Genetic Diversity Analysis of Sorghum [*Sorghum bicolor* (L.) Moench] Races in Ethiopia Using SSR Markers

Gamachu Olani Nagara

A Thesis Submitted to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biotechnology

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SCHOOL OF GRADUATE STUDIES

INSTITUTE OF BIOTECHNOLOGY

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This is to certify that the thesis prepared by, **Gamachu Olani** entitled **'Genetic Diversity of Ethiopian Sorghum Races using SSR Markers'** and submitted in partial fulfillment of the requirements for the degree of Masters of Science in Biotechnology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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GENETIC DIVERSITY ANALYSIS OF SORGHUM [*Sorghum bicolor* (L.) Moench] RACES IN ETHIOPIA USING SSR MARKERS

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ABSTRACT: Sorghum [Sorghum bicolor (*L.*) Moench], *a cereal crop of family Poaceae, is believed to be originated in Ethiopia and Sudan. It is an important food security crop mainly in semi arid and tropical parts of the African countries. Although many morphological and molecular diversity studies reveal the existence of genetic variations with sorghum populations, their distribution within basic races were not considered. Hence, the present study aimed to analyze the extent and distribution of genetic variation within basic Ethiopian sorghum landraces using SSR markers. A total of 107 landraces obtained from Ethiopian Biodiversity Institute (EBI) representing 12 ecological zones grouped according to their race types based on inflorescence and spiklet on field at their maturity time. Twelve SSR markers revealed a total of 110 alleles with average polymorphic content of 0.76 and the allele frequencies shows 42 of alleles were rare (less than 0.05), 22 ranged from 0.05 to 0.1, while 46 of them were higher than 0.1. Expected and observed heterozygosity were 0.78 and 0.2 respectively. The genetic differentiation between populations were also moderate* (FST=0.07 *for races and 0.13 for E/zones) indicating continuous exchange of genes among them. Partitioning the total genetic variation also indicated 61.38% and 55.17% of the variations were among individuals within racial and zonal populations respectively. Neighbor-Joining cluster analysis also indicated four major grouping of the landraces according to their racial groups where majority of race caudatum and durra form separate groups while intermediate durra-bicolor form two separate sub-clusters. Overall locus the intra-racial population diversity showed the greatest genetic diversity (He=0.77 and 0.75) among race bicolor and caudatum respectively. Information with sorghum races along their important agronomic traits could be used for conservation and future breeding programs of sorghum.*

Key words: *Sorghum bicolor*, races, Genetic diversity, SSR

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Lists of Abbreviations

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
BSA	Bovine Serum Albumin
dNTPs	Deoxynucleotide triphosphates
CSA	Central Statistical Agency
EBI	Ethiopian Biodiversity Institute
EST	Expressed Sequence Tags
FAO	Food and Agriculture Organization
GRIN	Germplasm Resource Information Network
NABRC	National Agricultural Biotechnology Research Center
ICRISAT	International Crop Research Institute for Semi-Arid Tropics
ISSR	Inter Simple Sequences Repeats
NPGS	National Plant Germplasm System
PAGE	Polyacrylamide Gel Electrophoresis
PGR	Plant Genetic Resources
PIC	Polymorphic Information Content
PVP	Polyvinylpyrrolidone
QTLs	Quantitative Trait Loci's
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], a cultivated diploid (2n = 20) tropical cereal C4 grass plant, is the fifth most important cereal crop grown in the world. It is a monocotyledon plant of tropical origin, belonging to Poaceae family. According to United State Department of Agriculture, (2016), USA was the world's largest producer of grain sorghum, followed by the Mexico and Nigeria. In developed countries, and increasingly in developing countries such as India, it is predominantly used as fodder for poultry and cattle. Besides it also used as staple food for humans in many parts of the world making its bread, syrup and its unmilled grain cooked to make various food types like cakes, soups, etc., and as most ingredients in various alcoholic beverages as malt. In addition, sorghum straw (stem fibers) can also be made into excellent wallboard for house building, as well as biodegradable packaging. Since it does not accumulate static electricity, it is also used in packaging materials for sensitive electronic equipment. The new direction now a day given a major attention was its use as a renewable bioethanol production. In this regards a sweet sorghum has been used as smart biofuel crops because of its sugar-rich stalk (Tesfaye Disasa *et al*., 2016) and currently, 12% of grain sorghum production in the US is used to make ethanol. It has one of the highest dry matter accumulation rates and it is the quickest maturing food plant. In its nutritional composition, sorghum is similar to maize. Starch is the major component followed by protein, fat, and fiber (National Research Council, 1996).

In Ethiopia, sorghum is one of the most important staple cereal crops after tef [*Eragrostis tef* (Zucc.) Trotter.] and maize (*Zea mays* L.). Tef, maize, sorghum and wheat (*Triticum aestivum* L.) are accounting for about 24.0, 16.8, 14.6, and 13% of the total cereal crop cultivated area, respectively (CSA, 2014). In Ethiopian agriculture sorghum holds the third largest share of total cereal production. Ethiopia is the second largest sorghum producer in Africa, after the Sudan (Mekonnen Demeke and Di Marcantonio, 2013). Ethiopia also ranks first among countries that have contributed many germplasm collections to the world collections of sorghum at both International Crop Research Institute for Semi-Arid Tropics (ICRISAT) (Rao *et al*., 1989) and Griffin by NPGS (GRIN, 2012), indicating that the country is a rich source of sorghum landraces. Likewise, Ethiopian Biodiversity Institute (EBI) collected large number of farmers' landraces though doesn't had a racial category.

Though it was difficult to determine when and where sorghum domestication occurred, according to the archeological studies, Dogget, (1965) suggests that the practice of cereal cultivation was introduced from Ethiopia to Egypt about 3000 B.C. In addition, Vavilov, (1951) suggested Ethiopia as a center of origin of sorghum due to the wide variation of the crop. Being the major producing area, it was believed that the habitat within these regions where the greatest genetic variation for both cultivated and wild sorghum is found could contribute much to its domestication (Doggett, 1991; House, 1985). The crop is a physiological marvel by adapting to a range of biotic and abiotic stresses, resulting in the evolution of many landraces cultivated in various sub regions (Roa *et al*., 2002).

Sorghum is also an indigenous crop to Ethiopia, it is believed that cultivated sorghum was first domesticated in north-eastern Africa and originated in Ethiopia due to the presence of their wide genetic variation (Vavilov, 1951; Masresha Fetene *et al*., 2011). All the five basic sorghum races except kafir also reported. In this regard, different researchers also indicated the presences of huge genetic and phenotypic variations, in both qualitative and quantitative traits, across the Ethiopian sorghum germplasms (Amsalu Ayana and Endashaw Bekele, 1998; Amsalu Ayana *et al*., 2000; Cuevas and Prom, 2013 and Missihoun *et al*., 2015). These also enable Ethiopian sorghum landraces as a source for an important agronomic trait (Doggett, 1991). Morphological variation study within Ethiopian landraces also showed panicle compactness and shapes had adapted to varying agroecological patterns which could serve as criteria for site of sorghum conservation (Amsalu Ayana and Endashaw Bekele, 1998). Though morphological race had a major effect on patterns of genetic diversity, the studies were mostly not involved the different racial grouping. Deu *et al.*, (1994) quantified, for the first time, the amount of diversity within race (based on a measure of gene diversity, mean number of alleles, its presence within the defined clusters of accessions) to differentiate 94 accessions selected on basis of their geographical origin and racial classification using restriction fragment length polymorphism markers (RFLP) noting that the greatest levels were found with the races bicolor and guinea. Missihoun *et al*., (2015) also reported separation of race guinea from other races, caudatum and durra while studying 61 landraces from Benin by 20 SSR. Both found out absence of race bicolor forming specific group and appeared highly variable and are scattered among the various clusters. Missihoun *et al*., (2015) also recommended integration of botanical

races and morpho physiological characters for better crop genetic resource preservation. Hence, the present study aimed at studying the extents and distributions of genetic variations within basic Ethiopian sorghum races using SSR markers.

2. Literature Review

2.1. Sorghum in the World

Sorghum is the 5th most important cereal crop globally, after maize, wheat, rice and barley with annual production of over 60 million tonnes (www.fao.org) and an important food grain crop in the semi-arid tropics. Sorghum is a critical food security crop for more than 100 million people in Africa. It predominantly grows in low-rainfall, arid to semi-arid environments due to its excellent tolerance to drought, high temperature stresses and low soil fertility. The crop displays relatively high water use efficiency compared to other cereals such as maize and wheat (Doggett, 1988). Its wide adaptation to harsh environments, tolerance to stress conditions, diverse germplasm collections and its small genome size (710Mb) made sorghum as an important botanical model crop for many tropical grasses with complex genomes, which employ C4 photosynthesis. Sorghum is also the first crop genome of African origin to be sequenced (Kresovich *et al*., 2005; Clarissa *et al*., 2013).

Hence, in Africa, mostly characterized by semi-arid and sub-tropical climatic conditions, it is the second most critical food security cereal grain after maize and the continent produces about 20 million tonnes of sorghum per annum, about one-third of the world crop. Along with Sudan and Ethiopia, Western African countries such as Nigeria and Burkina Faso are the leading sorghum producing countries, accounting for nearly 70% of Africa's production (FAO, 2015). It is one of the crops originated in the continent and it is generally, although not universally, considered to have first been domesticated in North

Africa, possibly in the Ethiopian regions (including Sudan) as recently as 3000 BC. The complex diversity of agro ecologies and the presence of wide genetic variation describe Ethiopia as a centre of origin of sorghum (Vavilov, 1951). Hence all lines of evidence point to the north-east quadrant of Africa, mainly Ethiopia, as the centre of domestication of sorghum. Therefore, the greatest genetic diversity for both cultivated and wild forms of sorghum is found in Ethiopia and the surrounding eastern African countries. It is also the second most important staple cereal crop after maize in the region, making a huge contribution to the domestic food supply chain and rural household incomes with a total acreage of 8.2 million hectares. In Ethiopia, it is the 2nd staple cereal after tef, *Eragrostis tef,* and in total national production it ranks 3rd after maize and tef (CSA, 2014). It is cultivated in almost all regions by subsistence farmers for various uses such as food, animal feed and to prepare local beverages. Further, the stalk is also used for animal feed and for house and fence construction (McGuire, 2008).

In addition, landraces from Ethiopia have been used as the source of valuable traits for sorghum improvement programs including a devastating resistant to pest, sorghum midge (*Contarinia sorghicola*) (Doggett, 1988), and high lysine and protein contents (National Research Council, 1996). Among the several lines identified resistance to sorghum midge was the SC lines mostly of zera-zera lines from Ethiopia (Doggett, 1988).

2.2. Taxonomic classifications and Races

It was believed that there were 20 to 30 species of genus *Sorghum* that are recognized until now, and these are classified into five sections: *Stiposorghum*, *Parasorghum*, *Eu-sorghum*, *Heterosorghum* and *Chaetosorghum*. Under the section Eu-sorghum, three species are recognized: *S. halepense* (L.) Pers. occurring in India, *S. propinquum* (Kunth) Hitchc found in Southeast Asia and *S. bicolor* (L.) Moench, which originated in Africa (De Wet, 1978). All classified under genus Sorghum. De Wet's recognized *S. bicolor* (L.) Moench representing all annual cultivated, wild and weedy sorghums along with two rhizomatous taxa, S. *halepense* and S. *propinquum*. All types of the *S. bicolor* (the primitives) propagated with seeds except the rhizomatous taxa's which reproduces through both seed and rhizome production. *Sorghum bicolor* was further broken down into three subspecies: *S. bicolor* subsp *bicolor*, *S. bicolor* subsp *drummondii* and *S. bicolor* subsp *verticilliflorum*. The cultivated sorghums are classified as *S. bicolor* subsp *bicolor* and represented by agronomic types such as grain sorghum, sweet sorghum, sudangrass and broomcorn.

All the cultivated sorghum taxa of the world have been classified by inflorescence type, grain and glumes into five races (Durra, Bicolor, Caudatum, Kaffir and Guinea) and intermediates involving all of the pair-wise combinations of the basic races (de Wet, 1978; Harlan and de Wet, 1972). The entire races were differentiated morphologically based on their inflorescence, grain and glumes (Figure 1). The race bicolor has its grain elongated, with glumes clasping the grain, which may be completely covered or exposed. This race is mostly grown west of the Rift valley and also on a minor scale almost

everywhere in Africa. Guinea is primarily West African with a secondary centre in Malawi and Tanzania. The grain is flattened dorso-ventrally, twisting at maturity 90 degrees between glumes that are nearly as long as or longer than the grain. The caudatum grain is asymmetrical, with glumes half the length of the grain or less. This race is most abundant in east Nigeria, Sudan and Uganda. Kafir is mostly a race of east and Southern Africa. It has symmetrical grain, with glumes of variable length clasping the grain. Durra is dominant in Ethiopia and westward across the continent, covering the driest parts near the Sahara. Its grain is rounded and the glumes are very wide (House, 1985).

Four of the five sorghum races and wild forms are present in Ethiopia except Kaffir (Awegechew Teshome *et al*., 1997). Stemler *et al*., (1977) described and discussed the diversity of sorghum grown in Ethiopia for the races Bicolor, Durra, Durra-Bicolor, Caudatum and Guinea based on Ethiopian sorghum collections and field observations. The geographic pattern of distribution of each race appears to be determined by the topography and climate variation present in Ethiopia. Accordingly, sorghum race Durra is the main crop of the eastern highland region and mid elevation terrace of the north, while Caudatum race is grown primarily in hot, dry valleys and lowland savannas in the south and west of Ethiopia. The intermediate race Durra-Bicolor predominates in the southwestern highland region, where cooler temperature and rain are higher than eastern and northern region. In contrast, Bicolor and Guinea races represent a very small part of Ethiopian sorghum diversity and both are mostly found in the Rift Valley region.

Qualitative morphology characterization work on Ethiopian landraces along with introduced lines of sorghum from abroad and neighboring Eritrean accessions also shows the differential distribution of these different panicle types. This indicated the adaptive significance of panicle compactness and shape reflected the distribution patterns of different races of sorghum in Ethiopia. In their studies, the patterns of distribution of the different panicle types appeared to follow the temperature, humidity and rainfall patterns of Ethiopia and Eritrea. The compact and semi-compact panicle types were more frequent in Eritrea and in relatively hot and dry regions of Ethiopia such as Harerge, Tigray and Wello. The loose panicle types with dropping branches occurred abundantly in relatively cool and wet regions of Ethiopia such as Wollega, Illubabor, Shewa and Sidamo (Amsalu Ayana and Endashaw Bekele, 1998).

2.3. Diversity Studies on *Sorghum bicolor*

For utilization of crop genetic resources for breeding and conservation, availability of adequate genetic variation is a prerequisite. The diversity or variations within a crop of interest could be attributed with their morphological traits or based on DNA sequence. Classification of germplasm accessions based on discrete morphological characters may not provide an accurate indication of their genetic divergence (Menkir *et al*., 1997). Molecular markers have been widely applied to characterize genetic diversity in sorghum germplasm collections and in breeding programs.

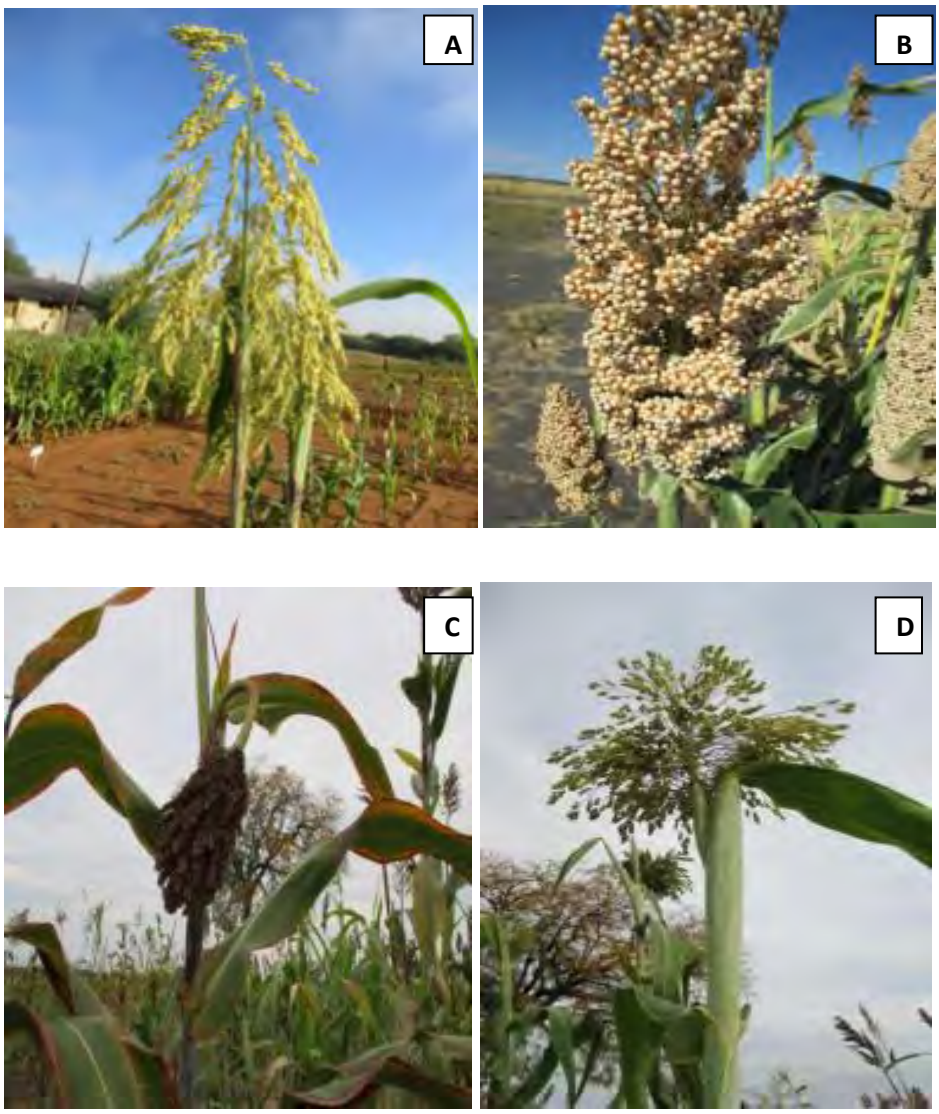

Figure 1. Sorghum basic races by their basic spiklet types (Harlan and de Wet, 1972) Photo by Motlandi, 2016 (A: Guinea, B: Caudatum, C: Durra and D: Bicolor).

Their direct application in applied breeding has been emphasized in reports on identification and characterization of quantitative trait loci (QTL) associated with important traits, such as resistance to diseases and insects, and tolerance to aluminum and drought stresses. Understanding the molecular basis of the essential biological phenomena in plants is needed for conservation, management and utilization of

germplasm collections as well as for establishing a core collection of germplasm for efficient utilization of plant genetic resources (Mondini *et al*., 2009).

In Sorghum, there have been lots of genetic diversity studies which include phenotypic, biochemical or molecular analysis. Previously, biochemical markers such as isozymes and storage proteins have been used extensively to characterize plant genetic resources. Currently, there exist a wide range of molecular markers that can be used to characterize genetic diversity. The different marker analysis applied so far for the identification of the genetic distances/ diversity between sorghum landraces includes allozyme (Amsalu Ayana *et al*., 2001), restriction fragment length polymorphism (RFLP) (Menz *et al.,* 2004), random amplified polymorphic *DNA (RAPD)* (Amsalu Ayana *et al*., 2000), amplified fragment length polymorphism (AFLP) (Nemera Geleta and Labuschagne, 2006), inter simple sequence repeats (ISSR) (Hailekiros Tadesse and Tileye Feyissa, 2013), simple sequence repeats (SSR) (Manzelli *et al.,* 2007; Amelework Beyene, 2012; Asfaw Adugna, 2014), expressed sequence tags (EST) (Ramu, 2009), and the like. All differ in their power of genetic resolution, quality of information content, extents of polymorphism, degree of environmental stability, number of loci and practicality. Therefore, no single method is adequate for assessing genetic variation in germplasm collections. It is common to use DNA based molecular markers, which are more reliable and robust methods for the characterization of genetic diversity (Amsalu Ayana *et al*., 2001 ; Singh *et al*., 1991).

SSR, also called microsatellites, short tandem repeats (STRs) are PCR-based markers. They are randomly tandem repeats of short nucleotide motifs (2-6 bp/nucleotides long). Di-, tri- and tetra-nucleotide repeats, e.g. (GT)n, (AAT)n and (GATA)n, are widely distributed throughout the genomes of plants and animals. The copy number of these repeats varies among individuals and is a source of polymorphism in plants. Because the DNA sequences flanking microsatellite regions are usually conserved, primers specific for these regions are designed for use in the PCR reaction. Even though the technique requires nucleotide information for primer design, labor-intensive marker development process and high start-up costs for automated detections, they are usually characterized by their hyper-variability, reproducibility, co-dominant nature locus-specificity, and random genome-wide distribution in most cases for genetic studies (Jiang, 2013). In addition, they have been widely applied for identification and fingerprinting of genotypes (Menz *et al.,* 2002), estimation of genetic distances between and within populations (Nemera Geleta and Labuschange, 2006; Asfaw Adugna, 2014), detection and mapping of QTLs, and gene tagging and mapping in the identification of agronomic traits in wild, traditional and improved germplasm through the dissection of quantitative traits using linkage map based approaches (Klein *et al.,* 2001, Tanksely and McCouch, 1997). Yet among DNA markers, SSR markers remain the markers of choice for practical breeding applications.

The studies in Ethiopia using already mentioned techniques shows the existence of huge sorghum diversity mainly within populations of different agro ecologies. In addition to their regions of origin, sorghum production areas were classified into three adaptation

zones, namely, lowland (< 1600 m above sea level (masl)), intermediate (1600–1900 masl) and highland (> 1900 masl) (Brhane Gebrekidan, 1981). The extent and patterns of phenotypic variation for 10 qualitative (categorical) traits in 415 sorghum germplasm collections from Ethiopia and Eritrea shows the presence of huge total variation within regions of origin and adaptation zones (86% and 96% respectively) than between regions and ecological adaptation zones. Greatest share of variation within regions that was 31 percent were carried by their panicle compactness and shape (Amsalu Ayana and Endashaw Bekele, 1998). The same study for their quantitative characters found that the discrimination of sorghum accessions was more pronounced when discriminant analysis was based on adaptation zones rather than regions of origin. The overall high diversity index (0.90) observed in this study is consistent with Doggett's long standing hypothesis that Ethiopia is not only one of the centers of diversity but also the centre of domestication of sorghum (Doggett, 1991; Doggett and Rao, 1995). Likewise, the variation assessed by RAPD for 80 germplasm accessions from Ethiopia and Eritrea shows great diversity within regions and ecologies and suggest a weak differentiation of the sorghum material both on regional and agro-ecological bases, ascribing it to the high rate of out crossing in cultivated sorghum and its free natural hybridization with its wild and weedy relatives, as well as to seed movement by humans (Amsalu Ayana and Endashaw Bekele, 1998; Amsalu Ayana *et al*., 2000).

Nemera Geleta and Labuschange, (2006) also showed the existence of high genetic diversity value among the 45 sorghum accessions using combinations of morphological traits, AFLP and SSR markers indicating the level of genetic diversity was not influenced

by breeding activities. Though the phenotypic variations do not reliably reflect genetic variation because of the role of environmental interaction in determining the phenotype, morphological traits show significant correlation with SSR (but no correlation with AFLP based data sets). Similarly, population structure and molecular diversity study among Ethiopian sorghum collections maintained at the USDA–ARS National Plant Germplasm System (NPGS) indicated high sorghum genetic diversity in the country with expected and observed heterozygosity of 0.78 and 0.23, respectively. The result also demonstrated the continuous exchange of genes among subpopulations of sorghum along with presence of highly related accessions (Cuevas and Prom, 2013). Additionally, several other studies also support the presence of wide genetic diversity of sorghum (bicolor) in Africa mainly in Eastern regions including the SSR diversity studies of Eritrean collections (Tesfamichael Abraha *et al*., 2014), Eastern Kenya (Muui *et al*., 2016), Benin landraces (Missihoun *et al*., 2015), and Zambia collections (N G'Uni *et al*., 2011) giving especial attention for the crops domestications and origins. The high diversity study among Ethiopian sweet sorghum collections could also suggest that the country may be the center of diversity for sweet sorghum germplasm as well (Tesfaye Disasa *et al*., 2016).

Factors assumed to have contributed to the increase in phenotype and genetic diversity includes the very diverse and different environmental conditions where sorghum is grown in Ethiopia, gene flow among cultivated sorghum and wild relatives, and out crossing among sorghum landraces (Stemler *et al.*, 1977; Yao *et al.*, 2004). Farmer's seed exchange and the practiced planting of varietal mixtures creating a heterogeneous farming system could also add a variation (Frew Mekbib, 2008). In addition, bulking

several plants (2–3) for DNA extraction could also add the variation among these plants since landraces usually are high genetically and phenotypically diverse, hence the observed heterozygosity as well (Nemera Geleta and Labuschagne, 2006; Cuevas and Prom, 2013). Apart, Cuevas and Prom noted the existence of other sorghum races in their sample collections other than durra and durra-bicolor which is the major in their sample (in Ethiopian collections) might also contribute to an increased observed genetic variation. In addition, Missihoun *et al*., (2015) recommended research on genetic diversity to integrate both botanical races and morpho-phsyiological characteristics of the crops for better preservation of sorghum genetic resources. Morphological variation studies of Ethiopian and Eritrean germplasm also indicated that panicle compactness and shapes had followed adaptive significance to the varying temperature, humidity and rainfall patterns of the countries and could serve as criteria in choosing sites for sorghum conservation strategy (Amsalu Ayana and Endashaw Bekele, 1998). Therefore, racially partitioned diversity studies among founding major basic races and representative of the whole collections of Ethiopian adaptation zones were lacking. Hence, the present study aimed at analyzing the genetic variation within basic Ethiopian sorghum races.

3. Objectives

3.1. General Objective

- To analyze the extent and distribution of genetic variation within basic Ethiopian sorghum races using SSRs,

3.2. Specific Objectives

- To determine the genetic distance among the five basic Ethiopian races (bicolor, caudatum, durra, intermediate durra-bicolor and guinea).
- To determine the genetic variation within twelve selected sorghum cultivating zones of Ethiopia,

4. Material and Methods

4.1. Germplasm Plant Materials

The accessions used for this study were landrace accessions collected by Ethiopian Biodiversity Institute (EBI). A total of 107 sorghum landrace accessions were selected (Table 1) based on phonological evaluation of inflorescence and spiklet types at their maturity time in order to define the racial classifications in 2015/16 cropping season at Arsi Negelle Research Station based on their passport data. The materials were received and planted along with other germplasm by Melkassa Sorghum Improvement Program. In addition to the difference in head morphology, geographical distribution of the sorghum races across the country were considered for selection of their adaptation zones (Table 2).

All basic sorghum races except kafir and from the intermediate types, widely distributed durra-bicolor were included. The selected 107 sorghum landrace collections were grouped based on their source of origins (Figure 2) into 12 populations, which each contained 9 landrace accessions for DNA extraction and genotyping study.

Table 1. Lists of Sorghum landraces obtained from EBI for Sorghum landrace diversity study

S/No.	Acc. #	E/Zone	Race	S/No.	Acc. #	E/Zone	Race
1.	73061	North Wello	D	55.	75123	Jimma	DB
2.	75448	North Wello	D	56.	75113	Jimma	DB
3.	73046	North Wello	D	57.	75118	Jimma	DB
4.	74098	North Wello	D	58.	70938	Jimma	DB
5.	73060	North Wello	D	59.	71395	Jimma	DB
6.	73089	North Wello	D	60.	70073	Jimma	DB
7.	73087	North Wello	D	61.	70604	Jimma	DB
8.	73040	North Wello	D	62.	234858	Jimma	DB
9.	73081	North Wello	D	63.	228183	Jimma	DB
10.	72458	South Wello	D	64.	245057	Illubabor	DB
11.	72437	South Wello	D	65.	245056	Illubabor	DB
12.	200653	South Wello	D	66.	69542	Illubabor	DB
13.	73107	South Wello	D	67.	245056	Illubabor	DB
14.	242048	South Wello	D	68.	228916	Illubabor	DB
15.	72558	South Wello	D	69.	245061	Illubabor	DB
16.	72451	South Wello	D	70.	245060	Illubabor	DB
17.	72497	South Wello	D	71.	228919	Illubabor	DB
18.	73102	South Wello	D	72.	71551	Illubabor	DB
19.	228836	East Harerge	D	73.	16111	West Wollega	DB
20.	228829	East Harerge	D	74.	69526	West Wollega	DB
21.	228843	East Harerge	D	75.	69295	West Wollega	DB
22.	200193	East Harerge	D	76.	16181	West Wollega	DB
23.	228833	East Harerge	D	77.	70584	West Wollega	DB
24.	200194	East Harerge	D	78.	16110	West Wollega	DB
25.	242384	East Harerge	D	79.	70816	West Wollega	DB
26.	15867	East Harerge	D	80.	70797	West Wollega	DB
27.	9123	East Harerge	D	81.	237793	North Shewa	G
28.	229834	Metekel	C	82.	237762	North Shewa	G
29.	229835	Metekel	C	83.	237763	North Shewa	G
30.	229833	Metekel	C	84.	74995	North Shewa	G
31.	229832	Metekel	C	85.	75001	North Shewa	G
32.	229831	Metekel	C	86.	237765	North Shewa	G
33.	69147	Metekel	C	87.	70758	North Shewa	G
34.	69154	Metekel	C	88.	69054	North Shewa	G
35.	211254	Metekel	C	89.	75002	North Shewa	G
36.	69165	Metekel	C	90.	74654	Bench Maji	G
37.	70029	Gambella (Z1)	C	91.	74653	Bench Maji	G
38.	69476	Gambella (Z1)	C	92.	74647	Bench Maji	G
39.	69475	Gambella (Z1)	C	93.	74685	Bench Maji	G
40.	70051	Gambella (Z1)	C	94.	200022	Bench Maji	G
41.	70064	Gambella (Z1)	C	95.	241724	Bench Maji	G
42.	69479	Gambella (Z1)	C	96.	74649	Bench Maji	G
43.	70035	Gambella (Z1)	C	97.	204622	Bench Maji	G

Table 1. (Continued…)

S/No.	Acc. #	E/Zones	Race	S/No.	Acc. #	E/Zones	Race

44.	69489	Gambella (Z1)	C	98.	210903	Bench Maji	G
45.	69483	Gambella (Z1)	C	99.	16479	East Shewa	B
46.	73785	Central & SouthTigray	C	100.	16475	East Shewa	B
47.	19623	Central & SouthTigray	C	101.	16482	East Shewa	B
48.	234112	Central & SouthTigray	C	102.	16450	East Shewa	B
49.	234079	Central & SouthTigray	C	103.	16436	East Shewa	B
50.	238403	Central & SouthTigray	C	104.	237547	East Shewa	B
51.	16057	Central & SouthTigray	C	105.	16438	East Shewa	B
52.	19622	Central & SouthTigray	C	106.	16163	East Shewa	B
53.	238388	Central & SouthTigray	C	107.	16437	East Shewa	B
54.	234086	Central & SouthTigray	C				

N.B: Acc. #- Accession number, Z1*-Zone 1, D-Durra, C-Caudatum, DB-intermediate Durra-bicolor, G- Guinea and B-Bicolor

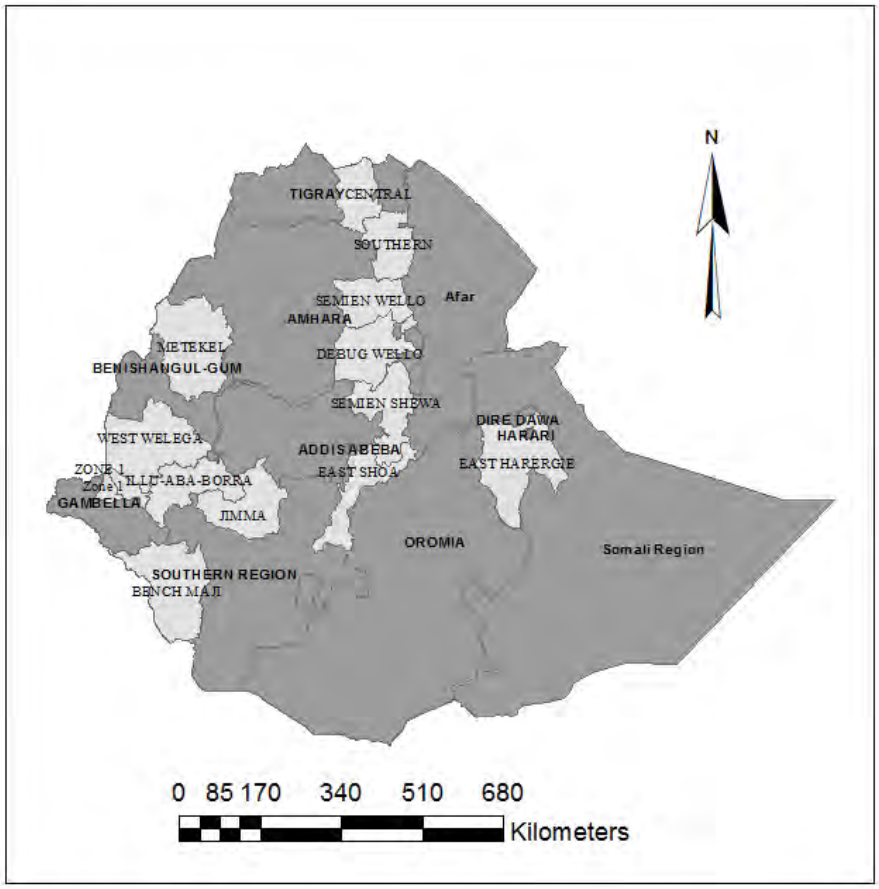

Figure 2. Maps of Ethiopia showing Ethiopian administrative zones from where the sorghum germplasms used in this study were collected.

Table 2. Ethiopian sorghum races presented according to their ecological distribution (Modified from Stemler et al., 1977).

Ethiopian sorghum races	Ecological distribution	Major growing regions/zones
DURRA	Eastern high land regions and mid-elevation terrace of the North	North Wello, South Wello, East Harerghe
CAUDATUM	Hot dry valleys and lowland savannas in N and W Ethiopia	Metekel, Central and South Tigray, Gambella (Z1)
DURRA-BICOLOR	S-W Highland regions where Temp. and raining higher than N and E regions	Jimma, Illubabor, West Wollega
BICOLOR and GUINEA	Rift valley regions of Ethiopia	North Shewa, Benchi Maji, East Shewa*

*E/Shewa representing the only bicolor populations in the study while N/Shewa and Benchi Maji is guinea

4.2. DNA extraction and PCR amplification

The seeds of collected sorghum genotypes were planted at National Agricultural Biotechnology Research Center (NABRC), Holetta, on seedling tray in greenhouse for germination. Genomic DNA was extracted from 2-week old seedlings using fresh leaves according to Xin *et al*., (2003) which is simple, low-cost, high throughput method of DNA extraction for PCR. The technique also utilizes only two ordinary buffers that made the genomic DNA available for PCR amplification reactions and avoiding a maceration of the tissues and other sequential centrifuging. Approximately 50 mm^2 single leaf sample per landraces was harvested to PCR plates for their DNA extraction. The technique utilizes two buffers: Buffer A made from 100 mM NaOH and 2% Tween 20,

which are made fresh from their stock solutions (10M NaOH and 20% Tween 20) and Buffer B consisting of 100mM Tris- HCl and 2mM EDTA, whose pH set to 2.0. Once the buffers are ready, the genomic DNA was extracted with the following procedures: - 1) Approximately 30 mm^2 leaf tissue transferred to 96-well plates; 2) 50 µL buffer A added and incubated for 10 minutes at 95°C in thermo cycler; 3) 50 µL buffer B was added and mixed at moderate speed; 4) Aliquot PCR mixture to 96-well plates at a reaction volume of 20 µL/well; 5) And finally transfer approximately 1.5 µL DNA from the crude DNA plates to PCR plates with a 96-pin applicator.

Twelve polymorphic SSR primer pairs (Table 3) were selected for genotyping the selected 107 sorghum landraces. PCR amplification was carried out in 20 µL reaction volume containing 1.5 µL crude genomic DNA, 2.25 µl PCR buffer with $MgCl_2$ (17.5 mM), 1.8 µl of dNTPs (10 mM), 0.45 µl each of forward and reverse primers (10 mM), 0.133 µl of Taq Polymerase (5U), 0.1% BSA (w/v) and 1% PVP (w/v). The amplifications were carried out with thermo cycler programmed for initial denaturation at 94 °C for 15 minutes, the second denaturation at 94 °C for 30 seconds, annealing at 50 °C for 1 minute, extension at 72 °C for 2 minute, final elongation at 72 °C for 20 minutes and holding temperature at 4 °C until conclusion. PCR products were analyzed by loading the 3µl PCR products along with a 3µL loading dye mixed with Gel Red (at a ratio of 1000:1) using 3.5% agarose gel electrophoresis run with 100V for three hours along with DNA Ladder (500bp bioline Hyperladder V).

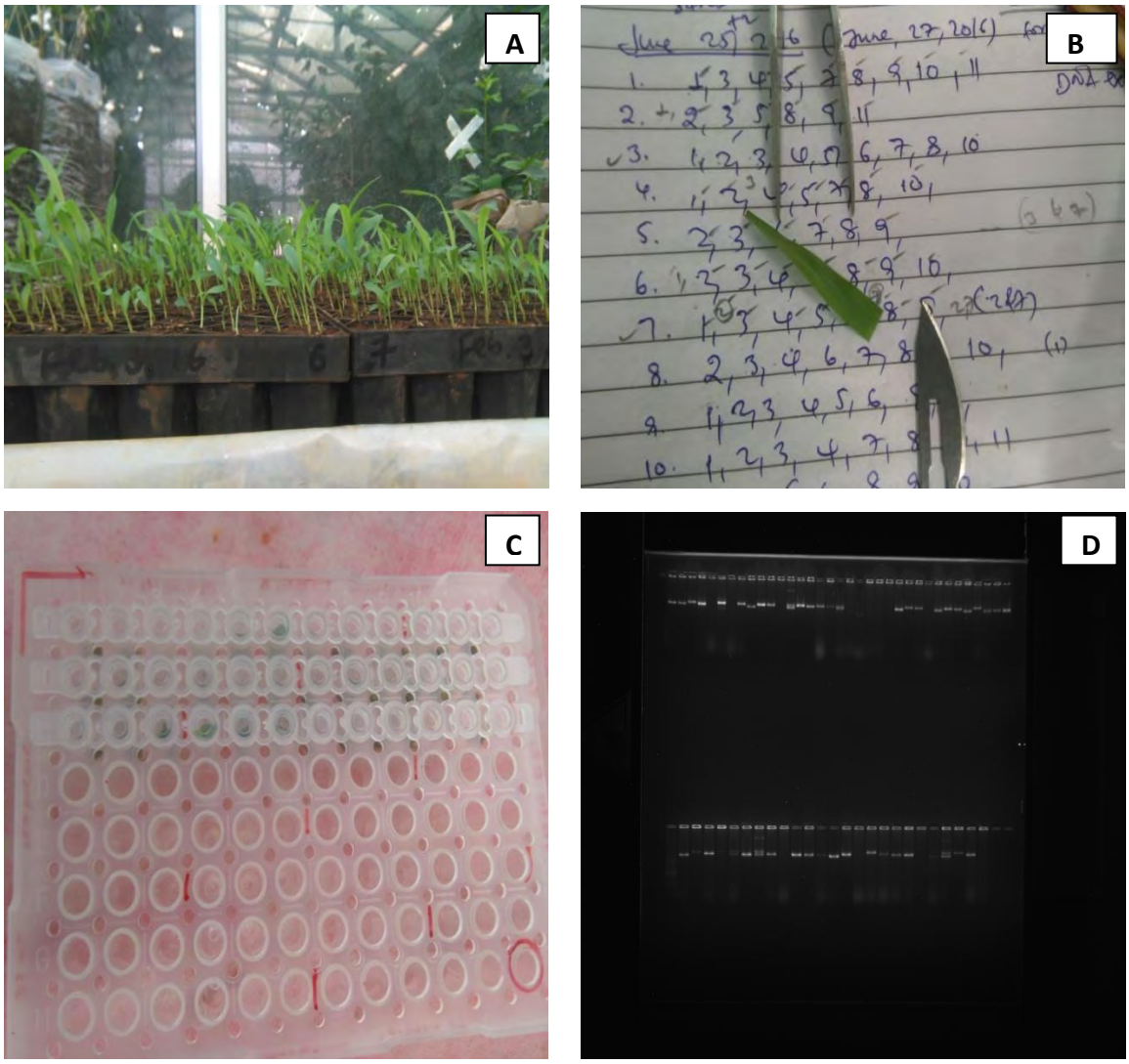

Figure 3. Procedures for the throughput (commonly called Dirty method) DNA extraction protocols (A: Sorghum seeds planted for their germination to be used for DNA extraction after two weeks, B: About 50 mm^2 single leaf per accession were used, C: the leaves were transferred to PCR plates sequentially and 50 µl Buffer A added to each before their incubation at 95ºC in thermocycler for 10 minutes and the same amount of Buffer B added, and briefly mixed to be used for PCR amplification, and D: Confirmation of PCR amplification using marker *xtxp258*).

Table 3. Lists of 13 Selected Sorghum SSR Markers used with their primer sequences

No.	SSR Marker name	Forward Primer sequence	Reverse Primer sequence	Repeat motif	Expected allele size
1.	*Xtxp211*	TCAACGGCCAATGATTTCTAAC	AGGTTGCGAATAAAAGGTAATGTG	(CT)23	206
2.	*Xtxp258*	CACCAAGTGTCGCGAACTGAA	GCTTAGTGTGAGCGCTGACCAG	(AAC)19	222
3.	*Xgap001*	TCCTGTTTGACAAGCGCTTATA	AAACATCATACGAGCTCATCAATG	(AG)16	240
4.	*Xtxp295*	AAATCATGCATCCATGTTCGTCTTC	CTCCCGCTACAAGAGTACATTCATAGCTTA	(TC)19	175
5.	*Xtxp008*	ATATGGAAGGAAGAAGCCGG	AACACAACATGCACGCATG	(TG)31	148
6.	*Xtxp012*	AGATCTGGCGGCAACG	AGTCACCCATCGATCATC	(CT)22	193
7.	*Xtxp312*	CAGGAAAATACGATCCGTGCCAAGT	GTGAACTATTCGGAAGAAGTTTGGAGGAAA	(CAA)26	154
8.	*Xtxp141*	TGTATGGCCTAGCTTATCT	CAACAAGCCAACCTAAA	(GA)23	152
9.	*Xtxp285*	ATTTGATTCTTCTTGCTTTGCCTTGT	TTGTCATTTCCCCCTTCTTTCTTTT	(CTT)11 CTC(CTT)1	231
10.	*Xtxp021*	GAGCTGCCATAGATTTGGTCG	ACCTCGTCCCACCTTTGTTG	(AG)18	172
11.	*Xtxp357*	CGCAGAAATACGATTG	GCTATCTGGAGTAACTGTGT	(GT)10	273
12.	*SbKAKG1*	AGCATCTTACAACAACCAAT	CTAGTGCACTGAGTGATGAC	(ACA)9	142

4.3. Data collection and Statistical Analysis

Once gel images were taken with Gel documentation, the PCR fragments were scored manually by estimating their base pair size comparing with known fragments size ladders that were run gel electrophoresis along with each accession. The number of alleles (N), major allele frequency (A), observed heterozygosity (Ho), expected heterozygosity/gene diversity (He) and polymorphism information content (PIC) for each SSR locus were analyzed. Gene diversity was defined by Weir (1996) as the probability of two randomly chosen alleles being different from a population. PIC was defined by Botstein *et al*., (1980) as the measure to calculate the discrimination power and informativeness of the SSR markers and calculated for a marker locus Ai, with a formula;

$$\mathbf{PIC} = 1 - (\sum_{i=1}^{n} pi^2) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2pi^2 pj^2$$

Where, PIC is polymorphic information content, *n* is number of alleles and their frequencies *pi*. All the above parameters were analyzed using PowerMarker 3.25 (Liu and Muse, 2005).

Pairwise genetic distance was calculated as given by Nei and Takezaki, (1994). Further, the allelic data were subjected to estimate the genetic distances using simple matching coefficients (10,000 bootstraps) and the genotypes were clustered using Neighbor Joining method. Both the clustering analysis and PCoA were done using DARwin software ver. 6.0.13. The data were tested for presence of population structure and analysis of

molecular variance (AMOVA) was performed to separate the total molecular variance into components between groups, within groups and intra population variation using Arlequin version 2.0 software (Excoffier *et al.*, 2005). Pairwise genetic differentiation between different groups was assessed with fixation index (Weir and Cockerham, 1984) as implemented in Arlequin software.

5. Results

Marker Polymorphism

A total of 110 alleles were separated using 12 SSR markers (Table 4.) The number of alleles per marker ranged from 5 (*xtxp298* and *xtxp258*) to 18 (*xtxp211*) with an average of 9.2 alleles per locus. *Xtxp298* and *xtxp012* hold the lowest and highest number of genotypes (6 and 19, respectively) with an average of 15.8 genotypes per locus. The allele frequencies varied from 0.005 for marker *xtxp285* to 0.648 for *xtxp312* with an overall average frequency of 0.109. Out of the total 110 alleles, specifically 42 alleles had frequencies below 0.05 (rare alleles), 22 alleles had a frequency within 0.05 to 0.10 (common alleles) while the rest 46 alleles had frequency higher than 0.10 becoming an abundant allele. While across all races average number of frequency ranged from 7.17 in intermediate durra-bicolor to 4.75 in race bicolor whereas mean gene diversity ranged from 0.32 (bicolor) to 0.22 (durra). Their mean number of alleles within the different races ranged from 7.17 (durra-bicolor) to 4.75 (bicolor). Likewise, their gene diversity ranged from 0.77 for durra-bicolor to 0.70 for durra, guinea and bicolor (Table. 5, Annex. 2).

Their polymorphic information content (PIC) varied from 0.51 (*xtxp312*) to 0.91 (*xtxp211*) with an average of 0.76 and the expected and observed heterozygosity (gene diversity and heterozygosity respectively) ranged from 0.54 (*xtxp312*) to 0.92 (*xtxp211*) and 0.014 (*xtxp298*) to 0.62 (*xtxp211*) respectively.

Table 4. Basic statistics of 12 SSR markers using powermarker V3.25 software

Marker	MAF	N_G	N_A	H_e	H_o	PIC
xtxp258	0.359	8.0	5.0	0.755	0.071	0.72
xtxp008	0.284	16.0	8.0	0.813	0.257	0.79
xtxp012	0.257	19.0	9.0	0.841	0.229	0.82
xtxp312	0.648	8.0	6.0	0.543	0.042	0.51
xtxp141	0.294	13.0	10.0	0.820	0.221	0.80
xtxp211	0.108	33.0	18.0	0.917	0.619	0.91
xtxp285	0.163	35.0	16.0	0.900	0.471	0.89
xtxp021	0.388	14.0	8.0	0.763	0.117	0.73
xtxp357	0.380	9.0	9.0	0.751	0.024	0.71
SbKAKG 1	0.383	14.0	9.0	0.758	0.107	0.73
xgap001	0.282	15.0	7.0	0.812	0.234	0.79
xtxp298	0.338	6.0	5.0	0.738	0.014	0.69
Mean	**0.324**	**15.8**	**9.2**	**0.784**	**0.200**	**0.76**

MAF- Major allele frequency; N_G- number of genotypes; N_A - Total number of Alleles; H_e- GeneDiversity; H_o- Heterozygosity; PIC-polymorphic information content

Table 5. Comparison of genetic diversity among the five Ethiopian sorghum races

Markers	Durra				Caudatum				Durra-bicolor intermediate				Guinea				Bicolor			
	N_A	R_A	C_A	A_A	N_A	R_A	C_A	A_A	N_A	R_A	C_A	A_A	N_A	R_A	C_A	A_A	N_A	R_A	C_A	A_A
xtxp258	5	1	4	-	5	-	5	-	5	-	5	-	4	-	3	1	2	-	1	1
xtxp008	6	3	3	-	6	1	5	-	7	1	6	-	5	-	4	1	6	-	6	-
xtxp012	6	2	4	-	7	-	7	-	5	-	5	-	7	3	4	-	4	-	3	1
xtxp312	4	-	3	1	3	-	2	1	5	1	3	1	5	1	3	1	2	-	1	1
xtxp141	4	-	4	-	7	1	6	-	9	1	8	-	6	2	4	-	5	-	5	-
xtxp211	10	3	7	-	12	6	6	-	13	7	6	-	10	2	8	-	8	-	8	-
xtxp285	11	5	6	-	9	1	8	-	12	3	9	-	11	2	9	-	9	-	9	-
xtxp021	4	2	1	1	6	1	5	-	7	4	3	-	6	1	5	-	7	-	7	-
xtxp357	6	2	3	1	6	2	4	-	6	3	2	1	3	-	2	1	2	-	1	1
SbKAKG1	3	-	3	-	6	2	4	-	6	1	5	-	5	-	4	1	4	-	4	-
xgap001	7	3	3	1	7	2	5	-	7	2	5	-	5	1	4	-	4	-	3	1
xtxp298	4	-	4	-	4	-	4	-	4	-	4	-	3	-	2	1	4	-	4	-
Total	**70**	**21**	**45**	**4**	**78**	**16**	**61**	**1**	**86**	**23**	**61**	**2**	**70**	**12**	**52**	**6**	**57**	**-**	**52**	**5**
Mean	**5.83**	**1.75**	**3.75**	**0.33**	**6.50**	**1.33**	**5.08**	**0.08**	**7.17**	**1.92**	**5.08**	**0.17**	**5.83**	**1.00**	**4.33**	**0.50**	**4.75**	**-**	**4.33**	**0.42**

N_A - Total number of Alleles; R_A - Rare alleles that were present in <5% of the landraces; C_A- Common alleles that were present in 5-50% of the landraces; A_A- Abundant alleles that were present in more than 50% of the landraces

AMOVA Analysis

Analysis of Molecular Variance was used to determine the extent of the total variation within and among populations. Partitioning the total variation of 107 Ethiopian sorghum landraces using 12 SSR markers revealed the presence of 61.38% and 55.17% variations explained by individual differences within race and their ecological zones, respectively. In contrast, the variations among the two populations are very small (6.86% among races and 12.9% among zones). A considerable amount of its total variation was recorded across the overall individual landraces that is 31.7% with a moderate degree of gene differentiation among racial populations in terms of allele frequencies, **FST**: 0.073 (Table 6).

Table 6. Analysis of Molecular Variance (AMOVA) using 12 SSR markers by Alrequin ver 3.5.1.3

Components	Source of variation	d.f.	Sum of squares	Variance component	Percentage variation
Sorghum Races	Among populations	4	31.93	0.22	6.86
	Among individuals within populations	102	500.81	1.95	61.38
	Within individuals	107	108.00	1.01	31.76
	Total	**213**	**664.76**	**3.18**	
Ecological Zones	Among populations	11	129.49	0.41	12.90
	Among individuals within populations	95	427.26	1.74	55.17
	Within individuals	107	108.00	1.01	31.93
	Total	**213**	**664.76**	**3.16**	

(*Average F-statistics across all loci becomes: *Population_race*, FIS: 0.66, FST: 0.07 and FIT: 0.68, and *population_E/Zones*, FIS: 0.63, FST: 0.13 and FIT: 0.68, *p*-value= 0.0001)

Cluster Analysis and Pairwise Genetic Dissimilarity

The Unweighted Neighbor-joining analysis of 107 Ethiopian landraces using 110 alleles indicated four major clusters (Figure 4 and 3). Cluster 1 representing the largest numbers of accessions of all races in scattered manner. It formed two sub-clusters inside, one with mainly of caudatum and another uniformly intermixed race. The second cluster most uniquely contained mainly durra race (18/27, 67% of the total population representing the race) along with rare numbers of caudatum and durra-bicolor (3 and 4). Exceptionally no bicolor race clustered under this group, while only a single guinea represented. Like the first cluster, the third cluster also formed two sub-clusters and most intermediate durra-bicolor structured in the one sub-cluster along with other race types. Under the final cluster 4, majority of race durra-bicolor contained along with a single caudatum and guinea race, and rare number (that is 4) of bicolor. Matrix of pairwise genetic distance of the racial population relationships (Table. 8) indicated the existence of the highest dissimilarity between race bicolor and durra (highest genetic distance, 0.19) while the lowest score was registered between intermediate durra-bicolor and guinea (0.06). Whereas, among populations of different ecological zones pairwise genetic distance ranged from 0.0096 to 0.286 between West Wollega and Illubabor, and between Central and South Tigray and East Harerge respectively (Table. 7).

Table 7. Population Pairwise FSTs_according to E/Zones (1:N/Wello, 2:S/Wello, 3:E/Harerge, 4:Metekel, 5:Gambella(Z1), 6:Cent_S/Tigray, 7:Jimma, 8:Illubabor, 9:W/Wellega, 10:N/Shewa, 11:Bench_Maji and 12:E/Shewa).

	1	2	3	4	5	6	7	8	9	10	11	12
1	0.0000											
2	0.16238	0.0000										
3	0.16930	0.25522	0.0000									
4	0.11781	0.07339	0.05757	0.0000								
5	0.22977	0.25775	0.17980	0.14032	0.0000							
6	0.27502	0.23017	**0.28600**	0.12754	0.14374	0.0000						
7	0.24439	0.24344	0.13635	0.10946	0.15578	0.22198	0.0000					
8	0.10194	0.16709	0.06590	0.07200	0.21133	0.20215	0.16575	0.0000				
9	0.12428	0.22139	0.15742	0.12568	0.25391	0.22957	0.19963	**0.00961**	0.0000			
10	0.18015	0.19762	0.25003	0.11359	0.17774	0.15225	0.20035	0.11829	0.15778	0.0000		
11	0.13568	0.12301	0.09620	0.09943	0.20278	0.21224	0.16786	0.08059	0.09937	0.13631	0.0000	
12	0.20226	0.23695	0.18089	0.10790	0.18632	0.18925	0.15120	0.06780	0.09728	0.12207	0.11949	0.0000

Average gene diversity over loci: 0.587146 ± 0.321973 and numbers in bold were the highest &lowest distance scored

Table 8. Pairwise genetic dissimilarity among sorghum races (Distance method: Pairwise differences).

	Durra	Caudatum	Durra_bicolor	Guinea	Bicolor
Durra	—				
Caudatum	0.08609	—			
Durra_bicolor	0.05741	0.08787	—		
Guinea	0.07009	0.07734	**0.05728**	—	
Bicolor	**0.18937**	0.11858	0.07156	0.09562	—

N.B. The bolded numbers shows the highest and lowest genetic distance scored between races

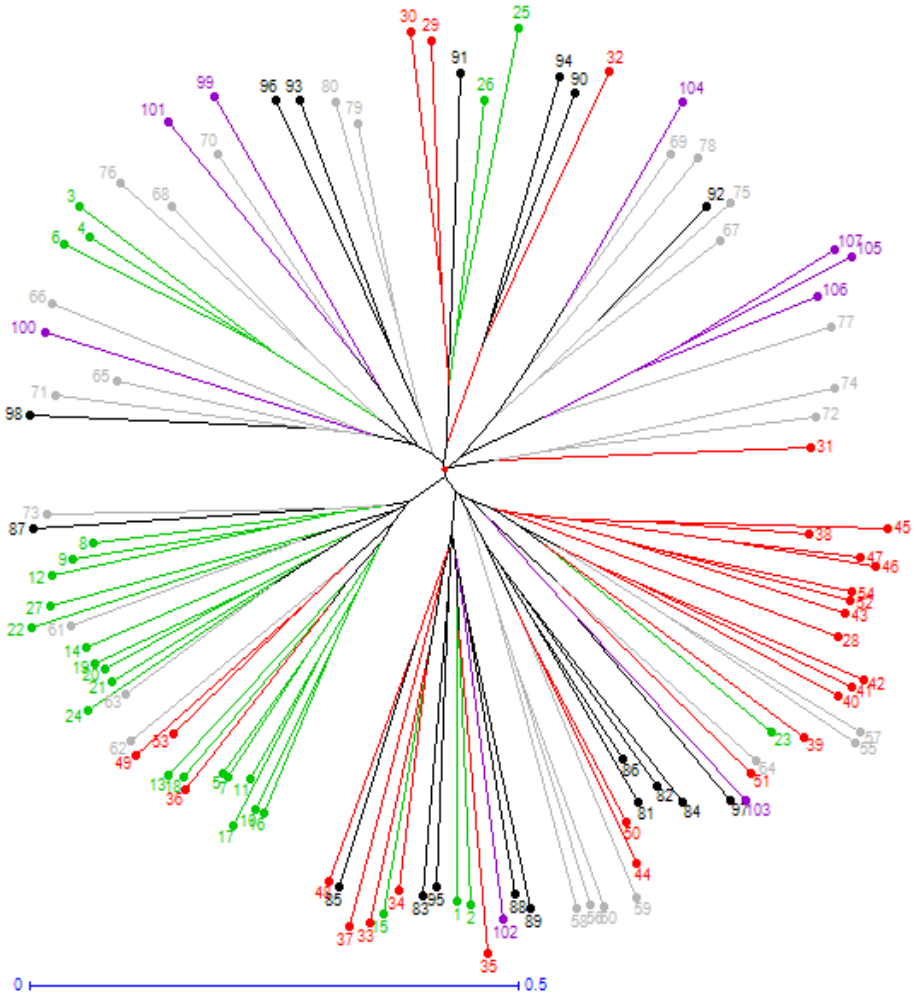

Figure 4. Cluster analysis of 107 Ethiopian sorghum landraces (Green: race durra, accession 1-27, Red: caudatum, accession 28-54, Gray: durra-bicolor, accessions 55-80, Black: race guinea, accession 81-98 and Purple: race bicolor, accessions from S/N 99 to 107).

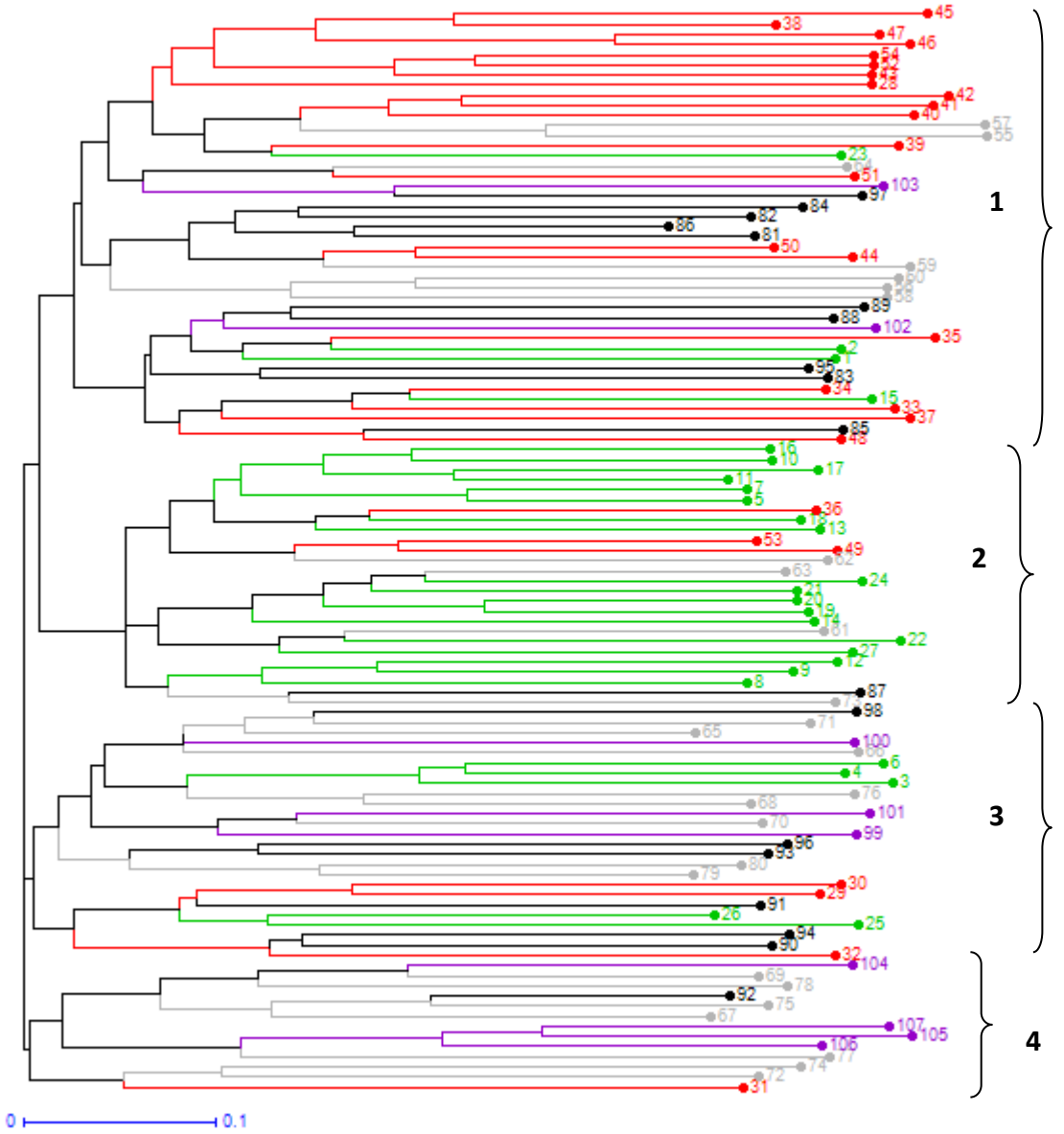

Figure 5. Dendogram for 107 Ethiopian landraces Based on Unweighted Neighbor Joining (Green: race durra, accession 1-27, Red: caudatum, accession 28-54, Gray: durra-bicolor, accessions 55-80, Black: race guinea, accession 81-98 and Purple: race bicolor, accessions from S/N 99 to 107).

6. Discussion

SSR allelic diversity

The result in the present study indicates the presence of high diversity among Ethiopian sorghum collections. Moreover, the mean number of alleles per locus (9.2) observed in this study was higher than similar SSR studies with accessions from North Eastern Benin, 7 (Missihoun *et al*., 2015), Zambia, 4.4 (Ng'Uni *et al*., 2011), Eastern Kenya, 5.05 (Muui *et al*., 2016), Eritrea, 4.8 (Tesfamichael Abraha *et al*., 2014) and Egypt, 7.3 (El-awady *et al*., 2008), and Ethiopian collections in combination with other countries (Agrama and Tuinstra, 2004). However, it is lower than Cuevas and Prom, (2013)'s population structure and diversity study for 137 Ethiopian germplasm conserved at USDA-ARS National Plant Germplasm System, that is 14 per locus.

The low polymorphism in the above similar studies may be the result of low numbers of accessions considered in their studies and sample collections represented are from specific areas of agro-ecologies. Higher polymorphism within the present Ethiopian landraces observed may be indication of the extensive and regular seed exchange farming system within farmers of Ethiopia (McGuire, 2000). This form of seed migrations also adds allelic variations and frequency changes with their movement adding variation and even rare alleles to landraces avoiding genetic drift. Thus the observed rare alleles could be useful as an additional source of important agronomic traits. In fact, Sorghum, a genus having evolved across a wide range of environments in Africa, exhibits a great range of phenotypic diversity and numerous resistances to abiotic and biotic stresses (Clarissa *et al*., 2013). It is cultivated in all regions of Ethiopia from 400 – 2500 masl. Hence, the

wider agro ecological diversity of Ethiopian climates from which all races and zonal sample collections represented and the presence of wider morphological variations observed within accessions from Ethiopia and Eritrea (Amsalu Ayana *et al*., 2000; McGuire, 2000; Dogget, 1965) might also contributed to its genetic variations. The high level of the observed average PIC (0.76) in this study also indicated the discriminatory power of the selected SSR markers. Similar findings were indicated in studies by Ceuvas and Prom, (2013) and to a certain extent Agrama and Tuinstra, (2004) who reported average PIC values 0.78 and 0.622, respectively. However, the observed PIC value is higher than that of Nemera Geleta and Labuschagne, (2006), Missihoun *et al*., (2015), and Muui *et al*., (2016) who reported 0.46, 0.33 and 0.49, respectively. Although sorghum is considered as self-pollinating species, cross pollinations between sorghum landraces are believed to be as high as 7%, and can even reach 70% in certain races in particular environments (Clarissa *et al*., 2013). The observed high allelic frequencies could also arise from outcrossing within wild and weedy relatives (House, 1985; Dogget, 1965). In addition to all these factors, our marker selection criteria which is based on their high levels of polymorphisms in the previous similar studies may also be considered as attributes to its observed heterozygosity. Its lower value than aforementioned study may be attributed by use of agarose gel electrophoresis for the separation of PCR products; while polyacrylamide gel electrophoresis (PAGE) has a capacity to unveil products' having up to 2 base pair differences. In addition, in the present study each accession was represented by a single leaf samples to avoid their variations within bulked samples since a landrace itself is genetically and phenotypically diverse.

Genetic Relationships

Partitioning the total variations according to their centers of zonal collections and races also indicated the presence of huge amount of variation among both populations (55.17% and 61.38% respectively). Similar results were observed within 80 germplasms from Ethiopia and Eritrea by 20 RAPD analysis though they are not grouped as of their latter population structure revealing 77% of total variation within their ecological zone of collections (Amsalu Ayana *et al*., 2000). Ng'Uni *et al*., (2011) and Muui *et al*., (2016) also observed large genetic variation (82% and 91.61%) within individual accessions of areal populations across Zambia and Kenya sorghum genotypes, respectively. The moderate genetic differentiation (FST= 0.07 for races and 0.13 for zones) among the populations in terms of allele frequency also indicated the continuous exchange of genes between them. This finding also supports earlier studies by Ceuvas and Prom, (2013) who found genetic differentiation of 0.10 among 137 Ethiopian sorghum maintained at NPGS. They also indicated the presence of highly related accessions within their samples by genetic redundancy analysis. However, Ganapathy *et al*., (2012) reported high estimate of fixation index (FST=0.35, P=0.001) when 82 Indian genotypes were structured as rainy and post-rainy season adaptations and a much higher estimate (FST=0.40) when they are classified as varieties, maintainers, restorers and germplasm lines. The difference in Fixation indices and genetic variations between the two populations in our case might be because of additive effect in population formations where 2-3 ecological zones were merged to represent a single race population.

Cluster Analysis and Pairwise Genetic Distance

Pairwise genetic distance in the present study indicated greatest genetic dissimilarity between race bicolor and durra (0.189), and the least record between guinea and intermediate durra-bicolor (0.0573). The intra-population diversity within Ethiopian races with overall locus showed the greatest genetic diversity among races bicolor and caudatum (He = 0.77 and 0.75 respectively). Similar result was reported by Deu *et al*., (2000) that the greatest amount of diversity within races were found between bicolor and guinea. The least genetic distance between West Wollega and Illubabor (0.0096) may be due to the close proximity of the two zones where free seed exchange might occur. In contrast, the greatest dissimilarity (0.286) recorded between central and South Tigray and East Harerghe zones (Table. 7).

The Unweighted Neighbor-joining cluster analysis and PCoA in the present study by genetic dissimilarity using Darwin grouped 107 Ethiopian sorghum landraces in to four major clusters. Their structure was according to racial difference where most of race caudatum and durra-bicolor formed sub-clusters within the first and third clusters respectively while race durra exceptionally formed its own cluster with 67% of the population being represented in cluster 2. In addition, some intermediate durra-bicolor formed sub-cluster in the fourth cluster. However the third cluster with most races durra-bicolor grouped along with other racial groups being scattered. Bicolor and guinea didn't form any group and found being scattered within other groups. Previous reports also show similar results (Deu *et al*., 2000; Missihoun *et al*., 2015; Hailekiros Tadesse and

Tileye Feyissa, 2013) where the individuals are structured according to their racial groups and geographical origins. UPGMA of Ethiopian accessions using ISSR showed a clear grouping and differentiation based on populations of ecological zones and their locality (Hailekiros Tadesse and Tileye Feyissa, 2013). Missihoun *et al.*, (2015) also found that the 61 samples from Benin structured according to their botanical race and morpho-physiological characteristics of sorghum grains using 20 SSR. In addition, Deu *et al*., (1994) reported the race bicolor appeared highly variable and didn't form a specific group while studying RFLP analysis on 94 accessions selected based on the basis of their geographic origins and racial classification. They also indicated race guinea being clustered in one group and divided into three sub-clusters inside as West Africans, Southern Africans and the *margaritiferum* subrace while other races such as kafir, durra and caudatum clustered in their respective groups.

The present finding and that of Deu and her colleagues (2000) also supported by Wang *et al*., (2013)'s 242 landrace mini-core collections who reported a structured classification both by their geographic origin and races with 390 SNPs. In contrast, cluster analysis of Ethiopian and Eritrean accessions failed to group the landraces of the same region and adaptation zones together when analyzed by RAPD (Amsalu Ayana *et al*., 2000). Dje *et al*., (2000) also found that accessions belonging to same race or geographic origins were scattered when matrix plot of individual sorghum accessions based on R_{ST} distance matrix. Likewise, UPGMA based cluster analysis for collections from Botswana and other Southern African countries like Namibia, Swaziland, Zambia and Zimbabwe didn't group the landraces as of their racial, ethnicity or agro-ecological zones (Motlhaodi,

2016). The scattered nature of bicolor race in the present study may be the indication of its most primitive and heterogeneous type of the race. Hence it was believed to be distributed wherever sorghum is grown (Wang *et al*., 2013). The most unique and clustered race in PCoA, race durra, is abundant in Ethiopian and Sudan as well and Harlan and de Wet, (1972) reported also settlers in warm highlands of Ethiopia have used the durra sorghum as their foundation of their agricultural system almost 500 years ago. These may be the reason why durra-bicolor intermediate race were also abundant in the country. In addition, caudatum, described as being adapted to harsh conditions, are found most commonly in areas receiving 250 to 1,300 mm of rain annually (Stemler *et al*., 1977). They formed a separate sub-cluster in the first group. The bicolor and guinea, representing the smallest parts of Ethiopian diversity, distributed evenly across all clusters except the bicolor race which didn't form a group within durra race cluster.

7. Conclusion and Recommendations

7.1. Conclusion

In general, the racial classification among *Sorghum bicolor* could be used for *in-situ* and *ex-situ* conservation and genetic dissimilarity with their respective agronomic characteristics favors the future crops germplasm breeding programs. In this regard, Ethiopian sorghum races were structured into four major clusters according to their racial difference except for race bicolor and guinea. Bicolor found being scattered within other groups. The greatest genetic distance found between race bicolor and caudatum, and between the Ethiopian zones Central and South Tigray and East Harerghe. There were also a huge variation observed among the populations of both racial classification and ecological zones (61.38% and 55.17%, respectively). The greater mean number of alleles per locus (9.2) and PIC value of 0.76 in the present study also indicate the presence of high genetic diversity among Ethiopian sorghum collections and the discriminatory power of the selected markers. There is also a moderate levels of genetic differentiation among races (FST= 0.07) and Ethiopian sorghum producing zones (FST=0.13). Hence, racial groups could also be used as representation of the germplasm collection along with the commonly known diverse agro-ecological and zonal collections and their adaptation zones.

7.2. Recommendations

In line with the present study, the future research areas should include molecular studies along with the morphological components using markers linked to specific agronomic traits to enable the use of racial groupings within sorghums in its breeding areas. In addition, classification of the national sorghum germplasm collections according to their race also needed since the Ethiopian landraces have been used as the source of important traits.

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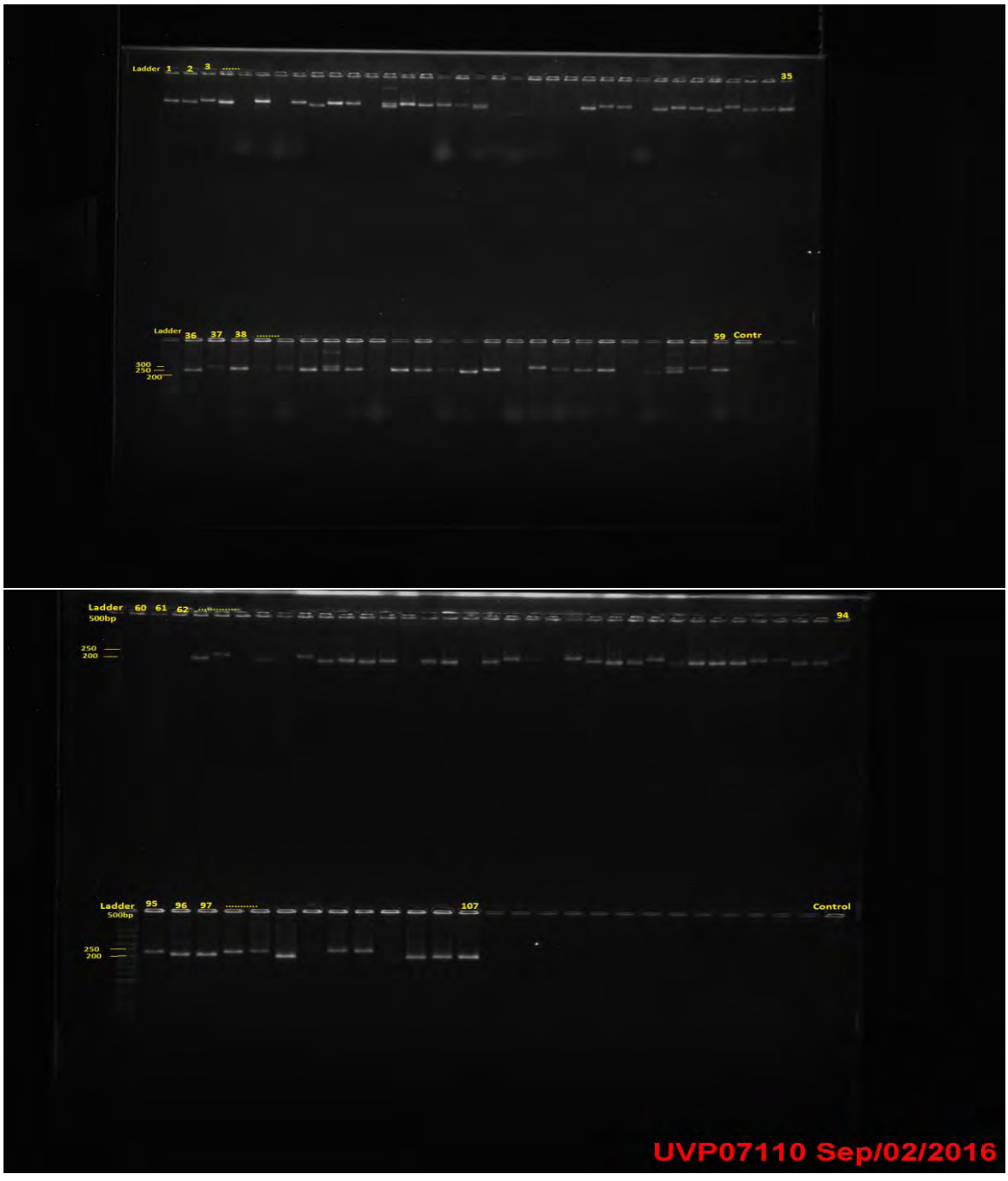
9. Appendices

Annex 1. PCR products with SSR primer xtxp258 (S/N 01 to 35 on the first lane, and 36-59 on the second with controls at the end), and On the second gel image Sample number 60 to 94 on top lane and 95-107 on the bottom lane



Annex 2. Summary of intra-population variation between sorghum races (# of Alleles and gene diversity, He).

	Locus#	durra	caudatum	durra_bicolor	guinea	bicolor	Mean	s.d.	Tot.
# of Alleles	1	5	5	5	4	2	4.20	1.30	5
	2	6	6	7	5	6	6.00	0.71	8
	3	6	7	5	7	4	5.80	1.30	9
	4	4	3	5	5	2	3.80	1.30	6
	5	4	7	9	6	5	6.20	1.92	10
	6	10	12	13	10	8	10.60	1.95	18
	7	11	9	12	11	9	10.40	1.34	16
	8	4	6	7	6	7	6.00	1.22	8
	9	6	6	6	3	2	4.60	1.95	9
	10	3	6	6	5	4	4.80	1.30	9
	11	7	7	7	5	4	6.00	1.41	7
	12	4	4	4	3	4	3.80	0.45	5
	Mean	5.83	6.50	7.17	5.83	4.75	6.02	0.90	9.17
	s.d.	2.48	2.32	2.82	2.48	2.34	2.49	0.20	4.02
He	1	0.71	0.76	0.76	0.61	0.53	0.67	0.10	0.76
	2	0.67	0.80	0.81	0.68	0.76	0.74	0.07	0.82
	3	0.80	0.81	0.78	0.83	0.62	0.77	0.09	0.85
	4	0.64	0.51	0.57	0.39	0.53	0.53	0.09	0.55
	5	0.73	0.74	0.84	0.78	0.83	0.78	0.05	0.83
	6	0.87	0.87	0.89	0.89	0.83	0.87	0.02	0.92
	7	0.83	0.86	0.90	0.90	0.89	0.88	0.03	0.90
	8	0.43	0.76	0.73	0.76	0.84	0.70	0.16	0.77
	9	0.62	0.75	0.66	0.52	0.53	0.61	0.10	0.76
	10	0.64	0.68	0.73	0.64	0.73	0.68	0.05	0.76
	11	0.70	0.74	0.76	0.74	0.62	0.71	0.06	0.82
	12	0.75	0.75	0.76	0.61	0.73	0.72	0.06	0.74
	Mean	0.70	0.75	0.77	0.70	0.70	0.72	0.03	0.79
	s.d.	0.12	0.09	0.09	0.15	0.13	0.12	0.03	0.09

Average gene diversity over loci (E/zones) = 0.59 ± 0.321973