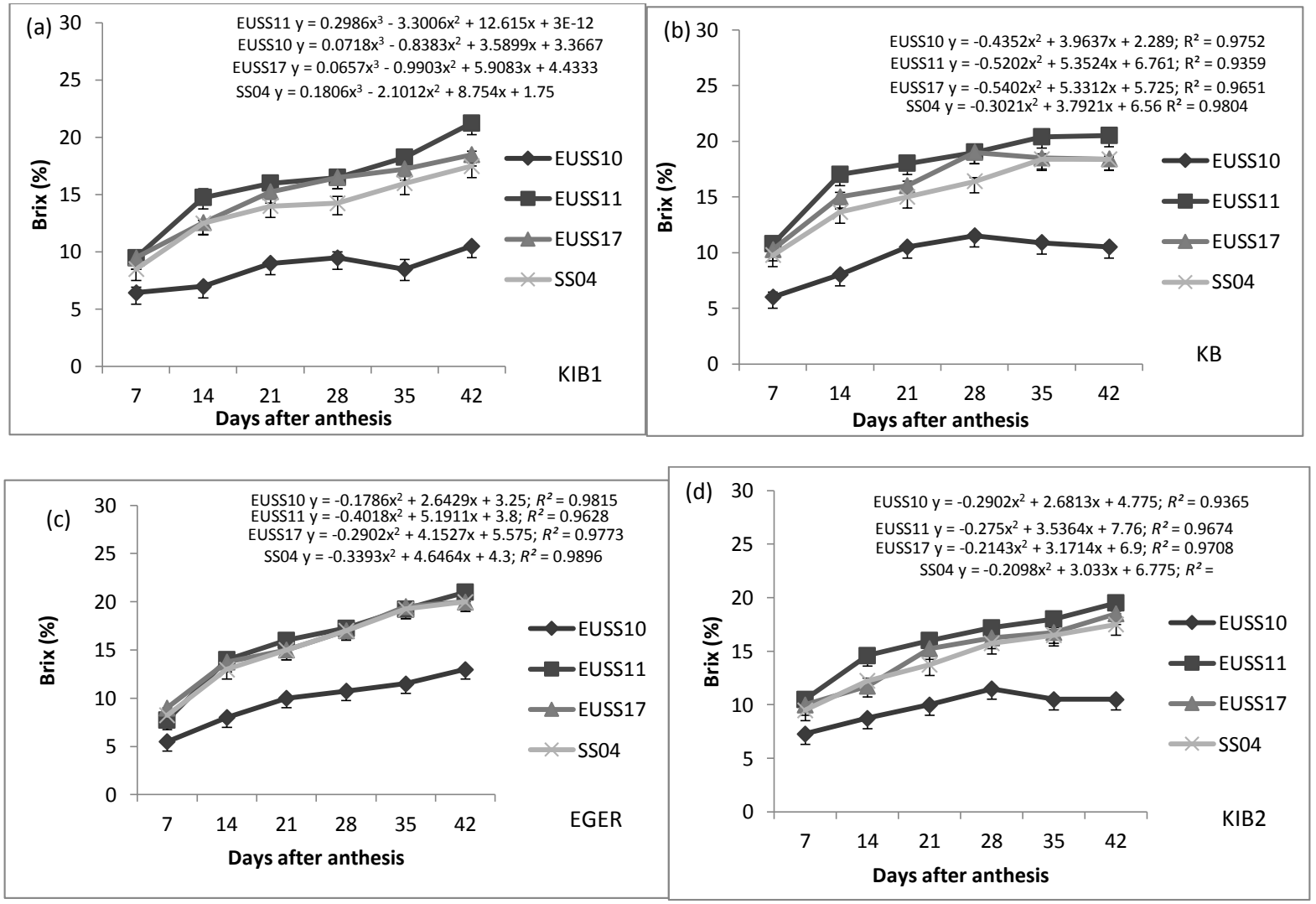

Figure 3.10 Brix at four environments [Kendu-bay (KB), Egerton (EGER), Kibos (KIB1 and KIB2) seasons]

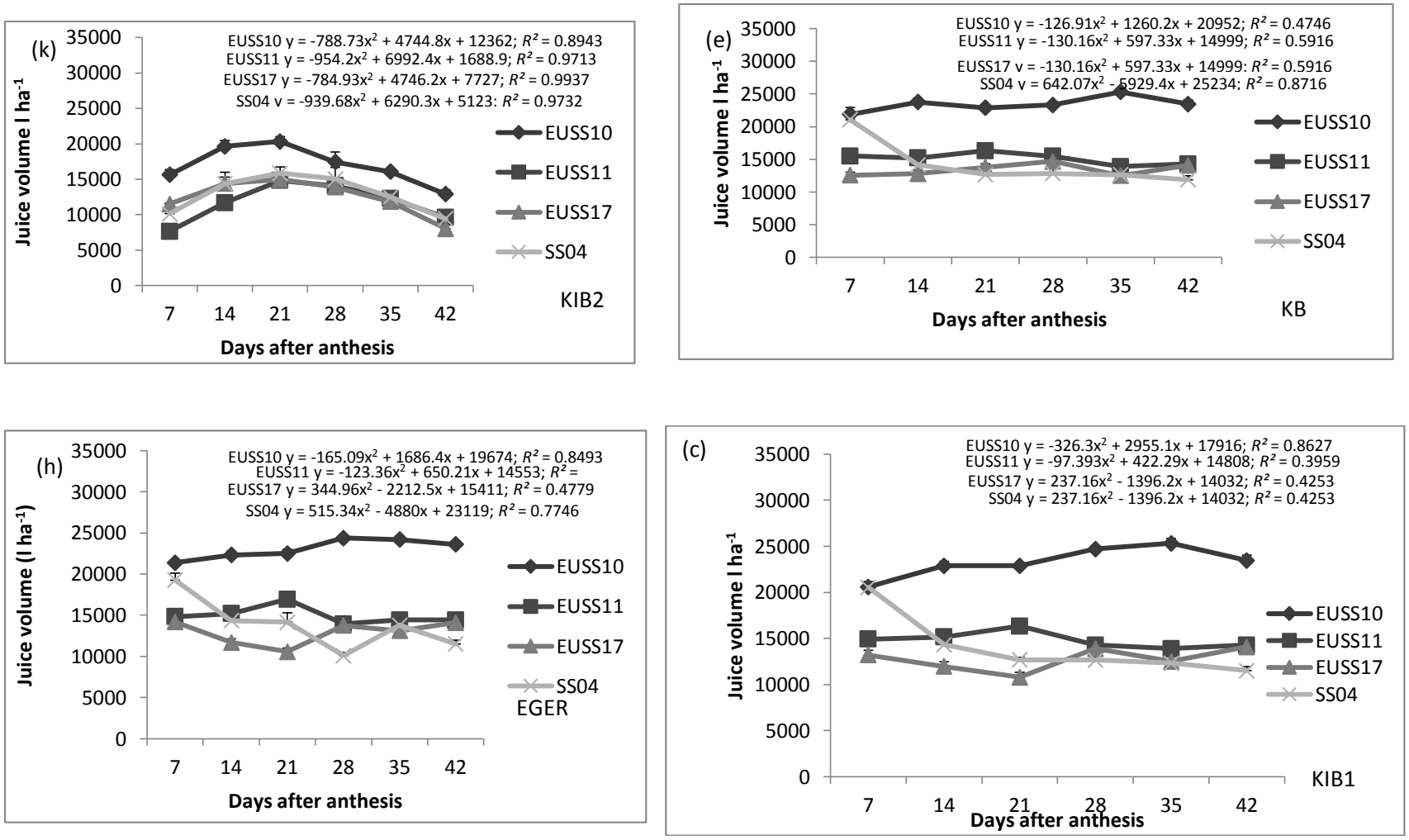

Figure 3.11 Juice volume at four environments [Kendu-bay (KB), Egerton (EGER), Kibos (KIB1 and KIB2) seasons]

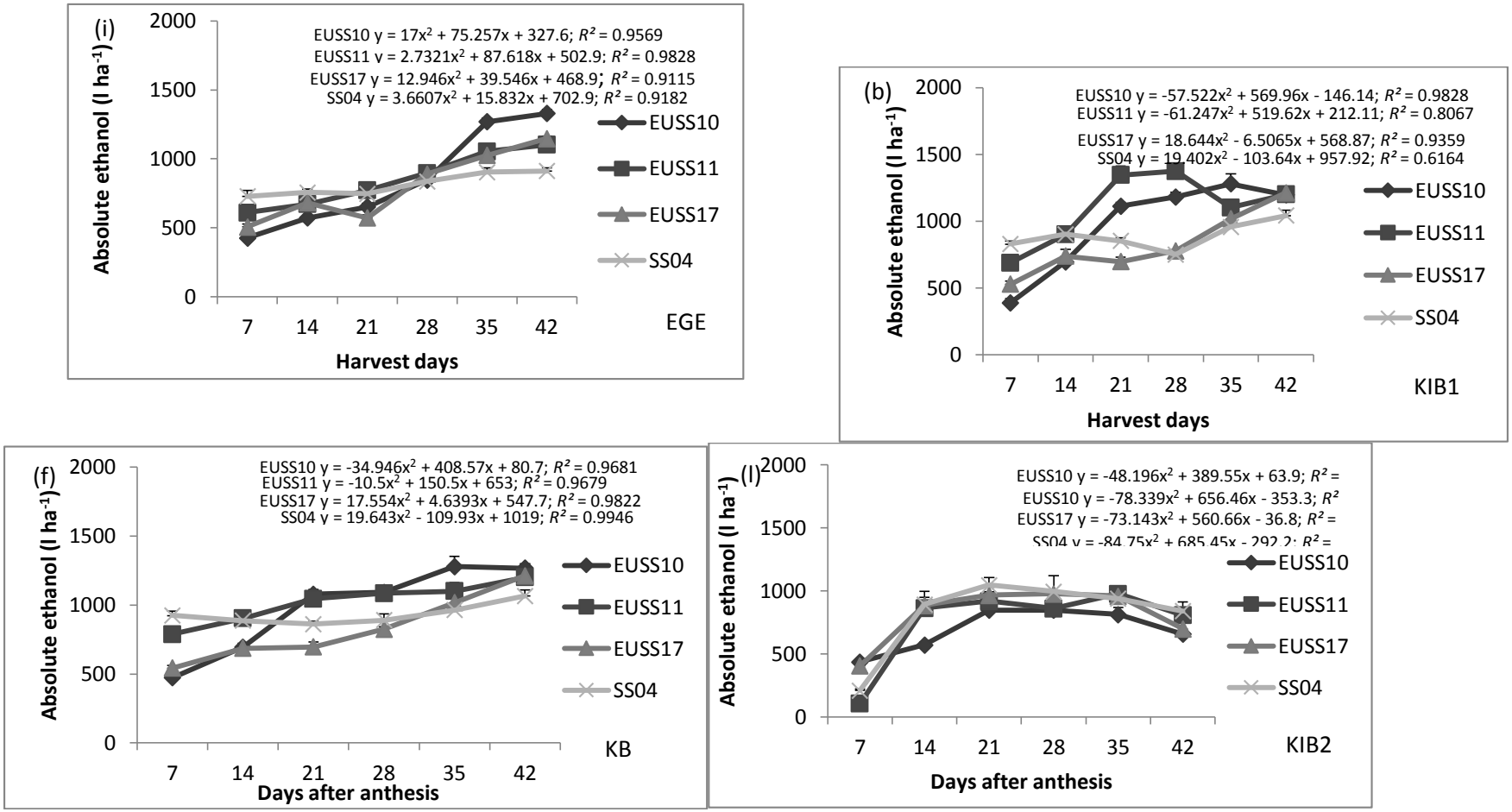

Figure 3.12 Ethanol observed at four sites [Kendu-Bay (KB), Egerton (EGE), Kibos (KIB1&KIB2) seasons]

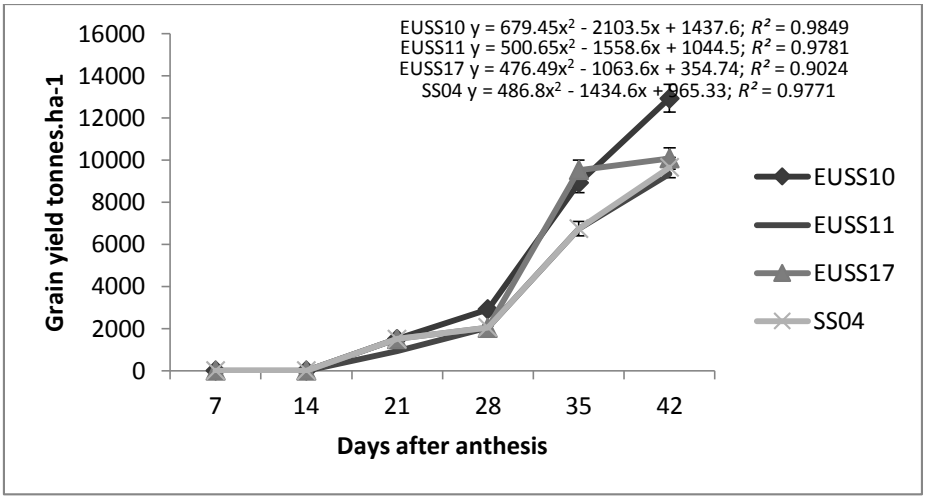

Figure 3.13 Effect of harvesting stage on grain yield among four sweet sorghum genotypes evaluated in three environments in Western Kenya.

3.8 Discussion

The environment and soils in western Kenya highly favour the growth and development of sweet sorghum and the sorghum crops in general. Early maturity was realized both at Kendu-Bay and Kibos with the same crops planted at Egerton University maturing late by a difference of more than 50 days. This is due to the low temperatures experienced at Egerton University. However, sweet sorghum performed competitively well at both high (Egerton) and low (Kibos and Kendu-Bay) altitude. EUSS10 and EUSS11 have been released as sweet sorghum varieties while, EUSS17 is still being developed by Egerton University. This study demonstrated that sorghum line EUSS17 reached anthesis stage earliest but EUSS10 took the longest duration from sowing to flowering. The variation in the height, the girth and flowering influenced juice volume, content of brix and ethanol production from the four sorghum genotypes. This is expected since both height and girth are yield components with regard to juice volume. Among the four genotypes tested, there were genotypic variations attributed to genetic background for most of the traits evaluated. Significant stage × genotype interaction suggests that stage of harvesting vary with sorghum genotype for girth, volume of juice, brix content, volume of ethanol and weight of the kernels. There were no differences in height probably due to environmental conditions that prevailed during growth period. Since accumulated biomass and cane yield is a function of height and girth, for improvement of sweet sorghum varieties, EUSS10 can be a good source of genes responsible for height as well as girth. EUSS10 had the highest volume among the genotypes at the 5^{th} stage. The height was

significantly different for all the genotypes probably due to plant architectural and morphological differences.

The observed significant differences for both agronomic and ethanol related traits among locations and the interaction between location and stage and location genotype suggests ethanol production for sreet sorghum is affected by many factors. For example, days to flowering was seen to be highest at Egerton. This is probably because of the low temperatures due to high altitude. Although it took long for the crops to flower (114 days) in the highlands (Egerton University), the grain filling period for both the highland and the lowland differed by much fewer days. In the highlands it took 57 days to physiological maturity while in the low lands it took 42 days. Kendu-Bay emerged to be the best performing location for ethanol production. This is probably due to high brix and high juice volume which was realized in the location. Although the crops took many days to mature in Egerton University fields, the low temperature is favorable during harvesting period as minimal juice is lost to evapotranspiration during harvesting.

Genotype EUSS10 produced the highest volume of juice when it was harvested at 21 days after anthesis suggesting high plant physiological activity. This could be due to the fact that this is the most demanding stage of plant development hence a lot of water has to be accumulated in the stem to support grain filling process. This indicated that harvesting stage and genotype has an effect in juice volume. However, this study clearly demonstrated that volume of juice depends on the genotype, the size of the cane and soil moisture related factors. The extracted juice contains the fermentable sugars that contribute to ethanol yield during fermentation process. High amount of juice volume together with content of brix directly impacts on ethanol production but should be balanced with accumulation of sugars which is predicted by the level of brix.

The observed increase in the volume of ethanol with the increase in the number of days after flowering was directly related to increased brix and volume of the juice. Even though juice volume is an important aspect in bioethanol production, low brix can undermine the production of ethanol making brix an important quality trait in ethanol production. Brix has been seen to increase with increasing number of days from flowering time and also vary with the genotypes. Among the genotypes, EUSS10 had the lowest accumulation rate of the brix as well as the lowest y-intercept. Like in the case of other studies, sugar content among the genotypes is highest at hard dough stage which corresponds to the harvest after 35 days after

anthesis (Almodares *et al*., 2008). In this study, it was evident that there was a decrease in the rate of brix accumulation in the stems of sweet sorghum varieties, and then increased again in all the genotypes except for EUSS10 which showed a relatively constant increase. This observation is due to the propotions of invertase and sucrose synthase. Although the content of juice in line ESS10 was generally high, concentration of brix which is important for fermentation was low. Since EUSS11 is high in sugar content while EUSS10 is high in juice volume it is important to determine inheritance pattern of sucrose accumulation genes and introgress them into line EUSS10 from line EUSS11 and other genetic stocks. A sound sorghum breeding programme with an objective of developing sweet stalk sorghum would aim at reconstituting genes for juice and large stem with those of accumulation of brix in the stem. Although EUSS10 had low brix, its higher volume of juice translated to high volume of ethanol than other genotypes which had high brix with very low volume of juice. This suggests that although brix is important, high juice volume with some small amount of brix would still yield high ethanol volume. The relationship between the low amount of brix, juice volume and ethanol yield is worth investigating.

The results from this study suggest that the rate of accumulation of sugar in the sweet sorghum stems decrease after flowering then increase towards maturity. Towards the maturity of sweet sorghum plant, the rate of accumulation of brix increases again. The physiological processes depend on the factors that support the productivity of the crop such as the activities of sucrose synthase and invertase enzymes. Accumulation of sugar in the stems is influenced by metabolic and transport processes as well as partitioning within the sink cells (Almodares *et al*, 2008). The rapid increase in the rate of accumulation of the brix in sweet sorghum is as a result of effect of carbohydrate partitioning. As the kernels mature, there is more carbohydrate retained in the stem of the plant, a factor that contributes to the concentration of the solutes in the stem hence increased brix. On the contrary, during grain filling, more carbohydrate is transferred to the grains leaving very little to be transferred to the stalk. This is similar to what was observed by McKinley *et al*. (2016) while looking at sucrose accumulation in sorghum. This explains why there is a decrease in accumulation of the brix and then an increase when grain filling is almost complete. Also, there is reduction in the uptake of water and this was indicated by the reduction in the juice volume as from 28 to 42 days after anthesis.

Kernels obtained from sweet sorghum can improve the food situation among the rural households within the tropics. The results indicate that there was a significant increase in the

weight of the kernels obtained per hectare for all the genotypes evaluated in this study. Determining the contents of the juice in terms of types of sugars in the juice and sorghum kernels was beyond the scope of this study. But a delay in harvesting by 14 to 21 days after anthesis of sweet sorghum would yield more kernels. Harvesting of sweet sorghum after 21, 28, 35, and 42 days after anthesis would give ethanol yields which were not significantly different although for kernel production in all the aforementioned stages there would be a significant increase. From this study, it can be hypothesized that harvesting sweet sorghum after 35 days after anthesis would be appropriate for production of both ethanol and kernels. For the four sorghum varieties evaluated; (EUSS11, SS04, EUSS10 and EUSS17), this would be between 92-117 days after sowing in Western Kenya.

The chlorophyll content showed a steady decline as the stages of stalk harvesting increased suggesting that it can be used to predict the time of harvesting of sweet sorghum. This is because the decrease in chlorophyll indicates lowered photosynthetic activity of the plant. As sweet sorghum approaches physiological maturity, chlorophyll content goes down. There was no significant difference in the chlorophyll levels in various environments. However, the rate of decline in the level of chlorophyll for EUSS10 was the lowest suggesting that it could be having stay green properties. This aspect of sorghum crop can be explored to improve forage sorghum that can be used in the animal feed industry. Even though each of the genotypes had its own level of chlorophyll at each stage, the same can be investigated to provide a way of detecting maturity of the sweet sorghum remotely using satellite images. Haboudane *et al*., (2002) suggested that chlorophyll levels can be used to increase the precision in agricultural practices. It is imperative that harvesting stage of sweet sorghum can be predicted using chlorophyll levels. From this study, harvesting of sweet sorghum can be done when chlorophyll levels attain 20 to 40 for all the genotypes as measured by SPAD 502. This can be further investigated for accuracy and precision especially with the variation of locations.

3.9 Conclusion

Harvesting stage of sweet sorghum is best at the hard dough stage of the grain or the fifth stage of harvesting. Results from this study showed that harvesting sweet sorghum after 104 to 117 days after planting in Western part of Kenya in the low lands and 150 to 200 days in the highlands would be appropriate for production of kernels and ethanol. However, these stages may be influenced by environmental conditions. The rate of sugar accumulation in the stems of sweet sorghum decrease then increase towards maturity.

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CHAPTER FOUR

HETEROSIS AND COMBINING ABILITY FOR ETHANOL AND BRIX IN SWEET SORGHUM [*Sorghum bicolor* (L.) MOENCH]

4.1 Abstract

Heterosis and combining ability are important in improving key traits of economic importance in sweet sorghum [*Sorghum bicolor* (L.) Moench]. Objectives of this study were to determine: (i) heterosis and (ii) Combining ability for ethanol related traits in sweet sorghum. A multi-location experiment was conducted across 3 environments in western Kenya to evaluate 30 sweet sorghum hybrids developed from four sweet sorghum lines and 14 grain sorghum cultivars for cane yield, brix, volume of juice and ethanol in a Randomized Complete Block Design (RCBD). Among the hybrids, 18 exhibited positive high parent heterosis for brix, within the range of 1.81% (BM23 × EUSS11) to 130.57% (GS007 × EUSS10), while 21 hybrids exhibited heterosis ranging from 7.97% (GS001 × SS04) to 122.05% (P40 × SS04) for juice volume and 0.01% (NYANGEZI × EUSS10) to 136.52% (P40 × SS04) for ethanol volume. Sorghum line IS25547 and tester EUSS10 showed the highest general combining ability (GCA) of 2885.5 L ha^{-1} and 280.01 L ha^{-1} respectively while the highest specific combining ability effects of 1441.96 L ha^{-1} was observed on BM39 × EUSS10. GCA effects accounted for a larger portion of the treatment sum of squares than SCA effects suggesting that additive gene effects are more pronounced than non-additive gene effects for the inheritance of sweet sorghum cane yield, brix, ethanol and volume of juice. This indicates that sweet sorghum ethanol related traits exhibit high heterosis, GCA and SCA and can be used in hybrid development.

Key words*: Better parent, Dominance, Epistasis, Mid parent, Over-dominance, Standard heterosis*

4.2 Introduction

Sweet sorghum [*Sorghum bicolor* (L.) Moench] has been known to be one of the most useful multipurpose crops with the capacity to produce food, fodder and bioethanol (Eggleston *et al.*, 2013). Being a C_4 crop, it can withstand adverse climatic conditions hence can be grown by farmers in marginal lands (Prakasham *et al.*, 2014). Heterosis refers to a phenomenon in which progeny of diverse species or varieties exhibit performance which is greater than that of the two parents (Sing, 1995). Heterosis has been used in crop improvement to decide the

performance of hybrid in various crops. In a study of two tomato (*Solanum lycopersicum*) mutant alleles, Krieger *et al.* (2010) observed the occurrence of single gene heterosis which was expressed in fruit brix. Although difference in allelic frequency and dominance are necessary for there to be heterosis, they are not sufficient in the case of multiple alleles (Cress, 1966). For multiple alleles segregating in a population, the lack of heterosis cannot be used to infer lack of genetic divergence between the parental populations (Lamkey and Edward, 1998).

Dominance and overdominance theories have been put forward in an attempt to explain heterosis. The dominance model which proposes that due to hybridization between genetically diverse individuals, the F_1 generation displays heterotic characteristics due to complementation of multiple slightly deleterious alleles from the genome of one parental line by superior dominant ones from the other genome was proposed (Birchler *et al.*, 2003). Over-dominance model of heterosis posits that synergistic allelic interactions at particular heterotic loci can lead to superior performance of the F_1 progeny (Birchler *et al.*, 2010). It is believed that the F_1 hybrids inherit varied alleles from both parents that act synergistically resulting into heterotic effects. However, single locus over-dominance has been observed (Mckeown *et al*., 2013). With an assumption that there is no heterosis, epistasis model proposes that heterosis can arise due to epistatic interactions between alleles at different loci. Both Meyer *et al*. (2010) and Riedelsheiner *et al.* (2012) working separately on Arabidopsis (*Arabidopsis thaliana*) and maize (*Zea mays*) respectively observed through molecular studies and Quantitative Trait Loci (QTL) dissection associated with heterosis that it is complex and highly multigenic.

Estimation of heterosis has been conducted using various formulas put into three categories as mid parent, better parent and standard heterosis (Lamkey and Edward, 1998). Mid parent heterosis is calculated by finding the mean of the two parents, better parent is the better performing parent and check refers to the commercial variety. Standard heterosis is considered important as it offers advantage over cultivated commercial variety (Lamkey and Edward, 1998). Investigation of heterosis using molecular and genomic tools alone did not yield results that conformed to the understanding of heterosis and suggested that further research should be conducted by integration of both classical and molecular tools (Lippman and Zamir, 2007).

Heterosis has been the major driving force in the production of hybrid seeds. In sorghum, it was first observed by Corner and Karper (1927) but remained unexploited until the discovery of cytoplasmic male sterility by Stephen and Holland in 1954. Exploitation of heterosis brought about the success in development of maize (*Zea mays*) hybrids (Crow, 1998;

Duvick, 2001), wheat (*Triticum estivum*) (Wang *et al*., 2006; Qi *et al*., 2012), rice (*Oryza sativa*) (Yu *et al*., 1997) and tomatoes (*Lycopersicum esculatum*) (Williams and Gilbert, 1960; Krieger *et al.,* 2010). Meshram *et al*. (2005) observed that in a cross between *A* lines and 10 restorer lines, overall heterosis was positive for plant height, cane yield and sugar yield. Sorghum cultivars vary considerably and exhibit both negative and positive heterosis which can be exploited for sorghum improvement (Umakanth *et al*., 2012). Sorghum production trippled after the adoption of hybrid sorghum cultivars and exploitation of hybrid vigor in USA was done (Kenga *et al*., 2004). Studies conducted in sorghum have indicated that positive heterosis in F_1 hybrids is higher in early maturity, high stripped stalk yield, percent cane juice extracted and grain yield which is suitable for dual purpose (Pothisoong and Jastil, 2014). In wheat, through classical breeding approaches heterosis has been exploited (Bailey *et al*., 1980). More intensive molecular approaches in wheat were used to explore heterosis (Li *et al*., 2014). High parent heterosis was observed in sorghum (Singhania, 1980; Springer and Stupar, 2007; Makanda *et al*., 2009). Other than plants, heterosis has also been observed in animals (Han *et al*., 2008). Welcker *et al.* (2005) assessed the implications of heterosis and combining ability for maize adaptation to tropical soils. Exploitation of heterosis in development of sweet sorghum hybrids is promising and can be rewarding for farmers in Kenya.

GCA is defined as the average performance of a line in a hybrid combination (Spague and Tatum, 1942). It informs a plant breeder that the trait under investigation is affected by additive gene action (Falconer, 1975). SCA refers to the better or poorer performance than expected of a given hybrid combination based on average performance and suggests that environment and interaction between the genotypes and environment affect phenotypic expression of the trait (Spague and Tatum, 1942). Both SCA and GCA were used in sesame (*Sesamum indicum*) breeding by estimation of heritabilities leading to the computation of Baker's ratio which is an indicator of the contribution made from the parent to the offspring (Anyanga *et al*., 2016). It is necessary to test hybrids in multilocational trials when SCA is seen to be significant. Line by tester mating design has been used to estimate GCA and SCA in maize (Shah *et al*., 2015), wheat (Fellahi, 2013) and cotton (*Gossypium hirsutum*) (Talpur *et al.,* 2016). Van Becelaere and Miller (2004) developed a sunflower cultivar which was resistant to sclerotinia head rot in sunflower.

Tadesse *et al*. (2008) estimated the combining abilities of introduced sorghum parental lines for major morphological and agronomic traits and found that GCA was significant for

height of the plant, yield of the kernels and length of the panicle while SCA effects were not significant. Combining ability for quantitative characters in sunflower (*Helianthus annus*) was estimated using half diallel mating design to predict their usefulness in hybrid development (Machikowa *et al*., 2011). A positive correlation of SCA and breeding values are sufficient to conduct selection for a breeding population in trees (Wu and Matheson, 2004). On the other hand, the effect of reciprocal recurrent selection on relative efficiency of genetic value was assessed using SCA and GCA in maize breeding population in which efficient process of selection was observed to be based on GCA effects (Makumbi *et al*., 2011). GCA and SCA have been used in identification of potential parental lines and a combination of hybrids in breeding programmes in maize hybrids in which consequences of tester of choice was seen to be significantly reduced (Lariepe *et al*., 2017).

Sorghum crop has high variability making improvement of the crop viable and readily achievable (Tester and Langridge, 2010). The main purpose of sweet sorghum is the provision of fermentable sugars which are accumulated in the stem (Davila-Gomez *et al*., 2012). Estimation of GCA and SCA of sorghum lines is an important step in sorghum hybrid development. Exploitation of heterosis is one such step towards increasing sorghum productivity in Kenya. Heterosis has been well documented in grain sorghum and related traits. However, reports on heterosis in sweet sorghum owing to their sugar traits and ethanol production are limited (Pfeiffer *et al*., 2010). The main objectives of this study were to determine heterosis, GCA and SCA for the agronomic and ethanol related traits of sweet sorghum in Kenya.

4.3 Materials and Methods

4.3.1 Genotypes

One sweet sorghum cultivar SS04 and three sweet sorghum lines (EUSS10, EUSS11 and EUSS17) were used as males in the development of sweet sorghum hybrids. NYANGEZI and thirteen lines (GS001, GS002, GS003, GS005, GS006, GS007, GS008, BM23, BM39, IS9203, IS25547, P23, P40) were used as females. The lines GS001 to GS008 were collected from western part of Kenya and are local varieties cultivated for kernel production. They are drought tolerant and less preferred by birds but have low volume of juice and sugar content in the stalks. BM23 and BM29 originated from Rwanda and have high biomas and can do well in high altitude areas. IS25547, IS9203, P23, P40 and SS04 were obtained from ICRISAT. EUSS10, EUSS11, EUSS17 are sweet sorghum lines under development by Egerton

University. Sweet sorghum lines are known to exhibit higher brix % in the stems than the local varieties or grain sorghum (Table 4.1). The resulting 30 hybrids were evaluated in replicated trials in two seasons in one location (Kibos 1 and Kibos 2) and on a separate location (Kendu-Bay). Agronomic traits such as height, girth, cane yield, days to flowering (DTF) and sugar traits thus brix, juice volume and ethanol volume of the F_1 plants were compared to that of the offspring.

4.4 Experimental site and environmental conditions

During the short rains of 2015 at Kibos Sugar Research Institute (SRI), crosses were made in a line by tester design which yielded 30 hybrids. Out of the 56 hybrids which were expected, only 30 were realized because some of the lines such as GS002 did not result to any seeds because pollination did not take place after the pollen was transferred. There was variation in the success of cross pollination since some lines developed very healthy seeds while some did not. The succeeding experiments were conducted in three environments thus Kendu-Bay (0.35^{o}13'07"S, 34^{o}07'44") in one season (September-December, 2016) and Kibos (0^{o}04'06"S, 34^{o}49'03"E) in two seasons (March-August 2016 and September 2016-January 2017). Kendu-Bay experimental site has an altitude of 1,132 m a.s.l, about 5 Km east of Kendu-Bay Township Southern shore of Nyanza Gulf, Lake Victoria. The area experiences an average rainfall of about 1,200 mm per annum with the onset of long rains in March while the short rains commence in September. The average temperature in this area is 27^{o}C with a minimum of 17^{o}C while the soil is predominantly sandy and drains very easily.

Sugar Research Institute experimental fields, Kibos has an altitude of 1,173 m a.s.l, about 8 km East of Kisumu City, in the western part of Kenya. This area experiences mean precipitation of 1,323 mm per annum with the onset of long rains in March while short rains commence in August with a gradual reduction towards September and about 374.4 mm in December. In general, the average maximum temperature of this location is 30^{o}C with a minimum of 15.5^{o}C. The soils in the experimental site are predominantly heavy black cotton type. Supplemental irrigation was done at both Kendu-Bay and Sugar Research Institute. Harvesting of the cane was done when the moisture in the soil was generally very low.

Table 4.1 Female and male sweet sorghum genotypes, origin of the seeds and phenotypic description of their agronomic and ethanol related traits.

Genotypes	Origin	Description
Female		
BM23	Rwanda	Highland sorghum; high biomas
BM39	Rwanda	Highland sorghum; high biomas
GS001	Western Kenya	Local variety; low juice; low sugar; drought resistant; less preferred by birds
GS002	Western Kenya	Local variety; low juice; low sugar; drought resistant; less preferred by birds
GS003	Western Kenya	Local variety; low juice; low sugar; drought resistant; less preferred by birds
GS005	Western Kenya	Local variety; low juice; low sugar; drought resistant; less preferred by birds
GS006	Western Kenya	Local variety; low juice; low sugar; drought resistant, less preferred by birds
GS007	Western Kenya	Local variety; low juice; low sugar; drought resistant; less preferred by birds
GS008	Western Kenya	Local variety; low juice; low sugar; drought resistant; less preferred by birds
IS25547	ICRISAT	High biomas; low sugar,
IS9203	ICRISAT	High biomas; low sugar,
NYANGEZI	DRC	High biomas; high juice volume; low sugar
P23	ICRISAT	High biomas; low sugar; late maturity
P40	ICRISAT	High biomas; low sugar; late maturity
Male		
EUSS10	Egerton University	Sweet sorghum line; high juice volume; low sugar
EUSS11	Egerton University	Sweet sorghum line; relatively low juice volume; high sugar
EUSS17	Egerton University	Sweet sorghum line; relatively low juice volume high sugar
SS04	ICRISAT	Sweet sorghum cultivar; high juice volume; high sugar

DRC- Democratic Republic of Congo

4.5 Generation of sweet sorghum crosses

Crosses between grain sorghum lines and sweet sorghum testers were made in a line by tester design as proposed by Kemphorne in 1957 and cited by Sharma (2006). All the crosses were done at SRI, Kibos during short rains of 2015. Land preparation and agronomic practices were as indicated in section 3.5 to the time of flowering. Planting of the testers was staggered (at intervals of 1 week for 3 weeks) for provision of pollen for both late and early flowering varieties due to the fact the sorghum lines and varieties used as females flower at different times.

4.6 Emasculation and pollination

Selected plants from among the 14 lines were tagged for cross pollination. Sorghum panicles were treamed to size by clipping the panicle tip with pair of scissors and removing the lower florets at the base of the panicle. The reduced panicle was covered with plastic bag to create high humidity inside the bag. Under such humidity the florets open, the anthers emerge but shed no pollen. After two to three days, anthers were detached by simple tapping. Pollen was obtained from the tester plants which had been bagged the previous night for pollination. The bags containing pollen grains from the male testers were transferred to the female parents which had been emasculated previously. This was done after tapping the panicle and removing the plastic bag. This was repeated for all the lines and all the testers and panicles were harvested at physiological maturity. All the panicles which were cross pollinated were harvested by cutting the panicle at the base of the paper bag which had been used to pollinate and protect the grains from the birds. A total of thirty sweet sorghum hybrids were developed and were used in further experiments.

4.7 Experimental procedure

Thirty hybrids developed from a line by tester mating design between four sweet sorghum cultivars and 14 sorghum varieties were planted at Kendu-Bay (0.35°13'07"S, 34°07'44") and at Sugar Research Institute (SRI) experimental plots in Kibos (0°04'06"S, 34°49'03"E). At Kendu Bay, the farm was under watermelon (*Citrullus lanatus*) in the previous season. The field was cleared, disc ploughed and harrowed twice to achieve the required tilth. SRI experimental field was under maize cultivation in the previous season. It was disc ploughed and harrowed twice to achieve a fine tilth suitable for planting sorghum. The experiments were conducted in Randomized Complete Block Design (RCBD) with three replications in all the exprerimental sites. Sorghum genotypes were planted at seeding rate of 10 kg ha^{-1} in an experimental unit measuring (3 × 5) m with inter row spacing of 60 cm and intra spacing of 15 cm. At planting time, each plot received an equivalent rate of 30 kg ha^{-1} of P, 10 kg ha^{-1} of K and 40 kg ha^{-1} N from NPK with the ratio 20:10:10, respectively in all the sites and in the two seasons. Two weeks after emergence, plants were thinned to one plant per hole, six weeks after planting, additional 40 kg ha^{-1} of N was supplied from CAN to each plot in all the experimental sites. Infestation by shoot fly (*Atherigona soccota*) on young seedlings was minimized by spraying a systemic insecticide Bulldock (*beta-cyfluthrin* 25 g l^{-1}) at 25 g ha^{-1} at intervals of 14 days for one month. Within the experimental plots, weed growth was restricted by manual

weeding. Weeding and inter cultivation operations was done twice between 5-leaf and when panicle emerges from the boot. Between booting stage to the end of anthesis, a second dose of Bulldock was applied at 25 g ha^{-1} to control sorghum midge (*Contarinia sorghicola*). After heading, the panicles were covered using paper bags to prevent them from birds' damage. These were done systematically at Kendu-Bay and for the two seasons at Kibos experimental site.

4.8 Data collection

Number of days sorghum takes to flower were determined when anthesis occurred on 50% of the plants. Measurements on height of the plants were taken just before harvesting in which height of three plants were taken at random and the average computed. Five plants were harvested from each plot when the grains were at hard dough stage. Girth measurements (mm) were taken on any three of the harvested canes per plot using vanier calipers at the fifth internode from the base of the plant. The stalks were stripped of the leaves and the mass of three stalks picked at random were weighed and the average computed. The stripped stalks were crushed using a three-roller crusher (FuanLiyuan, China, type YC 80B-4) by passing the canes once and the collected juice strained through a 1 mm sieve. The volume of the collected juice was taken. A single drop of the juice samples were put on hand held-refractometer to determine the brix. For each sample, 100 ml of juice was transferred to conical flasks (200 ml) for fermentation. Fermentation of the juice was done by adding approximately 1.5 g yeast *(Saccharomyces cerevisiae)* to the juice sample and incubating at 35^{o}C for 4 days. The fermented samples were then distilled by heating in a conical flask connected to a Liebig condenser and the ethanol content in the distillates was determined by measuring the refractive indices on a hand-held refractometer (model: standard line Alla made in France). Determinations were based on a standard curve drawn by measuring the refractive indices of absolute ethanol solutions (0, 5, 10, 15, 20, 25, and 30%) in distilled water.

4.9 Statistical analyses

Combined analysis of variance and combining ability were performed following a line by tester design (Sing and Chaudhary, 1985). Data were subjected to analysis of variance according to Steel and Torrie (1980) to determine significant difference among genotypes. Combining ability effects was computed according to the line and tester mating design proposed by Kemphtorne (1957). Line × Tester model:

$$Y_{ijkl} = \mu + E_i + R_{kl} + V_{ij} + (EV)_{ijl} + \boldsymbol{\varepsilon_{ijkl}} \quad (4.1)$$

Where Y_{ijkl} = observed value from each experimental unit; μ = population mean; E_i = environmental effect; R_{kl}= replication effect within each environment; V_{ij}= F_1 hybrid effect =g_i+g_j+S_{ij}. However, V_{ij} =g_i+g_j+$S_{ij,}$ where g_i = general combining ability (GCA) for the i^{th} parental line; g_i=GCA effect of the jth tester; S_{ij} = specific combining ability (SCA) for the ij^{th} F_1 hybrid and $(EV)_{ijl}$ = interaction effect between i^{th} F_1 hybrid and i^{th} location; and $\boldsymbol{\varepsilon}_{ijkl}$ = error component.

Data were analyzed using Proc GLM of SAS (Statistical Analysis System) Version 9.1 with replicates within environment, interactions between Lines × Testers, Lines × Environment, Testers × Environment, Lines × Testers × Environment included in the random statement and genotype and environment considered as fixed. During the analysis, hypothesis on environment was tested using Environment × Lines as the error term, the hypothesis on lines was tested using Lines × Testers as the error term, hypothesis on testers was done with Lines × Testers as the error term, hypothesis on Environment × Testers was tested using Environment × Line × Testers as the error term. For every trait under investigation, error variances and means were computed for lines, testers and environments. They were compared and declared significant or not at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$. The data were arranged on program editor of SAS as indicated on appendix I.

A further comparison was done between hybrids versus the males and the females to determine heterosis.

General formula for heterosis: $H_{F_1} = \mu F_1 - \frac{\mu P_1 + \mu P_2}{2}$ (4.2)

Where P_1 and P_2 represents the male and female parent respectively and H is heterosis.

$$\text{Mid parent heterosis } \frac{F_1 - MP}{MP} \times 100 \quad (4.3)$$

Where MP is the average of the two parents, F1 is the hybrid. High parent or better parent heterosis was calculated using the hybrids, and either male or female parent values depending on the one which was seen to be better.

$$\text{High parent heterosis or Heterobeltiosis} = \frac{F_1 - BP}{BP} \times 100 \quad (4.4)$$

Where BP is the better parent. Standard heterosis was computed using the hybrid and values observed for SS04 which was considered as a standard variety since it is currently grown commercially.

$$\text{Standard Heterosis} = \frac{F_1 - \text{Check}}{\text{Check}} \times 100 \quad (4.5)$$

Variance for general and combining abilities were tested against their respective error variance, derived from the analysis of Variance of the different traits as follows:

$$\text{Covariance of half sib of line} = \text{Cov.H.S} = \frac{M2 - M4}{rf} \quad (4.6)$$

$$\text{Covariance of half sib of tester} = \text{Cov.H.S} = \frac{M3 - M4}{rm} \quad (4.7)$$

Covariance of full sib = Cov.F.S

$$= \frac{M2 - Me + M3 - Me + M4 - Me + 6r\text{Cov.H.S} - r(l + t)\text{Cov.H.S}}{3r} \quad (4.8)$$

Cov.H.S. (average) were calculated by the formula:

$$= \frac{1}{r(2mf - m - f)}\left[\frac{(m-1)(M2) + (f-1)(M3)}{m + f - 2} - M4\right] \quad (4.9)$$

Where M_1 = mean square for replications, M_2 = mean square for lines, M_3 = mean square for testers; M_4 = mean square for lines x testers; Me = mean square error. With the assumption that there is no epistasis, variance due to GCA (σ^2_{gca}) and variance due to SCA (σ^2_{sca}) was calculated as follows:

$$\sigma^2_{gca} = \text{Cov.H.S} = \left(\frac{1+F}{4}\right)\sigma^2_A \quad (4.10)$$

$$\sigma^2_{sca} = \text{Cov.H.S} = \left(\frac{1+F}{2}\right)^2\sigma^2_D \quad (4.11)$$

(Modified from Sing and Chaudhary, 1985)

Additive and dominance genetic variance (σ^2_A and σ^2_D) was calculated by taking inbreeding coefficient (*F*) to be equal to one because both the lines and the testers are inbred lines. The GCA effects were calculated using the formula:

$$g_t = \frac{x_{j.}}{lr} - \frac{x_{...}}{ltr} \quad (4.12)$$

(source: Sing and Chaudhary, 1985)

Where *gt* is the GCA effect, *l* is the number of lines, *t* is the number of testers and *r* the number of replicates.

The SCA effects were calculated using the formula:

$$S_{ij} = \frac{x_{ij.}}{r} - \frac{x_{i..}}{tr} - \frac{x_{.j.}}{lr} - \frac{x_{...}}{ltr} \quad (4.13)$$

(source: Sing and Chaudhary, 1985)

Where S_{ij} is the SCA effect, *l* is the number of lines, *t* is the number of testers and *r* the number of replicates.

4.10 Results

4.10.1 Sweet sorghum hybrids

From crosses made during the short rains of 2015 at Kibos, it was observed that not all the crosses were successful. Only 30 out of the possible 56 hybrids were developed from the crosses in a line by tester design. Furthermore, line such as GS002 was not fertilized by all the testers resulting to no hybrid. Genotypes NYANGEZI and GS008 yielded hybrids for crosses made with testers EUSS10, EUSS11, EUSS17 and SS04. EUSS10 formed hybrids with GS001, GS005, GS006, GS007, GS008, BM39, IS9203 and NYANGEZI. EUSS11 formed hybrids with GS001, GS003, GS008, BM39, BM23, IS25547 and NYNGEZI. EUSS17 formed hybrids with GS001, GS005, GS007, GS008, P40, BM23 and NYANGEZI. Finally, SS04 formed hybrids with NYANGEZI, GS001, GS003, GS007, GS008, BM39, P40 and P23. There are some crosses that resulted to formation of seeds in each of the florets (GS008, NYANGEZI), thus 100% fertilization, while for others less than 30% fertilization was realized.

4.10.2 Environmental conditions

The growing period of the first season for experiments conducted at Sugar Research Institute, Kibos (0°04'06"S, 34°49'03"E) commenced from 14th April 2016 to July 2016. At the time of planting on 14th April, the seedbed was saturated with moisture after receiving 471.4 mm of rainfall. During this period, the highest (254.9 mm) rainfall was received in May

followed by June which received 52 mm. Relatively low temperatures were experienced with April mean at 16.9^{o}C, May 17^{o}C and June at 16^{o}C. Harvesting was done in July and during this period low temperature of up to 15^{o}C and rainfall of only 4 mm were experienced. For the second season of the experiments conducted at Sugar Research Institute, the growing season commenced on 26th September 2016 to 26th January 2017. At Kendu-Bay (0.35^{o}13'07"S, 34^{o}07'44"), the crop growing period commenced on 5th September and continued to December 2016. Unlike in the first season at SRI, the second season was characterized by low rainfall.

4.10.3 Analyses of variances of agronomic and ethanol related traits

Genotypes exhibited significant ($p \leq 0.05$) variation for the traits that were investigated (Table 4.2). Significant variations were also seen across the three environments thus; Kendu-Bay and Kibos and also between two seasons in the same location, Kibos in the first and second season for all the traits except girth, days to flowering and brix. The female parents were taller than the male parents and the male parents had higher brix than the female parents (Table 4.3). NYANGEZI × EUSS17 was taller (309.11 cm) than both NYANGEZI (253.78 cm) and EUSS17 (176.33 cm) suggesting heterosis.

There were significant ($p \leq 0.001$) differences due to the environments for volume of juice, cane yield and ethanol volume. Days to flowering, girth and brix did not show any significant variation. Interaction between Lines and testers showed significant differences for all the traits. However, the interaction between the environment and the lines, the environment and the testers did not show any significant difference for all the traits that were investigated. Interactions between the environment, lines and testers were significant only for cane yield, volume of the juice and ethanol volume. Bekar's ratio was seen to be high in all the traits (Table 4.2). High parent heterosis was observed for all the traits that were investigated (Table 4.4). Out of 30 hybrids, 17 had positive high parent heterosis for height raing from 1.86% (NYANGEZI × EUSS10) to 57.50%.

Table 4.2 Mean squares analysis of lines and testers evaluated for height, cane yield, girth, juice volume, brix, ethanol volume and flowering time for sweet sorghum hybrids developed in a line × tester design in three environments in Kenya in 2016.

Sources of Variation	Df	Expected Mean Squares	Overall height (cm)	Cane yield (ton ha^{-1})	Girth (mm)	Days to flowering
Environment	2	$\sigma^2_e+3\sigma^2_{SLT}+19.99\ \sigma^2_{ST}+6.36\ \sigma^2_{SL}+26.64\ \sigma^2_{RS}+79.93\ \sigma^2S$	18862.48***	5581.69***	12.40	0.45
Rep (Environment)	6	$\sigma^2_e+30\sigma^2_{SLT}$	265.33	71.95	3.15	0.40
Lines (GCA)	12	$\sigma^2_e+3\sigma^2_{SLT}+6.5\ \sigma^2_{ST}+9\sigma^2_{LT}+19.5\sigma^2_L$	5242.05***	2446.96***	133.61**	198.84
Testers (GCA)	3	$\sigma^2_e+3\sigma^2_{SLT}+17\ \sigma^2_{ST}+9\sigma^2_{LT}+51\sigma^2_T$	31812.84*	2928.03*	63.51*	541.67
Line × Testers (SCA)	14	$\sigma^2_e+3\sigma^2_{SLT}+9\sigma^2_{LT}$	6692.70***	2136.63	46.75	348.70***
Environment × Lines	24	$\sigma^2_e+3\sigma^2_{SLT}+6.5\sigma^2_{SL}$	86.03	36.99	1.97	0.20
Environment × Testers	6	$\sigma^2_e+3\sigma^2_{SLT}+17\sigma^2_{ST}$	73.23	67.82	2.91	0.27
Environment × Lines × Testers	28	$\sigma^2_e+3\sigma^2_{SLT}$	67.31*	38.72***	1.50*	0.33
Error	174	σ^2_e	36.86	12.52	0.84	0.40
Baker's Ratio			0.92	0.83	0.89	0.81
CV%			2.56	6.11	4.07	0.80
R^2			0.98	0.98	0.95	0.99

***Significant difference at $p \leq 0.001$; df = degrees of freedom

Test H= Environment, E= Environment × Lines; Test H = Lines, E= Lines × Testers; Test H= Testers, E= Lines × Testers; Test H= Environment × Testers E= Environment × Line × Testers

Continued on the next page

Table 4.2 *continue*

Sources of Variation	df	Expected Mean Squares	Volume of juice (L ha^{-1})	Brix	Ethanol Volume (L ha^{-1})
Envoronment	2	$\sigma^2_e+3\sigma^2_{SLT}+19.99\ \sigma^2_{ST}+6.36\ \sigma^2_{SL}+26.64\ \sigma^2_{RS}+79.93\ \sigma^2S$	358993724.00**	0.52	32194373.30***
Rep (Environment)	6	$\sigma^2_e+30\sigma^2_{SLT}$	760605.00	0.92	513868.00
Lines (GCA)	12	$\sigma^2_e+3\sigma^2_{SLT}+6.5\ \sigma^2_{ST}+9\sigma^2_{LT}+19.5\sigma^2_L$	356652585.00	212.56	46786849.40
Testers (GCA)	3	$\sigma^2_e+3\sigma^2_{SLT}+17\ \sigma^2_{ST}+9\sigma^2_{LT}+51\sigma^2_T$	216296841.00	209.58	43732672.50
Line ×Testers (SCA)	14	$\sigma^2_e+3\sigma^2_{SLT}+9\sigma^2_{LT}$	235385620.00***	177.31***	31236820.40***
Envoronment ×Lines	24	$\sigma^2_e+3\sigma^2_{SLT}+6.5\sigma^2_{SL}$	4687575.00	0.65	692398.00
Environment ×Testers	6	$\sigma^2_e+3\sigma^2_{SLT}+17\sigma^2_{ST}$	13947583.00*	0.61	1726336.60*
Environment × Lines × Testers	28	$\sigma^2_e+3\sigma^2_{SLT}$	4118624.00***	0.56	516085.60***
Error	174	σ^2_e	429184.00	0.57	155450.00
Baker's Ratio			0.83	0.83	0.85
CV%			3.49	4.48	6.96
R^2			0.99	0.98	0.98

Table 4.3 Means for agronomic and sugar traits for sweet sorghum hybrids, male and female parents grown in three environments in Kenya in 2016.

Genotypes	Agronomic and sugar traits of sweet sorghum					
	Height (cm)	Girth (mm)	Cane Yield (ton ha $^{-1}$)	Juice Volume (l ha^{-1})	Brix	Ethanol Volume (l ha^{-1})
GS001 × EUSS10	230.67 lnm	18.67 nmo	41.65 rq	15347.2 lm	17.88 ijk	4249.9 nml
GS005 × EUSS10	230.56 lnm	23.33 ef	47.28 nmo	13505.1 p	12.22 q	4217.5 lnm
GS006 × EUSS10	222.00 opq	26.33 b	77.56 bcd	27948.8 b	18.00 jl	8028.7 e
GS007 × EUSS10	281.44 b	23.11 efg	83.65 b	25355.7 d	22.78 c	8390.7 d
GS008 × EUSS10	230.22 lnm	18.00 op	37.07 tu	13845.3 no	16.11 n	3766.9 o
BM39 × EUSS10	261.11 e	27.78 a	99.85 a	34730.7 a	17.67 jklm	11257.2 a
IS9203 × EUSS10	263.56 cde	21.56 k	50.92 hijkl	14784.0 mn	17.00 m	4470.9 kl
NYANGEZI × EUSS10	258.89 ef	23.11 gef	52.09 ijk	17576.5 j	21.55 d	5353.7 gh
EUSS10	188.67 uv	21.46 k	49.28 lmn	22093.9 f	9.89 uv	5353.2 g
GS001 × EUSS11	218.33 rq	23.67 d	72.28 ed	22657.1 f	10.11 tu	5916.8 f
GS003 × EUSS11	262.00 ed	22.88 efgh	69.11 f	21049.6 g	19.67 ef	6195.8 f
GS008 × EUSS11	269.11 c	21.89 jk	55.38 ghij	17905.1 j	9.81 uv	4810.2 j
BM39 × EUSS11	249.89 gh	28.33 a	55.85 ghij	19113.6 i	9.67 uvw	5299.2 ghi
BM23 × EUSS11	220.22 pq	21.89 jk	46.58 mno	12507.7 r	19.11 fg	3941.8 no
IS25547 × EUSS11	238.89 kj	23.11 efg	47.63 mno	15699.2 kl	18.33 hij	4976.1 j
NYANGEZI × EUSS11	201.44 t	22.44 ghij	55.49 hi	17893.3 j	20.22 e	5309.9 ghi
EUSS11	181.56 wx	18.96 lm	38.37 st	13329.1 pq	18.77gh	4106.6 mn
GS001 × EUSS17	209.78 s	22.00 ijk	46.69 ijklmnopk	17494.4 j	16.11 n	5012.7 ij
GS005 × EUSS17	191.44 u	18.89 lm	36.73 tu	16039.5 k	17.22 klm	4191.7 lmn
GS007 × EUSS17	201.33 t	21.89 jk	49.52jk lmno	15077.3 lmn	23.7 b	4737.2 jk
GS008 × EUSS17	181.44 wx	21.89 jk	38.60 st	17341.9 j	19.67 ef	4449.5 kl
P40 × EUSS17	243.33 ij	22.67 fghij	46.35 jklmno	17822.9 j	19.89 e	5029.5 hij
NYANGEZI × EUSS17	309.11 a	24.78 d	83.66 bc	26916.3 c	21.33 d	3073.2 pq
BM23 × EUSS17	178.44 wx	22.78 efgh	53.62ghi ijk	14455.5 no	18.44 ghi	4334.1 lm
EUSS17	176.33 x	16.83 q	33.44 uv	12695.5 qr	17.72 ijk	3996.0 no
GS007 × SS04	267.78 cd	22.11 hijk	61.83 efg	15394.1 lm	14.44 o	4203.5 lmn
GS008 × SS04	278.78 b	18.22 mno	38.37 st	11111.5 t	23.35 bc	3337.5 p
BM39 × SS04	253.33 fg	25.78 bc	66.29 ef	24311.5 cde	9.67 uvw	7808.5 e
P40 × SS04	228.00 mno	25.44 cd	80.84 bc	27772.8 b	9.44 uvw	9479.2 c
GS001 × SS04	280.0 b	17.11 q	54.56 ghij	13505.1 p	16.05 n	3736.8 o
GS003 × SS04	229.33 lmn	15.77 r	46.11 op	11827.2 s	11.33 rs	3073.2 pq
P23 × SS04	201.22 t	24.67 d	58.08 fgh	19958.4 h	9.67 uvw	5988.9 f
NYANGEZI × SS04	234.89 kl	26.22 cb	84.95 b	24710.4 e	25.44 a	8553.5 d
SS04	176.33 x	16.83 q	31.33 wx	12507.7 r	17.02 lm	4007.7 no

Table 4.3 *Continue*

Genotypes	Agronomic and sugar traits of sweet sorghum					
	Height (cm)	Girth (mm)	Cane Yield (ton ha $^{-1}$)	Juice Volume (l ha^{-1})	Brix	Ethanol Volume (l ha^{-1})
Females						
BM23	213.78 rs	17.33 pq	37.67 st	10876.8 t	13.89 o	2728.0 rstu
BM39	225.44 nop	18.83 m	43.29 lmnopq	8976.0 u	9.22 vw	2557.9 stu
GS001	221.00 pq	15.05 rs	28.74 xy	5585.1 x	12.67 pq	2962.7 qr
GS002	246.00 hi	14.67 rst	34.38 rsuv	5866.7 x	12.56 pq	2485.1 tuv
GS003	231.22 lmn	14.17 st	25.11 zaa	7697.1 w	12.00 qr	2229.3 v
GS005	231.78 lm	18.89 lm	40.12 v	9879.5 u	5.44 x	2428.8 uv
GS006	193.89 u	14.33 st	24.28 aa	6160.0 x	9.11 x	2851.2 qrs
GS007	188.67 uv	12.33 u	20.29 bb	3848.5 y	9.89 uv	2704.5 rstu
GS008	177.00 wx	15.11 rs	27.69 yz	9668.3 u	9.89 uv	1795.2 w
IS25547	268.78 c	189.03 nop	37.43 st	7978.7 w	13.11 p	2595.4 stu
IS9203	268.78 c	16.67 q	39.07 opqrst	8987.7 v	9.22 vw	2792.5 qrst
NYANGEZI	253.78 fg	18.89 lm	42.01 lmnoqr	12542.9 r	10.67 st	2581.3 stu
P23	221.67 pq	18.82 mn	46.46 lmnop	13505.1 p	4.33 y	2422.9 uv
P40	267.44 cd	19.67 k	42.01 lmnopq	11792.0 s	12.67 pq	2634.1 stu
LSD 0.05	21.19	2.4	10.67	3582	1.28	935

Means denoted by the same letter are not statistically different.

Table 4.4 High parent heterosis for agronomic and ethanol related traits in sweet sorghum hybrids observed across three environments in Kenya in 2016.

Genotypes	Agronomic and sugar traits for sweet sorghum hybrids					
	Height	Girth	Cane yield	Juice volume	Brix	Ethanol volume
NYANGEZI × SS04	-7.44	38.80	102.21	97.01	49.47	113.43
GS001 × EUSS10	4.38	-13.84	-15.48	-30.54	41.12	-20.61
GS005 × EUSS10	-0.53	7.66	-4.06	-38.87	23.68	-21.22
GS006 × EUSS10	14.50	21.50	57.39	26.50	82.19	49.98
GS007 × EUSS10	49.17	6.65	69.70	14.77	130.57	56.74
GS008 × EUSS10	22.31	-16.94	-24.78	-37.33	63.06	-29.63
BM39 × EUSS10	15.82	28.20	102.58	57.20	78.85	110.29
IS9203 × EUSS10	-1.94	-0.51	3.33	-33.08	72.06	-16.48
NYANGEZI × EUSS10	1.86	6.65	5.70	-20.44	118.12	0.01
GS001 × EUSS11	-1.21	24.84	88.38	69.98	-46.14	44.08
GS003 × EUSS11	13.31	20.68	80.11	57.92	4.79	50.87
GS001 × SS04	26.70	-9.76	74.15	7.97	-5.70	-6.76
GS008 × EUSS11	48.22	15.45	44.33	34.33	-47.74	17.13
BM39 × EUSS11	10.85	49.42	29.01	43.40	-48.48	29.04
BM23 × EUSS11	3.01	15.45	21.40	-6.51	1.81	-4.01
IS25547 × EUSS11	-11.12	21.89	24.13	17.78	-2.34	21.17
NYANGEZI × EUSS11	-20.62	18.35	32.09	35.26	7.73	29.30
GS001 × EUSS17	-5.08	30.72	39.62	37.80	-9.09	25.44
GS005 × EUSS17	-17.40	0.00	-8.45	26.34	-2.82	4.90
GS007 × EUSS17	6.71	30.07	48.09	18.76	33.75	18.56
GS008 × EUSS17	2.51	30.07	15.43	36.60	11.00	11.35
P40 × EUSS17	-9.02	15.25	10.33	40.39	12.25	25.86
GS003 × SS04	-0.82	-6.30	47.18	-5.44	-33.43	-23.32
BM23 × EUSS17	-16.53	31.45	41.81	13.86	4.06	8.46
GS007 × SS04	41.93	31.37	97.35	23.08	-15.16	4.89
GS008 × SS04	57.50	8.26	22.47	-11.16	37.19	-16.72
BM39 × SS04	12.37	36.91	53.13	94.37	-43.18	94.84
P40 × SS04	-14.75	29.33	92.43	122.05	-44.54	136.52
P23 × SS04	-9.23	31.08	25.01	59.57	-43.18	49.43
NYANGEZI × EUSS17	21.80	31.18	102.21	112.01	20.37	-23.09

Twenty three genotypes showed positive high parent heterosis for girth ranging from 6.65% (NYANGEZI × EUSS10) to 49.42% (BM39 ×EUSS11). For cane yield, 26 hybrids exhibited positive high parent heterosis ranging from 3.3% (IS9203 × EUSS10) to 102.58% (BM39 × EUSS10). Positive high parent heterosis was observed for volume of the juice in 21 hybrids ranging from 7.97% (GS001 × SS04) to 122.05% (P40 × SS04). GS007 × EUSS10 had the highest (130.57%) positive high parent heterosis for brix followed by 118.12% (NYANGEZI × EUSS10). The highest (136.52%) positive high parent heterosis for ethanol volume was exhibited by BM39 × SS04 (Table 4.4).

Twenty eight out of the 30 hybrids had significant standard heterosis for height, 27 for girth and all the hybrids showed significant standard heterosis for cane yield. For juce volume the number of hybrids with significant ($p \leq 0.05$) standard heterosis reduced to 24. A further reduction in the number was seen for brix of which only 22 were significant. Ethanol volume was only significant for 20 hybrids (Table 4.5). Standard heterosis was observed across three environments (Table 4.6). The percent range for height was between -9.71% to 64.68% at Kendu-Bay and 3.56% to 74.77% during the second season at Kibos (Table 4.5). Brix mostly showed negative standard heterosis especially at Kibos during the second season where the range was -47.72% to 38.75%. Kendu-Bay had the highest heterosis for brix with a range of -47.73% to 51.35% (Table 4.6). Heterosis was observed for agronomic traits such as height, girth and cane yield (Table 4.8) while sugar traits such as brix, juice volume and ethanol volume showed low and mostly negative heterosis (Table 4.7). Better parent or high parent heterosis was relatively low in all the two sites and for two seasons on the same site for sugar traits (Table 4.7). Standard heterosis was positive for height at all the experimental sites (Table 4.8). Girth and cane yield also showed positive mid parent, better parent and standard heterosis in all the experimental sites and for two seasons at Kibos (Table 4.8). Out of 30 hybrids, 26 of them showed positive mid parent, better parent and standard heterosis in all the experimental sites and for the two seasons (Table 4.9). However, there was considerable variation in heterosis percent in all the 30 genotypes and for all the traits, (Table 4.10). For sweet sorghum, brix and juice volume are the most important traits. The hybrids exhibited both negative and positive sugar traits. Eighteen hybrids showed positive heterosis for ethanol volume while 12 showed negative heterosis (Figure 4.1).

Table 4.5 Standard heterosis for agronomic and sugar traits in sweet sorghum hybrids observed across three environments in Kenya in 2016.

Genotypes	Agronomic and sugar traits for sweet sorghum hybrids					
	Height	Girth	Cane yield	Juice Volume	Brix	Ethanol Volume
NYANGEZI × SS04	33.21***	55.79***	171.15***	97.56***	49.47***	113.43***
GS001 × EUSS10	30.82***	10.93***	32.94***	22.70***	5.05	6.04
GS005 × EUSS10	30.75***	38.62***	50.91***	7.97	-28.20***	5.23
GS006 × EUSS10	25.90***	56.45***	147.56***	123.45***	5.76	100.33***
GS007 × EUSS10	59.61***	37.31***	167.00***	102.72***	33.84***	109.36***
GS008 × EUSS10	30.56***	6.95	18.32***	10.69***	-5.35	-6.01
BM39 × EUSS10	48.08***	65.06***	218.70***	177.67***	3.82	180.89***
IS9203 × EUSS10	49.47***	28.10***	62.53***	18.20***	-0.12	11.56
NYANGEZI × EUSS10	46.82***	37.31***	66.26***	40.53***	26.62***	33.59***
GS001 × EUSS11	23.82***	40.64***	130.71***	81.15***	-40.60***	47.64***
GS003 × EUSS11	48.59***	35.95***	120.59***	68.29***	15.57***	54.60***
GS001 × SS04	58.79***	1.66	74.15***	7.97	-5.70	-6.76
GS008 × EUSS11	52.62***	30.07***	76.76***	43.15***	-42.36***	20.02***
BM39 × EUSS11	41.72***	68.33***	78.26***	52.81***	-43.18***	32.23***
BM23 × EUSS11	24.89***	30.07***	48.68***	0.00	12.28***	-1.64
IS25547 × EUSS11	35.48***	37.31***	52.03***	25.52***	7.70***	24.16***
NYANGEZI × EUSS11	14.24***	33.33***	77.11***	43.06***	18.80***	32.49***
GS001 × EUSS17	18.97***	30.72***	49.03***	39.87***	-5.35	25.08***
GS005 × EUSS17	8.57***	12.24***	17.24***	28.24***	1.18	4.59
GS007 × EUSS17	14.18***	30.07***	58.06***	20.54***	39.25***	18.20***
GS008 × EUSS17	2.90	30.07***	23.20***	38.65***	15.57***	11.02
P40 × EUSS17	38.00***	34.70***	47.94***	42.50***	16.86***	25.50***
GS003 × SS04	30.06***	-6.30	47.18***	-5.44	-33.43***	-23.32***
BM23 × EUSS17	1.20	35.35***	71.15***	15.57***	8.34***	8.14
GS007 × SS04	51.86***	31.37***	97.35***	23.08***	-15.16***	4.89
GS008 × SS04	58.10***	8.26***	22.47***	-11.16***	37.19***	-16.72***
BM39 × SS04	43.67***	53.18***	111.59***	94.37***	-43.18***	94.84***
P40 × SS04	29.30***	51.16***	158.03***	122.05***	-44.54***	136.52***
P23 × SS04	14.12***	46.58***	85.38***	59.57***	-43.18***	49.43***
NYANGEZI × EUSS17	75.30***	47.24***	167.03***	115.20***	25.32***	146.33***

***Significant at $p \leq 0.001$; Dunnett's test

Table 4.6 Standard heterosis for sweet sorghum hybrids observed across three environments in Kenya in 2016 for height, girth, brix and ethanol volume for all genotypes.

CODE	Genotypes	Height			Girth			Brix			Ethanol Volume		
		Kendu-Bay	Kibos 1	Kibos2	Kendu-Bay	Kibos 1	Kibos2	Kendu-Bay	Kibos 1	Kibos2	Kendu-Bay	Kibos 1	Kibos2
1	NYANGEZI × SS04	24.30	36.08	34.76	38.07	43.39	36.32	38.75	35.14	38.75	93.70	130.18	84.71
10	GS001 × EUSS10	19.44	33.46	35.14	-2.65	6.21	-6.16	-4.48	2.70	-8.10	-9.27	20.31	-8.65
11	GS005 × EUSS10	20.56	31.78	35.14	23.89	18.59	29.20	-31.51	-38.75	-31.51	-17.28	27.41	-10.05
12	GS006 × EUSS10	12.71	28.41	32.34	41.63	39.83	38.07	-4.48	-0.92	-2.70	87.26	85.66	98.32
13	GS007 × EUSS10	45.79	65.42	62.06	22.14	29.21	16.83	26.10	22.54	20.70	71.78	156.78	68.46
14	GS008 × EUSS10	18.88	36.26	31.78	-6.53	2.66	-9.71	-13.51	-11.73	-13.51	-16.94	0.81	-15.85
15	BM39 × EUSS10	34.58	54.77	49.91	48.69	46.94	46.94	-2.70	-6.32	-4.48	117.21	127.20	116.55
16	IS9203 × EUSS10	37.01	54.39	51.97	11.52	23.89	7.96	-8.11	-9.89	-6.32	-6.46	26.90	-2.32
17	NYANGEZI × EUSS10	34.39	51.22	49.91	20.33	23.89	24.11	15.29	17.14	17.13	8.47	44.50	26.53
18	GS001 × EUSS11	10.47	28.78	28.04	27.45	25.65	23.89	-47.73	-44.16	-44.16	35.58	52.97	32.44
19	GS003 × EUSS11	35.33	52.34	53.07	20.39	23.89	20.39	6.32	6.32	6.32	38.01	63.13	39.70
2	GS001 × SS04	48.98	62.43	59.63	-13.27	0.90	-15.03	-10.81	-17.14	-11.73	-19.54	2.48	-17.07
20	GS008 × EUSS11	40.19	60.75	51.78	18.58	15.08	15.08	-47.73	-44.16	-47.72	6.86	25.08	7.94
21	BM39 × EUSS11	31.03	45.98	43.36	55.76	43.38	52.25	-45.94	-47.73	-49.56	19.77	43.09	14.21
22	BM23 × EUSS11	10.65	30.47	29.35	16.83	16.83	15.08	4.48	4.48	0.918	-18.70	16.62	-5.59
23	IS25547 × EUSS11	22.43	41.13	38.32	23.89	25.70	18.58	51.35	-0.91	0.92	10.73	27.20	16.13
24	NYANGEZI × EUSS11	11.97	16.08	10.84	23.89	18.58	15.02	9.89	9.89	8.11	18.48	30.19	29.13
25	GS001 × EUSS17	3.36	23.74	25.79	16.83	18.58	15.08	-15.29	-11.73	-11.72	15.01	33.16	8.51
26	GS005 × EUSS17	-0.93	12.89	10.09	-2.65	7.96	-4.40	-6.32	-9.89	-4.48	-7.13	13.45	-8.07
27	GS007 × EUSS17	3.36	16.82	18.50	18.58	15.08	15.08	27.94	31.51	26.11	2.53	27.15	7.38
28	GS008 × EUSS17	-7.85	5.24	7.85	15.08	22.14	11.52	9.89	6.32	2.70	2.85	11.00	2.74
29	P40 × EUSS17	25.79	41.87	41.68	16.83	20.39	23.89	9.89	6.32	6.32	18.28	21.29	18.28
3	GS003 × SS04	20.56	31.22	34.02	-18.58	-11.47	-18.58	-38.75	-38.75	-38.75	-44.13	-3.73	-33.48
30	BM23 × EUSS17	-9.71	6.36	3.56	22.14	20.39	20.39	2.70	-0.91	-2.70	-9.65	24.41	-6.38
4	GS007 × SS04	35.88	56.64	57.94	15.08	23.89	13.28	-18.91	-21.78	-26.11	-11.24	21.47	-11.15
5	GS008 × SS04	45.23	64.12	59.44	-7.96	6.21	-7.97	26.11	24.32	27.95	-34.70	5.28	-33.11
6	BM39 × SS04	34.77	46.36	45.05	38.07	38.07	34.52	-47.71	-47.73	-47.72	74.71	98.36	82.51
7	P40 × SS04	15.89	36.08	31.59	38.07	32.76	34.51	-47.73	-49.56	-49.56	101.73	113.71	119.02
8	P23 × SS04	8.41	15.33	14.77	30.96	31.01	31.01	-47.73	-47.73	-47.72	28.58	61.31	36.23
9	NYANGEZI × EUSS17	64.68	80.56	74.77	34.51	32.76	27.45	18.92	13.51	13.51	119.60	111.25	121.55

Table 4.7 Standard heterosis observed at three environments in Kenya in 2016 for sugar traits: Juice volume, brix and ethanol volume classified by environment.

	Juice volume			Brix			Ethanol Volume		
	Kendu-Bay	Kibos (season 1)	Kibos (season 2)	Kendu-Bay	Kibos (season 1)	Kibos (season 2)	Kendu-Bay	Kibos (season 1)	Kibos (season 2)
NYANGEZI × SS04	88.35***	121.59***	-81.65***	38.75***	35.14***	38.75***	93.70***	130.18***	84.71***
GS001 × EUSS10	10.23***	46.31***	11.88***	-4.48	2.70	-8.10	-9.27	20.31***	-8.65
GS005 × EUSS10	-10.23	38.35***	-3.87	-31.51***	-38.75***	-31.51***	-17.28	27.41***	-10.05
GS006 × EUSS10	114.77***	134.38***	121.27***	-4.48	-0.92	-2.70	87.26***	85.66***	98.32***
GS007 × EUSS10	75.28***	159.66***	74.03***	26.10***	22.54***	0.70***	71.78***	156.78***	68.46***
GS008 × EUSS10	0.85	26.99***	4.42***	-13.51	-11.73	-13.51	-16.94	0.81	-15.85
BM39 × EUSS10	134.38***	212.78***	185.64***	-2.70	-6.32	-4.48	117.21***	127.20***	116.55***
IS9203 × EUSS10	4.91***	42.33***	7.73***	-8.11	-9.89	-6.32	-6.46	26.90***	-2.32
NYANGEZI × EUSS10	23.01***	62.22***	36.46***	15.29***	17.14***	17.13***	8.47***	44.50***	26.53***
GS001 × EUSS11	72.73***	100.28***	70.72***	-47.73***	-44.16***	-44.16***	35.58***	52.97***	32.44***
GS003 × EUSS11	56.53***	89.20***	59.39***	6.32***	6.32***	6.32***	38.01***	63.13***	39.70***
GS001 × SS04	-3.41	24.43***	-0.83	-10.81	-17.14	-11.73	-19.54	2.48	-17.07
GS008 × EUSS11	32.95***	61.65***	35.08***	-47.73***	-44.16***	-47.72***	6.86***	25.08***	7.94***
BM39 × EUSS11	45.17***	67.90***	45.58***	-45.94***	-47.73***	-49.56***	19.77***	43.09***	14.21***
BM23 × EUSS11	-13.64	26.70***	-12.71	4.48***	4.48***	0.918***	-18.70	16.62***	-5.59
IS25547 × EUSS11	20.17***	33.81***	22.65***	51.35***	-0.91	0.92***	10.73***	27.20***	16.13***
NYANGEZI × EUSS11	37.50***	46.02***	45.58***	9.89***	9.89***	8.11***	18.48***	30.19***	29.13***
GS001 × EUSS17	33.24***	59.94***	26.80***	-15.29	-11.73	-11.72	15.01***	33.16***	8.51***
GS005 × EUSS17	18.47***	46.59***	19.89***	-6.32	-9.89	-4.48	-7.13	13.45	-8.07
GS007 × EUSS17	10.23	39.49***	20.00***	27.94***	31.51***	26.11***	2.53***	27.15***	7.38***
GS008 × EUSS17	32.95***	47.16***	35.91***	9.89***	6.32***	2.70***	2.85	11.00	2.74
P40 × EUSS17	40.34***	45.74***	41.44***	9.89***	6.32***	6.32***	18.28***	21.29***	18.28***
GS003 × SS04	-25.85	23.01***	-13.26	-38.75***	-38.75***	-38.75***	-44.13***	-3.73***	-33.48***
BM23 × EUSS17	1.42***	41.19***	4.42***	2.70	-0.91	-2.70***	-9.65	24.41***	-6.38
GS007 × SS04	11.93***	46.02***	11.60***	-18.91***	-21.78***	-26.11***	-11.24	21.47***	-11.15
GS008 × SS04	-26.70***	20.74***	-27.07***	26.11***	24.32***	27.95***	-34.70***	5.28***	-33.11***
BM39 × SS04	85.80***	108.81***	88.67***	-47.71***	-47.73***	-47.72***	74.71***	98.36***	82.51***
P40 × SS04	100.00***	148.86***	117.40***	-47.73***	-49.56***	-49.56***	101.73***	113.71***	119.02***
P23 × SS04	47.44***	82.95***	48.62***	-47.73***	-47.73***	-47.72***	28.58***	61.31***	36.23***
NYANGEZI × EUSS17	100.28***	142.33***	103.31***	18.92***	13.51***	13.51***	119.60***	111.25***	121.55***

***Significant at $p \leq 0.001$ Dunnett's test.

Table 4.8 Standard heterosis for agronomic traits of sweet sorghum hybrids grown in three environments in Kenya in 2016.

Genotypes	Height			Girth			Cane Yield		
	Kendu-Bay	Kibos (season 1)	Kibos (season 2)	Kendu-Bay	Kibos (season 1)	Kibos (season 2)	Kendu-Bay	Kibos (season 1)	Kibos (season 2)
NYANGEZI × SS04	24.30***	36.08***	34.76***	38.07***	43.39***	36.32***	144.37***	217.11***	161.41***
GS001 × EUSS10	19.44***	33.46***	35.14***	-2.65***	6.21***	-6.16***	5.72***	81.85***	15.92***
GS005 × EUSS10	20.56***	31.78***	35.14***	23.89***	18.59***	29.20***	38.65***	77.30***	42.07***
GS006 × EUSS10	12.71***	28.41***	32.34***	41.63***	39.83***	38.07***	117.08***	172.78***	161.41***
GS007 × EUSS10	45.79***	65.42***	62.06***	22.14***	29.21***	16.83***	136.39***	206.88***	167.10***
GS008 × EUSS10	18.88***	36.26***	31.78***	-6.53	2.66	-9.71	5.68***	45.46***	7.98***
BM39 × EUSS10	34.58***	54.77***	49.91***	48.69***	46.94***	46.94***	196.64***	252.34***	218.24***
IS9203 × EUSS10	37.01***	54.39***	51.97***	11.52***	23.89***	7.96	40.91***	110.27***	42.07***
NYANGEZI × EUSS10	34.39***	51.22***	49.91***	20.33***	23.89***	24.11***	38.65***	115.95***	50.02***
GS001 × EUSS11	10.47***	28.78***	28.04***	27.45***	25.65***	23.89***	100.03***	167.10***	133.00***
GS003 × EUSS11	35.33***	52.34***	53.07***	20.39***	23.89***	20.39***	103.42***	150.05***	115.95***
GS001 × SS04	48.98***	62.43***	59.63***	-13.27**	0.90	-15.03	47.76***	110.27***	70.49***
GS008 × EUSS11	40.19***	60.75***	51.78***	18.58***	15.08***	15.08***	55.70***	115.95***	64.80***
BM39 × EUSS11	31.03***	45.98***	43.36***	55.76***	43.38***	52.25***	45.46***	119.34***	76.17***
BM23 × EUSS11	10.65***	30.47***	29.35***	16.83***	16.83***	15.08***	34.10***	80.72***	36.39***
IS25547 × EUSS11	22.43***	41.13***	38.32***	23.89***	25.70***	18.58***	39.78***	79.56***	42.07***
NYANGEZI × EUSS11	11.97***	16.08***	10.84***	23.89***	18.58***	15.02***	70.49***	90.93***	76.17***
GS001 × EUSS17	3.36***	23.74***	25.79***	16.83***	18.58***	15.08***	36.39***	51.15***	64.80***
GS005 × EUSS17	-0.93***	12.89***	10.09***	-2.65***	7.96***	-4.40***	2.29***	45.46***	7.98***
GS007 × EUSS17	3.36***	16.82***	18.50***	18.58***	15.08***	15.08***	39.78***	80.69***	59.12***
GS008 × EUSS17	-7.85	5.24	7.85	15.08***	22.14***	11.52***	10.24***	50.02***	13.66***
P40 × EUSS17	25.79***	41.87***	41.68***	16.83***	20.39***	23.89***	39.78***	78.43***	30.71***
GS003 × SS04	20.56***	31.22***	34.02***	-18.58	-11.47	-18.58	39.78***	64.80***	42.07***
BM23 × EUSS17	-9.71	6.36	3.56	22.14***	20.39***	20.39***	47.76***	106.85***	64.80***
GS007 × SS04	35.88***	56.64***	57.94***	15.08***	23.89***	13.28***	72.75***	133.00***	93.22***
GS008 × SS04	45.23***	64.12***	59.44***	-7.96***	6.21***	-7.97***	10.24***	53.44***	7.98***
BM39 × SS04	34.77***	46.36***	45.05***	38.07***	38.07***	34.52***	78.11***	150.05***	112.53***
P40 × SS04	15.89***	36.08***	31.59***	38.07***	32.76***	34.51***	137.52***	178.46***	167.10***
P23 × SS04	8.41***	15.33***	14.77***	30.96***	31.01***	31.01***	53.44***	121.63***	87.54***
NYANGEZI × EUSS17	64.68***	80.56***	74.77***	34.51***	32.76***	27.45***	119.34***	218.24***	172.78***

***Significant at $p \leq 0.001$; Dunnett's test.

Table 4.9 Sweet sorghum hybrids, male and female juice volume means with mid parent, high parent and standard heterosis observed at two environments in Kenya in 2016.

	Kendu-Bay						Kibos (Season 1)					
Genotypes	Juice volume (l ha^{-1})			Heterosis (%)			Juice volume (l ha^{-1})			Heterosis (%)		
	Hybrid	Male	Female	Mid parent	High Parent	%SS04	Hybrid	Male	Female	Mid parent	High Parent	%SS04
NYANGEZI × SS04	23337.6	12390.4	12264.0	89.32	88.35	88.35	27456.0	12390.4	11440.0	130.43	121.59	121.59
GS001 × EUSS10	13657.6	25027.2	6160.0	-12.42	-45.43	10.23	18128.0	25027.2	5420.8	19.08	-27.57	46.31
GS005 × EUSS10	11123.2	25027.2	10595.2	-37.55	-55.56	-10.23	17142.4	25027.2	9961.6	-2.01	-31.50	38.35
GS006 × EUSS10	26611.2	25027.2	6230.4	70.27	6.33	114.77	29040.0	25027.2	5913.6	87.71	16.03	134.38
GS007 × EUSS10	21718.4	25027.2	4012.8	49.58	-13.22	75.28	32172.8	25027.2	3696.0	124.02	28.55	159.66
GS008 × EUSS10	12496.0	25027.2	9750.4	-28.14	-50.07	0.85	15734.4	25027.2	9328.0	-8.40	-37.13	26.99
BM39 × EUSS10	29040.0	25027.2	6054.4	86.86	16.03	134.38	38755.2	25027.2	5104.0	157.24	54.85	212.78
IS9203 × EUSS10	12998.8	25027.2	9046.4	-23.70	-48.06	4.91	17635.2	25027.2	8764.8	4.38	-29.54	42.33
NYANGEZI × EUSS10	15241.6	25027.2	12264	-18.26	-39.10	23.01	20099.2	25027.2	11440.0	10.23	-19.69	62.22
GS001 × EUSS11	21401.6	13798.4	6160.0	114.46	55.10	72.73	24816.0	13798.4	5420.8	158.24	79.85	100.28
GS003 × EUSS11	19395.2	13798.4	8307.2	75.48	40.56	56.53	23443.2	13798.4	7920.0	115.88	69.90	89.20
GS001 × SS04	11968.0	12390.4	6160.0	29.03	-3.41	-3.41	15417.6	12390.4	5420.8	73.12	24.43	24.43
GS008 × EUSS11	16473.6	13798.4	9750.4	39.91	19.39	32.95	20028.8	13798.4	9328.0	73.21	45.15	61.65
BM39 × EUSS11	17987.2	13798.4	6054.4	81.21	30.36	45.17	20803.2	13798.4	5104.0	120.11	50.77	67.90
BM23 × EUSS11	10700.8	13798.4	10102.4	-10.46	-22.45	-13.64	15699.2	13798.4	9996.8	31.95	13.78	26.70
IS25547 × EUSS11	14889.6	13798.4	5948.8	50.80	7.91	20.17	16579.2	13798.4	5526.4	71.58	20.15	33.81
NYANGEZI × EUSS11	17036.8	13798.4	12264.0	30.74	23.47	37.50	18092.8	13798.4	11440.0	43.38	31.12	46.02
GS001 × EUSS17	16508.8	13024.0	6160.0	72.11	26.76	33.24	19817.6	13024.0	5420.8	114.89	52.16	59.94
GS005 × EUSS17	14678.4	13024.0	10595.2	24.29	12.70	18.47	18163.2	13024.0	9961.6	58.04	39.46	46.59
GS007 × EUSS17	13657.6	13024.0	4012.8	60.33	4.86	10.23	17283.2	13024.0	3696.0	106.74	32.70	39.49
GS008 × EUSS17	16473.6	13024.0	9750.4	44.67	26.49	32.95	18233.6	13024.0	9328.0	63.15	40.00	47.16
P40 × EUSS17	17388.8	13024.0	9046.4	57.58	33.51	40.34	18057.6	13024.0	9222.4	62.34	38.65	45.74
GS003 × SS04	9187.2	12390.4	8307.2	-11.22	-25.85	-25.85	15241.6	12390.4	7920.0	50.09	23.01	23.01
BM23 × EUSS17	12566.4	13024	10102.4	8.68	-3.51	1.42	17494.4	13024.0	9996.8	51.99	34.32	41.19
GS007 × SS04	13868.8	12390.4	4012.8	69.10	11.93	11.93	18092.8	12390.4	3696.0	124.95	46.02	46.02
GS008 × SS04	9081.6	12390.4	9750.4	-17.97	-26.70	-26.70	14960.0	12390.4	9328.0	37.76	20.74	20.74
BM39 × SS04	23020.8	12390.4	6054.4	149.62	85.80	85.80	25872	12390.4	5104.0	195.77	108.81	108.81
P40 × SS04	24780.8	12390.4	9046.4	131.20	100.00	100.00	30835.2	12390.4	9222.4	185.34	148.86	148.86
P23 × SS04	18268.8	1239.4	13516.8	147.61	35.16	47.44	22668.8	12390.4	13657.6	74.05	82.95	82.95
NYANGEZI × EUSS17	24816.0	25027.2	12264	33.09	-0.84	100.28	30025.6	25027.2	11440.0	64.67	19.97	142.33

Check variety SS04

Table 4.10 Sweet sorghum hybrids, male and female, brix means with mid parent, high parent and standard heterosis observed two environments in Kenya in 2016.

	Kendu-Bay						Kibos (Season 1)					
Genotypes	Brix			Heterosis (%)			Brix			Heterosis (%)		
	Hybrid	Male	Female	Mid	High	%SS04	Hybrid	Male	Female	Mid	Better	%SS04
NYANGEZI × SS04	25.67	18.50	10.00	80.14	38.76	38.76	25.00	16.00	11.33	82.95	56.25	35.14
GS001 × EUSS10	17.67	10.83	12.00	54.80	63.16	-4.49	19.00	18.17	14.33	16.92	4.57	2.70
GS005 × EUSS10	12.67	10.83	5.33	56.81	16.99	-31.51	11.33	18.17	6.00	-6.25	-37.64	-38.76
GS006 × EUSS10	17.67	10.83	8.67	81.23	63.16	-4.49	18.33	18.17	9.67	31.68	0.88	-0.92
GS007 × EUSS10	23.33	10.83	10.33	120.51	115.42	26.11	22.67	18.17	11.33	53.69	24.77	22.54
GS008 × EUSS10	16.00	10.83	9.33	58.73	47.74	-13.51	16.33	18.17	9.00	20.21	-10.13	-11.73
BM39 × EUSS10	18.00	10.83	9.33	78.57	66.20	-2.70	17.33	18.17	9.33	26.04	-4.62	-6.32
IS9203 × EUSS10	17.00	10.83	9.00	71.46	56.97	-8.11	16.67	18.17	11.33	13.02	-8.26	-9.89
NYANGEZI × EUSS10	21.33	10.83	10.00	104.80	96.95	15.30	21.67	18.17	14.33	33.35	19.26	17.14
GS001 × EUSS11	9.67	20.17	12.00	-39.88	-52.06	-47.73	10.33	18.17	11.67	-30.76	-43.15	-44.16
GS003 × EUSS11	19.67	20.17	12.33	21.05	-2.48	6.32	19.67	18.17	14.33	21.05	8.26	6.32
GS001 × SS04	16.50	18.50	12.00	8.20	-10.81	-10.81	15.33	16.00	14.33	1.09	-4.19	-17.14
GS008 × EUSS11	9.67	20.17	9.33	-34.44	-52.06	-47.73	10.33	18.17	11.33	-29.97	-43.15	-44.16
BM39 × EUSS11	10.00	20.17	9.33	-32.20	-50.42	-45.95	9.67	18.17	9.00	-28.82	-46.78	-47.73
BM23 × EUSS11	19.33	20.17	13.33	15.40	-4.16	4.49	19.33	18.17	11.33	31.05	6.38	4.49
IS25547 × EUSS11	28.00	20.17	11.67	75.88	38.82	51.35	18.33	18.17	14.00	13.96	0.88	-0.92
NYANGEZI × EUSS11	20.33	20.17	10.00	34.77	0.79	9.89	20.33	18.17	4.33	80.71	11.89	9.89
GS001 × EUSS17	15.67	18.67	11.33	4.47	-16.07	-15.30	16.33	17.50	14.33	2.61	-6.69	-11.73
GS005 × EUSS17	17.33	18.67	5.33	44.42	-7.18	-6.32	16.67	17.50	6.00	41.87	-4.74	-9.89
GS007 × EUSS17	23.67	18.67	10.33	63.24	26.78	27.95	24.33	17.50	9.67	79.09	39.03	31.51
GS008 × EUSS17	20.33	18.67	9.33	45.21	8.89	9.89	19.67	17.50	11.33	36.46	12.40	6.32
P40 × EUSS17	20.33	18.67	12.00	32.57	8.89	9.89	19.67	17.50	13.33	27.60	12.40	6.32
GS003 × SS04	11.33	18.50	12.33	-26.50	-38.76	-38.76	11.33	16.00	11.67	-18.11	-29.19	-38.76
BM23 × EUSS17	19.00	18.67	13.33	18.75	1.77	2.70	18.33	17.50	11.33	27.16	4.74	-0.92
GS007 × SS04	15.00	18.50	10.33	4.06	-18.92	-18.92	14.47	16.00	9.67	12.74	-9.56	-21.78
GS008 × SS04	23.33	18.50	9.33	67.66	26.11	26.11	23.00	16.00	11.33	68.31	43.75	24.32
BM39 × SS04	9.67	18.50	9.33	-30.51	-47.73	-47.73	9.67	16.00	9.00	-22.64	-39.56	-47.73
P40 × SS04	9.67	18.50	12.00	-36.59	-47.73	-47.73	9.33	16.00	13.33	-36.38	-41.69	-49.57
P23 × SS04	9.67	18.50	4.33	-15.29	-47.73	-47.73	9.67	16.00	4.33	-4.87	-39.56	-47.73
NYANGEZI × EUSS17	22.00	10.83	10.00	111.23	103.14	18.92	21.00	8.17	11.33	115.38	157.04	13.51

Check variety SS04.

Table 4.11 Specific combining ability (SCA) effects of 30 hybrids and overal mean for agronomic and sugar traits across three environments in Kenya in 2016.

Genotypes	Agronomic and sugar traits of sweet sorghum											
	Height (cm)		Girth (mm)		Cane yield (ton ha $^{-1}$)		Juice volume (l ha^{-1})		Brix		Ethanol volume (l ha^{-1})	
	Overal mean	SCA effects	Overal mean	SCA effects	Overal mean	SCA effects	Overal mean	SCA effects	Overal mean	SCA effects	Overal mean	SCA effects
SS04												
BM39	253.33	15.54	25.78	1.48	66.27	1.94	24311.47	1250.82	9.67	0.25	7808.53	752.46
BM23		-23.56		-2.11		-5.54		-2586.96		-2.19		-509.60
IS25547		-25.12		-2.21		-5.63		-1648.29		-1.42		-3984.62
IS9203		-27.17		-2.08		-5.90		-1572.03		-1.31		-192.52
P40	228.00	31.51	25.44	4.19	80.84	14.69	27772.80	5117.93	9.44	0.81	9479.24	2130.74
NYANGEZI	234.89	-10.61	26.22	0.41	84.94	3.64	24710.40	2881.75	25.44	1.22	8553.48	608.79
GS001	280.00	9.89	17.11	-1.37	54.56	-1.41	13340.80	-1630.13	16.15	0.45	3736.83	-150.71
GS002		-5.21		-0.28		-1.66		-340.03		0.11		180.05
GS003	231.22	30.29	15.78	1.75	46.11	4.11	11827.20	862.64	11.33	1.30	3073.19	432.04
P23	201.22	-40.37	24.67	-3.80	58.08	-8.66	19958.40	-2802.07	9.67	-2.34	5988.93	-520.72
GS005		45.10		5.88		12.86		4649.57		2.53		1677.29
GS006		-23.71		-2.48		-8.12		-2669.09		-1.39		-489.00
GS007	267.77	23.16	22.11	1.49	61.83	2.70	15394.13	139.08	14.44	-0.16	4203.46	136.92
GS008	278.78	0.25	18.22	-0.88	38.37	-2.99	11111.46	-1653.18	23.33	2.14	3337.55	-71.11
EUSS11												
BM39	249.88	19.77	28.33	2.58	55.85	0.65	19113.60	40.05	9.67	0.75	5299.48	-120.62
BM23	220.22	55.23	21.89	5.43	46.58	12.18	12507.73	2104.14	19.11	1.35	3941.81	767.60
IS25547	238.88	59.89	23.11	5.74	47.64	12.44	15699.20	4106.63	18.33	5.19	4976.11	1387.35
IS9203		-21.79		-1.83		-3.71		-1050.17		-0.80		-229.25
P40		-39.11		-4.04		-10.07		-3617.82		-1.83		-1065.74
NYANGEZI	258.89	2.77	23.11	-0.38	52.10	-5.12	17576.53	1025.65	21.56	0.42	5333.74	-501.19
GS001	218.33	-5.28	23.67	1.07	72.28	6.69	22657.07	1997.14	10.11	-1.03	5916.27	539.21
GS002		0.17		-0.04		0.53		181.83		0.61		143.32
GS003	262.00	46.56	22.89	4.37	69.11	13.97	21049.60	4458.63	19.67	4.59	6195.79	1436.17
P23		-34.99		-3.55		-6.47		-2280.22		-1.84		-557.45
GS005		-16.60		-2.09		-4.31		-1481.37		-0.19		-355.76
GS006		-18.33		-2.23		-5.93		-2147.24		-0.89		-525.73
GS007		-60.72		-5.63		-15.72		-4470.44		-4.47		-1300.96
GS008	269.11	12.41	21.89	0.60	55.38	4.87	17905.07	1133.20	9.89	-1.84	4810.20	383.04

Continued next page.

Table 4.11 *Continue*

Genotypes	Agronomic and sugar traits of sweet sorghum											
	Height (cm)		Girth (mm)		Cane yield (t.ha^{-1})		Juice volume (l ha^{-1})		Brix		Ethanol volume (l ha^{-1})	
	Overal mean	SCA effects	Overal mean	SCA effects	Overal mean	SCA effects	Overal mean	SCA effects	Overal mean	SCA effects	Overal mean	SCA effects
EUSS10												
BM39	261.11	16.10	27.78	1.96	99.85	12.39	34730.67	4793.17	17.67	2.36	11257.19	1441.96
BM23		-25.59		-2.30		-6.27		-2517.68		-2.75		-969.66
IS25547		-27.15		-2.41		-6.36		-1579.01		-1.98		-694.68
IS9203	263.55	58.65	21.55	4.91	50.92	10.34	14784.00	3425.26	17.00	3.80	4470.87	837.71
P40		-46.52		-4.49		-12.99		-4070.39		-2.90		-1489.07
NYANGEZI	309.11	12.10	24.78	-0.27	83.65	2.48	26916.27	-5285.77	21.33	-0.73	9871.96	588.22
GS001	230.66	-8.58	18.67	-1.04	41.65	-6.44	15347.20	-892.05	17.89	0.50	4249.93	-439.74
GS002		-7.24		-0.48		-2.39		-270.74		-0.45		-280.01
GS003		-48.19		-3.70		-11.99		-3010.48		-3.04		-1052.42
P23		34.44		3.78		6.37		1768.90		1.17		425.07
GS005	230.55	-24.01	23.33	-2.54	47.28	-7.23	13505.47	-1933.94	12.22	-1.26	4217.55	-779.09
GS006	222.00	48.26	26.33	6.10	77.55	17.00	27948.80	6716.46	18.00	4.05	8028.65	1727.16
GS007	281.44	25.68	23.11	1.63	83.65	9.25	25355.73	3528.90	22.78	2.06	8390.74	1072.62
GS008	230.22	-7.96	18.00	-1.15	37.08	-4.15	13845.33	-672.61	16.11	-0.83	3766.87	-388.06
EUSS17												
BM39		-51.42		-6.02		-14.98		-6084.03		-3.35		-1805.94
BM23	178.44	-6.07	22.78	-1.02	53.62	-0.36	14455.47	3000.50	18.44	3.58	4334.06	979.52
IS25547		-7.63		-1.13		-0.45		-879.32		-1.80		-190.19
IS9203		-9.68		-1.00		-0.72		-803.05		-1.69		-148.08
P40	243.33	54.11	22.67	4.35	46.34	8.37	17822.93	2570.28	19.89	3.92	5029.49	691.93
NYANGEZI	201.44	-4.27	22.44	0.23	55.50	-1.00	17893.33	1378.37	20.22	-0.91	5309.92	-427.96
GS001	209.78	3.97	22.00	1.35	46.70	1.15	17494.40	525.04	16.11	0.09	5012.95	319.10
GS002		12.28		0.80		3.52		428.95		-0.27		224.49
GS003		-28.67		-2.42		-6.08		-2310.79		-2.85		-547.93
P23		40.93		3.58		8.76		3313.39		3.02		920.96
GS005	191.44	-4.49	18.89	-1.26	36.72	-1.32	16039.47	-1234.25	17.22	-1.08	4191.73	-274.59
GS006		-6.22		-1.39		-2.94		-1900.12		-1.77		-444.56
GS007	201.33	11.87	21.89	2.50	49.52	3.78	15077.33	802.46	23.78	2.57	4737.22	359.28
GS008	181.44	-4.70	21.89	1.43	38.60	2.27	17341.87	1192.59	19.67	0.54	4449.51	343.98

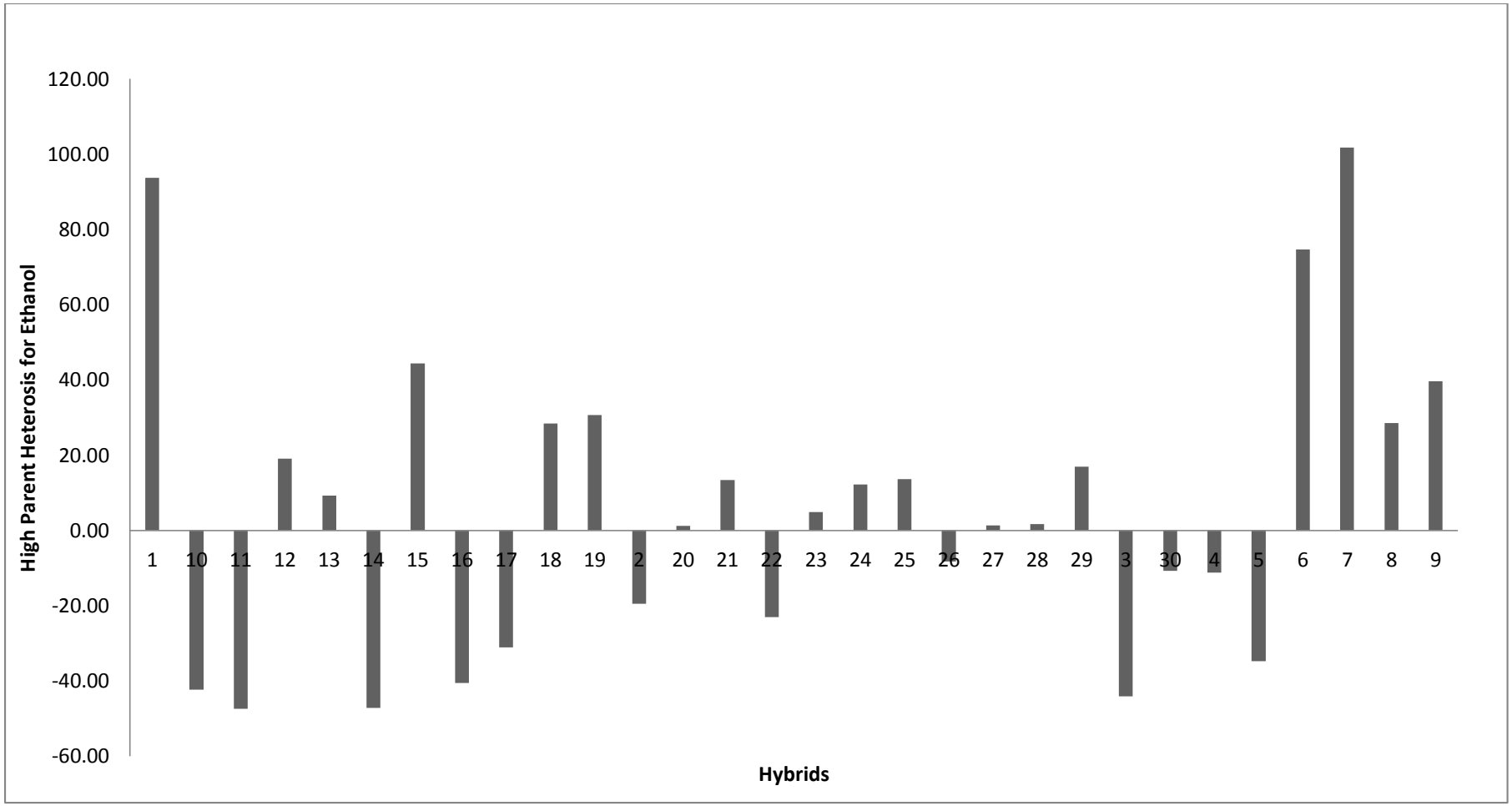

Figure 4.1 High parent heterosis for ethanol production of 30 hybrids planted across three environments in western part of Kenya.

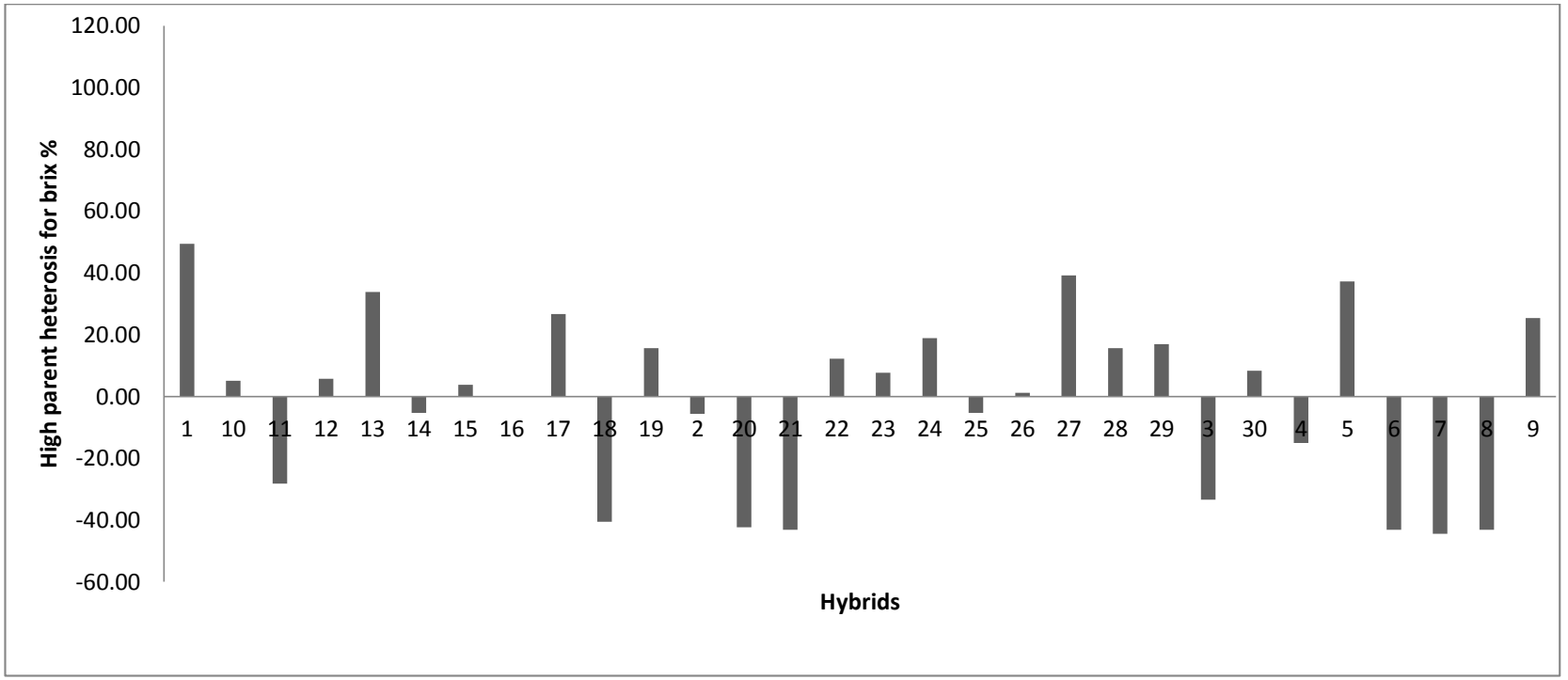

Figure 4.2 High parent heterosis for brix % of 30 hybrids planted across three environments in western part of Kenya.

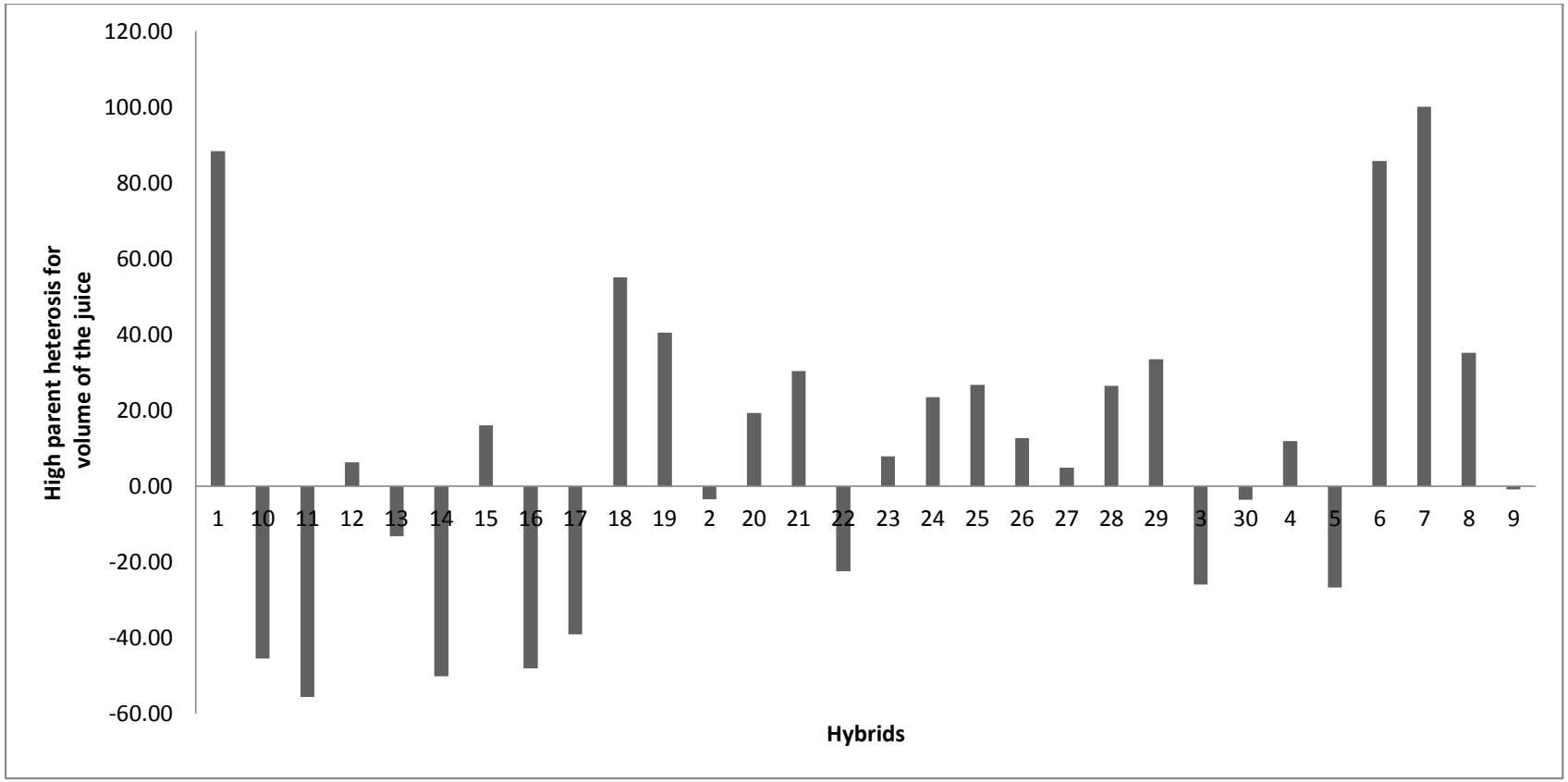

Figure 4.3 High parent heterosis for volume of the juice of 30 hybrids planted across three environments in western part of Kenya.

Table 4.12 General combining ability (GCA) effects of lines and testers for agronomic and sugar traits of sweet sorghum

Geonotype	Overall height (cm)	Cane yield (t. ha^{-1})	Girth (mm)	Volume of juice (L ha^{-1})	Brix	Ethanol Volume (L ha^{-1})
BM39	22.63	8.47	2.93	3319.04	0.13	750.83
BM23	-22.71	-6.15	-2.07	-947.01	-0.66	-589.95
IS25547	-21.15	-6.06	-1.96	-1885.67	-1.43	2885.07
IS9203	-19.10	-5.79	-2.09	-1961.94	-1.54	-907.03
P40	-1.78	0.57	0.12	605.70	-0.51	-70.54
NYANGEZI	42.63	12.99	4.16	1821.08	4.43	1142.82
GS001	37.17	7.90	2.90	2543.10	2.06	296.77
GS002	-41.06	-10.03	-3.89	-3193.94	-2.95	-1279.61
GS003	-0.12	-0.43	-0.67	-454.21	-0.37	-507.19
P23	-5.89	-3.03	-0.37	-731.90	-0.50	-578.83
GS005	-24.29	-5.19	-1.83	-1530.74	-2.15	-780.53
GS006	-22.56	-3.57	-1.70	-864.87	-1.45	-610.55
GS007	19.83	6.22	1.70	1458.32	2.13	164.68
GS008	36.40	4.09	2.78	1823.04	2.80	84.07
SS04	5.21	1.66	0.28	340.03	-0.11	87.80
EUSS11	-0.17	-0.53	0.04	-181.83	-0.61	-143.32
EUSS10	7.24	2.39	0.48	270.74	0.45	280.01
EUSS17	-12.28	-3.52	-0.80	-428.95	0.27	-224.49

Table 4.13 Correlation coefficients for Brix, cane yield, juice volume and ethanol volume.

Traits	Cane yield	Volume of Juice
Brix	0.01825	-0.028850
Volume of Ethanol	0.91778***	0.96995***

*** Significant at $p \leq 0.001$

Brix had the lowest number of hybrids with positive high parent heterosis with their percent relatively low (Figure 4.2). Like ethanol production, juice volume production exhibited high negative heterosis with 18 hybrids showing positive heterosis (Figure 4.3). This indicates that there is a correlation between the juice volume production and ethanol volume. The genotypes exhibited positive mid parent, better parent and standard heterosis for the two important traits.

The GCA effects of both the male and the female lines were significant ($p \leq 0.001$) (Table 4.2). The GCA effects of both lines and testers accounted for 55.19% of the treatment sum of squares. SCA effects were also significant ($p \leq 0.001$) for all the traits that were investigated and accounted for 29.51% of the treatment sum of squares. GCA effects greatly varied among the genotypes and for the traits (Table 4.10). GS008 had the highest positive GCA effect for height, NYANGEZI had the highest GCA effect on cane yield, girth and brix. BM39 had the highest GCA effect for volume of the juice. IS25547 had the highest GCA for ethanol volume. BM39 × EUSS10 had the highest SCA (Table 4.10) while line GS008 and

tester EUSS10 had the highest GCA (Table 4.11). Correlation analysis of cane yield, volume of the juice, volume of ethanol, and brix revealed that there was no relationship between cane yield and brix. The correlation between volume of the juice and brix was also found to be negative. However, there was a high correlation between cane yield and volume of ethanol as well as that of volume of juice and volume of ethanol produced.

4.12 Discussion

Both climatic and soil conditions at Kendu-Bay and Kibos favor growth and development of sorghum crop with temperature, rainfall and altitude almost similar at the sites. Sandy soil at Kendu-Bay Seka Farm, made the plants to be more prone to drought due to high drainage. There was higher rainfall during the first season at Kibos (March-August, 2016) than during the second season (September 2016-January, 2017). Cosequently, there was better performance at during the first season at Kibos than during the second. Sorghum is known to exhibit high drought tolerance as compared to other crops such as maize. However, this study has revealed that sweet sorghum performs better when the conditions are favourable than when they are not as observed between the first and second seasons at Kibos. Furthermore, harvesting sweet sorghum canes require relatively moist conditions as opposed to dry weather which is a requirement during harvesting of grain sorghum. Sweet sorghum is therefore more sensitive to drought than grain sorghum. Significant variations due to the environment were observed in all the traits that were investigated. This is because all the three environments varied considerably in the growth factors of sorghum such as soil, temperature and rainfall. Significant differences were also seen due to the genotypes. This observation indicates that each of the genotypes that were evaluated were unique and exhibited unique characteristics.

Heterosis is an important genetic phenomenon among many species of organisms. Sorghum species exhibit high variability which forms the basis of heterosis. Most of the hybrids exhibited positive mid parent, high parent and standard heterosis for height and girth. This observation is similar to what was observed by Crow (1998) and Duvick (2001) when they developed maize hybrids. Since both height and girth contribute to cane yield, the same also exhibited all the three types of heterosis. All the males were sweet sorghum and had contrasting characteristics with those of the females. SS04 was used as the standard parent. This is because it is a variety which is currently cultivated as sweet sorghum and was used as a check in this study. None of the hybrids exhibited similar percent heterosis among the traits that were investigated. For example, genotype GS006 × EUSS10 exhibited positive mid parent, better

parent and standard heterosis for girth and height but not for brix. Similarly, each of the genotypes exhibited varied range of heterosis for the traits that were investigated. This is an indication that the agronomic traits are controlled by poly genes. This phenotypic observation corroborates what was observed by Meyer *et al*. (2010) and Riedelsheiner *et al*. (2012).

Significant standard heterosis was observed among only nine hybrids for brix with a range (-49.56% to 38.75%). This study corroborates previous studies by Pfeiffer *et al*. (2010) who also observed that heterosis levels were higher for height than for brix levels while looking at ethanol related traits in sorghum. As compared to all the other traits, brix and juice volume are important for ethanol production. This study has demonstrated that heterosis of the agronomic tratits vary with the environment. It is most unlikely to realize significant yield improvement for sweet sorghum by making single crosses. This means that a cross between a sweet sorghum variety and a non sweet sorghum variety do not necessarily result to a sweet sorghum hybrid. This is evidenced by the fact that all the 30 hybrids responded differently and exhibited varied mid parent, high parent and standard heterosis. For example, in one particular cross, there could be high heterosis in juice volume but low heterosis in brix, yet both are important for ethanol production from sweet sorghum. Although individual traits for the genotypes may be varied in standard heterosis, the overall ethanol yield which is the ultimate purpose of growing sweet sorghum is seen to be positive. This indicates that it is possible to increase ethanol production from sweet sorghum by developing hybrids with higher ethanol potential.

Both juice volume and brix correlated well with ethanol production. This indicates that successful development of sweet sorghum hybrids for ethanol production can be achieved by increasing pressure on selection for high brix and high juice volume. High juice volume is accompanied by low brix making sweet sorghum improvement for ethanol production a complicated task. This study has revealed facts similar to what was observed by Reddy *et al*. (2013) that improvement of sweet sorghum can better be achieved by targeting the stalk yield which also has high correlation with volume of the juice.

Over-dominance, dominance and epistatic theories that have been used to explain heterosis have been manifested in this study. Development of an elite hybrid for sweet sorghum requires screening for the desired traits such as height, girth, juice volume and brix which is an indicator of sugar level. Inbred lines exhibiting these superior traits can be crossed in all possible combinations including reciprocal crosses. Three way cross, four way cross and back

cross breeding programmes for gene pyramiding towards the realization of an elite sweet sorghum hybrid to which this study has offered guideline.

The GCA of the lines and the testers and the SCA of the hybrids were seen to be significant in all the traits under investigation. Baker's ratios which reflect heritability in narrow sense to that of heritability in broad sense is high and closer to unity meaning that the parents contributed significantly to the traits in the offsprings. This is consistent with the findings by Anyanga *et al*. (2016) while working on sesame. Significant GCA effects of both the lines and the testers suggest that both the agronomic and sugar traits that were investigated are controlled by additive gene action. This implies that ethanol related traits such as volume of the juice, cane yield, brix and volume of ethanol traits have high heritability and can be easily selected for in breeding programmes. Large negative GCA effect values indicate that the genotype has negative contribution to the trait while large positive GCA effects indicate that the genotype has positive contribution for the trait in question. Most of the lines exhibited high positive GCA effects for height. For example, GS008 had the highest positive GCA effect for height. This means that making a cross with the testers will result to offsprings which are taller than the parents. NYANGEZI had the highest GCA effect on cane yield, girth and brix. This suggests that for improvement of brix, cane yield and girth can be selected. BM39 had the highest GCA effect for volume of the juice and IS25547 had the highest GCA for ethanol volume. A sweet sorghum improvement program can therefore be started by identification of cultivars with high GCA for the traits of intrest.

The SCA was seen to be significant in all the traits suggesting that both the agronomic and the sugar traits are affected by environment and are due to non additive gene action. For successful development of sweet soghum hybrids, it is necessary to conduct multilocational trials before rolling out a variety that can perform well across the locations as suggested by Spague and Tatum, (1942). They observed that significant SCA indicates that the observed phenotypic variations are mostly due to non additive gene action which is accompanied with low to no heritability hence not useful for a plant breeder. High correlation between cane yield and juice volume and consequently the volume of ethanol suggests that high biomas in terms of the cane yield can significantly increase ethanol production. High cane yield can be realized from genotypes with large girth, and height values. Similarly, high juice volume contributes to ethanol volume. A negative correlation was seen between volume of juice between brix indicating that canes with high juice volume had low brix. This could be due to the fact that

higher juice volume indicates high amount of water molecules which could probably dilute the solutes in the cane making it to have low brix due to the inverse relationships. Breeding for high ethanol production potential should focus on brix, cane yield and volume of juice due to their high correlation coefficient with volume of ethanol.

4.13 Conclusion

Even though manifestation of heterosis for important sugar traits appears complex, its exploitation remains important towards the development of elite hybrids. All the hybrids exhibited varied mid parent, high parent and standard heterosis validating the manifestation of heterosis in sweet sorghum. Agronomic traits such as height and girth show positive heterosis for more genotypes as compared to brix where majority of the genotypes exhibited negative heterosis. The GCA effects accounted for more than 50% of the treatment sum of squares suggesting that the additive gene action was more important that non additive gene action. Volume of juice, brix and cane yield have very high correlation with ethanol yield hence should be the main focus in sweet sorghum hybrid development program.

4.14 Recommendations

It is necessary to conduct multilocational trials before roling out hybrids as suggested by the high level of significance of SCA. For successful development of elite sweet sorghum hybrids, gene pyramiding by complex crosses and backcrosses is recommended since many traits which are controlled by many genes are involved. A further investigation should be done by crossing sweet sorghum varieties with other sweet sorghum varieties of diverse origin. It would be useful to investigate the mechanisms behind lack of compatibility of GS002 to any of the testers.

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CHAPTER FIVE

EVALUATION OF SWEET SORGHUM [*Sorghum bicolor* (L.) MOENCH] HYBRIDS USING GGE BIPLOT ANALYSIS IN WESTERN KENYA

5.1 Abstract

Improvement of sweet sorghum (*Sorghum bicolor* L.) for agronomic and ethanol related traits depends on the understanding of the influence of genotypes (G), environment (E) and their interactions (GE). The objective of this study was to select the best performing sweet sorghum hybrid(s) with high brix, juice and ethanol volume by using GGE biplot and Principal Component Analyses (PCA). Thirty sweet sorghum hybrids were developed from crosses between 4 sweet sorghum lines and 14 grain sorghum cultivars that were grown in three environments in Kenya in 2016. In a Randomized Complete Block Design, the agronomic variables; height, girth, cane yield and ethanol related variables; brix, volume of juice and ethanol were investigated. There was significant difference ($p \leq 0.05$) in both agronomic and ethanol related traits. The highest cane yield (99.85 t ha^{-1}), volume of juice (34730 L ha^{-1}) and ethanol (11257 L ha^{-1}) were obtained from BM39 × EUSS10 while NYANGEZI × SS04 had the highest brix (25.44). Hybrids GS001 × EUSS10, NYANGEZI × SS04 and IS9203 × EUSS10 were selected after conducting GGE biplot analysis and PCA. Sweet sorghum for ethanol production performed better during the first season at Kibos than in the other environments.

Key Words*: Environment, Genotypes, GGE Biplots, Principal Component Analysis*

5.2 Introduction

Demand for energy and growing concerns over climate change in the global environment, the use of biofuel such as bioethanol produced from sweet sorghum has become increasingly attractive (Gomez *et al*., 2008). Globally, sorghum has been used as a multipurpose crop from which excellent food, fodder and fuel has been obtained (Zegada-Lizarazu and Monti, 2012). Industrial bioethanol is currently produced in USA from sweet sorghum with other countries such as Australia and India following closely (Balat and Balat, 2009). Kenya has high sweet sorghum potential but productivity is low. Studies indicate that the low productivity of sorghum in Kenya is due to lack of improved cultivars (Muui *et al.,* 2013).

Environment influences crop productivity in various ways (Black *et al*., 2011). In some cases, flowering time, height, girth and even kernel quality vary depending on the environment (Ready *et al.,* 2003; Rai *et al*., 2006). Major, (1980) studied 9 different crops and observed that their flowering time was affected by photoperiod. Plant breeding exercises yield numerous hybrids most of which do not end up in the farmers' fields (Morris, and Heisey, 2003). This is because hybrids have to be tested in multilocations for the assessment of the effect of G × E interactions (Yan, 2001). In most cases, genotypes found not to be able to perfom well in multilocations are regarded as inferior and are discarded (Yan and Hunt, 1998). Good performance of hybrids across environments is an important aspect of germplasm evaluation (Fan *et al.*, 2007). Both agronomic and ethanol related traits of sweet sorghum vary considerably across environments (Shoemaker and Bransby, 2010). Riedelsheimer *et al*. (2012) observed that the performance of hybrids across environments varies with the traits under investigation. Due to the challenge encountered in phenotypic selection of important traits, Jordan *et al*. (2003) employed the use of molecular markers for the prediction of hybrid performance. Almodares and Hadi (2009) observed that brix from sweet sorghum do not vary with the environment but highly varied from one genotype to another. However, other agronomic traits such as height, cane yield and girth have been known to vary considerably with the environment since they are controlled by minor genes.

Kenya has five agroecological zones most of which are favourable for sorghum growing (Evenson and Mwabu, 2001). Finding a hybrid that can perform well across the ecological zones is an important ingredient in sweet sorghum hybrid development. Significant G × E interaction indicates that the traits are controlled by minor genes (Frascaroli *et al*., 2007) a condition which necessitates further evaluation before hybrid varieties can be released to the farmers. Observed phenotypic expression is as a result of genotype (G) and environment (E). Selection of best performing genotype is done with the consideration of genotype (G) and interaction between genotype and environment (GE). For effective selection of best genotypes, G and GE have been combined to form GGE which is used in analysis of multienvironment trials (Yan *et al*., 2000). Although some studies have suggested that single biplots are able to provide information on genotype and environment under investigation, Yan (2002) demonstrated that multiple biplots are required for consideration of various parameters under investigation. Selection of the best performing genotypes can be performed by univariate or multivariate statistical analysis tools (Flores *et al*., 1998). However, when there is a large

number of desirable traits involved like in the case of sweet sorghum where cane yield, volume of the juice, volume of ethanol and brix are involved, principal component analysis (PCA) is suitable for selection of the most desirable genotype (Sousa *et al.*, 2015). The objective of this study was to select elite sweet sorghum hybrids for ethanol production in Kenya.

5. 3 Materials and Methods

5.3.1 Experimental sites

This study was carried out at Kendu-Bay (0.35°13′07′′S, 34°07′44′′) and Kenya Agriculture and Livestock Research Organization (KALRO), Kibos (0°04′06′′S, 34°49′03′′E) station. KALRO-Kibos lies at an altitude of 1173 m a.s.l, about 8 km East of Kisumu City. This area experiences bimodal rainfall with mean precipitation of 1323 mm per year, the onset of long rains is usually in March (Hansen *et al*., 2011). The short rains usually commence in August with a drop in September, reaching the peak of 374.4 mm in December (SRI weather station no. 200). Generally, high precipitation is often experienced during the long rainy season. In general, the average maximum temperature of this location is 30 °C with a minimum of 15.5°C, while the soils are heavy black clay. There was considerable difference in rainfall and temperature during first and second season at Kibos in 2016 with the second season characterized by low rainfall and high temperatures. Kendu-Bay has an altitude of 1132 m a.s.l, and the farm was about 5 km east of Kendu-Bay Township. The area experiences an average rainfall of about 1200 mm per annum with the onset of long rains in March while the short rains commence in September. The average temperature in this area is 27 °C with a minimum of 17°C. The soil in this area is predominantly sandy and drains very easily. The study was conducted between September and December, 2016.

5.3.2 Genotypes

Genotypes included 30 hybrids (Table 5.1) which were developed by crosses in a line by tester mating design as explained in chapter 4.

5.3.3 Experimental procedure

KALRO-Kibos experimental field was under maize cultivation in the previous season. It was disc ploughed and harrowed twice to achieve a fine tilth suitable for planting sorghum. Thirty sweet sorghum genotypes (Table 5.1) were planted and tended to maturity. The experiment was conducted in Randomized Completely Block Design (RCBD) with three replications. Within the replicates, sorghum genotypes were planted at seeding rate of 10 kg

ha^{-1} in an experimental unit measuring 5 × 3 m with inter row spacing of 60 cm and intra row spacing of 15 cm. NPK fertilizer of the ratio 20:10:10 respectively was applied to supply nutrients at the rate of 30 kg ha^{-1} of P, 10 kg ha^{-1} of K and 40 kg ha^{-1} N. At growth stage, six weeks after planting, additional 40 kg ha^{-1} N was supplied to each plot as a top dresser. Infestation by shoot fly (*Atherigona soccota*) on young seedlings was minimized by spraying a systemic insecticide Bulldock (*beta-cyfluthrin* 25 g l^{-1}) at 25 g ha^{-1}at intervals of 14 days for one month. Within the experimental plots, weed growth was restricted by manual hand weeding. Weeding and inter cultivation operations were done twice between 5-leaf stage and panicle emergence. From booting stage to the end of anthesis, a second dose of Bulldock was applied at 25 g ha^{-1} to control sorghum midge (*Contarinia sorghicola*). After heading, the panicles were covered using paper bags to protect them from damage by birds. Harvesting of sweet sorghum canes was done at hard dough stage or 104 days after planting.

Table 5.1: Plant genotypes, hybrid code and grain colour.

Sr.No.	Hybrid Code	Genotype	Grain Color
1	1	GS001 × EUSS10	Brown
2	10	GS005 × EUSS10	Brown
3	11	GS006 × EUSS10	Red
4	12	GS007 × EUSS10	Brown
5	13	GS008 × EUSS10	Red
6	14	BM39 × EUSS10	Brown
7	15	IS9203 × EUSS10	Brown
8	16	NYANGEZI × EUSS10	Brown
9	17	GS001 × EUSS11	Red
10	18	GS003 × EUSS11	Cream
11	19	GS008 × EUSS11	Cream
12	2	BM39 × EUSS11	Brown
13	20	BM23 × EUSS11	Dark brown
14	21	IS25547 × EUSS11	Cream
15	22	NYANGEZI × EUSS11	Cream
16	23	GS001 × EUSS17	Brown
17	24	GS005 × EUSS17	Brown
18	25	GS007 × EUSS17	Red
19	26	GS008 × EUSS17	Red
20	27	P40 × EUSS17	Brown
21	28	NYANGEZI × EUSS17	Cream
22	29	BM23 × EUSS17	White
23	3	GS007 × SS04	White
24	30	GS008 × SS04	Brown
25	4	BM39 × SS04	Brown
26	5	P40 × SS04	Brown
27	6	GS001 × SS04	Red
28	7	GS003 × SS04	Red
29	8	P23 × SS04	Cream
30	9	NYANGEZI × SS04	Cream

5.4 Data collection

Measurements of plant height were taken from the base of the plant to the tip of the panicle using a graduated meter rule. Days to flowering was estimated by counting the days taken to panicle emergence. Five plants per row per plot were harvested by cutting the plant at the base and stripping off the leaves. Stalk juice was extracted in a three-roller crusher (Fuan Liyuan, China, type YC 80B-4) and strained through a sieve into a juice container. The stripped stalks were passed through the mill once and all extractable juice removed from stalks and measured immediately. The extracted juice was sieved to remove large solids and volumes computed. Juice yield was determined by measuring the volume of the juice extracted from 5 plants, getting the average and multiplying by the number of plants per hectare. Juice brix was determined using a digital hand-held refractometer (Model: Standard line Alla made in France). The fresh juice (100 ml) was transferred to conical flasks which were then prepared for the process of fertmentation.

5.5 Fermentation of juice

From each plot, juice was sampled and fermentation carried out in 250 ml conical flasks with working volume of 100 ml. Fermentation of the juice was done by adding approximately 1.5 g yeast (*Saccharomyces cerevisiae*) to 100 ml of juice sample and incubating at 35^{o}C for 4 days. The fermented samples were then distilled by heating in a conical flask connected to a Liebig condenser and the ethanol content in the distillates was determined by measuring the refractive indices on a hand held refractometer (Model: Standard line Alla made in France). Determinations were based on a standard curve drawn by measuring the refractive indices of absolute ethanol solutions (0, 5, 10, 15, 20, 25 and 30%) in distilled water.

5.6 Data analyses

Data were analyzed using Proc GLM of SAS (Statistical Analysis System) Version 9.1. For every trait under study, error variances were computed and means by genotype and location computed. During the analysis, both hypothesis on environment and that on genotype were tested using genotype × environment as an error term while the interaction between environment × genotype was tested using the random error. They were compared and declared significant or not at $p \leq 0.05$. Analysis of variance was done following the procedure for RCBD design for three environments using statistical model:

$$Y_{ijk}=\mu + E_i + R_{ji} + G_k + + GE_{ik} + \varepsilon_{ijk} \qquad (5.1)$$

where Y_{ijk} is observation, μ is the overall mean, E_i is the effect due to environment, R_{ji} is the effect due to replicate within location, G_k is effect due to k^{th} genotype, GE_{ki} is the effect due to G × E interaction and is due to k^{th} genotype and i^{th} location, and ε_{ijk} is the random error component as outlined by Gomez and Gomez (1984).

Means of genotypes were subjected to GGE biplot analysis and Principal Component Analysis (PCA) using GeneStat 15th edition. For the GGE biplot analysis, comparison biplots were constructed for height, volume of ethanol, volume of the juice and brix. Ethanol related traits were compared with the ideal genotype and ranking of plots constructed for performance and stability tests of the genotypes. GGE biplot analysis was also conducted to know the traits of the genotypes that performed best in various environments. Finally, PCA was conducted to obtain scores and Factorial Component Analysis was also done to show how the genotypes were performing in each location.

5.7 Results

5.7.1 Environmental conditions

The growing period of the first season for experiments conducted at KALRO-Kibos commenced from 14th April 2016 to July 2016. Planting was done on 14th April when the seedbed was saturated with moisture after receiving 471.4 mm of rainfall. The highest rainfall was received in the month of April and reduced systematically through May (254.9 mm) and June (52 mm). Relatively low temperatures were experienced with April mean at 16.9ºC, May 17ºC and June at 16ºC. Harvesting was done in July and during this period low temperatures (15ºC) and rainfall (4 mm) were experienced. During the second season for the experiments conducted at Kibos, the growing season commenced on 26th September 2016 to 26th January 2017. At Kendu-Bay, the growing commenced on 5th September to December 2016. Unlike in the first season at Kibos, the second season was characterized by low rainfall. Supplemental irrigation was done for the three environments. Harvesting of the cane was done during dry season when the moisture in the soil was generally very low. Although there were significant variations in the performance of sweet sorghum genotypes in the three environments, the first season in Kibos was seen to be better than the second season and Kendu Bay site (Figure 5.1).

5.7.2 Analysis of variance of sweet sorghum traits

There were significant ($p \leq 0.001$) differences due to genotype for height of the plant, girth, volume of the juice, brix, volume of ethanol (Table 5.2). Significant ($p \leq 0.001$) effects

due to genotype and genotype × environment interaction were detected for height, cane yield, girth, juice volume, brix, ethanol volume and days to flowering. Although significant effects due to genotype and environment were observed, no significant effects due to genotype × environment interactions were observed for days to flowering. Significant ($p \leq 0.001$) difference was observed among the environments for most of the sweet sorghum traits. Cane yield, girth, overall height, days to flowering, brix, and volume of ethanol were significantly different in all the three environments. Interaction between environment and stage was significant ($p \leq 0.001$) for days to flowering, volume of ethanol, girth, cane yield and brix. All the traits showed significant difference among the genotypes except for brix which did not show significant difference. For interaction between location and genotype, there was significant difference ($p \leq 0.001$) for cane yield, days to flowering, volume of ethanol, height and brix. The interaction between genotype and environment showed significant ($p \leq 0.001$) difference for all the traits (Table 5.2).

Table 5.2 Analysis of variance for height, cane yield, girth, juice volume, brix, ethanol volume and flowering time for sweet sorghum hybrids grown in three environments in Kenya in 2016.

Sources of Variation	Df	Expected mean squares	Overall height (cm)	Cane yield (ton ha^{-1})	Girth (mm)	Volume of juice (l ha^{-1})	Brix	Ethanol Volume (l ha^{-1})	Days to flowering
Environment	2	$\sigma^2_E+3\sigma^2_{GE}+48\sigma^2_{R(L)}+144\sigma^2_L$	24013.00***	3245.32***	11.45	240769040.00***	0.31	30162835***	871.20
Replication (Environment)	6	$\sigma^2_E+48\sigma^2_{R(L)}$	176.76	44.30	1.64	390065.00	0.98	350184	13.92
Genotype	47	$\sigma^2_E+3\sigma^2_{GE}+48\sigma^2_{R(L)}+\sigma^2_G$	10000.78***	2816.61***	131.90***	394250346.00***	233.23***	42859195***	3.21***
Genotype*Environment	94	$\sigma^2_E+3\sigma^2_{GE}$	507.87***	128.68***	10.02***	14083666.00***	1.81***	998560***	6.74***
Error	282		45.43	10.33	1.67	482914.00	0.57	4650.13	0.42
CV%			2.92	6.48	6.29	4.46	5.10	7.76	0.79
R^2			0.97	0.98	0.93	0.99	0.98	0.98	0.99

***Significant difference at $p \leq 0.001$; CV= coefficient of variation
Test H = Environment, E= Genotype; Test H = Genotype, E= Environment × Genotype

GS005 × EUSS17, NYANGEZI × EUSS10, GS001 × EUSS10 took the least (66.33, 68.11 and 72.89) number of days respectively to flower. Hybrids GS001 × EUSS11, P23 × SS04 and NYANGEZI × SS04 were the latest and took (91.11, 90.22, 89.44) days respectively (Table 5.3). Genotype NYANGEZI × EUSS17 attained the highest mean height of 309.11 cm, followed by GS007 × EUSS10 (281.44 cm) and GS005 × EUSS17 (191.44 cm) was the shortest. BM39 × EUSS10 had the highest girth (27.78 mm) while GS003 × SS04 had the lowest (15.77 mm). For cane yield, juice volume as well as ethanol production, BM39 × EUSS10 was the best nominaly among the hybrids (Table 5.3).

Sweet sorghum hybrids grown in Kibos during the first season were taller than those grown in the second season while Kendu-Bay had the shortest plants (Table 5.4). There was no significant difference in girth for the plants grown at Kendu-Bay and Kibos during the first season. The first season at Kibos produced the highest cane yield and juice volume followed by the second season. Both cane yield and volume of juice were lower at Kendu-Bay (Table 5.4). Suprisingly, there was no significant difference in the brix for all the three environments. Ethanol volume was highest at Kibos in the first season (Table 5.4). This was followed by Kendu-Bay and lastly by Kibos during the second season. The crops flowered faster at Kendu-Bay than at Kibos during the first and the second season (Table 5.4).

5.7.3 GGE Biplot analysis

The first two principal components were used to obtain GGE biplots by GGE software using the model:

$$Y_{ij} - \mu - \beta = \sum_{i=1}^{k} \lambda_l \xi_{il} \eta_{lj} + \varepsilon_{ij} \qquad (5.2)$$

Where Y_{ij} = the mean yield of genotypes i(=1,2…n) in the environment, μ= the grand mean, β=the main effect of environment j, ($\mu+\beta_j$) is the mean yield of environment j, λ_l = the singular value of the l^{th} principal component (PC), ξ_{il} = eigenvector of genotype *i* for PC 1 and 2, $\boldsymbol{\eta}_{lj}$ = the eigenvector of the environment *j* for PC 1 and 2, $\boldsymbol{\varepsilon}_{ij}$ = the residual associated with genotype *i* in the environment *j*.

In order to generate the biplots, the singular values can be partisionned into the genotype and environment eigenvectors transforming the above model (5.2) to:

$$Y_{ij} - \mu - \beta = g_{ij} e_{lj} + g_{i2} e_{2j} + \varepsilon_{ij} \qquad (5.3)$$

Where g_{ij} and e_{lj} are PC 1 and PC2 scores for genotype *i* and environment *j* respectively in a biplot, genotype *i* is displayed as a point displayed as a point defined by all g_i values and environment *j* is displayed as a point defined by all e_j values.

The PC 1 and 2 explained 99% of the total GGE variation (Figure 5.1-5.5). This suggests that biplots of PC1 and PC2 can be used to approximate the the environment centered data. GGE biplot analysis based on multienvironment trials was conducted to show the performance of genotypes per environments, the interrelationships among the test environments and the ranking of the means based on performance and stability. Figure 5.1 illustrates GGE biplot analysis of sweet sorghum traits relative to the ideal environment in which brix is seen not to be affected by the environment (Figure 5.1 c). Environmental effect was greater for height, volume of ethanol and juice (Figure 5.1 a, b, d) respectively. GGE comparison biplot analysis was done for sweet sorghum genotype traits relative to the ideal genotype. The patterns illustrate that there was more variability in height of the plant relevant to the ideal genotype. Most of the genotypes were concentrated close to the AEC (Figure 5.2). Height of the plant (Figure 5.2 a) volume of the juice (Figure 5.2 b) and ethanol (Figure 5.2 c) showed high variability since many genotypes lie away from AEC.

Table 5.3 Means for agronomic and ethanol related traits for sweet sorghum hybrids, male and female parents grown in three environments in Kenya in 2016.

Genotypes	Agronomic and sugar traits of sweet sorghum						
	Height (cm)	Girth (mm)	Cane Yield (t ha^{-1})	Juice Volume (L ha^{-1})	Brix	Ethanol Volume (L ha^{-1})	Days to flowering
GS001 × EUSS10	230.67 lnm	18.67 nmo	41.65 rq	15347.2 lm	17.88 ijk	4249.9 nml	68.11 z
GS005 × EUSS10	230.56 lnm	23.33 ef	47.28 nmo	13505.1 p	12.22 q	4217.5 lnm	78.33 st
GS006 × EUSS10	222.00 opq	26.33 b	77.56 d	27948.8 b	18.00 jl	8028.7 e	75.33 u
GS007 × EUSS10	281.44 b	23.11 efg	83.65 bc	25355.7 d	22.78 c	8390.7 d	74.11 u
GS008 × EUSS10	230.22 lnm	18.00 op	37.07 tu	13845.3 no	16.11 n	3766.9 o	78.39 s
BM39 × EUSS10	261.11 e	27.78 a	99.85 a	34730.7 a	17.67 jklm	11257.2 a	74.33 v
IS9203 × EUSS10	263.56 cde	21.56 k	50.92 kl	14784.0 mn	17.00 m	4470.9 kl	74.05 v
NYANGEZI × EUSS10	258.89 ef	23.11 gef	52.09 ijk	17576.5 j	21.55 d	5353.7 gh	72.89 w
GS001 × EUSS11	218.33 rq	23.67 d	72.28 e	22657.1 f	10.11 tu	5916.8 f	91.11 f
GS003 × EUSS11	262.00 ed	22.88 efgh	69.11 f	21049.6 g	19.67 ef	6195.8 f	80.56 nm
GS008 × EUSS11	269.11 c	21.89 jk	55.38 hi	17905.1 j	9.81 uv	4810.2 j	77.33 t
BM39 × EUSS11	249.89 gh	28.33 a	55.85 hi	19113.6 i	9.67 uvw	5299.2 ghi	77.56 t
BM23 × EUSS11	220.22 pq	21.89 jk	46.58 mno	12507.7 r	19.11 fg	3941.8 no	75.11 u
IS25547 × EUSS11	238.89 kj	23.11 efg	47.63 mno	15699.2 kl	18.33 hij	4976.1 j	86.33 i
NYANGEZI × EUSS11	201.44 t	22.44 ghij	55.49 hi	17893.3 j	20.22 e	5309.9 ghi	82.89 k
GS001 × EUSS17	209.78 s	22.00 ijk	46.69 mno	17494.4 j	16.11 n	5012.7 ij	79.22 pq
GS005 × EUSS17	191.44 u	18.89 lm	36.73 tu	16039.5 k	17.22 klm	4191.7 lmn	66.33 aa
GS007 × EUSS17	201.33 t	21.89 jk	49.52 lm	15077.3 lmn	23.7 b	4737.2 jk	77.11 t
GS008 × EUSS17	181.44 wx	21.89 jk	38.60 st	17341.9 j	19.67 ef	4449.5 kl	75.33 u
P40 × EUSS17	243.33 ij	22.67 fghij	46.35 no	17822.9 j	19.89 e	5029.5 hij	79.39 p
NYANGEZI × EUSS17	309.11 a	24.78 d	83.66 bc	26916.3 c	21.33 d	3073.2 pq	79.11 rq
BM23 × EUSS17	178.44 wx	22.78 efgh	53.62 ijk	14455.5 no	18.44 ghi	4334.1 lm	77.33 t
GS007 × SS04	267.78 cd	22.11 hijk	61.83 g	15394.1 lm	14.44 o	4203.5 lmn	80.22 no
GS008 × SS04	278.78 b	18.22 mno	38.37 st	11111.5 t	23.35 bc	3337.5 p	74.33 v
BM39 × SS04	253.33 fg	25.78 bc	66.29 f	24311.5 f	9.67 uvw	7808.5 e	82.44 k
P40 × SS04	228.00 mno	25.44 cd	80.84 c	27772.8 b	9.44 uvw	9479.2 b	77.17 t
GS001 × SS04	280.0 b	17.11 q	54.56 ij	13505.1 p	16.05 n	3736.8 o	75.22 u
GS003 × SS04	229.33 lmn	15.77 r	46.11 op	11827.2 s	11.33 rs	3073.2 pq	82.83 k
P23 × SS04	201.22 t	24.67 d	58.08 h	19958.4 h	9.67 uvw	5988.9 f	90.22 g
NYANGEZI × SS04	234.89 kl	26.22 cb	84.95 b	24710.4 e	25.44 a	8553.5 c	89.94 g
LSD	21.19	2.4	10.67	3582	1.28	935	1.52

Means with the same letters along the same column are not significantly different.

However, brix (Figure 5.2 d) did not show much variability and most of the genotypes were lying along AEC. Ranking of the genotypes depending on performance and stability revealed interesting results since only few genotypes were found on the extreme right and closer to AEC line (Figure 5.3). It can be seen that genotype 15 is in high ranking for juice volume (Figure 5.3 c) and ethanol volume (Figure 5.3 d). In determining the performance across environments, it can be seen that each of the three locations lie on the same sector of the six sectors divided by the rays. This indicates that all the three environments do not varry significantly (Figure 5.4). The genotypes at the vertex of the polygons is among the best for trait under investigation. Genotype 1 is the best in brix (Figure 5.4 d) and genotype 15 is the best in ethanol volume (Figure 5.4 c). Test on the relationship between environments revealed that there was a significant difference among the environments for height (Figure 5.5 a). Kendu-Bay and and the second season of Kibos was seen to be similar with respect to volume of the juice (Figure 5.5 b) and volume of ethanol (Figure 5.5 c).

5.7. 4 Principal component analysis

Principal component analysis was conducted following the model:

$$Y_{ij} = \mu + \sum_{n-1}^{h} k_n v_{ni} s_{nj} \text{ (Source: Crossa, 1990)} \qquad (5.4)$$

Where k_n is the singular value of the n^{th} axis (k_n^2 is the eingen value); v_{ni} is the eingen vector of the i^{th} genotype for the n^{th} axis; s_{nj} is the eingen vector of the j^{th} environment for the n^{th} axis, and $\Sigma v_{ni} = \Sigma s_{nj} = 1$.

Principal Component Analysis (PCA) revealed that there were two principal components with Eigen values greater than 1 explaining 43.93% and 18.6% of the total variance (Table 5.5). PCA results on Figure 5.6 indicate that there was a variation in all the three environments. The polygons are drawn by connecting the genotypes which lie furthest in a particular environment. All the other genotypes are left inside the polygon for a particular environment. From this study, the first season at Kibos was seen to be the best for most of the genotypes. PCA biplot with principal component 1 (PC1) and PC2 indicate that the first and the second principle component explains more than 65% of the variation observed (Figure 5.7). A scrutiny of the scree plot revealed a clear break after the first principal component (Figure 5.8). It was therefore decided to retain the first principal component which explained 43.93%

of the total variance. The scree plot has two lines in which the lower one shows the proportion of variance for each principal component while the upper one shows cumulative variance explained by the first components. The principle components are sorted in decreasing order of variance with the most important component coming first. Volume of the juice has been seen to be the most important component. Factorial Component Analysis illustrates distribution of the genotypes in the locations with the ones which are away from the origin leading in performance for the traits under investigation (Figure 5.9). The results from the principal component analysis are consistent with that of the analyses of variance.

Table 5.4 Means of agronomic and sugar traits observed across three environments in Kenya in 2016.

Environment	Height (cm)	Girth (mm)	Cane Yield (t ha^{-1})	Juice Volume (Lha^{-1})	Brix (%)	Ethanol Volume (Lha^{-1})	Days to flowering
Kendu Bay	216.70 c	20.36 b	45.45 c	14388.73 c	14.93 a	4389.59 b	81.12 b
Kibos season one	242.77 a	20.45 ab	54.76 a	16952.47 a	14.86 a	5178.90 a	81.37 b
Kibos season two	232.59 b	20.76 a	48.51 b	15376.53 b	14.84 a	4382.22 b	81.74 a
LSD 0.05	3.84	0.38	1.92	180.1	0.28	170.5	0.36

Means with the same letter along the same columns are not significantly different.

Table 5.5 Principal component analysis of sweet sorghum hybrid traits tested in three environments in western part of Kenya in 2016.

Variables	Principal Component loadings					
	1	2	3	4	5	6
Brix	-0.0116	**0.6682**	**0.6765**	0.2870	0.1022	0.0640
Cane Yield	0.0249	0.2999	-0.6006	**0.6899**	0.1077	-0.0112
Volume of Ethanol	**0.5739**	0.0071	0.1429	-0.0402	-0.3637	-0.7185
Girth	0.4799	-0.3393	0.2087	0.0348	**0.7805**	0.0244
Height of the plant	0.2269	0.5859	-0.3332	-0.66218	0.2326	0.0399
Volume of juice	**0.5718**	0.0715	0.0883	-0.0201	-0.4269	**0.6909**
Eigen values	2.1816	1.1160	0.9470	0.8000	0.2970	0.0250
% of eigen values	43.93	18.60	15.79	13.33	4.95	0.41

Figures printed in bold show strong contrast.

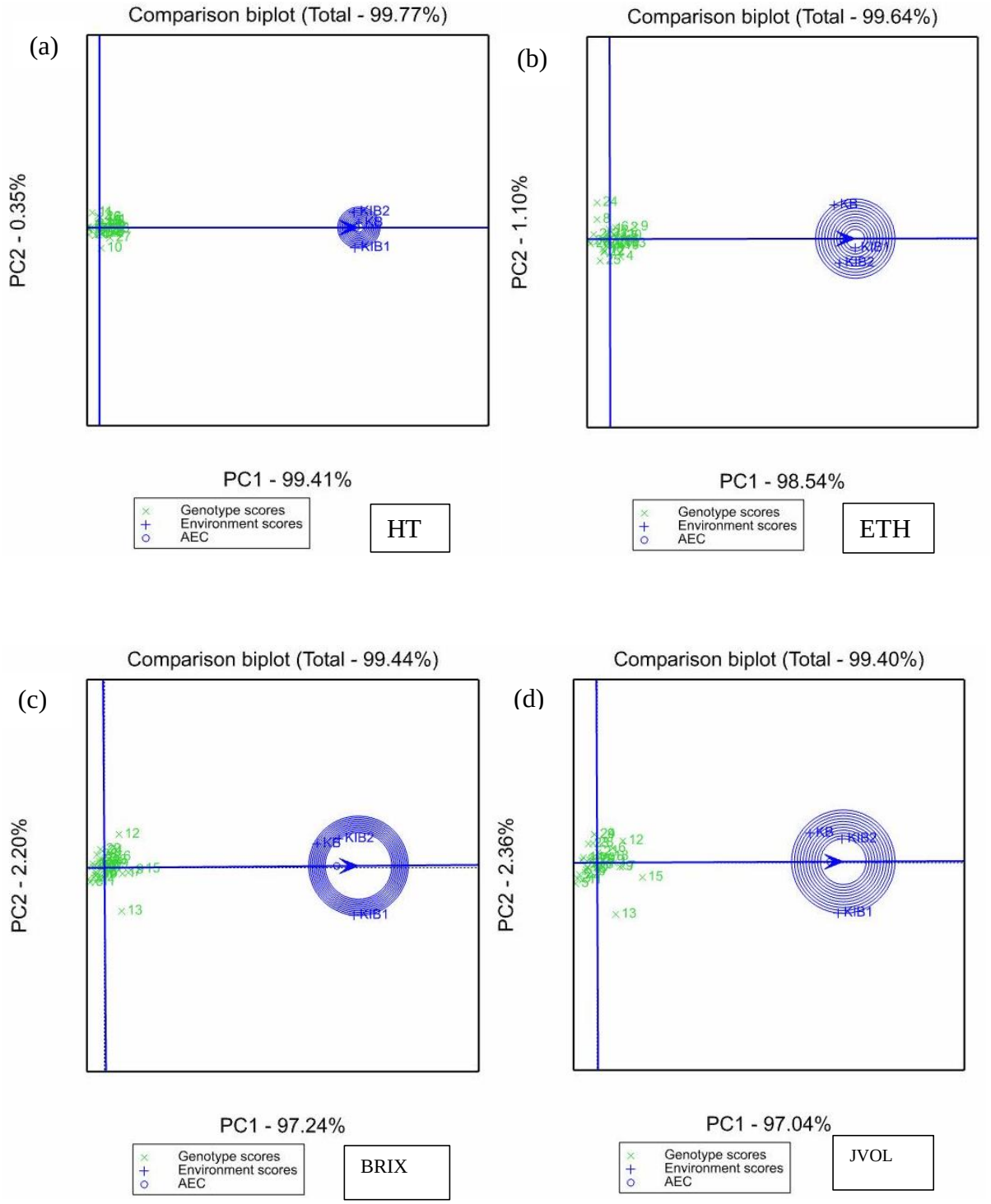

Figure 5.1 GGE biplot analysis of sweet sorghum traits relative to the ideal environment: (a) Height of the plant (b) Ethanol Volume (c) Brix % (d) Volume of the juice

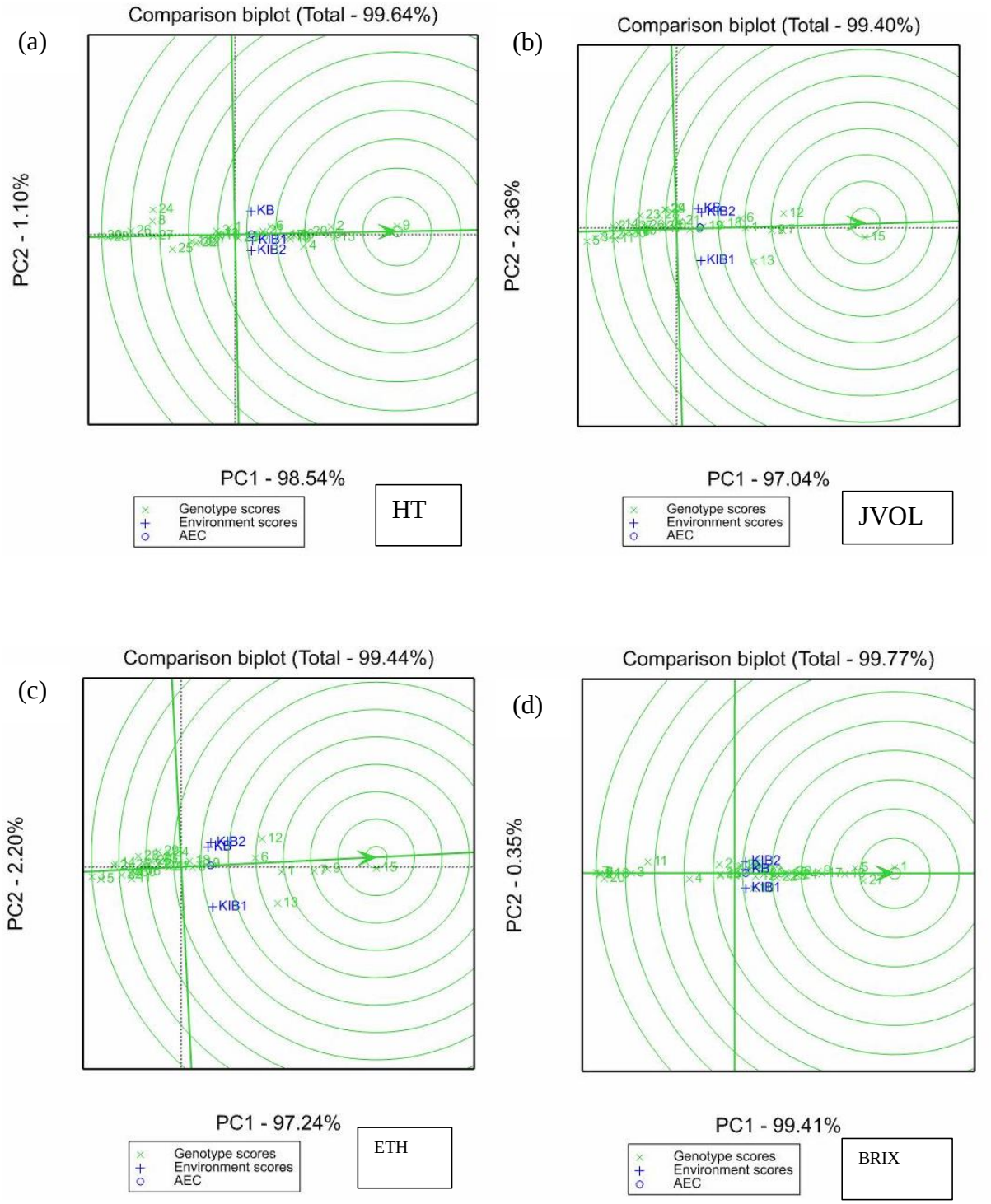

Figure 5.2 GGE comparison biplot analysis of sweet sorghum genotype traits relative to the ideal genotype: (a) Height of the plant (b) Volume of ethanol (c) Volume of the juice (d) Brix %

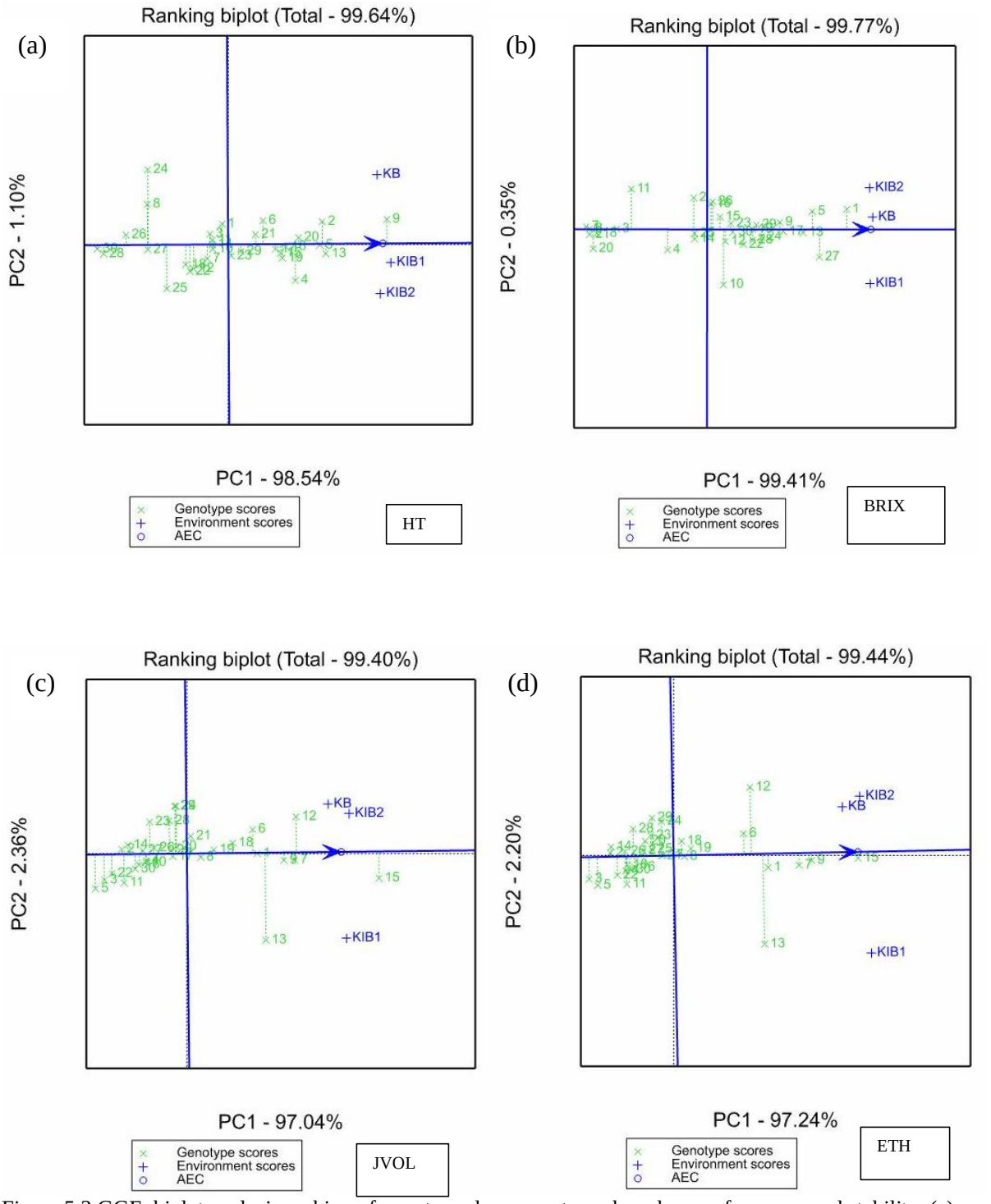

Figure 5.3 GGE biplot analysis ranking of sweet sorghum genotypes based on performance and stability (a) Height of the plant (b) Brix % (c) Volume of the juice (d) Volume of ethanol

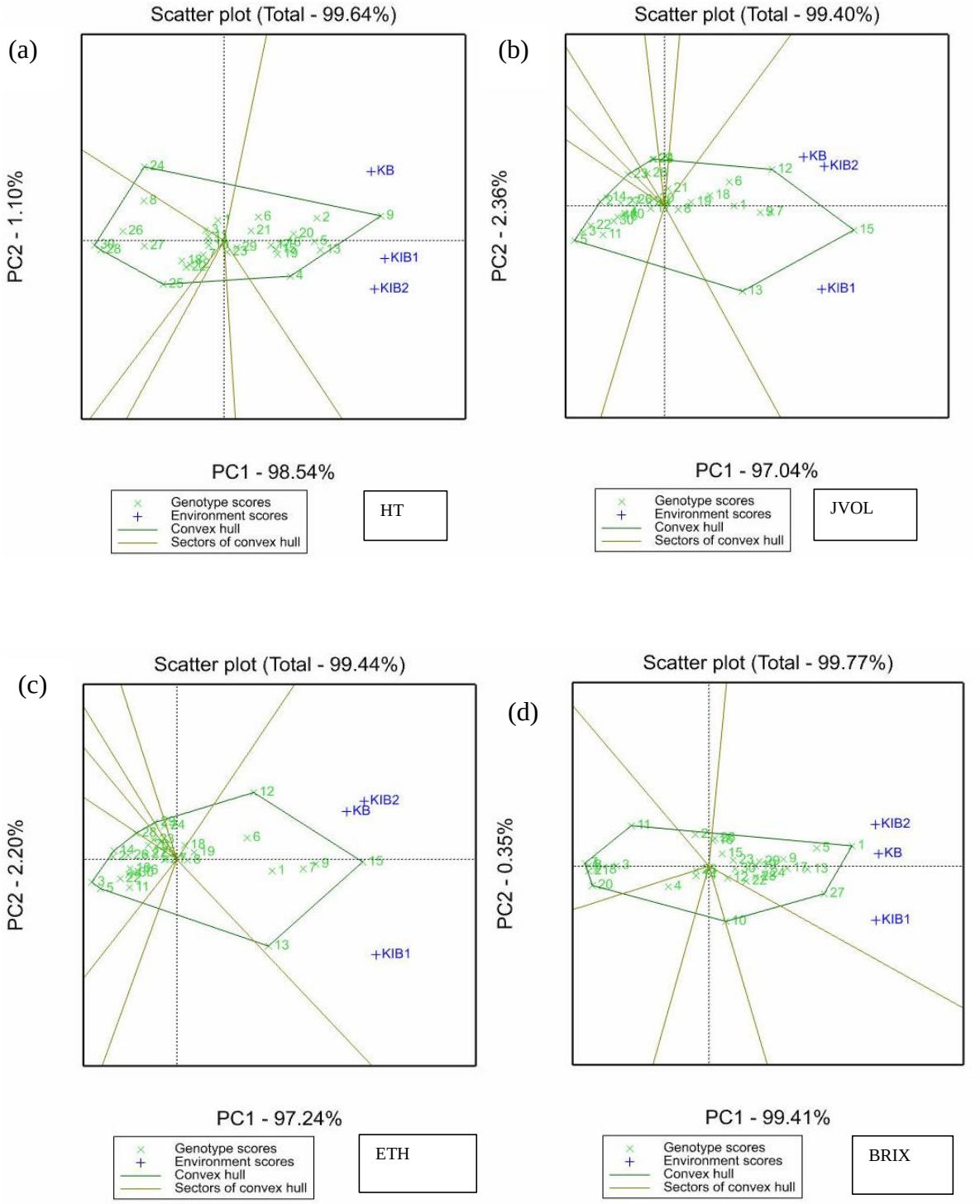

Figure 5.4 GGE biplot analysis showing sweet sorghum genotypes by traits which-won-where (a) Height of the plant (b) Volume of the juice (c) Volume of ethanol (d) Brix %

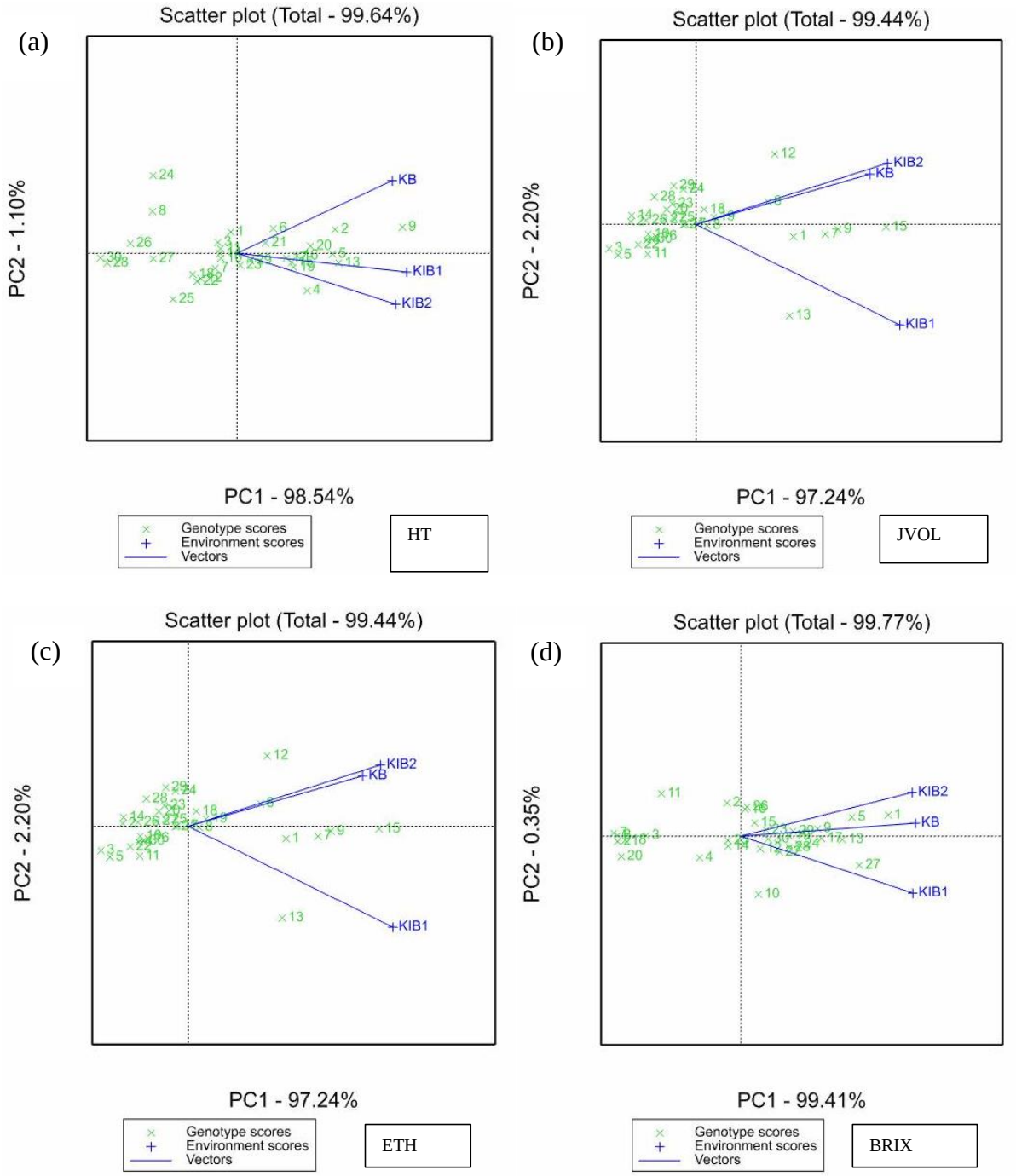

Figure 5.5 GGE biplot analysis showing the relationship of the test environments based on sweet sorghum genotypes traits (a) Height of the plant (b) Volume of the juice (c) Volume of ethanol (d) Brix %

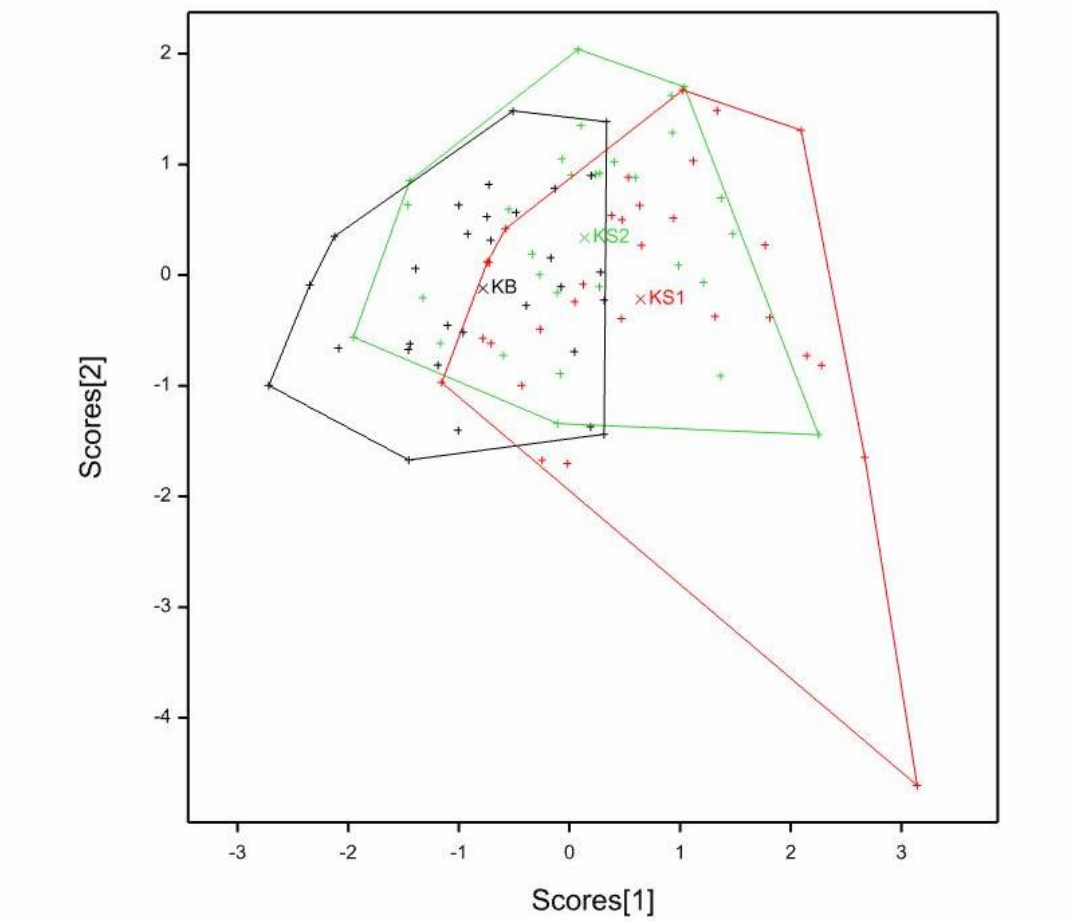

Figure 5.6 Principal Component Analysis scores plot of sweet sorghum genotypes across three environments (KB: Kendu-Bay; KS1: Kibos season 1; KS2: Kibos season 2) in western part of Kenya in 2016.

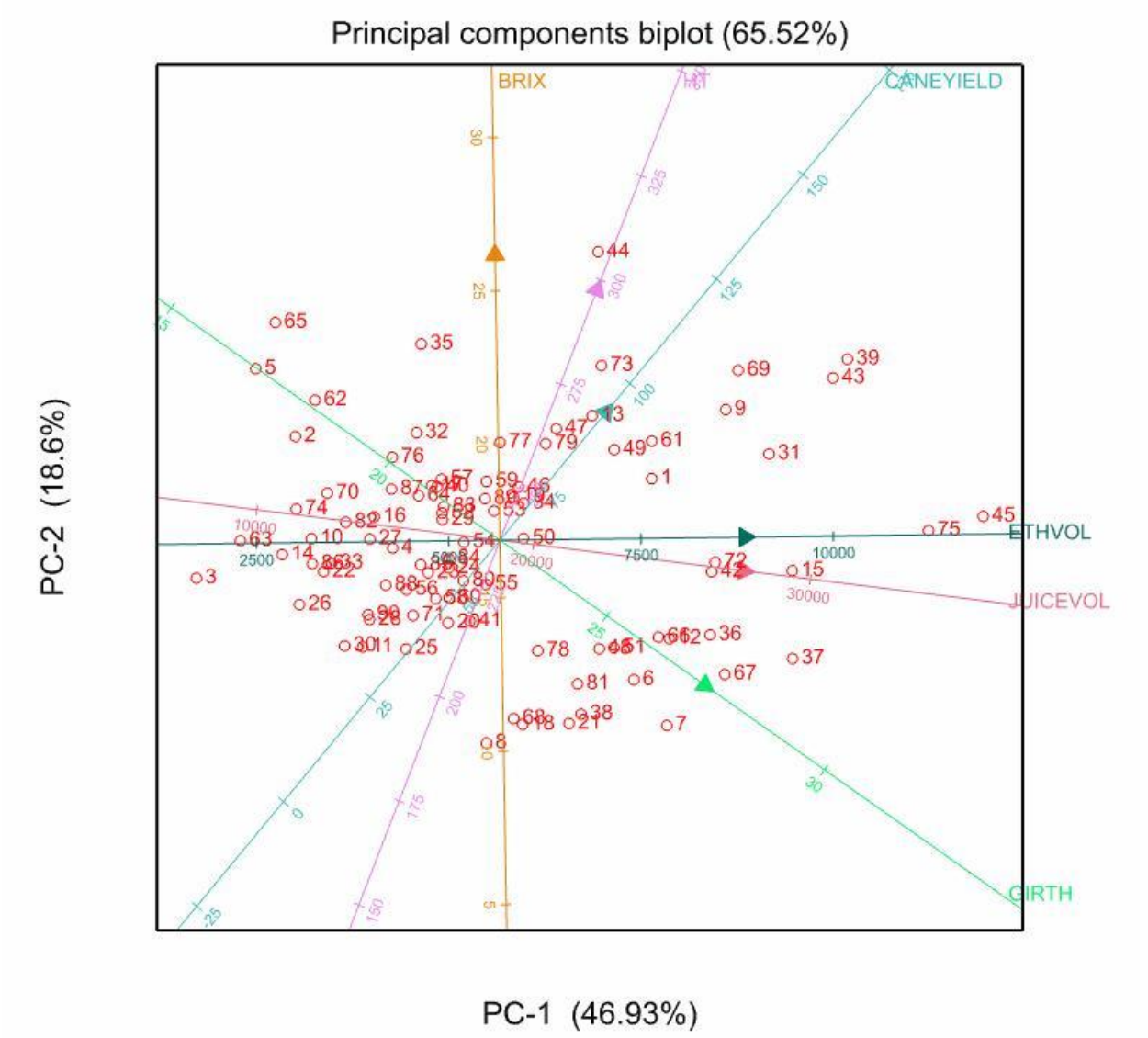

Figure 5.7 Principal Component Analysis biplot. PC1 and PC2 refer to eigen vectors relating to height, girth, juice volume, cane yield, brix and ethanol volume of sweet sorghum genotypes in three environments in western part of Kenya in 2016.

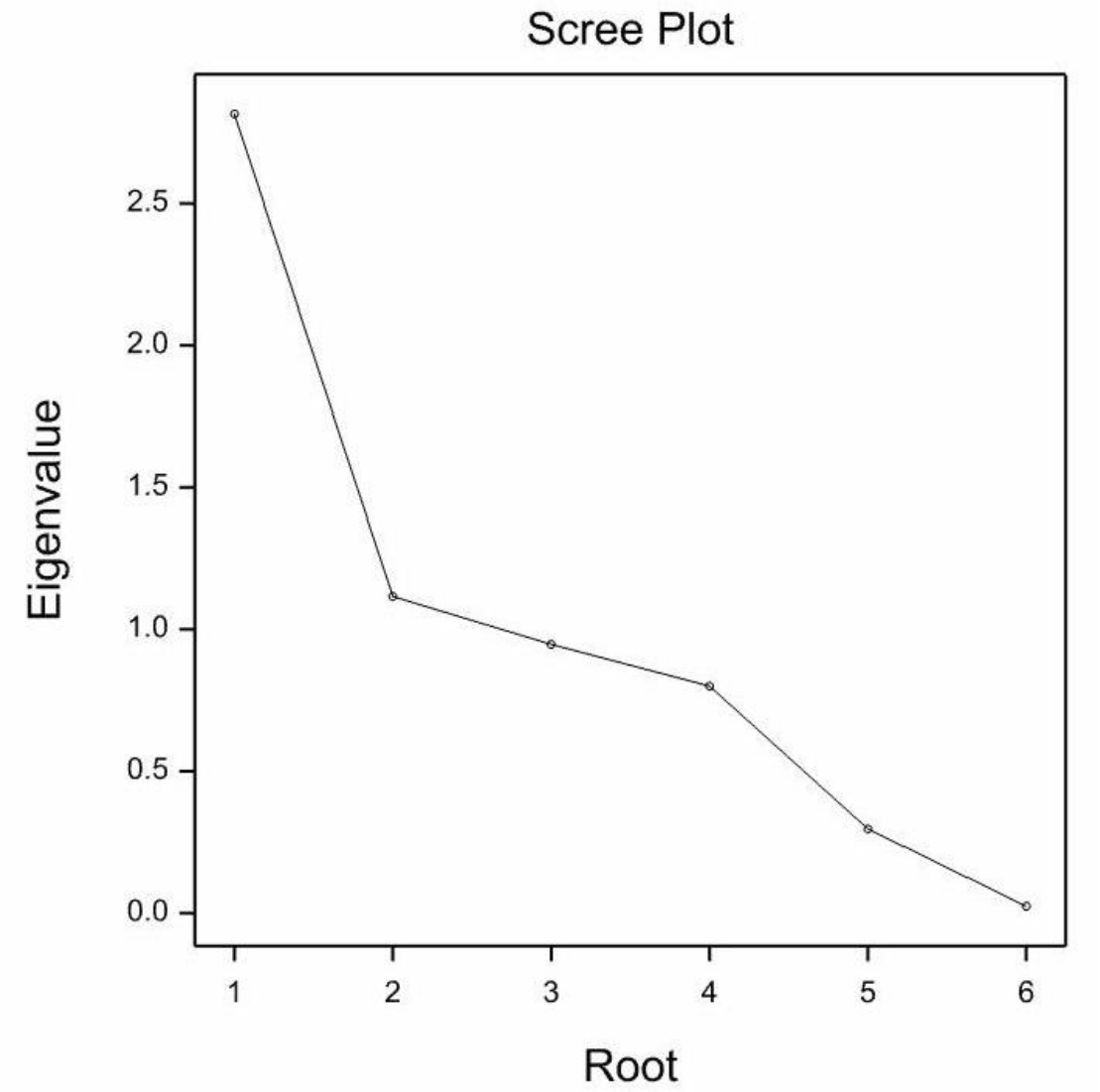

Figure 5.8 Scree plot for eigenvalues for principle component analysis of agronomic and ethanol related traits in sweet sorghum.

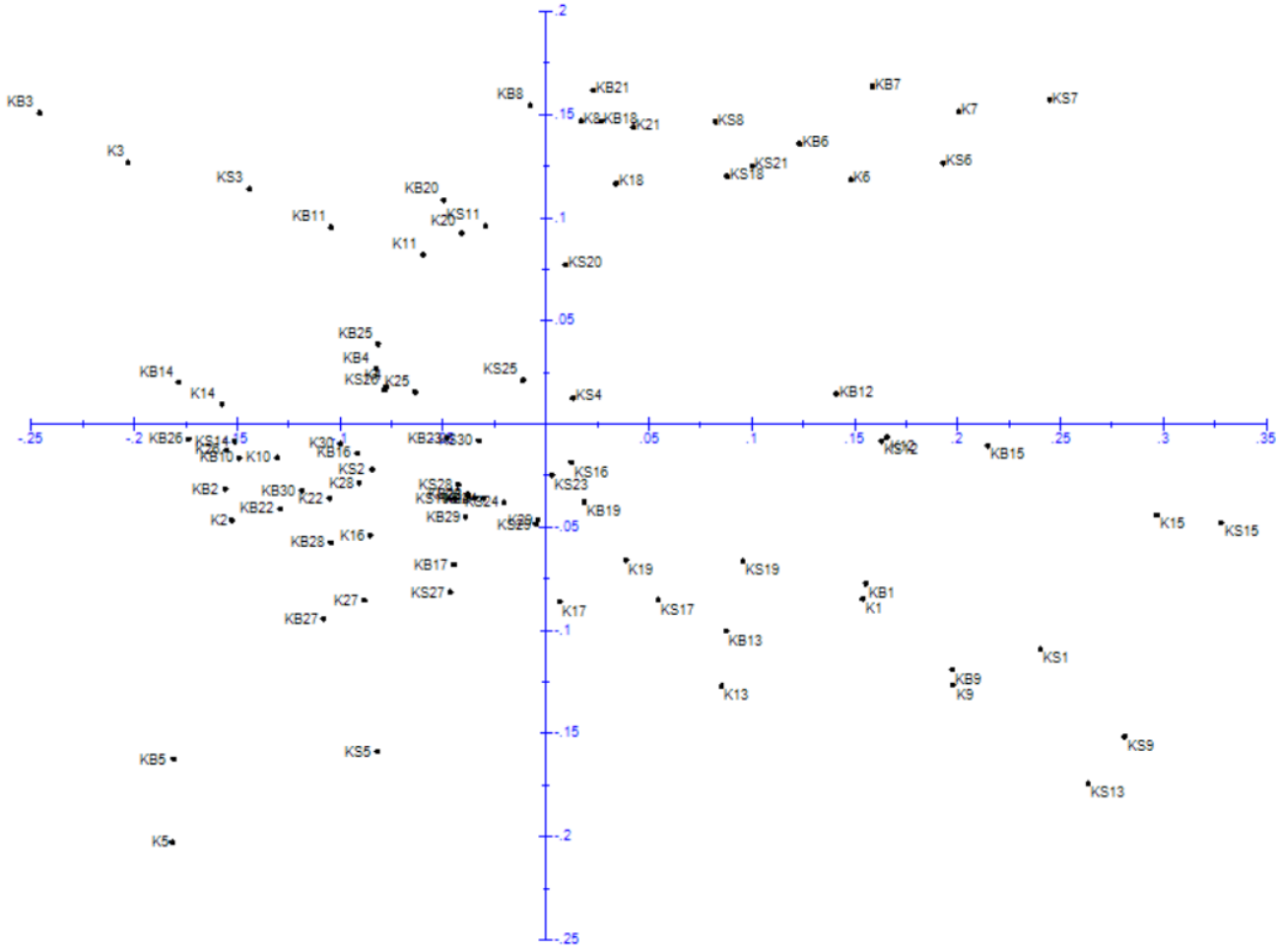

Figure 5.9 Factorial Component Analysis for sweet sorghum hybrids in three environments (KB: Kendu-Bay, KS: Kibos season 1; K: Kibos season 2).

5.8 Discussion

Sorghum is known to thrive well in almost all agro-ecological zones in Kenya. Lowlands such as coastal and western Kenya regions are particularly known for optimal performance. The experimental field at Kibos is among the best areas for sorghum production. Both climatic and soil conditions at Kendu-Bay and Kibos favor growth and development of sorghum crop with temperature, rainfall and altitude almost similar. However, the soils at Kendu-Bay are sandy making the crops more prone to drought due to high drainage. At Kibos, the first season of the year (March-August 2016) had higher rainfall than the second season (September 2016-January 2017). This explains why there was better performance among sweet sorghum hybrids during the first season at Kibos than both Kendu-Bay and the second season at Kibos. Although there was considerable drought during the second season of 2016, significant harvests were realized, but were not as good as the first season of long rains.

Sorghum is known to exhibit high drought tolerance as compared to other crops such as maize and its cultivation can ensure maximum utilization of land in the arid and semi-arid areas. Sweet sorghum however, requires relatively moist conditions during harvest as observed between the first and second seasons at Kibos.

Significant differences observed among the genotypes suggest that each of the genotypes had unique traits. Both agronomic and ethanol related traits among the hybrids were seen to be significantly different even those that were half sibs. Among the genotypes, days to flowering were significantly different. However, there was no significant difference observed for days to flowering for the interaction between genotype and environment. This is probably due to the fact that all the enviroments had more or less the same altitude, temperature and rainfall patterns. The main difference was the type of soil in which Kendu Bay had sandy soil while Kibos had black cotton soil. This is probably because the three environments lie almost along the same altitude. It would have been necessary to conduct multilocational trials considering both the high land and low land areas in Kenya.

There was no significant difference in brix observed in the three environments. However, there was significant difference among the genotypes which were evaluated. This indicates that the process of sugar accumulation in the stems of sweet sorghum is not affected by the environment. It is most probable that a particular genotype grown in different locations may exhibit similar brix levels indicating that brix could be controlled by major genes. This finding is consistent with the findings of Almodares, and Hadi, (2009). While working on sweet sorghum brix, they observed that brix was not affected by the environment. Since the environments under which the evaluation was conducted shares most climatic aspects, evaluation of the same hybrids in diverse environment is necessary.

For all the variables under study, PC1 and PC2 explained more than 99% of the total GGE variation suggesting that the biplots are a good approximation of environment dependent data. Height of the plants were seen to be more affected by the environment than all the other variables. Brix was the least affected by the environment; an observation which was consisted with the results from the analysis of variance. Consequently, the concentric circles on the brix were closer together. The comparison of the genotypes to the ideal genotype revealed interesting results. Athough there is nothing as an ideal genotype, the pattern reveals a point which is assumed to be the position of the ideal genotype. The genotypes which lie closer to the point is assumed to be the best. In this case, genotype 9, 15, and 1 are seen to be lying closer

to the ideal genotypes for height of the plant, volume of the juice and ethanol and brix respectively.

Performance and stability tests also put genotype 15, 9 and 1 in the lime light. This is because the genotypes that lie closer to the average environmental coordination (AEC) line are known to be the most stable and the ones that lie on the extreme right are known to be the best perfoming. The same genotypes identified are seen again to be the most stable and the best performing making them important in sweet sorghum hybrid development.

In an attempt to know which of the genotypes were the best for what in which areas, a pattern determining "which-won-where" for the traits illustrate that each of the three locations lie on the same sector. This indicates that there is no significant variation in the three environments which were tested. The genotypes at the vertex of the polygons are among the best for trait under investigation. Genotype 1 is the best in brix and genotype 15 is the best in ethanol volume. This suggests that the genotypes were the leading in the highlighted variables. For example, genotype 1 was leading in brix while genotype 15 was leading in volume of juice and consequently that of ethanol. The test environments were seen not to be significantly different especially for brix. There were slight variations in juice volume and ethanol production with sighificant variations in height.

Principal component analysis results suggest that the environment at Kibos during the first season was most suited for sweet sorghum. This was during the main season of between April and June. Although sorghum can tolerate drought conditions, better yields are obtained during long rains when the crops have not been subjected to moisture stress. Furthermore, sweet sorghum is grown for the juice in the stalks making soil moisture an important factor even during harvesting. The results from the present study have confirmed that volume of the juice is among the most important components for ethanol production.

For maximum ethanol production in sweet sorghum, a genotype with the highest juice volume, cane yield, and brix is preferred. This is due to the fact that the three aspects are the major factors that influence ethanol production. Genotype 14, (BM39 × EUSS10) hybrid was among the best but was not selected since it failed the stability test but was good due to its high cane yield and volume of juice. However, the brix was relatively low as compared to all the other hybrids. EUSS10 was seen to yielded higher ethanol volume than three other lines which were better in accumulation of brix. This suggests that sweet sorghum improvement

programme have to focus on all the traits that contribute to ethanol yield with particular intrest in volume of the juice.

The significant G × E interactions observed suggests that minor genes control the traits under investigation. This study is in agreement with the findings of Shoemaker, and Bransby, (2010) who observed that both agronomic traits and ethanol related traits of sweet sorghum are greatly influenced by environment. Since the traits are controlled by minor genes, it is necessary for the plant breeder conducting sweet sorghum improvement to pyramid the genes so as to realize the best hybrid genotype for ethanol production. The PCA performed on the thirty sweet sorghum hybrids produced the first principal component that encompassed eigenvectors relating to volume of juice and the second principal component that included eigenvectors relating to brix.

5.9 Conclusion

Sweet sorghum performance is greatly affected by the environment. Significant G × E interactions suggest that the traits under investigation are controlled by minor genes. This study has confirmed that brix accumulation is largely controlled by genotypes but not environment. Three hybrids have been found to be good for production of ethanol. For cane yield, juice volume as well as ethanol production, IS9203 × EUSS10 emerged to be among the most promising croses that can be developed further to establish an elite cultivar for bioethanol production. GS001 × EUSS10 is good for brix while NYANGEZI × SS04 is good for cane yield. GGE biplot analysis is effective in selection of best performing and stable genotypes.

5.10 Recommendation

Further trials should be conducted with inclusion of environments with highly varied characteristics for the selected hybrids before they can be developed for release as cultivars. Development of male sterile lines for mass hybrid production is highly recommended.

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CHAPTER SIX
GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

6.1 General discussion

The need for high yielding sweet sorghum genotypes cannot be overemphasized in attemps to meet the ever increasing energy and food demands globally. In Kenya, sweet sorghum production is insignificant due to lack of understanding of its utilization and un-availability of suitable varieties. The only remedy to this problem is the development of sweet sorghum hybrids. It is also interesting to note that many food crops are directed towards the supply of bioenergy which complicates the food security situation. This competition leaves many countries in limbo as far as food security is concerned. Sweet sorghum appears to be able to contribute to the provision of energy in the form of bioethanol from stalks and food from the kernels. Further benefits can be obtained by using sweet sorghum as fodder to support livestock industry which is important in food security. This multipurpose crop can change the lives of people living in the ASALs which form 80% of the land area in Kenya (Sombroek *et al*., 1982).

This study was aimed at developing sweet sorghum hybrids for ethanol production. The results have indicated that there is great potential in developing sorghum hybrids due to the diverse phenotypic expressions of the traits. The study has also indicated that hybridization between sweet sorghum and grain sorghum is feasible and results in high productivity. All the hybrids which were developed out performed their parents virtually in all the aspects that were investigated except for brix in which the sweet sorghum varieties which were used as testers exhibited higher brix. On the other hand, the rates of accumulation of sugars in the stem of sweet sorghum decreases then increases. This has been associated with the carbohydrate partitioning process that conveys them to the sink organs that are mostly in the stem and kernels of sweet sorghum. It was also interesting to note that brix accumulation did not vary with the environment in which the crops were grown. For example, brix exhibited by SS04 in Kendu Bay was similar at a particular stage to that of Kibos and Egerton. This indicates that brix accumulation is mostly controlled by genes and not environment.

From the findings of this study, sweet sorghum farmers are able to know their harvesting time by counting callender days which lie between 97 days and 123 days after sowing depending on the location and the variety. They are also able to choose whether they want to harvest cane only or kernels as well. Stage five and six have been recommended for the farmers who are interested in food and fuel.

Heterosis and combining abilities of sweet sorghum have been determined. However, the challenge with development of sweet sorghum is that not all the desirable traits are inherited together. For example, yield of sorghum canes and volume of the juice may not necessarily be inherited together with brix. This is probably because the traits could be located on different chromosomes hence the different pattern in inheritance. Gene pyramiding is recommended to be able to put together all the desirable traits which are necessary for ethanol production. The method of crosses that was used was very effective and resulted to near 100% hybrids. The emasculation procedure was effective since most of the harvested panicles resulted to hybrid seeds and not selfed seeds. This was noted when all the hybrids displayed better performance than the parents. However, some of the female lines for example GS002 failed to fertilize in the process. This observation is worth investigating since reports have indicated that there are no barriers to cross pollination of sorghum as they belong to the same species as was stated by Murray *et al*. (2008).

GGE biplot and Principle Component Analysis (PCA) has been conducted to aid the selection of hybrids. GGE biplot analysis enables a plant breeder to make selection of suitable cultivars by combining the effects due to the genotypes, environment and the interactions between the genotypes and the environment. Understanding these relationships is an asset for a plant breeder since they can select for performance and stability of the hybrids. Three hybrids IS9203 × EUSS10, GS001 × EUSS10 and NYANGEZI × SS04 were selected from among thirty hybrids based on their stability across environments and performance.

From the analysis of variance and table of means, the observations made for the genotypes are consistent with what was revealed by PCA. Volume of the juice has emerged as one of the most important components in the study. In Chapter three, although brix level of EUSS10 was low, production of ethanol was seen to be high since it had high volume of the juice. It can be confirmed that high ethanol volume can be realized if the volume of the juice is high. Breeding programs in sweet sorghum hybrids should therefore target high juice volume.

6.2 Conclusion

The hard dough stage of grain in sweet sorghum which occurs after 35 days after anthesis is the recommended harvesting stage as it gives both kernels for food and juice for high bioethanol. The selected crosses IS9203 × EUSS10, GS001 × EUSS10 and NYANGEZI × SS04 can be developed for use by the farmers in areas with conditions similar to the tested

environments. High general combining ability observed in the sorgum lines indicate that the traits under investigation are controlled by additive gene action and have high potential in hybrid development. Among the environments, the first season in Kibos had the highest potential for ethanol production. Sweet sorghum also performs well in the highlands represented by Egerton location but takes a longer time to reach maturity.

6.3 Recommednation

Further study using high performance liquid chromatography for the analysis of sweet sorghum juice is recommended. It is necessary to develop male sterile female lines of the selected hybrids so as to facilitate large scale production of the hybrid seeds. Gene pyramiding by multiple crosses is also recommended to be able to put together the desirable genes of interest that are responsible for high brix and high volume of juice. Focus on yield of the juice, brix and cane yield is recommended due to their high correlation with ethanol yield.

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APPENDIX I: PROGRAM EDITOR FOR LINE × TESTER ANALYSIS

data LxT1;
input Site REP Entry GENOTYPE$ PvsC$ Lines Testers HTG HTFL AVWT CYTONHA GIRTH JVOLml JVOLHaL BRIX
ETHVOLml ETHVOLHa DTF;
cards;

1	1	1	6×15	Cross	6	15	246	209	0.90	95.04	26.00	248.00	26188.80	25.00	34.00	8904.19	69.00
1	2	2	6×15	Cross	6	15	245	212	1.00	105.60	27.00	260.00	27456.00	24.00	37.00	10158.72	68.00
1	3	3	6×15	Cross	6	15	237	203	0.89	93.98	28.00	272.00	28723.20	26.00	35.00	10053.12	68.00
1	1	4	7×15	Cross	7	15	300	275	0.65	68.64	20.00	146.00	15417.60	15.00	28.00	4316.93	78.00
1	2	5	7×15	Cross	7	15	279	258	0.60	63.36	18.00	140.00	14784.00	16.00	27.00	3991.68	77.00
1	3	6	7×15	Cross	7	15	290	265	0.60	63.36	19.00	152.00	16051.20	15.00	29.00	4654.85	77.00
1	1	7	9×15	Cross	9	15	237	209	0.50	52.80	17.00	148.00	15628.80	11.00	26.00	4063.49	81.00
1	2	8	9×15	Cross	9	15	230	199	0.45	47.52	16.00	137.00	14467.20	12.00	28.00	4050.82	80.00
1	3	9	9×15	Cross	9	15	235	207	0.50	52.80	17.00	148.00	15628.80	11.00	26.00	4063.49	79.00
1	1	10	13×15	Cross	13	15	287	259	0.70	73.92	24.00	178.00	18796.80	14.00	28.00	5263.10	82.00
1	2	11	13×15	Cross	13	15	262	239	0.65	68.64	22.00	158.00	16684.80	15.00	29.00	4838.59	83.00
1	3	12	13×15	Cross	13	15	289	254	0.70	73.92	24.00	178.00	18796.80	15.00	28.00	5263.10	83.00
1	1	13	14×15	Cross	14	15	295	273	0.50	52.80	19.00	148.00	15628.80	22.00	30.00	4688.64	78.00
1	2	14	14×15	Cross	14	15	286	254	0.40	42.24	20.00	139.00	14678.40	23.00	29.00	4256.74	77.00
1	3	15	14×15	Cross	14	15	297	273	0.45	47.52	21.00	138.00	14572.80	24.00	30.00	4371.84	77.00
1	1	16	1×15	Cross	1	15	262	230	0.75	79.20	27.00	242.00	25555.20	9.00	31.00	7922.11	75.00
1	2	17	1×15	Cross	1	15	260	229	0.70	73.92	26.00	241.00	25449.60	10.00	34.00	8652.86	76.00
1	3	18	1×15	Cross	1	15	261	230	0.75	79.20	25.00	252.00	26611.20	10.00	32.00	8515.58	75.00
1	1	19	5×15	Cross	5	15	247	216	0.85	89.76	26.00	300.00	31680.00	9.00	35.00	11088.00	83.00
1	2	20	5×15	Cross	5	15	239	205	0.80	84.48	25.00	294.00	31046.40	10.00	36.00	11176.70	84.00
1	3	21	5×15	Cross	5	15	242	216	0.80	84.48	24.00	282.00	29779.20	9.00	33.00	9827.14	83.00
1	1	22	11×15	Cross	11	15	205	179	0.65	68.64	25.00	210.00	22176.00	10.00	30.00	6652.80	90.00
1	2	23	11×15	Cross	11	15	210	182	0.67	70.75	26.00	218.00	23020.80	9.00	31.00	7136.45	90.00
1	3	24	11×15	Cross	11	15	202	175	0.63	66.53	23.00	216.00	22809.60	10.00	29.00	6614.78	90.00
1	1	25	6×17	Cross	6	17	330	300	0.95	100.32	26.00	289.00	30518.40	22.00	38.00	11596.99	89.00
1	2	26	6×17	Cross	6	17	312	288	0.90	95.04	24.00	278.00	29356.80	20.00	35.00	10274.88	89.00
1	3	27	6×17	Cross	6	17	324	293	0.95	100.32	25.00	286.00	30201.60	21.00	37.00	11174.59	90.00
1	1	28	7×17	Cross	7	17	242	209	0.55	58.08	20.00	168.00	17740.80	18.00	28.00	4967.42	78.00
1	2	29	7×17	Cross	7	17	240	211	0.50	52.80	21.00	178.00	18796.80	19.00	27.00	5075.14	79.00

1	3	30	7×17	Cross	7	17	232	207	0.55	58.08	19.00	169.00	17846.40	20.00	29.00	5175.46	78.00
1	1	31	10×17	Cross	10	17	238	218	0.54	57.02	24.00	166.00	17529.60	13.00	32.00	5609.47	75.00
1	2	32	10×17	Cross	10	17	235	214	0.50	52.80	20.00	161.00	17001.60	10.00	30.00	5100.48	75.00
1	3	33	10×17	Cross	10	17	232	206	0.52	54.91	23.00	160.00	16896.00	11.00	32.00	5406.72	76.00
1	1	34	12×17	Cross	12	17	230	198	0.85	89.76	26.00	278.00	29356.80	18.00	30.00	8807.04	74.00
1	2	35	12×17	Cross	12	17	225	194	0.80	84.48	27.00	278.00	29356.80	18.00	20.00	5871.36	75.00
1	3	36	12×17	Cross	12	17	232	191	0.75	79.20	26.00	269.00	28406.40	19.00	31.00	8805.98	74.00
1	1	37	13×17	Cross	13	17	298	270	0.90	95.04	24.00	302.00	31891.20	23.00	34.00	10843.01	79.00
1	2	38	13×17	Cross	13	17	291	268	0.90	95.04	25.00	310.00	32736.00	22.00	32.00	10475.52	78.00
1	3	39	13×17	Cross	13	17	297	271	0.90	95.04	24.00	302.00	31891.20	23.00	35.00	11161.92	78.00
1	1	40	14×17	Cross	14	17	241	214	0.43	45.41	19.00	149.00	15734.40	16.00	27.00	4248.29	75.00
1	2	41	14×17	Cross	14	17	246	220	0.45	47.52	20.00	152.00	16051.20	17.00	28.00	4494.34	74.00
1	3	42	14×17	Cross	14	17	242	215	0.40	42.24	19.00	146.00	15417.60	16.00	26.00	4008.58	74.00
1	1	43	1×17	Cross	1	17	275	250	1.20	126.72	28.00	370.00	39072.00	18.00	34.00	13284.48	73.00
1	2	44	1×17	Cross	1	17	268	239	1.00	105.60	27.00	359.00	37910.40	16.00	32.00	12131.33	74.00
1	3	45	1×17	Cross	1	17	285	254	0.90	95.04	28.00	372.00	39283.20	18.00	31.00	12177.79	74.00
1	1	46	4×17	Cross	4	17	278	239	0.65	68.64	23.00	164.00	17318.40	17.00	30.00	5195.52	73.00
1	2	47	4×17	Cross	4	17	272	227	0.60	63.36	24.00	168.00	17740.80	16.00	29.00	5144.83	72.00
1	3	48	4×17	Cross	4	17	276	233	0.60	63.36	23.00	169.00	17846.40	17.00	32.00	5710.85	73.00
1	1	49	6×16	Cross	6	16	270	239	0.65	68.64	24.00	188.00	19852.80	22.00	31.00	6154.37	91.00
1	2	50	6×16	Cross	6	16	267	234	0.60	63.36	22.00	196.00	20697.60	21.00	29.00	6002.30	91.00
1	3	51	6×16	Cross	6	16	272	242	0.65	68.64	24.00	187.00	19747.20	22.00	31.00	6121.63	92.00
1	1	52	7×16	Cross	7	16	230	198	0.80	84.48	24.00	236.00	24921.60	10.00	26.00	6479.62	80.00
1	2	53	7×16	Cross	7	16	227	189	0.75	79.20	23.00	231.00	24393.60	11.00	27.00	6586.27	81.00
1	3	54	7×16	Cross	7	16	232	201	0.80	84.48	24.00	238.00	25132.80	10.00	25.00	6283.20	80.00
1	1	55	9×16	Cross	9	16	273	247	0.75	79.20	23.00	220.00	23232.00	20.00	29.00	6737.28	77.00
1	2	56	9×16	Cross	9	16	270	241	0.70	73.92	24.00	226.00	23865.60	19.00	30.00	7159.68	78.00
1	3	57	9×16	Cross	9	16	272	250	0.75	79.20	23.00	220.00	23232.00	20.00	29.00	6737.28	77.00
1	1	58	14×16	Cross	14	16	289	251	0.65	68.64	22.00	189.00	19958.40	10.00	27.00	5388.77	75.00
1	2	59	14×16	Cross	14	16	284	249	0.63	66.53	21.00	192.00	20275.20	11.00	26.00	5271.55	74.00
1	3	60	14×16	Cross	14	16	287	256	0.62	65.47	22.00	188.00	19852.80	10.00	26.00	5161.73	75.00
1	1	61	1×16	Cross	1	16	260	234	0.65	68.64	27.00	194.00	20486.40	10.00	28.00	5736.19	86.00
1	2	62	1×16	Cross	1	16	262	235	0.63	66.53	28.00	202.00	21331.20	9.00	29.00	6186.05	86.00
1	3	63	1×16	Cross	1	16	259	230	0.65	68.64	26.00	195.00	20592.00	10.00	30.00	6177.60	87.00
1	1	64	2×16	Cross	2	16	230	203	0.54	57.02	22.00	142.00	14995.20	19.00	33.00	4948.42	83.00
1	2	65	2×16	Cross	2	16	233	204	0.50	52.80	22.00	141.00	14889.60	20.00	30.00	4466.88	83.00

1	3	66	2×16	Cross	2	16	235	213	0.55	58.08	22.00	163.00	17212.80	19.00	31.00	5335.97	82.00
1	1	67	3×16	Cross	3	16	256	227	0.55	58.08	24.00	158.00	16684.80	18.00	32.00	5339.14	79.00
1	2	68	3×16	Cross	3	16	247	218	0.53	55.97	25.00	164.00	17318.40	19.00	33.00	5715.07	80.00
1	3	69	3×16	Cross	3	16	252	224	0.50	52.80	22.00	149.00	15734.40	18.00	32.00	5035.01	79.00
1	1	70	6×18	Cross	6	18	205	177	0.55	58.08	21.00	169.00	17846.40	20.00	30.00	5353.92	66.00
1	2	71	6×18	Cross	6	18	210	180	0.58	61.25	23.00	175.00	18480.00	21.00	31.00	5728.80	67.00
1	3	72	6×18	Cross	6	18	206	175	0.55	58.08	23.00	170.00	17952.00	20.00	30.00	5385.60	66.00
1	1	73	7×18	Cross	7	18	220	189	0.45	47.52	22.00	194.00	20486.40	16.00	28.00	5736.19	77.00
1	2	74	7×18	Cross	7	18	218	187	0.43	45.41	23.00	186.00	19641.60	17.00	29.00	5696.06	78.00
1	3	75	7×18	Cross	7	18	224	190	0.45	47.52	22.00	183.00	19324.80	16.00	28.00	5410.94	77.00
1	1	76	10×18	Cross	10	18	200	181	0.43	45.41	20.00	167.00	17635.20	17.00	26.00	4585.15	75.00
1	2	77	10×18	Cross	10	18	196	170	0.40	42.24	21.00	173.00	18268.80	16.00	27.00	4932.58	75.00
1	3	78	10×18	Cross	10	18	208	184	0.45	47.52	20.00	176.00	18585.60	17.00	26.00	4832.26	76.00
1	1	79	13×18	Cross	13	18	212	185	0.55	58.08	22.00	168.00	17740.80	24.00	32.00	5677.06	79.00
1	2	80	13×18	Cross	13	18	203	175	0.53	55.97	21.00	159.00	16790.40	25.00	30.00	5037.12	80.00
1	3	81	13×18	Cross	13	18	210	181	0.51	53.86	22.00	164.00	17318.40	24.00	31.00	5368.70	79.00
1	1	82	14×18	Cross	14	18	190	163	0.45	47.52	22.00	169.00	17846.40	20.00	26.00	4640.06	80.00
1	2	83	14×18	Cross	14	18	188	153	0.42	44.35	23.00	172.00	18163.20	19.00	25.00	4540.80	79.00
1	3	84	14×18	Cross	14	18	185	160	0.45	47.52	24.00	177.00	18691.20	20.00	26.00	4859.71	79.00
1	1	85	5×18	Cross	5	18	256	224	0.54	57.02	23.00	172.00	18163.20	20.00	28.00	5085.70	78.00
1	2	86	5×18	Cross	5	18	250	221	0.50	52.80	22.00	165.00	17424.00	19.00	29.00	5052.96	77.00
1	3	87	5×18	Cross	5	18	253	222	0.53	55.97	23.00	176.00	18585.60	20.00	28.00	5203.97	77.00
1	1	88	2×18	Cross	2	18	191	158	0.62	65.47	23.00	165.00	17424.00	18.00	30.00	5227.20	75.00
1	2	89	2×18	Cross	2	18	189	157	0.60	63.36	22.00	162.00	17107.20	19.00	31.00	5303.23	74.00
1	3	90	2×18	Cross	2	18	189	157	0.60	63.36	23.00	170.00	17952.00	18.00	29.00	5206.08	74.00
1	1	91	16	Parent	16	16	178	153	0.40	42.24	18.50	138.00	14572.80	19.50	31.80	4634.15	75.00
1	2	92	16	Parent	16	16	190	172	0.40	42.24	18.87	130.00	13728.00	18.50	29.40	4036.03	73.00
1	3	93	16	Parent	16	16	174	157	0.40	42.24	19.50	124.00	13094.40	16.50	35.80	4687.80	73.00
1	1	94	15	Parent	15	15	180	127	0.28	29.57	16.00	118.00	12460.80	14.00	35.40	4411.12	69.00
1	2	95	15	Parent	15	15	189	162	0.30	31.68	16.50	108.00	11404.80	17.00	32.80	3740.77	69.00
1	3	96	15	Parent	15	15	157	126	0.30	31.68	18.00	126.00	13305.60	17.00	33.80	4497.29	70.00
1	1	97	17	Parent	17	17	192	179	0.48	50.69	21.50	244.00	25766.40	8.00	26.00	6699.26	82.00
1	2	98	17	Parent	17	17	183	166	0.46	48.58	21.40	225.00	23760.00	5.50	25.00	5940.00	82.00
1	3	99	17	Parent	17	17	191	169	0.49	51.74	21.50	242.00	25555.20	11.00	28.40	7257.68	81.00
1	1	100	18	Parent	18	18	197	173	0.34	35.90	19.00	122.00	12883.20	17.50	34.20	4406.05	57.00
1	2	101	18	Parent	18	18	179	145	0.32	33.79	18.50	129.00	13622.40	16.50	33.00	4495.39	58.00

1	3	102	18	Parent	18	18	175	146	0.30	31.68	18.80	119.00	12566.40	18.50	31.00	3895.58	57.00
1	1	103	1	Parent	1	1	252	208	0.34	35.90	15.50	48.00	5068.80	10.00	24.50	2587.20	81.00
1	2	104	1	Parent	1	1	254	211	0.35	36.96	16.00	49.00	5174.40	9.00	24.00	2534.40	79.00
1	3	105	1	Parent	1	1	250	206	0.36	38.02	15.00	48.00	5068.80	8.00	25.00	2640.00	80.00
1	1	106	2	Parent	2	2	243	203	0.36	38.02	15.00	100.00	10560.00	11.00	24.50	2587.20	101.00
1	2	107	2	Parent	2	2	240	198	0.35	36.96	14.00	94.00	9926.40	11.00	25.00	2640.00	100.00
1	3	108	2	Parent	2	2	239	209	0.33	34.85	16.00	90.00	9504.00	12.00	26.00	2745.60	100.50
1	1	109	3	Parent	3	3	281	248	0.20	21.12	15.00	49.00	5174.40	14.00	24.20	2555.52	102.00
1	2	110	3	Parent	3	3	283	245	0.25	26.40	14.00	60.00	6336.00	13.00	24.20	2555.52	101.00
1	3	111	3	Parent	3	3	281	244	0.20	21.12	13.30	48.00	5068.80	15.00	24.20	2555.52	102.00
1	1	112	4	Parent	4	4	270	245	0.36	38.02	16.00	80.00	8448.00	9.00	26.00	2745.60	97.00
1	2	113	4	Parent	4	4	276	251	0.38	40.13	17.00	87.00	9187.20	10.00	27.00	2851.20	96.00
1	3	114	4	Parent	4	4	269	243	0.35	36.96	16.00	82.00	8659.20	9.00	26.00	2745.60	95.00
1	1	115	5	Parent	5	5	285	259	0.34	35.90	20.00	92.00	9715.20	13.00	25.00	2640.00	87.00
1	2	116	5	Parent	5	5	280	253	0.30	31.68	19.00	84.00	8870.40	14.00	24.00	2534.40	88.00
1	3	117	5	Parent	5	5	283	261	0.31	32.74	19.00	86.00	9081.60	13.00	24.50	2587.20	87.00
1	1	118	6	Parent	6	6	285	269	0.35	36.96	16.00	109.00	11510.40	11.00	24.00	2534.40	81.00
1	2	119	6	Parent	6	6	271	264	0.35	36.96	15.00	112.00	11827.20	12.00	24.00	2534.40	82.00
1	3	120	6	Parent	6	6	288	261	0.36	38.02	17.00	104.00	10982.40	11.00	24.00	2534.40	80.00
1	1	121	7	Parent	7	7	239	215	0.25	26.40	13.50	48.00	5068.80	14.00	28.00	2956.80	81.00
1	2	122	7	Parent	7	7	241	219	0.30	31.68	15.00	56.00	5913.60	15.00	28.00	2956.80	79.00
1	3	123	7	Parent	7	7	237	212	0.25	26.40	13.00	50.00	5280.00	14.00	28.00	2956.80	80.00
1	1	124	8	Parent	8	8	271	242	0.35	36.96	14.50	53.00	5596.80	13.00	23.60	2492.16	89.00
1	2	125	8	Parent	8	8	268	239	0.30	31.68	16.00	60.00	6336.00	13.00	23.60	2492.16	90.00
1	3	126	8	Parent	8	8	270	241	0.31	32.74	15.00	50.00	5280.00	14.00	23.60	2492.16	89.00
1	1	127	9	Parent	9	9	250	223	0.20	21.12	13.50	72.00	7603.20	12.00	21.00	2217.60	98.00
1	2	128	9	Parent	9	9	259	228	0.25	26.40	13.50	79.00	8342.40	11.00	21.00	2217.60	97.00
1	3	129	9	Parent	9	9	251	225	0.21	22.18	14.00	74.00	7814.40	12.00	21.00	2217.60	96.00
1	1	130	10	Parent	10	10	249	228	0.45	47.52	18.40	126.00	13305.60	4.00	24.50	2587.20	96.00
1	2	131	10	Parent	10	10	252	232	0.46	48.58	19.00	138.00	14572.80	4.00	24.50	2587.20	97.00
1	3	132	10	Parent	10	10	248	228	0.44	46.46	18.00	124.00	13094.40	5.00	24.50	2587.20	96.00
1	1	133	11	Parent	11	11	267	240	0.43	45.41	19.00	101.00	10665.60	6.00	25.00	2640.00	93.00
1	2	134	11	Parent	11	11	258	235	0.40	42.24	18.00	86.00	9081.60	6.00	25.00	2640.00	92.00
1	3	135	11	Parent	11	11	264	236	0.41	43.30	18.00	96.00	10137.60	6.00	25.00	2640.00	92.00
1	1	136	12	Parent	12	12	216	186	0.20	21.12	14.00	52.00	5491.20	9.00	27.00	2851.20	111.00
1	2	137	12	Parent	12	12	212	183	0.21	22.18	14.00	52.00	5491.20	10.00	27.00	2851.20	112.00

1	3	138	12	Parent	12	12	217	188	0.23	24.29	13.00	64.00	6758.40	9.00	27.00	2851.20	112.00
1	1	139	13	Parent	13	13	200	166	0.15	15.84	12.00	30.00	3168.00	10.00	25.50	2692.80	82.00
1	2	140	13	Parent	13	13	205	179	0.20	21.12	12.00	42.00	4435.20	9.00	26.00	2745.60	80.00
1	3	141	13	Parent	13	13	208	162	0.18	19.01	11.00	33.00	3484.80	10.00	25.50	2692.80	81.00
1	1	142	14	Parent	14	14	170	152	0.30	31.68	15.00	89.00	9398.40	11.00	17.00	1795.20	79.00
1	2	143	14	Parent	14	14	162	149	0.24	25.34	15.00	92.00	9715.20	12.00	18.00	1900.80	78.00
1	3	144	14	Parent	14	14	165	151	0.23	24.29	15.00	84.00	8870.40	11.00	16.00	1689.60	77.00
2	1	1	6×15	Cross	6	15	245	212	0.80	84.48	26.00	230.00	24288.00	26.00	36.00	8743.68	69.00
2	2	2	6×15	Cross	6	15	236	202	0.70	73.92	25.00	211.00	22281.60	25.00	33.00	7352.93	68.00
2	3	3	6×15	Cross	6	15	240	211	0.80	84.48	26.00	222.00	23443.20	26.00	31.00	7267.39	67.00
2	1	4	7×15	Cross	7	15	300	275	0.50	52.80	16.00	120.00	12672.00	16.00	28.00	3548.16	77.00
2	2	5	7×15	Cross	7	15	270	254	0.50	52.80	16.00	118.00	12460.80	17.00	27.00	3364.42	78.00
2	3	6	7×15	Cross	7	15	284	265	0.50	52.80	16.00	121.00	12777.60	16.00	28.00	3577.73	77.00
2	1	7	9×15	Cross	9	15	235	207	0.45	47.52	15.00	98.00	10348.80	11.00	26.00	2690.69	80.00
2	2	8	9×15	Cross	9	15	249	219	0.40	42.24	15.00	118.00	12460.80	11.00	26.00	3239.81	81.00
2	3	9	9×15	Cross	9	15	233	200	0.40	42.24	16.00	98.00	10348.80	12.00	24.00	2483.71	80.00
2	1	10	13×15	Cross	13	15	287	259	0.60	63.36	22.00	138.00	14572.80	14.00	28.00	4080.38	83.00
2	2	11	13×15	Cross	13	15	281	243	0.50	52.80	21.00	129.00	13622.40	14.00	26.00	3541.82	82.00
2	3	12	13×15	Cross	13	15	277	239	0.60	63.36	21.00	137.00	14467.20	13.00	25.00	3616.80	83.00
2	1	13	14×15	Cross	14	15	295	273	0.30	31.68	17.00	88.00	9292.80	24.00	30.00	2787.84	77.00
2	2	14	14×15	Cross	14	15	288	267	0.35	36.96	18.00	92.00	9715.20	23.00	31.00	3011.71	76.00
2	3	15	14×15	Cross	14	15	271	253	0.30	31.68	17.00	84.00	8870.40	24.00	30.00	2661.12	77.00
2	1	16	1×15	Cross	1	15	262	230	0.65	68.64	27.00	232.00	24499.20	10.00	34.00	8329.73	76.00
2	2	17	1×15	Cross	1	15	254	231	0.60	63.36	25.00	222.00	23443.20	9.00	32.00	7501.82	75.00
2	3	18	1×15	Cross	1	15	260	228	0.62	65.47	24.00	229.00	24182.40	10.00	30.00	7254.72	75.00
2	1	19	5×15	Cross	5	15	247	216	0.80	84.48	26.00	264.00	27878.40	9.00	35.00	9757.44	82.00
2	2	20	5×15	Cross	5	15	217	184	0.75	79.20	24.00	262.00	27667.20	10.00	30.00	8300.16	83.00
2	3	21	5×15	Cross	5	15	240	219	0.80	84.48	26.00	261.00	27561.60	9.00	35.00	9646.56	83.00
2	1	22	11×15	Cross	11	15	205	179	0.55	58.08	25.00	180.00	19008.00	10.00	30.00	5702.40	91.00
2	2	23	11×15	Cross	11	15	202	173	0.50	52.80	24.00	178.00	18796.80	9.00	31.00	5827.01	90.00
2	3	24	11×15	Cross	11	15	207	182	0.60	63.36	25.00	180.00	19008.00	10.00	30.00	5702.40	91.00
2	1	25	6×17	Cross	6	17	330	300	0.85	89.76	25.00	258.00	27244.80	22.00	38.00	10353.02	90.00
2	2	26	6×17	Cross	6	17	287	259	0.80	84.48	23.00	237.00	25027.20	21.00	34.00	8509.25	90.00
2	3	27	6×17	Cross	6	17	318	290	0.75	79.20	24.00	241.00	25449.60	20.00	36.00	9161.86	91.00
2	1	28	7×17	Cross	7	17	242	209	0.35	36.96	18.00	136.00	14361.60	18.00	27.00	3877.63	78.00
2	2	29	7×17	Cross	7	17	240	214	0.32	33.79	19.00	138.00	14572.80	17.00	28.00	4080.38	79.00

2	3	30	7×17	Cross	7	17	241	211	0.35	36.96	16.00	131.00	13833.60	16.00	26.00	3596.74	78.00
2	1	31	10×17	Cross	10	17	238	218	0.40	42.24	24.00	112.00	11827.20	13.00	32.00	3784.70	75.00
2	2	32	10×17	Cross	10	17	247	221	0.45	47.52	25.00	125.00	13200.00	12.00	30.00	3960.00	76.00
2	3	33	10×17	Cross	10	17	239	216	0.40	42.24	24.00	111.00	11721.60	13.00	31.00	3633.70	75.00
2	1	34	12×17	Cross	12	17	230	198	0.75	79.20	26.00	260.00	27456.00	17.00	30.00	8236.80	74.00
2	2	35	12×17	Cross	12	17	246	212	0.80	84.48	27.00	274.00	28934.40	18.00	29.00	8390.98	75.00
2	3	36	12×17	Cross	12	17	232	200	0.75	79.20	25.00	267.00	28195.20	19.00	30.00	8458.56	74.00
2	1	37	13×17	Cross	13	17	298	270	0.80	84.48	23.00	213.00	22492.80	23.00	34.00	7647.55	78.00
2	2	38	13×17	Cross	13	17	276	244	0.75	79.20	22.00	204.00	21542.40	21.00	30.00	6462.72	79.00
2	3	39	13×17	Cross	13	17	293	265	0.80	84.48	21.00	213.00	22492.80	23.00	32.00	7197.70	78.00
2	1	40	14×17	Cross	14	17	241	214	0.30	31.68	17.00	126.00	13305.60	17.00	27.00	3592.51	74.00
2	2	41	14×17	Cross	14	17	235	203	0.35	36.96	18.00	126.00	13305.60	16.00	26.00	3459.46	75.00
2	3	42	14×17	Cross	14	17	231	199	0.30	31.68	16.00	126.00	13305.60	15.00	27.00	3592.51	74.00
2	1	43	1×17	Cross	1	17	275	250	1.10	116.16	28.00	361.00	38121.60	18.00	34.00	12961.34	74.00
2	2	44	1×17	Cross	1	17	268	232	0.90	95.04	27.00	342.00	36115.20	16.00	30.00	10834.56	74.00
2	3	45	1×17	Cross	1	17	259	234	0.80	84.48	28.00	331.00	34953.60	19.00	32.00	11185.15	75.00
2	1	46	4×17	Cross	4	17	278	239	0.45	47.52	19.00	134.00	14150.40	17.00	30.00	4245.12	73.00
2	2	47	4×17	Cross	4	17	271	240	0.40	42.24	21.00	130.00	13728.00	18.00	30.00	4118.40	72.00
2	3	48	4×17	Cross	4	17	264	239	0.40	42.24	21.00	126.00	13305.60	17.00	30.00	3991.68	73.00
2	1	49	6×16	Cross	6	16	270	239	0.45	47.52	24.00	168.00	17740.80	22.00	31.00	5499.65	91.00
2	2	50	6×16	Cross	6	16	261	233	0.42	44.35	22.00	158.00	16684.80	21.00	30.00	5005.44	91.00
2	3	51	6×16	Cross	6	16	271	240	0.45	47.52	24.00	168.00	17740.80	22.00	31.00	5499.65	92.00
2	1	52	7×16	Cross	7	16	230	198	0.70	73.92	24.00	216.00	22809.60	10.00	26.00	5930.50	80.00
2	2	53	7×16	Cross	7	16	225	189	0.65	68.64	22.00	204.00	21542.40	11.00	25.00	5385.60	80.00
2	3	54	7×16	Cross	7	16	230	198	0.70	73.92	24.00	198.00	20908.80	10.00	26.00	5436.29	81.00
2	1	55	9×16	Cross	9	16	273	250	0.65	68.64	23.00	190.00	20064.00	20.00	29.00	5818.56	77.00
2	2	56	9×16	Cross	9	16	270	247	0.60	63.36	22.00	193.00	20380.80	19.00	28.00	5706.62	79.00
2	3	57	9×16	Cross	9	16	276	258	0.65	68.64	23.00	194.00	20486.40	20.00	30.00	6145.92	77.00
2	1	58	14×16	Cross	14	16	289	251	0.50	52.80	22.00	167.00	17635.20	10.00	27.00	4761.50	75.00
2	2	59	14×16	Cross	14	16	254	221	0.45	47.52	21.00	156.00	16473.60	9.00	26.00	4283.14	76.00
2	3	60	14×16	Cross	14	16	269	242	0.50	52.80	22.00	166.00	17529.60	10.00	28.00	4908.29	75.00
2	1	61	1×16	Cross	1	16	260	234	0.55	58.08	30.00	180.00	19008.00	9.00	24.00	4561.92	86.00
2	2	62	1×16	Cross	1	16	245	214	0.50	52.80	29.00	178.00	18796.80	10.00	26.00	4887.17	87.00
2	3	62	1×16	Cross	1	16	262	231	0.50	52.80	27.00	169.00	17846.40	9.00	28.00	4996.99	86.00
2	1	64	2×16	Cross	2	16	230	203	0.40	42.24	22.00	102.00	10771.20	19.00	33.00	3554.50	83.00
2	2	65	2×16	Cross	2	16	232	212	0.40	42.24	21.00	112.00	11827.20	18.00	30.00	3548.16	84.00

2	3	66	2×16	Cross	2	16	230	203	0.40	42.24	22.00	102.00	10771.20	19.00	31.00	3339.07	83.00
2	1	67	3×16	Cross	3	16	256	227	0.45	47.52	24.00	148.00	15628.80	18.00	32.00	5001.22	79.00
2	2	68	3×16	Cross	3	16	234	207	0.40	42.24	22.00	149.00	15734.40	19.00	30.00	4720.32	79.00
2	3	69	3×16	Cross	3	16	250	220	0.40	42.24	21.00	147.00	15523.20	19.00	32.00	4967.42	80.00
2	1	70	6×18	Cross	6	18	205	177	0.55	58.08	23.00	170.00	17952.00	20.00	30.00	5385.60	66.00
2	2	71	6×18	Cross	6	18	198	170	0.50	52.80	22.00	171.00	18057.60	21.00	28.00	5056.13	67.00
2	3	72	6×18	Cross	6	18	190	167	0.50	52.80	20.00	186.00	19641.60	19.00	30.00	5892.48	66.00
2	1	73	7×18	Cross	7	18	220	189	0.45	47.52	22.00	156.00	16473.60	16.00	28.00	4612.61	78.00
2	2	74	7×18	Cross	7	18	231	197	0.55	58.08	21.00	146.00	15417.60	17.00	29.00	4471.10	77.00
2	3	75	7×18	Cross	7	18	222	188	0.45	47.52	22.00	157.00	16579.20	16.00	28.00	4642.18	77.00
2	1	76	10×18	Cross	10	18	200	181	0.35	36.96	19.00	146.00	15417.60	17.00	26.00	4008.58	76.00
2	2	77	10×18	Cross	10	18	187	160	0.30	31.68	17.00	140.00	14784.00	18.00	23.00	3400.32	75.00
2	3	78	10×18	Cross	10	18	202	185	0.30	31.68	18.00	148.00	15628.80	18.00	27.00	4219.78	75.00
2	1	79	13×18	Cross	13	18	212	185	0.50	52.80	22.00	138.00	14572.80	24.00	32.00	4663.30	79.00
2	2	80	13×18	Cross	13	18	210	175	0.45	47.52	21.00	130.00	13728.00	22.00	31.00	4255.68	80.00
2	3	81	13×18	Cross	13	18	212	185	0.45	47.52	22.00	138.00	14572.80	24.00	32.00	4663.30	79.00
2	1	82	14×18	Cross	14	18	190	163	0.35	36.96	22.00	167.00	17635.20	20.00	26.00	4585.15	79.00
2	2	83	14×18	Cross	14	18	198	164	0.35	36.96	20.00	161.00	17001.60	19.00	24.00	4080.38	80.00
2	3	84	14×18	Cross	14	18	189	160	0.30	31.68	21.00	164.00	17318.40	18.00	25.00	4329.60	79.00
2	1	85	5×18	Cross	5	18	256	224	0.40	42.24	23.00	172.00	18163.20	20.00	28.00	5085.70	78.00
2	2	86	5×18	Cross	5	18	252	214	0.35	36.96	24.00	168.00	17740.80	19.00	27.00	4790.02	77.00
2	3	87	5×18	Cross	5	18	250	220	0.40	42.24	23.00	172.00	18163.20	20.00	28.00	5085.70	77.00
2	1	88	2×18	Cross	2	18	189	157	0.50	52.80	24.00	126.00	13305.60	18.00	30.00	3991.68	74.00
2	2	89	2×18	Cross	2	18	178	159	0.50	52.80	23.00	126.00	13305.60	17.00	28.00	3725.57	75.00
2	3	90	2×18	Cross	2	18	187	154	0.45	47.52	21.00	126.00	13305.60	19.00	31.00	4124.74	74.00
2	1	91	16	Parent	16	16	178	153	0.30	31.68	18.50	118.00	12460.80	19.00	31.80	3358.08	69.00
2	2	92	16	Parent	16	16	190	172	0.28	29.57	18.87	110.00	11616.00	18.00	29.40	3104.64	70.00
2	3	93	16	Parent	16	16	174	157	0.29	30.62	19.50	124.00	13094.40	17.00	35.80	3780.48	69.00
2	1	94	15	Parent	15	15	180	127	0.30	31.68	16.00	119.00	12566.40	16.00	35.40	3738.24	72.00
2	2	95	15	Parent	15	15	189	162	0.32	33.79	16.50	127.00	13411.20	17.00	32.80	3463.68	71.00
2	3	96	15	Parent	15	15	157	126	0.29	30.62	18.00	116.00	12249.60	17.00	33.80	3569.28	70.00
2	1	97	17	Parent	17	17	192	179	0.45	47.52	21.50	144.00	15206.40	11.00	26.00	2745.60	80.00
2	2	98	17	Parent	17	17	183	166	0.43	45.41	21.40	155.00	16368.00	10.00	25.00	2640.00	81.00
2	3	99	17	Parent	17	17	191	169	0.46	48.58	21.50	162.00	17107.20	11.00	28.40	2999.04	80.00
2	1	100	18	Parent	18	18	197	173	0.31	32.74	19.00	112.00	11827.20	16.00	34.20	3611.52	70.00
2	2	101	18	Parent	18	18	179	145	0.32	33.79	18.50	119.00	12566.40	17.00	33.00	3484.80	69.50

2	3	102	18	Parent	18	18	175	146	0.30	31.68	18.80	111.00	11721.60	18.00	31.00	3273.60	70.00
2	1	103	1	Parent	1	1	235	207	0.45	47.52	24.00	148.00	15628.80	10.00	22.50	2376.00	80.00
2	2	104	1	Parent	1	1	231	200	0.40	42.24	23.00	144.00	15206.40	9.00	23.00	2428.80	79.00
2	3	105	1	Parent	1	1	243	218	0.48	50.69	25.00	156.00	16473.60	9.00	26.00	2745.60	79.50
2	1	106	2	Parent	2	2	180	161	0.35	36.96	22.00	114.00	12038.40	17.00	28.00	2956.80	108.00
2	2	107	2	Parent	2	2	187	164	0.37	39.07	23.00	125.00	13200.00	16.00	27.50	2904.00	109.00
2	3	108	2	Parent	2	2	182	158	0.36	38.02	21.00	117.00	12355.20	18.00	26.00	2745.60	108.00
2	1	109	3	Parent	3	3	270	243	0.40	42.24	23.00	107.00	11299.20	14.00	24.00	2534.40	105.00
2	2	110	3	Parent	3	3	291	267	0.45	47.52	24.00	128.00	13516.80	13.00	26.00	2745.60	106.00
2	3	111	3	Parent	3	3	266	232	0.41	43.30	23.00	119.00	12566.40	14.00	25.20	2661.12	103.00
2	1	112	4	Parent	4	4	271	246	0.35	36.96	17.00	86.00	9081.60	10.00	27.00	2851.20	100.00
2	2	113	4	Parent	4	4	270	245	0.36	38.02	16.00	80.00	8448.00	9.00	26.00	2745.60	101.00
2	3	114	4	Parent	4	4	277	251	0.37	39.07	16.00	94.00	9926.40	9.00	24.00	2534.40	100.00
2	1	115	5	Parent	5	5	255	219	0.53	55.97	22.00	156.00	16473.60	13.00	25.00	2640.00	85.00
2	2	116	5	Parent	5	5	252	221	0.50	52.80	20.00	163.00	17212.80	12.00	27.00	2851.20	85.50
2	3	117	5	Parent	5	5	250	227	0.49	51.74	22.00	167.00	17635.20	13.00	26.00	2745.60	85.00
2	1	118	6	Parent	6	6	225	199	0.50	52.80	24.00	139.00	14678.40	11.00	24.00	2534.40	81.00
2	2	119	6	Parent	6	6	247	214	0.54	57.02	26.00	146.00	15417.60	10.00	25.00	2640.00	82.00
2	3	120	6	Parent	6	6	220	187	0.49	51.74	23.00	139.00	14678.40	11.00	26.00	2745.60	81.00
2	1	121	7	Parent	7	7	205	185	0.25	26.40	17.00	48.00	5068.80	12.00	28.00	2956.80	80.00
2	2	122	7	Parent	7	7	221	192	0.30	31.68	16.00	56.00	5913.60	12.00	28.00	2956.80	81.00
2	3	123	7	Parent	7	7	202	185	0.21	22.18	16.00	43.00	4540.80	11.00	28.00	2956.80	79.00
2	1	124	8	Parent	8	8	221	202	0.31	32.74	14.50	49.00	5174.40	13.00	23.60	2492.16	88.00
2	2	125	8	Parent	8	8	215	191	0.30	31.68	14.50	49.00	5174.40	13.00	23.60	2492.16	89.00
2	3	126	8	Parent	8	8	235	200	0.35	36.96	14.50	68.00	7180.80	13.00	23.60	2492.16	88.00
2	1	127	9	Parent	9	9	205	181	0.21	22.18	13.00	60.00	6336.00	12.00	21.00	2217.60	97.00
2	2	128	9	Parent	9	9	218	192	0.25	26.40	15.00	73.00	7708.80	12.00	21.00	2217.60	96.00
2	3	129	9	Parent	9	9	203	178	0.20	21.12	13.50	62.00	6547.20	12.00	21.00	2217.60	95.00
2	1	130	10	Parent	10	10	214	171	0.34	35.90	19.00	84.00	8870.40	5.00	22.00	2323.20	87.00
2	2	131	10	Parent	10	10	217	189	0.35	36.96	19.00	94.00	9926.40	5.00	23.00	2428.80	88.00
2	3	132	10	Parent	10	10	226	191	0.35	36.96	19.00	80.00	8448.00	5.00	22.00	2323.20	87.00
2	1	133	11	Parent	11	11	194	178	0.45	47.52	20.00	126.00	13305.60	4.00	19.00	2006.40	96.00
2	2	134	11	Parent	11	11	199	174	0.47	49.63	21.00	126.00	13305.60	5.00	20.50	2164.80	97.00
2	3	135	11	Parent	11	11	191	172	0.42	44.35	20.00	127.00	13411.20	4.00	20.00	2112.00	96.00
2	1	136	12	Parent	12	12	170	156	0.23	24.29	14.00	52.00	5491.20	9.00	27.00	2851.20	112.00
2	2	137	12	Parent	12	12	178	159	0.25	26.40	15.00	72.00	7603.20	10.00	28.00	2956.80	111.00

2	3	138	12	Parent	12	12	172	146	0.20	21.12	14.00	56.00	5913.60	9.00	27.00	2851.20	112.00
2	1	139	13	Parent	13	13	182	164	0.15	15.84	12.00	30.00	3168.00	10.00	25.50	2692.80	81.00
2	2	140	13	Parent	13	13	187	166	0.21	22.18	12.00	47.00	4963.20	9.00	26.00	2745.60	82.00
2	3	141	13	Parent	13	13	181	169	0.17	17.95	12.00	32.00	3379.20	10.00	25.50	2692.80	81.00
2	1	142	14	Parent	14	14	225	189	0.28	29.57	15.00	102.00	10771.20	9.00	18.00	1900.80	78.00
2	2	143	14	Parent	14	14	217	180	0.24	25.34	15.00	89.00	9398.40	9.00	17.00	1795.20	79.00
2	3	144	14	Parent	14	14	220	187	0.25	26.40	15.00	91.00	9609.60	9.00	16.00	1689.60	78.00
3	1	1	6×15	Cross	6	15	224	193	0.75	79.20	27.00	224.00	23654.40	25.00	35.00	8279.04	69.00
3	2	2	6×15	Cross	6	15	220	191	0.70	73.92	25.00	218.00	23020.80	26.00	36.00	8287.49	68.00
3	3	3	6×15	Cross	6	15	221	190	0.70	73.92	26.00	221.00	23337.60	26.00	34.00	7934.78	67.00
3	1	4	7×15	Cross	7	15	267	235	0.45	47.52	16.00	112.00	11827.20	17.00	28.00	3311.62	77.00
3	2	5	7×15	Cross	7	15	261	230	0.43	45.41	17.00	118.00	12460.80	16.00	29.00	3613.63	78.00
3	3	6	7×15	Cross	7	15	269	236	0.42	44.35	16.00	110.00	11616.00	16.50	28.00	3252.48	79.00
3	1	7	9×15	Cross	9	15	219	184	0.40	42.24	15.00	88.00	9292.80	11.00	26.00	2416.13	80.00
3	2	8	9×15	Cross	9	15	214	179	0.43	45.41	16.00	84.00	8870.40	12.00	27.00	2395.01	81.00
3	3	9	9×15	Cross	9	15	212	181	0.40	42.24	15.00	89.00	9398.40	11.00	24.00	2255.62	80.00
3	1	10	13×15	Cross	13	15	245	219	0.50	52.80	22.00	128.00	13516.80	14.00	28.00	3784.70	83.00
3	2	11	13×15	Cross	13	15	240	213	0.52	54.91	21.00	134.00	14150.40	15.00	26.00	3679.10	82.00
3	3	12	13×15	Cross	13	15	242	220	0.50	52.80	22.00	132.00	13939.20	16.00	27.00	3763.58	81.00
3	1	13	14×15	Cross	14	15	260	233	0.33	34.85	17.00	79.00	8342.40	24.00	30.00	2502.72	77.00
3	2	14	14×15	Cross	14	15	256	227	0.30	31.68	18.00	82.00	8659.20	23.00	31.00	2684.35	78.00
3	3	15	14×15	Cross	14	15	261	235	0.34	35.90	17.00	97.00	10243.20	23.00	30.00	3072.96	77.50
3	1	16	1×15	Cross	1	15	242	211	0.55	58.08	27.00	220.00	23232.00	9.00	34.00	7898.88	75.00
3	2	17	1×15	Cross	1	15	238	206	0.50	52.80	26.00	214.00	22598.40	10.00	32.00	7231.49	76.00
3	3	18	1×15	Cross	1	15	241	209	0.53	55.97	25.00	220.00	23232.00	10.00	30.00	6969.60	74.00
3	1	19	5×15	Cross	5	15	209	175	0.70	73.92	26.00	237.00	25027.20	9.00	35.00	8759.52	83.00
3	2	20	5×15	Cross	5	15	212	179	0.72	76.03	27.00	238.00	25132.80	9.00	33.00	8293.82	82.00
3	3	21	5×15	Cross	5	15	199	170	0.67	70.75	25.00	229.00	24182.40	11.00	35.00	8463.84	82.50
3	1	22	11×15	Cross	11	15	195	169	0.45	47.52	25.00	171.00	18057.60	10.00	30.00	5417.28	90.00
3	2	23	11×15	Cross	11	15	191	164	0.44	46.46	24.00	172.00	18163.20	9.00	28.00	5085.70	91.00
3	3	24	11×15	Cross	11	15	194	160	0.46	48.58	25.00	176.00	18585.60	10.00	31.00	5761.54	89.00
3	1	25	6×17	Cross	6	17	297	270	0.65	68.64	25.00	239.00	25238.40	22.00	38.00	9590.59	90.00
3	2	26	6×17	Cross	6	17	290	261	0.62	65.47	26.00	243.00	25660.80	21.00	36.00	9237.89	89.50
3	3	27	6×17	Cross	6	17	294	269	0.66	69.70	25.00	223.00	23548.80	23.00	38.00	8948.54	91.00
3	1	28	7×17	Cross	7	17	212	178	0.33	34.85	18.00	128.00	13516.80	18.00	28.00	3784.70	78.00
3	2	29	7×17	Cross	7	17	217	169	0.30	31.68	19.00	132.00	13939.20	17.00	29.00	4042.37	79.00

3	3	30	7×17	Cross	7	17	210	175	0.30	31.68	18.00	128.00	13516.80	18.00	27.00	3649.54	78.00
3	1	31	10×17	Cross	10	17	208	174	0.42	44.35	24.00	114.00	12038.40	12.00	32.00	3852.29	76.00
3	2	32	10×17	Cross	10	17	234	179	0.40	42.24	22.00	102.00	10771.20	13.00	30.00	3231.36	75.00
3	3	33	10×17	Cross	10	17	204	171	0.40	42.24	24.00	100.00	10560.00	13.00	32.00	3379.20	75.00
3	1	34	12×17	Cross	12	17	200	169	0.65	68.64	27.00	249.00	26294.40	18.00	30.00	7888.32	74.00
3	2	35	12×17	Cross	12	17	205	174	0.64	67.58	27.00	249.00	26294.40	18.00	29.00	7625.38	73.00
3	3	36	12×17	Cross	12	17	198	173	0.62	65.47	26.00	258.00	27244.80	17.00	30.00	8173.44	74.00
3	1	37	13×17	Cross	13	17	262	230	0.70	73.92	24.00	211.00	22281.60	23.00	34.00	7575.74	78.00
3	2	38	13×17	Cross	13	17	258	224	0.67	70.75	22.00	201.00	21225.60	23.00	32.00	6792.19	79.00
3	3	39	13×17	Cross	13	17	260	229	0.71	74.98	23.00	205.00	21648.00	24.00	34.00	7360.32	78.50
3	1	40	14×17	Cross	14	17	211	180	0.30	31.68	18.00	114.00	12038.40	16.00	27.00	3250.37	74.00
3	2	41	14×17	Cross	14	17	217	186	0.33	34.85	17.00	123.00	12988.80	17.00	29.00	3766.75	75.00
3	3	42	14×17	Cross	14	17	208	189	0.30	31.68	18.00	118.00	12460.80	15.00	28.00	3489.02	74.00
3	1	43	1×17	Cross	1	17	245	213	0.90	95.04	28.00	271.00	28617.60	18.00	34.00	9729.98	74.50
3	2	44	1×17	Cross	1	17	240	216	0.92	97.15	29.00	280.00	29568.00	17.00	32.00	9461.76	74.00
3	3	45	1×17	Cross	1	17	235	204	0.79	83.42	27.00	274.00	28934.40	19.00	33.00	9548.35	74.00
3	1	46	4×17	Cross	4	17	249	217	0.41	43.30	21.00	116.00	12249.60	17.00	30.00	3674.88	73.00
3	2	47	4×17	Cross	4	17	241	210	0.40	42.24	22.00	134.00	14150.40	16.00	31.00	4386.62	74.00
3	3	48	4×17	Cross	4	17	243	212	0.43	45.41	20.00	119.00	12566.40	18.00	30.00	3769.92	73.00
3	1	49	6×16	Cross	6	16	240	207	0.40	42.24	24.00	148.00	15628.80	23.00	31.00	4844.93	91.00
3	2	50	6×16	Cross	6	16	238	211	0.42	44.35	23.00	145.00	15312.00	20.00	29.00	4440.48	90.00
3	3	51	6×16	Cross	6	16	241	209	0.40	42.24	21.00	140.00	14784.00	21.00	30.00	4435.20	91.00
3	1	52	7×16	Cross	7	16	202	171	0.60	63.36	24.00	198.00	20908.80	10.00	26.00	5436.29	80.00
3	2	53	7×16	Cross	7	16	189	181	0.56	59.14	25.00	216.00	22809.60	10.00	28.00	6386.69	81.00
3	3	54	7×16	Cross	7	16	200	170	0.60	63.36	23.00	194.00	20486.40	9.00	26.00	5326.46	82.00
3	1	55	9×16	Cross	9	16	243	217	0.60	63.36	23.00	178.00	18796.80	20.00	29.00	5451.07	77.00
3	2	56	9×16	Cross	9	16	240	222	0.58	61.25	24.00	184.00	19430.40	19.00	32.00	6217.73	76.00
3	3	57	9×16	Cross	9	16	241	219	0.61	64.42	21.00	189.00	19958.40	20.00	29.00	5787.94	78.00
3	1	58	14×16	Cross	14	16	252	221	0.45	47.52	22.00	151.00	15945.60	10.00	27.00	4305.31	75.00
3	2	59	14×16	Cross	14	16	248	219	0.47	49.63	23.00	164.00	17318.40	9.00	28.00	4849.15	76.00
3	3	60	14×16	Cross	14	16	250	220	0.45	47.52	22.00	153.00	16156.80	10.00	27.00	4362.34	75.00
3	1	61	1×16	Cross	1	16	235	204	0.40	42.24	30.00	164.00	17318.40	10.00	26.00	4502.78	86.00
3	2	62	1×16	Cross	1	16	236	214	0.45	47.52	28.00	183.00	19324.80	9.00	30.00	5797.44	87.00
3	3	63	1×16	Cross	1	16	230	199	0.43	45.41	30.00	164.00	17318.40	11.00	28.00	4849.15	86.00
3	1	64	2×16	Cross	2	16	200	173	0.40	42.24	22.00	102.00	10771.20	19.00	33.00	3554.50	83.00
3	2	65	2×16	Cross	2	16	189	162	0.38	40.13	23.00	98.00	10348.80	20.00	30.00	3104.64	82.00

3	3	66	2×16	Cross	2	16	203	170	0.40	42.24	21.00	104.00	10982.40	19.00	33.00	3624.19	83.00
3	1	67	3×16	Cross	3	16	222	194	0.41	43.30	24.00	138.00	14572.80	18.00	32.00	4663.30	79.00
3	2	68	3×16	Cross	3	16	213	189	0.40	42.24	22.00	136.00	14361.60	17.00	30.00	4308.48	79.00
3	3	69	3×16	Cross	3	16	220	194	0.42	44.35	24.00	149.00	15734.40	19.00	32.00	5035.01	79.00
3	1	70	6×18	Cross	6	18	199	167	0.50	52.80	23.00	152.00	16051.20	20.00	30.00	4815.36	66.00
3	2	71	6×18	Cross	6	18	203	179	0.52	54.91	24.00	164.00	17318.40	21.00	28.00	4849.15	67.00
3	3	72	6×18	Cross	6	18	197	165	0.48	50.69	23.00	168.00	17740.80	20.00	30.00	5322.24	66.00
3	1	73	7×18	Cross	7	18	189	160	0.40	42.24	22.00	149.00	15734.40	16.00	28.00	4405.63	77.00
3	2	74	7×18	Cross	7	18	180	156	0.37	39.07	23.00	161.00	17001.60	15.00	32.00	5440.51	76.00
3	3	75	7×18	Cross	7	18	184	162	0.43	45.41	21.00	159.00	16790.40	16.00	28.00	4701.31	77.00
3	1	76	10×18	Cross	10	18	179	151	0.29	30.62	18.00	137.00	14467.20	17.00	26.00	3761.47	75.00
3	2	77	10×18	Cross	10	18	177	150	0.30	31.68	19.00	141.00	14889.60	18.00	28.00	4169.09	76.00
3	3	78	10×18	Cross	10	18	174	149	0.31	32.74	18.00	139.00	14678.40	17.00	26.00	3816.38	75.00
3	1	79	13×18	Cross	13	18	183	152	0.40	42.24	22.00	128.00	13516.80	24.00	32.00	4325.38	79.50
3	2	80	13×18	Cross	13	18	190	153	0.43	45.41	23.00	134.00	14150.40	23.00	31.00	4386.62	79.00
3	3	81	13×18	Cross	13	18	180	157	0.40	42.24	22.00	126.00	13305.60	24.00	32.00	4257.79	80.00
3	1	82	14×18	Cross	14	18	164	133	0.32	33.79	22.00	152.00	16051.20	20.00	26.00	4173.31	79.00
3	2	83	14×18	Cross	14	18	169	138	0.35	36.96	23.00	152.00	16051.20	20.00	27.00	4333.82	78.00
3	3	84	14×18	Cross	14	18	160	130	0.30	31.68	20.00	164.00	17318.40	21.00	26.00	4502.78	79.00
3	1	85	5×18	Cross	5	18	227	201	0.43	45.41	22.00	163.00	17212.80	20.00	28.00	4819.58	77.00
3	2	86	5×18	Cross	5	18	220	191	0.40	42.24	23.00	168.00	17740.80	21.00	30.00	5322.24	78.00
3	3	87	5×18	Cross	5	18	226	204	0.40	42.24	21.00	163.00	17212.80	20.00	28.00	4819.58	77.00
3	1	88	2×18	Cross	2	18	167	140	0.45	47.52	23.00	118.00	12460.80	18.00	30.00	3738.24	74.00
3	2	89	2×18	Cross	2	18	160	137	0.43	45.41	22.00	112.00	11827.20	20.00	31.00	3666.43	75.00
3	3	90	2×18	Cross	2	18	156	131	0.42	44.35	24.00	127.00	13411.20	19.00	30.00	4023.36	74.00
3	1	91	16	Parent	16	16	171	153	0.40	42.24	18.50	138.00	14572.80	21.00	31.80	4634.15	72.50
3	2	92	16	Parent	16	16	193	172	0.40	42.24	18.87	130.00	13728.00	20.00	29.40	4036.03	72.00
3	3	93	16	Parent	16	16	186	157	0.40	42.24	19.50	124.00	13094.40	19.50	35.80	4687.80	71.50
3	1	94	15	Parent	15	15	190	157	0.28	29.57	16.00	118.00	12460.80	18.00	35.40	4411.12	69.50
3	2	95	15	Parent	15	15	178	162	0.30	31.68	16.50	108.00	11404.80	18.50	32.80	3740.77	68.00
3	3	96	15	Parent	15	15	167	136	0.30	31.68	18.00	126.00	13305.60	19.00	33.80	4497.29	69.00
3	1	97	17	Parent	17	17	190	179	0.48	50.69	21.50	244.00	25766.40	11.00	26.00	6699.26	81.00
3	2	98	17	Parent	17	17	187	169	0.46	48.58	21.40	225.00	23760.00	11.50	25.00	5940.00	81.50
3	3	99	17	Parent	17	17	189	169	0.49	51.74	21.50	242.00	25555.20	10.00	28.40	7257.68	82.00
3	1	100	18	Parent	18	18	195	172	0.34	35.90	19.00	122.00	12883.20	18.50	34.20	4406.05	56.00
3	2	101	18	Parent	18	18	173	152	0.32	33.79	18.50	129.00	13622.40	19.50	33.00	4495.39	57.50

3	3	102	18	Parent	18	18	176	146	0.30	31.68	18.80	119.00	12566.40	18.00	31.00	3895.58	57.00
3	1	103	1	Parent	1	1	185	161	0.40	42.24	16.00	48.00	5068.80	9.00	24.00	2534.40	76.00
3	2	104	1	Parent	1	1	198	171	0.45	47.52	17.00	56.00	5913.60	9.00	24.50	2587.20	77.00
3	3	105	1	Parent	1	1	181	160	0.46	48.58	18.00	68.00	7180.80	10.00	24.50	2587.20	76.00
3	1	106	2	Parent	2	2	212	183	0.35	36.96	15.00	97.00	10243.20	13.00	25.00	2640.00	107.00
3	2	107	2	Parent	2	2	231	192	0.38	40.13	15.00	94.00	9926.40	14.00	24.50	2587.20	106.00
3	3	108	2	Parent	2	2	210	181	0.36	38.02	15.00	96.00	10137.60	13.00	26.00	2745.60	107.00
3	1	108	3	Parent	3	3	251	228	0.42	44.35	16.00	49.00	5174.40	12.00	24.20	2555.52	104.00
3	2	110	3	Parent	3	3	247	238	0.45	47.52	18.00	64.00	6758.40	11.00	25.00	2640.00	105.00
3	3	111	3	Parent	3	3	249	234	0.41	43.30	16.00	56.00	5913.60	12.00	24.20	2555.52	104.00
3	1	112	4	Parent	4	4	267	242	0.40	42.24	17.00	90.00	9504.00	9.00	27.00	2851.20	98.00
3	2	113	4	Parent	4	4	260	241	0.38	40.13	18.00	87.00	9187.20	9.00	28.00	2956.80	97.00
3	3	114	4	Parent	4	4	259	238	0.38	40.13	17.00	80.00	8448.00	9.00	27.00	2851.20	98.00
3	1	115	5	Parent	5	5	265	239	0.37	39.07	18.00	84.00	8870.40	12.00	24.50	2587.20	85.00
3	2	116	5	Parent	5	5	276	251	0.40	42.24	19.00	91.00	9609.60	12.00	24.00	2534.40	84.00
3	3	117	5	Parent	5	5	261	234	0.34	35.90	18.00	82.00	8659.20	12.00	24.50	2587.20	84.00
3	1	118	6	Parent	6	6	253	235	0.35	36.96	16.00	99.00	10454.40	10.00	24.00	2534.40	81.00
3	2	119	6	Parent	6	6	254	237	0.32	33.79	16.00	112.00	11827.20	10.00	25.00	2640.00	80.00
3	3	120	6	Parent	6	6	241	235	0.32	33.79	17.00	109.00	11510.40	10.00	24.00	2534.40	80.00
3	1	121	7	Parent	7	7	216	179	0.28	29.57	16.00	56.00	5913.60	12.00	28.00	2956.80	79.00
3	2	122	7	Parent	7	7	219	180	0.30	31.68	14.00	71.00	7497.60	12.00	28.50	3009.60	80.00
3	3	123	7	Parent	7	7	209	184	0.31	32.74	15.00	48.00	5068.80	12.00	28.00	2956.80	79.00
3	1	124	8	Parent	8	8	241	212	0.33	34.85	14.00	53.00	5596.80	11.00	23.60	2492.16	86.00
3	2	125	8	Parent	8	8	249	208	0.35	36.96	14.50	62.00	6547.20	12.00	23.00	2428.80	87.00
3	3	126	8	Parent	8	8	244	211	0.33	34.85	14.50	56.00	5913.60	11.00	23.60	2492.16	87.00
3	1	127	9	Parent	9	9	237	213	0.30	31.68	16.00	88.00	9292.80	12.00	21.00	2217.60	96.00
3	2	128	9	Parent	9	9	230	203	0.27	28.51	15.00	72.00	7603.20	13.00	22.00	2323.20	95.00
3	3	129	9	Parent	9	9	228	196	0.25	26.40	14.00	76.00	8025.60	12.00	21.00	2217.60	95.00
3	1	130	10	Parent	10	10	210	184	0.38	40.13	19.00	91.00	9609.60	5.00	22.00	2323.20	87.00
3	2	131	10	Parent	10	10	218	195	0.40	42.24	20.00	112.00	11827.20	5.00	21.00	2217.60	86.00
3	3	132	10	Parent	10	10	212	180	0.36	38.02	19.00	98.00	10348.80	6.00	22.00	2323.20	86.00
3	1	133	11	Parent	11	11	219	188	0.42	44.35	18.00	126.00	13305.60	4.00	24.50	2587.20	95.00
3	2	134	11	Parent	11	11	223	191	0.45	47.52	18.00	132.00	13939.20	4.00	24.50	2587.20	96.00
3	3	135	11	Parent	11	11	220	187	0.40	42.24	17.00	126.00	13305.60	5.00	24.50	2587.20	95.00
3	1	136	12	Parent	12	12	192	166	0.25	26.40	15.00	52.00	5491.20	9.00	27.00	2851.20	110.00
3	2	137	12	Parent	12	12	190	164	0.23	24.29	14.00	58.00	6124.80	9.00	27.00	2851.20	111.00

3	3	138	12	Parent	12	12	198	171	0.27	28.51	16.00	67.00	7075.20	8.00	26.00	2745.60	110.00
3	1	139	13	Parent	13	13	179	154	0.22	23.23	13.00	36.00	3801.60	10.00	25.50	2692.80	81.00
3	2	140	13	Parent	13	13	174	150	0.20	21.12	14.00	30.00	3168.00	10.00	25.50	2692.80	82.00
3	3	141	13	Parent	13	13	182	159	0.25	26.40	13.00	48.00	5068.80	11.00	25.50	2692.80	81.00
3	1	142	14	Parent	14	14	148	121	0.30	31.68	15.00	96.00	10137.60	9.00	18.00	1900.80	79.00
3	2	143	14	Parent	14	14	141	119	0.25	26.40	15.00	89.00	9398.40	10.00	17.00	1795.20	80.00
3	3	144	14	Parent	14	14	145	123	0.27	28.51	16.00	92.00	9715.20	9.00	16.00	1689.60	79.00

;

Run;
*STEP 1: 'Analysis of all genotypes comprising parents and crosses pooled over sites';
Data LxT1;set LxT1; **run**;
Proc glm;
Class Site REP Entry GENOTYPE PvsC Lines Testers;
Model HTG HTFL AVWT CYTONHA GIRTH JVOLml JVOLHaL BRIX ETHVOLml ETHVOLHa DTF = Site Rep(site) Genotype Genotype*Site;
random Site Rep(site) Genotype*Site/test;
means GENOTYPE/lsd E=Genotype*Site;
lsmeans Genotype/stderr;
Run;
*STEP 2: 'Analysis of parents across sites';
Data Parents; set LxT1;
If entry <**91** then delete;
parents = entry;
Proc glm;
Class Site REP Parents GENOTYPE PvsC Lines Testers;
Model HTG HTFL AVWT CYTONHA GIRTH JVOLml JVOLHaL BRIX ETHVOLml ETHVOLHa DTF = Site Rep(site) parents parents*site;
lsmeans parents/stderr;**Run**;
*STEP 3: 'Analysis of parents versus crosses - estimate of average heterosis';
Data LxT3; set LxT1;
Proc glm;
Class Site REP Entry GENOTYPE PvsC Lines Testers;
Model HTG HTFL AVWT CYTONHA GIRTH JVOLml JVOLHaL BRIX ETHVOLml ETHVOLHa DTF = Site Rep(site) PvsC PvsC*Site;lsmeans PvsC/stderr;
Run;
*STEP 4: 'Analysis of crosses across sites';
Data cross; set LxT1;
If entry >**90** then delete;
Cross = entry;
Proc glm;
Class Site REP cross GENOTYPE PvsC Lines Testers;
Model HTG HTFL AVWT CYTONHA GIRTH JVOLml JVOLHaL BRIX ETHVOLml ETHVOLHa DTF = Site Rep(site) cross cross*site;
lsmeans Cross/stderr;**Run**;
*STEP 5: 'Line x Tester Analysis - i.e., partitioning of the crosses';**Data** LxT2; set Lxt1;*Remove parents by activating any of the 2 statements below;
*if entry >90 then delete;If Lines = Testers then delete;**Proc glm**; Class Site REP Entry Genotype PvsC Lines Testers;
Model HTG HTFL AVWT CYTONHA GIRTH JVOLml JVOLHaL BRIX ETHVOLml ETHVOLHa DTF = Site Rep(site) Lines Testers Lines*Testers Lines*Site Testers*Site Lines*Testers*Site;
random Site Rep(site) Lines Testers Lines*Testers Lines*Site Testers*Site Lines*Testers*Site/test;
lsmeans Lines Testers Lines*Testers/stderr;
Run;Quit;
/*Then combine OUTPUTs from the 5 analyses into one table as follows:
Sources of variation: SitesRep(sites)Genotypes Parents Parents vs. Crosses Crosses Lines Testers Lines*Testers Error*/

APPENDIX II: PROGRAM EDITOR FOR COMBINED ANALYSIS

```
data comanalysis;
input GEN $ LOC $ STAGE REP HTG HTFL AVWT CYTONHA GIRTH JVOLml AEthVol
JWTg JVOLHaL BRIX WBWTg WBTONHA DBWT ETHVOLml ETHCONC ETHVOLHA
AbsEtHa DTF CHLOR GRAINWT AVGRAINWT;
twbw=(88660*WBWTg)/(1000*1000);
cards;
.
.
.
.
*Step 1: 'Combined Analysis of Variance across four Environments';
proc glm;
class GEN LOC STAGE REP;
model HTG HTFL AVWT CYTONHA GIRTH JVOLml AEthVol JWTg JVOLHaL BRIX
WBWTg twbw WBTONHA DBWT ETHVOLml ETHCONC ETHVOLHA AbsEtHa DTF CHLOR
GRAINWT AVGRAINWT=LOC REP(LOC) STAGE LOC*STAGE GEN GEN*LOC GEN*STAGE
LOC*GEN*STAGE/ss4;
random LOC REP(LOC) LOC*STAGE GEN*LOC GEN*STAGE LOC*GEN*STAGE/test;
means LOC /lsd E=STAGE;
means GEN/lsd E=GEN*STAGE;
Means STAGE/lsd E=GEN*STAGE;
means LOC GEN LOC*GEN STAGE STAGE*LOC GEN*STAGE LOC*GEN*STAGE/lsd;

/*Step 2: 'Individual Environmental Analysis';*/
/*data comanalysis;*/
proc sort; by LOC;
proc glm; by loc;
class GEN STAGE REP;
Model HTG HTFL AVWT CYTONHA GIRTH JVOLml AEthVol JWTg JVOLHaL BRIX
WBWTg WBTONHA DBWT ETHVOLml ETHCONC ETHVOLHA AbsEtHa DTF CHLOR GRAINWT
AVGRAINWT=REP STAGE GEN GEN*STAGE/ p ss4;
means GEN STAGE GEN*STAGE/lsd;
run;
```

APPENDIX III: PROGRAM EDITOR FOR HYBRID EVALUATION

```
data Hybrids;
input GENOTYPE $        ENV $   REP      HTG      HTFL AVWT CYTONHA GIRTH JVOLml
     JVOLHaL    BRIX    ETHVOLml  ETHVOLHa DTF;
Eff= (ETHVOLml/JVOLml)*100;
EffC=(ETHVOLml/AVWT)*100;
RBrix=(BRIX/ETHVOLml);
cards;
.
.
.
;
proc print;
proc glm;
class GENOTYPE ENV REP;
model HTG        HTFL    AVWT  CYTONHA GIRTH JVOLml JVOLHaL      BRIX    ETHVOLml
     ETHVOLHa DTF Eff EffC RBrix=LOC REP(LOC) GENOTYPE GENOTYPE*ENV/ss4;
test h= ENV E=GENOTYPE;
test h= GENOTYPE E=GENOTYPE*ENV;
random ENV REP(ENV) GENOTYPE*ENV/test;
means GENOTYPE/lsd E=GENOTYPE*ENV;
means ENV/lsd E=REP(ENV);
proc sort; by ENV;
proc glm; by ENV;
class Rep Genotype;
model HTG HTFL AVWT CYTONHA GIRTH JVOLml JVOLHaL BRIX ETHVOLml ETHVOLHa
     DTF Eff EffC RBrix=Rep Genotype;
means GENOTYPE  GENOTYPE/dunnett('SS04');
run;
```

APPENDIX IV: PUBLICATIONS