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Phenotypic diversity in Ethiopian durum wheat (*Triticum turgidum* var. *durum*) landraces

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ABSTRACT

The phenotypic diversity of 274 Ethiopian durum wheat accessions was analyzed, taking their geographic origins into account. The aim was to assess the extent and patterns of agronomically important phenotypic variation across districts of origin and altitude classes for major qualitative traits using diversity index and multivariate methods. Eight qualitative and three quantitative traits were scored for 2740 plants and analyzed for diversity. The Shannon–Weaver diversity (H') index was used to estimate phenotypic diversity. The estimated H' ranged from monomorphic for glume hairiness to highly polymorphic for other traits. The highest (0.86) H' was obtained for seed degree of shriveling, possibly indicating the differential responses of the genotypes to water deficit during later growth stages. With respect to district of origin, the highest (0.72) and lowest (0.44) H' values were obtained for the Bale and SNNP districts, respectively. With respect to altitude, the highest (0.76) and lowest (0.62) H' values were recorded for altitudes 1600–2000 and >3000 m above sea levels, respectively. Principal components analysis explained substantial variation contributed by district of origin and altitude range. Genotypes were clustered into three groups by districts of origin and altitude class, with relatively strong bootstrap values of 57 and 62 for the former and latter, respectively. It could be concluded that Ethiopian durum wheat landraces are very diverse both within and among districts of origin and altitude classes. This wealth of genetic diversity should be exploited for wheat improvement of yield and for resistance to biotic and abiotic stresses, particularly terminal drought.

1. Introduction

Ethiopian durum [*Triticum turgidum* var. *durum*, $2n = 4x = 28$] wheat is conspicuously diverse unexploited landraces. They harbor high variation, which is important for durum improvement of various traits [1–5]. Ethiopian durum wheat landraces are unique sources of useful traits [7,8], although collections have not been used to their full potential in breeding programs. The natural and artificial forces operating on the crop, including high ecological variation [3], isolation, differences in agricultural practices, and natural cross-fertilization [4] may explain this great diversity, which is molecularly largely uncharacterized.

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Use of crop diversity is one of several approaches to improving agricultural productivity and is a key to achieving global food security [9]. Knowledge of existing genetic diversity and its distribution in crop species is useful for germplasm conservation and selection of parents with diverse genetic background, thereby rendering crop improvement more efficient [10]. Crop landraces are described as geographically or ecologically distinct populations that show conspicuous diversity in their genetic composition both among populations (landraces) and within them [11] and display genetic variation for useful quantitative and qualitative characters [12]. These advantages pooled in landraces are not exploited by durum wheat improvement programs of Ethiopia, despite the country's large genetic diversity of durum wheat. These genetic resources, however, have contributed to world wheat improvement. For instance, Klindworth et al. [13] found that the Ethiopian durum wheat landrace *ST464* is one of the major sources of *Sr13*, the only known gene for resistance to *Ug99* or race *TTKS*, a new stem rust race currently threatening wheat production worldwide. The identification of Sr13 in Ethiopian durum wheat landraces and the fact that Ethiopia is one of the hot spots of *Ug99*, since it appeared in the country in 2003, make the country a center for stem rust screening and the primary durum wheat phenotyping site for the Durable Rust Resistance in Wheat Project that started in 2005.

Ethiopian farmers have been growing durum wheat for centuries, and as a result durum wheat covered 60–70% of the arable land under wheat cultivation in Ethiopia until the 1980s, with bread wheat (*Triticum aestivum*) covering only the remaining 30–40% [14]. However, the introduction of improved bread wheat from international breeding programs into Ethiopia and their wide adaptation with satisfactory yield potential has shifted the predominance to bread wheat and left durum wheat landraces almost an orphan crop. Now, approximately 80% of the wheat area in Ethiopia is planted to bread wheat [14], implying that 20% of the wheat area is planted to durum wheat. Tessema and Bechere [2] reported that improved durum varieties are grown on less than 20% of durum wheat cultivation area, because of a lack of a modern seed market and farmers' low purchasing power. The majority of durum wheat grown in Ethiopia is thus landraces consisting of large numbers of different genetic lines [15]. Information about the national annual production and productivity of durum wheat has not been separately documented in annual Ethiopian statistical abstracts published by the Ethiopian central statistical agency [35]. Data on the national annual average yields of durum wheat is still scanty. Yield reported by research institutions for improved durum wheat varieties in the central highland plateau of Ethiopia under researcher-managed conditions was encouraging, although it must not be considered to be the national average yield.

Consumer demand for wheat far exceeds domestic production, and wheat imports cost the country millions of dollars in foreign exchange [14,16]. Future gains in yield potential and quality standard of the produce are desirable and require exploitation of the largely untapped genetic diversity of durum wheat landraces housed in the national gene bank [17]. The geographic pattern of diversity of Ethiopian durum wheat has been documented [1,3,4,6,18–21]. However, these studies were limited to landraces collected from fairly restricted areas, mainly the central highlands of Ethiopia, and cannot give an overall picture of diversity across the country. The results from such studies are not dependable and are often misleading [6], as the contribution from geographical region of origin to total observed variability among the landraces is unknown. The aim of this study was to extend the assessment of the extent and patterns of phenotypic variation in Ethiopian durum wheat, sampled from major wheat growing regions of the country, to agro-morphological traits. Specifically, it aimed to determine the amount, extent and distribution of genetic variation in durum wheat landraces by district of origin and altitude class for selection of landraces to produce pre-breeding lines.

2. Materials and methods

2.1. Plant materials and data collection

A total of 274 durum wheat genotypes, 271 landraces and 3 improved varieties, representing the major wheat-growing areas of Ethiopia were studied (Table S1). The districts of collection of the landraces are shown as points in Fig. 1. Landraces from individual districts were considered independent populations except those collected from various districts of Southern Nations Nationalities and People (SNNP), which were pooled into a single population because their number was only eight (Table S1). The landraces were also classified based on the altitude of collection. They were collected from five altitude classes (Table S1). This altitude classification was also used by Hailu et al. [22]. Under rainfed conditions, wheat is grown mainly at altitudes ranging from 1800 to 3000 m.a.s.l. Consequently, a small number of landraces were sampled from the last altitude class. Two field experiments were conducted during the 2011/2012 main cropping seasons at Hagreselam Tigray (13°39′ N and 39°07′ E, 2590 m.a.s.l.) and Debre Zeit Agricultural Research Centre Station (8°46′ N and 39°00′ E, 1870 m.a.s.l.). At each site, an experiment was laid out in a partial lattice design with plots 2.5 m long and 1.2 m wide containing six rows with 20 cm inter-row spacing in two replications. Seed rate was adjusted to the recommended rate for each site (100 kg ha^{-1} for Hagreselam and 150 kg ha^{-1} for Debre Zeit). Fertilizer application was performed on the basis of 100 kg ha^{-1} DAP and 50 kg ha^{-1} UREA for the Tigray site (Hagreselam) and 100 kg ha^{-1} DAP and 100 kg ha^{-1} UREA for Debre Zeit. At each site, nitrogen fertilizer was applied in two splits: two thirds at planting and one third at knee stage. All agronomic practices were applied equally to experimental plots.

At each site, 10 representative spikes (five in each replication) were randomly sampled from each landrace, listed in Table S1, during harvesting and taken to a laboratory at Mekelle University for phenotyping for qualitative traits including spike density (SD), awn length (AL), kernel color (KC), kernel size (KS), glume color (GC), glume hairiness (GH), seed nature/texture or vitreousness (VT), beak awn (BA), and degree of seed shriveling (DSH). Scoring was performed for all 10 spikes based on the International Plant Genetic Resource Institute's (IPGRI) wheat descriptor list [32]. The numbers of phenotypic classes used for the Shannon–Weaver diversity index, which differed for each trait, are listed in Table 1. Data for days to 50% booting (DB) and days to maturity (DM) were recorded on a plot basis.

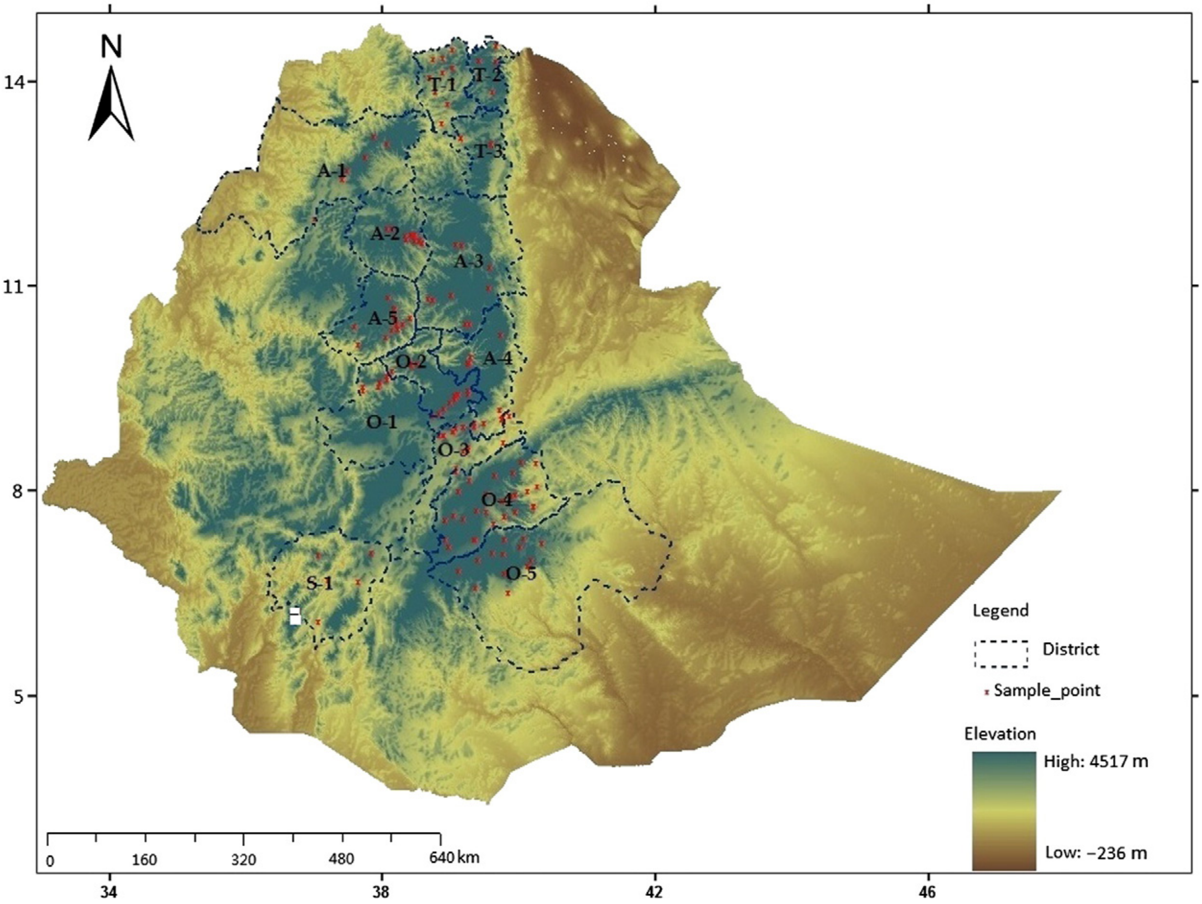

Fig. 1 – Map of Ethiopia showing the districts of origin of the studied durum wheat landrace accessions. Districts of collections are represented by combinations of letters and numbers. Districts of origin are T-1 (Central Tigray), T-2 (East Tigray), T-3 (Southern Tigray), A-1 (East Gojjam), A-2 (West Gojjam), A-3 (North Gonder), A-5 (South Gonder), A-4/O-2 (North Shoa), O-1 (West Shoa), O-3 (East Shoa), O-4 (Arsi), O-5 (Bale), and S-1 (SNNP).

2.2. Diversity index estimation

The numbers of phenotypic classes, which differed for each trait, used for the Shannon–Weaver diversity index are listed in Table 1. The Shannon–Weaver diversity index (H') used to characterize the phenotypic frequencies of the characters was defined as:

$$H = \sum_{i=1}^{n} p_i \ln p_i \qquad (1)$$

where n is the number of phenotypic classes for a character and p_i is the proportion of the total number of entries in the ith class. H was estimated for each trait, district of origin, and altitude class. Each value of H was standardized by conversion to a relative phenotypic diversity index (H') by division by $H_{\max} = \ln(n)$ in order to express the values of H' in the range of 0–1.

$$H' = H/H_{\max} \qquad (2)$$

The diversity index was classified as high ($H' \geq 0.60$), intermediate ($0.40 \leq H' \leq 0.60$), or low ($0.10 \leq H' \leq 0.40$), as described in Eticha et al. [21]. These normalized values were used in analysis of variance of diversity for individual characters, collection districts, and altitude classes, and hierarchical analysis of variance (ANOVA) pooled over characters as described in Tessema et al. [5].

2.3. Statistical analysis

Multivariate analysis was performed to discriminate districts of origin and altitude classes with hierarchical clustering and principal components analysis. Principal components analysis, operating on either sums of squares and products, a correlation matrix, or a matrix of variances and covariance, finds linear combinations of a set of varieties that maximize the variation contained within them, thereby describing most of the original variability in a smaller number of dimensions. Principal components that explained at least 5% of total variance and having eigenvalue at least 1 were retained for analysis. A correlation matrix was used to define the patterns of variation among landraces for both districts of origin and altitude classes using the Genstat-12.1 statistical package [33] in the principal components analysis. For cluster and principal components analysis the values for each trait were

Table 1 – Descriptors used for estimating phenological and spike-based trait diversity in durum wheat landraces, their numbers of classes, and proportion (%) of occurrence of each class, and estimated phenotypic diversity index (*H′*) for each trait.

Morphological trait	Observed phenotypic class [a]	Class	Proportion (%)	Diversity index (*H′*)
Spike density (SD)	Lax	4	2.95	0.85
	Intermediate		23.43	
	Dense		41.57	
	Very dense		32.05	
Glume color (GC)	Brown	7	35.00	0.71
	Brown–black		1.68	
	Dark brown		3.88	
	Light brown		1.74	
	Gray		7.25	
	White		40.62	
	White with black spot		2.75	
Glume hairiness (GH)	Absent	3	88.82	0.24
	Hairy (low)		5.34	
	Hairy (high)		5.84	
Days to 50% booting (DB)	Early: <68	3	9.85	0.43
	Medium: 68.1–75		84.24	
	Late: >75		5.91	
Days to maturity (DM)	Early: <117.58	3	14.28	0.41
	Medium: 118–130		66.5	
	Late: >130		19.22	
Kernel color (KC)	White/white–yellow	6	22.53	0.69
	Amber		4.28	
	Gray		6.02	
	Brown		44.58	
	Brown–purple		10.78	
	Purple		11.75	
Awn length (AL)	Awnletted (<3 cm)	3	3.05	0.53
	Awnletted (3–6 m)		20.45	
	Awned (>6 cm)		69.72	
Beak form (BF)	Pointed	4	30.54	0.81
	Acuminated		9.65	
	Intermediate		25.9	
	Awned		33.92	
Kernel size (KS)	Small (<5 mm)	4	14.04	0.82
	Intermediate (5–7 mm)		46.63	
	Large (7.1–9.0 mm)		31.02	
	Very large (>9 mm)		8.31	
Seed nature/texture or vitreousness (VT)	Soft	3	15.48	0.68
	Partly vitreous		24.34	
	Hard (vitreous)		60.18	
Degree of seed shriveling (DSH)	Plump	3	51.08	0.86
	Intermediate		39.22	
	Shriveled		9.70	

[a] Observed class defined based on IPGRI manual, Hailu et al. [22] and Eticha et al. [21].

standardized to unit variance and zero mean. Hierarchical clustering was performed using a numerical measure of similarity computed from standardized data, using Phylip-3.63 statistical software [34], to assess the patterns of diversity among districts and altitude classes. The standard genetic distances from the portion of phenotypic classes were used to construct a dendrogram by the unweighted pair group method based on arithmetic average (UPGMA) with bootstrap test of 100 replicates for the clustering tree generated. Hierarchical analysis of variance (ANOVA) pooled over districts of origin, altitude classes, and finally characters was also performed to test the significance of variation of the estimated diversity index for each trait, district of origin, and altitude class using Genstat-12.1.

3. Results

3.1. *Phenotypic diversity among landraces, between districts and altitude classes*

Large natural variations were found among the landraces for all investigated spike-based qualitative traits (Table 1). This variation is an indication of wider phenotypic diversity among Ethiopian durum wheat landraces. Estimated diversity (*H′*) for individual traits ranged from 0.24 for GH to 0.86 for DSH with overall means of 0.67, 0.57, and 0.64 for qualitative, quantitative, and grand diversity mean, respectively (Table 1). Traits showing high levels of polymorphism ($H' > 0.60$) included SD

($H' = 0.85$), KL ($H' = 0.82$), BL ($H' = 0.81$), KC ($H' = 0.69$) and VT ($H' = 0.68$). Lower levels of diversity were observed for GH and AL with H' value of less than 0.60.

On a district basis, high diversity indices ($H' \geq 0.60$, as used in [21]), pooled over traits, were obtained for landraces collected from all districts. The within-district H' depended on the indices of the measured traits. When H' of each character was considered, in most of the districts glume hairiness showed the lowest diversity index and was monomorphic ($H' = 0.00$) in Bale, South Gonder, and North Gonder. However, landraces from SNNP showed high diversity ($H' = 0.68$) for this trait. For other traits, high diversity indices ($H' \geq 0.60$) were obtained across the majority of districts with the highest ($H' = 0.99$) value for degree of shriveling in Southern and Central Tigray (Table S2). Tigray populations were very diverse for spike density. In contrast, very low diversity indices were obtained for seed vitreousness in SNNP ($H' = 0.11$) and awn length for North Shoa ($H' = 0.32$), Wollo ($H' = 0.38$) and North Gonder ($H' = 0.12$) landraces. Similarly, a very low diversity index ($H' = 0.34$) was obtained for beak length for landraces collected from the Bale district. The range of difference in diversity was not wider between than within districts (Table S2). The between-district estimate of diversity index was highest in Arsi ($H' = 0.72$) and lowest in improved varieties ($H' = 0.44$). Within-district diversity for the majority of the districts was in the high-diversity index range. This observation suggests the importance of within- compared to between-district diversity for Ethiopian durum wheat.

In most of the altitude classes, all traits but GH showed high diversity indices (Table 2). Glume hairiness consistently showed very low ($H' < 0.40$) across all altitude classes, showing that the landraces had low divergence for this trait. For the majority of the traits, the mean diversity index showed a declining trend with altitude, although the relationship was not linear. However, the mean diversity index for KS increased with elevation. Averaged over altitude classes, the highest mean diversity index ($H' = 0.90$) was observed for KS and the smallest ($H' = 0.22$) for GH. High diversity indices ($H' \geq 0.60$) pooled over traits were observed for altitude classes. The between-altitude diversity index was highest (0.76) for 1600–2000 m.a.s.l. and lowest (0.64) for 2801–3000 m.a.s.l.

3.2. Principal components (PCs) analysis

Principal components analysis effectively explained the variation among districts of origin and altitude classes, with the first five and four principal components accounting for 89% and 99%, respectively, of variation (Table 3). Characters VT, SD, AL, DSH, and DB were the most important traits contributing to Principal component one (PC1) of districts of origin. In principal component two (PC2), which described about 20% of the total variance of districts, DB, DM, and GH showed large contributions, whereas BF, GC, and KL accounted for much of the total variance in principal component three (PC3). BF and SD contributed most to principal component four (PC4).

In altitude classes, PC1 explained 50% of the total variance, with the main contributions from DB, DM, KS, and SD. Similarly, PC2 explained 25% of the total variation, with the main contributions from BF, DSH, and VT. The third and fourth PCs contributed 16% and 8% of total altitude variance, respectively. AL, GC, and KC were larger contributors to PC3, while the glume-associated traits, GC and GH, were major contributors to variance of the fourth PC. The distribution of districts of origin of the landraces along the first two principal component axes was concentrated around the origin except for the improved varieties and landraces from SNNP (Fig. 2). The extremes of the PC1 and PC2 axis were occupied by improved varieties and landraces, respectively, from SNNP, with low negative principal scores. In contrast, landraces from North Gonder were placed in the second quadrant of the principal components with high positive scores. This finding implies that improved varieties and landraces from SNNP differ greatly from others. Overlaying traits on districts showed that phenological (DB and DM) traits are more important for improved varieties than for landraces, although variability was associated more with spike traits than with phenological traits. GH and AL were important traits discriminating SNNP landraces from the rest. In general, KC, DSH, SD, BF, and GC were the most important traits for discriminating districts.

3.3. Cluster analysis

The dendrogram constructed to describe the relationship among districts of origin (Fig. 3-A) grouped the genotypes into four main clusters with bootstrap values of 57, 55, and 54. The first cluster combined improved released varieties and landraces from SNNP. The second and third clusters contained landraces from North Gonder, West Gojjam and Arsi, and East Tigray. Landraces from West Shoa, North Shoa, East Shoa, Central Tigray, Southern Tigray, Wollo, East Gojjam, Bale, and

Table 2 – Shannon–Weaver diversity index (H') estimated for qualitative traits of Ethiopian tetraploid wheat across altitude ranges.

Altitude class (m.a.s.l.)	Traits studied											$\overline{H'}$
	SD	GC	GH	KC	VT	DSH	BF	KS	AL	DB	DM	
1600–2000	0.96	0.67	0.26	0.78	0.64	0.89	0.93	0.84	0.86	0.56	0.71	0.74
2001–2400	0.94	0.79	0.26	0.80	0.68	0.89	0.94	0.96	0.58	0.64	0.68	0.74
2401–2800	0.91	0.70	0.24	0.80	0.81	0.79	0.76	0.83	0.68	0.51	0.59	0.69
2801–3000	0.72	0.67	0.33	0.60	0.61	0.92	0.84	0.91	0.40	0.58	0.49	0.64
>3000	0.75	0.75	0.00	0.62	0.66	0.79	0.65	0.96	0.58	0.56	0.45	0.62
$\overline{H'}$	0.86	0.72	0.22	0.72	0.68	0.86	0.82	0.90	0.62	0.57	0.58	

Traits are abbreviated as SD, spike density; GC, glume color; GH, glume hairiness; KC, kernel color; VT, vitreousness; DSH, degree of shriveling; BF, beak form; KS, kernel size; AL, awn length; DB, days to 50% booting; and DM, days to maturity.

Table 3 – Eigenvalues and eigenvectors of the most important principal components (PC) for variation among regions and four altitudinal classes of origin using 11 traits in durum wheat landrace accessions from Ethiopia.

Trait	Eigenvector								
	Districts of origin					Altitude classes			
	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4
SD	0.395	0.083	0.076	0.461	−0.169	0.397	−0.083	0.009	0.338
GC	0.210	−0.113	−0.407	−0.440	0.353	−0.253	0.043	0.435	−0.554
GH	−0.276	0.475	−0.255	0.180	−0.021	0.254	0.330	−0.190	−0.535
KC	−0.060	0.178	−0.435	0.012	−0.738	−0.222	0.214	0.557	0.224
VT	0.445	0.015	0.218	0.268	0.001	0.189	−0.492	0.147	−0.326
DSH	0.349	−0.346	0.044	0.160	−0.170	0.092	0.579	−0.125	−0.080
BF	−0.045	−0.161	−0.419	0.519	0.393	0.310	0.409	−0.051	0.091
KS	0.275	0.049	−0.548	0.097	0.122	0.405	−0.149	0.087	0.164
AL	−0.371	0.222	0.187	0.364	0.259	0.189	0.225	0.599	0.152
DB	0.318	0.471	0.104	−0.224	0.140	−0.408	0.023	−0.118	0.245
DM	0.288	0.551	0.049	−0.039	0.127	−0.396	0.133	−0.205	0.11
Eigenvalue	3.28	2.20	1.94	1.48	1.00	5.49	2.74	1.79	1.01
Variance explained (%)	29.77	19.97	17.59	13.44	8.59	49.93	24.87	16.24	7.96
Total variance (%)	29.77	49.74	67.33	80.77	89.36	49.93	47.80	91.04	99.00

South Gonder were clustered together in the fourth main cluster. Three subclusters formed the fourth cluster. The first comprised landraces from West Shoa and North Shoa, while the second combined landraces from Central Tigray, Southern Tigray, Wollo, and East Gojjam. The third subcluster was formed from landraces from Bale, East Shoa, and South Gonder. With respect to altitude classes, cluster analysis showed two main clusters, in the first of which the first (1600–2000 m.a.s.l.), second (2001–2400 m.a.s.l.) and third (2401–2800 m.a.s.l.) altitude classes were grouped together and the second contained the fourth (2801–3000 m.a.s.l.) and fifth (>3000 m.a.s.l.) classes (Fig. 3-B) with bootstrap values of 58 and 62, respectively.

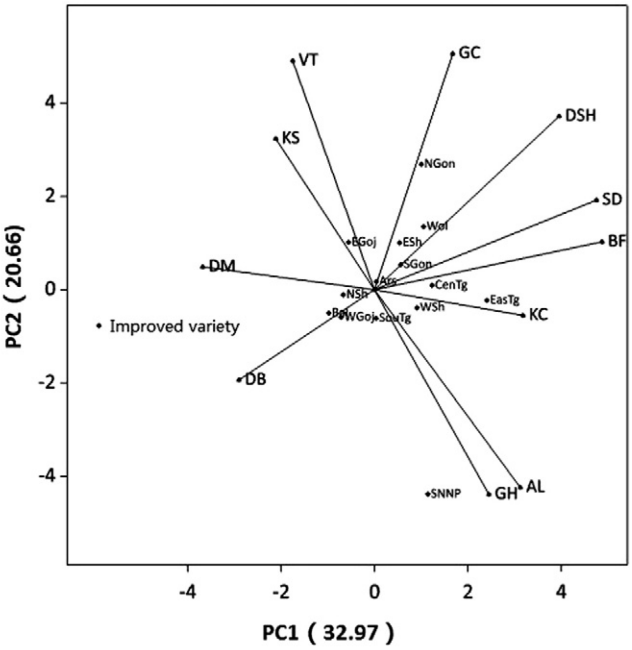

Fig. 2 – Principal component biplot showing phenotype overlaid on district of origin.

4. Discussion

4.1. Morphological markers for genetic diversity study

Information on available genetic resources, their geographical locations, and understanding of their relationships can be used to gain insight into population divergence. Such information is derived from phenotypic as well as genotypic studies using either morphological or molecular markers or a combination of both for rigorous characterization of genotypes. Morphological markers have been in use for such purposes, starting in the era of selection based plant breeding, and continue to play a role even today when molecular markers are being used. Morphological markers have been called the best alternative to their molecular counterparts for assessing genetic diversity [22]. Eleven morphological traits were used in the present study, to assess phenotypic diversity in Ethiopian durum wheat collected from different districts of origin and altitude classes. Previous studies, using these morphological traits, indicated that Ethiopian durum wheat collected from different regions and altitude classes are very diverse for morphological and phenological traits [3,6,19–23]. However, most of these studies characterized only a few samples collected from a few regions of Ethiopia, mainly the central highlands and northeastern parts.

Our study was designed to better understand the variation within and among populations of Ethiopian durum wheat randomly sampled from the national gene bank. Thorough characterization of these landraces is needed to use selected landraces as donor parents to develop varieties for different traits. A study by Negassa [6], for instance, showed that landraces obtained from *Gamugofa* (represented by SNNP in the current study) and Harrar were highly resistant to a virulent race of powdery mildew (*Erysiphe graminis* f. sp. *tritici*) and were potential parents for resistance breeding.

4.2. Qualitative trait distribution

All the studied morphological markers were polymorphic with 3 to 7 phenotypic classes across both districts of origin

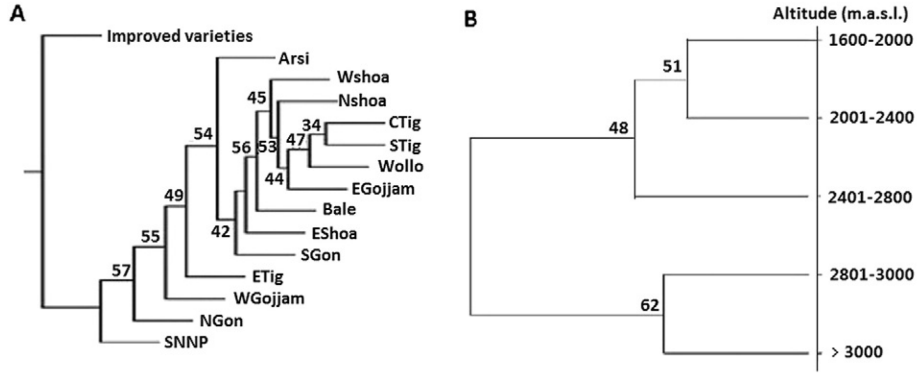

Fig. 3 – Unweighted pair group method based on arithmetic average (UPGMA) dendrogram constructed for A) districts of origin and B) altitudinal ranges based on the diversity index (*H′*). *H′* values were used to form a similarity matrix for hierarchical cluster analysis. Full representation of district name was given in Table S2.

and altitude classes (Table 1). However, the frequency distributions of phenotypic classes showed high variability. Spike density was highly polymorphic and the dense class was larger in landraces obtained from all districts but SNNP. The intermediate type predominated in SNNP, a result in agreement with previous findings [3,6,22,24]. This variation in spike density creates good opportunity for wheat breeders to develop varieties for different environmental conditions. For instance, in areas of high rainfall and low temperature, wheat varieties with lax spikes are reported to show more resistance to spike diseases than other variants [19]. With respect to altitude classes, lax and intermediate spike forms were dominant at lower altitudes compared to the very dense type. This finding is in contrast to that of Fassil et al. [23] indicating a lack of lax forms in the lower altitudes. However, their report supports the rarity of lax form in the high altitude classes.

Landraces from Bale and Gonder showed low diversity for glume hairiness, with the trait being fixed for non-hairy types. Similar findings were reported by others [3,19,21] indicating that the majority of Ethiopian durum wheat landraces have non-hairy glumes. Tessema et al. [5] also observed monomorphism for glume hairiness in many of their landraces. However, Bekele [18] reported a high level of polymorphism for hairiness in Ethiopian durum wheat. Though glume hairiness is usually rare to glabrous (non-hairy) it can be of interest to breeders engaged in resistance breeding [6]. Briggle and Sears [25] reported an association of powdery mildew resistance with a locus (H_g) conditioning the presence of glume pubescence. In contrast, GC showed a high level of polymorphism among both district of origin and altitude classes. Gray glume color was observed in landraces from Arsi, Wollo, and East Tigray districts and black-spotted mosaic colors in landraces from SNNP, Central Tigray, and East Shoa, but the black-spotted mosaic was rare. A study three decades ago of Jain et al. [26] showed the rarity of this color form in Ethiopian durum wheat, but Negassa [6] reported that gray and black colors are invariably associated with very low gluten quality. Beak form is an important trait discriminating *T. turgidum* from *T. durum* (Yemane Tsehaye, *personal communication*). Intermediate and long beak were dominant for both districts of origin and altitude classes.

With respect to AL, landraces from four districts such as Bale, North Shoa, East Shoa, and West Gojjam were all found to be awned, a result agreeing with previous findings [21,22,24]. Long-awned types were more frequent in all districts of origin and in all altitude classes and the proportion of long awned landraces increases with elevation. Long awns may offer an advantage to the crop in contributing assimilates to increase productivity, even under water-deficit conditions, given that awns photosynthesize. The prevalence of awned types may be associated with tolerance to water-stress conditions [27] and as an adaptive structure against rust attack. These considerations suggest taking into account the adaptive significance of awns for rust-affected areas and semiarid environments during parent selection to develop varieties for these purposes. The genotypes were highly polymorphic for kernel color and showed very wide variation for districts of origin and altitude classes. The presence of amber and white-to-yellow colors in only a few landraces and its total absence in Bale collections is in close agreement with previous findings [3,18,21]. The KC of 12% of the landraces was purple. Landraces with purple KC are classified as *T. turgidum* ssp. *aethiopicum* [21]. Zeven [28] reported that purple-seeded cultivated durum wheat are endemic to Ethiopia. The presence of many kernel colors in Ethiopian durum wheat could be associated with the cultural use value of different kernel color types for various traditional purposes, which could ensure the conservation of the different morphotypes by small-scale farming communities.

4.3. Estimation and analysis of phenotypic diversity

Previous studies, such as that of Fassil et al. [23], assessing geographic patterns of diversity among Ethiopian durum wheat found a high level of diversity, indicating the high genetic potential of Ethiopian durum wheat and the presence of many important genes for use in crop improvement programs and genetic studies. Our study also revealed large natural variation in Ethiopian durum wheat population collected from various districts and altitude classes. Estimated diversity indices for traits, districts of origin, and altitude classes are presented in Tables 1, 2, and S2. All traits but GH had diversity indices greater than 0.60, showing large genetic diversity among populations for each trait investigated. The estimated diversity index (H') for traits, pooled over districts and altitude classes, ranged from 0.24 for GH to 0.86 for DSH with an overall mean of 0.69. The best indices were observed for SD ($H' = 0.85$), DSH ($H' = 0.86$), BF ($H' = 0.81$), KL ($H' = 0.82$), and GC ($H' = 0.71$). In contrast, small H' values were observed for GH ($H' = 0.24$) and AL ($H' = 0.53$). However, Sourour and Hajer [29] reported a high diversity index ($H' = 0.80$) for GH in Tunisian durum wheat. Eticha et al. [21] reported high Shannon–Weaver indices of 0.75 and 0.72 for AL for durum wheat landrace populations from Wollo and Bale, respectively. The diversity indices estimated for SD are higher than those reported by Fassil et al. [23]. High diversity indices for the traits indicate the presence of high diversity in Ethiopian durum wheat and suggest that Ethiopia is the center of diversification for this crop. Differing levels of diversity in Ethiopian durum wheat from different geographic regions have been reported previously [5,6,18–21], and this pattern was repeated in the present study.

The overall mean H' for districts of origin, presented in Table 1, of the present study was high and comparable with previous findings. Some traits were fixed in some localities but polymorphic in others. For instance, landraces collected from Bale, North Gonder, and South Gonder were all glabrous (non-hairy), whereas hairy glume was represented in the SNNP collections. Similarly, landraces from North Gonder and East Tigray were monomorphic for phenological traits, indicating that the landraces have low diversity for DB and DM. A high mean diversity index pooled over traits was also found for altitude classes (Table S2). The highest ($H' = 0.76$) was found at the altitude range of 1600–2000 m.a.s.l. In contrast, [23] reported highest diversity at high altitude (>2501 m.a.s.l.).

The higher H' value implies that differences between districts and altitude classes contribute strongly to the variation among landraces. All traits within districts and altitude classes also showed highly significant differences ($P < 0.001$) among the landraces (Table 4). The hierarchical analysis of variance of diversity showed highly significant differences among districts of origin ($P < 0.001$) and altitude classes ($P < 0.01$) (Table 5). The within-district and -altitude class variability was also significant. These results suggested similar levels of mean diversity within and among districts and altitude classes. Similarly, different previous studies concluded that variation among populations from different geographical areas contributes more to the total variability than within-population differences [5,22]. In contrast, other reports [3,22] have concluded that variation among populations within region and altitude groups contribute most to variability. However, these conclusions were derived from studies of samples from narrower geographic areas. From such contrasting results, it appears that covering a large number of collection sites and avoiding lumping together wider geographic areas in a single population should be considered for further dissection of geographical patterns of variability of Ethiopian durum wheat.

Table 4 – Mean squares from the analysis of variance of H' for individual traits.

Trait	Altitude classes ($df = 4$)	Districts of origin ($df = 14$)
	Mean square	Mean square
SD	0.021**	0.115**
GC	0.005*	0.045***
GH	0.025*	0.093**
KC	0.016***	0.022*
VT	0.010**	0.089**
DSH	0.006**	0.048***
BF	0.024***	0.094***
KS	0.007**	0.010**
AL	0.045***	0.096***
DB	0.004*	0.134***
DM	0.021**	0.202***

* Significant at 0.05.
** Significant at 0.01.
*** Significant at 0.001.

Table 5 – Hierarchical analysis of variance of diversity (H') across districts of origin and altitude classes.

Source of variation	df	Mean square	F-prob.
Districts	14	0.012	<0.001
Traits within districts	10	0.011	<0.001
Altitude class	4	0.007	<0.001
Traits within altitude	10	0.746	<0.001

4.4. PCA and cluster analysis

The results of PCA and cluster analysis strongly support each other. Districts of origin clustered together in Fig. 3 were also placed near each other in the same quadrant except for improved varieties and SNNP (Fig. 2). The 11 morphological traits discriminated the districts of origin very well, with DB and DM separating improved varieties from the remaining landraces. Landraces from SNNP were well discriminated from the others by GH and AL. Kernel color was the most discriminant trait for landraces from East Tigray, but kernel size and vitreousness discriminated East Gojjam well from other districts. The implication is that different traits have different importance for and contribution to the variance explained by each PC, as reported by Johnson and Wichern [30].

The clustering of different districts of origin using morphological markers may be arbitrary, as consistency has been lacking among studies conducted using populations from the same districts. For instance, [22] grouped landraces from Tigray with improved varieties with a bootstrap value of 70 and subclustered Arsi and Bale together, while our study did not. Our result clustered improved varieties with landraces from SNNP with a bootstrap value of 57. The clustering of improved varieties and landraces from SNNP could be explained in two ways. First, landraces from SNNP may be included in the pedigrees of the improved varieties. The second reason may be the recollection of the improved varieties, as farmers usually consider improved varieties cultivated for longer periods in a given area as landraces. Consideration of different districts as independent sites of origin instead of lumping them together enabled the separation of landraces from Tigray, Gojjam, Gonder, and Shoa into different clusters. The findings of previous studies, such as [21–23,30], showed different patterns of clustering for landraces from the same sites of origin. The findings of Fassil et al. [23], however, showed different patterns of clustering when smaller sites (districts) of origin were considered than when landraces from larger areas were lumped together as a single population. Tsegaye et al. [19] reported that lumping of species together from larger areas not only affects the clustering pattern but may bias estimates of diversity.

The clustering of various districts of origins appeared to follow a propinquity-based trend, representing a greater probability of germplasm exchange among farmers in neighboring regions than among those in distant regions [22]. This trend may explain the clustering of North Gonder with West Gojjam and of West Shoa with North Shoa in the same cluster. However, the clustering of mutually distant districts could be due to the introduction of germplasm from one to the other, either formally or informally, sometimes long ago. For instance, the clustering of Arsi and East Tigray landraces in one subcluster and Bale and South Gonder in the other subcluster may be due to such movements. Hailu et al. [22] also suggested germplasm exchange among nonproximal farmers as an explanation for the clustering of landraces from Arsi and Wollo, mutually distant regions, in their study.

5. Conclusion

The great wealth of morphological diversity in Ethiopian durum wheat landraces may be attributed to the interacting effects of 1) the wide diversity in natural environments [18], 2) natural cross-fertilization as a result of growing mixed genotypes in the field [31], and/or 3) differences in agricultural practices of smallholding farmers [5]. This high genetic diversity should be exploited in improvement programs to reduce heavy reliance on exotic materials, which often fail to adapt to the wide agroecological and climatic variations of Ethiopia. The diversity among landraces for days to maturity and degree of seed shriveling enabled us to select a subset of characterized landraces for further study of behavior under terminal drought, a condition that leads to severe yield loss. Selection from the landraces for immediate access to locally adapted varieties through participatory varietal selection could help farmers adapt their crops to the harsh growing conditions of northern Ethiopia. For breeding purposes and further genetic studies, we have selected 50 diverse landraces, based on investigated traits from different districts of origin and altitude classes, and crossed them to “Asassa”, an elite improved durum wheat variety. We are currently growing F_6 populations of each cross with more than 130,000 individual variants.

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Supplementary data

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REFERENCES

[1] Y. Teklu, K. Hammer, Diversity of Ethiopian durum wheat germplasm: breeding opportunities for improving grain yield potential and quality traits, Plant Genet. Resour.-C 7 (2008) 1–8.

[2] T. Tessema, E. Bechere, Developing elite durum wheat landrace selections (composites) for Ethiopian peasant farm use: raising productivity while keeping diversity alive, Euphytica 102 (1998) 323–328.

[3] E. Bechere, G. Belay, D. Mitiku, A. Merker, Phenotypic diversity of durum wheat landraces from northern and north-central regions of Ethiopia, Hereditas 124 (1996) 165–172.

[4] T. Tessema, H. Becker, G. Belay, D. Mitiku, E. Bechere, S. Tsegaye, Performance of Ethiopian durum wheat landraces at their collection sites, Euphytica 71 (1993) 221–230.

[5] T. Tessema, G. Belay, M. Worede, Morphological diversity in durum wheat landrace populations from central highlands of Ethiopia, Hereditas 114 (1991) 171–176.

[6] M. Negassa, Estimates of phenotypic diversity and breeding potential of Ethiopian wheat, Hereditas 104 (1984) 41–48.

[7] A. Amri, J. Hatchett, T. Cox, M. Bouhassini, R. Sears, Resistance to Hessian fly from North African durum wheat germplasm, Crop Sci. 30 (1990) 378–381.

[8] K. Kubo, Y. Jitsuyama, K. Iwama, T. Hasegawa, N. Watanabe, Genetic difference in root penetration ability by durum wheat (*Triticum turgidum* L. var. *durum*) evaluated by a pot with paraffin-Vaseline discs, Plant Soil 262 (2004) 169–177.

[9] D. Tilman, P. Reich, J. Knops, D. Wedin, T. Mielke, Diversity and productivity in a long-term grassland experiment, Science 294 (2001) 843–845.

[10] Y. Teklu, K. Hammer, X. Huang, M. Roder, Analysis of microsatellite diversity in Ethiopian durum wheat landraces, Genet. Resour. Crop. Evol. 53 (2006) 1115–1126.

[11] A. Brown, Isozymes, plant population genetics structure and genetic conservation, Theor. Appl. Genet. 52 (1978) 145–157.

[12] J. Harlan, Our vanishing genetic resources, Science 188 (1975) 618–621.

[13] D. Klindworth, J. Miller, Y. Jin, S. Xu, Chromosomal of genes for stem rust resistance in monogenic lines derived from durum wheat accession ST464, Crop Sci. 47 (2007) 1012–1013.

[14] A. Negassa, J. Koo, K. Sonder, B. Shiferaw, M. Smale, H. Braun, D. Hodson, S. Gbegbelgbe, Z. Guo, S. Wood, T. Payne, B. Abeyo, The Potential for Wheat Production in Sub-Saharan Africa: Analysis of Biophysical Suitability and Economic Profitability, Pre-print galley for circulation at the conference “Wheat for food security in Africa: Science and policy dialogue about the future of wheat in Africa”, Addis Ababa, Ethiopia, Oct. 08–12 2012.

[15] G. Arega, M. Hussien, S. Harjit, Genetic divergence in selected durum wheat genotypes of Ethiopia, Afr. Crop. Sci. J. 15 (2007) 67–72.

[16] B. Abebe, Individual trade balance in some major agricultural products: evidence from Ethiopia, J. Pov. Invest. Dev. 6 (2014) 6–10.

[17] B. Skovmand, M. Reynolds, I. Delacy, Mining wheat germplasm collections for yield enhancing traits, Euphytica 119 (2001) 25–32.

[18] E. Bekele, Analysis of regional patterns of phenotypic diversity in Ethiopian durum and hexaploids wheat, Hereditas 100 (1984) 131–154.

[19] S. Tsegaye, T. Tesemma, G. Belay, Relationships among durum wheat (*Triticum turgidum* L.) landrace populations revealed by isozyme markers and agronomic characters, Theor. Appl. Genet. 93 (1996) 600–605.

[20] L. Pecetti, A. Damania, Geographic variation in durum wheat (*Triticum turgidum* ssp. *durum*) landraces from two provinces of Ethiopia, Genet. Resour. Crop. Evol. 43 (1996) 395–407.

[21] F. Eticha, E. Bekele, G. Belay, A. Börner, Phenotypic diversity in durum wheat collected from Bale and Wello regions of Ethiopia, Plant Genet. Resour. 3 (1) (2005) 35–43.

[22] F. Hailu, A. Merker, E. Johnsson, Patterns of phenotypic diversity for phenologic and qualitative traits in Ethiopian durum wheat germplasm, Genet. Resour. Crop. Evol. 53 (2010) 1089–1098.

[23] K. Fassil, T. Yemane, T. McNeilly, Diversity of durum wheat (*Triticum durum* Desf.) at in situ conservation sites in North Shewa and Bale, J. Agric. Sci. 136 (2001) 383–392.

[24] S. Al Khanjari, A. Filatenko, K. Hammer, Morphological spike diversity in Omani wheat, Genet. Resour. Crop. Evol. 55 (2008) 1185–1195.

[25] L. Briggle, E. Sears, Linkage of resistance to *Erysiphe graminis* f. sp. *tritici* (Pm3) and hairy glume (Hg) on chromosome IA of wheat, Crop Sci. 6 (1966) 559–561.

[26] S. Jain, C. Qualset, G. Bhatt, K. Wu, Geographical patterns of phenotypic diversity in a world collection of durum wheat, Crop Sci. 15 (1975) 700–704.

[27] L. Evans, J. Bingham, P. Jackson, J. Sutherland, Effect of awns and drought on the supply of photosynthate and its distribution within wheat ears, Ann. Appl. Biol. 70 (1972) 67–76.

[28] A. Zeven, Wheat with purple and blue grains: a review, Euphytica 56 (1991) 243–258.

[29] A. Sourour, S. Hajer, Distribution and phenotypic variability aspects of some quantitative traits among durum wheat landraces, Afr. Crop. Sci. J. 16 (2009) 219–224.

[30] R. Johnson, D. Wichern, Applied Multivariate Statistical Analysis, Prentice-Hall, Englewood Cliffs, 1988.

[31] M. Feldman, Wheat, in: N. Simmonds (Ed.), Evolution of Crop Plants, Longman Groups limited, London, UK 1976, pp. 120–128.

[32] International Board for Plant Genetic Resources (IBPGR), Revised Descriptor List for Wheat (*Triticum* spp.), 1985. (Rome, Italy).

[33] W. Payne, A. Harding, A. Murray, M. Soutar, B. Baird, I. Glaser, C. Channing, J. Welham, R. Gilmour, R. Thompson, R. Webster, The Guide to GenStat Release 12, Part 2: Statistics, VSN International, Hemel Hempstead, 2009.

[34] J. Felsenstein, Inferring Phylogenies, Sinauer Associates, Sunderland, Massachusetts, 2004.

[35] Central Statistical Agency (CSA). Agriculture: Statistical Abstracts. Addis Ababa, Ethiopia, 2003–2012.