Genetic Diversity Analysis and QTL Mapping of Selected Traits in Sorghum (*Sorghum bicolor* (L.) Moench)

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DEDICATION

This dissertation is dedicated to my mother for her remarkable sacrifice to raise me up

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ACRONYMS AND ABBREVIATIONS

Add = Additive effect

AFLP = Amplified fragment length polymorphism

AMOVA = Analysis of molecular variance

ANOVA = Analysis of variance

BC = Back cross

Chl = Chlorophyll content

CSA = Central Statistics Agency

CTAB = Cetyltriethylammonium bromide

CV = Coefficient of variation

DAP = Diammonium phosphate

DArT = Diversity Arrays Technology

DH = Double haploid

DMRT = Dunkan's Multiple Range Test

dNTPs = Deoxynucleotide triphosphate

Dom = Dominance effect

EDTA = Ethylenediaminetetraacetic acid

EIAR = Ethiopian Institute of Agricultural Research

FAO = Food and Agriculture Organization of the United Nations

FAOSTAT= the Food and Agriculture Organization Corporate Statistical Database

GBS = Genotyping by sequencing

GPS= Geographical positioning system

GWAS = Genome wide association studies

HARC= Holetta Agricultural Research Center

He = Expected heterozygosity

Ho = Observed heterozygosity

IBC = Institute of Biodiversity Conservation

IBPGR = International Board for Plant Genetic Resource

ICIM = Inclusive Composite Interval Mapping

ICRISAT = International Crops Research Institute for the Semi-Arid Tropics

IM = Interval Mapping

iTOL= Interactive Tree Of Life

LG = Linkage group

LOD = Logarism of the odds

MARC = Melkassa Agricultural Research Center

MCMC = Monte Carlo Markov Chain

METAR = Multi Environment Trial Analysis for R

N_A = Number of alleles

NILs = Near-isogenic lines

NJ = Neighbor Joining

PCA = Principal component analysis

PCR = Polymerase chain reaction

PCs = Principal components

PIC = Polymorphic information content

PVE = Phenotypic variance explained

QTL = Quantitative trait loci

RAPD = Random amplified polymorphic DNA

RFLP = Restriction fragment length polymorphism

RILs = Recombinant inbred lines

SAS = Statistical Analysis System

SBI = Sorghum bicolor chromosome

SD = Standard deviation

SMA = Single Marker Analysis

SNP = Single nucleotide polymorphism

SSR = Simple sequence Repeat

Stg = Stay-green

TASSEL = Trait Analysis by aSSociation Evolution Linkage

TSW = Thousand seed weight

UPGMA = Unweighted Pair-group Method Using Arithmetic Averages

USDA = United States Department of Agriculture

WARC = Werer Agricultural Research Center

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ABSTRACT

*Sorghum (*Sorghum bicolor *(L.) Moench) is one of the most important multipurpose crops grown globally. It can be used as food, feed, fiber and biofuel feedstock. In Ethiopia, sorghum is among the most important cereal crops accounting for 18 % of annual production and 16 % of land cover allocated to the entire grain crops including cereals, pulses and oilseeds. Despite this importance and huge potential as bioenergy feedstock, detailed study on characterization, identification and mapping of novel genes that code for sugar content related and drought tolerance traits using Ethiopian sorghum was lacking. Therefore, this study is aimed at germplasm collection, characterization, and identification and mapping of novel QTLs using both grain and sweet type of sorghum germplasm of Ethiopia. Both morphological and molecular evaluation of Ethiopian sweet sorghum germplasm was undertaken. Genotyping of 175 Ethiopian sweet sorghum genotypes alongside 27 improved accessions from eastern and southern Africa was undertaken. Two independent experiments were also carried out to identify and map QTLs associated to Brix and stay-green related traits using $F_{2:3}$ segregating mapping populations derived from a cross between grain sorghum with stay-green feature (Sorcoll 163/07) and sweet sorghum (Gambella). A genetic map was constructed using 192 F_2 populations genotyped with 76 SSR markers. Research was carried out to screen and compile the most informative SSR markers across some accessions. A total of 304 markers were used to identify the most polymorphic SSR markers across eleven farmers preferred sorghum genotypes. Combined analysis of variance for Brix and other morphological characters to evaluate 181 sweet sorghum accessions collected from the major producing regions of Ethiopia showed that there is a significant effect of the environment on all tested traits. Mean separation analysis revealed that collections from northern Ethiopia were found to be superior in terms of Brix degree. Collections from the rest of collection regions showed relatively low Brix mean value but characterized by higher biomass. Broad sense heritability estimate showed most characters are highly heritable except grain yield. Pearson correlation coefficients (r) of the seven traits presented Brix degree was negatively correlated to most of the traits. Cluster analysis based on the Brix and other morphological characters grouped the accessions into five clusters. The constructed dendrogram based on mean of collection zones for the tested traits also clearly put adjacent regions or zones together. All the tested markers detected 159 alleles and a high degree of polymorphism information content (PIC) averaging 0.69. A comparison between Ethiopian and improved accessions revealed higher allele numbers (124) in Ethiopian than improved accessions (92 alleles). More than half (65 out of 124) of the alleles observed in the Ethiopian accessions were rare (<5%) while 64 were private (only present within Ethiopian accessions) while in the improved accessions, 41% and 38% of the alleles detected were rare and private respectively. Both weighted Neighbor Joining-based clustering and hierarchical clustering grouped the 202 accessions into three major clusters based on geographical origin. Ethiopian accessions from the north (Wello and south Tigray) not only clustered separately from accessions from the west central and eastern Ethiopia, but were also distinct from most of the improved genotypes. A total of seven QTLs distributed across five*

linkage groups that controls Brix content were detected using Inclusive Composite Interval Mapping (ICIM). Each QTL contributed 17.2 to 44.3% of the total phenotypic variation. Using the same genotypic data ICIM detected two stay-green QTLs using $F_{2:3}$ populations evaluated under regulated irrigation supply. Four QTLs distributed on linkage groups SBI-01, SBI-06, SBI-08 and SBI-09 were detected under rain fed drought prone environment. Three QTLs that controls chlorophyll content also detected on SBI-03, SBI-06 and SBI-07. Out of the total SSRs used across eleven farmers preferred sorghum genotypes, nearly half of the markers 139 (45.7%) detected 543 alleles and a high degree of polymorphism information content (PIC) averaging 0.53.The overall observed heterozygosity (Ho) over loci varied from 0.00 to 1.00 with an average of 0.16. Nearly 60 % (83 markers) have showed zero value of the Ho. The gene diversity index (expected heterozygosity, He) ranged from 0.17 to 0.91 with a mean of 0.58. Weighted neighbor-joining cluster analysis grouped the genotypes into three distinct groups. All genotypes with stay-green future (B 35, Sorcoll 163/07, E 36-1 and Sorcoll 141/07) were clustered together. Genotypes such as Gambella, Macia, 76T#23, Meko and Melkam were distinct from the rest of the genotypes and grouped together. The third group consists of Teshale and Sorcoll 146/07. The results from both morphological and molecular based evaluation reveal an unexploited highly diverse sweet sorghum genetic resource from Ethiopia that can be more efficiently included in the regional breeding programs in order to efficiently optimize productivity. Co-localization of stay-green QTL with QTLs for chlorophyll content in two environments has the implication that the two traits were governed by genes found on same linkage group and useful QTLs to be used in breeding program in the future. The current study forms an essential foundation for future grain and sweet sorghum breeding programs of the country as well as for ongoing programs at International Crop Research Institutes for Semi-Arid Tropics.

1. GENERAL INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important grain crop globally after maize, wheat, rice, and barley (FAO, 2007). It is the main staple food crop for more than 500 million people in Africa, Asia and Latin America particularly in semi-arid tropical regions where drought is the major limitations to food production (Gebisa Ejeta, 2005). About two third of sorghum grain produced worldwide is used for human consumption in developing countries. Furthermore, it is the second most important feed grain as well as biofuel crop after maize in the United States (Dahlberg *et al.,* 1995; Paterson, 2008). Besides, sweet sorghum with high sugar content in its stem is considered as a promising crop for forage and silage purposes. Increased demand for limited fresh water supplies, increasing use of marginal farmland, and global climatic trends, all suggest that dryland crops such as sorghum will be of growing importance to feed the world's expanding population (Paterson, 2008). Since sorghum is a C4 grass, it has clear advantages over other grain crops because of its ability to return economic yields in hotter and drier environments (Bryden *et al*., 2009). Under favorable condition, sorghum has a high yield potential as compared to the other major cereals such as rice (*Oryza sativa*), wheat (*Triticum aestivum*) and maize (*Zea mays*) (Reddy *et al*., 2012).

In Ethiopia, sorghum is among the most important cereal crops, particularly in the lowland areas where rainfall is unreliable and crop failures due to recurrent drought are common. It plays a significant role for millions of food-insecure people living in such environments. It is a multipurpose crop because the grain is used to prepare food and beverages, and the juicy sweet stem of sorghum is often chewed by humans particularly in rural Ethiopia. The stem is also used

for various purposes such as animal feed, fencing and fuel for cooking. It can also be a potential source of ethanol production in the country that can be used as biofuel crop in the future.

Despite its huge economic importance, the productivity of sorghum in Africa remains very low (FAOSTAT, 2013). In Ethiopia, the national average sorghum productivity is estimated to be 2.3 tons per hectare (CSA, 2014) whereas the global average is 3.2 tons per hectare (FAO, 2005). Various biotic and abiotic factors contributed to the low productivity of sorghum. Among the abiotic factors, drought is the single most important cause of yield loss in sorghum production all over the world though sorghum is an excellent drought tolerant crop. Drought stress can cause total crop failure. It can devastate sorghum both at vegetative and reproductive growth stages. Hence, drought tolerance is particularly desirable trait in sorghum to sustain the life of several millions of people living in drought prone areas of Ethiopia and elsewhere.

Globally, there is increasing demand for bioenergy related crops because of the uncertainty of the fossil fuel supplies and the fluctuation and unpredicted price of international crude oil. The demand for biofuels is increasing also due to the concern to protect the environment against the expanding CO_2 emission resulted from the utilization of non-renewable energy. The overall advantage of using sweet sorghum crop as biofuel feedstock is very visible. The economic advantage is because of the cost of sweet sorghum production which is three times cheaper than the cost of sugarcane production (Reddy *et al*., 2005). In addition, bioethanol production processing of stem sugar involves shorter chemical reaction steps and minimum energy from primary feedstock to the end product than grain sorghums (Wang *et al*., 2009). Hence, there is imperative to focus on breeding *program*s with the objectives of selecting best varieties that are suitable for biofuel processing as well as improving traits related to biofuel production in sweet sorghum.

Crop improvement program requires understanding and proper assessment of genetic variability among germplasm collections and efficient utilization of such variability in breeding programs. It is an important step toward reliable grouping of accessions and identification of subsets of core accessions with possible utility for specific purpose. Besides, identification and utilization of novel genes adapted to water deficit environments and genes that code for high sugar content is a crucial step towards the development of superior grain and sweet sorghum varieties. Identification of these genes involves either the conventional breeding and/or the modern molecular approaches.

The conventional morphological based characterization alone has its own drawbacks due to limited number of markers which are also influenced by the environment (van Beuningen and Busch, 1997). On the other hand, molecular markers reflect genetic similarity and differences without being influenced by the environment. Furthermore, molecular marker based characterization does not require previous pedigree information (Bohn *et al*., 1999). In general, crop improvement through a conventional breeding method is very slow especially for traits coded by quantitative gene action like drought tolerance and sugar content. Hence, a faster breeding method which guarantees a timely and robust response to these traits is vital. Such breeding method provides an opportunity to develop sorghum varieties with multiple stress tolerance and desirable traits like high sugar content. In this regard, the recent advancement in molecular biology, genomics, gene mapping and bioinformatics in general, provide an excellent opportunity to support sorghum improvement program.

Molecular markers have played an important role in conservation, and use of plant genetic resource trait improvement program of various crops (Aldrich and Doebley, 1992; Whitkus *et al*., 1992; Rami *et al*., 1998; Deu *et al*., 2006; Wang *et al*., 2006; Morris *et al*., 2013). It also supported sorghum improvement programs in many aspects ranging from identification of diverse

lines to mapping of genomic regions controlling desirable traits and their use in marker-assisted breeding programs. Various marker systems such as Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Diversity Arrays Technology (DArT) and single nucleotide polymorphism (SNP) were used in sorghum research (Menz *et al*., 2002; Mace *et al*., 2009; Ramu *et al*., 2009). Simple sequence repeats (SSRs) markers have shown their great potential in detecting the genetic diversity and relationships of organisms due to its allele-specific and co-dominant nature. In addition, SSR markers are the preferred marker system for many sorghum genomics and molecular breeding applications due to its technical simplicity, high throughput level and potential for automation (Caniato *et al*., 2007; Ali *et al*., 2008; Deu *et al*., 2008).

Several morphological and molecular based studies have been carried out for different purposes using Ethiopian sorghum (Brhane Gebrekidan, 1982; Awegechew Teshome *et al*., 1997; Amsalu Ayana and Endashaw Bekele, 1998; 1999; Amsalu Ayana *et al*., 2000, 2001; Tesfaye Tesso *et al*., 2008; Asfaw Adugna *et al*., 2012; Amelawork Beyene *et al*., 2014; Asfaw Adugna, 2014). However, most of these studies focused on grain sorghums or their wild relatives, and there is no report on the intensive study of sweet sorghum germplasms collected from Ethiopia. In addition, no attempt has been done to identify and map markers associated with stay-green QTL from currently screened stay-green materials of Ethiopia though the commonly used stay-green genotypes such as *B 35* and *E 36-1* are initially screened from Ethiopia. Similarly, there are only few studies done globally to identify and map QTL associated with sugar content traits in sorghum using linkage mapping (Guan *et al.*, 2011; Shiringani *et al*., 2010 and Ritter *et al.*, 2008) and association mapping (Murray *et al.,* 2009, Upadhyaya *et al.,* 2012). There was no study

reported regarding QTL mapping of °Brix and related sugar content traits using Ethiopian germplasms.

Ethiopia being the center of diversity for sorghum is a potential area to look for novel genes that encode various relevant traits as was exhibited in identification of stay green genes (*E 36-1, B 35*) and other important traits (Haussmann *et al.,* 2002a; Harris *et al.,* 2007, Reddy *et al*., 2009). Hence, it is important to study sorghum accessions of Ethiopian origin extensively to identify novel genes that could encode traits such as drought, sugar and other related traits using both grain and sweet sorghum germplasms.

1.1. Objectives

1.1.1. Major objective

To study the genetic diversity of Ethiopian sweet sorghum landraces, and map QTLs associated with drought and sugar-related traits.

1.1.2. Specific objectives

I. To characterize Ethiopian sweet sorghum germplasms using °°Brix , morphological and yield traits

II. To study the genetic diversity and population structure of sweet sorghum germplams collected from a range of eco-geographical conditions in Ethiopia, and compare them to global sweet sorghum collections

III. To identify and map QTLs for °Brix trait and other morphological characters from Ethiopian sorghum germplasms

IV. To identify and map QTLs associated with drought tolerance related traits from Ethiopian sorghum germplasms

V. To screen and compile best set of informative microsatellite markers using key Ethiopian sorghum cultivars

2. LITERATURE REVIEW

2.1. Taxonomy

The genus sorghum belongs to the family Poaceae, tribe Andropogoneae, subtribe Sorghinae (Clayton and Renvoize, 1986). A number of authors had attempted to classify sorghum into different category since sixteenth century, however, the first detailed classification as well as clear description of sorghum was reported by Snowden (1936). Snowden (1955) documented that 31 cultivated species and 17 related wild species belong to the genus Sorghum. His study created an excellent opportunity for further taxonomic studies of sorghum. Similarly, detailed studies were conducted during early 1970s based on classification, origin and evolution of sorghum (Harlan and de Wet, 1972; de Wet and Harlan, 1971; de Wet and Huckabay, 1967). According to de Wet (1978), all cultivated, wild and weedy sorghums are grouped under *Sorghum bicolor* which is further classified into three subspecies: *S. bicolor* subsp. *bicolor*, *S. bicolor* subsp. *drummondii*, and *S. bicolor* subsp. *verticilliflorum*.

Cultivated sorghums are classified as *S. bicolor* subsp. *bicolor* which further sub grouped into five basic races and ten intermediate races (de Wet, 1978). The basic races include; (1) *bicolor*, (2) *guinea*, (3) *kafir*, (4) *caudatum*, and (5) *durra*. The race *bicolor* is considered as primitive type. Intermediate races are created by hybridization of these races and reveal characters of both parents. These hybrid races are *guinea-bicolor, caudatum-bicolor, kafir-bicolor, dura-bicolor, guinea-caudatum, guinea-kafir, guinea-durra, kafir-caudutum, durra-caudutum* and *kafir-durra*.

Wild sorghum is grouped under *S. bicolor* subsp. *drummondii* while *S. bicolor* subsp. *verticilliflorum* constitutes hybrid derivatives of cultivated and wild sorghums (De Wet, 1978).

The wild sorghums are further grouped into four varieties such as *S. arundinaceum*, *S. verticilliflorium, S. vergatum* and *S. aethiopicum*.

2.2. Domestication and geographic distribution of sorghum

Sorghum is believed to have been domesticated in Ethiopia and surrounding countries commencing around 4000 – 3000 BC (Dillon *et al*., 2007). There is evidence that the crop was first domesticated in a savanna between Chad and western Ethiopia (Doggett, 1988). This theory is further supported by the presence of a diverse number of wild sorghum relatives in the eastern African region, especially in Sudan and Ethiopia (Brhane Gebrekidan, 1982). Archaeological evidence also indicates that cereal domestication practice was introduced from Ethiopia to Egypt about 3000 BC (Doggett, 1965). It is possible that domestication of sorghum crops was started during that time. The existence of high genetic diversity of wild sorghum crops in Ethiopia (Amsalu Ayana *et al*., 2001; Tesfaye Tesso *et al*., 2008; Asfaw Adugna, 2012) has the implication that the country is most likely the center of origin and diversity.

Nowadays, *S. bicolor* is distributed widely around the world but Africa is the leading continent in terms of cultivated sorghums diversity (Kimber *et al*., 2013). The geographic distribution of the five races is summarized as follows:

***S. bicolor subsp. bicolor race durra**:*

This race is widely cultivated in Arabia and Asia Minor, and durra types are cultivated in India, Burma, along the Nile Valley, and in Ethiopia. There appear to be three centers of morphological diversity: the Ethiopian-Sudan region, the Near East, and India. Among these three centers of diversity, Doggett (1988) argued that the durra is Ethiopian in origin because the entire set of

wild-type *bicolor-durra* crosses is found in Ethiopia. According to Doggett (1988), the distributions of these adapted varieties were from the Horn of Africa through Yemen and Saudi Arabia to India.

S. bicolor subsp. bicolor race bicolor:

The greatest diversity of race *bicolor* is found in Asia but is also widely distributed in Africa. There are some cultivars found only in African, some are strictly Asian, a few occur in Southeast Asia, and some are found on the South China coast. The *bicolor* race is believed to have been originated in Eastern Africa from the variety *aethiopicum* though the high genetic diversity found in Asia which was arose after its introduction to the region (Kimber *et al*, 2013). This is because race *aethiopicum* is distributed throughout Kassala region of the Sudan, more sparsely to the west along the fringes of the Sahara, and in Ethiopia (Kimber *et al*., 2013). Based on the ecology of the wild races, the other wild type *S. verticilliflorum* is also widely distributed throughout the sorghum-growing areas, including the savanna zone of eastern and southern Africa.

S. bicolor subsp. bicolor race caudatum:

The race *caudatum* classification is based on *S. caudatum* and *S. nigricans* of subseries Caffra (de Wet, 1978). These are dominant in parts of Sudan, Chad, Nigeria, and most of Uganda. This race in combination with other races appears to be very important race agronomically. Because *Caudatum* is an old race of sorghum (de Wet, 1978).

The wild race *arundinaceum* is widely distributed in central and West Africa along stream banks which is characterized by wet and humid environment (Kimber *et al*., 2013). This environment is not suitable for the cultivated races.

S. bicolor subsp. bicolor race guinea

This race predominantly cultivated sorghum grown in West Africa region which receive high amount of rainfall annually (Kimber *et al*., 2013). The race is also widely distributed along the mountains of eastern Africa that also receive high amount of rainfall. It is well adapted to wet and humid type of environmental conditions in which other domesticated races fail to adapt. The morphological affinities and distribution indicate that the race *guinea* was probably derived from selection among wild members of the variety *arundinaceum.*

S. bicolor subsp. bicolor race kafir*:*

This race is the most commonly cultivated sorghum covering greater parts of the sorghum growing region of the world. The race is also widely grown in West Africa extending from northern Nigeria west to northern Ghana, where there is a gene flow between the races guinea and kafir (Kimber *et al*., 2013). According to the authors, there is no specific center of origin for race *kafir*, probably due to migrations of the Bantu people. Their migrations since man's earliest history have brought all of the major cultivars into contact. This race appears to be of strictly African origin and its distribution and morphological affinities suggest that it arose from variety *verticilliflorum.*

Sorghum was first introduced into the United States in 1857 suggesting that sorghum cultivation practice in the United States was started very recently though the crop was extensively used in the early 1900s for syrup (Doggett, 1965). Besides, USA is the leading sorghum producer country regardless of the new introduction of the crop.

2.3. Utilization and production of *S. bicolor*

Sorghum is used as human food, animal feed, biofuel feedstock and other industrial products. The larger proportion of produced sorghum in Africa and India is used as human food and beverages, which is estimated to account for about 55% of the annual total production (Kumar *et al.*, 2011). There is an increasing trend of sorghum consumption as human food which was earlier reported to be about 35% of total annual production (Dicko *et al.*, 2006). Sorghum is among the most important cereal crops particularly in food insecure regions and is estimated to be a staple food for more than 500million people in Africa, Asia and Latin America (Gebisa Ejeta and Grenier, 2005; Henley *et al.*, 2010). Sorghum is consumed as whole grain or processed into flour. Mexico and Nigeria rank first and second, respectively in terms of sorghum consumption.

Sorghum is the third important dominant cereal crops of Ethiopia next to corn and teff (*Eragrostis tef*) in terms of area coverage and production (USDA, 2012). Almost the total sorghum production in Ethiopia is consumed domestically either as food such as *Enjera, Kolo, Nifro* and *Genfo* or drinks like, *Tella, Arake, Borde* and so on. In Ethiopia, sorghum residues are fed to livestock and the stems are used as construction materials. It also serves as fuel for cooking.

In the United States and Australia, grain sorghum is mainly used for animal feed while sweet sorghum is used as an important crop to be used as bioenergy feedstock. It has been recognized widely for its high levels of sucrose accumulation in the stem juice which is comparable to sugar cane (Huligol *et al*. 2004). Currently, there is an increased interest in the utilization of sweet sorghum for biofuel production in countries like USA, Brazil and India. It is the best suited for ethanol production because of its higher reducing sugar content as compared to sugar cane. Ethanol can be produced from any sugary or starchy material such as sorghum, sugarcane and

corn. However, sugarcane and corn are very energy and water intensive. For drier climates, sweet sorghum is found to be an ideal crop because it requires limited amount of water. It is a very efficient source of bio-energy as it uses C4 photosynthesis to produce sucrose as a storage molecule, which can be directly fermented. This important trait, along with its suitability for mechanized crop production and seed propagation makes it the best alternative source of raw material for ethanol production. Hence, it is highly imperative to breed new cultivars of sweet sorghum with high sugar content varieties in combination with other desirable agronomic traits.

The United States of America is the leading sorghum producer accounting for about 15% of the world total annual production (FAOSTAT, 2013). The main goal of sorghum production in the United States of America is for the purpose of animal feed and biofuel feedstock. Nigeria and India follow in the second and third position contributing about 13% and 12% of the world annual production, respectively (FAOSTAT, 2013). Over 77% of the total annual world sorghum production is produced by ten countries, with the United States, Nigeria, India and Mexico together contributing about 50% of the world annual production (FAOSTAT, 2013). According to the report, Ethiopia contributes about 5% of total world production. Ethiopia is the third sorghum producing countries in Africa after Nigeria and Sudan.

2.4. Genetic diversity and population structure of *S. bicolor*

Genetic diversity study is the process by which variation among individuals or groups of individuals or populations is analyzed by specific method or a combination of methods. Several approaches ranging from morphological to DNA sequence level analysis can be used to assess the genetic diversity of the crops. In sorghum, a number of studies have been conducted using

morphological data, biochemical approach, DNA based marker data and sequence based diversity analysis.

Though morphological based characterization has its own limitations, it is an important step in complementing with molecular diversity study. Most of these studies focused on cultivated grain sorghum collected from a particular region of the country (Brhane Gebrekidan, 1973; Amsalu Ayana and Endashaw Bekele, 1998; 1999; Durrishahwar *et al*., 2012). Globally, limited studies using morphological based genetic diversity have also been carried out using sweet sorghum and wild sorghum germplasms (Amsalu Ayana *et al*., 2001; Evans Mutegi *et al*., 2009 and Asfaw Adugna *et al*., 2012).

Attempts have been made in using biochemical markers for the diversity study of sorghum. Amsalu Ayana *et al*. (2001) tried to investigate the geographic and altitudinal variation using allozymes among landraces collected from Ethiopia and Eritrea. Similarly, Dje *et al*. (1999) assessed population genetic structure of sorghum landraces from north-western Morocco using allozyme and microsatellite markers.

DNA markers are the most used type of markers as compared to both morphological and biochemical types of markers. This is because DNA markers reveal abundant and neutral variation at the DNA sequence level (Collard *et al*., 2005). In the past and recent years, a number of studies of genomic diversity of sorghum and QTL mapping of some traits have been undertaken using various types of molecular markers, RAPDs (Amsalu Ayana *et al*., 2001; Agrama and Tuinistra, 2003; Nkongolo and Nsapato, 2003; Uptmoor *et al*., 2003), RFLP (Ahnert *et al*., 1996), AFLP (Menz *et al*., 2004), SSRs (Haussmann *et al*., 2002a, Haussmann *et al*., 2002b; Anas and Yoshida 2004; Menz *et al*., 2004; Abu Assar *et al*., 2005; Casa *et al*., 2005;

Dillon *et al*., 2005; Folkertsma *et al*., 2005; Nemera Geleta *et al*., 2006; Barnaud *et al*., 2007; Caniato *et al*., 2007; Ali *et al*., 2008; Deu *et al*., 2008; Murray *et al*., 2009; Shehzad *et al*., 2009; Wang *et al*., 2009; Pei *et al*., 2010; Billot *et al*., 2013; Kapanigowda *et al*., 2013; Ramu *et al*., 2013), DArT (Mace *et al*., 2008; 2009) and SNPs (Zheng *et al*., 2011; Morris *et al*., 2013; Wang *et al*., 2013; Wubeshet Bekele *et al*., 2013). Billot *et al*. (2013) presented a large diversity survey in sorghum with 3367 accessions and using 41 reference nuclear SSR markers. The generation and use of these genomic resources have important implications as it contributes to our insights of sorghum diversity and also enhance the use of global genetic resources. Studies to quantify the amount of diversity within race showed the greatest levels of diversity within *bicolor* and *guinea* races (Deu *et al*., 2006). In contrast, race *kafir* had very limited diversity within the individuals. The finding likely reflects the classical view of this race and its recent origin and restricted geographic distribution.

Population structure refers to the non-random distribution of genotypes among individuals within population i.e., in structured population mating doesn't occur at random. It occurs from the unequal distribution of alleles among subpopulations of different ancestries. Correction for population structure is critical in a collection of genotypes, especially in a breeding program where genetic relationships among breeding lines are highly variable (Sukumaran and Yu, 2014). The study of population structure in *S. bicolor* has been investigated using SSR markers (Wang *et al*., 2009; Billot *et al*., 2013)

2.5. Drought tolerance in *S. bicolor*

In sorghum, drought stress during and after flowering stage results in premature leaf senescence which in turn leads to stalk lodging, stalk rot diseases, and significant yield loss (Rosenow and Clark, 1995). Drought is among the most important abiotic stress limiting crop production and productivity around the world. Drought stress significantly decreases grain yield particularly in the semiarid tropics, where the amount of rainfall is generally low and unevenly distributed throughout the growing period of the crop (Gebisa Ejeta *et al*., 2007). The major cause of leaf senescence is the loss of chlorophyll which results in progressive reduction in photosynthetic capacity. It causes poor grain quality as well as significant yield reduction of crop plants (Xu *et al.*, 2000).

Any mechanism that delays the onset of senescence and maintains the greenness of the leaves throughout the grain filling period will be of paramount importance. There are three general mechanisms of drought resistance in crop plants (Levitt, 1980). These include drought avoidance, tolerance, and escape. Drought avoidance mechanism is associated with the physiology of the plant in which the plant able to maintain cell turgor and cell water content under water-limiting environment. Several mechanisms may be involved like maintaining water uptake by the roots, regulation of transpiration rate by reducing water loss and developing thick waxy cuticle which reduces the loss of water in water-limiting environment. This is the common feature of most sorghum genotypes. Development of deep and massive root system also contribute to drought avoidance mechanism. Drought tolerance mechanism is the ability of the plant to maintain metabolic activity even under drought stress conditions. This can be achieved by regulation of plant water potential that involved accumulation of solutes or ions in their shoots and roots.

Drought escape mechanism is usually associated with early maturing genotypes to avoid drought stress.

The development of drought tolerant genotypes is attracting the interest of many sorghum breeders in order to address the problem of significant yield loss due to water shortage during grain filling period. Identification and testing of drought tolerant germplasms in a plant breeding program, however, is a complex and slow process. This is because drought tolerance is a complex trait controlled by many genes and hence the genetic and physiological mechanisms are not well understood (Gebisa Ejeta *et al*., 2007).

According to Reddy *et al*. (2012), drought stress can be grouped into four. These are: (1) Seedling emergence under deep planting and high temperature; 2) Early seedling drought; (3) Mid-season drought or pre-flowering drought; and (4) Post-flowering/terminal drought. Among these, the most serious and commonly occurring stresses are pre-flowering and post-flowering drought stresses. Therefore, varieties that adapt to these critical environments should be developed and utilized in moisture limited environment. Pre-flowering drought tolerance traits are usually characterized by high leaf photosynthetic rates (Lawlor and Cornic, 2002), reduced canopy temperature, enhanced panicle exertion (Lawlor and Cornic, 2002) and better pollen viability. Pre-flowering drought stress response in sorghum occurs when plants are under significant moisture stress prior to flowering, especially from panicle differentiation or shortly thereafter until flowering. This type of stress directly affects panicle size, grain number and grain yield. Post-flowering drought tolerance traits are characterized by the maintenance of green stems and upper leaves (Rajcan and Tollenaar, 1999; Borrell *et al*., 2000), enhanced rooting depth (Sharp *et al*., 2004; Hoad *et al*., 2001), extended grain filling period, better seed filling rate and improved seed weight (Tuinstra *et al*., 1997; Borrell *et al*., 2000; Harris *et al*., 2007). Post-flowering

drought stress causes premature leaf senescence leading to stalk lodging, stalk rot disease and significant yield loss in sorghum (Rosenow and Clark, 1995). Traits that control post-flowering drought tolerance are usually termed as "stay-green" trait. More than 40 sorghum genotypes which are considered as drought tolerance sources were identified at International Crops Research for Semi-Arid tropics (ICRISAT) (Reddy *et al*., 2012).

Opening and closing of stomata in plants plays an important role in controlling their leaf temperature. When there is high external temperature, the stomata will open and hence more water is moved up from the soil in order to cool the leaves. According to Sinclair *et al*. (2008), limitation on maximum transpiration rate (TR) at high air vapour pressure deficit (VPD) under water limiting environment may improve the yield of the plant. Such kind of traits is also called slow wilting (Sinclair *et al*., 2008).

Sorghum is among the most drought-tolerant grain crops with high genetic diversity for stress tolerance. Sorghum is a C4 plant and successfully adapted to a wide range of environmental conditions ranging from very wet and humid environment to post flowering drought stress (Gebisa Ejeta and Knoll, 2007). Sorghum withstands extreme environment and has the capability of remaining dormant during the driest periods (Gnansounou *et al.,* 2005) as compared to many other crops. The crop is also adapted to high temperature stress and various types of soil and requires low input of production. These unique characteristics make sorghum as an excellent model crops and considered as one of the most important food and feed crops in the arid and semi-arid regions of the world.

Ethiopia is the origin of the major source of stay-green sorghum *E 36-1* and *B 35* (Haussmann *et al.,* 2002b; Harris, 2007). These are excellent drought tolerant varieties known for their stay-

green characters and highly utilized in QTL mapping of drought tolerance traits (Crasta *et al*., 1999; Tuinstra *et al*., 1997; Subudhi *et al*., 2000; Xu *et al*., 2000; Haussmann *et al*., 2002b; Harris, 2007). Development and utilization of additional sorghum varieties adapted to water deficit environments is important for improving and stabilizing crop productivity.

2.6. Brix content in *S. bicolor*

Sweet sorghum which belongs to the same species to that of grain sorghum (*Sorghum biocolor*) is an important feedstock for ethanol production. The crop attracted the energy sector of the world due to its high level of sucrose accumulation in the juicy stem which is a potential source for ethanol production (Vietor and Miller, 1990). Unlike sugar cane, sweet sorghum is more tolerant to moisture stresses and requires very limited amount of water that is nearly one-third of that of sugar cane water requirement (Reddy *et al*., 2013). As compared to the cutting type of propagation used in sugarcane, sweet sorghum is very suitable for commercialization due to its seed based propagation nature. Since it is an annual crop, it can be harvested twice a year as compared to sugar cane which takes about 18 months to mature. Besides, sweet sorghum is adapted to a wide range of agro-ecologies from tropical to temperate climates, from subsistence agriculture to intensive agriculture, making it amenable for cultivation in many parts of the world.

Sweet sorghum accumulates similar level of sugars such as sucrose, glucose and fructose in its stalk to that of sugar cane (Reddy *et al*., 2013). °Brix is commonly used to describe the total soluble solids accumulated in the stem of the sweet sorghum crop. The measured °Brix value helps to estimate the total ethanol yield production from a hectare of land. Murray et al. (2008a) reported that there is high potential of total sugar production up to 13.2 metric tons which is equivalent to7682 liters of ethanol per hectare under favorable environment. Similarily, Miller

and Ottman (2010) measured juice sugars using high performance liquid chromatography (HPLC) and reported an average ethanol yield of 2726 liters per hectare. Zhao et al. (2009) also estimated a high ethanol yields ranged from 3533 to 5414 liters per hectare by measuring total stalk nonstructural sugars (i.e., not expressed juice). Relatively low ethanol yields ranged from 1968 to 2700 liters per hectare was estimated from measured °Brix values for using two sweet sorghum cultivars over 2 years without irrigation (Tamang *et al*., 2011).

Sweet sorghum juices usually contain approximately 16–18% fermentable sugar, which can be directly fermented into ethanol by yeast (Ratnavathi *et al*., 2011) Technical challenges of using sweet sorghum for biofuels are a short harvest period for highest sugar content and fast sugar degradation during storage. According to Ratnavathi *et al*. (2011), as much as 20% of the fermentable sugars can be lost in three days at room temperature because of activities of contaminating bacteria, which lead to significant increases in bacterial count and decrease in pH values. Storage of the fermentable sugar requires well functional refrigerator facilities in order to maintain its actuall PH value, sugar contents, and sugar profiles (Wu *et al*. 2010).

2.7. Sorghum linkage groups

The major idea behind the construction of alinkage map is that the frequency of recombination between two markers can be used as the measure of distance between them on a chromosome. The nearer the two markers on a chromosome, less is the frequency of recombination between them. Therefore, the markers in closeproximity on a chromosome are said to be linked. Each DNA marker occupies a specific position called marker locus on a chromosome. A linkage map is the linear order of DNA markers indicating the positions and relative genetic distances between them on a chromosome.

Genetic linkage maps are an essential component for studying the inheritance of both qualitative and quantitative traits, to develop markers for molecular breeding, for map-based gene cloning and for comparative genomic studies. The most important use of linkage maps is to identify genomic locations associated with the expression of genes or QTLs. In gene/QTL mapping studies, construction of a linkage map for a species is the first step for which development of a mapping population is essential. A suitable mapping population, appropriate marker system, and software for data analyses are key to the construct of a genetic map. Map construction requires (1) selecting the most appropriate mapping population(s); (2) genotyping with polymorphic markers and calculating pair-wise recombination frequencies; (3) establishing linkage groups and estimating map distances; and (4) determining loci order.

Construction of genetic linkage map in sorghum was started in the 1990's using RFLP marker (Hulbert *et al*., 1990). Later, diverse mapping populations and various molecular markers such as RFLPs, AFLPs, SSRs, and DArT were used to construct sorghum linkage maps. Most of these maps were constructued using RFLP (Pereita *et al*., 1994; Xu *et al*., 1994; Boivin *et al*., 1999; Peng *et al*., 1999; Bhattramakki *et al*., 2000; Haussmann *et al*., 2002; Menz *et al*., 2002), SSR (Bhattramakki *et al*., 2000; Kong *et al*., 2000; Menz *et al*., 2002; Wu *et al*, 2007) and DArT markers (Mace *et al*., 2008; 2009). Most of these studies luck common nomenclature system for sorghum linkage groups. In order to address this problem, Kem *et al*. (2005) conducted FISH-based karyotyping in concert with analysis of chromosome lengths, arm lengths, and arm ratios to establish a size-based nomenclature for sorghum chromosomes. They were able to align and orientate the linkage maps relative to the 10 chromosome pairs, and developed nomenclatures for chromosomes (SBI-01 to SBI-10) and linkage groups (LG-01 to LG-10) that are based on sorghum chromosome size.

A consensus genetic map of sorghum that integrates multiple component maps and high-throughput Diversity Array Technology (DArT) markers were found to be very useful to map a larger number of markers than possible in any individual map, to obtain a more complete coverage of the sorghum genome and to fill a number of gaps on individual maps (Mace et al., 2009). Nowadays SNP arrays are publically made available and their utilization in molecular breeding programs are becoming more visible.

2.8. Stay-green QTL mapping in *S. bicolor*

Stay-green is a term used to describe a post-flowering drought tolerant trait that gives plants resistance to premature senescence under severe soil moisture stress. Stay-green usually contributes to high grain yield production as well as yield stability under drought-stress conditions (Xu *et al*., 2000). In sorghum, the best characterized form of drought tolerance during crop growth stage is the stay-green (non-senescence or delayed senescence) under moisture stress which is considered as an important trait for sustaining yield under stress during grain filling (Borrell and Hammer, 2000, Sanchez *et al.,* 2002). In contrast, leaf senescence is the death of plant leaves which depends on the concentration of chlorophyll, the most important green pigment absorbing sunlight energy for photosynthesis. Leaf yellowing generally results from progressive breakdown of chlorophyll during senescence (Tuinstra *et al.,* 1997; Crasta *et al.,* 1999; Tao *et al.,* 2000; Yoo *et al*., 2007). Therefore, chlorophyll content of the leaves has direct implication on the drought tolerance of the plants.

The objective of many genetic mapping studies is to identify QTL that are responsible for phenotypic variation. Quantitative traits are traits that are controlled by many genes and show

continuous variation. Many agriculturally important traits such as yield, drought tolerance, sugar content and disease resistance are controlled by multiple genes. QTL are regions that are associated with a particular quantitative trait in a genome. These loci can be detected using genetic linkage analysis, which indicates the position and relative distance between markers along chromosome, based on the association between phenotype and marker of genotype (Tanksley, 1993). QTL mapping is based on the principle that genes and markers segregate via chromosome recombination during meiosis thus allowing their analysis in the progeny. Genes or markers that are close to each other or tightly-linked will be transmitted together from parent to progeny more frequently than genes or markers that are located further apart.

QTL map requires three major steps such as production of mapping population, identification of polymorphism, and linkage analysis of markers (Collard *et al*., 2005). Different types of mapping populations can be used for the purpose of linkage mapping. These include F_2 mapping population which are the progenies derived from selfing of F_1 individuals. Back cross (BC) populations can be obtained from a cross of F_1 population to the recurrent parents. Doubled haploid (DH) populations are mapping populations that are derived from anther, microspore or ovule culture of a single parent followed by *in vitro* doubling of the chromosomes using chlchicine treatement. These populations represent homozygous lines which are similar to recombinant inbred lines (RILs). The value of DH in plant breeding is in the rapid production of homozygous lines. Those homozygous lines are also evaluated as possible cultivars in the case of self-pollinated crops or as hybrid parents in the case of cross-pollinated crops. Near-isogenic lines (NILs) is another type of mapping population that can be developed by backcrossing the RILs into one of the parents.

The choice of these mapping populations mainly depends on the objective of the research, cost and time required. Highly contrasting parents for the trait of interest should be selected (Collard *et al*., 2005). Up to twelve quantitative trait loci (QTL) for terminal drought tolerance have been mapped and are being utilized to support the breeding program of sorghum (Harris, 2007). So far, various types of stay-green QTLs have been identified and documented (Table 2.1).

Table 2. 1. Some of the mapped stay-green QTLs in *S. bicolor* using different molecular markers (modified from Harris *et al*., 2007)

Type of molecular markers	Number of markers	Mapping populations	No of linkage groups	Type of Identified stay-green	References
AFLPs, RFLPs, SSRs, and RAPD	187	225 RILs	10	Stg1	Haussmann *et al*., 2002b
AFLPs, RFLPs, SSRs, and RAPD	228	226 RILs	12	Stg2, Stg3	Haussmann *et al*., 2002b
RAPD and RFLPs	170	98 RILs	17	Stg1, Stg3	Tuinstra *et al.,* 1996
RFLPs	128	96 RILs	14	Stg2, Stg4	Crasta *et al*., 1999
RFLPs, SSRs, and RAPD	236	98 RILs	10	Stg1, Stg2, Stg3, Stg4	Subudhi *et al*., 2000;
RFLPs and SSRs	311	152 RILs	14		Tao *et al*., 2000
RFLPs	145	98 RILs	10	Stg1, Stg2, Stg3, Stg4	Xu *et al*., 2000
RFLPs	144	125 RILs	10	Stg2, Stg4	Hirut Kebede *et al*., 2001
AFLPs	unknown	98 NILs	10	Stg1, Stg2, Stg3, Stg4	Sanchez *et al*., 2002

NILs = Near-isogenic lines, RILs=Recombinant Inbreed Lines, AFLPs = Amplified Fragment Length Polymorphisms, RFLPs = Restriction Fragment Length Polymorphisms, SSRs = Simple Sequence Repeats, and RAPD = Random Amplified Polymorphic DNA, Stg = stay-green loci

2.9. Sugar-related QTLs mapping in *S. bicolor*

Eventhough sweet sorghum is among the most preferred crops for bioethanol production, little attention has been given to sweet sorghum improvement program in general and identification of important QTLs associated with sugar-related traits in particular. Only limited researches have been done so far to study their genetic diversity (Casa *et al*., 2005; Wange *et al*., 2009), and identify and map QTLs conferring for bio-energy related traits (Casa *et al*., 2008; Ritter *et al*., 2008; Murray *et al*, 2008a, Murray *et al*, 2008b; Shiringani *et al*., 2010). Murray *et al*. (2009) performed association mapping for height and stem sugar (°Brix) traits using information on population structure and relatedness. Attempts have been done based on selected traits derived from contrasting parents such as grain and sweet sorghums to map stem sugar and grain nonstructural carbohydrates and identified 129 QTLs using 27 traits (Murray *et al*., 2008a). Murray *et al*. (2008b) also identified and mapped 110 QTLs controlling stem and leaf structural carbohydrates related traits. Shiringani *et al*. (2010) investigated QTL associated with the sugar components and sugar-related agronomic traits and mapped several QTLs controlling sugar related traits. Similarly, QTL mapping for eleven sugar-related agronomic traits were also conducted (Ritter *et al*., 2008). Most of the identified QTLs were found to be minor and explaining low percentage of phenotypic variation. Recently, association mapping for biomass and plant height traits was carried out using mini core collection of sorghum (Upadhyaya *et al.,* 2012).

2.10. Sorghum genome

Since recently, tremendous effort has been made to build the molecular and genomic foundation to increase our understanding of sorghum diversity in the genome and gene pool. The use of high-density genetic and physical maps (Menz *et al*., 2002; Mace *et al*., 2009), extensive sets of molecular markers for association (Casa *et al*., 2008) and diversity panels (Deu *et al*., 2006) are among the major scientific investigation and breeding efforts. The utilization of these genomic resources is well recognized in sorghum improvement program across the world. Sorghum is considered as the first crop genome of African origin to be sequenced (Paterson *et al*., 2009). It has smaller genome size (730 Mb) as compared to other C4 grasses other than rice (Paterson *et al***.,** 2009). Due to its small genome size combined with its high drought tolerance nature and high level of inbreeding ranging from 94 to 99.4% , sorghum is considered as an attractive model crop for functional genomics and association studies of Saccharinae and other C4 grasses (Hamblin *et al*., 2005; Paterson *et al***.,** 2009; Hariprasanna and Patil, 2015). Nearly 61% of the sorghum genome is characterized by repetitive sequence and contains 27,640 protein coding genes (Paterson *et al***.,** 2009). According to the authors, about 24 % of the genes are grass-specific whereas 7% of the genes are sorghum-specific. Drought tolerance nature of the crop may be as a result of the recent gene and microRNA duplications occurred in the sorghum genome (Paterson *et al***.,** 2009).

The availability of the genome sequence has tremendous importance in the sorghum improvement program particularly in the application of marker assisted breeding. Moreover, the genome sequence information will create an excellent foundation for the improvement of drought tolerance traits of other related cereal crops.

3. MATERIALS AND METHODS

3.1. Characterization of Ethiopian sweet sorghum accessions for °Brix, morphological and yield traits

3. 1. 1. Plant materials

Eventhough the Ethiopian Institute of Biodiversity Conservation is maintaining large number of sorghum accessions collected from various sorghum growing regions of Ethiopia, there was no distinct classification of grain and sweet types. Hence, independent sweet sorghum germplasm collection and characterization was an important step toward the improvement of the crop.

Collection of sweet sorghum germplasms was conducted in the major sweet sorghum cultivation regions of Ethiopia. These include Tigray, north and south Wello, east and west Hararge, west Shewa, east Wollega and Gojam. Geographical coordinates and elevation data were collected for each collection areas using a handheld Geographical Positioning System (GPS), Garmin. Later, coordinate information were used to map the collection regions (Fig. 3.1). A total of 175 sweet sorghum accessions were collected from all major sweet sorghum growing regions of Ethiopia. The passport description of the collected accessions is summarized in Table 3.1. One additional improved sweet sorghum genotype (AS 27) obtained from South Africa and five Ethiopian accessions from different sources were included in this experiment. A total of 181 accessions were used for phenotypic evaluation.

The unequal number of regional representation is due to the limited cultivation practice of the sweet sorghum accessions in some regions. Large numbers of accessions were collected from commonly cultivated regions while few accessions were obtained from regions where sweet sorghum cultivation was not common.

The collected accessions represented the three major agro-ecologies of sorghum (Amsalu Ayana, 2001). Eighty four of them were considered as lowland accessions according to their altitude of collection (< 1600 m.a.s.l.); forty two accessions were categorized as intermediate (1600-1900 m.a.s.l.) whereas the remaining forty nine accessions were grouped as highland accessions (> 1900 m.a.s.l.).

3. 1. 2. Seed multiplication and phenotypic evaluation

The accessions were planted at two locations, Ambo Plant Protection Research Center and Melkassa Agricultural Research Center (MARC) during the offseason as well as rainy season of 2011/12 in order to multiply and purify the seeds. Later, purified lines were planted at two locations: Melkassa Agricultural Research Center (39°21′E, 8°24′N with an altitude of 1550 m.a.s.l.) and its sub center, Arsi Negelle (38 ° 65′ E, 7 ° 35 ′N, with intermediate altitude of 1990 m.a.s.l.).

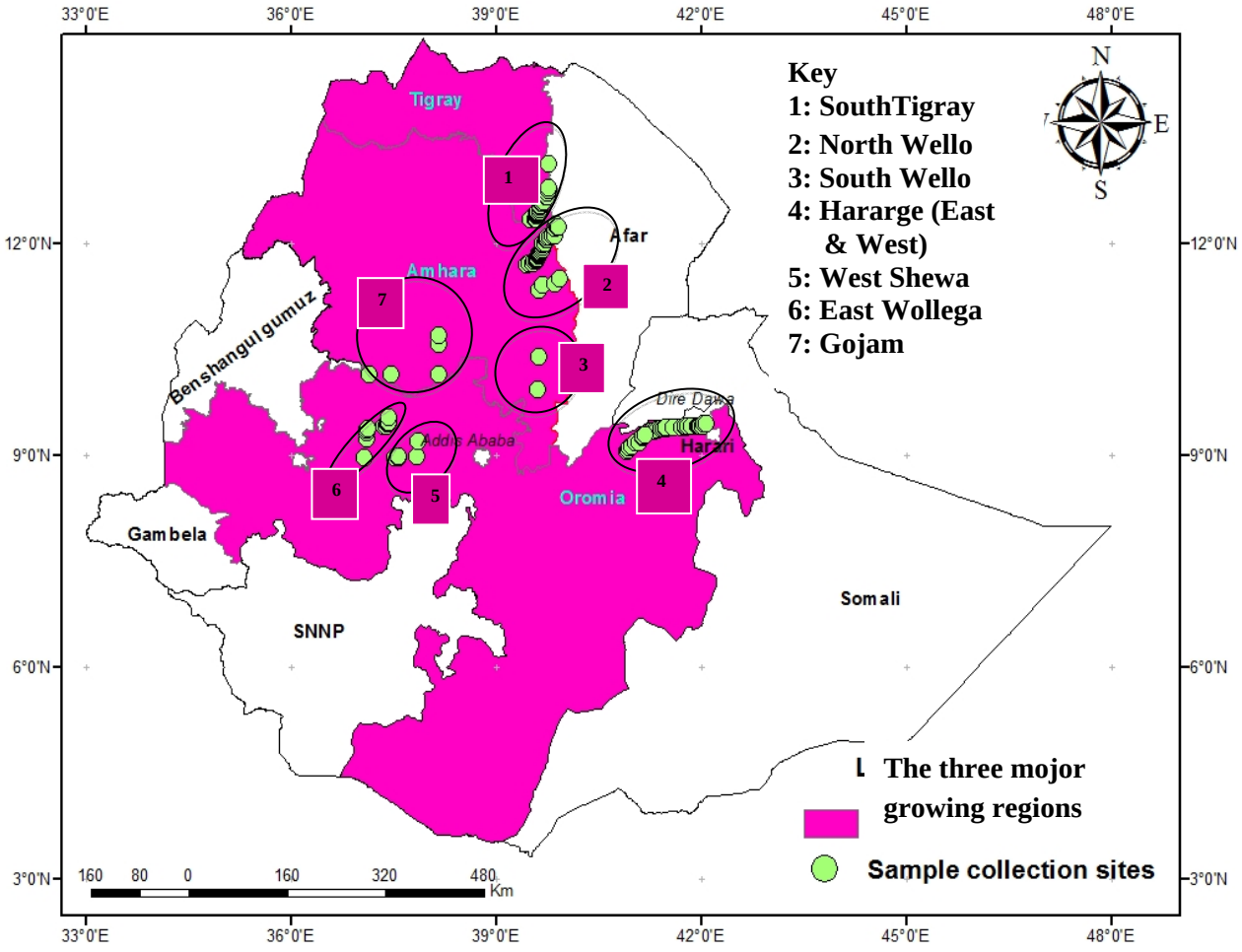


Fig. 3. 1. Map of Ethiopia showing sorghum collection sites and regions (based on geographic coordinate information /data)

Table 3. 1. Passport description of the collected accessions

No.	Accessions	Region	Zone	District	Altitude	Longitude	Latitude
1	Ssorcoll 001/11	Amahara	S.Wello	Kemise	1400	N09°55.459	E039°36.768
2	Ssorcoll 003/11	Amahara	S.Wello	Kemise	1498	N10°22.968	E039°37.333
3	Ssorcoll 004/11	Amahara	S.Wello	Kemise	1501	N10°22.968	E039°37.333
4	Ssorcoll 005/11	Amahara	S.Wello	Haik	1504	N11°19.890	E039°37.597
5	Ssorcoll 006/11	Amahara	S.Wello	Haik	1578	N11°19.890	E039°37.597
6	Ssorcoll 007/11	Amahara	S.Wello	Ambassel	1631	N11°24.213	E039°40.491
7	Ssorcoll 008/11	Amahara	S.Wello	Ambassel	1631	N11°24.345	E039°40.501
8	Ssorcoll 009/11	Amahara	S.Wello	Ambassel	1703	N11°26.014	E039°51.377
9	Ssorcoll 011/11	Amahara	S.Wello	Ambassel	1705	N11°29.440	E039°56.033
10	Ssorcoll 012/11	Amahara	S.Wello	Ambassel	1913	N11°26.015	E039°51.378
11	Ssorcoll 133/11	Amahara	S.Wello	Kalu	1913	N11°29.541	E039°56.134
12	Ssorcoll 013/11	Amahara	N.Wello	Marsa	1329	N11°41.789	E039°26.493
13	Ssorcoll 014/11	Amahara	N.Wello	Habro	1329	N11°42.300	E039°29.184
14	Ssorcoll 015/11	Amahara	N.Wello	Habro	1332	N11°42.300	E039°31.841
15	Ssorcoll 016/11	Amahara	N.Wello	Habro	1332	N11°44.418	E039°33.054
16	Ssorcoll 017/11	Amahara	N.Wello	Woldiya	1400	N11°45.222	E039°35.163
17	Ssorcoll 019/11	Amahara	N.Wello	Woldiya	1406	N11°45.222	E039°35.195
18	Ssorcoll 021/11	Amahara	N.Wello	Woldiya	1409	N11°46.084	E039°35.195
19	Ssorcoll 022/11	Amahara	N.Wello	Woldiya	1436	N11°46.084	E039°35.705
20	Ssorcoll 023/11	Amahara	N.Wello	Woldiya	1450	N11°46.950	E039°35.705
21	Ssorcoll 024/11	Amahara	N.Wello	Woldiya	1452	N11°47.473	E039°36.258
22	Ssorcoll 025/11	Amahara	N.Wello	Woldiya	1457	N11°47.473	E039°36.258
23	Ssorcoll 071/11	Amahara	N.Wello	Kobo	1468	N11°48.627	E039°37.092
24	Ssorcoll 072/11	Amahara	N.Wello	Kobo	1468	N11°48.627	E039°37.171
25	Ssorcoll 073/11	Amahara	N.Wello	Kobo	1481	N11°49.176	E039°37.171
26	Ssorcoll 074/11	Amahara	N.Wello	Kobo	1490	N11°50.096	E039°37.249
27	Ssorcoll 075/11	Amahara	N.Wello	Kobo	1490	N11°50.235	E039°37.628
28	Ssorcoll 076/11	Amahara	N.Wello	Kobo	1495	N11°50.648	E039°37.628
29	Ssorcoll 077/11	Amahara	N.Wello	Kobo	1496	N11°50.648	E039°37.636
30	Ssorcoll 078/11	Amahara	N.Wello	Kobo	1498	N11°50.766	E039°37.636
31	Ssorcoll 079/11	Amahara	N.Wello	Kobo	1507	N11°50.781	E039°38.174
32	Ssorcoll 080/11	Amahara	N.Wello	Kobo	1516	N11°50.905	E039°38.174
33	Ssorcoll 162/11	Amahara	N.Wello	Kobo	1516	N11°52.617	E039°38.205
34	Ssorcoll 081/11	Amahara	N.Wello	Kobo	1550	N11°52.761	E039°38.418
35	Ssorcoll 082/11	Amahara	N.Wello	Kobo	1570	N11°53.028	E039°39.288
36	Ssorcoll 083/11	Amahara	N.Wello	Kobo	1570	N11°53.425	E039°39.297
37	Ssorcoll 084/11	Amahara	N.Wello	Kobo	1610	N11°53.647	E039°39.350
38	Ssorcoll 085/11	Amahara	N.Wello	Woldiya	1631	N11°54.394	E039°40.124
39	Ssorcoll 086/11	Amahara	N.Wello	Woldiya	1634	N11°56.173	E039°40.330

Table 3.1. Continued

No.	Accessions	Region	Zone	District	Altitude	Longitude	Latitude
40	Ssorcoll 087/11	Amahara	N.Wello	Woldiya	1645	N11°58.272	E039°41.145
41	Ssorcoll 088/11	Amahara	N.Wello	woldiya	1645	N12°00.142	E039°41.158
42	Ssorcoll 089/11	Amahara	N.Wello	woldiya	1650	N12°00.142	E039°42.631
43	Ssorcoll 090/11	Amahara	N.Wello	Woldiya	1664	N12°03.270	E039°44.236
44	Ssorcoll 091/11	Amahara	N.Wello	Woldiya	1766	N12°03.270	E039°44.951
45	Ssorcoll 092/11	Amahara	N.Wello	Woldiya	1800	N12°04.842	E039°45.296
46	Ssorcoll 093/11	Amahara	N.Wello	Woldiya	1800	N12°05.267	E039°47.567
47	Ssorcoll 094/11	Amahara	N.Wello	Woldiya	1809	N12°06.505	E039°49.154
48	Ssorcoll 145/11	Amahara	N.Wello	Habro	1876	N12°06.738	E039°49.154
49	Ssorcoll 095/11	Amahara	N.Wello	Habro	1876	N12°06.738	E039°50.938
50	Ssorcoll 096/11	Amahara	N.Wello	Habro	1915	N12°12.905	E039°50.938
51	Ssorcoll 148/11	Amahara	N.Wello	Habro	1956	N12°16.279	E039°52.436
52	Ssorcoll 097/11	Amahara	N.Wello	Woldiya	1971	N12°16.279	E039°52.542
53	Ssorcoll 098/11	Amahara	N.Wello	Woldiya	1973	N12°13.738	E039°53.387
54	Ssorcoll 099/11	Amahara	N.Wello	Woldiya	1974	N12°13.634	E039°53.768
55	Ssorcoll 100/11	Amahara	N.Wello	Woldiya	2245	N12°13.846	E039°54.408
56	Ssorcoll 160/11	Amahara	N.Wello	Woldiya	1976	N12°13.967	E039°54.432
57	Ssorcoll 161/11	Amahara	N.Wello	Woldiya	1954	N12°13.478	E039°54.498
58	Ssorcoll 026/11	Tigray	S.Tigray	Raya Azebo	1375	N12°20.434	E039°29.834
59	Ssorcoll 027/11	Tigray	S.Tigray	Raya Azebo	1401	N12°20.434	E039°34.126
60	Ssorcoll 028/11	Tigray	S.Tigray	Raya Azebo	1401	N12°20.434	E039°35.267
61	Ssorcoll 029/11	Tigray	S.Tigray	Raya Azebo	1407	N12°23.294	E039°35.267
62	Ssorcoll 030/11	Tigray	S.Tigray	Raya Azebo	1439	N12°24.902	E039°35.267
63	Ssorcoll 031/11	Tigray	S.Tigray	Raya Azebo	1439	N12°24.902	E039°35.706
64	Ssorcoll 032/11	Tigray	S.Tigray	Raya Azebo	1450	N12°25.085	E039°35.706
65	Ssorcoll 033/11	Tigray	S.Tigray	Raya Azebo	1466	N12°25.085	E039°36.264
66	Ssorcoll 034/11	Tigray	S.Tigray	Raya Azebo	1466	N12°25.346	E039°36.264
67	Ssorcoll 146/11	Tigray	S.Tigray	Raya Azebo	1471	N12°26.065	E039°36.402
68	Ssorcoll 147/11	Tigray	S.Tigray	Raya Azebo	1471	N12°26.065	E039°36.402
69	Ssorcoll 035/11	Tigray	S.Tigray	Raya Azebo	1480	N12°26.135	E039°36.772
70	Ssorcoll 036/11	Tigray	S.Tigray	Raya Azebo	1480	N12°26.135	E039°36.772
71	Ssorcoll 037/11	Tigray	S.Tigray	Raya Azebo	1482	N12°26.135	E039°38.161
72	Ssorcoll 038/11	Tigray	S.Tigray	Raya Azebo	1482	N12°26.386	E039°38.161
73	Ssorcoll 039/11	Tigray	S.Tigray	Raya Azebo	1482	N12°26.740	E039°38.216
74	Ssorcoll 040/11	Tigray	S.Tigray	Raya Azebo	1490	N12°26.740	E039°38.585
75	Ssorcoll 041/11	Tigray	S.Tigray	Raya Azebo	1490	N12°26.740	E039°38.585
76	Ssorcoll 042/11	Tigray	S.Tigray	Raya Azebo	1493	N12°28.343	E039°38.633
77	Ssorcoll 043/11	Tigray	S.Tigray	Alamata	1493	N12°28.343	E039°38.633
78	Ssorcoll 044/11	Tigray	S.Tigray	Alamata	1493	N12°28.422	E039°38.743

Table 3.1. Continued

No.	Accessions	Region	Zone	District	Altitude	Longitude	Latitude
79	Ssorcoll 045/11	Tigray	S.Tigray	Alamata	1516	N12°28.422	E039°38.743
80	Ssorcoll 046/11	Tigray	S.Tigray	Alamata	1570	N12°30.633	E039°38.752
81	Ssorcoll 047/11	Tigray	S.Tigray	Alamata	1570	N12°30.633	E039°38.789
82	Ssorcoll 048/11	Tigray	S.Tigray	Alamata	1570	N12°30.843	E039°38.789
83	Ssorcoll 049/11	Tigray	S.Tigray	Alamata	1585	N12°32.529	E039°38.973
84	Ssorcoll 050/11	Tigray	S.Tigray	Alamata	1585	N12°32.529	E039°38.973
85	Ssorcoll 051/11	Tigray	S.Tigray	Alamata	1591	N12°33.660	E039°39.120
86	Ssorcoll 052/11	Tigray	S.Tigray	Alamata	1600	N12°33.660	E039°39.120
87	Ssorcoll 053/11	Tigray	S.Tigray	Alamata	1600	N12°33.660	E039°40.543
88	Ssorcoll 054/11	Tigray	S.Tigray	Alamata	1629	N12°34.803	E039°42.107
89	Ssorcoll 163/11	Tigray	S.Tigray	Alamata	1629	N12°35.481	E039°42.107
90	Ssorcoll 055/11	Tigray	S.Tigray	Alamata	1646	N12°35.481	E039°42.107
91	Ssorcoll 056/11	Tigray	S.Tigray	Alamata	1646	N12°39.031	E039°43.943
92	Ssorcoll 057/11	Tigray	S.Tigray	Alamata	1688	N12°39.906	E039°45.251
93	Ssorcoll 058/11	Tigray	S.Tigray	Alamata	1688	N12°39.906	E039°45.273
94	Ssorcoll 059/11	Tigray	S.Tigray	Alamata	1700	N12°42.661	E039°45.273
95	Ssorcoll 060/11	Tigray	S.Tigray	Alamata	1733	N12°42.661	E039°45.273
96	Ssorcoll 061/11	Tigray	S.Tigray	Alamata	1733	N12°44.917	E039°45.772
97	Ssorcoll 062/11	Tigray	S.Tigray	Alamata	1733	N12°44.917	E039°45.772
98	Ssorcoll 063/11	Tigray	S.Tigray	Alamata	1733	N12°46.694	E039°45.786
99	Ssorcoll 064/11	Tigray	S.Tigray	Alamata	1733	N12°46.694	E039°45.800
100	Ssorcoll 065/11	Tigray	S.Tigray	Alamata	1738	N12°47.440	E039°45.814
101	Ssorcoll 066/11	Tigray	S.Tigray	Alamata	1738	N12°47.440	E039°45.828
102	Ssorcoll 067/11	Tigray	S.Tigray	Alamata	1762	N13°06.959	E039°45.842
103	Ssorcoll 068/11	Tigray	S.Tigray	Alamata	1768	N12°47.441	E039°45.856
104	Ssorcoll 069/11	Tigray	S.Tigray	Alamata	1768	N13°06.960	E039°45.870
105	Ssorcoll 070/11	Tigray	S.Tigray	Alamata	1861	N12°47.442	E039°45.884
106	Ssorcoll 150/11	Tigray	S.Tigray	Alamata	1861	N13°06.961	E039°45.898
107	Ssorcoll 151/11	Tigray	S.Tigray	Alamata	2029	N12°47.443	E039°45.912
108	Ssorcoll 152/11	Tigray	S.Tigray	Alamata	1923	N13°06.962	E039°45.926
109	Ssorcoll 154/11	Tigray	S.Tigray	Alamata	1892	N12°47.444	E039°45.940
110	Ssorcoll 155/11	Tigray	S.Tigray	Alamata	1954	N13°06.963	E039°45.954
111	Ssorcoll 156/11	Tigray	S.Tigray	Alamata	1921	N12°47.445	E039°45.968
112	Ssorcoll 158/11	Tigray	S.Tigray	Alamata	1888	N13°06.964	E039°45.982
113	Ssorcoll 159/11	Tigray	S.Tigray	Alamata	1855	N12°47.446	E039°45.996
114	Ssorcoll 101/11	Amahara	W.Gojam	Jabi	1981	N10°07.962	E037°08.348
115	Ssorcoll 102/11	Amahara	W.Gojam	Dembecha	2042	N10°07.962	E037°27.296
116	Ssorcoll 103/11	Amahara	W.Gojam	Dejen	2042	N10°07.962	E038°09.565
117	Ssorcoll 164/11	Amahara	W.Gojam	Dejen	2042	N10°07.962	E038°09.565

Table 3.1. Continued

No.	Accessions	Region	Zone	District	Altitude	Longitude	Latitude
118	Ssorcoll 165/11	Amahara	W.Gojam	Dejen	2042	N10°07.962	E038°09.565
119	Ssorcoll 166/11	Amahara	W.Gojam	Dejen	2042	N10°34.473	E038°09.565
120	Ssorcoll 167/11	Amahara	W.Gojam	Dejen	2093	N10°41.862	E038°09.565
121	Ssorcoll 104/11	Oromiya	E. Hararge	Haromaya	2005	N09°19.761	E041°16.175
122	Ssorcoll 105/11	Oromiya	E. Hararge	Haromaya	2026	N09°20.650	E041°17.794
123	Ssorcoll 106/11	Oromiya	E. Hararge	Haromaya	2026	N09°20.650	E041°17.794
124	Ssorcoll 107/11	Oromiya	E. Hararge	Haromaya	2027	N09°20.824	E041°21.135
125	Ssorcoll 108/11	Oromiya	E. Hararge	Haromaya	2027	N09°20.824	E041°21.135
126	Ssorcoll 109/11	Oromiya	E. Hararge	Haromaya	2027	N09°22.537	E041°25.029
127	Ssorcoll 110/11	Oromiya	E. Hararge	Haromaya	2027	N09°22.537	E041°26.347
128	Ssorcoll 111/11	Oromiya	E. Hararge	Haromaya	2028	N09°22.650	E041°26.347
129	Ssorcoll 112/11	Oromiya	E. Hararge	Haromaya	2029	N09°22.650	E041°29.213
130	Ssorcoll 113/11	Oromiya	E. Hararge	Kersa	2031	N09°22.942	E041°29.213
131	Ssorcoll 114/11	Oromiya	E. Hararge	Kersa	2035	N09°22.968	E041°35.196
132	Ssorcoll 115/11	Oromiya	E. Hararge	Kersa	2038	N09°23.499	E041°42.609
133	Ssorcoll 116/11	Oromiya	E. Hararge	Kersa	2075	N09°23.499	E041°45.715
134	Ssorcoll 117/11	Oromiya	E. Hararge	Kersa	2075	N09°24.088	E041°45.715
135	Ssorcoll 118/11	Oromiya	E. Hararge	Meta	2086	N09°24.098	E041°47.954
136	Ssorcoll 119/11	Oromiya	E. Hararge	Meta	2086	N09°24.211	E041°51.644
137	Ssorcoll 120/11	Oromiya	E. Hararge	Goro Gutu	2086	N09°24.211	E041°56.232
138	Ssorcoll 121/11	Oromiya	E. Hararge	Goro Gutu	2098	N09°24.211	E041°57.417
139	Ssorcoll 122/11	Oromiya	E. Hararge	Goro Gutu	2098	N09°24.211	E041°59.363
140	Ssorcoll 123/11	Oromiya	E. Hararge	Goro Gutu	2241	N09°24.685	E042°01.245
141	Ssorcoll 124/11	Oromiya	E. Hararge	Goro Gutu	2241	N09°26.181	E042°02.324
142	Ssorcoll 125/11	Oromiya	E. Hararge	Goro Gutu	2265	N09°26.213	E042°02.324
143	Ssorcoll 126/11	Oromiya	E. Hararge	Goro Gutu	2265	N09°26.213	E042°02.324
144	Ssorcoll 127/11	Oromiya	E. Hararge	Goro Gutu	2268	N09°26.360	E042°02.324
145	Ssorcoll 128/11	Oromiya	E. Hararge	Goro Gutu	2315	N09°26.417	E042°04.160
146	Ssorcoll 129/11	Oromiya	E. Hararge	Goro Gutu	2504	N09°27.003	E042°02.325
147	Ssorcoll 157/11	Oromiya	E. Hararge	Goro Gutu	2593	N09°26.418	E042°04.161
148	Ssorcoll 130/11	Oromiya	W. Hararge	Doba	1794	N09°03.290	E040°54.445
149	Ssorcoll 131/11	Oromiya	W. Hararge	Doba	2150	N09°04.374	E040°55.563
150	Ssorcoll 132/11	Oromiya	W. Hararge	Doba	2190	N09°06.092	E040°57.077
151	Ssorcoll 133/11	Oromiya	W. Hararge	Doba	2195	N09°07.678	E040°58.879
152	Ssorcoll 134/11	Oromiya	W. Hararge	Tullo	2224	N09°10.185	E041°02.423
153	Ssorcoll 135/11	Oromiya	W. Hararge	Tullo	2245	N09°10.379	E041°05.809
154	Ssorcoll 136/11	Oromiya	W. Hararge	Tullo	2254	N09°15.132	E041°08.084
155	Ssorcoll 137/11	Oromiya	W. Hararge	Doba	2328	N09°15.433	E041°08.698
156	Ssorcoll 138/11	Oromiya	W. Hararge	Chiro	2364	N09°15.433	E041°08.698

Table 3.1. Continued

No.	Accessions	Region	Zone	District	Altitude	Longitude	Latitude
157	Ssorcoll 139/11	Oromiya	W. Hararge	Chiro	2364	N09°15.433	E041°08.698
158	Ssorcoll 140/11	Oromiya	W. Hararge	Chiro	2364	N09°16.861	E041°10.322
159	Ssorcoll 141/11	Oromiya	W. Shewa	Bako	1882	N08°57.930	E037°04.001
160	Ssorcoll 142/11	Oromiya	W. Shewa	Chalia	2109	N08°57.930	E037°30.999
161	Ssorcoll 143/11	Oromiya	W. Shewa	Toke Kutaye	2194	N08°58.175	E037°33.761
162	Ssorcoll 144/11	Oromiya	W. Shewa	Toke Kutaye	2194	N08°58.243	E037°34.208
163	Ssorcoll 168/11	Oromiya	W. Shewa	Ambo	2262	N08°58.549	E037°50.752
164	Ssorcoll 169/11	Oromiya	W. Shewa	Ambo	2302	N08°58.743	E037°50.752
165	Ssorcoll 170/11	Oromiya	W. Shewa	Ambo	2370	N09°11.327	E037°50.996
166	Ssorcoll 171/11	Oromiya	E.Wollega	Horo Guduru	2260	N09°13.875	E037°06.449
167	Ssorcoll 172/11	Oromiya	E.Wollega	Horo Guduru	2271	N09°19.419	E037°06.611
168	Ssorcoll 173/11	Oromiya	E.Wollega	Horo Guduru	2276	N09°20.891	E037°06.612
169	Ssorcoll 174/11	Oromiya	E.Wollega	Horo Guduru	2283	N09°22.396	E037°07.046
170	Ssorcoll 175/11	Oromiya	E.Wollega	Horo Guduru	2289	N09°24.038	E037°21.631
171	Ssorcoll 176/11	Oromiya	E.Wollega	Horo Guduru	2289	N09°24.734	E037°23.318
172	Ssorcoll 177/11	Oromiya	E.Wollega	Horo Guduru	2295	N09°26.081	E037°24.343
173	Ssorcoll 178/11	Oromiya	E.Wollega	Horo Guduru	2305	N09°26.459	E037°25.167
174	Ssorcoll 179/11	Oromiya	E.Wollega	Horo Guduru	2329	N09°27.874	E037°25.381
175	Ssorcoll 180/11	Oromiya	E.Wollega	Horo Guduru	2376	N09°32.017	E037°25.497

3. 1. 3. Experimental design and data collection

A total of 181 accessions including checks were planted in single row. An α-lattice design with three replications was used in both experimental locations. Each plot consisted of 5 m length spaced at 0.75 m. The spaces between replications were 2.0 m while spaces between blocks were 1.5 m. Planting was undertaken manually followed by thinning to 0.2 m spacing after 20 days of emergence (i.e., the space between plants was 0.2 m). After thinning each plot consisted of an average of 25 plants. Five plants from each row were randomly selected based on the type of traits to take measurements for the trait of interests. Recommended amount (100 kg/ha) of diammonium phosphate (DAP) was applied during planting whereas the same amount of urea was supplemented as a split application following the procedure used by Amsalu Ayana (2001). All the necessary agronomic practices were followed. Occurrence of pests was also regularly monitored. A minimum of five plants were selected immediately after flowering and the heads were covered with suitable and durable paper bags in order to protect from neighboring pollen contamination. This helps to fix and purify the seeds that can be used further in breeding program in the future. The panicles were further covered with selected cloth bags designed for the protection of seeds against devastating bird (*Quelea quelea*) damages.

The most important traits used in bioethanol improvement program are sugar content, biomass and yiled related traits. Therefore, data for seven quantitative traits: days to flowering, days to maturity, plant height, stalk diameter, grain yield, thousand seed weight and °Brix content were collected. All morpholgical and yeld related traits were collected based on sorghum agro-morphological descriptor as summarized in Table 3.2. In order to measure °Brix content, stalks were harvested two to three weeks before maturity stage. Stem juice was manually extracted and

°Brix was measured using a digital hand refractometer (TEC++ DR.VOLKER SCHMIDT GM BH, Deutschland). A volume of one to two drops of the extracted juice was used for °Brix measurement.

Table 3. 2. Sorghum agro-morphological descriptor (IBPGR and ICRISAT, 1993)

No	Parameter	Description of the character
1	Days to 50 % flowering (number of days)	The number of days from planting to when approximately 50% of the plants in a plot reached half flowering stage.
2	Days to maturity (number of days)	Recorded as the number of days from planting to when seeds on 50% of the plants in a plot exhibited black layer on the lower third of the panicle.
3	Plant height (cm)	The length of the plant from the ground to the panicle tip at physiological maturity
4	Stalk diameter (mm)	Stalk diameter was measured 20 cm above ground using digital caliper (World Precision Instruments, Shanghai Trading Co., Ltd).
5	Grain yield (g/plant)	Measured as the total weight of the grain harvested from each plot (from five randomly selected plants)
6	Thousand seed weight (g)	Measured as the weight of one thousand kernels from bulk seeds from all randomly selected heads in each plot. Seed counter was used to count the seeds

3. 1. 4. Morphological data analysis

Morphological diversity among and within accessions was studied through visual observation of based on the size, shape, compactness and color of the panicle.

Analysis of variance across the entire accessions was undertaken using SAS version 9.2 (SAS Institute, 2008). The PROC GLM procedure was used to estimate variance for all traits. All factors were treated as random variables. Normality of the data for the seven traits for combined locations was determined using the Minitab version 16 statistical software (Minitab Inc., 2010). Similarly, other descriptive statistics such as mean, standard deviation and percent coefficient of variation (CV) of the seven traits across the entire sweet sorghum accessions were analyzed using SAS software version 9.2.

In order to know whether there is significant variability for °Brix degree along the stalk position, experiment was carried out at three internodes of each plant representing bottom, mid and upper part of the plant. The height of the plant was measured by excluding the final internode found at the tip of the plant and divided to three equal portions. °Brix was manually extracted from each node of the stalk and measured separately using handheld digital refractometer. The °Brix values were compared using the mean value of 112 randomly selected accessions. Each line was represented by four randomly selected plants. Mean separation was conducted using Duncan's Multiple Range Test (Gomez and Gomez, 1984) implemented in SAS.

Broad-sense heritability was computed using METAR (Multi Environment Trial Analysis for R) Version 2.1 software. The Pearson correlation coefficient was also calculated for every pair of traits using the PROC CORR procedure. The correlation for each character was performed using

the mean values of all accessions as well as the mean value of collection regions following the procedure used by Amsalu Ayana (2001). The correlation of traits based on the entire accessions and geographic based classification were compared.

Cluster analysis, based on Euclidean distances as similarity measures and the unweighted pair-group method with arithmetic averages (UPGMA) was used to determine the genetic relationship among accessions. Dendrogram was constructed for both accessions as well as collection regions using the aforementioned method using SAS version 9.2. Principal component analysis considering eigenvalues and percentage variations was computed after transformation of the mean values of each trait using Minitab version 16 statistical software (Minitab Inc., 2010). This helped to determine the level of principal component and the major contributing traits among the selected traits contributing to the phenotypic variation of the accessions.

3. 2. Genetic diversity and population structure of Ethiopian sweet sorghum collections using SSR markers

3. 2. 1. Plant materials

A total of 175 sweet sorghum accessions collected from all major sweet sorghum growing regions of Ethiopia described in Table 3.1 were used for this study. Three additional improved genotypes Gambella, Sorcoll 163/07 and AS27 were included. Gambella is one of farmers'prefered variery released by EIAR in 1970s and obtained from Melkassa Agricultural Research Center (MARC). It is characterized by sweety stalk where as Sorcoll 163/07 is grain type and characterized by its drought tolerance (stay-greeness). The seeds were provided by Addis Ababa University through Bio-Innovate Project. A total of 24 improved sweet sorghum materials either released or lined up for release in the eastern and southern Africa were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi (Table 3.3).

Table 3. 3. Sweet sorghum genotypes obtained from the International Crops Research Institute for the Semi-Arid Tropics-Nairobi

No.	Accessions	Source	No	Accessions	Source
1	ICSB 324	ICRISAT-India	13	SDSL 90167	ICRISAT-Zimba.
2	ICSB 654	ICRISAT-India	14	SPV 422	ICRISAT-India
3	IESV 91104 DL	ICRISAT-Nairobi	15	IS 2331	South Sudan
4	IESV 92001 DL	ICRISAT-Nairobi	16	SPV 1411	ICRISAT-India
5	IESV 92008 DL	ICRISAT-Nairobi	17	ICSR 93034	ICRISAT-India
6	IESV 92021 DL	ICRISAT-Nairobi	18	E 36-1	Ethiopia
7	IESV 92028 DL	ICRISAT-Nairobi	19	ICSV 93046	ICRISAT-India
8	IESV 92165 DL	ICRISAT-Nairobi	20	ICSV 700	ICRISAT-India
9	Kari Mtama 1	KARI-Kenya	21	S 35	ICRISAT-India
10	IESV 91018 LT	ICRISAT-Nairobi	22	104GRD	ICRISAT-India
11	IESV 93042 SH	ICRISAT-Nairobi	23	Ent#64DTN	ICRISAT-India
12	IESV 92038 SH	ICRISAT-Nairobi	24	NTJ2	India

3. 2. 2. DNA extraction

After seed multiplication, seeds were sown in a greenhouse at Holetta Agricultural Research Center (HARC) of the Ethiopian Institute of Agricultural Research, while global collections were grown at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT-Nairobi). Leaf tissues were collected from two to three-week-old seedlings (Fig. 3.2) followed by genomic DNA extraction using Promega Kit (Madison, USA) and CTAB method for the Ethiopian and global collections respectively. Quantity and quality of the DNA was checked

using spectrophotometer and by running on 0.8% agarose gel stained with GelRed® (Biotium, USA), respectively. The final volume of the extracted DNA was diluted to the concentration of 10 ng/µl to be used for polymerase chain reaction (PCR).

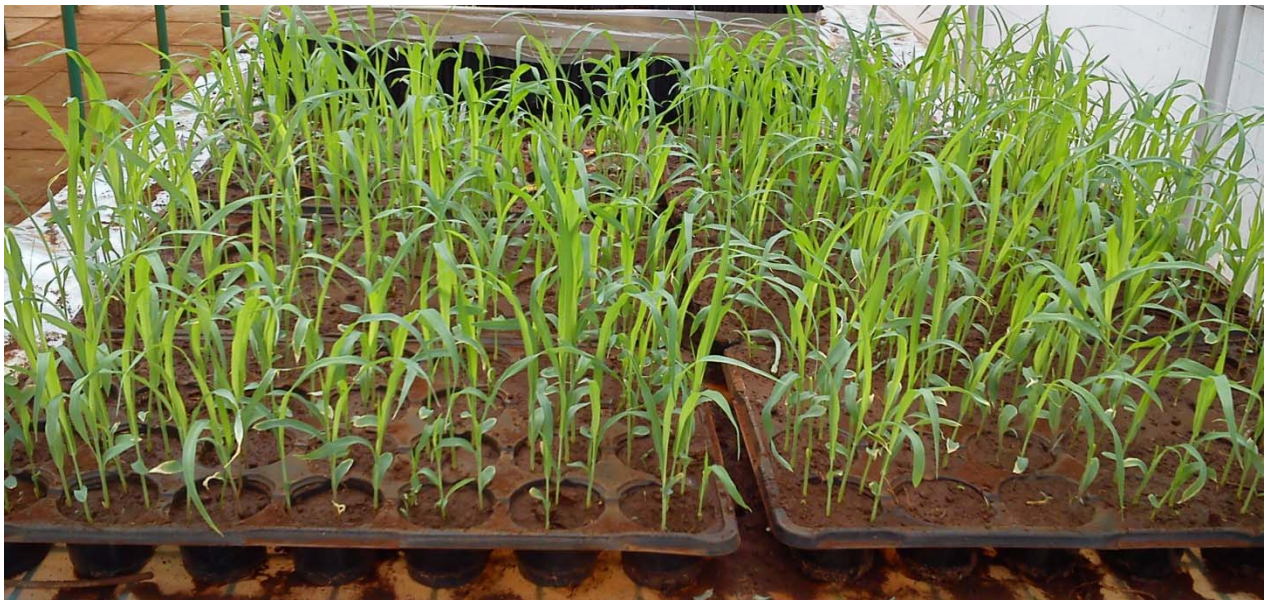


Fig. 3. 2. Two-week-old sweet sorghum seedlings ready for DNA extraction

3. 2. 3. Polymerase chain reaction and fragment analyses

Sixteen polymorphic SSR primers that were evenly distributed across the whole sorghum nuclear genome were selected for use in genotyping the 202 accessions. Three primers were excluded because of their poor amplification quality and the remaining thirteen primer pairs were listed in Table 3.4. All forward primers contained an M13-tag (5'- CACGACGTTGTAAAACGAC - 3') on the 5' end that was fluorescently labeled to allow detection of amplification products (Schuelke, 2000). PCR amplification was performed in 10 µl reaction volume comprising of 1 x PCR buffer (20 mM Tris-HCl, pH 7.6; 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 0.5% (w/v) Triton X-100; 50% (v/v) glycerol), 2 mM $MgCl_2$, 0.16 mM dNTPs, 0.16 µM fluorescent labeled M13-forward primer, 0.04 µM forward primer, 0.2 µM reverse primer, 0.2 units of Taq DNA polymerase (SibEnzyme Ltd, Russia) and 30ng template DNA. Forward primers were labeled with FAM, PET, NED or VIC (Applied Biosystems, USA). PCR was carried out in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems) programed for initial denaturation at 94°C for 15 min, followed by second denaturation at 94°C for 30 sec, annealing at 50°C for 1 min, extension at 72°C for 2 min and final elongation at 72°C for 20 min.

Table 3.4. List of selected primer pairs that succefully amplified the genomic DNA of sweet sorghum

Marker	Forward primer	Reverse primer	Repeat	LG
Xisep0108	GTACGTTCCCCATCCTTCCT	CTCCTGTTCTCTCCGCATTC	(GGC)5	SBI-08
Xisep0617	GGCTGGGAGAGCTAGGAAGA	GACGGCTCGTCCATCATC	(GATC)3	SBI-06
Xisep0938	TGCTGTTCTTGAACGTGTTTG	TTTTGCACAAAGTTGCGTGT	(TGGGT)6	SBI-02
Xisep1029	GACCCTCCTCCTCAACCACT	CATGCATGCACAAGCAGATT	(GCAT)3	SBI-05
Xtxp141	TGTATGGCCTAGCTTATCT	CAACAAGCCAACCTAAA	(GA)3	SBI-10
Xtxp205	CCTGCCGTGTCTTCC	TATATGCATGCCGTAGATTT	(GA)12	SBI-03
Xtxp211	TCAACGGCCAATGATTTCTAAC	AGGTTGCGAATAAAAGGTAATGTG	(CT)23	SBI-02
Xtxp284	CCAGATTGGCTGATGCATACACACT	AAGGGTAATTTATGCACTCCAAGGTAGGAC	(AG)5(GA)28	SBI-01
Xtxp012	AGATCTGGCGGCAACG	AGTCACCCATCGATCATC	(CT)22	SBI-04
Xtxp279	ATTCTGACTTAACCCACCCCTAAA	AGCTCATCAATGTCCCAAACC	(CTT)10	SBI-01
Xtxp312	CAGGAAAATACGATCCGTGCCAAGT	GTGAACTATTCGGAAGAAGTTTGGAGGAAA	(CAA)26	SBI-07
Xcup33	GCGCTGCTGTGTGTTGTTC	ACGGGGATTAGCCTTTTAGG	(AT)7	SBI-01
Xgap342	TGCTTGTGAGAGTGCCTCCCT	GTGAACCTGCTGCTTTAGTCGATG	(AC)25	SBI-07

LG = Linkage group

Successful amplification was confirmed by running 2.0 µl of the PCR products on a 2% (w/v) agarose gel stained with GelRed® (Biotium) and visualized under UV. Depending on the nature of fluorescent label and strength of the amplification bands used, a volume ranging from 2.5µl to 3.5µl of four different amplification products were co-loaded along with the internal size standard, GeneScan™ –500 LIZ® (Applied Biosystems) and Hi-Di™ Formamide (Applied

Biosystems, USA). The fragments were separated by capillary electrophoresis using an ABI Prism® 3730 Genetic analyzer (Applied Biosystems). PCR fragment sizes were manually scored using GeneMapper 4.0 software (Applied Biosystems). Power Marker v.3.25 (Liu and Muse, 2005) was used to compute PIC, heterozygosity (*Ho*) and gene diversity (expected heterozygosity, *He*) for each marker as well as the average across markers. Rare (<5%), common (5-50%), abundant (>50%) and private alleles (alleles only present in one group and not the other) were manually counted in MS Excel (Microsoft Inc., Seattle, USA) for improved (ICRISAT, Sorcoll163/07, Gambella and AS27) and Ethiopian accessions. Polymorphism information content (PIC) was calculated using the method of Botstein *et al.,* (1980).

$$PIC = 1 - \sum_{i=0}^{k} p_i^2 - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2\, p_i^2 p_j^2$$

Where, p_i and p_j are the frequencies of alleles *i* and *j*, respectively

Markers with a PIC value of more than 0.5 were considered highly informative, between 0.25 and 0.5 as informative and less than 0.25 as less informative.

Heterozygosity at a single locus was computed as:

$$H_l = 1 - \sum_{i=1}^{k} P_i$$

Where P_i = i^{th} allele frequency

Similarly, gene diversity was estimated using the following formula

$$H_e = (1 - \sum_{i=1}^{n} P_{il}^2)/(1 - \frac{1+f}{n})$$

Where Pi = i^{th} allele frequency

f = inbreeding coefficient

n = number of individuals

3. 2. 4. Phylogenetic analysis

To identify the pair-wise genetic relationship between accessions, a genetic dissimilarity matrix was analyzed using Neighbor Joining (NJ) method, as implemented in DARwin v5 (Perrier and Jacquemoud-Collet, 2006).

$$d_{ij} = 1 - \frac{1}{L}\sum_{i=1}^{L}\frac{mi}{\pi}$$

Where d_{ij} = dissimilarity between units i and j

L = number of loci

π = ploidy

mi = number of matching alleles for locus i

The dendrogram was constructed using online tree generator software called Interactive Tree Of Life; iTOL v.2 (Letunic and Bork, 2011). Genetic distance between groups was estimated by F_{ST} statistics. F statistics parameters were performed as follows based on the method of Wright (1951).

$$F_{ST} = (F_{IT} - F_{IS}) / (1-F_{IS})$$

Where,

F_{ST} is the fixation index describing the correlation of genes of different individuals in the same population;

F_{IS} is the inbreeding coefficient, describing the correlation of genes within individuals in the population;

F_{IT} is the overall inbreeding coefficient, describing the correlation of genes within individuals relative to the total population.

Analysis of Molecular Variance (AMOVA) was determined using Arlequin 3.11 software (Excoffier *et al.,* 2005). A genetic dissimilarity matrix was calculated using pair wise F_{ST} in order to determine pair-wise genetic relationships among regions. Principal component and coordinate analysis were computed using TASSEL v.3 (Bradbury *et al.,* 2007).

3. 2. 5. Population structure

Two complementary approaches, Bayesian clustering and Neighbor-Joining tree, were used to assess the genetic structure without defining a-priori populations. The genetic structure of sweet sorghum populations was determined using the admixture model based on Monte Carlo Markov Chain (MCMC) algorithm implemented in program STRUCTURE 2.3.3 software (Pritchard *et al.,* 2000). The admixture model with correlated allele frequencies was used, assuming that the genome of each individual resulted from the mixture of *K* ancestral populations. The estimated proportions of each individual's genotype originating from each of the *K* ancestral populations (q) was calculated for *K* ranging from 1 to 12 ancestral populations (or clusters), with fifteen runs for each *K* value. For each run, a burn-in period of 50000 and MCMC replications of 10000 was

used. The optimum *K* value was calculated using structure harvester http://taylor0.biology.ucla.edu/structureHarvester/, which computed the log likelihood of the data [LnP(D)] in the STRUCTURE output and an *adhoc* statistic *Δk* based on the rate of change in LnP(D) between successive *k* (Evanno *et al.,* 2005). Results from each replicate run were combined using the CLUMPP software (Jakobsson Rosenberg, 2007). The results of both the Bayesian clustering and Neighbor-Joining methods were then compared to check for consistency of the clusters.

3. 3. Identification and mapping of QTLs associated with sugar content and drought tolerance from Ethiopian sorghum collections

3. 3. 1. Identification and mapping of QTLs associated with °Brix content and biomass related traits

3. 3. 1. 1. Development of mapping populations

F_2 mapping populations were developed by crossing one of the selected grain sorghum (*Sorcoll 163/07*) as a female parent and sweet sorghum (*Gambella*) as a pollen source. *Sorcoll 163/07* was as inbred line characterized by drought tolerance potential with an average plant height (224.5 cm), stalk diameter (15.5 mm) and °Brix (6.8 %). *Gembella* is farmers preferred genotypes released as improved variety by EIAR and characterized by its sweet stalk with an average plant height (192.3 cm), stem diameter (14.0 mm), and °Brix (14.4 %).

Both parents were planted at Melkassa Agricultural Research Center during main rainy season in 2012. The field was supplied with irrigation as the area experiences frequent rain failure. All the necessary agronomic management practices were implemented. Crossing was carried out using hand pollination techniques with great care. The heads were tightly covered with appropriate paper bag in order to avoid contamination from neighboring pollen. Sufficient seeds from the cross were harvested and used to produce F_1 plants. Selfing of F_1 populations was undertaken at Werer Agricultural Research Center (WARC) during offseason in the same year using irrigation facilities. The seeds from F_1 populations were harvested and planted to produce F_2 mapping population for sugar-related traits and identify markers that are closely linked to the traits. A total of 200 F_1 seeds were planted in greenhouse of Holetta Agricultural Research Center, Ethiopia in

order to produce F_2 plants from which genotyping was undertaken. In order to test in multiple environments, each F_2 plant was selfed to produce F_2 derived F_3 plants ($F_{2:3}$).

3. 3. 1. 2. Experimental design and phenotypic evaluation

Seeds were harvested from 192 F_2 lines. Planting was undertaken using an α-lattice design with three replications across two locations: Melkassa Agricultural Research Center and Dhera Research Station. A single row per plant with a row length of 5 m was used. A total of 25 seeds per row were planted. The space between the lines and plants were 0.75 m and 0.2 m, respectively. The spaces between replications were 2.0 m while 1.5 m spacing was used to separate blocks. All the necessary agronomic practices were followed. Occurrence of pests and birds was also regularly monitored.

Phenotyping of sugar and biomass related traits like stem juice (°Brix %), stalk diameter and plant height were undertaken for the entire replications and both experimental locations. (1) The juice was extracted manually from five randomely selected plants just one to two weeks before complete maturity of the plant. One to two drop of the extracted juice was used to measure °Brix using a digital hand refractometer (TEC++ DR.VOLKER SCHMIDT GM BH, Deutschland). (2) stalk diameter was measured 20 cm above ground using digital caliper (World Precision Instruments, Shanghai Trading Co., Ltd) and (3) plant height measurement was undertaken as the length of the plant from the ground to the panicle tip at physiological maturity.

3. 3. 1. 3. Phenotypic data analysis

Phenotypic value and statistical anlaysis such as mean, variance, standard error, skewness, kurtosis, minimium, maximium and range were computed using Minitab version 16 (Minitab Inc.,

2010) and SAS software version 9.2 (SAS inst. 2008). Broad-sense heritability was computed using METAR (Multi Environment Trial Analysis for R) Version 2.1 software. The Pearson correlation coefficient was calculated for every pair of traits using the PROC CORR procedure implemented in SAS.

3. 3. 1. 4. Polymorphism screening

Leaf tissues from greenhouse-grown seedlings of *Sorcoll 163/07* and *Gambella* varieties were used for SSR analysis. Genomic DNA from these genotypes was extracted from leaf tissue using Promega Wizard® Genomic DNA Purification Kit (Madison, USA). Quantity and quality of the DNA was measured using spectrophotometry and by running on 0.8% agrose gel stained with GelRed® (Biotium, USA), respectively. The final volume of the DNA was diluted to the concentration of 30 ng/µl to be used for polymerase chain reaction (PCR).

Polymorphic markers were selected from previously published sorghum SSR markers (Brown *et al.,* 1996; Bhattramakki *et al.,* 2000; Kong *et al.,* 2000; Menz *et al.,* 2002; Wu *et al.,* 2006; Li *et al.,* 2009; Ramu *et al.,* 2009). A total of 304 sorghum SSR primers (Appendix 1) which are distributed across the ten linkage groups were selected to screen polymorphic markers across the parental lines. PCR was performed in a 10 µl reaction volume containing 3 µl DNA, a total of 30 ng/µl template DNA, 1x taq buffer, 2.0 mM $MgCl_2$, 0.16 mM each deoxynucleotide triphosphates (dNTPs), 0.16 pmol each fluorescent label, 0.04 pmol forward primer, 0.2 pmol reverse primer and 0.2 u Amplitaq. Forward primers were labeled with FAM, PET, NED or VIC. PCR was carried out in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, USA) programed for initial denaturation at 94 °C for 15 min, followed by second denaturation at 94 °C

for 30 sec, annealing at 50 °C for 1 min, extension at 72 °C for 2 min and final elongation at 72 °C for 20 min.

3. 3. 1. 5. Genotyping of F_2 mapping populations

Young leaves were collected from greenhouse grown seedlings of 192 F_2 populations. The same methodology used for genomic DNA extraction and quantification used in polymorphism screening across the two parents was used.

Eighty one SSR markers that showed polymorphism among the parents, *Sorcoll 163/07* and *Gambella*, were selected for genotyping the entire progenies and parents. Polymerase chain reaction and detection of PCR products were performed following the same procedure used in parental polymorphism screening. Fragment Scores were recorded as 2, 0 and 1 for P1 (*Sorcoll163/07)*, P2 (*Gambella)* and F1 (*heterozygous)*, respectively. Missing values were scored as –1 for each marker.

3. 3. 1. 6. Linkage map construction

Based on 76 successfully scored markers, the linkage map was constructed using IciMapping software v 4.0 (Li *et al*., 2007; http://www.isbreeding.net/software/?type=detail&id=3). The linkage groups identified was considered as linked if the logarithm of odds (LOD) score was greater than 3.0. Map distances (in Centi-Morgan units) was calculated using the Kosambi mapping function (Kosambi, 1944). The relationship between genetic distance in cM (*m*) and recombination frequency (*r*) is given by

$m = 25 \ln(\frac{1+2r}{1-2r})$ Or $r = \frac{1}{2}\frac{e^{4m}-1}{e^{4m}+1}$

The linkage map was compared and assigned to chromosomes according to sorghum censuses map (Mace *et al*., 2009).

The whole polymorphism screening of parents and F_2 genotyping activities were undertaken at ICRISAT-Nairobi, Kenya whereas DNA extraction was carried out at Holetta Molecular Biology Laboratory of the Ethiopian Institute of Agricultural Research (EIAR), Ethiopia.

3. 3. 1. 7. QTL analysis

LS means of each of the traits were used for QTL mapping. However, only traits that showed significant mean value difference between the progenies as well as the two parents were used in identifying the QTLs. Missing phenotypic data was scored as -100.00. Based on 70 SSR markers that were successfully assigned to their specific linkage groups and mean phenotypic data collected for the mapping populations, QTL identification and mapping for °Brix trait was undertaken. QTL analysis was computed using phenotypic data collected from each location separately followed by combined analysis using IciMapping software v 4.0. Relatively higher LOD threshold (3.5) was used in order to minimize false positive result. Inclusive Composite Interval Mapping (ICIM) implemented in ICIMapping software was used to run analysis.

3. 3. 2. Identification and mapping of QTLs associated with stay-green and yield related traits

3. 3. 2. 1. Phenotypic evaluation

Two ideal sites, Werer Agricultural Research Center and Dhera research station, were selected to screen the lines for drought tolerance. Werer Agricultural Research Center is located in Eastern Ethiopia, and is characterized by very high temperature with maximum temperature of 40.8 °C and minimum 26.7 °C and dry climate with annual average rainfall of 590 mm. Similarly, Dhera Research Station is mainly known for its high daily mean temperature and dry land semi-arid climate with a rainfall of less than 400 mm per annum, i.e. April to August. The station has silty loam soil type with a pH of nearly 7.8.

The same experimental design used in °Brix phenotypic evaluation was also used in this experiment. The seeds were planted at Werer Agricultural Research Center under irrigation with a regulated water supply. Irrigation was terminated immediately after flowering. Each row was labeled and characterized for its stay-green features (phenotyped) using appropriate descriptors (Table 3.5).

Table 3. 5. Description of the parameters for stay-green and related traits in sorghum

No.	Parameter	Description of the character
1	Chlorophyll content	Chlorophyll content was measured using Chlorophyll meter, SPAD-502 (Minolta Co. Ltd, Japan).
2	Leaf senescence (stay-greenness)	Recorded on a scale of 1-5, 1 being least senescing and 5 most senescing at physiological maturity
3	Plant height	The length of the plant from the ground to the panicle tip at physiological maturity
4	Panicle width (cm)	The average width of the panicle at its widest section
5	Panicle length (cm)	The length between the base and tip of a panicle
6	Panicle weight (g)	The weight of the un-threshed head
7	Grain yield (g/plant)	Grain yield in gram from each plant. The average of five plants was used.
8	Thousand seed weight (g)	Weight in gram of individual sample of 1000 seeds (gm)

The experiment was repeated in 2014 main season in order to validate the data in a drought prone area under normal rain fed condition using F_2 derived F_3 families. Seeds were planted at Dhera research station using the same design as in the previous year.

Normality and frequency distribution test was performed for each of the traits using Minitab version 16 statistical software (Minitab Inc., 2010) followed by analysis of variance in order to test any significant variation among progenies as well as the two parents using SAS version 9.2 (SAS Institute, 2008). LS means of traits calculated for each mapping populations were used in QTL analysis. Each phenotypic trait such as stay-green, chlorophyll content, plant height, panicle width, panicle length, panicle weight, thousand seed weight and grain yield was tested for any QTL associated to them both independently as well as in combined form.

3. 3. 2. 2. Genotyping of F_2 mapping populations

The markers selected for °Brix QTL analysis was also segregating for stay-greenness so that the same genotypic data was used to detect stay-green and related traits. The already constructed linkage map was also used for the QTL analysis of stay-green and other traits.

3. 3. 2. 3. QTL identification and analysis

QTL mapping for stay-green and related traits were undertaken. Due to its complexity of stay-green traits, a LOD threshold of 2.0 was used to confirm the presence of a QTL in a given genomic region for all stay-green related traits. Both Interval Mapping (IM) and Inclusive Composite Interval Mapping implemented in ICIMapping software were used and their outputs were compared.

3. 4. Screening and compiling of informative microsatellite sets for marker-assisted breeding of key Ethiopian sorghum cultivars

3. 4. 1. Plant materials

Eleven Ethiopian and introduced improved sorghum genotypes were used to screen and compile informative microsatellite sets that can be used for marker-assisted breeding of Ethiopian sorghum cultivars. These genotypes were *Teshale, Gambella, Meko, Melkam, Macia, 76T#1, B 35, E 36-1, Sorcoll 141/07, Sorcoll 146/07* and *Sorcoll 163/07*. They were selected for their essential traits such as: early maturity, seed quality, high yield, high sugar content and drought tolerance. *B 35* and *E 36-1* are useful sources of stay-green trait worldwide.

3. 4. 2. DNA extraction

Seeds of eleven selected sorghum genotypes were sown in a greenhouse at Holetta Agricultural Research Center (HARC) of the Ethiopian Institute of Agricultural Research. Leaf tissues were collected from two to three-week-old seedlings. Leaf tissues from five plants per genotype were bulked followed by genomic DNA extraction using Promega Kit (Madison, USA) and CTAB method (Mace *et al.,* 2003) in order to compare the two protocols for sorghum genotyping. Quantity and quality of the DNA was checked using *Qubit®2.0* (*Life Technologies***,** Grand Island, NY) and by running on 0.8% agarose gel stained with GelRed® (Biotium, USA), respectively.

3. 4. 3. Polymerase Chain Reaction and Fragment Analysis

A total of 300 polymorphic SSR primers that were evenly distributed across the whole sorghum nuclear genome were selected for use in genotyping the eleven genotypes. The markers were selected from previous reports (Brown *et al*., 1996; Bhattramakki *et al*., 2000; Kong *et al*., 2000;

Menz *et al*., 2002; Wu *et al*., 2006; Li *et al*., 2009; Ramu *et al*., 2009). All forward primers contained an M13-tag (5'- CACGACGTTGTAAAACGAC - 3') on the 5' end that was fluorescently labeled to allow detection of amplification products (Schuelke, 2000). PCR amplification was performed in 10 µl reaction volume comprising of 1 x PCR buffer (20 mM Tris-HCl, pH 7.6; 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 0.5% (w/v) Triton X-100; 50% (v/v) glycerol), 2 mM $MgCl_2$, 0.16 mM dNTPs, 0.16 µM fluorescent labeled M13-forward primer, 0.04 µM forward primer, 0.2 µM reverse primer, 0.2 units of Taq DNA polymerase (SibEnzyme Ltd, Russia) and 30 ng of template DNA. Forward primers were labeled with FAM, PET, NED or VIC (Applied Biosystems, USA). PCR was carried out in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems) programed for initial denaturation at 94°C for 15 min, followed by second denaturation at 94°C for 30 sec, annealing at 50°C for 1 min, extension at 72°C for 2 min and final elongation at 72°C for 20 min.

Successful amplification was confirmed by running 2.0 µl of the PCR products on a 2% (w/v) agarose gel stained with GelRed® (Biotium) and visualized under UV. Depending on the nature of fluorescent label and strength of the amplification bands used, a volume ranging from 2.5µl to 3.5µl of four different amplification products were co-loaded along with the internal size standard, GeneScan™ –500 LIZ® (Applied Biosystems) and Hi-Di™ Formamide (Applied Biosystems, USA). The fragments were separated by capillary electrophoresis using an ABI Prism® 3730 Genetic analyzer (Applied Biosystems). PCR fragment sizes were manually scored using GeneMapper 4.0 software (Applied Biosystems). Power Marker v.3.25 (Liu and Muse, 2005) was used to compute PIC, heterozygosity (*Ho*) and gene diversity (expected heterozygosity, *He*) for each marker as well as the average across markers. Polymorphism information content (PIC) was calculated using the method of Botstein *et al.* (1980).

$$PIC = 1 - \sum_{i=0}^{k} p_i^2 - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2\, p_i^2 p_j^2$$

Where, p_i and p_j are the frequencies of alleles *i* and *j*, respectively

3. 4. 4. Phylogenetic analysis

To identify the pair-wise genetic relationships among accessions, a genetic dissimilarity matrix was analyzed using Neighbor Joining (NJ) method, as implemented in DARwin v5 (Perrier and Jacquemoud-Collet 2006). The dendrogram was also constructed using the same software.

4. RESULTS

4. 1. Characterization of Ethiopian sweet sorghum accessions for °Brix and other morphological parameters

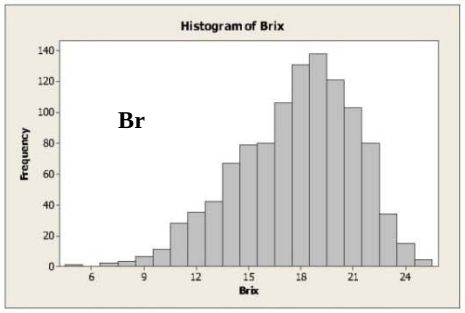
4. 1. 1. Visual classification and trait performance

Throughout the collection areas, high diversity of sweet sorghum was observed in terms of panicle color, compactness, shape and size among and within accessions (Fig. 4.1A). However, highly diverse sweet sorghum within the same locality was observed in collections from northern Ethiopia (Fig. 4.1B). Collections from a single farm in southern Wello (Fig. 4.1B) showed high level of panicle variation in terms of size, shape and color. However, there is no variation in terms of panicle compactness. Collections from Gojam, east Wollega and west Shewa showed similar type of panicles in terms of color, shape, compacteness and size.

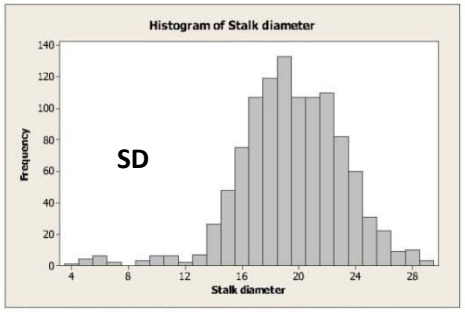
According to interviewed farmers, the sweetness of the stalk also varies from cultivar to cultivar. A number of sweet sorghum cultivars have been maintained by local farmers. Selection is mainly based on sweetness of the stalk and generally cultivated for chewing purposes. In northern Ethiopia, local cultivars such as *Hawaye, Jamiyo, Gegebsa, and Yiker Selat* were among the most preferred cultivars by the local communities for their sweetness of the juicy stalk. Some of these cultivar names tell the degree of the sweetness and preference for chewing. These cultivars were also showed high °Brix mean value as compred to other collections. Unlike the diversity of sweet sorghum cultivars in northern Ethiopia, farmers from other regions (Gojam, east Wollega and west Shewa) are cultivating a single cultivar. Similarly, farmers from east and west Hararge are using few sweet sorghum cultivars

Result from frequency distribution analysis showed that almost all the tested accessions of sweet sorghum had normal distribution for the seven traits measured with slight skewness for grain yield, days to 50 % flowering and stalk diameter traits (Fig. 4.2 and Table 4.1). The computed coefficient of variation (CV) for six tested traits is fairly small ranged from 3.83 to 13.02 % (Table 4.1). However, slightly higher CV was observed for grain yield (30.97 %).

Fig. 4. 1. Diversity in panicle color, shape, size and compactness among (A) and within (B) Ethiopian sweet sorghum accessions collected from different regions. (A) representing sorghum panicle collected from various regions while (B) representing collections from a single farm from southern Wello area.

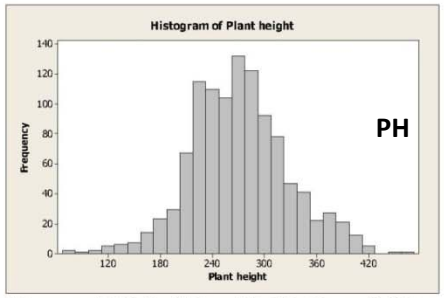


Mean = 17.7, SD = 3.34, P = <0.05

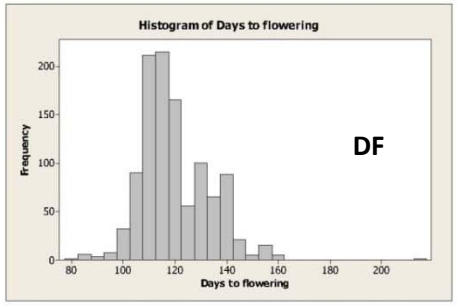


Mean = 19.5, SD = 3.63, P = <0.05

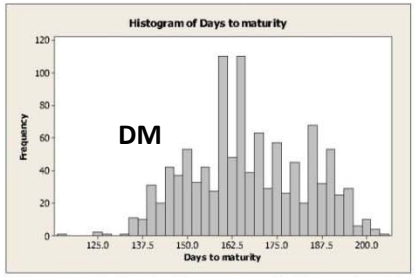
Quantitative traits	*Units*
Brix	*%*
Stalk diameter	*mm*
Plant height	*cm*
Days to flowering	*Number of days*
Days to maturity	*Number of days*
Grain yield	*g*
Thousand seed wt	*g*



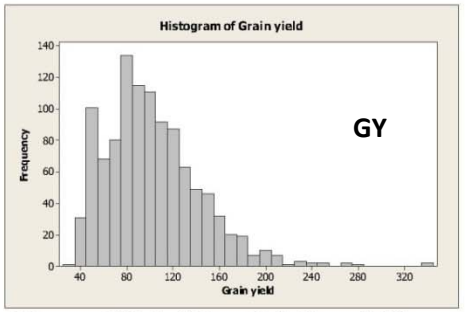
Mean = 270.3, SD = 55.63, P = <0.05



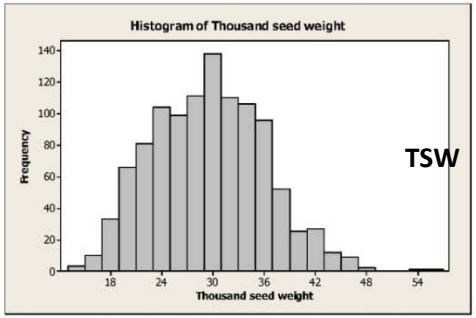
Mean = 119.6, SD =13.3, P = <0.05



Mean = 167.1, SD = 16.1, P = <0.05



Mean = 101.5, SD = 41.1, P = <0.05



Mean = 29.47, SD = 6.52, P = <0.05

Fig. 4. 2. Frequency distribution of the seven traits in 181 sweet sorghum accessions evaluated at combined locations (MARC and Arsi Negelle experimental sites). Where, Br = °Brix , GY = grain yield, PH = plant height, SD = stalk diameter, DF = days to flowering, TSW = thousand seed weight, DM = Days to maturity

Table 4. 1. Variance and descriptive statistics for seven traits of 181 sweet sorghum accessions of Ethiopia

Variable	Mean	Standard Error	Standard deviation	Variance	CV (%)	Skewness	Kurtosis
°Brix (%)	17.72	0.10	3.34	11.15	12.25	-0.48	-0.13
Stalk diameter (mm)	19.51	0.11	3.63	13.15	9.99	-0.60	1.87
Plant height (cm)	270.29	1.69	55.63	3094.53	12.90	0.12	0.48
Days to 50 % flowering (days)	119.63	0.40	13.25	175.49	4.51	0.84	2.15
Days to Maturity (days)	167.09	0.49	16.09	259.00	3.83	0.05	-0.71
Grain yield (g/plant)	101.48	1.25	41.09	1688.76	30.97	1.11	2.53
Thousand seed weight (g)	29.45	0.20	6.53	42.59	13.02	0.23	-0.13

°Brix and other traits characteristics

°Brix (%)

Significant variation was observed for °Brix degree among the collections ranging from 11.8 to 22.5 % with a mean value of 17.7 %. Significant variation for °Brix value was also observed among the regions of collections (Table 4.2). Collections from northern Ethiopia (south Tigray, north Wello and south Wello) showed highest °Brix values and significantly different from the rest of the region such as Hararge, west Shewa, east Wollega and Gojam. However, there was no significant difference within themselves (Table 4.2). The °Brix values for collections from south Tigray ranged from 12.8 to 22.4 % with a mean value of 18.2 %. Similarly, the lowest value for north Wello collections was 15.3 % while the highest °Brix value was 22.5 % with mean value of

19.2 %. Collections from south Wello also showed similar °Brix mean value (19.2 %) with the highest and lowest percentage of 21.7 and 13.5 %, respectively. In contrary, collections from Hararge, west Shewa, east Wollega and Gojam regions showed significantly lower °Brix mean value ranged from 15.1 % (west Hararge) to 16.8 % (east Wollega) as compared to collections from northern Ethiopia. There was no significant difference among accessions collected from eastern Ethiopia, west Shewa and east Wollega for °Brix content. In general, the highest °Brix value (22.5 %) was recorded from accessions collected from north Wello whereas the lowest value was obtained from accessions originated from east and west Hararge (11.8 % each).

Stalk diameter and plant height

An average of 19.51 mm stalk diameter was obtained across the accessions. The highest mean for stalk diameter was measured from accessions collected from west Hararge (23.4 mm) followed by east Hararge (22.8 mm), east Wollega (20.2 mm) and west Shewa (20.1 mm). In contrary, the lower mean value for thickness were observed in collection of north Wello (18.1 mm) and south Tigray (18.2 mm). There was no significant difference between accessions collected from west Shewa (20.1 mm), east Wollega (20.2 mm), east Hararge (22.8 mm) and west Hararge (23.4 mm) for stalk diameter character though they are found to be significantly thicker than the rest of collections. Collections from south Wello (19.0 mm) and Gojam (19.2 mm) area showed intermediate thickness as compared to south Tigray (18.2 mm) and north Wello (18.1mm) accessions which were thinner than the rest of the collections.

The tallest individual accession was collected from east Hararge (351.1 cm) while a mean score of 293.5 cm obtained for the entire entire accessions collected from east Hararge region. This mean value was similar to the mean value of accessions from Gojam (278.7 cm), west Shewa

(297.4 cm), east Wollega (293.7 cm) and west Hararge (282.3 cm).These accessions showed no significant difference for plant height. Collections from northern regions of Ethiopia were characterized as shorter accessions and significantly different from accessions collected from east Wollega, west Showa and east Hararge areas.

Days to flowering and maturity

Accessions from south Tigray, north Wello and south Wello were categorized as early flowering as well as maturing type and significantly different from the rest of the accessions (Table 4.2). In contrary, those collections representing the eastern Ethiopia (Hararge) were among the late flowering and maturing type while collections from east Wollega, Gojam and west Shewa grouped as intermediate type.

Yield and yield components

Both the lowest and highest grain yield was obtained from accessions from north Wello. However, there was no significant difference among the collections for grain yield trait except collections from east Wollega areas which showed the least amount of grain yield. The highest and significant mean value for thousand seed weight was obtained for accessions collected from north Wello (31.7 g), south Wello (31.3 g), south Tigray (30.5 g) and east Hararge (29.3 g). In contrary, collections from Gojam (24.4 g), east Wollega (22.7 g) and west Shewa (22.4 g) showed significant and lowest mean value for thousand seed weight trait.

Table 4. 2. Comparison of accessions based on the mean of their region of origin using seven selected traits

Parameter/ Region		°Brix (%)	Stalk diameter (mm)	Plant height (cm)	Days to flowering (days)	Days to maturity (days)	Yield (g/plant)	Thousand seed weight (g)
south Tigray	High	22.4	24.4	317.6	142.1	190.0	154.3	41.7
	Low	12.8	11.4	195.1	88.0	132.6	43.7	22.6
	Mean	**18.2^{a}**	**18.2^{c}**	**258.5^{b}**	**113.6^{d}**	**159.9^{c}**	**101.3^{ab}**	**31.3^{a}**
north Wello	High	22.5	22.5	316.9	119.5	169.2	160.9	38.3
	Low	15.3	9.3	152.6	100.1	140.3	42.3	31.4
	Mean	**19.2^{a}**	**18.1^{c}**	**258.3^{b}**	**112.4^{d}**	**158.7^{c}**	**98.1^{ab}**	**31.7^{a}**
south Wello	High	21.7	21.7	320.8	116.5	167.6	156.6	42.1
	Low	13.5	17.3	224.7	106.8	151.8	82.3	20.3
	Mean	**19.2^{a}**	**19.0^{bc}**	**266.2^{b}**	**112.9^{d}**	**159.2^{c}**	**113.0^{a}**	**30.5^{ab}**
Go jam	High	17.4	22.3	340.0	131.0	179.3	128.1	27.7
	Low	13.2	19	268.2	117.3	166.4	72.0	19.5
	Mean	**15.9^{bc}**	**19.2^{bc}**	**278.7^{ab}**	**121.7^{c}**	**171.5^{b}**	**91.5^{ab}**	**24.4^{cd}**
east Wollega	High	18.3	21.9	323.9	136.8	186.1	129.8	26.7
	Low	13.2	18.3	268.1	121.4	169.7	68.5	18.8
	Mean	**16.6^{bc}**	**20.2^{a}**	**293.7^{a}**	**128.0^{b}**	**177.5^{b}**	**87.8^{b}**	**22.7^{d}**
west Shewa	High	20.7	20.8	314.0	132.7	185.6	128.9	23.8
	Low	14.2	19.1	282.1	116.0	167.7	62.9	19.6
	Mean	**16.8^{b}**	**20.1^{a}**	**297.4^{a}**	**125.0^{bc}**	**175.5^{b}**	**95.8^{ab}**	**22.4^{d}**
east Hararge	High	20.5	25.9	351.1	149.2	195.3	170.6	40.9
	Low	11.8	17.2	199.1	113.0	157.6	38.3	21.9
	Mean	**15.4^{bc}**	**22.8^{a}**	**293.5^{a}**	**135.3^{a}**	**183.9^{a}**	**106.0^{ab}**	**29.3^{ab}**
west Hararge	High	18.6	24.9	313.0	138.9	190.8	153.2	37.9
	Low	11.8	19.2	265.2	136.5	188	97.5	23.5
	Mean	**15.1^{c}**	**23.4^{a}**	**282.3^{ab}**	**134.8^{a}**	**185.3^{a}**	**111.9^{a}**	**27.4^{bc}**

Means with the same letter within column are not significantly different at $P \leq 0.05$

Genotype by environment interaction

Result from combined analysis of variance showed that the accession effect was highly significant for all the studied traits (°Brix degree, stalk diameter, plant height, days to 50% flowering, days to maturity, grain yield and thousand seed weight) (Table 4.3). Similarly, the effect of environment and accession by environment interaction was highly significant for all the traits. Replication effect was also highly significant for traits such as stalk diameter, plant height, days to maturity and grain yield. Besides, highly significant effect among blocks was obtained for all the traits except grain yield and thousand seed weight.

Table 4. 3. Combined analysis of variance for seven traits across 181 sweet sorghum accessions evaluated at two locations

		Mean squares						
Source of variation	Degrees of freedom	°Brix	Stalk diameter	Plant height	Days to 50 % flowering	Days to maturity	Grain yield	Thousand seed weight
Environment	1	96.13**	2094.81**	1024145.95**	7653.49**	29384.16**	103540.35**	1289.54**
Replication	2	0.65	37.87**	6366.38**	60.84	622.43**	4878.25**	5.94
Block	21	12.37**	8.31**	2627.69**	87.23**	151.18**	1264.76	10.69
Accessions	180	29.57**	37.98**	5084.01**	637.74**	843.23**	3346.56**	142.67**
Environment x accessions	180	13.53**	8.00**	1807.31**	91.49**	165.40**	2002.52**	32.01**

** Significant at P < 0.01

4. 1. 2. Comparison of stalk position for °Brix degree

Result obtained from mean separation analysis showed that juice extracted from each part of the stalk had similar mean value for °Brix degree (Table 4.4). However, the juice extracted from the mid part of the stalk showed significantly higher mean °Brix percentage as compared to the bottom part of the stalk ($P \leq 0.05$). In contrary, there was no significant difference among the means using the juice extracted from the bottom and upper position of the stalk. Therefore, juice extraction for °Brix measurement in this experiment was done using the bottom and mid parts of the stalk in order to avoid overestimation or underestimation of the °Brix mean value.

Table 4. 4. Mean comparison of the stalk position for °Brix value.

Part of the stalk used	°Brix mean value (%)
Upper	17.1^{ab}
Mid	17.7^{a}
Bottom	16.6^{b}

Means with the same letter within column are not significantly different at $P \leq 0.05$, n = 112

4. 1. 3. Trait heritability and correlation

The highest broad-sense heritability was obtained from days to 50 % flowering (88.8%) followed by days to maturity (87.0 %) and stalk diameter (82.0 %) traits. °Brix , plant height and thousand seed weight showed 61.1 %, 68.7 % and 79.9 % of broad-sense heritability, respectively. Grain yield showed the least broad-sense heritability (42 %).

Pearson correlation coefficients (r) of the seven traits are shown in Table 4.5. °Brix content showed negative correlation with the rest of the traits except thousand seed weight (Table 4.5).

The strongest negative correlation (r = -0.582, P < 0.001) was also obtained between °Brix and days to maturity, followed by days to 50 % flowering (r = -0.576, P < 0.001), stalk diameter (r = -0.479, P < 0.001) and plant height(r = -0.267, P < 0.001). However, it was positively associated with thousand seed weight (r = 0.404, P < 0.001). Significant strong negative correlation between °Brix trait and altitude (r = -0.543, P < 0.001) was also obtained in this experiment.

Stalk diameter showed positive correlation with traits such as days to 50 % flowering (r = 0.797, P < 0.001), days to maturity (r = 0.816, P < 0.001) and grain yield (r = 0.304, P < 0.001). In contrary, it is negatively correlated with thousand seed weight (r = -0.236, P < 0.001). The trait is also positively correlated with altitude ((r = 0.485, P < 0.001).

There was also significant association between plant height, and days to 50 % flowering (r = 0.501) and days to maturity (r = 507). The strongest positive correlation was between days to 50% flowering and days to maturity (r = 0.980) whereas the strongest negative correlation was between thousand seed weight and days to maturity (r = -0.450) followed by days to 50 % flowering (r= -0.450).

The correlation of quantitative traits based on their mean values computed from each collection region also showed relatively stronger significant correlation as compared to the correlation computed based on the entire accessions (Table 4.6). The highest negative correlation was observed between °Brix trait and some other traits such as days to 50% flowering (r = -0.922, P < 0.001), days to maturity (r = -0.908, P < 0.001) and stalk diameter (r = -0.887, P < 0.001) while the highest positive correlation was obtained between days to 50% flowering and days to maturity (r=0.992, P < 0.001) followed by stalk diameter with days to 50% flowering (r=0.949, P < 0.001) and days to maturity (r=0.932, P < 0.001).

Table 4. 5. Correlation among traits of sweet sorghum accessions from Ethiopia. The correlation coefficient was computed using the mean values of the traits for each of 181 accessions

Traits	°Brix	Stalk diameter	Plant height	Days to 50% flowering	Days to maturity	Grain yield	Thousand seed weight	Altitude
°Brix	1.000							
Stem diameter	-0.479***	1.000						
Plant height	-0.267***	0.598 ^{NS}	1.000					
Days to 50% flowering	**-0.576***	0.**797***	0.**501***	1.000				
Days to maturity	**-0.582***	0.**816***	0.**507***	0.**980***	1.000			
Grain yield	-0.099^{NS}	0.304***	0.318***	0.073 ^{NS}	0.103 ^{NS}	1.000		
Thousand seed weight	0.404***	-0.236**	-0.112 ^{NS}	-0.440***	**-0.450***	0.439***	1.000	
Altitude	**-0.543***	0.485***	0.334***	0.**628***	0.**655***	-0.037	**-0.474***	

Significance levels are indicated by *, ** and *** for P = 0.05, 0.01 and 0.001, respectively. NS = non-significance

Table 4. 6. Correlation of the traits based on mean of the accessions collected from specific collection regions. The correlation coefficient was computed based on the mean of the nine regions of origin Tigray, Wello (north and south), Hararge (east and west), west Shewa, east Wollega and Gojam

Traits	°Brix	Stalk diameter	Plant height	Days to 50% flowering	Days to maturity	Grain yield	Thousand seed weight
°Brix	1.000						
Stem diameter	**-0.887*** **	1.000					
Plant height	-0.509 ^{NS}	0.517 ^{NS}	1.000				
Days to 50% flowering	**-0.922*** **	**0.949*** **	0.646 ^{NS}	1.000			
Days to maturity	**-0.908*** **	**0.932*** **	**0.717*** **	**0.992*** **	1.000		
Grain yield	0.017^{NS}	0.313 ^{NS}	-0.410 ^{NS}	0.105 ^{NS}	0.039 ^{NS}	1.000	
Thousand seed weight	0.493 ^{NS}	-0.311 ^{NS}	-0.828 *	-0.486 ^{NS}	-0.574 ^{NS}	0.602	1.000

Significance levels are indicated by *, ** and *** for P = 0.05, 0.01 and 0.001, respectively. NS = non-significance

4. 1. 4. Cluster and principal component analysis

Cluster analysis using the entire accessions showed five distinct groups, which was two clusters less than the result obtained from principal component analysis. The cluster formed three major groups that further sub grouped into five clusters (Table 4.7 and Fig. 4.3). Cluster two (C2) had the largest number (98) accessions. Collections from the northern part of Ethiopia completely grouped under this cluster. These include South Tigray and the entire Wello (south and north) collections. Cluster one (C1) also contained large number of accessions (60 accessions). This cluster (C1) had collections predominantly from eastern and west central Ethiopia such as east Hararge, west Hararge, west Shewa and east Wollega. One of the collections from Gojam also clustered here. The second and first clusters are considered as the major clusters as they contained a total of more than 150 accessions while C3 and C4 accommodated fewer numbers of accessions. Only ten and eleven sweet sorghum accessions were grouped under C3 and C4, respectively. Cluster three (C3) entirely constituted accessions from east Hararge except one intermixed from Gojam. Similarly, group four (C4) had accessions collected from the same region including west Hararge. Still there was co-clustering of a single individual accession from Gojam area. The last cluster (C5) had only two accessions of different origin, north Wello and south Tigray.

Interestingly enough, the second cluster which constituted the largest number of accessions is also characterized by the high percentage of soluble solids in stem (°Brix). In contrary, accessions that are clustered within the rest of the clusters were characterized by relatively low level of °Brix percentage.

Table 4. 7. Clustering of 181 sweet sorghum accessions into five clusters using mean of seven morphological characters

Cluster	No. of Access.	Name of accessions*
C1	60	Ssorcoll 175/11, Ssorcoll 176/11, Ssorcoll 165/11, Ssorcoll 166/11, Ssorcoll 124/11, Ssorcoll 129/11, Ssorcoll 169/11, Ssorcoll 171/11, Ssorcoll 146/11, Ssorcoll 149/11, Ssorcoll 152/11, Ssorcoll 161/11, Ssorcoll 137/11, Ssorcoll 142/11, Ssorcoll 178/11, Ssorcoll 134/11, Ssorcoll 140/11, Ssorcoll 177/11, Ssorcoll 182/11, Ssorcoll 125/11, Ssorcoll 127/11, Ssorcoll 145/11, Ssorcoll 168/11, Ssorcoll 133/11, Ssorcoll 174/11, Ssorcoll 184/11, Ssorcoll 160/11, Ssorcoll 148/11, Ssorcoll 136/11, Ssorcoll 144/11, Ssorcoll 138/11, Ssorcoll 162/11, Ssorcoll 167/11, Ssorcoll 183/11, Ssorcoll 141/11, Ssorcoll 154/11, Ssorcoll 157/11, Ssorcoll 180/11, Ssorcoll 143/11, Ssorcoll 156/11, Ssorcoll 150/11, Ssorcoll 147/11, Ssorcoll 163/11, Ssorcoll 179/11, Ssorcoll 155/11, Ssorcoll 139/11, Ssorcoll 170/11, Ssorcoll 173/11, Ssorcoll 131/11, Ssorcoll 159/11, Ssorcoll 120/11, Ssorcoll 126/11, Ssorcoll 128/11, Ssorcoll 132/11, Ssorcoll 103/11, Ssorcoll 158/11, Ssorcoll 181/11, Ssorcoll 121/11, Ssorcoll 153/11, Ssorcoll 164/11
C2	98	Ssorcoll 094/11, Ssorcoll 095/11, Ssorcoll 020/11, Ssorcoll 022/11, Ssorcoll 031/11, Ssorcoll 035/11, Ssorcoll 068/11, Ssorcoll 069/11, Ssorcoll 084/11, Ssorcoll 091/11, Ssorcoll 082/11, Ssorcoll 083/11, Ssorcoll 013/11, Ssorcoll 015/11, Ssorcoll 034/11, Ssorcoll 039/11, Ssorcoll 011/11, Ssorcoll 012/11, Ssorcoll 037/11, Ssorcoll 042/11, Ssorcoll 052/11, Ssorcoll 053/11, Ssorcoll 017/11, Ssorcoll 024/11, Ssorcoll 090/11, Ssorcoll 075/11, Ssorcoll 081/11, Ssorcoll 043/11, Ssorcoll 046/11, Ssorcoll 093/11, Ssorcoll 077/11, Ssorcoll 025/11, Ssorcoll 033/11, Ssorcoll 085/11, Ssorcoll 098/11, Ssorcoll 100/11, Ssorcoll 016/11, Ssorcoll 018/11, Ssorcoll 089/11, Ssorcoll 092/11, Ssorcoll 032/11, Ssorcoll 074/11, Ssorcoll 078/11, Ssorcoll 062/11, Ssorcoll 071/11, Ssorcoll 051/11, Ssorcoll 058/11, Ssorcoll 019/11, Ssorcoll 023/11, Ssorcoll 005/11, Ssorcoll 027/11, Ssorcoll 028/11, Ssorcoll 080/11, Ssorcoll 097/11, Ssorcoll 044/11, Ssorcoll 060/11, Ssorcoll 059/11, Ssorcoll 066/11, Ssorcoll 007/11, Ssorcoll 072/11, Ssorcoll 111/11, Ssorcoll 038/11, Ssorcoll 047/11, Ssorcoll 008/11, Ssorcoll 010/11, Ssorcoll 041/11, Ssorcoll 054/11, Ssorcoll 096/11, Ssorcoll 079/11, Ssorcoll 086/11, Ssorcoll 076/11, Ssorcoll 026/11, Ssorcoll 040/11, Ssorcoll 045/11, Ssorcoll 055/11, Ssorcoll 029/11, Ssorcoll 014/11, Ssorcoll 099/11, Ssorcoll 057/11, Ssorcoll 061/11, Ssorcoll 050/11, Ssorcoll 036/11, Ssorcoll 087/11, Ssorcoll 006/11, Ssorcoll 009/11, Ssorcoll 048/11, Ssorcoll 067/11, Ssorcoll 070/11, Ssorcoll 021/11, Ssorcoll 049/11, Ssorcoll 001/11, Ssorcoll 004/11, Ssorcoll 056/11, Ssorcoll 073/11, Ssorcoll 063/11, Ssorcoll 002/11, Ssorcoll 064/11, Ssorcoll 030/11,
C3	10	Ssorcoll 105/11, Ssorcoll 106/11, Ssorcoll 107/11, Ssorcoll 108/11, Ssorcoll 101/11, Ssorcoll 113/11, Ssorcoll 104/11, Ssorcoll 115/11, Ssorcoll 109/11, Ssorcoll 110/11
C4	11	Ssorcoll 112/11, Ssorcoll 116/11, Ssorcoll 119/11, Ssorcoll 130/11, Ssorcoll 114/11, Ssorcoll 117/11, Ssorcoll 135/11, Ssorcoll 122/11, Ssorcoll 118/11, Ssorcoll 123/11, Ssorcoll 102/11
C5	2	Ssorcoll 088/11, Ssorcoll 151/11

* The passport description of the accessions was documented in Table 3.1 under materials and methods

Similar result was obtained from constructed dendrogram based on mean of collection regions and seven morphological traits. The dendrogram clearly put adjacent regions or zones together (Fig. 4.4). For instance, collections from the eastern part of the country which were represented by east and west Hararge were grouped together. Similarly, collections from north Ethiopia such as south Tigray, north Wello and south Wello were clustered together. Collections from central and western Ethiopia that share common borders, west Shewa, east Wollega and Gojam were clustered into the same group. The five Ethiopian accessions included in this experiment and the one obtained from South Africa were also clusetered in this group.

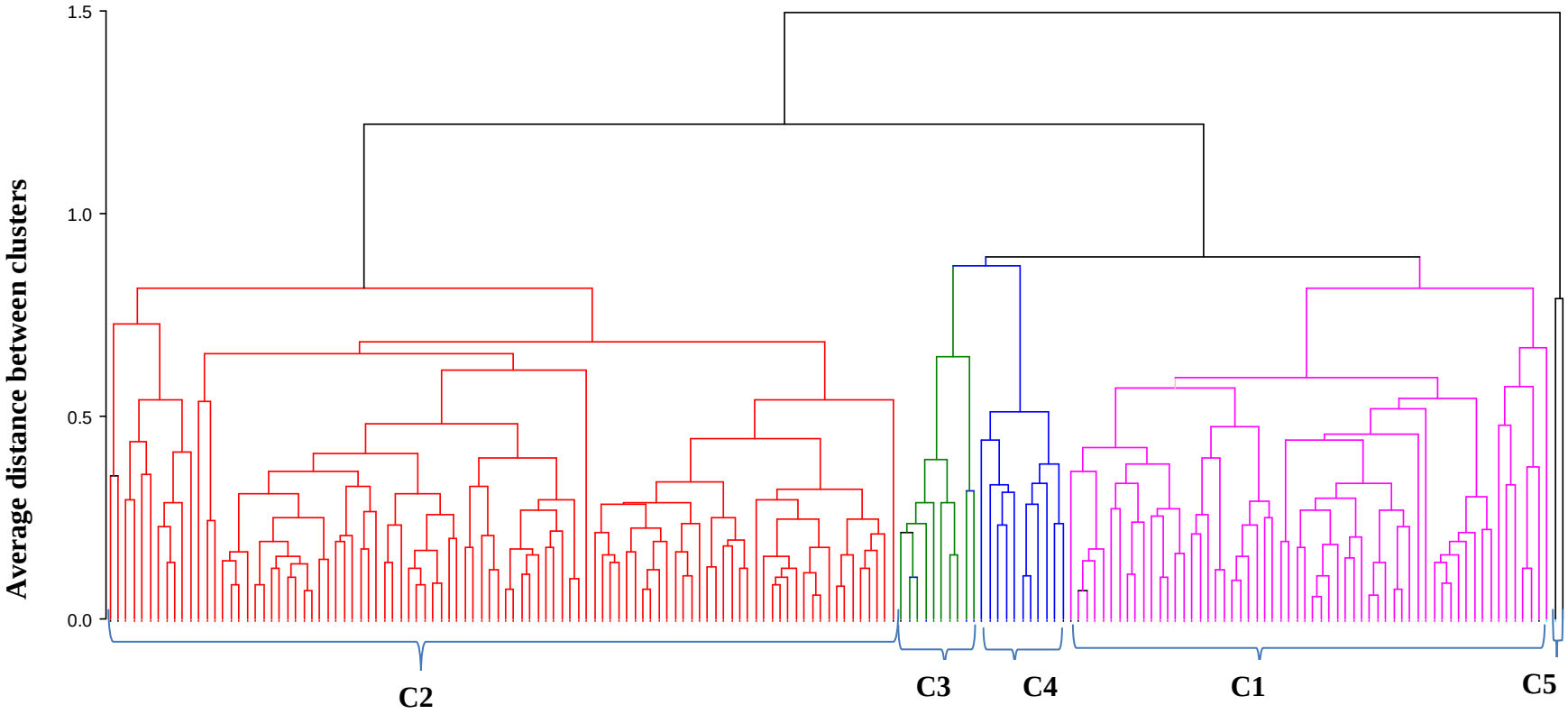


Fig. 4. 3. Dendrogram of 181 sweet sorghum accessions based on seven morphological traits

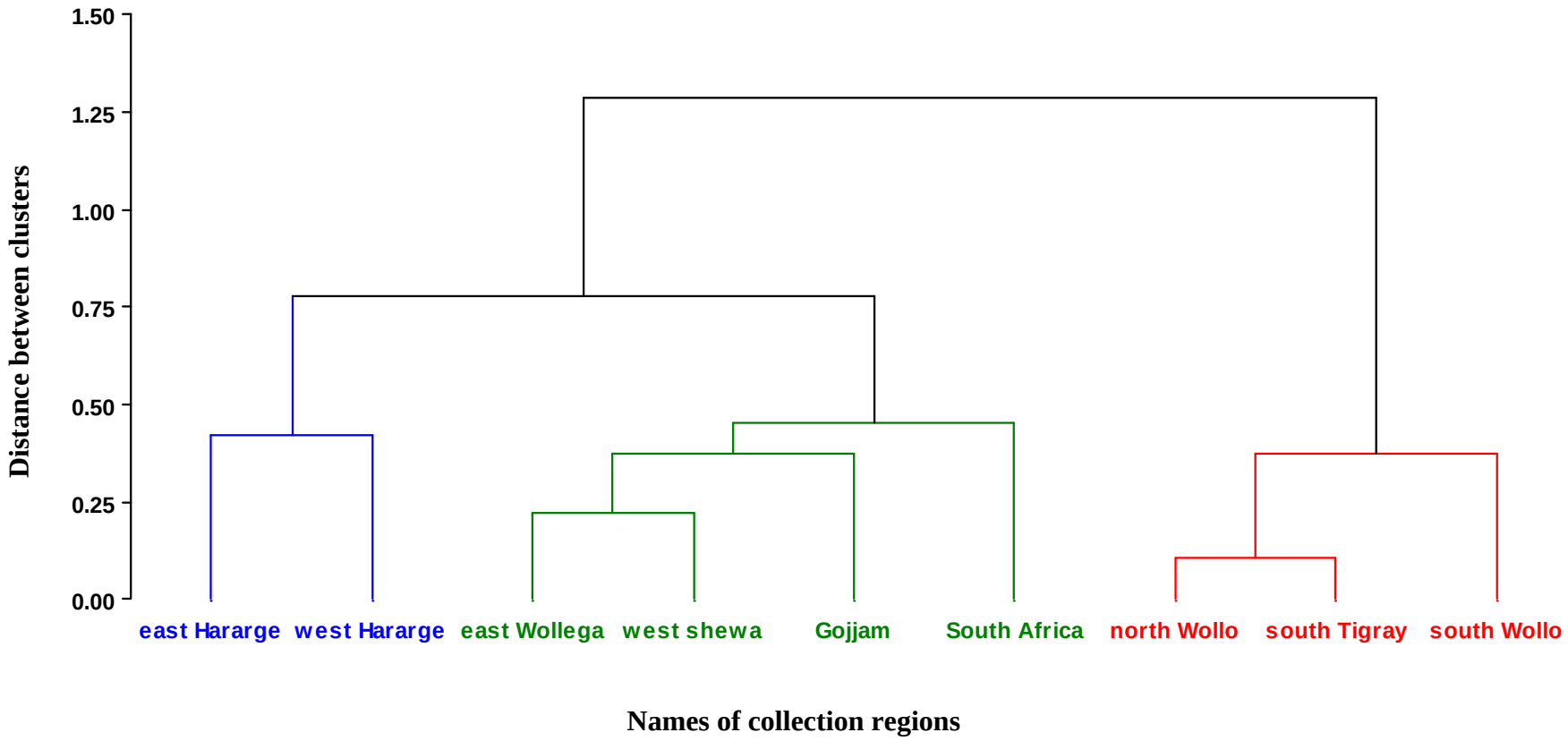


Fig. 4. 4. Dendrogram of eight major sweet sorghum growing regions of Ethiopia and one improved sweet sorghum genotype of South African origin

A total of seven principal components were observed using seven traits in 181 sweet sorghum accessions. The first five principal components were described in table 4.8. The first three principal components explained 85 percent of the total variation across the 181 sweet sorghum accessions for the seven traits studied (Table 4.8). The first principal component alone contributed 53.5 % of the total variation, while the second principal components accounted for 22.3 % of the total variation. The first and second principal component cumulatively contributed 75.8 % of the total variation.

Among the seven traits stalk diameter, days to 50 % flowering and days to maturity contributed the most to the first principal component; whereas grain yield and thousand seed weight were the major contributor to the second and fifth principal component. Similarly, °Brix , plant height and grain yield were the major contributing characters to the third principal component. Plant height was also the major contributor to the fourth principal component.

Table 4. 8. Eigenvalue, proportion and cumulative variances and eigenvectors on the first five principal components for seven traits in 181 sweet sorghum accessions

Variables	PC1	PC2	PC3	PC4	PC5
°Brix	-0.359	-0.128	**-0.745**	0.393	-0.375
Stalk diameter	**0.456**	-0.181	-0.113	0.251	-0.033
Plant height	0.335	-0.308	**-0.555**	**-0.644**	0.234
Days to 50 % flowering	**0.487**	0.064	-0.050	0.354	0.060
Days to maturity	**0.492**	0.051	-0.037	0.337	-0.009
Grain yield	0.093	**-0.702**	0.339	-0.093	**-0.591**
Thousand seed weight	-0.25	**-0.597**	0.074	0.348	**0.671**
Eigenvalue	3.744	1.560	0.664	0.496	0.325
Proportion	0.535	0.223	0.095	0.071	0.046
Cumulative	0.535	0.758	0.853	0.923	0.97

Biplot analysis showed that most of the traits that accounted for 80.5 % of the phenotypic variation were laid in the first principal component (Fig. 4.5) and 18.1 % variation was contributed to the second principal component. Grain yield with high loading value (Fig. 4.5) was found to be the top trait to discriminate the accessions. The second highest contributor to the phenotypic variation was thousand seed weight followed by the °Brix trait. The remaining traits such as plant height, stalk diameter, days to 50 % flowering and days to maturity contributed similar amount of phenotypic variation. Similarly, cluster analysis placed plant height, stem diameter, days to 50 % flowering and days to maturing in one group, and the other traits °Brix , thousand seed weight and grain yield in a second group.

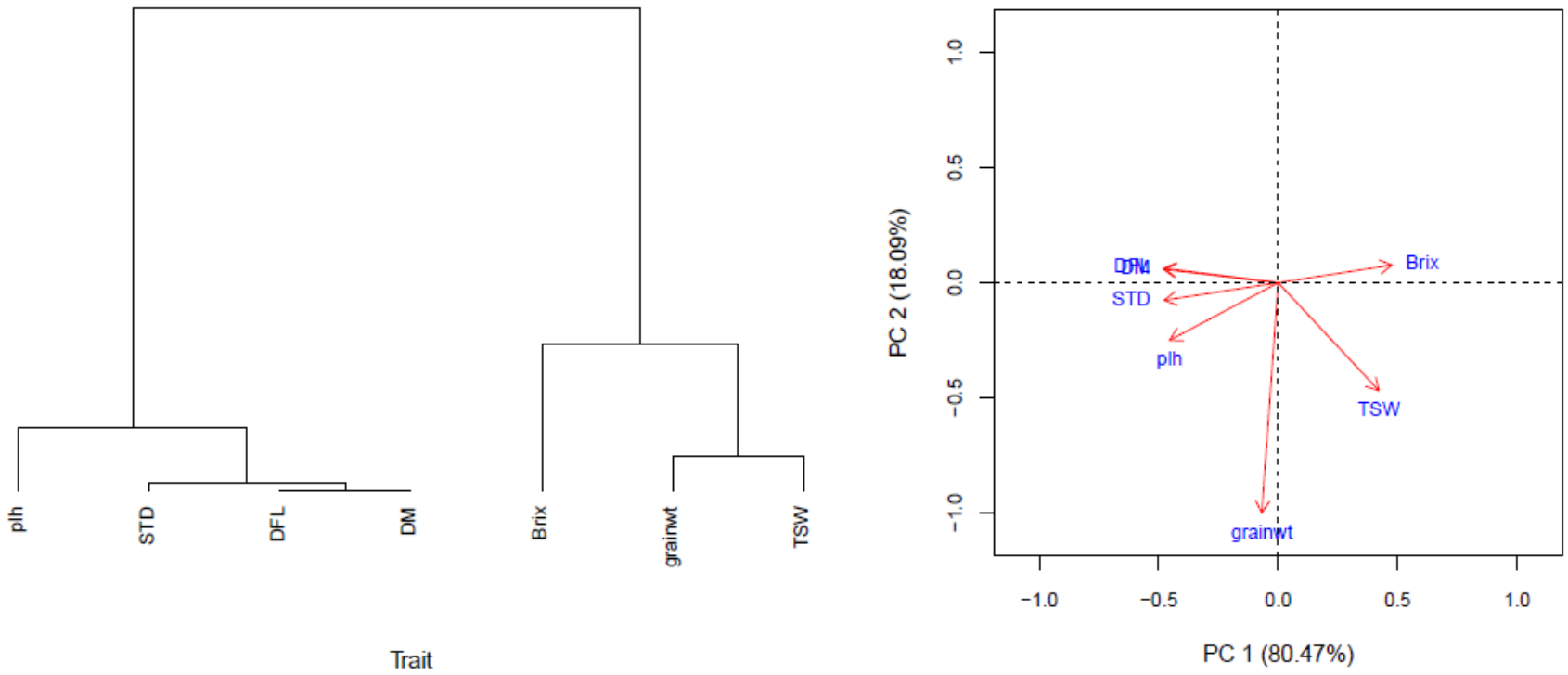


Fig. 4. 5. Cluster for distance matrix and biplot for principal component analysis for seven traits across 181 sweet sorghum accessions (Phl = plant height, STD = stalk diameter, DFL = days to 50 % flowering, DM = days to maturity, grainwt = grain yield, TSW = thousand seed weight)

4.2. Genetic diversity and population structure of Ethiopian sweet sorghum collections using SSR markers

4. 2. 1. SSR allelic diversity

Three SSRs, *Xtxp289, SbKAK01* and *Xcup53*, were excluded from the final analysis because of poor quality. Thirteen markers produced a total of 159 alleles, across all 202 sweet sorghum accessions with an average of 12.23 alleles per marker. Most of the SSR loci were highly polymorphic. The number of alleles per marker ranged from 5 to 17 (Table 4.9). The PIC values for the SSR loci ranged from 0.37 to 0.85 with an average of 0.69 (Table 4.9). The highest PIC value of 0.85 was obtained for marker *Xtxp211*, whereas the lowest PIC value of 0.37 was recorded for marker *Xisep1029*. The overall observed heterozygosity (*Ho*) over loci varied from 0.04 (*Xgap342*) to 0.16 (*Xisep0108*) with an average of 0.09. The gene diversity index (expected heterozygosity, *He*) ranged from 0.42 (*Xisep1029*) to 0.85 (*Xtxp141*) with a mean of 0.72. Marker *Xisep1029* presented the lowest gene diversity as well as PIC value.

Table 4. 9. Diversity statistics of 202 sweet sorghum accessions across 13 SSR loci

Marker	Repeat	Linkage groups	Avail	N_A	He	Ho	PIC
Xisep0108	(GGC)5	SBI-08	0.85	8.00	0.54	0.16	0.51
Xisep0617	(GATC)3	SBI-06	0.99	5.00	0.55	0.09	0.45
Xisep0938	(TGGGT)6	SBI-02	0.96	12.00	0.77	0.07	0.75
Xisep1029	(GCAT)3	SBI-05	0.99	6.00	0.42	0.06	0.37
Xtxp141	(GA)3	SBI-10	0.98	13.00	0.85	0.12	0.83
Xtxp205	(GA)12	SBI-03	1.00	10.00	0.79	0.13	0.75
Xtxp211	(CT)23	SBI-02	0.98	16.00	0.86	0.13	0.85
Xtxp284	(AG)5(GA)28	SBI-01	1.00	17.00	0.85	0.11	0.83
Xtxp012	(CT)22	SBI-04	0.99	17.00	0.76	0.05	0.75
Xtxp279	(CTT)10	SBI-01	1.00	12.00	0.69	0.10	0.64
Xtxp312	(CAA)26	SBI-07	0.97	17.00	0.83	0.06	0.81
Xcup33	(AT)7	SBI-01	0.98	9.00	0.69	0.11	0.64
Xgap342	(AC)25	SBI-07	1.00	17.00	0.80	0.04	0.77
Total			**N/A**	**159**	**N/A**	**N/A**	**N/A**
Mean			**0.98**	**12.23**	**0.72**	**0.09**	**0.69**

Avail Availability; NA = Number of Alleles; He = Gene diversity; Ho = Observed heterozygosity

Though the highest mean gene diversity of 0.69 was observed in improved genotypes that are a composition of sweet and grain sorghum, it cannot represent an independent population due to very limited number of samples used. The population from ICRISAT showed relatively higher mean gene diversity (*He* = 0.68) followed by populations of south Tigray, north and south Wello with a mean gene diversity values of 0.65, 0.65 and 0.62, respectively (Table 4.10). In contrary, populations from west Shewa (*He* = 0.48) and east Wollega (*He* = 0.32) showed the lowest mean gene diversity. The highest mean values of observed heterozygosity was also obtained from accessions collected from north Wello (*Ho* = 0.15), whereas the population from Gojam had the lowest mean value of observed heterozygosity (0.02).

Table 4. 10. Inter-population based molecular diversity analysis

Standard diversity indices	SW	NW	ST	Goj	EH	WH	WS	EW	ICR
Polymorphic loci	13.00	13.00	13.00	9.00	12.00	13.00	8.00	11.00	13.00
Mean number of alleles	4.01	6.69	7.20	2.80	5.50	3.40	2.60	3.00	6.30
Mean *Ho*	0.13	0.15	0.08	0.02	0.12	0.05	0.04	0.07	0.04
Average *He*	0.62	0.64	0.65	0.33	0.56	0.5	0.3	0.32	0.68

SW = south Wello, NW = north Wello, ST = south Tigray, Goj = Gojam, EH = east Hararge, WH = west Hararge, WS = west Shewa, EW = east Wollega, ICR= ICRISAT, He = Gene diversity, Ho = Observed heterozygosity

A comparison between Ethiopian accessions and the improved sweet sorghum revealed higher average number of alleles (9.5) in the former than in the latter (7.1) (Table 4.11). Ethiopian

accessions were also significantly richer in rare and private alleles even though the numbers of common alleles were comparable between the two groups (Table 4.11).

Table 4. 11. Comparison of genetic diversity of Ethiopian and improved sweet sorghum genotypes

	Ethiopian Accessions					**Improved Accessions**				
Marker	N_A	R_A	C_A	A_A	P_A	N_A	R_A	C_A	A_A	P_A
Xcup33	9	6	3	-	6	3	1	2	-	0
Xgap342	10	5	5	-	7	10	4	6	-	7
Xisep0108	6	3	2	1	1	7	3	4	-	2
Xisep0617	3	1	1	1	0	5	1	4	-	2
Xisep0938	12	6	6	-	9	3	1	1	1	0
Xisep1029	4	1	2	1	1	5	3	1	1	2
Xtxp012	16	10	6	-	10	7	3	3	1	1
Xtxp141	12	6	6	-	6	7	3	3	1	1
Xtxp205	5	1	4	-	2	8	3	5	-	5
Xtxp211	13	6	7	-	7	9	5	4	-	3
Xtxp284	12	6	6	-	4	13	6	7	-	5
Xtxp312	12	7	5	-	8	9	4	5	-	5
Xtxp279	10	7	3	-	3	6	1	5	-	2
Total	**124**	**65**	**56.0**	**3.0**	**64**	**92**	**38.0**	**50.0**	**4**	**35**
Mean	**9.5**	**5**	**4.31**	**1.0**	**4.9**	**7.1**	**2.9**	**3.8**	**1**	**2.7**

N_A - Total number of Alleles; R_A - Rare alleles that were present in <5% of the accessions; C_A- Common alleles that were present in 5-50% of the accessions; A_A- were present in more than 50% of the accessions; P_A - Private alleles that were only found in the respective population set

4. 2. 2. Genetic relationships

Phylogenetic analysis revealed clustering of accessions based on their geographical origin with a few discrepancies (Fig. 4.6). Three major clusters were observed; one for accessions from the northern Ethiopia (Wello and Tigray); a second one for accessions from the eastern (east Hararge and west Hararge) and west central (Shewa, Wollega and Gojam) parts of Ethiopia; and a third cluster of improved cultivars (Fig. 4.6). The dendrogram showed that accessions collected from different zones of northern Ethiopia were strongly differentiated from the rest of the collections. Similarly, collections from ICRISAT showed close similarity with accessions from Hararge suggesting that most of the breeding lines probably shared ancestry with accessions originally selected from Hararge. One particular improved line *ICSV93046* (ICR_19) clustered with Ethiopian accessions from Tigray and also showed potential duplication with improved cultivar *Sorcoll163/07* (PI2) from Ethiopia and high similarity with *Gambella* (PI3) (Fig. 4.6). ICRISAT line *ICSV93046* and Ethiopian grain type accessions *Sorcoll 163/07* could be originated from the same region. There was also another potential duplication of two ICRISAT lines that clustered close to accessions from Gojam (Fig. 4.6).

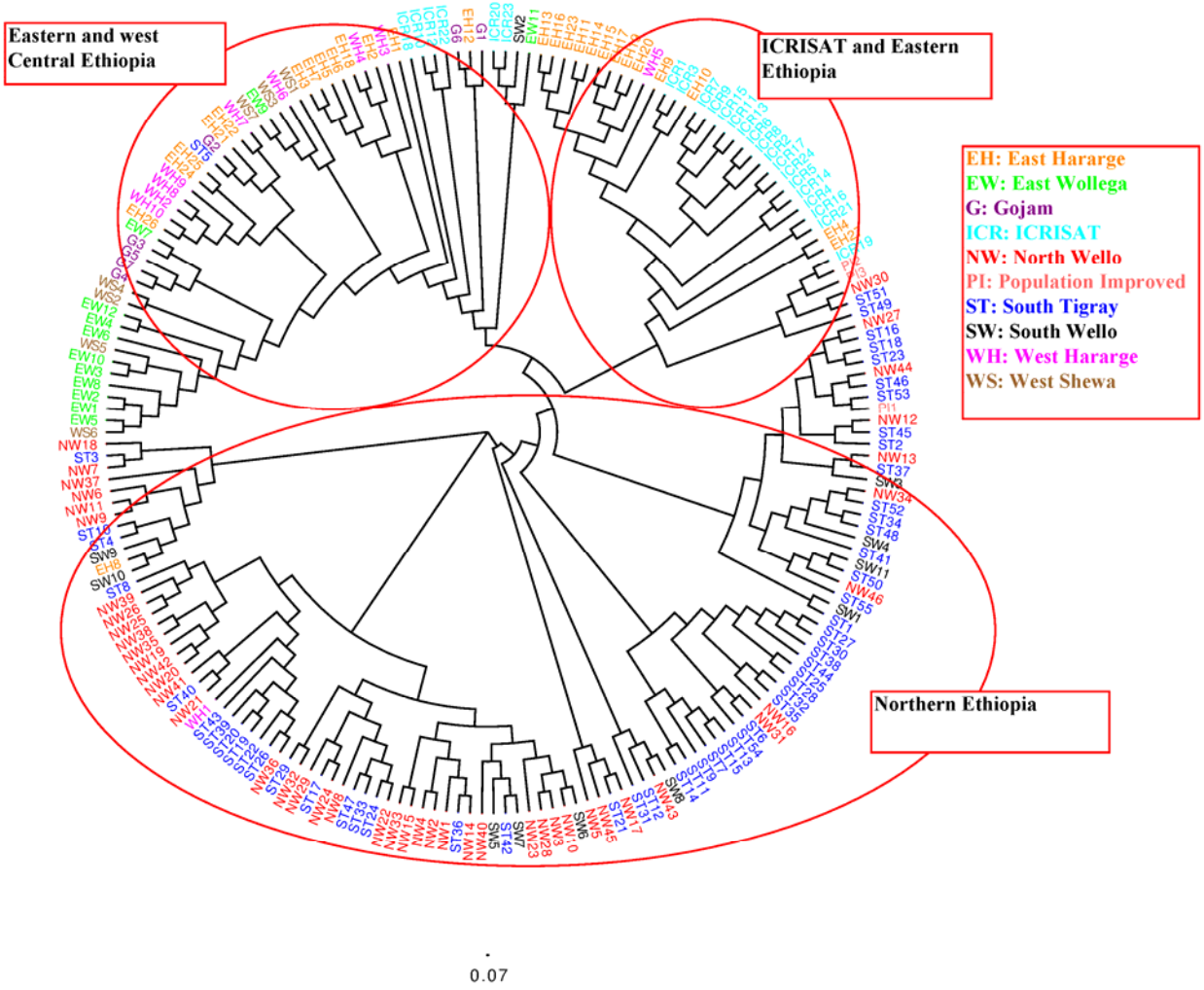


Fig. 4. 6. Unweighted Neighbor-Joining tree showing relatedness among the 202 sweet sorghum accessions. The key is shown with respective colour codes. The respective accessions for the codes provided are shown in Tables 3.1 and 3.3. Three major clusters were observed with accessions from the northern Ethiopia clustering together and those from the central region forming another cluster. Improved materials received from ICRISAT formed an independent cluster although six of the lines clustered with the central Ethiopian material. One ICRISAT improved line ICR19 (ICSV93046) clustered with Ethiopian improved materials Sorcoll163/07 and Gambella among the accessions collected from the north.

The highest population differentiation was observed among improved genotypes and the rest of the Ethiopian accessions from different regions (Table 4.12). Expectedly, the lowest population differentiation was observed among south Tigray and Wello, which are adjacent to each other and the GPS coordinates also show that the collections were made around the same area. The lowest population differentiation was therefore observed among accessions from south Tigray and north Wello (F_{ST} = 0.03), followed by south Tigray and south Wello (F_{ST} = 0.08). The highest population differentiation was observed between the three improved genotypes (*Sorcoll163/07, Gambella, AS27*) and accessions from east Wollega (F_{ST} = 0.55), east Shewa (F_{ST} = 0.52) and Gojam (F_{ST} = 0.52) (Table 4.12). Results of AMOVA showed significant genetic difference in the sampled populations with 76.9% genetic variation within collection regions (populations) and 23.1 % among collection regions (Table 4.13).

Table 4. 12. Estimate of pair wise F_{ST} among different geographic regions

Pair wise F_{ST}	South Wello	North Wello	South Tigray	Gojam	East Hararge	West Hararge	West Shewa	East Wollega	ICRISAT
North Wello	0.08***								
South Tigray	0.08*	0.03***							
Gojam	0.37***	0.37***	0.37***						
East Hararge	0.19***	0.24***	0.24***	0.32***					
West Hararge	0.24***	0.29***	0.28***	0.31***	0.10*				
West Shewa	0.32***	0.36***	0.36***	0.33***	0.23***	0.14*			
East Wollega	0.35***	0.38***	0.37***	0.22***	0.25***	0.20***	0.11		
ICRISAT	0.15***	0.19***	0.18***	0.34***	0.17***	0.24***	0.35***	0.36***	
Improved	0.23*	0.24***	0.21**	0.52**	0.31***	0.40***	0.52***	0.55*	0.19***

Pairs were significant at **P* value < 0.05, ***P* value < 0.01, ****P* value < 0.001

Table 4. 13. Analysis of molecular variance of 202 sweet sorghum accessions genotyped using 13 polymorphic SSR markers

Source of Variation	*Df*	Sum of squares	Variance components	Percentage of variation	*P* value
Among populations	9	422.76	1.16	23.1	< 0.001
Within populations	394	1513.90	3.84	76.9	< 0.001
Total	403	1936.66	5.00		

df Degrees of freedom

Principal coordinate analysis also showed accessions collected from neighboring regions were clustered within the same coordinate (Fig. 4.7). Principal component analysis using 202 Ethiopian and global accessions and 13 informative microsatellite markers, resulted in 13 PCs (Fig. 4.8). Unlike the seven principal components obtained using morphological characters, molecular marker based analysis generated relatively large number of principal components. However, greater contribution (67.5 %) was obtained from the first five principal components with Eigenvalue > 1 (Table 4.14). The first principal component contributed 27.1% of the total variation whereas the second principal component contributed 11.6 % of the total variation. Similar percentage of variation was contributed by the third principal component (10.3 %). The fourth principal component contributed 9.7 % of the variation while the fifth one accounted for 8.8 % of the variation. The individual contribution by the last six principal components such as PC8, PC9, PC10, PC11, PC12 and PC13 was found to be very small as each of them contributed to less than 5 % of the variation and they also had Eigenvalue < 1.

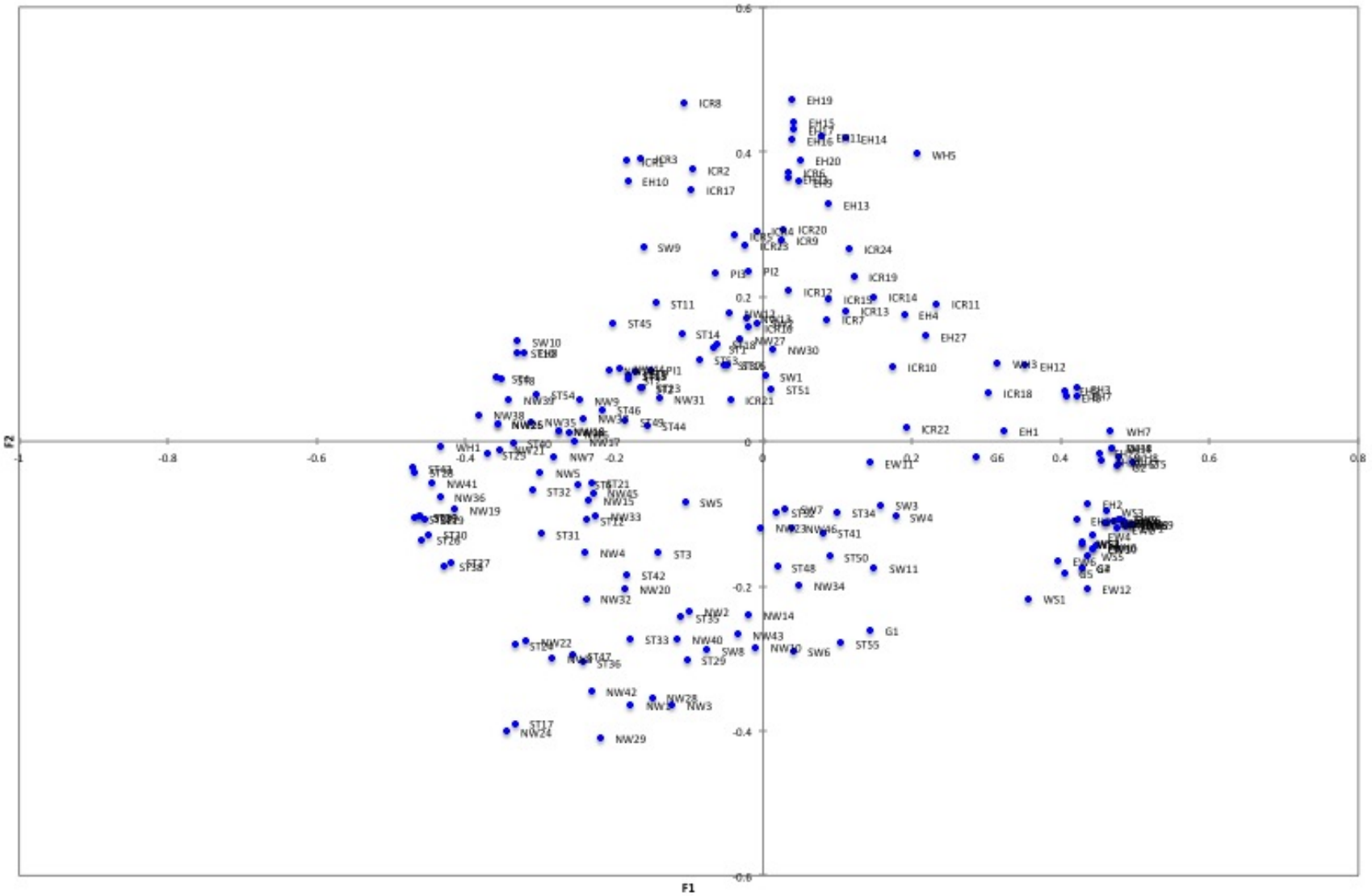


Fig. 4. 7. Principal coordinate analysis of 202 sorghum accessions using 13 SSR markers

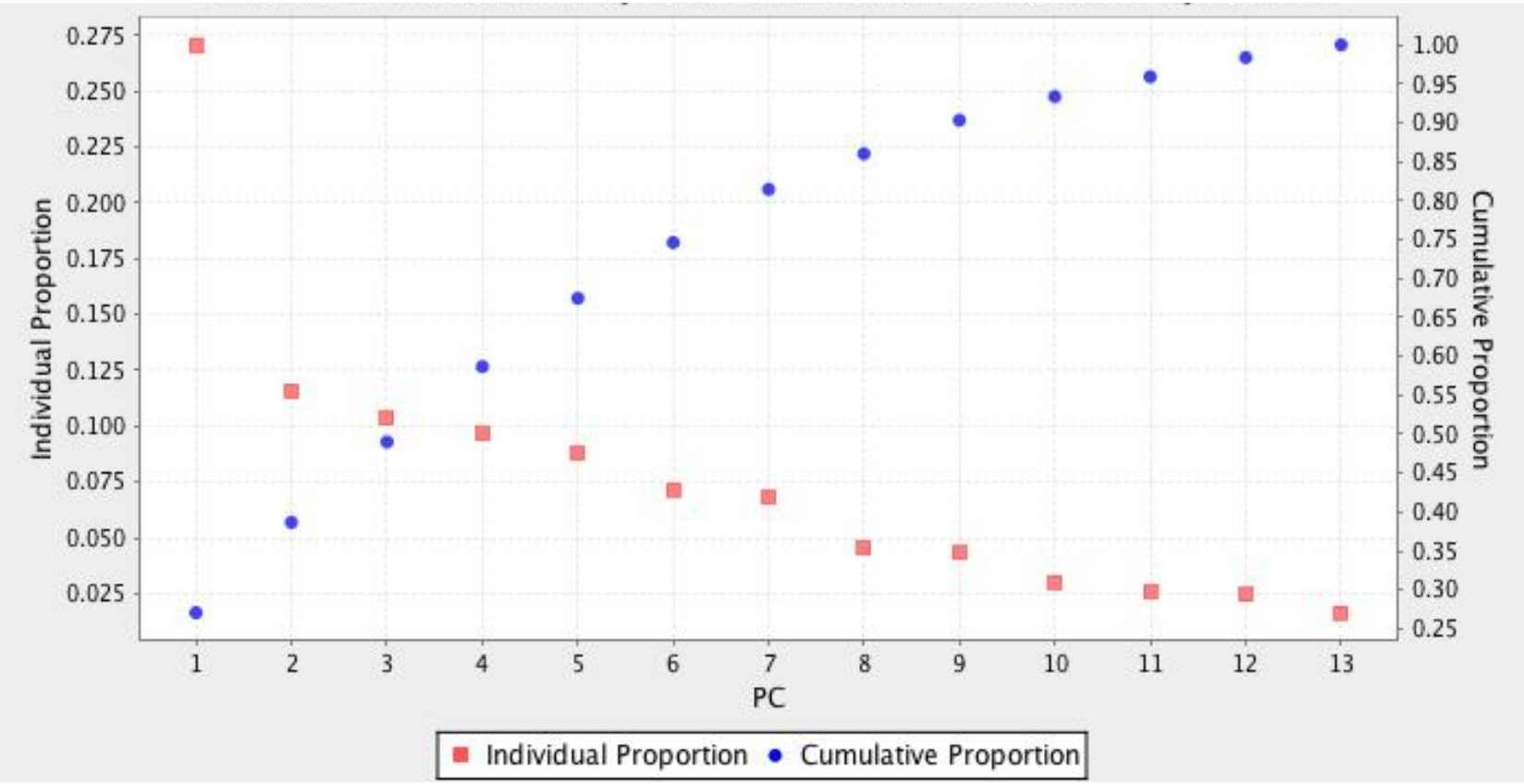


Fig. 4. 8. Individual and cumulative proportion contributed by each principal component using13 polymorphic SSRs in 202 sweet sorghum accessions

Table 4. 14. Eigenvalue, individual and cumulative contribution on the first five principal components using 13 polymorphic SSRs in 202 sweet sorghum accessions (only those with Eigenvalue > 1 were listed)

PC	Eigenvalue	Individual contribution	Cumulative contribution
1	3.520	0.271	0.271
2	1.507	0.116	0.387
3	1.345	0.103	0.490
4	1.255	0.097	0.587
5	1.143	0.088	0.675

4. 2. 3. Population structure

Analysis of population structure was done using 13 polymorphic SSR markers using STRUCTURE. A sharp peak was detected in ΔK at K=3 (Fig. 4.9) suggesting the presence of 3 major populations (Evanno *et al.,* 2005). The 3 detected sub-populations (Fig. 4.10) followed a similar clustering pattern as seen in Fig. 4.6 with two major Ethiopian sub-populations and one sub-population of improved genotypes. Population I consisted mainly of accessions from the eastern and west central part of Ethiopia (Hararge, Shewa, Gojam, Wollega), while population II was composed mainly of improved accessions. Population III contained the highest numbers of accessions, which were mainly from the northern part of Ethiopia (Tigray and Wello) (Fig. 4.10).

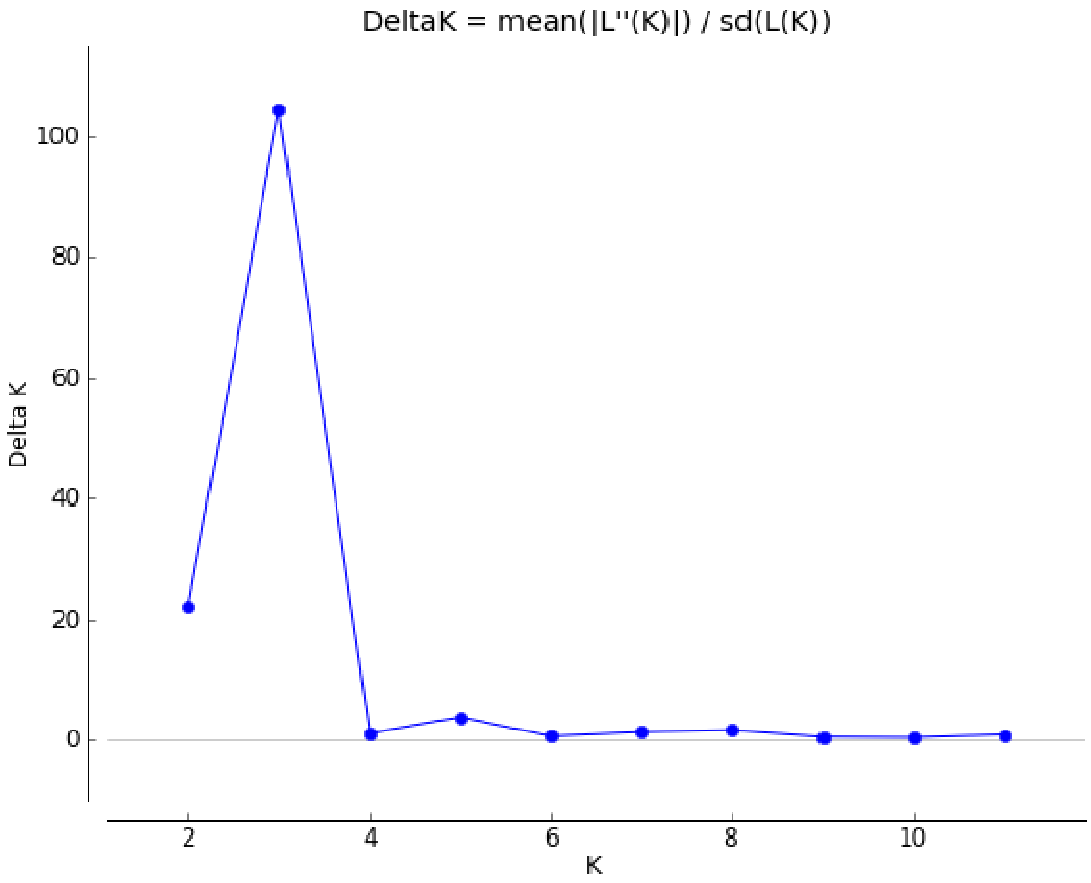


Fig. 4. 9. The modal value of this distribution is the true K. The Delta k of 15 repeats based on STRUCTURE calculation using SSR data. The optimum number of predicted sub- population K= 3 was calculated from structure Harvester (http://taylor0.biology.ucla.edu /structureHarvester/)

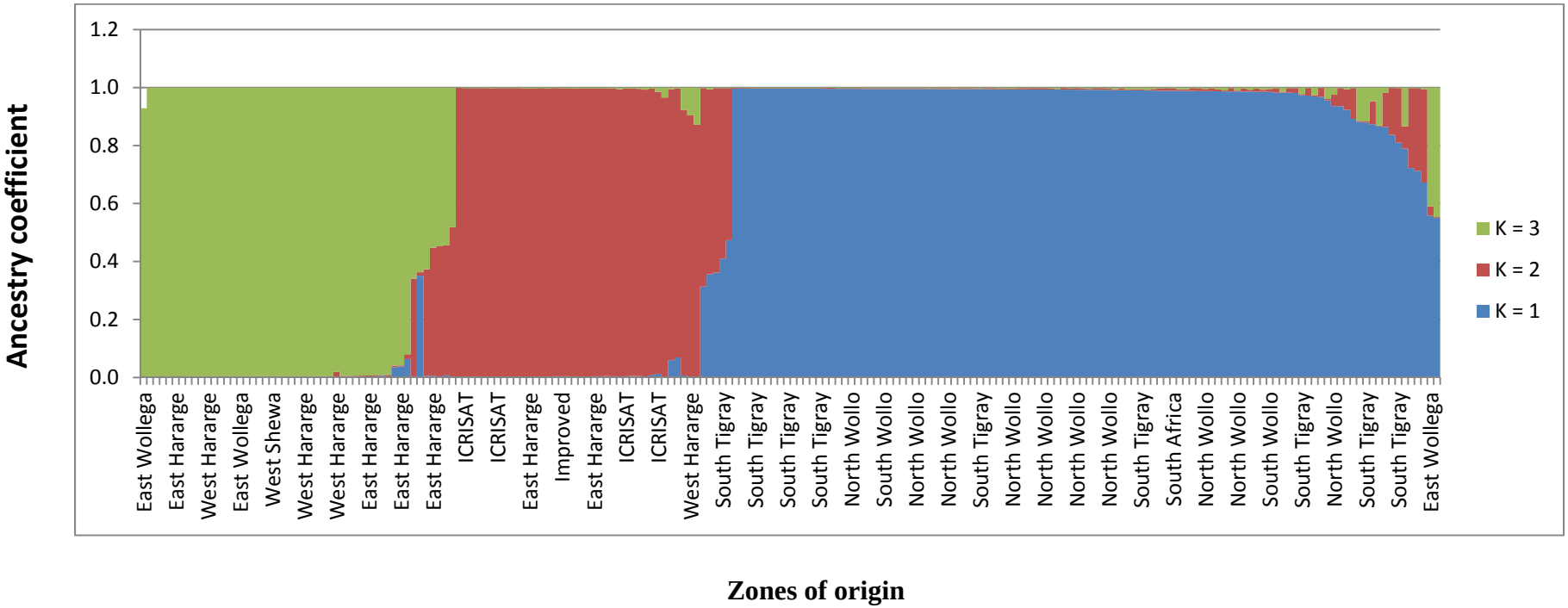


Fig. 4. 10. Bar plots of the population structure analysis of Ethiopian sweet sorghum lines with global collection. Populations were arranged based on regions of origin

4. 3. Identification and mapping of QTLs associated with sugar content and drought tolerance traits from Ethiopian sorghum collections

4. 3. 1. Identification and mapping of QTLs associated with °Brix content and biomass related traits

4. 3. 1. 1. Phenotypic evaluation

For traits such as plant height and stem diameter, there was no significant difference between the two parents as well as the progenies in both environments (Appendix 2). However, °Brix value measured for *Gambella* was significantly ($P < 0.01$) higher than that of *Sorcoll 163/07*. The phenotypic value of °Brix showed continuous variation across the progenies in both locations and normally distributed with a moderate value of skwenness and kurtosis (Appendix 2). Similar level of environmental means for °Brix were 8.9 and 9.1% in the two locations was observed. The highest (15.8 %) and lowest (4.5%) °Brix values were recorded among the progenies at Dhera and Melkassa stations, respectively.

The broad sense heritability of the traits °Brix, stalk diameter and plant height were H = 0.88, H = 0.41 and H = 0.65, respectively. °Brix showed a high degree of heritability whereas the stalk diameter and plant height showed moderate to high heritability. Pearson's correlation coefficients analysis showed that there was no significant correlation between °Brix and plant height but a slight negative correlation was observed between °Brix and stalk diameter ($r = -0.320$, $P < 0.05$). The result also showed that there was no significant correlation between plant height and stalk diameter traits.

4. 3. 1. 2. Construction of linkage map

Among the screened SSR markers, twenty seven percent (81 out of the 304) revealed polymorphism between the two parents. Genotyping 192 F_2 mapping populations using 76 polymorphic markers helped to construct linkage map. Most of 76 SSR markers used here showed good segregation pattern, which did not significantly deviate from the expected 1:2: 1 segregation ratio (Appendix 3). The distributions of markers more or less appear to be evenly distributed across the 10 linkage groups except few numbers of markers on some of the linkage groups due to the difference in length of chromosomes (Fig. 4.11). Nine markers were grouped to Linkage group SBI-01. The second linkage group, SBI-02, was composed of eleven markers. The minimum number of markers was observed in linkage groups SBI-03, SBI-05, and SBI-0, each of them consisting of five markers.

Six polymorphic markers (6%) were failed to group into any of the linkage groups and excluded from the analysis (Fig. 4.11). These markers (unknown) may be wrongly documented in previous study. The final map consisted of 70 markers that were distributed on 10 linkage groups (Fig. 4.12).

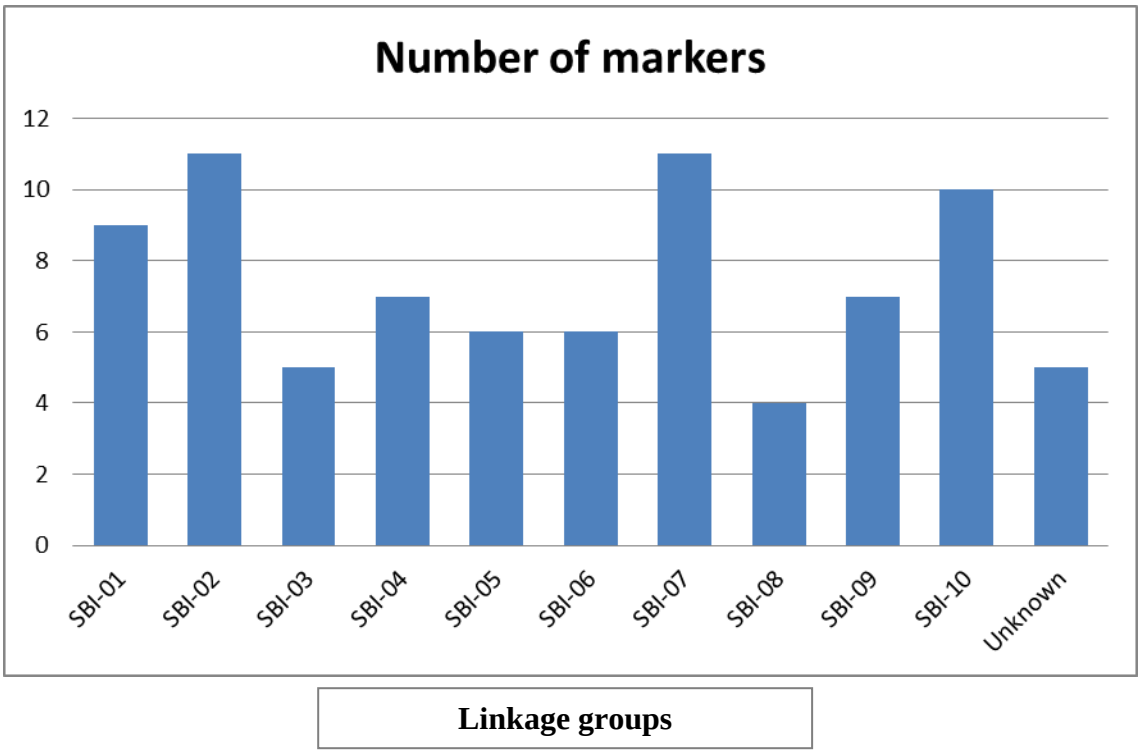


Fig. 4. 11. Distribution of polymorphic SSR markers across the ten linkage groups

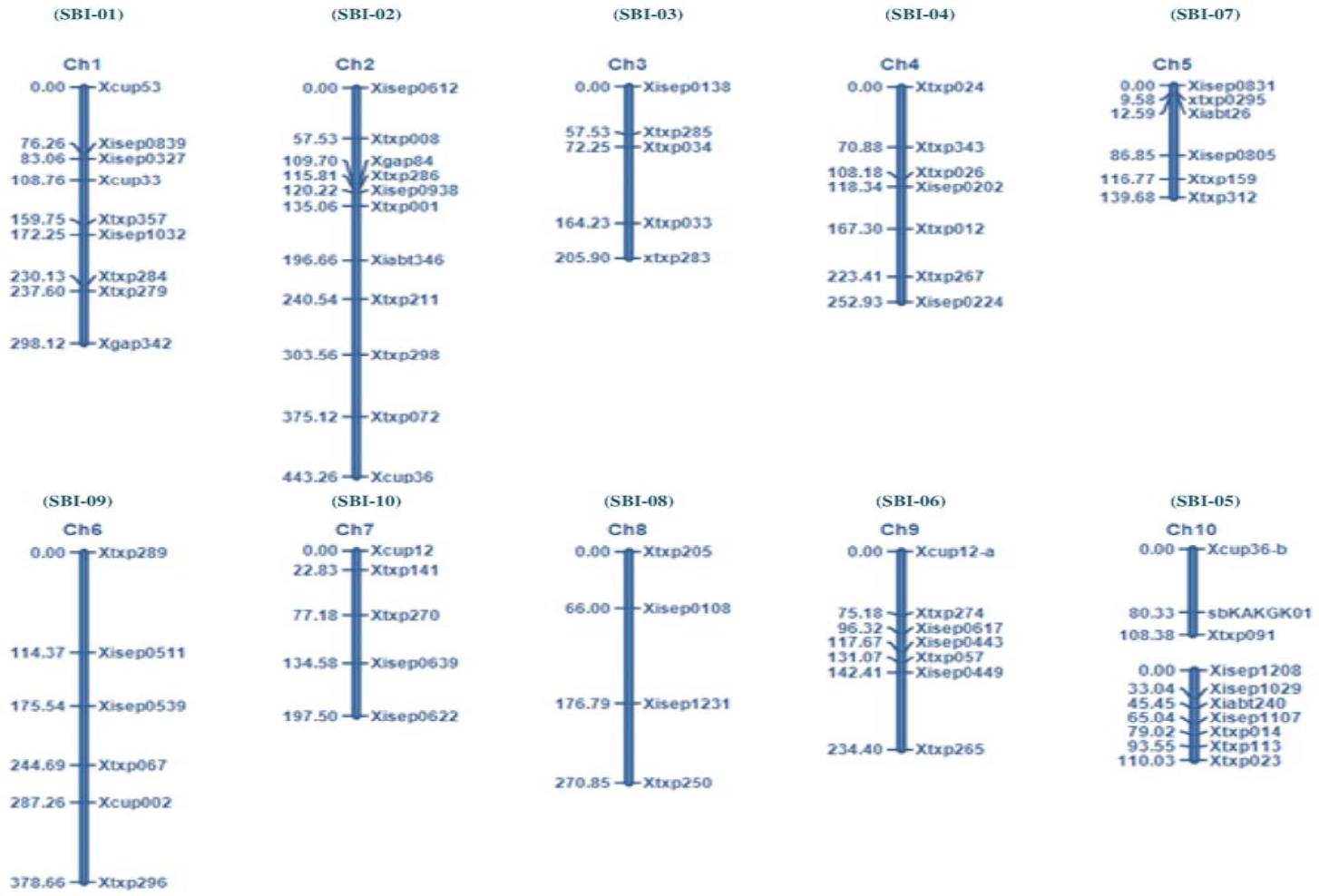


Fig. 4. 12. Constructed genetic linkage map using 192 F_2 mapping populations genotyped with 76 SSR markers. Linkage group designations are identical to those described in Hausmann et al. (2002) and Menze et al. (2002). Therefore the order of the chromosomes and their corresponding linkage groups are [(Ch1=A, SBI-01), (Ch2=B, SBI-02), (Ch3=C, SBI-03), (Ch4=D, SBI-04), (Ch5=E, SBI-07), (Ch6=F, SBI-09), (Ch7=G, SBI-10), (Ch8=H, SBI-08), (Ch9=I, SBI-06) and (Ch10=J, SBI-05)].

The figures in the right of each linkage groups represent the loci and the corresponding numbers (left) represent the distance position in Centi Morgan.

4. 3. 1. 3. QTL identification and analysis

Two major QTLs influencing the °Brix were detected on linkage group SBI-07 and SBI-9 at a LOD threshold of 3.5 using the data collected from Melkassa Agricultural Research Center. As expected, QTLs influencing plant height and stem diameter were not detected at similar LOD cut value (Fig. 4.13). The two QTLs associated with °Brix were found to be major QTLs each contributed 40.0 and 44.7 % of the total phenotypic variation (Table 4.15). More than two QTLs could be detected at a LOD threshold of 3.0 (Fig. 4.13). The first QTL on the SBI-07 linkage group lies between *Xiabt26* and *Xisep0805* with a LOD value of 3.5 whereas the second QTL on the linkage group SBI-09, located between *Xtxp289* and *Xisep0511* with a LOD value of 5.0. The additive effect of each QTL on linkage group SBI-07 and SBI-09 was -1.59 % and -1.93 %, respectively. Similarly, the dominance effect was -2.97 % and -2.67 %.

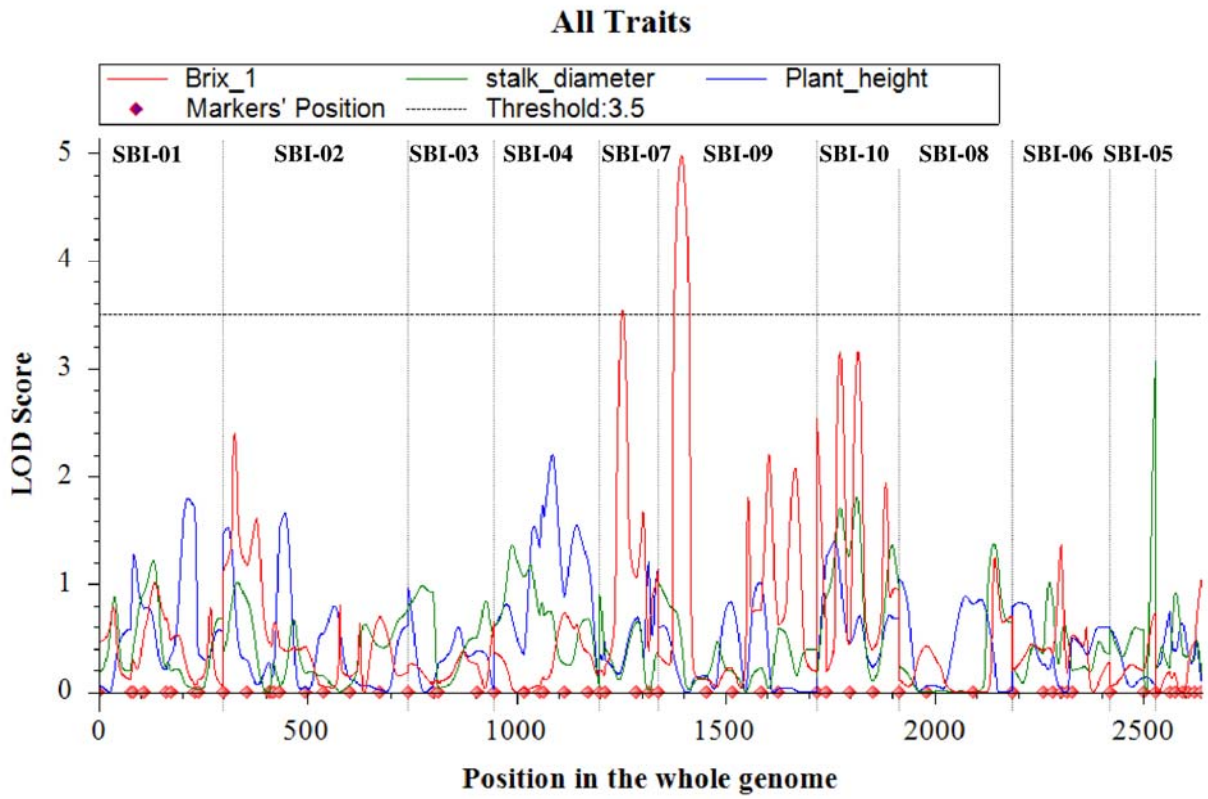


Fig. 4. 13. Position of detected quantitative trait loci in the whole genome for °Brix trait in sorghum at LOD threshold 3.5 (at MARC environment)

Seven significant QTLs that are associated with the °Brix content (LOD threshold $\geq$ 3.5) were detected from the experiment evaluated at Dhera station (Fig. 4.14). Similar to Melkassa environment, no QTLs were detected that are associated with plant height and stem diameter traits. The QTLs were distributed across five linkage groups; SBI-02, SBI-10, SBI-09, SBI-07, and SBI-10 (Table 4.15). The LOD values of the QTLs were ranging from 3.5 to 5.5 while each QTL was explaining a phenotypic variation ranging from 17.2 to 44. 3 %. The additive effect of each QTL ranged from -2.5 to 2.3 % whereas dominant effect ranged from -3.0 to -1.7 % of the °Brix content.

Table 4. 15. Linkage groups, position and flanking markers of detected quantitative trait loci associated to °Brix trait evaluated at MARC and Dhera environments.

Locations	Linkage Groups	Position	Left Marker	Right Marker	LOD	PVE (%)	Add	Dom
MARC	SBI-07	54	Xiabt26	Xisep0805	3.54	39.97	-1.59	-2.97
	SBI-09	55	Xtxp289	Xisep0511	4.97	44.68	-1.93	-2.67
Dhera	SBI-02	80	*Xtxp008*	*Xgap84*	3.52	25.69	-1.32	-2.21
	SBI-02	165	*Xtxp001*	*Xiabt346*	3.57	39.94	-1.77	-2.38
	SBI-05	40	*Xcup36*	*sbKAKGK01*	3.51	41.11	1.69	-2.53
	SBI-07	121	*Xtxp159*	*Xtxp312*	4.33	17.21	-2.50	-2.14
	SBI-09	58	*Xtxp289*	*Xisep0511*	4.40	44.27	2.34	-1.68
	SBI-09	329	*Xcup002*	*Xtxp296*	3.87	38.39	-1.74	-2.47
	SBI-10	98	*Xtxp270*	*Xisep0639*	5.45	39.61	-2.36	-2.13

PVE = Phenotypic variance explained, Add = Additive effect, Dom = dominant effect

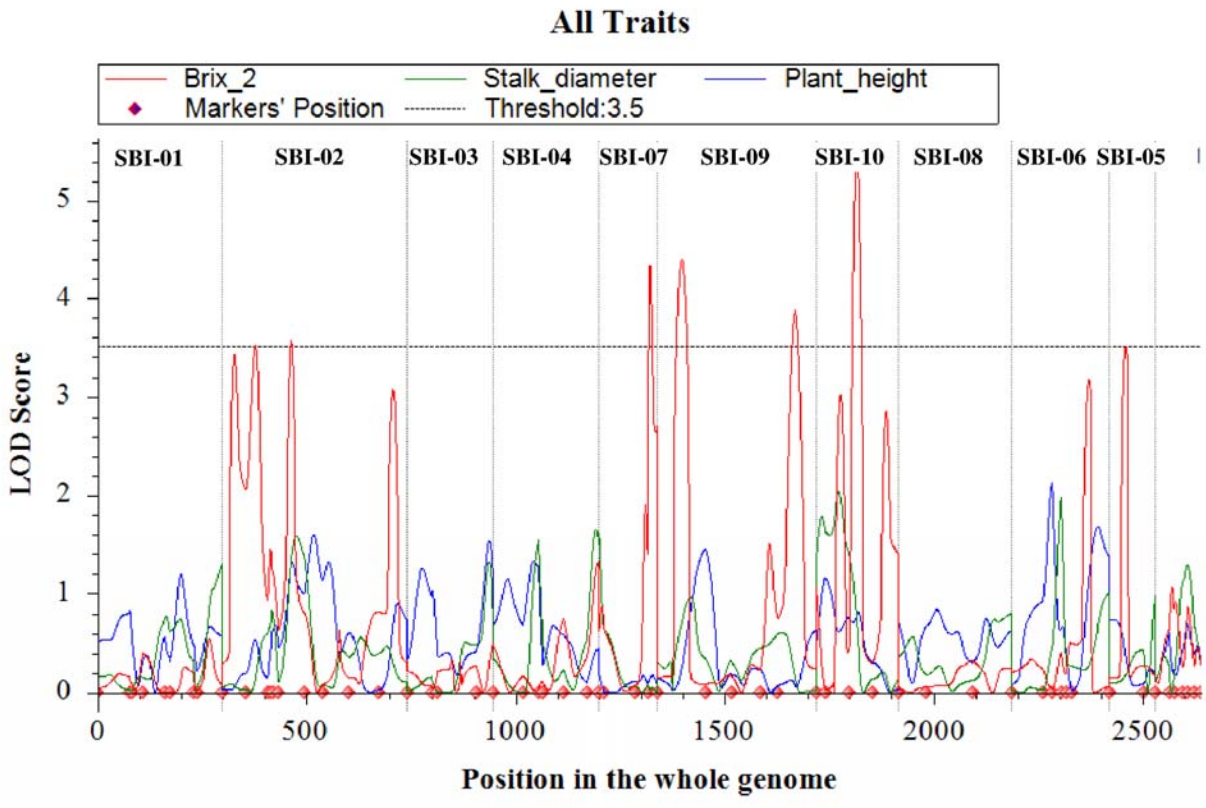


Fig. 4. 14. Detected quantitative trait loci for °Brix trait in sorghum at LOD threshold 3.5 (at Dhera environment)

Brix QTLs

A total of seven QTLs were detected using the combined data collected from two locations using LOD threshold ≥ 3.5 whereas no QTLs influencing plant height and stem diameter were detected (Fig. 4.15). Two of the QTLs conferring for total soluble solids (°Brix) were consistent across the two environments and located on the same linkage groups (SBI-07, SBI-09). The remaining QTLs were located on linkage group SBI-02, SBI-05 and SBI-10 with a LOD value ranging from of 3.5 to 5.4 (Table 4.16). Three QTLs were detected on linkage group SBI-10 with pairs of flanking markers *Xtxp141-Xtxp270, Xisep0639-Xisep0639* and *Xisep0639- Xisep0622.* The three QTLs are considered as independent because the distance between them is greater than 20 centi-morgan (Shiringani *et al*., 2010). The QTL on the SBI-06 linkage group was detected between markers *Xisep0449* and *Xtxp265*. Almost all the identified QTLs are considered as major QTLs contributing high percentage of phenotypic variance which ranged from 38.5 to 49.8 % of the phenotypic variation with the additive effect ranging from -2.3 to 2.1 %. Besides, the dominance effect ranged from -3.0 to -2.3 %.

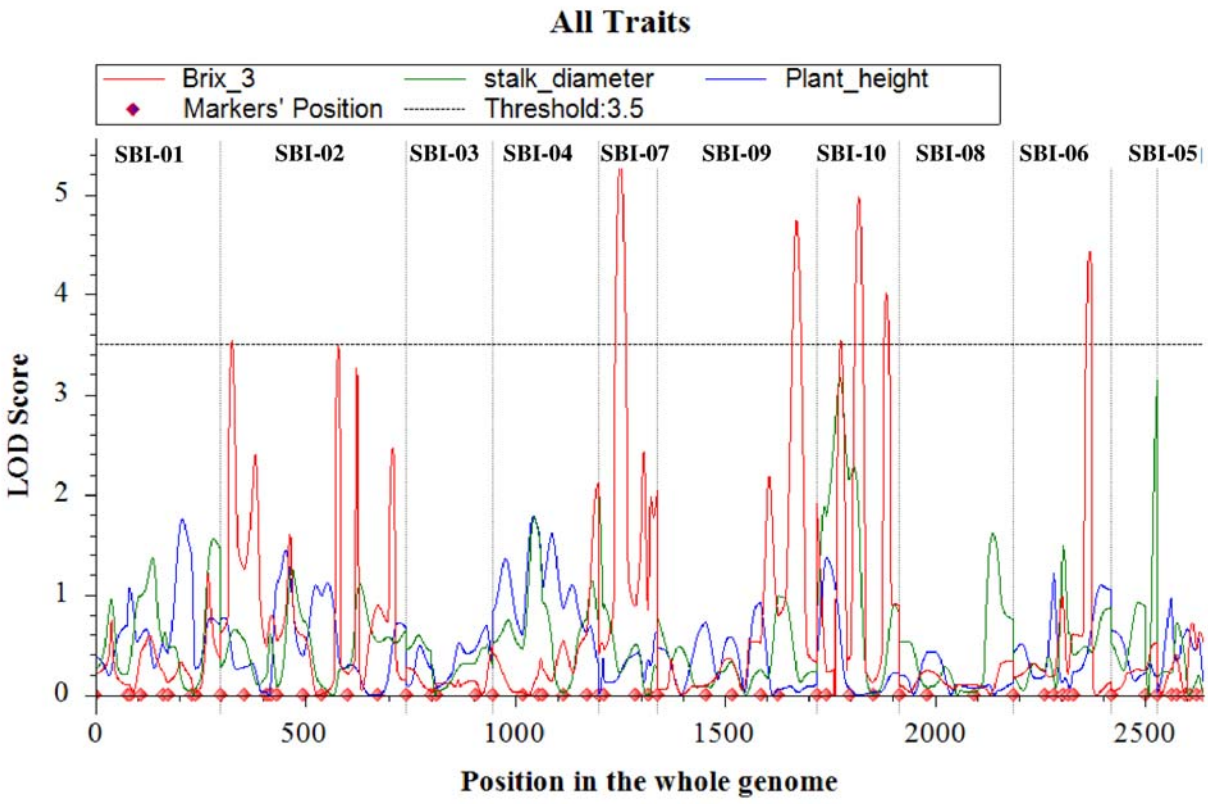


Fig. 4. 15. Detected quantitative trait loci for °Brix trait in sorghum at LOD threshold 3.5 combined locations (Melkassa and Dhera)

Table 4. 16. Linkage groups, position and flanking markers of quantitative trait loci associated to °Brix trait evaluated at MARC and Dhera environments (combined locations)

Linkage Groups	Position	Left Marker	Right Marker	LOD	PVE (%)	Add	Dom
SBI-02	27	*Xisep0612*	*Xtxp008*	3.54	40.13	-1.92	-2.48
SBI-06	182	*Xisep0449*	*Xtxp265*	4.43	49.77	2.12	-2.26
SBI-07	51	*Xiabt26*	*Xisep0805*	5.38	46.76	-1.87	-2.54
SBI-09	331	*Xcup002*	*Xtxp296*	4.74	49.14	-2.10	-2.25
SBI-10	58	Xtxp141	Xtxp270	3.54	38.51	-2.25	-2.24
SBI-10	101	*Xtxp270*	*Xisep0639*	4.97	45.35	-1.99	-2.75
SBI-10	167	*Xisep0639*	*Xisep0622*	4.01	43.59	-1.83	-3.00

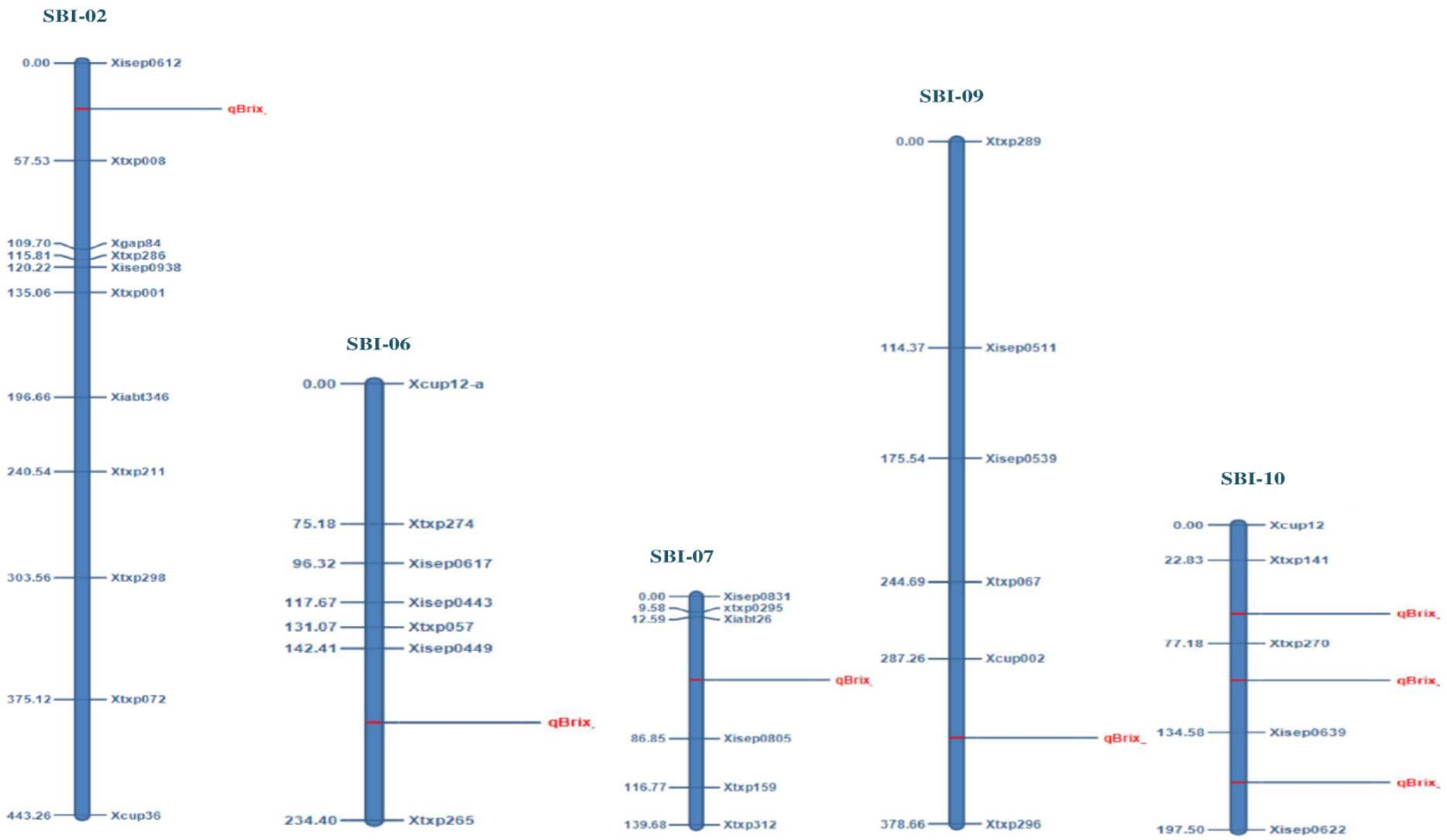


Fig. 4. 16. Map of QTLs controlling °Brix using F_2 genotypic data and combined phenotypic data of $F_{2:3}$ mapping populations using a LOD cutoff 3.5

4. 3. 2. Identification and mapping of QTLs associated with stay-green and yield related traits

4. 3. 2. 1. Phenotypic evalution

Subsequent crossing and selfing of parents resulted in successful development of F3 mapping populations. A clear segregation pattern for stay-green trait among the populations was observed with a mean value of 2.5. The significant variation among $F_{2:3}$ families for eight traits such as grain yield, thousand seed weight, plant height, panicle weight, panicle length, panicle width, chlorophyll content and stay-green feature were obtained. The mean score for stay-green source parent, *Sorcoll163/07*, was 1.5 whereas average mean value of 3.3 for *Gambella* (susceptible) parent was scored. Similarly, a mean value of 38.4 % and 23.5% chlorophyll content was obtained for the stay-green parent and non-stay-green parents, respectively. The mean chlorophyll content of the $F_{2:3}$ families ranged from 18.2 to 55.8 % with an average value of 33.4 %. There was significant grain yield variation among the two parents as well as progenies. The mean grain yield for the *Sorcoll163/07* was 79.6 g/plant whereas the mean grain yield for Gambella was found to be 44.8 g/plant.

The frequency distribution of all traits was found to be nearly normally distributed (Fig. 4.17). Only few traits showed slightly skewed type of distribution. The F_2 population showed a clear segregation for drought tolerance, which was observed during grain filling period (Fig. 4.18)

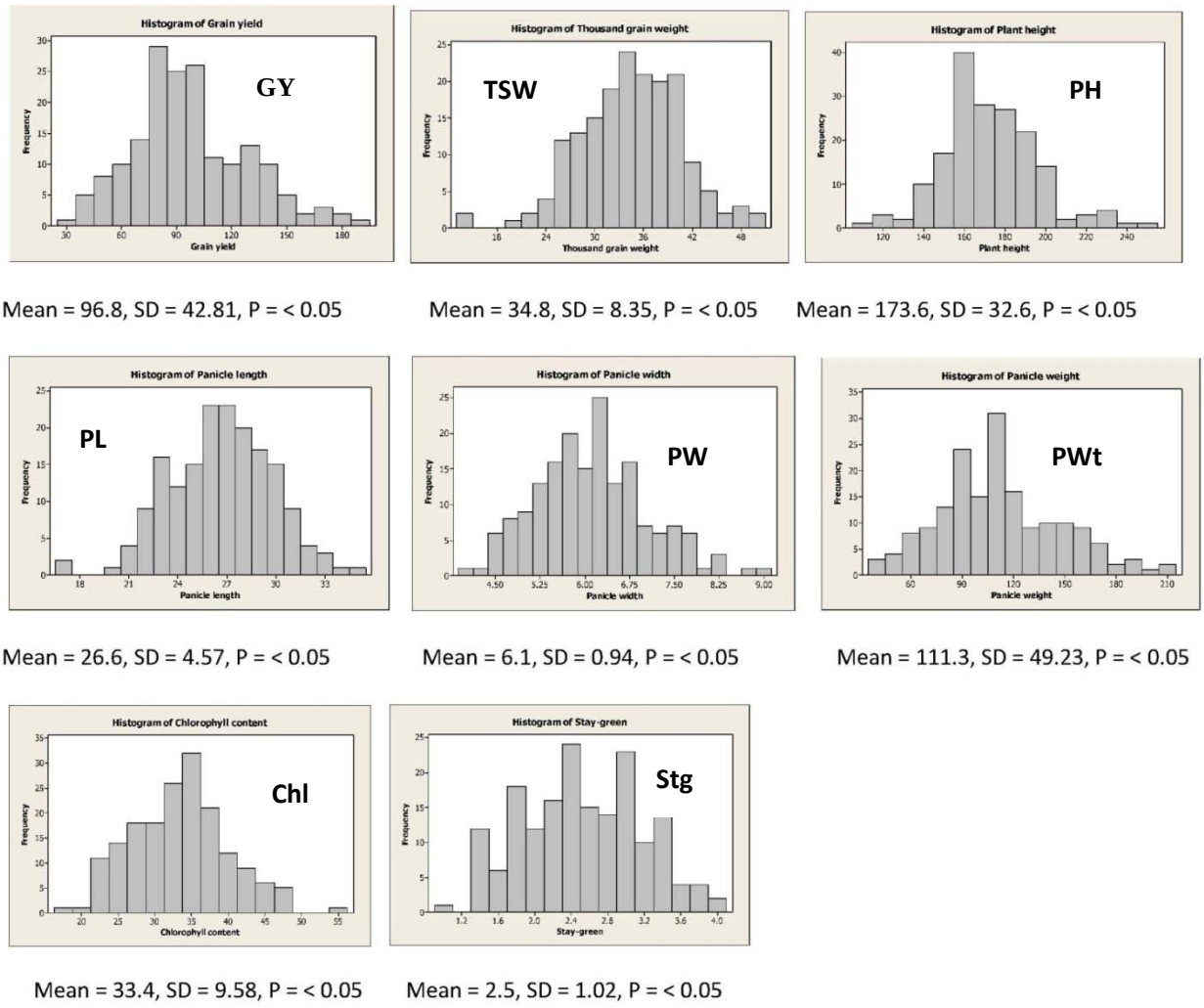


Fig. 4. 17. Frequency distributions of the $F_{2:3}$ mapping populations for eight selected traits (GY = grain yield, TSW = thousand seed weight, PH = plant height, PL = panicle length, PW = panicle width, PWt = panicle weight, Chl = chlorophyll content, Stg = stay-green)

Pearson correlation coefficients (r) of stay-green and yield related traits are shown in Table 4.17. Stay-green correlated significantly with three traits such as chlorophyll content, panicle length and plant height. The strong correlation was observed between chlorophyll content and stay-green feature (r = -0.770), followed by grain yield (r = -0.371) and plant height (r = -0.371). Negative sign doesn't mean that they are negatively correlated rather the negative sign was due to the reverse score (1-5 scale) given to plants with stay-green features. It is also moderately correlated with panicle width (r = -0.366), plant height (r = -0.276) and thousand grain weight (r = 0.238). Slightly positive correlation was observed between stay-green character and panicle length (r = 0.173). Strongest positive correlation was observed between grain yield and panicle weight (r = 1.000). Furthermore, grain yield was strongly correlated with the rest of the traits. Thousand seed weight showed significant strong positive correlation with panicle weight (r = 0.535), followed by panicle width (r = 0.389) and stay-green feature (r = 0.238). The strongest correlation of plant height with the rest of the traits was observed in panicle weight (r = 0.366)

Fig. 4. 18. $F_{2:3}$ segregating populations (stay-green plants left and non-stay green plants right) evaluated at Werer Agricultural Research Center, Ethiopia using regulated irrigation supply.

Table 4. 17. Correlation among seven selected traits based on the 192 $F_{2:3}$ mapping populations

Traits	Grain yield	Thousand seed weight	Plant height	Panicle length	Panicle width	Panicle weight	Chlorophyll content
Thousand seed weight	**0.535*****						
Plant height	0.366***	0.230**					
Panicle length	0.461***	0.170*	0.213**				
Panicle width	**0.692*****	0.389***	0.318***	**0.507*****			
Panicle weight	**0.990*****	**0.535*****	0.366***	0.461***	**0.692*****		
Chlorophyll content	0.316***	0.130	0.176*	0.142	0.263***	0.316***	
Stay-green	-0.371***	-0.238**	-0.276***	-0.173*	-0.366***	-0.371***	**-0.770*****

Significance levels are indicated by *, ** and * for P = 0.05, 0.01 and 0.001, respectively. Stay-green trait is positively correlated with the rest of the traits; the negative sign was due to giving low scale for the most stay-green plants.**

4. 3. 2. 2. QTL detection and mapping

Phenotypic variation of chlorophyll content, stay-green, grain yield, thousand seed weight, panicle length, panicle width and panicle weight were computed. The stay-green source parent, Sorcoll 163/07, was characterized by its relatively higher chlorophyll content and remains green during post-flowering drought stress condition, whereas Gambella which is among the farmers preferred improved genotypes for its seed quality as well as yield potential, was found to be relatively drought susceptible and categorized as non-stay green parent in this study.

A total of fourteen QTLs controlling various traits were successfully mapped on seven linkage groups with a LOD value ranging from 2.09 to 3.79 (Table 4.18, Fig.4.19). The pattern of those QTLs that influence stay-green, chlorophyll content and grain yield follow appeared in similar fashion (Fig. 4.20).

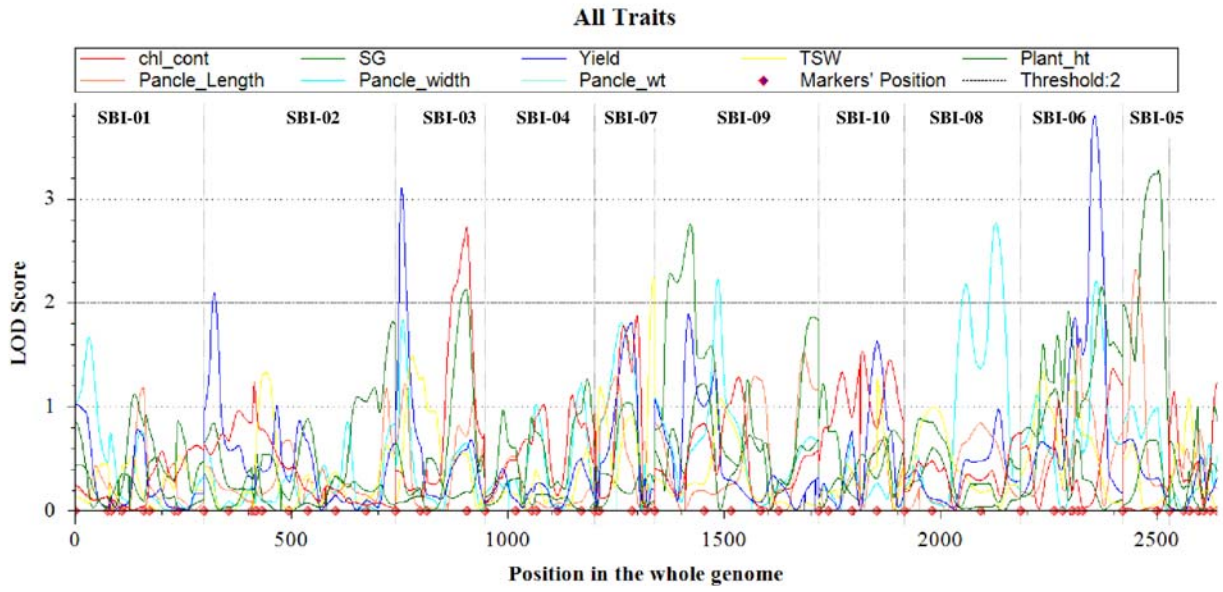


Fig. 4. 19. Position of detected QTLs in the whole genome and LOD scores for eight traits across the whole linkage groups.

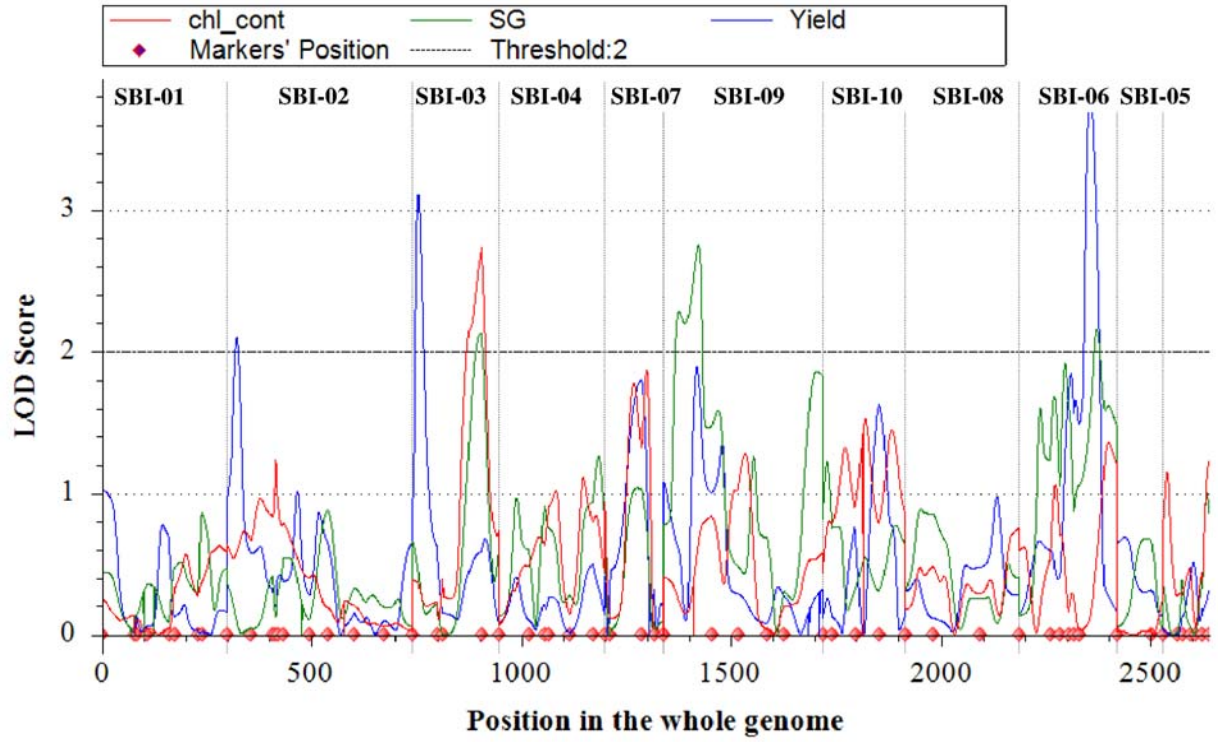


Fig. 4. 20. LOD scores for three traits across the whole linkage groups.

4. 3. 2. 3. QTL detection for stay-green

Two QTLs were identified on linkage groups SBI-03 (flanked by *Xtxp034-Xtxp033* markers) and SBI-09 (flanked by *Xtxp289- Xisep0511* markers) based on average performance of each $F_{2:3}$ mapping population evaluated for their drought tolerance using regulated irrigation supply. Attempt to validate these QTLs under drought prone areas, resulted in four QTLs distributed on various linkage groups (SBI-01, SBI-06, SBI-08 and SBI-09). One QTL which is located on SBI-09 was detected in both environments. This QTL was also categorized as a major QTL explaining 15.2% of the phenotypic variance with both additive and dominance effect of 0.05 and 0.97, respectively.

4. 3. 2. 4. QTL for chlorophyll content

A QTL responsible for chlorophyll content is co-localized with stay-green on SBI-03 linkage group flanked by the same flanking markers (*Xtxp034-Xtxp033).* This individual QTL explained 7.3 % of the phenotypic variation with additive and dominant effect of -3.1 and 1.4, respectively. Result from the second environment showed that two different QTLs controlling chlorophyll content on linkage group SBI-09 and SBI-10 flanked by *Xtxp289- Xisep0511* and *Xcup12-Xtxp141* markers, respectively (Fig. 4.21). Similar to the first experiment, the QTL for chlorophyll content coexisted with stay green QTL at both positions with the same flanking markers (Figs. 4.21 and 4.22), which is an indication of the high trait correlation. Furthermore, one of the chlorophyll content QTLs detected on linkage group SBI-09 also share the same flanking marker with QTL that influence grain yield (Fig. 4.22).

4. 3. 2. 5. QTLs for yield and yield components

Based on the evaluated phenotypic performance from the first experiment during 2013, Inclusive Composite Interval Mapping (ICIM) detected three QTLs responsible for grain yield. These QTLs were located on SBI-02 (*Xisep0612-Xtxp008),* SBI-03 (*Xisep0138- Xtxp285)* and SBI-06 (*Xisep0449- Xtxp265)* with a LOD value of 2.09, 3.11 and 3.19, respectively. Each of them contributed to significant phenotypic variation of 30.33 % (SBI-02), 46.66 % (SBI-03) and 47.10% (SBI-06) with additive effects of -18.61, 4.37 and 21.95, respectively. The additive effect of QTLs was -28.20 (SBI-02), 49.98 (SBI-03) and -29.05 (SBI-03). One additional QTL was detected using mean performance of the second environment (2014) which is mapped to linkage group SBI-03.

ICIM also identified one minor QTL for thousand seed weight located on linkage group SBI-07 (*Xtxp159-Xtxp312)* with a LOD value of 2.25. The QTL contributed 5.86 % of the total phenotypic variation and considered as minor QTLs. Both the additive and dominance effect of values were positive 1.80 and 1.44, respectively. The QTL is much closer to *Xtxp312* than *Xtxp159* (Fig. 5.21). In contrary, one major QTL controlling panicle length on SBI-05 with a LOD value of 2.32 was detected and responsible for 40.57 % of the phenotypic variation. The QTL is flanked by *Xcup36- sbKAKGK01*markers and relatively closer to *Xcup36.*

Three QTLs controlling panicle width of sorghum were mapped on linkage groups and their respective flanking markers such as SBI-09 *(Xisep0511- Xisep0539)*, SBI-08 *(Xisep1231-Xtxp250)* and SBI-05 (*Xisep0449-Xtxp265)* with a LOD value of 2.23, 2.76 and 2.22, respectively. All of them were found to be major QTLs and explain 28.47 %, 40.21 % and 38.45 % of the phenotypic variance, respectively. Similarly, three QTLs controlling panicle weight were detected on SBI-02 *(Xisep0612-Xtxp008)*, SBI-03 (*Xisep0138-Xtxp285*) and SBI-09 (*Xisep0449-Xtxp265*) each contributing 46.63%, 30.33%, and 47.10 % of the phenotypic variance, respectively. The LOD values ranged from 2.09 to 3.79 with additive effect ranging from -21.40 to 25.24. Very strong dominance effect with high positive value and negative values were obtained in this experiment (Table 4.18).

Table 4. 18. Mapped quantitative trait loci for stay-green and yield related traits under the post-flowering drought stress in sorghum

Trait Name	LG	Posit.	L. Marker	R. Marker	LOD	PVE (%)	Add	Dom
Chlorophyll content	SBI-03	164	*Xtxp034*	*Xtxp033*	2.73	7.34	-3.08	1.37
Stay-green	SBI-03	163	*Xtxp034*	*Xtxp033*	2.13	6.18	0.28	-0.17
Stay-green	SBI-09	82	*Xtxp289*	*Xisep0511*	2.75	15.16	0.05	0.97
Grain yield	SBI-02	24	*Xisep0612*	*Xtxp008*	2.09	30.33	-18.61	-28.20
Grain yield	SBI-03	14	*Xisep0138*	*Xtxp285*	3.11	46.66	4.37	49.98
Grain yield	SBI-06	169	*Xisep0449*	*Xtxp265*	3.79	47.10	21.95	-29.05
1000 seed weight	SBI-07	139	*Xtxp159*	*Xtxp312*	2.25	5.86	1.80	1.44
Panicle length	SBI-05	31	*Xcup36-b*	*sbKAKGK01*	2.32	40.57	1.35	3.58
Panicle width	SBI-06	173	*Xisep0449*	*Xtxp265*	2.22	38.45	0.58	-0.85
Panicle width	SBI-08	212	*Xisep1231*	*Xtxp250*	2.76	40.21	-0.64	-0.84
Panicle width	SBI-09	146	*Xisep0511*	*Xisep0539*	2.23	28.47	-0.50	-0.75
Panicle weight	SBI-02	24	*Xisep0612*	*Xtxp008*	2.09	30.33	-21.40	-32.43
Panicle weight	SBI-03	14	*Xisep0138*	*Xtxp285*	3.11	46.63	5.03	57.46
Panicle weight	SBI-06	169	*Xisep0449*	*Xtxp265*	3.79	47.10	25.24	-33.41

L = Left, R = Right

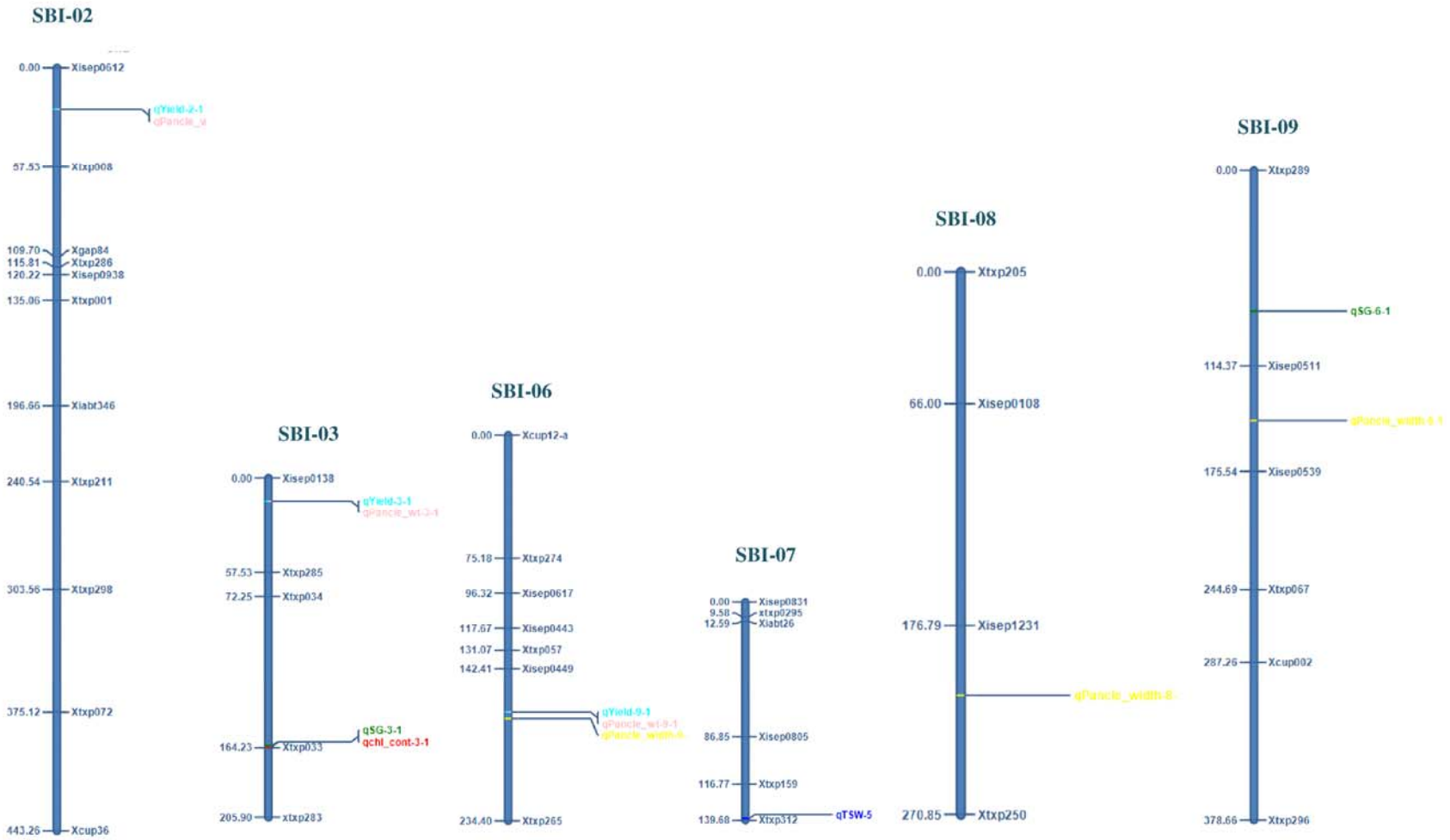


Fig. 4. 21. Mapped quantitative trait loci based on 192 $F_{2:3}$ mapping population derived from *Sorcoll163/07* x *Gambella.* QTLs were detected using a LOD threshold of 2.0 (Werer station). **Light blue = yield trait, Red = chlorophyll content, Yellow = Pancle width, Green = stay-green, Brown = pancle weight, Deep blue= thousand seed weight**

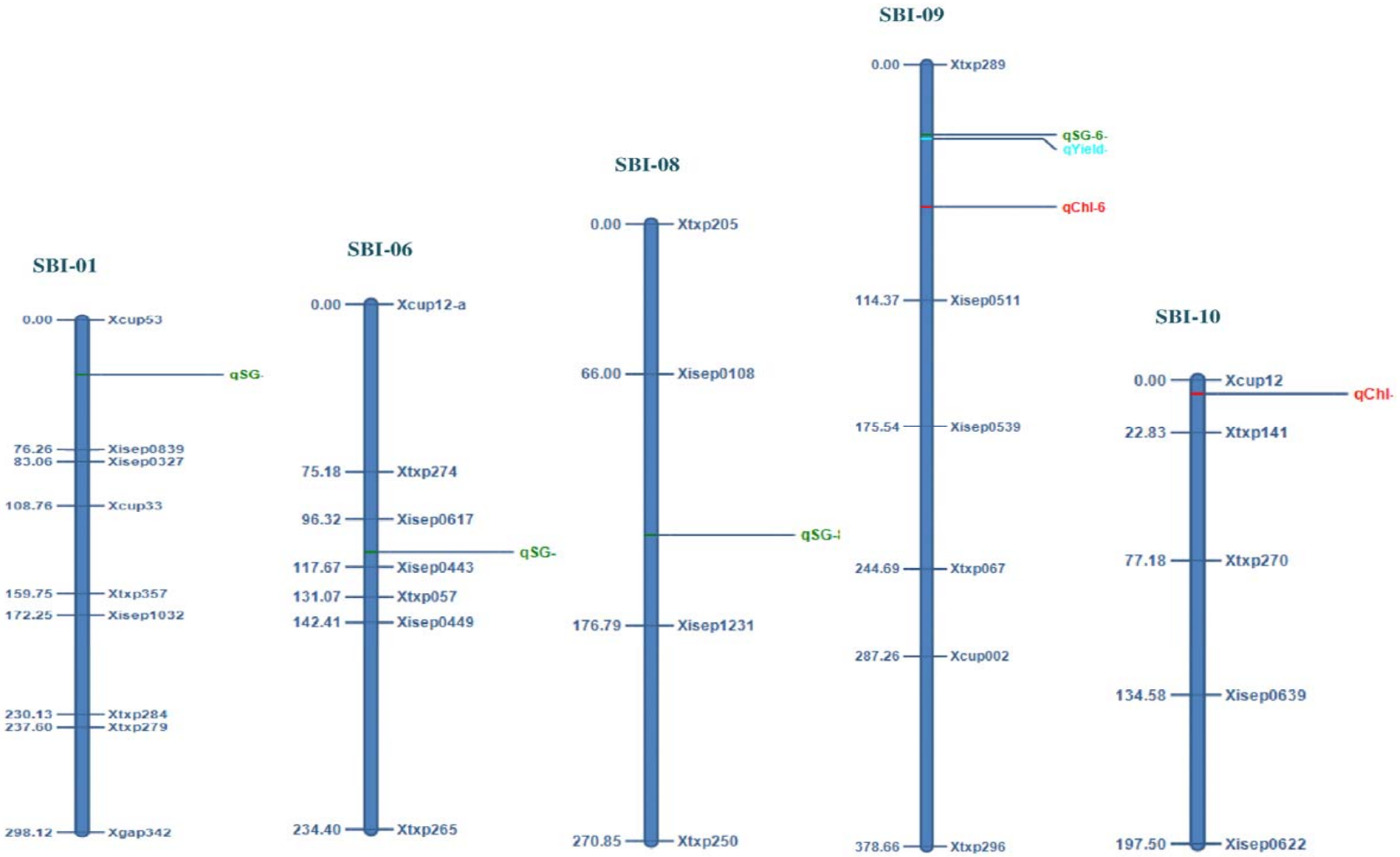


Fig. 4. 22. Mapped quantitative trait loci mapping based on 192 F2:3 mapping population derived from Sorcoll163/07 x Gambella (Dhera station)

4. 4. Screening and compiling of informative microsatellite sets for marker-assisted breeding of Key Ethiopian sorghum cultivars

4. 4. 1. Comparison of DNA isolation protocol

High quality genomic DNA was obtained using both CTAB and Kit methods (Table 4.19, Fig. 4.23). Both methods yielded high concentration of DNA. The concentration obtained using CTAB method is reasonably high ranging from 123.1 to 433.3 ng/µl (Table 4.19). The concentration obtained using Kit method is nearly double as compared to CTAB method of DNA isolation with the lowest and highest score of 434.7 ng/µl for *76T#23* and 758.6 ng/µl for *Sorcoll 163/07* genotypes, respectively. The ratio of 260 to 280 obtained using CTAB method was between 1.83 and 2.01 which is within the expected range of good quality DNA free from impurities. Similarly, the quality of genomic DNA isolated using Kit method is high with a 260 to 280 ratio ranging from 1.78 to 1.89 (Table 4.19).

The genomic DNA isolated using the two approaches worked very well and showed no difference during microsatellite analysis (Fig. 4.24). The modified CTAB method will be cost effective method for sorghum and related species.

Table 4. 19. Concentration test of genomic DNA extracted using CTAB and Kit method (the precise concentration was quantified using *Qubit®2.0 (Life Technologies*, Grand Island, NY) while 260nm/280nm absorbance ratios was measured using spectrophotometer in order to monitor the purity of DNA)

Genotypes	CTAB Method		Kit Method	
	Conc. (ng/µl)	260nm/280nm	Conc. (ng/µl)	260nm/280nm
Sorcoll 146/07	221.4	1.83	460.1	1.82
Teshale	235.8	1.91	491.8	1.82
76T#23	286.8	1.83	434.7	1.78
Sorcoll 163/07	283.5	1.83	758.6	1.84
Melkam	123.1	1.98	634.6	1.82
Meko	353.7	1.86	700.3	1.86
Macia	179.8	1.95	488.9	1.87
Gambella	160.2	1.81	424.6	1.82
B 35	433.3	1.87	647.5	1.88
Sorcoll 141/07	155.0	1.88	538.8	1.80
E 36-1	155.4	2.01	446.3	1.89

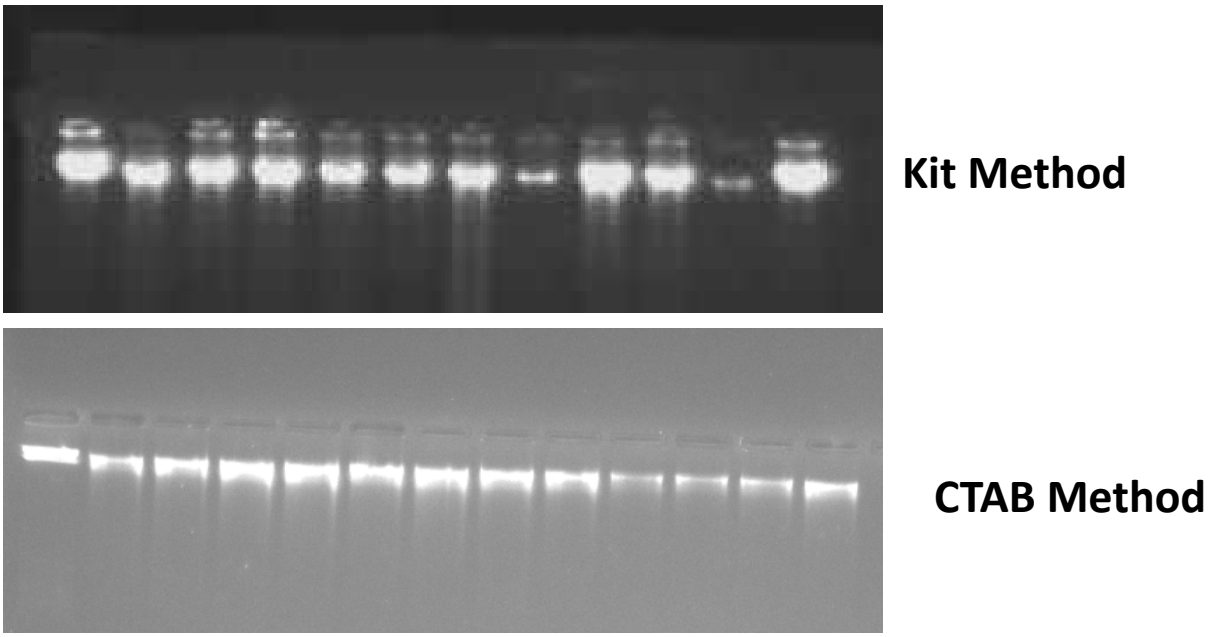


Fig. 4. 23. Comparison of CTAB and Kit based DNA isolation method for quality of genomic DNA tested using 0.8% Agarose gel electrophoresis

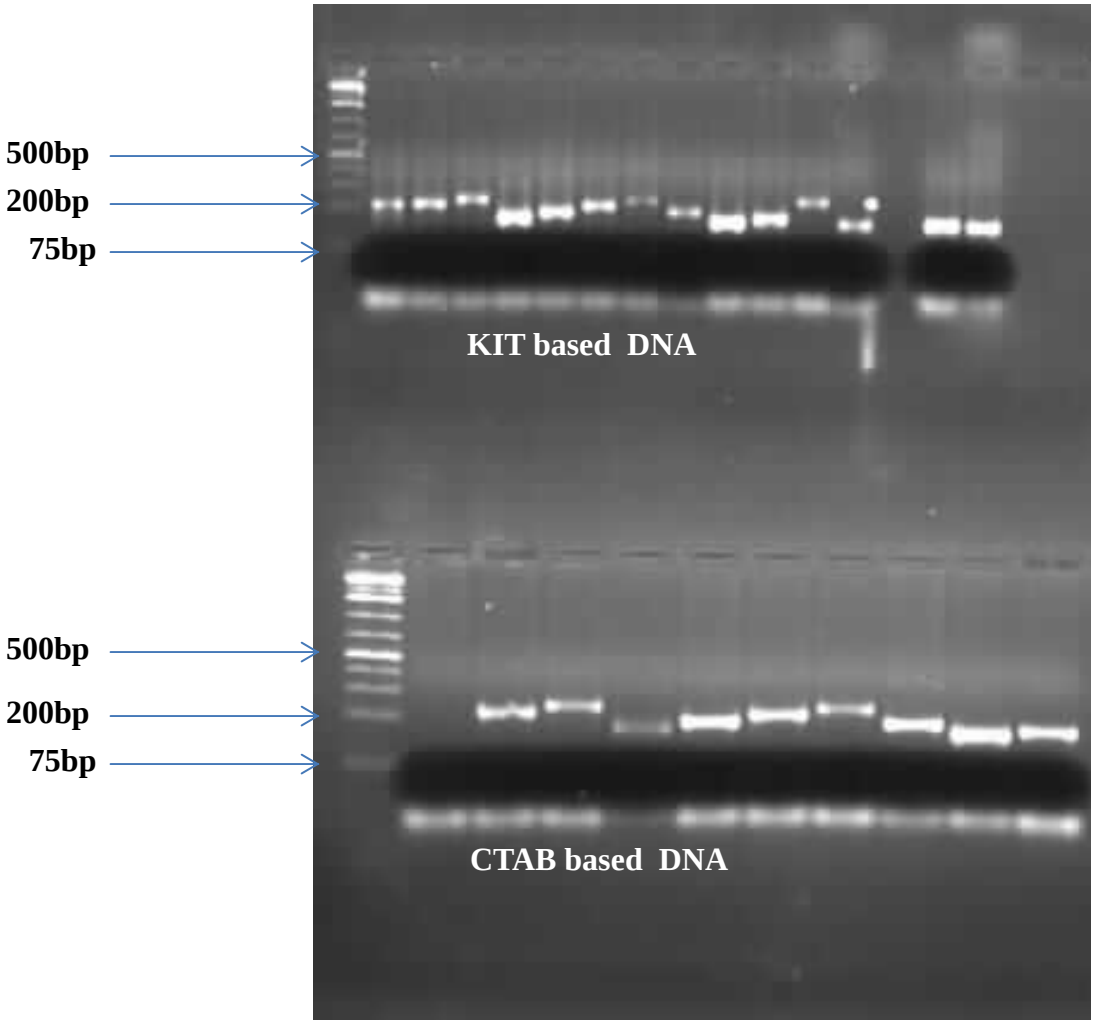


Fig. 4. 24. Comparison of CTAB and Kit based genomic DNA isolation protocol for quality PCR product separated on 2 % (w/v) agarose gel electrophoresis.

4. 4. 2. SSR polymorphism

Fragment analysis result showed that among 300 SSR primer pairs tested, a total of 142 polymorphic markers evenly distributed across all linkage groups (Table 4.20). However, three

markers were excluded due to their poor quality. A total of 139 (45.7%) showed clear band and found to be polymorphic across the selected eleven sorghum genotypes. In contrary, 97 (31.9%) were unable to discriminate the genotypes and considered as monomorphic markers and the remaining 68 (22.4%) didn't work at all. Most of the polymorphic and monomorphic markers were clearly separated during fragment scoring (Fig. 4.26).

The majority of the markers (40 %) had fragment size between 201 to 250 bp (Fig. 4.25). Similarly, second highest frequency (29 %) was observed for allele size ranged from 151 to 200 bps. Only very few markers (6 %) have showed fragment size between 300 and 400 bps.

Table 4. 20. Distribution of the polymorphic markers across the ten linkage groups

No	Chromosomes (Linkage groups)										
	A (SBI-01)	**B (SBI-02)**	**C (SBI-03)**	**D (SBI-04)**	**E (SBI-07)**	**F (SBI-09)**	**G (SBI-10)**	**H (SBI-08)**	**I (SBI-06)**	**J (SBI-05)**	**Not well known**
1	*Xcup33*	*Xtxp008*	*Xtxp033*	*Xtxp012*	*Xtxp159*	*Xtxp0289*	*Xtxp0141*	*Xisep108*	*Xisep0429*	*Xisep1029*	*Xisep0643*
2	*xgap342*	*Xgap084*	*Xtxp285*	*Xtxp041*	*Xisep704*	*Xtxp0296*	*Xgap001*	*Xisep809*	*Xisep0444*	*Xisep1127*	*Xisep0648*
3	*Xtxp032*	*Xisep310*	*Xisep0114*	*Xtxp021*	*Xisep0716*	*Xgap032*	*Xisep314*	*Xisep0815*	*Xisep0502*	*Xtxp014*	*Xisep0830*
4	*Xtxp357*	*Xisep0612*	*Xisep0117*	*Xtxp024*	*Xisep0829*	*Xisep110*	*Xisep0621*	*Xisep1150*	*Xisep346*	*sbKAKG1*	*Xisep0901*
5	*Xisep327*	*Xisep0938*	*Xisep0132*	*Xisep202*	*Xisep831*	*Xisep0517*	*Xisep0622*	*Xisep1225*	*Xisep0422*	*Xisep1208*	*Xisep0905*
6	*Xisep0839*	*Xisep1013*	*Xisep138*	*Xisep224*	*Xisep0328*	*Xisep537*	*Xisep0624*	*Xtxp205*	*Xisep423*	*Xtxp303*	*Xisep1717*
7	*Xisep1032*	*Xcup26*	*Xcup24*	*Xisep0234*	*Xisep0805*	*Xisep0539*	*Xisep0639*	*Xisep1231*	*Xisep0443*	*Xisep1107*	*Xtxp113*
8	*Xisep1039*	*Xcup40*	*Xcup61*	*Xisep0242*	*Xtxp312*	*Xisep0543*	*Xcup50*	*Xtxp0210*	*Xisep0449*	*Xisep1140*	*Xtxp160*
9	*Xisep1046*	*Xtxp211*	*Xisep0101*	*Xcup048*	*SBGEF06*	*Xcup002*	*Xisep0630*		*Xisep0617*	*Xtxp091*	*Xtxt267*
10	*Xtxp080*	*Xtxp315*	*Xtxp205*	*Xtxp328*		*Xisep511*	*Xtxp270*		*Xcup12*	*Xtxp023*	*Xtxp355*
11	*Xtxp329*	*Xtxp348*	*SABGEF08*	*Xisep1103*		*Xisep0506*	*SBGE01*		*Xisep0435*	*Xtxp283*	*Xtxp307*
12	*Xcup53*	*SABAGA04*	*Xisep1042*	*Xtxp343*		*Xisep0550*	*Xcup49*		*Xtxp274*		*Xtxp361*
13	*Xcup22*	*SBAGAB03*	*Xtxp031*	*Xtxp026*		*Xtxp324*					
14	*Xtxp279*	*Xcup36*	*Xtxp034*			*Xtxp067*					
15	*Xtxp284*	*Xisep1145*				*Xtxp258*					
16	*Xtxp335*	*Xtxp072*				*Xtxp324*					
17		*Xtxp286*				*Xtxp358*					
18		*Xtxp298*									
Total	**16**	**18**	**14**	**13**	**9**	**17**	**12**	**8**	**12**	**11**	**12**

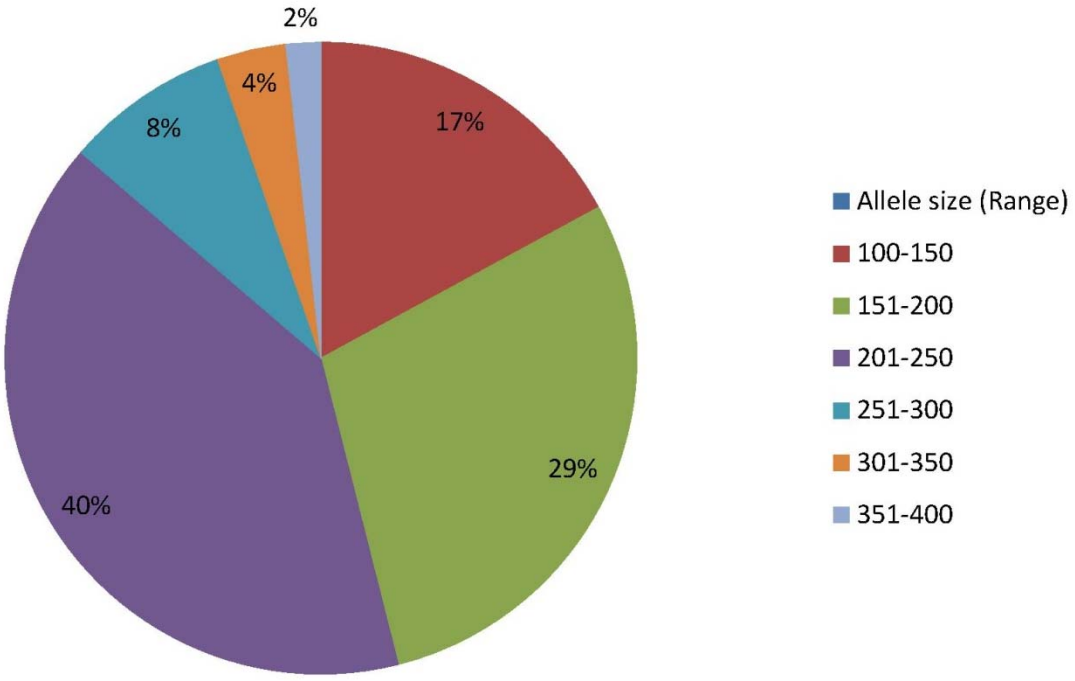


Fig. 4. 25. Frequency distribution of alleles based on their fragment size (in bp)

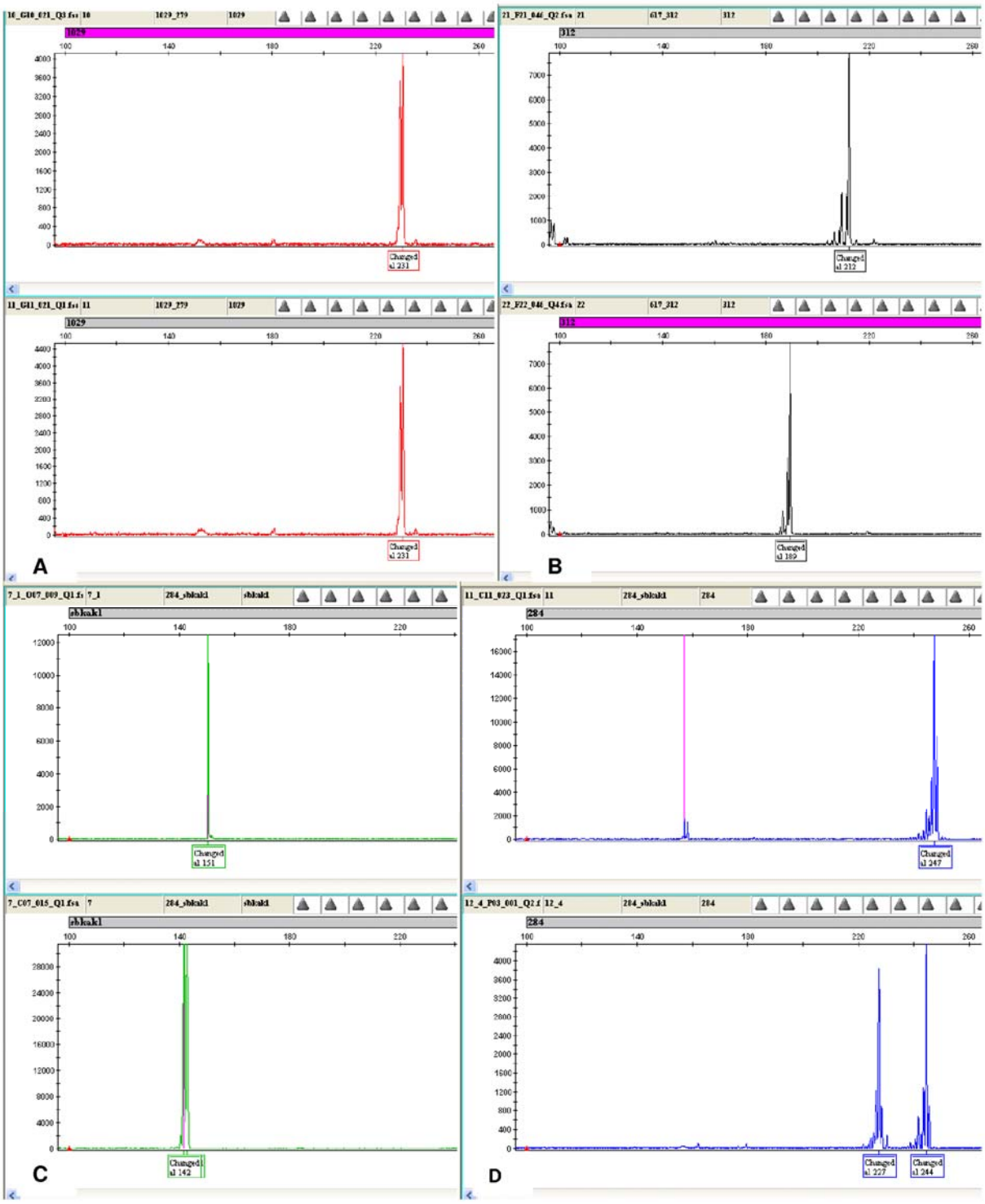


Fig. 4. 26. Scored bins showing (A) a monomorphic marker, unable to discriminate the genotypes and (B, C and D) polymorphic markers. Each marker was labeled M-13 tagged forward primer labels (A = PET, B = NED, C = VIC and D = FAM)

One hundred thirty nine informative SSR markers produced a total of 543 alleles across the eleven key Ethiopian genotypes with an average of 3.91 alleles per marker. The observed number of alleles ranged from two to ten per primer pair. *Xtxp008* produced the highest number of alleles (10) followed by *Xtxp032* (9) and *Xtxp033* (8). These markers also presented higher values of gene diversity (*He* = 0.9).

Majority of the markers (56 %) have a PIC value of greater than 0.5 and considered as very informative. Similarly 58 out of 139 markers (42 %) showed PIC value between 0.25 and 0.5 which is categorized as informative markers. Only three markers *Xisep0805*, *Xisep0444* and *Xisep0815* grouped as less informative. The highest PIC values of the scored SSR loci were obtained from *Xtxp343* which was 0.91 whereas the lowest PIC value (0.15) was obtained from *Xisep0815* with an average value of 0.53 (Table 4.21).

Lowest mean value (0.16) for observed heterozygosity (*Ho*) was obtained in this experiment (Table 4.21). Nearly 60 % (83 markers) have showed zero value of the *Ho*. Only four markers *Xisep0938, Xisep0704, Xtxp159* and *Xtxp283* had *Ho* value of 1.0. In contrast, the gene diversity index (expected heterozygosity, *He*) was ranging from 0.17 for *Xisep 0805* to 0.91 for *Xtxp343* with a mean value of 0.58. The lowest gene diversity index and PIC value was scored from *Xisep0805*. Among the tested SSR markers, primers named as Xtxp showed higher PIC as well as highly informative features.

Table 4. 21. Diversity statistics of key Ethiopian sorghum genotypes computed with 139 polymorphic SSR loci

Marker	Maj. Alle. Freq.	Allele No.	Avail.	Gene Diversity	Hetero.	PIC
Xcup033	0.55	4.00	1.00	0.59	0.09	0.52
Xgap342	0.36	5.00	1.00	0.74	0.00	0.70
Xtxp008	0.20	10.00	0.91	0.88	0.20	0.87
Xtxp012	0.45	4.00	1.00	0.64	0.73	0.58
Xtxp041	0.50	4.00	1.00	0.65	0.36	0.59
Xtxp021	0.45	3.00	1.00	0.63	0.00	0.55
Xtxp024	0.41	7.00	1.00	0.77	0.18	0.74
Xtxp032	0.20	9.00	0.91	0.87	0.80	0.85
Xtxp033	0.14	8.00	0.64	0.87	0.43	0.85
Xtxp0141	0.45	4.00	1.00	0.64	0.00	0.58
Xtxp159	0.50	4.00	1.00	0.61	1.00	0.53
Xtxp285	0.73	4.00	1.00	0.45	0.00	0.42
Xtxp0289	0.50	4.00	0.73	0.66	0.00	0.60
Xtxp0296	0.36	4.00	1.00	0.69	0.00	0.64
Xtxp357	0.45	3.00	1.00	0.63	0.00	0.55
Xgap001	0.30	6.00	0.91	0.79	0.10	0.75
Xgap032	0.25	7.00	0.73	0.84	0.00	0.82
Xgap084	0.71	3.00	0.64	0.45	0.00	0.41
Xisep0110	0.73	2.00	1.00	0.40	0.00	0.32
Xisep0114	0.82	3.00	1.00	0.31	0.00	0.29
Xisep0117	0.82	3.00	1.00	0.31	0.00	0.29
Xisep0132	0.38	4.00	0.73	0.71	0.13	0.66
Xisep0138	0.40	4.00	0.91	0.66	0.20	0.59
Xisep0202	0.40	5.00	0.91	0.67	0.30	0.60
Xisep0224	0.60	3.00	0.91	0.54	0.00	0.47
Xisep0234	0.50	4.00	0.91	0.64	0.00	0.58
Xisep0242	0.38	4.00	0.73	0.69	0.00	0.63
Xisep0310	0.39	5.00	0.82	0.75	0.33	0.71
Xisep0314	0.25	6.00	0.73	0.81	0.00	0.79
Xisep0327	0.65	3.00	0.91	0.49	0.10	0.41
Xisep0429	0.50	3.00	0.82	0.55	0.11	0.45
Xisep0444	0.89	2.00	0.82	0.20	0.00	0.18
Xisep0502	0.50	3.00	0.82	0.61	0.11	0.54
Xisep0517	0.38	3.00	0.73	0.66	0.00	0.58
Xisep0537	0.80	3.00	0.91	0.34	0.00	0.31
Xisep0539	0.43	3.00	0.64	0.61	0.00	0.53
Xisep0543	0.60	3.00	0.91	0.56	0.00	0.50

Table 4.21. Continued

Marker	Maj. Alle. Freq.	Allele No.	Avail.	Gene Diversity	Hetero.	PIC
Xisep0621	0.40	4.00	0.91	0.72	0.20	0.67
Xisep0622	0.50	4.00	0.73	0.66	0.00	0.60
Xisep0624	0.56	3.00	0.82	0.54	0.78	0.44
Xisep0643	0.50	5.00	0.73	0.67	0.75	0.63
Xisep0648	0.75	3.00	0.55	0.40	0.33	0.36
Xisep0704	0.50	4.00	0.73	0.60	1.00	0.53
Xisep0716	0.50	3.00	1.00	0.60	0.91	0.52
Xisep0809	0.63	2.00	0.73	0.47	0.00	0.36
Xisep0815	0.88	2.00	0.73	0.22	0.00	0.19
Xisep0829	0.50	3.00	0.91	0.58	0.00	0.49
Xisep0830	0.60	2.00	0.91	0.48	0.00	0.36
Xisep0831	0.60	3.00	0.91	0.54	0.00	0.47
Xisep0839	0.50	2.00	0.91	0.50	0.00	0.38
Xisep0901	0.63	2.00	0.73	0.47	0.00	0.36
Xisep0905	0.64	3.00	0.64	0.52	0.14	0.46
Xisep0938	0.40	4.00	0.91	0.69	1.00	0.63
Xisep1013	0.50	2.00	0.73	0.50	0.00	0.38
Xisep1029	0.56	4.00	0.82	0.62	0.00	0.57
Xisep1032	0.73	4.00	1.00	0.44	0.09	0.41
Xisep1039	0.60	2.00	0.91	0.48	0.00	0.36
Xisep1046	0.80	3.00	0.91	0.34	0.10	0.30
Xisep1127	0.82	2.00	1.00	0.30	0.00	0.25
Xisep1150	0.33	4.00	0.55	0.72	0.00	0.67
Xisep1717	0.50	3.00	0.82	0.62	0.78	0.55
Xtxp080	0.43	5.00	0.64	0.73	0.00	0.70
Xtxp329	0.44	4.00	0.82	0.69	0.00	0.64
Xcup002	0.64	3.00	1.00	0.51	0.00	0.44
Xcup024	0.50	3.00	0.91	0.62	0.00	0.55
Xcup048	0.57	4.00	0.64	0.61	0.00	0.57
Xcup053	0.60	5.00	0.91	0.60	0.00	0.57
Xcup061	0.59	2.00	1.00	0.48	0.09	0.37
Xisep0101	0.30	5.00	0.91	0.78	0.00	0.74
Xisep0108	0.60	4.00	0.91	0.58	0.00	0.54
Xisep0328	0.70	3.00	0.91	0.46	0.00	0.41
Xisep0346	0.73	3.00	1.00	0.43	0.00	0.39
Xisep0422	0.45	3.00	1.00	0.63	0.00	0.55
Xisep0423	0.45	4.00	0.91	0.63	0.30	0.55
Xisep0443	0.45	5.00	1.00	0.71	0.00	0.67
Xisep0612	0.38	4.00	0.73	0.72	0.00	0.67

Table 4.21. Continued

Marker	Maj. Alle. Freq.	Allele No.	Avail.	Gene Diversity	Hetero.	PIC
Xisep0449	0.55	2.00	1.00	0.50	0.00	0.37
Xisep0511	0.41	7.00	1.00	0.76	0.36	0.73
Xisep0639	0.27	7.00	1.00	0.83	0.00	0.80
Xtxp014	0.30	6.00	0.91	0.80	0.00	0.77
sbKAKG01	0.55	4.00	1.00	0.63	0.00	0.58
Xcup22	0.73	2.00	1.00	0.40	0.00	0.32
Xcup26	0.82	3.00	1.00	0.31	0.00	0.29
Xcup40	0.55	3.00	1.00	0.56	0.00	0.48
Xcup50	0.45	3.00	1.00	0.64	0.09	0.57
Xisep0506	0.64	3.00	1.00	0.51	0.00	0.44
Xisep0550	0.73	4.00	1.00	0.45	0.00	0.42
Xisep0617	0.70	2.00	0.91	0.42	0.00	0.33
Xisep0630	0.56	3.00	0.82	0.59	0.00	0.53
Xisep0805	0.91	2.00	1.00	0.17	0.00	0.15
Xisep1208	0.50	3.00	0.73	0.59	0.00	0.51
Xtxp211	0.27	7.00	1.00	0.80	0.55	0.77
Xtxp270	0.55	5.00	1.00	0.64	0.00	0.61
Xtxp279	0.27	6.00	1.00	0.81	0.00	0.78
Xtxp284	0.36	6.00	1.00	0.78	0.00	0.75
Xtxp303	0.68	3.00	1.00	0.48	0.27	0.42
Xtxp312	0.27	6.00	1.00	0.81	0.00	0.78
Xtxp315	0.45	3.00	1.00	0.58	0.00	0.49
Xtxp328	0.75	3.00	0.73	0.41	0.00	0.37
Xtxp348	0.70	2.00	0.91	0.42	0.00	0.33
SABAGAH04	0.27	7.00	1.00	0.79	0.55	0.76
SABGEF08	0.64	2.00	1.00	0.46	0.18	0.36
SBAGAB03	0.60	5.00	0.91	0.57	0.40	0.52
SBGE01	0.72	5.00	0.82	0.46	0.22	0.43
SBGEF06	0.61	2.00	0.82	0.48	0.56	0.36
Xcup12	0.64	4.00	1.00	0.55	0.00	0.50
Xcup36	0.32	5.00	1.00	0.78	0.36	0.74
Xcup49	0.77	3.00	1.00	0.38	0.09	0.34
Xisep1042	0.68	3.00	1.00	0.48	0.09	0.43
Xisep1103	0.82	2.00	1.00	0.30	0.00	0.25
Xisep1107	0.73	3.00	1.00	0.43	0.00	0.39
Xisep1140	0.45	4.00	0.91	0.69	0.70	0.63
Xisep1145	0.85	3.00	0.91	0.27	0.10	0.25

Table 4.21. Continued

Marker	Maj. Alle. Freq.	Allele No.	Avail.	Gene Diversity	Hetero.	PIC
Xisep1225	0.68	5.00	1.00	0.50	0.18	0.46
Xtxp343	0.18	5.00	1.00	0.91	0.73	0.91
Xtxp026	0.27	7.00	1.00	0.83	0.00	0.80
Xtxp067	0.64	2.00	1.00	0.46	0.00	0.36
Xtxp072	0.33	7.00	0.82	0.80	0.78	0.77
Xtxp091	0.45	5.00	0.91	0.71	0.60	0.67
Xtxp113	0.45	5.00	1.00	0.71	0.82	0.67
Xtxp160	0.68	5.00	1.00	0.51	0.36	0.48
Xtxp205	0.70	2.00	0.91	0.42	0.20	0.33
XtXt267	0.56	5.00	0.82	0.64	0.00	0.61
Xtxp335	0.56	4.00	0.82	0.57	0.11	0.50
Xisep1231	0.60	2.00	0.91	0.48	0.00	0.36
Xisep0435	0.77	2.00	1.00	0.35	0.45	0.29
Xtxp021	0.55	3.00	1.00	0.60	0.00	0.53
Xtxp023	0.56	3.00	0.82	0.59	0.00	0.53
Xtxp031	0.67	3.00	0.55	0.50	0.00	0.45
Xtxp034	0.64	5.00	1.00	0.56	0.00	0.53
Xtxp258	0.27	5.00	1.00	0.76	0.00	0.72
Xtxp274	0.45	6.00	1.00	0.73	0.00	0.70
Xtxp283	0.50	3.00	1.00	0.60	1.00	0.52
Xtxp286	0.73	3.00	1.00	0.43	0.00	0.39
Xtxp298	0.33	5.00	0.82	0.77	0.00	0.73
Xtxp307	0.65	5.00	0.91	0.55	0.10	0.52
Xtxp324	0.59	3.00	1.00	0.57	0.45	0.50
Xtxp358	0.56	5.00	0.73	0.63	0.13	0.60
Xtxp361	0.36	6.00	1.00	0.76	0.00	0.73
Total	**74.49**	**543**	**124.96**	**81.24**	**22.07**	**73.49**
Mean	**0.54**	**3.91**	**0.90**	**0.58**	**0.16**	**0.53**

4. 4. 3. Genetic relationship

The 139 polymorphic markers discriminated the selected Ethiopian sorghum genotypes with stay-green features in one group including the worldwide known stay-green sources of sorghum, *B 35* and *E 36-1* (Figs.4.27, 4.28). Neighbor-joining cluster analysis grouped the genotypes into

three distinct groups. The first group constituted genotypes with stay-green feature, *B 35, Sorcoll 163/07, E 36-1 and Sorcoll 141/07*. The second group composed of farmers preferred Ethiopian genotypes *Gambella, Macia, 76T#23, Meko* and *Melkam*. The third group includes *Teshale* and *Sorcoll 146/07*.

The highest genetic distance (0.86) was observed between genotypes *Sorcoll 141/07* and *76 T1#23* followed by *Sorcoll 141/07* and *Meko* (0.79). In contrary, the lowest genetic distance was found between *Gambella* and *Melkam* (0.20). Most of them appeared to be distantly related and they have genetic distance of greater than 0.50 (Table 4.22).

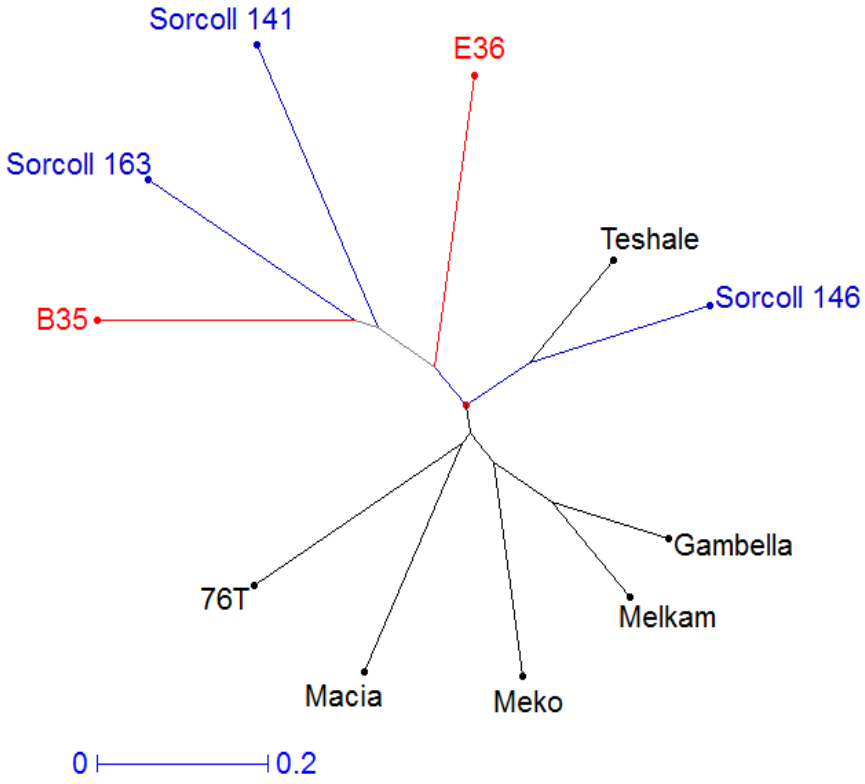


Fig. 4. 27. Neighbor-joining cluster analysis dendrogram showing the genetic relationship among the farmers preferred sorghum genotypes based on 139 SSR markers

Table 4. 22. Pair wise population Nei's genetic distance showing the magnitude of genetic differentiation among key Ethiopian sorghum genotypes

Genotypes	*Sorcoll 141/07*	*Sorcoll 146/07*	*Sorcoll 163/07*	*76T1#23*	*B-35*	*E 36*	*Gambella*	*Macia*	*Meko*	*Melkam*	*Teshale*
Sorcoll 141/07	0.000										
Sorcoll 146/07	0.667	0.000									
Sorcoll 163/07	0.619	0.676	0.000								
76T1#23	0.855	0.565	0.731	0.000							
B-35	0.594	0.730	0.495	0.743	0.000						
E 36-1	0.690	0.620	0.757	0.648	0.716	0.000					
Gambella	0.779	0.505	0.728	0.509	0.743	0.643	0.000				
Macia	0.754	0.599	0.750	0.487	0.746	0.648	0.492	0.000			
Meko	0.786	0.514	0.654	0.464	0.728	0.662	0.380	0.578	0.000		
Melkam	0.763	0.518	0.756	0.517	0.725	0.623	0.204	0.512	0.395	0.000	
Teshale	0.681	0.227	0.638	0.565	0.667	0.581	0.462	0.496	0.496	0.452	0.000

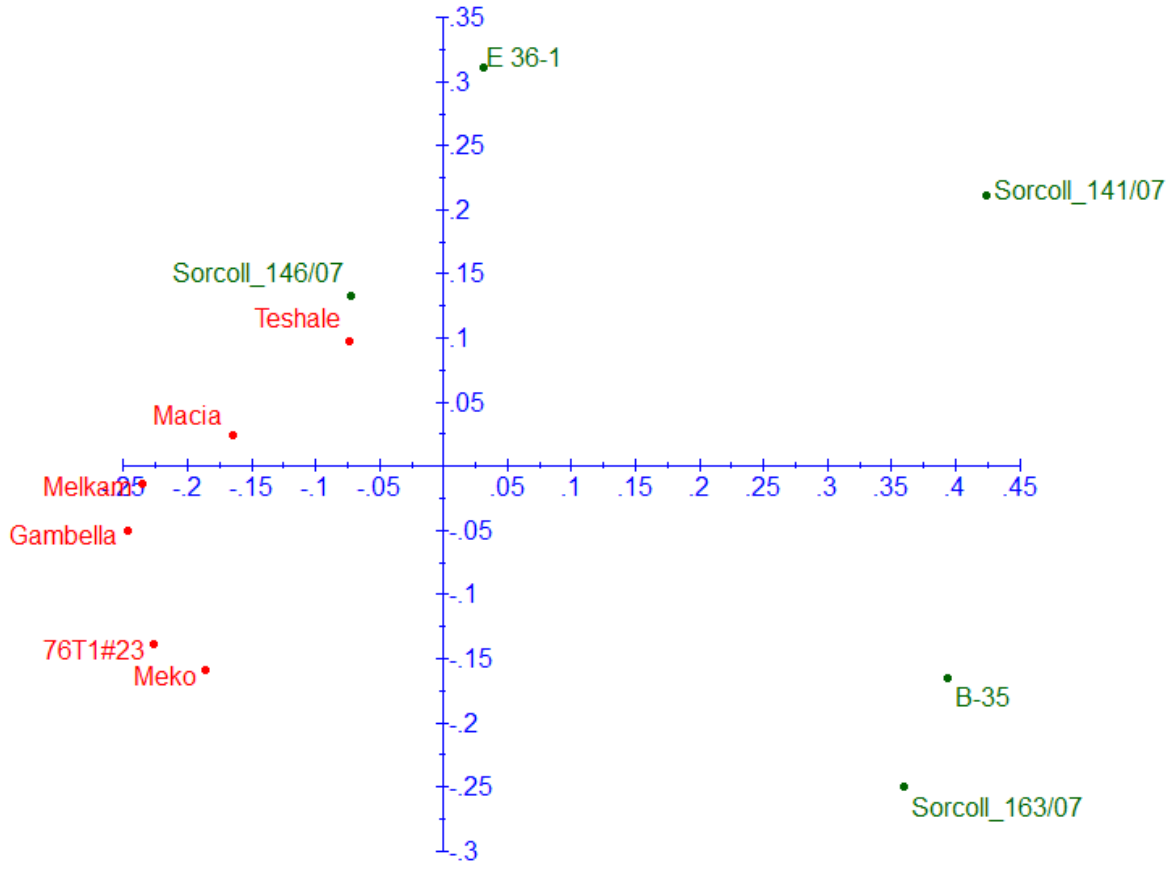


Fig. 4. 28. Biplot of the axis 1 and 2 of the principle coordinate analysis based on the dissimilarity of 139 SSR markers for key Ethiopian sorghum genotypes

5. DISCUSSION

5.1. Characterization of Ethiopian sweet sorghum germplasms for °Brix and other morphological parameters

5. 1. 1. Visual classification and trait performance

Large-scale cultivation of sweet sorghums is not common in most regions of Ethiopia. In most cases, small amount of seeds are mixed with grain sorghum or maize and planted in a limited plot of land. However, in east and west Hararge farmers grow sweet sorghums on an entire field. The intention of cultivation in these areas is not for the juicy stalk rather for grain production. According to local farmers, the grain harvested from sweet sorghum has a better grain quality than grain sorghum. Though sweet sorghum cultivation is limited to small scale, it is an important source of income for resource poor farmers in some areas such as south Tigray, north Wello, Gojam and west Shewa. Some farmers in these areas usually sale the stalk for chewing while majority of them are cultivating for children consumption at home. The sweet type stalk also more preferred by livestock as compared to grain type stalks.

Distribution of various types of panicles in terms of compactness, size, shape and color that were collected from the same places of northern part of Ethiopia (Wello and Tigray) have the implication that the regions have diverse set of sweet sorghum germplasm as compared to the other regions which are limited to one to few sweet sorghum cultivars. The existence of many farmers preferred local cultivars for their sweetness within a particular area also strengthend expectation that high diversity of sweet sorghum landraces were found in these regions. This may demand further study using large and equal sample size collected from the entire sorghum growing regions in order to estimate microcenter of diversity of the particular region.

The normal distribution pattern of the traits implies the existence of high genetic variation among sweet sorghum accessions in Ethiopia. This is because diverse accessions are characterized by normal pattern of distribution. ANOVA also generated significant high genetic diversity that exists among the collected accessions based on °Brix and other morphological traits. This is in agreement with the previously documented studies based on morphological characters (Awegechew Teshome *et al*., 1997; Amsalu Ayana, 2001) who reported the existence of high genetic diversity in Ethiopian sorghum collections. Ethiopia is believied to be the center of origin and diversity because of the existence of high genetic variation for sorghum which is favoured by its unique geographic and climatic nature. High variation among the landraces can also be attributed to seed exchange practice among farmers.

The wide variation for °Brix value ranging from 11.8 to 22.5 has an implication that modern cultivar development for sugar related traits can be possible through hybridization in the future. Reddy *et al*. (2005) reported similar type of diversity among sweet sorghum genotypes from India with °Brix value ranged from 13 to 24 %. The mean value of °Brix (17.7) obtained in this experiment is higher than most of globally known sweet sorghum varieties such as *L-Tian* (Guan *et al*., 2011), *R9188* (Ritter *et al*., 2008) and *SS79* (Shiringani *et al*., 2010). The overall mean for °Brix is a little bit smaller than the mean °Brix value of *Rao* cultivar (Murray *et al*., 2008a) but it is similar to the mean values computed for the accessions from Northern Ethiopia (Tigray and Wello) which showed significantly higher °Brix percentage as compared to the rest of collection regions. Accessions from northern Ethiopia were found to be superior in terms of °Brix value than some improved sweet sorghum cultivars *ICSV 93046, ICSV 25274, ICSV 700, ICSSH 39* and *ICSSH 39* that have been released recently in India (Reddy *et al*., 2013). It is also little bit

higher than commercially released sweet sorghum cultivars with °Brix mean value of 17-18% (Reddy *et al*., 2013).

Plant height is among the important traits for the improvement of bioenergy related traits. Development of sweet sorghum cultivars with maximum plant height with high sugar content is the interest of many breeders. Most of the commercially released cultivars have relatively higher mean for plant height as compared to grain sorghum cultivars (Reddy *et al*., 2013). The mean value ranged from 259-297 cm obtained in this study is similar to the commercial variety released in India *SSV 84* (265 cm) but shorter than *CSH 22SS* (280-350 cm), the first high yielding sweet sorghum hybrid in India (Reddy *et al*., 2013).

Collection from Tigray and Wello showed significantly shorter mean for plant height than the rest of the regions. This is in agreement with previous report using grain sorghum collections by Amsalu Ayana (2001) who documented significantly shorter mean for collections from these regions. He also reported that collections from Wollega area were found to be the tallest and significantly different from the rest of the collection regions which is in complement with the current study. However, the mean plant height for this collection region is not significantly different from collections from Hararge, west Shewa and Gojam.

The thickness of the stalk also contributed to the juice yield of the traits. Unfortunately, those accessions with high °Brix value were characterized by thin stalk diameter and vis versal. Murray *et al*. (2008a, b) also reported that sweet sorghum stalks are much thinner than the stalks of grain sorghums which had lower °Brix value. This creates difficulty to undertake direct selection for sugar related traits based on the thickness of the stalks. It requires different breeding strategies in order to develop varieties that posses the two characteristics together.

In general, accessions from eastern Ethiopia (Hararge), west Shewa and east Wollega were known for having significantly thicker stalk diameter as compared to the rest of accessions. Similarly, Amsalu Ayana (2001) also reported significantly thicker accessions collected from Hararge and Wello areas. However, the mean value of the foregoing result showed that collections from Wello and Tigray were characterized as the thinnest stalks.

Collections from northern Ethiopia are characterized by early maturity (five months in average) though they are characterized by shorter plant height as compared to late maturing type. However, early maturity may not be a desirable characteristic in sweet sorghum variety development program as the plant accumulates more biomass and store energy in its stalk throughout the growing period (Natoli *et al*., 2002; Murray *et al*., 2008a; Murray *et al*., 2008b; Ritter *et al*., 2008; Shiringani *et al*., 2010). Obviously, collections from highland areas such as east and west Hararge followed by east Wollega, west Shewa and Gojam were characterized by late maturity due to the the adaptation of the crop to different environment.

The significant high °Brix value observed in collections from both Tigray and Wello regions would suggest that these accessions will be potential sources to develop superior sweet sorghum varieties with high °Brix degree.

Sweet sorghum is usually characterized by higher stalk yields but low grain yields as compared to grain sorghum (Murray *et al*., 2008a; Murray *et al*., 2008b; Ritter *et al*., 2008; Wang *et al*., 2009; Shiringani *et al*., 2010; Reddy *et al*., 2013). Unlike many other bioenergy feedstocks, the crop can be used as dual purpose because it accumulates sugar in its stalk and also produces grains that can be used for various purposes like animal feed.

The current study showed that collections from north Wello have highest and lowest grain yield mean values which would be an implication of high genetic diversity of the accessions within the region. These accessions also showed higher °Brix mean values with relatively smaller stalk biomass as compared to the rest of collections.

5. 1. 2. Comparison of °Brix degree in three stalk positions

The variation obtained from °Brix degree analysis tested for three positions of the stalk (bottom, mid and upper) indicated that juice extraction from a specific position (internode) alone may result in overestimation or underestimation of the actuall °Brix value. Though the ideal position for juice extraction was the whole stalk, practically it is difficult to extract the juice from the entire plant in field condition. Our result showed that at least juice extraction from the mid and bottom parts of the stalk is necessary for °Brix measurement since there was no significant difference between the juice extracted from the upper and bottom part of the stalk (Table 4.4).

5. 1. 3. Trait heritability and correlation

In this experiment, the observed broad sense heritability for °Brix trait (61.1 %) is similar to the previous studies (59.0 %) reported by Shiringani *et al*. (2010) but slightly smaller than (65.0 %) which was documented by Murray *et al*. (2008a). This slightly higher heritability nature of the crop will facilitate the selection for the trait in sweet sorghum improvement program. In contrary, grain yield trait showed slight lower broad-sense heritability (42.4 %) thus making it difficult to carry out a selection for this trait in sweet sorghum.

Highly significant negative correlation between °Brix and stalk diameter ($r = -0.479$, $P < 0.001$) was observed in this study. This suggests that thicker stem may accumulate less concentration of

sugar as compared to thinner stalks. However, Murray *et al*. (2008a) and Shiringani *et al*. (2010) reported non-significant correlation between the two traits. Therefore, selection for thicker genotype may not improve the sugar concentration of the crop but it may improve the overall °Brix yield per hectare.

Result from the current study also showed that there was a slight negative correlation between °Brix and plant height traits ($r = -0.267$, $P < 0.001$) while most of the past study showed significant positive correlation between the traits (Murray *et al*., 2008a; Murray *et al*., 2009; Guan *et al*., 2011). The deviation may be due to the different types of germplasms used. The current study was focused on landraces collected from wide range ecogeographic regions whereas most of the past studies focused on recombinant inbred lines derived from two highly contrasting inbred parents such as grain and sweet sorghums.

Though the current study showed non-significant correlation between °Brix and grain yield traits, some studies documented significant negative correlation between °Brix and grain yield (Ritter *et al*., 2008; Murray *et al*, 2008a; Guan *et al*, 2011). In this study all accessions were sweet type and the correlation analysis was done among themselves. However, most of the previously reported studies were based on different types of populations (grain versus sweet) which are obviously sweet type sorghums known for their low grain yield potential suggesting that they are negatively correlated due to the dependence on same photosysnthetic source.

Plant height and days to flowering showed significant positive correlation suggesting that taller plants tended to flower later. This is in agreement with the past studies (Murray *et al*., 2008; Tesfaye Tesso *et al*., 2011). As expected, there was also a significant positive correlation between plant height and days to maturity which is in complement with the study done by

Tesfaye Tesso *et al*. (2011). Plant height also showed non-significant correlation with stalk diameter which is in consistent with Murray (2008). Amsalu Ayana (2001) also reported non-significant correlation between the two traits using the mean value of eleven collection regions. He also documented significant positive correlation between the two traits based on the mean value of 415 sorghum accessions. The positive significant correlation between plant height and grain yield is in line with the previous study (Tesfaye Tesso *et al*., 2011) indicating that improvement for stalk biomass as well as yield potential is possible.

°Brix value is also negatively correlated with the altitudes of collection regions. This implies that collection from lowland area had better °Brix value than landraces collected from highland regions. However, the entire germplasms are evaluated under the same environment and the variation in sugar concentration could be due to adaptation differences between genotypes of highland and lowland regions rather than environmental factors. Focusing on lowland germplasm for the improvement of sugar concentration has some comparative advantages but they are not desirable for their biomass accumulation due to their earliness as well as shorter plant height nature of the accessions.

In general, selection of genotypes with high sugar potential plays an important role for the improvement program of sweet sorghum germplasm. This is because the total stem sugar yield per hectare is dependent on two traits (Murray *et al*., 2008a), sugar concentration in the stem and stem juice yield per hectare. Most research activities focused on increasing sugar concentration in order to increase energy density. This will help to reduce processing and transportation costs.

5. 1. 4. Cluster and principal component analysis

All the accessions were grouped into five clusters which is in line with the past study conducted by Abdi *et al*. (2002) who grouped thirty four landraces collected from north Shewa and south Wello into five clusters. However, the number of accessions and type of characters used in both experiments are different. In contrary, Amsalu Ayana (2001) employed cluster analysis using 415 accessions representing different regions of Ethiopia, Eritrea and a group of introduced lines and grouped them into ten clusters. In the present study, accessions collected from both Tigray and Wello clustered in one group suggesting that these accessions are more similar than the other collections. Both locations are also predominantly characterized as lowland environment. This is in agreement with the previous study reported by Amsalu Ayana (2001) who documented most lowland accessions distributed in the same clusters. The constructed dendrogram based on trait mean values from each collection zones also grouped the accessions according to their locations which are in agreement with the cluster analysis.

Result from principal component analysis showed that the first two principal components have an Eigenvalue greater than one suggesting that traits such as stalk diameter, days to 50 % flowering, days to maturity, grain yield and thousand seed weight are useful traits and had higher contribution to the total variation of the accessions.

5.2 Genetic diversity and population structure of Ethiopian sweet sorghum collections using SSR markers

5. 2. 1. SSR allelic diversity

In this study, 13 polymorphic markers that are evenly distributed across the sorghum genome were used to determine the extent of diversity among 202 sweet sorghum accessions collected from Ethiopia including improved lines either released or lined up for release in the eastern and southern Africa. The PIC values ranged from 0.37-0.85, which was comparable to a similar recent study of Ethiopian sorghum collections (Asfaw Adugna, 2014) in which a PIC range of 0.39-0.85 was observed using 12 SSRs across 160 accessions. We report better results than those observed using global sweet sorghum germplasm (142 accessions) obtained from various gene banks using 29 polymorphic SSR markers (Lekgari and Dweikat, 2014) in which the PIC ranged from 0.221 to 0.75. Overall, the average PIC value of the current study (0.69) was higher than most of the previously reported values using both grain and sweet sorghum (Agrama and Tuinstra, 2003; Cania *et al.*, 2007; Ali *et al.*, 2008; Wang *et al.*, 2009; Ramu *et al*., 2013) although lower in comparison to other studies (Muraya *et al*., 2011; Asfaw *et al.*, 2012) that included wild sorghum. Wild sorghum has been reported to be more genetically diverse resulting in higher numbers of alleles per locus of SSRs studied (Mutegi *et al.*, 2011; Ramu *et al.*, 2013; Fernandez *et al.*, 2014) than in cultivated sorghum.

The higher numbers of rare and private alleles observed among Ethiopian collections in comparison with improved sweet sorghum revealed that the Ethiopian accessions have preserved the most rare and private alleles, and therefore are very important sources for future sweet sorghum improvement. Our results further confirm a report by Mace *et al.* (2013) who observed an untapped genetic potential in indigenous sorghum (both wild and cultivated) for crop

improvement. Although the significance of the rare alleles cannot be speculated from the current results, their abundance in the Ethiopian sweet sorghum accessions suggests that this diverse set of germplasm has not been extensively exploited to improve sweet sorghum material in eastern and southern Africa. We observed that the 27 improved genotypes also contained 35 private alleles indicating their uniqueness to the Ethiopian sweet sorghum gene pool. This is not surprising, especially for ICRISAT improved lines, which have made use of global collections available at the gene bank in India. Future genome wide association studies (GWAS) will be required to determine the exact contributions of these private and rare alleles to traits of agronomic importance in sorghum. Such studies will also guide the implementation of various conservation decisions that will be necessary to ensure these important alleles are not genetically eroded.

The high numbers of rare and private alleles may also be indicative of gene flow from wild to cultivated Ethiopian accessions. Ethiopia being a center of diversity for sorghum harbors abundant wild accessions that grow side by side with cultivated accessions resulting in regular hybridization between the different gene pools. It would therefore be expected that unimproved sorghum collections from the region would have a high proportion of rare and private alleles when compared to improved ones. This is further confirmed by other studies that characterized Ethiopian sorghum accessions maintained in Ethiopia (Asfaw Adugna, 2014) or elsewhere (Cueva and Prom, 2013) as well as those from Kenya (Mutegi *et al.*, 2011) that reported comparable proportions of rare alleles. Although an overall high level (78.7%) of rare alleles has been reported in global composite germplasm collection (GCGC) of sorghum (Billot *et al.*, 2013), the proportion of rare alleles reported for Indian sorghum collections have been lower than in the current study (Madhusudhana *et al.*, 2011).

5. 2. 2. Genotypic diversity

The Ethiopian sweet sorghum collections showed distinct clustering among collections grown in the north and those from the eastern and west central Ethiopia. The results are in agreement with past studies conducted for Ethiopian (Amelework *et al.,* 2014) and global sorghum collections (Madhusudhana *et al.,* 2011; Ramu *et al.,* 2013; Lekgari and Dweikat, 2014) in which clustering based on geographic origin was observed for both grain and sweet sorghum. Other studies that included different sorghum races (Wang *et al.*, 2013) of sorghum and assessed biological status (Billot *et al.*, 2013) further reported clustering based on geographic origin and race (Kitavi *et al.*, 2009; Wang *et al.,* 2013; Sagnard *et al.*, 2009) and also according to biological status (Billot *et al.,* 2013). There are also other studies in sorghum that did not observe geographical origin or racial clustering pattern (Motlhaodi *et al.,* 2014) although low numbers of accessions used may have been a major contributing factor.

The specific grouping of accessions obtained from ICRISAT is consistent with results of another diversity study carried out on Chinese sweet sorghum breeding lines (Zhan *et al.*, 2012) that reported clustering of genotypes bred from the same institute. However, this unique clustering of breeding lines points to underutilization of the available genetic resources needed to maximize outputs for sweet sorghum in the region, and therefore should be a major concern to the relevant breeding institutions, especially ICRISAT. The clustering process also revealed the existence of putative duplicate genotypes, some of which were improved lines. In the case of Ethiopian accessions, the duplicates may be a result of exchanges of seeds between farmers of different regions, which subsequently get renamed and treated as different genotypes. In the case of improved lines, the duplicate genotypes may be the same genotype released in different countries under different names, or selections from the same background that might be differing only at a

few loci. The clustering results will therefore aid in future breeding programs to select parental lines that are genetically diverse if heterosis is to be achieved. The high level of diversity obtained within few principal component will be a good implication for the use of the accessions for further breeding purposes.

5. 2. 3. Population structure

Analysis of molecular variance showed high genetic diversity within population than among populations. This means that most genetic differentiation was observed when all the 202 accessions were analyzed together than when collections from different regions were analyzed independently. Our observations are similar to previous studies on grain sorghum (Asfaw Adugna *et al.,* 2012; Ramu *et al.,* 2013; Asfaw Adugna, 2014) and suggest that farmers from the same region tend to grow the same genotypes (Labeyrie *et al.*, 2014). The genetic similarity of materials collected from Gojam and east Wollega could be geographic as the two regions share a common boundary, which could enhance regular informal seed exchange.

The 202 accessions collected from 10 different groups were further classified into three distinct sub-populations (delta K = 3) using STRUCTURE. The sub-populations generated were complementary to the Neighbor-Joining (NJ) tree analysis that also grouped the 202 sweet sorghum accessions into three clusters. The similarity in the clustering of genotypes using Neighbor Joining and STRUCTURE further validates the representative nature of the markers selected for this study. Although no known genetic diversity studies have been done in Ethiopia focusing on sweet sorghum accessions, a study by Murray *et al.* (2009) also showed a minimum of 3 distinct sweet sorghum populations while studying a diverse panel.

5. 3. Identification and mapping of QTLs associated with sugar content and drought tolerance from Ethiopian sorghum collections

5. 3.1 Identification and mapping of QTLs associated with °Brix content and biomass rlated traits

5. 3. 1. 1. Phenotypic evaluation

°Brix combined with other sugar related traits are the most important traits for the utilization of sorghum crops as a bioenergy feedstock. Biomass related traits such as plant height and stalk diameter are also important agronomic traits for the production of ethanol from sweet sorghum crop. Any positive correlation between these traits helps to improve the yield of the stalk juice of the crop. According to Murray *et al*. (2008a), the total stem sugar yield per hectare is dependent on two traits, sugar concentration in the stem and stem juice yield per hectare. Increasing sugar concentration would be an important step to increase energy density and reduce processing and transportation costs. Identification and introgression of QTLs that are associated with both sugar concentration and stem juice yield into elite varieties will be an important step. Therefore, QTLs that influence °Brix content would be good breeding target for biofuel improvement program in the future since °Brix has strong positive correlation with the sugar concentration and stem juice yield traits (Ritter *et al*., 2008).

Our result showed that non-significant correlation between °Brix and plant height which deviates from previously reported study whereby most of sugar related traits showed significantly positive correlation with plant height (Ritter *et al*., 2008, Murray *et al*., 2009). The deviation may be due to non-contrasting parent (no significant variation between the two parents) for plant height trait and stem diameter used to develop mapping populations.

The high heritability (H = 0.88) estimate for °Brix trait was in complement with previous study conducted by Ritter *et al*. (2008) using the same trait (H = 0.86). However, the result is higher than the study reported by Murray *et al*. (2008a), Shiringani *et al*. (2010) and Guan *et al*. (2011) who reported H = 0.65, H = 0.59 and H = 0.76, respectively. This high heritability estimate nature of the trait has an implication that the trait is suitable for QTL mapping studies as compared to traits with low heritability potential.

5. 3. 1. 2. QTL identification and analysis

All of the detected QTLs were found to be major QTLs explaining phenotypic variation ranging from 38.5 to 49.8 % and greater than most of the studies conducted in the past using grain and sweet sorghum genotypes (Ritter *et al*., 2008; Shiringani *et al*., 2010; Guan *et al*., 2011). However, direct comparison is difficult due to the difference in type of mapping populations and number of markers used in the study.

Two similar QTLs located on SBI-07, SBI-09 which influenced °Brix traits were detected in both MARC and Dhera environments. Similar studies were reported by Ritter *et al*. (2008) who detected similar QTLs across two environments for many agronomic traits. This is a good indication of the high heritability nature of the trait. The repeatability of these QTLs has also an implication that they are useful for the introgression of these QTLs into elite breeding lines due to their stability in different environments.

Similar result was reported by Ritter *et al*. (2008) on a linkage group SBI-06 with QTL of major effect. Our findings are also partly inconsistent with the previously reported result by Guan *et al*. (2011) who detected four QTLs out of which two of them were located on linkage group SBI-02 and SBI-07 whereas the remaining two QTLs were detected on linkage group SBI-02 and SBI-07

across two environments. In general, the current study results support most of the identified QTLs at different times on various linkage groups such as SBI-02 (Shiringani *et al*. 2010, Guan *et al*., 2011), SBI-06 (Murray *et al*., 2008a; Ritter *et al*., 2008), SBI-07 (Murray *et al*., 2008a; Guan *et al*., 2011) and SBI-10 (Bian *et al*., 2006).. The coexistence of these linkage groups is may be due to their large proportion coverage of the genome. However, there is no report on QTLs located on linkage group SBI-09. Similarly, some of the previously identified QTLs were located on different linkage groups which were not detected in this study such as SBI-01 (Guan *et al*., 2011), SBI-03 (Murray *et al*., 2008a; Guan *et al*., 2011), SBI-04 (Bian *et al*., 2006; Shiringani *et al*., 2010) and SBI-05 (Ritter *et al*., 2008).

The major goal of many sweet sorghum breeders is to develop cultivars with improved stem sugars both interms of sugar concentration and stem juice yield. QTLs that control these important traits are usually controlled by many genes and require dissection of the region using selected molecular markers. Successful identification and mapping of these novel QTLs plays a significant role to introgress the QTLs into locally adapted sweet sorghum genotypes. Major QTLs with high repeatability across locations were identified and mapped in this study.The high °Brix heritability observed in this experiment has implication that successful utilization of these novel QTLs in breeding program in the future. Therefore, the current study will serve as a foundation to start advanced sweet sorghum breeding program in Ethiopia through marker assisted breeding approaches.

5. 3. 2. Identification and mapping of QTLs associated with stay-green and yield related traits

5. 3. 2. 1. Phenotypic evaluation, QTL detection and mapping

Stay-green QTL

Nowadays, genetic improvement of crops for drought tolerance is the interest of many breeders due to the challenge of the changing environment. However, drought tolerance trait is controlled by many complex genes and the development of varieties to drought stress may require a lot of efforts and preparation. Identification and introgression of the QTLs that are responsible for drought tolerance into farmers preferred varieties is among the top priority areas by breeders.

Identification , mapping and validation of QTLs and/or markers associated with stay-green trait has been widely studied in sorghum (Tuinstra *et al.,* 1997, 1998; Crasta *et al.,* 1999; Subudhi *et al.,* 2000; Tao *et al.,* 2000; Xu *et al.,* 2000, Haris *et al*., 2007, Kassahun Bentie *et al.,* 2010). Almost all of them used selected RIL populations that were derived from commonly known sorghum genotypes for their stay-green characteristics such as B-35, SC56, and E36-1. Most of these studies identified and mapped four major QTLs (*Stg1, Stg2, Stg3* and *Stg4*).

QTLs that are directly or indirectly associated with drought tolerance traits were identified and mapped in this experiment using $F_{2:3}$ mapping population derived from a cross between *Sorcoll 163/07* and *Gambella*, Ethiopian genotypes. Two QTLs influencing stay-green traits were detected on linkage group SBI-03 and SBI-06 using regulated water supply at Werer Agricultural Research Center. Similarly, Xu *et al*. (2000) mapped two QTLs on linkage groups SBI-03 out of the four identified QTLs influencing stay-green traits. The two QTLs were distributed on different linkage groups SBI-02 and SBI-05. Our study is also in agreement with that of Subudhi

et al. (2000) to some extent, who reported five stay-green QTLs out of which three QTLs were detected on linkage group SBI-02 and SBI-03. However, our findings was different from some of the previously reported studies on linkage groups SBI-01 and SBI-10 (Crasta *et al*., 1999, Hirut Kebede *et al.,* 2001, Haussmann *et al*., 2002a), SBI-04 (Crasta *et al*., 1999) and SBI-05 (Hirut Kebede *et al*., 2001).

Attempt to validate the identified stay-green QTL under rain fed condition using $F_{2:3}$ mapping population resulted in detection of three stay-green QTLs distributed on linkage groups SBI-09, SBI-08 and SBI-05. One of the detected QTLs located on SBI-09 is common across the two environments despite the differences in environments as well as mapping populations. This implies that the QTL is highly stable at different environments.

Chlorophyll content

In many cases plants with stay-green features have relatively high chlorophyll content in their leaves during post-flowering drought stress. Report showed that there is significant linear relationship between chlorophyll content and visual stay-green rating in sorghum (Xu *et al*., 2008). The current finding further strengthen this idea due to the significant strong correlation between the two traits ($r = -0.77$, $P < 0.001$). Similar strong correlation between the leaf chlorophyll content and stay green rating ($r = -0.91$, $P < 0.001$) using F7 RIL populations derived from B 35 and T x 7000 (Xu *et al*., 2000). Slightly higher significant correlation between stay-green rating and chlorophyll index ($r = -0.57$, $p < 0.05$) was also reported by Crasta *et al*. (1999) using pooled data collected from different environment. The negative sign does not mean that the two traits are negatively correlated rather the negative sign is due to the scoring of the stay-green trait in reverse order. Therefore, measurement of chlorophyll content can be used along with

visual rating in order to minimize the personal biasness and validate the visual scoring of stay-greenness in plants (Wonous *et al*., 1991).

Three QTL influencing chlorophyll content was detected on linkage SBI-03, SBI-09 and SBI-10. The first QTL was detected during 2013 while the second and third QTLs were identified during 2014. One of these QTLs detected on linkage group SBI-10 is in agreement with recent findings by Reddy *et al*. (2014) who identified and mapped five QTLs distributed across five linkage groups (SBI-01, SBI-02, SBI-07, SBI-09, and SBI-10). However, none of the detected QTLs in this experiment co-localized with previously reported QTLs by Xu *et al*. (2000) who detected three QTLs (*Chl1 Chl2* and *Chl3*) on linkage groups SBI-01 and SBI-04. Subudhi *et al*. (2000) also detected three QTLs (Chl1 Chl2 and Chl3) on the same linkage group to that of Xu *et al*. (2000). Two of these QTLs *(Chl2* and *Chl3)* were located on the identical position while one of the QTL (*Chl1*) was located on different position but at very close position to the one identified by Xu *et al*. (2000). The consistency of one QTL on linkage group SBI-09 in two different environments may suggest that the detected QTL is stable, real and new. Besides, the co-localization of this QTL with one of the stay-green QTL on the same position is also a good implication that the QTLs are highly correlated and very useful in sorghum breeding.

Yield and yield components

Like stay-green traits, grain yield in sorghum is a complex trait controlled by a number of genes (Beil and Atkins, 1967). The two traits showed somewhat interrelation because plants with stay-green features usually possess relatively higher grain yield as compared to non-stay-green parents during drought stress condition. This is due to the fact that stay-green plants remain filling their grain during post-flowering stress through reduction of tillering and the size of upper

leaves, which reduces canopy size at anthesis which in tern reduces the transpiration leaf area conserved soil and water before anthesis for use during grain filling (Borrell *et al*., 2014).

Extensive attempts have been made to identify and map QTLs associated to yield and its component traits since 1980s (Rami *et al*., 1998; Feltus *et al*., 2006; Srinivas *et al*., 2009). The current study showed that slight significant correlation between stay-green and grain yield traits ($r= 0.371$, $P < 0.001$). Likewise, strong significant positive correlation between grain yield and its component traits such as between grain yield and panicle weight ($r = 0.990$, $P < 0.001$), grain yield and panicle length ($r = 0.461$, $P < 0.001$), grain yield and panicle width (($r = 0.692$, $P < 0.001$), grain yield and thousand seed weight ($r = 0.535$, $P < 0.001$) and grain yield and plant height ($r = 0.366$, $P < 0.001$). This is complement with many studies in the past which reported significant strong correlation between yield and its yield components (Rami *et al.,* 1998, George-Jaeggli *et al.,* 2011; Takai *et al.,* 2012; Zou *et al.,* 2011 and Raddy *et al.,* 2013).

Four major QTLs (three from the first experiment (Werer) and one from the second experiment (Dhera) controlling grain yield) were detected. Each of the first three QTLs is explaining 30.33%, 46.66% and 47.10% of the phenotypic variation, respectively. This has an implication that future application of the materials in breeding program is likely to be effective. Besides, the developed recombinant inbred lines (RILs) will be important source of breeding materials to be used further in sorghum improvement program.

Four QTLs were detected on linkage groups SBI-02, SBI-03, SBI-06 and SBI-09. Raddy *et al*. (2013) also reported that six QTLs distributed across four linkage groups (SBI-03, SBI-04, SBI-06 and SBI-09). Out of them, three QTLs were located on linkage group SBI-09. Similarly,

Srinivas *et al*. (2009) reported one QTL consistently on SBI-06 using three individual environments and combined analysis.

Three QTLs controlling panicle weight were also detected on linkage groups SBI-02, SBI-03 and SBI-06. Relatively large number of QTLs for panicle weight that is distributed on various linkage groups were previously detected using multiple environment analysis (Reddy *et al*., 2013). Out of the ten QTLs reported, three of them were located on SBI-09 and one QTL on SBI-03 which is more or less inconsistent with the currently detected QTL position. In this study, the position of detected QTLs controlling panicle weight is identical to the position QTLs controlling grain yield. This is in complement with Mace and Jordan (2011) who co-located with the QTL for two important grain yield components, panicle weight and grain number. Srinivas *et al*. (2009) also documented that QTL for panicle weight was exactly located on same genomic region to that of grain yield QTL in all environments and combined locations tested. Similarly, on linkage group SBI-06, the QTL for grain yield was also present with panicle width. This co-location of different traits on the same genomic region may be due to the common phenomenon that phenotypically correlated traits can be mapped together (Hittalmani *et al*., 2002) and hence influenced by the same gene.

One minor QTL (PVE = 5.9 %) influencing thousand seed weight was detected on linkage group SBI-07 which is different from the previously reported QTLs on linkage groups SBI-01, SBI-04 and SBI-06 (Srinivas *et al*., 2009). It is also different from the genomic region (SBI-01) identified by Tuinstra *et al*. (1998) using AFLP markers. Unlike the previous reports (Rami *et al*., 1998, Srinivas *et al*., 2009 and Reddy *et al*., 2013), a single QTL controlling panicle length which resides on linkage group SBI-05 was mapped in this experiment. Srinivas *et al*. (2009) detected four QTLs that significantly affect panicle length on linkage groups SBI-02, SBI-06 and

SBI-07. Furthermore, about twelve QTLs for the same trait was identified and mapped to SBI-01, SBI-02, SBI-03, SBI-04, SBI-06, SBI-07 and SBI-09 (Reddy *et al*., 2013). Validation of the currently identified QTL for panicle length trait using advanced lines like NILs should be undertaken before using into breeding programs.

Three QTLs controlling panicle width residing on linkage groups on SBI-06, SBI-08 and SBI-09 was detected and mapped in this experiment. The position of the detected QTLs were somewhat different from previously mapped QTLs (Hart *et al*., 2000) who documented seven QTLs distributed on linkage groups A (SBI-01) , C (SBI-03), G (SBI-10), I (SBI-06) and J (SBI-05). Only QTL on the SBI-06 coexisted with the current findings. This trait is among the least studied yield components in sorghum as far QTL detection is concerned.

Most of the identified QTLs have shown strong stability across diverse sorghum lines which are evaluated in two environments and found to be important component to be utilized in breeding program of sorghum.

5. 4. Screening and compiling of informative microsatellites sets for marker-assisted breeing of key Ethiopian sorghum cultivars

5. 4. 1. Comparison of DNA isolation protocol

Isolation of good quality and high concentration of DNA is the major steps in study of molecular biology and related disciplines. It is always important to look for protocols that meet this intended quality with low cost. The effectiveness of each protocol varies from plant species to species. Some crop plants will give a very good DNA with the commonly used protocol whereas others require a sophisticated protocol to isolate genomic DNA. Our result showed that there was no significant difference between CTAB and KIT based method on genomic DNA quality suggesting that CTAB method would be an ideal method to isolate sorghum genomic DNA for SSR analysis. This is because CTAB method may not demand sophisticated and expensive method. Therefore, genomic DNA extracted using simplified CTAB protocol can also be used for genotyping by sequencing which requires relatively good quality DNA with fair concentration.

In the current study, attempt has been made to screen and compile the most polymorphic markers using some selected Ethiopian sorghum genotypes to be used in marker assisted breeding of Ethiopian sorghum germaplasm in the future. Large number of informative markers were found to be very suitable for genotyping Ethiopian sorghum germplasm and the majority of them detected allele size lie between 100 and 300 bp which is in agreement with many studies undertaken in the past (Bhattramakki *et al*., 2000; Kong *et al*., 2000; Menz *et al*., 2002; Scholes *et al*., 2002; Wu *et al*., 2006; Li *et al*., 2009; Ramu *et al*., 2009). Besides most of the markers that their fragment sizes lie in this range showed clear and longer beaks while scoring using

GenMapper software (Fig. 4.26) indicated that they should be the choice of marker for the application in sorghum improvement program.

5. 4. 2. SSR polymorphism

SSR markers have been extensively used to detect the variability in grain sorghum and to evaluate their genetic diversity (Ali *et al*., 2008; Muraya *et al*., 2011; Asfaw *et al*., 2012; Billot *et al*., 2013). The polymorphic information content values of markers play an important role in estimating the discrimination power in a set of accession based on the number of alleles as well as the frequencies of each allele (Smith *et al*., 2000). Most of the markers used in the present study were highly informative as well as highly polymorphic. The average PIC value of the current study (0.53) was very close to most of the previously reported values using both grain and sweet sorghum (Agrama and Tuinstra, 2003; Cania *et al*., 2007; Ali *et al*., 2008; Wang *et al*., 2009; Ramu *et al*., 2013). However, the result was somewhat lower than the average PIC value reported in wild sorghum population (Muraya *et al*., 2011; Asfaw *et al*., 2012). This is possibly due the polymorphic nature of the selected SSR markers used for genotyping as well as type of germplasm studied. Since the selected markers are highly polymorphic, they will serve as underground markers for further molecular characterization and mapping of Ethiopian sorghum germplasms in the future.

The mean number of alleles per locus (3.91) detected in this study was lower than the average number of alleles reported by Wang *et al*. (2009) but slightly higher than the result documented by Ali *et al*. (2008) who documented 3.2 in average. The result was also smaller than mean value obtained from genotyping of large number of sorghum accessions collected around the world

(Billot *et al*., 2013). This variation is most probably due to the diverse and massive accession used for genotyping and the polymorphism nature of the selected SSR markers. The overall mean gene diversity (0.58) in this study is slightly smaller than previously reported studies (Due *et al*., 2008; Asfaw *et al*., 2012; Billot *et al*., 2013). However, this shouldn't be used for comparison purposes because estimates of such type of genetic parameters depends on various factors such as the type of marker used (Barakat *et al*., 2011), the size of the SSR repeats and the location of the SSR on the genome (between coding or non-coding DNA regions), the sampling schemes (single plant or DNA bulk) and the number of surveyed SSR (Due *et al*., 2008).

5. 4. 3. Genetic relationship

Molecular based knowledge of the genetic relationships among these selected sorghum genotypes is very essential to intensively utilize further in breeding program. This helps to validate phenotypically characterized genotypes for the trait of interest like drought tolerance in sorghum. Zelalem Mengistie (2009) reported that three accessions (*Sorcoll 163/07, Sorcoll 141/07* and *Sorcoll 146/07*) collected from different parts of Ethiopia showed stay-green features after evaluating phenotypically in different water deficit environment. It was very important to compare these genotypes with globally known stay-green sorghum genotypes using large number of molecular markers.

The principle coordinate analysis based on the dissimilarity of 139 SSR markers and Cluster analysis clearly showed that *Sorcoll 163/07* strongly clustered with *B 35* (Fig. 4.28). Similarly Neighbor-joining cluster analysis grouped the accessions (*Sorcoll 163/07* and *Sorcoll 141/07)* in between the two stay-green genotypes (*E 36-1* and *B 35*). This suggests that the accessions have similar genome composition to that of the stay-green materials and may be originated from the

same environment. Detection of marker polymorphism among these genotypes will play a significant role in selecting contrasting parents to be used as mapping population and hence improves the efficiency of marker assisted selection program.

6. CONCLUSION AND RECOMMENDATIONS

6. 1. Conclusions

To our knowledge, the current study which aimed at collection, characterization and QTL mapping for some useful traits using Ethiopian sweet and grain sorghum accessions is the first report and useful information for further sweet and grain sorghum improvement program.

Wide variability was observed among the accessions collected from different regions for °Brix and other morphological characters. Collections from northern part of Ethiopia (Wello and Tigray) showed significantly high °Brix mean value while collections from the rest of the regions expressed higher biomass for stem sugar yield. Besides, the °Brix mean value for collections from Wello and Tigray regions was found to be higher than the °Brix mean value of most globally known sweet sorghum cultivars and hence these germplasms can be a potential source of genes that code for sugar concentration and related traits. In addition, the information is useful to develop superior sweet sorghum varieties by crossing accession with high °Brix degree to that of genotypes with higher biomass features and other desirable characteristics.

The current molecular based Ethiopian and global sweet sorghum characterization study has significant implication for accession conservation and sweet sorghum breeding program in the region. It highlights the under-exploited nature of sweet sorghum diversity from Ethiopia in the regional sweet sorghum breeding program at ICRISAT. The global efforts on generation and subsequent utilization of green energy call for optimum breeding and exploitation of available genetic resources around the world. The current study forms not only an essential foundation for future sweet sorghum breeding programs but also for ongoing programs such as ICRISAT. Both the Ethiopian sweet sorghum breeding program and that of ICRISAT will stand to gain from

each other as they each represent different genetic groups. The information generated from both molecular and morphological based studies greatly facilitates genetic mapping and plant breeding in sweet sorghum crops.

QTLs that are responsible for °Brix content were easily identified with higher LOD score as compared to QTLs that code for stay-green traits. This implies that QTLs controlling °Brix trait seems to be coded by few genes while stay-green QTLs are complex traits that are controlled by many genes. The information will help to design suitable mapping methods for the two traits in the future. Stay-green and chlorophyll content traits are controlled by genes located on similar position on the same linkage group (SBI-06) and these genes could be inherited together.

Large number of best set of microsatellite markers are screened and made available that can be used in the future for various purposes such as characterization, QTL mapping and marker assisted selection of Key Ethiopian sorghum genotypes. These markers were also successfully used in validating *Sorcoll 163/ 07* and *Sorcoll 141/ 07* accessions that were reported to be potential source of stay-green source.

6. 2. Recommendations

The present study was only limited to accessions collected from major sweet sorghum growing regions with variable number of accessions collected and characterized from each region. However, there may be accessions with high sugar content and related traits distributed throughout the country other than the major producing regions. Therefore, future studies should focus on extensive collection and analysis by surveying a larger number of accessions in order to cover the entire regions. Besides, equal sample size from the major growing regions should be included in the study in order to estimate the micro-center of diversity for the Ethiopian sweet sorghum collections. The current study focused on °Brix degree and some biomass related traits but future study should focus on detailed analysis of sugar related traits such as sucrose content, glucose content, fructose content, sugar content, sucrose yield and sucrose to sugar ratio.

A future global detailed study on the genetic structure and diversity of sweet sorghum will be necessary for more efficient and sustainable utilization of the genetic resources for sweet sorghum breeding. The abundance of rare and private alleles also calls for careful germplasm conservation strategies in order to ensure that these important alleles are not lost. The specific contributions of these alleles to important agronomic traits will need to be studied in more details to ensure superior genotypes with desirable allele combinations are created.

The collected germplasm (together with their passport descriptions) will be preserved in Institute of Biodiversity Conservation (IBC) which can be used as public resource in the future. Furthermore, $F_{2:3}$ mapping populations that were advanced to F_6 Recombinant Inbred Lines (RILs) will be a potential source material for further use in fine mapping and other breeding related studies.

The sorghum genome contains 730 Mbp of DNA (Paterson *et al*., 2009). With 76 SSR markers, the coverage in this study averaged one marker in 9.6 Mbps which needs additional polymorphic markers in order to saturate the regions. Therefore, future study should include large number of SSR or SNP markers using either by PCR based genotyping or genotyping by sequencing (GBS) of the RIL populations derived from the same parents.

High quality combined with high concentration of genomic DNA isolation is an important step in any molecular biology related activities. Most researchers are interested in a cheaper protocol that can generate good quality with fair concentration of genomic DNA. The result from DNA extraction protocol comparison reveal that both CTAB and kit method generated high quality and quantity which had no significant difference on the quality of PCR products. Therefore, CTAB could be an ideal method for isolation of sorghum genomic DNA particularly in developing countries like Ethiopia due to its lower cost.

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8. APPENDICES

Appendix 1. Selected SSR primer pairs for parental (*Sorcoll 163/07 and Gambella*) screening

Marker name	Forward primer sequence	Motif	Reverse primer sequence	Exp. size
SbAGA01	CGAACCATGATAAATGACTG	(AG)33	ATCCGTTTCACAAAAAAAGT	94
SbAGB03	GTGTGTGTAGCTTCTTGGG	(AG)41	ACGTAGGAGTAGTTTCTAGGATT	117
SBAGD02	AGCACTGCTTGACACTCC	(AG)32	CTTTGAAACCCTGAACTCAT	98
SbAGE01	GACCGATCTAATGATGCAG	(AG)30	ACGGTAGAGAAAGACCCATC	207
SbAGF06	GTTAAACGACCAATCACCC	(AG)35	TAGAGGTGTCACTGATGAGC	152
SbAGF08	ATGGTCGTCTGTCCAGGT	(AG)34	CAGTTGCTAATCTTTGACCG	131
SbAGG02	ATCCATGCATATATTCCGAC	(AG)41	TTCGTCACCCACAACATAC	172
SbAGH04	GGCACTCATGGAGTCACA	(AG)39	TTTATCCAAATCAAACCGG	133
SbKAKGK1	AGCATCTTACAACAACCAAT	(ACA)9	CTAGTGCACTGAGTGATGAC	142
SvPEPCAA	GCAGCTCAGGGACAAATAC	(AT)10	CTGCTTCAGGTAAGGATCG	206
Xcup02	GACGCAGCTTTGCTCCTATC	(GCA)6	GTCCAACCAACCCACGTATC	199
Xcup05	GGAAGGTTTGCAAGAACAGG	(GA)8	CCAGCCCAACAAGTGCTATC	215
Xcup07	CTAGAGGATTGCTGGAAGCG	(CAA)8	CTGCTCTGCTTGTCGTTGAG	246
Xcup12	TGTTACAGAGACGCGCAGAG	(TG)7	GGCTGGTTGCTACCTTGTTC	126
Xcup13	TCTCCTCCACCTTGTCAACCC	(CCGG)5	CCTTGCCATCGACCACTC	209
Xcup14	TACATCACAGCAGGGACAGG	(AG)10	CTGGAAAGCCGAGCAGTATG	213
Xcup24	AAACTGGATGCCACACCAAG	(TA)9	AGCTATACCAACACGGGCAG	214
Xcup28	GGTGTGAGACTGTGAGCAGC	(TGAG)5	TATAGCACGGTTGTTGTGCC	164
Xcup33	GCGCTGCTGTGTGTTGTTC	(AT)7	ACGGGGATTAGCCTTTTAGG	269
Xcup36	TGAGCTGATAATGGCTGCTG	(CA)8	GCGTCACGGAAGTTGGAC	174
Xcup47	TGAGCAATGAACTTAGGGGG	(GA)21	CTACCCTTTGATGGCAGTACC	238
Xcup48	TCACTAGCGCCTCCAAAATC	(AT)7	TCCAATCCTTCCTGTGCTTC	283
Xcup49	TCCACCTCCATCATCTTTCC	(GGAT)6	CTCCACCACCTCCATGACTC	151
Xcup50	TGATTGATTGAGGCAGGCAC	(ACAGG)5	TTCCGGTCTCTGTCCATTTC	145
Xcup53	GCAGGAGTATAGGCAGAGGC	(TTTA)5	CGACATGACAAGCTCAAACG	193
Xcup57	CTGCAGAGAGCTAATTGTGC	(TAGC)5	TCTTGGAAGAGACGGACCTG	174
Xcup64	TATTGACACGCAGGTAACGC	(TA)9	GAGGACGAGTGCATGATGAG	215
Xcup67	GGTCAGTGCTTACACAGATTCC	(TA)6	GGGGATTGCAGGTGTCATAG	260
Xcup70	GGAGGAACACGCACAAAAAG	(TTGTT)5	CACTCTAGCTATGGCCTGGG	150
Xcup73	GGTTCTGTCGTCATCACCAG	(TA)10	ATCTTTAGCCGCCACATGAC	204
Xcup74	GTCGCCATTGTGATGAAGAG	(TG)9	CAGTAGTCCAGCAAAACGGC	226
Xgap010	GTGCCGCTTTGCTCGCA	(AG)27	TGCTATGTTGTTTGCTTGTCCCTTCTC	274
Xgap015	GCTGCTAAGCCGTGCTGA	(AG)16	TTATTTGGGTGAAGTAGAGGTGAACA	210
Xgap022	TGAGCCGAAAACCGTGAG	(ACGAC)4(AG)6	CCCAAAACCAAGAGGGAAGG	316
Xgap032	CTCGGCGGTTAGCACAGTCAC	(AG)15	GCCCATAGACAGACAGGAAAGCC	197
Xgap036	GCATAATGACGGCGTGCTC	(AG)19	CTTCCAAGTGAAAGAAACCATCA	182
Xgap057	ACAGGGCTTTAGGGAAATCG	(AG)18	CCATCACCGTCGGCATCT	301
Xgap084	CGCTCTCGGGATGAATGA	(AG)14	TAACGGACCACTAACAAATGATT	191
Xgap085	AGACGCTTTTCTCCTCTCTCTCTCTCTCTCT	(AG)12	TAGCCCCTGCCGCATACTGAATG	
Xgap121	GAAAAATCTCCGTCAATCCCAAAATAA	(AC)14	CGCTGAACAACGAAAGGAATAAGTG	231
Xisep0101	CAGATCTCCGGTTGAAGAGC	TG(9)	TGAGCCGAGCTCAACATACA	205

Appendix 1. Continued

Marker nan	Forward primer sequence	Motif	Reverse primer sequence	Exp. size
Xisep0102	CGCTGGAGTACCAGAGGAAG	AAG(4)	AACAAAATCCGAGCCTGTTG	680
Xisep0107	GCCGTAACAGAGAAGGATGG	TGG(4)	TTTCCGCTACCTCAAAAACC	201
Xisep0108	GTACGTTCCCCATCCTTCCT	GGC(5)	CTCCTGTTCTCTCCGCATTC	190
Xisep0110	GAGGGGAAGCTGGAGACC	CG(6)	TCAAGTGTACAACGCATCCAG	480
Xisep0114	CTTCGCCGCCTAGATCTATTT	GT(10)	GGGGATCATCAGATCACACA	200
Xisep0117	GGATGTACCAGCACCAGCTC	CCT(7)	GAGAACAGCCGAGGGAGAG	270/145
Xisep0120	CACGAGGCACATCTATCCAC	CCGT(4)	CTCGCTCCAGCAATCCTC	245
Xisep0122	TCGATCGAGCTCCAAAGAAC	CGA(5)	ACAGCAGCACCAGCTTCC	190
Xisep0123	CGACGACCTCAGGAGACG	AGG(7)	CTTCCGCGAGATGGTCAC	-
Xisep0125	TCAACAAGAACAACGCCAAC	CAA(6)	GGCTCTTGAACCTCTTGTCG	198
Xisep0131	TCAGTCTTGACACAAGCAAGC	CTGCT(4)	CGCTTCTTCCTGAGCTTGAG	225
Xisep0132	CGTCGATGAGTTCGTCAAGA	CAG(5)	CTGAGCAGAGTTGTCGGTTG	198
Xisep0138	GAGATCGAGAGGCACTTTGG	TA(6)	CAGCGACAAGCCAATACCA	210
Xisep0146	CGACCGAGCTTGAGAGTGAT	CGG(5)	CGATCTTGACGTCGTAGCAG	300
Xisep0202	CAACCTGTGATTGACCCATTT	TGA(7)	AAACATGTCCAGATTCATCAAGG	195
Xisep0203	CGATGGTGAGGATGGGTAAC	ATAC(3)	TTCTGCACAACCATCTTTGG	205
Xisep0209	GAGCCACGAGCCTAACAAAA	GCCG(4)	ACAGCATCGTGTCGTGAGTC	200
Xisep0210	ACGAGACACGACTCCTCCAT	GA(8)	CGAGGAGGTCGAGTAGAACG	197
Xisep0224	ACTGGGGTTCCTTTTCCTGT	CTG(4)	TCCCTGATTTCCCCTCTTTT	200
Xisep0228	GACATGGCCAGCTAAGAGGA	GAGG(3)	CCATGCAGTGATCGTTGTGT	220
Xisep0234	GCCTCCCTTCCCTTCCTT	CT(10)	CCTCTGCCTCTTCACGTTTC	140
Xisep0242	GCTGGAGAAGCTCAAGGAGA	TACC(3)	TCGTTGAATGTTGGAGTGGA	205
Xisep0247	GGCCAGATCATGCACCTC	CGC(4)	CAGACATCTCCGGCTCATCT	-
Xisep0303	GGTGCGTAGGGAGTACCAGA	GT(7)	GAGCCCGCGGTACTAGACT	195/230
Xisep0310	TGCCTTGTGCCTTGTTTATCT	CCAAT(4)	GGATCGATGCCTATCTCGTC	203
Xisep0314	GTTCGACGACGACGACCT	GCC(4)	GTCCTCGAACCCGACCTT	405
Xisep0317	AAGGTTATCCCGGGAAGGA	CCG(4)	CAATAGGCAGCAACAGCAAA	405
Xisep0318	CTGCGACGACTGGGCTAC	CGC(7)	AGCGAGAGGGAGTGGTACTG	345
Xisep0320	GGCCACCATGAACTCCTACT	CTC(6)	AGATGTCACCGACCATGGAG	180/360
Xisep0325	GCCACACTACCTGCCTCTCT	GGC(6)	TCCTTGAACCTCGCAGTCTT	1400
Xisep0327	CTGTTTGTGCTTGCAACTCC	GTT(4)	TCATCGATGCAGAACTCACC	200/210
Xisep0328	CATCTTCCTCCGTCAACCAT	AAG(4)	ATCCTGCGACCCTTCTCAC	300
Xisep0332	GCACAGGACACTAGGGAAGC	GGC(7)	AGCAGCCTGGTGCTACTACTG	400/500
Xisep0334	TCCAAAAATCCAAAGCCATC	GCT(4)	AAGGTGAGCAGCAGGAAGAG	-------
Xisep0346	CGCTCCTCAGGCTCCTCT	CCT(4)	TCCTCGAGCACCTGGTTG	250
Xisep0347	GATCGGCCAACATCAACC	GGC(6)	AACATGTCCCAGTGCTGCTT	160/210
Xisep0348	AAGCTCAACTTCCCCTCCTC	CCG(4)	GCTGCTCTTGTTCCTCTTGG	210
Xisep0412	CACTCTGCCATGAGCTTTGA	TG(7)	TGAGACTGAGACACCCGTATCAT	180/250
Xisep0413	CGCTGTTGCTCTCCTCTGTT	GCG(4)	GGTACAGCCGCTCGTTCTC	150/280
Xisep0417	GAGGGAGCTGGTTGCGTA	GCC(4)	GTACTTGACGCGCACCTTG	260
Xisep0422	TGCCCGTAATTAAGCCCATA	GCAT(3)	CCCACTGCTCCAGGTAAGAA	300

Appendix 1. Continued

Marker nan	Forward primer sequence	Motif	Reverse primer sequence	Exp. size
Xisep0423	GCTACCACCTCTTCGTCACC	ACG(4)	GCGAGGTTGATCCTCATCAT	290/500
Xisep0427	AAGCGGCGGAAAGAGAAG	GA(6)	GAGCGAGAGGCTGAGGACT	200/220
Xisep0429	GTCGTCTGGAAGCAACAGC	GCG(4)	TGGGGGTAGTTGGTGGTG	210/250
Xisep0432	GCATCTTCAACGCCTCGT	CGC(4)	AGGCTGCAACCAATCTGTCT	200
Xisep0435	GAGGGAAGGCAGCTCTCAG	GCCG(3)	CCTAGCAGCCAGCTCTGC	170/200
Xisep0436	TTTCTGTGCGACGAGAACC	CGC(6)	GTCGGCTAATGCCTTTGACT	180/280
Xisep0439	TAGTCGAAGCAGCAGTCGTG	GAC(4)	GTGTTCAAGTTCGACGAGCA	620
Xisep0442	AAACCCTAGCCTTGCTGCTT	GCG(4)	TCTCCACATCCATAGCAGAGG	300
Xisep0443	TCATGTACAGAGGCGACACG	GCA(7)	AGGTCGCAACAGACACCTTC	190
Xisep0444	ATGATCCGTCGGAGTTAGCA	TG(7)	GGATGCAGGACAGCATCTCT	200
Xisep0449	CCGCTCATCAGTCATCACAT	TCA(7)	ACAAAATCCATCCCACAACG	200
Xisep0502	GTATACCCCATGCCATACGC	GCC(4)	AAGCACAACAATGACTGACCA	200
Xisep0503	TTGAAGGAAGCTGTGGAAGG	CGT(4)	CGTAGGGGGACACGTAGAAG	355
Xisep0504	GCTCAAGACCATCGAGAAGC	CTGC(6)	TGATTGTGAAATAACAGCAGGAG	190
Xisep0506	CGTGCAAGTTTGGAATTTGTC	AACG(3)	CGGGCAGGTATAAGGTGTTG	225/430
Xisep0510	GCCCTCTCCAGTCTTCCTG	GGA(4)	CCGACGCATTTGCTTACATA	470
Xisep0511	CCTCGCCCAAAACCCTAC	CCG(5)	GAGGATCACCTCATCGTGCT	160
Xisep0513	GAGGGAAGAAGAAAACCCAGA	GGC(5)	AGCCTCTCCTCCTCCTCCT	300
Xisep0515	AGCTCGAGGGAGAGGAAGAT	AGG(4)	GTCCGAGCACTCCTCCAAG	300
Xisep0517	AGCAAAGGTGCCGAAGAAG	GCG(4)	TCCGGTTTTTCTTGTTGTCC	360
Xisep0518	ACGTGCTGTCCTCCATCG	CGG(4)	AGACCGACGTCGTCACCTT	150/170
Xisep0519	CACCACCACCACCACCTC	GCG(4)	GTACATCTTGGGGTCGAGGA	250
Xisep0522	TCATGGACCGTGTCATCG	CAG(8)	GCGTACTTGCTCCACCTCTC	330
Xisep0523	ACGACATGGACGACATCAGA	TGC(4)	AACAAAAACACACGGGAAGG	220/305
Xisep0524	CCCTAAACCCTCGGTTTCC	CGG(4)	GCGTCCTTCCACCTCTCC	420/430
Xisep0537	CACGAGGGACCTGCACTC	CTG(4)	TGAAGGCAAATCCTTTCAGC	130/280
Xisep0539	GACCCCATCTCTTCCTTTCC	GCT(4)	AGACTGAGGCACCGCTTG	210/240
Xisep0543	CGAGGGTTTTTCTTCTGTGG	CGC(4)	GACATCGGAGACCTTGAGGA	185
Xisep0549	TTCTCCTCCCCCACCAAT	AGCG(3)	AGCTTCATGAGGAGCGAGAG	280
Xisep0550	GCGGCGAGAGAGAGAGTTC	GA(11)	CGAGCTTGATCTTCTCGTTGA	185
Xisep0603	GTTCTCGATCGGCTTCTCAG	CGG(6)	GTGCCTTCCTTCACCTTCCT	295
Xisep0604	GCACCTACGGCTTTTACTGC	CTC(15)	ACGGTGGATAATCGAGGATG	240
Xisep0607	CACGAGGATTTCACCAAACC	AGA(4)	TGCACGTGTTCGAAATAGGA	195
Xisep0608	TTTCACCAAACCAAGCTAAGG	AGA(4)	GTAGAGGCAGCCCTTCTCCT	205
Xisep0609	AGGTGAAGCAGAAGCTGAGG	CCG(4)	CGTGAAGATGCATCCGTAGA	190/290
Xisep0611	GAGCCGTGCTGATGATCC	GCG(5)	CTCTGCAGCGTGTTGGACT	200
Xisep0612	CTCTCTGTCCTCCTCGTCGT	GCT(4)	CCTGCTTCTTGGACACCTTC	202
Xisep0614	CCCCCAACACATACAAGAGC	CGA(5)	GTTCACCAGCAGCTTCGTC	250/400
Xisep0617	GGCTGGGAGAGCTAGGAAGA	GATC(3)	GACGGCTCGTCCATCATC	200
Xisep0621	CAGTCGCGGTGGTAGACAT	GCG(4)	GCCGAGTCGTCAGAAGAAGA	205
Xisep0622	GAGGATCGGAGGAAGAGACC	TA(5)	TCTCCCATTCTCCCCTCTTT	220/235

Appendix 1. Continued

Marker nan	Forward primer sequence	Motif	Reverse primer sequence	Exp. size
Xisep0624	TCCTCCTCCTCCTTCTCCTC	CCG(4)	TGTAAGCATGGTGCCAGAAC	180/695/705
Xisep0625	CTAGCAGCAGCAGCAGTCAC	TCC(4)	GCCTTTGCTTGCTTTGATTT	185
Xisep0627	CAGACCAACAGCCCCAAC	CGA(8)	GCTTGGTCGTACGTGGATCT	180
Xisep0630	GATCGAGTCGTTCGTCGAGT	GTC(5)	AAATCCATCGACCAATCAGC	240/280
Xisep0632	AGAGAGGAGGTCCCAAATGC	CATG(4)	TTAAGGCCCAAACAAACTGG	195
Xisep0634	GCATAGCCACCAGATCTTCC	CAG(5)	AATCATGCTTGCACACTTGC	200/280
Xisep0639	TCGGACGGAGTCATCAGATA	TCT(6)	GCCTTCGTGTCTTCTGTCCT	200
Xisep0641	ATAACAAGGCCATTGCTGCT	AGG(5)	ATGGTCCATCGTCTCAGAGC	620
Xisep0643	CTCACCTTGGGAGCTGAATC	TC(7)	GGAGGACCTAGCAAGCAAGA	230
Xisep0648	GAGAAGTTGGAGCGGAGGA	GCC(4)	AACACCCAGATCAGCGAAAC	100
Xisep0701	CGGTGGGAGAGACAGAGAGA	TTCTT(3)	CCAATCAATACCACTCCTGTGA	200
Xisep0646	AGAGGAGGACGAGGAGGAAG	GGA (5)	ACAGGGTGAGCTGGTTGGT	520
Xisep0704	CAAGTCCGTCGTGCTAGAGG	GT(5)	CCCTTTAATTAGCCCCAAACA	200
Xisep0712	GGTCGGCAGGAAGAGGTC	ACG(4)	GCCGATGATCTGGTCGAG	190/230
Xisep0713	AGACAAGCACCTCGAGAAGG	GGC(4)	GGTGTCTTCTCCGTCAGAGC	120/450
Xisep0714	TACCGCAAAATTCGATGTGA	TGCA(3)	GAGATGAGCTTGAGCGAGGT	190/220
Xisep0716	GAGCACGGGCACGTAGAC	CCG(4)	CCTCTGCTCACCGATCTCAC	140/710
Xisep0720	AGGCGTGGAAGAGAGGGTA	GAG(9)	CAAGTGTCAACACCCACCAG	220
Xisep0728	AGGAGGAGGAGAAGCACCAC	AGC(4)	TGAAGAGCGCGTTCACCT	190
Xisep0730	ACCACCACCACCACAACTC	CGA(4)	CTCTTGCCCTTGATGGTGAT	205/250
Xisep0733	GTGATGCATCTCACGGACAG	TGTAA(5)	GCAGCTACTGCATGCTTGTC	400
Xisep0739	GCCCAAATCATCCAAATGAG	GAA(5)	ATGAAACCGTCCATCCAGAG	200
Xisep0746	GAGCTGCTGAGGAGGTGAAG	TTTG(4)	TCGCAAGGATTCTTCTTCGT	505
Xisep0747	AGGCAGCCTGCTTATCACAA	TCC(5)	ACAAGCTCAGGTGGGTGGT	210
Xisep0805	CTCCCCCGTGATTTGATCT	GT(8)	TAAGCAAAAGCACCATCAGC	190
Xisep0806	GCATGCCCCTGGTACAGTAG	CAG(4)	CTGCCAGCCAGCTTTTAATC	220
Xisep0809	GGAAACTCTTGTGGGTTGGA	TATG(4)	TTGACCTCTCTACAAATGATCCAC	200
Xisep0815	GCATATTCACATCGACCAAGG	TG(10)	TTTTGGTAGCGCACAGACAG	210
Xisep0819	GAGTACGACGTGGACGAGTG	CGG(4)	GTAGGTGGCCGTCGACTC	390
Xisep0824	TCCTGAAAGAAACGCACACA	CCG(4)	GAGGAGGGTGTGGAGGTGTA	200
Xisep0829	CGCTGCCAAAATCTAAGCTC	AG(6)	CACGGTGGTCACATCAGAAG	205/243
Xisep0831	TCCATGACCTTGAGGAGGAG	AAAAG(3)	TTGAAGCAGGACAACACACC	200
Xisep0838	TCGTGCCTAGCCAGTCTTCT	TCG(4)	CCCAGAAGTGGGTCGTCTT	200
Xisep0839	TACGCATAGCGCCTTTCAAT	ATTAC(4)	ATTTCATTATGCCGGTCTCG	200
Xisep0841	TAGGAATGACGACACCACCA	GCA(10)	CAAAGGCAAGGGTTTTGCTA	210
Xisep0843	CCCAAACATTCCCACGTAAC	AGCTG(3)	GTGAACAGAGGAGGCAGAGG	200
Xisep0844	GTGTTCAAGTTCGACGAGCA	CGT(4)	TAGTCGAAGCAGCAGTCGTG	600
Xisep0845	CAGCAAGCAACATCAACCAT	CT(5)	GAGCTCGAAGAACGACGAAC	160
Xisep0901	ACCGTCTCTCCTGCTCCAC	GGC(4)	ATCTCCGCCGTACCAAAAG	240
Xisep0905	GCACGAGGCACATACACAAC	GCC(4)	GAGAACGGACCCAGGTACAG	220

Appendix 1. Continued

Marker nan	Forward primer sequence	Motif	Reverse primer sequence	Exp. size
Xisep0910	TTCCTTCTCGCGATCTCACT	GGC(7)	GAACCGCAGCTTACCGAAC	610
Xisep0918	CTGGAGCTGTCAGGCTGTG	AGC(5)	GTGCCGTTCCGTTTCACT	185
Xisep0938	TGCTGTTCTTGAACGTGTTTG	TGGGT(6)	TTTTGCACAAAGTTGCGTGT	225
Xisep0941	TCACCATCATCACCATGGAC	GCG(4)	CTAGCCGCATGCATAAATCC	200/280
Xisep0948	AGGCCGAATCACAATAATGG	TA(5)	AGTGCATGAACAGGGCATC	205
Xisep0949	CAGTGCCAATAAGCTCGTCTC	GCA(5)	CATCGATCTCTGCTTCTGCTT	100
Xisep1001	GGTAGGCTGGTGGACGACTA	GAT(4)	ATGAGGGCCAAGCATCACT	205
Xisep1008	GATGCGCAAGCAGAACAAG	CAG(7)	CAGCAATGGAAATAGCTCAGG	340
Xisep1009	CCCCTTCTGCTAATCCTCGT	CGC(4)	GGGATGGCCAAAGTAGGTCT	205
Xisep1011	GGAGAAGGAGGTGCAGGAG	GT(5)	CACTGACTGACCACGAGCTT	260
Xisep1012	TAGCAAGCAGAAATCGACCA	TC(40)	ACCATTGTCCCTCACTCCTG	120
Xisep1013	CGGTTACGGCGGATTATTAC	CGG(7)	ATGGTGGCGATGCAGACTA	220
Xisep1014	ACCGCCGACGTCATAGTAAG	GT(5)	GGCAGTAACATAGCATCCATCA	240
Xisep1025	ACCTTCTCGTCCTCGTCCTC	GCG(5)	AGAACATGACCGGATCGAAG	490
Xisep1028	CAGCGACCATGAGGATGAC	GCA(4)	TGGCATGCATCAAACAAGAT	200
Xisep1029	GACCCTCCTCCTCAACCACT	GCAT(3)	CATGCATGCACAAGCAGATT	190/210
Xisep1031	TGCTCCTGCCTCGTTCTC	CGG(6)	TAGTCCTCGGTCGACTCCAT	690
Xisep1032	GCAAGCTCTACGGGATCTTC	TTCAG(3)	GCAGCTGGAAAATAATCGAAA	200
Xisep1035	CACTTTCTACCGCTCCTTCG	TGAT(5)	AGTGATGATGATGACCGAACC	160
Xisep1038	GGGCTCTAATCCTCCTCAGC	GCT(4)	GCTACCACTGCCTCCATTGT	200
Xisep1039	GTGGATTCAAATCCGCTGAC	CCTG(5)	GGCAATTTGGCAAGCAAT	200
Xisep1042	GGAGGCAAGTTCAGGAAGTG	CGTA(5)	TGTGTGCAGTGCATGCTTAG	160
Xisep1046	CGCAATGGAAGAGGACTGAT	GCTC(4)	CTACATCCTTTGCCCCAAAC	200
Xisep1103	CTCTTCGAGGACACCAACCT	TCG(7)	AAGGCAAAGCACAAAGCCTA	200
Xisep1107	GGATAATCTGCAGGCGACTT	GCA(6)	CCATCTGCTGCTCTGACTTG	210
Xisep1109	CACAAGATCACGGAGGAGGT	CGG(5)	AGGTCCGGAAAAGGGACTTA	295
Xisep1110	CCCGAGCATACTCCTCTCAT	AGTG(3)	CCCTCCTTGAACTTCACCAG	200
Xisep1111	CCTCCTCTCCTCATCCCTCT	CGAG(3)	ATGGGTAGCGGGTTTCTTG	220
Xisep1127	GCTGGAGGAGGAGTTCAAGA	GCG(4)	CCATCCGTCCAGATTGTCTC	220
Xisep1128	GGCGGGAAAAAGTTCCTTTA	AT(6)	CGCACACCCATTTCAATTC	220
Xisep1129	CCTCCAGCCTACAACTCTGC	GGCC(4)	TGCCTTATTGGCTTTCTGCT	210
Xisep1130	GCATGACGAGGAGAAGAAGG	CG(6)	CCACGAGGAAGACGAAGG	130/230
Xisep1133	CGATGCAGCTCCAACTCATA	CCA(5)	GTGTATGTCGCCGAAGTGG	220
Xisep1139	CACGACTTCCTCGGCTTC	CGG(5)	GGCAGGTGAGCACCAGAG	260
Xisep1140	TGGGAGTACTACCCGGAGGT	GAC(4)	CGCACGTACACCCTTAATCTT	225
Xisep1145	GAGGACGAGTGCATGATGAG	AT(8)	GGACGGGAACAGAGAAAGAA	190
Xisep1150	GTATTGTACGGCGCCCTTT	TCTA(15)	ATGCACTAACCGGGGACATA	210
Xisep1202	CTACCTCGTGCACCAAATGA	ATA(6)	CGCAAACAGATCCTTGCTTT	202
Xisep1208	TCCAAACACACAGACCGTTT	TCGAA(4)	TCCGATGGTTGAGAGCTTGT	180
Xisep1213	AGGTCAGCGTCTTGCAATCT	GAA(5)	ACAAATTGAAAGGGCGAGAG	202
Xisep1218	TGCTCTGGGCTTACTTCCTC	TA(8)	TACACGGTGCTCATCACTGC	200

Appendix 1. Continued

Marker nan	Forward primer sequence	Motif	Reverse primer sequence	Exp. size
Xisep1220	CGTCGTCGCTGGAGAGAT	GGT(4)	CACCATGACCGATCCTTTTT	198
Xisep1225	AATTCCAGTTGCTCGCTCTC	CTC(8)	CCTCCCTCCCCCTACTACAC	200
Xisep1226	ATCGATCCATGGAGGGTGT	AGG(4)	CAACCACCACCGCTACAATA	290
Xisep1231	CTGCTTATGCGCTTCGATTT	GT(11)	CATAATGGGTGCACTCTAGCC	222
Xisep1237	AATCATGCCAACGAGAGGAC	GGT(4)	CACCAACACCACCACCATAG	200
Xisep1241	GAGGGCGAGACAGAGGAGAT	AGCTG(5)	CTACCTTTGAGCCCACCGTA	190
Xisep1248	AGCAAAAGGCAGCAGGAAT	GAG(6)	CCGTCTAGCTCGCAGGTCT	520
Xtxp001	TTGGCTTTTGTGGAGCTG	(AG)34	ACCCAGCAGCACTACACTAC	188
Xtxp003	AGCAGGCGTTTATGGAAG	(CT)8(CT)36	ATCCTCATACTGCAGGACC	206
Xtxp004	AATACTAGGTGTCAGGGCTGTG	(GA)23	ATGTAACCGCAACAACCAAG	173
Xtxp006	ATCGGATCCGTCAGATC	(CT)33	TCTAGGGAGGTTGCCAC	141
Xtxp007	ACATCTACTACCCTCTCACC	(CT)14	ACACATCGAGACCAGTTG	229
Xtxp008	ATATGGAAGGAAGAAGCCGG	(TG)31	AACACAACATGCACGCATG	148
Xtxp010	ATACTATCAAGAGGGGAGC	(CT)14	AGTACTAGCCACACGTCAC	143
Xtxp012	AGATCTGGCGGCAACG	(CT)22	AGTCACCCATCGATCATC	193
Xtxp012	AGATCTGGCGGCAACG	(CT)22	AGTCACCCATCGATCATC	165
Xtxp014	GTAATAGTCATGACCGAGG	$(GA)_{15}$	TAATAGACGAGTGAAAGCCC	149
Xtxp018	ACTGTCTAGAACAAGCTGCG	(AG)21	TTGCTCTAGCTAGGCATTTC	229
Xtxp020	TCTCAAGGTTTGATGGTTGG	(AG)21	ACCCATTATTGACCGTTGAG	202
Xtxp021	GAGCTGCCATAGATTTGGTCG	(AG)18	ACCTCGTCCCACCTTTGTTG	179
Xtxp021	GAGCTGCCATAGATTTGGTCG	(AG)18	ACCTCGTCCCACCTTTGTTG	172
Xtxp024	TTGTGTAGTCCATCCGATGC	(TC)21	TTCTAAGCCCACCGAAGTTG	146
Xtxp025	CCATTGAGCTTCTGCTATCTC	(CT)12	CATTTGTCACCACTAGAACCC	139
Xtxp026	AAGTGTAGTAGCAGTTTAGTCTC	(TG)9(AG)12	TAGGTATCAAAGGACCAAGG	184
Xtxp027	AACCTTGCCCTATCCACCTC	(AG)37	TATGATGAATCAAGGGAGAGG	303
Xtxp030	AAAAAGGACGCGCAGCTG	(AAT)25	CTGGTCTCCACCATCCGTAG	273
Xtxp031	TGCGAGGCTGCCCTACTAG	(CT)25	TGGACGTACCTATTGGTGC	219
Xtxp032	AGAAATTCACCATGCTGCAG	(AG)16	ACCTCACAGGCCATGTCG	138
Xtxp037	AACCTAAGAGGCCTATTTAACC	(TC)23	ACGGCGACTCTGTAACTCATAG	177
Xtxp038(lg)	ACAAACCGCGACGAAGTAAC	(AG)17	ACAAGGCAAAGCACAAAGC	370
Xtxp041	TCTGGCCATGACTTCTCAC	(CT)19	AAATGGCGTAGACTCCCTTG	281
Xtxp043	AGTCACAGCACACTGCTTGTC	(CT)28	AATTTACCTGGCGCTCTGC	169
Xtxp045	CTCGGCGGCTCCCTCTC	(CT)5 + (GA)25	GGTCAAAGCGCTCTCCTCCTC	202
Xtxp056	TGTCTTCGTAGTTGCGTGTTG	(GA)39	CCGAAGGAGTGCTTTGGAC	326
Xtxp058	TTCCCTTGCTGTTGCTTGTG	(AG)13 + (GA)16	TTCCCTTGCTGTTGCTTGTG	160
Xtxp063	CCAACCGCGTCGCTGATG	(GA)24	GTGGACTCTGTCGGGGCACTG	170
Xtxp067	CCTGACGCTCGTGGCTACC	(GA)28	TCCACACAAGATTCAGGCTCC	175
Xtxp069	ACACGCATGGTTTGACTG	(TC)12	TTGATAATCTGACGCAACTG	214
Xtxp072	TTATGGAAGCAAAATGAC	(GA)36	CGAATCCTAATTGAGGTAAGC	123
Xtxp075	CGATGCCTCGAAAAAAAAACG	(TG)10	CCGATCAGAGCGTGGCAGG	161
Xtxp080	GCTGCACTGTCCTCCCACAA	(CT)13 + (ACG)3	CAGCAGGCGATATGGATGAGC	287

Appendix 1. Continued

Marker nan	Forward primer sequence	Motif	Reverse primer sequence	Exp. size
Xtxp083(Ka	CGCTAGTCAAAATTGAGGAG	(GT)9(AT)12	CAAACCGATAAACAAGTATGA	274
Xtxp088	CGTGAATCAGCGAGTGTTGG	(AG)31	TGCGTAATGTTCCTGCTC	119
Xtxp091	GGTCTCTGGATACTAGGTG	(AG)19	AAGTAATAGACGAGTGAAAGC	170
Xtxp095	TCTCCGTTTGCCCGCCAG	(GA)18(GC)4	CACCGTACCGCCTCCCGAATC	90
Xtxp096	GCTGATGTCATGTTCCCTCAC	(GA)24	CATTCGTGGACTCTGTCGG	177
Xtxp098	GCCAGCTTATCGGAAACAAG	(CTGT)5	GTGTCCACTAGAGCGGCTATG	215
Xtxp113	CTCAGCTAATTTAGCCATAG	(GA)11	CAAGTAATAGACGAGTGAAAG	198
Xtxp120(Cb	AAAGCTCGGCGTTAGAAATA	(AT)18	CGCTTAACAACTCCTACCATC	217
Xtxp123(Ka	TCGGCGAGCATCTTACA	$(CAA)_9$	TACGTAGGCGGTTGGATT	279
Xtxp130(Pe	TGGGAAGCAGCTCAGG	(AT)10	AGGGTGGTGATGTAGGGA	247
Xtxp141	TGTATGGCCTAGCTTATCT	(GA)23	CAACAAGCCAACCTAAA	152
Xtxp145	GTTCCTCCTGCCATTACT	(AG)22	CTTCCGCACATCCAC	226
Xtxp159	ACCCAAAGCCCAAATCAG	(CT)21	GGGGGAGAAACGGTGAG	174
Xtxp160	AGTCGGCTGGTAAAGTGG	(TG)14	GGATATGGGATGGTTTGG	158
Xtxp162	CCCGATATCTCTCCTGAC	(GGA)6	CCCCAGTTTAGCCAAGT	213
Xtxp177	GCCGGTTGTGACTTG	(CT)7 (GT)8	TTAAAGCGATGGGTGTAG	167
Xtxp201	GCGTTTATGGAAGCAAAAT	(GA)36	CTCATAAGGCAGGACCAAC	183
Xtxp205	CCTGCCGTGTCTTCC	(AG)12	TATATGCATGCCGTAGATTT	211
Xtxp207	ACACATCTACTACCCTCTCACCCT	(CT)14	TGATAGACTTGTGAGCAGCTCC	180
Xtxp210	CGCTTTTCTGAAAATATTAAGGAC	(CT)10	GATGAGCGATGGAGGAGAG	194
Xtxp211	TCAACGGCCAATGATTTCTAAC	(CT)23	AGGTTGCGAATAAAAGGTAATGTG	206
Xtxp217	GGCCTCGACTACGGAGTT	(GA)23	TCGGCATATTGATTTGGTTT	175
Xtxp225	TTGTTGCATGTTGGTTATAG	(CT)9	CAAACAAGTTCAGAAGCTC	165
Xtxp228	ACAGGTTGGCGATGTTTCTCT	(TC)12	TTCTTTTTCGAATTCATTCCTTTT	235
Xtxp230	GCTACCGCTGCTGCTCT	(GA)28	AGGGGGCATCCAAGAAAT	191
Xtxp248	GGGTGTCCAATGTTGTCTGC	(AG)5 (GA)28	GGCCGTTACTGTCCCTTACTCA	223
Xtxp250	GCACATCCTCTAAAACTACTTAGT	(AAG)17	GAACAGGACGATGTGATAGAT	282
Xtxp258	CACCAAGTGTCGCGAACTGAA	(AAC)19	GCTTAGTGTGAGCGCTGACCAG	222
Xtxp265	GTCTACAGGCGTGCAAATAAAA	(GAA)19	TTACCATGCTACCCCTAAAAGTGG	191
Xtxp273(Pbl	GTACCCATTTAAATTGTTTGCAGTAG	(TTG)20	CAGAGGAGGAGGAAGAGAAGG	222
Xtxp274	GAAATTACAATGCTACCCCTAAAAGT	(TTC)19	ACTCTACTCCTTCCGTCCACAT	194
Xtxp279	ATTCTGACTTAACCCACCCCTAAA	(CTT)10(CTT)3(CT	AGCTCATCAATGTCCCAAACC	275
Xtxp283	CGCCCGAACTCTTCTTAAATCT	(TTC)12	ATTATGCCCTAACTGCCTTTGA	281
Xtxp284	CCAGATTGGCTGATGCATACACACT	(AAG)19	AAGGGTAATTTATGCACTCCAAGGTAGGAC	219
Xtxp285	ATTTGATTCTTCTTGCTTTGCCTTGT	(CTT)11 CTC(CTT)	TTGTCATTTCCCCCTTCTTTCTTTT	231
Xtxp287	GCAAGCGAGCTGACTTATGTAACGAGA	(AAC)21	CAAAGTGCTACTAAACCTATGCAGGGTGAA	367
Xtxp289	AAGTGGGGTGAAGAGATA	(CTT)16(AGG)6	CTGCCTTTCCGACTC	289
Xtxp292	GTACCCATTTAAATTGTTTGCAGTAG	(AC)12	CATTCCTGACTGCCCTCTCC	332
Xtxp295	AAATCATGCATCCATGTTCGTCTTC	(TC)19	CTCCCGCTACAAGAGTACATTCATAGCTTA	175
Xtxp296	CAGAAATAACATATAATGATGGGGTGAA	(CA)18	ATGCTGTTATGATTTAGAGCCTGTAGAGTT	165
Xtxp297	GACCCATATGTGGTTTAGTCGCAAAG	(AAG)24	GCACAATCTTCGCCTAAATCAACAAT	220

Appendix 1. Continued

Marker nan	Forward primer sequence	Motif	Reverse primer sequence	Exp. size
Xtxp298	GCATGTGTCAGATGATCTGGTGA	(AGA)23	GCTGTTAGCTTCTTCTAATCGTCGGT	202
Xtxp299	CTCTCCCCCTTTGTCATCCATC	(CAT)19	TCTTGCCCACCAGGACTTCTC	210
Xtxp302	TAGGTTCTGGACCACTTTTCTTTTTGTGTT	(TGT)8	GAATCAACTATGTGCTTGCATTGTGCT	193
Xtxp303	AATGAGGAAAATATGAAACAAGTACCAA	(GT)13	AATAACAAGCGCAACTATATGAACAATAAA	160
Xtxp304	ACATAAAAGCCCCTCTTC	(TCT)42	CTTTCACACCCTTTATTCA	290
Xtxp312	CAGGAAAATACGATCCGTGCCAAGT	(CAA)26	GTGAACTATTCGGAAGAAGTTTGGAGGAAA	154
Xtxp316	CCAGCTTCACTTACGAGGAGATG	(AGA)12	ATGCCCGTTTTCTAATTCTTCTACT	376
Xtxp319	TAGACATCTGAATTAAGGAGC	(TC)17	CATGCCCCTGAAAGAGA	376
Xtxp320(Phy	TAAACTAGACCATATACTGCCATGATAA	(AAG)20	GTGCAAATAAGGGCTAGAGTGTT	274
Xtxp321	TAACCCAAGCCTGAGCATAAGA	(GT)4+(AT)6+(CT)2	CCCATTCACACATGAGACGAG	205
Xtxp324	GCAAGAGTAACCTGCATTAAATTA	(TA)18	ACATAACACAAGCAGGGAGAG	202
Xtxp325(Ka	GGGGCGACTCTTTGTAAAACATA	(AAT) 23	ATCGTGTCACTATCTACATCTAAATCGG	220
Xtxp327	ACCACTGCTCACGCTCAC	(TAG)3(GA)22	GCGGTGTACAGCTTCGTC	158
Xtxp328	ACGACGACGAGGTGG	(CT)6 + (GGAC)6	TTCAACAAAGGAAAGGATTC	245
Xtxp329	ACTACGAAGGTGTTTAGTTTAAGGG	(ATC)8 + (CTT)22	CATTCATAAAACTAAACGAAAAACG	162
Xtxp331	AACGGTTATTAGAGAGGGAGA	(GAT)32	AGTATAATAACATTTTGACACCCA	226
Xtxp335	TATTTCCTCTTGAAAGAATCAGGG	(GT)12	TATTCATCGAGCAAAAGGCA	174
Xtxp343	CGATTGGACATAAGTGTTC	(AGT)21	TATAAACATCAGCAGAGGTG	198
Xtxp344	ACAGAGAAGTAGGTTGAAGA	(TAG)14(CAG)5	CTTCTATTTTCTACTCTCATCC	177
Xtxp348	CGACATCAGCGTTGTCTTTCTA	(TAA)37	GCTTACGAATAGGGCAAAAGAACT	294
Xtxp354	TGGGCAGGGTATCTAACTGA	(GA)21	GCCTTTTTCTGAGCCTTGA	159
Xtxp355	TGGTTGGAAAGATAATCAAG	(CT)7CG(CT)15(CT	GCCCTAATGAGTCCTCAC	289

Appendix 2. Phenotypic values and statistical analysis of °Brix , stalk diameter and plant height values evaluated in different environments (the data was used to map QTLs for the aforementioned traits)

Locations	Traits	Mean	Variance	Std Error	Skewness	Kurtosis	Min	Max	Range	P-value
	°Brix (%)	**8.88**	**8.56**	**2.93**	**0.46**	**-1.06**	**4.50**	**14.90**	**10.40**	**0.00**
MARC	Stalk diameter (mm)	16.19	5.23	2.29	0.11	-0.35	11.40	23.30	11.90	0.36
	Plant height (cm)	199.34	1811.43	42.56	-0.06	-0.48	104.60	306.30	201.70	0.13
Dhera sub	**°Brix (%)**	**9.10**	**6.89**	**2.62**	**0.76**	**-0.54**	**5.70**	**15.80**	**10.10**	**0.00**
station	Stalk diameter (mm)	13.12	2.76	1.66	0.14	-0.21	9.10	17.30	8.20	0.44
	Plant height (cm)	203.67	1414.54	37.61	-0.24	-0.55	90.30	287.60	197.30	0.08
	°Brix (%)	**8.99**	**6.84**	**2.61**	**0.61**	**-0.96**	**5.50**	**14.90**	**9.40**	**0.00**
Combined	Stalk diameter (mm)	14.66	2.52	1.59	0.10	-0.36	11.00	19.10	8.10	0.59
location	Plant height (cm)	201.50	1156.04	34.00	-0.13	-0.29	108.20	285.40	177.20	0.56

Appendix 3. Pattern of marker segregation using 76 polymorphic SSR markers across 192 F_2 populations derived from *Sorcoll 163/07* X *Gambella* parents (H = Hetrozygous/F_1, A = *Sorcoll 163/07*, B = *Gambella*, - = missing data)

dividual	Xcup53	Xisep0839	Xisep0327	Xcup33	Xtxp357	Xisep1032	Xtxp284	Xtxp279	Xgap342	Xisep0612	Xtxp008	Xgap84	Xtxp286	Xisep0938	Xtxp001	Xiabt346	Xtxp211	Xtxp298	Xtxp072	Xcup36	Xisep0138	Xtxp285	Xtxp034	Xtxp033	Xtxp283	Xtxp024	Xtxp343	Xtxp026	Xisep0202	Xtxp012	Xtxp267	Xisep0224	Xisep0831	Xtxp0295	Xiabt26	Xisep0805	Xtxp159	Xtxp312	Xtxp289
T_100	H	B	H	-	-	H	A	-	B	H	A	-	A	-	A	H	A	A	A	H	A	A	-	-	-	H	A	A	A	A	B	H	H	-	A	A	B	-	A
T_101	H	B	B	B	B	H	B	B	H	B	A	H	H	H	H	H	A	A	H	A	A	H	H	A	H	B	H	H	H	H	H	H	A	A	A	H	A	-	B
T_102	H	A	A	H	H	A	H	H	H	H	A	A	A	-	A	H	B	B	H	A	B	B	B	H	H	A	A	H	H	A	B	A	B	B	B	H	H	-	H
T_103	A	H	H	B	B	H	H	B	B	A	H	H	H	H	H	H	H	A	H	B	A	H	H	B	B	B	B	B	H	A	H	H	A	A	A	H	A	H	B
T_104	B	H	H	H	H	H	H	H	H	H	H	-	-	H	H	B	B	B	A	H	A	H	H	H	H	A	A	H	H	A	H	H	B	B	B	B	H	H	H
T_105	H	H	A	H	H	H	H	B	B	H	B	B	B	B	B	H	A	B	H	A	H	H	H	B	H	B	H	B	B	H	B	B	H	H	H	H	B	A	A
T_106	H	H	H	H	H	H	A	A	H	H	-	B	B	B	B	B	H	B	A	H	A	A	A	B	H	H	B	H	H	B	H	H	B	H	H	-	H	H	H
T_107	B	B	B	B	H	H	B	B	A	A	B	B	B	B	B	H	H	B	H	A	H	H	H	H	H	H	H	A	A	H	A	A	H	H	H	B	B	B	A
T_108	H	A	A	A	H	H	B	B	H	A	A	B	B	B	H	B	H	B	H	H	A	B	H	B	B	H	B	H	H	B	H	A	H	H	H	H	H	A	B
T_109	B	H	H	A	A	A	A	A	B	H	A	B	B	B	B	H	H	B	H	B	A	A	A	H	B	H	A	A	A	A	A	H	B	B	B	A	H	H	H
T_11	H	H	B	B	H	H	-	H	H	H	-	A	A	H	H	B	A	B	A	B	H	H	H	H	H	H	H	H	H	H	H	A	H	H	H	A	H	A	A
T_110	H	H	H	H	H	H	A	A	A	H	H	B	B	B	B	B	B	B	A	H	A	A	A	H	H	H	A	A	A	A	A	B	H	H	H	B	H	H	H
T_111	B	A	H	A	B	A	-	B	A	A	H	-	-	B	A	H	B	H	H	A	A	B	B	-	-	H	-	A	H	A	H	H	B	B	H	A	A	-	A
T_112	H	B	H	-	B	H	H	H	H	H	A	B	B	B	B	H	H	A	B	H	H	H	A	A	B	H	B	H	H	A	H	H	H	H	H	H	A	-	B
T_114	-	A	A	A	H	H	H	H	B	A	A	B	B	B	B	A	H	B	A	H	A	H	H	A	H	B	H	A	A	H	A	A	H	H	H	B	H	B	B
T_115	A	H	H	A	H	H	B	B	H	H	-	A	A	H	B	B	H	B	A	H	A	A	A	-	H	H	H	H	H	H	H	H	H	H	H	B	H	H	H
T_116	B	B	B	B	H	H	H	H	A	H	-	B	H	H	H	H	H	B	H	H	A	A	A	H	H	A	A	A	A	A	A	B	B	B	B	A	A	A	B
T_117	B	H	H	-	H	H	H	B	B	H	H	-	-	H	B	B	B	B	B	B	A	H	B	A	H	H	H	H	B	H	B	H	A	A	A	H	A	-	A
T_118	H	B	B	H	A	H	H	H	H	A	A	H	H	H	-	H	H	A	H	H	A	H	B	H	B	B	A	H	H	A	H	H	H	H	H	H	A	H	B
T_119	B	B	H	B	A	A	A	A	B	H	H	H	H	H	H	H	H	B	H	H	H	H	H	H	H	A	H	B	B	H	B	B	H	H	H	H	H	H	-
T_120	B	B	B	H	H	H	A	A	A	A	A	H	H	H	H	H	H	B	H	H	B	H	H	H	B	B	H	A	A	H	A	A	A	A	A	A	A	A	B
T_121	A	B	H	-	B	H	A	-	A	H	B	H	A	-	A	H	A	B	A	A	-	-	B	A	B	H	A	A	H	A	H	A	H	A	H	H	A	-	-
T_122	A	H	H	B	B	B	H	H	H	A	-	H	H	A	-	H	B	B	H	B	H	H	H	H	H	A	H	H	A	H	A	H	A	H	H	H	H	-	B
T_123	A	B	B	H	H	H	H	H	A	H	-	B	H	B	B	H	A	H	H	B	H	H	H	H	H	H	B	A	A	B	A	A	A	H	H	H	H	H	B
T_124	B	B	B	B	H	H	H	H	H	A	H	H	H	H	H	H	H	B	A	A	A	A	A	B	H	A	B	H	H	B	H	H	H	H	H	H	H	B	A
T_125	B	A	H	H	A	A	A	A	A	H	H	H	H	H	H	A	A	H	B	H	B	H	H	B	H	H	A	A	A	A	A	H	H	H	H	H	H	H	H
T_126	B	B	B	H	H	H	H	H	H	H	A	H	H	H	H	H	H	B	H	H	B	H	H	H	H	A	H	H	H	H	H	H	H	H	H	B	H	H	B

T_127	H	H	H	H	H	H	H	B	B	A	H	H	H	H	H	A	H	B	B	H	H	H	H	H	H	B	H	B	B	H	B	B	A	A	A	H	A	A	B
T_128	B	H	H	H	H	H	A	A	H	H	H	-	-	H	H	H	H	H	B	A	A	A	A	H	B	H	A	H	A	A	A	H	H	H	B	H	H	H	B
T_129	A	H	H	H	H	H	A	A	H	H	B	-	-	H	H	H	H	B	H	A	A	A	H	H	H	A	A	H	H	A	H	H	H	H	A	A	H	H
T_130	A	H	H	H	H	H	H	A	H	H	-	H	A	A	A	H	H	B	H	H	H	H	H	B	H	B	B	H	H	B	H	H	H	H	A	H	H	H
T_131	H	H	H	B	H	H	B	B	H	A	A	A	A	A	A	H	H	B	H	B	B	H	H	H	H	A	H	H	H	H	H	H	H	H	H	H	H	B
T_132	H	H	H	B	B	B	H	H	H	A	-	-	-	A	A	A	H	B	B	B	B	B	B	H	B	A	A	H	H	A	H	H	H	H	B	H	A	B
T_133	H	A	A	A	A	A	A	H	B	A	H	A	A	A	A	A	A	A	B	H	H	H	H	B	H	-	B	B	B	B	B	B	B	H	H	H	H	B	B
T_134	A	A	H	-	B	H	H	H	H	H	A	H	H	A	B	H	A	A	B	B	A	H	B	H	H	H	B	H	B	B	B	H	H	H	H	A	A	B	B
T_135	B	H	H	H	H	H	A	H	H	A	A	H	H	-	H	A	A	B	B	H	H	B	B	B	H	H	B	H	H	B	H	H	B	B	B	H	H	H	B
T_136	H	B	B	H	A	A	H	H	B	H	B	B	B	B	B	B	H	-	A	A	B	B	B	B	H	A	B	B	-	B	H	B	B	B	B	H	H	A	B
T_137	A	B	B	B	H	H	H	H	H	A	A	A	A	A	A	H	H	B	H	H	H	B	B	B	B	B	B	H	H	B	H	H	H	B	B	B	H	B	B
T_138	A	A	A	A	B	B	H	H	B	H	-	B	B	B	B	H	A	B	A	H	B	H	H	B	B	H	H	B	B	H	B	H	B	B	B	H	A	A	B
T_139	H	B	B	H	A	A	A	A	H	H	H	H	H	H	H	H	H	B	H	A	B	B	B	B	B	H	H	H	H	H	H	H	H	B	B	B	H	H	B
T_14	A	H	H	B	B	B	B	H	B	H	A	H	A	A	A	H	A	H	H	H	H	H	H	H	B	B	H	B	B	H	B	B	A	A	A	H	A	A	H
T_140	B	B	H	A	H	H	A	A	H	H	H	H	H	H	H	H	H	B	A	H	A	A	A	B	H	A	H	H	H	H	H	H	A	A	A	H	H	H	B
T_141	H	H	H	H	H	H	H	A	H	H	H	A	A	A	H	B	H	H	H	H	B	B	B	B	H	A	B	H	H	B	H	H	H	H	B	H	H	B	B
T_142	B	A	A	H	H	H	H	H	A	H	B	A	A	A	H	A	A	B	B	A	H	H	H	B	B	H	A	A	A	A	A	H	H	H	H	H	H	A	A
T_143	B	B	B	B	B	H	H	H	A	A	H	B	B	B	B	A	H	B	H	H	A	H	A	B	B	A	H	A	A	H	A	A	B	B	B	A	A	A	H
T_144	H	H	H	A	B	H	H	H	H	A	H	B	B	B	B	B	B	B	B	A	H	A	H	B	H	H	H	H	H	H	H	B	B	B	B	H	A	A	B
T_145	A	B	B	B	H	H	H	H	H	H	A	-	-	B	B	A	A	B	B	-	A	B	B	B	H	H	A	H	H	A	H	A	H	H	H	A	A	A	B
T_146	-	H	H	H	A	A	H	A	A	H	-	H	H	H	H	H	H	B	H	H	A	H	H	H	B	H	H	A	A	H	A	A	A	A	A	B	H	B	B
T_147	H	B	B	H	B	B	H	H	A	H	-	H	H	H	H	H	H	B	H	H	A	H	H	H	B	-	H	A	A	H	A	A	H	H	H	H	H	H	B
T_148	H	H	H	H	A	A	A	A	H	A	A	B	B	B	B	H	H	B	A	A	A	A	A	B	H	H	B	H	H	B	H	H	A	A	A	A	H	H	B
T_149	H	H	H	H	H	H	A	A	A	A	A	B	B	B	B	H	H	B	H	H	B	H	H	A	H	H	A	A	A	A	A	H	H	B	B	B	H	B	B
T_15	H	H	H	B	H	H	H	H	H	A	A	A	-	A	A	B	B	B	-	H	A	B	H	H	H	B	H	H	H	H	H	H	A	H	H	H	A	H	H
T_150	H	A	A	A	A	A	A	A	-	A	A	H	H	H	H	B	H	B	A	B	A	H	B	B	H	H	H	-	B	H	B	B	B	B	B	A	A	A	B
T_151	H	A	A	A	H	H	B	-	H	H	B	H	H	H	H	H	H	H	B	A	A	A	H	B	H	B	B	H	H	B	H	H	B	B	B	B	H	B	B
T_152	B	H	A	A	A	A	H	H	A	H	H	H	H	H	H	B	H	B	H	H	B	H	A	B	H	B	B	A	A	B	A	A	H	H	H	H	H	B	B
T_153	H	A	A	A	B	B	B	B	H	H	B	-	-	H	H	H	H	B	H	A	A	H	A	B	H	B	B	H	H	H	H	H	H	H	H	H	H	B
T_154	-	H	H	H	H	H	B	-	H	H	-	H	H	H	H	H	H	B	H	H	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
T_155	H	H	H	H	H	H	H	B	H	H	A	A	A	A	A	H	B	H	B	H	B	B	B	B	H	A	B	H	H	B	H	H	H	H	H	B	H	H	B
T_156	H	B	B	B	H	H	A	A	H	A	A	-	-	H	A	H	H	B	H	H	A	H	H	A	H	H	H	H	H	H	H	H	B	B	B	H	H	H	H
T_157	A	H	H	H	H	H	H	H	B	H	A	H	H	H	H	H	B	B	B	A	A	H	H	B	B	A	B	B	B	B	B	H	H	H	H	H	H	H	B

T_158	B	B	B	H	A	A	H	H	H	H	H	B	B	B	B	A	A	B	H	H	B	B	B	H	H	B	H	H	H	H	H	H	A	A	A	H	H	H	B
T_160	A	H	H	B	B	B	H	H	H	H	A	H	H	H	H	H	H	B	H	H	B	B	B	B	B	A	H	H	H	H	H	H	A	A	-	H	H	H	B
T_161	H	H	H	-	B	B	H	B	B	A	B	H	-	H	H	A	H	B	B	A	A	A	A	H	B	H	H	B	A	H	A	B	H	H	H	A	A	A	B
T_162	H	A	A	B	B	A	A	A	A	H	H	B	H	B	B	H	H	B	H	H	A	H	B	A	B	H	A	A	H	H	H	A	H	H	H	H	A	A	B
T_163	-	B	B	B	B	B	B	B	H	H	-	H	A	H	H	B	B	B	A	A	A	A	A	H	H	H	A	H	H	A	H	H	A	A	A	B	H	B	B
T_164	H	A	A	A	H	H	H	H	H	A	A	A	A	A	A	H	H	B	H	H	A	H	H	H	H	B	H	H	H	H	H	A	B	B	B	H	H	H	B
T_165	B	H	H	H	B	B	B	B	H	A	H	-	-	H	B	H	B	B	H	A	A	A	A	H	H	A	H	H	B	H	B	B	B	B	B	B	H	B	B
T_166	B	A	A	A	A	A	H	H	B	H	B	-	-	H	H	H	H	B	H	H	A	H	H	A	B	H	A	H	H	A	H	H	B	B	B	A	A	A	-
T_167	H	B	H	H	H	H	A	A	H	H	A	H	H	A	A	H	H	B	H	H	A	A	A	H	H	H	H	H	H	H	H	A	H	H	H	A	H	B	B
T_168	B	H	H	H	H	H	B	B	H	H	H	B	B	B	H	A	H	B	B	H	B	B	B	B	H	H	A	H	B	A	B	H	H	H	H	B	H	H	B
T_169	H	B	B	B	H	H	B	H	H	A	A	A	A	A	A	H	H	H	A	H	A	B	B	H	H	H	H	H	A	H	A	H	H	H	H	A	A	H	H
T_17	A	H	H	B	H	H	H	H	B	H	-	-	-	B	B	A	H	B	A	H	A	A	H	B	B	B	B	B	B	B	B	B	B	B	B	B	H	B	H
T_170	H	A	A	A	B	B	B	B	A	A	A	A	A	A	A	H	B	B	B	B	H	A	B	B	H	H	H	-	A	A	H	A	H	B	B	-	H	A	-	A
T_171	A	A	A	A	H	H	A	A	A	A	H	H	H	H	H	A	A	B	H	H	A	B	B	H	H	H	A	A	A	A	B	H	H	H	H	H	H	H	B
T_172	H	H	H	H	A	A	H	H	H	H	A	-	-	B	B	H	A	B	H	A	A	H	H	H	H	H	A	H	H	A	H	B	H	B	B	A	A	H	H
T_173	H	H	H	-	A	H	A	A	H	B	H	H	A	H	H	B	H	B	H	H	A	H	B	H	B	H	H	H	H	H	H	H	H	H	A	A	A	A	B
T_174	B	A	-	H	H	H	H	H	H	B	B	H	H	H	H	B	H	B	B	H	A	H	H	H	B	H	H	H	H	H	H	B	H	H	H	H	H	H	B
T_175	B	H	B	-	H	A	A	H	H	H	B	H	H	-	H	B	A	A	A	H	A	A	B	H	A	H	H	H	-	B	-	H	H	B	-	A	A	-	A
T_176	H	H	H	B	H	H	A	A	A	A	B	B	B	B	B	B	B	B	B	H	A	H	H	B	B	H	H	A	A	H	A	A	H	H	H	A	A	A	B
T_177	B	H	B	-	H	A	H	B	A	A	A	H	B	-	A	B	A	A	A	B	A	H	-	A	-	H	A	A	B	A	B	A	H	A	A	H	A	-	H
T_178	H	A	A	B	H	A	H	-	H	B	A	B	H	H	H	B	H	H	B	H	A	H	-	H	B	A	A	H	-	H	-	B	H	H	H	H	A	B	A
T_179	A	B	B	H	B	B	B	B	A	A	-	A	A	A	A	A	H	H	B	-	B	H	H	H	H	H	H	A	A	H	A	H	H	H	H	H	H	A	B
T_18	B	B	B	-	H	H	A	A	H	A	A	A	-	H	H	H	-	B	A	A	A	H	A	H	H	H	H	H	H	H	H	H	H	A	A	A	A	A	-
T_180	H	H	H	-	-	H	B	B	B	B	A	H	B	H	B	B	B	H	A	B	A	A	A	B	B	A	B	B	B	B	B	B	H	H	H	H	H	H	A
T_181	H	A	A	B	B	B	B	B	H	A	A	A	A	A	H	B	H	B	H	A	A	H	B	-	H	A	B	H	H	B	H	H	B	B	B	H	H	H	B
T_182	H	B	B	B	A	A	A	A	A	B	H	H	H	H	H	H	B	B	-	H	A	H	H	A	B	B	H	A	H	H	H	H	H	B	B	H	H	H	B
T_183	B	H	H	A	H	H	A	A	H	B	H	H	H	H	B	H	H	A	H	H	B	B	B	B	B	H	B	H	H	B	H	H	B	B	B	H	H	B	-
T_184	H	A	A	A	A	H	B	B	A	H	H	-	-	B	H	H	H	H	H	A	A	A	B	H	B	B	H	A	A	H	A	B	A	A	A	A	H	H	H
T_185	B	H	H	H	B	B	H	H	H	H	H	H	H	H	H	H	B	H	H	H	A	H	H	H	B	B	B	H	H	B	H	B	H	B	B	B	A	H	B
T_186	A	A	A	A	H	H	A	A	A	A	H	B	B	B	B	H	B	B	B	H	A	H	H	H	H	H	H	H	H	H	H	H	A	A	A	A	A	A
T_187	H	A	A	-	H	A	H	H	H	A	H	H	-	A	B	B	A	A	A	B	B	B	B	H	B	B	H	H	A	H	A	H	H	H	H	H	A	H	B
T_188	H	A	A	H	B	B	A	A	H	H	A	A	A	A	A	B	B	B	H	A	A	A	A	H	H	H	H	H	H	H	H	B	B	B	B	H	B	B
T_189	H	H	H	H	H	H	H	H	A	H	A	H	H	H	H	H	A	B	H	H	A	H	H	B	H	B	B	A	A	B	A	A	B	B	B	H	H	A	H

T_19	H	A	A	-	H	H	A	A	B	A	B	A	-	H	H	H	-	B	H	H	A	H	H	H	H	B	H	B	B	H	B	B	B	B	B	H	H	H	A
T_190	H	H	H	H	H	H	H	H	B	A	A	-	-	A	H	B	H	B	B	H	A	H	H	B	B	B	B	B	B	B	B	H	H	B	B	H	H	H	H
T_191	B	H	H	A	H	A	A	A	A	H	B	H	H	H	A	A	A	A	A	B	A	H	H	H	B	B	H	A	A	H	A	A	H	H	H	H	H	H	B
T_192	H	A	H	B	B	H	A	A	H	A	H	B	B	B	B	H	H	A	B	B	A	-	H	H	B	H	A	H	H	A	H	H	H	H	H	A	A	A	H
T_193	B	B	B	H	H	H	H	H	A	H	B	H	H	H	A	A	H	H	H	A	A	A	A	H	H	A	A	A	B	A	B	B	H	A	A	H	A	A	H
T_194	H	A	A	A	B	H	H	H	A	H	H	H	H	H	H	H	H	B	A	A	A	B	B	H	H	B	A	A	A	A	A	A	H	H	H	A	A	A	A
T_195	H	A	A	A	A	A	A	A	B	A	A	A	A	A	H	B	H	B	H	H	A	H	H	H	H	H	B	B	B	B	B	H	B	B	B	A	H	H	B
T_196	B	H	H	H	B	H	A	A	H	A	H	H	H	H	B	H	B	H	A	A	A	B	B	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A
T_197	H	A	H	-	H	A	-	-	-	H	-	A	H	-	A	B	A	B	B	B	A	-	B	H	-	H	H	-	A	-	A	B	B	B	H	H	A	-	B
T_198	H	H	H	H	H	H	B	B	B	A	A	H	H	H	H	A	H	B	B	H	A	H	H	H	B	B	B	B	H	B	H	B	A	A	A	H	H	H	B
T_199	H	A	A	-	H	A	B	H	A	H	B	H	-	A	H	A	B	B	H	B	B	H	B	A	B	H	H	A	B	H	B	A	H	B	B	H	A	H	H
T_20	A	B	B	-	H	B	H	A	A	H	A	H	H	H	A	H	A	A	H	H	A	B	B	H	B	A	A	A	H	H	H	A	H	H	A	B	H	B	B
T_200	A	A	A	B	B	B	A	A	H	A	B	H	H	A	H	H	H	-	B	A	A	H	B	H	H	H	A	H	H	A	H	H	H	H	H	A	A	A	
T_201	B	H	H	H	H	H	A	A	H	H	-	A	A	A	A	H	H	H	A	H	B	H	H	H	H	A	A	H	H	A	H	B	A	A	A	H	H	H	A
T_202	H	A	H	A	A	A	A	A	B	H	A	H	H	H	A	B	H	B	H	H	B	A	A	H	H	B	H	B	B	B	B	A	H	H	H	A	A	A	B
T_203	H	H	H	H	H	H	H	H	H	H	A	A	A	A	A	H	A	B	B	B	A	H	H	H	B	A	H	H	H	H	H	B	H	H	H	H	A	A	A
T_204	A	H	H	B	H	H	H	H	A	A	A	H	H	H	B	H	B	B	B	B	A	A	A	A	B	H	A	A	A	A	A	B	A	A	A	B	H	B	B
T_205	A	B	B	H	H	H	H	H	B	B	A	H	A	A	A	A	B	H	H	H	A	H	H	-	H	A	B	B	B	B	B	B	A	H	H	B	H	B	H
T_206	H	H	H	-	H	H	A	A	A	B	H	A	A	A	B	H	A	A	A	H	A	H	H	A	B	A	A	A	A	A	A	A	H	H	H	H	H	B	B
T_207	H	B	B	-	B	B	A	A	H	A	A	H	-	-	H	B	A	A	A	A	A	H	H	B	B	H	A	H	B	H	B	H	H	H	H	H	A	-	A
T_208	H	B	H	H	A	A	A	A	H	H	A	H	H	H	H	B	B	H	A	H	A	H	A	H	H	H	A	H	H	A	H	H	H	H	H	H	H	B	A
T_209	H	B	B	-	A	H	H	H	H	H	A	H	H	A	H	B	B	H	H	H	A	A	A	H	B	A	H	H	A	H	A	H	B	B	B	H	H	H	A
T_21	A	B	B	H	H	H	B	B	B	B	H	A	A	A	A	H	A	H	B	B	A	H	-	B	B	H	B	B	B	H	B	B	H	H	H	B	H	B	B
T_210	A	H	H	A	H	H	B	B	A	H	A	H	H	H	A	A	H	B	H	A	B	H	H	H	B	B	H	A	A	H	A	A	H	A	A	B	H	B	A
T_211	-	A	A	A	H	H	H	H	H	A	A	B	B	B	B	A	H	B	B	H	A	A	A	H	H	H	H	H	H	H	H	A	A	A	B	H	-	A	
T_25	A	A	A	A	A	A	B	B	H	A	H	A	A	A	A	A	A	A	B	H	A	H	H	B	B	A	H	H	H	H	H	A	B	H	H	A	H	H	H
T_26	A	A	A	A	H	H	H	H	B	H	H	B	H	H	A	B	H	H	A	H	A	B	B	H	B	H	H	B	B	H	B	B	H	A	A	H	H	H	B
T_27	H	H	H	B	H	H	H	H	H	H	H	A	A	A	A	A	H	B	B	B	A	A	A	H	B	B	H	H	H	H	H	B	A	A	A	A	A	A	B
T_28	H	H	H	H	A	A	H	H	B	A	H	-	-	H	H	H	B	B	H	H	A	B	B	H	H	A	H	B	B	H	B	B	A	A	A	A	A	A	B
T_29	A	A	A	A	H	H	B	B	H	B	A	A	A	A	H	H	B	B	H	H	B	B	B	H	H	A	B	H	H	H	H	A	B	B	B	A	A	A	H
T_30	H	A	A	B	B	B	A	-	A	H	H	H	-	H	H	B	H	B	A	A	A	B	-	B	H	H	H	A	A	H	A	H	H	H	H	H	H	B	B
T_31	B	H	H	H	B	B	H	H	H	H	B	B	B	B	B	B	H	H	A	A	B	H	H	H	B	B	H	H	H	H	H	H	B	B	B	A	A	A	B
T_32	A	H	B	H	H	H	H	A	H	H	H	A	A	A	-	H	B	B	-	H	A	H	H	B	B	B	H	H	H	H	H	H	-	B	B	H	A	A	-

T_33	H	A	A	A	H	H	H	H	A	A	H	-	-	H	H	H	H	H	B	H	B	B	B	B	B	B	A	A	A	A	A	H	H	H	H	H	H	H	A
T_34	B	H	H	H	B	A	A	-	B	A	B	A	A	A	A	A	B	B	B	A	A	H	H	H	H	H	A	B	A	A	A	H	B	B	B	B	H	-	B
T_35	H	B	B	B	B	B	H	H	B	H	H	A	A	A	A	A	A	B	B	A	A	H	B	H	H	B	H	B	B	H	B	B	A	A	A	H	H	H	B
T_36	H	B	B	H	B	B	A	-	H	A	A	H	H	H	A	H	H	B	H	B	A	H	H	H	H	H	H	H	H	H	H	A	H	H	H	A	A	A	B
T_37	H	B	B	H	H	A	A	-	B	H	B	B	B	B	H	A	H	B	B	A	A	B	B	H	H	B	H	B	B	H	B	B	H	H	H	H	H	B	H
T_38	H	H	H	-	-	H	A	A	-	H	A	A	A	-	H	A	A	A	-	A	A	-	-	A	-	B	A	-	H	H	H	B	-	-	B	H	A	-	B
T_39	A	H	B	H	H	B	H	H	A	B	-	H	H	H	H	A	A	B	B	A	A	A	A	H	H	H	H	A	H	H	H	A	B	B	H	H	A	H	B
T_40	B	B	B	B	B	H	A	A	A	A	A	A	A	A	H	H	H	H	H	B	A	B	H	H	H	-	H	A	A	H	A	A	H	H	H	A	A	A	B
T_41	A	A	A	H	H	H	H	H	H	B	A	B	B	B	B	A	H	H	B	H	A	B	B	H	B	H	A	H	H	A	H	H	H	H	H	A	A	A	B
T_42	A	H	H	H	H	A	H	H	B	B	B	H	H	H	H	H	A	B	A	H	A	B	B	B	B	H	B	B	B	B	B	H	A	A	H	H	H	H	B
T_43	H	H	H	H	B	B	H	H	B	H	H	H	H	H	H	B	H	B	A	H	A	H	H	B	H	H	B	B	B	B	B	H	H	H	H	H	H	B	B
T_44	A	H	H	A	H	H	B	B	H	A	H	H	H	H	H	B	H	B	A	H	A	A	B	H	H	H	H	H	H	H	H	H	B	B	B	A	A	A	A
T_45	H	H	H	A	A	A	A	-	H	B	B	H	H	H	H	H	A	B	H	B	B	B	B	B	H	H	H	H	H	H	H	H	B	B	B	H	H	H	B
T_46	H	H	H	H	H	H	B	B	H	A	H	H	-	H	H	H	H	B	H	B	A	B	B	H	H	H	B	H	H	B	H	H	H	H	B	H	A	A	B
T_47	B	H	H	B	H	H	A	A	B	B	A	A	A	A	A	H	H	A	B	H	A	B	H	-	B	-	A	B	B	A	B	B	H	H	H	H	H	H	B
T_48	A	A	A	A	B	B	B	H	B	A	A	A	H	A	A	B	H	-	H	H	B	H	B	H	H	A	H	B	B	H	B	H	A	H	H	H	H	H	B
T_49	A	B	B	B	H	H	H	A	A	H	B	-	-	B	B	H	H	A	H	B	B	H	H	H	H	H	H	A	A	H	A	A	B	H	H	A	A	A	B
T_50	B	H	B	H	A	A	A	-	H	B	H	A	A	A	B	B	H	B	A	H	A	B	H	H	H	H	H	H	H	H	H	B	A	A	-	B	H	H	-
T_51	A	A	A	A	A	A	B	B	H	B	H	B	B	B	B	A	H	B	B	A	A	B	B	B	H	A	B	H	H	H	H	H	H	H	H	B	H	B	-
T_53	B	A	A	A	B	B	H	H	B	A	H	B	B	B	B	A	B	B	B	H	A	H	H	H	H	A	H	B	B	H	B	H	H	B	B	H	A	A	B
T_55	H	A	H	B	H	H	A	-	A	H	H	A	H	-	B	H	A	A	B	B	A	H	B	A	B	B	A	A	B	A	B	H	H	H	H	H	-	-	B
T_56	B	A	A	-	B	B	A	-	A	H	H	H	H	H	H	B	H	H	A	A	A	H	H	H	B	B	A	A	A	A	A	A	H	H	H	B	H	B	H
T_57	A	A	A	A	A	A	B	B	H	H	H	H	H	H	H	H	H	H	H	H	A	H	H	B	B	B	-	H	H	H	H	H	H	H	H	A	A	A	H
T_58	A	B	B	B	A	A	B	B	H	A	-	H	H	H	B	B	B	A	A	H	B	B	B	H	H	H	H	H	H	H	H	B	B	B	B	H	H	B	H
T_60	H	H	H	H	H	H	B	B	B	A	-	A	A	A	H	B	H	A	A	A	A	H	B	H	H	B	A	B	B	A	B	B	H	B	B	B	H	B	B
T_61	H	A	A	A	H	H	B	B	H	H	H	H	H	H	-	H	H	A	H	H	B	H	H	H	-	H	H	H	H	H	H	H	H	H	A	A	A	B
T_62	H	H	H	-	B	B	A	A	H	A	H	A	A	A	A	B	B	-	H	A	A	A	A	B	H	B	B	H	H	B	H	H	H	H	H	H	H	B
T_63	A	H	H	H	H	H	B	B	H	B	H	H	H	H	H	H	B	H	H	B	A	A	A	H	H	A	H	H	H	H	H	A	H	H	H	B	H	B	H
T_64	H	B	B	H	H	H	A	H	A	B	A	A	A	A	A	A	H	B	B	B	A	A	A	H	B	H	H	A	A	H	A	H	H	B	B	B	H	B	B
T_65	H	A	A	H	B	B	H	H	H	A	H	H	H	H	H	A	H	H	B	H	A	H	H	H	B	B	H	H	H	H	H	A	H	H	H	H	H	H	B
T_66	A	H	H	H	H	H	B	B	B	H	-	B	B	B	A	B	H	B	H	H	A	H	H	H	H	H	B	B	B	B	B	H	H	H	H	B	H	B	H
T_67	H	A	A	H	B	B	B	B	H	A	H	H	H	H	H	H	H	A	A	H	B	H	B	B	H	H	H	H	H	B	H	H	H	H	H	B	H	B	B
T_68	H	B	B	A	A	A	A	A	H	B	A	H	H	H	H	H	H	B	H	A	A	H	H	H	H	H	A	H	H	A	H	H	B	B	B	A	A	A	B

T_69	H	B	B	H	A	A	H	B	B	A	H	H	H	H	H	B	H	B	H	H	A	A	H	B	B	H	B	B	B	B	B	B	B	H	H	B	H	B	B
T_70	H	H	H	H	A	A	H	H	H	B	B	B	-	B	B	B	B	H	A	A	A	H	H	H	H	H	A	H	H	A	H	A	B	B	B	H	H	B	B
T_71	H	A	A	H	H	H	H	H	B	B	H	H	A	A	A	H	H	B	H	B	A	A	B	B	B	H	B	B	B	H	B	B	A	A	A	H	A	A	B
T_72	B	H	H	H	A	A	H	H	B	A	H	H	H	H	H	B	H	B	H	B	B	B	B	H	H	H	H	B	B	H	B	B	B	B	B	H	H	B	A
T_73	A	H	H	H	B	B	H	H	H	H	A	H	H	H	H	H	B	B	H	H	A	H	H	H	H	A	A	H	H	A	H	H	B	B	B	H	H	H	B
T_74	B	H	H	H	A	A	A	A	H	A	A	A	A	A	A	H	H	B	H	A	A	H	H	B	H	B	H	H	H	H	H	H	H	H	H	B	H	H	B
T_75	B	H	H	H	A	A	B	B	H	A	A	H	H	H	H	A	H	B	B	H	A	A	A	H	H	H	H	H	H	H	H	B	H	H	H	H	H	H	B
T_76	H	A	A	A	H	H	B	B	A	A	-	H	H	H	H	H	H	B	H	A	B	B	B	H	H	B	H	A	A	H	A	A	A	A	A	A	A	-	B
T_77	H	H	H	B	H	H	A	A	H	A	-	H	H	H	H	B	B	H	H	B	A	A	A	A	H	A	H	H	B	H	B	H	B	B	B	H	H	H	A
T_78	H	B	B	H	B	B	H	H	H	B	H	H	H	H	H	A	A	B	B	B	A	A	A	B	B	H	B	H	H	B	H	H	B	B	B	A	A	A	A
T_79	B	A	A	A	A	A	H	H	B	A	A	H	H	H	H	A	H	B	H	A	A	H	H	H	H	H	A	A	A	A	A	A	A	A	A	H	H	H	H
T_80	A	H	H	H	B	B	B	B	A	H	H	H	H	H	H	H	H	B	A	B	B	A	H	H	B	A	A	A	H	A	H	H	H	H	H	A	A	A	H
T_81	-	A	A	A	H	H	A	B	B	B	H	A	A	A	A	A	A	B	B	A	A	H	H	H	H	H	B	B	H	B	B	B	B	B	B	H	B	B
T_82	B	H	H	H	H	H	A	A	H	A	-	B	B	B	B	B	H	H	A	H	A	B	H	B	H	H	B	H	H	B	H	H	B	B	B	B	H	B	H
T_83	H	B	B	B	B	B	A	H	H	B	A	A	A	A	A	A	H	B	B	H	A	H	H	H	H	B	H	H	H	H	H	A	A	H	H	H	H	H	B
T_84	B	B	B	B	A	A	H	-	A	A	H	A	H	H	A	H	H	B	H	H	A	A	H	H	H	H	H	A	A	H	A	A	H	B	B	H	H	H	B
T_85	H	H	H	H	B	H	B	B	H	H	A	H	-	H	H	H	H	B	H	H	A	H	H	B	H	H	B	H	B	B	B	B	B	B	B	A	A	A	B
T_86	H	H	H	H	B	A	A	A	A	H	H	H	-	H	H	B	B	B	A	H	A	H	B	B	H	H	H	A	A	H	A	H	B	B	B	B	H	H	A
T_87	H	B	B	B	H	H	A	A	H	H	A	B	B	-	H	-	H	B	B	H	A	H	-	B	B	A	A	H	-	H	B	H	B	B	B	H	A	-	B
T_88	B	H	H	H	A	H	A	A	A	H	B	B	B	B	B	H	B	B	H	H	A	H	-	H	B	A	H	A	A	H	A	A	H	H	H	B	A	A	B
T_89	H	H	H	B	A	H	H	H	H	B	B	B	B	B	B	B	B	-	A	A	A	H	H	B	H	H	H	H	H	H	H	H	B	B	B	A	A	A	H
T_90	A	H	H	H	H	H	B	B	H	H	-	H	H	H	H	B	B	B	A	H	B	H	B	H	B	H	B	H	H	H	H	A	H	B	A	H	H	H	B
T_91	H	H	H	H	B	B	A	A	A	H	H	B	B	B	B	B	H	A	A	H	B	A	H	B	H	B	H	A	A	B	A	A	H	H	H	H	H	H	H
T_92	B	H	H	H	A	A	A	A	H	A	H	A	A	A	A	H	H	B	H	H	A	H	A	H	B	H	H	H	H	H	H	H	B	B	B	B	H	B	H
T_93	H	B	H	-	H	A	A	-	B	B	H	A	H	-	A	-	H	B	H	B	A	H	A	B	A	A	H	B	A	B	A	H	A	B	H	A	B	-	B
T_94	H	A	A	H	H	B	A	A	B	H	B	H	H	H	A	A	H	B	B	B	A	H	H	H	H	H	H	B	B	H	B	B	H	H	H	B	H	B	-
T_95	B	B	A	H	-	A	H	H	A	A	H	A	A	A	A	H	H	B	H	H	A	B	B	H	H	H	A	A	A	A	A	H	B	B	B	H	H	B	B
T_96	B	H	H	H	B	A	H	H	H	A	H	H	H	H	H	B	H	A	A	H	B	H	B	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H
T_97	B	H	H	H	H	A	H	H	H	H	A	H	H	H	B	B	H	B	H	H	A	H	B	A	H	A	H	H	H	H	H	H	H	H	H	H	H	H	-
T_98	B	H	H	H	B	B	H	H	H	B	-	A	A	A	A	-	H	B	H	H	A	H	H	B	B	B	H	H	H	H	H	H	H	H	H	A	A	A	-
T_99	H	H	H	H	A	A	H	H	B	B	A	H	H	H	H	-	H	H	A	A	A	B	B	A	H	H	H	A	A	H	A	A	H	H	H	B	H	H	-
P1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
P2	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

Individual	Xisep0511	Xisep0539	Xtxp067	Xcup002	Xtxp296	sbAGE01	Xisep0639	Xisep0622	Xcup12-a	Xtxp270	Xtxp141	Xcup12	Xtxp141	Xtxp270	Xisep0639	Xisep0622	Xtxp205	Xisep0108	Xisep1231	Xtxp250	Xcup12-a	Xtxp274	Xisep0617	Xisep0443	Xtxp057	Xisep0449	Xtxp265	Xcup36-b	sbKAKGK01	Xtxp091	Xisep1208	Xisep1029	Xiabt240	Xisep1107	Xtxp014	Xtxp113	Xtxp023
T_100	A	H	A	B	-	A	H	B	A	A	A	A	A	A	H	B	H	A	H	-	A	-	H	H	B	B	H	H	H	B	B	A	B	A	H	A	-
T_101	H	H	H	H	A	H	B	A	B	H	B	B	B	H	B	A	B	H	A	H	B	-	A	A	A	H	H	A	A	A	B	B	B	B	B	B	B
T_102	B	H	A	A	B	B	A	A	A	H	H	A	H	H	A	A	H	B	A	B	A	H	H	H	H	H	A	A	A	H	B	B	B	A	A	H	H
T_103	A	H	B	H	-	B	B	B	A	H	B	H	B	H	B	B	B	H	B	H	A	A	B	B	B	B	B	B	H	H	H	A	A	A	H	H	H
T_104	H	B	H	B	B	H	H	A	A	H	H	H	H	H	H	A	H	H	B	A	A	H	A	A	A	A	H	H	-	H	A	A	A	H	H	H	H
T_105	H	B	B	H	A	A	H	A	A	A	B	B	B	A	H	A	A	H	B	H	A	A	A	H	H	H	A	A	B	B	H	A	A	H	A	A	A
T_106	H	H	H	B	B	H	H	-	A	H	H	H	H	H	H	-	H	A	H	H	A	-	B	H	H	H	H	H	H	H	B	B	B	B	H	H	H
T_107	H	A	H	H	B	A	H	A	B	H	A	A	A	H	H	A	H	H	H	H	B	A	B	A	A	A	B	A	A	H	H	A	A	A	H	H	H
T_108	H	B	H	A	H	B	H	B	A	H	H	A	H	H	H	B	A	B	H	A	A	A	A	A	A	A	B	H	H	H	A	H	H	H	H	H	H
T_109	H	B	H	H	A	H	H	B	A	H	A	H	A	H	H	B	A	H	H	B	A	B	B	B	H	B	A	B	A	A	H	H	H	H	B	B	B
T_11	A	H	H	H	H	H	B	A	H	H	H	A	H	H	B	A	B	B	B	H	H	H	H	H	H	A	H	B	H	A	B	H	B	B	B	B	B
T_110	H	H	H	H	B	H	H	A	A	H	A	B	A	H	H	A	H	A	H	A	A	H	H	H	A	H	B	H	A	A	H	H	H	B	B	B	B
T_111	A	H	H	A	-	A	-	B	A	B	A	H	A	B	-	B	B	A	B	A	A	H	A	B	-	-	H	A	H	B	H	B	B	H	B	A	-
T_112	H	H	A	A	A	A	B	H	H	A	H	H	H	A	B	H	H	H	H	-	H	B	H	H	H	H	B	H	H	H	H	A	H	H	H	H	H
T_114	A	A	A	A	H	H	H	A	H	H	B	A	B	H	H	A	H	B	H	A	H	H	H	H	A	A	H	H	A	A	B	B	B	B	B	B	B
T_115	A	H	A	A	A	H	A	H	B	H	H	H	H	H	A	H	A	A	H	B	B	H	H	H	H	H	H	H	B	B	A	A	A	A	A	A	H
T_116	H	B	B	A	H	H	A	A	B	H	A	B	A	H	A	A	H	H	H	B	B	H	H	H	H	H	B	H	B	B	B	B	B	H	H	A	H
T_117	H	H	H	B	A	-	H	A	H	H	H	H	H	H	H	A	B	H	B	B	H	-	B	H	H	H	H	B	B	B	B	B	H	H	A	A	-
T_118	A	A	A	A	A	B	B	B	H	H	H	H	H	H	B	B	B	B	H	H	H	H	H	H	H	B	B	H	H	H	B	A	H	H	H	H	H
T_119	A	A	H	A	-	B	H	A	H	A	B	B	B	A	H	A	H	H	H	H	H	H	H	B	B	B	H	H	H	A	H	H	H	H	B	B	B
T_120	H	H	H	H	H	H	B	B	A	H	A	A	A	H	B	B	H	B	H	A	A	H	B	B	B	B	H	H	H	H	B	H	H	H	H	H	H
T_121	A	B	-	B	-	B	-	B	A	A	A	A	A	A	-	B	H	H	A	B	A	-	-	B	-	H	B	A	H	-	H	-	B	A	-	H	-
T_122	H	H	A	A	B	H	A	A	H	H	H	H	H	H	A	A	B	B	H	H	H	H	B	B	B	H	A	B	H	A	H	A	A	A	A	A	-
T_123	H	H	B	H	A	H	B	A	H	H	A	A	A	H	B	A	B	H	B	B	H	H	H	H	H	A	B	B	B	A	A	B	B	B	B	B	-
T_124	H	H	A	H	H	H	A	A	B	H	H	H	H	H	A	A	A	A	B	H	B	A	A	H	A	H	A	A	H	H	B	B	B	B	H	H	H
T_125	B	B	A	H	A	B	H	A	A	A	A	H	A	A	H	A	H	H	B	B	A	H	H	H	H	H	H	H	B	B	B	H	A	A	A	A	A
T_126	A	A	H	B	H	H	H	A	A	A	H	H	H	A	H	A	H	H	B	H	A	H	H	H	H	H	A	H	H	H	H	A	A	A	A	H	A
T_127	H	H	B	B	H	H	A	A	H	A	B	B	B	A	A	A	B	H	B	A	H	H	H	H	H	H	B	H	A	H	H	A	A	A	A	H	H
T_128	H	H	H	H	H	H	A	H	A	H	H	H	H	H	A	H	B	A	A	H	A	A	A	A	H	A	A	H	A	-	A	B	B	B	H	H	H	B
T_129	B	B	A	A	A	H	H	A	A	H	H	H	H	H	H	A	A	H	H	A	A	A	A	A	H	A	A	H	A	-	H	B	B	H	H	H	H	B

T_130	B	H	H	H	A	H	A	A	H	A	H	H	H	A	A	A	H	H	H	B	H	H	H	B	H	H	H	H	H	H	H	B	H	H	H	H	H
T_131	H	A	A	A	A	H	H	A	B	H	H	H	H	H	H	A	H	B	H	H	B	B	B	B	B	B	H	B	H	H	B	B	B	H	A	H	H
T_132	B	H	A	A	H	B	B	H	B	H	H	H	H	H	B	H	B	B	H	H	B	B	B	B	B	H	H	B	-	H	H	A	A	A	A	H	H
T_133	B	H	B	B	A	H	A	A	H	H	B	B	B	H	A	A	H	H	A	H	H	B	H	H	H	H	H	H	H	H	H	B	B	H	H	H	B
T_134	A	H	H	B	A	A	A	A	H	-	H	H	H	-	A	A	H	H	A	A	H	B	H	B	A	H	B	B	B	B	H	-	A	A	A	H	A
T_135	H	B	H	H	A	B	H	A	A	H	H	H	H	H	H	A	H	A	H	H	A	H	H	H	H	H	A	H	A	H	H	H	H	B	H	H	H
T_136	H	H	H	H	H	B	H	A	A	H	B	B	B	H	H	A	A	H	H	B	A	A	H	H	-	B	H	A	H	A	B	B	H	B	H	A	H
T_137	A	A	H	B	H	B	A	B	B	H	H	H	H	H	A	B	H	H	H	B	B	H	H	H	H	H	B	H	A	A	H	B	B	B	B	B	B
T_138	H	H	B	B	A	H	B	B	H	H	B	H	B	H	B	B	B	H	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
T_139	A	B	H	B	A	B	B	H	A	H	H	H	H	H	B	H	H	H	H	A	A	A	H	H	H	H	A	A	A	H	H	A	A	A	A	H	H
T_14	B	H	A	A	A	H	B	B	B	H	B	B	B	H	B	B	H	A	B	H	B	H	B	H	H	H	A	H	H	B	B	H	H	H	A	A	H
T_140	H	H	A	H	H	H	A	A	H	H	H	H	H	H	A	A	B	B	H	H	H	H	H	H	H	B	H	H	B	B	B	H	H	A	A	A	A
T_141	A	H	B	H	H	H	H	A	H	A	H	H	H	A	H	A	H	B	A	H	H	H	A	H	H	H	B	H	H	A	A	H	B	B	B	B	H
T_142	A	H	H	H	H	A	H	H	H	H	A	H	A	H	H	H	B	H	H	A	H	A	H	H	H	H	H	A	B	B	B	B	A	A	A	A	A
T_143	H	B	H	H	A	H	B	H	B	H	B	A	B	H	B	H	B	B	H	H	B	H	A	H	H	H	A	H	H	A	H	B	B	B	B	B	H
T_144	A	H	H	A	A	A	H	A	H	A	H	B	H	A	H	A	H	A	H	H	H	A	A	H	H	H	H	A	H	A	H	B	B	B	B	B	H
T_145	H	H	H	H	H	B	B	A	B	H	H	A	H	H	B	A	H	B	H	A	B	H	H	H	H	H	H	-	-	B	B	B	H	A	A	A	A
T_146	H	H	B	H	B	H	B	H	A	H	A	A	A	H	B	H	H	H	A	B	A	H	H	H	H	H	B	H	B	H	B	A	H	H	H	H	H
T_147	H	A	A	A	A	H	-	B	B	H	A	A	A	H	-	B	A	H	H	A	B	H	H	H	H	H	B	H	H	H	H	A	A	A	H	H	H
T_148	A	B	H	B	H	H	A	A	A	A	H	H	H	A	A	A	B	H	H	H	A	A	A	A	-	A	H	A	H	H	H	H	H	H	H	H	H
T_149	A	A	H	H	B	H	A	A	A	A	A	H	A	A	A	A	H	H	H	A	A	B	B	H	H	H	A	H	H	H	A	A	A	A	H	H	H
T_15	B	H	H	A	H	B	H	A	A	H	H	H	H	H	H	A	A	B	H	H	A	-	H	H	A	A	H	H	-	A	B	H	H	H	H	H	B
T_150	A	A	H	H	A	H	-	A	H	H	-	B	-	H	-	A	H	H	H	H	H	A	A	A	-	H	H	B	H	H	H	H	H	A	H	A	A
T_151	H	H	A	B	A	H	H	B	A	A	H	H	H	A	H	B	B	H	H	H	A	A	A	H	A	A	A	A	B	B	B	A	A	A	A	A	A
T_152	H	A	B	H	H	B	H	A	A	A	A	A	A	A	H	A	H	H	H	H	A	H	H	H	H	H	B	H	H	H	H	H	H	A	A	H	H
T_153	H	A	H	H	B	H	A	A	A	H	H	H	H	H	A	A	H	H	H	B	A	A	A	A	A	A	B	A	-	A	H	B	B	H	B	B	H
T_154	H	B	A	A	A	A	B	A	H	H	H	H	H	H	B	A	A	H	H	H	H	H	H	B	B	B	H	H	B	B	A	A	A	A	A	A	A
T_155	H	A	H	H	B	B	H	A	A	A	H	H	H	A	H	A	B	H	B	B	A	H	B	B	B	B	A	H	H	B	H	H	H	H	H	A	A
T_156	B	H	H	B	H	B	H	A	H	H	H	H	H	H	H	A	A	B	H	H	H	H	H	H	B	B	H	H	-	-	H	A	A	H	H	H	B
T_157	A	B	A	A	B	H	H	H	A	H	B	H	B	H	H	H	-	A	H	B	A	A	A	A	A	A	H	A	H	-	H	H	A	A	H	H	H
T_158	B	H	B	H	A	B	H	A	A	H	H	H	H	H	H	A	A	H	H	B	A	B	A	A	A	A	A	H	B	-	A	A	A	A	A	A	A
T_160	B	H	A	H	H	B	B	H	B	H	H	H	H	H	B	H	H	H	H	H	B	H	H	H	H	H	A	H	H	A	H	H	-	H	H	A	A
T_161	B	B	H	A	A	A	H	H	A	A	B	B	B	A	H	H	H	B	A	B	A	-	A	H	A	A	H	A	A	-	A	B	B	H	B	B	B

T_162	A	H	H	-	A	H	B	H	A	A	A	A	A	A	B	H	A	A	H	H	A	B	H	B	B	B	B	H	B	-	B	B	B	B	B	B	H
T_163	H	A	B	H	B	H	H	A	B	A	H	H	H	A	H	A	H	H	H	H	B	A	A	H	H	H	B	A	H	A	H	B	B	B	B	B	H
T_164	B	A	H	A	H	B	H	A	A	H	H	A	H	H	H	A	A	H	H	A	A	H	H	H	A	A	H	H	B	B	A	A	A	A	A	A	A
T_165	B	H	H	H	B	H	H	A	A	H	H	B	H	H	H	A	B	B	H	H	A	A	H	H	H	H	H	A	-	B	H	A	A	A	A	A	H
T_166	B	A	H	A	A	H	B	B	H	H	H	H	H	H	B	B	A	H	H	A	H	H	H	H	H	H	H	H	-	A	A	A	H	B	B	B	B
T_167	H	A	H	H	A	H	H	A	H	H	H	A	H	H	H	A	H	B	A	H	H	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H
T_168	B	A	B	H	-	B	A	A	H	A	H	H	H	A	A	A	H	H	H	B	H	H	H	H	H	H	B	H	H	H	H	A	A	A	A	H	H
T_169	H	H	H	H	A	-	H	A	H	A	H	H	H	A	H	A	H	B	B	H	H	-	H	A	A	A	H	H	H	B	B	B	H	H	H	H	A
T_17	H	A	B	H	A	H	A	A	B	H	B	B	B	H	A	A	A	H	H	A	B	-	B	H	H	H	A	H	H	H	A	H	H	H	H	H	H
T_170	H	A	H	H	B	H	A	A	H	A	A	H	A	A	A	A	H	A	A	H	H	B	-	H	H	H	H	H	H	H	B	H	B	B	H	H	B
T_171	B	H	B	B	A	B	H	A	A	H	A	H	A	H	H	A	B	H	H	H	A	H	H	B	B	B	H	H	B	H	B	B	B	H	H	H	H
T_172	H	H	H	B	A	B	H	A	A	A	H	B	H	A	H	A	B	H	H	A	A	A	A	H	H	H	H	A	-	B	H	A	A	H	H	A	A
T_173	H	B	H	H	A	H	H	A	A	H	H	H	H	H	H	A	H	H	H	A	A	-	H	H	H	B	A	H	B	H	H	A	H	A	A	A	A
T_174	H	H	H	B	A	B	B	A	A	H	H	B	H	H	B	A	H	A	A	B	A	H	B	H	H	H	H	H	H	H	B	B	B	B	B	B	B
T_175	H	H	H	A	A	B	H	A	A	A	H	H	H	A	H	A	H	H	B	H	A	A	A	A	A	-	A	H	H	B	B	A	-	H	A	A	A
T_176	B	H	H	H	B	H	A	A	H	H	A	A	A	H	A	A	H	H	H	H	H	B	H	H	H	H	H	H	A	H	B	B	B	B	B	B	B
T_177	A	A	B	-	H	H	H	B	A	A	A	A	A	A	H	B	H	B	A	A	A	H	H	H	-	H	H	B	H	A	B	-	-	A	A	A	A
T_178	B	H	H	H	-	H	A	B	A	A	H	B	H	A	A	B	A	B	B	A	A	A	-	B	-	H	A	H	H	A	H	A	H	H	H	A	B
T_179	A	A	A	H	A	B	H	A	A	H	A	H	A	H	H	A	H	H	H	B	A	H	A	A	-	H	H	-	B	H	H	H	H	H	A	A	H
T_18	H	A	B	A	-	H	A	A	A	H	H	H	H	H	A	A	B	H	H	B	A	-	H	H	H	H	A	A	B	H	A	A	H	H	H	H	B
T_180	H	H	H	B	-	A	A	A	A	A	B	B	B	A	A	A	H	H	B	-	A	-	H	H	H	H	A	B	H	H	H	H	H	H	H	A	B
T_181	H	H	H	H	H	B	B	A	H	H	H	H	H	H	B	A	H	A	H	B	H	-	A	H	H	H	A	A	B	B	H	H	H	H	H	A	A
T_182	H	H	B	H	B	B	A	A	H	H	A	H	A	H	A	A	B	H	H	A	H	H	H	H	H	H	A	H	H	A	B	B	H	A	H	H	H
T_183	H	B	H	H	A	B	H	B	A	H	H	H	H	H	H	B	A	A	A	H	A	-	B	B	H	H	H	H	B	B	B	A	H	H	-	H	H
T_184	H	H	A	A	A	H	A	H	A	H	B	B	B	H	A	H	H	H	A	B	A	H	H	H	H	H	B	A	-	H	H	A	A	H	H	H	B
T_185	H	A	H	H	B	H	A	H	A	H	H	B	H	H	A	H	B	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
T_186	H	H	H	H	B	H	H	A	A	H	H	H	H	H	H	A	B	H	A	H	A	H	H	H	A	H	H	H	H	H	B	B	B	H	H	H	H
T_187	B	H	A	H	A	A	A	H	A	H	H	H	H	H	A	H	A	B	H	-	A	-	H	B	H	H	A	B	B	B	H	A	H	A	A	A	A
T_188	A	A	H	H	B	H	H	A	A	H	H	H	H	H	H	A	A	B	H	A	A	A	A	H	H	H	B	A	H	A	A	H	H	H	B	B	B
T_189	A	A	H	H	-	H	A	A	A	H	A	A	A	H	A	A	B	H	H	A	A	H	A	A	A	A	B	H	H	H	B	-	H	H	H	H	H
T_19	B	H	B	H	-	B	A	A	A	H	B	B	B	H	A	A	H	B	H	B	A	-	H	H	B	H	B	H	-	B	H	A	A	A	A	A	A
T_190	H	A	H	H	A	H	H	A	A	H	B	H	B	H	H	A	H	B	H	H	A	H	H	H	H	H	H	H	-	A	H	H	H	H	H	A	A
T_191	B	H	H	H	A	B	B	A	A	H	A	A	A	H	B	A	H	H	H	H	A	A	B	B	B	H	B	B	H	H	H	B	B	H	H	H	H

T_192	A	A	H	H	-	H	H	H	A	A	H	H	H	A	H	H	H	A	H	A	A	-	B	B	B	B	B	B	H	H	H	H	H	H	H	H
T_193	H	A	A	A	A	A	-	A	A	A	A	B	A	A	-	A	H	H	A	A	A	A	A	A	A	H	B	A	A	A	H	B	B	A	B	B	B
T_194	B	B	H	H	B	B	A	A	A	H	A	A	A	H	A	A	H	H	H	H	A	A	H	H	H	H	H	A	H	A	A	A	A	H	B	B	B
T_195	A	A	A	H	B	H	H	A	A	H	B	H	B	H	H	A	H	H	H	H	A	H	H	H	H	H	A	H	H	B	H	H	A	A	A	A	H
T_196	H	H	H	H	H	B	H	A	A	H	H	H	H	H	H	A	H	B	H	-	A	B	A	H	-	H	H	A	H	H	H	H	H	H	H	H	H
T_197	A	H	H	-	B	-	B	B	A	H	-	B	-	H	B	B	H	H	B	H	A	-	A	B	-	H	A	B	A	A	A	H	B	A	A	A	-
T_198	A	B	B	B	H	H	B	H	A	H	B	B	B	H	B	H	H	A	H	H	A	H	H	H	H	H	H	H	B	B	A	A	A	A	A	A	H
T_199	B	A	H	B	H	A	B	A	A	H	A	A	A	H	B	A	A	H	A	A	A	-	H	H	H	H	B	B	H	H	A	H	H	A	H	H	H
T_20	B	H	H	B	A	H	B	A	A	A	A	A	A	A	B	A	H	B	B	B	A	B	H	A	A	H	B	H	A	A	B	B	-	B	B	B	B
T_200	B	A	H	H	H	B	A	A	H	H	H	H	H	H	A	A	B	H	H	H	H	-	A	A	H	H	B	A	H	A	H	B	B	B	B	B	B
T_201	H	B	-	B	A	H	A	A	A	H	H	B	H	H	A	A	H	H	H	B	A	H	A	A	A	A	H	H	H	H	H	H	H	H	H	H	H
T_202	H	H	H	A	H	A	H	A	A	H	B	A	B	H	H	A	B	B	H	H	A	B	H	H	A	H	H	H	H	H	H	A	A	A	A	H	H
T_203	B	H	A	A	A	H	H	A	A	H	H	B	H	H	H	A	B	A	H	B	A	B	B	B	B	B	A	B	H	H	A	A	A	H	H	H	H
T_204	H	H	H	H	B	H	B	-	B	H	A	B	A	H	B	-	A	H	A	A	B	B	B	B	B	B	H	B	H	A	H	H	H	H	H	H	H
T_205	A	A	H	B	B	A	H	A	A	H	B	B	B	H	H	A	H	H	A	H	A	B	B	B	H	H	A	H	H	A	B	B	B	B	H	H	H
T_206	H	B	H	B	-	H	H	A	A	A	A	A	A	A	H	A	H	A	A	H	A	H	H	H	H	B	B	H	H	A	H	H	H	B	B	B	H
T_207	-	H	H	B	B	-	H	A	A	H	H	H	H	H	H	A	H	H	B	A	A	H	H	B	A	H	H	A	A	H	H	A	H	H	H	H	B
T_208	H	B	B	H	B	A	A	A	A	H	H	H	H	H	A	A	H	H	H	H	A	H	H	H	H	H	H	H	H	H	B	B	B	H	H	H	H
T_209	A	A	B	A	-	B	H	A	A	H	H	H	H	H	H	A	H	H	H	A	A	B	H	A	A	A	H	H	A	A	B	B	B	B	B	B	B
T_21	B	A	A	A	A	H	A	A	A	H	B	B	B	H	A	A	H	H	B	B	A	H	H	H	H	H	H	B	H	H	H	A	A	H	H	H	H
T_210	B	A	H	H	A	H	H	H	A	H	A	A	A	H	H	H	H	H	H	B	A	H	A	H	H	H	H	A	H	A	B	B	B	B	B	B	H
T_211	H	H	B	B	H	H	H	A	A	H	H	H	H	H	H	A	H	H	H	A	A	B	A	A	A	A	H	H	A	A	H	B	B	B	B	B	B
T_25	B	H	H	A	A	B	H	A	A	H	H	A	H	H	H	A	H	H	B	H	A	A	A	A	H	H	A	H	A	H	H	H	H	H	H	H	B
T_26	H	B	B	H	H	H	B	H	A	H	B	B	B	H	B	H	A	H	B	B	A	H	H	H	H	B	H	H	A	H	B	H	H	H	H	H	H
T_27	H	A	H	H	H	B	A	H	H	H	H	B	H	H	A	H	A	B	B	H	H	B	B	H	H	H	H	B	H	H	H	H	H	H	H	H	H
T_28	B	B	H	A	B	B	B	A	A	H	B	B	B	H	B	A	B	B	A	H	A	H	H	A	A	A	A	H	-	A	H	B	B	B	B	B	B
T_29	A	H	A	B	B	B	H	A	A	H	H	A	H	H	H	A	H	H	A	A	A	H	H	H	H	H	B	H	H	B	H	H	H	H	A	A	H
T_30	B	H	B	H	A	B	A	A	H	H	A	H	A	H	A	A	H	B	A	H	H	H	A	H	H	H	-	A	A	H	H	B	B	B	H	H	B
T_31	B	H	H	H	A	H	A	H	A	H	H	H	H	H	A	H	A	B	H	B	A	A	B	H	A	A	H	A	A	H	B	H	-	H	H	H	B
T_32	H	B	A	H	B	H	A	H	A	H	H	H	H	H	A	H	H	B	H	A	A	H	H	A	H	H	B	H	B	B	A	A	A	A	A	A	H
T_33	A	H	A	H	H	B	B	H	A	H	A	H	A	H	B	H	H	A	H	B	A	H	H	A	A	A	B	H	-	H	A	A	H	H	H	H	B
T_34	H	H	H	A	B	B	H	H	H	H	B	H	B	H	H	H	A	A	H	B	H	H	H	H	H	A	H	A	B	-	B	B	B	B	B	-	B
T_35	H	H	H	A	A	H	A	A	H	H	B	B	B	H	A	A	B	B	H	A	H	A	H	H	H	H	H	A	H	B	H	A	A	A	A	A	A

T_36	H	B	A	B	A	B	H	A	A	H	H	A	H	H	H	A	A	B	H	B	A	-	B	B	H	H	H	B	B	-	H	H	H	B	B	-	H
T_37	B	A	B	B	H	B	H	A	A	H	B	B	B	H	H	A	H	H	H	H	A	A	A	H	A	H	H	A	B	H	H	B	B	H	H	H	H
T_38	B	A	H	-	B	-	B	-	A	H	-	B	-	H	B	-	B	H	A	B	A	H	H	H	-	H	-	A	B	A	H	-	-	-	A	H	-
T_39	B	H	H	B	A	H	B	A	B	H	A	A	A	H	B	A	B	H	A	B	B	H	H	A	A	A	H	A	H	A	H	H	H	B	B	B	H
T_40	H	B	H	H	A	-	H	B	H	H	A	A	A	H	H	B	H	H	B	H	H	B	B	B	B	B	B	B	B	B	A	A	A	A	H	H	H
T_41	A	H	B	H	H	B	B	A	A	H	H	H	H	H	B	A	A	A	B	A	A	H	H	A	A	A	B	H	H	H	B	H	H	A	A	H	H
T_42	B	H	H	B	A	B	H	A	A	H	B	H	B	H	H	A	A	H	B	A	A	A	A	A	A	A	B	H	A	A	B	B	B	B	B	B	B
T_43	H	B	H	B	H	B	H	A	A	H	B	H	B	H	H	A	B	B	B	A	A	H	H	H	H	A	B	H	A	H	B	B	B	H	H	H	B
T_44	A	H	H	B	A	H	H	A	A	H	H	H	H	H	H	A	A	A	B	H	A	H	A	A	A	A	H	H	H	A	H	H	H	H	H	H	H
T_45	B	A	H	B	A	B	H	A	A	H	H	H	H	H	H	A	A	B	B	H	A	B	B	H	H	H	H	B	H	A	A	A	H	H	B	B	H
T_46	A	A	H	B	H	B	H	A	A	H	H	H	H	H	H	A	H	A	A	H	A	H	H	B	A	A	H	B	H	H	H	A	A	A	H	H	H
T_47	H	B	B	H	H	B	B	H	B	H	B	B	B	H	B	H	H	H	H	B	B	H	H	H	A	H	H	H	B	H	H	B	B	B	H	H	H
T_48	A	H	H	H	A	H	H	A	A	H	B	H	B	H	H	A	H	H	H	B	A	B	B	B	B	H	A	H	H	B	B	B	H	H	H	A	A
T_49	B	H	H	H	H	B	B	A	H	H	A	A	A	H	B	A	H	A	A	H	H	B	B	B	B	H	A	B	-	H	A	H	H	H	H	H	H
T_50	B	B	B	B	H	B	A	A	A	H	H	B	H	H	A	A	H	B	A	B	A	A	B	B	B	B	H	H	A	H	B	B	-	A	A	H	H
T_51	A	A	H	A	A	B	H	A	A	H	H	H	H	H	H	A	B	H	A	H	A	A	A	H	A	H	H	A	B	B	B	B	B	B	B	A	A
T_53	H	B	H	B	B	H	-	A	A	H	B	H	B	H	-	A	H	H	A	H	A	H	A	A	A	A	A	H	H	B	H	H	H	A	A	A	A
T_55	B	H	H	-	B	-	B	A	A	-	A	H	A	-	B	A	H	H	A	H	A	B	B	H	-	H	H	B	H	H	H	A	B	A	H	H	-
T_56	H	H	A	H	H	H	B	H	B	B	A	A	A	B	B	H	B	H	B	H	B	A	H	H	B	B	B	A	B	B	B	B	H	A	A	A	H
T_57	B	B	B	H	H	H	B	H	A	H	H	H	H	H	B	H	B	H	A	A	A	H	H	H	H	H	B	H	H	A	H	H	H	B	H	B	B
T_58	B	A	H	A	B	B	H	A	H	H	H	B	H	H	H	A	A	A	A	H	H	H	B	B	B	B	B	H	A	A	H	B	B	B	H	B	B
T_60	H	H	H	B	H	H	B	A	A	H	B	B	B	H	B	A	B	H	A	B	A	A	A	A	H	H	B	A	A	A	B	B	B	B	H	B	B
T_61	H	A	B	B	H	H	A	B	-	H	H	H	H	H	A	B	A	B	A	A	-	B	H	H	H	B	H	H	H	H	H	A	A	H	H	H	B
T_62	A	A	A	H	B	A	H	A	A	H	H	H	H	H	H	A	A	A	B	H	A	A	H	H	H	B	A	A	H	H	H	H	H	H	H	H	H
T_63	A	A	B	H	B	H	H	A	A	A	H	A	H	A	H	A	B	B	B	H	A	B	B	B	B	B	H	B	H	H	H	A	A	A	H	H	H
T_64	B	H	H	A	H	H	H	H	A	H	A	H	A	H	H	H	B	H	B	A	A	B	B	B	B	B	H	B	A	H	H	A	H	A	H	A	H
T_65	H	B	B	A	H	A	H	H	A	B	H	A	H	B	H	H	A	H	B	B	A	H	H	H	H	H	B	H	B	B	-	H	H	H	H	A	A
T_66	H	H	H	H	H	B	A	A	A	H	B	H	B	H	A	A	B	B	B	H	A	H	H	H	H	H	B	H	H	H	H	H	H	H	H	H	H
T_67	H	A	H	B	H	B	H	A	A	H	H	H	H	H	H	A	B	H	B	H	A	H	H	B	B	B	B	H	H	A	A	B	H	B	B	B	B
T_68	B	B	B	B	A	B	A	A	A	H	H	H	H	H	A	A	A	H	B	H	A	A	A	A	A	A	A	H	A	H	B	A	A	A	A	A	A
T_69	B	H	H	H	A	H	B	B	A	H	B	B	B	H	B	B	B	B	B	A	A	H	H	H	H	H	H	H	H	H	H	B	B	B	H	A	H	H
T_70	H	A	A	A	B	H	H	A	A	H	H	A	H	H	H	A	H	H	A	A	A	A	A	A	A	A	A	A	A	H	H	H	H	H	H	H	H
T_71	A	B	B	H	H	A	B	B	A	H	B	B	B	H	B	B	H	H	A	A	A	B	B	B	B	B	A	B	H	H	B	B	H	A	A	H	H

T_72	B	B	A	A	A	B	A	A	A	H	B	B	B	H	A	A	H	H	A	H	A	B	B	B	H	H	H	B	B	B	H	A	A	A	H	A	A
T_73	H	A	B	B	B	B	H	A	A	H	H	H	H	H	H	A	A	B	A	A	A	H	H	H	H	A	A	H	H	B	H	A	A	H	H	A	A
T_74	H	A	H	A	B	H	H	A	A	H	H	H	H	H	H	A	H	B	B	H	A	A	A	H	A	A	H	A	H	A	H	B	B	B	H	B	H
T_75	B	B	A	A	H	B	H	A	A	H	H	B	H	H	H	A	A	A	A	H	A	H	H	H	H	H	H	H	A	A	A	H	H	H	H	B	B
T_76	H	H	H	B	A	B	A	A	A	B	A	A	A	B	A	A	H	B	B	H	A	A	A	A	A	H	H	A	H	B	B	H	H	H	H	A	A
T_77	B	H	A	H	B	H	H	A	H	H	H	H	H	H	H	A	A	B	A	B	H	H	H	H	H	H	H	B	A	A	H	B	B	B	B	B	B
T_78	H	B	H	H	A	H	B	A	B	H	H	H	H	H	B	A	B	B	A	H	B	B	B	B	B	B	H	B	H	A	H	H	H	B	B	B	H
T_79	B	H	B	H	A	H	H	A	A	A	A	A	A	A	H	A	B	H	A	A	A	A	A	H	H	H	H	A	H	H	B	H	H	H	H	-	H
T_80	A	H	A	A	B	H	H	B	A	H	A	H	A	H	H	B	H	H	B	H	A	B	H	H	H	H	A	B	H	H	A	A	A	H	H	H	H
T_81	H	H	A	A	A	H	H	A	A	H	B	B	B	H	H	A	H	B	A	H	A	A	A	A	A	A	H	A	H	A	H	B	B	B	B	B	B
T_82	A	A	A	H	H	B	H	A	A	H	H	H	H	H	H	A	A	B	H	H	A	H	H	B	A	H	A	H	H	B	B	B	B	B	H	A	A
T_83	A	B	B	A	H	H	H	A	H	H	H	A	H	H	H	A	H	H	H	H	H	H	A	H	H	H	H	H	B	B	H	A	A	A	A	H	A
T_84	H	H	-	H	A	B	H	A	B	A	A	A	A	A	H	A	H	H	H	A	B	H	H	B	B	B	H	H	H	H	H	A	H	H	H	H	-
T_85	B	H	H	B	A	B	B	B	A	H	H	B	H	H	B	B	H	A	H	H	A	B	B	H	H	H	H	H	B	B	A	A	A	H	H	A	A
T_86	B	H	H	H	B	H	A	A	B	A	A	H	A	A	A	A	H	H	H	B	B	A	B	A	A	H	H	H	H	H	H	H	-	H	H	H	H
T_87	B	B	A	A	A	B	H	A	A	A	H	H	H	A	H	A	A	H	A	B	A	H	H	A	-	A	A	H	B	H	H	B	H	H	H	B	H
T_88	H	H	H	H	B	H	H	B	A	H	B	A	B	H	H	B	B	H	H	H	A	H	H	A	A	A	B	H	H	B	H	H	H	H	H	B	H
T_89	H	H	B	B	B	B	A	A	B	H	H	H	H	H	A	A	A	B	H	A	B	A	H	A	H	H	A	A	H	H	H	H	H	H	H	H	B
T_90	A	A	A	H	B	B	B	B	A	H	H	B	H	H	B	B	H	B	A	H	A	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	B
T_91	H	A	A	B	H	H	A	A	B	H	A	A	A	H	A	A	H	H	A	A	B	H	H	A	A	A	H	H	A	H	H	H	B	H	H	H	B
T_92	A	B	B	B	H	B	A	B	A	H	H	H	H	H	A	B	H	H	A	A	A	H	H	H	H	A	H	H	B	H	B	H	H	H	H	H	H
T_93	B	B	H	H	A	-	A	B	A	A	B	H	B	A	A	B	A	A	B	B	A	-	H	-	-	A	-	B	H	B	A	H	A	A	B	H	-
T_94	A	A	A	H	H	H	B	A	A	H	B	B	B	H	B	A	B	B	A	B	A	B	A	A	A	A	A	B	H	H	A	A	A	A	A	H	H
T_95	A	H	B	A	A	B	H	A	A	A	A	H	A	A	H	A	B	A	-	H	A	H	A	A	H	H	A	H	A	A	B	B	B	B	B	B	B
T_96	A	A	A	A	H	H	H	A	A	H	H	H	H	H	H	A	H	A	A	A	A	H	H	H	B	H	A	H	H	H	H	H	H	H	H	H	H
T_97	A	H	H	A	A	H	B	A	A	B	H	H	H	B	B	A	B	B	A	A	A	A	A	H	B	H	H	H	H	A	H	A	H	H	H	H	H
T_98	A	A	H	H	A	H	A	B	A	A	H	H	H	A	A	B	H	H	H	A	A	B	H	H	H	H	B	H	A	H	B	B	B	H	H	H	H
T_99	A	B	H	H	H	B	A	A	A	H	A	A	A	H	A	A	H	H	H	H	A	H	H	B	B	B	B	A	H	H	B	A	A	A	A	H	H
P1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
P2	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

9. DECLARATION

I, the undersigned, declare that this Dissertation is based on my original work and that it has not previously been submitted for any other degree or diploma in any other university. All sources of materials have been duly acknowledged.

Tesfaye Disasa (PhD candidate) Signature: _____