MARKER ASSISTED SELECTION FOR POST-FLOWERING DROUGHT TOLERANCE IN *SORGHUM BICOLOR* [L.] MOENCH

By

SISSOKO SORY

10359707

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY PLANT BREEDING DEGREE

WEST AFRICA CENTRE FOR CROP IMPROVEMENT

COLLEGE OF BASIC AND APPLIED SCIENCES

UNIVERSITY OF GHANA

LEGON

December 2015

DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

.....

SISSOKO SORY

(Student)

.....

PROF. VERNON GRACEN

(Supervisor)

.....

PROF. ERIC Y. DANQUAH

(Supervisor)

.....

DR. SIDI BEKAYE COULIBALY

(Supervisor)

ABSTRACT

Sorghum is the second most important cereal crop after pearl millet in terms of area planted, production, and per capita consumption in Mali. Production of sorghum is declining due to several reasons including lack of sorghum hybrids and adapted varieties tolerant to drought. Evaluation of sorghum genotypes under different stresses would be useful for identifying genotypes that combine stability with high yield potential for stress-prone areas. The general objective of the study was to improve sorghum grain yield, through the development of high yielding hybrids tolerant to post flowering drought. Among the specific objectives were to: **(i)** assess sorghum production constraints, importance and farmers' sorghum variety preference criteria in Mali, **(ii)** introgress genes (QTLs) for tolerance to post-flowering drought into elite B-lines of sorghum by backcrossing and **(iii)** identify potential parents and hybrids tolerant to post flowering water stress. A Participatory Rural Appraisal consisting of focus group discussions followed by individual interviews was conducted in different communities, where sorghum is among the main starchy staples. The focus group discussions were conducted among a total of 74 small-scale farmers whilst 265 households were involved in the interviews. The results indicated that in Mali, the main reasons for cultivating sorghum are for grain, used for human consumption, while the crop residues are used for feeding animal, fencing and composting. The two major sorghum production constraints identified were drought and *Striga* infestation. Since drought was a major concern for farmers, sorghum hybrids tolerant to post-flowering drought were developed. To accomplish that, the parental stay green lines (B and R) were identified. To generate stay green B-line populations, three elite lines from the Malian sorghum program, 98-BE-F5P-82B, 03-SB-F5DT-134B and 09PR-3009B (senescent, drought susceptible), were crossed

with B35 (stay green donor). Marker-assisted backcrossing method was used to incorporate stay green genes (QTL) into B-lines. Finally, a total of twelve individuals were selected. To identify stay green Rf-lines, the cytoplasmic male sterile line 02-F5DT-12A (12-A) was used as female parent in test crosses and with 120 selected lines from BCNAM's populations for testing the cytoplasmic male sterility reaction. Ten stay green restorer lines (Rf-lines), useful for developing hybrids for wider adaptation in semi-arid regions were identified. Among them, five lines were used for hybrid seed production. A set of twelve female (B-lines) and five male lines (R-lines) were sown for making crosses in North Carolina Design II mating design in the experimental area of Agriculture Research Centre of Sotuba in Mali. A total of 60 F_1 hybrids were obtained. These F_1 hybrids were planted along with six sorghum lines used as check. A total of 66 genotypes of sorghum were evaluated in both drought and well-watered conditions. The split-plot design with drought intensity as the main plots were used. To achieve the main goal of this study, different methods of analysis were used: combining ability estimation, GGE biplot analysis, and stress tolerance indices using multivariate analysis. Results revealed three hybrids (B35//134B)-F3-44/BCNAM-76-2, (B35//82B)-F3-64/BCNAM-45-1 and (B35//82B)-F3-104/BCNAM-76-2 as the most drought tolerant genotypes with high yield stability in both contrasting environments. Two female B-lines parents (B35//134B)-F3-44 and (B35//82B)-F3-64 and one male R-line parent BCNAM-76-2 had highest grain yield in both environments. The hybrids and parental lines can be introduced as post-flowering drought tolerant genotypes after further evaluation in the semi-arid regions of Mali.

DEDICATION

This thesis is dedicated to my parents, my wife Assitan TRAORE and children Fatoumata and Cheick Boukhadary.

ACKNOWLEDGEMENTS

I am grateful to the Alliance for Green Revolution in Africa for sponsoring my PhD studies.

I thank the Management and Staff of West Africa Centre for Crop Improvement (WACCI), University of Ghana, for identifying me as potential doctoral candidate and for their support and the effective administration of my scholarship.

I express my sincere gratitude to my supervisors, Prof. Vernon Gracen and Prof. Eric Danquah, for their guidance and valuable inputs in this thesis. My sincere thanks go to my in-country supervisor, Dr. Sidi Bekaye Coulibaly for his close supervision and valuable comments. My thanks to memory of Dr. Charles *The*, may his soul rest in perfect peace.

I am thankful to Dr. Abdoulaye Gaoussou Diallo, Dr. Niaba Teme, Mr. Abocar Oumar Toure and Mr. Abdoul Wahab Toure from Sorghum program of IER-Mali for their support and constructive criticism. I sincerely thank Dr. Mamourou Djourté Head of sorghum program, Dr. Aboubacar Toure, Prof. Alhousseini Bretaudeau, Prof. Assetou Foune Samake and Dr. Mamadou Mory Coulibaly for their pieces of advice.

I thank Mr. Brehima Camara and Mr. Sohoiya Djigue from CRRA of Sotuba who assisted me in the field work.

My special thanks to Dr. Daniel Fonceka and Mr. Joël Romaric from CERAAS of Thies-Senegal who assisted me in the genotyping works in Senegal.

I thank my colleagues at University of Sciences of Technics and Technologies of Bamako (USTTB), IER Mali, WACCI, all my friends for their encouragements and support, and everyone who may have contributed in one way or the other to this work.

I am also grateful to my family, my parents and siblings, for their love, encouragements and prayers. My sincere thanks go to my wife, Assitan Traore and our children, Fatoumata and Cheick Boukhadary, for their endurance, love and encouragement throughout my studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS

ABA:	Abscisic Acid
AD:	Anno Domini
AFLP:	Amplified Fragment Length Polymorphism
AGRA:	Alliance for Green Revolution in Africa
ANOVA:	Analysis of Variance
B.C.:	Before Christ
BC:	Backcross
BCNAM:	Backcross Nested Association Mapping
bp:	Base pairs
CE:	Capillary Electrophoresis
CERAAS:	Centre d'Etude Régional pour l'Amélioration de l'Adaptation à la Sécheresse
CIAA:	Chloroforme-isoamyl alcohol
CMS:	Cytoplasmic Male Serility
CRRA:	Centre Régional de Recherche Agronomique
DAP:	Di-ammonium Phosphate
DArT:	Diversity Array Technology
DNA:	Deoxyribonucleic acid
DNA-Mali:	Direction Nationale de l'Agriculture du Mali
DP:	Donor Parent
FAO:	Food and Agriculture Organization
FAOSTAT:	Statistical Database of the Food and Agriculture of the United Nations

FG:	Focus Group
FL-1:	Fag leaf
GCA:	General Combining Ability
GDPs:	Gross Domestic Products
GGE:	Genetics, Genetics×Environment
GS:	Growth Stage
ICRISAT:	International Crops Research Institute for the Semi-Arid Tropics
IER:	Institut d'Economie Rurale
INTSORMILL:	International Sorghum and Millet
IRDye:	Infrared Dye
LG:	Linkage Group
MAB:	Marker Assisted Breeding
MABC:	Marker Assisted Backcrossing
MARS:	Marker Assisted Recurrent Selection
MAS:	Marker Assisted Selection
MATAB:	Mixed Alkyltrimethylammonium bromide
Mb:	Million bases
MDR :	Ministère du Développement Rural
MET:	Multi Environnent Trial
MPa:	Mega Pascals
mRNA:	Messenger Ribo Nucleic Acid
N:	Nitrogen
NARS:	National Agricultural Research Systems
NCD:	North Carolina Design

ng:	Nanogram
NIL:	Near-Isogenic Lines
OA:	Osmotic Adjustment
OPV:	Open Pollinated Varieties
PAGE:	Polyacrylamide Gel Electrophoresis
PC:	Principal Component
PCA:	Principal Component Analysis
PCR:	Polymerase Chain Reaction
PRA:	Participatory Rural Appraisal
QTL:	Quantitative Trait Loci
RAPD:	Randomly Amplified Polymorphic
RF:	Restorer of Fertility
RFLP:	Restriction Fragment Length Polymorphism
RP:	Recurrent Parent
SCA:	Specific Combining Ability
SG:	Stay Green
SNP:	Single Nucleotide Polymorphism
SPAD:	Soil-Plant Analysis Development
SSR:	Simple Sequence Repeats
STMS:	Sequence Tagged Microsatellite Sites
STR:	Short Tandem Repeat
UN:	United Nation
USA:	United States of America
USTTB:	Univesité des Sciences Techniques et de Technologies de Bamako

UV:	Ultra Violet
WACCI:	West Africa Centre for Crop Improvement
WECARD/CORAF:	West and Central African Concil for Agricultural Research and Development/Conseil Ouest et Centre Africain pour la Recherche et le Développement Agricoles
WFP:	World Food Program
WUE:	Water Use Efficiency
µL:	Micro Liter

CHAPTER ONE

1.0. GENERAL INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench), a C4 grass (Kresovich *et al*. 2005) and a close relative of maize (Doebley 1990; Paterson *et al*., 1995), is a member of the family Poaceae and the tribe Andropogoneae. *Sorghum bicolor* (diploid 2n =2x= 20) belongs to the section *Sorghum*, together with the two perennial species *Sorghum halepense* ((2n=4x= 40)) and *Sorghum propinquum* (2n=2x=20). There are 20 – 30 species of sorghum. Cultivated sorghums are divided into seven basic races (agronomic types): Kafir (South Africa), Milo-Caudatums (East Africa), Feterita-Guineas (Sudan), Durra (East Africa, Middle East and India), Sballu (India), Koaliang (China), and Hegari (Sudan). Sorghum was domesticated around 3,000 B.C. in the savanna zone of Africa, south of the Sahara and north of the equator. From there, it spread through Arabia around 1000 to 800 B.C., through India (1st century AD) and through China (3rd century, AD). It was introduced to the United States with the slave trade. Ethiopia can be considered as a center of diversity for sorghum, but it is not the sole center of origin or diversity (*http://ecampus.oregonstate.edu*).

In terms of human consumption, sorghum is the fifth most important cereal crop in the world after wheat, maize, rice, and barley. It is the dietary staple of more than 500 million people in more than 30 countries and it is grown on 42 million ha in 98 countries of Africa, Asia, Oceania and Americas (FAOSTAT, 2013). The average world sorghum production is about 61 million tons, produced on 42 million hectares (FAOSTAT, 2013). According to FAO estimates for 1980 through 2013, both the world production of sorghum grain and the harvested area have been decreasing.

In Africa, sorghum ranks second after maize (*Zea mays)* in terms of production and consumption with the most important producing countries being Nigeria, Mali, Burkina Faso, Mauritania and Gambia. The continent of Africa produces about 25 million tons of sorghum per year, slightly more than 40% of world sorghum production (FAOSTAT, 2013). Sorghum and millet (*Pennisetum glaucum* (L.) R. Br.), are more important crops in areas where drought causes frequent failure of other crops. Both crops are usually grown under moisture and temperature stress conditions in the semi-arid environments (Atokple, 2003). Adaptation to drought prone environments makes sorghum an important food and feed crop in the arid and semi-arid regions. This has resulted in the development of extensive genetic variation for drought tolerance in sorghum (Blum, 1979; Doggett, 1988), which makes it a crop model of choice for studying the genetic and physiological mechanisms of drought tolerance.

Drought is the most significant cause of crop yield loss (Boyer and Westgate, 2004), especially in water limited areas where most of the world's poorest farmers live. Up to 45% of the world's agricultural lands, where 38% of the world's population resides, are subjected to continuous or frequent drought (Ashraf and Foolad, 2007). Development of drought tolerant crops will enhance food production and the livelihood of farmers in these areas. Moreover, as the world population continues to grow and water resources for crop production decline, development of drought tolerant cultivars and water use efficient crops is becoming a major strategic priority.

The world production of sorghum grain have been decreasing and the average yield trends are also downwards. Paramount among the yield reducing factors are predominant cultivation of inherently low yielding varieties, poor soil fertility, drought, *Striga*, pests and diseases. Exploitation of host plant resistance through genetic enhancement has always been

the first approach and forms the basis of an integrated control package in addressing these constraints (Olembo, 2010).

In Mali, sorghum is among the major crops along with pearl millet, rice, maize, cotton, peanut, and cowpea. It ranks second to millet in terms of production (819,606 tons), occupies about 30% of total harvested agricultural area and 26% of the total production of cereal of the country (DNA-Mali, 2015).

The areas cultivated to sorghum in Mali have extended widely on all the agricultural areas of the humid regions to the arid ones. Different types of production of sorghum exist in Mali by season: the rain-fed and the "decrue" season. The main production areas of decrue sorghum include the floodplains of the Senegal and Niger rivers (Jordi and Helena, 2005).

Production of sorghum, however, does not meet demand of the growing population in the country. Grain sorghum yields are very low (0.8 to 1.3 tons/ha) compared to countries like the USA (3.7 tons/ha), Argentina (4.1 tons/ha), and China (3.7 tons/ha), (FAOSTAT, 2013). These yields are well below the genetic potential of sorghum, which, under favorable conditions, can yield up to 13 tons/ha (Balole and Legwaila, 2006). Toure (1979) indicated that three major sorghum races exist in Mali: guinea, durra and caudatum. The Guinea sorghums constitute the dominant race grown by farmers and represent about 70% of sorghum races in Mali. They are well known for their high grain quality, good weathering, pest resistance, and storage qualities (Toure *et al.*, 1998). However, the yield potential of most cultivars is relatively low and they do not respond well to mineral fertilizer.

The adoption of improveded varieties from sorghum breeding programs is very low, less than 5% of the cultivated area (Traore *et al.*, 2000 and IER-Mali and PSA-2-3sor., 2006). This is due the poor adaptation of the available varieties to the farmers' environments, the

poor food quality, poor storability of grain and the lack of information on the existence and utility of new varieties (Deb et *al.*, 2004).

The adoption of hybrid sorghum is also very low by Malian sorghum producers. However hybrids have contributed significantly to increased grain and forage yields in most countries around the world. From 1960s to 1990s, sorghum grain productivity increased by 47% in China and by 50% in India because of the adoption of hybrids in these countries (Reddy *et al.*, 2006). Toure (1980) and Diallo (2013) reported that the exploitation of hybrids began in Mali in 1979. Initial work in Mali on hybrids using local cultivars as male parents and introduced Texas cytoplasmic male sterile lines ATx623, ATx2219, ATx2277, as female lines, demonstrated that hybrids could provide higher yields, but must be based on parents with appropriate grain characteristics and adaptation to be commercially viable (Touré *et al.*, 1982; Touré and Scheuring, 1998). Between 1986 and 1990 the initial hybrid materials in Mali were lost from the Malian sorghum collection (Diallo, 2013).

The "Institut d'Economie Rural" (IER), Mali and ICRISAT-Mali have pursued different but complimentary, approaches to hybrid-parent development over the past decade. The IER program produced a series of inter-racial parental lines by crossing Guinea-race landrace parents and Caudatum race breeding lines, followed by repeated hybridization and backcrossing to obtain genotypes with adapted panicle, grain and glume characteristics (Diallo, 2013). ICRISAT, in contrast, emphasized use of Guinea landrace varieties and random-mating populations based on Guinea germplasm from the West African region to produce a series of parental lines that have more Guinea-race background. The first *guinea* landrace CMS A-lines were based on Malian varieties Fambe, IPS001 and CSM-219 (developed by ICRISAT), and seven inter-racial derivatives of a cross [*guinea* landrace (Bimbiri Soumale) × *caudatum* varieties] (developed by IER). The *guinea* landrace-based

A-lines are tall, photoperiod sensitive, and possess typical *guinea* grain and panicle architecture, but the inter-race A-lines are dwarf, basically photoperiod insensitive and possess relatively small grain (Reddy *et al.,* 2006).

The low production of sorghum in Mali is due to several reasons including agronomic practices that do not maintain soil fertility, climatic risks such as drought, diseases, adoption of low yielding landrace varieties and cereal breeding programs that have not accounted for the micro variability observed across the region. Among these reasons, drought is one of the major limiting factors in agriculture in Mali where the rainy season has become shorter over the last 20 years (Traore *et al.,* 2007). Periodic drought cause considerable hardship in Mali and often destroy all the production. Terminal drought (post-flowering) needs serious attention because it is the most recurrent form in arid and semi-arid regions of Mali.

Sorghum has long been recognized as tolerant to heat and water stresses, and this has led to its extensive production in drought-prone environments. Grain sorghum productivity under such environments can be increased by management systems that are environmentally safe and sustainable. Deployment of drought tolerant grain sorghum hybrids is one such approach.

Drought tolerance can be assessed based on relative yield performance of different varieties (compared to a non-stressed control moisture regimes) in a particular drought scenario where drought escape is not a major factor (Nguyen, 1999). This drought tolerance involves genotypic differences and is, therefore, useful for improving and stabilizing plant productivity.

Drought can reduce sorghum yield at all growth stages, germination and seedling emergence, post-emergence or early seedling, midseason or pre-flowering, and terminal or post-flowering stages (Kumar, *et al.,* 2011). Drought response in sorghum has been studied at two

distinct stages, pre-flowering and post-flowering (Rosenow and Clark, 1981). Most sorghum cultivars used for grain production have pre-flowering drought tolerance but do not have any significant post-flowering drought tolerance (Subudhi *et al.*, 2000). Drought stress during the post-flowering stage is referred to as "terminal drought" and needs serious consideration because its negative impact on yield can be very severe. Genotypes sensitive to this type of drought are characterized by premature senescence, stalk collapse and lodging, charcoal rot, and reduced grain number and size. In sorghum, the best indicator of drought tolerance during this stage of crop growth is the "stay-green" (SG) trait. SG is defined as the ability to resist premature plant senescence, retain green leaf area, fill grain normally, and resist charcoal rot and lodging under post-flowering drought stress conditions (Rosenow *et al*., 1983). When water is limiting during the grain-filling period, genotypes possessing this trait maintain photosynthetically active leaf area better than genotypes that do not possess this trait. SG results from the balance between N demand by the grain and N supply from the roots during grain filling (Borrell and Hammer, 2000a).

Several sorghum genotypes have been identified that exhibit the stay-green characteristic including B35 (BTx642), KS19, SC56, and E36-1 (Rosenow *et al*., 1983; Borrell *et al*., 2000b; Kebede *et al*, 2001; Haussmann *et al*., 2002b). The most common source of stay green trait has historically been the sorghum line B35, a member of the durra race. This genotype has been particularly useful as a source of stay green trait in public and commercial breeding programs in Australia (Henzell *et al*, 2001).

Several stay green QTL associated with post-flowering drought tolerance have been mapped (Tuinstra, and Goldsbrough, 1998; Crasta *et al*., 1999; Subudhi *et al*., 2000; Tao *et al*., 2000; Xu *et al*., 2000) and molecular markers linked to these QTL are thus available (Harris *et al*., 2007; Kassahun *et al*., 2009). Four major stay green QTL designated as *Stg1*, *Stg2*, *Stg3* and

Stg4 as well as many additional minor QTL including *StgA* and *StgB* have been identified. The stay green QTL *Stg1* and *Stg2* were mapped on sorghum linkage group (LG) 03, which corresponds to chromosome three (3). These two QTLs (*Stg1* and *Stg2*) explain approximately 13 – 20% and 20 – 30% of the phenotypic variance, respectively (Sanchez *et al.*, 2002; Harris *et al.*, 2007). The other two stay green QTLs: *Stg3* and *Stg4* were located on LG-02 (chromosome 2) and LG-05 (chromosome 5), accounting for 16 and 10% of the phenotypic variance, respectively (Sanchez et al. 2002; Harris et al. 2007).

Research has been conducted in Mali targeting tolerance to drought in sorghum, but no research has been conducted into tolerance to recurrent post flowering drought. To the best of my knowledge, no research using molecular markers to develop new varieties of sorghum tolerant to post flowering drought has been conducted so far.

Molecular markers have been used to improve sorghum tolerance to *Striga hermonthica*. Molecular markers are also being used to develop sorghum varieties for harsh environments and for better grain yield and quality. Both marker assisted recurrent selection and backcross nested association mapping to detect QTL linked of important traits linked to grain yield and grain quality are being conducted (Teme *et. al.*, 2012 and 2013).

A positive impact of stay green characteristic on grain yield QTL under terminal drought has been observed (Borrell, *et al.*, 1999; Jordan *et al.*, 2003; Kassahun *et al*., 2009). Tuinstra *et al.* (1997) reported co-localization of stay green and grain yield QTL under drought stress, suggesting that the gene(s) underlying stay green may also result in enhanced yield performance under drought stress. Tuinstra and Goldsbrough, (1998), using near-isogenic lines (NIL), found positive associations between these two traits reinforcing the potential for indirect selection based on stay green for improving grain yield under drought stress in sorghum.

The aim of the present study was to develop parental lines and hybrids tolerant to post flowering drought stress using stay green QTL.

The research is described in a dissertation that has nine chapters: General introduction, literature review, participatory rural Appraisal, revelopment of stay green sorghum B-lines, identification of stay green sorghum Rf-lines, development of post flowering drought tolerant hybrids and parental lines, stability analysis of grain yield of hybrid and parental lines, study of drought effects on genotypes and general conclusions and recommendations.

OBJECTIVES

General objective

The general objective of the present study was to improve sorghum grain yield through the development of hybrids tolerant to post flowering drought in Mali.

Specific objectives

The specific objectives were to:

- assess sorghum production constraints, importance and farmers' sorghum variety preference in Mali
- introgress genes (QTLs) for tolerance to post-flowering drought into elite B-lines of sorghum by backcrossing.
- develop isogenic stay green, male sterile A-lines and maintainer B-lines from the Malian germplasm sources
- identify stay green R-lines from the Malian germplasm sources

- analyze combining ability of B and R-lines under well-watered and post flowering water stress conditions.
- evaluate drought effects on yield and its various components.
- examine the stability of grain yield performance of parents and hybrids under stressed and non-stressed environments.
- identify high yielding parents and hybrids with tolerance to post flowering water stress.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Background information

2.1.1. Description of sorghum

Sorghum [*Sorghum bicolor* (L.) Moench] ($2n = 2x = 20$) is a member of the family *Poaceae* (*Gramineae*) and the tribe *Andropogoneae.* Sorghum is an important target for plant genomics studies due to its adaptation to harsh environments and relatively small genome size (Menz *et al.*, 2002). The genome size of sorghum is approximately 740 Mb (Paterson *et al.*, 2009).

Sorghum is an annual cereal grass species, with a plant height of up to five meters with one or many tillers. Roots of sorghum concentrate in the top 90 cm layers of the soil but sometimes extend to twice that depth, spreading laterally up to 1.5 meters. The solid stem (culm) of sorghum is usually erect. It has simple alternate leaves, leaf sheath 15 – 35 cm long, often with a waxy bloom, a band of short white hairs at the base near attachment, and reddish in some cultivars used for dyes.

The inflorescence or panicle is 15 - 60 cm long depending on variety and growing conditions. Panicles are erect, sometimes recurved, compact, semi loose or loose. They are looser in forage types. Rachis is short or long, with primary, secondary and sometimes tertiary branches, with spikelets in pairs and in groups of three at the ends of branches. The fruit is a caryopsis (grain), usually partially covered by glumes, 4 – 8 mm in diameter, rounded and bluntly pointed; that may or may not be removed by threshing.

Sorghum is predominantly self-pollinating and classical breeding procedures include hybridization, mass selection, pedigree selection and backcrossing. Cross-pollination may

range from 5 to 25%, depending on the cultivar and climatic conditions (House, 1985). Doggett (1988) reported up to 50% cross-pollination. Due to the high levels of cross-pollination, controlled selfing through bagging is necessary to maintain pure lines.

2.1.2. Domestication of sorghum

Sorghum is an old crop. It is difficult to determine when and where domestication occurred (De Wet et *al.*, 1970). Murdock (1959) has suggested that the Mande people around the headwaters of the Niger River may have domesticated sorghum. Doggett (1965a) indicated that archaeological evidence suggests that the practice of cereal domestication was introduced from Ethiopia to Egypt about 3000 BC. It is possible that domestication of sorghum began about that time.

De Wet *et al.* (1970) suggested that sorghum had diverse origins and probably arose from *Sorghum verticilliflorum*. This species is usually found in areas where sorghum is cultivated. Snowden (1936) and Porteres (1951) suggested that races durra, guinea, and kafir are closely allied and may have arisen from *S. aethiopicum, S. arundinaceum,* and *S. verticilllflorum,* respectively. This is difficult to demonstrate experimentally.

Morphological differences between races may have arisen because of ethnic isolation. Kafir is widely grown in Bantu Africa, while durra is not found there. Caudatums are most common in central Sudan, and Guinea are found primarily in West Africa. The distribution of sorghum indicates that the races kafir and caudatum were derived from *S. verticilliflorum,* and that durra possibly could have come from *S. bicolor.* Guinea corn is quite distinct, but it is questionable that it could have come from *S. arundinaceum* (which is a grass of tropical forests and not found where sorghum is extensively cultivated).

Introgression studies indicate that cultivated sorghums probably developed through disruptive selection (Doggett, 1965b). Crossing easily occurs between wild and cultivated types; however, these types form distinct populations. It is speculated that, as man began to select, there was substantial gene flow between improved and unimproved types. This gene flow would decrease as field sizes became larger. The selection by man and nature would provide a disruptive force resulting in diverse populations (polymorphic populations). These disruptive forces have been continuously active through time (and are still active), influencing cultivated and wild populations. Most intermediate forms do not exist long in nature: those backcrossed by cultivated crops would tend to contribute genes in the direction of cultivated types; and those backcrossed to wild types would tend to contribute genes to the wild population. Polymorphic populations would tend to be maintained and change over the years. New forms would arise, leading to the types of sorghum now in cultivation. Ethnic isolation would help this process (Doggett, 1965b).

2.1.3. Botanical classification of sorghum

Sorghum belongs to the family Graminae, tribe Andropogoneae. It was first described by Linnaeus (1753) under the name *Holcus.* In 1974 Moench separated the genus *Sorghum* from the genus *Holcus* (Clayton, 1961). Several authors have discussed the systematics, origin, and evolution of the crop (Snowden, 1936; De Wet, *et al.*, 1972; Harlan, 1975a) and Doggett (1988) provided an excellent overview of the present day classification. Snowden (1936) presented the most complete classification of cultivated sorghums, and classification schemes since then have been based on this monumental work. Harlan, and De Wet, (1972) proposed a simplified classification of cultivated sorghums that most present day breeders have come to recognize and utilize. Harlan, and De Wet, (1972) partitioned the primary gene

pool, *Sorghum bicolor* (L.) Moench into five major races; *bicolor, guinea, caudatum, kafir*, and *durra* and ten intermediate races or all combinations of the basic races.

The 5 basic races are:

– **Bicolor:** the most primitive cultivated sorghum, characterized by open inflorescences and long clasping glumes that enclose the usually small grain at maturity. They are frequently found in wet conditions.

– **Caudatum:** characterized by turtle-backed grains that are flat on one side and curved on the other; the panicle shape is variable and the glumes are usually much shorter than the grain.

– **Durra:** characterized by compact inflorescences, characteristically flattened sessile spikelets, and creased lower glumes; the grain is often spherical. It is the most specialized and highly evolved of all races and many useful genes are found in this type. Durra cultivars range in maturity from long to short season. Most of them are drought resistant.

– **Guinea:** characterized by usually large, open inflorescences with branches often pendulous at maturity; the grain is typically flattened and twisted obliquely between long gaping glumes at maturity. Many subgroups can be distinguished with cultivars especially adapted to high or low rainfall regimes. In the past, the grain was often used as ship's provisions because it stored well.

– **Kafir:** characterized by relatively compact panicles that are often cylindrical in shape, elliptical sessile spikelets and tightly clasping glumes that are usually much shorter than the grain. Kafir landraces tend to be insensitive to photoperiod and most commercially important male-sterile lines are derived from kafir type sorghum.

Hybrids exhibit various combinations and intermediate forms of the characteristics of the 5 basic races.

Durra-bicolor is found mainly in Ethiopia, Yemen and India, Guinea-caudatum is a major sorghum grown in Nigeria and Sudan, Guinea-kafir is grown in East Africa and India. Kafir-caudatum is widely grown in the United States and almost all of the modern North American hybrid grain cultivars are of this type.

The wild sorghum, *Sorghum halepense* is a perennial plant with well developed, creeping rhizomes. It is a native of southern Eurasia, east to India. Introgression with cultivated sorghums in the U.S. has created one of the most toxic weeds, Johnson grass. *Sorghum propinquum* is another perennial with stout rhizomes and occurs primarily in Sri Lanka and southern India (Dahlberg, 1995).

The weedy plants are usually considered as hybrids between subsp. *bicolor* and subsp. *verticilliflorum*, and named subsp. *drummondii*.

2.1.4. Production

Sorghum (*Sorghum bicolor* L. Moench) is the 5th most important cereal grain crop in the world, after wheat, maize, rice and barley. FAO (2014) estimates the average world production of sorghum grain in 2013 amounted to 61 million tons from 42 million hectares with an average yield of 1280 kg/ha worldwide.

Sorghum is often recommended to farmers operating in harsh environments where other crops do poorly as it can grow with limited rainfall and often without application of any fertilizers or other inputs. Sorghum constitutes the principal source of energy, protein, vitamins, and minerals for millions of the poorest people in these regions (Carl and Latha, 2009).

In sub-Saharan Africa, sorghum is the second most widely grown cereal crop after maize. Sorghum production and cultivated areas in sub-Saharan Africa have steadily increased from

around 10 million tons from 13 million hectares in the early 1960s to about 25 million tons from 26 million hectares in 2012 (FAOSTAT, 2013). Sorghum is largely grown in arid and semi-arid areas where annual rainfall is very low (Kahiu, 2012). In these marginal areas, the grain yields of sorghum are very low mainly due to abiotic and biotic stresses such as drought stress, *Striga* infestation, poor soil fertility, pests and diseases.

2.1.5. Sorghum uses

Sorghum is grown for food and feed (grains and forage), and for alternative renewable bio-fuel in the semi-arid tropics of Asia, Africa, the Americas and Australia. The location of production often defines the ultimate end use and the specific types of sorghum that will be grown. For example, in many regions of Africa, sorghum is a vital food grain and the stem and leaves are valued for construction (building) and forage. In these production systems, small scale farmers prefer pure-line cultivars that are tall with specific food quality parameters and stable production under stress. In developed countries, sorghum is grown as a feed grain with high input and management. The production system is mechanized and demands sorghum hybrids with high yield potential, relatively short plants, lodging resistance, and responsiveness to favorable environmental conditions (Rooney, 2004).

Sorghum grain is nutritionally superior to fine cereals like rice because of its high mineral and fiber content. Because of its slower digestibility, sorghum is also recommended for diabetic, jaundice affected, and obese people. Sorghum does not have gluten and therefore becomes a very good gluten free energy source as some people are allergic to gluten. However, due to the difficulties in processing for different foods, its usage is limited in urban areas. It may be feasible to use sorghum for the preparation of different traditional and non-traditional foods replacing rice and wheat as the crop has superior nutritional qualities and

recipes for sorghum foods that can be easily prepared among rural and urban population are known (Seetharama, 2007).

2.2. Drought resistance and plant response

Drought limits agricultural production by preventing plants from expressing their full genetic potential and it is considered as the most important cause of yield reduction in crop plants (Boyer, 1982).

Drought resistance can be evaluated based on the relative yield or survival of a genotype, compared with other genotypes subjected to the same drought, and where drought escape is not a major factor (Hall, 1993). Drought tolerance refers to the ability of a crop plant to produce its economic product with minimum loss in a water deficit environment (Blum, 1988; Zhang *et al.*, 1999). Plant resistance to water deficit may arise from escape, avoidance and desiccation tolerance strategies (Levitt, 1972; Turner, 1986). In most cases, plants may combine a range of response types (Chaves *et al.*, 2003).

2.2.1. Drought escape

Drought escape relies on successful reproduction before the onset of severe stress. The plants combine short life cycles with high rates of growth and gas exchange, using maximum available resources while moisture in the soil lasts (Moony *et al.*, 1987).

2.2.2. Drought avoidance

Drought avoidance involves minimizing water loss and maximizing water uptake (Chaves *et al.*, 2003). Water loss is minimized by closing stomata, reducing light absorbance through rolled leaves, and decreasing canopy leaf area. Increasing the size of the root system,

reallocation of nutrients stored in older leaves, and higher rates of photosynthesis maximize water uptake.

2.2.3. Drought tolerance

Drought tolerance appears to be the result of coordination of physiological and biochemical factors at the cellular and molecular levels. This may involve osmotic adjustment (Morgan, 1984), more rigid cell walls, or smaller cells (Wilson *et al.*, 1980). Changes occurring rapidly at the mRNA and protein levels lead to tolerance (Ingram and Bartels, 1996).

2.2.4. Physiological responses to water stress

Plants subjected to water stress display a number of physiological responses at the molecular, cellular, and whole plant levels (Bartels *et al.*, 1996; Chaves *et al.*, 2003). Two physiological mechanisms, most relevant will be discussed.

2.2.4.1. Water use efficiency

Water use efficiency (WUE) is a key factor determining plant productivity under limited water supply. In agronomic terms, it is defined as the ratio between total dry matter produced or yield harvested and water used (Jones, 1993). In physiological terms, however, WUE is defined as the ratio between the rate of carbon fixed and the rate of water transpired. The WUE index compares the number of moles of CO_2 assimilated by photosynthesis per mole of water transpired by the plant (Araus *et al.*, 2003).

Water use efficiency, describes the relationship between water use and crop production. In water-limiting conditions, it is important to produce a high amount of biomass, which contributes to crop yield, using a low or limited amount of water.

Plant resistance to drought stress can be improved through drought avoidance or drought tolerance, among which drought avoidance mechanisms tend to conserve water by promoting WUE (Amudha and Balasubramani, 2011).

2.2.4.2. Osmotic Adjustment

Osmotic Adjustment (OA) is the process of accumulation of solutes in leaves in response to increasing water deficits in the soil, thereby maintaining turgor or reducing the rate of turgor loss with decreasing water potential (Turner and Jones, 1980). Osmotic adjustment has been shown to maintain stomatal conductance and photosynthesis at low water potential, delay leaf senescence and death (Hsiao *et al.*, 1984), reduce flower abortion and improve root growth and water extraction from soil as water deficit develops (Morgan, 1984).

When water deficit develops, various solutes accumulate in cells and subsequently tissue osmotic potential is reduced. Osmotic adjustment is the net increase in cellular osmolality caused by the accumulation of solutes such as various ions (mainly potassium), sugars, poly-sugars (e.g. fructan), amino acids (e.g. proline), and glycinebetaine. Osmotic adjustment occurs when cellular water deficit exceeds a certain threshold, which is not universally known. Nor has the exact signaling for OA been resolved. Osmotic adjustment is a slow process requiring time, and very rapid desiccation in experiments or even in the field may not allow for OA to be expressed. Ideally the rate of plant dehydration should not be faster than about 0.1 Mega Pascals (MPa) per day. Practically, it should take about 2 weeks from fully hydrated state to wilting in order for the full capacity and impact of OA to be expressed in whole plants, depending on species and the growth history of the specific plant (Gebre, 2000). The rate of OA varies greatly among species and cultivars (Blum, 1996). Some crop plants generally tend to be better at OA than others. Cowpea, Japonica rice and maize

generally having lower rates while Indica rice, sorghum and wheat tend to express higher rates.

Osmotic adjustment enables a plant to sustain growth and productivity at lower plant water status. Irrespective of the effect on turgor maintenance, the accumulated solutes can protect cellular proteins, various enzymes, cellular organelles, and cellular membranes against desiccation injury. Hence, cell and tissues may continue to function despite the progressing desiccation. This is why the accumulated osmotic solutes are sometimes defined as "protectants". One consequence of OA at the whole plant level is the continued growth of roots and the extraction of deeper soil moisture. Finally, OA is crucial for the conservation of meristem viability under desiccation which is necessary for the recovery of function upon rehydration. Osmotic adjustment in different crops (wheat, sorghum,) is positively associated with biomass and/or yield under drought stress.

Upon rehydration, the various solutes are recycled and metabolized to the extent that the accumulated sugars, for example, are considered as an important energy resource for recovery growth (*http://www.plantstress.com*).

2.3. Drought resistance and crop yield

Blum, (2011) indicated that, breeding for drought resistance has long been part of the breeding process in most crops that are grown under dry land environments. During the period of pre-scientific agriculture, the genetic improvement of plant adaptation to dry environments was basically attained by repeatedly selecting plants that appeared to do well when drought stress occurred. As a result of many generations of selection by generations of farmers, varieties that are defined as "landraces" of the crop were developed. Such landraces possess distinct drought resistance traits. Later, as scientific agriculture developed

and following the emergence of Mendelian genetics, elaborate biometrical and statistical methods for quantitative genetics analysis were developed to enable selection for yield and yield stability more effectively and efficiently. An important factor of yield stability involves coping with drought and other abiotic plant stresses (Blum, 2011). As crop physiology emerged and developed, yield-based selection programs were augmented by observing plants under carefully managed stress environments, followed by the development of physiological selection criteria for stress resistance. More recently, molecular methods, such as marker-assisted selection, are being adopted to facilitate more efficient selection for distinct components of abiotic stress resistance. Finally, genetic transformation opens the way for additional genetic solutions to breeding for drought resistance.

2.4. Drought stress in sorghum

Even though sorghum possesses excellent drought resistance compared to most other crops, drought stress is the principal factor that reduces sorghum production worldwide (Rosenow *et al.,* 1997). Sorghum is commonly grown in regions of the world where water is limiting and the crop commonly experiences periods of extreme water stress at some point within the growing season. Its rich genetic diversity for stress tolerance makes it an excellent crop model and choice for studying the genetic and physiologic mechanisms of drought tolerance. Drought stress response in sorghum is dependent on the stage of growth in which the drought stress occurs. The classification of growth stages is particularly useful in studying drought reaction in each stage, the drought resistance reaction is controlled by different genetic mechanisms (Rosenow *et al.,* 1997). Growth stage 1 (GS1) is the vegetative stage that begins with germination and ends at panicle differentiation. Growth stage 2 (GS2) is the pre-

flowering or reproductive phase of growth ranging from panicle differentiation until the cessation of anthesis. Growth stage 3 (GS3) is the post-flowering or grain fill phase that begins immediately after anthesis and continues until physiological maturity of the grain.

There have been relatively few reports on variation within sorghum for seedling drought tolerance. Smith *et al.* (1989) indicated that differences in germination and emergence among genotypes were observed at different levels of soil water stress. Wenzel (1991) reported that additive effects controlled variation for seedling drought tolerance and that the trait was highly heritable. However, the relative magnitude of this effect was minimal compared to the variation observed for soil temperature effects.

The pre-flowering stress response occurs when the plant encounters significant drought stress during GS2 prior to anthesis. Sorghum susceptible to pre-flowering drought stress exhibits symptoms such as leaf rolling, leaf tip burn, delayed flowering, poor panicle exertion, panicle blasting, and reduced panicle size (Rosenow *et al.,* 1997). In a breeding nursery, pre-flowering susceptibility is evident when a characteristic "saddle effect" is observed where panicle development occurs only at the ends of a plot. Because pre-flowering stress occurs during panicle development, it affects yield potential by influencing panicle size and seed number.

Post-flowering water stress results from drought stress that is encountered at GS3 during grain fill. Sorghum is most vulnerable to drought stress at post-flowering stage when reduction of grain yields by more than 50% can occur (FAO, 1999).

Sorghum susceptible to post-flowering drought stress exhibits symptoms such as reduced kernel size, significant leaf and stem death and lodging (Rosenow *et al.,* 1997). The increase in lodging is due to the plant remobilizing carbohydrate from the stem in an attempt to complete the grain filling process.

Sorghum is better adapted to water limiting conditions than other cereal crops because some genotypes have the ability to withstand post-flowering drought stress by retaining high chlorophyll content in their leaves and by maintaining the ability to carry out photosynthesis longer. These genotypes, aptly called 'stay-green', also show reduced stalk lodging and are resistant to charcoal rot disease (Rosenow and Clark, 1981). The physiological basis of stay-green is not clear, though the trait is associated with high cytokinnins and sugar levels (Kahiu *et al.*, 2013).

The stay-green trait has recently been characterized in a few genotypes such as B35 and E-36-1 where the QTL have been mapped (Rosenow and Clark, 1981; Kebede *et al.*, 2001; Haussmann *et al.*, 2002b). These QTLs are now being used in MAS programs to introgress these regions into elite but drought susceptible parental lines (Nguyen *et al*., 1997; Borrell *et al.*, 2000b and Coulibaly, 2002).

The post-flowering drought tolerance is the result of several phenotypic and physiological traits that have been identified and characterized by sorghum physiologists. Traits that have been associated with drought tolerance include heat tolerance, osmotic adjustment (Basnayake *et al.*, 1995), transpiration efficiency (Muchow *et al.*, 1996), rooting depth and patterns (Jordan and Miller, 1980), and epicuticular wax (Maiti *et al.*, 1984). All of these traits have been associated with drought tolerance in sorghum but most have not been of any practical use in improvement programs because of the difficulty in evaluation and selection.

2.5. Breeding for drought tolerance

Crop production in drought prone environments may be improved and stabilized by the development and use of crop species and varieties that can tolerate or avoid water deficit.

Selection for tolerance, while maintaining maximum overall productivity, has been a challenge (Rosenow *et al.,* 1983). There are several explanations for this.

First, drought tolerance can be expressed in several ways and the lack of a simple screening procedure has slowed the selection of better genotypes. Some researchers use grain yield per se to quantify drought tolerance, but selecting for grain yield under drought conditions is not efficient (Clarke *et al.,* 1992). Selection for drought tolerance should ideally integrate high yield potential with stability of agronomic performance across drought prone areas. The second problem in selecting for drought tolerance is that genotypes must be screened for tolerance in controlled environments where drought can routinely be imposed. Testing under dry land conditions is difficult because specific drought conditions cannot be easily and reproducibly imposed. Finally, drought tolerance is subject to strong environmental variation and genotype x environment interaction (Clarke *et al.,* 1992).

Yield is not an effective selection criteria for drought tolerance as naturally occurring environments are variable and unrepeatable and the precision of measurement of genotypic differences in yield is often poor because of low heritability (Blum, 1985).

Blum, *et al.* (1983) suggested that selection for drought tolerance must combine selection for yield potential in favorable conditions with selection under stress for the expression of traits thought to be associated with drought tolerance. Sinha (1987) argued that traits representing phenological and morphological adaptations are more effective than physiological and biochemical adaptations for drought resistance. Stay-green is one such trait.

Phenotypic selection for stay-green is not simple as the trait is complex (Van Oosterom *et al.,* 1996) and its expression is affected both by the degree of stress during grain filling and by the sink size (nitrogen demand) of the panicle.

In breeding programs, selection for stay-green is carried out by visually rating the proportion of green leaf area in plants that have encountered post-flowering water stress.

The trait is likely to be more easily manipulated using marker-assisted breeding approach for selection for specific alleles at molecular loci linked to genomic regions contributing to the stay-green trait, identified in carefully managed, replicated, multi-environment tests (Kassahun *et al*, 2010).

2.6. What is stay-green?

According to Tenkouano *et al.* (1993), stay-green is a mechanism of delayed leaf and plant death that circumvents the detrimental effects of reduced soil moisture combined with high temperatures during drought stress that occurs during post-anthesis growth.

2.6.1. Stay Green and Yield

Borrell *et al.* (1999) observed that stay-green and yield were positively associated in a range of studies conducted in both Australia and India, demonstrating the value of retaining green leaf area under post-anthesis drought. Grain yield is the product of grain number and grain size (Borrell *et al.*, 1999). Grain number is generally the main determinant of differences in grain yield for sorghum, grown under post-anthesis drought stress in southern India. Grain size is independent of green leaf area at anthesis. However, the retention of photosynthetic capacity under water-limited conditions of stay-green hybrids ensures continued availability of new assimilates and is associated with increased N uptake during grain filling (Borrell and Hammer, 2000a), potentially improving grain size. This was illustrated in a recombinant inbred line study in which Borrell *et al.* (1999) found that grain size was correlated with relative rate of leaf senescence during grain filling and that reducing rate of leaf senescence

from 3 to 1% loss of leaf area per day resulted in the doubling of the grain size from about 15 to 30 mg. Thus stay-green can increase grain yield by improving grain number and grain filling ability.

2.6.2. Stay green trait in sorghum

Stay green in sorghum is characterized by the plant's ability to tolerate post-flowering drought stress, thereby delaying the premature leaf and plant death (Subudhi *et al.*, 2000). Stay-green lines produce two to three more basal tillers per plant at black layer, have a greater stem diameter, have higher sugar concentrations at the base of the stem, maintain greater green leaf area longer, have a greater leaf area index than senescent lines, have a higher leaf relative water content, have higher specific leaf nitrogen, contain a higher level of cytokinins, and have enhanced transpiration efficiency (Duncan *et al.*, 1981; Ambler *et al.* 1987; Dahlberg, 1992; Borrell, and Hammer, 2000a). Moreover, stay-green genotypes do not show reduced yield under well-watered conditions, thus stay-green genotypes are productive on both irrigated and non-irrigated condition (Borrell *et al.*, 2000b). Sorghum is an annual plant but stay-green lines can survive for years through the generation of new tillers from the old plant base (Thomas and Smart, 1993). Numerous sorghum genotypes (B35, SC56, E36-1, KS19 etc.) have been identified that exhibit the stay-green trait (Rosenow *et al.*, 1983; Kebede *et al.*, 2001; Haussmann *et al.*, 2002b).

The genotype B35 (BT×642) has been an especially useful source of stay green for research (Tuinstra, and Goldsbrough, 1998; Crasta, *et al.*, 1999; Tao, *et al.*, 2000) and the development of commercial hybrids (Henzell *et al.*, 2001). Genetic studies show that the B35 has genes that confer the stay green trait and that they express various levels of dominance (Walulu, *et al.*, 1994) or act in an additive manner (Van Oosterom *et al.*, 1996).

Several stay green QTL mapping studies have been conducted using B35 as one of the parents (Tuinstra *et al.*, 1996; Crasta, *et al.*, 1999; Subudhi *et al.*, 2000; Tao *et al.*, 2000; Xu *et al.*, 2000). These studies identified four major QTL designated *Stg1*, *Stg2*, *Stg3*, and *Stg4* and many additional minor QTL (*StgA, StgB*) that can modulate expression of the stay-green trait. *Stg1* and *Stg2* are located on linkage group 03 (LG-03) and explain 20% and 30% of the phenotypic variability, respectively (Xu *et al.*, 2000; Sanchez *et al.*, 2002). *Stg3* is located on LG-02 and *Stg4* on LG-05, accounting for 16% and 10% of the phenotypic variance, respectively (Sanchez *et al.*, 2002). Three QTLs for chlorophyll content (*Chl1, Chl2*, and *Chl3*), explaining 25 - 30% of the phenotypic variability were also identified under post-flowering drought stress (Xu *et al., 2000*). They all corresponded with the three stay green QTL regions (Stg1, Stg2, and Stg3) accounting for 46% of the phenotypic variation.

Xu, *et al.* (2000) noted that the *Stg1* and *Stg2* regions also contain the genes for key photosynthetic enzymes, heat shock proteins, and an abscisic acid (ABA) responsive gene. Such spatial arrangement shows that LG-03 is important for drought and heat stress tolerance and yield production in sorghum.

Borrell and Hammer, (2000a) indicated that sorghum hybrids containing the stay green trait retain more photosynthetically active leaves under drought than do hybrids that do not contain this trait. Since the longevity and photosynthetic capacity of a leaf are related to its nitrogen (N) status, it is important to clarify the role of N in extending leaf greenness in stay-green hybrids.

Stay green in sorghum has been associated with improved grain yields, particularly in environments in which available water during grain filling is not adequate to support potential transpiration (Borrell and Hammer, 2000a). It is a valuable trait that improves a genotypes adaptation to post flowering drought stress. The trait is best expressed in

environments in which the crop is dependent upon stored soil moisture and where this is only sufficient to meet part of the transpiration demand (Mahalakshmi and Bidinger, 2002). Expression of stay-green has been reported in several crops; maize, *Zea mays L* (Crafts-Brandner, *et al.,* 1984a, Gentineta *et al.,* 1986; Subedi and Ma, 2005); rice, *Oryza sativa L.* (Mondal and Choudhuri, 1985; Wada and Wada, 1991; Jiang *et al.,* 2004); oat, *Avena sativa L.* (Helsel and Frey, 1978); and pearl millet, *Pennisetum glaucum L.* A stay-green mutant of the pasture grass *Festuca pratensis Huds* has been identified and subsequently studied leading to a better understanding of the biochemistry of senescence (Thomas and Smart, 1993).

2.6.3. Inheritance of Stay-green in sorghum

Stay green expression is strongly influenced by environmental factors such as drought, heat, and nitrogen deficiency. The primary expression of leaf senescence is the breakdown of chlorophyll and the subsequent cessation of photosynthesis (*http://www.plantstress.com/articles/drought).* Due to varied expression, field environments often do not offer ideal conditions for selection, and molecular markers associated with this trait may offer a better alternative (Crasta *et al.,* 1999; Xu *et al.,* 2000).

Difference in patterns of senescence exist in many species that exhibit stay green, differences occur even among genotypes that may retain the same level of leaf area at maturity (Thomas and Howarth, 2000), This is influenced by both the time of onset and the rate of development of stress in an individual environment. There is more than one mechanism by which leaves stay green (Thomas and Howarth, 2000) and these are likely to be controlled by different genes that are triggered by specific patterns of stress development (Dunwell, 2000).

Broad and narrow sense heritability estimates for the stay green were 0.8 and 0.6, respectively (Walulu *et al.,* 1994), indicating that the stay green is heritable and progress from selection can be attained. Van Oosterotn *et al.,* (1996) observed that the inheritance of onset of senescence in sorghum was additive, but a slow senescence rate was dominant over a fast rate.

2.7. Quantitative traits

Falconer and Mackay, (1996) and Lynch and Walsh, (1998) defined quantitative traits as traits that have a continuous phenotypic distribution. Variances of these characters are often controlled by the segregation of many loci, called quantitative trait loci (QTL). Therefore, quantitative traits are often synonymously called polygenic traits. Another characteristic of quantitative traits is that environmental variations can play a large role in determining the phenotypic variance (Xu, 2002). Rukam *et al.* (2010) indicated that quantitative traits are typically controlled by many genes, each contributing only a small part to the observed variation. Selection for quantitative traits is difficult, because the relation between observed trait values in the field (the phenotype) and the underlying genetic constitution (the genotype) is generally not straightforward (Rukam *et al.*, 2010). The environmental variance resulting from differences in growing conditions further obscures the relation between phenotype and genotype.

The polygenic nature and the modification of expression by the environment make the study of genetic basis for quantitative traits more difficult than that for monogenic traits. Traditional methods of quantitative genetics that use only the phenotypic and pedigree information cannot separate the effects of individual loci but examine the collective effect of all QTL. With the rapid development of molecular technology, a large number of

molecular markers (DNA variants) can be generated with ease. Most molecular markers are functionally neutral, but they normally obey the laws of Mendelian inheritance. Therefore, the relative positions of the markers along the genome (called the marker map) can be reconstructed using observed recombinant events. The joint segregating patterns of markers, in conjunction with phenotypic and pedigree information, provides additional information about the genetic basis of quantitative traits, including the number and chromosomal locations of QTL, the mode of gene action, and sizes (effects) of individual QTL. A complete description of the properties of QTL is called the genetic architecture.

2.8. Molecular Markers

A molecular marker is a particular sequence of deoxyribonucleic acid (DNA) that is identifiable within the context of an entire genome. According to Jiang (2013) genetic markers are the biological features that are determined by allelic forms of genes or genetic loci and can be transmitted from one generation to another, and thus they can be used as experimental probes or tags to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene.

Xu (2010), and Jiang (2013), classified genetic markers used in genetics and plant breeding into two categories: classical markers and DNA markers. Classical markers include morphological markers, cytological markers and biochemical markers. Morphological markers characterized phenotypic characters such as flower colour, seed shape, and pigmentation. Biochemical markers are differences in gene products, proteins, or enzymes (isozymes) that are detected by electrophoresis and specific staining. The major disadvantages of morphological and biochemical markers are that they may be limited in number and are influenced by environmental factors or the developmental stage of the plant

(Winter and Kahl, 1995). Despite these limitations, morphological and biochemical markers have been extremely useful to plant breeders (Eagles *et al.*, 2001).

Molecular markers can be thought of as constant landmarks in the genome. They are identifiable DNA sequences, found at specific locations of the genome, and transmitted by the standard laws of inheritance from one generation to the next (Rajib *et al.*, 2014). DNA markers can be used to detect polymorphism between different genotypes or alleles of a gene for a particular sequence of DNA in a population or gene pool. Such fragments are associated with a certain location within the genome and may be identified by means of molecular technology. There are two basic methods to detect the polymorphism: Southern blotting, a nuclear acid hybridization technique (Southern, 1975), and PCR, a polymerase chain reaction technique (Mullis, 1990). Using PCR or molecular hybridization followed by electrophoresis e.g. PAGE (Polyacrylamide Gel Electrophoresis), AGE (Agarose Gel Electrophoresis) and CE (Capillary Electrophoresis), the variation in DNA samples or polymorphism for a specific region of DNA sequence can be identified based on the product features, such as band size and mobility.

DNA markers that do not discriminate between genotypes are called monomorphic markers. Polymorphic markers discriminate between genotypes and may also be described as co-dominant or dominant. This description is based on whether markers can discriminate between homozygotes and heterozygotes. Co-dominant markers indicate differences in size whereas dominant markers are either present or absent. The different forms of a DNA marker (e.g. different sized bands on gels) are called marker 'alleles'. Co-dominant markers may have many different alleles whereas a dominant marker has only two alleles (Rajib *et al.*, 2014).

The use of DNA-based markers for the genetic analysis and manipulation of important agronomic traits has become an increasingly useful tool in plant breeding (Ejeta and Knoll, 2007 and Jiang, 2013). DNA markers have the potential to enhance the operation of a plant breeding program through a number of ways, ranging from finger printing of elite genetic stocks, assessment of genetic diversity, increasing the efficiency of selection for difficult traits, to making environment neutral selection possible.

However, their greatest potential appears to be in accelerating the rate of gain from selection for desirable genotypes and in the manipulation of quantitative trait loci (QTL) that condition complex economic traits. DNA markers also permit plant breeders to correctly map or place the various interacting genes that condition complex agronomic traits. Genetic mapping is essential for effective manipulation of important genes.

According to Collard *et al.,* (2005) based on different polymorphism detecting techniques or methods (southern blotting nuclear acid hybridization, PCR polymerase chain reaction, and DNA sequencing), DNA markers have developed into many systems such as RFLP, AFLP, RAPD, SSR, and SNP.

2.8.1. Restriction Fragment Length Polymorphism (RFLP) markers

The RFLP markers are the first generation of DNA markers and one of the important tools for plant genome mapping. They are a type of Southern-Blotting-based markers (Jiang, 2013). For comparative and synteny mapping, RFLP markers are powerful tools. Most RFLP markers are co-dominant and locus specific. RFLP genotyping is greatly reproducible, and the procedure is simple and no special equipment is required (Konieczny and Ausubel, 1993). Another advantage of RFLP is that the sequence used as a probe need not be known. The disadvantages of RFLP are the requirement of relatively large amounts of pure and intact

DNA and the tedious experimental procedure (Botstein *et al.,* 1980; Weising *et al.,* 2005; Edwards *et al.,* 2007). Radioactive autography involved in genotyping and physical maintenance of RFLP probes limit its use and sharing between laboratories (Jiang, 2013).

2.8.2. Randomly-Amplified Polymorphic DNA (RAPD) markers

The RAPD is a PCR-based marker system. In this system, the total genomic DNA of an individual is amplified by PCR using a single, short and random primer (Williams *et al.* 1990). The primer, which binds to many different loci, is used to amplify random sequences from a complex DNA template that is complementary to it (Jiang, 2013). The DNA amplification products are visualized by gel electrophoresis. Because the primers are randomly chosen, no prior knowledge of the DNA sequence is needed. A disadvantage of RAPD markers is the fact that the polymorphisms are detected only as the presence or absence of a band of a certain molecular weight, with no information on heterozygosity. Besides being dominantly inherited, RAPDs also show some problems with reproducibility of data. Their major advantages are the technical simplicity and the independence of any prior DNA sequence information (Weising *et al.,* 2005; Edwards, *et al.,* 2007).

2.8.3. Amplified Fragment Length Polymorphism (AFLP) markers

The AFLPs are PCR-based marker system. This technique combines elements of RFLP and RAPD. It is based on the selective PCR amplification of restriction fragments (Sarah and Maria 2011). The AFLP *markers* are very reliable, robust and immune to small variations in PCR amplification parameters (e.g., thermal cycles, template concentration), and it also can

produce a high marker density. The AFLP products can be separated in high resolution electrophoresis systems (Jiang, 2013).

In general, AFLP assays can be conducted using relatively small DNA samples (1-100 ng per individual). AFLP has a very high multiplex ratio and genotyping throughput, and is relatively reproducible across laboratories. Another advantage is that it does not require sequence information or probe collection prior to generating the fingerprints, and a set of primers can be used for different species. This is especially useful when DNA markers are rare. However, AFLP assays have some limitations also. For instance, polymorphic information content for bi-allelic markers is low (the maximum is 0.5). High quality DNA is required for complete restriction enzyme digestion (Edwards *et al.*, 2007).

2.8.4. Simple Sequence Repeats (SSRs) markers

The SSRs markers, also called microsatellites, short tandem repeats (STRs) or sequence-tagged microsatellite sites (STMS), are PCR-based markers. They are randomly tandem repeats of short nucleotide motifs (2-6 bp/nucleotides long). Di, tri and tetra-nucleotide repeats, e.g. (GT)n, (AAT)n and (GATA)n, are widely distributed throughout the genomes of plants and animals. The copy number of these repeats varies among individuals and is a source of polymorphism in plants. One of the most important attributes of microsatellite loci is their high level of allelic variation, thus making them valuable genetic markers (Jiang, 2013).

SSR markers are characterized by their hyper variability, reproducibility, co-dominant nature, locus-specificity, and random genome wide distribution in most cases. The advantages of SSR markers are that they can be readily analyzed by PCR and easily detected by PAGE or AGE. SSR markers can be multiplexed, have high throughput genotyping and

can be automated (Powell *et al.* 1996; and Weising *et al.* 2005). SSR assays require only very small DNA samples (100 ng per individual) and low start-up costs for manual assay methods. However, SSR technique requires nucleotide information for primer design, labor intensive marker development process and high start-up costs for automated detections. Since the 1990s, SSR markers have been extensively used in constructing genetic linkage maps, QTL mapping, marker-assisted selection and germplasm analysis in plants. In many species, SSR markers have been developed and are available. Because of their abundance, high throughput ability and cheaper price, they are very useful in plant breeding.

2.8.5. Single Nucleotide Polymorphism (SNP) markers

A SNP is a single nucleotide base difference between two DNA sequences or individuals. SNPs can be categorized according to nucleotide substitutions either as transitions (C/T or G/A) or transversions (C/G, A/T, C/A or T/G). In practice, single base variants in cDNA (mRNA) are considered to be SNPs as are single base insertions and deletions (indels) in the genome. SNPs provide the ultimate/simplest form of molecular markers as a single nucleotide base is the smallest unit of inheritance, and thus they can provide the maximum number of markers. SNPs occur very commonly in animals and plants. Typically, SNP frequencies are in a range of one SNP every 100-300 bp in plants (Edwards *et al.*, 2007; Xu, 2010). SNPs may be present within coding sequences of genes, non-coding regions of genes or in the intergenic regions between genes at different frequencies in different chromosome regions.

SNPs are co-dominant markers, often linked to genes and present in the simplest/ultimate form for polymorphism, and thus they have become very attractive as potential genetic markers in genetic studies and breeding. Moreover, SNPs can be very easily automated and

quickly detected, with a high efficiency for detection of polymorphism. Therefore, SNPs will be increasingly used for various purposes, particularly as whole DNA sequences become available for many species (e.g., rice, soybean, and maize,). However, high costs for start-up or marker development, high quality DNA required and high technical (equipment) demands limit, to some extent, the application of SNPs in some laboratories and practical breeding programs. Because of their abundance, high throughput ability and cheaper price, they are very useful to plant breeders.

2.9. Molecular markers and their applications in sorghum

Conventional cereal breeding is time consuming and dependent on environmental conditions. Breeding a new variety takes between eight and twelve years and requires testing over multiple environments. Hence, Breeders are interested in new technologies that could make this procedure more efficient.

Molecular markers play a vital role in many aspects of crop breeding ranging from identification of diverse breeding lines to mapping of genomic regions controlling desirable traits useful in marker assisted breeding (MAB) programs. The availability of markers is not the limiting factor in crops like sorghum, hence it is time to explore the extent to which marker technology is likely to be useful in future sorghum breeding. Use of molecular markers in sorghum began in the 1990's. The RFLP marker system was greatly exploited in sorghum (Whitkus *et al.,* 1992; Xu *et al.,* 1994; Pereira and Lee, 1995; Dufour *et al.,* 1997; Tao *et al.,* 1998). Later, other marker systems came in to existence including AFLP (Boivin *et al.,* 1999; Klein *et al.,* 2000; Menz *et al.,* 2002), RAPD (Xu *et al.,* 2001; Haussmann, *et al.,* 2002a), SSRs (Taramino *et al.,* 1997; Bhattramakki *et al.,* 2000; Klein *et al.,* 2000; Kong *et al.,* 2000; Subudhi *et al.,* 2000; Menz *et al.,* 2002; Schloss *et al.,* 2002; Bowers *et al.,*

2003; Wu and Huang, 2006) and DArT markers were exploited by Mace et *al.*, (2008). All these marker systems were used in sorghum for different purposes like fingerprinting, assessing genetic diversity and QTL mapping but none were very useful for breeding new varieties.

2.11. Marker Assisted Selection

Marker assisted selection (MAS) is a process whereby an indicator (marker) is used to identify indirectly (indirect selection) a genetic determinant or determinants of a trait of interest (e.g. productivity, disease resistance, abiotic stress tolerance, and quality). The process is used in plant and animal breeding and involves selecting individuals based on their marker pattern (genotype) rather than their observable traits (phenotype). The method is based on selecting specific alleles at marker loci that are known to be linked to the genes that cause the desired trait (Patrick and Kelley 2005). Marker assisted selection has great potential to improve both intrinsic and operational yields of crop species (Edgerton, 2009; Gruian-Sherman, 2009).

DNA markers linked to the genomic regions of interest serve as an important tool, enabling breeders to conduct early-stage selection on the basis of genotype rather than phenotype (De Vicente and Tanksley, 1993; Abdurakhmonov *et al*., 2011a and b). Marker assisted selection provides many advantages over conventional breeding (Collard and Mackill, 2008; Kumpatla *et al*., 2012).

The most important requirement for a MAS program is the availability of a sufficient number of polymorphic markers evenly covering the target genome and associated with a trait of interest, mapping details with flanking loci, the extent of linkage disequilibrium blocks, donor genotypes bearing the QTL of interest, and breeder's ability and capacity to use

available molecular resources. User friendly automated genotyping platforms are vital to perform large scale MAS programs (Collard, and Mackill, 2008; Abdurakhmonov, *et al*., 2011a and b; Kumpatla, *et al*., 2012).

The MAS approach is considered to be an efficient breeding tool to improve crops. Some early successful applications of MAS were improvements in maize (Ragot, *et al*., 2007) and soybeans (Crosbie *et al*., 2003; Cahill, and Schmidt, 2004; Kumpatla, *et al*., 2012),

Broadly, MAS can be divided into two categories, that of marker-assisted backcrossing (MABC) or introgression and that of marker-assisted recurrent selection (MARS) or population improvement. In the former, the goal is to incorporate one or few major genes or QTL into elite breeding lines. The second involves using markers to improve the overall genetic value of a population with respect to some complementary traits. Of the two, marker-assisted backcrossing, particularly of a single gene, is the easiest to put into practice.

Backcross breeding enables breeders to transfer a desired trait, such as disease resistance or drought tolerance, from one variety considered as donor parent (DP) into another considered as recurrent parent (RP), (Vogel, 2009). Backcross breeding is an effective method to transfer one or a few genes controlling a specific trait from one line into a second, usually elite breeding line (Matthew, 2012). The parent with the desired trait, called the donor parent, provides the desired trait but may not perform as well as an elite variety in other areas. The elite line, called the recurrent parent, usually performs well in all other areas but lacks the desired trait (Matthew, 2012). Backcrossing involves crossing the donor and recurrent parents. The resultant F_1 progeny have 50% of their genetic material of each parent. F_1 individuals are crossed to the recurrent parent to develop a backcross one (BC_1) population. Individuals from the BC_1 population are once again crossed to the recurrent parent to obtain BC_2 and so on. Each generation of backcrossing reduces the proportion of the donor parent

present in the population by half. This cycle of backcrossing progeny to the recurrent parent continues until a new line that is identical to the recurrent parent, but with the desired gene or trait from the donor parent, is created. By the BC_4 generation, the lines are 96% identical to the recurrent parent (Matthew, 2012). The backcrossing process can often be accelerated using marker-assisted backcrossing, also known as background selection.

Marker-assisted backcrossing increases efficiency in introgression programs by permitting simultaneous foreground selection for introgression of an exotic allele with potential to improve performance for a trait of interest and background selection for the desired recurrent parent genotype in other genomic regions. While considerable work has been done on the identification of stay green genotypes and mapping of QTLs associated with the trait and identification of molecular makers linked to these QTLs, there are few reports on the actual transfer of these QTLs into elite cultivars and the assessment of the expression and consequences of the stay green trait in different backgrounds.

2.12. Cytoplasmic Male Sterility in Sorghum

Cytoplasmic male sterility (CMS) adversely affects development of specific cells in the anthers during some stage of microsporogenesis to cause male sterility. Its inheritance is non Mendelian in fashion. It is maternally inherited and causes complete male sterility under normal environmental conditions (Horner and Palmer, 1995). Some plants have CMS systems that contain nuclear restorer genes that override the CMS condition. These systems are designated genic-cytoplasmic male sterility *(g-cms)*. Cytoplasmic male sterility, is controlled by mitochondrial genes and CMS rf plants are male sterile. Nuclear fertility restoration genes, RF, restore pollen fertility in CMS RF plants.

2.12.1. Appraisal of sterility types in sorghum

Male sterility arising from physical and chemical emasculation is not practical in a commercial setting because sorghum flowers are tiny and significant numbers cannot be emasculated by hand. The hot water technique (Stephens, 1937) and chemical emasculation (Robinson, 1987) can emasculate florets on masse, but they lack efficiency. Many fertile flowers always remain. Hybrids produced that way would be a mixture of primarily selfed lines and hybrids (non-uniform) (Robinson, 1987). Bags used in the hot water technique pose increased cost of seed production. Cytoplasmic male sterility is the most pratical means to produce commercial sorghum hybrids. It is efficient because all the female plants and florets are male sterile due to the male sterile cytoplasm resulting in production of pure hybrid seed. RF genes in the male parent restore the F_1 seed to male fertility in the farmer's field.

2.12.2. Development of cytoplasmic male sterile and male fertile lines

The cms (male sterile) line is cytoplasmic male sterile and referred to as A-line. The B-line is the same nuclear genotype as the A-line but is fertile because it has a non-sterile cytoplasm. The B-line can be used to increase seed on the cms A-line. It is called a maintainer line. A third line that contains the nuclear male fertility restorer gene, RfRf, (is used as the male to cross onto the female CMS A-line to produce a fertile hybrid (Horner and Palmer, 1995). This line is called the R-line. Thus, it is possible to both maintain and perpetuate male sterility in the sterile line through crossing with a maintainer line to reproduce the sterile line and to restore fertility of a hybrid by using an R-line which restores fertility to the sterile cytoplasm. Sorghum hybrids are produced with these three types of lines. According to Andrews *et al.* (1996), once a sterile cytoplasm is identified, it is used to identify maintainer and restorer lines in sorghum populations. New A-lines are developed by introgression of

the A-line into desired male sterile lines until the two lines are isogenic, differing only in cytoplasm. A cross of the sterile cytoplasm with a line that restores fertility identifies a new restorer (R-lines).

2.13. Hybrid vigour (heterosis)

Heterosis is a situation whereby the F_1 hybrid is superior to its parents. Heterosis leads to increase in yield, reproductive ability, adaptability, disease and insect resistance, general vigour, and quality. For most of the characters, the desirable heterosis is positive, but for some characters like earliness, height in cereals and toxic substances, negative heterosis is desirable.

Although heterosis was demonstrated as early as 1927 in sorghum (Conner and Karper, 1927), its commercial exploitation was not possible until the discovery of a stable and heritable cms system (Stephens and Holland, 1954). This cms system has been designated as A1 (*milo*). A large number of hybrids have been developed and released (marketed) for commercial cultivation in Asia, the Americas, Australia and Africa.

Two types of heterosis have been reported (Niehaus and Pickett, 1966). In one type, plants were associated with gross changes in maturity, size and height but in the other type, plants were associated with general vigour without gross changes in size.

Heterosis varied with germplasm and plant traits (Kirby, and Atkins, 1968; Haussmann, *et al.*, 1998). Heterosis was displayed in stalk diameter and number of leaves (Kirby, and Atkins, 1968), in number of sorghum heads per plot and seed size (Kirby, and Atkins, 1968; Haussmann, *et al.*, 1998), in biomass, harvest index and stalk lodging (Haussmann, *et al.*, 1998), in seed density, growth rate, head exsertion (Kambal, and Webster, 1966) and in protein content (Collins, and Pickett, 1972). Grain yield heterosis ranged between 22% and

79.7%. Low performing parents generally had higher heterosis values than high performing parents (Miller, and Lee, 1964; Kirby, and Atkins, 1968).

The basis of heterosis is complementary heterotic parental groups (Pollack, *et al.*, 1991; Andrews, *et al.*, 1996). Various causes of heterosis have been proposed (Niehaus, and Pickett, 1966). They are allelic dominance, overdominance and epistatic gene action.

2.14. The combining ability

The concept of combining ability was first used by maize breeders in the USA in the 1930s to predict parental breeding value from their progenies (Simmonds, and Smartt, 1999). General combining ability (GCA) is the average contribution an inbred makes to hybrid performance in a series of hybrid combinations in comparison to other inbreds in the same series of hybrid combinations. The specific combining ability (SCA) is the contribution of an inbred to hybrid performance in a cross with a specific other inbred in relation to its contributions in crosses with an array of other inbreds.

General combining ability is a measure of additive genetic variance and SCA is a measure of non-additive genetic variance (Falconer, and Mackay, 1996). Both additive and non-additive genetic variances can be estimated. Combining ability is necessary for identification of good parental lines in hybrid breeding programs (Kambal, and Webster, 1965). Variance of GCA was more important than variance of SCA in grain sorghum yield (Kambal, and Webster, 1965; Beil, and Atkins, 1967). The ratio GCA: SCA was used to express the importance of GCA over SCA effects (Niehaus, and Pickett, 1966). Kambal, and Webster, (1965), used the ratios of male GCA to the sum male GCA plus male GCA x location interaction and female GCA to the sum female GCA plus female GCA x location interaction to express stability of GCA of males and GCA of females over locations.

CHAPTER THREE

3.0. FARMERS' VARIETAL PREFERENCES AND IMPLICATIONS FOR THE IMPROVEMENT OF SORGHUM

3.1. Introduction

Participatory rural appraisals (PRAs) have been used worldwide to solicit farmers' views on various agricultural resource management options necessary to ensure household food security and improvement in their welfare (Chambers, 1992). These have resulted in community based action plans being implemented for the farmers' benefit.

Sorghum is among the major crops in Mali and it occupies about 30% of total harvested agricultural area and 26% of the total production of cereals (DNA-Mali, 2015).The productivity of sorghum is constrained by numerous biotic and abiotic stresses. Agronomic practices employed by most farmers do not maintain soil fertility. Farmers manage their crops to produce annual harvests, relying mostly on farmer varieties in contrast to modern varieties developed by professional plant breeders (Soleri, and Cleveland, 2004). Professional plant breeders have not developed varieties for marginal environments, partly because of an incomplete understanding of why farmers choose the varieties they grow (Weltzien, *et al.*, 1998; Ceccarelli, and Grando, 2002; Christinck, 2002; Vom Brocke, *et al.*, 2003). One reason for this misunderstanding is the common assumption by breeders that modern varieties selected in optimal environments will also out yield farmer varieties in farmers' marginal environments (Cleveland, 2001; Ceccarelli, and Grando, 2002). Understanding farmer varietal choice should help formal research and extension programs better serve the needs of resource poor farmers. This can also support collaboration between

farmers and plant breeders in meeting these goals (Cleveland, 2001; Ceccarelli, and Grando, 2002).

A participatory rural appraisal was used to assess farmers' varietal preferences and the implications in the improvement of sorghum productivity in Mali.

The objectives of this study were to:

- rank the importance of sorghum in selected localities.
- determine sorghum production constraints in semi-arid regions of Mali.
- identify the criteria used by farmers in choosing which variety to grow.

3.2. Materials and methods

3.2.1. The study area

The focus group (FG) discussions were conducted in three villages namely, Diedougou, Wacoro and Kenioroba (Figure 3.1). Individual interviews (survey) were conducted in Keniroba and Diedougou while Wacoro was replaced by M'Pessoba because of similarity of agronomical and ecological conditions of Wacoro and Diedougou.

The study (PRA) was conducted between December 2012 and May 2013 when most smallholder farmers were less busy.

3.2.1.1. Diedougou

In this location situated between the 12°47′80″N and 6°40′75″W, agricultural production is dominated by cotton, maize, sorghum and millet.

3.2.1.2. Wacoro

Wacoro is located at 12°36′03″N and 6°41′36″W and crops grown are mainly sorghum, maize and cotton.

3.2.1.3. Kenioroba

Situated between the 13°07′22″N and 9°48′41″W, Kenioroba lies in the major sorghum growing belt of Mali, where sorghum covers between 60 and 80% of cultivated land.

3.2.1.4. M'Pessoba

M'Pessoba is situated between 12°66'93″N and 5°72′15″W. Agricultural production in M'Pessoba area is mostly dominated by sorghum and pearl millet. Maize and cotton are grown mainly as cash crop.

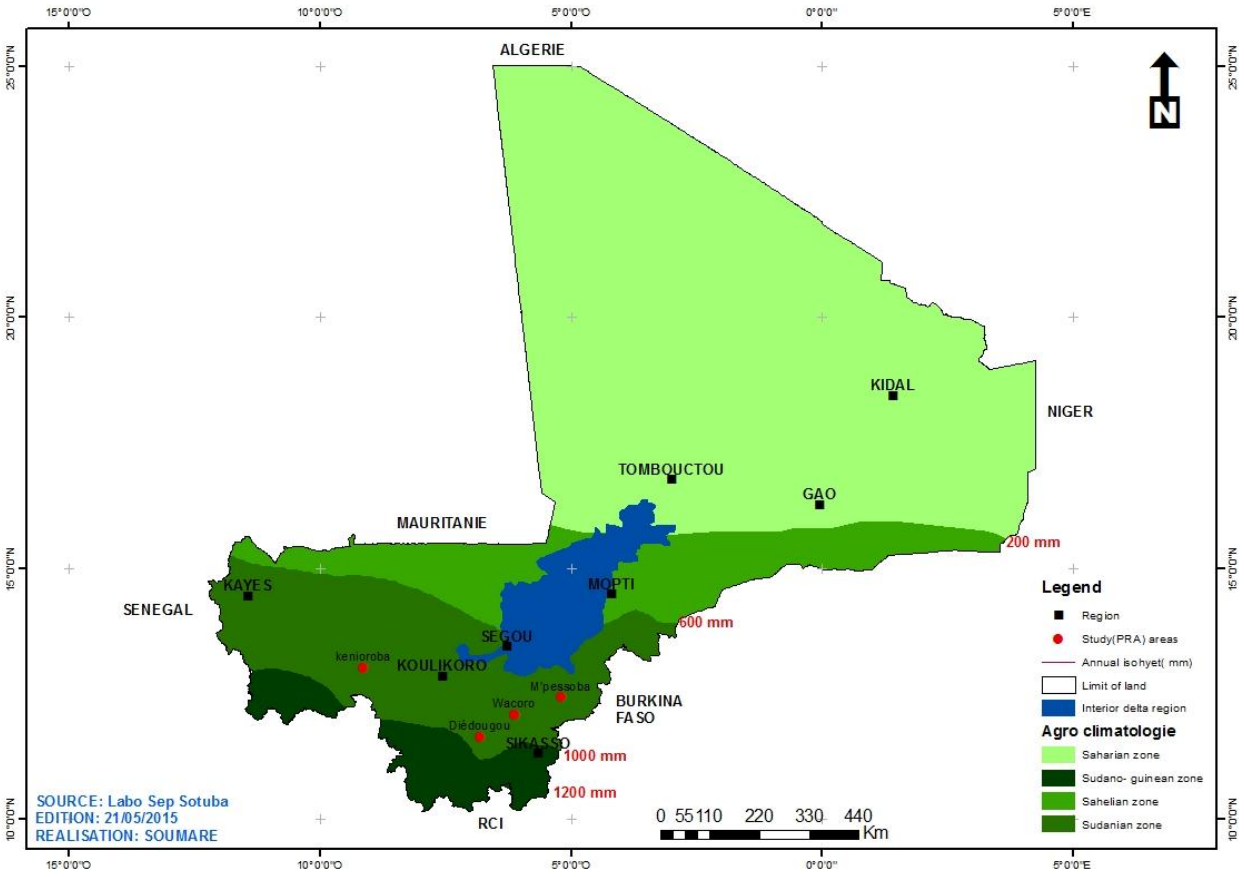


Figure 3. 1. Map of Mali with sites where PRA was conducted
(Sources. Soumare M. 2015)

3.2.2. Methodology for focus group discussions

The FG discussions were conducted with a total of 74 small-scale farmers (Table 3.1.) identified through local agricultural extension workers and randomly selected without any bias towards age, gender, experience in farming, or status. The number of farmers interviewed were 30 in Diedougou, 27 in Kenioroba and 17 in Wacoro.

Thirty-two per cent (32%) of participants were women, of which 15 were in Diedougou and 9 in Kenioroba. There were no female participants in Wacoro (Table 3.1). Among the participants, 46% were above 40 years old and 54% were young adults between 40 to 16 years.

Table 3. 1. Selected districts, villages and number of participants

Districts	**Villages**	**Number of farmers**	
		Female	Male
Beleco	Diedougou	15	15
Dioila	Wacoro	0	17
Kita	Kenioraba	9	18
Total		**24**	**50**

The opening of PRA meetings in all the three villages followed a standard procedure starting with formal discussions with the leaders of farmers' association or the village chief about the reason for the study and introduction of the PRA team by the Agricultural extension Officers of each district. The team was composed of two extentionists (PRA-Facilitators) and a PhD student

During the meetings farmers were encouraged to use the local languages so they could express themselves more clearly.

A combination of various PRA methods was adopted (Figure 3.2 a. & b.) including focus group discussions, pairwise rankings, preference rankings and scoring (Chambers, 2002). The identification and ranking of constraints related to sorghum production and use were done using pair wise ranking and scoring techniques. This is a tool that facilitates priority setting. Rankings were done by key informants or groups that represented a mixture of interests (Luigi, 2003). Farmers were also asked to give their criteria (preferences) for choosing which variety to grow using preference ranking. This method of ranking involves participants assessing different items or options, using criteria that they themselves have identified (World Bank, 2003).

a. Focus group (FG) discussion method ***b. Preference rankings and scoring method***

Figure 3. 2. Different participatory rural appraisals (PRA) methods.

3.2.3. Results of focus group discussions

3.2.3.1. Sorghum uses (importance) identified during FG discussions

3.2.3.1.1. Sorghum uses in Diedougou

In Diedougou, farmers listed six main uses of sorghum (Table 3.2). Of these money was the first, followed by animal feed (fodder), and human consumption. According to the farmers

in this area, maize is relatively more important for human consumption than sorghum. There is contract with World Food Program (WFP) to produce sorghum for cash.

Besides the contributions of sorghum to the economy and food security, it plays an important role in the socio-cultural and religious aspects of the farmers in this area. Sorghum is used as dowries during weddings, for therapeutic purposes and for brewing local beers. These uses were ranked fourth, fifth and sixth (Table 3.2).

Table 3. 2. Pairwise ranking of sorghum utilization by Diedougou farmers

Usages	Consum	Cash	Fodder	Dowry	Beer	Therap	Score	Rank
Consumption		Cash	Fodder	Consum	Consum	Consum	3	**3rd**
Cash			Cash	Cash	Cash	Cash	5	**1st**
Fodder				Fodder	Fodder	Fodder	4	**2nd**
Dowry (wedding)					Dowry	Dowry	2	**4th**
Beer						Therap	0	**6th**
Therapeutic							1	**5th**

3.2.3.1.2. Sorghum utilization in Wacoro

Farmers in Wacoro listed five main uses of sorghum (Table 3.3); these are consumption, fodder, cash, sacrify and construction (roofing, fence etc.). According to the farmers in Wacora, sorghum is important for home consumption because of its high yield for cooking.

Table 3. 3. Pairwise ranking of sorghum utilization by Wacoro farmers

Usages	Consum	Cash	Fodder	Sacrif	Construc	Score	Rank
Consumption		Consum	Consum	Consum	Consum	4	**1st**
Cash			Fodder	Cash	Cash	2	**3rd**
Fodder				Fodder	Fodder	3	**2nd**
Sacrifices (Religion)					Sacrif	1	**4th**
Construction						0	**5th**

3.2.3.1.3. Sorghum utilization in Kenioroba

In Kenioroba, four main uses of sorghum were listed. Consumption, fodder, cash and sacrifices were ranked first, second, third and fourth, respectively (Table 3.4). According to the farmers in Kenioroba, sorghum is important for home consumption because the yield in cooking is important, food with sorghum grains have the best (taste) and grains can be used in many local dishes such as sorghum-rice, djouka, and couscous.

Table 3. 4. Pairwise ranking of sorghum utilization by Kenioroba farmers

Usages	Consum	Cash	Fodder	Sacrifices	Score	Rank
Consumption		Consum	Consum	Consum	3	**1st**
Cash			Fodder	Cash	1	**3rd**
Fodder				Fodder	2	**2nd**
Sacrifices					0	**4th**

In conclusion, the results of survey showed that, except for Diedougou, sorghum is an important human food and animal fodder for farmers in the PRA areas. According to farmers, sorghum grain is used primarily at home for preparing local foods such as “tou” (thick porridge), couscous, porridge, sorghum-rice and many other dishes. Green forage is used for animal feeding. In addition, sorghum plays an important part in the socio-cultural and

religious aspects of farmers' lives. It is used for dowry in wedding, as sacrifice in funerals, and as beverage (local beer) in rituals. Leaves and grain are also used as medicine (therapeutic) to heal several illnesses in the areas.

3.2.3.2. Constraints to sorghum production

A pairwise ranking exercise was conducted to identify and rank sorghum production constraints as presented in Tables 3.5 and 3.6. In Diedougou, sorghum production constraints rankings varied from women to men farmers. Nine constraints were identified and ranked (Table 3.5). The three top constraints for men were high cost of inputs (fertilizer), lack of equipment and lack of training. These were given equal rankings as the most important constraint. Women ranked lack of land as the top constraint, followed by lack of equipment and high cost of inputs (Table3.5). Both men and women agreed on lack of equipment and high cost of inputs but men did not have a problem with lack of land. Drought was ranked fourth by men but women ranked drought, low soil fertility and lack of training of farmer as equal (fourth) followed by *Striga* damage and lack of markets. The lack of improved seeds is not a major problem for women (ranked 9th) whereas men ranked it at the fifth position (Table 3.5). The women explained that all the improved seeds they grow are obtained from a Non-governmental organization (NGO) as part of "food security pack". The low soil fertility, *Striga*, lack of market and lack of land were ranked by men as sixth, seventh, eighth and ninth.

In Diedougou, sorghum is mainly a cash crop (Table 3.5) but lack of market is not a major constraint for men and women because all their production is bought by the World Food Program (WFP) of United Nation (UN).

There were no women participants at Wacoro and in Kenioroba because there were not enough people to allow two groups. During FG discussion at these two sites, ten constraints were identified and ranked (Table 3.6).

At Wacoro, high cost of inputs was ranked first. Low soil fertility, lack of equipment and lack of training were ranked second. Drought and *Striga* were ranked fifth and lack of improved seed, bird damage, diseases (charcoal rot) and lack of markets for payment were ranked seventh, eighth, ninth and tenth. At Keniroba, the top constraints were lack of training (first), high cost of inputs (second), while lack of improved seed and drought were ranked third. Farmers ranked lack of equipment as fifth position followed by low soil fertility. *Striga* and charcoal rot disease were ranked seventh. Lack of market and bird damage were ranked ninth and tenth, respectively.

Table 3. 5. Pairwise ranking of constraints to sorghum production in Diedougou (Men and Women)

		MEN										
WOMEN	Constraints	LSF	Dght	Stga	HCI	LE	LM	LF	LIS	LT	**Score**	**Rank**
	Low soil fertility (LSF)		Dght	LSF	HCI	L.E	LSF	LSF	LIS	LT	**3**	**6th**
	Drought (Dght)	LSF		Dght	HCI	LE	Dght	Dght	Dght	LT	**5**	**4th**
	Striga (Stga)	LSF	Dght		HCI	LE	Stga	Stga	LIS	LT	**2**	**7th**
	High cost of inputs/fertilizer (HCI)	HCI	Dght	HCI		HCI	HCI	HCI	HCI	LT	**7**	**1st**
	Lack of equipment (LE)	LE	LE	LE	LE		LE	LE	LE	LE	**7**	**1st**
	Lack of markets (LM) for payment	LSF	Dght	Stga	HCI	LE		LM	LIS	LT	**1**	**8th**
	Lack of field (LF)	LF	LF	LF	LF	LF	LF		LIS	LT	**0**	**9th**
	Lack of improved seed (LIS)	LSF	Dght	Stga	HCI	LE	LM	LF		LT	**4**	**5th**
	Lack of training (LT)	LT	LT	LT	HCI	LE	LM	LF	LT		**7**	**1st**
	Score	**4**	**4**	**2**	**5**	**7**	**2**	**8**	**0**	**4**		
	Rank	**4th**	**4th**	**7th**	**3rd**	**2nd**	**8th**	**1st**	**9th**	**4th**		

Ranking Men Ranking Women

Table 3. 6. Pairwise ranking of constraints to sorghum production in Kenioroba and Wacoro

WACORO	KENIOROBA											
	Constraints	LSF	Dght	Stga	Dis	Bds	HCI	LE	LM	LIS	LT	**Score**	**Rank**
	Low soil fertility (LSF)		Dght	LSF	LSF	LSF	HCI	LE	LSF	LIS	LT	**4**	**6th**
	Drought (Dght)	LSF		Dght	Dght	Dght	HCI	Dght	Dght	LIS	LT	**6**	**3rd**
	Striga (Stga)	LSF	Stga		Stga	HCI	LE	Stga	Stga	LIS	LT	**3**	**7th**
	Charcoal disease (Dis)	LSF	Dght	Stga		Dis	HCI	LE	Dis	LIS	LT	**3**	**7th**
	Birds (Bds)	LSF	Dght	Stga	Bds		HCI	LE	LM	LIS	LT	**0**	**10th**
	High cost of inputs/fertilizer (HCI)	HCI	HCI	HCI	HCI	HCI		HCI	H.C.I	HCI	LT	**7**	**2nd**
	Lack of equipment (LE)	LE	LE	LE	LE	LE	HCI		LE	LE	LT	**5**	**5th**
	Lack of markets (LM) for payment	LSF	Dght	Stga	Dis	Bds	HCI	LE		LIS	LT	**1**	**9th**
	Lack of improved seed (LIS)	LSF	Dght	Stga	LIS	Bds	HCI	LIS	LIS		LT	**6**	**3rd**
	Lack of training (LT)	LT	Dght	LT	LT	LT	HCI	LT	LT	LIS		**9**	**1st**
	Score	**6**	**5**	**5**	**1**	**3**	**9**	**6**	**0**	**4**	**6**		
	Rank	**2nd**	**5th**	**5th**	**9th**	**8th**	**1st**	**2nd**	**10th**	**7th**	**2nd**		

Ranking at Kenioroba Ranking at Wacoro

In all communities, three main constraints of sorghum production were identified during FG discussion. They were lack of training, high cost of inputs, and lack of equipment except that women in Diedougou felt that lack of land was more important than lack of training. The men pointed out that, in Malian tradition, women are not land owners. It is men (husbands) who hand over to them part of their land, usually the part with low fertility. The lack of improved seeds was replaced by lack of equipment in third position at Kenioroba. After training, cost of inputs, and lack of equipment, drought, *Striga*, low soil fertility and lack of improved seeds were the most important. Lack of markets, charcoal rot disease and bird damage were the least important constraints.

The increasing demand for training was due to the introduction new agronomic practices (fertilizers, pesticide, field managements etc.) and new improved varieties by research institutions that were not known by most farmers in rural areas.

3.2.3.3. Varieties of sorghum grown in each of the three sites

The farmers grow local cultivars, improved varieties and hybrids. The choice of a variety depends mainly on the morphological, agronomic and organoleptic (gastronomic) characteristics as well as the ecological and socio-economic conditions where a variety is grown. During FG discussion, 28 varieties were named across all areas. The repartition between sites is shown in Table 3.7.

Table 3. 7. Repartition of varieties and hybrids between the sites

Site	Number of local varieties	Number of improved varieties	Number of Hybrids
Diedougou	5	5	-
Wacoro	6	3	3
Kenioroba	4	2	-
Total	**15**	**10**	**3**

The local landraces have been cultivated by many farmers for 30 – 60 years, and most of these varieties belong to the Guinea race of *Sorghum bicolor (L.) Moench* (Table 3.8). This preference for local varieties is based on food quality (taste), grain quality, adaptation to low soil fertility, photoperiod response and storage quality. The major limitation of the local landraces are their relatively low yield, susceptibility to *Striga* and lodging.

The improved varieties were introduced, from research institutions and have been grown by farmers for the last 10 years (Table 3.8). The varieties have good yield and forage quality but they have poor grain quality, low adaptation to local environments, susceptibility to diseases, storage problems and are prone to bird damage.

Sorghum hybrids were grown only in Wacoro (Table 3.7), these hybrids were introduced from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) not long ago. The hybrids have very good yield, but are sensible to photoperiod, susceptible to grain mold and small grains.

Table 3. 8. Varieties of sorghum grown in villages of Diedougou, Wacoro and Kenioroba

Villages	Variety name	Year grow	Seed type	Advantages	Weaknesses
Diedougou	Niobleni	30 years	Local landrace	Early maturity, Good taste of the tô	Low yield
	Bandonaka	> 20 years	Local landrace	Very early maturity, good tô.	Low yield, lodging
	N'Guegnefing	30 years	Local landrace	Medium maturity, high yield, good tô	Susceptible to *Striga*, fall of grain before harvest.
	Fanbekoun	>60 years	Local landrace	Yield, seed weight.	Late maturity and Susceptible to *Striga*.
	Nioble	4 years	Local landrace	Yield, seed weight, good tô.	Lodging
	Tiandougou	3 years	Improved var.	*Striga* and drought tolerance, yield, forage.	Difficult conservation after shelling, fall of grain.
	Tiandougou Coura	3 years	Improved var.	*Striga* and drought tolerance, yield, forage, good taste of the tô.	Difficult conservation after shelling, fall of grain.
	Soumba	4 years	Improved var.	Early maturity, yield	Low dormancy
	Grinkan	7 years	Improved var.	Yield, grain quality, forage. Good for couscous	Storage
	Seguifa	3 years	Improved var.	High yield, forage.	Susceptible to mildew
Wacoro	Tienkouraba	>60 years	Local landrace	High yield, good storage	Very late maturity.
	Seguetana djie	>40 years	Local landrace	Adapted to the poor soils, tolerant to the *Striga* and white color of grain.	Relatively low yield and bird's damage.
	Tiemarifing	>40 years	Local landrace	Medium maturity, good storage, white color of grain.	Susceptible to *Striga* and lodging
	Solotine	>40 years	Local landrace	Adapted to the area, birds tolerance, and good storage.	Relatively low yield
	Bandoka	30 years	Local landrace	Early maturity	Bad storage and relatively low yield.

Table 3.8: cont'd

Villages	Variety name	Year grow	Seed type	Advantages	Weaknesses
Wacoro	CSM 63E (Kalamani)	7 years	Improved var.	Very early maturity, Good taste of the tô	Low yield
	Fambe	10 years	Improved var.	Photosensitive, Medium yield.	High Vitreousness of grain
	Soumba (Nio-courouni)	7 years	Improved var.	Yield and forage	Bad taste of tô, bad storage.
	Bobodie	4 years	Improved var.	Good grain quality, good yield	Late maturity.
	Pablo	2 years	Hybrid	High yield, Photoperiod-sensitive.	Don't like the strong densities, The yield is bound to the photosensitivity
	Lata	3 years	Hybrid	Very productive, Photoperiod-sensitive.	Susceptible to Mildew (mold)
	Fada	3 years	Hybrid	High yield, Photoperiod-sensitive.	Very small grains.
Kenioroba	Sounoukoura Oulé	> 60 years	Local landrace	Adapted to the area, Early, good to, good yield	Susceptible to *Striga.*
	Sounoukoura Fing	> 60 years	Local landrace	Adapted to the area, Early, good to, good yield	Susceptible to *striga.*
	Boybagalawili	> 40 years	Local landrace	Good yield to thrashing, *Striga* tolerance, White grain, vitreousness	Late maturity
	Nioniteli	6 years	Local landrace	Very early, good yield to thrashing.	Bird's damage
	CSM 388 (Keniké blé)	15 years	Improved var.	Good yield	Susceptible to *Striga*
	Tiandougou Coura	2 years	Improved var.	Good yield, *Striga* tolerance, forage and good quality grains	Low yield in shelling, susceptible to abundant rain

3.2.3.4. Farmers' criteria (preferences) for choosing varieties

The major criteria for varietal preferences, in the three study areas, are presented in Table 3.9.

In Diedougou, 14 criteria were given by men for choosing varieties to grow. High yielding was the first preference, followed by adaptation to environment, lack of photoperiod sensitivity, ablility to produce yield even when rains stop early, *Striga* tolerance, drought tolerance, grain color, and vitreousness (hard) grain. Other criteria of less importance were biomass (fodder), lodging resistance, high yield for cooking and easy dehulling (seed coat). The least important traits were plant height, non-shattering (indehiscent), tolerance to bird domage, and excess water tolerance (abundant rain).

Women in Diedougou listed 9 preferences and ranked grain color (White) as first preference followed by high yield, drought tolerance, early maturity, good taste, easy dehulling, high yield in cooking, *Striga* tolerance and bird tolerant.

In Wacoro, high yield, early maturity, grain color (white), drought tolerance and good taste were considered the most important criteria. Of lesser importance were biomass production (fodder), lodging resistance, adaptation to environment, *Striga* tolerance, tolerance to bird domage, high yield in cooking, and charcoal rot tolerance.

In Kenioroba, the five top preferences were high yield, early maturity, drought tolerance, grain color (white) and *Striga* tolerance. The least important traits were good taste, biomass production (fodder), high yield in cooking, vitreousness (hard grain), charcoal rot tolerance, easy shelling (seed coat), medium plant height, and non-shattering (indehiscent).

During FG discussion, except for the group of women in Diedougou, the first trait preferred was high yield followed by earliness or adaptation to environment. Earliness enables the crop to escape late season drought (Post-flowering drought). Drought is one

of the main reasons given for selecting early maturing varieties by several farmers. However, Farmers believe that the majority of early maturing varieties have some disagreeable qualities, such as lower yields, poor grain and stem qualities. Farmers also expressed interest in drought tolerant varieties and ranked these as third and fourth preference depending on the sites. The desire of farmers to use more drought tolerant varieties is probably a result of the drought that has affected the semi-arid areas of Mali during the past twenty years.

Table 3. 9. Farmer's varietal preferences at different PRA sites

Preferences	Diedougou Men (n = 15)` Rank	Diedougou Women (n = 15)` Rank	Wacoro Men (n =17) Rank	Kenioroba Men & Women (n = 27) Rank
Adapted to environment	2nd	*	8th	*
Biomass (fodder)	7th	*	6th	7th
Birds tolerant	12th	9th	10th	*
Charcoal tolerant	*	*	12th	10th
Drought tolerant	4th	3rd	4th	3rd
Early maturity	*	4th	2nd	2nd
Easy dehulling (seed coat)	10th	6th	*	11th
Indehiscent	12th	*	*	13th
Good taste	*	4th	4th	6th
Grain color (White)	4th	1st	3rd	3rd
High yielding	1st	2nd	1st	1st
High Yield in Cooking	9th	7th	11th	8th
Lodging resistance	8th	*	7th	*
Medium plant height	11th	*	*	12th
Striga tolerant	3rd	8th	9th	5th
Vitreousness grains	6th	*	*	9th
Water tolerance (abundant rain)	14th	*	*	*

* = Preferences not listed in site or in group
N = Number of farmers that participated to the PRA

3.2.4. Methodology of interview questionnaires (survey)

Farmers' perceptions on sorghum production collected from the focus group discussion were used to develop an individual interview questionnaires. The individual interview questionnaires allowed farmers to provide input into the process of new variety development by professional plant breeders and to contribute to the improvement of sorghum productivity. The survey questionnaire was composed of 20 questions (Appendix 1).

The questionnaire was administered to 265 households in 25 villages or communities of three districts: seven villages in the Beleco district (Diedougou area), eleven villages in the Kita district (Kenioroba area) and seven villages in the M'Pessoba district (M'Pessoba area).

The survey areas were selected on the basis of the importance of sorghum and for their agro-ecological and socio-economic conditions as well as ethnic and cultural multiplicity.

Surveys were not done in the district of Dioila (Wacoro areas) because it has agro-ecological and socio-economic conditions similar to the Beleco district (Biedougou areas).

3.2.4.1. Data collection and analysis

The PRA questionnaire was completed at the time of the individual interviews (survey) and data were recorded in Microsoft Excel, and then imported into IBM SPSS (Statistical Package for Social Science) version 20. Data were analyzed using nonparametric statistics and summarized into averages, frequencies or percentages as shown in the forms of tables and graphs.

3.2.5. Results of interview questionnaires

3.2.5.1. Households Characterization

Households were selected in each village (or community) on a voluntary basis from households experienced in sorghum cultivation. Individual interviews were conducted in these households with a farmer respondent who is the head of the household or others in the household. In total, 265 surveys were conducted with 239 male respondents (90%) and 26 female respondents (10%) with age ranging between 18-78 years.

The respondents had grown sorghum for 3 to 70 years. The minimum number of members in activities in the household was one and the maximum was 41 with an average of 9, (Table 3.10). The average number of women per household was four (Table 3.10). Both men and women were involved in sorghum production. Women play an important role in sorghum production as they help in planting, weeding, harvesting, threshing, carting and cooking.

The mean total area of the households surveyed was nine hectares and the average planted to sorghum was 2.73 ha, which is about 29% of total area. The households are poorly equipped interms of farm equipment. On average, each farm has none or one piece of farm equipment (plows, seeder plows, gang plows and donkey or horse carts), two cows for plowing and less than ten cattle.

Farm equipment reduces the time needed for the planting and weeding, transporting and broadcasting manure and usually increases the type of crops grown and crop hectares.

Table 3. 10. Household Characterization

Characteristics	Mini.	Maxi.	Mean	Std. Deviation
Persons belonging to the household:	1	76	16	11.04
Persons in activities in the household	1	41	9	6.26
Women in activities in the household	0	26	4	3.30
Age of Respondent	18	78	46.74	12.56
Years of growing sorghum	3	70	28.27	14.92
Total area of household (ha)	0.25	56	9.49	6.84
Area under sorghum (ha)	0.25	20	2.73	2.11
Number of cultivator plows	0	6	1.15	0.87
Number of Grain drills (Seeders)	0	2	0.74	0.60
Number of Gang plows	0	5	0.86	0.83
Number of donkey carts	0	3	0.96	0.61
Number of cattle	0	100	8.83	11.67
Number of cows for plowing	0	12	2.15	1.81
Number of donkeys	0	4	1.09	0.738

N = Frequency
Mini. = Minimum
Maxi. = Maximum
Std. deviation = Standard deviation

3.2.5.2. Sources of livelihood of households

The main sources of revenue of surveyed households are different. Forty-one per cent get most of their income from selling cash crops such as cotton, peanut and sesame, 28% get most from selling sorghum and 19% from selling other food crops such as maize, millet, root, and tuber crops. In addition, 10% get most of their incomes from selling livestock and two per cent (2%) from others sources such as fishing, trading, monthly salary, and farm labor (Figure 3.3).

Overall, sorghum occupied second place in economic status of farmers in the study areas.

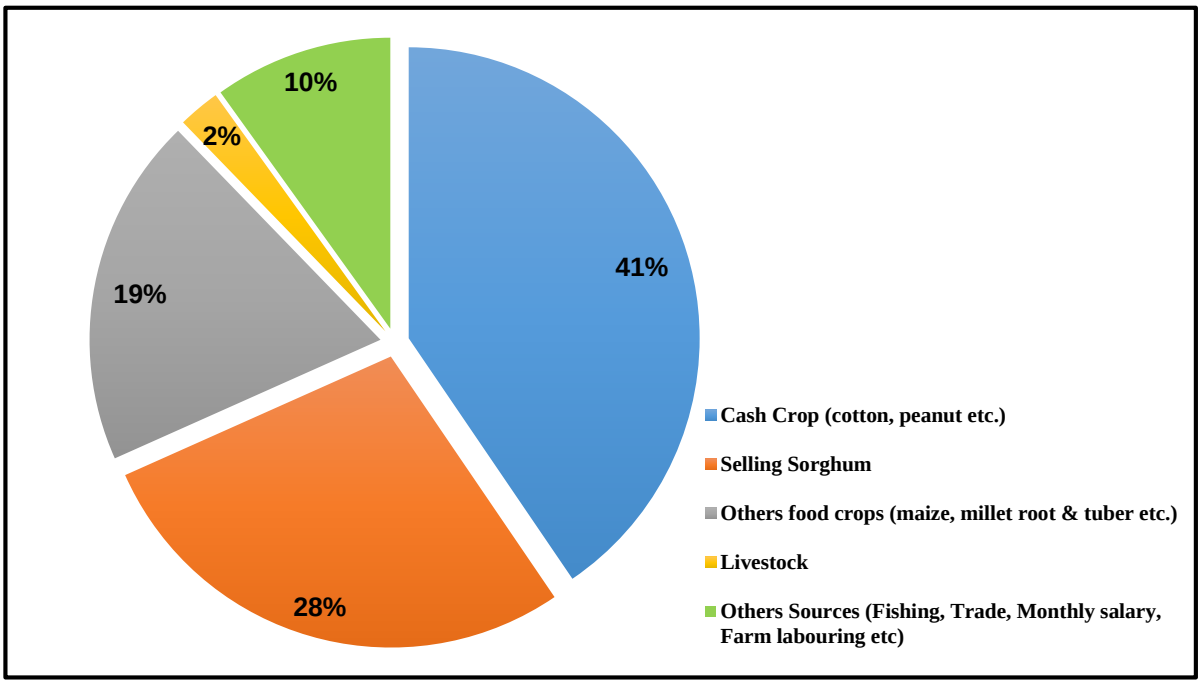


Figure 3. 3. Sources of livelihood of households

3.2.5.3. Crops cultivated in the surveyed households and main reasons for growing these crops.

All surveyed households grow sorghum (100% of households) and it is the major crop for 31% of growers (Table 3.11). After sorghum, 77% of households grow cowpea generally associated with sorghum, maize or others crops. Maize and groundnut are grown by 72% of farmers and are the major crops for 29.8% and 11%, respectively. Pearl millet and cotton are grown by 69.8% and 60.8% of farmers. Pearl millet is the major crop for 22.7% while cotton is the first crop for 29% of the farmers. Rice and fruit and vegetables are grown by few households (27.5% and 26.4%, respectively) (Table 3.11). Sorghum, maize, pearl millet, rice and cowpea are grown mainly for household consumption while cotton and groundnuts are the main cash crops.

Table 3. 11. Crops cultivated in the surveyed households

Crops	Number of farmers who grow (N)	Percent (%)	Rank first crop (%)
Cotton	161	60.8%	29.2%
Maize	191	72.1%	29.8%
Millet	185	69.8%	22.7%
Sorghum	265	100.0%	31.3%
Rice	73	27.5%	5.5%
Cowpea	205	77.4%	0.0%
Groundnut (Peanut)	191	72.1%	11.0%
Fruit & Vegetables	70	26.4%	4.3%
Other crops	156	58.9%	3.8%

Sorghum is primarily grown by 68.7% of the households for consumption, 28.3% for cash and 2.6% for others reasons such as dowry, therapeutic, sacrifices and beverage (Figure 3.4).

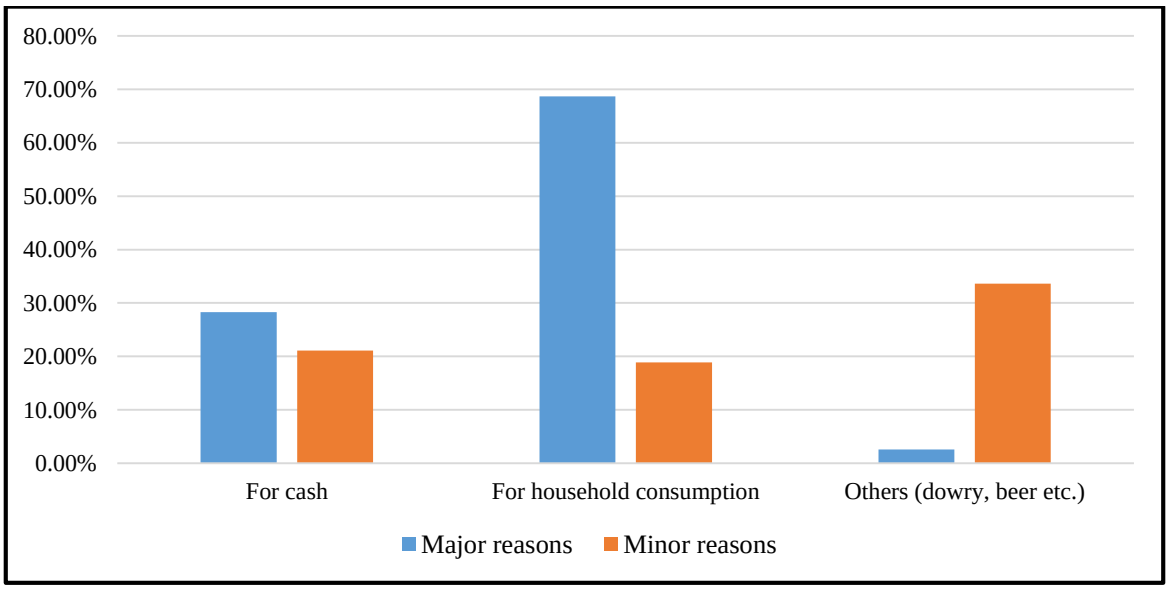


Figure 3. 4. Main reasons for growing sorghum in households

3.2.5.4. Varieties grown in surveyed households

Twenty-seven varieties were identified to be grown in surveyed areas; four improved varieties, four improved local cultivars and 19 local land races. Seventy per cent of the sorghum cultivated are local landraces, 15% are improved local landraces and 15% are improved varieties.

The majority of households grow one variety of sorghum but some households grow two to three varieties. They plant a combination of improved and local varieties or medium and early maturing varieties to secure and optimize yield, for grain qualities and for managing drought.

3.2.5.5. Main sources of seed for planting

The main source of seeds for planting is saved seeds with about 70% of households using their own seeds saved from previous crop harvest (Figure 3.5). The second most important source of seeds are exchanges among farmers (between neighbors, relatives, and friends). Other sources of seeds are Non-Governmental Organizations (NGOs). These organizations provide improved seed to farmers as part of a food security pack and for diffusion of information about the new improved varieties generated by research.

The access and adoption of seeds from seed companies or research institutes is poor. About 70 to 80% of farmers have never bought seed from the seed companies or research institutes.

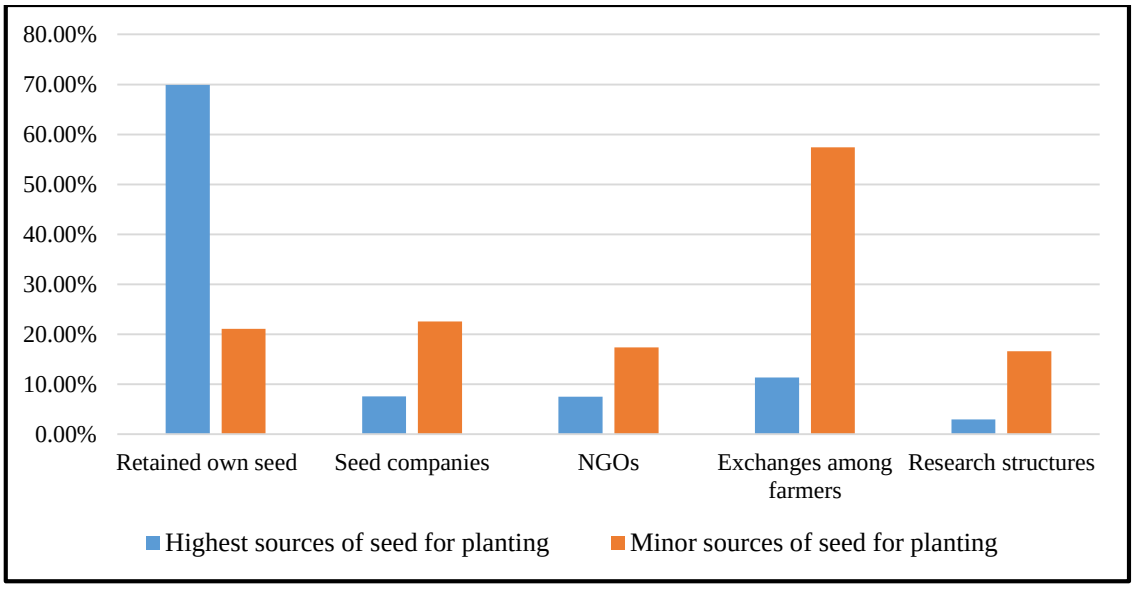


Figure 3. 5. Main sources of seed for planting in households

3.5.5.6. Crop management practices in the field

Sorghum cultivation relies mainly on traditional methods. Many households have few farm equipment and little income to buy inputs. Mostly family labor is used for planting, weeding and harvesting.

More than 20% of households don't do land preparation such as plowing before sowing, 41% don't use seed treatment, 70% don't use herbicides, 60% don't apply mineral fertilizer and 75.8% apply the organic fertilizer (Figure 3.6).

In households which apply fertilizer, the dose of fertilizer (mineral and organic) is low.

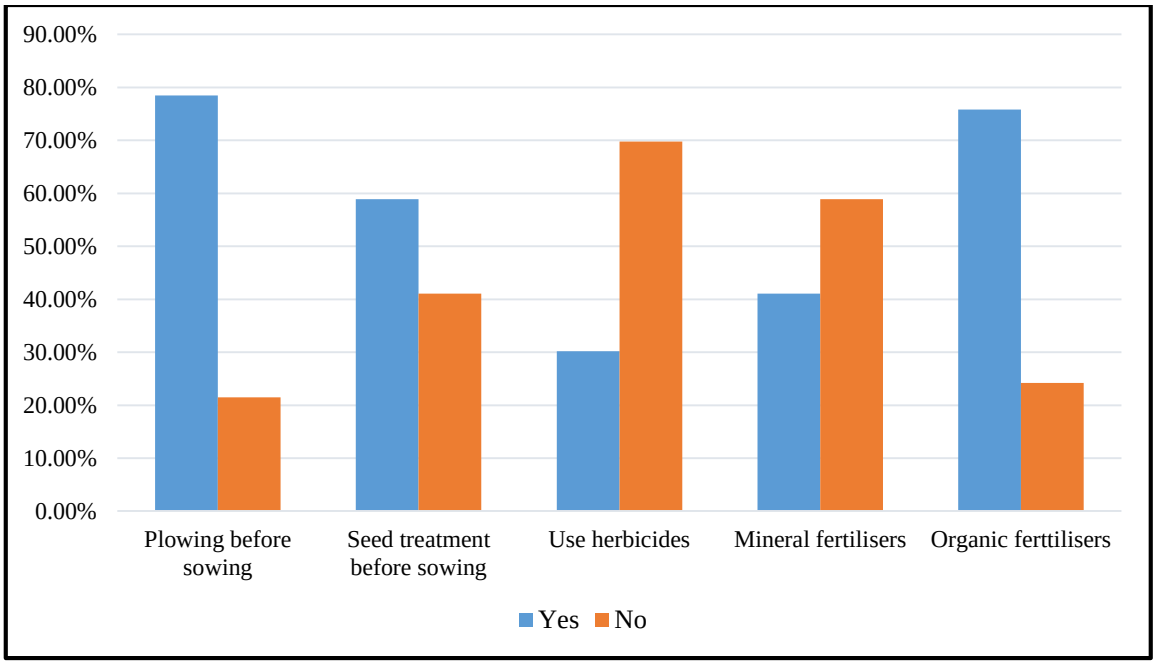


Figure 3. 6. Crop management practices in households

3.2.5.7. The level of yield for the different types of seeds

The yields recorded in the surveyed area are divided into four groups: Group I: 700 kg/ha, Group II: 701 to 1100 kg/ha, Group III: 1101 to 1500 kg/ha and Group IV: 1500 kg/ha. The lowest yielding group (Group I) comprised 62.3% of local landraces, and 37.7% of improved varieties. The highest yielding group (group IV) is dominated by improved varieties, over 70%, and 22.2% of local landraces (Figure 3.7).

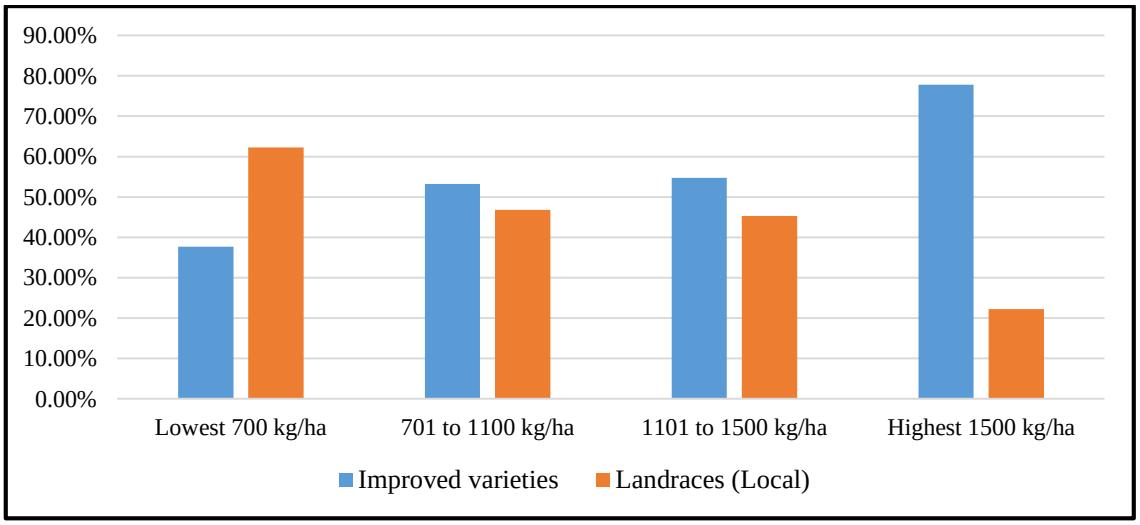


Figure 3. 7. The level of yield for different seed types

3.2.5.8. The main criteria used by farmers for selecting varieties

The farmers were asked to give three main criteria for selecting a variety and to rank them (Appendix 2).

a. Criteria for selecting varieties

About 34.3 % of farmers select a variety on the basis of yield potential, 25.7% select for earliness, 14.7% for grain color (white). 7.9% for drought tolerance, 6.4% for *Striga* tolerance (6.4%), 5.7% for grain size and 5.4 % for adaptation to the environment (Figure 3.8).

In total, 7 characteristics were cited by different farmers as the major criteria for selecting a variety. The differences in the preferences among groups reflected the different uses of the varieties (consumption, cash, etc.) and the agro-ecological conditions in which varieties are grown (poor soil, drought prone area, unpredictable rainy season, etc.).

A large number of farmers are interested in early maturing varieties because they consider earliness to be a form of drought tolerance because earliness enables the crop to escape late season drought and early maturing varieties provide an early source of food to reduce hunger.

b. The second criteria for selecting varieties

Twenty three percent of farmers favored the yield potential followed by white color of grain (15.1%), drought tolerance (12.5%), earliness (11.7%) and grain taste (9.1%) (Figure 3.8). The grain quality was the most frequently indicated preference criteria by farmers in graphic 3.8. This implies that the main reasons for growing sorghum is for use in preparing local foods such as tô, couscous, sorghum-rice, and porridge. The white color and good taste of grain generally is preferred by farmers for food. The vitreous grain (hard grain) is preferred for good food quality, floury grain for couscous and grain size for sorghum-rice and porridge.

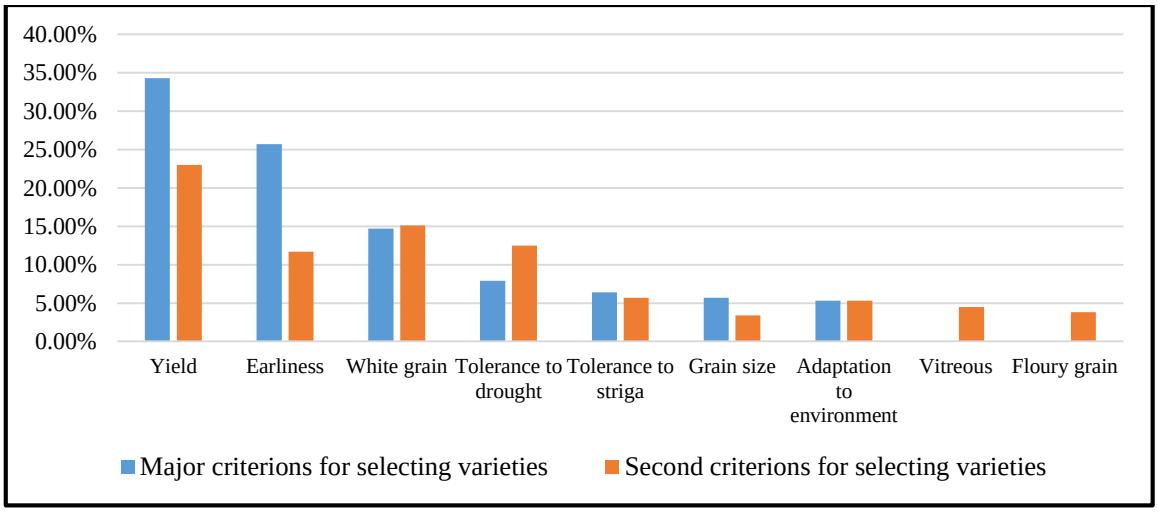


Figure 3. 8. The criteria for selecting which varieties to grow

3.2.5.9. Farmers preferences for sorghum grain

The sorghum grain is used principally in the households to prepare local foods and grain quality plays a significant role in the criteria for selecting which variety to grow. Nine characteristics of the sorghum grain were identified (Figure 3.9). The first preference was for grain color (56.6%) followed by organoleptic quality (taste) of grain for 11.7%. Most farmers considered vitreous grain, yield in cooking, grain size, floury endosperm (soft corneous seed) and storage quality as important characteristics of grains. The red color of grain are preferred for 3% and 6.8% of farmers because in some areas the red grains of sorghum are substituted for fonio in the preparation of the local food “Djouka” and also in some areas red grains are preferred for brewing a local beer.

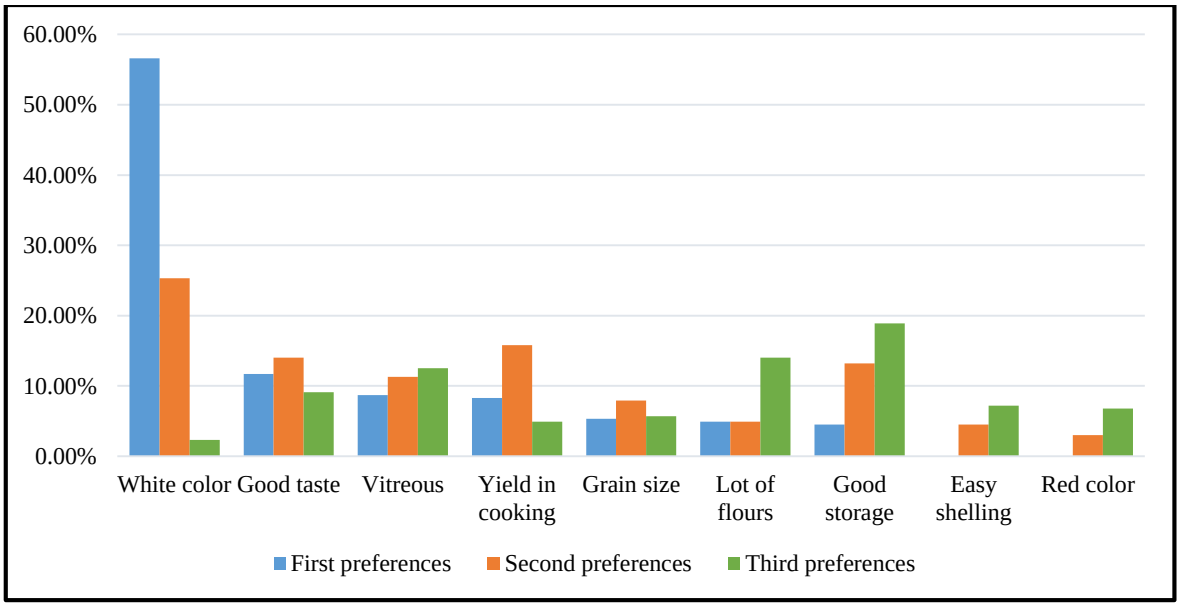


Figure 3. 9. Famers' preferences for sorghum grain

3.2.5.10. Farmers preferences for sorghum's stem

Sorghum is grown in Mali exclusively for the grain but the crop residues such as the stalks are a resource vital for animal feeding and for other uses such as fencing, thatching and firewood.

Seven characteristics are preferred for stems (Figure 3.10). The majority of farmers prefer medium stems height (25.7%) followed by high stems (23.4%). They are not familiar with short sorghum as all landraces are mostly tall.

The characteristics such as green stem, robust stem and biomass are considered as important characteristics of stem. Lodging tolerance is also an essential preference cited by many farmers.

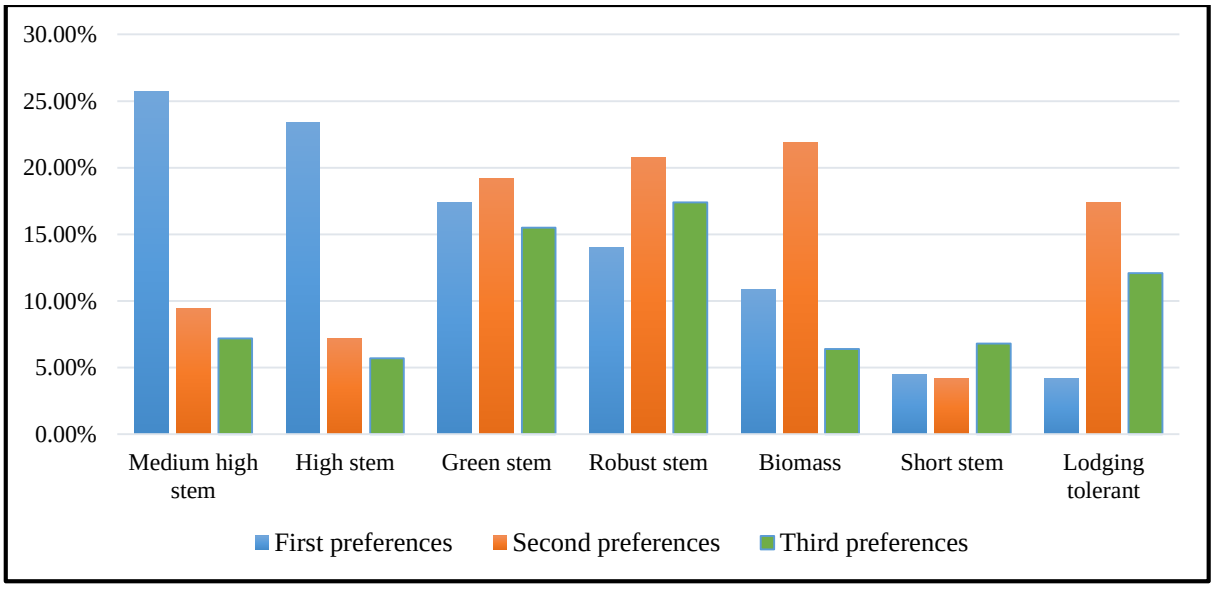


Figure 3. 10. Farmers' preferences for sorghum stem

3.2.5.11. Do the varieties that farmers grow satisfy the preferences cited?

The farmers were asked if the varieties that they grow fit the preferences cited above. The survey showed that 7% of farmers are largely satisfied with varieties that they grow whereas 73% of farmers responded that the varieties do not fit their preferences (Figure 3.11). These farmers hope to find other varieties.

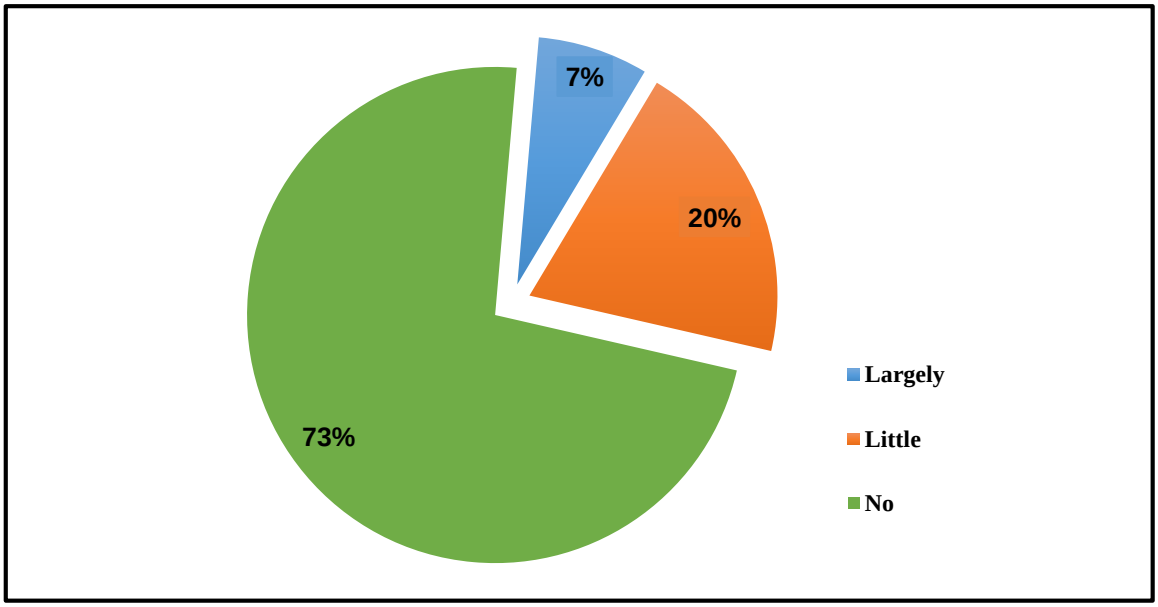


Figure 3. 11. The varieties that farmers grow satisfy the preferences cited?

3.2.5.12. Causes of low yield of sorghum

Farmers cited 11 reasons for the low yield in sorghum production (Figure 3.12). Twenty six percent of farmers indicated that the low yield is mainly due to low soil fertility while 21% think it is due to lack of equipment. These two factors are socio-economic details rather than breeding problems. Drought (13.2%) and poor rainfall (11.7%) were among the main reasons for low yield. The poor rainfall is concidered by the majority of respondent as lack of water or drought but some farmers consider it as unpredictable rains. These two factors could be combined and ranked as the major constraint followed by *Striga* infestation. The farmers mentioned also soil type (sandy), late sowing and lack of improved seed as reasons for low yield.

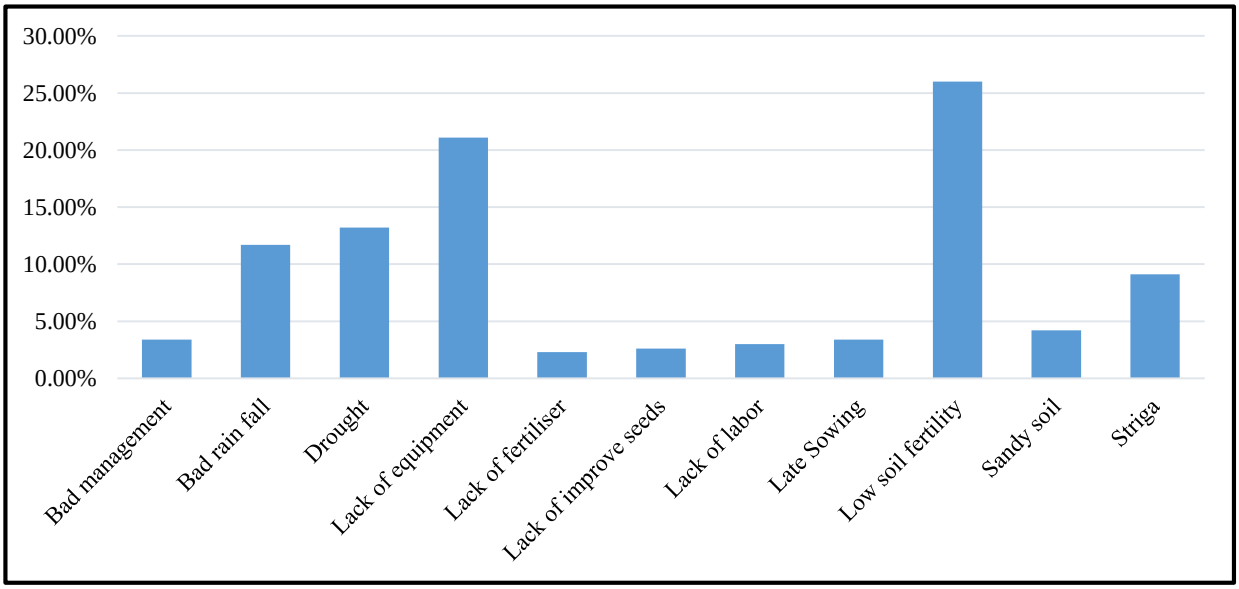


Figure 3. 12. Main reasons of the low yield of the sorghum in the households

3.2.5.13. Proposals by farmers to improve sorghum yield

Farmers suggested 10 solutions for improvement of sorghum (Figure 3.13). These include socio-economic, the three top solutions were supplying mineral and organic fertilizers (18.5%), providing equipment (18.1%) and providing adapted varieties (17%).

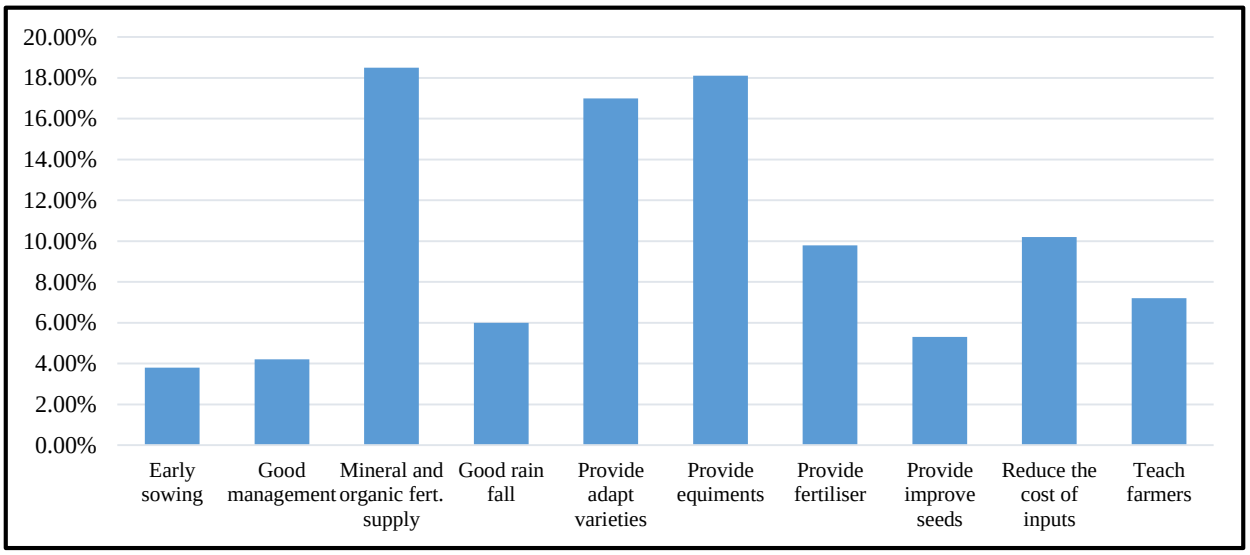


Figure 3. 13. Proposed solutions to improve sorghum yield

3.3. Breeding opportunities

The study identified several breeding opportunities for improvement of sorghum yield. Drought and *Striga* are considered the most important factors limiting sorghum production in Mali. Farmers link *Striga* with poor soil fertility and manage it by providing fertilizers and weeding. For management of drought, farmers use early maturing varieties, early sowing and planting in the low lands. These methods cannot always overcome drought because early maturing varieties are not always drought tolerant, and drought can occur at any stage of the growing season. In addition, the yield potential of some early maturing varieties are low compared with late maturing varieties.

Farmers' major criteria for selecting varieties were high yield, grain quality (color, taste, size etc.), and drought tolerance. Further strategies improvement of sorghum must take these selection criteria into consideration.

3.4. Discussion

The main reasons to grow sorghum in Mali are for use as grain for human consumption, while the crop residues are used in feeding animals, fencing, thatching, for firewood and compost (organic fertilizer). Similar finding were reported by Mathieu (2005) in the study "Pour une gestion paysanne de l'agro-biodiversité: le cas du sorgho au Mali". Also Ketema (2008), reported that sorghum is a high priority staple crop ranked as 1st, 2nd and 3rd in Ethiopia, Sudan and Uganda, respectively.

The major sorghum production constraints are low soil fertility and high cost of inputs, lack of farm equipment, drought, poor rainfall pattern and lack of training of farmers.

Traore *et al.* (2007), reported that, among the different constraints to pearl millet (*Pennisetum glaucum* L.) production, low soil fertility is one of the most important limiting yield.

The soil fertility problems (decreasing of fertility and cost of inputs) and lack of farm equipment are among the main constraints of sorghum production in Mali and sub-Saharan Africa (INSORMIL-CRSP, 2006). The report of the Minister of Rural Development (MDR) of Mali (2002) indicates that soil degradation, characterized by decrease in fertility and crop productivity is a major constraint in all eco-systems. Very little mineral fertilizers are used in sorghum production. Indeed, without subsidies, the prices of inputs (fertilizers) compared to the income from agricultural such as pearl millet, maize, and sorghum doesn't make of the use of these fertilizers cost-effective. The use of the organic manure is limited because most farmers are without sufficient farm equipment. Few farmers have donkey carts for transportation of organic manure. The lack of farm equipment in Mali was also reported by Mathias (2010).

Drought and unpredictable rains are important factors limiting sorghum production in Mali. According to the Intergovernmental Panel on Climate Change IPCC (2001) and Siart et *al.* (2005), the length of the rainy season has decreased since the severe droughts of the early 1970s in Mali. Boyer and Westgate, (2004) also reported that drought is the most important cause of crop yield loss, especially in water-limited areas where most of the world's poorest farmers live.

The lack of training of farmers, mentioned by farmers in F.G. discussions, is also a serious problem because of climate change. To combat the effects of climate change, many varieties and new production and management skills have been suggested by Malian research institutes and ICRISAT to improve productivity, but farmers have very limited information on these new varieties and management skills. Juma (2011) reported that Africa's productivity is also contrained by lack of diffusion of improved technology for agricultural production.

An important finding of this study was that the majority of sorghum varieties grown by farmers are local early maturing landraces. This is due to their adaptation, grain qualities and pest resistance. This is consistent with the results of Toure *et al.* (1998).

The adoption and access to improved sorghum varieties is still poor in the study areas. The main seed sources in households are farmers' own production, as also reported by Siart *et al.* (2005) in "Mandé" in southern Mali.

The criteria for selecting varieties are highly variable, which is due to the size of the production area of sorghum in Mali. It spreads across all the agricultural humid regions to Sahelian (semis arid) regions sorghum is grown by several ethnic groups which very diverse socio-economic and agro-ecological conditions. The five top criteria for selecting a variety were high yield potential, earliness, grain quality (color, taste and size), the rest

are tolerance to drought and *Striga.* Studies conducted in southern Mali (Dissan/Bougouni) by Lacy *et al.* (2006) showed similar finding.

To improve sorghum yield, the farmers proposed the use of mineral fertilizers and organic manure, using equipment and reducing of the cost of inputs. Which have also been reported in the MDR of Mali (2002). Farmers also recommended the use of improved adapted varieties and training in farm management. The development of improved varieties adapted to the farmer's production area, provision of improved seed, and training farmers how to produce seed using new field management skills are major objectives of the strategic plans of the agronomic research institutes in Mali.

The need for the involvement of farmers in the development of new crop varieties for smallholder farmers was highlighted by DeVries and Toenniessen, (2001). They advocated that farmers should be involved in all aspects of variety development including priority setting, early generation selection, variety testing and selection so that breeders obtain regular input from farmers that enables them to structure their selection indices accurately.

3.5. Conclusions

The PRA activities underscored the importance of sorghum and identified production constraints in the study areas. It also collected information about the sorghum varieties grown by farmers and their agronomic practices (management).

The study identified selection criteria (preferences) of farmers for varieties and suggestions from farmers on how to improve sorghum yield. Understanding farmers' preferences will help plant breeders to know the needs of farmers.

The results of the PRA will be used in research for the development of high yielding sorghum hybrids tolerant to post flowering drought and with good grain qualities.

CHAPTER FOUR

4.0. DEVELOPMENT OF STAY GREEN INBRED SORGHUM "B-LINES" USING MORPHOLOGICAL AND DNA-BASED MARKERS

4.1. Introduction

Agriculture is an important sector in the Sahelian countries, given its multiple roles in food security, employment and contribution to national Gross Domestic Products (GDPs). The paradox, however, is that agriculture in these Sahelian countries remains a highly under-developed sector, characterized by an almost total dependency on rainfall; low use of external inputs such as improved seeds and fertilizers; absence of mechanization; and poor linkage to markets. This makes agriculture highly vulnerable to climate change (Serigne *et al.*, 2006). Yet, people who depend on this activity for their livelihoods have faced a large variety of shocks to which they have responded, based on traditional knowledge or by devising innovative measures when faced with new sets of constraints. Also, research over the last few decades has devoted a lot of efforts on the development of useful technologies in response to the various constraints and stresses facing agriculture in the Sahel region (Serigne *et al.*, 2006).

Cereal yields in Mali have been stagnating or trends have been downwards over the last 30 years. A number of reasons exist for this phenomenon, such as climatic risks or climate change (Coulibaly, 1996). In Mali, climate change is expected to have drastic consequences. The trend of decreasing rainfall that has been going on for the last few decades will be exacerbated with the following consequences: reduction in cereal yield due to drought and declining soil fertility; reduction of livestock numbers due to the shrinking of grazing areas; reduction of fauna and fishing resources; expansion of cultivated areas to compensate for low yields with encroachment into low potential areas. Vulnerability and adaptation studies have been carried out in the cereal crops, mainly

sorghum. Different temperature rise scenarios, coupled with rainfall reduction in relation to the 1961–1990 period, have been used to predict the behavior of sorghum at the 2025 horizon (Butt *et al.*, 2003). The results show that sorghum yield will drop by 2 to 26% depending on the scenario and model considered. To adapt to the changing climatic conditions, the government of Mali focuses its adaptation strategies on several aspects with emphasis on identifying or developing varieties and or hybrids tolerant to the post-flowering drought (Coulibaly, 1996; Toure, *et al.*, 2005).

Sorghum hybrids have the potential to significantly increase the yield of sorghum in Mali as they have done in many other areas of the world. Hybrids must be produced using CMS to be economically viable. This requires the development of hybrid parents which involves two steps: identification of potential B-lines which maintain CMS and R-lines which restore pollen fertility; and development of A-lines which are male sterile.

Considering the success of CMS-based hybrid technology in sorghum, continued research investments have been made on male-sterile A-lines and restorer R-lines and hybrid development in sorghum improvement programs of the ICRISAT and the National Agricultural Research systems (NARS).

In Mali, hybrid research was initiated in 1982 by the sorghum program of IER at Sotuba research center in collaboration with ICRISAT. The results of this research indicated that the *guinea*-based landraces showed high frequency of lines that maintain CMS, but there were no lines converted into male sterile A-lines at that time (Belum, *et al.*, 2006).

Initially, individual plants that are tolerant to specific biotic/abiotic stresses and which did not restore male sterility were selected at the F6 generation within high yielding families. These were converted into A-lines after testing for GCA (Belum, *et al.*, 2006).

There is a high frequency of maintainers of CMS in landrace varieties from the Sudanian zone of West Africa (33% of the accessions from Burkina Faso, Mali and Senegal)

Many of these CMS maintainer lines have tolerance to post flowering drought, an important constraint of yield of sorghum in Mali. The stay green trait has been shown to have a positive impact on grain yield under terminal drought. Several studies (Borrell, *et al.*, 1999; Jordan *et al.*, 2003; Kassahun *et al.*, 2009 and Tuinstra *et al.*, 1997) have reported co-localization of stay green and grain yield QTL under drought stress, suggesting that the gene(s) underlying stay green may also result in enhanced yield performance under drought stress. Tuinstra and Goldsbrough, (1998), using near-isogenic lines (NIL), found positive associations between these two traits reinforcing the potential for indirect selection based on stay green for improving grain yield under drought stress in sorghum.

The major objectives of this study are to develop stay green lines (B-lines) that are useful for developing hybrids for wider adaptation to the semi-arid regions of Mali.

The specific objectives are to:

- develop isogenic, male sterile A-lines and maintainer B-lines that tolerate post flowering drought stress using stay green QTLs.
- introgress stay green QTLs into elite varieties using the backcross method
- identify genotypes with stay green QTLs
- characteriz genotypes morphologically for other traits such as plant architecture (plant height, good head exsertion, and good seed set,) and farmers' preferences for other characteristics identified in PRA.
- develop inbred B-lines with stay green QTL and appropriate morphological characters.

4.2. Materials and methods

4.2.1. Materials

4.2.1.1. Experimental Sites

The development of the sorghum "B-lines" with the stay green trait was carried out at ; (i) Regional Center for Agronomic Research (CRRA) of Sotuba in Mali (field experiments) and (ii) Regional Study Center for the Improvement of the Adaptation to Drought (CERAAS) in Thies Senegal (genotyping).

4.2.1.1.1. Regional Center for Agronomic Research (CRRA) of Sotuba

The field research was conducted at the Regional Center for Agronomic Research (CRRA) of Sotuba, IER Mali. The research center of Sotuba is located in Bamako at the west bank of river Niger and lies in the Sudano-sahelian zone. Coordinates are 12°39'47''North 7°54'50'' West on altitude 320 m and isohyet 600 – 1000 mm.

The average annual precipitation collected at CRRA weather station from 2000 to 2014 year is presented in Figure 4.1. The soils at Sotuba are sandy with low water holding capacity, low inherent soil fertility and organic matter content.

The average temperature was 28º C with variations between 15 to 42 ºC; and the hottest temperatures were recorded in April and May, while the minimal values were observed in December and January. The average annual relative humidity was 42.8% and average monthly relative humidity ranged from 16% in February to 74% in August (Station météo de Sotuba, 2014).

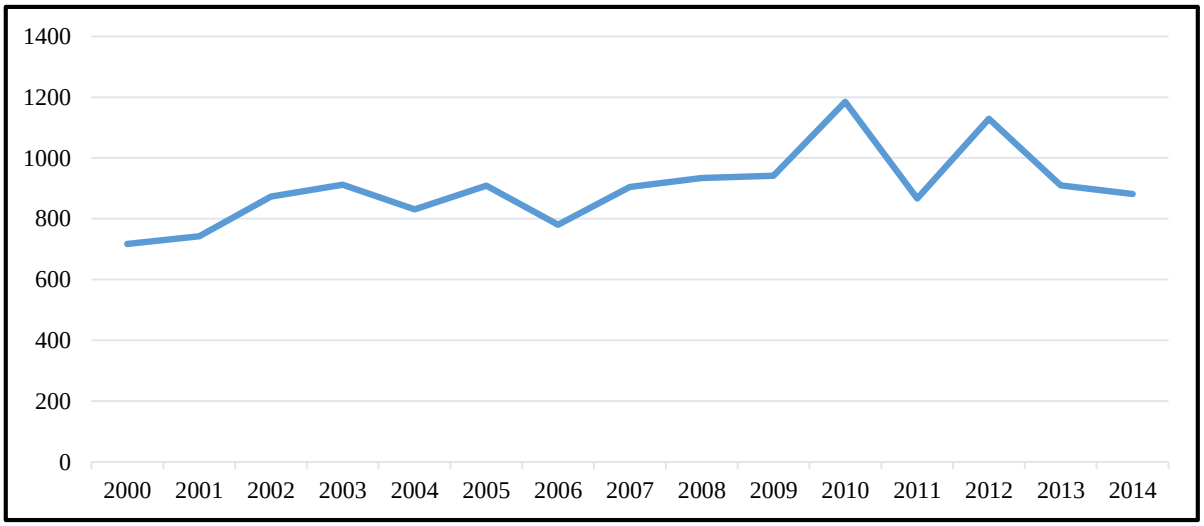


Figure 4. 1. Rainfall pattern from 2000 to 2014 at CRRA of Sotuba Metrologic station
(Source: Station météo de Sotuba, 2014)

4.2.1.1.2. Genotyping at the Regional Study Center for the Improvement of the Adaptation to Drought (CERAAS) of Thies.

The molecular analysis was done at the Regional Study Center for the Improvement of the Adaptation to Drought (CERAAS) of Thies in Senegal.

The general objective of CERAAS is to develop plant varieties adapted to drought methods of analysis and decision-making tools which contribute to improving and/or stabilizing agricultural production in arid and semi-arid zones. (*http://www.fao.org CERAAS*).

4.2.1.2. Irrigation system used at the experimental site

In selection of irrigation method, several factors such as land slope, water intake, crops water tolerance, water availability and wind action are usually considered (Melvin and Payero, 2007). Irrigation methods are broadly divided into two systems, they either fall under surface or non surface irrigation systems (Athuman, 2014). Surface irrigation systems are those whereby water is applied into the ground and flow by gravity over the surface of irrigated field, for instance furrow irrigation, flood irrigation, border irrigation and basin irrigation. Non surface irrigation is the one whereby water applied does not flow by gravity, this systems include sprinkler irrigation, trickle or drip irrigation (Athuman, 2014).

Of all the methods of irrigation, drip irrigation is mostly preferable in arid and semi arid areas due to scarcity of water and high rate of evapotranspiration (Liz *et al*., 2005). The method is popular in areas with limited water supply and salt problems (Abdrabbo and Abou, 2009). Drip irrigation consists of an extensive network of pipes small in diameter to deliver filtered water directly to the soil nearby plant. The systems apply water slowly to keep the soil moisture within the desired range for plant growth. Therefore conventional losses as deep percolation, runoff and soil water evaporation are minimized (Franken, 2005). Water emission is delivered in form of small droplets, continuously droplets or tiny streams. With drip irrigation; only active feeding zone of the crop is wetted, and kept moist without being saturated. Because of the high potential that is maintained at the root zone throughout the growing season, adverse effects of the salinity is significantly low when drip irrigation is used (Liz *et al*., 2005).

In this study the drip irrigation was used. The irrigation pipes was installed between the rows, the plots were irrigated at two days interval.

4.2.1.3. Fertilizer application

During the study, the fields received five tons per hectare of organic fertilizer and pre-sowing fertilization consisting of 100 kg/ha Di-ammonium phosphate (DAP) [NPK: 18-46-0] and nitrogen in the form of urea was applied 40 days after sowing at 50 kg/ha.

4.2.1.2. Plant material

Four inbred B-lines of sorghum were used in this study; one non-senescent line used as donor parent of stay green QTL was crossed with three senescent lines used as recurrent parents. The characteristics of these inbreds are presented in Table 4.1.

Table 4. 1. Characteristics of the inbred lines used in the backcrossing program

N°	Name	Code Name	Origin	Race	Reaction to drought
1	BTx642	B35	Ethiopia	Durra	Non-senescent
2	98-BE-F5P-82B	82B	Mali	Caudatum	Senescent
3	03-SB-F5DT-134B	134B	Mali	Guinea	Senescent
4	09PR-3009B	3009B	Mali	Guinea-Caudatum	Senescent

4.2.1.3. Markers used in the study

Two kinds of markers were used in the study: morphological and DNA-based markers.

4.2.1.3.1. Morphological markers

Morphological markers were used in the study to characterize and identify genotypes for traits such as plant height, good head exsertion, presence or absence of a testae, good seed set, grain color and grain qualities. These markers are detectable by simple visual inspection.

4.2.1.3.2. DNA-based markers

DNA-based markers were used for identification of genotypes with stay green QTLs, using SSR markers. Three genomic SSR marker series were used: *Xtxp* series (Kong *et*

al., 2000; Bhattramakki *et al.*, 2000), *Xcup* series (Schloss *et al.*, 2002), and *msbcir* series (Agropolis-Cirad-Genoplante, France) (Table 4.2).

Table 4. 2. List of primers used for genotyping

N°	Markers	QTLs	cM (range)	Tm	References
1	Msbcir 225	stg1	134.6 – 137.4	54.26	Cirad-Genoplante
2	Msbcir 314	stg1 & stg2	112	54.755	Cirad-Genoplante
3	Msbcir 276	stg2	76.3	53.47	Cirad-Genoplante
4	Msbcir 224	stg2	59.8 – 62.8	56.13	Cirad-Genoplante
5	Msbcir 339	stg3	122.7	54.985	Cirad-Genoplante
6	Xcup 29	stg3	60.5	60	Schloss et al. (2002)
7	Msbcir 312	stg3	97.7 – 104.2	56.49	Cirad-Genoplante
8	Msbcir 222	stg4	98.8	53.9	Cirad-Genoplante
9	Xtxp 123	stg4	93.1	53	Bhattramakki et al. (2000)

QTL = Quantitative trait locus
cM (range) = Centi-Morgan
Tm = Annealing (Melting) temperature

4.2.2. Methods

4.2.2.1. B-line population development (Pool B-lines)

4.2.2.1.1. Introgression of stay green QTLs using Backcross method

For introgression of stay green QTL, marker-assisted backcrossing (MABC) was used to develop B-line populations. The goal of MABC method was to incorporate one or a few major genes or QTLs into elite breeding lines. B35 (Donor) was crossed with 98-BE-F5P-82B, 03-SB-F5DT-134B and 09PR-3009B (recurrent parents). All four lines are maintainers of CMS (B-lines).

- **F_1 population development**

To generate F_1 populations for the study, B35 (Donor) was crossed to 98-BE-F5P-82B, 03-SB-F5DT-134B and 09PR-3009B (recurrent). Two crossing blocks were planted at different times at Sotuba during the off-season (December 2012) for synchronization of

flowering dates. The first crossing block was planted on 6th and the second the 18th December 2012. The goal of these crossing blocks was to introgress genes for tolerance to post-flowering drought (stay green) into the three sorghum lines. The crossing blocks consisted of three rows of 3 m length of each recurrent parent planted between two rows of donor parent. A total of six crossing plots of five rows each were planted. The spacing between the rows was 0.75 m and between hills within the row was 0.30 m. and between crossing plots was 1.5 m.

Hand emasculation was carried out on the 3 recurrent parents and pollen from B35 transferred to the stigma of emasculated florets to produce 250 to 350 F_1 seeds per crossing plot. Reciprocal crossing was also done.

- **BC_1F_1 population development**

To generate BC_1F_1 populations for the study, each F_1 population was crossed to the appropriate recurrent parent (98-BE-F5P-82B, 03-SB-F5DT-134B and 09PR-3009B). The F_1 and recurrent parents' seeds were sown in three crossing blocks in May 2013 at Sotuba research center. Three rows of 3 m each of F_1 and each recurrent parent were sown side by side. The spacing was the same as above. A total of nine crossing plots of six rows each were planted.

Hand emasculation was done on the three recurrent parents and each F_1. The emasculated flowers were pollinated with the F_1 pollen and reciprocal crosses with recurrent parents' pollen were also made to produce three BC_1F_1 populations. About 350 to 450 BC_1F_1 seeds per population were obtained.

- **BC_1F_2 population development**

To generate BC_1F_2 populations, about 130 – 150 plants of each BC_1F_1 were planted at the end of the rainy season (October 2013). Two rows of 20 m length per population were

arranged side by side. The spacing between rows was 0.75 m, between hills within a row was 0.30 m. Each plant was selfed to produce three different BC_1F_2 populations.

- **BC_1F_3 population development**

Each BC_1F_2 population was sown in two rows of 50 m during the off season, February 10, 2014 at Sotuba research center. From 150 – 200 plants per population and their parents, leaf samples were collected 45 days after sowing. The samples were preserved in plastic bags containing 4 to 6 g of silica gel in order to dry the leaves. The samples were sent to the CERAAS in Senegal for molecular analysis using SSR markers and to identify lines with QTLs from the donor parent (foreground screening). At flowering each plant was selfed to produce BC_1F_3 seeds.

4.2.2.1.2. Identification of genotypes with stay green QTLs

Genotyping of the leaf samples of the three BC_1F_2 populations and their parents was done at CERAAS) in May 2014 as follows:

a. DNA extraction

Young leaves were harvested from 40 day old plants and were stored in silica gel before DNA extraction. DNA was extracted from 20 mg of dried leaves following a slightly modified mixed alkyl trimethyl ammonium bromide (MATAB) protocol described by Cardoso *et al*. (1998). Preparation and processing were done as follows: 20 mg of dried leaves per sample were put in an Eppendorf tube of 2 ml with two steel balls of 5 mm diameter per tube. Grinding was done using the Vibro-grinder RETSCH at 30 pulsations per second for 8 minutes. Alter grinding, a volume of 750 µl of MATAB buffer preheated to 65°C was added in the Eppendorf tube (for the deterioration of the nuclear membrane and liberation of the DNA) and then the tube was fixed in a locking device and incubated at +65°C in a water bath for 20 minutes with occasional manual shaking and then cooled for 5 minutes at room temperature. Then, 750 µL of chloroform-isoamyl alcohol (CIAA,

24:1) was added to each sample. All samples were homogenized by gently shaking 50 times, before centrifugation at 13,000 rpm for 20 minutes. The supernatant was collected and the DNA was precipitated with 600 µL of isopropanol. After centrifugation, DNA pellets were washed with 300 µL of 70% ethanol, air dried and dissolved in 300 µL of TE 1X.

DNA for 150 samples per population and that of one sample for each parental line (B35, 98-BE-F5P-82B, 03-SB-F5DT-134B and 09PR-3009B) were extracted. In all a total of 454 DNA samples were extracted.

b. Checking DNA quality and quantity

The objective was to determine the quality and the quantity (concentration) of DNA obtained per sample. After isolating the DNA, samples were loaded into 0.8% agarose gel electrophoresis and DNA content of the samples was estimated by comparison with λ DNA standards. The gel was run 30 minutes after which the quality was checked under UV and the migration photographed. A clear band of DNA indicated good quality (See, Figure 4.2). Then the samples were diluted to final working concentration of about 2 to 2.5 ng per µl.

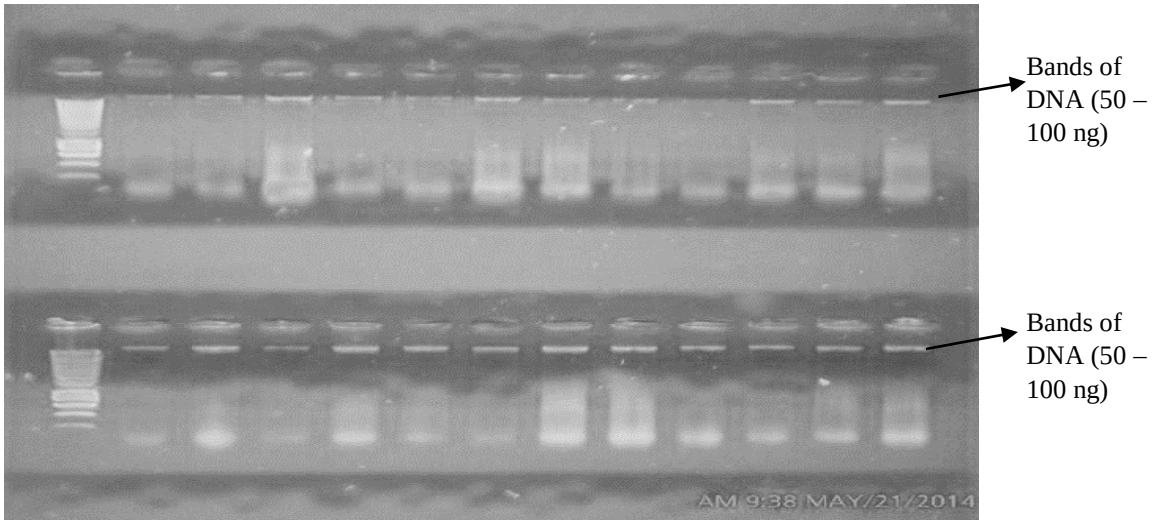


Figure 4. 2. Agarose gel electrophoresis image showing the quality of the sorghum DNA extraction.

c. PCR amplifications and revelation of SSR markers on sequencer Li-Cor system.

Initial screening of parents was carried out for polymorphism before genotyping BC_1F_2 populations. For this, parental DNA from B35 (donor), 98-BE-F5P-82B, 03-SB-F5DT-134B and 09PR-3009B (recurrent) were subjected to PCR amplification with a set of 20 foreground SSR markers of Xtxp series (Kong et al., 2000; Bhattramakki et al., 2000) and Xcup series (Schloss *et al.,* 2002) localized in regions of stay green QTLs (Stg1, Stg2, Stg3 and Stg4). PCR reactions were conducted in 96 wells plates with a reactional volume of 10 µl per well containing the DNA extracts, forward "F" and reverse "R" primer with M-13 tailed primers marked to the IRDye 700 or the IRDye 800. In addition, others reagents such as the buffer 10X, $MgCl_2$, dNTP, Taq polymérase and mineral oil were also used. These reagents make possible and stabilize the reactions, act as source of magnesium ion for PCR, supply nucleotides and prevent evaporation of the sample.

Plates were placed in a DNA thermocycler with Touchdown PCR program during 1 hour 30 mns to 2 hours 30 mns for denaturation and amplification of sequences of microsatellite (SSR) from the DNA. Then, PCR products were loaded down on the 6.5% gel acrylamide for separation by capillarity using Li-Cor 4300 DNA Sequencer. Alter running PCR products, two markers showed the highest polymorphism between donor parent B35 and the other three recurrent parents (98-BE-F5P-82B, 03-SB-F5DT-134B and 09PR-3009B) on the screen of a computer joined to the Li-Cor. Six markers were monomorphic and twelve were not functional.

A new set of markers were selected the basis of the consensus sorghum map elaborated by Mace *et al.,* (2009). The projection of each QTL (stg1, 2, 3 and 4) location onto the consensus sorghum map in the different linkage groups (SBI-01, SBI-02, SBI-03 and SBI-5), identified other sets of microsatellite markers located in the same genetic region

containing the target QTLs (Appendix 3). A set of 33 markers of msbcir, gpsb, SBKAFGK (unpublished, Agropolis Cirad Genoplante France) series and some working series of Xtxp, were chosen for initial screening.

Alter running the PCR, products exhibited successful amplification. The highest polymorphism was observed between donor parent B35 and the recurrent parents with 21 markers (Figure 4.3).

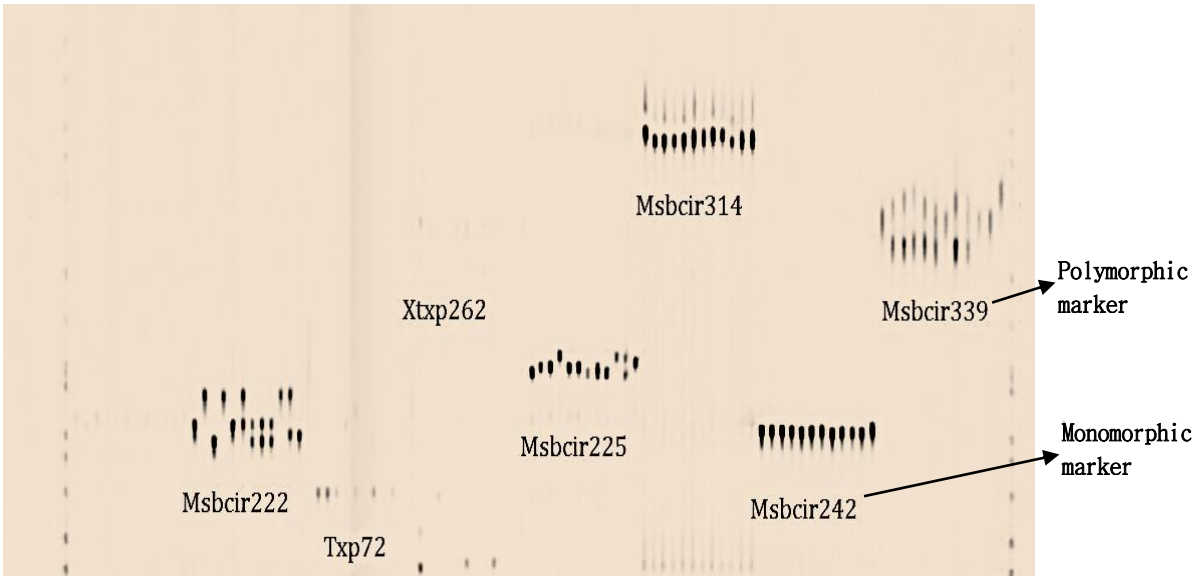


Figure 4. 3. Parental survey to identify polymorphic SSR markers for stay green QTL (stg1, 2, 3 & 4) in sorghum

For foreground screening (genotyping) of BC_1F_2 families, nine markers were selected (Table 4.2). After running of the 6.5% gel acrylamide, PCR products showed successful amplification in sequencer Li-Cor 4300 system. When the migration was finished, pictures were recorded directly in the computer for scoring and analyses. A total of 100 BC_1F_2 DNA samples including the donor parent and recurrent parent for each population were genotyped (Figure 4.4 a, b and c).

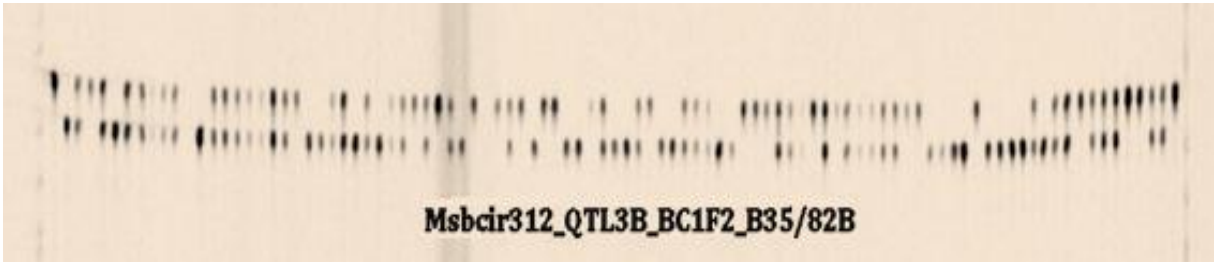


Figure 4.4a. Segregation of SSR marker msbcir312 in the BC1F2 (B35//82B) population for stg3

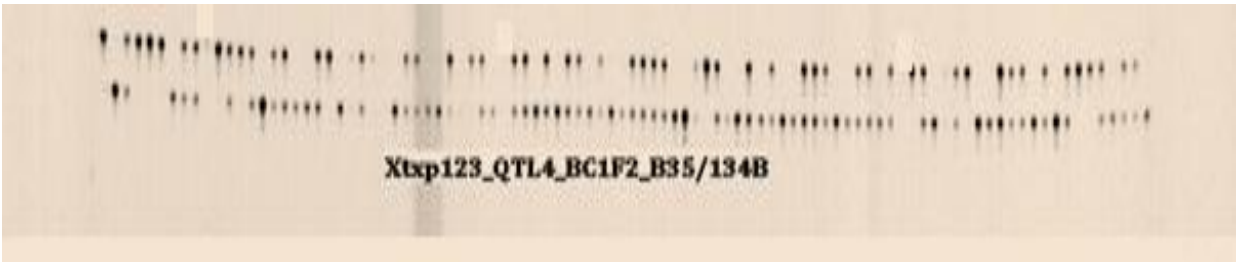


Figure 4.4b. Segregation of SSR marker xtxp123 in the BC1F2 (B35//134B) population for stg 4

Figure 4.4c. Segregation of SSR marker msbcir314 in the BC1F2 (B35//3009B) population for stg 1.

Figure 4. 4. Segregation of SSR markers

d. Data scoring

The software Jelly XL2007_2.0b17 was used to determine the number of the different alleles for every locus. Each PAGE gel for SSR markers was imported in the Jelly XL2007_2.0b17 application for reading the gels and, data points were marked for all populations BC_1F_2 and parents on the basis of the amplicons size.

For a given locus the following letters were assigned:

"A" = Allele for the recurrent parent

"B" = Allele for the donor parent

"H"= Heterozygous (presence of both parental alleles)

"D" = Off Type

"X" = Missing data (failed amplification)

Microsoft Excel was used to arrange and rank the alleles identified in each region of QTLs.

4.2.3. Results

The results for part of the scoring sheet for the (B35//82B)-F2, (B35//134B)-F2 and (B35//3009B)-F2 populations are presented in Appendix 4a to 4c.

- **Choice of lines with stay green introgression**

To advance to BC_1F_3 population, families that had all four major stay green QTLs in the BC_1F_2 plants or families with the two major QTLs (*stg1* and *stg2*) in homozygous form in the plant were identified.

Before making the selection of individuals (lines) to be advanced, three criteria were defined based on a lines' possession of stay green QTLs in the order given in Table 4.3.

Table 4. 3. Rank of three criteria of choice

Rank	Criteria of possession
1st	Homozygous for *stg1, stg2, stg3 and stg4*
2nd	Homozygous for *stg1, stg2* and *stg3 or stg4*;
3rd	Homozygous for *stg1* and *stg2* and heterozygous for others QTLs

On the basis of these criteria, 23 individuals were chosen from the (B35//82B)-F2 population, 39 individuals from (B35//134B)-F2 and 11 individuals from (B35//3009B)-F2. A total of 73 individuals were found possessing the target QTLs in homozygous form (Table 4.4).

Table 4. 4. Number of individuals by criteria of choice and by population

Choices	Number of individuals by criteria of choice and by population			Total choice
	(B35//82B)F2	(B35//134B)F2	(B35//3009B)F2	
1st	1	2	1	**4**
2nd	12	22	7	**41**
3rd	10	15	3	**28**
Total	**23**	**39**	**11**	**73**

- **Choice of lines for morphological traits**

The 73 introgression lines were evaluated using morphological markers. The individuals were selected to advance based on plant architecture (plant height, good head exsertion, and good seed set,) and farmers' preferences for others characteristics identified in PRA. Using these selection criteria, four individuals were selected per population for a total of twelve individuals (Table 4.5). The selected plants were selfed and BC_1F_3 seeds were produced.

Table 4. 5. List of B lines selected to generate BC_1F_3 seeds

N°	Lines	Stay green QTLs	Height (cm)	Grain color
1	(B35//82B)F2-64	Homozygous for *stg1*, *stg2* and *stg3*; heterozygous for stg4	170	A little red
2	(B35//82B)F2-104	Homozygous for stg1, stg2 and *stg4*; heterozygous for stg3	203	A little red
3	(B35//82B)F2-114	Homozygous for *stg1*, *stg2* and *stg3*; heterozygous for stg4	180	Dirty white
4	(B35//82B)F2-136	Homozygous for *stg1*, *stg2* and *stg3*; heterozygous for *stg4*	201	Dirty white
5	(B35//134B)F2-44	Homozygous for *stg1*, *stg2* and *stg3*; heterozygous for *stg4*	200	White
6	(B35//134B)F2-64	Homozygous for *stg1*, *stg2* and *stg3*; heterozygous for *stg4*	150	White
7	(B35//134B)F2-89	Homozygous for stg1, stg2 and stg4; heterozygous for stg3.	200	White
8	(B35//134B)F2-125	Homozygous for *stg1*, *stg2* and *stg4*; heterozygous for *stg3*	140	White
9	(B35//3009B)F2-16	Homozygous for *stg1*, *stg2* and *stg4*; heterozygous for *stg3*.	250	White
10	(B35//3009B)F2-24	Homozygous for stg1, stg2 and stg3; heterozygous for stg4	210	White
11	(B35//3009B)F2-51	Homozygous for *stg1*, *stg2* and *stg4;* heterozygous for *stg3*.	180	White
12	(B35//3009B)F2-90	Homozygous for *stg1*, *stg2*, *stg3* and *stg4*	192	White

4.3. Discussion

Different cultivars of sorghum have different morphological and physiological modifications to overcome the various environmental stresses encountered during the crop growth. One such modification is stay-green or non-senescence. In this study, MABC was used to introgress genomic regions from a stay-green donor parent, B35, into non-senescence recurrent parents: 98-BE-F5P-82B, 03-SB-F5DT-134B and 09PR-3009B. Three populations were developed: (B35//82B)-F2, (B35//134B)-F2 and (B35//3009B)-F2. For foreground screening (genotyping) of these populations, nine markers linked to stay-green QTL regions to be transferred from donor to recurrent parents were selected (Table 4.2).

According to the criteria established for advancement to the next generation presented in Table 4.3, one plant from (B35//82B)-F2 population, two plants from (B35//134B)-F2 population and one plant from (B35//3009B)-F2 population were selected. These were homozygous for the donor parent (B35) alleles at all the tested polymorphic SSR marker loci in the *stg1, stg2, stg3* and *stg4* QTL regions (Table 4.4). Twelve individuals from (B35//82B)-F2 population, twenty-two from (B35//134B)-F2 population and seven from (B35//3009B)-F2 population were selected. These were homozygous for the donor parent (B35) alleles at all the tested polymorphic SSR marker loci in the *stg1, stg2, stg3 or stg4* QTL regions (Table 4.4). Ten individuals from (B35//82B)-F2 population, fifteen from (B35//134B)-F2 population and three from (B35//3009B)-F2 population that were homozygous for the donor parent (B35) alleles at all the tested polymorphic SSR marker loci in the *stg1, stg2,* and heterozygous for *stg3* and *stg4* QTL regions were selected (Table 4.7). A total of 73 individuals with two to four target stay green QTLs were selected and advanced to the next generation. The advantages of MABS was reported by DeVicente and Tanksley, (1993) and by Abdurakhmonov *et al*. (2011b). DNA markers

linked to the genomic regions of interest serve as an important tool, enabling breeders to conduct early-stage selection on the basis of genotype rather than phenotype. Concibido *et al.* (1996) defined MABS as an indirect selection method based on genotype rather than plant phenotype. They reported that MABS can be used to accelerate the rate at which new cultivars are developed. Lande (1992) reported that MABS may be especially useful in the early generations of pedigree selection or in selection for characters that are difficult or expensive to measure such as drought and pest resistance. MABS creates the opportunity for increasing selection intensity in the early generations of pedigree selection (Lande, 1992). Marker-assisted selection could also be applied to individual plants in succeeding generations without the necessity of extensive progeny tests in the field. The marker has to be closely linked with the desired trait for MAS to be used in plant breeding (Mohan *et al.,* 1997).

In this study, the 73 individuals selected for advancement to the next generation using DNA makers for stay green trait were evaluated again in the field using morphological markers (phenotyping). They were then selected for advancement to the next generation based on plant architecture (plant height, good head exsertion, and good seed set,) and farmers' preferences for others characteristics identified in PRA. Four individuals were selected per population for a total of twelve individuals (Table 4.8). The selected plants were selfed and BC seeds were produced to develop early generation stay-green BC_1F_3 populations. Morphological markers were also used to supplement DNA markers for the other traits. In plant breeding, morphological markers are extremely useful to plant breeders and DNA markers usually complement the morphological markers. A similar conclusion was reached by Knapp (1998) who reported that MAS has emerged as a strategy to complement phenotypic selection and should in theory yield greater selection gains than phenotypic selection alone. Paterson *et al.* (1988) and William *et al.* (1992)

reported that plant breeders and molecular geneticists can integrate molecular biology and traditional plant breeding methodology to identify QTLs and use the probes to conduct marker-assisted selection (MAS).

4.4. Conclusions

In the present study, the main objective was to develop parental B-lines with tolerance to post flowering drought stress using stay green QTL. Three elite lines from the Malian sorghum program, 98-BE-F5P-82B, 03-SB-F5DT-134B and 09PR-3009B (non-stay green, drought susceptible), were crossed with B35 (stay green source, drought tolerant). MABC method was used to incorporate two to four major stay green genes or QTL into elite breeding lines. Three populations were developed: (B35//82B)-F2, (B35//134B)-F2 and (B35//3009B)-F2 and genotyped using nine markers linked to stay-green QTL regions.

Plants that had all four major stay green QTLs (stg1, stg2, stg3 and stg4), or had two major QTLs (stg1 and stg2) in homozygous form, in the BC_1F_2 populations were advanced to BC_1F_3 population. A total of 73 individuals with two to four target stay green QTLs were evaluated in the field using morphological markers (phenotyping). They were then selected based on plant architecture and farmers' preferences for other characteristics identified in PRA. Finally, four individuals were selected per population for a total of twelve individuals. These were selfed and seeds of BC_1F_3 families of stay-green lines that are maintainers of CMS (B-lines) have been produced.

CHAPTER FIVE

5.0. IDENTIFICATION OF STAY GREEN SORGHUM Rf-LINES

5.1. Introduction

Sorghum is a major crop in Mali but grain yields are low and decreasing due to drought stress. The development of F_1 hybrids with tolerance to drought could substantially improve grain yields. The government of Mali has developed strategies to develop hybrid sorghums tolerant to the post-flowering drought (Coulibaly, 1996; Toure *et al.*, 2005).

Sorghum is the first self-pollinated cereal staple crop, in which F_1 hybrids have been commercially exploited to improve productivity. This is due to the availability of a stable and heritable cytoplasmic male sterility (CMS) system. Utilization of CMS systems for hybrid development requires the development of restorer lines that possess nuclear genes that restore pollen fertility to CMS lines.

At ICRISAT-Bamako, Mali, several CMS restorer lines have been identified from a sub-sample of guinea-core collections. A number of inter-racial R-lines have been identified by the IER-Mali program. However, these restorer lines need to have tolerance to post flowering drought, an important constraint affecting sorghum grain yield in Mali. The stay green trait has been shown to have positive impact on grain yield QTL under terminal drought.

The major objective of this study was to identify stay green restorer lines (R-lines) that would be useful for developing hybrids for wide adaptation in semi-arid regions of Mali.

The specifics objectives are to:

- test the cytoplasmic restoration reaction of F_1 sorghum populations derived from crossing lines from Backcross Nested Association Mapping (BCNAM) project with a cytoplasmic male sterile (CMS) line 02-F5DT-12A (12-A).

- characterized the fertility restoring lines for stay green and others traits such as plant architecture and grain qualities.
- identify lines with stay green and others farmers' preferred traits identified in the PRA.

5.2. Materials and methods

5.2.1. Materials

Information on the experimental site used, the irrigation system, and details on fertilizer application have been described in the earlier section 4.2.

5.2.1.1. Plant material

5.2.1.1.1. Populations from BCNAM

The populations of Backcross Nested Association Mapping (BCNAM) project of "Institut d'Economie Rurale" (IER) and ICIRISAT Mali were used in the study to identify Rf-lines (fertility restorer line).

The BCNAM design is multi-parental design that combines high resolution population development for genetic analysis of complex traits, genetic basis enlargement and direct breeding applications and to the use of advanced-backcross populations with an adapted elite recurrent parent (RP). This complex multi-parental crossing scheme involves 3 elite varieties obtained from participatory breeding in Mali (Lata, Grinkan and Keninkeni) as recurrent parents and 29 donor parents either specific or common to one or several of the recurrent parents. A total of 50 BC_1F_3 populations were obtained representing a total of approximately 6300 individuals (Teme *et al.*, 2010).

For this study, 20 lines (individuals) each were selected from six BCNAM populations (BCNAM-02, BCNAM-03, BCNAM-08, BCNAM-11 BCNAM-40 and BCNAM-42) giving a total of 120 lines. The list of selected populations and their sources of

combinations is presented in Appendix 5. The cytoplasmic male sterile (CMS) line 02-F5DT-12A was used as female parent in testcrosses. It was crossed with the 120 selected lines from BCNAM's populations for testing the cytoplasmic male sterility reaction.

5.2.2. Methods of testcross

5.2.2.1. Identification of R-lines (Pool R-lines)

5.2.2.1. Testcross

To identify R-lines, the 120 lines from six populations of BCNAM project were used as pollen parents or pollinators (Appendix 5). A CMS line, 02-F5DT-12A, was used as female parent and was crossed to each of the 120 lines. The crossing blocks were established at CRRA of Sotuba, during off-season (December 2012). Two rows length of 2 m composed of one row for the line and one for the CMS line 02-F5DT-12A (rows plots of 0.75 x 0.30) were sown. The pollen from each of the 120 lines was transferred to the stigma of 2 to 3 panicles of CMS line 02-F5DT-12A to generate F_1 seeds and the panicles covered with paper bags before anthesis. Some crosses could not be made due to lack of synchronization of flowering because the line 02-F5DT-12A was earlier than some male lines. A total of 162 crosses were obtained.

5.2.2.2. Test of cytoplasmic reaction of F_1

The hybrids obtained by crossing 120 pollinator lines with a male-sterile line, 02-F5DT-12A, were evaluated for the sterility maintenance or fertility restoration. The evaluation was done in two rows of 2 m each per F_1 panicle, at Sotuba during rainy season (July 2013). At flowering, 3 panicles per F_1 plant were covered with paper bags to check for cytoplasmic male sterility. The paper bags were removed at harvest and seed set percentage was scored for classifying the hybrids as male-sterile or male-fertile.

Fertility was scored using the sorghum descriptor and converted to 1 – 6 scale as follows.

- **Score of phenotypes (%** seed setting**)**

  1= 0 grain

  2= 1-5 grains

  3= 25% of seed filled spikelet

  4= 26-50% of seed filled spikelet

  5= 51-75% of seed filled spikelet

  6= 76-100% of seed filled spikelet

5.2.3. Results of testcross

Based on the seed production, 98 plants of restorer lines (R-lines) were identified which had percentage seed set that ranging from 51 – 100% (Table 5.1) and could serve as male parents to produce F_1 hybrids. Three plants with sterility-maintenance ability (B-lines) were identified. These could serve as a new source of CMS. Nineteen lines exhibited partial seed set and were rejected as they could neither serve as restorers nor as maintainers.

Table 5. 1. List of lines identified in testcross

N°	Number of lines	Score	Seed setting (%)	Line type
1	98	5 - 6	51% – 100 %	R-line
2	3	2	< 25%	B-line
3	19	3	25%	R/B-line

5.2.4. Morphological characterization and seed multiplication of R-lines

The self-pollinated seed generated from 120 lines of BCNAM project were sown in July 2013 at Sotuba research center for morphological characterization and seed

multiplication. Each genotype was planted in one row of 4 m length with and inter and intra row spacing of 75 cm and 30 cm respectively.

For characterization of genotypes, the target traits were stay green, lodging tolerance, plant height, grain quality and flowering date. The major criteria for selecting R-lines for hybrid development were stay green and grain quality Data were taken on three plants randomly selected per genotype for each row.

- **Stay green**

For stay green, the total number of green leaves at maturity per plant were counted and recorded.

- **Lodging tolerance**

Lodging tolerance was evaluated by allowing the plants to remain in the field after maturity and throughout the winter to apply uniform lodging pressure.

The scoring for lodging was done as in sorghum descriptor and converted to a scale of 1 – 5 as follows.

Scores of phenotypes (% lodging)

1. = All plants in the plot completely upright
2. = 1 - 10% of plants in the plots completely lodged
3. = 11 - 25% of plants in the plots completely lodged
4. = 26 - 40% of plants in the plots completely lodged
5. = > 40% of plants in the plots completely lodged

- **Grain appreciation**

The grain appreciation was done by visual appraisal of grain color and grain size. It was recorded on the following scale

Scores of phenotypes

5 = Very good

4 = Good

3 = Average

2 = Below average

1 = Poor (rejected)

- **Plant height**

The plant height was recorded by measuring the distance from ground level to the tip of the panicle at physiological maturity and expressed in centimeters.

- **Days to 50% flowering.**

This was recorded as number of days from the date of sowing to the date when half the panicles were in bloom.

5.2.5. Results of morphological characterization

After characterization, ten genotypes were selected based on their morphological traits (Table 5.2) for hybrid development.

Table 5. 2. List of Rf-lines selected for hybrid development

Genotypes	Pedigree/ origin	Pop. source	Race	TNGL	L.T	GA	Height (cm)	Mat. cycle
BCNAM 27-2	Fara Fara//Keninkeni	03	Guinea	5	1	4	247	Early
BCNAM 44-1	Grinkan//E 36-1	08	Caudatum	7	1	5	184	Early
BCNAM 45-1	Grinkan//E 36-1	08	Caudatum	6	1	5	161	Early
BCNAM 57-1	Grinkan//E 36-1	08	Caudatum	5	2	5	222	Medium
BCNAM 55-2	Grinkan//E 36-1	08	Caudatum	5	2	5	247	Late
BCNAM 76-2	Grinkan//IS15401	11	C-Guinea	6	1	5	160	Early
BCNAM 67-2	Grinkan//IS15401	11	C-Guinea	5	1	3	207	Early
BCNAM 79-2	Grinkan//IS15401	11	Guinea	5	2	4	155	Medium
BCNAM 80-2	Grinkan//IS15401	11	Guinea	5	2	4	279	Late
BCNAM 84-1	Grinkan//CSM388	40	Guinea	7	2	3	222	Early

Pop. Population sources, **TNGL**: Total number of green leaves at maturity, **LT:** Lodging tolerance and **GA**: Grain Appreciation

5.3. Discussion

The key objective of this study was to identify stay green and fertility restoring lines (R-lines) useful for developing hybrids for wide adaptation in semi-arid regions of Mali.

Based on the seed produced, 98 individuals with restorer reaction (R-lines) and three individuals with sterility-maintenance ability (B-lines) were identified. Nineteen F_1 plants exhibited partial seed set (R/B lines). The corresponding plants, with partial seed set, were rejected from the program as they served neither as restorers nor as maintainers. A similar procedure was used by Murty *et al.* (1994), to identify B- and R-lines at ICRISAT Patancheru, India. Several improved varieties developed at ICRISAT-Patancheru were found to be restorers on the A1 CMS system using the testcross method and they were added to the restorer gene pool. Belum *et al.* (2006) have reported that several lines with restorer reactions have been identified from a sub-sample of *guinea*-core collections at ICRISAT-Bamako, Mali.

In this study, the genotypes with restorer reaction (R-lines) identified were characterized morphologically. The target traits were stay green, lodging tolerance, plant height, grain quality and flowering date. On the basis of morphological evaluations, ten lines were selected among the lines with restorer reactionwhich could serve as male parents for hybrid production.

5.4. Conclusions

In this study to identify R-lines, a CMS line, 02-F5DT-12A, was crossed with 120 line pollinators from BCNAM project. The hybrids obtained were evaluated for the percentage of the seed set to classify the male lines as sterility maintenaners (B-lines) or fertility restoratorers (R-lines).

Based on the number of seed set, 98 individuals with restorer reactions (R-lines) and three individuals with sterility-maintenance ability (B-lines) were identified. Nineteen F_1 plants exhibited partial seed set (R/B lines). The genotypes with restorer reactions (R-lines) were characterized morphologically.

The morphological characterization identified ten stay green restorer lines (R-lines) useful for developing hybrids for wider adaptation in semi-arid regions of Mali.

CHAPTER SIX

6.0. DEVELOPMENT OF POST FLOWERING DROUGHT TOLERANT HYBRIDS

6.1. Introduction

Sorghum is classified predominantly as a self-pollinated crop, however heterosis has been commercially exploited to improve its productivity. Although heterosis was demonstrated as early as 1927 in sorghum (Conner and Karper, 1927), its commercial exploitation was possible only after the discovery of a stable and heritable cytoplasmic-nuclear male-sterility (CMS) mechanism (Stephens and Holland, 1954). This CMS system has been designated as A1 (*milo*). Since then, a large number of hybrids have been developed and released for commercial cultivation in Asia, the Americas, Australia and Africa. The hybrids have contributed significantly to increased grain and forage yields in several countries. Currently, over 95% of the sorghum area is planted to the hybrids in USA, Australia and China. Over 85% of the rainy season sorghum area is planted to hybrids in India.

Sorghum production in Africa is widespread with low yields due to low inputs and the lack of sorghum hybrids. The low production of sorghum in Mali is due to several reasons including lack of sorghum hybrids and adapted varieties with tolerance to stresses such as drought. The adoption of hybrid sorghum is very low by Malian sorghum producers.

The objective of this study was to develop post-flowering drought tolerant hybrids.

Specific objectives were to:

- produce F_1 hybrid seeds by crossing B and R-lines obtained in Chapters 4 and 5.
- determine combining ability (GCA and SCA) of B and R-lines.
- identify potential high yielding inbred parents and hybrids tolerant to post flowering drought.

6.2. Materials and methods

6.2.1. Materials

Information on the experimental site used, the irrigation system, and details on fertilizer application have been described in the earlier section 4.2.

6.2.1.1. Plant material

The plant materials were the twelve inbre B-lines and ten R-lines, developed during this thesis used as parents of hybrids. In addition, six inbred lines from the Malian sorghum program were used as checks. The list of the inbred lines is presented in Table 6.1.

Table 6. 1. List of plant material

N°	Lines	Type	Origin
1	(B35//82B)-F3-64	B-line, parent	Developed in this thesis research
2	(B35//82B)-F3-104	B-line, parent	Developed in this thesis research
3	(B35//82B)-F3-114	B-line, parent	Developed in this thesis research
4	(B35//82B)-F3-136	B-line, parent	Developed in this thesis research
5	(B35//134B)-F3-44	B-line, parent	Developed in this thesis research
6	(B35//134B)-F3-64	B-line, parent	Developed in this thesis research
7	(B35//134B)-F3-89	B-line, parent	Developed in this thesis research
8	(B35//134B)-F3-125	B-line, parent	Developed in this thesis research
9	(B35//3009B)-F3-16	B-line, parent	Developed in this thesis research
10	(B35//3009B)-F3-24	B-line, parent	Developed in this thesis research
11	(B35//3009B)-F3-51	B-line, parent	Developed in this thesis research
12	(B35//3009B)-F3-90	B-line, parent	Developed in this thesis research
13	BCNAM 27-2	R-line, parent	Identified in this thesis research
14	BCNAM 44-1	R-line, parent	Identified in this thesis research
15	BCNAM 45-1	R-line, parent	Identified in this thesis research
16	BCNAM 57-1	R-line, parent	Identified in this thesis research
17	BCNAM 55-2	R-line, parent	Identified in this thesis research
18	BCNAM 76-2	R-line, parent	Identified in this thesis research
19	BCNAM 67-2	R-line, parent	Identified in this thesis research
20	BCNAM 79-2	R-line, parent	Identified in this thesis research
21	BCNAM 80-2	R-line, parent	Identified in this thesis research
22	BCNAM 84-1	R-line, parent	Identified in this thesis research
23	B35 (BTx642)	B-line, check	Ethiopie
24	98-BE-F5P-82B (82B)	B-line, check	Mali
25	03-SB-F5DT-134B (134B)	B-line, check	Mali
26	09PR-3009B (3009B)	B-line, check	Mali
27	02-SB-F4DT-275 (Grinkan)	R-line, check	Mali
28	CSM 388 (Jiguiseme)	R-line, check	Mali

6.2.2. Methods

6.2.2.1. Hybrid seed production (B-lines by *R-lines)*

A set of twelve female (B-lines) and ten male lines (R-lines) were planted for making North Carolina Design II crosses at CRRA of Sotuba. The crossing block for F_1 hybrid seeds production was planted on two dates: the 18th and 30th July 2014.

The crossing blocks were consisted of three rows of 3 m length of each female parent (B-line) and were between female rows, two rows of 3 m of different male parents (R-line). The spacing between rows was 0.75 m and between the hills was 0.30 m.

By this stage of the study, male sterile female parents (A-lines) were not available. Therefore hand emasculation was used on the female parents and reciprocally to make these crosses. At flowering, the normal bisexual florets of the twelve female lines were hand emasculated and pollen from one of the ten male R-lines was transferred to the stigma of emasculated florets (and reciprocally) according to the mating design NCD II to generate F_1 hybrids seeds.

The problem of synchronization of flowering between the B and R lines was encountered. The B-lines were generally earlier than the R lines. In addition, sorghum flowers are tiny and significant numbers could not be emasculated by hand. As a result, there were not enough seeds of all hybrids (12 x 10) produced and the number R-lines was reduced from ten to five. A total of 60 F_1 hybrids were obtained from the NCD II (12 x 5 = 60).

6.2.2.2. Identification of potential parents and hybrids under well-watered and post flowering water stress conditions

During December 2014 to May 2015, the F_1 hybrids were grown in the CRRA of Sotuba during the off season. The 60 F_1 hybrids were planted along with six sorghum lines used as checks (Appendix 6). A total of 66 sorghum genotypes were evaluated under both drought stress and well-watered conditions.

The trial was sown on the 15th December 2014 in a split-plot design with drought intensity as the main plots were used (well-watered and water stressed). The sub-plots composed different genotypes arranged in an alpha lattice design [(22 x 3) x2]. Each genotype was sown in two rows of 2 m length. Plant to plant and row to row distances were 30 and 75 cm, respectively. The distance between water-stressed and well-watered experiments was 5 m. For irrigation management, the drip system was installed between rows. Before the stress imposition, all the plots were irrigated at two day intervals. Once 50% of the plants in the plots had visible heads with flowers, post-flowering drought was imposed by withholding irrigation in the drought stressed trials. The well-watered trials continued to receive irrigation up to crop maturity.

About 100 kg/ha Di-ammonium phosphate (DAP) [NPK: 18-46-0] and Nitrogen in the form of urea were applied 40 days after sowing at 50 kg/ha.

Three plants per row were tagged randomly for each genotype for the above observations.

6.2.2.3. Data collection

Data were collected on the following parameters:

- **Seedling vigor**

Early seedling vigor was recorded at 15 days after emergence of seedlings on the scale of 1 to 5, where 1 = Poor seedling vigor and 5 = Very good seedling vigor.

- **Days to 50% flowering**

This was recorded as number of days from the date of sowing to the date when half the panicles were in bloom.

- **Leaf chlorophyll index**

A Minolta chlorophyll meter (SPAD-502) was used to measure the greenness of the leaf below the flag leaf (FL-1) from three tagged plants in each row at flowering and at maturity. Three measurements were taken down one side of the leaf at the base, centre and tip, approximately 1 cm from the leaf edge.

- **Plant height:** Measured as described in section 4.3.1.2
- **Lodging tolerance:** Determined as described in section 4.3.1.2
- **Number of leaves per plant**

Total number of leaves (TNL) at maturity were recorded, thereafter the total number of green leaves (TNGL) were recorded.

- **Thousand grains weight**

The 1000 grains were randomly counted and weight recorded in grams.

- **Vitreousness**

The endosperm varies from 100% soft tissue with a little corneous portion to a solid corneous seed. It was rated at physiological maturity of the seed on the scale of 1 to 10, where 1 = Very solid corneous seed and 10 = Very soft corneous seed (floury endosperm).

- **Panicle appreciation**

Panicle appreciation was recorded at physiological maturity on the visual scale of 1 to 5, where 1 = Very loose drooping panicle and 5 = Very compact panicle.

- **Grain appreciation.** Recorded as described in section 4.3.1.2.
- **Grain yield per plant**

Well-dried panicles were threshed and seeds separated. Weight of the seeds in grams was recorded as grain yield per plant.

- **Biomass**

After panicles were harvested, each plant of an experimental plot was harvested manually by cutting the plant at the base and then dried. After drying, the samples were weighed and used to estimate the dry biomass.

6.2.2.4. Statistical analysis

Analysis of variance (ANOVA) was performed on plot means for grain yield and other agronomic characters for each environment and across environments using GENSTAT 12th edition. In the combined analysis, water regimes, replications and blocks were treated as random effects while entries were considered as fixed effects.

The analysis of combining ability was based on the model described by Kempthorne, (1957) and Comstock and Robinson, (1952). The GCA and SCA effects were examined for each environment and across environments.

a. Model of combining ability for each environment

$$Yijk = \mu + rk + fi + mj + (f \times m)ij + eijk$$

- **Yijk** : the observed measurement for the k^{th} replication of the ixjth progeny;
- **μ**: experimental mean;
- ***fi* :** is the effect of the i^{th} female (GCAfemale*i*); I = 1,2,3….21
- ***tj*:** is the effect of the j^{th} male (GCAmale*j*); j = 1,2,3
- **(f x m)*ij***: is the interaction effect of the i^{th} female with the j^{th} male (SCA*ij*);
- **rk**: effect replication within environment; k = 1,2
- **e*ijk*** : is the error effect associated with the ijk^{th} observation;

b. Model of combining ability for across environments

$$Yijkm = \mu + rk + fi + mj + (f \times m)ij + (f \times s)im + (m \times s)jm + (f \times m \times s)ijm + eijkm$$

- **Yijkm** : the observed measurement for the k^{th} replication at the m^{th} environment of the ixj^{th} progeny;
- **(f x s)*im***: is the interaction effect of the i^{th} female and m^{th} environment; im = 1….n

- **(m x s)*jm***: is the interaction effect of the j^{th} male and m^{th} environment; jm = 1……n
- **(f x m x s)*ijm***: is the interaction effect of the i^{th} female and j^{th} male at the m^{th} environment; ijm = 1..n
- **rk:** effect replication within environment; k = 1,..n
- **e*ijkm*** : is the error effect associated with the $ijkm^{th}$ observation; (Hede et al., 1999)

c. <u>Estimation of GCA and SCA effects</u>

- **GCA will be computed as:**

  GCA_f = X_f – μ and

  GCA_m = X_m – μ

X_f and X_m = Mean of female and male respectively

GCA_f and GCA_m = General combining ability of female and male respectively

μ = Overall mean of crosses in the trial

- **SCA will be computed as:**

  SCAij = Xi – Ej **= SCAij** = Cross (ij) mean – [GCAfemalei + GCAmalej + μ]

Xi = Observed mean value of the cross

Ej = Expected mean value of the cross based on the 2 GCAs of its parents

Ij = crosses, ij= 1…n

6.3. Experimental results

6.3.1. Experimental conditions

The experiment was conducted during the off season from December 2014 to May 2015 at Sotuba Research Center. The weather conditions during this period are presented in Figures 6.1 and 6.2. There was no rainfall during crop growth.

Increasing temperatures, under shelter, over surface of ground and under-ground to 10 cm, were recorded from February to April. The minimal values were observed in January (Figure 6.1). Evaporation increased steadily from 243.2 mm/month during January 2015 to 318.7 mm/month during April 2015 (Figure 6.2). The increased evapotranspiration (ET) levels, coupled with lack of rainfall during the post-flowering crop growth stage, created ideal moisture stress conditions. During January to April it was recorded also the increase of insolationat (Figure 6.2).

Effects of the weather conditions during the growth period made it difficult to conserve soil moisture in the plots after irrigation due to the high temperature (heat) and the dry wind. This induced the abortion of flowers in several genotypes at flowering stage.

Eight F_1 plants and two check varieties were photoperiod sensitive or underwent an abortion at flowering (Appendix 6) and were excluded from further analysis.

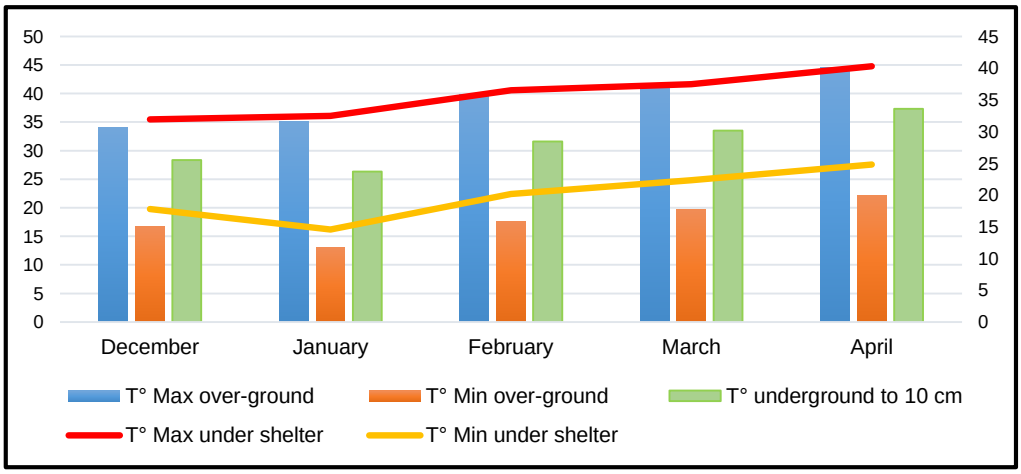


Figure 6. 1. Temperatures (Under shelter, over surface ground and underground to 10 cm) during crop growth period at Sotuba.

(Soure: Station météo de Sotuba, 2015)

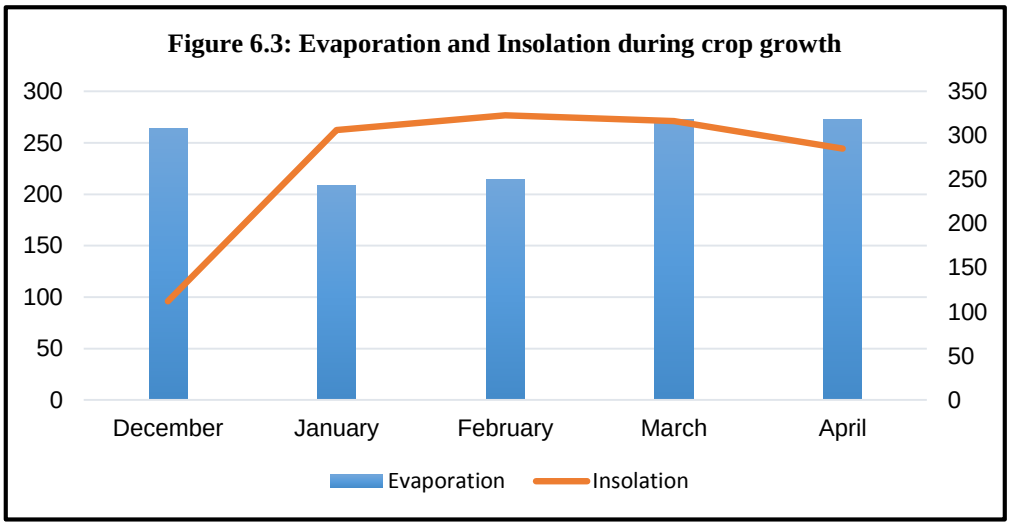


Figure 6. 2. Evaporation and Insolation during crop growth period at Sotuba.

(Soure: Station météo de Sotuba, 2015)

6.3.2. Combining ability analysis and gene action for various quantitative traits of parents and their F_1 hybrids

6.3.2.1. Mean squares of combining ability across well-watered and water stress plots for various quantitative traits of parents and their F_1 hybrids.

The analyses of variance (ANOVA) for combining ability of females and males evaluated for grain yield (kg/ha), total number of green leaves (TNGL) at maturity and SPAD chlorophyll meter readings at maturity (SCMR), across water management plots (females x males x water management) were significantly different (at P = 0.05 and P = 0.01) (Table 6.2).

There were significant differences among female parents for all traits except for biomass in tons/ha (BIOM). Significant differences among males was found for biomass, Total number leaves (TNL) at maturity and plant height (height). But there were no significant differences for grain yield (Yield kg/ha), days to 50 per cent flowering (50FL), SPAD chlorophyll meter readings at maturity (SCMR), total number of green leaves (TNGL) and thousand grains weight (TGW).

General combining ability mean squares for females (GCA_f) were greater than males GCA_m mean squares for grain yield indicating that the major contribution was additive variance for this trait and was due the female parents. SCA_{fm} mean squares differed significantly for all traits except for days to 50 per cent flowering (50FL). This indicates the importance of both additive and non-additive variance for these traits (Table 6.2).

Table 6. 2. Mean squares of GCA and SCA across well-water and water stress management for various quantitative traits of parents and their F_1 hybrids

Source of variations	d.f.	Yield (kg/ha)	50FL (day)	Biom (t/ ha)	height (cm)	T.N.L	TNGL	SCMR MAT	TGW (g)
Whole plot	1	80334620**	248.0**	713.0**	69753.4**	2.5**	636.1**	11179.7**	1194.2**
Female (GCA_f)	11	1595995**	154.8**	6.5^{ns}	3522.6**	7.9**	10.6**	46.9^{ns}	18.1**
Male (GCA_m)	4	135759^{ns}	111.4^{**}	18.9**	6625.5**	6.5**	0.9^{ns}	22.1^{ns}	2.8^{ns}
Whole plot x Female	11	380948^{ns}	12.7^{ns}	5.6^{ns}	$1067,0^{ns}$	1.4^{ns}	4.2^{ns}	56.2*	4.5^{ns}
Whole plot x Male	4	402084^{ns}	43.0^{ns}	6.0^{ns}	778.1^{ns}	0.6^{ns}	3.2^{ns}	21.8^{ns}	1.3^{ns}
Female x Male (SCA_{fm})	36	992951**	39.0	10.3**	1817.6**	2.6*	3.7*	61.4**	14.8**
Whole plot x Female x Male	**36**	**605851****	$\mathbf{16.1^{ns}}$	$\mathbf{3.9^{ns}}$	$\mathbf{854.1^{ns}}$	$\mathbf{1.0^{ns}}$	**4.0***	**50.1***	$\mathbf{6.6^{ns}}$
Residual	78	275133	28.8	4.4	655.8	1.6	2.4	28.9	5.9
Total	181	1012429	38.3	9.9	1641.9	2.1	7.1	103.7	14.9
S.E.		524.5	5.4	2.1	25.6	1.3	1.6	5.4	2.4
CV%		30.0	7.0	21.7	15.3	11.3	36.3	14.1	13.8

df = Degree of freedom, **Yield (kg/ha)** = Grain yield, **50 FLO (day)** = Days to 50 per cent flowering, **SCMR MAT** = SPAD chlorophyll meter readings at maturity, **BIOM. (t/ha)** = Biomass, **T.N.L** = Total number leaves, **TNGL** = Total number of green leaves, **Height (cm)** = Plant height, **TGW (g)** = Thousand grains weight.
.** Significant at the 0.05 probability level; *.** Significant at the 0.01 probability level; **ns:** not significant
Whole_plot. = Water Management

6.3.2.2. SCA effects of female parents crossed with male parents for various traits in sorghum under well-water conditions and water stress conditions

Estimates of specific combining ability effects (SCA) and mean data of genotypes for grain yield (kg/ha), SCMR at maturity and TNGL at maturity are presented in Table 6.3. The best check for grain yield (kg/ha), under well-watered conditions was the genotype 09PR-3009B with value 2115 kg/ha (Appendix 7) and the Least significant difference (Lsd 5%) of 1165. In the well-watered environment the best combinations, for gain yield were (B35//134B)-F3-44/BCNAM-27-2 with mean yield 4227 kg/ha followed by (B35//134B)-F3-44/BCNAM-76-2 with mean yield of 4164 kg/ha. The cross combination (B35//134B)-F3-125/BCNAM-44-1, (B35//82B)-F3-64/BCNAM-45-1, (B35//134B)-F3-89/BCNAM-45-1 and (B35//82B)-F3-104/BCNAM-76-2 followed with mean yield ranging from 3803 kg/ha to 3477 kg/ha (Table 6.3).

Under water stress conditions the best check in terms of grain yield was B35 (BTx642), with yield of 786 ± 958 kg/ha (Appendix 7). Under the water stress conditions, entries (B35//134B)-F3-44/BCNAM-44-1 (1795 kg/ha), (B35//82B)-F3-64/BCNAM-44-1 (1786 kg/ha) and (B35//3009B)-F3-16/BCNAM-84-1 (1758 kg/ha) were higher yielding hybrid in water stress environment (Table 6.3).

Based on the yield data in Table 6.3, the best hybrids under both environments were (B35//134B)-F3-44/BCNAM-76-2 with mean yields of 4164 and 1698 kg/ha, (B35//82B)-F3-64/BCNAM-45-1 with yields of 3616 and 1415 kg/ha, (B35//82B)-F3-104/BCNAM-76-2 with yields of 3477 and 1442 kg/ha. These three hybrids also had high SCA values under both environments respectively as +1077 and +443, +678 and +228 and +646 and +105 respectively and all showed positive value for SCA (Table 6.3). For SCMR at maturiry the best check was B35 in all environments with the values 57.3 and 46.3. There were no combinations that performed better than the check for this trait

under both well-watered and water stress environments (Appendix 7). However between combinations the hybrids (B35//3009B)-F3-16/BCNAM-45-1 had values of 54.0 and 34.1, (B35//3009B)-F3-24/BCNAM-27-2 with values of 51.4 and 36.7, showed positive values for SCA under both environments (Table 6.3).

For TNGL at maturity, the positive combinations under well-watered and water stress conditions were (B35//134B)-F3-125/BCNAM-84-1 with 9 and 4 green leaves at maturity, followed by (B35//82B)-F3-114/BCNAM-44-1 with 7 and 5 green leaves and (B35//82B)-F3-114/BCNAM-45-1 with 8 and 4 green leaves at maturity (Table 6.3).

Table 6. 3. SCA effects of females crossed to male for various traits in sorghum under well-watered and water stressed conditions

Genotypes	Grain Yield (kg/ha)		SCA for Yield		SCMR MAT		SCA SCMR MAT		TNGL MAT		SCA TNGL MAT	
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
(B35//134B)F3-125/BCNAM-27-2	1627	1309	-768	22	47.8	40.2	-0.9	8.7	8.5	2.7	0.1	-0.5
(B35//134B)F3-44/BCNAM-27-2	4227	753	1215	-631	52.3	24.9	3.1	-6.5	6.2	0.5	-0.9	-2.5
(B35//134B)F3-89/BCNAM-27-2	1287	1135	-1387	-251	45.7	23.7	-1.9	-6.4	4.3	2.0	-2.8	-2.0
(B35//3009B)F3-16/BCNAM-27-2	3251	633	660	-608	50.5	25.2	0.2	-5.1	5.0	0.2	-2.6	-3.2
(B35//3009B)F3-24/BCNAM-27-2	2650	478	60	-925	51.4	36.7	2.6	4.7	8.5	1.7	2.2	-1.4
(B35//3009B)F3-51/BCNAM-27-2	2213	998	-148	-343	47.7	19.6	-0.3	-14.2	3.2	2.0	-5.0	-1.5
(B35//3009B)F3-90/BCNAM-27-2	1470	358	-1034	-857	46.6	41.9	-1.0	4.2	7.2	1.3	-0.2	-1.7
(B35//82B)F3-114/BCNAM-27-2	3182	418	723	-879	42.6	21.0	-5.1	-10.3	7.3	0.0	0.5	-4.2
(B35//82B)F3-136/BCNAM-27-2	1213	188	-1990	-1446	49.3	24.4	0.2	-8.3	4.5	0.0	-2.1	-4.5
(B35//82B)F3-64/BCNAM-27-2	2386	625	-524	-764	42.2	34.4	-5.7	2.2	7.3	2.0	0.3	-1.2
(B35//134B)F3-125/BCNAM-44-1	3803	428	1421	-619	41.7	34.7	-4.9	1.5	7.2	1.7	-1.1	-0.7
(B35//134B)F3-44/BCNAM-44-1	3018	1795	19	651	47.0	38.9	-0.1	5.8	5.8	2.5	-1.0	0.3
(B35//134B)F3-64/BCNAM-44-1	2848	609	344	-440	43.3	14.0	-3.7	-24.0	7.2	2.3	0.7	0.0
(B35//134B)F3-89/BCNAM-44-1	2007	1577	-654	431	49.6	20.9	4.0	-10.9	6.0	1.0	-0.9	-2.2
(B35//3009B)F3-16/BCNAM-44-1	1967	806	-611	-195	45.8	26.8	-2.4	-5.2	5.8	2.5	-1.6	-0.1
(B35//3009B)F3-24/BCNAM-44-1	1518	1081	-1059	-82	39.5	27.8	-7.1	-5.9	3.5	0.7	-2.6	-1.6
(B35//82B)F3-104/BCNAM-44-1	2269	505	-474	-721	44.0	23.4	-5.8	-9.7	7.5	0.0	1.5	-2.9
(B35//82B)F3-114/BCNAM-44-1	1407	278	-1039	-779	42.4	18.6	-3.2	-14.4	6.8	4.5	0.2	1.1
(B35//82B)F3-136/BCNAM-44-1	1458	736	-1732	-658	40.7	25.4	-6.2	-9.0	4.8	1.7	-1.6	-2.0
(B35//82B)F3-64/BCNAM-44-1	2820	1786	-77	637	46.6	36.5	0.9	-7.2	3.7	3.0	-3.2	0.6
(B35//134B)F3-44/BCNAM-45-1	1413	748	-1627	-434	38.1	20.7	-8.6	-12.3	2.0	1.8	-5.0	-0.4
(B35//134B)F3-64/BCNAM-45-1	1728	278	-817	-809	38.2	16.4	-8.5	-21.5	4.5	0.0	-2.1	-2.3
(B35//134B)F3-89/BCNAM-45-1	3591	1157	889	-27	40.4	33.5	-4.9	1.8	4.5	2.7	-2.6	-0.5
(B35//3009B)F3-16/BCNAM-45-1	2187	1013	-432	-26	54.0	34.1	6.1	2.2	4.5	2.0	-3.1	-0.6
(B35//3009B)F3-24/BCNAM-45-1	2169	201	-449	-1000	55.9	19.4	9.5	-14.2	8.0	1.3	1.7	-1.0
(B35//3009B)F3-51/BCNAM-45-1	2164	1304	-225	165	38.9	23.2	-6.8	-12.2	4.3	1.7	-3.8	-0.9
(B35//82B)F3-114/BCNAM-45-1	2962	1648	475	553	40.6	33.6	-4.8	0.7	7.8	3.7	1.0	0.3
(B35//82B)F3-136/BCNAM-45-1	1573	260	-1658	-1172	47.0	27.5	0.4	-6.8	9.2	1.0	2.5	-2.7
(B35//82B)F3-64/BCNAM-45-1	3616	1415	678	228	46.7	33.0	1.3	-0.8	5.3	3.7	-1.7	1.3
(B35//134B)F3-125/BCNAM-76-2	1660	340	-810	-818	48.5	25.6	2.5	-7.0	7.3	0.8	-0.9	-2.1
(B35//134B)F3-44/BCNAM-76-2	4164	1698	1077	443	52.5	31.8	6.0	-0.7	5.8	3.7	-1.0	1.0
(B35//134B)F3-64/BCNAM-76-2	2046	1444	-546	284	44.9	31.0	-1.6	-6.4	7.7	3.2	1.2	-0.5
(B35//134B)F3-89/BCNAM-76-2	1362	625	-1387	-632	40.8	37.6	-4.2	6.4	5.5	0.0	-1.4	-3.7
(B35//3009B)F3-16/BCNAM-76-2	3016	308	350	-804	40.8	24.8	-6.8	-6.6	3.8	2.0	-3.6	-1.1
(B35//3009B)F3-24/BCNAM-76-2	2803	1296	138	22	39.6	21.4	-6.5	-11.7	5.7	4.5	-0.5	1.7
(B35//3009B)F3-51/BCNAM-76-2	2628	955	192	-257	49.3	29.7	3.8	-5.2	4.7	4.0	-3.3	0.8

Table 6.3. Cont'd

Genotypes	Yield (kg/ha)		SCA for Yield		SCMR MAT		SCA SCMR MAT		TNGL MAT		SCA TNGL MAT	
	WW	**WS**	**WW**	**WS**	**WW**	**WS**	**WW**	**WS**	**WW**	**WS**	**WW**	**WS**
(B35//3009B)F3-90/BCNAM-76-2	2335	1158	-244	72	43.3	36.2	-1.7	-2.6	8.2	2.3	1.0	-0.4
(B35//82B)F3-104/BCNAM-76-2	3477	1442	646	105	40.7	33.7	-8.5	1.2	5.3	4.3	-0.7	0.9
(B35//82B)F3-114/BCNAM-76-2	1100	820	-1434	-348	51.9	31.5	6.8	-0.9	6.5	4.2	-0.1	0.3
(B35//82B)F3-136/BCNAM-76-2	1760	1173	-1518	-332	42.1	25.4	-4.4	-8.4	4.2	0.3	-2.3	-3.9
(B35//82B)F3-64/BCNAM-76-2	2756	1590	-229	330	45.3	35.1	0.1	1.8	8.2	2.8	1.3	-0.1
(B35//134B)F3-125/BCNAM-84-1	2387	1353	-150	252	45.4	23.7	-1.6	-8.3	9.3	3.8	1.1	1.4
(B35//134B)F3-44/BCNAM-84-1	2111	648	-1043	-550	41.8	38.7	-5.7	6.8	5.8	2.0	-1.0	-0.2
(B35//134B)F3-64/BCNAM-84-1	2007	1093	-652	-10	46.7	25.4	-0.7	-11.4	5.8	2.3	-0.6	0.1
(B35//134B)F3-89/BCNAM-84-1	1757	1162	-1059	-38	46.9	32.8	1.0	2.2	5.0	0.0	-1.9	-3.2
(B35//3009B)F3-16/BCNAM-84-1	2405	1758	-328	703	46.4	38.8	-2.1	8.0	3.5	2.0	-3.9	-0.6
(B35//3009B)F3-24/BCNAM-84-1	1280	655	-1452	-562	43.6	24.8	-3.5	-7.7	4.7	3.0	-1.5	0.7
(B35//3009B)F3-90/BCNAM-84-1	2707	1273	61	244	44.1	33.8	-1.9	-4.4	5.8	2.5	-1.3	0.3
(B35//82B)F3-104/BCNAM-84-1	2444	1686	-454	406	36.6	35.8	-13.5	3.9	4.8	4.2	-1.2	1.3
(B35//82B)F3-114/BCNAM-84-1	2427	1076	-174	-35	47.1	29.0	1.1	-2.8	4.3	4.3	-2.3	0.9
(B35//82B)F3-136/BCNAM-84-1	1355	198	-1990	-1250	37.1	23.9	-10.2	-9.3	5.2	0.0	-1.3	-3.7
(B35//82B)F3-64/BCNAM-84-1	2843	255	-209	-948	44.7	20.1	-1.5	-12.6	9.3	0.0	2.5	-2.4
SE	**581**	**384**	**579**	**393**	**5.4**	**5.3**	**5.4**	**5.4**	**1.8**	**1.0**	**1.8**	**1.0**

Yield (kg/ha) = Grain yield, **SCMR** = SPAD chlorophyll meter readings at maturity, **TNGL** = Total number of green leaves per plant at maturity.
WW: well-watered conditions; **WS**: water stress conditions; **S.E.** = Standard error.

6.3.2.3. GCA effects of female parents for various traits under drought stress (WS) and well-watered conditions (WW).

The GCA effect of the female parents under drought and well-watered conditions are presented in Table 6.4. Under well-watered conditions, three female parents showed positive GCA for grain yield and produced the higher yield in hybrid combination. These were (B35//134B)-F3-44, (B35//82B)-F3-64, (B35//82B)-F3-104, with a gain on yield raning from +406 - +662 kg/ha (Table 6.4). In this environment the females parents (B35//3009B)-F3-16, (B35//134B)-F3-125 and (B35//3009B)-F3-51 had positive estimates of GCA but did not produce the high yielding hybrids (Table 6.3).

Under drought stress, two female parents (B35//82B)-F3-64 and (B35//134B)-F3-44 exhibited positive GCA values for grain yield (Table 6.4) and produced high yielding hybrids (Table 6.3). The parents (B35//82B)-F3-104 and (B35//134B)-F3-89 had positive estimates of GCA but did not produce high yielding hybrids under water stress environments (Table 6.3). Under both well-watered and drought stress conditions, the parents (B35//134B)-F3-44 with +662 and +184 followed by (B35//82B)-F3-64 with +560 and +189 had positive GCA and produced high yielding hybrids as well.

The best parent that produced hybrid with high value SCMR under well-watered conditions was (B35//3009B)-F3-16. However, under water stressed conditions, the best genotype was (B35//3009B)-F3-90 and produced hybrids with high SCMR in drought stress environment (Table 6.4). It was noted in this study that among all female parents only the parent (B35//3009B)-F3-90 had the four major stay green QTLs in homozygous form. This parent could be invaluable donor of stay green trait.

The parent (B35//134B)-F3-125 exhibited a positive GCA values under well-watered conditions for TNGL at maturity. On the other hand, (B35//82B)-F3-114 was the best parent under water stressed conditions for TNGL at maturity (Table 6.4).

The parent (B35//82B)-F3-64 had positive GCA value for all three characters studied under drought stress and well-watered conditions (Table 6.4). Similarly the parent (B35//134B)-F3-125 had positive GCA value for all three characters studied under drought stress and well-watered conditions except for yield under drought stress. Except for the value of TNGL at maturity, the parent (B35//134B)-F3-44 had positive value for all three characters and the same was noted for the parent (B35//82B)-F3-104 except for SCMR at maturity under well-watered conditions (Table 6.4).

Table 6. 4. GCA effects of female parents for various traits in sorghum under drought stress and well-watered conditions.

Female Parents	Characters					
	Yield kg/ha		SCMR MAT		TNGL MAT	
	WW	WS	WW	WS	WW	WS
(B35//134B)F3-125	45	-87	1.1	2.3	2.2	0.2
(B35//134B)F3-44	662	184	1.6	2.2	-0.8	0.0
(B35//134B)F3-64	-167	-89	-1.5	-7.1	0.4	-0.1
(B35//134B)F3-89	-324	186	-0.1	0.9	-0.9	-1.0
(B35//3009B)F3-16	241	-41	2.7	1.1	-1.4	-0.4
(B35//3009B)F3-24	-240	-203	1.2	-2.8	0.1	0.1
(B35//3009B)F3-51	11	141	0.5	-4.6	-1.9	0.5
(B35//3009B)F3-90	-154	-15	-0.1	8.5	1.1	0.0
(B35//82B)F3-104	406	266	-4.3	2.2	0.0	0.7
(B35//82B)F3-114	-109	-97	0.2	-2.1	0.6	1.2
(B35//82B)F3-136	-853	-434	-1.5	-3.5	-0.4	-1.5
(B35//82B)F3-64	560	189	0.3	3.0	0.8	0.2
SE	**260**	**194**	**2.4**	**2.6**	**0.8**	**0.5**

Yield (kg/ha) = Grain yield, **SCMR MAT**= SPAD chlorophyll meter readings at maturity, **TNGL MAT** = Total number of green leaves per plant at maturity.
WW: well-watered conditions; **WS**: water stress conditions; **S.E.** = Standard error.

6.3.2.4. The GCA effects of male parents for various traits of sorghum under drought stress and well-watered conditions.

Under water stress conditions the parents BCNAM-76-2 and BCNAM-84-1 had positive GCA (Table 6.5) and produced hydrids with high yield. Only the male parent BCNAM-76-2, exhibited positive GCA and produced best combinations for grain yield under both well-watered and water stressed conditions (Table 6.5).

The results of this study that the male parent BCNAM-76-2 had positive GCA value for all three characters studied under drought stress and well-watered conditions.

Table 6. 5. GCA effects of male parents for various traits in sorghum under drought stress and well-watered conditions

Male Parents	Characters					
	Yield kg/ha		SCMR MAT		TNGL MAT	
	WW	WS	WW	WS	WW	WS
BCNAM-27-2	26	-255	2.8	0.4	0.3	-0.9
BCNAM-44-1	-13	15	-0.7	-2.1	-0.1	-0.1
BCNAM-45-1	54	-53	-0.4	-2.0	-0.3	-0.1
BCNAM-76-2	101	126	0.2	1.5	0.1	0.6
BCNAM-84-1	-168	69	-1.1	0.9	-0.1	0.1
SE	**174**	**141**	**1.6**	**1.9**	**0.5**	**0.4**

Yield (kg/ha) = Grain yield, **SCMR MAT**= SPAD chlorophyll meter readings at maturity, **TNGL MAT** = Total number of green leaves per plant at maturity.
WW= well-watered conditions; **WS=** water stress conditions; **SE** = Standard error.

6.5. Discussion

The objective of the present chapter was to examine the combining abilities of new B and R-lines under both water stressed and well-watered environments and to evaluate the effect of drought on yield and its various components. The analyses of variance (ANOVA) for combining ability of females and males evaluated for grain yield (kg/ha), total number of green leaves (TNGL) at maturity and SPAD chlorophyll meter readings at maturity (SCMR), across water management plots (females x males x water management) were significantly different (at $P = 0.05$ and $P = 0.01$).

For grain yield, three combinations (B35//134B)-F3-44/BCNAM-76-2, (B35//82B)-F3-64/BCNAM-45-1, (B35//82B)-F3-104/BCNAM-76-2) were idendified as possessing high yield and positive SCA under well-watered and water stress environments. The mean squares of males x female contribution (SCA) for grain yield was greater than male plus females' percentage contribution (GCA) for grain yield. The highly significant male x female mean squares for grain yield indicated that non-additive gene effects must be considered if maximum improvement of yield is to be achieved. Similar results have been

reported in maize by Abdel-Moneam *et al*. (2009) who showed that grain yield was governed by genes acting non-additively. Shiri *et al.* (2010) also reported in maize that the effect of gene action could be both non additive and additive in the expression of grain yield. However, non-additive genetic variance was more important in the expression of grain yield than additive variance.

Under both well-watered and drought stress conditions, the parents (B35//134B)-F3-44 and (B35//82B)-F3-64 had positive GCA and produced higher yielding hybrids. The study revealed that the GCA_f female parents mean squares were significant (at P=0.05 P=0.01) for all traits except biomass and SCMR at maturity. This indicates the presence of genotypic variability among cultivars used as female parents. The significance of GCA mean squares indicated the importance of additive gene effects. These results are in agreement with those obtained by Diallo (2013) who found significant GCA mean squares of grain yield for female parents in across and several environments, which indicated the importance of additive gene effects governing the inheritance of grain yield. Only the male parent, BCNAM-76-2, exhibited positive GCA and produced best hybrid combinations for grain yield and under both well-watered and water stressed conditions in the study.

The GCA_m mean squares for male parents were non-significant for all traits except biomass, plant height and total number of leaves (Table 6.2).

GCA_f females' mean squares were greater than GCA_m males' mean square for all traits except biomass. This indicated that the major contribution of additive variance for traits was due to the female parents. This presumably was due to the fact that the number of female lines used in this study was greater than males and that they represented a wider range of genotypes than the male lines. Omanga (1983) reported in pigeonpea (*cajanus*

cajan (la) millsp.) that the contribution of males was consistently larger than that of the females when the number of male lines used in study was greater than females.

SCA_{fm} females' and males' mean squares differed significantly for all studied traits except for days to 50 per cent flowering (50FL). The results showed that for the majority of the characters studied, both general and specific combining ability effects contributed significantly with the GCA effects being more pronounced than the SCA effects. This indicates that additive as well as non-additive effects were important. This is consistent with the findings of Nevado and Cross, (1990) and Choukan (2008) that GCA and SCA are the most important indicators for expressing the potential value of lines. The non-additive gene effects are estimates of specific combining ability while additive gene effect are estimates of general combining ability. Nguyen *et al*. (1997) has been reported that GCA and SCA effects were highly significant for all the measured traits. They found that some parents heading which were considered as good combiners had high positive GCA for grain yield and low or negative GCA for culm length and days to maturity. It has also been reported that estimates of general and specific combining ability effects indicate the presence of both additive and dominance gene action for yield per plant, number of leaves per plant, plant height and days to 50 per cent flowering in seven *sorghum bicolor* genotypes and their F_1s (Bhadouriva and Saxena, 1997)

6.6. Conclusions

Sixteen hybrids and six inbred lines were evaluated under well-watered and water stress environments. Results showed that under well-watered environments, the hybrids (B35//134B)-F3-44/BCNAM-27-2 , (B35//134B)-F3-44/BCNAM-76-2, (B35//134B)-F3-125/BCNAM-44-1, (B35//82B)F3-64/BCNAM-45-1, (B35//134B)F3-89/BCNAM-45-1 and (B35//82B)F3-104/BCNAM-76-2 had high yield and positive SCA. Under water stress conditions three hybrids (B35//134B)-F3-44/BCNAM-44-1, (B35//82B)-F3-64/BCNAM-44-1 and (B35//3009B)-F3-16/BCNAM-84-1 were higher yielding with positive SCA. The best hybrids under both environments were (B35//134B)-F3-44/BCNAM-76-2, (B35//82B)-F3-64/BCNAM-45-1, and (B35//82B)-F3-104/BCNAM-76-2, with high SCA values under both environments.

Under well-watered conditions, three female parents, (B35//134B)-F3-44, (B35//82B)-F3-64, (B35//82B)-F3-104, produced the highest yielding hybrids and showed positive GCA for grain yield. In contrast, under drought stress conditions, two female parents, (B35//82B)-F3-64 and (B35//134B)-F3-44, produced high yielding hybrids and exhibited positive GCA.

Under both under well-watered conditions and drought stress, the parents (B35//134B)-F3-44 and (B35//82B)-F3-64 produced higher yielding hybrids with positive GCA.

The best hybrid for SCMR at maturity under well-watered and water stressed conditions, was (B35//3009B)-F3-16/BCNAM-45-1. For the TNGL at maturity, the best hybrid was (B35//134B)-F3-125/BCNAM-84-1.

The highest SCMR and positive value of GCA for SCMR at maturity was recorded for female parent (B35//3009B)-F3-16 under well-watered conditions. Whilst under water stressed conditions, the highest value was recorded for parent (B35//3009B)-F3-90. This

parent can be a suitable donor for stay green trait after complementary experiments due to the preseence of four stay green QTL.

For the TNGL per plant, the highest value was recorded for parent (B35//134B)-F3-125 under well-watered conditions Whilst under water stress conditions, the highest positive value was for (B35//82B)-F3-114.

The parent (B35//82B)-F3-64 had positive GCA value for all three characters studied under drought stress and well-watered conditions. Except for the value of TNGL at maturity, the parent (B35//134B)-F3-44 had positive value for all three characters.

Under well-watered and water stress conditions, the male parent BCNAM-76-2 had positive GCA values for SCMR and TNGL at maturity.

CHAPTER SEVEN

7.0. STABILITY ANALYSIS FOR GRAIN YIELD PERFORMANCE OF PARENTS AND HYBRIDS SORGHUM IN STRESSED AND NON-STRESSED ENVIRONMENTS

7.1. Introduction

Sorghum bicolor (L) Moench is the second most important cereal food, after maize, for millions of people living in the semi-arid and sub-tropical regions of Africa (Taylor and Dewar, 2003). Although globally sorghum is ranked fifth in importance (after wheat, maize, rice, and barley), its critical role as a source of energy and dietary protein to food insecure people of sub-Saharan Africa cannot be overemphasized (Taylor and Dewar, 2003). Most of the world's grain sorghum production occurs in arid or semi-arid climates without supplementary irrigation. Drought of variable duration and intensity often associated with above average temperatures in these regions is the major cause of low grain yield.

Mali is one of the largest sorghum producing countries in sub-Saharan Africa. Its staple crops are millet, sorghum, maize, rice and fonio but production of these cereals is failing to keep up with the needs of the growing population. Furthermore, rainfall is erratic and drought is a recurring problem. Sorghum and pearl millet are the most important cereal crops in terms of area planted, production, and per capita consumption.

The cultivation areas of sorghum in Mali have extended widely from all the agricultural areas of the humid regions to the arid ones. Different types of production of sorghum exist in Mali by season: the rain-fed and the "decrue" season. (Jordi, and Helena, 2005). The considerable variation in crop conditions, because of climatic situations and different soil constituents, cause large annual variations in yield performance of crops. This is mainly because of low heritability of yield. Thus, grain yield could be affected by not

only genotype, but also by environment and genotype×environment interactions (Mortazavian, *et al.*, 2014).

Generally, different genotypes behave differently because of differences in gene responses in different environments (Brandiej and Meverty, 1994). G×E interaction decreases the correlation between genotype and phenotype, which in turn reduces the progress of genotype selection, especially under drought stress conditions. Stability analysis is the most important method used to discover the nature of G × E interaction by which stable and consistent genotypes can be identified and selected (Perkins and Jinks, 1971 and Cornelius and Crossa, 1999).

Genetics, genetics by environment (GGE) biplot analysis allows visual examination of the relationships among the test environments, genotypes, and the genotype × environment (G×E) interactions (Ding, *et al.*, 2008). One of the advantages of yield stability analysis using GGE biplot method is that in the output of this software, the environments or the studied experimental conditions, which are considered as an environment, are also being tested, and the environments with no significant difference form a bigger group as mega environment (Yarnia *et al*., 2011).

The main purposes of this study was to analyze G × E interaction using the GGE biplot method to evaluate sorghum hybrids, environments and the relationships between hybrids and environments, as well as to identify ideal hybrids and parents that are suitable for different water regimes (environments).

The specifics objectives were to:

- conduct yield stability analysis for female and male parents using GGE biplot method
- conduct yield stability analysis for hybrids using GGE biplot method

7.2. Materials and methods

7.2.1. Materials

Information on the experimental site used, the irrigation system, and details on fertilizer application have been described in the earlier section 4.2.

7.2.1.1. Plant material

Sixty F1 sorghum hybrids plus six checks were used in the present study (Appendix 7).

7.2.2. Method

A split plot design with two levels of moisture regimes, well-watered and drought stressed was used. Each water regime was regarded as an environment.

The genetype plus genetype by environment (GGE) biplots (Yan *et al.*, 2000 and Yan and Tinker, 2006) was employed to analyze the Multi Environment Trial (MET) data and to examine the "which-won-where" pattern of MET data (Ding, *et al.*, 2008). The ideal genotype was identified based on both mean performance and stability across environments (Aina *et al.*, 2009). In this study, the GGE biplot analyses were performed using GENSTAT 12th edition.

The GGE biplot model used is:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varphi_{ij}$$

- Y_{ij} is the measured mean of i^{th} genotype in j^{th} environment
- μ is the grand mean
- τ_i is the main effect of i^{th} genotype
- β_j is the main effect of j^{th} environment
- φ_{ij} is interaction between i^{th} genotype and j^{th} environment

7.2.3. Results

7.2.3.1. Grain yield performance of hybrids and checks under well-watered and water stress conditions.

The Grain yield (kg/ha^{-1}) performance of the hybrids and checks under well-watered and water stress conditions is presented in Table 7.1. The best hybrids were indentified on the basis of mean yield of best check plus LSD in each environment.

Under the well-watered environment, the best hybrids gain yield were (B35//134B)-F3-44/BCNAM-27-2 with mean yield 4227 kg/ha followed by (B35//134B)-F3-44/BCNAM-76-2 with mean yield of 4164 kg/ha, (B35//134B)-F3-125/BCNAM-44-1 mean yield of 3803 kg/ha, (B35//82B)F3-64/BCNAM-45-1 yield 3616 kg/ha, (B35//134B)F3-89/BCNAM-45-1 yield 3591 kg/ha and (B35//82B)F3-104/BCNAM-76-2 mean yield of 3477 kg/ha.

Under water stress conditions, the best hybrids were B35//134B)-F3-44/BCNAM-44-1 (1795 kg/ha), (B35//82B)-F3-64/BCNAM-44-1 (1786 kg/ha) and (B35//3009B)-F3-16/BCNAM-84-1 (1758 kg/ha).

The most outstanding hybrid in term of grain yield were (B35//134B)-F3-44/BCNAM-76-2 with mean yields 4164 and 1698 kg/ha, (B35//82B)-F3-64/BCNAM-45-1 mean yieds of 3616 and 1415 kg/ha, (B35//82B)-F3-104/BCNAM-76-2 with yields of 3477 and 1442 kg/ha, (B35//134B)F3-44/BCNAM-44-1 with yields of 3018 and 1795 kg/ha (Table 7.1).

Table 7. 1. Grain yield performance of hybrids and parents under well-watered and water stress conditions.

Code	Genotypes	Grain Yield (kg/ha)	
		Well-water	Water stress
G1	(B35//134B)F3-125/BCNAM-27-2	1627	1309
G2	(B35//134B)F3-44/BCNAM-27-2	4227	753
G3	(B35//134B)F3-89/BCNAM-27-2	1287	1135
G4	(B35//3009B)F3-16/BCNAM-27-2	3251	633
G5	(B35//3009B)F3-24/BCNAM-27-2	2650	478
G6	(B35//3009B)F3-51/BCNAM-27-2	2213	998
G7	(B35//3009B)F3-90/BCNAM-27-2	1470	358
G8	(B35//82B)F3-114/BCNAM-27-2	3182	418
G9	(B35//82B)F3-136/BCNAM-27-2	1213	188
G10	(B35//82B)F3-64/BCNAM-27-2	2386	625
G11	(B35//134B)F3-125/BCNAM-44-1	3803	428
G12	(B35//134B)F3-44/BCNAM-44-1	3018	1795
G13	(B35//134B)F3-64/BCNAM-44-1	2848	609
G14	(B35//134B)F3-89/BCNAM-44-1	2007	1577
G15	(B35//3009B)F3-16/BCNAM-44-1	1967	806
G16	(B35//3009B)F3-24/BCNAM-44-1	1518	1081
G17	(B35//82B)F3-104/BCNAM-44-1	2269	505
G18	(B35//82B)F3-114/BCNAM-44-1	1407	278
G19	(B35//82B)F3-136/BCNAM-44-1	1458	736
G20	(B35//82B)F3-64/BCNAM-44-1	2820	1786
G21	(B35//134B)F3-44/BCNAM-45-1	1413	748
G22	(B35//134B)F3-64/BCNAM-45-1	1728	278
G23	(B35//134B)F3-89/BCNAM-45-1	3591	1157
G24	(B35//3009B)F3-16/BCNAM-45-1	2187	1013
G25	(B35//3009B)F3-24/BCNAM-45-1	2169	201
G26	(B35//3009B)F3-51/BCNAM-45-1	2164	1304
G27	(B35//82B)F3-114/BCNAM-45-1	2962	1648
G28	(B35//82B)F3-136/BCNAM-45-1	1573	260
G29	(B35//82B)F3-64/BCNAM-45-1	3616	1415
G30	(B35//134B)F3-125/BCNAM-76-2	1660	340
G31	(B35//134B)F3-44/BCNAM-76-2	4164	1698
G32	(B35//134B)F3-64/BCNAM-76-2	2046	1444
G33	(B35//134B)F3-89/BCNAM-76-2	1362	625
G34	(B35//3009B)F3-16/BCNAM-76-2	3016	308
G35	(B35//3009B)F3-24/BCNAM-76-2	2803	1296
G36	(B35//3009B)F3-51/BCNAM-76-2	2628	955

Table 7. 1. Cont'd

Code	Genotypes	Grain Yield (kg/ha)	
		Well-water	Water stress
G37	(B35//3009B)F3-90/BCNAM-76-2	2335	1158
G38	(B35//82B)F3-104/BCNAM-76-2	3477	1442
G39	(B35//82B)F3-114/BCNAM-76-2	1100	820
G40	(B35//82B)F3-136/BCNAM-76-2	1760	1173
G41	(B35//82B)F3-64/BCNAM-76-2	2756	1590
G42	(B35//134B)F3-125/BCNAM-84-1	2387	1353
G43	(B35//134B)F3-44/BCNAM-84-1	2111	648
G44	(B35//134B)F3-64/BCNAM-84-1	2007	1093
G45	(B35//134B)F3-89/BCNAM-84-1	1757	1162
G46	(B35//3009B)F3-16/BCNAM-84-1	2405	1758
G47	(B35//3009B)F3-24/BCNAM-84-1	1280	655
G48	(B35//3009B)F3-90/BCNAM-84-1	2707	1273
G49	(B35//82B)F3-104/BCNAM-84-1	2444	1686
G50	(B35//82B)F3-114/BCNAM-84-1	2427	1076
G51	(B35//82B)F3-136/BCNAM-84-1	1355	198
G52	(B35//82B)F3-64/BCNAM-84-1	2843	255
G53	02-SB-F4DT-275 (Grinkan)	1052	528
G54	B-35 (BTx642)	1380	786
G55	98-BE-F5P-82B (82B)	1335	201
G56	09PR-3009B (3009B)	2115	436
Least significant difference (Lsd)		1165	958
Standard error (SE)		581	384

7.2.3.2. Stability analysis for grain yield performance of hybrids sorghum using GGE biplot method.

The yield stability of the cultivars was examined using the GGE biplot method, the result is indicated in the Figure 7.1. The GGE biplot analysis reveals that PC1 and PC2 together accounted for 100% of the total variance for grain yield across test environments, with PC1 explaining 77.23 % of the total variance while PC2 explaining the rest.

In this analysis, drought levels (well-watered and stressed) were considered as environments. It is obvious that both environments, well-watered (+E1) and water

stressed (+E2), are located in different quadrants, indicating that they are significant different and could be considered as two different environments (Figure 7.1).

The hybrids (B35//134B)-F3-44/BCNAM-27-2 (code G2), (B35//134B)-F3-125/BCNAM-44-1 (G11), (B35//134B)-F3-89/BCNAM-45-1 (G23), with mean grain yields 4227 Kg/ha, 3803 Kg/ha, and 3591 Kg/ha respectively (Table 7.1), are scattered in the well-watered environment (+E1). These genotypes produced higher yields in these environments and have better adaptation than other cultivars for well-watered (+E1) environments. In contrast, the genotypes (B35//134B)-F3-44/BCNAM-44-1 (code G12), (B35//82B)-F3-64/BCNAM-44-1 (G20), (B35//3009B)-F3-16/BCNAM-84-1 (G46), with mean grain yields 1795 Kg/ha, 1786 Kg/ha, and 1758 Kg/ha, respectively (Table 7.1), are located in the drought stress environment (+E2) and exhibited high yield in this environment, indicating that they have better adaptation than other cultivars in this environment.

Genotypes (B35//134B)-F3-44/BCNAM-76-2 (G31) yield 4164 and 1668 Kg/ha, (B35//82B)-F3-64/BCNAM-45-1 (G29) yield 3616 and 1415 kg/ha, and (B35//82B)F3-104/BCNAM-76-2 (G38) yield 3477 and 1442 Kg/ha (Table 7.1), are located between yield under well-watered and stress conditions, indicating that they are correlated with both environment. These genotypes had a much higher yield than other genotypes in both environments and were indentified as the most tolerant genotypes with high yield stability across both environments.

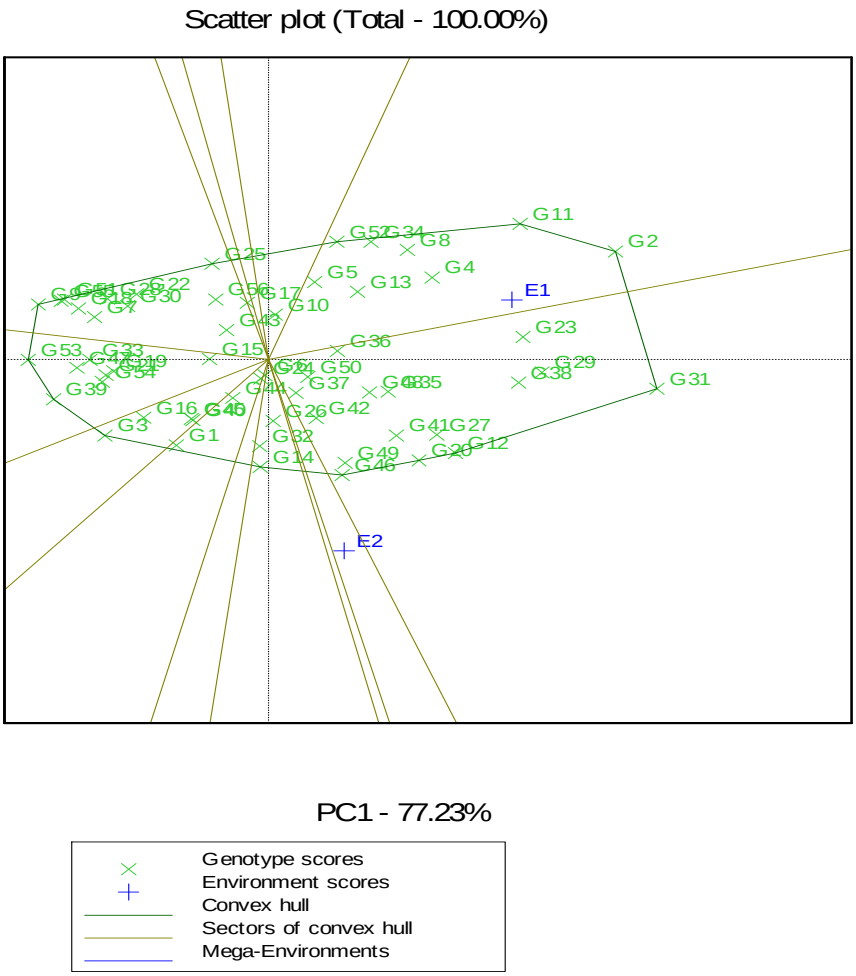


Figure 7. 1. The GGE biplot showing which hybrids yielded best in each environment for grain yield (See codes of hybrids in Table 7.1 chapter 7)

7.2.3.3. General combining effects of parental lines for yield trait

The GCA effects of female and male parents are presented in Table 7.2. Under well-watered conditions six female and three male parents had positive GCA. Whereas under water stress conditions five female and three male parents give positive estimates of GCA (Table 7.2).

Table 7. 2. General combining ability effect of parental lines for yield

Genotypes	GCA of parental lines for Yield kg/ha^{-1}	
	Well-Water	**Water Stress**
Female parents		
(B35//134B)F3-125	45	-87
(B35//134B)F3-44	662	184
(B35//134B)F3-64	-167	-89
(B35//134B)F3-89	-324	186
(B35//3009B)F3-16	241	-41
(B35//3009B)F3-24	-240	-203
(B35//3009B)F3-51	11	141
(B35//3009B)F3-90	-154	-15
(B35//82B)F3-104	406	266
(B35//82B)F3-114	-109	-97
(B35//82B)F3-136	-853	-434
(B35//82B)F3-64	560	189
Male parents		
BCNAM-27-2	26	-255
BCNAM-44-1	-13	15
BCNAM-45-1	54	-53
BCNAM-76-2	101	126
BCNAM-84-1	-168	69

7.2.3.4. The yield stability analysis of Female (GCA_f) and) Male (GCA_m) parents using GGE biplot method

The yield stability analysis helps to identify which parent combined best in each environment and was the most stable in term of grain yield.

a) ***The GGE biplot of the GCA of Females*** **(GCA_f)**

The polygon view of the twelve female parents under two irrigation regimes is presented in Figure. 7.2. Both drought regimes, well-watered (+E1) and water stressed (+E2) were located in the different quadrants, suggesting that they were significantly different and could considered as two different environments. The GGE biplot analysis of GCA of Females revealed that PC1 and PC2 together account for 100% of the total variance for

GCA across test environments. The PC1 explained 93.04% of the total variance while PC2 explained 6.96% (Figure 7.2).

Figure 7.2 showed the parents (B35//134B)-F3-44 (+662 and +184), (B35//82B)-F3-104 (+406 and +266) and (B35//82B)-F3-64 (+560 and 189) were located between +E1 and +E2, indicating that they are correlated with both environments. These parents also produced hybrids with high yield in both environments and were identified as the most stable and drought tolerant female parents.

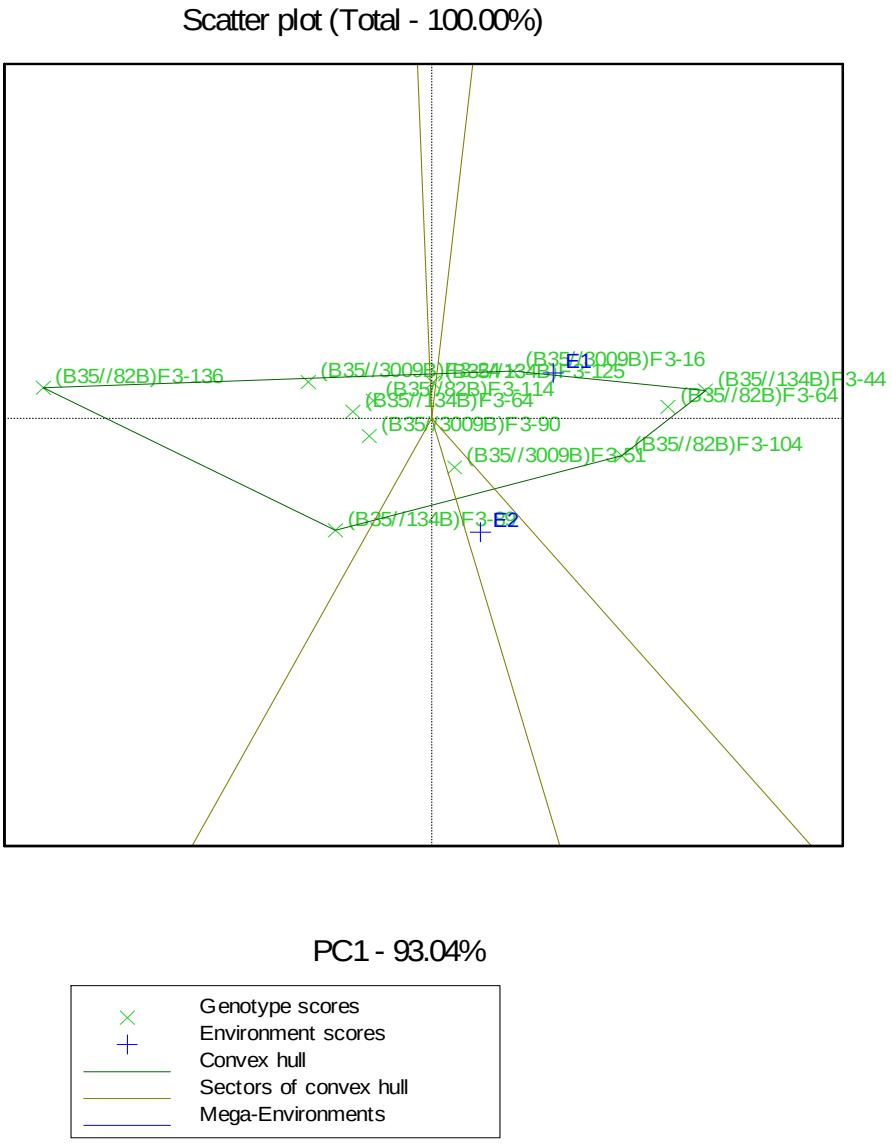


Figure 7. 2. GGE biplot of GCA of Female parents showing which parent combined best in each environment for grain yield.

*b) **The GGE biplot** (**GCA$_m$**) **of males***

The GGE biplot analysis of GCA of males revealed that PC1 and PC2 together accounted for 100% of the total variance for GCA across tested environments. The PC1 explained 68.52% of the total variance while PC2 explained 31.48% (Figure 7.3).

Figure 7.3 showed that well-watered (+E1) and water stressed (+E2) environments were located in the same part part of scatter plot, implying that these environments had a strong positive correlation, and therefore was no significant difference among them so that they could be considered as one environment. Only one parent BCNAM-76-2 had high GCA estimate (Table 7.2) and produced high yielding and stable hybrids in this environment (Figure 7.3).

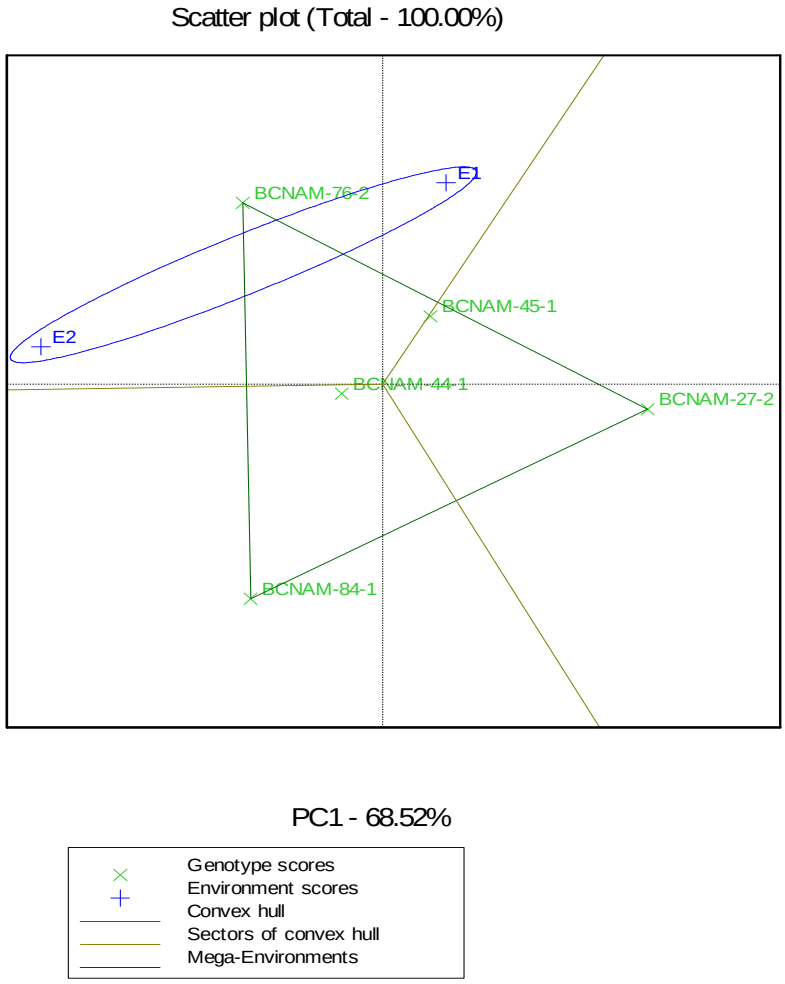


Figure 7. 3. GGE biplot of GCA of Male parents showing which parent combined best in each environment for grain yield.

7.3. Discussion

GGE biplot was used to study the yield stability of hybrids and parents. An important objective of this study was to examine the adaptation and stability of hybrids and their parents in different environments. The results showed that the hybrids (B35//134B)-F3-44/BCNAM-27-2, (B35//134B)-F3-125/BCNAM-44-1, (B35//134B)-F3-89/BCNAM-45-1 were more stable and had superior performance only in the well-watered (+E1) environments. (B35//134B)-F3-44/BCNAM-44-1, (B35//82B)-F3-64/BCNAM-44-1, (B35//3009B)-F3-16/BCNAM-84-1 had high yield and better adaptation than other cultivars for water stress environment (+E2). The genotypes (B35//134B)-F3-44/BCNAM-76-2 (G31), (B35//82B)-F3-64/BCNAM-45-1, and (B35//82B)-F3-104/BCNAM-76-2, were located between yield under well-watered and stress conditions (+E1 and +E2) and had a much higher yield than other genotypes in both environments. These hybrids were identified as the most drought tolerant genotypes with high yield stability under both conditions.

GGE biplot method has been used to study GE interaction and yield stability of different crops in semiarid areas of Mali. Diallo (2013) identified parents and hybrids of sorghum with superior performance in different environments, high and low level of phosphorous (P). Several studies have been reported using GGE biplots for genotypic stability analysis through the world. Shiri (2013), identified ideal maize hybrid genotypes among seven maize hybrids evaluated under four irrigation regimes (well-watered, water deficit at the vegetative growth stage, water deficit during flowering and water deficit during grain-filling) in northwestern Iran.

The GGE biplot method showed that the performance of the female parents (B35//134B)-F3-44, (B35//82B)-F3-104 and (B35//82B)-F3-64 were correlated in both environments

and that they produced stable and high yielding hybrids both environments. They were indentified as the most stable and drought tolerant female parents in both environments. Only one male parent BCNAM-76-2 had positive GCA and produced the most outstanding hybrids with good stability and adaptation to the contrasting environments. Shiri (2013) reported, similar result in a study of yield stability of maize (*Zea mays* L.) hybrids under different drought stress conditions, that the environments E1 (well-watered) and E4 (water deficit during grain-filling) had a strong positive correlation with each other and the results of environment E1 (well-watered) could be could be pertinent to environment E4 (no irrigation during grain-filling). Yan and Kang, (2003), also reported that in the event of a strong positive correlation between two or more environments, experiments could be conducted in one environment and the results obtained extrapolated to the others.

7.4. Conclusions

The present study was conducted to identify grain yield performance and yield stability of selected sorghum hybrids and parents under two water regimes.

Through stability analysis for grain yield performance of hybrids using the GGE biplot method, three hybrids (B35//134B)-F3-44/BCNAM-76-2, (B35//82B)-F3-64/BCNAM-45-1, and (B35//82B)-F3-104/BCNAM-76-2 were identified as high yielding and stable in well-watered and water stress envirionments. The study also identified two superior female parents (B35//134B)-F3-44 and (B35//82B)-F3-64 and one male parent BCNAM-76-2 that are outstanding parents for both water regimes. These genotypes can be considered as superior and adapted to the test environments and may be considered for introduction as tolerant to post-flowering drought after further testing on experiment stations and in farmers' fields.

CHAPTER EIGHT

8.0. GENETIC ANALYSIS OF VARIOUS TRAITS CORRELATED WITH DROUGHT TOLERANCE

8.1. Introduction

Sorghum is one of the most important dry land crops of semi-arid tropics Drought is one of the most important abiotic stresses and causes considerable yield loss in sorghum each year in different regions of the world. Hence, developing drought tolerant varieties is a major breeding objective. Drought stress during vegetative phase, flowering and grain filling periods cause considerable decrease in yield of sorghum (Rosenow, *et al.*, 1997). Several drought stress indices or selection criteria, such as stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), stress susceptibility index (SSI), stress tolerance index (STI) have been proposed as ways to identify genotypes with good stress tolerance.

One of the main objectives of this study was to compare the usefulness of several drought stress indices for the identification of genotypes with better performance under different levels of water stress.

The specific objectives were to:

- Study drought effect on various traits
- Identify hybrids with appropriate performance in semi-arid regions of Mali.

8.2. Materials and methods

8.2.1. Materials

Information on the experimental site used, the irrigation system, and details on fertilizer application have been described in the earlier section 4.2. The same plant material of section 7.2.1.4 were used.

8.2.2 Method

8.2.2.1. Data collection (Idem section 6.2.2.3 chapter six)

- **Observations on traits used in drought tolerance indices**

Six selection indices including stress susceptibility index, SSI (Fischer and Maurer, 1978), stress tolerance index, STI (Fernandez, 1992), tolerance index, TOL, (Hossain *et al.*, 1990), mean productivity, MP (Hossain *et al.*, 1990), geometric mean productivity, GMP (Fernandez, 1992) and percentage reduction (Re %) were calculated based on grain yield under drought-stress and well-watered conditions. Stress tolerance indices were calculated by the formula:

$SSI = [1- (Ys) /(Yp)] / SI$.

SI is the stress intensity and was calculated as:

$SI = [1- (\bar{Y}s) /(\bar{Y}p)]$,

$STI = [(Yp) \times (Ys)/(Yp)^2]$,

$GMP = \sqrt{(YP \times YS)}$

$TOL = (Yp - Ys)$ and

$MP = (Yp + Ys) / 2$

Reduction (%) = (Yp – Ys)/Yp (Choukan *et al.*, 2006)

Where Ys and Yp are the yields of genotypes evaluated under stress and non-stress conditions and $\bar{Y}s$ and $\bar{Y}p$ are the mean yields over all genotypes evaluated under stress and non-stress conditions.

8.2.2.2. Statistical analysis

Multivariate statistical analyses such as the principal component analysis and cluster analysis were performed using GENSTAT 12th edition and "R" software version 3.1.2.

8.3. Experimental results

8.3.1. Mean squares from the Analysis of variance (ANOVA) across well-watered and water stressed conditions for various traits

Thirteen traits were measured and analyzed across water managements (stress and well-watered) using GENSTAT 12th edition (Table 8.1). The analysis of variance of genotypes evaluated across water management levels showed interaction (at $P = 0.05$ and $P = 0.01$) between genotype by water management of mean squares for traits such as Grain yield (kg/ha), SPAD chlorophyll meter readings at maturity, and total number of green leaves (Table 8.1). No interaction (ns) between genotype (G) x water management was detected for Days to 50 per cent flowering, SPAD chlorophyll meter readings at 50 % Flowering, Biomass, total number of leaves, plant height**,** thousand grains weight, panicle appreciation, grain appreciation and vitreousness.

No significant differences were seen for genotypes across water managements for other traits (Table 8.1). This indicates that, for some traits, there was no effect of drought on the genotypes and they behave the same in the two environments. The mean squares for genotypes (Entries) were significant (at $P = 0.05$ and $P = 0.01$) for all traits except grain vitreousness (Table 8.1). This shows that, for each environment (water management) separately, a statically significant difference occurs between genotypes.

Table 8. 1. ANOVA of Mean squares from across well-water and water stress management for various traits of genotypes

Source of variations	d.f.	Yield (kg/ha)	Biom (t/ha)	50FLO (day)	SCMR 50FLO	SCMR MAT	Height (cm)	TNL	TNGL	TGW (g)	G.A.	VITR	P.A.	LT	S.V.
Rep	1	1015	5.9	94.0	28.8	3.1	2505.9	5.9	1.3	2.0	0.7	8.0	1.0	0.1	0.1
Whole plot	1	83018538**	763**	301**	85*	11796**	72961**	4^{ns}	703**	1344**	47**	3^{ns}	2.5**	0.1	0.1^{ns}
Rep x Whole plot	1	347445^{ns}	23.3*	243.4*	180.8*	0.01^{ns}	1091.2^{ns}	6.7*	0.7^{ns}	5.4^{ns}	0.01^{ns}	2.1^{ns}	2.4*	0.1	5.5**
Entry	55	1116223**	591**	79.0**	53.8**	74.6**	2933.5**	4.2**	5.0**	14.1**	1.1**	4.9^{ns}	0.9**	0.0*	1.0**
Entry x Whole plot	**55**	**517187****	**3.7^{ns}**	**16.0^{ns}**	**18.0^{ns}**	**48.4***	**811.8^{ns}**	**1.0^{ns}**	**3.9***	**5.6^{ns}**	**0.3^{ns}**	**2.4^{ns}**	**0.4^{ns}**	**0.1^{ns}**	**0.2^{ns}**
Residual	82	271050	4.4	22.7	17.4	29.2	615.8	1.4	2.4	5.7	0.5	5.5	0.3	0.0	0.2
Total	195	1002208	10.0	39.6	29.1	107.5	1707.9	2.1	7.2	14.9	0.9	4.4	0.6	0.0	0.4
Mean		**1700**	**9.6**	**76.8**	**43.6**	**38.2**	**165.6**	**11.1**	**4.4**	**17.6**	**3.7**	**6.0**	**3.4**	**1.0**	**4.3**
LSD		1154	4.7	10.6	9.2	12.0	55.0	2.7	3.5	5.3	1.6	5.2	1.3	0.3	0.9
SE		520.6	2.1	4.8	4.2	5.4	24.8	1.2	1.6	2.4	0.7	2.3	0.6	0.1	0.4
%CV		30.6	22.1	6.2	9.6	14.2	15.0	11.0	35.8	13.7	20.1	39.2	17.5	13.0	9.8

df = Degree of freedom, **Yield (kg/ha)** = Grain yield, **50 FLO (day)** = Days to 50 per cent flowering, **SPAD 50 FLO** = SPAD chlorophyll meter readings at 50 % Flowering, **SPAD MAT** = SPAD chlorophyll meter readings at maturity, **BIOM. (T/ha)** = Biomass, **TNGL** = Total number of green leaves, **Height (cm)** = Plant height**, P.A.** = Panicle appreciation, **G.A.** = Grain appreciation, **VITR** = Vitreousness, **TGW (g)** = Thousand grains weight, LT = lodging, **S.V.** = Seedling vigor,
******. Significant at the 0.01 probability level; *****. Significant at the 0.05 probability level; **ns:** not significant
Entry = Genotypes**; Whole_plot.** = Water Management; **Rep** = Repetitions.

8.3.2. Grain yield and drought tolerance indices

Different drought tolerance indices were calculated on the basis of grain yield of the genotypes under well-watered (Yp) and stressed (Ys) conditions.

Based on SSI index (table 8.2) the hybridss (B35//82B)-F3-64/BCNAM-84-1 (yields 2843 and 255 Kg/ha), (B35//3009B)-F3-24/BCNAM-45-1 (2169 and 201 Kg/ha), (B35//3009B)-F3-16/BCNAM-76-2 (3016 and 308 Kg/ha) and (B35//134B)-F3-125/BCNAM-44-1 (3803 and 428 Kg/ha) with high SSI values (1.5) were found to be the most sensitive genotypes to stress whereas genotypes (B35//134B)-F3-89/BCNAM-44-1 (2007 and 1577 Kg/ha), (B35//3009B)-F3-16/BCNAM-84-1 (2405 and 1758 Kg/ha) and (B35//82B)-F3-104/BCNAM-84-1 (2444 and 1686 Kg/ha), with low value (0.4 to 0.5) were found to be tolerant to drought stress (Table 8.2). In this study, the results of SSI indices in selection of genotypes were similar to Re (%) index.

For the TOL index, the highest values were recorded for hybryds (B35//134B)-F3-44/BCNAM-27-2 (4227 and 753 Kg/ha), (B35//134B)-F3-125/BCNAM-44-1 (3803 and 428 Kg/ha) and (B35//82B)-F3-114BCNAM-27-2 (3182 and 418 Kg/ha) which were considered as susceptible, to drought stress, whereas the (B35//134B)-F3-89/BCNAM-27-2 (1287 and 1135 Kg/ha), (B35//134B)-F3-125/BCNAM-27-2 (1627 and 1309 Kg/ha), (B35//134B)-F3-89/BCNAM-44-1 (2007 and 1577 Kg/ha) and (B35//3009B)-F3-24/BCNAM-44-1 (1518 and 1081 Kg/ha) with low values were considered as tolerant genotypes but mostly with low value also of grain yields in both environments (Table 8.2).

The highest MP and GMP were recorded for genotypes (B35//134B)-F3-44/BCNAM-76-2 (4164 and 1698 Kg/ha) and (B35//82B)-F3-64/BCNAM-45-1 (3616 and 1415 Kg/ha), they were considered as tolerant genotypes with high yield stability under both conditions (Table 8.2).

Yield Based on STI index , the genotypes (B35//134B)-F3-44/BCNAM-76-2 (4164 and 1698 Kg/ha), (B35//134B)-F3-44/BCNAM-44-1 (with mean grain yields of 3018 and 1795 Kg/ha), (B35//82B)-F3-64/BCNAM-45-1 (3616 and 1415 Kg/ha), (B35//82B)-F3-64/BCNAM-44-1 (2820 and 1786 Kg/ha), (B35//82B)-F3-104/BCNAM-76-2 (3477 and 1442 Kg/ha) and (B35//82B)-F3-114/BCNAM-45-1 (3616 and 1415 Kg/ha) were considered as the most tolerant genotypes with high yield under both conditions, with highest STI value (1.0 – 1.4). Based on this result the STI index appears to be the best drought tolerance index.

Table 8. 2. Drought stress indices and yield under normal and drought stress conditions

Genotypes	Yp	Ys	TOL	MP	GMP	SSI	STI	Red
(B35//82B)F3-64/BCNAM-76-2	2756	1590	1166	2173	2093	0.7	0.9	42.3
(B35//82B)F3-64/BCNAM-44-1	2820	1786	1034	2303	2244	0.6	1.0	36.7
(B35//82B)F3-64/BCNAM-45-1	3616	1415	2201	2516	2262	1.0	1.0	60.9
(B35//82B)F3-64/BCNAM-27-2	2386	625	1762	1505	1221	1.2	0.3	73.8
(B35//82B)F3-64/BCNAM-84-1	2843	255	2588	1549	851	1.5	0.1	91.0
(B35//82B)F3-104/BCNAM-76-2	3477	1442	2035	2460	2239	1.0	1.0	58.5
(B35//82B)F3-104/BCNAM-44-1	2269	505	1765	1387	1070	1.3	0.2	77.8
(B35//82B)F3-104/BCNAM-84-1	2444	1686	758	2065	2030	0.5	0.8	31.0
(B35//82B)F3-114/BCNAM-76-2	1100	820	281	960	949	0.4	0.2	25.5
(B35//82B)F3-114/BCNAM-44-1	1407	278	1129	842	625	1.3	0.1	80.3
(B35//82B)F3-114/BCNAM-45-1	2962	1648	1314	2305	2209	0.7	1.0	44.4
(B35//82B)F3-114/BCNAM-27-2	3182	418	2764	1800	1153	1.4	0.3	86.9
(B35//82B)F3-114/BCNAM-84-1	2427	1076	1351	1751	1616	0.9	0.5	55.7
(B35//82B)F3-136/BCNAM-76-2	1760	1173	587	1466	1437	0.6	0.4	33.4
(B35//82B)F3-136/BCNAM-44-1	1458	736	722	1097	1036	0.8	0.2	49.5
(B35//82B)F3-136/BCNAM-45-1	1573	260	1314	916	639	1.4	0.1	83.5
(B35//82B)F3-136/BCNAM-27-2	1213	188	1025	700	477	1.4	0.0	84.5
(B35//82B)F3-136/BCNAM-84-1	1355	198	1157	776	518	1.4	0.1	85.4
(B35//134B)F3-44/BCNAM-76-2	4164	1698	2466	2931	2659	1.0	1.4	59.2
(B35//134B)F3-44/BCNAM-44-1	3018	1795	1224	2406	2327	0.7	1.1	40.5
(B35//134B)F3-44/BCNAM-45-1	1413	748	666	1080	1028	0.8	0.2	47.1
(B35//134B)F3-44/BCNAM-27-2	4227	753	3474	2490	1784	1.4	0.6	82.2
(B35//134B)F3-44/BCNAM-84-1	2111	648	1463	1379	1169	1.2	0.3	69.3
(B35//134B)F3-64/BCNAM-76-2	2046	1444	603	1745	1719	0.5	0.6	29.4
(B35//134B)F3-64/BCNAM-44-1	2848	609	2239	1729	1317	1.3	0.3	78.6

Table 8.2. Cont'd

Genotypes	Yp	Ys	TOL	MP	GMP	SSI	STI	Red
(B35//134B)F3-64/BCNAM-45-1	1728	278	1450	1003	693	1.4	0.1	83.9
(B35//134B)F3-64/BCNAM-84-1	2007	1093	914	1550	1481	0.8	0.4	45.5
(B35//134B)F3-89/BCNAM-76-2	1362	625	738	993	922	0.9	0.2	54.1
(B35//134B)F3-89/BCNAM-44-1	2007	1577	431	1792	1779	0.4	0.6	21.4
(B35//134B)F3-89/BCNAM-45-1	3591	1157	2434	2374	2039	1.1	0.8	67.8
(B35//134B)F3-89/BCNAM-27-2	1287	1135	152	1211	1208	0.2	0.3	11.8
(B35//134B)F3-89/BCNAM-84-1	1757	1162	595	1459	1429	0.6	0.4	33.9
(B35//134B)F3-125/BCNAM-76-2	1660	340	1321	1000	751	1.3	0.1	79.5
(B35//134B)F3-125/BCNAM-44-1	3803	428	3375	2115	1276	1.5	0.3	88.7
(B35//134B)F3-125/BCNAM-27-2	1627	1309	318	1468	1459	0.3	0.4	19.5
(B35//134B)F3-125/BCNAM-84-1	2387	1353	1034	1870	1797	0.7	0.6	43.3
(B35//3009B)F3-16/BCNAM-76-2	3016	308	2708	1662	964	1.5	0.2	89.8
(B35//3009B)F3-16/BCNAM-44-1	1967	806	1161	1387	1259	1.0	0.3	59.0
(B35//3009B)F3-16/BCNAM-45-1	2187	1013	1174	1600	1488	0.9	0.4	53.7
(B35//3009B)F3-16/BCNAM-27-2	3251	633	2618	1942	1434	1.3	0.4	80.5
(B35//3009B)F3-16/BCNAM-84-1	2405	1758	647	2081	2056	0.4	0.8	26.9
(B35//3009B)F3-24/BCNAM-76-2	2803	1296	1507	2050	1906	0.9	0.7	53.8
(B35//3009B)F3-24/BCNAM-44-1	1518	1081	437	1300	1281	0.5	0.3	28.8
(B35//3009B)F3-24/BCNAM-45-1	2169	201	1968	1185	661	1.5	0.1	90.7
(B35//3009B)F3-24/BCNAM-27-2	2650	478	2172	1564	1125	1.4	0.2	82.0
(B35//3009B)F3-24/BCNAM-84-1	1280	655	625	967	915	0.8	0.2	48.9
(B35//3009B)F3-51/BCNAM-76-2	2628	955	1673	1791	1584	1.1	0.5	63.7
(B35//3009B)F3-51/BCNAM-45-1	2164	1304	861	1734	1680	0.7	0.6	39.8
(B35//3009B)F3-51/BCNAM-27-2	2213	998	1215	1605	1486	0.9	0.4	54.9
(B35//3009B)F3-90/BCNAM-76-2	2335	1158	1177	1746	1644	0.8	0.5	50.4
(B35//3009B)F3-90/BCNAM-27-2	1470	358	1112	914	725	1.3	0.1	75.7
(B35//3009B)F3-90/BCNAM-84-1	2707	1273	1434	1990	1856	0.9	0.7	53.0
02-SB-F4DT-275 (Grinkan)	1052	528	524	790	745	0.8	0.1	49.8
B-35 (BTx642)	1380	786	594	1083	1042	0.7	0.2	43.0
98-BE-F5P-82B (82B)	1335	201	1134	768	518	1.4	0.1	84.9
09PR-3009B (3009B)	2115	436	1679	1276	961	1.3	0.2	79.4
Overall mean	**2263**	**901**	**1362**	**1582**	**1428**	**1.0**	**0.4**	**58.3**

Yp = yield under irrigated conditions, **Ys** = yield under drought-stressed, **TOL** = tolerance index, **MP** = Mean productivity, **GMP** = Geometric mean productivity, **SSI** = stress susceptibility index, **STI** = stress tolerance index, **Red** = percentage reduction.

8.3.2.1. Correlation between grain yield and drought tolerance indices

Correlation coefficients were used to identify the best criterion for selecting drought tolerant genotypes. As shown in Table 8.3, indices including GMP, MP and STI were

highly correlated with each other as well as with Yp and Ys. A positive correlation between TOL and Yp and the negative correlation between TOL and Ys (Table 6.8) suggested that selection based on TOL will lead to reduction of yield under well-watered conditions. SSI showed a negative correlation with Ys while no significant correlation was detected between Yp and SSI. Thus SSI index is suitable for identification of genotypes with low yield and tolerance to drought stress. The results of SSI indices in selection of genotypes were similar to Re (%) index in this study. SSI has been widely used by researchers for selecting drought tolerant genotypes (Shirazi, and Naroui-Rad, 2011). However, TOL and SSI were not strongly correlated with indices GMP, MP and STI (Table 8.3). TOL and SSI ranked differently from the other selection.

Table 8. 3. Coefficients of correlation between grain yields and drought stress indices under stress and well-watered conditions

Indices	Yp	Ys	TOL	MP	GMP	SSI	STI	Red
Yp	1.000							
Ys	0.359**	1.000						
TOL	0.762**	-0.280*	1.000					
MP	0.903**	0.702**	0.440**	1.000				
GMP	0.686**	0.911**	0.113	0.917**	1.000			
SSI	0.222^{ns}	-0.793**	0.768**	-0.178^{ns}	-0.501**	1.000		
STI	0.686**	0.911**	0.113^{ns}	0.917**	1.000**	-0.501**	1.000	
Red	0.222^{ns}	-0.793**	0.768**	-0.178	-0.501**	1.000**	-0.501**	1.000

*, ** = significant at 5 and 1% of probability level, **Yp** = yield under irrigated conditions, **Ys** = yield under drought-stressed, **TOL** = tolerance index, **MP** = Mean productivity, **GMP** = Geometric mean productivity, **SSI** = stress susceptibility index, **STI** = stress tolerance index, **Red** = percentage reduction.

8.3.2.2. Multivariate analysis

a) Principal component analysis

Principal component analysis was used to classify the genotypes using GenStat 12th Edition software. The first principal axis (PC1) explained 58.43% of the variation and

had positive correlation with Yp, Ys, MP, GMP and STI (Table 8.4). Thus, the first dimension can be considered as the yield potential and drought tolerance. Genotypes possessing high values of PC1, could be high yielding under stressed and well-watered environments. The second PCA (PC2) explained 39.48% of the total variability and correlated positively with Yp, TOL, SSI and Red but had a negative correlation with yield under stressed conditions (Table 8.4). Therefore, the stress susceptibility dimension was able to separate the drought-susceptible cultivars. Hence, selection of genotypes that have high PCA1 and low PCA2 would result in genotypes good in both stressed and non-stressed conditions.

In order to obtain the biplot diagram of principal components (Figure 8.1), the "R" software version 3.1.2 was used. In the biplot diagram of principal components analysis, angles between the vectors show a correlation between them. The Figure 8.1 revealed that SSI and Red indices were similar and GMP was strongly correlated with STI.

Table 8. 4. Principal component analysis of potential yield (YP), yield under stress (YS) and drought tolerance indices.

Principal component	**Dim1**	**Dim2**
Eigenvalues	4.68	3.16
Percentage of variance	58,43	39,48
Yp	0.27	0.45
Ys	0.45	-0.14
TOL	-0.01	0.55
MP	0.40	0.27
GMP	0.46	0.08
SSI	-0.28	0.43
STI	0.44	0.10
Red	-0.28	0.43

Yp = yield under well-watered conditions, **Ys** = yield under drought-stress, **TOL** = tolerance index, **MP** = Mean productivity, **GMP** = Geometric mean productivity, **SSI** = stress susceptibility index, **STI** = stress tolerance index, **Red** = percentage reduction.

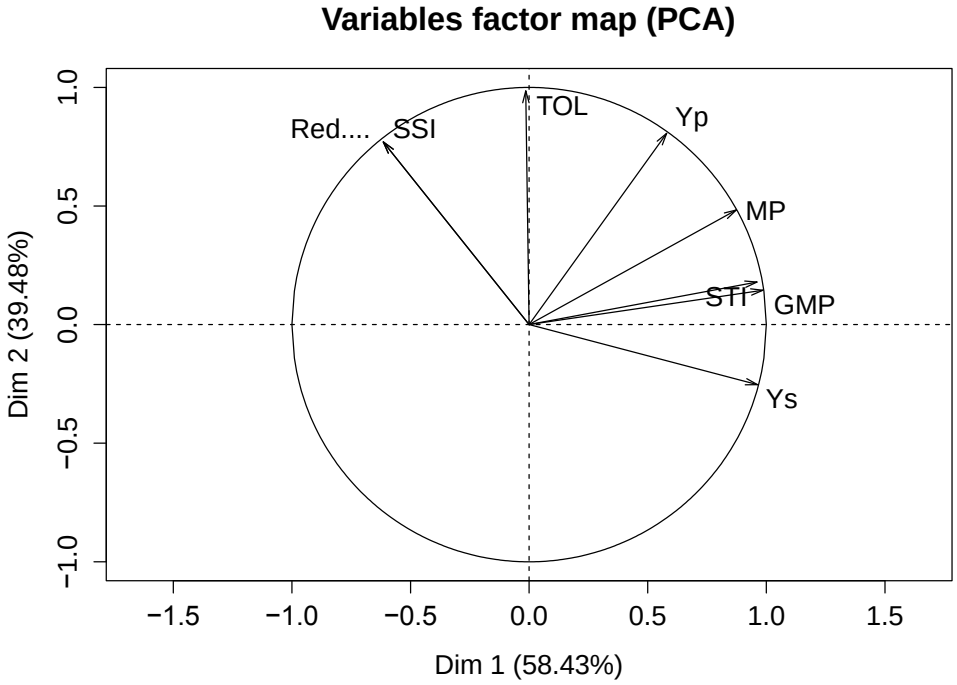


Figure 8. 1. The biplot diagram of principal components analysis of genotypes according to yield under well-watered (Yp) and stress (Ys) conditions and drought tolerance indices

b) Cluster analysis

Cluster analysis was done to study the variation between genotypes based on drought tolerance indices. Cluster analyses were based on drought tolerance indices using "R" software version 3.1.2. The dendrogram (Figure 8.2) shows three clusters (groups) of genotypes based on yield under well-watered and stress conditions, tolerance index, mean productivity, geometric mean productivity, stress susceptibility index, stress tolerance index, and percentage yield reduction.

Cluster I includes genotypes that had high yield under well-watered and low yield in stressed conditions. These genotypes in most cases, had the highest values of TOL, and SSI indices. Cluster II includes genotypes that had low yield in well-watered and stressed conditions. Hence, genotypes in this cluster could be stable in non-stressed environments but low yielding. The genotypes belonging to cluster III had high yield in well-watered and stressed conditions and, in the most cases, they had the highest values of STI, and GMP indices.

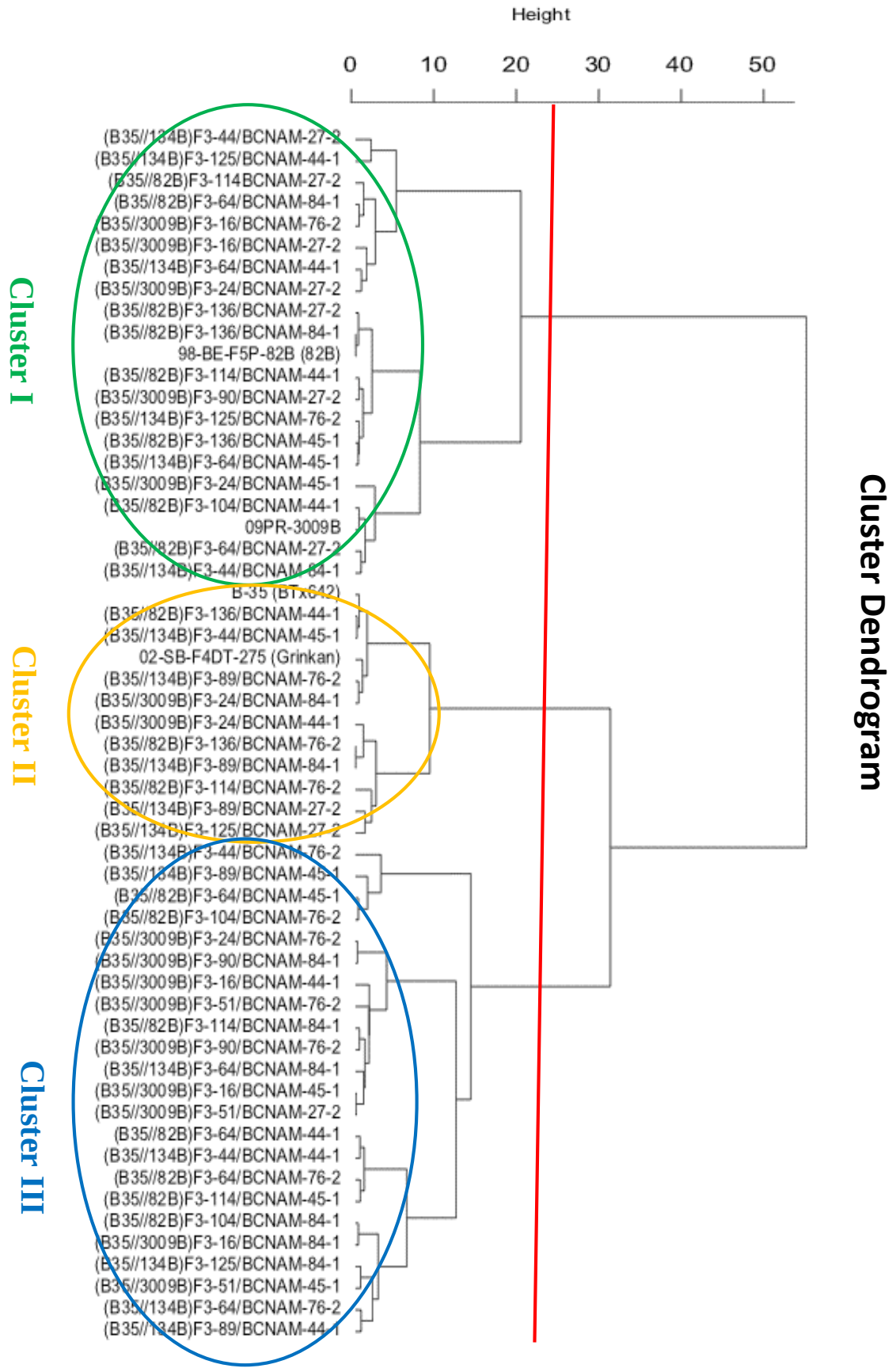


Figure 8. 2. Dendrogram from cluster analysis based on drought tolerance indices and grain yield of genotypes in both normal and stress environments.

8.3.3. Drought effects on chlorophyll index (SCMR) at maturity

The ANOVA of genotypes evaluated across water managements showed significant differences ($P < 0.05$) between genotype (G) by water management for the SCMR at maturity. This indicates that the genotypes reacted differently well-watered and water stress conditions.

The overall means of chlorophyll contents were 28.6 and 44.9 under drought stress and under well-watered conditions. The genotypes B35 (BTx642), (B35//3009B)F3-90/BCNAM-27-2, (B35//134B)F3-125/BCNAM-44-1, (B35//134B)F3-44/BCNAM-44-1, (B35//134B)F3-44/BCNAM-84-1 and (B35//3009B)F3-16/BCNAM-84-1 showed the highest SCMR at maturity under drought stress conditions (Figure 8.3).

The magnitude of the per cent reduction of SCMR (Figure 8.3) showed that drought effects were lower at maturity for the genotypes (B35//82B)-F3-104/BCNAM-84-1, (B35//3009B)-F3-90/BCNAM-27-2, (B35//134B)-F3-44/BCNAM-84-1 and (B35//134B)-F3-89/BCNAM-76-2 and were very high for the genotypes (B35//134B)-F3-64/BCNAM-44-1, (B35//3009B)-F3-24/BCNAM-45-1, 98-BE-F5P-82B, (B35//3009B)F3-51/BCNAM-27-2, (B35//134B)F3-89/BCNAM-44-1, (B35//82B)F3-114/BCNAM-44-1, (B35//82B)F3-64/BCNAM-84-1, (B35//134B)-F3-64/BCNAM-45-1 and (B35//134B)-F3-44/BCNAM-27-2.

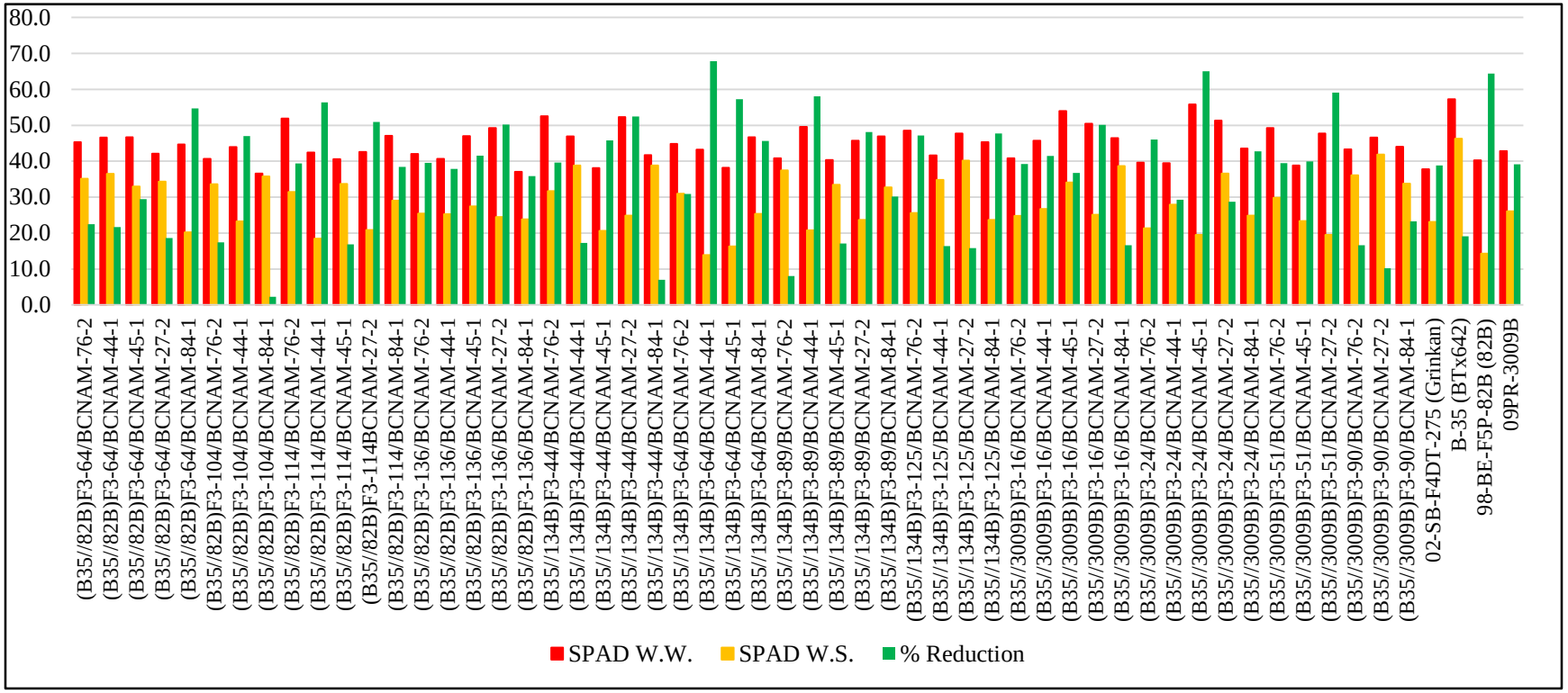


Figure 8. 3. SPAD Chlorophyll pattern at maturity under WW and WS conditions

SPAD W.W. = SPAD Chlorophyll reading at maturity under well-watered conditions,

SPAD W.S. = SPAD Chlorophyll reading at maturity under water stress conditions

8.3.4. Drought effects on number of leaves under water stress conditions at maturity

Entries showed highly significant (P <0.01) differences in total number of green leaves at maturity under water stress (Appendix 7). The overall means of total number of leaves (TNL) per plant and total number of green leaves (TNGL) at maturity were 11 and 2 under drought stress conditions, respectively.

The magnitude of per cent reduction between total number of leaves and total number of green leaves at maturity under stress conditions (Figure 8.4) showed that drought effects were important for many genotypes; the ones most serely affected (100% senescent) were (B35//82B)F3-64/BCNAM-84-1, (B35//82B)F3-104/BCNAM-44-1, (B35//82B)F3-114BCNAM-27-2, (B35//82B)F3-136/BCNAM-27-2, (B35//82B)F3-136/BCNAM-84-1, (B35//134B)F3-64/BCNAM-45-1, (B35//134B)F3-89/BCNAM-76-2, (B35//134B)F3-89/BCNAM-84-1 and 98-BE-F5P-82B. The genotypes that had the lowest effect of drought on greenness of leaves were B35 (BTx642), (B35//82B)F3-104/BCNAM-76-2, (B35//3009B)F3-51/BCNAM-76-2, (B35//3009B)F3-24/BCNAM-76-2, (B35//82B)F3-114/BCNAM-84-1, (B35//82B)F3-64/BCNAM-45-1, (B35//134B)F3-44/BCNAM-76-2 and (B35//134B)F3-89/BCNAM-45-1.

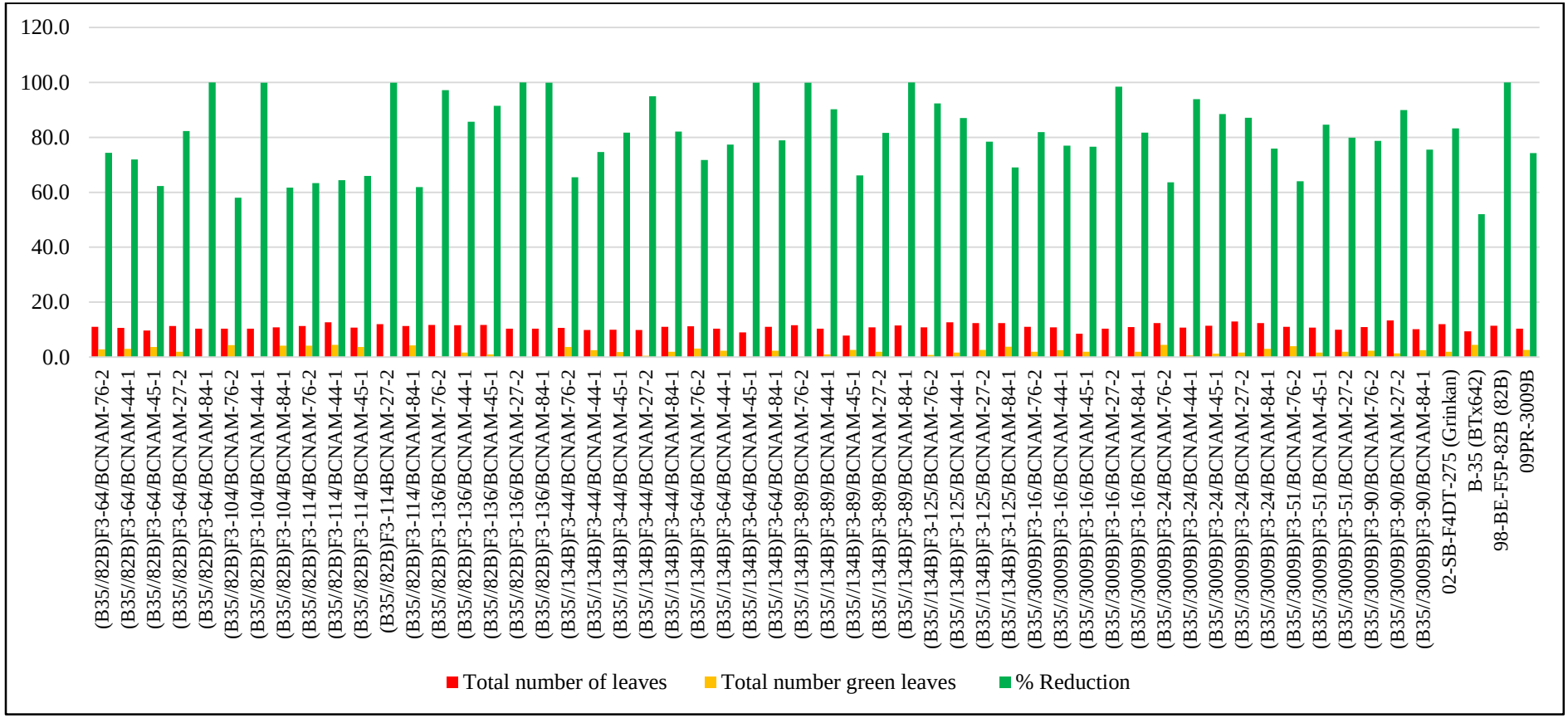


Figure 8. 4. Drought effect on number of leaves

8.3.5. Other measured traits

During the experiment, all plants in the plot were completely standing under drought stress and under irrigated conditions, except one (98-BE-F5P-82B) that was susceptible to lodging in the non-stress plot with (25% of plants in the plots completely lodged). Most genotypes were not susceptible to lodging under either water stressed or well-watered conditions (Appendix 8). But mainly this tolerance to lodging could be due to manifestation of stay green QTL in the genotypes. This will need to be to confirmed in future experiments.

The ANOVA across water management regimes showed no significant differences for the thousand grain weight (Table 8.1). This indicates that, for this trait, there was no effect of drought on the genotypes and in the two environments. This may be attributable to the presence of the stay green QTL in the genotypes. There is also a need to confirm this in future experiments.

The traits such as date Biomass (t/ha), 50 % flowering, SCMR at 50% flowering, plant height, grain appreciation, vitreousness, panicle appreciation, seedling vigor and total number of leaves per plant showed no significant differences across water managements (Table 8.1).

The performance of genotypes across water regimes is presented in Appendix 8. The genotypes having hight biomass were (B35//3009B)-F3-90/BCNAM-76-2, (B35//134B)-F3-125/BCNAM-27-2, (B35//134B)-F3-89/BCNAM-44-1, (B35//82B)-F3-114/BCNAM-76-2 and (B35//3009B)-F3-24/BCNAM-27-2 with more than 12 ton/ha^{-1}. The hybrids that flowered earlie include (B35//134B)-F3-89/BCNAM-45-1 (68 days). The highest value SCMR at 50% flowering was for hybrid (B35//3009B)-F3-24/BCNAM-45-1 (Appendix 8). The tallest genotype was (B35//134B)-F3-89/BCNAM-27-2 (239 cm). The genotypes (B35//134B)-F3-125/BCNAM-84-1 and (B35//3009B)-F3-90/BCNAM-27-2 had highest

number of leaves per plant. The panicles of (B35//134B)-F3-44/BCNAM-84-1 and (B35//134B)-F3-89/BCNAM-44-1 and the grains of hybrids (B35//82B)-F3-104/BCNAM-76-2, (B35//134B)-F3-125/BCNAM-27-2 and (B35//3009B)-F3-16/BCNAM-45-1 were the most appreciated.

8.4. Discussion

The main objective of the present study was to evaluate the effect of drought stress on sorghum grain yield and other traits of the sorghum genotypes and to study relevant drought tolerance indices, and to identify drought tolerant genotypes.

Different drought tolerance indices were calculated on the basis of grain yield of the genotypes under well-watered (Yp) and stressed (Ys) conditions (Table 8.2). Considering SSI and TOL indices, some genotypes with high SSI and TOL values were indentified ((B35//82B)-F3-64/BCNAM-84-1, (B35//3009B)-F3-24/BCNAM-45-1, (B35//3009B)-F3-16/BCNAM-76-2 (B35//134B)-F3-125/BCNAM-44-1, (B35//134B)-F3-44/BCNAM-27-2, and (B35//82B)-F3-114BCNAM-27-2) and were considered the most sensitive genotypes to drought. The genotypes (B35//134B)-F3-89/BCNAM-44-1, (B35//3009B)-F3-16/BCNAM-84-1, (B35//82B)-F3-104/BCNAM-84-1, (B35//134B)-F3-89/BCNAM-27-2, (B35//134B)-F3-125/BCNAM-27-2, and (B35//3009B)-F3-24/BCNAM-44-1) were identified as tolerant to drought, but had mostly low grain yields in both environments.

These results are in agreement with those reported by Mehrdad *et al.* (2011) in a study involving evaluation of drought tolerance indices among some winter rapeseed cultivars.

It has been noted that some genotypes with high or low yields in both well-watered and water stressed environments had the same SSI or TOL values (Naeemi *et al.*, 2008). They described SSI as deceptive. They believed that since the formula for this index involved the

proportion of a certain cultivar's yield under stress conditions to that of the non-stress conditions and to the proportion of the yield under stress conditions to that of the non-stress conditions in all experimental cultivars, two cultivars with high or low yields in both environments could have equal SSI values. Furthermore, Moghaddam *et al.* and Hadizadeh, (2002) in studying drought tolerance indices in corn, stated that low TOL did not necessarily mean a cultivar's high yield in a stress environment because a cultivar's yield might be low under irrigation conditions, but would entail a smaller drop under stress conditions that might result in a low TOL and it could be introduced as a drought-tolerant cultivar.

Furthermore, Fernandez (1992) reported that TOL index was efficient in improving yield under stressed condition but the selected genotypes performed poorly under non-stressed conditions. Fernandez (1992) favored the use of the STI index which discriminates genotypes with high yield and stress tolerance potentials. A high STI value indicated a high tolerance to stress.

Based on STI index in this study, genotypes (B35//134B)-F3-44/BCNAM-76-2, (B35//134B)-F3-44/BCNAM-44-1, (B35//82B)-F3-64/BCNAM-45-1, (B35//82B)-F3-64/BCNAM-44-1, (B35//82B)-F3-104/BCNAM-76-2 and (B35//82B)-F3-114/BCNAM-45-1 were considered as most tolerant genotypes with high yield stability in both environments.

To identify the best criterion for selecting drought tolerant genotypes, correlation coefficients were used in the present study. The STI, MP and GMP were significantly correlated with the seed yield under well-watered and stress conditions. Farshadfar *et al.* (2001) reported that the most suitable index for selecting stress-tolerant cultivars is an index which has a relatively strong correlation with the seed yield under stress and non-stress

conditions. Therefore, evaluating correlations between stress tolerance indices and the seed yield in both environments can lead to identification of the most suitable index.

Most genotypes were not susceptible to lodging under both water stressed and well-watered conditions and showed no significant differences for thousand grain weight across water management regimes. This result is consisten with the finding of Rosenow and Clark, (1981) who reported that the stay green can have a major impact on reducing lodging and is associated with increased grain size in sorghum.

8.5. Conclusions

The analysis of variance of genotypes evaluated across water management level showed interaction (at $P = 0.05$ and $P = 0.01$) between genotype (G) by water management for grain yield (kg/ha), SCMR and TNGL at maturity. Suggesting that the behavior of genotypes were different from one environment to another.

The results of evaluation of the effect of drought stress on grain yield using stress tolerance indices suggested that breeders should choose the indices on the basis of stress severity in the target environment. STI, MP and GMP are useful indicators for selection of tolerant genotypes. Based on of these indices, the genotypes (B35//134B)-F3-44/BCNAM-76-2, (B35//82B)-F3-64/BCNAM-45-1, and (B35//82B)-F3-104/BCNAM-76-2 were found to be most drought tolerant genotypes with high yield stability in the well-watered and drought conditions. These genotypes could be commercialized as tolerant genotypes after further evaluations on station trials and in farmers' fields.

CHAPTER NINE

9.0. GENERAL CONCLUSIONS AND RECOMMENDATIONS

9.1. Conclusions

Post-flowering drought stress is the most important environmental constraint contributing to grain yield instability in sorghum (*Sorghum bicolor* L. Moench). Evaluation of sorghum genotypes under different stresses would be useful for identifying genotypes that combine stability with high yield potential for stress-prone areas.

The goal of this study was to improve post-flowering drought stress tolerance in sorghum by incorporating the stay-green trait into drought susceptible elite sorghum B-lines through the use of molecular marker-assisted backcrossing and to identify stay green sorghum inbred R-lines using the test cross approch. Then development of stay green and high yielding parents and hybrids tolerant to post-flowering drought could be done.

The results of this study showed that, the main reasons to grow sorghum are for use as grain for human consumption, while the crop residues are used in feeding animals, fencing, thatching, for firewood and compost (organic fertilizer). The major sorghum production constraints are low soil fertility and high cost of inputs, lack of farm equipment, drought, *Striga* infestation, poor rainfall and lack of training of farmers. Majority of sorghum varieties grown by farmers in Mali are landraces due to their better adaptation, grain quality and pest resistance. Farmers' criteria for selecting varieties are highly variable. The main farmers' varietal preferences are high yield potential, earliness, grain quality, and tolerance to drought and *Striga* infestation.

The genomic regions of the stay-green donor parent B35 were introgressed into non-senescence recurrent parents: 98-BE-F5P-82B, 03-SB-F5DT-134B and 09PR-3009B using

MAS backcross method and three populations were developed: (B35//82B)-F2, (B35//134B)-F2 and (B35//3009B)-F2.

Nine molecular markers linked to stay-green QTL regions were selected for foreground screening on these populations.

A set of B-lines was developed with twelve B-lines containing the target stay green QTL, better morphology (plant architecture), and farmers' preferences for various traits as identified in PRA.

A set of R-lines was developed with ten R-lines with stay green and farmers' preferences for various traits as identified in the PRA.

Crosses were made between a set of twelve female parents (B-lines) and five male parents (R-lines) using the North Carolina Design II mating design ($12 \times 5 = 60$). A total of 60 F_1 hybrids were obtained.

Combining ability, GGE biplot, and stress tolerance indices using multivariate analysis were used to identify ideal hybrids and their parents

Threr hybrids (B35//134B)-F3-44/BCNAM-76-2, (B35//82B)-F3-64/BCNAM-45-1 and (B35//82B)-F3-104/BCNAM-76-2 were found to be most drought tolerant with high yield stability under the well-watered and drought conditions.

The female parents (B35//134B)-F3-44 and (B35//82B)-F3-64 and male parent BCNAM-76-2 were identified as suitable parents with good adaptation to all the test environments.

The identified hybrids and parents can be introduced as post-flowering drought tolerant genotypes after futher evaluation on station and in farmers' fields.

9.2. Recommendations

In other to overcome the constraints for sorghum production identified th, it is recommended to utilize the female B-lines (B35//134B)-F3-44 and (B35//82B)-F3-64, and male R-line BCNAM-76-2, identified as having high yield and good GCA at all the test environments, as parental lines in the hybrid breeding program.

There is also the need to convert the female B-lines to A-lines or cytoplasmic-nuclear male-sterility (CMS) lines.

The three highest yielding hybrids, (B35//134B)-F3-44/BCNAM-76-2, (B35//82B)-F3-64/BCNAM-45-1 and (B35//82B)-F3-104/BCNAM-76-2 should be included in multi-locational trials to assess the consistency of performance for probable release.

With the present climate change, improving germplasm for drought tolerance is important, therefore, the National Breeding Program in Mali should, in the near future, be able to register drought tolerant parents and hybrids as products of this study.

REFERENCES

Abdel-Moneam, M.A., Attia, A.N., El-Emery, M.I., and Fayed, E.A. 2009. Combining ability and heterosis for some agronomic traits in crosses of maize. Pak. J. *Biol. Sci.*, 12: 433-438.

Abdrabbo, A., and Abou, K. 2009. Comparison among different irrigation systems for deficit irrigated corn in the Nile valley. *Agricultural Engineering International*: the CGR E-journal. Manuscript LW 08 010. Vol. XI: 122-138 pp.

Abdurakhmonov, I.Y., Buriev, Z.T., Shermatov, S.E, Kushanov, F.N., Makamov, A., Shopulatov, U., Turaev, O., Norov, T., Akhmedov, C., Mirzaakhmedov, M. and Abdukarimov, A. 2011a. Utilization of natural diversity in Upland cotton (*G. hirsutum*) germplasm collection for pyramiding genes via marker-assisted selection program. Presentation in 5 Asian Cotton Research and Development Network Meeting, Pakistan.

Abdurakhmonov, I.Y., Buriev, Z.T., Shermatov, S.E. 2011b. Marker-assisted selection for complex fiber traits in cotton. World Cotton Research Conference-5, Spaecial session of ICGI, Mumbai, India, November 7-12.

Aina, O., Dixon, A., Paul, I. and Akinrinde, E. 2009. G×E Interaction Effects on Yield and Yield Components of Cassava (Landraces and Improved) Genotypes in the Savanna Regions of Nigeria. Afr. J. *Biotechnol.*, 8 (19): 4933-4945.

Ambler, J.R., Morgan, P.W., and Jordan, W.R. 1987. Genetic regulation of senescence in a tropical grass, pp.43-53 in Plant Senescence: Its Biochemistry and Physiology, edited by Thomson, W.W. Nothnagel, E.A. and Huffaker, R.C. American Society of Plant Physiologists, Rockville, MD.

Amudha, J. and Balasubramani, G. 2011. Recent molecular advances to combat abiotic stress tolerance in crop plants. *Biotechnology and Molecular Biology Review* Vol. 6 (2), pp. 31-58.

Andrews, D.J., Ejeta, G., Gilbert, M., Goswami, P., Kumar, K.A., Maunder, AB., Porter, K., Rai, K.N., Rajewski, J.F., Reddy, V.S., Stegmeier, W. and Talukdar. B.S. 1996. Breeding hybrid parents. In: proceedings of the international conference on the genetic improvement of sorghum and pearl millet (D. Rosnow et al. (Eds.) p.173- 185, September 23-27, 1996- Lubbock, Texas.

Araus, J.L., Bort, J., Steduto, P., Villegas, D. and Royo, C. 2003. Breeding cereals for Mediterranean conditions: Ecophysilogy clues for biotechnology application. Ann. *Appl. Biol.*, 142: 129-141.

Ashraf, M. and Foolad, M. 2007. Role of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot* 59: 206-216.

Athuman, J.M. 2014. Effect of drip irrigation on the production and economic returns of sorghum (S*orghum bicolor*) in semi-arid areas of Tanzania. Department of Land

Resource Management and Agricultural Technology (LARMAT). University of Nairobi. 94pp.

Atokple, I.D. 2003. Sorghum and millet breeding in West Africa in practice. CSIR-Savanna Agricultural Research Institute, Tamale, Ghana. 11pp.

Balole, T.V. and Legwaila, G.M. 2006. *Sorghum bicolor* (L.) Moench. PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. Available at *http://www.prota4u.org.*

Bartels, D. Furini, A., Ingram, J. and Salamini, F. 1996. Responses of plant to dehydration stress: A molecular analysis. *Plant Growth Reg.,* 20: 111-118.

Basnayake, J., Cooper, M., Ludlow, M.M., Henzell, R.G. and Snell, P.J. 1995. Inheritance of osmotic adjustment to water stress in three grain sorghum crosses. *Theor. Appl. Genet.* 90, 675–682.

Beil, G.M. and Atkins, R.E. 1967. Estimates of general and specific combining ability in F_1 hybrids for grain yield and its components in grain sorghum, *sorghum vulgare* Pers. *Crop Sci.* 7:225-228.

Belum, V.S., Sharma, H.C., Thakur, R.P., Ramesh, S., Rattunde F. and Mgonja, M. 2006. Sorghum Hybrid Parents Research at ICRISAT, Strategies, Status, and Impacts. ICRISAT. Patancheru 502 324, Andhra. Volume 2. Issue 1. 24 pp.

Bhadouriva, N.S. and Saxena, M.K. 1997. Combining ability studies in sorghum through diallel analysis. *J. Crop.* Res. Hisar., 14: 253-256.

Bhattramakki, D., Dong, J., Chhabra, A. and Hart G. 2000. An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. *Genome* 43:988-1002

Blum, A. 1979. Genetic improvement of drought resistance in crop plants: a case for sorghum. Stress Physiology in Crop Plants. New York: *Academic:* 429-445.

Blum, A. 1985. Breeding crop varieties for stress tolerance. CRC Critical *Reviews in Plant Sciences* 2: 198-238

Blum, A. 1988. Plant breeding for stress environments. CRS Press. Boca Roton, Florida.

Blum, A. 1996. Crop responses to drought and the interpretation to adaptation. *Plant Growth Reg.,* 20: 135-148.

Blum, A. 2011, entitled "Plant Breeding for Water Limited Environments", published by Springer Available at *htt//www.plantstress.com/articles/drought.*

Blum, A., Piorkova, R., Golan, G. and Mayer, J. 1983. Chemical desiccation of wheat plants as simulator of post-anthesis stress. I. Effects on translocation and kernel growth. *Fld Crops Res.* 6, 51-58.

Boivin, K., Deu, M., Rami, J., Trouche, G. and Hamon, P. 1999. Towards a saturated sorghum map using RFLP and AFLP markers. *Theor Appl Genet* 98:320-328

Borrell, A.K. and Hammer, G.L. 2000a. Nitrogen dynamics and the physiological basis of stay-green in sorghum. *Crop Science,* 40 5: 1295-1307.

Borrell, A.K., Hammer, G.L. and Henzell, R.G. 2000b. *Crop Sci.* 40:1037-1048.

Borrell, A.K., Bidinger, F.R. and Sunitha, K. 1999. Int. Sorg. Mill. News. 40:31-34.

Botstein, D., White R., Skolnick M. and Davis R. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am. J. Hum. Genet.* 32: 314-331.

Bowers, J.E., Abbey, C., Anderson, S., Chang, C. and Draye, X. 2003. A high-density genetic recombination map of sequence-tagged sites for sorghum, as a framework for comparative structural and evolutionary genomics of tropical grains and grasses. *Genetics* 165:367-386

Boyer, J., and Westgate, M. 2004. Grain yields with limited water. *Journal of Experimental Botany* 55:2385-2394.

Boyer, J.S. 1982. Plant productivity and environment. *Science,* 228:444-448.

Brandiej, E. and Meverty, B. 1994. Genotype × environmental interaction and stability of seed yield of oil rapeseed. *Crop Sci.* 18: 344-353.

Butt, T., McCarl, B., Angerer, J., Dyke, P. and Stuth, J. 2003. Food security implication of climate change in developing countries: findings from a case study in Mali. Department of Agricultural Economics, Texas A&M University

Cahill, D.J. and Schmidt, D.H. 2004. Use of marker assisted selection in a product development breeding program. Proc. 4th Int. *Crop. Sci.* Cong. Vol. 26, No., ISSN.

Cardoso, M.A., Provan, J., Powell, W., Ferreira, P.C. and de Oliveira D.E. 1998. High genetic differentiation among remnant populations of the endangered Caesalpinia echinata Lam. (*Leguminosae Caesalpinioideae*). *Molecular Ecology*, 7, 601–608.

Carl, E.P. and Latha, N. 2009. Pearl Millet and Sorghum Improvement in India. The International Food Policy Research Institute (IFPRI) Discussion Paper 00919. 36 pp.

Ceccarelli, S., and Grando, S. 2002. Plant breeding with farmers requires testing the assumptions of conventional plant breeding: Lessons from the ICARDA barley program. CAB International, Wallingford, Oxon, UK, pp. 297–332.

Chambers R. 2002. Relaxed and Participatory Appraisal: Notes on practical approaches and methods for participants in PRA/PLA-related familiarisation workshops. IDS, Brighton, UK.

Chambers, R. 1992. Rapid Appraisal: Rapid, Relaxed and Participatory. Institute of Development Studies discussion paper 311. University of Sussex, England, 90pp.

Chaves, M.M., Maroco, J.P. and Pereira, J.S. 2003. Understanding plant responses to drought from genes to the whole plant. Funct. *Plant Biol.*, 30: 239-264.

Choukan, R. 2008. Methods of genetical analysis of quantitative traits in plant breeding. 1st Edn. Agricultural Extension, Education and Research Organization, 270 pp.

Choukan, R., Taherkhani, T., Ghannadha, M.R. and Khodarahmi, M. 2006. Evaluation of drought tolerance in grain maize inbred lines using drought tolerance indices. *Iran. J. Agric. Sci.* 8 (1): 79-89.

Christinck, A. 2002. "This seed is like ourselves." A case study from Rajasthan, India, on the social aspects of biodiversity and farmers' management of pearl millet seed, Margraf Verlag, Weikersheim, Germany.

Clarke, J.M., DePauw R.M., and Townley-Smith T.F. 1992. Evaluation of methods for quantification of drought tolerance in wheat. *Crop Science* 32: 723-728

Clayton, W.D. 1961. Proposal to conserve the generic name *Sorghum Moench*. (*Gramineae*) versus *Sorghum Adans (Gramineae).* Taxonomy 10:242.

Cleveland, D.A. 2001. Is plant breeding science objective truth or social construction? The case of yield stability. *Agriculture and Human values* 18 (3): p. 251–270.

Collard, B.C. and Mackill, D.J. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* Vol. 363, No. 1491: 557-72.

Collard, B.C., Jahufer, M.Z., Brouwer, J.B. and Pang, E.C. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica* 142, 1, 169-196.

Collins, F.C. and Pickett, R.C. 1972. Combining ability for protein and lysine in an incomplete diallel of *sorghum bicolor* (L) Moench. *Crop Sci.* 12:5-6.

Comstock, R.E. and Robinson, H.F. 1952. Average Dominance of Genes in Heterosis. Iowa State College Press, *Ames:* pp. 494-516.

Concibido, V.C., Dennis, R.L., Lange, D.A., Orf, J.H. and Young, N.D. 1996. RFLP mapping and marker-assisted selection of soybean cyst nematode resistance in PI 209332. *Crop Sci.* 36: 1643-1650.

Conner, A.B. and Karper, R.E. 1927. Hybrid vigor in sorghum. Texas Experiment Stations Bulletin no 359. Texas A&M University, Texas, USA.

Cornelius, P., and Crossa, J. 1999. Prediction assessment of shrinkage estimators of multiplicative models for multi-environment cultivar trials. *Crop Sci.* 39: 998-1009

Coulibaly, S.B. 1996. Rapport annuel, Agrophysiologie Sorgho. Programme Sorgho, Institut d'Economie Rurale (IER) Mali. 30 pp.

Coulibaly, S.B. 2002. Evaluation of backcross progenies and recombinant inbred line populations of sorghum (*Sorghum bicolor* (L.) Moench). Ph.D. Dissertation. Texas Tech University, Lubbock, TX. 151 pp.

Crafts-Brandner, S.J., Blow, F.E., Harper, J.E. and Hageman, R.H. 1984a. Differential senescence of maize hybrids following ear removal. I. Whole plant. Plant Physiology, 74: 360-367.

Crasta, O., Xu, W., Rosenow, D., Mullet, J. and Nguyen, H. 1999. Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. Molecular and General Genetics MGG 262:579-588.

Crosbie, T.M., Eathington, S.R., Johnson G.R., Edwards, Sr,M., Reiter, R., Stark, S., Mohanty, R.G., Oyervides, M., Buehler, R.E. and Walker, A.K. 2003. *Plant breeding: Past, present, and future.* p. 3-50.

Dahlberg, J.A. 1995. African Crop Science Journal, African Crop Science Society ISSN: 1021-9730 *EISSN:* 2072-6589. *Vol. 3. No.2,* pp. 143-151.

Dahlberg, J.A., 1992. Variation of C Assimilate Export and Partitioning in Reduced Progressive Senescent and Senescent Sorghums [*Sorghum bicolor* (L.) Moench] and the Potential Use of Anatomical Features as a Genetic Marker for Variation in Sorghum. Ph.D. dissertation, Texas A&M University, College Station, TX.

Darvishzadeh, R. Pirzad, A. Hatami M.H., Poormohammad K.S. and Sarrafi A. 2010. Evaluation of the reaction of sunflower inbred lines and their F_1 hybrids to drought conditions using various stress tolerance indices. Span. *J. Agric.* Res. 8:1037-1046.

De Vicente, M.C. and Tanksley, S.D. 1993. QTL analysis of transgressive segregation in an interspecific tomato cross. *Genetics.* 134:585–96

De Vries, J. and Toenniessen, G. 2001. Securing the Harvest: Biotechnology, breeding and seed systems for African crops. CABI Publishing. The Cromwell Press, Trowbridge, Wiltshire, UK.

De Wet, J.M., Harlan, J.R. and Kurmarohita, B. 1972. Origin and evolution of guinea sorghums. *East African Agriculture and Forestry Journal* 38:114-119.

De Wet, J.M., Harlan, J.R. and Price, E.G. 1970. Origin of variability In the Spontanea complex of *Sorghum bicolor*. *American Journal of Botany* 57 (6): 704-707.

Deb, U.K., Bantilan, M.C., Hash, C.T. and Ndjeunga, J. 2004. Adoption of improved sorghum cultivars. Patancheru 502 32.4, Andhra Pradesh, India: *International Crops Research Institute for the Semi-Arid Tropics*, pp. 181-198

Diallo, A.G. 2013. Etude de l'Aptitude à la Combinaison et la performance agronomique des hybrides de Sorgho de type Guinea dans divers environnements au Mali. USTTB. Thèse de Doctorat. 92 pp.

Ding, M. Tier, B., Yan, W., Wu, H., Powell M. and Mcrae T. 2008. Application of GGE biplot analysis to evaluate genotype (G), environment (E), and G×E interaction on *Pinus radiata*: a case study. *New Zealand Journal of Forestry Science* 38 (1): 132–142.

DNA-Mali. 2015. Evolution des superficies et des productions. *Rapport bilan champagne* 2013/2014.

Doebley, J. 1990. Molecular evidence for gene flow among *Zea* species. *BioScience* 40:443–448

Doggett, H. 1965a. The development of the cultivated sorghums. Pages 50-69 in Essays on crop plant evolution (Eds. J.B. Hutchinson *et al*.). London: Cambridge University Press.

Doggett, H. 1965b. Disruptive selection in crop development. Nature 206 (4981): 279-280.

Doggett, H. 1988. Sorghum. Second edition. UK: Tropical Agricultural Series, Longman Scientific and Technical Publishers; and Ottawa, Canada: *International Development Research Centre.* 512 pp.

Dufour, P. Deu, M., Grivet, L., D'Hont, A., Paulet, F., Bouet, A., Lanaud, C., Glaszmann, J. and Hamon, P. 1997. Construction of a composite sorghum genome map and comparison with sugarcane, a related complex polyploid. *Theor Appl Genet* 94:409-418

Duncan, R.R., Bockholt, A.J. and Miller, F.R. 1981 Descriptive comparison of senescent and nonsenescent sorghum genotypes. *Agron. J*. 73: 849-853.

Dunwell, J.M. 2000. Transgenic approaches to crop improvement. *J. Exp. Bot.* 51 Spec No: 487-496.

Eagles, H., Bariana, H., Ogbonnaya, F., Rebetzke, G., Hollamby, G., Henry, R., Henschke, P. and Carter, M. 2001. Implementation of markers in Australian wheat breeding. Aust. *J. Agric.* Res., 52: 1349-1356.

Edgerton, M.D. 2009. Increasing crop productivity to meet global needs for feed, food, and fuel. *Plant Physiol.* 149:7-13.

Edwards, D., Forster J., Chagne D., and Batley J. 2007. What is SNPs? In: Oraguzie, N.C., Rikkerink, E.H., Gardiner S.E. and de Silva, H.N. (eds.), Association Mapping in Plants. *Springer, Berlin, pp*. 41-52.

Edwards, J. and McCouch, S. 2007. Molecular Markers for Use in Plant Molecular Breeding and Germplasm Evaluation. In: Guimaraes, E. P., Ruane, J., Scherf, B. D., Sonnino, A. and Dargie, J. D. (Eds.): Marker-assisted Selection - Current Status and Future Perspectives in Crops, Livestock, Forestry and Fish. Rome FAO: 29-49.

Ejeta, G. and Knoll, J.E. 2007. Marker-Assisted Selection in Sorghum. *Genomics Assisted Crop Improvement.* Vol 2. pp. 187-205.

Falconer, D.S. and Mackay, T.F. 1996. Introduction to Quantitative Genetics. Longman Group, Pearson Education Ltd, London. p. 356.

FAOSTAT. 1999. Statistical Database of the Food and Agriculture of the United Nations.

FAOSTAT. 2013. Statistical Database of the Food and Agriculture of the United Nations.

FAOSTAT. 2014. Statistical Database of the Food and Agriculture of the United Nations.

Farshadfar, E. and Sutka, J. 2002. Screening drought tolerance criteria in maize. Acta Agronomica Hungarica 50(4): 411-416.

Farshadfar, E., Zamani, M., Motallebi, M. and Imamjomeh, A. 2001. Selection for drought resistance in chickpea lines. Iran. *J. Agris. Sci.* 32 (1): 6577.

Fernandez, G.C. 1992. Effective selection criteria for assessing stress tolerance. In: Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress Tolerance, Asian Vegetable Research and Development Centre, Taiwan, pp. 257-270.

Fischer, R.A. and Maurer, R. 1978. Drought resistance in spring wheat cultivars. I. Grain yield response. Aust. *J. Agric.* Res., 29: 897–907.

Franken, K. 2005. Water report 29, in Africa in figures- AQUASTAT survey. Rome, Italy: Food and Agricultural Organization of the United Nations.

Gebre, G.M. 2000. Role of Osmotic Adjustment in plant productivity. Environmental sciences, Colorado State University. 34 pp.

GenStat 12th Edition, (2009). GenStat Procedure Library Release PL20.1.

Gentinetta, E., Lepori, C., Perico, G., Motto, M. and Salamini, F., 1986, A major gene for delayed senescence in maize. Pattern of photosynthesis accumulation and inheritance. *Plant Breeding*, 97:193-203.

Gurian-Sherman, D. 2009. Failure to yield: performance of genetically engineered crops. Union of Concerned Scientists (available at www.ucsusa.org, verified on November 20, 2013).

Hall, A.E. 1993. Is dehydration tolerance relevant to genotypic differences in leaf senescence and crop adaptation to dry environments? In: Plant Responses to Cellular Dehydration

during Environmental Stress. Eds. Close, T.J. and Bray, E.A., *American Society of Plant Physiology, Rockville, Md.* pp. 1-10.

Harlan, J.R. 1975a. Geographic patterns of variation in some cultivated plants. *Journal of Heredity* 66:184-191.

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

Harris, K., Subudhi, P., Borrell A., Jordan D., Rosenow D., Nguyen H., Klein P., Klein R., and Mullet, J. 2007. Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. *Journal of Experimental Botany*, Vol. 58, No. 2, pp. 327–338,

Haussman, B.I., Obilana, A.B., Ayiecho, P.O., Schipprack W. and Geiger, H.H. 1998. Hybrid performance of sorghum and its relationship to morphological and physiological traits under variable drought stress in Kenya, *Plant Breeding* 117: 223-229.

Haussmann, B.I., Hess, D.E., Seetharama, N., Welz, H.G. and Geiger, H.H. 2002a. Construction of a combined sorghum linkage map from two recombinant inbred population using AFLP, SSR, RFLP, and RAPD markers, and comparison with other sorghum maps. *Theor Appl Genet* 105:629-637

Haussmann, B.I., Mahalakshmi, V., Reddy, B., Seetharama, N., Hash, C. and Geiger, H. 2002b. QTL mapping of stay-green in two sorghum recombinant inbred populations. TAG *Theoretical and Applied Genetics* 106:133-142.

Helsel, G.B. and Frey, K.J. 1978. Grain yield variations in oats associated with differences in leaf area duration among oat lines. *Crop Science*, 18:765-769.

Henzell, R.G., Jordan, D.R., Fletcher, D.S., McCosker, A.N., Bunker, G. and Persley, D.S. 2001. Sorghum breeding in Australia: public and private endeavours. In: Borrell, A.K. Henzell, R.G. Eds. Proceedings of the 4th Australian sorghum conference Kooralbyn, Australia.

Horner, H.T. and Palmer. 1995. Mechanisms of genic male sterility. *Crop Science* 35: 1527-1535.

Hossain, A.B., Sears, A.G., Cox, T.S. and Paulsen, G.M. 1990. Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. *Crop. Sci.* 30: 622-627.

House, L.R. 1985. A guide to sorghum breeding. Vol. II. Patancheru, A.P., 502 324, India: International Crops Research Institute for the Semi-Arid Tropics. 206 pp.

Hsiao, T.C., O'Toole, J.C., and Turner N.C. 1984. Influence of Osmotic Adjustment on leaf rolling and tissue death in rice (*Oryza sativa* L). *Plant Physiol.* 75: 338-341

http://ecampus.oregonstate.edu/forms/contact.aspx. Oregon State University; Crop Science Dept. (Accessed 04 February 2012).

http://plantbreeding.coe.uga.edu/index.php?title=12. Translational Breeding by Charles Brummer; Department of Crop & Soil Sciences, University of Georgia (Accessed May 2014).

http://www.fao.org.CERAAS: A regional tool of WECARD/CORAF (Accessed June 2015).

http://www.plantstress.com; The Mitigation of Drought Stress (Accessed 30 March 2012)

IER-Mali and PSA-2-3sor., 2006. Développement et utilisation de variétés et hybrides de sorgho à double usage et conservation de la diversité génétique des sorghos au Mali. Available at http://www.globalplanofaction.org.

Ingram, J. and Bartels, D. 1996. The molecular basis of dehydration tolerance in plants. Ann. Rev. Plant Physiol. *Plant Mol. Biol.,* 47: 377-403.

INSORMIL-CRSP. 2006. Sorghum, Millet, and Other Grains Collaborative Research Support Program, SMOG CRSP. Leader with Associates Cooperative Agreement No. EPP-A-00-06-00016-00. University of Nebraska. 90 pp.

Intergovernmental Panel on Climate Change (IPCC). 2001. Working group science. Climate Change. Impacts, Adaptation and Vulnerability. Cambridge.

Jiang, G., Xu, C., Li, X., and Zhang, Q. 2004. The genetic basis of stay-green in rice analyzed in a population of doubled haploid lines derived from an *indica* by *japonica* cross. *Theoretical and Applied Genetics,* 108: 688- 698.

Jiang, G.L. 2013. Molecular Markers and Marker-Assisted Breeding in Plants. Plant Science Department, South Dakota State University, Brookings, USA 40 pp.

Jones, H.J. 1993. Drought tolerance and water-use efficiency. In: Water deficits, plant response from cell to community (Smith JAC, Griffiths H Eds.). Oxford, UK: BIOS Scientific Publishers, pp. 193-203.

Jordan, D.R., Tao Y., Godwin I.D., Henzell R.G., Cooper M. and McIntyre C.L. 2003. Prediction of hybrid performance in grain sorghum using RFLP markers. *Theoretical and Applied Genetics* 106, 559–567.

Jordan, W.R., and Miller, F.R. 1980. Genetic variability in sorghum root systems: implications for drought tolerance. In "Adaptation of Plants to Water and High Temperature Stress" (Turner, P.C. and Kramer, P.J. Eds.), pp. 383–399.

Jordi, C. and Helena, G.M. 2005. La culture du sorgho de décrue en Afrique de l'Ouest et du Centre : Situation actuelle et définition d'un Plan d'Action Régional. Agence Espagnole de Coopération Internationale Avec la collaboration de la FAO, p.24

Juma, C. 2011. The New Harvest: Agricultural Innovation in Africa. Oxford University Press, Oxford.

Kahiu, N. 2012. Marker Assisted Selection for Drought Tolerance and *Striga* Resistance: Introgressing Quantitative Trait Loci (QTL) in farmer preferred varieties of sorghum. LAP Lambert Academic. Broché: 116 pp.

Kahiu, N., Wilson, K., Dan, K., Eunice, W. and Mutitu. 2013. "Improving Drought Tolerance in *Sorghum bicolor* L. Moench: Marker-Assisted Transfer of the Stay-Green Quantitative Trait Loci (QTL) from a Characterized Donor Source into a Local Farmer Variety" International *Journal of Scientific Research in Knowledge* (IJSRK), 1 (6), pp. 154-162

Kambal, A.E. and Webster, O.J. 1965. Manifestation of hybrid vigour in grain sorghum and the relations among the components of yield, weight per bushel and height. *Crop Sci.* 6:513-515.

Kambal, A.E. and Webster, O.J. 1966. Estimates of general and specific combining ability in grain sorghum, *sorghum vulgare* Pers. *Crop Sci*. 5:521-523.

Kassahun, B., Bidinger, F, Hash, C. and Kuruvinashetti, M. 2010. Stay-green expression in early generation sorghum [*Sorghum bicolor* (L.) Moench] QTL introgression lines. *Euphytica* 172.3 : 351-362.

Kassahun, B., Ramu, P., Senthilvel, S., Kumar, C., Jayashree, B., Folkertsma, R., Reddy, L., Kuruvinashetti, M., Haussmann, B. and Hash, C. 2009. Exploiting rice and sorghum synteny for targeted development of EST-SSRs to enrich the sorghum genetic linkage map. TAG *Theoretical and Applied Genetics* 119:1193-1204.

Kebede, H.S., Rosenow, D.T. and Nguyen, H.T. 2001. Quantitative trait loci influencing drought tolerance in grain sorghum. *Theoretical and Applied Genetics* 103:266-276.

Kempthorne, O. 1957. An Introduction to Genetical Statistics. John Wiley and Sons, Champman and Hall, London.

Ketema, S. 2008. Strategic choices and research priorities for the Asareca sub-region: Food crops, livestock, natural resources management, policy and information, 760 pp.

Kirby, J.S. and Atkins, R.E., 1968. Heterosis response for vegetative and mature plant characters in grain sorghum, *Sorghum bicolor* (L,), Moench, *Crop Sci*, 8, 335–339.

Klein, P.E., Klein, R.R., Cartinhour, S.W., Ulanch, P.E., Dong J., Obert, J.A., Morshige D.T., Schlueter, S.D., Childs, K.L., Ale, M. and Mullet, J.E. 2000. A high-throughput AFLP-based method for constructing integrated genetic and physical maps: Progress toward a sorghum genome map. *Genome Res* 10:789-807

Knapp, S.J. 1998. Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. *Crop Sci.* 38: 1164-1174.

Kong, L. Dong, J. and Hart, G. 2000. Characteristics, linkage-map positions and allelic differentiation of *Sorghum bicolor* (L.) Moench DNA simple sequence repeats (SSRs). *Theor Appl Genet* 101:438-448

Konieczny, A., and Ausubel, F. 1993. A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR based markers. *The Plant Journal* 4: 403-410.

Kresovich, S, Barbazuk, B., Bedell J.A., Borrell, A. and Buell A.R. 2005. Towards sequencing the sorghum genome. A U.S. National Science foundation-sponsored workshop report. *Plant Physiol* 138:1898-1902

Kumar, A.A., Sharma, H.C. and Hash, C.T. 2011. Recent Advances in Sorghum Genetic Enhancement Research at ICRISAT. *American Journal of Plant Sciences* 2:589-600.

Kumpatla, S.P., Buyyarapu, R., Abdurakhmonov, I.Y. and Mammadov, J.A. 2012. In: Ibrokhim, I.Y. (Eds.). Genomics-Assisted Plant Breeding in the 21st Century: Technological Advances and Progress. *Plant Breeding*, *ISBN*: 978-953-307-932-5, InTech, Croatia.

Lacy, S.M., Cleveland, D.A., and Soleri, D. 2006. Farmer Choice of Sorghum Varieties in Southern Mali. Human Ecology, Vol. 34, No. (3), 23 pp.

Lande, R. 1992. Marker-assisted selection in relation to traditional methods of plant breeding. P. 437-452. In Stalker, H.T. and Murphy, J.P. (Eds.). Plant breeding in the 1990s. CAB International. Wallingford, UK.

Levitt, J. 1972. Responses of plants to environmental stresses. Academic Press, New York.

Linnaeus, C. 1753. Species Plantarum (Latin for "The Species of Plants"). Vol.1

Liz, H., Bad, F. Chris O'Neill., and Warren, M. 2005. Maize under sprinkler, drip and furrow irrigation. CSRO land and Water, Griffith, Charles Sturt University, CRC for irrigation future. IREC Farmers Newsletter, No. 170 pp.

Luigi, C. 2003. Participatory Rural Appraisal (PRA) Concepts Methodologies and Techniques. Universita' Degli Studi di Padova. Facolta' di agraria. 38 pp.

Lynch, M. and Walsh, B. 1998. Genetics and Analysis of Quantitative Traits. Sinauer Associates.

Mace, E.S. and Jordan, D.R. 2010. Location of major effect genes in sorghum (*Sorghum bicolor* (L.) Moench). *Theor Appl Genet.* DOI 10.1007/s00122-010-1392-8

Mace, E.S. and Jordan, D.R. 2011. Integrating sorghum whole genome sequence information with a compendium of sorghum QTL studies reveals uneven distribution of QTL and of gene rich regions with significant implications for crop improvement. *Theor Appl Genet* DOI 10.1007/s00122-011-1575-y.

Mace, E.S., Rami, J.F, Bouchet S., Klein P.E, Klein R.R., Kilian A., Wenzl P., Xia L., Halloran K. and Jordan D.R. 2009. A consensus genetic map of sorghum that integrates multiple component maps and high-throughput Diversity Array Technology (DArT) markers. BMC *Plant Biology* 2009 9:13. DOI: 10.1186/1471-2229-9-13.

Mace, E.S., Xia, L., Jordan, D.R., Halloran, K., Parh, D.K., Huttner, E., Wenzl, P. and Kilian, A. 2008. DArT markers: diversity analyses and mapping in *Sorghum bicolor*. BMC *Genomics* 9:26

Mahalakshmi, V. and Bidinger, F. 2002. Evaluation of stay green sorghum germplasm lines at ICRISAT. *Crop Science* 42: 965 -974

Maiti, R.K., Rao, K.E., Raju, P.S., House, L.R., and Prasada-Rao, K.E. 1984. The glossy trait in sorghum: its characteristics and significance in crop improvement. *Field Crops Res.* 9, 279–289.

Mathias, F.F. 2010. Agricultural mechanization in Mali and Ghana: strategies, experiences and lessons for sustained impacts. Food and Agriculture Organization of the United Nations Rome. 69 pp.

Mathieu, T.D. 2005. Pour une gestion paysanne de l'agro biodiversité: le cas du sorgho au Mali. Université Paris 7. Mémoire de fin d'étude 103 pp.

Matthew, R. 2012. Backcrossing, Backcross (BC) Populations, and Backcross Breeding. Plant Breeding and Genomics. The Ohio State University. Available at *http://www.extension.org/*

Mehrdad, Y., Narges, A., Farrokh, R.K. and Peiman, Z. 2011. Evaluation of drought tolerance indices among some winter rapeseed cultivars. *African Journal of Biotechnology* Vol. 10. 53 pp.

Melvin, S. R., and, J.O. 2007. Irrigation water conservation strategies for corn. Proceedings of the 19th Annual Central Plains Irrigation Conference and Expansion.53-61 pp.

Menz, M.A., Klein, R.R., Mullet, J.E., Obert, J.A., Unruh, N.C. and Klein, P.E. 2002. A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP and SSR markers. *Plant Mol Bio* 48:483-499

Miller, P.A. and Lee, J.A. 1964. Heterosis and combining ability in varietal top crosses of upland cotton, *Gossypium hirsutum* L. Crop Sci. 4:646-649.

Ministère du Développement Rural (MDR) du Mali. 2002. Plan national pour la gestion intégrée de la fertilité des sols au Mali. 82 pp.

Moghaddam, A, and Hadizadeh, M. 2002. Response of corn (*zea mays* L.). Hybrids and their parental lines to drought using different stress tolerance indices (autumn 2002). *Seed and plant improvement J.* 18(3): 255-275.

Mohan, M., Nair S., Bentur J.S., Bhagwat A., Krishna T.G., Yano M., Bhatia O.R., and Sasaki T. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding* 3: 87-103.

Mondal, W.A. and Choudhuri, M.A. 1985. Comparison of phosphorous mobilization during monocarpic senescence in rice cultivars with sequential and non-sequential leaf senescence. *Physiologica Planta*, 65:221-227.

Moony, H.A., Pearcy, R.W. and Ehleringer, J. 1987. Plant Physiology ecology today. *BioScience*, 37: 18-20.

Morggan, J.M. 1984. Osmoregulation and water stress in higher plants. Annu. Rev. *Plant Physiol.* 35: 299-319

Mortazavian, S.M., Nikkhah, H.R., Hassani, F.A., Sharif-al-Hosseini, M., Taheri, M. and Mahlooji M. 2014. GGE Biplot and AMMI Analysis of Yield Performance of Barley Genotypes across Different Environments in Iran. *J. Agr.* Sci. Tech. Vol. 16: 609-622

Muchow, R.C., Cooper, M. and Hammer, G.L. 1996. Characterizing environmental challenges using models. In "Plant Adaptation and Crop Improvement" (Cooper M. and Hammer, G.L. Eds.), CAB International, Wallingford, UK. pp. 349–364.

Mullis, K. 1990. The unusual origin of the polymerase chain reaction. *Scientific American* 262 (4): 56–61, 64–65.

Murdock, G.P. 1959. Staple subsistence crops of Africa. *Geographical Review* 50:521-540.

Murty, D.S., Tabo, R. and Ajayi, O. 1994. Sorghum hybrid seed production and management. Information Bulletin no. 41. Patancheru 502 324, Andhra Pradesh, India: ICRISAT.

Naeemi, M., Akbari, G., Shirani-Rad A.H., Modares S.A., Sadat-Nuri S.A. and Jabari H. 2008. Evaluation of drought tolerance in different Canola cultivars based on stress evaluation indices in terminal growth duration. Electronic *J. Crop Prod.* 1 (3): 83-98.

Nevado, M.E. and Cross, H.Z. 1990. Diallel analysis of relative growth rates in maize synthetics. *Crop Sci.*, 30: 549-552.

Nguyen, D.C., Nakamura, S. and Yoshida T. 1997. Combining ability and Genotype x Enviromental Interaction in early maturing grain sorghum for summer seeding. Japaness J. *Crop Sci.*, 66: 698-705.

Nguyen, H. 1999. Molecular dissection of drought resistance in crop plants: from traits to genes. Molecular approaches for the genetic improvement of cereals for stable production in water-limited environments. CIMMYT, Mexico DF: 36-40.

Niehaus, M.H. and Pickett, 1966. Heterosis and combining ability in a diallel cross in *sorghum vulgare* Pers, *Crop Sci* 6: 33-36.

Olembo, K.N., Kiplagat, S., Sitieney, J.K. and Oyugi, F.K. 2010. Sorghum breeding in Sub-Saharan Africa: the success stories. The African Biotechnology Stakeholders Forum Nairobi, Kenya: 40 pp.

Omanga, P.G. 1983. Studies on the magnitude and nature of hybrid vigor utilizing male sterile and fertile lines in pigeonpea (*cajanus cajan* (la) millsp.). Master of Science in Agriculture. Andhra Pradesh Agricultural University. 143 pp.

Paterson, A.H., Bowers, J.E., Bruggmann, R., Dubchak I., Grimwood, J., Gundlach, H., Haberer, G., Hellsten, U., Mitros, T., Poliakov, A., and *et al.* 2009. The *Sorghum bicolor* genome and the diversification of grasses. *Nature* 457:551-556

Paterson, A.H., Lander, E.S., Hewitt, J.D., Peterson, S., Lincoln, S.E., and Tanksley, S.D. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* 335: 721-736.

Paterson, A.H., Lin Y.R., Li, Z., Schertz, K.F., Doebley, J.F., Pinson, S.R., Liu, S.C., Stansel, J.W. and Irvine, J.E. 1995. Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. *Science* 269:1714-1718

Patrick, B. and Kelley, R. 2005. Marker-Assisted Selection. Objectives and Overview. Available at *http://www.plantandsoil.unl.edu*

Pereira, M.G., and Lee, M. 1995. Identification of genomic regions affecting plant height in sorghum and maize. *Theoretical and Applied Genetics* 90:380-388.

Perkins, J.M., and Jinks, J.L. 1971. Environmental and genotype environment components of variability. III. Multiple line and crosses. *Hered.* 23: 339-356.

Pollack, L.M., Cardona S.T. and Sotomayor-Rios. A. 1991. Evaluation of heterotic patterns among Caribbean and tropical x temperate maize populations. *Crop Sci.* 31:1480-1483.

Portéres, R. 1951. Géographie alimentaire, berceaux agricoles et migration des plantes cultivées en Afrique intertropicale. C. r. Somm. Séanc. Soc. Biogéogr. 28:16–21.

Powell, W., Machray, G. and Provan, J. 1996. Polymorphism Revealed by Simple Sequence Repeats. Trends in *Plant Science* 1: 215-222.

"R" version 3.1.2, (2014). "Pumpkin Helmet" The R Foundation for Statistical Computing Platform: i386-w64-mingw32/i386.

Ragot, M., Lee, M., Guimarães, E., Ruane, J., Scherf, B., Sonnino, A. and Dargie, J. 2007. Marker-assisted selection in maize: current status, potential, limitations and perspectives from the private and public sectors. Marker-Assisted Selection, Current Status and Future Perspectives in Crops, Livestock, Forestry and Fish. Vol., No., pp. 117–150, ISSN.

Rajib, R., Abdelmoumen T., Khalid R.H., Mohamed R.A. and Jagatpati T. 2014. Molecular Marker-Assisted Technologies for Crop Improvement. I.K. International Publication House Pvt. Ltd. pp. 242-258.

Reddy, B.V., Sharma H.C., Thakur R.P., Ramesh S., Rattunde F. and Mgonja M. 2006. Sorghum Hybrid Parents Research at ICRISAT. Strategies, Status, and Impacts. SAT ejournal. Volume 2. Issue 1. 24 pp.

Robinson, R.A. 1987. Host management in crop pathosystems. Macmillan publishing company 866 third Avenue, New York, NY 10022 pp. 204-221.

Rooney, W.L. 2004. Sorghum improvement integrating traditional and new technology to produce improved genotypes. Advances in Agronomy. Department of Soil and Crop Science, Texas A&M University, College Station, Texas 77843-2474, USA. Volume 83. pp. 38

Rosenow, D.T., and Clark, L.E. 1981. Drought tolerance in sorghum In Loden, H.D. and Wilkinson, D. (ed.) Proc. 36th Annu. Corn and Sorghum Industry Res. Conf. Chicago IL. 9-11 Dec 1981. Am: pp. 18-31.

Rosenow, D.T., Ejeta, G., Clark, L.E., Gilbert, M.L., Henzell, R.G., Borrel, A.R. and Muchow, R.C. 1997. Breeding for pre and post-flowering drought stress resistance in sorghum, Proc, Intern. Can! Genetic Improvement of Sorghum and Pearl Millet. pp. 406-411.

Rosenow, D.T., Quisenberry, C., and Clark, L.E. 1983. Drought tolerant sorghum and cotton germplasm. *Agricultural Water Management* 7:207-222.

Rukam, S.T. Manoj, V.P., Sunil V.P. and Golakiya, B.B. 2010. Molecular Markers and Plant Biotechnology, New India Pub. Agency, pp. 152-153

Sanchez, A.C., Subudhi, P.K, Rosenow, D.T. and Nguyen, H.T. 2002. Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). *Plant Mol Biol* 48:713–726

Sarah, B. and Maria, R., 2011. Applications and potentials of marker assisted selection (MAS) in plant breeding. BfN-Skripten 298 Federal Agency for Nature Conservation pp. 4-10

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

Seetharama, N. 2007. Newsletter on sorghum from National Research Centre for Sorghum (NRCS), India (Hyderabad), Jowar Samachar, Volume 3 No. 1, pp. 2 – 8.

Serigne, T., Louis, V., Jens, M. 2006. Climate Change and Variability in the Sahel Region: Impacts and Adaptation Strategies in the Agricultural Sector. Word Agroforestry Centre (ICRAF), and United Nations Environment Programme (UNEP). 58 pp.

Shirazi, M.A. and Naroui-Rad, M.R. 2011. Evaluation of sorghum genotypes under drought stress conditions using some stress tolerance indices. *African Journal of Biotechnology* Vol. 10 (61), pp. 13086-13089.

Shiri, M.R. 2013. Grain yield stability analysis of maize (*Zea Mays* L.) hybrid under different drought stress conditions using GGE biplot analysis. *Crop Breeding Journal,* 3, 107—112.

Shiri, M.R., Aliyev R.T. and Choukan, R. 2010. Water Stress Effects on Combining Ability and Gene Action of Yield and Genetic Properties of Drought Tolerance Indices in Maize. *Research Journal of Environmental Sciences,* 4: 75-84.

Siart, S., Eva, W., Moussa, K. and Volker, H. 2005. Understanding a local seed system. The example of Sorghum in Southern Mali. Stuttgart-Hohenheim. Conference on International Agricultural Research for Development. 7 pp.

Simmonds, N. and Smartt, J. 1999. Principles of crop improvement, 2nd edn, 81 ackwellscience Inc., New York, chapter 4, pp. 101-113.

Sinha, S.K. 1987 "Drought resistance in crop plants a critical physiological and bio-chemical assessment" Wiley inter-science Newyork

Smith, R. L., Hoveland, C. S., and Hanna, W. W. 1989. Water stress and temperature in relation to seed germination of pearl millet and sorghum. Agron. J. 81, 303–305.

Snowden, J.D. 1936. Cultivated races of sorghum. London: Adlard and Sons. 274 pp.

Soleri, D. and Cleveland, D. 2004. Farmer selection and conservation of crop varieties. In Goodman, R.M. (ed.), Encyclopedia of Plant & Crop Science. Marcel Dekker, New York, pp. 433–438.

Soumare, M. 2015. Zones Agroclimatiques du Mali. Labo Sep Sotuba. Système d'Information Geographique (SIG) IER-Mali.

Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* 98: 503-517.

Station Météo Sotuba, 2014. Relevés météoritiques du CRRA de Sotuba.

Station Météo Sotuba, 2015. Relevés météoritiques du CRRA de Sotuba.

Stephens, J.C. 1937. Male sterility in sorghum: Its possible utilization in production of hybrid seed. *J. Am. Soc. Agron.* 29:690–696.

Stephens, J.C., and Holland, R.F. 1954. Cytoplasmic male-sterility for hybrid sorghum seed production. *Agron. J.* 46, 20–23.

Subedi, K.D. and Ma, B.L. 2005. Nitrogen uptake and partitioning in stay green and leafy maize hybrids. *Crop Sci* 45: 740-747.

Subudhi P.K, Rosenow D.T, Nguyen H.T, 2000. Quantitative trait loci for the stay green trait in sorghum (*Sorghum bicolor* L. Moench): consistency across genetic backgrounds and environments. *Theoretical and Applied Genetics,* Vol. 101, Issue 5-6, pp 733-741

Tao, Y., Henzell, R., Jordan, D., Butler, D., Kelly, A. and McIntyre, C. 2000. Identification of genomic regions associated with stay green in sorghum by testing RILs in multiple environments. TAG *Theoretical and Applied Genetics* 100:1225-1232.

Tao, Y.Z., Jordan, D.R., Henzell, R.G. and McIntyre, C. L. 1998. Identification of genomic regions for rust resistance in sorghum. *Euphytica* 103, 287–292.

Taramino, G., Tarchini, R., Ferrario, S., Lee, M. and Pe, M.E. 1997. Characterization and mapping of simple sequence repeats (SSRs) in *Sorghum bicolor. Theor Appl Genet* 95:66–72

Taylor, J. and Dewar, J. 2003. Fermented products: Beverages and porridges. In 'Sorghum: Origin, History, Technology, and Production', (Wayne Smith C. and Frederiksen, R.A. Eds.), John Wiley & Sons, New York (2000) pp. 751-795.

Teme, N. Toure A. Diallo A. Hash C. Weltzein E. Rattunde F. Rami J. Kouressy M. Vaksmann M. Coulibaly S. 2010. Enhancing sorghum grain yield and quality for the Sudano-Sahelian zone of West Africa using the Backcross Nested Association Mapping (BCNAM) approach. Annual Technical Update Report. IER Mali 12 pp.

Teme, N., Coulibaly, S.B., Diallo, A.G., Vaksmann, M., Dagno K., Tekete, M.L., Hash, C.T., Weltzein, E. Rattunde, F., Rami, J.F., Shah, T. and Sissoko, I. 2012. Enhancing sorghum grain yield and quality for the Sudano-Sahelian zone of West Africa using the Backcross Nested Association Mapping (BCNAM) approach. IER-Mali. Rapport de recherche de la campagne 2011/2012.

Teme, N., Coulibaly, S.B., Diallo, A.G., Vaksmann, M., Dagno, K., Tekete, M.L., Hash, C.T., Weltzein, E. Rattunde, F., Rami, J.F., Shah, T. and Sissoko, I. 2013. Enhancing sorghum grain yield and quality for the Sudano-Sahelian zone of West Africa using the Backcross Nested Association Mapping (BCNAM) approach. IER-Mali. Rapport de recherche de la campagne 2012/2013. 54 pp.

Tenkaouno, A., Miller, F.R., Frederiksen, R.A. and Rosenow, D.T., 1993, Genetics of non-senescence and charcoal rot resistance in sorghum, Theoretical and Applied Genetics, 85:644-648.

Thomas, H, and Howarth, C.J. 2000. Five ways to stay green. *Journal of Experimental Botany*

Thomas, H., and Smart, C. 1993. Crops that stay-green. *Ann. Appl. Biol.* 123: 193-233.

Toure, A. 1980. Etude de l'effet Hétérosis Chez le Sorgho au Mali, Mémoire de fin d'études cycle Ingénieur d'Agriculture, Institut Polytechnique Rural de Katibougou, Mali.

Touré, A. and Scheuring J. 1982. Présence de gènes mainteneurs de l'androsterilité cytoplasmique parmi les variétés locales de sorgho au Mali. *Agron. Trop.* (Paris) 37:362–365.

Toure, A.K., Coulibaly, S.B., Touré, A.O. and Diallo, A.G. 2005. Drought research on sorghum in Mali: breeding for drought prone environments. The Inter Drought-II, the 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production under Drought Stress Bologna Rome, Italy.

Toure, A.K., Traore, S., Bengaly A., Scheuring, J.F., Rosenow, D.T. and Rooney L.W. 1998. The potential of local cultivars in sorghum improvement in Mali. *African Crop Science Journal,* Vol. 6(1):1-7.

Toure, S. 1979. Evaluation de la Collection Malienne de Sorgho: prospection 1978-1979. Mémoire de fin d'études. Cycle Ingénieur d'Agriculture. Institut Polytechnique Rural de Katibougou, Mali.

Traore, S., Coulibaly B., Kone, A., Bagayoko, M. and Kouyate, Z. 2007. Increasing the productivity and sustainability of Millet based cropping systems in the sahelian zones of West Africa. *Journal springer* 567-574.

Traore, S.B., Reyniers, F.N., Vaksmann, M., Kone, B., Sidibe, A., Yorote, A., Yattara, K. and Kouressy, M. 2000. Adaptation à la sécheresse des écotypes locaux de sorghos du Mali. Science et changements planétaires /Sécheresse. Volume 11, Numéro 4, 227-37,

Tuinstra, M.R. and Goldsbrough, P.B. 1998. Evaluation of near isogenic sorghum lines contrasting for QTL markers associated with drought tolerance. *Crop Science* 38:835-842.

Tuinstra, M.R. Grote, E.M., Goldsbrough, P.B., and Ejeta. G. 1997. Genetic analysis of post-flowering drought tolerance and components of grain development in sorghum. *Molecular Breeding* 3:439-448.

Tuinstra, M.R., Grote, E.M. Goldsbrough, P.B. and Ejeta. G. 1996. Identification of quantitative trait loci associated with pre-flowering drought tolerance in sorghum. *Crop Science* 36:1337-1344.

Turner, N.C. 1986. Adaptation to water deficits: a changing perspective. *Austral. J. Plant Physiol.* 13: 175 -190.

Turner, N.C. and Jones, M.M. 1980. Turgor maintenance by osmotic adjustment. In: Turner N.C., Kramer, P.J., eds. Adaptation of plants to water and high temperature stress. New York: Wiley, 87–103.

Van Oosterom, E.J., Jayach, A.R. and Bidinger F.R. 1996. Diallele analysis of the stay-green trait and its components in sorghum. *Crop Science* 36: 549-555

Varshney, R.K., Graner, A. and Sorrells, M.E. 2005. Genic microsatellite markers in plants: features and applications. *Trends Biotechnol* 23:48-55

Vogel, K.E. 2009. Methods Mol Biol. Backcross breeding; 526:161-9. Doi: 10.1007/978-1-59745-494-0-14.

Vom Brocke, K., Weltzien, E., Christinck, A., Presterl, T. and Geiger, H. 2003. Effects of farmers' seed management on performance and adaptation of pearl millet in Rajasthan, India. *Euphytica* 130:267–280.

Wada, Y. and Wada, G. 1991. Varietal differences in leaf senescence during ripening period of advanced *indica rice*. *Japanese Journal of Crop Sciences*, 60:529-636.

Walulu, R.S., Rosenow, D.T., Wester, D.B. and Nguyen H.T. 1994. Inheritance of stay green trait in sorghum. *Crop Science* 34: 970-972

Weising, K., Nybom, H., and Kahl, G. 2005. DNA Fingerprinting in Plants - Principles, Methods, and Applications. Boca Raton (CRC Press).

Weltzien, R.E., Whitaker, M.L., Rattunde, H.F., Dhamotharan, M. and Anders, M.M. 1998. Participatory approaches in pearl millet breeding. In Witcombe, J., Virk, D., and Farrington, J. (Eds.), Seeds of Choice, Intermediate Technology Publications, London, pp. 143–170.

Wenzel, W.G. 1991. The inheritance of drought resistance characteristics in grain sorghum seedlings. S. *Afr. J. Plant Soil Sci.* 8, 169–171.

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

William, J.G., Hanafey, M.K., Rafalski, J.A., and Tingey, S.V. 1992. Genetic analysis using random amplified Polymorphic DNA markers. In Xu, R. (Eds.) Methods in enzymology. Academic Press, San Diego.

Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA Polymorphisms Amplified by Arbitrary Primers are Useful as Genetic Markers. *Nucleic Acids Research* 18: 6531-6535.

Wilson, J.R., Ludlow, M.M., Fisher, M.J. and Schulze, E.D. 1980. Adaptetion to water stress of the leaf water relation of four tropical forage species. *Aust. J. Plant Physiol.*, 7: 207-220.

Winter, P. and Kahl, G. 1995. Molecular marker technologies for plant improvement. *World J. Microbiol. Biotechnol.* 11: 438-448.

World Bank. 2003. Participatory Rural Appraisal, Available at web site: *http://go.worldbank.org.*

Wu, Y.Q. and Huang, Y. 2006. An SSR genetic map of *Sorghum bicolor* (L.) Moench and its comparison to published genetic map. *Genome* 50:84-89

Xu, G.W., Magill, C.W., Schertz, K.F. and Hart, G.E. 1994. A RFLP linkage map of *Sorghum bicolor* (L.) Moench. *Theor. Appl. Genet.* 89:139-145.

Xu, G.W., Subudbi, P.K., Crasta, O.R., Rosenow, D.T., Mullet, J.E. and Nguyen, H.T. 2000. Molecular mapping of QTLs conferring staygreen in grain sorghum (*Sorghum bicolor* L. Moench). *Genome* 43, 461-469.

Xu, J.C. Weerasuriya, Y.M. and Bennetzen, J.L. 2001. Construction of genetic map in sorghum and mapping of the germination stimulant production gene response to *Striga asiatica*. *Acta Genetica Sinica* 28:870-876

Xu, S. 2002. QTL Analysis in Plants. Quantitative Trait Loci. *Methods in Molecular Biology*. Vol.195, pp 283-310.

Xu, Y. 2010. Molecular plant breeding. CAB International. pp. 195-336.

Yan, W.K and Kang, M.S. 2003. GGE biplot analysis: a graphical tool for breeders. In: Kang, M.S., ed., Geneticists, and Agronomist. CRC Press, Boca Raton, FL. data. *Crop Sci.* 42: 21-30.

Yan, W.K, Hunt, L.A., Sheng Q. and Szlavnics Z. 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Sci.* 40: 597-605.

Yan, W.K. and Tinker, N.A. 2006. Biplot analysis of multi-environment trial data: Principles and applications. *Canadian Journal of Plant Science*, 623-645.

Yarnia, M., Arabifard, N., Khoei, F.R. and Zandi, P. 2011. Evaluation of drought tolerance indices among some winter rapeseed cultivars. *African Journal of Biotechnology* Vol. 10. 53 pp.

Zhang, J., Klueva, N.Y. and Nguyen, H.T. 1999. Plant adaptation and crop improvement for arid and semiarid environments. In: Proceedings of the Fifth International Conference on Desert Development: The Endless Frontier. Eds. Traylor, I.R., Dregne, H. and Mathis, K., 12- 17 August 1996. Lubbock, TX., USA. pp. 34-42.

APPENDICES

Appendix 1: PRA questionnaires

I. Identification

Questionnaire number…….Date of interview:
……/……/201……
Name of interviewer: …….. Function:
……...
Location of interview…….

II. Respondent and household Characterization

Name: ……. Gender: Male [ ] Female [ ]
Age: ……. Ethnic group:
……....
Function: Head of the household [ ]; Responsible of works [ ]; Others [ ]
Educational level: Illiterate [ ]; Primary [ ]; Secondary [ ]; Superior school [ ]
Name of respondent village: ……

1. How many persons belong to your household: [ ]
2. How many persons assets in the household: [ ]
3. How many women assets in the household: [ ]
4. How much land does your household own: [ ]/hectare
5. The proportion in area of sorghum: [ ]/hectare
6. How many plow: [ ]
7. How many grain drill: [ ]
8. How many gang plow: [ ]
9. How many cart (donkey or ox cart): [ ]
10. How many cows for plowing: [ ]
11. How many donkeys (for cart): [ ]
12. How many cattle [ ]
13. What are the main sources of livelihood of your household?

	Most income	Some income	Very little	No income
Selling of cash crop	[ ]	[ ]	[ ]	[ ]
Selling of sorghum	[ ]	[ ]	[ ]	[ ]
Selling of others food crops (Maize, millet, root & tuber etc.)	[ ]	[ ]	[ ]	[ ]
Livestock	[ ]	[ ]	[ ]	[ ]
Others (Fishing, Trade, Monthly salary, Farm labouring etc.)	[ ]	[ ]	[ ]	[ ]

14. What are the crops of your household and rank?

	1st rank	2nd	3rd	4th	5th	6th & more	No grow
Maize	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Millet	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Sorghum	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Cotton	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Rice	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Cowpea	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Fruits and vegetables	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Peanut	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]
Others (Soy/Sesame etc.)	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

III. Questions on the sorghum

1. How many year you grown the sorghum? [ ]
2. What is your main reason for growing Sorghum?

	Largely	Little	Very little	No
Household consumption	[ ]	[ ]	[ ]	[ ]
For cash	[ ]	[ ]	[ ]	[ ]
Others (beer, Therapeutic, sacrifices etc.)	[ ]	[ ]	[ ]	[ ]

3. Do you use stem (forage) for animal feeding?

	Largely	Little	Very little	No
Use stem (forage) for animal feeding	[ ]	[ ]	[ ]	[ ]

4. Which varieties of sorghum are you growing? (Name and type of varieties)

	Local cultivar	Improve	Improved local cultivar	Hybrid	Yield (kg/ha)
1……	[ ]	[ ]	[ ]	[ ]	{ }
2……	[ ]	[ ]	[ ]	[ ]	{ }
3……	[ ]	[ ]	[ ]	[ ]	{ }

5. Where did you get seed for planting?

	Largely	Some time	No
Retained own seed	[ ]	[ ]	[ ]
Seed company	[ ]	[ ]	[ ]
Provided by NGO	[ ]	[ ]	[ ]
Research Structure (IER)	[ ]	[ ]	[ ]
Exchange with other farmers	[ ]	[ ]	[ ]
Others	[ ]	[ ]	[ ]

✓ Crop management strategies in households

6. On which kind of soil are you growing sorghum?

Clayey [ ]; gravel [ ]; Sandy [ ]; Clayey/Sandy [ ]; Others [ ]

7. Are you doing the plowing before sowing?
   Yes [ ] No [ ] Why not?

……

8. Are you doing seed treatment before planting?
   Yes [ ] No [ ] Why not?

……

9. Do you apply the mineral fertilizer? Yes [ ] No [ ] Why not?
   ……..
10. Do you apply the organic fertilizer? Yes [ ] No [ ] Why not?
11. Do you use herbicides? Yes [ ] No [ ] Why not?

12. How many manual weeding do you make? [ ]
13. Do you plant sorghum with other crops (Mixe)? Yes [ ] No [ ]

✓ **Level yields in households**

14. What is the level (group) of sorghum yield in your household?

Group yields	**Level**	
Very good (> 1500 kg/ha)	[ ]	……
Good (1101 – 1500 kg/ha)	[ ]	……
Acceptable (701 – 1000 kg/ha)	[ ]	……
Bad (< 700 kg/ha)	[ ]	……

✓ **The criteria (preferences) farmers use for selecting sorghum varieties**

15. What are your criteria (pretences) to select the variety? (Rank: 1 to 5)

1st ……
2nd ……
3rd ……
4th ……
5th ……

16. Which grain qualities you prefer? (Rank: 1 to 3)

1st ……..
2nd ……..
3rd ……..

17. Which stems qualities you prefer? (Height, short, robust, green, etc.) (Rank: 1 to 3)

1st ……..
2nd ……..
3rd ……..

18. The varieties that you grow answer at preferences (criteria of choice) listed above?

	Largely	**Little**	**No**
Varieties answer	[ ]	[ ]	[ ]

19. According to you what are the main reasons of the low yield of the sorghum? (Rank: 1 to 3)

……
……

20. Which solutions (or advises) to improve sorghum yield?

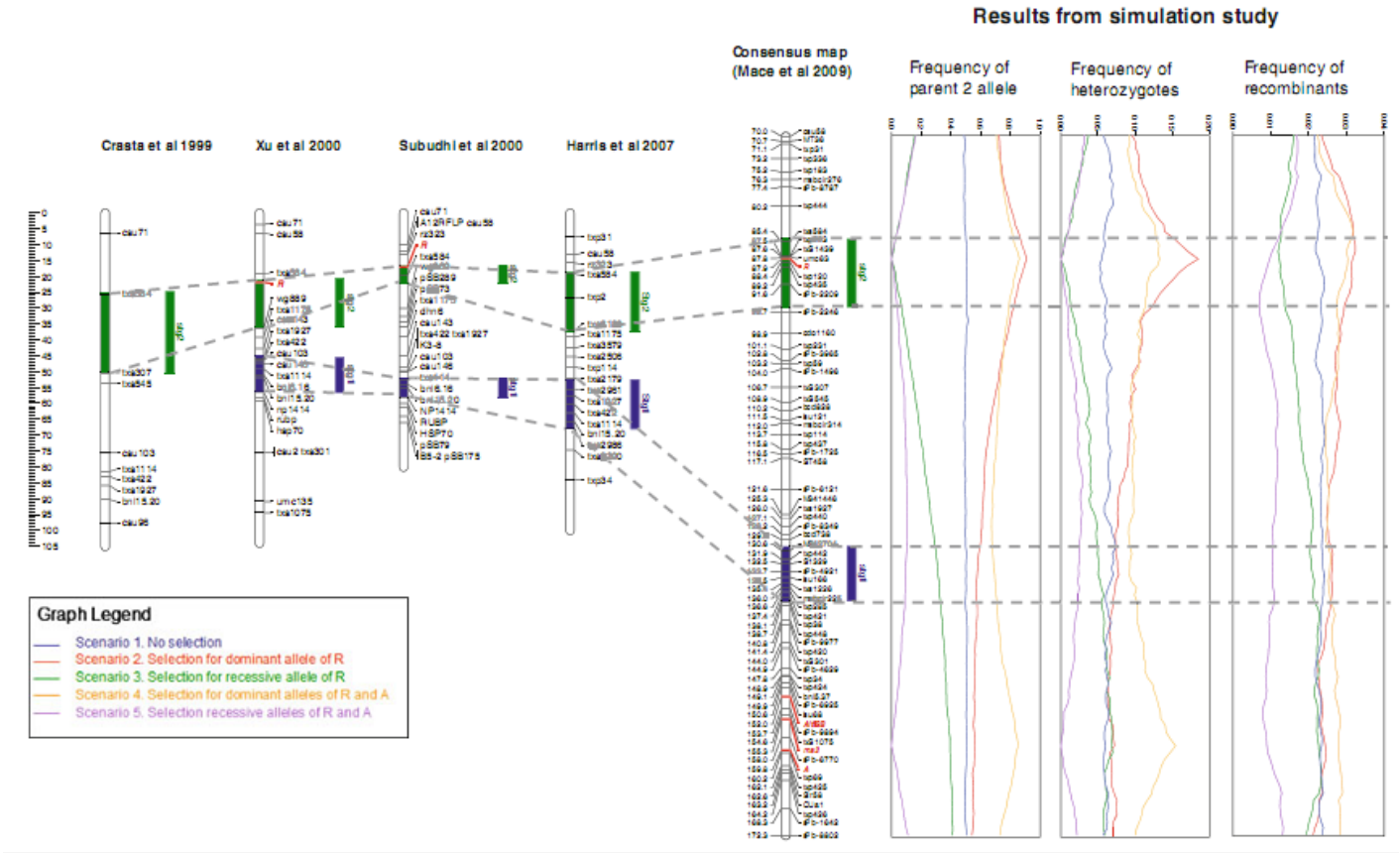
……
……

Appendix 2: Farmers' varietal preferences and criteria for selection

Preferences	1st Pref		2nd Pref		3rd Pref		4th Pref		5th Pref	
	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%
Adaptation to environment	14	5.3	14	5.3	*	*	*	*	*	*
Biomass (Fodder)	*	*	*	*	5	1.9	29	10.9	19	7.2
Birds tolerant	*	*	*	*	2	.8	9	3.4	13	4.9
Diseases tolerant	*	*	*	*	*	*	18	6.8	10	3.8
Drought tolerant	21	7.9	33	12.5	18	6.8	9	3.4	3	1.1
Earliness	68	25.7	31	11.7	20	7.5	*	*	*	*
Easy shelling	*	*	*	*	*	*	12	4.5	10	3.8
Good storage	*	*	*	*	16	6.0	16	6.0	20	7.5
Good taste	*	*	24	9.1	25	9.4	19	7.2	13	4.9
Grain size	15	5.7	9	3.4	18	6.8	5	1.9	4	1.5
Green stem	*	*	*	*	13	4.9	20	7.5	24	9.1
High stem	*	*	*	*	14	5.3	14	5.3	8	3.0
Lodging tolerant	*	*	*	*	6	2.3	15	5.7	11	4.2
Lot of flours	*	*	10	3.8	11	4.2	3	1.1	16	6.0
Middle high stem	*	*	*	*	17	6.4	19	7.2	9	3.4
Middle maturity	*	*	16	6.0	14	5.3	6	2.3	4	1.5
Robust stem	*	*	*	*	*	*	13	4.9	16	6.0
Short stem	*	*	*	*	*	*	2	.8	7	2.6
Striga Tolerant	17	6.4	15	5.7	19	7.2	7	2.6	4	1.5
Vitreousness	*	*	12	4.5	14	5.3	17	6.4	13	4.9
Water tolerant	*	*	*	*	*	*	*	*	2	.8
White grain	39	14.7	40	15.1	23	8.7	11	4.2	4	1.5
Yield	91	34.3	61	23.0	13	4.9	*	*	*	*
Yield in Cooking	*	*	*	*	17	6.4	10	3.8	25	9.4
Total	265	100.0	265	100.0	265	100.0	254	95.85	235	88.68

Pref = preference
Freq = frequency (Number of answers)
% = percent
* = No listed or no answers

Appendix 3: Sections of SBI-03 with stay-green QTL compared across four previous publications and aligned to the consensus map.



Appendix 3. Sections of SBI-03 with stay-green QTL compared across four previous publications and aligned to the consensus map, in addition to the results from the simulation study. Source: Mace *et al. BMC Plant Biology* 2010.

Appendix 4a: Part of scoring sheet for the (B35//82B)-F2 population

N°	Genotypes	stg1 and stg2, LG-03 foreground		stg3, LG-02 foreground			stg4, LG-05 foreground	
		Msbcir225	Mbscir314	Xcup29	Mbscir339	Msbcir312	Xtxp123	Msbcir222
1	98-BE-F5P-82B	A	A	A	A	A	A	A
2	B35	B	B	B	B	B	B	B
3	(B35//82B)F2_4	H	H	H	H	H	H	H
4	(B35//82B)F2_5	H	H	B	B	B	H	H
5	(B35//82B)F2_6	H	H	H	H	H	B	A
6	(B35//82B)F2_7	B	B	A	A	A	D	D
7	(B35//82B)F2_8	B	B	B	B	H	B	B
8	(B35//82B)F2_9	B	B	H	H	H	A	H
9	(B35//82B)F2_10	H	H	H	X	H	H	H
10	(B35//82B)F2_11	A	D	H	H	H	D	B
11	(B35//82B)F2_12	H	H	B	B	H	H	A
12	(B35//82B)F2_14	H	X	H	H	H	A	A
13	(B35//82B)F2_16	B	B	H	H	A	H	H
14	(B35//82B)F2_19	A	A	B	X	H	H	H
15	(B35//82B)F2_20	H	A	H	H	H	H	H
16	(B35//82B)F2_21	B	B	H	H	H	B	H
17	(B35//82B)F2_22	X	A	H	H	H	H	A
18	(B35//82B)F2_23	H	H	H	H	H	H	H
19	(B35//82B)F2_27	A	A	H	A	H	H	A
20	(B35//82B)F2_28	A	H	H	H	H	A	A
21	(B35//82B)F2_29	H	H	H	H	B	H	H
22	(B35//82B)F2_31	H	H	B	X	B	A	H
23	(B35//82B)F2_32	B	B	H	H	A	H	H
24	(B35//82B)F2_33	D	A	A	D	A	X	D

Appendix 4a: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground		stg3, LG-02 foreground			stg4, LG-05 foreground	
		Msbcir225	Mbscir314	Xcup29	Mbscir339	Msbcir312	Xtxp123	Msbcir222
25	(B35//82B)F2_34	B	H	H	H	H	B	H
26	(B35//82B)F2_36	H	H	H	H	H	H	H
27	(B35//82B)F2_39	H	A	A	A	A	H	A
28	(B35//82B)F2_40	B	B	H	X	H	H	H
29	(B35//82B)F2_42	A	A	A	H	H	B	H
30	(B35//82B)F2_43	H	D	H	A	H	D	B
31	(B35//82B)F2_44	H	H	H	H	H	H	H
32	(B35//82B)F2_45	H	D	H	H	B	B	B
33	(B35//82B)F2_46	H	D	X	H	H	D	B
34	(B35//82B)F2_47	H	B	B	B	B	H	D
35	(B35//82B)F2_50	H	H	X	H	H	H	H
36	(B35//82B)F2_51	H	H	A	A	A	H	B
37	(B35//82B)F2_55	B	B	B	B	B	H	A
38	(B35//82B)F2_56	B	H	H	H	X	X	B
39	(B35//82B)F2_60	H	D	H	H	B	D	D
40	(B35//82B)F2_62	H	H	A	A	H	H	H
41	(B35//82B)F2_64	B	B	B	B	B	B	H
42	(B35//82B)F2_65	A	H	A	A	A	H	H
43	(B35//82B)F2_66	H	H	H	H	B	H	H
44	(B35//82B)F2_67	H	D	B	B	B	D	D
45	(B35//82B)F2_70	H	A	A	A	A	H	H
46	(B35//82B)F2_71	H	H	A	A	A	B	H
47	(B35//82B)F2_72	H	D	B	H	B	D	D
48	(B35//82B)F2_73	H	H	H	H	H	B	H

Appendix 4a: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground		stg3, LG-02 foreground			stg4, LG-05 foreground	
		Msbcir225	Mbscir314	Xcup29	Mbscir339	Msbcir312	Xtxp123	Msbcir222
49	(B35//82B)F2_74	B	D	A	A	A	H	H
50	(B35//82B)F2_78	H	H	B	B	A	D	D
51	(B35//82B)F2_79	H	H	B	B	H	H	B
52	(B35//82B)F2_81	H	H	H	H	B	H	A
53	(B35//82B)F2_82	B	H	A	A	A	H	H
54	(B35//82B)F2_83	A	A	A	A	A	A	B
55	(B35//82B)F2_87	H	H	B	B	H	H	H
56	(B35//82B)F2_91	H	H	H	H	H	A	B
57	(B35//82B)F2_92	H	H	X	X	H	H	H
58	(B35//82B)F2_93	B	B	A	A	A	D	H
59	(B35//82B)F2_94	H	H	A	A	A	B	B
60	(B35//82B)F2_95	B	D	H	H	B	H	H
61	(B35//82B)F2_96	H	H	H	H	B	H	A
62	(B35//82B)F2_97	H	X	H	H	B	H	H
63	(B35//82B)F2_98	A	H	A	H	H	A	B
64	(B35//82B)F2_99	H	H	A	A	H	A	A
65	(B35//82B)F2_100	H	A	H	H	H	H	B
66	(B35//82B)F2_101	X	B	X	H	H	X	B
67	(B35//82B)F2_102	B	B	H	H	B	H	B
68	(B35//82B)F2_103	B	B	B	B	H	H	H
69	(B35//82B)F2_104	B	B	H	H	B	A	A
70	(B35//82B)F2_106	A	H	A	A	H	B	D
71	(B35//82B)F2_108	X	D	H	X	H	B	B
72	(B35//82B)F2_109	H	H	A	A	H	A	B

Appendix 4a: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground		stg3, LG-02 foreground			stg4, LG-05 foreground	
		Msbcir225	Mbscir314	Xcup29	Mbscir339	Msbcir312	Xtxp123	Msbcir222
73	(B35//82B)F2_110	A	A	B	B	H	H	B
74	(B35//82B)F2_111	H	H	H	H	H	H	A
75	(B35//82B)F2_112	H	D	B	B	B	B	D
76	(B35//82B)F2_114	B	B	A	B	B	H	H
77	(B35//82B)F2_115	H	A	H	A	A	A	H
78	(B35//82B)F2_116	H	D	A	A	A	D	D
79	(B35//82B)F2_117	H	B	A	A	A	H	B
80	(B35//82B)F2_118	D	A	A	D	A	A	A
81	(B35//82B)F2_123	H	D	H	H	B	B	D
82	(B35//82B)F2_125	H	H	B	H	A	H	B
83	(B35//82B)F2_127	D	A	A	D	A	A	D
84	(B35//82B)F2_128	H	H	X	A	A	H	A
85	(B35//82B)F2_129	A	X	A	A	A	H	B
86	(B35//82B)F2_131	H	B	H	H	H	A	H
87	(B35//82B)F2_132	B	H	H	H	A	B	H
88	(B35//82B)F2_134	H	A	H	H	H	A	H
89	(B35//82B)F2_135	H	B	B	H	H	H	H
90	(B35//82B)F2_136	B	B	B	B	B	H	H
91	(B35//82B)F2_137	A	A	H	H	H	H	H
92	(B35//82B)F2_138	A	B	H	H	H	H	D
93	(B35//82B)F2_139	A	A	B	B	H	A	H
94	(B35//82B)F2_140	A	A	B	B	B	B	H
95	(B35//82B)F2_141	H	H	B	B	B	H	B
96	(B35//82B)F2_142	H	H	H	B	H	A	B

Appendix 4a: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground		stg3, LG-02 foreground			stg4, LG-05 foreground	
		Msbcir225	Mbscir314	Xcup29	Mbscir339	Msbcir312	Xtxp123	Msbcir222
97	(B35//82B)F2_143	A	A	B	B	H	A	H
98	(B35//82B)F2_146	A	D	B	B	H	A	A
99	(B35//82B)F2_147	B	B	B	B	B	B	H
100	(B35//82B)F2_150	A	B	H	H	A	H	H

Appendix 4b: Part of scoring sheet for the (B35//134B)-F2 population

N°	Genotypes	stg1 and stg2, LG-03 foreground			stg3, LG-02 foreground			stg4, LG-05 foreground	
		Msbcir225	Msbcir314	Msbcir276	Xcup29	Msbcir312	Mbscir339	Xtxp123	Msbcir222
1	03-SB-F5DT-134B	A	A	A	A	A	A	A	A
2	B35	B	B	B	B	B	B	B	B
3	(B35//134B)F2_1	A	A	A	B	B	X	H	A
4	(B35//134B)F2_4	H	H	H	A	H	A	B	A
5	(B35//134B)F2_5	A	A	A	A	H	A	B	H
6	(B35//134B)F2_6	H	H	H	A	A	A	B	H
7	(B35//134B)F2_8	H	H	H	B	H	B	A	A
8	(B35//134B)F2_9	A	H	B	A	H	A	H	B
9	(B35//134B)F2_10	A	H	H	A	H	A	H	B
10	(B35//134B)F2_13	D	A	A	A	A	A	D	D
11	(B35//134B)F2_14	H	H	B	B	B	B	B	A
12	(B35//134B)F2_17	A	A	A	B	B	B	H	A
13	(B35//134B)F2_19	X	B	B	H	H	H	B	A
14	(B35//134B)F2_21	B	B	H	H	X	A	H	A
15	(B35//134B)F2_22	H	H	H	H	H	H	A	B

Appendix 4b: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground			stg3, LG-02 foreground			stg4, LG-05 foreground	
		Msbcir225	Msbcir314	Msbcir276	Xcup29	Msbcir312	Mbscir339	Xtxp123	Msbcir222
16	(B35//134B)F2_25	H	H	H	H	B	H	H	H
17	(B35//134B)F2_27	B	B	B	H	B	B	B	B
18	(B35//134B)F2_29	X	A	A	H	X	X	A	A
19	(B35//134B)F2_37	B	H	H	A	B	A	A	A
20	(B35//134B)F2_40	B	A	B	H	B	B	A	A
21	(B35//134B)F2_41	B	B	B	A	H	H	H	H
22	(B35//134B)F2_42	X	H	H	H	B	H	B	A
23	(B35//134B)F2_43	A	A	A	A	A	A	A	H
24	(B35//134B)F2_44	B	B	B	A	A	A	H	H
25	(B35//134B)F2_45	H	H	H	A	B	A	H	A
26	(B35//134B)F2_46	A	A	A	A	A	A	B	B
27	(B35//134B)F2_47	X	A	A	H	H	H	B	B
28	(B35//134B)F2_48	H	H	A	H	H	H	A	A
29	(B35//134B)F2_51	H	H	H	A	H	H	H	B
30	(B35//134B)F2_52	X	H	B	H	H	H	H	B
31	(B35//134B)F2_53	A	A	A	A	H	H	A	B
32	(B35//134B)F2_56	B	B	B	A	H	A	A	B
33	(B35//134B)F2_58	H	H	H	A	A	A	H	H
34	(B35//134B)F2_59	X	A	H	H	H	A	A	H
35	(B35//134B)F2_60	H	H	H	A	B	A	B	A
36	(B35//134B)F2_62	H	H	H	A	A	A	H	A
37	(B35//134B)F2_64	B	B	B	B	B	B	H	H
38	(B35//134B)F2_65	D	A	A	A	A	A	D	D

Appendix 4b: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground			stg3, LG-02 foreground			stg4, LG-05 foreground	
		Msbcir225	Msbcir314	Msbcir276	Xcup29	Msbcir312	Mbscir339	Xtxp123	Msbcir222
39	(B35//134B)F2_66	B	B	H	A	H	A	H	A
40	(B35//134B)F2_67	A	A	A	A	A	A	H	A
41	(B35//134B)F2_68	H	H	H	H	H	H	A	H
42	(B35//134B)F2_70	A	A	A	B	H	B	H	A
43	(B35//134B)F2_71	H	H	H	B	B	B	A	H
44	(B35//134B)F2_72	A	H	H	H	H	A	H	A
45	(B35//134B)F2_73	A	A	A	A	H	A	H	A
46	(B35//134B)F2_77	H	H	H	H	X	H	A	B
47	(B35//134B)F2_80	A	B	B	A	H	A	H	A
48	(B35//134B)F2_82	H	A	A	H	H	H	A	H
49	(B35//134B)F2_83	B	B	H	A	H	A	A	A
50	(B35//134B)F2_84	B	B	B	H	A	H	H	A
51	(B35//134B)F2_85	A	H	H	A	B	A	H	H
52	(B35//134B)F2_87	A	H	H	B	H	B	H	H
53	(B35//134B)F2_88	H	H	A	A	H	A	H	A
54	(B35//134B)F2_89	B	B	B	H	H	H	A	A
55	(B35//134B)F2_92	A	A	A	A	A	A	A	H
56	(B35//134B)F2_94	X	B	B	H	B	H	H	H
57	(B35//134B)F2_95	H	H	H	A	H	A	B	H
58	(B35//134B)F2_96	H	H	H	A	A	A	H	A
59	(B35//134B)F2_97	H	H	H	H	H	H	A	B
60	(B35//134B)F2_100	H	B	B	A	H	A	A	A
61	(B35//134B)F2_101	H	A	A	B	B	B	H	H
62	(B35//134B)F2_103	H	A	A	H	H	H	A	H

Appendix 4b: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground			stg3, LG-02 foreground			stg4, LG-05 foreground	
		Msbcir225	Msbcir314	Msbcir276	Xcup29	Msbcir312	Mbscir339	Xtxp123	Msbcir222
63	(B35//134B)F2_104	H	H	B	X	A	A	H	A
64	(B35//134B)F2_105	B	B	B	A	H	A	A	A
65	(B35//134B)F2_106	X	B	B	H	H	H	A	H
66	(B35//134B)F2_107	A	A	A	A	B	A	H	B
67	(B35//134B)F2_108	H	H	H	B	B	B	H	B
68	(B35//134B)F2_109	B	B	H	A	H	X	H	A
69	(B35//134B)F2_110	H	H	H	X	H	H	A	H
70	(B35//134B)F2_146	A	A	A	H	A	H	A	B
71	(B35//134B)F2_112	B	H	H	H	H	H	H	B
72	(B35//134B)F2_113	A	B	H	A	B	A	H	A
73	(B35//134B)F2_114	B	H	A	H	H	H	A	H
74	(B35//134B)F2_115	B	B	B	H	H	H	H	H
75	(B35//134B)F2_116	D	A	A	A	A	A	D	D
76	(B35//134B)F2_118	X	H	H	B	B	B	B	B
77	(B35//134B)F2_120	H	H	H	A	B	A	H	H
78	(B35//134B)F2_121	H	B	B	A	A	A	A	H
79	(B35//134B)F2_122	H	H	H	A	H	A	H	H
80	(B35//134B)F2_123	X	A	H	A	H	A	H	A
81	(B35//134B)F2_125	B	B	B	H	H	H	B	B
82	(B35//134B)F2_126	H	B	B	B	B	B	A	A
83	(B35//134B)F2_127	A	A	A	H	H	H	A	A
84	(B35//134B)F2_128	A	A	A	H	H	H	H	A
85	(B35//134B)F2_129	H	H	H	A	A	A	H	A
86	(B35//134B)F2_130	H	H	H	A	H	A	H	H

Appendix 4b: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground			stg3, LG-02 foreground			stg4, LG-05 foreground	
		Msbcir225	Msbcir314	Msbcir276	Xcup29	Msbcir312	Mbscir339	Xtxp123	Msbcir222
87	(B35//134B)F2_131	X	A	A	B	H	B	A	A
88	(B35//134B)F2_132	X	A	A	A	A	A	H	A
89	(B35//134B)F2_134	H	H	A	B	A	H	B	H
90	(B35//134B)F2_135	H	X	A	B	H	B	A	H
91	(B35//134B)F2_136	H	A	A	A	A	A	H	A
92	(B35//134B)F2_138	A	A	A	B	B	B	B	H
93	(B35//134B)F2_139	H	H	A	H	B	H	B	H
94	(B35//134B)F2_140	B	A	A	H	H	H	H	H
95	(B35//134B)F2_141	H	H	H	A	A	A	A	A
96	(B35//134B)F2_142	H	H	H	A	H	A	H	H
97	(B35//134B)F2_143	H	H	A	A	H	A	H	H
98	(B35//134B)F2_145	A	A	A	H	H	H	A	H
99	(B35//134B)F2_147	B	H	H	B	H	B	A	B
100	(B35//134B)F2_149	H	H	A	H	A	H	A	H

Appendix 4c: Part of scoring sheet for the (B35//3009B)-F2 population

N°	Genotypes	stg1 and stg2, LG-03 foreground			stg3, LG-02 foreground		stg4, LG-05 foreground	
		Msbcir225	Msbcir314	Msbcir224	Xcup29	Msbcir339	Xtxp123	Msbcir222
1	09PR-3009B	A	A	A	A	A	A	A
2	B35	B	B	B	B	B	B	B
3	(B35//3009B)F2_2	H	H	H	H	H	H	H
4	(B35//3009B)F2_3	H	H	B	H	H	H	H
5	(B35//3009B)F2_4	B	B	H	H	A	A	H
6	(B35//3009B)F2_5	H	H	H	H	H	B	A
7	(B35//3009B)F2_7	H	H	H	A	A	H	H
8	(B35//3009B)F2_8	D	A	H	H	H	B	B
9	(B35//3009B)F2_9	H	H	H	H	H	A	H
10	(B35//3009B)F2_10	H	H	A	A	A	H	B
11	(B35//3009B)F2_11	D	A	B	H	H	D	B
12	(B35//3009B)F2_12	H	H	B	H	H	H	H
13	(B35//3009B)F2_13	H	B	A	H	H	D	B
14	(B35//3009B)F2_14	D	A	A	A	A	H	H
15	(B35//3009B)F2_15	H	X	H	H	A	X	H
16	(B35//3009B)F2_16	B	B	B	H	H	B	B
17	(B35//3009B)F2_17	A	A	B	B	B	B	H
18	(B35//3009B)F2_18	A	A	A	H	H	B	H
19	(B35//3009B)F2_19	H	B	A	H	A	A	A
20	(B35//3009B)F2_20	X	A	H	A	A	H	H
21	(B35//3009B)F2_22	D	A	H	B	B	B	B
22	(B35//3009B)F2_23	X	H	A	B	B	H	H
23	(B35//3009B)F2_24	B	B	B	B	B	H	H
24	(B35//3009B)F2_27	A	A	H	A	A	B	A

Appendix 4c: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground			stg3, LG-02 foreground		stg4, LG-05 foreground	
		Msbcir225	Msbcir314	Msbcir224	Xcup29	Msbcir339	Xtxp123	Msbcir222
25	(B35//3009B)F2_28	H	B	H	A	A	B	H
26	(B35//3009B)F2_30	A	A	A	A	A	H	A
27	(B35//3009B)F2_31	B	H	H	H	H	A	H
28	(B35//3009B)F2_32	A	H	B	H	H	B	H
29	(B35//3009B)F2_33	H	H	H	H	H	H	B
30	(B35//3009B)F2_34	H	B	H	H	H	B	B
31	(B35//3009B)F2_35	X	B	H	H	H	H	B
32	(B35//3009B)F2_36	D	A	H	H	H	H	H
33	(B35//3009B)F2_37	H	H	H	H	H	H	B
34	(B35//3009B)F2_38	H	H	A	H	H	B	A
35	(B35//3009B)F2_39	H	H	A	H	H	B	A
36	(B35//3009B)F2_40	H	H	B	A	A	H	B
37	(B35//3009B)F2_42	X	A	B	H	H	D	B
38	(B35//3009B)F2_43	A	A	H	B	B	A	A
39	(B35//3009B)F2_44	A	B	A	H	H	H	B
40	(B35//3009B)F2_45	D	B	H	H	H	D	B
41	(B35//3009B)F2_46	H	A	A	H	H	B	H
42	(B35//3009B)F2_47	D	A	B	B	B	H	B
43	(B35//3009B)F2_48	X	A	A	H	H	D	H
44	(B35//3009B)F2_49	D	B	B	B	B	H	B
45	(B35//3009B)F2_50	H	H	H	A	H	H	B
46	(B35//3009B)F2_51	B	B	B	H	H	A	A
47	(B35//3009B)F2_53	H	H	B	H	H	H	A
48	(B35//3009B)F2_54	A	A	A	A	A	D	D

Appendix 4c: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground			stg3, LG-02 foreground		stg4, LG-05 foreground	
		Msbcir225	Msbcir314	Msbcir224	Xcup29	Msbcir339	Xtxp123	Msbcir222
49	(B35//3009B)F2_55	H	H	H	H	H	H	B
50	(B35//3009B)F2_56	D	A	H	H	H	A	H
51	(B35//3009B)F2_57	A	H	H	H	H	A	B
52	(B35//3009B)F2_59	H	H	H	H	H	H	B
53	(B35//3009B)F2_61	A	A	H	H	H	A	B
54	(B35//3009B)F2_63	H	H	H	A	A	B	B
55	(B35//3009B)F2_67	A	H	B	H	H	H	A
56	(B35//3009B)F2_70	H	B	H	H	H	X	A
57	(B35//3009B)F2_73	A	H	H	A	A	H	H
58	(B35//3009B)F2_74	A	H	H	D	H	H	B
59	(B35//3009B)F2_76	H	H	B	B	B	H	H
60	(B35//3009B)F2_80	H	H	A	B	B	H	B
61	(B35//3009B)F2_82	D	A	H	H	H	B	H
62	(B35//3009B)F2_83	H	H	B	D	B	B	B
63	(B35//3009B)F2_84	B	H	H	H	H	B	B
64	(B35//3009B)F2_90	B	B	B	B	B	B	B
65	(B35//3009B)F2_92	H	A	A	H	H	A	H
66	(B35//3009B)F2_95	A	A	B	H	H	H	H
67	(B35//3009B)F2_97	H	H	H	H	H	A	H
68	(B35//3009B)F2_98	D	H	H	H	D	B	B
69	(B35//3009B)F2_102	H	A	B	H	D	B	B
70	(B35//3009B)F2_101	B	B	H	B	H	H	A
71	(B35//3009B)F2_104	A	A	H	A	A	H	H
72	(B35//3009B)F2_108	A	A	A	A	A	H	H

Appendix 4c: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground			stg3, LG-02 foreground		stg4, LG-05 foreground	
		Msbcir225	Msbcir314	Msbcir224	Xcup29	Msbcir339	Xtxp123	Msbcir222
73	(B35//3009B)F2_110	D	H	A	H	D	B	B
74	(B35//3009B)F2_111	B	B	H	H	H	B	H
75	(B35//3009B)F2_112	A	A	A	A	A	D	D
76	(B35//3009B)F2_113	B	H	A	H	H	B	H
77	(B35//3009B)F2_115	H	A	H	A	A	H	H
78	(B35//3009B)F2_116	B	B	H	A	A	B	H
79	(B35//3009B)F2_118	H	H	H	D	B	H	B
80	(B35//3009B)F2_119	B	B	B	B	B	B	H
81	(B35//3009B)F2_121	B	B	B	H	H	B	B
82	(B35//3009B)F2_124	A	H	H	A	A	B	B
83	(B35//3009B)F2_125	A	A	H	H	H	A	H
84	(B35//3009B)F2_127	H	H	H	B	B	B	B
85	(B35//3009B)F2_128	A	A	A	H	A	D	D
86	(B35//3009B)F2_129	H	H	B	H	H	H	B
87	(B35//3009B)F2_130	H	H	H	X	D	H	B
88	(B35//3009B)F2_131	D	H	H	H	H	H	B
89	(B35//3009B)F2_132	H	A	H	H	X	H	B
90	(B35//3009B)F2_134	A	A	A	H	H	H	B
91	(B35//3009B)F2_135	D	A	H	H	H	X	A
92	(B35//3009B)F2_136	H	H	H	B	B	A	A
93	(B35//3009B)F2_137	B	H	A	H	H	H	A
94	(B35//3009B)F2_138	A	A	H	H	H	H	B
95	(B35//3009B)F2_140	H	H	A	H	H	A	H
96	(B35//3009B)F2_141	D	H	A	B	B	D	B

Appendix 4c: cont'd

N°	Genotypes	stg1 and stg2, LG-03 foreground			stg3, LG-02 foreground		stg4, LG-05 foreground	
		Msbcir225	Msbcir314	Msbcir224	Xcup29	Msbcir339	Xtxp123	Msbcir222
97	(B35//3009B)F2_142	H	H	H	B	B	H	B
98	(B35//3009B)F2_143	A	A	A	A	A	B	H
99	(B35//3009B)F2_144	D	B	H	B	B	D	B
100	(B35//3009B)F2_148	A	H	H	B	H	A	H

Appendix 5: List of BCNAM populations used in the present study

N°	Population source	Selected lines	Combination sources
1	BCNAM-02	4	Grinkan // Fara Fara
2	BCNAM-02	8	Grinkan // Fara Fara
3	BCNAM-02	11	Grinkan // Fara Fara
4	BCNAM-02	13	Grinkan // Fara Fara
5	BCNAM-02	15	Grinkan // Fara Fara
6	BCNAM-02	16	Grinkan // Fara Fara
7	BCNAM-02	17	Grinkan // Fara Fara
8	BCNAM-02	18	Grinkan // Fara Fara
9	BCNAM-02	39	Grinkan // Fara Fara
10	BCNAM-02	49	Grinkan // Fara Fara
11	BCNAM-02	50	Grinkan // Fara Fara
12	BCNAM-02	55	Grinkan // Fara Fara
13	BCNAM-02	65	Grinkan // Fara Fara
14	BCNAM-02	79	Grinkan // Fara Fara
15	BCNAM-02	89	Grinkan // Fara Fara
16	BCNAM-02	91	Grinkan // Fara Fara
17	BCNAM-02	96	Grinkan // Fara Fara
18	BCNAM-02	118	Grinkan // Fara Fara
19	BCNAM-02	119	Grinkan // Fara Fara
20	BCNAM-02	138	Grinkan // Fara Fara
21	BCNAM-03	11	Fara Fara // Keninkeni
22	BCNAM-03	12	Fara Fara // Keninkeni
23	BCNAM-03	13	Fara Fara // Keninkeni
24	BCNAM-03	15	Fara Fara // Keninkeni
25	BCNAM-03	18	Fara Fara // Keninkeni
26	BCNAM-03	20	Fara Fara // Keninkeni
27	BCNAM-03	23	Fara Fara // Keninkeni
28	BCNAM-03	24	Fara Fara // Keninkeni
29	BCNAM-03	25	Fara Fara // Keninkeni
30	BCNAM-03	34	Fara Fara // Keninkeni
31	BCNAM-03	86	Fara Fara // Keninkeni
32	BCNAM-03	93	Fara Fara // Keninkeni
33	BCNAM-03	99	Fara Fara // Keninkeni
34	BCNAM-03	125	Fara Fara // Keninkeni
35	BCNAM-03	149	Fara Fara // Keninkeni
36	BCNAM-03	158	Fara Fara // Keninkeni
37	BCNAM-03	159	Fara Fara // Keninkeni
38	BCNAM-03	161	Fara Fara // Keninkeni

Appendix 5: cont'd

N°	Population source	Selected lines	Combination sources
39	BCNAM-03	163	Fara Fara // Keninkeni
40	BCNAM-03	169	Fara Fara // Keninkeni
41	BCNAM-08	3	Grinkan // E 36-1
42	BCNAM-08	13	Grinkan // E 36-1
43	BCNAM-08	14	Grinkan // E 36-1
44	BCNAM-08	15	Grinkan // E 36-1
45	BCNAM-08	24	Grinkan // E 36-1
46	BCNAM-08	27	Grinkan // E 36-1
47	BCNAM-08	34	Grinkan // E 36-1
48	BCNAM-08	49	Grinkan // E 36-1
49	BCNAM-08	58	Grinkan // E 36-1
50	BCNAM-08	69	Grinkan // E 36-1
51	BCNAM-08	71	Grinkan // E 36-1
52	BCNAM-08	72	Grinkan // E 36-1
53	BCNAM-08	77	Grinkan // E 36-1
54	BCNAM-08	78	Grinkan // E 36-1
55	BCNAM-08	89	Grinkan // E 36-1
56	BCNAM-08	133	Grinkan // E 36-1
57	BCNAM-08	134	Grinkan // E 36-1
58	BCNAM-08	136	Grinkan // E 36-1
59	BCNAM-08	140	Grinkan // E 36-1
60	BCNAM-08	142	Grinkan // E 36-1
61	BCNAM-11	3	Grinkan // IS15401
62	BCNAM-11	12	Grinkan // IS15401
63	BCNAM-11	13	Grinkan // IS15401
64	BCNAM-11	27	Grinkan // IS15401
65	BCNAM-11	30	Grinkan // IS15401
66	BCNAM-11	34	Grinkan // IS15401
67	BCNAM-11	37	Grinkan // IS15401
68	BCNAM-11	48	Grinkan // IS15401
69	BCNAM-11	71	Grinkan // IS15401
70	BCNAM-11	72	Grinkan // IS15401
71	BCNAM-11	74	Grinkan // IS15401
72	BCNAM-11	76	Grinkan // IS15401
73	BCNAM-11	80	Grinkan // IS15401
74	BCNAM-11	89	Grinkan // IS15401
75	BCNAM-11	129	Grinkan // IS15401
76	BCNAM-11	131	Grinkan // IS15401

Appendix 5: cont'd

N°	Population source	Selected lines	Combination sources
77	BCNAM-11	134	Grinkan // IS15401
78	BCNAM-11	151	Grinkan // IS15401
79	BCNAM-11	163	Grinkan // IS15401
80	BCNAM-11	164	Grinkan // IS15401
81	BCNAM-40	10	Grinkan // CSM388
82	BCNAM-40	31	Grinkan // CSM388
83	BCNAM-40	32	Grinkan // CSM388
84	BCNAM-40	42	Grinkan // CSM388
85	BCNAM-40	49	Grinkan // CSM388
86	BCNAM-40	60	Grinkan // CSM388
87	BCNAM-40	70	Grinkan // CSM388
88	BCNAM-40	71	Grinkan // CSM388
89	BCNAM-40	83	Grinkan // CSM388
90	BCNAM-40	86	Grinkan // CSM388
91	BCNAM-40	89	Grinkan // CSM388
92	BCNAM-40	102	Grinkan // CSM388
93	BCNAM-40	127	Grinkan // CSM388
94	BCNAM-40	138	Grinkan // CSM388
95	BCNAM-40	140	Grinkan // CSM388
96	BCNAM-40	149	Grinkan // CSM388
97	BCNAM-40	162	Grinkan // CSM388
98	BCNAM-40	177	Grinkan // CSM388
99	BCNAM-40	197	Grinkan // CSM388
100	BCNAM-40	198	Grinkan // CSM388
101	BCNAM-42	6	Grinkan // White Kaura
102	BCNAM-42	9	Grinkan // White Kaura
103	BCNAM-42	10	Grinkan // White Kaura
104	BCNAM-42	12	Grinkan // White Kaura
105	BCNAM-42	34	Grinkan // White Kaura
106	BCNAM-42	60	Grinkan // White Kaura
107	BCNAM-42	67	Grinkan // White Kaura
108	BCNAM-42	70	Grinkan // White Kaura
109	BCNAM-42	71	Grinkan // White Kaura
110	BCNAM-42	77	Grinkan // White Kaura
111	BCNAM-42	80	Grinkan // White Kaura
112	BCNAM-42	84	Grinkan // White Kaura
113	BCNAM-42	88	Grinkan // White Kaura
114	BCNAM-42	89	Grinkan // White Kaura

Appendix 5: cont'd

N°	Population source	Selected lines	Combination sources
115	BCNAM-42	95	Grinkan // White Kaura
116	BCNAM-42	98	Grinkan // White Kaura
117	BCNAM-42	112	Grinkan // White Kaura
118	BCNAM-42	113	Grinkan // White Kaura
119	BCNAM-42	123	Grinkan // White Kaura
120	BCNAM-42	124	Grinkan // White Kaura

Appendix 6: List of entries for hybrids trial.

No	Entry Name	Type	Remark
1	(B35//82B)F3-64/BCNAM-76-2	Hybrid	
2	(B35//82B)F3-64/BCNAM-44-1	Hybrid	
3	(B35//82B)F3-64/BCNAM-45-1	Hybrid	
4	(B35//82B)F3-64/BCNAM-27-2	Hybrid	
5	(B35//82B)F3-64/BCNAM-84-1	Hybrid	
6	(B35//82B)F3-104/BCNAM-76-2	Hybrid	
7	(B35//82B)F3-104/BCNAM-44-1	Hybrid	
8	(B35//82B)F3-104/BCNAM-45-1	Hybrid	O.S.
9	(B35//82B)F3-104/BCNAM-27-2	Hybrid	O.S.
10	(B35//82B)F3-104/BCNAM-84-1	Hybrid	
11	(B35//82B)F3-114/BCNAM-76-2	Hybrid	
12	(B35//82B)F3-114/BCNAM-44-1	Hybrid	
13	(B35//82B)F3-114/BCNAM-45-1	Hybrid	
14	(B35//82B)F3-114BCNAM-27-2	Hybrid	
15	(B35//82B)F3-114/BCNAM-84-1	Hybrid	
16	(B35//82B)F3-136/BCNAM-76-2	Hybrid	
17	(B35//82B)F3-136/BCNAM-44-1	Hybrid	
18	(B35//82B)F3-136/BCNAM-45-1	Hybrid	
19	(B35//82B)F3-136/BCNAM-27-2	Hybrid	
20	(B35//82B)F3-136/BCNAM-84-1	Hybrid	
21	(B35//134B)F3-44/BCNAM-76-2	Hybrid	
22	(B35//134B)F3-44/BCNAM-44-1	Hybrid	
23	(B35//134B)F3-44/BCNAM-45-1	Hybrid	
24	(B35//134B)F3-44/BCNAM-27-2	Hybrid	
25	(B35//134B)F3-44/BCNAM-84-1	Hybrid	
26	(B35//134B)F3-64/BCNAM-76-2	Hybrid	
27	(B35//134B)F3-64/BCNAM-44-1	Hybrid	
28	(B35//134B)F3-64/BCNAM-45-1	Hybrid	
29	(B35//134B)F3-64/BCNAM-27-2	Hybrid	O.S.
30	(B35//134B)F3-64/BCNAM-84-1	Hybrid	
31	(B35//134B)F3-89/BCNAM-76-2	Hybrid	
32	(B35//134B)F3-89/BCNAM-44-1	Hybrid	
33	(B35//134B)F3-89/BCNAM-45-1	Hybrid	
34	(B35//134B)F3-89/BCNAM-27-2	Hybrid	
35	(B35//134B)F3-89/BCNAM-84-1	Hybrid	
36	(B35//134B)F3-125/BCNAM-76-2	Hybrid	
37	(B35//134B)F3-125/BCNAM-44-1	Hybrid	

Appendix 6: cont'd

No	Entry Name	Type	Remark
38	(B35//134B)F3-125/BCNAM-45-1	Hybrid	O.S.
39	(B35//134B)F3-125/BCNAM-27-2	Hybrid	
40	(B35//134B)F3-125/BCNAM-84-1	Hybrid	
41	(B35//3009B)F3-16/BCNAM-76-2	Hybrid	
42	(B35//3009B)F3-16/BCNAM-44-1	Hybrid	
43	(B35//3009B)F3-16/BCNAM-45-1	Hybrid	
44	(B35//3009B)F3-16/BCNAM-27-2	Hybrid	
45	(B35//3009B)F3-16/BCNAM-84-1	Hybrid	
46	(B35//3009B)F3-24/BCNAM-76-2	Hybrid	
47	(B35//3009B)F3-24/BCNAM-44-1	Hybrid	
48	(B35//3009B)F3-24/BCNAM-45-1	Hybrid	
49	(B35//3009B)F3-24/BCNAM-27-2	Hybrid	
50	(B35//3009B)F3-24/BCNAM-84-1	Hybrid	
51	(B35//3009B)F3-51/BCNAM-76-2	Hybrid	
52	(B35//3009B)F3-51/BCNAM-44-1	Hybrid	O.S.
53	(B35//3009B)F3-51/BCNAM-45-1	Hybrid	
54	(B35//3009B)F3-51/BCNAM-27-2	Hybrid	
55	(B35//3009B)F3-51/BCNAM-84-1	Hybrid	O.S.
56	(B35//3009B)F3-90/BCNAM-76-2	Hybrid	
57	(B35//3009B)F3-90/BCNAM-44-1	Hybrid	O.S.
58	(B35//3009B)F3-90/BCNAM-45-1	Hybrid	O.S.
59	(B35//3009B)F3-90/BCNAM-27-2	Hybrid	
60	(B35//3009B)F3-90/BCNAM-84-1	Hybrid	
61	02-SB-F4DT-275 (Grinkan)	Control	
62	B35 (BTx642)	Control	
63	98-BE-F5P-82B (82B)	Control	
64	03-SB-F5DT-134B (134B)	Control	O.S.
65	09PR-3009B (3009B)	Control	
66	CSM 388 (Jiguiseme)	Control	O.S.

O.S = out of studied (due to photoperiod sensitive or abortion at flowering period)

Appendix 7: Performance of genotypes for measured traits under well-watered and water stressed regimes.

Genotypes	Yield kg/ha		SCMR		TNGL		BIOM t/ha^{-1}		TNL		TGW (g)	
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
(B35//82B)F3-64/BCNAM-76-2	2756	1590	45.3	35.1	8.2	2.8	12.0	7.9	11.8	11.0	20.0	16.1
(B35//82B)F3-64/BCNAM-44-1	2820	1786	46.6	36.5	3.7	3.0	9.7	8.0	11.0	10.7	27.0	18.1
(B35//82B)F3-64/BCNAM-45-1	3616	1415	46.7	33.0	5.3	3.7	13.5	5.2	11.5	9.7	22.0	17.6
(B35//82B)F3-64/BCNAM-27-2	2386	625	42.2	34.3	7.3	2.0	8.9	8.0	11.0	11.3	19.0	12.4
(B35//82B)F3-64/BCNAM-84-1	2843	255	44.7	20.2	9.3	0.0	12.8	6.7	11.3	10.4	20.5	12.7
(B35//82B)F3-104/BCNAM-76-2	3477	1442	40.7	33.6	5.3	4.3	9.6	8.4	10.7	10.3	20.5	17.4
(B35//82B)F3-104/BCNAM-44-1	2269	505	44.0	23.3	7.5	0.0	10.7	6.1	11.0	10.3	20.0	14.4
(B35//82B)F3-104/BCNAM-84-1	2444	1686	36.6	35.8	4.8	4.2	7.8	5.4	11.2	10.9	22.0	16.6
(B35//82B)F3-114/BCNAM-76-2	1100	820	51.9	31.5	6.5	4.2	14.8	9.3	11.8	11.4	18.0	14.1
(B35//82B)F3-114/BCNAM-44-1	1407	278	42.4	18.5	6.8	4.5	13.8	9.5	12.3	12.7	19.5	11.4
(B35//82B)F3-114/BCNAM-45-1	2962	1648	40.6	33.7	7.8	3.7	13.7	7.2	11.7	10.7	25.0	19.7
(B35//82B)F3-114BCNAM-27-2	3182	418	42.6	20.9	7.3	0.0	7.7	6.4	13.3	12.0	21.5	12.4
(B35//82B)F3-114/BCNAM-84-1	2427	1076	47.1	29.0	4.3	4.3	7.8	4.8	11.3	11.4	23.0	14.6
(B35//82B)F3-136/BCNAM-76-2	1760	1173	42.1	25.4	4.2	0.3	7.9	5.9	11.0	11.7	19.0	15.6
(B35//82B)F3-136/BCNAM-44-1	1458	736	40.7	25.3	4.8	1.7	13.0	6.6	11.7	11.7	18.0	13.4
(B35//82B)F3-136/BCNAM-45-1	1573	260	47.0	27.5	9.2	1.0	12.2	10.3	12.7	11.7	17.0	12.6
(B35//82B)F3-136/BCNAM-27-2	1213	188	49.3	24.5	4.5	0.0	14.2	8.2	13.0	10.4	15.0	11.7
(B35//82B)F3-136/BCNAM-84-1	1355	198	37.1	23.8	5.2	0.0	7.4	5.0	11.0	10.3	17.0	11.4
(B35//134B)F3-44/BCNAM-76-2	4164	1698	52.5	31.7	5.8	3.7	12.9	10.1	11.0	10.7	23.5	16.4
(B35//134B)F3-44/BCNAM-44-1	3018	1795	47.0	38.9	5.8	2.5	13.4	5.4	10.8	9.9	17.0	16.6
(B35//134B)F3-44/BCNAM-45-1	1413	748	38.1	20.7	2.0	1.8	8.5	5.2	10.8	10.0	15.5	13.1
(B35//134B)F3-44/BCNAM-27-2	4227	753	52.3	24.9	6.2	0.5	13.0	5.8	10.8	9.9	23.5	14.6
(B35//134B)F3-44/BCNAM-84-1	2111	648	41.8	38.8	5.8	2.0	10.7	7.1	10.8	11.1	21.5	13.7
(B35//134B)F3-64/BCNAM-76-2	2046	1444	44.9	31.0	7.7	3.2	11.6	8.4	13.0	11.2	22.0	17.1
(B35//134B)F3-64/BCNAM-44-1	2848	609	43.3	13.9	7.2	2.3	10.7	7.0	9.7	10.3	18.5	11.4
(B35//134B)F3-64/BCNAM-45-1	1728	278	38.2	16.3	4.5	0.0	10.3	7.6	11.3	9.0	15.0	10.4
(B35//134B)F3-64/BCNAM-84-1	2007	1093	46.7	25.4	5.8	2.3	8.0	6.9	10.8	11.0	17.5	16.1
(B35//134B)F3-89/BCNAM-76-2	1362	625	40.8	37.5	5.5	0.0	10.3	6.1	11.5	11.7	18.5	10.4
(B35//134B)F3-89/BCNAM-44-1	2007	1577	49.6	20.8	6.0	1.0	14.4	10.4	11.0	10.3	21.5	16.4
(B35//134B)F3-89/BCNAM-45-1	3591	1157	40.4	33.5	4.5	2.7	9.4	5.4	8.0	7.9	21.5	14.6
(B35//134B)F3-89/BCNAM-27-2	1287	1135	45.7	23.7	4.3	2.0	8.8	6.8	11.3	10.9	17.5	15.6
(B35//134B)F3-89/BCNAM-84-1	1757	1162	46.9	32.8	5.0	0.0	13.7	7.3	10.2	11.5	14.0	13.1
(B35//134B)F3-125/BCNAM-76-2	1660	340	48.5	25.6	7.3	0.8	12.7	7.9	11.0	10.9	23.0	13.6
(B35//134B)F3-125/BCNAM-44-1	3803	428	41.7	34.8	7.2	1.7	14.8	7.6	12.3	12.7	21.0	13.7
(B35//134B)F3-125/BCNAM-27-2	1627	1309	47.8	40.2	8.5	2.7	14.8	10.1	10.7	12.4	19.0	16.1
(B35//134B)F3-125/BCNAM-84-1	2387	1353	45.4	23.7	9.3	3.8	12.9	5.5	13.8	12.4	23.0	14.6

Appendix 7: cont'd

Genotypes	Yield kg/ha		SCMR		TNGL		BIOM t/ha^{-1}		TNL		TGW (g)	
	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
(B35//3009B)F3-16/BCNAM-76-2	3016	308	40.8	24.8	3.8	2.0	8.6	8.2	11.2	11.0	20.5	14.6
(B35//3009B)F3-16/BCNAM-44-1	1967	806	45.8	26.8	5.8	2.5	11.4	6.5	10.8	10.9	16.0	14.6
(B35//3009B)F3-16/BCNAM-45-1	2187	1013	54.0	34.1	4.5	2.0	12.5	8.8	8.0	8.5	23.0	15.1
(B35//3009B)F3-16/BCNAM-27-2	3251	633	50.5	25.2	5.0	0.2	11.3	7.9	11.2	10.4	21.5	14.1
(B35//3009B)F3-16/BCNAM-84-1	2405	1758	46.4	38.7	3.5	2.0	10.0	9.2	12.0	11.0	17.0	16.4
(B35//3009B)F3-24/BCNAM-76-2	2803	1296	39.6	21.4	5.7	4.5	9.3	7.8	10.8	12.4	22.0	15.6
(B35//3009B)F3-24/BCNAM-44-1	1518	1081	39.5	27.9	3.5	0.7	13.6	6.1	12.0	10.7	17.0	13.7
(B35//3009B)F3-24/BCNAM-45-1	2169	201	55.9	19.5	8.0	1.3	14.6	8.3	10.0	11.4	21.0	12.7
(B35//3009B)F3-24/BCNAM-27-2	2650	478	51.4	36.6	8.5	1.7	14.5	9.1	11.5	13.0	20.0	13.4
(B35//3009B)F3-24/BCNAM-84-1	1280	655	43.6	24.9	4.7	3.0	9.4	6.3	12.5	12.4	17.0	14.7
(B35//3009B)F3-51/BCNAM-76-2	2628	955	49.3	29.8	4.7	4.0	9.9	9.4	10.2	11.1	19.0	13.7
(B35//3009B)F3-51/BCNAM-45-1	2164	1304	38.9	23.3	4.3	1.7	11.2	8.4	9.0	10.7	19.0	14.7
(B35//3009B)F3-51/BCNAM-27-2	2213	998	47.7	19.5	3.2	2.0	11.7	5.8	6.7	10.0	19.5	16.4
(B35//3009B)F3-90/BCNAM-76-2	2335	1158	43.3	36.1	8.2	2.3	14.9	10.0	11.5	11.0	16.5	14.4
(B35//3009B)F3-90/BCNAM-27-2	1470	358	46.6	41.9	7.2	1.3	8.8	7.9	12.7	13.3	21.0	11.4
(B35//3009B)F3-90/BCNAM-84-1	2707	1273	44.1	33.8	5.8	2.5	10.1	8.2	12.0	10.2	21.5	15.1
02-SB-F4DT-275 (Grinkan)	1052	528	37.8	23.1	6.8	2.0	13.3	8.3	12.3	12.0	19.0	13.4
B-35 (BTx642)	1380	786	57.3	46.3	8.3	4.5	11.1	6.3	9.0	9.4	23.5	13.6
98-BE-F5P-82B (82B)	1335	201	40.3	14.3	7.0	0.0	7.1	5.1	13.2	11.4	20.0	14.7
09PR-3009B (3009B)	2115	436	42.8	26.1	6.8	2.7	8.5	6.5	11.0	10.4	18.5	13.6
Overall mean	**2263**	**901**	**44.9**	**28.6**	**6**	**2**	**11.2**	**7.4**	**11**	**11**	**19.8**	**14.4**
Genotypes	**	**	ns	**	*	**	**	ns	**	ns	**	*
Least significant difference (Lsd)	1165	958	11	13	3.5	2.1	4.5	3.8	2.6	2.0	5.4	3.2
Coefficient of variation (cv %)	25.7	40.5	12.0	18.0	29.4	46.5	19.3	25.4	11.6	9.1	13.7	10.6
Standard error (se)	581	384	5.4	5.3	1.8	1.0	2.2	1.8	1.3	1.0	2.7	1.5

Yield (kg/ha) = Grain yield, **BIOM. (t/ha)** = Biomass, **SCMR** = SPAD chlorophyll meter readings at maturity, **TNGL** = Total number of green leaves per plant at maturity, TNL = Total number leaves at maturity, **TGW (g)** = Thousand grains weight.
WW: well-watered conditions; **WS**: water stress conditions; **S.E.** = Standard error.

Appendix 8: Performance of genotypes for measured traits across water managements

Genotypes	Biom t/ha	TGW (g)	50FLO (day)	SPAD 50FLO	Height (cm)	T.N.L	P.A.	G.A.	VITR.	S.V.
(B35//82B)F3-64/BCNAM-76-2	10.2	18.3	72.0	45.8	162.6	11.5	4.2	3.6	6.5	4.7
(B35//82B)F3-64/BCNAM-44-1	9.0	23.2	74.0	46.7	151.9	10.9	3.3	3.9	6.9	5.0
(B35//82B)F3-64/BCNAM-45-1	9.9	20.1	77.5	42.9	157.9	10.7	3.6	3.6	5.5	4.8
(B35//82B)F3-64/BCNAM-27-2	8.5	16.2	80.1	41.3	143.3	11.1	2.9	3.6	5.2	3.9
(B35//82B)F3-64/BCNAM-84-1	10.2	17.1	78.8	45.9	168.2	10.9	3.0	3.3	2.8	4.1
(B35//82B)F3-104/BCNAM-76-2	9.1	19.2	77.4	38.1	155.4	10.5	3.2	4.6	4.5	4.6
(B35//82B)F3-104/BCNAM-44-1	8.7	17.6	79.0	43.9	180.4	10.7	3.2	3.8	4.3	4.6
(B35//82B)F3-104/BCNAM-84-1	6.7	19.7	73.2	41.7	158.4	11.0	3.5	3.1	6.0	4.8
(B35//82B)F3-114/BCNAM-76-2	12.4	16.3	82.1	43.9	185.6	11.6	3.2	3.4	3.8	4.5
(B35//82B)F3-114/BCNAM-44-1	11.9	16.0	76.9	44.6	144.8	12.5	2.6	3.0	5.2	4.2
(B35//82B)F3-114/BCNAM-45-1	10.9	22.7	80.6	42.7	132.5	11.3	4.1	4.3	8.5	3.4
(B35//82B)F3-114BCNAM-27-2	7.1	17.6	87.1	36.2	171.8	12.8	3.8	3.6	5.7	4.0
(B35//82B)F3-114/BCNAM-84-1	6.5	19.4	79.8	37.1	148.2	11.3	3.0	3.6	7.8	4.4
(B35//82B)F3-136/BCNAM-76-2	7.0	17.5	76.8	45.6	133.8	11.3	3.1	3.3	3.6	4.1
(B35//82B)F3-136/BCNAM-44-1	10.3	16.0	81.0	39.5	149.5	11.7	2.8	4.3	7.3	3.9
(B35//82B)F3-136/BCNAM-45-1	11.4	15.1	75.9	45.6	155.9	12.2	2.5	3.1	5.9	4.5
(B35//82B)F3-136/BCNAM-27-2	11.6	13.6	85.6	40.7	175.5	11.9	3.2	3.5	7.1	4.4
(B35//82B)F3-136/BCNAM-84-1	6.4	14.6	78.6	40.8	202.1	10.7	2.9	3.0	5.6	2.9
(B35//134B)F3-44/BCNAM-76-2	11.7	20.5	73.1	46.8	172.3	10.9	4.2	4.3	6.3	4.5
(B35//134B)F3-44/BCNAM-44-1	10.0	16.8	69.7	45.6	192.2	10.4	4.1	4.4	5.5	4.8
(B35//134B)F3-44/BCNAM-45-1	7.1	14.4	73.0	43.3	161.6	10.5	3.2	4.4	5.6	4.5
(B35//134B)F3-44/BCNAM-27-2	9.9	19.7	73.0	49.9	231.6	10.4	3.5	3.8	5.5	5.0
(B35//134B)F3-44/BCNAM-84-1	9.2	18.1	73.2	49.0	208.5	10.9	4.5	3.9	5.5	5.1
(B35//134B)F3-64/BCNAM-76-2	10.2	19.9	80.7	41.8	160.4	12.2	3.7	3.1	6.6	4.7
(B35//134B)F3-64/BCNAM-44-1	9.1	15.5	74.9	41.3	136.4	10.0	2.9	3.8	5.3	4.2
(B35//134B)F3-64/BCNAM-45-1	9.1	13.0	74.4	42.8	157.8	10.3	2.2	3.7	8.3	4.6
(B35//134B)F3-64/BCNAM-84-1	7.5	16.9	74.2	46.5	175.1	10.9	3.6	3.9	4.4	4.8
(B35//134B)F3-89/BCNAM-76-2	8.5	15.0	80.4	37.4	132.2	11.6	2.9	3.3	7.4	3.9
(B35//134B)F3-89/BCNAM-44-1	12.7	19.3	78.6	47.2	144.0	10.7	4.3	4.1	6.9	3.9
(B35//134B)F3-89/BCNAM-45-1	7.7	18.5	68.2	44.5	139.5	7.9	2.6	4.4	7.2	3.5
(B35//134B)F3-89/BCNAM-27-2	7.9	16.7	72.2	45.2	237.8	11.1	3.5	4.3	7.9	4.7
(B35//134B)F3-89/BCNAM-84-1	11.0	13.6	74.0	38.6	140.6	10.7	3.3	3.6	5.5	4.5
(B35//134B)F3-125/BCNAM-76-2	10.6	18.9	77.7	41.7	185.5	10.9	3.6	2.5	6.8	4.7
(B35//134B)F3-125/BCNAM-44-1	11.7	17.9	79.9	38.6	120.1	12.5	4.0	3.6	7.1	3.7
(B35//134B)F3-125/BCNAM-27-2	12.8	17.7	78.0	45.9	213.0	11.4	4.2	4.5	5.7	5.0
(B35//134B)F3-125/BCNAM-84-1	9.7	19.4	82.2	38.4	191.3	13.2	3.8	4.1	7.1	4.2
(B35//3009B)F3-16/BCNAM-76-2	8.4	17.9	77.4	47.3	152.2	11.1	3.3	4.1	5.8	3.5
(B35//3009B)F3-16/BCNAM-44-1	9.3	15.4	73.9	46.3	169.4	10.8	3.5	3.6	6.8	3.4

Appendix 8: cont'd

Genotypes	Biom t/ha	TGW (g)	50FLO (day)	SPAD 50FLO	Height (cm)	T.N.L	P.A.	G.A.	VITR	S.V.
(B35//3009B)F3-16/BCNAM-45-1	10.9	19.6	68.5	46.1	196.0	8.2	3.2	4.5	5.4	4.3
(B35//3009B)F3-16/BCNAM-27-2	9.8	18.3	72.8	51.7	186.9	10.8	3.3	4.1	6.2	4.2
(B35//3009B)F3-16/BCNAM-84-1	9.7	16.7	73.1	47.0	206.4	11.6	3.6	3.6	7.2	3.6
(B35//3009B)F3-24/BCNAM-76-2	8.7	19.2	80.0	40.4	189.2	11.5	3.5	3.1	6.1	4.7
(B35//3009B)F3-24/BCNAM-44-1	10.4	15.6	78.0	44.1	195.1	11.5	3.4	3.9	5.8	4.1
(B35//3009B)F3-24/BCNAM-45-1	11.9	17.4	73.3	52.8	173.9	10.6	2.7	3.5	5.7	4.0
(B35//3009B)F3-24/BCNAM-27-2	12.2	17.2	84.4	43.1	219.3	12.1	4.2	3.6	7.3	4.9
(B35//3009B)F3-24/BCNAM-84-1	8.0	16.0	74.9	39.8	169.9	12.5	3.7	4.3	5.2	3.8
(B35//3009B)F3-51/BCNAM-76-2	9.7	16.7	79.0	42.0	136.3	10.5	3.2	3.9	6.7	4.4
(B35//3009B)F3-51/BCNAM-45-1	10.0	17.1	73.8	44.5	143.0	9.7	2.8	3.3	6.1	4.1
(B35//3009B)F3-51/BCNAM-27-2	9.1	18.2	77.7	47.3	170.8	8.1	3.8	3.6	5.0	2.9
(B35//3009B)F3-90/BCNAM-76-2	12.8	15.6	85.7	38.3	167.2	11.3	3.1	2.6	5.0	3.9
(B35//3009B)F3-90/BCNAM-27-2	8.4	16.9	89.9	42.5	133.1	13.0	2.6	3.6	5.9	4.6
(B35//3009B)F3-90/BCNAM-84-1	9.3	18.7	75.9	41.0	145.5	11.2	3.5	4.3	4.8	3.5
02-SB-F4DT-275 (Grinkan)	11.1	16.6	87.6	34.3	158.0	12.2	2.9	3.3	6.3	3.9
B-35 (BTx642)	9.0	19.2	67.8	52.0	96.6	9.2	2.8	2.1	4.6	5.0
98-BE-F5P-82B (82B)	6.3	17.7	73.0	42.8	113.9	12.4	2.8	2.9	6.7	5.1
09PR-3009B (3009B)	7.6	16.4	80.2	44.7	191.1	10.7	3.0	4.1	4.2	4.6
Overall Mean	**9.6**	**17.5**	**77.1**	**43.5**	**165.9**	**11.1**	**3.3**	**3.7**	**6.0**	**4.3**
Genotypes	**	**	**	**	**	**	**	**	ns	**
CV%	22.0	13.7	6.2	9.6	15.0	10.9	17.5	20.1	39.2	9.8
SE	2.1	2.4	4.8	4.2	24.8	1.2	0.6	0.7	2.3	0.4
LSD	3.0	3.4	6.8	5.9	35.3	1.7	0.8	1.0	3.3	0.6

BIOM. (t/ha) = Biomass, **TGW (g)** = Thousand grains weight, TNL = Total number leaves at maturity, **S.V.** = Seedling vigor, **50 FLO (day)** = Days to 50 per cent flowering, **SCMR 50 FLO** = SPAD chlorophyll meter readings at 50 % Flowering, **Height (cm)** = Plant height**, P.A.** = Panicle appreciation, **G.A.** = Grain appreciation, **VITR** = Vitreousness.