NOVEL SOURCES OF THE STAY GREEN TRAIT IN SORGHUM AND ITS INTROGRESSION INTO FARMER PREFERRED VARIETIES FOR IMPROVED DROUGHT TOLERANCE

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PLANT BREEDING AND BIOTECHNOLOGY

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION FACULTY OF AGRICULTURE

THE UNIVERSITY OF NAIROBI

2022

DECLARATION

I declare that this thesis is my original work and has not been presented for any award of degree in any other university.

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DEDICATION

This thesis is dedicated to my parents, Vincent Ochieng Oyieng and Consolater Auma Ochieng, for their prayers, support and confidence in the value of higher education.

ACKNOWLEGEMENTS

Most appreciation to God for life, good health and sufficient grace throughout the period of this research. This research was made possible by the financial support from the National Research Fund (NRF) under the project ―Harnessing the genetic potential of Sorghum for arid and semi-arid areas, and the International Crops Research Institute for Semi-Arid tropics (ICRISAT). Sincere gratitude for the technical support by the Kenya Agricultural and Livestock Research Organization (KALRO) Kiboko Centre and the staff at the ICRISAT genomics laboratory Nairobi. I wish to express my sincere gratitude to Prof. Kahiu Ngugi, my lead supervisor for giving me the opportunity to work under this project and his constructive scholarly criticism and professional help throughout the project. I am grateful to Dr. Damaris Odeny (ICRISAT) for going above and beyond to ensure the laboratory work was excellently done, I appreciate her mentorship and the exposure to the science world that she gave me during my time in ICRISAT. I am also thankful to my supervisors' Dr. Eric Manyasa (ICRISAT) and Dr. Lydia Wamalwa (UoN); I sincerely appreciate your strict valuable criticism and the prompt response you always gave; I appreciate the valuable suggestions and discussions along the completion of this project. Many thanks are also extended to Mr. Patrick Sheunda, Mr. Samuel Manthi and Millicent Avosa (ICRISAT) whose advice and assistance were instrumental. I would also like to acknowledge the technical assistance of Mr. Paul Nzalu and his team at KALRO Kiboko. Many thanks also to the technologist from the Department of Plant Science and Crop Protection Mr. Alexander Shivachi, for sharing his expertise and knowledge in the laboratory.

I would also like to acknowledge my parents Eng. Vincent Ochieng Oyieng and Mrs. Consolater Auma Ochieng, you are my pillars of support, strength and inspiration. I would not have successfully accomplished the project without your financial help and constant encouragement. You have sacrificed a lot to build my capacity and I promise to live to your expectations to my very best.

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
BC_1F_1	Backcross one F_1
CAPS	Cleaved amplification polymorphic sequences
CWR	Crop wild relatives
DArT	Diversity Array Technology
DFL	Days to flowering
GBK	Gene Bank of Kenya
GCV	Genotypic coefficient of variation
GLAM	Number of green leaves at maturity
HSW	Hundred seed weight
ISSR	Inter simple sequence repeats
FLA	Flag leaf area
MABC	Marker Assisted Backcrossing
MAS	Marker Assisted Selection
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variation
PHT	Plant height
PWT	Panicle weight
QTL	Quantitative Trait Loci
RAPD	Random amplified polymorphic DNA
RCC	Relative chlorophyll content
RFLP	Restriction fragment length polymorphism
RP	Recurrent parent
SCAR	Sequence characterized amplification region
SNP	Single nucleotide polymorphism
SPAD	Soil plant analysis development
SSR	Simple sequence repeats markers
STG	Stay Green
YLD	Grain Yield

GENERAL ABSTRACT

Drought is an important abiotic stress in the tropics that highly constrains sorghum production. Sorghum landraces and wild relatives have been known to harbor sources of novel genes but there is hardly any information about their drought tolerance performance during the post flowering period based on the stay green trait. There is need to characterize this stay green expressed drought tolerance and transfer the mapped QTLs into drought susceptible farmer preferred varieties. This research aimed at identifying sorghum genotypes that have the stay green trait through phenotypic and molecular characterization and subsequently, introgress the stay green QTL from mapped donor lines into farmer preferred varieties. This characterization was done phenotypically and also with Diversity array technology (DArT) molecular markers in genotypes grown under well irrigated and induced drought stress conditions. The trials in the field were set in an alpha lattice design of 12*8 replicated three times. The backcross progenies were genotyped using DArT markers. The genotypes and water regimes used had effects on various traits and helped to identify stay green genotypes. Nine genotypes, namely OKABIR, LODOKA, IESV92043 DL, IESV21400 DL, IESV23010 DL, IESV23006 DL, AKUOR-ACHOT, GBK 016109, GBK 048156 outperformed the check varieties, B35 and E36-1 and in their relative chlorophyll content, whereas the genotypes namely, IBUSAR, LODOKA, GBK 047293 AKUOR-ACHOT, OKABIR, F6YQ212, GBK 048917 had more green leaves at maturity than B35 and E36-1 in drought induced conditions. Ten genotypes, namely, AKUOR-ACHOT, LODOKA, GBK 045827, GBK 047293, WAHI IESV23010 DL, IESV23006 DL, IESV92043 DL, GBK 016114, OKABIR that outperformed B35 when ranked using Relative chlorophyll content measurements yielded higher than both B35 and E36-1 which were the check varieties. LODOKA a landrace, recorded the highest chlorophyll content, highest number of green leaves at maturity and a yielded 2.2 tons $ha^{-1.}$ The accessions whose yield was higher than B35 and E36-1 and B35 and also had higher GLAM and RCC values were chosen as novel sources of stay green. The results also indicated the possibility of

finding stay-green alleles from wild genotypes with five wild genotypes, namely, GBK016114, GBK045827, GBK016109, GBK048922, GBK047293 that also clustered separately from B35 and E36-1 in the Neighbor Joining tree. The high significant positive correlation coefficients observed between the relative chlorophyll content and number of green leaves at maturity confirmed that the stay green trait was exhibited as functional stay green. High broad sense heritability estimates of the relative chlorophyll content (0.61) and the number of green leaves at maturity (0.64), indicated the influence **of additive gene effects. T**he narrow sense heritability estimates for the quantity of green leaves at maturity (0.52) and for the relative chlorophyll content (0.45) also indicated the likelihood of a high positive response to selection**.** This study also identified 20 informative SNP markers that were highly polymorphic and were well distributed across the genome. The F_2 genotypes from parental lines, ICSV 111 IN and LODOKA gave high general combining ability (GCA) for relative chlorophyll content and number of green leaves at maturity. Backcrossing for the stay green trait from mapped donor lines into farmer preferred varieties was successful with over 50% of the genotypes having greater than 75% recovery of the genome of the recurrent parent in the first backcross. These genotypes will form a strong basis for selection of superior drought tolerant sorghum varieties and the potential of improving susceptible sorghum genotypes for drought tolerance through marker assisted breeding.

Keywords: Sorghum, drought, stay green, diversity analysis, marker assisted backcrossing

CHAPTER ONE: INTRODUCTION

1.1 Background information

Sorghum (*sorghum bicolor L. Moench)* is a grass that uses the C4 pathway (Kresovich et al., 2005). Sorghum is part of the Poaceae family and the Andropogonea tribe. Sorghum is diploid (2n=2x=20), belongs to the genus Sorghum together with the two perennial species *Sorghum halepense* (2n=4x=40) and *Sorghum propinquum* (2n=2x=20). Sorghum is highly diverse and it is made of five botanical races (Durra, Bicolor, Caudatum, Kafir and Guinea) characterized according to the different inflorescence types (Harlan and Dewet, 1922). In order of importance of cereal crops worldwide, sorghum is the fifth (FAO, 2005) and ranks second in the semi-arid tropics. Sorghum is important for food security (Kidanemaryam et al., 2018) for many people in Asian and sub-Saharan African countries (Mindaye et al., 2016). Sorghum provides proteins, vitamins and minerals (Kumar et al., 2011), this could be due to its wide adaptability in comparison to other field crops like wheat and maize (Ali et al., 2009).

Over the last seven years, global sorghum production has fluctuated between 57 and 66 MMT. The USA currently leads with an annual output of around 9 million metric tons, followed by Nigeria (6.9 MMT), Ethiopia (5.0 MMT), Mexico (5.0 MMT), India (4.5 MMT), and China (3.6 MMT) although by area, more than 90% of the world's sorghum is from the developing countries, in Africa and Asia (USDA 2019). The top sorghum producing countries in Africa are Nigeria (6.9 MMT), Ethiopia (5.2 MMT), Sudan (4.0 MMT), Niger (1.9 MMT) and Burkina Faso (1.8 MMT), Kenya produces 0.15 MMT (USDA 2019).

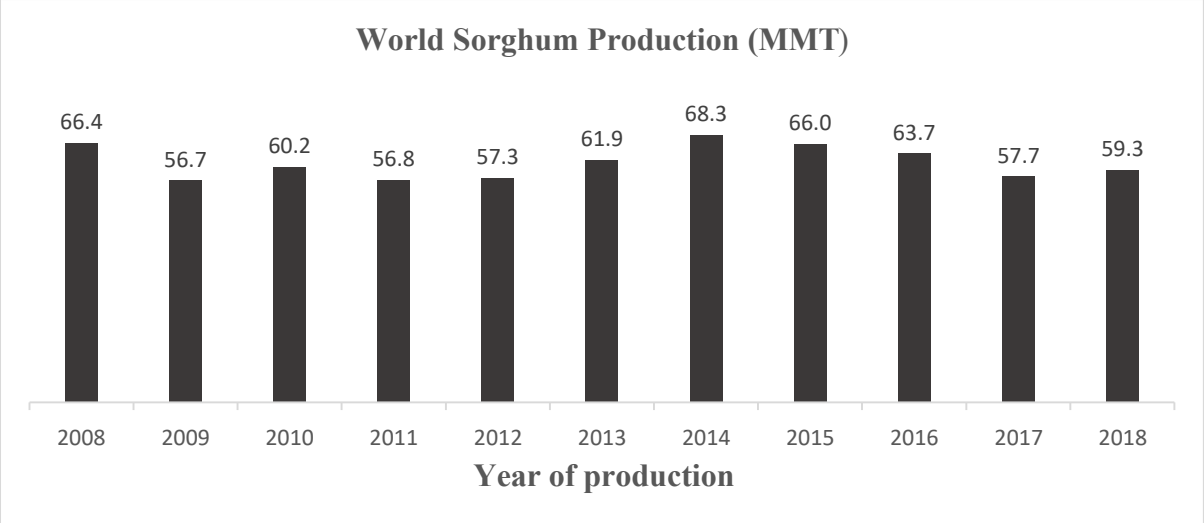


Figure 1. 1: (Source: FAOSTAT 2018)

1.2 Constraints in Production

Sorghum production is limited by biotic and abiotic constraints, numerous pests and diseases, water deficit and low soil fertility (Orr et al., 2020). Together these may significantly reduce yields. To address most of these constraints, genetic enhancement through exploiting host plant resistance is the best approach used that forms a basis for integrated control programs (Olembo, 2010).

Drought is a very significant cause of crop yield losses (Boyer and Westgate, 2004), drought prone areas are apparently where most of the resource poor farmers are found. In these areas with moisture and temperature stress, sorghum and millets are important crops due to their ability to cope (Atokple, 2003). Sorghum is better adapted to drought prone areas, extensive studies on drought tolerance in sorghum have been done (Blum, 1979; Doggett, 1988), therefore making it a model crop used in studies for various mechanisms of drought tolerance.

1.3 Statement of the Problem

Drought is a complex natural hazard which affects all climates and results in socio-economic impacts, the extent of which varies depending on several factors and conditions. Agriculture is the first and most drought affected sector.

According to (FAO, 2020), a direct impact of drought is the reduced water levels which cause reduced crop productivity. A reduction in crop productivity usually impacts the livelihoods of local populations resulting in less income for farmers, hunger and mass starvation, increased food prices, unemployment, and migration. Responding to drought after the impacts have taken their toll is commonly referred to as crisis management. It is known to be untimely, poorly coordinated and ineffective (FAO, 2020).

Drought in the tropics has significantly limited the sorghum yield potential (Kidanemaryam et al., 2018). Changes in climate are definitely going to accelerate the occurrence and intensity of episodes of drought in many African countries. For example, by 2050, limited water availability is expected to affect about a large proportion of the population which will lead to severe food insecurity (UNESCO, 2017). Drought is unpredictable, it can happen during any crop stage, the stage of anthesis and grain filling are the most critical stages in sorghum, drought occurring at this point is able to cause severe yield losses. For sorghum, drought tolerance is quantified by the plants ability to avoid senescing prematurely often called stay-green which most sorghum genotypes currently lack.

1.4 Justification of the study

Sorghum genotypes grown in semi-arid areas either have to deploy drought escape or inherent drought tolerance to maintain their yields in drought (Ngugi et al., 2013). The drought escape mechanism helps in managing drought stress however in most cases it is accompanied by yield penalties. An important drought tolerance trait that ensures non senescence of the leaves and consistent yields even in drought conditions is the stay green. Genotypes having this trait express it during post flowering drought tolerance period by maintaining a larger leaf area which is photosynthetically active. In comparison to the non-stay green genotypes, stay green genotypes are able to continue grain filling normally under drought stress conditions.

The contribution of this trait is reported for a number of crops, its utilization has led to increased grain yields and established tolerance to drought and heat.

The challenge to produce sorghum under water scarcity conditions requires integrated actions and strategies to remodel the crop genetic background and the cropping systems. Landraces and wild relatives of crops have been established to be reservoirs of useful genes for crop breeding including drought tolerance. Utilization of landraces from dry habitats has been used successfully in breeding maize for water-limited environments, and wild species that are relatives of cultivated crops have been on the agenda as possible donors for drought tolerance (Xu et al., 2009). It is important to limit depending on few stay green sources, this is the current case in many breeding programs currently. There is therefore need to gather, characterize and identify genotypes possessing the stay green trait among the wild relatives and local landraces in order to alleviate negative effects of drought stress on grain yield. These stay green alleles can be introduced into sorghum genotypes grown in drought prone agro ecologies of Eastern Africa to harness adaptation to drought. Having sorghum genotypes that have the stay green trait will improve sorghum yield under moisture stress conditions, this will limit the adverse effects of drought on food security.

1.5 Objectives

The main objective of the research was to contribute to improved sorghum production in drought prone areas in Kenya through identification of new drought tolerant genotypes among wild relatives and local landraces.

1.5.1 Specific objectives

1. To identify new stay green genotypes in sorghum wild relatives and local landraces
2. To introgress the stay-green alleles from two mapped donor sources into two drought susceptible farmer preferred varieties through marker assisted backcrossing.

1.5.2 Hypothesis

1. There is no genetic variation for drought tolerance among the sorghum wild relatives and local landraces.
2. Marker assisted backcrossing is not effective in the introgression of drought tolerance alleles from mapped donor sources into recipient farmer preferred varieties.

CHAPTER TWO: LITERATURE REVIEW

2.1 Sorghum production and utilization

Sorghum bicolor (L.) Moench (sorghum) is mainly grown in sub-tropical and semiarid regions. Annually, sorghum produces about 60 million metric tons of grain (Sanders et al., 2019). The annual rainfall requirement ranges between 420 mm - 630 mm for good growth and production. In Kenya, sorghum is mainly produced in the former Rift valley, Nyanza, Western and Eastern provinces (Republic of Kenya, 2003) under traditional farming systems by small scale producers (Iren, 2004). These areas account for 99% of the total grain sorghum produced in Kenya (Ngugi and Maswili 2010; Ngugi et al., 2013). Sorghum is grown for fodder and grain in Asia and Africa (Hariprasanna and Rakshit 2016; Mace et al., 2013; Paterson et al., 2009; Sanders et al., 2019). The grain flour is used in making *ugali*, injera, porridge and malt (Calder, 1955). Sorghum stalks are also used in biofuels production (ICRISAT, 2007; Laopaiboon et al., 2007).

Table 2. 1: Sorghum production in Kenya

Market Year	Production (1,000 MT)	Growth Rate (%)
2009	99	83.3
2010	164	65.6
2011	160	-2.4
2012	167	4.4
2013	169	1.2
2014	178	5.3
2015	189	6.2
2016	117	-38.1
2017	125	6.8
2018	180	44.0
2019	150	-16.7

Source: USDA 2019

2.2 Constraints to sorghum production

Yield gaps are of concern in Eastern Africa, the average on farm yields of sorghum range between 0.6-1.5 t/ha while worldwide average sorghum yields are 4.5 t/ha and above (Ngugi

and Maswili, 2010). Many biotic and abiotic factors contribute to these severe yield losses but drought stress ranks highest of abiotic stresses globally since it is unpredictable especially for the semi-arid areas where farming relies highly on rainfall (Beyene et al., 2015; Dalawai, 2017). Intense episodes of drought that affects sorghum mostly at the final growth stages, causes reduced biomass and grain yields (Borrell et al., 2014; O'Donnell et al., 2013; Prasad et al., 2008). Drought is a valid abiotic stress concern due to its complexity in timing, duration and intensity and it is often the foundational cause of other constrains to production. Drought is able to limit the availability of nutrients by reducing the rate at which nutrients diffuse from the soil to roots. Pests and diseases like stalk rot diseases (charcoal rot, Fusarium stalk rot, and sorghum ergot), are facilitated by drought causing weak growth and defense system (Assefa et al., 2010).

2.3 Drought response in Sorghum.

Under drought stress conditions, plants would urgently need to increase water uptake to efficiently utilize the soil water (Xiong et al., 2006). There have been efforts to identify and understand the mechanism of tolerance to drought in sorghum. These mechanisms include the plants having a root system that is prolific and their ability to maintain the stomata closed when the leaf water is inadequate and having low levels of osmotic adjustment under drought (Rajendran et al., 2011). In moisture stress conditions, the roots sense the water deficit and the synthesis of abscisic acid starts in the roots followed by the transportation of the abscisic acid via xylem within minutes to hours, Varieties of sorghum with deeper roots are more drought tolerant (Kaydan and Yagmur, 2008; Leishman and westoby, 1994). According to Dhanda et al. (2004), the stability of the leaf segment is important in drought tolerance and also the ratio of roots to shoots. It is vital to exploit the water sensitive stages to compare genotypes on how they resist water deficiency, this was undertaken in wheat (Dhanda et al., 2004), Sorghum (Gill et al., 2002, Bibi et al., 2010) and in Maize (Mohammadkhani and Heidari, 2008).

Adaptations leading to drought resistance in Sorghum are divided into: drought escape, avoidance and tolerance (Ludlow and Muchow, 1990).

2.3.1 Drought avoidance

Plants using this mechanism are able to prevent tissue water potential reduction in water deficit conditions (Amelework et al., 2012). The plants maximize water uptake through the root and minimize water loss through the stomata (Balko et al., 1975; Tadesse et al., 2008). For plants that use the C4 photosynthetic pathway, drought avoidance is a water conservation mechanism while for some other plants, drought avoidance makes them enhance water collection from underground water resources (Tesfamichael, 1999). The water conservation genotypes can have morphological changes represented by reduced leaf area or increased stomatal and cuticular resistance to reduce water loss while the water collection genotypes may develop deep root systems to enhance water uptake (Staggenborg, 2010).

2.3.2 Drought escape

Drought escape mechanism ensures that plants get to maturity before severe drought (Manavalan et al., 2017). Drought escaping genotypes are early maturing and have morphological modifications such as dwarfing to enhance the water use efficiency and development plasticity to escape drought stress conditions (Rao and Nigam, 2003). In drought escape, so as to ensure improved stability under drought conditions, there are yield penalties involved. Through this mechanism, plants that are short season annuals like sorghum can germinate, grow rapidly and complete their lifecycle before soil water is exhausted.

2.3.3 Drought tolerance

Drought tolerance traits include canopy temperatures, transpiration efficiency and chlorophyll content (Rohacek et al., 2008; Harris et al., 2007; Talebi, 2011; Kapanigowda, 2011; Kumar et al.,2008; Liu et al., 2010; Mutava et al., 2011). An improvement of these secondary traits can increase the potential for sorghum to tolerate drought (Prasad et al., 2006; Borell et al., 2010).

Genotypes that can tolerate drought can withstand low water potential during water deficit conditions and they can cope with stress and tolerate desiccation to survive longer (Hsiao, 1982). Drought tolerant plants can employ osmotic adjustment through accumulating compatible solutes within cells, lowering the osmotic potential and help to maintain turgor of the roots and shoots (Nguyen et al., 1997).

Various plants have different drought tolerant mechanisms during different development stages. In sorghum, the two distinct stages for drought tolerance are post-flowering and pre-flowering (Tainstra et al., 1997). Drought affects the development of panicles and grain yield in pre-flowering drought tolerant genotypes (Subudhi et al., 2000; Subudhi et al., 2002; Tuberosa et al., 2003; Ramesh et al., 2006). Genotypes that can tolerate drought stress that occurs after flowering can also maintain active photosynthesis when there is drought during the grain-filling period, they are high yielding under moisture stress conditions. These genotypes are classified as possessing the "stay-green" trait (Rosenow et al., 1983).

Crop improvement to reduce water stress effects is achievable if traits associated with drought resistance are carefully selected alongside yield (Borrell et al., 2000a; Sanchez et al., 2002). The yield traits, yield components and the stay green trait are controlled by mapped QTLS (Sanchez et al., 2002). QTL mapping has been done on SC56, B35 and E36-1 (Haussmann et al., 2002; Kebede et al., 2001). On the B and I linkage groups, there are two stay green QTLs identified (Tao et al., 2000). The *Stg1* and *Stg2 QTLs* mapped on the A linkage group and *Stg3* and *Stg4* QTLS mapped on the D and J linkage groups respectively were identified by (Xu et al., 2000) and (Crasta et al., 1999). These QTLs have been mapped on merit basis as *Stg2, Stg1, Stg3 and Stg4*. It was established that the map positions of the QTLs *Chl1*, *Chl2* and *Chl3* that have been mapped by (Xu et al., 2000) for the content of chlorophyll align with map positions for the stay green QTLs.

2.4 The stay green trait/ non-senescence

This is the capability of a plant having photosynthetically active leaf area after physiological maturity (Borrell et al., 2014) and an integrated drought adaptation trait resulting from the plant having good moisture balance in post flowering drought stress (Borrell et al.,2014). Functional stay green results to greater biomass accumulation and improved productivity in water deficit situations (Jordan et al., 2003; Jordan et al., 2012). Some stay green phenotypes are cosmetic or non-functional because they are not photosynthetically active. Stay-green can be due to alterations in the root architecture and canopy development occurring earlier in crop growth (Borrell et al., 2009; Jordan et al., 2012). The contribution of this trait has been reported in various crops, it has contributed to increased yields (Silva, 2005; Adu et al., 2011).

The QTLs responsible for stay green are: Stg1, Stg2, Stg3 and Stg4. These QTLs explain 20%, 30%, 16% and 10%, respectively, of the phenotypic variability for retention of green leaf area during grain filling under post-anthesis drought (Xu et al., 2000; Sanchez et al., 2002). Most of these studies have been undertaken using B35/BTx642 (Rosenow et al., 1983) some have used SC56 (Kebede et al., 2001) and E36-1 (Haussmann et al., 2002).

2.5 Wild relatives of sorghum

The genetic diversity existing in crop wild relatives' species (CWR) has been less explored (Brozynska et al., 2016; Hajjar and Hodgkin, 2007). Through natural selection, wild accessions have evolved to be tolerant and productive across diverse environments (Dillon et al., 2007b; Lazarides et al., 1991). Undomesticated sorghum species have the adaptability to survive in varied soil and moisture conditions in many microenvironments. This adaptability in many undomesticated Sorghum species enables them to develop resistance to several diseases and pests affecting sorghum grain production.

Landraces and wild relatives of crops (CWR) have been found to be sources of important genes for improving crops (Nyamongo et al., 2018; Brar and Khush, 2018; Kyratzis et al., 2019). Screening and characterizing the novel alleles in these germplasms would be the first step towards exploiting them (Hokanson et al., 2010).

Estimates of genetic diversity among and within the species has gained importance for crop improvement. They can help reduce population bottlenecks, threats to genetic losses and determining how landraces vary across various geographic regions.

Sorghum is originally from Ethiopia, from where it was disseminated to other areas of varying agroclimatic conditions (Zhou et al., 2010), Sorghum cultivars are diverse both phenotypically and genotypically (Kong et al., 2000; Hart et al., 2001). The breeder is able to choose the best parents for the breeding program and for introgressions with related germplasm when there is proper understanding of how genetically diverse a crop is.

2.5.1 Morphological characterization

Morphological descriptors are used in distinguishing accessions. Records of qualitative and quantitative phenotypic characters have provided a good basis for characterization and evaluation (Geleta and La buschagne, 2005). Morphological traits are a conventional way for analysis of genetic diversity, the traits are easy and cheap to score. Variation has been reported in sorghum accessions for qualitative traits characterized using traits like leaf midrib color, endosperm texture, glume color, pericarp color, pericarp thickness, grain color, awns, leaf trichomes, panicle compactness and testa color by (Abdi et al. (2002).

Quantitative traits such as plant height, 100 grain weight, days to maturity, panicle length, leaf area, number of leaves, leaf width and length, grain number per panicle, grain size, grain yield per plant, number of primary branches per panicle, panicle width, and panicle weight are also important in determination of genetic diversity (Punitha et al.,2010). (Borras and Gambin,

2011) have also reported a great variation in patterns of grain filling for various sorghum genotypes. Morphological characterization is essential in designing breeding programmes, studying the genetic variation patterns, identification of duplicates and the relationship between agronomic traits for selection.

2.5.2 Molecular markers

These markers are used to detect the differences in genetic information in two or more individuals. These markers have more heterozygosity with a high multiplex ratio making them have greater utility (Weising et al., 1995), the development stage of the plants and environment rarely affects their usage (Tanksley, 1983). Molecular markers could be DNA based or not. DNA based markers include the Random amplified polymorphic DNA markers (RAPD), simple sequence repeats (SSR) and Restriction fragment length polymorphism markers (RFLP). Crop characterization using various techniques like easily assayable markers has accelerated introduction of desirable traits into sorghum genomes (O'Kennedy et al., 2006). Molecular markers enable accurate identification of genotypes and thus genetic improvement is made easy without the environmental effect. Marker assisted selection used in sorghum accelerates the genetic gain, and the number of generations are reduced since selection uses the genetic values (Meuwissen et al., 2001). In elite sorghum inbreds, SSRs have been used to study genetic diversity (Menz et al., 2004), for the germplasm collections obtained from varied geographic locations (Muraya et al., 2011). SSRs are important in assessing the relatedness within or among landraces and the population genetic structure (Folkertsma et al., 2005). SSRs have been used in sorghum hybrids to determine the existing genetic diversity (Smith et al., 2010). The sorghum genome has also been mapped using SSRs comparing it to existing genetic linkage maps (Wu and Huang, 2006). To increase the efficiency of SSRs, other molecular techniques can be used with them (Geleta et al., 2006). Currently, genetic characterization of accessions is done using single nucleotide polymorphisms (SNPs) utilizing approaches like

genotyping-by- sequencing (Elshire et al., 2011), which promotes the analysis of the diversity level in the germplasm collection and genetic relationships among the germplasm collection (Romay et al., 2013; Morris et al., 2013; Munoz et al., 2014). The SNPs have a high reproducibility which gives a chance for comparing various sets of data from a number of collections of germplasm, make selections of the accessions based on genotypic information, and associate regions of the genome with useful economic traits.

2.5.2.1 Hybridization based molecular markers

Restriction Fragment Length Polymorphisms (RFLP) are widely used hybridization-based markers. Restriction enzymes reveal pattern differences in fragments of DNA for individual organisms. Restriction enzymes digest DNA resulting in fragments with varying size and numbers among individuals. RFLP markers are codominant markers and can be converted to SCARS. The disadvantage of these markers is that they cannot be automated, have a lower level of polymorphism, require radioactively labelled probes and are time consuming and very laborious to use (Allard, 1960).

2.5.2.2 PCR based markers

DNA polymerase enzymes are used to amplify small quantities of DNA through the polymerase chain reaction. A PCR based marker can consist of PCR techniques that are targeted to specific sites and are developed from known sequences of DNA like the Expressed sequence tags (ESTS), cleaved amplification polymorphism sequences (CAPS), simple sequence repeats (SSR) and the sequence characterized amplification region (SCAR) (Semayn et al., 2006a). The second type of PCR based markers can be developed even when the sequence information is unavailable and they consist of semi arbitrary or completely arbitrary primed PCR techniques.

2.5.2.4 Micro-array hybridization-based markers (DArT Markers)

Diversity Arrays Technology (DArT) is a high-throughput marker system that does not depend on prior sequence information (Jaccoud et al., 2001).

DArT is able to do scoring for the presence or absence of DNA fragments in representations of genomic DNA by generating medium-density genome scans. It is able to determine thousands of polymorphic loci simultaneously in one assay (Jaccoud et al., 2001; Wenzl et al., 2004). DArT was initially developed for rice, it has been employed in barley for assessment of diversity and genetic mapping (Wenzl et al., 2007 and also in Sorghum (Mace et al., 2008). This marker system has also been useful in non-model organisms for the study of pan-genomic evolution (James et al., 2008).

2.6 Marker assisted selection

This involves selecting plants with genomic regions that are responsible for expressing a given trait (Choudhary et al., 2008). For polygenic traits and quantitative trait loci, this is utilized because there are available markers and concentrated genetic maps (Semayn et al., 2006a). Selection assisted by markers helps to improve the efficiency of breeding done in foreground selection as this ensures accurate transfer of the regions of interest into the genome. Marker assisted selection used in background selection accelerates the rate at which the genome of the recurrent parent is recovered (Semayn et al., 2006; Choudhary et al., 2008; Ibitoye and Akinidowa, 2010). The breeding process can be improved by exploiting the various advantages of selection based on markers (Ribaut and Hoisington 1998; Morris et al., 2003).

There are five main considerations when using DNA markers in Marker assisted selection: reliability; quality and amount of DNA required; expertise for marker assay; polymorphism; and affordability (Mackill and Ni 2000; Mohler and Singrun, 2004).

In production of hybrids, especially Sorghum and Maize, they have identified heterotic groups through DNA markers which have been utilized in exploiting heterosis. Within a genome, shifts in the frequency of alleles is essential for breeders as they can monitor specific alleles of haplotypes for designing suitable strategies for breeding (Steele et al., 2004). The selected regions in the genome can be used in quantitative trait loci analysis or in validating associations of markers and traits that had been detected previously (Jordan et al., 2004). Effective marker assisted breeding requires the availability of sufficient polymorphic markers that cover the target genome evenly and are linked to the trait of interest. Early successful application of Marker assisted breeding has been used in Maize (Roget et al., 2007) and soybeans (Crosbie et al., 2003; Cahill and Schmidt, 2004; Kumpatla et al., 2012). Marker assisted breeding has been employed successfully in sorghum and pearl millet for stover yield quality, foliar disease resistance, and in vitro estimates of the nutritive value of various stover fractions for ruminants. In sorghum, ICRISAT has focused on initiating a large-scale high-throughput marker-assisted backcrossing program for the stay-green component of terminal drought tolerance. Marker assisted breeding can either be through backcrossing or recurrent selection of improving the population.

CHAPTER THREE; PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF SORGHUM WILD RELATIVES AND LOCAL LANDRACES

3.1 Abstract

Sorghum (*Sorghum bicolor [L] Moench*) is fifth most important cereal crop in the world, in Kenya, it ranks second after maize in Kenya. Sorghum is a critical food security crop in the dry areas were drought hampers crop production. Identifying sources of drought tolerance is key to improving sorghum grain yields. In this study, the objective was to screen wild relatives of sorghum and local landraces for drought tolerance. The accessions used included 44 landraces and wild relatives of sorghum and 52 F_2 populations generated between the landraces, these genotypes were screened under well irrigated and drought conditions in a 12 x 8 alpha lattice design that was replicated three times. One set of these materials received regular irrigation from planting to physiological maturity whereas the second set had water withheld at 14 days and irrigation applied at 40 and 55 days after sowing. Stay- green was determined using the relative chlorophyll content and number of green leaves at maturity. Under drought, some genotypes demonstrated drought escape whereas others were drought tolerant. Drought tolerant genotypes had high stay green traits score and were high yielding. The most drought tolerant stay green genotypes were, GBK044058, GBK 047293, GBK016114, Lodoka and Akuor-Achot. Stay green trait was positively correlated with panicle weight (r=0.82), hundred seed weight (r=0.85) and grain yield (r=0.78). The results showed that wild relatives and landraces of sorghum can be utilized as sources of drought tolerance to improve productivity.

Key words: Drought escape, drought tolerance, stay green, grain yield

3.2 Introduction

Sorghum (*Sorghum bicolor* [L.] Moench) 2n=2x=20, is an important food security crop for millions of inhabitants of Africa (Kidanemaryam et al., 2018). In semi-arid areas, drought is a major abiotic stress affecting sorghum production, the drought conditions are exacerbated by water scarcity in the ASALs. The stage of plant development determines the impact of moisture stress on crop yield. In sorghum, moisture deficiency that occurs at the anthesis and grain filling stages may cause reduced yields or total crop failure (Kidanemaryam et al., 2018. There are sorghum genotypes that possess the stay green trait which enables them to handle the harmful effects of moisture stress. Stay green is expressed through delayed leaf senescence, and is due to improved water balance in the plant (Borrell et al., 2014). When the stay green phenotype is associated with greater biomass accumulation and increased crop yields in water limiting conditions, it is considered to be functional (Jordan et al.,2012). Some stay green phenotypes are considered to be cosmetic or non-functional because they are photosynthetically inactive. In drought conditions, functional stay green lines are able to keep filling grain normally in comparison to senescent ones.

In several crops, employment of stay green has brought increase in grain yield and plant tolerance to drought (Silva, 2005; Adu et al., 2011). Studies by (Kassahun et al*.,* 2010) in sorghum, associated stay-green with drought tolerance, high grain yield potential and delayed senescence.

Estimates of genetic diversity within or among plant species have gained importance in crop improvement and therefore it is important to establish the importance and function of crop alleles within local sorghum wild germplasm (Hokanson et al., 2010). Such studies have not been undertaken in sorghum and hence this study was proposed.

3.3 Materials and Methods

3.3.1 Plant material and experimental layout

Ninety-six sorghum genotypes (44 wild sorghum relatives and local landraces, and 52 F_2 segregating populations) were used in the study (Table 3.1). The F_2 populations were derived from some of the 44 landraces and cultivated accessions. The experiment was set up at KALRO Kiboko field station, Kenya (2.15^o S and longitude 37.75^{o}E) in July 2017. The experiment was laid in two blocks, one block was well irrigated the other block was drought stressed to evaluate the drought response among the genotypes. The trials were set up in a 12 x 8 alpha lattice design replicated thrice. The trial had 2 row plots of 2m length, an inter row spacing of 0.75m and intra row spacing of 0.25m.

Table 3. 1: Sorghum genotypes used in the study

#	Genotype	Source	Status
1.	GBK 044058	GeRRI	Wild
2.	GBK 044336	GeRRI	Wild
3.	GBK 048922	GeRRI	Wild
4.	GBK 047293	GeRRI	Wild
5.	GBK 048916	GeRRI	Wild
6.	GBK 016085	GeRRI	Wild
7.	GBK 048917	GeRRI	Wild
8.	GBK 016114	GeRRI	Wild
9.	GBK 044063	GeRRI	Wild
10.	GBK 048156	GeRRI	Wild
11.	GBK 016109	GeRRI	Wild
12.	GBK 044120	GeRRI	Wild
13.	GBK 040577	GeRRI	Wild
14.	GBK 048921	GeRRI	Wild
15.	GBK 044448	GeRRI	Wild
16.	GBK 045827	GeRRI	Wild

#	Genotype	Source	Status
17.	GBK 048152	GeRRI	Wild
18.	GBK 044065	GeRRI	Landrace
19.	GBK 043565	GeRRI	Landrace
20.	GBK 044054	GeRRI	Landrace
21.	OKABIR	ICRISAT-Nairobi	Landrace
22.	IS 9830	ICRISAT-Nairobi	Landrace
23.	SRN39	ICRISAT-Nairobi	Landrace
24.	IBUSAR	ICRISAT-Nairobi	Landrace
25.	AKUR-ACHOT	ICRISAT-Nairobi	Landrace
26.	LODOKA	ICRISAT-Nairobi	Landrace
27.	E36-1	ICRISAT-Nairobi	Mapped donor
28.	B35	ICRISAT-Nairobi	Mapped donor
29.	N13	ICRISAT-Nairobi	Landrace
30.	KARIMTAMA-1	ICRISAT-Nairobi	Improved variety
31.	GADAM	ICRISAT-Nairobi	Improved variety
32.	F6YQ212	ICRISAT-Nairobi	Improved variety
33.	MACIA	ICRISAT-Nairobi	Improved variety
34.	FRAMIDA	ICRISAT-Nairobi	Improved variety
35.	KAT/ELM/2016 PL82 KM32-2	ICRISAT-Nairobi	Improved variety
36.	KAT/ELM/2016 PL1 SD15	ICRISAT-Nairobi	Improved variety
37.	IESV23006DL	ICRISAT-Nairobi	Improved variety
38.	ICSV 111 IN	ICRISAT-Nairobi	Improved variety
39.	HAKIKA	ICRISAT-Nairobi	Improved variety
40.	CR35 ' 5	ICRISAT-Nairobi	Improved variety
41.	IESV92043DL	ICRISAT-Nairobi	Improved variety
42.	WAHI	ICRISAT-Nairobi	Improved variety
43.	IESV21400DL	ICRISAT-Nairobi	Improved variety
44.	IESV23010DL	ICRISAT-Nairobi	Improved variety
45.	LODOKA X OKABIR	ICRISAT-Nairobi	F_2 generation
46.	IBUSAR X ICSVIIIN	ICRISAT-Nairobi	F_2 generation
47.	LODOKA X ICSVIIIN	ICRISAT-Nairobi	F_2 generation
48.	OKABIR X AKUR ACHOT	ICRISAT-Nairobi	F_2 generation

#	Genotype	Source	Status
49.	ICSV 111 IN X MACIA	ICRISAT-Nairobi	F_2 generation
50.	ICSV 111 IN X LANDWHITE	ICRISAT-Nairobi	F_2 generation
51.	B35 X LODOKA	ICRISAT-Nairobi	F_2 generation
52.	ICSV 111 IN X B35	ICRISAT-Nairobi	F_2 generation
53.	B35 X AKUR ACHOT	ICRISAT-Nairobi	F_2 generation
54.	LODOKA X LANDWHITE	ICRISAT-Nairobi	F_2 generation
55.	ICSV 111 IN X E36 – 1	ICRISAT-Nairobi	F_2 generation
56.	B35 X F6YQ212	ICRISAT-Nairobi	F_2 generation
57.	OKABIR X ICSV 111 IN	ICRISAT-Nairobi	F_2 generation
58.	F6YQ212 X B35	ICRISAT-Nairobi	F_2 generation
59.	B35 X E36-1	ICRISAT-Nairobi	F_2 generation
60.	OKABIR X B35	ICRISAT-Nairobi	F_2 generation
61.	LANDIWHITE X MACIA	ICRISAT-Nairobi	F_2 generation
62.	B35 X ICSV 111 IN	ICRISAT-Nairobi	F_2 generation
63.	IBURSAR X LANDWHITE	ICRISAT-Nairobi	F_2 generation
64.	B35 X LANDIWHITE	ICRISAT-Nairobi	F_2 generation
65.	F6YQ212 X LODOKA	ICRISAT-Nairobi	F_2 generation
66.	E36-1 X MACIA	ICRISAT-Nairobi	F_2 generation
67.	LANDIWHITE X B35	ICRISAT-Nairobi	F_2 generation
68.	ICSV 111 IN X LODOKA	ICRISAT-Nairobi	F_2 generation
69.	IBURSAR X E36-1	ICRISAT-Nairobi	F_2 generation
70.	AKUR-ACHOT X ICSVIIN	ICRISAT-Nairobi	F_2 generation
71.	E36-1 X IBUSAR	ICRISAT-Nairobi	F_2 generation
72.	AKUOR-ACHOT X OKABIR	ICRISAT-Nairobi	F_2 generation
73.	ICSV 111 IN X OKABIR	ICRISAT-Nairobi	F_2 generation
74.	E36-1 X LANDIWHITE	ICRISAT-Nairobi	F_2 generation
75.	ICSV 111 IN X E36-1	ICRISAT-Nairobi	F_2 generation
76.	AKUOR-ACHOT X B35	ICRISAT-Nairobi	F_2 generation
77.	F6YQ212 X OKABIR	ICRISAT-Nairobi	F_2 generation
78.	AKUOR-ACHOT X LODOKA	ICRISAT-Nairobi	F_2 generation
79.	AKUOR-ACHOT X IBUSAR	ICRISAT-Nairobi	F_2 generation
80.	AKUOR-ACHOT X ICSV 111 IN	ICRISAT-Nairobi	F_2 generation

#	Genotype	Source	Status
81.	E36-1 X AKUOR-ACHOT	ICRISAT-Nairobi	F_2 generation
82.	IBUSAR X MACIA	ICRISAT-Nairobi	F_2 generation
83.	F6YQ212 X E36-1	ICRISAT-Nairobi	F_2 generation
84.	IBUSAR X E36-1	ICRISAT-Nairobi	F_2 generation
85.	B35 X OKABIR	ICRISAT-Nairobi	F_2 generation
86.	OKABIR X LODOKA	ICRISAT-Nairobi	F_2 generation
87.	LANDIWHITE X E36-1	ICRISAT-Nairobi	F_2 generation
88.	E36-1 X LODOKA	ICRISAT-Nairobi	F_2 generation
89.	LODOKA X E36-1	ICRISAT-Nairobi	F_2 generation
90.	ICSV 111 IN X LODOKA	ICRISAT-Nairobi	F_2 generation
91.	F6YQ212 X IBUSAR	ICRISAT-Nairobi	F_2 generation
92.	E36-1 X B35	ICRISAT-Nairobi	F_2 generation
93.	ICSV 111 IN X F6YQ212	ICRISAT-Nairobi	F_2 generation
94.	B35 X OKABIR	ICRISAT-Nairobi	F_2 generation
95.	LANDI WHITE X F6YQ212	ICRISAT-Nairobi	F_2 generation
96.	IBUSAR X MACIA	ICRISAT-Nairobi	F_2 generation

#-Serial number, GeRRI- Genetic Resources Research Institute (Kenya), F_2- Second filial generation, ICRISAT-International Crops Research Institute for the semi-Arid Tropics

3.3.2 Data collection

Agronomic data was taken from 6 plants selected randomly in the 2 center rows from all replications according to the methodology described by IBPGR and ICRISAT (1993). Data was collected on; stem girth (mm), plant height (cm), days to 50% flowering (counts), panicle exertion (cm), panicle length (cm), number of green leaves at maturity (count), productive tillers (counts), panicle width (cm), panicle weight (kg), hundred seed mass (g), leaf area (m^2), relative chlorophyll content (spad readings), and grain yield (t ha^{-1}). Grain yield data was determined at plot basis as suggested by IBPGR and ICRISAT (1993).

Diammonium Phosphate (18% N, 46% P_2O_5) fertilizer was used when planting at a rate of 100kg ha^{-1}, urea was used in top dressing the crop 21 days post emergence at a rate of 40kg ha^{-1} 30 days after emergence, earthing up was done. The crop was raised according to the recommended standard agronomic practices.

Table 3. 2: Agronomic traits measured in the study

Trait	Description/Measurement
Days to 50% flowering(counts)	The number of days from planting to when 50% of the plants in the plot were in flowering
Stem girth(mm)	Measured by a Vernier calipers on the upper sheath of fourth leaf at grain filling
Plant height(cm)	Plant length from ground level to tip of panicle at physiological maturity
Leaf area (m^2)	Calculated from the leaf length and width at anthesis
Green leaves at Maturity (counts)	Number of non-senescent leaves at maturity
Relative chlorophyll content (spad)	Measured on the fourth middle leaf at physiological maturity
No. of productive tillers (counts)	No. of Basal tillers with mature panicles at maturity
Panicle excersion (cm)	Measured from the sheath of flag leaf to the bottom of the panicle
Panicle length(cm)	Measured from the base to tip of longest panicle at physiological maturity
Panicle width(cm)	Measured as distance across center of longest panicle at physiological maturity
Panicle weight(kg)	Measured for dried harvested samples at physiological maturity
Grain yield (t ha-1)	The mass of dry grain per plot at 12.5% moisture content converted to tones/hectare
100-grain mass (g)	Mass of 100 grains at 12.5% moisture

Source: IBPGR (1985)

3.3.3 Drought screening

The trial that was well watered was irrigated three times each week. The duration of each irrigation was 3 hours and the amount supplied per irrigation was 25mm of water, this was done from sowing to soft dough stage. The water stressed trial had water withdrawn at 14 days post sowing. At 40 days after sowing, 50mm of water was applied in two applications, then the trial was exposed to 20 days of drought and the final flush of irrigation of 50mm given at 60 days after sowing. Drought stress conditions were thereafter maintained until maturity.

3.4 Statistical analysis

Analysis of variance (ANOVA), variances and averages of the quantitative traits was done by GenStat v14.1 (VSN International, 2011). Treatment means were compared using Fisher's protected least significant differences at $P \leq 0.05$. The estimates of genotypic and phenotypic variance, phenotypic and genotypic coefficients of variation were done based on the formula proposed by (Syukur et al., 2012).

Genotypic variance;

$$\sigma_g^2 = \frac{MS_g - MS_e}{r}$$

Phenoypic variance;

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

where: σ_g^2= Genotypic variance; σ_p^2= Phenotypic variance; σ_e^2= environmental variance (error mean square from the analysis of variance);

MS_g = mean square of genotypes; MS_e = error mean square; r = number of replications.

Genotypic coefficient of variation;

$$[GCV] = \left[\left\{ \sqrt{\sigma_g^2} \right\} \Big/ \bar{x} \right] \times 100$$

Phenotypic coefficient of variation;

$$[PCV] = \left[\left\{ \sqrt{\sigma_P^2} \right\} \Big/ \bar{x} \right] \times 100$$

where: σ_g^2 = Genotypic variance; σ_P^2 = Phenotypic variance; $\bar{x}$ is grand mean of a character.

Estimations of broad sense heritability (H^2) of all traits were calculated according to the formula described by Allard (1960):

$$H^2\, bs = \left[\sigma_g^2 / \sigma_p^2 \right] \times 100$$

$H^2\, bs$ = heritability in broad sense; σ_g^2 = Genotypic variance; σ_p^2 = Phenotypic variance.

Estimation of broad sense heritability (H^2) assuming selection intensity of 5% for individual and combined analysis of variance were computed using the formula adopted from (Johnson et al., 1955). H^2 scores were classified according to (Robinson et al., 1949) as follows: 0 – 30% = low; 30 – 60% = moderate; > 60% = high.

Simple linear correlation coefficient (Pearson, 1986) was calculated to understand the relationship among the studied agronomic traits as below

$$PX,Y = \frac{cov(x,y)}{\sigma_x \sigma_Y}$$

Where cov is the covariance, σ_x is the standard deviation of x, σ_y is the standard deviation of Y

3.5 Genotyping, diversity estimation and quality control (QC) panel

At seedling stage, the leaf tissues of the 43 diverse accessions were collected, ISOLATE II Genomic DNA extraction kit (Bioline Pty Ltd, Nottingham, UK) was used for the genomic DNA extraction as per the manufacturer's instructions. The purity and quantity of the DNA was estimated by an agarose gel electrophoresis and a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA) respectively. For library construction and DArT-sequencing (DArTseq) (https://www.diversityarrays.com/products-and-services/applications/), the DNA was sent to the Integrated Genotyping Service and Support (IGSS) at the Bioscience eastern

and central Africa (BecA) Lab at the International Livestock Research Institute (ILRI) hub, as previously described (Wójcik-Jagła et al., 2018).

The GBS pipeline of the Trait Analysis by Association, Evolution and Linkage (TASSEL) 5.2.58 program (Bradbury et al., 2007) was used to process the resulting raw reads from the DArT sequencing. Raw SNPs were filtered using minor allele frequency of ≥ 0.05, taxa and SNP minimum call rate of 50% and 70% respectively were used for drawing the Neighbor Joining dendrogram, the dendrogram was drawn using the Darwin 6.0.20 software with 1000 bootstraps (Perrier and Jacquemoud-Collet, 2006). For developing the QC panel, the SNPs were filtered using a minor allele frequency ≥0.05 and SNP call rate of 100%. A set of 20 most informative SNPs was extracted from the filtered SNP set using a java script (Ignacio, 2019) for future use as QC panel.

3.6 RESULTS

3.6.1 Phenotypic variation of traits and heritability among diverse sorghum accessions

Phenotypic data was collected for 37 genotypes out of the 44 diverse genotypes and 44 F_2 lines out of 52 that did not senesce during drought stress. Table 3.4 and Table 3.5 shows that the Analysis of variance revealed significant ($P \leq 0.05$; $P \leq 0.01$) differences among the 44 diverse genotypes for all the studied traits under drought and well-watered conditions respectively. The interaction of the various water regimes and genotypes also led to significant differences in the traits observed, except in the case of flag leaf area as sown in Table 3.6.

Table 3. 3: ANOVA of mean squares across 37 genotypes under drought stress conditions

SOV	DF	PHT	SG	RCC	GLAM	DFL	FLA	VT	PEX	PAL	PAW	HSW	PWT	YLD
Rep	2	1929	2.37	46.77	1.413	34.48	0.002481	9.5	12	27.6	19.3	0.1641	0.001125	0.0005
Genotypes	36	3555.8***	1.53***	66.37***	6.133**	179.82***	0.0137***	1.87***	38.46***	37.5***	12.3***	0.675***	0.005708***	0.997***
Residual	71	730.1	0.2	26.66	2.796	2.888	0.003	7.4	5.3	5.6	2.8	0.1516	0.001071	0.1299

SOV: Sources of variation, DF: Degrees of freedom, PHT: Plant height, SG: Stem girth, RCC: Relative chlorophyll content, GLAM: No. of green leaves at maturity, DFL: Days to flowering, FLA: Flag leaf area, PEX: Panicle exertion, PAL: Panicle length, PAW: Panicle width, HSW: Hundred seed weight, PWT: Panicle weight, YLD: Grain yield, **: P< 0.01, ***: P<0.001

Table 3. 4: ANOVA of mean squares across 37 sorghum genotypes under well-watered conditions

SOV	DF	PHT	SG	RCC	GLAM	DFL	FLA	VT	PEX	PAL	PAW	HSW	PWT	YLD
Rep	2	3648.5	1.23	210.11	123.36	4.199	0.000484	6.1	10.21	17.5	15.2	0.0477	0.027492	0.02749
Genotypes	36	7892.2***	1.02***	50.86**	6.504**	94.66***	0.00066**	1.36***	28.62***	30.1***	8.3***	0.8205***	0.0026**	0.6139***
Residual	71	421.2	0.1	25	3.024	3.897	0.000307	5.2	4.1	3.8	1.6	0.2497	0.2497	0.00185

SOV: Sources of variation, DF: Degrees of freedom, PHT: Plant height, SG: Stem girth, RCC: Relative chlorophyll content, GLAM: No. of green leaves at maturity, DFL: Days to flowering, FLA: Flag leaf area, PEX: Panicle exertion, PAL: Panicle length, PAW: Panicle width, HSW: Hundred seed weight, PWT: Panicle weight, YLD: Grain yield, **: P< 0.01, ***: P<0.001

Table 3. 5: Combined ANOVA of mean squares under well-watered and drought stress conditions

SOV	DF	PHT	SG	RCC	GLAM	DFL	FLA	VT	PEX	PAL	PAW	HSW	PWT	YLD
Rep	2	2478.80	4.13	226.53	75.50	31.05	0.19	0.58	37.83	87.81	156.72	0.05	0.20	0.02
Water regimes(W)	1	101724.5***	8.3***	55.63***	199.49***	376.33***	0.25***	498.17***	3053.66***	1505.14**	1035.81**	0.11ns	0.31ns	0.18ns
Genotypes(G)	35	8394.5***	0.53***	5.62**	9.82***	215.43***	0.11***	4.85***	184.52***	70.92***	50.68***	0.93***	0.57***	1.22***
W X G	35	3053.5***	0.13***	162.81*	3.82*	59.05***	0.22ns	93.43***	86.41***	42.38***	34.23***	0.57***	0.26***	0.39***
Residual	144	611.20	0.04	2.24	3.56	3.45	0.02	1.03	37.62	11.12	8.54	0.20	0.16	0.15

SOV; source of variation, DF: Degrees of freedom, PHT: Plant height, SG: Stem girth, RCC: Relative chlorophyll content, GLAM: No. of Green leaves at maturity, DFL: Days to flowering, FLA: Leaf area, PEX: Panicle exertion, PAL: Panicle length, PAW: Panicle width, HSW: Hundred seed weight, PWT: Panicle weight, YLD: Grain yield

3.6.2 Comparison of mean values of growth-related parameters of the diverse accessions under well-watered and drought stress conditions and the effect of drought

The mean comparison under both water regimes showed significant differences among the genotypes for growth related parameters (Table 3.6). Under drought stress conditions, the genotypes recorded reduction in the overall mean compared to well-watered conditions. The genotypes that were severely affected by drought stress (completely senescent) were: Gadam, SRN39, GBK 044120, GBK 044063, GBK 016085, GBK 048916 and GBK 044336. (Table 3.6) Plant height recorded an overall mean of 204.12cm under well-watered conditions compared to 117.33cm under drought stress conditions (Table 3.6), the genotype that was least affected by drought (12.48%) was GBK048917 that had a height of 176cm under drought and 201cm under well-watered conditions. The height of genotype F6YQ212 was most affected by drought (66.30%), it recorded 79.80cm under drought compared to 236.80cm under well-watered conditions. The overall effect of drought on plant height was 42.52% (Table 3.6). Stem girth recorded an overall mean of 1.76mm under well-watered conditions compared to 1.39mm under water stress conditions (Table 3.6). The most affected genotype was Okabir (37.29%), its stem girth reduced from 1.25mm under well-watered conditions to 0.82mm in water stress conditions. The least affected genotype in water stress was IESV23010 DL (0.92%) that recorded 2.17mm under well-watered compared to 2.15mm in water stress conditions. The overall effect of drought stress on stem girth was 21.02% (Table 3.6). The relative chlorophyll content had an overall mean of 46.97 under well-watered conditions compared to 36.71 under drought stress conditions (Table 3.6). The most affected genotype under drought for the relative chlorophyll content was Framida (44.52%) which had a mean reduction of 52.39 to 28.64 under drought stress. The overall effect of drought stress on levels of relative chlorophyll content was 21.84% (Table 3.6). The number of green leaves at maturity had an overall mean of 13.48 under well-watered conditions compared to 7.90 under water stressed conditions (Table 3.6).

The most affected genotype was GBK 048156 (66.98%) which recorded a reduction from 9.71 in well-watered compared to 7.00 green leaves at maturity in water stressed conditions. The least affected genotype was E36-1 (8.27%) that had 12 green leaves at maturity under water stressed conditions and 13 under well-watered conditions. The overall effect of drought on number of green leaves at maturity was 41.40% (Table 3.6). The days to 50% flowering was averagely 50.52 in well-watered conditions and 41.01 under water stressed conditions (Table 3.6). The genotype that had severe reduction in the days it took to get to 50% flowering under water stressed conditions compared to well-watered conditions was GBK 044054 (28.24%), the days to flowering reduced from 52.33 days to 37.55 days. Genotype Okabir was least affected by drought stress (2.35%), it took 53.71 days to get to 50% flowering under drought stress compared to 55.00 days under well-watered conditions. The overall effect of drought stress on days to 50% flowering was 18.82% (Table 3.6). The mean flag leaf area under drought stress conditions was $0.04m^2$ and $0.06m^2$ in well-watered conditions (Table 3.6). The most affected genotype in leaf area was GBK 045827 (181.87%), Genotype ICSV 111 IN was least affected by drought stress (6.23%). The overall effect of drought stress on flag leaf area was 33%. (Table 3.6)

Table 3. 6: Mean comparisons for growth related parameters in the diverse genotypes and effect of drought stress

GENOTYPE	PHT			SG			RCC			GLAM			DFL			LFA		
	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect
GBK 044058	163.5	249	0.34	1.93	1.8	-7.28	50.61	54.41	6.98	8.71	11.69	25.55	49.11	54	9.06	0.05	0.06	24.21
GBK 048922	170.7	217.1	0.21	1.55	2.02	23.02	47.74	51.17	6.7	8.08	12.32	34.38	64.96	55	-18.11	0.05	0.06	11.03
GBK 047293	137.2	170.7	0.2	1.59	1.77	10.55	48.84	36.16	-35.07	12.32	14.39	14.37	50.54	54	6.41	0.05	0.06	6.93
GBK 043565	192.8	240.3	0.2	0.88	1.25	34.59	28.64	52.39	45.33	8.19	16.05	48.97	45.51	54	15.72	0.02	0.02	31.16
GBK 048917	176	201.1	0.12	1.93	1.13	-71.4	25.63	36.08	28.96	12.33	15.47	20.3	41	52	21.15	0.05	0.06	23.44
GBK 016114	153.9	194.2	0.21	1.26	1.14	-11.07	51.88	46.63	-11.25	9.66	12.82	24.64	48.52	53.33	9.02	0.03	0.04	14.3
GBK 044054	156	199	0.22	0.96	1.4	31.76	47.85	52.37	8.63	7	14.71	52.39	37.55	52.33	28.24	0.01	0.05	73.08
GBK 048156	158.7	249.8	0.36	1.06	1.41	24.75	52.23	39.1	-33.58	5.08	15.4	66.98	39.27	53	25.91	0.02	0.06	62.07
GBK 016109	142	249.6	0.43	1.34	1.77	24.39	52.51	47.6	-10.32	8.25	12.61	34.54	46.79	53.67	12.82	0.04	0.07	46.37
GBK 044065	122.8	230.1	0.47	1.97	1.34	-47.23	39.07	50.9	23.24	6.5	14.97	56.58	46	54.33	15.33	0.04	0.05	11.07
GBK 045827	130.7	156.5	0.16	1.2	1.48	18.75	49.68	50.66	1.93	7.67	11.4	32.76	30	35	14.29	0.05	0.02	-181.87
GBK 048152	131.8	179.1	0.26	1.53	1.62	5.56	36.35	46.49	21.81	7.25	13	44.23	43	53.67	19.88	0.05	0.06	23.29
GBK 040577	152.2	300.7	0.49	1.26	1.65	23.27	41.34	52.79	21.69	8.81	11.09	20.6	52.46	54	2.85	0.05	0.06	14.59
GBK 048921	202.1	277.4	0.27	1.18	1.54	23.18	33.8	41.19	17.94	8.33	13.34	37.59	54.51	52.67	-3.49	0.03	0.04	7.37
GBK 044448	169.8	281.4	0.4	1.42	1.57	9.85	35.77	35.22	-1.56	6.78	14.21	52.25	50.71	43	-17.93	0.04	0.05	27.37
GBK 044336	0	190.4	100	0	2.01	100	0	40.56	100	0	12.52	100	0	48.43	100	0	0.03	100
GBK 048916	0	163.6	100	0	1.72	100	0	43.42	100	0	12.03	100	0	46.32	100	0	0.02	100
GBK 016085	0	201.5	100	0	1.53	100	0	39.86	100	0	13.52	100	0	53.54	100	0	0.04	100
GBK 044063	0	176.8	100	0	1.68	100	0	41.53	100	0	10.83	100	0	52.31	100	0	0.03	100
GBK 044120	0	170.6	100	0	1.13	100	0	44.25	100	0	12.44	100	0	47.89	100	0	0.03	100
SRN39	0	142.2	100	0	1.45	100	0	40.05	100	0	10.31	100	0	51.39	100	0	0.08	100
GADAM	0	120.4	100	0	1.52	100	0	36.43	100	0	15.54	100	0	56.27	100	0	0.06	100

GENOTYPE	PHT			SG			RCC			GLAM			DFL			LFA		
	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect
KAT/ELM/2016 PL1 SD-15	120.1	211.3	43.16	1.53	1.76	13.0682	31.46	41.5	24.19	7.44	11.15	33.27	50.27	41.72	-20.49	0.031	0.05	40.5
KARI MTAMA 1	145.2	181.7	0.2	1.5	2.27	33.91	34.32	39.56	13.25	9.56	16.24	41.12	39.47	46.33	14.81	0.05	0.07	23.68
IESV23006DL	106.42	128.95	0.17	2.14	2.43	11.93	55.34	53.28	-3.87	11.03	12.85	14.16	49	52	5.77	0.04	0.07	42.37
N13	156.7	205.1	0.24	1.29	1.38	6.32	36	44.41	18.94	10.75	14.63	26.55	36	45.33	20.58	0.02	0.04	55.08
E36-1	114.1	162.1	0.3	1.57	1.69	7.56	52.01	53.21	2.26	12.15	13.25	8.27	49.53	46.67	-6.13	0.05	0.03	-57.09
B35	65	82.45	0.21	1.65	1.55	-6.65	42.16	54.72	22.95	11.11	13.92	20.2	41.56	46.5	10.62	0.04	0.04	-7.59
ICSV III IN	112.9	182.6	0.38	2.16	1.86	-16.12	35.72	57.12	37.46	6.37	13.25	51.93	35	41	14.63	0.05	0.05	6.23
F6YQ212	79.8	236.8	0.66	1.87	1.47	-27.61	36.89	43.56	15.31	13.02	10	-30.13	55.55	52.67	-5.47	0.03	0.04	43.96
MACIA	104.1	186.2	0.44	2.08	1.61	-28.93	44.24	50.42	12.26	6.26	11.16	43.89	58.12	53.67	-8.29	0.05	0.07	24.03
OKABIR	157.6	286.5	0.45	1.63	2.6	37.29	54.6	43.63	-25.14	10.6	14.82	28.5	53.71	55	2.35	0.06	0.04	-39.19
LODOKA	90.3	232.7	0.61	1.8	2	9.98	55.84	49.67	-12.42	15.4	17.17	10.33	66	62	-6.45	0.04	0.06	31.23
FRAMIDA	149.8	197.8	0.24	1.86	1.81	-2.65	30.61	55.17	44.52	8.43	12.42	32.07	36.02	43	16.23	0.04	0.17	77.78
AKUOR-ACHOT	181.9	287.6	0.37	1.5	1.6	5.89	52.11	49.43	-5.42	13.5	15.13	10.81	53.51	43.67	-22.53	0.05	0.07	26.43
HAKIKA	130	255.5	0.49	1.64	1.17	-40.22	49.7	51.64	3.76	5.16	13.72	62.38	63.79	46.67	-36.68	0.05	0.04	-9
IBUSAR	235.7	287.5	0.18	1.9	2.23	14.78	32.01	45.43	29.54	14.42	18.43	21.76	44	48	8.33	0.06	0.09	34.13
IS 9830	113.4	271.2	0.58	1.87	2.45	23.67	34.52	55.9	38.25	12.16	16.08	24.41	59.53	52.67	-13.02	0.05	0.08	41.67
IESV21400DL	113.5	134.5	0.16	2.28	2.52	9.51	55.62	41.43	-34.25	11.5	15.87	27.54	50	52	3.85	0.05	0.07	19.53
IESV23010DL	132.6	185.4	0.28	2.15	2.17	0.92	55.32	55.41	0.16	10.26	14.92	31.25	48.52	50	2.96	0.07	0.1	32.14
IESV92043DL	141.7	185.3	0.24	1.9	2.16	11.97	55.34	53.21	-4	9.05	11.88	23.76	52.03	55	5.4	0.07	0.09	24.18
WAHI	89.7	115.4	0.22	2	2.32	13.83	49.6	52.12	4.83	7	11.45	38.89	59	64	7.81	0.05	0.07	38.63
CR35 ' 5	123.7	148.4	0.17	1.85	2.06	9.88	40.13	45.31	11.43	8.28	12.04	31.23	49.4	53	6.79	0.05	0.09	39.58
KAT/ELM/2016 PL82 KM32-2	138.1	254.8	0.46	1.86	2.22	16.34	39.59	51.5	23.13	9.24	11.96	22.7	54.51	41.67	-30.81	0.06	0.08	25.7
Mean	117.33	204.12	17.15	1.39	1.76	20.39	36.71	46.97	22.92	7.9	13.48	41.84	41.01	50.52	18.44	0.04	0.06	32.01
SE±	5.78	7.88		0.21	0.16		1.49	0.94		0.2	0.27		1.43	0.8		0.02	0.01	
CV%	23.5	33.54		26.52	13.23		32.14	9.63		19.23	12.53		9.6	12.7		18.54	9.43	

PHT: Plant height, SG: Stem girth, RCC: Relative chlorophyll content, GLAM: Number of Green leaves at maturity, DFL: Days to 50% flowering, LFA: Leaf area

3.6.2.1 Comparison of mean values of Yield related parameters of the diverse accessions under well-watered and drought stress conditions and the effect of drought

There was an overall decrease in the means of all yield related traits under drought conditions (Table 3.7). The genotypes that were completely senescent under drought stress were: Gadam, SRN39, GBK 044120, GBK 044063, GBK 016085, GBK 048916 and GBK 044336 (Table 3.7). The mean of productive tillers was 3.32 under well-watered conditions and 0.77 in drought stress (Table 3.7) The overall effect of drought stress on number of productive tillers was 80.72%. The genotype that was most affected in number of viable tillers was GBK 048921 (96.29%), that had 14 viable tillers under well-watered to 0.52 tillers in drought stress conditions. The least affected genotype was ICSV 111 IN (12.04%) that had 0.88 viable tillers under drought stress compared to 1.00 in well-watered conditions (Table 3.7. The panicle exertion an overall mean reduction of 15.10cm in well-watered conditions compared to 8.65cm under drought stress (Table 3.7), the overall effect of drought stress was 42.71%, the most affected genotype was Okabir (91.68%) which had 16.23cm under well-watered compared to 1.35cm under water stress conditions. Genotype GBK 044058 was the least affected by (0.58%) with an overall panicle exertion of 19.00cm under drought stress and to 19.11cm in well-watered conditions (Table 3.7). Panicle length had an overall mean of 24.53cm under water stress compared to 29.17cm under well-watered conditions. (Table 3.7) The overall effect of drought on panicle length was 29.84%. The most affected genotype was GBK 044065 (47.83%) with 41.21cm under well-watered compared to 21.50cm in water stress conditions. The least affected genotype was N13 (1.39%) with 13.44cm under well-watered compared to 13.63cm in water stress conditions (Table 3.7). The overall panicle width was 9.31cm under water stress compared to 12.56cm in well-watered (Table 3.8). The overall effect of drought on panicle width was 25.88%.

The most affected genotype was F6YQ212 (80.23%), the panicle width was 8.55cm in well-watered compared to 1.69cm in water stressed conditions (Table 3.7).

The least affected genotype was ICSV 111 IN (2.51%) with 11.54cm in well-watered conditions compared to 11.25cm under water stressed conditions. Panicle weight had an overall mean of 0.26kg under well-watered compared to 0.23kg under water stress conditions (Table 3.7). The most affected genotype by drought stress was GBK 043565 (41.86%), The mean panicle weight reduced to 0.22kg in drought stress compared to 0.23kg in well-watered conditions. The overall effect of drought was 5.18%. The most affected genotype was GBK 043565(41.86%), the panicle weight reduced from 0.21kg in well-watered conditions to 0.12 kg in drought stress conditions. The least affected genotype in panicle weight was IESV 23006 DL (5.71%) that had a panicle weight of 0.33kg under drought stress compared to 0.35kg in well-watered conditions (Table 3.7). The overall means of hundred seed weight under drought stress was 1.79g compared to 1.96g in well-watered conditions (Table 3.7). The overall effect was 8.44%. The most affected genotype under drought stress was Framida (66.80%) that had a reduction from 3.47 to 1.15, the least affected genotype was KAT/ELM/2016 PL1 SD 15 (2.04%) that had 2.14g in water stressed and 2.19g under well-watered conditions (Table 3.7). The overall yield under drought stress conditions was 1.12tons/ha compared to 1.33tons/ha under well-watered conditions (Table 3.7), the overall effect of drought was 15.80%. The most affected genotype by drought stress was GBK 048917 (85.71%), with the yield being reduced from 0.21 to 0.03t/ha in drought stress. The least affected genotype was CR35`5 (0.93%), the yield was 0.76tons/ha under well-watered and 0.75t/ha in drought stress conditions (Table 3.7)

Table 3. 7: Mean comparisons for yield related parameters in the diverse genotypes

	VT(counts)			PEX(cm)			PAL(cm)			PAW(cm)			PWT(kg)			HSW(g)			YLD(tons/ha)		
GENOTYPE	**WS**	**WW**	**% Effect**	**WS**	**WW**	**% Effect**	**WS**	**WW**	**% Effect**	**WS**	**WW**	**% Effect**	**WS**	**WW**	**% Effect**	**WS**	**WW**	**% Effect**	**WS**	**WW**	**% Effect**
GBK 044058	0.97	4	75.7	19	19.11	0.58	32.8	34.85	5.88	21.86	11.08	-97.29	0.32	0.19	-67.15	1.98	1.32	-50.3	2.31	2.29	-0.87
GBK 048922	0.7	1.5	53.6	25.13	12.66	-98.89	34.88	26.91	-29.62	37.95	27.23	-39.37	0.26	0.19	-36.72	2.59	0.62	-320.65	0.89	1.92	53.65
GBK 047293	0.91	2.83	67.88	13.31	9.05	-47.07	21.69	15.22	-42.51	12.36	7.67	-61.15	0.32	0.15	-108.64	2.62	0.43	-504.85	2.23	2.27	1.76
GBK 043565	0.83	2.92	71.55	11.56	23.62	51.06	34	35.07	3.05	6.52	8.5	23.29	0.12	0.21	41.86	0.31	0.58	46.86	0.26	0.32	18.96
GBK 048917	0.41	2.89	85.81	11.52	16.56	30.43	33.65	38.14	11.77	7.33	10.68	31.37	0.09	0.15	41.83	0.27	0.43	37.69	0.03	0.21	85.71
GBK 016114	0.44	3.58	87.61	18.02	33.02	45.43	29.65	32.88	9.82	17.07	24.45	30.18	0.27	0.19	-40.86	2.21	1.02	-117.77	1.79	1.83	2.09
GBK 044054	0.88	9.15	90.38	13.87	11.96	-15.97	29.17	38.19	23.62	15.12	11.38	-32.86	0.35	0.22	-60.43	2.14	1.47	-45.57	0.96	0.5	-92.4
GBK 048156	0.29	11.25	97.41	7.26	20.92	65.3	31.24	39.14	20.18	3.24	13.57	76.12	0.32	0.19	-69.09	1.91	0.58	-227.6	0.61	0.46	-32.87
GBK 016109	0.46	2.78	83.33	24.62	35.4	30.45	25.42	39.01	34.84	10.41	22.45	53.63	0.3	0.2	-49.66	2.33	2.07	-12.74	0.8	0.52	-53.41
GBK 044065	0.98	7.67	87.18	14.92	24.29	38.58	21.5	41.21	47.83	13.8	29.41	53.08	0.2	0.26	23.08	1.42	1.23	-15.38	1.4	0.5	-179.4
GBK 045827	0.59	8	92.63	6.45	17.98	64.13	18.67	29.48	36.67	5.95	7.68	22.53	0.29	0.19	-56.83	2.14	0.63	-237.11	1.48	0.3	-394
GBK 048152	0.67	0.87	22.99	3.45	7.46	53.75	10.33	18.97	45.55	6.46	10.75	39.91	0.32	0.15	-104.24	2.31	0.43	-431.92	0.79	1.03	23.5
GBK 040577	0.8	1.17	31.21	5.43	12.47	56.46	27.56	35.33	21.99	6.37	15.26	58.26	0.31	0.23	-32.55	2.08	1.02	-104.49	1.22	0.69	-77.16
GBK 048921	0.52	14	96.29	13.5	29.24	53.83	29.91	33.43	10.53	8.32	14.72	43.48	0.23	0.15	-50.47	2.94	0.43	-577.31	1.31	0.94	-38.83
GBK 044448	1	3.92	74.44	21.85	23.8	8.19	24.84	38.35	35.23	21.52	6.25	-244.32	0.28	0.2	-43.85	1.9	0.75	-153.6	0.48	0.48	0.24
GBK 044336	0	1.21	100	0	9.54	100	0	23.53	100	0	12.21	100	0	0.19	100	0	2.31	100	0	0.59	100
GBK 048916	0	3.42	100	0	17.24	100	0	27.61	100	0	9.83	100	0	0.21	100	0	2.22	100	0	1.03	100
GBK 016085	0	2.31	100	0	10.41	100	0	30.42	100	0	11.53	100	0	0.19	100	0	1.97	100	0	1.21	100
GBK 044063	0	4.41	100	0	7.43	100	0	40.04	100	0	9.82	100	0	0.17	100	0	2.36	100	0	0.89	100
GBK 044120	0	3.59	100	0	13.12	100	0	37.33	100	0	17.23	100	0	0.18	100	0	1.92	100	0	0.86	100
SRN39	0	2.38	100	0	11.05	100	0	29.43	100	0	9.81	100	0	0.28	100	0	3.02	100	0	1.96	100
GADAM	0	1.52	100	0	15.62	100	0	21.17	100	0	10.65	100	0	0.31	100	0	3.11	100	0	2.14	100
KARI MTAMA 1	1.02	0.94	-8	0.62	1.66	62.65	19.89	21.34	6.79	6.23	7.47	16.6	0.23	0.3	25.31	1.96	2.55	23.18	1.35	1.77	23.58
IESV23006DL	1.02	3.54	71.19	6.24	11.56	46.02	24	25.33	5.25	10.82	11.18	3.22	0.33	0.35	5.71	3.19	3.15	-1.27	2.36	2.39	1.26
KAT/ELM/2016 PL1 SD-15	0.57	0.84	32.14	2.51	4.63	45.79	20.14	28.12	28.38	5.18	9.21	43.76	0.2	0.3	31.61	2.14	2.19	2.04	2.01	2.13	5.62

	VT(counts)			PEX(cm)			PAL(cm)			PAW(cm)			PWT(kg)			HSW(g)			YLD(tons/ha)		
	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect	WS	WW	% Effect
N13	0.33	0.78	57.69	10	13.2	23.9	13.4	13.63	1.39	12.6	11.42	-10.51	0.2	0.26	22.53	1.95	2.08	6.54	1	1.96	46.94
E36-1	1.06	1.83	42.35	6.73	14.1	52.4	16.9	24.45	31	10.4	9.01	-15.09	0.16	0.18	11.11	1.38	2.68	48.72	1.4	1.42	3.76
B35	1.08	2.67	59.63	25	16.4	-52.1	21	21.64	2.87	15.2	10.05	-51.64	0.18	0.23	21.74	1.64	1.73	5.67	0.8	0.96	19.34
ICSV III IN	0.88	1	12.04	11	17.4	36.97	24.7	30.1	17.94	11.3	11.54	2.51	0.26	0.29	10.44	1.31	3.27	59.99	0.9	1.83	51.84
F6YQ212	0.63	1.58	60.21	11.5	12.2	5.73	14.5	22.17	34.82	1.69	8.55	80.23	0.25	0.25	-1.13	2.65	3.7	28.35	1.1	0.9	-27.14
MACIA	0.66	0.42	-57.15	7.01	5.08	-37.99	18.1	19.89	8.8	4.34	9.59	54.74	0.3	0.29	-2.66	2.15	2.32	7.33	1	1.52	34.64
OKABIR	0.89	3.5	74.68	1.35	16.2	91.68	39.6	27.26	-45.3	20.5	13.53	-51.44	0.27	0.23	-18.27	2.63	2.39	-10.04	1.4	0.58	-139.3
LODOKA	1.03	1.28	19.79	3.34	9.59	65.17	20.9	20.84	-0.24	12.3	9	-36.22	0.28	0.18	-56.89	2.08	1.98	-4.82	2.2	1.93	-15.03
FRAMIDA	0.5	0.86	41.86	7.1	9.18	22.66	32.5	33.46	2.9	10.5	8.86	-17.95	0.23	0.36	36.72	1.15	3.47	66.8	0.4	0.9	54.01
AKUR-ACHOT	1.19	2.28	47.85	3.06	26.4	88.39	17.6	23.87	26.27	12.4	9.5	-30.74	0.29	0.23	-26.09	3.5	2.81	-24.56	2.3	2.05	-13.66
HAKIKA	0.82	6.5	87.35	7.01	16.2	56.67	25.1	38.96	35.47	3.52	12.78	72.46	0.37	0.25	-47.49	2.53	2.73	7.62	0.9	1.47	42.18
IBUSAR	1.14	4.21	73.01	21.1	31.6	33.02	20.5	22.51	8.88	17.4	18.34	5.07	0.26	0.28	9.4	1.68	1.74	3.84	1.7	1.72	1.74
IS 9830	0.35	1.11	68.5	6.43	11.3	43.15	24.8	38.66	35.8	7.45	13.5	44.81	0.17	0.28	40.12	2.45	3.13	21.81	1.5	1.71	11.02
IESV21400DL	1.09	4.45	75.43	7.39	12.5	41.07	25	26.65	6.19	11.7	11.81	0.59	0.29	0.16	-81.25	3.19	3.15	-1.27	0.9	0.76	-21.05
IESV23010DL	1.24	4.35	71.43	10.2	13.5	24.46	24.9	27.45	9.18	13.4	19.52	31.25	0.36	0.34	-5.88	2.66	2.42	-10.04	2.5	2.35	-4.26
IESV92043DL	0.22	0.79	72.04	4.18	7.15	41.54	23.3	30.75	24.16	4.59	10.56	56.53	0.29	0.23	-26.09	3.45	2.63	-31.18	2.4	2.32	-1.29
WAHI	0.2	0.45	55.33	3.79	8.93	57.56	26	30.25	14.05	7.57	12.43	39.1	0.3	0.28	-7.64	2.34	2.36	0.89	1.9	2	6.59
CR35 ' 5	1.04	6.46	83.9	11.6	17.9	35.18	20.7	26.54	22	10.2	13.45	24.24	0.31	0.25	-26.1	1.54	1.58	2.1	0.8	0.76	0.93
KAT/ELM/2016	0.77	0.94	18.49	3.51	5.63	37.66	24.4	28.02	12.88	5.98	9.02	33.7	0.22	0.32	29.61	2.15	2.17	0.91	2	2.12	4.9
Mean	0.64	3.32	80.72	8.65	15.1	42.71	20.5	29.26	29.84	9.31	12.56	25.88	0.22	0.23	5.18	1.79	1.96	8.44	1.1	1.33	15.8
SE±	3.87	3.43	6.82	5.7	3.23	2.87	3.13	3.65	0.12	0.12	0.12	0.14	0.1	0.1							
CV%	32.7	29.6	30	27.4	21.4	11.01	28.7	25.33	28.45	12.33	23.3	21.5	34	30.5							

VT; Number of viable tillers, PEX; Panicle excersion, PAL; Panicle length, PAW; Panicle width, PWT; Panicle weight, HSW; Hundred seed weight, YLD; Grain yield

3.6.3 Genetic variation among sorghum accessions

Out of 38 diverse genotypes (samples from 6 genotypes failed QC), 26,291 raw SNPs were generated. Filtering for quality SNPs was done and 8,101 SNPS were retained for assessing the genetic diversity of the 38 genotypes. Two major clusters were observed, one dominated by wild accessions; and another by cultivated accessions (Figure 3.1). There were 3 sub-clusters of improved varieties within cluster B which suggested that there were high similarities among the improved genotypes. A number of the "wild" accessions clustered with cultivated ones. B35, the most common source of stay-green alleles, clustered between the cultivated and wild accessions while E36-1 which is also a common source of stay green clustered together with cultivated accessions. The selected set of accessions that were used in the study was diverse and likely to enhance the value of the improved varieties if integrated in breeding programs.

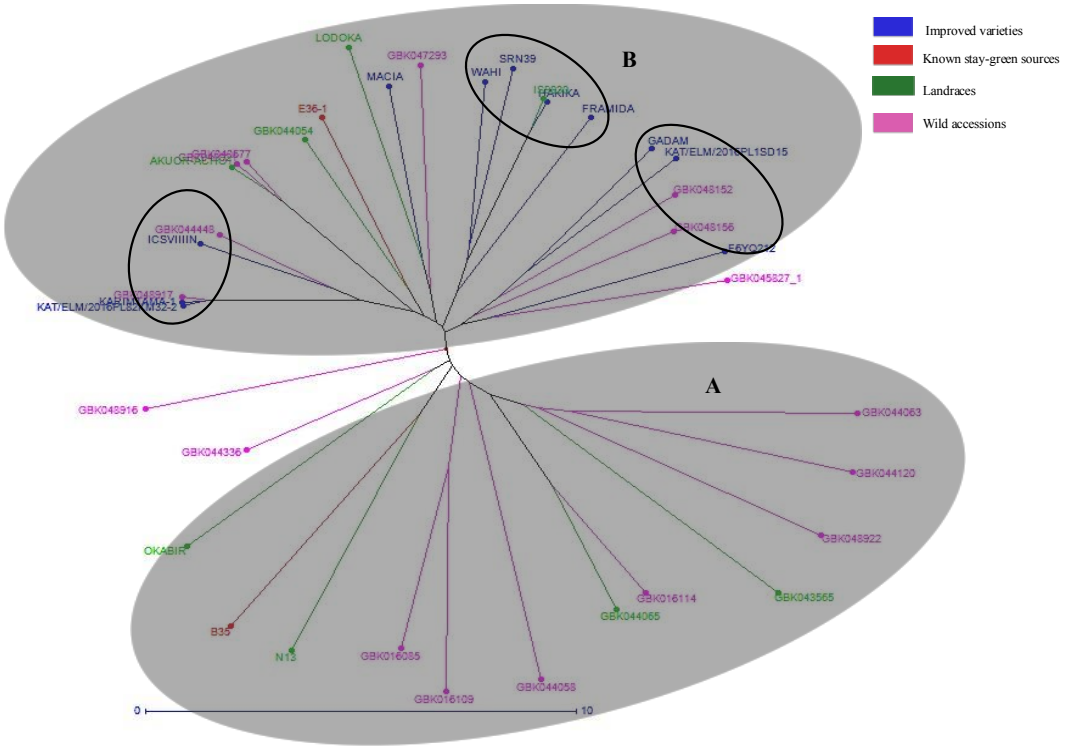


Figure 3. 1: A dendrogram illustrating two major clusters of the 38 genotypes analyzed.

Cluster A had wild and landrace accessions, while cluster B had all of the improved varieties plus some of the wild genotypes and landraces. Three sub-clusters of improved varieties are seen within cluster B.

3.6.4 Molecular markers for Quality Control (QC) and marker-assisted backcrossing (MABC)

Out of the 8,101 SNP markers used for assessing genetic diversity (Figure 3.1), further filtering for no missing SNP data yielded 803 markers, which were used to select a final set of 20 markers which were the most informative for the 38 accessions (Table 3.8). The 20 markers were selected to ensure they were well distributed across the genome and highly polymorphic.

Table 3. 8. The selected set of 20 most informative SNP markers for the 38 accessions

Chromosome	Position	Variant
1	21279335	C/G
1	45984426	C/T
2	7803138	C/T
2	77523709	G/A
3	13455829	A/G
3	57242431	A/G
4	1389787	T/A
4	2600536	C/T
4	3295616	C/G
4	56353491	C/G
4	59715414	T/C
4	65176114	C/G
5	53835336	T/C
5	56501094	G/A
5	61381207	A/G
6	49278472	G/T
6	50201989	C/G
6	57826860	G/A
8	54998738	A/C
9	786078	T/C

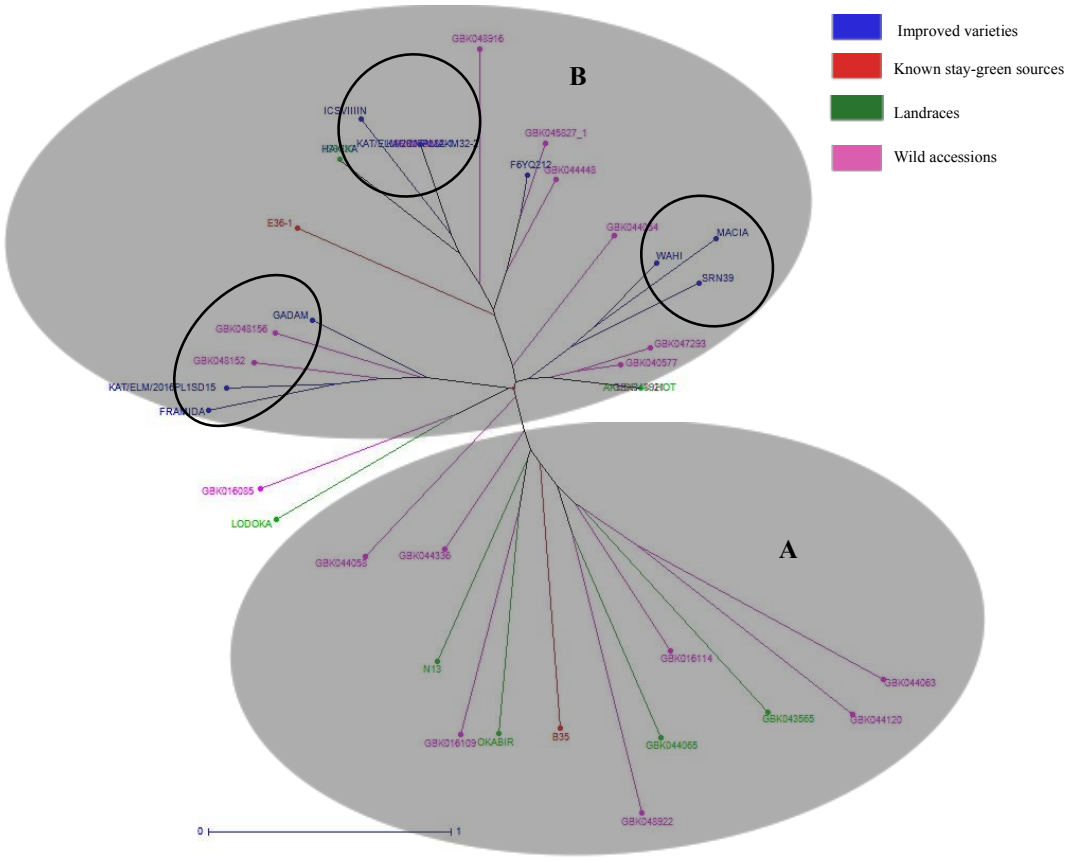


Figure 3. 2: A dendrogram drawn using the 20 selected informative markers. Two clusters previously identified with 803 SNP markers (Figure 3.1) were still revealed.

3.6.5 New sources of Drought tolerance

Nine genotypes (IESV21400 DL, LODOKA, IESV23006 DL, IESV92043 DL, OKABIR, GBK 016109, GBK 048156, IESV23010 DL, AKUOR-ACHOT) outperformed both E36-1 and B35 with respect to their RCC at maturity (Figure 3.3A), while 7 genotypes (LODOKA, OKABIR, IBUSAR, F6YQ212, AKUOR-ACHOT, GBK 047293, GBK 048917) had more GLAM than E36-1 and B35 under drought conditions (Figure 3.3B). Ten (IESV23010 DL, IESV23006 DL, IESV92043 DL, AKUOR-ACHOT, GBK 047293, LODOKA, WAHI, GBK 016114, GBK 045827, OKABIR) of the 18 genotypes that had outperformed B35 when ranked using RCC also yielded better than both E36-1 and B35 (Figure 3.3C). The landrace genotype, LODOKA, stood out as having the highest GLAM and RCC and was also a top yielder, with a yield of 2.2 tons ha^{-1} out of the highest recorded yield of 2.45 tons ha^{-1}. All genotypes that yielded better than E36-1 were considered as potential new sources of functional stay-green.

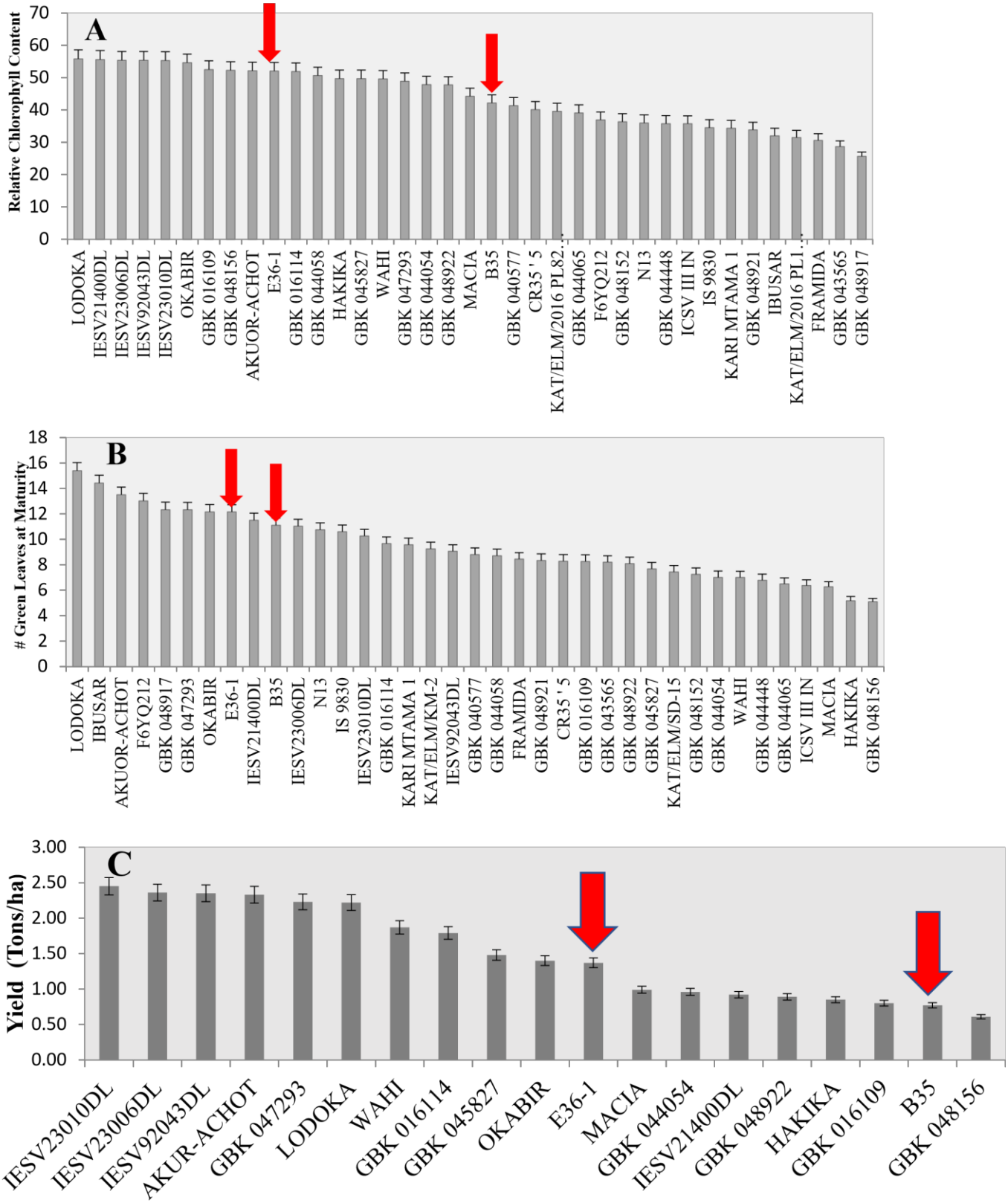


Figure 3. 3: Performance of 37 out of the 44 sorghum genotypes that did not senesce under water stress conditions in comparison with known stay-green sources, E36-1 and B35 as measured using RCC (A), GLAM (B) and Yield (C).

3.6.6 Genotypic and phenotypic variation and heritability estimate

For the 13 quantitative traits, estimates of genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and broad sense heritability across the water regimes were determined (Table 3.9). The PCV estimates were higher than GCV estimates for all the traits. The highest PCV was recorded for productive tillers (99.40%) and the lowest was in grain yield (2.63%), the highest GCV was recorded in days to flowering (66.52%), the lowest GCV was in grain yield (2.46 %). Days to 50% flowering had the highest broad sense heritability estimate of 98.40% while the lowest was recorded in the number of productive tillers (44.32%).

Table 3. 9: Heritability, Phenotypic and genotypic variation estimates of all traits measured under drought stress conditions

	Range	σ^2G	σ^2P	GCV (%)	PCV (%)	H^2 bs (%)
PHT	65-235.7	2594.43	2798.17	33.63	34.92	92.72
YLD	0.26-2.45	0.36	0.41	2.46	2.63	87.68
RCC	25.63- 55.84	1.13	1.87	63.89	82.38	60.14
HSW	0.31-3.50	0.24	0.31	4.54	5.13	78.41
PWT	6.08-11.50	0.14	0.19	3.38	1.98	72.66
LFA	0.01-0.07	0.03	0.04	12.78	14.27	80.15
GLAM	6.08-11.50	2.08	3.27	3.45	4.33	63.71
DFL	30-103	70.66	71.81	66.52	67.06	98.4
SG	0.82-2.28	0.16	0.18	24.79	25.88	92.45
VT	0.2-1.24	0.27	0.62	66.18	99.4	44.32
PEX	0.62-25.18	48.96	61.5	64.85	72.68	79.61
PAL	10.33-39.61	19.93	23.64	18.59	20.24	84.32
PAW	1.69-37.95	14.05	16.89	32.51	35.65	83.15

PHT: Plant height, YLD: Grain yield, RCC: Relative chlorophyll content, HSW: Hundred seed weight, PWT: Panicle weight, FLA: Leaf area, GLAM: Green leaves at maturity, DFL: Days to 50% flowering, SG: Stem girth, PEX: Panicle exertion, PAL: Panicle length, PAW: Panicle width

3.6.7 Correlation Analysis

Under drought stress conditions, plant height was positively and significantly correlated to stem girth (0.31), leaf area (0.28), green leaves at maturity (0.48), panicle exertion (0.52), panicle length (0.42), panicle width (0.38), hundred seed weight (0.63), grain yield (0.64), relative chlorophyll content (0.75), days to flowering (0.55) and negatively significantly correlated to panicle weight (-0.81) (Table 3.10).

Stem girth was positively significantly correlated to panicle exertion (0.43), panicle length (0.39), panicle width (0.43), panicle weight (0.40), grain yield (0.28), relative chlorophyll content (0.33) and days to flowering (0.40) (Table 3.10)

Number of productive tillers was positively significantly correlated to panicle width (0.25), grain yield (0.36) and negatively significantly correlated to relative chlorophyll content (-0.29) (Table 3.10)

Leaf area was positively significantly correlated to number of green leaves at maturity (0.44), and negatively significantly correlated to panicle exertion (-0.36), panicle length (-0.33), panicle width (-0.20), panicle weight (-0.38), grain yield (-0.34), relative chlorophyll content (-0.64), days to 50% flowering (-0.54) (Table 3.10)

The number of green leaves at maturity was positively significantly correlated to panicle exertion (0.42), panicle length (0.53), panicle width (0.44), panicle weight (0.61), hundred seed weight (0.66), grain yield (0.55), relative chlorophyll content (0.71) and days to flowering (0.68) (Table 3.10)

Panicle exertion was positively significantly correlated to panicle length (0.52), panicle width (0.65), panicle weight (0.37), hundred seed weight (0.33), grain yield (0.45), relative chlorophyll content (0.46) and days to 50% flowering (0.38) (Table 3.10)

Panicle length was positively significantly correlated to panicle width (0.64), panicle weight 0.83, hundred seed weight (0.51), grain yield 0.65**, relative chlorophyll content (0.82), and days to 50% flowering (0.82) (Table 3.10)

Panicle width was positively and significantly correlated to panicle weight (0.47), grain yield (0.31), relative chlorophyll content (0.31) and days to flowering (0.48) (Table 3.10)

Hundred seed weight was significantly positively correlated to grain yield (0.73), relative chlorophyll content (0.85) and days to 50% flowering (0.42) (Table 3.10)

Grain yield was significantly positively correlated to relative chlorophyll content (0.78), days to 50% flowering (0.35) (Table 3.10). The relative chlorophyll content was positively significantly correlated to days to 50% flowering (0.66) (Table 3.10)

Table 3. 10: Phenotypic correlations of the traits under drought stress conditions

	PHT	SG	VT	FLA	GLAM	PEX	PAL	PAW	PWT	HSW	YLD	RCC
PHT												
SG	0.31*											
VT	0.14ns	0.10ns										
FLA	0.28*	0.13ns	0.16ns									
GLAM	0.48*	0.25*	0.18ns	0.44*								
PEX	0.52**	0.43	0.08ns	-0.36*	0.42*							
PAL	0.42*	0.39*	0.13ns	-0.33*	0.53**	0.52**						
PAW	0.38*	0.43*	0.25*	-0.20*	0.44*	0.68**	0.64**					
PWT	-0.81***	0.40*	-0.02ns	-0.38*	0.61***	0.37*	0.83**	0.47*				
HSW	0.63***	0.21	0.11ns	-0.19ns	0.66***	0.33*	0.51*	0.19ns	0.74***			
YLD	0.64***	0.28*	0.36*	-0.34*	0.55***	0.45*	0.65**	0.31*	0.66***	0.73***		
RCC	0.75***	0.33*	-0.29*	-0.64**	0.71***	0.46*	0.82***	0.39*	0.82***	0.85***	0.78***	
DFL	0.55**	0.40*	0.13ns	-0.54**	0.68***	0.38*	0.82***	0.48**	0.36*	0.42*	0.35*	0.66***

$P \leq 0.05$; * $P \leq 0.01$; ** $P \leq 0.001$***

3.6.8 Performance of F_2 Genotypes for key agronomic traits under drought stress conditions

The mean value of F_2 genotypes is presented in (Table 3.11). Earliness in days to 50% flowering was exhibited in the F_2 cross; ICSV 111 IN*LANDIWHITE (35 days), the latest flowering was IBUSAR*LANDIWHITE (82 days). The largest flag leaf area was exhibited for the AKUOR-ACHOT*IBUSAR cross (0.08m^2) and the smallest was in LODOKA*ICSV 111 IN (0.01 m^2) show in Table 3.11. The number of green leaves at maturity was highest in LODOKA*LANDIWHITE (15.67 leaves) and lowest in AKUOR-ACHOT*ICSV 111 IN (5.23 leaves) Table 3.11. The relative chlorophyll content was highest in B35 X LODOKA (51.53) and lowest in F6YQ212 X LODOKA (33.17) Table 3.11. Panicle weight was highest in B35 X LODOKA (0.35kg) and lowest in ICSV 111 IN X MACIA (0.22KG) Table 3.11. The highest hundred seed weight was in OKABIR X AKUOR-ACHOT (3.27g), the lowest was in B35 X LANDIWHITE (1.1.) Table 3.11. Grain yield was highest in AKUOR- ACHOT X LODOKA (2.88t/ha) and lowest in LODOKA X OKABIR (0.42t/ha) Table 3.11

Table 3. 11: Mean performance of F_2 genotypes under drought stress conditions

GENOTYPE	PHT	GLAM	DFL	RCC	FLA	PWT	HSW	YLD
LODOKA X OKABIR	116.17	10.83	52.00	38.68	0.04	0.27	1.42	0.65
LODOKA X ICSV 111 IN	158.17	9.17	41.00	47.15	0.05	0.33	2.38	0.88
LODOKA X LANDWHITE	249.00	15.67	79.00	50.18	0.04	0.29	3.03	2.88
LODOKA X E36-1	181.33	11.67	51.00	47.55	0.06	0.33	2.38	1.79
ICSV 111 IN X MACIA	136.87	6.55	79.00	35.55	0.04	0.22	1.27	0.45
ICSV 111 IN X LANDWHITE	145.67	9.67	35.00	44.03	0.03	0.28	2.23	1.54
ICSV 111 IN X B35	102.33	7.50	55.00	45.30	0.05	0.33	2.47	1.54
ICSV 111 IN X E36 - 1	132.17	9.33	36.00	40.05	0.04	0.32	2.00	0.52
ICSV 111 IN X LODOKA	206.50	12.00	48.00	41.27	0.07	0.27	2.13	1.79
ICSV 111 IN X OKABIR	231.00	11.50	53.00	39.77	0.08	0.24	2.78	1.13
ICSV 111 IN X LODOKA	232.00	11.00	49.00	40.00	0.04	0.28	2.73	0.65
ICSV 111 IN X F6YQ212	116.33	8.83	38.00	33.58	0.03	0.27	2.22	4.25
B35 X LODOKA	141.50	13.00	54.00	51.53	0.04	0.35	3.12	0.69
B35 X AKUOR ACHOT	148.83	11.67	45.00	40.63	0.05	0.32	2.18	1.69
B35 X F6YQ212	60.00	10.83	55.00	39.63	0.04	0.24	2.78	0.80
B35 X LANDIWHITE	147.17	12.00	50.00	33.43	0.03	0.23	1.15	0.42
B35 X E36-1	133.83	11.00	55.00	44.22	0.05	0.33	2.47	1.38
B35 X ICSV 111 IN	131.33	8.57	41.00	42.25	0.03	0.26	1.61	0.92
B35 X OKABIR	190.00	14.33	52.00	37.68	0.05	0.23	1.33	0.71
LANDIWHITE X E36-1	163.83	10.33	57.00	44.03	0.02	0.33	2.50	0.96
LANDIWHITE X MACIA	115.17	9.50	38.00	43.28	0.03	0.33	2.05	0.92
LANDIWHITE X B35	123.00	11.17	42.00	42.75	0.03	0.27	2.07	0.92
LANDIWHITE X F6YQ212	127.33	11.33	51.00	42.02	0.03	0.27	1.92	0.71
OKABIR X ICSV 111 IN	179.53	11.00	41.00	42.82	0.05	0.27	2.07	0.92
OKABIR X LODOKA	159.50	5.83	40.00	43.67	0.01	0.29	2.35	1.71
OKABIR X B35	162.54	8.55	45.00	43.60	0.06	0.29	2.35	0.88
OKABIR X AKUOR ACHOT	186.34	7.77	38.00	33.30	0.03	0.25	3.27	0.76
F6YQ212 X B35	74.50	5.25	47.00	45.97	0.03	0.26	2.31	1.03
F6YQ212 X OKABIR	131.33	10.83	52.00	44.87	0.04	0.34	2.50	0.92
F6YQ212 X E36-1	103.00	8.17	40.00	39.73	0.05	0.24	2.78	1.94
F6YQ212 X IBUSAR	207.50	10.33	48.00	44.15	0.04	0.34	2.53	2.19
F6YQ212 X LODOKA	163.67	11.50	52.00	33.17	0.04	0.23	1.15	0.42
IBURSAR X LANDWHITE	172.54	12.00	82.00	40.58	0.04	0.32	2.00	0.87
IBUSAR X ICSV 111 IN	154.17	9.17	51.00	40.43	0.02	0.26	2.05	0.92

GENOTYPE	PHT	GLAM	DFL	RCC	FLA	PWT	HSW	YLD
IBURSAR X E36-1	125.63	11.00	46.00	49.50	0.01	0.29	2.58	0.96
IBUSAR X MACIA	138.67	12.17	53.00	41.23	0.04	0.27	2.13	1.69
E36-1 X MACIA	126.67	12.33	56.00	41.57	0.02	0.27	2.13	1.79
E36-1 X IBUSAR	119.25	10.00	48.00	43.18	0.06	0.29	2.35	0.88
E36-1 X LANDIWHITE	227.67	11.50	53.00	39.10	0.04	0.24	2.68	0.85
E36-1 X AKUOR ACHOT	196.00	11.33	49.00	39.43	0.07	0.24	2.68	1.79
E36-1 X LODOKA	206.83	9.77	47.00	47.35	0.03	0.34	2.39	0.85
E36-1 X B35	142.00	13.67	52.00	42.47	0.06	0.27	1.90	0.88
AKUOR ACHOT X ICSVIIN	202.00	5.23	41.00	39.10	0.02	0.23	2.53	1.00
AKUOR ACHOT X OKABIR	212.17	12.67	53.00	45.13	0.05	0.33	2.47	1.38
AKUOR ACHOT X B35	190.33	12.83	52.00	42.17	0.07	0.27	1.98	1.67
AKUOR ACHOT X LODOKA	226.33	12.67	59.00	39.25	0.05	0.24	2.68	0.85
AKUOR ACHOT X IBUSAR	224.67	12.50	51.00	44.47	0.07	0.34	2.40	1.29
Mean	160.05	10.54	50.04	41.93	0.04	0.28	2.27	1.20
CV	38.43	25.23	28.55	25.67	19.63	27.52	31.64	36.42

3.6.8.1 General combining ability

In Table 3.12, General combing ability estimates for the traits varied among the parents exhibiting both positive and negative estimates. For days to 50% flowering, significant ($P<0.05$) GCA estimates among the female parents ranged from -2.27 (B35) to 4.03 (Ibusar). GCA estimates for number of green leaves at maturity ranged between 0.58 (Lodoka) to 0.01 (Landiwhite). A hundred seed weight GCA estimates ranged from 0.31(Akuor-Achot) to -0.26 (B35). Leaf area recorded significant ($P<0.05$) GCA estimates ranging from 0.74 (Ibusar) and -0.68 (F6YQ212). GCA estimates for plant height ranged from 2.68 (Akuor-Achot) to -1.68 (B35). GCA estimates for panicle weight ranged from 2.57 (Akuor-Achot) to -2.74 (ICSV 111 IN). GCA estimates for the relative chlorophyll content ranged from 0.78 (LODOKA) to -0.79 (F6YQ212). GCA estimates for grain yield ranged from 1.19 to -1.09 (B35) (Table 3.12).Among the male parents (Table 3.12), significant ($P<0.05$) GCA effects for days to 50% flowering ranged between -3.54 (ICSV 111 IN) to 3.16(Landiwhite). Number of green leaves at maturity ranged between

0.13 (Landiwhite), and -0.04 (F6YQ212). Hundred seed weight ranged between 1.82 (Ibusar) and -1.57 (ICSV 111 IN). Leaf area ranged between 0.03 (Ibusar) and -0.05 (ICSV 111 IN).

Plant height ranged between 1.56 (Lodoka) and -2.29 (B35). Panicle weight estimates ranged between 6.53 (Okabir) and -4.27 (Landiwhite). Relative chlorophyll content ranged from 0.53 (Ibusar) to -1.14 (ICSV 111 IN). Grain yield ranged between 3.01 (Okabir) and -2.95 (ICSV 111 IN).

Table 3. 12: General combining ability estimates

	DFL	GLAM	HSW	FLA	PHT	PWT	RCC	YLD
GCA FEMALES								
AKUOR-ACHOT	2.75**	0.08**	0.31**	0.04	2.68**	2.57**	0.04**	1.16**
B35	-2.27**	0.07	-0.26**	0.13	-1.68**	-2.54*	-0.29*	-1.09*
E36-1	-1.61**	0.37	0.19	-0.48	0.93*	1.28*	0.57**	0.89*
F6YQ212	-1.55**	0.49**	0.15	-0.68**	-0.83	1.01	-0.79**	0.41
ICSV 111 IN	6.57	0.31	0.19**	-0.51	-1.41	-2.74**	-0.31	-0.82**
LODOKA	4.04	0.58**	0.09	0.62**	0.04*	-1.15	0.78**	-0.33
OKABIR	3.04	0.54	-0.07	-0.55	0.13	-1.12	0.64	-0.23
LANDIWHITE	5.24	0.01**	0.08	-0.11	0.92	0.27**	0.64	0.24**
IBUSAR	4.03	0.23**	-0.12	0.74**	0.78	-0.72	0.43	-0.24
GCA MALES								
AKUOR-ACHOT	2.26*	-0.02**	-0.53	0.01	0.45**	1.64*	0.10**	0.57*
B35	-0.21	-0.05	-0.36	-0.01*	-2.29**	-3.15	0.03**	-0.86
E36-1	-1.14*	0.01	0.56	-0.02	-1.57	1.41	0.34**	0.90**
F6YQ212	-0.57	-0.04*	0.51	0.01*	-0.73**	2.64**	0.19*	0.95
ICSV 111 IN	-3.54**	0.11**	-1.57**	-0.05**	-0.11*	7.28**	-1.14*	-2.95**
LODOKA	0.05**	0.03**	0.48	-0.02	1.56**	2.73**	0.18**	0.75**
OKABIR	1.33	0.05**	1.79	0.01*	1.35**	6.53**	0.11	3.01**
LANDIWHITE	3.16**	0.13**	1.05**	-0.04	0.95**	-4.27**	0.23	-1.53
IBUSAR	0.06	0.03**	1.82**	0.03***	1.07**	5.90**	0.53**	2.86**

* Significance at P< 0.05, ** Significance at P< 0.01, DFL: Days to flowering, GLAM: Number of green leaves at maturity, HSW: Hundred seed weight, FLA: Leaf area, PHT: Plant height, PWT: Panicle weight, RCC: Relative chlorophyll content, YLD: Grain yield

3.6.8.2 Specific combining ability

Specific combining ability effects were different among crosses for each trait (Table 3.13). Specific combining ability (SCA) effects in the desirable direction were recorded in the crosses for the various traits. Positive SCA effects were desirable for all traits except for days to flowering. The cross involving F6YQ212 X ICSV 111 IN parental lines had the highest significant (P<0.05) positive SCA effects for grain yield (1.08), whereas AKUOR-ACHOT X OKABIR had significant (P<0.05) positive SCA effects for 100 seed weight (0.45). Significant (P<0.05) positive SCA effects for relative chlorophyll content was recorded in LODOKA X B35 (3.26) and in ICSV 111 IN X LODOKA for panicle weight (0.14). The cross involving LODOKA X AKUOR- ACHOT recorded the highest significant (P<0.05) positive SCA effects for plant height (32.16). Significant (P<0.05) negative SCA effects were recorded in the cross involving B35 X OKABIR (-0.28) for days to flowering (Table 3.13).

Table 3. 13: specific combing ability effects

GENOTYPES	DFL	GLAM	HSW	FLA	PHT	PWT	RCC	YLD
AKUOR-ACHOT X B35	-2.24**	-0.05	-0.06**	0.02	-18.79	0.13*	-0.68	0.12
AKUOR-ACHOT X E36-1	-1.47**	0.17	0.14	0.13	20.29**	0.23**	0.33	0.17
AKUOR-ACHOT X OKABIR	-7.69**	-1.15	0.45**	-0.14*	13.66**	-0.15	-4.28*	-0.31
IBUSAR X AKUOR-ACHOT	1.01	1.41	-0.18	0.08	31.16**	0.12	-0.53	-0.17
IBUSAR X E36-1	2.18	-0.98	-0.19	0.03	-23.7	-0.10*	-0.91	-0.29
IBUSAR X F6YQ212	-2.88*	0.75	0.05	0.07	28.32**	0.01	2.64**	0.33
B35 X E36-1	3.23	1.57	-0.17	0.01**	-12.58	0.32**	0.31	-0.14*
B35 X F6YQ212	-5.33*	-3.47**	-0.07	0.14	-20.94	-0.11	1.16	-0.20
B35 X ICSV 111 IN	3.23	-1.57	0.09	0.16	-32.69	0.22**	1.67	0.14
B35 X OKABIR	-0.28**	-0.59	0.14	0.09	5.82**	0.17	2.03	-0.20
B35 X LANDIWHITE	-2.61**	0.48	0.08	0.04	-17.95	0.19	1.47	-0.27
E36-1 X F6YQ212	-1.55**	-0.42	0.18	0.06	-29.57	0.13	-1.23	0.34
E36-1 X ICSV 111 IN	-10.11**	-0.17	-0.12	0.07	-16.36	0.24*	-1.05	-0.45
E36-1 X LODOKA	4.67	1.56	0.32**	0.01**	8.73	0.25*	3.02**	0.49**
E36-1 X LANDIWHITE	4.28*	0.43	-0.16	0.14	25.45**	0.21	-0.63**	-0.27
F6YQ212 X ICSV 111 IN	-9.00**	-0.59	0.03	0.13	-22.17	0.24	-1.41	1.08**

F6YQ212 X LANDIWHITE	3.06	0.41	0.07	0.12	20.59**	-0.11	-0.67**	-0.27
ICSV 111 IN X AKUOR-ACHOT	-5.13**	-3.56**	0.14	-0.01**	20.89**	-0.12	-1.49	-0.17
ICSV 111 IN X IBUSAR	1.09	-0.94	-0.05	0.16	1.85**	-0.21	-2.88	0.03
ICSV 111 IN X LODOKA	-4.75**	-0.89	0.05	0.15	-2.89**	0.14	2.62*	-0.24

GENOTYPES	**DFL**	**GLAM**	**HSW**	**FLA**	**PHT**	**PWT**	**RCC**	**YLD**
ICSV 111 IN X OKABIR	-3.19**	0.71	-0.15	0.21**	-11.88	0.13	0.28	-0.05
LODOKA X AKUOR-ACHOT	2.18	1.22	-0.13	0.23**	32.16**	-0.22	-2.43	0.19
LODOKA X B35	10.35**	2.27**	0.23	0.16	-13.67	0.26*	3.26**	-0.03
LODOKA X F6YQ212	0.24	-0.44	-0.3	0.13	1.17	0.22	-1.97	-0.36
LODOKA X OKABIR	-7.15**	-2.76**	0.31	0.11	-3.15**	0.23*	1.38	0.13
MACIA X IBUSAR	-0.66**	2.49**	0.14	0.18**	9.41	0.31**	1.73	0.57**
MACIA X E36-1	1.67	1.27	-0.06	-0.01	-21.34	-0.30*	-0.52	0.26
MACIA X ICSV 111 IN	19.95**	-2.36**	-0.44*	0.13	-13.89**	-0.28	-3.37	-0.49
MACIA X LANDIWHITE	-7.28**	-1.24	-0.11	-0.23	2.68**	0.25	0.64	-0.22
OKABIR X F6YQ212	1.97	0.14	0.22	0.01*	-6.89	0.19	2.87**	0.35
OKABIR X ICSV 111 IN	-0.37**	0.98	0.35	0.02*	31.68**	-0.14**	-2.51	-0.22
OKABIR X LODOKA	-0.37**	0.13	-0.38	0.06	-27.98	0.16	-1.76	-0.38
LANDIWHITE X IBUSAR	10.27*	0.89	-0.03	0.05	-18.77	0.23**	0.26	-0.02**
LANDIWHITE X ICSV 111 IN	-10.74**	0.19	-0.05**	0.03	-5.72**	0.25**	0.20	0.41**
LANDIWHITE X LODOKA	18.05**	3.28***	0.06	0.16**	23.35**	0.28**	2.25*	0.29*

3.7 Narrow Sense Heritability

For the 13 traits, the narrow-sense heritability ranged from 26.39% -58.18% (Table 3.14). The highest heritability estimate (58.18%) was recorded for Panicle length, the lowest heritability estimate (26.39%) was recorded for stem girth.

Table 3. 14: Narrow sense heritability estimates

Trait	Narrow sense heritability(h^2)
PHT	47.02
SG	26.39
FLA	37.73
RCC	44.77
GLAM	51.74
DFL	52.37
VT	55.92
PEX	54.75
PAL	58.18
PAW	54.72
PWT	41.70
HSW	43.21
YLD	45.82

PHT: Plant height, RCC: Relative chlorophyll content, YLD: Grain yield, RCC: Relative chlorophyll content, HSW: Hundred seed weight, PWT: Panicle weight, FLA: Leaf area, GLAM: Green leaves at maturity, DFL: Days to 50% flowering, SG: Stem girth, Viable tillers, PEX: Panicle exertion, PAL: Panicle length, PAW: Panicle width

3.8 DISCUSSSION

Phenotypic characterization

Analysis of variance revealed significant ($P \leq 0.05$; $P \leq 0.01$) differences among the genotypes for the studied traits in the individual trials. The various water regimes also led to significant differences in the traits observed, except in the case of flag leaf area (FLA),

this could be as a result of the units of measurement and the stage of induction of the water stress. The function of flag leaf during drought is still unknown for cereals, although the flag leaf functionality is known to be a prerequisite for grain filling (Biswal and Kohli, 2013). To understand the relationship between yield and FLA, results from this study still need validation.

The landrace LODOKA showed superior performance under drought in respect to RCC and GLAM, however it was not the highest yielding (Figure 3.3A). The improved varieties; IESV23006 DL IESV203010 DL, and IESV92043 DL had the highest yields (Figure 3.3C). To improve the yields of these drought tolerant landraces, there is need to enhance their overall genetics. This is possible through improving the agronomic traits of the landraces or selecting them under intense cultivation. Of the genotypes that yielded better under drought, most were wild or landraces. suggesting there are other traits in these accessions that could have influenced their performance. These traits will need to undergo further studies to generate more supporting data generated before making conclusions. Other factors responsible for yield losses under well irrigated conditions need further investigation. The stay-green related traits RCC (0.60) and GLAM (0.64) had high heritability values, this is an indication of a high genetic control. In various crops, the heritability estimates have been variable, in a study involving progenies of B35, (Walulu et al., 1994) and (Mkhabela, 1995) gave reports of high and low heritability values respectively. These variations could be due to the parameters measured and environmental inconsistencies. This high heritability found in our study will need validation using larger bi-parental populations created using the new sources identified.

For the RCC, GLAM and yield related traits, the high significant positive correlation coefficients observed are consistent with previous reports in both sorghum (Borrell et al., 2000; Kassahun et al., 2010; Jordan et al., 2012; Borrell et al., 2014; Kamal et al., 2019) and other cereals (Bekavac et al., 2006; Kamal et al., 2019), they confirm that the identified stay-green genotypes are functional.

Molecular characterization

This study revealed the importance of including wild and landrace accessions for improving important traits in breeding programs. In determining the genetic relationship among the germplasm used through molecular characterization, two major clusters were observed (Figure 3.1, Figure 3.2), with Cluster A containing landrace and wild accessions, cluster B had all of the improved varieties and some wild accessions and landraces. This revealed the genetic relationship existing between landraces, wild and improved genotypes which will be important in future parental selection aiming at improving the diversity within the breeding programs. This in in line with a recent study in Sorghum by (Disasa et al., 2016) where the genetic analyses showed that the unexploited landrace collections and commonly used breeding lines were similar (Mofokeng et al., 2014; Disasa et al., 2016; Upadhyaya et al., 2019) .The results in this study showed clustering of wild and cultivated germplasm (Figure 3.1, Figure 3.2), some studies have reported this, (Mutegi et al., 2010; Mutegi et al., 2011; Sagnard et al., 2011) this indicates the gene flow between the two groups, this could have agronomic and ecological issues (Ohadi et al., 2018). The ability of the wild relatives' accessions used in this study to cluster differently (Figure 3.1 and Figure 3.2) shows that wild sorghum is highly diverse, previous studies by Sagnard et al., (2011), Billot et al., (2013) and Mace et al., (2013) have also revealed this, this has made wild Sorghum an important source of resistance to biotic (Wang et al., 2014; Mbuvi et al., 2017) and abiotic traits (Cowan et al., 2020), including staygreen.

From the molecular characterization results in this study, they illustrate the possibility of finding more stay-green sources from wild accessions with the 5 (GBK045827, GBK016114, GBK048922, GBK016109, GBK047293) promising wild accessions clustering differently from B35 and E36-1. The oldest of stay green source is B35, which is a BC1 derivative of IS12555, durra sorghum from Ethiopia (Subudhi et al., 2000) while E36-1 was derived from

the Ethiopian zera-zera germplasm collection (Thomas and Ougham, 2014). There is need to study and fully understand the genetic control of stay-green in these new sources. In table 3.8, we identified a set of 20 SNP markers that are highly polymorphic and well distributed across the genome, linkage drag is a valid issue in utilization of the wild accessions (Zamir, 2001), the SNP marker set developed here for QC, will help reduce linkage drag to some extent until markers linked to relevant QTLs have been identified for the new sources of stay-green identified in this study. These markers will promote parentage verification, germplasm characterization and confirming the purity of genotypes. These markers need to be developed into quick assays as has been done in other crops (Chen et al., 2016; Ertiro et al., 2015; Ndjiondjop et al., 2018; Gemenet et al., 2020) for better application

General Combing Ability effects

A decreasing GCA effect would be desirable for maturity, an increasing effect would be best for traits related to grain yield. Desirable significant negative GCA effects for days to flowering were detected in female parent E36-1 and male parent ICSV 111 IN. Negative significant GCA effects for days to flowering suggests that parents have the earliness trait that can be exploited for developing drought stress evading hybrids. Previous research has reported negative GCA effects for days to flowering (Girma et al., 2011; Sally et al., 2017, Mengf et al., 1988, Siddiqual and Baig, 2001) and have advocated for use of genotypes with significant negative GCA effects to confer earliness in sorghum. The GCA effects prediction can be used in selection of potential parents for desired traits hence the best crosses could be obtained from parents with high desirable GCA effects (Baker, 1978). This was illustrated in the crosses with high relative chlorophyll content and number of green leaves at maturity mean scores which had Lodoka (with desirable GCA for relative chlorophyll content and green leaves at maturity) as female parent. Female line B35 and male line ICSV 111 IN had the best desirable negative GCA effects for imparting earliness. Desirable positive GCA effects for panicle weight and grain

yield were exhibited by male parent Okabir and female parent Akuor- Achot which correspondingly had the best crosses with higher means for panicle weight and grain yield. Male parent Ibusar and female parent AkuorAchot with desirable significant GCA effects for 100 grain mass would be ideal for breeding for improvement of this trait. The genotype Ibusar had significant desirable positive GCA effects for leaf area as both male and female. Medium and short statured cultivars are preferred for addressing difficulties in harvesting. Female parent B35 had significant negative GCA effects for plant height, this would be the best for breeding for height reduction. This study revealed that none of the genotypes used is a good combiner for all the traits evaluated. This is an indication of the genetic diversity that was present among the genotypes.

This is in agreement with (Panwar et al., 2005, Singh et al., 2007) who reported similar findings. With a large unexploited regional Sorghum gene pool, it is possible to find better parents to generate genotypes with desirable drought tolerance traits, high yield and early to medium maturity as this study has shown.

Specific Combining Ability Estimates

Specific combining ability estimates (SCA) provide the measure of deviation of a cross from the mean performance of the parental genotypes (Sally and Odongi, 2017). Selection of a cross will therefore depend on it's per se grain yield, high SCA effects and positive effects of yield components. On this basis, the cross of OKABIR X ICSV 111 IN with significant negative SCA effects for days to flowering would be best to advance for earliness. The Cross of F6YQ212 X ICSV 111 IN had high significant positive SCA effects for grain yield and would be the best for selection for grain yield. The cross of AKUOR-ACHOT X B35 had high significant negative SCA estimates for plant height and would be the best for selection for short plant stature. The cross of LODOKA X B35 would be best for selection for high relative chlorophyll content under drought while ICSV 111 IN X AKUORACHOT will be best for

selection for high number of green leaves at maturity due to the high positive significant SCA estimates. The AKUOR- ACHOT X B35 cross gave high SCA effects for leaf area, ICSV 111 IN X LODOKA gave high SCA effects for panicle weight,

AKUOR-ACHOT X OKABIR had the highest SCA effects for hundred seed weight making them the best for selection for these traits under drought stress conditions.

3.9 CONCLUSION

These results revealed that there is great potential to discover more sorghum genotypes that are drought tolerant in eastern Africa. Wild accessions and local landraces will still be a valid source of novel alleles in the region for stay-green, and other important traits too. The developed SNP markers will act as the first molecular toolkit for most of the breeders in the region, who currently use mainly classical breeding. This work has been published in the crop science journal (https://doi.org/10.1002/csc2.20300)

CHAPTER FOUR; MARKER ASSISTED BACKCROSSING TO INTROGRESS STAY GREEN FROM MAPPED DONOR LINES INTO FARMER PREFERRED VARIETIES

4.1 Abstract

Drought remains an important abiotic stress factor in crop production. Drought tolerance is a quantitative trait which is often affected by the environment and conventional breeding for it is difficult. Deploying molecular markers that are linked to the stay green QTLs which confer drought tolerance into conventional breeding programs helps to accelerate the efficiency of conventional breeding. In the present study, the objective was to introgress the stay green QTLs from mapped donor lines (E36-1 and B35) into the genetic backgrounds of Kari Mtama1 and Gadam in a backcrossing program. Hand emasculation was used in generation of the crosses. Three independent crosses were made which included Kari Mtama1 X E36-1, Kari Mtama1 X B35 and Gadam X B35. The F_1 genotypes generated were used to pollinate the respective recurrent parents to get BC_1F_1 progenies. A total of 46 BC_1F_1 progenies were generated, and these were genotyped by DArT markers to identify QTL introgression and percentage recovery of the recurrent parents used. From the cross of Kari Mtama1 X E36-1 BC_1F_1, 12 out of 22 backcrosses had a high percentage recovery of the recurrent parent genome that ranged from 22%-30%, 6 progenies from the cross of Kari Mtama1 X B35 BC_1F_1 had 22%-31% recovery of the recurrent parent genome while 2 progenies of Gadam X B35 BC_1F_1 had 29-40% recurrent parent recovery. The 20 genotypes that had the introgressed stay green QTL and had a high recovery proportion of the recurrent parent genome will be advanced to generate more backcross generations.

Key words: Stay green, DArT, QTL Introgression, Marker assisted backcrossing, Recurrent parent, Donor parent

4.2 Introduction

Sorghum [Sorghum bicolor (L.) Moench] is a valuable source of food and nutrition to many people in the semi-arid regions. (Reddy et al., 2010). For the introgression of drought tolerance genes to sorghum, conventional breeding has been successful but the strong environmental influence on its expression has made the process complex (Ejeta, 2007). Efforts in crop genomics have led to identifying molecular markers that are linked to the trait(s) of interest, these can be used in selecting superior lines in a breeding program. Selection using markers is good for improving breeding prospects especially for assessing complex agronomically important traits (Robert et al., 2001) like drought. The DArTseq (Diversity Array Technology sequencing) marker system has been developed, it is based on the principle of genome complexity reduction through the right choice of methylation-sensitive restriction enzymes (Elshire et al., 2011, Cruz et al., 2013). It is able to deliver thousands of SNP markers in a short time, it is affordable and efficient in identifying associations between the traits and markers across the whole genome (Varshney et al., 2014). Screening in the field is often complex, in breeding for drought, integrating marker assisted selection can accelerate the progress in breeding. This study aimed at improving sorghum productivity in drought prone areas in Kenya through introgression of the stay green QTLs. A marker assisted backcross scheme was initiated using DArT markers, with the aim of Introgressing the QTL regions controlling stay green from mapped donor sources (E36-1 and B35) into two Kenyan sorghum varieties (KARI Mtama-1 and Gadam)**).** The outcome of the MAS program was assessed for the trait recovery from the donor parent and recovery of recurrent parent genotype.

4.3 Materials and Methods

4.3.1 Experimental Site

The site description remains as discussed in section 3.3.1

4.3.2 Plant material

One population of backcrosses (BC_1F_1) was generated from crosses between B35 and E36-1 (used as donor parents) with two sorghum cultivars, (Kari mtama-1 and Gadam) as reccurent parents. Genotype B35 has been extensively used as a donor parent for stay green, many studies have reported four major QTLs in B35 three of which are shared with E36-1. The recurrent parents (KARI Mtama-1 and Gadam) are both high- yielding farmer varieties that are susceptible to drought.

4.3.3 Generation of crosses

The two target cultivars (Karimtama-1 and Gadam) were used as female parents to make independent crosses (Karimtama-1×B35; Gadam×B35), (Karimtama-1× E36-1; Gadam × E361). The F_1 plants were used donor parents to make the first backcross (BC_1F_1).

Hand emasculation technique was used in this study, Emasculation of the sorghum florets was undertaken a day before anthesis, a sharp object was inserted between the outer glumes of the sessile spikelets to tease out the anthers. The fertile spikelets that remained on the panicle were removed and the sorghum heads bagged with a *khaki* bag that was inscripted with the emasculation date to avoid foreign pollen from landing on the stigmas (Reddy and Kumar 2008).

Pollen harvesting was undertaken in the mornings hours when anthers dehisce, the bag that had the harvested pollen was placed on top of the emasculated sorghum head, tapped to release the pollen to the stigma.

4.3.4 DNA extraction and DArT genotyping

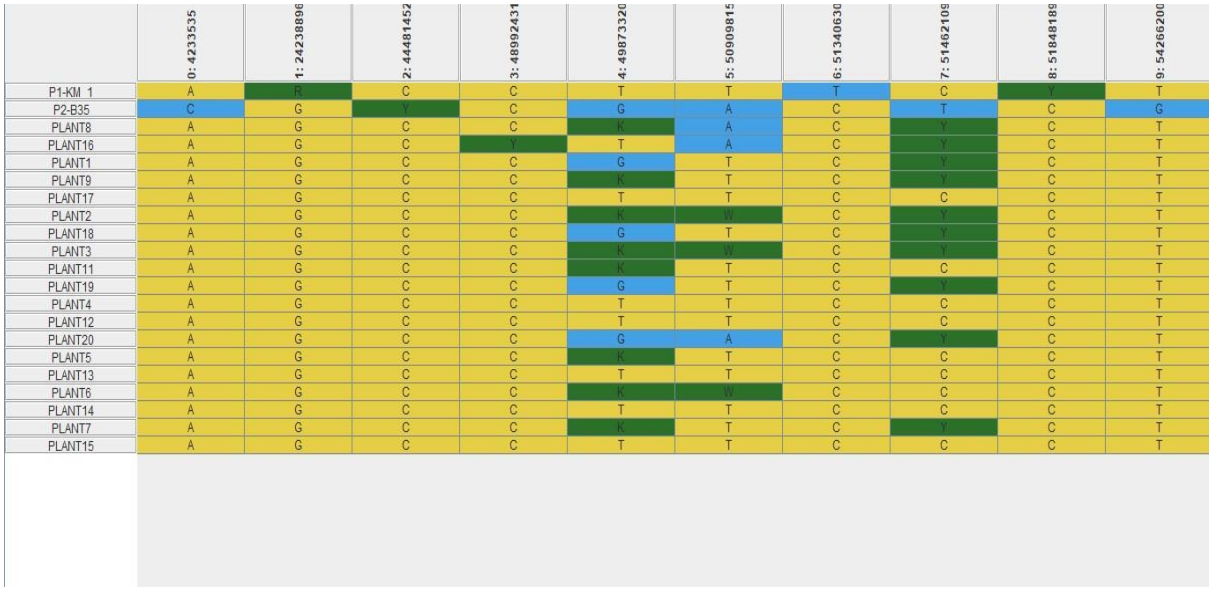
Leaf tissues of the 46 BC_1F_1 progenies and recurrent parents were collected two weeks after sowing, the extraction of the genomic DNA and further processing was done as described in section 3.5

4.3.4.1 Genotyping with DArT markers

DArT arrays were developed using the genomic representation from the backcross progenies and parental lines. The complexity reduction method described by Wenzl et al. (2007) was used to prepare genomic representation. A total of 287, 263 and 578 Diversity array technology (DArT markers) linked to stay green QTL were used for selection for the Kari Mtama1 X E36-1 BC_1F_1, Kari Mtama1 X B35 BC_1F_1 and Gadam X B35 BC_1F_1 crosses respectively. A total of 145, 59 and 177 most informative DArT markers were used for analysis and to select genotypes that had the recovered a large portion of the recurrent parent genome for estimation of the recurrent parent genome that was recovered.

4.4 RESULTS

3324, 1124 and 3832 raw SNPs were generated from crosses of Kari Mtama1 X E36-1 BC_1F_1, Kari Mtama1 X B35 BC_1F_1 and Gadam X B35 BC_1F_1 crosses respectively. After filtering for quality SNPs, 287,263 and 578 SNPs were retained for assessing the heterozygous backcrosses between the genotypes. The backcross progenies were assigned to their recurrent parent based on DArT polymorphism. DArT clones were uniquely used to differentiate between B35, E36-1 and the recurrent parents, the resulting 12 progenies of the cross (Kari Mtama1 X E36-1 BC_1F_1) possessed high frequency of Karimtama-1 alleles, while 6 progenies of (Kari Mtama1 X B35 BC_1F_1) possessed high frequency of Karimtama_1 allele. Only two progenies out of 5 of the crosses (Gadam X B35 BC_1F_1) possessed high frequency of Gadam alleles. The proportions of minor alleles (Donor parent alleles) and the major alleles (Recurrent parent alleles) are as shown in the figures below.



	0: 4233535	1: 24238896	2: 44481452	3: 48992431	4: 49873320	5: 50909815	6: 51340630	7: 51462109	8: 51848189	9: 54266200
P1-KM 1	A	R	C	C	T	T	T	C	T	T
P2-B35	C	G	Y	C	G	A	C	T	C	G
PLANT8	A	G	C	C	K	A	C	Y	C	T
PLANT16	A	G	C	Y	T	A	C	Y	C	T
PLANT1	A	G	C	C	G	T	C	Y	C	T
PLANT9	A	G	C	C	K	T	C	Y	C	T
PLANT17	A	G	C	C	T	T	C	C	C	T
PLANT2	A	G	C	C	K	W	C	Y	C	T
PLANT18	A	G	C	C	G	T	C	Y	C	T
PLANT3	A	G	C	C	K	W	C	Y	C	T
PLANT11	A	G	C	C	K	T	C	C	C	T
PLANT19	A	G	C	C	G	T	C	Y	C	T
PLANT4	A	G	C	C	T	T	C	C	C	T
PLANT12	A	G	C	C	T	T	C	C	C	T
PLANT20	A	G	C	C	G	A	C	Y	C	T
PLANT5	A	G	C	C	K	T	C	C	C	T
PLANT13	A	G	C	C	T	T	C	C	C	T
PLANT6	A	G	C	C	K	W	C	C	C	T
PLANT14	A	G	C	C	T	T	C	C	C	T
PLANT7	A	G	C	C	K	T	C	Y	C	T
PLANT15	A	G	C	C	T	T	C	C	C	T

Figure 4. 1: A snapshot of some informative SNPS in the backcross progenies of Kari Mtama1 X B35 BC_1F_1

	0: 1212900	1: 1403114	2: 1571397	3: 2181200	4: 4302594	5: 4834040	6: 4959943	7: 5603240	8: 6478804	9: 6478858	10: 7586891	11: 8709727	12: 9147715	13: 10482716	14: 12337672	15: 13283874	16: 16660903	17: 20216753	18: 58258662	19: 59115174	20: 59175397	21: 59885167
PLANT14	Y	K	R	G	M	A	T	W	G	R	G	S	K	C	T	G	G	M	G	S	G	A
PLANT22	Y	T	R	G	M	A	T	A	R	R	K	G	T	C	W	G	G	C	G	S	S	A
KM 1	T	T	A	G	C	A	T	T	G	G	G	G	T	C	T	G	G	C	G	G	G	A
PLANT15	Y	K	R	G	M	A	K	W	R	R	K	G	K	Y	T	G	G	C	G	S	S	W
PLANT23	T	T	A	G	C	A	T	T	G	G	G	G	T	C	T	G	G	C	G	G	G	A
E36 1	C	G	G	G	A	A	G	A	A	A	T	C	G	Y	A	G	G	A	G	S	C	T
PLANT8	T	T	A	G	C	A	K	W	R	R	K	G	K	Y	T	G	G	C	G	C	S	W
PLANT16	Y	G	G	G	M	A	K	T	R	R	K	S	T	Y	W	S	G	M	G	S	S	W
PLANT24	Y	T	R	G	M	A	K	W	R	R	K	S	K	Y	W	G	G	M	G	S	S	W
PLANT17	Y	G	A	R	C	A	T	T	G	G	G	G	T	C	T	G	G	C	G	G	S	W
PLANT2	Y	K	R	G	M	A	T	W	R	G	G	S	K	Y	T	C	R	M	G	S	S	W
PLANT10	T	T	A	G	C	A	T	T	G	G	G	G	T	C	T	G	G	C	G	G	G	A
PLANT18	T	K	R	G	M	A	K	W	R	R	G	S	K	Y	W	G	G	M	G	S	S	A
PLANT26	Y	K	R	G	M	G	K	W	R	R	K	S	K	Y	T	G	G	M	G	S	S	W
PLANT3	Y	T	R	G	M	A	K	W	R	G	G	C	K	Y	W	G	G	M	G	S	S	W
PLANT19	T	K	A	G	M	A	T	W	G	R	G	G	T	C	W	G	G	M	G	G	G	A
PLANT27	C	G	R	G	M	A	T	A	A	G	G	C	T	C	T	G	G	M	R	G	G	A
PLANT4	Y	T	R	G	M	A	K	A	R	R	K	C	K	Y	W	G	G	C	G	S	S	A
PLANT12	T	T	A	G	C	A	T	T	R	G	G	S	T	C	W	S	G	M	G	S	S	W
PLANT28	Y	T	R	G	M	A	K	W	R	R	K	S	T	Y	T	G	G	C	G	S	G	W
PLANT5	T	K	R	G	M	A	K	W	G	G	K	G	K	Y	W	G	G	M	G	G	S	W
PLANT13	Y	T	R	G	C	A	K	W	R	R	G	G	K	C	W	G	G	C	G	S	S	W
PLANT21	T	K	A	G	C	A	T	T	G	G	G	G	T	C	T	G	G	C	G	G	G	A
PLANT29	Y	T	G	G	M	G	K	T	R	R	K	S	K	Y	T	G	G	C	G	S	G	W

Figure 4. 2: A snapshot of some informative SNPS in the backcross progenies of Kari Mtama1 X E36-1 BC_1F_1

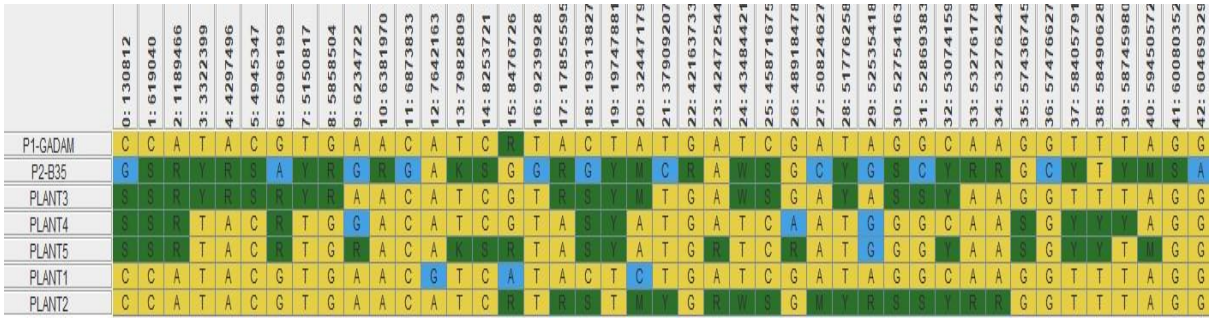


Figure 4. 3: A snapshot of some informative SNPS in the backcross progenies of Gadam X B35 BC_1F_1

Table 4. 1: Background screening of the backcross progenies

These genotypes were selected for advancement to the next generations due to their high proportion of the recurrent parent genomes

Genotypes	RP Loci	HT Loci	DP Loci	% RP	% HT Loci	% RP Loci + HT Loci
Kari Mtama1 X E36-1						
BC_1F_1						
KM_1 x E36_1_14	43	101	1	30	70	100
KM_1 x E36_1_22	52	87	6	36	60	96
KM_1 x E36_1_15	39	103	3	27	71	98
KM_1 x E36_1_16	39	101	5	27	70	97
KM_1 x E36_1_24	32	112	1	22	77	99
KM_1 x E36_1_18	42	101	2	29	70	99
KM_1 x E36_1_26	24	119	2	17	82	99
KM_1 x E36_1_3	34	109	2	23	75	98
KM_1 x E36_1_12	32	112	1	22	77	99
KM_1 x E36_1_28	41	104	0	28	72	100
KM_1 x E36_1_13	42	98	5	29	68	97
KM_1 x E36_1_29	42	99	4	29	68	97
Kari Mtama1 X B35						
BC_1F_1						
KM_1 X B35_1	16	37	6	27	63	90
KM_1 X B35_9	18	36	5	31	61	92
KM_1 X B35_2	18	36	5	31	61	92
KM_1 X B35_3	15	39	5	25	66	91
KM_1 X B35_20	13	37	9	22	63	85
KM_1 X B35_6	17	37	5	29	63	92
Gadam X B35 BC_1F_1						
Gadam X B35_ 3	70	103	4	40	58	98
Gadam X B35_ 2	51	123	3	29	69	98

RP: Recurrent parent, HT: Heterozygous, DP: Donor parent

4.5 DISCUSSION

To support and increase the efficiency of conventional breeding, deploying molecular markers linked to QTLs or any gene, governing the trait of interest and transferring of these QTLs/gene is advocated for (Kumar et al., 2013). Marker-assisted backcrossing has been proven to be a quick way to improve one or two traits in existing preferred cultivars in several crops (Varshney et al., 2010). This approach can be used to generate cultivars with desired characters in less time and high precision (Varshney et al., 2010). Both the recurrent parents selected in this study were agronomically elite and preferred by the farmers and researchers.

Knowledge of parental polymorphism is a pre-requisite to initiate any backcrossing program. Polymorphic parents help in efficient selection of plants carrying the trait of interest in progenies in each generation. The parents used in this crossing program belonged to different racial backgrounds with diverse geographic origins, therefore the diversity among the parents was higher which manifested in the form of polymorphism. The two donor parents used (B35 and E36_1) had also shown good heritability and combining ability for stay green-related traits, this has been shown to be a good prerequisite for any crossing program for development of hybrids (Sory et., al 2015).

In this study, the stay green QTLs from B35 and E36_1 were introgressed into genetic backgrounds of Kari Mtama1 and Gadam. DArT markers spanning all the 10 sorghum chromosomes were used for selection of plants with maximum recurrent parent genome. Chi-square goodness-of-fit test of associations between the proportions of A, B and H alleles gotten in comparison to the expected proportions, showed that most of the genotypes had about 75% level of heterozygosity as was expected and were selected to be true backcrosses. In this study, selection was done in the early backcross generations (BC_1F_1) hence reducing the time that would have been taken to do field evaluations in case of conventional breeding methods, this is in line with Morris et al. (2003), who established that Marker-assisted selection is able to reduce the time required for selection of desirable genotypes. Also, with conventional breeding, it is complex to reliably differentiate heterozygous individuals. In this study, it was with precision that the true

BC_1F_1 genotypes were identified when exotic donor sources were used for introgression. In this era of molecular biology, introgression can be done with precision when markers are used. Marker assisted introgression from exotic donor parents has also been achieved in using N13 as a donor line for striga resistance into a farmer preferred sorghum variety by (Mohamed et al., 2014).

From this study, we realized that this initial investment of using markers to make early generation selections is worthwhile. This is in line with findings by (Morris et al., 2003) who confirmed that early selections assisted by markers accelerates the rate of release of improved varieties.

4.6 CONCLUSION

Marker-assisted backcrossing can be used to accurately introgress stay green genes into genetically diverse sorghum varieties grown in areas prone to drought. The stay-green QTLs were introgressed into Kari Mtama1 and Gadam and with further validation, they will be utilized in enhancing productivity under drought conditions.

CHAPTER FIVE; GENERAL DISCUSSION, CONCLUSION AND RECCOMENDATIONS

5.1 GENERAL DISCUSSION

This study aimed at identifying new sources of drought tolerance from characterization of wild and local sorghum landraces with respect to stay green. Genotypes used revealed the genetic relationship between wild, landraces and cultivated genotypes which will form a basis for future parental selection. The most drought tolerant stay green genotypes from this study were wild relatives; GBK044058, GBK 047293, GBK016114 and local landraces; Lodoka and Akuor-Achot. The results show that wild relatives and landraces of sorghum can be utilized as sources of drought tolerance to improve productivity. The wild genotypes; GBK045827, GBK016114, GBK048922, GBK016109, GBK047293 clustered differently from the two mapped donor sources (E36_1 and B35), this is an indication of the potential of discovering many stay green alleles from wild genotypes. Stay green trait was positively significantly correlated with panicle weight (r=0.82), hundred seed weight (r=0.85) and grain yield (r=0.78) which showed that the identified sources of stay green are functional. The genotypes that had longer panicles, high panicle weight, high hundred seed weights and were also stay green were also high yielding, these genotypes could be used as high yielding breeding lines under drought stress conditions. Genotypes selected based on their yield potential alone could be misleading as yield is controlled by many genes (Ramakrishnan et al., 2006), in addition, this revealed that simultaneous improvement of all these traits is possible.

Results of the general combing ability estimates showed that genotypes; Lodoka, Akuor-Achot and ICSV 111 IN had very high general combing ability for stay green traits and yield. These genotypes contributed to progenies with good specific combining ability estimates for stay green and yield related traits.

According to Gakunga et al., (2012), in identifying gene actions responsible for control of traits of interest, GCA and SCA estimates are useful factors for consideration in crop improvement, therefore these parents can be selected for yield improvement in sorghum breeding programs.

Marker assisted backcrossing for the stay green trait was possible under this study, stay green lines with introgressed stay green QTLs and high proportion of recovered recurrent parent were identified which will form a good basis for future backcrossing to enhance release of sorghum drought tolerant lines.

5.2 CONCLUSION

The productivity of sorghum is severely affected by drought, causing substantial yield losses. Given the inadequacy of classical breeding techniques to substantially manage this, there was need for complementing conventional breeding with molecular techniques in developing drought tolerant sorghum varieties. Molecular and phenotypic work was undertaken and it identified potential novel sources of drought tolerance and successful introgression of the stay green into drought susceptible sorghum varieties. The conclusion from this research is that Marker Assisted Selection (MAS) is a successful tool for identification of drought tolerance alleles and introgression of drought tolerance alleles into drought susceptible sorghum varieties.

5.3 RECOMMENDATIONS

1. The new identified sources of resistance will have to be characterized so as to understand the genetics of stay-green to ensure their deployment in breeding programs.
2. The marker set of SNPs that was developed for quality control (QC) will have to be made into quick assays for more efficient application.
3. The BC_1F_1 material developed needs to be evaluated in replicated field trials to assess the potential usefulness of the introgressed stay green QTLs and the stability of the introgressed genes.

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APPENDICES

Appendix I: Allele summary in the genotypes from the cross of KM1 X E36-1 BC_1F_1

Alleles	Number	Proportion	Frequency
C	1610	0.23374	0.23374
G	1607	0.2333	0.2333
T	788	0.1144	0.1144
A	747	0.10845	0.10845
R	671	0.09742	0.09742
Y	579	0.08406	0.08406
S	275	0.03992	0.03992
K	224	0.03252	0.03252
M	214	0.03107	0.03107
W	173	0.02512	0.02512
C: T	68	0.23693	NaN
G: A	68	0.23693	NaN
A: G	35	0.12195	NaN
T: C	28	0.09756	NaN
G: C	15	0.05226	NaN
C: A	13	0.0453	NaN
T: G	13	0.0453	NaN
C: G	12	0.04181	NaN
G: T	11	0.03833	NaN
T: A	11	0.03833	NaN
A: C	8	0.02787	NaN
A: T	5	0.01742	NaN

Appendix II: Allele summary from the cross of KM1 X B35 BC_1F_1

Alleles	Number	Proportion	Frequency
G	1545	0.27974	0.27974
C	1367	0.24751	0.24751
T	819	0.14829	0.14829
A	613	0.11099	0.11099
Y	345	0.06247	0.06247
R	312	0.05649	0.05649
S	196	0.03549	0.03549
K	168	0.03042	0.03042
M	81	0.01467	0.01467
W	77	0.01394	0.01394
C: T	63	0.23954	
G: A	59	0.22433	
T: C	34	0.12928	
A: G	28	0.10646	
G: C	21	0.07985	
T: G	14	0.05323	
C: G	10	0.03802	
A: C	8	0.03042	
G: T	8	0.03042	
T: A	7	0.02662	
C: A	6	0.02281	
A: T	5	0.01901	

Appendix III: Allele summary from the cross of GADAM X B35 BC_1F_1

Alleles	Number	Proportion	Frequency
C	763	0.18858	0.18858
G	712	0.17598	0.17598
T	682	0.16856	0.16856
A	646	0.15966	0.15966
Y	356	0.08799	0.08799
R	320	0.07909	0.07909
S	191	0.04721	0.04721
M	150	0.03707	0.03707
K	116	0.02867	0.02867
W	110	0.02719	0.02719
T:C	87	0.15052	
A: G	84	0.14533	
C: T	84	0.14533	
G: A	74	0.12803	
C: G	42	0.07266	
G: C	41	0.07093	
C: A	33	0.05709	
T: G	29	0.05017	
A: C	28	0.04844	
A: T	26	0.04498	
G: T	26	0.04498	
T: A	24	0.04152	