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GENETIC IMPROVEMENT OF LOCAL SORGHUM (*Sorghum bicolor* L. Moench) VARIETIES FOR DROUGHT TOLERANCE

By

Nofou OUEDRAOGO

(10359703)

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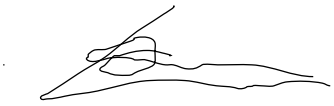
DECLARATION

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.



……

OUEDRAOGO Nofou
Student

……

Prof. Vernon GRACEN
Supervisor

……

Prof. Pangirayi Tongoona
Supervisor

……..

Prof. Eric Yirenkyi Danquah
Supervisor

……

Dr. Jacob SANOU
Supervisor

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ABSTRACT

Drought is the major abiotic factor limiting crop production worldwide. Sorghum is one of the most drought tolerant grain crops that have the ability of addressing food insecurity in semi-arid areas. However, it is exposed recurrently to severe forms of drought and, therefore, requires improvement to cope with moisture stress. The present investigation was undertaken to improve local sorghum varieties for drought tolerance in Burkina Faso. A participatory Rural Appraisal was conducted to identify farmers' constraints and to determine their preferred sorghum varieties. One hundred and ten accessions, including improved and introduced materials, were assessed in full irrigated and terminal drought conditions in an alpha lattice design to identify drought tolerant and high yielding genotypes. A molecular characterization using 26 SSRs markers was conducted with the same accessions to determine the genetic diversity, genetic differentiation and the level of homozygosity of these accessions. Finally, marker-assisted backcrossing was carried out to introgress stay-green QTLs into four farmers' preferred varieties. The major constraint limiting sorghum production in Burkina Faso was *Striga*, a parasitic weed. Drought was the second most important factor reducing sorghum yield and the most important abiotic constraint. The study revealed that terminal moisture stress was the most frequent type of drought but has less effect on yield than pre-flowering drought. Forty seven per cent of farmers rely preferentially on their local landraces. A few preferred improved varieties derived from local landraces, over high yielding exotic (introduced) varieties. Among released varieties, farmers grew more Kapelga in all different climatic zones of the country. There was a strong correlation between grain filling rates, yield under stress, grain number, Stress Tolerance Index (STI) and

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Geometric Mean Productivity (GMP). This indicates that improvement can be achieved using accessions with high STI and GMP indices. Classification based on yield and its components, divided accessions into three main groups. Grinkan was the highest yielding and most drought tolerant genotype in the study. A group of high yielding and drought susceptible accessions were identified including local improved varieties and landraces that performed well under non-stressed conditions and were susceptible to terminal drought. Low yielding and drought tolerant accessions were also identified. Genotypes with high STI indices could be used by farmers to ensure optimal production in drought prone areas and in breeding programs. There was low gene diversity of 0.34 compared to previous molecular studies in Burkina Faso. The Fixation index (*Fst*) value exhibited low genetic differentiation among populations and moderate genetic differentiation between local population and exotic materials. The local accessions have a high level of homozygosity.

Five stay-green QTLs (Stg1, Stg2, Stg3, Stg4 and StgB) were incorporated into four recurrent (Kapelga, Sariaso09, Sariaso01 and Grinkan) backgrounds. The BC_1F_1 lines which carried at least one QTL included eighteen (18) progenies from Sariaso09 and two from Kapelga which were heterozygous at all marker loci flanking the target QTLs. Four, three and four progenies, respectively, from Grinkan, Kapelga and Sariaso01 carried four QTLs. Only one plant, GB7, carried three major QTLs (Stg1, Stg2 and Stg4). Seven, four, three and one progenies, respectively, from Sariaso09, Kapelga, Grinkan and Sariaso01 carried two stay-green QTLs. Fifteen, one and one progenies, respectively, from Sariaso09, Kapelga and Sariaso01 carried one QTL for stay-green. The screening using background markers identified progenies with different recovery rates of the

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recurrent parent genome. A majority of the progenies recovered less than 75% of the recurrent parent genome. Two progenies (GB8 and S1B3) recovered 75% of their recurrent parent genome. Three (S9B73, KB4 and GB7), two (S9B48 and KB16) and one (S1B6) progenies had respectively recovered 78%, 81% and 83% of the recurrent parent. The best 30 accessions identified through phenotypic evaluation and the promising advanced introgression lines should be evaluated in different locations for possible release as commercial varieties. In the future, drought tolerant lines developed through MABC and high yielding lines could help stakeholders to improve their sorghum production, and, therefore, contribute to ensure food security in Burkina Faso.

INTEGRI PROCEDAMUS

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DEDICATION

To my parents for their assistance during my childhood and prayers throughout this study. To my brothers and sisters for their different supports and indefatigable care. To my children and wife for their patience.

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LIST OF ABREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of variance
APS	Ammonium Per Sulphate
BC1F	Backcross and self-pollination generation
BET	Bromure of Ethidium
CIRAD	Centre International de la Recherche Agricole et du Développement
CVS	Collection Voltaïque du Sorgho
DGESS	Direction General de l'Economie et des Statistique
DNA	DesoxyriboNucleic Acid
DNTP	DesoxyriboNucleic Acid Tri Phosphate
FAO	Food and Agriculture Organization of United Nations
FAOSTAT	Food and Agriculture Organization of United Nations, Statistics Department
GB	Backcross progenies derived from Grinkan and B35
GLA	Green leaf area
GMP	Geometric Means productivity
GS	Growth stage
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
INERA FKB	Institute de l'Environnement et de recherche Agricole
KB	Backcross progenies derived Kapelga and B35
LG	Linkage group
LNC	Leaf nitrogen concentration
MABC	Markers Assisted Backcross
MAS	Markers Assisted-Selection
MASA	Minister de l'Agriculture et de la Sécurité Alimentaire
MATAB	Mixed Alkyl Trimethyl Ammonium Bromide
NIL	Near isogenic line
PCA	Principal Component Analysis

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PCR	Polymerase Chain Reaction
PRA	Participatory Rural Appraisal
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphism DNA
RNA	RiboNucleic Acid
REE	Région de l'Est Ecotype
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred line
ROE	Région de l'Ouest Ecotype
RSOE	Région du Sud Ouest Ecotype
SAG	Senescence Associated Genes
SAS	Statistical Analysis Software
SAT	Semi-Arid Tropic
SLN	Specific leaf Nitrogen
SSR	Simple Sequence Repeat
SNP	Single Nucleotide Polymorphism
STI	Stress tolerance index
S9B	Backcross progenies derived Sariaso09 and B35
S1B	Backcross progenies derived Sariaso09 and B35
SWC	Soil and Water Conservation
TE	Transpiration Efficiency
TBE	Tris Borate EDTA
TE	Tris EDTA
TEMED	Tetra Methylene Diamine
TM	Maximal Temperature

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CHAPTER I: Introduction

1.1. Importance of sorghum in semi-arid areas

Sorghum (*Sorghum bicolor* L. Moench) is the fifth most important cereal after wheat, rice, maize, and barley in the world. It is a staple crop in developing countries of sub-Saharan Africa, Central America and South Asia. Sorghum is the main food of about 500 million of people in 30 countries (Dahlberg *et al*., 2011) but, in developed countries such as USA and Australia, it is used for industrial purposes and as fodder. More recently, the sweet sorghum has been used as a bio fuel crop in the form of ethanol production.

World sorghum production was estimated to be 61.3 million tons on 42.1 million hectares in 2013 and more than 70% of the cultivated lands are located in Africa and India (FAOSTAT, 2013). Sorghum is mostly grown in the semi-arid and arid zones where other cereals cannot be produced successfully. Burkina Faso is a semi-arid country where sorghum occupies 45.36% of cultivated lands and constitutes a staple food for about 80% of country's population (MASA/DGESS, 2014). Sorghum plays an important socioeconomic role in Burkina Faso. Sorghum has a large range of adaptability and can be cultivated in a vast series of environments (Ali *et al*., 2009). Sorghum production is faced by several biotic and abiotic constraints that reduce its yield. *Striga hermontica* (a parasitic weed) is the most important biotic stress affecting sorghum production while drought remains the main abiotic factor limiting the crop cultivation resulting in yield instability and food shortage (Abdalla and Gamar, 2011). Drought is the most important abiotic constraint to sorghum production in semi-arid tropics where rainfall is very low with an erratic distribution (Zougmoré, 2003).

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Drought occurrence is erratic and may occur at any stage of growth. However, drought that happens at pre-flowering and post-flowering stages of growth is more severe than the juvenile stages of growth and has the most reduction effects on yield (Kebede *et al*., 2001). In Burkina Faso, terminal drought is more frequent and this hampers considerably sorghum production. It disturbs sorghum plants physiology and leads to barrenness, kernel abortion or shriveled grain which reduces the grain yield (Prasad *et al*., 2008). Its effect can be alleviated only by irrigation or by supplying farmers with varieties that could withstand terminal drought. Unfortunately, smallholder farmers cannot afford irrigation facilities (Derera, 2005), therefore, breeding for drought tolerance is most sustainable alternative to address that major constraint. Developing varieties with post-flowering drought tolerant traits should be a breeding target in order to meet farmers' desires in Burkina Faso where terminal drought is the most frequent.

In sorghum, stay-green is the best characterized form of drought tolerance (Rosenow, 1987). Subudhi *et al*. (2000) reported that post-flowering drought adaptation in sorghum is linked to the stay-green phenotype, which is characterized by the maintenance of green stems and upper leaves under water limitation after flowering. Many researches have been undertaken to find suitable molecular markers linked to post-flowering (stay-green) drought resistance loci. Several stay-green QTLs associated with stay-green have been mapped (Tuinstra *et al*., 1997, 1998; Crasta *et al*., 1999; Tao *et al*., 2000; Xu *et al*., 2000; Subudhi *et al*., 2000; Kebede *et al*., 2001; Haussmann *et al*., 2002; Sanchez *et al*., 2002) and molecular markers linked to these QTLs are available (Hash *et al*., 2003; Harris *et al*., 2007; Kassahun *et al*., 2009). The post-flowering studies identified four major stay-green QTLs designated as Stg1, Stg2, Stg3 and Stg4 which were consistently identified in

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a range of different environments (Subudhi *et al*., 2000; Tao *et al*., 2000) and in different genetic backgrounds (Subudhi *et al*., 2000). The same study revealed also two minor QTLs designed as stgA and stgB. Stg1 and Stg2 were mapped to sorghum chromosome 3, and explain approximately 20 and 30% of the phenotypic variance, respectively (Xu *et al*., 2000; Sanchez *et al*., 2002; Harris *et al*., 2007). At these loci, the stay-green alleles are from BTx642 (B35), the most common source of stay-green alleles. This information could be used in order to improve local sorghum to drought tolerance in Burkina Faso.

Until recently, research activities on sorghum improvement were not given much attention as in other crop such maize, rice or cotton. Farmers rely on their low yielding landraces to ensure their production. Farmers prefer their local varieties to improved or introduced varieties for several reasons such as grain quality for producing the main dish, farming system, susceptibility to diseases and insect damage. Earlier breeders did not take into consideration farmers preferences in their breeding programmes. Therefore, the improved varieties released had poor adoption by farmers due to the incongruence with their needs. It is therefore necessary to undertake a farmer participatory varieties selection in order to identify the constraints and interests of other stakeholders in the sorghum value chain.

To identify different constraints that limit crop production and ensure the fulfillment of farmers' desires, genetic resources should develop genetic material farmers can use. Genetic materials are a resource that plays an important role in agriculture and, in scientific research by supplying breeders or other users with genes of interest. Genotypes identified with a gene of interest could be improved and used for production or could be used as donor parents for this particular gene in order to improve a recurrent elite line.

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It is important to study the variation in the important crops like sorghum. Several studies have been conducted on local sorghum accessions but these studies were limited to agro-morphological and genetic characterization on grain sorghum (Zongo, 1991; Barro-Kondombo, 2010) and sweet sorghum (Nebié *et al.,* 2012; Sawadogo *et al.,* 2014). Some of these accessions have shown potential for breeding, however, only a few have been used in genetic improvement programmes. An investigation has been undertaken by the National Research Program in Burkina Faso to collect and safely store the local sorghum genetic resources. This investigation focus on local sorghum accessions of two agro-ecological zones (Sudanian and Sudano-sahelian climatic areas) for a genetic diversity study and to identify potential drought tolerant genotypes. A part of this activity consists of incorporating stay-green QTLs into local varieties farmers' preferred in order to provide sorghum lines that could lessen terminal drought effect on production.

1.2. Objectives of the study

The overall objective was to improve grain yield of local sorghum varieties under terminal drought stress conditions.

The specific objectives were to:

1. identify farmers' perceptions on impact of drought and determine their preference for varieties and traits;
2. determine sorghum accessions for stay-green characteristics and identify drought tolerant genotypes;
3. assess the genetic diversity among accessions from Sudanian and Sudano-Sahelian climatic zones, and
4. incorporate the stay-green QTLs into four farmers' preferred lines.

The hypotheses tested in the study were:

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- ✓ farmers in Burkina Faso recognize different constraint limiting grain sorghum production and have specific preference trait for sorghum cultivar;
- ✓ local sorghum accessions possess stay-green traits and are drought tolerant traits;
- ✓ accessions from Sudanian and Sudano-sahelian are not really genetically distant, and;
- ✓ stay-green QTLs can be incorporated into farmer's elite sorghum lines.

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Chapter II: Literature Review

2.1 Introduction

This review includes information on the origin, domestication, taxonomy and distribution of sorghum. It also includes sorghum cultivation and production constraints with emphasis on the drought problem in Burkina Faso. Cropping systems on drought prone areas and farmers' preferences for cultivars and traits are reviewed. The effect of drought stress at different growth stages in sorghum, gene expression of leaf senescence, different types of stay-green (stay-green trait, its physiology and correlation to yield in sorghum) are enlightened. Molecular markers, sorghum genome mapping, genetic distance or polymorphism, genetic diversity studies in sorghum, mapping of stay-green QTLs and Molecular Marker Assisted Selection (MAS) are described.

2.2 Origin and domestication of sorghum

Some authors such as Snowden (1936) suggest that races *guinea*, caffra and *durra* are closely related and may have originated from *S. arundinaceum, S. verticilliflorum* and *S. aethiopicum*, but these relationhips are not clear and could not be experimentally proven due to morphological differences among races. House (1985), according to distribution patterns, indicated that *S. verticilliflorum* was the ancestor of races caudatum and caffra, and that durra probably could have been derived from *S. bicolor*. *Guinea* sorghum, primarily found in West Africa is quite difficult to be allied to *S. arundinaceum*. De Wet *et al.* (1975) supported the idea that sorghum had a multiple origin and the race *verticilliflorum* of S*orghum bicolor* may probably be the primitive parent of cultivated sorghum. *S. verticilliflorum* is a plant found in savanna regions, major sorghum areas

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whereas the desert is the habitat of *S. aethiopicum* and *S. virgatum.* *S. arundinaceum* is found in the tropical forests (De Wet *et al.,* 1975). However, excavation studies at an early Holocene archeological site near the Egyptian-Sudanese border (Nabta playa) (Wendorf *et al*., 1992), Dahlberg and Wasylikowa (1996) revealed carbonized sorghum seeds with consequent radiocarbon dates of 8,000 years BP. Mann *et al.* (1983) indicated that sorghum originated and domesticated about 5,000 years ago in northeastern Africa precisely in the north of the equator and east of 10°E latitude.

2.3 Taxonomy of *S. bicolor*

Linnaeus classified three species of cultivated sorghum as follows: *Holcus sorghum*, *H. saccaratus* and *H. bicolor*. Later, Moench (1794) named the genus *Sorghum* and removed it from *Holcus* and made the combination of *Sorghum bicolor*. The present official taxonomic concept of sorghum genus and species agree with the one established by Moench. From this time, all specific names given by different taxonomists who attempted the classification of sorghum genus are taken as synonymous to *S. bicolor* (L) Moench (House, 1985). Snowden (1936) divided the sorghums genus into sections, and subsections. Garber (1950) described five sections: Sorghum (*Eu-sorghum*), *Stiposorghum, Parasorghum, Chaetosorghum* and *Heterosorghum*. The section Sorghum contains *S. bicolor* corresponding to all annual cultivated, wild and weedy sorghums along with two rhizomatous taxa, *S. halepense* and *S. propinquum*. In the continuation of his work, Snowden (1955) subdivided the section sorghum into subsection *Arundinacea* composed of 31 cultivated species and 17 wild related species, and subsection *Halepensia* which comprises 4 weedy species. Overall, he considered as species 48 different types that were well defined by a number of noticeable characters, but he

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admitted that cultivated sorghums could be regarded as races of one vast species. Later on, De wet and Huckabay (1967) put together the 52 species (described by Snowden) into a single species based on the nonexistence of genetic barriers among the Sorghum taxa. *Sorghum bicolor* was fragmented further into three subspecies: *S. bicolor* subsp. *verticilliflorum, S. bicolor* subsp. *drummondii*, and *S. bicolor* subsp. *bicolor*. De Wet (1978), after two decades of biosystematics research, revised the status of sorghums and listed 28 taxa in *subsp. bicolor*, 7 in *subsp. drummondii* and 13 in *subsp. arundinaceum*. Dahlberg (2000) has developed a new integrated classification system which describes the variation found within cultivated sorghums.

2.4 Cultivation and distribution of *S. bicolor* in Africa

Grain sorghums originated from human selection and cultivation (de Wet and Huckabay, 1967) and belong to *S. bicolor* subsp. *Bicolor.* Fifteen agronomic categories have been identified based on five essential spikelet forms (Harlan and de Wet 1972) and they were recognized as races (de wet and Huckabay, 1967). However, based on the aforementioned inflorescence criteria, they are now divided into five basic races (*bicolor, guinea, caudatum, kafir, durra*) and ten intermediate or hybrid races derived from possible combinations between the primary races (*guinea-bicolor*, *guinea-caudatum, guinea-kafir, guinea-durra, caudatum-bicolor, kafir-bicolor, kafir-caudatum, kafir-durra, durra-bicolor, durra-caudatum*). All the basic races are found in Africa (de Wet *et al*, 1970) and their repartitioning is somewhat reliable in the continent (Harlan, 1972). It is possible to differentiate the cultivated sorghums, each in a fundamental location:

The race *kafir* is the commonly grown sorghum of 5° north latitude and east of 20° east longitude (de Wet and Huckabay, 1967) and the name is derived from the Arabic call of

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nonbeliever Bantu "kafr". In Africa, it is cultivated from northern Ghana to northern Nigeria where there is an evident genetic drift from *kafir* into *guinea* races. According to Harlan and Stemler (1967), that race is also grown south of the equator, in South Africa, specifically in the eastern areas where rainfall is over 600 mm per year. Unlike the other races, *kafir* is not fixed to an apparent centre of domestication due probably to migrations of Bantu people. During their historical migrations events, Bantu people came into contact with all the major cultivars.

Bicolor are grown throughout the range of sorghum cultivation in Africa and Asia but are seldom an important crop (de Wet, 1978). They have an impressive diversity in Asia and are also extensive in Africa but, only some cultivars are from Africa. They are low yielding and are usually cultivated for their sour grains used as flavoring for sorghum beer. It appears that this race originated in east Africa from selections out of variety *aethiopicum* (de Wet, 1978).

Durra sorghums: the appellation "*durra*" comes from the Arabic name of sorghum, and its distribution in Africa is associated with Islamic people. This race is broadly cultivated in Asia Minor, Arabia and a few types in Burma and India. In Africa, it is grown along the Nile valley and in Ethiopia where it constitutes the most economically important race of sorghum (de Wet *et al*, 1975). It is also found across arid West Africa, along the periphery of southern Sahara. *Durra* sorghum, according to morphological diversity study, originated from three centres, one in Sudan-Ethiopian region, the second in the near east and the third one in India. The same study suggests that this race is derived from selections from the race *kafir* and were introduced into Arabia and India from East Africa.

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Caudatum race is an old race of grain sorghum and is linked with people who speak languages of the Chari-Nile group (de Wet *et al*, 1975). *Caudatum* sorghums constitute the basic cereal crop in Chad, Sudan, northern Nigeria, Cameroon and Uganda. It is an important farming race, particularly in hybridization with other races and it is usually cultivated by people who raise cattle. *Caudatum* race and the three aforementioned races are not very important economically in Burkina Faso. The few *caudatum* cultivars grown in Burkina Faso are mostly used as sources of germplasm for the improvement of locals' *guinea* landraces.

Race *guinea* was initially found in tropical West Africa with a secondary centre in East African rift valley from Malawi south to Swaziland. It is widely grown in areas where annual rainfall is over 1000 mm and some cultivars are used in décrue agriculture (Harlan and Pasquereau, 1969). It is extensively cultivated in West Africa and some hybrids with drought-tolerant kinds of *durra* are found in northern Nigeria where the range of var. *arundinaceum* overlaps with the range of var. *aethipicum* (de Wet and Huckabay, 1967). Along the eastern edge of cultivation of *guinea*, gene flow between *guinea* and *Kafir* forms give rise to complex intermediate forms that spread out from eastern Africa to south Zululand. *Guinea* races constitute the landraces used in cultivation in Burkina Faso.

2.5 Sorghum production constraints

2.5.1 Sorghum production constraints in Burkina Faso

Sorghum is the most commonly grown crop in Burkina Faso with respectively 44% and 48% of harvested area and cereal production (FAOSTAT, 2013). However, sorghum cultivation is hampered by various biotic and abiotic constraints (Néya and le Normand, 1998, Ratnadass *et al*., 2003) that considerably reduce its yield to around 0.9 t ha^{-1}.

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Biotic constraints includes diseases, weeds and insect pests. Sorghum anthracnose caused by *Colletotrichum graminicola* is the most important seed-borne disease in Burkina Faso and causes yield losses up to 46% (Néya and le Normand, 1998). This fungus causes grain, stalk and leaf anthracnose as well as head. Leaf blight due to *Exserohilum turcicum* is a harmful foliar disease of sorghum which causes significant grain losses by reducing the photosynthetic leaf area (Bergquist, 2000). The disease is severe when it occurs before flowering and can reduce susceptible cultivars' yield up to 50%. When, the infection happens late, the pathogen development is slower and harvest losses are minimal (Hennessy *et al*., 1990).

Rust (*Puccinia purpurea* Cooke) is another foliar pathogen that causes injury to sorghum by decreasing flour quality and grain yield. It is wide spread and occurs everywhere sorghum is cultivated. Under favorable conditions, the pathogen development is fast and influences panicle exsertion and grain development which results in poor grain yield. Its presence facilitates the occurrence of other important diseases such as charcoal rot, stalk rot and grain on sorghum (Frederiksen, 1986).

Among the insect pests, sorghum midge is the major pest in the world as well as in Burkina Faso where it occurs particularly in the South, Central-Southern and Eastern zones of the country (Bonzi, 1979 and Dakuo, 2001). This pest is responsible for yield losses up to 33% that represents about 135 000 tons per year (Dakuo, 2001).

In terms of weed, *Striga Sp* remains the main biotic constraint in Sub-Sahara Africa. This weed has infested up to 20 to 40 million hectares and grain yield losses of host crops is estimated at 10,7 million tons per year (Gressel *et al*., 2004). These parasitic weed infestations are higher on marginal lands where wetness and soil nutrients are limited.

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Striga affects the smallholder' farmers more because they have limited means of controlling it (Marechera *et al*, 2011).

Concerning abiotic constraints, photoperiodism, drought (Tenkouano, 1995 and Trouche *et al*, 2001), low soil fertility and limited external inputs are responsible for low productivity in Sub-Sahara Africa (SSA). In semi-arid areas of West Africa, water stress depends on rainfall patterns which sometimes are characterized by short uni-modal rainy season. The rainy season can be characterized by erratic rainfall with risk of sporadic water stress during juvenile, pre-flowering or post-flowering growth stages. It can also be an intensive rainfall which causes run-off losses and therefore contributes to considerable top soil loss through erosion. The rainfall can also be characterized by a high evaporative demands occurring at the beginning and at the end of the rainy season with evident risk of drought stress during planting and grain-filling stages (Matlon, 1990).

2.5.2 Drought

Drought occurs when there is lack of rainwater for a successful growth of crops (Encarta, 2008). It results in an unexpected and persistent moisture shortage that has undesirable effects on plants. Drought is the most important limiting factor which affects crop production and remains the major cause of yield shortage and food insecurity worldwide (Abdalla and Gamar, 2011). It is more severe in arid and semi-arid tropics areas where the climate is characterized by an erratic distribution of rainfall (Zougmoré, 2003). The risk of drought occurrence may arise in the future due to the climate change which could lead to an increase of temperatures, evapotranspiration losses and to decreased rainfall (World Bank, 2007). Therefore, development of drought tolerant crops is necessary to improve food production in drought prone environments.

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In agriculture, drought tolerance is the ability of a plant to produce its economic product with minimum loss in drought prone environments compared to non-stressed production areas. Crop agronomic performance under drought conditions is due to complex interactions including: drought avoidance (dehydration), drought escape and desiccation tolerance mechanisms (Zhang *et al*., 1999). Crop plant tolerance to drought can be evaluated based on the relative yield or the survival of a genotype compared with other genotypes screened in the same drought condition, and where drought escape is not a major factor (Hall, 1993), or drought escape and yield potential are otherwise accounted for (Bidinger *et al*., 1987).

2.5.3 Sorghum response to drought

Sorghum plants have a wide range of physiological responses to regulate their water status for environmental adaptation, thus to ensure their reproduction and survival. During drought stress, growth reduction, water movement modification linked to the metabolism adjustment, the stomata closure and the reduction of photosynthesis are observed on susceptible cultivars. Drought is unpredictable and may occur at any stage of growth. Rosenow *et al*. (1996) reported that water stress may happen during the early seedling vegetative stage, during panicle development (pre-flowering stage), during the post-flowering stage, within the period of grain filling and physiological maturity. Drought that happens at pre-flowering (GS2) and post-flowering stages of growth (GS3) are more severe and has the more undesirable effects on yield (Kebede *et al*., 2001) than drought at earlier stages of growth (GS1). Post-flowering (terminal) drought is more responsible for yield losses in semi-arid areas of Burkina Faso.

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Pre-flowering drought stress occurs when sorghum plants are under significant water stress from panicle initiation until flowering. Susceptible cultivars exhibit reduced plant height, unusual leaf erectness, leaf rolling, leaf bleaching, leaf firing, delayed flowering, floret abortion, poor panicle exertion, reduced panicle size and reduced seed set. According to Blum *et al*. (1994), at this stage, the formation of generative organs occurs, therefore, it may directly affect the final yield by reducing the photosynthetic efficiency and stem reserve accumulation. Lines tolerant to moisture stress at this stage show good panicle development, normal leaf morphology, and good seed set (Tuinstra *et al*., 1997).

Under post-flowering stages of growth (post-anthesis), drought leads to rapid premature leaf and stalk senescence, barrenness, kernel abortion or shriveled grain, all reduce the seed weight, thus total grain yield (Prasad *et al*., 2008). As a consequence, premature leaf and stalk senescence result in stalk lodging, development of charcoal rot and significant yield loss. This type of stress accelerates chlorophyll loss and leads to a progressive decline of photosynthetic capacity. Early onset of senescence affects assimilation and grain filling. Tolerance to post-anthesis drought is manifested by a normal grain filling with leaves and stems staying-green. Delaying leaf and stem senescence or stay-green has been recognized as the major mechanism of tolerance to post flowering drought stress in sorghum (Rosenow *et al.*, 1996).

2.5.4. Crop production and drought management in semi-arid drought prone areas in Burkina

In Burkina Faso, most of the crop production is subsistence agriculture which depends on the erratic rainfall that varies from the south (more than 1000 mm/ year) to the north (less than 400 mm/year). Over the past 50 years, this distribution pattern has decreased

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latitudinally with the lowest rainfall in the north of the country going from 500 to 400 mm/year and the highest going from 1200 to 1100 mm /year in the south. This indicates clearly a reduction of 100 mm/year of rain throughout the country (Some *et al*., 2004). Because of this reduction in rainfall, managing drought is a prerequisite to improve agriculture production in such climatic condition.

Several drought management strategies to improve crop yields are available and are mostly combined with a wide range of soil and water conservation techniques (SWC). These include some crop management techniques as well. Some of these strategies tend to maximize water availability in the plant root areas while others aim at maximizing plant water uptake capacity. The first set of strategies focus on increasing water availability and soil management through practices that reduce surface runoff such as mulching, stone lines, hal-moons or semi-circular hoops, planting pits or pitting (recognized as zaï system in West Africa), and vegetative bunds. These techniques are easy to design, cheap, easily replicable and adaptable. They also include erosion control and can be constructed on almost any slope. The second set focuses on practices which involves crop management techniques such as optimum crop geometry, optimum crop rotation, intercropping and improvement of crop varieties. These agricultural practices contribute to reduced drought impact on the crop through improvement of water uptake capacity and adequate edaphic growth conditions but are subject to climate risks. For insistence, in years with very low rainfall, the water shortage cannot be compensated by these strategies. They can also be a source of conflict between downstream and upstream water users. Prinz (2002) noted that these techniques can cause damage to flora and fauna adapted to wetlands and running water. The focus on crop management techniques to reduce drought effect is on crop improvement because most of the fresh, innovative

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investment opportunities are in this area. There is an emphasis on a new selection system based on plant genome options. Comprehensive evaluations of this tool speeds breeding approach in the creation of new varieties.

2.5.5. Participatory variety selection status in Burkina Faso

Earlier breeding programmes in Burkina Faso focused on the improvement of high yielding varieties derived from exotic material such as *caudatum* and *kafir* races but it failed to have much impact (Ouédraogo, 2005). These varieties did not fit with local farming systems and therefore were not accepted by farmers. Furthermore, their cost of production was high due to their requirement for fertilizer and improved cultural practices such as plowing and weeding compared to local *guinea* landraces. The opacity of the panicle made them susceptible to diseases such as grain mold and insect damage (Barro-Kodombo, 2010). Matlon (1985) reported a low adoption rate of exotic varieties in West Africa, indicating that farmers still prefer local landraces for their sorghum production. For instance, in Mali, 30% of the sorghum was the improved cultivars. In Niger, about 17.10% and 16% are reported using improved sorghum and millet varieties. In Burkina Faso, less than 5% of farmers have reported using improved sorghum varieties (Olembo *et al*., 2010). Vom Brocke *et al*. (2010) argued that the breeding objectives were not enough directed towards farmers' needs and preferences, and did not target prevailing growing conditions of Burkina Faso. The study revealed clearly that farmers can select for traits while pursuing specific agronomic aims such as adaption. In Mali, Yapi *et al*. (2000) reported that a slight increase (15%) in adaptation of improved sorghum varieties in the early 1990 was mostly due to varieties derived from selections in local germplasm and not improved varieties derived from exotic materials. In Burkina Faso, *guinea* local

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landraces are predominantly used in traditional farming systems (Sapin*, 1984;* Zongo, 1991) due to their adaptability to the environment (Barro-Kondombo *et al., 2008*).

2.6. Gene expression of leaf senescence

Leaf senescence is a degenerative process by which cells promote their own death via natural activation of self deterioration system. Once, the leaf reaches the top of assimilatory capacity, mesophyll tissue starts to lose its greenness and turns red or yellow. The color alteration is due both to the synthesis of new compounds such as anthocyanins and phenolics, and to the degradation of chlorophyll (Matile, 1992). During the senescence process, the photosynthetic apparatus is broken down and nutrients are relocated from deteriorating cells to growing young leaves, storage tissues, or developing seeds. For example, nitrogen from leaves of deciduous trees is transferred to the stems where it is used for the synthesis of storage proteins that will support development of new cells during the next season (Clause and Apel, 1991). Gan and Amasino (1997) also reported that tissues (tissues close to the vascular system) involved in nutrient exportation are the last to senesce in many species. Leaf senescence ends by foliar abscission in some species and requires clearly localized and timed senescence in the petiole.

The initiation of leaf senescence is regulated by both external (environmental) and internal factors. The external factors refer to abiotic and biotic stresses such as nutrient deficiency, pathogen attack, wounding, shading, salinity, heat and drought, whereas the internal causes are due to development stage, reproductive stage, age and hormone levels. Drought accelerates leaf senescence (Gan and Amasino, 1997) and also limits yield in crop production by reducing the photosynthesis' effect. After the onset of senescence, a set of genes regulates the process until leaf death. The process proceeds along with the

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decreased expression of some genes related to photosynthesis, decreased protein synthesis and the increased expression of senescence associated genes (SAGs) (Lim *et al*., 2007). Overall, six broad categories of genes whose expression influences the pattern of senescence are known and listed below:

1. Genes regulating the primary metabolic activities of viable cells, e.g. respiration components, ribosomal RNA and protein synthesis;

2. Genes expressed earlier during the leaf life that become active later such as homeotic genes which may act on following genes expression by indicating the parts of the genome that can be accessed, and genes which encode messenger RNA or proteins that become active later during the life of the leaf e.g. zymogens and vacuolar enzymes;

3. Genes controlling growth or carbon assimilation components and which contribute to the progress of senescence by switching off, e.g. nuclear and plastid genes for Calvin cycle enzymes and thylakoid proteins;

4. Genes specifically activated at the initiation of senescence and control its timing and rate of progress. Such genes activation may be due to environmental and internal causes. They can be classified as 'causal';

5. Genes encoding proteins induced *de novo* or showing increased expression during senescence, e.g. catabolic enzymes. These genes may be designated as 'consequential';

6. Genes encoding proteins involved in the remobilization of storage products, e.g. enzymes of gluconeogenesis. These genes play an important role in other similar stages of the plant life cycle, e.g. seed germination.

Numerous other regulatory genes of leaf senescence have been found through functional identification of some *SAG*s and through genetic screening of senescence mutants. Lim *et*

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al., (2007) reported that more than 800 genes are distinctively upregulated during senescence and involved in alteration of cellular physiology that underlies leaf senescence (Smart, 1994).

2.6.1. Different types of stay green

The term stay-green is delayed senescence of leaves and is characterized by the maintenance of green stem and upper leaves compared with standard reference genotypes. Different greenness (stay-green) phenotypes could arise due to alteration within each class of senescence related genes. It is essential to highlight that the stay-green trait in one genetic line may have only a superficial resemblance to the same trait in another genotype and may arise from different physiological and biochemical changes. Five distinctive types of stay-green (Type A, Type B, Type C, Type D, Type E) were reported by Thomas and Howarth (2000).

Types A and B are functionally stay-green and could arise after modification of genes involved, respectively, in the timing of the initiation of senescence and the regulation of its rate of progress. In type A stay-green senescence is initiated late but then proceeds at the normal rate. This assumes a strong correlation between chlorophyll content and net carbon output leading to it being classified as quantitative stay-green behavior (Thomas and Howarth, 2000). Type B stay-green starts senescence on schedule, but, afterwards, senesce progresses relatively slowly. These stay-green types (A and B) continue their photosynthetic activities longer than normal and are supposed to show a higher yield in crops for which carbohydrate is a major component of the harvest (Thomas and Smart, 1993).

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Types C and D stay-green, in other hand, are nonfunctional forms of stay-green. Plants with these types appear green but lack photosynthetic competence. Type C stay-green plants may arise after alteration of genes involved in the regulation of chlorophyll catabolism and a type D stay-green plant are characterized by a premature death in herbaceous species.

Another type of stay-green is characterized by late maturing types in which leaves tend to be greener at maturity than those of early genotypes. Greenness preservation in this type may be simply because its chlorophyll rate that has further to fall. This type of staying green may be classified as type E.

Stay-green classification in this way is helpful for understanding the kinds of physiological processes or modified genes that control of the phenotype although, in practice, stay-green can be the result of combination of two or more different functional types (Thomas and Howarth, 2000). For insistence, Thomas and Smart (1993) reported that genes responsible for type A and B stay-green lines come probably from class 4 of the classification of senescence-related genes, whereas genes involved in type C derived more likely from classes 5 and 6.

2.6.2. Gene action conditioning stay-green in sorghum

Genes represent the basic units of inheritance and control the expression of characters, either individually or in combinations. Gene action is the way genes express themselves and can be studied through the biometrical approach in which genetic components are divided into additive, dominance variance and epitasis (Robinson *et al*., 1949; Falconer, 1981). Different types of gene action control stay-green and its associated traits.

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To ascertain the genetics of non-senescence and charcoal rot in sorghum, Tenkouano *et al*. (1993) evaluated families from a diallel crosses between two non-senescent, charcoal rot resistant inbred lines (B35 and SC599-11E) and two senescent, charcoal rot susceptible inbreds (BTx378 and BTx623) under controlled and field conditions. They found that non-senescence was controlled by dominant and recessive epistatic interactions between non senescent inducing loci and a third locus with modifying effects. They also concluded that non-senescence and charcoal rot resistance are not different manifestations of a single trait and must not be evaluated with each other.

Walulu (1994), in order to find the mode of gene action for the stay-green trait in sorghum, evaluated stay green inbred line (B35), non stay-green inbred line (RTx7000), and their F_1, F_2, and backcrosses progenies in moisture stress conditions at the grain filling period in the field and rainout shelter. Stay-green was evaluated on an individual plant basis by visual scoring of leaf and plant death at or soon after physiological maturity. Their results suggest that a major gene influences the stay-green trait in B35, and that this gene exhibits varied levels of dominant gene action depending on the environment where the evaluation is made. They also found that the frequency distribution of BC_1F_1 population grown in field indicated complete dominance of this single major gene.

Khizzah *et al*. (1995) studied through a diallel mating design the genetic and physiological components of post-flowering drought tolerance by using four parental lines [two parental lines with high water and high osmotic potential (BT×3179 and B35) and two contrasting parental lines with low water and low osmotic potential (RTx430, and RTx7000)] and their progenies (F1 and F2) under irrigated and non-irrigated conditions. They suggested that inheritance of drought tolerance was under the control of

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multiple genes with dominant and recessive epistatic effects. According to the heritability estimates, they proposed either B35 or BTx3179 to be used as parents for improvement of drought tolerance. They also found important correlations between dry matter accumulation, water potential and percent moisture. They also reported correlation between relative water content and water potential.

Van Oosterom *et al*. (1996) used a full diallel analysis to describe stay-green and its components. According to their result, inheritance of stay-green is a function of its components and suggested that the inheritance of the onset of senescence was additive whereas the inheritance of the rate of senescence was completely dominant for the slow rate over the fast rate. Thus, the relative green leaf area duration, being the sum of an additively and a dominantly inherited trait, expressed partial dominance for a large green leaf area duration. They also determined that the expression of heterosis for non-senescence was stable across experiments (environments).

2.6.2.1. The stay-green trait, physiology and correlation to yield in sorghum

Sorghum is known to be particularly adapted to semi-arid areas, and contains various mechanisms to escape and withstand drought. Understanding the physiological basis of such drought resistance mechanism is useful to develop new varieties that are better adapted to dry conditions. Keeping functional leaves alive is a fundamental strategy for increasing crop production, specifically under water-limited conditions (Borrell *et al.,* 2000a).

A review of physiological studies about stay-green and its relation to yield in sorghum is described in paragraphs below. Some authors relate the stay-green to the nitrogen content

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of the vegetative parts (leaves and stems), some to transpiration capability while some refer to the genetic ability of particular genotypes.

Muchow (1988) investigated the effect of variable nitrogen supply on leaf initiation, expansion and senescence and also on leaf nitrogen content in sorghum and maize. The study indicates that N uptake and allocation to leaves was enough to keep Specific Leaf nitrogen (SLN) above 1.0 g m^{-2} and showed that maize was less responsive to N than sorghum. The effect of nitrogen in leaf initiation was not significant between sorghum and maize. SLN was more important at higher rates of N applied in maize than sorghum. This effect declined during the grain filling and remained more important in maize than in sorghum. Leaf senescence was more noticeable in sorghum than maize at low rates of applied N during grain filling.

Rosenow *et al*. (1983), McBee (1984), Wolfe *et al*. (1988) and Borrell *et al*. (2000a) found that sorghum hybrids containing the stay-green trait kept more photo-synthetically active leaves than do hybrids that do not contain it. Borrell and Hammer (2000) determined that the expression of the stay-green trait under terminal drought is as a consequence of balance between demand of nitrogen (N) by the grain and N supply of translocation from vegetative plant parts (stems and leaves) based on the fact that leaf longevity and photosynthetic capacity are related to its N status (Thomas and Rogers, 1990). Borrell and Hammer (2000) reported that N concentration in stem, leaves and panicle was lower in senescent genotypes than in stay-green ones. In addition, for sorghum plants grown under terminal water stress, they discovered positive correlation between above-ground N content and green leaf area index at anthesis (r = 0.8), mid-grain filling (r = 0.47) and maturity (r = 0.69). In that study, they also found that leaf

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nitrogen concentration was correlated with onset (r=0.75) and rate (r=-0.78) of leaf senescence. They proposed that genotypes stay-green longer due to the content of N in their leaves based on the higher status of specific leaf nitrogen (SLN) in stay-green genotypes than in intermediate and senescent genotypes from the anthesis to maturity. At early stage of growth (27 days after emergence), they also observed differences in leaf N status between stay-green and non stay-green genotypes. This clearly shows that the leaves of stay-green types contained more N even before the trait was expressed.

Renuka and Chimmad (2006) studied the effect of irrigation at post anthesis stages on stay-green trait on sorghum and determined that stay-green (E36-1, TSLG-262) and drought tolerant (C-43) genotypes maintained higher N content in different plant parts at all stages of growth and they attributed the increase of N content to their capacity to absorb more nitrogen from the soil.

Lemaire *et al.* (2008) determined a diagnostic tool for plant and crop N status at the vegetative growth stage based on the determination of the critical N dilution curve for each crop species considered. This study included C3, C4, monocots and dicots plants and they found out that plant N concentration decreased as crop grew due to the ontogenetic decline in leaf area per unit of plant mass, and the remobilization of N from leaves at the bottom of the canopy to leaves at the top. The study showed that the N accumulated in crop biomass (within leaf and stem) at the end of this period (at anthesis) determines the potential yield through the number of grains (about the 2/3 third of the amount of N is available for grain filling) for annual crops. The remaining N being absorbed during the grain filling is used more for grain protein synthesis.

Moll *et al*. (1994) reported that a delayed remobilization of N from leaves having a larger pool of N in stay-green genotypes would uphold photosynthetic capacity longer, and

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consequently continue to supply carbohydrate to the developing grain which possibly results in higher grain yield. Therefore, they suggested that a prolonged accumulation of dry matter and N uptake from maize roots during grain filling are important characteristics related to high yields.

For indirect appreciation of nitrogen status in leaf, Chapman and Barreto (1997) used a chlorophyll content meter (SPAD) to estimate specific leaf nitrogen (SLN) as chlorophyll content is correlated with nitrogen concentration. Xu *et al*. (2000), also used the SPAD meter and a spectrophotometric method and found that the SPAD value had a significant linear relationship with total leaf chlorophyll (R2 = 0.91) and with visual stay-green rating (R2 = 0.82).

During grain filling, a daily decrease in the rate of leaf senescence from 3-1% of leaf area resulted in doubling of grain size from about 15 mg to 30 mg. From that result, Borrell *et al*. (1999) suggested that sorghum grain size was correlated with the relative rate of leaf senescence, therefore the stay-green trait has potential to increase sorghum grain yield by improving both grain number and grain filling ability. Under post-flowering drought, Borrell *et al*. (2000) found that sorghum yield was positively correlated with green leaf area at maturity and noticed that stay-green sorghum hybrids produced 47% more biomass than their non-stay-green counterparts under terminal moisture deficit conditions at post-anthesis stage. From the strong correlation observed between leaf N concentration (LNC) at anthesis and grain yield under drought, Borrell *et al*. (2000b) suggested the use of LNC at flowering stage as a selection index for drought resistance in sorghum breeding programmes.

Unfortunately, little is known about how the stay-green trait works to absorb more N during grain filling. Borrell and Hammer (2000) suggested that a range of responses

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including transpiration efficiency is initiated by higher SLN. Transpiration efficiency (TE) enables the plant to have high yield potential which eventually leads to important grain yield and lodging resistance under post-anthesis drought stress conditions.

Henderson *et al*. (1997) reported that the transpiration efficiency (TE), the amount of biomass the sorghum crop produces per unit of water it captures for transpiration, varies among cultivars, but the differences were small. However, Hammer *et al*. (1997) examined the same parameter through a simulation study and, found that a small increase in TE may have great influence on sorghum yield in some environments. Borrell *et al*. (2000) noticed that differences in biomass of nine sorghum genotypes when grown under post-anthesis water deficit were related to variation in transpiration and transpiration efficiency for sources of stay-green (A35 and RQL12). The transpiration efficiency varied up to 45% among the nine genotypes, ranging from 3.3 g kg^{-1} (AQL39/R69264) to 6.0 g kg^{-1} (A35/RQL36), although, it remained similar for 7 of 9 genotypes. Some studies have also revealed that TE is correlated with plant mineral or ash content (Masle *et al*., 1992), though the physiological basis of this association remains unknown.

Van Oosterom *et al*. (1996) indicated that the ability of the leaves to maintain greenness during post-anthesis drought has a genetic basis in sorghum but they emphasized that the external factors affect significantly the expression of the character. Sanchez *et al*. (2002), Harris *et al*. (2007) studied Quantitative Trait Loci (QTL) by using recombinant inbred lines (RILs) and, near-isogenic lines (NILs) and they determined that tolerance to pre-flowering and post-flowering drought stress were associated with a number of genomic regions. From RIL population developed with B35 and Tx7000, they identified four genomic regions associated with the stay-green. Payton *et al*. (2003) used the same population [stay-green parent (B35), senescent parent (RTx7000) and derived mapping

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populations (RILs and NILs)] containing specific stay-green QTLs to analyze the physiology of stress plants which included gas-exchange analysis for photosynthesis, transpiration, stomatal conductance, leaf greenness, and chlorophyll content. They linked drought response in sorghum to an internal (genetic) factor that must be identified and used in improvement for drought tolerance.

More recently, Borrell *et al*. (2014), tried to elucidate the physiology and genetic mechanism underpinning the stay-green trait by studying NILs developed from RTx7000. They determined that stay-green QTLs reduced canopy size at anthesis by decreasing the number of tillers and upper leaf sizes. This reduction in transpirational leaf area allows the conservation of soil water before anthesis for use during grain filling. The increase in water uptake ability during grain filling of stay-green NILs resulted in greater post-anthesis biomass production and grain yield.

2.7. Application of molecular markers in genetic analysis of sorghum

Genetic markers are measurable characters that can detect the existence of dissimilarity in a trait, a protein or DNA sequence. They can be defined as an allele, a DNA sequence, a chromosomal landmark, a cytogenetic segment, an enzyme, a protein or a trait that allows for tracing a precise individual or organism. A difference in a phenotype or genotype may act as a genetic marker if it identifies characteristics of an individual genotype or phenotype and if its inheritance can be followed through different generations. There are three main types of genetic markers: (i) morphological markers are phenotypic traits or characters which are evident to the naked eye. They are also known as visible or classical markers; (ii) biochemical markers or allozyme markers are allelic forms of enzymes called isozymes. These markers are based on protein polymorphisms and can be

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separated on electrophoretic gels and then detected by staining the gels; and (iii) DNA or molecular markers are those that revealed the genetic variation at the DNA level (Jones *et al*., 1997a; Winter and Kahl, 1995).

Morphological markers have been utilized as earlier tools for germplasm management and, breeding (Eagles *et al*., 2001; Weeden *et al.*, 1994), but they have a number of weaknesses such as low polymorphism, low heritability, late expression and vulnerability to environmental impact (Smith and Smith, 1992). Like morphological markers, the biochemical markers show some disadvantages. They are influenced by environmental factors and limited in number. Their major hindrance is that each of the proteins that are being scored may be expressed at different times in development and by different tissues. Unlike morphological and biochemical markers, DNA markers are almost non-limited in number and are not affected by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995). These markers are discerningly neutral because they are mostly located in non-coding regions of the DNA. Among the genetic markers, DNA markers are the most commonly used due to their abundance. They have various utilizations in plant breeding such as evaluation of diversity level within germplasm, identification of cultivars, their application in different selection procedures and their use in the construction of linkage maps (Weising *et al*., 1995; Baird *et al*., 1997; Henry, 1997; Jahufer *et al*., 2003).

Molecular markers can be divided into three classes based on their method of detection. These include: (i) hybridization based markers such as Restriction Fragment Length Polymorphism (RFLP); (ii) PCR-based markers such as Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphism DNA (RAPD) and microsatellite or Simple Sequence Repeat (SSR); and (iii) sequence based markers such

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as Single Nucleotide Polymorphism (SNP) (Winter and Kahl, 1995; Jones *et al*., 1997b; Gupta *et al*., 2001).

2.7.1. Genome mapping

The discovery of molecular markers and their combination with the polymerases chain reaction has become a suitable device for plant breeding programmes. This has facilitated the visualization of the composition of organism at DNA level and has allowed obtaining the genetic fingerprint. Sorghum (2n=2x=20) is the first cereal crop of African origin whose genome has been sequenced (Paterson *et al*., 2009) and complete genome sequence (8x scaffold) information with ~10.5 million reads is now available for public use (Sorbi v1.0) (http://genome.jgi-psf.org/Sorbi1/Sorbi1.info.html). That study revealed a high quality genome sequence obtained from homozygous sorghum genotypes genome (BTx623) by using the Whole Genome Shotgun (WGS) sequence. Its genome size is about 730 Mb within which 98% of the genes are located in their chromosomal context and genetic recombination is mostly restricted to one third of its genome. Sorghum genome size is relatively small compared to that of maize (one third of maize genome size) but larger than that of rice (~75%) (Paterson *et al*., 2009). This investigation revealed that 24% of genes are grass-specific and only 7% are sorghum specific. Gene and microRNA duplication could be responsible for drought tolerance in sorghum. Many studies relative to sorghum genome sequencing were conducted and they used BTx623 as a reference for sorghum genome (Nelson *et al*., 2001; Zheng *et al*., 2011; Thurber *et al*., 2013; Mace *et al*., 2013)**.** These assembled genome sequencing projects provide a resource for genetic improvement of sorghum through the progress made to correlating genes to their function.

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2.7.2. Genetic distance analysis

Genetic distance stands for genetic differences that exist among genotypes. It can also be perceived at phenotypic and enzymatic level. The genetic difference is important for diversity study, for QTLs detection via linkage map construction and for QTLs introgression. For instance, Langridge *et al*. (2001) reported that useful markers in breeding should reveal polymorphism in different populations derived from a wide range of different parental genotypes. Methods of estimating genetic differences include microsatellite markers for polymorphic loci. It is necessary that sufficient polymorphism exists between genotypes at flanking marker loci for a particular trait for MAS. In many cases, parents that provide adequate polymorphism are selected on the basis of the level of genetic diversity between them (Anderson *et al*., 1993 and Collard *et al*., 2005). The choice of DNA markers used for Marker Assisted Selection may depend on the availability of characterized markers or the appropriateness of particular markers for particular traits. Once polymorphic markers have been identified, they must be screened across the entire breeding population (F_1 hybrid, BC_1, BC_2 etc) including the parents. This is known as marker 'genotyping' of the population.

2.7.3. Genetic diversity analysis

Genetic diversity stands for the variation of inheritable characters existing among alleles of genes within individuals of species' populations and plays an important role in evolution by allowing adaptation in new environments (Charrier *et al*., 1997). Intensive investigations, either phenotypic, genotypic or the combination of both, have been conducted on sorghum diversity. Chantereau *et al*. (1989) reported on the characterization of world sorghum collections based on phenotypic assessment. The use

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of biochemical markers grouped sorghum by race and origin (Ollittrault, 1987, Zongo, 1991). Several generations of DNA based-markers (RFLP, RAPD, AFLP, SSR and SNPs) were used and improved the assessment (Billot *et al*., 2013).

In Burkina Faso, morphological and biochemical markers have been used by Zongo (1991) and Zongo *et al*. (2005) to characterize the local collections. The assessment of sorghum from three ecological zones (Centre-South, Centre-North and Western part of the country) was studied by use of molecular markers (Barro-Kodombo *et al*., 2008). SSRs markers were later used to assess the diversity of sweet sorghum (Nebie, 2014; Sawadogo, 2015). Diversity assessment in sorghum germplasm has been done using several molecular markers (Tao *et al*., 1993; Vierling *et al*., 1994; Brown *et al*., 1996; Taramino *et al*., 1997; Ghebru *et al*., 2003; Uptmoor *et al*., 2003; Paterson *et al*., 2009; Billot *et al*., 2013). Comparative studies in crop plants revealed that microsatellite markers provide more variation than other markers (Taramino and Tingey, 1996; Powell *et al*., 1996a; Pejic *et al*., 1998). According to Powel *et al*. (1996b); Dean *et al*. (1999); Dje *et al*. (2000); Scott *et al*. (2000); Kong *et al*. (2000), SSR markers displayed high discrimination level between closely related individuals in some cases where even when a few number of loci were used. Apart from characterization studies, no markers have been used in sorghum breeding programmes such as markers assisted selection. This thesis is a pioneer on the use of molecular markers in sorghum breeding in Burkina Faso.

2.7.4. Mapping of stay-green QTLs and Molecular Marker Assisted Selection (MAS)

Most economically important traits in crop plants such as yield and some forms of diseases are polygenic in nature and phenotyping selection for such characters is

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complex. The genetic loci linked to complex traits are known as quantitative trait loci which are chromosomal regions expected to contain a gene or genes that contribute to the expression of a quantitative trait (Brumplop and Sarah, 2011). The approximate numbers and chromosomal regions influencing polygenic phenotypes can be estimated by experimental mapping approaches. In plants, two approaches can be used to identify genomic regions underlying expression of quantitative traits. The most common approach is the identification of QTL in a segregating population through QTL mapping (Shehzad *et al*., 2009). QTL mapping is a process of constructing linkage maps and conducting QTL analysis to identify genomic regions associated with the traits. Alternatively, association studies (association mapping) with candidate genes based on the use of diverse populations to identify associations between allele frequencies and phenotypic variation can also be applied for QTLs identification. The first association between a genetic marker and genes that influence a quantitative trait in plants was observed by Sax (1923) and the event has since then been observed for a variety of traits in a range of crops (Hamblin *et al*., 2005).

Genetic mapping of QTLs conferring stay-green trait resulted in the detection of many genomic regions associated with terminal moisture stress tolerance and constitutes an important step leading to the development of drought tolerant elite lines and hybrids. Tuinstra *et al*. (1997) were the first to explore QTLs map constructed by using 98 Recombinant Inbred lines (RILs) obtained from cross between inbred lines B35 (post-flowering drought tolerant, pre-flowering drought susceptible) and RTx7078 (pre-flowering drought tolerant, post-flowering drought susceptible). Thirteen (13) genomic regions associated with post-flowering moisture tolerance were found and most of them were linked to the expression of the stay-green trait. Two QTLs (SBI-10 on LG G and

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SBI-09 on LG F) were identified with major effect on grain yield and stay-green under terminal moisture stress conditions. Crasta *et al*. (1999) used RILs derived from B35 and Tx430 and detected seven QTLs for stay-green. Xu *et al*. (2000) used 98 RILs obtained from the cross of B35 and RTx7000 to map QTLs that control stay-green and chlorophyll content. They detected four stay-green QTLs located on three linkage groups. Two QTLs (Stg1 and Stg2) were located on LG C and the other (Stg3 and Stg4) were located, respectively, on LG B and J. Using RILs derived from the same parents [(B35 and RTx7000) already mentioned by Xu *et al*. (2000); Subudhi *et al*. (2000)], Sanchez *et al*. (2002) reported also four QTLs associated with stay-green trait.

Besides B35, many other sources of post-flowering drought tolerant lines were also used to identify genomic regions associated with stay-green QTLs. For instance, Tao *et al*. (2000) searched for stay-green QTLs from RILs developed from cross of QL39 and QL41. They identified five regions on distinct linkage groups associated with stay-green expression. Kebede *et al*. (2001) used composite interval mapping from RILS derived from cross of SC56 and RTx7000 and identified nine stay-green QTLs distributed on different linkage groups. Three of these QTLs (SBI-01 on LG A; SBI-05 on LG J and SBI-10 on LG G) were consistent across different terminal moisture stress environments. Haussman *et al*. (2002) investigated two Recombinant Inbred Populations (RIPs) developed from E36-1 xIS9830 (RIP1) and E36-1xN13 (RIP2) to identify stay-green QTLs and, detected three QTLs (SBI-01 on LG A, SBI-07 on LG E and SBI-10 on LG G) that were common to both RIP1 and RIP2.

These above studies have made available six candidates stay-green QTLs designated as Stg1, Stg2, Stg3, Stg4, StgA and StgB. Four (Stg1, Stg2, Stg3 and Stg4) were recognized

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as major QTLs and were consistently identified in a range of different environments (Subudhi *et al*., 2000; Tao *et al*., 2000) and in different genetic backgrounds (Subudhi *et al*., 2000). Stg1 and Stg2 were mapped to sorghum chromosome 3, and explained approximately 20 and 30% of the phenotypic variance, respectively (Xu *et al*., 2000; Sanchez *et al*., 2002; Harris *et al*., 2007). The interaction of Stg2 and Stg3 resulted in explanation of 49.8% phenotypic variation (Subudhi *et al.,* 2000). At these loci, the alleles are from the most common source of stay-green, BTx642 (B35). The incorporation either individually or in combination of some of these QTLs through marker assisted backcrossing (MABC) would provide elite lines with better stay-green expression and good response to terminal moisture stress.

Markers are used for selecting, through MAS, qualitative as well as quantitative traits that are difficult to assay phenotypically. MACBC is one of the breeding strategies for which MAS is applied. This method consists of incorporating desired (favorable) traits from a donor parent into an elite genotype (recurrent parent) through repeated crosses in which the original offspring is crossed back to the recurrent parent until the elimination of unwanted genes derived from the donor parent except for the desired gene/s. However, the offspring (backcrossed descendants) can carry along with the target allele an important fragment from the donor chromosome and marker essays can be useful to minimize that linkage drag (Frisch *et al*., 1999). In this situation, markers can be applied either to control the target gene (foreground selection) or to ensure rapid reconstruction of the recurrent genotype (background selection). Application of background selection may reduce the time for the reconstitution of the recurrent parent genotype to three generation (BC3Fn) while it requires more than six generation (BC6Fn) in conventional breeding (Tanksley *et al*., 1989). Frisch *et al*. (1999) confirmed that result in a computer

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simulation and showed that MAS can reconstruct a level of recurrent parent genome in BC3 which would only be reached in BC7 in traditional breeding.

Several studies showed that genetic markers have been used for the introgression of genes from the stay-green donors into sorghum elite lines. The post-flowering drought tolerant line (E36-1) has been used as a donor parent to incorporate stay-green traits in Ochuti (a Kenyan farmer's preferred line) (Ngugi *et al*., 2010). The most common stay-green donor parent (B35) has been used to improve Ethiopian local elite lines by transferring the stay-green trait via MAS. Field trials results showed that most of the introgression lines were comparable to B35 in terms of green leaf area percentage (%GLA) during the critical stage of grain filling and maintained more green leaf than the recurrent parent. It is also reported that the introgression lines had similar grain yield as the recurrent parent (Kassahun *et al*., 2009).

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Chapter III: Farmers' perception on impact of drought and, their preference for sorghum cultivars and traits in two agro ecological (Sudanian and Sudan-Sahelian) zones of Burkina Faso

3.1. Introduction

Small-scale farmers in the Semi Arid Tropical (SAT) Sub-Saharan Africa dominate production of sorghum. In Burkina Faso, it is cultivated mainly under rain-fed conditions (June- October) in Burkina Faso. Sorghum is a major staple for about 80% of the rural population. Unfortunately its production is threatened by frequent and unpredictable drought around the flowering stage (pre-flowering and post-flowering) of growth during the rainy season. Drought remains the major constraint to sorghum production worldwide and poor, small-scale farmers cannot afford irrigation facilities to lessen the negative impact on their production (Derera, 2005).

Breeding research was conducted to solve drought problems in the early 1970 s in Burkina Faso as well as in other semi-arid countries of West Africa. The objectives of this research was the improvement of high yielding varieties derived from exotic material such as *caudatum* and *kafir* races but it failed to have much impact (Ouédraogo, 2005). These exotic varieties did not meet farmers' needs. The varieties did not fit with local farming systems and therefore were not accepted by farmers. Furthermore, their cost of production was high due to their requirement for fertilizer and improved cultural practices such as plowing and weeding compared to local *guinea* landraces. The compactness of the panicle made them susceptible to diseases such as grain mold and insect damage (Barro-Kodombo, 2010). Matlon (1985) reported a low adoption rate (less

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5%) of exotic varieties in West Africa, indicating that farmers still prefer local landraces for their sorghum production.

Sorghum grain yield is low due to the variable production environment, stress and limited access to essential inputs (Banziger and de Meyer, 2002). Until quite recently, breeders did not involve farmers in their selection programme. Therefore, they did not solve farmers problem which persist until now. An efficient breeding programme should be based on clear identification of farmers' constraints and their preferences for varieties through participatory research and breeding. This would strengthen the relationship between farmers, extension services and breeders and also support formulate decision-making concerning what to breed for highest impact. In participatory crop selection, farmers and extension workers provide key information on plant types, desired traits and insight into trade-offs they are willing to make in designing cultivars for their area (Sperling *et al*., 1993). The current study reports on a survey carried out in two agro ecological zones of Burkina Faso on farmers' perceptions on drought effects on sorghum. The objective of the survey was to define farmers' perceptions on drought management, their knowledge on drought effects on sorghum production and identify their preferred traits and cultivars.

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3.2. Materials and Methods

3.2.1. Study area

The study was conducted in twelve sites (villages) located in six districts (Houet, Poni, Kossi, Bale, Zoundweogo and Sanguie) (Figure 3.1). The six districts are situated in two different climatic zones where sorghum is the major cereal crop. The districts Houet, Boromo and Poni are located in the South Sudanian (Sudan savanna) climatic area with an annual rainfall between 900-1100 mm with five full months of cropping season from June to October. The remaining districts (Kossi, Zoundweogo and Sanguie) are located in the North Sudanian (Sudano-sahelian) or transition climatic zone characterized by an annual rainfall between 700-900 mm with four months (July to October) of cropping season. The average temperature is 25°C during the cold season (December- February) and 33°C during the hot season (April-June). These regions are characterized by irregular climatic conditions where the total annual rainfall is decreasing with a poor distribution in space and time and also with many spells of drought during the cropping season (Nicou *et al*., 1990, Guillobez and Zougmoré, 1991 and Zougmoré, 2003). The amount of rainfall over 30 years is displayed in Figure 3.2. Not long ago, the World Bank (2007) confirmed that the climate change will potentially lead to increased temperatures, evapotranspiration and in decreased rainfall, which, according to Rijsberman (2006) and Lobell *et al* (2008), may have a negative impact on crop production in developing countries of Africa and Asia.

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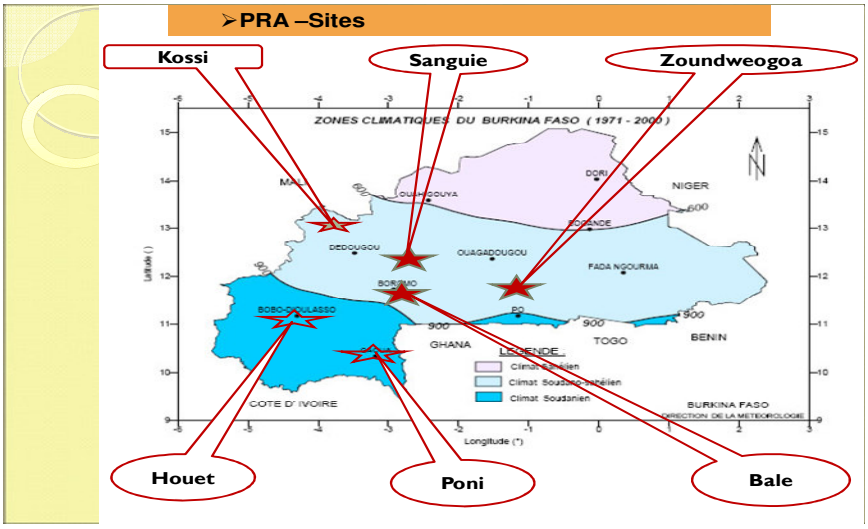

Figure3. 1: Location of the present study sites in Burkina Faso

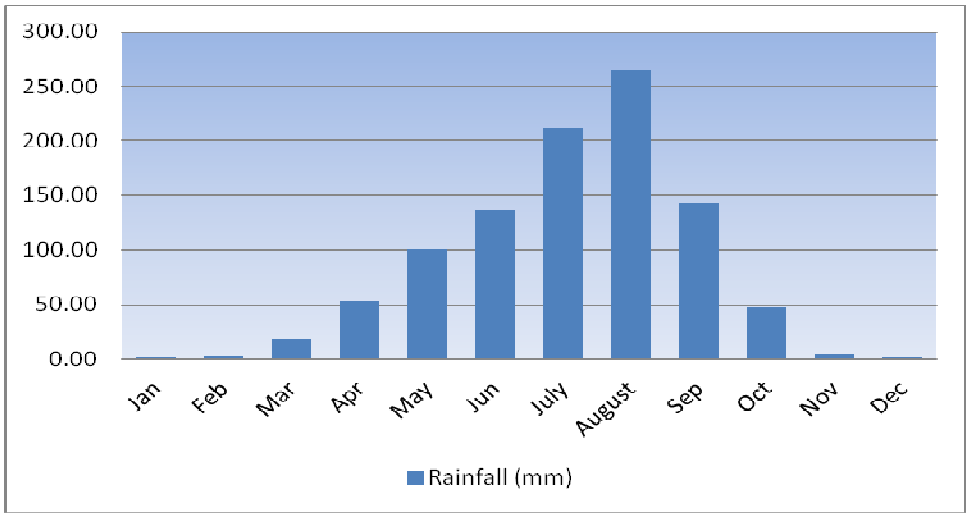

Figure3. 2: Amount of rainfall over thirty years (1983-2013) in Sudanian climatic areas

3.2.2. Data collection

Primary and secondary data were collected. Primary data were collected through both formal household surveys and Participatory Rural Appraisal (PRA) tools. Prior to the survey itself, a meeting was organized with all the farmers in the village to explain the objective of the survey. Local extension staff and village headmen facilitated the survey by creating a friendly relationship with local people. Famers were mobilized for the focus group discussions and were introduced to the formal survey. The PRA involved focus

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group discussions and interviews with key informants such as agricultural extension staff and technicians in research stations. The technique employed consisted of problem listing, analyses and ranking by key informants using semi-structured questionnaires. Questionnaires were used to guide the discussions and to provide the focus group a chance to come out with their own issues. Farmers were asked to list uses of sorghum and identify competing cultivated cereal crops in their areas. They were also asked to list and rank key constraints to sorghum production and to list cultivars they had grown, rank them and identify traits or characteristics they preferred. Additionally, seed and fertilizer issues were briefly discussed. A local extension staff member guided the discussions. Thirty respondents were sampled per village making a total of sixty (60) questionnaires per district except in the district of Zoundweogo where the number of questions was reduced to forty (40). Overall, a total of three hundred and forty (340) respondents' were involved in this study. Secondary data were collected from research articles and the "Direction Regional de l'Agriculture".

3.2.3. Data analysis

Statistical analyses of data were performed with Excel 2007 and SphinxME (le Sphinx plus^2) version 4.5 (Ganassali, 2003) computer package. Descriptive statistics, analysis of variance and means were determined between districts.

3.3. Results

Cereals grown in the PRA sites included sorghum, pearl millet, maize, rice and fonio (MASA/DGESS, 2014). All of the crops listed were used for food but sorghum was the most important cereal used as a staple food.

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3.3.1. Sorghum production constraints

The parasitic weed *(Striga hermontica*), drought, low soil fertility and diseases were respectively the most important limiting constraints to sorghum production. In four (Houet, Kossi, Zoundweogo and Poni) out of the six districts, farmers ranked *Striga* first, followed by drought. Only in one district (Sanguie), was drought mentioned first, followed by *Striga*. In the district of Bale *Striga* was ranked at the same level as drought. Low soil fertility was the third ranked constraint. The remaining constraints were of lesser importance and were ranked as follows: diseases in fourth position followed by insect attack, bird damage, and finally seed quality (Figure 3.3). The factorial correspondence analysis highlights the different productions' constraints in the different sites (Figure 3.4). In addition to the major constraints (*Striga*, drought and poor soil fertility), the analysis revealed that disease was a concern in Kossi, bird damage in Houet and seed quality in two districts (Poni and Zoundweogo) (Figure 3.3).

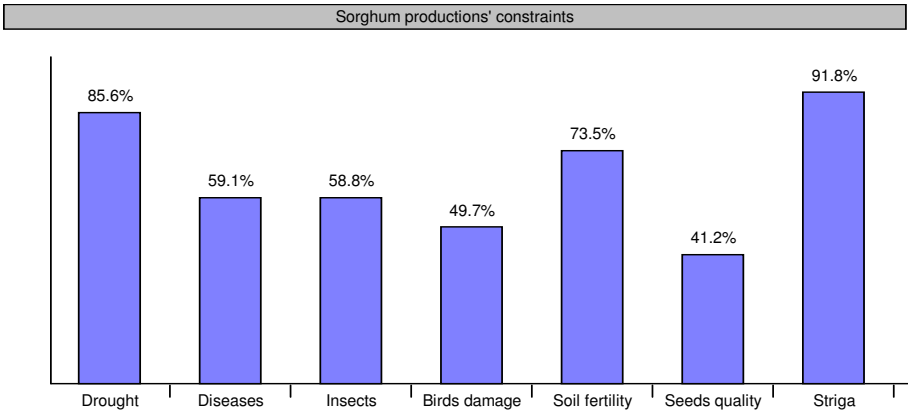

Figure3. 3: Sorghum productions' constraints in PRA sites

The Chi-square test for constraints to sorghum production was significant (X^2 = 103.75, df = 7, 1-p = >99.99%). X^2 is calculated by using theoretical effective equal to each modality. Interval of confidence at 95% is given for each modality. Number of citations is larger than number of observations due to question type (multiple answer questions).

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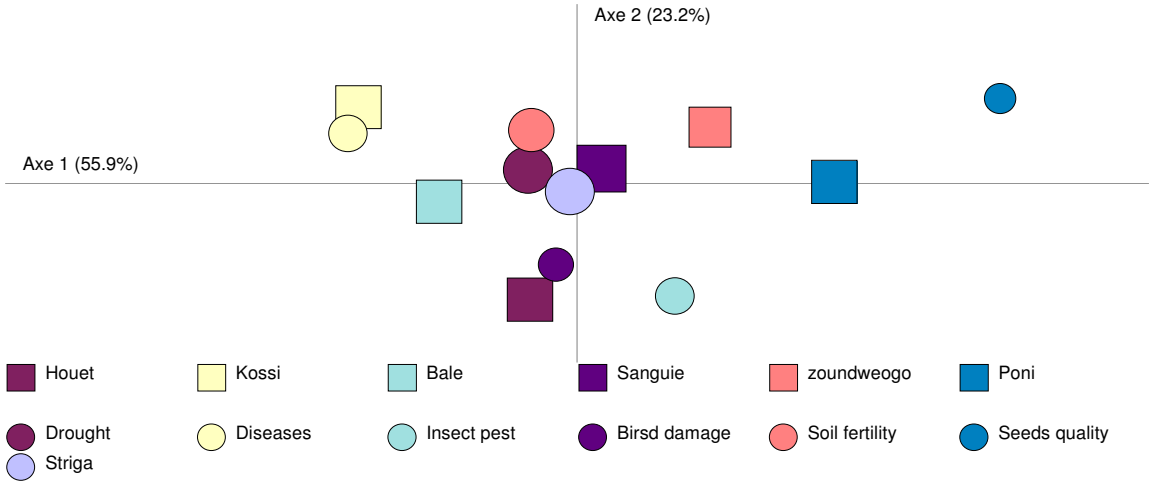

Figure3. 4: The factorial correspondence analysis of different constraints at different sites

Overall, drought was the second most important constraint according to farmers in the PRA sites and the most important abiotic constraint to production.

3.3.2. Farmers knowledge of drought

The majority of farmers reported that moisture stress during the grain filling and maturation (GS3) was the most common type of drought during the rainy seasons. Pre-flowering (GS2) moisture stress was not quite as frequent as the terminal stress but occurrs more than early type of drought (GS1). Only a few farmers (3.53%) thought that different types of drought have the same chance to occur (Table 3.1). The majority of the respondents (62.05%) indicated that GS3, even if it occurs more frequently than GS2 and GS1, was less harmful to sorghum production than drought at GS2 but is more damaging than GS1 (Table 3.1).

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Table 3. 1: Farmers' perception on recurrent type and severe form of drought.

Type of drought	Recurrent drought		Severe form of drought	
Grow stage	Nb.cit	Freq (%)	Nb.cit	Freq (%)
GS1	86	25.29	39	11.47
GS2	105	30.88	211	62.06
GS3	137	40.29	90	26.47
Ind	12	3.529	0	0
Total Obs	340	100	340	100

Nb.Cit: Number of farmers cited

3.3.3. Farmers' perception on impact of drought in sorghum production

Farmers listed different effects of moisture stress on sorghum plants but reduced yield was the major effect. Almost all of the famers thought that lack of moisture reduced sorghum yield to different extents depending on the plant growth stage. Most of them (304 famers ≡ 86.1<89.4<92.7 of confidence interval) thought stress at GS2 was most damaging, followed by GS3 with 296 farmers (83.5<87.1<90.6) and then GS1 with a number of 180 farmers (47.6<52.9<58.2). Farmers reported that leaf yellowing (mentioned by 238 farmers) was the most visible aspect when moisture stresses occurred at GS1. At GS2, they pointed out barenness (57.5<62.6<67.8) and delayed flowering (46.7<52.1<57.4) as the major effects, while during GS3, malformation of grain (56.9<62.1<67.2) and leaf senescence (45.3<50.6<55.9) were reported. The remaining traits (germination rate, growing vigor, development vigor, leaf rolling, reduction of panicle height, LPE, reduction of panicle size, stem senescence and lodging) were less important.

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Table 3. 2: Traits affected by stress at different growth stages.

GS1			GS2			GS3		
Obs	Nb ct	Confidente inter	Obs	Nb ct	Confidente inter	Obs	Nb ct	Confidente inter
Ger R	118	29.6<34.7<39.8	Lf rol	75	17.7<22.1<26.5	Lf sn	172	45.3<50.6<55.9
Gr Vg	105	26.0<30.9<35.8	Re Ph	48	10.4<14.1<17.8	S Sn	109	27.1<32.1<37.0
De Vg	103	25.4<30.3<35.2	D Fl	177	46.7<52.1<57.4	RePS	56	12.5<16.5<20.4
Lf yg	238	65.1<70<74.9	Barn	213	57.5<62.6<67.8	Gasp	211	56.9<62.1<67.2
Lw yd	180	47.6<52.9<58.2	LPE	77	18.2<22.6<27.1	Ldg	129	32.8<37.9<43.1
other	13	1.8<3.8<5.9	Re PS	130	33.1<38.2<43.4	lw yd	304	86.1<89.4<92.7
-	-	-	lw yd	296	83.5<87.1<90.6	-	-	-
Total Obs	340			340			340	

GS1: Growth stage1 (germination rate=Ger R, growing vigor=Gr vg, development vigor=De vg, leaf yellowing=Lf yg, Low yielding= Lw yd); GS2: growth stage2 (leaf rolling=Lf rol, reduction of plant height=Re Ph, delaying flowering=DFl, barenness=Barn, LPE, reduction of panicle size=Re Ps, low yielding=Lw yd); GS3:Growth stage3 (Leaf senescence=Lf Sn, stem senescence=S Sn, reduction of panicle size=Re ps, grain aspec=Gr asp, lodging=Ldg, low yielding=Lw yd).

3.3.4. Monitoring drought on sorghum production

Figure 3.4 shows different methods employed by farmers' to lessen the impact of drought during the rainy season. Majority of the farmers used agronomics practices (such as weeding, hoe weeding, hilling), manure, soil conservation and restoration, and planting in humid soil to lessen drought effect on sorghum field during the rainy season. Some of them (less than 10%) used early maturing cultivars and early planting at the beginning of rainy season. A small number prayed for rain. Result is showed in Figure 3. 5.

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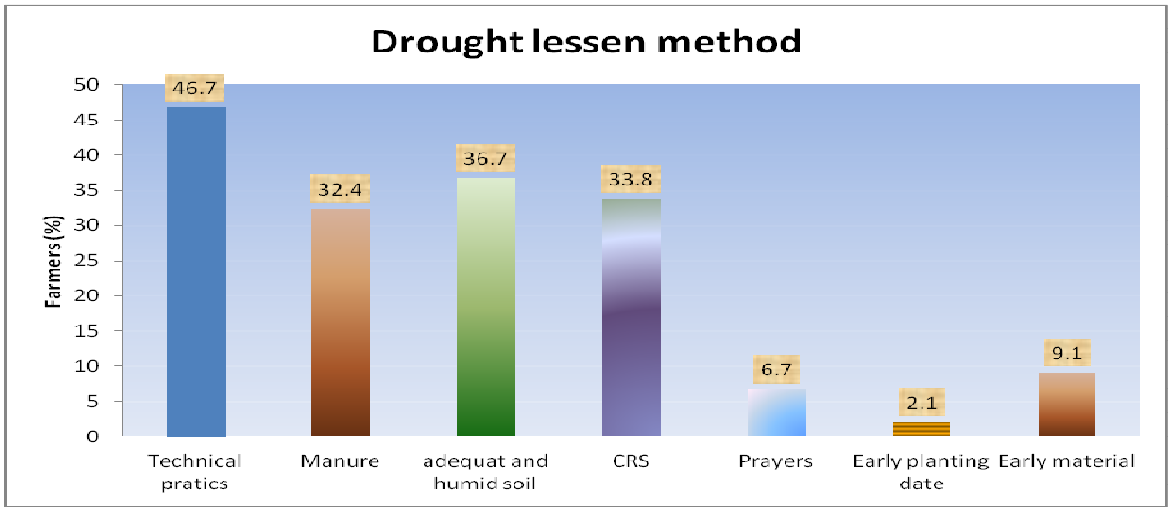

Figure3. 5: Methods used to lessen the negative impact of drought

CRS: Conservation and restoration of Soil

3.3.5. Released sorghum cultivars and farmers' preferences

The main sorghum varieties grown in the six districts included the improved varieties [Sariaso01 (INERA, 1990), Sariaso02 (INERA, 1990), Sariaso14 (INERA), Framida (ICRISAT), Ouedzoure (INERA), Grinkan (IER/Mali, 2011), ICSV1049 (ICRISAT) and Kapelga (INERA, 1998)], and local landraces (Table 3.3). Overall, local landraces were grown more often by farmers (47.19%) in the six districts compared to the improved varieties. Each district had one local landrace on which farmers relied for production. The improved varieties were grown by a small number of farmers. Kapelga was the most popular improved variety grown by farmers (19.39%) in the six districts followed by Framida (10.39%) and Sariaso01 (11.49%). The remaining varieties (Ouedzoure, Grinkan, ICSV1049 and other Sariaso varieties) were grown by less than 0.2% of farmers. Specifically, Kapelga was grown more often in three districts (Sanguie, Poni and Zoundweogo) while Framida was grown more often in two districts (Sanguine and Zoundweogo) and Sariaso 01 was mainly grown in the district of Houet. Grinkan was

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mentioned only once, in the district of Bale, and Sariaso 09 was not mentioned by any of the farmers.

Table 3. 3: Sorghum varieties grown in each district included in the PRA study

	District													
	Houet		Kossi		Bale		Sanguie		Zoundweogo		Poni		Mean	Gl rank
variety	Nb	Rank	Nb	Rank	Nb	Rank	Nb	Rank	Nb	Rank	Nb	Rank		
Sar01	59	1	1	3	0	-	0	-	0	-	5	3	10.83	3
Framida	5	3	0	-	0	-	15	3	36	1	2	4	9.7	4
Ouedz	2	4	0	-	0	-	0	-	0	-	0	-	0.33	6
Kapelga	0	-	3	2	1	3	58	1	21	3	25	2	18	2
Grinkan	0	-	0	-	1	3	0	-	0	-	0	-	0.17	8
Sar09	0	-	0	-	0	-	0	-	0	-	0	-	0	9
Boromoloc	1	5	0	-	60	1	0	-	0	-	0	-	1.67	5
ICSV1049	0	-	0	-	0	-	0	-	1	4	0	-	0.17	8
Sar14	0	-	0	-	0	-	0	-	1	4	1	5	0.33	6
Sar02	1	4	0	-	0	-	0	-	0	-	0	-	0.17	8
Ecot loc	47	2	59	1	22	2	43	2	35	2	58	1	44	1

Nb: Number

3.3.6. Criteria for selecting sorghum variety

Overall, farmers in the six districts indicated the edibility as the most important criterion followed by high yielding ability and drought tolerance for varietal selection. The remaining criteria were ordered as follows: grain color as fourth, grain size as fifth, the tolerance to disease at sixth and marketability of grain. In Kossi, tolerance to drought was co-ranked with marketability as the second most important criterion while it occupied the third place in Zoundweogo and fourth place in Bale. In Sanguie and Poni, it was ranked fifth and was sixth factor in Houet. Aside from the edibility, drought tolerance, marketability and grain size, there were significant differences in ranking criteria farmers preferred within districts (Table 3. 4).

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Table 3. 4: Criteria used to select varieties by district

	District													
	Houet		Kossi		Bale		Sanguie		Zoundweogo		Poni		Mean	F. prob
Criteria	Nb	Rank	Nb	Rank	Nb	Rank	Nb	Rank	Nb	Rank	Nb	Rank		
High yield	53.5	2	30.5	5	27.5	2	25.5	6	36.5	1	42.5	1	36	0.032
Edible	54	1	55	1	29.5	1	58	1	34	2	31	2	43.58	0.257
R Disease	25	7	7.5	8	23.5	5	56	3	2	8	7	7	20.17	0.0001
T drought	38	6	40	2	25	4	51	5	30	3	17.5	5	34.25	0.1970
Market	44.5	5	40	2	25.5	3	15	7	19	6	18	4	27	0.4236
GrCo	50	3	36	4	21.5	7	58	1	25.5	4	6	8	32.83	0.0001
Grsize	48	4	10	7	19.5	8	47.5	4	19.5	5	20	3	27.42	0.0737
Other	6	8	28.5	6	23	6	0	8	7.5	7	12.5	6	12.91	0.2873
Total number of respondents														340

Resistance to disease: R disease; Resistance to drought:R drought; GrCo:Grain color; Grsize: Grain size; Nb: number

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3.4. Discussions

3.4.1. Production constraints

Striga hermontica was the most important and drought the second most important constraint for sorghum production in all districts, except Sanguie where farmers ranked drought as the main constraint. However, the remaining constraints (low soil fertility, diseases, insect attack, bird's damage, and seeds quality) were not negligible. Overall, *Striga* was mentioned by almost all the farmers (91.8%) whereas drought was ranked as the second major hindrance (about 85.6%) following by soil fertility (73.5%) as the third one. This is an indication that the most important constraint to sorghum production in the studied area is drought.

Famers considered *Striga* as the leading constraint due its presence in all the agro-ecological zones in Burkina Faso (Ouedraogo, 1986). It infests 70% of the cultivated land for cereal production (Lagoke *et al*., 1991). This indicates that most farmers are farming in *Striga* infested fields. *Striga* infests up to 20 to 40 million ha of arable land and is responsible for grain yield losses of host crops up to 10.7 million tons per year (Gressel *et al*., 2004). In Burkina Faso, yield losses due to *Striga* vary between 30% and 50% (Aggarwal and Ouédraogo, 1989) and may reach 100% on heavily infested fields (Parker and Riches, 1993) or on marginal lands where wetness and soil nutrients are limited as in the PRA sites included in this study. The *Striga* problem is permanent and affects smallholder farmers more because they have limited means for controlling it (Marechera *et al*., 2011).

Drought was perceived as the major factor limiting sorghum production in two districts (Sanguie and Bale) and the second, after *Striga*, in the remaining districts (Houet, Kossi,

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Poni and Zoundweogo). This is due to the unpredictable climatic conditions that characterize the semi-arid areas. The districts of Sanguie and Bale are located in the transition (Sudano-sahelian) climatic zone which is less wet than the Sudanian zone. Compared to others districts (Kossi, Zoundweogo) in the same areas, farmers' opinions can be justified by the fact that the concerned districts have received less rainfall than others districts as explained by Zougmore (2003) who reported that rainfall is decreasing with a poor distribution in these areas.

The poor soil fertility is due to exposure to wind and water erosion after destruction of the vegetative cover due to rapid population growth and demand of arable land. As a consequence, the soils are fragile and have mostly low to moderate inherent fertility (Bationo, 1998; Zougmore, 2003; Ouedraogo, 2004).

Farmers in Kossi reported more damage from diseases than other areas while farmers in Houet complained about bird damage. Seed quality was a problem in Poni and Zoundweogo. The majority of the varieties farmers grew do not have awns (arista) to protect them from bird damage and unfortunately, farmers did not use any control methods to protect their sorghum field against diseases or other pests.

3.4.2. Farmers' perceptions on impact of drought in sorghum production

Many farmers (40.3%) reported that terminal moisture stress (GS3) occurred more frequently than pre-flowering (30.87%) moisture stress and the early season stress (25.29%). However, they thought that pre-flowering moisture stress was more damaging to sorghum than terminal or early season moisture stress. Farmers' opinions about GS3 as the recurrent type of drought are in agreement with meteorological data that show a

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reduction of total annual rainfall followed by a decreased duration of the rainy season (Some, 1989; Zougmoré, 2003; Some *et al*., 2004). As a result, drought is worsening due the farmers persistence of growing local late cultivars.

Overall, farmers indicated yield loss as the major consequence of drought no matter the growth stage at which the stress happened. Besides the yield loss, farmers noted significant leaf yellowing at GS1, barenness and delayed flowering at GS2 and leaf senescence and grain malformation at GS3 as morphological damage when drought occurred during these growth stages. Farmers thought that, during drought stress, yield was reduced more at GS2 than in GS3 due to abortion or barenness. According to their analysis, the reduction of yield in GS3 was due to loss of grain weight as the consequence of grain mal-formation and the reduction of the panicle size. Cakir (2004) reported that drought around the flowering stage is responsible for maximum damage and yield reduction.

Farmers used several methods to lessen drought impact on their sorghum fields. These methods were essentially based on agronomics practices including weeding (hand and hoe weeding), hilling, applying manure, planting in humid and adequate moisture soil and also the application of soil and water conservation (SWC) techniques such as stone lines, planting pits, half-moon-structures and mulching. Smallholder farmers rely on these methods to reduce moisture stress impact at pre-flowering and post-flowering stages of growth because they cannot afford irrigation facilities (Derera, 2005). Some of the farmers (10%) reported the use of early maturing varieties to escape drought. In Houet, farmers mentioned the availability of early maturing landraces that allowed them to withstand hunger before the maturity of their local late maturing varieties. A few of

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them mentioned early planting on humid and adequate moisture soils. The six districts are located in different types of soil and some of these soils have the ability to retain more rainfall water than others. Smart farmers recognize that kind of soil and therefore exploit it for sorghum production.

3.4.3. Farmers' varietal preference and selection criteria

Farmers' preferences for landraces over improved varieties suggests that either farmers lack awareness about the improved varieties or they are not satisfied with them. The unavailability of information on varieties is because of an absence of a real extension service. For instance, Sariaso01 is derived from Boromo local (local landrace) but the majority of farmers in Boromo do not have knowledge about that improved variety and are still growing their local landrace (Boromo local). Since 1980, breeders have released a number of sorghum varieties and even introduced some to meet farmers demand, but, they failed to have much positive impact (Ouedraogo, 2005). In fact, breeders have introduced high yielding caudatum and kafir varieties based on photo period insensitive materials without taking into account farmers' preferences. However, in Burkina Faso, *guinea* local landraces are predominantly used in traditional farming systems (Sapin*, 1984;* Zongo, 1991) due to their adaptability to the environment (Barro-Kondombo *et al., 2008*). According to Harlan (1975), the strong adoption of the *guinea* race by West African farmers is historical because West Africa is a centre of diversification of *guinea* race and, furthermore, Burkina Faso seems to be the heart of that centre (Zongo, 1991).

The few improved varieties (Kapelga and Sariaso01) grown by farmers have been derived from selection of local landraces except Framida. Kapelga and Sariaso 01 were derived from improvement of local *guinea* race collected respectively in south centre and

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southern part of the country whereas Framida is an exotic material from ICRISAT that originated from South Africa. Farmers in the districts of Sanguie, Poni and Zoundweogo grew Kapelga because they were familiar with it and liked its medium crop growth cycle. Sariaso01 is more accepted in the district of Houet due to the promotion it received during its release and because its varietal cycle fits with the rainy season cycle. Yapi *et al*. (2000) found similar results and linked the increase in use of improved varieties to the fact that these varieties were derived from selection from local germplasm. Framida is grown for its ability to produce local beer. The rate of adoption of local improved varieties is low compared to landraces, but they are more readily adopted than improved exotic materials.

The low rate of adoption of improved exotic varieties is probably due to breeder's objectives to release high yielding caudatum varieties with the possibility to intensify the production. Unfortunately, these varieties are not satisfactory for the local farming system in economic terms and their adaptation to the environment. The cost of production is high due to the need to use fertilizer and improved cultural practices such as ploughing, weeding, etc. The opacity of the panicle makes them susceptible to diseases such as grain mold and insect damage (Barro-Kodombo, 2010). Furthermore, the grain quality is not as good for preparing the staple food (Tô) in the rural zones compared to *Guinea* varieties (Stoop *et al*., 1981). Malton (1985) showed that less than 5% of farmers cultivate improved varieties in West Africa. Ceccarelli and Grando (2007) reported that some staple crops of many developing countries have also met resistance to the introduction of modern improved varieties.

Farmers preferred highly edible varieties consumed as staple food. Their preference for *Guinea* landraces can be linked to its suitability for producing the main dish (Kodombo,

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2010). Grain from *guinea* race is more vitreous and better for hand pounding to decorticate than grain from the *caudatum* race. Banziger and de Meyer (2002) reported that farmers like firm endosperm types of maize for ease of pounding and husk cover for protection against ear rots and storage pest. Farmers in Houet and Bale preferred cultivars with good edible quality and also with high yielding ability whereas farmers in Poni and Zoundweogo preferred high yielding ability and then edibility. Farmers in Kossi preferred drought tolerance after edibility in choosing the varieties they grew. Concerning grain color, farmers desired tannin (red) sorghum varieties that could allow them to obtain white flour for confection of the local food (Tô) than high anthocyanin varieties that are used for local beer.

Farmers showed little interest in tolerance to disease and big grain size. Smallholder farmers thought that improved management was for industrial crops such as cotton and sugar cane and the majority of them limited their sorghum field management to land preparation and weeding.

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3.5. Conclusions and perspectives

The study investigated famers' perceptions on sorghum production constraints with emphasis on drought impact, on sorghum varieties farmers grew and their traits of choice. The major constraint limiting sorghum production was the parasitic weed, *Striga*, which infests almost all the arable land of the country. Drought was the second factor reducing sorghum yield and it is the most important abiotic constraint during the rainy season. Except for *Striga* and drought, sorghum' production constraints varied among districts.

Farmers showed a good empirical knowledge about drought and indicated that terminal drought occurred more often than other types of moisture stress but was less damaging to sorghum production than the pre-flowering drought. To alleviate drought effect, farmers employed different methods including cultural practices, soil and water conservation systems.

Farmers in Burkina Faso relied more on their local landraces for sorghum production than the improved varieties derived from selection of local landraces and the exotic materials. Farmers lacked information about research concerning released varieties due to the absence of an effective extension service in rural areas. Apart from local landraces, farmers grew Kapelga in all different agro-ecological zones of the country and Sariaso01 in the Southern part of country. Farmers preferred white grain varieties with high edibility, high yielding potential and also that withstand drought. This would improve sorghum production and improve food security in the rural areas of the country. In conclusion, the result shows that participatory variety selection is the best way to identify farmers' needs in order to conduct a breeding programme to overcome the constraints facing their production.

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Chapter IV: Diversity study and identification of potential drought tolerant materials within local sorghum landraces in Burkina Faso

4.1. Introduction

Genetic resources are indispensable for agriculture, medicine, industry and constitute a guarantee for adaptation in changing environments (Barro-Kodombo, 2010). The evolution and genetic improvement of species depend on genetic diversity existing in crop germplasm (Charrier *et al*., 1997). Genetic diversity is variation among alleles of genes within individuals of species'. During evolution, cultivated plants acquire some traits enabling them to survive or to be adapted to new environmental conditions.

According to FAO (2009), the biological basis of world food security for agriculture and consumption relies on genetic resources. The world future food production depends on domesticated species (CDB, 1996). However, only 3% (about 7 000) of plants species suitable for cropping are utilized in agriculture over 250 000 species available (Vernooy, 2003). The cultivated species have been grown for many centuries and have been kept through good agricultural practices. The use of these resources remains low due to lack of knowledge on their potential and compare to world population need for feed. They also contain many undesirable genes and are useful primarily as sources of a few desirable genes they possess.

In Africa, particularly in Sub-Saharan African countries, the majority of the population lives in rural zones and rely mainly on crop production for subsistence. Unfortunately, agriculture is limited by many constraints that hamper socio-economic development (Assogbado *et al*., 2009). In semi-arid areas, rural populations are constantly faced by a reduction and erratic distribution of rainfall during cropping seasons and, as a

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consequence, their food security status is weakened. According to Assogbado *et al*. (2009) the problem could be solved through diversification of agriculture via the use of important genetic resources in development of new durable production systems.

In Burkina Faso, after the food shortage due to drought of the 1970, sorghum breeding programs were started to provide farmers with high yielding varieties in order to avoid another food crisis. The objectives were focused on the release of exotic *caudatum* varieties in place of local *guinea* varieties. The *caudatum* race is known to be more productive than the *guinea* race, respond better to intensified management and new sorghum production systems (Barro-Kodombo, 2010). This initiative failed to make measurable impact due to lack of interest of stakeholders. The c*audatum* varieties did not fit with local farming systems and were not adequate for farmers' edibility qualities compared to *guinea* varieties. Furthermore, their cost of production was high due to the requirement for inputs that farmers cannot afford and their panicle structure (opacity) made them susceptible to diseases such as grain mold and insect damage (Barro-Kodombo, 2010).

There are limitations on introducing exotic materials and breeders need to search for adequate breeding material within available local sorghum germplasm in order to fulfill farmers' needs. Knowledge about the variation existing in local germplasm of a crop is essential to a rational management and use of that crop (Somé, 1989). It is therefore necessary to screen in our local germplasm and identify lines that may respond to ecological, climatic constraints and that are suitable for farmers' requirement. The probability of getting appropriate material is high due the *guinea* genetic diversification. According to Harlan (1975) West Africa is a centre of diversity of the *guinea* race and Burkina Faso may probably be at the heart of that centre (Zongo, 1991).

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Many studies have been conducted to identify sorghum diversity in Burkina Faso. For instance, agro-morphological characterization (Zongo, 1991; Barro-Kodombo *et al*., 2008), enzymatic characterization (Zongo *et al*., 2005), molecular characterization (Barro-kodombo *et al*., 2008) on grain sorghum and sweet sorghum (Nebie, 2014; Sawadogo, 2014) have been reported on this crop. The molecular characterizations were focused on grain sorghum of three ecological zones (centre-south, centre-north and Centre-western part of the country) and sweet sorghum characterization only. Until now the local sorghum accessions from west, south-west and Eastern part of the country have not yet been characterized with molecular tools. A collection of sorghum germplasm from these regions was been made in 1999 by the sorghum breeding programme of INERA/FKB. Morphological characterizations have been done on these accessions. Unfortunately, phenotypic characterization is not indicative of genetic variation due to environmental interaction. Therefore, a combination of phenotypic and molecular techniques is required in order to optimize the characterization efficiency (Yada *et al*., 2010). Numerous types of molecular markers are available and among them, Simple Sequence Repeats (SSRs) have been widely used for population genetic analysis of both animals and plants (Li *et al*., 2009). The present study aims to assess diversity of 186 sorghum accessions from three regions located in two agro-ecological zones in Burkina Faso.

The objective was to characterize the sorghum germplasm using some morphological descriptors related to drought and SSR markers.

The specific aims of the study were to: (1) define the existing diversity among the population using 15 morphological characters and 26 SSR markers; (2) to indentify, within local sorghum germplasm, drought tolerant material for breeding purposes.

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4.2. Materials and methods

4.2.1. Plants materials

One hundred and eighty six (186) accessions were collected in 1999 in three districts located in two agro-ecological zones of the country. Ninety were collected in the west, 46 six in the south western part and 40 in the eastern part of the country. The accessions were self-pollinated regularly for seeds regeneration and conservation. Many accessions are highly photo sensitive and 50% flowering date of the majority of the collected accessions does not fit during the rainy season. Four [two post-flowering drought tolerant lines (B35, a *durra* line; ET36-1, a *guinea-caudatum*, from Ethiopia) and two pre-flowering drought tolerant lines (RTx7000, RTx7078, from USA)] were obtained from ICRISAT. Three (Grinkan, Tiandougou and Tiandougou-coura) drought tolerant lines were obtained from Mali and three (SAMURAI1&2 and Pahat) drought tolerant mutant lines were obtained from Indonesia.

4.2.2. Agro-morphological characterization

4.2.2.1. The experiment

Field trial was established during the post rainy season (from November 2013 to March 2014) and repeated a second time from October 2014 to February 2015. Based on a prior evaluation, some early maturity accessions were identified and were planted three weeks after planting date of late maturity accessions. An alpha lattice design (11x10) in 3 replicates was used in two environments (full irrigated conditions and terminal drought condition). Gravity irrigation was used to supply water. In full irrigation environment, water was supplied until the physiological maturity was reached while terminal drought

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was achieved in water stressed regime by withholding water from 50 % flowering for three weeks.

The materials were planted in a single row of 4 m spaced 80 cm. Three seeds were planted per hole spaced 40 cm in each row. The experiment was conducted at Valley du kou (INERA station of Bobo) located at 11°22' N Latitude, 4°22 W Longitude, on a ferruginous acid soils with silty texture and, where irrigation facilities are available.

4.2.2.2. Data collection

Agro morphological data according to the sorghum descriptor (IBPGR and ICRSAT, 1993) were collected on 5 plants from the middle portion of the row:

- ✓ **Days to 50% flowering (Flo):** recorded as the number of days from emergence to when 50% of plants have started flowering.
- ✓ **Plant height (PH):** height in centimeters of main stalk at 50% flowering from ground level to the flag leaf at maturity.
- ✓ **Chlorophyill content (Spad):** chlorophyll value measured using a chlorophyll content meter.
- ✓ **Panicle length (PL):** measurement of panicle length from the peduncle to the tip of the panicle.
- ✓ **Panicle weight (PWe):** measurement of the dry panicle weight in grams.
- ✓ **Grain weight/panicle (GrWP):** total dry weight in grams of panicle harvested in each plot. This value was used later for determination of grains yield in kilograms per hectare.
- ✓ **100 grains weight (100GrWe):** dry weight of 100 grains from each plot.
- ✓ **Number of grains per panicle (NbGrP):** average of five panicles grain in the plot.
- ✓ **Yield under full irrigated condition (Yirr):** yield of a given line under full irrigated condition.

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- ✓ **Yield under stress condition (Ystr):** yield of a given line under terminal drought stress condition.
- ✓ **Leaf greenness (Stg):** greenness aspect ranking from 1 to 5 (1 greater green leaf, 5 weak green leaf aspect).
- ✓ **Senescence or wilting (SNT):** estimation of death leaves and stalks at time of grain maturity [1 not senescent (no death of leaves), 5 completely senescent (leaves and stalks gead).
- ✓ **Grain filling rate (GFR):** percentage estimate of grain filling rate at maturity.

Two drought tolerance indexes were also calculated: Stress tolerance index (**STI**) and the Geometric Mean productivity (**GMP**).

$$STI = \frac{Ystr \times Yirr}{Xp^2}$$

$$GMP = \sqrt{Ystr \times Yirr}$$

Ystr is the grain yield of a given genotype under drought condition. Yirr is the grain yield of a given genotype under non stress condition, and Xp is the mean grain yield averaged across all the genotypes tested under non stress condition.

4.2.2.3. Data analysis

The SAS computer program SAS 9.3 (SAS institute, Cary, NC, USA) was used to analyze the morphological data. The general linear model following was used.

$y_{ijk} = \mu + \tau_i + \rho_j + \beta_{jk} + e_{ijk}$

Where y_{ijk} is the value of the observed trait for i^{th} treatment received in the k^{th} block within j^{th} replicate (superblock),

τ_i is the fixed effect of the i^{th} treatment (i = 1, 2… t);

ρ_j is the effect of the j^{th} replicate (superblock) (j = 1, 2… r);

β_{jk} is the effect of the k^{th} incomplete block within the j^{th} replicate (k = 1, 2…s)

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e_{ijk} is an experimental error associated with the observation of the i^{th} treatment in the k^{th} incomplete block within the j^{th} complete replicate.

Analysis of variance was performed to identify differences within genotypes (accessions) and Pearson correlation was run to identify correlations among parameters. Principal Component Analysis was used to examine the structure of correlations between parameters. The eigen values and eigenvectors of the correlation matrix were derived and, were used to reduce the number of parameters in the statistical analyses (Daulfrey, 1976). Cluster analyses were performed to group parameters together using the Euclidean distance. Data points with smaller distance between them grouped together and a dendrogram was plotted from these computed clusters as a graphical relationship among accessions. The drought tolerance index (STI) and Geometric Mean productivity were used to rank the thirty top accessions.

4.2.3. Genetic diversity study of local accessions

4.2.3.1. Samples collection and DNA extraction

Leaf samples were collected from three weeks old plants and dried in a stove at 40° during three days. After drying leaf samples were ground using a Geno-grinder (RETSCH) at 500 strokes per minute for 9 minutes (3 tmes, 3 minutes each) and then DNA was extracted following a Mixed Alkyl Trimethyl Ammonium Bromide (MATAB) method (Frost *et al.,* 2007). A pre-heated (65°) 750µl of MATAB tampon was added to each sample and then incubating at 65° in a shaking water bath for 20 minutes. After incubation, 750µl of chloroform-isoamylalcohol (24:1) was added to each sample and agitated manually 50 times before being centrifuged at 13000 rpm for 20 minutes. 600 µl of the aqueous layer was transferred in a new tube of 1500 µl then 600 µl of cold isopropanol was added, mixed and kept at -20° for two hours. The samples were

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centrifuged at 13000rpm for 20 minutes and the supernatant was decanted and pellets were washed with 500 µl of 70% ethanol and centrifuged at 13000rpm for 20 minutes. The supernatant was decanted and pellets were air dried during the night or 45 minutes in a stove at 40°. Finally, the samples were dissolved in 150 µl of TE (TE1X) and kept at ambient temperature overnight and then stored at -20°. The working concentration of about 5 ng per µl was obtained from dilution of initial DNA solution after checking DNA concentrations and quality on 0.8% agarose gels.

4.2.3.2. Polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis

PCR was done in 10 µl reaction volumes containing 25ng of genomic DNA template, 0.1mM of dNTPs, 1x buffer, 200µM of MgCl2, 0.1µM of both forward and reverse primers, 0.1µM of IRdye, 0.1U of ampliTaq polymerase enzyme and double distilled water. The PCR were realized in 35 cycles using a thermocycler (MWG AG Biotech). Thermocycler was programmed as follows: denaturation at 94°C lasting 4 min, followed by 10 cycles composed of 45s of denaturation at 94°C and annealing at TM-5°C with a reduction of 0.5°C/cycle for 1min and at last an extension at 72°C for 1 min15s. This was followed by 25 cycles. Each cycle was a chain of denaturation (94°C during 45s) and, annealing (at TM- 5°C for 1min) and then an extension (at 72°C for 1 min15s). Finally, the reaction ended by 5 min extension at 72°C. PCR products were subjected to electrophoresis in 6.5% polyacrylamide gels with a licor 4300 DNA Analyser system. Electrophoresis was carried out using 20 ml of polyacrylamide 6.5% polyacrylamide, 200ml 10X TBE diluted to 1 L, 175 µl ammonium per-sulphate (APS), and 25 µl TEMED). The gel was run at 1500V and 35 mA constant power supply, for 2 hours using a licor DNA analyser unit. For polymorphism assessment, the bands on the gel were

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coded as ‘‘1’’, ‘‘2’’, ‘‘3’’, ‘‘4’’ “5” and “6” based on their allele position. The missing data were scored as ‘‘x’’.

4.2.3.3. Data analysis

4.2.3.3.1. Descriptive parameters for genetic diversity

- **Polymorphism rate**

Polymorphism rate (P) is an estimate of the number of polymorphic loci compared to the whole studied loci. Polymorphism or intra-specific diversity is defined as the presence at a locus of two or several allelic forms (Pernes, 1984). It is obtained by the following formulas: **P=Number of polymorphic loci/Number of studied loci**. A locus is considered to be polymorphic when the most common allele has a frequency below 95%.

- **Allelic diversity**

Allelic diversity is an estimate of the average number of alleles per locus**. Na= Total number of alleles/Number of studied locus**. Allelic diversity depends on the population size.

N is the total number of allele in a population whereas **Na** is the average number of allele per locus.

- **Heterozygosity rate**

Heterozygosity rate is a measure of Nei genetic diversity (Nei, 1978).

The observed heterozygosity (*Ho*) represents the proportion of the average observed heterozygous individuals in the population: Ho=Number of heterozygotes/Number of individuals.

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The expected heterozygosity (*He*) is the theoretical proportion of expected heterozygous individuals at a locus when Hardy-Weinberg equilibrium is reached. It is obtained by calculation: $He=1-\sum_{i=1}^{n} Pi^2$; where *Pi* is the frequency of the i^{th} allele for the population and $\Sigma P i^2$ is sum of the squared population allele frequencies.

4.2.3.3.2. Structure of genetic diversity

Three fixation indexes (F-statistic) based on heterozygosity rate (*Ho, He*) have been defined by Wright (1965) to describe genetic structure of populations:

- ✓ $\boldsymbol{F_{is}}$ measures the heterozygosity deficit in a sub-population and indicates the intra –population genetic differentiation. It is obtained by calculation: *Fis* = (Mean *He* - Mean *Ho*)/Mean *He*. It ranges from -1 (heterozygote population) to 1 (homozygote population).

- ✓ $\boldsymbol{F_{st}}$ measures the genetic differentiation among sub-populations and it is calculated as follows: *Fst* = (*Ht* - Mean *He*)/*Ht* where *Ht* is the total expected heterozygotes. It ranges from 0 (identical population) to 1(population totally different).

- ✓ **Nei** genetic distance was determined among the population.

- ✓ **Shannon's Information Index**; I = -1* Sum (pi * Ln (pi)).

An Analysis of Molecular Variance (AMOVA) was performed to explain the genetic variation. Its estimation is based on genetic distance within individuals and takes account of information released by all studied markers.

All descriptive parameters involved in the diversity study were analyzed using GenAlex version 6.41 (Rockall and Smouse PE, 2006). Darwin software, version 5.0.158 (Perrier

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and Jacquemoud-Collet, 2006) was used to display graphical genetic relationship (Factorial analysis and cluster analysis for diversity structure). The factorial analysis was performed using Rogers-Tanimoto dissimilarity index and the cluster was obtained using the "Neighbor-Joining" method.

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4.3. Results and discussions

4.3.1. Results

4.3.1.1. Morphological characterization for drought trait tolerance, yield and yield components

- **Analysis of variance**

Differences within genotypes were highly significant (P<0.001) for all the morphological traits evaluated. Environmental effect (different moisture stress) displayed high difference for the same genotypes (P<0.001) for chlorophyll value (Spad), panicle height, rate of grain filling, panicle weight, number of grain/panicle and the yield. The environment effect did not affect the 50% flowering date and plant height. The genotype x water regime interaction was significant for all parameters. The ANOVA result is presented in Table 4.1.

Table 4. 1: Result of combined ANOVA of F value for various traits

Source	DF	Flo	Spad	Stg	PH	PL	GFR	GrWP	Nbgrpa	Yield
RH	**1**	**3.37ns**	**67.75*** **	**139*** **	**0.51ns**	**189.63*** **	**1315.49*** **	**2889.99*** **	**2997.75*** **	**2894.73*** **
Rep	**2**	**3.55ns**	**6.2ns**	**3.32ns**	**3.12ns**	**1.28ns**	**6.22ns**	**4.49ns**	**3.9ns**	**5.95ns**
Block(Rep)	**27**	**3.61ns**	**2.13ns**	**2.26ns**	**2.02ns**	**1.03**	**5.10ns**	**7.70ns**	**5.64ns**	**5.19ns**
Geno	**109**	**11.26*** **	**4.10** **	**9.06** **	**68.77*** **	**26.22** **	**20.35*** **	**49.23*** **	**57.07*** **	**49.20*** **
RH*Geno	**109**	**7.26*** **	**4.471** **	**8.96** **	**6.14** **	**5.69** **	**10.74*** **	**38.46*** **	**39.25*** **	**38.27** **

***P<0.001; **P<0.01; *P<0.05; Flo= 50% flowering date; Spad=chlorophyll content value; PH= Plant height; PL= panicle Length; GFR=grain filling rate; GrWP=grain weight/panicle; Nbgrpa=number of grain/panicle; Yield= grain yield; Geno= Genotypes; RH=Hydric Regime; RH*Geno= interaction genotype by moisture regime.

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❖ **Pearson correlation**

The chlorophyll content (Spad) was not correlated with the grain filling rate, yield and its components (panicle size, grain weight/panicle and number of grain/panicle). However, it was correlated with the panicle size, leaf senescence and plant height. The visual scoring of stay-green was positively and significantly correlated with all parameters except the stress tolerance index and the geometric mean productivity. Plant height was positively correlated with leaf senescence and panicle size but was not correlated with the grain weight per panicle, the grain number and yield. In contrast, it was negatively correlated with grain filling rate, STI and GMP. The panicle length was negatively and significantly ($P<0.001$) correlated with grain weight per panicle, yield under stress (Ystr), grain filling rate, STI and GMP. The grain filling rate was highly, positively correlated ($P<0.001$) with the yield under stress and its components. The yield under stress was highly correlated with its components and STI and GMP. The correlation results are presented in Table 4.2.

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Table 4. 2 : phenotypic correlation between traits recorded for 110 accessions including checks in two moisture regimes

	Flo	Spad	Stg	PH	SNF	PL	GrFR	GrWP	Nbgrpa	Ystr	STI
Flo											
Spad	**0.28***										
Stg	**0.18NS**	**0.479*****									
PH	**0.65*****	**0.44*****	**0.23***								
SNF	**0.31****	**0.36*****	**0.48*****	**0.22***							
PL	**0.49****	**0.45*****	**0.24***	**0.66*****	**0.36*****						
GrFR	**-0.01NS**	**-0.09NS**	**0.20***	**-0.15NS**	**0.16NS**	**-0.22*****					
GrWP	**0.045NS**	**0.12 NS**	**0.31****	**0.14NS**	**-0.24*****	**-0.26*****	**0.59*****				
Nbgrpa	**0.05NS**	**0.11NS**	**0.29****	**0.11NS**	**0.02NS**	**0.18NS**	**0.59*****	**0.95*****			
Ystr	**0.04NS**	**0.12NS**	**0.31****	**0.14NS**	**-0.25***	**-0.26*****	**0.59*****	**0.99*****	**0.96*****		
STI	**-0.25****	**-0.10 NS**	**0.13NS**	**-0.27****	**0.09NS**	**-0.6*****	**0.46*****	**0.70*****	**0.67*****	**0.70*****	
GMP	**-0.29****	**-0.12 NS**	**0.10NS**	**-0.28****	**0.05NS**	**-0.11*****	**0.54*****	**0.73*****	**0.69*****	**0.73*****	**0.95*****

***P<0.001; **P<0.01; *P<0.05; Flo= 50% flowering date; Spad=chlorophyll content value; PH= Plant height; PL= panicle Length; GFR=grain filling rate; GrWP=grain weight/panicle; Nbgrpa=number of grain/panicle; Ystr= grain yield under terminal moisture condition; Yirr= grain yield in full irrigated condition; STI= Stress Tolerance index; GMP=Geometric mean Productivity.

- **Determination of principal variable through a Principal Components Analysis (PCA)**

From the correlation analysis, the results revealed that phenotypic (Flo, Spad, stg, PH, SNF, GrFR) traits were mostly redundant and the yield components (PWe, GrWP, Nbgrpa, Ystr, STI, GMP) also had redundancy within them. Principal components analysis was performed to determine the most important variables that should be used for the final discrimination of genotypes under terminal drought condition. The scree plot test illustrated in Figure 4.1 indicated that three principal components should be used and the proportion (78.5%) of the total variation confirms that criterion. The PC1 accounted for 45.76% with yield, its components (Ystr, PWe, GrWP, Nbgrpa, Ystr), STI and GMP as the major variables that explain variation among genotypes. The PC2 accounted for

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25.46% variation and is associated with flo, Spad, PH, SNF and PS The third PC3 accounted for 7.27% of the variation and it was associated with traits such as leaf senescence (SNF) and grain filling rate.

Table 4. 3 : Eigen vectors of three principal components used to determine principal variables

Traits	PC1	PC2	PC3
Flo	**0.010**	**0.432**	**0.005**
Spad	**0.054**	**0.366**	**0.267**
PH	**0.035**	**0.493**	**-0.290**
SNF	**0.127**	**0.268**	**0.791**
PS	**0.095**	**0.462**	**-0.238**
GrFR	**0.275**	**-0.148**	**0.348**
PWe	**0.383**	**0.138**	**-0.092**
GrWP	**0.417**	**0.029**	**-0.099**
Nbgrpa	**0.403**	**0.011**	**-0.105**
Ystr	**0.417**	**0.031**	**-0.099**
STI	**0.341**	**-0.221**	**-0.020**
GMP	**0.350**	**-0.243**	**-0.032**
Eigen value	**5.49**	**3.055**	**0.87**
Variance (%)	**45.76**	**25.46**	**7.27**
Cumulative	**45.76**	**71.23**	**78.5**

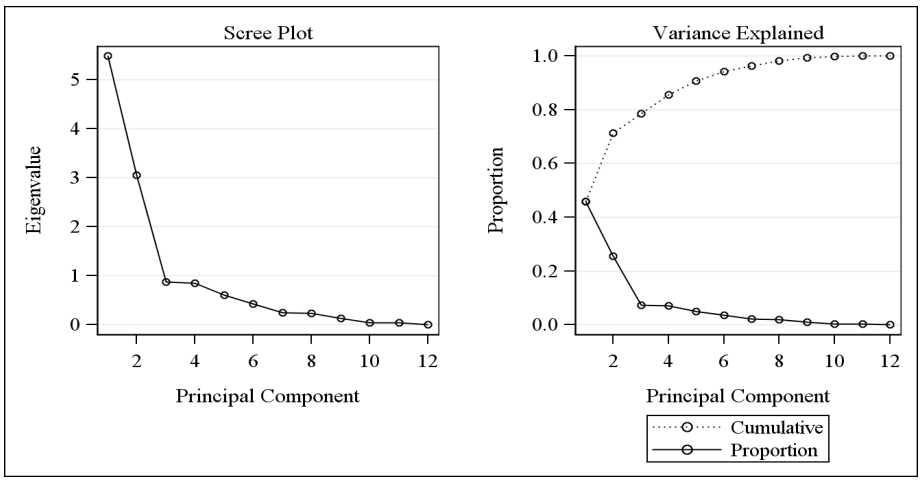

Figure4. 1: Scree plot of the principal components with their Eigen value and the proportion of explained variance.

- **Cluster analysis**

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From the principal components analysis, the variables associated with PC1 (yield, yield components, stress tolerance index and geometric mean productivity) were used for hierarchical cluster analysis. The dendrogram was constructed to group accessions according to their root mean square distance (Figure 4.2). The minimum root mean square distance (0.038) was recorded between PSE58 and Sariaso09 and the maximum (3.55) was recorded between Grinkan and B35. The accessions were grouped into three major clusters. Cluster I could be considered as an outlier as it contained only one accession (Grinkan a drought tolerant check). Cluster II was composed of three sub-clusters. The first sub-cluster contained one drought tolerant check (Tiandougou-coura), one local variety (Ouedzoure) and 12 landraces. The second sub-cluster contained 45 accessions within which were six local varieties (Sariaso04, Sariaso07, Sariaso5, V1, V2, and Kapelga), three introduced materials (ICSV1049, Framida, SAMURAI: drought tolerant check) and 36 landraces. The third sub-cluster contained 6 landraces and 1 improved variety (RTx7000). The cluster III comprised three sub-clusters. The first sub-cluster contained 13 landraces, the second 11 landraces and 3 improved varieties (Sariaso04, Sariaso09 and RTx7078). The third sub-cluster contained 19 accessions composed of 15 landraces and 3 local improved varieties and B35. The accessions were classified according to their yield and yield components under terminal drought conditions. They were ranked from the higher yielding to the low yielding accessions. Accessions in cluster I exhibited high yielding capability and accessions in cluster II showed moderate yielding potential whereas accessions in cluster III had low yielding potential under drought conditions. The classification was done according to the difference of root mean square of accessions.

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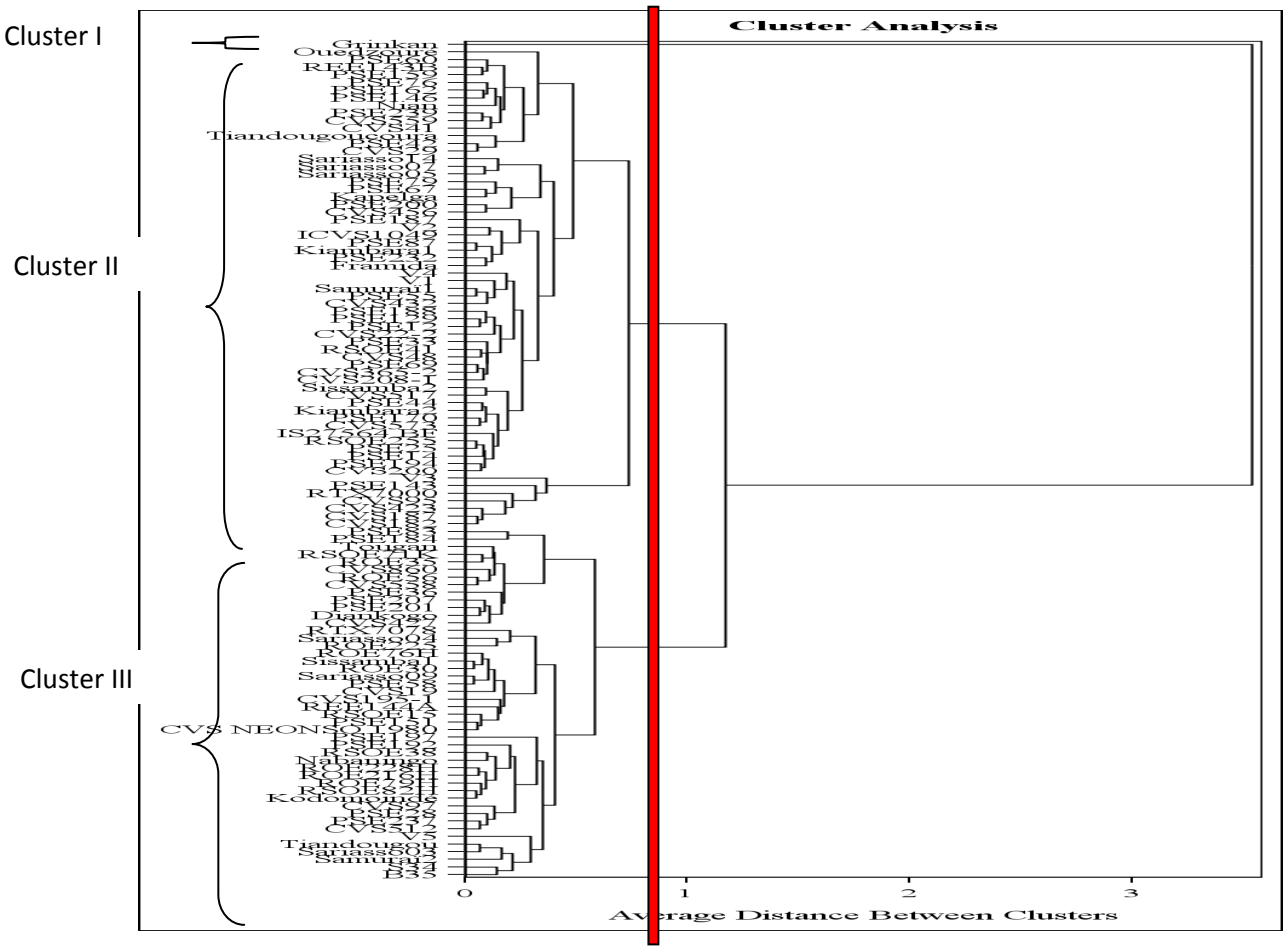

Figure4. 2: Dendrogram of the 110 sorghum accessions revealed by cluster analysis based on Root Mean Square (RMS) distance of their STI value.

❖ **Performance of 30 top accessions according to their stress tolerance index**

From cluster analysis, accessions ranged from higher to the lower yield and yield components value. The 30 top accessions were selected based on their stress tolerance index, geometric mean productivity and Spad value. Grinkan was the top accession with higher value of STI and GMP (Table 4.4). Six landraces (Diankogo, S34, PSE184, CVS860, CVS427 and RSOEK71) and some drought tolerant checks, Tiandougou, SAMURAI2, Tiandougou-coura, showed moderate values of STI and GMP which were higher than that of B35 and they were ranked among the fifteen top accessions. For the Spad value, B35 showed the highest chlorophyll content (55.7) in leaves two weeks after

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flowering under terminal drought conditions. Grinkan was not among the 30 top accessions for this trait. The 29 accessions with important Spad value ranking below B35 in the top 30 were all local landraces.

Table 4. 4: Performance of the 30 top genotypes according to their Spad value, STI and GMP

Rank	Geno	STI	GMP	Rank	Geno	Spad
1	Grinkan	4.8	4.0	1	B35	55.7
2	Diankogo	3.7	3.5	2	Ouedzouré	55.3
3	S34	2.3	2.8	3	ROE225	53.4
4	Tiandougou	2.1	2.6	4	PSE143	51.1
5	Samuraï2	1.8	2.5	5	RSOE15	50.0
6	PSE187	1.6	2.3	6	PSE36	49.9
7	CVS860	1.6	2.3	7	CVS456	49.2
8	Tiandougoucoura	1.5	2.2	8	PSE60	48.3
9	CVS427	1.4	2.2	9	CVS22-2	48.3
10	V5	1.3	2.1	10	IS27564 BF	48.1
11	Sariasso04	1.2	2.0	11	PSE33	47.8
12	RSOE71K	1.2	2.0	12	CVS559	47.5
13	B35	1.2	2.0	13	CVS29	47.2
14	ROE79H	1.1	1.9	14	PSE159	47.2
15	PSE36	1.1	1.9	15	PSE197	47.1
16	REE143B	1.1	1.9	16	ROE76H	47.0
17	V2	1.0	1.9	17	ROE216H	46.1
18	ICVS1049	1.0	1.9	18	CVS432	46.0
19	ROE56	1.0	1.9	19	CVS558	45.5
20	ROE76H	1.0	1.9	20	PSE44	45.1
21	PSE232	1.0	1.8	21	PSE194	45.1
22	Framida	0.9	1.8	22	CVS208-1	44.9
23	PSE28	0.9	1.8	23	RSOE82H	44.9
24	CVS512	0.9	1.8	24	PSE188	44.7
25	PSE42	0.9	1.8	25	REE144A	44.4
26	CVS558	0.9	1.8	26	PSE58	44.4
27	RSOE15	0.9	1.7	27	RSOE38	44.3
28	Sariasso03	0.9	1.7	28	PSE201	44.3
29	RTx7078	0.9	1.7	29	ROE79H	44.2
30	Kiambara2	0.8	1.7	30	PSE76	44.2
	Means	1.4	2.1		Means	47.4

Geno= genotypes; STI= Stress Tolerance index; GMP=Geometric mean Productivity.

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4.3.1.2. Molecular characterization of accessions using SSRs markers

4.3.1.2.1. Genetic polymorphism and allelic diversity

- **Polymorphism level of SSR markers used for characterization**

The characterization results are presented in Table 4.5. Twenty-six SSRs markers were used in the present study. The polymorphism rate was 86.54%. The number of alleles ranged from 2 to 6 with an average of 3.5 alleles per locus. A total of 93 alleles were identified. The unbiased expected heterozygosity ranged from 0.061 (Msbcir 225) to 0.613 (Gpsb032) with a mean of 0.367. The expected heterozygosity (gene diversity) ranged from 0.021 (Msbcir 225) to 0.553 (Gpsb032). The lower genetic differentiation was showed at Msbcir 225 with a *Fst* value of 0.026 and the higher distance was revealed by Xtxp225 with a *Fst* value of 0.601. Intra-population genetic differentiation (*Fis*) was very high (0.963) and showed a low rate of heterozygous individuals (0.013).

- **Allelic diversity patterns across population**

The allelic frequency was very low (<5%) and ranged from 2.23% (exotic accessions) to 2.69% (local accessions of Burkina). The rate of number of common alleles was low in exotic material (23.1%) and relatively high within accessions of local populations (Eastern Region=69.2% Western and South-west Regions showed 80.8%). Twenty-two rare alleles were observed in local accessions. Five rare allelic forms were observed in Eastern Region at locus Xtxp15. Seventeen rare alleles were also observed in accessions of Western Region, respectively, five at Gpsb14 locus, 6 at Xtxp123 locus and 6 at Xtxp285 locus. No rare allele was observed in exotic accessions. Figure 4 3 illustrates the

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allelic pattern across populations and Figure 4.4 shows an output of alleles as revealed by the marker Gpsb014.

Table 4. 5. Genetic diversity values provided by 26 SSRs markers

Markers	N	Na	I	Ho	He	Fis	Fst
Msbcir238	4	2.333	0.485	0.000	0.284	1.000	0.337
Gpsb014	5	3.000	0.722	0.002	0.401	0.995	0.242
Gpsb079	3	2.333	0.591	0.000	0.362	1.000	0.394
Gpsb098	4	3.000	0.790	0.006	0.459	0.987	0.181
Gpsb123	4	2.833	0.696	0.007	0.427	0.983	0.385
Gpsb133	2	1.500	0.239	0.000	0.165	1.000	0.327
Gpsb136	3	2.333	0.616	0.023	0.401	0.943	0.332
Gpsb158	3	2.167	0.478	0.004	0.286	0.988	0.456
Msbcir188	3	2.500	0.623	0.002	0.368	0.995	0.390
Msbcir222	3	1.833	0.152	0.000	0.071	1.000	0.034
Msbcir243	3	2.333	0.413	0.006	0.246	0.977	0.190
Sbagb02	3	2.000	0.460	0.000	0.313	1.000	0.264
Xcup11	2	2.000	0.510	0.000	0.339	1.000	0.243
Xcup43	3	2.333	0.590	0.010	0.365	0.974	0.331
Xtxp15	5	2.833	0.492	0.004	0.274	0.987	0.168
Xtxp23	6	3.833	0.992	0.059	0.553	0.872	0.141
Gpsb032	3	2.667	0.873	0.016	0.553	0.893	0.056
Xtxp03	3	2.333	0.635	0.004	0.407	0.972	0.280
Msbcir225	4	2.333	0.145	0.017	0.060	0.991	0.026
Msbcir314	3	2.333	0.622	0.000	0.405	0.710	0.229
Sb5-236	4	2.667	0.500	0.005	0.279	1.000	0.092
Xtxp55	4	2.833	0.712	0.011	0.428	0.981	0.102
Xtxp72	3	2.000	0.448	0.004	0.278	0.975	0.328
Xtxp123	4	3.167	0.824	0.061	0.478	0.987	0.305
Xtxp225	3	2.167	0.285	0.028	0.157	0.872	0.601
Xtxp285	6	3.667	1.010	0.000	0.513	0.820	0.325
Mean	3.5	2.513	0.573	0.01	0.341	1.000	0.260
SE		0.095	0.01	0.003	0.02	0.963	0.027

N= Number of alleles; Na=Allelic diversity; Ho=Observed heterozygosity; He= Expected heterozygosity; I=Shanon index; *Fis* =Fixation index measuring intra-genetic diversity; *Fs*= Fixation index measuring genetic diversity among population

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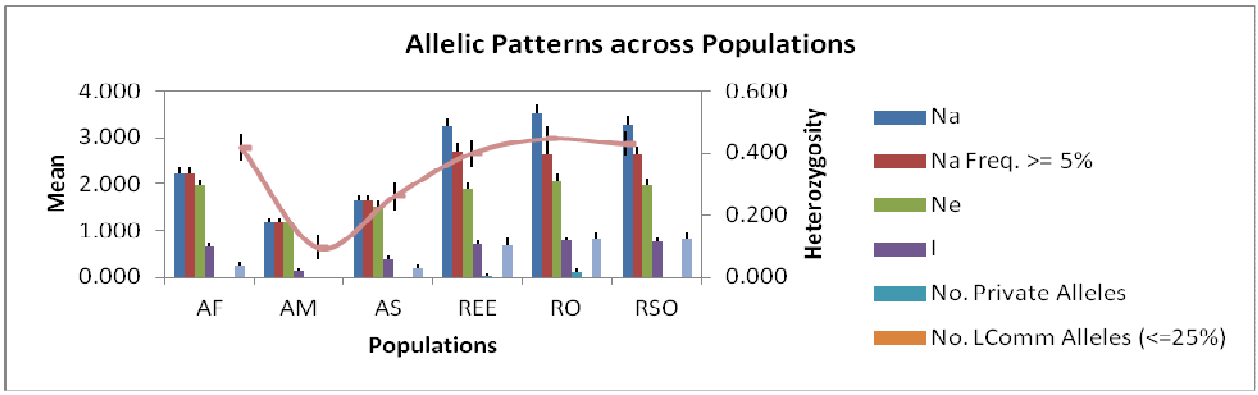

Figure4. 3: Picture illustrating the allelic diversity patern

AF: Accessions from Africa; AM: Accessions from America; AS: Accessions from Asia; REE: Eastern Region' accessions; RO: Western region' accessions and RSO: South-western region' accessions

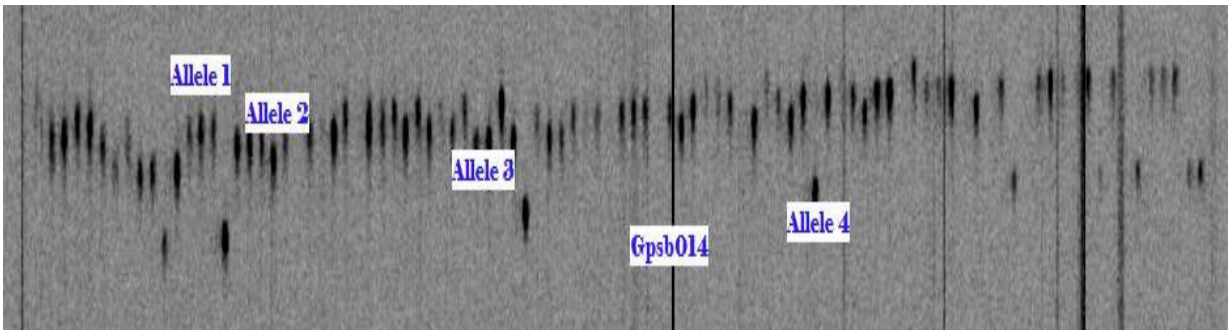

Figure4. 4: Picture illustrating the allelic diversity patern as revealed by the gel at the locus Gpsb014

- **Expected Heterozygosity or Genetic diversity**

Genetic diversity (*He*) for the total accessions was 0.34 and varied among populations. It was important in two populations of Burkina (respectively South-west Region=0.43 West Region=0.45) and relatively low in population of Eastern Region (0.39). The exotic accessions also exhibited important gene diversity (0.42). The Shanon Index value ranged from 0.6 exotic accessions and 0.8 in local accessions of Burkina. The polymorphism rate was high in local accessions (from 96.15% to 100%) but relatively low in exotic material (53.84%) compared to local accessions. However, genetic diversity was very low among regions (refer Table 4.6).

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Table 4. 6: Genetic diversity values per collected areas of 186 accessions

Pop	I	Ho	He	%P
Exotic	0.6	0.0	0.42	53.84%
ER	0.7	0.01	0.39	96.15%
WR	0.8	0.01	0.45	100.00%
SWR	0.7	0.01	0.43	96.15%
Mean	0.6	0.01	0.34	86.54%
SE	0.03	0.003	0.02	11.32%

Exotic accessions (Accessions from Africa, America and Asia), ER: Eastern Region, WR: Western region and WSR: South-west region, SE: Standard Error. Ho=Observed heterozygosity; He= Expected heterozygosity; I=Shanon index; P=Polymorphism rate

4.3.1.2.2. Description of genetic diversity

- **Genetic differentiation of pairwise populations**

The overall *Fst* value was 0.260 and was sufficient to exhibit differences among populations at a probability of P<0.05. The lower genetic distance or differentiation was shown by Msbcir 225 with a *Fst* value of 0.026 and the higher genetic distance was revealed by Xtxp225 with a *Fst* value of 0.601. The pairwise population *Fst* values show that there was no significant differentiation among populations from the regions (West, South-West and East) but the result indicated a slight genetic distance between exotic accessions and populations of the three regions. The analysis of Nei genetic identity revealed that accessions of the three populations were closer to each other and exhibit significant similarity with exotic material. The result is summarized in Table 4.7.

Table 4. 7: *Fst* and Nei distance matrix for Pairwise Population characterisation

Matrix of *Fst* value					**Matrix of Nei Genetic identity**				
Sudano-sahelian		**Sudanian**			**Sudano-sahelian**		**Sudanian**		
Exotic	**ER**	**WR**	**SWR**		**Exotic**	**ER**	**WR**	**SWR**	
0				**Exotic**	1				**Exotic**
0.17*	0			**ER**	0.70	1			**ER**
0.12*	0.015	0		**WR**	0.79	0.97**	1		**WR**
0.12*	0.022	0.011	0	**SWR**	0.78	0.96**	098**	1	**SWR**

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Exotic accessions (Accessions from Africa, America and Asia), ER: Eastern Region, WR: Western region and WSR: South-west region.

❖ **Analysis of molecular variance**

The analysis of molecular variance (Table 4.8) attributes 4% of genetic variation to the differentiation among populations, 93% due to differentiation among individuals and 3% was due to difference within individuals in a population. These variations were significant (P<0.05) according to the probability value of F-*Fst* (0.04).

Table 4. 8: Result of molecular variance analysis with 186 accessions

Source	df	SS	MS	Est. Var.	P(%)	F-stat *Fis*	F-stat *Fst*	Prob
Among Pops	5	114.55	22.9	0.24	4%	0.973	0.04	0.01
Among Indiv	180	2037.08	11.31	5.58	93%			
Within Indiv	186	28.5	0.153	0.153	3%			
Total	371	2180.13		5.97	100%			

Df=Degree of freedom; SS= Sum of square; MS=Mean square; P=Polymorphism rate

❖ **Structure of genetic diversity through factorial analysis**

Factorial analysis of 186 accessions was performed based on Rogers-Tanimoto dissimilarity index. The two first axes accounted for 34.23% of the total variation with respectively 27.91% for axis 1 and 6.35% for axis 2. Three major groups are displayed (Figure 4.6). Group1 and 2 are mainly composed of landraces from the three regions and group 3 includes exotic improved materials and local improved varieties from Burkina Faso. An exotic material (B35) was isolated from the remaining accessions. The goup1 and 2 comprised mostly local *guinea* landraces whereas group3 comprised three races (*caudatum*, *bicolor* and *durra*) and an intermediate race (*caudatum-guinea*). The *Caudatum* race was represented by local improved varieties from Burkina (Sariaso03, Sariaso06, Sariaso07 and Sariaso08). Six *caudatum-guinea* were represented in that group (Saraiso11, Sariaso14, Tiandougou, V2, V4 and V5). Four materials were of the

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bicolor race (RTx7000, TRx7078, Samurai2, and Pahat) and one was a *durra* (B35). This result showed that local landraces are very closely related and still have many alleles in common. The analysis revealed that improved varieties were also closely related but, slightly distinct from local accessions.

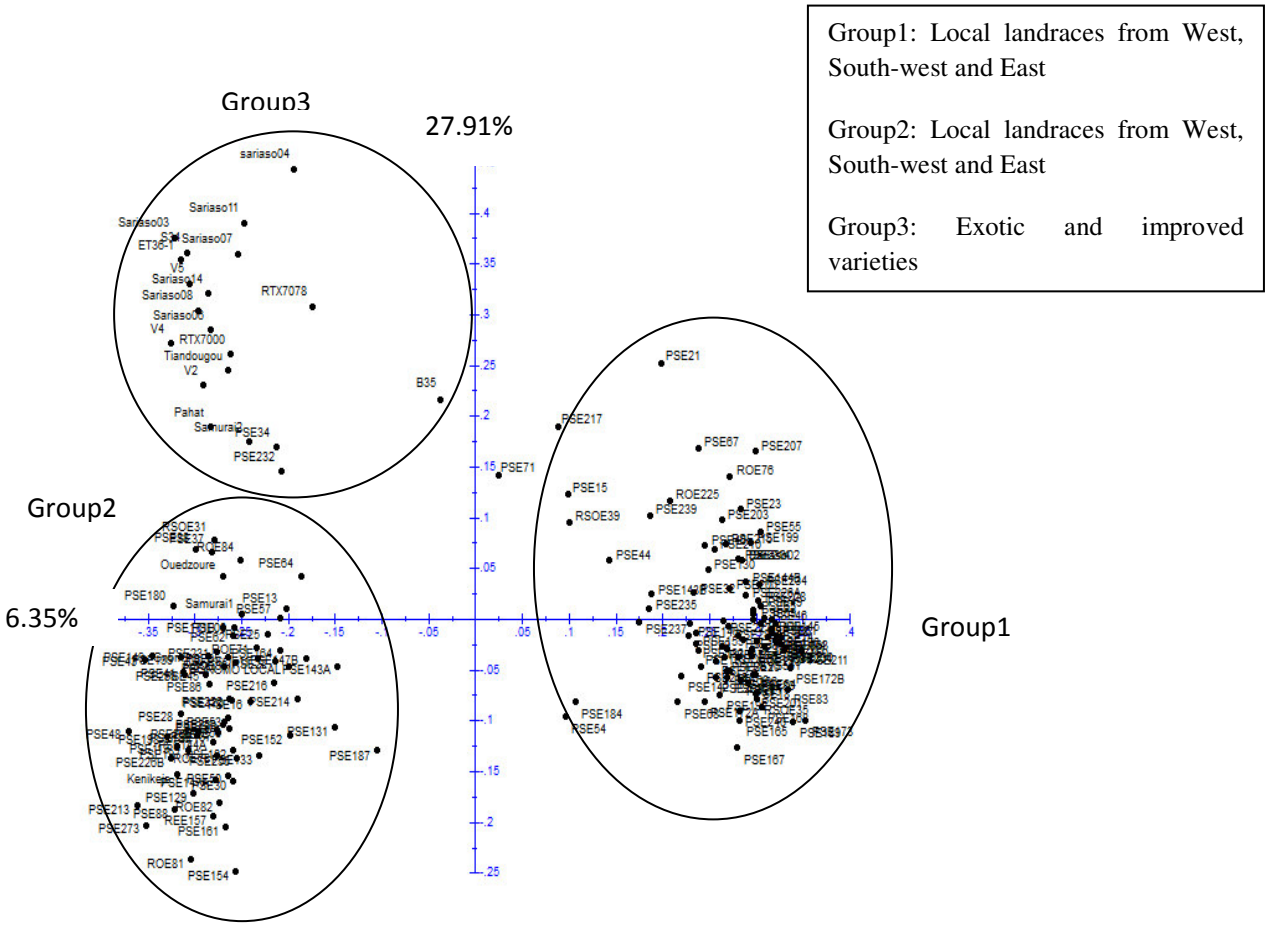

Figure4. 5: Plot of PC1 (27.96%) and PC2 (6.35%) from principal coordinate analysis illustrating the grouping of local sorghum accessions of three regions of Burkina Faso and some exotic material

- **Structure of genetic diversity through "Neighbor-Joining" method**

Genetic structure using "Neighbor-Joining" method displayed three major groups. Group A contained two sub-groups. The sub-group A1 contained mainly landraces from Eastern region and the sub-group A2 contained landraces from Western and South-western regions. The group B contained three sub-groups. The sub-group B1 contained landraces

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from Western and South-western regions with some accessions from Eastern region. The sub-groups B2 and B3 were mostly composed of exotic improved materials. The group C was composed of an isolated material (B35). The groups A and B contained mostly local Guinea landraces. The result is presented in the figure 4 6.

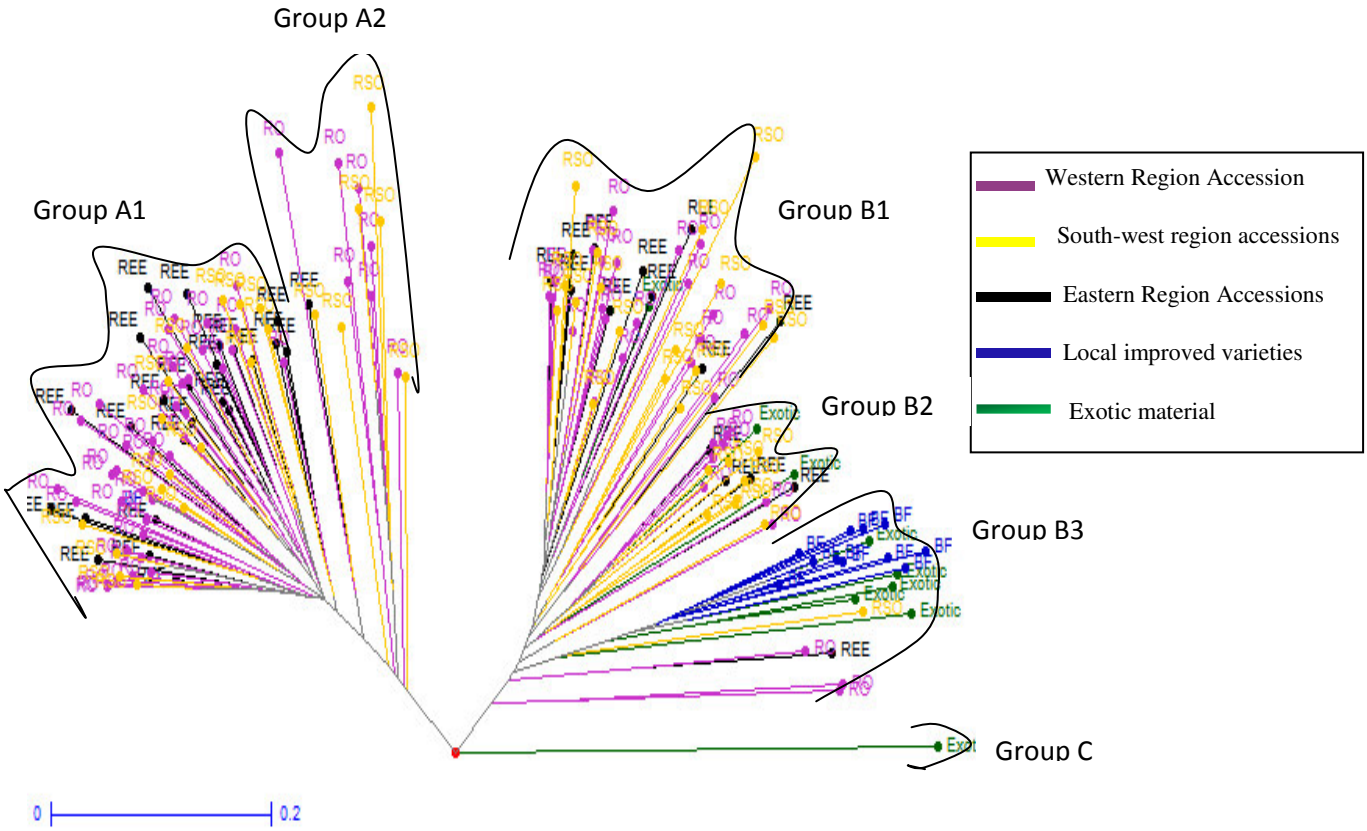

Figure4. 6: Dendrogram illustrating the genetic diversity of 186 accessions with 26 SSRs markers using "Neighbor Joining" method

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4.4. Discussions

Genetic improvement of species depends on variability existing in available genetic resources (Charrier *et al*., 1997). In this study, focus was on the genetic variation of agro-morphological traits related to drought, yield and yield components in order to identify materials with high yielding capability under terminal drought conditions. The analysis of variance revealed that evaluated accessions exhibited significant differences for all agro-morphological traits. Zongo (1991), Barro-Kodombo *et al*. (2008), using agro-morphological parameters of grain sorghums, reported that local sorghum accessions were highly diversified. Nebie *et al*., (2012) and Sawadogo *et al*. (2014) investigated Burkina Faso sweet sorghum diversity, and reported the existence of high variation among morphological characters that pointed out great variability among local accessions. The differences within materials showed that some of the accessions performed well under drought stress conditions while some did not. This indicated high probability to find drought tolerant genotypes within local accessions.

The lack of correlation between chlorophyll content and grain filling rate, yield and its components may be due to the fact that most of the local accessions did not have functional stay-green genes such as some checks (B35 and ET36-1). The positive correlation of visual scoring of the stay-green with the majority of the traits except the STI and GMP confirmed that greenness exhibited by local accessions did not have a positive impact on yield under stress conditions. This greenness was probably due to types C and D stay-green types which, according to Thomas and Smart (1993), are nonfunctional forms of stay-green and plants of these types appear green but lack photosynthetic competence. There was no correlation between plant height, grain weight

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per panicle, grain number and the yield and plant height was negatively correlated with the STI and GMP. Mutava *et al*. (2011) reported a negative correlation between plant height and grain weight, grain numbers and yield in their investigation of sorghum genotypes for traits related to drought tolerance. The analysis showed also a negative correlation between panicle size and grain filling rate, yield under stress conditions, STI and GMP. This indicated that panicle sizes were more important than weak grain filling rate, low STI and GMP indexes. Prasad *et al*., (2008) found that moisture stress from flowering to seed-set decreased percent seed-set and seed dry weight. The strong correlation between grain filling rates, yield under stress, grain numbers, STI and GMP indicate that improvement can be feasible using these accessions. Craufurd and Peacock (1993) reported that sorghum grain yield was highly and positively correlated with seed numbers and treatment effects affected during booting and flowering.

The principal components result identified the main variables responsible for high discrimination among genotypes. PC1 explained most of the variation observed in the yield (Ystr), the yield components (Nbgrpa, GrWP, PWe) and different indexes (STI and GMP) associated to the yield whereas PC2 was linked to morphological traits related to drought (PS, PH, Spad and Flo). The PC3 was also important and was correlated to two morphological parameters (GrFR and SNF) related to drought. The remaining components explained less than 7% each of the variation observed among accessions. This indicated that major variables allowing differentiation among genotypes were associated with PC1 and within variables associated to PC1, GrWP and Ystr contributed (0.417) more to the variation. Then Nbgrpa, PWe, GMP and STI contributed, respectively, as follow: 0.403, 0.383, 0.350 and 0.341 to the variation exhibited by PC1. The PC2 and PC3 explained secondly the variation among genotypes and were. The

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analysis showed that the yield and its components were more important sources of variation among genotypes than morphological parameters related to drought. The results suggest that focus should be directed toward yield and its components while selecting directly for drought tolerance under moisture stress conditions. Ibrahim *et al*. (2014) found that grain yield exhibited strong and positive phenotypic and genotypic correlation with its components. Vaisi and Golparvar (2013), in their investigation to improve grain yield and seed weight in oats, found that selection of panicle and grain number per panicle was relevant. However the morphological parameters such as chlorophyll content, leaf senescence, panicle size and grain filling rate could play important roles indirectly by allowing accessions to withstand moisture stress and lessen somehow yield loss. Tolk *et al*. (2012) found that during terminal moisture stress, panicle growth rate was linearly related to seed number for senescent and stay-green (non-senescent) hybrids but they concluded that stay-green hybrids produced 10% more seed than senescence hybrids. They concluded that stay-green hybrids maintained yield by retaining greater seed numbers.

The result of principal components analysis gave a hierarchical classification by using variables associated with the major components (PC1). The accessions were grouped into different clusters according to the similarity index described by Vilain (1999) and Fernandez (1992). High yielding and drought tolerant accessions were grouped in cluster I. In cluster II high yielding accessions and susceptible to drought were grouped while in Cluster III low yielding and drought tolerant accessions were grouped. Cluster I contained only the drought tolerant material (Grinkan) that performed well under both conditions (stress and non stress-conditions). It exhibited the highest STI and GMP

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values. Cluster II was primarily composed of local varieties (Sariaso04, Sariaso07, Sariaso5, V1, V2, Ouedzoure and Kapelga) with high yielding potential in research stations but known to be susceptible to terminal drought. Within the exotic material, some post-flowering drought susceptible genotypes (RTx7000, SAMURAI1) were found. Their presence in that group demonstrated that accessions in cluster II are susceptible to terminal drought. The cluster III included 46 accessions including six local improved varieties, one pre-flowering drought tolerant line (RTx7078) and one post-flowering drought tolerant line (B35). B35 is the most common source of stay-green used in breeding programmes and is recognized as a low yielding material. Its presence in this group illustrated the low yielding character of these accessions and their potential to withstand terminal drought. Sariaso04 and Sariaso09 are local high yielding improved varieties but they were found in the low yielding group in this study. Although these materials did not yield well in this study, they are normally high yielding and drought susceptible materials. RTx7078 is known to be a pre-flowering drought tolerant line. According to ranking of Hernandez (1992) and Vilain (1999), these materials should be classified within cluster II accessions.

The ranking of thirty top sorghum accessions was done according the stress tolerance index, geometric mean productivity and Spad value. Grinkan had highest value of STI and GMP. Some local landraces (Diankogo, S34, PSE184, CVS860, CVS427, V5 and RSOEK71) also exhibited high STI and GMP values. Except Grinkan that performed well under both conditions, the remaining accessions were grouped in cluster III. This indicated that accession with high STI and GMP were less productive but yielded more than B35. Most accessions with high STI and GMP values were ranked below B35 for

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stress tolerance index and were classified into cluster II meaning that they yielded better under non-stress conditions than under moisture stress conditions. This indicates that high yielding potential is not necessarily linked to drought tolerance ability. Sio-Se Mardeh *et al*. (2006) and Talebi *et al*. (2009) found similar results working on durum wheat. Belko *et al*. 2014 reported the same findings working on cowpea.

For Spad value, B35 showed the highest chlorophyll content level (55.7) in leaves two weeks after flowering under terminal drought conditions. Ouedzoure, a local improved variety, as well as four landraces (CVS 558, PSE197, REE144 and RSOE38) displayed high Spad values and yielded more under moisture stress condition than non-stress conditions. The greenness exhibited by B35 was due to the expression of functional stay-green genes. However, these landraces did not display premature leaf senescence and yielded more under stress conditions. This result may be due to the impact of greenness on the yield under moisture stress conditions of these genotypes. Borell *et al*. (1999) found that, during grain filling, the decrease in the rate of leaf senescence from 3% to 1% loss of leaf area per day resulted in doubling of grain size from about 15 mg to 30 mg. They suggested that sorghum grain size was correlated with the relative rate of leaf senescence, therefore stay-green trait has potential to increase sorghum grain yield by improving both grain number and grain filling ability. Rosenow (1984), henzell *et al*. (1992), Tuinstra *et al*. (1998), reported a positive correlation between stay-green and grain yield. Kebede *et al*. (2001) reported that stay-green lines maintained photosynthetic capacity during grain filling with the upper canopy leaves remaining active photosynthetically even after physiological grain maturity. Rosenow *et al*. (1983), McBee (1984); Wolfe *et al*. (1988) and Borrell *et al*. (2000a) found that sorghum hybrids containing the stay-green trait keep more photo-synthetically active leaves than do

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hybrids that do not contain it. These local varieties may need further investigation to determine whether they have functional stay-green genes or not.

In Burkina Faso, SSR markers have been used for diversity assessment of grain sorghum (Barro-Kodombo, 2008) and sweet sorghum (Nebie, 2014 and Sawadogo, 2015). These investigations were limited to particular regions of the country or to a particular type of sorghum. In the present investigation, 26 SSR markers were used to genotype 186 sorghum accessions collected in three regions from two different agro-ecological zones of the country including some exotic materials.

The study revealed a high rate of polymorphism (86.54%) compared to that (79.3%) obtained by Barro-Kodombo *et al*. (2008) using 28 SSRs markers on grain sorghum and was close to the polymorphism rate (87.5%) reported by Sawadogo (2015) on sweet sorghum and slightly below the rate (87%) reported by Missihoun *et al*., (2012) while working on 11 sorghum accessions with 8 SSRs markers. The polymorphism rate found in this work was higher than that reported by Smith *et al*. (2000) (P=65%) and Wang *et al*. (2009) (61%) using respectively 15 markers to characterize 50 elite sorghum lines and 95 SSR markers to assess diversity of 96 accessions. It was relatively low compared to the polymorphism rate (100%) reported by Nebie (2014) working on sweet stem sorghum. However, the rates found in the three different regions separately were high compared to the previous aforementioned studies.

The total allele numbers found was similar to those observed in other studies. Barro-Kodombo *et al*. (2008) found almost the same number of alleles per locus using Gpsb123 (4 alleles), Xcup11 (2 alleles), Sbagb02 (4 alleles) but Xtxp15 (6 allele) exhibited one allele more. This study revealed more allelic forms at Sbagb02 and Xtxp15 and fewer at Gpsb123 than reported by Sawadogo (2015) on sweet sorghum. However, Nebie (2014)

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found on sweet sorghum an important number of allelic forms at Sbagb02 (20 alleles) with an average of 6.79 alleles per locus. Billot *et al*. (2013) found 19.2 alleles per locus with 39 allelic forms at Sbagb02.

The gene diversity (*He*) mean value was 0.34. Msbcir225 exhibited the lowest gene diversity (*He*=0.021) while Gpsb032 exhibited the highest gene diversity (*He*=0.553) within accessions. The genetic diversity values ($2<N<6$; $N_m=3.5$; Nt=93; P=86.54%; *He*=0.34) were relatively low in this study compared to previous studies. The gene diversity was close to that (*He*=0.37) reported by Barro-Kodombo *et al*. (2008) on grain sorghum and, below the value (0.47 and 0.61) reported, respectively, by Nebie (2014) and Sawaodgo (2015) on sweet sorghum. Ghebru *et al.* (2003) Deu *et al*. (2006), Barnaud *et al*. (2007), Sagnard *et al*. (2011) Billot *et al*. (2013) found the following gene diversity 0.77, 0.61, 0.57 and 0.674 working on grain sorghum accessions from Eritrea, Niger, Cameroon, Mali and accessions representing world collection. The gene values reported in these investigations were all beyond gene diversity values found in this study. The weak diversity may be due to the relatively small number of accessions compared to the huge number (3367) of accessions used by Billot *et al*. (2013). It may also be attributed to the number and type of microsatellite markers involved in the present study. The number of markers was low compared to that reported by Wang *et al*. (2009) who utilized 95 SSR markers and Billot *et al*. (2013) who used 41 SSR markers for molecular characterization. More diversity reported by Ghebru *et al*. (2003) may be explained by the origin of accessions. Eritrea is of one the centers of domestication and diversification of this crop.

The observed heterozygosity mean was *Ho*=0.01 and this indicates that accessions collected from these regions contain few heterozygous individuals. This is primarily due to the conservation and the reproduction mode of the crop. Sorghum is a self-pollinated

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crop with only a small percentage of cross-fertilization. Since the collection in 1999, the accessions were regularly regenerated by self-pollination for conservation purpose and to be used as sources of breeding material for different traits of interest. This information shows that the majority of the accessions in the collection is homozygous and could be used as pure lines in a breeding programme.

The *Fst* value was low but significant exhibiting differentiation among populations, especially between exotic material and local populations. Most of the local populations were composed of *guinea* landraces while exotic materials included some *bicolor* race (RTx7000, RTx7078, SAMURAI1&2 and PAHAT), *durra* (B35), and some intermediates *guinea-caudatum* (Grinkan, Tiandougou, Tiandougou-coura and ET36-1). The racial difference is probably the source of differentiation within local *guinea* accessions and exotic materials. Genetic differentiation between regions and climatic zones was very low (0.011 to 0.022). Populations from West and South-west were more closely related (*Fst*=0.0.11) than populations from East. The Eastern region is geographically distant from the two other regions and, ecologically, it is located in the Sudano-sahelian climatic zone whereas the other regions are located in the Sudanian climatic zone. The Nei genetic identity was in agreement with this finding and confirmed the proximity among local populations compared to exotic material. The proximity among local populations could be due to the common historical origin of the guinea gene pool (Barro-Kodombo *et al*., 2008). The closeness among populations from West and Southwest could be due to the result of gene flow as the consequence of intensive exchange of accessions within farmers from these regions. The analysis of molecular variance fits with the above explanation as only 4% of genetic variation was attributed to

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the differentiation among populations, and 3% to the variation within individuals. The important part of genetic variation (93%) was due to differentiation among individuals.

Sorghum accessions from the three different regions and exotic material were separated into three main groups using 26 SSRs markers for genetic structure. A strong correlation existed between the factorial analysis and Neighbor-joining clusters in the grouping of the accessions. Most of the accessions in groups 1 and 2 from the factorial distribution matched, respectively, with the accessions in groups A and B displayed by the Neighbor-joining cluster. However, the exotic materials from group 3 were found in sub-group B2 and sub-group B3. Groups 1 and 2 matches with groups A and B1 which were composed of local *Guinea* landraces whereas group 3 matched with groups B2, B3 and C which were composed of exotic and local improved varieties. The last group contained mostly intermediate races (*guinea-caudatum*), *bicolor* and *durra*. The unique *durra* line was totally isolated from other accessions. The genetic structure found in this study appears to be more strongly linked with the accessions' race than other factors. Nebie (2014) found similar result working on sweet stem sorghum collections from Burkina Faso. Deu *et al*. (2008) and Murray *et al*. (2009) reported a racial genetic structure in Niger accessions and a representative world sorghum core collection. In contrast with this result, Barro-Kodombo *et al*. (2008) found a genetic structure linked to the kernel colour. Ghebru *et al*. (2002) reported that local varieties with similar identification characters were clustered in the same group.

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4.5. Conclusions

Local sorghum accessions exhibited many differences in agro-morphological traits related to drought tolerance. Among traits related to drought tolerance, yield and its components were the major variables that displayed major differences between accessions and they were more prominent than morphological traits in identifying drought tolerant genotypes. However, morphological parameters had an important role as key factors that contributed indirectly to increase the yield potential under moisture stress conditions. High yielding and drought tolerant as well as high yielding and drought susceptible material were identified. Low yielding and drought tolerant material were also seen. The study identified genotypes with high STI index that could be used by farmers to ensure optimal production in drought prone areas and may be useful in breeding programmes.

SSRs markers displayed genetic diversity among local populations, between local population and exotic material and within accessions. An important finding was the presence of private alleles in some local accessions genomes. These alleles were found particularly in stay-green chromosomal regions and may probably play an important role for this phenological drought trait. Another finding was the low level of heterozygosity revealed by the study. It showed a high homozygosity level of accessions and, therefore, confirmed the homozygous status of the majority of the accessions. This information was great news as it showed that local accessions were stable and can be used for breeding purposes.

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Chapter V: Introgression of Stay-green QTLs into farmers preferred sorghum varieties for drought tolerance and yield

5.1. Introduction

Sorghum [*Sorghum bicolor* (L) Moench] is the most important cereal crop grown in Burkina Faso and constitutes the staple food for the rural population (about 80% of the country's population). It is a crop of hot, semi-arid, tropical environments (with 400 - 600 mm rainfall) too dry for other cereals but is also grown in temperate regions and at altitudes of up to 2300 meters in the tropics. In Burkina Faso, sorghum is cultivated only under rain-fed conditions and over all of the different climatic zones of the country.

Sorghum grain is used in diverse ways. It is consumed in the form of stiff or thin porridges, as a steam-cooked product, such as couscous, or as a beverage in the areas where it is the main staple of people's diet. Jaggery and syrup, which are very useful in confectionaries, are obtained from sweet sorghum stems and the starch obtained from the waxy sorghum is useful sizing in the textile industry (Kassahun, 2006). More recently, sweet sorghum has been used as a potential bioenergy crop for ethanol production all over the world. Stems and foliage are used as green chop, hay, silage and pasture. Plant stems can also be used as building materials and bioenergy source for cooking by the poorest farmers in semi-arid areas.

Overall, sorghum production has moderately increased since the beginning of its breeding programs in Burkina Faso. This increase was partly due to the impact of improved varieties but mostly due to the expansion of sorghum cultivated surface. The slight increase of the yield was attributed to the replacement of local or traditional tall cultivars with the semi-dwarf varieties (Araus *et al*., 2008) and improved agronomic practices

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appropriate for specific climatic zones. In fact, early breeding programmes, in collaboration with some international institutes (ICRISAT and CIRAD), have released many local varieties and allowed the introduction of exotic material with high yielding potential. Unfortunately, most of these varieties performed better in research stations than in farmers' fields where grain yield is estimated to be 863 kg/ha (FAO, 1999). The poorest farmers usually cannot afford to apply fertilizer and depend totally on their rain-fed crop cultivars with low but stable yields.

In addition to farmers' precarious condition, sorghum production faces some constraints of abiotic and biotic stresses. The most damaging biotic stress is the parasitic weed, *Striga* which is harmful to sorghum during its life cycle while drought remains the main abiotic limitation to crop cultivation worldwide and the major source of yield instability and food shortage (Abdalla and Gamar, 2011). Drought is important in semi-arid tropics where the rainfall is very low with an erratic distribution (Zougmoré, 2003).

Drought is unpredictable and may occur at any stage of growth. Rosenow *et al*. (1996) highlighted that water stress may occur during the early vegetative seedling stage, panicle development (pre-flowering stage), the post-flowering stage, the period of grain filling and at physiological maturity. Whenever it occurs, it affects negatively the yield quantity and quality. However, drought that happens at pre-flowering and post-flowering stages of growth is more severe than the earlier stages of growth and has the most undesirable effect on yield (Kebede *et al*., 2001). Cakir (2004) reported that drought causes maximum damage and yield reduction around the flowering stage. In advanced stages of the growth cycle, during flowering and anthesis (post-flowering stage), drought leads to barrenness, kernel abortion or shriveled grain which reduces the grain yield (Prasad *et al*., 2008).

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Drought at early stages of growth (seedling) can be alleviated by replanting while, at flowering stage, its effect can only be lessened by irrigation, which unfortunately cannot be afforded by many smallholder farmers (Derera, 2005). Therefore, breeding for drought stress tolerance in sorghum should be targeted around the flowering stage in order to mitigate substantial drought induced loss. It should be focused on selecting cultivars with post-flowering drought tolerant traits.

Over the past decades, many studies have been conducted to set selection criteria for drought tolerance. A variety of morphological and physiological changes have been identified in response to drought stress in plants. Many traits whose presence or expression is associated with plant adaptability to drought-prone environments have been identified. They involve root morphology and rooting depth, plant architecture, variation in leaf cuticle thickness, stomatal regulation, osmotic adjustment, antioxidant capacity, hormonal regulation, desiccation tolerance (membrane and protein stability), and maintenance of photosynthesis through persistent green leaf area (stay-green). Plants expressing a variety of genes associated with these morphological and physiological traits tolerant to abiotic stresses have been identified (Bohnert *et al*., 1995; Bray 1997; Nguyen *et al*., 1997). Nevertheless, direct selection of these traits for improving yield potential under drought-stressed conditions through conventional breeding has been hampered by their polygenic control, epistasis, significant genotype by environment (G × E) interaction and quantitative trait loci to environment (QTL× E) interaction. Therefore, breeding for drought tolerance has resulted in limited success in developing cultivars with high yields under variable drought conditions. Application of molecular marker technology coupled

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with conventional breeding is a promising approach for crop improvement for complex traits, such as drought tolerance.

Marker-Assisted Selection (MAS) relies on genetic improvement through selection of traits that are putatively related to higher yield and/or to improved behavior of the crop when grown under stress environments. Subudhi *et al*. (2000) reported that post-flowering drought adaptation in sorghum is linked to the stay-green phenotype, which is characterized by the maintenance of green stems and upper leaves under water limitation after flowering. Considerable research has been undertaken to investigate suitable molecular markers linked to post-flowering (stay-green) drought resistance loci. Several stay-green QTLs associated with post-flowering drought tolerance have been mapped (Tuinstra *et al*., 1997, 1998; Crasta *et al*., 1999; Tao *et al*., 2000; Xu *et al*., 2000; Subudhi *et al*., 2000; Kebede *et al*., 2001; Haussmann *et al*., 2002; Sanchez *et al*., 2002) and molecular markers linked to these QTLs are available (Hash *et al*., 2003; Harris *et al*., 2007; Kassahun *et al*., 2009). The post-flowering studies identified four major Stay green QTLs designated as Stg1, Stg2, Stg3 and Stg4 which were consistently identified in a range of different environments (Subudhi *et al*., 2000; Tao *et al*., 2000) and in different genetic backgrounds (Subudhi *et al*., 2000). Stg1 and Stg2 were mapped to sorghum chromosome 3, and explain approximately 20 and 30% of the phenotypic variance, respectively (Xu *et al*., 2000; Sanchez *et al*., 2002; Harris *et al*., 2007). At these loci, the Stg alleles are from BTx642 (B35), the most common source of stay-green alleles.

Molecular information can be utilized to improve farmers' preferred cultivars in the semi-arid areas of Burkina Faso for drought tolerance and grain yield improvement. This study

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therefore seeks to improve drought adaptation of local elite cultivars through introgression of major (consistent) QTLs for stay-green.

5.2. Specific Objectives of the study

- ✓ To determine genetic distance and flanking polymorphism markers between stay-green donor QTL (B35) and local elite lines;
- ✓ To introgress and identify genotypes with favorable QTLs tightly linked to stay-green traits into farmers' elite varieties;
- ✓ To select BC_1F_1 progenies with favorable donor alleles and the recurrent parent background.

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5.3. Materials and methods

5.3.1. Plants materials

The parental lines (varieties) used for this breeding program were B35 (donor parent) and four local varieties (Kapelga, Grinkan, Sariaso01 and Sariaso09) that were used as recurrent parents.

B35 is a BC_1 derivative of *durra* sorghum germplasm accession IS 12555 from Ethiopia (Rosenow *et al*., 1983). It is dwarf, has purple-colored plant with awned semi-open panicles enclosing grains with a thick mesocarp and yellow pericarp. It is a low yielding plant due to its short life cycle, short plant height, small panicle size, and low grain number per panicle. However, it is recognized as the most common source of stay-green alleles for terminal drought tolerance in sorghum. Several studies identified six stay-green QTLs within which four (Stg1, Stg2, Stg3, Stg4,) are major and the remaining are considered as minor (StgA and, StgB).

Kapelga is an improved variety from Burkina Faso. It is a tan line obtained in 1998 by mass selection of a local *guinea* landrace collected in the middle south of the country. It is tall, resistant to lodging, moderately tolerant to midge, to weed such as *Striga*, highly sensitive to photoperiod and terminal drought. It has a potential yield of 2.5 t/ha and is produced for local consumption. It is cultivated all over the different climatic zones of the country but the most suitable area for its cultivation is from 600 mm to 900 mm of rainfall. It is recommended by the ministry of agriculture when the rainy season is characterized by an early drought at seedling level.

Grinkan is an improved variety from Mali and has been released in Burkina Faso as double usage material. It is an intermediate tan sorghum race originated from a cross

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between a local *guinea* landrace and a *caudatum* race. It is resistant to lodging, not sensitive to photoperiod, has a long cycle (120 days) and can be grown in rainfall areas of 800mm to 1000mm which correspond to the Sudanian climatic zone in Burkina Faso. This sorghum variety has medium plant height, possesses a semi-loose panicle type and high grain number. Grinkan has been introduced in Burkina Faso and has been adopted by sorghum breeders and farmers as a highly productive variety for its yield as well as for its foliage.

Sariaso 01 is an improved variety from Burkina Faso. It is an anthocyanin line obtained in 1990 by mass selection of a local *guinea* landrace (Boromo local). It is tall, resistant to lodging, sensitive to midge, *Striga*, highly sensitive to photoperiod, and post-flowering moisture stress. It is a high yielding variety produced for local consumption. Sariaso01 is cultivated only in the Western and Southern parts of Burkina Faso where the rainfall can reach up to 900 mm/year.

Saraiaso 09 is an improved variety from Burkina Faso. It is an anthocyanin line obtained in 1990 by mass selection of a local *guinea* landrace collected in the central-south region of Burkina Faso (Koumbissiri). It is also called ‘‘nazongala’’ in local language (Moore). This variety is tall, resistant to lodging, sensitive to midge, *Striga*, highly sensitive to photoperiod and post-flowering moisture stress. It is a high yielding variety produced for local consumption. Sariaso09 is cultivated in the western, southern and central parts of Burkina Faso. In contrast to Sariaso01 and Sariaso02, it has an intermediate cycle and it is cultivated in the central, western, southern and eastern part of the country.

The materials used to develop the introgression lines are presented in table 5.1.

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Table 5. 1: Pedigree information of lines used in the study

Lines	Germplasm source	Pedigree	Adaptation	Maturity	Status to water stress
B35	ICRISAT	IS12555 (BT✕643)	600<mm<900	3 months	Terminal drought tolerance
Grinkan	Mali	Line	600<mm<900	3-4months	Terminal drought susceptible
Kapelga	Burkina Faso	Line	600<mm<900	3-4months	Terminal drought susceptible
Sariaso01	Burkina Faso	Line	600<mm<900	4-5months	Terminal drought susceptible
Saraiso09	Burkina Faso	Line	600<mm<900	4-5months	Terminal drought susceptible

5.3.2. Methodology

5.3.2.1. Polymorphism assessment

Genetic distance among the parents [donor (B35) and recurrent (Kapelga, Grinkan, Sariasao01 and Sariaso09)] was assessed using 74 SSR markers. Thirty three (33) and 41 markers were used to identify polymorphic markers respectively in region of target QTLs and in non target regions of the genome of the recurrent parents. Polymorphic markers were then used in the target regions for foreground selection to choose individuals that have incorporated the donor allele and non-target QTLs were used to detect progenies that have adequate recovery rate of the recurrent parent genome. List of markers is presented in Table 5. 2. The primers sequences and source are in appendices 2 and 3.

5.3.2.2. Foreground and background selection

Five QTLs (Stg1, Stg2, Stg3, Stg4 and StgB) associated with the stay-green trait and located on three linkage groups (LG2, LG3 and LG5) (Crasta *et al*., 1999; Xu *et al*., 2000; Subudhi *et al*., 2000) were selected as the target QTLs for introgression into local farmers' preferred varieties through Marker-Assisted Backcrossing (MABC) technology. The donor parents were crossed to the recurrent parent and the resulting F_1 plants were backcrossed to the recurrent parent to generate the BC_1F_1 populations. The progenies

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were screened using QTL-flanking markers for donor parent alleles (foreground selection). Markers located other than in the target stay-green QTLs regions were used to screen individuals for recurrent parent alleles (background selection) and identify those having good recovery of the recurrent parent genome. The SSR markers used in this study are shown in Table 5.2.

Table 5. 2: List of SSR markers used for foreground and background selection

Markers for foreground selection				
N°	Stay-green	Markers	LG	Position
1	**Stg3**	Msbcir238	Sb02_1	45.23764
2	**StgB**	Xtxp72	Sb02_1	49.39548
3	**StgB**	Xtxp55	Sb02_1	51.59249
4	**StgB**	Xtxp428	Sb02_1	
5	**Stg3**	Msbcir339	Sb02_1	87.83764
6	**Stg3**	Stgnshsmbm36	Sb02_1	
7	**Stg2**	Msbcir222	Sb03_1	
8	**Stg2**	Sb5-236	Sb03_1	77.16271
9	**Stg1&2**	Msbcir314	Sb03_1	102.9627
10	**Stg1**	Xtxp285	Sb03_1	124.0096
11	**Stg2**	Msbcir225	Sb03_1	122.3127
12	**Stg4**	Xtxp225	Sb05_1	53.75
13	**Stg4**	Xtxp15	Sb05_1	61.2
14	**Stg4**	Xtxp23	Sb05_1	80.2
15	**Stg4**	Xtxp14	Sb05_1	60.75
16	**Stg4**	Xtxp123	Sb05_1	102.5001
17	**Stg4**	Gpsb32	Sb05_1	118.3

LG=Linkage Group

Markers for background selection				
N°	Markers	LG	Position	chromosome
1	Gpsb158	Sb01_1	10.41076	Top
2	Msbcir243	Sb01_1	68.2923	Middle
3	Gpsb129	Sb01_1	164.6245	Bottom
4	Gpsb178	Sb02_1	11.88764	Top
5	Gpsb14	Sb02_1	120.0082	Bottom
6	Xcup11	Sb03_1	3.445377	Top
7	Xcup14	Sb03_2	136.17	Bottom
8	Msbcir188	Sb04_1	19.02932	Top
9	Gpsb098	Sb04_1	111.6645	Bottom
10	Gpsb136	Sb06_1	93.19133	Bottom
11	Xtxp159	Sb07_1	14.36734	Top
12	Sbagb02	Sb07_1	95.6	Bottom
13	Gpsb041	Sb08_1	5.39999	Top
14	Gpsb123	Sb08_1	92.3587	Bottom
15	Msbcir191	Sb09_1	16.437	Top
16	Gpsb079	Sb09_1	83.78589	Bottom
17	Xcup49	Sb10_1	0.535191	Top
18	Xcup43	Sb10_1	145.7377	Bottom

5.3.2.3. Samples collection and DNA extraction

Leaf samples were collected from three weeks old plants and dried in an oven at 40°C for three days. After drying leaf samples were ground using a Geno-grinder (RETSCH) at 500 strokes per minute for 9 minutes (3 times, 3 minutes each) and then DNA extraction was conducted according to the Mixed Alkyl Trimethyl Ammonium Bromide (MATAB) procedure (Frost *et al.,* 2007). A pre-heated (65°C) 750µl of MATAB tampon was added

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to each sample and then incubated at 65°C in a shaking water bath for 20 minutes. After incubation, 750µl of chloroform-isoamylalcohol (24:1) was added to each sample and agitated manually 50 times before being centrifuged at 13000 rpm for 20 minutes. 600 µl of the aqueous layer was transferred in a new tube of 1500 µl then 600 µl of cold isopropanol was added, mixed and kept at -20°C for two hours. The samples were centrifuged at 13000rpm for 20 minutes and the supernatant was decanted and pellets were washed with 500 µl of 70% ethanol and centrifuged at 13000rpm for 20 minutes. The supernatant was decanted and pellets were air dried over a night or 45 minutes in a stove at 40°C. Finally, the samples were dissolved in 150 µl of TE (TE1X) and kept at ambient temperature for a night and then conserved at -20°C. The working concentration of about 5ng per µl was obtained from dilution of initial DNA solution after checking DNA concentrations and quality on 0.8% agarose gels.

5.3.2.4. Polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis

PCR was performed in a 10 µl reaction volume containing 25ng of genomic DNA template, 0.1mM of dNTPs, 1x buffer, 200µM of $MgCl_2$, 0.1µM of both forward and reverse primers, 0.1µM of IRdye, 0.1U of ampliTaq polymerase enzyme and double distilled water. The PCR were realized in 35 cycles using a thermal cycler (MWG AG Biotech). Thermal cycler were programmed as followed: a denaturation at 94°C for 4 min, followed by 10 cycling composed of 45s of denaturation at 94°C and annealing at TM-5°C with a reduction of 0.5°C/cycle for 1min and extension at 72°C for 1min15s. The process was repeated in 25 cycles. Each cycle was a chain of denaturation (94°C during 45s) and, annealing (at TM- 5°C for 1min) and then an extension (at 72°C for 1min15s). Finally, the reaction ended by 5 min extension at 72°C.

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PCR products were subjected to electrophoresis in 6.5% polyacrylamide gels with a licor 4300 DNA Analyser system. Electrophoresis was carried out using 20ml of polyacrylamide 6.5% polyacrylamide, 200ml 10X TBE completed to 1L, 175 µl ammonium per sulphate (APS), and 25µl TEMED). The gel was run at 1500V and 35mA constant power supply, for 2 hours using a licor DNA analyser unit. For polymorphism assessment, the bands on the gel were coded as ‘‘a’’, ‘‘b’’, ‘‘c’’, ‘‘d’’ based on their allele position compared with that of the donor parent allele. For BC1 progenies, the bands were coded as ‘‘a’’, ‘‘b’’, ‘‘h’’ according to their pattern compared to those of the parents. The code ‘‘a’’ indicated homozygote plant for allele from non stay-green parents (Kapelga, Grinkan, Sariaso01 and Sariaso09). Code ‘‘b’’ indicated homozygote plant for allele from donor parent (B35) and code ‘‘h’’ indicated heterozygote plant with both parental allele for a marker loci. The missing data were scored as ‘‘x’’.

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5.4. Results and discussions

5.4.1. Results

5.4.1.1. Polymorphism assessment and selection of useful markers

Out of the 33 flanking markers, 17 polymorphic markers were retained and out of 41 markers in non-target regions, 18 polymorphic markers were retained for background screening. The list of polymorphic markers is presented in Table 5 2. The number of alleles per locus varied from 2 to 6 and allelic diversity was low and estimated to be 2.34.

5.4.1.2. Marker- assisted Introgression of Stay-green QTLs

Foreground selection was focused on five consistent stay-green QTLs (stg1, stg2, stg3, stg4 and stgB) located on three linkage groups (SB02-1 on LG2, SB03-1 on LG3 and SB05-1on LG 5). These QTLs were selected as targets for MABC into four elite senescent backgrounds (Kapelga, Sariaso01, Sariaso09 and Grinkan). The results of the screening with foreground markers are presented in Tables 5.3, 5.4 and 5.5.

Out of 107 backcross progenies, 40 (37.38%) incorporated at least one QTL from the donor parent (B35). Eighteen (18) progenies (S9B1, S9B40, S9B52, S9B68, S9B74, S9B88, S9B39, S9B50, S9B51, S9B7, S9B66, S9B67, S9B18, S9B65, S9B91, S9B24, S9B97 and S9B107) were heterozygous at all marker loci flanking the target QTLs. Seven plants (S9B43, S9B46 S9B85, S9B87, S9B34, S9B38, and S9B73) incorporated two QTLs (Stg3and stgB). Five heterozygous plants (S9B37, S9B85, S9B73, S9B17 and S9B41) with stg1 QTLs and ten plants (S9B37, S9B43, S9B46, S9B85, S9B64, S9B13, S9B38, S9B44, S9B48 and S9B73) with stg4 were also selected. The genotyping data of backcross progenies derived from Sariaso09xB35 are presented in Table 5.3 and an output exhibiting heterozygous individuals with the donor and the recurrent parent alleles of the marker Xtxp123 is shown in the Figure 5.1.

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The screening of BC_1F_1 derived from cross of Kapelga x B35 during foreground genotyping, identified ten (10) progenies carrying at least one stay-green QTL. Only two progenies (KB4 and KB5) were heterozygous at all loci flanking markers (carrying the five stay-green QTLs). Three plants (KB8, KB11 and KB12) incorporated four stay-green QTLs (Stg1, Stg2, Stg3 and StgB). Four plants (KB9, KB17, KB18 and KB19) were identified with two stay-green QTLs (Stg1 and Stg2) and only plant one (KB16) incorporated one QTL (Stg4).

In the BC_1F_1 population derived from the cross of Grinkan x B35, no plants were identified with all target loci. The genotyping identified four plants with four different QTLs. GB1and GB8 carrying QTLs (Stg2, Stg3, StgB and Stg4) and GB2 and GB4 carrying the same QTLs (Stg1, Stg2, Stg3 and StgB). GB7 incorporated three QTLs (Stg1, Stg2 and Stg4) and GB3, GB5 and GB9 incorporated two QTLs (Stg1 and Stg2).

In the population developed from cross of Sariaso01 x B35, four plants were selected through the foreground genotyping. Among the selected plants, three (S1B1, S1B3) were identified with four QTLs of Stg (Stg2, Stg3, Sgt4 and stgB) and one S1B4 carrying two QTLs of Stg (Stg2 and Stg4). S1B6 was identified with Stg4 QTL regions in its genome.

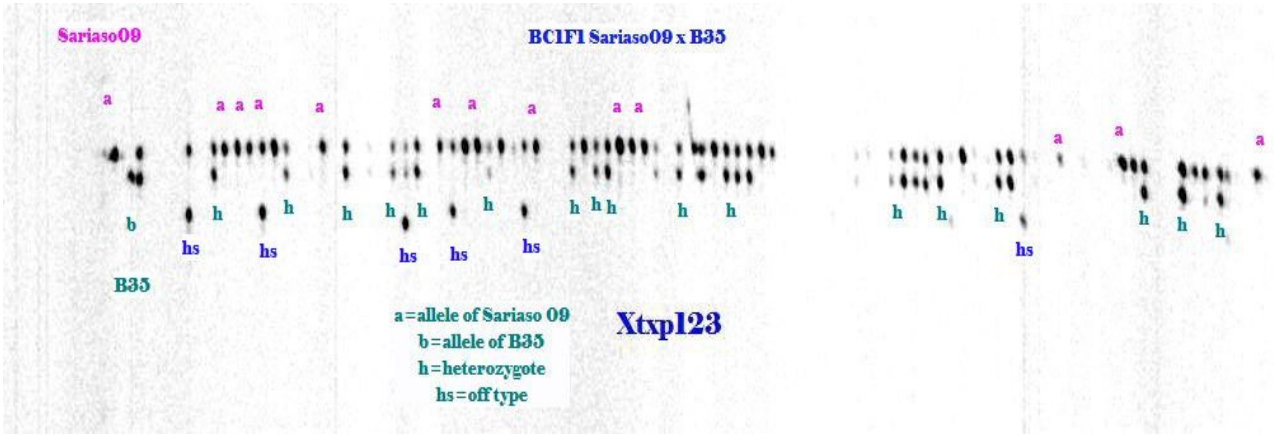

Figure5. 1: Output showing heterozygous individuals with the donor and the recurrent parent alleles of the marker Xtxp123.

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Table 5. 3: Genotyping data for foreground selection in population one (BC_1F_1 progenies derived from cross of Sariaso09 x B35)

Markers	Stg	Chro	Position	Sar09	B35	S9B1	S9B40	S9B52	S9B68	S9B74	S9B88	S9B39	S9B50	S9B51	S9B7	S9B66	S9B67	S9B18	S9B65
Msbcir238	**Stg3**	Sb02_1	62.01687	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Xtxp72	**StgB**	Sb02_1	70.50211	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Xtxp55	**StgB**	Sb02_1	71.86763	a	b	h	h	h	h	h	h	h	a	h	h	x	h	h	h
Msbcir339	**Stg3**	Sb02_1	117.4172	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Sb5-236	**Stg2**	Sb03_1	80.19823	a	b	h	h	h	h	h	h	h	h	h	h	h	h	x	h
Msbcir314	**Stg1&2**	Sb03_1	116.8419	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Xtxp285	**Stg1**	Sb03_1	145.4819	a	b	h	h	h	h	h	h	h	h	a	h	h	h	h	a
Xtxp225	**Stg4**	Sb05_1	60.07417	a	b	h	h	h	h	h	h	h	h	h	h	a	a	a	a
Xtxp15	**Stg4**	Sb05_1	76.06783	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Xtxp23	**Stg4**	Sb05_1	78.50456	a	b	h	h	h	h	h	h	h	h	h	x	h	h	h	h
Xtxp14	**Stg4**	Sb05_1	79.23723	a	b	h	h	h	h	h	h	x	h	h	a	h	h	h	h
Xtxp123	**Stg4**	Sb05_1	90.92021	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Gpsb32	**Stg4**	Sb05_1	113.2587	a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Stgnshsmbm36	**Stg3**	Sb02_1		a	b	h	h	h	h	h	h	h	h	h	h	h	h	h	h
Total						**14**	**14**	**14**	**14**	**14**	**14**	**13**	**13**	**13**	**12**	**12**	**13**	**12**	**12**

Table 5.3 continued:

Markers	Stg	S9B91	S9B24	S9B37	S9B43	S9B46	S9B85	S9B64	S9B13	S9B34	S9B38	S9B44	S9B48	S9B73	S9B20	S9B30	S9B61	S9B90	S9B16
Msbcir238	**Stg3**	h	x	a	h	h	h	h	h	h	h	h	a	h	x	x	h	h	a
Xtxp72	**StgB**	h	h	a	h	h	h	a	h	h	h	a	a	h	x	a	x	a	a
Xtxp55	**StgB**	x	x	a	h	h	x	h	x	h	h	h	a	b	x	x	x	x	a
Msbcir339	**Stg3**	h	h	h	h	a	h	a	a	h	h	a	a	a	a	h	a	a	a
Sb5-236	**Stg2**	h	h	h	a	a	h	h	h	a	a	b	a	h	a	x	h	h	h
Msbcir314	**Stg1&2**	h	h	h	a	h	h	a	h	h	a	a	h	h	x	x	x	a	h
Xtxp285	**Stg1**	h	h	h	h	a	a	a	a	h	a	a	a	x	h	h	x	h	h
Xtxp225	**Stg4**	a	x	h	h	a	a	x	a	a	h	x	a	a	x	x	x	x	a
Xtxp15	**Stg4**	h	h	h	h	h	h	h	a	a	h	h	h	h	h	h	h	a	a
Xtxp23	**Stg4**	x	x	a	h	a	x	x	x	a	a	h	h	a	x	a	x	h	a
Xtxp14	**Stg4**	h	h	h	h	h	x	h	a	a	x	x	h	x	h	x	x	a	a
Xtxp123	**Stg4**	h	h	h	a	h	h	h	h	a	a	h	h	h	h	h	h	h	a
Gpsb32	**Stg4**	h	h	h	a	h	h	h	h	a	a	a	h	h	x	x	x	a	h
Stgnshsm36	**Stg3**	h	h	a	h	h	h	a	h	h	h	h	a	a	h	h	h	a	a
Total		**11**	**10**	**9**	**10**	**9**	**9**	**7**	**7**	**7**	**7**	**6**	**6**	**7**	**5**	**5**	**5**	**5**	**4**

a = recurrent parent allele; b=donor parent allele; h=heterozygote

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Table 5. 4: Genotyping data for foreground selection in population two (BC_1F_1 progenies derived from cross of Kapelga x B35)

Sample	Stg	Chr	Kap	B35	Kb4	Kb5	Kb8	Kb12	Kb11	Kb16	Kb17	Kb19	Kb18	Kb9	Kb7	Kb14	Kb2	Kb13	Kb15	Kb1	Kb6	Kb10
Msbcir238	Stg3	Sb02_1	a	b	h	a	h	h	**h**	h	a	**a**	a	a	a	a	**h**	a	a	a	a	a
Xtxp55	StgB	Sb02_1	a	b	h	a	h	h	a	a	a	a	a	a	a	h	h	h	a	a	a	a
Xtxp72	StgB	Sb02_1	a	b	h	a	a	a	**a**	a	a	**a**	a	a	a	a	**a**	a	a	a	a	a
Msbcir225	Stg2	Sb03_1	a	b	h	h	h	h	**h**	h	h	**h**	h	h	a	a	**a**	a	a	a	a	a
Msbcir314	Stg1&2	Sb03_1	a	b	h	h	h	h	**a**	a	h	**h**	h	h	a	a	**a**	a	a	a	a	a
Sb5-236	Stg2	Sb03_1	a	b	h	h	h	h	**h**	a	h	**h**	h	h	a	a	**a**	a	a	a	a	a
Xtxp285	Stg1	Sb03_1	a	b	h	a	h	h	**h**	h	h	**h**	h	h	a	a	**a**	a	a	a	a	a
Gpsb32	Stg4	Sb05_1	a	b	h	h	a	a	**h**	a	h	**a**	h	a	a	a	**a**	a	a	a	a	a
Xpxp123	Stg4	Sb05_1	a	b	h	a	a	h	**h**	h	h	**a**	a	a	a	a	**a**	a	a	a	a	a
Xtxp14	Stg4	Sb05_1	a	b	h	h	a	a	**a**	h	a	**a**	a	a	a	a	**a**	a	a	a	a	a
Xtxp15	Stg4	Sb05_1	a	b	h	h	a	a	**a**	h	a	**a**	a	a	a	a	**a**	a	a	a	a	a
Xtxp23	Stg4	Sb05_1	a	b	h	h	a	a	**a**	a	a	**a**	a	a	a	a	**a**	a	a	a	a	a
Msbcir222	Stg4	Sb08_1	a	b	h	h	a	a	**a**	a	a	**h**	a	a	a	a	**a**	a	a	a	a	a
Stgnhm36	Stg3		a	b	h	h	h	a	**h**	a	a	**a**	a	a	h	a	**a**	a	a	a	a	a
xtxp428	StgB		a	b	h	h	h	h	**h**	a	a	**a**	a	a	h	h	**a**	a	h	a	a	a
Total			0	0	15	10	8	8	8	6	6	5	4	2	2	2	2	1	1	0	0	0

Table 5. 5: Genotyping data for foreground selection in population three (BC_1F_1 progenies derived from cross of Grinkan x B35)

Sample	Stg	Chro	Position	Grinkan	B35	GB1	GB8	GB2	GB4	GB7	GB3	GB5	GB9	GB10	GB11	GB12	GB6
Msbcir238	Stg3	Sb02_1	62.01687	a	b	h	h	h	h	h	a	a	a	a	a	a	a
Xtxp55	StgB	Sb02_1	71.86763	a	b	h	h	h	h	h	a	a	a	a	a	a	a
Xtxp72	StgB	Sb02_1	70.50211	a	b	a	a	h	a	a	a	a	a	a	a	a	a
Msbcir225	Stg2	Sb03_1	141.6202	a	b	h	h	a	a	a	h	h	h	a	h	a	a
Msbcir314	Stg1&2	Sb03_1	116.8419	a	b	a	a	h	h	a	h	h	h	a	h	h	a
Sb5-236	Stg2	Sb03_1	80.19823	a	b	h	h	h	h	h	h	a	a	a	a	a	a
Xtxp285	Stg1	Sb03_1	145.4819	a	b	a	a	a	h	h	a	a	h	h	a	a	a
Gpsb32	Stg4	Sb05_1	113.2587	a	b	h	a	h	a	a	a	h	a	h	h	h	a
Xpxp123	Stg4	Sb05_1	90.92021	a	b	h	h	a	a	h	a	h	a	a	a	a	h
Xtxp14	Stg4	Sb05_1	79.23723	a	b	h	h	a	h	h	h	a	a	a	a	-	a
Xtxp15	Stg4	Sb05_1	76.06783	a	b	h	h	h	h	a	a	h	a	h	a	a	a
Xtxp23	Stg4	Sb05_1	78.50456	a	b	a	h	a	a	a	h	a	a	a	a	a	a
Msbcir222	Stg4	Sb08_1	90.61926	a	b	a	a	a	a	a	a	a	a	a	h	a	a
Stgnhmsbm36	Stg3			a	b	a	a	a	a	a	a	a	h	a	a	a	a
xtxp428	StgB			a	b	a	a	a	a	a	a	a	h	h	a	a	a
Total				0	0	8	8	7	7	6	5	5	5	4	4	2	1

a = recurrent parent allele; b=donor parent allele; h=heterozygote

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5.4.1.3. Marker-assisted background selection

Genotyping of the BC_1F_1 plants identified progenies that incorporated either five, four, three, two or one stay-green QTLs in their recurrent parent background. The backcross genotyping aimed at identifying progenies with stay-green QTLs and that have an optimal recovery rate of the recurrent parent genome. Eighteen markers (two in each linkage group) were used. One marker was located at top or near top end and the other was located at bottom or near bottom end of each linkage group.

Among the BC_1F_1 progenies derived from cross of Sariaso9 x B35, thirty (S9B40, S9B51, S9B67, S9B68, S9B85, S9B88, S9B96, S9B1, S9B16=56%, S9B18, S9B24, S9B34, S9B37=58%, S9B38=56%, S9B39, S9B40, S9B41, S9B43, S9B44, S9B46, S9B50, S9B51, S9B52, S9B64, S9B65, S9B66, S9B67, S9B68, S9B74, S8B85, S9B88, S9B91, S9B96, S9B97, S9B107) descendants had low recovery rate (below 75%) of the recurrent parent (Sariaso09). Two progenies (S9B73, S9B48) recovered 78% and 83% of the genome of the recurrent parent. Results are presented in Table 5.5. An output showing homozygous individuals with the recurrent parent alleles of the marker Gpsb136 is represented by Figure 5.2.

Background markers used to screen the backcross progenies identified descendants with different recovery rates. Fifteen descendants from Kapelga, Grinkan and Sariaso 01 (KB5, KB8, KB12, KB18, KB19, KB11, KB19, GB1, GB2, GB3, GB4, GB5, GB8, GB9 and S1B4) had a recovery rate of the recurrent parent genome ranging from 42% to 69%. Two progenies (GB8 and S1B3) had recovered 75% of the recurrent parent genome and four individuals (KB4, KB16, GB7 and S1B6) had also got back approximately 78% to 83% of their recurrent parent genome, respectively (Kapelga, Grinkan and Sariaso01). The results are presented in Table 5.6 and 5.7.

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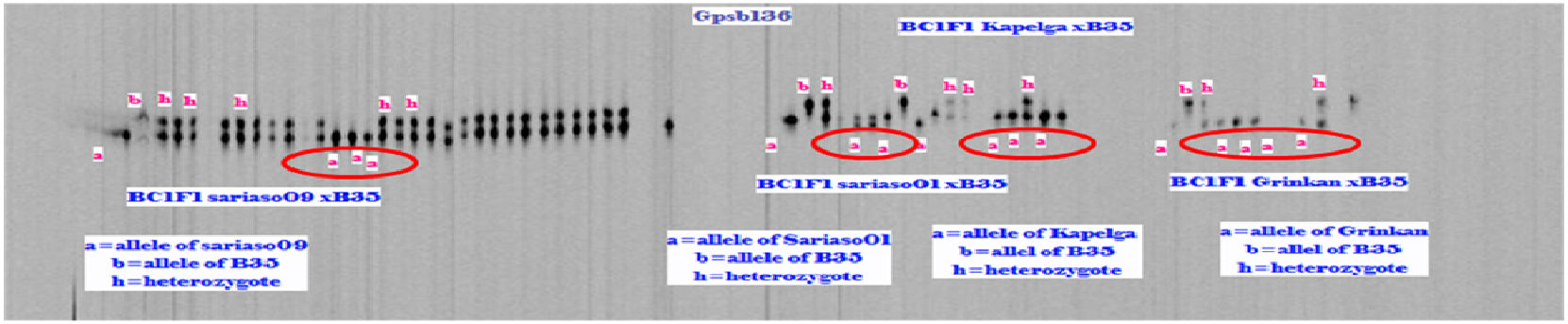

Figure5. 2: Output showing some homozygous individuals with only the recurrent parent alleles of SSRs Marker Gpsb136

Table 5. 6: Genotyping data for background selection in population one (BC_1F_1 progenies derived from cross of Sariaso09 x B35)

Markers	LG	Position		Sar09	B35	S9B1	S9B7	S9B16	S9B17	S9B18	S9B24	S9B34	S9B37	S9B38	S9B39	S9B40	S9B41	S9B43	S9B44
Gpsb158	Sb01_1	10.41	Top	a	b	h	h	h	h	h	h	h	h	a	a	h	h	a	h
Msbcir243	Sb01_1	68.29	Middle	a	b	h	a	h	h	h	h	h	a	a	h	h	a	h	a
Gpsb129	Sb01_1	164.62	bottom	a	b	a	h	h	a	a	a	a	a	a	a	a	h	h	h
Gpsb178	Sb02_1	11.89	Top	a	b	a	a	a	a	a	a	h	h	h	h	h	h	a	a
Gpsb14	Sb02_1	120.01	bottom	a	b	a	a	a	h	a	h	h	a	a	a	a	a	a	a
Xcup11	Sb03_1	3.45	Top	a	b	h	h	a	h	h	a	a	h	h	h	h	a	h	h
Xcup14	Sb03_1	136.17	bottom	a	b	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Msbcir188	Sb04_1	19.03	Top	a	b	h	h	h	h	a	h	h	h	a	h	h	a	a	h
Gpsb98	Sb04_1	111.66	bottom	a	b	a	a	a	a	h	a	a	a	h	a	a	h	a	a
Gpsb136	Sb06_1	93.19	bottom	a	b	h	h	h	h	h	a	h	h	h	h	a	a	a	a
Xtxp159	Sb07_1	14.37	Top	a	b	a	h	a	a	a	a	h	a	a	a	h	h	h	a
Sbagb02	Sb07_1	95.60	bottom	a	b	h	a	h	h	h	h	h	a	h	h	a	a	a	h
gpsb41	Sb08_1	5.40	Top	a	b	h	a	a	h	h	a	a	h	h	h	a	h	h	a
Gpsb123	Sb08_1	98.75	bottom	a	b	a	h	h	a	a	h	a	a	a	a	h	a	a	h
Msbcir191	Sb09_1	16.44	Top	a	h	h	a	a	a	h	a	a	a	a	h	a	a	a	a
gpsb79	Sb09_1	83.79	bottom	a	b	a	h	h	h	a	h	h	h	h	a	h	h	h	h
xcup49	Sb10_1	0.54	Top	a	b	h	a	h	h	a	a	h	h	h	h	a	h	h	h
Xcup43	Sb10_1	145.74	bottom	a	b	a	h	a	a	h	h	a	a	a	a	h	a	a	a
Recovery rate of Recurrent Parent						0.53	0.75	0.56	0.47	0.53	0.53	0.53	0.58	0.56	0.53	0.47	0.53	0.53	0.53

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Table 5. 6: continued

Markers	LG		S9B46	S9B48	S9B50	S9B51	S9B52	S9B64	S9B65	S9B66	S9B67	S9B68	S9B74	S9B85	S9B88	S9B91	S9B96	S9B97	S9B107	S9B73
Gpsb158	Sb01_1	Top	h	h	a	h	h	h	a	a	h	h	h	h	h	h	h	h	a	a
Msbcir243	Sb01_1	Middle	h	a	a	h	h	a	h	a	a	h	h	h	h	a	h	a	a	a
Gpsb129	Sb01_1	bottom	a	h	h	a	a	a	h	h	a	a	a	a	a	a	a	a	h	a
Gpsb178	Sb02_1	Top	h	a	h	h	h	h	h	h	h	h	a	a	a	a	h	h	a	h
Gpsb14	Sb02_1	bottom	a	a	a	a	a	a	a	a	h	h	a	h	h	h	a	a	h	a
Xcup11	Sb03_1	Top	a	h	h	h	h	h	h	h	h	h	h	h	h	h	h	h	a	a
Xcup14	Sb03_1	bottom	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Msbcir188	Sb04_1	Top	h	a	a	h	a	h	a	a	h	h	h	a	h	h	a	h	h	h
Gpsb98	Sb04_1	bottom	a	a	a	a	h	a	a	a	a	a	a	h	a	a	h	a	a	a
Gpsb136	Sb06_1	bottom	h	h	h	h	a	h	h	h	h	h	h	h	h	h	a	h	a	a
Xtxp159	Sb07_1	Top	a	a	a	a	h	h	h	a	h	a	h	h	a	a	a	a	h	a
Sbagb02	Sb07_1	bottom	a	a	a	h	a	a	a	h	a	h	h	a	h	h	h	a	a	a
gpsb41	Sb08_1	Top	h	a	h	h	a	a	a	a	a	a	a	h	a	h	a	h	h	a
Gpsb123	Sb08_1	bottom	h	a	a	a	h	a	a	a	h	h	a	a	h	a	h	a	a	a
Msbcir191	Sb09_1	Top	a	h	h	h	a	a	h	a	a	a	h	a	h	a	a	h	a	a
gpsb79	Sb09_1	bottom	h	a	a	h	h	h	a	h	h	h	a	h	a	h	h	a	h	a
xcup49	Sb10_1	Top	a	a	a	a	a	a	a	h	h	h	a	a	a	h	a	h	h	h
Xcup43	Sb10_1	bottom	h	h	h	a	a	a	h	a	a	a	h	h	h	a	h	a	a	a
Recovery rate of RP			0.53	0.81	0.64	0.47	0.53	0.64	0.58	0.64	0.47	0.42	0.64	0.47	0.47	0.53	0.47	0.58	0.58	0.78

a = recurrent parent allele ;b=donor parent allele; h=heterozygote; x= missing data

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Table 5. 7: Genotyping data for background selection in population two (BC_1F_1 progenies derived from cross of Kapelga x B35)

Markers	LG	Position		Kapelga	B35	KB4	KB5	KB8	KB12	KB16	KB18	KB19	KB11	KB19
Gpsb158	Sb01_1	10.41	Top	a	b	a	h	h	h	a	a	a	h	h
Msbcir243	Sb01_1	68.29	Middle	a	b	a	h	a	h	h	a	a	a	a
Gpsb129	Sb01_1	164.62	bottom	a	b	a	a	a	a	a	h	h	a	h
Gpsb178	Sb02_1	11.89	Top	a	b	a	a	h	a	h	a	h	a	a
Gpsb14	Sb02_1	120.01	bottom	a	b	h	h	a	a	a	h	a	a	h
Xcup11	Sb03_1	3.45	Top	a	b	a	h	a	h	a	a	h	h	h
Xcup14	Sb03_1	136.17	bottom	a	b	a	a	h	a	h	h	a	a	a
Msbcir188	Sb04_1	19.03	Top	a	b	a	a	h	h	a	a	a	a	h
Gpsb98	Sb04_2	111.66	bottom	a	b	a	h	h	a	a	a	h	h	a
Gpsb136	Sb06_1	93.19	bottom	a	b	a	a	h	a	h	a	a	h	a
Xtxp159	Sb07_1	14.37	Top	a	b	h	h	h	a	a	h	h	h	h
Sbagb02	Sb07_1	95.60	bottom	a	b	a	a	a	h	a	a	a	a	a
gpsb41	Sb08_1	5.40	Top	a	b	a	a	h	a	a	a	a	a	a
Gpsb123	Sb08_1	98.75	bottom	a	b	a	h	a	a	a	a	a	a	h
Msbcir191	Sb09_1	16.44	Top	a	b	h	a	a	a	h	h	h	a	a
gpsb79	Sb09_1	83.79	bottom	a	b	a	h	h	a	a	a	a	a	h
xcup49	Sb10_1	0.54	Top	a	b	a	a	h	a	a	a	a	a	h
Xcup43	Sb10_1	145.74	bottom	a	b	h	h	a	a	a	h	h	h	h
Recovery rate of the recurrent parent						0.78	0.47	0.47	0.64	0.81	0.64	0.58	0.69	0.42

a = recurrent parent allele; b=donor parent allele; h=heterozygote; x=missing data

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Table 5. 8: Genotyping data for background selection in population three and four (BC_1F_1 progenies derived from cross of Grinkan x B35)

Markers	LG	Position		Grinkan	B35	GB1	GB2	GB3	GB4	GB5	GB7	GB8	GB9	S1B1	S1B3	S1B4	S1B6
Gpsb158	Sb01_1	10.41	Top	a	b	h	h	a	a	a	a	a	a	h	h	h	a
Msbcir243	Sb01_1	68.29	Middle	a	b	h	a	a	a	a	a	h	h	h	a	a	a
Gpsb129	Sb01_1	164.62	bottom	a	b	a	a	h	h	h	a	a	h	a	a	h	a
Gpsb178	Sb02_1	11.89	Top	a	b	h	a	a	a	a	h	h	a	a	a	a	a
Gpsb14	Sb02_1	120.01	bottom	a	b	a	h	a	h	a	h	a	a	h	a	h	h
Xcup11	Sb03_1	3.45	Top	a	b	a	a	a	a	a	a	a	a	a	h	h	h
Xcup14	Sb03_1	136.17	bottom	a	b	a	a	h	a	h	a	a	a	h	a	a	a
Msbcir188	Sb04_1	19.03	Top	a	b	h	h	a	a	h	a	a	a	a	h	h	a
Gpsb98	Sb04_2	111.66	bottom	a	b	a	a	h	a	a	a	a	a	h	a	a	a
Gpsb136	Sb06_1	93.19	bottom	a	b	h	a	a	a	a	a	a	h	a	a	a	a
Xtxp159	Sb07_1	14.37	Top	a	b	a	a	a	h	a	a	h	a	h	a	a	a
Sbagb02	Sb07_1	95.60	bottom	a	b	h	h	a	a	a	a	a	h	h	a	a	a
gpsb41	Sb08_1	5.40	Top	a	b	a	a	h	a	a	a	a	h	a	h	a	a
Gpsb123	Sb08_1	98.75	bottom	a	b	a	h	a	h	h	a	a	a	h	a	a	a
Msbcir191	Sb09_1	16.44	Top	a	b	h	h	h	a	h	h	h	h	h	a	a	a
gpsb79	Sb09_1	83.79	bottom	a	b	a	a	a	h	a	a	a	a	a	a	a	a
xcup49	Sb10_1	0.54	Top	a	b	a	h	a	a	h	a	a	a	a	a	a	a
Xcup43	Sb10_1	145.74	bottom	a	b	h	a	h	h	a	h	a	h	h	a	a	a
Recovery rate of the RP						0.58	0.64	0.69	0.69	0.64	0.78	0.75	0.64	0.47	0.75	0.64	0.83

a = recurrent parent allele; b=donor parent allele; h=heterozygote; x=missing data

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5.5. Discussions

Low levels of polymorphism were observed between the donor parent (B35) and the four recurrent parents (Kapelga, Grinkan, Sariaso01 and Sariaso09). Allelic diversity was 2.34 indicating that there were more than two alleles per locus. The highest number of allele/locus was 6 and the lowest number was 2. The genotypes displayed a common allele in several loci meaning that they were not polymorphic at these precise loci. However, they showed polymorphism at 35 loci. These alleles indicated dissimilarity between genotypes and exhibited the presence of small genetic distance that is important for QTLs introgression. According to Langridge *et al*. (2001), useful markers in breeding should reveal polymorphism in different populations derived from a wide range of different parental genotypes.

Foreground selection is conducted to identify the marker allele (s) of donor parent at the target locus in order to keep it at heterozygous state until the backcross procedure is completed. This study is the first step of a backcross breeding program and aims to ascertain that some BC_1F_1 progenies within the four populations have incorporated one or more stay-green QTLs. The results of foreground genotyping revealed that 62 (or 43%) progenies respectively 40 (or 37.38%) from crosses of sariaso09 x B35, 10 (or 52.63%) from crosses of Kapelga x B35, eight and four (or 66.66%) from crosses of Grinkan x B35 and crosses of Sariaso09 x B35 had incorporated at least one stay-green QTL in their genomes. The rate of introgression of the target locus was higher than expected in populations two, three and four but in population one it was a little below the normal rate. Overall, the number of progenies was low but the majority of them had incorporated the target QTLs from the donor parent. A few did not carry chromosomal regions with stay-green QTLs at each BC generation. According to Hospital (2003), the expected number

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of individuals at BC_1 generation is 70 and individuals carrying a portion of chromosome with target locus must reach 27 (or 38.4%). Ngugi *et al*. (2010) reported that 22 genotypes (less than 27) in BC_1F_1 were enough to capture one QTL with about 95% confidence. Results from this study revealed rates beyond the expected and beyond that obtained by Ngugi *et al.* (2010). This may be attributed to the suitability of the recurrent parents to receive the donor pollen or it may be linked to the efficiency of the technology (hand emasculation) employed to develop these populations.

Twenty progenies, 18 from backcross populations derived from the cross of Sariaso09 x B35 and 2 from the cross derived Kapelga x B35, were heterozygous at all loci and eventually incorporated the five target QTLs (Stg1, Stg2, Sg3, Stg4 and StgB). The results indicate that these progenies had introgressed 55.40 cM on LG2, 65.28 cM on LG3 and 53.18 cM on LG5. Ten progenies (3 from backcross population 2 and 4 from backcross population 3 and 4 from population 4) incorporated the four following stay-green QTLs (Stg2, Stg3, StgB and Stg1/Stg4). This is an indication that they had respectively 36.64 cM on LG3, 55.40 cM on LG2 and 28.64/53.18 cM on LG3/LG5. Fifteen progenies have incorporated double QTLs and fifteen others carried single QTLs. Individuals selected with double stay-green QTLs located on the same linkage group such as Stg1&2 (LG3) and Stg3&B (LG2) had respectively incorporated 55.40 cM and 65.28 cM whereas individuals selected with single QTL such as Stg1, Stg2, Stg3 and stg4 had introgressed respectively 28.64 cM, 36.64 cM, 1.36 cM, 53.18 cM from the donor parent genome according to the distance within the flanking markers. The distance (53.18 cM) for stg4 is important due to the number (6) of markers used for its screening. However, individuals selected with the flanking markers Xtxp15 & Xtxp23, Xtxp23 & Xtxp14 and

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Xtxp15 & Xtxp14 have kept respectively 2.43 cM, 0.73 cM and 3.17 cM of B35 genome. According to the consensus map constructed by Mace *et al*. (2009), the markers Xtxp285, Msbcir225 are located near the peak of Stg1 QTL and SB5-236 is close to the peak of Stg2 QTL. In that portion of the chromosome the recombination frequency is very low, meaning that individuals with these markers have high chance to keep these QTLs in advanced backcross generations.

Supposedly, all the QTLs contributing to a trait of interest could be taken into account (Collard *et al*., 2005; Jiang, 2013), but, concerning MAS for multiple genes or QTLs, it was suggested to limit the number of genes in a selection procedure to three or four in case they are QTLs selected on the basis of linked markers, and five to six if they are known loci selected directly (Hospital, 2003). Ribaut and Bertrand, (1999) reported that three QTLs are considered as appropriate and feasible for MAS, although, Lecomte *et al*. (2004) used five QTLs in the improvement of fruit quality traits in tomato via marker-assisted introgression. Kassahun *et al*. (2009) used B35 as the donor parent and found backcross families that carried four putative stay-green (Stg1, Stg3, Stg4 and StgB) QTLs in the BC_2F_1 generation. Ngugi *et al*. (2010), used another donor parent (E36-1) and identified at BC_1F_1 generation, individuals with single stay-green QTL (Stg1 or 2).

In this study, importance is given to progenies that have the major QTLs (Stg1, Stg2 and Stg3). In fact, previous studies revealed that Stg1 and Stg2 were incorporated in different genetic background (Subudhi *et al*., 2000) which explain respectively about 20 and 30% of the phenotypic variance (Xu *et al*., 2000; Sanchez *et al*., 2002; Harris *et al*., 2007). Their (stg1 and 2) interaction resulted in explanation of 49.8% phenotypic variation (Subudhi *et al.,* 2000). Stg3 and Stg4 accounted respectively for 16% and 10% of the

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phenotypic variation (Sanchez *et al*., 2002). The introgression of such QTLs into local varieties will surely confer in them the capacity to withstand drought particularly at terminal stage of growth.

The background markers have detected individual with high (83%), adequate (78%) and low recovery rates (47%) of the recurrent parent genome. Two progenies [S9B48 and S1B6] with one incorporated QTLs (Stg4) were able to restore the recurrent parent genome to 81% and 83% respectively. Plants carrying single or double QTLs incorporated less of the donor parent genome. Forty five individuals with a low proportion of the recurrent parent genome had incorporated many QTLs (four or five QTLs). This reduces the chance to identify plants carrying all donor alleles with high background genomes of the recurrent parent, therefore, this limits the number of progenies to be selected in the next generation. Sebolt *et al*. (2000) reported that the rate of success decreases when large numbers of QTLs are selected. According to Newbury (2003), if many QTLs are incorporated, the proportion of undesirable genes will be larger due to linkage drag. Furthermore, undesirable genes are mostly located on non-target chromosomes in early BC generations.

In practical MAS, breeders are concerned about the population size, the number of generations to be conducted and the number of markers. In many cases, marker screening is performed for two to four generations in a segregating population. Bonnett *et al*. (2005) reported that efficient implementation of MAS involves several issues, e.g. breeding systems or schemes, population sizes, number of target loci, etc. Their strategies include F2 enrichment, backcrossing, and inbreeding.

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In case of conventional selection, breeders need a larger number of individuals at each backcross generation to be sure that there are sufficient plants for background selection after the foreground and recombinant selection has been performed. It also requires to evaluate the material at each generation to select those carrying the target gene in order to avoid blind backcrossing with the recurrent parent. In such conditions, conventional backcrossing is time consuming whereas in MAS, if markers are used in close proximity to the QTL or gene of interest, fewer generations are needed.

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5.6. Conclusion

Low levels of polymorphism were detected between the stay-green trait donor parent (B35) and the recurrent parents (Kapelga, Grinkan, Sariaso01 and Sariaso09). The number of alleles varied from two to six per locus. Seventeen polymorphic markers were used for foreground selection to indentify BC_1F_1 individuals that had incorporated the target alleles. The screening allowed the selection of sixty two plants (forty, ten, eight and four plants, respectively, from populations one, two, three and four) with at least one stay-green QTL. Twenty plants (eighteen and two, respectively, from populations one and two) have incorporated all five stay-green QTLs (Stg1, Stg2, Stg3, Stg4 and StgB). Nine plants (three, four and two) respectively, from populations two, three and four, were heterozygous at four target regions containing stay-green QTLs. One plant from population three has been identified with three QTLs. Fifteen plants (seven, four, three and one) respectively, from populations one, two, three and four had stay-green QTLs. Seventeen plants (fifteen, one and one) respectively, from family one, three and four have one stay-green QTL. Two markers such as Msbcir225, Xtxp285 were located near the peak of Stg1 and Sb5-236 (around 7 cM) was close to the peak of Stg2. These markers enable selection of plants with successful introgression of QTLs region in genetic background of different recurrent parents. In this study, the use of background markers allowed quite rapid recovery of the recurrent parent's genomes. In one backcross generation some individuals had recovered their recurrent' parent genome up to 83%. A few progenies were identified with the normal rate (79%) of the genome recovery and the majority carried large proportions of donor parent genome. The promising lines need to be selfed and progenies with major QTLs selected and then the phenotyping evaluation would be conducted to confirm the impact of the introgressed character.

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Chapter VI: Conclusions and recommendations

6.1. Conclusions

The PRA revealed that the major constraint limiting sorghum production in Burkina Faso was *Striga* in the studied area. Drought was the second major constraints reducing sorghum yield and the most important abiotic constraint during the rainy season. Soil fertility was the third factor all over sorghum cultivated areas. Some minor factors such as diseases, insects, and birds' damages contributed also to lessen sorghum yield. The investigation on farmers' perceptions on the major abiotic constraint indicated that terminal moisture stress was the most frequent type of drought but has less effect on yield than pre-flowering drought. Different drought management methods (such as agronomic practices, soil and water conservation systems) were employed by farmers to mitigate drought effects on sorghum production. In the driest part of the country, specific methods, such as planting pits (locally named zaï), stone lines, half and full moons are used to increase soil wetness during drought. Most of the farmers (47%) relied preferentially on their local landraces. Less than 20% of them preferred improved varieties derived from local landraces. Less 15% preferred high yielding exotic varieties. Among released varieties, farmers grew Kapelga more frequently in all different climatic zones of the country.

Providing farmers with sorghum varieties that can withstand drought is necessary and Kapelga for drought tolerance would be the suitable variety to be improved for farmers' needs.

The field study indicated that local accessions were agro-morphologically distant. The stay-green trait exhibited by local accessions was not correlated with the yield probably due to its non-functional aspect. No correlation was also observed between plant height,

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grain weight per panicle, grain number and the yield while plant height was negatively correlated with STI and GMP. Negative correlations were observed between panicle size and grain filling rate, yield, STI and GMP under stress conditions. However, a strong correlation between grains filling rates, yield under stress, grain numbers, STI and GMP indicated that breeding progress can be feasible using these accessions. Major variables that displayed differences among accessions were yield, its components, STI and GMP. The second most important sources of differentiation were morphological parameters linked to drought. Classification using yield and its components assigned accessions to three main groups. Grinkan was the highest yielding and most drought tolerant genotype identified in this study. This is consistent with its use as a high yielding and drought tolerant check. A group of accessions that were high yielding and drought susceptible were identified. This group contained most of the local improved varieties that perform better under non-stressed conditions but were susceptible to terminal drought. Low yielding and drought tolerant materials were also observed. The study identified genotypes with high STI index that could be used by farmers to ensure optimal production in drought prone areas and which may be useful in breeding programmes.

SSRs markers analysis revealed a polymorphism rate of 86.54%. The number of alleles per locus was low and ranged from 2 to 6 with an average of 3.5 alleles/locus and total alleles of 93. There were rare alleles (27) located in stay-green chromosomal regions of some local accessions' genomes is an important finding. There was a high level of homozygosity of local accessions. The characterization indicated a lower gene diversity of 0.34 in Burkina Faso. The *Fst* value was low but significant enough to exhibit genetic

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differentiation among the population. The local populations were genetically closely related but they were genetically distant from the exotic material.

Marker-Assisted Backcrossing methodology allowed the introgression of five stay-green QTLs (Stg1, Stg2, Stg3, Stg4 and StgB) into four recurrent (Kapelga, Sariaso09, Sariaso01 and Grinkan) parent backgrounds. The BC_1F_1 lines selected have at least one QTL during foreground screening:

- ✓ Eighteen (18) plants (S9B1, S9B40, S9B52, S9B68, S9B74, S9B88, S9B39, S9B50, S9B51, S9B7, S9B66, S9B67, S9B18, S9B65, S9B91, S9B24, S9B97 and S9B107) from Sariaso09 progenies were heterozygous at all marker loci flanking the target QTLs. Only two plants (KB4 and KB5), from Kapelga progenies, were heterozygous at all loci flanking markers (carrying the five stay-green QTLs).
- ✓ Three plants (KB8, KB11 and KB12) had incorporated four stay-green QTLs (Stg1, Stg2, Stg3 and StgB). Four plants from Grinkan progenies had incorporated four different QTLs. GB1and GB8 carried QTLs (Stg2, Stg3, StgB and Stg4) and GB2 and GB4 carried the same QTLs (Stg1, Stg2, Stg3 and StgB). Among the selected plants, three (S1B1, S1B3) from Sariaso01 progenies were identified with four QTLs of Stg (Stg2, Stg3, Stg4 and StgB).
- ✓ Only one plant GB7 had incorporated three major QTLs (Stg1, Stg2 and Stg4).
- ✓ Seven plants (S9B43, S9B46 S9B85, S9B87, S9B34, S9B38, and S9B73) had incorporated two QTLs (Stg3and StgB). Four plants (KB9, KB17, KB18 and KB19) had been identified with two stay-green QTLs (Stg1 and Stg2). GB3, GB5 and GB9 had incorporated two QTLs (Stg1 and Stg2). S1B4 carries two QTLs of Stg (Stg2 and Stg4).

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- ✓ Five heterozygous plants (S9B37, S9B85, S9B73, S9B17 and S9B41) with Stg1 QTL and ten plants (S9B37, S9B43, S9B46, S9B85, S9B64, S9B13, S9B38, S9B44, S9B48 and S9B73) with stg4 were also selected. Only plant KB16 has incorporated one QTL (stg4). S1B6 was identified with Stg4 QTL regions in its genome.

The screening using background markers had identified progenies with different recovery rates of the recurrent parent genome. The majority of the progenies had recovered less than 75% of the recurrent parent genome. Two progenies (GB8 and S1B3) had recovered 75%. Six progenies (S9B73, S9B48, KB4, KB16, GB7 and S1B6) had recovered 78% and 83% of the genome of the recurrent parent.

6.2. Recommendations

- ✓ Farmers' priorities should be taken into account in the breeding program to facilitate the adoption of released varieties in the future.
- ✓ High yielding local varieties, identified in the experiments should be stabilized and used for breeding purposes.
- ✓ Clarification of the identified private alleles should be done for their effective use.
- ✓ BC_1F_1 plants that have incorporated major QTLs should be further backcrossed and then genotyped to identify individuals that still carry different QTLs and then phenotype to confirm the function of the introgressed characters.

The best 30 accessions identified through phenotypic evaluation and the promising advanced introgression lines should be evaluated in different locations for possible release as improved farmers preferred varieties. Drought tolerant lines developed

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should be tested through MABC and high yielding lines could be released to ensure food security in Burkina Faso.

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REFRENCES

Abdalla, H. M. and Gamar, Y. A. 2011. Climate change: Selection of sorghum genotype with wide adaptation, AG-17,for rain-fed areas of Sudan. *International Journal of AgriScience* Vol. 1(3): 144-555.

Aggarwal, V. D. and Ouédraogo, J. T. 1989. Estimation of cowpea yield loss from *Striga* infestation. *Tropical Agriculture,* 66: 91-92.

Ali, M. A., A. Abbas., S. Niaz., M. Zulkiffal. and S. Ali, 2009. Morpho-physiological criteria for drought tolerance in sorghum (*Sorghum Bicolor*) at seedling and post-anthesis stages. *International Journal of Agriculture and Biology*, 11: 674–680.

Anderson, J.A., Sorrells, M. E. and Tanksley, S. D. 1993. RFLP analysis of genomic regions associated with resistance to pre-harvest sprouting in wheat (Triticum aestivum). *Crop Science,* 33:453-459.

Araus, J. L., Slafer, G. A., Royo, C. and Serret, D. M. 2008. Breeding for Yield Potential and Stress Adaptation in Cereals. *Critical Reviews in Plant Science*, 27:377–412.

Amasion, R. M. and Gan, S. 1997. Molecular Genetic Regulation and Manipulation of Leaf Senescence. *Plant physiology*. 113: 313-319.

Assogbado, A. E., Amadji, G., Glélé-Kakaï, R., Mama, A., Sinsin, B. and Van Damme, P. 2009. Evaluation écologique et ethnobotanique de *Jatropha curcas* L. au Bénin. *International. Journal of Biology and Chemical Sciences*. 3(5): 1065-1077.

Baird, V., Abbott, A., Ballard, R., Sosinski, B. and Rajapakse, S. 1997. DNA Diagnostics in Horticulture, p. 111–130 In: P. Gresshoff (Ed.), Current Topics in Plant Molecular Biology: Technology Transfer of Plant Biotechnology. CRC Press, Boca Raton.

Banziger, M. and de Meyer, J. 2002. Collaborative maize cultivar development for stress-prone environments in Southern Africa. In: Cleveland, D.A and D. Soleri. (ed.) 2002. *Farmers, Scientists and Plant Breeding*. CAB International. pp 269-296.

University of Ghana http://ugspace.ug.edu.gh

Barnaud, A., Deu, M., Garine, E., McKey, D. and Joly, H. 2007. Local genetic diversity of sorghum in a village in northern Cameroon: structure and dynamics of landraces. *Theoretical and Applied Geneticst,* 114: 237-248.

Barro-Kondombo, P. C., Vom-Brocke, K., Chantereau, J., Sagnard, F. and Zongo, J. D., 2008. Variabilité phénotypique des sorghos locaux de deux régions agricoles du Burkina Faso: la Boucle du Mouhoun et le Centre-Nord. *Cahiers Agricultures*, 17: 107-113.

Barro-Kondombo, P.C., Chantereau, j., Gozé, E., Weltzien, E., Trouche, G. and Brocke, V,K. 2010. Participatory variety development for sorghum in Burkina Faso: Farmers' selection and farmers' criteria. *Field Crops Research* 119:183–194.

Barro-Kondombo, P.C. 2010. Diversité agro-morphologique et génétique de variétés locales de sorgho *(Sorghum bicolor* [L.] Moench) au Burkina Faso. Eléments pour la valorisation des ressources génétiques locales. Thèse de doctorat, Université de Ouagadougou, 112 p.

Bationo, A., Lompo, F. and Koala, S. 1998. Research on nutriënt flows and balances in West Africa: state of the art. *Agriculture, Ecosystems and Environment* 71:19-35.

Belko, N., Cisse, N., Diop, N. N., Zombre, G., Thiaw, S., Muranaka, S. and Ehlers, D.J. 2014. Selection for Postflowering Drought Resistance in Short- and Medium-duration Cowpeas Using Stress Tolerance Indices. *Crop Science,* 54:1–9 (2014). doi: 10.2135/cropsci2012.12.0685.

Bergquist, R. R. 2000. Leaf blight. Pages 9-10 *in*: Compendium of Sorghum Diseases. Frederiksen, R. A and Odvody, G. N. eds. *American Phytopathological Society*, St. Paul, MN.

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

Bidinger, F.R., Mahalakshmi, V. and Rao, P.D.G. 1987. Assessment of drought resistance in pearl millet [*Pennisetum americanum* (L.) Leeke]. I. Factors affecting yield under stress. *Australian Journal of Agriculture Research*. 38: 37–48.

Billot, C., Ramu, P., Bouchet.S., Chantereau,J., Deu, M., Gardes, L., Noyer,J-L., Rami, J.F., Rivallan, R., Li,Y., Lu, P., Wang,T., Folkertsma, R. T., Arnaud,E.C.,

University of Ghana http://ugspace.ug.edu.gh

Upadhyaya, H.D, Glaszmann, J.C. et Hash, T. 2013. Massives sorghum collection genotyped with markers to enhance use of global genetic resources. *PLOS one*. www.plosone.org. Volume8. Issue4. Doi:10.1371/*journal.pone*.0059714.

Blum A., Sinmena B., Mayer J., Golan G. and Shpiler L. 1994. Stem reserve mobilisation supports wheat grain filling under heat stress. *Australian Journal of Plant Physiology* 21, 771–781.

Bohnert, H.J., Nelson, D.E. and Jensen, R. G. 1995. Adaptations to environmental stresses. *Plant Cell* 7:1099–1111.

Bonnett, D. G., Rebetzke, G. J. and Spielmeyer, W. 2005. Strategies for Efficient Implementation of Molecular Markers in Wheat Breeding. - *Molecular Breeding* 15:75-85.

Bonzi M. *La cécidomyie du sorgho,* Contarinia sorghicola *Coq. en Haute Volta, Possibilités de lutte.* Comptes rendus du congrès sur la lutte contre les insectes en milieu tropical, Chambre de Commerce et d'Industrie de Marseille, 13-16 mars 1979 : 531-41.

Borrell, A. K., Bindinger, F. R. and Sunitha, K. 1999. Stay-green trait associated with yield in recombinant inbred sorghum lines varying in rate of leaf senescence. *International Sorghum and Millets Newsletter,* 40: 31-34.

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

Borrell, A.K., Hammer, G. L. and Douglas, A. C. L. 2000a, Does maintaining green leaf area in sorghum improve yield under drought? I. Leaf growth and senescence. *Crop Science,* 40:1026-1037.

Borrell, A. K., Hammer, G. L. and Henzel, R. G. 2000b, Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. *Crop Science* 40:1037-1048.

Borrell, A. K., Van Oosterom, E. J., Mullet, J. E., George-Jaeggli, B., Jordan, D. R., Klein, P. E and Hammer, G. L. 2014. Stay-green alleles individually enhance grain yield in sorghum under drought by modifying canopy development and water uptake patterns. *New Phytol*. 203 (3): 817-30. doi: 10.1111/nph.12869.

University of Ghana http://ugspace.ug.edu.gh

Botstein, D., White, R. L., Skolnick, M. and Davis, R. W. 1980. Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms. – *American Journal of Human Genetics* 32: 314-331.

Bray, E. A. 1997. Plant responses to water deficit. Trends in Plant Sci 2:48–54.

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

Brumlop, S and Finckh, M. R. 201. Applications and potentials of marker assisted selection (MAS) in plant breeding BfN-Skripten 298. Federal Agency for Nature Conservation. 178p.

Cakir, R. 2004. Effect of water stress at different development stages on vegetative and reproductive growth of corn. *Field Crops Research*, **89:** 1-16.

CDB, 1996. La diversité biologique agricole. Note préparée par le secrétariat. Distr. Générale UNEP/CBD/SBSTTA/2/10 le 12 août 1996.

Ceccarelli, S and Grando, S. 2007. Decentralized-participatory plant breeding: an example of demand driven research. *Euphytica* 155, 349–360.

Chambers, G. K and MacAvoy, E. S. 2000. Microsatellites: consensus and controversy. Comparative *Biochemical Physiology* (Part B) 126:455–476.

Chantereau J., Arnaud M., Ollitrault P., Nabayaogo P., Noyer J. L., 1989. Etude de la diversité morphologique et classification des sorghos cultivés. *L'Agronomie tropicale*, 44 (3) :223-232.

Chapman, S. C. and Barreto, M. J. 1997. Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. *Agronomy Journal*, 89:557-562.

Charrier, A., Jaquot, M., Hamon, S. et Nicolas D., 1997. L'amélioration des plantes tropicales. CIRAD-ORSTOM. Montpellier, France, 401-427.

Clausen, S. and Apel, K. 1991. Seasonal changes in the concentration of the major storage proteins and its mRNA in xylem ray cells of poplar trees. *Plant Mo1ecular Biology,* **17:** 669-678

University of Ghana http://ugspace.ug.edu.gh

CIRAD :http://sat.cirad.fr/sat/sorghum_SSR_kit/data/msat_char.html Agropolis-Cirad-Genoplante.

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

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

Craufurd, P. Q Peacock, J. M. 1993. Effect of heat and drought stress on sorghum (Sorghum bicolor). II. Grian yield. *Experimental Agriculture*.29.77-86.

Dahlberg, J., Berenji, J., Sikora, V. and Latkovic, D. 2011. Assessing sorghum [*Sorghum bicolor* (L) Moench] germplasm for new traits: food, fuels and unique uses. *Maydica* 5-17506

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

Dahlberg, J. A. 2000. Classification and characterization of *Sorghum*. Pp. 99–130 in: Smith, C.W. (ed.), *Sorghum: Origin, History, Technology, and Production*. John Wiley & Sons, Inc., New York.

Dakouo D. La cécidomyie du sorgho : bioécologie et pertes. In : Ratnadass A, Ajayi O, Marley PS, Akintayo I, eds. *Les insectes ravageurs du sorgho en Afrique de l'Ouest et du Centre*. Actes de l'atelier de formation ROCARS-Icrisat-Cirat, 14-23 octobre 1996, Samanko (Mali). Colloques. Montpellier : Centre de coopération internationale en recherche agronomique pour le développement (Cirad, 2001 ; 4 p. (*cédérom*).

Daulfrey, S. 1976. Principal component analysis. *Concepts and Techniques in Modern Geography*. 8:50.

University of Ghana http://ugspace.ug.edu.gh

Dean, R. E., Dahlberg, J. A., Hopkins, M.S., Mitchell, S.E and Kresovich, S. 1999. Genetic redundancy and diversity among "orange" accessions in the U.S. national sorghum collection as assessed with simple sequence repeat (SSR) markers. *Crop Science,* 39: 1215-1221

Derera, J. 2005. Genetic Effects and Associations between Grain Yield Potential, Stress Tolerance and Yield Stability in Southern African Maize (*Zea mays* L.)Base Germplasm. A PhD thesis in Plant Breeding African Centre for Crop Improvement (ACCI) School of Biochemistry, Genetics, Microbiology and Plant Pathology Faculty of Science and Agriculture University of KwaZulu-Natal Republic of South Africa, 189p.

Deu, M., Rattunde, F and Chantereau, J. 2006. A global view of genetic diversity in cultivated sorghum using a core collection. *Genome*, 49: 168-180.

Deu, M., Sagnard, F., Chantereau, J., Calatayud, C., Hérault, D., Mariac, C., Pham, J. L., Vigouroux, Y., Kapran, I., Traoré, P. S., Mamadou, A., Gérard, B., Ndjeunga, J. and Bezançon G. 2008. Niger-wide assessment of in situ sorghum genetic diversity with microsatellite markers. *Theoretical and Applied Genetics,* 116: 903-916.

De Wet, J.M.J. 1978**.** Systematics and evolution of *Sorghum* sect. *Sorghum (Gramineae). American Journal of Botany* 65(4):477-4 **1.**

De Wet J. M. J and Huckabay J. P, 1967. The origin of Sorghum bicolor. II. Distribution and domestication. *Evolution* 21: 787-802.

De Wet, J. M. J., 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.

De Wet, J. M. J., Harlan R. J. and Stemler, L. B. A. 1975. Evotionary History of cultivated sorghums (Sorghum bicolor [Linn.] Moench) of Ethiopia. *Bulletin of Torrey Botanical Club,* Vol. 102, No. 6. Pp325-333.

Dje, Y., Heuertz, M., Lefebvre, C. and Vekemans, X. 2000. Assessment of genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellite markers. *Theoretical and Applied Genetics*, 100: 918-925.

University of Ghana http://ugspace.ug.edu.gh

Eagles, H., Bariana, H., Ogbonnaya, H., Rebetzke, G., Hollamby, G., Henry, R., Henschke, P. and Carter, M. 2001. Implementation of markers in Australian wheat breeding. *Australian Journal of Agriculture Research,* 52: 1349–1356.

Encarta, 2008. Microsoft student DVD with Encarta Premium.

Falconer, D.S. 1981. Introduction to quantitative genetics second edition. Longman. England

FAO, 1999.FAO Quart. Bull. Statis. 12 (1/2):33.

FAO, 2009. The state of food insecurity in world. 61p.

FAOSTAT, 2013. FAO Statistics Data base (FAOSTAT). *http://www.faostat.fao.org*.

Farooq, S. and Azam, F. 2002. Molecular markers in plantbreeding – 1: concepts and characterization. Pakistan journal of biological sciences 5 (10): 1135- 1140.

Fernandez, G. C. J. 1992. Effective selection criteria for assessing plant stress tolerance. *In Proceedings of Symposium. Taiwan, 13-16 August*: 257-270.

Frederiksen, R. A. 1986. Compendium of Sorghum Disease. *American Phytopathological Society*, St. Paul, MN.

Frisch, M., Bohn, M. and Melchinger, A. E. 1999. Comparison of Selection Strategies for Marker-Assisted Backcrossing of a Gene. - *Crop Science* 39: 1295-1301.

Frost, R., Xi, Y and He, H. 2007. Modification of the surfaces of Wyoming montmorillonite by the cationic surfactants alkyl trimethyl, dialkyl dimethyl and trialkyl methyl ammonium bromides. *Journal of Colloid and Interface Science* 305(1):pp. 150-158.

Gan, S and Amasino, R. M. 1997. Molecular Genetic Regulation and Manipulation of Leaf Senescence: Making Sense of Senescence[1]. *Plant Physiology*. Pp313-319.

Ganassali, S. 2003: Enquête et analyse de données avec Sphinx, Ed Pearson.

Garber, E. D. 1950. Cytotaxonomic studies In the genus Sorghum. University of Califronia Publications, *Botany* 23:283-261.

Ghebru, B., Schmidt, R. J. and Bennetzen, J. L. 2003. Genetic diversity of Eritrean sorghum landraces assessed with simple sequence repeat (SSR) markers. *Theoretical and Applied Genetics* 105: 229-236.

University of Ghana http://ugspace.ug.edu.gh

Gressel, J., Hanafi, A., Head, G., Marasas, W., Obilana, A.B., Ochanda, J., Souissi, T and Tzotzos, G. 2004. Major heretofore intractable biotic constraints to African food security that may be amenable to novel biotechnological solutions. *Crop Protection*, **23**: 661-689.

Guillobez, S. and Zougmoré, R. 1991.Etude du ruissellement et de ses principaux paramètres à la parcelle (Saria, Burkina Faso). In: John Libbey (ed) Bilan hydrique agricole et sécheresse en Afrique tropicale. *Eurotext*, Paris, pp 319-329.

Gupta, P. K., Roy, J. K. and Prasad, M. 2001. Single Nucleotide Polymorphisms: A New Paradigm for Molecular Marker Technology and DNA Polymorphism Detection With Emphasis on Their Use in Plants. - *Currrent Science* 80: 524-535.

Hall, A. E. 1993. Is dehydration tolerance relevant to genotypic differences in leaf senescence and crop adaptation to dry environments? In T.J. Close, and E.A. Bray (eds.), *Plant Responses to Cellular Dehydration during Environmental Stress*. Rockville, Md: American Soc. Plant Phys.

Hamblin, M.T., Maria G., Fernandez, S., Casa, A.M., Mitchell, S.E., Paterson, A.H. and Kresovich, S. 2005. Equilibrium processes cannot explain high levels of short and medium range linkage disequilibrium in the domesticated grass Sorghum bicolor. *Genetic*. 171: 1247-1256.

Hammer, G. L., Farquhar, G. D. and broad, I.J. 1997. On the extent of genetic variation for transpiration efficiency in sorghum. *Journal of Agricultural Research*, 48: 649-55.

Harlan, J. R. 1975. Les plantes cultivées et l'homme (Crops and man. trad. Franç., 1987). Collection techniques vivantes, PUF, 414 p.

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

Harlan, J, R. and Pasquereau, J. 1969. Décrue agriculture in Mali. *Econ. Bot*. 23: 70-74.

Harlan, J, R. and Stemler, A. 1967. The races of sorghum in Africa. *In* origin of African plant domestication. Harlan, J,R. de Wet, J,M,J and Stemler, A. eds Mouton, the Hague.

Harris, K., Subudi, P. K., Borrell, A., Jordan, D., Roseonw, D., Nguyen, H., klein, R., Klein, P. and Mullet, J. 2007. Sorghum stay-green QTL individually reduce post-

University of Ghana http://ugspace.ug.edu.gh

flowering drought-induced leaf senescence. *Journal of Experimental Botany*. 58(2): 327:38.

Hash,C.T., Bhaskerraj, A.G., Lindup, S., Sharma, A., Beniwal., C.R., Folkertsma, R.T., Mahalakshmi, V., Zerbini, E. and Blummel, M. 2003. Opportunities for marker-assisted selection (MAS) to improve the feed quality of crop residues in pearl millet and sorghum. *Field Crops Research,* 84: 79–88.

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

Henderson, S.A., Von Caemmerer, S., Farquhar, G.D., Wade, L, J. and Hammer, G. L., 1997, Correlation between carbon isotope discrimination and transpiration efficiency in lines of the C4 species *Sorghum bicolor* in the glasshouse and the field. *Australian Journal of Plant Physiology*, 25:111-123.

Hennessy, G. G., de Milliano, W. A. J., and McLaren, C. G. 1990. Influence of primary weather variables on sorghum leaf blight severity in southern Africa. *Phytopathology* 80:943-945.

Henry, R., 1997. Molecular markers in plant improvement. In: Practical Applications of Plant Molecular Biology, pp. 99–132, Chapman and Hall, London.

Henzell, R.G., Brengman, R. L., Fletcher, D. S., McCoster, A. N. 1992. Relationship between yield and non-senescence (stay-green) in some grain sorghum hybrids grown under terminal drought stress. In: Proc 2nd Aust sorghum conf, 4-6 February, 1992. Gatton, Queensland. Australia Institute of Agricultural Sciences, Melbourne, *Occasional Publication* No. 68, pp355-358.

Hospital, F. 2003. Marker-assisted breeding. In: Newbury HJ (ed) Plant molecular breeding, Blackwell Scientific, London, pp 30-56.

House, L. R. 1985. *A guide to sorghum breeding* 2nd edition. ICRISAT, Patancheru, P.O. Box, Andhra Pardesh 502 324, India, 206p.

Ibrahim, B. E., Abdalla, H. W. A., Ibrahim, A. E. and El Naim, M. A. 2014. Association between yield components of sorghum [*Sorghum biocolor* L. (Moench)] under different watering intervals. *International Journal of Sustainable Agricultural Research*, 1(3): 85-92.

University of Ghana http://ugspace.ug.edu.gh

IBPGR and ICRISAT, 1993. Descriptors for sorghum [*Sorghum bicolor* (L) Moench]. International board for Plant genetic resources, Rome, Italy; International Crops Research Institute for the semi-Arid tropics, Patancheru, India. ISBN 92-9043-135-0, 45p.

Jahufer, M., Barret, B., Griffiths, A. and Woodfield, B. 2003. DNA fingerprinting and genetic relationships among white clover cultivars. In: J. Morton (Ed.), Proceedings of the NewZealand Grassland Association, Vol. 65, pp. 163–169, Taieri Print Limited, Dunedin.

Jiang, L-G. 2013. Moelcular markers and Marker-Assisted Breeding in Plants. Plant Science department, South Dakota State University, brookings, USA. 40p. Http://dx.doi.org/10.5772/52583

Jones, N., Ougham, H., Thomas, H. and Pasakinskiene, I. 2009: Markers and Mapping Revisited: Finding Your Gene. - *New Phytologist* 183: 935-966.

Jones, N., Ougham. H. and Thomas, H. 1997a. Markers and mapping: We are all geneticists now. *New Phytol,* 137: 165–177.

Jones, C. J., Edwards, K. J., Castaglione, S., Winfield, M. O., Sala, F., Wiel, C. van de., Bredemeijer, G., Vosman, B., Matthes, M., Daly, A., Brettschneider, R., Bettini, P., Buiatti, M., Maestri, E., Malcevschi, A., Marmiroli, N., Aert, R., Volckaert, G., Rudea, J., Linacero, R., Vazquez, A. and Karp, A. 1997b. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding* 3**:** 381–390

Johnson, B. F. 1958. The staple food economics of western Tropical Africa. Standford univ, press California.

Kassahun, B. B. 2006. Mapping of simple sequence repeats (SSRs) and Marker-Assisted Introgression of quantitive trait loci (QTLs) of stay-green in sorghum [sorghum bicolor (L) Moench]. Thesis of Doctor of Philosophy in plant biotechnology. Dharwad university of agricultural sciences. 180p.

Kassahun, B. B., Bidinger, R.F, Hash, T.C and Kuruvinashetti, S.M. 2009. Stay-green expression in early generation sorghum [*Sorghum bicolor* (L.) Moench] QTL introgression lines. *Euphytica*. 172:351-362.

University of Ghana http://ugspace.ug.edu.gh

Kebede, H., P. K. Subudhi., D. T Rosenow. and H.T. Nguyen, 2001.Quantitative trait loci influencing drought tolerance in grain sorghum (Sorghum bicolor L. Moench). *Theoretical and Applied Genetics*, 103: 266–276.

Khizzah, W.B., Miller, R.F. and Newton, J.R. 1995. Genetic and physiological components of post-flowering drought tolerance in sorghum. *African Crop Science Journal*, Vol.3, No.1, pp.15-21.

Kong, L., Dong, J. and Hart, G.E. 2000. Characteristics, linkage-map positions and allelic differentiation of [*Sorghum bicolor* (L) Moench] DNA simple sequence repeats (SSRs). *Theoretical Applied Genetics,* 101: 438–448.

Lagoke, S. T. O., Parkinson, V and Agunbiade, R. M. 1991. Parasitic weeds and control methods in Africa. *In* "Proceedings of international Workshop on combating *Striga* in Africa" *(S.* K. Kim, Ed.), pp3-14, lITA Ibadan Nigeria.

Langridge, P., Lagudah, E. S., Holton, T. A., Appels, R., Sharp, P.J. and Chalmers, K. J. 2001. Trends in genetic and genome analyses in wheat: a review. *Australian Journal of Agricultural Research*, **52**, 1043–1077.

Lecomte, L., Duffe, P., Buret, M., Servin, B., Hospital, F. and Causse, M. 2004. Marker-Assisted Introgression of Five QTLs Controlling Fruit Quality Traits Into Three Tomato Lines Revealed Interactions Between QTLs and Genetic Backgrounds. - *Theoretical and Applied Genetics*, 109: 658-668.

Lemaire, G., Jeuffroy, H. M and Gastal, F. 2008. Diagnosis tool for plant and Crop N status in vegetative stage Theory and practices for crop n management. *European Journal of Agronomy* 28, 614-624.

Li, P., Wang, Y., Sun, X. and Han, J. 2009. Using microsatellite (SSR) and morphological markers to assess the genetic diversity of 12 falcata (*Medicago sativa* spp. *falcata*) populations from Eurasia. *African Journal of Biotechnology,* 8(10):2102-2108

Lim, O.P., Kim, J.H and Nam, G.H. 2007. Leaf Senescence. *Annu. Rev. Plant Biology*. 2007. 58:115–36.

University of Ghana http://ugspace.ug.edu.gh

Lobell, D. B., Burke, M. B., Tebaldi, C., Mastrandrea, M. D., Falcon, W. P. and Naylor, R.L. 2008.Priorizing climate change adaptation needs for food security in 2030. *Nature* 319: 607–610.

Mace, E. S. and Jordan, D. R. 2010. Location of major effect genes in sorghum (*Sorghum bicolor* (L.) Moench), *Theoretical and Applied Genetics*, 121:1339–1356.

Mace, E. S., Tai, S, Gilding, K. E., Li, Y., Prentis, P.J., Bian, L., Campbell, C B., Hu, W, Innes, D. J., Han, X., Cruickshank, A., Dai, C., Frere, C., Zhang, H., Hunt, C.H., Wang, X., Shatte, T., Wang, M., Su, Z., Li,J., Lin, X., Godwin, I. D., Jordan, D. R. and Wang, J. 2013. Whole-genome sequencing reveals untapped genetic potential in Africa's indigenous cereal crop sorghum. Nature communications | 4:2320 | DOI:10.1038/ncomms3320 | www.nature.com/nature communications.

Malse, J., Farquhar, G. D. and Wong, S. C., 1992, Transpiration ratio and plant mineral content are related among genotypes of a range of species. *Australian Journal of Plant Physiology,* 19: 709-721.

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

Marechera, G., Mbwika, J.M., Odme, H and and Ngugi, E. 2011. Feasibility study on striga control in sorghum. African Agricultural Technology Foundation, Nairobi. 78p.

MASA/DGESS. 2014. Résultats *définitifs de la campagne agricole et de la situation alimentaire et nutritionnelle 2013/2014*. Ouagadougou, Burkina Faso.43P.

Matile, P. 1992. Chloroplast senescence. In ; Baker N, Thomas H, eds. *Crop photosynthesis : spatial and temporal determinants.* Amsterdam: *Elsevier*, 413-440.

Matlon, P. J. 1985. A critical review of objectives, methods and progress to date in sorghum and millet improvement: case study of ICRISAT/Burkina Faso. Appropriate technologies for farmers in Semi-Arid Africa. In: Ohm, H.W., Nagy, J.G. (Eds.), International Programs in Agriculture. Purdue University, West Lafayette, USA, pp. 154–178.

University of Ghana http://ugspace.ug.edu.gh

Matlon, P. J. 1990. Improving productivity in sorghum and pearl millet in semi-arid Africa. *Food Research Institute studies*, Vol. XXII, No. 1, 44p.

McBee, G.G., 1984, Relation of senescence, non senescence, kernel maturity to carbohydrate metabolism in sorghum. In: Sorghum Root and Stalk Diseases: A Critical Review. Proceedings of the consultative discussion of Research Needs and Strategies for Control of Sorghum Root and Stalk Diseases. Ed. Mughogho, L. K., Bellagio, Italy, 27 November–2 December 1983, ICRISAT, Patancheru, India, pp. 119–129.

Missihoun, A. A., Agbangla, C., Adoukonou-Sagbadja, H., Ahanhanzo, C. et Vodouhè, R. 2012. Gestion traditionnelle et statut des ressources génétiques du sorgho (*Sorghum bicolo1r* L. Moench*)* au Nord-Ouest du Bénin. *International Journal of Biology and Chemical Sciences*, 6(3): 1003-1018.

Moench, C. 1794. Methodus plantas horti botanici et agri., 207 p.

Moll, R.H., Jackson, W.A. and Mikkelsen, R.L., 1994, Recurrent selection for maize grain yield: Dry matter and nitrogen accumulation and partitioning changes, *Crop Science,* 34:874-881.

Muchow, R. C. 1988. Effect of nitrogen supply on the comparative productivity of maize and sorghum in a semi-arid tropical environment. I. Leaf growth and leaf nitrogen. *Field Crops Research*, 1-16.

Murray, S. C., Rooney, W. L., Hamblin, M. T., Mitchell, S. E. and Kresovich, S. 2009. Sweet sorghum genetic diversity and association mapping for brix and height. *The Plant Genome*, 2 (1): 15 p.

Mutava, R. N. Prasada, P. V. V. Tuinstra, M. R. Kofoid, K. D. Yua, . J. 2011. Characterization of sorghum genotypes for traits related to drought tolerance. *Field Crop Research*, 10-11.

Nelson, C. J., Wang, S., Wu, Y., Li, X., Antony, G., White, F. F. and Yu, J. 2001. Single- nucleotide polymorphism discovery by high-throughput sequencing in sorghum. Research article Open Access. *BMC Genomics*, 12:352 http://www.biomedcentral.com/1471-2164/12/352.

University of Ghana http://ugspace.ug.edu.gh

Nebié, B. Diversité génétique des sorghos à tiges sucrée [*Sorghum bicolor* (L) Moench] du Burkina Faso. Thèse unique, Université de Ouagadougou, UFR/SVT, 118p.

Nebié, B., Gapili, N., Traoré, R. E., Nanema, K. R, Bationo-Kando, P., Sawadogo, M. et Zongo, J. D., 2012. Diversité phénotypique des sorghos à grains sucrés du centre nord du Burkina Faso. *Sciences et techniques, sciences naturelles et agronomie* vol 32, N° 1 et 2.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small numberof individuals. *Genetics,* 89: 583-590.

Néya, A. and Le Normand, M. 1998. Response of sorghum genotypes to leaf anthracnose (*Colletotrichum graminicola*) under field conditions in Burkina Faso. *Crop Protection,* 17; 47-53.

Newbury, H. J. 2003. Plant molecular breeding, Blackwell Scientific, (ed) London, pp 30-56.

Ngugi, k., Kimani,W and Kiambi, D. 2010. Introgression of stay-green trait into a Kenyan farmer prefered sorghum variety. *African Crop Science Journal, Vol. 18, No. 3, 141-146 pp.*

Nguyen, H. T., Babu, R. C. and Blum, A. 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations.*Crop Sciences,* 37:1426–1434.

Nicou, R., Ouattara, B. and Somé, L. 1990.Effets des techniques d'économie de l'eau a la parcelle sur les cultures céréalières (sorgho, maïs, mil) au Burkina Faso. *L'Agronomie Tropicale*, 45:43-57.

Olembo, K. N., M'mboyi, F., Kiplagat, S., Sitieney, J. K. and Oyugi, F. K. 2010. Sorghum breeding in Sub-Saharan Africa: The success stories. African Biotechnology Stakeholders forum (ABSF) 40p.

Ollitrault, P. 1987. Evaluation génétique des sorghos cultivés (Sorghum bicolor L. Moench) par l'analyse conjointe des diversités enzymatique et morpho-physiologique ; relation avec les sorghos sauvages. Thèse de doctorat en science de la vie, université ParisXI, Orsay, 187p.

Ouédraogo, E. 2004. Soil quality improvement for erop production in semi arid West Africa. Tropical resource management papers no 51. Wageningen University and

University of Ghana http://ugspace.ug.edu.gh

Research centre, Department of Environmental Science, Erosion, Soil and Water conservation groups, The Netherlands, 193 pp.

Ouédraogo, O. 1986. *Striga* in Burkina Faso. Improved Management in Africa: Proc. of the *FAO/OAU* AII-Africagouverment consultation on *Striga* control (Edited by Robson T.D. and Broad H.R.) pp 34-36.20-24 October 1986, Maroua Cameroon.

Ouédraogo, S. 2005. Intensification de l'agriculture dans le plateau central du Burkina Faso: Une analyse des possibilités à partir des nouvelles technologies. Thesis, Groningen University, 322 pp.

Parker, C. and C. Riches. 1993. Parasitic Weeds of the World: Biology and control. Wallingford: CAB International.

Paterson, A. H., Bowers, J. E., Bruggmann, R., Dubchak, I., Grimwood, J., Gundlach, H., Haberer, G., Hellsten, U., Mitros, T., Poliakov, A., Schmutz, J., Spannag, l.M., Tang, H., Wang, X., Wicker., T, Bharti, A.K., Chapman, J., Feltus, F.A, Gowik, U., Grigoriev, I.V., Lyons, E., Maher, C.A, Martis, M., Narechania, A., Otillar, R.P., Penning, B.W., Salamov, A.A., Wang, Y., Zhang, L. and Carpita, N.C. 2009. The Sorghum bicolor genome and the diversification of grasses. *Nature* 2009, 457:551-556.

Payton, S. P., Cartor, R., Kayla, M. C., Pathsn, M. S., Rosenow, D. T. and Nguyen, H. T., 2003, Transcriptional profiling of drought response in sorghum, Abs: 76, Poster: Functional Genomics. *American Society of Plant Biologists Annual Meeting*, August 8, 2002, Texas.

Pejic, I., Ajmone-Marsan, P., Morgante, M., Kozumplick, V., Taranimo, G and Motto, M 1998. Comparative analysis of genetic similarities among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. *Theoretical and Applied Genetics*, 97:1248- 1255.

Pernes, J. 1984. Gestion des ressources génétiques des plantes. ACCT, Paris, Volume II, 346p.

Perrier, X. et Jacquemoud-Collet J. P. 2006. DARwin software. http://darwin.cirad.fr/darwin

University of Ghana http://ugspace.ug.edu.gh

Powell, W., Morgante, M. C ., Hanafey, M., Tingey, S. and Rafaski, A. 1996a. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*, 2: 225-238.

Powell, W., Marchray, G. C. and Provan, J. 1996b. Polymorphism Revealed by Simple Sequence Repeats. - *Trends in Plant Science* 1: 215-222.

Prasad, P.V.V., S.R. Pisipati, R.N. Mutava and M.R. Tuinstra, 2008.Sensitivity of grain sorghum to high temperature stress during reproductive development. *Crop Sciences,* 48: 1911–1917.

Prinz, D. 2002. The Role of Water Harvesting in Alleviating Water Scarcity in Arid Areas. Keynote Lecture, Proceedings, International Conference on Water Resources Management in Arid Regions. 23-27 March, 2002, Kuwait Institute for Scientific Research, Kuwait, (Vol. III, 107-122).

Ratnadass, A., Butler, D.R., Marley, P. S., Bandyopadhay, R., Hess, D.E., akintayo, I. 2003. Sorghum head bugs and grain mold in west and central Africa; II. Relationships between weather, head-bugs and mold damage on sorghum grains. *Crop Protection*, 22: 853-858.

Renuka, K. and Chimmad, P, V. 2006. Effect of Irrigation at Different Post Anthesis Stages on Stay Green Trait in *Rabi* Sorghum Genotypes in Relation to Chlorophyll, Nitrogen and Sugar Content. *Karnataka Journal of Agricultural Science, 19(3):* 523- 528.

Ribaut, J. M. and Betran, J. 1999. Single large-scale marker-assisted selection (SLS-MAS). *Molecular Breeding*. 5: 531-541.

Rijsberman, F. R. 2006. Water scarcity: Fact or fiction? Agric. Water Manage. 80:5-22.

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

Rockall and Smouse PE, 2006. Genalex version 41.6Xla.

Rosenow, D., Quisenberry, J.E., Wendt, C.E. and Clark, L.E., 1983, Drought tolerant sorghum and cotton germplasm.*Agricultural Water Management,* 7: 207–222.

Rosenow, D.T. 1984. Breeding for resistance to root and stalk rots in Texas. In: sorghum root and stalk rots: a critical review, Proc Consultative Group, Discussion on

University of Ghana http://ugspace.ug.edu.gh

Research needs and Strategies for Control of Sorghum Root and Stalk Rot Diseases- Nov 27- Dec-2, 1983, Bellagio, Italy, ICRISAT, patancheru, AP 502 324, pp209-218.

Rosenow, D. T. 1987. Breeding sorghum for drought resistance. In: *International Drought Symposium.-Food and Grain Production in Semiarid Africa Proceedings*. Eds. Menyonga, J.M., Bezuneh, T. and Youdeowei, A., Nairobi, Kenya, pp.72-78.

Sagnard, F., Deu, M., Dembélé, D., Leblois, R., Touré, L., Diakité, M., Calatayud, C., Vaksmann, M., Bouchet, S., Mallé, Y., Togola, S and Traoré P. C. S., 2011. Genetic diversity, structure, gene flow and evolutionary relationships within the *Sorghum bicolor* wild-weedy-crop complex in a western African region. *Theorical and Applied Genetics*, 16 p.

Rosenow, D.T., Ejeta, G., Clark, L. E., Gilbert, M. L., Henzell, R. G., Borrell, A. K., Muchow, R. C. 1996. Breeding for pre- and post-flowering drought stress resistance in sorghum. In: Rosenow DT, Yohe JM (eds) Proceedings of the international conference on genetic improvement of sorghum and pearl millet (Lubbock, TX, 22–27 September 1996), ICRISAT, Lubbock, India, pp 400–411.

Sanchez, A.S., Subudi, P.K., Rosenow, D.T and Nguyen, H.T. 2002. Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). *Plant Molecular Biology*. 48 (5-6): 713-26.

SAS Institute Inc., 2015. SAS/STAT Software: Changes and Enhance-ment through Release 9.3. SAS Institute Inc., Cary.

Sapin, P. 1984. Le sorgho et son amélioration. Synthèse Haute Volta 1961-1981, Irat, Montpellier, 86 P.

Sawadogo, N. 2015. Diversité génétique des sorghos à grains sucrés [*Sorghum bicolor* (L) Moench] du Burkina Faso. Thèse Unique, Université de Ouagadougou, UFR/SVT, 185p.

Sawadogo, N., Nebié, B., Kiébré, M., Bationo-Kando, P., Nanema, K. R., Traoré, R. E., Gapili, N, Sawadogo, M et Zongo, J. D., 2014. Caractérisation agromorphologique des sorghos à grains sucrés [*Sorghum bicolor* (L.) Moench]

University of Ghana http://ugspace.ug.edu.gh

du Burkina Faso. *International Journal of Biology and Chemical Sciences.* 8 (5):*2183-2197:* 15p.

Sax, K. 1923. The association of size differences with seed coat pattern and pigmentation in Phaseolus vulgaris. *Genetics* 8:552–560

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

Scott, K. D., Eggler, P., Seaton, G., rosseto, M., Ablettem, M., lee, L. S. and Henry, R.J. 2000. Analysis of SSRs derived from grape ESTs. *Theoretical and Applied Genetics,*100:723–726.

Sebolt, A. M., Shoemaker, R. C. and Diers, B.W 2000. Analysis of a quantitative trait locus allele from wild soybean that increases seed protein concentration in soybean. *Crop Science,* 40: 1438-1444.

Shehzad, T., Iwata, H. and Okuno, K., 2009. Genome-wide association mapping of quantitative traits in sorghum (Sorghum bicolor (L.) Moench) by using multiple models. *Breeding Sciences*. 59: 217-227.

Sio-Se Mardeh, A., Ahmadi, A., Poustini, K. and Mohammadi, V. 2006. Evaluation of drought resistance indices under various environmental conditions. *Field Crops Research*. 98:222–229. doi:10.1016/j.fcr.2006.02.001.

Smart, C. M. 1994. Gene expression during leaf senescence. *New Phytology* **126,** 419-48.

Smith, J.S.C and Smith, O.S. 1992. Fingerprinting crop varieties. *Adv. Agron*. 47: 85-140.

Some, L., Lodun, T. and Some, B. 2004. Les facteurs agro écologiques dans la conservation in situ de la biodiversité agricole. Enhancing the use of crop genetic diversity in: To manage abiotic stress in agricultural production systems (D. Jarvis, I. Mar and L. Sears). Compte-rendu des travaux de l'atelier scientifique organisé par le 23-27 May 2005, Hunagry, Budapest.

Somé, L. 1989. Diagnostic agroclimatique du risque de sécheresse au Burkina Faso. Etude de quelques techniques agronomiques améliorant la résistance pour les

University of Ghana http://ugspace.ug.edu.gh

cultures de Sorgho, Mil, Maïs. Thèse de doctorat, Université de Montpellier II, 312 p.

Snowden, J.D. 1936. Cultivated races of sorghum. London: Adlard and Sons. 274 pp.

Snowden, J. D. 1955. The wild fodder sorghums of the section Eu-sorghum**.** *Journal of the Linnean Society of London: Botany* 1955, **55:**191-260.

Sperling, L., Loevinsohn, M. E. et Ntabomvuras, B. 1993. Rethinking the farmer's role in plant breeding: local bean experts and on station selection in Rwanda. *Experimental Agriculture*. 29, 509–519.

Stoop, W. A., Pattanayak, C. M., Matlon, P. J. and Root, W. R., 1981. A strategy to raise the productivity of subsistence farming systems in the west African semi-arid tropics. *In Proceedings, sorghum in the Eighties ICRISAT 2-7 November 1981, Patancheru 502 324, A.P., India. Stratégie Mondiale de la Biodiversité*, 19-26.

Subudhi, P. K., Rosenow, D.T. and 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,* 101:733–741 123.

Tanksley, S. D., Young, N. D., Paterson, A. H. and Bonierbale, M. W. 1989. RFLP Mapping in Plant Breeding: New Tools for an Old Science. *Nature Biotechnology*, 7: 257-264.

Talebi, R., Fayaz, F and Naji, M.A. 2009. Effective selection criteria for assessing drought stress tolerance in durum wheat (*Triticum durum* Desf.). *General and Applied Plant Physiology*. 35:64–74.

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. *Theoretical and Applied Genetics,* 100:1225–1232.

Tao, Y.Z., Manners, J. M. and Henzell, R.G. 1993. DNA polymorphism in grain sorghum (*Sorghum bicolor (L) Moench). Theoretical and Applied Genetics,* 86:679-688.

University of Ghana http://ugspace.ug.edu.gh

Taramino, G and Tingey, S., 1996, Simple sequence repeats for germplasm analysis and mapping in maize. *Genome,* 39:277-287.

Taramino, G., Tarchini, R., Ferrario, S., Lee, M. and Pe, M.E. 1997. Characterization and mapping of simple sequence repeats (SSRs) in *Sorghum bicolor*. *Theoretical and Applied Genetics,* 95: 66–72.

Tenkouano, 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.

Tenkouano, A. 1995. Current trends in regional integration of sorghum research in Western and Central Africa. *Int. Sorghum Millets Newsl*. (SICNA and ICSIRAT, ed.) pp. 36:41-44.

Thomas, H and Howarth, J.C. 2000. Five ways to stay-green. *Journal of experimental botany*, vol. 51, GMP Special issue, PP.329-337.

Thomas, H and Rogers, L.J. 1990. Turning over an old leaf. University of Wales R*eview of Science & Technology,* 6:29-38.

Thomas, H. and Rogers, J.L. 1990. Turning over an old leaf. *University of Wales Review Science and Technology* 6: 29–38.

Thomas, H and Smart, M.C.1993. Crops that stay-green. *Annals of applied biology* 123, 193-1219.

Trouche, G., Da, S., Pale, G., Sohoro, A., Ouedraogo, O., and Gosso, G. 2001. Evaluation participative de nouvelles variétés de sorgho, pp. 36-55, *in* H. Hocdé´, J. Lançon and G. Trouche (eds.). Sélection Participative. CIRAD, Montpellier.

Thurber, S.C., Ma, M.J., Higgins, H.R and Brown, J.P. 2013. Retrospective genomic of analysis of sorghum adaptation to temperate zone grain production. Research open access. *Genome Biology*, 14:R68. http://genomebiology.com/2013/14/6/R68.

Tolk, A. J., Howella, A. T. and Miller, R. F. 2012. Yield component analysis of grain sorghum grown under water stress. *Field Crop Research* 145, 44-51.

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 bicolor (L.)Moench. *Molecular Breeding*, 3:439–448.

University of Ghana http://ugspace.ug.edu.gh

Tuinstra, M. R., Ejeta, G. and Goldsborough, P. B. 1998. Evaluation of nearisogenic sorghum lines for QTL markers associated with drought tolerance. *Crop Science* 38, 835–842.

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

Vaisi, H. and Golparvar, R. A. 2013. Determination of the best indirect selection criteria to improve grain yield and seed weight in oat (*Avena sativa* L.) genotypes. *International Journal of Farming and Allied Sciences*. Pp 747-750. Available online at www.ijfas.com.

Van-Oosterrom, E. J., Jyachandram, R. and Bidinger F.R., 1996, Diallel Analysis of the stay-green trait and its components in sorghum. *Crop Science,* 36:549-555.

Vierling, G.R.A., Xiang, Z., Jochi, C.P., Gilbert, M. L and Nguyen, H.T. 1994. Genetic diversity among elite sorghum lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs. *Theoretical and Applied Genetics*, 87:816-820.

Vilain, M. 1999. Méthodes expérimentales en agronomie. Pratique et analyse. Les bases de l'agriculture. Ph. Prevost, 2^{e} edition, 337p.

Vernooy, R. 2003. *Les semences du monde*. L'amélioration participative des plantes. CRDI (http://www.irdc.ca/openbooks/015-2#page vii).

Vom Brocke, K., Trouche, G., Weltzien, E., Barro-Kodombo, P. C., Goze, E. and Chantereau, J. 2010. Participatory variety development for sorghum in Burkina Faso: Farmers' selection and farmers' criteria. *Field Crops Research*, 119, pp183-194.

Walulu, R. S., Rosenow, D. T., Wester, D. M. and Nguyen, H.T., 1994, Inheritance of stay- green trait in sorghum, *Crop Science*, 34:970-972.

Wang, M. l., Zhu, C., Barkley, N. A., Chen, Z., Erpelding, J. E., Seth, C., Murray, S. C., Tuinstra, M. R., Tesso, T., Gary, A., Pederson, G. A. and Yu, J. 2009. Genetic diversity and population 127 structure analysis of accessions in the US historic

University of Ghana http://ugspace.ug.edu.gh

sweet sorghum collection. *Theoretical and Applied Genetics,* 120:13–23, DOI 10.1007/s00122-009-1155-6.

Weeden, N., Timmerman, G. and Lu, J. 1994. Identifying and mapping genes of economic significance. *Euphytica* 73: 191–198.

Weising, K., Nybom, H., Wolff, K and Meyer, W. 1995. Applications of DNA Fingerprinting in Plants and Fungi DNA Fingerprinting in Plants and Fungi, CRC Press, Boca Raton.

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

Winter, P. and Kahl., G. 1995. Molecular marker technologies for plant improvement. *World Journal of Microbiology & Biotechnology* 11: 438–448.

Wright, S., 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution*, 19 : 395-420.

Wolfe, D.W., Henderson, D.W., Hsiao, T.C and Albino, A. 1988. Interactive water and nitrogen effects on senescence of maize. II. Photosynthetic decline and longevity of individual leaves. *Agronomy Journal,* 80:865-870.

World Bank 2007.World Development Report 2008. Agriculture for Development. Washington DC. http://siteresources.worldbank.org/INTWDR2008/Resources/WDR00 book.pdf.364 pp.consulted the 06/08/2012.

Xu, W., Subudhi, P. K., Crasta, O. R., Rosenow, D. T., Mullet, J. E. and Nguyen, H.T. 2000. Molecular mapping of QTLs conferring stay-green in grain sorghum (Sorghum bicolor L. Moench). *Genome*, 43:461–469.

Yada, B., Tukamuhabwa, P.S, Wanjala, B., Kim, D.-J., Skilton R.A., Alajo, A. and Mwanga R.O.M., 2010. Characterization of Ugandan sweetpotato germplasm using fluorescent labeled simple sequence repeat markers. *Horticultural science,* 45(2):225-230.

Yapi, A. M., Kergna, A. O., Debrah, S. K., Sidibé, A. and Sanogo, O. 2000. Analysis of the Economic Impact of Sorghum and Millet Research in Mali. Impact Series no.

University of Ghana http://ugspace.ug.edu.gh

8. Patancheru 502 324. International Crops Research Institute for the Semi-Arid Tropics, Andhra Pradesh, India, 60 pp.

Zhang, J., Klueva, Y. N. and Nguyen, T. H. 1999. Plant adaptation and crop improvement for arid and semiarid environments. *In* I.R. Traylor, H. Dregne, and K. Mathis (eds.), *Proceedings of the Fifth International Conference on Desert Development: The Endless Frontier*, 12- 17 August 1996. Lubbock, TX. (USA): Texas Tech University, Vol. II. Pp. 604–624.

Zheng, L.Y., Guo, X.S., He, B., Sun, L.J., Peng, Y., Dong, S.S., Liu, T.F., Jiang, S., Ramachandran, S., Liu, C.M and Jing, H. C. 2011. Genome-wide patterns of genetic variation in sweet and grain sorghum (Sorghum bicolor). *Genome Biology*, open access. 12:R114

Zongo, J.D., 1991. Ressources génétiques des sorghos (Sorghum bicolor L. Moench) du Burkina Faso : Evaluation agro-morphologique et génétique. Thèse de doctorat, Université d'Abidjan, 175 p.

Zongo, J. D., Gouyon, P. H., Sarr, A. et Sandmeier M. 2005. Genetic diversity and phylogenic relations among Sahelian sorghum accessions. *Genetic Resources and Crop Evolution*, 52 : 869 – 878.

Zougmoré, R. 2003. Integrated water and nutrient management for sorghum production in semi-arid Burkina. Tropical resources management papers no 45, Wageningen University and Research center, Wageningen, the Netherlands, 205 pp.

University of Ghana http://ugspace.ug.edu.gh

APPENDICES

Appendix 1: Sequences of SSRs for diversity study

Primers	Forward	Reverse	LG	Reference
mSbCIR243	AATTATGCGTCCATTTTG	CCCAGTATCCTTGAACAG	Sb01_1	CIRAD
gpsb158	GAATTGAGTGGGATTTGT	GTCAGAGAATGGGTTCAT	Sb01_1	CIRAD
msbcir238	AGAAGAAAAGGGGTAAGAGC	CGAGAAACAATTACATGAACC	Sb02_1	CIRAD
gpsb014	GGCAGGCATCGTTTTCT	AGGGTGAATAGCGTGAGC	Sb02_1	CIRAD
Xtxp3	AGCAGGCGTTTATGGAAG	ATCCTCATACTGCAGGACC	Sb02_1	Kong et al. (2000)
Xtxp55	TCATGGCATGGGACTATTG	AAGGTTGGCGTAGAAATGTGT	Sb02_1	Kong et al. (2000)
Xtxp72	TTATGGAAGCAAAATGAC	CGAATCCTAATTGAGGTAAGC	Sb02_1	Kong et al. (2000)
mSbCIR225	AATCCGAGAATCCCAAATA	CGCAATGATTAGGAACACTA	Sb03_1	CIRAD
mSbCIR314	ATACAAAAGGTGGTGCTTTC	CCATTCAGTGTTTTGGAACT	Sb03_1	CIRAD
gpsb133	GCGTGTGGGTATGACT	GTGAGATGAACCGTGTG	Sb02_1	CIRAD
Xcup11	TACCGCCATGTCATCATCAG	CGTATCGCAAGCTGTGTTTG	Sb03_1	Schloss et al. 2002
Xtxp285	ATTTGATTCTTCTTGCTTTGCCTTGT	TTGTCATTTCCCCCTTCTTTCTTTT	Sb03_1	Bhattramakki et al. 2000
sb5-236	GCCAAGAGAAACACAAACAA	AGCAATGTATTTAGGCAACACA	Sb02_1	CIRAD
mSbCIR188	GGGCTTAGATGCTTGAACTA	GCATAATGAAAGTGTTGTGG	Sb04_1	CIRAD
gpsb098	CTATTGTATGGATGGGCT	GACTGAAGACTGAATTGCT	Sb04_1	CIRAD
msbcir222	CCTCATTTCTCTAGCTCCTG	CGGGAACGACATTATTTT	Sb03_1	CIRAD
gpsb032	ATGCTGCCTTTTTCTTTG	TATGTGCTGCCGTGCT	Sb05_1	CIRAD
Xtxp15	CACAAACACTAGTGCCTTATC	CATAGACACCTAGGCCATC	Sb05_1	Kong et al. (2000)
Xtxp123	TCGGCGAGCATCTTACA	TACGTAGGCGGTTGGATT	Sb05_1	Bhattramakki et al. (2000)
Xtxp225	TTGTTGCATGTTGGTTATAG	CAAACAAGTTCAGAAGCTC	Sb05_1	Bhattramakki et al. 2000
Xtxp23	AATCAACAAGAGCGGGAAAG	TTGAGATTCGCTCCACTCC	Sb05_1	Kong et al. (2000)
gpsb136	ACAGGAGGAAAGGACAA	GGTTTGAATAACGGCA	Sb06_1	CIRAD
SbAGB02	CTCTGATATGTCGTTGTGCT	ATAGAGAGGATAGCTTATAGCTCA	Sb07_1	Taramino et al., 1997
gpsb123	ATAGATGTTGACGAAGCA	GTGGTATGGGACTGGA	Sb08_1	CIRAD
gpsb079	AGGACAGAACAGGAAGG	GTGAATTTGAGTTGGAAAG	Sb09_1	CIRAD
Xcup43	GCCTAACTCCCTTGTGATGC	GTCAGTGGATGTGGATGTGC	Sb010_1	Schloss et al. 2003

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Appendix 2: Sequences of SSRs flanking markers for foreground selection and their references

Primers	Stay-green	Forward	Reverse	Reference
msbcir238	Stg3	AGAAGAAAAGGGGTAAGAGC	CGAGAAACAATTACATGAACC	CIRAD
Xtxp55	StgB	TCATGGCATGGGACTATTG	AAGGTTGGCGTAGAAATGTGT	Kong et al. (2000)
Xtxp72	StgB	TTATGGAAGCAAAATGAC	CGAATCCTAATTGAGGTAAGC	Kong et al. (2000)
mSbCIR339	Stg3	AGCACAGTCTTGGTGATAGG	CACAAGTTTGGAGCTGTATTT	CIRAD
Xtxp428	StgB	CACTGGCCAAGGTTTCACTT	CATGGAATGCAACATAGCAA	
Stgnhsbm36	Stg3	CTTTCGCCTGGTCGTACACT	AGAAGAACGCCTCGCTCTC	
mSbCIR225	Stg2	AATCCGAGAATCCCAAATA	CGCAATGATTAGGAACACTA	CIRAD
mSbCIR314	Stg1&1	ATACAAAAGGTGGTGCTTTC	CCATTCAGTGTTTTGGAACT	CIRAD
Xtxp285	Stg1	ATTTGATTCTTCTTGCTTTGCCTTGT	TTGTCATTTCCCCCTTCTTTCTTTT	Bhattramakki et al. (2000)
sb5-236	Stg2	GCCAAGAGAAACACAAACAA	AGCAATGTATTTAGGCAACACA	Taramino et al., 1997
msbcir222	Stg2	CCTCATTTCTCTAGCTCCTG	CGGGAACGACATTATTTT	CIRAD
gpsb032	Stg4	ATGCTGCCTTTTTCTTTG	TATGTGCTGCCGTGCT	CIRAD
Xtxp15	Stg4	CACAAACACTAGTGCCTTATC	CATAGACACCTAGGCCATC	Kong et al. (2000)
Xtxp123	Stg4	TCGGCGAGCATCTTACA	TACGTAGGCGGTTGGATT	Kong et al. (2000)
Xtxp225	Stg4	TTGTTGCATGTTGGTTATAG	CAAACAAGTTCAGAAGCTC	Bhattramakki et al. (2000)
Xtxp23	Stg4	AATCAACAAGAGCGGGAAAG	TTGAGATTCGCTCCACTCC	Kong et al. (2000)
Xtxp14	Stg4	GTAATAGTCATGACCGAGG	TAATAGACGAGTGAAAGCCC	Kong et al. (2000)

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Appendix 3: Sequences of SSRs for background selection and their references

Primers	Stay-green	Forward	Reverse	References
gpsb014	Non stg	GGCAGGCATCGTTTTCT	AGGGTGAATAGCGTGAGC	CIRAD
gpsb079	Non stg	AGGACAGAACAGGAAGG	GTGAATTTGAGTTGGAAAG	CIRAD
gpsb098	Non stg	CTATTGTATGGATGGGCT	GACTGAAGACTGAATTGCT	CIRAD
gpsb123	Non stg	ATAGATGTTGACGAAGCA	GTGGTATGGGACTGGA	CIRAD
gpsb133	Non stg	GCGTGTGGGTATGACT	GTGAGATGAACCGTGTG	CIRAD
gpsb136	Non stg	ACAGGAGGAAAGGACAA	GGTTTGAATAACGGCA	CIRAD
gpsb158	Non stg	GAATTGAGTGGGATTTGT	GTCAGAGAATGGGTTCAT	CIRAD
SbAGB02	Non stg	CTCTGATATGTCGTTGTGCT	ATAGAGAGGATAGCTTATAGCTCA	CIRAD
Xcup11	Non stg	TACCGCCATGTCATCATCAG	CGTATCGCAAGCTGTGTTTG	Schloss et al. 2002
Xcup43	Non stg	GCCTAACTCCCTTGTGATGC	GTCAGTGGATGTGGATGTGC	Schloss et al. 2003
Xtxp3	Non stg	AGCAGGCGTTTATGGAAG	ATCCTCATACTGCAGGACC	Kong et al. (2000)
mSbCIR243	Non stg	AATTATGCGTCCATTTTG	CCCAGTATCCTTGAACAG	CIRAD
gpsb129	Non stg	CTCAACTCAACGCCTAC	ACTACTCCAATCCAATCC	CIRAD
gpsb178	Non stg	TGTTTTCCTTGACCTTCT		CIRAD
Xcup14	Non stg	TACATCACAGCAGGGACAGG	CTGGAAAGCCGAGCAGTATG	Schloss et al. 2002
mSbCIR188	Non stg	GGGCTTAGATGCTTGAACTA	GCATAATGAAAGTGTTGTGG	CIRAD
gpsb136	Non stg	ACAGGAGGAAAGGACAA	GGTTTGAATAACGGCA	CIRAD
Xtxp159	Non stg	ACCCAAAGCCCAAATCAG	GGGGGAGAAACGGTGAG	Bhattramakki et al. 2000
gpsb041	Non stg	TGAGGCACACCAAAGT	TGCTAAGAAAAGGAGAAT	CIRAD
mSbCIR191	Non stg	TTTGTCCATGTTCATCAAA	TTTGCACTTAGCACCATATC	CIRAD
Xcup49	Non stg	TCCACCTCCATCATCTTTCC	CTCCACCACCTCCATGACTC	Schloss et al. 2002

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List of accessions used in the field evaluation

N°	Accessions	Origin	N°	Accessions	Origin	N°	Accessions	Origin
01	**B35**	Ethi	38	**PSE129**	BF	75	**ROE228H**	BF
02	**CVS NEONSO1980** BF		39	**PSE14**	BF	76	**ROE30**	BF
03	**CVS182**	BF	40	**PSE143**	BF	77	**ROE35**	BF
04	**CVS187**	BF	41	**PSE146**	BF	78	**ROE56**	BF
05	**CVS19**	BF	42	**PSE151**	BF	79	**ROE76H**	BF
06	**CVS195-1**	BF	43	**PSE159**	BF	80	**ROE79H**	BF
07	**CVS200**	BF	44	**PSE162**	BF	81	**RSOE07**	BF
08	**CVS208-1**	BF	45	**PSE170**	BF	82	**RSOE15**	BF
09	**CVS22-2**	BF	46	**PSE187**	BF	83	**RSOE255**	BF
10	**CVS29**	BF	47	**PSE188**	BF	84	**RSOE38**	BF
11	**CVS365-2**	BF	48	**PSE192**	BF	85	**RSOE41**	BF
12	**CVS41**	BF	49	**PSE194**	BF	86	**RSOE71K**	BF
13	**CVS423**	BF	50	**PSE197**	BF	87	**RTX7000**	USA
14	**CVS427**	BF	51	**PSE200**	BF	88	**RTX7078**	USA
15	**CVS432**	BF	52	**PSE201**	BF	89	**S34**	BF
16	**CVS456**	BF	53	**PSE207**	BF	90	**Samouraï1**	INDO
17	**CVS48**	BF	54	**PSE232**	BF	91	**Samouraï2**	INDO
18	**CVS512**	BF	55	**PSE237**	BF	92	**Sariaso03**	BF
19	**CVS517**	BF	56	**PSE239**	BF	93	**Sariaso04**	BF
20	**CVS558**	BF	57	**PSE25**	BF	94	**Sariaso05**	BF
21	**CVS559**	BF	58	**PSE28**	BF	95	**Sariaso07**	BF
22	**CVS573**	BF	59	**PSE33**	BF	96	**Sariaso09**	BF
23	**CVS860**	BF	60	**PSE36**	BF	97	**Sariaso14**	BF
24	**CVS95**	BF	61	**PSE42**	BF	98	**Sepon82**	ICRISAT
25	**CVS97**	BF	62	**PSE44**	BF	99	**Sissamba1**	BF
26	**Diankogo**	BF	63	**PSE45**	BF	100	**Sissamba2**	BF
27	**Framida**	ICRISAT	64	**PSE55**	BF	101	**Tiandougou**	Mali
28	**Grinkan**	Mali	65	**PSE58**	BF	102	**Tiandougoucoura**	Mali
29	**ICSV1049**	BF	66	**PSE60**	BF	103	**Tougan**	BF
30	**IS27564 BF**	BF	67	**PSE67**	BF	104	**V1**	BF
31	**Kapelga**	BF	68	**PSE69**	BF	105	**V2**	BF
32	**Kiambara1**	BF	69	**PSE76**	BF	106	**V3**	BF
33	**Kiambara2**	BF	70	**PSE79**	BF	107	**V4**	BF
34	**kodomoindé**	BF	71	**PSE87**	BF	108	**V5**	BF
35	**Nabaningo**	BF	72	**REE143B**	BF	109	**PSE12**	BF
36	**Nian**	BF	73	**REE144A**	BF	110	**ROE225**	BF
37	**Ouedzouré**	BF	74	**ROE216H**	BF			

BF= Burkina Faso, **CVS=**collection voltaïque de sorgho, **PSE**= prospection Eotype sorgho, **ROE**= région de l'Ouest (Western region) Ecotype, **RSOE=** région du Sud-Ouest Ecotype (Southwestern region), **REE=** région de l'Est Ecotype (Estern region)

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List of accessions used for the molecular characterization

Num	Zone	Sample	Num	Zone	Sample	Num	Zone	Sample
1	AF	B35	39	ER	PSE166	77	WR	PSE196
2	AF	Kenikeje	40	ER	PSE167	78	WR	PSE197
3	AF	Tiandougou	41	ER	PSE168	79	WR	PSE198
4	AF	ET36-1	42	ER	PSE170	80	WR	PSE199
5	AM	RTX7000	43	ER	PSE171A	81	WR	PSE200
6	AM	RTX7078	44	ER	PSE171B	82	WR	PSE201
7	AS	Pahat	45	ER	PSE172A	83	WR	PSE203
8	AS	Samurai1	46	ER	PSE172B	84	WR	PSE204
9	AS	Samurai2	47	ER	PSE173	85	WR	PSE205
10	ER	PSE130	48	ER	PSE174	86	WR	PSE206
11	ER	PSE137	49	ER	REE157	87	WR	PSE207
12	ER	PSE138	50	WR	CVS561	88	WR	PSE208
13	ER	PSE139	51	WR	S34	89	WR	PSE210
14	ER	PSE140	52	WR	Sariaso02	90	WR	PSE211
15	ER	PSE142	53	WR	Sariaso03	91	WR	PSE213
16	ER	PSE143A	54	WR	sariaso04	92	WR	PSE214
17	ER	PSE143B	55	WR	Sariaso06	93	WR	PSE214
18	ER	PSE144	56	WR	Sariaso07	94	WR	PSE215
19	ER	PSE144A	57	WR	Sariaso08	95	WR	PSE216
20	ER	PSE144B	58	WR	Sariaso11	96	WR	PSE217
21	ER	PSE145	59	WR	Sariaso14	97	WR	PSE218
22	ER	PSE146	60	WR	V2	98	WR	PSE220
23	ER	PSE147A	61	WR	V4	99	WR	PSE222
24	ER	PSE147B	62	WR	V5	100	WR	PSE226A
25	ER	PSE148	63	WR	BOROMO LOCAL	101	WR	PSE226B
26	ER	PSE149	64	WR	Gnonfing	102	WR	PSE227
27	ER	PSE150	65	WR	Ouedzoure	103	WR	PSE228
28	ER	PSE152	66	WR	PSE180	104	WR	PSE230
29	ER	PSE153	67	WR	PSE182	105	WR	PSE231
30	ER	PSE154	68	WR	PSE183	106	WR	PSE232
31	ER	PSE155	69	WR	PSE184	107	WR	PSE233
32	ER	PSE158	70	WR	PSE187	108	WR	PSE234
33	ER	PSE16	71	WR	PSE188	109	WR	PSE235
34	ER	PSE160	72	WR	PSE189	110	WR	PSE237
35	ER	PSE161	73	WR	PSE189	111	WR	PSE238
36	ER	PSE163	74	WR	PSE190	112	WR	PSE239
37	ER	PSE164	75	WR	PSE192	113	WR	PSE240
38	ER	PSE165	76	WR	PSE195	114	WR	PSE255

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Num	Zone	Sample	Num	Zone	Sample
115	WR	PSE273	151	SWR	PSE21
116	WR	PSE288	152	SWR	PSE22
117	WR	PSE60	153	SWR	PSE23
118	WR	PSE61	154	SWR	PSE25
119	WR	PSE62	155	SWR	PSE25
120	WR	PSE64	156	SWR	PSE27
121	WR	PSE64	157	SWR	PSE28
122	WR	PSE65	158	SWR	PSE29
123	WR	PSE67	159	SWR	PSE30
124	WR	PSE68	160	SWR	PSE32
125	WR	PSE69	161	SWR	PSE33
126	WR	PSE71	162	SWR	PSE34
127	WR	PSE80	163	SWR	PSE35
128	WR	PSE83	164	SWR	PSE37
129	WR	PSE85	165	SWR	PSE38
130	WR	PSE86	166	SWR	PSE40
131	WR	PSE88	167	SWR	PSE41
132	WR	ROE223	168	SWR	PSE43
133	WR	ROE225	169	SWR	PSE44
134	WR	ROE71	170	SWR	PSE45
135	WR	ROE76	171	SWR	PSE46
136	WR	ROE78	172	SWR	PSE48
137	WR	ROE81	173	SWR	PSE49
138	WR	ROE84	174	SWR	PSE50
139	WR	ROE82	175	SWR	PSE51
140	SWR	PSE06	176	SWR	PSE53
141	SWR	PSE119	177	SWR	PSE54
142	SWR	PSE129	178	SWR	PSE55
143	SWR	PSE13	179	SWR	PSE57
144	SWR	PSE131	180	SWR	ROE56
145	SWR	PSE133	181	SWR	RSOE07
146	SWR	PSE14	182	SWR	RSOE11
147	SWR	PSE15	183	SWR	RSOE31
148	SWR	PSE18	184	SWR	RSOE35
149	SWR	PSE19	185	SWR	RSOE38
150	SWR	PSE20	186	SWR	RSOE39

SWR : South Eastern Region ; WR : Western Region: ER Eastern Region