GENETIC DIVERSITY AND SELECTION EFFICIENCY OF ETHIOPIAN DURUM WHEAT (*Triticum durum Desf.)* LANDRACE COLLECTIONS

BY

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Abstract

Durum wheat (Triticum durum Desf) is an economically important crop because of its unique characteristics and end products like semolina for pasta making. In Ethiopia, durum wheat is among the most important Triticum species with higher morphological variability among landraces. Knowledge of the extent and pattern of genetic diversity within and among populations is very crucial to identify useful breeding materials and design appropriate collection and conservation strategies. Genetic diversity of 160 durum wheat accessions were studied using 17 morpho-agronomic traits and 12 SSR markers. A field and laboratory experiments were also undertaken in 2014-2016 in Ethiopia to characterize and evaluate durum wheat germplasm for attributes of morphological and molecular characterization. The field studies for morphological characterization was undertaken at Adadi Maryam and Ginchi using randomized complete block design with two replications while the molecular characterization study was conducted in Holeta national agricultural biotechnology laboratory (HNABL) at Holetta in Ethiopia. The average linkage technique of clustering produced a more understandable portrayal of the 160 durum wheat accessions by grouping them into seven clusters with inter-cluster D^2 values ranged from 13to 235 . According to genotypic correlation grain yield had highly significant positive correlation coefficient with harvest index, biomass yield, grain filling efficiency, biomass production rate and economic growth rate. In the present study spike length, grain yield, stem rust disease reaction, harvest index, economic growth rate, biomass production rate and biomass yield exhibited relatively higher broad sense heritability. In genetic progress study of 19 released varieties, empirical experimental evidence of gains in durum wheat yield showed that at average rate of increase in yield potential per year of release over 46 years period from the slope of linear regression shown in the graph was 30.99 kg/ha (0.74%) per year over the oldest variety Arendato. Analysis Molecular of Variance (AMOVA) showed higher variation within populations (81%) while the remaining 19% attributed for among population. The degree of polymorphism between populations varied from 74% for the collections from Eastern Tigray to 98.86 % for collections from Harergie, with the average of 88%. The dendogram of cluster analysis based on neighbor-joining algorithm categorized the 160 durum wheat accessions into three major clusters consisting of 47, 64, and 49 of the accessions in cluster I, II, and III, respectively. The highest values of genetic distance ranges from North Gonder and East Tigray (0.576) to smallest genetic distance between accessions from Bale and Harergie (GD=0.092).The HWE test for 12 loci were exhibited that loci (BARC 153, CFD 257, WMS 493, and WMS 516) were in Hardy-Weinberg equilibrium in all the populations analyzed. However, significant deviation from HWE was observed in some of the locus through tested populations. In 2D, most individuals of the respective populations were observed to form three major cluster groups with some sub cluster groups with higher intermixing from the other population. Thus, most of accessions from all 15 populations were intermixed with one another.

Key Words: *Triticum durum Desf*, Genetic Diversity, Trait association, Heritability, SSR primers, Polymorphism, Disease resistance.

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LIST OF ACRONYMS

AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
CIMMYT	International Maize and Wheat Improvement center
DAP	Diammonium phosphate
DMRT	Dunkan's Multiple Range Test
DZARC	Debre Zeit Agricultural Research Center
EBI	Ethiopian Biodiversity Institute
EIAR	Ethiopian Institute of Agricultural Research
EST-SSR	Express Sequence Tag derived Simple Sequence Repeats
FAO	Food and Agriculture Organization (of the United Nations)
GA	Genetic advance from selection
HNABL	National Agricultural Biotechnology Research Center
ICARDA	The International Center for Agricultural Research in the dry areas
MSE	Mean square of error
NF H2O	Nuclease free water
NJ	Neighbor Joining
NPL	Number of polymorphic loci
PAGE	Polyacrylamide Gel Electrophoresis
SAS	Statistical Analysis System

1. INTRODUCTION

Durum wheat (*Triticum durum Desf.*) is a member of the Gramineae family which belongs to the Triticeae tribe. It is an allotetraploid (two genomes: AABB) with a total of 28 chromosomes (2n = 4x = 28). *Triticum durum* is believed to be originated thousands of years ago from a hybridization between the wild diploid *T. monococcum L. subsp. Boeoticum (Boiss.) (*A *genome donor) (Synonym: Triticum urartu:* AA) and the donor of the B genome which, according to morphological, geographical and cytological evidence, has been recognized as *T. speltoides* (Tausch) Gren. Or its closely related species Colomba and Gregorini (2011) and Friebe and Gill (1996), Von Buren (2001).

There are six types of *Triticum* species of which *Triticum aestivum* and *Triticum turgidum* are the most dominantly grown species in Ethiopia. Durum wheat is grown in Ethiopia since antiquity because of its wide adaptation to the different agro-ecology of the country, and resistance to biotic and a biotic stresses. Zohary (1970) considered Ethiopia as the center of origin for the crop, whereas Purseglove (1975) reported existence of adequate genetic diversity in landraces of durum wheat grown in the country. The proposition that Ethiopia is the center of origin for durum wheat has been controversial because of the absence of ancestral forms and wild relatives Pecetti *et al*. (1992).

In Ethiopia, durum wheat is traditionally planted on heavy black clay soils (vertisols) of the highlands between 1800-2800 meters above sea level (masl) Tesemma and Belay (1991). It is not only the main source of semolina for the production of pasta, couscous, burghul and other local end-use products, but also provides many beneficial traits, including resistance, environmental stability, yield potential, and high quality for bread wheat improvement Singh (2002).

Genetic divergence arises either as a result of geographic separation or genetic barriers to crossability Singh (2002). Knowledge of the extent and pattern of genetic diversity within and between populations (accessions) is very important for the identification of useful materials for plant breeding purposes and also to better understand the crop to design appropriate collection and conservation strategies. It is believed that crosses between genetically diverse parents are likely to produce higher heterosis, desirable genetic recombination and segregation in progenies Chahal and Gosal (2002), Singh (2002). The presence of adequate genetic diversity and divergence among populations for some traits may also indicate the effectiveness of selection to improve the traits. The

other benefit is also to select possible accession(s) for *in situ* and/or *ex situ* conservation and to have evidence of the evolutionary forces influencing natural populations Semagn (2002). Joshi and Dhawan (1966) suggested the concept that differences in geographical origins of crop plants may serve as an index of genetic distance in parental selection.

The inception of wheat breeding in Ethiopia dated back to the early 1960's and, as the result of the efforts made hitherto, a number of improved varieties have been developed and released to farmers. Ethiopia has also benefited from the country-wide scaling up of improved varieties of a limited number of cereals dominantly maize (*Zea mays*) and bread wheat Gebre Mariam (1991). The scaling up programs so far proved existence of a tremendous genetic advance of improved varieties of bread wheat over the old-age durum wheat landraces but, despite the importance in terms of boosting crop productivity, the expansion of bread wheat at the expense of durum wheat had its own negative side effects in terms of agricultural sustainability. As the result of modern wheat breeding, which substituted durum wheat with bread wheat, it is believed that the genetic diversity in wheat, particularly durum wheat, has been increasingly dwindling Uddin and Boerner (2008). Narrow genetic diversity is a major problem for breeding varieties with better adaptation to different agro-ecologies, resistant/tolerant to biotic stresses like diseases, and abiotic stresses like drought.

Despite the importance of knowledge of genetic diversity in Ethiopian durum wheat landraces for morphological and molecular markers, however, the available is not holistic and sampling is not systematic to cover better representation. The available information revealed that tetraploid wheat's are genetically and morphologically diverse but evolution under domestication has not been fully elucidated Matsuoka (2011). First, most of the studies that have dealt so far with the variation in the Ethiopian wheat landraces were carried out elsewhere out of their natural habitat Jain *et al*. (1975); Bekele (1984); Negassa (1986), In some instances, the samples did not include major production regions like northern and eastern parts of the country but just concentrated on the central highlands and they were evaluated together with hexaploid wheat Negassa (1986). Second, even though some of the studies were inclusive in terms of area coverage, they were limited to either morphological or molecular traits but not both. Nevertheless, the studies so far have reported a high amount of variation for morphological traits. It is, therefore, necessary to evaluate the diversity of durum wheat accessions from major durum wheat producing areas along with the released varieties to properly determine the magnitude and the pattern of genetic diversity using both morphological

and molecular markers. Similarly, there is no detailed information on the extent of interrelationship among characters and their selection efficiency. Therefore, this study was designed with the following general and specific objectives

1.1. General Objective

To study the extent of genetic diversity and pattern of distribution using both molecular and morphological markers, and investigate character associations and selection efficiency in durum wheat landraces and released varieties of Ethiopia.

1.2. Specific Objective

- To assess the extent of genetic diversity and pattern of distribution within durum wheat landraces and released varieties both at phenotypic and genotypic levels;
- To determine geographical hotspot sites of divers populations of Ethiopian durum wheat landrace;
- To determine the magnitude of association between yield and yield components thereby estimate selection efficiency of the traits.

2. LITERATURE REVIEW

2.1. Origin and Distribution of Durum Wheat

Wheat may have been the first crop to be domesticated and cultivated by human beings. The process began possibly between 18,000 and 12,000 BC, when wheat was apparently grown in the Middle East and Abyssinian region Faris (2011). The first domesticated wheat recorded is from the Tigris-Euphrates Valley. Human beings have used wheat as food since prehistoric times and some of the evidence indicates that it was first used in a parched form Bechere *et al*. (2000), Reif *et al*. (2005) and Reitz (1967). Within tetraploid wheat, cultivated emmer (*T. dicoccum*) was the first to be domesticated. Others such as *T. durum, T. turgidum, T. aethiopicum and T. polonicum* might have originated from cultivated emmer through mutation(s) that reduced the toughness of the glumes to attain free-threshing Morris & Sears (1967).

Wheat (*Triticum spp*.) is the second major food crop of the world in its importance next to rice. Bousba, *et al*. (2012). Durum wheat (*Triticum turgidum L. sub sp. durum*) is the only tetraploid (AABB, 2n=4x=28) species of wheat of commercial importance that is widely cultivated today. It originated thousands of years ago from hybridization (pollen exchange) of the wild diploid *T. monococcum L*. (A genome) and the donor of the B genome which, according to morphological, geographical and cytological evidence, has recently been recognized as *Triticum speltoides (Tausch) Gren* or a closely related species Von Buren (2001).

Tetraploid wheat has been under cultivation in Ethiopia for thousands of years, and has acquired a diverse set of characteristics that makes the country a center of diversity for that species. However, it is not known when and from where tetraploid wheat was first introduced to Ethiopia Tesemma and Bechere (1998). It has been speculated that emmer (*Triticum dicoccon Schrank*) was the first wheat to arrive in Ethiopia and that this may have occurred around 5,000 years ago. Some studies suggest that the free threshing tetraploid wheat landraces of Ethiopia were separate introductions after emmer wheat. Bayush *et al.* (2007).

2.2. Agro-ecologies of The Crop

Durum wheat is best adapted to regions having a relatively dry climate, with hot days and cool nights during the growing season Gebre Maryam (1991). Durum is spring wheat, although winter durum is grown Gebre Maryam (1991). Durum wheat is predominantly grown in central, northwestern and northeastern parts of Ethiopia, ranging from 1800-2800 meter above sea level. Arega *et al*. (2013). Farmers commonly call the tetraploid wheat 'yekoticha sinde', meaning ''wheat of the heavy black soils''. Major production areas include the former Shewa, Gojam, Gonder, Wello, Tigray, and the highlands of Harergie, Arsi and Bale Bechere *et al*. (2000) and Tesemma (1991). Today, both the tetraploid wheat (with genomic designation of AABB), and hexaploid types (having genomic designation of AABBDD) are grown in the country. Compared to tetraploid wheat, the hexaploid (bread wheat) varieties are later introductions and generally show limited diversity Bechere *et al*. (2000).

Landraces are thus still largely cultivated in Ethiopia as well as in the world. One important characteristic of these landraces is their relevant variation for various qualitative and quantitative traits, with the result of a good adaptability to changing environmental conditions. These factors make the Ethiopian durum wheat germplasm extremely interesting for genetic studies and as a source of genes and gene complexes Harlan (1969) and Porceddu (1973).

2.3. Concept of Genetic Diversity

Genetic diversity is the level of biodiversity refers to the total number of characteristics in the genetic makeup of a species. It is distinguished from genetic variability, which describes the tendency of genetic characteristics to vary Hartl and Clark (1997). Genetic diversity is the sequence variation within species. Information on genetic diversity and relationships among and between individuals, populations, plant varieties, animal breeds and species is of importance to plant and animal breeders for the improvement of crop plants and animal breeds, for conservation biology and for studying the evolutionary ecology of populations. It is essentially the first step of plant breeding for crop improvement which is immediately available from Germplasm which is considered as the reservoir of variability for different characters Vavilov (1951).

Genetic diversity studies can identify alleles that might affect the ability of the organism to survive in its existing habitat, or might enable it to survive in more diverse habitats. This knowledge is valuable for germplasm conservation, individual, population, variety or breed identification.

2.3.1. Genetic diversity for crop improvement

Genetic variability, which is due to the genetic differences among individuals within a population, is the core of plant breeding because proper management of diversity can produce permanent gain in the performance of plant and can buffer against seasonal fluctuations Welsh (1990) and Sharma (1998).The evaluation of the level and structure of genetic diversity in crops is a prerequisite for plant breeding and genetic resource conservation programs. Genetic diversity is the basis for genetic improvement. Knowledge of germplasm diversity has a significant impact on the improvement of crop plants. The analysis of genetic diversity among registered/advance lines/stocks can be a useful tool to get information about the genetic variability of the varieties/stocks and possibly change the direction of breeding programs and strategies Kleshtkina *et al*. (2004). The major breeding efforts in the twentieth century have been a strong force in the reduction of crop genetic diversity Gepts (2006). It is generally thought that continuous selection among crosses of genetically related cultivars has led to a narrowing of the genetic base of the crops on which modern agriculture is based, contributing to the genetic erosion of the crop gene pools on which breeding is based Plucknett *et al.* (1987).

Genetic variability is essentially the first step of plant breeding for crop improvement which is immediately available from germplasm which is considered as the reservoir of variability for different characters Vavilov (1951). In the past, genetic uniformity of crops has led to several devastating attacks of pests and diseases Mark van de Wouw *et.al*. (2011). Well-known examples are the potato blight epidemic in Ireland in the 1840s, and the corn leaf blight which devastated maize production in the USA in the 1970s Lopez (1994). New strains of old diseases like (ug99) of stem rust might threaten future agriculture productivity. This (ug99) race of stem rust is now a cause of concern to wheat growers Singh *et al*. (2006) and it is feared that the global banana production will face severe losses in the near future due to a new strain of Panama disease which is attacking

the very widely planted 'Cavendish' banana clone Ploetz (2006). Even though, the reduction of genetic diversity in crops is becoming narrowed, still it didn't get enough focus.

2.3.2. Genetic diversity for selection efficiency

Wheat landraces are composed of traditional crop varieties developed by farmers through years of natural and human selection and are adapted to local environmental conditions and management practices. As distinct plant populations, landraces are named and maintained by traditional farmers to meet their social, economic, cultural, and environmental needs. They are alternately called farmers' varieties or folk varieties to indicate the innovative role of farmer communities in their development and maintenance.

For a successful breeding program, the presence of genetic diversity and variability play a vital role. Genetic diversity is essential to meet the diversified goals of plant breeding such as breeding for increasing yield, wider adaptation and desirable quality. Genetic divergence analysis estimates the extent of diversity existed among selected accessions Mondni (2012).

The major objective of crop breeding is to create new improved accessions with features that contribute to greater yield potential, increased yield stability and improved product quality Poehlman and Sleper (1995). To make the crossing program effective, parents should belong to different genetic clusters. The more distant the parents within overall limits of fitness are the greater the chances of obtaining higher amount of heterotic expression in F1's and broad spectrum of variability in segregating populations Reddy (1988). However, crossing of accessions belonging to the same genetic cluster would not be expected to yield desirable recombinants. These factors make the advanced test entries and popular cultivars extremely interesting for genetic variability studies using molecular and morphological markers.

2.3.3. Measurement of genetic diversity

Measurements of genetic diversity were initially based on co-ancestry and pedigree records Kim & Ward (1997). There are now different means that can be used to assess the genetic diversity. The most widely employed measures of variation within populations are gene diversity or heterozygosity, the number of alleles per locus, the percent of polymorphic loci, allele and

genotypic frequencies. For any ploidy level and reproductive system, the Nei (1973) gene diversity index can be used to estimate the diversity that exists in a population.

Most of the studies to date on Ethiopian wheat landraces have been based on the diversity of agro-morphological characters, which are highly heritable Bechere *et al*. (1996) and Eticha *et al*. (2005). There are specific endemic characters for some of the tetraploid wheat species in Ethiopia, such as violet grain and beardless or half-bearded hard durum wheat, which shows great variation from place to place Belay *et al*. (1993), Jain *et al*. (1975), Teklu & Hammer (2006) and Tesemma *et al*. (1991).

When we measure the genetic diversity of crops, it is necessary to partition the observed overall phenotypic variation into heritable and non-heritable components using suitable design which enable us to know whether the superiority of selection is inherited by the progenies. Information regarding the genetic parameters such as variation coefficient, heritability, expected genetic advance, degree of association between the various characters, direct and indirect effects of characters contributing to total grain yield are significance in formulating appropriate breeding strategy and exploiting the inherent variability of the experimental materials.

2.4. Heritability and Genetic Advance

Heritability estimates provide the information about index of transmissibility of the quantitative characters of economic importance and are essential for an effective crop breeding strategy. Heritability in broad sense can be defined as the proportion of the total genetic variability to the total phenotypic variance Allard (1960). Heritability in broad sense refers to the portion of phenotypically expressed variation, within a given environment and it measures the degree to which a trait can be modified by selection Christiansen and Lewis (1982). Since broad sense heritability doesn't give a clear picture of transmissibility of variation from generation to generation, (because the genetic variation includes the fixable and non-fixable dominance and epistatic variation), its utilization is limited in crop improvement. Estimate of heritability in a narrow sense give a clear picture. According to Falconer and Mackay (1996), heritability in narrow sense is defined as "the ratio of additive genetic variance to phenotypic variance". Estimation of heritability as a ratio of genotypic to phenotypic variance may vary greatly depending upon the unit for which variance is

considered Johnson *et al*. (1955). The greater the proportion of the total variability that is due to environment, the more difficult will it be to select for inherited differences. On the other hand, if environmental variability is small in relation to genotypic differences, selection will be efficient because the selected character will be transmitted to its progeny.

Generally, heritability indicates the effectiveness with which selection of accessions can be based on phenotypic performance. If heritability were 100%, that is genotypic variance (σ2g) is equal to phenotypic variance (σ2p), then phenotypic performance would be a perfect indication of genotypic value Johnson *et al*. (1955), but, even in such a hypothetical situation, the heritability value in itself provides no indication of the amount of genetic progress that would result from selecting the best individuals. However, genetic progress would expect from selection increase with an increase in the genotypic variance. Therefore, the utility of estimates of heritability is increased when they are used in conjunction with the selection differential leading to concomitant estimate of genetic advance expectations from selection.

The magnitude of heritability also helps in predicting the behavior of succeeding generations by devising the appropriate selection criteria and assessing the level of genetic improvement. Similarly, genetic advance gives clear picture and precise view of segregating generations for possible selection. Higher estimates of heritability coupled with better genetic advance confirm the scope of selection in developing new accessions with desirable characteristics. Ajmal *et al*. (1995), Singh *et al*. (1999), Ghimirary and Sarkar (2000) and Shazly *et al.* (2000) found high heritability estimates along with greater values of genetic advance for number of spikes per plant, number of grains per spike, 100 grain weight, grain yield and plant height. However Afiah *et al.* (2000) reported low to high estimates of heritability and genetic advance for these traits except plant height.

2.5. Character Association Study in Durum Wheat

Determination of correlation coefficients between various characters helps to obtain best combinations of attributes in wheat crop for obtaining higher return per unit area. The studies conducted by Monral *et al*. (1997), Simane *et al.* (1993) states that according to path analysis in durum wheat accessions, number of seeds per spike, 1000 seed weight and number of tillers have direct and positive effects on

yield. In other ways Kumar and Gupta (1984) reported direct positive but little effect of plant height, number of seeds per spike, 1000 seed weight and number of tiller on yield.

The following findings are observed about the association of some important traits of Ethiopian durum wheat landraces. Environment also plays an important role in the correlation. In some cases, environment affects both the traits simultaneously in the same direction or some time in different directions. The genetic and environmental causes of correlation combine together and give phenotypic correlation.

The dual nature of phenotypic correlation makes it clear that the magnitude of genetic correlation cannot be determined from phenotypic correlation. Therefore, estimation of degree of genotypic and phenotypic correlation of grain yield with yield components is very important to utilize the available genetic variability through selection Singh *et al*. (1998).

In some previous studies some traits are positively correlated and some are negatively correlated. Akram *et al*. (2008) and Mohammed *et al*. (2011), indicated that significant positive association was observed for grain yield with days for heading, spike number per spike and harvest index. However, Negative but non-significant correlation was observed on plant height with number of seed per spike and grain yield. Similar association was observed by Dorgan (2009). The negative correlation of some important character with yield leads to some undesirable selection depending on whether negative association is due to linkage or pleiotrophic effect. To improve yield components with negative association with other, suitable recombination may be obtained through bi-parental mating, mutation breeding or di-allele selective mating by breaking undesirable linkages. In other ways, biomass was positively and moderately related with spikelet per spike, and negatively correlated with harvest index. The negative and significant correlation was observed between biomass and harvest index. The same result was found by Jamali *et al*. (2003) and Villareal *et al*. (1992).

2.6. Marker Systems Used in Genetic Diversity

2.6.1. Morphological markers

The morphological traits used as markers had several advantages. Firstly, they are easily describable and facilitate enumeration. Secondly, detection and use of morphological markers are simple and cheap Chittaranjan and Albert (2008). The main shortcomings of this approach are the number of characters that can be detected is few (lower polymorphism), and cover only a limited genetic fraction of the organism. The existence of inter-allelic interactions in many morphological characters may lead to deviation of di-hybrid segregation ratios which in turn limits their application in linkage analysis. It is also impossible to distinguish homozygous dominant and heterozygous individuals because of dominant intra-allelic interactions Chittaranjan and Albert (2008). Moreover, some morphological traits are observed to be very plastic in nature, which could be easily influenced by environmental change and affect the exact relationships of plant species and/or populations Vithanage *et al*. (1995). Despite their limitations, morphological markers are well established tools for taxonomy, variety classification and breeding. The limitation to their application can be overcome by the application of biochemical and molecular markers.

2.6.2. Molecular marker

It is a gene or DNA sequence with a known location on a chromosome that can be used to identify individuals or species. It can be described as a variation (which may arise due to mutation or alteration in the genomic loci) that can be observed. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism), or a long one, like minisatellites.

The uses of molecular markers are based on the naturally occurring DNA polymorphism, which forms basis for designing strategies to exploit for applied purposes. A marker must to be polymorphic i.e. it must exist in different forms so that chromosome carrying the mutant genes can be distinguished from the chromosomes with the normal gene by a marker it also carries. Genetic polymorphism is defined as the simultaneous occurrence of a trait in the same population of two discontinuous variants or accessions. DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers DNA markers segregate as single genes and they are not affected by the environment. DNA is easily extracted from plant materials

and its analysis can be cost and labor effective. The first such DNA markers to be utilized were fragments produced by restriction digestion the restriction fragment length polymorphism (RFLP) based genes marker. Consequently, several markers system has been developed.

2.6.2.1. Simple sequence repeat (SSR)

Microsatellites are tandemly repeated short DNA sequences that are favored as molecular-genetic markers due to their high polymorphism index Mun *et al*. (2006). Tandem repeat in DNA is a sequence of two or more contiguous, approximate copies of a pattern of nucleotides and tandem repeats occur in the genomes of both eukaryotic and prokaryotic organisms Sokol *et al*. (2006). Microsatellite markers are the best DNA markers so far used for genetic diversity studies and fingerprinting of crop varieties. Microsatellites motifs are conserved in species and their unique behavior abundance, co-dominance, robustness and easiness for PCR screening make them the best DNA markers for the evaluation of crop genetic diversity. Tandem repeats are usually in the size range of 100-500 base pairs composed of di-nucleotide and tri-nucleotide repeats. They are very polymorphic, scattered throughout genomes. Genomes typically contain thousands of SSRs. They are detected by PCR using primers flanking the repeats and resolved on gels.

Microsatellites are ideal genetic markers for detecting differences between and within species. These are heritable, useful to monitor gene flow, excellent for parentage determination and ideally suitable for analysis via multiplexing with highly reproducible profiles Farooq and Azam (2002). The variation in the number of tandem repeated unit results in highly polymorphic banding patterns Junjian *et al*. (2002). Microsatellites may even be used across species and genus boundaries. Microsatellites are not limited to the nuclear genome. They occur in chloroplast as well as in mitochondrial genome Soranto *et al*. (1999). Despite their advantage, initial isolation of useful SSR loci can be a time consuming and expensive process Chittaranjan and Albert (2008), FAO (2003). Polymorphism is revealed by PCR-amplification from total genomic DNA, using two unique primers that flank and hence define the microsatellite locus. Amplification products obtained from different individuals can be resolved on gels to reveal polymorphism that are detected based on size differences due to differences in the number of repeats.

Microsatellites have a wide range of applications: determination of paternity and kinship, studying the structure of populations, determination of hybridity etc. Brufood and Wayne (1993) Schlotterer

and Pemberton (1994). Microsatellite markers have been applied for genetic diversity studies in many crop plants including wheat Alamerew *et al*. (2004) Khlestkina *et al*. (2004), Faris *et al*. (2005), Teklu *et al*. (2007), rice Zeng *et al*., (2004), pearl millet Kapila *et al*. (2008). Microsatellite markers have many advantages for tracing pedigrees because they represent single loci and avoid the problems associated with multiple banding patterns obtained with other marker systems Powell *et al*. (1996). However, developing microsatellite markers for a plant species requires prior knowledge of its genomic sequences, lack of which makes this technology very expensive and time consuming Yu *et al*. (2009).

3. MATERIALS AND METHODS

3.1. Experiment I. Phenotypic Diversity

3.1.1. Plant materials

One hundred forty one durum wheat germplasm accessions collected from various eco-geographical zones in Ethiopia and nineteen released varieties were considered for this study. The germplasm accessions were all received from the Ethiopian Biodiversity Institute whereas the released varieties were received from Debre Zeit Agricultural Research Center. The germplasm accessions were initially collected from the major durum wheat producing zones of Oromia, Tigray and Amhara regions including Arsi, Bale, West Shewa, East Shewa, East Harergie, West Harergie, Semen Shewa, North Gonder, South Gondar, South Wello, North Gojam, South Gojam, Northern Tigray, Central Tigray and Eastern Tigray. The detailed descriptions of the test accessions are given in Table 1 below.

Table 1. Description of the test accessions and released varieties

Sr. No	Accession /Genotype	Origin Region	Zone	Locality	Sr. No.	Accession /Genotype	Origin Region	Zone	Locality
1	231623	Amhara	South Wello	Mekdela	81	226180	Oromiya	Harerge	Kersa
2	231597	Amhara	South Wello	Debre Sina	82	226183	Oromiya	Harerge	Kersa
3	231600	Amhara	South Wello	Debre Sina	83	222708	Oromiya	Harerge	Goro Gutu
4	222855	Amhara	South Wello	Debre Sina	84	231471	Oromiya	Harerge	Girawa
5	226094	Amhara	South Wello	Debre Sina	85	203690	Oromiya	Harerge	Kurfa Chele
6	8185	Amhara	South Wello	Legambo	86	231603	Oromiya	Harerge	Kombolcha
7	8186	Amhara	South Wello	Legambo	87	5730	Oromiya	Harerge	Kurfa Chele
8	214590	Amhara	South Wello	Debre Sina	88	203854	Oromiya	Harerge	Haromaya
9	214550	Amhara	South Wello	Debre Sina	89	226179	Oromiya	Harerge	Kersa
10	213149	Amhara	South Wello	Wereilu	90	231606	Oromiya	Harerge	Kurfa Chele
11	206573	Amhara	South Gondar	Lay Gayint	91	231613	Oromiya	Harerge	Kurfa Chele
12	222621	Amhara	South Gondar	Lay Gayint	92	210808	Oromiya	East Shewa	Gimbichu
13	222641	Amhara	South Gondar	Lay Gayint	93	5429	Oromiya	East Shewa	Akaki
14	216614	Amhara	South Gondar	Lay Gayint	94	216651	Oromiya	East Shewa	Ada`aChukala
15	222655	Amhara	South Gondar	Lay Gayint	95	5314	Oromiya	East Shewa	Ada`aChukala
16	222608	Amhara	South Gondar	Lay Gayint	96	203748	Oromiya	East Shewa	Shashemene
17	222613	Amhara	South Gondar	Lay Gayint	97	5300	Oromiya	East Shewa	Gimbichu
18	7412	Amhara	South Gondar	Lay Gayint	98	5248	Oromiya	East Shewa	Lome
19	216474	Amhara	South Gondar	Lay Gayint	99	5180	Oromiya	East Shewa	Lome
20	226951	Amhara	South Gondar	Lay Gayint	100	5736	Oromiya	East Shewa	Akaki
21	222616	Amhara	South Gondar	Lay Gayint	101	214313	Oromiya	East Shewa	Ada`aChukala
22	203750	Amhara	West Gojam	Dembecha	102	226959	Oromiya	East Shewa	Minjarna
23	203922	Amhara	West Gojam	Dembecha	103	231528	Oromiya	West Shewa	Ginde Beret
24	203893	Amhara	West Gojam	Dembecha	104	222457	Oromiya	West Shewa	Dendi
25	5487	Amhara	West Gojam	Dembecha	105	222461	Oromiya	West Shewa	Dendi
26	208212	Amhara	West Gojam	Jabi Tenhan	106	231557	Oromiya	West Shewa	Wolisona Goro

27	203757	Amhara	West Gojam	Dembecha	107	231526	Oromiya	West Shewa	Ginde Beret
28	208189	Amhara	East Gojam	Enarji Enawga	108	214328	Oromiya	West Shewa	Ejere
29	208195	Amhara	East Gojam	Enarji Enawga	109	5454	Oromiya	West Shewa	Ambo
30	210821	Amhara	East Gojam	Dejen	110	5144	Oromiya	West Shewa	Ejere
31	226833	Amhara	East Gojam	Dejen	111	6101	Oromiya	West Shewa	Cheliya
32	226844	Amhara	East Gojam	Enemay	112	7206	Oromiya	West Shewa	Dendi
33	231617	Amhara	East Gojam	Enemay	113	227020	Oromiya	West Shewa	Meta Robi
34	231618	Amhara	East Gojam	Enemay	114	214343	Tigray	Southern	Enda Mehoni
35	214515	Amhara	East Gojam	Huletej Enefse	115	7956	Tigray	Southern	Enderta
36	8333	Amhara	East Gojam	Enarji Enawga	116	207854	Tigray	Southern	Enda Mehoni
37	8328	Amhara	East Gojam	Enemay	117	206551	Tigray	Southern	Enderta
38	214517	Amhara	East Gojam	Huletej Enefse	118	206554	Tigray	Southern	Enderta
39	222515	Amhara	North Gondar	Wegera	119	223257	Tigray	Southern	Alaje
40	203840	Amhara	North Gondar	Wegera	120	226199	Tigray	Southern	Enderta
41	5217	Amhara	North Gondar	Gondar Zuria	121	206558	Tigray	Southern	Enderta
42	204340	Amhara	North Gondar	Gondar Zuria	122	238113	Tigray	Southern	Ofla
43	6856	Amhara	North Gondar	Alefa	123	226245	Tigray	Southern	Enderta
44	216492	Amhara	North Gondar	Dabat	124	238114	Tigray	Central	Adwa
45	216545	Amhara	North Gondar	Debark	125	238118	Tigray	Central	Werie Lehe
46	226207	Amhara	North Gondar	Gondar Zuria	126	238121	Tigray	Central	Werie Lehe
47	226117	Amhara	North Gondar	Gondar Zuria	127	238122	Tigray	Central	Werie Lehe
48	216440	Amhara	North Gondar	Wegera	128	238123	Tigray	Central	Werie Lehe
49	208265	Oromiya	North Shewa	Kuyu	129	238124	Tigray	Central	Werie Lehe
50	208278	Oromiya	North Shewa	Degem	130	238125	Tigray	Central	Adwa
51	208286	Oromiya	North Shewa	Degem	131	238126	Tigray	Central	Adwa
52	208310	Oromiya	North Shewa	Girar Jarso	132	238136	Tigray	Central	Degua Tenben
53	208312	Oromiya	North Shewa	Girar Jarso	133	238137	Tigray	Central	Tahtay
54	208317	Oromiya	North Shewa	Girar Jarso	134	238127	Tigray	Eastern	Ganta Afeshum
55	208491	Oromiya	North Shewa	Girar Jarso	135	238128	Tigray	Eastern	Ganta Afeshum
56	226375	Oromiya	North Shewa	Yaya Gulele	136	238129	Tigray	Eastern	Ganta Afeshum
57	5679	Oromiya	North Shewa	Mulona	137	238130	Tigray	Eastern	Hawzen
58	5739	Oromiya	North Shewa	Kembbit	138	238131	Tigray	Eastern	Atsbi
59	226892	Oromiya	North Shewa	Girar Jarso	139	238133	Tigray	Eastern	Atsbi
60	222421	Oromiya	Arssi	Robe	140	238134	Tigray	Eastern	Atsbi
61	222422	Oromiya	Arssi	Robe	141	238135	Tigray	Eastern	Atsbi
62	222428	Oromiya	Arssi	Robe	142	Ginchi	-	-	-
63	226868	Oromiya	Arssi	Jeju	143	Yerer	-	-	-
64	226356	Oromiya	Arssi	Tena	144	Worer	-	-	-
65	7073	Oromiya	Arssi	Amigna	145	Mangude	-	-	-
66	214498	Oromiya	Arssi	Jeju	146	Arendato	-	-	-
67	7022	Oromiya	Arssi	Robe	147	Assasa	-	-	-
68	226273	Oromiya	Arssi	Sherka	148	Denbi	-	-	-
69	227013	Oromiya	Arssi	Seru	149	Tob	-	-	-
70	5927	Oromiya	Arssi	Sherka	150	LD-357	-	-	-
71	231467	Oromiya	Bale	Goba	151	Hitosa	-	-	-
72	222324	Oromiya	Bale	Gaserana	152	Mukaye	-	-	-
73	222338	Oromiya	Bale	Gaserana	153	Killinto	-	-	-
74	204349	Oromiya	Bale	Sinana	154	Quamy	-	-	-
75	204370	Oromiya	Bale	Agarfa	155	Gerardo	-	-	-
76	227060	Oromiya	Bale	Sinana	156	Foka	-	-	-

77	204357	Oromiya	Bale	Goba	157	Cocorit	-	-	-
78	214503	Oromiya	Harerge	Tulo	158	Boohie	-	-	-
79	203695	Oromiya	Harerge	Kuni	159	Bichena	-	-	-
80	203886	Oromiya	Harerge	Kuni	160	Metyaya	-	-	-

3.1.2. Test locations

The accessions were evaluated at two locations, Ginchi and Adadi, in Ethiopia during the year 2014. The locations were assumed to represent the major durum wheat production areas of Ethiopia. The detailed descriptions of the test locations in terms of geographical position, mean annual rainfall, mean annual temperature and soil characteristics is given in table 2 below.

Table 2. Description of the test locations for geographical position and physico-chemical properties of the soils

Descriptor	Locations	
	Ginchi	Adadi
Latitude	09°30' N	08°31' N
Longitude	38°30' E	38°13' E
Altitude (m a. s. l.)	2200	2383
Mean annual rain fall (mm)	1139	1105
Mean annual temperature ($°C^0$)	16.3	16.9
Soil type	Black Vertisol	Light brown
Soil drainage	Poorly drained	Well drained
Soil pH	6.18	7.62
% clay	65.83	61.19
% silt	20.42	25.91
% sand	13.75	12.66
Organic C(%)	1.30 (low)	1.16
N (%)	0.103 (low)	0.15
P (ppm*)	4.49 (low)	8.70
K (ppm)	2.483 (high)	39.75
PH (H_2O)	6.18	6.32
EC (µs)	547.33	405.63

Source: Keneni et al. (2012)

3.1.3. Experimental design and layout

Sound seeds of all accessions and improved varieties were planted in a plot of 1 row 1m long with 30 cm between rows. A blanket basal application of nitrogen and phosphorus fertilizer was made to all plots at the recommended rate of N_2:P_2O_5 Ginchi (64:46) and Adadi (60:69). All other crop management practices were applied uniformly to all plots as required so that the test accessions could express their full genetic potential for the traits under consideration. The experiment was laid

down in a RCBD with 2 replications. The accessions were assigned to plots at random within each block.

3.1.4. Data Collection

Data were collected either on plot basis or from randomly selected five plants (IBPGR, 1993) on:

- Days to heading (DH):the number of days from planting to a stage when 50% of the plants in a plot have produced spikes;
- Days to maturity (DM): the number of days from planting to a stage when 50% of the plants in a plot have reached maturity;
- Stem rust scoring: incidence and severity of the disease scored at two times;
- Number of tillers/plant (TPP): the average number of effective tillers per plant;
- Number of spikelets/spike (SPPS): the average number of spikelets per spike from five typical spikes selected from a genotype;
- Grain yield/plant (GYP): the weight in gram of grain yield obtained from randomly selected five plants and its tillers;
- Harvest index (HI%): the ratio of dried grain yield to the biomass yield and multiplied by 100;
- Biomass/Plant (BMP): The total above ground biological yield (grains and all other parts) in grams per plant
- Plant height (PH): the average height of the plants in cm from the ground level to the tip at maturity excluding the awn;
- Leaf area index at maturity (LAM): it is defined as the one-sided green leaf area per unit ground surface area (LAI = leaf area /ground area, m^2/m^2);
- Grain filling period (GFP): the number of days from the heading to maturity;
- Grain production efficiency (GPE): grain filling duration divided by duration of vegetative; period and then multiplied by grain yield;
- Economic growth rate (EGR): grain weight divided by grain filling duration and then multiplied by 100);

- Thousand kernel weight (TKW): the weight in gram of 1000 seeds; and
- Spike length (SL): the average length of a spike, in cm from its base to the tip excluding awns.

3.1.5. Data Analysis

3.1.5.1. Analysis of variance

Data of stem rust scoring at two stages were subjected to arcsine transformation as per the standard procedure set by Gomez and Gomez (1984) prior to performing statistical analysis in order to normalize the distribution.

For combined analysis of variance the homogeneity of error variance was tested using F-max test method of Hartley (1950), which is based on the ratio of the larger mean square of error (MSE) from the separate analysis of variance to the smaller mean square of error given by the following formula:

$$F\text{-}_{max} = \frac{\text{Largest MSE}}{\text{Smallest MSE}}$$

If the calculated value of $F\text{-}_{max}$ was less than three means that the ratio of the highest error mean square is not three fold larger than the smallest error mean square which indicate that the variance was considered homogenous and thus it was possible to proceeding combined analysis of variance Gomez and Gomez (1984). Then the data were subjected to the analyses of variance (ANOVA) and pooled analysis of variance over locations was done using the SAS statistical package (SAS, 2002). Components due to genotype (σ_g^2), location (σ_l^2), genotype by location interaction (σ_{gl}^2) variances, broad-sense heritability (h^2) and the expected genetic gain from selection (GA) were calculated from the pooled analyses of variance by assuming various observed mean squares equal to their expected mean squares (Table 2) Singh and Chaudhary (1985) as:

$$\sigma^2_g = [(\sigma^2_e + R\sigma^2_{gl} + RL\sigma^2_g - (\sigma^2_e + R\sigma^2_{gl})]/RL$$
$$= (MS3-MS4)/RL$$
$$\sigma^2_e = MS5$$
$$\sigma^2_{ge} = [(\sigma^2_e + R\sigma^2_{gl}) -(\sigma^2_e)]/R$$
$$= (MS4-MS5)/R$$

$\sigma^2_p = \sigma^2_g + \sigma^2_{gl} + \sigma^2_e$

Where σ_g^2 = genotypic, σ_{gl} = genotype by location interaction and σ_e^2 = environmental variances.

Table 3. Combined Analysis of Variance for collection of landraces based on randomized complete block design

Source of variation	Degree of freedom	Mean Square (MS)	Expected Mean Square (EMS)[1]
Locations	$L-1$	MS1	$\sigma_e^2 + G\sigma_r^2 + GRL\sigma_l^2$
Replications/location	$L(R-1)$	MS2	$\sigma_e^2 + G\sigma_r^2$
Genotypes	$G-1$	MS3	$\sigma_e^2 + R\sigma_{gl}^2 + RL\sigma_g^2$
Genotype × Location	$(G-1)(L-1)$	MS4	$\sigma_e^2 + R\sigma_{gl}^2$
Error	$L(G-1)(R-1)$	MS5	σ_e^2

The coefficients of variations at phenotypic and genotypic levels were estimated using the formula adopted by Johnson *et al.* (1955) as:

$PCV = [\sigma_p / \bar{x}] \times 100$

$GCV = [\sigma_g / \bar{x}] \times 100$

Where σp = phenotypic standard deviation (σg + σe), σg = genotypic standard deviation, σe = environmental standard deviation and $\bar{x}$ = grand mean for the character x; PCV and GCV = phenotypic and genotypic coefficients of variation, respectively.

Broad-sense heritability (h^2) for all they were quantified using pooled analyses of variance over locations using the following model:

$h^2 = \sigma^2_g / [\sigma^2_g + \sigma^2_{gl}/L + \sigma^2_e/(RL)]$

Where σ^2_g = genotypic variance, σ^2_{gl} = genotype by location interaction variance, σ^2_e = environmental variance, L = number of locations and R = number of replications.

Genetic advance in absolute unit (GA) and percent of the mean (GAM), assuming selection of the superior 5% of the accessions, was estimated in accordance with the methods illustrated by Johnson *et al*. (1955) as:

$GA = k\sigma_p h^2$

$GAM = (GA/\bar{x}) \times 100$

Where k = the standardized selection differential at 10% selection intensity (k =2.06), σ_p = phenotypic standard deviation, h^2 = heritability and $\bar{x}$ = grand mean.

3.1.5.2. Comparison of secondary traits as determinants of grain yield

Correlation coefficients between characters were estimated based on the standard procedure as:

$r = Cov\ xy/sqrt\ [\sigma x^2 + \sigma y^2]$

Where Cov(xy)= co-variance of traits x and y, σx^2 = variance of x and σy^2 = variance of y. The level of significance of correlation coefficients was determined from r table (correlation table) at appropriate degrees of freedom and probability level Gomez and Gomez (1984). The genotypic correlation between grain yield and yield components were estimated based on the following formula Singh and Chaundhary (1986), and Falconer (1989): $r_{g = COV(xy)/sqrt\ (\sigma(x^2) + \sigma(y^2).}$ The genotypic COV (xy) = genotypic covariance of traits x and y.

3.1.5.3. Comparison of direct and indirect selection for grain yield

The predicted response of grain yield and yield components to direct selection (DR) were calculated assuming a standard selection differential of 1.0 Falconer (1989) as:

$DR = \delta . \sigma . h$

Where δ is selection differential, σ and h are the square root of variances and heritability of a given trait. The response of grain yield to indirect selection (IR) for a secondary trait (spike length, spike lets per spike, thousand kernel weight, and productive tiller) was calculated as suggested by Wricke and Weber (1986) as:

$IR = \delta . r_g . \sigma_y . h_x$

Where, δ is selection differential, r_g the genetic correlation coefficient between the secondary traits and grain yield, σ_y and h_x are the square roots of variances of grain yield and heritability of the

secondary traits, respectively. The relative efficiency of indirect selection (IR) for a secondary trait as compared to direct selection (DR) for grain yield was calculated as:

$RE = r_{g.} h_{y.}/h_x$ or IR/DR

Where RE is relative efficiency of indirect selection through the component traits, h_y and h_x are the square roots of heritability of grain yield and the secondary traits, respectively. The value of 1.0 indicates that direct selection was predicted to be equally efficient as indirect selection. A value of less than 1.0 indicates that direct selection was more efficient and a value of more than 1.0 indicates that indirect selection was more efficient Banziger and Edmeads (1997), Banziger *et al*. (1997), Banziger and Lafitte (1997).

3.1.5.4. Analysis of genetic divergence

The data on quantitative traits was standardized to a mean of zero and a variance of unity before clustering, and principal component analysis to avoid differences in scales used to measure different traits Manly (1986). Clustering of the accessions into different homogeneous groups based on multiple traits was conducted following the average linkage method. Genetic diversity between clusters based on correlation matrix was calculated based on Mahalanobis's D^2 statistic Mahalanobis's (1936) using the SAS software package SAS Institute (2002). The important traits in each principal component that significantly contributed to the variation observed were identified as suggested by Johnson and Wichern (1988).

3.1.5.5. Cluster analysis

Clustering of the accessions and improved varieties was performed by average linkage method of SAS software SAS Institute (2002). Points where local peaks of the pseudo F statistic join with small values of the pseudo t^2 statistic followed by a larger pseudo t^2 for the next cluster fusion was examined to decide the number of clusters SAS Institute (1996). The dendrograms were built using MINITAB 14.

3.1.5.6. Distance analyses

Genetic distances between clusters as standardized Mahalanobis's D^2 statistics was calculated as:

$D^2_{ij} = (xi - xj)' \, cov^{-1}(xi - xj)$

Where, D^2ij = the distance between cases i and j; xi and xj = vectors of the values of the variables for cases i and j; and cov^{-1} = the pooled within groups variance-covariance matrix. Principal components based on correlation matrix will be calculated using the same software as in clustering.

The D^2 values obtained for pairs of clusters were considered as the calculated values of Chi-square (X^2) and were tested for significance both at 1% and 5% probability levels against the tabulated values of X^2 for 'P' degree of freedom, where P is the number of characters considered Singh and Chaudhary (1985).

3.1.6. Comparison of Selected Accessions with the Original Population

The best performing 5% of the accessions were selected, their means were independently computed for each character and compared with the original population. The absolute value of Student's t test was used to compare performances of the 5% best selected accessions with the base population as:

$$t = \frac{\overline{x} - \mu_0}{s/\sqrt{n}}$$

Where $\overline{x}$ is mean of selected accessions, μ is mean of the base populations, S is the standard deviation of the sample and n is the number of accessions selected from the base population for better performance. The significance of the difference between the population parameter (mew) and sample mean ($\overline{x}$) was tested using t- table, i.e. when the calculated value of t-test was more than the tabulated t- value, the difference was considered significant Singh (2001).

3.1.5.6. Genetic Progresses from Durum Wheat Breeding

The mean performances of released varieties were regressed on years of release starting from 1966, using the first released/recommended variety as a base Cox *et al*. (1988). A linear model was fitted between grain yield as a dependent variable Y and year of release as an independent variable X as:

$Y = bx + a,$

Where Y = the mean value of the dependent variable, x = the mean value of the independent variable, a = the intercept and b= the regression coefficient (the slope of the line).

Annual rate of gain (b) = Cov_{xy}/ Var_X, Where, X = the year of variety release, Y = the mean value of each character for each variety, Cov_{xy} = covariance of x and y and Var_X= variance of X. The relative annual gains achieved over the last 46 years (1966-2012) was determined as the ratio of genetic gain to the corresponding estimated values of the oldest variety and expressed as percentage.

3.2. Experiment II. Molecular Diversity

3.2.1. Plant materials and DNA extraction

The same accessions used for the first experiment were used for this study (Table 1). Sound seeds of each genotype were planted in the greenhouse of the National Agricultural Biotechnology Laboratory at Holetta, Ethiopia. Three weeks after planting, approximately equal amount of bulk leaf samples were collected from 3-5 plants of each entry and placed in 50 ml autoclaved and labeled falcon tubes with enough amount of yellow silica gel as a desiccant. After the leaves were totally dried, about 50 mg of dry leaves were ground using a mixer mill (Retsch RM2000) and genomic DNA was extracted using the cetyltriethyl ammonium bromide (CTAB) method Borsch *et al*. (2003). A total of 700 µl of CTAB (CTAB2%, 1.4M NaCl, 2%Beta mercapto ethanol, 20mM EDTA PH=8), 100mMTris HCL(PH=8) and 0.5% PVP) was added to each sample to break open plant cells and solubilize the contents. The samples were shortly mixed with the CTAB solution by thoroughly shaking by hand and incubate in water bath (GFL 1086) at 65 ^{0}c for 30 minutes. After the samples were taken out of the water bath, they were centrifuged with Eppendorf centrifuge 5810R at 13000 rpm for seven minutes. The supernatant was transferred to a new labeled 1.5 ml eppendorf tube. Chlorophyll and some denatured proteins were removed from the samples by dissolving in 600µl chloroform and the organic phase was separated by centrifugation for 7 minutes at 13000 rpm. These steps were repeated until a clear supernatant was recovered. About 325µl of iso-propanol was added and the DNA was precipitated by centrifugation. The iso-propanol was removed and the DNA was cleaned by repeated washing with 70% ethanol. Then the pellet was air-dried at room temperature. In order to get the clean genomic DNA subsequent washing with ammonium acetate and sodium acetate with a total of one and half hour centrifugation were done. Finally, the pellet was air-dried and dissolved in 100 µl of 1X TE. To remove RNA and get pure genomic DNA, 7 µl of 10mM RNAase was added to each sample and incubated in water bath at 37^{o}c.

3.2.2. Genomic DNA quality and quantity measurement

Genomic DNA of 5 µl from each sample with 3µl of 6x DNA loading dye mixed with gel red was loaded on 1% agarose gel. The DNA samples were electrophoresed at a constant voltage of 150V for about 20 minutes visualized using a UV- Trans illuminator (JYSPBT) and gel pictures were taken. The quality and quantity of the same DNA samples were confirmed with the use of spectro-nano-gram optimizer (ND-8000). Samples with high band intensity and lesser smear having concentrations of above 500 ng/µl were maintained for polymerase chain reaction (PCR) but samples with low qualities were suspended for re-extraction. The selected genomic DNA was further diluted to 1:9 (i.e. 1 primer**:** 9 NF H_2O) for PCR.

3.2.3. Primer selection and optimization

A total 53 simple sequence repeat (SSR) primers previously reported to be polymorphic with other sets of durum wheat genetic materials Bousba *et al*. (2012), Mondini (2012), Abouzied *et al*. (2013) were pre-screened for polymorphism in this specific population and then 12 primers which showed high variability and reproducibility were selected for use in PCR (Table 1)

Table 4. Description (forward and reverse sequences) of the polymorphic microsatellite markers used in the analysis of the durum wheat accessions

Microsatellites	**Primer sequences**	
	Forward	**Reverse**
CFA2278 2BS	AAGTCGGCCATCTTCTTCCT	GCCTCTGCAAGTCTTTACCG
Wms493 3BS	GGAACATCATTTCTGGACTTTG	TTCCCATAACTAAAACCGCG
Wmc516 4AS	GACTCGCAACTAGGGGT	GGGCCACGAATAAACAG
Wms5 3AL	GCCAGCTACCTCGATACAACTC	AGAAAGGGCCAGGCTAGTAGT
Wmc532 3AS	GATACATCAAGATCGTGCCAAA	GGGAGAAATCATTAACGAAGGG
Wms120 2BL	GATCCACCTTCCTCTCTCTC	GATTATACTGGTGCCGAAAC
WMC516	GGGCCACGAATAAACAG	GACTCGCAACTAGGGGT
WMS269	TGCATATAAACAGTCACACACCC	TTTGAGCTCCAAAGTGAGTTAGC
CFD257	TCTCAACTTGCAACTGCCAC	CCCTCCATGGATTCTTGCTA
BARC153	CGCGCCTTGCTTTATTAGTATTAGTATT	GCGGCATGCACATATAATTCTCATTGACT
WMS234 5BL)	GAGTCCTGATGTGAAGCTGTTG	CTCATTGGGGTGTGTACGTG
WMS375 4BL)	ATTGGCGACTCTAGCATATACG	GGGATGTCTGTTCCATCTTAGC
Xgwm1361AS)	GACAGCACCTTGCCCTTTG	CATCGGCAACATGCTCATC

3.2.4. Procedures of polymerase chain reaction (PCR)

PCR reaction was performed with a thermal cycler (Gene Amp @PCR System 9700) in a total volume of 25 µl containing 6.25 µl green promega master mix containing all components of PCR

except genomic DNA and primers, 3.75 µl, 1.25 µl forward and 1.25 µl reverse primer and 12.5 nuclease free water. The PCR was programmed at an initial denaturation step of 2 minutes at 94°C followed by 30 cycles of 30 seconds denaturation at 94°C, annealing at 51 to 60°C (depending on the requirements of the primers) for 30 seconds, initial extension at 72°C for 1minute and final extension at 72°C for 10 minutes. At the end of the reaction, the PCR products were stored at -20^0 C and were later subjected to agarose gel ectrophoresis to ensure amplification.

3.2.5. Polyacrylamide gel electrophoresis (PAGE)

After amplification, 4 µl of the SSR amplified samples were mixed with 2.5 µl 6x DNA loading dye containing gel red. Then samples were loaded on 40% polyacrylamide gel containing (70 ml ddH_2O, 20 ml 40% polyacrylamide gel, 5 ml 10XTBE, 1100 µl APS prepared by mixing 1g of APS in 10 ml of ddH_2O), 60 µl TEMED). Electrophoresis was carried out on a vertical electrophoresis set up (CS-500V) at a constant voltage of 300V for 1:30 h using a standard DNA ladder (HyperLadder ^{TM}500-2000 bp) with known reference band to estimate the molecular weight of each amplified product. Amplification was visualized under 3UV-trans illuminator. Primers with unclear and missing bands were sorted out and repeated. Non-polymorphic, missing, faint and distorted gels were overlooked at scoring and only records of twelve markers with clear polymorphic bands were considered for statistical analysis (Figure.4).

3.2.6. Scoring and data analysis

Clearly resolved and unambiguous bands were scored visually for their presence or absence for each primer and sample Seetharam *et al*. (2004). The intensity of the bands was not taken into account and the fragments with the identical mobility were considered to be identical fragments and treated as a unit character. Each fragment was scored independently based on the expected frogment size of each primer. Bands with the same molecular weight and mobility were treated as identical fragments. The total numbers of bands, number of polymorphic bands in a set of accessions, and genetic diversity of all individuals and across each primer were calculated Sarla, *et al*. (2005).

3.2.6.1. Analysis of molecular variance and F-statistics

The genotypic data were subjected to various measures of the genetic relationships within and among the durum wheat accessions using GeneAlex version 6.5 Peakall and Smouse (2007).

Pairwise Fst and Nm values were also computed using the same software. Genetic parameters such as total number of alleles per locus (Na), number of effective alleles per locus (Ne), Shannon's Information Index (I), and gene diversity were determined according to Nei and Li (1979). The F-statistics such as genetic differentiation (FST), fixation index or inbreeding coefficient (FIS), and overall fixation index (FIT) were calculated according to Wright's original derivation Wright (1951). The non-parametric Analysis of Molecular Variance (AMOVA) was conducted by using Gene Alex version 6.5 software package based on Euclidian distance Song *et al*. (1999). Gene flow (Nm) was calculated as: Nm = ¼*[(1−FST)/FST)] where FST is genetic differentiation Slatkin and Barton (1989). Polymorphic information content (PIC) was estimated using Power marker version 3.25.

3.2.6.2. Genetic distance and cluster analysis

To examine the degree of population differentiation among the study materials the Nei unbiased genetic distance and genetic identity were computed according to Nei's (1978) using GeneAlex version 6.5 Peakall and Smouse (2007) software package. The neighbor joining (NJ) method Saitou and Nei (1987), Studier and Keppler (1988) was used to compare individual accessions and evaluate patterns of genotypic clustering.

3.2.6.3. Hardy-Weinberg equilibrium test

The expected heterozygosity (He) for all SSR markers was calculated by relating the observed allele frequencies to the expected frequency of heterozygotes based on Hardy-Weinberg Equilibrium. Genalex version 6.5 software package Peakall and Smouse (2012) was used to carry out a chi-square (χ^2) test for each SSR marker to determine deviation from Hardy-Weinberg equilibrium (HWE) and the statistical significance was tested at 5 % probability level. For a single locus with two alleles X and Y whose frequencies arep (X) and q(Y), where p + q = 1, the frequencies of the three possible accessions are given by$p^2 + 2pq + q^2 = 1$. Therefore, the expected heterozygosity (He) was calculated as:Expected Heterozygosity (He) $= 1 - \Sigma$ pi2 Nei (1973)

3.2.6.4. Principal co-ordinate Analysis

The Principal Component Analysis (PCO) was performed by the Gene Alex version 6.0 in order to examine the patterns of variation among individual samples and highlight the resolving power of ordination Seetharam *et al*. (2004). Jaccard's similarity coefficient Jaccard (1908) matrix was used

for principal coordinate analysis. The calculation of Jaccard's coefficient, variance, cumulative variance, Eigen values and two dimensional coordinates were performed using Gene Alex software version 6.0. The first three axes were later used to plot a three dimensional plot with STATISTICA version 5.5 software Hammer *et al*. (2001), Statistica soft Inc. (2001).

4. RESULTS

5. 4.1. Experiment I. Phenotypic Diversity

4.1.1. Performance Evaluation

The pooled analysis of variance over two locations exhibited statistically significant ($p \leq 0.05$) differences among the location and accessions for all phenotypic traits considered in this study but non-significant effects for leaf area index, number of tillers/plant and spike lets per spike and grain filling duration. Genotype by environment interaction exhibited non significance values for the plant height, maturity date, number of tillers/plant, number of spike lets per spike and grain filling duration. Grain filling duration showed non-significance difference between the two locations and among the accessions (Table 5).

Table (5). Combined analysis of variance (over locations) for mean performances of genotypes tested at two locations

Traits	Mean square			CV(%)
	L	G	L*G	
Plant height (cm)	**	**	NS	12.31
Days to heading	**	*	**	5.76
Days to maturity	**	*	NS	2.77
Leaf area at maturity	NS	NS	*	30.89
Stem rust (first score)	**	**	**	29.82
Stem rust (second score)	**	**	**	21.25
Number of tillers/plant	**	NS	NS	25.99
Thousand kernel weight (g)	**	**	**	19.12
Harvest index (%)	**	**	**	9.91
Grain yield (g/plant)	**	**	**	10.59
Biomass yield (g/plant)	**	**	**	10.57
Grain filling duration	*	NS	NS	7.82
Spike length (cm)	**	**	NS	17.64
Number of spike lets per spike	**	NS	NS	18.24
Grain production efficiency (g/plant)	**	**	**	22.21
Biomass production rate (g/plant)	**	**	**	11.10
Economic growth rate (%)	**	**	**	14.75

** = highly significant (P < 0.01), * = significant (P < 0.05) and NS = non-significant (P > 0.05)

4.1.1.1. Effect of genotypes

There was significant ($P < 0.01$ or $P < 0.05$) differences among the accessions for all traits except for leaf area index at maturity, number of tillers/plant, grain filling duration and number of spikelet per spike (Table 5). There were larger coefficients of variation (CV) values (>20%) observed in many traits including leaf area index at maturity, stem rust, number of tillers/plant and grain production efficiency may be because there was moisture stress during certain periods of the cropping season. Differences among the treatments were more pronounced for economic growth rate followed by grain production efficiency; the minimum level of variation being in number of tillers/plant, number of spike lets per spike and spike length (Table 5).

According to grain yield there was no big difference between released varieties and landraces (Table 6). But relatively better grain yield was recorded from the released variety Mangud. Landraces accession 231467 exhibited relatively better yielding potential than others. Some landraces exhibited better performance for thousand kernel weight rather than the varieties. Accession 206554 was the least in grain yield. Assasa and Gerardo had shown the least potential in grain yield from the released ones. Complete resistance stem rust was not observed in any of the current durum wheat accessions studied. Four accessions 231618, 5487, 203922, 216545, and 203748 were found to have relatively better resistance for stem rust disease. From released varieties Yerer, Bichena, Quamy, Gerardo, Metetyaya, Assasa and kilinto have shown better resistance for stem rust incidence and severity.

Table 6. Average performances of the 160 durum accessions for two morpho-agronomic traits

Accession	G.yield	S.rust 2	Accession	G. yield	S.rust 2	Accession	G. yield	S.rust 2
231623	101.1L-Z	42.5A-P	5679	100.7L-Z	28.8E-P	227020	85.23a-o	38.14 A-P
231597	48.8X-Z3	38.3A-P	5739	109.56F-T	37.5 A-P	214343	131.77BC	37.75 A-P
231600	63.3s-y	42.8A-P	226892	67.29p-w	46.7A-O	7956	105.86I-V	54.21A-G
222855	116.4C-K	44.0A-P	222421	90.93U-Z	47.6A-M	207854	59.3U-Z3	56.65A-D
226094	119.2B-J	46.2A-P	222422	88.12L-Z	33.0B-P	206551	32.9E-G3	53.37A-H
8185	100.37l-z	51.6A-I	222428	95.53o-z	39.6A-P	206554	27.62G3	54.68A-F
8186	86.23z	51.2A-K	226868	80.60f-q	42.5 A-P	223257	90.28v-z	56.02A-E
214590	29.5B-G3	49.9A-L	226356	113.53E-M	43.4 A-P	226199	29.83FG3	52.94A-I
214550	102.3J-Z	27.2G-P	7073	58.11u-z	43.7 A-P	206558	82.73d-p	49.24A-L
7529	106.05I-V	42.5A-P	214498	54.06v-z	32.3C-P	238113	81.39e-p	50.39A-L
213149	69.3n-v	50.7A-L	7022	103.05j-z	36.7 A-P	226245	76.33l-s	61.86A
206573	101.2L-Z	46.7A-O	226273	85.25a-o	42.3 A-P	238114	40.1B-G3	46.42 A-P
222621	101.4L-Z	41.3A-P	227013	101.5k-z	39.4 A-P	238118	94.78o-z	38.99 A-P
222641	81.8f-q	45.2A-P	5927	58.57U-Z3	33.0B-P	238121	93.61s-z	49.82A-L
216614	124.5B-F	50.3A-L	231467	115.6D-L	39.2 A-P	238122	108.9G-T	52.88 A-I

222655	123.8B-H	49.9 A-L	222324	46.61Z	32.3C-P	238123	92.58t-z	46.34 A-P
222608	108.7G-T	52.94A-I	222338	98.28l-z	35.4A-P	238124	88.31x-z	43.96 A-P
222613	108.2 G-T	45.0A-P	204349	61.58s-z	50.5A-L	238125	96.27n-z	60.26AB
7412	105.3I-X	46.2 A-P	204370	93.54s-z	33.0B-P	238126	78.64h-s	57.22A-C
216474	73.1k-u	44.3 A-P	227060	102.60j-z	32.1C-P	238136	90.41u-z	55.31A-E
226951	97.6m-z	44.9 A-P	204357	90.11w-z	29.2D-P	238137	85.94z	55.73A-E
222616	109.9F-S	37.4 A-P	214503	72.08l-u	34.3B-P	238127	64.51q-w	48.10A-M
203750	99.9L-Z	44.9 A-P	203695	94.48p-z	40.9 A-P	238128	94.07r-z	46.30 A-P
203922	47.5yz3	25.5 J-P	203886	87.23z	39.4 A-P	238129	63.27s-y	50.80A-L
203893	110.9F-R	41.4 A-P	226180	68.71o-v	42.2 A-P	238130	63.53r-y	38.96 A-P
5487	110.7F-R	25.7I-P	226183	130.51B-D	31.2C-P	238131	39.5B-G3	50.62A-L
208212	104.5I-Y	44.7 A-P	222708	93.59s-z	39.4 A-P	238133	36.9C-G3	51.44A-J
208195	110.7F-R	41.3 A-P	231471	49.48X-Z3	42.9 A-P	238134	56.9U-Z3	50.03A-L
210821	84.4b-o	43.4 A-P	203690	69.65m-v	37.6 A-P	238135	95.58o-z	47.03A-N
226833	127.5B-E	50.4A-L	231603	106.29I-V	32.7C-P	Ginchi	106.26I-V	26.05H-P
226844	49.8X-Z3	33.2B-P	5730	103.1J-Z	31.5C-P	Yerer	111.4E-P	19.80N-P
231617	92.5T-Z	43.9A-P	203854	99.2l-z	33.0B-P	Worer	111.2E-Q	32.65C-P
231618	111.5E-O	39.2 A-P	226179	116.1C-L	34.6 A-P	Mangude	163.9A	23.66K-P
214515	115.4D-L	40.9 A-P	231606	84.15c-o	46.8A-O	Arendato	114.5D-M	46.04 A-P
8333	88.1yz	24.1J-P	231613	80.81f-q	44.6 A-P	Assasa	35.4D-G3	23.84K-P
8328	88.5X-Z	39.4 A-P	210808	79.9g-r	42.9 A-P	Denbi	105.7I-V	32.66C-P
214517	45.61Z3	43.9 A-P	5429	120.9B-I	41.0 A-P	Tob	70.28m-v	27.66F-P
222515	84.7b-o	39.6 A-P	216651	91.1u-z	40.0 A-P	LD-357	88.63x-z	27.36F-P
203840	107.2H-V	44.2 A-P	5314	118.6B-H	29.2D-P	Hitosa	64.42q-w	25.53I-P
5217	85.93z	32.8B-P	203748	113.2E-N	27.6F-P	Mukaye	55.1V-Z3	27.22F-P
204340	59.4U-Z3	41.0 A-P	5300	44.9A-F3	44.5 A-P	Killinto	86.28z	25.9H-P
6856	72.9l-u	46.6 A-P	5248	80.5f-q	45.0 A-P	Quamy	63.50r-y	23.35L-P
216492	92.54t-z	39.2 A-P	5180	110.2F-S	34.6 A-P	Gerardo	36.5C-G3	20.93M-P
216545	86.4z	30.3C-P	5736	95.7o-z	46.6 A-P	Foka	92.73t-z	29.59D-P
226207	94.24q-z	30.3C-P	214313	116.9C-K	28.5E-P	Cocorit	116.3C-L	30.9C-P
226117	114.6D-M	27.2F-P	226959	151.4A	40.6 A-P	Boohie	110.9F-R	27.4F-P
216440	133.4B	36.3 A-P	231528	78.9h-s	44.3 A-P	Bichena	52.2W-Z3	19.2P
208265	107.4H-U	36.5 A-P	222457	91.11u-z	37.7 A-P	Metyaya	94.62o-z	19.4O-P
208278	109.3F-T	37.5 A-P	222461	87.28z	40.35 A-P			
208286	93.5s-z	28.8E-P	231557	93.96r-z	36.25 A-P			
208310	80.9f-q	38.1 A-P	231526	125.7B-F	35.87 A-P			
208195	69.29n-v	49.6A-L	214328	82.20d-p	40.35 A-P			
208312	30.13B-F	44.3 A-P	5454	78.34i-s	37.86 A-P			
208317	28.08G3	42.4 A-P	5144	78.76h-s	47.59A-M			
208491	87.51yz	43.1 A-P	6101	81.47e-p	34.62 A-P			
226375	76.66j-s	49.0A-L	7206	86.07z	36.33 A-P			

4.1.1.2. Genotype by location interaction effects

Genotype by location interaction effects, i.e. the differential response of accessions at different locations or the differential discrimination of the locations or both, were significant for a number of traits including days to heading, leaf area at maturity, stem rust scores, seed size, harvest index, grain and biomass yields, grain production efficiency, biomass production rate and economic

growth rate but non-significant for other traits including plant height, days to maturity, number of tillers/plant, grain filling duration, spike length and number of spike lets per spike (Table 5).

This clearly showed that the two locations were distinctly different or the accessions responded differently to the two locations for these traits or both (Figure 1). Hence, for traits with significant genotype by location interaction effects, the best genotype under one location may not be the best under the other and a separate breeding program may be required. This may indicate the importance of spatial replication of trials in order to get accurate data.

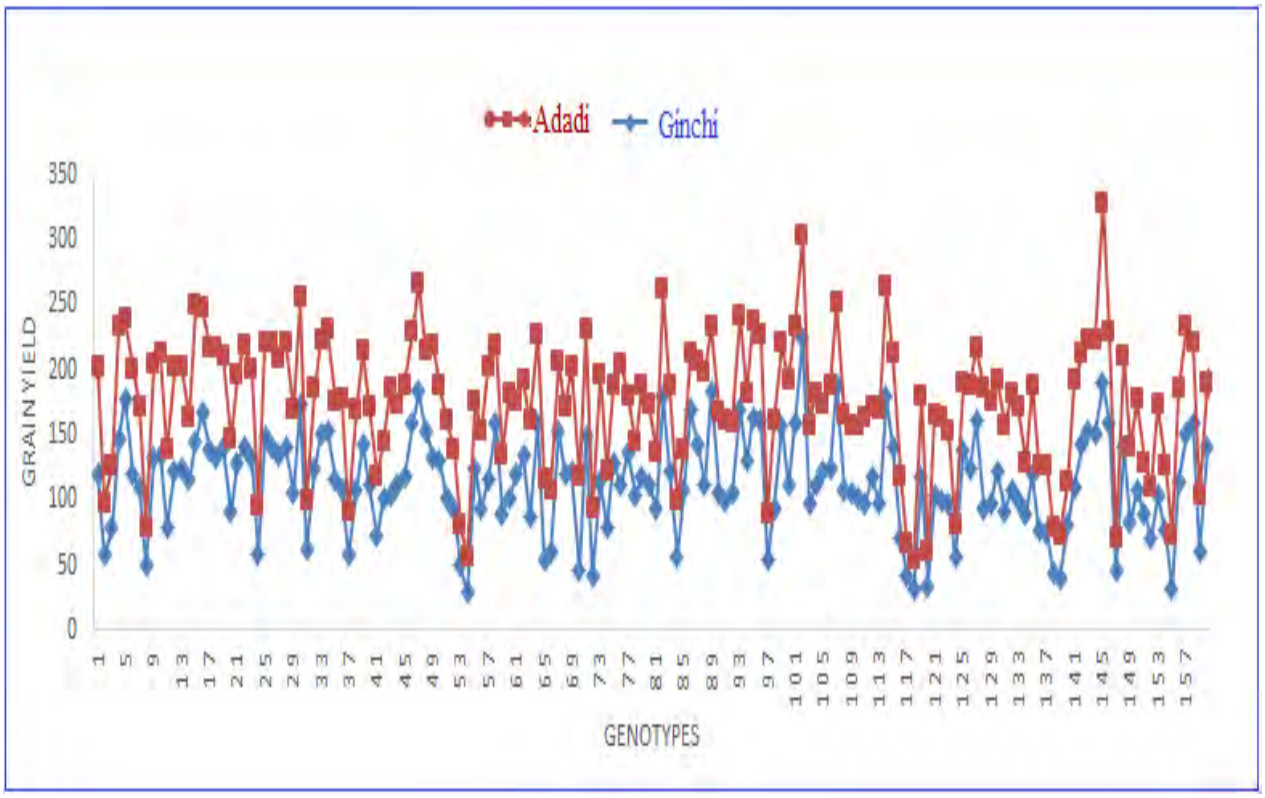

Figure 1. Responses of 160 durum wheat accessions to Grain yield at two locations showing the existence of crossover type of genotype by location interaction.

In such cases, the appropriateness of varieties for release in terms of performance consistency across a range of physical environments (spatial variability) and years (temporal variability) is usually confirmed by multi-location evaluation of varieties even if it is laborious, costly and time consuming Keneni *et al*. (2012). The non-significant effect of location by interaction effects may

indicate that accessions selected for better performance for these traits at one location may display a similar relative performance at another location. If accessions perform similarly across the locations, there may be no need for spatial replication as evaluation at a single location may be sufficient for the purpose.

4.1.2. Cluster and distance analysis

4.1.2.1. Cluster analysis

Cluster analysis of the 160 accessions distinguished them into seven different clusters (Figure 2 and Table 7), members within a single cluster or in clusters with non-significant distances being considered as similar or as having more close relationships with each other than they are with those in significantly distant clusters. The first cluster (C1, n = 113) had the largest number of accessions collected from three regions in Ethiopia. The second cluster (C2, n = 25) was the second largest in number of accessions constituted from all over the regions, the fourth and six clusters, with only a single genotype each, being the least in terms of number of accessions. The fifth and seventh clusters took the third and fourth ranks in terms of numbers of accessions.

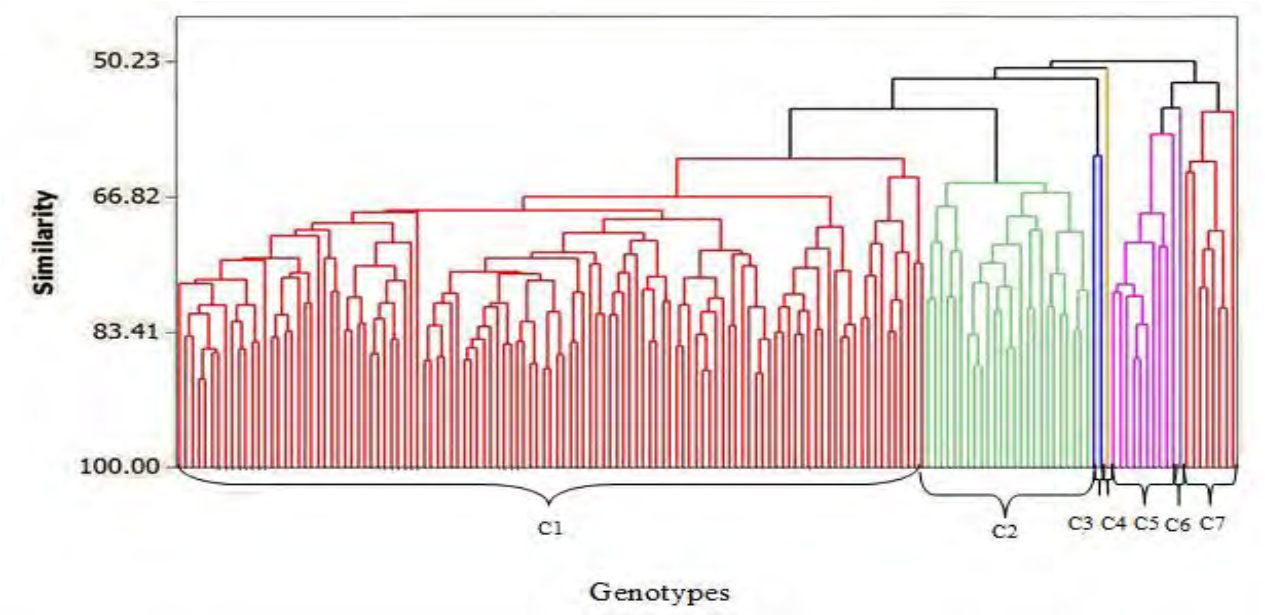

Figure 2. A dendrogram of 160 durum wheat accessions developed by average linkage method based on Euclidean distance using 17 morpho-agronomic characters.

Table 7. Clustering of 160 durum wheat accessions from different origins into seven clusters using 17 morpho-agronomic characters

Cluster	No. of Accessions	Accessions included in the cluster	Sources of accessions	Origins of accessions
1	113	231623,206573,222613,222616,203893,208212,238122,214550 ,216440,7022,226180,5217,208310,222421,204370,6856, 14328,8186, 231606, 226868,238118,238123, 238126, 238137,5314,214343, 216545, 226207,222608,222621, 5736,7412, 226356, 231617, 216492, 222515, 203695, 231618, 238129, 222641, 226273, 214515, 214503, 222422, 238124, 231557, 204357, 8185, 238136, 5248, 5679, 5180,231467, 231603, 222708, 226183, 226833, 226951, 238135, 216614, 7956, 222855, 208278, 214517, 226844, 231600,5144, 231528, 8328, 210808, 203886, 222338, 238125, 231613, 238128, 7206, 223257, 206558, 203748, 227060, 203750, 203757, 7529, 227013, 226094, 208195, 5739208265, 238130, 208286, 208491, 5454, 227020, 204340, 216474,226892, 208189, 226375, 222428, 216651, 238113, 226245, 5730, 203854, 222457, 6101, 231557, 5487, 226179, 214313, 5429, 226959	Collection	Tigray, Amhara and Oromiya
C2	25	231597,7073,206554,238133,226199,207854,231471, 927,213149, 8333, 238131, 203922, 222324, 238127, 208312, 204349, 203840, 208317,214590, 214590, 238114, 210821, 206551,5300, 214498	Collection	Tigray, Amhara and Oromiya
C3	2	222655, 238121	Collection	Amhara and Tigray
C4	1	238134	Collection	Tigray
C5	10	Ginchi, Cocorit, Arendato, Yerer, Kilinto, Foka, Boohie	Released V.	DZARC
C6	1	Mangud	Released .V	DZARC
C7	8	Assasa Gerardo Tob Hitosa Muka Quamy Bichena	Released V. LD-357	DZARC

4.1.2.2. Magnitude of phenotypic diversity

Genetic distances (D^2) between the clusters of 160 durum wheat accessions are presented in Table 8. Inter-cluster D^2 values ranged from 13 (between clusters C1 and C2) to 253.89 (between clusters C3 and C6). The maximum pairwise generalized squared distances (D^2) were found between clusters C3 and C6. Cluster C3 constituted landraces collected from Tigray and Amhara whereas cluster C6 constituted of released varieties. The minimum inter-cluster distances was observed between clusters C1 and C2. Cluster 3 was the most divergent group from all clusters formed by landrace collections. It showed higher distances with cluster 6 (253.89), cluster 5 (235.51) and cluster4 (149.69) respectively (Table 8).

Table (8). Pair-wise generalized squared distances between seven clusters constituting 160 durum wheat accessions

	Clusters						
Clusters	C1	C2	C3	C4	C5	C6	C7
C1	0.00	13.72^{NS}	128.80**	33.35**	60.26**	71.62**	57.35**
C2		0.00	149.94**	35.17**	73.20**	104.32**	45.88**
C3			0.00	149.69**	235.51**	253.89**	231.05**
C4				0.00	101.83**	135.74**	100.55**
C5					0.00	40.54**	29.55*
C6						0.00	75.95**
C7							0.00

* =Significant, **= highly significant and NS = Non significant

Cluster I: In the first cluster consisted of 113 landraces collected from the entire regions of collection. Members exhibited medium plant height and late mature than other clusters. They showed medium leaf area index and moderately resistant and moderately susceptible disease reaction in the first time of stem rust score and finally became more susceptible at last in the second time of score. This cluster was good in tillering capacity and relatively better in thousand kernel weights. From the first four clusters made by landraces, cluster one was the second in harvest index, grain yield and biomass yield next to cluster three. In all the characters, this cluster showed the medium performance from other clusters.

Cluster II: This cluster had consisted 25 landraces collected from all region of collection. Which were medium in plant height and relatively late matured than accessions of other clusters. Accessions in this cluster were characterized by lower in harvest index, grain and biomass yield. They were medium in grain filling duration but they showed lower efficiency in grain production and had small rate of biomass production and economic growth.

Cluster III: Consisted of two landraces collected from Amhara and Tigray region. It was characterized by late maturing and it needs longer grain filling period than others. It was more susceptible to stem rust disease. Accessions in this cluster showed lower thousand kernel weight, medium in grain yield and biomass yield. From the clusters made by landraces, this cluster had shown high biomass production and economic growth rate.

Cluster IV: This cluster had long height, late matured, large leaf area index, susceptible to stem rust, lower tillering capacity and harvest index. This cluster was the least performed cluster in terms of yield and yield components than the remaining clusters. Other clusters were relatively better in harvest index grain yield and biomass yield. This cluster had lower rate of biomass production and economic growth rate.

Cluster V: Consisted 10 released varieties. They were characterized by short plant height, relatively early matured and it required short time for grain filling. It has exhibited moderately resistance to stem rust disease reaction. It was the second cluster in grain yield performance next to cluster six. This cluster had better performance in harvest index, grain production efficiency, biomass production rate and economic growth rate.

Table 9. Differences among the three clusters of 160durum wheat accessions for mean performance of 17 characters

Character	Cluster							Grand Mean
	C1	C2	C3	C4	C5	C6	C7	
Plant Height (PHT)	107.25	107.74	108.75	117.5	85.91	90.00	88.89	100.86
Heading Date (HD)	62.86	62.39	56.83	62	63.95	62.25	63.19	61.92
Maturity Date (MD)	126.97	127.22	127.83	125.75	124.41	124.25	124.56	125.86
Leaf Area Index (LAI)	96.95	92.86	101.5	147.75	77.87	72.33	84.98	96.32
Stem Rust first score (SR1)	30.43	30.73	36.8	39.46	18.85	10.80	15.56	26.09
Stem Rust second score (SR2)	41.21	43.31	49.85	50.03	28.16	23.66	23.8	37.15
Tillering (TIL)	7.9	8.18	8.92	5.25	7.61	7.00	8.56	7.63
Thousand Kernel Weight (TKW)	29.83	25.42	19.93	24.54	27.21	34.23	30.3	27.35
Harvest Index (HI)	77.29	60.19	78.53	67.43	83.82	89.10	64.34	74.39
Grain Yield (GLD)	96.36	46.51	103.67	56.88	103.27	163.99	55.7	89.48
Biomass Yield (BM)	122.88	75.58	129.32	81	123.27	183.77	83.36	114.17
Grain Filling Duration (GFD)	64.1	64.55	71	63.75	60.02	62.00	60.61	63.72
Spike length (SL)	9.71	9.82	10.33	9.75	5.64	6.50	5.61	8.19
Spike lets Per Spike (SLPS)	20.25	20.08	18.83	16	17.07	17.75	19.75	18.53
Grain Production Efficiency (GPE)	99.89	49.06	159.75	60.45	95.26	165.03	53.75	97.60
Biomass Production Rate (BPR)	97.23	59.61	101.51	64.68	99.61	148.57	67.29	91.21
Economic Growth Rate (EGR)	151.37	72.01	146.87	88.68	175.56	264.88	91.57	141.56

Cluster VI: This cluster had consisted only one released variety which is characterized by best performance in terms of thousand kernel weight, harvest index, grain yield and biomass yield. It was resistant to stem rust disease reaction. It was early matured and it required shorter grain filling period. It had relatively short spike length with dense spike type. It was also characterized by high grain yield and biomass yield production. The only member included in this cluster was variety Mangud which was better in yield and yield components than released varieties and landrace

collections as well. It was more efficient in grain production and it has high biomass production and economic growth rate.

Cluster VII: This was the last cluster which consisted of 8 released varieties. They were small in yield performance than other clusters except cluster two. They were characterized by early maturing and short plant height. They also showed that smaller spike length with dense spike shape. It was moderate resistant to stem rust but it was lower in grain yield and biomass yield and also harvest index. The grain production efficiency, biomass production rate and economic growth rate were also lower for this cluster.

4.1.2.3. Pattern of phenotypic diversity

The local landraces were distributed over clusters C1-C4 but mostly fell into clusters C1 and C2 whereas the released varieties fell into three clusters C5, C6 and C7. Generally, the pattern of distribution of the accessions from different origins over different clusters was apparently random, showing that there was no clear association between geographic sources of origin and genetic diversity. Some accessions from the same places of origin fell into different clusters and *vice versa*. Similar results were found from previous works Gashaw *et al*. (2007).

Landraces collected from West Shewa and West Gojam zones showed limited phenotypic diversity in that their members were entirely grouped only into cluster C1. The landraces collected from the other zones, with the exception of accessions from Central Tigray, Eastern Tigray, South Gondar and South Wello which were either distributed over distantly divergent or more than two clusters, fell into the first two clusters (C1 and C2) with a non-significant distance. The detailed distribution of the accessions over the clusters is given in table 10.

Table 10. Clustering pattern of Durum wheat accessions from different origins over seven clusters based on mean performance of 17 response characters at two locations.

Origin	No.of accessions	No. of accessions in each cluster						
		C1	C2	C3	C4	C5	C6	C7
North Shewa	11	9	2	-	-	-	-	-
Arssi	11	8	3	-	-	-	-	-
Bale	7	5	2	-	-	-	-	-
West Harergie	3	3	-	-	-	-	-	-
East Harergie	11	9	2	-	-	-	-	-
East Shewa	11	10	1	-	-	-	-	-

West Shewa	11	11	-	-	-	-	-	-
South Wello	11	8	-	3	-	-	-	-
South Gonder	11	10	-	1	-	-	-	-
West Gojam	6	6	-	-	-	-	-	-
East Gojam	11	9	2	-	-	-	-	-
North Gonder	9	8	1	-	-	-	-	-
Southern Tigray	10	5	5	-	-	-	-	-
Central Tigray	10	9	-	1	-	-	-	-
Eastern Tigray	8	4	3	-	1	-	-	-
Released Varieties	19	-	-	-	-	10	1	8

4.1.2.4. Principal component analysis

Five of the 17 principal components accounted for more than 76.98% of the total variation in the Ethiopian durum wheat accessions. The first principal components accounted for 32% of the total variations. The corresponding value for the second principal components was 20% (Table 11). The first two principal components (PRIN1 and PRIN2) contributed about 52.93% of the total variation. Generally, the traits included in the first principal component had small effects of individual contribution (-0.089 to 0.416) to the variation in PRIN1 but characters with relatively larger absolute values of eigenvector weights in PRIN1 had the largest contribution to the variation of the accessions into clusters, as it is normally assumed that characters with larger absolute values closer to unity within the first principal component influence the clustering more than those with lower absolute values closer to zero Chahal and Gosal (2002). Therefore, the differentiation of the accessions into different clusters was rather dictated by the cumulative effects of a number of characters Keneni *et al*. (2013).

Characters with relatively greater positive weights of eigenvectors in PRIN1 include grain yield, economic growth rate, biomass production rate, biomass yield and harvest index. Selection based on these characters may be effective because of the higher comparative variability. Other characters like days to heading and number of spike lets per spike had smaller negative/ positive eigenvector values contributed the least share to the total variation of accessions in the first principal component. Spike length, grain filling duration, plant height, stem rust scores and leaf area index had relatively larger positive contribution to the second principal component. Different characters also contributed to the variation in the third fourth and five principal components (PRIN3 PRIN4, and PRIN5) but both components accounted for relatively smaller total variations of 9.7%, 8% and 6.2%, respectively.

Table (11). The Eigen values and vectors of the correlation matrix for 17 traits of 160 *Triticum turgidum L.* landraces of Ethiopia.

Parameter		PRIN1	PRIN2	PRIN3	PRIN4	PRIN5
Eigen value		5.60	3.19	1.67	1.31	1.14
% variance		32.91	20	9.7	8	6.2
Cumulative		32.91	52.93	62.71	70.77	76.98
Character	Eigen vectors					
Plant Height (PHT)		-0.069	0.358	0.141	0.124	-0.156
Heading Date (HD)		-0.003	-0.222	0.497	0.248	0.413
Maturity Date (MD)		-0.072	0.315	-0.237	0.027	0.434
Leaf Area Index (LAI)		-0.054	0.303	0.201	0.231	-0.026
Stem Rust first score (SR1)		-0.089	0.325	0.437	-0.164	-0.074
Stem Rust second score (SR2)		-0.119	0.353	0.291	-0.247	-0.089
Tillering (TIL)		-0.062	0.037	0.014	-0.302	0.560
Thousand Kernel Weight (TKW)		0.116	-0.060	-0.143	0.558	-0.255
Harvest Index (HI)		0.348	0.053	0.009	-0.073	0.115
Grain Yield (GLD)		0.416	0.083	0.037	-0.027	0.047
Biomass Yield (BM)		0.403	0.094	0.062	-0.008	0.016
Grain Filling Duration (GFD)		-0.048	0.377	-0.517	-0.156	0.012
Spike length (SL)		-0.075	0.418	0.102	0.294	-0.087
Spike lets Per Spike (SLPS)		-0.017	0.150	-0.137	0.505	0.443
Grain Production Efficiency (GPE)		0.384	0.184	-0.129	-0.080	-0.038
Biomass Production Rate (BPR)		0.405	0.069	0.076	-0.013	-0.018
Economic Growth Rate (EGR)		0.414	0.014	0.124	-0.012	0.061

PRIN1, PRIN2, PRIN3, PRIN4 and PRIN5 = Principal component 1, 2, 3, 4 and 5 respectively

4.1.3. Interrelationships between characters

4.1.3.1. Phenotypic Correlations

Interrelations between the characters revealed strong associations in a number of cases both in positive and negative directions. Plant height showed highly significant positive phenotypic correlation with days to maturity (r = 0.39), stem rust (r = 0.40) and (r=0.41), grain filling duration (r = 0.79) and biomass yield (r = 0.53). Highly significant negative associations were observed between stem rust disease and thousand kernel weight (r = 0.41) and (r = 0.32) at two levels respectively.

Table 12. Phenotypic Correlation coefficients (r) between agronomic characters in 160durum wheat accessions grown at two locations in Ethiopia.

	PH	HD	MD	LAI	SR1	SR2	TIL	TKW	HI	GLD	BM	GFD	SL	SLPS	GPE	BPR	EGR
PH	1																
HD	-0.73 **	1															
MD	0.39 **	-0.46 **	1														
LAI	0.58 **	-0.67 **	0.41 **	1													
SR1	0.40 **	-0.44 **	0.13 NS	0.53 **	1												
SR2	0.47 **	-0.48 **	0.35 **	0.63 **	0.71 **	1											
TIL	-0.26 **	0.27 **	-0.15 NS	-0.38 **	-0.41 **	-0.32 **	1										
TKW	-0.31 **	0.44 **	-0.26 **	-0.35 **	-0.27 **	-0.27 **	0.22 **	1									
HI	-0.09 NS	0.14 NS	-0.10 NS	-0.17 *	-0.19 *	-0.13 NS	0.17 *	0.77 **	1								
GLD	0.00 NS	0.03 NS	-0.22 **	-0.13 NS	-0.19 *	-0.18 *	0.18 *	0.59 **	0.94 **	1							
BM	0.53 **	-0.55 **	0.33 **	0.36 **	0.15 NS	0.25 **	-0.07 NS	0.06 NS	0.42 **	0.60 **	1						
GFD	0.79 **	-0.92 **	0.48 **	0.72 **	0.49 **	0.51 **	-0.32 **	-0.45 **	-0.17 *	-0.08 NS	0.60 **	1					
SL	-0.22 **	0.29 **	-0.14 NS	-0.21 *	-0.21 *	-0.19 *	0.09 NS	0.05 NS	-0.06 NS	-0.08 NS	-0.17 *	-0.24 **	1				
SLPS	-0.23 **	0.24 **	-0.16 NS	-0.26 **	-0.20 *	-0.20 *	0.19 *	0.75 **	0.92 **	0.86 **	0.35 **	-0.26 **	0.00 NS	1			
GPE	0.05 NS	-0.10 NS	0.02 NS	-0.04 NS	-0.15 NS	-0.05 NS	0.15 *	0.54 **	0.90 **	0.97 **	0.65 **	0.04 NS	-0.10 NS	0.81 **	1		
BPR	-0.14 NS	0.19 *	-0.13 NS	-0.23 **	-0.25 **	-0.16 *	0.21 *	0.77 **	0.97 **	0.93 **	0.37 **	-0.26 **	-0.07 NS	0.87 **	0.89 **	1	
EGR	0.02 NS	0.07 NS	-0.09 NS	-0.16 NS	-0.22 **	-0.10 0.NS	0.20 *	0.58 **	0.75 **	0.80 **	0.52 **	-0.11 NS	-0.04 NS	0.66 **	0.78 **	0.77 **	1

*PH = plant height, HD = heading date, MD = maturity date, LAI = leaf area index, SR1and SR2 = stem rust first and second score, TIL = number of tillers per plant, HI = harvest index, GLD = grain yield, BM = biomass yield, GFD = grain filling duration, SL = spike length, SLPS = number of spike lets per spike, GPE = grain production efficiency, BPR = biomass production rate, EGR = economic growth, ** highly significant, * significant, NS non-significant.

4.1.3.2. Genotypic correlations

Based on the values of genotypic correlation coefficient plant height was negatively correlated with heading date (r = -0.18), number of tiller per plant (r = -0.02), thousand kernel weight (r = -0.02), harvest index (r = -0.15), biomass production rate (r = -0.06) and economic growth rate (r = -0.15). It has positive but non-significant correlation coefficients with grain (r = 0.09), biomass yields (r = 0.04). On the contrary, plant height showed highly significant positive correlation coefficients at genotypic levels with stem rust diseases (r = 0.44) and spike length (r = 0.56). Significant positive genotypic correlation coefficients were also observed between grain yield and two other traits, namely thousand kernel weight (r = 0.19) and harvest index (r = 0.83). Grain yield had negative non-significant genotypic correlation with spike length (r = -0.06) and spike lets per spike (r=-0.02). biomass yield (r = 0.97), grain production efficiency (r = 0.94), biomass production rate (r = 0.96) and economic growth rate (r=0.98) but significant negative correlation with stem rust (r = -0.26) and grain filling duration (r = -0.24), which indicated that accessions with the ability to fill their grains within shorter periods of time produced better yield than those with longer grain filling periods. Biomass yield had significant positive genotypic correlation coefficients with grain filling duration (r = 0.15) and grain production efficiency (r = 0.91). Spike length had non-significant but negative correlation with both grain yield (r = 0.06) and biomass yield (r = 0.03). Early accessions that are able to complete their life cycle within a short duration could escape the effect of terminal moisture stress and may perform better than late accessions in areas with short growing duration. In some cases, environment affects both the traits simultaneously in the same direction or some time in different directions. Genetic and environmental causes of correlation combine together and give phenotypic correlation. The dual nature of phenotypic correlation makes it clear that the magnitude of genetic correlation cannot be determined from phenotypic correlation. Therefore, estimation of degree of genotypic and phenotypic correlation of grain yield with yield components is very important to utilize the available genetic variability through selection Singh *et al*. (1998).

Table13. Genotypic correlation coefficients (r) between agronomic characters in 160 Ethiopian durum wheat accessions.

Traits	PH	HD	MD	LAI	SR1	SR2	TIL	TKW	HI	GLD	BM	GFD	SL	SLPS	GPE	BPR	EGR
PH	1																
HD	-0.18 *	1															
MD	0.27 **	-0.02 **NS**	1														
LAI	0.12 NS	-0.02 **NS**	0.06 *	1													
SR1	0.35 **	0.01 **NS**	0.17 *	0.22 **	1												
SR2	0.44 **	-0.13 **NS**	0.25 **	0.02 NS	0.73 **	1											
TIL	-0.02 **	0.01 NS	0.06 *	-0.01 NS	0.08 NS	0.08 NS	1										
TKW	-0.02 NS	0.00 NS	-0.12 NS	-0.05 NS	-0.21 *	-0.29 **	-0.18 *	1									
HI	-0.15 NS	0.02 NS	-0.01 NS	-0.07 NS	-0.09 NS	-0.12 NS	-0.10 NS	0.12 NS	1								
GLD	0.09 NS	-0.01 NS	0.71 **	-0.04 NS	-0.18 *	-0.26 **	-0.11 NS	0.19 *	0.83 **	1							
BM	0.04 NS	-0.02 NS	0.36 *	-0.03 NS	-0.07 NS	-0.14 NS	-0.09 NS	0.20 *	0.69 **	0.97 **	1						
GFD	0.32 **	-0.72 **	0.23 *	0.05 NS	0.11 NS	0.27 **	0.03 NS	-0.08 NS	-0.02 NS	-0.24 *	-0.15 *	1					
SL	0.56 **	-0.17 *	0.04 NS	0.34 **	0.43 **	0.45 **	-0.03 NS	0.03 NS	0.09 NS	-0.06 NS	-0.03 NS	0.37 **	1				
SLPS	0.17 *	-0.05 NS	-0.17 NS	0.04 NS	0.03 NS	0.05 NS	0.14 NS	0.15 NS	0.03 NS	-0.02 NS	0.00 NS	0.19 *	0.33 **	1			
GPE	0.01 NS	-0.30 **	-0.17 NS	0.00 NS	-0.06 NS	-0.08 NS	-0.10 NS	0.18 *	0.77 **	0.94 **	0.91 **	0.24 **	0.05 NS	0.03 NS	1		
BPR	-0.06 *	-0.02 NS	0.71 **	-0.04 NS	-0.08 NS	-0.15 NS	-0.10 NS	0.21 *	0.69 **	0.96 **	1.00 **	-0.10 NS	-0.06 NS	-0.02 NS	0.90 **	1	
EGR	-0.15 NS	0.12 NS	0.36 *	-0.06 NS	-0.12 NS	-0.20 *	-0.10 NS	0.19 *	0.82 **	0.98 **	0.95 **	-0.21 *	-0.13 NS	-0.06 NS	0.87 **	0.96 **	1

*PH = plant height, HD = heading date, MD = maturity date, LAI = leaf area index, SR1and SR2 = stem rust first and second score, TIL = number of tillers per plant, HI = harvest index, GLD = grain yield, BM = biomass yield, GFD = grain filling duration, SL = spike length, SLPS = number of spike lets per spike, GPE = grain production efficiency, BPR = biomass production rate, EGR = economic growth, ** highly significant, * significant, NS non-significant.

4.1.4. Comparison of selected accessions with the original population

Comparison of the average performance for respective characters of the selected subsets of the top 5% accessions with the average performances of the whole population revealed possibilities for different level of improvements through selection. Selected accessions were short in plant height, early matured and moderately resistant for stem rust compared to the population means (Table 14). The t-test showed significant differences between means of the selected subsets of the top 5% best accessions ($\overline{X}$) and the population parameters (μ) for traits of thousand kernel weight, harvest index, grain yield, biomass yield, grain production efficiency, biomass production rate and economic growth rate ranging from 0.10% for heading date to 39.92% for economic growth rate. Plant height, heading date, maturity date tillering capacity, spike length and stem rust disease reaction were not significantly changed through selection (Table 14).

It should be noted that selection of superior accessions based on phenotype in a single environment may not repeat under another set of environments at least for the characters that showed genotype by environment interaction effects. This is because the phenotype is the result of not only genetic but also environmental and genotype by environment interaction effects Singh (2002). When selecting for phenotypic performance in the presence of significant genotype by environment interaction, particularly for primary traits of economic importance like grain yield, the plant breeder should weigh the importance of performance consistency of accessions across environments to its average performance over environments, i.e. performance stability Keneni *et al*., (2012).

Table 14. Comparison of the mean performances of selected subsets of the 5% best accessions with their characters and with the average performances of the whole population (μ) of 160 Durum wheat accessions.

Characters	Population parameter (μ)	Mean of selected accessions ($\bar{X}$)	Change through selection (/μ- $\bar{X}$/)	Change as % of population parameter (μ)	t
Plant height	107.59	107.26	0.33	0.31	0.15 NS
Heading date	62.71	62.77	0.06	0.10	0.14 NS
Maturity date	127.05	126.69	0.36	0.28	0.80 NS
Leaf area index	96.85	95.07	1.78	1.84	0.51 NS
Stem rust first score	30.71	29.42	1.29	4.20	0.76 NS
Stem rust second score	41.86	38.80	3.06	7.31	1.54 NS
Tillering	7.97	7.63	0.34	4.27	1.47 NS
Thousand kernel weight	28.97	32.88	3.91	13.50	2.59 **
Harvest index	74.25	82.92	8.67	11.68	4.11 **
Grain yield	87.49	121.49	34.00	38.86	6.05 **
Biomass yield	114.50	145.80	31.30	27.34	5.87 **
Grain filling duration	64.34	63.92	0.42	0.65	0.65 NS
Spike length	9.75	9.61	0.14	1.44	0.44 NS
Spike lets per spike	20.19	20.33	0.14	0.69	0.42 NS
Grain prod. efficiency	91.60	126.77	35.17	38.40	5.69 **
Biomass production rate	90.55	115.62	25.07	27.69	5.88 **
Economic growth rate	136.98	191.53	54.55	39.82	5.98 **

The 5% best landrace accessions for grain yield were found to be superior by 17.86- 43.23% over Mukye and 9.21%-32.72% to the mean grain yield performance of all the released varieties (Table 15).

Landraces (226959) collected from Oromia region East Shewa zone Minjrna Shenkora woreda and (216545) collected from North Gonder Debark, were the first in five best high yielder accessions, (214343) from Southern Tigray Enda- mehoni, (226183) from Harargie Kersa, (208189) from East Gojjam Enarj Enawga woreda, (231526) from west Shewa Gende Beret, (216614) from South Gonder Lay Gayint were the 5% best accessions in their yielding potential. According to this study, all of the three regions had high yielder landraces which can be the parental material for future durum wheat improvement program.

Table 15. Comparison of mean performances of 5% of the accessions selected for best Agronomic performance with Mukye a recently released variety, and with mean performances of released varieties.

Acce. No.	Mean of selected accession	Comparative advantage over		Acce. No.	Mean of selected Acce.	Comparative advantage over	
		Mukye	MRV			Mukye	MRV
Grain Yield				Biomass Yield			
226959	151.35	43.23	32.72	208195	151.67	31.19	8.88
216545	133.39	26.23	16.97	226959	153.69	32.94	10.33
214343	131.77	24.70	15.55	5429	154.13	33.32	10.65
226183	130.51	23.51	14.44	226207	155.22	34.26	11.43
208189	127.47	20.63	11.78	214343	160.37	38.72	15.13
231526	125.72	18.97	10.24	226183	176.46	52.63	26.68
216614	124.54	17.86	9.21	231467	179.49	55.25	28.85
Mean	132.11	25.02	15.84	Mean	161.57	39.76	15.99
Mukye	105.67	---	-7.33	Mukye	115.61	-----	-17.01
MRV	114.04	7.92	---	MRV	139.30	2049	----
Acce. No.	Mean of Selected Acc.	Comparative advantage over		AcceNo accession	Meanof Selected Acc.	Comparative advantage over	
		Mukye	MRV			Mukye	MRV
Harvest index				Grain production Efficiency			
226844	111.25	40.24	40.75	238121	163.90	84.95	57.17
214515	89.07	12.28	12.69	226207	158.18	78.49	51.69
231617	88.67	11.77	12.18	222655	157.68	77.93	51.21
6614	87.87	10.77	11.17	226959	144.02	62.51	38.11
222855	86.08	8.51	8.91	226094	140.48	58.52	34.71
227013	86.05	8.47	8.87	226183	136.06	53.53	30.48
5487	84.94	7.07	7.46	208195	135.23	52.60	29.68
Mean	90.56	14.16	14.58	Mean	147.93	66.93	41.86
Mukye	79.33	---	0.33	Mukye	88.62	---	-15.02
Acce. No.	Mean of selected acc.	Comparative advantage over		Acce. No.	Mean of selected acc.	Comparative advantage over	
		Mukye	MRV			Mukye	MRV
Biomass Production Rate				Economic Growth Rate			
226959	139.88	48.51	22.25	226959	247.98	59.96	25.62
208195	138.84	47.40	21.34	231526	216.66	39.75	9.75
5429	130.20	38.23	13.79	214343	209.64	35.23	6.20
226183	123.85	31.49	8.24	226183	206.60	33.26	4.66
226207	122.41	29.96	6.98	214313	200.46	29.30	1.55
231467	121.15	28.62	5.88	226356	198.03	27.74	0.32
226179	120.91	28.37	5.67	226207	195.43	26.06	-1
Mean	128.18	36.08	12.02	Mean	210.68	35.90	6.73
Mukye	94.19	---	-17.68	Mukye	155.03	---	27.34
MRV	114.42	21.48	---	MRV	197.41	-21.46	---

4.1.5. Heritability and Genetic Advance

Heritability is a significant parameter for the selection of an efficient population improvement method Abinasa *et al*. (2011). Single plant selection and that in the earlier generations may be much effective for a character that is highly heritable as compared to that a character which is less heritable. Furthermore, environment may also interact with the genotypic constitution to influence heritability Raiz (2003). In the present study spike length, grain yield, stem rust disease reaction, harvest index, economic growth rate, biomass production rate and biomass yield exhibited relatively higher broad sense heritability.

Relatively high expected genetic advance estimates were obtained for grain production efficiency, grain yield and biomass yield. Relatively intermediate to high estimates of heritability and high estimates of genetic advance (as percentage of mean) was observed in the present study for grain yield, grain production efficiency and economic growth rate. Broad sense heritability values ranged from 10 -78% and the genetic advance was ranged from (0-55%). Based on the values of genotypic and phenotypic coefficient of variance, most of the traits were significant except leaf area index at maturity, tillering and spike lets per spike. Maximum PCV was observed from number of tillers/plant (41%) followed by thousand kernel weight (35.28). The higher PCV and GCV values for some of the characters (EGR, GFD, Tillering and thousand kernel weight) might be evidence for the existence of a wide range of variation for such characters. In other way, the PCV values of some characters like plant height, heading date, maturity date, biomass yield spike lets per spike and grain production efficiency were closer than the corresponding GCV values showing little environment effect on the expression of these characters. Selection on a phenotypic basis may be effective for the genetic improvement of such traits. But the PCV values of some of the characters were far from the values of GCV. This indicates that the higher environmental effect on the expression of these characters. Selection on the phenotypic basis may not be effective in this case. The smallest value has been showed from heading date, maturity date, and leaf area index at maturity and biomass production rate.

Table 16. Mean Square, genotypic, environment and phenotypic coefficient of variability, heritability and expected genetic advance for different characters of 160 durum wheat accessions

Variable	Mean square	GCV (%)	PCV (%)	h^2 (%)	GA	GAM
Plant height	394.26**	6.61	9.44	49	10.04	9.54
Heading date	17.26*	0.00	3.31	0	0	0
Maturity date	16.74*	0.88	1.61	29	1.22	0.96
Leaf area index	1004.65NS	0.00	16.69	0	0	0
Stem rust first score	446.78**	23.73	26.81	65	14.17	55.58
Stem rust second score	824.75**	14.46	22.74	42	12.44	30.57
Tillering	4.40NS	33.49	41.45	13	0.28	3.54
Thousand kernel weight	190.88**	22.80	35.28	10	1.43	4.93
Harvest index	374.24**	4.65	13.18	64	12.77	17.14
Grain yield	2647.18**	7.47	23.89	65	34.5	39.53
Biomass	2387.48**	10.41	12.99	55	27.72	24.36
Grain filling duration	34.83**	23.68	29.47	28	1.7	2.67
Spike length	9.24**	15.92	21.47	78	2.45	26.44
Spike length per spike	9.90NS	2.43	4.62	0	0	0
Grain production efficiency	3200.38**	14.49	16.43	57	33.27	36.87
Biomass production rate	1524.06**	0.00	7.89	55	22.15	24.55
Economic growth rate	6987.61**	23.63	31.35	63	54.33	39.41

*, ** significant at 5 and 1% level of probability, respectively; ns Non significant

High heritability magnitude indicates the reliability with which the high chance of the genotype to be recognized by its phenotypic expression Chandrababu and Sharma (1999). Zero heritability values were observed for heading date, leaf area index and spike lets per spike. In addition, Low heritability values were exhibited for thousand kernel weight, tillering, grain filling duration and maturity date (Table.16). High heritability percentage was observed from spike length, grain yield, harvest index, biomass yield, grain production efficiency, economic growth rate and also from plant height. Maximum expected genetic advance as percentage of mean was observed on economic growth rate, grain yield and grain production efficiency. Table.16 indicating the presence of additive gene effects; while the minimum was for heading date, maturity date, leaf area index, spike lets per spike and grain filling duration.

4.1.6. Comparison of four secondary traits as determinants of grain yield, study of yield contributing components

From the analysis of variance two of the secondary traits showed non significance difference and the remaining two were significant both at $P<0.05$ and $p<0.01$ (Table.17). This means accessions

have not varied for number of tillers/plant and spikelets per spike. But they varied significantly for thousand kernel weight and spike length. But, accessions were varied for spike length, thousand kernel weight and grainyield.

Table 17. Significance of combined genotype effects for grain yield quintal /ha and yield components in durum wheat experiments conducted at Ginchi and Adadi Maryam.

Traits	Mean Squares		
	Adadi	Ginchi	Combined
Spike length	**	**	**
Spike lets per spike	NS	NS	NS
Thousand Kernel weight (g)	*	**	**
Number of tillers/plant	NS	NS	NS
Grain yield	**	**	**

NS, * and** represents non-significant, significant at (p<0.5%) and tat (p<.01%).

Genetic variance, broad sense heritability, and response for direct selection for each trait are given (table 18). Phenotypic variance in secondary traits was high for thousand kernel weight (47.72) but for other traits it was small. According to genotypic variance grain yield was high rather than all the secondary traits. Broad sense heritability was high for spike length. Selection based on phenotypic performance will be effective to improve this trait Keneni *et al*. (2001). Spike lets per spike has zero broad sense heritability. The genetic variance for this trait was zero and the phenotypic variance was 2.48. Its broad sense heritability was zero. This indicates that the trait was more influenced by environmental effects than genotype effects. In other way the accessions were not as such variable for this trait. Selection is difficult for the traits which have low heritability because of that the environment effect can influence the genetic effects Keneni *et al*. (2001).

Table 18. Combined Mean performance, phenotypic, genotypic variances, broad sense heritability and expected genetic gain from direct selection for yield and yield other three traits of durum wheat experiment conducted in 2014.

Traits	Mean	PV	GV	h^2	DR
Spike length	9.25	2.31	1.8	0.78	1.18
Spikelet per spike	19.95	2.48	0	0	0
Thousand kernel weight (g)	28.92	47.72	4.67	0.10	0.69
Number of tillers/plant	7.96	1.1	0.14	0.13	0.15
Grain yield	87.28	661.8	427.24	0.65	16.72

According to this study, the genotypic correlation coefficient thousand kernel weight and grain yield exhibited positive significant association. But the other traits of spike length, spikelets per spike and number of tillers/plant have non- significant association with the grain yield.

Table 19. Correlation coefficients (r) of grain yield with yield components in durum wheat

Traits	Genetic correlation
Spike length	-0.06 non- significant
Spike lets per spike	-0.02 non -significant
Number of tillers/plant	-0.11 non- significant
Thousand Kernel weight (g)	0.19 significant

Relative efficiencies between direct selection for grain yield and indirect selection for yield components were listed on the above table. In direct selection of yield through selecting yield components have low relative efficiencies than selecting directly by grain yield itself. The above result indicates that selection based on, all of the traits of yield components showed lower relative efficiency than selection directly for grain yield. Spike lets per spike has zero relative efficiency. This due to that it has low (zero) heritability and very small genotypic and phenotypic variances. In direct selection of yield through thousand kernel weight the relative efficiency was 0.19. In general, based on the relative efficiency of the above traits, selection of accessions for yielding potential would be effective by selecting directly for grain yield itself rather than selecting secondary traits.

Table 20. Response of yield to indirect selection (IR) and efficiency of indirect selection (IR/DR) as compared to direct ones.

Trait	IR	IR/DR
Spike length	-0.08	-0.06
Spike lets per spike	0	0
Number of tillers/plant	-0.24	-0.35
Thousand kernel weight	0.131	0.19

4.1.7. Genetic progress from breeding

Grain yield is the first priority in durum wheat improvement program. In this experiment, therefore, genetic progresses achieved from breeding efforts since the last 46 years were discussed in terms of all the characters recorded but with great focus on grain yield as the most important economic traits.

4.1.7.1. Grain yield

The mean grain yield of individual accessions from the combined mean of two tested environments ranged from 6199.63 kg/ha (Mangud) to 4167.88 kg /ha (Assasa). The second best genotype in terms of grain yield was Mukye with a yield of 6054.25 kg/ha. Disregarding variety Worer, which was released for specific adaptation to moisture stressed area (i.e 450-1200 masl.), the yield range remained the same with the above. The two locations have differed in their range and accessions also differ in their rank too. At the first location Adadi the highest yield was scored from variety Worer (Mangud kg/ha). At Ginchi the high yielder genotype was Mangud (Worer). In the case of low yielder accessions Assasa was for Adadi with yield of 2913.13kg/ha and Kilinto was for Ginchi with 3141.12kg/ha grain yield.

Table 21. Mean grain yields (kg ha-1) and their rank orders of the test varieties at each location

Locations						
No.	Varieties	Adadi	Rank	Ginchi	Rank	Mean
1	Arendato	2105.3	18	2108.08	17	4213.38hi
2	Cocorit	2154.63	19	2357.87	19	4512.5 fgh
3	Gerardo	2575.13	15	2449.12	18	5024.25 ef
4	LD - 357	2214.61	9	2216.64	16	4431.25 gh
5	Boohie	2381.87	17	2161.88	9	4543.75fg
6	Foka	2219.35	10	2417.28	13	4636.63 fg
7	Kilinto	2253.6	8	2060.53	14	4314.13gh
8	Tob	2607.07	3	2407.06	12	5014.13 ef
9	Bichena	2904.38	16	2704.37	3	5608.75 c
10	Quamy	2637.5	12	2437.50	10	5075 e
11	Assasa	2053.26	13	2114.62	6	4167.88 i
12	Ginchi	2506.5	14	2506.50	8	5013 ef
13	Yerer	2484.57	5	2684.56	2	5169.13 de
14	Meteyaya	2444.25	7	2586.63	15	5030.88 ef
15	Hitosa	2910.44	6	2310.44	4	5220.88 c-e
16	Dendi	2541.69	4	2741.69	5	5283.38 cd
17	Worer	2816.07	2	3144.06	11	5960.13 bc**
18	Mangud	3195.82	1	3003.81	1	6199.63 a
19	Mukye	2913.13	11	3141.12	7	6054.25 ab
	Grand mean	2522.06		2502.83		
	CV (%)	13.93		9.21		
	R^2	0.52		0.49		

** Varieties released for moisture stress and varieties with the same letters have not significant difference varieties with different letters have significant difference.

The annual rate of gain in yield potential was estimated from linear regression of mean grain yields of varieties on year of release expressed as the number of years since 1966, the period when the oldest variety Arendato was released. Experimental evidence of gains in Durum wheat yield showed that at average rate of increase in yield potential per year of release over 46 years period from the slope of linear regression shown in the graph was 30.99 kg/ha (0.74%) per year over the oldest variety Arendato. If estimation is made from equation Y= 30.99X + 4213.38, the estimated grain yield at initial year (1960s) was 4213.38 kg ha-1 compared with 4523.28 kg ha-1 at 10 years (1970s), 4833.08 kg ha-1 at 20 years (1980s), 5143.38 kg ha-1 at 30 years (1990s), 5453.38kg/ha at 40 years (2000s) and 5763.38kg/ha at 50 years (2010s). The genetic progress of grain yield obtained since 1966 using the good performed variety (Mangud) as compared to one of the oldest varieties (Arendato) was 1125.5 kg/ha in 46 year or close to 31kg/ha per year. Varieties released in 1970s (Cocorit, Gerardo and LD-357) exhibited the yield increment of 442.62 kg/ha over the oldest variety Arendato (4213.38). They have 10.5% yield increment compared with the oldest variety Arendato. Boohie was released in 1982 and it has 330.37 kg/ha or 7.84 in percentage yield increment over Arendato. The 1990s released varieties (Foka, Kilinto, Tob, Bichena, Quamy and Assasa) have showed that the genetic progress of grain yield was 589.37kg/ha. That was 14.10% from the oldest variety Arendato.

In other way the recent varieties released in 2000s (Ginchi, Yerer, Meteyaya, Hitosa, Dendi and Worer) were exhibited genetic progress in terms of grain yield which was 1066.19kg/ha and this was 25.30 % over the oldest variety Arendato. The two varieties (Mangud and Mukye) were released in 2012. They are latest varieties of durum wheat. They showed better genetic progress of grain yield (2000.56 kg/ha) compared with Arendato (4213.38kg/ha) which was released in 1966. Relatively lowest progress (330.37kg/ha) was achieved when we compare the oldest variety Arendato with variety Boohie which was released in 1982. Accessions which were released in 2012 (Mangud and Mukye) showed relatively genetic progress in terms of grain yield. Their mean grain yield was much higher than the Arendato. Arendato was the second low yielder variety next to Assasa. There was a progressive yield increment in a series of varieties released from 1966 to 2012.The existence of non- significant positive correlation between mean grain yield of serious of released varieties (0.71 $P<0.01$) and their respective years of release that confirmed the same thing.

Table 22. Trends in genetic progresses in grain yield from breeding of durum wheat released in 1960's 1970s, 1980s, 2000's and 2012.

Varieties	Year of release	Mean grain yield (kg ha-1)	Increment in (Kg a-1) over Arendato	Increment (%) over Arendato
Arendato	1966	4213.38		
Cocorit Gerardo LD – 357	1970s	4656	442.62	10.5
Boohie	1982	4543.75	330.37	7.84
Foka Kilinto Tob Bichena Quamy Assasa	1990s	4802.75	589.37	14.10
Ginchi Yerer Meteyaya Hitosa Dendi Worer	2000s	5279.57	1066.19	25.30
Mangud Mukye	2012	6126.94	2000.56	47.48

Grain yield was significantly correlated with most of the traits. Based on phenotypic correlation, it was positively associated with TKW (gm), harvest index, biomass yield and also with spike lets per spike. Based on the table 23, the relative annual genetic gain of variety means over mean of the oldest variety Arendato in terms of grain yield was 811.51kg/ha. It was also 915.39kg/ha for biomass yield.

Table 23. Estimates of the mean annual relative genetic gain in (%) of Arendato, correlation coefficient of all traits with thousand Kernel weight(r (TKW)) and grain yield (r(GLD))

Traits	Over all mean of Traits	Mean of traits of Arendato	RGG (%) year-1	Correlation Coefficient r (TKW)	r (GLD)
Plant Height	89.08	87.50	1.58	-0.31**	0.00NS
Heading Date	63.82	66.75	-2.93	0.44**	0.03NS
Maturity Date	124.63	126.00	-1.37	-0.26**	-0.02NS
Leaf Area index	82.18	64.28	17.90	-0.35**	-0.13NS
stem Rust first score	11.47	24.75	-13.28	-0.27**	-0.19*
Stem Rust Second score	23.37	51.75	-28.38	0.27**	-0.08NS
Tillering	8.08	8.75	-0.67	0.22**	0.18*
Thousand Kernel weight	29.49	23.30	6.19	1.00	0.59**
Harvest Index	92.73	84.12	8.62	0.77**	0.94**
Grain Yield	5024.89	4213.38	811.51	0.59**	1.00
Biomass Yield	5392.10	4476.72	915.39	0.06NS	0.60**
Grain Filling duration	60.82	59.25	1.57	-0.45**	-0.08NS
Spike Length	5.76	6.00	-0.24	0.05NS	-0.08NS
Spike Lets Per Spike	18.43	18.00	0.43	0.75**	0.86**
Grain production Efficiency	4805.50	3739.97	1065.53	0.54**	0.97**
Biomass Production Rate	4329.10	3552.95	776.15	0.77**	0.93**
Economic Growth Rate	8271.82	7111.19	1160.63	0.58**	0.80**

Grain yield and biomass yield were highly significant based on the regression coefficient values with their respective year of release. This indicates that varieties released starting from 1966 up to 2012 exhibited good genetic progress in terms of grain yield and biomass yield as well. The regression coefficient of grain yield was 30.99. This indicates that change in grain yield was 50.03 % due to change in their respective year of release. The lowest the R2 value implies that the lower the contribution of years for change in grain yield and other economical traits. The reverse is true for high R2 values.

Table 24. Estimates of mean values, coefficient of determination (R2), regression coefficient (b), intercept and correlation coefficient of all traits with year of release (r yr) of the varieties.

Trait	**Mean**	**R^2**	**b**	**Intercept**	**$r_{(yr)}$**
Plant Height	89.08	9.46	-0.21	505.44	-0.31
Heading Date	63.82	24.4	-0.07	203.56	-0.49*
Maturity Date	124.63	1.53	0.02	94.23	0.12
Leaf Area index	82.18	0.68	0.07	-56.35	0.08
stem Rust first score	11.47	10.51	-0.14	284.31	-0.32
Stem Rust Second score	23.37	12.8	-0.25	526.65	-0.36
Number of tillers/plant	8.08	15.2	-0.03	76.27	-0.39
Thousand Kernel Weight	29.49	8.39	0.12	-200.19	0.29
Harvest Index	92.73	14.38	0.15	-198.67	0.38

Grain Yield kg/ha	5024.89	50.03	30.99	-56797.00	0.71**
Biomass Yield kg/ha	5392.10	39.21	31.80	-58047.00	0.63**
Grain Filling duration	60.82	21.76	0.09	-109.33	0.47 *
Spike Length	5.76	0.04	0.00	7.45	-0.02
spike Lets Per Spike	18.43	3.84	0.03	-48.07	0.20
Grain production Efficiency	4805.50	60.45	41.32	-77629.00	0.78 **
Biomass Production Rate	4329.10	35.67	24.99	-45528.00	0.60**
Economic Growth Rate	8271.82	29.56	39.15	-69821.00	0.50*

*,** Significant, highly significant

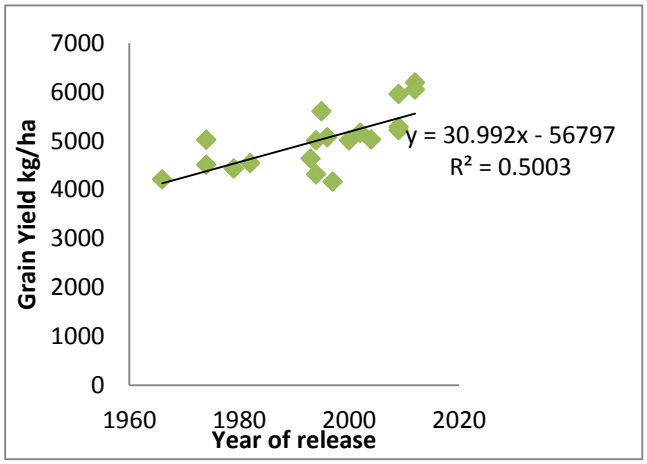

Figure (3). Plot of grain yield of the varieties against year of release

The annual increment of grain yield 1966 – 2012 was 30.99kg/ha. Starting from the release of the first variety (Arendato) in 1966 up to 2012 the yield increment was almost 31kh/ha per year. The total yield gain from the past 46 years were 1471.5kg/ha. There was a progressive yield increment year after year. In general, durum wheat varieties showed progressive increment for the last 46 years.

4.2. Experiment II. Genetic Diversity study as revealed by SSR markers

4.2.1. Genetic polymorphism of SSR markers

A total of 12 SSR markers were selected for diversity analysis of 141durum wheat landraces and19 released cultivars. Table 25 summarizes the genetic diversity parameters of the 12 SSR markers. The difference between the longest and shortest amplified fragment size ranged from 100 to 350 bp. The highest variation in fragment size was observed for primer *CFD 257* (250–350 bp) and the lowest was for primer *CFA2278* (100–180 bp). All the 12 SSR primer pairs were polymorphic and

generated a total of 74 alleles. The number of effective alleles (Ne) ranged from 3.63 for marker WMS493 to 5.75 for marker *WMS234,* with a mean of 4.81 (Table 25). The observed heterozygosity (Ho) varied from 0.99 for marker *CFA2278* to 1.00 for all the remaining locus, with a mean Ho of 0. 99. Gene diversity (He) of the markers ranged from 0.62 to 0.77 for*WMS493* and *WMS 234*, respectively, with a mean of 0.72.

Table 25. Summary of genetic parameters for Durum wheat landraces and its wild released varieties using 12 selected SSR markers.

Locus	Genetic parameters							
	Na	Ne	I	Ho	He	uHe	F	PIC
BARC153	6	4.09	1.49	0.82	0.74	0.78	-0.35	0.75
CFA2278	7	3.72	1.38	0.97	0.72	0.76	-0.38	0.82
CFD 257	5	3.57	1.32	0.78	0.70	0.74	-0.46	0.74
WMS53	9	4.12	1.49	0.80	0.75	0.78	-0.35	0.87
WMS120	6	4.05	1.49	0.81	0.74	0.78	-0.36	0.78
WMS234	8	4.46	1.58	0.83	0.77	0.81	-0.30	0.85
WMS269	7	4.30	1.55	0.77	0.76	0.80	-0.31	0.89
WMS375	5	3.59	1.35	0.78	0.71	0.75	-0.42	0.74
WMS493	5	2.78	1.10	0.73	0.62	0.66	-0.63	0.68
WMS516	6	3.40	1.30	0.79	0.69	0.73	-0.46	0.76
WMS 532	6	3.60	1.37	0.81	0.70	0.74	-0.44	0.78
XGWM136	6	3.42	1.30	0.77	0.69	0.73	-0.45	0.72
Mean	6	3.76	1.39	0.79	0.72	0.76	-0.41	0.76
SE	0.54	0.45	0.13	0.00	0.04	0.04	0.09	0.22

Nm= gene flow Na=number of alleles; Ne=number of effective alleles; I=Shannon's information index; Ho=observed heterozygosity; He=unbiased expected heterozygosity; F=fixation index; Nm=gene flow; PIC=polymorphic information content; SE=standard error.

The results obtained in the present study which was 160 durum wheat landraces and released varieties with 12 SSR primer pairs only 74 alleles were detected with an average of 6 alleles per locus.

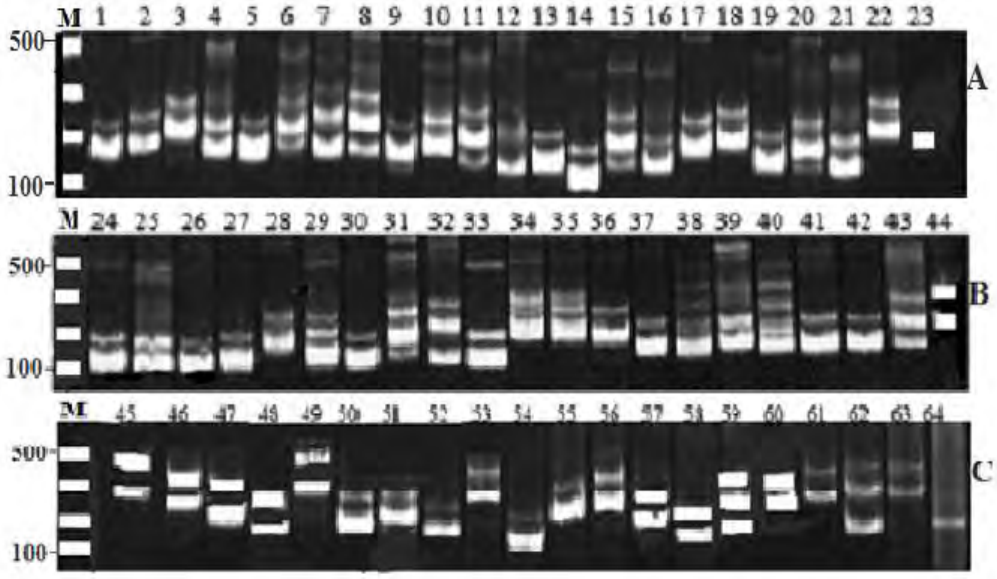

Figure (4). Electrophoretic bands of polymorphic SSR marker on 64 durum wheat accessions

The high heterozygosity and low genetic fixation in the current study signals high genetic variability within the presently sampled durum wheat accessions. This also implies that from 160 durum wheat accessions sampled for this study 141 were landraces collected from 14 zones of Tigray, Oromia and Amhara regions and the remaining were released varieties. The highest heterozygosity might be due to wider geographic origin of the accessions. Some loci were more monomorphic (*WMS493, WMS516, and WMS532*) with less gene flow than other loci such as *WMS269* and *WMS234* (Table 25).

The genetic diversity contribution of the SSR marker was measured by the polymorphic information content (PIC). According to Vaiman *et al.* (1994), loci polymorphism can be considered high, medium or low if PIC>0.5, 0.5>PIC>0.25 and PIC<0.25, respectively. The polymorphic information content (PIC) of loci ranged from 0.69 (*WMS493*) to 0.89 *WMS269*), with a mean of 0.76 (Table 25). All of the 12 locus used in the present study were highly informative throughout the genome of 160 durum wheat accessions. Four microsatellites (*CFA2278, WMS53, WMS234, and WMS269*), each with more than 6 alleles per loci, had PIC values higher than 0.80.

4.2.2. Patterns of Diversity and genetic relationship

The genetic diversity parameters between the studied durum wheat accessions are presented in Table 26. Knowledge on frequency and distribution of alleles is important to determine the relationship among and diversity within populations of a species. The numbers of observed and effective alleles were higher for the landraces than improved varieties. Relatively the highest Shannon diversity index of was recorded for the landraces collected from south Gonder (1.73) while population collected from Bale (1.17) was showed the least variability. The mean value of Shannon diversity index was 1.39. Heterozygosity values (expected and observed hetrozygosities) are used to determine the genetic variation within accessions under study. The expected heterozygosity can be explained as the probability of an individual in a population to be heterozygous at a given locus or it is a probability of nonidentity of two randomly chosen genes in a population Nei (1973). The observed heterozygosity tells the proportion of individuals found to be heterozygous at a given locus. The gene diversity (observed hetrozygosity) of accessions included in this study were ranged from 0.79 (West Shewa) to 0.99 for (East Gojam) with the mean genetic diversity of 0.92 (Table 26). The high genetic variation detected in this study shows the existence of ample variability that can be exploited for future durum wheat breeding program as a source of valuable traits.

Table 26. Mean of genetic parameters for fifteen groups of the 160 durum wheat accessions studied using 12 selected SSR markers

Geographical regionof origin	N	Na	Ne	I	Ho	He	uHe	F	P%
South Wello	11	5.92±0.313	4.66±0.260	1.38±0.078	0.92±0.00	0.71±0.024	0.74±0.025	-0.43±0.06	91.63%
South Gonder	11	6.5±0.195	5.29±0.26	1.73±0.054	0.94±0.00	0.76±0.016	0.79±0.017	-0.33±0.02	98.65%
West Gojam	6	5.25±0.329	4.53±0.24	1.31±0.079	0.96±0.00	0.69±0.024	0.76±0.026	-0.45±0.06	92.67%
East Gojam	11	6.17±0.207	5.06±0.313	1.46±0.072	0.99±0.00	0.73±0.023	0.77±0.025	-0.38±0.05	98.53%
North Gonder	9	6.3±0.188	5.01±0.28	1.49±0.061	0.91±0.00	0.74±0.019	0.78±0.021	-0.36±0.04	91.67%
North Shewa	11	5.92±0.336	4.69±0.253	1.38±0.076	0.89±0.00	0.71±0.024	0.75±0.025	-0.43±0.06	90.70%
Arsi	11	6.08±0.29	4.59±0.29	1.36±0.076	0.93±0.00	0.70±0.024	0.73±0.025	-0.45±0.05	83.33%
Bale	7	4.92±0.39	3.13±026	1.178±0.095	0.94±0.00	0.65±0.029	0.70±0.032	-0.57±0.08	75.00%
Harergie	14	6.83±0.207	5.59±0.218	1.37±0.055	0.97±0.00	0.71±0.018	0.74±0.019	-0.42±0.04	98.86 %
East Shewa	11	6.17±0.207	5.82±0.248	1.43±0.059	0.95±0.00	0.72±0.019	0.76±0.020	-0.39±0.04	75.00%
West Shewa	11	5.33±0.310	4.44±0.224	1.29±0.079	0.79±0.01	0.69±0.026	0.72±0.027	-0.46±0.07	75.00%
Southern T.	10	6.08±0.193	5.07±0.205	1.47±0.051	0.90±0.00	0.75±0.016	0.78±0.017	-0.35±0.03	90.67%
Central T.	10	6.5±0.174	4.79±0.139	1.43±0.028	0.94±0.00	0.73±0.010	0.77±0.011	-0.37±0.02	91.65%
Eastern T.	8	5.75±0.329	4.60±0.252	1.36±0.075	0.80±0.00	0.71±0.022	0.75±0.023	-0.433±0.05	74.00%
Released V.	19	5.33±0.376	5.01+0.239	1.47±0.056	0.93±0.00	0.74±0.014	0.76±0.015	-0.354±0.03	91.79%
Mean± SE	11	5.91±0.076	4.76+0.07	1.39±0.02	0.92±0.01	0.72±0.006	0.75±0.006	-0.411+0.013	88±2.41

Na=number of alleles; Ne=number of effective alleles; I=Shannon's information index; Ho=observed heterozygosity; He=unbiased expected heterozygosity; F=fixation index; and SE=standard error.

The degree of polymorphism between populations varied from 74% for the collections from Eastern Tigray to 98.86 % for collections from Harergie, with the average of 88%. Landraces collected from Harergie, South Gonder and East Gojam had exhibited 98.86%, 98.65 %and 98.53% polymorphism, respectively. This is higher than the rest of the collection areas. Lower percentage of polymorphism were observed from the landraces collected from Eastern Tigray, East and West Shewa and Bale zones 74%, 75%, 75% and 75% respectively. The remaining collection areas showed moderate polymorphism that ranged from 83 to 92%. The polymorphism percentage of improved varieties was 91.67% which is higher than some of the local collections (Table 26). It appears that the level of polymorphism among the local collections increased as one goes from North (Eastern Tigray) to central high lands (East and west Shewa) then to east and southeast (Harergie, Bale, Arsi) towards North (Tigray, Gonder, Gojam and Wello) to eastern parts of the country.

4.2.3. Population differentiation and partitioning the genetic diversity

Analysis of molecular variance (AMOVA) showed significant differences among the populations. A low level of variations was observed among populations (19%), while high levels of variation were revealed within population (81%) (Table 27). The relatively higher with in population variance we obtained could be attributed to the presence of landraces collected from divers' geographic locations of 14 zones that can be grouped in to three major durum wheat producer regions (Tigray, Amhara and Oromiya). Moreover, seed exchange among neighboring durum growing regions could also contribute for the observed higher within population variation.

Table 27. Analysis of Molecular Variance (AMOVA)* showing the distribution of genetic diversity within and among populations of durum wheat accessions from different sources of origins.

Sources of variation	df	SS	MS	Variation		Statistics	Values	P	F-statistics
				Estimated	%				
Among populations (AP)	14	289.60	20.69	1.398	19%				Fis=0.398
Within population (WP)	145	849.36	5.86	5.858	81%	PhiPT	0.193	0.001	Fit=0.26
Total	159	1138.96	----	7.255	100%				Fst=0.10

df stands for degrees of freedom; SS for sum of square and MS for mean of squares PhiPT = AP / (WP + AP) = AP / TOT

The F-statistics values across each locus and over all loci was computed and summarized in Table 27. The values above the table showed the F-statistics values of the whole population at each locus. It can be inferred from this table that all of the locus exhibited low level of inbreeding (0.39). Inbreeding results in homozygosity, which can increase the chances of offspring being affected by recessive or deleterious traits Jiménez, *et al*. (1994). Low inbreeding value of all locus for the tested durum wheat accessions indicated that there were the wider genetic distance between the accessions or there were lower effects of non- random mating. Fixation index (F_{ST}) is a measure of population differentiation due to genetic structure. The mean genetic differentiation estimated by F-statistics at all loci was moderately high Hartel and Clark (1997) (0.10) that can be due to high levels of variation among individuals. The mean gene flow of 12 loci for the tested 160 accessions was 0.26. The observed levels of gene flow indicated that there was moderate gene exchange between areas of collections which could be facilitated via short distance slow seed movements between neighboring areas.

4.2.4. Clustering analysis and pattern of genetic diversity

The dendogram of cluster analysis based on neighbor-joining algorithm categorized the 160 durum wheat accessions into three major clusters consisting of 47, 64, and 49 of the accessions in cluster I, II, and III, respectively (Fig. 5). The populations collected from 14 zones of three regions (Tigray, Amhara and Oromiya) and released varieties were grouped into 3 clusters and 9 sub clusters of distinct genetic populations (Fig. 5). These grouping of accessions in to different distinct clusters and sub-clusters showing that they had evolved from different lines of ancestry or derived from independent events of evolutionary forces (genetic drift, mutation, migration, selection and in flux/out flux of genes in the form of germplasm exchange) that separated them into related but different gene pools Keneni *et al.* (2012).

Table 28. Estimated probabilistic memberships of durum wheat populations from different geographic origins to the 3 clusters

Geographical region of origin	Number of accessions	Clusters		
		C I	C II	C III
South Wello	11	0.819	0.182	0.000
South Gonder	11	0.637	0.182	0.182
West Gojam	6	0.000	0.833	0.167
East Gojam	11	0.182	0.727	0.091
North Gonder	9	0.444	0.556	0.000
North Shewa	11	0.727	0.000	0.243
Arsi	11	0.000	0.000	1.000
Bale	7	0.000	0.000	1.000
Harergie	14	0.143	0.071	0.785
East Shewa	11	0.636	0.000	0.364
West Shewa	11	0.364	0.000	0.636
Southern T.	10	0.400	0.400	0.400
Central T.	10	0.200	0.300	0.500
Eastern T.	8	0.125	0.125	0.1667
Released V.	19	0.052	0.842	0.105

The first cluster consisted most of the accessions collected from South Wello, South Gonder, North and East Shewa. Most of the released varieties and half of the landraces of north Gonder and most of East Gojam landrace collections were grouped in cluster two. Cluster three on the other hand consisted most of the accessions collected from Harergie, West Shewa, Central and Eastern Tigray and all of the landraces collected from Arsi and Bale were grouped in cluster three (Table 28). The highest number of accessions (62) out of the total 160 were included in this cluster. It can be observed from Fig. 5 that landraces collected from South Wello share a portion of their ancestral gene pool with landraces collected from the same region of North Gonder. Accessions collected from West Shewa shared some part of ancestral gene pool with the accessions from the adjoining regions of East Shewa on one side and with Harergie on the other side. Accessions collected from South Wello, East Gojam, Harergie, East and West Shewa fall into all the three clusters. This indicated that, there were high genetic diversity between accessions collected from these areas. This is also partly confirmed by Shannon index.

In contrary, accessions collected from Arsi and Bale areas were grouped only in cluster three. The accessions of the two areas exhibited low genetic variability and this may be due to they shared similar ancestral gene pool. Moreover, the introduction of high yielding dwarf bread wheat in Bale and Arsi could also contributed towards narrowing the local gene pool in this region. Most of the improved varieties were included in cluster two and shared similar ancestral gene pool with the

accessions collected from West Gojam East Gojam and North Gonder. This might be due to the distribution of released varieties for farmers of those areas and gene-flow to landraces or admixture. The genetic variability of landraces collected from South Wello and East Gojam were lower than the Harergie and most of the Tigray areas.

4.2.5. Genetic distances

Based on the magnitude of the genetic distance (GD), more differentiations were revealed between accessions the different populations from different geographical regions of Ethiopia with GD range of 0.092 - 0.576 (Table 29) while improved accessions from the national durum wheat improvement program vs landraces showed 0.245 to 0.518. The largest interregional distance range was observed between accessions from Eastern Tigray and those from the rest of the sources (GD= 0.212– 0.576) (Table 29), which could be explained by geographic isolation of the region couple with dominant sorghum and millet based production system. The highest values of GD (0.576, 0.550, 0.528 and 0.518) were recorded between accessions from North Gonder and East Tigray, Bale, and Arsi and between the improved varieties and accessions of South Wello in respective order. This could be due to low level of gene exchange coupled with geographic distance between those areas or due to wide genetic differentiation between accessions of these areas. The smallest genetic distance (GD=0.092) was observed between accessions from areas of Bale and Harergie. Previously these two areas were under one administrative area. This may promote high gene flow between two areas.

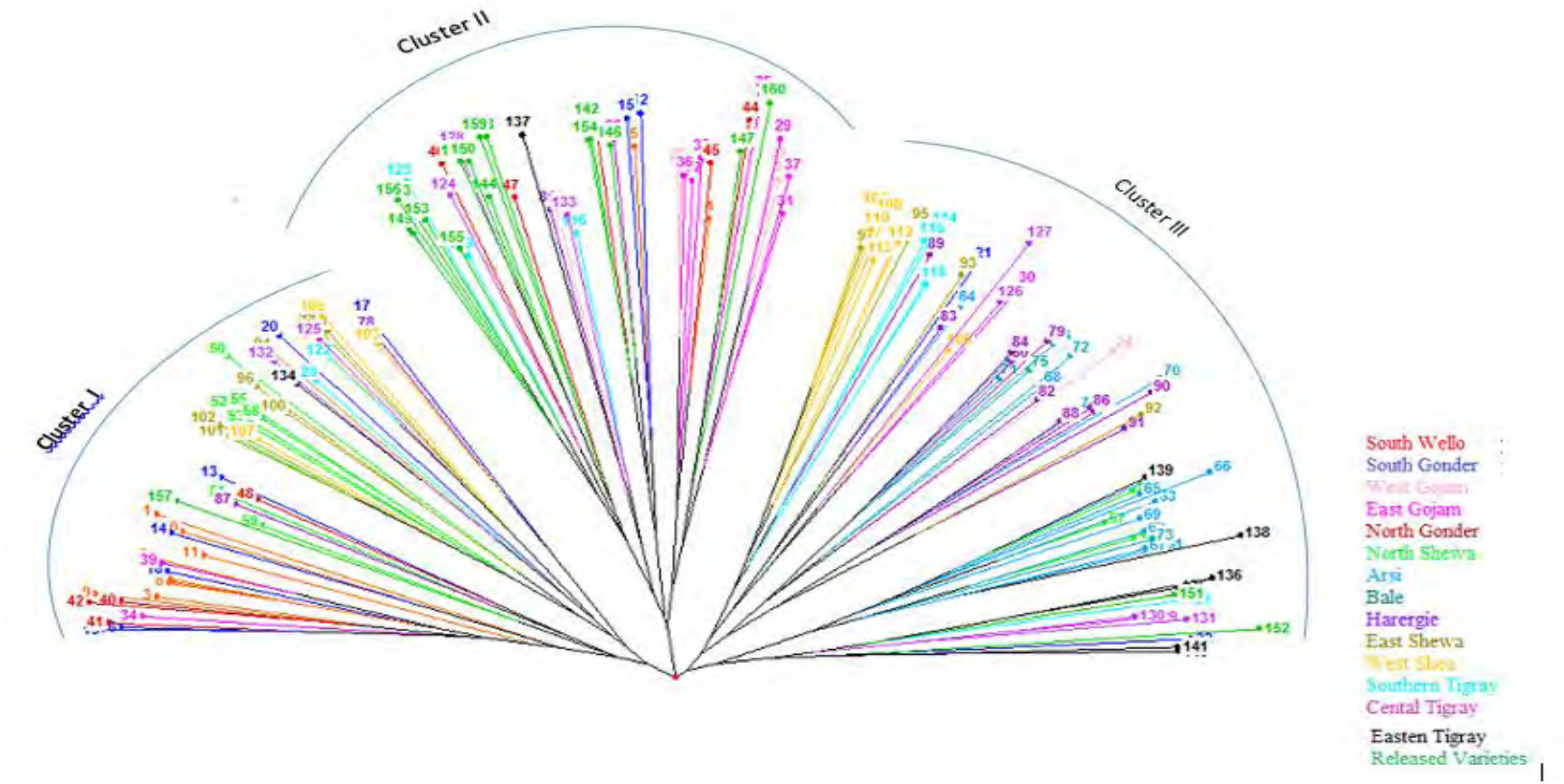

Figure (5). Dendogram of 160 durum wheat landraces collections and released varieties.

Table (29). Pairwise population Nei's genetic distance showing the magnitude of genetic differentiation between Durum wheat landraces from different sources and released varieties.

Geographical origin of region	S.Wello	S.Gonder	W.Gojam	E.Gojam	N. Gon	N.Shewa	Arssi	Bale	Harergi	E.Shewa	W.Shewa	SouthernT.	Central T.	Eastern T.	Released V.
South Wello	0.000														
South Gonder	0.112	0.000													
West Gojam	0.448	0.469	0.000												
East Gojam	0.274	0.252	0.157	0.000											
N.Gonder	0.190	0.180	0.317	0.142	0.000										
North Shewa	0.279	0.220	0.517	0.376	0.398	0.000									
Arsi	0.456	0.306	0.475	0.351	0.528	0.154	0.000								
Bale	0.487	0.406	0.454	0.480	0.550	0.480	0.359	0.000							
Harergie	0.413	0.322	0.341	0.365	0.426	0.365	0.271	0.092	0.000						
East Shewa	0.217	0.228	0.426	0.442	0.304	0.192	0.406	0.483	0.370	0.000					
West Shewa	0.404	0.363	0.484	0.488	0.439	0.392	0.451	0.382	0.235	0.246	0.000				
Southern T.	0.358	0.268	0.410	0.381	0.343	0.211	0.276	0.305	0.246	0.198	0.127	0.000			
Central T.	0.329	0.240	0.396	0.427	0.323	0.328	0.432	0.492	0.391	0.257	0.248	0.122	0.000		
Eastern T.	0.467	0.384	0.475	0.513	0.576	0.276	0.212	0.349	0.361	0.460	0.500	0.276	0.300	0.000	
Released V.	0.518	0.407	0.289	0.307	0.245	0.394	0.497	0.457	0.409	0.405	0.418	0.282	0.275	0.314	0.000

4.2.6. Hardy Weinberg equilibrium (HWE) test

The Hardy Weinberg equilibrium states that for independently segregating alleles, the allelic and genotypic frequencies remain unchanged from generation to generation for a very large, randomly mating population in the absence of natural selection, mutation, gene flow and immigration Hartl and Clark (1997) It is considered as a simple model that serves as a starting point for examining the genetic structure of populations. The HWE test for 12 locus exhibited that loci *BARC 153, CFD 257, WMS 493, and WMS 516* were in Hardy-Weinberg equilibrium in all the populations analyzed. However, significant deviation from HWE was observed in the entire 12 loci through tested populations.

The HWE test for 12 loci exhibited that loci BARC 153, CFD 257, WMS 493, and WMC 516 were deviate from Hardy-Weinberg equilibrium in all the populations analyzed. However, significant deviation from HWE was observed in all of the 12 locus through tested populations. Only one locus (WMS 234) was at hardy-Weinberg equilibrium for most of the populations used in this analysis, while loci WMS234. WMS 53 and WMS375 were at hardy- Weinberg equilibrium for landraces collected from South Wello. Moreover, locus WMS269 was at hardy Weinberg equilibrium for only two populations (West Gojam and Central Tigray), but it was deviated from HWE for the rest of populations. This implied that those populations and locus that shows some deviation from HWE continued to evolve in a condition of absence of mutation, drift, random mating migration and gene flow etc. On the other hand, South Gonder, East Gojam and Harerge showed deviation from HWE at all of the loci. This also indicated that all alleles of these loci did not transfer generation to generation without change in allelic frequency. This may be due to the presence of one or more of the above evolutionary forces and mixed matting system facilitated by wind or insects. Loci WMS120 and WMS532 were also at HWE for landraces collected from West Gojam Bale and Eastern Tigray. Landraces collected from North Gonder, North Shewa and released varieties were showed deviation from Hardy Weinberg equilibrium at all of the loci except WMS 234. They showed consistency for HWE only at WMS234 loci. Of all the populations that considered in this study, West Gojam showed better consistency in HWE for 7 analyzed loci, however, the rest 5 loci showed deviation from HWE. On the other way, most of the landraces collected from the remaining 13 areas and released varieties had exhibited deviation with HWE in

most of the locus analyzed for this study. The summery of HWE test of the population at each locus is given in Table 30. In general, deviation from HWE might be due to the violation of one or more of the assumptions, via, large population size, random mating, absence of natural selection, absence of mutation and gene flow. However, the most considerable, reason for the observed deviation in the study could be due to presence of natural selection that disrupts allelic frequencies, especially for those genes related to fitness, gene flow, migration through gene exchange and mutation which might have introduced bias into the parameter estimate Hartl and Clark (1997), Wiggin ton et al. (2005). Accessions as homozygotes because of the allelic drop out during PCR amplification might have played a vital role in the observed deviation from Hardy-Weinberg equilibrium. Rehman and Khan (2009).

Table 30. Summary of chi-square tests for Hardy-Weinberg equilibrium with their probability values

	Geographical Origin														
Locus	S.Welo	S.Gonder	W.Gojam	E.Gojam	N. Gon	N.Shewa	Arssi	Bale	Hareg i	E.Shewa	W.Shewa	SoutherT.	CentralT.	Eastern T.	Released V.
Barc153	0.000 ***	0.000 ***	0.006 **	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.002 **	0.000 ***	0.057 *	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***
CFA2278	0.000 **	0.003 **	0.055 ns	0.000 ***	0.003 **	0.021 *	0.002 **	0.006 **	0.030 *	0.004 ns	0.063 ns	0.082 ns	0.042 *	0.072 ns	0.000 **
CFD 257	0.000 ***	0.000 ***	0.014 *	0.000 ***	0.003 **	0.000 ***	0.000 ***	0.008 **	0.000 ***	0.000 ***	0.000 ***	0.001 ***	0.000 ***	0.008 **	0.000 ***
Wms53	0.005 ns	0.000 ***	0.263 ns	0.005 **	0.028 *	0.043 *	0.082 ns	0.072 ns	0.000* **	0.005 **	0.000 ***	0.010 *	0.000 ***	0.001 ***	0.000 ***
Wms120	0.030 *	0.035 *	0.112 ns	0.000 ***	0.029 *	0.000 ***	0.005 **	0.137 ns	0.000* **	0.005 **	0.000 ***	0.012 *	0.001 ***	0.065 ns	0.000 ***
Wms234	0.230 ns	0.008 **	0.006 **	0.000 ***	0.168 ns	0.108 ns	0.005 **	0.021 *	0.008 **	0.258 ns	0.327 ns	0.206 ns	0.725 ns	0.544 ns	0.123 ns
Wms269	0.000 ***	0.005 **	0.280 ns	0.015 *	0.029 *	0.000 ***	0.005 **	0.021 *	0.000 ***	0.005 **	0.000 ***	0.000 ***	0.054 ns	0.008 **	0.000 ***
Wms375	0.210 ns	0.007 **	0.095 ns	0.042 *	0.007 **	0.015 *	0.088 Ns	0.120 ns	0.000* **	0.000 ***	0.088 ns	0.001 ***	0.001 ***	0.001 ***	0.000 ***
Wms493	0.000 ***	0.000 ***	0.006 **	0.000 ***	0.003 **	0.000 ***	0.000 ***	0.008 **	0.000* **	0.000 ***	0.001 ***	0.000 ***	0.001 ***	0.001 ***	0.000 ***
Wms516	0.000 ***	0.000 ***	0.006 *	0.001 **	0.032 *	0.004 **	0.000 ***	0.002 **	0.000* **	0.000 ***	0.000 ***	0.001 ***	0.001 ***	0.008 **	0.000 ***
Wms 532	0.000 **	0.005 **	0.260 ns	0.000 ***	0.021 *	0.000 ***	0.000 ***	0.072 ns	0.000* **	0.005 **	0.012 *	0.001 ***	0.001 ***	0.065 ns	0.001 ***
Xgwm136	0.000 ***	0.000 ***	0.090 ns	0.000 ***	0.000 ***	0.003 **	0.000 ***	0.072 ns	0.000* **	0.187 ns	0.051ns	0.011 *	0.001 ***	0.046 *	0.000 ***

4.2.7. Principal coordinate analysis

PCoA was carried out on all the data obtained from 12 SSR primers using Jaccard's coefficient of similarity for all the 160 accessions. The first three coordinates of the PCO having Eigen values of 17.28, 16.54 and 14.58 respectively, were used to show the grouping of individuals using two and three coordinates Fig. 6 and 7. The first three coordinates of PCO accounted 9.77%, 9.35 % and 8.25 % for the total multi loci standardized variations.

A two-dimensional scatter plot and three dimensional coordinates involving all 160 accessions shown in Fig.6 and 7. The broad pattern of distribution of accessions in the PCoA plot revealed three major clusters with sub clusters in two-dimensional and three dimensional coordinates. The groupings identified through PCoA analysis were also similar to those identified in cluster analysis (Fig. 4) which explains about conformity of results obtained from the cluster analysis.

In 2D, most individuals of the respective populations were observed to form three major cluster groups with some sub cluster groups with higher intermixing from the other population. Thus, most of accessions from all 15 populations were intermixed with one another. There was no separate group formed by a single population. However, most of the accessions collected from south Wello and North Shewa were included in a single group. In this group, there were other accessions collected from the remaining populations. Using 2D coordinates, almost all individuals of each population were spread all over the plot. In some cases, accessions of the same population spread in different groups even in different sub groups as formed by NJ in clustering. In NJ almost all individuals of each population spread all over the plot. Lack of association of the individual accessions into their respective populations in case of NJ may indicate seeds exchange (high level of gene flow) among populations.

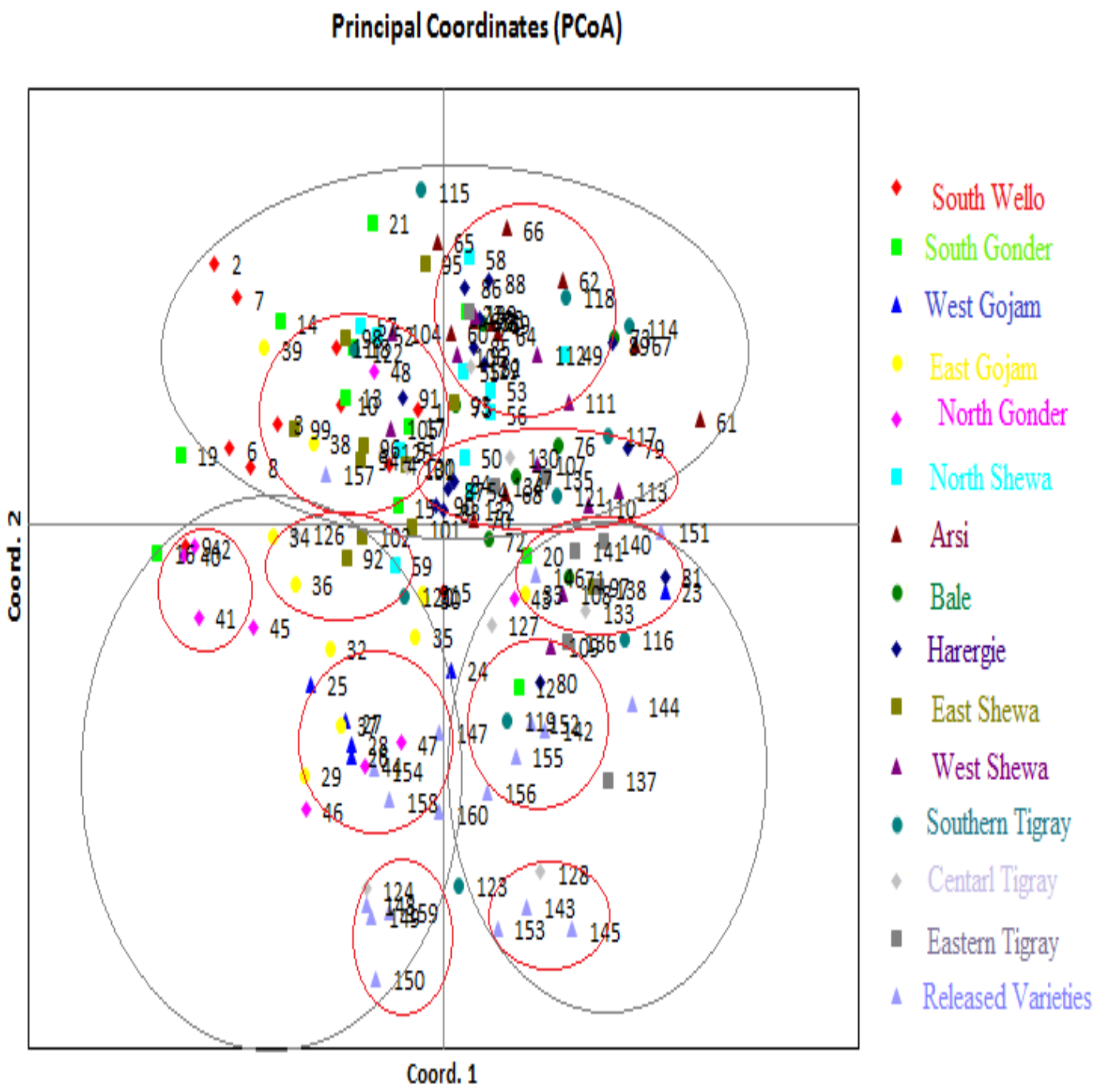

Figure (6). Two dimensional representations of PCO analysis of 160 landraces and released durum wheat varieties of Ethiopia based on Jaccard's similarity coefficients using coordinates 1 and 2.

5. Discussion

5.1. Experiment I. Phenotypic Diversity

5.1.1. Cluster and distance analysis

On the basis of agro-morphological traits, the tetraploid wheat landraces and improved varieties were clustered in to seven cluster groups consisting of 1 up to 113 accessions. However, all of the improved varieties were clustered in to three separate clusters from landraces. This indicated most of Ethiopian durum wheat landraces share similar ancestral gene pool and the genetic diversity of durum wheat has become narrowed and there is a duplication of germplasms in Ethiopian collection. This result is in agreement with the previous studies on Ethiopian tetraploid wheat Faris (2011). On the other hand, the current result is in contrary with the previous studies Yifru *et al*. (2006), Pecetti & Damania (1996), Negassa (1986), Teklu & Hammer (2008) and Hamrick & Godt (1989) indicated that there was high genetic diversity among Ethiopian durum wheat landraces.

The first most divergent cluster group were clusters C3 and C6 (D^2= 253.89) which constituted local landraces collected from Amhara and Tigray regions and released varieties, respectively. Since maximum genetic recombination and variation in the subsequent generation is expected from crosses that involve parents from the clusters characterized by maximum distances, crosses between the landraces and introduced accessions constituted in divergent clusters are expected to provide relatively better genetic recombination and segregation in their progenies Singh (2002) On the other hand, the least divergent groups were clusters C1 and C2 (D^2 = 13.72).

The minimum inter-cluster distances was observed between clusters C1 and C2, indicating that members of these clusters were closely related and, therefore, crossing of accessions from these two clusters may not produce good level of heterotic expression in the F1's with broad-spectrum of variability in segregating (F2) populations Allard (1960). On other hand, the maximum inter-cluster distance between clusters C3 and C6 indicated that the members of these clusters were most divergent and, hence, crossing of accessions from these clusters may be successful in terms of producing higher heterotic expression in the first filial generation and better variability in the segregating generations thereof. Parents for hybridization could be selected on the basis of large inter-cluster distance for isolating useful recombinants in the segregating generations Allard

(1960). Increasing parental distance implies a greater number of constraining alleles at the desired loci, and then to the extent that these loci recombine in the F2 and F3 generations following a cross of distantly related parents, the greater will be the opportunities for successful selection for any character of yield interest Ghaderi *et al*. (1984), Gashaw *et al*. (2007).

5.1.2. Interrelationships between characters

Yield is the prime concern of the plant breeder and is the ultimate factor on which selection programs are to be envisaged. All changes in crop yield must be accompanied by a shift in one or more traits Graffius (1964). All the shift in the traits need not, however, be expressed by changes in yield. This could be due to varying levels of positive or negative correlations between yield and its component traits and among the components themselves Izge (2012). The study of association between traits helps in the selection of accessions and also promote a way forward for a simultaneous selection scheme in more than trait.

Based on the values of genotypic correlation coefficient plant height was negatively correlated with heading date (r = -0.18), number of tiller per plant (r = -0.02), thousand kernel weight (r = -0.02), harvest index (r = -0.15), biomass production rate (r = -0.06) and economic growth rate (r = -0.15). This result did not agree with the previous study by Ajmal (2009) who found that plant height was positively correlated with the traits above. It has positive but non-significant correlation coefficients with grain (r = 0.09), biomass yields (r = 0.04). On the contrary, plant height showed highly significant positive correlation coefficients at genotypic levels with stem rust diseases (r = 0.44) and spike length (r = 0.56). Significant positive genotypic correlation coefficients were also observed between grain yield and two other traits, namely thousand kernel weight (r = 0.19) and harvest index (r = 0.83). This result agreed with previous reports where significant positive correlation coefficients were observed between grain yield and numbers of kernel per spike and spikelet per spike in wheat Akram *et al*. (2008), Amsal *et al*. (2011). Grain yield had negative non-significant genotypic correlation with spike length (r = -0.06) and spike lets per spike (r=-0.02). biomass yield (r = 0.97), grain production efficiency (r = 0.94), biomass production rate (r = 0.96) and economic growth rate (r=0.98) but significant negative correlation with stem rust (r = -0.26) and grain filling duration (r = -0.24), which indicated that accessions

with the ability to fill their grains within shorter periods of time produced better yield than those with longer grain filling periods. This result is in agreement with the work of Amin *et al*. (1992) and Arega *et al.* (2007). Biomass yield had significant positive genotypic correlation coefficients with grain filling duration (r = 0.15) and grain production efficiency (r = 0.91). Spike length had non-significant but negative correlation with both grain yield (r = 0.06) and biomass yield (r = 0.03).The negative correlation of important traits with grain yield may lead to undesirable selection depending on whether negative association is due to linkage or pleiotrophic effects Amsal *et al*. (2011). Existence of strong correlation coefficients between different traits generally implies presence of linkage disequilibrium, pleiotrophic gene actions and epistatic effect of different genes Falconer (1985). Environment also plays an important role in conditioning the magnitude and direction of relationships between morpho-agronomic characters. For instance, days to maturity may be positively associated with grain yield under highly suitable environment as observed here (r = 0.71) at genotypic level. Care must be taken that this conclusion must not contradict with changes in environment as it could be possible in another resource poor as short season location, the result could have been different. The negative correlation of important traits with grain yield may lead to undesirable selection depending on whether negative association is due to linkage or pleiotrophic effects Amsal *et al*. (2011).

5.1.3. Heritability and Genetic Advance

In the present study spike length, grain yield, stem rust disease reaction, harvest index, economic growth rate, biomass production rate and biomass yield exhibited relatively higher broad sense heritability values this is partially in line with the work of Abinasa *et al.* (2011). Maximum PCV was observed from number of tillers/plant (41%) followed by thousand kernel weight (35.28). this is partially agreed with Tsegaye *et al*. (2012). In other way, the PCV values of some characters like plant height, heading date, maturity date, biomass yield spike lets per spike and grain production efficiency were closer than the corresponding GCV values showing little environment effect on the expression of these characters. Similar results were also observed by Mohammed *et al.* (2011) and Subhashchandra *et al*. (2009). Selection on a phenotypic basis may be effective for the genetic improvement of such traits. But the PCV values of some of the characters were far from the values of GCV. This indicates that the higher environmental effect on the expression of

these characters. Selection on the phenotypic basis may not be effective in this case. The smallest value has been showed from heading date, maturity date, and leaf area index at maturity and biomass production rate. This implies that the difficulties of improving these traits through simple selection. Similar results were observed from works of Tsegaye *et al.* (2012). The PCV and GCV value for thousand kernel weight showed the wide range and this result is in line with the work of Al-Marakby *et al*. (1994) and Tazeen *et al*. (2009). But it is contradicting with the result of Tsegaye *et al*. (2012). High heritability magnitude indicates the reliability with which the high chance of the genotype to be recognized by its phenotypic expression Chandrababu and Sharma (1999). High heritability magnitude indicates the reliability with which the high chance of the genotype to be recognized by its phenotypic expression Chandrababu and Sharma (1999). Zero heritability values were observed for heading date, leaf area index and spike lets per spike. In addition, Low heritability values were exhibited for thousand kernel weight, tillering, grain filling duration and maturity date (Table.16). Similar results of low heritability have been found from the previous studies of Tsegaye *et al*. (2012) for tillering and maturity date. This implies that selection for these characters would not be effective due to pre-dominant effects of non-additive genes in this population Tsegaye *et al*. (2012). High heritability percentage was observed from spike length, grain yield, harvest index, biomass yield, grain production efficiency, economic growth rate and also from plant height. According to grain yield this result is contradicted with the work of Paul *et al.* (2006) and Kahrizi *et al*. (2010). They had got lower heritability values for grain yield.

Maximum expected genetic advance as percentage of mean was observed on economic growth rate, grain yield and grain production efficiency. Table.16 indicating the presence of additive gene effects; while the minimum was for heading date, maturity date, leaf area index, spike lets per spike and grain filling duration. Similar results for maturity date were recorded in other findings Maniee *et al*. (2009); Kahrizi *et al*. (2010). High heritability and genetic gain was recorded for thousand grain weight, spike length and number of spike lets per spike indicating selection for these characters would be more effective Kashif *et al*. (2003).

5.1.4. Comparison of four secondary traits as determinants of grain yield, study of yield contributing components

Selection criteria takes into account the information on interrelationship among agronomic characters, their relationship with grain yield as well as their direct influence on grain yield. Yet, selection for yield via highly correlated characters becomes easy if the contribution of different characters to yield is quantified Dewey and Lu (1959). Information regarding interrelationships between quantitatively inherited plant traits and their direct and indirect effects on grain yield is of great importance for success in selections to be conducted in breeding programs Khan *et al*. (2010).

The genetic variance for this trait was zero and the phenotypic variance was 2.48. Its broad sense heritability was zero. This indicates that the trait was more influenced by environmental effects than genotype effects. In other way the accessions were not as such variable for this trait. Selection is difficult for the traits which have low heritability because of that the environment effect can influence the genetic effects Keneni *et al*. (2001).

According to this study, the genotypic correlation coefficient thousand kernel weight and grain yield exhibited positive significant association. But the other traits of spike length, spikelets per spike and number of tillers/plant have non- significant association with the grain yield. This indicates that the selection based on better weight of thousand kernels of durum wheat can lead to selection of best grain yielding accessions. On the other hand, selection of accessions based on their spike length, tillering capacity and spikelets per spike may not improve productivity. The previous study on faba been also indicated that selection for traits which have weak association, in most of the cases, indicating that, there is independent genetic control between the two traits and that improvement in any of the two would have little effect to the other Keneni *et al.* (2001).

5.1.5. Genetic progress from breeding

The annual increment of grain yield 1966 – 2012 was 30.99kg/ha. Starting from the release of the first variety (Arendato) in 1966 up to 2012 the yield increment was almost 31kh/ha per year. The total yield gain from the past 46 years were 1471.5kg/ha. There was a progressive yield increment

year after year. There were the previous studies on genetic progress of durum and bread wheat showed almost similar trends with this study these are, genetic progress in grain yield and nitrogen use efficiency (NUE) of CIMMYT semi-dwarf spring wheat in Mexico. Gains in both grain yield and NUE during 1950 to 1985 were 1.1%, 1.0%, 1.2%, and 1.9% year–1on a relative basis or 32, 43, 59, and 89 kg per hectar per year on an absolute basis, when provided with 0, 75,150, and 300 kg ha–1nitrogen, respectively Ortiz-Monasterio *et al*. (1997) . This indicated that modern semi-dwarf wheat varieties produce higher yields than the older tall varieties under both high and low nitrogen fertility condition in contradiction to the notion that modern semi-dwarf varieties require more nitrogen than the older varieties. On the other hand the finding of Tahir *et al*. (2000) on genetic improvement on seven released bread wheat varieties in Sudan and yield potential increased from 2524 kg ha-1in 1960 to 3295 kg ha-1in 1990 with genetic gain of 26 kg ha-1year-1in grain yield. Similarly Cox *et al*. (1988) evaluated thirty-five hard winter wheat varieties developed between 1874 and 1987 in Kansas to estimate genetic progress. Linear regression of variety means on year of release (YoR) showed increase of 16.2 kg per hectar per year-in grain yield. Tarekegn (1994) also reported that yield potential gain of bread wheat varieties released since 1949 indicating grain yield potential increment at an average rate of 77 kg ha-1 (2.21%) per year at Holetta and 50 kg ha-1 (1.77%) per year at Kulumsa.

In the same way grain yield potential of durum wheat varieties over 25 years (1967 to 1992) increased at an average annual rate of 64 kg ha-1 (2.01%) Perry and D'Antuono (1989) evaluated twenty eight wheat varieties representing series from the 1860 to 1982 in Australia that yield has increased from 1022 kg ha-1to 1588 kg ha-1which represent a rate of increase of 5.8 kg ha-1 (0.57%) year-1 since 1884. Similar investigation was executed to identify traits associated with gain in grain yield of winter wheat from 1940s to 1990s in USA that grain yield improvement ranging from 2718 kg ha-1for earlier variety to 4987 kg ha-1for released varieties with 15 mean genetic gain of 0.16 % year-1for early accessions and 0.63 % year-1for recent wheat accessions Donmez *et al*. (2001). In general, durum wheat varieties showed progressive increment for the last 46 years.

5.2. Experiment II. Genetic Diversity study as revealed by SSR markers

5.2.1. Genetic polymorphism of SSR markers

The number of alleles per locus ranged from 4 for SSR markers *WMS 375, WMS 493*, and *CFD257* to 9 for markers *WMS53*, with a mean of 6 alleles per locus. This was in agreement with an average 4–6 alleles per locus reported by Porceddu *et al*. (2012).

The results obtained in the present study which was 160 durum wheat landraces and released varieties with 12 SSR primer pairs only 74 alleles were detected with an average of 6 alleles per locus. This is relatively low when we compared with those earlier published studies based on SSR markers for Ethiopian tetraploid wheat Alamerew *et al*. (2004), Teklu *et al.* (2006). Alamerew *et al*. (2004) analyzed the genetic diversity of 135 wheat accessions of Ethiopian gene bank collection maintained at IPK using 22 wheat microsatellites and detected 286 alleles with 7.9 alleles per locus. The diversity of Ethiopian tetraploid wheat landraces was assessed using 29 SSR markers and revealed 320 alleles with an average number of alleles per locus 11.03 Teklu *et al*. (2006). Diversity of 10 tetraploid wheat landrace populations collected from different localities in the central highlands of Ethiopia were also studied using isozyme marker and a total of 18 alleles were detected at nine polymorphic loci Tsegaye *et al*. (1996). Moreover, recent diversity analysis by Jemanesh *et al*. (2012) on 58 landrace collections with 31 neutral SSR primer pairs identified a total of 286 alleles, with 9.2 alleles per locus.

Four microsatellites (*CFA2278, WMS53, WMS234, and WMS269*), each with more than 6 alleles per loci, had PIC values higher than 0.80. This indicates the high discrimination ability of the microsatellites for the studied durum wheat accessions. Moreover, these are useful markers for further genetic analysis and marker-assisted selection in durum wheat. Likewise, these markers may be used to determine relationships and genetic diversity in durum wheat germplasm for breeding and germplasm conservation. Similar results on polymorphic information content were observed from the previous studies of (Bousba *et al*., 2012) on durum wheat accessions through SSR markers.

5.2.2. Patterns of Diversity and genetic relationship

The high variability noted within the tested improved varieties (0.93) indicated that the durum wheat breeding program in Ethiopia has been utilizing diverse source of genes to develop new varieties. The present results are also in agreement with the high genetic variability of durum wheat reported by Ratiba *et al*. (2012).

5.2.3. Population differentiation and partitioning the genetic diversity

A low level of variations was observed among populations (19%), while high levels of variation were revealed within population (81%). The relatively higher with in population variance we obtained could be attributed to the presence of landraces collected from divers' geographic locations of 14 zones that can be grouped in to three major durum wheat producer regions (Tigray, Amhara and Oromiya). This result was in accordance with the results of the previous studies (Mondini *et al*., 2012; Abuzied *et al*., 2012) whereby they detected among and within variation, respectively.

5.2.4. Clustering analysis and pattern of genetic diversity

The clustering pattern showed the existence of definite pattern of relationships between geographical origins and genetic diversity for microsatellite markers. Populations from the same geographical origin fall in a single or different clusters similarly, accessions from different origins were grouped in a single cluster or in different cluster. Some clusters contained populations from the same geographical origin while others had populations from different geographical origins and the number of accessions varied from cluster to cluster. The cross border similarities between a few adjoining regions may be attributed, at least in part, to seed movements among neighboring regions. Ratiba, *et al*. (2012) also observed definite relationship between sources of geographic origin and genetic diversity in durum wheat collections from different countries. Similarly observation was found from the previous study of chickpea germplasms by Gemechu, *et al.* (2012).

The genetic variability of landraces collected from South Wello and East Gojam were lower than the Harergie and most of the Tigray areas. This lower variability of accessions and sharing of ancestral gene with improved varieties implied that the oldest accessions from those areas were replaced by the improved varieties. These results indicate that the diversity of Ethiopian durum wheat varieties that are released over the last 43 years is relatively low. This result is in agreement

with the results of Tesfaye *et al*. (2005), who assessed the genetic diversity of durum wheat varieties based on gliadin alleles. Ijaz and Khan (2009) reported the same scenario after their study of 48 accessions and 15 varieties that the cluster analysis separated the accessions and the landraces in to two distinct groups Atintas *et al.* (2008). Similarly, Jemanesh *et al.* (2012) also evaluated 58 tetraploid wheat accessions including landraces and advanced improved varieties by 31 neutral SSR markers and observed low variability among the released cultivar.

5.2.5. Genetic distances

The largest interregional distance range was observed between accessions from Eastern Tigray and those from the rest of the sources. The smallest genetic distance (GD=0.092) was observed between accessions from areas of Bale and Harergie. Thus, crossing of accessions from these two clusters may not produce a high amount of heterotic expression in the F1's and broad-spectrum of variability in segregating (F2) populations. Parents for hybridization could be selected on the basis of large inter-cluster distance for isolating useful recombinants in the segregating generations. Increasing parental distance implies a greater number of constraining alleles at the desired loci, and then to the extent that these loci recombine in the F 2 and F3 generations following a cross of distantly related parents, the greater will be the opportunities for successful selection for any character of yield interest Ghaderi *et al*. (1984). Higher genetic distance was observed between landraces and released varieties. This result was in line with the previous studies Gashaw *et al.* (2007)

6. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusion

- Morphological and molecular characterization of the durum wheat accessions showed the existence of moderate genetic diversity with large number of duplications in Ethiopian durum wheat germplasm collections. Genotypes collected from Tigray, North Gonder and Harergie showed high genetic diversity b/c of this those areas were considered as hot spot for genetic diversity.
- Despite the fact that the diversity analysis indicated the existence of variability among the durum wheat accessions evaluated but low overall variability. The low genetic diversity found among the Ethiopian accessions indicated that the accessions are closely related or populations are intermixed due to seed exchange.
- Genetic correlation coefficient analysis indicated that important agronomic traits (maturity date, harvest index, biomass yield) were positively correlated with grain yield. This suggests a common genetic/physiological basis among these traits. Hence, simultaneous improvement of these traits would be possible.
- Biomass and harvest index showed significant positive correlation with grain yield and can be considered as suitable selection criteria for the development of high yielding durum wheat varieties.
- According to this study the genotypic correlation coefficient thousand kernel weight and grain yield were exhibited the positive significant association. But the other traits of spike length, spike lets per spike and tillering have non- significant association with the grain yield.
- Based on the estimation of relative efficiency, selection of accessions based on their spike length, number of tillers / plant and spike lets per spike may not improve productivity. Generally, direct selection of accessions for grain yield is better so as to get high yielder accessions.

- The breeding progress of improved varieties exhibited a progressive yield increment in a series of varieties released from 1966 to2012. The annual gain of grain yield was (30.99kg/year) from the oldest variety Arendato to the latest variety Mangud.

5.2. Recommendations

- The national durum wheat program should plan and implement good breeding strategy to improve the genetic gain via releasing new high yielder varieties.
- There was high duplications in the durum wheat germplasm collections. This needs core collection should be implemented to represent the duplicated accessions.
- Landraces which showed high genetic diversity (collected from Eastern Tigray, North Gonder and Harergie) may use as sources of important traits for future durum wheat breeding program.
- The eco-geographic pattern of distribution of genetic diversity established in this study may help as a source of information for further genetic diversity study.
- For the future further analysis of durum wheat genotypes by high resolution markers like SNP and sequencing are recommend.

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Appendices

Appendix 1. Average performances of the 160 durum wheat accessions in agronomic characters

Accessions	PH	HD	MD	LAI	GFD	TIL	SL
231623	105.00b-l	66.00a-g	130.50a-d	86.43e-x	64.50b-q	7.50f-m	9.50b-j
231597	108.75b-k	63.50a-l	130.50a-d	103.70b-v	67.00a-k	11.50ab	10.25a-h
231600	112.50a-i	64.25a-k	126.75a-k	104.90b-u	62.50f-t	8.00e-m	11.25a-d
222855	118.75a-e	62.50a-l	127.50a-k	78.25k-x	65.00b-o	8.25d-l	10.25a-h
226094	106.25b-l	58.50j-m	126.25a-k	70.45q-x	67.75a-i	9.25b-i	9.25c-j
8185	116.25a-f	63.75a-l	123.75f-k	101.60b-w	60.00l-u	8.50c-l	9.25c-j
8186	108.75b-k	60.75d-m	127.25 a-k	101.60b-w	66.50a-m	6.50i-m	10.00b-i
214590	117.50a-f	62.50a-l	128.75 a-k	69.02s-x	66.25a-m	7.75f-m	8.75e-k
214550	110.00a-j	58.75i-m	127.50 a-k	99.55b-x	68.75a-f	7.50f-m	11.00a-e
213149	105.00b-l	63.00a-l	127.25 a-k	90.68d-x	64.25b-q	9.25b-i	10.00b-i
206573	115.00a-g	60.50d-m	127.75 a-k	104.23b-v	67.25a-k	8.75b-k	9.25c-j
222621	112.50a-i	60.75d-m	130.75a-c	86.15e-x	70.00a-c	8.25d-l	10.50a-g
222641	108.75b-k	62.50a-l	128.25 a-k	83.23h-x	65.75a-n	8.25d-l	9.00d-j
216614	115.00a-g	63.00a-l	129.00a-j	92.07d-x	66.00a-n	8.50c-l	8.75e-k
222655	121.25a-d	62.75a-l	127.75 a-k	94.54d-x	65.00b-o	6.25j-m	9.75b-j
222608	102.50b-m	60.00f-m	132.50a	115.90a-m	72.50a	8.50c-l	10.25a-h
222613	111.25a-j	61.25c-m	129.25a-i	98.85b-x	68.00a-h	8.50c-l	9.50b-j
7412	105.00b-l	61.25c-m	127.75 a-k	69.63r-x	66.50a-m	7.00g-m	9.75b-j
216474	116.25a-f	64.50a-j	128.50 a-k	77.48l-x	64.00b-r	7.50f-m	9.25c-j
226951	96.25e-m	65.00a-j	127.25 a-k	94.65d-x	62.25f-u	6.00k-m	9.00d-j
222616	106.25b-l	62.25a-l	130.00a-f	79.55j-x	67.75a-i	9.00b-j	9.50b-j
203750	107.50b-k	62.75a-l	126.50 a-k	85.38f-x	63.75b-r	7.75f-m	10.00b-i
203922	105.00b-l	63.00a-l	130.75a-c	89.40d-x	67.75a-i	7.75f-m	9.50b-j
203893	100.00c-m	61.25c-m	127.25 a-k	66.23v-x	66.00a-n	9.00b-j	9.00d-j
5487	116.25a-f	61.25c-m	128.00 a-k	73.83n-x	66.75a-l	7.25f-m	10.25a-h
208212	108.75b-k	62.00a-l	126.25 a-k	113.28a-o	64.25b-q	8.00e-m	10.50a-g
203757	102.50b-m	61.75b-m	127.75 a-k	97.40b-x	66.00a-n	7.50f-m	10.00b-i
208189	123.75a-c	62.00a-l	131.50ab	96.27c-x	69.50a-e	8.00e-m	9.50b-j
208195	111.25a-j	62.75a-l	130.00a-f	83.30h-x	67.25a-k	7.00j-m	9.25c-j
210821	116.25a-f	62.75a-l	129.00a-j	82.53i-x	66.25a-m	8.75b-k	9.25c-j
226833	102.50b-m	64.25a-k	125.50b-k	99.96b-x	61.25h-u	6.75h-m	8.75e-k
226844	112.50a-i	61.50c-m	128.25 a-k	88.48d-x	66.75a-l	7.50f-m	9.00d-j
231617	103.75b-l	63.00a-l	128.75 a-k	89.40d-x	65.75a-n	7.00g-m	10.25a-h
231618	93.75f-n	62.75 a-l	126.25 a-k	82.88h-x	63.50b-r	7.25f-m	8.25g-l
214515	101.25c-m	62.75 a-l	127.75 a-k	101.42b-w	65.00b-o	7.75f-m	10.75a-f
8333	98.75c-m	63.75 a-l	128.50 a-k	106.55b-t	64.75b-p	8.25d-l	10.25a-h
8328	116.25a-f	63.00 a-l	129.00a-j	93.90d-x	66.00a-n	6.75h-m	10.75a-f
214517	101.25c-m	64.00 a-l	127.00 a-k	120.20a-i	63.00d-s	8.75b-k	9.25c-j
222515	96.25c-m	61.75b-m	130.25a-e	106.73b-t	68.50a-g	6.75h-m	9.75b-j
203840	101.25c-m	57.75k-m	127.50 a-k	88.98d-x	69.75a-d	8.00e-m	9.75b-j
5217	121.25ad	60.50d-m	127.25 a-k	127.08a-d	66.75a-l	7.25f-m	10.75a-f

Appendix 1. continued							
Accessions	PH	HD	MD	LAI	GFD	TIL	SL
204340	118.75ae	64.00 a-l	129.00a-j	98.78b-x	65.00b-o	7.75f-m	12.50a
6856	102.50b-m	62.00 a-l	126.50 a-k	110.31a-q	64.50b-q	7.25f-m	10.25a-h
216492	103.75b-l	61.75b-m	127.75 a-k	97.65b-x	66.00a-n	8.25d-l	9.50b-j
216545	103.75b-l	61.25c-m	128.00 a-k	111.22a-p	66.75a-l	6.50i-m	10.50a-g
226207	100.00c-m	59.50g-m	125.25b-k	99.95b-x	65.75a-n	8.00e-m	10.50a-g
226207	111.25a-j	60.00f-m	127.50 a-k	117.75a-k	67.50a-j	7.75f-m	10.50a-g
216440	106.25b-l	61.25c-m	126.25 a-k	125.33a-f	65.00b-o	8.00e-m	7.75i-m
208265	87.50j-o	61.50c-m	127.50 a-k	110.14a-q	66.00a-n	5.75l-m	9.50b-j
208278	93.75f-n	57.75k-m	128.00 a-k	111.10a-p	70.25b-q	8.25d-l	10.50a-g
208286	116.25a-f	62.25a-l	128.00 a-k	100.63b-x	65.75a-m	7.00g-m	11.00a-e
208310	116.25a-f	64.50a-j	128.75 a-k	77.50l-x	64.25b-q	9.00b-j	9.50b-j
208312	106.25b-l	63.25 a-l	129.75a-g	100.50b-x	66.50a-m	8.00e-m	11.25a-d
208317	108.75b-k	64.75a-j	129.75a-g	85.63f-x	65.00b-o	7.50f-m	10.00b-i
208491	100.00c-m	64.25a-k	129.75a-g	81.10i-x	65.50b-n	8.50c-l	9.00d-j
226375	108.75b-k	62.75 a-l	128.25 a-k	108.48a-s	65.50b-n	9.00b-j	10.25a-h
5679	112.50a-i	66.50a-f	129.75a-g	101.43b-w	63.25c-r	9.25b-i	10.00b-i
5739	100.00c-m	61.75b-m	128.50 a-k	104.90b-u	66.75a-l	8.00e-m	10.25a-h
226892	106.25b-l	64.75a-j	126.00b-k	73.27o-x	61.25h-u	8.00e-m	10.25a-h
222421	123.75a-c	63.25 a-l	128.50 a-k	76.75m-x	65.25b-n	7.50f-m	10.00b-i
222422	116.25a-f	63.00 a-l	125.75b-k	103.03b-v	62.75e-t	6.75h-m	8.75e-k
222428	111.25a-j	62.75 a-l	126.75 a-k	105.75b-u	64.00b-r	10.00a-f	9.25c-j
226868	106.25b-l	63.25 a-l	126.00b-k	66.98t-x	62.75e-t	7.25f-m	9.50b-j
226356	103.75b-l	66.00a-g	127.25 a-k	79.09k-x	61.25h-u	9.00b-j	9.50b-j
7073	118.75a-e	60.75d-m	129.25a-i	82.70h-x	68.50a-j	10.00a-f	10.25a-h
214498	106.25b-l	63.75 a-l	126.25 a-k	92.29d-x	62.50f-t	7.25f-m	9.00d-j
7022	93.75f-n	62.25 a-l	126.50 a-k	111.00a-p	64.25b-q	7.50f-m	8.75e-k
226273	113.75a-l	64.00 a-l	128.00 a-k	87.83d-x	64.00b-r	8.75b-k	10.50a-g
227013	111.25a-j	62.25 a-l	125.75b-k	70.52q-x	63.50b-r	8.50c-l	9.75b-j
5927	105.00b-l	60.25e-m	125.00c-k	104.18b-v	64.75b-g	10.75a-e	8.50f-k
231467	118.75a-e	64.50a-j	125.00c-k	126.33a-e	60.50k-u	9.00b-j	10.00b-i
222324	102.50b-m	61.75b-m	125.25b-k	93.25d-x	63.50b-r	8.75b-k	10.75a-f
222338	111.25a-j	62.50 a-l	125.00c-k	90.69d-x	62.50f-t	7.25f-m	8.50f-k
204349	100.00c-m	62.75 a-l	127.75 a-k	72.40p-x	65.00b-o	8.00e-m	9.25c-j
204370	102.50b-m	62.75 a-l	127.50 a-k	96.05c-x	64.75b-p	7.50f-m	11.75ab
227060	105.00b-l	65.25a-i	126.75 a-k	89.47d-x	61.50h-u	8.00e-m	10.00b-i
204357	102.50b-m	61.50c-m	126.25 a-k	87.95d-x	64.75b-p	8.25d-l	9.75b-j
214503	103.75b-l	62.50 a-l	128.00 a-k	100.85b-w	65.50b-n	8.25d-l	9.00d-j
203695	110.00a-j	61.75b-m	129.75a-j	122.90a-h	68.00a-h	7.50f-m	10.25a-h
203886	121.25a-d	61.50c-m	125.50b-k	117.78a-k	64.00b-r	7.75f-m	9.75b-j
226180	108.75b-k	63.25 a-l	127.00b-k	90.95d-x	63.75b-r	7.00g-m	10.00b-i
226183	103.75b-l	61.25c-m	125.00c-k	91.51d-x	63.75b-r	7.00g-m	10.25a-h
222708	105.00b-l	57.50lm	123.00i-k	113.86a-n	65.50b-n	6.75h-m	10.00b-i
231471	103.75b-l	66.50a-f	124.25b-k	96.86c-x	57.75q-u	7.75f-m	10.25a-h
203690	96.25e-m	60.75d-m	125.00c-k	81.55i-x	64.25b-q	8.00e-m	10.25a-h

Appendix 1. continued							
Accessions	PH	HD	MD	LAI	GFD	TIL	SL
231603	111.25a-j	67.75a-c	123.25h-k	92.83d-x	55.50u	8.25d-l	9.00d-j
5730	97.50d-m	66.00a-g	128.00 a-k	108.06a-s	62.00f-u	7.75f-m	8.00h-l
203854	106.25b-l	64.00 a-l	126.75 a-k	119.50a-j	62.75e-t	8.50c-l	11.00a-e
226179	103.75 b-l	61.50c-m	125.25b-k	109.10a-s	63.75b-r	6.50i-m	9.00d-j
231606	105.00 b-l	62.75 a-l	127.00 a-k	83.75h-x	64.25b-q	8.75b-k	10.50a-g
231613	103.75 b-l	60.25e-m	127.00 a-k	68.94s-x	66.75a-l	6.00k-m	9.00d-j
210808	93.75f-n	61.50c-m	125.00c-k	108.10a-s	63.50b-r	8.00e-m	9.25c-j
5429	111.25a-j	61.75b-m	123.00 a-k	62.29w-x	61.25h-u	7.50f-m	8.75e-k
216651	108.75b-k	63.75 a-l	125.75b-k	97.18v-x	62.00f-u	9.25b-i	10.50a-g
5314	102.50b-m	64.25a-k	128.00 a-k	103.43b-v	63.75b-r	6.00k-m	8.75e-k
203748	107.50b-k	65.25a-i	125.75b-k	101.43b-w	60.50k-u	7.75f-m	10.00b-i
5300	103.75b-l	63.50 a-l	127.75 a-k	115.93a-m	64.25b-q	9.00b-j	10.75a-f
5248	91.25g-o	65.25a-i	126.00b-k	89.03d-x	60.75j-u	8.25d-l	9.50b-j
5180	112.50a-i	65.00a-j	129.00a-j	96.55c-x	64.00b-r	8.25d-l	9.75b-j
5736	101.25c-m	62.75 a-l	126.00b-k	87.93d-x	63.25c-r	7.25f-m	10.25a-h
214313	101.25c-m	63.50 a-l	123.50g-k	100.57b-x	60.00l-u	6.25j-m	9.75b-j
226959	110.00a-j	65.50a-h	126.50 a-k	120.13a-i	61.00i-u	8.00e-m	10.25a-h
231528	112.50a-i	62.75 a-l	130.75a-c	96.88c-x	68.00a-h	9.25b-i	9.50b-j
222457	106.25b-l	68.25ab	126.00b-k	105.55b-u	57.75q-u	8.25d-l	9.00d-j
222461	110.00a-j	64.00 a-l	126.25 a-k	97.19b-x	62.25f-u	7.50f-m	9.75b-j
231557	106.25b-l	68.50 a	126.75 a-k	117.70a-l	58.25o-u	7.00g-m	11.50a-c
231526	101.25c-m	67.00a-d	124.25d-k	101.63b-w	57.25r-u	7.50f-m	9.50b-j
214328	107.50b-k	64.75a-j	126.00b-k	88.54d-x	61.25h-u	7.00g-m	8.50f-k
5454	105.00b-l	64.75a-j	128.50 a-k	110.15a-q	63.75b-r	9.25b-i	8.50f-k
5144	108.00b-k	63.00 a-l	129.75a-j	105.91b-u	66.75a-l	8.00e-m	8.50f-k
6101	106.25b-l	66.25a-f	124.50c-k	102.48b-w	58.25o-u	7.00g-m	10.25a-h
7206	113.75a-h	63.75 a-l	124.25d-k	88.38d-x	60.50k-u	8.50c-l	7.75i-m
227020	97.50d-m	63.25 a-l	127.00 a-k	106.83b-t	63.75e-r	8.50c-l	9.75b-j
214343	98.75d-m	64.75 a-j	128.25 a-k	95.50d-x	63.50b-r	7.50f-m	7.50j-m
7956	102.50b-m	63.25 a-l	127.75 a-k	90.52d-x	64.50b-q	8.00e-m	8.75e-k
207854	91.25g-o	63.75 a-l	128.50 a-k	95.00d-x	64.75b-p	8.75b-k	9.75b-j
206551	106.25b-l	63.50 a-l	129.00a-j	85.03g-x	65.50b-n	7.00g-m	8.75e-k
206554	106.25b-l	63.50 a-l	129.50a-h	91.96d-x	66.00a-n	7.75f-m	10.50a-g
223257	88.75i-o	65.00a-j	125.00c-k	110.55a-q	60.00l-u	8.00e-m	10.50a-g
226199	102.50b-m	64.75a-j	126.50a-k	136.15a-c	61.75j-u	9.50a-h	10.00b-i
206558	108.75b-k	60.25e-m	125.00c-k	137.23ab	64.75b-p	8.00e-m	9.75b-j
238113	101.25c-m	60.75d-m	125.25b-k	93.82d-x	64.50b-q	7.50f-m	9.00d-j
226245	110.00a-j	61.75b-m	124.00c-k	88.20d-x	62.25f-u	9.00b-j	9.50b-j
238114	126.25ab	62.00 a-l	125.25b-k	76.21m-x	63.25c-r	7.25f-m	11.50a-c
238118	111.25a-j	63.00 a-l	126.00b-k	125.42a-f	63.00d-s	11.25a-c	9.75b-j
238121	121.25ad	55.25m	125.50b-k	94.30d-x	70.25ab	9.75a-j	10.50a-g
238122	116.25a-f	62.50 a-l	127.50 a-k	88.40d-x	65.00b-o	9.25b-i	8.75e-k
238123	116.25a-f	62.00 a-l	124.25d-k	99.75b-x	62.25f-u	9.00b-j	9.75b-j
238124	115.00a-g	60.25e-m	125.75b-k	94.53d-x	65.50b-n	7.50f-m	10.00b-i

Appendix 1. continued							
Accessions	PH	HD	MD	LAI	GFD	TIL	SL
238125	115.00a-g	62.00 a-l	124.25d-k	72.07p-x	62.25f-u	6.75h-m	9.75b-j
238126	115.00a-g	62.00 a-l	124.50c-k	97.03b-x	62.50f-t	8.00e-m	10.25a-h
238136	108.75b-k	62.25 a-l	124.50c-k	86.72e-x	62.25f-u	11.00a-d	10.00b-i
238137	105.00b-l	60.75d-m	122.75j-k	96.00c-x	62.00f-u	8.25d-l	9.50b-j
238127	132.50a	59.25h-m	126.00 b-k	76.38m-x	66.75a-l	8.00e-m	9.50b-j
238128	106.25b-l	64.25a-k	125.75 b-k	98.70b-x	61.50h-u	8.00e-m	10.75a-f
238129	107.50b-k	62.25 a-l	125.75 b-k	124.67a-j	63.50b-r	8.50c-l	10.00b-i
238130	108.75b-k	62.25 a-l	126.00 b-k	109.38a-r	63.75b-r	7.50f-m	10.75a-f
238131	113.75a-h	62.50 a-l	127.75a-k	110.58a-q	65.25b-n	7.50f-m	10.50a-g
238133	98.75d-m	60.25e-m	125.00c-k	87.53d-x	64.75b-p	7.50f-m	8.50f-k
238134	117.50a-f	62.00 a-l	125.75 b-k	147.75a	63.75b-r	5.25m	9.75b-j
238135	107.50b-k	64.25a-k	128.00a-k	102.68b-v	63.75b-r	7.00g-m	9.50b-j
Ginchi	80.00m-o	61.50c-m	124.75c-k	84.46g-x	63.25c-r	7.00g-m	6.50k-n
Yerer	90.00h-o	65.25a-i	123.25h-k	84.45g-x	58.00p-u	6.75h-m	6.00mn
Worer	70.00o	63.50 a-l	126.00 b-k	60.48x	62.50f-t	8.75b-k	5.50mn
Mangude	90.00h-o	62.25 a-l	124.25d-k	72.33p-x	62.00f-u	7.00g-m	6.50k-n
Arendato	87.50j-o	66.75a-e	126.00 b-k	64.28v-x	59.25n-u	8.75b-k	6.00l-n
Assasa	100.00c-m	64.25a-k	130.25a-e	89.30d-x	66.00a-n	8.50c-l	6.00l-n
Denbi	85.00k-o	67.00a-d	125.25 b-k	81.55i-x	58.25o-u	8.25d-l	5.50mn
Tob	95.00e-m	62.00 a-l	123.25h-k	71.90p-x	61.25h-u	8.00e-m	4.50n
LD-357	87.50j-o	63.00 a-l	124.50c-k	77.81k-x	61.50h-u	12.25a	5.50mn
Hitosa	72.50n-o	64.00 a-l	123.75f-k	84.47g-x	59.75m-u	8.25d-l	6.00l-n
Mukaye	90.00h-o	60.50d-m	124.00e-k	112.10a-p	63.50b-r	8.25d-l	5.50mn
Killinto	100.00c-m	63.25 a-l	125.50 b-k	78.51k-x	62.25f-u	7.25f-m	5.00n
Quamy	85.00k-o	64.00 a-l	124.00e-k	86.58e-x	60.00l-u	7.50f-m	6.50k-m
Gerardo	100.00c-m	67.75a-c	123.75f-k	97.85b-x	56.00t-u	8.75b-k	5.50mn
Foka	102.50b-m	62.50 a-l	123.00i-k	78.10k-x	60.50k-u	6.75h-m	5.50mn
Cocorit	80.00m-o	66.25a-f	122.50k	89.35d-x	56.25s-u	8.50c-l	6.50k-n
Boohie	97.50d-m	63.25a-l	123.75f-k	86.35e-x	60.50k-u	7.50f-m	5.50mn
Bichena	97.50d-m	62.75 a-l	124.25d-k	72.94p-x	61.50h-u	8.00e-m	6.50k-n
Metyaya	82.50l-o	62.75 a-l	126.00b-k	88.60d-x	63.25c-r	7.50f-m	5.00n
Mean	105.39	62.85	126.77	95.14	63.91	8.0	9.28
Appendix 1. continued							
Accessions	TKW	HI	BM	GPE	BPR	EGR	SLPS
231623	29.33d-t	83.30b-n	121.33a-i	106.30	93.11	156.11a-r	21.50a-h
231597	24.13y-z	62.16i-v	78.63a-i	50.89	60.40	73.81d-u	21.50a-h
231600	30.50o-z	71.80e-v	87.19a-i	62.69	69.20	101.59k-u	21.25a-i
222855	24.96d-v	86.08b-j	133.17a-i	120.02	104.87	183.52a-s	24.25a
226094	22.90h-z	79.06b-m	145.14a	140.48	116.72	178.54a-r	20.75a-k
8185	30.38d-x	81.28b-o	123.29a-i	97.20	99.90	167.03a-u	18.00d-l
8186	30.71j	77.83q-v	109.63a-i	93.08	87.21	132.78a-s	19.50a-l
214590	18.21j	50.39b-o	58.68c-i	31.14	45.71	45.00p-v	19.50a-l
214550	30.00f-z	80.25b-r	125.97a-i	128.88	99.94	150.69c-u	21.00a-j

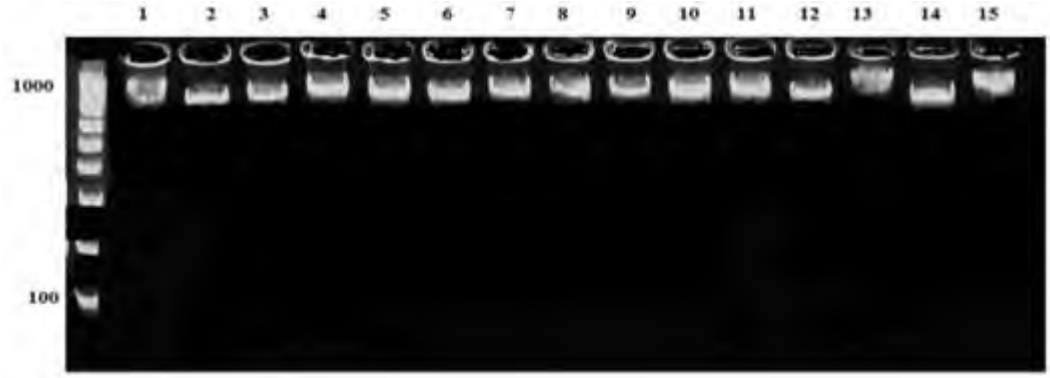
Appendix 1. continued							
Accessions	TKW	HI	BM	GPE	BPR	EGR	SLPS
213149	36.00d-x	83.22b-v	126.88a-i	108.96	100.63	166.90a-q	19.75a-l
206573	27.67q-z	71.35b-m	97.65a-i	78.73	76.54	102.93c-u	19.50 a-l
222621	21.79d-x	82.62b-m	121.26a-i	117.17	92.66	144.17b-u	20.00 a-l
222641	32.00d-v	82.22b-v	121.98a-i	107.18	95.62	155.74a-s	20.50a-k
216614	29.42i-z	71.87b-f	109.11a-i	84.59	85.14	126.09a-t	19.50 a-l
222655	29.50c-o	87.87b-j	142.71a-i	133.27	112.05	190.43a-u	19.25b-l
222608	18.79h-z	82.60b-j	148.92a-e	157.68	113.15	172.17a-i	18.75c-l
222613	20.00d-t	83.89b-l	128.98a-i	121.43	100.99	162.04a-r	21.75a-g
7412	21.50e-z	82.79b-l	129.74a-i	121.62	102.03	162.79a-s	18.50c-l
216474	21.28f-z	82.35b-p	125.83a-i	102.70	99.04	169.18a-m	18.75c-l
226951	27.28d-v	81.11b-v	90.86a-i	69.37	71.84	119.58c-u	19.25b-l
222616	29.67h-z	77.49b-v	124.29a-i	106.02	96.18	145.66a-u	20.25a-l
203750	26.13d-x	80.24b-l	135.71a-i	114.56	107.99	172.26a-s	18.50c-l
203922	25.95i-z	79.20b-t	124.73a-i	108.36	95.35	147.06a-s	18.75c-l
203893	35.40a-j	59.92i-v	78.57a-i	50.87	61.94	72.98i-u	21.50a-h
5487	35.71e-y	82.89b-l	132.21a-i	121.23	104.83	168.71a-u	21.50a-h
208212	44.75d-v	84.94b-l	129.45a-i	117.34	103.31	172.88a-r	22.00a-g
203757	41.71d-v	83.18b-o	124.57a-i	116.29	98.04	158.54a-r	21.50a-h
208189	29.58c-p	83.97b-l	132.05a-i	123.27	100.63	160.47a-r	20.25a-l
208195	45.98d-x	80.32b-v	104.46a-i	91.64	80.12	124.62b-u	21.75a-g
210821	25.77o-z	71.53b-j	179.49ab	135.23	138.84	192.04a-o	19.75 a-l
226833	25.58a-j	59.17j-v	83.97a-i	47.88	67.20	82.07d-u	19.75 a-l
226844	28.28d-v	81.26b-v	111.45 a-i	101.95	87.26	138.51c-u	19.75 a-l
231617	31.13b	111.25b-l	112.31 a-i	120.34	87.92	168.59a-s	19.25b-l
231618	30.16c-p	88.67b-k	129.98 a-i	118.01	103.92	183.81a-n	19.25b-l
214515	30.83o-z	73.29b-v	117.19 a-i	91.62	91.94	135.95a-t	23.75ab
8333	21.58c-m	89.07b-v	102.42 a-i	90.01	80.22	137.65b-u	22.00a-g
8328	19.13o-z	68.24k-v	66.49 a-i	47.94	51.85	69.47b-u	20.25a-l
214517	18.00o-z	76.52b-v	109.66 a-i	82.11	86.66	137.38d-u	21.25a-i
222515	25.08d-u	80.97b-l	130.07 a-i	120.60	100.36	157.07a-t	20.00a-l
203840	22.46k-z	75.91b-v	111.73 a-i	104.59	88.64	125.73a-t	20.50a-k
5217	37.42l-z	69.16e-v	84.81 a-i	66.50	66.83	89.88j-u	19.00b-l
204340	20.50t-z	67.13b-v	103.95 a-i	71.74	81.32	116.80c-u	19.50 a-l
6856	38.67g-z	77.26b-v	120.34 a-i	97.59	95.71	143.66e-u	19.75 a-l
216492	33.38i-z	78.29b-v	108.55 a-i	94.55	85.24	130.34a-u	19.75 a-l
216545	29.21e-z	80.45b-v	117.72 a-i	108.06	92.55	140.81a-u	20.50a-k
226207	43.50d-v	80.96b-l	138.43 a-i	133.45	111.24	172.83a-q	21.25a-i
226207	37.04d-x	84.31b-e	155.22a-e	158.18	122.41	195.43a-m	20.00 a-l
216440	26.46d-s	84.39b-r	124.82 a-i	119.97	99.92	163.65a-n	20.50a-k
208265	23.88e-z	80.60b-l	134.82 a-i	123.67	105.87	168.81a-u	20.50a-k
208278	31.58h-z	76.22b-v	119.37 a-i	123.58	93.78	132.42a-s	19.25b-l
208286	27.50l-z	74.12b-v	108.24 a-i	84.89	84.95	124.17c-u	20.00 a-l

Appendix 1. continued							
Accessions	TKW	HI	BM	GPE	BPR	EGR	SLPS
208310	21.86y-z	63.98d-v	106.03 a-i	67.41	83.17	112.29c-u	20.00 a-l
208312	33.96a-j	49.85u-v	61.15 a-i	31.81	47.20	45.30r-u	19.75 a-l
208317	31.21a-j	55.29t-v	51.08 e-i	28.20	39.36	43.17u	21.25a-i
208491	28.75q-z	70.77b-v	117.87 a-i	90.62	90.82	133.76d-u	20.75a-k
226375	45.84n-z	70.55b-v	108.14 a-i	80.63	84.77	117.67e-u	20.75a-k
5679	30.59k-z	78.10b-o	128.07 a-i	94.90	98.98	161.82a-u	19.25b-l
5739	27.58d-w	84.72b-o	125.85 a-i	115.07	99.03	168.92a-l	20.75a-k
226892	35.37n-z	70.09c-v	95.35 a-i	62.92	76.29	112.27c-u	23.00a-c
222421	30.04o-z	75.54b-v	120.53 a-i	95.08	93.91	139.16e-u	21.75a-g
222422	25.96h-z	76.14b-v	113.63 a-i	87.76	91.15	141.96a-s	20.75a-k
222428	20.09f-z	79.98b-v	118.03 a-i	97.33	93.36	150.69a-t	20.50a-k
226868	44.96l-z	71.23b-v	113.32 a-i	80.02	90.19	128.72d-u	20.00 a-l
226356	43.38d-x	80.34b-m	139.19a-g	105.25	109.79	198.03a-h	20.00 a-l
7073	23.21q-z	65.67e-v	89.32 a-i	65.66	69.67	85.88j-u	21.75a-g
214498	22.67z	60.39g-v	89.31 a-i	52.76	70.97	87.66j-u	21.00a-j
7022	23.25h-z	78.98b-t	124.34 a-i	105.64	99.43	163.31a-r	19.00b-l
226273	29.58k-z	75.33b-v	109.66 a-i	84.17	86.26	135.53b-u	22.00a-g
227013	39.46c-r	86.05b-o	119.59 a-i	106.39	95.78	159.21b-u	21.50a-h
5927	24.83o-z	65.62e-v	88.89 a-i	62.45	71.07	90.83f-u	19.25b-l
231467	28.29h-z	77.19b-l	151.67i	108.88	121.15	191.95a-n	21.50a-h
222324	28.75u-z	63.58i-v	73.17 a-i	48.11	58.46	73.59f-u	20.75a-k
222338	23.79m-z	73.59b-o	134.07 a-i	98.39	107.26	157.59b-u	20.75a-k
204349	28.71w-z	67.26e-v	89.72 a-i	64.67	70.18	94.26c-u	22.50a-e
204370	36.17g-z	76.18b-v	121.29 a-i	95.78	95.35	145.99b-u	19.25b-l
227060	24.43h-z	75.40b-l	136.33 a-i	96.87	107.82	168.37a-u	20.75a-k
204357	48.29o-z	69.46b-v	122.45 a-i	98.24	98.09	139.13a-o	21.50a-h
214503	28.00o-z	71.89b-v	97.22 a-i	76.74	75.52	109.35e-u	20.50a-k
203695	24.71g-z	79.96b-v	116.62 a-i	103.78	90.30	140.09c-u	22.50a-e
203886	25.34l-z	74.38b-v	115.73 a-i	91.40	92.31	136.16a-u	20.00a-l
226180	26.46o-z	68.07c-v	99.16 a-i	71.44	78.22	106.55c-u	20.00a-l
226183	41.74d-v	83.70b-f	153.69a-h	136.06	123.85	206.60a-n	18.00d-l
222708	46.34o-z	73.10b-l	125.84 a-i	117.16	102.31	140.06a-u	22.25a-f
231471	28.17d-v	60.46i-v	82.08 a-i	42.38	66.34	87.07e-u	20.25a-l
203690	27.68q-z	65.97d-v	99.71 a-i	76.95	80.19	107.46e-u	21.25a-i
231603	28.54o-z	71.14b-v	137.85 a-i	87.42	111.54	190.59a-u	18.75c-l
5730	24.96i-z	77.67b-r	129.44 a-i	97.67	100.70	164.88a-t	19.75a-l
203854	24.67o-z	75.14b-n	131.78 a-i	96.80	104.12	158.81a-u	18.50c-l
226179	40.33q-z	73.53b-q	150.03a-d	124.92	120.91	181.00a-e	19.75a-l
231606	43.46e-x	78.04b-v	108.38 a-i	88.10	85.81	130.90d-u	19.50a-l
231613	24.71o-z	68.93b-v	115.33 a-i	95.35	90.99	119.69c-u	20.50a-k
210808	27.740-z	69.84b-v	111.66 a-i	85.50	89.14	123.88c-u	22.00a-g
5429	35.08oh-z	74.21b-l	160.37a-f	130.59	130.20	191.76a-p	20.25 a-l

Appendix 1. continued							
Accessions	TKW	HI	BM	GPE	BPR	EGR	SLPS
216651	21.21o-z	73.59b-v	119.68 a-i	89.71	95.08	145.87a-q	19.50 a-l
5314	31.78d-v	81.26b-l	145.96 a-i	119.71	113.56	184.00a-q	20.25 a-l
203748	30.21d-v	83.41b-l	131.09 a-i	105.09	104.21	187.98a-k	20.00 a-l
5300	22.17t-z	63.50l-v	70.52 a-i	45.49	55.35	69.97j-u	19.75 a-l
5248	21.83h-z	72.27b-v	111.54 a-i	75.90	88.63	131.90f-u	19.75 a-l
5180	26.06k-z	77.14b-n	138.83 a-i	106.39	108.75	176.09a-m	21.50a-h
5736	28.29o-z	71.51b-s	134.75 a-i	96.51	106.76	151.14a-m	18.75c-l
214313	32.79e-y	82.21b-l	139.97 a-i	107.87	113.78	200.46a-f	18.00d-l
226959	39.92d-t	83.34ab	176.46a	144.02	139.88	247.98a	21.75a-g
231528	31.50h-z	71.43b-v	112.42 a-i	85.94	86.97	116.96a-u	21.25a-i
222457	37.00l-z	72.94b-v	124.01 a-i	78.72	98.50	157.37a-u	20.50a-k
222461	33.75o-z	70.12b-v	122.75 a-i	85.52	97.09	139.45a-r	20.25a-l
231557	33.96f-z	75.49b-v	126.06 a-i	83.16	98.96	160.52a-r	21.25a-i
231526	39.17a-j	82.83b-i	141.76 a-i	113.94	114.41	216.66a-d	22.25a-f
214328	27.71j-z	73.04b-v	113.44 a-i	77.40	90.68	135.76a-u	22.25a-f
5454	28.67v-z	68.06b-v	111.66 a-i	76.66	86.95	123.69b-u	20.25a-l
5144	23.92s-z	67.87b-v	114.87 a-i	82.73	88.80	119.36d-u	20.75a-k
6101	33.46o-z	70.53b-v	118.96 a-i	71.02	95.91	141.18c-u	19.25b-l
7206	25.58o-z	71.85b-v	117.44 a-i	82.31	94.83	142.89a-s	18.75c-l
227020	27.42l-z	73.84b-v	117.69 a-i	85.87	93.44	135.49c-u	21.50a-h
214343	28.33c-r	84.16b-g	154.13abc	127.99	120.40	209.64a-g	21.25a-i
7956	39.83h-z	78.73b-o	132.67 a-i	108.29	104.39	165.00a-o	20.25 a-l
207854	25.08z	62.24e-v	94.99b-i	60.36	74.19	92.01c-u	22.00a-g
206551	20.63a-j	54.72q-v	59.41c-i	35.23	46.33	50.04n-u	18.75c-l
206554	19.13ij	52.61v	52.34 a-i	28.67	40.43	41.91s-u	17.50f-l
223257	17.13p-z	77.11b-v	114.69h-i	83.70	91.69	151.48o-u	18.50c-l
226199	24.63ij	53.92p-v	55.99 a-i	28.25	44.45	48.91c-u	18.50c-l
206558	21.38o-z	71.89b-v	115.72 a-i	93.49	93.33	127.11p-u	20.25a-l
238113	23.46o-z	73.94b-v	111.17 a-i	87.53	89.27	126.23b-u	16.75h-l
226245	24.8o-z	68.49b-v	110.40 a-i	79.97	89.38	121.53c-u	18.75c-l
238114	22.13c-j	54.13d-v	74.52 a-i	42.86	60.07	62.76e-u	20.25a-l
238118	21.63o-z	75.38b-v	120.45 a-i	94.47	96.51	154.22h-u	18.75c-l
238121	22.21k-z	76.50b-v	119.52 a-i	163.90	95.69	134.23a-u	19.00b-l
238122	22.67k-z	75.03b-o	137.73 a-i	112.21	108.73	170.01a-u	18.25c-l
238123	23.04i-z	74.41b-t	125.84 a-i	92.97	101.31	148.96a-t	19.25b-l
238124	44.25h-z	76.90b-v	117.48 a-i	96.77	93.58	135.03a-t	20.25a-l
238125	39.29l-z	75.05b-v	127.77 a-i	97.94	102.87	154.14a-u	20.00a-l
238126	25.21l-z	71.36b-v	108.98 a-i	79.59	87.71	126.09b-u	19.00b-l
238136	20.38a-j	78.57b-v	114.88 a-i	92.59	92.59	144.38a-u	19.75a-l
238137	18.08m-z	76.35b-v	112.43 a-i	89.48	91.86	138.77a-u	20.25a-l
238127	23.30r-z	69.20e-v	91.31 a-i	77.15	73.33	95.77a-u	19.25b-l
238128	29.88d-x	80.97b-u	114.60 a-i	88.74	91.50	156.01e-u	18.25c-l

Appendix 1. continued							
Accessions	TKW	HI	BM	GPE	BPR	EGR	SLPS
238129	27.83o-z	66.73c-v	93.39 a-i	66.01	74.66	99.64c-u	19.25b-l
238130	33.75o-z	70.92b-v	90.20 a-i	66.03	71.77	99.31f-u	21.50a-h
238131	26.13y-z	54.32n-v	74.64 a-i	41.06	58.75	60.89l-u	21.50a-h
238133	22.79a-j	56.05m-v	67.13b-i	41.39	53.69	56.64q-u	18.25c-l
238134	24.54t-z	67.43h-v	81.00 a-i	60.45	64.68	88.68p-u	16.00k-l
238135	24.21c-i	79.85b-l	119.33c-i	93.93	93.49	152.58d-u	19.75a-l
Ginchi	22.78c-i	80.49b-g	130.37c-i	108.51	105.97	171.43d-u	17.50f-l
Yerer	33.43cde	83.99a-e	129.84g-i	96.37	105.82	197.17a-m	17.25g-l
Worer	27.98c-f	83.28b-e	131.64g-i	102.79	105.88	189.80abc	16.50i-l
Mangude	34.23bc	89.10a	183.77d-i	165.03	148.57	264.88a-g	17.75e-l
Arendato	23.30a	118.56a-d	114.54f-i	97.96	91.89	199.74ab	18.00d-l
Assasa	31.05z	51.57s-v	67.60 a-i	35.74	52.91	55.64c-u	17.75e-l
Denbi	24.25c-g	82.85b-h	124.98g-i	88.58	100.75	188.75c-u	21.25a-i
Tob	26.43f-z	68.07b-v	102.43e-i	69.72	83.38	115.13a-s	22.00a-g
LD-357	25.65c-l	78.60b-r	112.45e-i	84.98	91.03	147.54c-u	22.50a-e
Hitosa	23.25d-s	73.33b-v	85.71f-i	63.70	69.67	106.09b-u	22.75a-d
Mukaye	37.15k-z	63.85f-v	85.95c-i	58.48	69.74	86.93g-u	22.00a-g
Killinto	29.28c-n	78.00b-v	110.31c-i	84.26	88.10	140.11d-u	15.50l
Quamy	30.13h-z	66.25d-v	95.22f-i	58.92	76.97	107.59c-u	17.75e-l
Gerardo	35.10l-z	63.01r-v	59.06i	30.40	47.66	64.89m-u	17.50f-l
Foka	29.23c-k	79.33b-m	115.61e-i	88.62	94.19	155.03e-u	16.75h-l
Cocorit	24.30cd	84.76abc	135.83i	97.86	111.23	209.57a-n	16.25j-l
Boohie	24.48c-h	81.68b-e	131.51e-i	104.05	106.55	186.17a-n	17.25g-l
Bichena	40.68m-z	62.79i-v	82.76c-i	51.45	66.60	84.69a-u	18.00d-l
Metyaya	37.58c-q	74.54b-l	121.00c-i	94.63	97.23	153.32d-u	16.00k-l
Mean	29.00	74.62	114.19	90.62	90.53	136.46	19.98

Appendix 2. Sample Genomic DNA bands



Appendix 3. Sample of jel picture of PCR