ADDIS ABABA UNIVERSITY SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY

GENETIC VARIATION IN LONG MATURING SORGHUM [*Sorghum bicolor* (L.) Moench] LANDRACES FROM WELLO AND HARARGE REGIONS OF ETHIOPIA

BY

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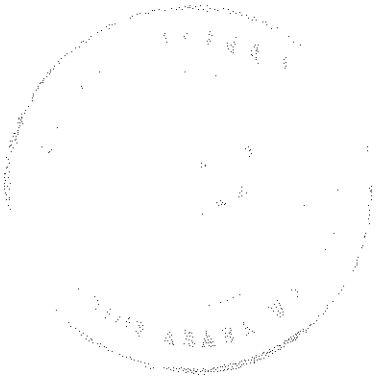

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ABSTRACT

A study on the extent and pattern of genetic variability among long maturing sorghum genotypes collected from Wello and Hararge areas of Ethiopia was conducted using morphological and random amplified polymorphic DNA (RAPD) markers. The field experiment was conducted at the experimental field of Melkassa Agricultural Research Center and the molecular work was done at Armauer Hanson Research Institute laboratory, AddisAbaba. A total of 27 morphological traits were recorded from all 26 genotypes included in the study. RAPD marker analysis was conducted on fourteen genotypes sub-sampled, from the different clusters generated using morphological data. Distribution of various qualitative traits between regions and altitudes zones showed variation from total absence to high level of variation from one morphological trait to the other. Mean Shannon-Weaver diversity index (H) varied from 0.04 for grain plumpness to 0.99 for seed color with over all mean of 0.60. Highest heritability and genetic advance was observed for leaf area, plant height, number of primary branches and number of seeds per head, indicating that selection progress will be expected to be high for these traits. High heritability coupled with high expected genetic gain may be due to high additive gene effects implying that selection applied for these traits would lead to yield in an improvement in the crop of study. Variation within accessions, regions of origin and altitude classes accounted for a large portion of the total variation for morphological data. But, Nie's diversity index from molecular marker data fails to reflect the within accessions and region variation, rather it showed relatively higher variability between populations and regions of origin. Cluster analysis from morphological data fail to group genotypes either based on their region of origin or altitude classes while cluster from molecular marker tend to arrange genotypes based on their regions of origin regardless of their altitude classes.

1. INTRODUCTION

Genetic variability is the diversity with in a species and it is the raw material which allows the species the opportunity to evolve under changing environments and selection pressures. Analysis of the extent and distribution of genetic variation within a species is essential for understanding the genetic relationship between accessions and to sample genetic resources in a more systematic fashion for breeding and conservation purposes (Ayana, 2001; Gebru, *et al.*, 2002; Geleta, 2003).

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important grain crop in the world, in terms of production, after wheat, maize, rice and barely, and the second most important cereal crop (after maize) in sub-Saharan Africa (Dogget, 1988; Zidenga, 2004). The greatest variability of sorghum is found in North East quadrant of Africa, and most evidence points to this area as the likely primary center of origin and domestication (Vavilov, 1951; Dogget, 1970; House, 1985; Dogget, 1988; Deu *et al.*, 1994; Zidenga, 2004). According to Gebrekidan (1973, 1981) sorghum, in Ethiopia, exists in tremendous diversity throughout the major growing areas which includes pockets of isolation with an extremely broad and valuable genetic base for potential breeding and improvement. Adequate information on the extent and pattern of genetic variability among sorghum accessions is an important element in the improvement of yield and biotic and abiotic stress tolerance of the crop.

Accurate, fast, reliable and cost effective identification of plant population and varieties is essential in agriculture (Geleta, 2003). Traditionally, taxonomists classify genetic resources in sorghum based on morphological traits (Ejeta *et al.*, 1999). This usually involves description

of variation for morphological traits, particularly morpho-agronomic characteristics of direct interest to users (Geleta, 2003). But, classifying germplasm accessions based solely on morphological characters may not provide an accurate evidence of the genetic variability among the cultivated genotypes of sorghum (Ejeta *et al.*, 1999; Ayana, 2001). To obtain detailed information about the amount and distribution of genetic variation in a species, combination of methods including cytogenetic analysis; biochemical such as isozyme/allozyme and molecular techniques that analyze polymorphism at the DNA level directly is essential (Teshome *et al.*, 1997; Ejeta, *et al.*, 1999; Zidenga, 2004). DNA based techniques have been used successfully in DNA finger printing of plant genomes and in genetic diversity studies (Agrama and Tuinstra, 2003). Among them random amplified polymorphic DNA (RAPD) analysis is fast technique (Colombo *et al.*, 1998; Fahima *et al.*, 1999; Dahiberg *et al.*, 2002; Agrama and Tuinstra, 2003), comparatively easy to perform and well adapted to non-radioactive DNA finger printing of genotypes (Cao *et al.*, 1999) but, with low level of reproducibility and only with dominant inheritance.

In this study the level of genetic diversity was determined among 26 long maturing sorghum genotypes obtained from national variety trials of the Sorghum Research Coordinating Center, Melkassa Agriculture Research Center (MARC), using 17 quantitative and 10 qualitative morpho-agronomic traits and random amplified polymorphic DNA (RAPD) marker technique. The genotypes are originally collected from Wello and Hararge regions by the then Institute of Bio-diversity Conservation (IBC) and by researchers from MARC.

1.1. Objective

1.1.1. General Objective

To determine the amount and pattern of distribution of genetic diversity in samples of long maturing sorghum landraces using morphological and random amplified polymorphic DNA (RAPDs) markers.

1.1.2. Specific Objectives

1) To asses the genetic similarity among long maturing sorghum landraces collected from Hararge and Wello regions of Ethiopia.
2) To enrich the genetic stock of sorghum improvement program and gene pool of the country and provide researchers with detailed information about the extent and pattern of distribution of genetic diversity of long maturing sorghum landraces.
3) To estimate heritability and genetic advance for quantitative traits of long maturing sorghum landraces.

2. LITERATURE REVIEW

2. 1. Adaptation, Production and Economic Importance of Sorghum

Sorghum is widely adapted to wide environmental conditions but it is particularly adapted to the semi-arid parts of the world (Dogget, 1988; ICRISAT, 1999). A number of morphological and physiological characteristics contribute to its adaptation to dry conditions. It is also tolerant to water logging and can be grown in high rainfall areas; however, it is primarily a crop of hot semi arid tropical environments with 400-600 mm rainfall that are too dry for maize (ICRISAT, 1999). It is also widely grown in temperate regions and altitude of up to 2300 m in the tropics (ICRISAT, 1999; Dogget, 1988).

In Ethiopia, sorghum is the major crop both in terms of production and food and occupies, on average, 6.75 million hectares of land annually and 7.74 million tones of production (CSA, 2004). It is produced on subsistence basis under rainfed conditions in traditional farming systems. Sorghum grain is mainly used for human food, preparing traditional beverages, the grain and stalk are also used for animal feed, and the stalk is used for fuel and construction. As the crop has the potentiality to grow under adverse condition, minimum input and care its cultivation is extended all over the country. Based on their growing zones within the country, cultivated sorghum is grouped into highland, intermediate and lowland sorghum. Highland sorghums grow mainly at an altitude of greater than 1900 masl, intermediate sorghums at an altitude range of 1600-1900 masl and those of lowlands grow in areas of altitude less than 1600 masl (Alemayehu, 2003).

2.2. The Races of Sorghum in Ethiopia

Harlan and DeWet (1972) classified *Sorghum bicolor* ssp. into five basic races (bicolor, caudatum, dura, guinea and kafir) and 15 derivative races of the basic races. All the basic races, except kafir, are present in Ethiopia (Harlan and deWet, 1972).

2.3. Studies of Sorghum Diversity in Ethiopia

Information on genetic diversity within and among closely related crop species is essential for rational use and management of genetic resources. It is particularly useful in characterizing individual accessions and cultivars, in detecting genetic materials with novel gene and thereby rescuing them from erosion, and as general guide in selecting parents for crossing in breeding program.

Efforts have been made to identify the different accessions of Ethiopian origin sorghum germplasm based on morphological characters (Ayana and Bekele, 1998; Teshome et al., 1997; Geleta,2003; Abebe and Wech, 1982; Gebrekidan and Menkir, 1979). However, qualitative traits are influenced by environmental factors and show variation resulting, in low heritability and high environment by genotype interactions. Consequently, it is difficult to accurately determine genetic variability. However, a continued use of morphological data to describe cultivar indicates that these data retain popularity as descriptor (Smith and Smith, 1992). Due to their limited number of detectable loci, allozyme markers also did not clearly separate the various races of cultivated and wild sorghum accessions into distinct classes (Ayana, 2001).

2.4. Assessment of Genetic Diversity in sorghum

The wide rage of environmental conditions under which sorghum is produced in Ethiopia has given rise to tremendous range of genetic variability (Dogget, 1988). But the indigenous germplasm of sorghum has been threatened due to a series of adverse rainfall situation, which is forcing farmers to shift to short season crops like teff (*Eragrostis tef*) and early maturing uniform sorghum cultivars and the practice for relief agencies to supply farmers with seeds of uniform varieties for rehabilitation purpose. In addition, farmers' decision in process of planting, managing, harvesting and processing their crops affect the genetic variability of the crop.

The progenitors (wild sorghum) of cultivated sorghums are widely distributed across many African countries, including Ethiopia (Ayana *et al.*, 2000). As in the case elsewhere, Ayana *et al.* (2000), wild sorghum in Ethiopia is rapidly disappearing, largely because more and more of its habitat is used for agriculture, grazing, human settlement, construction etc.

Among the earliest markers used in scientific investigations and are still in use in germplasm management were the qualitative morphological traits such as: leaf midrib color, glume color, glume hairiness, grain covering, grain form, panicle compactness and shape, endosperm texture, and presence or absence of glume (Breting and Widerlechner, 1995). The determinations of center of diversity by Vavilvo for various species were based on an extensive field studies and observation of phenotypic traits.

The study of genetic variability using morphological traits that is of interest for the breeder is extremely important to speed up the breeding programs. These morphological traits are easy to

score, quick and simple to evaluate, frequently without requiring high level of technical skill unlike molecular and biochemical markers (Teshome *et al.*, 1998). Morphological traits, however, have got their own short comings that many of the traits are polygenic and influenced by the environment, the phenotype of which can only be determined at the whole plant level, and require growing of plants to suitable stages before certain characters can be scored (Powell 1992; Seifu, 1997; Ayana, 2001).

Molecular markers have been used to characterize genetic diversity represented by elite inbred genotypes and cultivated races of sorghum (Menkir *et al.*, 1997; Dean *et al.*, 1998). In contrast to morphological markers, which are based on visible traits and biochemical markers (Isozyme /allozyme), which are based on proteins produced by genes, molecular markers relay on a DNA assay (Ayana, 2001; Agrama and Tuinstra, 2003; Tefera, 2003).

There are many different kinds of molecular markers including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs), microsatellites, and single nucleotide polymorphisms (SNPs). There are merits and demerits of each type of marker, depending on the specific objectives of the particular study (Table 1).

Table 1. Comparison of the most commonly used marker systems in cereals

Feature	RFLPs	RAPDs	AFLPs	SSRs	SNPs
DNA required (μg)	10	0.02	0.5-1.0	0.05	0.05
DNA quality	High	High	Moderate	Moderate	high
PCR-based	No	Yes	Yes	Yes	Yes
Number of polymorph	1.3-3.0	1.5-50	20-100	1.0-3.0	1.0
Loci analyzed	Not easy	Easy	Easy	Easy	Easy
Ease of use amenable to automation	Low	Moderate	Moderate	High	High
Reproducibility	High	Unreliable	High	High	High
Development cost	Low	Low	Moderate	High	High
Cost per analysis	High	low	moderate	low	low

Source: Korzun (2002).

Faced with such marker technologies, several factors may need to be considered in choosing the marker of interest; the degree of polymorphism revealed by the markers, the reproducibility of the experiment, time and cost required for running the analysis, etc (Agrama and Tuinstra, 2003; Tefera, 2003).

As an extension to the variety of existing techniques using polymorphic DNA markers, the random amplified polymorphic DNA (RAPD) technique can be used in molecular diversity studies to determine taxonomic identity, asses genetic relationship, analyze mixed genome samples, and create specific probes. According to Hadrys *et al.*, (1992), the main advantages of RAPD technology include: (1) suitability for work for anonymous genomes, (2) applicability to problems where only limited quantities of DNA are available, (3) efficiency

and low expense.

Generally, form the plant breeding perspective, genetic variability is very important since the potential for cultivar improvement is directly influenced by the amount of genetic variability present within sorghum genotypes. Hence, evaluation of genetic variability among adapted, elite sorghum genotypes may help to estimate the degree of heterosis in progeny of certain parental combinations. It can also provide a predictive estimate of performance of pure line cultivars that could be developed from the segregating populations (Tefera, 2003).Therefore, in the present study, morphological markers and RAPD analysis were used to determine the amount of genetic variability among long maturing sorghum land races.

3. MATERIAL AND METHODS

3.1 Morphological Data

3.1.1 Description of the Study Area

The field experiment was conducted in moisture deficient low land areas of Oromia at Melkassa Agricultural Research Center (MARC), 117 km south of Addis Ababa. Around MARC unexpected crop failure due to short rainfall is a common occurrence. MARC, with growing period of 85-130 days, is located 8^0 30'N 39^0 21'E, and it is about 1500 meter above sea level with average annual rain fall of less than 600 mm, and with an average temperature of 21 ^{0}C.

3.1.2 Plant Materials

A total of 26 long maturing sorghum (5-8months depending on the location) landraces collected from Wello (10 genotypes) and Hararge (15 genotypes) and one local check from the experimental site (Melkassa), were used for the present study (Table 2). All of the 26 accessions are from the research materials (Long Cycle Sorghum National Variety Trial) of National Sorghum Research Coordinating Center, MARC. All the accessions were obtained from the Coordinating Center of Sorghum Research in Ethiopia, MARC; and the passport data for the accessions were obtained from Institute of Biodiversity Conservation. These landraces, which are commonly planted in the first rains of April in the moisture stressed area of both Wello and Hararge. After their germination, these cultivars have to face the dry period of May and June.

The genotypes were planted at Melkassa Agricultural Research Center on 5 meter long three raw plots. The distance between rows and plants were 0.75 and 0.15, respectively. The experiment was laid in RCBD replicated four times. For each accession a total of 396 (33 plants in a single row and 3 rows with in a block and 4 blocks) individual plant were planted. Fertilizer was applied at the rate of 100 kg per hectare Diamonium Phosphate (DAP) and 100kg urea per hectar as recommended for sorghum production in the country. Other agronomic practices (weeding, land cultivation, bird scaring, thinning etc...) were done accordingly. On the bases of altitude of the collection site of each genotypes the 26 accessions were divided into three sets. These were lowland (< 1600masl), intermediate (1600 to 1900 masl) and highland (≥ 1900masl) (Gebrekidan, 1981; Kebede and Menkir, 1987).

For the 26 accessions a total of 2080 individual plants (20 plants in one block for all 26 genotypes) were marked with tag for data recording for both quantitative and qualitative (Table 3 and 4) characters. For each accession 80 individual plants (7 plants in a single row) were selected and tagged/labeled randomly. For both qualitative and quantitative characters, data was recorded based on Sorghum Descriptors (IBPGR/ICRISAT, 1993).

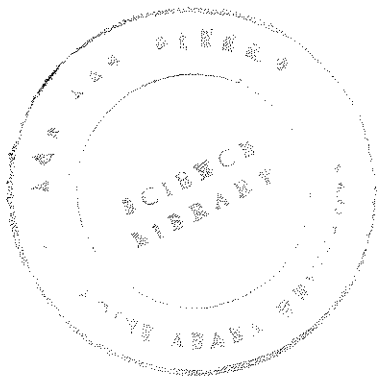


Table 2. List of Sorghum landraces with their respective administrative zone and altitude

		Place of collection		
Entry No.	Accession No.	Zone	District	Altitude
1	PGRC/E Acc # 69245	Hararge		1360
2	PGRC/E Acc # 69184	Hararge	Chercher	1480
3	PGRC/E Acc # 69189	Hararge	Chercher	1510
4	PGRC/E Acc # 69208-1	Wello	Awsa	1670
5	PGRC/E Acc # 69227	Hararge	Chercher	1750
6	PGRC/E Acc # 69228	Hararge	Chercher	1750
7	PGRC/E Acc # 69230	Hararge	Chercher	1760
8	PGRC/E Acc # 228252	Wello	Yidju	1975
9	PGRC/E Acc # 69239	Hararge	Chercher	1480
10	PGRC/E Acc # 69185	Hararge	Chercher	1520
11	PGRC/E Acc # 69208-2	Hararge	Awsa	1670
12	PGRC/E Acc # 69237	Hararge	Chercher	1640
13	PGRC/E Acc # 69241	Hararge	Chercher	1800
14	Wello Coll # 007	Wello	Ambassel	1950
15	Wello Coll # 012-1	Wello	Ambassel	1700
16	Wello Coll # 023	Wello	Ambassel	1598
17	Harar Coll # 121	Hararge	Chiro	1670
18	Harar Coll # 144	Hararge	Chiro	1950
19	Harar Coll # 166	Hararge	Chiro	1920
20	Wello Coll # 012-2	Wello	Ambassel	1700
21	Wello Coll # 038	Wello	Kobo	1850
22	Wello Coll # 041	Wello	Kobo	1905
23	Wello Coll # 050	Wello	Kobo	1595
24	Wello Coll # 072	Wello	Bati	1680
25	Harar Coll # 170	Hararge	Chiro	1675
26	Local Check (Rufie)	Test site	Melkassa	1555

Table 3. List of quantitative characters used for the study

Character	Code	Description
Leaf number (Count)	NL	Count of total leaves per plant
Leaf length(cm)	LL	Length of the fourth leaf from the flag leaf
Leaf width(cm)	LW	Width of the fourth leaf from the flag leaf
Leaf area(cm^2)	LA	Area of the fourth leaf from the flag leaf (LL X LW x 0.75)
Length of leaf sheath(cm)	LSL	Length measured on the fourth internode from the surface
Internode length(cm)	IL	Length measured on the fourth internode measured from the surface
Stalk diameter(cm)	SD	Diameter measured on the fourth internode from the surface
Plant height(cm) (Figure 1)	PH	Height from the surface to the tip of the panicle
Peduncle exertion(cm) (Figure 2)	PE	1. Well exerted more than 10cm between ligule of the flag and ear base 2. Exertion 2cm to 10cm between flag leaf and ear base 3. less than 2cm, but ligule definitely below the panicle base 4. Peduncle recurved, but panicle is below the ligule and clearly exposed, splitting the leaf sheath 5. Ear covered by the leaf sheath
Days to flowering(count)	DF	Days from planting to when 50% of the panicle Flowered half way dawn the panicle
Panicle width(cm)	PW	Width of the panicle in middle
Panicle length(cm)	PL	Length of the panicle from its base to tip
Hundred seed weight(g)	HSW	Weight of 100 seed counts
Number of primary branches per panicle(Count)	NPBPP	Count of primary branches per panicle
Length of primary branches per panicle(cm)	LPBPP	Length of primary branches per panicle
Grain yield per head(g)	GYPH	Weight of thrashed grain per panicle
Number of seeds per head(count)	NSPH	Number of seeds per panicle (GYPH/HSW)

Table 4. List of qualitative characters used for the study

Character	Code	Description
Leaf midrib color	1	White
	2	Dull green
	3	Yellow
	4	Brown
Waxy bloom	3	Slightly present
	5	Medium present
	7	Mostly present
Panicle shape and compactness (Figure 3)	1	Very lax panicle
	2	Very loose erect primary branches(2E)
	3	Very loose drooping primary branches(2D)
	4	Loose erect primary branches(3E)
	5	Loose drooping primary branches(3D)
	6	Semi- loose erect primary branches (4E)
	7	Semi- loose drooping primary branches(4D)
	8	Semi compact elliptic (5)
	9	Compact elliptic (6)
	10	Compact oval(7)
	11	Half broom corn(8)
	12	Broom corn(9)
Awn at maturity	0	Absent
	1	Present
Glume color	1	White(W)
	2	Yellow (S-sienna)
	3	Brown (Mahogany-M)
	4	Purple(P)
	5	Black(B)
	6	Gray(G)
Grain covering	0.25	Glume cover 25% of the length of the grain
	0.50	Glume cover 50% of the length of the grain
(Figure 4)	0.75	Glume cover 75% of the length of the grain
	1	Glume cover 100% of the length of the grain
Seed color	1	White
	2	Yellow
	3	Red
	4	Brown
	5	Buff
Grain plumpness (Figure 5)	3	Dimple (D)
	7	Plump(P)
Grain sub-coat	0	Sub-coat color is absent(A)
	1	Sub-coat color is Present(P)
Endosperm texture	1	Completely corneous
	2	Almost corneous
	3	Partly corneous
	4	Almost starchy
	5	Completely starchy

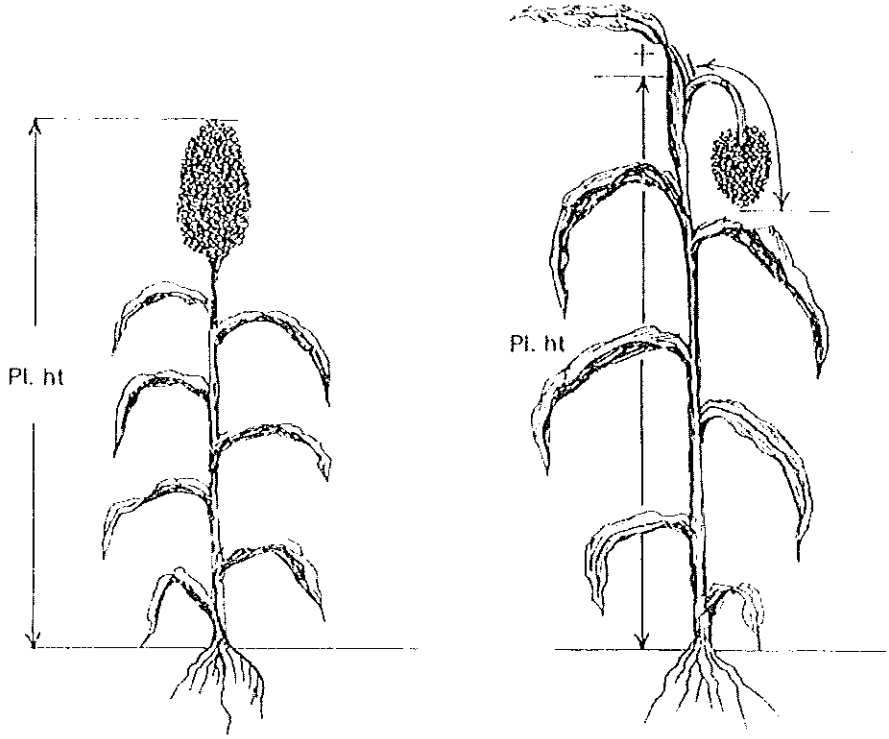


Figure 1. Plant height measure in sorghum (Source: House, 1985).

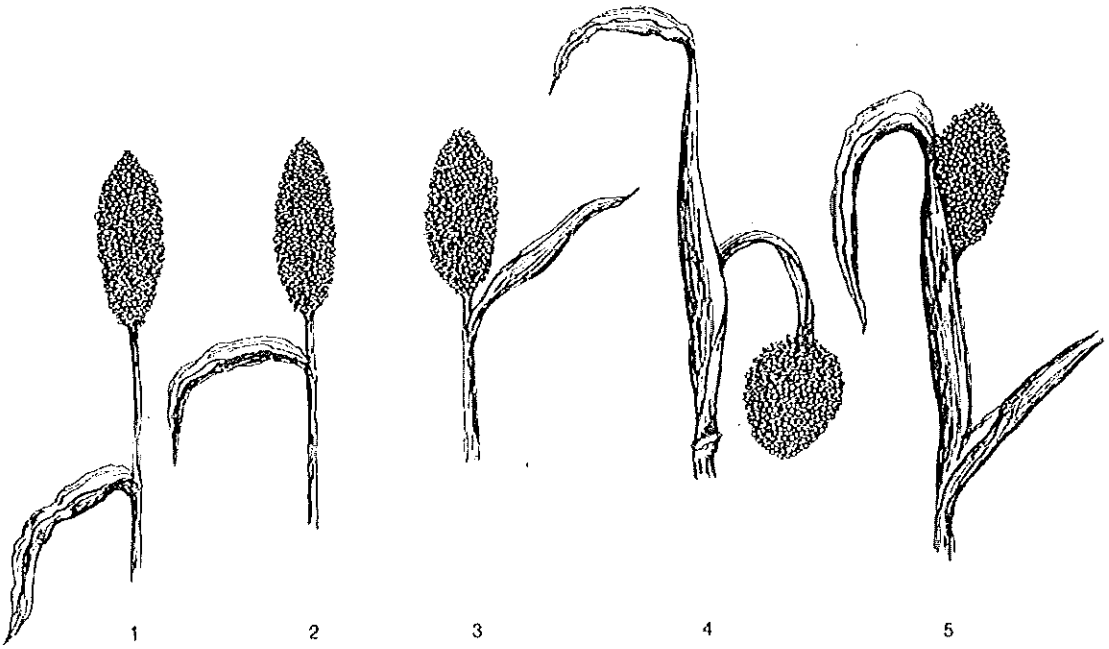


Figure 2. Peduncle exertion and recurving in sorghum (Source: House, 1985).

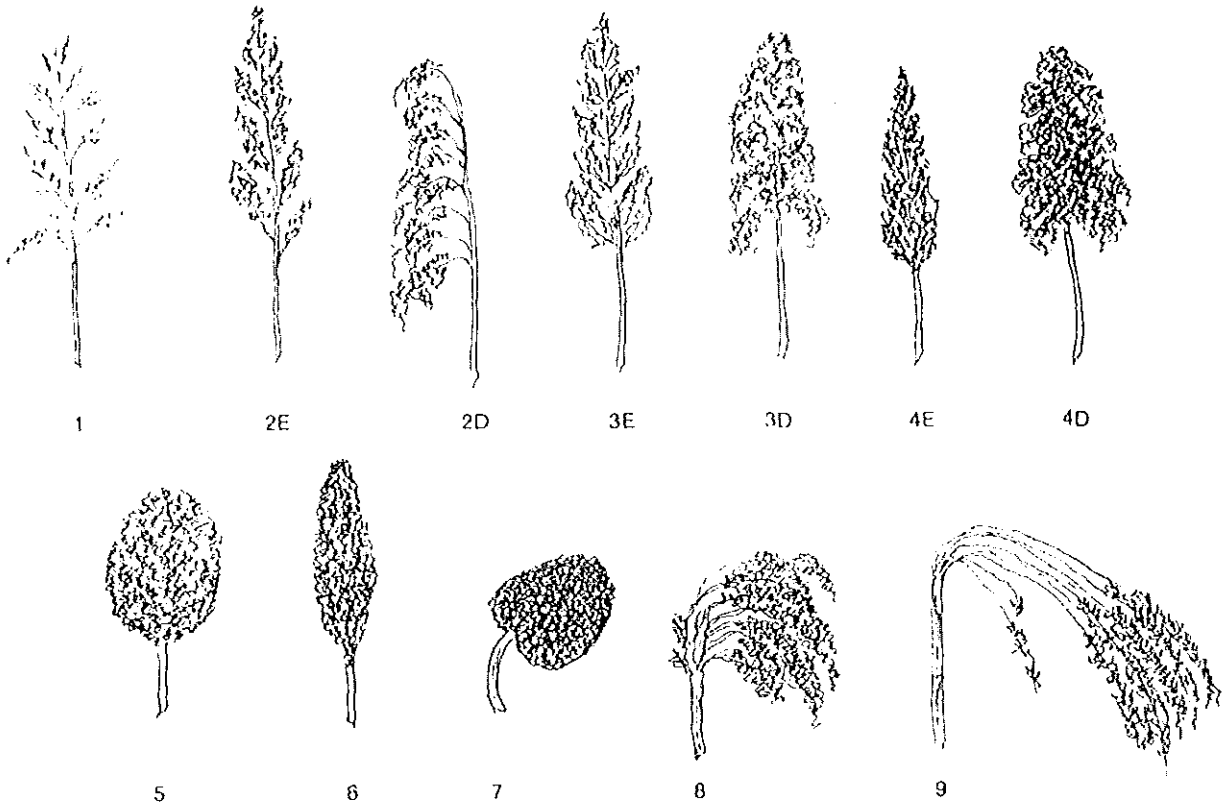


Figure 3. Panicle shape and compactness in sorghum (Source: House, 1985).

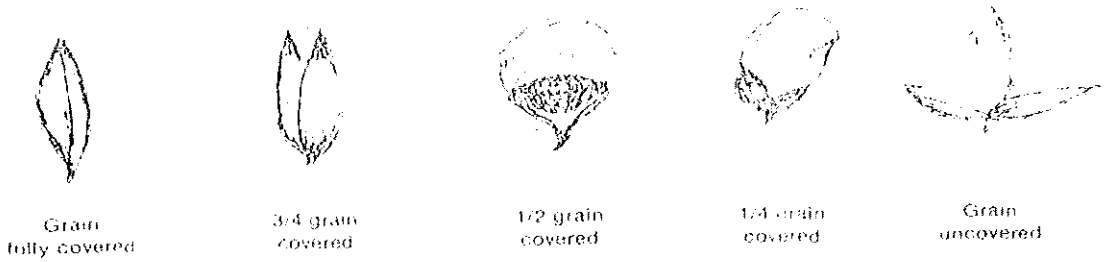


Figure 4. Grain covering in sorghum (Source: House, 1985).

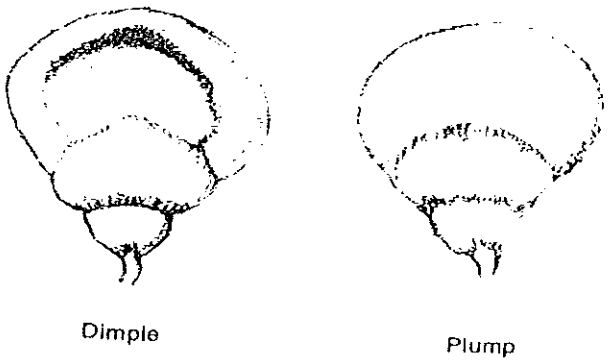


Figure 5. Grain form of sorghum seed (Source: House, 1985).

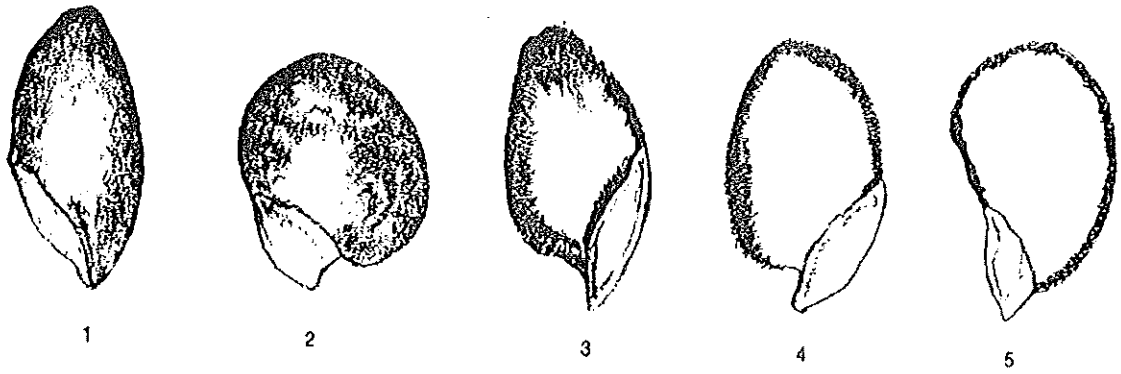


Figure 6. Endosperm texture of sorghum seeds (Source: House, 1985).

3.3 RAPD Data

3.3.1 Plant Material

A total of 14 sorghum genotypes consisting of seven from Hararge, six from Wello and one local from the test site as a local check, were used for this study (Table 5). The genotypes were sampled from the 26 accessions that were grouped in six clusters based on 17 quantitative characters. Accessions from a cluster were chosen to represent region of origin and the three adaptation zones. Summary of the 14 sorghum genotypes selected for RAPD assay shown in Table 5.

Table 5. List of sorghum landraces used for RAPD analysis with their respective region of origin and adaptation zones

*Entry No.	Accession No.	Zone	District	Altitude
1	PGRC/E Acc # 69245	Hararge		1360
4	PGRC/E Acc # 69208-1	Wello	Awsa	1670
6	PGRC/E Acc # 69228	Hararge	Chercher	1750
8	PGRC/E Acc # 228252	Wello	Yidju	1975
10	PGRC/E Acc # 69185	Hararge	Chercher	1520
12	PGRC/E Acc # 69237	Hararge	Chercher	1640
13	PGRC/E Acc # 69241	Hararge	Chercher	1800
15	Wello Coll # 012-1	Wello	Ambassel	1700
17	Harar Coll # 121	Hararge	Chiro	1670
19	Harar Coll # 166	Hararge	Chiro	1920
21	Wello Coll # 038	Wello	Kobo	1850
22	Wello Coll # 041	Wello	Kobo	1905
24	Wello Coll # 072	Wello	Bati	1680
26	Local check(Rufie)	From test site	Melkassa	1555

3.3.2. DNA Extraction

A total of 70 individual plants (five individual plants represented an accession/genotype) were used for DNA extraction. Seed from selected genotypes were germinated in a green house pots at Melkassa Agricultural Research Center (Figure 9). Fresh leaves of individual plants were harvested from 7 to 14 day old seedlings for DNA extraction. Total genomic DNA was extracted from 125 mg leaf samples according to the method of Thomson and Henry (1993) with some modifications. The modification was that in this work 125 gram leaf sample was

used instead of 100 gram leaf sample. DNA quantification was done using spectrophotometric measurement of UV absorption at wavelengths 230, 260 and 280 nm. Measures of DNA purity was determined by the A260:A280 and A260:A230 ratios. These ratios provided indications of protein, and polyphenol and carbohydrate contamination, respectively (Manning, 1991). The DNA showed a clear absorbance peak at 260 nm. The A260 value provided a measure of concentration (roughly 1.0 reading at A260 is equivalent to 50 mg/ ml).

3.3.3 Primer Screening

A total of six oligonucleotide primers (OPC-01, OPA-02, OPA-17, OPA-18, OPC-05 and OPA-13) from TIB MOLBIOL (Berlin, Germany) were first screened using a sample of five sorghum accessions. Four primers (OPC-01, OPA-02, OPA-17, and OPA-18) were selected for generating reproducible polymorphic bands and used for DNA amplification.

3.3.4 PCR Amplification Conditions

The amplification reactions was performed in a final volume of 25 µl consisting of puReTaq Ready-to-Go PCR Beads containing stabilizer, BSA, dATP, dCTP, dGTP, dTTP, ~ 2.5 µl units of puReTaq DNA polymerase and reaction buffer, and 50 to 100 ng genomic DNA. When a bead was reconstituted into a 25 µl final volume, the concentration of each dNTP was 200 µM in 10 mM Tris-HCl (p^H 9 at room temperature), 50 mM, KCl and 1.5 mM $MgCl_2$.

pH

Amplification was performed using a Hybaid Omnigene thermocycler with the simulated tube control function set at 25 µl. The amplification were programmed for 3 minutes at 95^0C for initial strand separation followed by 45 cycles of 1 minute at 94^0C to denature template DNA;

1 minute at 37^0C to allow the primer to anneal to their target sequences and then raised to the optimum temperature, 2 minutes at 72^0C, for DNA polymerase activity (chain elongation), using the fastest possible transition times between each temperature. The last cycle was followed by additional extensions at 72^0C for 10 minutes to ensure that the primer extension reaction was completed. Finally, hold time at 4^0C was set until samples were collected.

3.3.5 Agarose Gel Electrophoresis

After amplification, products were resolved by electrophoresis on 1.2 % agarose gels run in 50 X TAE buffer, p^H 8.0, for 2 hours at 100 volt. The gel was stained with ethidium bromide (0.5 mg/ml) and the DNA fragments (bands) were visualized under UV trans-illumination and picture was taken under computerized and programmed camera system and bands recorded as absent and present from the picture. Only those fragments/bands that amplified reproducibly were included in the data analysis.

3.4. Statistical Analysis

3.4.1. Morphological Data

Raw data of morphological parameters were entered into Microsoft Excel spreadsheet and then calculated for each accession and the mean values were imported into the CPCS Systems (Harjinder and Balwant, 2000) program. The 17 quantitative traits data were arranged as rows and the replication as column. Results were obtained as ANOVA table for each genotype.

3.4.1.1. Qualitative Data

Percentage frequency distribution of qualitative traits across altitude and between regions was calculated using SPSS (SPSS, 1989-2001), version 12 for window.

A) Estimation of Diversity Index

Genetic diversity index was estimated to measure the diversity of each qualitative trait employed in this study. The amount of genetic variation was determined using the Shannon diversity index, which is given as described by (Hutchenson, 1970):

$$H' = -\sum_{i=1}^{n} P_i \log_e(P_i)$$

Where P_i = the proportion of total number of entries in the i^{th} class of an ''N'' class trait. N is the number of phenotypic classes for a character. Each value of H′ is divided by its maximum value, $\log_e n$, and normalized in order to keep the values between zero and one.

3.4.1.2. Quantitative Data

Analysis of variance (ANOVA) was computed for all quantitative traits to detect the variability present among 26 sorghum genotypes. The variance was analyzed following the standard procedure applicable to randomized block design as suggested by Gomez and Gomez (1984) using SPSS version 12 for window. From this analysis expected mean square was estimated.

a) Genotypic and Phenotypic Variances

The variability of each quantitative morphological trait was estimated by simple statistical measures such as mean, range, phenotypic and genotypic variances and coefficient of variation.

The phenotypic and genotypic variance and coefficient of variations were calculated following the formula suggested by Singh and Chaundhar (1977) as:

$\sigma^2p = \sigma^2g + \sigma^2e$ Where, σ^2p = phenotypic variance

σ^2g = genotypic variance

σ^2e = environmental variance

$\sigma^2g = (MS_t - MS_e)/ r$ Where, MS_t = mean square of treatments

MS_e = mean square of error

r = number of replication

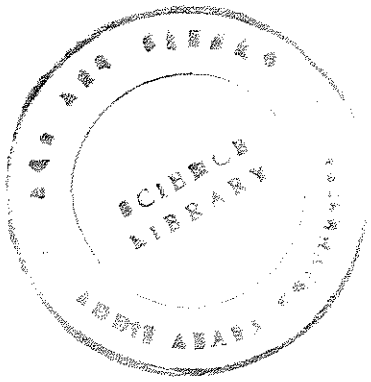
$PCV = [(\sigma^2_p)^{1/2} / \bar{X})] X 100$

Where, PCV = phenotypic coefficient of variation

$\bar{X}$ = population mean

$GCV = [(\sigma^2_g)^{1/2} / \bar{X})] X 100$

Where, GCV = genotypic coefficient of variation



b) Heritability and Genetic Advance

Broad sense heritability (H^2) was obtained according to Sing and Chaundhar (1977) by dividing genotypic variance by phenotypic variance:

$$H^2 = Vg/Vp.$$

Expected genetic advance was also computed by the formula:

$$GA = I X h^2 * (Vp)^{1/2} \text{ and}$$

$$GA (\% \text{ of mean}) = (GA \% X m) X 100\%$$

Where, m = mean value, Vp = phenotypic variance, Vg = genotypic variance, H^2 = heritability in broad sense, i = selection differential that varies depending upon the selection intensity and stands at 2.06 for selecting 5% of the genotypes.

c) Estimation of Correlation Coefficient

Correlation coefficient between two variables was estimated using the formula suggested by Falconer and Mackay (1996).

$$\text{Correlation coefficient (r)} = \frac{\sigma_{pxy}}{(\sigma^2_{px} X \sigma^2_{py})^{1/2}}$$

Where, σ_{pxy} = co-variance between traits X and Y

σ_{px} = variance of trait X

σ_{pxy} = variance of trait Y

The correlation coefficients between all possible pairs of traits were tested for their significance.

d) Analysis of Genetic similarity

In the present study a total of 17 quantitative morphological characters were used for cluster analysis. The quantitative data collected for each character were subjected to cluster analysis in order to quantify the variability among the genotypes. Dendrogram was constructed using STATISTICA (1984-2001) software based on Euclidian genetic distance by hierarchical clustering called unweighted pair group method with arithmetic average (UPGMA). An appropriate number of clusters were determined at a cut point of 5.0 Euclidian distances.

e) Estimate of Genetic Distance

Euclidian distance was used to measure the genetic distance between each genotype. The Euclidian dissimilarity measure was computed from raw quantitative traits using SPSS (SPSS, 1989-2001) for windows computer program for investigating the relative dissimilarity among genotypes.

3.4.2 RAPD Data Analysis

Representative genotypes, both for region and altitude, from each cluster obtained from quantitative data were taken for further random amplified polymorphic DNA (RAPD) analysis. For example, from the sixth cluster which contains ten individual genotypes, three genotypes were selected to represent the sub-clusters with in the main cluster.

3.4.2.1. Data Scoring

Following Lynch and Milligan (1994) assumptions, each amplified RAPD band was treated as an independent character of locus and assigned numbers in order of decreasing molecular weight. The size of each band was estimated using the 1 kb DNA molecular weight marker (100 base pair ladder). A band was scored as present (1) and absent (0). Based on Lynch and Milligan (1994) recommendation only reliably scored bands were used for the analysis

Estimation of Genetic variability

Genetic variation in total populations, between and within populations, across adaptation zones, between and within region of origin was analyzed using Nei's and Shannon diversity index,

which was computed using POPGENE (FRANCIS, 1997) software that was designed for population genetic analysis.

Average Nei's gene diversity (Hs) was estimated following the formula described as:

$$Hs = \sum(1 - \sum(P_i^2))/ n$$

The gene diversity in total population was also computed following the formula described by Nei(1975; 1976):

$$Ht = 1 - \sum(P_m^2)$$

Where "$1 - \sum(P_m^2)$ " is gene diversity for a given locus ; "$\sum(P_i^2)$)" is gene identity or homozygosity of a locus; "P_m" is mean frequency of 'n' loci ; "n" is the number of loci analyzed ;

The magnitude of genetic variation was estimated using the Shannon-weaver information measure described as

$$H' = \sum_{i=1}^{n} p_i \ln p_i$$

Where Pi = proportion of amplified bands among accessions in the i^{th} region of origin or i^{th} adaptation zone and n is the number of phenotypic classes for a character.

Partitioning of Genetic Diversity

Proportion of variation attributed to difference between and within populations was determined using the formula described by Nei (1975; 1976):

$$Dst = H_t - H_s$$

$$Gst = (Ht - Hs)/ Ht$$

$$Dm = Hs/ Ht$$

Where Dm = proportion of genetic variation within population or regions; Gst = proportion of genetic variation among population or regions; Hs = mean genetic variation of population or regions; Dst = mean gene diversity between population or regions; Ht = Gene diversity in the total population or region.

Estimation of Genetic Distance

The pair-wise genetic distance among populations and regions of origin was estimated using Nei's unbiased genetic distance (Nei, 1978) based on the allele frequency data matrix. The genetic distance among populations and regions of origin were estimated using the formula (Nei, 1978):

$$D = - \ln [G_{xy}/ (G_x G_y)^{1/2}]$$

Where G_x and are G_y the average of $(2n_x J_x - 1)/ (2n_x - 1)$ over the total loci studied, respectively, and $G_{xy} = J_{xy}$.

J_{xy}, J_x and J_y are the arithmetic mean of j_{xy}, j_x and j_y, respectively

$$j_{xy} = \sum X_i Y_i \qquad j_x = \sum X_i^2 \qquad \text{and,} \quad j_y = \sum Y_i^2$$

Where X_i Y_i represent the frequencies of the i^{th} allele in population X and Y

n_x and n_y = the number of individuals in X and Y, respectively.

3.5. Clustering of the Genotypes from RAPD Data

Cluster analysis was performed using POPGENE (FRANCIS, 1997) software by hierarchical method of clustering called un-weighted pair group method with Arithmetic Average (UPGMA) to estimate the relation among the accessions. Dendrogram was constructed among accessions based on their Nei's genetic distance between accessions of sorghum genotypes

4. RESULTS AND DISCUSSION

4.1. Morphological Diversity Based on Qualitative Traits

Qualitative data obtained from 26 genotypes, each containing 80 individual plants, was analyzed based on region of origin, adaptation zones and Shannon-weaver diversity index.

4.1.1. Regional Distribution of Qualitative Traits

The regional frequency distribution for the ten qualitative characters is presented in Table 6. Out of 26 accessions, nine (38.32%) had compact elliptic with goose peduncle which is in line with the result of Ayana (2001). They comprised seven accessions from Hararge and two from Wello. Most of the accessions from Wello mostly have semi-compact elliptic panicle shape followed by compact elliptic and semi loose panicles respectively. About half of the accessions from Hararge contained genotypes with compact elliptic panicle shape with goose peduncle. Compact elliptic panicle shape with goose peduncle was predominant in populations of the two regions. The local check was half broom corn. The number of awnless accessions was much greater (77.88%) than accessions with awn (22.12%).

The distribution of awn remains the same (i.e, percentage of accessions with awn are greater than awnless accessions with in the two region of origin) accessions for each region, awn less accessions being the predominant. The local check was awnless. The extent of the distribution for grain covered by glume remains almost equal for both the entire accessions and form the two regions origin. The local check was 75% covered with glume.

The most abundant glume color was white for the entire accessions followed by gray. White glume was more frequent followed by gray glume for accessions from Wello. For accessions

from Hararge white glume was the predominant followed by brown glume. The glume color for accession from test site was purple. Yellow seeded accessions were more frequent (36.7%) followed by red white seeded (36.4%) accessions for the entire accessions. The distribution of seed color remains the same for Wello and Hararge as of the total population. Ayana (2001) has also indicated that yellow seed color was more frequent among the accessions from Hararge and Wello. Almost all the accessions had dimple seeds and similar trend was found for all accessions from both regions, almost all (92%) of the accessions were with starchy endosperm texture following the same trend for the regions. In contrary to Ayana (2001) 60% of the accessions have no grain sub coat the remaining 40% have grain sub coat with the same pattern for the regions. Leaf midrib color distribution shows equal pattern with colorless midrib and yellow midrib color both for the entire accessions and for the two regions.

4.1.2. Distribution of Qualitative Traits Across Adaptation Zones

The compact elliptic panicles were most frequent in all adaptation zones (Table 7). In line with the regions, the distribution of awnless panicle (77%) was high for the entire accessions (Table 7). The distribution of 25% grain covering increases as one move from low altitude to high altitude areas. For the entire accession, 50% grain covering was evenly distributed in the three adaptation zones. White glume color was predominant for all adaptation zones. All the adaptation zones were predominantly occupied by dimple grains. Yellow and red grain color were equally distributed in all the accessions and white and yellow grain colors increase as one goes from low altitude to the high altitude adaptation zone.

Table 6. Percentage frequency distribution of qualitative morphological characters by region

Region	Leaf midrib color			Waxy bloom			Panicle shape & compactness						
	1	2	3	3	5	7	4	5	6	8	9	10	11
Hararge	52	2	46	23	50	27	0	4	15	13	52	15	0
Wello	46	0	54	0	67	33	4	4	23	31	27	10	0
Local check	100	0	0	0	100	0	0	0	0	100	0	0	0
All Population	51	1	48	12	60	29	2	4	18	21	38	13	4

Table 6.Continued

Region	Awn at maturity		Glume color						Grain covering			Seed color		
	0	1	1	2	3	46	5	6	0.25	0.5	0.75	1	2	3
Hararge	73	27	46	0	25	0	6	23	42	58	0	25	19	56
Wello	81	19	60	2	21	0	0	17	44	56	0	31	59	10
Local check	100	0	0	0	0	100	0	0	0	0	1	0	0	100
All Population	78	22	1	0	22	4	3	19	42	55	0	27	37	36

Table 6.Continued

Region	Grain plumpness		Grain sub-coat		Endosperm texture			
	1	2	0	1	1	2	3	4
Hararge	98	2	69	31	0	8	0	92
Wello	100	0	54	46	4	2	2	92
Local check	100	0	25	75	0	0	0	100
All Population	99	1	60	40	2	5	1	92

Table 7. Percentage frequency distribution of qualitative morphological characters by altitude

Altitude	Leaf midrib color			Waxy bloom			Panicle shape & compactness						
	1	2	3	3	5	7	4	5	6	8	9	10	11
Low altitude	45	2	52	27	59	14	0	5	21	16	34	16	9
Mid altitude	47	0	53	0	69	31	5	5	20	30	38	2	0
High altitude	71	0	29	0	41	59	5	5	20	30	38	2	0
All populations	51	1	48	12	60	29	2	4	18	21	38	13	4

See table 3. for the the representation of the numbers in each qualitative character.

Table 7.Continued

Altitude	Awn at maturity		Glume color						Grain covering			Seed color		
	0	1	1	2	3	6	7	8	0.25	0.5	0.75	1	2	3
Low altitude	77	23	33	0	32	9	0	26	34	56	9	18	30	52
Mid altitude	85	15	52	2	20	0	7	18	46	54	0	35	41	25
High altitude	64	36	87	0	5	0	0	8	49	51	0	31	44	26
All populations	78	22	51	1	22	4	3	19	42	55	4	27	37	36

Table 7.Continued

Region	Grain sub-coat		Endosperm texture				Grain plumpness	
	0	1	1	2	3	5	1	2
Low altitude	68	32	0	0	0	100	100	0
Mid altitude	43	57	5	10	0	85	98	2
High altitude	80	20	0	5	5	90	99	1
All populations	60	40	2	5	1	92	99	1

4.1.3. Estimate of Diversity

The estimates of Shannon Weaver diversity index 'H' by regions and adaptation zones for morphological qualitative characters are summarized and presented in Table 8. For the entire accessions mean, Shannon diversity index varied from 0.04 for grain plumpness to 0.99 for seed color with overall mean of 0.57. The diversity of leaf midrib color increases as one goes from high altitude to low altitude. Among all the characters, grain covering showed the highest variability (1.00) in all adaptation zones. Mean diversity index for seed color and grain sub-coat, 0.96 and 0.87, respectively, showed higher variability for all the adaptation zones. There was almost equal variability for all characters within adaptation zones, the mean of H' ranged from 0.57 for high altitude accessions and 0.62 for both mid and low-altitude accessions. It seems that the variability for the qualitative character states remain more or less the same for the three adaptation zones 0.04 for grain plumpness up to 0.99 for grain sub-coat. The variability of qualitative morphological characters between regions varied from 0.01 for the local check to 0.65 and 0.66 for Hararge and Wello, respectively. The diversity of seed color (0.99) contributed higher H' for between regions. It seems both Hararge and Wello have the same diversity index (0.65) for qualitative characters. Ayana (2001) has also discussed that 86% of the variation was found within region and the remaining 14% found between regions.

Table 8. Estimates of Shannon-Weaver diversity index, H', for ten qualitative morphological characters by region and adaptation zone

Altitude	LMC	WB	PSC	AM	GC	GRC	SC	GP	GSC	ET	Mean H'
Lowland	0.71	0.86	0.74	0.37	0.72	1	0.92	0	0.9	0	0.62
Mid Alt.	0.63	0.56	0.73	0.29	0.52	1	0.98	0.14	0.99	0.37	0.62
Highland	0.55	0.62	0.73	0.45	0.26	1	0.97	0.08	0.72	0.28	0.57
Mean	0.63	0.68	0.73	0.37	0.5	1	0.96	0.07	0.87	0.22	0.6
Std. Error of Mean	0.05	0.09	0	0.05	0.13	0	0.02	0.04	0.08	0.11	0.06
All	0.67	0.83	0.82	0.76	0.71	0.11	0.99	0.04	0.48	0.25	0.57
Region											
Harar	0.71	0.94	0.52	0.58	0.68	0.98	0.90	0.14	0.89	0.20	0.65
Wello	0.63	0.58	0.67	1.04	0.57	0.99	0.82	0.00	1.00	0.26	0.66
Test Site	0	-1	0	0	0	0	0	0	0.81	0.31	0.01
Mean	0.45	0.17	0.40	0.54	0.42	0.66	0.57	0.05	0.90	0.26	0.44
Std. Error of Mean	0.22	0.60	0.20	0.30	0.21	0.33	0.29	0.05	0.06	0.03	0.23
All	0.68	0.84	0.82	0.76	0.71	0.12	0.99	0.04	0.49	0.25	0.57

4.2. Morphological Diversity Based on Quantitative Traits

4.2.1. Mean, Range and Coefficient of Variation (CV)

Mean, range and coefficient of variation (CV) in agronomic traits are widely used to determine variations available in populations. The analysis in the present study for these values showed that there is high variability (CV > 20%) associated with both regions (Appenices 1) and altitude (Appendice 2) classes, except for the local check, for peduncle exertion, panicle width, number of primary branches per panicle, length of primary branches per panicle, grain yield per head and number of seeds per head. Regions and altitude classes with high coefficient of variation (CV > 20%) had high variability for the traits under consideration. These characters showed high variation with CV ranging from 20.09% for peduncle exertion in high altitude class to 58.95% for length of primary branches in the low altitude class and 20.70% for number of primary branches per panicle to 34.70% for grain yield per head in Hararge. Coefficient of variation of all these characters computed from all populations pooled together varied from 22.73% for peduncle exertion to 69.99% for length of primary branches per panicle.

Generally, least CV (< 10%) was observed for days to flowering, leaf width, leaf length and leaf sheath length in the two regions suggesting that there is no variability for these characters in both regions and these traits have no input as character of population selection. Least coefficient of variation was also observed for number of leaves per plant, leaf length, leaf width, leaf sheath length and days to flowering in low altitude class; leaf width, leaf area, leaf sheath length and days to flowering in the mid altitude class and; leaf sheath length, plant height, days to flowering in the high altitude class.

Populations from Hararge were characterized by high, number of leaves per plant, internode length, stalk diameter, plant height, days to flowering, panicle length, and hundred seed weight while low means for the other traits (Appenice 2). These populations were also characterized by high ranges (maximum - minimum) for most of the panicle characters (grain yield per head, length of primary branches, number of primary branches per panicle, panicle length), days to flowering and plant height.

For altitudinal classes highest mean was obtained for almost all traits in high altitude class while low mean for low altitude class. On the contrary, the range (Maximum-minimum) for most traits did not follow regular pattern in the altitudinal classes.

Analysis of variance (ANOVA) for quantitative morphological traits showed highly significant difference between sorghum genotypes except for leaf width (Table 9).

Table 9. Mean square for quantitative morphological traits[a] of sorghum genotypes as obtained from ANOVA

	d.f	NL	LL	LW	LA	LSL	IL	SD	PH	PE
Replication	3	2.5	82.6	1.2*	12063.42*	1.79	5.71	0.18	4094.90	0.39
Populations	25	10.15**	69.18**	0.52	6966.26*	8.49**	45.65**	0.21**	8128.61**	2.20**
Error	75	0.61	25.06	0.31	3557.82	0.81	5.39	0.07	602.12	0.20
CV (%)		11.06	7.85	6.77	12.62	7.07	14.73	13.94	15.07	22.73
Mean		15.60	77.98	9.21	539.41	23.26	26.45	2.32	333.79	3.67

[a]Refer to table 4 for the abbreviation of the characters.

* and ** Correlation is significant at the 0.05 level and 0.01 level respectively.

Table 9. Continued

	d.f	DF	PW	PL	HSW	NPBPP	LPBPP	GYPH	NSPH
Replication	3	32.35	5.43	2.46	0.19	22.60	1.56	752.11	606941.35
Populations	25	230.61**	122.99**	23.23**	0.93**	1608.95**	64.12**	1870.23**	1958772.35**
Error	75	12.18	2.93	3.99	0.09	40.79	1.55	313.36	308777.82
CV (%)		6.02	28.21	14.86	17.23	25.00	49.13	32.28	32.09
Mean		134.75	20.10	19.75	3.17	82.05	8.33	82.19	2640.84

[a]Refer to table 4 for the abbreviation of the characters.

* and ** Correlation is significant at the 0.05 level and 0.01 level respectively

4.2.2. Phenotypic and Genotypic Variance

The effectiveness of selection in any crop depends on the extent and nature of genotypic variability present in different agronomic traits of population (Arora, 1991).

Generally, genetic parameters including genotypic coefficient of variation (GCV), heritability (H^2) and genetic advance (GA) are prerequisite for genetic improvement of crops (Khorgade *et al.* 1985). Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) values < 10%, 10-20% and > 20% are considered to be low, intermediate, and high, respectively (Khorgade *et al.*, 1985). High genetic coefficient of variation indicates availability of high genetic variation. From the present study, there was a wide range of variation in both GVC and PVC for number of primary branches per panicle, length of primary branches per panicle, grain yield per head, panicle width and number of seeds per head. In addition, high PVC was observed for peduncle exertion. All other traits, except internode length (12.04), plant height (13.05), peduncle exertion (19.25) and panicle length which had medium value of GVC, had lower value (<10%) of GVC. In case of PVC, six traits were observed having medium PVC and for the remaining five traits lower value of PVC was observed (Table 10). The lower value of variation indicates that selection will not be effective for particular character because of the narrow genetic variability (Pandy and Tiwari, 1983; Arora, 1991). Selection for those traits with high variation (GCV > 20%) will be effective. Accordingly, selection for peduncle exertion, number of primary branches per panicle, length of primary branches per panicle, grain yield per head and number of seeds per head will be effective. Arora (1991), in his study in chick pea, indicated that moderately high PCV with low GVC suggests a relatively high environmental influence for traits and vice versa. In the present study, the PVC value for peduncle exertion PVC value was slightly higher than GCV implying

that, in addition to genetic factors, other factors such as environmental factors influence the variation. On the other hand, for grain yield per head and number of seeds per head the value of GCV was relatively much lower than the PVC values signifying that environmental factors contributed reasonable effect to estimating the variation for the traits.

4.2.3. Heritability and Genetic Advance

Information on heritability and genetic advance of yield attributing traits and their association with seed helps plant breeders to identify characters for effective selection (Misra, 1999). Heritability (H^2) is an important factor to determine the response of selection in breeding program. Its estimation is an important aspect of inheritance of quantitative traits as it indicates the genetic gains that may be achieved through selection (Pandey and Tiwari, 1983). The present study, summarized in Table 10, showed that all the traits had higher broad heritability values, except leaf width (14.41%), ranging from 30% - 91%. Selection based on traits with highest value of heritability such as panicle width (91.08%) number of primary branches per panicle (90.58%) and length of primary branches per panicle (91%) will be relatively successful. Generally, according to the results of Arora (1991) on his chick pea research, those traits with the highest heritability value were less affected by environmental factors. Therefore, traits with high heritability could be utilized in breeding program.

Highest broad heritability and genetic advance was observed for leaf area, plant height, number of primary branches and number of seeds per head, indicating that selection progress will be expected to be high for these traits. High heritability, coupled with high expected genetic gain, may result due to high additive gene effects and thus selection applied on such traits lead to yield improvement in the crop of study (Arora, 1991; Misra, 1991). On the contrary, for those

traits such as, number of leaves, leaf sheath length, peduncle exertion and hundred seed weight, with high value of heritability and low expected genetic gain selection is expected to be less effective due to the presence of non-additive gene action. In such traits, most of the variation is environmental, leading to low heritability and low expected genetic gain from selection and eventually results in low progress of selection.

Table10. Mean, genotypic (GCV) and Phenotypic (PCV) coefficient of variation, Genotypic (GV), Phenotypic (PV) and environmental (EV) variance, Heritability (H^2) and genetic gain (GG as % of mean) of quantitative traits of the sorghum genotypes

Traits*	Mean	CV	GV	PV	EV	GCV	PCV	H2	GA
NL	15.63	4.99	2.39	2.99	0.61	9.88	11.06	79.73	2.85
LL	77.76	6.42	11.03	36.09	25.06	4.27	7.73	30.57	3.78
LW	9.21	6.05	0.05	0.36	0.31	2.48	6.54	14.41	0.17
LA	533.94	8.33	943.48	2926.14	1982.66	5.75	10.13	32.24	35.93
LSL	23.26	3.88	1.92	2.73	0.81	5.96	7.11	70.26	2.39
IL	26.35	8.78	10.06	15.46	5.39	12.04	14.92	65.11	5.27
SD	2.33	10.03	0.03	0.09	0.05	7.95	12.81	38.56	0.21
PH	332.51	7.35	1881.64	2483.73	602.1	13.05	14.99	75.76	77.78
PE	3.67	12.33	0.5	0.7	0.2	19.25	22.85	70.99	1.23
DF	134.94	2.59	54.61	66.8	12.18	5.48	6.06	81.76	13.76
PW	20.11	8.52	30.01	32.95	2.93	27.25	28.55	91.09	10.77
PL	19.76	10.12	4.81	8.8	3.99	11.1	15.02	54.64	3.34
HSW	3.17	9.58	0.21	0.3	0.09	14.44	17.32	69.44	0.79
NPBPP	82.09	7.78	392.04	432.83	40.79	24.12	25.34	90.58	38.82
LPBPP	8.33	14.94	15.64	17.19	1.55	47.48	49.77	91.00	7.77
GYPH	82.22	21.54	389.22	702.57	313.35	23.99	32.24	55.40	30.25
NSPH	2642.34	21.04	412496.8	721278.7	308781.9	24.31	32.14	57.19	1000.54

* Refer to table 4 for the abbreviation of the characters.

4.3. Multivariate Analysis of Morphological Data

4.3.1. Analysis of Correlation Coefficient

Association among traits are useful for selecting genotypes possessing group of desired characters, although, such correlation coefficients could vary with genotypes studied and the environment where the test is carried out (Hadjichristoundolou, 1987). In the present study, phenotypic correlation coefficients for 17 quantitative characters were computed for the entire data (Table 11). The majority of the correlation coefficients were positively and highly significant. Highest and positively significant correlation was observed between number of seeds per head and grain yield per head ($r = 0.873$), leaf length and leaf area ($r = 0.772$) (Table 11). Ayana (2001) also reported significant correlation between leaf area and leaf length in sorghum accessions from the two regions.

Table 11. Correlation among seventeen quantitative[a] traits of sorghum genotypes

Character	NL	LL	LW	LA	LSL	IL	SD	PH	PE
NL	1	-0.04	0.055	.059(*)	0.026	-0.044	.303(**)	.379(**)	.081(**)
LL	-0.04	1	.089(**)	.722(**)	.100(**)	0.035	.141(**)	0.014	0.05
LW	0.055	.089(**)	1	.328(**)	-.088(**)	-.055(*)	.093(**)	-0.001	.059(*)
LA	.059(*)	.722(**)	.328(**)	1	.056(*)	-.060(*)	.218(**)	0.012	.074(**)
LSL	0.026	.100(**)	-.088(**)	.056(*)	1	.278(**)	0.051	.175(**)	.077(**)
IL	-0.044	0.035	-.055(*)	-.060(*)	.278(**)	1	-.086(**)	.270(**)	-0.014
SD	.303(**)	.141(**)	.093(**)	.218(**)	0.051	-.086(**)	1	.280(**)	.213(**)
PH	.379(**)	0.014	-0.001	0.012	.175(**)	.270(**)	.280(**)	1	0.033
PE	.081(**)	0.05	.059(*)	.074(**)	.077(**)	-0.014	.213(**)	0.033	1
DF	.288(**)	-0.025	-0.009	-0.028	.218(**)	.243(**)	.238(**)	.276(**)	.093(**)
PW	-.108(**)	.141(**)	0.038	.129(**)	-0.024	-.209(**)	-0.038	-.211(**)	-.092(**)
PL	0.026	-0.001	0.015	0.009	-0.008	-0.038	0.028	.162(**)	0.042
HSW	-0.044	0.023	-0.032	-.060(*)	-0.026	.095(**)	-.075(**)	.105(**)	-.178(**)
NPBPP	.107(**)	-0.021	-0.049	-.067(*)	-0.025	.166(**)	.106(**)	.265(**)	-.211(**)
LPBPP	-.139(**)	0.052	0.045	.100(**)	-0.024	-.302(**)	-.079(**)	-.313(**)	.069(*)
GYPH	-0.041	-0.017	0.038	0.005	-0.037	-.084(**)	0.004	.071(*)	0.026
NSPH	-0.054	0.006	.061(*)	.064(*)	-0.031	-.170(**)	0.026	-0.044	.115(**)

[a]Refer to table 4 for the abbreviation of the character.

Table 11ontinued

Character	DF	PW	PL	HSW	NPBPP	LPBPP	GYPH	NSPH
NL	.288(**)	-.108(**)	0.026	-0.044	.107(**)	-.139(**)	-0.041	-0.054
LL	-0.025	.141(**)	-0.001	0.023	-0.021	0.052	-0.017	0.006
LW	-0.009	0.038	0.015	-0.032	-0.049	0.045	0.038	.061(*)
LA	-0.028	.129(**)	0.009	-.060(*)	-.067(*)	.100(**)	0.005	.064(*)
LSL	.218(**)	-0.024	-0.008	-0.026	-0.025	-0.024	-0.037	-0.031
IL	.243(**)	-.209(**)	-0.038	.095(**)	.166(**)	-.302(**)	-.084(**)	-.170(**)
SD	.238(**)	-0.038	0.028	-.075(**)	.106(**)	-.079(**)	0.004	0.026
PH	.276(**)	-.211(**)	.162(**)	.105(**)	.265(**)	-.313(**)	.071(*)	-0.044
PE	.093(**)	-.092(**)	0.042	-.178(**)	-.211(**)	.069(*)	0.026	.115(**)
DF	1	-.223(**)	-.073(**)	-.125(**)	0.025	-.227(**)	-.218(**)	-.196(**)
PW	-.223(**)	1	-0.008	-.082(**)	-0.029	.435(**)	.112(**)	.212(**)
PL	-.073(**)	-0.008	1	.126(**)	.194(**)	-0.055	.354(**)	.293(**)
HSW	-.125(**)	-.082(**)	.126(**)	1	.245(**)	-.267(**)	.253(**)	-.115(**)
NPBPP	0.025	-0.029	.194(**)	.245(**)	1	-.352(**)	.161(**)	0.005
LPBPP	-.227(**)	.435(**)	-0.055	-.267(**)	-.352(**)	1	0.04	.266(**)
GYPH	-.218(**)	.112(**)	.354(**)	.253(**)	.161(**)	0.04	1	.873(**)
NSPH	-.196(**)	.212(**)	.293(**)	-.115(**)	0.005	.266(**)	.873(**)	1

Refer table 3 for to the abbreviation of the characters

* and ** Correlation is significant at the 0.05 level and 0.01 level respectively

4.3.2. Cluster Analysis

The clustering using normalized quantitative data (Figure 7) revealed two major clusters where one genotype represents the first cluster and the remaining genotypes in the second cluster. However, when 5.0 Euclidean genetic distances was taken as a cutting point for clustering, there were six clusters. The first and the second cluster consist of only one genotype each. The third cluster contains six genotypes. While the fourth and the fifth cluster contains four genotypes each. The sixth cluster contains the, remaining ten genotypes. The pattern of clustering in the sixth cluster reveals that the genotypes tend to group based on their region of origin and adaptation zones. As seen from Figure 7, the sub-clusters within cluster six contain materials from the same region of origin and adaptation zone (for instance in the third sub-cluster low altitude adapted genotypes from Hararge were grouped together). Generally the genotypes with in the cluster fail to group either based on their region of origin or adaptation zones.

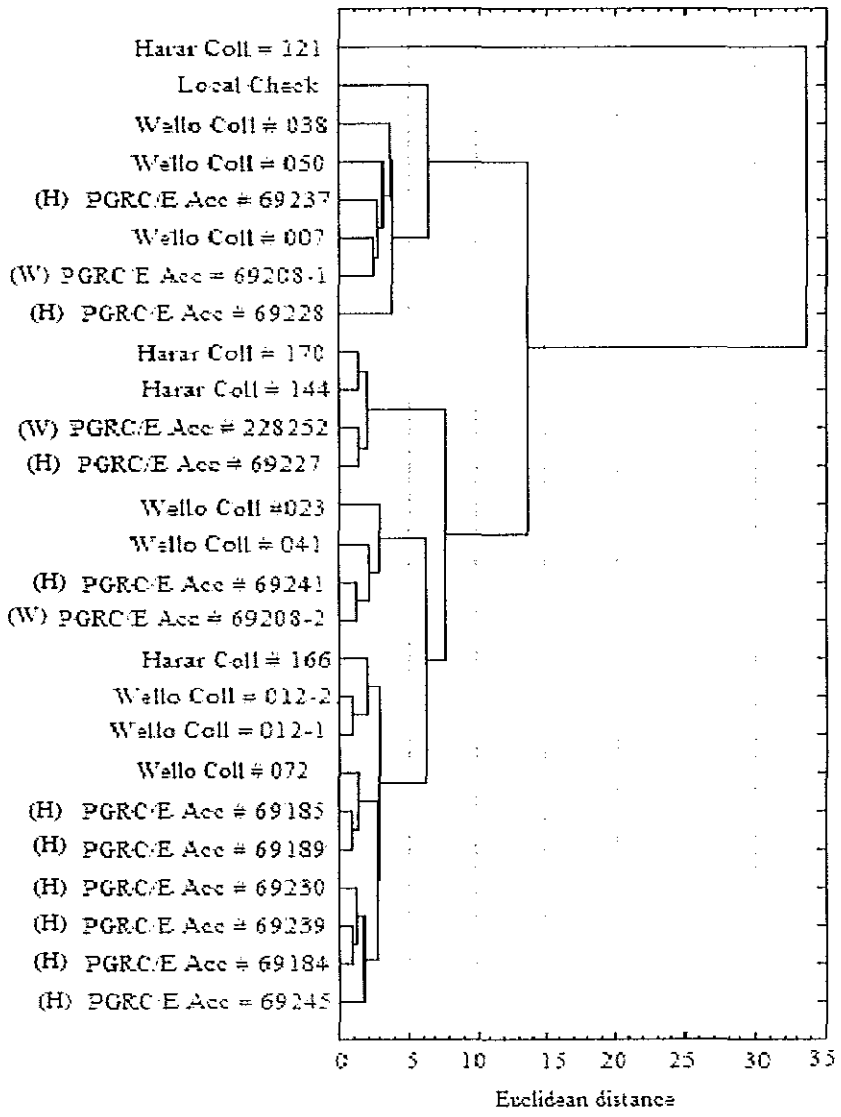


Figure 7. Dendrogram showing the clustering pattern of 26 sorghum genotypes from quantitative morphological data. The letters attached with the accessions represent the regions of origin for each accession ('H' stand for Hararge and 'W' for Wello).

4.4. RAPD Data Analysis

4.4.1. DNA Polymorphism

Six oligoncleotide primers were first screened using a sample of five sorghum accessions. Four primers were selected for generating reproducible polymorphic bands (OPA-02, OPA-018, OPC-01, and OPA-013). These four primers generated a total of 55 RAPD bands, all of which were polymorphic across 70 individual samples from fourteen genotypes studied (Table 12 A band (locus) was considered as polymorphic if the band differentiated at least any, two of the 14 accessions. The number of amplification products per primer varied from 13 to 19 with a mean of 16. The size of the amplified DNA fragments ranged from 100 to 2200 base pairs. In his study on genetic diversity of sorghum, Ayana *et al* (2000) has also indicated that, the number of polymorphic band per primer varied from 8 to 12 with amplified DNA fragment ranging from 400 to 4200 base pairs. The RAPD band profiles obtained with OPA-18 and OPC-01 is shown in Figure 8. The proportion of polymorphic loci and average heterozygosity (Gene diversity) per locus are used for measuring genetic variation for a population. A locus is defined as polymorphic if any of the individual plant lacks it (Ayana, 2001). Summary of DNA polymorphism and number of polymorphic band of sorghum genotypes is presented in table 15.

The lowest polymorphism percent was recorded for PGRC/E Acc # 69245, Harar Coll # 121, and Harar Coll # 166 with no polymorphism to 20 % for Harar Coll # 166. On the other hand, Ayana (2001) has reported the percent of polymorphic bands varied from 39 to 69 % for sorghum populations he studied. This implies that there was low percent of polymorphism for all genotypes used for this study.

Regional number of polymorphic bands for the entire sorghum population varied from 41 to 46 for Wello and Hararge regions, respectively (Table 12. Percent polymorphism ranged from 49.49 % for Wello to 77.97% for Harage regions and 93.22% for the total population. Ayana (2001) also reported 48 to 100 % regional percent of polymorphic bands. The number of polymorphic bands found to vary from 20 to 51 for low altitude genotypes and intermediate genotypes, respectively. Percentage polymorphism across adaptation zones ranged from 47.46% for low altitude to 86.44% for both intermediate and high altitude adaptation zone.

Table 12 Number and proportion of polymorphic loci and mean genetic diversity with their standard error of mean four the 14 sorghum genotypes in population

Accession Number	Number of Polymorphic loci	Percent of Polymorphic loci	Shannon Index ± S.E	Nei's genetic Diversity ± S.E
PGRC/E Acc # 69245	0.0	0.00	0.00 ± 0.00	0.00 ± 0.00
PGRC/E Acc # 69208-1	2.0	3.39	0.01 ± 0.01	0.01 ± 0.02
PGRC/E Acc # 69228	1.0	1.69	0.01 ± 0.02	0.01 ± 0.02
PGRC/E Acc # 228252	3.0	5.08	0.01 ± 0.02	0.02 ± 0.02
PGRC/E Acc # 69185	4.0	6.78	0.02 ± 0.02	0.03 ± 0.03
PGRC/E Acc # 69237	2.0	3.39	0.02 ± 0.02	0.02 ± 0.03
PGRC/E Acc # 69241	4.0	6.78	0.02 ± 0.02	0.03 ± 0.03
Wello Coll # 012-1	0.0	0.00	0.00 ± 0.00	0.00 ± 0.00
Harar Coll # 121	0.0	0.00	0.00 ± 0.00	0.00 ± 0.00
Harar Coll # 166	12.0	20.34	0.06 ± 0.03	0.09 ± 0.05
Wello Coll # 038	3.0	5.08	0.01 ± 0.01	0.02 ± 0.02
Wello Coll # 041	3.0	5.08	0.01 ± 0.02	0.02 ± 0.03
Wello Coll # 072	7.0	11.86	0.05 ± 0.04	0.07 ± 0.05
Local check (Rufie)	3.36	5.69	0.02 ± 0.02	0.03 ± 0.03
Mean ± S.E	3.17 ±0.30	5.37 ± 0.31	0.26 ± 0.04	0.41 ± 0.06
All population	55.0	93.22	0.26 ± 0.04	0.41 ± 0.06

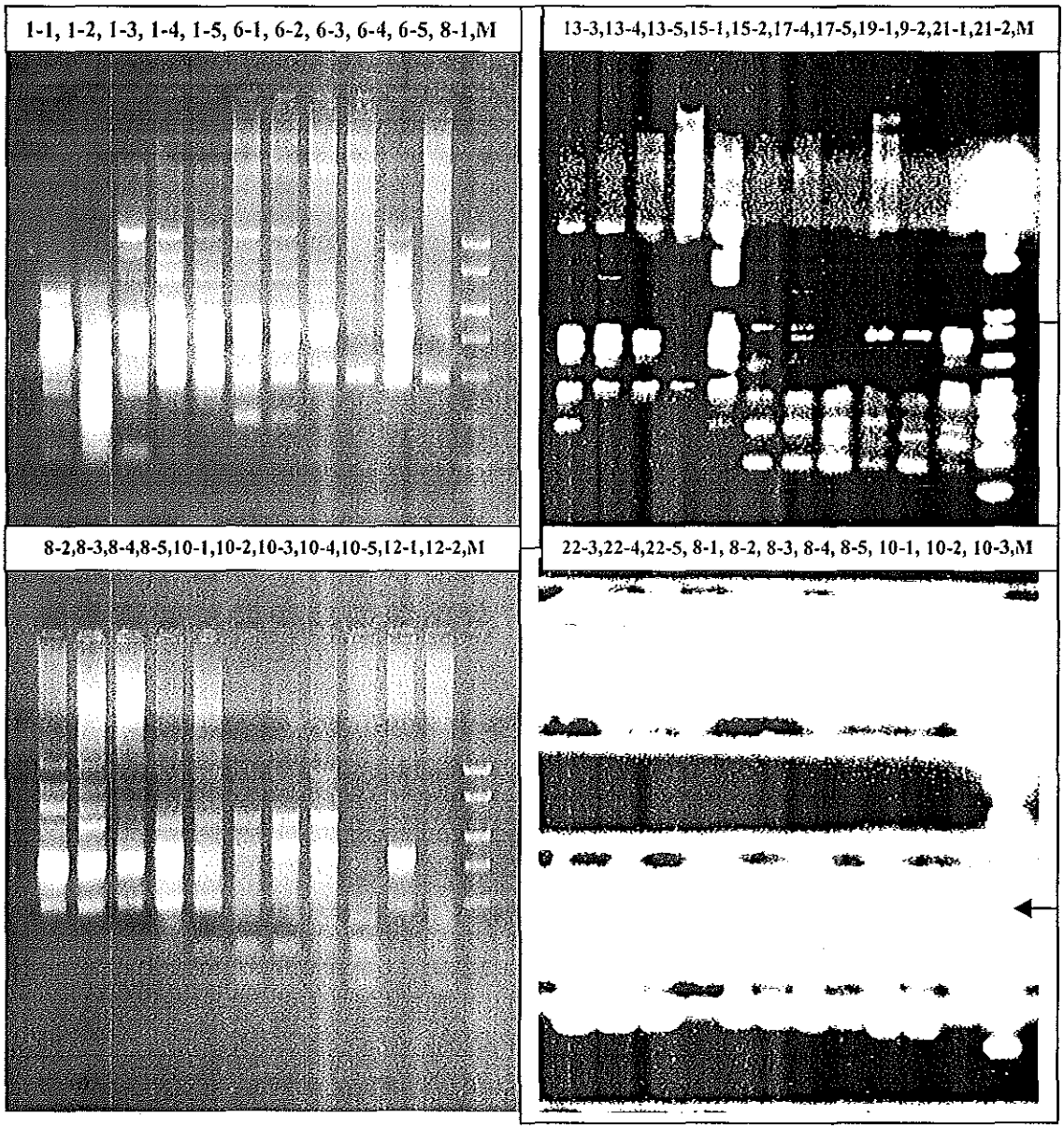


Figure 8. Amplification products from genomic DNA of sorghum accessions used for RAPD study amplified with primer OPA-18 and OPC-01.The molecular weight marker (1Kb DNA ladder) is shown in the right lane (M) the arrow indicates 850 bp band and the lane numbers indicate accessions and individual plants of each accession refer table 2 for the genotypes represented by each lane number : Lane No. 1-1, 1-2, 1-3, 1-4, 1-5, 6-1, 6-2, 6-3, 6-4, 6-5, 8-1,M-with OPA-18; Lane No. 8-2,8-3,8-4,8-5,10-1,10-2,10-3,10-4,10-5,12-1,12-2,M; Lane No. 13-3,13-4,13-5,15-1,15-2,17-4,17-5,19-1, 9-2,21-1,21-2,M with OPA-18 and Lane 22-3,22-4,22-5,8-1,8-2,8-3,8-4,8-5,10-1,10-2,10-3,M with OPC-01.

4.4.2. Estimate of Genetic Diversity

I) Population Genetic Diversity

Nei's unbiased genetic diversity for the whole population varied from 0.0 to 0.09 with a mean of 0.41 ± 0.06 while that of Shannon information index varied from 0.0 to 0.06 with mean of 0.26 ± 0.04 implying that there was low variation in the sorghum genotypes Table 12 The genetic diversity estimated from overall entire data pooled together was 0.41 ± 0.06 and 0.26 ± 0.04 for Nei's and Shannon diversity, respectively. There was no gene diversity observed for PGRC/E Acc # 69245, Harar Coll # 121 and Harar Coll # 166 for both diversity indexes. Nei's and Shannon information index was relatively high for accessions PGRC/E Acc # 69208-1, local check (Rufie) and Wello Coll # 038 as compared to other sorghum populations implying that there was relatively higher variability for these genotypes Table 12 Ayana *et al.* (2000) has also reported the genetic variation of sorghum, which was assessed with Shannon-Weaver diversity index, varied from 0.2 to 0.47 with an average of 0.37.

II) Regional Gene Diversity.

The overall mean of Nei's genetic diversity and Shannon index of the two regions was 0.24 ± 0.13 and 0.36 ±0.19, respectively (Table 13). Shannon versity index varied from 0.32 ± 0.20 for Wello to 0.39 ± 0.18 for Hararge region, while that of Nei's diversity ranged from 0.22 ± 0.14 for Wello to 0.26 ± 0.13 for Hararge region indicating that Hararge has relatively higher genetic diversity than Wello for sorghum genotypes. Generally, Hararge showed the higher gene variability for both Shannon information index and Nei's gene diversity. The diversity index obtained from RAPD data was lower than genetic diversity estimated from Shannon diversity from qualitative morphological data. Different results and reviews indicated

disagreement between morphological and RAPD for some crops (Gepts, 1995; Dullo *et al.*, 1997; Person *et al.*, 2000) as well as agreement between the two markers for some other crops or plants (Gepts, 1995; Dullo *et al.*, 1997; Ruiz *et al* 1997; Aga *et al.*, 2004). These different methods sample genetic variation at different level and hence, differ in their power of genetic resolution as well as the quality of the information they generate (Ruiz *et al.,* 1997).

III) Genetic Diversity Across Adaptation Zones

The overall mean of Nei's genetic diversity and Shannon index of the three adaptation zones was 0.22 ± 0.12 and 0.32 ± 0.18, respectively (Table 14). Shannon diversity index varied from 0.25 ± 0.17 for lowland to 0.41 ± 0.14 for intermediate, while that of Nei's diversity ranged from 0.17 ± 0.12 for lowland to 0.22 ± 0.10 for intermediate indicating that populations adapted to mid-altitude have relatively higher genetic diversity than low-altitude adapted sorghum populations. Generally, populations from mid-altitude showed the higher genetic variability for both Shannon information index and Nei's gene diversity.

Table 13 Regional gene diversity, number of polymorphic bands and percent of polymorphic bands of fourteen sorghum genotypes computed from RAPD data.

Region	Number of Polymorphic loci	Percent of Polymorphic loci(%)	Diversity Indexes	
			Nei's ± S.E	Shannon's ± S.E
Hararge	46	77.97	0.26 ± 0.13	0.39 ± 0.18
Wello	41	69.49	0.22 ± 0.14	0.32 ± 0.20
Mean ± S.E	44 ± 0.08	73.73 ± 0.32	0.24 ± 0.13	0.36 ±0.19
All population	55	93.22	0.26 ± 0.12	0.41 ± 0.29

Table 14 Gene diversity, number of polymorphic bands and percent of polymorphic bands of fourteen sorghum genotypes by adaptation zones computed from RAPD data

Adaptation Zone	Number of Polymorphic loci	Percent of Polymorphic loci	Diversity Indexes	
			Nei's ± S.E	Shannon's ± S.E
Lowland	28	47.46	0.17 ± 0.12	0.25 ±0.17
Intermediate	51	86.44	0.27 ± 0.10	0.41 ± 0.14
Highland	30	86.44	0.22 ± 0.13	0.31 ± 0.96
Mean ± SE	36.3 ± 3 7.36	61.58 ± 12.99	0.22 ± 0.12	0.32 ± 0.18
All population	55	93.22	0.26 ± 0.12	0.41 ± 0.29

IV) Partitioning of Genetic Diversity

The genetic variability in the total population (Ht) was analyzed by Nei's (Nie, 1975) method. The gene diversity in the total population (Ht) was divided into genetic diversities within and between subpopulations (genotypes), signified by Hs and Dst (Dst = Ht-Hs), respectively. The relative and absolute magnitude of gene differentiation among genotypes was measured by Gst (Gst = Dst/Ht) and Dm (Dm = Hs/Ht), respectively. Analysis of gene diversity in 14 sorghum genotypes for 59 loci is presented in Appendix 3

Locus OPA-18 had the maximum gene diversity in total population (0.50), followed by OPC01-5(0.498), OPA02-3(0.494) and OPA13-3 with a value of (0.49), at which there were high effective number of alleles. The minimum Ht was 0.0 at locus OPA18-14, OPA18-15, OPC18-17, and OPC01-13. These loci were stable and had few effective number of alleles. Gst ranged from 0.092 (OPA18-10) to 1.000 (for 17 loci, Appendix 3) for almost all loci above 0.500, indicating that gene diversity at a single locus is mainly contained between populations than within population. Average Gst calculated from 59 RAPD loci was 0.929, and revealed 7.10% and 92.9% of gene diversity contained within and among populations, respectively. Average Dm from 59 loci was 0.071, meaning that an estimate of the net gene codon differences between populations and independent of the gene variability within population was 0.071.

Analysis of genetic variability in region of origin and adaptation zones in the 14 sorghum genotypes is presented in Table15. Mid-altitude adaptation zone had the maximum gene diversity in total population (0.265), followed by high-altitude, at which there were high

percent of polymorphic loci. The minimum Ht was 0.169 at low-altitude adaptation zone. The minimum Gst (0.880) was recorded in the low-altitude adaptation zone and the maximum Gst (0.930) from the high-altitude adaptation zone, both the minimum and maximum above 0.500, indicating that gene variability at a one adaptation zone is mainly contained between genotypes. In case of the two regions of origin, Hararge and Wello, Ht observed was 0.257 and 0.215, respectively. The respective Gst for the two regions of origin was also 0.969 and 0.881, having the same implication as that of adaptation zones.

Generally, such results imply that there is high genetic variation between the populations and regions than within populations and regions of origin. This result suggests that sampling from few sites for breeding or conservation may not capture large proportion of variation within sorghum populations. In other words, for breeding or conservation purposes, one has to sample sorghum genotypes in a large number of sites to effectively represent the variations present between populations.

Table 15. Partitioning of genetic diversity into within and between populations and regions of sorghum genotypes

	Partitions of Nei's genetic variation				
Region	Ht	Hs	Gst	Dst	Dm
Hararge	0.257	0.008	0.969	0.249	0.031
Wello	0.215	0.026	0.881	0.190	0.119
Mean	0.236	0.017	0.925	0.219	0.075
Adaptation Zon					
Low-altitude	0.169	0.020	0.880	0.149	0.120
Mid-altitude	0.265	0.020	0.927	0.246	0.073
High-altitude	0.218	0.015	0.930	0.203	0.070
Mean	0.218	0.018	0.912	0.199	0.088
All Population	0.261	0.019	0.929	0.243	0.071

Where: Ht = total gene diversity in the population or regions; Hs = Mean gene diversity within region or population; Gst = gene diversity between population or region; Dm = Proportion of gene diversity within population or region; and Dst = Proportion of gene diversity between population or region.

4.4.3. Clustering of sorghum Genotypes from RAPD Data

The result of clustering obtained from RAPD data of sorghum genotypes is different from cluster obtained from morphological data (Figure 9). However, the cluster from molecular data showed that there was such a pattern of clustering in the sorghum genotypes based on their region of origin than their adaptation zones. But in the case of cluster based on morphological data genotypes fail to cluster either based on their center of origin and adaptation zones

As observed from UPGMA dendrogram constructed using Nei's genetic distance (Nie, 1978), there are three major clusters at the Euclidean distance of 16.0 where the two major clusters further sub-clustered at varying genetic distance. In the first cluster, there is only one genotype; PGRC/E Acc # 69241, which is from Hararge adapted to intermediate altitude adaptation zone. This accession had high genetic distance with Harar Coll # 121 (0.67), with PGRC/E Acc # 69228 (0.54), with PGRC/E Acc # 69245(0.51), and with Acc # 69185 (0.51) which were higher than the mean genetic distance (0.25). Even the lowest genetic distance (0.33) with Harar Coll # 166 was higher than the mean genetic distance.

The second cluster contains five genotypes representing the three regions (including the genotype from the test site) and the three adaptation zones. This cluster also sub-clustered into two at 16 Nei's genetic distance where three accessions from Wello and one from the test site were included. The accessions represent the three adaptation zones.

The third cluster was the one containing the higher number of genotypes which were more or less clustered to represent their center of origin but not adaptation zones. These accessions were sub-clustered into four at 9.00 Nei's genetic distance where the clustering based on their center of origin than adaptation zone was clearly reflected

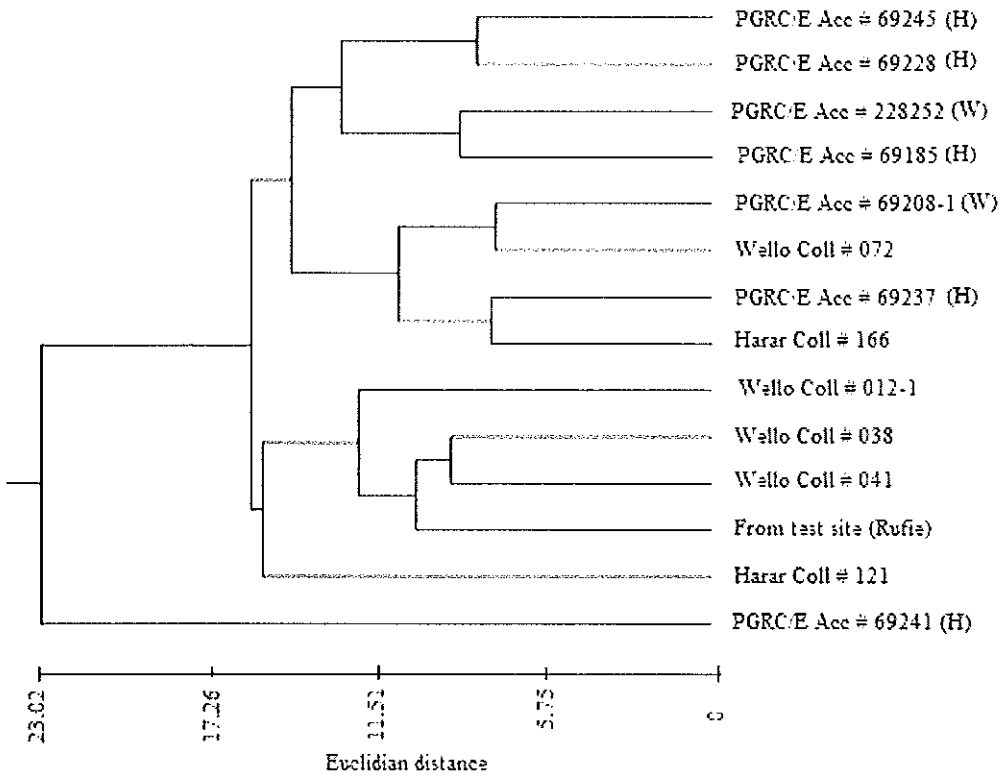


Figure 9. Dendrogram of fourteen sorghum genotypes from RAPD data. The letters attached with the accessions represent the regionof origin for each accession ('H' stand for Hararge and 'W' for Wello)

4.4.4. Estimation of Genetic Distance and Genetic Identity

The pair-wise genetic distances calculated among the 14 sorghum genotypes based on Nei's genetic distance varied from 0.149 to 0.665 with a mean of 0.25 (Table 16) .The higher Pair-wise genetic distances were observed for PGRC/E Acc # 692418 with the other genotypes such as Harar Coll # 121, PGRC/E Acc # 69228, and PGRC/E Acc # 69245. The lowest pair wise genetic distance (0.15) was obtained between PGRC/E Acc # 69208-1 and Wello Coll # 072,

which are under the third cluster. Generally, the pair wise genetic distance follows the pattern of clustering from UPGMA dendrogram constructed using Nei's genetic distance.

Most of sorghum genotypes had high genetic identity varying from 0.515 to 0.862 with an over all mean of 0.8075 (Table16). Some genotypes had high genetic identity. For instance, genetic identity between PGRC/E Acc # 69208-1 and Wello Coll # 072, PGRC/E Acc # 69245 and PGRC/E Acc # 69208-1, PGRC/E Acc # 228252 and Acc # 69185 is high (Relatively higher than the mean). Relatively low genetic identity was observed between PGRC/E Acc # 692418 and Harar Coll # 121, PGRC/E Acc # 69228 and PGRC/E Acc # 692418, PGRC/E Acc # 69245 and PGRC/E Acc # 692418, PGRC/E Acc # 69185 and PGRC/E Acc # 692418. Generally, genotypes with higher genetic identity tend to cluster together and vice versa. It was observed that genetic distance is the mirror reflection of genetic identity.

Table16. Nei's Unbiased Measures of Genetic Identity and Genetic distance with Nei's genetic identity (above diagonal) and genetic distance (below diagonal) for fourteen sorghum genotypes.

Pop ID*	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	****	0.7818	0.8505	0.7739	0.8288	0.7182	0.6000	0.6502	0.6780	0.6949	0.7431	0.6457	0.7248	0.7222
2	0.2462	****	0.7506	0.7883	0.8017	0.8573	0.6643	0.7498	0.7722	0.7739	0.7176	0.7588	0.8620	0.7685
3	0.1619	0.2868	****	0.7218	0.7768	0.7000	0.5814	0.7349	0.7278	0.7108	0.7210	0.6957	0.7409	0.7814
4	0.2563	0.2379	0.3260	****	0.8404	0.7638	0.6829	0.7209	0.7569	0.6717	0.7428	0.6528	0.7702	0.7884
5	0.1878	0.2211	0.2526	0.1739	****	0.8193	0.6003	0.7389	0.7129	0.7264	0.7771	0.6770	0.8255	0.7703
6	0.3309	0.1540	0.3567	0.2695	0.1993	****	0.7225	0.7914	0.6934	0.8596	0.6637	0.7298	0.8226	0.7073
7	0.5108	0.4089	0.5424	0.3814	0.5104	0.3250	****	0.6873	0.5145	0.7026	0.6066	0.6276	0.6293	0.6169
8	0.4304	0.2880	0.3080	0.3273	0.3026	0.2339	0.3751	****	0.7396	0.7405	0.7563	0.7907	0.8129	0.8044
9	0.3887	0.2585	0.3177	0.2786	0.3384	0.3662	0.6646	0.3017	****	0.6441	0.6826	0.7614	0.7886	0.7552
10	0.3640	0.2563	0.3413	0.3979	0.3196	0.1513	0.3529	0.3004	0.4400	****	0.6508	0.7175	0.7715	0.6681
11	0.2970	0.3318	0.3271	0.2974	0.2522	0.4100	0.4999	0.2793	0.3819	0.4296	****	0.8354	0.7746	0.8198
12	0.4374	0.2760	0.3628	0.4265	0.3900	0.3150	0.4658	0.2348	0.2726	0.3320	0.1799	****	0.7983	0.8118
13	0.3219	0.1485	0.2999	0.2611	0.1918	0.1952	0.4631	0.2072	0.2375	0.2594	0.2555	0.2253	****	0.8075
14	0.3255	0.2633	0.2466	0.2377	0.2610	0.3463	0.4831	0.2177	0.2808	0.4034	0.1987	0.2085	0.2138	****

* 1= PGRC/E Acc # 69245, 2= PGRC/E Acc # 69208-1, 3= PGRC/E Acc # 69228 4 = PGRC/E Acc # 228252, 5 = PGRC/E Acc # 69185,6 = PGRC/E Acc # 69237 ,7 = PGRC/E Acc # 692418, 8 = Wello Coll # 012-1, 9= Harar Coll # 121, 10 = Harar Coll # 166 ,11 = Wello Coll # 038 , 12 = Wello Coll # 041 , 13= Wello Coll # 072, 14 = From test site (Rufie).

5. CONCLUSION

From the present study of sorghum genetic diversity the following conclusion remarks are made:

1) The sorghum genotypes studied showed a wide range of variation for both quantitative and qualitative traits with some of the traits such as panicle compactness and shape, having adaptive significance in region of origin and adaptation zones distribution.

2)Percentage frequency of qualitative traits across region and adaptation zone was so variable that it is ranging from being absent to high percentage.

3) Shannon diversity index for the entire genotypes studied showed low variation for grain plumpness to high variability for grain covering.

4) More than three-fourth of the variation for Shannon diversity index was found within regions and the remaining between regions.

5) Highly significant variability (CV> 20%) was associated with both for regions and altitude classes for peduncle exertion, panicle width, number of primary branches per panicle, length of primary branches per panicle grain yield per head and number of seeds per head.

6) For altitude classes, higher mean was obtained for almost all traits in high altitude classes while low mean for low altitude classes.

7) Traits with high genotypic variation such as peduncle exertion, number of primary branches per panicle, grain yield per head and number of seeds per head will have high input as selection traits for further breeding.

8) High heritability and genetic advance was observed for leaf area, plant height, number of primary branches and number of seeds, implying that selection progress will be high for these traits.

9) Positive and significant correlation was observed between number of seeds per head and grain yield per head, leaf length and leaf area.

10) The variability obtained from morphological data was within region and adaptation zones. But, the variability from RAPD data was between region of origin and adaptation zones.

11) The cluster analysis from molecular data in general failed to arrange genotypes either based on their region of origin or altitude classes. Morphological data relatively tend to arrange genotypes based on their region of origin.

12) Partitioning of genetic variation from RAPD data indicated that there is more variability between populations and regions than within population and region of origin. This result suggests that one has to sample sorghum genotypes in a large number of sites to effectively represent the variation present between populations and between regions.

13) The genetic distance and identity recorded among genotypes from RAPD data were so variable that genotypes with higher genetic distance and low genetic identity tend to come

from different regions of origin. This implies that the relative differentiation is found to be higher between regions of origin than within regions and adaptation zones.

The entire above conclusion was derived from results of studies conducted at one location and RAPD markers. For effective utilization of sorghum genotypes for breeding and conservation purposes, it is essential to assess such variation in breeding environments as well as additional molecular markers.

6. RECOMMENDATIONS

This study was conducted on limited number of accession from the Long Cycle Sorghum National Variety Trial (LCSNVT) genetic stocks of sorghum improvement program of MARC. Comprehensive study of a range of long maturing sorghum genotypes grown in different parts of Ethiopia is suggested in order to enhance their effective utilization by national and regional breeding programs.

Clustering from this study provides preliminary structure for further germplasm collection for genetic breeding or agronomic studies on long maturing sorghum genotypes where information on multiple quantitative traits is required.

Knowledge of genetic diversity among these long maturing sorghum populations and its quantitative assessment helps breeders in choosing desirable parents for breeding program as selection of parents on the basis of divergence analysis would be more effective.

This study provides specific information for sorghum breeders on which long maturing sorghum genotypes to concentrate for further crossing and hybrid development

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8. LIST OF APPENDICES

Appendice 1. Mean, range, and coefficient of variation (CV) of altitude groups* of quantitative traits in different adaptation zone.

Altitude		NL	LL	LW	LA	LSL	IL	SD	PH	PE
Low Altitude	Mean	15.56	76.25	9.22	531.15	22.72	25.39	2.25	329.18	3.55
	CV (%)	8.43	6.6	7.87	14.22	6.4	14.96	11.49	16.01	29.04
	Minimum	12.05	62.19	8.3	436.53	20.14	17.38	1.2	221.29	1
	Maximum	19.29	83.86	13.29	920.25	25.86	32	2.63	495.19	4.95
Mid Altitude	Mean	15.26	79.56	9.05	536.7	23.19	26.91	2.28	327.33	3.69
	CV (%)	13.15	5.9	5.76	9.16	7.15	14.35	12.8	16.84	15.59
	Minimum	10.57	69.43	8.12	460.15	18.52	17.38	1.47	215.62	2.29
	Maximum	19.24	89.4	10.45	650.53	26.81	36.67	2.79	415.62	4.48
High Altitude	Mean	16.38	78.59	9.48	563	24.61	27.86	2.55	356.87	3.87
	CV (%)	10.73	11.92	5.04	14.31	5.16	13.37	16.14	6.28	20.09
	Minimum	13.48	66.76	8.35	420.4	22.05	19.29	2.06	317.86	2.19
	Maximum	21.81	111.57	10.49	814.07	26.43	33.05	3.95	400.29	5
Total	Mean	15.66	78.03	9.19	621.46	23.26	26.76	2.71	337.26	3.63
	CV (%)	10.23	5.43	3.90	69.99	6.39	11.40	34.47	12.55	20.24
	Minimum	11.82	68.79	8.63	471.09	20.44	19.30	1.89	227.36	1.35
	Maximum	18.55	87.12	9.90	2701.41	26.15	30.87	5.33	401.54	4.42

Appendice 1. Continued

Altitude		DF	PW	PL	HSW	NPBPP	LPBPP	GYPH	NSPH
Low Altitude	Mean	132.66	19.56	19.81	3.16	76.58	8.88	84.88	2784.14
	CV (%)	5.06	23.53	11.10	19.01	30.72	58.95	25.57	30.99
	Minimum	118.00	12.90	16.52	1.63	21.95	4.88	49.34	1474.75
	Maximum	143.43	28.86	25.05	4.09	135.00	25.67	142.23	4800.91
Mid Altitude	Mean	135.86	20.91	19.55	3.11	82.35	8.30	75.34	2447.90
	CV (%)	6.37	27.71	18.87	13.50	19.61	34.80	36.09	32.97
	Minimum	119.24	14.38	12.52	2.09	56.57	5.07	31.51	1093.08
	Maximum	150.48	36.24	29.90	3.82	118.57	15.80	139.10	4241.81
High Altitude	Mean	137.15	19.65	20.01	3.30	93.50	7.18	89.97	2711.48
	CV (%)	6.60	37.79	13.85	19.38	18.11	42.97	36.09	31.70
	Minimum	124.10	13.30	13.59	2.10	62.71	4.17	42.44	1584.92
	Maximum	152.71	35.05	24.52	4.44	129.00	13.33	139.11	4320.60
Total	Mean	134.75	20.10	19.75	3.17	82.05	8.33	82.19	2640.84
	CV (%)	6.02	28.21	14.86	17.23	25.00	49.13	32.28	32.09
	Minimum	118.00	12.90	12.52	1.63	21.95	4.17	31.51	1093.08
	Maximum	152.71	36.24	29.90	4.44	135.00	25.67	142.23	4800.91

Refer to table 4 for the abbreviation of the characters.

Appendice 2. Mean, range and coefficient of variation (CV) by Region groups of quantitative traits* from the entire data

Region		NL	LL	LW	LA	LSL	IL	SD	PH	PE
Harar	Mean	16.19	77.27	9.22	530.57	23.67	27.09	2.40	340.14	3.84
	CV	10.63	7.06	7.78	10.30	6.92	11.28	12.16	12.54	18.06
	Minimum	12.33	62.19	8.30	420.40	20.71	21.33	1.87	222.38	1.00
	Maximum	21.81	89.40	13.29	694.35	26.81	36.67	3.95	495.19	4.71
Wello	Mean	14.98	78.99	9.16	548.89	22.73	26.34	2.23	333.59	3.36
	CV	10.16	8.89	5.45	15.27	6.59	16.24	15.77	16.30	27.16
	Minimum	10.57	66.76	8.12	444.25	18.52	17.38	1.20	215.62	1.00
	Maximum	17.52	111.57	10.49	920.25	24.76	33.10	2.95	414.05	5.00
	Range	6.95	44.81	2.37	475.99	6.24	15.71	1.75	198.43	4.00
Local check	Mean	14.27	76.60	9.60	558.84	23.43	18.73	2.21	247.08	4.55
	CV	2.55	3.34	3.15	3.48	8.82	0.84	7.01	7.68	7.58
	Minimum	14.05	73.57	9.33	533.78	20.90	18.57	1.98	221.29	4.24
	Maximum	14.81	79.81	10.00	580.45	25.86	18.90	2.33	263.00	4.95
Total	Mean	15.60	77.98	9.21	539.41	23.26	26.45	2.32	333.79	3.67
	CV	11.06	7.85	6.77	12.62	7.07	14.73	13.94	15.07	22.73
	Minimum	10.57	62.19	8.12	420.40	18.52	17.38	1.20	215.62	1.00
	Maximum	21.81	111.57	13.29	920.25	26.81	36.67	3.95	495.19	5.00

Table 2. Continued

Region		DF	PW	PL	HSW	NPBPP	LPBPP	GYPH	NSPH
Harar	Mean	137.93	17.43	20.22	3.23	84.06	6.69	77.20	2386.63
	CV	5.45	17.88	14.72	13.66	20.70	30.03	34.70	30.21
	Minimum	120.38	12.90	12.52	2.09	58.38	4.17	31.51	1093.08
	Maximum	152.71	26.43	29.90	4.09	135.00	16.85	142.23	4320.60
Wello	Mean	131.19	22.82	19.39	3.21	84.83	9.02	89.32	2821.45
	CV	5.73	28.63	14.77	16.33	20.00	32.73	29.04	29.47
	Minimum	118.00	14.06	13.59	2.10	56.57	4.98	42.44	1775.78
	Maximum	148.43	36.24	24.52	4.44	129.00	15.80	139.11	4624.04
Local check	Mean	129.49	27.48	17.10	1.77	23.40	23.64	73.62	4213.17
	CV	0.74	3.63	3.40	6.47	5.71	10.39	11.01	11.29
	Minimum	128.52	26.48	16.57	1.63	21.95	20.34	66.16	3646.97
	Maximum	130.33	28.86	17.67	1.90	25.10	25.67	83.69	4800.91
Total	Mean	134.75	20.10	19.75	3.17	82.05	8.33	82.19	2640.84
	CV	6.02	28.21	14.86	17.23	25.00	49.13	32.28	32.09
	Minimum	118.00	12.90	12.52	1.63	21.95	4.17	31.51	1093.08
	Maximum	152.71	36.24	29.90	4.44	135.00	25.67	142.23	4800.91

*Refer to table 3 for the abbreviation of the characters

Appendice3. Gene diversity and diversity indexes at 59 RAPD loci of 14 genotypes of sorghum from Hararage and wello

Locus	Ht	Hs	Gst	Dst	Dm	h*	i
OPA02-1	0.015	0.014	0.099	0.002	0.900	0.015	0.044
OPA02-2	0.415	0.014	0.968	0.401	0.033	0.415	0.605
OPA02-3	0.494	0.025	0.950	0.469	0.050	0.494	0.687
OPA02-4	0.473	0.033	0.930	0.440	0.070	0.473	0.666
OPA02-5	0.133	0.000	1.000	0.133	0.000	0.133	0.257
OPA02-6	0.402	0.014	0.966	0.388	0.034	0.402	0.591
OPA02-7	0.133	0.000	1.000	0.133	0.000	0.133	0.257
OPA02-8	0.197	0.035	0.821	0.162	0.179	0.197	0.348
OPA02-9	0.365	0.033	0.909	0.332	0.091	0.365	0.552
OPA02-10	0.298	0.035	0.882	0.263	0.118	0.298	0.475
OPA02-11	0.217	0.066	0.694	0.151	0.306	0.217	0.375
OPA02-12	0.172	0.038	0.777	0.134	0.223	0.172	0.314
OPA02-13	0.146	0.014	0.907	0.132	0.093	0.146	0.276
OPA18-1	0.500	0.027	0.946	0.473	0.054	0.500	0.693
OPA18-2	0.256	0.014	0.947	0.242	0.053	0.256	0.423
OPA18-3	0.256	0.014	0.947	0.242	0.053	0.256	0.423
OPA18-4	0.337	0.000	1.000	0.337	0.000	0.337	0.520
OPA18-5	0.133	0.000	1.000	0.133	0.000	0.133	0.257
OPA18-6	0.402	0.014	0.966	0.388	0.034	0.402	0.591
OPA18-7	0.337	0.000	1.000	0.337	0.000	0.337	0.520
OPA18-8	0.337	0.000	1.000	0.337	0.000	0.337	0.520
OPA18-9	0.463	0.014	0.971	0.450	0.029	0.463	0.656
OPA18-10	0.030	0.027	0.092	0.003	0.909	0.030	0.078
OPA18-11	0.267	0.025	0.907	0.243	0.093	0.267	0.438
OPA18-12	0.015	0.014	0.099	0.002	0.900	0.015	0.044
OPA18-13	0.256	0.014	0.947	0.242	0.053	0.256	0.423
OPA18-14	0.000	0.000	0.000	0.000	****	0.000	0.000
OPA18-15	0.000	0.000	0.000	0.000	****	0.000	0.000
OPA18-16	0.133	0.000	1.000	0.133	0.000	0.133	0.257
OPA18-17	0.000	0.000	0.000	0.000	****	0.000	0.000

Appendice 3. Continued

Locus	Ht	Hs	Gst	Dst	Dm	h*	i
OPA18-18	0.172	0.038	0.777	0.134	0.223	0.172	0.314
OPA18-19	0.222	0.060	0.728	0.162	0.272	0.222	0.381
OPC01-1	0.245	0.000	1.000	0.245	0.000	0.245	0.410
OPC01-2	0.448	0.066	0.852	0.381	0.148	0.448	0.640
OPC01-3	0.015	0.014	0.099	0.002	0.900	0.015	0.044
OPC01-4	0.456	0.069	0.850	0.387	0.150	0.456	0.648
OPC01-5	0.498	0.035	0.929	0.463	0.071	0.498	0.691
OPC01-6	0.133	0.000	1.000	0.133	0.000	0.133	0.257
OPC01-7	0.379	0.035	0.907	0.343	0.093	0.379	0.566
OPC01-8	0.429	0.033	0.923	0.396	0.077	0.429	0.621
OPC01-9	0.015	0.014	0.099	0.002	0.900	0.015	0.044
OPC01-10	0.488	0.069	0.860	0.420	0.140	0.488	0.681
OPC01-11	0.337	0.000	1.000	0.337	0.000	0.337	0.520
OPC01-12	0.032	0.025	0.213	0.007	0.785	0.032	0.082
OPC01-13	0.000	0.000	0.000	0.000	****	0.000	0.000
OPC01-14	0.133	0.000	0.000	0.133	0.000	0.133	0.257
OPA02-1	0.186	0.050	0.732	0.136	0.269	0.186	0.333
OPA02-2	0.160	0.025	0.844	0.135	0.156	0.160	0.297
OPA13-3	0.490	0.000	1.000	0.490	0.000	0.490	0.683
OPA13-4	0.133	0.000	1.000	0.133	0.000	0.133	0.257
OPA13-5	0.490	0.000	1.000	0.490	0.000	0.490	0.683
OPA13-6	0.133	0.000	1.000	0.133	0.000	0.133	0.257
OPA13-7	0.485	0.025	0.949	0.460	0.051	0.485	0.678
OPA13-8	0.355	0.025	0.930	0.330	0.070	0.355	0.540
OPA13-9	0.490	0.000	1.000	0.490	0.000	0.490	0.683
OPA13-10	0.245	0.000	1.000	0.245	0.000	0.245	0.410
OPA13-11	0.415	0.014	0.968	0.401	0.033	0.415	0.605
OPA13-12	0.256	0.014	0.947	0.242	0.053	0.415	0.423
OPA13-13	0.408	0.000	1.000	0.408	0.000	0.408	0.598
Mean	0.261	0.019	0.929	0.243	0.071	0.261	0.405

Where: Ht = total gene diversity in the population or regions; Hs = Mean gene diversity within region or population; Gst = gene diversity between population or region; Dm = Proportion of gene diversity within population or region; nd Dst = Proportion of gene diversity between population or region * h = Nei's (1973) gene diversity * I = Shannon's Information index.