Morphology, Biochemical and Genomic Diversity of Hexaploid Wheat (*Triticum aestivum* L.) Varieties in Ethiopia: A Prospective Study

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***Abstract*:** Hexaploid Wheat (*Triticum aestivum* L.) is one of the most important food cereals grown in many areas worldwide. World population is to increase by 2.6 billion in 2050. Ethiopia is one of the world's nine countries needed to increase food production. Since few studies on genetic diversity & in adequate evaluation of bread wheat varieties; my objective the project is to study genetic diversity of bread wheat varieties using morphological, SSRs, good baking quality, grain quality performance across environments, and compare and contrast all the mentioned characters. 121 bread wheat varieties, arranged in 11x11 simple lattice design, will be studied at 3 agro ecological regions of Ethiopia for 2013/14 using 25 morphological/phenotypic parameters as a preliminary genetic diversity study from the field; 10 baking and milling quality parameters of the varieties, seed storage protein using SDS electrophoresis banding patterns, and DNA finger printing microsatellite markers of each varieties in laboratory. Hence, the extent and nature of genetic diversity, grain quality, environmental effects on seed storage proteins, genetic variation using SSRs markers, and potential varieties for further breeding and improvement of nutritional and baking quality suggested. Genetic diversity, plant breeders rely on during selection in cultivar development, is one of the key factors for the improvement of many crop plants including wheat. This research is of great interest and is in line with the current Global Wheat Program, will contribute to the increasing of food security, improve productivity and profitability of wheat farming and sustain natural resources in the developing world.

Key words: Genetic diversity, SSRs, *Triticum aestivum* L., Varieties.

Introduction

Wheat is one of the most important cereals worldwide and it is grown in many areas [1}. A rapid increase in global wheat production has taken place during the last five decades, mainly due to increased productivity rather than an expansion of the

cultivated area, with average global yields having risen from 1 t/ha in the 1950s to about 2.5 t/ha at the turn of the century [1].

In global terms, wheat is the leading source of cereal proteins in human food, having higher protein content than maize or rice. In total, 16% and 26% of total dietary calories in developing and developed countries, respectively, come from wheat [2]. In Sub-Saharan Africa, Ethiopia ranks 2nd next to South Africa in terms of total wheat area and production. According to CSA [3], wheat in Ethiopia is an important cereal crop and it ranks third in total production next to teff and maize. It is largely grown in the highlands of the country and constitutes roughly 20-30% of the annual cereal production and plays an appreciable role of supplying the production with carbohydrates, proteins and minerals [4].

Within the total area of wheat under cultivation in Ethiopia, it has been reported that hexaploid and tetraploid species each occupy approximately 50% of the area [5]. However, a change in the relative proportions of wheat types grown has been reported more recently, with e.g. hexaploid and tetraploid species occupying approximately 70% and 30%, respectively, of the total wheat area under cultivation [3].

Origin, Taxonomy and Distribution of Hexaploid Wheat

Wheat belongs to the genus *Triticum* and the tribe *Triticeae* of the family Poaceae (*Gramineae*) [6] and it evolved from wild grasses found in the Eastern Mediterranean and Near East regions. It was probably domesticated around 10,000 to 15,000 B.C. in the bordered areas of the countries known as Iran, Iraq, Syria, and Turkey; a mountainous hilly region in the upper reaches of the Tigris and Euphrates drainage basin [7].

There are fifteen recognized species within the genus *Triticum.* About 90% of the world's wheat production consists of three species: *Triticum aestivum* (common wheat), *Triticum compactum* (club wheat) and *Triticum durum* (durum or macaroni wheat).

The species of the genus *Triticum* and their close relatives can be divided into diploid, tetraploid and hexaploid groups, with chromosome numbers of 2n = 14, 28and 42 respectively, in which the basic chromosome number is x = 7. The wild species are diploids (2n = 2x = 14), e.g. with the genome designation AA (*T.monococcum*), DD (*T. tauschii*, syn. *Aegilops squarossa*), and SS (*T. speltoids*), or tetraploids (2n = 2x = 28), e.g. with the genomes AABB (*T. durum* or *T. turigidum*) or AAGG (*T. timopheevii).* The most common cultivated wheat (bread wheat) now a day is hexaploid, *T. aestivum*, AABBDD, (2n= 6X= 42) [8].

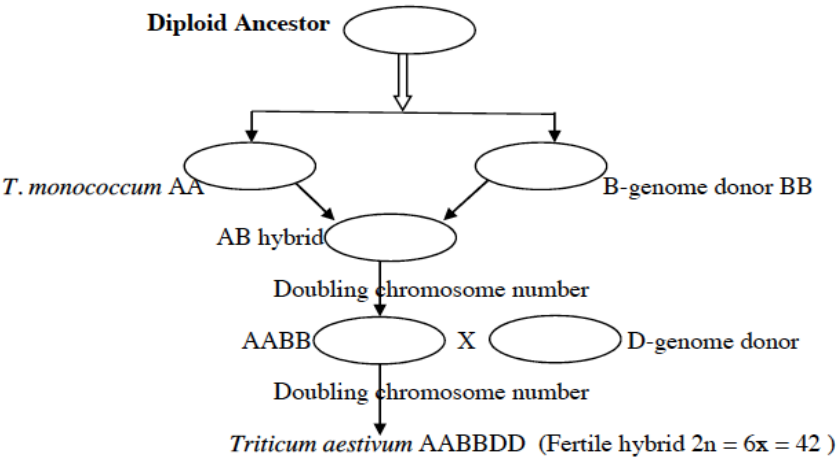

Fig. 1. Possible origins of Triticum aestivum (after van Buren, 2001; Kerby & Kuspira, 1987). *(Each capital letter represents a genome composed of seven chromosomes).*

Assessing Genetic Diversity

Genetic diversity is one of the key factors for the improvement of many crop plants including wheat. Plant breeders rely on the availability of genetic diversity during selection in cultivar development. The efficiency of genetic gain by selection can be improved if the patterns of genetic diversity within a population of breeding lines are known. Genetic similarity/distance estimates among genotypes are helpful in the selection of parents to be used in a breeding program. Varieties developed with wider genetic base may be helpful in enhancing the yield under various agro-climatic conditions. Diverse genetic base may also resist the spread of diseases, in approved varieties. Genetic diversity can be accessed from pedigree analysis, morphological traits or using molecular markers. However, diversity estimates based on pedigree analysis have generally been found inflated and unrealistic [9]. Genetic diversity estimates based on morphological traits, on the other hand, suffer from the drawback that such traits are limited in number and are influenced by the environment Molecular markers are useful tools for estimating genetic diversity as these are not influenced by environment, are abundant and do not require previous pedigree information [10].

Morphological diversity

Agro-morphological characterization (phenotyping) is a first step towards diversity and conservation of plant genetic resources. When assessing genetic diversity, the use of agro-morphological variation provides greater complementary information to molecular markers characterization. Agro-morphological criteria such as the color and structure of seeds, glume nature, spike density, days to maturity and heading, plant height,

thousand kernel weight, etc. can be used to study the variation among the hexaploid wheat varieties. For successful diversity and conservation, knowledge on the nature and extent of the available variation is important. Different studies have dealt with the variability in the Ethiopian wheat landraces for agronomic and morphological characters [11-13]. In most cases the studies showed the presence of variation within and between populations and geographical regions.

Biochemical diversity

It is the investigation of variation that may exist within and between population in wheat by the use of biochemical markers that involves the nutritional contents of the wheat like hectoliter weight, thousand grain weight, grain protein percentage, zeleny sedimentation, gluten content, grain starch percentage, grain hardness, and the seed storage proteins and isozymes [14-15]. These techniques utilize enzymatic functions and are comparatively inexpensive yet power full method of measuring allele frequencies for specific genes.

Biochemical analysis can also be based on the separation of proteins into specific banding patterns. It is a fast method, which requires only small amounts of biological material. However, only a limited number of enzymes are available and thus, the resolution of diversity is limited.

This information can be used to measure population subdivision, genetic diversity, gene flow, genetic structure of species, and comparisons among species out-crossing rates, population structure and population divergence, such as in the case of crop wild relatives.

Molecular diversity

It is the divergence or similarity of crops based on the molecular markers work by highlighting differences (polymorphisms) within a nucleic acid sequence between different individuals. These differences include insertions, deletions, translocations, duplications and point mutations. They do not, however, encompass the activity of specific genes. In addition to being relatively impervious to environmental factor, molecular markers have the advantage of: (i) being applicable to any part of the genome (introns, exons and regulation regions); (ii) not possessing pleiotrophic or epistatic effects; (iii) being able to distinguish polymorphisms which not produce phenotypic variation and finally, (iv) being some of them co - dominant. The different techniques employed are based either on restriction-hybridization of nucleic acids or techniques based on Polymerase Chain Reaction (PCR), or both.

Criteria for the estimation of genetic diversity can be different: pedigree records, morphological traits or molecular markers. The use of molecular markers for the evaluation of genetic diversity is receiving much attention. Many wheat

scientists have studied genetic diversity in common wheat using different molecular markers [16-18]. However, most of these marker systems show a low level of polymorphism in wheat, especially among cultivated lines and/or cultivars [19].

Because SSRs are multiallelic, they have high potential for use in evolutionary studies [20-21] and studies regarding genetic diversity and relationships. At present, microsatellites are one of the most promising molecular-marker types able to identify or differentiate genotypes within a species. Their co-dominant inheritance, high level of polymorphism and easy handling make them extremely useful for many different applications [19].

Seed Storage Proteins in Wheat

Protein is considered the most important nutrient for humans and animals. The protein content of wheat grains varies between 10%-18% of the total dry matter. Wheat proteins are classified according to their extractability and solubility in various solvents. Classification is based on the classic work of T. B. Osborne [22] at the turn of the last century. In his procedure, sequential extraction of ground wheat grains result in the following protein fractions [23-24].

Statement of Problem

World population is expected to increase by 2.6 billion over the next 45 years, from 6.5 billion today to 9.1 billion in 2050. Ethiopia is one of the nine countries predicted to account for the 2.6 billion increases. There is a pressing need for an astonishing increase in food production to feed this population. Wheat is among the major cereal crops grown in Ethiopia. It grows on an area of about 1.69 million hectares, and ranks third in area and second in total production (FAO 2005). It is an important commodity crop, which could contribute a major part in achieving the country's agricultural objective of food grain self-sufficiency). Despite the country having potential environments for wheat culture and being the centre of diversity for wheats, the average national yield is low (1.8 t/ha). The national average of wheat productivity in the country is 1.4 tons/ha, which is still 24% and 48% that of the South Africa and the world’s averages respectively [25]. The major wheat yield limiting factors in Ethiopia which resulted in such low yield levels, compared to any other part of the world, are diseases, weeds, poor soil fertility, lack of cultivar choice, frost occurrence in the highlands, terminal drought stress and water logging in the intermediate altitudes, and drought stress in the lowlands. Moreover, many of the variability studies [12][26] conducted so far are based on morphological traits, which are largely influenced by environmental factors. The few studies performed using microsatellites [11][27], isozymes [28], and glutenine and gliadine storage protein and AFLP [11]

considered either few accessions or focused mainly in the central highlands of Ethiopia. Thus, it was felt that because wheat varieties have not been adequately evaluated, their genetic resource remains largely unexploited.

For a successful breeding program, the presence of genetic diversity and seed storage protein play a vital role. Genetic diversity based on morphological & biochemical, seed storage protein and SSR are essential to meet the diversified goals of plant breeding such as breeding for increasing yield, wider adaptation, desirable quality, pest and disease resistance.

Research on bread wheat genotypes for genetic and molecular diversity study based on seed storage protein and microsatellite markers is limited. Moreover, study in baking quality of hexaploid wheat varieties is lacking. Comparative study to show the inter and intra population variation in Ethiopian bread wheat using morphologic and biochemical characters, seed storage protein and DNA markers is not available. Hence, the objective of this study is to ascertain the genetic diversity of bread wheat (*Triticum aestivum* L.*)* varieties using morphological, grain quality, seed storage protein, and SSR markers in Ethiopia.

Materials and Methods

The experiments will be conducted in the field as well as in laboratory in the following manner:

Experimental Materials

121bread wheat varieties released at various times by different agricultural research centers will be used in the study. The varieties include advanced lines. These varieties will be obtained from Kulumsa and Adet Agricultural Research Centers, which are National Bread Wheat Research Coordination Center and Regional Research Center, respectively. Most released varieties are adapted to 1900-2800m.a.s.l.

Field Experiments

Agronomic and morphological characters

Experimental site: The phenotypic characterization will be conducted at three sites viz., Adet Agricultural Research Center (west Gojam, Amhara), Debretabor sub-center (North Gondor, Amhara) and Kulumsa Agricultural research Center (Arsi zone, Oromia).

***Experimental Design*:** The treatments will be arranged in an 11x11 simple lattice design with plot size of 2 m with 4

rows of 2 m length, and 20 cm intra row spacing. Seed and fertilizer rates will be as recommended by respective research centers and testing sites. DAP will be applied at planting and urea will be splitted i.e., half at planting and half at late tillering stages.

Sowing will be done by hand drilling. The trial will be planted around mid of June, 2014 G.C.

Morphological Data Collection

The following data will be collected based on two central rows to minimize boarder effect:

Plant height (PH), Maturity Date (MD), Number of tillers per plant (NTPP), Plant Stand (PS) or Stand percent (SP)%, Spike length (SL), Number of seeds per spike (NSPS), Grain yield(GY) or Grain Dry Weight (GDW) Kg/plot, Biomass yield (BY) or Total Dry Weight (TDW) Kg/plot, Harvest index, Spike density(5 samples), Days to flower, Number of spike lets per spike, Tillering capacity, Awn presence (awnedness) Characteristics, 1000grain weight(gm), (DH) days to heading is number of days from sowing to emergence of spike, Days to 50% germination, Glume Characteristics, Seed Characteristics, Grain Characteristics, Disease occurrence.

Seed Storage Protein analysis

In this analyses there are two possible characters to be determined, one is the determination of quality traits - characterization of wheat germplasm for composition of high molecular weight (HMW) ─ glutenin subunits, low molecular weight (LMW) ─ glutenin subunits and gliadins. Secondly determination of variation of bread wheat varieties as it can be done in molecular markers using band determination as given below in the data analysis part. Banding patterns of the varieties will be investigated for their variability using the gel documentation system (Damania et al., 1983), followed by Gel Documentation and - Analysis.

Analysis using Simple Sequence Repeats (SSR)

Tissue harvest and DNA extraction

Young leaves will be collected separately from 5 randomly selected individual plants per accession after four weeks of planting and dried in silica gel. Approximately equal amounts of the dried leaf samples will be bulked for each accession and ground with pestle and mortar. Total genomic DNA will be isolated from about 0.4g of the pulverized leaf sample using modified triple Cetyl Trimethyl Ammonium Bromide (CTAB) extraction technique as described by [29]; followed by Primer selection and optimization, PCR and gel electrophoresis, gel documentation and analysis.

Statistical analysis

Morphological and Biochemical Data: Analysis of variance using SAS software (v. 9.1) will be used to determine all morphological and biochemical components.

Protein Analysis*:* The similarity matrix generated will be converted to a dissimilarity matrix [30]. All analysis will be carried out using a statistical package NTSYS -pc, version 1.8 [31] and STATISTICA.

SSR Profiles/Bands will be scored visually for each individual accession from the gel photograph. The bands will be recorded as discrete characters, presence '1' or absence '0' and '?' for missing data if any. Based on recorded bands different software will be used for analysis. POPGENE version1.32 software [32] will be used to calculate genetic diversity for each population as number of

polymorphic loci, percent polymorphism, Gene diversity (H) and Shannon diversity index (I). Analysis of molecular variance (AMOVA) will be used to calculate variation among and within population using Harlequin version 3.01. NTSYS- pc version 2.02 [33] and Free Tree 0.9.1.50 [34] software will be used to calculate Jaccard's similarity coefficient which is calculated with the formula:-

Expected Research Outcome

a) The extent and nature of genetic diversity within Ethiopian bread wheat varieties analyzed.

b) The grain quality characteristics of the varieties determined.

c) Environmental effects on the quality of seed storage proteins determined.

d) Composition of high molecular weight (HMW) glutenin subunits, low molecular weight (LMW) glutenin subunits and gliadins characterized.

e) Genetic variation using SSRs molecular markers determined

f) Varieties of required potential for further breeding and improvement of nutritional and baking quality suggested

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