Analysis of Genetic Diversity and Population Structure of Tetraploid Wheat Accessions Using Simple Sequence Repeat (SSR) Markers

Addisalem Moges Sime

A Thesis Submitted to the Department of Applied Biology,

School of Applied Natural Science,

Presented in Partial Fulfillment of the Requirement for the Degree of Master of Science in Applied Biology (Specialization in Biotechnology).

Office of Graduate Studies

Adama Science and Technology University

March, 2023

Adama, Ethiopia

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DECLARATION

I hereby declare that this Master Thesis entitled “Analysis of Genetic Diversity and Population Structure of Tetraploid Wheat Accessions Using Simple Sequence Repeat (SSR) Markers” is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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I/we, the advisor(s) of this thesis, hereby certify that I/we have read the revised version of the thesis entitled “Analysis of Genetic Diversity and Population Structure of Tetraploid Wheat Accessions Using Simple Sequence Repeat (SSR) Markers” prepared under my/our guidance by Addisalem Moges Sime submitted in partial fulfillment of the requirements for the degree of Masters of science in Applied Biology (Specialization Biotechnology). Therefore, I/we recommend the submission of a revised version of the thesis to the department following the applicable procedures.

Major advisor	signature	Date
Co-advisor	Signature	Date
Co-advisor	Signature	Date
Co-advisor	Signature	Date

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We, the undersigned, members of the Board of Examiners of the thesis by Addisalem Moges Sime have read and evaluated the thesis entitled "Analysis of Genetic Diversity and Population Structure of Tetraploid Wheat Accessions Using Simple Sequence Repeat (SSR) Markers" and examined the candidate during the open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied biology (Biotechnology).

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LIST OF ACRONYMS AND ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
CIMMYT	International center for agricultural research in the dry areas
CTAB	Cetyl- trimethyl ammonium bromide
DNA	Deoxyribonucleic Acid
ICARDA	International maize and wheat improvement center
ISSR	Inter simple sequence repeat
NJ	Neighbor joining
PCOA	Principal Coordinate Analysis
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeat
UPGMA	Unweighted pair group with arithmetic mean
USAD	United states department agriculture

ABSTRACT

Wheat is one of the most important cereals world-wide and it is grown in many areas. In order to assess the genetic diversity and population structure of tetraploid wheat in Ethiopia and infer the possible breeding, conservation and management measures, 102 tetraploid wheat germplasm accessions representing five populations were analyzed using 11 simple sequence repeat (SSR) markers. A wide range of diversity indices including number of alleles, effective number of alleles, Shannon's information index, percentage of polymorphic loci (%P) and polymorphic information content (PIC) were computed. All SSR markers were highly polymorphic, resulting an average of polymorphic information content (PIC) value of 0.69 with an overall mean of 7 alleles per locus. The populations showed a high mean observed heterozygosity (0.47), expected heterozygosity (0.6), Shannon's Information Index (1.20) and percentage of polymorphic loci (94%). Analysis of molecular variance (AMOVA) statistically significant high genetic differentiation (Fst = 0.22) *where 78% of the total genetic variation was accounted within populations and 22% for the genetic variation accounted among populations. Gene flow accounted to 0.89. The highest and lowest pairwise genetic differentiation and Nei's genetic distance were observed between the emmer populations and international durum wheat lines, and Oromia and Amhara regions, respectively. The Neighbor-joining (NJ) clustering and principal coordinate analysis (PCoA) grouped the accessions into two major clusters the Emmer population on one hand and durum population on the other hand. In addition, Bayesian model-based population STRUCTURE analysis revealed four genetic groups with genetic admixture. Overall, this study confirmed the existence of high genetic diversity in tetraploid wheat which may use as a source for the improvement of wheat through selection and conservation. Tetraploid wheat germplasm from Amhara, Oromia and CIMMYT showed high gene diversity and hence, useful sources of breeding relevant alleles. Thus, proper germplasm management strategies need to be deployed for their conservation.*

Key words: AMOVA, Genetic diversity, SSR marker, Tetraploid wheat

1. INTRODUCTION

1.1. Background of the study

Cereals are the world's most significant food crops, feeding 70% of the world's population (Bousba *et al*., 2012). The world's most widely consumed agricultural plant and the staple cereal of old world agriculture is wheat, which is followed by rice and maize (FAOSTAT, 2011). Between 8000 and 10,000 years ago, wheat (*Triticum L.)* was one of the most important cultivated cereal crops in the Fertile Crescent (Lev-Yadun *et al*., 2000). Wheat is one of the most essential cereals, providing nourishment to more than half of the global population (Kashif *et al*., 2020). Tetraploid wheat domestication took place about 12,000 years ago in the Fertile Crescent, when ancient farmers selected among cultivated forms of wild emmer (*Triticum turgidum* ssp. *dicoccoides*) (Kabbaj *et al*., 2017).

East African countries cultivate around 2 million hectare of wheat, of which only 630,000 hectare are farmed with durum wheat (Tidiane *et al*., 2019). Wheat was the fifth most important cereal crop in Ethiopia until the 1990s, followed by teff, maize, barley, and sorghum in terms of area coverage and production (Lev-Yadun, 2000). Currently ranks fourth after teff, maize and sorghum in area coverage and third after maize and teff in total production (Adugnaw Anteneh and Dagninet Asrat, 2020). USDA recent report indicate that Ethiopian wheat consumption (6.7 million tons) quickly outpaced production (>5 million tons) due to rapid urbanization, population growth, and changing eating patterns (Esterhuizen and Bonus, 2020). The demand for wheat grain has expanded tremendously because of the growing interest in commercial and staple foods such as local bread, porridge (genfo), local beer (tela), roasted grain (Kolo), boiled grain (nifro), pasta, and various confectionery items (Wuletaw Tadesse *et al.*, 2018).

Durum wheat {*Triticum turgidum L. var. durum*) is the 10th most important crop worldwide owing to its annual production of 37 million tons (Kabbaj *et al*., 2017), mostly farmed in the Mediterranean region's arid land under demanding environmental conditions (Damania *et al*., 1992). Ethiopia is the major durum wheat producer in sub-Saharan Africa (SSA), with a durum acreage of 0.6 million hectares (Kefyalew Negisho *et al*.,,2021). It is far cultivated in a range of environments, besides in very arid and lowland regions. Durum wheat is traditionally grown inside the more marginal vital and northern parts of the country, at the

same time bread wheat (*T. aestivum L.)* is grown inside the extra favorable, wetter crucial, and southern components (Belay Simane *et al*., 1993).

Cultivated emmer wheat (*Triticum dicoccon* Schrank) is one of the world's oldest crops that has been used as a main source of food for millennia (Curna and Lacko, 2017). According to molecular genetics and archaeological evidence emmer wheat is emerged from wild emmer wheat in the fertile crescent region, 8,000 years ago (Zommita *et al*., 2022). In 2016, 49,200 tons of emmer wheat were produced on an estimated 24,000 hectares of land in Ethiopia, where it is still grown. About 7% of the Ethiopian national wheat production is emmer wheat, which is mostly cultivated on marginal land areas including Bale, Arsi, Shewa, Harerege, Wollo, Gojam, Gondar and Tigray for domestic use (Bethlehem Melese *et al.,* 2019).

The knowledge of genetic diversity of a crop species helps a breeder in choosing parents for breeding program to improve desirable agronomic traits such as yield, yield related traits, biomass traits, and biotic and abiotic stress resistances and tolerances (Allard, 1999). Nowadays, molecular marker technology are widely used to unfold the genetic structure of crop plants so far, several DNA markers including RFLP,RAPD,AFLP,ISSR,SSR,SNPs etc. have been developed, and widely used to disclose the genetic structure of crop plant. These markers systems vary in their principle, quantity of polymorphism, value and reproducibility (Qadir *et al*., 2020). Among these marker system SSR marker is considered to be the marker of choice for genetic and trait analysis due to its (Nadeem *et al*., 2017), multi allelic nature , evenly distributed all over the genome, co-dominant, highly polymorphic, highly specific, very reputable. SSR markers are cheap, easy to run and require small amount of DNA to run the analysis. It is also semi-automated and performs without the need of radioactivity (Tanmay and Soumen, 2016).

1.2. Statement of the problem

Tetraploid wheats (2n =4×=28) have been under cultivation in Ethiopia for thousands of years, and have acquired a diverse set of characteristics that makes the country a center of diversity for these species (Perrino and Porceddu, 1990). For effective conservation and use of genetic sources, evaluation of genetic variant within collections can be dramatically enhanced by using DNA molecular markers (El Hussein *et al*., 2014). Amongst specific markers developed today, SSR markers have been widely used to investigate the genetic diversity and other tetraploid wheats (Gupta and Varshney, 2000, Vieire *et al*, 2016).

Al Khanjari *et al*. (2006) studied the molecular diversity of 6 Omani tetraploid wheat landraces using 30 SSR markers. A collection of 85 Kazakhstan tetraploid wheat accessions was studied using seven SSR markers (Zatybekov *et al*., 2020). Oliveira *et al*. (2012) also studied the taxonomy, evolution and genetic diversity of tetraploid wheat landraces form the Mediterranean basin using microsatellite markers.

In Ethiopia, SSR markers are also used to profile the genetic structure of tetraploid wheat. Jemanesh Kifetew *et al.* (2013) assessed the genetic diversity of Ethiopian tetraploid wheat landraces and improved durum wheat varieties using SSR markers linked with stem rust resistance and neutral SSR markers. Yifru Teklu *et al.* (2006) analyzed 141 Ethiopian tetraploid wheat landraces consisting of three species *Triticum durum* Desf., *T. dicoccon* Schrank and *T. turgidum* L using 29 SSR markers and found a high level of polymorphism and detected a large number of alleles that are unique for each species. Alayachew Shiferaw and Kassahun Tesfaye (2017) studied the genetic diversity of 85 Ethiopian emmer wheat landraces using seed storage proteins markers. However, the so far diversity studies on tetraploid wheat in Ethiopia are relatively low as compared to the huge germplasm collections the country is endowed with. The researchers that have been conducted so far are either limited in scope or not exhaustive, the present work fulfill the gaps by analyzing the genetic diversity of tetraploid wheat using SSR markers. Thus, the present study was carried out to investigate the genetic diversity and population structure of existing tetraploid wheat accessions in Ethiopia using SSR markers to generate valuable information for wheat improvement through breeding and its conservation. Therefore, the present study was designed with the following general and specific objectives.

1.3. Objectives

1.3.1. General objective

The general objective of the present study is to characterize the genetic diversity and population structure of tetraploid wheat accessions using simple sequence repeat markers.

1.3.2. Specific objectives

- To analyze the genetic diversity of tetraploid wheat accessions using simple sequence repeat markers.
- To determine the population structure of tetraploid wheat accessions using simple sequence repeat markers.

1.4. Significance of the study

This study generated valuable information on the genetic diversity and population structure in the existing tetraploid wheat accessions in Ethiopia. The information is useful for breeders in general and plant science researchers to select appropriate parental lines in further tetraploid wheat breeding program. The information also has great implication in designing appropriate tetraploid wheat genetic resource management strategies. Moreover, the highly informative markers used in this study are helpful genetic tools for further genetic analysis in wheat. As the SSR markers used in this study are believed to be very informative, the generated data could be extrapolated for the exploitation of other tetraploid wheat genetic resources.

2. LITERATURE REVIEW

2.1. Origin, domestication and distribution of tetraploid wheat

Wheat cultivation is started in the Mesopotamian Fertile Crescent about 12,000 years ago (Kabbaj *et al*.,2017), and from there it apparently spread to the center of East, North Africa, Asia, and ultimately Europe (Harlan, 1981). All domesticated varieties of the tetraploid wheat *T. turgidum* were descended from wild emmer, according to genetic and historical evidence, and their domestication occurred in or nearby to the Fertile Crescent's southwest corner, which is the present geographic range where wild emmer resides (Feldman and Kislev, 2007).

Most tetraploid wheats (AABB, 2n = 4× = 28) are derived from wild emmer through hybridization between a wild grass, *Triticum uratu* (2n = 2× = 14, A genome source) and unknown source of the B genome (most likely Aegliops speltoides 2n= 2× = 14), long time ago (0.5 - 0.36 million years ago) via domestication (Hancock, 2004) and derived by natural selection. Then, the human-driven tetraploid wheat evolution process led to the domestication of emmer wheat. The evolution was due to the accumulation of mutations that occurred during the cultivation of wild emmer which formed the basis for selection. Hence, continued evolution under domestication evolved domesticated emmer wheat to other closely related tetraploid wheat species such as *T. durum, T. aethiopicum* and *T. polonicum* through mutations (Morris and Sears, 1967).

Triticum durum wheat originated thousand years ago the domesticated shape of a wild species named emmer wheat (*Triticum dicoccum Koern)* (Tidiane *et al*., 2019) from a hybridization between the wild diploid *T. monococcum* L. (A genome donor) and the donor of the B genome which, according to morphological, geographical and cytological evidence, has been recognized as *T. speltoides* or a closely related species (Figure 1) (Faris Hailu, 2011, Huang *et al*., 2002). Emmer (*T. turgidum L. spp. dicoccum Schrank ex Schubler*) is a tetraploid wheat (AABB; 2n = 4x = 28) which is a domesticated form of *T. turgidum spp. dicoccoides* (wild emmer wheat) (Arzani and Ashraf, 2017).

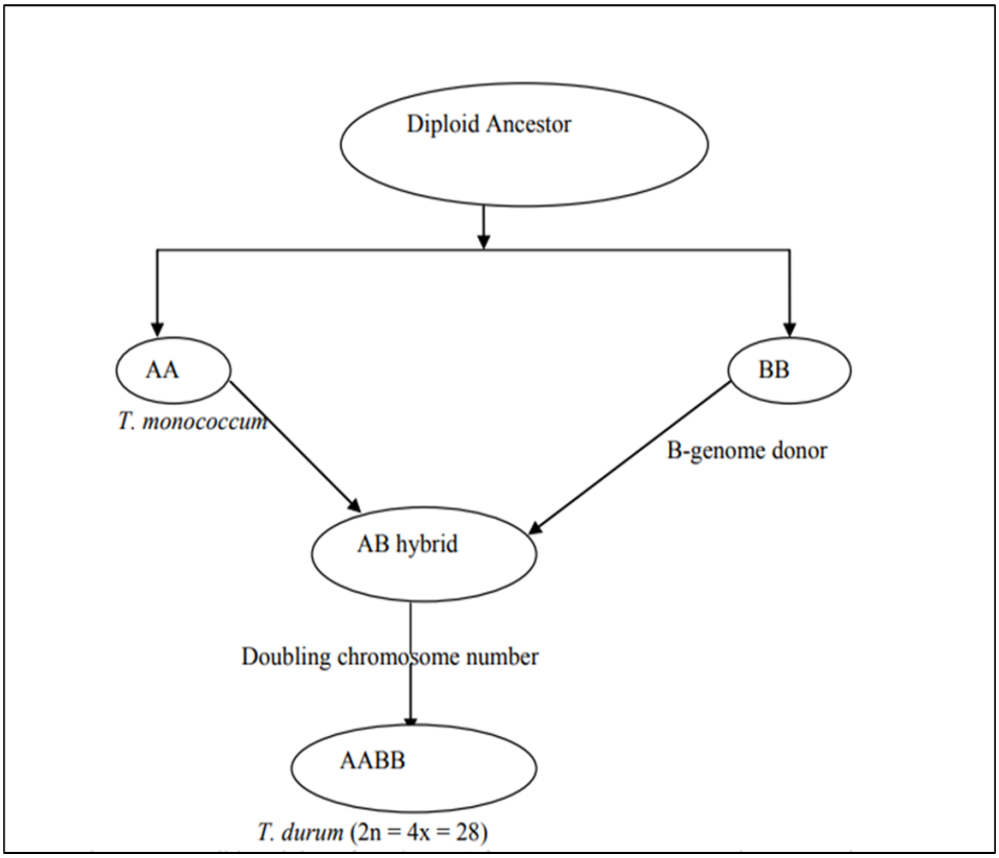

Figure 1. Possible origins of *T. durum* (Von Buren 2001 and Kerby and Kuspira (1987).

2.2. Taxonomy and biology of tetraploid wheat

Wheat taxonomy has a long history. The classification of the *Triticum L.* (wheat) genus is phylum: Angiospermatophyta, class: Monocotyledonopsida, order: Poales (Glumiflorae), family: Poaceae (Gramineae), subfamily: Pooideae, tribe: Triticeae, subtribe: Triticinae, genus: Triticum L (Balint *et al*.,2000). The species of Triticum are grouped into three ploidy 2X =14), tetraploid (2n=4X =28), and hexaploid (2n=6X=42). Three genomes (A, B, and D) comprise the polyploid series of wheat (Acquaah, 2007). The taxonomy of tetraploid wheat is controversial. Triticum has been recognized as a distinct genus in the Triticeae since Linnaeus's time (Goncharov *et al.* 2015). On the basis of morphological, cytological, or genetic characteristics, several classification schemes have been developed for genetic diversity study of *Triticum turgidum*. All varieties of *Triticum turgidum* are regarded as subspecies by both Van Slageren (1994) and MacKey(1998). For example, in the Van Slageren system durum is *T. turgidum subsp. durum*. Conversely, Dorofeev and colleagues classify all tetraploid wheat forms as individual species *(T. durum*) (Oliveira *et al.*,2012).

Wheat is predominantly self-pollinated plant with about 1– 4% natural cross pollination (Acquaah,2007).

2.3. Importance of tetraploid wheat

Wheat has played a fundamental role in human civilization and improved food security at the global and regional levels. It provides about 19% of calories and 21% of protein needs of daily human requirements at the global level (Braun *et al*., 2010). Wheat is one of the main food crops of the world, providing nearly 55% of the carbohydrates consumed globally (Gupta *et al*., 1999). Between 1970 and 2010, increased wheat imports met more than half of the increase in wheat consumption, and several developing countries became completely dependent on wheat imports (FAO 2013). Amongst all cultivated wheat, *Tritícum aestívum* and *Triticum durum* are the most vital cereal crops in the globe (Elias, 1995). Tetraploid wheats have played a critical role in human history (Olivira *et al*., 2012). Among tetraploid wheat, durum wheat (*Triticum durum Desf.)* is an essential food crop with 36 million ton of annual worldwide production (Tidiane et al., 2019). The wheat preferred for making pasta products is durum (Sissons, 2016). Durum wheat semolina and durum flour are used to fabricate pasta and non-pasta food products. Pasta products are manufactured by using blending water with semolina or durum wheat flour to form unleavened dough, that's shaped into distinctive shapes both by lamination or more generally extrusion (Hammami, 2020). The ensuing product can be cooked and eaten, so-referred to as clean pasta, or dried under controlled temperature and humidity conditions as dried pasta for later intake after its cooking. Pasta and couscous are paste products (Pollini et al. 2012). Other products from durum wheat encompass bulgur (cracked durum wheat) and frekeh (parched immature wheat kernel), each on-paste meals products (Elias, 1995, Hammami, 2020).

One of the promising ways to enrich macaroni products with dietary fibers and give them functional properties is the use of non-traditional plant raw materials for this type of product. For instance, - emmer wheat flour, in particular, which has a number of advantages in terms of nutrient composition compared to wheat flour, as well as the introducing dietary fibers in the recipe (Curna and Lacko-Bartosova, 2017). Emmer wheat (*Triticum dicoccum,* amel corn) belongs to the ancient chaffy wheat, is unpretentious to the cultivation conditions, resistant to agricultural diseases, fast-growing and has a rich chemical composition. Emmer wheat exceeds soft wheat in the content of dietary fibers (by 2.65 times), as well as protein,

reducing sugars, polyunsaturated fatty acids, some vitamins (pantothenic, folic acids and choline) and minerals (magnesium, phosphorus, zinc, manganese) (Khmeleva *et al*., 2021).

2.4. Production and productivity of tetraploid wheat

Tetraploid wheats have played a critical role in human history. Durum is the primary wheat for pasta and semolina production and the second most cultivated wheat after bread wheat (*Triticum aestivum L.)* (Oliveira, 2012). Now a day‘s durum wheat is cultivated in developed countries largely to support the food processing industries mainly to produce pasta and macaroni. Europe, South America, and the USA are the leading producers and consumers of pasta in the world, and Africa accounts only for 14.1% of total pasta production, mainly in Tunisia, Egypt and South Africa (IPO, 2021).

Durum wheat is one of the major industrial crops in Ethiopia. he pasta production covers about 20-30 % of the production of the country as a whole (150,000 tons) (Brasesco *et al*., 2019). However, according to the Italian Agency for Development Cooperation, such production does not meet the pasta demand of the country as 30,000 tons of pasta was imported in 2014 (Brasesco *et al.*, 2019). As Ethiopia is producing 90% of the durum cultivated in SSA, it is the only country capable of producing pasta using locally grown grain, while all other SSA countries import 483 million euros worth of durum grain in 2013 from Canada, Turkey, and the USA for pasta production requirements (Sall *et al*., 2019).

2.5. Wheat production constraints in Ethiopia

Wheat production and productivity in SSA, especially Ethiopia, are impacted by the complex and intricate interactions of biotic and abiotic elements as well as socioeconomic problems, particularly in the smallholder agricultural systems (Mulu Nigus *et al*., 2022). Diseases, insects, and weeds are the three main biotic restrictions that have a negative impact on Ethiopia's ability to produce wheat. The most important abiotic stresses are drought, (especially in the lowlands), soil acidity, erosion, poor soil fertility, water-logging, and pre-harvest sprouting (Wuletaw Tadesse *et al*.,2022). Other factors that contributed to low productivity of wheat in Ethiopia are Lack of access to improved varieties, outdated agronomic techniques, usage of marginal agricultural land, and terminal drought stress (Mulu Nigus *et al*., 2022).

2.6. Genetic diversity study

Variety in plant genetic resources (PGR) provides the possibility for plant breeders to broaden new and advanced cultivars with appropriate characteristics, which consist of both farmers favored traits (yield capacity and massive seed, and many others.) and breeders favored traits (pest and ailment resistance and photosensitivity, and so forth.) (Govindaraj, 2015). Diversity analysis can be performed through morphological, cytological, biochemical, and molecular characterization. In the beginning, morphological markers have been used for variety evaluation and are still in use.

2.6.1. Morphological characterization

Morphological traits had been the various earliest markers used in germplasm management, but they have some limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental impacts (Smith and Smith, 1992) also reflect variant of expressed regions of the genome(Mariana *et al*., 2019). The morphological characterization does no longer require highly-priced technology however massive tracts of land are regularly required for these experiments, making it probably more high priced than molecular assessment these trends are regularly vulnerable to phenotypic plasticity; conversely, this allows assessment of variety inside the presence of environmental variation (Mondini *et al*.,2009).

2.6.2. Cytological characterization

It includes a study of cytological functions like chromosome length, secondary constriction in chromosomes, the role of centromere, arm ratio, constitutive heterochromatic patterns, banding characteristics , DNA content, general genomic chromosome duration, chromosome volume, and many others. Different cytological capabilities were implemented to assess genetic variety inside and among species in maize, in potato, in lentil, in radish, and so forth. However, those have limited packages in genetic range evaluation on account of their restricted range and low decision (Bhandari, 2017).

2.6.3. Biochemical characterization

Earlier than the DNA revolution, Biochemical markers which include isozymes had been the primary molecular device for use for genetic characterization. Biochemical analysis is based on the separation of proteins into precise banding styles. It is a quick technique that requires

only small quantities of biological material. However, only a restricted number of enzymes are to be had, and thus, the resolution of variety is limited (Mondini et al., 2009). The application of these markers became restrained because the proteins and isozymes aren't genetic materials. They're products of gene expression so that they could be suffering from the environmental element (AlSamarai and Alkazaz, 2015) and also, they exhibit low polymorphism (Kumar, 2018). With the appearance of genomic tools, molecular markers became the technique of preference for genetic diversity evaluation (Bhandari, 2017).

2.6.4. Molecular characterization

Molecular genetics made it possible to study genetic variation in the natural population at the DNA level (Kumar *et al*., 2009). There may be various classes of markers, depending on the method employed for marker detection and amplification. Restrictions site alterations in the target DNA and subsequent hybridization with probe DNA are the foundation of restriction fragment length polymorphism (RFLP). The target DNA's restriction site alterations and the mutation at the primer annealing site are the basis of amplified fragment length polymorphism (AFLP). Simple sequence repeat (SSR), Inter simple sequence repeat (ISSR), and single nucleotide polymorphism (SNP) markers are also available (Datta *et al.*, 2011).

Restriction fragment length polymorphisms (RFLP) bring their definition in their name. A restriction fragment is a piece of DNA this is excised from the larger molecule through a protein known as a restriction enzyme or restriction endonuclease (Robert, 1989). It plays a crucial role in allowing scientists to map the human genome, in addition, to providing data on genetic illnesses via finding out in which the precise gene for diseases lies on chromosome. The RFLP was also one of the first techniques used for genetic fingerprinting despite that it is still slow and more tedious and requires a larger pattern size than different sorts of DNA analysis techniques (AlSamarai and Al-Kazaz, 2015).

RAPD for Random Amplification of Polymorphic DNA, is a kind of PCR reaction, but the segments of DNA which are amplified are random (Nachimuthu and Guruswami, 2011). RAPD markers generate less information per locus information per locus examined. The disadvantage of the usage of the RAPD markers method is generating less information per locus information per locus examined and the reproducibility among different gel runs that's due to the short primer length and low annealing temperature (Tanmay and soumn, 2016).

AFLP markers have emerged as a prime new form of genetic marker with broad software in systematic, pathotyping, population genetics, DNA fingerprinting, and quantitative trait loci (QTL) mapping (Muller and wolfenbarger , 1999). AFLP, molecular genetic polymorphisms are identified by means of the presence or absence of DNA fragments following restriction and amplification of genomic DNA (Blears, 1998).

Simple sequence repeats (SSR), additionally known as microsatellites, are DNA segments with a tandem repeat motif of 1-6 nucleotides (Bhattarai, 2021). SSR markers have wide applicability for genetic analysis in crop improvement strategies. They are widely used in plants because of their abundance, hyper-variability, and suitability for high throughput analysis (Datta *et al*., 2011). Inter simple sequence repeat (ISSR) marker is a PCR-based method that amplifies DNA segments between two identical microsatellite repeat regions that are orientated in the opposite way (Blears, 1998). A 200–2000 bp-long amplified product can be generated using the quick, easy, and effective ISSR method and also useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping, and evolutionary biology (Reddy *et al.*,2002).

2.6.4.1. SSR markers in genetic diversity study of wheat

The application of molecular markers in plant systems, extensive genetic maps were first developed in tomato and maize. Thereafter, this map was produced in a variety of other plant systems, including several cereal crops like rice, barley, and wheat Information on genetic diversity, relatedness and distance is a foundation for plant breeding for desired traits and DNA markers for alleles and traits are valuable tools for it (Poudel *et al*., 2019). Wheat serves as a model system for the study of polyploid cytogenetics because of the ease of chromosome manipulation (Gupta *et al*., 1999).

SSRs are preferred markers for genetic diversity studies, linkage mapping, association studies, and marker-assisted selection due to their high reproducibility, multi-allelic nature, co-dominant inheritance, robust amplification (Roder *et al*. 1998, Kumar *et al*., 2009) and have been extensively used for the assessment of genetic diversity and molecular genetic mapping of wheat (Poudel *et al*.,2019) because of this appealing character SSR markers are the best from other molecular markers (AFLP, RAPD, RFLP, etc.) for genetic diversity studies and fingerprinting of the crop (Mun *et al*., 2006). Despite their benefits, SSR are time-consuming, species-specific primers, flanking sequence information is needed (Kumar

et al., 2009) and low requirements of knowledge and instrumentation are some of the appealing features of SSR markers (Bhattarai,2021).

3. MATERIALS AND METHODS

3.1. Plant materials

This study was conducted using a total of one hundred two (102) wheat accessions (Appendices 4), 90 durum wheat and 12 emmer wheat. The germplasm was collected from Ethiopian Biodiversity Institute. Among the 90-durum wheat, 18 were landraces from Amhara, 32 from Oromia, 24 from International Maize and Wheat Improvement Center (CIMMYT) wheat collection, and 16 were from International Center for Agricultural Research in the Dry Areas (ICARDA) wheat collection. These wheat materials were grown on pots at greenhouse of Addis Ababa University main campus. A 15 days old young leaves were harvested in plastic bags containing silica gel for drying purpose.

3.2. Genomic DNA extraction

Fifty milligram of dried leaf sample was taken from each accession and grinded using Geno-grinder machine. Genomic DNA was extracted using CTAB protocol (Doyle and Doyle , 1987). The quantity and quality of the genomic DNA were checked out using Nano-drop spectrophotometer machine and 1% agarose gel electrophoresis (Appendices 2). Samples with high molecular weight DNA were maintained for polymerase chain reaction (PCR) and samples with low DNA quality and quantity were subjected to re-extraction.

3.3. Primer selection and optimization

Fifteen simple sequence repeat (SSR) markers were initially tested for their polymorphism and from which 11 primers that showed polymorphism and reproducibility were selected for this study (Table 1).

Table 1. List of SSR markers used in the study.

No.	Chromosme location	Locus	Tºa	Expected size	Sequence
1	1AS	Xwmc 24	52	152	Fw: GTGAGCAATTTTGATTATACTG Rv: TACCCTGATGCTGTAATATGTG
2	1BL	Xbarc 240	60	267	Fw: AGAGGACGCTGAGAACTTTAGAGAA Rv: GCGATCTTTGTAATGCATGGTGAAC
3	4AL	Xbarc 155	59	182	Fw: GCGAGTATTGACGTCTTATTTTTGAAF Rv: GCGTCATGAATTCTAACAATGTGCATA
4	6BL	XBarc178	59	266	Fw: GCGTATTAGCAAAACAGAAGTGAG Rv: GCGACTAGTACGAACACCACAAAA
5	7BS	Xgwm 46	60	187	Fw: GCACGTGAATGGATTGGAC Rv: TGACCCAATAGTGGTGGTCA
6	6AL	Xwmc256	61	191	Fw: CCAAATCTTCGAACAAGAACCC Rv: ACCGATCGATGGTGTATACTGA
7	5BL	Xgwm371	61	191	FW: GACCAAGATATTCAAACTGGCC RV: AGCTCAGCTTTGCTTGGTAC
8	6AL	Xwmc256	61	117	Fw: CCAAATCTTCGAACAAGAACCC Rv: ACCGATCGATGGTGTATACTGA
9	3AS	Xbarc 12	56	200	Fw: CGACAGAGTGATCACCCAAATATA Rv: CGACAGAGTGATCACCCAAATATAA
10	7A	Xwmc83	50	160	Fw: TGGAGGAAACACAATTGGATGCC Rv: GAGTATCGCCGACGAAAGGGAA
11	1BS	Xbarc137	52	260	Fw: GGCCCATTTCCCACTTTCCA Rv: CCAGCCCCTCTACACATTTT

Footnote: AL is A-genome long arm; AS is A-genome short arm; BL is B-genome long arm; BS is B-genome short arm; Fw is forward; Rv is reverse; Tºa is annealing temperature.

3.4. PCR amplification and electrophoresis

The extracted DNA concentration was diluted to 100 ng/µl using nuclease free sterile water before being used in the PCR reaction. For genotyping, a total of 11 polymorphic primers

were used (Table 1). The annealing temperature of primers were optimized using gradient PCR machine (HIMEDIA – Prima-96™ Thermal Cycler 96 wells block). The thermocycling program was optimized at initial denaturation of 94°C for 4 minutes followed by 40 cycles for 30 second at 94°C denaturation, 40 second of annealing at optimized temperature (52-63°C), primer extension at 72°C at 90 seconds followed by final extension at 72°C for 9 minutes and then stored at 4°C. The amplicons were mixed with DNA loading dye and fractionated on a 3% agarose gel stained with ethidium bromide solution (2 µl), in 1x TBE running buffer in a horizontal gel tank and operated at 100 volts for one hour and thirty minutes. A standard 100 base pair (bp) DNA ladder was used to estimate the molecular weight of amplified products. The electrophoresed gel was visualized under UV light and subsequently photographed using Bio rad Gel-Doc EZ gel documentation machine (Bio-Rad Laboratories,

3.5. Band scoring and data analysis

Clearly resolved and unambiguous bands on an electrophoresed gel were scored visually for their presence (1) or absence (0) for each primer and sample (Appendices 3). In addition, the fragment size of bands on an electrophoresed gel in base-pairs were obtained using PyElph software package (Pavel and Vasile, 2012).

3.5.1. Molecular data analysis

The scored marker data were used to analyze genetic diversity indices and infer population structure using different analytical software. Power-marker version 3.25 software (Liu and Muse, 2005), GenALEx version 6.5 software (Peakall and Smouse, 2012), MEGA version X software (Kumar *et al*., 2018) and STRUCTURE version 2.3.4 software (Pritchard *et al.*, 2010) were used for the data analysis.

Power marker version 3.25 software (Liu and Muse, 2005) were used to compute the number of alleles per locus (Na), major allelic frequency (MAF), gene diversity (h) and polymorphic information content (PIC) of SSR markers. The remaining locus-based diversity indices i.e., number of alleles (Na), gene diversity (h), effective number of alleles (Ne), Shannon's Information index (I), observed heterozygosity (Ho), expected heterozygosity (He), Fixation index (F) and gene flow were computed using GenAlEx ver. 6.501 software (Peakall and Smouse, 2006).

Population diversity indices that mean allelic frequency, number of alleles (Na), effective number of alleles (Ne), Shannon's Information index (I), number of private alleles (NPA) and Percentage of Polymorphic Loci (%P), were computed using GenAlEx ver. 6.501 software (Peakall and Smouse, 2006). This software was also used to calculate the pairwise Nei's genetic distance, pairwise genetic differentiation, pairwise gene flow among populations.

The Nei genetic distance across entire loci accessions were computed using Power-Marker v3.25 software (Liu and Muse, 2005) using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method and the cluster dendrogram was plotted in MEGA software (Kumar *et al.*, 2018). The Nei genetic distance across accessions were computed using Power-Marker v3.25 software (Liu and Muse, 2005) using Neighbor-Joining (NJ) method and the cluster dendrogram was plotted in MEGA software (Kumar *et al.*, 2018). Principal coordinate analysis (PCoA) and analysis of molecular variance (AMOVA) was computed using GenALEx software (Peakall and Smouse, 2012) from the genetic distance matrix.

Population structure and admixture patterns were determined using STRUCTURE software ver. 2.3.4 with a Bayesian model-based clustering algorithm (Pritchard *et al.* , 2000). To estimate the true number of population cluster (K), a burn-in period of 100,000 was used in each run, and data were collected over 200,000 Markov Chain Monte Carlo (MCMC) replications for K = 1 to K = 10 using 20 iterations for each K. The optimum K value was predicted following the simulation method of Evanno *et al.*(2005) using the web-based STRUCTURE HARVESTER ver. 0.6.92 (Earl, 2012). A bar plot for the optimum K was determined using Clumpak beta version (Kopelman *et al.* , 2015).

4. RESULTS

4.1. Polymorphism of the SSR markers

The molecular genetic diversity analysis of 102 tetraploid wheat genotypes were evaluated using 11 SSR markers and produced a total of 84 alleles with an average of 7.6 alleles per locus (Table 2) The genetic diversity (h) ranged from 0.48(Xgwm46) to 0.87 (Xbarc178 and Xbarc137) with the overall mean of 0.73. The highest and lowest major allele frequency (MAF) was obtained from locus Xgwm46 (0.69) and Xbarc137 and Xbarc178 (0.18) with the overall mean of 0.38(Table 2). The polymorphic information content (PIC) ranged from 0.44(Xgwm46) to 0.86 (Xbarc137**)** with the mean of 0.69. The number of alleles (Na) per locus ranged from 4 to 11 with an average of 7 and also the number of effective alleles ranged from 1.5 to 5.27 with average number of 3.32. The lowest and highest Na and Ne were observed in the locus Xbarc240, Xgwm46 and Xbarc137 respectively. Locus Xgwm371 and Xbarc240 recorded the highest and lowest gene flow (Nm) respectively whereas locus Xbarc137 showed the highest Shannon's information index. Locus Xbarc240 (0.25) and Xbarc137 (0.75) scored the lowest and highest value for expected heterozygosity (He), respectively, and Xbarc12 (0.15) and Xbarc178 (0.82) showed the lowest and highest value for observed heterozygosity (Ho) **(Table 2).**

Table 2. Diversity indices of the 11 simple sequence repeat (SSR) loci across 102 tetraploid wheat accessions.

Marker	MAF	Na	Ne	I	H	He	Ho	F	Nm	PIC
Xbarc240	0.60	4.00	1.50	0.42	0.57	0.25	0.19	0.172	0.20	0.52
Xbarc178	0.18	9.00	4.68	1.48	0.87	0.67	0.82	-0.104	0.89	0.85
XCf2257.1	0.35	7.00	3.48	1.30	0.78	0.66	0.35	0.462	1.76	0.75
Xgwm135	0.28	7.00	3.33	1.28	0.80	0.63	0.51	0.163	1.08	0.77
Xbarc12	0.28	8.00	3.47	1.37	0.82	0.70	0.15	0.819	1.41	0.80
Xwmc256	0.40	6.00	2.60	1.04	0.68	0.59	0.56	0.031	1.75	0.62
Xgwm46	0.69	4.00	1.52	0.47	0.48	0.30	0.27	0.052	0.39	0.44
Xwmc83	0.57	5.00	3.88	1.41	0.55	0.72	0.26	0.181	2.06	0.46
Xbarc137	0.18	11.00	5.27	1 .67	0.87	0.75	0.57	0.225	1.76	0.86
Xgwm371	0.37	8.00	2.95	1.27	0.75	0.65	0.50	0.133	2.53	0.72
XWmc24	0.28	8.00	3.88	1.45	0.80	0.72	0.72	-0.012	2.53	0.77
Mean	**0.38**	**7.00**	**3.32**	**1.20**	**0.73**	**0.60**	**0.45**	**0.196**	**1.49**	**0.69**

MAF: major allele frequency; Na: number of different alleles; Ne: number of effective alleles; I: Shannon information index; h: gene diversity; He: expected heterozygosity, Ho: observed heterozygosity; F: Fixation index; Nm: gene flow; PIC: polymorphism information content.

4.2. Genetic diversity analysis within population

A summary of different genetic diversity estimates over all investigated loci across populations is presented in (Table 3) The average number of alleles (Na) and effective number of alleles (Ne) between populations was 4.76 and 3.32, respectively. The highest Na, Ne and I observed in Oromia population with the value of 6.09, 4.08, and 1.42, respectively. The lowest Na, Ne, I were showed in emmer wheat population with the value of 2.7, 1.88 and 0.65, respectively. From overall populations the highest observed heterozygosity (Ho) was from Amhara wheat population (Ho = 0.55) whereas lowest was from emmer population with the value of 0.36. CIMMYT lines had the highest He of 0.69 and the lowest was from emmer wheat populations which was 0.38. The average within populations percentage of polymorphic loci (%PPL) was 94.5%. The Amhara, CIMMYT and Emmer populations showed three private alleles while the Oromia population showed two private alleles but ICARDA didn't show private allele. The Locus and allele size of Amhara population that

showed private alleles are Xbarc178; 260, Xbarc137;180, and Xbarc371; 185 respectively. Locus XCf2257.1;170 and locus Xwmc83;170 are the Oromia population which showed private alleles. Locus Xwmc83 was the CIMMYT population that exhibited three private alleles at size of 140, 190, 200 bp. The locus Xwmc256 resulted three private alleles in Emmer wheat populations at size of 150 and 180, and locus Xwmc83 at size of180 bp.

Table 3. Mean diversity indices of 5 populations over 11 SSR loci.

Pop	N	Na	Ne	I	Ho	He	F	%P
Amhara	18.00	5.91	3.90	1.41	0.55	0.68	0.19	100.00%
Oromia	31.73	6.09	4.08	1.42	0.45	0.66	0.32	100.00%
ICARDA	15.82	3.82	3.09	1.12	0.54	0.61	0.08	90.91%
CIMMYT	23.36	5.27	3.66	1.38	0.47	0.69	0.34	100.00%
Emmer	11.36	2.73	1.88	0.65	0.36	0.38	0.00	81.82%
Mean	**20.06**	**4.76**	**3.32**	**1.20**	**0.47**	**0.60**	**0.20**	**94.55%**

N: sample Size; Na: Number of alleles per locus; Ne: Number of effective alleles; I: Shannon information index; Ho: observed heterozygosity; He: expected heterozygosity; F: fixation index and %P: percent polymorphic loci.

4.3. Genetic relationship between population

The pairwise Nei's standard genetic distance of each population from the other populations ranged from 0.21 to 1.05 (Table 4). The lowest genetic distance (0.21) was observed between the wheat population of Oromia and Amhara, and the highest genetic distance (1.05) was observed between Emmer and ICARDA. Moderate to high genetic distance was observed between Emmer and Oromia (0.81), Emmer and Amhara (0.64), Emmer and CIMMYT (0.68) populations.

Table 4. Pairwise Population Matrix of Nei Genetic Distance

	Amhara	Oromia	ICARDA	CIMMYT	Emmer
Amhara	0.00				
Oromia	0.21	0.00			
ICARDA	0.42	0.42	0.00		
CIMMYT	0.29	0.32	0.38	0.00	
Emmer	0.64	0.81	1.05	0.68	0.00

4.4. Population genetic differentiation

The pairwise coefficient of genetic differentiation between the populations ranged from 0.05 (between Amhara and Oromia) to 0.28 (between Emmer and ICARDA) (Table5). Genetic differentiation exhibited between ICARDA and Amhara was 0.10, ICARDA and Oromia was 0.09, which is similar to genetic differentiation of CIMMYT and ICARDA.

Table 5. Pairwise population matrix of Fst values

	Amhara	**Oromia**	**ICARDA**	**CIMMYT**	**Emmer**
Amhara	0				
Oromia	0.05	0			
ICARDA	0.10	0.09	0		
CIMMYT	0.05	0.07	0.09	0	
Emmer	0.19	0.22	0.28	0.19	0

4.5. Analysis of molecular variance

The results of AMOVA (Table 6) revealed statistically significant high population differentiation (Fst = 0.219, $P< 0.0001$) where 78% of the total genetic variation resides within populations, leaving 22% for the among populations. The overall gene flow was low (Nm= 0.89) (Table 6).

Table 6. AMOVA showing the partitioning of genetic variation within and among populations.

Source	Df	SS	MS	Est. Var.	%	Fst	p-value	Nm
Among Pops	4	245.4	61.4	2.626	22%	0.219	0.001	0.89
Within Pops	97	907.5	9.4	9.356	78%			
Total	101	1152.9		11.982	100%			

Df: degree freedom; SS: sum of squares; MS: mean square; %: percentage of variation; Est. Var.: estimation of variations; Fst: genetic differentiation; Nm: gene flow.

4.6. Cluster analysis

The Neighbor-Joining tree (NJ) based clustering of 102 tetraploid wheat genotypes comprised the study materials into two equal major clusters (Figure 2). Cluster 1 composed of genotype from all populations except individuals from Emmer population. Cluster 2 also comprised of individuals from all population confirming existence of intermixing of accessions from various populations. Emmer population exclusively clustered at sub-clusters level under Cluster 2.

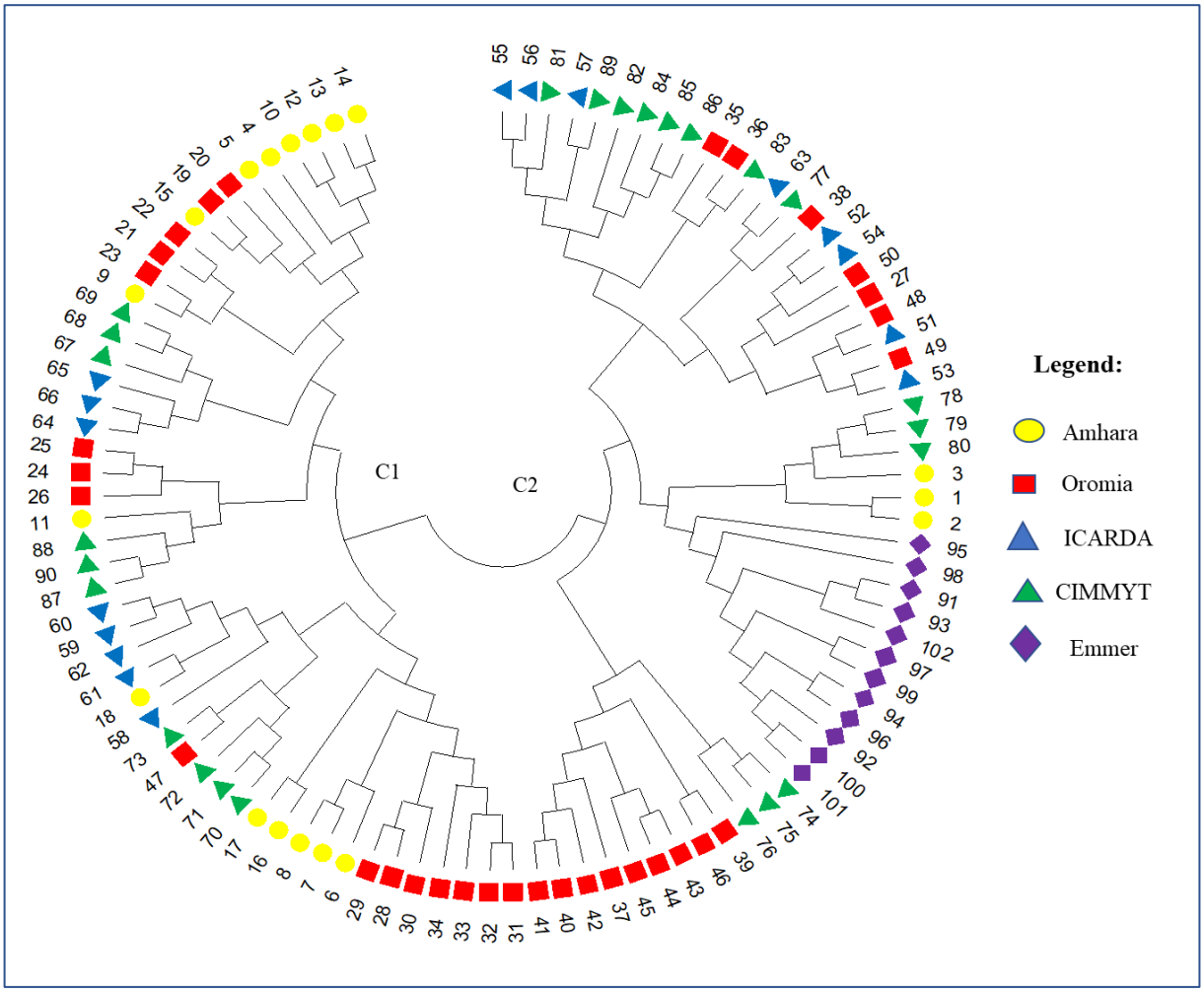

Figure 2. Neighbor-Joining dendrogram showing the pattern of genetic diversity among 102 genotypes based on the 11 SSR markers. C1 and C2 are cluster 1 and cluster 2, respectively.

The cluster dendrogram among populations grouped the five populations into two major clusters (cluster I, and cluster II) where the first cluster was composed of only a single population (Emmer), and cluster II composed of four populations in different sub-clusters. ICARDA and CIMMYT populations were grouped under different sub-clusters whereas Amhara and Oromia are in the same subcluster (Figure 3).

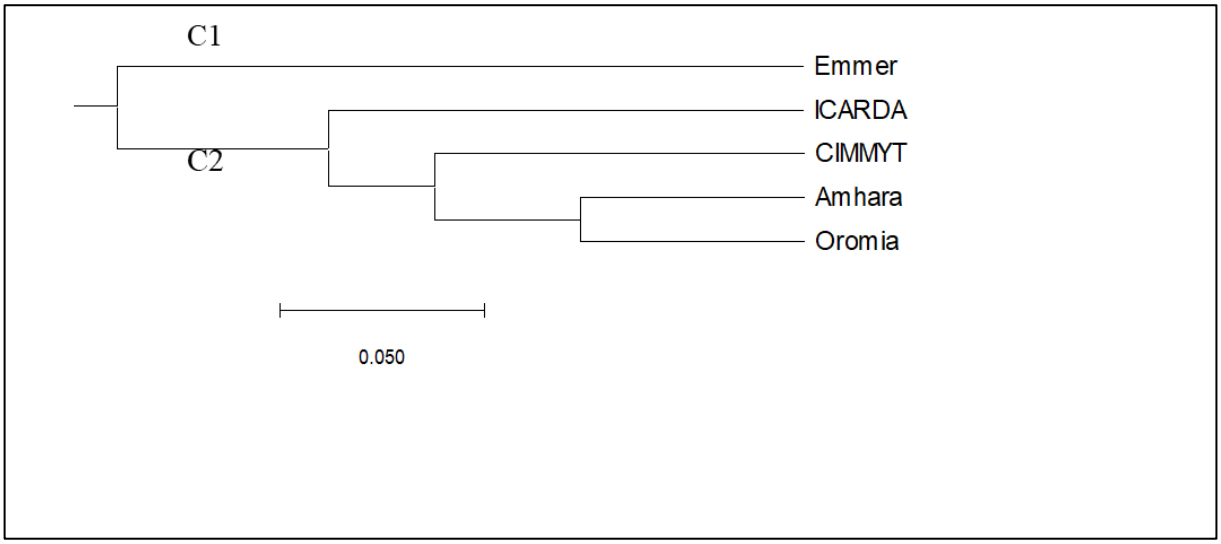

Figure 3. UPGMA based dendrogram showing genetic relationships among the five tetraploid populations of 102 samples based on Nei's genetic distances.

4.7. Principal coordinate analysis

The first three coordinates, PCo1 (14.97 %), Pco2 (10.68 %) and Pco3 (8%) explained 34.16% of the total genetic variation and the first two principal coordinates grouped the genotypes into two main groups (Figure 4). This distribution and grouping of genotypes on the PCoA have confirmed the presence of admixture similar to NJ clustering.

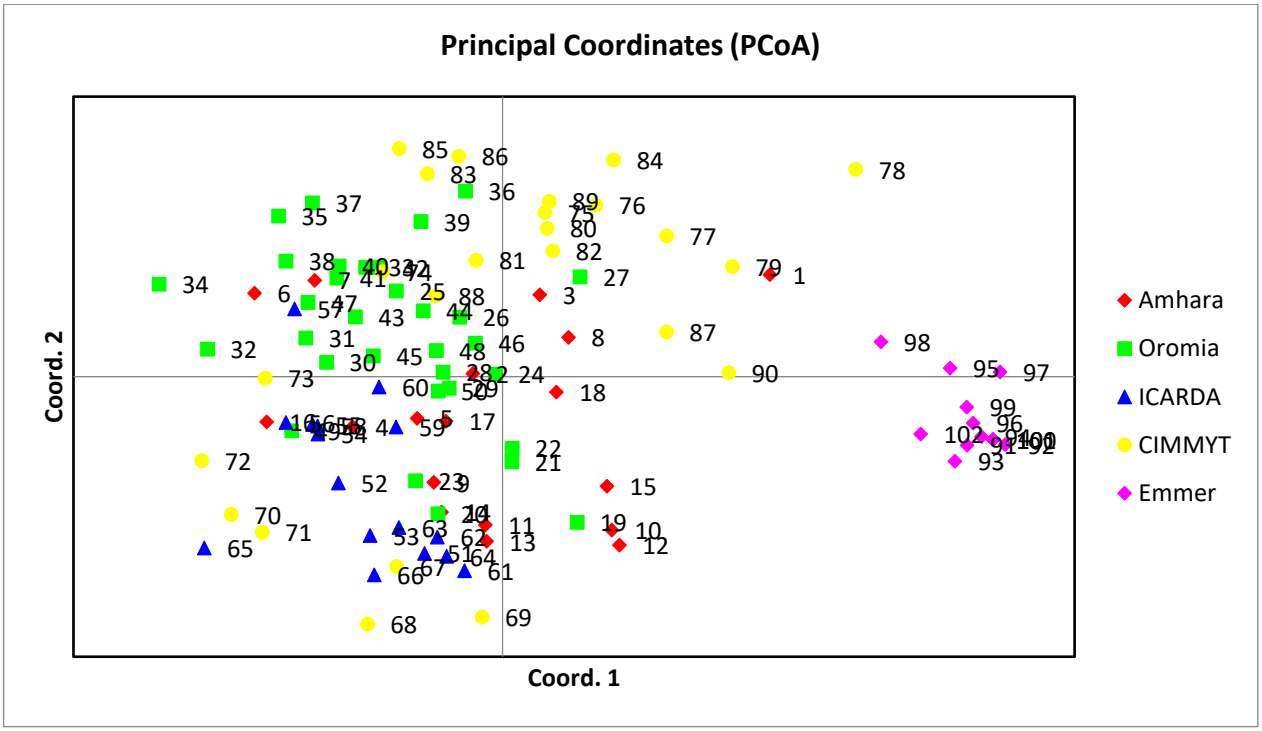

Figure 4. PCoA of the 102 tetraploid wheat accessions as revealed by 11SSR markers. samples coded with the same symbol and color belongs to the same population.

4.8. Population structure

The output of the Structure Harvester revealed that the delta K (ΔK) values reached a sharp peak at K = 4 (Figure 5) indicating that the optimal number of genetic groups are four.

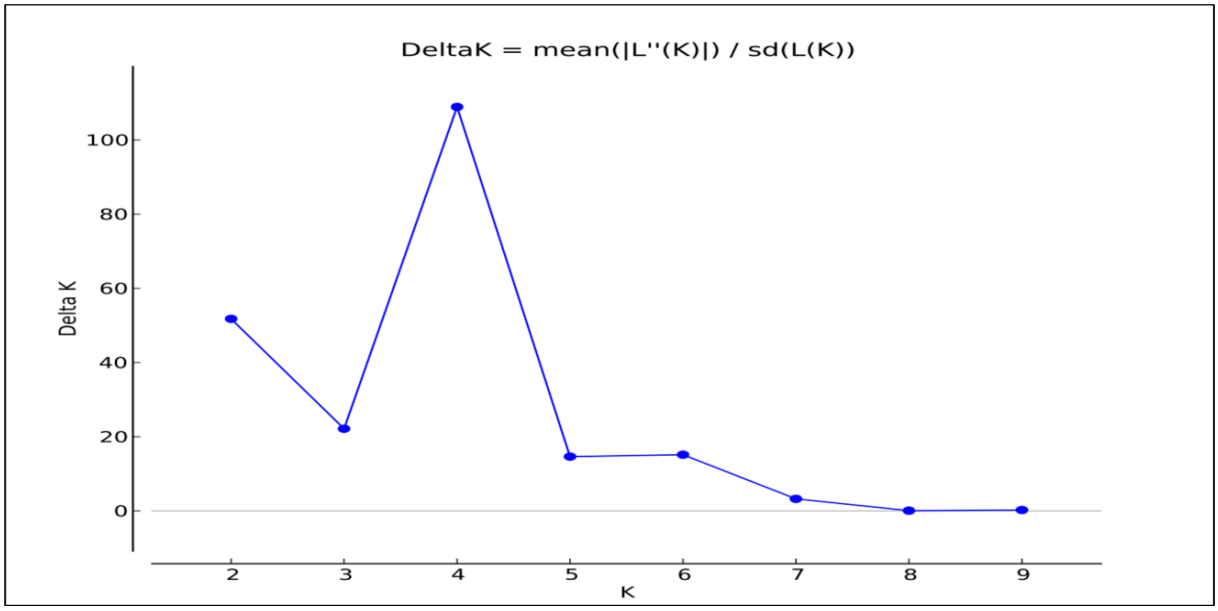

Figure 5. Biplot detected the best delta K value of 4 (the optimal number of clusters) based on Evanno *et al*. (2005) method.

The Clumpak result (bar plot) identified the intermixing of one genetic group with another genetic group, and hence, there was no clear geographic origin-based structuring in the populations (Figure 6). Admixture of genetic groups was observed for Amhara, Oromia, and CIMMYT lines.

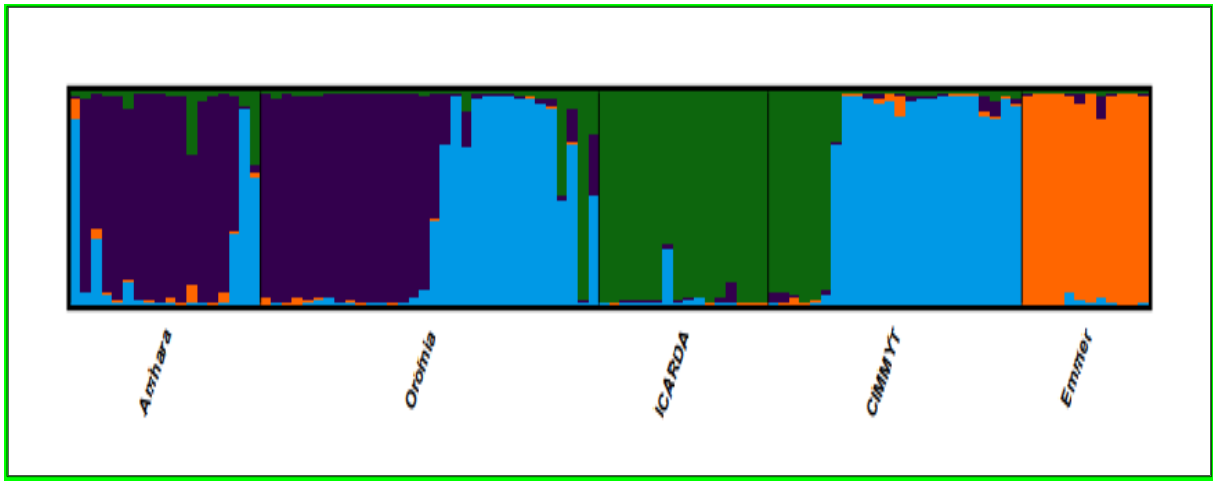

Figure 6. Structure bar graph of 102 populations at K = 4.

5. DISCUSSION

5.1. Polymorphism level of the SSR markers

In this study, 11 microsatellite markers were used to determine the extent of diversity among one hundred two tetraploid wheat accessions. Each locus showed four to eleven number of alleles per locus with overall number of seven alleles per locus loci. The frequency of major alleles across each locus ranged in between 0.18(Xbarc178) and 0.68 (Xgwm46) which shows that all markers were polymorphic. The average number of alleles observed in the present study was significantly higher than the level reported by Kirouani *et al*. (2018), who described an average of 3 alleles per locus among 14 durum wheat accessions. Nikolai *et al.* (2022) reported comparative mean number of alleles per locus (6.88) from 90 modern durum wheat accessions. While, Laido *et al*. (2013) reported high (16.8) mean number of alleles per locus in 230 tetraploid wheat accessions using 26 microsatellite markers. The observed variation is likely due to the diverse nature of accessions used for the study and the polymorphism level of the selected SSR markers.

PIC measures the informativeness of the genetic markers, and markers with a PIC value of more than 0.5 are considered to be highly informative, between 0.25 and 0.5 as moderate informative and less than 0.25 as less informative. According to this all the SSR markers used in this study were informative, and hence, useful genetic markers for genetic analysis. The PIC obtained in the present study (PIC=0.68) was comparable with the level reported in previous studies by Al Khanjari *et al*. (2006) (PIC = 0.64), Zatybekov *et al*. (2020) (PIC= 0.62), and Meseret Asemamaw *et al*. (2020) (PIC= 0.7) using 28, 7 and 12 microsatellites for 38, 85 and 141 tetraploid wheat genotypes, respectively.

The average PIC value of the current study was higher than some of the previously reported values in Alegirian, Bulgarian and Tunisian durum wheat varieties (Kirouani, *et al*., 2018; Nikolai *et al*.,2022; Slim *et al*., 2019) which were 0.39, 0.52, and 0.57, respectively. This could be due to the polymorphic nature of the selected SSR markers used for genotyping and also genotype differences. In the current study, the gene diversity (h) ranged from 0.57 (Xbarc240) to 0.82 (Xbarc240) with an overall mean of 0.72. This average gene diversity in this study was greater than the reports of Nikolai, *et al*. (2022).

5.2. Population genetic diversity

The high mean number of alleles per locus (4.76), effective number of alleles (3.3), Shannon diversity index (1.195), and high percent of polymorphic loci (94%) observed across the 5 populations demonstrate the existence of high genetic diversity within wheat populations. Among tetraploid populations, the study of Abouzied and Abdellatif (2013) found a mean Na of 2.00, Ne of 1.40, Ho of 0.28, and I of 0.45, which is lower than the present study. Meseret Asmamaw's report shows from 160 durum wheat 5.91 (Na), 4.76 (Ne) and 1.39 (I) which is higher than present study (Meseret Asmamaw *et al.*, 2019). Mondini *et al.* (2010) reported a high percentage of polymorphism (91%), 4.87 numbers of alleles per locus and low observed heterozygosity (0.14) across 9 populations. The high levels of variation on different study of tetraploid wheat illustrates the presence of an extensive genetic diversity of tetraploid accessions across the world that represented different geographic regions. The observed highest molecular diversity for Na (6), Ne (4) and I (1.4) in Oromia population is may resulted due to high number of accessions from Oromia were used in the study.

5.3. Genetic relationship and population structure

Eleven SSR markers were utilized to define the relationship between genotypes, and the AMOVA, the NJ based clustering and PCoA were employed to determine the genetic relatedness of genotypes**.** AMOVA showed that most (78%) of the total genetic variation of the populations resides within the population leaving 22% for among population. Similar low among population genetic variation in tetraploid wheat was reported by Meseret Asmamaw's (2020), Mondini *et al*. (2010) (18.76%), in Tunisia by Slim *et al.* (2020) (22%). The high genetic variation within the population observed in this study could be attributed to mutation and/or recombination. The higher genetic differentiation Fst = 0.22 limited gene flow (Nm) = 0.89 among the studied populations. Gene flow (Nm) value less than one is an indication of limited gene exchange as it was suggested by Frankham *et al*. (2002).

The NJ method of clustering demonstrates that all 90 durum wheat accessions and 12 emmer wheat accessions clusters to two clusters with greater degree of admixture. Except for emmer wheat populations which showed distinct grouping at the sub-levels, none of individuals of the populations showed sharp distinct clustering following their sample sources likely due to the presence of some sort of gene flow. UPGMA dendrogram showed the presence of genetic relationships among the five populations and clustered the five populations in to two clusters. The present result was contrary to the finding of Oliveira *et al* (2012) where SSR

based PCoA, STRUCTURE and UPGMA dendrograms, separate all the hulled tetraploid wheats (emmer and wild emmer) from the naked tetraploid wheats (durum and rivet) (Oliveira *et al*.,2012).

Similar to the present result where PCoA sharply distinguished *T. durum* from *T. dicoccum*, Zatybekov *et al*. (2020) reported that *T. carthlicum* and *T. durum* from *T. dicoccum* and *T. dicoccoides*. Likewise, with PCoA Kefyalew Negisho *et al.* (2021) observed two population clustering among that consisted of released, advanced, and CIMMYT lines on one hand and the landraces and other major populations on the other using SNP markers.

The population structure analysis detected sub-populations with some degree of intermixing among the different subgroups. It was observed that tetraploid wheat from Amhara and Oromia had high genetic similarity, indicating that they share alleles from common ancestor. CIMMYT lines share almost all genetic structure of other populations particularly with Oromia. This might be due to the presence of increased mixing, which could result from past seed exchanges between local and national farming communities through an informal seed system and due to similar genetic background. In Ethiopia since 1970 until recent time, CIMMYT is the major source for most of the improved durum wheat materials (Dejene Mengistu *et al*., 2015) which may lead to admixture of the varieties during germplasm collection. Similar population structuring was reported by Laido *et al*.(2013) for 230 wheat accessions analyzed using a Bayesian approach on implemented in the STRUCTURE software.

Likewise, Oliveira *et al*. (2012) found well-supported population structure among wheat with a degree of admixture between clusters. In addition, among 319 Indian durum wheat varieties, Arora et al. (2014) discovered a highly structured (K = 7) and genetically admixture-rich sub-populations. In addition, six population clusters with little mixing were discovered by Marzario *et al*. (2018) among the 136 landraces and 28 variations of Italian durum wheat genotypes.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The present study was conducted to explore the genetic diversity and population structure in tetraploid wheat accessions using eleven SSR markers. The microsatellite markers-based profiling revealed the presence of considerable genetic variation in tetraploid wheat accessions. The high average number of alleles per locus (7) and PIC value of 0.69 in the present study confirmed that the used SSR markers are informative. Presence of high within population gene diversity (h) indicates promising possibility to improve desired agronomic traits through breeding. AMOVA confirmed the presence of significant population differentiation (Fst=0.22) where most (78%) of the total genetic variation was accounted for among populations leaving, likely due to mutation and sexual recombination. NJ based clustering, PCoA and population structuring did not sharply distinctly cluster the 102 durum wheat genotypes following their geographical areas of sampling or seed source, likely due to the presence of seed exchange. The high expected hetrozygosity in Amhara (He =0.68), CIMMYT (He=0.69) and Oromia (He=0.66), indicates that these populations are hotspots for tetraploid wheat genetic diversity, and hence, promising source for breeding relevant alleles for wheat improvement, and also conservation purposes. Therefore, the present study had generated valuable information for wheat improvement through breeding, wise utilization and conservation of the available genetic resources. Genetic diversity and population structure analysis in the existing tetraploid wheat in Ethiopia have enormous importance in enhancing breeding effort and for sustainable conservation.

6.2. Recommendations

Based on the study, the following recommendations were made:

- The SSR markers used in this study showed a higher resolution power in terms of polymorphic information content and other diversity parameters, indicating their relevance for similar future genetic structure analysis.
- Similar studies should be carried out on other populations from the remaining parts of the country using dense markers to generate a nationwide representation of the genetic structure of tetraploid wheat in Ethiopia.

- Tetraploid wheat germplasm from Amhara, Oromia and CIMMYT showed high gene diversity and hence, useful sources of breeding relevant alleles. Thus, proper germplasm management strategies need to be deployed for their conservation.

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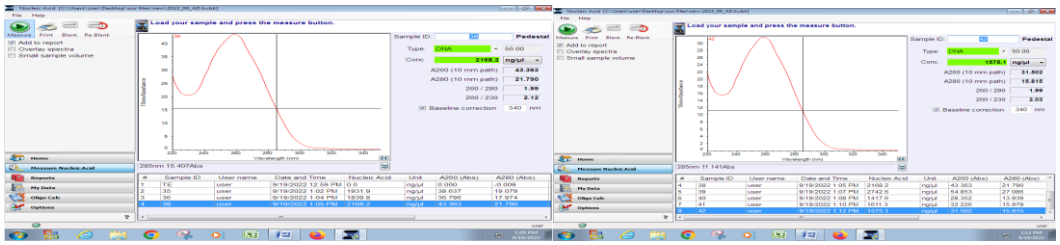
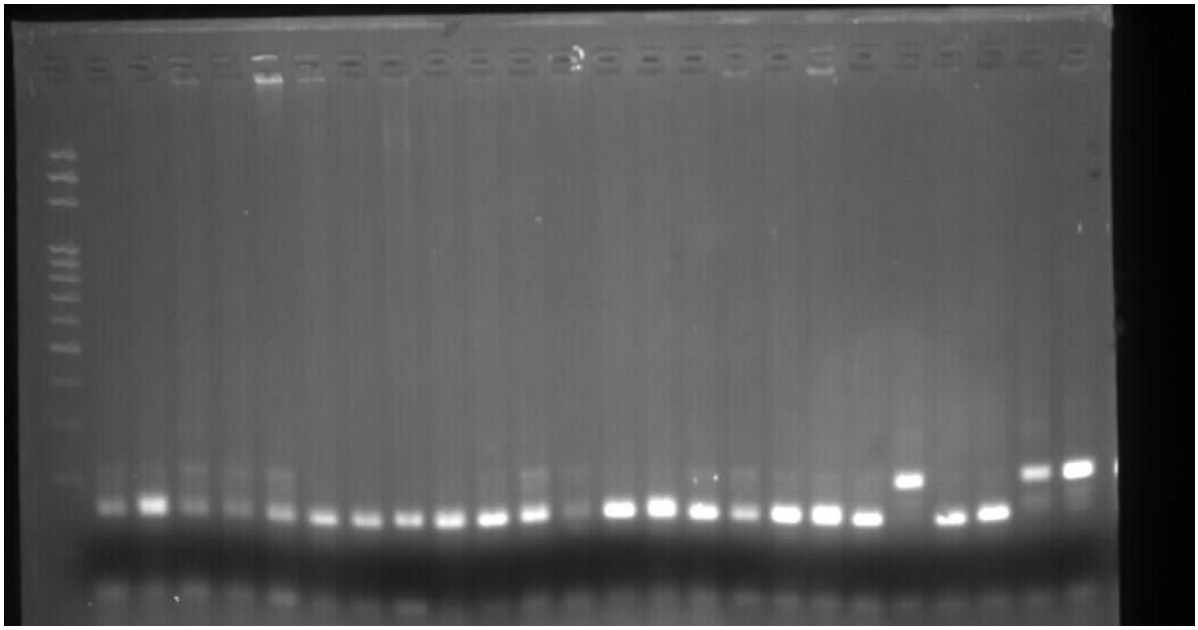
LIST OF APPENDICES

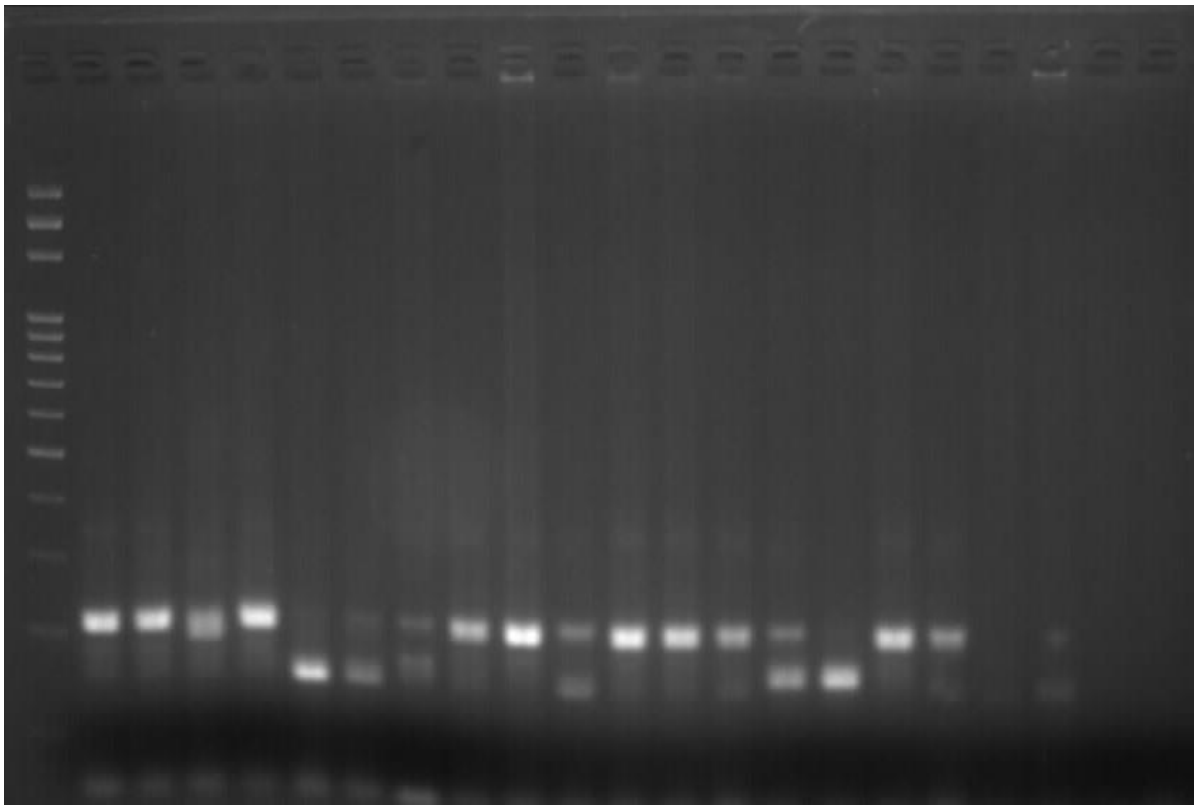
Appendices 1. Representative pictures of laboratory work starting from collection of the plant to drying, extraction and PCR process.

Appendices 2. Quantification and qualification of genomic DNA using Nano drop 2000



Appendices 3. Gel doc image of SSR marker Xbarc12.





Appendices 4. Tetraploid wheat accessions used in this study. The description of the sixteen ICARDA lines, twenty-four CIMMYT lines and twelve Emmer wheat landraces used in this study

Code	Acc	Region	Zone	Altitude	Longitude	Latitude
1	31126	Amhara	Misrak Gojam		41-48-00-E	09-26-00-N
2	31154	Amhara	Semen Gonder	3020	37-45-00-E	12-55-00-N
3	31158	Amhara	Mirab Gojam	2145	37-29-00-E	10-34-00-N
4	31172	Amhara	Semen Shewa	2810	39-29-00-E	09-39-00-N
5	31201	Amhara	Semen shewa	3010	39-05-00-E	10-20-00-N
6	31669	Amhara	Semen Gonder	2650	37-44-00-E	12-56-00-N
7	31681	Amhara	Semen Gonder	2004	37-24-00-E	12-33-00-N
8	31683	Amhara	Semen Wello	1900	39-36-00-E	11-46-00-N
9	31686	Amhara	Debub Wello	1790	39-45-00-E	11-06-00-N
10	31689	Amhara	Debub Gonder	3130	38-27-00-E	11-39-00-N
11	31691	Amhara	Debub Gonder	3040	38-32-00-E	11-38-00-N
12	31696	Amhara	Debub Gonder	2900	38-30-00-E	11-38-00-N
13	31698	Amhara	Debub Gonder	2750	38-32-00-E	11-38-00-N
14	31703	Amhara	Debub Gonder	3120	38-29-00-E	11-44-00-N
15	31745	Amhara	Semen Wello	2920	39-10-00-E	11-34-00-N
16	31287	Amhara	Weldia		39.56667	11.6
17	31230	Amhara	Gonder		37.33	12.4
18	31383	Amhara	Deberemarkos		39.5	9.68

Code	Acc	Region	zone	Altitiude	Longitude	Latitude
19	31129	Oromia	Misrak Harer		41-48-00-E	09-26-00-N
20	31132	Oromia	Bale	2583	39-24-00-E	07-00-00-N
21	31137	Oromia	Misrak Harerge		42-07-00-E	09-35-00-N
22	31146	Oromia	Misrak Harerge			
23	31181	Oromia	Arsi	2565	39-52-00-E	08-22-00-N
24	31209	0romia	Arsi	2520	39-36-00-E	07-55-00-N
25	31215	Oromia	Arsi	2740	39-07-00-E	07-15-00-N
26	31218	Oromoa	Semen Shewa	2800	38-45-00-E	09-46-00-N
27	31222	Oromia	Semen Shewa	2640	38-48-00-E	09-22-00-N
28	31550	Oromiya	Bale	2460	40-11-00-E	07-04-00-N
29	31553	Oromiya	Bale	1900	40-28-00-E	06-59-00-N
30	31609	Oromiya	Mirab Shewa		38-20-00-E	09-01-00-N
31	31637	Oromia	Misrak Shewa	2500	38-59-00-E	08-58-00-N
32	31646	Oromia	Mirab Shewa	2450	37-58-00-E	08-57-00-N
33	31652	Oromia	Mirab Shewa	2310	37-51-00-E	08-53-00-N
34	31654	Oromia	Semen Shewa	2500	38-49-00-E	09-03-00-N
35	31656	Oromia	Semen Shewa		38-43-00-E	14-07-00-N
36	31354	Oromia	Gimbichu			

Code	Genotype name	Region	Pedigree
37	**DWLRC19-20#25**	Oromia	DWLR
38	**DWLRC19-20#39**	Oromia	DWLR
39	**DWLRC19-20 # 48**	Oromia	DWLR
40	**DWLRC19-20 # 16**	Oromia	DWLR
41	**DWLRC19-20 # 20**	Oromia	DWLR
42	**DWLRC19-20 # 94**	Oromia	DWLR
43	**DWLRC19-20 # 89**	Oromia	DWLR
44	**DWLRC19-20 # 92**	Oromia	DWLR
45	**DWLRC19-20 # 79**	Oromia	DWLR
46	**DWLRC19-20 # 84**	Oromia	DWLR
47	**DWLRC19-20 # 85**	Oromia	DWLR
48	**DWLRC19-20 # 88**	Oromia	DWLR
49	**DWLRC19-20 # 96**	Oromia	DWLR

Gen code	Genotypes	source	Pedigree/ID
50	Werer	ICARDA	1346/LAHN//BICRE/LOUKOS-4
51	**Mangudo**	ICARDA	MRF1/STJ2|/3/1718/BT//KARIM, TUN
52	**Mekuye**	ICARDA	STJ3//BICRE/LOUKOS-4/3/TER3
53	FIGSDRYWET016	ICARDA	RUSS199/ID-82244
54	FIGSDRYWET078	ICARDA	AFG68::77/ID-90233
55	FIGSDWHOTCLD015	ICARDA	ETH732::91/ID-81510
56	FIGSDRYWET028	ICARDA	ETH64:112/ID-83807
57	DURUM_PANEL_UNIBO-054	ICARDA	D333/GAVIOIA/4/AVETORO//ALUINCO/D/EIOD/ID-MARJANA MOROCCO
58	FIGSDRYWET001	ICARDA	OMN87:113/ID-43315
59	DURUM_PANEL_UNIBO-048	ICARDA	INRA1807 /ID-CHAOUI MOROCCO
60	FIGSDWHOTCLD009	ICARDA	ETH74::56/ID-79653
61	FIGSDRYWET0144	ICARDA	IRNS382/ID-119026
62	DURUM_PANEL_UNIBO-081	ICARDA	ID-BRAVADUR,SW USA
63	FIGSDRYWET091	ICARDA	ETH731::112/ID-90482
64	FIGSDRYWET108	ICARDA	IRNS294/ID-98797
65	ICARASHA2	ICARDA	Stj3/Ber/Lks4/3/Ter3

66	Hitosa	CIMMYT	CHEN/ALTAR-84
67	Tesfaye	CIMMYT	ARMENT//SRN-NIGRIS 4/3/CANED-9.1/4/TOSKA 26RASCON-37//SNITSN/5/PLAYERO
68	Fetan	CIMMYT	ARMENT//2*SOOTY-9/RASCON-37/4/CNDO/PRIMADUR//HAI-OU-17/3/SNITAN
69	Denbi	CIMMYT	AJAIA/BAUSHEN[3589];
70	Ude	CIMMYT	CHEN/ALTAR-84//JORI
71	Yerer	CIMMYT	CHEN/TEZONTLE/3/GUILLEMOT//CANDEAL-II
72	Toltu	CIMMYT	4/B/R9096#21001(980SN Patho
73	CD13DZOS F6SR 2013 MS DZLS/93	CIMMYT	K0FA/9/USDA595/3/D67.3/RABI//CRA/4/AL0/5/HUI/YAV-1/6/ARDENTE/7/HUI/YAV79/8/…
74	CD13DZOS F6SR 2013 MS DZLS/106	CIMMYT	RBC/HULITA/5/M0HAWK/3/GUANAY//TIL0-1/L0TUS-4/4/ARMENT//SRN-3/NIGRIS-4/3/…
75	CD13DZOS F6SR 2013 MS DZLS/81	CIMMYT	CF420S/4/YAZI-1/AKAKI-4//S0MAT 3/3/AUK/GUIL//GREEN/5/CANEL0-9.1//SHAKE-3/…
76	CD13DZOS F6SR 2013 MS DZLS/111	CIMMYT	RBC/5/ARMENT//SRN-3/NIGRIS-4/3/CANEL0-9.1/4/LIR0-3/L0TAIL-6/6/C F4 20S/4/YAZI-1/…

77	CD13DZOS F6SR 2013 MS DZLS/83	CIMMYT	CF420S/4/YAZI-1/AKAKI-4//S0MAT-3/3/AUK/GUIL//GREEN/5/CANEL0-9.1//SHAKE-3/…
78	CD13DZOS F6SR 2013 MS DZLS/87	CIMMYT	C F4 20S/4/YAZI-1/AKAKI-4//S0MAT-3/3/AUK/GUIL//GREEN/5/CANEL0-9.1//SHAKE-3/…
79	CD13DZOS F6SR 2013 MS DZLS/80	CIMMYT	C F4 20S/4/YAZI-1/AKAKI-4//S0MAT-3/3/AUK/GUIL//GREEN/5/CANEL0-9.1//SHAKE-3/…

80	CD15DZ_ELT/off/ 1006/2015	CIMMYT	JUPARE C2001*2/RBC/6/STORLOM/3/RASCON-37/TARRD-2//RASCON-37/4/D00003A/5/…
81	CD15DZ_ELT/off/ 989/2015	CIMMYT	TRIDENT /3*KUCUK/7/CMH83 2578/4/D88059//WARD/YAV 79/3/AC089/5/2*SOOTY-9/RASCON-37/6/…
82	CD15DZ_ELT/off/ 989/2015	CIMMYT	TRIDENT/3*KUCUK/7/CMH832578/4/D88059//WARD/YAV 79/3/AC089/5/2*SOOTY-9/RASCON-37/6/…
83	CD15DZ_ELT/off/ 303/2015	CIMMYT	AGI /2*ACO89//2*UC1113/3/5*KOFA/5KOFA/4/DUKEM-1//PATKA-7/YAZI-1/3/PATKA-7/…

84	CD15DZ_ELT/off/1035/2015	CIMMYT	CMH83.2578/4/D88059//WARD/YAV79/3/AC089/5/2*S00TY-9/RASCON-37/6/1A.1D 5+1-06/…
85	CD15DZ_ELT/off/1038/2015	CIMMYT	WID22289/10/SWAHEN-2/KIRKI-8//PROZANA-1/4/ADAMAR-15//ALBIA-1/LTAR84/3/SNITAN/9/…
86	CD15DZ_ELT/off/1115/2015	CIMMYT	JUPARE C2001*2/IM/5/KOFA/4/DUKEM-1//PATAK-7YAZI-1/3/PATKA/7/YAZI-1/6/ALAS/…
87	CD15DZ_ELT/off/1037/2015	CIMMYT	BOLENGA/10/KOFA/9/USDA595/3/D67.3/RABI//CRA/4/ALO/5/HUI/YAV-1/6/ARDENTE/7/HUI/…
88	CD15DZ_ELT/off/1069/2015	CIMMYT	CHAM1/4/INRAM-1005//SOMAT-4/INTER/8/3/SOOTY-9/RASOON-37//TILO-1/LOTUS-4/5/…
89	CD15DZ_ELT/off/1079/2015	CIMMYT	CMH83.2578/4/D88059//WARD/YAV79/3/AC089/5/2*S00TY-9/RASCON-37/6/1A.1D 5+1-06/…
90	CD15DZ_ELT/off/1079/2015	CIMMYT	CMH83.2578/4/D88059//WARD/YAV79/3/AC089/5/2*S00TY-9/RASCON-37/6/1A.1D 5+1-06/…

Code	Accession	Region	Zone	Altitude	longitude	Latitude
91	7984	Oromia	Mirab Shewa	2000	37-58-00-E	08-27-00-N
92	8072	Oromia	Bale	1980	40-43-00-E	07-08-00-N
93	8420	Oromia	Bale	1950	40-20-00-E	07-01-00-N
94	28665	Oromia	Bale	1985	40-38-03-E	07-09-53-N
95	28666	Oromia	Bale	1985	40-39-03-E	07-09-53-N
96	28667	Oromia	Bale	1921	40-43-03-E	07-10-50-N
97	28668	Oromia	Bale	1955	40-38-39-E	07-16-22-N
98	286669	Oromia	Bale	1989	40-38-29-E	07-16-50-N
99	28670	Oromia	Bale	1916	40-40-60-E	07-16-42-N
100	28671	Oromia	Bale	1938	40-41-17-E	07-16-48-N
101	28672	Oromia	Bale	1934	40-41-31-E	07-16-81-N
102	28673	Oromia	Bale	1958	40-42-51-E	07-14-53-N