



**GENETIC DIVERSITY, RELATION BETWEEN
CHARACTERS, AND ALUMINIUM TOXICITY
TOLERANCE OF AVENA SPECIES FROM
ETHIOPIA**

DOCTORAL DISSERTATION

BY

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ADDIS ABABA

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GENETIC DIVERSITY, RELATION BETWEEN CHARACTERS, AND ALUMINIUM TOXICITY TOLERANCE OF AVENA SPECIES FROM ETHIOPIA

BY

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**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES,
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DECLARATION

I, the undersigned, declare that the thesis submitted to the Department of Microbial, Cellular, and Molecular Biology, Addis Ababa University for the Degree of Doctor of Philosophy (PhD) in Biology (Applied Genetics) is my own work and has not been submitted at another university. The materials obtained from other sources are duly acknowledged in the thesis.

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Signed on November, 2018, Department of Microbial, Cellular, and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University, Arat Kilo.

DEDICATION

This piece of work is dedicated to the eternal love and memories of my hero and beloved mother, **Tiru Zeru Tessema**. She goes away without seeing my achievement. My dedication also goes to my uncle, **Getachew Zeru**, and his impressive sons (**Kassa and Simeneh Getachew**) for nursing me with affection and love and for their firm dedication in the success of my life.

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Acronyms and Abbreviations

AFLP:	Amplified Fragment Length Polymorphism
AMOVA:	Analysis of Molecular Variance
ANOVA:	Analysis of Variance
AHC	Agglomerate Hierarchical Cluster
CSA:	Central Statistics Agency
DAP:	Diammonium Phosphate
GD:	Genetic Distance
H	Shannon diversity index
IBPGR:	International Board for Plant Genetic Resources
ISSR:	Inter Simple Sequence Repeats
MAS:	Marker Assisted Selection
PC:	Principal Component
PCA:	Principal Component Analysis
PCoA:	Principal Coordinate Analysis
PIC:	Polymorphic Information Content
RAPD:	Random Amplified Polymorphic DNA
RFLP:	Restriction Fragment Length Polymorphism
SAS:	Statistical Analysis System
SCAR:	Sequence Characterized Amplified Region
SMs:	Sum of Squares;
SSR:	Simple Sequence Repeats
UPGMA:	Unweighted pair group method with arithmetic mean

ABSTRACT

GENETIC DIVERSITY, RELATION BETWEEN CHARACTERS, AND ALUMINIUM TOXICITY TOLERANCE OF AVENA SPECIES FROM ETHIOPIA

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Oat is used for animal feed and human food. It is well adapted to a wide range of environments, but it grows well in cool-temperate and tropical African regions, particularly Ethiopia. Ethiopia is one center of origin for *A. abyssinica* and *A. vaviloviana* oat species, but in Ethiopia oat is a minor crop and only a few cultivars have been used. Despite the many benefits oat has, the production and productivity of the crop have been highly constrained by the inadequacy of improved varieties and narrow genetic base of the germplasm used in breeding. Aluminium toxicity is a substantial agronomic problem in Ethiopia and developing Al tolerant oat cultivar is crucial. Therefore, this study was primarily aimed to assess the genetic diversity, population structure, relation between characters and evaluating the aluminum toxicity tolerance potential of oat accessions in Ethiopia. A total of 176 accessions from five *Avena* species namely, *A. sativa* (82), *A. fatua* (18), *A. sterilis* (12), *A. abyssinica* (44), and *A. vaviloviana* (20) were used for morpho-agronomic experiment and molecular genetic diversity study. Except for the number of accessions of *A. sativa* obtained from USA, the Netherlands, and Australia all the accessions were from Ethiopia. The morpho-agronomic experiment was conducted using RCBD design at Debre Brehan and Holetta Agricultural Research Centers. Data were collected on 22 qualitative and 12 quantitative traits. The Shannon diversity index was analyzed for qualitative traits. The mean, ANOVA, heritability, genetic advance, principal component analysis, clustering, cluster distance, and association of traits were analyzed for the quantitative trait. The molecular genetic diversity analysis was carried out at ILRI-BecA Hub,

Nairobi, Kenya, using 19 SSR markers with fluorescently labeled forward primers. The analysis of the allelic pattern and distribution, MANOVA, clustering, PCoA, population structure, and differentiation were analyzed. The hydroponic experiment was conducted at Addis Ababa University to evaluate aluminum toxicity tolerance of the accessions. The hydroponic experiment was carried out on 150 accessions including accessions from, *A. sativa* (74), *A. fatua* (16), *A. sterilis* (5), *A. abyssinica* (40), and *A. vaviloviana* (15). Regarding morpho-agronomic traits, accession had low to high heritability and genotypic advance of mean. The wide range of heritability and genotypic advance of mean were found in five *Avena* species. Similarly, wide range correlation of grain yield with other traits were observed. The Agglomerate hierarchical clustering at a phenotypic level among five *Avena* species revealed three clusters. The first two clusters were predominantly consisted of *A. sativa* accessions with only a few wild *Avena* accessions and the third one cluster with two sub-clusters consisted accessions of wild species. Most accessions of *A. abyssinica* and *A. fatua* were clustered together in one group (sub-cluster 1) regardless to their geographical origin, and similarly *A. sterilis* and *A. vaviloviana* accessions were found together in another group (sub-cluster 2). The distances between clusters of species were different and relatively the highest cluster distance was observed between *A. sativa* and *A. abyssinica* accessions. Results of analysis of molecular variance (AMOVA) showed that 26% variation was between the wild and the cultivated species. Calculated F_{ST} value between wild and cultivated species was high ($F_{ST} = 0.080$), with moderate gene flow ($N_m = 0.943$). In addition, 33% of genetic variation was due to the differences among oat species. The calculated F_{ST} value among oat species was high ($F_{ST} = 0.325$) with a moderate gene flow ($N_m = 0.519$). We found F_{ST} value of *A. sativa* populations and *A. abyssinica* populations to be 0.051 and 0.034, respectively. Unweighted pairwise method with arithmetic mean (UPGMA) clustering and Principal Coordinate analyses (PCoA) revealed that accessions from different collection sites clustered together. Three groups of clusters were recognized in the UPGMA Dendrogram, the first cluster contained *A. abyssinica* and *A. fatua*, the second cluster contained *A. vaviloviana* and *A. sterilis*, and the third cluster contained only *A. sativa* accessions. Not only the UPGMA clustering but also had the PCoA clustered revealed the wild and cultivated species grouped separately. At $K=2$ (optimal gene pool number), two

gene pools were found; one dominated by the cultivated oat and the second by wild oat species. Predominantly, *A. sativa* had one gene pool type, whereas, *A. sterilis* and *A. vaviloviana* had an admixture of the two gene pools. *A. abyssinica* and *A. fatua* accessions had the second gene pool type (wild gene pool). The oat species was variable in Al^{3+} toxicity tolerance to acid soil and the majority of the accessions were tolerant to aluminium toxicity. On the basis of the species type, ploidy level, and genome type, that a comparison was made and it was found more similarity between species of different ploidy levels and genome type, for example, *A. sterilis* and *A. vaviloviana* and between *A. abyssinica* and *A. fatua* than species having similar genome and ploidy level. *A. sativa* had relatively high genetic distance to the four wild *Avena* species, especially *A. abyssinica* and *A. fatua*. Low differentiation among populations may imply significant gene flow through the exchange of planting materials among farmers. The high genetic diversity from morpho-agronomic traits within a population would be harnessed for oat improvement. That the majority of the accessions are aluminium toxicity tolerance shows the potential of the oat to produce cultivars tolerant to acidic soil. The best top 5% performing accessions for grain yield and other important agronomic traits and the best top 5% aluminium toxicity tolerant accessions were identified. It is recommended that the breeder would be consider these for the future improvement of oat crop. It is recommended that future research would be for production stability of identified accessions across a wide range of environment and their Al toxicity potential in the actual field condition. Thus, it is considered this study will provide useful information for the breeders and conservationists for proper conservation management, and utilization of oat germplasm and improve oat production and productivity to sustain food security.

Keywords: Morpho-agronomic, *Avena* species, oat, SSR marker, aluminium toxicity tolerance, and genetic diversity

1. INTRODUCTION

1.1 Background and justification

The genus *Avena* is a cereal grain of the family Gramineae (Poaceae) with an unknown center of origin, but most likely in the Mediterranean basin or the Middle East (Rinesl *et al.*, 2006). It consists of about 32 species (Chawade *et al.*, 2010), of which a few are cultivated species including *Avena sativa* L. The majority of the species are wild, including *A. abyssinica*, *A. vaviloviana*, *A. sterilis*, and *A. fatua* (Evanno *et al.*, 2005; Fu *et al.*, 2007; Liu and Muse, 2005; Loskutov, 2004; Loskutov and Rines, 2011).

The cultivated oat (*A. sativa* L.) is an economically important crop in the world and it ranks sixth in cereal production after wheat, rice, maize, barley, and sorghum (Stewart and McDougall, 2014). Oat is a food for humans and feeds to livestock (Ahmad *et al.*, 2014). The crop is an important source of essential nutrients for human consumption (Boczkowska and Tarczyk, 2013). Oat is also used as a multipurpose crop for pasture and forage. Oat has considerable importance in Ethiopia as fodder and grain for animal feed particularly calves and young stock, horses, poultry and sheep with sufficient nutritive value (Bilal *et al.*, 2017). The presence of an excellent growth habit, palatable, succulent and nutritive value, oat becomes one of the most important cereal fodder crop (Ali *et al.*, 2016). Now days, due to oat nutritious quality fodder for livestock and its grains as animal feed with high net energy gains (Kujur *et al.*, 2017; Ruwali *et al.*, 2013a), it has fascinated the attention of breeders for it oat improvement. The oat crop is a heavy fodder yielder and the average yield varies from 45 to 49 tons of green fodder per hectare (Getnet Assefa *et al.*, 2003).

Wild oats (*A. fatua* and *A. sterilis*) are closely related to the cultivated species and seems a potential source of desirable genes useful for oat breeding (Matiello *et al.*, 1999). Wild oat species contain genes for characters of adaptation to changing environments such as drought, acid soil, disease and pest

resistance (Leonova, 2013; Leonova *et al.*, 2013) that directly or indirectly affects both crop yield and quality.

Oat can be grown on a variety of soils. Moderately fertile and well-drained soil is ideal for oat crop at favorable temperature and moisture, although maximum oat yields can be achieved by adding sufficient lime to bring the soil pH in the range between 5.3-5.7. Oat can tolerate acid soils with a pH near about 4.5. It is mostly grown under cool and moist conditions in cool-temperate regions. In tropical Africa, it is mostly grown in mid - to high-altitude areas (1600–3000 m asl). It shows excellent growth habit, good regeneration capacity after cutting and excellent quality forage (Choudhary, 2013). Its fodder is highly nutritious, highly palatable, succulent and highly nutritious (Bilal *et al.*, 2017).

Genetic diversity, also known as genetic divergence or genetic distance reveals the heritable variation within and among populations of a species. Wild and weedy relatives of cultivated species have the highest potential as a source of genetic diversity for the improvement of cultivated species. Accurate evaluation of the levels and patterns of the genetic diversity is helpful in breeding programs (Fu, 2015): (a) it helps in the identification of diverse parental combinations in hybridization programmes (Barrett and Kidwell, 1998). (b) it is useful to introgress desirable genes from diverse germplasm into the available genetic base (Thompson *et al.*, 1998), and (c) it can assist in the classification of accessions and documentation of subset of core accessions with possible utility for a specific breeding purpose.

Priority should be given to research aimed at seeking new sources of novel genes from wild relatives of oats, because cultivated species, in evolution have usually lost the characters that initially belonged to their wild ancestors. There are many genetic approaches for the characterization of genetic diversity in germplasm accessions, including morpho-agronomic characters, and DNA markers (Mishra and Tomar, 2014; Mondini *et al.*, 2009; Naeem, 2014; Paredes and Gepts, 1995).

Morpho-agronomic characterization of the germplasm is always liable to be affected by the environment thus it may not solely be an accurate approach to evaluate the germplasm (Moose and Mumm, 2008). Morphological characterization has been relatively used for the analysis of the diversity of oat germplasm collections and their relatives. Limited success has been achieved alone with morphological diversity analysis in the selection of parental genotypes for hybridization schemes because of phenotypic plasticity (Kumar *et al.*, 2015) and the environmental influence on morphological traits characterization (Karp, 1997).

Molecular markers have become an important genetic diversity analysis tool for enhancing oat breeding efficiency. Several molecular marker types in different crops have been developed and each has its advantages and disadvantages. Globally, some molecular marker/biochemical techniques have been used in oat genetic diversity studies, including isozymes (Podyma *et al.*, 2017), restriction fragment length polymorphism, random amplified polymorphic DNAs (Abbas *et al.*, 2008; Ruwali *et al.*, 2013b), amplified fragment length polymorphisms (Achleitner *et al.*, 2008), inter-simple sequence repeats (Paczos-Grzeda and Bednarek, 2014; Ruwali *et al.*, 2013b), and simple sequence repeat markers (Sood *et al.*, 2016).

Aluminum (Al) toxicity is the primary limitation of crop production in acid soils or fields with subsoils with a pH below 5 (Von Uexküll and Mutert, 1995). Almost 50% of the total world's potentially arable land, consists of acidic soils. Fertilizers and acid rains further aggravate the problem of Al toxicity.

Tolerance to Al toxicity is observed in some plant species and varies among genotypes (Nava *et al.*, 2006). Oat is considered one of the most Al tolerant small grains. Understanding the physiological mechanisms underlying stress tolerance is a prerequisite for enhancement of crop stress tolerance (Ladizinsky, 1998; Loskutov *et al.*, 2017; Loskutov, 1998, 2008). External and internal mechanisms

may cope with Al toxicity (Kochian, 1995; Kochian *et al.*, 2004). As a consequence, the maintenance of related wild and weedy genotypes is important for conservation and transfers of genes of agronomic importance into cultivated germplasm.

Development of varieties adapted to specific marginal growing environments, such as acid soils, has not yet obtained adequate research attention. However, identification of Al-tolerant oat genotypes makes it possible to introduce these genes from wild relatives into Al-sensitive cultivars, producing crops with high Al tolerance.

To date, only limited efforts have been made to assess the genetic diversity, population structure, and acid soil tolerance variability of accessions of Ethiopian oats germplasm. The present study aims at addressing these limitations through morphological, Al toxicity tolerance screening, and molecular markers approaches.

2. OBJECTIVES

2.1 General objective

- The general objective is to analyze the genetic diversity, relation between characters, and aluminium toxicity tolerance within and among cultivated and wild accessions of oat species from Ethiopia and exotic sources using morpho-agronomic traits, SSR markers, and hydroponic culture.

2.2 Specific objectives

The specific objectives are to:

- determine the magnitude and pattern of genetic diversity of morpho-agronomic traits in oat accessions from local and exotic sources
- evaluate the relation between character in wild and cultivated oat
- evaluate the magnitude and pattern of genetic diversity and population structure of oat accessions from local and exotic sources using SSR markers;
- assess variability in Al toxicity tolerance of oat accessions from Ethiopia and exotic sources using hydroponic experiment.

3 LITERATURE REVIEW

3.1 Origin, domestication and taxonomy of oat

3.1.1 Origin of oat

Oat is a cereal grain of the grass family with no well-established center of origin, but most likely its center lies in the western part of the Mediterranean region. The origin of the cultivated oat (*A. sativa*) is in the Asia Minor (Ladizinsky, 2012b). *A. sativa* spread like a weed of wheat and barley from the Fertile Crescent (a region extending from Israel to western Iran) to Europe (Ladizinsky, 2012a; Leggett and Thomas, 1995). *A. sativa* has wide range of adaption of cultivated oat may be due to polyploidy and subsequent divergence and specialization (Esvelt Klos *et al.*, 2016). *A. abyssinica* and *A. vaviloviana* are endemic to Ethiopia (Ladizinsky, 1975).

3.1.2 Oat domestication

Domestication of oat was next to wheat and barley. *A. sativa* (the cultivated oat) first appeared on the global agricultural scene probably in the last 4,000 years (Holland, 1997). Before this time, oat presumably existed as a wild, weedy plants, and well adapted to cultivated wheat and barley fields in the Middle East. Most likely, domestication of oat occurred outside its center of diversity in northern Europe where the man had migrated following the Neolithic revolution (Murphy and Hoffman, 1992). During this migration, northern Europe was probably selected non-shattering grain from the weedy oat present as a contaminant in wheat and barley fields from the Middle East; as these species were introduced and grown as cereal crops. The extent of oat domestication in Europe paralleled the horse population in the region, although oat was also grown as a staple crop primarily by farmers in Scotland, Wales, and Irelands from the time of the Middle Ages (Murphy and Hoffman, 1992).

The Abyssinian oat (*A. abyssinica* Hochst) is also referred to as a domesticated form. Ladizinsky (1975), elucidated, that *A. abyssinica* is very widespread as a weed in Ethiopia. It is assumed that *A. abyssinica* originated from *A. barbata* by adaptation to the practice of barley growing by the Ethiopian farmers. Although *A. abyssinica* possesses some domesticated features, it is never grown purposely as a crop plant (Millet, 2012). In rainy years, the farmers weed it out but in dry years it is harvested, threshed and consumed together with barley (Ladizinsky, 1975). Similarly, *A. sativa* in Europe originated from the weedy forms of *A. sterilis*. The latter species is a wild hexaploid oat species which is native in the Mediterranean regions (Harlan, 1977).

3.1.3 Oat taxonomy

The genus *Avena* belongs to the family Poaceae (Gramineae) and contains about 32 recognized species (Baum, 1977; Fu, 2018; Ladizinsky, 1998). The genus is foremost in importance in the order Cyperales; class Liliopsida (monocotyledon); and division (flowering plant). The genus is commonly named oat. It comprises species of three ploidy levels: diploid, tetraploid and hexaploid, with a basic chromosome number of seven. The present study includes two tetraploid (*A. abyssinica*, and *A. vaviloviana*) and three hexaploid species (*A. sativa*, *A. sterilis* and *A. fatua*). Of these, the hexaploid species, *A. sativa* is cultivated species all the rest are wild oat. The progenitor of the cultivated oat was *A. sterilis*.

Ladizinsky and Zohary (1971) and Baum (1977) suggested that all hexaploid oat be considered as one biological species because they share the same genome (AACCDD), and they are inter-fertile. But now they have been classified into a number of taxonomic species. The biology of oat species investigated in the present study is presented below.

1) *A. sativa* L. ($2n = 6x = 42$)

It has 2–3 florets per spikelets, non-disarticulates at maturity. In addition, it usually has a yellow lemma and awns may be present but reduced in size or absent. *A. sativa* is inter-fertile with other hexaploid oat and shares dentate lemma tips. It is a domesticated form, extremely variable as a result of human selection and breeding. This gives this crop a chance for wide geographic distribution as compared to the other oat species (Ladizinsky, 2012b).

2) *A. sterilis* L. ($2n = 6x = 42$)

A. sterilis L. is commonly called ‘sterile oat’. It is annual. It has erect to prostrate growth habit at the juvenile stage; erect flowering stems; plant height of 30–145 cm; equilateral panicle; large, sized V shaped spikelets containing 3–5 florets, and bidentate lemma tips (Loskutov and Rines, 2011). In addition, this species has only the lowest disarticulates florets at maturity; awn inserted at lower 1/2 of the slightly moderate pubescent lemma (Loskutov and Rines, 2011).

3) *A. fatua* L. ($2n = 6x = 42$)

A. fatua L. is commonly called ‘common wild oat. It is annual. It has erect to prostrate growth habit at juvenile stage ; erect flowering stems; plant height 40–150 cm tall; equilateral panicle, very drooping; 20–25mm equal glume sized; hairy or glabrous lemma and bidentate lemma tip (Coffman, 1977; Loskutov and Rines, 2011). It differs from *A. sterilis* mainly by the mode of spikelet disarticulation, which occurs at each floret.

4) *A. vaviloviana* (Malz.) Mordv. ($2n = 4x = 28$)

It is an annual plant. Has the following features, prostrate to erect growth habit at the juvenile stage: erect flowering stems; plant height of 80–110 cm; unilateral panicle; medium-sized awned spikelet and containing 2–3 florets. In addition, *A. vaviloviana* has equal glume longer than florets; 20–25 mm long with 8 veins: Lemma tips biaristulate 1 mm long. All florets disarticulate at maturity. Awn inserted at 1/2 of the lemma. Callus short, oval, about 3–5 mm long (Ladizinsky, 2012a).

5) *A. abyssinica* H. ($2n = 4x = 28$)

It is an erect, annual grass growing up to 1.5 meters tall. Glumes equal or almost equal in size, Lemma tips biaristulate, callus blunt, glumes shorter, lemmas glabrous, florets 2–4 not disarticulate, and all have awned inserted at or just below the middle of the valve. Grain tightly embraced, long, hairy all over (Ladizinsky, 2012b).

3.1.4 Geographical distribution of oat

Avena species are widely distributed throughout the temperate regions of the world. They are generally concentrated between latitudes 35 to 65°N, and 20 to 46°S (Webster, 2016).

A. sterilis is extensively spread in the most area of Eurasia and all countries of northern Africa. *A. sterilis*, is rare in Ethiopia. Small dislocated populations of *A. sterilis* are found along roadsides and edges of cultivations in Shewa and Tigray regions of Ethiopia and in Eritrea. At present several forms of *A. sterilis* are known to be found in Japan and South Korea (Ladizinsky, 1975; Loskutov, 2008).

A. fatua is the most widely distributed oat species and it occurs mainly in Europe, Asia, North America, and also on other continents. *A. fatua* has penetrated almost all agricultural areas around the globe extending from the Americans, across Eurasian into Mongolia, Japan, and South Korea; and spread throughout southern and northern Africa, Australia, and New Zealand (Ladizinsky, 2012b).

A. vaviloviana and *A. abyssinica* are endemic species in Ethiopia, Eritrea, and Yemen. In these countries, *A. vaviloviana* occurs widely distributed and is very common on the Ethiopian Plateau at altitudes between 2,200 and 2,800 m asl, mostly on cultivated fertile lands (Ladizinsky, 1975; Loskutov *et al.*, 2017). *A. abyssinica* is confined to Ethiopia where it grows as tolerated weed in barley fields. It does not shatter its seeds at maturity and in that sense can be regarded a domesticated form, but it is never purposely planted. It endures because the Ethiopian farmers cannot select them out by their threshing-winnowing

methods (Harlan, 1977). Therefore, this oat is planted, harvested, and consumed together with barley (Harlan, 1977) .

3.2 Genome and polyploidy of oat

The genus *Avena* has four genomes (A, B, C, and D) at three natural ploidy levels (Fu, 2018; Thomas *et al.*, 1975) and Table 1. *A. sativa* L. is hexaploid with $2n = 6x = 42$ consisting of three genomes (AACCCDD). Diploid species have two distinct genome types AA and CC. Based on cytogenetic evidence, the D genome of the hexaploid oat is quite similar to the A genome (Katsiotis *et al.*, 1996; Rajhathy and Morrison, 1959; Singh and Kumar, 2016; Thomas *et al.*, 1975) and is probably only a recent variant of it. No extant DD genome diploids have been identified.

There are three distinct types of tetraploid genomes. The first, AABB tetraploid: Three tetraploid oat species, *A. barbata*, *A. abyssinica* and *A. vaviloviana* have AABB genomes. The B genome chromosomes are quite similar to the A genome chromosomes (Katsiotis *et al.*, 1997), suggestive of an autopolyploid origin of these species (Leggett *et al.*, 2004). The second, AACCC tetraploids: three tetraploid species, *A. maroccana*, *A. murphyi* and *A. insularis*, likely the progenitors of the AACCCDD. The third, CCCC tetraploid: the tetraploid, *A. macrostachya*, is an exceptional perennial *Avena* species (Welch, 2012). *A. macrostachya* is the only known natural autotetraploid oat.

Table 1. Chromosome number, genomes, and ploidy of *Avena* species, used in the present study, source (Leggett and Thomas, 1995)

<i>Avena</i> species	Chr. No (2n)	Genome
<i>A. vaviloviana</i>	$2n=4x=28$	AB
<i>A. abyssinica</i>	$2n=4x=28$	AB
<i>A. fatua</i>	$2n=6x=42$	ACD
<i>A. sterilis</i>	$2n=6x=42$	ACD
<i>A. sativa</i>	$2n=6x=42$	ACD

The genome size of *Avena* species is larger than that of most cereals, such as wheat, barley, and corn (Yan *et al.*, 2016). According to Yan *et al.* (2016), the genome size of diploid, tetraploid and hexaploid species were 9.23, 18.08, and 25.75 pg, respectively. According to Yan *et al.* (2016), the C genome diploids had higher C-values (≥ 10.18 pg) than all A genome diploids (≤ 9.23 pg), and tetraploids containing the AC genomes (18.51 to 18.70 pg) had higher C-values than those containing the AB genomes (16.38 to 17.49 pg), whereas the single CC genome tetraploid (21.78 pg) had an evidently higher C-value than any other tetraploid species (1 pg = 978 Mbp).

3.2.1 Possible origin of polyploid oat

Two or more genomes are brought together into the same nucleus, usually by hybridization followed by chromosome doubling to form polyploidy. Polyploidy is an evolutionary process. As a result a new species is formed, and the new polyploid is genetically isolated from its diploid progenitor(s). The hexaploid taxa are believed to trace back to a founding polyploidization event demanding the fusion of an ancestral diploid and ancestral tetraploid (Ladizinsky, 2012a). Going back farther in time, the ancestral tetraploid must have resulted from the fusion of two ancestral diploids.

3.3 Reproduction and gene pool of oat (*A. sativa* L.)

3.3.1 Reproduction of oat

Oat is self-fertilized and so opening of the florets is not essential to achieve pollination except for *A. macrostachya*, which is perennial out-crossing oat (Leggett and Thomas, 1995). In other words, all the cultivated and wild oat are autogamous allopolyploids with the exception of *A. macrostachya*, which is allogamous autotetraploid (Sheidai *et al.*, 2002).

3.3.2 The gene pool of oat

Loskutov (2004), classified the genus *Avena* into primary, secondary and tertiary gene pools with respect to their ease of crossability with *A. sativa* (the cultivated oat).

The Primary Gene pool: The primary gene pool consists of all the hexaploid oat. Ladizinsky and Fainstein (1977), grouped all the hexaploid oat into a single biological species because they are inter-fertile. The lack of fertility barrier and the presence of vast genetic diversity within some of the hexaploid species are the major advantage to oat breeding programs. Hybrids between wild and cultivated hexaploid taxa can be produced easily. Introgression of desired traits is easy through conventional breeding strategies.

The Secondary Gene pool: The secondary gene pool consists of the tetraploid species of AACC genome. According to Leggett and Thomas (1995), the secondary gene pool consists of three species, *A. maroccana*, *A. murphyi* and *A. insularis* (all are AACC genomes) and *A. macrostachya* (CCCC genome) . For this reason, these species have two genomes potentially homologous with the genome of the cultivated hexaploid species. Pentaploid F1 hybrid from crosses between these tetraploid and the hexaploid species are completely self-sterile, but occasional seeds are produced when pollinated by either parental species (Ladizinsky and Fainstein, 1977).

The Tertiary Gene pool: The tertiary gene pool consists of diploid species and tetraploid species of AABB genome. Leggett and Thomas (Leggett and Thomas, 1995) defined the tertiary gene pool as those *Avena* species that do not readily hybridize with *A. sativa*. These often need embryo rescue to produce an F1 hybrid, which then cannot be backcrossed to the *A. sativa* without the use of colchicine to double the chromosome number of the hybrid.

3.4 Oat ecology and adaptation

Oat grows mainly in marginal environments, including cool, wet climates, and low fertile soils (Sood *et al.*, 2016). This versatile ecological adaptation of cultivated oat may be due to polyploidy and subsequent divergence and specialization (Esvelt Klos *et al.*, 2016). It can be grown in a wide range of environments on diverse soil types (Stevens *et al.*, 2004). Oat is a useful crop from the perspective of agricultural sustainability, it reduces soil erosion, and controls diseases, insects, and weeds.

Oat, in comparison with other food cereals, is resistant to foliar diseases, and comparatively, needs a few pesticides (Stewart and McDougall, 2014). Many diseases cause either serious direct damage, mainly by reduction of the grain and fodder yield, or indirect damage, by compromising the quality of the product. Among those causing a severe direct damage are a crown, stem rusts and leaf blotch can reduce crop yield by as much as 50% in susceptible cultivars (Stewart and McDougall, 2014). Barley yellow dwarf disease (Stewart and McDougall, 2014), which has aphids as its primary vector, is considered the most important viral disease of oat, and currently, none of the oat cultivars is highly resistant to barley yellow dwarf virus. Other diseases, such as scab and ergot, can also produce toxins in the grains and make them unsuitable for consumption by either animals or humans.

3.4.1 Oat adaptation to aluminium toxicity

Cereals differ greatly in their responses to the presence of aluminium in the soil. Even though different *Avena* species have different aluminium tolerance capacity, in general, all species can tolerate acidic soils as low as pH of 4.5 but the yield is lower. The highest yield is obtained over a soil pH range of 5.3 to 5.7 but less tolerant than rye (Sánchez-Martín *et al.*, 2014). Oat is more tolerant of acid soil than wheat, maize, and barley (Nava *et al.*, 2015; Radmer *et al.*, 2012). However, only a few oat cultivars have been characterized specifically for their response to Al stress resulting in a lack of knowledge of the extent of Al resistance in oat. For oat, Sánchez-Chacón *et al.* (2000) concluded that the trait

tolerance to toxic aluminium level is an inheritable trait, controlled by one gene and with dominant gene action.

3.5 Oat production

3.5.1 Global oat production

Oat production currently ranks sixth in the world grain production statistics, following wheat, rice, maize, barley and sorghum (Webster, 2016). Oat is considered a minor coarse grain and account for only 5-7% of world coarse grain production (Ahmad *et al.*, 2014). According to FAOSTAT (2017) report, global productions of oat were 21.2, 23.8, 22.8, 22.4 and 22.9 million tonnes in the year 2012, 2013, 2014, 2015, and 2016, respectively.

FAOSTAT (2017), elucidated that in the year from 2014 to 2016, the Russian Federation, Canada, Poland, Australia, United States of America, and Finland, were the large oat producers with the annual production of 4.9, 3.1, 1.3, 1.3, 1.1, 1.0 million tonnes, respectively. These accounted for about 56.5% of oat production in the three successive years.

3.5.2 Oat production in Ethiopia

Oat production in Ethiopia fluctuated substantially over years, with a decreasing trend through 1996-2016 period, ending at 49,180 tonnes of annuals production in 2016 (FAOSTAT, 2017) (Figure 1). In the year 2016, Ethiopia ranked 37th in global oat production and it was the first in Africa.

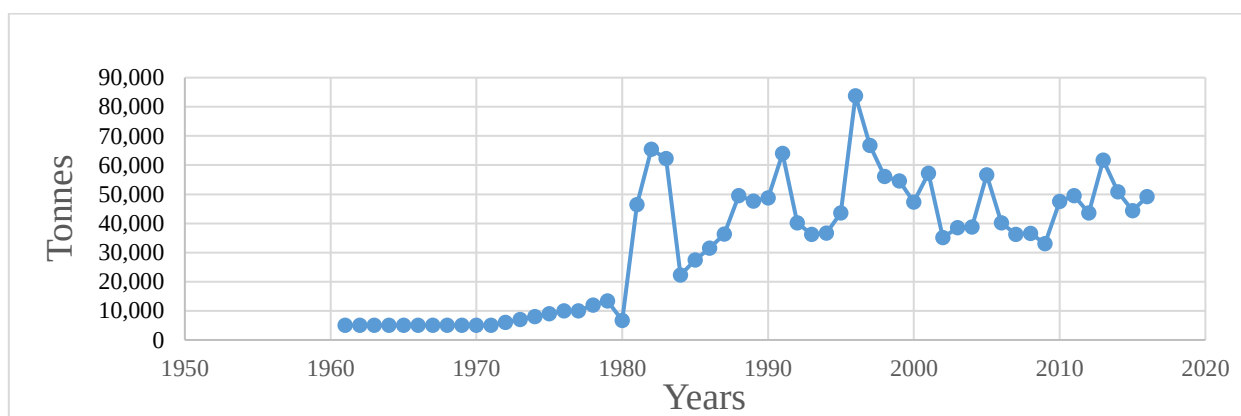


Figure 1. Oat production in Ethiopia from 1961-2016 (source: FAOSTA, 2017).

3.6 Oat in Ethiopia

3.6.1 Oat introduction in Ethiopia

There are five *Avena* species in Ethiopia (Phillips, 1995) which include the two tetraploid species that have been originated in Ethiopia (*A. abyssinica* and *A. vaviloviana*) and three hexaploid species (*A. sativa*, *A. fatua* and *A. sterilis*) which were first introduced by a private dairy farm owner called San George in the early 1960s for feed, but farmers in central highland of Shewa, in this area also have been growing oat as a food crop since the mid-1980s (Gezahagn Kebede *et al.*, 2007). In the early 1970`s, following introductions of about 9,054 lines of oat collected from over 55 countries of the world, conventional research on the species was initiated (Kebede Wuletaw *et al.*, 2017). In the mid-1970`s, after rigorous screening and evaluation, about six promising varieties were identified and recommended for forage production in the highlands of the country by conventional researchers. In the mid-1980`s, about 40 additional dual purposes (forage and/or grain) oat varieties were also introduced from CIMMYT, out of which some ten varieties have been selected for better overall performance in the highlands (Getnet Assefa *et al.*, 2003)

3.6.2 Oat distribution in Ethiopia

According to Engels *et al.* (1991) two wild (weedy) tetraploid oat species (*A. abyssinica* and *A. vaviloviana*) occur in the 'cereal belt' between altitude 2,200 and 2,800 m of Ethiopia.

North Shewa zone is one of the most oat growing areas in the highlands from 1650 to 3000 m asl of Ethiopia (Phillips, 1995). It is also grown to a considerable scale in other parts of the country like Arsi, Bale, Gojam, and Gondar (Kebede Wuletaw *et al.*, 2017). Nearly 20,000 farm households or more than 100,000 people in Selale area, north Shewa, either partially or totally depend on oat for their staple food supply (Kebede Wuletaw *et al.*, 2017).

Cultivated oat was informally distributed to the farming community by different livestock development projects of the Ministry of Agriculture. North Shewa has been one of the beneficiaries of various dairy development projects and oat belonging to either of the six varieties recommended in the mid 1970`s, was introduced to the region along with the projects. Although the initial aim of oat introduction to the smallholders was for feed production, it has been realized that it is also being extensively grown as a food grain. However, it has been perceived that farmers have no awareness of the existence of different oat varieties with different merits. The extent of horizontal expansion and utilization trend (forage vs grain), socio-economic factors governing production and utilization of oat, available improvement opportunities and the overall prospect of oat in the region have not been clearly understood.

3.7 Importance of oat

The cultivated oat (*A. sativa* L.) is a dual purpose crop which is important for animal feed and human food. The nutritive value of oat fodder is high and has dry matter digestibility (Ayub *et al.*, 2011). It is principally fed to horses, mules, turkeys and dairy cattle. The straws have a similar chemical

composition, but oat straw is more acceptable to livestock compared to that of other cereal crops. The important nutritional value of oat lies in its protein, lipid, and fiber content.

Oat is a very good source of protein, starch, unsaturated fatty acids, and dietary fiber, as soluble and insoluble fractions, and minerals (Butt *et al.*, 2008). Among the cereals, oat is a grain crop with high protein content. The average protein content of oat is 11–15% in the kernel with a hull (Nieto, 2015). Oat grain has low lipid contents, rich in protein and fiber and it is, especially preferred for its β -glucans, which are important to reduce the cholesterol and sugar levels in the blood, weight loss as well as cancer prevention (Anderson *et al.*, 2009; Ie, 2014; Newell, 2011; Satija and Hu, 2012). Furthermore, oat is a source of several natural antioxidants such as vitamin E (tocols), phytic acid, phenolic compounds, flavonoids, and sterols (Butt *et al.*, 2008; Marmouzi *et al.*, 2016). Other micronutrients that are found in oat are folates, zinc, iron, selenium, copper, manganese, carotenoids, betaine, choline, and sulphur containing amino acids (Butt *et al.*, 2008). The phenolic content in oat reveals that oat may serve as an excellent dietary source of natural antioxidants with antiradical potential (Marmouzi *et al.*, 2016).

Cultivated oat grain is an ingredient in a wide range of food products including breakfast cereals, porridge, cookies, bread, crackers and snacks, beverages, meat extenders, and baby foods. In Ethiopia, *A. sativa* is used mixed with barley to make ‘injera’ (pancake-like bread), ‘tella’ (traditional beer) and the grain is also eaten roasted as a snack (‘kollo’). Nutritionally, oat is generally considered "healthy", or a health food (Marmouzi *et al.*, 2016). Oat is used as porridge for patients, and mother who delivered new babies. The green plant of oat is good forage; it makes good hay, silage, and grazing by animals. According to Engels *et al.* (1991) besides being sometimes noxious weeds and adjusting themselves well to changing conditions, the Ethiopian oat (*A. abyssinica*) is of no commercial value.

3.8 Soil acidity, Al toxicity and its distribution

3.8.1 Distribution of acid soil

Global distribution of acid soil: Soil acidity is one of the most important factors that affect crop production worldwide. Acid soils (pH < 5.5 in surface layer) constitute 3,950 million ha or over 40 % of the arable land in the world has problems of soil acidity. About 67% of the world's acid soil has crop production constraint associated with Al-toxicity (Eswaran *et al.*, 1997) and it is critical in developing countries. The American continents account for 40.9% of the world's acid soils, followed by Asia (26.4%), Africa (16.7%), Europe (9.9%), Australia, and New Zealand (6.1%) (Abdenna Deressa, 2013; Eswaran *et al.*, 1997; Reynolds *et al.*, 1998).

It has been assessed that more than half of the world's possibly arable terrains are acidic (Valencia *et al.*, 2012; Vardar and Ünal, 2007). Up to 60% of the corrosive soils on the planet happen to occur in South America, Africa, and Southeast Asia, where nourishment generation is basic (Vardar and Ünal, 2007). In spite of the fact that the poor fruitfulness of acidic soils is because of a blend of mineral toxicities (Al and Mn) and inadequacies (P, Ca, Mg and Mo), Al is the most imperative factor (Eswaran *et al.*, 1997).

Distribution of acid soil in Ethiopia: Ethiopia is believed to be affected by soil acidity of differing magnitude (Abreha Kidanemariam *et al.*, 2012; Ermias Abate *et al.*, 2017; Mesfin Abebe, 2007). Of these, 13.2% is strong to moderate acidic soils (pH < 5.5) and 27.7 % is moderate to weak acid soil (pH 5.5 - 6.7) (Ermias Abate *et al.*, 2017). The western and southern parts of Ethiopia overwhelmingly have soils with pH < 5.5 (Ermias Abate *et al.*, 2017). Similarly, regions or areas such as Gozamin and Senan Woreda in eastern Gojam and Awi zone in West Amhara district have problems of acidic soil.

According to Abdenna Deressa (2013), the degree of soil acidity is increasing from central Ethiopia (West Shewa) to western Ethiopia (West Welega). According to Abdenna Deressa *et al.* (2007), the acidity problem in East and West Welega zones of the Oromia region is becoming very critical; and reached a level that affects crop productivity.

3.8.2 Soil acidity

Soil is a complex environment made up of organic and mineral particles, fluids, gases and a different scope of natural creatures. Numerous agents impact the soil acidity including its pH, for example, edaphic, climatic, and biological factors (Redondo, 2013). Soil acidity is determined by the amount of hydrogen (H^+) activity in soil solution (Reynolds *et al.*, 1998).

3.8.2.1 Development of soil acidity

As the soil becomes more acidic, Al is solubilized into a phytotoxic form such as $Al(OH)^{2+}$, $Al(OH)_2^+$, and $[Al(H_2O)_6]^{3+}$ (Simões *et al.*, 2012). The $[Al(H_2O)_6]^{3+}$, which is known as Al^{3+} , and it is dominant in acidic soil below pH 5 and is the most toxic form. Al toxicity is the main growth-limiting factor for plants in acid soils (Foy, 1992) and is most severe in soils with low base saturation, poor in Ca and Mg (Vardar and Ünal, 2007).

Acidity and Al-toxicity develop as a consequence of leaching of cations in soils of high rain fall areas that have rapid drainage. Acidifying effect of acid forming nitrogen fertilizers, poor nutrient recycling and mining of soil cations via harvested crops, runoff loss, and acid rain also make contributions for development of soil acidity (Rao *et al.*, 1993; Wagatsuma and Kaneko, 1987).

Negatively charged clay particles can trap Al^{3+} from the soil solution, and, therefore, can lessen its toxicity to plants. Similarly, organic matter with many negative charges of carboxyl ($-COOH$), hydroxyl ($-OH$) and carbonyl ($-CO$) functional groups can also strongly retain Al from soil solution

by forming natural complexes (Vitorello *et al.*, 2005). Other complexing inorganic anions along with SO_4^{2-} , PO_4^{3-} and so forth and natural anions like citrate, malate, oxalate and so forth can have a similar impact (Delhaize and Ryan, 1995). The concentration of cations at a given pH also considerably upsets Al toxicity (Wang *et al.*, 2006).

3.8.3 Al toxicity

Aluminium (Al) is the most abundant metal and the third most common element in the earth's crust (Vitorello *et al.*, 2005). In soils with normal pH; Al is present in the insoluble form and hence causes no harm to plants. In neutral and alkaline soils the solubility of Al is too low to be toxic to plants. In the acidic soils, it becomes soluble and (i) enters root where it inhibits root growth and development, (ii) reacts with soluble phosphorus (available to plants) and converts it to insoluble aluminium phosphate which is not available to plants. Even micromolar concentrations of aluminium in the soil solution can rapidly inhibit the root growth of many species (Pattanayak and Pfukrei, 2013).

3.8.3.1 Effect of aluminium toxicity in plants

The most important short-term symptom of Al toxicity is inhibition of root growth which is expressed within few minutes to few hours after exposure to micromolar concentrations of Al (Barcelo and Poschenrieder, 2002). In root inhibition, the primary and lateral roots thicken and turn brown in color (Inostroza *et al.*, 2008). The distal transition zone of root tip where the cells switch from cell division to cell elongation is the most sensitive part of roots (Barcelo and Poschenrieder, 2002). Callose formation and lignin deposition in cortical cells of roots are also reported as one of the early symptoms of Al toxicity in different plant species (Barcelo and Poschenrieder, 2002). Reduced fine roots branching, suppression of root hairs development and abnormal root morphology are consequences of long-term exposure of Al-sensitive plants to the toxic concentration of Al (Inostroza *et al.*, 2008). These effects directly influence nutrient uptake as well as water absorption. Consequently, the

deficiency to calcium, magnesium, potassium, iron, molybdenum, and phosphorus is a common symptom in plants grown on soils with Al toxicity problem (Inostroza *et al.*, 2008). Inhibitory effect of Al on root development also hinders absorption of nutrients from subsoil (Samac and Tesfaye, 2003). Some of the indirect effects of Al toxicity include increased susceptibility to stress and to diseases and vulnerability to the drought of even short dry spell due to restricted root growth (Chen, 2013). Generally, the outcome of Al toxicity is significantly expressed on biomass and grain yield of crops. The growth of several tropical crops in acid soil areas is reduced by 50% or more when compared to plants grown on lime soil (Jain *et al.*, 2013). Gallardo *et al.* (1999), also reported 50% and 30% reduction of grain yield in Al-sensitive and tolerant varieties, respectively.

The majority of Al associated with roots is bound in the apoplasm; only a relatively small fraction enters the symplasm where it can interact with intracellular targets. Aluminium exposure results in alterations to the surface, disruption of cytoskeletal dynamics, changes in homeostasis and signaling, peroxidative damage, induction of reactive oxygen species and mitochondrial dysfunction leading to Al mediated inhibition of root growth (Apel and Hirt, 2004).

3.8.3.2 Physiological mechanisms of Al tolerance patterns in plants

Aluminium stimulated secretion of organic acid anions (OA) from plant roots has two different patterns (Ma, 2000; Ma *et al.*, 2001). In barley and wheat (Pattern I), the secretion occurs immediately after the addition of Al; this stimulation could occur in one of three ways: (1) Al^{3+} interacts directly with the channel protein to trigger its opening; (2) Al^{3+} interacts with a specific receptor on the membrane surface or with the membrane itself to initiate a secondary messenger cascade that then activates the channel or; (3) Al^{3+} enters the cytoplasm and activates the channel directly, or indirectly via secondary messengers. However, in rye, sorghum, rice and maize (Pattern II), there is a delay between the secretion and exposure to Al^{3+} . In the Pattern II response, Al interacts with the cell,

perhaps via a receptor protein on the plasma membrane, to activate the transcription of genes that encode proteins involved with the metabolism of organic acids or their transport across the plasma membrane. Organic acid anions develop a stable complex with Al^{3+} , thereby detoxifying Al^{3+} in the rhizosphere. Rapid secretion in the Pattern I species does not need gene induction, while gene induction is required for the delayed secretion in Pattern II species. Moreover, the components of the mechanism that undergoes the Pattern I have been identified, but the components that are involved in the Pattern II are not yet identified, but speculated (Ma *et al.*, 2001).

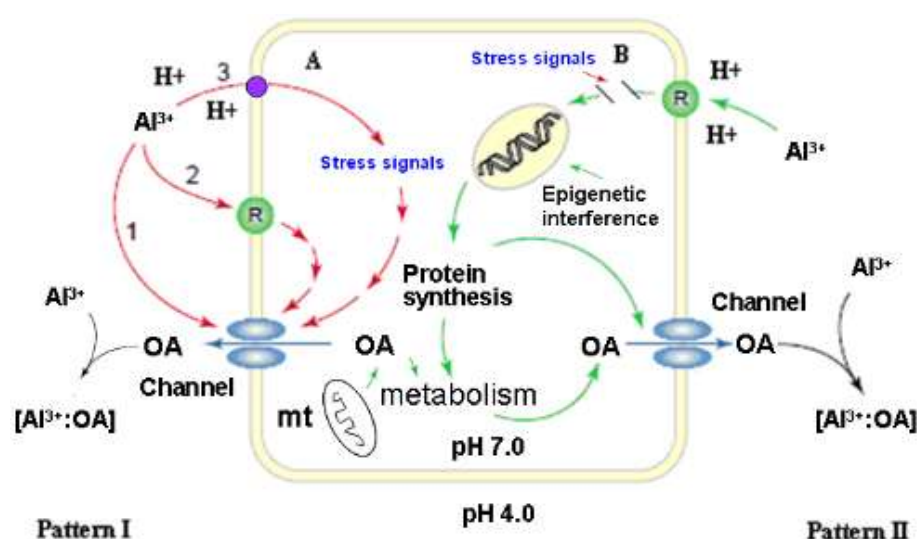


Figure 2. Pattern of aluminium toxicity tolerance of plants, source (Ma *et al.*, 2001).

Key, Al^{3+} : Aluminium ions, OA: organic Acid, H^+ : hydrogen ion and mt: mitochondria

3.8.3.3. Resistance and tolerance strategies of aluminium toxicity

Aluminium resistance mechanisms have been classified into internal and external (Ryan and Delhaize, 2010). The two main Al resistance mechanisms in plants are exclusion of Al ions from root apical cells (i.e., apoplastic mechanisms) (Vázquez *et al.*, 1999) and detoxification of Al in the root and shoot (i.e., symplastic mechanisms) (Kochian *et al.*, 2004; Kochian *et al.*, 2015; Ma, 2000; Ma *et al.*, 2014). In cereals, grass species, the exclusion mechanism is the most common mechanism.

3.8.3.3.1 Exclusion mechanisms

Apoplastic mechanisms are also known as Al exclusion mechanisms. Plants have developed many strategies to detoxify Al externally. Some of them are:

- 1) secretion of Al-chelating substances from the roots including organic acid anions, phenolic compounds (Piñeros *et al.*, 2005; Singh and Chauhan, 2011), and mucilage (Ramos *et al.*, 2012);
- 2) increasing the pH of rhizosphere to reduce the level of toxic Al^{3+} (Larsen *et al.*, 1998);
- 3) accumulation of silicon in their system that can release the silicon so as to detoxify Al by forming aluminosilicate compounds in root apoplast (Brunner and Sperisen, 2013);
- 4) decreasing the ratio of phospholipids to β^5 -sterols in the plasma membrane to lower the negative binding site for Al binding (Kobayashi *et al.*, 2013);
- 5) cell wall modification is another mechanism: For Al^{3+} that are able to traverse the OA barrier in the rhizosphere, meaning that the root cell wall constitutes the next site of Al interaction with the plant and
- 6) increasing methylation of pectin to reduce Al binding to the cell wall (Yang *et al.*, 2013); the primary root cell wall is a complex and heterogeneous structure consisting of cellulose microfibrils embedded in a matrix of pectins and hemicellulose. Al^{3+} binds to pectins due to its high affinity for the pectins' negatively charged carboxylic groups (Pattanayak and Pfukrei, 2013). Al-resistant cultivars exhibit greater levels of methylated pectin, and under Al stress, the resistant cultivars have lower pectin methylesterase activity (Jones and Kochian, 1997). This results in a lower level of net negative charge with the cell wall, which reduces the accumulation of binding Al^{3+} in the cell wall.

3.8.3.3.2 Internal detoxification

In symplastic mechanisms, Al enters the cytoplasm and is detoxified inside the cell by complexation with organic compounds (Delhaize and Ryan, 1995). In cereals, internal detoxification is a less popular mechanism as compared to the exclusion mechanism. Several compounds can form stable complexes

with Al inside the cell, including organic acids such as citrate, oxalate, malate (Zheng *et al.*, 2014), and proteins (Zheng *et al.*, 2014). Free Al^{3+} or Al complexes with chelating agents can be transported to cell vacuoles, where they are stored without causing toxicity (Kochian *et al.*, 2004). Suicidal death of cells affected by Al is also reported as the detoxification mechanism in wheat (Delhaize *et al.*, 2009). The internal detoxification of crops is maximized through the following mechanisms.

- 1) Reducing the negative charge of the plasma membrane, lowers Al activity and phytotoxicity, because of the lower amount of Al that may attach to the membrane. Tolerant genotypes can maintain membrane integrity and electrical balance through a net inflow of H^+ and K flux compared to susceptible genotypes.
- 2) Low cation exchange capacity could contribute to a lower accumulation of Al in the symplast because of a low reactivity of Al^{3+} on the surface of the plasma membrane (Silva, 2012). Cell walls with a lower content of pectin and a high degree of methylation are more resistant to damage from Al toxicity.

In many cases, high levels of Al are not toxic to the plant because it is present as non-toxic organometallic complexes, located in some instances in cellular vacuoles (Maejima *et al.*, 2014; Morita *et al.*, 2009). In cells of the root apex at the primary site of Al toxicity, only small vacuoles are present and according to Silva (2012), these small vacuoles accumulate significant amounts of Al-P and Al-Si, in maize genotypes tolerant to aluminium. This vacuolar compartmentalization is accompanied by a reduction in the concentration of apoplast Al and improved root elongation (Morita *et al.*, 2009). Al transport to the vacuole and formation of aluminium complexes with proteins, permit full or partial inactivation of toxic aluminium (Gupta *et al.*, 2013).

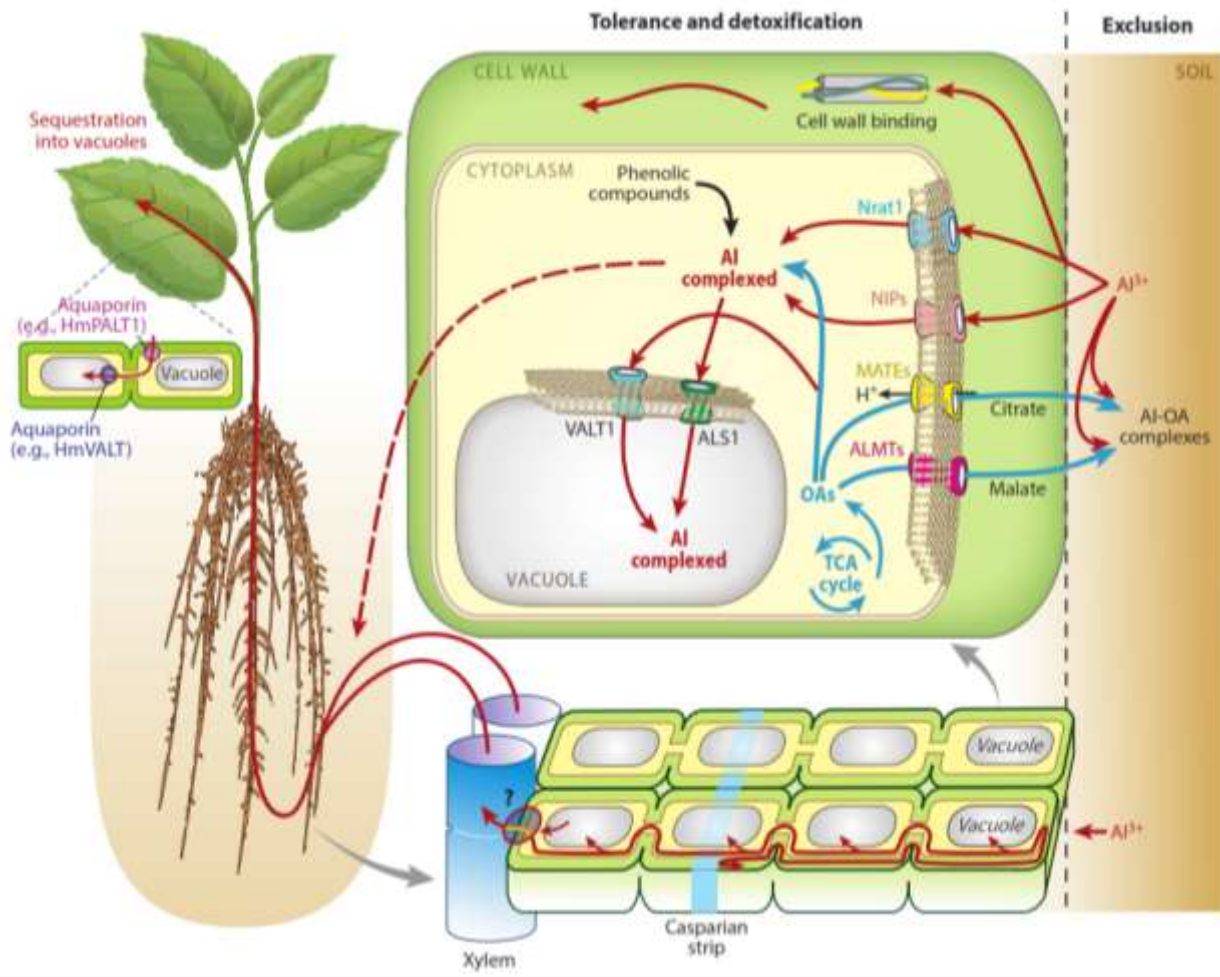


Figure 3. General model illustrating mechanisms of Al resistance (Kochian *et al.*, 2015).

General model illustrating mechanisms of Al resistance (Al exclusion and Al tolerance/detoxification) that plants employ as Al is taken up from the soil solution by the root and some of the absorbed Al is translocated to and stored in the shoot. Where, ABC, ATP-binding cassette; Al, aluminium; *ALMT*, Al-activated malate transporter; *ALS1*, Al-sensitive 1; *MATE*, multidrug and toxic compound extrusion; *NIP*, nodulin 26-like intrinsic protein; *Nramp*, natural resistance-associated macrophage protein; *Nrat1*, *Nramp* Al transporter 1; OA, organic acid; *PALT1*, plasma membrane Al transporter 1; TCA, tricarboxylic acid; *VALT*, vacuolar Al transporter.

3.8.3.4 Gene and the genetics of aluminium toxicity

The majority of Al tolerance genes identified in several species come from two distinct gene families that encode for transport proteins involved in Al activated organic acid release, specifically citrate and malate. As explained below, malate efflux is encoded by members of the Aluminium-activated malate transporter (*ALMT*) gene family and citrate efflux is encoded by members of the multidrug and toxic compound exudation (*MATE*) gene family.

3. 8. 3.4 .1 The *ALMT* family

Aluminium-activated malate transporter (*ALMT*) proteins are a family of anion transporters in plants (Delhaize *et al.*, 2012). The action of genes encoding aluminium-activated malate transporters (*ALMT*) family is based on malate exudation. *ALMT*-type proteins are a small family of protein unique to plants, which mediate passive transport of both organic and inorganic anions. The genes encode protein of anion channels but differ in substrate specificity, membrane location, and function (Kochian *et al.*, 2015). *ALMT* genes have been characterized for several species but only a few are known to confer Al tolerance. Members of the *ALMT* family appear to be involved in several physiological processes including Al tolerance, mineral nutrition and guard cell regulation (Liu *et al.*, 2009).

3. 8.3.4.2 *MATE* gene family

The *MATE* (multi-drug and toxic compound extrusion) family of proteins are encoded by a large and diverse group of genes found in all living organisms. In plants, there are a large number of genes encoding *MATE* proteins suggesting an array of biological functions. Only a few have been functionally characterized as secondary transporters, exporting a wide variety of substrates and organic compounds. The first *MATE* genes functioned to export cationic drugs and toxic compounds, hence their name. Several plant *MATE* proteins have recently been found to mediate citrate transport, iron transport, and other secondary metabolites. Others have specificity for substrates such as flavonoids, xenobiotics, and alkaloids (Inostroza *et al.*, 2012; Liu *et al.*, 2014). A *MATE* transporter found in wheat, *TaMATE 1*, is thought to mediate root citrate release as a possible secondary mechanism to confer Al tolerance.

AtMATE in *Arabidopsis*, a close homolog of *SbMATE*, was discovered through mutant analysis and it confers citrate release. It is a minor Al tolerance mechanism that functions with *AtALMT1* malate release to confer the full range of *Arabidopsis* Al tolerance. Al tolerance in *Arabidopsis* was not associated with increased expression of genes encoding OA synthesis but with differential expression

of transporters (Maron *et al.*, 2008). The transcription factor, STOP1, regulates expression of both *AtALMT1* and *AtMATE1* (Maron *et al.*, 2008). Mutational analysis also identified *AtALS1* and *AtALS3* genes which encode ‘half’ ABC transporters required for Al tolerance by redistributing Al away from sensitive tissues.

Two maize *MATE*, *ZmMATE1* and *ZmMATE2*, were co-localized with two major Al tolerance QTL on maize chromosomes 6 and 5, respectively (Maron *et al.*, 2008; Piñeros *et al.*, 2005). *ZmMATE1* encodes a transmembrane protein that is highly similar to *SbMATE* (Magalhaes, 2010), and its over expression in *Arabidopsis* results in increased citrate exudation as well as higher Al tolerance (Maron *et al.*, 2008; Piñeros *et al.*, 2005). In contrast, *ZmMATE2* expression, which was not induced by Al, was similar between Al-tolerant and Al-sensitive genotypes (Maron *et al.*, 2008; Piñeros *et al.*, 2005). In addition, association between *ZmMATE2* and citrate exudation has not been found, raising questions to a possible role for *ZmMATE2* in maize Al tolerance.

Plants differ in their ability to grow in acidic soils (Kochian, 1995) and the mechanism of tolerance is either quantitative or qualitative inheritance and it is not the same in all species. Moreover, in some species a combination of mechanisms may be operating simultaneously to produce tolerance, hence the genetic control of tolerance can be very complex and involve many genes (Valencia *et al.*, 2012). Other reports indicate that, by altering the synthesis of citrate, transgenic plants can produce aluminium tolerance (Delhaize *et al.*, 2001). Differential tolerance has been attributed to a single dominant gene, although complex inheritance has also been suggested, as in soybean, alfalfa, tobacco, rye, sorghum and maize (Eshel and Beeckman, 2013).

Al tolerance in barley (Furukawa *et al.*, 2007), wheat (Aniol, 1990), sorghum (Magalhaes, 2010; Magalhaes *et al.*, 2007) and oat (Nava *et al.*, 2006) is largely controlled by a single dominant gene,

while Al tolerance in maize (Krill, 2013) and rice (Wu *et al.*, 2000) is a quantitative trait controlled by multiple genes. In addition, according to Ryan *et al.* (2009), wheat has a second Al resistance mechanism (in addition to *TaALMT1*-mediated malate release) that involves citrate exudation controlled by a *MATE* homolog. This shows that there is more diversity in wheat Al resistance than initially thought. This suggests that even in the case of major genes in crop species, allelic variation at auxiliary loci may give rise to polygenic inheritance of Al resistance. In diverse range of genotypes, a major tolerance at Al activated malate for Al-activated plasma membrane protein that allows efflux of malate from root apices up on exposure to Al (Sasaki *et al.*, 2004). Recently, with discovery of a new mechanism of Al-tolerance that involves efflux of citrate in root apices of Brazilian wheat cultivars, another gene has been identified (Ryan *et al.*, 2009). These authors indicated that the citrate efflux was controlled by single gene which could explain 50% of the phenotypic variation in citrate efflux. However, unlike the *TaALMT1*, this gene belonged to a gene encoding a multidrug and toxic compound extrusion protein and is designated as *TaMATE1* (Ryan *et al.*, 2009).

Although in-depth intraspecific investigations emphasized the importance of *TaALMT1* and *SbMATE* in wheat and sorghum Al resistance, respectively (Mickelbart *et al.*, 2015), other important Al resistance genes may remain unidentified in those species. Perhaps the clearest case of monogenic inheritance of Al resistance is found in barley, where Al resistance in genotypes of distinct genetic origins has long been known to be due largely to an allelic series at the *Alp* locus, resulting in little potential for improvement beyond this locus. However, although a whole genome sequencing in barley confirmed that Al plays an important role in the cultivated gene pool, possibly play a part in Al resistance in wild Tibetan barleys (Cai *et al.*, 2013). Therefore, Al resistance in crop plants should be considered intrinsically quantitative in nature and should be tackled as such from genetic and physiological perspectives.

3.9 Markers in genetic diversity analysis

Understanding the molecular basis of the essential biological phenomena in plants is crucial for plant breeding, effective conservation management, and efficient utilization of plant genetic resources. The continuous infusions of gene from wild relatives and traditional varieties depend on crop genetic resources used of modern breeding techniques. These processes all require an assessment of plants with markers at some level, to select resistant, and highly productive varieties. The following are the commonly used markers for the above purpose

3.9.1 Morphological markers

Morphological markers are based on visually accessible traits such as flower color, seed shape, growth habits, and pigmentation, and do not require expensive technology. These types of markers have an advantage, and they are required for distinguishing the adult plants (Govindaraj *et al.*, 2015). This allows the assessment of genetic diversity in the presence of environmental variation (Ashenafi Alemu *et al.*, 2018).

There are several undesirable factors that are associated with morphological markers. The first is that they are highly influenced by environmental factors. These morphological traits are often susceptible to phenotypic plasticity. Customarily, the conditions under which a plant is grown can influence the expression of these markers and lead to a false determination. Performing breeding experiments with these markers are time-consuming, labor intensive and require handling massive populations of plants. This needs large plots of land and/or greenhouse space in which to be grown (Govindaraj *et al.*, 2015).

3.9.2 Biochemical markers: isozyme and allozymes

Isozymes are metabolic enzymes which have the same function but result from different genes. Also called isoenzyme. Isozymes are enzymes that are detected by electrophoresis (Kumar *et al.*, 2009) and

specific staining. Isozyme markers are codominant in nature, and has simple inheritance. They help to detects diversity at the functional gene level. This technique requires only small amounts of plant material. However, only a limited number of enzyme markers are available. However, this enzyme has complex structural and special problems; thus, the resolution of genetic diversity is limited to explore. Isozymes have been used to distinguish phylogenetic relationships, to estimate genetic variability and taxonomy, to study population genetics and developmental biology, to characterize plant genetic resources management and plant breeding (Staub *et al.*, 1996).

Allozymes are genetically controlled allelic variants of enzymes. Low level of polymorphism and abundance are the major weakness of allozymes (Krieger and Ross, 2002).

3.9.3 Molecular (DNA) markers

The discovery of restriction enzymes (Kelly Jr and Smith, 1970) and the polymerase chain reaction (PCR) (Mullis and Faloona, 1987) have created the opportunity to visualize the composition of DNA (Kearsey *et al.*, 1997; Naeem, 2014). According to Roychowdhury *et al.* (2014) a molecular (called DNA) marker is defined as: a) chromosomal landmark or allele that allows for the tracing of a specific region of DNA, b) a specific DNA portion with a known position on the genome, or (c) a gene whose phenotypic expression is usually easily discerned, used to identify an individual or a cell that carries it, or as a probe to mark a nucleus, chromosomes, or locus. Molecular markers offer numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or resistance status of the cell are not confounded by the environment, pleiotropic and epistatic effects (Mishra and Tomar, 2014; Mondini *et al.*, 2009; Ullah *et al.*, 2011; Weising *et al.*, 2005).

3.9.3.1 Restriction fragment length polymorphism (RFLP)

The first DNA based molecular markers system called the Restriction Fragment Length Polymorphism (RFLP), was developed in early 1980 (Jeffreys, 1987) RFLPs can be applied in the study of diversity (Dubreuil *et al.*, 1996), phylogenetic (Fang and Roose, 1997), and hybridization and introgression, including studies of gene flow between crops and weeds (Clausen and Spooner, 1998).

3.9.3.2 Randomly amplified polymorphic DNA (RAPD) marker

RAPDs were the first PCR-based molecular markers to be employed in genetic variation analyses (Williams *et al.*, 1990). Using short primers, RAPD markers are generated through the random amplification of genomic DNA. The use of short primers is suitable for annealing and able to find homologous sequences. As this approach requires no prior knowledge of the genome analyzed, it can be employed across species using universal primers (Bardakci, 2001).

Variability analysis and individual-specific genotyping has largely been carried out by RAPD marker but is less popular due to problems such as faint or fuzzy products, poor reproducibility, and bands scoring difficulty, which lead to unfortunate inferences. RAPDs have been used for many purposes such as the study of, genetic identity and gene mapping (Hadrys *et al.*, 1992).

3.9.3.3 Amplified fragment length polymorphism (AFLP)

To overcome the limitation of reproducibility associated with RAPD, AFLP technology was developed by Vos *et al.* (1995) through digestion of DNA with restriction endonuclease enzymes and use PCR technology. This technique involves a restriction digestion with a combination of one frequent cutter (3 bp recognition site) and one rare cutter (6 bp recognition site). A subset of the total fragments is then ligated to the oligonucleotide adaptors. The ligating fragments are then subjected to PCR amplification under stringent conditions with specific primers, which are complementary to the adaptors. Usually,

50 to 150 fragments are amplified with each primer combination. AFLP fragments separation is performed on highly resolved denaturing polyacrylamide gel using autoradiography, silver staining or fluorescence methods (Vos *et al.*, 1995).

AFLPs can be applied in studies involving genetic identity, phylogenetic studies of closely related species, parentage and identification of clones and cultivars, QTL analysis, and genetic mapping. Generally, this marker has random distribution throughout the genome and high genomic abundance make AFLPs a useful technology for gene mapping studies (Vos *et al.*, 1995).

3.9.3.4 Microsatellites or simple sequence repeat (SSR)

SSR are sets of repeated sequences found within eukaryotic genomes (Fu *et al.*, 2007; Kalia *et al.*, 2011; Wasala and Prasanna, 2013). SSRs are sections of DNA, consisting of mono-, di-, tri-, tetra- or penta-nucleotide tandemly repeating units and found throughout the genomes of most eukaryotic species (Powell *et al.*, 1996). These markers are PCR-based, co-dominant (Litt and Luty, 1989; Senan *et al.*, 2014) and consist of short tandem repeats of between 1 and 6 base pairs (Gupta *et al.*, 1996; Senan *et al.*, 2014), because of these, this marker is used extensively in studies of variability and genotype characterization (Cabral *et al.*, 2012). According to Jannink and Gardner (2005) analysis with SSRs are rapid and cost-effective than RFLP and AFLP markers.

3.9.3.5 Inter-simple sequence repeats (ISSR)

ISSRs are located between adjacent, oppositely oriented microsatellite regions and the fragments range from 100-3000 bp. This technique was reported by Zietkiewicz *et al.* (1994). Primers based ISSRs are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16-18bp). About 10-60 fragments from

multiple loci are generated simultaneously, separated by gel electrophoresis and scored as the presence or absence of fragments of a particular size (Vijayan, 2005).

Genetic identity, parentage, clone and strain identification, and taxonomic studies of closely related species can be analyzed using ISSR marker. In addition, ISSRs are considered useful in gene mapping studies because of the multilocus fingerprinting profiles capacity (Vijayan, 2005).

3.9.3.6 Single nucleotide polymorphism (SNP) markers

SNP is a single nucleotide (A, T, G or C) differs that occurs in DNA sequence among members of a species. SNP is the most abundant marker system both in animal and plant genomes and has recently emerged as the new generation of molecular markers for various applications. Being binary or co-dominant status, they are able to efficiently discriminate between homozygous and heterozygous genotypes (Foster *et al.*, 2010). The more evolutionarily conserved nature of SNPs makes them less subject to the problem of homoplasy (Brumfield *et al.*, 2003). Most importantly, SNPs are amenable to high throughput automation, allowing rapid and efficient genotyping of large numbers of samples (Tsuchihashi and Dracopoli, 2002). In plants, SNPs can be designed from ESTs (Jiménez-Gómez and Maloof, 2009) and single-stranded pyrosequencing. A high-throughput genome analysis method called diversity array technology (DArT), based on a microarray platform, has been developed for the analysis of plant DNA polymorphism (Arif *et al.*, 2010). Arif *et al.* (2010), described a novel SNP genotyping technique, SNP wave. Chip-based SNP arrays use thousands of oligonucleotide probes attached to a solid surface (e.g., glass, silicon wafer) allowing for a large number of SNPs to be introgressed simultaneously (Arif *et al.*, 2010). Insertions, deletions, and SNPs are important in determining sequence variation between individuals.

3.10 Oat genetic diversity

Genetic diversity and its utilization are topics for a lot of different research (Von Bothmer, 2003), since genetic diversity of crops is considered to be one of the main resources supporting human life. Genetic diversity of any crop species is defined as the amount of genetic information within and among individuals of a population, a species, an accumulation, or a community, arising due to recombination, mutations, and introgressions (Ashenafi Alemu *et al.*, 2018; Hawkes, 1991). The use of highly diverse germplasm in breeding, increases the chances for success in developing highly productive new cultivars with good quality properties over a long period of time (Acquaah, 2009). Genetic diversity of related wild species or crop ancestors can also be important to use in breeding in order to solve problems related to crop failure such as pest and disease management, and improving biotic and abiotic stresses resistance (Mulatu Geleta *et al.*, 2007). Many alleles conferring disease resistance have been introgressed from wild oat germplasm into cultivated oat (Rooney *et al.*, 1994; Thomas *et al.*, 1975). Other traits such as plant height and spikelet number (Loskutov and Rines, 2011) show potentially useful genetic variation in wild oat species.

3. 10.1 Genetic diversity of oat based on morpho-agronomic characters

Morpho-agronomic assessment of a germplasm collection must articulate its genetic diversity and depict agronomically valuable variation. Assessing the morphological traits were analyzed using descriptors. According to Bhandari *et al.* (2017) assessing germplasm collection for key agronomic traits, seed quality and defensive traits, flowering, maturity, plant height, protein content, oil content, primary branches, resistances to pests and diseases, drought and cold tolerances and or other worthwhile traits is necessary before getting on a breeding programme.

Rodgers *et al.* (1983), valued the genetic associations among, and grain yields of spring oat (*A. sativa* L.) cultivars released in the north central and northeastern USA between 1941 and 1980.

Studied genetic diversity based on quantitative and qualitative agronomic traits as characters and coefficient of parentage (CP) in oat using plant traits and demonstrated the relationship between population genetic variance for agronomic traits. CP was the best predictor of genetic variance (Souza and Sorrells, 1991a). Variance for CP was positively and correlated with biomass.

Singh and Mishra (1996), reported the genetic diversity of 2576 oat germplasm lines using 11 quantitative traits such as days to heading, leaf length, leaf width, plant height, and days to maturity, panicle length, grains per panicle, seed length, seed width, 100-grain weight, 100-groat weight using a multivariate technique of principal component analysis. All lines were clustered into 12 non-overlapping clusters.

Using morphological characters such as panicle type, panicle erectness, panicle density, lemma color, dorsal awn of a lemma, plant height, flag leaf length, green fodder yield, and dry matter yield (Diederichsen *et al.*, 2001) reported the genetic diversity of oat germplasm.

Cowen and Frey (1987) studied nine varieties of cultivated oat using characters such as plant height (cm), number of tillers per meter row, number of leaves per tiller, stem thickness (cm), green fodder yield (t ha^{-1}) and leaf area (cm^2). The data recorded were statistically analyzed and who found significant differences for plant height, number of tillers per meter row, number of leaves per tiller and green fodder yield while the non-significance association was observed for stem thickness and leaf area.

Diederichsen (2007) studied 10,105 accessions of hexaploid cultivated oat from 85 countries for eight environmentally stable morphological traits. The 10,105 accessions represented 118 morphological

groups. The number of accessions in each morphological group was randomly distributed with the eight most frequent morphological groups.

Travlos and Giannopolitis (2010), surveyed 151 fields and evaluated 158 accessions of wild oat using morphological traits such as awn length, plant height, number of leaves per plant, number of tillers per plant, lemma hairiness. Qualitative characters observed, such as lemma color, being intermediate in *A. fatua* and having a high variation ranging from yellowish to dark brown in *A. sterilis*. The data revealed an overall higher diversity in *Avena sterilis* than *Avena fatua*.

Ahmed *et al.* (2011) studied genetic diversity in 75 germplasm lines of oat and contributing traits such as days to 50% flowering, plant height, number of spikelet per panicle, panicle length, and number of leaves per plant, leaf length, leaf width, green fodder yield, and grain yield. Analysis of variance revealed the significant difference among the germplasm lines and clustering pattern (nine clusters) using ten yields. A high number of lines in a single cluster depicts that the lines had less genetic variation among them and are more closely related. A crossing within these clusters will be of less use in the breeding programme. They also revealed using principal component analysis that the first four most informative components accounted for 98.33% variance.

Bibi *et al.* (2012), evaluated 108 accessions of oat (*A. sativa* L.) including five checks for green fodder yield. Analysis of variance of 108 accessions of oat revealed highly significant differences ($p < 0.01$) for morpho-agronomic traits such as plant height, internode length, number of leaves per plant and leaf area. The highest values of genotypic and phenotypic coefficient of variance were recorded for the number of tillers per meter row while lowest for the number of leaves per plant. The highest heritability values were recorded for most traits. Internode length, leaf area, number of tillers and dry matter yield

had a positive and significant correlation with green fodder yield. Green folder yield had negative and non-significant correlation with days to 50% flowering, days to 90% maturity and plant height.

3. 10.2 Genetic diversity of oat based on molecular markers

Various biochemical and molecular markers such as isozyme and allozyme analysis, RFLPs, RAPDs, AFLPs, and SSRs have been used in genetic diversity analysis of oat.

Gene-targeted markers, a novel marker system called SCoT. Using 18 SCoT primers markers 153 different fragments were amplified of which 67 were polymorphic with an average number of 3.72 polymorphic fragments per genotype (Balážová *et al.*, 2017). Genetic polymorphism was characterized based on polymorphic information content (PIC).

Moser and Lee (1994), analyzed nine parental lines of oat (*A.sativa*) with 24 RFLP markers. The genealogical distance was found from the coefficient of parentage. Many modern high-throughput strategies for DNA extraction and particularly modern high-throughput marker genotyping platforms have been created (Syvänen, 2005). A current trend in a few crops is the selection of high throughput genotyping device for SSR markers (Brennan *et al.*, 2005).

Microsatellite (or SSR) markers have demonstrated to be vital devices in crop genetics (Gao *et al.*, 2003; Li *et al.*, 2000; Yu *et al.*, 2004) and germplasm research (Fu *et al.*, 2003; Karp, 2002). For *Avena* species and oat cultivars (Li *et al.*, 2000) built oat microsatellite libraries, to confine microsatellite arrangements and assess their level of polymorphism. They separated and sequenced one hundred clones from three oat microsatellite- libraries enhanced for either (AC/TG)_n, (AG/TC)_n or (AAG/TTC)_n repeats in which 78 clones contain microsatellites. In their study, about 44 preliminary sets were utilized to amplify the DNA from 12 *Avena* species and 20 *A. sativa* cultivars among them 82% of the primers revealed polymorphism among the *Avena* species, but 36% among the cultivars.

An average of four alleles with polymorphism information content (PIC) of 0.57 per primer set was detected among the *Avena* species, and 3.8 alleles with a PIC of 0.55 among the cultivars. They also tested 54 barley microsatellite primers in *Avena* species and 26% of the primers amplified microsatellites from oat. Utilizing microsatellite polymorphisms, dendrograms were developed showing phylogenetic relationships among *Avena* species and hereditary connections among *A. sativa* cultivars.

Baohong *et al.* (2003), used RAPDs for measuring and comparing genetic diversity in Chinese and European oat accessions. Fu *et al.* (2004), demonstrated genetic diversity in Canadian oat using AFLPs.

Paczos-Grzeda (2004), compared AFLP and RAPD markers for accessing genetic diversity in oat varieties registered in Poland. They found that polymorphic RAPD and AFLP markers could distinguish all analyzed cultivars, demonstrating the usefulness of both methods for cultivar identification with better correlation (0.50) between similarities estimates derived from both markers. Based on pedigree and molecular analyses using a neighbor-joining algorithm (NJ) separate dendrogram were made. They conclude that both molecular techniques can be conveniently used for genetic characterization of oat cultivars; however, AFLP would be the method of choice due to the higher efficiency and reproducibility.

Fu *et al.* (2005), screened 670 accessions representing 79 countries and one group of uncertain origin using applied AFLP technique. For each accession, they scored 170 AFLP polymorphic bands detected by five AFLP primer pairs. The average frequencies of polymorphic bands were 0.72 and ranged from 0.11 to 0.99. Mainly 89.9% of the AFLP variation were found within accession in each country than among accessions of major geographic regions. Achleitner *et al.* (2008), found a significant association of 23 AFLP markers with agronomic traits (grain yield, panicle emergence,

plant height, lodging, and kernel quality traits) using simple regression model and calculated genetic similarity by Dice coefficient for cluster analysis.

Abbas *et al.* (2008), used RAPD markers for diversity analysis for ten oat germplasm lines and they found 303 DNA fragments, giving an average of 30.3 alleles per genotype. Also by genetic dissimilarity analyses, they showed a high level of genetic diversity (average G.D. ranging from 15 - 81%) existed in 10 genotypes used.

Cabral *et al.* (2012), assessed microsatellite (SSR) polymorphism across 90 diploid *A. strigosa* and tetraploid *A. barbata* using 105 genomic SSRs. They identified eleven polymorphic SSRs that detected 69 alleles in 90 accessions, which were chosen from a larger set of 385 accessions based on geographical source-diversity. They obtained eight diploid and eight tetraploid clades among the 90 accessions. Both species formed a distinct cluster in the dendrogram, a few instances of diploids showing high similarity with tetraploids and vice versa were observed. They revealed 86 % of the total genetic variation to be distributed within the two oat species, while between-species differences accounted for only 14 %.

Montilla-Bascón *et al.* (2013), used 31 SSRs to characterize and evaluate the genetic diversity in the landraces and commercial varieties of oat collection and found a high level of polymorphism, showing 454 alleles. They recognized and classified 138 alleles out of 454 detected as rare alleles due to their low frequency (<0.03), 194 were classified as “common,” with frequencies between 0.03 and 0.2, and 122 were classified as “more frequent” with frequencies >0.2 . Based on PIC values gotten, most SSRs, with the few exceptions were considered informative markers ($PIC > 0.7$). From the dendrogram generated, the 177 accessions could be classified into four major clearly separated commercial

cultivars. The average PIC of 0.80 for the SSR loci indicated the usefulness of many of the SSR for genotype identification.

Ruwali *et al.* (2013a), analyzed genetic variability among 15 oat genotypes comprising fodder and dual-purpose oat varieties from diverse topographical locales by RAPD marker strategy. Their result, evidenced high genetic diversity among the oat genotypes examined. Fifteen primers of *Avena* microsatellite amplify 259 clear and identifiable groups, of which 250 groups were polymorphic, accounting for 96.52% genetic polymorphism. They particularly separated all the oat genotypes considered into two major groups with the genetic distance level at 0.46 by cluster examination based on the Jaccard's coefficient of similarity. They found several polymorphic bands in different genotypes which helped in molecular diversity analysis of these genotypes.

In another study, Ruwali *et al.* (2013b) appraised equivalence between phenotypic and ISSR marker to analyze the genetic diversity of 20 high yielding genotypes representing to diverse topographical zones of the world. Genotypes in morphological character based dendrogram were clustered into their particular geographic groups, whereas an irregular clustering was observed in dendrogram based on the ISSR markers.

Nowosielska and Nowosielski (2009), studied 64 oat germplasm lines for diversity analysis based on 25 morphological traits (23 qualitative and two quantitative) and studied DNA polymorphism in these varieties using AFLP and RAPD markers. Cluster analysis of 64 oat varieties on the basis of morphological characters has proved that mutual similarity of the studied objects.

4 MATERIALS AND METHODS

4.1 Morpho-agronomic Diversity

4.1.1 Experimental materials

The plant material used for this study are presented in Table 2 and 3 and the study sites are shown on Figure 4. One hundred seventy six accessions of five *Avena* species, (four wild: *A. abyssinica*, *A. vaviloviana*, *A. fatua*, and *A. sterilis* and one cultivated: *A. sativa* L.) were used for the study. Of these, 115 accessions were collected during the present study from Gojam, Gondar, Wello, Shewa, Arsi, Bale, and Welega regions of Ethiopia (Figure 4). In addition, 34 *Avena* species accessions were provided by Ethiopian Biodiversity Institute (EBI), 32 of which had been collected from Ethiopia and 2 obtained from the Netherlands. Additional 27 *A. sativa* accessions were received from the International Livestock Research Institute (ILRI), Addis Ababa. The origin of the ILRI accessions was Ethiopia (1), USA (23) and Australia (3) (Figure 4 and Table 2 and 3). Totally, the number of accessions in each of the five *Avena* species are 82 belonged to *A. sativa*, 18 to *A. fatua*, 12 to *A. sterilis*, 44 to *A. abyssinica* and 20 to *A. vaviloviana*. Accession number and other related information about the accessions are presented in Tables 2 and 3.

Table 2. Number of accessions of the five species of oat used in the present study by region/country of origin

Species	Arsi	Bale	Gojam	Gondar	Shewa	Welega	Wello	USA	Netherland + Australia	Total
<i>A. sativa</i>	3	1	10	6	22	7	5	23	2+3	82
<i>A. fatua</i>	1		1		14		2			18
<i>A. sterilis</i>	3			7	2					12
<i>A. abyssinica</i>	9	4	1	4	18	5	3	.		44
<i>A. vaviloviana</i>	10	5	1	1	2	1				20
Total	26	10	13	18	58	13	10	23	5	176

The details of the species name, accession code, region of origin for each of 176 accessions of oat studied is given in Table 3. The taxonomic identification of the material was done at the National Herbarium of the Addis Ababa University.

Table 3. Species of oats, their accessions number, and region/country used

S. No	Acc ID	Origin of acc	Species	S.No	Acc ID	Origin of acc	Species
1	57	Arsi	<i>A. sativa</i>	46	236854	Shewa	<i>A. sativa</i>
2	59	Arsi	<i>A. sativa</i>	47	5432	USA	<i>A. sativa</i>
3	65	Arsi	<i>A. sativa</i>	48	5433	USA	<i>A. sativa</i>
4	9675	Gojam	<i>A. sativa</i>	49	5436	USA	<i>A. sativa</i>
5	9677	Gojam	<i>A. sativa</i>	50	5438	USA	<i>A. sativa</i>
6	9678	Gojam	<i>A. sativa</i>	51	5440	USA	<i>A. sativa</i>
7	9680	Gojam	<i>A. sativa</i>	52	5441	USA	<i>A. sativa</i>
8	9682	Gojam	<i>A. sativa</i>	53	5443	USA	<i>A. sativa</i>
9	9683	Gojam	<i>A. sativa</i>	54	5452	USA	<i>A. sativa</i>
10	9685	Gojam	<i>A. sativa</i>	55	5454	USA	<i>A. sativa</i>
11	212664	Gojam	<i>A. sativa</i>	56	5465	USA	<i>A. sativa</i>
12	238234	Gojam	<i>A. sativa</i>	57	5475	USA	<i>A. sativa</i>
13	238235	Gojam	<i>A. sativa</i>	58	5489	USA	<i>A. sativa</i>
14	8	Gondar	<i>A. sativa</i>	59	5490	USA	<i>A. sativa</i>
15	15	Gondar	<i>A. sativa</i>	60	5492	USA	<i>A. sativa</i>
16	16	Gondar	<i>A. sativa</i>	61	5493	USA	<i>A. sativa</i>
17	26	Gondar	<i>A. sativa</i>	62	5499	USA	<i>A. sativa</i>
18	28	Gondar	<i>A. sativa</i>	63	5507	USA	<i>A. sativa</i>
19	52018	Gondar	<i>A. sativa</i>	64	5509	USA	<i>A. sativa</i>
20	15152	Australia	<i>A. sativa</i>	65	5510	USA	<i>A. sativa</i>
21	15153	Australia	<i>A. sativa</i>	66	5513	USA	<i>A. sativa</i>
22	16101	Australia	<i>A. sativa</i>	67	5527	USA	<i>A. sativa</i>
23	235519	Netherland	<i>A. sativa</i>	68	5529	USA	<i>A. sativa</i>
24	235521	Netherland	<i>A. sativa</i>	69	5538	USA	<i>A. sativa</i>
25	14	Shewa	<i>A. sativa</i>	70	200	Welega	<i>A. sativa</i>
26	76	Shewa	<i>A. sativa</i>	71	203	Welega	<i>A. sativa</i>
27	79	Shewa	<i>A. sativa</i>	72	204	Welega	<i>A. sativa</i>
28	98	Shewa	<i>A. sativa</i>	73	205	Welega	<i>A. sativa</i>
29	104	Shewa	<i>A. sativa</i>	74	206	Welega	<i>A. sativa</i>
30	105	Shewa	<i>A. sativa</i>	75	207	Welega	<i>A. sativa</i>
31	113	Shewa	<i>A. sativa</i>	76	208	Welega	<i>A. sativa</i>
32	116	Shewa	<i>A. sativa</i>	77	2	Wello	<i>A. sativa</i>
33	123	Shewa	<i>A. sativa</i>	78	3	Wello	<i>A. sativa</i>
34	1000	Shewa	<i>A. sativa</i>	79	22	Wello	<i>A. sativa</i>
35	2000	Shewa	<i>A. sativa</i>	80	23	Wello	<i>A. sativa</i>
36	3000	Shewa	<i>A. sativa</i>	81	52003	Wello	<i>A. sativa</i>
37	6708	Shewa	<i>A. sativa</i>	82	6207	Bale	<i>A. sativa</i>
38	6710	Shewa	<i>A. sativa</i>	83	33	Arsi	<i>A. abyssinica</i>
39	8237	Shewa	<i>A. sativa</i>	84	52	Arsi	<i>A. abyssinica</i>
40	52001	Shewa	<i>A. sativa</i>	85	54	Arsi	<i>A. abyssinica</i>
41	52004	Shewa	<i>A. sativa</i>	86	58	Arsi	<i>A. abyssinica</i>
42	52012	Shewa	<i>A. sativa</i>	87	60	Arsi	<i>A. abyssinica</i>
43	236851	Shewa	<i>A. sativa</i>	88	62	Arsi	<i>A. abyssinica</i>
44	236852	Shewa	<i>A. sativa</i>	89	132	Arsi	<i>A. abyssinica</i>
45	236853	Shewa	<i>A. sativa</i>	90	133	Arsi	<i>A. abyssinica</i>

Table 3 continued ...

S. No	Acc ID	Origin of acc	Species	S. No	Acc ID	Origin of acc	Species
91	52020	Arsi	<i>A. abyssinica</i>	134	93	Shewa	<i>A. fatua</i>
92	137	Bale	<i>A. abyssinica</i>	135	94	Shewa	<i>A. fatua</i>
93	140	Bale	<i>A. abyssinica</i>	136	95	Shewa	<i>A. fatua</i>
94	141	Bale	<i>A. abyssinica</i>	137	96	Shewa	<i>A. fatua</i>
95	142	Bale	<i>A. abyssinica</i>	138	97	Shewa	<i>A. fatua</i>
96	7	Gondar	<i>A. abyssinica</i>	139	117	Shewa	<i>A. fatua</i>
97	9	Gondar	<i>A. abyssinica</i>	140	52011	Shewa	<i>A. fatua</i>
98	10	Gondar	<i>A. abyssinica</i>	141	52014	Shewa	<i>A. fatua</i>
99	161	Gondar	<i>A. abyssinica</i>	142	52006	Wello	<i>A. fatua</i>
100	13	Shewa	<i>A. abyssinica</i>	143	52010	Wello	<i>A. fatua</i>
101	18	Shewa	<i>A. abyssinica</i>	144	17	Arsi	<i>A. sterilis</i>
102	20	Shewa	<i>A. abyssinica</i>	145	30	Arsi	<i>A. sterilis</i>
103	21	Shewa	<i>A. abyssinica</i>	146	37	Arsi	<i>A. sterilis</i>
104	101	Shewa	<i>A. abyssinica</i>	147	1	Gondar	<i>A. sterilis</i>
105	103	Shewa	<i>A. abyssinica</i>	148	5	Gondar	<i>A. sterilis</i>
106	107	Shewa	<i>A. abyssinica</i>	149	6	Gondar	<i>A. sterilis</i>
107	108	Shewa	<i>A. abyssinica</i>	150	12	Gondar	<i>A. sterilis</i>
108	109	Shewa	<i>A. abyssinica</i>	151	19	Gondar	<i>A. sterilis</i>
109	111	Shewa	<i>A. abyssinica</i>	152	24	Gondar	<i>A. sterilis</i>
110	118	Shewa	<i>A. abyssinica</i>	153	25	Gondar	<i>A. sterilis</i>
111	119	Shewa	<i>A. abyssinica</i>	154	77	Shewa	<i>A. sterilis</i>
112	121	Shewa	<i>A. abyssinica</i>	155	78	Shewa	<i>A. sterilis</i>
113	122	Shewa	<i>A. abyssinica</i>	156	32	Arsi	<i>A. vaviloviana</i>
114	126	Shewa	<i>A. abyssinica</i>	157	36	Arsi	<i>A. vaviloviana</i>
115	127	Shewa	<i>A. abyssinica</i>	158	41	Arsi	<i>A. vaviloviana</i>
116	128	Shewa	<i>A. abyssinica</i>	159	45	Arsi	<i>A. vaviloviana</i>
117	52015	Shewa	<i>A. abyssinica</i>	160	48	Arsi	<i>A. vaviloviana</i>
118	145	Welega	<i>A. abyssinica</i>	161	51	Arsi	<i>A. vaviloviana</i>
119	146	Welega	<i>A. abyssinica</i>	162	53	Arsi	<i>A. vaviloviana</i>
120	147	Welega	<i>A. abyssinica</i>	163	63	Arsi	<i>A. vaviloviana</i>
121	148	Welega	<i>A. abyssinica</i>	164	71	Arsi	<i>A. vaviloviana</i>
122	201	Welega	<i>A. abyssinica</i>	165	131	Arsi	<i>A. vaviloviana</i>
123	52005	Wello	<i>A. abyssinica</i>	166	134	Bale	<i>A. vaviloviana</i>
124	52008	Wello	<i>A. abyssinica</i>	167	135	Bale	<i>A. vaviloviana</i>
125	52009	Wello	<i>A. abyssinica</i>	168	136	Bale	<i>A. vaviloviana</i>
126	52019	Gojam	<i>A. abyssinica</i>	169	138	Bale	<i>A. vaviloviana</i>
127	219512	Gojam	<i>A. fatua</i>	170	139	Bale	<i>A. vaviloviana</i>
128	73	Shewa	<i>A. fatua</i>	171	212663	Gojam	<i>A. vaviloviana</i>
129	83	Shewa	<i>A. fatua</i>	172	11	Gondar	<i>A. vaviloviana</i>
130	86	Shewa	<i>A. fatua</i>	173	80	Shewa	<i>A. vaviloviana</i>
131	88	Shewa	<i>A. fatua</i>	174	81	Shewa	<i>A. vaviloviana</i>
132	89	Shewa	<i>A. fatua</i>	175	152	Welega	<i>A. vaviloviana</i>
133	92	Shewa	<i>A. fatua</i>	176	64	Shewa	<i>A. sativa</i> ; AGM

Where, acc: accessions, S.No: serial number, and ID: Identification number

4.1.2 Experimental sites

The field experiment for morpho-agronomic traits study was carried out at Holetta Agricultural Research Center (9 ° 3' N, 38° 30' E, altitude 2400 m asl) and Debre Berhan Agricultural Research Center (9° 32'N, 39° 35' E 2780 m asl.) in the year of 2014/2015 and 2015/2016 cropping seasons. At each site, the germplasm accessions were planted with a randomized block design with two replications see Figure 4 below.

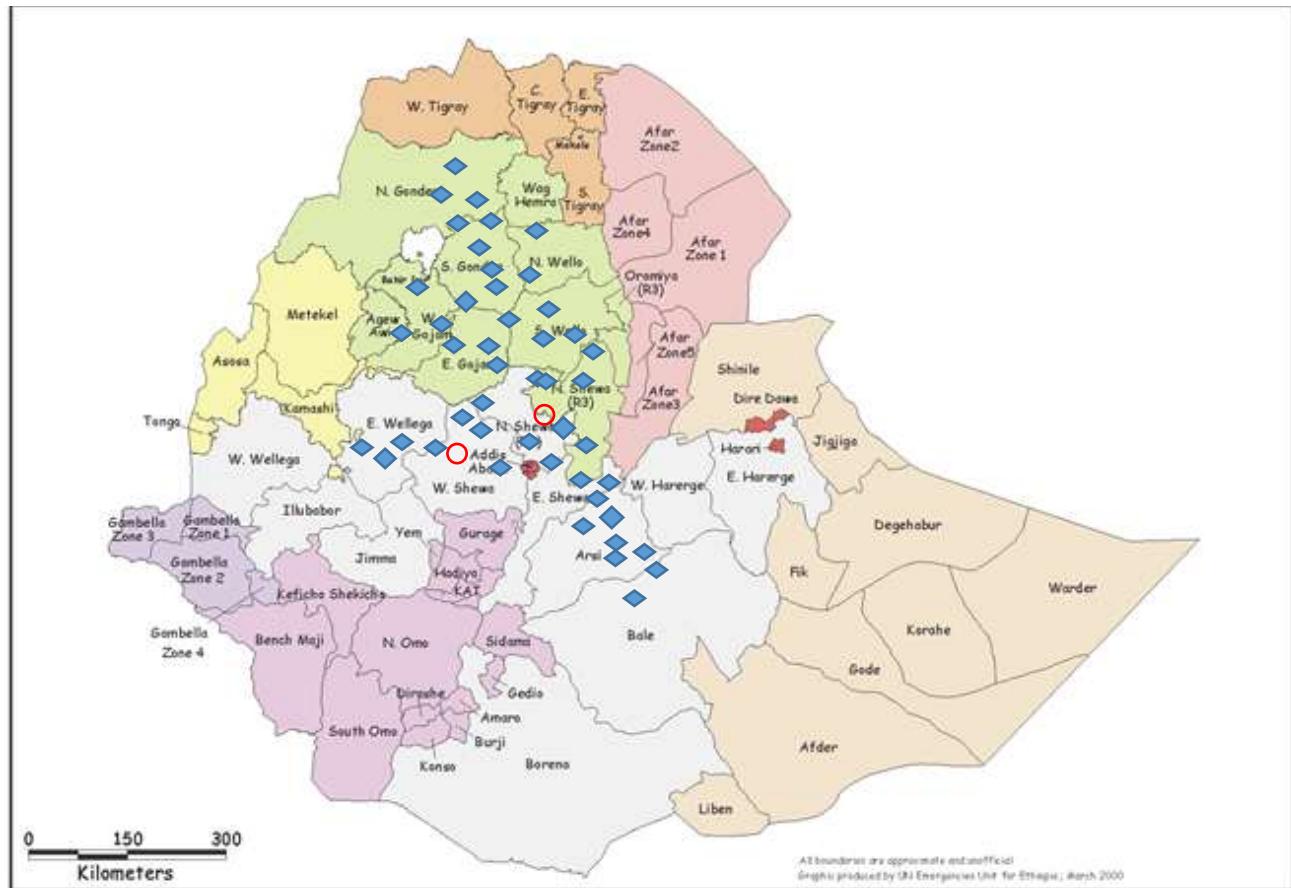


Figure 4. Map of Ethiopia showing the geographical distribution of collection sites (◆) of a 148 of the local oat accessions collected from Ethiopia and the geographical location of experimental sites (○), the map was adopted from (Tamene *et al.* 2006).

Holetta: The annual average precipitation at Holetta Agricultural Research Center was 1044 mm, and mean relative humidity was 60.6%. The mean daily temperature ranged from the minimum of 6.1°C to the

maximum 22.2°C (Bedasa Mekonnen *et al.*, 2015). The soil of the experimental field is red-brown loam nitisols with a pH of 4.92 (Fekede Feyissa *et al.*, 2008; Tiegist Dejene *et al.*, 2010).

Debre Berhan: The annual rainfall of Debre Berhan Agricultural Research Center was 1060 mm while the average annual minimum and maximum temperatures of the area were 2.6°C and 16.9°C, respectively (Fedaku Wondimu *et al.*, 2014). The soil of experimental site is classified as Vertisol (Beyene *et al.*, 2015).

4.1.3 Experimental design and agronomic practices

The field experiment was carried out during the main rainy seasons of 2014/15 and 2015/2016 at the two locations. Each accession was grown in a single row plot of 1 m long and 0.2 m wide and plant to plant spacing was 4 cm in a row, with two replications in a randomized complete block design. In each plot there were 25 plants. Each replication had two blocks, at the end of each block two border rows were used to minimize the bordering effect. Terraces were formed over the block to protect the plot from soil erosion and weeds were manually regulated.

Sowing was done by hand at 85 kg ha⁻¹. Sowing plump seed at the right depth is an important first step towards achieving vigorous, healthy seedlings. Planting should be deep enough to provide uniform coverage of the seed and to help maintain moist conditions for germination. The recommended depth for most oat varieties is 3-6 cm into moist soil. Fertilizer application was done at 41 kg N and 46 kg P₂O₅ ha⁻¹ at planting.

4.1.4 Data collection

4.1.4.1 Qualitative characters

Each accession was classified based on qualitative morpho-agronomic traits, using oat descriptor (IBPGR, 1985). The qualitative traits scores, codes, and descriptors used are listed and described below

(Table 4). Some quantitative traits like stem thickness, stem length and stem width of the second leaf from the top were recorded and converted to scalar qualitative using reference of the three cultivars (Jassary, Acc no 1000, 2000, and 3000). Based on the mean average of these cultivars the quantitative traits of the other accessions were converted to scalar qualitative forms.

Table 4. Qualitative traits scored, code and descriptors used for characterization.

Character	Code	Cha. state observed	Phenotypic classes and their c.odes
Growth habit	GH	3	At juvenile stage, angle of the tillers from the vertical and quantified as, prostrate (3), semi prostrate (5), and erect (7)
Stem thickness	ST	3	Relative to Jassary (accession No = 1000); thin (3), intermediate (5), and thick (7)
Angle of flag leaf to culm	AFLC	3	Acute < 90° (3), intermediate about 90° (5), and obtuse > 90° (7)
Rigidity of flag leaf	RFL	3	Bent (3), Slightly bent (5), and Stiff (7)
Shape of panicle	SP	2	Unilateral (1), and Equilateral (2)
Erectness of panicle	EP	3	Drooping (3), Semi-erect (5), and Erect (7)
Lemma color	LC	5	White (1), Yellow (2), Grey (3), Red (4), and Black or brown-black (5)
Glume color	GuC	3	White (1), Red to brown (2), and Purple to black (3)
Glume size	GuS	4	Small (3), Intermediate (5), Large (7), and Very large (9)
Seed size	SeS	4	Small (3), Intermediate (5), Large (7), and Very large (9)
Seed color	SC	5	White (1), Yellow (2), red (3), Grey (4), and brown-black (5)
Hairiness of lemma	HL	4	Glabrous (0), Slightly pubescent (3), Moderately pubescent (5), and Highly pubescent (7)
Length of second leaf from top	LSL	3	Relative to specified to Jassary (3), Medium (5), and Long (7)
Width of second leaf from top	WSL	3	At widest point. Relative to Jassary; Narrow (3), Medium (5), and Broad (7)
Lodging at immature and or mature stage	LoGI/ LoGM	5	A state of permanent displacement of a stem crop from its upright position at immature or mature stage. It is measured as Upright (all plants) (0), Minor lodging (3), Intermediate (5), Lodged (7), and Lodged (all plants) (9)
Awedness	A	3	Recorded on basal floret: No awns (0), Weak awns (3), and Strong awns (7)
Awn type	AT	3	Recorded on basal floret Straight (1), Geniculate (2), and Other (3)
Relation maturity of grain to straw	RGS	3	Straw ripe before grain (3), Simultaneously (5), and Grain ripe before the straw (7)
Hairiness of leaf sheath (lower leaves)	HLS	4	Glabrous (0), Slightly pubescent (3), Moderately pubescent (5), and Highly pubescent (7)
Susceptible to diseases	SD	4	Low (3), Intermediate (5), High (7), Very high (9)
Panicle compactness	PCom	3	Compact (3), Intermediate (5), Loose (7)

4.1.4.2 Quantitative traits

Data on twelve morpho-agronomic quantitative traits were scored on plot basis and plant basis. Data collected on an individual plant basis include the number of effective/fertile tillers, internode length and flag leaf length, length of panicle, number of spikelet/panicle, and plant height. Randomly selected five plants that have approximately equal interval were labeled for data collection.

Table 5. Quantitative traits scored, code and descriptors used for characterization

Character	Code	Descriptors
Grain yield/m ² (g)	GY	Seeds obtained from the dried plants in the plot were threshed and weighed.
Biological yield/m ² (g)	BY	The above ground parts of all plants in a plot were harvested, air-dried for five days, and oven dried at 45-58°C. The resulting dry weight was added to the grain yield to obtain the biological yield.
Harvest index (%)	HI	The ratio of weight of dried grain yield per plot in grams adjusted to 14% moisture content obtained from each plot to the dried total weight of above ground biological yield per plot expressed in percent.
Number of effective or fertile tillers (number)	NET	Number of shoots, which arise from the base of the stem (main stalk), that bear spike.
Number of Spikelet/panicle (number)	NSP	Fertile number of spikelet for five sampled, plants were counted and recorded.
Length of panicle (cm)	LP	Length of panicle from its base to tip
Plant height (cm)	PH	The distance between the ground level to the tip of the terminal spikelet of the main stock
Flag leaf length (cm)	FLL	The length of uppermost leaf on the oats stem measured and recorded
Internode length (cm)	IL	Length of internode between 3 rd and 4 th nodes of the main tiller was measured and recorded
Node number/plant (number)	NN	Count of total of nodes per plant (main stalk)
Days to 50% flowering/heading (days)	HDE	Number of days required to 50% panicles/tassel exertion from the flag leaf of grasses or cereals in a field or study.
Days to 90% maturity (days)	DM	Number of days required from seeding to seed/grain ripening

Data collected on plot basis were for grain yield, biological yield, harvest index, days to 50% heading, and days to 90% maturity (Table 5).

4.1.5 Data analysis of morpho-agronomic traits

Phenotypic frequency distributions of the qualitative characters were calculated on percentage basis on cultivated versus wild accessions (Savova and Valcheva, 2017) and genetic diversity index (H') was calculated by Shannon-wiener index (Savova and Valcheva, 2017):

$$H' = -\sum P_i \ln P_i, \text{ and } P_i = N_i/N,$$

Where, N is the number of the same sample

Analysis of variance of quantitative data was made using the SAS statistical package with the GLM procedure of SAS. Using Levene's test homogeneity of variances between environments were tested. Location, years, genotypes, and their interactions were all considered to be random (Peterson *et al.*, 2005). Variance components attributed to genotypes and their interactions were computed using the VARCOMP procedure of SAS. Analysis of variance over location was performed on data collected for all experiments using the statistical model:

$$Y_{ijkl} = \mu + b_i + g_j + l_k + y_l + (gl)_{jk} + (gy)_{jl} + (gyl)_{jkl} + e_{ijkl}$$

Where, b = effect of block i , g = effect of genotype j , l = effect of location k , y = effect of year l , gl = effect of interaction of genotype j by location k , gy = the effect of interaction of genotype j by year l , gyl = effect of interaction of genotype j , by year l , and location k , and e = effect of interaction of genotype by block i , genotype j , location k , and year l .

Table 6. Mean square (MS) and expected mean square (EMS) for the ANOVA of genotype, location, and year and the interaction of different environmental factors, interaction of environment with genotype, and experimental error

Source	df	MS	EMS
Replication within location and year (b)	LY(r-1)	M ₁	$\sigma^2_e + g\sigma^2_r$
Years (y)	(y-1)	M ₂	$\sigma^2_e + r\sigma^2_{gly} + rg\sigma^2_{ly} + rl\sigma^2_{gy} + rlg\sigma^2_y$
Locations (l)	(l-1)	M ₃	$\sigma^2_e + r\sigma^2_{gly} + rg\sigma^2_{ly} + ry\sigma^2_{gl} + rgy\sigma^2_l$
Genotypes (g)	(g-1)	M ₄	$\sigma^2_e + r\sigma^2_{gyl} + rl\sigma^2_{gy} + ry\sigma^2_{gl} + ryl\sigma^2_g$
Year x Location	(y-1) (l-1)	M ₅	$\sigma^2_e + r\sigma^2_{gly} + rg\sigma^2_{ly}$
Genotype x Year	(g-1) (y-1)	M ₆	$\sigma^2_e + r\sigma^2_{gly} + rl\sigma^2_{gy}$
Genotype x Location	(g-1) (l-1)	M ₇	$\sigma^2_e + r\sigma^2_{gly} + ry\sigma^2_{gl}$
Genotype x Year x Location	(g-1) (y-1) (l-1)	M ₈	$\sigma^2_e + r\sigma^2_{gly}$
Residual	ly(g-1) (r-1)	M ₉	σ^2_e

The genotypic value (σ^2_g) phenotypic value (σ^2_p) were compliment of variables estimated from the combined ANOVA assuming the actual mean square were equal to the expected mean square

Table 7. Parameters of variability

Variance component	Function
σ^2_g	(M ₄ -M ₆ -M ₇ +M ₈)/rl
σ^2_y	(M ₂ -M ₅ -M ₇ +M ₈)/rlg
σ^2_l	(M ₃ -M ₅ -M ₇ +M ₈)/ryg
σ^2_{gl}	(M ₇ -M ₈)/ry
σ^2_{gy}	(M ₆ -M ₈)/rl
σ^2_{ly}	(M ₅ -M ₈)/rg
σ^2_{gyl}	(M ₈ -M ₉)/r
σ^2_e	M ₉
σ^2_p	$\sigma^2_g + \frac{\sigma^2_{gl}}{l} + \frac{\sigma^2_{gy}}{y} + \frac{\sigma^2_{gyl}}{ly} + \frac{\sigma^2_r}{lyr}$

Where, σ^2_g : genotypic variance, and σ^2_p : phenotypic variance, l: location, y: year: E: residual. σ^2_{gl} : variance of genotypic and location interaction, σ^2_{gy} : variance of genotypic and year interaction, σ^2_{gyl} : variance of genotypic, year, and location interaction, and σ^2_e : residue

i) Heritability (broad sense)

Heritability in broad sense (H^2 or h^2) was estimated according to Falconer *et al.* (1996) and Visscher *et al.* (2008) .

Broad-sense heritability is calculated as:
$$h^2 = \frac{\sigma^2_g}{\sigma^2_p}$$

Where, h^2 : Heritability; σ^2_g : genotypic variance, and σ^2_p : phenotypic variance

ii) Expected genetic advance from selection

Genetic advance (GA) was calculated: $GA = K * \sigma_p * h^2$

Where: **GA** = genetic advance. **K**: constant = 2.06 at 5% selection intensity, **σ_p** : square root of phenotypic variance, and **h^2** : Heritability.

Genetic advance as percent of mean: Genetic advance in percentage of mean was calculated using the formula given by Robinson *et al.* (1949):

$$GA \text{ as \% of mean (GAM)} = (GA/\bar{x}) * 100$$

Where, **GAM** = genetic advance as percent of mean, **GA** = expected genetic advance, and $\bar{x}$ = grand mean of a character.

iii) Phenotypic and genotypic correlations

Phenotypic and genotypic correlations were estimated using the standard procedure suggested by Hanson *et al.* (1956) from the corresponding variance and covariance components.

Phenotypic correlation coefficient: $r_{pxy} = \frac{\sigma_{pxy}}{\sqrt{(\sigma^2_{px} * \sigma^2_{py})}}$

Genotypic correlation coefficient: $r_{gxy} = \frac{\sigma_{gxy}}{\sqrt{(\sigma^2_{gx} * \sigma^2_{gy})}}$

Where, r_{pxy} = phenotypic correlation coefficient between characters X and Y and
 r_{gxy} = Genotypic correlation coefficients between characters X and Y.

iv) Multivariate analysis

A. Cluster and distance analysis

Cluster analysis was made using xlstat Software ver 17 (Sasirekha and Baby, 2013). For multivariate analysis, the combined over location means of each quantitative character was standardized. This helps to avoid differences in scales on the different quantitative characteristics used in the recorded data. Dendrograms were built using xlstat Ver 18 (Sasirekha and Baby, 2013). The largest gap between two successive combination similarities is the cutting point of the dendrogram. Arguably large gaps indicate “natural” clustering. The quality of the clustering significantly decreases with adding one more cluster, so cutting before this steep is desirable. The accessions historical background also impose to determine

the cutting point of a cluster. The xlstat software automatically determines the cluster number and the cutting point based on the above criteria.

Principal Component Analysis (PCA) was performed to determine the contribution to each character to the total variation. PCA was computed on the standardized data using the correlation matrix.

B. Euclidean distance

Hierarchical clustering algorithms are either top-down or bottom-up. Bottom-up algorithms treat each document as a singleton cluster at the outset and then successively merge (or agglomerate) pairs of clusters until all clusters have been merged into a single cluster that contains all documents.

The generalized distance between any two populations are given by the formula:

$$\text{Euclidean distance: } \|a-b\|_2 = \sqrt{\sum_i (a_i - b_i)^2}$$

v) Comparison of performance of selected genotypes

To make a comparison of the top 5% best performing genotypes within the whole genotypes utilized in the present study, the materials were sorted and means were independently computed for each character. The absolute value of Student's z test was calculated to compare genotypic values of the 5% best selected genotypes with the base population as:

$$Z = \frac{\bar{x} - \mu}{\sigma / \sqrt{n}}$$

Where $\bar{x}$ is mean of selected genotypes, μ is mean of the base populations, σ is the standard deviation calculated for the base populations and n is the number of genotypes selected from the base population for better performance.

The significance of the difference between the population parameter (μ) and sample mean ($\bar{x}$) was tested using z Table by comparing calculated value of z-test with that of tabulated z value. The present study was aimed to analyze the genetic diversity among oat species and accessions using morphological and SSR molecular markers. Geographical origins of accessions and species type were the basis for this study. Detail information on the structure of data and the methods used is presented below under three main sections. Morpho-agronomic diversity study, molecular marker diversity study,

and hydroponic experiment for screening Al toxicity tolerance. The materials and methods used are explained under the individual sub-sections.

4.2 Molecular genetic diversity and population structure

4.2.1 Plant materials

One hundred seventy-six accessions shown in section 4.1 (Table 3) were grown in a greenhouse at Addis Ababa University, College of Natural and Computational Sciences. Fifteen plants per accession were planted in a pot in March 2015. From each accession, healthy young leaf tissues from 10 randomly chosen plants were bulked and dried in silica gel.

4.2.2 DNA extraction

Five randomly selected leaves were used from 10 randomly chosen plants. Total genomic DNA was extracted using zymo plant/seed DNA extraction kit protocol (Appendix 2). DNA quantity, quality, and integrity were evaluated by 1% agarose gel for 1 hour with 80 V using 100 bp λ -DNA marker. In addition, the quality and quantity of all DNA samples were determined at A260/A280 ratio with a typical spectral pattern for nucleic acids. The DNA samples were diluted to a working concentration of 20 μ M for PCR reaction.

4.2.3 PCR primers and amplification

Preliminary a set of 20 SSR primer pairs were used. Out of the 20 SSR markers, one was dropped after preliminary evaluation, because of producing no specific amplification. The primer came from the nuclear genome of oat, wheat and barley microsatellites. The microsatellites are abbreviated as ‘AM’ for *Avena*, CWM for wheat and HVMwG for barley. From *Avena*, barley, and wheat, we took 17, 2, and 1 microsatellite, respectively. They were developed by Li *et al.* (2000) and Pal *et al.* (2002) for *Avena*, by (Gao *et al.*, 2003,2007) for wheat and by Liu *et al.* (1996) and Russell *et al.*(2000) for barley. Sequences, repeat motifs and color (dye) of the markers are presented in Table 36.

For PCR amplification 5' labeled forward primers of PET (red), 6 FAM (blue), VIC (green), and NED (yellow) and unlabeled reverse primers (Table 36), were used in this study. The PCR was performed in a total reaction volume of 10 μ L using the Accupower PCR premix tube. It contains lyophilized 1U top Taq DNA polymerase, 200 μ M dNTPs, 10 mM Tris-HCl (pH 9.0), 2.0-2.5 mM $MgCl_2$ and a tracking and stabilizing dye (Bioneer, Daejeon, North Korea). To the premix was added 0.2 μ L of 0.5 pM of primers, 2.0-2.5 μ L of 20 ng/ μ L DNA template, and 2.0-2.6 μ L of double-distilled water. The PCR program consisting of initial denaturation at 95 °C for 3 minutes followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 54-67 °C for 1 minute and extension at 72 °C for 1 minute and the final extension at 72 °C for 20 minutes was used.

4.2.4 Pooling of PCR products and SSR fragment analysis

PCR products were pooled by combining 0.8-1.5 μ L of each amplified product diluted with double-distilled water. Dilution was based on the relative intensities of amplification resolved on a 0.8% TBE agarose gel and the relative fluorescence unit on the ABI3730 sequencer. A standard cocktail mix for fragment analysis was prepared by mixing 9.0 μ L of 500 LIZ size standard and 1,000 μ L Hi-Di Formamide. Then 1.0 μ L of each pooled PCR product was mixed with 9.0 μ L of the cocktail mix for a 96-well PCR plate. The PCR plates were gently vortexed and spun down at 3,000 rpm for 2 min. The mix was denatured at 95°C for 3 min and quickly chilled on ice for 5 min and then loaded into the ABI 3730 sequencer for fragment analysis.

SSR fragment sizing was done using Genemapper software Ver.4.1 (Applied Biosystems), which performed peak detection and fragment size matching from the reference data. Allele calls were automatically made when a peak from a data sample matched the location of a bin. Completed results were run in AlleloBin software (Prasanth and Chandra, 1997) to correct any errors in the scored alleles. The errors resulted from the slippage of DNA polymerase during PCR that leads to stutter peaks

(Schlötterer and Tautz, 1992). The allele calls were then converted to binary data operating ALS-Binary software (Prasanth and Chandra, 1997) for the successive analyses.

4.2.5 SSR marker data analysis

Power marker Ver 3.25 (Liu and Muse, 2005), was used to calculate genetic diversity parameters, such as gene distance (GD), number of alleles (Na), number of effective alleles (Ne), number of private alleles (Npa), major allele frequency; and polymorphic information content (PIC). Polymorphic Information Content (PIC) was calculated based on the following formula:

$$PIC = 1 - (\sum_{i=1}^n P_i^2)$$

Where, P_i is the frequency of the i^{th} alleles and n is the number of alleles and this value is called heterozygosity and gene diversity (Anderson *et al.*, 1993; Salami *et al.*, 2016; Vivotík *et al.*, 2017).

The genetic diversity among the germplasm lines was computed using Computer Software Programme-DARwin. Dissimilarly matrix for SSR primers was constructed using Dice coefficient of associations to discover genetic relationships. The data were subjected to unweighted pair-groups' method with arithmetic mean (UPGMA) analysis to generate dendrogram. Data from 19 primers were used to estimate the dissimilarity based on the number of shared amplified bands:

$$d_{ij} = \frac{b+c}{2a+(b+c)}$$

Where, **dij**: dissimilarity between units i and j ; x_i , x_j : variable values for units i and j ; a : number of variables where $x_i = \text{presence}$ and $x_j = \text{presence}$; b : number of variables where $x_i = \text{presence}$ and $x_j = \text{absence}$; c : number of variables where $x_i = \text{absence}$ and $x_j = \text{presence}$; and 'a' represents matched fragments, b and c are unmatched fragments. The $2a + (b+c)$ are the total amplified bands in a particular set.

In population genetics, the most widely applied measurements, besides heterozygosity are F -statistics, or fixation indices, to measure allelic fixation by genetic drift. The F -statistics are related to heterozygosity and genetic drift. Since inbreeding increases the frequency of homozygotes, as a consequence, it decreases the frequency of heterozygotes and genetic diversity.

$$F_{IT} = 1 - \frac{H_I}{H_T}, \quad F_{IS} = 1 - \frac{H_I}{H_S}, \quad F_{ST} = 1 - \frac{H_S}{H_T}$$

Where, F_{IT} : Individual with the total Population, F_{IS} : Individual within the total population, F_{ST} : Subpopulation within the total population. H_I is the individual heterozygosity in the total population, H_S is the average heterozygosity of the subpopulation, and H_T is of the total heterozygosity of the total population.

GenAlEx 6.503 Peakall and Smouse (2016), was used to calculate genetic diversity parameters, like gene distance, number of alleles (N_a), number of private alleles (N_{pa}), Analysis of Molecular Variance (AMoVA), population differentiation (F_{ST}), gene flow (N_m), and Principal Coordinate Analysis (PCoA).

Genetic associations were also determined with Darwin V. 6.5 using the unweighted pair with genetic distance algorithm. Gene flow among populations were estimated with N_m , the number of migrants per generation between pairs of populations, using the following formula (Slatkin, 1995).

$$N_m = \frac{1}{4F_{ST}} - \frac{1}{4}$$

The N_m values were grouped into three categories: high ($N_m \geq 1$), intermediate (0.250- 0.99) and low (0.00-0.249) (Govindaraju, 1988; Slatkin, 1981; Slatkin and Takahata, 1985).

$$\text{Average } \mathbf{PhiPT} = \frac{VAP}{VWP+VAP} = \frac{VAP}{VT},$$

Where, $\mathbf{PhiPT}$ coefficient denotes the proportion of the variance among populations relative to the total variance, VAP is the variance among populations, VWP the variance within populations and VT is the total variance or the VAP + VWP.

Pairwise between populations :

$$\mathbf{PhiPT} = \frac{VAP+VAR}{VWP+VAP+VAR} = \frac{VAP+VAR}{VT},$$

Where $\mathbf{PhiPT}$, VAP and VWP are as mentioned above; VAR stands for variance between geographical regions and VT is the total variance or the VAP + VWP + VAR.

Expected and unbiased expected heterozygosity values were estimated based on Nei (1978) assuming each band to correspond with a single biallelic locus but the genetic interpretation of individuals as

heterozygous/ homozygous for a particular locus was not attempted throughout because each sample from an accession constituted of a bulk of leaves from different individual plants.

$$H_e = 1 - \sum p^2,$$

Where, H_e is the expected heterozygosity, assuming Hardy- Weinberg Equilibrium where an amplified allele (band present) has frequency $p = 1 - q$, a null allele (band absent) has frequency $q = 1 - p$, frequency of genotype with null allele is $q^2 = \text{frequency of absence} = 1 - \text{frequency of presence}$ and $q = \sqrt{(\text{frequency of absence})}$

$$U H_e = \frac{2N}{2N-1} * H_e,$$

Where, $U H_e$ is unbiased expected heterozygosity and N is the number of population.

$$\% P = \frac{NPL}{TNL} \times 100,$$

Where, % P is percent polymorphism, NPL is the number of polymorphic bands and TNL is total of bands.

4.2.6 Analysis of population structure

The Bayesian model-based clustering approach to reveal the genetic structure in the oat accessions were performed using Structure 2.3.4 (Pritchard *et al.*, 2000). The structure algorithm was run using the admixture model, with 9 independents replicate runs per K value (number of clusters) ranging from one to nine. Each run involved 50 000 and 100000 Markov Chain Monte Carlo (MCMC) iterations and a post burning simulation length of 100,000. Determination of the most likely number of clusters K was performed with the Structure Harvester. The $K = 2$ analyses were done with a particular interest of distinguishing between cultivated and wild oat accessions.

4.3 Aluminium toxicity tolerance of *Avena* species

4.3.1 Plant materials and germination conditions

Seeds of the 150 accessions of five *Avena* species (Table 3) were used (*A. sativa*, *A. abyssinica*, *A. fatua*, *A. sterilis*, and *A. vaviloviana*). Twenty randomly selected seeds of each accession were surface sterilized by wrapping with soft paper soaked in 70% ethanol for 2 minute. The surface sterilized seeds were moistened with distilled water and germinated in a tissue paper in Petri dishes for about 48 hours under dark condition.

4.3.2 Hydroponics experimental setup

4.3.2.1. Equipment setup

The hydroponic equipment was improvised using the lower half of half liter plastic water bottles:

- The plastic bottles were cut into two halves and the lower half was kept,
- The half bottles were perforated with tiny holes for aeration and circulation of nutritional solution during the hydroponic treatment,
- Each half part of the bottle was partitioned into two equal parts by placing a flat plastic partition in the middle (Figure 5 B),
- Six such bottles were attached forming a set (Figure 5 B),
- Two sets of bottles were attached together, as a half batch (Figure 5C), A pair of the half batch was used on a treatment unit (single setup) (Figure 5D),
- The treatment unit was immersed in a large bowl in which six liter of the media was placed,
- Seedlings to be treated were placed in each of the two compartments of the individual half bottle, and
- The set up was aerated by using aeration pump.

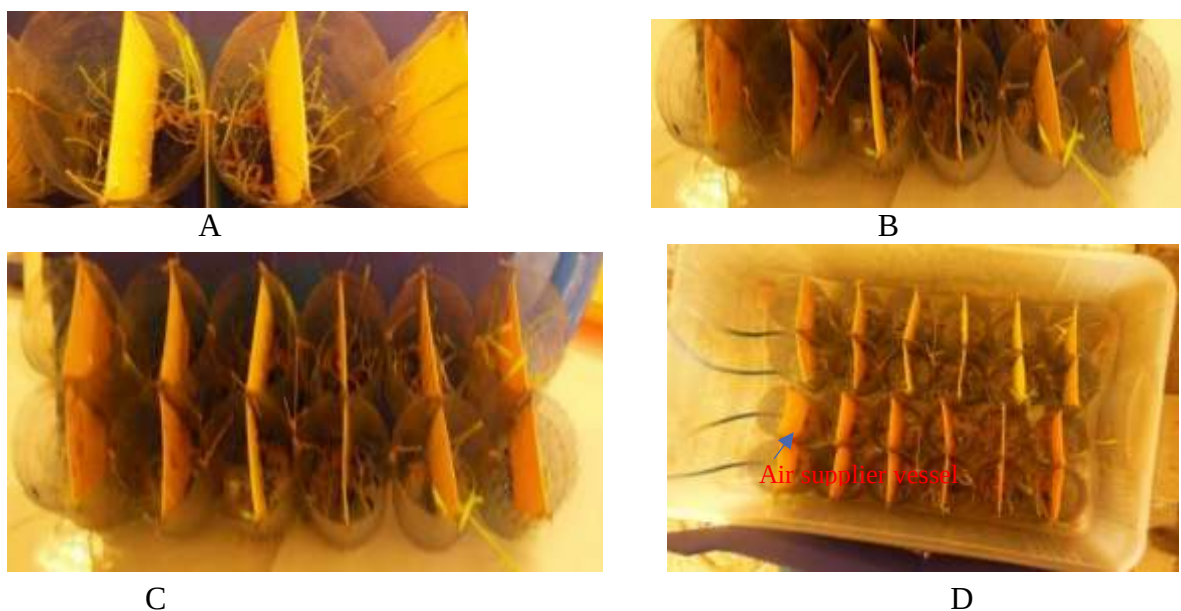


Figure 5. Schematic representation of hydroponic setup.

A. A half bottle divided into two parts, B. A set of six attached half bottles, C. Two set of six half bottle each (half treatment unit), and D. Four sets of six bottles each (a single treatment unit)

4.3.2.2. Culture nutrient solution

A. Stock solution preparation

The following solutions were prepared separately

- 1) 1 M CaCl_2 was prepared by dissolving 55.04g of CaCl_2 in 0.5L distilled H_2O ;
- 2) 1 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was prepared by dissolving 60.18g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 0.5L distilled H_2O ;
- 3) 1 M NH_4NO_3 was prepared by dissolving 40.04g of NH_4NO_3 in 0.5L distilled H_2O ;
- 4) 0.1 M FeCl_3 was prepared by dissolving 8.1g of FeCl_3 in 0.5L distilled H_2O ;
- 5) 1 M KNO_3 was prepared by dissolving 50.5516 g of KNO_3 in 0.5L distilled H_2O ;
- 6) 1 M KH_2PO_4 was prepared by dissolving 68g of KH_2PO_4 in 0.5L distilled H_2O , and
- 7) Different concentrations of $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ was prepared by different $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ in 0.5L distilled H_2O .

B. Preparing 6-liters of treatment solution from the stock solution

- I. From each CaCl_2 , NH_4NO_3 , and KNO_3 , stock solutions three thousand microliters were added to the six-liter treatment solution.
- II. From the remaining three stock solutions 900 μL $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 96 μL FeCl_3 , and 60 μM KH_2PO_4 and
- III. Variable microliter (0.28, 0.57, 1.75, 2.84, 5.69, 6.40, and 8.53 μL of $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$) were added to prepare six-liter treatment solution of variable concentration.

C. Nutrient concentration in the treatment solution

Nutrient solution for hydroponic treatment was prepared according to Delhaize *et al.* (2004) with simple modification. It is composed of 500 μM KNO_3 , 500 μM CaCl_2 , 500 μM NH_4NO_3 , 100 μM KH_2PO_4 , 150 μM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2 μM FeCl_3 and varying concentrations of $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$. This was prepared from the stock solution according to the above procedure. The pH of the nutrient was adjusted to 4.3 (the minimum suitable acid level of Al) by using 1M HCl and the solution was renewed every 24hrs.

The experiment was laid down in RCBD (Randomized Complete Block Design) with three replications. Nine in-vitro germinated seedlings of each accession, having approximately equal length and size, were placed in each compartment and the setup was subjected in a large bowl filled with hydroponic solution. For a setup containing 48 samples, 6 little hydroponic solutions were used. Air pump was used to aerate and to circulate the nutrient solution through the setup. After 72 h of the treatment, the seedling was removed from aluminium containing treatment solution, thoroughly washed with water for 2-3 minutes, and transferred for an Al-free medium for 48 hours. The control experiment also contained all the above nutrients except $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$. The pH of the nutrient was adjusted to 4.3 using 1M HCl. The nutrient solution was renewed every 24 hours.

4.3.2.3. Optimization of Al^{3+} concentration and number of days for seedling growth

To determine the root growth inhibition optimal concentration of Al^{3+} , evaluation was conducted at different concentrations of aluminium, using 0, 5, 10, 25, 50, 100, 112.5, 150, and 200 μM of $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$. This initial optimization experiment showed 112.5 μM of $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ as optimum Al^{3+} concentration and, therefore, this concentration was used for further preliminary screening and characterization tests.

And the optimum number of days at which seedlings can be differentiated into different classes of tolerance (tolerant, intermediate or susceptible) was determined. The three classes of tolerance were determined by assessing the performance of seedlings of the accessions after treatment for 5, 6, 8, and 10 days. This preliminary experiment showed that seedlings grown for 8 days were better differentiated into three tolerance classes.

4.3.3 Data recording and statistical analysis

Root length of nine seedlings per accession was measured using a ruler. Means and analysis of variance (ANOVA) under each optimization, screening and characterization of $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ for each accession grown under different batch was conducted using SPSS software Ver 20. Least significance difference tests were used to compare the mean of each accession under control and Al treated conditions. Tukey Honesty Significance test was used to make a pairwise mean comparison of each germplasm under control and Al-treated conditions. Relative Tolerance Index related to root length bring in the pairwise mean comparison of each germplasm under control and Al treated conditions was estimated for accession categorization following the method of Mendes and Paula (1984).

$$\text{RTI} = \frac{\text{Root length under treatment (Al)}}{\text{Root length under control (without Al)}}$$

$\text{RLI} = (1 - \text{RTI}) * 100 \%$; Where; RTI is root tolerance index and RLI= Root length inhibition

5. RESULTS

5.1 Agronomic traits

5.1.1 Qualitative trait variability

The morphological qualitative traits showed a wide range of phenotypic variability in all the studied accessions. Distributing accessions for twenty-two morphological characters is presented in Table 8. Traits such as awnedness, covered grain, and 2-4 fertile spikelet per floret were uniform across accessions, which mean that is all the accessions had awns at maturity, covered grain and 2-4 fertile spikelet per floret.

Among the qualitative characters scored, the descriptors were observed from invariant to mutivariate across the *Avena* species (Table 8). The Shannon diversity index of *A. abyssinica* ranged from low to high. Lodging at immature stage and seed color had high Shannon diversity index, whereas shattering habit, relation between maturity of grains and straw, grain size, glume size, awnedness and awn type, panicle compactness and shape, rigidity of flag leaf, hairiness of leaf sheath (lower leaves), and growth habit had low Shannon diversity index. The remaining traits showed moderate Shannon diversity index. The Shannon diversity index of *A. vaviloviana* ranged from low to high. Majority of the accessions showed low Shannon diversity index but lemma color and lodging at immature stage showed high Shannon diversity index. The remaining traits showed intermediate. Stem thickness, seed color, lodging at mature stage had high Shannon diversity index in *A. fatua* species whereas, lodging at immature stage, lemma color, seed size, glume size, erectness of spikelet, width of second leaf from top, length of second leaf from top, and angle of flag leaf to culm, were moderate ; the remaining traits showed low Shannon diversity index. Susceptibility to disease, lodging at mature stage, glume size, and lemma color showed high Shannon diversity index in *A. Sterilis*; whereas, growth habit, rigidity of flag leaf, shape of panicle, panicle compactness, awnedness and types, grain size, and hairiness of lemma showed low. The remaining traits shown moderate Shannon diversity index.

Table 8. Qualitative traits showing the predominant state observed, distribution (%) and the calculated Shannon diversity indices (H') for each descriptor scored for 176 oat accessions, cultivated versus wild

character	Character state observed	Wild								cultivated		Total	
		<i>A. abyssinica</i>				<i>A. vaviloviana</i>				<i>A. sativa</i>			
		%	H'	%	H'	%	H'	%	H'	%	H'	%	H'
Growth habit	Erect	97.7%	0.11	94.4%	0.22		0.11		0.00	80.5%	0.49	0.716	0.79
	Semi prostrate	2.3%	L	5.6%	L				L	19.5%	M	0.125	H
	prostrate					100.0%		100.0%		0.0%		0.159	
Stem thickness	Thin	70.5%	0.69	100.0%	0.00	58.3%	0.96	85.0%	0.42	18.3%	0.95	0.477	0.97
	Intermediate	27.3%				25.0%		15.0%		59.8%	M	0.409	H
	Thick	2.3%	M		L	16.7%	H		M	22.0%		0.114	
Angle of flag leaf to culm	Acute	52.3%	0.69	22.2%	0.53	83.3%	0.45	70.0%	0.61	86.6%	0.39	0.659	0.64
	Intermediate	47.7%		77.8%		16.7%		30.0%		13.4%	L	0.341	M
	obtuse		M		M		M		M	0.0%		0	
Rigidity of flag leaf	Bent	6.8%	0.33		0.00	16.7%	0.45		0.00	0.0%	0.63	0.023	0.54
	Slightly bent	93.2%		100.0%	L	83.3%		100.0%	L	67.1%	M	0.818	M
	Stiff	2.3%	L				M			32.9%		0.159	
Hairiness of leaf sheath (lower leaves)	Glabrous	88.6%	0.35	100.0%	0.00	91.7%	0.29	95.0%	0.20	92.7%	0.26	0.494	0.75
	Slightly pubescent	11.4%			L	8.3%	L	5.0%	L	7.3%	L	0.494	H
	Moderately pubescent		L							0.0%		0.011	
Length of second leaf from top	Short	52.3%	0.69	88.9%	0.35	75.0%	0.56	70.0%	0.61	9.8%	0.63	0.381	0.85
	Medium	47.7%	M	11.1%		25.0%	M	30.0%		80.5%	M	0.517	H
	Long				L				M	9.8%		0.045	
Width of second leaf from top	Narrow	79.6%	0.51	100.0%	0.00	83.3%	0.45	65.0%	0.65	19.5%	0.68	0.523	0.78
	Medium	20.5%	M		L	16.7%	M	35.0%	M	75.6%	M	0.455	H
	Broad									4.9%		0.023	
Shape of panicle	Unilateral		0.00		0.00	100.0%	0.00	100.0%	0.00	0.0%	0	0.176	0.47
	Equilateral	100.0%	L	100.0%	L		L		L	100.0%	L	0.818	M
Erectness of spikelets	Drooping		0.40	22.2%	0.22	50.0%	0.45	40.0%	0.58	0.0%	0.26	0.063	0.88
	Semi-erect	86.4%	M	94.4%	L	83.3%	M	50.0%	M	7.3%	L	0.466	H
	Erect	13.6%		5.6%		16.7%		10.0%		92.7%		0.472	

Table 8. Continued...

Panicle Compactness	Loose	100.0%	0.00	100.0%	0.00	100.0%	0.00	100.0%	0.00	0.0%	0.23	0.534	0.8
	Semi compact		L		L		L		L	93.9%	L	0.438	H
	Compact									6.1%		0.028	
Awnedness	Weak Awn	100.0%	0.00	100.0%	0.00		0.00		0.00	100.0%	0	0.818	0.47
	Strong Awn		L		L	100.0%	L	100.0%	L	0.0%	L	0.182	M
Awn types	Straight	100.0%	0.00	100.0%	0.00		0.00		0.00	100.0%	0	0.818	0.47
	Geniculate		L		L	100.0%	L	100.0%	L	0.0%	L	0.182	M
Glume size	Small	86.4%	0.40	94.4%	0.22	75.0%	0.56	70.0%	0.75	39.0%	1.07	0.563	0.98
	Medium	13.6%	M	5.6%	L	25.0%	M	25.0%	H	37.8%	H	0.256	H
	Large							5.0%		23.2%		0.176	
Grain size	Small	93.2%	0.29	100.0%	0.00	75.0%	0.56	95.0%	0.20	13.4%	0.94	0.511	0.98
	Medium	4.6%	L		L	25.0%	M	5.0%	L	58.5%	H	0.347	H
	Large	2.3%								28.0%		0.136	
Hairiness of lemma	Glabrous	18.2%	0.71	11.1%	0.35		0.00	10.0%	0.33	95.1%	0.23	0.54	1.13
	Slightly pubescent	75.0%	H	88.9%	L		L	90.0%	L	2.4%	H	0.267	H
	Moderately pubescent	6.8%								2.4%		0.125	
	Highly pubescent					100.0%				0.0%		0.068	
Lemma color	White	47.7%	0.43	33.3%	0.73		0.55		0.73	17.1%	0.89	0.233	1.53
	Yellow					66.7%				68.3%	H	0.352	H
	Grey	2.3%	M	33.3%	H	8.3%	M	15.0%	H	13.4%		0.131	
	Red	50.0%		33.3%				10.0%		0.0%		0.142	
	Brown- black					25.0%		75.0%		1.2%		0.142	
Seed color	White	47.7%	0.85		0.69	25.0%	1.04	0.0%		74.4%	0.65	0.483	1.32
	Yellow	47.7%				50.0%	H	0.0%		2320.0%	M	0.261	H
	Grey	4.6%	H	50.0%	M	25.0%		20.0%		2.4%		0.114	
	Red			50.0%				40.0%		0.0%		9.7	
	Brown- black							40.0%		0.0%		4.5	
Lodging at immature stage	Upright (all plants)	68.2%	0.93	27.8%	1.24	58.3%	0.68	95.0%	0.20	69.5%	0.91	0.659	0.96
	Minor lodging	18.2%	H	27.8%	H	41.7%	M	5.0%	L	14.6%	H	0.182	H
	Intermediate	9.1%		38.9%						12.2%		0.125	
	Lodged	4.6%		5.6%						3.7%		0.034	

Table 8. Continued...

Lodging at mature stage	Upright (all plants)	77.3%	0.69	83.3%	0.56	66.7%	0.82	45.0%	1.26	41.5%	1.1	0.574	1.02
	Minor lodging	11.4%		11.1%		25.0%		25.0%		41.5%	H	0.273	H
	Intermediate	11.4%	M	5.6%	M	8.3%	H	20.0%	H	14.6%		0.131	
	Lodged							10.0%		2.4%		0.023	
Relation between maturity of grains and straw	Grain ripe before straw	100.0%	0.00	100.0%	0.00	100.0%	0.00	100.0%	0.00	15.9%	0.5	0.557	0.86
	Simultaneous		L		L		L		L	82.9%	M	0.386	H
	Straw ripe before grain									1.2%		0.057	
Shattering Habit	Low		0.00		0.00		0.00		0.00	87.8%	0.37	0.409	1.04
	Intermediate		L		L		L		L	12.2%	L	0.182	H
	High	100.0%		100.0%		100.0%		100.0%		0.0%		0.409	
Susceptibility to disease	Low	79.6%	0.58	94.4%	0.22	100.0%	0.00	40.0%	1.09	93.9%	0.29	0.881	0.46
	Medium	18.2%	M	5.6%	L		L	30.0%	H	1.2%	L	0.023	M
	High	2.3%						30.0%		3.7%		0.085	

Where, L low; M: moderate, and H: high

In cultivated oat glume size, lodging at mature and immaturity stage, lemma color and hairiness, and size of the glume and grain showed high Shannon diversity index, whereas, stem thickness, rigidity of flag leaf, length of second leaf from top, width of second leaf from top and lodging at lodging at mature stage showed moderate. The remaining traits showed low Shannon diversity index.

Overall, the Shannon diversity index in qualitative traits was high with an average index of 0.79. In wild oat, low diversity were obtained only in the following three characters rigidity of flag leaf, panicle compactness and relation between grain and straw maturation. Similarly, *A. sativa* had low Shannon diversity index in the following traits, angle of flag leaf length to the culm, erectness of the panicle, panicle compactness, awnness, awn type, and hairiness of lemma. The remaining traits ranged from moderate to high Shannon diversity index in both cultivated and wild oat.

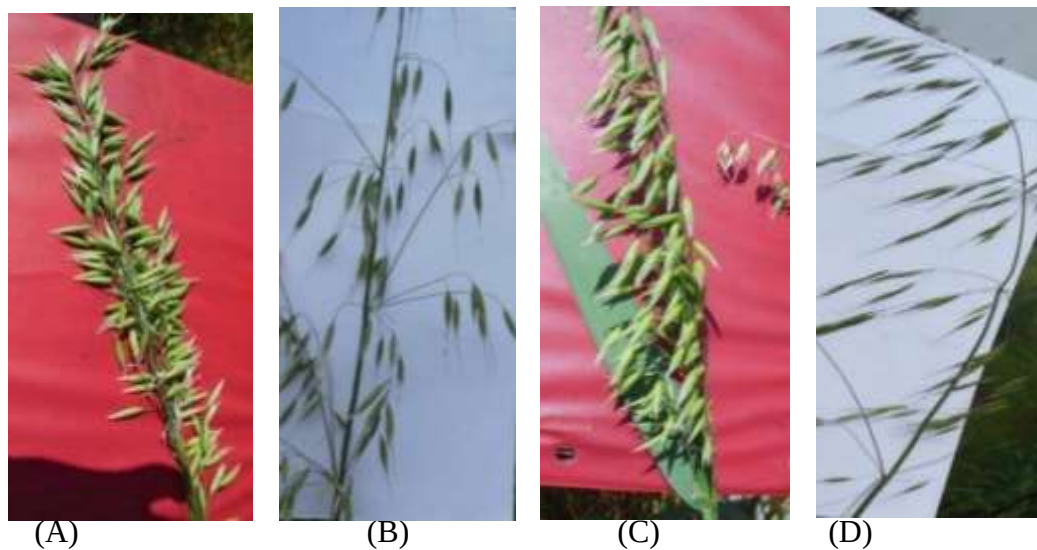


Figure 6. Diversity in panicle type, shape and compactness: A. Equilateral, erect and compact panicle of *A. sativa* B. Equilateral, erect and loose panicle of *A. abyssinica*, C. Unilateral, erect and compact panicle of *A. sativa* , and D. Unilateral droopy and loose panicle of *A. sterilis*.

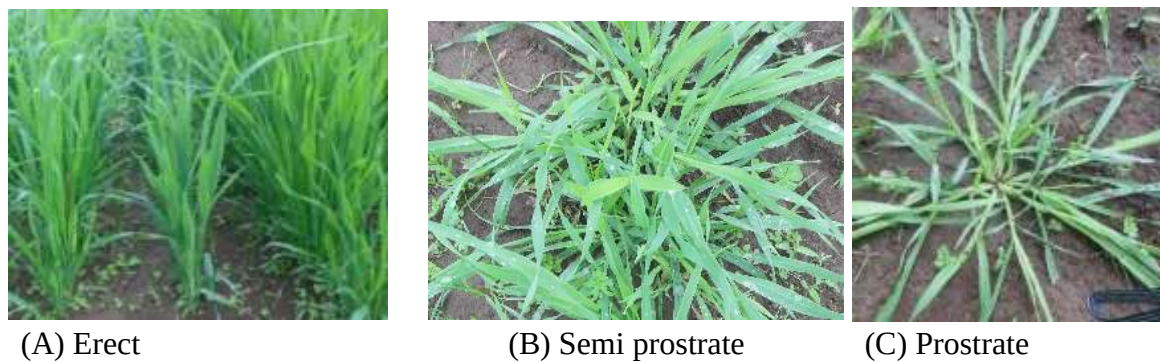


Figure 7. Diversity in growth habit: A. Erect growth habit of *A. sativa* and B. Semi prostrate growth habit of *A. fatua*, and C. Prostrate growth habit of *A. sterilis*.



Figure 8. Diversity in disease susceptibility: A. Susceptible leaves and B. Susceptible panicles.

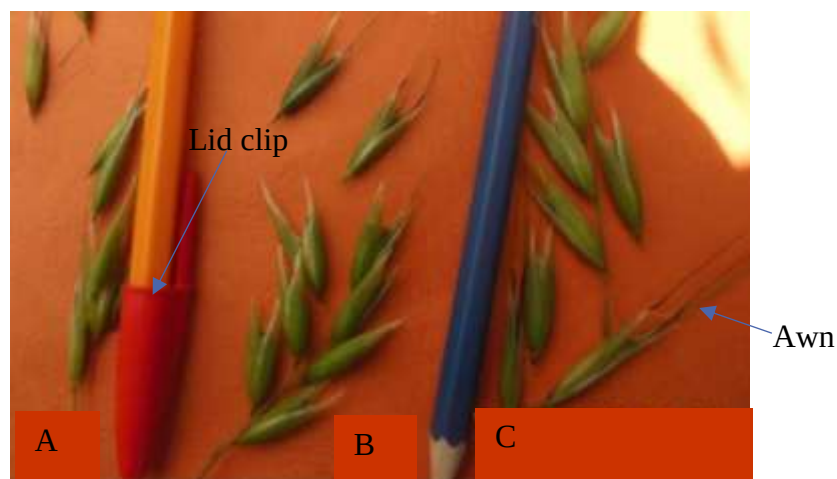


Figure 9. Diversity in glume size and awn length: A. Small glume size and small grain length, B. Medium glum size and small awn length, and C. Large glume size and small and large awn length.

The scale: 1: 1.25



Figure 10. Diversity of morphological feature of oats in the field at different stage: A. Juvenile stage, B. Heading stage, and C. Maturity stage.



Figure 11. Diversity in seed color: A. White (*A. sativa*), B. Yellow (*A. sativa*), C. Red (*A. fatua*), and D. Brown black (*A. fatua*.)

Scale: (A) 1: 3.67; (B) 1: 2.75; (C) 1: 4.4; and (C) 1: 2.2.



Figure 12. Diversity in grain size A. Large size (*A. sativa*), B. Medium size (*A. sativa*), and C. Small size (*A. abyssinica*).

Scale: (A) 1: 2.2; (B) 1: 2.2; and (C) 1: 2.0

5.1.2 Quantitative traits

5.1.2.1 Analysis of variance

Variance components attributed to genotypes and their interactions presented using the VARCOMP procedure of SAS and the result of the mean sum of squares of quantitative characters indicated the presence of highly significant differences among genotypes. The result is presented in Table 9-13.

Table 9. Mean squares values of 12 quantitative traits of *A. abyssinica* within, location, years, replication, accessions, accession interactions with year, accessions interaction with location, accession interaction with location and year, and error with their significance level

Source	Rep (Year*L)	Year	L	Year*L	Acc	Acc* Year	Acc*L	Acc*Year* L	Error
DF	4	1	1	1	43	43	43	43	172
NET	28.9*	18	9.6	0.3	9.8	12.6*	8.8	8.4	8.6
NN	1.9	1.2	15*	1.7	0.7	0.4	0.5	0.4	0.7
IL	42.1	7.6	88.2	179	17.7	15.3*	18	10.8	18.1
FLL	85.3	343*	255	43.4	277**	184**	96.8	77.3	82.9
LP	43.4	33.9	21.7	62.9	131	129*	110	78.6	85.2
NSP	1983	1629	175.1	774	634.8	519	452.1	293.6	346.2
PH	1007.8	376.6	2275.6	1323.2	527.8	700.5	417.4	631	370
DHE	304	181.6	122.3	6.4	738.6	400.1	209.3	380.4	302.6
DM	746	28.3	298.9	96.6	635.9	399.8	270.8	422.5	308.6
By	1059930	16732204	6414609	39391	532957	643145.8	483227	645812.7	571451
GY	34046.4	182895.4	91.5	1130.3	16294.5	12577.5	10457.2	10814.5	12761.9
HI	0.003	0.002	0.017	0.007	0.002	0.004	0.004	0.003	0.003

Where, * and **, are 5% and 1% level of significance, respectively.

Where, DF: degree of freedom, L: location, Rep: replication, Acc: accession, NET: number of effective tiller per plant, NN: number of node per plant, IL: internode Length, FLL: flag leaf length, LP: Length of panicle, NSP: number of spikelet per panicle, PH: plant height, DHE: days to 50% heading, DM: days to 90% maturity, BY: biological yield, and GY: grain yield

Table 10. Mean squares values of 12 quantitative traits of *A. vaviloviana* within, location, years, replication, accessions, accession interactions with year, accessions interaction with location, accession interaction with location and year, and error with their significance level

Mean square of variables	DF	NET	NN	IL	FLL	LP	NSP	PH	DHE	DM	BY	GY	HI
Rep (Year*L)	3	3.4	0.4	12.0	243.6	61.4	1151.3	93.3	451.6	755.9	2047944.7*	20747.4	0.0042
Year	1	18.2	1.5	0.8	793.4*	4.4	980.6	354.9	584.8	1501.9	3388404.1*	150411.8***	0.0078
L	1	0.1	3.0*	27.8	609.6*	0.3	2299.0	312.8*	2068.6*	3252.6*	6901621.8*	120752.6*	0.0009*
Year*L	1	7.8	4.4	101.0	19.5	599.9	114.1	1194.1	1689.4*	1573.8*	130119.7	83.4	0.0026
Acc	19	8.6	0.3	25.1	202.9***	166.9***	618.3**	653.6**	312.7**	372.8	801194.5***	19926.0	0.0016
Year*Acc	19	12.1*	0.6	17.3	315.4*	264.5*	807.8*	811.2*	778.2	737.9	339139.4	16693.3	0.0038
L*Acc	1	4.5	0.1	17.8	460.7	6.2	1217.2	3.5	1354.3	1394.8	162091.1**	22228.9	0.0060
Year*L*Acc	19	8.0	0.4	29.4	72.3	117.8	399.7	221.0	354.8	342.4	681932.4**	22070.9	0.0021
Error	76	2.7	0.3	22.1	79.4	76.9	476.1	284.0	428.1	422.7	438186.0	13135.0	0.0022

Where, *, **, and ***, are 5%, 1%, and 0.1% level of significance, respectively.

Where, DF: degree of freedom, L: location, Rep: replication, Acc: accession, NET: number of effective tiller per plant, NN: number of node per plant, IL: internode Length, FLL: flag leaf length, LP: Length of panicle, NSP: number of spikelet per panicle, PH: plant height, DHE: days to 50% heading, DM: days to 90% maturity, BY: biological yield, and GY: grain yield

Table 11. Mean squares values of 12 quantitative traits of *A. sterilis* within, location, years, replication, accessions, accession interactions with year, accessions interaction with location, accession interaction with location and year, and error with their significance level

Mean square variables	DF	NET	NN	IL	FLL	LP	NSP	PH	DHE	DH	BY	GY	HI
Rep(Year*L)	3	3.6	1.4	14.1	213.2	8.8	470.6	1738.0*	765.3	1588.3	2289102.9*	54750.6	0.004
Year	1	0.4	4.5*	55.2	29.3	5.2	894.9	2470.5	605.0	194.1	4044092.1*	128166.2	0.000
L	1	0.0	1.4	6.4	153.5	27.8	41.0	927.5	410.9	174.7	106371.9	756.6	0.000
Rep	1	13.7	1.1	10.7	4.3	25.3	898.5	103.8	401.8	1059.3	3623549.3	49345.3	0.001
Year*L	1	1.6	0.0	38.8	107.5	5.9	152.8	3.8*	414.2	666.2	93376.9	379.6	0.002
Acc	11	4.2	0.9	19.7	356.0**	85.7	324.8	329.5*	642.9*	703.5	934616.3	27457.9	0.006
Year*Acc	11	5.5	0.4	19.0	117.7	42.4	200.6	412.5	288.9	281.5	801476.1	18081.5	0.002
L*Acc	11	5.1	0.6	14.2	185.7	40.8	351.1	440.5	220.6	253.8	531526.0	11242.7	0.004
Year*L*Acc	11	13.5	0.9	13.2	141.5	24.6	284.4	388.6	493.3	393.7	1367210.6	13227.2	0.003
Error	44	7.4	0.7	16.1	94.9	45.9	327.7	534.2	342.6	337.9	749192.8	17759.3	0.003

Where, * and ** are 5% and 1% level of significance, respectively.

Where, DF: degree of freedom, L: location, Rep: replication, Acc: accession, NET: number of effective tiller per plant, NN: number of node per plant, IL: internode Length, FLL: flag leaf length, LP: Length of panicle, NSP: number of spikelet per panicle, PH: plant height, DHE: days to 50% heading, DM: days to 90% maturity, BY: biological yield, and GY: grain yield

Table 12. Mean squares values of 12 quantitative traits of *A. fatua* within, location, years, replication, accessions and accession interactions with year, accessions interaction with location, accession interaction with location and year, and error with their significance level

Mean square of the variables	DF	NET	NN	IL	FLL	LP	NSP	PH	DHE	DM	BY	GY	HI
Rep (Year*L)	3	18.0	1.1	67.8	39.1	61.9	1241.7	5883.5*	337.1	827.2*	935997.6*	7771.4	0.000
Year	1	1.7	0.5*	277.7	198.8	41.2	13.7	3447.9*	452.2	113.1	5151102.1*	78296.6*	0.000
L	1	17.3	10.2	6.0	284.6	143.3	5153.1	14278.2	1.0	304.0	918360.1	79714.6	0.012
Rep	1	5.6	0.3	73.3	222.9	32.8	296.7	8516.3	263.1	32.6	3028176.2	9682.6	0.004
Year*L	1	21.2	0.5	263.7	21.5	183.3	850.1	2946.8	0.2	112.5	2766145.1	13682.4	0.003
Acc	17	7.5	0.7	16.1	308.5	223.8	630.1	903.6	811.9**	803.6	328268.0	15557.5	0.004
Year*Acc	17	4.4	0.9	33.6	212.5	123.8	459.9	565.7	551.9	475.8	472817.6	43002.5	0.004
Loc*Acc	17	4.9	0.8	22.0	89.0	106.2	416.3	1119.3	309.6	345.3	675647.4	34667.0	0.005
Year*L*Acc	17	5.4	0.7	11.1	197.0	130.1	419.3	1006.8	377.8	418.5	400487.4	13742.4	0.003
Error	68	7.0	0.9	19.0	92.7	70.3	384.2	663.5	315.4	309.6	443811.6	20606.8	0.003

Where, *and **, are 5% and 1% level of significance, respectively.

Where, DF: degree of freedom, L: location, Rep: replication, Acc: accession, NET: number of effective tiller per plant, NN: number of node per plant, IL: internode Length, FLL: flag leaf length, LP: Length of panicle, NSP: number of spikelet per panicle, PH: plant height, DHE: days to 50% heading, DM: days to 90% maturity, BY: biological yield, and GY: grain yield

Table 13. Mean squares values of 12 quantitative traits of *A. sativa* within, location, years, replication, accessions, accession interactions with year, accessions interaction with location, accession interaction with location and year, and error with their significance level

Traits	Rep(Year*L)	Year	L	Rep	Year*L	Acc	Year*Acc	L*Acc	Year*L*Acc	Error
DF	3	1	1	1	1	81	81	81	81	324
NET	31.5*	3.2	17.7	1.6	5.2	6.4	8	7.2	6.3	6.9
NN	6.8*	14.9	22.4***	2.7	0.1	0.7***	0.7	0.7	0.6	0.6
IL	112.0*	10.9	240.8***	58.2	8.2	18.8	20.5	22.5*	15.8	16.5
FLL	125.9	777.5*	161	195.2	75.7	348.2***	151.7	109.2	107.7	116.7
LP	288.6*	14.1	96.5	455.9	1.8	199.6***	109.3	99.2	116.6*	86.4
NSP	2419.8	2073.2*	621.7	51.5	74.8	537.4	431	389	465.4*	422.4
PH	1914.9**	0.5	2	1187.3	6224.0***	815.6***	548.7	411.8	487.7	446.3
DHE	903.2	42.6*	917.4*	1554.5	550.3	551.4	530.8*	323.2	342.6	371.3
DM	1374.6*	1579.3*	3230.3*	1599.5	1404.1*	596.0**	549.5	283.5	302.4	364.9
BY	2119454.7*	42249000.6***	763169.2	574649.5	2798867.7	986940.5*	945989.2	652828.7	658060.5	729354.5
GY	40818.3	624093.6***	30555.8	37324.5	10793.3	20135.1	19980.7	17106.1	17916.7	18219.9
HI	0.040*	0.013*	0.009	0.002	0.006	0.003	0.003	0.004	0.004	0.004

Where, *, **, and ***, are 5%, 1%, and 0.1% level of significance, respectively.

Where, DF: degree of freedom, L: location, Rep: replication, Acc: accession, NET: number of effective tiller per plant, NN: number of node per plant, IL: internode Length, FLL: flag leaf length, LP: Length of panicle, NSP: number of spikelet per panicle, PH: plant height, DHE: days to 50% heading, DM: days to 90% maturity, BY: biological yield, and GY: grain yield

5. 1.2.2 Mean performance of quantitative traits

The average values of all 12 quantitative characters for 176 accessions *Avena* species were different. The traits are presented in (Table 14) hereunder.

Grain yield: Among the agronomic traits, the means of grain yield were variable but species ranged from 91.2 g/m² for *A. sterilis* to 284.1g/m² for *A. sativa* with overall mean of 200.7 g/m². The grain yield of *A. sativa* is higher than all the wild species. The mean grain yield of individual species of *A. abyssinica*, *A. fatua*, and *A. vaviloviana* was less than the overall mean of all the studied species (Table 14).

Yield related traits: The range for the mean of the biological yield varied from 756.2 g/m² for *A. sterilis* to 2314.1 g/m² for *A. sativa* with grand mean of 1674.2 g/m². The mean biological yield of *A. fatua* (1147.9g/m²), *A. abyssinica* (1133.7g/m²), and *A. vaviloviana* (1264.3 g/m²) were lower than the grand mean. The LSD value of this trait is 337.8. All the five species had significance difference from the grand mean but among the three species such as *A. fatua*, *A. abyssinica*, and *A. vaviloviana* had non-significant difference. A narrow range difference was observed Harvest index (%) with the minimum value being 11.2% and the highest 12.3% in *A. abyssinica* and *A. sativa*, respectively. The grand mean of harvest index was 11.9% with LSD value 1.0. The overall average length of panicle was 30.7 cm with the range from 32.0 cm in *A. vaviloviana* to 27.8 cm in *A. fatua* with LSD value of 2.5. *A. fatua* had significantly lower mean value than the overall mean. The maximum (147.5 cm) mean plant height was recorded in *A. sativa* and the minimum (119.2 cm) in *A. fatua* accessions. The overall mean was recorded as 141.8 cm. *A. sativa* had values greater than the overall mean, while the *A. fatua* accessions had the mean value significantly less than the overall mean but the remaining three species (*A. abyssinica*, *A. sterilis*, and *A. vaviloviana*) had non-significant difference from the grand mean. The number of spikelet per panicle ranged from 37.1 in *A. sterilis* to 61.9 in *A. abyssinica* accessions, and had the overall mean of 52.8. In *A. sterilis* the mean value of number of spikelets per panicle was least

which was followed by and the mean value of *A. vaviloviana* whereas, the mean value *A. abyssinica* was high. *A. sativa* and *A. fatua* had almost equal number spikelet per panicle and the difference is non-significant. The mean value of the number of effective tillers/plant ranged from 5.3 in *A. sterilis* to 7.5 in *A. fatua* with the grand mean of 5.9. Accessions of *A. abyssinica* and *A. vaviloviana* had almost the same the mean value of number of effective tiller and the difference is non-significant, whereas the mean of tiller number of *A. sativa* and *A. sterilis* is significantly lower than the grand mean. These two species had no significance difference. Leaves raised from the nodes, thus number of leaves are directly proportional to number of nodes. Leaves made an important role for making and provide food materials during photosynthesis. The number of leaves per plant ranged from 5-6.3.

Phenological traits: These includes day to 50% flowering and days to 90% maturity. The maximum (114.8 days) and the minimum (104.5 days) for 50% flowering were observed in *A. sativa*, and *A. fatua*, respectively, with an overall means of 112.6 days and LSD value of 3.5. The number of days to 50% flowering in *A. abyssinica* and *A. fatua* was significantly less than the overall mean. The maximum (145.9 days) and the minimum (128.5 days) as number of days to 90% maturity were recorded in *A. sativa* and *A. fatua*, respectively. The overall mean for this character was 141 days with LSD value of 2.3. The mean number of days to 90% maturity in *A. abyssinica* and *A. fatua*, was significantly less compared to the overall mean value whereas *A. vaviloviana* had values significantly higher than the overall mean value.

Table 14. Mean of 12 quantitative traits in 176 accessions of wild and cultivated *Avena* species

Character	Wild species				Cult'd spp	Overall	LSD
	<i>A. abyssinica</i>	<i>A. fatua</i>	<i>A. sterilis</i>	<i>A. vaviloviana</i>	<i>A. sativa</i>		
NET	6	7.5	5.3	6.2	5.4	5.9	0.6
NN	4.5	4.6	4.6	4.4	5	4.7	0.3
IL	21.7	18.3	19.1	20	19.8	20.1	1.4
FLL	27.5	24.1	27.7	27.9	30.1	28.4	2.2
LP	30.9	27.8	30.3	32	30.9	30.7	2.5
NSP	61.9	53.1	37.1	43.1	52.4	52.8	5.9
PH	141.6	119.9	138.1	141.2	147.5	141.8	5.9
DHE	110	104.5	113.5	115.3	114.8	112.5	3.5
DM	135.5	128.5	141.9	143.6	145.9	141	2.3
BY	1133.7	1147.9	756.2	1264.3	2314.1	1674.2	377.5
GY	127	130.9	91.5	150.5	284.6	200.8	23.8
HI (%)	11.20%	11.40%	12.10%	11.90%	12.30%	11.90%	1.00

Where, spp: species, var: variable, NET: number of effective tiller per plant, NN: number of node per plant, IL: internode length, FLL: flag leaf length, LP: Length of panicle, NSP: number of spikelet per panicle, PH: plant height, DHE: days to 50% heading, DM: days to 90% maturity, BY: biological yield, GY: grain yield, and HI: harvest index

5.1.2.3. Mean value quantitative trait of *A. sativa* accessions by regions/ populations

The mean performance of accessions from Gojam region, on trait number of effective tiller per plant was the highest and significant, whereas flag leaf length, number of spikelets per panicle, and plant height mean values significantly lower than the other regions. Accessions from Gondar region none of the traits had significantly lower mean value than the grand mean value. From Shewa region, only trait of flag leaf length had means significantly higher than the grand mean. From Welega region, traits such as biological yield and grain yield had significantly better mean performance than the grand mean value. From Wello region, traits such as, number of spikelets per panicle, and plant height had a significant high mean performance than the grand mean value with LSD 6.8 and 8.1 respectively. However, traits such as the number of effective tillers, and biological yield mean performance were significantly higher than the grand mean value.

For the seed collected in USA, majority of the traits such as number of nodes, number of spikelets per panicle, plant height, biological yield, and grain yield had mean performance significantly lower than the grand mean. The other traits had non-significant difference from the grand mean.

Table 15. Mean performance of *A. sativa* accessions with 12 quantitative traits by geographical origin

Region	Characters											
	NET	NN	IL	FLL	LP	NSP	PH	DHE	DM	BY	GY	HI
Gojam	6.6	5.3	18.0	25.7	29.0	42.0	139.8	113.5	144.7	2353.3	296.1	12.6%
Gondar	5.1	5.5	18.7	31.2	32.9	63.2	161.3	116.1	145.8	2555.0	313.7	12.3%
Shewa	5.2	5.1	20.7	32.3	33.8	57.7	157.0	116.5	147.2	2468.9	301.6	12.2%
Welega	5.3	5.0	20.6	31.3	30.0	50.9	144.2	114.6	145.4	2891.0	338.3	11.8%
Wello	4.6	5.3	18.2	30.1	32.5	61.5	159.9	115.7	147.1	2055.0	254.0	12.4%
USA	5.5	4.5	20.2	29.0	28.3	46.0	136.0	113.8	146.4	1981.6	245.4	12.4%
Overall	5.4	5.1	19.5	29.9	31.1	53.6	149.7	115.0	146.1	2384.1	291.5	12.30%
LSD	0.4	0.4	1.7	2.1	2.9	6.8	8.1	3.00	2.55	303.4	42.1	.01

Where, NET: number of effective tiller per plant, NN: number of node per plant, IL: internode length, FLL: flag leaf length, LP: Length of panicle, NSP: number of spikelet per panicle, PH: plant height, DHE: days to 50% heading, DM: days to 90% maturity, BY: biological yield, GY: grain yield, and HI: harvest index.

The number of effective tiller per plant was highest in Gojam and lowest in Wello accessions (Table 15). The number of nodes had low difference from the grand mean except USA and Gondar accessions which was lowest and the highest respectively. Accessions from Shewa had high flag leaf length, but accessions from Gojam had the least mean value. Better biological and grain yield were observed in the accessions that were collected from Welega and least value were recorded in Wello and USA accessions. Similarly, grain yield traits had better mean value in the accessions Welega, but the least found in USA accessions. Traits like internode length, panicle length, days to 50% flowering, days to 90% maturity and harvest index had no significant variation across regions.

5.1.2.4 Mean value of 12 traits of population of *A. abyssinica* accessions

The majority of traits mean performance of *A. abyssinica* accessions across regions were non-significant and all regions except from Bale had non-significant difference from the grand mean (Table 16). The Arsi, Bale, Gondar, Shewa, and Welega population contained nine, four, four, eighteen, and five accessions, respectively. All regions had lowest and highest mean value compared to the grand mean but the difference are non-significant from the grand mean except the Bale region accessions had the lowest value in number of spikelets per panicle compared to the grand mean (Table 16).

Table 16. Mean performance of *A. abyssinica* accessions with 12 quantitative traits by geographical origin

Region	Characters											
	NET	NN	IL	FLL	LP	NSP	PH	DHE	DM	BY	GY	HI
Arsi	6.4	4.6	23.3	28.6	31.8	58.3	147.3	111.8	138.3	1039.0	113.8	10.9%
Bale	6.1	4.2	23.6	26.8	29.5	56.5	135.1	109.7	135.5	991.0	112.3	11.3%
Gondar	4.7	4.5	21.1	27.0	29.2	68.7	134.0	111.2	136.7	1196.0	131.2	10.9%
Shewa	5.7	4.5	21.0	27.2	31.0	64.5	142.1	109.6	134.9	1172.3	130.6	11.1%
Welega	7.2	4.4	22.5	29.1	34.4	60.7	147.6	109.4	133.8	1142.5	133.7	11.8%
Overall	6.0	4.5	22.0	28.0	31.6	63.1	142.8	110.5	135.9	1137.5	127.3	11.2%
LSD	1.58	0.38	NS	NS	3.35	6.16	12.19	NS	3.42	NS	NS	NS

Where, NET: number of effective tiller per plant, NN: number of node per plant, IL: internode length, FLL: flag leaf length, LP: Length of panicle, NSP: number of spikelet per panicle, PH: plant height, DHE: days to 50% heading, DM: days to 90% maturity, BY: biological yield, GY: grain yield, and HI: harvest index. The bold figure showed significance difference from the grand mean.

5.1.2.5 Heritability and genetic advance of oat accessions

The estimates of broad sense heritability and genetic advance in all characters of the accessions of five *Avena* species studied are listed in Table 17-21. The calculated heritability was classified into three groups as suggested by (Johnson *et al.*, 1955): 0-30% = Low, 30-60% = Moderate, and > 60% = High.

According to Johnson *et al.* (1955), genetic advance as per cent of mean was categorized as per the formula, 0-10 % = Low, 10-20 % = Moderate, and > 20 % = High

Low broad-sense heritability values ranging less than 30 were observed for the characters for *A. sativa* and *A. sterilis* accessions. The low heritability value was recorded in all of the traits in *A. sativa*. In *A. fatua* species, grain yield and flag leaf length traits showed moderate heritability but the remaining traits showed low heritability. Likewise, *A. vaviloviana* accessions showed moderate heritability in traits of number of effective tiller, number of nodes, flag leaf length, and length of panicle. Furthermore, *A. abyssinica* had moderate heritability in flag leaf length, days to 50% heading, and days to 90% maturity.

The high value (>20%) of genetic advance as percent of means were obtained in *A. sativa* accessions for six four of the characters number of effective tiller, flag leaf length, length of panicle, number of spikelet per panicle, biological yield, and grain yield. Number of nodes and harvest index had low genetic advance as percent of means and the remaining traits had moderate genetic advance as percent of means (Table 21). In *A. fatua* the majority traits had high genetic advance as percent of the mean except number of effective tiller, number of nodes, plant height, and days to 90% maturity which had moderate genetic advance as percent of the mean (Table 20). Only Harvest index had low genetic advance as percent of the mean. For *A. sterilis* moderate genetic advance as percent of the mean were observed in days to 90% maturity, plant height, number of effective tiller, and number of nodes traits whereas, low genetic advance as percent of the mean was observed in harvest index (Table 19).

The remaining traits showed high genetic advance as percent of the mean (Table 19). Majority of the traits had high genetic advance as percent of the mean in *A. abyssinica* and *A. vaviloviana* species. Moderate genetic advance as percent of the mean were observed in internode length for *A. vaviloviana* and in plant height, number of nodes, and internode length for *A. abyssinica* species (Table 17 and 18)

Table 17. Results of variance components and heritability with standard error for *A. abyssinica* accessions

Traits	μ	σ^2g	σ^2gy	σ^2gl	σ^2gyl	σ^2e	σ^2p	H ² b (%)	GA	GAM (%)	R-Squ.	cv
NET	6	9.8	12.6	8.8	8.4	8.6	48.2	20.3%	2.9	48.5	0.6	49
NN	4.5	0.7	0.4	0.5	0.4	0.7	2.7	25.9%	0.9	19.5	0.5	17
IL	21.7	17.7	15.3	18	10.8	18.1	79.9	22.2%	4.1	18.8	0.5	21.5
FLL	27.5	277	184	96.8	77.3	82.9	718	38.6%	21.3	77.4	0.7	32.7
LP	30.9	131	129	110	78.6	85.2	533.8	24.5%	11.7	37.8	0.6	29.7
NSP	61.9	634.8	519	452.1	293.6	346.2	2245.7	28.3%	27.6	44.6	0.6	35.1
PH	141.6	527.8	700.5	417.4	631	370	2646.7	19.9%	21.1	14.9	0.6	13.7
DHE	110	738.6	400.1	209.3	380.4	302.6	2031	36.4%	33.8	30.7	0.6	15.3
DM	135.5	635.9	399.8	270.8	422.5	308.6	2037.6	31.2%	29.0	21.4	0.6	12.4
By	1133.7	532956.9	643145.8	483226.5	645812.7	571451.1	2876593	18.5%	647.3	57.1	0.6	36.8
GY	127	16294.5	12577.5	10457.2	10814.5	12761.9	62905.6	25.9%	133.8	105.4	0.5	43.2
HI	0.11.20	0.002	0.004	0.004	0.003	0.003	0.016	12.5%	0.0	105.4	0.549	38.1

Where, σ^2gy :variance of genotype interaction with year, σ^2gl : variance of genotype interaction with location, σ^2gyl : variance of genotype with location and year, σ^2g : genotypic variance, σ^2p : phenotypic variance, σ^2e variance of error, h^2b :heritability in broad sense; GA: genetic advance; GAM: genetic advance percentage of mean; NET: number of effective tillers per plant; NN: Number of nodes per plant, IL: internode length; FLL: flag leaf length; LP: Length of panicle; NSP: number of spikelet per panicle; PH: plant height; DHE: days to 50% heading; DM: days to 90% maturity; BY: biological yield; GY: grain yield; and HI: Harvest index.

Table 18. Results of variance components and heritability with standard error for *A. vaviloviana* accessions

Traits	μ	σ^2_g	σ^2_{gy}	σ^2_{gl}	σ^2_{gyl}	σ^2_e	σ^2_p	H ² b (%)	GA	GAM (%)	R-Squ.	cv
NET	6.2	12.1	4.5	8	2.7	7.3	34.6	35.0%	4.2	68.3	0.5	47.3
NN	4.4	0.6	0.1	0.4	0.3	0.5	1.9	31.6%	0.9	20.4	0.5	14.2
IL	20	17.3	17.8	29.4	22.1	25.1	111.7	15.5%	3.4	16.9	0.5	24.5
FLL	27.9	315.4	460.7	72.3	79.4	107.9	1035.7	30.5%	20.2	72.4	0.7	37.3
LP	32	264.5	6.2	117.8	76.9	84.7	550.1	48.1%	23.2	72.6	0.7	29.4
NSP	43	807.8	1217.2	399.7	476.1	283.5	3184.3	25.4%	29.5	68.6	0.7	32.3
PH	141.2	811.2	3.5	221	284	280.8	1600.5	50.7%	41.8	29.6	0.6	11.9
DHE	115.3	778.2	1354.3	354.8	428.1	315	3230.4	24.1%	28.2	24.5	0.6	15.6
DM	143.6	737.9	1394.8	342.4	422.7	312.9	3210.7	23.0%	26.8	18.7	0.7	12.4
By	1264.3	339139.4	162091.1	681932.4	438186	413797.6	2035147	16.7%	489.7	38.7	0.7	31.8
GY	150.5	16693.3	22228.9	22070.9	13135	11824	85952.1	19.4%	117.3	77.9	0.7	37.2
HI	11.90%	0.38%	0.60%	0.21%	0.22%	0.22%	1.63%	23.3%	6%	52%	56%	31.3%

Where, σ^2_{gy} :variance of genotype interaction with year, σ^2_{gl} : variance of genotype interaction with location, σ^2_{gyl} : variance of genotype with location and year, σ^2_g : genotypic variance, σ^2_p : phenotypic variance, σ^2_e variance of error, h^2_b :heritability in broad sense; GA: genetic advance; GAM: genetic advance percentage of mean; NET: number of effective tillers per plant; NN: Number of nodes per plant, IL: internode length; FLL: flag leaf length; LP: Length of panicle; NSP: number of spikelet per panicle; PH: plant height; DHE: days to 50% heading; DM: days to 90% maturity; BY: biological yield; GY: grain yield; and HI: Harvest index.

Table 19. Results of variance components and heritability with standard error for *A. sterilis* accessions

Traits	μ	σ^2g	σ^2gy	σ^2gl	σ^2gyl	σ^2e	σ^2p	H ² b (%)	GA	GAM (%)	R-Squ.	cv
NET	5.3	5.5	5.1	13.5	3.6	7.4	35.1	15.7%	1.9	36.1	0.5	48.4
NN	4.6	0.4	0.6	0.9	1.4	0.7	4	10.0%	0.4	9.0	0.6	16.4
IL	19.1	19	14.2	13.2	14.1	16.1	76.6	24.8%	4.5	23.4	0.6	20.1
FLL	27.7	117.7	185.7	141.5	213.2	94.9	753	15.6%	8.8	31.9	0.7	33.2
LP	30.3	42.4	40.8	24.6	8.8	45.9	162.5	26.1%	6.9	22.6	0.5	23.6
NSP	37.1	200.6	351.1	284.4	470.6	327.7	1634.4	12.3%	10.2	27.6	0.5	35.4
PH	138.1	412.5	440.5	388.6	1738	534.2	3513.8	11.7%	14.3	10.4	0.5	16.6
DHE	113.5	288.9	220.6	493.3	765.3	342.6	2110.7	13.7%	13.0	11.4	0.6	16.6
DH	141.9	281.5	253.8	393.7	1588.3	337.9	2855.2	9.9%	10.9	7.6	0.6	13.2
BY	756.2	801476.1	531526	1367211	2289103	749192.8	5738508	14.0%	689.2	91.1	0.6	42
GY	91.5	18081.5	11242.7	13227.2	54750.6	17759.3	115061.3	15.7%	109.8	120.0	0.6	49
HI	0.121	0.002	0.004	0.003	0.004	0.003	0.016	12.5%	0.0	26.9	0.579	38.3

Where, σ^2gy :variance of genotype interaction with year, σ^2gl : variance of genotype interaction with location, σ^2gyl : variance of genotype with location and year, σ^2g : genotypic variance, σ^2p : phenotypic variance, σ^2e variance of error, h^2b :heritability in broad sense; GA: genetic advance; GAM: genetic advance percentage of mean; NET: number of effective tillers per plant; NN: Number of nodes per plant, IL: internode length; FLL: flag leaf length; LP: Length of panicle; NSP: number of spikelet per panicle; PH: plant height; DHE: days to 50% heading; DM: days to 90% maturity; BY: biological yield; GY: grain yield; and HI: Harvest index.

Table 20. Results of variance components and heritability with standard error for *A. fatua* accessions

Traits	μ	σ^2_g	σ^2_{gy}	σ^2_{gl}	σ^2_{gyl}	σ^2_e	σ^2_p	H ² b (%)	GA	GAM (%)	R-Squ.	cv
NET	7.5	4.4	4.9	5.4	18	7	39.7	11.1%	1.4	19.2	0.5	46.9
NN	4.6	0.9	0.8	0.7	1.1	0.9	4.4	20.5%	0.9	19.2	0.6	17.7
IL	18.3	33.6	22	11.1	67.8	19	153.5	21.9%	5.6	30.5	0.6	23
FLL	24.1	212.5	89	197	39.1	92.7	630.3	33.7%	17.4	72.3	0.7	33.7
LP	27.8	123.8	106.2	130.1	61.9	70.3	492.3	25.1%	11.5	41.3	0.7	27.7
NSP	53.1	459.9	416.3	419.3	1241.7	384.2	2921.4	15.7%	17.5	33.0	0.6	36.1
PH	119.9	565.7	1119.3	1006.8	5883.5	663.5	9238.8	6.1%	12.1	10.1	0.7	19.4
DHE	104.5	551.9	309.6	377.8	337.1	315.4	1891.8	29.2%	26.1	25.0	0.6	16.1
DM	128.5	475.8	345.3	418.5	827.2	309.6	2376.4	20.0%	20.1	15.6	0.6	12.8
BY	1147.9	472817.6	675647.4	400487.4	935997.6	443811.6	2928762	16.1%	569.1	49.6	0.6	31.1
GY	130.9	43002.5	34667	13742.4	7771.4	20606.8	119790.1	35.9%	255.9	195.5	0.6	53.4
HI	11.9	0.004	0.005	0.003	0	0.003	0.015	26.7%	0.1	0.6	0.578	43.4

Where, σ^2_{gy} :variance of genotype interaction with year, σ^2_{gl} : variance of genotype interaction with location, σ^2_{gyl} : variance of genotype with location and year, σ^2_g : genotypic variance, σ^2_p : phenotypic variance, σ^2_e variance of error, h^2_b :heritability in broad sense; GA: genetic advance; GAM: genetic advance percentage of mean; NET: number of effective tillers per plant; NN: Number of nodes per plant, IL: internode length; FLL: flag leaf length; LP: Length of panicle; NSP: number of spikelet per panicle; PH: plant height; DHE: days to 50% heading; DM: days to 90% maturity; BY: biological yield; GY: grain yield; and HI: Harvest index.

Table 21. Results of variance components and heritability with standard error for *A. sativa* accessions

Traits	μ	σ^2_g	σ^2_{gy}	σ^2_{gl}	σ^2_{gyl}	σ^2_e	σ^2_p	H^2b (%)	GA	GAM (%)	R-Squ.	cv
NET	5.4	8	7.2	6.3	31.5	6.9	59.9	13.4%	2.1	39.4%	0.5	46.1
NN	5	0.7	0.7	0.6	6.8	0.6	9.4	7.4%	0.5	9.4%	0.6	16.4
IL	19.8	20.5	22.5	15.8	112	16.5	187.3	10.9%	3.1	15.6%	0.6	20.4
FLL	30.1	151.7	109.2	107.7	125.9	116.7	611.2	24.8%	12.6	42.0%	0.6	38.3
LP	30.9	109.3	99.2	116.6	288.6	86.4	700.1	15.6%	8.5	27.5%	0.6	30.4
NSP	52.4	431	389	465.4	2419.8	422.4	4127.6	10.4%	13.8	26.4%	0.5	38.6
PH	147.5	548.7	411.8	487.7	1914.9	446.3	3809.4	14.4%	18.3	12.4%	0.6	15
DHE	114.8	530.8	323.2	342.6	903.2	371.3	2471.1	21.5%	22.0	19.2%	0.6	17
DM	145.9	549.5	283.5	302.4	1374.6	364.9	2874.9	19.1%	21.1	14.5%	0.6	13.6
BY	2314.1	945989.2	652828.7	658060.5	2119455	729354.5	5105688	18.5%	862.4	37.3%	0.6	39
GY	284.6	19980.7	17106.1	17916.7	40818.3	18219.9	114041.7	17.5%	121.9	42.8%	0.5	47.4
HI	12.3	0.003	0.004	0.004	0.04	0.004	0.055	5.5%	0.0	0.2%	0.519	43.88

Where, σ^2_{gy} :variance of genotype interaction with year, σ^2_{gl} : variance of genotype interaction with location, σ^2_{gyl} : variance of genotype with location and year, σ^2_g : genotypic variance, σ^2_p : phenotypic variance, σ^2_e variance of error, h^2b :heritability in broad sense; GA: genetic advance; GAM: genetic advance percentage of mean; NET: number of effective tillers per plant; NN: Number of nodes per plant, IL: internode length; FLL: flag leaf length; LP: Length of panicle; NSP: number of spikelet per panicle; PH: plant height; DHE: days to 50% heading; DM: days to 90% maturity; BY: biological yield; GY: grain yield; and HI: Harvest index.

5.1.3. Correlation analysis

5.1.3.1. Correlation analysis of the 5 *Avena* species

At phenotypic level, grain yield had a positive and significant correlation with biological yield, days to 50% heading, number of nodes, and harvest index, while number of days to 90% maturity, and number of effective tillers showed negative and significant correlation with grain yield. The number of effective tillers per plant and harvest index had a significant negative correlation with the biological yields and days to 50% heading. The number of spikelets per panicle has a negative and significant correlation with panicle length and flag leaf length (Table 22). A significant positive association was observed for both plant height and days to 90% maturity with biological yield; length of panicle significant negative correlation with flag leaf length and number of spikelet per panicle (Table 22). Traits like days to 50% heading had a positive and highly significant correlation with biological yield, and days to 90% maturity. Similarly, days to 90% maturity had a highly significant positive correlation with days to 50% heading, and biological yield (Table 22).

At genotypic level, for grain yield a positive and significant association was observed with days to 50% heading, biological yield and harvest index; whereas, grain yield showed a negative and significant associations with plant height (Table 22). In the majority of the traits the genotypic coefficient of variation is greater than the phenotypic coefficient of variation, i.e., only few traits showed higher phenotypic coefficient than genotypic coefficient of variation.

Table 22. Estimate phenotypic (above the diagonal) and genotypic (below the diagonal) correlation coefficient of combined mean of 12 quantitative traits for 176 oat accessions tested at Holetta and Debre Berhan agricultural research centers during 2014/15 and 2015/2016 main cropping season

Variable	NET	NN	IL	FLL	LP	NSP	PH	DHE	DM	BY	GY	HI
NET	1	0.004	0.003	-0.003	0.001	0.001	0.001	-0.140***	0.053	0.004	-0.081**	0.012
NN	0.709***	1	-0.007	-0.013	0.001	0.003	0.032	0.208***	-0.106***	-0.008	0.081**	-0.001
IL	0.000	-0.012	1	-0.003	-0.003	0.003	0.003	-0.118***	0.047	0.019	0.018	-0.010
FLL	-0.108	-0.073	0.008	1	-0.284***	-0.213***	0.016	-0.049	-0.013	0.011	-0.005	-0.029
LP	-0.025	-0.041	-0.030	-0.289***	1	-0.220***	-0.011	0.027	-0.012	-0.044	0.013	-0.050
NSP	0.0862	0.1034	0.0545	-0.214**	-0.221**	1	-0.0310	0.0016	0.0445	-0.0156	-0.0177	0.0254
PH	-0.039	0.025	-0.003	0.044	-0.027	-0.089	1	-0.042	0.136***	0.073**	-0.032	0.010
DHE	-0.007	-0.059	0.031	-0.127	0.075	0.000	-0.047	1	-0.229***	0.038	0.251***	0.306***
DM	-0.110	-0.144	0.003	-0.043	-0.043	0.134	0.103	-0.192*	1	-0.085**	-0.027	0.110***
BY	0.055	-0.007	-0.009	0.013	-0.093	-0.031	0.041	0.087	-0.190*	1	0.363***	0.451***
GY	0.134	0.089	0.104	-0.020	0.036	-0.041	-0.219**	0.266***	-0.138	0.420***	1	0.582***
HI	0.105	0.045	0.069	-0.083	-0.122	0.063	-0.062	0.310***	0.040	0.628***	0.693***	1

Where, *, **, and *** are significant at 5%, 1 %, and 0.1% level of probability, respectively

Where, NET: Number of effective tillers per plant; NN: Number of node per plant; IL: Internode length; FLL: Flag leaf length; LP: Length of panicle; NSP: Number of spikelet per panicle; PH: Plant height; DHE: Days to 50% heading; MD: Days to 90% maturity; BY: Biological yield; GY: Grain yield; and HI: harvest index

Table 23. Estimate phenotypic (above the diagonal) and genotypic (below the diagonal) correlation coefficient of combined mean of 12 quantitative traits for 176 oat accessions tested at Holetta and Debre Berhan agricultural research centers during 2014/15 and 2015/2016 main cropping season

Variable	NET	NN	IL	FLL	LP	NSP	PH	DHE	DM	BY	GY	HI
NET	1	0.004	0.003	-0.003	0.001	0.001	0.001	-0.140***	0.053	0.004	-0.081**	0.012
NN	0.709***	1	-0.007	-0.013	0.001	0.003	0.032	0.208***	-0.106***	-0.008	0.081**	-0.001
IL	0.000	-0.012	1	-0.003	-0.003	0.003	0.003	-0.118***	0.047	0.019	0.018	-0.010
FLL	-0.108	-0.073	0.008	1	-0.284***	-0.213***	0.016	-0.049	-0.013	0.011	-0.005	-0.029
LP	-0.025	-0.041	-0.030	-0.289***	1	-0.220***	-0.011	0.027	-0.012	-0.044	0.013	-0.050
NSP	0.0862	0.1034	0.0545	-0.214**	-0.221**	1	-0.0310	0.0016	0.0445	-0.0156	-0.0177	0.0254
PH	-0.039	0.025	-0.003	0.044	-0.027	-0.089	1	-0.042	0.136***	0.073**	-0.032	0.010
DHE	-0.007	-0.059	0.031	-0.127	0.075	0.000	-0.047	1	-0.229***	0.038	0.251***	0.306***
DM	-0.110	-0.144	0.003	-0.043	-0.043	0.134	0.103	-0.192*	1	-0.085**	-0.027	0.110***
BY	0.055	-0.007	-0.009	0.013	-0.093	-0.031	0.041	0.087	-0.190*	1	0.363***	0.451***
GY	0.134	0.089	0.104	-0.020	0.036	-0.041	-0.219**	0.266***	-0.138	0.420***	1	0.582***
HI	0.105	0.045	0.069	-0.083	-0.122	0.063	-0.062	0.310***	0.040	0.628***	0.693***	1

Where, *, **, and *** are significant at 5%, 1 %, and 0.1% level of probability, respectively

Where, NET: Number of effective tillers per plant; NN: Number of node per plant; IL: Internode length; FLL: Flag leaf length; LP: Length of panicle; NSP: Number of spikelet per panicle; PH: Plant height; DHE: Days to 50% heading; MD: Days to 90% maturity; BY: Biological yield; GY: Grain yield; and HI: harvest index

5.1.3.2. Correlation analysis of *A. sativa* accessions

Grain yield exhibited a positive correlation with all of the traits (Table 24), but only biological yield, harvest index, and panicle length had high to very high significant correlation with the grain yield. Yield-related components such as biological yield exhibited a high to very high significant and positive correlation with flag leaf length, and length of panicle, whereas number of effective tillers per plant revealed a very significant and negative correlation with plant height, flag leaf length, length of panicle, and number of spikelet per panicle, in addition, number of spikelet per panicle showed a positive and significant correlation with the majority of the accessions (Table 24). Days to 50% heading traits showed positive and significant correlation with traits like the length of panicle, flag leaf length, number spikelets of panicle, plant height, and days to 90% maturity.

Table 24. Pearson correlation for quantitative traits in *A. sativa*

Traits	NET	NN	IL	FLL	LP	NSP	PH	DHE	DM	BY	GY
NN	-0.211										
IL	-0.060	-0.310**									
FLL	-0.474***	0.105	0.176								
Lp	-0.351***	0.342**	-0.040	0.672***							
NSp	-0.417***	0.421***	0.007	0.572***	0.584***						
PH	-0.426***	0.574***	0.052	0.539***	0.590***	0.657***					
DHE	-0.192	0.165	-0.124	0.283**	0.386***	0.328**	0.264**				
DM	-0.105	-0.005	0.007	0.155	0.135	0.105	0.033	0.761***			
BY	0.097	0.206	0.024	0.254*	0.354***	0.131	0.145	0.151	0.039		
GY	0.140	0.206	0.025	0.213	0.352***	0.105	0.144	0.131	0.024	0.979***	
HI	0.182	-0.069	-0.003	-0.270*	-0.134	-0.188	-0.092	-0.164	-0.084	-0.359 ^c	-0.165

Where, *, **, and *** are significant at 5%, 1 %, and 0.1% level of probability, respectively

Where, NET: Number of effective tillers per plant; NN: Number of node per plant; IL: Internode length; FLL: Flag leaf length; LP: Length of panicle; PH: Plant height; NSP: Number of spikelet per panicle; BY: Biological yield; GY: Grain yield; DHE: Days to 50% heading; and MD: Days to 90% maturity

5.1.3.3. Correlation analysis of *A. abyssinica* accessions

Grain yield had highly significant and positive relationships with biological yield and harvest index, and harvest index showed significant positive associations with grain yield. All the remaining traits

showed non-significant associations with grain yield (Table 25). Days to 90% maturity showed positive and significant correlation with the number of nodes, internode length, plant height, and days to 50% heading. Except for flag leaf length with internode length, the remaining traits had a non-significant correlation.

Table 25. Pearson correlation for 12 quantitative traits in *A. abyssinica* accessions

Trait	NET	NN	IL	FLL	LP	NSP	PH	DHE	DM	BY	GY
NN	0.219										
IL	0.044	-0.128									
FLL	0.168	0.126	0.382**								
LP	0.072	-0.091	-0.037	0.174							
NSP	-0.192	0.079	-0.050	0.090	-0.040						
PH	0.058	0.257	0.522***	0.475***	0.389**	-0.035					
DHE	-0.055	0.264	0.287	0.175	-0.065	0.190	0.267				
DM	0.030	0.306*	0.337**	0.289	-0.010	0.138	0.349**	0.869***			
BY	0.004	0.136	-0.071	-0.057	0.029	0.136	0.098	-0.131	-0.111		
GY	0.078	0.108	-0.111	-0.078	0.036	0.183	0.020	-0.133	-0.159	0.955***	
HI	0.325*	-0.081	-0.204	-0.095	0.121	0.069	-0.224	-0.083	-0.240	0.076	0.358**

Where, *, **, and *** are significant at 5%, 1 %, and 0.1% level of probability, respectively.

Where, NET: number of effective tiller per plant, NN: number of node per pant, IL: internode length, FLL: flag leaf length, LP: Length of panicle, NSP: number of spikelet per panicle, PH: plant height, DHE: days to 50% heading, DM: days to 90% maturity, BY: biological yield, GY: grain yield, and HI: harvest index.

5.1.3.4 Correlation analysis of *A. vaviloviana*, *A. sterilis*, and *A. fatua* accessions

Grain yield had positive and significant correlation were with biological yield in all the three species. Grain yield had a negative correlation with number of nodes interned length, flag leaf length, number of spikelets of the panicle, and plant height for *A. sterilis*. Similarly, number of effective tiller, internode length, number of spikelet, and days to 50% heading and 90% maturity had negative correlations with grain yield in *A. vaviloviana* species. From these

traits days to 50% heading, days to 90% maturity showed significant correlation. For *A. fatua*, traits like number of effective tiller, internode length, flag leaf length, and length of panicle had negative correlation with grain yield.

Table 26. Pearson correlation for 12 quantitative traits in *A. vaviloviana* accessions

Traits	NET	NN	IL	FLL	LP	NSp	PH	DHE	DM	DH	BY	GY
NN	-0.216											
IL	0.2	-0.117										
FLL	0.333	0.194	0.227									
LP	0.104	-0.131	-0.029	0.011								
NSp	-0.156	-0.265	-0.011	-0.067	0.429							
PH	-0.032	0.309	0.101	0.493	-0.012	0.128						
DHE	0.353	-0.689	0.146	-0.173	0.173	0.26	-0.004					
DM	0.303	-0.762***	0.238	-0.303	0.13	0.298	-0.123	0.912***				
DH	0.32	-0.635**	0.11	-0.562**	0.137	0.233	-0.34	0.798***	0.86***			
BY	-0.229	0.592**	-0.124	-0.073	0.046	-0.112	-0.054	-0.524	-0.688***	-0.435		
GY	-0.17	0.627	-0.094	-0.016	0.024	-0.2	-0.06	-0.567**	-0.724***	-0.498*	0.977***	
HI	0.28	0.22	0.133	0.231	-0.114	-0.464	-0.077	-0.245	-0.249	-0.317	0.036	0.247

Where, *, **, and * are significant at 5%, 1 %, and 0.1% level of probability, respectively.

Where, NET: number of effective tiller per plant, NN: number of node per pant, IL: internode length, FLL: flag leaf length, LP: Length of panicle, NSP: number of spikelet per panicle, PH: plant height, DHE: days to 50% heading, DM: days to 90% maturity, BY: biological yield, GY: grain yield, and HI: harvest index.

Table 27. Pearson correlation for 12 quantitative traits in *A. sterilis* accessions

Traits	NET	NN	IL	FLL	LP	NSp	PH	DHE	DM	BY	GY
NN	0.288										
IL	0.211	-0.386									
FLL	0.24	-0.025	0.572								
LP	-0.135	-0.445	0.102	-0.066							
NSp	0.08	0.581*	-0.529	-0.227	0.189						
PH	0.181	0.742**	-0.235	-0.014	-0.054	0.426					
DHE	0.407	0.138	0.026	-0.157	0.385	0.306	0.249				
DM	0.432	0.146	0.021	-0.179	0.37	0.297	0.242	0.994***			
BY	0.29	-0.215	-0.057	-0.195	-0.05	-0.154	-0.37	0.186	0.24		
GY	0.292	-0.202	-0.14	-0.248	0.033	-0.052	-0.33	0.206	0.266	0.983***	
HI	0.012	0.055	-0.464	-0.339	0.458	0.543	0.199	0.104	0.142	-0.136	0.047

Table 28. Pearson correlation for 12 quantitative traits in *A. fatua* accessions

Traits	NET	NN	IL	FLL	LP	NSp	PH	DHE	DM	BY	GY
NN	-0.365										
IL	0.007	-0.655**									
FLL	0.281	-0.033	-0.192								
LP	0.125	-0.17	0.467*	0.048							
NSp	-0.03	0.197	0.013	0.183	0.557*						
PH	0.052	-0.106	0.4	0.096	0.825***	0.529*					
DHE	-0.033	-0.049	0.054	0.178	0.56*	0.562*	0.557*				
DM	-0.034	-0.02	0.051	0.095	0.496	0.572*	0.531*	0.972***			
BY	-0.582*	0.324	-0.013	0.034	-0.033	0.197	0.245	0.416	0.427		
GY	-0.579*	0.363	-0.043	-0.007	-0.032	0.235	0.233	0.399	0.407	0.991**	
HI	-0.085	0.336	-0.156	-0.309	0.055	0.423	-0.021	0.009	0.003	0.15	0.277

Where, *, **, and * are significant at 5%, 1 %, and 0.1% level of probability, respectively.

Where, NET: number of effective tiller per plant, NN: number of node per pant, IL: internode length, FLL: flag leaf length, LP: Length of panicle, NSP: number of spikelet per panicle, PH: plant height, DHE: days to 50% heading, DM: days to 90% maturity, BY: biological yield, GY: grain yield, and HI: harvest index.

5.1.4 Cluster analysis

5.1.4.1 Cluster analysis of accessions of five *Avena* species

The 176 oat accessions were grouped into three clusters using agglomerate hierarchical clustering of unweighted pair group average mean (Figure 13). As shown in Table 29, the number of accessions per cluster varied from 23 to 87 in Cluster 2 and Cluster 3, respectively. Cluster 1 and cluster 2 contains only *A. sativa* accessions and they came from all regions/country except accessions from Welega region, whereas, in cluster 2, the majority of the accessions came from Shewa, Welega, Gojam, and Gondar. In cluster 2, a single accession from Australia and Bale were represented. Cluster 3 contains accession of all four wild species, in addition, it contains a single accession from cultivated oat (Acc No. 2). Cluster 3 exhibited two sub-clusters, Sub-cluster 1 consisted of *A. vaviloviana* and *A. sterilis* variance of genotype interaction with year and sub-cluster 2 *A. fatua* and *A. abyssinica* that came from different regions of Ethiopia. In general, the result revealed, accessions of same species clustered together regardless of their source of origin (Table 29 and Figure 13). The variance percentage within and among clusters were 11.5 and 88.5%, respectively. The variance observed within cluster 1, cluster 2, and cluster 3 was 3 48234.4, 60163.7, and 61388.7, respectively. Relatively, cluster 3 had the highest variance

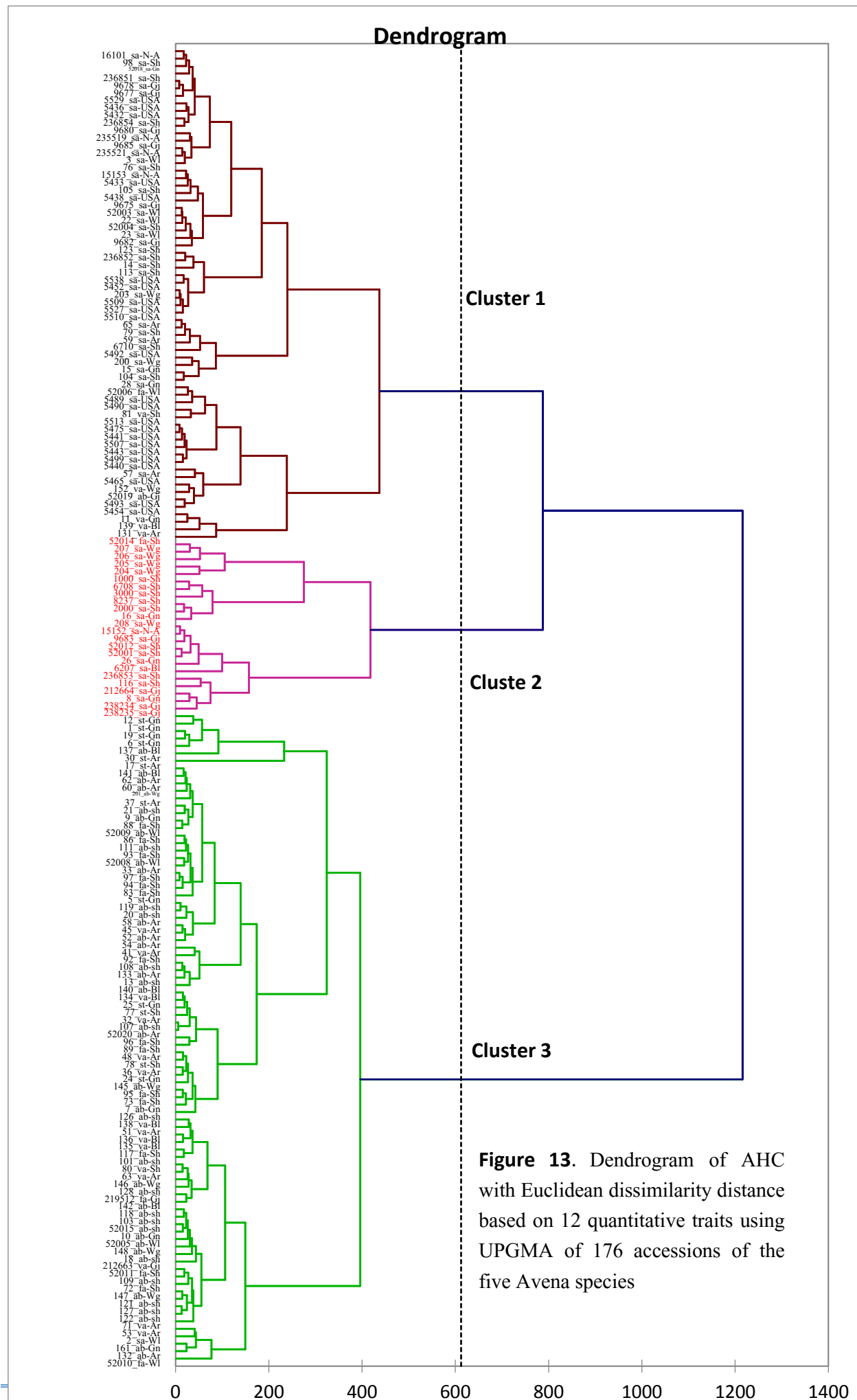


Table 29. Total accessions in the cluster, accession number with their respective source of origin of 176 oat accessions

Cluster 1 (#66)			Cluster 2 (# 23)	Cluster 3 (# 87)			
9675_sa-Gj	5432_sa-USA	5538_sa-USA	9683_sa-Gj	2_sa-Wl	103_ab-Sh	73_fa-Sh	25_st-Gn
9677_sa-Gj	5433_sa-USA	200_sa-Wg	212664_sa-Gj	33_ab-Ar	107_ab-Sh	83_fa-Sh	77_st-Sh
9678_sa-Gj	5436_sa-USA	203_sa-Wg	238234_sa-Gj	52_ab-Ar	108_ab-Sh	86_fa-Sh	78_st-Sh
9680_sa-Gj	5438_sa-USA	3_sa-Wl	238235_sa-Gj	54_ab-Ar	109_ab-Sh	88_fa-Sh	32_va-Ar
9682_sa-Gj	5440_sa-USA	22_sa-Wl	8_sa-Gn	58_ab-Ar	111_ab-Sh	89_fa-Sh	36_va-Ar
9685_sa-Gj	5441_sa-USA	23_sa-Wl	16_sa-Gn	60_ab-Ar	118_ab-Sh	92_fa-Sh	41_va-Ar
15_sa-Gn	5443_sa-USA	52003_sa-Wl	26_sa-Gn	62_ab-Ar	119_ab-Sh	93_fa-Sh	45_va-Ar
28_sa-Gn	5452_sa-USA	57_sa-Ar	116_sa-Sh	132_ab-Ar	121_ab-Sh	94_fa-Sh	48_va-Ar
52018_sa-Gn	5454_sa-USA	59_sa-Ar	1000_sa-Sh	133_ab-Ar	122_ab-Sh	95_fa-Sh	51_va-Ar
14_sa-Sh	5465_sa-USA	65_sa-Ar	2000_sa-Sh	52020_ab-Ar	126_ab-Sh	96_fa-Sh	53_va-Ar
76_sa-Sh	5475_sa-USA	15153_sa- Aus	3000_sa-Sh	137_ab-BI	127_ab-Sh	97_fa-Sh	63_va-Ar
79_sa-Sh	5489_sa-USA	16101_sa- Aus	6708_sa-Sh	140_ab-BI	128_ab-Sh	117_fa-Sh	71_va-Ar
98_sa-Sh	5490_sa-USA	235519_sa-Nth	8237_sa-Sh	141_ab-BI	52015_ab-Sh	52011_fa-Sh	134_va-BI
104_sa-Sh	5492_sa-USA	235521_sa-Nth	52001_sa-Sh	142_ab-BI	145_ab-Wg	52010_fa-Wl	135_va-BI
105_sa-Sh	5493_sa-USA	52019_ab-Gj	52012_sa-Sh	7_ab-Gn	146_ab-Wg	17_st-Ar	136_va-BI
113_sa-Sh	5499_sa-USA	52014_fa-Sh	236853_sa-Sh	9_ab-Gn	147_ab-Wg	30_st-Ar	138_va-BI
123_sa-Sh	5507_sa-USA	52006_fa-Wl	204_sa-Wg	10_ab-Gn	148_ab-Wg	37_st-Ar	212663_va-Gj
6710_sa-Sh	5509_sa-USA	131_va-Ar	205_sa-Wg	161_ab-Gn	201_ab-Wg	1_st-Gn	80_va-Sh
52004_sa-Sh	5510_sa-USA	139_va-BI	206_sa-Wg	13_ab-Sh	52005_ab-Wl	5_st-Gn	
236851_sa-Sh	5513_sa-USA	11_va-Gn	207_sa-Wg	18_ab-Sh	52008_ab-Wl	6_st-Gn	
236852_sa-Sh	5527_sa-USA	81_va-Sh	208_sa-Wg	20_ab-Sh	52009_ab-Wl	12_st-Gn	
236854_sa-Sh	5529_sa-USA	152_va-Wg	6207_sa-BI	21_ab-Sh	219512_fa-Gj	19_st-Gn	
			15152_sa- Aus	101_ab-Sh	72_fa-Sh	24_st-Gn	

Where, #: number, acc: accessions, sa: *A. sativa*, ab: *A. abyssinica*, fa: *A. fatua*, va: *A. vaviloviana*, and st: *A. sterilis*, Ar: Arsi, BI: Bale. Gj: Gojam, Gn: Gondar, Sh: shewa, N: The Netherlands, Aus: Australia, Wg: Welega, Wl: Wello, and USA: Unite States of America. Note: the number before under score is the accessions number

Cluster mean analysis of accessions of five *Avena* species

Cluster 1 had the highest mean value in nine traits. The low mean value in cluster 1 was recorded in the values of effective tiller per plant, internode length, and the number of spikelets per panicle. In cluster 2, the traits including, effective tiller per plant, internode length, and the number of spikelets per panicle had high mean value (Table 30). Cluster 3 had relatively high mean value only in days to 50% heading, day to 90% maturity, and plant height. The remaining traits in this cluster possessed low mean value.

Table 30. Means of 12 quantitative traits in three clusters in five *Avena* species

Variable/ traits	Cluster 1	Cluster 2	Cluster 3	μ	std
Number of effective tiller/plant (count)	5.4	6.6	5.3	6.1	0.8925
Number of node (count)	5.0	4.5	4.6	4.6	0.2016
Internodes length (cm)	19.8	20.0	19.1	19.8	1.2879
Flag leaf length (cm)	30.1	26.5	27.5	27.4	2.1414
Length of panicle (cm)	30.9	30.4	30.3	30.5	1.4740
Number of spikelet/ panicle	52.4	52.8	37.1	49.6	9.6696
Plant height (cm)	147.4	134.2	138.1	137.6	10.2657
Days to 50% heading (days)	114.8	110.0	113.5	111.6	4.4742
Days to 90% maturity (days)	145.9	135.9	141.9	139.1	7.0880
Biological yield per m2(g)	2314.1	1180.5	756.2	1322.3	586.34
Grain yield per m2(g)	284.1	136.7	91.2	157.1	74.2145
Harvest index	12.3%	11.5%	12.1%	11.8%	0.0045

Where, μ : grand mean, and std: standard deviation

The distance between the cluster centroids ranged from 786.9 (between C2 and C3) to 1798.9 (between C 1 and C 3). The average cluster centroid distance was found between cluster 1 and cluster 2, it was recorded 1012.1.

Generally, the five *Avena* species were clustered in three groups. Cluster 1 and cluster 2 contained *A. sativa* and cluster 3 contained *A. abyssinica*, *A. fatua*, *A. sterilis*, and *A. vaviloviana* the analysis was done by taking the mean average of 12 quantitative traits. The distance between and among clusters account 1.7% and 98.3%, respectively.

5.1.4.2. Cluster analysis in 73 *A. sativa* accessions

The 73 *A. sativa* accessions were grouped into three clusters with a Euclidean distance of unweighted pair group average means using agglomerate hierarchical clustering. The variance within clusters ranged from 19.0% (in Cluster 3) to 47.4% (in cluster 2) and cluster 2 had 33.7% variance within the cluster. The number of accessions varied from 12 in cluster 3) to 45 (in cluster 2). In clusters 3, the accessions are USA materials. In cluster 2, the majority of accessions came from Shewa and Welega. Cluster 1 had had accessions originated from all regions and sub-clustered into two sub-clusters (Figure 14).

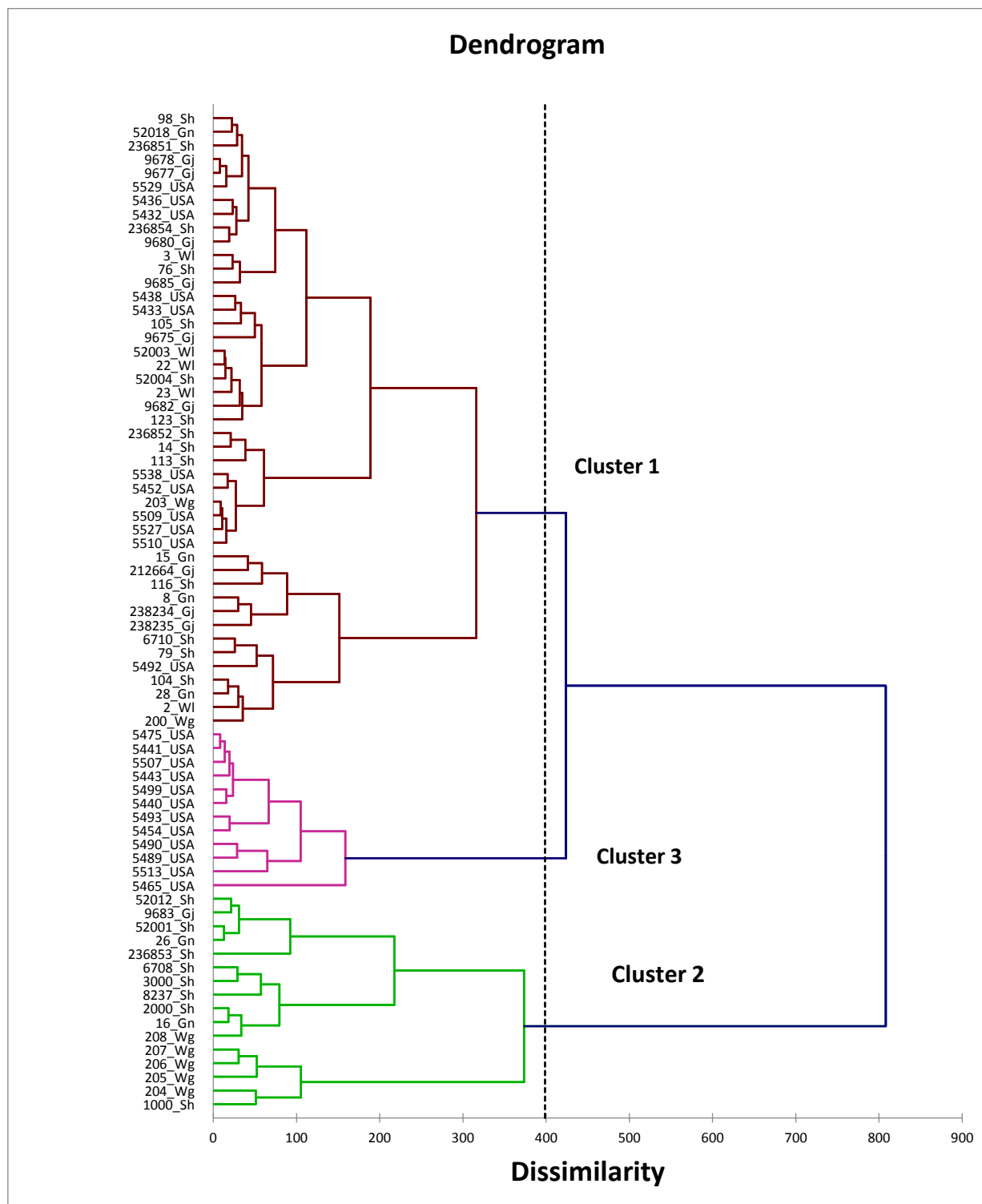


Figure 13. Dendrogram of AHC with Euclidean dissimilarity distance of 73 *A. sativa* accessions based on 12 quantitative traits using UPGMA by *xlstat*.

When a geographical pattern of phenotypic diversity, is considered accessions originating from all regions were found in either of the two clusters. Accessions from Gondar, Gojam, Shewa, Wello, and Welega were found in cluster 1 and cluster 2. However, accessions from the USA were found in clusters 1 and 3. In cluster 1 the USA accessions are mixed with accessions from other sources, whereas in cluster 3 all the accessions are from USA.

Table 31. Means of different traits in the three clusters for 73 *A. sativa* accessions by 12 quantitative characters

Variable/ traits	Cluster 1	Cluster 2	Cluster 3	μ	SD
Number of effective tiller/plant (count)	5.4	5.5	5.7	5.5	0.7
Number of node (count)	5.0	5.1	4.5	5.0	0.4
Internodes length (cm)	19.6	20.2	20.4	19.9	2.0
Flag leaf length (cm)	29.6	31.8	28.8	30.0	2.9
Length of panicle (cm)	31.1	32.8	27.5	30.9	3.4
Number of spikelet/ panicle	52.3	54.1	47.5	51.9	9.7
Plant height (cm)	149.1	150.2	136.7	147.3	12.3
Days to 50% heading (days)	115.3	115.3	113.0	114.9	2.6
Days to 90% maturity (days)	146.3	146.6	145.8	146.3	2.7
Biological yield per m ² (g)	2244.9	2959.3	1824.9	2332.4	403.4
Grain yield per m ² (g)	276.9	354.8	226.8	285.8	45.7
Harvest index	12.3%	12.0%	12.4%	12.3%	0.4%

Where μ : grand mean and C: Cluster

Key: The bold figure refers to a value greater than the grand mean. Cluster 1 showed relatively high mean value plant height, days to 50% heading, number of spikelet/ panicle, and length of panicle. Cluster 2 showed relatively better in all traits except two traits number of effective tiller per plant and harvest index. Cluster 3 exhibited better mean value in two of the quantitative traits number of effective tiller per plant and harvest index Table 20).

Distance between clusters of *A. sativa* accessions

Agglomerate hierarchical clustering of unweighted pair group arithmetic mean with Euclidean distance were analyzed, the cluster centroid distance between clusters ranged from 423.2 (between cluster 1 and cluster 3) to 1141.7 (between cluster 2 and Cluster 3). Cluster 1 and Cluster 2 exhibited a centroid

distance of 718.2. The variance within a cluster and among clusters was recorded 17.7 and 82.3%, respectively.

A. sativa accessions were grouped counter to their geographical proximity, for example, populations of Gondar and Shewa are found in the same cluster, but these two populations are not geographically closely related. Similarly, the Welega population was distantly related to its geographically proximate populations (Figure 15).

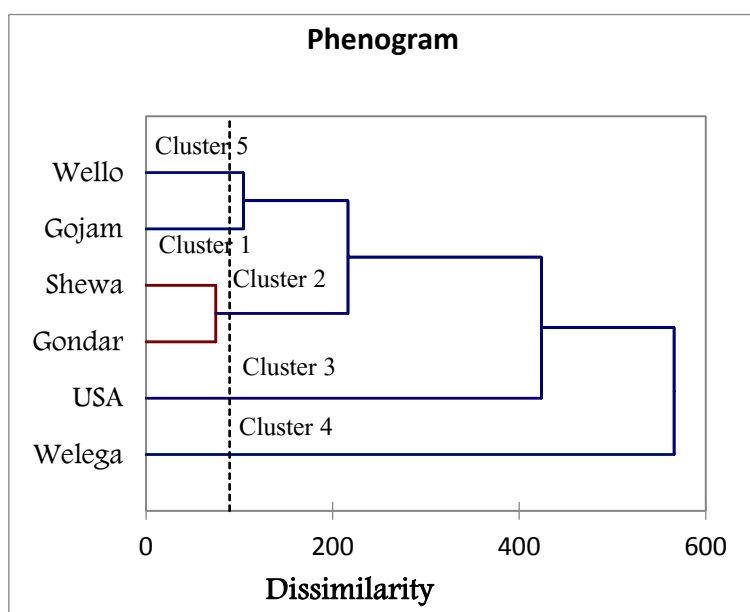


Figure 14. Phenogram of AHC with Euclidean dissimilarity distance of 73 *A. sativa* accessions based on 12 quantitative traits using UPGMA based on their geographical origin by *xlstat*..

5.1.4.3. Cluster analysis of *A. abyssinica* accessions

The accessions were grouped into three clusters of AHC with Euclidean distance of unweighted pair group mean average. The number of accessions in the cluster varied from 3 to 21 in clusters 3 and 2, respectively (Figure 16).

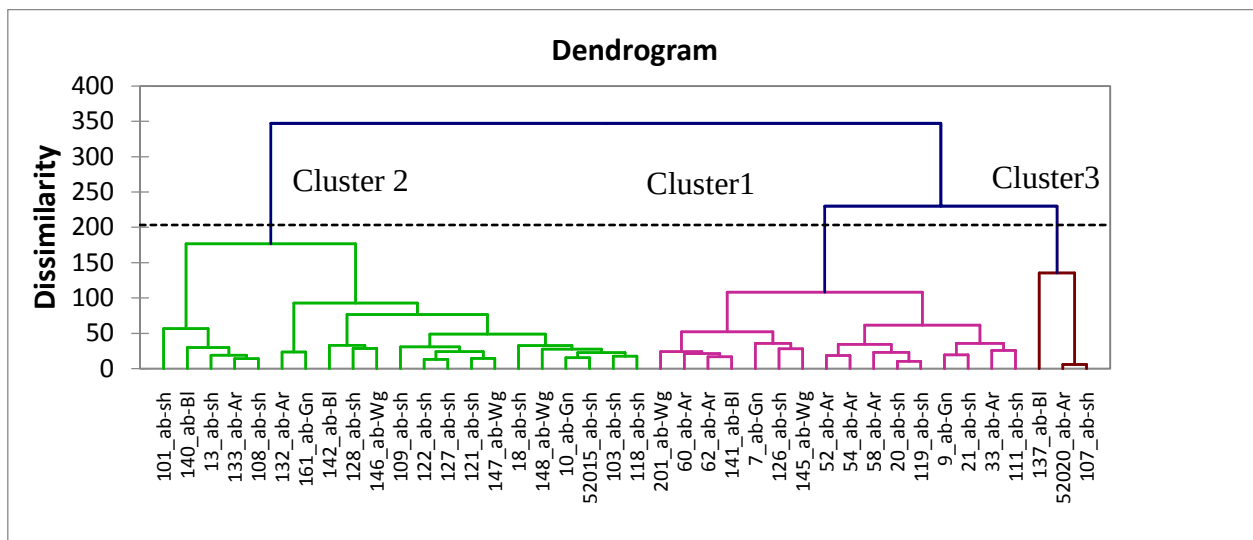


Figure 15. Dendrogram of AHC with Euclidean dissimilarity distance of 40 *A. abyssinica* accessions based on 12 quantitative traits using UPGMA by *xlstat*.

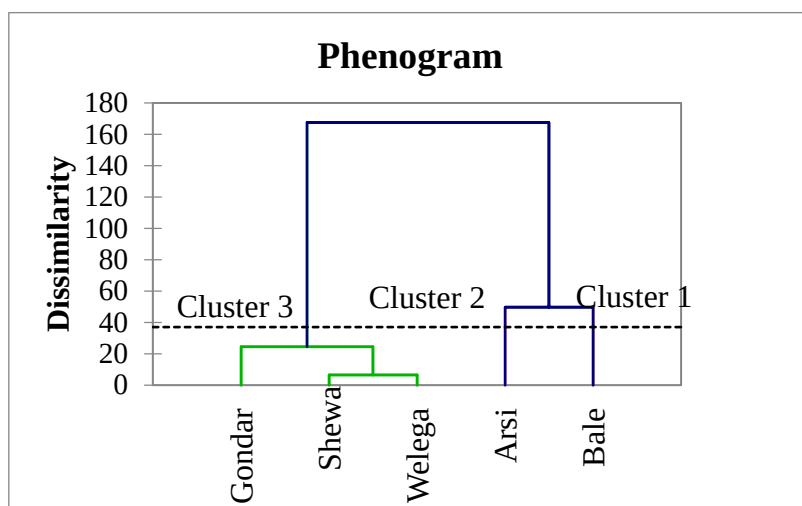
Cluster mean of 40 *A. abyssinica* accessions

Cluster 1 had low centroid mean value in the number of spikelet per panicle, biological yield, and grain yield (Table 32). In the above three traits, cluster 2, unlike cluster 1, had high grand centroid mean value. The remaining traits showed similar value in the two clusters. Even though the sample size was not equal, accessions from all source origin were grouped in all three clusters except Wello and Welega accessions, the former in clusters 2 and 3 and the latter in cluster 3 only.

The cluster of Euclidean distance calculated using unweighted pair group average mean, the variation within and among the clusters were 14.9% and 85.1% respectively. The centroid distance between clusters varied from 229.2 (between cluster 1 and 3) to 540.0 (between cluster 2 and 3). The centroid distance between cluster 1 and cluster 2 was 310.8. The population was grouped into three clusters. Cluster 1(Arsi) and cluster 3 (Bale) had only one population. Cluster 2 had three populations, namely, Gondar, Shewa, and Welega. Clustering of populations shown Figure 17 did not show on the origin of geographical proximity.

Table 32. Cluster means of the 12 quantitative traits in different clusters of *A. abyssinica* accessions

Variable	Cluster 1	Cluster 2	Cluster 3	μ	SD
Number of effective tiller/plant (count)	6.2	5.9	5.7	6.0	1.2
Number of node (count)	4.5	4.4	4.4	4.5	0.3
Internodes length (cm)	22.1	21.8	22.4	22.0	2.4
Flag leaf length (cm)	28.2	27.5	26.2	27.7	3.4
Length of panicle (cm)	31.5	31.4	29.7	31.3	3.1
Number of spikelet/ panicle	60.0	64.2	60.6	62.2	6.8
Plant height (cm)	143.3	142.4	138.3	142.4	8.9
Days to 50% heading (days)	111.4	109.5	109.4	110.2	3.1
Days to 90% maturity (days)	136.9	135.1	134.4	135.8	3.0
Biological yield per m2(g)	973.2	1281.3	745.4	1117.9	198.2
Grain yield per m2(g)	107.7	144.7	81.9	125.2	24.1
Harvest index	11.1%	11.2%	11.0%	11.2%	0.6%

**Figure 16.** Phenogram of AHC with Euclidean dissimilarity distance of *A. abyssinica* populations in relation to their source of origin based on 12 quantitative traits using UPGMA.

5.1.5 Principal component analysis

5.1.5.1 The PCA of wild versus cultivated *Avena* species

The first four principal components of the eigenvalue were almost equal to unity and accounted for 74.4% of the total variation estimated. PC1 had eigenvalue of 39.0% of the total variation estimated, followed by PC2 (16.7%), PC3 (11.1%) and PC4 (7.6%). The most important contributing traits for PC1 were number of days to 90% maturity, grain yield, days to 50% heading, biological yield, harvest

index, plant height, flag leaf length, number of nodes, and number of effective tiller per plant. The remaining eigenvalue had a little contribution and positive effect. All factors of PC1, except number of effective tiller, the number of spikelet per panicle and flag leaf length showed positive values.

Table 33. Eigen vectors and eigenvalues of the first four principal components for 176 oat accession with 12 quantitative traits

Variable	PC 1	PC 2	PC 3	PC 4
Eigenvalue	4.6819	2.0046	1.3321	0.9116
Proportion %	39.0%	16.7%	11.1%	7.6%
Cumulative %	39%	55.7%	66.8%	74.4%
Eigenvector				
	PC1	PC2	PC3	PC4
Number of effective tiller/plant (count)	-0.25	-0.109	0.088	0.151
Number of node (count)	0.268	-0.107	0.493	-0.196
Internodes length (cm)	-0.006	0.44	-0.315	0.631
Flag leaf length (cm)	0.315	0.256	-0.047	0.13
Length of panicle (cm)	0.19	0.372	-0.019	-0.465
Number of spikelet/ panicle	0.032	0.478	0.472	0.032
Plant height (cm)	0.343	0.335	0.034	-0.115
Days to 50% heading (days)	0.347	0.005	-0.409	-0.191
Days to 90% maturity (days)	0.389	-0.111	-0.359	-0.038
Biological yield per m ² (g)	0.372	-0.171	0.255	0.366
Grain yield per m ² (g)	0.379	-0.204	0.232	0.348
Harvest index	0.24	-0.394	-0.092	-0.029

For the second principal component, the most predominant characters were the number of spikelet per panicle, internode length, length of panicle, plant height, flag leaf length, grain yield, and harvest index. Eigenvectors for the number of spikelet per panicle, internode length, length of panicle, plant height, flag leaf length, and days to 50% heading exhibited positive values. The remaining traits revealed negative eigenvector values.

The third principal component explained 11.1% of the total variation, traits with better contribution being the number of nodes, number of spikelet per panicle, biological yield, grain yield, days to 50% heading, internode length, number of spikelet per panicle, number of tiller per plant, and days to 90%

maturity. Eigenvectors for effective tillers, internodes length, spikelet per panicle, plant height, biological yield, and grain yield were the major contributor for PC1.

Using the first two PCs we constructed a scatter plot (Figure 18). The PC1 and PC 2 account for 55.7% of the cumulative variance. The scatter plot displayed by PC 1 and PC 2 had clear groups. Group1 (green) contains *A. sativa*, group 2 (blue) contains *A. abyssinica*, group 3 (dark red) contains *A. fatua*, accessions, and group 4 (purple and brown) contains an admixture of *A. vaviloviana* and *A. sterilis* accessions.

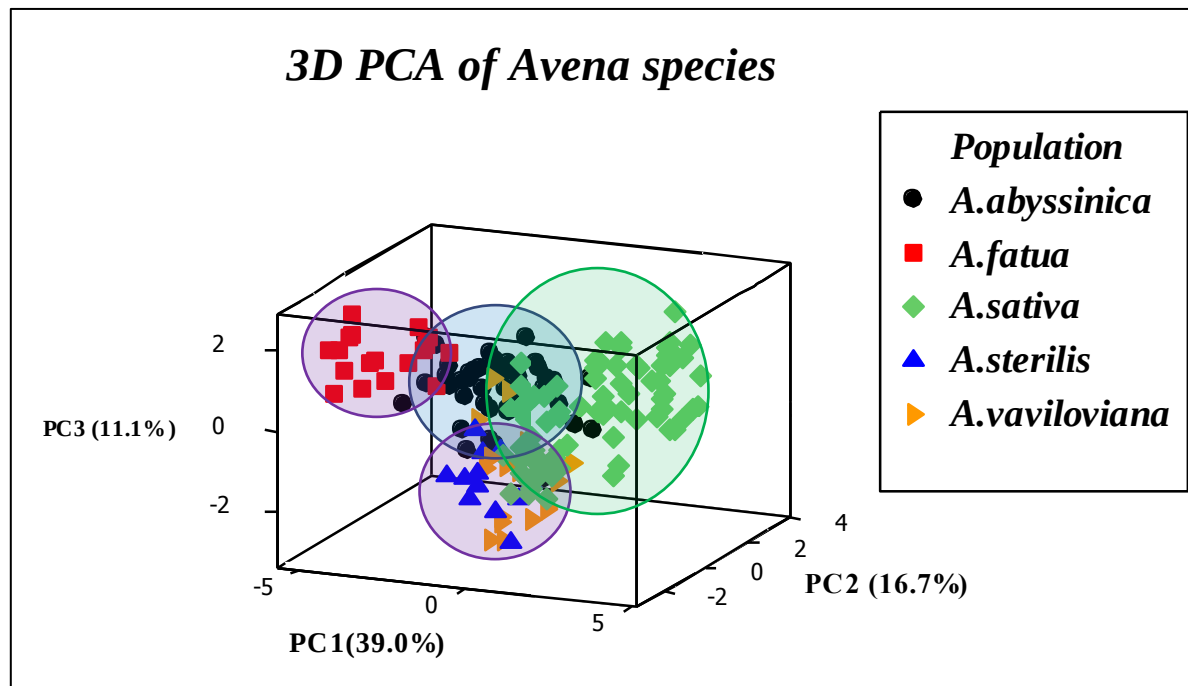


Figure 17. Score plot of the first two principal components of 176 accessions of oat based on species type using minitab17 with average Euclidean distance similarity with 12 morpho-Agronomic quantitative traits.

5.1.5.2. Principal component analysis of 73 *A. sativa* accessions

The first four principal components for *A. sativa* accessions alone, with eigenvalues greater than or equal to one, explained 75.7% of the total variation among accessions for the 12 quantitative traits

(Table 34). The relative magnitude of Eigenvectors from the first principal component (35.5%) indicated that length of panicle, flag leaf length, plant height, days to 90% maturity, days to 50% heading, biological yield, number of node, followed by number of spikelet's per panicle and internode length were the important contributing traits, whereas number of effective tiller had negative factor loading. From the second principal component, which contributed 15.5% of the total variation, the major contributing traits were, days to 90% maturity, days to 50% heading, number of effective tiller per plant and followed by biological yield and grain yield. Number of spikelet per panicle and number of node per plant showed negative eigenvalues in PC2.

Table 34. Eigenvectors and Eigen values of the first four principal components of *A. sativa* with 12 quantitative traits

Variable	PC1	PC2	PC3	PC4
Eigenvalue	4.2636	1.8553	1.5847	1.3836
% variance	35.5%	15.5%	13.2%	11.5%
% commutative	35.5%	51%	64.2%	75.7%
Eigenvector				
	PC1	PC2	PC3	PC4
No of effective tiller/plant (count)	0.239	0.424	0.177	-0.056
Number of node (count)	-0.258	0.145	-0.178	-0.538
Internodes length (cm)	-0.011	-0.172	-0.114	0.652
Flag leaf length (cm)	-0.362	-0.164	-0.062	0.334
Length of panicle (cm)	-0.394	-0.008	-0.12	0.001
Number of spikelet/ panicle	-0.389	-0.195	-0.095	-0.107
Plant height (cm)	-0.387	-0.163	-0.267	-0.164
Days to 50% heading (days)	-0.271	-0.117	0.578	-0.141
Days to 90% maturity (days)	-0.152	-0.168	0.691	0.001
Biological yield per m2(g)	-0.288	0.553	0.041	0.191
Grain yield per m2(g)	-0.265	0.569	0.019	0.145
Harvest index (%)	0.197	-0.074	-0.109	-0.234

The third principal component accounted for 13.2% of the total variation and factors mainly connected to the traits were grain yield, biological yield, and internode length. Number of node, number of days to 50% heading and number of days to 90% maturity showed negative eigenvalues. The fourth principal

component, contributed 11.5% of the total variation, and the major contributing traits were internode length, biological yield, grain yield and flag leaf length.

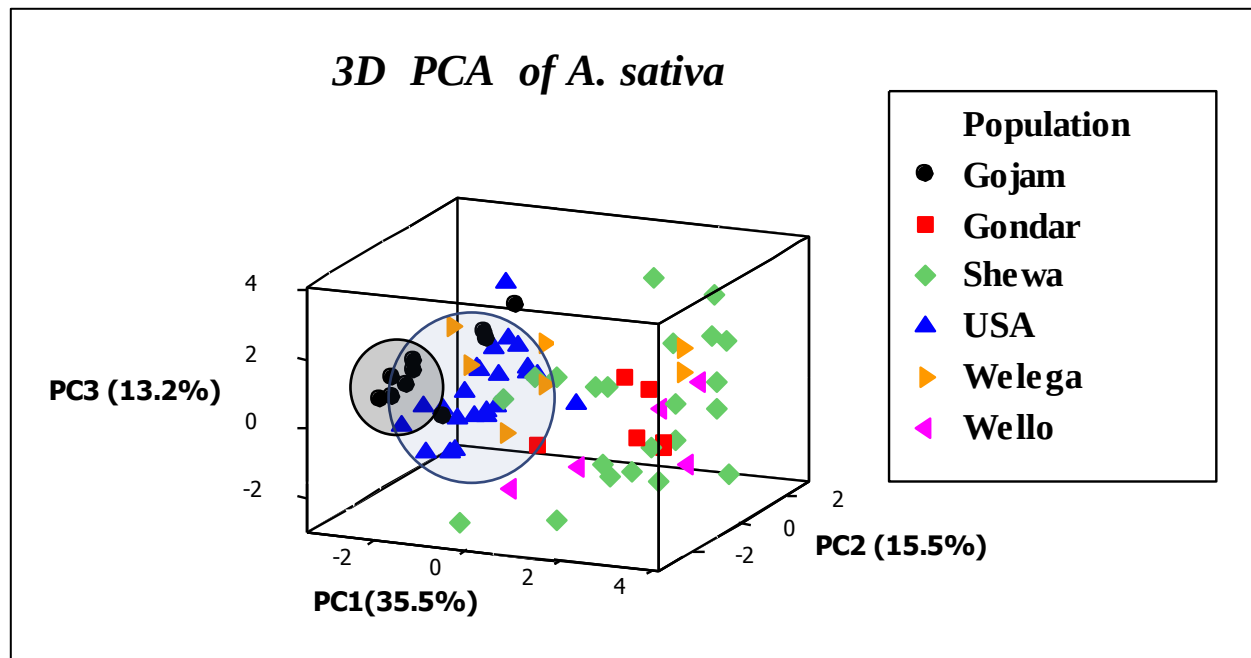


Figure 18. Score plot of the first two principal components of 73 *A. sativa* accessions using average Euclidian distance similarity on 12 morpho-agronomic quantitative traits based on their geographical origin by Minitab 14.

The score plot revealed three groups, the black (accessions from Gojam), the blue (accessions from USA), and the rest of the color represent all the rest of the accessions from different geographical origin.

5.1.5.3. Principal component analysis of 40 *A. abyssinica* accessions

The six populations of *A. abyssinica* showed non-clear grouping. The populations showed admixture of accessions from different regions.

Table 35. Eigen vectors and eigenvalues of the first five principal components of *A. abyssinica* with 12 quantitative traits.

Variables	PC1	PC2	PC3	PC4	PC5
Eigenvalue	2.8679	2.1858	1.5683	1.3413	0.9929
% variance	23.8%	18.3%	13.1%	11.2%	8.3%
% commutative	23.8%	42.1%	55.2%	66.4%	74.6%
	Eigenvector				
	PC1	PC2	PC3	PC4	PC5
No of effective tiller/plant (count)	0.029	0.097	0.352	0.613	0.292
Number of node (count)	0.204	0.164	-0.178	0.407	-0.223
Internodes length (cm)	0.357	0.09	0.208	-0.288	0.543
Flag leaf length (cm)	0.335	0.143	0.288	-0.03	0.022
Length of panicle (cm)	0.044	0.187	0.436	-0.019	-0.676
Number of spikelet/ panicle	0.021	0.266	-0.448	-0.07	-0.214
Plant height (cm)	0.388	0.263	0.322	-0.176	-0.148
Days to 50% heading (days)	0.433	0.068	-0.361	0.176	0.011
Days to 90% maturity (days)	0.483	0.076	-0.274	0.146	0.001
Biological yield per m2(g)	-0.178	0.578	-0.076	-0.224	0.114
Grain yield per m2(g)	-0.232	0.601	-0.071	-0.056	0.149
Harvest index (%)	-0.241	0.233	0.086	0.483	0.098

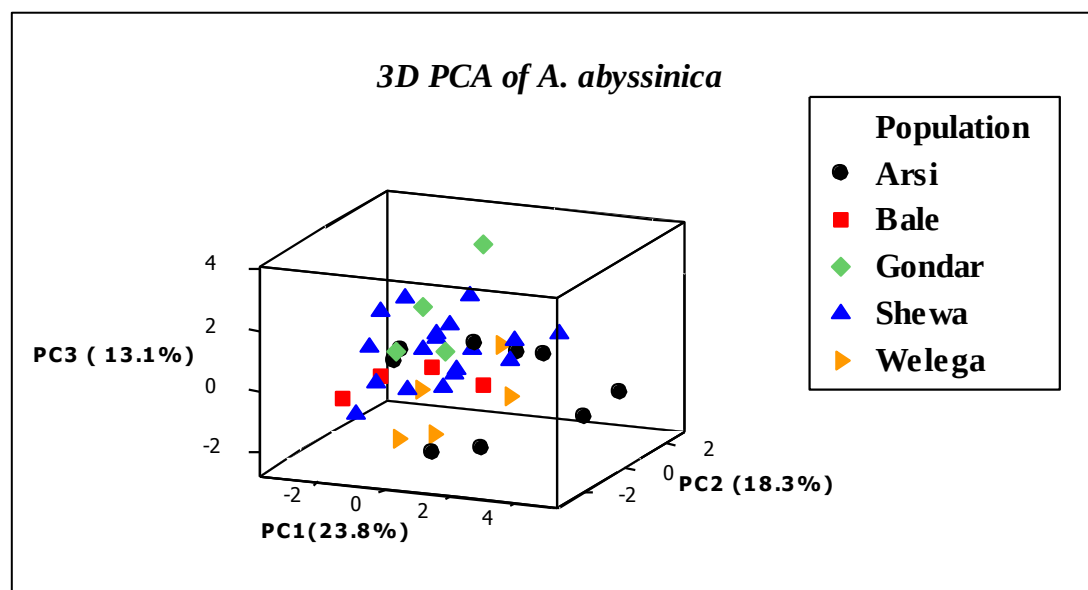


Figure 19. Score plot of the first two principal components of 40 accessions of *A. abyssinica* with 12 morpho-agronomic quantitative traits using average Euclidean distance similarity by Minitab 14.

5.1.6 Agronomic performance of *A. sativa*

Differences among the genotypes were significant for several characters (Table 36). The comparison of the different genotypes with the average of *A. sativa* accessions showed the superior performances of several Ethiopian origins for many agronomic attributes. The best top 5% performing accessions for grain yield include Acc. No. 1000, 204, 2000, 16, 205, 206, 208, 207, and 3000.

But comparisons at the levels (with average *A. sativa* accessions performances) showed there were no exotic accessions comparable to the average oat genotypes for the number of node per plant and number of spikelet per panicle grain yield, and biological yield (Table 36).

Although there was no single accession exhibiting consistent superiority for grain yield and other desirable characters, the Ethiopian accessions were among the top 5% best performers compared to the exotic accessions for many attributes (Table 36). This was exemplified by Acc. No. 1000 that displayed the best character in grain yield and biomass yield. Another accession, Acc. No. 207 was also among the top performers for the number of nodes per plant, number of spikelets per panicle, grain yield, biological yield, and harvest index. Several other superior accessions for multiple traits of agronomic significance including grain yield were also identified (Table 36).

5.1.6.1 Comparison of selected accessions with the original population

The Z-test revealed significant differences among means of the selected top 5% best genotypes ($\bar{x}$) and the population parameters (μ) for all traits at the phenotypic level. Comparison of the average performance for all characters of the selected subsets of the top 5% best accessions with the average performances of the whole population of *A. sativa* for agronomic characters revealed possibilities for the different level of improvements through selection, ranging from 2% for harvest index to 34% for grain yield. Biological yield can also be improved by 29 % (Table 36).

Performance evaluation at phenotypic level is the effect of environment, genotype and the interaction of genotype by the environment (Gemechu Keneni and Musa Jarso, 2009). As a result superior accessions may not be found across all environments. To be certain about the consistency of the superiority of the accessions, a plant breeder should have to analyze the genotype by environment interaction over a range of environments (Gemechu Keneni and Musa Jarso, 2009). The plant breeder should measure performance stability across a wide range of environments on the primary trait like grain yield, to its average performance over environments.

Table 36. Accessions for response of 12 quantitative traits with the average performances of the whole population (μ) of 73 *A. sativa* accessions

Characters	Population parameter (μ)	Mean of selected accessions ($\bar{x}$)	Change through selection $ (\mu - \bar{x}) $	Change as % of population Parameter (μ)	Z
No of effective tiller/plant (count)	5.4	6.6	1.2	22%	2.9*
Number of node (count)	5	5.6	0.6	12%	1.6*
Internodes length (cm)	19.8	23.5	3.7	19%	2.5*
Flag leaf length (cm)	30.1	34.7	4.6	15%	2.0*
Length of panicle (cm)	30.9	36.1	5.2	17%	2.2*
Number of spikelet/ panicle	52.4	68.2	15.8	30%	4.0*
Plant height (cm)	147.5	166	18.5	13%	1.7*
Days to 50% heading (days)	114.8	119.2	4.4	4%	0.5*
Days to 90% maturity (days)	146	150.3	4.3	3%	0.4*
Biological yield per m ² (g)	2309.8	3101.2	791.4	34%	4.5*
Grain yield per m ² (g)	283.5	366.4	82.9	29%	3.9*
Harvest index (%)	12%	12.9%	0.007	7%	0.7*

* = significant ($P < 0.05$)

5.1.6.2. Effect of location

The two locations showed significant differences between cultivated and wild oat accessions for several agronomic characters (Table 37). The overall mean of some of the traits in the two locations had significant difference, these are number of nodes, internode length, days to 50% heading and days to 90% maturity, whereas, the remaining traits showed non-significant difference.

Grain yield, biological yield, number of nodes, and number of spikelet per panicle were higher at Holetta than Debre Berhan. However, the number of days for flowering and for maturity were high at Debre Berhan. Grain yield and biological yield showed non-significance difference at Holetta and Debre Berhan sites.

Table 37. Mean performances of 176 oat accessions for agronomic performance at two locations in Ethiopia

L	type	specie	Traits											
			NET	NN	IL	FLL	LP	NSP	PH	DHE	DM	BY	GY	HI
Debre Berhan	WILD	<i>A. abyssinica</i>	5.8	4.6	20.3	28.8	31.3	52.2	142.9	114.1	142.7	1923.7	262.5	0.1
		<i>A. fatua</i>	5.8	4.4	16	26.6	27.2	50	112.4	111.6	133.8	1732.1	212.8	0.1
		<i>A. sterilis</i>	5.8	5.4	18.9	30.3	28.1	51.7	144.5	114.3	140.7	1732.5	221.7	0.1
		<i>A. vaviloviana</i>	5.5	4.7	20.4	26.4	31.7	48	143.9	118.6	146.8	1636.6	222.7	0.1
	CULT'D	<i>A. sativa</i>	5.3	4.8	20.4	30.4	31.3	50.9	147.7	116.3	148.5	2269.4	276.1	0.122
	Total		5.7	4.7	21.6	28.4	30.7	51.3	139.9	114.1	142.6	2039.7	267.5	0.131
Holetta	WILD	<i>A. abyssinica</i>	6.1	5.1	19.3	26.8	31	53.7	137.8	113.3	141.2	2191.7	260.6	0.1
		<i>A. fatua</i>	5.6	4.8	19.9	29.3	31.2	55.6	139.3	109.8	138.9	2277.6	287.7	0.1
		<i>A. sterilis</i>	5.5	4.8	20.6	28.7	29	50.9	136.7	109.5	138.7	2255.6	302.1	0.1
		<i>A. vaviloviana</i>	5.8	4.7	20.4	28.3	31.1	53.4	140.3	112.5	141	2150	315.9	0.2
	CULT'D	<i>A. sativa</i>	5.5	5.2	19.2	29.8	30.6	53.9	147.2	113.2	143.3	2358.8	292.1	0.124
	Total		5.9	5	19.4	28	30.5	54.7	139.7	111.7	139.3	2208.3	287	0.13
Combined	WILD	<i>A. abyssinica</i>	6.0	4.9	19.8	27.8	31.2	53.0	140.4	113.7	142.0	2057.7	261.6	0.1
		<i>A. fatua</i>	5.7	4.6	18.0	28.0	29.2	52.8	125.9	110.7	136.4	2004.9	250.3	0.1
		<i>A. sterilis</i>	5.7	5.1	19.8	29.5	28.6	51.3	140.6	111.9	139.7	1994.1	261.9	0.1
		<i>A. vaviloviana</i>	5.7	4.7	20.4	27.4	31.4	50.7	142.1	115.6	143.9	1893.3	269.3	0.2
	CULT'D	<i>A. sativa</i>	5.4	5.0	19.8	30.1	31.0	52.4	147.5	114.8	145.9	2314.1	284.1	0.1
	Total		5.8	4.9	20.5	28.2	30.6	53.0	139.8	112.9	141.0	2124.0	277.3	0.1
LSD			0.6	0.3	1.4	2.2	2.5	5.9	5.9	3.4	2.3	377.5	23.8	0.01

Where, L location, μ : grand total, and LSD: least significance difference

5.2 SSR marker analysis

5.2.1 Molecular genetic variation

All selected markers showed greater than 0.5 PIC values ranging from 0.59 - 0.98 believed to be informative markers to study variability. The highest PIC value was recorded at locus AM31 and the lowest PIC value was recorded at locus AM21. The expected size (bp) of the loci ranged from 115 - 183bp to 254-297bp. The expected size ranges from 115bp in AM4 to the maximum 297 in AM22, both have a dinucleotide motif. The majority of core SSR sequence repeat motifs used in this study are dinucleotide, and trinucleotides, except CWM204 ((AT)₅(AC)₅(AC)₅, AM21 ((ATGT)₆) and AM102 ((AC)₃(AG)₄) repeated motif (Table 38). The major allele frequency value varied from 0.0591 in AM31 to 0.59 in AM23.

Table 38. The 19 SSR markers used in this study

No.	Marker	F - Primer 5' to 3' R - Primer 5' to 3'	Motif	F - Primer
1	AM3	CTGGTCATCCTCGCCGTTCA CATTTAGCCAGGTTGCCAGGTC	(AG)35	NED
2	AM4	GGTAAGGTTTTCGAAGAGCAAAG GGGCTATATCCATCCCTCAC	(AG)34	NED
3	AM6	AATGAAGAAACGGGTGAGGAAGTG CCAGCCCAGTAGTTAGCCCATCT	(AG)20	6-FAM
4	AM7	GTGAGCGCCGAATACATA TTGGCTAGCTGCTTGAAACT	(AG)21	PET
5	AM14	GTGGTGGGCACGGTATCA TTGGCTAGCTGCTTGAAACT	(AC)21	6-FAM
6	AM15	GTGACCGTAAACGATAACAAC AAGCAAGACGCGAGAGTAGG	(AC)14	VIC
7	AM21	ACGTTGGTCTCGGGTTGG TTGGCTAGCTGCTTGAAACT	(AT)5(AC)5(AC)5	NED
8	AM22	ATTGTATTTGTAGCCCCAGTTC AAGAGCGACCCAGTTGTATG	(AC)22	PET
9	AM23	TCTTTAAGGATTGGGTGGAG AAGAGCGACCCAGTTGTATG	(AC)19	6-FAM
10	AM30	TGAAGATAGCCATGAGGAAC GTGCAAATTGAGTTTCACG	(GAA)14	VIC
11	AM31	GCAAAGGCCATATGGTGAGAA CATAGGTTTGCCATTCGTGGT	(GAA)23	NED
12	AM42	GCTTCCCGCAAATCATCAT CATAGGTTTGCCATTCGTGGT	(GAA)16	6-FAM
13	AM53	TCGCCATTAATAAGAGGGAAGG GCTGCTGTTGGGTGGTTAGTG	(AC)10	VIC
14	AM87	GAGCAAGCTCTGGATGGAAG CCCGTTTATGTGGTTGTTAGC	(AC)13	6-FAM
15	AM89	GGCGGTTGGAGAGTGTCT AGGTGAAGGCGAGTGGAAG	(AC)16	VIC
16	AM102	TGGTCAGCAAGCATCACAAT TGTGCATGCATCTGTGCTTA	(AC)3(AG)4	PET
17	CWM48	GATCGGCGACTTCCTCCCTCAT ACCCCGCTCTTTCCCAATAAT	(ATG)7	NED
18	CWM204	GCTACAAACCAGTCAGCAA AACCGACCCTCCTCCTTC	(ATGT)6	VIC
19	HVM _w G	TCCAATGGCATCTACAGGACGGCAA GCAGGTTGAGCTGCGCAAAGTCGTCG	(AT)9	PET

Table 39. Summary of evaluation of 19 SSR markers, major allele frequency, length of fragments, annealing temperature (Ta), in major allele frequency, number of alleles, gene diversity (GD), and polymorphism information content (PIC) on oat accessions

No.	SSR Marker	Length	Ta (°C)	Major Allele. Freq	No Allele	G D	PIC
1	AM3	132-203	55	25.8%	45	88.1%	87.3%
2	AM4	115-181	58	9.7%	70	95.8%	95.7%
3	AM6	175-203	58	34.4%	22	76.2%	73.0%
4	AM7	150-200	58	17.7%	81	95.2%	95.0%
5	AM14	149-209	65	26.3%	36	87.2%	86.2%
6	AM15	209-224	55	29.6%	41	86.9%	86.0%
7	AM21	183-214	55	38.7%	23	75.1%	71.9%
8	AM22	254-297	65	24.7%	95	93.0%	92.8%
9	AM23	133-166	65	52.7%	12	63.7%	58.8%
10	AM30	178-231	55	16.1%	53	94.1%	93.8%
11	AM31	152-210	59	5.9%	122	98.5%	98.4%
12	AM42	158-207	57	16.1%	63	94.6%	94.4%
13	AM53	251-259	59	37.6%	11	73.1%	68.7%
14	AM87	148-166	64	30.1%	23	84.2%	82.7%
15	AM89	195-267	64	18.8%	74	94.8%	94.6%
16	AM102	121-181	64	21.0%	51	92.5%	92.1%
17	CWM48	148-225	65	11.8%	118	97.3%	97.2%
18	CWM204	155-225	54	24.2%	23	87.9%	86.9%
19	HVMwG	142--214	57	15.6%	90	95.1%	94.9%

5.2.1.1. SSR markers characterization and levels of genetic diversity of five *Avena* species

A total of 317 alleles were detected from 19 primers and the average allele per locus is listed in Table 29. The majority of primer pairs were highly informative, except AM23 (58.8%) and AM53 (68.7%). All 19 loci assayed exhibited a high level of polymorphism, with the number of alleles per locus ranging from 11 for AM53 to 122 for AM31. The gene distance (heterozygosity) ranged from 63.7% for marker AM23 to 98.5% for marker AM31.

5.2.1.2. Regional variation of SSR markers for five *Avena* species

In this study, the percentage of polymorphic loci indicated that the Shewa region had the highest percentage of polymorphic loci (82.97%), while the lowest was observed in Netherland and Australian materials (25.24%). Other populations had almost nearly equal percentage of polymorphic loci. This revealed that the observed and expected heterozygosity, allelic range, and number of gene copies were similar in the material originated from eight-geographical regions. Low values were recorded for Netherlands and Australia materials (Figure 20).

As compared to that of other regions, the Netherland and Australian materials, showed the lowest number of the band (87) over all loci while Shewa had the highest number of bands (263). Private bands have been recorded for populations from Shewa 4, USA 5, Arsi 1, Gondar 1 and Welega 1 (Figure 20).

Band patterns of the populations of five *Avena* species: The band pattern is almost uniform in all the populations (Figure 20). The number of bands were very high in materials from Arsi, Shewa, and Gondar populations. A number of bands, with frequencies greater or equal to five, is the same number of bands in the region of Bale, Gojam, Gondar Welega, and Wello. A number of bands having the frequency of less than or equal to 50% are high in the region of Arsi, Shewa, Gondar, and USA (Figure 20). USA and Shewa have highest number of private alleles and the Arsi, Gojam, Gondar, and Welega regions have a single private allele.

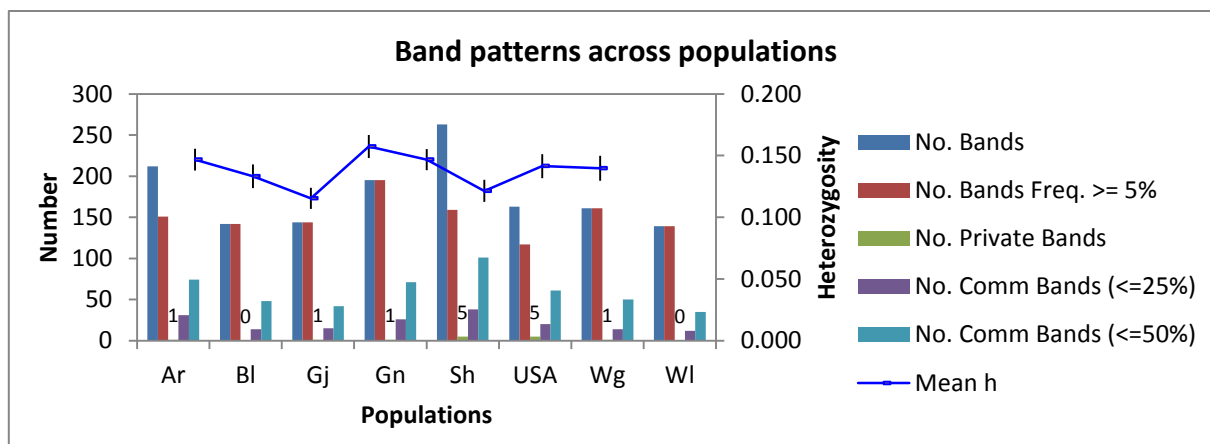


Figure 20. Patterns of allelic variation observed in population of 5 *Avena* species.

Where, Ar= Arsi, Bl= Bale, Gj= Gojam, Sh= Shewa, USA= United States of America, Wg=Welega, Wl= Wello, and h= heterozygous alleles

Band patterns of the population of *A. sativa*: The band pattern is almost uniform in all populations, except Shewa and USA populations. These two populations (Shewa and USA) contain the highest number of alleles, but some alleles in these populations had frequencies less or equal to 5%. Gondar, Gojam, Welega, and Wello regions had alleles where frequencies are greater or equal to 5%. All the populations contain private bands, but Shewa had the highest and followed by the USA and then Wello (Figure 21).

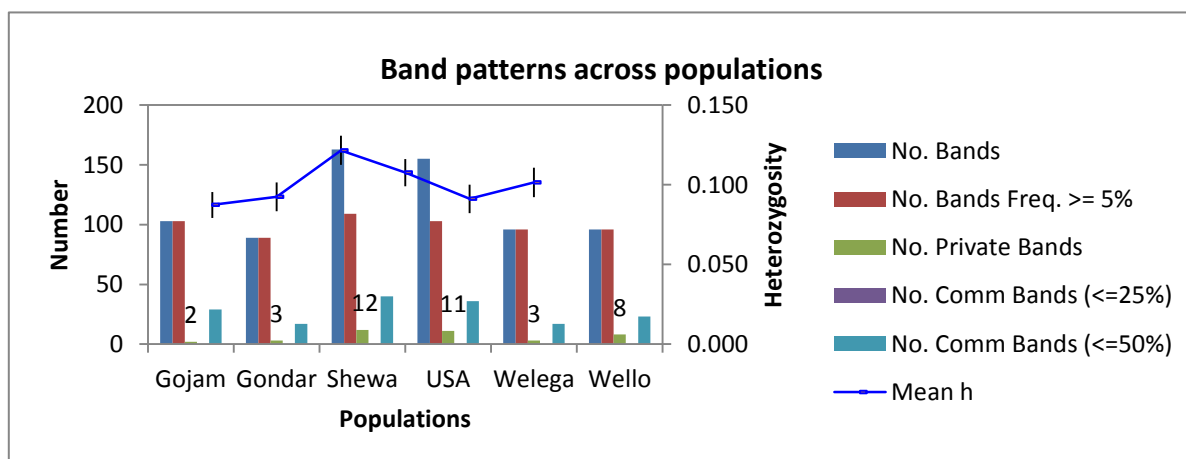


Figure 21. Patterns of allelic variation observed in *A. sativa* populations.

Where, h= heterozygous alleles

Band patterns of the population of *A. abyssinica*: The band pattern is almost uniform in all the populations, and the frequency of the bands greater or equal to 5% were observed in all regions. The highest private allele frequency was observed in Shewa region, followed by Arsi, and then Gondar and Welega (Table 22).

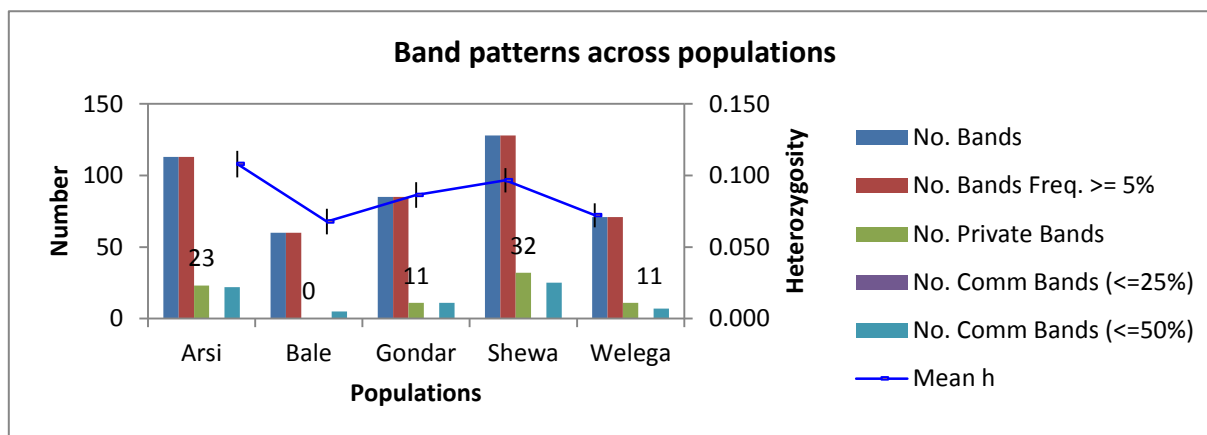


Figure 22. Patterns of allelic variation observed in *A. abyssinica* populations by regions.

5.2.2 Analysis of molecular variance (AMOVA) and genetic distances

5.2.2.1 Analysis of molecular variance (AMOVA)

AMOVA can be used to characterize the diversity of populations in terms of variances regardless of the marker type. The analysis of molecular variation (AMOVA) revealed that 8% of the variation resulted from the difference among populations and 92% of the variation from within populations (Table 40). This implies there is high genetic diversity within regions/populations than among the regions/populations. Nei (1978), developed a variance-based technique analysis of molecular variance (AMOVA) to calculate analogous indices to F_{ST} , which they called Φ_{PT} .

The overall population differentiation among regions were significantly ($P < 0.001$) with F_{ST} value of 0.08, which indicated moderate differentiation. The contribution of seed dispersal to overall gene flow

can be estimated by comparing levels of inter-regional differentiation (i.e., F_{ST}) and the gene flow among these populations was estimated to be 2.875 (Table 40).

AMOVA of *Avena* species revealed that 33% of variation resulted among *Avena* species, 67% variation within species and F_{ST} was 0.325, and gene flow was 0.519 (Table 40).

Table 40. AMOVA showing the distribution of genetic diversity within and among species from different sources of origins

Source	Df	SS	MS	Est. Var.	%	F_{ST}	p-value
Among species	4	1182.5	295.62	9.046	33%	0.325	0.001
Within species	171	3156.8	18.461	18.461	67%		
Total	175	4339.3		27.507	100%		
Gene flow: $Nm = \frac{1}{4F_{ST}} - \frac{1}{4} = 0.519$							

Where, df = Degrees of freedom; SS = Sum of squares; MS = Mean Square; Est. Var = Estimated Variability and F_{ST} : the amount of genetic variation that is due to population difference

Similarly, AMOVA of wild versus cultivated *Avena* species revealed 27% of variation 73% variation from within wild *Avena* species and cultivated *Avena* species. The F_{ST} was 0.265, with a gene flow between cultivated and wild oat of 0.943 (Table 41).

Table 41. AMOVA showing the distribution of genetic diversity within and among cultivated versus wild oat species

Source	df	SS	MS	Est. Var.	%	F_{ST}	p-value
Among wild and cultivated	1	685.010	685.010	7.581	27%	0.265	0.001
Within wild and cultivated	174	3654.280	21.002	21.002	73%		
Total	175	4339.290		28.582	100%		
Gene flow: $Nm = \frac{1}{4F_{ST}} - \frac{1}{4} = 0.693$							

Where, df: degree of freedom, SS: sum of the square, MS: mean of the square, Est.Var: estimated variance, and F_{ST} : the amount of genetic variation that is due to population difference

AMOVA of *A. sativa* accessions revealed about 5% of the variation to be among the populations, 95% of variation to be within populations. The F_{ST} was 0.051 with a gene flow between *A. sativa* populations of 4.65 (Table 42).

Table 42. AMOVA showing the distribution of genetic diversity within and among *A. sativa* populations

Source	df	SS	MS	Est. Var.	%	F_{ST}	p- value
Among Populations	5	146.06	29.21	0.97	5%	0.051	0.001
Within Populations	67	1224.22	18.27	18.27	95%		
Total	72	1370.27		19.24	100%		
Gene flow: $Nm = \frac{1}{4F_{ST}} - \frac{1}{4} = 4.651$							

Where, df: degree of freedom, SS: sum of the square, MS: mean of the square, Est.Var: estimated variance, and F_{ST} : the amount of genetic variation that is due to population difference

AMOVA of *A. abyssinica* accessions revealed about 3% of variation to be among the populations, 97% of the variation within populations. The F_{ST} was 0.034 with a gene flow between *A. abyssinica* populations of 7.102 (Table 43).

Table 43. AMOVA showing the distribution of genetic diversity within and among *A. abyssinica* populations

Source	df	SS	MS	Est. Var.	%	F_{ST}	p- value
Among Populations	4	83.64	20.91	0.58	3%	0.034	0.018
Within Populations	35	583.19	16.66	16.66	97%		
Total	39	666.83		17.25	100%		
Gene flow: $Nm = \frac{1}{4F_{ST}} - \frac{1}{4} = 7.102$							

Where, df: degree of freedom, SS: sum of the square, MS: mean of the square, Est.Var: estimated variance, and F_{ST} : the amount of genetic variation that is due to population difference

5.2.2.2 Genetic distances

Average unbiased genetic distances among the accessions were estimated using Nei unbiased genetic distance as presented in Table 44. According to the results in Table 44, the genetic distances recorded ranged from 0.000 (between accessions in region of Wello and Shewa, Wello and Gondar, and between Wello and Welega) to 0.047 (between accessions from Bale and USA). Noticeably, the USA population showed comparatively high genetic distance from all the rest of the populations (Table 44).

Table 44. Average Nei's unbiased genetic distance calculated among 176 accessions from different geographical origin

	Arsi	Bale	Gojam	Gondar	Shewa	USA	Welega
Bale	0.001	0.000					
Gojam	0.023	0.028	0.000				
Gondar	0.001	0.006	0.016	0.000			
Shewa	0.007	0.009	0.018	0.002	0.000		
USA	0.039	0.047	0.019	0.028	0.034	0.000	
Welega	0.010	0.013	0.013	0.001	0.003	0.015	0.000
Wello	0.009	0.015	0.013	0.000	0.000	0.023	0.000

Key: Bold values indicate relatively high genetic distance between regions

Pairwise genetic distance of *A. sativa* population: The pairwise genetic distance of *A. sativa* populations and *A. abyssinica* populations had similar result. Gojam population showed relatively high unbiased pairwise genetic distance to the other populations, except Wello population (Table 45).

Table 45. Average Nei's unbiased genetic distance calculated among *A. sativa* populations

	Gojam	Gondar	Shewa	USA	Welega
Gondar	0.019	0.000			
Shewa	0.017	0.011	0.000		
USA	0.013	0.000	0.005	0.000	
Welega	0.016	0.000	0.009	0.000	0.000
Wello	0.000	0.000	0.000	0.000	0.000

Pairwise genetic distance of *A. abyssinica* population: The minimum and maximum pairwise unbiased Nei genetic distance ranged from 0.014 (between Shewa and Arsi) to 0.033 (between Arsi and Welega). Welega populations had relatively high unbiased pairwise gene distance from all other populations (Table 46). In *A. abyssinica*, populations had relatively, lower pairwise genetic distance.

Table 46. Average Nei's unbiased genetic distance calculated among *A. abyssinica* populations

	Arsi	Bale	Gondar	Shewa
Bale	0.029	0.000		
Gondar	0.027	0.025	0.000	
Shewa	0.014	0.018	0.018	0.000
Welega	0.033	0.021	0.022	0.019

Key: Bolded values indicate relatively high genetic distance values observed in the study.

5.2.3 Cluster analysis

5.2.3.1. Cluster analysis of five *Avena* species

Based on the geographic location, species cluster was constructed as illustrated in Figure 24. Overall, Ethiopian accessions that came from the different locations were distributed over three clusters whereas the USA accessions were clustered in cluster III only. The Pairwise Nei's unbiased genetic distances between geographical origins of oat were assessed as shown in Table 47. Based on the geographical origin of accessions, the Arsi population is closely related to accessions of all the regions, except that of Gojam and the USA. The genetic distance between regions ranged from 0.012 (between Arsi and Gondar and between Shewa and Wello) to 0.073 (between Bale and the USA). Considering the geographical source of origin, the population from Shewa revealed lower gene distance to populations from all the rest of the regions, whereas, the population of the USA showed high genetic distance, especially to Gondar and Gojam populations.

In the three clusters shown (I, II, and III), *A. fatua* and *A. abyssinica* accessions were grouped in cluster I, whereas, cluster II had two species *A. sterilis* and *A. vaviloviana*, but, cluster III contained only *A. sativa*.

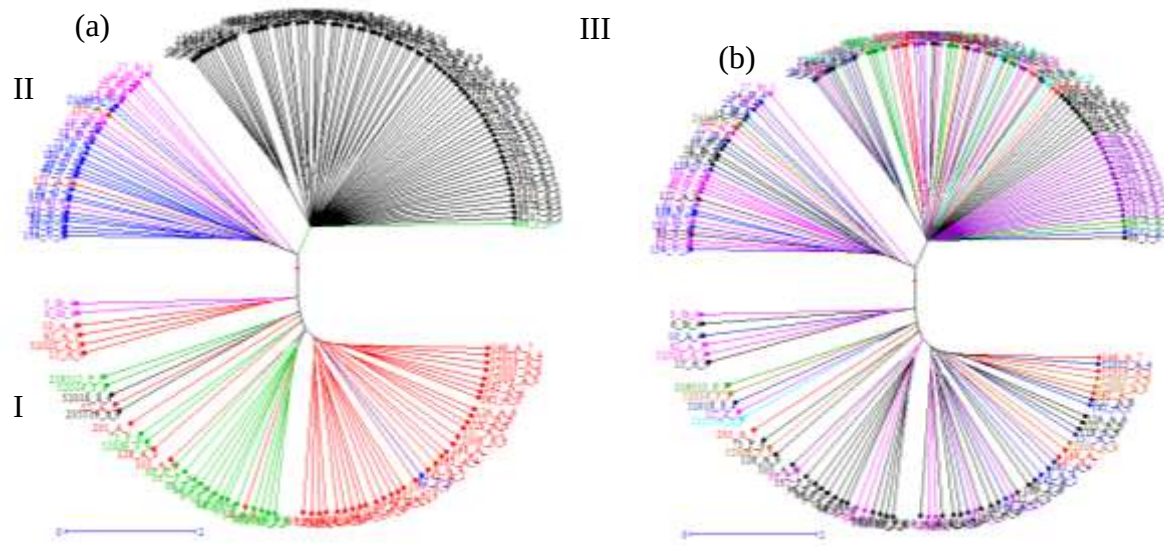


Figure 23. Cluster of oat accessions using Darwin v 6.5 program.

Where, (a) cluster of 5. *Avena* species based on species type shows three clear clusters; cluster I contains *A. vaviloviana* (pink color) and *A. sterilis* (blue color) and cluster II contains *A. fatua* (green color) and *A. abyssinica* accessions (red color); and cluster III contains only *A. sativa* accessions (b) cluster of 5. *Avena* specie based on their geographical origin. Eight of the populations (regions) with different colors had clear pattern of groupings.

The minimum and maximum pairwise unbiased Nei is genetic distance ranged from 0.013 (between *A. abyssinica* and *A. fatua*) to 0.108 (between *A. abyssinica* and *A. sativa*). *A. abyssinica* accessions had moderately pairwise gene distance from *A. sterilis* and *A. vaviloviana*. Similarly, *A. fatua* had high pairwise gene distance from *A. sativa* and *A. sterilis*. *A. sativa* had moderate pairwise genetic distance from *A. sterilis* and *A. vaviloviana*, but *A. sativa* had high pairwise genetic distance from *A. fatua*, and *A. abyssinica*.

Table 47. Pairwise species unbiased Nei genetic distance values

<i>Avena species</i>	<i>A. abyssinica</i>	<i>A. fatua</i>	<i>A. sativa</i>	<i>A. sterilis</i>
<i>A. fatua</i>	0.013	0.000		
<i>A. sativa</i>	0.108	0.104	0.000	
<i>A. sterilis</i>	0.094	0.102	0.076	0.000
<i>A. vaviloviana</i>	0.084	0.095	0.064	0.027

5.2.3 Principal Coordinate Analysis (PCoA) for five *Avena* species

The principal coordinate analysis (PCoA) was performed to understand the structure and the diversity of oat accessions (Figure 25). The first three principal components explained 46.13% of SSR variation (30.95, 10.79, and 4.39 %, respectively). The first axis, which accounted for 30.95% of the variance, separated the accessions assigned to the first and second clusters by Minitab 17. The second axis, accounted for 10.79% of the variance, grouped the landraces from the third cluster into another, the most diverse group. Results obtained by these two methods in an independent manner, and so complement each other.

Generally, accessions are ungrouped based on their geographical origin but rather they are clustered by species type (Figure 25). The first two coordinates displayed 41.74% of the total variation. The PCoA grouped the accessions into three distinct clusters (I, II, and III); cluster I containing *A. sativa*, cluster II *A. sterilis* and *A. vaviloviana*, and cluster III *A. abyssinica* and *A. fatua*.

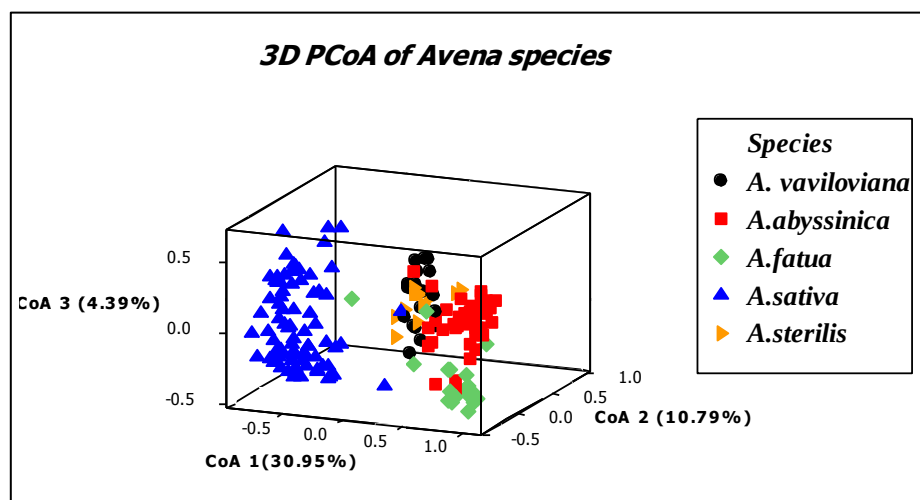


Figure 24. Principal coordinate analysis of accessions of five *Avena* species.

Where, dark green triangle is *A. sativa*, gray x mark color is *A. sterilis*, blue diamond is *A. abyssinica*, dark square is *A. fatua*, and blue star is *A. vaviloviana*

Principal coordinate analysis (PCOA) for *A. sativa*: The first three principal components explained 31.51% of SSR variation (12.34, 11.81, and 7.36%, respectively) (Figure 26). Samples from Gojam, Netherlands and Australia, and Gondar were clustered in their own groups. However, the PCO analysis revealed samples from the remaining populations had no clear groups.

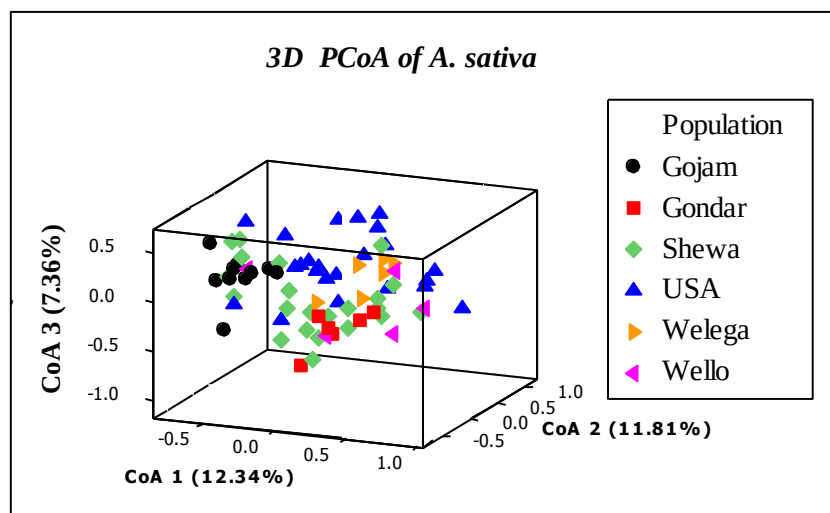


Figure 25. Principal coordinate analysis of accessions of *A. sativa* by regions.

Principal coordinate analysis (PCOA) for *A. abyssinica*: Similar to *A. sativa* populations, *A. abyssinica* populations do not show clear groups (Figure 26). The first three coordinates accounts to 47.00% of variation. The first and the second principal coordinates accounted for 27.59% and 11.76% of the variation, respectively.

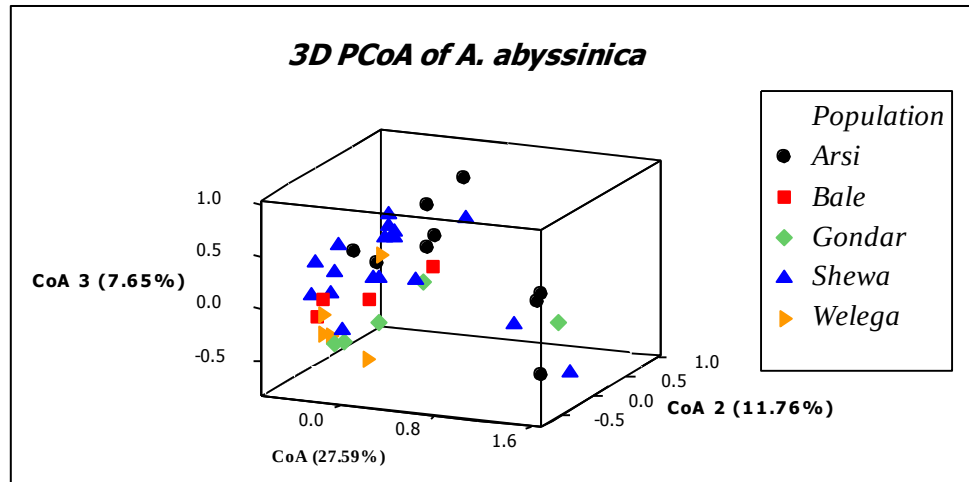


Figure 26. Principal coordinate analysis of *A. abyssinica* by regions.

5.2.4. Genetic diversity and population differentiation

The criteria used to define the number of subpopulations in the oat accessions, which are the position of a break point in the $L(k)$ curve and a peak in the k distribution, supported values of $k = 2$ and $k = 4$ (data not shown). For both k values, most accessions were assigned by STRUCTURE to subpopulations. With $k = 2$, it was possible to distinguish between the cultivated oat and the wild accessions.

The entire oat accessions (9 regions/populations) were successfully assigned into two groups (subpopulations) ($K: 2$, $DK: 560.85$). The first (blue) group (sub-population) are the USA and Netherlands and Australia accessions, while the second (red) subpopulation is Ethiopian accessions. Furthermore, the Ethiopian accessions showed the admixture of the two subpopulations. Likewise, based on species type, with $k = 2$, it was possible to distinguish between the cultivated and the wild oat accessions. With

$k = 4$, on species basis a finer sub-grouping of all species shown different gene pools. *A. sativa* had three gene pools in unequal proportion, but the more abundant gene pool in this species was rare in other species. *A. sterilis* had three gene pool and one shares with *A. sativa* and the other one was shared with *A. vaviloviana*, while the third was shared with all the species with smaller proportion.

Similarly, *A. vaviloviana* had three gene pools and the more abundant was shared with *A. sterilis* gene pool. Comparatively, *A. abyssinica* and *A. fatua* shared much more similar gene pools than the other species of oat studied here. The result was also supported by cluster analysis, PCA, and PCoA (Figure 24).

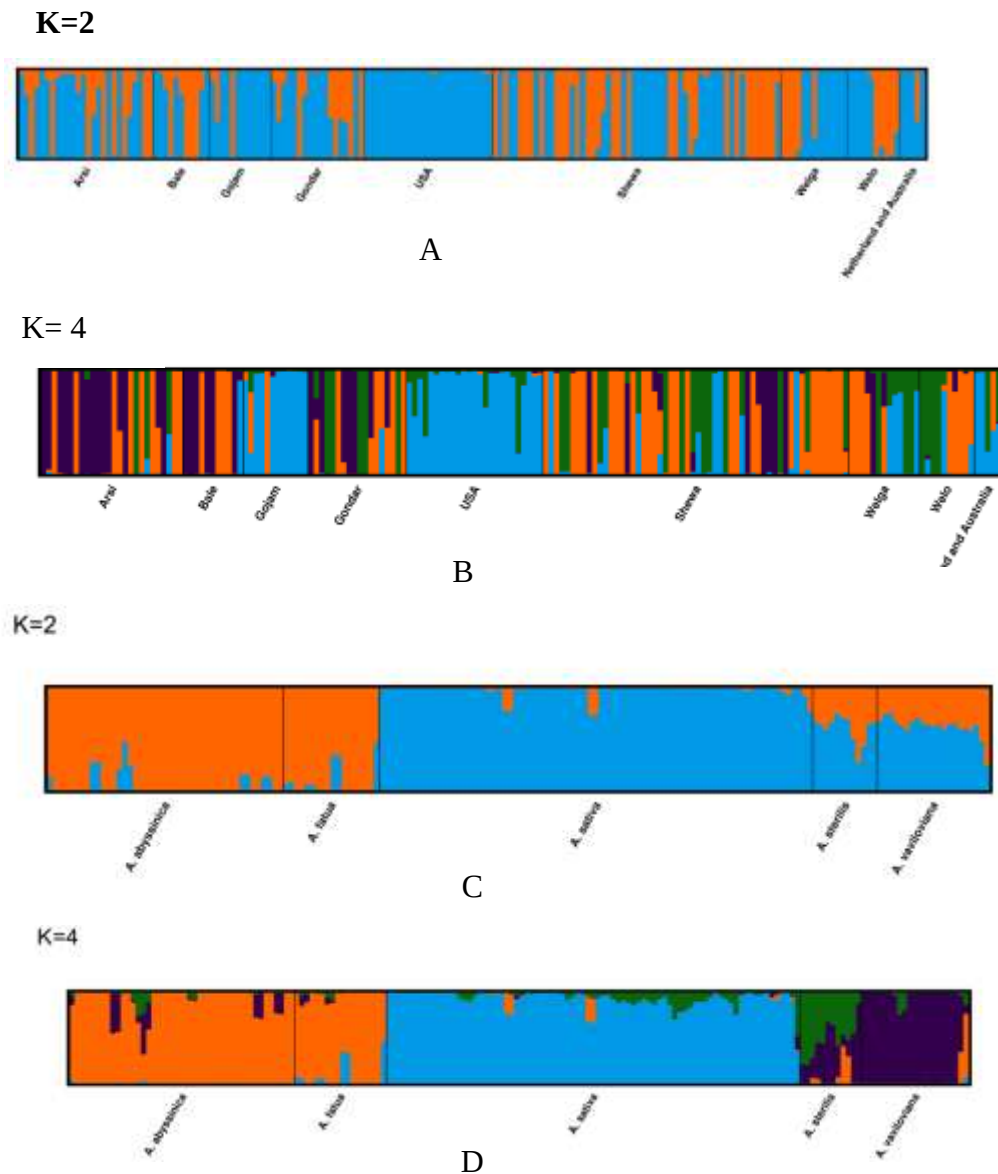


Figure 27. Bayesian cluster analysis of the optimum ($K = 2$ and 4) cluster of A and B Population structure of five *Avena* species based on geographical origin and C and D population structure of five *Avena* species based on species type at $k= 2$ and $K=4$, respectively.

Note: A) All Ethiopian and Netherland and Australia accessions show admixture of gene pools, whereas USA accessions had only one gene pool. B) The majority of the gene pools of *A. abyssinica*, and *A. fatua* are one, and *A. sativa* contain but the one gene pool differs from the first two species. However, *A. sterilis* and *A. vaviloviana* contain two gene pools in large proportion.

With $k=4$, population wise, the USA population showed two gene pools, and Gojam and Netherland-Australia population had three gene pools. These three populations consist almost only of *A. sativa* accessions. Other populations like Arsi, Bale, Shewa, and Gondar shared same gene pools. Shewa population had the most gene pool with equal proportion unlike the other populations (Figure 28).

5.3 Aluminium toxicity tolerance of oat accessions

5.3.1 Optimizing Al concentration for oat accession evaluation

Analysis of variance revealed non-significant ($P < 0.05$) differences between oat accessions at 25, 50, 150, 200 and 250 μM of Al^{3+} concentrations. At lower concentration, all accessions grow properly and at higher concentrations all accessions were highly suppressed. However, 100, 112.5, and 125 μM Al^{3+} concentration showed significant (< 0.05 and $p < 0.01$) Al induced stress in all accessions. Therefore, 112.5 μM Al^{3+} was selected as optimum concentration for extensive screening activities due to its multiple advantages. Initially, it allows to accurately discriminate between susceptible, tolerant and intermediate genotypes at the highest accuracy level (i.e. $p < 0.01$, then the solution of lower concentration levels). Second, its usage entails minimum cost as compared with solution strengthen than 112.5 μM Al^{3+} . At Al^{3+} concentration of 150 μM and above, the growth of roots of all the accessions were vulnerable if there was nearly no difference between them.

5.3.1.1 Al tolerance in oat accession as revealed by root length inhibition

Genotypes with tolerance inhibition index less than 50% are tolerant, whereas those with tolerance inhibition index greater than 75% are considered sensitive, while those with values between 50% and 75% inhibition index are intermediate. Relatively, *A. abyssinica*, *A. sterilis* and *A. vaviloviana* were better tolerant, while *A. sativa* and *A. fatua* showed lower tolerance levels. About 78 (52%) of the accessions were classified as tolerant, 18 (12%) accessions as sensitive and 54 (36%) accessions as intermediate.

5.3.1.2 Effect of Al-toxicity on oat accessions

The present screening was done using 112.5 μM solutions of Al^{3+} which showed weak root length inhibition on 78 (52%) of the oat accessions out of the total of 150 accessions tested.



Figure 28. The three-root growth category of oat accessions, that showed relative root length of tolerant (*A. sativa*), intermediate (*A. abyssinica*) and sensitive (*A. sterilis*) based on the root length at 112.5 μM Al^{3+} hydroponics culture.

Table 48. Effect of Al toxicity on root length (cm) of 150 oat accessions grown under treatment with 112.5µM Al³⁺ concentration in hydroponics solution

SNo	Acc. No	Spp	Pop	Treatment	Control	RTI	RLI	Cat	SNo	Acc. No	Spp	Pop	Treatment	Control	RTI	RLI	Cat
1	33	1	1	1	2.64	0.379	0.621	I	38	52005	1	7	1.22	2.65	0.46	0.54	I
2	52	1	1	3.17	4.6	0.689	0.311	T	39	52008	1	7	2.07	3.5	0.591	0.409	T
3	54	1	1	2.38	3.81	0.624	0.376	T	40	52009	1	7	0.06	1.49	0.04	0.96	S
4	60	1	1	1.54	2.97	0.518	0.482	T	41	219512	2	3	0.52	2.03	0.255	0.745	I
5	62	1	1	1.84	3.27	0.563	0.437	T	42	73	2	5	0.74	2.26	0.33	0.67	I
6	52020	1	1	0.06	1.49	0.04	0.96	S	43	83	2	5	0.33	1.84	0.181	0.819	S
7	137	1	2	0.94	2.37	0.398	0.602	I	44	86	2	5	1.24	2.75	0.451	0.549	I
8	141	1	2	1.97	3.4	0.58	0.42	T	45	89	2	5	0.31	1.82	0.168	0.832	S
9	142	1	2	0.78	2.21	0.353	0.647	I	46	92	2	5	1.64	3.15	0.52	0.48	T
10	52019	1	3	1.78	3.21	0.554	0.446	T	47	93	2	5	2.52	4.03	0.625	0.375	T
11	7	1	4	3.03	4.46	0.679	0.321	T	48	94	2	5	0.75	2.26	0.332	0.668	I
12	9	1	4	1.41	2.84	0.496	0.504	I	49	95	2	5	1.81	3.32	0.544	0.456	T
13	10	1	4	1.58	3.01	0.525	0.475	T	50	96	2	5	2.42	3.93	0.615	0.385	T
14	13	1	5	1.31	2.74	0.478	0.522	I	51	97	2	5	1.06	2.57	0.411	0.589	I
15	18	1	5	0.6	2.03	0.295	0.705	I	52	117	2	5	0.03	1.54	0.021	0.979	S
16	20	1	5	3.02	4.45	0.679	0.321	T	53	52011	2	5	1.72	3.23	0.533	0.467	T
17	21	1	5	2.59	4.02	0.644	0.356	T	54	52014	2	5	0.52	2.03	0.255	0.745	I
18	101	1	5	1.97	3.4	0.58	0.42	T	55	52006	2	7	1.08	2.59	0.416	0.584	I
19	103	1	5	0.67	2.1	0.318	0.682	I	56	52010	2	7	2.28	3.79	0.601	0.399	T
20	107	1	5	2.71	4.14	0.655	0.345	T	57	57	3	1	3.24	5.22	0.621	0.379	T
21	108	1	5	0.11	1.54	0.069	0.931	S	58	65	3	1	2.46	4.43	0.554	0.446	T
22	109	1	5	3.23	4.66	0.693	0.307	T	59	6207	3	2	1.53	3.5	0.436	0.564	I
23	111	1	5	1.6	3.03	0.528	0.472	T	60	9675	3	3	2.83	4.81	0.589	0.411	T
24	118	1	5	0.83	2.26	0.368	0.632	I	61	9677	3	3	3.22	5.2	0.62	0.38	T
25	119	1	5	2.25	3.68	0.611	0.389	T	62	9678	3	3	0.28	2.25	0.123	0.877	S
26	120	1	5	1.9	3.33	0.57	0.43	T	63	9680	3	3	1.31	3.28	0.398	0.602	I
27	121	1	5	3.13	4.56	0.687	0.313	T	64	9682	3	3	3.77	5.74	0.656	0.344	T
28	122	1	5	0.69	2.12	0.327	0.673	I	65	9683	3	3	3.68	5.65	0.651	0.349	T
29	126	1	5	2.14	3.57	0.599	0.401	T	66	9685	3	3	0.78	2.75	0.283	0.717	I
30	127	1	5	1.28	2.71	0.473	0.527	I	67	212664	3	3	3.83	5.81	0.66	0.34	T
31	128	1	5	0.5	1.93	0.259	0.741	I	68	238234	3	3	1.89	3.86	0.489	0.511	I
32	52015	1	5	1.75	3.18	0.55	0.45	T	69	238235	3	3	3.71	5.69	0.653	0.347	T
33	145	1	6	0.11	1.54	0.072	0.928	S	70	8	3	4	1	2.98	0.336	0.664	I
34	146	1	6	4.03	5.46	0.738	0.262	T	71	15	3	4	3.52	5.5	0.641	0.359	T
35	147	1	6	1.09	2.52	0.433	0.567	I	72	16	3	4	2.98	4.96	0.602	0.398	T
36	148	1	6	3.36	4.79	0.701	0.299	T	73	26	3	4	1.93	3.91	0.494	0.506	I
37	201	1	6	0.14	1.57	0.089	0.911	S	74	28	3	4	1.18	3.16	0.374	0.626	I

Table 4 continued...

S No	Acc. No	Spp	Pop	Treatment	Control	RTI	RLI	Cat	S No	Acc. No	Spp	Pop	Treatment	Control	RTI	RLI	Cat
75	161	3	4	2.26	4.24	0.534	0.466	T	113	5443	3	8	1.48	3.46	0.428	0.572	I
76	52018	3	4	0.67	2.64	0.252	0.748	I	114	5452	3	8	0.16	2.13	0.073	0.927	S
77	14	3	5	2.25	4.23	0.533	0.467	T	115	5454	3	8	1.13	3.11	0.364	0.636	I
78	76	3	5	1.94	3.92	0.496	0.504	I	116	5465	3	8	1.33	3.3	0.402	0.598	I
79	79	3	5	3.41	5.39	0.633	0.367	T	117	5475	3	8	2.75	4.73	0.582	0.418	T
80	98	3	5	2.72	4.7	0.579	0.421	T	118	5489	3	8	2.75	4.73	0.582	0.418	T
81	104	3	5	4.22	6.2	0.681	0.319	T	119	5490	3	8	3.71	5.68	0.652	0.348	T
82	105	3	5	1.1	3.08	0.358	0.642	I	120	5492	3	8	3.28	5.25	0.624	0.376	T
83	113	3	5	2.8	4.23	0.662	0.338	T	121	5493	3	8	1.64	3.61	0.454	0.546	I
84	114	3	5	0.07	2.05	0.035	0.965	S	122	5499	3	8	3.34	5.32	0.629	0.371	T
85	116	3	5	0.81	2.79	0.291	0.709	I	123	5507	3	8	2.65	4.63	0.573	0.427	T
86	123	3	5	3.28	5.25	0.624	0.376	T	124	5509	3	8	0.64	2.61	0.244	0.756	S
87	1000	3	5	0.63	2.61	0.243	0.757	S	125	5513	3	8	2.67	4.65	0.575	0.425	T
88	2000	3	5	1.12	3.1	0.362	0.638	I	126	5527	3	8	0.34	2.32	0.148	0.852	S
89	6708	3	5	2.28	4.25	0.536	0.464	T	127	15153	3	9	2.59	4.57	0.568	0.432	T
90	6710	3	5	3.64	5.62	0.648	0.352	T	128	16101	3	9	2.5	4.48	0.559	0.441	T
91	8237	3	5	0.98	2.96	0.332	0.668	I	129	235519	3	9	3.96	5.94	0.667	0.333	T
92	52001	3	5	4.17	6.14	0.678	0.322	T	130	235521	3	9	2.42	4.39	0.55	0.45	T
93	236851	3	5	1	2.98	0.336	0.664	I	131	37	4	1	2.19	3.84	0.572	0.428	T
94	236852	3	5	4.01	5.99	0.67	0.33	T	132	1	4	4	0.78	1.42	0.553	0.447	T
95	236853	3	5	0.22	2.2	0.101	0.899	S	133	12	4	4	1.07	1.7	0.628	0.372	T
96	236854	3	5	0.57	2.55	0.224	0.776	S	134	24	4	4	1.32	1.96	0.676	0.324	T
97	200	3	6	2.41	4.38	0.549	0.451	T	135	25	4	4	0.03	0.66	0.042	0.958	S
98	203	3	6	3.69	5.66	0.651	0.349	T	136	32	5	1	2.94	4.58	0.641	0.359	T
99	204	3	6	1.72	3.7	0.466	0.534	I	137	36	5	1	1.3	2.73	0.476	0.524	I
100	205	3	6	0.96	2.93	0.326	0.674	I	138	42	5	1	1.06	2.7	0.391	0.609	I
101	207	3	6	1.48	3.45	0.428	0.572	I	139	45	5	1	0.9	2.54	0.353	0.647	I
102	208	3	6	3.17	5.14	0.616	0.384	T	140	46	5	1	1.47	3.12	0.473	0.527	I
103	2	3	7	2.83	4.8	0.589	0.411	T	141	48	5	1	1.69	3.34	0.508	0.492	T
104	3	3	7	2.08	4.05	0.513	0.487	T	142	51	5	1	1.97	3.62	0.546	0.454	T
105	22	3	7	3.25	5.23	0.622	0.378	T	143	63	5	1	2.44	4.09	0.598	0.402	T
106	23	3	7	1.61	3.58	0.448	0.552	I	144	71	5	1	1.71	3.35	0.51	0.49	T
107	5432	3	8	1.48	3.45	0.428	0.572	I	145	131	5	1	1.93	3.58	0.541	0.459	T
108	5435	3	8	1.94	3.92	0.496	0.504	I	146	134	5	2	2.43	4.08	0.597	0.403	T
109	5436	3	8	1.07	3.04	0.351	0.649	I	147	135	5	2	2.5	4.14	0.604	0.396	T
110	5438	3	8	0.87	2.85	0.306	0.694	I	148	136	5	2	1.37	3.01	0.454	0.546	I
111	5440	3	8	0.65	2.63	0.248	0.752	S	149	80	5	5	1.36	3	0.453	0.547	I
112	5441	3	8	1.76	3.73	0.471	0.529	I	150	152	5	6	1.47	3.12	0.473	0.527	I

Where, spp = species (1= *A. abyssinica*, 2 = *A. fatua*, 3 = *A. sativa*, 4 = *A. sterilis*, and 5 = *A. vaviloviana*); Pop= Population (1= Arsi, 2=Bale,3= Gojam, 4 = Gondar, 5, Shewa, 6, Welega, 7= Wello, 8= USA, 9= Netherland -Australia);, RLT= Root length tolerance, RLI= Root length inhibition, and cat = category (T = tolerant, I= intermediate, and S = sensitive)

Table 49. Mean value of root length of oat accessions on species basis at 0 and 112.5 μ l of Al³⁺ concentration with root length tolerant index (RLT) and root length inhibition (RLI)

Species	Mean Treatment	Mean Control	Mean RLT	Mean RLI	Category						No of accession
					T	%	I	%	S		
<i>A. abyssinica</i>	1.65	3.08	0.47	0.53	22	55.0%	13	32.5%	5	12.5%	40
<i>A. fatua</i>	1.19	2.70	0.39	0.61	6	37.5%	7	43.8%	3	18.8%	16
<i>A. sativa</i>	2.13	4.10	0.48	0.52	38	51.4%	27	36.5%	9	12.2%	74
<i>A. sterilis</i>	1.08	1.92	0.49	0.51	4	80.0%		0.0%	1	20.0%	5
<i>A. vaviloviana</i>	1.77	3.40	0.51	0.49	8	53.3%	7	46.7%	0	0.0%	15
Total	1.56	3.04	0.47	0.53	78	52.0%	54	36.0%	18	12.0%	150
LSD	0.48	0.70	NS	NS							

Where, LSD: least significant difference, T: Tolerant, I: intermediate, S: sensitive, %: percentage, and NS: non-significant

Proportionally accessions of all species revealed tolerance to Al³⁺, except accessions of *A. fatua*. The top 10% tolerant accessions were found in *A. abyssinica* and *A. sativa* accessions (Table 49).

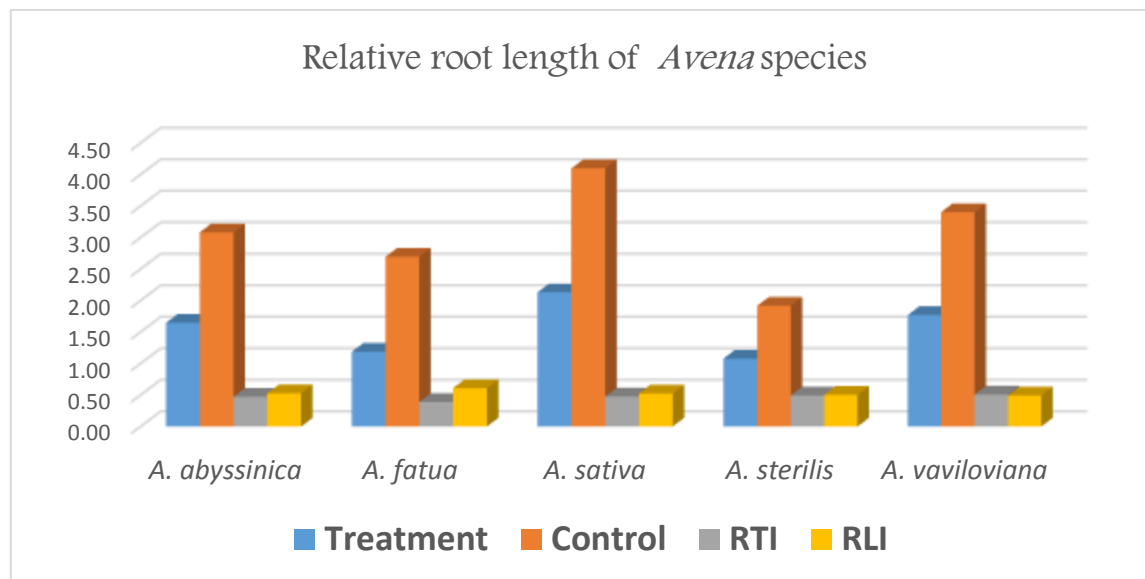


Figure 29. Mean root length of five *Avena* species grown at 0 and 112.5 μ l Al³⁺ concentration.

Table 50. The relative root length mean values on the basis of geographical origin of oat accessions at 0 and 115.5µl of Al³⁺ concentration

Region	Mean of Treatment	Mean of Control	Mean of RLT	Mean of RLI	Category						No Acc (Wild + Cult'd)
					T	%	I	%	S	%	
Arsi	1.86	3.47	0.51	0.49	13	68.4%	5	26.3%	1	5.3%	19
Bale	1.65	3.24	0.49	0.51	3	42.9%	4	57.1%		0.0%	7
Gojam	2.30	4.19	0.49	0.51	7	58.3%	4	33.3%	1	8.3%	12
Gondar	1.63	3.10	0.49	0.51	8	57.1%	5	35.7%	1	7.1%	14
Shewa	1.70	3.35	0.45	0.55	26	50.0%	18	34.6%	8	15.4%	52
Welega	1.92	3.64	0.46	0.54	5	38.5%	6	46.2%	2	15.4%	13
Wello	1.83	3.52	0.48	0.52	5	55.6%	3	33.3%	1	11.1%	9
USA	1.78	3.76	0.43	0.57	7	35.0%	9	45.0%	4	20.0%	20
Total	1.83	3.53	0.47	0.53	74	49.3%	54	36.0%	18	12.0%	150
LSD	NS	NS	NS	NS							

Where, RLT: root length tolerant index, RLI: root length inhibition, LSD: least significant differences, T: Tolerant, I: intermediate, S: sensitive, %: percentage, and NS: non-significant

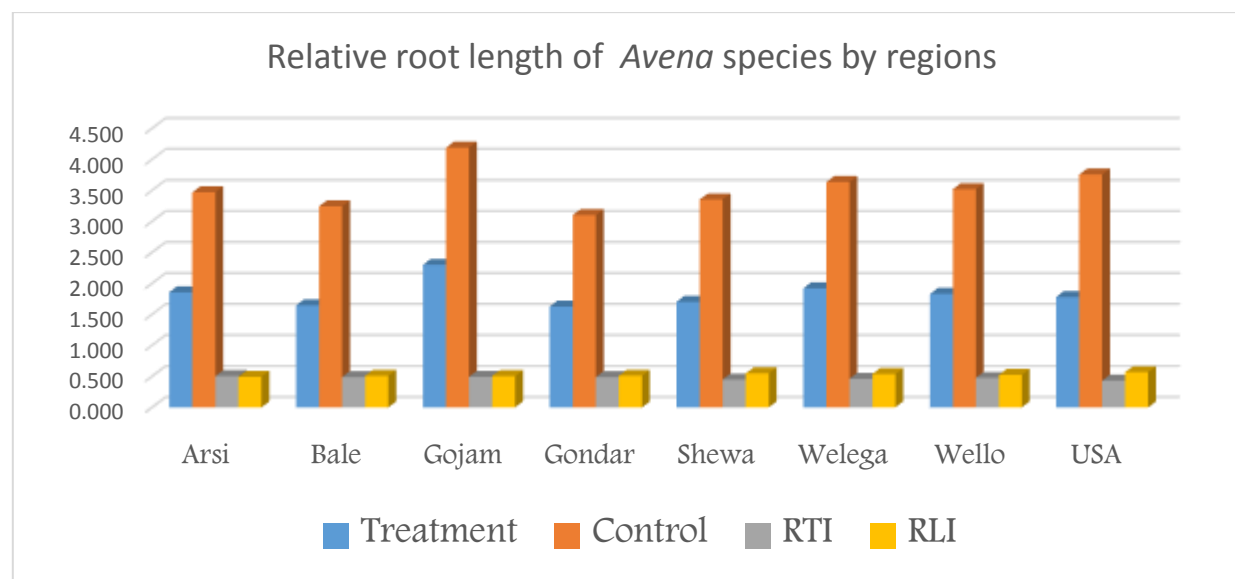


Figure 30. Root length of oat in 8 regions/populations of all five *Avena* species.

More than 50% of accessions showed tolerant, except Bale, Welega, and USA region. But the highest tolerant accessions were found in Welega. Generally, the accessions from Welega, Gojam and Shewa were predominant the top 10% tolerant accessions (Table 48).

6. DISCUSSION

In this study, 176 Ethiopian oat accessions of five *Avena* species were studied. Most accessions of the five species were collected from Ethiopia and a few accessions of *A. sativa* were originated from USA, the Netherlands and Australia and the non-Ethiopian materials were acquired through EBI and ILRI. Ethiopian accessions of *A. sativa* and *A. abyssinica* were collected from seven and five regions, respectively. Materials from individual regions were considered as populations for *A. sativa* and *A. abyssinica* accessions. The *A. sativa* accessions from USA were one population and the accessions from the Netherlands and Australia were not treated as a population for morpho-agronomic analysis. The *A. sativa* accessions of these two countries (the Netherlands and Australia) were used as one group for SSR marker study to see the distance of Ethiopian accessions from the exotic accessions. The data recorded from accessions of these 8 populations were subjected to various diversity analyses. Morphological data were run for multivariate analysis and the SSR markers data were also used to generate information about genetic variability on species basis and region/population basis. Morphological data were run for multivariate analysis and the SSR markers data were also used to generate information about genetic variability on species bases and region/population bases. Likewise *A. sativa* accessions and *A. abyssinica* accessions the data recorded from 7, and 6 regions/populations were used for data analysis for *A. sativa* and *A. abyssinica*, respectively. The remaining species (*A. vaviloviana*, *A. fatua* and *A. sterilis*) were not subjected to population based data analysis.

In this study, data of 12 quantitative and 22 qualitative morpho-agronomic traits were scored and analyzed for genetic diversity of the accessions. All the studied morpho-agronomic traits of the accessions revealed significant variation among accessions, over years and experimental sites. This was assessed in the 176 accessions. Similar results were observed in the populations of *A. sativa* and *A. abyssinica*. Similar finding was also reported on the *A. sativa* by Akcura *et al.* (2006), regarding the

effects of years, locations, cultivars and their interactions for grain yield. This variation is indispensable to run plant breeding programs intended to improve existing germplasm. Morpho-agronomic description has become good potential to select and conserve genotypes for breeding and agronomic research programmes (Boczkowska *et al.*, 2016). In addition to these benefits, morpho-agronomic traits are useful for phylogenetic analysis. Loskutov (2007), reported the phylogenetic relationships of the genus *Avena* using morpho-agronomic traits. This will assist us to improve the species classification.

6.1 Agronomic traits

Plant breeders are conventionally interested to characterize accessions with morpho-agronomic traits. The efficiency of a plant breeding programme is determined by genetic variability and selection techniques. The present investigation understood the variability pattern and association with different characters of different genotypes.

6.1.1. Qualitative traits variability

Regarding oat growth habit the majority of the accessions were found to possess erect growth habit (71.0%) followed by prostrate (18.8%) and semi-prostrate (10.2%). The existence of such variation for this trait is important to select genotypes with favorable growth habit. Each type of growth habits has its own merit, it would be selected. For example, plants that have erect growth habit are, erect plants can stand densely and have better air circulation among them, thus reducing the chance of fungal infection, etc. The erect growth habit is more common in *A. sativa*, *A. fatua* and *A. abyssinica* accessions. But prostrate growth habit is common in *A. sterilis* and *A. vaviloviana* accessions. The prostrate growth habit is important for grazing and it can also prevent or reduce soil erosion. As we understand from the literature, different types of growth habit occur in the same species with different proportions. For instance, regarding *A. sativa*, Boczkowska *et al.* (2014) reported that erect growth habit was predominant in Polish cultivars, his report agrees with our findings from Ethiopian *A. sativa*

accessions. Contrary to our finding Arora (2013) reported that semi-prostrate and prostrate types were predominant in *A. sativa* materials. The difference between his report and our result could be due to different genotypes being studied.

Our finding on white and yellow lemma color of *A. sativa* and *A. abyssinica* was in line with the finding of Tang *et al.* (2014) report that white and yellow lemma color are predominant in *Avena* species and are equally frequent. Likewise for grain color, similar to the present finding Tang *et al.* (2014) reported that in *A. sativa* and *A. abyssinica* yellow grain color was predominant. On the contrary, the present finding does not match with their finding regarding brown- black grain color, and this may be attributed to the difference in the accessions used. The second most probable reason for the more frequent abundance of white and yellow color is the unconscious and deliberate selection pressure during domestication and breeding by breeders for both grain and lemma color of *A. sativa* and *A. abyssinica* accessions.

Regarding lodging, accessions had significant variation both at immature and mature stages. This finding was in line with Ali *et al.* (2016) about evaluating oat phenol-physiological traits. Lodging decreases oat productivity and quality because the lodged plant can't support its inflorescence upright. In addition, lodging prevents adequate air circulation in the plot and enhances disease transmission through contact. This result provides plant breeders the potential to breed lodging resistant varieties of oat. Traits like thick stem and equilateral panicle shape played a significant role in the prevention of lodging. Lodging is influenced by environmental conditions such as the presence and strength of the wind, humidity of the soil surface, fertilizer and the sowing rate. These factors interact with the genetically based characteristics such as plant height, size, and properties of tissue mechanics of the lower internodes, straw flexibility, resistance to culm base diseases, and development of adventitious roots (Berry, 1998).

The majority of accessions were categorized into equilateral panicle shape and semi-compact panicle. These were common in cultivated oat while non-equilateral panicle shape and the non-compact panicle type were common in wild oat. These probably are due to the interference of human selection pressure for equilateral panicle shape which has lodging resistance. The types of panicles were observed and the predominant type are unilateral panicle type. This is in line with Nowosielska and Nowosielski (2009) that 55 of 64 varieties showed unilateral panicle types. Majority of the studied oat accessions were characterized by drooping erection of spikelets. This is in line with Nowosielska and Nowosielski (2009) that all studied oat breeding varieties showed drooping erection of spikelets.

Larger glume and grain size were more predominant in cultivated oat but exceptionally the wild oat *A. sterilis* had large glume and grain size. These traits were very important because they are related to grain yield. These helped oat breeders to select superior genotypes to use in a programme aimed at breeding to enhance the genetic potential of yield. Selecting traits that has contribution for the improvement of yield and quality characteristics of oat. This in line with Kumari *et al.* (2018) that an important step in plant breeding is selecting traits which suggested ample scope of improvement by inclusion of yield and quality characteristics of oat.

All accessions had either straight or geniculated awn type. This study is in line with Sumathi and Balamurugan (2014) that all varieties except one out of 12 varieties of *A. sativa* had awn. Short and straight type was dominant in *A. sativa* *A. abyssinica* and *A. fatua*, whereas strong and geniculate types were observed in *A. sterilize* and *A. vaviloviana*. On the other hand, this finding contradicts with Arora (2013), who reported that analyzed 70 accessions had awn from 110 *Avena* accessions, while only 40 accessions hadn't awn. The short and straight awn is the more preferred for fodder type due to smoothness feature. The reason is the genotype difference for his study and this study.

The majority of the accessions showed low infection levels. Especially, *A. sterilis* 100% non-susceptible to disease. This finding agrees with Okoń *et al.* (2014), reported wild relatives of *A. sativa* and *A. sativa* have many source of resistance to powdery mildew. In addition, our result was in line with Boczkowska *et al.* (2016), who reported a low infection level by crown rust in *A. sativa* accessions. Low infection levels observed might have been caused partly by weather conditions unfavorable to the pathogen and low disease pressure, rather than being due to plant resistance. To be sure the pathogen resistance of accessions have to be done to control experiment by treating the accessions with pathogens.

6.1.2 Quantitative trait variability

The goal of our study was also to describe the variability patterns of important morpho-agronomic traits in the Ethiopian and exotic *Avena* accessions. Based on the data obtained, the diversity of the accessions was estimated.

The mean, heritability, genetic advance and genetic advance as per cent of mean are presented. The mean values of the genotypes showed a wide range for all the characters, these are agrees with Jaipal and Shekhawat (2016).

The major factor contributing to forage yield of different crops is plant height. The oat plant height variability has been recorded by different investigators, for example, Buerstmayr *et al.* (2007), Khan *et al.* (2014) and Boczkowska *et al.* (2016), reported that plant height ranged from the average height of 80 to 140 cm. The observed variability in cultivated and wild oat accessions, would provide a large opportunity for breeders to develop new desirable types of cultivar through selection and hybridization. The predominance of short plant height in the wild species, for example, wild oat could probably be the due absence of artificial selection in this group. But cultivated oat is tall plant, probably because of

artificial selection aimed to increase plant height to maximize the forage yield. The plant breeder wants to develop genotypes with modified morpho-physiological traits such as plant height, maturity, and high fertility. These traits increase the grain yield potential of cultivars. A significant range of plant height were observed, this was in line with Ali *et al.* (2016) that plant height was ranged from 88 to 124 cm. The stem height can be influenced by the genotype, soil, climatic condition, and used agronomic practice. Tall plants have great impact to lodging. Taller stem is highly prone to lodging. According to Spasova *et al.* (2017) medium height and firm stem are the most suitable for production of oat varieties.

The maximum and the minimum number of days to 50% flowering were recorded in *A. sativa* and *A. fatua*, respectively. From the plant breeding point of view, early heading confers advantages for plants to pass harsh environmental conditions within a short time. The existence of variability of this nature in Ethiopian oat accessions has a great potential value to improve oat cultivars. Early flowering is directly related to low number of days to 50% flowering and late maturing plants are related to more number of days to maturity. The longer photoperiod promotes early flowering, and this response is greater in varieties from higher latitudes (Holland *et al.*, 2002). Decreasing the length of the vegetation period with a simultaneous increase of grain yield is one of the main breeding goals. Due to a relatively moderate heritability of the heading date, selection in early flowering and maturing generations should be effective.

The overall means of the 90% days to maturity was 141 days. Days to 90% maturity of *A. abyssinica* and *A. fatua*, was lower than the overall mean value unlike to the other two wild species. They had higher mean value than the overall mean values. Some findings, for example, Sheikhehpour *et al.* (2014) was at variance with our findings regarding the range and the average number of days to maturity in *A. sativa* accessions. These variations may be due to the differences between the genotypes

or accessions investigated and environmental condition under which the experiment was conducted. For increasing the grazing time of the livestock, varieties of oat would have valuable features which are long vegetative period of intermediate and late-maturing. Select high-yielding cultivars with late maturity is important for forage oat. Due to this the today's forage oat varieties releases are much higher yielding and much later to flower than the dual-purpose varieties.

The mean value of the number of effective tillers per plant in *A. sativa* (5.4) accessions was less than the overall mean value (5.9). This finding agreed with Premkumar *et al.* (2017), who reported that the number of effective tiller ranged from 3.67 to 12.67 with the mean of 6.56 in 48 oat genotypes. The range of number of effective tiller per plant agreed with that of Khan *et al.* (2014), who reported the number of tiller per plant of *A. sativa* cultivars varied from 6.33 to 7.78 with slight difference. This could be due to the variation of number of plant per plot, genotype, and environmental condition at the experimental site. The high number of effective tiller per plant was positively associated with the productivity of grain and biological yield. The existence of variability in the number of tillers could allow breeders to develop cultivar of high tiller number as desired.

The number of leaves, plays a vital role in growth, development, and yield of the plant. The number of leaves depends on number of nodes per main stem of a plant. The increase or decrease in the number of leaves (nodes) per main culm directly affects the yield, particularly of forage crops. Accessions of *A. sativa* had lower number of leaves per culm. This finding agrees with Ali *et al.* (2016), reported the number of leaves ranges from 5 to 6.8. However, this finding a bit contradicts with that of Khan *et al.* (2014), who reported a range of 7.50 to 5.93, with an average 6.9 number of leaves per culm for *A. sativa*. The difference may be attributed to genotypic differences and difference in the experimental conditions of the location. The experimental condition are, the amount of fertilizer used, soil fertility, the weather conditions and so on

Yield is a complex characteristic, and its components are strongly influenced by a large number of environmental factors (Doehlert *et al.*, 2001). The present finding of grain yield of *A. sativa* (287.4 g/m²) is lower than the findings of Sheikhehpour *et al.* (2014). He reported that the grain yield of *A. sativa* ranges from 209.30 to 689.2 g/m², with an average mean of 421.23 g/m². The high grain yield of *A. sativa* relative to that of wild species is expected because there may be unconscious and deliberate selection for increased grain yield during its domestications as well as during the breeding programme.

The mean of the biological yield (2314.1g/m²) was recorded in *A. sativa* accessions. Significant variation in biological yield of oat was also reported by Khan *et al.* (2014). These investigators reported that the biological yield of *A. sativa* ranged from 740g/m² to 1095g/m². Even though, there is genotypic and environmental difference between the two experiments, the *A. sativa* accessions used in the present study showed better performance in biological yield.

The range of number of spikelets per panicle is in line with Premkumar *et al.* (2017), who reported that spikelets per panicle ranged from 32.00-74.33, with the mean of 51.47 in 48 oat genotypes. In addition, our finding of panicle length was in agreement with Premkumar *et al.* (2017) who reported that the length of panicle ranged from 16.27-30.63 cm with the mean of 24.15 cm of 48 oat genotypes.

The length of flag leaf ranged from maximum (30.8) in *A. sativa* and minimum (24.1) in *A. fatua*. In *A. sterilis*, *A. vaviloviana*, and *A. abyssinica* the mean value of flag leaf length was about equal to the overall means. This finding is different from that of Sheikhehpour *et al.* (2014), who reported a range of 12.86 to 64.27 cm and average of 26.24 cm in 20 *A. sativa* accessions.

6.1.2.1 Heritability

The concept of heritability explains how much of the variation observed among individuals arose because of differences in their genetic makeup relative to environmental factors. According to Surek (1996) heritability estimates can be placed in these categories: low heritability ($< 30\%$), medium heritability (30-60%), and high ($> 60\%$). If heritability of a character is high, say 60% or more, selection for the character could be easy. This is because there would be a close correspondence between the genotype and the phenotype due to the relatively small contribution of the environment to the expression of a trait. Traits that exhibited high heritability values indicate that the effect of the environment on the phenotypic expression is small and the effectiveness of selection in the improvement of these traits is large. The result showed the scope for selection in these characters (Nidhi *et al.*, 2014). Hence, estimates of heritability are essential pre-requisites for the formulation of an effective selection method for genetic improvement. The record of low to high heritability observed in the present study is similar to the findings of Kumar (2002), Dubey (2011), and Premkumar *et al.* (2015).

6.1.2.2 Expected genetic advance

Genetic advance under selection (GA) refers to the extent by which the mean of a character of the new population is improved compared to the mean of the base population under one cycle of selection at a given selection intensity. Low to high genetic advance as percent of the mean was observed at different traits in different species of oat. All traits exhibited moderate to high genetic advance. This finding is in proximity with that of Bibi *et al.* (2012) for spikelet per panicle and biological yield; (Krishna *et al.*, 2014) for spikelet per panicle, and grain yield; and, (Dubey, 2011) for spikelet per panicle, and number of nodes per plant. The DHE moderate value of GAM was similar to the finding of Poonia *et al.* (2018), who reported that low to moderate GAM were recorded in DHE in 92 oat genotypes.

Low to high heritability with high genetic advance was observed in most traits in different *Avena* specie. High heritability indicates these characters seem reasonably heritable and can be improved by selection. This finding is in line with Choudhary (2013), who reported, that days to 50% flowering showed moderate heritability with moderate genetic advances as a percent of the mean.

High heritability, accompanied with high genetic advance, indicates that the heritability is largely due to additive gene effects. Selection may be an effective and reliable prediction of the genetic gain under selection. This can be made when high heritability is coupled with high genetic advance than heritability estimates alone. Low genetic advance indicates the predominance of non-additive gene action. Selection for characters with low heritability may not be rewarding. However, it does not necessarily mean that all characters showing high heritability will also exhibit high genetic advance (Johnson *et al.*, 1955). This is because sometimes the heritability value could be large due to non-additive gene action such as dominance and epistasis rather than due to additive gene effect.

The present study revealed low to high heritability, high heritability coupled with high genetic advance as percent of the mean in different traits in different species. These traits might be largely under the control of additive gene effects and, therefore, they can be effectively improved through selection. Better information could be provided by the combination of high heritability and of high genetic advance than any other parameters alone. The results agree with the findings of Krishna *et al.* (2014) for spikelet per panicle.

6.1.2.3 Correlation analysis

The correlation coefficient estimates the degree and the direction of the association between pairs of characters. It is useful for simultaneous improvement of the correlated traits through selection for one

of the two traits. These measures the mutual relationship among plant characters and determine the component characters on which selection can be made for the genetic improvement of the yield.

Correlation studies clearly picture the extent of association between pairs of characters due to linkage or pleiotropy. The linkage is a cause of transient correlation and it is found in populations derived from crosses between diverse species. If the cause is a linkage, the correlation relationship between two characters can be reversed due to crossing over effect in subsequent generations. If the cause of the correlation is pleiotropy, it is impossible to reverse the correlation relationship. The trait remains either positively or negatively correlated.

Correlation coefficients are calculated at genotypic, phenotypic and environmental levels to understand the masking influence of the environment on the genotypic expression of the character under study. Despite the lesser importance of the environmental correlation coefficient to the breeder, it will help to understand the extent traits that can be influenced by environmental factors respective to their genetic control.

The magnitudes of a genotypic coefficient of correlation were greater than corresponding phenotypic coefficient correlation, which is indication of the absence of environmental influence to the inherent association among various traits. These findings were in agreement with Singh *et al.* (2018). The correlations between different traits vary. Grain yield showed positive and significant correlation with different traits in different *Avena* species oat accessions. For example, we observed a positive and significant correlation of days to 50% heading and biological yield with grain yield. These results were similar to Bibi *et al.* (2012); Tewari and Pandey (2014); Krishna *et al.* (2014) and Kumar *et al.* (2017). Likewise, in *A. sativa* accessions, grain yield showed a significant positive correlation with flag leaf length, panicle length, and biological yield. In addition, grain yield of oat accessions showed a positive

correlation with all of the 12 morpho-agronomic traits, except harvest index, these were in line with Ahmed *et al.* (2013), Tewari and Pandey (2014), Jaipal and Shekhawat (2016) and Kumar *et al.* (2017). For *A. sativa* accessions, high magnitude and maximum positive direct effects on grain yield per meter square was exerted by biological yield per meter square, length of panicle, number of leaves per plant followed by leaf width, plant height, total numbers of tillers per plant, day to 50% flowering, indicating the true relationship between these characters as good contributors to grain yield. Like *A. sativa* accessions, *A. abyssinica* accessions grain yield had a significant positive correlation with days to 50% heading and biological yield. But grain yield differently correlated with other traits unlike to *Avena* species accessions.

One of selection criteria for breeding, the breeder should consider the association desirable traits which had importance for grain yield improvement. Undesirable relationship of the component traits might act as limiting for the formulation of a comprehensive selection program. Therefore, while formulating a comprehensive selection program, these traits must be considered with a caution.

6.1.2.4 Cluster Analysis

Analysis of clusters helps to establish potential relationships. The UPGMA dendrogram grouped the oat accessions mainly by species types rather than their geographical origin. Accessions of the five *Avena* species were grouped into a separate and distinct clusters. Based on the morpho-agronomic traits clustering of accessions didn't show distinct regional grouping pattern that is accessions from the same or adjacent regions appeared in different clusters. This implies that the variation exists within the population was higher than the variation among the population. The variations of different genotypes found among different regions were small, this implies that all populations of *A. sativa* and *A. abyssinica* are almost the same. Selection of different genotypes within a population is enough for the

breeding program. But all regions are important because the regions have some unique morpho-agronomic traits.

A. sativa accessions which originated from 4 countries were not placed in a separate cluster rather they were distributed in most of clusters. This finding is in line with Tang *et al.* (2014), who reported that accessions of *A. sativa* belonging to different countries and regions were scattered in a different cluster. The accessions originated from the same region, a result they grouped the same cluster. The second reason is the presence of gene flow so that accessions are not differentiated.

Grouping accessions into morphologically similar clusters are useful for selecting parents for crossing (Souza and Sorrells, 1991b). Crossing accessions found to different clusters of large Euclidean distance could maximize opportunities for heterosis, because unrelated genotypes could contribute unique desirable alleles at different loci. Similar findings were reported by Ahmed *et al.* (2011), who reported that a crossing cluster with low Euclidean distance will be of less useful in the improvement programme. Thus, the cluster pattern observed in our material shows a great opportunity to get hybrid vigor through hybridization, since the distance between the clusters are very high.

Comparative evaluation of cluster means suggested that for improving specific character, the genotypes should be taken from the cluster having high mean value. Clusters with better cluster mean for many characters might be considered better for selecting genotypes. The result of the present investigation also agrees with that of previous studies carried out on oat by several workers, for example (Poonia and Phogat, 2017). Crossing genotypes belonging to different clusters by large inter-cluster distances and having high cluster mean for one or other characters will likely be more useful. In this study, we found great cluster distances between clusters involving the total 176 oat accession. This distance is

greater than the observed cluster distance of *A. sativa* and *A. abyssinica* accessions when considered separately. This implies that other species should also be considered for crossing distant clusters.

Another main finding of the present study is of genome similarity in oat species. Accessions in all the five *Avena* species, few individuals were clustered together in one or a few cluster groups, and this indicates the presence of genomic similarity among species. This could be due to their co-evolution, or they probably have common ancestors. Loskutov (2008), analyzed diversity and found phylogenetic relationships and co-evolution in 26 *Avena* species.

6.1.2.5 Principal component analysis

The principal component analysis is a well-known data reduction technique developed by Hotelling (1933). The use of principal component analysis is to identify the minimum number of components, which can explain the maximum variability.

The first principal component accounted for 39% of the total variability. The remaining components accounted for progressively lesser and lesser amount of variation. The first four principal components that have an Eigenvalues greater than or equal to 0.9116, elucidated 76% of the total variation.

The present result agrees with the findings of Ahmed *et al.* (2011), who showed several traits contributed to variation in PC1 and PC, in oat accessions. Kujur *et al.* (2017), reported in oat about the contribution of the number of spikelet per panicle, seed and biological yield to PC1. He concluded that the selection of accessions from the maximum (PC1) than accessions in other PCs is useful (Savova and Valcheva, 2017).

When we separately considered *A. sativa* accessions, the first principal component contributed 35.5% of the total variation. The contribution of the first four principal components value progressively

decreased from the first principle component. Due to this the fourth principal components accounts for 11.5% of the total variation. The first four principal components have an Eigenvalues greater than or equal to 1.5 explained 75.7% of the total variation.

6.2 Molecular genetic diversity of oat accessions

Evaluation of genetic diversity of oat accessions is essential for oat breeding. Outcomes of assessments provide a general guide for choosing parental lines to make appropriate cross combinations for particular breeding purposes. Evaluating genetic variation among oat accessions collected from Ethiopia and the few accessions acquired from USA, the Netherlands, and Australia are discussed.

6.2.1 Allelic patterns/diversity of oat accessions

In this study, 317 alleles were generated from 19 primers, and the mean number of alleles per locus was 55.42. The average number of alleles detected per locus ranged from 11 to 122 in 176 oat accessions belonging to five species.

Fu *et al.* (2004) detected 125 alleles of 25 SSRs in 125 *A. sativa* samples; Choudhary (2013) reported 129 alleles of 40 primers in 96 *A. sativa* genotypes with an average of 3.22 alleles per locus. In addition, Nersting *et al.* (2006), who reported that 66 alleles of *A. sativa* from seven primers studied using 84 cultivars and landraces.

The majority of the primer pairs generated greater than 0.7 PIC and they were highly informative. Cabral *et al.* (2012), reported in *A. strigosa* and *A. barbata* that 62% of the SSRs tested were polymorphic among *Avena* species. Boczowska and Tarczyk (2013), also reported in *A. sativa* that 59.5% of the SSRs tested were polymorphic among *Avena* species.

Similar result in fragment size of the allele was reported by Fu *et al.* (2007) and Li *et al.* (2007). They reported that the allelic size varied greatly, ranging from 35 to 378 bp, depending on the length of repeat motif. The usefulness of markers is determined mainly by the percentage of polymorphic fragments. High levels of polymorphism show that the distinct species under study are genetically diverse.

On unique bands a similar finding was reported by Fu *et al.* (2007) and Nikoloudakis *et al.* (2015). They reported that 55 alleles (44%) were present in a modest proportion of the accessions (with frequencies less or equal to 0.05) in *A. sativa* landraces. In the present study, the minimum number and the maximum number of alleles observed ranges from 11(AM53) to 122 (AM31). Choudhary (2013) reported that a total amplified fragment for each primer ranged from 2-6 in *A. sativa* accessions. The result showed high number of amplified bands, because the primers are different.

The number of polymorphic bands observed for the accessions ranged from 87 to 262 with an average of 54 bands. The mean band frequency ranged from 0.023 to 0.319 with an average of 0.111. The number of polymorphic band variation in geographical origin of accessions ranged from 2.3 to 31.9 with an average of 11.1.

In cultivated crop species, geographical distribution patterns reflect both the specific selection pressures prevailing in a particular environment and the history of selection and production (Hawtin *et al.*, 1996). Hence, in diversity study, including genotypes collected from different geographic areas has been adopted as a strategy to capture all sorts of allelic diversity of a particular crop plant. In order to have all genetic resource with unique alleles, the breeders should have to conserve all genotypes collected from all regions for the further selection process. The study could not identify regions that

need special attention (hot spots) for conservation. For the breeding program, the plant breeder should have to give special attention to accessions that are found in different clusters.

6.2.2.1 Analysis of molecular variance of oat accessions

AMOVA was conducted for regions with the assumption of the existence of high variation among regions as compared to within regions. The results of AMOVA showed low (5%) genetic variation among populations, and high genetic variation (95%) was revealed within populations. Similarly, a fairly low contribution of regions to total variation as compared to within a region was reported for oat of Greek and Eastern European (Nikoloudakis *et al.*, 2015)

Generally, moderate differentiation within the five *Avena* species indicates high gene flow among accessions of different regions and low fixation index. It is expected because crossability species are easy in the same ploidy level and for those that have different ploidy level may it be their genome similarity. F_{ST} value and population differentiation have an inverse relationship with gene flow (Nm). The F_{ST} value of the *A. sativa* population obtained in this study was 0.051, which is a moderate population differentiation. The gene flow among the populations was high ($Nm = 4.70$). This implied significant exchange of seed even for wild species among farmers in the studied regions, even at the country level.

Considering the partitioning of diversity within and between populations, Wright's Fixation index (F_{ST}), which is calculated from allele frequencies, plays an important role in diversity studies (Lowe *et al.*, 2009). Besides measuring the partitioning of diversity among and within populations, it can be interpreted as a measure of differentiation among subpopulations, and also as the reduction of heterozygosity of subpopulations due to random genetic drift. In this respect, F_{ST} offers the possibility to calculate gene flow (Nm) between populations according to the formula $Nm = (1 - F_{ST}) / 4F_{ST}$, which

can be interpreted as the number of migrants between populations per generation. A value of F_{ST} lying 0 to 0.05 indicates low genetic differentiation, 0.05 to 0.15 moderate differentiation, and 0.15 to 0.25 above great differentiation

6.2.2.2 Genetic distances based on geographical origin of accessions

The overall magnitude of genetic distance recorded in this study was lower compared to reported values based on seed characters (Rezai and Frey, 1988), isozyme markers (Hunter, 1995), RFLP markers (Goffreda *et al.*, 1992) and AFLP (Fu *et al.*, 2005). The diversity observed within accessions indicated a large proportion of variation within the accessions of the species. However, some traits are region specific still regions are important depending on the target traits. For instance, materials with lodging resistance and large grain size were found from the USA, Shewa, Gojam, Welega, and Wello accessions. Grain and biological yield play a major role since farmers need oat for the market and household consumptions especially in Shewa.

Regarding genetic distances among *Avena* species, our assumption was the small genetic distance would found within tetraploid and hexaploid species as well as high genetic distance were found between tetraploid and hexaploid species because of the their ploidy level and genome difference. This study showed the smallest Nei genetic distance between *A. abyssinica* (tetraploid) and *A. fatua* (hexaploid) despite their having different genomes. The genetic distance between the hexaploid *A. sterilis* and tetraploid *A. vaviloviana* is small. But the two-tetraploid species, *A. abyssinica* and *A. vaviloviana*, showed moderate genetic distance, relative to the other hexaploid species. The genetic distance between the three hexaploid species were 0.104 between *A. fatua* and *A. sativa* 0.102, between *A. fatua* and *A. sterilis* and 0.076 between *A. sativa* and *A. sterilis*. This may imply that the accessions, with the D and B genomes, were derived from the A genome (Katsiotis *et al.*, 1997), and so still share similar features. The B genome found in tetraploid and D genome found in hexaploid species are thought to have been originated from the A genome. Thus, the distance between tetraploid and

hexaploid oat is not as large as expected. The other possible reason may be that the alleles are found in the same genome.

6.2.3 Clustering and population differentiation

6.2.3.1 Clustering

The *A. sativa* and *A. abyssinica* populations were not strongly differentiated from one another on the basis of their geographical origin. In a cluster, accessions of different geographical origin were grouped together. Moreover, different *Avena* species were grouped in the same or two/more clusters, this confirms that accessions of the different region of origin were not differentiated. These results may reflect the impact of seed exchange between farmers and the introduction of similar germplasm to different regions. Likewise, the wild oat species were moved along with cultivated oat, wheat, and barley seeds. However, the cluster had some species-wise pattern in which the cultivated and wild species were separated. It was found that materials of similar genomes did not cluster together but species with different genomes cluster together. This is because of the genome B and D were derived from A genome. The second reason is the primers maybe found in the same genome across different species. This finding is not in agreement with the finding of Achleitner *et al.* (2008), who found that material with a similar genome of *Avena* species clusters together. Regarding *A. sativa*, USA populations showed a little differentiation from the other population of this species. Studies in other cereals (Assefa *et al.*, 2007) also indicated that geographical origins do not have defined relationship with the pattern of genetic diversity

Oat accessions from the different source of origin had no equal diversity. Many genotypes in a single cluster depict these genotypes are more closely related and had less genetic variation among them. It further implies, using genotypes occupying a common cluster will be of little use and those from diverse clusters are beneficial for hybridization programme in oat improvement.

Our findings of a clear distinction between the wild and cultivated species agree with previous reports (Fu *et al.*, 2005; Newell, 2011; O'Donoghue *et al.*, 1994) which support the hypothesis that *A. sativa* was domesticated independently from wild progenitors (Zohary *et al.*, 2012).

6.2.3.2 Principal coordinate analyses (PCoA)

A PCoA analysis of the complete data matrix (Figure 25) shows that the collective SSR markers showed a clear partitioning of wild vs. cultivated accessions in the scatter plot. Clustering on the primary axes is somewhat correlated with species type of the germplasm. The accessions of *A. sativa* alone did not cluster based on their geographical origin proximity. Accessions of one region found in the different cluster groups. The populations are not differentiated. This pattern of clustering is observed in the accessions *A. abyssinica* and *A. sativa* species. This result disagreed with that of Achleitner *et al.* (2008), who reported accessions are grouped based on their geographical origin using AFLP markers. The difference may be the marker and accessions used for the study.

Relatively, USA accessions showed slight differentiation that is the majority of the USA accessions were assigned to a single cluster. This pattern of clustering was followed by Gojam, and the Netherland and Australia accessions grouped in the first cluster. Accessions from Shewa, Arsi, Gondar, Wello and Welega had high admixture among clusters. Accessions from these regions are not differentiated.

6.2.4 Genetic differentiation

Based on allele frequencies, genotypes were assigned to populations in STRUCTURE analyses, if populations were admixed and allele frequencies were correlated due to shared ancestry and/or migration. The population of most, but not all, species showed levels of genetic structuring, which may be due to a variety of exclusive agents. Genetic structure of populations may be shaped and influenced with environmental barriers, historical processes, and life histories (e.g. mating system). Bayesian clustering

based on this model with admixture, assuming each individual have inherited some proportion of its ancestry from each of K populations (Pritchard *et al.*, 2000). As defined by Evanno *et al.* (2005) analysis to determine number of K clusters using STRUCTURE has resulted in $K = 9$.

A graphic representation of the estimated membership coefficients to the $K = 2$ to $K = 9$ clusters for each individual obtained running structure was shown in Figure 28. The figure showed no distinct structure among populations, but it revealed the admixture of populations. Each accession in the graph was represented by a single vertical line broken into K colored segments, with lengths proportional to each of the K inferred clusters. Each color represents the proportion of the membership of each accession, represented by a vertical line, to the nine clusters. At $K=2$ from the graph, we observe the USA, the Netherlands and Australia materials were less admixed as compared to others while Arsi and Bale materials showed high admixtures. This analysis supports the result obtained from the Neighbor-Joining tree.

6.3 Hydroponic screening of oat accessions

6.3.1 Optimizing Al concentration

Lower and higher aluminium concentration did not discriminate the Al^{3+} toxicity proficiency variability of oat. At both lower and higher concentrations, analysis of variance revealed non-significant ($P < 0.05$) difference among oat accessions. The optimum concentration was $112.5 \mu M Al^{3+}$; and this has at least two advantages: 1) It helps to group aluminium tolerance variability of oat accessions as tolerant, intermediate and sensitive categories; and 2) It had better statistical discrimination value for the control and treatment group.

6.3.1.1 Evaluation of oat species for aluminium toxicity tolerance

Aluminium toxicity potency variability test of 150 oat accessions was done using hydroponic experiment. Of the total 150 accessions screened, 40, 16, 74, 5, and 15 accessions belong to *A. abyssinica*, *A. fatua*, *A. sativa*, *A. sterilis* and *A. vaviloviana*, respectively. Based on the root length 78 (52%) accessions were tolerant, 54 (36%) intermediate, and 18 (12%) sensitive for aluminium toxicity. The majority of the accessions showed tolerance, which indicates a high potential of genetic resource that can be utilized to produce varieties that withstand aluminum toxicity. A plant breeder could have to test these tolerant accessions in the field under the actual condition and select the superior accessions across a wide range of environment and acidic soils.

Genotypes with root length inhibition index (RLI) less than 50% were tolerant and those with greater than 75% were sensitive, while root length inhibition index values between 50% and 75% were intermediate. All species had better tolerance, and non-significance difference exist among the species. The treatment and the control were significant in all *Avena* species. Seventy eight accessions (22 from *A. abyssinica*, eight from *A. vaviloviana*, six from *A. fatua*, four from *A. sterilis*, and 38 from *A. sativa*) were classified tolerant, 54 intermediate (13 from *A. abyssinica*, seven from *A. vaviloviana*, seven from *A. fatua*, and 27 from *A. sativa*), and 18 susceptible or sensitive (five from *A. abyssinica*, three from *A. fatua*, one from *A. sterilis*, and nine from *A. sativa*).

Majority of the accessions that were collected from different regions were tolerant, except region, of Welega, Bale, and USA. However, the tolerant accessions that found in region Welega had highest RLI. From 78 tolerant accession the top 10% of Al toxicity tolerant were predominantly obtained from *A. abyssinica* and *A. sativa* accessions and most of these accessions were collected from Welega, Gojam, and Shewa regions. Soil acidity in these regions are high as a result the accessions from these regions have developed Al toxicity tolerance capacity.

6.3.1.2 Effect of Al-toxicity and variation among oat accessions

The optimal aluminium tolerance concentration level of 112.5 μM suggested for screening oat in the study and this optimal concentration of Al was higher than 30 μM suggested for barley (Echart *et al.*, 2002), 30 μM for wheat (Hede *et al.*, 2002), 20 μM for maize; (Boscolo *et al.*, 2003; Piñeros *et al.*, 2005). This is because the optimum concentration for screening aluminium tolerance is crops specific.

7. CONCLUSION AND IMPLICATIONS OF PRESENT STUDY FOR FUTURE PROSPECTS

At the genus level, accessions of all five *Avena* species, *A. sativa* and *A. abyssinica* were analyzed on regional basis using morpho-agronomic data. The qualitative and quantitative traits variations were observed and fairly distributed across their geographical origin of accessions. These indicated low incidence of genetic diversity in oat accessions across their geographical origin (regions), rather greater variation within the populations were observed than among the populations. *A. sativa* accessions were not differentiated based on their geographical origin. A little differentiation was observed between Ethiopian and USA materials for *A. sativa* accessions. *A. abyssinica* accessions did not show clear differentiation among populations/ regions. The Dendrogram of AHC with Euclidean dissimilarity distance of unweighted pair group arithmetic average analysis revealed a clear grouping of wild and cultivated oat in separate cluster. The accessions from one region were grouped in different clusters both in *A. sativa* and *A. abyssinica* accessions. The top 5% superior accessions with grain yield were identified, and these are Acc No.1000, 204, 2000, 16, 205, 206, 208, and 207, and 3000. All top % best performance accessions are Ethiopian origin.

The result at molecular data analysis showed similar results in differentiation of the five *Avena* species. We observed clear differentiation between wild and cultivated oat accessions, whereas the accessions were not differentiated on the basis on their geographical origin. The close relationship was observed between *A. vaviloviana* and *A. sterilis* species, similarly, *A. abyssinica* and *A. fatua* are closely related. Comparatively *A. vaviloviana* and *A. sterilis* had a short distance to *A. sativa* than the distance between *A. abyssinica* and *A. fatua* to *A. sativa*.

Both morpho-agronomic and SSR markers resulted in the presence of little differentiation between wild and cultivated oat and no differentiation of accessions based on their geographical origin. The result at both molecular marker and morpho-agronomic traits more similarity between of *A. abyssinica* with *A. fatua* species, likewise *A. vaviloviana* and *A. sterilis* showed more similarity. Relatively, *A. vaviloviana* and *A. sterilis* had low distance to *A. sativa*.

The hydroponic experiment revealed the presence of high aluminium toxicity tolerance of oat accessions. The variability of oat accessions on aluminium toxicity tolerance had no significant difference among species and regions.

The present study of the phenotypic and molecular genetic diversity of oat in Ethiopia generate very useful information to understand the population structure, differentiation, and gene pools of oat accessions. These results may play their own role for the future improvement of crop production and productivity to stable the suitability of the food security. The impact of the study are listed in the following paragraphs.

- Knowledge about existing variability and divergence pattern among accessions/ species is helpful in selecting parents for a crossing programme and determining a particular breeding procedure to follow. The best top 5% grain yield performing accessions could be used as parents for the development of superior breeding lines. Association study among different characters helps the breeders to select better accessions based on desirable economically important character. This might have a great encouragement on oat production improvement in the future.
- The study provided ample information for oat breeders to develop Al^{3+} toxicity tolerant lines. The study showed the possibility of developing lines and genotypes that can tolerate aluminum toxicity, so the tolerant accessions in the Ethiopian context can support agricultural development in acidic soil area in the country in the future.

- Accessions within the region/country showed high genetic variation than among regions/countries, showing that each region has a great potential of genetic resource which might be used for oat improvement in the future. Therefore, we should have to conserve accessions that were collected at each region, for utilization of germplasm for the future breeding program.

8. RECOMMENDATIONS

Traits found in distantly related wild *Avena* species are very imperative for cultivars development. Utilization of genes from hexaploid wild relatives is the choice for introgressing genes to oat cultivars (*A. sativa*). Thus, this study is of foremost important for the improvement of oat cultivars in Ethiopia. Generally, the following recommendations can be made from the results:

- The study revealed that for individual species more variability exists within regions than among regions/country. It is recommended that breeders can use material from any of the regions.
- The study also revealed the existence of moderate genetic variability among species. It is recommended that breeders introgressed desirable traits from the wild to the cultivated species.
- The 19 SSR markers used in the present study were found to be highly polymorphic and reproducible. We recommended these SSR marker for use in the study of genetic diversity of the other oat accessions not included in the present study.
- Even though the distribution of allele bands were uniform across regions, each region had private alleles in different *Avena* species. It is recommended to conserve the accessions from all regions in the Ethiopian gene bank.
- Through hydroponic culture screening methods for aluminium toxicity tolerance of oat are fast, they cannot totally replace the actual field experiment in acidic soils. It is recommended that the tolerant 78 (52%) accessions screened should be tested under field condition.
- We recommend that breeders should consider the best top 5% grain yield performing accessions to utilize in oat breeding.
- We also recommend that oat breeders consider the top 5% aluminium toxicity tolerance accessions of oat for utilization in developing varieties.

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10. Appendix

Appendix 1: Quick-DNA™ Plant/Seed 96 Kit Catalog No. D6021

Protocol

For optimal performance, add beta-mercaptoethanol (user supplied) to the **Genomic Lysis Buffer** to a final dilution of 0.5%(v/v) i.e., 250 µl per 50 ml or 500 µl per 100 ml.

1. Add up to 150 mg of finely cut plant or seed sample¹ to a **ZR BashingBead™ Lysis Tube (2.0 mm)**. Add 750 µl **BashingBead™ Buffer** to the tube and cap tightly.
2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for ≥ 5 minutes.

Note: Processing time will vary based on sample input and bead beater. Times may be as little as 5 minutes when using high-speed cell disrupters (FastPrep® -24) or as long as 20 minutes when using lower speeds (e.g., Disruptor Genie®).
3. Centrifuge the **ZR BashingBead™ Lysis Tube (2.0 mm)** in a microcentrifuge at ≥10,000 x g for 1 minute.
4. Transfer up to 400 µl supernatant to a **Zymo-Spin™ III-F Filter** in a **Collection Tube** and centrifuge at 8,000 x g for 1 minute. Discard the **Zymo-Spin™ III-F Filter**.
5. Add 1,200 µl of **Genomic Lysis Buffer** to the filtrate in the **Collection Tube** from Step 4. Mix well.
6. Transfer 800 µl of the mixture from Step 5 to a **Zymo-Spin™ IIC Column²** in a **Collection Tube** and centrifuge at 10,000 x g for 1 minute.
7. Discard the flow through from the **Collection Tube** and repeat Step 6.
8. Add 200 µl **DNA Pre-Wash Buffer** to the **Zymo-Spin™ IIC Column** in a new **Collection Tube** and centrifuge at 10,000 x g for 1 minute.
9. Add 500 µl **g-DNA Wash Buffer** to the **Zymo-Spin™ IIC Column** and centrifuge at 10,000 x g for 1 minute.
10. Transfer the **Zymo-Spin™ IIC Column** to a clean 1.5 ml microcentrifuge tube and add 100 µl (50 µl minimum) **DNA Elution Buffer** directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.
11. Place a **Zymo-Spin™ III-HRC Filter** in a clean **Collection Tube** and add 600 µl **Prep Solution**. Centrifuge at 8,000 x g for 3 minutes.
12. Transfer the eluted DNA to a prepared **Zymo-Spin™ III-HRC Spin Filter** in a clean 1.5 ml microcentrifuge tube and centrifuge at exactly 16,000 x g for 3 minutes.

The filtered DNA is now suitable for PCR and other downstream applications.

¹Dried samples may be hydrated in water prior to processing to improve DNA extraction efficiency.

²The **Zymo-Spin™ IIC Column** has a maximum capacity of 800 µl.

About the author

Ashenafi Alemu was born in June 28, 1973, in the Amhara region, Bahir Dar. He first went to Ayalewu Mekonnen Elementary and Junior Secondary School. Then after, he attended at Tana Haik compressive high school to pursue his high school education. In September 2000, he joined the then Dilla University and graduated in a BED in Biology in July 2004. After completing his undergraduate education, he was employed by Ethiopian Ministry of Education (MoE) in July 2004. During his occupation as a high school teacher from July 2004 to September 2007, he served as a teacher of Biology with various demanding responsibilities, such as Head of the Biology Departments and program coordinator at Ayer Tena High School, Addis Ababa. In April 2005, he started his postgraduate studies at Addis Ababa University and graduated in M.Sc. Degree in Applied Genetics in July 2008. Upon completing his postgraduate education, he has joined the University of Gondar (UoG). Since October 2009, I have been serving as a lecturer and researcher at UoG to date. I served at various demanding responsibilities, including offering more than 11 different courses for the undergraduate and 4 different courses for postgraduate students, at the post of the Focal Person of Natural and Computational Sciences Faculty such as Curriculum Reviewer, Quality Assurance and Auditing, and Gender and anti-HIV-AIDS coordinator. Consequently, he joined the Department of Microbial, Cellular, and Molecular Biology in Addis Ababa University (AAU) to pursue his PhD education in Applied Genetics. In December 2018, he completed his PhD education. During his research and graduate/post-graduate education career, he has produced various journal manuscripts for local and international journals. Further, he is transferring the technology the adoption of oats, *A. sativa*, production in Amhara Region, doing mega research, and offering courses to undergraduate and MSc students at University of Gondar.



Ashenafi Alemu Tiruneh: The son of Tiru Zeru Tessema and Alemu Tiruneh