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Revisiting the ignored Ethiopian durum wheat (*Triticum turgidum* var. *durum*) landraces for genetic diversity exploitation in future wheat breeding programs

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The majority of Ethiopian durum wheat are farmers' varieties. Durum wheat varieties under production in Ethiopia are less productive due to the effect of climate change and marginalism of agricultural inputs. Recognizant to these, revisiting farmers varieties, *ex-situ* conserved, could be among the best options to adapt these changes. This study has thoroughly characterized a set of 49 durum wheat varieties to assess the level of phenotypic diversity through multivariate approaches for traits of importance. The study was aimed at estimating the extent of genetic diversity among the genotypes and to identify traits maximally contributing to the observed phenotypic variations. The statistical analyses have confirmed that the genotypes have shown very large (*p<0.001*) variations for most traits considered, except for number of effective tillers. The estimated broad – sense heritability (h^2) has ranged from 20% for grain yield to 78% for spike length. The genotypes were grouped into six clusters with significant inter-cluster distances ($\chi^2=15.51, p<0.05$). The clustering revealed that the landraces are genetically far from the improved varieties. Genotypes consisted in a particular cluster are superior for a trait or more compared to the other clusters. For instance, genotypes in the first cluster are superior for grain and biomass yields. Early maturing and second high yielding genotypes were consisted in the fifth cluster. The genotype – by – traits biplot analysis has explained 82% of the total variation where 65.5% was explained by PC1, indicating that most of the observed variation is inherited. It can be concluded that the landraces are quite divergent from the improved varieties and the within landraces diversity was also high indicating that they represent important gene pool for important traits. Hence, revisiting landraces, which are *Ex-situ* conserved, and their exploitation in durum wheat breeding programs for grain yield improvement, earliness and other traits of importance is crucial.

Key words: Revisiting, ignored, Durum wheat, landrace, genetic variation, quantitative trait.

INTRODUCTION

Durum wheat (*Triticum turgidum* var. *durum Desf*) accounts for 8% of global wheat production and its cultivation is concentrated in the Mediterranean basin, the North American Great Plains, India, and the former

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USSR (Palanarchuk, 2005). Out of these regions, durum wheat is the oldest traditional crop in Ethiopia covering significant proportion of arable land devoted to national wheat production. Negassa et al. (2012) indicated that durum wheat covers about 20% of total area under wheat production, and estimated to contribute between 18 to 20% to the national wheat production with average productivity of 1.8t/ha (Teklu and Hammer, 2008). Durum wheat grown in Ethiopia is constituted by a few improved varieties and a large number of traditional farmers varieties, commonly referred as landraces (Bechere et al., 1996; Belay et al., 1993; Eticha et al., 2005). These landraces represent a large reservoir of genetic variability, even though their genetic constitution is mostly unknown, attributed to a number of factors including the natural and artificial selections (Bechere et al., 1996), diverse agro-ecologies (Mondini et al., 2010) and different local farming systems (Myers, 1994; Teklu and Hammer, 2008).

At present, more than 7000 traditional durum wheat accessions are housed in national gene bank of Ethiopia (Basazin, *personal communication*) for conservation as well as sustainable exploitation. These traditional landraces, conserved in the gene bank, could represent a useful reservoir of genes and alleles to be used in novel breeding programs aimed at the development of new genotypes adapted to different production conditions. Landraces are generally considered endemic to a particular region to which they are well adapted (Royo et al., 2014). Landraces are regarded 'variety with a high capacity to tolerate biotic and abiotic stress resulting in high yield stability and an intermediate yield level under a low input agricultural system' (Zeven, 1998). Despite such merits endowed in the landraces, their cultivation was progressively shrinking with the advent of new, improved and genetically uniform modern varieties derived from breeding programs. The landraces were seldom, if any, utilized in modern durum wheat breeding efforts to improve production and productivity. For instance, only less than 2% of the improved durum wheat varieties cultivated in Ethiopia were composed gene from Ethiopian landraces as evidenced from their pedigree records (CIMMYT, 2014). The remaining 98% of the improved durum wheat varieties are introductions of exotic materials from international breeding blocks. This is actually associated with the lack of comprehensive characterization of the landraces for quantitative traits including their yielding potential and performance for yield related traits. Previous studies entirely focus on spike based qualitative characterizations (Belay et al., 1993; Bechere et al., 1996; Eticha et al., 2005; Teklu and Hammer, 2008; Mengistu et al., 2015) and failed to show the potential of the landraces.

Besides, the Ethiopian durum wheat breeding program is highly centralized to the extent that the major agro-ecologies suitable for wheat production were not embraced. As evidenced by this study, varieties developed under highly centralized breeding systems with target to pockets of potential areas usually fail to perform in marginal areas, like that of northern Ethiopia. Referring to Ethiopian condition, the synergetic effects of these two approaches has been, and continued, to limit the production and productivity of durum wheat. Of the various possible solutions that can be sought, exhaustive characterization and exploitation of existing genetic diversities (Lopes et al., 2015) and decentralized breeding approaches (Ceccarelli and Grando, 2007; Dawson et al., 2008) could be applied to improve its production and productivity for the targeted agro-ecology. A rational decision to use genetic resource, however, depends on an extensive phenotypic characterization and on a deeper knowledge of the degree of genetic variation exists in them. This requires precise information on the nature and degree of genetic diversity in a set of genotypes to choose an appropriate parent for purposeful hybridization (Samsuddin, 1985). In this study, a set of 49 durum wheat genotypes, 45 landraces plus four improved modern varieties was used, where the landraces were collected from 15 zones of origin with the aim of (i) estimating the genetic variations in Ethiopian durum wheat genotypes, ii) to identify traits which contribute the most to the estimated genetic variability, and iii) to identify promising candidate landraces to be used in future durum wheat breeding programs

MATERIALS AND METHODS

Field experiments

Forty five landraces and 4 modern varieties were collected (Table 1), used as reference, grown during two years (2012 and 2013) at the Mekelle University Research site (13°28'N, 39°29'E and 2,212 m above sea level) in Tigray, Northern Ethiopia. The soil is clayloam with 28.7 and 17.8 volume percent, averaged over a soil depth of 0 to 40 cm, water holding nature at field capacity and permanent wilting points, respectively as measured by gravimetric method. The rainfall of the area is characterized by erratic temporal distribution, with a long term mean annual amount of 450 mm, with July and August contributing more than 80% of the rainfall in the main rainy season (Araya and Stroosnijder, 2010). The long term average seasonal temperature of the area is 18.5°C with maximum and minimum of 26 and 12.5°C, respectively (Araya and Stroosnijder, 2010).

Experiments consisted of a replicated plots of 3 m^2 (comprising six 2.5 m long rows, spaced 0.2 m apart), arranged in 7×7 simple lattice design with two replications in each year and conducted under rain fed condition in both years. Each genotype was planted at seeding rate of 100 kg ha^{-1}, the blanket recommendation of the area. Nitrogen and phosphorus fertilizers were applied at the rate of 41 kg N ha^{-1} and 20 kg P ha^{-1}, respectively. Phosphorus was applied at sowing in a single distribution, whereas nitrogen was split applied with two –third at sowing and the remaining one – third was top dressed at full tillering stage. Weed control was done manually following standard practices.

The phenological data such as days to 50% booting (number of days from sowing to the stage at which fifty percent of the plants within a plot boot, Zadoks stage 45), days to 50% flowering (number of days from sowing to the stage at which fifty percent of the plants within a plot flower, Zadoks stage 65) (Zadoks et al.,

Table 1. Description of the 49 wheat genotypes characterized in the study

S/N	Genotype	Form	Origin	S/N	Genotype	Form	Origin	S/N	Genotype	Form	Origin
1	206551	Landrace	Southern Tigray	18	231580	Landrace	South Gonder	35	Quamy	Improved	CIMMYT
2	206567	Landrace	South Gonder	19	238125	Landrace	Central Tigray	36	8019	Landrace	East Shoa
3	208234	Landrace	East Gojjam	20	238555	Landrace	North Shoa	37	210820	Landrace	East Gojjam
4	208286	Landrace	North Shoa	21	Hitosa	Improved	CIMMYT	38	222352	Landrace	West Gojjam
5	213310	Landrace	Southern Tigray	22	5679	Landrace	North Shoa	39	203989	Landrace	East Shoa
6	214494	Landrace	Arsi	23	8208	Landrace	North Shoa	40	206576	Landrace	North Shoa
7	214599	Landrace	Eastern Tigray	24	208315	Landrace	North Shoa	41	208227	Landrace	East Gojjam
8	Assasa	Improved	CIMMYT	25	208373	Landrace	North Shoa	42	208276	Landrace	North Shoa
9	214877	Landrace	Wollo	26	208482	Landrace	North Shoa	43	Ude	Improved	CIMMYT
10	222297	Landrace	North Gonder	27	214502	Landrace	West Hararghe	44	208309	Landrace	North Shoa
11	222360	Landrace	East Gojjam	28	214585	Landrace	Bale	45	208746	Landrace	North Shoa
12	222660	Landrace	South Gonder	29	215276	Landrace	North Shoa	46	222834	Landrace	Wollo
13	222666	Landrace	South Gonder	30	228762	Landrace	West Shoa	47	236269	Landrace	Southern Tigray
14	226826	Landrace	Bale	31	228862	Landrace	West Hararghe	48	236979	Landrace	East Gojjam
15	226840	Landrace	East Gojjam	32	236276	Landrace	Southern Tigray	49	238519	Landrace	West Shoa
16	226973	Landrace	South Gonder	33	238525	Landrace	East Shoa				
17	228593	Landrace	East Gojjam	34	238567	Landrace	North Shoa				

1974) and days to maturity [days from sowing to physiological maturity (yellowish uppermost internodes occur)] were recorded on plot basis, whereas data for plant height (PH), number of effective tillers (NET) and spike length (SPL) were recorded from five randomly sampled plants from the four central rows. The other two traits, above ground biomass yield (BY) and grain yield (GY), were recorded, after plants were mechanically harvested, the four central rows and then converted into a hectare basis. Above ground biomass was measured using hanging balance in the field during harvesting, whereas grain yield and 1000 grains weight (TGW) were obtained by weighing harvested grain and counted 1000 grains on an analytical balance. Finally, GY and TGW were adjusted to 12.5% moisture content.

Statistical analysis

Raw field data were fitted to a linear mixed model with the genotypes as fixed effects and the replication, the row number and column number as random effect (Payne et al., 2009). Restricted maximum likelihood (REML) was used to estimate the variance components and to produce the best linear unbiased predictors (BLUPs) for the data of each genotype each year using Genstat-12.1 (Payne et al., 2009), which was used for all subsequent multivariate analyses. Analysis of variance was conducted to evaluate the genotypic diversity for phenotypic traits and effect of years on the traits. The genetic divergence of the genotypes was further assessed using cluster analysis and pattern analysis. The cluster analysis was performed using MINTAB (vr.14) that adopts Squared Euclidean distance (D^2) as a measure of dissimilarity and Ward's method as the clustering algorism (Ward, 1963). All variables were standardized by subtracting the means and dividing by the standard deviation before calculating the distance matrix. Inter - cluster distances, D^2, and cluster mean values for each trait were used to compute genetic divergence, as described in Ahlawat et al. (2008) and Arega et al. (2007). Significance of the squared distances of each cluster was validated using the chi-square (χ^2) test at (t-1) degrees of freedom at alpha level of 5%, where t represents the number of traits used for clustering genotypes. Genotypes - by - trait (GT) biplot analysis was applied to assess the patterns of relations among measured traits, the genotypes and their interaction. Biplot analysis, using GGE biplot software, was conducted in the first two principal components (PC1 and PC2) dimension, using a singular – value decomposition (SVD) procedure (Yan and Rajcan, 2002). In this analysis, traits are represented as vector and genotypes as points; the origin of the biplot represents the average values for all traits (DeLacy et al., 2000).

The broad - sense heritability for the combined dataset (two years data) was estimated for all traits using variance estimated for each component by restricted maximum livelihood (REML) analysis. All factors except replication were treated as random variables. Heritability was calculated according to the formula suggested by Vargas et al. (2013) as:

Table 2. Analysis of variance (ANOVA) for quantitative traits of various tetraploid wheat genotypes tested in 2012 and 2013.

Source of var.	D.f.	Variants								
		DB	DF	DM	TN	PH	SPL	GY	BY	TGW
Rep	1	2.5	22.2	2.45	563.5	87.9	1.2	0.2	3.4	9.8
Rep/bloc	12	11.7***	14.9***	11.3***	9.3	243.0***	1.4***	0.4*	5.1***	13.1**
Year (Y)	1	632.2***	3088.2***	1611.5***	1383.8***	4666.8***	6.7***	1.2*	3.8ns	455.1***
Genotype (G)	48	25.7***	35.3***	46.1***	4.8ns	305.0***	4.0***	1.3***	8.8***	55.5***
Y×G	85	7.2***	11.0***	27.9***	3.3ns	85.9***	0.9***	0.8***	6.9***	13.5***
Error	195	1.8	4.7	3.2	9.6	41.3	0.3	0.2	1.4	4.4
Total		12.8	30.3	28.6	16.8	153.6	1.5	0.6	4.8	22.1

DB = Days to 50% booting, DF = days to 50% flowering, DM = Days to maturity, PH = plant height, NET = number of effective tillers, SPL = spike length, GY = grain yield, BY = biomass yield, HI = harvest index.

$$h^2 = \frac{\delta_g^2}{\delta_g^2 + \dfrac{\delta_{ge}^2}{nyrs} + \dfrac{\delta_{er}^e}{nyrs \times nreps}}$$

(1)

Where δ_g^2, δ_{ge}^2 and δ_{er}^2 are the genotypic, genotype by year interaction and error variance components, respectively, and nyrs and nreps are the number of years and replicates, respectively.

$$GA = K.\sqrt{\delta_p^2}.h^2$$

(2)

where GA is the genetic advance, K is a constant with value of 2.06 at 5% selection intensity, $\sqrt{\delta_p^2}$ is the square roots of phenotypic variance and h^2 is broad - sense heritability.

Since in natural population such as our wheat landraces, natural selection is expected, a 5% of natural selection intensity ($K = 2.06$) was assumed to calculate the genetic advance, as suggested by Allard (1960) and Singh and Chaudhary (1985).

RESULTS

Overall diversity

The analysis of variance has revealed a significant diversity within the genotypes for all the traits studied except for number of effective tillers (Table 2). There is also a significant variation in genotypes performance across years, but not for the above ground biomass. Importantly, the interaction between genotypes and year (G×E) has a similar pattern as that of genotypes, indicating that the effect due to genotypes is stronger than that of the year. The genotypes have showed great variation in performance. The use of grand mean (μ) and standard deviation (STDEV) enabled to classify the genotypes into five performance groups (Table S1). More than 57% of the genotypes performed averagely for all the traits while the remaining has shown either superior or inferior performance. Multivariate analyses were also supported the findings of analysis of variance as both clustering and biplot analyses showed that contribution from genotype to the total variance is large (Figures 1 and 2). The performance of the genotypes was also observed to vary across test years for all traits, except for biomass yield (Table 2 and Figure 3). The existence of such genetic diversity for phenological and yield and yield related traits could contribute greatly to efforts made to adapt with the global changing climate.

Diversity for phenological traits

The ANOVA for phenological (DB, DF and DM) data showed that the year, the genotype and their interaction significantly affected the phenological development of the genotypes (Tables 2, S1 and Figure 4). The landraces showed wider range for days to flowering (12 days between early and late flowered genotypes) and days to maturity (~18 days between the early and the late) than the improved varieties, which showed less than seven days difference for both days to flowering and maturity. As presented in Table S1 very interesting landraces were identified in this study. Six and seven landraces have flowered and matured, respectively in fewer days than the grand mean for the 49 genotypes to reach these stages and these landraces could be classified as very early to the area. There were also about 22.5 and 16.3% of the genotypes categorized as late flowering and maturing respectively. The elasticity in days to flowering and maturity was also observed during the two cropping seasons (Figure 4). Earliness alone is not a desired trait as other traits such as the final grain yield matters for adoption of varieties by farming communities. Interestingly, some of the identified early maturing landraces were found to give average to higher grain yield which make them desirable as they combined two important but mostly contradicting traits.

Diversity for yield and associated traits

There were significant diversity manifested by genotypes
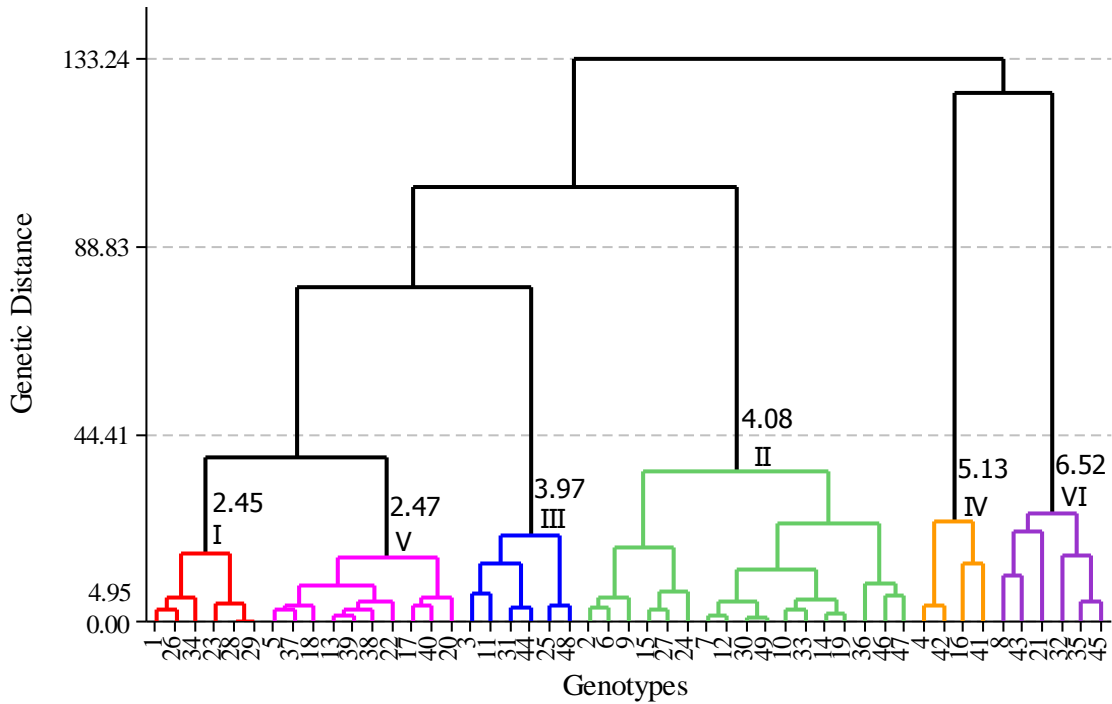
Figure 1. Dendrogram, based on genetic distance, showing the clustering of the 49 tetraploid wheat genotypes on the base of 9 morpho-agronomic traits. Name of genotypes are given in Table 1. Numbers on top of each cluster represent intra cluster distance.
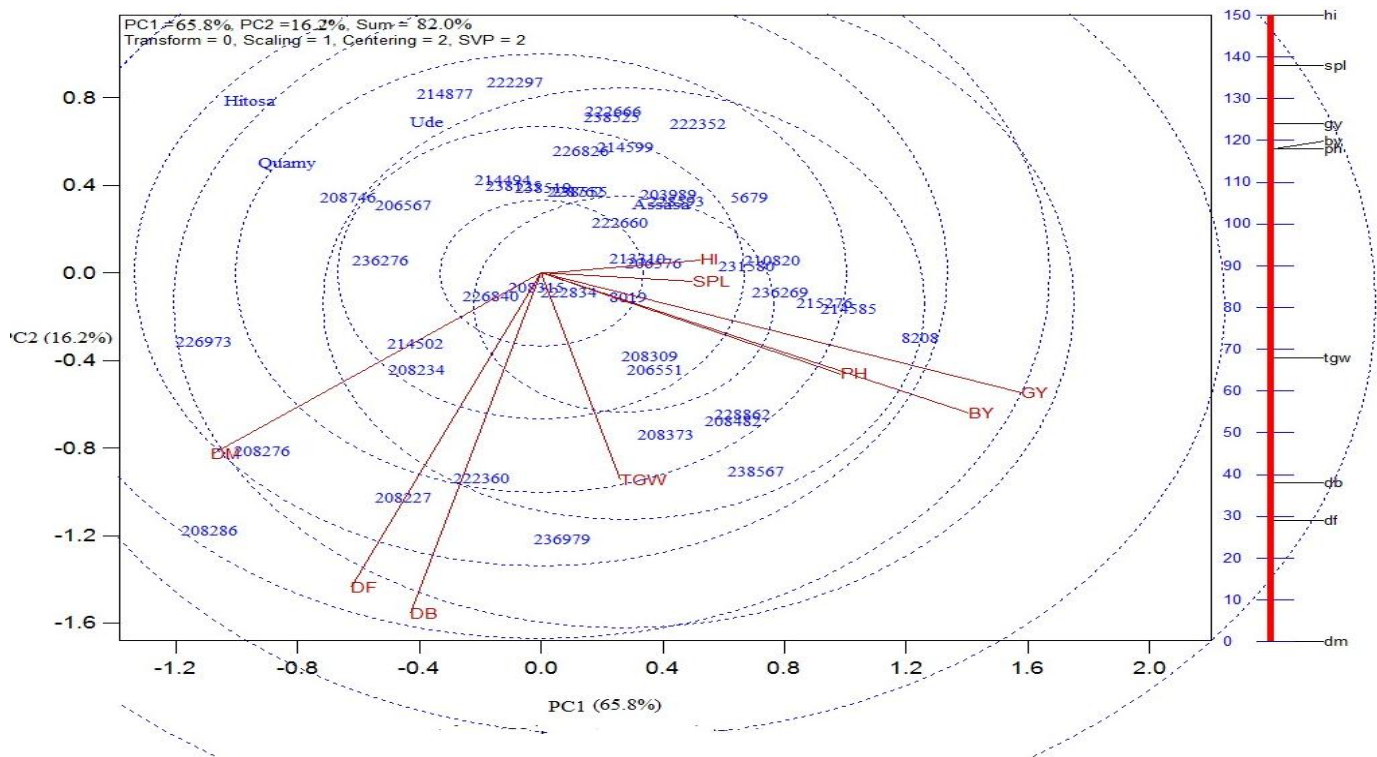
Figure 2. GGE-Biplot showing the distribution of the 49 genotypes across the first two axes and the overlay with the investigated traits. The vertical bar at left side shows the relative association of the traits in biplot.
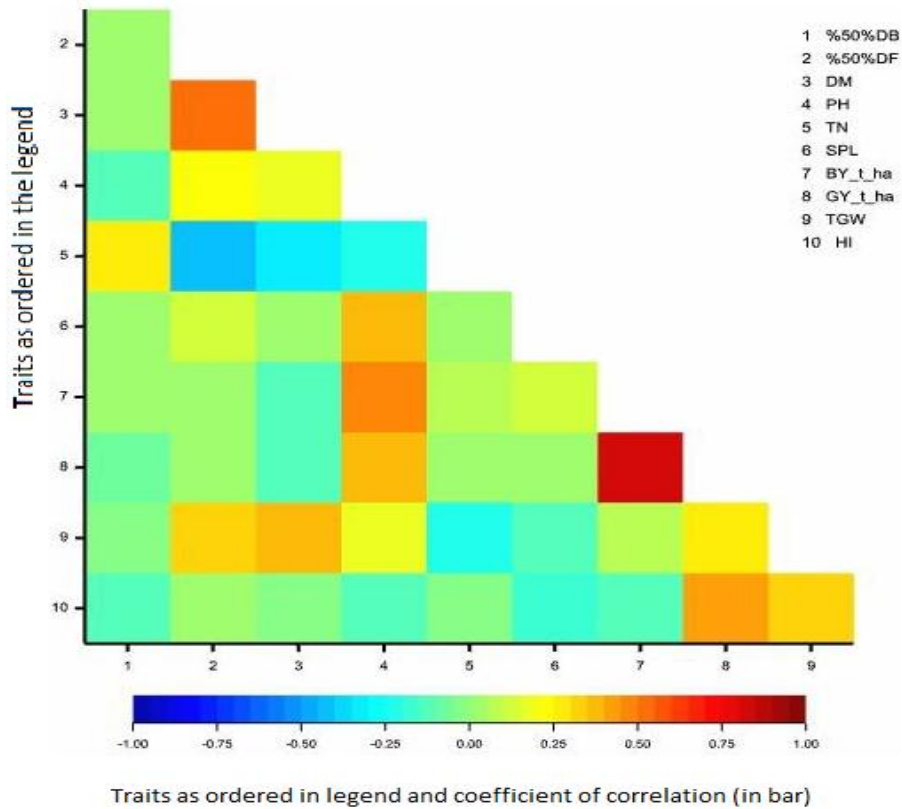
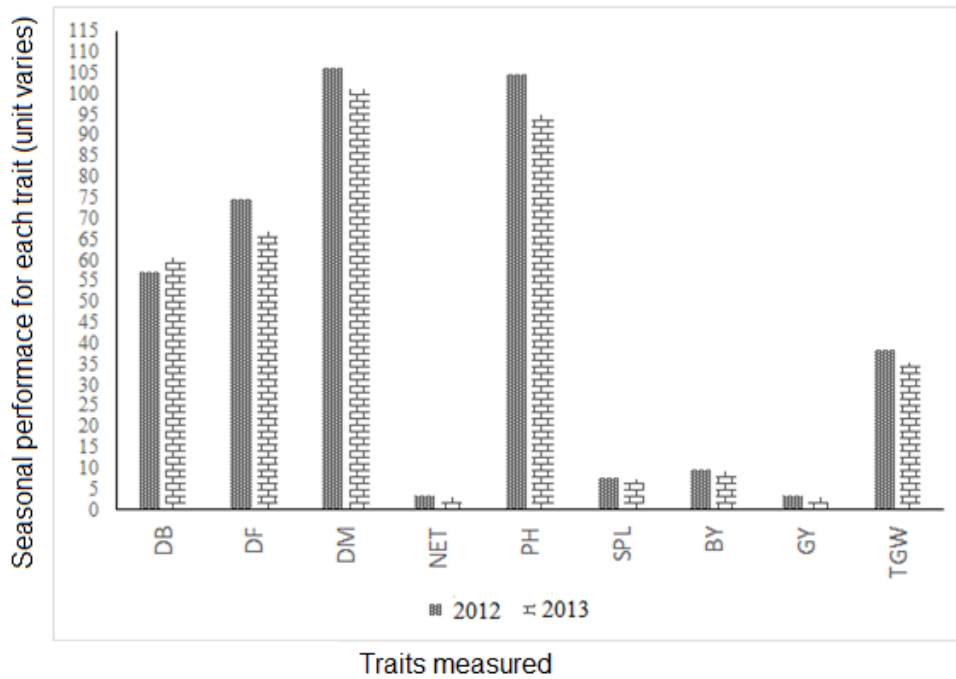**Figure 3.** Correlation matrix between pairs of traits.

Figure 4. Temporal effect on genotypes average performance for days to booting, days to flowering, days to maturity, number of effective tillers, plant height, spike length, biomass yield, grain yield and 1000 - grain weight.

Table 3. Grain yield, rank and seasonal effect difference of 10 top ranked genotypes and minimum, maximum and average yield of the whole set of tested genotypes during 2012 and 2013 cropping seasons.

S/N	2012				2013			
	Genotype	GY(t ha^{-1})	Rank	ΔGY	Genotype	GY(t ha^{-1})	Rank	ΔGY
1	238567[≠]	4.790	**1**	1.302	208482	4.417	**1**	0.823
2	Asassa	4.570	**2**	1.820	8208[≠]	4.352	**2**	0.153
3	214585	4.489	**3**	1.066	228593	3.861	**3**	1.164
4	215276	4.319	**4**	0.928	222360	3.756	**4**	1.227
5	8208[≠]	4.199	**5**	-0.153	208309	3.712	**5**	0.268
6	228862[≠]	4.199	**6**	0.586	213310	3.666	**6**	0.455
7	206551	4.186	**7**	1.158	5679	3.649	**7**	0.110
8	210820	3.931	**8**	0.521	228862[≠]	3.613	**8**	-0.586
9	208315	3.885	**9**	2.273	206576[≠]	3.510	**9**	-0.323
10	206576[≠]	3.833	**10**	0.323	238567[≠]	3.488	**10**	-1.302
Minimum[†]		**1.420**				**1.610**		
Maximum[†]		**4.790**				**4.420**		
Average[†]		**3.170**				**3.010**		

≠ Landraces ranked in the top 10 performers in both years; † Values determined from the wholes set (49) of tested genotypes; Negative sign indicates yield loss in that particular year.

for yield and yield associated traits as presented in Table 2 as well as other tables and figures. The BLUPs value for each trait, of course averaged over years, was presented in Table 3. The number of productive tillers, which contributes to the number of plants per squared meter, ranged from 2.0 to 4.3 with average mean of 3.1. Either the genotypes or their interaction with test years did not show significant variation for this trait. In terms of height, the genotypes showed huge variation ranging from 70.4 to 119.5 cm with overall average of 99.7 cm where the tallest was the landrace, 208227, and the shortest was an improved variety, *Hitosa*. Twenty five (51%) of the genotypes, of which 24 are landraces, are taller than the average height. Spike length is another trait for which large and significant variation was observed among the genotypes, year and their interaction. The magnitude of variation for this trait was ranged from 4.9 to 9.2 cm with average length of 7.3 cm. Of the tested genotypes, 49% of them had produced spike taller than 7.3 cm and all the 24 genotypes with taller spike were landraces. The diversity in the landraces was also reflected in their kernel weight, which ranged from 30.4 to 44.6 g per 1000 grains with overall mean of 36.8 g. Nineteen landraces and three improved varieties have produced grains heavier than the average weight. Interestingly, two landraces, 236979 and 228862, were produced heavier grains than the improved varieties. The diverse performance of the genotypes was also reflected in the final important traits: biomass and grain yields. The variation for biomass yield ranged from 5.8 t ha^{-1} (from improved variety *Quamy*) to 12.6 t ha^{-1} (for landrace 214585) with overall mean of 9.5 t ha^{-1}. Twenty three (47%) of the landraces have shown superior performance with biomass yield exceeding the overall mean.

Regarding to grain yield, the variation among the genotypes ranged from 2 to 4.3 t ha^{-1} with overall average of 3.1 t ha^{-1}. Landraces that are characterized with higher biomass yield have repeated themselves for grain yield as well. Twenty two (45%) of landraces have produced grain yield exceeding the overall mean. Except "*Asassa*", the improved varieties have performed below the overall average for both biomass and grain yields.

Temporal variation in genotypes performance

The seasonal effect on the performance of the genotypes was significant (*p<0.001*) with respect to all investigated traits (Table 2). The temporal variation for all the traits, averaged over genotypes was presented in Figure 4. It can be inferred that the overall performance of the genotypes was better in 2012 than in 2013. Cognizant to the effect of environment, that is, temporal and spatial variation, the concept of performance stability attracted attention of breeders and is a key determinant of crop adoption. We attempted to present top ten genotypes based on their grain yield for both years as presented in Table 3. Though the ranking varies across years, landraces have dominated top performing position both for biomass and grain yields. Considering the whole set of genotypes, change in yield due to temporal variation ranges from 2.27 t ha^{-1} for the landrace 208315 to 0.014 t ha^{-1} for the landrace 214599, which demonstrates the diversity for performance stability. Table 3 presents the yield and ranking of top 10 genotypes during the two cropping seasons. Four (40%) of the top 10 ranked genotypes, 8208, 206576, 228862 and 238567, maintained their superiority though the ranking varies

greatly (Table 3 and S1). Interestingly, the landraces repeated their top performance across years while the improved varieties fail to appear as top yielders. Looking more into the four landraces enables to roughly judge their consistent performance during the two cropping seasons. The deviation in yield ranges from 0.153t ha^{-1} (for 8208) to 1.302t ha^{-1} (for 238567) indicating that landrace 8208 is more stable than 238567. The higher and stable yields from these landraces make them suitable for cultivation in the semiarid of northern Ethiopia over the less adaptable improved varieties of durum wheat. Unexpectedly, the improved varieties were found inferior to the top performing landraces with respect to GY and other traits (Tables 3, 4 and S1). Of the four improved varieties tested, only *Asassa*, ranked 2th, appeared in the top 10 genotypes with a mean performance of 4.57 t ha^{-1} during 2012 but none of them appeared in the top 10 during the later cropping season.

Heritability and genetic advance estimation

In most traits, a large proportion of phenotypic variance was accounted for by the genetic constitution. The estimated heritability ranged from a minimum of 0.2 for biological yield to a maximum of 0.78 for spike length (SPL) (Table 5). The h^2 for days to 50% booting, days to 50% flowering, plant height and 1000-grain weight exceeded 70%. DB, DF, PH, SPL and TGW. However, the estimated h^2 for days to maturity, number of effective tillers, grain yield and biomass yields was moderately low, less than 50%. The expected genetic advance was positive for all traits ranging from 0.61 for grain yield to 17.24 for plant height (Table 4). Traits such as DB, DF, PH and TGW had high heritability accompanied by high genetic advance while SPL had high heritability coupled with a low genetic advance. Traits with high h2 value exhibited strong and positive association among each other (Figure 3).

The genetic diversity in the genotypes revealed by cluster analysis

The UPGMA dendrogram showing genetic relationship using morphological traits among the 49 durum wheat genotypes is presented in Figure 1. At a genetic distance of (44.41), the tested genotypes were divided into six main groups with significant inter-cluster distance ($\chi^2 = 15.51; p<0.05$). Inter cluster D^2 values ranged from 6.95 between clusters I and V to 74.38 between clusters IV and VI. Inter clusters genetic distances were proven statistically significant except between clusters I and V and II and V (Table 6) which might be associated with the closer cluster mean value of traits included (Table 7). The number of genotypes per cluster ranged from a minimum of 4 to a maximum of 17. Genotypes grouped in the same cluster are characterized by very low genetic distances and vise-versa. All the four improved varieties, together with two landraces, clustered together, characterized by a very low genetic distance. Though clustering was done based on the standardized value of all the 9 traits investigated, we described the clusters only taking into consideration grain yield as it is a crucial trait for the economic success of the crop and as a synthetic parameter for genotype performance. The first cluster comprises six landraces, of which half of them (8208, 214585 and 215276) were consistently ranked in the top 15 high yielding genotypes (Table 3). This cluster formed from two sub-clusters where both sub-clusters were formed of from three landraces. The second sub-cluster composed of high yielding landraces (Figure 1). Seventeen landraces were grouped in the second cluster, which are less yielder compared to the top 15 high yielding genotypes. The seventeen landraces were sub-grouped into four sub-clusters.

Five of the six landraces constituted the third cluster were in the top 15 high yielding genotypes in either one year or both. This cluster further subdivided into three sub-cluster each consisting 2 landraces. Similar to the second cluster, the fourth cluster composed of poor yielding genotypes except one (208284) which are subdivided into two sub-clusters. Ten genotypes, all landraces, were grouped together in the fifth cluster which further subdivided into four sub-clusters. The first sub-cluster of this cluster consisted four landraces of which two are in the top 10 yielding group in both years. The second and third sub-clusters were consisted three landraces each. All the four improved varieties were assembled together in the sixth cluster, which contains also landraces 208746 and 236276. The resemblance of the two landraces with the improved varieties might be due to the shorter plant height and spike length (Table 7). Further Table 7 presents the mean value, averaged over genotypes and years, of each trait in each cluster. The first cluster is composed of genotypes mature earlier the average maturity time for all genotypes and at the same time taller and high yielding genotypes. Genotypes constituted in the second cluster are featured with long spike and earliness while genotypes in the third clusters are characterized by just above average grain yield and heavier seed weight. The fourth cluster comprises genotypes characterized by late maturing, low yielding and below average performance for other traits except plant height. The inter-cluster distance between clusters I, II and V was not significant but they are separated (Table 6). However, the composition of genotypes characterized by very light seed weight is the special feature of cluster V. The sixth cluster, on the other hand, has composed of mainly the improved varieties and distinguished by early growth vigor (DB), shorter plants and shorter spike length.

Genotypes - by - traits biplot analysis

A biplot ordination with respect to the first (PC1) and

Table 4. Mean performance of the 49 durum wheat genotypes combined over the two seasons.

S/N	Genotype	DB	DF	DM	NET	PH	SPL	BY	GY	TGW
1	203989	57.3	69.6	100.8	3.3	101.5	6.7	10.0	3.3	35.1
2	206551	60.0	72.9	104.0	3.6	102.8	7.3	10.3	3.6	38.3
3	206567	59.1	70.0	106.0	2.5	92.9	7.8	9.0	2.5	31.9
4	206576	58.1	70.0	104.5	3.7	91.3	7.9	10.8	3.7	34.3
5	208227	63.4	78.1	107.0	2.3	119.5	8.3	9.9	2.3	35.3
6	208234	62.0	74.0	105.5	2.9	88.9	7.6	8.7	2.9	39.4
7	208276	63.7	74.2	108.8	2.4	88.3	6.4	8.6	2.4	40.8
8	208286	66.4	78.1	109.0	2.4	94.8	5.8	8.0	2.4	39.5
9	208309	59.6	71.9	102.0	3.6	97.9	6.5	9.4	3.6	43.3
10	208315	59.5	69.3	103.5	2.7	104.2	6.9	9.8	2.7	39.9
11	208373	61.7	70.4	108.2	3.5	111.1	7.7	10.3	3.5	42.3
12	208482	60.1	72.9	106.2	4.0	104.7	7.7	12.2	4.0	37.9
13	208746	56.3	70.1	107.2	2.4	88.8	6.2	7.1	2.4	39.2
14	210820	58.7	69.0	101.0	3.7	107.7	6.9	11.4	3.7	34.4
15	213310	59.0	69.6	103.8	3.4	106.1	6.1	10.0	3.4	34.0
16	214494	58.2	68.4	104.2	2.5	107.2	7.8	9.3	2.5	32.1
17	214502	62.4	72.7	104.8	2.7	94.5	6.8	9.9	2.7	33.7
18	214585	58.1	69.7	100.8	4.0	107.8	6.7	12.6	4.0	38.2
19	214599	56.0	66.4	103.8	2.9	108.1	8.1	9.3	2.9	35.7
20	214877	57.7	67.6	100.8	2.1	98.0	8.6	8.1	2.1	32.2
21	215276	58.0	69.2	100.8	3.9	107.1	6.7	12.3	3.9	38.4
22	222297	56.7	68.7	99.0	2.5	93.5	8.5	7.5	2.5	33.1
23	222352	54.5	68.7	99.0	3.2	103.1	7.0	10.0	3.2	33.8
24	222360	63.4	77.6	104.2	3.1	93.6	7.4	8.4	3.1	41.8
25	222660	57.6	67.9	104.2	2.9	112.2	7.8	9.1	2.9	36.7
26	222666	56.5	66.5	101.1	3.0	99.4	6.5	9.0	3.0	34.1
27	222834	58.6	74.6	101.8	3.1	94.9	8.8	9.8	3.1	35.3
28	226826	57.0	68.2	101.5	2.8	99.0	8.4	8.3	2.8	35.8
29	226840	61.2	69.5	106.5	3.0	98.0	7.1	9.8	3.0	34.3
30	226973	62.3	75.7	108.8	2.0	102.7	6.9	7.3	2.0	31.2
31	228593	58.2	69.5	101.5	3.3	99.3	8.5	10.4	3.3	31.3
32	228762	58.1	67.0	102.8	2.9	102.0	6.9	9.0	2.9	36.9
33	228862	59.8	74.9	100.0	3.9	101.9	7.3	10.5	3.9	43.5
34	231580	57.2	68.9	103.5	3.6	110.2	7.0	10.2	3.6	38.7
35	236269	59.0	70.3	99.0	3.4	107.1	9.2	11	3.4	38.2
36	236276	57.0	69.6	116.0	3.1	83.7	6.7	9.1	3.1	36.6
37	236979	62.2	75.1	110.5	3.5	109.1	8.0	9.5	3.5	44.6
38	238125	57.8	68.5	105.2	2.6	103.2	9.0	8.1	2.6	34.1
39	238519	56.8	68.8	102.8	2.8	98.1	7.4	8.6	2.8	38.2
40	238525	58.0	67.3	98.2	2.8	98.4	8.0	8.8	2.8	33.4
41	238555	59.1	70.7	100.5	3.3	88.8	7.1	9.2	3.3	30.4
42	238567	63.4	74.8	102.0	4.1	98.1	7.7	12.6	4.1	36.7
43	5679	56.9	69.5	99.0	3.7	100.3	7.1	10.3	3.7	35.7
44	8019	60.1	72.2	99.5	3.1	102.1	8.9	8.3	3.1	40.0
45	8208	59.0	69.9	100.5	4.3	116.1	7.3	12.0	4.3	39.3
46	Assasa	54.5	69.4	103.8	3.7	101.9	5.2	9.4	3.7	39.8
47	Hitosa	56.1	74.1	101.8	2.5	70.4	5.3	6.5	2.5	32.1
48	Quamy	55.6	67.9	108.0	2.0	96.9	5.3	5.8	2.0	43.3
49	Ude	54.5	69.1	101.5	2.8	77.2	4.9	7.9	2.8	40.9
	Maximum	66.4	78.1	116.0	4.3	119.5	9.2	12.6	4.3	44.6
	Minimum	1.0	1.5	1.4	0.4	4.5	0.5	0.8	0.3	1.4

Table 4. Contd.

Grand mean	58.9	70.8	103.6	3.1	99.7	7.3	9.5	3.1	36.8
STDEV	3.6	7.4	5.3	4.1	12.4	1.2	2.2	0.8	4.7
SED	1.0	1.5	1.4	0.4	4.5	0.5	0.8	0.3	1.4

Table 5. Broad - sense heritability (h^2) and genetic advance (GA) of traits Ethiopian durum wheat.

Traits	h^2	GA
Days to 50% booting (DB)	0.73	5.67
Days to 50% flowering (DF)	0.72	9.54
Days to Maturity (DM)	0.35	4.13
Number of productive tillers (NET)	0.37	2.61
Plant height (PH)	0.72	17.24
Spike length (SPL)	0.78	1.75
Grain yield (GY)	0.44	0.61
Biological yield (BY)	0.20	0.77
1000 grain weight (TGW)	0.72	6.69

Table 6. Intra - cluster (*diagonal*) and inter - cluster (*triangular*) genetic distance (D^2) values among the six clusters of wheat genotypes.

Cluster	I	II	III	IV	V	VI
I	**2.45**					
II	18.32*	**4.08**				
III	17.20*	18.88*	**3.97**			
IV	59.34**	39.72**	34.23**	**5.13**		
V	6.95ns	11.21^{ns}	24.44**	64.52**	**2.47**	
VI	47.49**	43.45**	36.53**	74.38**	35.84**	**6.52**

*,** and ^{ns} represents significant at 5, 1% and non –significant difference; χ^2 = 15.51 and 20.10 at 5 and 1% significance level, respectively

second (PC2) principal components helped to further visualize the relative distance between the 49 durum wheat genotypes and the traits most contributing to their differentiation (Figure 2). The distribution of the genotypes along the first two PCs axes has been supported by 82.0% of the total variations in the data set. This ordination has produced a number of concentric circles in which the genotypes and traits were overlaid. The core or central concentric circle has contained six landraces viz. 8019; 222834; 226840; 201815; 213310; 222660 and 206576. The genotypes are average performer for all the investigated traits. Genotypes placed far from the origin of the biplot are interpreted either as superior performer with respect to the traits based on the length and direction of traits vector in the biplot. Most genotypes in the upper side of the biplot could have average to poor performance for most of the traits while those in the lower portion of the biplot could be treated as best performers. For instance, genotypes such as 8208;

214585; 215276, 228862 and 208482 are best for grain yield, biomass yield and are taller in height compared to genotypes placed opposite side of these traits. The orientation of genotypes and the traits agrees with Table 3.

DISCUSSION

Genotypes characterization and evaluation

The various analyses used have revealed that the studied genotypes showed significant (p<*0.001*) variation for the majority of investigated traits. The level of genetic diversity within the landraces and between the landraces and improved varieties is extraordinarily high. The 49 [45 landraces + 4 improved] genotypes used in this study showed great variability in phenological and agronomic performances. Despite the difference in number of

Table 7. Cluster mean and standard deviation of the nine agronomic traits of tetraploid wheat, computed as average of genotypes and years 2012 and 2013).

| Traits | Cluster | | | | | | Grand $\bar{x}$ |
	I	II	III	IV	V	VI	
DB	59.7 ± 0.8	58.4 ± 0.4	61.5 ± 0.6	63.9 ± 0.8	57.6 ± 0.4	55.8 ± 0.4	59.5
DF	71.5 ± 0.9	69.3 ± 0.8	74.0 ± 1.0	76.8 ± 0.8	69.1 ± 0.4	70.0 ± 0.9	71.8
DM	102.5 ± 0.9	102.6 ± 0.6	105.2 ± 1.6	108.4 ± 0.5	101.5 ± 0.6	106.0 ± 1.8	104.3
PH	106.0 ± 2.5	100.9 ± 1.4	100.0 ± 3.6	101.1 ± 6.3	101.1 ± 2.3	86.2 ± 4.9	99.2
SPL	7.2 ± 0.2	8.0 ± 0.2	7.4 ± 0.2	6.8 ± 0.5	7.1 ± 0.2	5.6 ± 0.3	7.0
TGW	38.3 ± 0.4	35.3 ± 0.7	42.5 ± 0.8	36. 6 ± 2.3	34.3 ± 0.7	38.6 ± 1.6	37.6
GY	4.0 ± 0.1	2.8 ± 0.1	3.4 ± 0.2	2.3 ± 0.1	3.4 ± 0.1	2.7 ± 0.2	3.1
BY	12.0 ± 0.4	9.0 ± 0.2	9.5 ± 0.4	8.5 ± 0.6	10.2 ± 0.2	7.6 ± 0.6	9.5
HI	0.33 ± 0.01	0.31 ± 0.01	0.37 ± 0.01	0.27 ± 0.02	0.34 ± 0.01	0.36 ± 0.01	0.33

individual studies, landraces have shown larger ranges to reach flowering and maturity days than improved varieties. Early flowering is one of the most common physiological mechanisms of drought escape (Royo et al., 2014). Some of the early maturing landraces, such as 228862, 5679 and 222297, presumably employed a sort of developmental plasticity without yield penalty as evidenced by short grain filling period (difference between maturity and flowering dates) and high grain yield (Table S1). Earliness is an advantage, even with some yield penalty in drought prone areas, though it turns disadvantageous in non-stressed environments (Blum, 2011). The landrace 8208 is the best for grain yield with average flowering and maturity period. Landraces that combined earliness and high yield are ideal for areas prone to terminal drought, like that of Tigray- Ethiopia and elsewhere in the world, to cope with the ever changing global climate. The magnitude and effect of climate change is projected to surpass human-kinds effort to identify, select, reproduce and eventually use genetic resources in the field to cope with the climate change (FAO, 2015).

Regarding to grain and biomass yields, more than 10% of the landraces have absolutely outperformed the best performed improved varieties (Tables 3 and 6) which might remind that varieties developed for high potential areas could not perform well under marginal growing conditions like that of Tigray, a region characterized by erratic rainfall and recurrent terminal drought. When the average performance of each genotype analyzed over years, the amount of outperforming landraces even increases. In any case, the expected yield advantage from the improved varieties was not realized in this study. Similar conclusion was reported by Abay and Bjørnstad (2008), who compared the performance of local farmers' varieties with nationally released improved barley varieties under Tigray conditions. The positive advantage in grain and biological/straw yields obtained from landraces over the improved varieties could grant farmers in marginal areas with alternative varieties that satisfy

their variety demand both for grain yield and straw yield. In mixed farming systems, crop plus livestock enterprises, straw yield is equally valued as grain yield as it serves as animal feed and other purposes. The superiority of landraces over improved varieties was reported in Ethiopian as well as Jordanian wheat (Rawashden et al., 2007). Some of the landraces such as 8208, 208482, 214585, 228862 and 238567 have produced higher grain and biomass yields (Table 4) and have shown relatively consistent performance across seasons (Table 3).

The wider diversity in our tested panel of genotypes is an opportunity for wheat breeding programs. The diversity could be utilized either through direct selection or hybridization breeding. Interestingly, the outperformance of landraces in marginal environments alarms breeding institutions to adopt decentralized breeding strategies to address the specific needs of specific environments. Furthermore, landraces are important source of specific traits such as drought tolerance than the improved varieties (Kara et al., 2000) and could warranty adaptation to the global changing climate if properly characterized and utilized. Cluster analysis, performed considering all measured traits, successfully differentiates the genotypes into various groups and cluster mean performance for each trait was presented in Table 7. Both phenological and agronomic traits have best discriminated the clusters. The first cluster composed of genotypes with very desirable traits such as earliness, higher grain (4 t ha^{-1}) and straw (12 t ha^{-1}) yields and heavier seeds weight (38.3 g/1000 grains). Genotypes in the third cluster were next high yielder (3.4 t ha^{-1}) with heavier TGW (42.5 g) but were differentiated from those in the first cluster by phenological traits (late flowering and maturing).

Considering phenological traits, which were strongly and positively associated (Figure 3) particularly maturity days, clusters IV and V have composed of contrasting genotypes where selection for phenology breeding could benefit more from these clusters to produce early

maturing varieties for areas prone to terminal drought like that of Tigray. Ahlawat et al. (2008) have also reported the clear differentiation of late maturing genotypes from early maturing ones in Pakistani bread wheat. It can be inferred that clusters consisting contrasting genotypes for traits of importance could serve as valuable sources for parental selection for future national and international durum wheat breeding programs for earliness, yield and seed weight. The genetic analysis of quantitative traits is a prerequisite for plant breeding programs, which can lead to a systemic method of design and to the appropriate planning of plant breeding strategies. The current study confirms that the h^2 was higher with higher genetic gain (GA) for most traits (Table 5). The quantitative traits DB, DF, PH, SPL and TGW revealed more than 70% heritability, which could indicate the presence of additive gene action. This also showed that selection based on these traits would be more effective and efficient than the use of other traits for segregating generations in future breeding programs, which indicated an exploitable amount of variation. The knowledge on heritability and genetic advance enables to make appropriate parental selection (Tuhina-Khatun et al., 2007), predict the nature of succeeding generations and assess the magnitude of genetic improvement through selection (Yadav et al., 2015). Such genetic diversity in crops also underpins adaptations to various climate change related stresses such as heat and water deficit and improves agricultural production and productivity in the face of global climate change threat.

Promising landraces for future use in durum wheat breeding programs

The analysis of modern wheat varieties pedigree revealed that landraces are the backbone of breeding programs of national agricultural research systems and many giant wheat breeding organizations like CIMMYT (Virchow, 2013). The genetic diversity in stored landraces is recognized for use in future breeding though the direct use of these landraces in many breeding programs is low (Bellon, 2009). The current study has identified landraces of durum wheat that can be exploited in durum wheat breeding programs for various purposes. Taking into account the two economically important traits, grain and straw yields, as proxy traits for selection of the genotypes, landraces in the first, third and fifth clusters could be of particular importance for the high yielding. Accordingly, landraces with accession no. 8208, 238567, 228862, 214585, 215276 and 208482 are interesting materials to evaluate in various contrasting environments. Furthermore, landraces resided in the third cluster could be given special attention for their heavier seeds, an important contributor to the final yield. Breeding programs targeting drought prone areas could utilize landraces 5679, 8019, 203989, 222352, 231580 and 236269 for earliness as well as higher yield (Table 4). Earliness is

one of the determinants for adoption of crop varieties by farmers under changing climate as adaptation mechanism (Westengen and Brysting, 2014). Hence, this study recommends using the already identified potential durum wheat landraces in Ethiopian and other nationals' durum wheat breeding programs for yield improvement and terminal drought adaptation through escaper varieties.

Conclusion

The various analyses confirmed that the variation for quantitative phenological and agronomic traits in the studied Ethiopian durum wheat genotypes is quite high. This reveals that these genotypes could be a valuable genetic resource for durum wheat improvement programs in Ethiopia and elsewhere in the world. Exploitation of the identified landraces could bring about improvement for various traits such as grain and biomass yields, earliness to escape terminal drought and loading resistance through wider hybridization of genotypes from contrasting clusters. It is evident that direct selection, after conducting multi-environment trials, of the superior landraces will enable to make a much faster variety release which could shorten the time needed for variety development and release through the conventional crossing methods. It is also obvious that genetic variation is mandatory to start breeding programs of any level. The characterized landraces are revealed to possess diversified traits for future wheat improvement under the challenging global climate. Observant to the variations from this research work, we have selected and used five very divergent landraces, 8208 (cluster I), 222834 (cluster II), 208309 (cluster III), 208286 (cluster IV), 213310 (cluster V) and one improve variety "*Asassa*" from cluster VI, in our durum wheat breeding program with the aim of producing nested association mapping populations (NAM) for genetic study as well as breeding purpose.

Conflict of Interests

The authors have not declared any conflict of interests.

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Supplementary table

S1. Number of individual genotype falling into the five performance classes for each trait. The mean value is the average of 2012 and 2013.

Traits	Classes				
	<μ-2STDEV	μ-2STDEV to μ-STDEV	μ-STDEV to μ + STDEV	μ+ STDEV to μ+2STDEV	> μ+2STDEV
DB	0	7	33	9	1
Number and list of genotypes in the class		208746, 214599, 222352, Asassa, Hitosa, Quamy and Ude	203989, 206551, 206567, 208286, 208309, 208373, 208746, 210820, 213310, 214502, 214599, 214877, 215276, 222360, 222660, 222666, 222834, 226826, 226973, 228593, 228762, 228862, 231580, 236269, 231580, 236269, 236979, 238125, 238519, 238525, 238567, 5679 and 8019	206576, 208227, 208234, 208315, 214494, 222352, 226840, 236276 and 238555	208276
DF	0	6	32	8	3
Number and list of genotypes in the class		214599, 214877, 222660, 222666, 228762 and 23852	203989, 206551, 206567, 206576, 208315, 208373, 208482, 208746, 210820, 213310, 214494, 214502, 214585, 215276, 222297, 222352, 226826, 226840, 228593, 231580, 236269, 236269, 236276, 238125, 238519, 238555, 5679, 8019, 8208, Asassa, Quamy & Ude	208234, 208276, 222834, 226973, 228862, 236979, 238567 and Hitosa	208227, 208286 and 222360
DM	0	7	35	6	2
Number and list of genotypes in the class		222297, 222352, 228862, 236269, 238525, 5679 and 8018	203989, 206567, 206576, 208227, 208234, 208309, 208315, 208482, 210820, 214502, 214585, 214599, 214877, 215276, 222360, 222666, 222834, 226840, 228593, 231580, 238125, 238519, 8208, Asassa, Hitosa and Ude	208276, 208286, 208373, 208746, 226973 and Quamy	236276 and 236979
NET	1	10	27	9	1
Number and list of genotypes in the class	Quamy	206567, 208227, 208276,208286, 208746, 214494, 214877,222297, 226973 and Hitosa	203989, 206551, 208234, 208309, 208315, 208373, 213310, 214502, 214599, 222352, 222360, 222666, 222834, 226826, 226840, 228593, 228762, 231580, 236269, 236276, 236979, 238125, 238519, 238525, 238555, 8019 and Ude	206576, 208482, 210820, 214585, 215276, 228862, 238567, 5679 and Asassa	8208
PH	2	5	36	5	1
Number and list of genotypes in the class	Hitosa and Ude	208234, 208276, 208746, 236276 and 238555	203989, 206551, 206567, 206576, 208286, 208309, 208315, 208482, 210820, 213310, 214494, 214502, 214585, 214599, 214877, 215276, 222297, 222352, 222360, 222666, 222834, 226826, 226840, 226973, 228593, 228762, 228862, 236269, 238125, 238519, 238525, 238567, 5679, 8019, Asassa and Quamy	208373, 222660, 231580, 236979 and 8208	208227
SPL	3	4	32	10	0

S1. Contd.

Number and list of genotypes in the class	Asassa, Quamy and Ude	208286, 208746, 213310 and Hitosa	203989, 206551, 206567, 206576, 208234, 208276, 208309, 208315, 208373, 208482, 210820, 214494, 214502, 214585, 214599, 215276, 222352, 222360, 222660, 222666, 226840, 226973, 228762, 228862, 231580, 236276, 236979, 238519, 238525, 238555, 238567, 5679 and 8208	208227, 214877, 222297, 222834, 226826, 228593, 236269, 236269, 238125 & 8019	
BY	2	5	34	6	2
Number and list of genotypes in the class	Hitosa and Quamy	208286, 208746, 222297, 226973 and Ude	203989, 206551, 206567, 206576, 208227, 208234, 208276, 208309, 208315, 208373, 213310, 214494, 214502, 214599, 214877, 222352, 222360, 222660, 222666, 222834, 226826, 226840, 228593, 228762, 228862, 231580, 231580, 236276, 236979, 238125, 238519, 238525, 238555, 5679, 8019 and Asassa	208482, 210820, 215276, 236269, 236269 and 8208	214585 and 238567
GY	1	10	28	9	1
Number and list of genotypes in the class	Quamy	206567, 208227, 208276, 208286, 208746, 214494, 214877, 222297, 226973 and Hitosa	203989, 206551, 208234, 208309, 208315, 208373, 213310, 214502, 214599, 222352, 222360, 222660, 222666, 222834, 226826, 226840, 228593, 228762, 231580, 236269, 236276, 236979, 238125, 238519, 238525, 238555, 8019 and Ude	206576, 208482, 210820, 214585, 215276, 228862, 238567, 5679 and Asassa	8208
TGW	0	8	33	7	1
Number and list of genotypes in the class		206567, 214494, 214877, 222297, 226973, 228593, 238555 & Hitosa	203989, 206551, 206576, 208227, 208234, 208286, 208315, 208482, 208746, 210820, 213310, 214502, 214585, 214599, 215276, 222352, 222660, 222666, 222834, 226826, 226840, 228762, 231580, 236269, 236269, 236276, 238125, 238519, 238525, 238567, 5679, 8019 and 8208	208276, 208309, 208373, 222360, 228862, Quamy and Ude	236979

The mean value is the average of 2012 and 2013 cropping seasons.