Genetic Diversity Assessment and Evaluation of the Concentration and Stage of Application of a Male Gametocide for Hybrid Development in Sweet Stem Sorghum for Bioethanol Production

By

Precious Mangena

BSc Agriculture (Hons) (UKZN); MSc Plant Breeding (UKZN)

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy (PhD) in Plant Breeding

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

May, 2018

Thesis abstract

Sweet stem sorghum is a form of grain sorghum that has been increasingly used for the industrial production of bio-ethanol. In comparison with the world's principal biofuel crops, sweet stem sorghum exhibits a number of valuable traits, such as its ability to grow under relatively harsh growing conditions, and its high biomass production. The sweet stem sorghum enterprise for bioenergy production requires highly productive, open pollinated or hybrid cultivars, delivered through a dedicated breeding program. Therefore, the objectives of this study were: i) to determine the phenotypic variability present among diverse sweet stem sorghum genotypes based on ethanol production and related agronomic traits to select promising breeding lines; ii) to evaluate the genetic interrelationships among selected sweet stem sorghum genotypes using polymorphic simple sequence repeat (SSR) markers to complement the phenotypic data; iii) to investigate the concentration, stage of application and frequency of application of ethyl 4'fluorooxanilate (E_4FO) for inducing male sterility of sweet stem sorghum without affecting female fertility for hybrid breeding; and iv) to determine the combining ability between selected sweet stem sorghum testers and lines, and to assess heterosis in sweet stem sorghum hybrids for bio-ethanol production and related traits.

One hundred and ninety sorghum genotypes were phenotypically evaluated. Data collected included days to 50% flowering, plant height, stem diameter, fresh biomass yield, dry matter yield, fibre content, stalk brix and ethanol yield. Data were subjected to analysis of variance, cluster analysis, correlation analysis, path coefficient analysis and principal component analysis. Significant differences were detected among tested genotypes for all measured traits. The best genotypes for ethanol productivity were AS203, AS391, AS205, AS251 and AS448, which provided estimated mean ethanol yields of 5474 l ha^{-1}, 4509 l ha^{-1}, 4315 l ha^{-1}, 4205 l ha^{-1} and 3816 l ha^{-1}, in that order. Days to flowering, plant height, stalk brix and stem diameter exerted the greatest indirect effects on ethanol production through higher biomass production. Biomass yield had the greatest direct effect on ethanol production.

Eighteen phenotypically divergent sweet stem sorghum genotypes were evaluated using 25 polymorphic simple sequence repeat (SSR) markers. The results revealed the presence of clear genetic differentiation among the studied sweet stem sorghum genotypes. The polymorphic information content (PIC) values for all markers ranged from 0.00 to 0.85, with a mean value of 0.56, implying that the markers were highly informative and discriminatory. Sixty eight percent of the markers used had a PIC value > 0.50. Analysis of molecular variance revealed highly significant differences ($P<0.001$) among the test population. Among and within individual variances contributed to 78% and 21% of the total

genetic variance, respectively. This allowed selection of representative and well-differentiated sweet stem genotypes such as AS391, SS27, AS204 and AS244.

Two experiments were conducted to investigate the concentration, stage of application and frequency of application of a putative male gametocide, ethyl 4'fluorooxanilate (E_4FO), for the induction of male sterility in sweet stem sorghum parents, without affecting female fertility. Three sweet stem sorghum genotypes were tested at three application stages, with five E_4FO doses during the first experiment. In the second experiment, the frequency of application of E_4FO was determined using three sweet stem sorghum genotypes, three E_4FO doses, and six frequencies of application. Data on male sterility was inferred, based on seed set and seed count from the treated plants. To determine female fertility, controlled crosses were performed, seed set was assessed and the number of seeds on cross pollinated plants were counted. Male sterility and female fertility were assessed against comparative control treatments. High levels of male sterility were achieved when E_4FO was applied during the heading stage using the following rates: 1000 mg l^{-1}, 1500 mg l^{-1} and 2000 mg l^{-1}, with more than one application. Applying E_4FO twice during the heading stage at a rate of 2000 mg l^{-1} would induce male sterility in the tested sweet stem sorghum genotypes.

Eight selected sweet stem sorghum lines and four testers were crossed using a Line x Tester mating design. The F1 hybrids and parental lines were evaluated for bioethanol yield and related traits. Data were subjected to analysis of variance, combining ability and heterosis analyses. Tested sweet stem sorghum genotypes showed ethanol yields varying from 787 l ha^{-1} to 5470 l ha^{-1}, with a mean of 2055 l ha^{-1}. Four hybrids (AS246 x AS391, AS251 x AS204, AS79 x AS204, AS74 x AS204) expressed the best ethanol productivity with positive better-parent heterosis (>30 %). Lines AS253, AS246, AS 105 and testers AS391 and SS27 had highly positive general combining ability (GCA) effects for almost all the traits in a desirable direction. Due to its consistent, significant and positive GCA effect across majority of the traits, line AS253 can be recommended for sweet stem sorghum hybrid breeding. Among the new hybrids or test parents, ethanol productivity had significant and positive correlations with plant height, stem diameter and biomass.

Overall, the study established the existence of considerable genetic diversity among sweet stem sorghum genotypes morphologically and genotypically. The selected promising parental genotypes and experimental hybrids are recommended for bio-ethanol production and breeding.

Declaration

I, **PRECIOUS MANGENA**, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

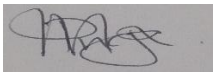
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

   a. Their words have been re-written but the general information attributed to them has been referenced

   b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.

5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.



……

Precious Mangena (Candidate)

Date: 25 May 2018

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

……

Professor Hussein Shimelis (Supervisor)

……

Professor Mark Laing (Co-supervisor)

Acknowledgements

My sincere gratitude and appreciation goes to the following people and institutes:

- My main supervisor, Prof Hussein Shimelis, and co-supervisor, Prof Mark Laing, for their guidance, support, patience and ensuring the success and completion of this study.
- The Technology Innovation Agency (TIA), South Africa for sponsoring my study.
- The National Research Foundation (NRF) for funding my third year of PhD study.
- Mr Ian Doidge and technical staff for field work support.
- The administrators, Margie Bowen, Ziyanda Ndlovu, and Noxolo Mncwabe for administrative support.
- Incotec (Pty) Ltd for assistance with the SSR data analysis.
- My mother, brother and grandmother: thank you for your love, prayers, sacrifices, support, understanding and encouragement. May the Lord continue to shower us with His blessings.
- My colleagues Amelework, Learnmore, Admire and Isack: thank you for your assistance throughout the study.

Dedication

To HIM who began a good thing in me and continues to cause all things to work together for my good.

Publications emanating from this thesis

1. Mangena, P., Shimelis, H. and Laing, M. 2017. Characterisation of sweet stem sorghum genotypes for bio-ethanol production. *Acta Agriculturae Scandinavica, Section B — Soil and Plant Science* 68:323-333.

2. Mangena, P., Shimelis, H., Laing, M. and Amelework, B. 2018. Genetic interrelationship of sweet stem sorghum genotypes assessed through simple sequence repeat markers. *In Press South African Journal of Plant and Soil Science*. https://doi.org/10.1080/02571862.2018.1446224

Table of contents

List of figures

List of tables

Introduction to thesis

Sorghum (*Sorghum bicolor* L. Moench) is the fifth important cereal crop after wheat, rice, maize and barley (Deepak *et al*., 2018). Sorghum originates from Ethiopia and its cultivation has spread from Africa to Asia, Australia and the Americas (Doggett, 1988). It has been documented that sorghum was grown in the Near East as early as 700 BC (Ziggers, 2006). At least 86 countries grow sorghum in an area of 38 million hectares and with an annual grain production of about 58 million tons. The mean productivity under smallholder production systems reaches up to 1.5 t ha^{-1} (FAO, 2016), which is well below the potential yields of 4 to 5 t ha^{-1}.

Sorghum is a C4 crop and is well adapted to grow in semi-arid and arid agro-ecologies (Hons *et al*., 1986). Studies have shown that when sorghum is grown in arid conditions, it sustains physiological activity comparable to plants with adequate moisture by increasing root length, density, and water-use efficiency (Zegada-Lizarazu *et al*., 2012). Depending on genotype and location, sorghum can grow up to a height of 3 m, and can produce fresh biomass yields of 45–112 t ha^{-1} (Shuklaa *et al.,* 2017).

Taxonomically, the genus *Sorghum* has only one species, *bicolor*. Two types of sorghum are widely recognised, grain sorghum and sweet stem sorghum (Doggett, 1988). Sweet stem sorghum is a variant of grain sorghum. It belongs to *S. bicolor* subsp. *bicolor*. Sweet stem sorghum is characterised by juicy and sweet stalks, accumulating high concentrations of soluble sugars (10–25%) in the stem as sap or juice. Traditionally, sweet stem sorghum has been used for sugar production (Ou *et al.,* 2016; Shuklaa *et al*., 2017; Li *et al.,* 2018). In some countries, sweet stem sorghum is cultivated as a forage crop or a raw material for the paper industry (Koeppen *et al*., 2009; Oyier *et al.,* 2017). More recently, sweet stem sorghum has been established as promising crop for use in the bioenergy industry. Compared with maize, a traditional biofuel cereal crop, sweet stem sorghum produces 23% more fermentable carbohydrates, and requires 37% less nitrogen fertilizer and 17% less irrigation water (Hills *et al.,* 1990; Putnam *et al*., 1991; Mullet *et al*., 2014). Sweet stem sorghum can potentially produce up to 8000 l ha^{-1} of ethanol, which is twice the amount that can be potentially produced from maize, and 30% greater than that obtainable from sugarcane (Hunter and Anderson, 1997).

Biofuels are defined as renewable energy that is produced from organic matter in the form of biomass. The use of biofuels has been driven by the need to establish energy sustainability,

lessen import costs, and strengthen domestic agricultural development (Kovarik, 2013; Araújo, 2017). The use of biofuels in the transportation industry has been minimal (2.5%). This is because of the high cost involved in the production of maize and sugarcane as the primary feedstocks for the production of biofuels (Searchinger and Heimlich, 2015). Lately, plant biomass-based transport fuels have become a strategic focus for countries aiming to reduce vehicle emissions, and to counter climate change (REN21, 2016). Also, researchers are now focusing on emerging biofuel sources that are less expensive, aiming to produce lignocellulosic biomass, and plants that can be grown on marginal lands (e.g., sweet stem sorghum) (Searchinger and Heimlich, 2015). The global biofuels supply has increased by 8% of the world's transport fuels from 2000 to 2015 (BP, 2016; REN21, 2016). Moreover, positive characteristics such as its short growth cycle, easy propagation from seed, fully mechanized production, dual purpose cropping for both stem sugar and grain, high water and nutrient use efficiency, and its wide adaptability to different environments, make sweet stem sorghum a biofuel of choice (Fernandez and Curt, 2005; Reddy *et al*., 2005; Mullet *et al.,* 2014). Furthermore, ethanol from sweet stem sorghum is carbon neutral, harmless to the environment owing to low sulphur content, low biological, and chemical oxygen demand, and has a high octane rating (Reddy *et al*., 2006).

Some of the major problems that the world is facing include the global energy crisis, climate change and food insecurity. Global energy security, independence from fossil fuels, reduction of greenhouse gases emissions, and mitigation of adverse global climatic change are a few of the motives driving the use of renewable energies worldwide. Conventional first generation biofuels produced mainly from maize, soybean and sugarcane are also used for food. Hence, using them for biofuel aggravates global food insecurity. Sweet stem sorghum, on the other hand, is a second-generation biofuel crop, implying that it is a dual-purpose crop that can be used for both biofuel and food without compromising on either (Daystar *et al.,* 2014). Sweet stem sorghum has been underutilized as a bio-fuel crop despite its evident potential.

Collection of seed of many genotypes, and characterisation of germplasm, are two important procedures in developing a gene pool for an under-researched crop for breeding. Genetic groups can be established, based on data on geographical origin, agronomical traits, pedigree data and on molecular marker data (Melchinger, 1999). Traditionally, analysis of morphological traits has been used to establish genetic diversity in a given population. Given the relative simplicity of phenotyping in assessing the extent of diversity, it is a useful tool for preliminary germplasm evaluation. Several researchers have estimated genetic diversity in cultivated sorghum using morphological traits (Dahlberg, 2002; Shehzad *et al.*, 2009; Adugna,

2014; Mangena *et al*., 2017). Genetic variability estimates among genotypes are helpful in selecting parental combinations for creating segregating populations to harness genetic diversity in a breeding program (Menz *et al*., 2004; Becelaere *et al*., 2005).

Genetic diversity can also be established by molecular markers, which are not influenced by the environment, and do not require previous pedigree information (Bohn *et al*., 1999), which is especially valuable for crops where there is little or no pedigree information. Molecular markers have played a noteworthy role in the preservation and use of sorghum genetic resources (Morris *et al.,* 2013), in recognition of diverse lines, mapping of genomic regions controlling economic traits and their use in marker-assisted breeding. Many studies have endorsed the use of simple sequence repeat (SSR) markers in genetic diversity analysis owing to their high polymorphism, abundance, codominance, being multiallelic, and chromosome-specificity (Parker *et al.,* 2002; Geleta *et al.,* 2006; Ali *et al*., 2008; Shehzad *et al*., 2009). SSR markers are currently the preferred marker system in genomic analysis and molecular breeding of sorghum (Ali *et al*., 2008; Muraya *et al.,* 2011).

Traditionally, seed of sorghum hybrids has been produced using an expensive and slow system called Cytoplasmic Male Sterility (CMS), which uses three parental populations, the A, B and R parents (Guilford *et al*., 1992). Recently, researchers have started using chemical hybridizing agents (CHAs) or male gametocides to sterilise pollen in designated female lines in crops such as wheat, maize and tef (Chakraborty *et al*., 2001; Ghebrehiwot *et al.,* 2015). Chemical hybridizing agents are chemicals that stop pollen development, rendering the treated plants male sterile but female fertile. Hence, all seed found on the treated plants are the result of cross-pollination. Application of CHAs on sweet stem sorghum has not been evaluated previously. The use of CHAs has many advantages over the conventional CMS, including reducing the time taken to develop new hybrid varieties by 5-10 years, thereby increasing hybrid seed production. This approach would reduce the costs of production of a sorghum hybrid dramatically.

In sorghum hybrid development, it is essential to comprehend the genetic nature of the parental genotypes (Makanda *et al*., 2010). Identification of suitable parental genotypes to be used in a hybridization program can be carried out through combining ability studies. Combining ability estimates also forecast the relative performance of various genotypes in hybrid combinations. Knowledge of combining ability helps in optimizing the breeding strategy. Use of the recurrent selection procedure is important when general combining ability (GCA) effects are predominant. This approach ensures the accumulation of desirable unfixable or fixable gene effects (Nadarajan and Gunasegaram, 2005). In contrast, hybrid breeding is important when

specific combining ability (SCA) effects are predominant. GCA effects are attributed to preponderance of genes with additive effects and SCA indicates predominance of genes with non-additive effects (Kenga *et al.,* 2004).

Research aim

The principal aim of the proposed research was to develop superior sweet stem sorghum hybrids over a short period of time, using a male gametocide, which can be used for bio-fuel production and related activities.

Research objectives

The specific objectives of the study were to:

i) investigate the phenotypic variability present among diverse sweet stem sorghum genotypes based on their estimated ethanol production and related agronomic traits;
ii) evaluate the genetic interrelationships among phenotypically selected sweet stem sorghum genotypes using simple sequence repeat (SSR) markers;
iii) investigate the concentration, stage of application and frequency of application of ethyl 4'fluorooxanilate (E_4FO) for the induction of male sterility in sweet stem sorghum plants, without affecting female fertility;
iv) investigate the combining ability between the selected sweet stem sorghum testers and lines; and
v) assess heterosis in sweet stem sorghum hybrids for bio-ethanol production and related traits.

Structure of the thesis

Table 0.1 shows the thesis outline. The thesis is written in the form of discrete research chapters, each following the format of a stand-alone research paper (whether or not, the chapter has already been published). This is the dominant thesis format adopted by the University of KwaZulu-Natal. As such, there is unavoidable repetition of some references and some introductory information between chapters.

Table 0.1: Thesis outline

Chapter	Objective
-	
Chapter 1	Literature Review
Chapter 2	Characterization of sweet stem sorghum genotypes for bio-ethanol production
Chapter 3	Genetic interrelationship of sweet stem sorghum genotypes assessed through simple sequence repeat markers
Chapter 4	Preliminary investigation of the effect of ethyl 4'fluorooxanilate as male gametocide in sweet stem sorghum
Chapter 5	Combining ability and heterosis of sweet stem sorghum genotypes for bioethanol yield and related traits

References

Adugna, A. 2014. Analysis of in situ diversity and population structure in Ethiopian cultivated *Sorghum bicolor* (L.) landraces using phenotypic traits and SSR markers. *SpringerPlus* 3: 1–14.

Ali, M.L., Rajewski, J.F., Baenziger, P.S., Gill, K.S., Eskridge, K.M., and Dweikat, I. 2008. Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. *Molecular Breeding* 21: 497‑509.

Araújo, K. 2017. Low Carbon Energy Transitions: Turning Points in National Policy and Innovation. Oxford University Press, New York, USA.

Becelaere, G.V., Edward, L.L., Paterson, A.H. and Chee, P.W. 2005 Pedigree-vs. DNA marker-based genetic similarity estimates in cotton. *Crop Science* 45: 2281–2287.

Bohn, M., Utz, H.F. and Melchinger, A.E. 1999 Genetic similarities among winter wheat cultivars determined on the basis of RFLPs and SSRs and their use for predicting progeny variance. *Crop Science* 39: 228–237.

British Petroleum, BP. 2016. Statistical Review of World Energy. BP, London, UK.

Chakraborty, K., Devakumar, C., Kumar, J. and Kumar, R. 2001. Effect of anilates on pollen sterility of chickpea (*Cicer arietinum* L. var. Pusa 240). *Tropical Agriculture Resolution 13:* 239—248.

Dahlberg, J.A., Zhang, X., Hart, G.E. and Mullet, J.E. 2002. Comparative assessment of variation among sorghum germplasm accessions using seed morphology and RAPD measurements. *Crop Science* 42: 291–296.

Daystar, J., Gonzalez, R., Reeb, C., Venditti, R., Treasure, T., Abt, R. and Kelley, S. 2014. Economics, environmental impacts, and supply chain analysis of cellulosic biomass for biofuels in the southern US: pine, eucalyptus, unmanaged hardwood, forest residues, switchgrass, and sweet sorghum. *Bio Resources* 9: 393-444.

Deepak R.P., Pahuja, S.K., Verma N.K. and Chaudhary, S. 2018. Morphological characterization of sorghum [*Sorghum bicolor* (L.) Moench] germplasm for DUS traits. *International Journal of Current Microbiology and Applied Sciences* 7: 2058-2071.

Doggett, H.1988. Sorghum. Longman Scientific and Technical. London, UK.

Fernandez, J. and Curt, M.D. 2005. New energy crops for bioethanol production in the Mediterranean region. *International Sugar Journal* 107: 622-627.

Food and Agriculture Organization Crop Production Statistics (FAOSTAT). 2016. World Sorghum Production and Utilization. FAO, Rome, Italy.

Geleta, N., Labuschagne, M.T. and Viljoen, C.D. 2006. Genetic diversity analysis in sorghum germplasm as estimated by AFLP, SSR and morpho‐agronomical markers. *Biodiversity and Conservation* 15: 3251‐3265.

Ghebrehiwot, H., Burgdorf, R., Shimelis, H. and Laing, M. 2015. The efficacy of four gametocides for induction of pollen sterility in *Eragrostis tef* (Zucc.) Trotter. *African Journal of Biotechnology 14(9):* 774-780.

Guilford, W.J., Patterson, T.G., Vega, R.O., Fang, L., Liang, Y., Lewis, H.A. and Labovitz, J.N. 1992. Synthesis and pollen suppressant activity of phenylcinnoline-3-carboxylic acids. *Journal of Agriculture and Food Chemistry* 40: 2026–2032.

Hills, F.J., Lewellen, R.T. and Skoyen, I.O. 1990. Sweet sorghum cultivars for alcohol production. *California Agriculture* 44: 14-16.

Hons, F.M., Moresco, R.F., Wiedenfeld, R.P. and Cothren, J.T. 1986. Applied nitrogen and phosphorus effects on yield and nutrient uptake by high energy sorghum produced for grain and biomass. *Agronomy Journal* 76: 1069-1078.

Hunter, E.L. and Anderson, I.C. Sweet sorghum. Horticultural Reviews. John Wiley and Sons, Oxford, UK, pp. 73-104.

Jinks, J.L. 1954. Analysis of continuous variation in diallel cross of *Nicotiana rustica* varieties. *Genetics* 39: 767-788.

Kenga, R., S.O. Alabi, and S.C. Gupta. 2004. Combining ability studies in tropical sorghum [*Sorghum bicolor* (L.) Moench]. *Field Crops Research* 88: 251–260.

Koeppen, S., Reinhardt, G. and Gaertner, S. 2009. Assessment of energy and greenhouse gas inventories of sweet sorghum for first and second generation bioethanol. FAO Environmental and Natural Resources Service Series, No. 30, FAO, Rome, Italy.

Kovarik, B. 2013. History of Biofuels. Biofuels Crops. Center for Bioscience International (CABI), Wallingford, UK.

Li, Y., Yuan, F. and Wang, B. 2018. Changes in the sugar content of sweet sorghum stems under natural conditions during winter in saline soil of the Yellow River Delta. IOP Conference Series: Earth and Environmental Science 113. 012109

Makanda, I., Tongoona, P., Derera, J., Sibiya, J., and Pedro, F. 2010. Combining ability and cultivar superiority of sorghum germplasm for grain yield across tropical low- and mid-altitude environments. *Field Crops Research* 116: 75–85.

Mangena, P., Shimelis, H. and Laing, M. 2017. Characterisation of sweet stem sorghum genotypes for bio-ethanol production. *Acta Agriculturae Scandinavica, Section B — Soil and Plant Science* 68:323-333.

Melchinger, A.E. 1999 Genetic diversity and heterosis. The Genetics and Exploitation of Heterosis in Crops. American Society of Agronomy, Madison, Wisconsin, USA, pp 99–118.

Menz, M.A., Klein, R.R., Unruh, N.C., Rooney, W.L., Klein, P.E. and Mullet, J.E. 2004. Genetic diversity of public inbreds of sorghum determined by mapped AFLP and SSR markers. *Crop Science* 44: 1236–1244.

Morris, G.P., Ramu, P., Deshpande, S.P., Hash, C.T., Shah, T., Upadhyaya, H.D., Riera-Lizarazu, O., Brown, P.J., Acharya, C.B., Mitchell, S.E., Harriman, J., Glaubitz, J.C. Buckler, E.S. and Kresovich, S. 2013. Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proceedings of the National Academy of Science, USA. 110: 453-458.

Mullet, J., Morishige, D., McCormick, R., Truong, S., Hilley, J., McKinley, B., Anderson, R., Olson, S.N. and Rooney, W. 2014. Energy sorghum—a genetic model for the design of C_4 grass bioenergy crops. *Journal of Experimental Botany* 65: 3479–3489.

Muraya, M.M., de Villiers, S., Parzies, H.K., Mutegi, E., Sagnard, F., Kanyenji, B.M., Kiambi, D. and Geiger, H.H. 2011. Genetic structure and diversity of wild sorghum populations (*Sorghum* spp.) from different eco-geographical regions of Kenya. *Theoretical and Applied Genetics* 123: 571–583.

Nadarajan, N. and Gunasegaram, M. 2005. Quantitative Genetics and Biometrical Techniques in Plant Breeding. Kalyani Publishers, New Delhi, India.

Ou, M.S., Awasthi, D., Nieves, I., Wang, L., Erickson, J., Vermerris, W., Ingram, L.O. and Shanmugam, K.T., 2016. Sweet sorghum juice and bagasse as feedstocks for the production of optically pure lactic acid by native and engineered *Bacillus coagulans* strains. *Bioenergy Research* 9: 123–131.

Oyier, M.O., Owuoche, J.O., Oyoo, M.E., Cheruiyot, E., Mulianga, B. and Rono, J. 2017. Effect of harvesting stage on sweet sorghum (*Sorghum bicolor* L.) genotypes in western Kenya. *The Scientific World Journal*. https://doi.org/10.1155/2017/8249532

Parker, G.D., Fox, P.N., Langridge, P., Chalmers, K., Whan, B. and Ganter, P.F. 2002. Genetic diversity within Australian wheat breeding programs based on molecular and pedigree data. *Euphytica* 124:293–306.

Putnam, D.H., Lueschen, W.E., Kanne, B.K. and Hoverstad, T.R. 1991. A comparison of sweet sorghum cultivars and maize for ethanol production. *Journal of Production Agriculture* 4: 377-381.

Reddy, B.V.S, Kumar, A.A. and Ramesh, S. 2006. Sweet Sorghum: a Water Saving Bioenergy Crop. ICRISAT, Patancheru, Andhra Pradesh, India.

Reddy, B.V.S. Ramesh, S. Reddy, P.S. Ramaiah, B., Salimath, P.M. and Rajashekar, K. 2005. Sweet sorghum—A potential alternate raw material for bio-ethanol and bioenergy. *International Sorghum and Millets Newsletter* 46: 79-86.

Renewable Energy Network 21 (REN21). 2016. Global Status Report; REN21: Paris, France.

Searchinger, T. and Heimlich, R. 2015. Avoiding bioenergy competition for food crops and land. Instalment 9 Creating a Sustainable Food Future. World Resources Institute, Washington, DC, USA.

Shehzad, T. Okuizumi, H. Kawase, M. and Okuno, K. 2009. Development of SSR-based sorghum (*Sorghum bicolor* (L.) Moench) diversity research set of germplasm and its evaluation by morphological traits. *Genetic Resources and Crop Evolution* 56: 809–827.

Shuklaa, S., Felderhoffa, T.J., Saballosb, A. and Vermerrisa, W. 2017. The relationship between plant height and sugar accumulation in the stems of sweet sorghum (*Sorghum bicolor* (L.) Moench). *Field Crops Research* 203: 181–191.

Zegada-Lizarazu, W., Zatta, A. and Monti, A. 2012. Water up- take efficiency and above- and below-ground biomass development of sweet sorghum and maize under different water regimes. *Plant and Soil* 351: 47-60.

Ziggers, D. 2006. Sorghum—the multipurpose grass. *Feed Technology* 5: 20–23.

Chapter 1

Literature review

1.1 Introduction

The review of literature in this chapter aims to provide information associated with development of sweet stem sorghum (*Sorghum bicolor* L.) hybrids using various approaches. The review covers characterization of sweet stem sorghum, its potential as a biofuel crop and breeding procedures involved in producing superior sweet stem sorghum hybrids. The chapter concludes with a summary of the findings and the knowledge gaps relevant to the current study.

1.2 Sorghum characterization

According to Mann *et al*. (1983) sorghum was first cultivated some 5 000 years ago in northeastern Africa, north of the Equator and east of 10˚E latitude. Conversely, carbonized seeds of sorghum with radiocarbon dates of 8,000 years BP were unearthed at Nabta Playa near the Egyptian- Sudanese border (Wendorf *et al.,* 1992; Dahlberg and Wasylikowa, 1996). Despite the initial cultivation of sorghum being in North Africa, the domestication events may also have taken place elsewhere and more than once.

Wider genetic assortment of sorghum was generated by means of disruptive selection and via isolation and recombination in the tremendously wide-ranging environments of northeast Africa and the migration of people to other parts of the continent (Miller, 1982). The largest diversity of cultivated and wild sorghum is in Africa (Doggett, 1970; de Wet and Harlan, 1971; de Wet, 1977). Moreover, sorghum is considered a crop with universal value because it can be grown in tropical, subtropical, temperate, and semi-arid regions of the world (Davila-Gomez *et al.,* 2011). Plant breeding progress has brought about the innovation of sorghum genotypes that are adapted to numerous geographic and climatic regions of the world, consequently extending their production area from the initial region of adaptation.

Knowledge of the biological background of sorghum is vital for plant breeding and conservation. Sorghum is a self-pollinated diploid (2n=2x=20) with outcrossing reaching up to 15%. Sorghum is categorized under the genus *Sorghum* (Clayton and Renvoize, 1986). De Wet, (1978) documented that *S. bicolor* denotes all annual cultivated, wild and weedy sorghums together with two rhizomatous taxa, namely *S. halepense* and *S. propinquum*. Cultivated

sorghums are classified as *S. bicolor* subsp. *bicolor*. Harlan and de Wet (1972) divided the cultivated sorghums (subspecies *bicolor*), into five basic races: Bicolor, Guinea, Caudatum, Kafir, and Durra, and 10 hybrid races that are combinations, and really stable intermediate hybrids, of the basic races. These include Guinea-Bicolor, Guinea-Caudatum, Guinea-Kafir, Guinea-Durra, Caudatum-Bicolor, Kafir-Bicolor, Kafir- Caudatum, Kafir-Durra, Durra-Bicolor, and Durra-Caudatum. Sorghum is a herbaceous annual grass that is planted from seed, accumulates a substantial quantity of sugar with moderate use of water and reaches maturity in 90 to 180 days. Moreover, sorghum is a C_4 grass with a high photosynthetic efficiency under hot conditions (Doggett, 1988).

1.3 Sweet stem sorghum

The value of sorghum can be attributed to the fact that its photosynthates can be utilized in numerous ways. Sorghum has been developed into four categories: grain, sweet, broom and grass sorghum. The main use of grain sorghum is for human consumption and is frequently exploited as a raw material for alcoholic beverages, sweets and glucose industries. It is estimated that more than 300 million people from developing countries essentially rely on sorghum as a source of energy (Dicko *et al.,* 2006). In contrast, broom sorghum is for crafting brooms, whereas grass sorghum is used as a source of forage and silage for livestock production (Mwadalu and Mwangi, 2013). Sweet stem sorghum has been successfully used for the production of bio-ethanol (Anami *et al*., 2015a).

1.4 Production constraints to sorghum

Regardless of its high photosynthetic efficiency, there are production constraints that inhibit sorghum's production potential. The yield and quality of sorghum products is affected by an extensive array of biotic and abiotic stresses. Biotic stresses which affect sorghum include weeds, pests and diseases. There are numerous diseases of sorghum and they can be categorized into bacterial, fungal, and viral diseases. Widespread fungal diseases include anthracnose, leaf blight, sorghum downy mildew, zonate leaf spot, rough spot, sorghum rust, charcoal rot, and stalk rot/grain mold (van den Berg and Drinkwater, 1997). Mosaics are the main viral diseases of sorghum with maize dwarf mosaic disease causing the most damage. Bacterial leaf stripe is the most common bacterial diseases of sorghum (Kucharek, 1992). On the other hand, pests of sorghum can be split into groups including soil and seedling insects (wireworms, white grubs, beetle larvae, rootworms, cornstalk borers, cutworms, and chinch bugs), leaf and stalk boring insects (aphids, green bugs, whorl-worms, budworms, fall armyworms, grasshoppers, mites, stalk boring moth caterpillars), and panicle and seed insects (sorghum midge, corn earworms, fall

armyworm, sorghum webworm, stink bugs, false chinch bugs) (Buntin, 2012). To control diseases and insect pests, some of the measures taken include selection of resistant genotypes, planting of disease-free seed at the appropriate soil temperatures, avoiding field operations when foliage is wet, proper crop rotation, control of weeds, and removing or burying crop debris (Buntin, 2012). Abiotic stresses are non-living factors with a negative impact on production that can also limit sorghum. Soils which are acidic and water logging usually affect sorghum productivity negatively. Conversely, sorghum is a crop of significant value due to its tolerance to arid and saline growing conditions.

1.5 Production trends of sorghum

Sorghum is the fifth most important cereal crop of the worldwide production next to wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.) (Shahwar *et al.,* 2012; Mace *et al.,* 2013; Cuevas *et al.,* 2014). At least 86 countries grow sorghum in an area of 38 million hectares and with an annual grain production of about 58 million tons. The mean productivity under smallholder production systems reaches up to 1.5 t ha^{-1} (FAO, 2016). According to the Food and Agricultural Organization (FAO), the mean productivity of sorghum globally has increased over the years. This can be attributed to improvements in production technology and knowledge generation. Productivity in South Africa is higher than that observed in Africa and globally. However, more and greater fluctuations in productivity are also observed in South Africa compared to Africa and the world at large. Considering the area allocated to sorghum production, Africa has increased the quantity of sorghum production over the years, whereas globally sorghum production has remained more or less constant and has decreased in South Africa.

1.6 Sweet stem sorghum: a biofuel crop

Sweet stem sorghum has attracted the attention of the scientific and industrial community because it possesses a number of traits that make it suitable as a biofuel crop. In comparison with other principal biofuel crops globally, sweet stem sorghum exhibits a number of valuable traits. Adaptation of sweet stem sorghum for bioenergy purposes will require the identification and incorporation of advantageous traits into breeding programs. Recent reviews have made the case for sweet sorghum as a bioenergy crop (Vermerris, 2011; Calvino and Messing, 2012; Mullet *et al*., 2014; Prakasham *et al.,* 2014; Anami *et al*., 2015a; Anami *et al.,* 2015b).

The high photosynthetic efficiency of sweet stem sorghum has been attributed to its physiological qualities such as its anatomical structure and physiological performance. The

response to environmental conditions of CO_2 of C_4 plants differs from those of C_3 plants. Firstly, the compensation point of the concentration of C_4 plants such as sweet sorghum is close to zero; while the saturation point is very high. Additionally, its photorespiration is extremely efficient and is difficult to measure, while in other crops such as soybean and sugar beet, 47-75% and 34-55% of photosynthetic products may be consumed by their photorespiration, respectively. Also, sweet stem sorghum has very high light saturation point. Lastly, under high temperature, the photosynthetic capacity of a C_4 plant is twice that of a C_3 plant. Therefore, the photosynthetic efficiency of sweet sorghum is over two times than that of C_3 plants such as soybean, sugar beet or wheat (Dajue, 1997; Xu *et al.,* 2011).

In addition to its high photosynthetic efficiency, sweet stem sorghum has the aptitude to yield a high biomass per hectare on marginal lands that are unsuitable for food and feed production (Lipinsky and Kresovich, 1980; Rosenow and Clark, 1995; Saballos, 2008; Vermerris and Saballos, 2013). Researchers dedicated to sweet stem sorghum as a bioenergy crop have reported yields of 14.1-17.6 ton ha^{-1} of dry biomass, 3.9 ton ha^{-1} of grain and 8.14 ton ha^{-1} of hexose sugars from the stem juice (Murray *et al.,* 2008; Tew *et al.,* 2008). Ligno-cellulosic biomass for ethanol production can attain a yield of 12 340 l ha^{-1}. High yields are likely to be improved further through breeding directed towards traits advantageous for bioenergy production. Another trait that qualifies sweet stem sorghum for biofuel production is that it will not impede food production, making it a third-generation biofuel crop.

Sweet stem sorghum stalk juice can be used productively for the production of syrup, fuel-grade ethanol, specialty and bulk organic chemicals, industrial alcohol, etc. Also, it can be cultivated on less fertile lands, is drought tolerant, has a wide adaptability (e.g. it grows in different types of soils with pH = 5 - 8.5) and has waterlogging tolerance. Moreover, sweet sorghum possesses readily available fermentable sugars within its stem (McBee *et al*., 1988; Sipos *et al.,* 2009). Sweet stem sorghum's short growth period (3–5 months), makes it an ideal short maturing biofuel crop (Rooney *et al.,* 2007; Carpita and McCann, 2008; Vermerris, 2011; Mullet *et al*., 2014).

Sweet stem sorghum has proven to have better biofuel production related characteristics when compared to sugarcane, currently the main biofuel crop. Sweet stem sorghum is sown with seed (at a rate of 4.5-7.5 kg ha^{-1}) and it can be sown by machine, while sugarcane is propagated with stem cuttings at a rate of 4,500-6,000 kg ha^{-1} and it is not suitable for machine planting.

Paterson *et al.* (2009) and Masood *et al.* (2015) reported sweet stem sorghum as an ideal model bioenergy crop due to its relatively small genome (735 Mbp), the availability of the sorghum genome sequence, its diploid character and its close evolutionary relationship to other crops being considered for bioenergy purposes, such as sugarcane and maize. Sweet sorghum-based ethanol is sulfur-free and cleaner than ethanol produced by most biofuel crops when mixed with gasoline. Table 1.1 compares sweet stem sorghum with two other biofuel crops. Figure 1.1 further illustrates the wide geographic suitability of sweet stem sorghum as a biofuel crop compared to sugarcane and sugar beet. Numerous traits have been identified which make sweet sorghum the ideal biofuel crop but that there is need to improve existing varieties.

Table 1.1: Comparative advantages of sweet sorghum vs. sugarcane and sugar beet for ethanol production (Adapted from Almodares and Hadi, 2009)

Parameter	Sweet sorghum	Sugarcane	Sugar Beet
Crop duration (months)	3.5	12-13	5-6
Growing seasons / year	1 Temperate areas 2 Tropical areas	1	1
Soil requirement	All types of drained soil	Loamy soil	Sandy loam Tolerates alkalinity
Water management (m^3 ha^{-1})	12000	36000	18000
Crop management	Simple Little fertilizer Less pests and diseases	Complex	Moderate High fertilizer
Yield (ton ha^{-1})	54 - 69	70 - 80	30-40
Sugar content on weight basis (%)	8-10	10-12	15-18
Sugar yield (ton ha^{-1})	6-8	7-8	5-6
Ethanol production directly from juice (l ha^{-1})	3000	3000-5000	5000-6000
Harvesting	Very simple Both manual and mechanical	Difficult Laborious	Very simple Normally manual

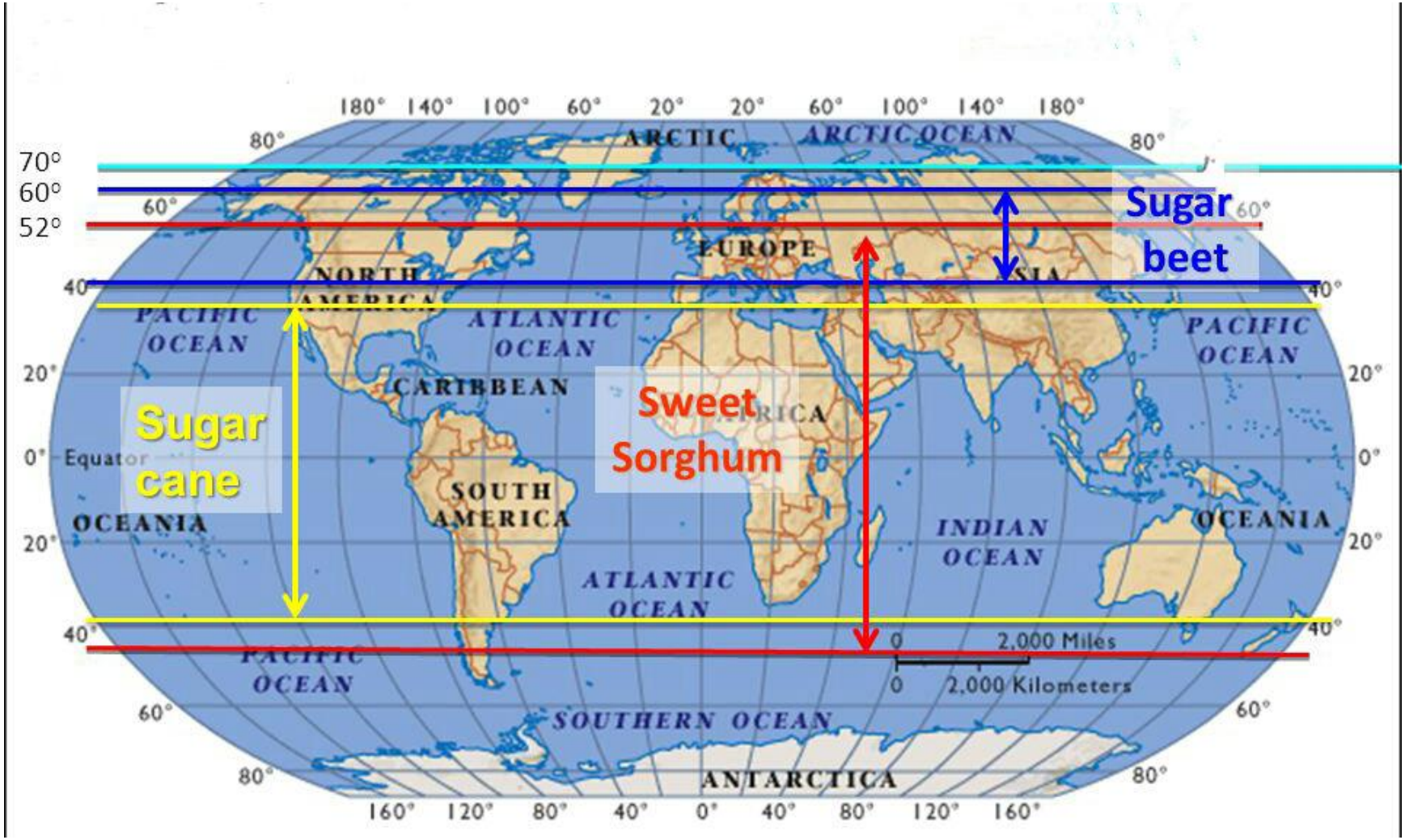


Figure 1.1: World map depicting approximate areas where sweet stem sorghum, sugarcane and sugar beet can be grown (Adapted from Debor, 2009)

1.7 Sweet stem sorghum breeding

1.7.1 Diversity analysis in sweet stem sorghum

1.7.1.1 Morphological diversity analysis

Success of a breeding program through devising appropriate breeding strategies is of paramount importance and is based on the genetic variation in a breeding population. Genetic variability for agronomic traits is a principal component of breeding programs for the reason that it broadens the gene pool of crops. Diversification of the genetic base of cultivars is achieved by intercrossing genetic sources of diverse origin. Sorghum is endowed with high variability due to its wide range of adaptation in tropical and temperate climates and free gene exchange among various races (Elangovan *et al.,* 2014).

Superior sorghum varieties can be attained through breeding, as long as adequate genetic variation is present in the diversity spectrum or by exploiting transgressive segregation or heterosis. An appreciable amount of genetic variability within a population is obligatory to facilitate and sustain an effective and durable plant breeding program (Ukaoma *et al.,* 2013). Sizeable genetic variation in sorghum germplasm has been comprehensively documented making breeding for superior sorghum germplasm feasible. Warkad *et al*. (2008); Elangovan *et al.* (2014); Kamatar *et al.* (2015); Tesfamichael *et al.* (2015) and Salih e*t al.* (2016) have reported variation in days to maturity, days to 50% flowering, plant height, stem diameter and dry fodder weight. The authors used both phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) to study variability. Variability depends on heritable and non-heritable components, and coefficient of variation measures the magnitude of variability present in a population.

In self-pollinating crops, germplasm improvement has been made through selection alone. Selection followed by hybridization has been confined to cross-pollinating crops such as maize. In recent developments, hybridization has been successfully used to develop hybrid cultivars in self-pollinating crops such as rice and sorghum (Makanda, 2009). This has enabled the exploitation of both additive genes and non-additive genes with the computation of combining abilities and heritability becoming more important in sorghum breeding. For its extensive economic significance and its adaptation to diverse agro-ecological environments, sorghum is a valuable species (Melil *et al*., 2013). The attainment of superior sorghum hybrids does not

exclusively depend on the magnitude of genetic variation but also on the heritability of traits under improvement.

The ratio of genetic variance to the total phenotypic variance, also known as heritability, is used for assessing the breeding value of population. Heritability estimates can be either in the narrow-sense, where estimates take into account the fraction of total phenotypic variance that is due to additive effects or in the broad-sense which measures total genetic effects (additive, dominance, and epistatic effects) as a function of total phenotypic variance. Since only additive effects are transmitted from parents to offspring, narrow-sense heritability is more valuable than broad-sense heritability. Nevertheless, estimation of narrow-sense heritability requires special mating designs that may not be commonly used in breeding programs (Mangena, 2014). Estimates of heritability are important preliminary steps in any breeding program as they provide information needed to estimate the relative practicality of selection as they represent the genetic potential of a trait (Kamatar *et al.,* 2015). Knowledge of heritability influences the choice of selection procedures used by the plant breeder to decide which selection methods would be the most useful to improve the character, to predict gain from selection and to determine the relative importance of genetic effects (Tesfamichael *et al.,* 2015). Consequently, it can be concluded that evaluations of the components of variation and heritability are among characters that will facilitate improvement of crops such as sorghum.

Success in breeding selections can be enhanced by studies of correlations between the traits. Several sweet stem sorghum populations have been studied for genetic variability and correlation of bio-fuel related traits (Murray *et al.,* 2008; Ritter *et al.,* 2008; Srinivas *et al.,* 2009; Shiringani *et al.,* 2010). For the reason that many morphological traits are related to ethanol production, it is useful to ascertain their correlation thereby establishing improved selection approaches of breeding. Assessment of one or more traits using the performance of another trait is made possible by employing correlation analysis, which essentially creates a way to perform indirect selection. Indirect selection is important at the initial stages of a breeding for traits that are not easily measured or are associated with low heritability (Carvalho and Cruz, 1996; Rios *et al.,* 2012). Correlation studies can also reduce the time and resources directed towards a breeding program as selection for one trait will automatically select for all the traits positively correlated to the trait of selection. Focusing on correlation studies alone has proven to be inadequate as this approach is limited to only two traits a time. Additionally, simple correlation estimates may not represent the actual association between two traits, since there may be interference by a third

trait, or group of traits, that might distort the correlation estimates (Wright, 1921). For these reasons, Wright (1921) proposed Path Analysis.

Path analysis separates phenotypic correlations into the direct and indirect effects of the traits on a chief variable, making for greater dependability in the choice of selection traits (Cruz *et al.,* 2014). It allows a more practical study of the association between groups of traits by the breakdown of the simple correlation coefficients into direct and indirect effects of a group of traits over a basic or main variable. The knowledge of the direct and indirect effects of the explanatory variables on a key trait might help in deciding on to use a correlated response or progress in selection (Rios *et al.,* 2012). Despite the importance of this analysis, studies that used path analysis in sweet stem sorghum are scarce (Kumar *et al.,* 2012). The use of path analysis should enable the acquisition of knowledge on the relationships among the main agro-industrial traits in sweet sorghum in order to provide the necessary information to establish the optimal breeding program strategies.

1.7.1.2 Diversity analysis using molecular marker technology

Morphological descriptors and molecular marker technology have been used to map out the genetic diversity in crop plants (Mace *et al.,* 2005). Molecular markers are nucleotide sequences corresponding to a physical position in the genome, and their polymorphisms between accessions allow the pattern of inheritance to be traced (Schulman, 2006). Genetic distance estimates among genotypes are important in selecting parental combinations for creating segregating populations so as to maintain genetic diversity in a breeding program (Becelaere *et al.,* 2005) and the classification of germplasm into heterotic groups for hybrid crop breeding (Menz *et al.,* 2004).. Molecular markers can play a significant role in the conservation and use of sorghum genetic resources (Aldrich and Doebley, 1992; Whitkus *et al.,* 1992; Rami *et al.,* 1998; Deu *et al.,* 2006; Wang *et al.,* 2006; Kumar *et al.,* 2011; Morris *et al.,* 2013b) and also in many aspects of sweet stem sorghum improvement programs ranging from identification of diverse lines, to mapping of genomic regions controlling desirable traits and their use in marker-assisted breeding. Molecular markers are also used to assess the identity in order to identify putative duplicate accessions, and also to establish relationships and genetic structure with the main aim of determining how variation is distributed among individuals (Westman and Kresovich, 1997). Because DNA markers are not affected by environmental factors, they are considered better than morphological markers as cultivar descriptors (Bowditch *et al.,* 1993). Moreover, they can be detected in all tissues at all stages of development, contrary to morphological markers (Soriano *et al.,* 2005). Various types of molecular markers are available for genome analysis.

Several diversity studies on sorghum have been done using restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), simple sequence repeats (SSRs), amplified fragment length polymorphisms (AFLPs), and single nucleotide polymorphisms (SNPs) (Klein *et al.,* 2008; Smith *et al.,* 2010; Morris *et al.,* 2013a). These marker systems are available for assessing the genetic diversity though they differ in principle, application, number of polymorphisms detected and also the time required. Simple sequence repeats are short repeated DNA sequences in the genome of 2 to 4 nucleotides in length (Reisch, 1998; Tawanda, 2004). They are abundant in genomes of plants where they are thought to be a source of genetic variation (Mahalakshmi *et al.,* 2002). Simple sequence repeat markers are the preferred marker system for many sorghum genomics and molecular breeding applications because they are highly polymorphic even among closely related cultivars, which demonstrates that they are highly informative (Uptmoor *et al.,* 2003; Caniato *et al.,* 2007; Ali *et al.,* 2008; Deu *et al.,* 2008; Muraya *et al.,* 2011). They are also multi-allelic and chromosome-specific (Ahmad, 2002; Huang *et al.,* 2002; Parker *et al.,* 2002). These markers are co-dominant and can be analyzed by a rapid, technically simple, specific and inexpensive polymerase chain reaction (PCR) based assay that requires only small amounts of DNA. The SSR markers have proved to be a valuable asset for breeding programs and have been used for a wide range of application and this include measuring of genetic diversity (Xiao *et al.,*1996), in assigning lines to heterotic groups (Senior et al., 1998), in the genetic analysis of breeding schemes, genetic distance analysis (Chen *et al.,* 1997) and in population genetic fingerprinting for legal protection of cultivars and parental lines, and in establishing genome relationship in species with putative interspecific parents.

1.7.2 Male sterility system in sorghum

In self-pollinated crops such as sweet stem sorghum, with the male and female organs in the same flower, selective sterilization of pollen is a prerequisite for crossing. There is typically 5 to 15% outcrossing in sweet stem sorghum, depending upon the wind direction, nature of genotype, and humidity (House 1985), which makes it amenable for use in population improvement and hybrid development to exploit the heterosis. The establishment of a male sterile line renders any crop variety readily adaptable to hybridization with virtually any male line having desired characteristics. Male sterile lines may be established in a number of ways. Hand emasculation which is not feasible for the large-scale emasculation of species. Genetic male sterility is also a known trait, usually inherited as a recessive and monogenic trait that is used to produce hybrid seed of barley, tomato, pepper, marigold, zinnia, and other crops that have a shortcoming of being ales than 100% male sterile. Cytoplasmic male sterility provides an

alternative mechanism whereby the genetic factors controlling male sterility are found in the cytoplasm.

In sorghum, Stephens and Holland (1954) discovered cytoplasmic male sterility (CMS). This has made it easier to create hybrid seed on a large scale (House 1985). However, this methodology is beset with many obstacles and limitations such as the non-availability of breeding stocks containing CMS and restorer systems, the instability of the CMS in various environments, and the laborious method of heterosis breeding. Besides being tedious and time-consuming, this technique sometimes becomes untenable because of the lack of a consistent restorer system for the genetic restoration of fertility (Guilford *et al*., 1992).

A method of producing male sterile lines which circumvents the difficulties of genetic induction is the use of chemical sterilization agents. The principle involved here is that the chemical acts as a gametocide, selectively sterilizing the male gamete, i.e., pollen, by inducing physiological abnormalities, which in turn prevent pollen development, pollen shed, or pollen viability (McDaniel, 1992).

In efforts to develop superior hybrids in self-pollinating crops, chemically induced male sterility is one avenue that plant breeders have pursued. Chemical-hybridizing agents (CHAs) emasculate treated plants by killing the male gametes, spores or organs and the selective elimination of the male sex has been termed chemical male sterility (Kaul, 1988). The first report of the effects of CHAs on crops was a study on the effects of maleic hydrazide on suppressing pollen development in spring wheat (Hoagland *et al.,* 1953). McRae (1985) reported that chemical male sterility (gametocidal activity) and the production of hybrid seed could be traced to as early as 1957.

Four main groups of CHAs can be distinguished regarding the mode of action. Growth regulators suppress the development of floral primordia; while metabolism inhibitors, pollen germination inhibitors and microsporogenesis inhibitors, prevent self-pollination and promote fertilization by an outside pollen source, thus offering opportunities to develop hybrids (Dotlacil and Apltauerova, 1978; Wong *et al.,* 1995; Blouet, 1999).

Considering how difficult it is to develop hybrids in self-pollinating crops, the use of chemical hybridizing agents could to be valuable as it enables plant breeders to manipulate self-pollinating crops more efficiently. Compared with CMS, an effective CHA allows for the production of a large number of parental combinations and allows for the evaluation of a great number of lines for combining capacity and genetic value. The time required for hybrid development is consequently

lessened substantially (Bruns and Peterson, 1998). Moreover, the use of CHAs includes the use of simple protocols of plant improvement, as in this system neither the conversion and maintenance of an androsterile line (line A) nor the incorporation of factors of fertility restoration in male progenitors is required (Cross and Schulz, 1997; Cisar and Cooper, 2003). Even the genotypes with short extrusion of stamens may be used as male progenitors. The evaluation of a great number of genotypes for general and specific combining capacity as well as for characteristics of seed production are relatively easy. Heterogeneous populations may be developed or improved. Using gametocides, one can develop a large pool of heterotic combinations with various traits, including higher productivity (Cross and Ladyman, 1991). This large pool could then provide an array of wheat hybrids. The unique advantage CHAs offer is that any variety or crop can be used for induction of male sterility in order to develop a hybrid with a pollen donor. In chemical induction of male sterility, only parental components of a hybrid variety (a double-component system) are needed. In addition to this, the advantage of a chemical induction of male sterility is also the absence of complex genetic engineering and facilitation of hybrid seed production in the F_1 generation without backcrossing. Chemically induced male sterility obviates the use of genetic and gene-cytoplasmic male sterility (Virmani, 2003).

The characteristics of a perfect CHA have been characterized by Liable (1974), Virmani and Edwards (1983) and Pickett (1993). The primary characteristics induction of male sterility only, without affecting female fertility; production of easily recognizable androsterility; lacking phytotoxic effects on the treated progenitor; consistent performance on all genotypes of a species; consistent activity in a wide range of environments; systemic activity and persistence; sterilizing early and late flowers of all the plants of the treated population; flexibility in the application stage to overcome adverse climatic conditions; wide dose flexibility to allow a secure margin of error; achieving sterility with only one treatment; no effect on the quality of F_1 seed or the vigor of either F_1 plantlets or plants; activity on several genera; being cheap to synthesize and practical to apply; being a non-toxic compound with no negative toxicological effects on humans or the environment. A gametocide with all the above mentioned traits will be able to fulfill all the benefits that chemical hybridizing agents could bring to the plant breeding world.

Ethyl 4-fluoro oxanilate (E_4FO) has been successfully used on various crops with minimal phyto-toxic effects (Table 1.2). It acts systemically. It has been reported to induce male sterility >95% should which is required for an effective production of hybrid seeds. This guarantees a high level of seed purity, where the resulting seed may be classified as pure hybrid (Wassel and Weaver, 1995; Nesvadba and Vyhnánek, 2001; Nesvadba *et al.,* 2001).

Table 1.2: Previous studies on the use of Ethyl 4-fluoro oxanilate as a chemical sterilizing agent

Crop	Dose /ppm	Time of application	Frequency of application	Success/failure	References
Oryza sativa	1500	Meiosis	1	High pollen and spikelet sterility Wide spectrum of varieties Low phytotoxity	Ali *et al.,* 1999
Eragrostis tef	1000	Panicle initiation	1	86.41% pollen sterility No phytotoxicity symptoms	Ghebrehiwot *et al.,* 2015
Eragrostis tef	1500	Panicle initiation	1	95.83% pollen sterility No phytotoxicity symptoms	Ghebrehiwot *et al.,* 2015
Eragrostis tef	2000	Panicle initiation	1	98.61% pollen sterility No phytotoxicity symptoms	Ghebrehiwot *et al.,* 2015
Eragrostis tef	3000	Panicle initiation	1	99.5% pollen sterility Floret dryness and early premature senescence	Ghebrehiwot *et al.,* 2015
Triticum aestivum	1000	Pre-meiotic	1	99.97 % pollen sterility	Chakraborty and Devakumar, 2006
Triticum aestivum	1500	Pre-meiotic	1	99.54 % pollen sterility	Chakraborty and Devakumar, 2006
Cicer arietinum	1000	One week before flowering	1	100 % pollen sterility 16.4 % basal flower sterility 89.6 % total flower sterility	Chakraborty *et al.,* 2001
Cicer arietinum	2000	One week before flowering	1	100 % pollen sterility 18.2 % basal flower sterility 92.6 % total flower sterility	Chakraborty *et al.,* 2001

1.7.3 Combining ability and gene action

General combining ability (GCA) and specific combining ability (SCA) are concepts proposed by Sprague and Tatum (1942), using single crosses of maize. The ability of an inbred line to transmit desirable performance to its offspring is termed combining ability. Falconer (1989) defined GCA as the mean performance of a genotype when crossed with a series of other genotypes. The performance of a cross can deviate from the mean GCA of two parental lines due to genetic effects that are specific to that cross, and this deviation is referred to as SCA (Bernardo, 2014). The term SCA is used to designate those cases in which certain combinations do better than would be expected on the basis of the mean performance of the lines involved (Deepak, 2014). Sprague and Tatum (1942) defined SCA as the performance of two specific inbreds in a particular cross combination.

Variations in GCA effects have been accredited to additive, the interaction of additive x additive, and the higher-order interactions of additive genetic effects in the base population, while discrepancies in SCA effects have been attributed to non-additive genetic variance. The analysis of combining ability, therefore, allows broad inferences on the nature of gene effects for a trait under selection (Yan and Hunt, 2002). The information on the nature and magnitude of gene action is important in understanding the genetic potential of a population and deciding the breeding procedure to be adopted in a given population (Tariq *et al.,* 2014). Kenga *et al.* (2004) reported significant variation due to GCA and SCA effects in sorghum for days to anthesis and plant height. Haussmann *et al.* (1999) reported similar results. The significance of these findings is that both additive and non-additive gene action is important for these traits. Zhou *et al.* (2005) reported that inheritance of stalk biomass, Brix % and stalk weight were subject to both additive gene effects and non-additive gene effects, but were mainly controlled by non-additive genes. Schlehuber (1945) reported that genes with partial dominance action controlled sucrose content in hybrids. However, Baocheng *et al.* (1986) reported that genes with additive and dominance effects influenced stem sugar accumulation. In contrast, Guiying *et al.* (2000) reported that recessive genes exhibiting additive effects controlled stem sugar accumulation in sorghum.

The success of any breeding program is dependent on combining ability studies of the parents because they assist in recognizing suitable parents which can be used for hybridization to produce superior hybrids. The estimates of combining ability are useful to predict the relative performance of different lines in hybrid combinations. Combining ability studies capture

knowledge on the genetic mechanisms regulating the inheritance of quantitative traits, and facilitate the selection of parents for their enhancement or their exploitation in hybrid breeding (Makanda *et al.,* 2010; Thakare *et al.,* 2014). The relative magnitude of GCA and SCA assist in selecting the appropriate procedure to breed for superior genetic gain (Justin *et al.,* 2015). Recurrent selection should be employed when GCA effects are predominant; inbreeding followed by cross breeding should be used when SCA effects are predominant; recurrent selection followed by hybridization should be used if both are important (Singh *et al.,* 2014). Combining ability studies provide useful information regarding the selection of suitable parents for effective hybridization programs and indicate the nature and magnitude of various types of gene action involved in the expression of quantitative characters (Bernardo, 2014). Combining ability studies also help in ensuring accumulation of desirable unfixable or fixable gene effects (Nadarajan and Gunasegaram, 2005).

A line × tester mating design was developed by Kempthorne (1957), and is one of the most powerful tools for predicting the GCA of parents and selecting of suitable parents and crosses with high SCA. Line × tester analysis provides information about the combining ability effects of genotypes and knowledge regarding the genetic mechanism controlling yield components (İştipliler *et al.,* 2015). The design has been widely used in sorghum breeding and continues to be utilized in quantitative genetic studies in sorghum.

1.7.4 Heterosis

The estimation of GCA effects helps to ascertain which genotypes may be hybridised to exploit heterosis and to select better crosses for further breeding (Singh and Chaudhary, 1985). Heterosis is the augmented vigor of the F_1 generation of a cross for size, duration or yield of economic product over the mean of the parents or better parent is concerned (Hayes *et al.,* 1955). Having progeny that out-performs both parental genotypes is the ultimate goal of all hybridization programs. In 1927, the first demonstration of heterosis was carried out by Corner and Karper (1927). The commercial exploitation of heterosis in sorghum, on the other hand, only became feasible when a stable and heritable cytoplasmic nuclear male-sterility (CMS) mechanism was developed (Stephens and Holland, 1954). Heterosis of the F_1 generation is expressed over mid parent, better parent or available standard check variety. Significant heterosis over mid parent indicates partial dominance, while significant heterosis over better parent indicates over-dominance. For profitability reasons, most breeders aiming for standard heterosis over best check variety when developing high yielding hybrids. Pfeiffer *et al.,* 2010 reported that most sweet

sorghum cultivars are still inbred lines. Sweet sorghum has not been a major focus of commercial breeding programs; hybrids have been developed between grain and sweet sorghum (Murray *et al.,* 2009). Increasing stalk sugar yields and hybridizing sweet sorghum has only gained impetus recently in efforts to increase bioenergy production and resolve the climate change crisis. There is heterosis in sorghum for most traits that are related to biofuel production, such as earlier blooming, increased height, larger stems and high biomass production (Quinby *et al.,* 1963; Haussman *et al.,* 1999; Makanda *et al.,* 2010). It is therefore essential to direct biofuel production studies in sweet stem sorghum towards hybrid production. There has been little research on heterosis in sweet stem sorghum so far (Pfeiffer *et al.,* 2010), although this area is now an area of intense research activity.

1.8 Summary

In conclusion, extensive genetic variation has to be established in the base population if a successful breeding program is to be realized. Variation can be analyzed morphologically or through the use of molecular markers. various hybridization systems have been proposed, including recessive male sterility genes combined with chemical restoration of fertility (Wilson, 1984); male nuclear sterility (MNS), as the XYZ system of Driscoll (1985), or photo thermal-sensible systems that require different photoperiods and thermal regimes (He *et al.,* 1998; Murray, 1998). With the continuous development of biotechnology, the development of hybridization systems via genetic engineering may be feasible. Nevertheless, the use of CHAs to develop improved populations and hybrids, seems efficient and may be necessary (Cisar and Cooper, 2003). There is substantial literature on dosage, application time and frequency of application time of ethyl 4-fluoro oxanilate that can induce pollen sterility without a phytotoxic response in crops. However, no studies that have been done on sweet stem sorghum using CHAs. There is a need to generate information on GCA of parents, SCA in cross combinations, the extent of heterosis to identify promising heterotic crosses for sugar related traits, yield and yield components in sweet sorghum.

References

Ahmad, M. 2002. Assessment of genomic diversity among wheat genotypes as determined by simple sequence repeats. *Genome* 45:646–651.

Aldrich, P.R. and Doebley, J. 1992. Restriction fragment variation in the nuclear and chloroplast genomes of cultivated and wild *Sorghum bicolor. Theoretical and Applied Genetics* 85:293-302.

Ali, A.J., Devakumar, C., Zaman, F.U. and Siddiq, E.A. 1999. Identification of potent gametocides for selective induction of male sterility in rice. *Indian Journal of Genetics 59*: 429—436.

Ali, M.L., Rajewski, J.F., Baenziger, P.S., Gill, K.S., Eskridge, K.M. and Dweikat, I. 2008. Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. *Molecular Breeding* 21: 497-509.

Almodares, A. and Hadi, M.R. 2009. Production of bioethanol from sweet sorghum: A review. *African Journal of Agricultural Research 4*: 772 – 780.

Anami, S.E., Zhang, L.M., Xia, Y., Zhang, Y.M., Liu, Z.Q. and Jing, H.C. 2015a. Sweet sorghum ideotypes: genetic improvement of the biofuel syndrome. *Food and Energy Security 4:* 159–177.

Anami, S.E., Zhang, L.M., Xia, Y., Zhang, Y.M., Liu, Z.Q. and Jing, H.C. 2015b. Sweet sorghum ideotypes: genetic improvement of stress tolerance. *Food and Energy Security 4*: 3–24.

Baocheng, C., Qiaoying, L., Hong, J. and Lerong, L. 1986. An analysis of genetic effects of the sugar containing on different stems in sorghum. *Acta Agronomica Sinica, (China)* 12: 39-42.

Becelaere, G.V., Edward, L.L., Paterson, A.H. and Chee, P.W. 2005. Pedigree-vs. DNA marker-based genetic similarity estimates in cotton. *Crop Science* 45:2281–2287.

Bernardo, R. 2014. Essentials of Plant Breeding. Stemma Press, Woodbury, Minnesota.

Blouet, A. Streiff, K. and Guckert, A. 1999. Possibilities for hybrid seed production in wheat. In: Heterosis and Hybrid Seed Production in Agronomic Crops. Food Products Press®: Binghamton, NY, USA: 81–108

Bowditch, B.M., Albright, D.G., Williams, J.G.K. and Braun, M.J. 1993. Use of random amplified polymorphic DNA (RAPD) in comparative genome studies. *Methods in Enzymology* 224: 294–309.

Bruns, R., and Peterson, C.J. 1998. Yield and stability factors associated with hybrid wheat. Wheat: Prospects for Global Improvement. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Buntin, D.G. 2012. Grain sorghum insect pests and their management. Cooperative Extension Bulletin 1283. University of Georgia, Griffin, USA. http://www.caes.uga.edu/publications/pubDetail.cfm?pk_id=7797 (accessed 13 October 2015)

Calvino, M., and Messing, J. 2012. Sweet sorghum as a model system for bioenergy crops. *Current Opinion Biotechnology 23*: 323–329.

Caniato, F., Guimara˜es, C., Schaffert, R., Alves, V., Kochian, L., Borém, A., Klein, P.E. and Magalhaes, J.V. 2007. Genetic diversity for aluminum tolerance in sorghum. *Theoretical and Applied Genetics* 114: 863-876.

Carpita, N.C., and McCann M.C. 2008. Maize and sorghum: genetic resources for bioenergy grasses. *Trends Plant Science 13*: 415–420.

Carvalho, S.P. and Cruz, C.D. 1996. Diagnosis of multicollinearity: assessment of the condition of correlation matrices used in genetic studies. *Brazilian Journal of Genetics* 19: 479-484.

Chakraborty, K. and Devakumar, C. 2006. Chemical hybridizing agents for chickpea (*Cicer arietinum* L.): leads from QSAR analysis of ethyl oxanilates and pyridones. *Journal of Agricultural Food Chemistry 54:* 1868-1873.

Chakraborty, K., Devakumar, C., Kumar, J. and Kumar, R. 2001. Effect of anilates on pollen sterility of chickpea (*Cicer arietinum* L. var. Pusa 240). *Tropical Agriculture Resolution 13:* 239—248.

Chen, X., Temnykh, S., Xu, Y., Cho,Y. G., and McCouch, S.R. 1997. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 95: 553-567.

Cisar, G., and Cooper, D.B. 2003. Hybrid wheat. Bread Wheat Improvement and Production. FAO Plant Production and Protection Series, No 30. FAO, Rome, Italy.

Clayton, W.D. and Renvoize, S.A. 1986. Genera of Gramineae grasses of the world. *Kew Bulletin Additional Series XIII, Royal Botanic*: 338-345.

Corner, A.B. and Karper, R.E. 1927. Hybrid vigour in sorghum. *Texas Agriculture Experiment Station Bulletin* 359: 1-23.

Cross, J. and Schulz, P. 1997. Chemical induction of male sterility. Pollen Biotechnology for Crop Production and Improvement. Cambridge University Press. Cambridge, England.

Cross, J.W. and Ladyman, J.A.R. 1991. Chemical agents that inhibit pollen development: Tools for research. *Sexual Plant Reproduction 4*: 235- 243.

Cruz, C.D., Carneiro, P.C.S. and Regazzi, A.J. 2014. Modelos Biométricos Aplicados ao Melhoramento Genético. 3rd edition. UFV, Viçosa, Brasilia.

Cuevas, H.E., Prom, L.K., Erpelding, J.E. and Brotons, V. 2014. Assessments of genetic diversity and anthracnose disease response among Zimbabwe sorghum germplasm. *Plant Breeding 133*: 234–242.

Dahlberg, J.A. and Wasylikowa, K. 1996. Image and statistical analyses of early sorghum remains (8000 B.P.) from the Nabta Playa archaeological site in the Western Desert, southern Egypt. *Vegetation History and Archaeobotany 5*: 293-299.

Dajue, L.1997. Proceedings of First International Sweet Sorghum Conference, Beijing, China.

Davila-Gomez, F., Chuck-Hernandez, C., Perez-Carrillo, E., Rooney, W. and Serna-Saldivar, S. 2011. Evaluation of bioethanol production from five different varieties of sweet and forage sorghums (*Sorghum bicolor* (L) Moench). *Industrial Crops Production 33*: 611–616.

de Wet, J.M.J. 1977. Domestication of African cereals. *African Economic History 3*: 15–32.

de Wet, J.M.J. 1978. Systematics and evolution of sorghum Sect. (Gramineae). *American Journal of Botany 65*: 477-484.

de Wet, J.M.J. and Harlan, J.R. 1971. The origin and domestication of *Sorghum bicolor*. *Economic Botany 25*: 128–135.

Deepak, G.C. 2014. Line × Tester analysis across environments for stalk sugar yield traits in sweet sorghum [*Sorghum bicolor* (L.) Moench]. MSc. Thesis. University of Agricultural Sciences, Dharwad, India.

Deu, M., Sagnard, F., Chantereau, J., Calatayud, C., He´rault, D., Mariac, C., Pham, J.L., Vigouroux, Y., Kapran, I., Traore, P.S., Mamadou, A., Gerard, B., Ndjeunga, J., Bezançon, G. 2008. Niger-wide assessment of in situ sorghum genetic diversity with microsatellite markers. *Theoretical and Applied Genetics* 116: 903-913.

Dicko, M.H., Gruppen, H., Traoré, A.S., Voragen, A.G.J. and van Berkel, W.J.H. 2006. Sorghum grain as human food in Africa: relevance of content of starch and amylase activities. *African Journal of Biotechnology 5*: 384-395.

Doggett, H. 1970. Sorghum (Tropical Agriculture Series). Longmans, London,United Kingdom.

Doggett, H. 1988. Sorghum. Longman Scientific and Technical, United Kingdom.

Dotlacil, L. and Apltauerova, M. 1978. Pollen sterility induced by ethrel and its utilization in hybridization of wheat. *Euphytica 27*: 353–360.

Driscoll, C.J. 1985. Modified XYZ system of producing hybrid wheat. *Crop Science 25*: 1115-1116.

Elangovan, M., Kiran babu, P., Seetharama, N. and Patil, J.V. 2014. Genetic diversity and heritability characters associated in sweet sorghum [*Sorghum bicolor* (L.) Moench]. *Sugar Tech 16:* 200–210.

Falconer, S.P. 1989. Introduction to Quantitative Genetics. The Ronald Press Company, New York, USA.

Food and Agriculture Organization Crop Production Statistics (FAOSTAT). 2016. World Sorghum Production and Utilization. FAO, Rome.

Ghebrehiwot, H., Burgdorf, R., Shimelis, H. and Laing, M. 2015. The efficacy of four gametocides for induction of pollen sterility in *Eragrostis tef* (Zucc.) Trotter. *African Journal of Biotechnology 14:* 774-780.

Guilford, W.J., Patterson, T.G., Vega, R.O., Fang, L., Liang, Y., Lewis, H.A. and Labovitz, J.N. 1992. Synthesis and pollen suppressant activity of phenylcinnoline-3-carboxylic acids. *Journal of Agriculture and Food Chemistry* 40: 2026–2032.

Guiying, L., Weibin, G., Hicks, A. and Chapman, K. R. 2000. A Training Manual for Sweet Sorghum. FAO/CAAS/CAS, Bangkok, Thailand, Available online at http://ecoportorg/ep?SearchType=earticleView&earticleId=172&page=2273

Harlan, J.R. and de Wet, J.M.J. 1972. A simplified classification of cultivated sorghum. *Crop Science* 12:172-176.

Haussmann, B.I.G., Obilana, A.B., Ayiecho, P.O., Blum, A., Schipprack, W. and Geiger, H.H. 1999. Quantitative genetic parameters of sorghum [*Sorghum bicolor* (L) Moench] grown in semi-arid areas of Kenya. *Euphytica* 105: 109-111.

Hayes, H.K., Immer, F.R. and Smith, D.C. 1955. Methods of Plant Breeding. Mc Graw Hill Book Co., Inc., New York, USA.

He, Z.H., Du, Z.H. and Zhuang, Q.S. 1998. Progress of wheat breeding research in China. Wheat: Prospects for Global Improvement. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hoagland, A.R., Elliott, F.C. and Rasmussen. L.W. 1953. Some histological and morphological effects of maleic hydrazide on spring wheat. *Agronomy Journal 45*: 468-472.

House, L.R. 1985. A Guide to Sorghum Breeding, 2nd edition. International Crops Research Institute for the Semi-Arid Tropics. Patancheru 502 324, Andhra Pradesh, India.

Huang, X.Q., Bo¨rner, A., Ro¨der, M.S. and Ganal, M.W. 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theoretical and Applied Genetics* 105: 699–707.

İştipliler, D., İlker, E., Aykuttonk, F., Çivi, G. and Tosun, M. 2015. Line × tester analysis and estimating combining abilities for yield and some yield components in bread wheat. *Turkish Journal of Field Crops 20:* 72-77.

Justin, R., Were, B., Mgonja, M., Santosh, D., Abhishek, R., Emmarold, M., Agustino, O. and Samuel, G. 2015. Combining ability of some sorghum lines for dry lands and sub-humid environments of East Africa. *African Journal of Agricultural Research 10:* 2048-2060.

Kamatar, M.Y., Biradar, B.D., Sowmya, H.H., Brunda, S.M., Deepakkumar, G.S., Sanjeevsingh, R. and Ramaling, H. 2015. Genetic variability and selection for grain mold resistance in F_3 progenies of sorghum. *International Journal of Farming and Allied Sciences 4*: 334-340.

Kaul, C.L. and Singh, S.P. 1967. Effects of some growth-regulators with gametocidal properties on *Cajanus cajan* (L.) Mitlsp. *Indian Journal of Agriculture Science 37*: 69-76.

Kempthorne, O. 1957. An Introduction to Genetic Statistics. Jonh Wiley and Sons, New York, USA.

Kenga, R., Alabi, S.O. and Gupta, S.C. 2004. Combining ability studies in tropical sorghum [*Sorghum bicolor* (L.) Moench]. *Field Crops Research* 88: 251–260.

Klein, R.R., John, E.M., David, R.J., Frederick, R.M., William L.R., Monica A.M., Cleve D.F., and Patricia, E.K. 2008. The Effect of tropical sorghum conversion and inbred development on genome diversity as revealed by high-resolution genotyping. *Crop Science 48*: 5–12.

Kucharek, T. 1992. Foliar and head diseases of sorghum in Florida. Circular 1073. Cooperative Extension Service, Gainesville, Florida, USA.

Kumar, A.A., Reddy, B.V.S., Sharma, H.C., Hash, C.T., Rao, P.S., Ramaia, B. and Reddy, P.S. 2011. Recent advances in sorghum genetic enhancement research at ICRISAT. *American Journal of Plant Sciences* 2: 589–600.

Kumar, C.V.S., Umakanth, A.V., Kotastane, T.V. and Sreelakshmi, C.H. 2012. Character association and path analysis for qualitative traits in sweet sorghum (*Sorghum bicolor* L. Moench). *Journal of Research ANGRAU* 40: 90-93.

Liable, C.A. 1974. Chemical methods for pollen control. Proceedings 29th Annual Corn and Sorghum Resolution Conference Publication 29: 174-182.

Lipinsky, E. and Kresovich, S. 1980. Sorghums as energy crops. Proceedings, Bio-Energy'80, World Congress and Exposition. The Bio-Energy Council, Atlanta, Georgia, USA.

Mace, E., and Jordan, D. 2013. Location of major effect genes in sorghum (*Sorghum bicolor* (L.) Moench). *Theoretical and Applied Genetics* 121: 1339–1356.

Mace, E.S., Mathur P.N., Godwin, I.D., Hunter, D., Taylor, M.B., Singh, D., DeLacy, I.H. and Jackson, G.V.H. 2005. Development of a regional core collection (Oceania) for taro, *Colocasia esculenta* (L.) Schott., based on molecular and phenotypic characterization. The Global Diversity of Taro: Ethnobotany and Conservation. Minpaku (National Museum of Ethnology), Osaka, Japan.

Mace, E.S., Tai, S., Gilding, E.K., Li, Y., Prentis, P.J., Bian, L., Campbell, B.C., Hu, W., Innes, D.J., Han, X., *et al*. 2013. Whole-genome sequencing reveals untapped genetic potential in Africa's indigenous cereal crop sorghum. *Nature Communications* 4: 1–9.

Mahalakshmi, V., Aparna, P., Ramadevi, S. and Rodomiro, O. 2002. Genomic sequence derived simple sequence repeats markers. A case study with *Medicago* spp. *Electronic journal of Biotechnology* 5:233-242.

Makanda, I. 2009. Combining Ability and Heterosis for Stem Sugar Traits and Grain Yield Components in Dual-Purpose Sorghum (*Sorghum bicolor* L. Moench) Germplasm. PhD thesis. University of KwaZulu-Natal, South Africa.

Makanda, I., Tongoona, P., Derera, J., Sibiya, J. and Fato P. 2010. Combining ability and cultivar superiority of sorghum germplasm for grain yield across tropical low and mid-altitude environments. *Field Crops Research* 116: 75-85.

Mangena, P. 2014. Breeding Gains, Cytoplasmic Effects and Path Coefficient Analysis in Pro-Vitamin A Maize Hybrids. M.Sc dissertation, University of KwaZulu-Natal, South Africa.

Mann J.A., Kimber, C.T. and Miller, F.R. 1983. The origin and early cultivation of sorghums in Africa. *Texas Agricultural Experiment Station Bulletin 1454.*

Masood, S.A., Awan, Z.K., Naseem, Z., Fazal, A., Aslam, N., Tahir, M.H.N. and Ali, Q. 2015. Genetic variability and trait association for relative growth rate and green fodder yield in *Sorghum bicolor* L. *International Journal of Biology, Pharmacy and Allied Sciences 4*: 5566-5576.

Mcbee, G.G., Creelman, R.A. and Miller, F.R. 1988. Ethanol yield and energy potential of stems from a spectrum of sorghum biomass types. *Biomass 17*: 203–211.

McDaniel, R.G. Chemical Hybridizing Agents. United States patent 5,113,617. May 19,1992.

McRae, D.H. 1985. Advances in chemical hybridization. *Plant Breeding Reviews 3*: 169-191.

Melil, K.C., Njoka, M.F., Ombakho, A.G. and Omari, M.O. 2013. Genetic variability analysis for growth and yield parameters in double cross maize (*Zea mays*) genotypes in Kitale county of Kenya. *Journal of Plant Breeding Genetics* 1: 7-11.

Menz, M.A., Klein, R.R., Unruh, N.C., Rooney, W.L., Klein, P.E. and Mullet, J.E. 2004. Genetic diversity of public inbreds of sorghum determined by mapped AFLP and SSR markers. *Crop Science* 44:1236–1244.

Miller, F.R. 1982. Genetic and environmental response characteristics of sorghum. International Crops Research Institute for the Semi-Arid Tropics Symposium on Sorghum. Patancheru, India.

Morris, G.P., Punna, R., Santosh P., Deshpande, C., Thomas, H., Trushar, S., Hari, D. Upadhyaya, O.R. *et al*. 2013. Population genomic and genome-wide association studies of agroclimatic traits in sorghum. *Proceedings of the National Academy of Sciences* 110: 453–458.

Morris, G.P., Ramu, P., Deshpande, S.P., Hash, C.T., Shah, T., Upadhyaya, H.D., Riera-Lizarazu, O., Brown, P.J., Acharya, C.B., Mitchell, S.E., Harriman, J., Glaubitz, J.C., Buckler, E.S. and Kresovich, S. 2013. Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proceedings of the National Academy of Sciences of the United States of America 110:453-458.

Mullet, J., Morishige, D., McCormick, R., Truong, S., Hilley, J., McKinley, B., Anderson, R., Olson, S.N. and Rooney, W. 2014. Energy Sorghum—a genetic model for the design of C_4 grass bioenergy crops. *Journal of Experimental Botany* 65: 3479–3489.

Muraya, M.M., de Villiers, S., Parzies, H.K., Mutegi, E., Sagnard, F., Kanyenji, B.M., Kiambi, D. and Geiger, H.H. 2011. Genetic structure and diversity of wild sorghum populations (*Sorghum* spp.) from different eco-geographical regions of Kenya. *Theoretical and Applied Genetics* 123: 571–583.

Murray, K. 1998. F_1 seed production efficiency by using photoperiod-sensitive cytoplasmic male sterility and performance of F_1 hybrid lines in wheat. *Breeding Science 48*: 35-40.

Murray, S. C., Rooney, W. L., Hamblin, M. T., Mitchell, S. E. and Kresovich, S. 2009. Sweet sorghum genetic diversity and association mapping for brix and height. *The Plant Genome* 2: 48-62.

Murray, S.C., Sharma, A., Rooney, W.L., Klein, P.E., Mullet, J.E., Mitchell, S.E. and Kresovich, S. 2008. Genetic improvement of sorghum as a biofuel feedstock: I. QTL for stem sugar and grain nonstructural carbohydrates. *Crop Science* 48: 2165-2179.

Mutengwa, C.S., Tongoona, P., Mabasa, S. and Chivinge, O.A. 1999. Resistance to *Striga asiatica* (L.) Kuntze in sorghum: Parent characterization and combining ability analysis. *African Crop Science Journal* 7: 321–326.

Mwadalu, R. and Mwangi, M. 2013. Potential role of sorghum in enhancing food security in semi-arid Eastern Kenya. *Journal of Applied Biosciences 71*: 5786– 5799.

Nadarajan, N. and Gunasegaram, M. 2005. Quantitative Genetics and Biometrical Techniques in Plant Breeding. Kalyani Publishers, New Delhi, India.

Nesvadba, Z., and T. Vyhnánek. 2001. Induction of hybrid wheat using the gametocide Genesis® in the Czech Republic. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* 49: 27-35.

Nesvadba, Z., Vyhnánek, T. and Vendar, J. 2001. The testing of efficacy of CHA Genesis® for production of hybrid wheat and hybrid triticale. *Acta Fytotechnica et Zootechnica 4*: 266-268.

Parker, G.D., Fox, P.N., Langridge, P., Chalmers, K., Whan, B. and Ganter, P.F. 2002. Genetic diversity within Australian wheat breeding programs based on molecular and pedigree data. *Euphytica* 124:293–306.

Paterson, A.H., Bowers, J.E. and Bruggmann, R. 2009. The *Sorghum bicolor* genome and the diversification of grasses. *Nature* 457: 551–556.

Pfeiffer, T.W., Bitzer, M.J., Toy, J.J. and Pedersen, J.F. 2010. Heterosis in sweet sorghum and selection of a new sweet sorghum hybrid for use in syrup production in Appalachia. *Crop Science* 50: 1788-1794.

Pickett, A.A. 1993. Hybrid Wheat: Results and Problems. Paul Parey Scientific Publishers, Berlin and Hamburg, Germany.

Prabhakar. 2001. Variability, heritability, genetic advance and character association in rabi sorghum. *Journal of Maharashtra Agricultural Universities* 26: 188-189.

Prakasham, R.S., Nagaiah, D., Vinutha, K.S., Uma, A., Chiranjeevi, T., Umakanth, A.V., *et al*. 2014. Sorghum biomass: a novel renewable carbon source for industrial bio-products. *Biofuels 5*:159–174.

Quinby, J.R. 1963. Manifestations of hybrid vigor in sorghum. *Crop Science* 3:288-291.

Rami, J.F., Dufour, P., Trouche, G., Fliedel, G., Mestres, C., Davrieux, F. 1998. Quantitative trait loci for grainquality, productivity, morphological and agronomical traits in sorghum (*Sorghum bicolor* L. Moench). *Theoretical and Applied Genetics* 97: 605–616.

Reisch, B.I. 1998. Molecular markers: The foundation for grapevine genetic mapping, DNA fingerprinting and genomics. Proceedings of the 7th International Symposium of Grapevine Genetics and Breeding. New York State Agricultural Experiment Station, Cornell University, Geneva, NY, USA.

Rios, A.S., Borem, A., Guimarães, P.E.O. and Paes, M.C.D. 2012. Análise de trilha para carotenoides em milho. *Revista Ceres* 59: 368-373.

Ritter, K.B., Jordan, D.R., Chapman, S.C., Godwin, I.D., Mace, E.S. and McIntyre, C.L. 2008. Identification of QTL for sugar related traits in a sweet × grain sorghum (*Sorghum bicolor* L. Moench) recombinant inbred population. *Molecular Breeding* 22:367-384.

Rooney, W.L., Blumenthal, J., Bean, B. and Mullet, J.E. 2007. Designing sorghum as a dedicated bioenergy feedstock. *Biofuels, Bio-production Bio-refinery 1*: 147–157.

Rosenow, D., and Clark, L. 1995. Proceedings of the 50th annual corn and sorghum industry research conference. Drought and lodging resistance for a quality sorghum crop. Chicago,USA.

Saballos, A. 2008. Development and utilization of sorghum as a bioenergy crop. Genetic Improvement of Bioenergy Crops. Springer Science and Business Media, LLC, New York, NY, U.S.A.

Salih, A.I.S., Ibrahim, N., Shahbaz, K.B., Sana, U.B. and Waseem, B. 2016. Genetic variability and estimates of heritability in sorghum (*Sorghum bicolor* L.) genotypes grown in a semiarid zone of Sudan. *Archives of Agronomy and Soil Science 62*: 139-145.

Schlehuber, A.M. 1945. Inheritance of stem characters in certain sorghum varieties and their hybrids. *Journal of Heredity* 36: 219–222.

Senior, M.L., Murphy, J.P., Goodman, M.M. and Tuber, C.W. 1998. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Science* 38:1088-1098.

Shahwar, D., Noor, M., Hidayat-ur-Rahman, N., Ullah, I., Ia, S., Ali, F., Shah, S.M.A. and Mehmood, N. 2012. Characterization of sorghum germplasm for various morphological and fodder yield parameters. *African Journal Biotechnology 11*: 11952–11959.

Shiringani, A.L., Frisch, M. and Friedt, W. 2010. Genetic mapping of QTLs for sugar-related traits in a RIL population of *Sorghum bicolor* L. Moench. *Theoretical and Applied Genetics* 121: 323-336.

Singh, P.K., Ranjan, R., Shahi, J.P and Singh, A.K. 2014. Studies on combining ability analysis in white kernel color of quality protein maize [*Zea mays* L.). *Agricultural Science Digest* 34: 21 – 25.

Singh, R.K. and Chaudhary, B.D. 1985. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers, New Delhi, India.

Sorriano, M.J., Romero, C., Vilanova, S., Lla`cer, G. and Badenes, M.L. 2005. Genetic diversity of loquat germplasm (*Eriobotrya japonica* [Thunb] Lindl) assessed by SSR markers. *Genome* 48: 108-114.

Sprague, G.F. and Tatum, L.A. 1942. General versus specific combining ability in single crosses of corn. *Journal of American Society of Agronomy* 22: 320–326.

Srinivas, G., Satish, K., Madhusudhana, R., Reddy, R.N., Mohan, S.M., and Seetharama, N. 2009. Identification of quantitative trait loci for agronomically important traits and their association with genic-microsatellite markers in sorghum. *Theoretical and Applied Genetics* 118:1439-1454.

Stephens, J.C. and Holland, P.F. 1954. Cytoplasmic male sterility for hybrid sorghum seed production. *Agronomy Journal 46*: 20–23.

Tariq, A.S., Akram, S., Shabbir, G., Khan, K.S., Mahmood, T., and Iqbal, M.S. 2014. Heterosis and combining ability evaluation for quality traits in forage sorghum (*Sorghum bicolor* L.). *SABRAO Journal of Breeding and Genetics 46:* 174-182.

Tawanda, Z. 2004. DNA-Based Methods in Sorghum Diversity Studies and Improvement. Plant Biotechnology Center, Ohio State University, USA.

Tesfamichael, A., Githiri, S.M., Kasili, R., Woldeamlak, A. and Nyende, A.B. 2015. Genetic variation among sorghum (*Sorghum bicolor* L. Moench) landraces from Eritrea under post-flowering drought stress conditions. *American Journal of Plant Sciences 6*: 1410-1424.

Tew, T.L., Cobill, R.M. and Richard, E.P. 2008. Evaluation of sweet sorghum and sorghum X sudangrass hybrids as feedstocks for ethanol production. *Bioenergy Research* 1: 147–152.

Thakare, D.P., Ghorade, R.B. and Bagade, A.B. 2014. Combining ability studies in grain sorghum using line X tester analysis. *International Journal of Current Microbiology and Applied Science 3:* 594-603.

Ukaoma, A.A., Okocha, P.I. and Okechukwu, R.I. 2013. Heritability and character correlation among some rice genotypes for yield and yield components. *Journal of Plant Breeding Genetics* 1: 73-84.

Uptmoor, R., Wenzel, W., Friedt, W., Donaldson, G., Ayisi, K. and Ordon, F. 2003. Comparative analysis on the genetic relatedness of *Sorghum bicolor* accessions from southern Africa by RAPDs, AFLPs and SSRs. *Theoretical and Applied Genetics* 106:1316-1325.

van den Berg, J. and Drinkwater, T.W. 1997. Field guide to identification of sorghum pests in Southern Africa. Agricultural Research Council-Grain Crops Institute, Potchefstroom, South Africa.

Vermerris, W. 2011. Survey of genomics approaches to improve bioenergy traits in maize, sorghum and sugarcane free access. *Journal of Integrated Plant Biology 53*: 105–119.

Vermerris, W., and Saballos, A. 2013. Genetic enhancement of sorghum for biomass utilization. Genomics of the Saccharinae. Springer, New York, USA.

Virmani, S.S. and Edwards. I.B. 1983. Current status and future prospects for breeding hybrid rice and wheat. *Advances in Agronomy 36*: 145-214.

Virmani, S.S., Sun, Z X., Mou, T.M., Jauhar Ali, A. and Mao, C.X. 2003. Male sterility systems in rice. Two-Line Hybrid Rice Breeding Manual. International Rice Research Institute: Los Baños, Philippines.

Wang, Y.H., Upadhyaya, H.D., Burrell, A.M., Sahraeian, S.M.E., Klein, R.R. and Klein P.E. 2013. Genetic structure and linkage disequilibrium in a diverse, representative collection of the C4 model plant, *Sorghum bicolor*. *Genes Genome Genetics* 3: 783-793.

Warkad, Y.N., Potdukhe, N.R., Dethe, A.M., Kahate, P.A. and Kotgire, R.R. 2008. Genetic variability, heritability and genetic advance for quantitative traits in sorghum germplasm. *Agricultural Science Digest* 28: 165 – 169.

Wassell, L., and Weaver, V. 1995. Benefits of hybrid wheat. HybriTech Product Development Report. Monsanto and HybriTech Seed International Inc. St. Louis, MO, USA.

Wendorf, F., Close, A.E., Schild, R., Wasylikowa, K., Housley, R.A., Harlan, J.R. and Królik, H. 1992. Saharan exploitation of plants 8,000 years BP. *Nature 359*: 721–724.

Westman, A.L. and Kresovich, S. 1997. Use of Molecular Marker Techniques for Description of Plant Genetic Variation. Plant Genetic Resources. Conservation and Use. CAB Int., Wallingford, United Kingdom.

Whitkus, R., Doebley, J., Lee, M. 1992. Comparative genome mapping of sorghum and maize. *Genetics* 132:1119-1130.

Wilson, J.A. 1984. Hybrid wheat breeding and commercial seed development. *Plant Breeding Reviews 2*: 303-319

Wong, M., Blouet, A. and Guckert, A. 1995. Effectiveness of SC2053 as a chemical hybridizing agent for winter wheat, importance of developmental stages and doses of application. *Plant Growth Regulation 16*: 243-248.

Wright, S. 1921.Correlation and causation. *Journal of Agricultural Research* 20: 557-585.

Xiao, J., Li, L., Yuan, L., McCouch, S.R. and Tanksley, S.D. 1996. Genetic diversity and its relationship to hybrid performance in heterosis in rice as revealed by PCR-based markers. *Theoretical and Applied Genetics*, 92:637-643.

Xu, F., Shi, Y.C., Wu, X., Theerarattananoon, K., Staggenborg, S. and Wang, D. 2011. Sulfuric acid pretreatment and enzymatic hydrolysis of photoperiod sensitive sorghum for ethanol production. *Bioprocess Bio-systems Engineering 34*: 485–492.

Yan, W. and Hunt, L. A. 2002. Biplot analysis of diallel data. *Crop Science Journal* 42: 21-30.

Zhou, H., Zuhu, D., Jun, L. and Zhang, M., 2005. Analysis on combining ability for the energy sugar stalk breeding. *Acta Agriculturae Universitatis Jiangxiensis* 27: 14-20.

Chapter 2

Characterization of sweet stem sorghum genotypes for bio-ethanol production

Abstract

In an effort to characterize and select promising sweet stem sorghum genotypes with enhanced biofuel productivity, the present study investigated the phenotypic variability present among diverse sorghum genotypes based on ethanol production and related agronomic traits. One hundred and ninety sorghum genotypes were evaluated at Ukulinga Research Station in South Africa. Data collected included days to 50% flowering, plant height, stem diameter, fresh biomass yield, dry matter yield, fibre content, stalk brix and ethanol yield. Data were subjected to analysis of variance, cluster analysis, correlation analysis, path coefficient analysis and principal component analysis. Significant differences ($P < 0.01$) were detected among tested genotypes for all measured traits. Days to flowering varied from 62 to 152 with a mean of 93 days. The genotypes with delayed maturity associated with increased biomass production were AS46, AS434, AS443, AS441 and AS205. Plant height varied from 90 to 420 cm with a mean of 236 cm. The tallest genotypes were AS442, AS443, AS447, AS448 and AS441. Stem diameter ranged from 7 to 31cm with a mean of 16 cm. Genotypes with the thickest stalks included AS143, AS441, AS251, AS250 and AS442. Biomass yield varied from 6.668 to 111.2 t ha^{-1} with a mean of 30 t ha^{-1}. Genotypes AS203, AS205, AS448, AS443, AS251 had the highest biomass production. Stalk dry matter content ranged from 17.2 to 44.2 % with a mean of 29.8 %, while fibre content varied from 8.92 to 34.8 % with a mean of 17.2 %. The stalk brix yield of genotypes varied from 3.3 to 18.9 % with a mean of 12.1 %. Ethanol productivity ranged from 240.9 to 5500 l ha^{-1} with a mean of 1886 l ha^{-1}. The best genotypes for ethanol productivity were AS203, AS391, AS205, AS251 and AS448 providing mean yields of 5474 l ha^{-1}, 4509 l ha^{-1}, 4315 l ha^{-1}, 4205 l ha^{-1} and 3816 l ha^{-1}, in that order. Days to flowering, plant height, stalk brix and stem diameter exerted the greatest indirect effects on ethanol production through higher biomass production. Biomass yield had the greatest direct effect on ethanol production. Therefore, the above traits should be considered during breeding sorghum for bio-ethanol production. Also, the traits had high heritability values, hence selection should provide for good genetic gains. Overall, the above sweet stem sorghum genotypes are useful genetic resources for breeding of sorghum with enhanced bio-ethanol production.

Keywords: correlation, heritability, genetic advance, genetic coefficient of variation, morphological diversity, path coefficient analysis, phenotypic coefficient of variation, principal component analysis

2.1 Introduction

Sweet stem sorghum possesses a number of adaptive and constituent traits rendering it suitable as a biofuel crop. In comparison with the other biofuel crops such as sugarcane and sugar beet, sweet stem sorghum exhibits more valuable traits such as lower water and fertilizer requirements, greater drought tolerance and salt tolerance, with greater adaptability to tropical, subtropical and temperate climates. The crop has a short harvesting period that lies in the intermittent sugar-harvesting period allowing for crop rotation (Ratnavathi *et al.,* 2011; Eggleston *et al.,* 2013; Morrissey and Thoma, 2017). Several recent studies have made the case for using sweet stem sorghum as a bioenergy crop (Vermerris, 2011; Calvino and Messing, 2012; Mullet *et al*., 2014; Prakasham *et al.,* 2014; Anami *et al*., 2015 a and b).

Genetic diversity is a vital prerequisite for selecting suitable parents for creating genetic diversity or for developing hybrid cultivars. Genetic enhancement of crops through conventional breeding is viable if substantial genetic variation is present and the desirable traits are heritable. Development of sweet stem sorghum cultivars for bioenergy requires efficient identification and incorporation of suitable traits from complementary genotypes. A number of morphological traits such as thick stems, talk stalks, late maturity and high biomass are regarded as important attributes of the crop for increased bioethanol production (Elangovan *et al.*, 2014; Lekgari and Dweikat, 2014; Regassa and Wortmann, 2014; Tesfamichael *et al.*, 2015). For example, Mathur *et al.* (2017) in their review on the potential of sweet stem sorghum as a biofuel crop indicated the high potential of the crop for biofuel production attributable to increased biomass yields, thicker and fleshier stems, with high juice yields reaching up to 78% of the total biomass, and Brix content ranging from 14 to 23% (Tesfamichael *et al.*, 2015). Breeding for adaptation to tropical and temperate climates, and gene exchange among the five races of sorghum, have endowed sweet stem sorghum with a high level of genetic variability (Elangovan *et al.,* 2014).

Given that most breeding endeavors are time consuming and cost intensive, it is necessary to undertake simultaneous selection of several traits and to investigate the inter-relationships of phenotypic traits. Understanding of the associations among traits will subsequently reduce time and funds invested in breeding programs for the reason that concurrent enhancement of a several traits can be pursued if they are positively correlated (Mangena, 2014; Lombardi *et al.,* 2015). Studies on the correlation of traits are instrumental for assessing the feasibility of mutual selection of two or more traits, based on calculating the influence of selection for secondary traits on genetic gain for the primary trait under consideration. In contrast, if

characters are negatively associated, then it is difficult to implement concurrent selection (El Naim *et al.,* 2012). Path coefficient analysis, originally proposed by Wright (1921) and later illustrated by Dewey and Lu (1959), permits apportioning of correlation coefficients into direct and indirect effects of traits on a dependent variable and accordingly assists in evaluating the cause-effect relationship for effective selection (Ezeaku and Mohammed, 2006; Ali *et al.,* 2011). Path coefficient analysis has not been not widely applied in sweet stem sorghum selection programs (Kumar *et al.,* 2012). Its application has gained momentum recently (Tesfaye *et al.,* 2014; Kassahun *et al.,* 2015) mainly due to the upsurge in research on sweet stem sorghum as a biofuel crop.

Phenotypic variability is a reflection of both genetic and non-genetic components, hence, approximation of genetic parameters in the context of trait characterization is an indispensable component for prospective sweet stem improvement programs (Sami *et al.,* 2013; Yaqoob *et al.,* 2015). To initiate a successful breeding program, it is of paramount significance to have a clear comprehension of variability using parameters like genetic co-efficient of variation, heritability and genetic advance (Govindaraj *et al.,* 2010; Govindaraj *et al.,* 2011; Kassahun *et al.,* 2015). Genetic coefficient of variation concurrently with heritability values give the best estimates of the extent of response anticipated from selection (Akhtar *et al.,* 2007).

The African Centre for Crop Improvement (ACCI) of University of KwaZulu-Natal (UKZN) is actively engaged in breeding sweet stem sorghum for bio-fuel production in South Africa. The center assembled a collection of over 190 sweet stem sorghum varieties from various sources. The objective of the present study was to determine the phenotypic variability present among diverse sweet stem sorghum genotypes based on ethanol production and seven related agronomic traits. Information presented in the study may assist in selecting promising sweet stem sorghum genotypes with enhanced biofuel productivity for direct production or for future breeding programs.

2.2 Materials and methods

2.2.1 Plant materials and experimental design

The study used 190 sorghum genotypes. The genotypes were sourced from various origins including from Zimbabwe, South Africa, the Netherlands, Australia, India, Ethiopia, Mozambique, Kenya, United States of America, Sudan, Zambia, Malawi and Mexico. The South African genotypes were collected from the following provinces: KwaZulu-Natal, Eastern Cape and

Limpopo. The test genotypes included Urja and Sugargraze that were used as standard checks. Urja is a commercial sweet stem sorghum hybrid being commercialized by Praj Industries in India. Sugargraze also referred to as SS120 is a three-way hybrid developed in Australia. Detailed information about each genotype is given in Table 2.1. Experiments were laid out in a lattice design containing 22 incomplete blocks with two complete blocks. Nine genotypes were allocated in each incomplete block. Each entry was planted in two-row plots of 3.0 m length with inter-row and intra-row spacing of 80 cm and 20 cm, respectively.

Table 2.1: Description of the 190 sweet stem sorghum varieties used in the study

Name	Pedigree	Origin	Name	Pedigree	Origin	Name	Pedigree	Origin
AS1	KARI Mtama-1	Kenya	AS197	SA landrace LP 43	-	AS49	MN 1618 (tall selection)	-
AS10	MN 1812(Cream)	USA	AS198	SA landrace LP 44	-	AS5	Pirira	Malawi
AS100	Sefofo	-	AS199	SA landrace LP 45	-	AS50	MN 1705	-
AS101	ICSB 478	-	AS2	Thar	Kenya	AS51	MN 1812	-
AS102	RTX 436	-	AS200	SA landrace LP 46	-	AS52	MN 2365	-
AS103	MN 2332	-	AS202	SA landrace LP 48	-	AS53	MN 2500	-
AS105	FPR(168 x GS70)	-	AS203	SA landrace LP 49	-	AS54	MN 2622	-
AS106	KAT-369 x Makueni local	-	AS204	SA landrace LP 50	Polokwane	AS55	MN 4002 (short selection)	-
AS107	SDS 5232	-	AS205	SA landrace LP 51	-	AS56	MN 4002 (tall selection)	-
AS108	P9504B	-	AS219	Imfe Bulawayo	Zimbabwe	AS57	MN 4137	-
AS109	P9511B	-	AS240	AS19	Ukulinga	AS58	MN 4320 (short selection)	-
AS110	P9538B	-	AS241	AS79	Ukulinga	AS59	MN 4320 (tall selection)	-
AS111	P9539B	-	AS242	AS1	Ukulinga	AS6	Serena	Kenya
AS112	BKS24ms3/BON34	-	AS243	AS2	Ukulinga	AS62	MN 4519	-
AS113	TX2737/91BE7414	-	AS244	AS2	Ukulinga	AS63	Nus 34	-
AS114	BTx3197	-	AS245	AS77	Ukulinga	AS64	Nus 34 (2nd gen.)	-
AS115	BTx631	-	AS246	AS97	Ukulinga	AS65	P9528	-
AS116	01Aphid207	-	AS247	AS79	Ukulinga	AS66	BTX 378	-
AS117	01Aphid148	-	AS248	AS72	Ukulinga	AS67	SDL 89473	-

Table 2.1 continued

Name	Pedigree	Origin	Name	Pedigree	Origin	Name	Pedigree	Origin
AS12	CR35.5 x IESV8820 x Serena	Kenya	AS249	AS72	Ukulinga	AS68	GV 3020	Zambia
AS121	Kat 369 x EX-1 Chira	-	AS250	AS97	Ukulinga	AS69	ICSR 91030	-
AS122	KSV 12	-	AS251	AS97	Ukulinga	AS7	Seredo	Kenya
AS124	02mn4034-(K70647-1-1/pl1	-	AS252	AS97	Ukulinga	AS70	SDS 3978	-
AS127	Tx2737	-	AS253	AS72	Ukulinga	AS71	Dwarf Wonder	-
AS128	Tx2883	-	AS254	AS72	Ukulinga	AS72	KAT-487	-
AS129	KARI Mtama 1 x ICS 3-1	-	AS255	AS72	Ukulinga	AS73	IRAT-204	-
AS13	Gadam El Hamam	Sudan	AS256	AS72	UKZN	AS74	ICSV 111	-
AS130	Gambella 1107	-	AS257	AS72	UKZN	AS75	SADC entry 35	-
AS131	WK#1025 Sudan	-	AS258	AS72	UKZN	AS76	CR35.5xIS-882	-
AS132	Parc 1260793	-	AS259	AS72	UKZN	AS77	SDSH 90162	-
AS133	Marimanti Co 1110	-	AS260	AS72	UKZN	AS78	IS 8193xAF 28	-
AS134	P6 NQ#23 Sudan	-	AS261	AS72	UKZN	AS79	P9513B	USA
AS135	Dinkmash	-	AS262	AS72	UKZN	AS80	P9521	-
AS136	FLO (107) x GS 3541	-	AS263	AS72	UKZN	AS81	P9526	-
AS137	IESV 92022 DL	-	AS264	AS13	Mtentu	AS82	ICSR 93034	India
AS138	Mugeta	-	AS265	AS79	Mtentu	AS83	ICSV 700	-
AS14	NUS 18 SA4455	SA	AS271	AS79 x SS27	Ukulinga	AS84	NTJ 2	-
AS140	Kaguru	-	AS28	NUS28 - SA4470	South Africa	AS85	Ent 64 DTN	-
AS141	Kiboko local	-	AS3	ZSV 3	Zambia	AS86	ICSV 574	-
AS143	Red Swazi	-	AS308	AS204 x AS138	Ukulinga	AS87	ICSB 323	-
AS145	AWN98	-	AS391	SS 27 C	Ukulinga	AS88	SDSL 89569	-
AS146	GV 3017	Zambia	AS393	AS138 x SS27	Ukulinga	AS89	Skalane	Zimbabwe

Table 2.1 continued

Name	Pedigree	Origin	Name	Pedigree	Origin	Name	Pedigree	Origin
AS147	MRS94	-	AS395	AS103 x AS97	Ukulinga	AS9	Nus 35(2nd gen.)	-
AS148	SDS 3472	-	AS4	Macia	Mozambique	AS90	ICSB 724	-
AS15	Lanet	Kenya	AS41	MN 1408	-	AS91	ICSB 5	-
AS150	SDSL89572	-	AS42	MN 1435	-	AS92	SV 1	-
AS152	01MN1589	-	AS421	#5 235466	Ethiopia	AS93	SAR 29	India
AS153	Mul	-	AS43	MN 1439	-	AS94	ICSB731(ICSV1171 BF)	-
AS154	SDSH 409	-	AS432	#14 235929	Ethiopia	AS95	SPV 1411	-
AS155	ICSV 91085 x IESV 9105 DL	-	AS434	#3 243684	Ethiopia	AS96	ICSB 4	-
AS158	964063 x Seredo	-	AS436	N-13strigaesistant	Ethiopia	AS97	E 36-1	Ethiopia
AS16	SDS 342	-	AS44	MN 1500	-	AS98	ICSVP 3046	-
AS161	KAT 369 x PP 290	-	AS440	White Degalet	Ethiopia	AS99	S 35	-
AS162	IESV 920220L	-	AS441	Red Degalet 1	Ethiopia	PEX40287	61(07S)-35	-
AS165	IS 155	-	AS442	Red Degalet 2	Ethiopia	SS120	Sugargraze	Australia
AS167	NUS 2 - SA4433	-	AS443	Red Degalet 3	Ethiopia	SS17	SS17	SA
AS17	ICSV 3	-	AS444	Yellow Degalet	Ethiopia	SS27	SS27	SA
AS173	Sinankhomo	Malawi	AS445	IBRO 1	Ethiopia	SS44	SS44	SA
AS18	Mexican R Line 5	Mexico	AS447	IS11167	Ethiopia	SS49	SS49	SA
AS19	Mexican R Line 15	Mexico	AS448	IS11758	Ethiopia	SS52	SS52	SA
AS192	Msinga imphe	-	AS45	MN 1557	-	SS56	SS56	SA
AS194	Mtentu imphe	-	AS46	MN 1557	-	Urja	URJA	India
AS195	SA landrace LP 41	-	AS48	MN 1618	-	WD103	W Head 3	Netherlands
AS196	SA landrace LP 42	-						

-=Unknown origin; SA= South Africa

2.2.2 Trial establishment and maintenance

Experimental genotypes were planted in seedling trays at the greenhouse facility of Controlled Environment Research Unit (CERU) at the University of KwaZulu-Natal. At four weeks after planting seedling plants were transplanted to Ukulinga Research Farm at the University of KwaZulu-Natal, in South Africa (29°37′S 30°22′E; 596 m above sea level). The trial was conducted during the 2015/2016 summer season (November to April). The trial site received 581 mm moisture through rainfall and supplemental irrigation. The mean minimum temperature for the season was 10.2ºC and the mean maximum temperature was 28.4ºC. Experimental plots were fertilized at 0.006 kg per plot with 2:3:2 (N:P:K) granular fertilizer. At the flowering stage plants were side dressed with 0.002 kg per plot of urea (46 % N) granular fertilizer. After transplanting the trial was treated with 100 ml ha^{-1} of lamda cyhalothrin (Karate) to control a wide range of pests. When plants reached 60 cm height plots were treated with 120 ml ha^{-1} of lamda cyhalothrin to control pests.

2.2.3 Data collection

The following quantitative traits were measured during the study: days to flowering were counted as the date of seeding to the time that 50 % of the plants started flowering (Vanderlip and Reeves, 1972). Plant height was measured from the base of the plant to the tip of the panicle and expressed in cm at 50% flowering. Stem diameter (cm) was measured using a vernier caliper on the three mid-internode sections. Stalk biomass ($t\ ha^{-1}$) was measured by removing leaves and heads, then cutting at ground level and weighing the stems at 50% maturity. Samples of chopped stalks were weighed green and re-weighed after oven drying at 60ºC for 30 h. Dry matter was calculated using the formula:$Dry\ Matter\ \% = \frac{Dry\ mass}{Wet\ mass} \times 100\ \%$. Fibre content was calculated using the formula: $Fibre\ \% = Dry\ matter\ \% - 0.005 - \frac{Stalk\ Brix}{100}$. Stalks were cut using a chaff cutter and a representative sample was analyzed for % Brix using hand-held refractometer method. Ethanol productivity was calculated using the formulae below:

$$Total\ Brix = Biomass\ t\ ha^{-1} \times \frac{Stalk\ Brix}{100}$$

$$Total\ fermentable\ sugars = Total\ Brix\ \times 0.85$$

$$Ethanol\ l\ ha^{-1}\ = \frac{Total\ fermentable\ sugars\ \times 0.46}{0.79} \times 1000$$

2.2.4 Data analyses

General analyses of variance were performed for all quantitative data using the REML program of GenStat 17th edition with number of plants as the covariate using a model by Cochran and Cox (1957). Multiple comparisons among variety means were conducted by Fisher's unprotected least significant difference (LSD) test at 5% levels of significance (Fisher, 1935). Cluster analysis was performed using Genstat 18th edition to establish genetic relationships among genotypes. Principal component analysis (PCA) based on the correlation matrix was performed using Genstat to identify influential traits for selection. A PCA bi-plot was plotted using GenStat to show the associations among genotypes based on observed traits. The Pearson's phenotypic correlation analysis was performed in GenStat 17th Edition (Payne et al., 2011) to describe the relationship among the morphological traits. Path coefficient analysis was used to calculate direct and indirect effects of traits on ethanol production using the PathSAS program (SAS Institute, 2010) developed by Cramer et al. (2000). Variance components were partitioned using the restricted maximum likelihood (REML) approach in Genstat 17th. *Heritability* in the broad- sense was calculated using Hallauer and Miranda (1981) as follows:$H^2 = \frac{\delta_g^2}{\delta_p^2}$; where: H^2 = broad-sense heritability; δ_g^2 = genotypic variance; δ_p^2 = phenotypic variance. The heritability % was categorized as low, moderate and high in accordance with Robinson *et al.* (1949) as follows: 0-0.3, low; 0.3-0.6, moderate and >0.6, high. *Genetic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV)* were computed according to Singh and Chaudhary (1979) and expressed as percentage as follows: $GCV = \frac{\sqrt{\delta_g^2}}{\bar{x}} \times 100$ and $PCV = \frac{\sqrt{\partial^2{}_p}}{\bar{x}} \times 100$; where: δ_g^2 = genotypic variance; δ_p^2 = phenotypic variance; $\bar{x}$ = grand mean of trait x. *Predicted genetic gain (PG) was calculated by* selecting 13 % of the superior genotypes and calculated using the following formula (Singh and Chaudhary, 1979): $PG = iH^2\sqrt{\partial^2{}_p} \times 100$; where: i = selection differential (1.627) at 13 % selection intensity; H^2 = broad-sense heritability; $\partial^2{}_p$= phenotypic variance. Finally, *predicted genetic gain % (PG %) was* calculated as percent of the mean calculated according to Shukla *et al*. (2006): $PG\ \% = \frac{PG}{\bar{x}} \times 100$; where: $\bar{x}$ = grand mean of trait x.

2.3 Results and discussion

2.3.1 Analysis of variance

Genotypes showed highly significant ($p<0.001$) differences for all measured traits (Table 2.2). The significant differences indicated the existence of high degree of variability among the genotypes that could be exploited for sweet stem sorghum improvement (Tesfamichael *et al.*, 2015).

Table 2.2: Partial analysis of variance and significant tests for eight bioethanol related traits of 190 sweet stem sorghum genotypes

Source	DF	DTF	PH	SD	BM	DM	FC	SB	EP
Rep	1	262.1*	0.256	5.03*	50.05	0.0027	0.00301	0.035	142233
Genotype	189	497**	0.44**	31.1**	276.1**	0.003**	0.0034**	12.1**	1374556**

* significant at 5 %, ** significant at 1 %, Rep= replication, DF= degrees of freedom, DTF=days to flowering, PH= plant height, SD=stem diameter, BM=biomass, DM= dry matter, FC= fibre content, SB= stalk brix, EP= ethanol

2.3.2 Mean response of test genotypes for eight bioethanol related traits

Table 2.3 shows the mean response of test genotypes for eight bioethanol related traits. Days to flowering varied from 62 to 152 with a mean of 93 days. The genotypes with delayed maturity associated with increased biomass production were AS46, AS434, AS443, AS441 and AS205. Plant height varied from 90 cm to 420 cm with a mean of 236 cm. The tallest genotypes were AS442, AS443, AS447, AS448 and AS441. Stem diameter ranged from 7 cm to 31.33 cm with a mean of 16 cm. Genotypes with the thickest stalks included AS143, AS441, AS251, AS250 and AS442. Biomass yield varied from 6.668 to 111.2 t ha^{-1} with a mean of 30 t ha^{-1}. Genotypes AS203, AS205, AS448, AS443, and AS251 had the highest biomass yield. Stalk dry matter content ranged from 17.2 to 44.2% with a mean of 29.8%, while fibre content varied from 8.92 to 34.8% with a mean of 17.2%. The stalk brix of genotypes varied from 3.3 to 18.9% with a mean of 12.1%. Ethanol productivity ranged from 240.9 to 5500 l ha^{-1} with a mean of 1886 l ha^{-1}.

The best ethanol yielding genotypes were AS203, AS391, AS205, AS251 and AS448 providing mean yields of 5474 l ha^{-1}, 4509 l ha^{-1}, 4315 l ha^{-1}, 4205 l ha^{-1} and 3816 l ha^{-1}, in that order. The top ethanol producing genotype AS 203 also had the highest biomass yield of 84 t ha^{-1}. However, this genotype was slightly shorter, thinner and had lower stalk brix than genotype AS205. A similar trend was observed for the bottom yielding genotype. Genotype AS12 was the

lowest yielding only in terms of ethanol production. But genotype AS 12 was thicker, taller and later maturing than genotypes AS71 and AS194. The trend in ethanol production was not affected by other quantitative traits.

Ethanol productivity of some of the tested genotypes were similar to the yields to the report of Regassa and Wortmann (2014). They reported that the highest ethanol productivity that can be anticipated from sweet stem sorghum as 6 000 l ha^{-1}. However, stalk brix, plant height and stem diameter of the experimental genotypes did not reach the maximum of 24%, 480 cm and 45 mm, respectively, reported by the same authors. Wide variability in plant height (90 cm to 270 cm) and days to flowering (50 to 80 days) were reported by Tesfamichael *et al.*, 2015. Elangovan *et al.* (2014) reported large variation among genotypes in days to flowering (68–100 days), plant height (232–497 cm) and stalk brix (4 to 22 %). Lekgari and Dweikat (2014) also reported a wide range in days to anthesis of 70 to 147 days, and plant height of 76 cm to 423.8 cm.

Ample genetic variability was detected among the tested sweet stem sorghum genotypes allowing for selection for all the measured traits. Most traits had a low standard deviation (SD) from the mean, with plant height having an SD of 4 cm from a mean of 240 cm and stem diameter having an SD of 4.9 cm from a mean of 15.8 cm. On the other hand, biomass yield had a relatively high SD of 15 t ha^{-1} from a mean of 30 t ha^{-1}. A relatively low coefficient of variation (< 17%) was detected for all traits except for biomass and ethanol production. The high coefficient of variation for both biomass and ethanol production denoted susceptibility to environmental factors influencing their expression (Sinha and Kumaravadivel, 2016). The selected top 13% of genotypes produced ethanol yields ranging from 3000 l ha^{-1} to 5 500 l ha^{-1} (Table 2.3). On the other hand, the bottom 13 % produced only 240 l ha^{-1} to 880 l ha^{-1}.

2.3.3 Clustering of sweet stem genotypes based on eight bioethanol related traits

Table 2.4 presents clustering of test genotypes. Most high ethanol producing genotypes were allocated in Cluster III. Sub-clusters a and b in Cluster III contained genotypes that did not produce much ethanol. All Sub-cluster III-b genotypes were among the top five high ethanol producing genotypes. All the sub-clusters had at least 1 high ethanol producing genotype (in the top 13 %) except Sub-clusters I-a, I-b and II-a. This is an indication of a high level of morphological diversity among the tested population. Sub-cluster II-a contained three of the five late maturing genotypes (AS434, AS441, AS443) and four of the five tallest genotypes (AS441, AS442 AS 443, AS 447).

Table 2.3: Means of eight bioethanol and related traits of 190 sweet stem sorghum genotypes

Name	DTF	PH	SD	BM	DM	FC	SB	EP	Name	DTF	PH	SD	BM	DM	FC	SB	EP
AS203	106	345	22.8	83.8	27.5	13.5	13.5	5474	AS69	79.0	143	13.5	22.7	28.1	13.0	14.6	1639
AS391	116	305	13.7	62.8	28.1	13.1	14.5	4509	AS53	147.0	310	27.3	40.3	29.8	20.7	8.6	1621
AS205	148	350	23.5	70.5	26.2	18.6	14.1	4315	AS444	140.0	375	18.8	38.9	27.4	18.5	8.4	1618
AS251	136	385	29.2	64.2	31.1	17.5	13.1	4205	AS198	128.0	240	14.3	30.2	23.4	11.8	11.1	1615
AS448	132	405	27.5	66.7	31.8	20.0	11.3	3816	SS120	87.5	260	16.7	31.7	26.7	16.0	10.2	1598
AS44	130	300	19.2	56.1	31.2	17.0	13.8	3800	AS94	84.0	190	14.0	24.9	23.9	10.6	12.8	1571
AS56	133	280	17.3	51.1	30.0	14.6	14.9	3761	AS14	74.0	190	13.5	29.1	22.7	11.4	10.8	1554
AS246	87	305	17.2	48.7	33.0	17.2	15.3	3743	AS16	77.0	285	13.3	28.3	30.9	19.4	11.0	1542
AS105	89	235	15.8	47.5	29.3	13.4	15.4	3619	AS162	77.0	160	17.8	24.6	29.4	16.3	12.6	1532
AS59	106	285	20.8	54.7	32.3	18.3	13.5	3574	AS442	136.5	410	27.8	53.6	29.5	23.3	5.7	1524
AS111	101	240	13.8	47.8	29.8	14.2	15.1	3570	AS202	89.5	240	17.2	31.4	22.2	12.1	9.6	1519
AS113	85	210	18.5	43.4	30.6	13.7	16.4	3548	AS252	114.0	345	24.8	37.3	33.0	24.2	8.3	1517
AS253	107	315	22.8	53.3	25.8	12.2	13.2	3476	AS85	82.5	180	15.0	19.9	29.4	13.6	15.3	1494
AS254	99	330	19.8	57.7	27.6	15.2	12.0	3418	AS440	147.0	365	22.3	41.0	30.5	22.6	7.4	1481
AS79	113	210	21.2	43.0	30.2	14.2	15.6	3313	AS248	86.0	310	21.5	30.9	25.0	15.0	9.6	1462
AS195	107	270	18.3	51.3	25.4	11.9	13.0	3310	AS265	107.5	330	15.3	32.6	35.0	25.8	8.7	1451
AS58	105	290	19.8	47.2	30.5	16.0	14.1	3286	AS3	76.5	220	14.7	25.6	32.3	20.4	11.4	1449
AS255	127	360	20.0	48.5	28.7	14.5	13.7	3278	AS247	90.0	320	22.5	28.2	41.6	30.7	10.4	1446
AS82	101	230	10.3	39.7	33.0	15.8	16.7	3260	AS436	81.0	210	11.8	23.3	27.7	14.9	12.3	1410
AS127	82	245	9.0	42.0	29.1	13.2	15.5	3181	AS146	91.5	190	18.2	23.3	31.5	19.1	11.9	1395
AS245	131	305	24.2	48.7	25.5	12.2	12.8	3152	AS88	85.0	150	12.8	18.7	29.5	14.1	14.9	1363
AS74	75	220	14.8	36.9	33.0	15.5	17.1	3117	AS421	121.5	295	20.0	31.3	35.8	26.7	8.7	1341
AS98	127	250	19.0	44.5	29.1	14.5	14.1	3105	AS441	148.0	400	30.5	46.1	25.9	19.6	5.8	1326
AS443	149	405	23.8	65.0	30.7	21.0	9.2	3021	AS77	73.0	185	11.3	20.7	28.4	14.7	13.2	1324
AS78	128	275	19.7	42.7	41.8	27.1	14.2	3021	AS52	106.0	370	20.7	36.8	33.3	25.5	7.3	1297

Table 2.3 continued

Name	DTF	PH	SD	BM	DM	FC	SB	EP	Name	DTF	PH	SD	BM	DM	FC	SB	EP
SS27	97	275	13.3	42.0	30.8	15.8	14.6	3008	AS18	86.0	170	14.3	19.0	27.6	13.6	13.5	1272
AS83	95	245	13.5	40.3	29.2	14.3	14.4	2869	AS51	86.5	305	20.7	27.2	34.0	24.3	9.2	1264
AS45	119	345	17.8	47.1	24.3	11.5	12.3	2864	AS19	81.5	193	12.7	20.0	27.6	14.5	12.6	1242
AS447	141	405	23.3	51.3	30.2	18.5	11.2	2813	AS1	83.5	170	13.3	19.2	28.3	14.8	13.0	1236
AS86	107	230	20.7	37.0	32.9	17.3	15.1	2770	AS271	104.0	280	17.8	30.8	20.1	11.5	8.1	1230
AS10	92	310	20.0	49.6	38.5	26.5	11.5	2723	AS243	120.0	330	20.7	36.3	35.3	28.0	6.8	1227
AS122	86	200	13.8	35.6	30.9	15.1	15.3	2674	AS55	88.0	170	12.3	20.5	29.9	17.4	12.0	1214
AS103	88	250	13.0	43.2	27.3	14.4	12.4	2667	SS44	78.0	190	13.3	17.8	29.5	15.1	13.9	1203
AS158	100	185	12.0	36.3	31.2	15.7	15.0	2655	AS89	85.0	200	8.8	27.8	20.0	10.6	8.9	1201
AS244	87	290	19.5	39.6	27.5	14.0	13.0	2548	AS15	125.5	275	22.2	29.9	34.1	25.7	7.9	1195
AS95	106	255	16.2	35.7	30.1	15.1	14.5	2547	AS197	112.5	230	13.5	23.2	23.9	13.6	9.8	1173
AS204	87	255	12.2	37.3	26.4	12.4	13.5	2517	AS242	82.0	265	26.0	23.1	25.2	14.5	10.2	1169
AS97	86	175	15.0	33.7	28.7	13.4	14.8	2471	AS109	85.0	140	12.8	18.2	29.1	15.7	12.9	1159
AS2	80	185	11.3	41.7	22.6	10.3	11.9	2448	AS100	86.0	255	17.5	25.9	35.4	26.0	8.9	1134
AS72	87	185	14.3	27.0	33.3	15.0	17.8	2391	PEX40287	76.0	165	18.0	18.8	23.0	12.0	10.5	1131
AS445	134	390	21.7	46.4	34.4	23.5	10.4	2387	AS432	114.5	295	18.8	26.7	34.3	25.2	8.6	1127
AS108	84	245	20.0	31.5	34.6	18.8	15.3	2386	AS80	80.0	140	8.8	17.3	26.6	13.3	12.8	1096
AS70	78	225	15.2	33.9	34.6	20.2	13.9	2348	AS154	83.5	165	15.3	19.4	26.6	14.8	11.3	1085
AS106	87	300	23.3	39.2	30.2	17.7	12.0	2326	AS150	78.5	180	8.8	24.2	23.9	14.5	9.0	1084
AS395	114	305	18.3	45.3	25.2	14.8	9.9	2321	AS65	79.0	135	10.2	16.2	27.5	13.6	13.5	1071
AS199	84	220	16.0	33.3	29.1	14.6	14.0	2301	AS117	84.5	140	12.5	18.4	26.0	13.8	11.8	1065
AS200	80	235	10.8	34.9	27.6	13.8	13.4	2285	AS165	74.0	160	12.8	18.9	32.3	20.6	11.2	1046
AS240	106	280	15.8	32.0	31.4	16.4	14.5	2285	AS262	83.0	190	14.2	20.0	27.1	16.1	10.5	1038
AS101	89	200	13.8	32.3	29.3	14.7	14.1	2256	AS140	73.0	205	10.0	16.4	39.5	26.3	12.7	1014

Table 2.3 continued

Name	DTF	PH	SD	BM	DM	FC	SB	EP	Name	DTF	PH	SD	BM	DM	FC	SB	EP
AS128	77	180	18.3	28.4	33.2	16.7	16.0	2236	AS145	103.0	290	13.8	30.0	36.0	29.1	6.4	1014
Urja	97	295	15.3	27.4	31.5	14.8	16.2	2193	AS28	62.0	200	11.0	15.9	32.9	19.7	12.7	995
AS259	87	290	18.8	35.9	28.2	15.5	12.3	2185	AS167	85.0	285	13.7	29.3	34.8	27.9	6.4	957
AS147	86	190	19.3	26.3	30.6	13.4	16.7	2178	AS99	75.0	170	9.8	14.2	29.3	16.3	12.6	919
AS258	75	170	11.3	32.6	28.6	14.6	13.5	2176	AS5	81.0	135	11.3	16.6	23.8	12.1	11.2	916
AS84	85	205	8.7	28.8	30.8	15.0	15.3	2176	AS173	85.5	315	15.2	23.8	40.2	32.2	7.5	909
AS41	129	340	16.2	38.6	29.7	17.7	11.6	2154	AS68	79.0	140	9.0	12.6	27.5	12.4	14.6	906
AS263	92	290	13.7	34.0	28.6	16.3	11.9	2134	AS393	106.0	280	16.0	24.7	23.2	15.7	7.0	887
AS148	79	185	17.0	27.8	29.6	13.6	15.5	2129	AS73	82.5	123	7.3	12.1	25.8	11.0	14.3	851
AS87	88	160	12.5	27.6	30.2	14.2	15.5	2121	AS194	74.0	160	11.0	13.7	38.1	25.1	12.5	849
AS7	84	180	18.3	27.0	34.1	17.8	15.8	2113	AS64	74.5	185	12.3	18.5	31.4	21.6	9.3	848
SS17	74	275	14.0	28.2	30.8	15.0	15.3	2098	AS4	80.0	125	13.5	19.2	20.4	10.6	9.3	842
AS196	100	270	13.7	28.0	30.2	14.4	15.3	2096	AS71	73.5	120	8.2	18.7	27.2	17.6	9.2	837
AS6	93	195	21.0	28.8	33.4	18.0	14.9	2083	AS81	90.5	120	15.2	18.4	22.2	12.9	8.8	803
AS107	81	215	12.5	28.7	32.3	17.4	14.4	2045	SS56	68.0	170	9.2	15.4	28.7	18.1	10.1	770
AS260	90	295	22.2	36.2	33.8	21.9	11.4	2037	AS116	85.5	110	11.0	19.7	22.2	13.8	7.9	769
AS112	88	160	17.3	24.6	35.5	18.5	16.5	2011	AS91	81.0	130	10.0	15.9	25.7	16.1	9.2	736
AS155	83	200	12.2	23.8	37.2	19.9	16.9	1993	SS49	76.5	170	13.5	8.9	33.0	24.4	8.1	716
AS135	82	190	13.3	24.6	29.9	13.1	16.3	1987	AS62	83.5	225	12.5	29.5	18.7	13.4	4.9	707
AS131	79	205	13.0	25.2	31.0	14.7	15.8	1942	AS42	120.0	270	18.3	20.5	39.9	32.2	7.2	701
AS249	88	295	25.0	36.1	23.3	12.3	10.5	1925	AS43	119.0	275	17.0	19.7	34.1	26.5	7.2	672
AS136	82	190	18.2	25.7	31.7	16.1	15.1	1924	AS13	76.5	125	9.2	11.0	24.8	12.1	12.2	663
AS121	88	215	11.3	28.4	32.5	18.5	13.5	1922	AS49	77.5	190	8.7	12.9	32.8	22.7	9.6	615
AS48	131	295	18.5	34.0	35.4	23.5	11.4	1919	AS114	78.0	120	8.3	14.5	22.7	14.2	8.0	605

Table 2.3 continued

Name	DTF	PH	SD	BM	DM	FC	SB	EP	Name	DTF	PH	SD	BM	DM	FC	SB	EP
AS129	79	190	13.8	24.1	31.7	15.3	15.9	1899	AS9	84.0	160	9.3	17.8	23.8	16.5	6.8	599
AS110	87	150	8.3	36.4	25.7	14.7	10.5	1889	AS57	86.5	200	14.0	12.7	27.5	18.0	9.0	567
AS241	122	300	21.7	33.5	33.4	21.5	11.4	1887	AS264	83.5	335	12.8	18.1	30.5	23.7	6.3	556
AS75	74	215	9.8	27.7	33.3	18.9	13.9	1883	AS115	92.5	110	8.7	17.1	26.0	19.8	5.7	482
AS141	70	175	14.0	25.3	31.2	15.6	15.2	1862	AS96	86.0	125	8.5	11.7	21.0	12.3	8.3	481
AS17	75	190	14.3	29.8	25.8	12.9	12.4	1853	AS124	84.0	95	12.2	10.6	18.7	12.5	5.7	363
AS143	84	195	31.0	27.9	26.1	12.4	13.3	1829	AS54	64.5	195	11.0	10.1	30.5	25.0	5.0	309
AS250	88	305	29.0	35.3	25.8	14.8	10.6	1816	AS12	74.5	165	12.0	8.4	38.5	31.9	6.1	241
AS137	83	190	13.5	24.1	30.8	15.1	15.2	1815	AS102	83.5	*	12.2	*	*	*	*	*
AS92	81	155	14.2	25.0	29.2	14.1	14.7	1792	AS152	78.5	90	10.8	7.8	*	*	*	*
AS134	86	210	18.3	28.3	31.2	18.3	12.4	1781	AS161	83.0	*	17.7	*	*	*	*	*
AS50	95	335	21.3	39.8	31.7	22.2	9.1	1777	AS192	79.0	200	10.5	15.5	*	*	*	*
AS434	150	360	18.0	42.0	26.8	17.7	8.6	1773	AS219	96.5	290	17.5	*	*	*	*	*
AS67	78	160	15.8	22.6	33.4	16.8	16.1	1769	AS256	72.5	230	9.7	8.4	*	*	*	*
AS63	78	220	15.3	23.8	31.4	16.0	14.9	1758	AS257	73.0	200	9.5	6.7	*	*	*	*
AS90	81	155	14.3	23.0	29.0	13.3	15.2	1744	AS261	68.5	180	14.3	16.6	*	*	*	*
AS76	72	205	24.3	23.5	36.5	21.0	15.0	1741	AS93	103.0	*	12.0	*	*	*	*	*
AS66	77	172	13.7	23.4	29.2	13.6	15.1	1734	SS52	73.5	130	9.7	8.8	*	*	*	*
AS308	94	295	15.3	31.5	26.4	14.8	11.1	1733	WD103	62.5	220	13.7	7.9	*	*	*	*
AS130	82	225	14.3	24.4	30.9	16.5	13.9	1721	Mean	92.9	236	15.8	30.1	29.8	17.2	12.1	1886
AS153	81	210	11.2	25.8	30.8	16.9	13.5	1720	Std D	20.9	4	5.0	14.4	4.8	5.2	3.2	1006
AS46	151	310	20.0	37.0	35.3	25.4	9.5	1717	Min	62.0	90	7.0	6.7	17.2	8.9	3.3	240.9
AS133	82	205	17.7	24.9	28.6	14.2	13.9	1693	Max	152.0	420	31.3	111.2	44.2	34.8	18.9	5474
AS138	73	190	12.8	22.6	31.1	15.2	15.5	1688	% CV	22.5	1.7	31.6	47.8	16.1	30.2	26.4	53.3
AS132	79	190	19.0	20.9	31.0	14.4	16.1	1653	LSD	19.7	64	1.9	19.5	8.6	8.3	4.7	

DTF=days to flowering, PH= plant height, SD=stem diameter, BM=biomass, DM= dry matter, FC= fibre content, SB= stalk brix, EP= ethanol, Std D= standard deviation, Min= minimum, Max= maximum, CV= coefficient of variation, LSD= least significant difference, *= missing result, Bold text denote high ethanol producing genotypes selected at 13 % selection intensity.

Table 2.4: Clusters and sub-clusters of 190 sweet stem genotypes based on eight bioethanol related traits

Cluster	Sub-cluster	Name of genotype
I	a	AS1, AS109, AS110, AS114, AS115, AS116, AS117, AS124, AS13, AS14, AS146, AS147, AS148, AS150 AS152 AS154, AS17, AS18, AS19, AS2, AS262, AS4, AS436, AS5, AS55, AS57, AS62, AS65, AS68, AS73AS77, AS80, AS81, AS89, AS9, AS91,AS94, AS96, AS99, PEX40287, SS44, SS52, SS56, AS71
	b	AS12, AS140, AS165, AS192, AS194, AS256, AS257
	c	AS101, AS102, AS103, AS105, AS107, AS108, AS111, AS112, AS113, AS121, AS122, AS127, AS128, AS129, AS130, AS131, AS132, AS133, AS134, AS135, AS136, AS137, AS138, AS141, AS153, AS155, AS158, AS16, AS196, AS199, AS200, AS204, AS240, AS258, AS3, AS6, AS63, AS66, AS67, AS69, AS7, AS70, AS74, AS75, AS76, AS82, AS83, AS84, AS85, AS87, AS88, AS90, AS92, AS93, AS95, AS97, AS162, AS161, AS72, SS17, SS27, Urja
	d	AS106, AS197, AS198, AS202, AS219, AS242, AS244, AS248, AS249, AS250, AS259, AS263, AS271, AS308, AS393, SS120
II	a	AS41, AS434, AS440, AS441, AS442, AS443, AS444, AS445, AS447, AS53
	b	AS78
	c	AS10, AS100, AS145, AS15, AS167, AS254, AS260, AS264, AS265, AS42, AS173, AS241, AS243, AS247, AS252, AS421, AS43, AS432, AS46, AS48, AS50, AS51, AS52
III	a	AS246, AS391, AS58, AS59, AS79, AS86
	b	AS203, AS205, AS251, AS448
	c	AS195, AS245, AS253, AS255, AS45, AS56, AS98, AS395, AS44
IV	a	AS143

2.3.4 Phenotypic correlation among bioethanol related traits

The correlation coefficients of the eight quantitative traits assessed are presented in Table 2.5. Correlations among quantitative traits were analyzed in terms of significance, strength and nature of correlation. Several traits showed highly significant ($p \leq 0.001$) correlations such as days to flowering with all traits; biomass with ethanol yield and plant height; ethanol with all traits except dry matter; plant height with all traits. Kumar *et al.* (2012) reported that plant height exhibited significant positive association with, days to flowering, and brix. A strong correlation was recorded between biomass with ethanol production ($r = 0.83$). Biomass was strongly correlated to days to flowering, ethanol production, plant height and stem diameter. These results were in agreement with Ganesh *et al.* (1995) and Prasad *et al.* (2013). Their studies indicated that high biomass with total sugar content is a pre-requisite for high ethanol production. Hence, these traits could be utilized in the sweet sorghum breeding programs for ethanol production. Selection for these traits would help in achieving highest ethanol productivity. Other strong correlations were detected between days to flowering with plant height and stem diameter, plant height with stem diameter, fibre content with dry matter. From these results, it is evident that these traits are associated with ethanol production and are inter-correlated among them. Thus, the selection in any one of these ethanol yield attributing traits will lead to increase in the other traits, thereby enhancing ethanol production. Stalk brix had a relatively weak positive association with biomass, agreeing with the reports of Bangarwa *et al.* (1989) and Kumar *et al.* (2012).

Table 2.5: Pearson's correlation coefficients, among quantitative traits of sweet stem sorghum genotypes (n =190)

	DTF	PH	SD	BM	DM	FC	SB	EP
DTF								
PH	0.7285**							
SD	0.5948**	0.6877**						
BM	0.6653**	0.7331**	0.6014**					
DM	0.0765	0.2381**	0.3726*	0.041				
FC	0.2386**	0.1714**	0.2198**	-0.0281	0.7811**			
SB	-0.2339**	-0.2272**	-0.0824*	0.1411	0.1886**	-0.4564**		
EP	0.3727**	0.4427**	0.3777**	0.8335**	0.109	-0.2403**	0.5701**	-

* significant at 5 %, ** significant at 1 %, DTF=days to flowering, PH= plant height, SD=stem diameter, BM= biomass, DM= dry matter, FC= fibre content, SB= stalk brix, EP= ethanol

2.3.5 Path coefficient analysis

Table 2.6 is a presentation of the direct and indirect effects of the component traits on ethanol productivity. Few traits exhibited significant ($p \leq 0.001$) direct effects on ethanol production including plant height, stem diameter and biomass. Biomass had the greatest direct effect on ethanol production. Hence, it may be concluded that these traits could enhance ethanol production more than other traits. Days to flowering, plant height, stalk brix and stem diameter showed the greatest indirect effects on ethanol production through biomass. Similar results have been reported by Kumar *et al.* (2012). Since biomass had the highest direct effect on ethanol production, these traits should be considered during future selection programs. This is in agreement with Naphade (1972), Potdukhe *et al.* (1994) and Kumar *et al.* (2012), who reported that days to flowering had an indirect positive association with biomass through plant height, while plant height had an indirect positive effect on biomass through days to flowering, which indicated that there is a real association between days to flowering and plant height. Hence, these traits should be considered during progeny selection. The greatest indirect effect on ethanol production via biomass was exerted by plant height. The greatest indirect effects on ethanol production via dry matter was fibre content and vice versa.

Table 2.6: Path coefficients showing direct (bold) and indirect effects of quantitative traits on ethanol production in 190 sweet stem sorghum genotypes

Traits	DTF	PH	SD	BM	DM	FC	SB
DTF	**-0.052**	0.005	-0.041	0.559	-0.005	0.020	-0.114
PH	-0.038	**0.007***	-0.047	0.616	-0.016	0.031	-0.110
SD	-0.031	0.005	**-0.068***	0.506	-0.012	0.018	-0.040
BM	-0.035	0.005	-0.041	**0.841****	-0.003	-0.002	0.069
DM	-0.004	0.002	-0.012	0.034	**-0.068**	0.065	0.092
FC	-0.012	0.003	-0.015	-0.024	-0.053	**0.083**	-0.222
SB	0.012	-0.002	0.006	0.119	-0.013	-0.038	**0.486**

* significant at 5 %, ** significant at 1 %, DTF=days to flowering, PH= plant height, SD=stem diameter, BM= biomass, DM= dry matter, FC= fibre content, SB= stalk brix

2.3.6 Principal component analysis (PCA)

Table 2.7 is a presentation of the rotated component matrix that displays the allocation of the entire variance explained by various principal components and their associations with quantitative traits. Principal component analysis suggested that only 3 first components (eigen value >1) are important, and account for 88 % of the total variation. This result differed from the studies of Mujaju and Chakuya (2008), Ali *et al.* (2011) and Tesfamichael *et al.* (2015) who reported on different agro-morphological traits in sweet stem sorghum. Ali *et al.* (2011) reported that out of a total of 21 PCs, seven had Eigen values >1 and these seven PCs contributed 77.653% of the total variability amongst the sorghum genotypes assessed for various morpho-physiological traits. Tesfamichael *et al.* (2015) reported that out of seven PCs the first 4 explained the majority of the total variation. These four PCs with Eigen value >1 contributed 74.6% of the total variability amongst the sorghum genotypes assessed for various morpho-physiological traits. In the present study principal component-1 (PC-1) and PC-2 were highly significant, contributing 71% to the total variation. The sign of the loading signifies whether the relationship is positive or negative. The first principal component, which accounted for about 45% of the variation, was strongly associated with plant height, stem diameter, biomass and days to flowering. Therefore selection of tall, thick, high biomass and late maturing genotypes is essential for the breeding program. Fibre content had a high positive loading into the second principal component while ethanol yield and stalk brix had negative loading into the second principal component. Traits with a high correlation to the third principal component in a negative direction were dry matter and stalk brix. Principal component-3 explained only 18 % of the total variation.

Figure 2.1 is a presentation of the principal component bi-plot analysis of eight phenotypic traits of 190 sweet stem sorghum genotypes. The bi-plot is illustrative of the associations of the phenotypic traits and the genotypes, with the principal components. According to Mwadzingeni *et al.* (2016) a high correlation of traits in discriminating genotypes can be observed from traits that have small angles between dimension vectors in the same direction. In the current study, such relationships can be observed between stem diameter, plant height and days to flowering, between ethanol, biomass and stalk brix and between fibre content and dry matter. Genotypes excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector, often on the vertices of the convex hull (Mwadzingeni *et al.,* 2016). According to the bi-plot the following genotypes: AS391, AS203 and AS205, were the best performers for ethanol yield and biomass because they were closest to these traits and located on the convex hull. Most of the genotypes were scattered in the negative sides of PC-1 and PC- 2.

Table 2.7: Rotated component matrix of 8 phenotypic traits of 190 sweet stem sorghum genotypes

Trait	PC-1	PC-2	PC-3
Days to flowering	0.43148	0.08430	0.25804
Plant height	0.47908	0.13052	0.11948
Stem diameter	0.41913	0.05609	0.09837
Biomass	0.47148	-0.24558	0.05053
Dry matter	0.16168	0.32890	-0.69421
Fibre content	0.16176	0.60491	-0.27921
Stalk Brix	-0.00608	-0.49564	-0.54753
Ethanol productivity	0.36606	-0.43893	-0.21733
Explained variance (eigenvalue)	3.548	2.121	1.397
Proportion of total variance (%)	44.35	26.51	17.47
Cumulative variance (%)	44.35	70.86	88.33

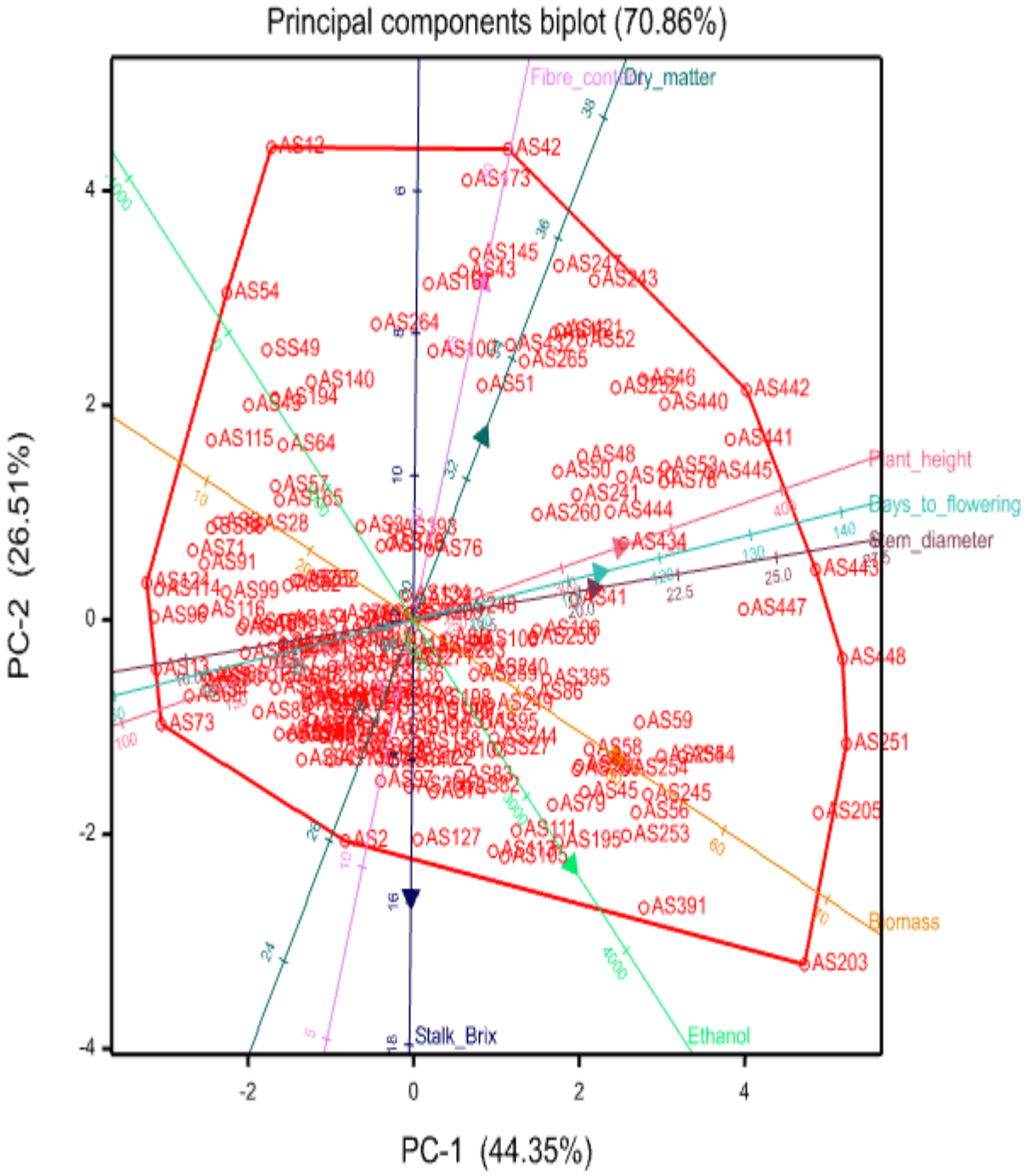


Figure 2.1: Bi-plot analysis of eight phenotypic traits of 190 sweet stem sorghum genotypes evaluated (PC= principal component)

2.3.7 Genetic parameters

In this study all the traits had high heritability (>0.5) (Table 2.8). According to Insan *et al.* (2016), the characters that have broad genetic variability and high heritability estimates could be influenced by additive gene action. High heritability may not result in high levels of genetic gain (Johnson *et al.* 1955). Therefore it is necessary to oversee heritability estimates alongside genetic advance. In this case, high heritability estimates did not guarantee high predicted gain as the low predicted gain of < 30 % for most traits did not match the high heritability of > 0.8 for most traits. Similar results of low predicted gain were observed by Insan *et al.* (2016) and such results may be attributed to the non-additive gene effects. On the other hand, high heritability coupled with high levels of genetic gain for traits such as ethanol productivity signified the existence of additive gene action, hence a strong response to selection for ethanol productivity (Panse and Sukhatme 1964, 1989). Medium heritability and low genetic gain indicate the presence of epistatic gene action (Elangovan *et al.,* 2014).

Burton (1952) advocated for the use of genotypic coefficient of variation (GCV) in addition to the heritability estimate in advancing the efficacy of the selection. The phenotypic coefficient of variation (PCV) was higher than the GCV for all traits, indicating the role of environmental factors influencing the expression of the traits to some extent (Godbharle *et al.,* 2010; Elangovan *et al.,* 2014). Differences in coefficients of variations (both genotypic and phenotypic) were exhibited with GCV ranging from 12 to 43.2% and PCV varying from 14 to 48.2%. Considering ethanol productivity, the higher GCV and PCV values suggested strong opportunity to select for superior genotypes for ethanol productivity. The lower or equal GCV and PCV observed for dry matter, plant height and days to flowering signified that enhancement of these particular traits would be limited(Elangovan *et al.,* 2014). Similar results of equal GCV and PCV for plant height in sweet stem sorghum genotypes have also been reported by other researchers (Reddy *et al.,* 2009).

Table 2.8: Predicted genetic gain for eight bioethanol related traits of sweet stem sorghum genotypes selected at 13% selection intensity

Traits	δ^2_g	h^2	GCV	PCV	Predicted gain	Predicted gain %
Days to flowering	277.23	0.91	17.93	18.76	25.89	27.87
Plant height	0.25691	0.91	21.51	22.57	0.79	33.37
Stem diameter	20.9478	0.99	29.00	29.19	7.40	46.89
Biomass	128.26	0.82	37.69	41.53	16.72	55.64
Dry matter	0.001415	0.79	12.62	14.24	0.05	18.20
Fibre content	0.001594	0.80	23.21	25.95	0.06	33.77
Stalk Brix	6.522	0.85	21.09	22.85	3.84	31.67
Ethanol productivity	663924	0.80	43.20	48.21	1188.08	62.99

δ^2_g = genetic variation, h^2 =heritability, GCV =Genetic coefficient of variation, PCV =Phenotypic coefficient of variation

2.4 Conclusions

The present study selected the best ethanol yielding genotypes including AS203, AS391, AS205, AS251 and AS448 that provided mean yields of 5474 l ha^{-1}, 4509 l ha^{-1}, 4315 l ha^{-1}, 4205 l ha^{-1} and 3816 l ha^{-1} in that order. Days to flowering, plant height, stalk brix and stem diameter had the strongest indirect effect on ethanol production through biomass. Since biomass had the strongest direct effect on ethanol production, these traits should be considered during a selection program.

References

Akhtar, M.S., Oki, Y., Adachi, T. and Khan, M.H.R. 2007. Analyses of the genetic parameters (variability, heritability, genetic advance, relationship of yield and yield contributing characters) for some plant traits among brassica cultivars under phosphorus starved environmental cues. *Journal of the Faculty of Environmental Science and Technology* 12: 91-98.

Ali, M.A., Abbas, A., Awan, S.I., Jabran, K. and Gardezi, S.D.A. 2011. Correlated response of various morpho-physiological characters with grain yield in sorghum landraces at different growth phases. *The Journal of Animal and Plant Sciences* 21: 671-679.

Ali, M.A., Jabran, K., Awan, S.I., Abbas, A., Zulkiffal, M., Acet, T., Farooq, J. and Rehman, A. 2011. Morpho-physiological diversity and its implications for improving drought tolerance in grain sorghum at different growth stages. *Australian Journal of Crop Science 5*: 311-320.

Amin, M.R., Barma, N.C.D. and Razzague, M.A. 1992. Variability, heritability, genetic advance and correlation study in some quantitative characters in durum wheat. *Rachis Newsletter* 11: 30-32.

Anami, S.E., Zhang, L.M., Xia, Y., Zhang, Y.M., Liu, Z.Q. and Jing, H.C. 2015a. Sweet sorghum ideotypes: genetic improvement of the biofuel syndrome. *Food and Energy Security 4:* 159-177.

Anami, S.E., Zhang, L.M., Xia, Y., Zhang, Y.M., Liu, Z.Q. and Jing, H.C. 2015b. Sweet sorghum ideotypes: genetic improvement of stress tolerance. *Food and Energy Security 4*: 3-24.

Arega, G., Hussein, M. and Harjit, S. 2010. Genotypic variability, heritability, genetic advance and associations among characters in Ethiopian durum wheat (*Triticum durum* Desf.) Accessions. *East African Journal of Sciences* 4: 27-33.

Arunkumar B., Biradar B.D. and Salimath, P.M. 2004. Genetic variability and character association studies in rabi sorghum. *Karnataka Journal of Agricultural Science* 17: 471-75.

Bangarwa, K.S., Grewal, R.P., Lodhi G.P. and Thakral, N.K. 1989. Association analysis for some quantitative traits in forage sorghum. *Indian Journal of Heredity* 21: 25-28.

Burton, G.W. 1952. Quantitative inheritance in grasses. Proceeding of the 6th Grassland Conference 7: 227–283.

Calvino, M., and Messing, J. 2012. Sweet sorghum as a model system for bioenergy crops. *Current Opinion Biotechnology 23*: 323-329.

Cochran, W.G. and Cox, M. 1957. Experimental Designs. John Wiley and Sons, Inc, New York: pp611.

Cramer, C.S. and Wehner, T.C. 2000. Path analysis of the correlation between fruit number and plant traits of cucumber populations. *Horticultural Science* 35: 708-711.

Dewey, D.R. and Lu, K.H. 1959. A correlation and path coefficient analysis of components of crested wheatgrass seed production. *Agronomy Journal* 51: 515-518.

Eggleston, G., Cole, M. and Andrzejewski, B. 2013. New commercially viable processing technologies for the production of sugar feedstocks from sweet sorghum (*Sorghum bicolor* L. Moench) for manufacture of biofuels and bioproducts. *Sugar Technology* 15: 232-249.

El Naim, A.M., Ibrahim, I.M., Abdel Rahman, M.E. and Ibrahim, E.A. 2012. Evaluation of some local sorghum (*Sorghum bicolor* L. Moench) genotypes in rain-fed. *International Journal of Plant Research* 2: 15-20.

Elangovan, M., Kiran Babu, P., Seetharama, N. and Patil, J.V. 2014. Genetic diversity and heritability characters associated in sweet sorghum (*Sorghum bicolor* (L.) Moench). *Sugar Technology* 16: 200–210.

Ezeaku, I.E. and Mohammed, S.G. 2006. Character association and path analysis in grain sorghum. *African Journal of Biotechnology* 5: 1337-1340.

Fisher, R.A. 1935. The Design of Experiments. Oliver and Boyd, Edinburgh and London.

Ganesh, S., Fazlullah, A.K., Suresh, M. and Senthil, N. 1995. Character associated for ethanol yield in sweet sorghum. *Madras Agricultural Cultural Journal* 82: 361-363.

Godbharle, A. R., More, A. W. and Ambekar, S.S. 2010. Genetic variability and correlation studies in elite 'B' and 'R' lines in kharif sorghum. *Electronic Journal of Plant Breeding* 1: 989-993.

Gonza´lez. M.D., Pollak, L.M. and Goggi, A.S. 2012. Genotype × environment interactions in populations possessing Ga1-s and ga1 alleles for cross incompatibility in maize. *Euphytica* 185: 377-384.

Govindaraj, M., Selvi, B., Rajarathinam, S. and Sumathi, P. 2011. Genetic variability and heritability of grain yield components and grain mineral concentration in India's pearl millet (*Pennisetum glaucum* (L) R. Br.) accessions. *African Journal of Food, Agriculture, Nutrition and Development* 11: 4758-4772.

Govindaraj, M., Shanmugasundaram, P. and Muthiah, A.R. 2010. Estimates of genetic parameters for yield and yield attributes in elite lines and popular cultivars of India's pearl millet. *African Journal of Agricultural Research* 5: 3060-3064.

Hallauer, A. and Miranda, J. 1981. Quantitative Genetics in Maize Breeding. Press, Iowa State University, Iowa.

Insan, R.R., Wirnas, D. and Trikoesoemaningtyas. 2016. Estimation of genetic parameters and selection of sorghum (*Sorghum bicolor* (L.) Moench) RILS F5 derived from single seed descent. *International Journal of Agronomy and Agricultural Research* 8: 95-103.

Johnson, H. W., Robinson, H.F. and Comstock, R.E. 1955. Estimates of genetic and environmental variability in soybean. *Agronomy Journal* 47: 314-318.

Kalpande, H.V., Chavan, S.K., More, A.W., Patil, V.S. and Unche, P.B. 2014. Character association, genetic variability and component analysis in sweet sorghum (*Sorghum bicolor* (L. Moench)). *Journal of Crop and Weed* 10:108-110.

Kassahun, A., Habtamu, Z. and Geremew, B. 2015. Variability for yield, yield related traits and association among traits of sorghum (*Sorghum bicolor* (L.) Moench) varieties in Wollo, Ethiopia. *Journal of Plant Breeding and Crop Science* 7: 125-133.

Kumar, C.V.S., Umakanth, A.V., Kotastane, T.V. and Sreelakshmi C.H. 2012.Character association and path analysis for qualitative traits in sweet sorghum (*Sorghum bicolor* L. Moench). *Journal of Research* 40: 90-93.

Laghari, K.A., Sial, M.A., Afzal Arain, M.A., Mirbahar, A.A., Pirzada, A.J., Dahot, M.U. and Mangrio, S.M. 2010. Heritability studies of yield and yield associated traits in bread wheat. *Pakistan Journal of Botany* 42: 111-115.

Lekgari, A. and Dweikat, I. 2014. Assessment of genetic variability of 142 sweet sorghum germplasm of diverse origin with molecular and morphological markers. *Open Journal of Ecology* 4: 371-393.

Lombardi, G.M.R., Nunes, J.A.R., Parrella, R.A.C., Teixeira, D.H.L., Bruzi, A.T., Durães, N.N.L. and Fagundes, T.G. 2015. Path analysis of agro-industrial traits in sweet sorghum. *Genetics and Molecular Research* 14: 16392-16402.

Mangena, P. 2014. Breeding gains, cytoplasmic effects and path coefficient analysis in pro-vitamin A maize hybrids. M.Sc Dissertation. University of KwaZulu-Natal, South Africa.

Masood, S.A., Awan, Z.K., Naseem, Z., Fazal, A., Aslam, N., Tahir, M.H.N. and Ali, Q. 2015. Genetic variability and trait association for relative growth rate and green fodder yield in *Sorghum bicolor* L. *International Journal of Biology, Pharmacy and Allied Sciences 4*: 5566-5576.

Mathur, S., Umakanth, A. V., Tonapi, V.A., Sharma, R. and Sharma, M.K. 2017. Sweet sorghum as biofuel feedstock: recent advances and available resources. *Biotechnology Biofuels* 10:146-165.

Morrissey, K. and Thoma, D. 2017. Life cycle assessment of sweet sorghum as feedstock for second-generation biofuel production. Chemical engineering undergraduate Honors Thesis. University of Arkansas, Fayetteville.

Mujaju, C. and Chakuya, E. (2008) Morphological variation of sorghum landrace accessions on-farm in semi-arid areas of Zimbabwe. *International Journal of Botany 4*: 376-382.

Mullet, J., Morishige, D., McCormick, R., Truong, S., Hilley, J., McKinley, B., Anderson, R., Olson, S.N. and Rooney, W.2014. Energy sorghum—a genetic model for the design of C_4 grass bioenergy crops. *Journal of Experimental Botany* doi:10.1093/jxb/eru229.

Mwadzingeni, L., Shimelis, H., Tesfay, S. and Tsilo, T.J. 2016. Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. *Frontiers in Plant Science 7*:1276.

Naphade, D.S. 1972. Correlation and path analysis for some characters contributing to fodder yield in sorghum. *Indian Journal of Agricultural Sciences* 42:790-791.

Panse, V.G. and Sukhatme, P.V. 1989. Statistical Methods for Agricultural Workers. (Revised by Sukhatme, P.V. and Amble, V.N.). Indian Council of Agricultural Research, New Delhi, India.

Panse, V.G., and Sukhatme, P.V. 1964. Statistical Methods for Agricultural Workers, 2nd ed. Indian Council of Agricultural Research, New Delhi, India.

Payne, R.W., Murray, D.A., Harding, S.A., Baird, D.B. and Soutar, D.M. 2011. An Introduction to GenStat for Windows (14th Edition). VSN International, Hemel Hempstead, UK.

Potdukhe, N.R., Shekar, V.B, Thote, S.G, Wanjari, S.S and Ingle R.W.1994. Estimates of genetic divergence, correlation coefficient and path analysis in grain yield in sorghum (*Sorghum bicolor* (L.) Moench). *Crop Research* 7:402-406.

Prakasham, R.S., Nagaiah, D., Vinutha, K.S., Uma, A., Chiranjeevi, T., Umakanth, A.V., *et al*. 2014. Sorghum biomass: a novel renewable carbon source for industrial bio-products. *Biofuels 5*:159-174.

Prasad, S., Kumar, A. and Muralikrishna, K.S. 2013. Assessment of ethanol yield associated characters in sweet sorghum. *Maydica* 58: 299-304.

Ratnavathi, C., Chakravarthy, S., Komala, V., Chavan, U. and Patil, J. 2011. Sweet sorghum as feedstock for biofuel production: A review. *Sugar Technology* 13: 399-407.

Reddy, D.C.S., Audilakshmi, S. and Seetharama, N. 2009. Genetic variability and divergence for DUS testing traits in sorghum (*Sorghum bicolor*). *Indian Journal of Agricultural Sciences* 79: 286-290.

Regassa, T.H. and Wortmann, C.S. 2014. Sweet sorghum as a bioenergy crop: Literature review. *Biomass and Bioenergy* 64: 348-355.

Robinson, H., Comstock, R.E. and Harvey, P. 1949. Estimates of heritability and the degree of dominance in corn. *Agronomy Journal* 41: 353-359.

Sami, R. A., Yeye M. Y., Usman I. S. Hassan L. B. and Usman M. 2013. Studies on genetic variability in some sweet sorghum (*Sorghum Bicolor* L Moench) genotypes. *Academic Research Journals* 1: 1-6.

Shukla, S., Bhargava, A. Chatterjee, A., Sirivastava, J., Singh, N. and Singh, N.P. 2006. Mineral profile and variability in vegetable amaranth (*Amaranthus tricolor*). *Foods Human Nutrition* 61: 23-28.

Singh, R.K. and Chaudhary, B.D. 1979. Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, India.

Sinha, S. and Kumaravadivel, N. 2016. Understanding genetic diversity of sorghum using quantitative traits. *Scientifica*. http://dx.doi.org/10.1155/2016/3075023

Tesfamichael A., Githiri, S. M., Kasili, R., Araia, W. and Nyende, A. B. 2015. Genetic variation among sorghum (*Sorghum bicolor* L. Moench) landraces from Eritrea under post-flowering drought stress conditions. *American Journal of Plant Sciences* 6: 1410-1424.

Tesfaye, W.M., Adugna, W., Tsige, G. 2014. Correlation and path coefficient analysis among yield component traits of Ethiopian mustard (*Brassica Carinata* a. Brun) at Adet, Northwestern, Ethiopia*. Journal of Plant Science* 2: 89-96.

Vanderlip, R.L. and Reeves, H.E. 1972. (*Sorghum bicolor* (L.) Moench.) Growth stages of sorghum. *Agronomy Journal* 64:13-16.

Vermerris, W. 2011. Survey of genomics approaches to improve bioenergy traits in maize, sorghum and sugarcane free access. *Journal of Integrated Plant Biology 53*:105-119.

Vermerris, W., and Saballos, A. 2013. Genetic enhancement of sorghum for biomass utilization. *Genomics of the Saccharinae*: 391-425.

Waqar-Ul-Haq, M., Malik, F., Rashid, M., Munir, M. and Akram, Z. 2008. Evaluation and estimation of heritability and genetic advancement for yield related attributes in wheat lines. *Pakistan Journal of Botany* 40: 1699-1702.

Wright, S.1921. Systems of mating. *Genetics* 6: 111-178.

Yaqoob, M., Hussain, N. and Rashid, A. 2015. Genetic Variability and heritability analysis for yield and morphological traits in sorghum (*Sorghum Bicolor* L. Moench) genotypes. *Journal of Agricultural Research* 53: 331-343.

Chapter 3

Genetic interrelationship of sweet stem sorghum genotypes assessed through simple sequence repeat markers

Abstract

Knowledge of the magnitude of genetic relatedness within and among breeding populations is valuable for a successful breeding program. The objective of this study was to evaluate the genetic interrelationships among 18 phenotypically selected sweet stem sorghum genotypes using 25 polymorphic simple sequence repeat (SSR) markers. The results revealed the presence of clear genetic differentiation among the studied sweet stem sorghum genotypes. The number of alleles detected per marker ranged from 1 to 10, with a mean of 4.64 per locus. The number of effective alleles varied from 1 to 6.75, with a mean of 2.9. The observed heterozygosity varied from 0.00 to 0.39 with a mean of 0.16. The inbreeding coefficient varied from 0.11 to 1.00, with a mean of 0.7, suggesting a considerable level of homozygosity existed among the tested genotypes. The polymorphic information content (PIC) values for all markers ranged from 0.00 to 0.85, with a mean value of 0.56, implying that the markers were highly informative and discriminatory. Sixty eight percent of the markers used had a PIC value > 0.50. Analysis of molecular variance revealed highly significant differences ($P<0.001$) among the test population. Among and within individual variances contributed to 78% and 21% of the total genetic variance. This suggested that selection of representative and well-differentiated genotypes should be effective in broadening the genetic base of sweet stem sorghum genotypes used for targeted breeding for biofuel production. The study identified genetically unique sweet stem sorghum genotypes such as AS391, SS27, AS204 and AS244 that can be recommended for direct production or further breeding for the bio-fuel industry.

Keywords: bio-fuel, SSR markers; *Sorghum bicolor*; yield components

3.1 Introduction

Knowledge on the genetic variation and interrelationship among breeding populations is vital for effective breeding or strategic conservation of valuable germplasm. Sweet stem sorghum, a variant of grain sorghum, is successfully used for the production of bio-ethanol (Anami *et al.,* 2015). Sweet stem sorghum thrives under relatively harsh growing conditions, providing high biomass yield per hectare compared to other food and feed crops (Lipinsky and Kresovich, 1980; Rosenow and Clark, 1995; Vermerris *et al.,* 2007; Saballos, 2008; Vermerris and Saballos, 2013). Various studies conducted on sweet stem sorghum as a bioenergy crop have reported yields of 45 to 75 t ha^{-1} fresh biomass, 14.1 to 17.6 t ha^{-1} of dry biomass, 0.05 to 3.9 t ha^{-1} of grain, and 0.9 to 8.14 t ha^{-1} of hexose sugars from the stem juice (Li, 1997; Murray *et al.,* 2008; Tew *et al.,* 2008; Atokple *et al.,* 2014).

Biofuel production from sweet stem sorghum does not compromise grain production for food, making it a second-generation bio-energy source (Daystar *et al.,* 2014; Morrissey and Thoma, 2017). According to the Food and Agricultural Organization, biofuels make up 98% of the renewable energy produced in Southern Africa (FAO, 2016). In comparison to other biofuel crops such as sugarcane and maize, sweet stem sorghum exhibits advantageous traits including a high sugar content in its stem that is directly fermentable. Further, the crop has lower water and fertilizer requirements, is more drought and salt tolerant, with wider adaptability to tropical, subtropical and temperate climates. Also, it has a short harvesting period that lies in the intermittent sugarcane-harvesting period, allowing for crop rotation to extend the combined window for harvesting (Ratnavathi *et al.,* 2011; Eggleston *et al.,* 2013; Morrissey and Thoma, 2017).

For effective exploitation of parent populations, germplasm conservation and successful establishment of breeding programs, it is essential to assess the genetic relatedness present in the base population. Information on the genetic relationships among genotypes is essential for developing appropriate breeding strategies. Subudhi *et al. (*2002) noted that the determination of the genetic identity of crop collections is essential for conserving, evaluating and utilizing genetic resources. This will enable the exploitation of the diversity of the available germplasm as potential sources of genes that can enhance the productivity of future varieties.

Genetic interrelationship can be established using morphological markers of crop germplasm. However, molecular markers are efficient in exploring the genetic constitution present among selections at a DNA level, which can assist conventional breeding in many aspects (Jain and Kharkwal, 2004; Iqbal *et al.,* 2010). Several studies have also reported that estimation of genetic

relatedness using pedigree, agronomic and morphological traits can be less precise due to environmental and genotype by environment interactions (Cox *et al.,* 1986; Van Beuningen and Busch, 1997; Bohn *et al.,* 1999; Almanza-Pinzon *et al.,* 2003; Fufa *et al.,* 2005). Furthermore, the application of molecular markers is effective in classifying lines into heterotic groups to create hybrids or breeding populations (Menz *et al.,* 2004; Becelaere *et al.,* 2005), and for developing mapping populations for detecting quantitative trait loci (QTLs) (Varshney, 2011). Further, molecular marker techniques are discrete, co-dominant or dominant, and free from epistatic gene action (Tanksley *et al.,* 1989; McIntyre *et al.,* 2001).

Various molecular assays have been applied in genetic analysis of sorghum, such as restriction fragment length polymorphism (RFLP) (Botstein *et al.,* 1980), random amplified polymorphic DNA polymorphism (RAPD) (Williams *et al.,* 1990; Prakash *et al.,* 2008; Iqbal *et al.,* 2010; ShivjeeSah and Khanna, 2010), simple sequence repeat polymorphism (SSR) (Tautz, 1989; Shehaz *et al.,* 2009; Rajput *et al.,* 2012; Reddy *et al.,* 2012), single nucleotide polymorphism (SNP) (McCormick et al., 2017) and amplified fragment length polymorphism (AFLP) (Zabeau and Vos, 1993). Of all molecular techniques, SSR markers have proved to be effective in genetic characterization, and assessing genetic relationships and population structures among genotypes (Amelework *et al.,* 2015). Most researchers have opted for SSR markers due to the following advantages: ease of detection through polymerase chain reaction (PCR); reasonably cheap; and high level of polymorphism (Brown *et al.,* 1996; Powell *et al.,* 1996). SSR can be detected easily without the use of radioisotopes techniques needed with RFLPs (Burr, 1994; Schloss *et al.,* 2002). Furthermore, SSRs have advantages over AFLPs and RFLPs because they are highly discriminative, co-dominant, locus-specific, highly reproducible and highly informative (Powell *et al.,* 1996; Bruford *et al.,* 1998; Agrama and Tuinstra, 2003; Missiaggia and Grattapaglia, 2006).

In an attempt to develop improved sweet stem sorghum varieties for bio-fuel production, the African Centre for Crop Improvement of the University of KwaZulu-Natal in South Africa acquired over 200 accessions from farmers' fields in southern Africa, from national breeding programs in Africa and introductions from India and Australia. The germplasm collection was assessed and showed considerable variability for agro-morphological traits and sugar yields. However, these accessions were not previously genotyped using molecular markers to establish their genetic relationship for effective breeding for bio-fuel production. In light of this, the objective of this study was to evaluate the genetic interrelationships present among selected sweet stem sorghum genotypes using 25 polymorphic simple sequence repeat (SSR) markers. Results of the study

may assist to objectively identify genetically unique collections for development of new sweet stem sorghum genotypes for biofuel production in South Africa or similar agro-ecologies.

3.2 Materials and methods

3.2.1 Plant materials

Eighteen sweet sorghum genotypes with distinct morphological attributes and greater ethanol production potential were selected from among 190 accessions assembled by the ACCI at UKZN, South Africa (Table 3.1). The test genotypes were originally collected from the USA, India, South Africa and Australia. Lines were selected based on their superior agro-morphological performances and higher levels of ethanol production. Table 3.1 presents descriptions of the genotypes including pedigree, origin and agro-morphological attributes, comprising of days to flowering, stem diameter, plant height, biomass, dry matter, fibre content, stalk brix content, and ethanol yield. The selected genotypes constituted self-fertilized entries or lines resulted from continuous controlled selfing, or open pollinated varieties (OPVs), constituting natural self-fertilizing populations.

Table 3.1: Description of 18 sweet stem sorghum varieties used in this study.

Genotype	Pedigree	Origin	Type	Days to flowering	Plant height (cm)	Stem diameter (mm)	Biomass (t ha^{-1})	Dry matter (%)	Fibre content (%)	Stalk Brix content (%)	Ethanol yield (l ha^{-1})
AS79	P9513B	USA	Line	113	210	21.17	42.96	30.24	14.19	15.55	3313
AS82	ICSR93034	India	Line	100.5	230	10.33	39.69	33.02	15.82	16.7	3260
AS103	MN2332	-	Line	87.5	250	13	43.21	27.25	14.4	12.35	2667
AS129	KARI Mtama X ICS 3-1	-	OPV	79	190	13.83	24.12	31.68	15.28	15.9	1899
AS138	Mugeta	-	Line	73	190	12.83	22.61	31.14	15.19	15.45	1688
AS204	SA Landrace LP50	South Africa	Line	87	255	12.17	37.3	26.41	12.41	13.5	2517
SS27	SS27	South Africa	Line	96.5	275	13.33	42	30.82	15.77	14.55	3008
SS120	Sugargraze	Australia	Line	87.5	260	16.67	31.65	26.71	16.01	10.2	1598
AS244	AS2 OPV	South Africa	OPV	86.5	290	19.5	39.58	27.51	14.01	13	2548
AS246	AS97 OPV	South Africa	OPV	86.5	305	17.17	48.73	32.99	17.19	15.3	3743
AS247	AS97 OPV	South Africa	OPV	90	320	22.5	28.23	41.55	30.65	10.4	1446
AS251	AS97 OPV	South Africa	OPV	135.5	385	29.17	64.21	31.11	17.51	13.1	4205
AS259	AS72 OPV	South Africa	OPV	87	290	18.83	35.88	28.21	15.46	12.25	2185
AS271	AS79 X SS27	South Africa	OPV	104	280	17.83	30.75	20.08	11.48	8.1	1230
AS308	AS204 X AS138	South Africa	OPV	93.5	295	15.33	31.54	26.39	14.84	11.05	1733
URJA	URJA	India	Line	97	295	15.33	27.35	31.49	14.79	16.2	2193
AS391	SS27 OPV	South Africa	OPV	116	305	13.67	62.82	28.1	13.1	14.5	4509
AS393	AS138 X SS27	South Africa	OPV	106	280	16	24.69	23.23	15.73	7	887

OPV=open pollinated variety

3.2.2 DNA extraction, purification, and quantification

Seeds of the 18 sweet sorghum genotypes were planted in seedling trays under greenhouse condition at the Controlled Environment Research Unit (CERU), UKZN, Pietermaritzburg, South Africa. Four weeks after planting, ten leaves from each of 18 genotypes were sampled and sent to the INCOTEC PROTEIOS Laboratory (Incotech South Africa Pty Ltd, Pietermaritzburg, South Africa) for genetic profiling using simple sequence repeat markers (SSR). DNA was extracted from all samples using a cetyltrimethylammonium bromide (CTAB)-based method according to Mace *et al.,* (2003). Twenty-five selected SSR markers were used for the analysis (Table 3.2). The markers used in this study were selected from an SSR diversity kit (Billot *et al.,* 2012) from all the linkage groups of sorghum. PCR products were fluorescently labelled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, SA).

Table 3.2: Description of the simple sequence repeat (SSR) primers used for sweet stem sorghum genetic diversity analysis

Marker [a]	Primer sequences (5′–3′)	Repeat motif	T (°C)	Reference
gpsb067	F:TAGTCCATACACCTTTCA R:TCTCTCACACACATTCTTC	(GT)10	49	Billot *et al.* (2012)
gpsb069	F:CCCATAATACTTGACCTTC R:ACTTACTCCCTCTGTCCC	(TC)12	50	Billot *et al.* (2012)
gpsb089	F:ATCAGGTACAGCAGGTAGG R:ATGCATCATGGCTGGT	(TG)9	50	Billot *et al.* (2012)
gpsb123	F:ATAGATGTTGACGAAGCA R:GTGGTATGGGACTGGA	(CA)7(GA)5	50	Mutegi *et al.,* (2011)
gpsb148	F:CAACCACAAACCAAGAG R:ATAGAAATGGGGTGGAG	(TC)3(CA)5	50	Billot *et al.* (2012)
gpsb151	F:ATACCAAGTTTCCTTTACCT R:GTTGGGGGAGAGTTTT	(CT)12	50	Billot *et al.* (2012)
mSbCIR223	F:CGTTCCAATGACTTTTCTTC R:GCCAATGTGGTGTGATAAAT	(AC)6	55	Billot *et al.* (2012)
mSbCIR238	F:AGAAGAAAAGGGGTAAGAGC R:CGAGAAACAATTACATGAACC	(AC)26	55	Mutegi *et al.,* (2011)
mSbCIR240	F:GTTCTTGGCCCTACTGAAT R:TCACCTGTAACCCTGTCTTC	(TG)9	55	Mutegi *et al.,* (2011)
mSbCIR246	F:TTTTGTTGCACTTTTGAGC R:GATGATAGCGACCACAAATC	(CA)7	55	Mutegi *et al.,* (2011)
mSbCIR248	F:GTTGGTCAGTGGTGGATAAA R:ACTCCCATGTGCTGAATCT	(GT)7	56	Mutegi *et al.,* (2011)
mSbCIR262	F:GCACCAAAATCAGCGTCT R:CCATTTACCCGTGGATTAGT	(CATG)3	57	Mutegi *et al.,* (2011)
mSbCIR276	F:CCCCAATCTAACTATTTGGT R:GAGGCTGAGATGCTCTGT	(AC)9	53	Mutegi *et al.,* (2011)
mSbCIR283	F:TCCCTTCTGAGCTTGTAAAT R:CAAGTCACTACCAAATGCAC	(CT)8(GT)8	54	Billot *et al.* (2012)
mSbCIR286	F:GCTTCTATACTCCCCTCCAC R:TTTATGGTAGGATGCTCTGC	(AC)9	55	Billot *et al.* (2012)
mSbCIR300	F:TTGAGAGCGGCGAGGTAA R:AAAAGCCCAAGTCTCAGTGCTA	(GT)9	61	Mutegi *et al.,* (2011)
mSbCIR306	F:ACATGGGGAGGAAGATGA R:GCTATTCAGGAGCCATGC	(CATG)3(GT)7	56	Billot *et al.* (2012)
mSbCIR329	F:GATCTTCACCAGGAACAGG R:ATGAGAGGAAAACATTGCTG	(AC)9	55	Billot *et al.* (2012)
sb4-72	F:TGCCACCACTCTGGAAAAGGCTA R:CTGAGGACTGCCCCAAATGTAGG	(AG)16	55	Brown *et al.,* (1996)
sb5-206	F:ATTCATCATCCTCATCCTCGTAGAA R:AAAAACCAACCCGACCCACTC	(AC)13(AG)20	55	Brown *et al.,* (1996)
Xcup02	F:GACGCAGCTTTGCTCCTATC R:GTCCAACCAACCCACGTATC	(GCA)6	54	Schloss *et al.,* (2002
Xisep0107	F:GCCGTAACAGAGAAGGATGG R:TTTCCGCTACCTCAAAAACC	(TGG)4	59	Ramu*et al.,* (2009)
Xisep0310	F:TGCCTTGTGCCTTGTTTATCT R:GGATCGATGCCTATCTCGTC	(CCAAT)4	60	Ramu*et al.,* (2009)
Xtxp12	F:AGATCTGGCGGCAACG R:AGTCACCCATCGATCATC	(CT)22	55	Kong *et al.,* (2000).
Xtxp57	F:GGAACTTTTGACGGGTAGTGC R:CGATCGTGATGTCCCAATC	(GT)21	55	Kong *et al.,* (2000).

NA = not available; T= annealing temperature.

3.2.3 Data analysis

Two approaches were adopted to investigate the genetic structure and relatedness among the sweet stem sorghum genotypes. In the first approach, polymorphisms were treated as binary data (presence or absence). In this case, each amplified fragment was considered as one locus and evaluated as dominant markers. However, to determine the genetic structure within and among genotypes, a second approach based on the co-dominant nature of the marker was adopted, using GenAlex version 6.5 (Peakall and Smouse, 2012). Genotypic data were subjected to analyses with various measures of genetic diversity within and among genotypes using GenAlex software version 6.5 (Peakall and Smouse, 2012). The χ^2 test was performed to determine the differences in allele frequencies among the SSR markers.

Genetic diversity parameters such as total number of alleles per locus (N_a), number of effective alleles per locus (N_e), Shannon's information index (I), observed heterozygosity (H_o), gene diversity (H_e), number of putative alleles (P_a), percent polymorphism (%P), and inbreeding coefficient (F_{IS}) were determined using the protocol of Nei and Li (1979). Other parameters such as differentiation and polymorphic information content (PIC) were estimated using GenAlex software.

The binary data were used to obtain a dissimilarity matrix using the Jaccard's index. The matrix was used to run a cluster analysis based on Neighbor-joining employing the software DARwin 5.0 (Perrier and Jacquemoud-Collet, 2006). A dendrogram was then generated on the dissimilarity matrix. Bootstrap analysis was performed for node construction using 10,000 bootstrap values to estimate the liability of the clustering pattern.

Based on Jaccards distances, analysis of molecular variance (AMOVA) was conducted using GenAlex software to partition total genetic variation into within and among groups of genotype based on type so as to quantify the level of diversity and genetic relationship among genotypes.

3.3 Results

3.3.1 Characteristics of the SSR markers

A summary of the genetic diversity parameters of the 25 SSR markers used in the study is given in Table 3.3. Overall, the markers amplified 106 putative alleles of different sizes among the sweet stem sorghum genotypes studied. The total number of alleles (N_a) amplified per locus across genotypes ranged from 1 (marker Xisep0310) to 10 (markers mSbCIR238 and Xgap206), with a mean of 4.64. All the markers except Xisep0310 were polymorphic. Ten markers (40%) had more alleles than the mean number of alleles per locus. The number

effective alleles (N_e) detected varied from 1 (Xisep0310) to 6.75 (Xgap206), with a mean of 2.9 per locus. The effective numbers of alleles for 32% of the loci were less than 2 and 36% of the loci had N_e values of more than 3. Furthermore, the observed heterozygosity (H_o) varied from 0.00 (Xisep0310 and mSbCIR286) to 0.39 (Xgap206), with a mean of 0.16. The expected heterozygosity (H_e) had values ranging from 0.00 (Xisep0310) to 0.88 (Xgap206) with a mean of 0.58. The inbreeding coefficient (F_{IS}) varied from 0.11 (Xisep0107) to 1.00 (mSbCIR286) with a mean of 0.7. The polymorphic information content values for all markers ranged from 0.00 (Xisep0310) to 0.85 (Xgap206), with a mean value of 0.56. Sixty eight percent and 28% of the markers had a PIC value greater than 0.50 and 0.70, respectively. Marker mSbCIR238 was the second most polymorphic locus with a PIC value of 0.84 followed by mSbCIR283 and Xtxp57 both with a PIC value of 0.78.

3.3.2 Genetic interrelationship within and among populations

Table 3.4 presents the genetic interrelationships within and among the 18 sorghum genotypes. The analysis classified genotypes into two populations based on their status as either lines or OPVs. Both the lines and OPVs showed a non-significant difference in most of the genetic parameters such as number of alleles per locus (N_a), Shannon diversity index (I), expected heterozygosity (H_o) and percentage of polymorphic loci (% P). As expected, both populations confirmed the dominant nature of self-fertilization in sorghum. Lines had a higher number of effective alleles (2.70) than OPVs which had 2.59 effective alleles per locus. The mean number of effective alleles was 2.65 per locus. Observed genetic diversity was 0.25 and 0.10 for the lines and OPVs, respectively even though they both had an expected genetic diversity of 0.57. Lines had an inbreeding coefficient of 0.52, while OPVs had an inbreeding coefficient of 0.80, giving a mean of 0.66, which was higher than the observed mean of 0.17. Test lines had high PIC values of 0.53 and 0.57, respectively, providing a mean of 0.53.

Table 3.3: Genetic relatedness within and among 18 sweet stem sorghum genotypes based on 25 microsatellite markers

Loci	Genetic parameter					
	N_a	N_e	H_o	H_e	F_{IS}	PIC
gbsb067	4	1.42	0.11	0.30	0.63	0.30
gpsb069	9	4.66	0.11	0.81	0.86	0.79
gpsb089	4	3.07	0.17	0.69	0.75	0.67
gpsb123	5	2.95	0.33	0.68	0.50	0.66
gpsb148	3	1.18	0.06	0.16	0.64	0.16
gpsb151	7	3.32	0.17	0.72	0.76	0.70
mSbCIR223	3	1.48	0.06	0.33	0.83	0.32
mSbCIR238	10	6.23	0.17	0.86	0.80	0.84
mSbCIR240	5	2.60	0.17	0.63	0.73	0.62
mSbCIR246	3	1.71	0.06	0.43	0.87	0.42
mSbCIR248	3	2.34	0.11	0.59	0.81	0.57
mSbCIR262	2	1.90	0.18	0.49	0.63	0.47
mSbCIR276	2	1.78	0.06	0.45	0.87	0.44
mSbCIR283	6	4.56	0.22	0.80	0.72	0.78
mSbCIR286	5	3.52	0.00	0.74	1.00	0.72
mSbCIR300	3	2.07	0.17	0.53	0.68	0.52
mSbCIR306	3	2.23	0.11	0.57	0.80	0.55
mSbCIR329	3	2.56	0.17	0.63	0.73	0.61
Xcup02	4	2.48	0.33	0.61	0.44	0.60
Xgap206	10	6.75	0.39	0.88	0.54	0.85
Xgap72	4	2.26	0.22	0.57	0.60	0.56
Xisep0107	2	1.46	0.28	0.32	0.11	0.31
Xisep0310	1	1.00	0.00	0.00	N/A	0.00
Xtxp12	8	4.44	0.22	0.80	0.71	0.77
Xtxp57	7	4.47	0.22	0.80	0.71	0.78
Overall mean	4.64	2.90	0.16	0.58	0.70	0.56
SE	0.51	0.31	0.02	0.05	0.04	0.04

N_a, total number of alleles per locus; N_e, number of effective alleles per locus; H_o, observed gene diversity within genotypes; H_e, average gene diversity within genotypes; F_{IS}, inbreeding coefficient; PIC, polymorphic information content; SE, Standard error.

Table 3.4: Genetic interrelationship within and among the 18 sweet stem sorghum genotypes classified into lines and open pollinated varieties (OPV)

Populations	Genetic parameter									
	N	N_a	N_e	I	H_o	H_e	F	PIC	N_{pa}	%P
Lines	8.00	3.76	2.70	0.99	0.25	0.57	0.52	0.533	22.00	96.00%
OPV	10.00	3.76	2.59	0.99	0.10	0.57	0.80	0.517	22.00	96.00%
Overall mean	8.96	3.76	2.65	0.99	0.17	0.57	0.66	0.525	22.00	96.00%
SE	0.15	0.25	0.18	0.07	0.02	0.03	0.04	0.002	0.00	0.00%

N =number of individual within each population; N_a =total number of alleles per locus; N_e =number of effective alleles per locus; I =Shannon's information index; H_o =observed gene diversity within genotypes; H_e =average gene diversity within genotypes; N_{pa} =number of putative alleles
%P =percentage of polymorphic loci; SE =Standard error

3.3.3 Genetic interrelationships among sweet stem sorghum genotypes using neighbour-joining algorithm and principal coordinates analysis

The genetic relationship among the 18 sweet stem sorghum genotypes was assessed using neighbour-joining algorithm with the unweighted pair group method (UPGMA). Figure 3.1 presents a dendrogram of clustering patterns of the 18 genotypes. Cluster analysis revealed the presence of three distinct major clusters (Clusters I, II and III). Cluster I was made up of 10 genotypes, of which 60% are OPVs. Cluster II consisted of seven genotypes of which 57% are lines. Cluster III contained only one genotype which is an OPV.

Principal coordinates analysis (PCoA) was performed on the genetic distance matrix using DARwin software. The first two principal components were plotted for visual examination of the clustering pattern of the genotypes. The results of the principal coordinate analysis of the 18 genotypes is presented in Figure 3.2. This analysis showed four major groups. Groups 1, 2, 3 and 4 containing ten, two, three and three genotypes, respectively. There was high concordance between the neighbour-joining clustering and the principal coordinate analysis in assigning lines into distinct groups. Group 1 of the principal coordinate analysis contained the exact genotypes as those in Cluster I of UWPGM. Group 2 of the principal coordinate analysis was made up of the genotypes SS27 and URJA which were found in sub-cluster Y of Cluster II of UWPGM. Moreover, the genotypes of Group 4 of the principal coordinate analysis consisted of AS204, AS271 and AS308 which were in sub-cluster X of Cluster II of UWPGM. Genotypes AS391, AS244, SS27 and AS204 were the highest ethanol producing varieties allocated in Groups 1, 2, 3 and 4, respectively.

3.3.4 Analysis of molecular variance (AMOVA)

The analysis of molecular variance (AMOVA) among sweet stem sorghum populations is shown in Table 3.5. AMOVA revealed non-significant variation among populations of sweet stem sorghum genotypes (P = 0.736). However, there was a highly significant difference (P ≤ 0.001) in molecular variance among and within individuals. AMOVA apportioned the total molecular variances into among populations, within individuals and among individuals. The highest genetic variability (73 %) was attributable to variation among individuals, while 27 % of variation was explained by within individual's variation.

Table 3.5: Analysis of molecular variance among 18 sweet stem sorghum genotypes collected from two populations when tested using 25 SSR markers

Source	df	SS	MS	Est. Var.	Per. Var	F-prob.
Among Populations	1	11.17	11.17	0.00	0%	0.736
Among individuals	16	206.19	12.89	5.43	73%	0.001
Within individuals	18	36.50	2.03	2.03	27%	0.001
Total	35	253.86		7.46	100%	

df= Degree of freedom, SS= sum of squares, MS= mean sum of squares, Est. var. = estimated variance, Per. Var. = Percentage variation

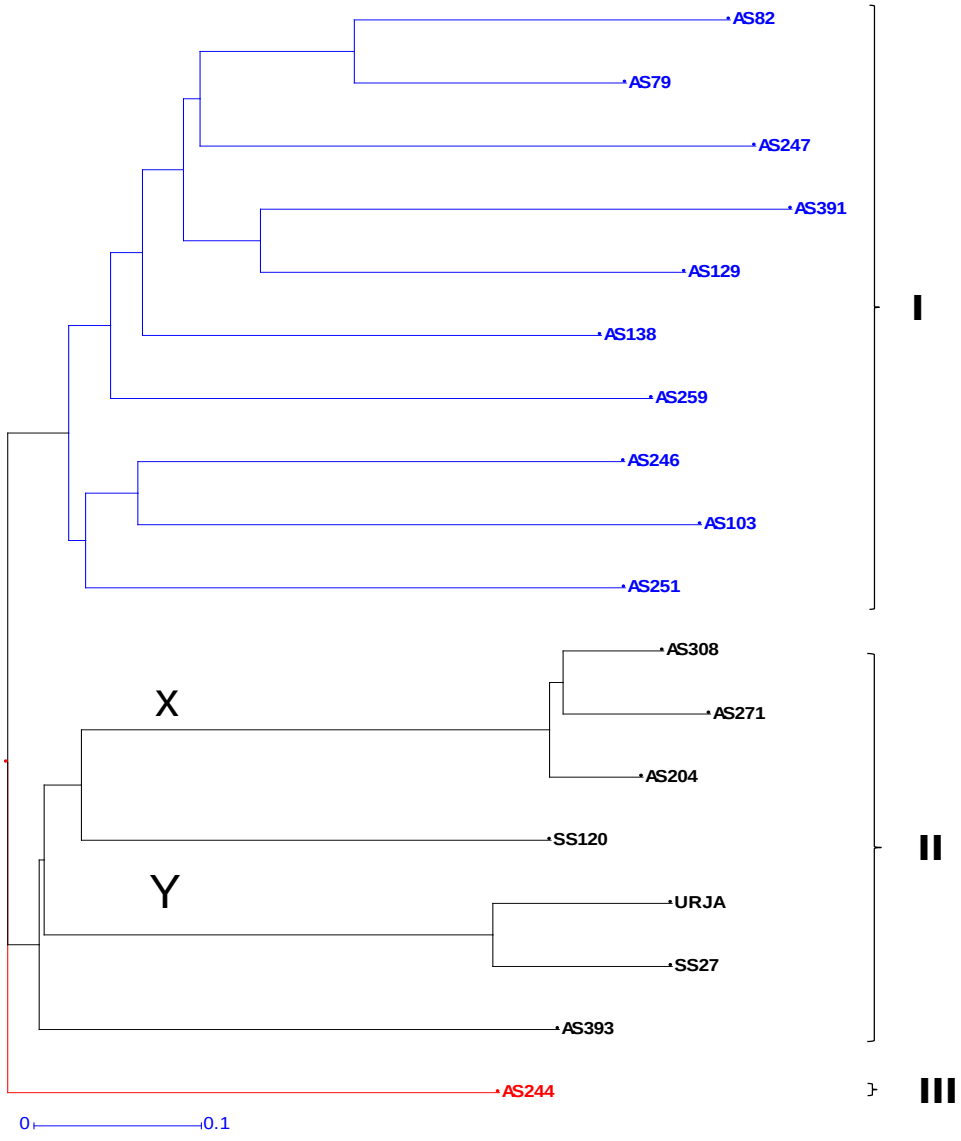


Figure 3.1: Un-weighted pair group method dendrogram showing the genetic relationships of the 18 sweet stem sorghum genotypes determined using 25 selected SSR markers

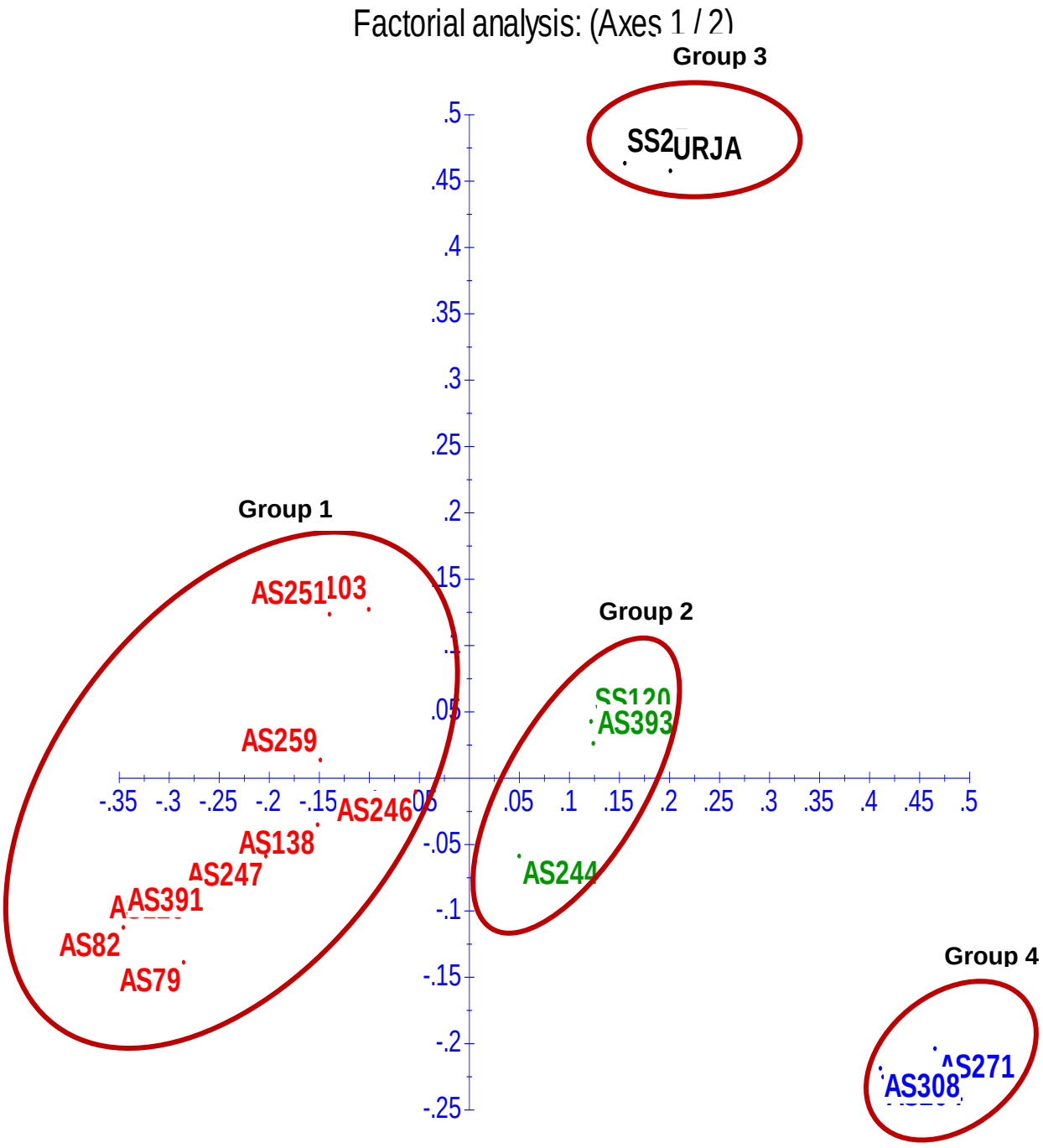


Figure 3.2: Principal coordinate analysis of the 18 sweet stem sorghum genotypes using 25 SSR markers

3.4 Discussion

3.4.1 SSR markers and allelic richness

The total number of fragments detected in this study was higher than the number found by Lekgari and Dweikat. (2014), who reported a total of 84 alleles in assessing 142 sweet stem sorghum germplasm using 33 SSR markers. Moreover, Olweny *et al*. (2014) studied 86 sweet stem genotypes using 11 SSR markers and reported a total of 86 alleles. On the other hand, the total number of alleles generated was lower than Ji *et al.* (2011) and Pei *et al.* (2010), who reported a total of 174 and 228 alleles using 63 and 46 markers on 29 and 47 elite sweet stem sorghum genotypes, respectively. Ali *et al.* (2008) reported 132 alleles using 41 SSR markers in 72 sweet stem sorghum accessions.

In the current study, the mean number of alleles per locus was 4.64. This result is comparable to Pei *et al.* (2010) and Wang *et al*. (2013), who reported a mean of 4.96 and 4.6 alleles per locus, respectively. Lower numbers of alleles were documented by Lekgari and Dweikat (2014) and Ali *et al.* (2008) who recorded a mean of 2.9 and 3.2 alleles per locus, respectively, implying low allelic richness. Wang *et al.* (2009) reported a mean of 7.6 allele per locus using 95 SSRs in 96 sweet stem sorghum accession. Olweny *et al*. (2014) reported a mean of 8 alleles per locus. The variation in the mean number of allele reported by different researches may be attributed to the variation in the number and type of SSR markers used, and the number and variable genetic background of genotypes. The higher number of alleles generated by SSR markers suggest the presence of extensive genetic diversity that can be utilized for breeding (Mashilo *et al.,* 2016). The high number of alleles and allelic richness found in this study may indicate the presence of significant genetic variation among sweet sorghum genotypes which would benefit supplementary systematic breeding (Ngailo *et al.,* 2016). Twenty-eight percent of the markers used contributed to half of the total numbers of observed alleles in the study, suggesting the existence of significant polymorphism among the markers.

As a self–pollinating species, sorghum is expected to have low level of heterozygosity. In this study, the mean observed heterozygosity of 18 sweet sorghum genotypes was 0.16, signifying that 84% of the loci were fixed and reached to acceptable level of homozygosity. However, the mean genetic diversity was 0.58, suggesting that the gene pool as a whole maintained a high level of allelic variation (Muui *et al.,* 2016). Other researchers also observed similar trends in sorghum, for example, Wang *et al.* (2009) and Olweny *et al*. (2014) reported a mean genetic diversity of 0.58 and 0.56, respectively. Ali *et al.* (2008) reported a lower genetic diversity of 0.4, while Wang *et al.* (2013) and Pei *et al.* (2010) reported higher genetic

diversity of 0.64 and 0.73, respectively. Low heterozygosity is associated with a high fixation of alleles in the population and the stability of genotypes in the absence of gene flow and genetic drift. This was supported by a mean inbreeding coefficient of 0.7, indicating the presence of appreciable levels of homozygosity among the experimental genotypes. Gene diversity reflects substantial genetic diversity that will enhance selection efficiency (Mashilo *et al.,* 2016). The moderate mean PIC of 0.56 found in the present study implies that half of the SSR markers used for analysis were informative, with strong discriminatory power; hence the markers can be used in analyzing the genetic diversity and relationship of sweet stem sorghum genotypes. These results are in agreement with the study by Wang *et al.,* (2009), Pei *et al.* (2010), Wang *et al.,* (2013) and Olweny *et al*. (2014).

3.4.2 Genetic structure in sweet stem sorghum genotypes

Genetic diversity analysis within a population is vital because it provides insights into the evolutionary aspects of species to employ for effective conservation strategies, and for the establishment of breeding programs (Li *et al*., 2011). In this study, the genetic relationship among the 18 sweet sorghum genotypes were assessed using a neighbor-joining algorithm, and principal coordinate analysis. Cluster analysis using UWPGM grouped the 18 genotypes into three distinct clusters, while the PCoA clustered the genotypes into four groups. The results indicated that there is strong correspondence between the two analyses in terms of number and types of genotypes assigned in each cluster. The clustering patterns were independent of the genotypes status as either being a line or OPV, signaling the true breeding nature of sorghum owing to its self-fertilization.

Genotypes AS391, AS244, SS27 and AS204 were the best ethanol producing varieties in each of Groups 1, 2, 3 and 4, respectively. These genotypes can be selected for future breeding program for biofuel production because of their high genetic diversity and good ethanol production potential.

There was a non-significant difference between the two populations in terms of the genetic parameters used in the study except for the observed heterozygosity and inbreeding coefficient. The OPVs had a higher fixation index and lower observed heterozygosity. This resulted from the differences in the maintenance of the two populations. The lines are maintained by controlled selfing, eliminating any alien pollen and should attain complete homozygosity since sorghum is self-pollinated crop. The lack of significant population differentiation was also supported by AMOVA revealing that 73 % of variation was among individuals, while 27 % of variation was explained by variation within individuals. This signifies that the genotypes made small but significant contributions to the total molecular variances detected and that the maximum variation was among the genotypes. Similar results were

reported by Olweny *et al.* (2014). The genetic structure of sweet stem sorghum genotypes in the current study revealed high genetic diversity and low population divergence.

3.5 Conclusions

Knowledge of genetic diversity within and among populations provides essential information in employing appropriate management strategies for germplasm conservation and for establishing a successful breeding program. The 25 SSR markers used were highly polymorphic and adequately demarcated the genetic relatedness of 18 sweet sorghum genotypes. In this study relatively few genotypes were used. However, theoretical population genetics predicts that large populations tend to maintain high allelic diversity. Therefore, collection of sweet sorghum genotypes adapted to extreme environments will improve the genetic base of the crop and allow mining of alleles from a diverse genetic background. Provided the high genetic diversity among genotypes and low population divergence observed in this study, selection of a few genetically diverse genotypes will be essential for future breeding programs and broaden the genetic base of the crop. Based on the SSR analysis and other suitable agronomic traits (e.g. high sugar yield), genetically unique genotypes such as AS391, SS27, AS204 and AS244 were selected to breed for sugar yield for bio-fuel production.

References

Alamnza-Pinzon, M.I., Khairullah, M., Fox, P.N. and, Warburton, M.L. 2003. Comparison of molecular markers and coefficients of parentage for the analysis of genetic diversity among spring bread wheat accessions. *Euphytica, 130*: 77-86.

Ali, M.L., Rajewski, J.F., Baenziger, P.S., Gill, K.S., Eskridge, K.M. and Dweikat, A.E. 2008. Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. *Molecular Breeding* 21:497–509.

Amelework, B., Shimelis, H., Tongoona, P., Laing, M., Mengistu, F. 2015. Genetic variation in lowland sorghum (*Sorghum bicolor* (L.) Moench) landraces assessed by simple sequence repeats. *Plant Genetic Resources: Characterization and Utilization* 13: 131-141.

Anami, S.E., Zhang, L.M., Xia, Y., Zhang, Y.M., Liu, Z.Q. and Jing, H.C. 2015. Sweet sorghum ideotypes: genetic improvement of the biofuel syndrome. *Food and Energy Security 4:* 159–177.

Atokple, I.D.K., Oppong, G.K. and Chikpah, S.K. 2014. Evaluation of grain and sugar yields of improved Sweet Sorghum (*Sorghum bicolor*) varieties in the Guinea Savanna Zone of Ghana. *Pinnacle Agricultural Research & Management* 2: 2-5.

Becelaere, G.V., Edward, L.L., Paterson, A.H. and Chee, P.W.2005. Pedigree-vs. DNA marker-based genetic similarity estimates in Cotton. *Crop Science.*45: 2281-2287.

Billot, C., Rivallan, R., Sall, M.N., Fonceka, D., Deu, M., Glaszmann, J.C., Noyer, J.L., Rami, J.F., Risterucci, A.M., Wincker, P., Ramu, P. and, Hash, C.T.2012. A reference microsatellite kit to assess for genetic diversity of *Sorghum bicolor* (Poaceae). *American Journal of Botany* 99: 245-250.

Bohn, M., Utz, H.F. and Melchinger, A.E. 1999. Genetic similarities among winter wheat cultivars determined on the basis of RFLPs and SSRs and their use for predicting progeny variance. *Crop Science* 39: 228-237.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32:314-31.

Brown, S. M., Hopkins, M.S., Mitchell, S.E., Senior, M.L., Wang, T.Y., Duncan, R.R., Gonzalez-Candelas, F. and Kresovich, S. 1996. Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum (*Sorghum bicolor* (L.) Monech). *Theoretical and Applied Genetics* 93: 190–198.

Bruford, M.W. and Beaumont, M.A. 1998. Binary data analysis. Molecular Tools for Screening Biodiversity. London, UK.

Burr, B. 1994. Some concepts and new methods for molecular mapping in plants. DNA-based Markers in Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Cox, T.S., Murphy, J.P., Rodgers, D.M. 1986. Changes in genetic diversity in the red winter wheat regions of the United States. PNAS, USA.

Daystar, J., Gonzalez, R., Reeb, C., Venditti, R., Treasure, T., Abt, R. and Kelley, S. 2014. Economics, environmental impacts, and supply chain analysis of cellulosic biomass for biofuels in the southern US: pine, eucalyptus, unmanaged hardwood, forest residues, switchgrass, and sweet sorghum. *Bio Resources* 9: 393-444.

Eggleston, G., Cole, M. and Andrzejewski, B. 2013. New commercially viable processing technologies for the production of sugar feedstocks from sweet sorghum (*Sorghum bicolor* L. Moench) for manufacture of biofuels and bioproducts. *Sugar Technoogy* 15: 232-249.

Food and Agriculture Organization Crop Production Statistics (FAOSTAT). 2016. World Sorghum Production and Utilization. FAO, Rome.

Fufa, H., Baenziger, P.S., Beecher, B.S., Dweikat, I., Graybosch. L., and Eskridge, K.M.(2005). Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. *Euphytica,* 145: 133-146.

Iqbal, A., Sadia, B., Khan A.I., Awan, F.S., Kainth, R.A. and,Sadaqat, H.A. 2010. Biodiversity in the sorghum (*Sorghum bicolor* L. Moench) germplasm of Pakistan. *Genetics and Molecular Research*. 9:756-764.

Jain, H.K. andKharkwal, M.C. 2004. Plant Breeding: Mendelian to Molecular Approaches. Narosa Publishing House, New Delhi.

Ji, G.S., Song, Y.F., Liu, G.Q., Du, R.H. and Hao, F.W. 2011. Genetic analysis of sorghum resources from China using SSRs. *Journal of SAT Agricultural Research* 9:1-7.

Lekgari, A. and Dweikat, I. 2014. Assessment of genetic variability of 142 sweet sorghum germplasm of diverse origin with molecular and morphological markers. *Open Journal of Ecology* 4: 371-393.

Li, D. 1997. Developing sweet sorghum to accept the challenge problems on food energy and environment in 21st century. Proceedings of First International Sweet Sorghum Conference. Institute of Botany, Chinese Academy of Sciences, Beijing, China.

Li, G., Ra, W.H., Park, J.W., Kwon, S.W., Lee, J.H., Park, C.B., Park, Y.J. 2011. Developing EST-SSR markers to study molecular diversity in Liriope and Ophiopogon. *Biochemical Systematic Ecology* 39:241–252.

Lipinsky, E. and Kresovich, S. 1980. Sorghums as energy crops. Proceedings of Bio-Energy'80, World Congress and Exposition. The Bio-Energy Council, Atlanta, USA.

Mace E.S., Hutokshi K., Buhariwalla H.K., and Crouch J.H. 2003. A high-throughput DNA extraction protocol for tropical molecular breeding programs. *Plant Molecular Biology Repository* 21: 459-46.

Mashilo, J., Shimelis, H. and Odindo, A. 2016. Genetic diversity of bottle gourd (*Lagenaria siceraria* (Molina) Standl.) landraces of South Africa assessed by morphological traits and simple sequence repeat markers. *South African Journal of Plant and Soil*: 1–12.

McCormick, F.C., Truong, S.K., Sreedasyam, A., Jenkins, J., Shu, S., Sims, D., Kennedy, M., n Amirebrahimi, M., Weers, B., McKinley, B., Mattison, A., Morishige, D., Grimwood, J., Schmutz, J. and Mullet, J. 2017. The Sorghum bicolor reference genome: improved assembly and annotations, a transcriptome atlas, and signatures of genome organization. https://doi.org/10.1101/110593

McIntyre, C.L., Tao, D.R., Jordan, D.R. and Henzell, R.G. 2001. Current status of molecular marker research in sorghum. Fourth Australian Sorghum Conference, Queensland, Australia.

Menz, M.A., Klein, R.R., Unruh, N.C., Rooney, W.L., Klein, P.E. and Mullet, J.E.2004. Genetic diversity of public inbreds of sorghum determined by mapped AFLP and SSR markers. *Crop Science* 44: 1236-1244.

Missiaggia, A. and Grattapaglia, D. 2006. Plant microsatellite genotyping with 4-color fluorescent detection using multiple-tailed primers. *Genetics and Molecular Research* 5:72-78.

Morrissey, K. and Thoma, D. 2017. Life Cycle Assessment of Sweet Sorghum as Feedstock for Second-generation Biofuel Production. Chemical Engineering Undergraduate Honors Theses. University of Arkansas, Fayetteville, USA.

Murray, S. C., Rooney, W.L., Mitchell, S.E., Sharma, A., Klein, P.E., Mullet, J.E. *et al.* 2008. Genetic improvement of sorghum as a biofuel feedstock: II. QTL for stem and leaf structural carbohydrates. *Crop Science 48*: 2180–2193.

Muui, C.W., Muasya, R.M., Kirubi, D.T., Runo, S.M. and Karugu, A. 2016. Genetic variability of sorghum landraces from lower Eastern Kenya based on simple sequence repeats (SSRs) markers. *African Journal of Biotechnology* 15: 264-271.

Nei, M., and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of National Academic Science, USA.

Ngailo, S., Shimelis H., Sibiya, J., Amelework, B. and Mtunda, K. 2016. Genetic diversity assessment of Tanzanian sweetpotato genotypes using simple sequence repeat markers. *South African Journal of Botany* 102: 40–45.

Olweny, C., Jamoza, J.,Dida, M.M.,Kimani, W.,Njuguna, J.,Githae, D.,Kiawa, B.,Yao, N., Kosambo, L.,C. Sally, C. and Okori. P. 2014. High genetic diversity for improvement of sweet sorghum (*Sorghum bicolor* (L.) Moench) genotypes for sugar and allied products. *Molecular Plant Breeding* 5: 29-35

Peakall, R. and Smouse, P.E. 201207. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288-295.

Pei, Z., Gao, J., Chen, Q., Wei, J., Li, Z., Luo, F., Shi, L., Ding, B. and Sun, S. Genetic diversity of elite sweet sorghum genotypes assessed by SSR markers. *Biologia Plantarum* 54: 653‑658.

Perrier X. and Jacquemoud-Collet J.P. 2006. DARwin software. Dissimilarity Analysis and Representation for Windows. Available from http://www.darwin.cirad.fr/darwin.html

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding,* 3:225-238.

Prakash, S.P.J, Biji, K. R., Gomez, S.M., Murthy, K.G, and Babu, R.C. 2008. Genetic diversity analysis of sorghum (*Sorghum bicolor* L. Moench) accessions using RAPD markers. *Indian Journal. Crop Science* 1(1-2):109-112.

Rajput, C.B., Mehtre, S.P., Dames. D.N., Kadam, B.P. 2012. Assessment of genetic diversity of sorghum [*Sorghum bicolor* (L.) Moench] using SSR. *Advanced Plant Science* 25:95-102.

Ratnavathi, C., Chakravarthy, S., Komala, V., Chavan, U. and Patil, J. 2011. Sweet sorghum as feedstock for biofuel production: A review. *Sugar Technology* 13(4): 399-407.

Reddy, D.C., Audilakshmi, S., Madhusudhana, R.,andSeetharama, N.2012. Comparative analysis of genetic similarity among sorghum (*Sorghum bicolor* (L.) Moench) lines as revealed by morphological and molecular markers. *Plant Genetics Resoucres.* 10:49-58.

Rosenow, D., and Clark, L. 1995. Drought and lodging resistance for a quality sorghum crop. Proceedings of the 50th Annual Corn and Sorghum Industry Research Conference. Chicago, USA.

Saballos, A. 2008. Development and utilization of sorghum as a bioenergy crop. *Genetic Improvement of Bioenergy Crops*: 211–248.

Schloss, S.J., Mitchell, S.E., White, G.M., Kukatla, R., Bowers, J.E., Paterson, A.H., and Kresovich, S. 2002. Characterization of RFLP probe sequences for gene discovery and SSR development in *Sorghum bicolor* (L.) Moench. *Theoretical and Applied Genetics* 105: 912-920.

Shehaz, T., Okuizumi, H., Kawase, M., and Okuno, K. 2009. Development of SSR-based sorghum (*Sorghum bicolor* (L.) Moench) diversity research set of germplasm and its evaluation by morphological traits. *Genetics Resources on Crop Evolution.* 56(6):809-827.

Shivjee, S., Khanna, V.K. 2010. Detection of diversity in germplasm of sorghum (*Sorghum bicolor*) using RAPD analysis. *Pantnagar Journal of Research* 8:170-174.

Subudhi P.K., Nguyen H.T., Gilbert M.L. and Rosenow D.T. 2002. Sorghum improvement: past achievements and future prospects. Crop Improvement: Challenges in the Twenty-First Century. The Haworth Press, Binghamton, New York, USA.

Tanksley, S.D., Young, N.D., Paterson, A.H., and Bonierbale, M.W. 1989. RFLP mapping in plant breeding: New tool for an old science. *Biotechnology* 7:257-264.

Van Beuningen, L.T., and Busch, R.H.1997. Genetic diversity among North American spring wheat cultivars: III. Cluster analysis based on quantitative morphological traits. *Crop Science*. 37: 981-988.

Varshney, R.K. 2011. Application of next generation sequencing and genotyping technologies to develop large-scale genomic resources in SAT legume crops. Genomics and Crop Improvement: Relevance and Reservations. Institute of Biotechnology, Acharya NG Ranga Agricultural University, Rajendranagar, Hyderabad, India.

Vermerris, W. 2011. Survey of genomics approaches to improve bioenergy traits in maize, sorghum and sugarcane free access. *Journal of Integrated Plant Biology 53*:105–119.

Vermerris, W., and Saballos, A. 2013. Genetic enhancement of sorghum for biomass utilization. *Genomics of the Saccharinae*: 391–425.

Wang, L., Zhu, C., Barkley, N.A., Chen, Z., Erpelding, J.E., Murray, S.C., Tuinstra, M.R., Tesso, T., Pederson, G.A and Yu, J. 2009. Genetic diversity and population structure analysis of accessions in the US historic sweet sorghum collection. *Theoretical and Applied Genetics* 120:13–23.

Wang, L., Jiao, S., Jiang, Y., Yanb, H., Sub, D., Sunb, G., Yana, X. and Sun, L. 2013. Genetic diversity in parent lines of sweet sorghum based on agronomical traits and SSR markers. *Field Crops Research* 149: 11–19.

Chapter 4

Preliminary investigation of the effect of ethyl 4'fluorooxanilate as a male gametocide of sweet stem sorghum

Abstract

An effective male sterility system enables targeted crosses between parent plants with desired and complementary characteristics. The use of chemical hybridizing agents (CHAs) to induce male sterility is quicker and more efficient than manual emasculation. This study investigated the concentration, stage of application and frequency of application of ethyl 4'fluorooxanilate (E_4FO) for inducing male sterility of sweet stem sorghum without affecting female fertility. Trials were conducted in the Controlled Environment Facility (CEF) at the University of KwaZulu-Natal. In Trial 1, the stage of application and dose rate of E_4FO were determined to optimize male sterility. In this experiment three genotypes were tested at three application stages and five E_4FO dose rates. In Trial 2 the frequency of application of E_4FO was determined using three sweet stem sorghum genotypes, three E_4FO doses, and six frequencies of application. Data on male sterility was inferred based on seed set and seed count from the treated plants. To determine female fertility, controlled crosses were performed and seed set was assessed and the number of seeds on cross pollinated plants were counted. Male sterility and female fertility were assessed against comparative control treatments. Male sterility was achieved when E_4FO was applied during heading stage using the following rates: 1000 mg l^{-1}, 1500 mg $l^{-1}$and 2000 mg l^{-1}, with more than one application. Applying E_4FO twice during the heading stage at a rate of 2000 mg l^{-1} would induce male sterility in the tested sweet stem sorghum genotypes, a result that could be useful in hybrid breeding programs. There is a need for further studies involving various yield components and the diverse responses of a range sweet stem sorghum genotypes.

Keywords: chemical hybridizing agent, ethyl 4'fluorooxanilate, male sterility and sweet stem sorghum.

4.1 Introduction

United States of America, Nigeria, and India are the three major producers of sweet stem sorghum, a multi-purpose, annual, C_4 crop (REN21, 2016). Among its many uses, sweet stem sorghum has emerged as a useful crop for the production of sugar as well as lignocellulosic biofuel feedstock (Mathur *et al.,* 2017). Sweet stem sorghum bred for high biomass production can be converted to biofuels (Codesido *et al.,* 2013). A lack of self-sufficiency in non-renewable energy resources, import costs of petro-chemicals, and the need to boost agricultural development are some of the reasons that biofuel production has become important during the past 10 years (Kovarik, 2013; Araújo, 2017). According to Schaffert (1992), the crisis in the supplies of fuel oil that occurred in the 1970s was the genesis of the production of ethanol from sweet stem sorghum. Since the year 2000, the global biofuels supply has increased by a factor of 8% (REN21, 2016; BP, 2016). The utilization of sweet stem sorghum for biofuel production has since increased owing to its environment friendliness: low sulfur content, low biological and chemical oxygen demand, and high octane rating (Reddy *et al.,* 2006). Enhancing the quantity and quality of the stalk juice are the chief drivers of sweet stem sorghum breeding. Meeting this goal not only requires extensive germplasm screening but also a well-defined strategy that takes less time because global climate change is an serious problem that calls for urgent mitigation strategies.

Sweet stem sorghum is predominately a self-pollinated crop with a low but quantifiable incidences of outcrossing (Schertz and Dalton, 1980; Pedersen *et al.,* 1998). For this reason, male sterility is essential for the production of hybrid sweet stem sorghum cultivars. Replacing sweet stem sorghum inbred lines with hybrid cultivars enables the exploitation of heterosis or hybrid vigor to increase stalk and sugar yields, and to protect breeder's right. Sweet stem sorghum inbred lines are also known for their high ethanol productivity owing to their inherent genetic potential as pure line cultivars.

Establishment of male sterility systems enables crosses between chosen parents having desirable and complementary characteristics. This is especially important in sorghum and other self-pollinating crops. In sweet stem sorghum both male and female flowers are found in the same spikelet. In the past, development of hybrid sweet stem sorghum cultivars has mainly relied on a cytoplasmic-genetic male sterility system (CMS), which requires male-sterile line (A-line), a sterility-maintainer line (B-line), and a fertility-restorer line (R-line). This system has been used for decades but it has various shortcomings: the non-availability of breeding stocks containing CMS and restorer systems, their instability and the laborious method of heterosis breeding using CMS. Besides being tedious and time-consuming, this technique

sometimes becomes untenable because of the lack of a consistent restorer system for the genetic restoration of fertility (Guilford *et al.,* 1992; Pfeiffer *et al.,* 2010).

A feasible alternative to the male sterile-maintainer-restorer based on three-line hybrid breeding is two-line hybrid breeding that exploits chemical sterilization (McRae, 1985; Guilford *et al.,* 1992). Chemical hybridizing agents (CHAs) can be used to develop a large pool of heterotic combinations expressing various traits (Mogensen and Ladyman, 1989). Induction of physiological deformities in the male gamete prevent pollen development, pollen shed or pollen viability is the modus operandi of most CHAs (Cross and Ladyman, 1991). Since the revolutionary studies on the gametocidal property of maleic hydrazide on gladiolus (Moore, 1950), a wide range of chemicals have been screened. Some of them selectively induce male sterility in crops. Oxanilates have been reported to selectively impair the pollen formation in monoecious and hermaphrodite plants (Batch *et al.,* 1980).

Triticum aestivum (L.) (Chakraborty and Devakumar, 2006), *Eragrostis tef* (Zucc.) (Ghebrehiwot *et al.,* 2015), *Oryza sativa* (L.) (Ali *et al.,* 1999), *Helianthus annuus* (L.)(Tripathi and Singh, 2008) and *Cicer arietinum* (L.) (Chakraborty *et al.,* 2001) are some of the crops that have been emasculated by ethyl 4'fluorooxanilate (E_4FO). In contrast, there is no data on its effects on sweet stem sorghum. In order to use CHAs in sorghum hybrid breeding, it is essential to identify effective and safe chemical male gametocides (Amelework *et al.,* 2016). The current study aimed at investigating the concentration, stage of application and frequency of application of E_4FO to induce male sterility in sweet stem sorghum without affecting female fertility.

4.2 Materials and methods

4.2.1 Chemical formulation and application

The CHA used in the current trial was E_4FO. The E_4FO, formulated as a white powder emulsion, was prepared by first dissolving at a 1:6 w/v ratio with dimethyl sulfoxide (DMSO) and adding 2% Tween 80 as a surfactant. Spray emulsions of 1000, 1500, 2000, 2500 and 3000 mg l^{-1} concentrations were prepared by diluting the solution with water. Chemical spraying was done with a hollow cone (HCX) 80° nozzle, using sprayer (Figure 4.1). Spraying was done in the early morning. The spray mist was directed to the top of the head until run-off occurred. The quantity of the liquid sprayed per plant was approximately 8 to 10 ml. Distilled water was used to spray the control treatment.

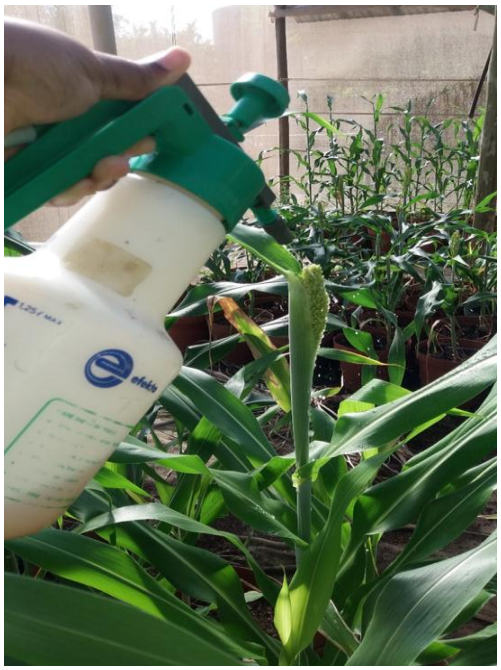


Figure 4.1: Illustration of the administration of E_4FO on to the plants

4.2.2 Sweet stem sorghum genotypes and experimental design

Trial 1: *Determination* of CHA concentration and stage of application

In this trial, the time and dose of E_4FO was determined to discern maximum male sterility. In this experiment three genotypes were tested using three application times and five E_4FO doses. In the trial, the following three sweet stem sorghum genotypes were used: Kari Mtama, Dwarf Wonder and KAT 487, labelled as AS1, AS71, and AS72, respectively. This was aimed to determine the most conducive stage to apply the CHA and ascertain the most effective concentration of the CHA. Experimental unit comprised of four pots. Each experimental unit was replicated thrice and arranged using a randomized blocks design. Trial 1 had 54 experimental units or treatments (six E_4FO concentrations × three sweet stem sorghum genotypes × three application stages) and the control. The three sorghum genotypes were treated with six different concentrations of E_4FO comprising of 0 mg l^{-1} (distilled water), 1000 mg l^{-1}, 1500 mg l^{-1}, 2000 mg l^{-1}, 2500 mg l^{-1}, 3000 mg l^{-1}. The aqueous solution of the E_4FO was sprayed before heading (when the heads were released halfway from the flag leaf), during heading and after heading.

Trial 2: *Determination* of the frequency of application of E_4FO

Trial 2 aimed at determining the frequency of application of E_4FO required for male sterility using three sweet stem sorghum genotypes, three E_4FO doses, and six frequencies

of application. Trial 2 was designed based on the results, deductions and conclusions of Trial 1 which indicated that E_4FO needed to be sprayed more than once. To establish the frequency of application, AS71 was used together two other sweet stem sorghum genotypes, ICSV 3 and SDSL 89569, denoted as AS17 and AS88 respectively. Each experimental unit comprised of four pots. Each experimental unit was replicated thrice. The experimental units were arranged in a completely random fashion. Trial 2 also had 54 experimental units or treatments (three E_4FO concentrations × three sweet stem sorghum genotypes × six application frequencies). Three sweet stem sorghum genotypes, AS17, AS71 and AS88, were treated with three concentrations of E_4FO of 1000 mg l^{-1}, 1500 mg $l^{-1}$and 2000 mg l^{-1}. All the E_4FO applications were carried out during the heading period. Figure 4.2 illustrates the various E_4FO application stages. Three plants of each experimental unit were bagged, and cross pollinated manually two days after the last E_4FO application. This was done to test how each treatment affected female fertility of the sweet stem sorghum plants.

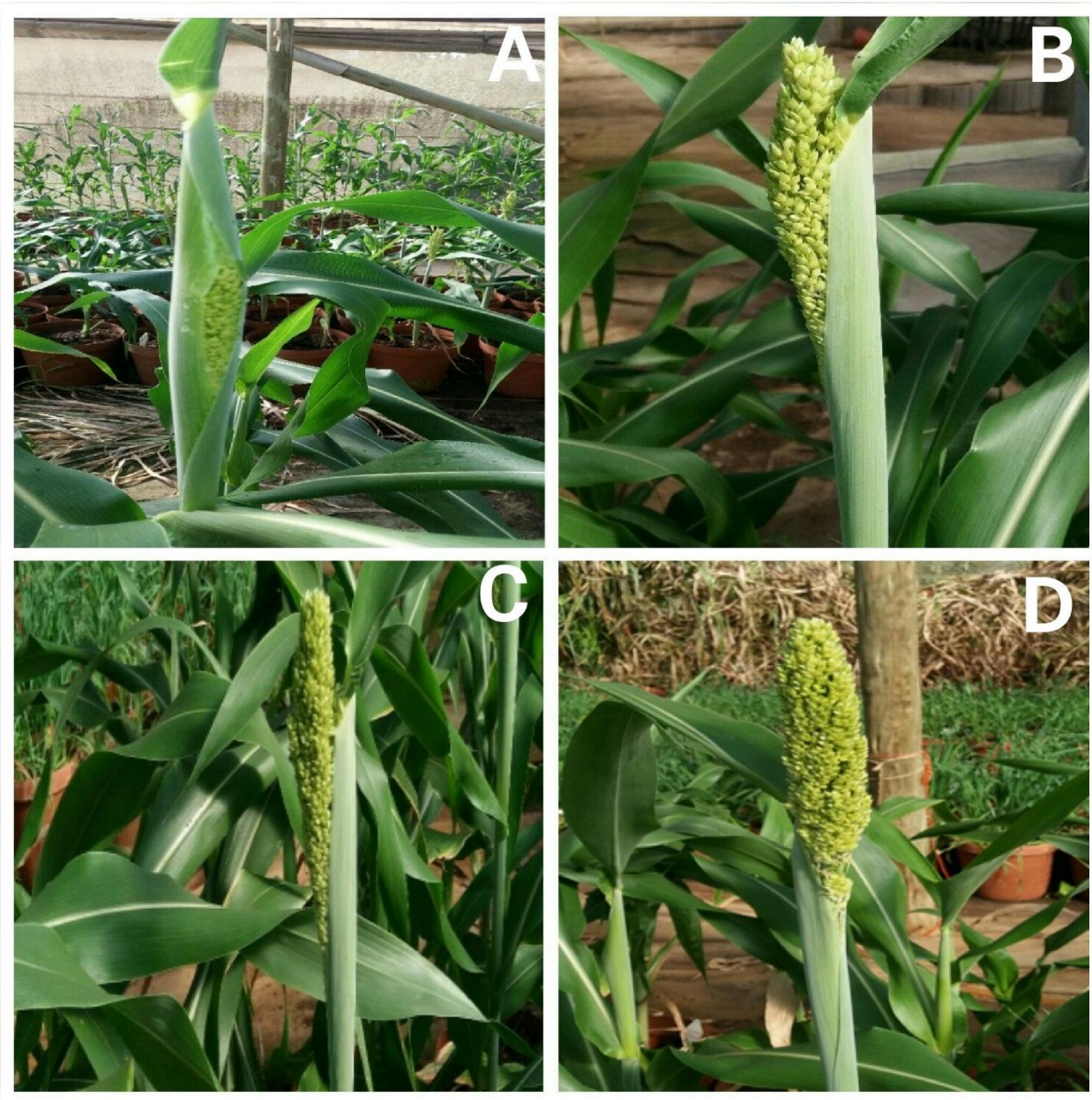


Figure 4.2: Illustration of the different growth stages of the sweet sorghum head at which the E_4FO was applied in Trial 2. A, B, C and D were approximately 0, 3, 6 and 9 days in the head protrusion process. E_4FO application time combinations were termed as T1 (A+B+C+D); T2 (B+C+D); T3 (A+B+C); T4 (A+C+D); T5 (B+D) and T6 (Control).

4.2.3 Trial establishment

The trial was conducted at the Controlled Environment Facility (CEF), University of KwaZulu-Natal. Experiments were conducted in an environmentally controlled greenhouse maintained at an air temperature of 28 ± 2.5°C. The sweet stem sorghum plants were grown from seeds sown directly into plastic pots (300 mm in diameter and 280 mm in height) filled with Gromor potting media (http://www.gromor.co.za). The plants were fertilized with Agchem

hydroponic water-soluble fertilizer (http://www.agchem.co.za). The plants were irrigated four times a day for 3 min. Four seeds per pot were sown, then thinned-out to two plants per pot at three weeks after germination. All lateral tillers were constantly clipped off, allowing only one main tiller to grow to flowering. Weeds were hand controlled. The control plots were maintained at a distance of 3m to circumvent chemical drift and pollen contamination.

4.2.4 Data collection and data analysis

At the end of the trial season, seeds produced from the plants were manually collected, counted and recorded. Data for each replication was collected based on average measurements of four plants. To study female fertility, the number of seeds on cross pollinated plants were counted. Both male sterility and female fertility were calculated by comparison to the control plants. The following formulae were applied:

$$Male\ sterility = \frac{number\ of\ seeds\ on\ control\ plant - number\ of\ seeds\ on\ E4FO\ treated\ plant}{number\ of\ seeds\ on\ control\ plant} \times 100$$

$$Female\ fertility = \frac{number\ of\ seeds\ on\ pollinated\ plant - number\ of\ seeds\ on\ E4FO\ treated\ plant}{number\ of\ seeds\ on\ control\ plant - number\ of\ seeds\ on\ E4FO\ treated\ plant} \times 100$$

Data was subjected to analysis of variance (ANOVA) using GenStat 17th edition Inc. (Payne *et al.*, 2014). Means separation of treatments was Fisher's LSD.

4.3 Results and discussion

4.3.1 Determination of CHA concentration and stage of application for effective sterilization of sweet stem sorghum in Trial 1

4.3.1.1 Analysis of variance

In Trial 1, data from two application stages (before and after heading) were excluded from analysis because no E_4FO effect was observed. This can be attributed to the fact that pollen development in sweet stem sorghum only occurs during heading and a CHA can induce male sterility in this period only.

Table 4.1 is a presentation of the analysis of variance (ANOVA) for male sterility. In Trial 1 treatment with six different concentrations of E_4FO on three different sweet stem sorghum genotypes showed that concentration of E_4FO had a highly significant ($p<0.001$) effect on male sterility. However, genotype and the interaction of genotype and concentration had non-significant effects on male sterility. From these results, it can be deduced that E_4FO was not genotype specific as all the genotypes had roughly the same levels of male sterility at all the concentrations. This can be attributed to the fact that all the three genotypes are

morphologically similar, with short plants because the study targeted shorter varieties that could fit in a greenhouse, and for ease of hand spraying the E_4FO. These results are similar to those of Amelework *et al.* (2016) who studied male emasculation in sorghum and reported highly significant emasculation due to variety and concentration. However, interaction was also significant.

Table 4.1: Analysis of variance for seed set involving three sweet stem sorghum varieties and six E_4FO concentrations in relation to induced pollen sterility

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	9.21	4.6	0.43	
Concentration	5	63353.82	12670.76	1181.08	<.001
Genotype	2	8.63	4.32	0.4	0.672
Concentration*Genotype	10	97.49	9.75	0.91	0.536
Residual	34	364.75	10.73		
Total	53	63833.91			

df =degrees of freedom, ss= sum of squares, ms= mean square, vr= variance ratio, F pr= F probability

4.3.1.2 Mean responses of male sterility and treatment combinations

Table 4.2 summarizes the degree of male sterility achieved by each treatment combination. As observed by Amelework *et al*. (2016), levels of pollen sterility increased with an increase in the concentration of test chemicals. Trial 1 showed that almost complete emasculation was achieved with both 2500 mg l^{-1} and 3000 mg l^{-1}. However, these concentrations were phytotoxic causing premature senescence of the plants. Similar results were obtained by Ghebrehiwot *et al.* (2015) on tef, who reported that near complete male sterility was achieved by E_4FO at rates ranging between 1500 mg l^{-1} and 3000 mg l^{-1}. They also reported floret dryness and early premature senescence when E_4FO was applied at 3000 mg l^{-1}. Amelework *et al*. (2016) reported that complete pollen sterility was caused by E_4FO at concentrations ranging between 2000 to 3000 mg l^{-1}. No phytotoxicity was observed for concentrations of 1000 mg l^{-1}, 1500 mg $l^{-1}$and 2000 mg l^{-1}which is similar to that Ghebrehiwot *et al.* (2015). However, the male sterility for these concentrations were low (<60%). This can be attributed to the fact that pollen on a sweet stem sorghum head are at different development stages at any given time. Consequently, depending on the application time of the CHA, it can sterilize some of the pollen and leave the rest unsterilized. For this reason, it was essential to conduct Trial 2, which applied three concentrations (1000 mg l^{-1}, 1500 mg $l^{-1}$and 2000 mg l^{-1}) and apply them more than once.

Chakraborty and Devakumar (2006) explained that when using E_4FO, the CHA technology needed to be optimized in terms of variation in genotype, choice of CHA, stage of

spray, numbers of spray, types of formulation, and dose. Although there were no significant differences among the responses of the genotypes, the highest level of male sterility was observed on genotype AS71 for all concentrations except 1000 mg l^{-1} and 1500 mg l^{-1} where AS1 and AS72 had the highest levels of male sterility respectively.

Table 4.2: Mean seed set of three sweet stem sorghum genotypes after treatment with E_4FO at six concentrations

Concentration (mg l^{-1})	Genotype			Observation
	AS1	AS71	AS72	
1000	43.33	38.56	36.56	No phytotoxicity symptoms observed
1500	47.19	48.95	50.72	No phytotoxicity symptoms observed
2000	56.79	56.84	54.39	No phytotoxicity symptoms observed
2500	97.85	98	96.9	Early, premature plant senescence
3000	98.7	99.11	99.44	Early, premature plant senescence
Control	0	0	0	

4.3.2 Determination of the optimum frequency of application of E_4FO to sterilize sweet stem sorghum in Trial 2

4.3.2.1 Analysis of variance

Trial 2 is an extension of Trial 1 whereby the frequency of application was increased to more than once. Tables 4.3 and 4.4 present the ANOVA for male sterility and female fertility in sweet stem sorghum observed in Trial 2, respectively. It can be observed that all the treatment factors and their interactions were highly significant ($p<0.001$). Contrary to Trial 1, in Trial 2 E_4FO was a genotype-specific CHA. The change in genotype specificity can be attributed to the fact that genotypes varied morphologically. Morphological variation affects the impact of surfactants that favor the penetration of gametocides into the plant (Parodi and Gaju, 2009; Amelework *et al.,* 2016). Similar female fertility results were obtained by Amelework *et al.* (2016) who reported that in the bagged panicles, a highly significant difference was observed between varieties, concentrations, and all their interactions.

Table 4.3: Analysis of variance for seed set among three sweet stem sorghum genotypes to three E_4FO concentrations at six frequencies in relation to induced pollen sterility

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	4.823	2.411	2.41	
Genotype	2	105.687	52.844	52.7	<.001
Concentration	2	4938.493	2469.246	2462.71	<.001
Frequency	5	182343.538	36468.708	36372.1	<.001
Genotype*Concentration	4	303.718	75.929	75.73	<.001
Genotype*Frequency	10	1810.251	181.025	180.55	<.001
Concentration*Frequency	10	5083.764	508.376	507.03	<.001
Genotype*Concentration*Frequency	20	4471.158	223.558	222.97	<.001
Residual	106	106.282	1.003		
Total	161	199167.713			

df =degrees of freedom, ss= sum of squares, ms= mean square, vr= variance ratio, F pr= F probability

Table 4.4: Analysis of variance of seed set among three sweet stem sorghum genotypes to three E_4FO concentrations at six frequencies in relation to female fertility

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	42.063	21.032	3.76	
Genotype	2	211.644	105.822	18.92	<.001
Concentration	2	619.633	309.817	55.38	<.001
Frequency	5	51591.872	10318.374	1844.51	<.001
Genotype*Concentration	4	534.05	133.512	23.87	<.001
Genotype*Frequency	10	966.138	96.614	17.27	<.001
Concentration*Frequency	10	1987.541	198.754	35.53	<.001
Genotype*Concentration*Frequency	20	2116.044	105.802	18.91	<.001
Residual	106	592.973	5.594		
Total	161	58661.958			

df =degrees of freedom, ss= sum of squares, ms= mean square, vr= variance ratio, F pr= F probability

4.3.2.2 Mean responses of pollen sterility and female fertility

Levels of male sterility and female fertility for all treatment combinations are presented in Table 4.5. Male sterility increased with increase in concentration. Moreover, the level of male sterility increased with the frequency of application. The highest level of male sterility observed was 99.6%, a result of applying 2000 mg l^{-1} four times (T1) and the lowest level of male sterility was for 1000 mg l^{-1} applied twice (T5). Similar results were also found in rice where 100% male sterility was induced by E_4FO (Ali *et al.,* 1999). For successful hybrid seed production on a commercial level mass emasculation is essential. Ghebrehiwot *et al.* (2015) noted that the

lack of seed production together with high level of pollen sterility found in plants treated with E_4FO were indicative of the effectiveness of E_4FO in mass emasculation.

The high levels of male sterility associated with higher frequency of application, however, came at a cost of lower female fertility. Female fertility increased with a reduction in concentration. The highest levels of female fertility was observed for AS88 sprayed with 1000 mg l^{-1} twice (T5), while the lowest female fertility was for AS71 sprayed four times (T1) at 2000 mg l^{-1}. Applying the CHA twice (T5) resulted in the highest levels of female fertility (desirable) for all genotype x concentration combinations. For genotype AS17, when CHA was applied twice (T5), the highest level of male sterility was observed at 2000 mg l^{-1}. Hence, the recommendation for high male sterility with functional female fertility for genotype AS17 is 2000 mg l^{-1} sprayed twice (T5). The same high level of male sterility with uncompromised female fertility was also achieved by applying the CHA at 2000 mg l^{-1} twice for both genotypes AS71 and AS88. Having considered all scenarios, the best balance between high levels male sterility without compromising female fertility were produced by applying the E_4FO twice (T5) at a dose of 2000 mg l^{-1} (98.6% male sterility and 96% female fertility). Such male sterility percentages comparable to the 95% male sterility considered satisfactory for the production of hybrid seed (Parodi and Gaju 2009). Similar results were obtained by Chakraborty and Devakumar (2006) on wheat. They reported that E_4FO induced high levels of male sterility (99.76 ± 0.37% at 0.15% concentration over 29 diverse wheat genotypes), and little reduction of female fertility (96.78 ± 2.07% in E_4FO treated plants). Ghebrehiwot *et al.* (2015) reported that 96–99% male sterility was achieved without a significant reduction in female fertility in tef using E_4FO at 1000–1500 mg l^{-1}. Research has conveyed that E_4FO not only induces a very high degree of male sterility, but also modifies the reproductive biology in such a fashion to ensure cross-pollination in the cleistogamous wheat flowers and increase the probability of the development of hybrids (Chakraborty and Devakumar, 2006). Ali *et al.* (1999) concluded that, of the CHAs they tested, E_4FO was the most promising and capable of inducing higher levels of pollen sterility than sodium methyl arsenate, their check gametocide.

Table 4.5: Male sterility and female fertility as measured by seed set in three sweet stem sorghum genotypes after treatments with three E_4FO concentrations at six frequencies

Genotype	Concentration (mg l^{-1})	Frequency of application											
		Male sterility						Female fertility					
		T1	T2	T3	T4	T5	T6	T1	T2	T3	T4	T5	T6
AS17	1000	91.9	87.1	88.4	86.1	48.8	0	49.7	74.5	78.2	85.4	99.2	100
	1500	95.5	92.6	94.3	94.8	90.5	0	48.6	63.8	88.4	78.6	95.4	100
	2000	98.8	98.6	98.7	98.5	98.8	0	43.4	58.6	72.8	68.5	94.7	100
AS71	1000	87.8	85	86.6	86.8	62.1	0	56.5	73.2	60.7	85.1	96.4	100
	1500	96.2	58.5	94	94.2	94.6	0	47.6	54.7	63.6	66.2	96.8	100
	2000	99.1	98.6	98.8	97.2	98.8	0	45.5	54.1	71.6	84.6	95.3	100
AS88	1000	90.2	85.3	87.4	62.3	78.6	0	54.8	76.8	65.4	73.9	99.5	100
	1500	97.8	93.4	76.5	92.1	94.9	0	50.7	60.3	63	87.1	98.8	100
	2000	99.2	75.3	98.9	98.6	98.4	0	44.5	58.6	86.2	72.1	97.6	100

E4FO application time combinations are termed as T1 (A+ B+C+D); T2 (B+C+D); T3 (A+B+C); T4 (A+C+D); T5 (B+D) and T6 (Control). A, B, C and D are approximately days 0, 3, 6 and 9 in the head protrusion process.

4.4 Conclusions

Emasculation of sweet stem sorghum without disrupting female fertility was achieved by the application of E_4FO twice during heading at 2000 mg l^{-1} for all the test genotypes. However, it is recommended that the effect of the CHA on the other genotypes of sweet sorghum be tested. It is also essential to carry out large scale trial in the field to produce hybrids that can be evaluated. This will also be an opportunity to ascertain the logistics of mass emasculation for commercial settings using E_4FO.

References

Ali, A.J., Devakumar, C., Zaman, F.U. and Siddiq, E.A. 1999. Identification of potent gametocides for selective induction of male sterility in rice. *Indian Journal of Genetics 59*: 429-436.

Amelework, A., Laing, M., Shimelis, H. 2016. Evaluation of effective gametocides for selective induction of male sterility in sorghum. *Czech Journal of Genetics and Plant Breeding* 52: 163–170.

Araújo, K. 2017. Low Carbon Energy Transitions: Turning Points in National Policy and Innovation. Oxford University Press: New York, NY, USA.

Batch, J.J., Parry, K.P., Rowe, C.F., Lawrence, D.K. and Brown, M.J. 1980. Method of controlling pollen formation in monoecious and hermaphrodite plants using oxanilates and their derivatives. Plant Growth Regulators and Herbicides, Antagonists- Recent Advances. Noyes Data Corporation. New Jersey, USA.

British Petroleum (BP). 2016. Statistical Review of World Energy. BP: London, UK.

Chakraborty, K. and Devakumar, C. 2004. Evaluation of chemical compounds for induction of male sterility in wheat (*Triticum aestivum* L.). *Euphytica 16:* 243-248.

Chakraborty, K. and Devakumar, C. 2006. Ethyloxanilates as specific male gametocides for wheat (*Triticum aestivum* L.). *Plant Breeding 125*: 441—447.

Chakraborty, K., Devakumar, C., Kumar, J. and Kumar, R. 2001. Effect of anilates on pollen sterility of chickpea (*Cicer arietinum* L. var. Pusa 240). *Tropical Agricultural Research 13:* 239—248.

Chakraborty, K., Devakumar, C., Tomar, S.M.S. and Kumar, R. 2003. Synthesis and quantitative structure-activity relationships (QSAR) of oxanilates as chemical hybridizing agents (CHAs) for wheat (*Triticum aestivum* L.). *Journal of Agriculture and Food Chemistry 51*: 992—998.

Codesido, V., Vacas, R., Macarulla, B., Gracia, M.P. and Igartua, E. 2013. Agronomic and digital phenotyping evaluation of sweet sorghum public varieties and F1 hybrids with potential for ethanol production in Spain. *Maydica* 58:42–53.

Cross, J.W. and Ladyman, J.A.R. 1991. Chemical agents that inhibit pollen development: Tools for research. *Sexual Plant Reproduction 4*: 235.

Ghebrehiwot, H., Burgdorf, R., Shimelis, H. and Laing, M. 2015. The efficacy of four gametocides for induction of pollen sterility in *Eragrostis tef* (Zucc.) Trotter. *African Journal of Biotechnology 14:* 774-780.

Guilford, W.J., Patterson, T.G., Vega, R.O., Fang, L., Liang, Y., Lewis, H.A. and Labovitz, J.N. 1992. Synthesis and pollen suppressant activity of phenylcinnoline-3-carboxylic acids. *Journal of Agriculture and Food Chemistry* 40: 2026–2032.

House, L.R. 1985. A Guide to Sorghum Breeding, 2nd Edition. International Crops Research Institute for the Semi-Arid Tropics. Patancheru 502 324, Andhra Pradesh, India.

Kaul, C.L. and Singh, S.P. 1967. Stamina and functional male sterility induced by chemical treatment in papilionaceous plants. *Indian Journal of Agriculture Science* 37: 264-269.

Kovarik, B. 2013. History of biofuels. Biofuels Crops. Center for Bioscience International (CABI): Wellington, UK.

Loussaert, D. 2004. Trihalogenated methylsulfonamides as specific male gametocides. *Sexual Plant Reproduction* 16: 299—307

Mathur, S., Umakanth, A.V., Tonapi, V.A., Sharma, R. and Sharma, M.K. 2017. Sweet sorghum as biofuel feedstock: recent advances and available resources. *Biotechnology and Biofuels* 10:146.

McRae, D.H. 1985. Advances in chemical hybridization. *Plant Breeding Reviews 3*: 169-191.

Mizelle, M.B., Sethi, R. Ashton, M.E. and Jensen, 1989. Development of the pollen grain and tapetum of wheat (*Triticum aestivum*) in untreated plants and plants treated with chemical hybridizing agent RH 0007. *Sexual Plant Reproduction 2*: 231–252

Mogensen, H.L. and Ladyman, J.A.R. 1989. A structural study on the mode of action of CHATM chemical hybridizing agent in wheat. *Sexual Plant Reproduction* 2: 173-183.

Moore, R.H. 1950. Several effects of maleic hydrazide on plants. *Science* 112: 52-53.

Nesvadba, Z., and T. Vyhnánek. 2001. Induction of hybrid wheat using the gametocide Genesis® in the Czech Republic. *Acta Univ. Agric. et Silvic. Mendel. Brun. 49*: 27-35.

Nesvadba, Z., Vyhnánek, T. and Vendar, J. 2001. The testing of efficacy of CHA Genesis® for the production of hybrid wheat and hybrid triticale. *Acta Fytotechnica et Zootechnica 4*: 266-268.

Parodi P.C. and Gaju M.A. 2009. Male sterility induced by the chemical hybridizing agent Colfencent on wheat, *Triticum aestivum* and *T. turgidum* var. *durum*. *Ciencia e Investigación Agraria*, 36: 267–279.

Pederson, J.F., Toy, J.J. and Johnson, B. 1998. Natural outcrossing of sorghum and sudangrass in the central great plains. *Crop Science* 38: 937-939.

Pfeiffer, T.W., Bitzer, M.J., Toy, J.J. and Pedersen, J.F. 2010. Heterosis in sweet sorghum and selection of a new sweet sorghum hybrid for use in syrup production in Appalachia. *Crop Science* 50: 1788-1794.

Reddy, B.V.S., Kumar, A.A. and Ramesh, S. 2006. Sweet sorghum: a water saving bioenergy crop. In: ICRISAT, Patancheru, Andhra Pradesh, India; p. 1–12. http://www.iwmi.cgiar.org/EWMA/files/papers/PaperforBioenergyandwater- Accessed 13 August 2017.

Renewable Energy Network 21 (REN21). 2016. Global Status Report; REN21: Paris, France.

Schaffert, R.E. 1992. Sweet sorghum substrate for industrial alcohol in: Utilization of sorghum and millets. Proceedings of the International Workshop on Policy Practice and Potential Relating Uses of Sorghum and Millets. ICRISAT Center, Bulawayo, Zimbabwe.

Schertz, K.F. and Dalton, L.G. 1980. Sorghum. Hybridization of Crop Plants. American Society of Agronomy and Crop Science Society of American, Madison, USA.

Stephens, J.C. and Holland, P.F. 1954. Cytoplasmic male sterility for hybrid sorghum seed production. *Agronomy Journal 46*: 20–23.

Tripathi, S.M. and Singh, K.P. 2008. Hybrid seed production in detergent induced male sterile *Helianthus annuus* L. *Helia 31*: 103-112.

Wassell, L., and Weaver, V. 1995. Benefits of hybrid wheat. HybriTech Product Development Report. Monsanto and HybriTech Seed International Inc., St. Louis, USA.

Chapter 5

Combining ability and heterosis of sweet stem sorghum genotypes for bioethanol yield and related traits

Abstract

Sorghum hybrids can provide enhanced bioethanol yield if genetically complementary parents and progenies are developed using efficient crossing and selection methods. The objective of this study was to determine the combining ability of sweet stem sorghum genotypes, and to assess heterosis among F1 hybrids for ethanol production and associated traits. Eight sweet stem sorghum lines and four testers were crossed using a line x tester mating design. The F1 hybrids and parental lines were evaluated for bioethanol yield and related traits. Data were subjected to analysis of variance, combining ability and heterosis analyses. Tested sweet stem sorghum genotypes had estimated ethanol yields of 787 l ha^{-1} to 5470 l ha^{-1}, with a mean of 2055 l ha^{-1}. Stem diameter varied from 0.4 cm to 2.4 cm, with a mean of 1.53 cm, while plant height varied from 128 cm to 215 cm, with a mean of 163 cm. Biomass production of test populations varied from 11.6 t ha^{-1} to 68 t ha^{-1}, with a mean of 30 t ha^{-1}. Six hybrids were among the top ten biomass producing genotypes. Fibre content varied from 12 to 20%, with a mean of 16%, while dry matter varied from 25 to 34%, with a mean of 31%. Stalk brix varied from 9.9 to 16.4%, with a mean of 13.8%. Four hybrids (AS246 x AS391, AS251 x AS204, AS79 x AS204, AS74 x AS204) expressed greater ethanol productivity with positive better-parent heterosis (>30 %). Lines AS253, AS246 and AS 105, and testers AS391 and SS27 had highly positive general combining ability (GCA) effects for almost of the traits in a desirable direction. Due to its consistent, significant and positive GCA effects across majority of the traits, line AS253 is recommended for utilization in sweet stem sorghum hybrid programs. Among the studied hybrids or test parents, ethanol productivity had significant and positive correlations with plant height, stem diameter and biomass.

Keywords: sweet stem sorghum, line x tester analysis, combining ability, heterosis, ethanol productivity

5.1 Introduction

Sweet stem sorghum is a variant from of grain sorghum that is being promoted as a feedstock for biofuel production. It has great potential for the biofuel industry and may contribute to solving the twofold challenges of sustainable energy and environmental security, on a global scale. Ethanol yield gains can be achieved through dedicated sorghum breeding programs. Understanding of the gene action of traits like bioethanol production that are controlled by polygenes with complex gene action is essential (Jain and Patel, 2014). Knowledge of the gene action also helps plant breeders in choosing suitable breeding approaches.

The ability of a parent to transmit its desirable genes to its progeny in crosses has been referred to as combining ability. For effective hybridization programs and perception of the nature and magnitude of various types of gene action involved in the expression of quantitative characters, combining ability studies have proven beneficial because they result in the selection of suitable parents (Bernardo, 2014). Falconer (1989) described general combining ability (GCA) as the mean performance of a genotype when crossed with a series of other genotypes. Falconer also defined specific combining ability (SCA) as the deviation from the average general combining ability of two parental lines due to genetic effects that are specific to that cross. Additive gene effects and higher order additive interactions result in differences in GCA, while non-additive gene effects bring about differences in SCA (Kenga *et al.,* 2004).

Knowledge of combining ability helps in optimizing the breeding strategy. Use of the recurrent selection procedure is important when GCA effects are predominant. This will drive the accumulation of desirable unfixable or fixable additive gene effects (Nadarajan and Gunasegaram, 2005). In contrast, back cross breeding is important when SCA effects are predominant.

In order to fully realize the potential of sweet stem sorghum for ethanol production, it is essential to exploit hybrid vigour or heterosis. In the preliminary evaluation of germplasm for use in hybridization, line × tester analysis is valuable in recognising good combiners that may be used to increase a population with favourable fixable genes for increased ethanol production. The line × tester mating design helps in assessing the combining ability of potential parents resulting in the selection of superior parents, as well as cross combinations (Sprague and Tatum, 1942). Line x tester analysis is one of the most efficient approaches of assessing the genetic worthiness of a large number of inbred lines for their combining ability (Kempthorne, 1957). Line x tester analysis has been dubbed the most fitting technique to investigate the combining ability for a number of parents (Dehinwal *et al.,* 2017), consequently,

simplifying commercial development of hybrids. Therefore, the objective of this study was to determine the combining ability of selected sweet stem sorghum genotypes, and to assess heterosis among the resultant F1 hybrids for ethanol production and associated traits, using line x tester analysis.

5.2 Materials and methods

5.2.1 Plant materials, crosses and experimental design

The study used four sorghum genotypes as testers and eight genotypes as lines. These provided 12 sweet stem sorghum genotypes for crosses. Lines were selected based on their previously established high ethanol producing abilities. The testers were selected based on their genetic diversity, coupled with their ability to produce ethanol (Mangena *et al*., 2017).

Based on a Simple Sequence Repeat (SSR) analysis, and suitable agronomic traits (e.g. high sugar yield), genetically unique genotypes, including AS391, SS27, AS204 and AS244, were selected as male parents (tester parents) (Table 5.1). These were crossed with eight selected South African adapted lines that were used as female parents. The crosses were carried out in accordance with a Line x Tester mating scheme to generate 32 hybrids. Detailed information about each genotype is given in Table 5.1.

The chemical hybridising agent (CHA), ethyl 4'fluorooxanilate (E_4FO), was applied (see Chapter 4, Section 2) twice at 2000 mg l^{-1} to induce male sterility in the female genotypes selected for this study. Because male sterility induction was through a novel CHA system, the amount of seed set was minimal per cross combination, hence the F1 hybrids were only evaluated at one location and during one season. The 32 experimental hybrids and 12 parents were field evaluated. Experiments were laid out using a randomised complete blocks design with two replications. Each entry was planted in two-row plots of 3.0 m length with inter-row and intra-row spacing of 80 and 20 cm, respectively.

Table 5.1: Description of the 12 sweet stem sorghum varieties used in the study

Name	Pedigree	Origin	Role in cross
AS105	FPR(168 x GS70)	-	Line
AS111	P9539B	-	Line
AS113	TX2737/91BE7414	-	Line
AS246	AS97	South Africa	Line
AS251	AS97	South Africa	Line
AS253	AS72	South Africa	Line
AS74	ICSV 111	-	Line
AS79	P9513B	USA	Line
AS391	SS 27 C	South Africa	Tester
SS27	SS27	South Africa	Tester
AS204	LP 50	South Africa	Tester
AS244	AS2	South Africa	Tester

-not available

5.2.2 Trial establishment and maintenance

Seed of the experimental genotypes were planted in seedling trays at the greenhouse facility of the Controlled Environment Facility (CEF) at the University of KwaZulu- Natal. Four weeks after planting, the seedlings were transplanted to the Ukulinga Research Farm of the University of KwaZulu-Natal, South Africa (29°37′S 30°22′E; 596 m above sea level). The hybrid evaluation trial was conducted from August 2017 to February 2018. The trial site received 526 mm moisture through rainfall and supplemental irrigation. The mean minimum temperature for the season was 6°C and the mean maximum temperature was 32°C. Experimental plots were fertilised at 375 kg ha^{-1} with 2:3:2 (N:P:K) granular fertiliser. At the flowering stage, plants were side dressed with 130 kg ha^{-1} of urea (46% N) granular fertiliser. Due to the fact that the trial was conducted off-season, growth of the crop was limited by the cold hence it was essential to boost growth by fertilization. After transplanting, the trials were treated with 100 ml ha^{-1} of lamda cyhalothrin (Karate) to control a wide range of pests, including cutworm. When the plants reached 60 cm height, the plots were treated with 120 ml ha^{-1} of lamda cyhalothrin to control lepidopteran pests.

5.2.3 Data collection

The following quantitative traits were measured during the study: plant height was measured from the base of the plant to the tip of the panicle and expressed in cm. Stem diameter (cm) was measured using a Vernier calliper on the midsection of three plants. One

row was harvested from each plot at dough stage. Biomass (t ha^{-1}) was measured at maturity by first removing the seed heads, then cutting the stems off at ground level, and weighing the stems and leaves.. Samples of chopped stalks were weighed green and reweighed after oven drying at 60°C until constant mass was reached, to gravitationally estimate dry mass (Hlophe 2014). Dry matter was calculated using the formula: $Dry\ matter\ \% = \frac{Dry\ mass}{Wet\ mass} \times 100\ \%$.

Fibre content was calculated using the formula: $Fibre\ \% = Dry\ matter\ \% - 0.005 - \frac{Stalk\ brix}{100}$.

Stalks were cut using a chaff cutter and a representative sample was analysed for % Brix using the hand-held refractometer method.

Given the same brix reading, this calculation results in an upward adjustment of juicy cultivars over dry ones because at equal brix readings, juicier cultivars have more sugar compared to drier ones (Makanda 2009). Ethanol productivity was calculated using the formulae below:

$$Total\ Brix = Biomass\ t\ ha^{-1} \times \frac{Stalk\ Brix}{100}$$

$$Total\ fermentable\ sugars = Total\ Brix\ \times 0.85$$

$$Ethanol\ l\ ha^{-1}\ = \frac{Total\ fermentable\ sugars \times 0.46}{0.79} \times 1000$$

5.2.4 Data analyses

The hybrid variation was partitioned into Line and Tester main effects, giving two independent estimates of general combining ability (GCA) effects. The Line × Tester interaction effects were used to estimate the specific combining ability (SCA) effects (Halluer and Miranda, 1988). The model for Line x Tester analysis within reciprocals was as follows:

$Y_{ijk} = \mu + L_i + T_j + (L \times T)_{ij} + r_k + \varepsilon_{ijk}$

where; Y_{ijk} = observed hybrid response; μ = grand mean; L_i= line main effect; T_j= tester main effect; $(L \times T)_{ij}$ = interaction between line and tester; r_k = replications main effect; and ε_{ijk} = random experimental error.

General combining ability effects

The GCA effects for parents, when used both as male and female parents, were calculated according to Kearsey and Pooni (1996) as follows:

$GCA_L = X_L - \mu$ and $GCA_T = X_T - \mu$

where: GCA_L and GCA_T = GCA of line and tester, respectively; X_L and X_T = mean of the lines and testers averaged over its crosses, respectively; μ = grand mean of all crosses.

The standard error (SE) for line and tester GCA effects was calculated according to Dabholkar (1999) separately because the numbers of males and females were not balanced. This was as follows:

$SE_L = \sqrt{\frac{MSE}{T \times r}}$ and $SE_T = \sqrt{\frac{MSE}{L \times r}}$

where: MSE = mean square error; r = number of replications; L and T = number of lines and testers, respectively.

T-tests were calculated to determine the significance of Lines, Testers and Line by Tester interaction effects as follows:

$t_X = \frac{GCA_X}{SE_X}$

where: t_X = t-statistic of either Line, Tester or Line × Tester interaction analysis; GCA_X = general combining ability for either line or tester; and SE_X = standard error of either line or tester.

Specific *combining* ability effects

The SCA effects of the crosses were computed according to Kearsey and Pooni (1996) as follows:

$SCA_X = X_X - E(X_X) = X_X - (GCA_L + GCA_T + \mu)$

where: SCA_X = SCA effects of the two parents in the cross; X_X = observed mean value of the cross; $E(X_X)$ = expected value of the cross based on the GCA effects of the two parents; GCA_L and GCA_T = GCA of Line and Tester parents, respectively.

The standard error (SE) for the SCA effects was calculated according to Dabholkar (1999) as follows:

$SE = \sqrt{\frac{MSE}{r}}$

where: MSE = mean square error; r = number of replications;

T-tests were calculated to determine the significance of Lines, Testers and Line by Tester interaction as follows:

$t_X = \frac{SCA_X}{SE_X}$

where: SCA_X = specific combining ability for the cross and SE_X = standard error of the SCA effects of the cross.

GCA and SCA *variance* estimates

The variance components for GCA effects (Lines and Testers) and that of SCA effects were calculated. Heritability in the broad- sense was calculated using Hallauer and Miranda (1981) as follows:

$$H^2 = \frac{\delta_g^2}{\delta_p^2},$$

where: H^2 = broad-sense heritability; δ_g^2 = genotypic variance; δ_p^2 = phenotypic variance.

The heritability % was categorized as low, moderate and high, in accordance with Robinson *et al.* (1949) as follows: 0-0.3, low; 0.3-0.6, moderate and >0.6, high.

Heterosis

Better-parent heterosis (%) was computed according Alam *et al.* (2004) as follows:

$$Better\ parent\ heterosis\ \% = \frac{X_X - X_{BP}}{X_{BP}} \times 100\ \%$$

where: X_X = observed mean value of the cross; X_{BP} = mean of the better parent.

Mid parent heterosis (%) was computed as follows:

$$Mid\ parent\ heterosis\% = \frac{X_X - X_{MP}}{X_{MP}} \times 100\%$$

where: X_X = observed mean value of the cross; X_{MP} = mean of both parents.

Associations among traits

The Pearson's phenotypic correlation analysis was performed in GenStat 18th Edition to describe the relationship among the morphological traits.

5.3 Results

5.3.1 Analysis of variance

Table 5.2 shows the analysis of variance for ethanol productivity and associated traits of sweet stem sorghum hybrids. The general analysis of variance for all genotypes showed that genotypes were significantly different for all traits except dry matter. Table 5.3 shows the combining ability analysis of variance. The lines exhibited significant differences for all traits except dry matter while testers were not significantly different for dry matter and fibre content.

The Line by Tester interaction was only significantly different for biomass, stalk brix and ethanol production.

Table 5.2: Standard ANOVA showing mean squares and significance tests for ethanol productivity and associated traits of sweet stem sorghum hybrids

Sources of variation	df	SD	PH	BM	FC	DM	SB	EP
Genotypes	43	0.24703***	786.2***	410.3***	0.0007292*	0.001087	5.043**	2621866***
Replication	1	0.00047	320.4	131.2	0.0022977*	0.016316***	63.677***	112048
Error	41	0.08584	263.8	133	0.000437	0.001017	2.391	882200

df = degrees of freedom, SD = stem diameter, PH = plant height, BM = biomass, FC = fibre content, DM = dry matter, SB = stalk brix, EP = ethanol productivity. ***, **, * significant at p≤0.001, p≤0.01 and p≤0.05, respectively.

Table 5.3: Line x Tester ANOVA mean squares and significance tests for ethanol productivity and associated traits of sweet stem sorghum

Sources of variation	df	SD	PH	BM	FC	DM	SB	EP
Replication	1	0.0016	140.7	522	0.003711	0.019712	63.173	437193
Testers	3	0.2179*	1221.4**	50.5*	0.000592	0.001298	3.314*	244931*
Lines	7	0.5102***	2724.8***	1236.4***	0.00149**	0.002023	9.564**	7512952***
Lines x Testers	21	0.0930	191.2	122.7*	0.000503	0.000824	3.054*	598690*
Error	29	0.1022	237.7	132.9	0.000474	0.001119	2.442	772082

df = degrees of freedom, SD = stem diameter, PH = plant height, BM = biomass, FC = fibre content, DM = dry matter, SB = stalk brix, EP = ethanol productivity. ***, **, * significant at p≤0.001, p≤0.01 and p≤0.05, respectively

5.3.2 Mean performance of 32 hybrids and parents for seven bioethanol related traits

Table 5.4 shows the mean performances of all the hybrids and their parents for seven traits measured in the study. Ethanol productivity varied from 787 l ha^{-1} to 5470 l ha^{-1}, with a mean of 2055 l ha^{-1}. The top ten ethanol producing genotypes included six hybrids (AS253 x AS244, AS246 x AS391, AS253 x AS204, AS251 x SS27, AS253 x SS27 and AS253 x AS391). In terms of stem diameter, genotypes varied from 0.4 cm to 2.4 cm, with a mean of 1.53 cm, while plant height varied from 128 cm to 215 cm, with a mean of 163 cm. The top ten genotypes with thick stem comprised of nine hybrids (AS253 x AS391, AS253 x SS27, AS253 x AS244, AS105 x AS204, AS79 x SS27, AS246 x AS391, AS251 x AS391, AS251 x AS204 and AS111 x SS27), while the top ten tallest genotypes included eight hybrids (AS253 x AS244, AS251 x SS27, AS253 x SS27, AS246 x AS244, AS251 x AS244, AS253 x AS391, AS111 x AS244 and AS246 x SS27). Biomass production varied from 11.6 t ha^{-1} to 68 t ha^{-1},

with a mean of 30 t ha^{-1}. Six hybrids (AS253 x AS244, AS253 x AS391, AS251 x AS391, AS253 x SS27, AS253 x AS204 and AS246 x AS391) were among the top ten biomass producing genotypes. Fibre content varied from 12 to 20%, with a mean of 16%, while dry matter varied from 25 to 34%, with a mean of 31%. Lastly, stalk brix varied from 9.9 to 16.4%, with a mean of 13.8%. Of the top ten genotypes, only five were hybrids (AS253 x AS204, AS253 x SS27, AS105 x AS244, AS79 x AS204 and AS253 x AS391).

Table 5.4: Means of seven ethanol productivity related traits of 32 sweet stem sorghum hybrids and their 12 parents

	Traits						
Genotypes	SD	PH	BM	FC	DM	SB	EP
AS 253*	1.5	177	67.00	13	30	16.2	5468.6
AS253 x AS244	2	215	68.00	15	29	14.2	4802.7
AS246 x AS391	1.8	160	37.25	15	29	13.2	4622.2
SS 27*	1.55	175	57.17	17	33	15.7	4562.0
AS253 x AS204	1.7	170	48.38	12	29	16.6	3858.9
AS 391*	1.6	185	46.88	17	31	13.45	3121.3
AS251 x SS27	1.45	205	36.08	15	26	10.5	3018.6
AS253 x SS27	2.35	197.5	49.63	14	31	16.5	2857.4
AS253 x AS391	2.4	185	53.17	14	30	15.1	2814.5
AS 246*	0.4	172.5	42.13	15	29	13.3	2779.8
AS251 x AS391	1.8	164.5	52.25	15	29	13.3	2564.0
AS 105*	1.4	147.5	29.21	17	34	15.95	2322.0
AS 113*	1.05	135	28.42	17	34	16.4	2296.8
AS246 x AS244	1.1	190	33.25	17	31	13.35	2197.8
AS 244*	1.4	170	29.54	19	33	14.05	2055.7
AS105 x AS204	1.9	147.5	30.00	15	29	13.8	2019.0
AS105 x AS244	1.6	157.5	25.46	16	33	15.55	1983.3
AS79 x AS244	1.45	157.5	28.21	17	31	13.8	1973.1
AS105 x SS27	1.6	150	22.46	17	30	12.15	1945.5
AS111 x SS27	1.8	170	34.88	16	31	14.3	1943.5
AS251 x AS204	1.8	166	26.88	19	33	13.75	1816.1
AS79 x AS204	1.7	136	23.79	17	33	15.2	1762.9
AS 111*	1.8	157.5	21.92	18	34	15.95	1729.3
AS79 x AS391	1.75	147.5	22.08	19	34	14.7	1535.3
AS246 x SS27	1.25	180	35.58	14	25	9.9	1499.5
AS111 x AS204	1.65	160	20.71	17	32	14.45	1472.6
AS113 x SS27	1.45	149	17.75	15	29	13.3	1471.7
AS105 x AS391	1.4	152.5	23.08	19	33	12.9	1469.8
AS251 x AS244	1.4	189	24.04	20	33	12.25	1441.7
AS 79*	1.25	130	21.00	13	27	13	1351.2
AS246 x AS204	1.05	172.5	20.63	12	25	12.7	1312.3
AS 251*	1.2	162.5	22.88	17	29	11.5	1298.7
AS74 x SS27	1.25	165	25.92	16	30	13.65	1298.0
AS113 x AS391	1.7	150	16.25	17	32	15.1	1296.2
AS111 x AS391	1.6	164.5	17.71	17	32	14	1250.4

Table 5.4 continued

Genotype	SD	PH	BM	FC	DM	SB	EP
AS74 x AS204	1.2	147.5	22.17	16	28	11.35	1230.5
AS74 x AS244	1.25	155.5	16.88	18	32	14.05	1159.8
AS113 x AS244	1.45	142.5	16.13	15	29	13.7	1139.0
AS74 x AS391	1.7	160	17.13	14	28	13.25	1112.2
AS111 x AS244	1.8	180	15.33	17	32	14	1050.7
AS 204*	1.3	147.5	17.96	20	31	10.75	954.7
AS79 x SS27	1.85	130	12.79	19	34	13.95	952.8
AS113 x AS204	1.35	127.5	12.96	17	30	12.65	816.7
AS 74*	1.1	146	11.63	18	32	13.6	786.5
Mean	1.53	162.5	29.6	16	31	13.8	2054.9

SD = stem diameter, PH = plant height, BM = biomass, FC = fibre content, DM = dry matter, SB = stalk brix, EP = ethanol productivity, * = parental genotype

5.3.3 General combining ability effects

The GCA effects of parental lines utilised in the study are shown in Table 5.5. General combining ability effects were significant for all lines, with the exception of genotypes AS105, AS251 and AS79. Of the five parental lines with significant ethanol production, only AS246 and AS253 had positive GCA effects. Genotype AS 253 had the highest GCA effect for ethanol production of almost 2100 l ha^{-1} , making it the best general combiner. Regarding stem diameter, only three genotypes (AS253, AS391 and AS244) had positive significant GCA effects. AS253 had the highest GCA effect at 0.64. Genotypes AS251, AS253 and AS244 had significant GCA effects for plant height, AS253 being the highest with GCA of 35.8. Genotype AS253 also had the highest positive significant GCA effects for biomass (33), although only one other genotype (AS 251) had a positive significant GCA effect. In terms of fibre content, two parental lines (AS246 and AS253) had significant negative GCA effects. Only AS79 had a significant positive GCA effect for dry matter. The parental line AS253 had a significant positive GCA effect for stalk brix. The parental line AS253 had significant GCA effects for all traits with the exception of dry matter. No tester parents had significant GCA effects for biomass, fibre content, dry matter and ethanol production.

Table 5.5: General combining ability (GCA) effects for the sweet stem sorghum parents for ethanol productivity and associated traits

	Parents	SD	PH	BM	FC	DM	SB	EP
Lines	AS 105	0.15	-4.25*	2.89	0.0010	0.002	0.07	366.17
	AS 111	0.24	12.50	-0.20*	-0.0003	0.006	0.65	-58.92*
	AS 113	0.01	-13.88**	-6.58**	-0.0099	-0.008	0.15	-307.33**
	AS246	-0.17**	19.50*	9.32	-0.0223*	-0.035**	-1.25**	919.71*
	AS 251	0.14	25.00**	12.46*	0.0050*	-0.006	-1.08*	721.87
	AS 253	0.64**	35.75**	32.44**	-0.0288**	-0.008	2.07**	2095.13**
	AS 74	-0.12**	0.88	-1.83*	-0.0076	-0.012	-0.46	-288.08*
	AS 79	0.21	-13.38**	-0.64*	0.0118**	0.021*	0.88*	67.79
	SE	0.103586	5.742386	4.077377	0.007392	0.011275	0.546695	332.0768
Testers	AS 204	0.07	-2.75**	3.33	-0.0126	-0.010	0.28	297.91
	AS 244	0.03*	17.25*	6.06	0.0012*	0.004	0.33	480.28
	AS 391	0.30**	4.38	7.51	-0.0047	-0.001	0.41	594.86
	SS 27	0.15	12.19	7.03	-0.0094	-0.014	-0.50*	385.13
	SE	0.073246	4.06048	2.883141	0.005227	0.007973	0.386571	234.8138

SD = stem diameter, PH = plant height, BM = biomass, FC = fibre content, DM = dry matter, SB = stalk brix, EP = ethanol productivity, SE = standard error, * = significant (t- statistic ≥ 2), **= highly significant (t- statistic > 3)

5.3.4 Specific combining ability effects

Table 5.6 presents the SCA effects of each sweet stem sorghum hybrid developed in the study. Each trait had a few hybrids with significant SCA effects. The only highly significant SCA effect, although negative, was for stem diameter when AS105 was crossed with AS391. All of the five significant SCA effects for stem diameter were in an undesirable direction. The same result was observed for plant height, biomass and stalk brix. Hybrid AS246 x AS391 exhibited a significant positive SCA effect of 1620 l ha^{-1}.

Table 5.6: Specific combining ability (SCA) effects for the sweet stem sorghum hybrids for ethanol productivity and associated traits

Hybrid	SD	PH	BM	FC	DM	SB	EP
AS105 x AS204	0.20	-1.6	1.4	-0.007	-0.008	-0.08	-133.3
AS111 x AS204	-0.13	-5.9	-4.8	0.011	0.011	-0.02	-254.6
AS113 x AS204	-0.21	-12.0	-6.1	0.019	0.006	-1.32	-662.1
AS246 x AS204	-0.32*	-0.4	-14.4*	-0.013	-0.012	0.13	-1393.5*
AS251 x AS204	0.12	-12.4	-11.3	0.026*	0.036*	1.02	-691.9
AS253 x AS204	-0.48*	-19.1*	-9.7	-0.004	0.004	0.72	-22.4
AS74 x AS204	-0.22	-6.8	-1.7	0.016	-0.004	-2.00*	-267.6
AS79 x AS204	-0.02	-4.0	-1.3	0.003	0.009	0.51	-91.0
AS105 x AS244	-0.06	-11.6	-5.8	-0.006	0.010	1.62	-351.4
AS111 x AS244	0.05	-5.9	-12.9*	0.004	-0.001	-0.52	-858.9
AS113 x AS244	-0.07	-17.0	-5.7	-0.009	-0.012	-0.32	-522.2
AS246 x AS244	-0.23	-2.9	-4.5	0.021	0.028	0.73	-690.4
AS251 x AS244	-0.25	-9.4	-16.8*	0.030*	0.024	-0.53	-1248.7*
AS253 x AS244	-0.15	5.9	7.2	0.006	-0.011	-1.73*	739.1
AS74 x AS244	-0.13	-18.8*	-9.7	0.016	0.022	0.65	-520.6
AS79 x AS244	-0.27	-2.5	0.4	-0.011	-0.021	-0.94	-63.2
AS105 x AS391	-0.52**	-3.8	-9.7	0.027*	0.016	-1.11	-979.4
AS111 x AS391	-0.41*	-8.5	-12.0	0.010	0.004	-0.60	-773.7
AS113 x AS391	-0.08	3.4	-7.0	0.013	0.023	1.00	-479.5
AS246 x AS391	0.20	-20.0*	-1.9	0.012	0.017	0.50	1619.4*
AS251 x AS391	-0.11	-21.0*	9.9	-0.016	-0.011	0.44	-240.9
AS253 x AS391	-0.01	-11.3	-9.1	0.010	0.001	-0.91	-1363.8*
AS74 x AS391	0.05	-1.4	-10.9	-0.015	-0.017	-0.24	-682.8
AS79 x AS391	-0.23	0.4	-7.1	0.010	0.009	-0.12	-615.6
AS105 x SS27	-0.18	-14.1	-9.8	0.012	0.002	-0.95	-294.1
AS111 x SS27	-0.06	-10.8	5.7	-0.0004	0.006	0.61	129.1
AS113 x SS27	-0.19	-5.4	-5.1	0.003	0.004	0.11	-94.4
AS246 x SS27	-0.20	-7.8	-3.1	0.007	-0.012	-1.89*	-1293.6*
AS251 x SS27	-0.31*	11.7	-5.8	-0.014	-0.029	-1.45	423.4
AS253 x SS27	0.09	-6.6	-12.2	0.013	0.027	1.40	-1111.1*
AS74 x SS27	-0.25	-4.2	-1.6	0.009	0.019	1.08	-287.2
AS79 x SS27	0.01	-24.9*	-16.0*	0.023*	0.023	0.04	-988.4
SE	0.207171	11.48477	8.154753	0.014783	0.02255	1.093389	664.1536

SD = stem diameter, PH = plant height, BM = biomass, FC = fibre content, DM = dry matter, SB = stalk brix, EP = ethanol productivity, SE = standard error, * = significant (t- statistic ≥ 2), **= highly significant (t- statistic > 3)

5.3.5 GCA and SCA variance estimates

Table 5.7 shows that the variance due to GCA (lines) was higher than SCA variance for all traits studied. On the other hand, the variance due to GCA (testers) was lower than SCA variance for all traits with the exception of plant height. The variance ratio of general to specific effects (δ^2 GCA / δ^2 SCA) is above one for all traits. Plant height showed the highest δ^2 GCA / δ^2 SCA ratio compared to other characters. In this study all the traits except fibre content and dry matter had high heritability values (>0.5).

Table 5.7: Estimates of genetic components for the measured traits in sweet stem sorghum

Variance components	SD	PH	BM	FC	DM	SB	EP
$\delta^2 GCA_{line}$	0.063993	335.3368	151.9244	0.000189	0.000255	1.186071	647220.9
$\delta^2 GCA_{tester}$	0.013525	77.19368	3.486027	3.57E-05	7.34E-05	0.180026	16267.25
$\delta^2 SCA_{line\ x\ tester}$	0.019178	40.29615	33.15803	0.000124	0.000228	0.681521	169826.8
δ^2 GCA / δ^2 SCA ratio	4.042021	10.23746	4.686964	1.803933	1.436172	2.004482	3.906851
Error mean squares	0.1022	237.7	132.9	0.000474	0.001119	2.442	772082
h^2	0.660245	0.671522	0.680606	0.411048	0.076782	0.534925	0.670486

SD = stem diameter, PH = plant height, BM = biomass, FC = fibre content, DM = dry matter, SB = stalk brix, EP = ethanol productivity, variance components for general (δ^2 GCA) and specific (δ^2 SCA) genetic effects, h^2 = narrow sense heritability

Table 5.8 shows that for all traits the order of contribution was lines > lines x testers > testers except for plant height, where the contribution of testers was greater than that of lines x testers. The contribution by lines was greater than 50% for all traits, with that of biomass being more than 80%. For biomass, stalk brix and ethanol productivity, the contribution of testers was less than 10%.

Table 5.8: Contribution of lines, testers, and lines x testers to the total variance for seven traits in sweet sorghum

Trait	Contribution (%)		
	Lines	Testers	Lines x Testers
Stem diameter	66.18	13.99	19.83
Plant height	74.05	17.05	8.90
Biomass	80.57	1.85	17.58
Fibre content	54.10	10.24	35.66
Dry matter	45.76	13.20	41.04
Stalk brix	57.92	8.80	33.28
Ethanol productivity	77.67	1.95	20.38

5.3.6 Heterosis

Only five traits had significant differences for better-parent heterosis values as presented in Table 5.9. Of the 18 hybrids that had positive better-parent heterosis for stem diameter, 11 had values above 10%. The highest was 52% for cross AS253 x SS27. Plant height had nine hybrids exhibiting positive better-parent heterosis, with five of those being above 10%, the highest being 21% by AS253 x AS244. Biomass and stalk brix exhibited comparable highest better-parent heterosis values of 23% and 21%, although they had 4 and 2 hybrids above 10%, respectively. Ethanol productivity had only four hybrids with positive better-parent heterosis, and all of them were 30% and above, the highest being AS246 x AS391 at almost 50%. Hybrid AS251 x AS204 had positive better-parent heterosis for all five traits. Hybrid AS246 x SS27 was in the bottom five for three of the traits.

All hybrids had positive mid-parent heterosis for stem diameter, with the exception of hybrids AS74 x SS27, AS111 x AS391 and AS105 x AS391. The highest mid-parent heterosis values for stem diameter, plant height, biomass, stalk brix and ethanol productivity were 80 %, 24 %, 50 %, 28 % and 61 %, respectively. Hybrids AS251 x AS204, AS253 x AS204, AS79 x AS244 and AS111 x AS204 had positive mid-parent heterosis for all five traits (Table 5.10).

Table 5.9: Sweet stem sorghum hybrids exhibiting positive better-parent heterosis for five ethanol productivity related traits

Hybrid	SD	Hybrid	PH	Hybrid	BM	Hybrid	SB	Hybrid	EP
AS253 x SS27	52%	AS253 x AS244	21%	AS74 x AS204	23%	AS251 x AS204	20%	AS246 x AS391	48%
AS253 x AS391	50%	AS251 x SS27	17%	AS251 x AS204	17%	AS79 x AS204	17%	AS251 x AS204	40%
AS251 x AS204	38%	AS253 x SS27	12%	AS79 x AS204	13%	AS79 x AS391	9%	AS79 x AS204	30%
AS105 x AS204	36%	AS251 x AS244	11%	AS251 x AS391	11%	AS253 x AS204	2%	AS74 x AS204	29%
AS253 x AS244	33%	AS246 x AS244	10%	AS105 x AS204	3%	AS253 x SS27	2%		
AS79 x AS204	31%	AS111 x AS244	6%	AS253 x AS244	1%				
AS79 x SS27	19%	AS246 x SS27	3%						
AS105 x AS244	14%	AS251 x AS204	2%						
AS253 x AS204	13%	AS111 x AS204	2%						
AS246 x AS391	13%								
AS251 x AS391	13%								
AS79 x AS391	9%								
AS113 x AS391	6%								
AS74 x AS391	6%								
AS113 x AS204	4%								
AS79 x AS244	4%								
AS113 x AS244	4%								
AS105 x SS27	3%								
Bottom Five									
AS105 x AS391	-13%	AS113 x AS244	-16%	AS111 x AS391	-62%	AS105 x AS391	-19%	AS113 x AS204	-64%
AS246 x AS204	-19%	AS105 x AS391	-18%	AS74 x AS391	-63%	AS113 x AS204	-23%	AS246 x SS27	-67%
AS246 x SS27	-19%	AS113 x AS391	-19%	AS113 x AS391	-65%	AS105 x SS27	-24%	AS113 x SS27	-68%
AS74 x SS27	-19%	AS79 x AS391	-20%	AS113 x SS27	-69%	AS251 x SS27	-33%	AS74 x SS27	-72%
AS246 x AS244	-21%	AS79 x SS27	-26%	AS79 x SS27	-78%	AS246 x SS27	-37%	AS79 x SS27	-79%

SD = stem diameter, PH = plant height, BM = biomass, FC = fibre content, DM = dry matter, SB = stalk brix, EP = ethanol productivity

Table 5.10: Sweet stem sorghum hybrids exhibiting positive mid-parent heterosis for five ethanol productivity related traits

Hybrid	SD	Hybrid	PH	Hybrid	BM	Hybrid	SB	Hybrid	EP
AS246 x AS391	80%	AS253 x AS244	24%	AS74 x AS204	50%	AS79 x AS204	28%	AS251 x AS204	61%
AS253 x AS391	55%	AS251 x SS27	21%	AS251 x AS391	50%	AS251 x AS204	24%	AS246 x AS391	57%
AS253 x SS27	54%	AS251 x AS244	14%	AS253 x AS244	41%	AS253 x AS204	23%	AS79 x AS204	53%
AS251 x AS204	44%	AS253 x SS27	12%	AS251 x AS204	32%	AS79 x AS391	11%	AS74 x AS204	41%
AS105 x AS204	41%	AS246 x AS244	11%	AS105 x AS204	27%	AS111 x AS204	8%	AS253 x AS244	28%
AS253 x AS244	38%	AS111 x AS244	10%	AS79 x AS204	22%	AS251 x AS391	7%	AS105 x AS204	23%
AS79 x AS204	33%	AS246 x AS204	8%	AS253 x AS204	14%	AS246 x AS204	6%	AS253 x AS204	20%
AS79 x SS27	32%	AS251 x AS204	7%	AS79 x AS244	12%	AS105 x AS244	4%	AS251 x AS391	16%
AS251 x AS391	29%	AS79 x AS244	5%	AS111 x AS204	4%	AS253 x SS27	3%	AS79 x AS244	16%
AS113 x AS391	28%	AS111 x AS204	5%			AS105 x AS204	3%	AS111 x AS204	10%
AS246 x SS27	28%	AS253 x AS204	5%			AS79 x AS244	2%	AS251 x SS27	3%
AS74 x AS391	26%	AS246 x SS27	4%			AS253 x AS391	2%		
AS246 x AS204	24%	AS74 x SS27	3%			AS74 x AS244	2%		
AS79 x AS391	23%	AS111 x SS27	2%			AS113 x AS391	1%		
AS246 x AS244	22%	AS253 x AS391	2%						
AS253 x AS204	21%	AS74 x AS204	1%						
AS113 x AS244	18%								
AS113 x AS204	15%								
AS105 x AS244	14%								
AS111 x AS244	13%								
AS113 x SS27	12%								
AS79 x AS244	9%								
AS105 x SS27	8%								
AS251 x AS244	8%								
AS111 x SS27	7%								
AS111 x AS204	6%								
AS251 x SS27	5%								

Table 5.10 continued

Bottom Five									
AS74 x AS204	0%	AS105 x SS27	-7%	AS105 x SS27	-48%	AS105 x AS391	-12%	AS74 x SS27	-51%
AS74 x AS244	0%	AS105 x AS391	-8%	AS111 x AS391	-49%	AS113 x SS27	-17%	AS113 x AS391	-52%
AS74 x SS27	-6%	AS113 x AS204	-10%	AS113 x AS391	-57%	AS251 x SS27	-23%	AS113 x SS27	-57%
AS111 x AS391	-6%	AS246 x AS391	-10%	AS113 x SS27	-59%	AS105 x SS27	-23%	AS246 x SS27	-59%
AS105 x AS391	-7%	AS79 x SS27	-15%	AS79 x SS27	-67%	AS246 x SS27	-32%	AS79 x SS27	-68%

SD = stem diameter, PH = plant height, BM = biomass, FC = fibre content, DM = dry matter, SB = stalk brix, EP = ethanol productivity

Table 5.11: Pearson's correlations for the observed traits for experimental sweet stem sorghum genotypes for hybrids (above diagonal) and parents and hybrids (below diagonal)

Trait	SD	PH	BM	FC	DM	SB	EP
SD	-	0.1867	0.4385**	-0.1227	0.1337	0.3912*	0.5241**
PH	0.1809	-	0.6950**	-0.2482*	-0.1817	-0.0241	0.6353**
BM	0.2488*	0.6260**	-	-0.2904*	-0.1518	0.0812	0.9619**
FC	-0.0629	-0.2398*	-0.3226*	-	0.8497**	0.3097*	-0.2196
DM	0.1365	-0.1722	-0.0978	0.8075**	-	0.7645**	0.0359
SB	0.3000*	-0.0093	0.2103*	0.1879	0.731*	-	0.3333*
EP	0.2883*	0.5425**	0.966**	-0.2659*	0.0726	0.4284**	-

SD = stem diameter, PH = plant height, BM = biomass, FC = fibre content, DM = dry matter, SB = stalk brix, EP = ethanol productivity. **, * significant at $p \leq 0.001$ and $p \leq 0.05$, respectively

5.3.7 Associations among observed traits

Table 5.11 is a presentation of Pearson's correlations for the observed traits for experimental sweet stem sorghum hybrids, parents and hybrids. In terms of the significance of correlation coefficients, there was not much difference between the analyses involving the two sets. For both the hybrid only and the hybrid and parents analyses, stem diameter was significantly correlated (P<0.001) with biomass (r=0.4385 and 0.2488, respectively), stalk brix (r=0.3912 and 0.3, respectively) and ethanol productivity (r=0.5241 and 0.2883, respectively). For both the hybrid only, and the hybrid and parents analyses, plant height was significantly (P<0.001) correlated to biomass (r=0.695 and 0.626, respectively), fibre content (r=-0.2482 and -0.2398, respectively) and ethanol productivity (r=0.6353 and 0.5425, respectively). For both the hybrid only and hybrid and parents analyses, biomass was also significantly correlated to fibre content (r= -0.2904 and -0.3226, respectively) and ethanol productivity (r= 0.9619 and 0.966, respectively). Biomass was not significantly correlated to stalk brix when considering crosses alone. Fibre content was significantly correlated to dry matter and ethanol productivity. For hybrids alone, fibre content was also significantly correlated to stalk brix (r=0.3097). Stalk brix was significantly correlated to dry matter (r=0.7645 and 0.731 for hybrids only and hybrid plus parent analyses, respectively) and ethanol productivity (r=0.3333 and 0.4284 for hybrids only and hybrid plus parent analyses, respectively). On the other hand, considering the strength of the correlations, the hybrid only analysis was notably different from the analysis that included parental genotypes. For the analysis excluding parents, there were strong (>0.5) correlations between biomass and plant height; dry matter with biomass, fibre content and stalk brix; ethanol productivity with stem diameter; plant height and biomass. For the hybrid plus parents analysis, the strong correlations were that of ethanol productivity with plant height and biomass; dry matter with stalk brix and fibre content; plant height and biomass.

5.4 Discussion

5.4.1 Mean performance and heterosis

Overall, the sweet stem sorghum genotypes tested, including the parents, performed slightly lower than expected for all traits. For example, the results were slightly lower in comparison to the morphological characterisation trial of the parental lines that was carried out in the summer season of 2015/2016 (Mangena *et al.,* 2017). The authors observed highest stem diameter, plant height, biomass, stalk brix and ethanol productivity of 31 mm, 420 cm, 111 t ha^{-1}, 18.9% and 5500 l ha^{-1}, respectively, compared to 24 mm, 215 cm, 68 t ha^{-1}, 16.4% and 5470 l ha^{-1}, respectively, observed in the current study. This can be attributed to the fact

that the trial was conducted off-season with weather conditions that do not encourage sweet stem sorghum to exhibit maximum growth potential. The genotypes were mostly affected by the low temperatures experienced from August 2017 to November 2017 with some days getting a mean minimum temperature as low as 3°C. However, the observed productivity was still more than that recorded in other studies. For example, Tsuchihashi and Goto (2004) and Umakanth *et al.* (2012) recorded their highest biomass of 39 and 54 t ha^{-1}, respectively, implying that heterosis breeding could be exploited to increase the biomass yields, owing to the importance of non-additive gene action in determining this character. On the other hand, for all traits, the majority of the best performing genotypes were hybrids (Table 5.4). This can be attributed to heterosis, reflecting the assumption that that sorghum hybrids are usually superior to most parental genotypes (Makanda, 2009). Similar conclusions were made by Corn (2008). For the parental genotypes that were in the top ten for the different traits, Makanda (2009) associated this phenomenon with the idea that the best parents still have a role to play in the hybrid breeding to be conducted in each region.

To increase ethanol productivity in sweet stem sorghum it is imperative to exploit heterosis. Quantitative genetic theory states that heterosis is a function of increasing genetic diversity among the parents (Falconer, 1989). Significant and positive heterosis is an indication of the presence of non-additive gene action (dominance and epistasis). One way to increase ethanol production derived from sweet stem sorghum is to manipulate the crosses with positive heterosis for ethanol productivity in future breeding programs (Tariq *et al.,* 2014). Six and twelve hybrids had positive better-parent and mid-parent heterosis for stalk brix, , respectively (Table 5.9 and Table 5.10). Although the heterosis for these hybrids is positive, the magnitudes were low. The same observation was also made by Bunphan *et al.* (2015), who reported that of 12 hybrids tested, only one showed a positive heterosis of 3.6%. They concluded that it was unclear why positive heterosis for stalk brix is relatively rare in hybrid sweet stem sorghum. Likewise, in a study conducted by Umakanth *et al.* (2012)on 16 hybrids, only one hybrid showed a positive mid-parent heterosis of 9%. Other researchers have identified quantitative trait loci (QTL) for stalk brix or sugar concentration, although they were predominantly of small effects (Murray *et al.,* 2008; Murray *et al.,* 2009; Ritter *et al.,* 2008). On the other hand, Yun-long *et al*. (2006) identified a QTL that explained up to 25% of the phenotypic variance in sugar concentration, also documenting an overdominance effect and insinuating that heterosis in hybrids should be anticipated. Tariq *et al.* (2014) also reported the highest better-parent heterosis of 35% with the second highest having 34% better-parent heterosis.

5.4.2 Combining ability

If line or tester GCA effects are significant, then it is an indication of either the line or tester could produce valuable hybrids for breeding programs for the specific traits. The existence of such genetic variation can be exploited for the production of superior ethanol producing sweet stem sorghum hybrids. The fact that AS246 and AS253 were the only parental genotypes with significant GCA effects that were positive for ethanol production implies that future ethanol production breeding programs could include AS246 and AS253 because they will positively influence their hybrids for ethanol production. Moreover, crossing two parents showing the highest general combining ability for a desirable trait may produce the best performing cross due to an increased frequency of favourable genes. On the other hand, the non-significant ethanol production GCA effects for all the testers indicated that better ethanol producing F1 hybrids could have been developed if different testers had been used in the study. On the other hand, Testers AS244 and AS391 had positive significant GCA effects for stem diameter and plant height (Table 5.5). These testers can be included in future ethanol breeding programs because stem diameter and plant height influence ethanol productivity (Mangena *et al*., 2017). For its positive significant GCA effects for almost all the traits, Line AS253 should be used in future sweet stem sorghum breeding programs. It is therefore, essential to develop hybrids involving Line AS253 and the better combining testers, AS244 and AS391, or to utilise them in recurrent selection programmes. Small numbers of parents with favourable GCA effects can be used to generate hybrids for sweet stem sorghum improvement because additive variance is associated with effective response to selection (Valiolla, 2012; Chikuta *et al.,* 2017). However, significance contributions of both lines and testers for stem diameter, plant height, biomass, stalk brix and ethanol production (Table 5.2) implied that genes with additive effects were important for the traits, and therefore, that breeding progress could be achieved through selection of good parents (Makanda *et al*., 2010). Similarly, Makanda *et al*., (2010) reported significant male and female parent GCA effects for stem diameter, plant height, biomass and stalk brix. Chikuta *et al.* (2017) reported significant GCA effects for both male and female genotypes for plant height and biomass.

The significance of SCA effects for biomass, stalk brix and ethanol productivity, suggested that further gains can be achieved through hybridisation, capitalising on non-additive gene effects (Makanda *et al*., 2010). Knowledge of the SCA of inbred lines is essential in hybrid-oriented programs because it accounts for good combinations of inbred lines, which can produce superior hybrids. Genotype AS253 had the best GCA effects for the majority of the traits, and it did not have significant SCA effects for most traits in most of its crosses. This is in agreement with Mwije *et al.* (2014) who reported that parents with the best GCA effects did not automatically develop hybrids with desirable SCA effects.

For stem diameter, plant height, biomass and ethanol productivity, both additive and non-additive gene actions are important, with the preponderance of additive gene actions. Moreover, the magnitude of GCA/SCA variance ratio was high (< 4) for stem diameter, plant height, biomass and ethanol productivity (Table 5.7), indicating the relative importance of additive gene action in these traits (Gupta *et al.* 1976). The high heritability estimates observed in most traits implies that they could be influenced by additive gene action (Insan *et al.*, 2016).

5.4.3 Associations among observed traits

Ethanol productivity had significant and strong correlations with plant height and biomass. Similar results were reported by Prasad *et al.* (2013) and Mangena *et al.* (2017). Ethanol productivity was also strongly correlated to stem diameter agreeing to the results of Ganesh *et al.* (1995).

5.5 Conclusions

Both additive and non-additive gene effects were shown to be important in controlling biomass, stem brix, and ethanol production in sweet stem sorghum. Due to its consistent, significant and positive GCA effects across majority of the traits, the line AS253 is recommended for utilization in sweet stem sorghum hybrid programs. For ethanol production improvement centred programs, genotypes AS253 and AS246 can be recommended because they both exhibited significant GCA effects for six out of seven traits. Hybrid AS246 x AS391 should also be further tested because it was the only one with a significant positive SCA effect.

References

Alam, M.F., Khan, M.R., Nuruzzaman, M., Parvez, S., Swaraz, A.M. Alam, I. and Ahsan, N. 2004. Genetic basis of heterosis and inbreeding depression in rice (*Oryza sativa* L.). *Journal of Zhejiang University-Science* 5: 406-411.

Bernardo, R. 2014. Essentials of Plant Breeding. Stemma Press, Woodbury, Minnesota, USA.

Bunphan, D., Jaisil, P., Sanitchon, J., Knoll, J.E. and Anderson, W.F. 2015. Heterosis and combining ability of F1 hybrid sweet sorghum in Thailand. *Crop Science* 55:178–187.

Chikuta, S., Odong, T., Kabi, F. and Rubaihayo, P. 2017. Combining ability and heterosis of selected grain and forage dual purpose sorghum genotypes. *Journal of Agricultural Science* 9: 122-131.

Corn, R. 2008. Sweet sorghum heterosis. Joint Annual Meeting., Houston, Texas, George R. Brown Convention Centre. http://a- cs.confex.com/crops/2008am/webprogram/Paper42644.html Accessed 11 March 2018.

Dabholkar, R.R. 1992. Elements of Biometrical Genetics. Ashok Kumar Mittal Concept Publishing Company, New Delhi, India.

Dehinwal, A.K., Pahuja, S.K., Shafiqurrahaman, M., Kumar, A. and Sharma. P. 2017. Studies on combining ability for yield and its component traits in forage sorghum. *International Journal of Pure and Applied Bioscience* 5: 493-502.

Falconer, S.P. 1989. Introduction to Quantitative Genetics. The Ronald Press Company, New York, USA.

Ganesh, S., Fazlullah, A.K., Suresh, M. and Senthil, N. 1995. Character associated for ethanol yield in sweet sorghum. *Madras Agricultural Journal* 82: 361-363.

Girma, M., Ayana, A. and Belete, K. 2010. Combining ability of yield and its components in Ethiopian sorghum landraces. *East African Journal of Sciences* 4: 34-40.

Gupta, S.C., Paliwal, R.L. and Nanda, J.S. 1976. Combining ability for forage yield and quality characters in sorghum. (*Sorghum bicolor* L. Moench). *Egyptian Journal of Genetics and Cytology* 5: 89-97.

Hallauer, A. and Miranda, J. 1981. Quantitative Genetics in Maize Breeding. Iowa State University Press, Iowa City, USA.

Hlophe, H.B. 2014. Sweet sorghum (*Sorghum bicolor* (L.) Moench) response to supplemental irrigation in different growth stages. MSc thesis. University of Pretoria, Pretoria, RSA.

Insan, R.R., Wirnas, D. and Trikoesoemaningtyas. 2016. Estimation of genetic parameters and selection of sorghum (*Sorghum bicolor* (L.) Moench) RILS F5 derived from single seed descent. *International Journal of Agronomy and Agricultural Research* 8: 95-103.

Jain, S.K. and Patel, P.R. 2014. Combining ability and heterosis for grain yield, fodder yield and other agronomic traits in sorghum (*Sorghum bicolor* (L) Moench). *Journal of Plant Breeding* 5: 152-157.

Kearsey, M.J. and Pooni, H.S. 1996. The Genetical Analysis of Quantitative Traits. Chapman and Hall, London, UK.

Kempthorne, O. 1957. An Introduction to Genetic Statistics. John Wiley and Sons, New York, USA.

Kenga, R., Alabi, S.O. and Gupta, S.C. 2004. Combining ability studies in tropical sorghum [*Sorghum bicolor* (L.) Moench]. *Field Crops Research* 88: 251–260.

Makanda, I. 2009. Combining ability and heterosis for stem sugar traits and grain yield components in dual-purpose sorghum (*Sorghum bicolor* L. Moench) germplasm. PhD thesis, University of KwaZulu-Natal, Pietermaritzburg, RSA.

Makanda, I., Tongoona, P., Derera, J., Sibiya, J. and Fato, P. 2010. Combining ability and cultivar superiority of sorghum germplasm for grain yield across tropical low and mid-altitude environments. *Field Crops Research* 116: 75-85.

Mangena, P. 2014. Breeding gains, cytoplasmic effects and path coefficient analysis in pro-vitamin A maize hybrids. MSc thesis, University of KwaZulu-Natal, Pietermaritzburg, RSA.

Mangena, P., Shimelis, H. and Laing, M. 2017. Characterisation of sweet stem sorghum genotypes for bio-ethanol production. *Acta Agriculturae Scandinavica, Section B — Soil and Plant Science* 68:323-333.

Murray, S.C., Rooney, W.L., Hamblin, M.T., Mitchell, S.E. and Kresovich, S. 2009. Sweet sorghum genetic diversity and association mapping for brix and height. *Plant Genetics* 2: 48–62.

Murray, S.C., Sharma, A., Rooney, W.L., Klein, P.E., Mullet, J.E., Mitchell, S.E. and Kresovich, S. 2008. Genetic improvement of sorghum as a biofuel feedstock: I. QTL for stem sugar and grain non-structural carbohydrates. *Crop Science* 48: 2165–2179.

Mutengwa, C.S., Tongoona, P., Mabasa, S. and Chivinge, O.A. 1999. Resistance to *Striga asiatica* (L.) Kuntze in sorghum: Parent characterization and combining ability analysis. *African Crop Science Journal* 7: 321–326.

Mwije, A., Mukasa, S.B., Gibson, P. and Kyamanya, S. 2014. Heritability analysis in putative drought adaptation traits in sweet potato. *African Crop Science Journal* 22: 79-87.

Nadarajan, N. and Gunasegaram, M. 2005. Quantitative Genetics and Biometrical Techniques in Plant Breeding. Kalyani Publishers, New Delhi, India.

Prasad, S., Kumar, A. and Muralikrishna, K.S. 2013. Assessment of ethanol yield associated characters in sweet sorghum. *Maydica* 58: 299-304.

Ritter, K.B., Jordan, D.R., Chapman, S.C., Godwin, I.D., Mace, E.S. and McIntyre, C.L. 2008. Identification of QTL for sugar-related traits in a sweet × grain sorghum [*Sorghum bicolor* (L.) Moench] recombinant inbred population. *Molecular Breeding* 22: 367–384.

Robinson, H., Comstock, R.E. and Harvey, P. 1949. Estimates of heritability and the degree of dominance in corn. *Agronomy Journal* 41: 353-359.

Sprague, G.P. and Tatum, L.S. 1942. General vs specific combining ability in six crosses of corn. *Journal of American Society of Agronomy* 34: 923- 932.

Tariq, A.S., Akram, Z., Shabbir, G., Khan, K.S., Mahmood, T. and Iqbal, M.S. 2014. Heterosis and combining ability evaluation for quality traits in forage sorghum (*Sorghum bicolor* L.). *SABRAO Journal of Breeding and Genetics* 46: 174-182.

Tsuchihashi, N. and Y. Goto. 2004. Cultivation of sweet sorghum (*Sorghum bicolor* (L.) Moench) and determination of its harvest time to make use as the raw material for fermentation, practiced during rainy season in dry land Indonesia. *Plant Production Science* 7: 442–448.

Umakanth, A.V., Patil, J.V., Rani, C.H., Gadakh, S.R., Kumar, S.S., Rao, S.S. and Kotasthane, T.V. 2012. Combining ability and heterosis over environments for stalk and sugar

related traits in sweet sorghum (*Sorghum bicolor* (L.) Moench.). *Sugar Technology* 14: 237–246.

Valiolla, R. 2012. Combining ability analysis of plant height and yield components in spring type of rapeseed varieties (*Brassica napus* L.) using line × tester analysis. *International Journal of Agriculture and Forestry* 2: 58-62.

Yun-long, B., Seiji, Y., Maiko, I. and Hong-wei, C. 2006. QTLs for sugar content of stalk in sweet sorghum [*Sorghum bicolor* (L.) Moench]. *Agricultural Science China* 5: 736–744.

Thesis overview

This chapter summarizes the major findings from the completed research. It gives a general overview of the conclusions deduced and the way forward. Recommendations on what future studies should focus on are also outlined in this chapter.

To recap, the objectives of this study were to:

i) investigate the phenotypic variability present among diverse sorghum genotypes based on ethanol production and related agronomic traits;
ii) evaluate the genetic interrelationships among phenotypically selected sweet stem sorghum genotypes using polymorphic simple sequence repeat (SSR) markers;
iii) investigate the concentration, stage of application and frequency of application of ethyl 4'fluorooxanilate (E_4FO) for inducing male sterility of sweet stem sorghum without affecting female fertility;
iv) investigate the combining ability between the selected sweet stem sorghum testers and lines; and
v) assess heterosis in sweet stem sorghum hybrids for bio-ethanol production and related traits.

Major findings

i) Characterization of sweet stem sorghum genotypes for bio-ethanol production

One hundred and ninety sweet stem sorghum genotypes were phenotypically evaluated. Agronomic and ethanol yield related traits were collected. Data were subjected to analysis of variance, cluster analysis, correlation analysis, path coefficient analysis and principal component analysis.

The main findings of the study were:

- The study demonstrated that there was high phenotypic diversity among tested genotypes for all measured traits;
- At least 20 experimental genotypes out-performed the checks for all observed traits;
- Ethanol productivity ranged from 240.9 to 5500 l ha^{-1} with a mean of 1886 l ha^{-1}. The best genotypes for ethanol productivity were AS203, AS391, AS205, AS251 and AS448 providing mean yields of 5474 l ha^{-1}, 4509 l ha^{-1}, 4315 l ha^{-1}, 4205 l ha^{-1} and 3816 l ha^{-1}, in that order;

- Days to flowering, plant height, stalk brix and stem diameter exerted the greatest indirect effects on ethanol production through higher biomass production;
- Biomass yield had the greatest direct effect on ethanol production; and
- All traits had high heritability values.

ii) Genetic interrelationship of sweet stem sorghum genotypes assessed through simple sequence repeat markers

Eighteen phenotypically divergent sweet stem sorghum genotypes were evaluated using 25 polymorphic simple sequence repeat (SSR) markers. Genetic diversity parameters such as total number of alleles per locus (N_a), number of effective alleles per locus (N_e), Shannon's information index (I), observed heterozygosity (H_o), gene diversity (H_e), number of putative alleles (P_a), percent polymorphism (%P), and inbreeding coefficient (F_{IS}) were determined. Other parameters such as differentiation and polymorphic information content (PIC) were estimated. The binary data were used to obtain a dissimilarity matrix using the Jaccard's index. Based on Jaccard's distances, analysis of molecular variance (AMOVA) was conducted.

The core findings of the study were:

- Genetic differentiation was observed among the studied sweet stem sorghum genotypes;
- The polymorphic information content (PIC) values for all markers ranged from 0.00 to 0.85, with a mean value of 0.56, implying that the markers were highly informative and discriminatory; and
- Genetically unique sweet stem sorghum genotypes such as AS391, SS27, AS204 and AS244 were identified in the study.

iii) Preliminary investigation of the effect of ethyl 4'fluorooxanilate as a male gametocide of sweet stem sorghum

Two experiments were conducted to investigate the concentration, stage of application and frequency of application of ethyl 4'fluorooxanilate (E_4FO) for inducing male sterility of sweet stem sorghum without affecting female fertility. Three sweet stem sorghum genotypes were tested at three application stages and five E_4FO dose rates during the first experiment. In the second experiment the frequency of application of E_4FO was determined using three sweet stem sorghum genotypes, three E_4FO doses, and six frequencies of application. Data on male sterility was inferred based on seed set and seed count from the treated plants. To determine female fertility, controlled crosses were performed, seed set was assessed and the number of seeds on cross pollinated plants

were counted. Male sterility and female fertility were assessed against comparative control treatments.

The main findings of the study were:

- Male sterility was achieved when E_4FO was applied during heading stage using the following rates: 1000 mg l^{-1}, 1500 mg $l^{-1}$and 2000 mg l^{-1}, with more than one application; and
- Applying E_4FO twice during the heading stage at a rate of 2000 mg l^{-1} would induce male sterility in the tested sweet stem sorghum genotypes.

iv) Combining ability and heterosis of sweet stem sorghum genotypes for bioethanol yield and related traits

Eight selected sweet stem sorghum lines and four testers were crossed using a line x tester mating design. The F1 hybrids and parental lines were evaluated for bioethanol yield and related traits. Data were subjected to analysis of variance, combining ability and heterosis analyses.

The main findings of the study were:

- Tested sweet stem sorghum genotypes showed high phenotypic variation;
- Ethanol yields varied from 787 l ha^{-1} to 5470 l ha^{-1} with a mean of 2055 l ha^{-1};
- Hybrids AS246 x AS391, AS251 x AS204, AS79 x AS204, AS74 x AS204 expressed greater ethanol productivity with positive better-parent heterosis (>30 %);
- Lines AS253, AS246, AS 105 and testers AS391 and SS27 had highly positive general combining ability (GCA) effects for almost all the traits in a desirable direction; and
- Among the studied hybrids or test parents ethanol productivity had significant and positive correlations with plant height, stem diameter and biomass.

Implications of the research findings for breeding sweet stem sorghum for bio-ethanol production

The following implications and future directions were identified:

i) The substantial amount of variation among the genotypes implicates that they can be used in breeding programs aiming for different better attributes in bioethanol production;

ii) Multiyear data are essential to establish stability and the Genotype × Environment Interaction patterns that are repeatable across years;

iii) Due to their consistent, significant and positive GCA effects across majority of the traits, lines AS253, AS246, AS 105 and testers AS391 and SS27 with high GCA effects are ideal parents for subsequent crosses with commercial varieties using the gametocide methodology to produce high bioethanol yielding varieties; and

iv) It is essential to standardise crossing genotypes using the gametocide methodology in a commercial capacity considering the different flowering dates and plant height that can be exhibited by different sweet stem sorghum genotypes.

Conclusively, the study established the existence of considerable genetic diversity among sweet stem sorghum genotypes morphologically and genotypically. The selected promising parental genotypes and experimental hybrids are recommended for bio-ethanol production and breeding.