

# Diversity of Ethiopian tetraploid wheat germplasm: breeding opportunities for improving grain yield potential and quality traits

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## Abstract

In this paper, Shannon–Weaver diversity indices were employed to examine the phenotypic diversity in 271 Ethiopian tetraploid wheat accessions in relation to characters, regions of origin and altitude. Moreover, review of genetic diversity studies in Ethiopian tetraploid wheat was made to explore breeding opportunities. The diversity index varied widely across regions. Among the four altitudinal classes, the highest (0.72) and lowest (0.61) mean diversity indices were observed in altitude classes II and IV, respectively. The diversity index ( $H'$ ) showed that most traits are polymorphic. The partitioning of the total phenotypic diversity into within- and among-region diversity indicated that 71% of the total variation was attributed to the within-region diversity. Principal component analysis was computed to examine the regional and altitudinal patterns of variation. On regional bases, the first four axes, whose eigenvalues are greater than 1, explained about 82% of the observed phenotypic diversity in the 271 tetraploid wheat accessions. On altitudinal bases, however, only the first two principal components explained 89.7% of the total variation. In general, phenotypic diversity showed considerable differences for each trait in different geographical regions and altitudinal classes which could be utilized in wheat improvement programmes. Breeding opportunities and strategies are suggested.

**Keywords:** breeding opportunities; Ethiopia; genetic diversity; tetraploid wheats

## Introduction

World population is expected to increase by 2.6 billion over the next 45 years. Ethiopia is one of the nine countries predicted to have the largest increase. Therefore, there is a pressing need to increase food production to feed this increasing population. Wheat is an important commodity crop that could contribute a major part in achieving the Ethiopia's agricultural objective of food grain self-sufficiency. In its strategic plan, the Ethiopian Agricultural Research Organization (EARO) considered

wheat as no. 1 priority crop among cereals (EARO, 2000). Despite having high potential environments and being rich in diversity, the average national yield of wheat is low (1.8t/ha).

The demand for wheat is continuously increasing. The estimated 2.2Mt national wheat grain requirement is 50% greater than the total production, and therefore Ethiopia is a net importer of wheat (Eshetu, 2002) suggesting the need to increase production. A dual strategy based on increasing wheat productivity through development of high yielding varieties and increase in area under wheat crops through expanding wheat cultivation to new areas was suggested to meet this demand (EARO, 2000). Although Ethiopia has potential environments for

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expanding wheat, meeting expected demands by continued expansion of agricultural production into marginal areas might be difficult as the economic costs of establishing new farms are high (Skovmand *et al.*, 2001). Therefore, the country has to work more towards increasing productivity and total production thereof. Future gains in yield potential require exploitation of the largely untapped sources of genetic diversity housed in collections of wheat landraces and wild relatives (Skovmand *et al.*, 2001). Even in the age of genomics, genetic diversity remains the cornerstone of crop improvement (Sneller *et al.*, 2005).

In Ethiopia, large amounts of wheat germplasm (about 12,000 accessions) have been maintained in the Institute of Biodiversity Conservation (IBC). Landraces constitute the lion's share of these collections. Landraces, which are locally adapted genotypes that have evolved because of natural and artificial selection forces over the millennia are one of the invaluable heritages that traditional farmers have given us (Myers, 1994). Landraces may be used as starting populations for cultivar development (Lakew *et al.*, 1997) or as sources for the introgression of genes and quantitative trait loci conferring resistance to biotic (Huang *et al.*, 1997) and abiotic stresses (Forster *et al.*, 2000). Lakew *et al.* (1997) confirmed the presence of individual genotypes within landraces that have a yield potential comparable with the best breeding lines. Various authors (Vavilov, 1951; Porceddu *et al.*, 1973; Amri *et al.*, 1990; Belay *et al.*, 1993; Kubo *et al.*, 2004) reported the uniqueness of the Ethiopian tetraploid wheat germplasm for different useful traits. However, it is felt that these collections have not been fully utilized in the breeding programmes. Efficient utilization of the genetic potential held in the germplasm collections requires a better knowledge of the collected material including morphological and phenological characterizations. Although several authors (Jain *et al.*, 1975; Bekele, 1984; Negassa, 1986a, b; Bechere *et al.*, 1996; Pecetti and Damania, 1996; Eticha *et al.*, 2005; Hailu *et al.*, 2006) have conducted variability studies in Ethiopian tetraploid wheats, there is still a need for more information on population structure and about their potential input for breeding. Hence, in this study, phenotypic diversity was investigated using 271 accessions collected from all over the country. Moreover, the breeding opportunities prevailing in Ethiopian tetraploid wheat have been summarized and strategies to enhance yield and quality traits were suggested.

## Materials and methods

### Plant materials and data collection

A total of 271 tetraploid wheat landraces collected from all geographical regions of Ethiopia were used. Because only 1, 6 and 6 accessions, respectively, were represented each

region of Ilubabur, Kefa and Sidamo, accessions from these three regions were pooled together and the three provinces were designated as IKS. Figure 1 shows the different regions of Ethiopia and its neighbours. The accessions have also been classified based on the four altitudinal classes: I [2000 meters above sea level (masl)], II (2001–2500 masl), III (2501–3000 masl) and IV (> 3000 masl). The number of accessions belonging to these four altitudinal classes is 47, 123, 89 and 12, respectively. In Ethiopia, wheat is mainly grown under rainfed condition at altitudes ranging from 1800 to 2800 masl. In some parts of the country, it is known to grow at above 3000 masl. Consequently, a larger number of accessions were sampled from altitude classes II and III.

The experiment was conducted during 2002/2003 and 2003/2004 main cropping seasons at the Alemaya University research site (rare), which is located at 1980 masl. A randomized complete block design with two replications was used. Each plot consisted of two rows, each 1 m long and 20 cm apart. The distance between the blocks and the spacing between plots were 1.5 m and 0.5 m, respectively. Fertilization of experimental plots and all other cultural management were done following the recommended cultural practices.

Thirteen quantitative phenological and morpho-agronomic traits were measured (Table 3). Data for days from emergence to anthesis (DTH) and from emergence to maturity (DTM) were determined on plot basis, and grain filling period (GFP) was determined as days between these two phenological traits. Plant height (PH) in cm and spike length (SL) in cm and spikes/plant (SP), spikelets/spike (SS), kernels/spike (KS) and kernels/plant (KP) in numbers were determined based



Fig. 1. Map showing the different regions of Ethiopia and its neighbours.

on five randomly selected plants per plot. Grain yield per plant (GY) and biomass yield per plant (BY), both in grams, and harvest index were assessed on ten randomly selected plants per plot and 1000-kernel weight (TKW) in grams was determined from dried samples of 1000 grains.

### Statistical analysis

All measured variables were subjected to analysis of variance procedures to assess differences among varieties. Mean value over the 2 years for each character was used to determine descriptive statistics such as the range, arithmetic means and standard errors of means for each of the variables. The Shannon–Weaver (1949) diversity index ( $H'_C$ ) was used as a measure of phenotypic diversity for each trait after their transformation into classes. It is estimated using  $H'_C = -\sum_{i=1}^n p_i \log_e p_i$  where for a given character C,  $n$  is the number of phenotypic classes and  $p$  is the proportion of observation in the  $i^{\text{th}}$  class. The diversity index ranges from 0 to 1, where 0 indicates complete evenness and 1 shows complete unevenness. The index was estimated for each character over all accessions and for each character within a region and altitudinal class. Due to its additive property (Kent and Coker, 1992), Shannon–Weaver diversity indices obtained for each character were pooled for each region and altitudinal class over the respective number of accessions. To avoid the effect of the different numbers of phenotypic classes while comparing indices obtained for the different characters, a standardized index (SDIc) was calculated as  $\text{SDIc} = H'_C / \log e^n$ . The diversity index computed based on the whole dataset ( $H_t$ ) was partitioned into within region or altitude and between region of origin and between altitudinal classes following the procedure of Paul *et al.* (1997). The within-region ( $H_r$ ) and within-altitude class ( $H_a$ ) diversity indices refers to the average diversity index of each character estimated based on the regions of origin and altitudinal classes, respectively. The between-region ( $\text{Dst}_r$ ) and between-altitude class ( $\text{Dst}_a$ ) diversity index were computed as  $H_t - H_r$  and  $H_t - H_a$ , respectively.  $(\text{Dst}_r)/H_t$  and  $(\text{Dst}_a)/H_t$  are the coefficients of gene differentiation based on the regions and altitudinal classes, respectively. Principal component analysis on the diversity index was conducted using the computer program NTSYS-pc (Numerical and Taxonomy and Multivariate Analysis system version 2.0; Rohlf, 1998).

## Results

### Descriptive statistics

Descriptive statistics such as the minimum, maximum, mean and standard error of means from the combined

analysis of variance for 13 quantitative traits were presented in Table 2. The combined analysis of variance results revealed that the genotypic differences among genotypes were highly significant ( $P \leq 0.05$ ) for all the characters. Also, the range of variation, which is the difference between the maximum and minimum values of each character, with regard to the 13 quantitative traits indicated the presence of wide variation for all quantitative variables. Among traits, GY per plant ranged from 3.7 to 9.2 g/plant with a mean value of 8.3 g. DTM, DTH and GFP have shown range values of 30, 31 and 22 days, respectively. High differences between the maximum and minimum mean values were also found for the other traits.

### Estimates of diversity

The overall diversity index estimated based on the entire dataset is 0.72 (Table 3). Among regions, the range of mean index of diversity varied from 0.68 for the accessions from Hararghe to 0.40 for the accessions from Gamugofa. The diversity indices differed among regions for specific characters. For instance, traits such as SS, SL, seeds/spike, spikes/plant and KP showed high polymorphism in Arsi, Hararghe and Shewa. The highest diversity index (0.75) for PH was recorded in collections from Arsi. Hararghe and Shewa also exhibited high polymorphism for BY and harvest index (Table 1).

Among the three altitudinal classes, the highest (0.72) and lowest (0.61) mean diversity indices were noted in altitude classes II and IV, respectively. The diversity indices differed among altitudinal classes for specific characters. In altitude class I, all characters except GY and biomass showed diversity index of more than 0.70. In altitude classes II and III, most of the traits except GY and SS in the former and GY and days to maturity in the latter displayed a diversity index value of more than 0.70. In altitude class IV, however, only five traits have exhibited a diversity index of greater than 0.70 (Table 1), indicating low polymorphism for traits in this altitudinal class than others.

### Partitioning of phenotypic diversity

Subdividing the variation into its components may facilitate genetic resources conservation and utilization, by determining the relative contribution of the different levels of variability to the total diversity available in any one area. To determine the significance of the different regional components, the total variation was partitioned into within- and among-region diversity. The coefficient of regional diversity differentiation is 0.29 implying that 71% of the total variation was explained

**Table 1.** Geographic region, number of sampled accessions (N) and estimates of the Shannon–Weaver diversity index ( $H'$ ) for the 13 metric characters across geographical regions and altitudinal classes

Geographical regions	N	CY	BY	HI	DTM	DTH	GFP	PH	SS	SL	KS	SP	KP	TKW	Mean
Atisi	24	0.47	0.62	0.68	0.51	0.64	0.73	0.75	0.74	0.73	0.71	0.75	0.69	0.75	0.67
Bale	24	0.6	0.64	0.68	0.67	0.64	0.67	0.6	0.5	0.71	0.19	0.49	0.45	0.41	0.56
Gamugofa	7	0.23	0.4	0.59	0.37	0.73	0.4	0.37	0.37	0.12	0.47	0.26	0.47	0.37	0.40
Gojam	19	0.53	0.66	0.65	0.62	0.52	0.53	0.62	0.71	0.73	0.6	0.69	0.64	0.71	0.63
Gondar	27	0.45	0.6	0.73	0.54	0.62	0.75	0.54	0.63	0.74	0.73	0.73	0.65	0.73	0.65
Hararghe	29	0.5	0.7	0.7	0.69	0.49	0.63	0.69	0.75	0.73	0.73	0.7	0.75	0.72	0.68
Shewa	75	0.57	0.71	0.72	0.45	0.48	0.72	0.45	0.73	0.74	0.75	0.74	0.71	0.74	0.65
IKS	13	0.34	0.52	0.61	0.65	0.57	0.73	0.65	0.71	0.71	0.62	0.51	0.46	0.71	0.60
Tigray	26	0.53	0.66	0.69	0.38	0.47	0.67	0.38	0.28	0.7	0.58	0.69	0.58	0.7	0.56
Welega	10	0.52	0.66	0.47	0.61	0.61	0.69	0.61	0.74	0.74	0.67	0.61	0.61	0.74	0.64
Welo	17	0.42	0.64	0.63	0.63	0.66	0.63	0.49	0.74	0.56	0.74	0.7	0.7	0.49	0.62
<i>Altitudinal classes (m)</i>															
I		0.56	0.31	0.7	0.74	0.74	0.73	0.75	0.74	0.72	0.74	0.74	0.73	0.74	0.69
II		0.6	0.74	0.73	0.73	0.74	0.75	0.68	0.74	0.75	0.75	0.73	0.72	0.75	0.72
III		0.57	0.71	0.72	0.65	0.7	0.72	0.73	0.75	0.74	0.74	0.74	0.71	0.75	0.71
IV		0.37	0.59	0.53	0.73	0.62	0.73	0.54	0.73	0.37	0.64	0.73	0.57	0.73	0.61

by the within-region diversity (Table 3). Among the 13 traits, BY, SL, HI, GFP and SL showed high within-region variation indicating their relative significance for differentiating accessions within regions. On the other hand, the two phenological traits, DTM and DTH, and PH contributed more to the between-region variation. The partitioning of between- and within-altitude diversity revealed that 95 and 5% of the total variation was attributed to the within- and between-altitude class variation, respectively (Table 3). The contribution of individual characters to the within-altitude diversity revealed that SS, TKW, GFP and BY have slightly stronger effect than others. In Ethiopian tetraploid wheat, studies (Bekele, 1984; Bechere *et al.*, 1996; Pectti and Damania, 1996) showed greater contributions of the lower (within populations and among populations within regions and altitude zones) than the higher (among regions and altitude zones) level hierarchies to the total phenotypic variation.

Phenotypic diversity was also examined using multivariate analyses. To this end, principal component analysis was computed on the diversity index of regions of origin and altitudinal class for the 13 quantitative traits to examine the regional and altitudinal patterns of variation. On regional bases, the first four axes, whose eigenvalues are greater than 1, explained about 82% of the observed phenotypic diversity in the 271 tetraploid wheat accessions (data not shown). The first and second axes accounted for about 29.8 and 20.5% of the total variation, respectively. Johnson and Wichern (1988) suggested that coefficient or eigenvector greater than half divided by the standard deviation of the eigenvalue of the respective PC is more important in explaining the overall variation. Following this criteria, traits such as days to heading, KS, SL and SP accounted for much of the variation on these axes. On altitudinal bases, only the first two principal components, which produced eigenvalues greater than 1, explained 89.7% of the total variation (data not shown).

## Discussion

### *Genetic diversity: a raw material and an opportunity for breeding*

Efficient utilization of the genetic potential held in the germplasm collections requires, among other things, a better knowledge of the genetic diversity present in the collected material. Knowledge of the extent of variability for plant traits and association of specific traits with geographic origin not only facilitates breeding programmes but also helps to define needs and locations for future collections of germplasm. In this study, results of analysis

**Table 2.** Summary of descriptive statistics from combined analysis of variance

Trait	Descriptive statistics			
	Minimum	Maximum	Mean	SE of a mean
Grain yield	3.70	9.20	7.93	0.05
Biomass yield	11.50	30.00	24.35	0.10
Harvest index	15.86	48.31	36.78	0.20
Days to maturity	127.0	157.00	119.17	0.16
Days to heading	86.00	117.00	98.16	0.18
Grain filling period	32.00	54.00	41.04	0.11
Plant height	70.20	99.90	91.08	0.20
Spikelets/plant	16.00	27.40	20.26	0.08
Spike length	9.10	14.30	8.89	0.04
Kernels/spike	25.00	51.80	10.53	0.18
Spikes/plant	3.00	8.90	5.99	0.05
Kernels/plant	75.00	243.70	133.50	2.11
TKW	23.10	51.90	35.11	0.14

of variance and descriptive statistics indicated that the genotypes differed significantly for all the traits revealing the presence of a considerable diversity, which could be utilized in developing high yielding cultivars through selection breeding. The overall Shannon–Weaver diversity index for all traits was 0.74. Because Shannon–Weaver diversity index is sensitive to both the type of phenotypic descriptor and the number of descriptor classes used (Grenier *et al.*, 2004), direct comparison of Shannon–Weaver diversity indices from different studies involving different descriptors and descriptor classes need caution. In Ethiopian tetraploid wheats, Jain *et al.* (1975), Negassa (1986a), Bechere *et al.* (1996) and

Eticha *et al.* (2005) employed the same method to investigate phenotypic diversity and reported a Shannon–Weaver diversity index of 0.70, 0.81, 0.87 and 0.71, respectively. As with the findings of phenotypic diversity studies, the presence of appreciable genetic diversity in Ethiopian tetraploid wheats has been reported from variability studies using microsatellites (Messele, 2001; Alamerew *et al.*, 2004; Teklu *et al.*, 2006a, b), cytological markers (Belay and Merker, 1999), isozymes (Tsegaye *et al.*, 1994, 1996), glutenine and gliadine storage protein and amplified fragment length polymorphisms (Messele, 2001). Many microsatellite loci might be significantly linked to agronomically important traits (Teklu *et al.*,

**Table 3.** Partitioning of the phenotypic diversity within and between geographical regions of collections and altitudinal classes

Characters	Geographical regions of origin				Altitudinal classes		
	$H_t$	$H_r$	$Dst_r$	$Gst_r$	$H_a$	$Dst_a$	$Gst_a$
Grain yield	0.59	0.40	0.19	0.33	0.53	0.06	0.12
Biomass yield	0.61	0.52	0.09	0.14	0.59	0.02	0.03
Harvest index	0.74	0.55	0.19	0.26	0.67	0.07	0.10
Days to maturity	0.73	0.47	0.26	0.36	0.71	0.02	0.03
Days to heading	0.75	0.49	0.26	0.34	0.70	0.05	0.07
Grain filling period	0.74	0.55	0.19	0.26	0.73	0.01	0.01
Plant height	0.73	0.47	0.26	0.35	0.68	0.05	0.07
Spikelets/spike	0.75	0.53	0.22	0.29	0.74	0.01	0.01
Spikelet length	0.74	0.55	0.19	0.25	0.65	0.09	0.13
Kernels/spike	0.75	0.52	0.23	0.30	0.72	0.03	0.04
Spikes/plant	0.75	0.53	0.22	0.29	0.74	0.01	0.02
Kernels/plant	0.73	0.52	0.21	0.30	0.68	0.05	0.07
1000 kwt	0.75	0.54	0.21	0.27	0.74	0.01	0.01
Mean	0.72	0.51	0.21	0.29	0.68	0.04	0.05

$H_t$ , diversity index for each character computed from the whole dataset;  $H_r$  and  $H_a$ , the average diversity indices of each character estimated based on regions of origin and altitudinal classes, respectively.  $Dst_r$  and  $Dst_a$  are the proportions of diversity within regions and altitudinal classes, respectively.  $Gst_r$  and  $Gst_a$  are the coefficients of gene differentiation based on regions and altitudinal classes, respectively.



2006a, b). Various authors also confirmed the uniqueness of the Ethiopian tetraploid wheat germplasm for different useful traits. For example, they have valuable features such as early ripening, short culm, long coleoptiles and low tillering (Porceddu *et al.*, 1973); high degree of allelic variation for quality traits such as the seed storage proteins (glutenins and gliadins; Messele, 2001); resistance to powdery mildew and glume blotch (Negassa, 1986a); Hessian fly (Amri *et al.*, 1990) and stripe rust and moderate resistance to pH and drought (Porceddu *et al.*, 1973). Vavilov (1951) found Ethiopian tetraploid wheat that had 20% protein. Ethiopian tall type (*rht*) landraces of durum wheat (*Triticum durum* Desf.) showed higher root penetration ability than semi-dwarf (*rht*) varieties bred in North America (Kubo *et al.*, 2004). High variation was also reported for seed colour, kernel texture, flour colour, seed size and protein content (Negassa, 1986b).

### Strategies to utilize genetic diversity

The best way to exploit the diversity contained in landraces and wild relatives is to introduce the valuable variation for qualitative and quantitative traits into adapted breeding materials using wide-crossing (Skovmand *et al.*, 2001) and complex crosses (Vetelainen, 1994). In barley, the study of Vetelainen (1994) indicated that hybrids from the complex crossing programme exceeded parents in earliness and TKW. Also, it is useful to consider the paradigm shift proposed by Tanksley and McCouch (1997) in which the most divergent accession(s) relative to elite cultivars are used to increase genetic variability for the improvement of quantitative traits. This strategy was used successfully to improve quality traits in tomatoes (Fulton *et al.*, 2000).

Natural populations harbour rich genetic diversity, which is eco-geographically structured and largely adaptive (Nevo, 1988). As a result, landraces provide a valuable resource for plant breeding as well as for the preservation of genetic diversity. Under Ethiopian condition, where wheat landraces cultivation is predominant (Tessema *et al.*, 1993; Bechere *et al.*, 1996; Tessema and Bechere, 1998), the first step in breeding should be the utilization of indigenous materials (Tessema, 1991). Enhancing the yield of landraces, while maintaining an appreciable level of genetic diversity, is crucial to improve their competitiveness with modern varieties and maximize their utilization (Tessema and Bechere, 1998). A modification of phenotype mass selection by selecting pure lines from genetically mixed landrace populations through yield testing and then bulking two or more superior pure lines has been suggested as one of the best strategies to improve the productivity of the landrace cultivars grown by the farmers (Tessema, 1991).

The huge diversity contained in the large amounts of wheat germplasm that have been collected and maintained mainly in the Ethiopian IBC has not been exploited effectively in cultivar development. Hoisington *et al.* (1999) described this phenomenon as valuable genetic resources are essentially 'sitting on the shelf' in what have been dismissively termed 'gene morgues'. The most attributed reason could be the large numbers of accessions, which make it difficult and time consuming to evaluate for all useful yield traits in the field trials and choose the most promising ones with which to work. Development of wheat core collections based on species could facilitate utilization of the huge diversity stored in genebanks in Ethiopia. Core collections could provide plant breeders a manageable number of accessions to use in the search of new characters or character combinations and a structured way to evaluate whole collections (Rao and Hodgkin, 2002). In addition, germplasm enhancement may be one of the keys for maximizing utilization of germplasm. It has become an important tool for the genetic improvement of breeding populations by gene introgression or incorporation of wild and landrace genetic resources into respective crop breeding pools.

### Conservation

In crop improvement, it is not only working with the existing genetic variation that is central, but also parallel and periodic assessment of the threat of loss of diversity is necessary.

Using the calculation scheme, gene erosion = 100% – gene integrity, i.e. the still extant landraces, a genetic erosion up to 100% was detected in *T. durum*, *Triticum dicoccon* and *Triticum turgidum* in some districts of eastern Ethiopia (Teklu and Hammer, 2006). Other reports (Worede, 1983; Hailu, 1991; FAO, 1996; Tsegaye and Berg, 2007) also reported the problem of genetic erosion in Ethiopian tetraploid wheats. Therefore, priority should also be placed on collection and conservation of landraces, which are irreplaceable materials, if lost. The best method of conservation is the use of complementary approach of the different *ex situ* and *in situ* conservation techniques. Apart from conservation, creation of sustainable agricultural systems that actively use as much biodiversity as possible should remain the major goal. The guiding principle of 'conservation through use' should be respected because only in use can diversity be appreciated enough to be saved, only in use it can continue to evolve and thus retain its value.

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