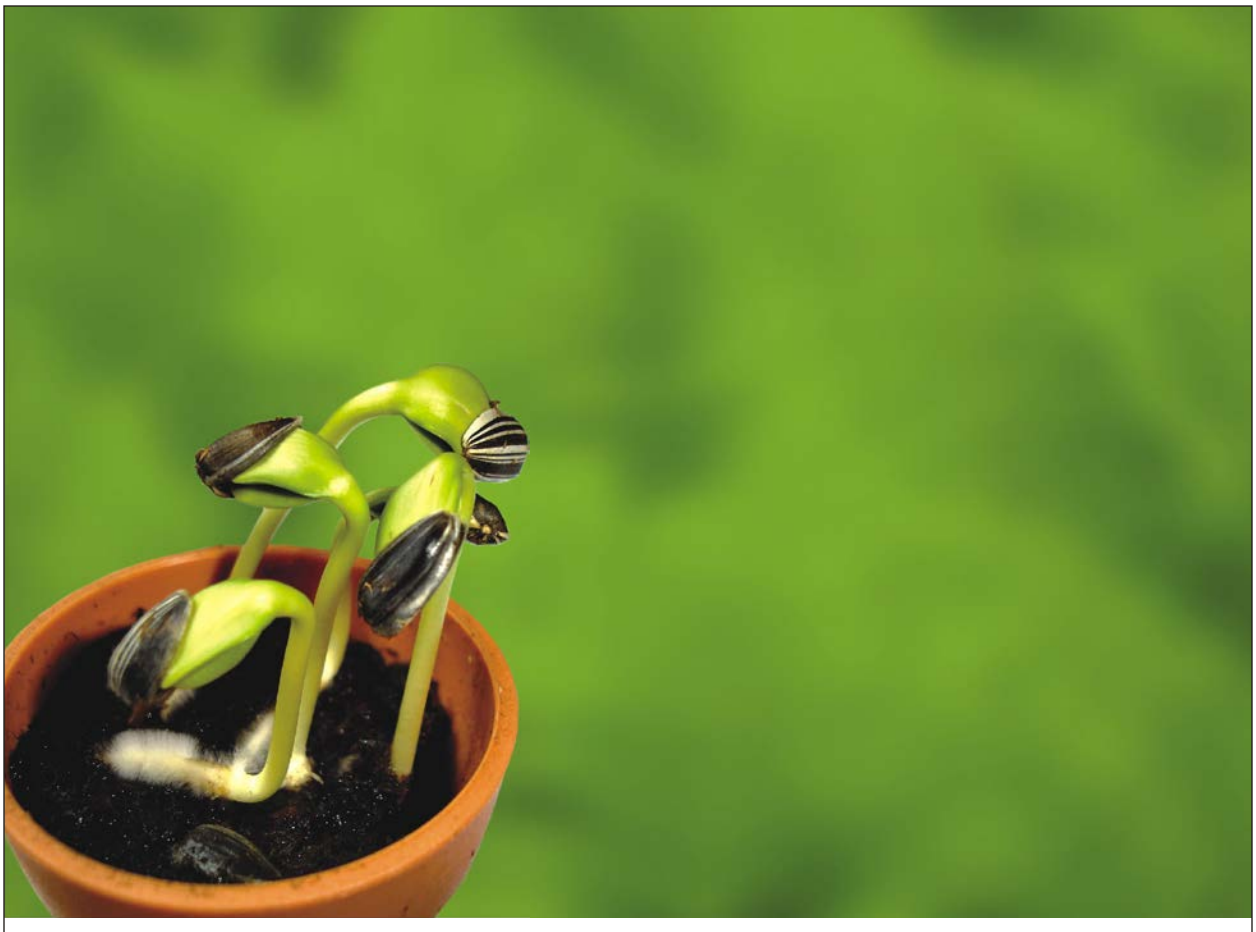

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Genotypic Diversity and Interrelationship of Characters in Ethiopian Food Barley (*Hordium vulgare* L.) Landraces

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ABSTRACT

An experiment was conducted at Holetta Agricultural Research Center with the objective of analyzing genotypic diversity and interrelationship of characters in Ethiopian food barley (*Hordium vulgare* L.) landraces. One hundred two barley accessions and five checks were evaluated using augmented design plots consisting of four complete blocks in 2012 main cropping season. Ten quantitative characters were recorded. Analysis of variance showed significant difference ($p<0.01$) among accessions for plant height, awn length, peduncle extrusion, thousand seed weight, number of seeds per spike, days to 50% flowering and days to maturity. Phenotypic correlation coefficient among characters showed that days to maturity had significant correlation with plant height ($r = 0.31$), number of seeds per spike ($r = 0.30$), days to 50% flowering ($r = 0.81$) and peduncle length ($r = -0.31$). Altitude had positive and significant correlation with number of seeds per spike ($r = 0.40$), days to 50% flowering ($r = 0.47$) and days to maturity ($r = 0.44$). Cluster analysis grouped accessions in to five distinct classes with maximum number of accessions 44 in cluster (I) and minimum 2 in cluster (V). Principal component analysis showed that variances of 30, 17, 15 and 10% were extracted from the first four principal components, respectively which contributed 72% of the total variation among accessions. Discriminant analysis indicated that around 49% (50 of the 102) and 53% (54 of 102) of the studied accessions were correctly classified to their respective regions of origin and altitude groups, respectively. Days to flowering, days to maturity and number of seeds per spike were the most characters which contributed variances among accessions. In general, this study demonstrated the existence of variation patterns in barley accessions of their regions of origin and altitude groups. Existence of diversity implies further attention and analysis on these accessions.

Key words: Cluster analysis, discriminant analysis, phenotypic correlation, principal component analysis

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an annual cereal crop which belongs to the genus *Hordeum* in the Tribe Triticeace of grass family Poaceae which contains about 350 wild species and within *Hordeum* genus there are 32 species, all with a basic chromosomal number of x = 7 (Amanda, 2008). Among crops of the Poaceae family, besides oat and barley shows a clear differentiation of grain type which can be hulled or naked (Barabaschi *et al*., 2012). It is thought

to have originated in the Fertile Crescent area of the Near East from the wild progenitor *Hordeum spontaneum* over 10,000 years ago (Zentani, 2005; Azhaguvel and Komatsuda, 2007; Dai *et al*., 2012).

In Ethiopia, barley is the fifth most important cereal crop both in area coverage and production with around 1,013,623.72 ha and 18,155,830.29 qt, respectively (CSA, 2012). It is grown both in Meher (June-September) and Belg (March-April) seasons. The diversity in soils, climate, altitude and topography together with geographical isolation for long periods are considered to be the main factors influenced the large diversity in Ethiopian barley (Harlan, 1976). Social factors also play an important part in the diversification, thus the morphological, biochemical and molecular groups in Ethiopian barley are the result of accumulated long-term mutations, hybridization, gene recombination and natural and human selection in heterogeneous environments (Lakew and Alemayehu, 2011).

Ethiopian barley landraces have useful traits, especially for resistance to diseases such as: Powdery mildew, barley yellow dwarf virus, net blotch, scald and loose smut. Other useful characteristics of Ethiopian barley include high tillering capacity, tolerance to marginal soil conditions, barley shoot fly, aphids and frost, vigorous seedling establishment and quick grain filling period. The knowledge of the population structure of the Ethiopian barley landraces together with a deeper understanding of the nature and extent of their variation are important prerequisites to efficiently use and conserve the existing plant material and it is important to study the potential of the available barley landraces and document information enabling barley researchers to design strategic germplasm enrichment and improvement programs aimed at stable barley production. Assessing genetic variation is a crucial for varietal development and genetic resource conservation (Lule *et al*., 2012).

Barley landraces comprise the major genetic resources cultivated in Ethiopia. Several studies have been conducted on Ethiopian barley landraces, but majority of the studies were focused on the level and structural diversity of Ethiopia barley within a given region, not focused on within and between region of origin and altitude around the country in a single study and also extensive collections have been made by the IBC/E to conserve genetic diversity. However, most of collected and preserved landraces at the gene centre are not yet studied for their genetic diversity (Alemayehu and Parlevliet, 1997; Abdi, 2011). Therefore, the objectives of the present study were (1) To analyze genotypic diversity and interrelationship of characters in Ethiopian food barley landraces, (2) To assess the extent of morphological variation in barley accessions in respect to regions of origin and altitudes of collection and (3) To cluster the accessions into relatively homogenous groups and to identify the major characters contributing to the overall diversity of the germplasm.

MATERIALS AND METHODS

Experimental site: The experiment was conducted at the Holetta Agricultural Research Center, Ethiopia during the main cropping season of 2012 under rain fed condition. Holetta Agricultural Research Center is located at 9°3'N, 38°30'E with an altitude of 2400 m.a.s.l. and characterized with annual rainfall of 1044 mm, mean relative humidity of 60.6% and mean maximum and minimum temperature of 22.1 and 6.2°C, respectively.

Experimental materials and design: A total of 102 barley accessions were obtained from the Institute of Biodiversity Conservation, Addis Ababa, Ethiopia. The accessions were selected based

on their region of origin and altitude (Table 1). Five standard checks (controls) (HB-42, Ardu, Shege, HB1307 and Balami) that were obtained from the Holetta Agricultural Research Center were included (a total of 107 genotypes were used in this study). The experiment was laid out in augmented randomized complete block design consisting of four blocks in which the 102 accessions were planted in un-replicated plots and the five checks were replicated four times (ones in each block) to estimate error variance. The plot size used was one row with 2.5 m length and 0.4 m between rows. Seeds were planted by hand with a seeding rate of 100 kg ha^{-1}. Plots were kept free from weeds. The experiment was conducted at Holetta Agricultural Research Center.

Data collection: Based on the IPGRI descriptor list (IPGRI, 1994), ten quantitative characters were recorded (Table 2) for each accession, 10 randomly selected individual plants were used for recording quantitative characters, except days to 50% flowering, days to maturity and thousand seed weight which were recorded on plot basis.

Table 1: Region of origin, altitude and number of accessions used for this study

	No. of accessions by altitude groups (m.a.s.l)				
Regions	Group I (1500-2000)	Group II (2001-2500)	Group III (2501-3000)	Group IV (3001-3500)	Total No. of accessions
Arsi	3	3	3	3	12
Bale	3	3	2	3	11
Gojam	2	4	3	2	11
Gonder	2	0	7	2	11
Shewa	3	4	3	2	12
Sidamo	3	3	4	1	11
Tigray	3	4	4	1	12
Wellega	3	4	4	0	11
Wello	3	2	3	3	11
Total	25	27	33	17	102

Table 2: List of quantitative characters recorded along with their code and definition

Characters	Code	Character definition
Awn length (cm)	AWL	Distance from the tip of the spike to the end of the awn
Days to 50% flowering (count)	DFL	Number of days from planting to the day when 50% of the heads fully flower (heading) emerge from the boot of flag leaf in each row
Days to maturity (count)	DMA	Number of days starting from planting to the days when peduncles of the spikes in each row become complete yellow and mature
Number of fertile tillers per plant (count)	NFTPP	Number of fertile tillers (spike bearing) of randomly selected plants per plant, counted at maturity
Number of seeds per spike (count)	NSPS	Number of seed per spike on randomly selected plants counted at maturity
Peduncle extrusion length (cm)	PEDext	Distance from the auricle of flag leaf to the base of spike
Peduncle length (cm)	PDL	Distance from last node to base of the spike
Plant height (cm)	PLH	Length of randomly selected plants measured from the ground to the tip of the spike excluding awns at maturity
Spike length (cm)	SPL	Length measured from base of spike to top of spikelets excluding the awns at maturity
Thousand seed weight (g)	TSW	The weight of 1000 seeds taken from each row in gram

Statistical data analysis

Analysis of variance: All quantitative data were analyzed using SAS v 9.1.3 Software (SAS, 2004). A mixed model in which standard checks effect were considered as fixed and accessions effect as random effect was adopted as:

$$Y_{ij} = \mu+\alpha_i+\beta_j+e_{ij}$$

where, Y_{ij} is response variable, μ is general mean, α_i is the fixed effect of i-th standard checks and random effect of accessions, β_j is the random effect of jth block and e_{ij} is random errors (Table 3). Mean squares were calculated as shown in Table 3. Estimates of σ_e^2, σ_g^2 and σ_b^2 were obtained by equating the obtained sum of squares to their expectancies and solving the resulting system equations:

$$\sigma_g^2 = \frac{\text{Genotypes MS-Error MS}}{\text{Blocks}} \quad \sigma_c^2 = \frac{\text{Genotypes MS-Error MS}}{\text{Blocks}}$$

$$\sigma_t^2 = \frac{\text{Test (Accessions) MS-Error MS}}{\text{Blocks}}$$

Cluster analysis: Before undertaking multivariate analysis of variance in which two or more variables were analyzed at a time, the data was standardized to mean of zero (0) and a variance of one (1) to avoid differences in scales.

One hundred two accessions and nine regions of origin were grouped into respective classes. The values of pseudo F statistic (PSF) and Hotellin's pseudo T^2 statistic were used for defining optimum number of clusters. Cluster analysis was made using the hierarchical cluster analysis. The PROC CLUSTER procedure of SAS V9.1.3 (SAS, 2004) using Unweighted Pair Group Method using Arithmetic Average linkage (UWPGMA) was employed. The results of the cluster analysis were presented in the form of dendrogram to depict degree of similarity and inter-relationships among characters.

Principal component analysis: The Principal Component Analysis (PCA) was computed to reduce the number of variables into a few correlated components that can explain much of the variability. It was performed using the correlation matrix to define the patterns of variation among

Table 3: ANOVA table for sum of squares and their expectancies for the statistical genotypic model (Federer and Ragavarao, 1975)

Source of variation	Degree of freedom	Mean square	Expected mean square
Blocks (b)	b-1	MSb	-
Genotypes (g)	g-1	MSg	$\sigma_e^2+\sigma_g^2$
Tests (accessions) (t)	t-1	MSt	$\sigma_e^2+\sigma_t^2$
Controls (c)	c-1	MSc	$\sigma_e^2+\sigma_c^2$
Tests vs. controls (t vs. c)	1	MSt vs. c	$\sigma_e^2+\sigma_t^2+\sigma_c^2$
Error	(b-1)(c-1)	MSe	σ_e^2
Total	n-1	-	$\sigma_e^2+\sigma_g^2+\sigma_b^2$

MSg: Mean square of genotypes, MSb: Mean square of blocks, MSt: Mean square of test (accessions), MSc: Mean square of controls, MSt vs. c: Mean square of tests vs. controls, MSe: Mean square of error, σ_e^2: Expected error variance (MSe), σ_g^2: Genotypic variance component, σ_t^2: Accessions variance component. Genotypes = accessions+checks (controls)

landraces based on the mean of quantitative characters. And also helps to identify characters that load the most in explaining the observed variation. The PROC PRINCOMP Procedure of SAS V9.1.3 (SAS, 2004) was used for principal component analysis.

Discriminant analysis: To assess whether the accessions membership conformed to their regions of origin and altitudinal groups, discriminant analysis was used by using The PROC DISCRIM Procedure of SAS V9.1.3 (SAS, 2004).

RESULTS

Analysis of variance: The analysis of variances indicated significant difference ($p<0.01$) among genotypes, accessions, controls and accessions vs. controls for all quantitative characters except awn length in controls, peduncle length in accessions vs. controls, spike length in genotypes, accessions and accessions vs. controls, number of fertile tiller per plant in controls and accessions vs. controls and day to maturity in controls (Table 4). Hence, the result indicated the existence of high morphological variation in Ethiopian barley landraces in their regions of origin and altitude groups.

Genotypic variation within regions: Estimate of genotypic variance for regions of origin among accessions showed highly significant difference ($p<0.01$) for plant height, peduncle extrusion, spike length, thousand seed weight, numbers of seed per spike, days to 50% flowering and days to maturity in all regions. Similarly, awn length from Arsi, Sidamo and Wellega; peduncle length from Bale, Shewa, Sidamo, Wellega and Wello; number of fertile tillering per plant from Arsi and Tigray significantly varied (Table 5 and 6). Analyses of diversity pattern among accessions from different regions for quantitative characters revealed existence of morphological diversity within regions indicating differences in agro-ecological conditions across regions contributing for the observed morphological diversity.

Table 4: Analysis of variance for ten quantitative characters

Source of variation	Mean square									
	DF	PLH	AWL	PDER	PDL	SPL	TSW	NSPS	NFTPP	DFL	DMA
Block	3	470.87	0.16	4.10	43.48	1.57	70.17	79.13	0.53	50.61	19.51
MSg	106	107.45**	0.86**	5.83**	46.31*	1.05^{ns}	28.09**	185.81**	0.39*	86.20**	130.72**
MSt	101	98.66**	0.87**	5.47**	45.87*	0.88^{ns}	26.60**	162.52**	0.40*	69.52**	101.43**
MSc	4	302.99**	0.56^{ns}	11.54**	58.81*	5.29**	63.63**	555.81**	0.25^{ns}	14.84*	1.12^{ns}
MSt vs. c	1	214.27**	1.09*	19.37**	42.07^{ns}	1.44^{ns}	36.79**	1056.57**	0.59^{ns}	2047.01**	2240.60**
MSE	12	19.24	0.22	1.24	18.41	0.62	1.52	15.39	0.16	3.32	1.60
Cort'd.total	121	13033.45	95.24	645.75	5260.68	124.13	3206.77	20118.48	45.83	9329.60	13934.57
Mean		104.78	11.45	13.89	37.69	8.09	45.23	37.63	3.71	78.82	125.80
SE		4.70	0.51	1.19	4.60	0.93	1.32	4.20	0.44	1.95	1.35
CV (%)		4.18	4.17	8.02	11.38	9.79	2.73	10.42	11.04	2.31	1.01

*,**,ns: Indicates significant at p = 0.05 level, p = 0.01 and non-significant, respectively. MSg: Mean square of genotypes (controls+accessions), MSt: Mean square accessions, MSc: Mean square of control, MSt vs. c: Mean square of accessions vs. control, MSE: Mean square of error (error variance), Cort'd total: Corrected total, SE: Standard error, CV(%): Coefficient of variation, PLH: Plant height, AWL: Awn length, PDER: Peduncle extrusion, PDL: Peduncle length, SPL: Spike length, TSW: Thousand seed weight, NSPS: No. of seed per spike, NFTPP: No. of fertile tiller per plant, DFL: Days to 50% flowering, DMA: Days to maturity. Genotypes = accessions+checks (controls)

Table 5: Estimates of genotypic variance, environmental variance and heritability

Characters	σ_g^2	σ_t^2	σ_e^2	h^2_{Bg}±SE	h^2_{Bt}±SE
Plant height	22.05**	19.85**	19.24	0.53±4.70	0.50±4.70
Awn length	0.16**	0.16**	0.22	0.42±0.51	0.42±0.51
Peduncle extrusion	1.14**	1.06**	1.24	0.47±1.19	0.46±1.19
Peduncle length	6.97*	6.86*	18.41	0.27±4.60	0.27±4.60
Spike length	0.10^{ns}	0.06^{ns}	0.62	0.13±0.93	0.08±0.93
Thousand seeds weight	6.64**	6.27**	1.52	0.81±1.32	0.80±1.32
Number of seed per spike	42.60**	36.78**	15.39	0.73±4.20	0.70±4.20
Number of fertile tillers per plant	0.05*	0.06*	0.16	0.23±0.44	0.27±0.44
Day to 50% flowering	20.72**	16.55**	3.32	0.86±1.95	0.83±1.95
Day to maturity	32.28**	24.95**	1.60	0.95±1.35	0.93±1.35

*,**,ns: Indicates significant at p = 0.05, p = 0.01 and non-significant, respectively. σe^2: Error variance, σg^2: Genotypic variance in genotypes (control+accession), σ_t^2: Genotypic variance of accessions, h^2_{Bg}: Broad sense heritability of genotype (test+accession), h^2_{Bt}: Broad sense heritability of accessions, SE: Standard error

Table 6: Estimate of genotypic variances for nine regions of origin and four altitude groups based on ten quantitative characters

Region	PLH	AWL	PDER	PDL	SPL	TSW	NSPS	NFTPP	DFL	DMA
Arsi	19.06**	0.09*	0.87**	4.58^{ns}	0.27*	6.77**	54.59**	0.13**	19.18**	33.52**
Bale	24.51**	0.02^{ns}	1.22**	8.33*	0.26*	6.66**	58.59**	0.01^{ns}	32.20**	43.46**
Gojam	35.38**	0.02^{ns}	1.31**	5.51^{ns}	0.24*	6.36**	63.94**	0.03^{ns}	34.63**	28.93**
Gonder	40.41**	0.02^{ns}	0.88**	1.74^{ns}	0.34*	9.64**	45.06**	0.01^{ns}	11.23**	25.56**
Shewa	33.41**	0.07^{ns}	1.34**	9.96*	0.47**	8.11**	63.52**	0.03^{ns}	15.39**	36.98**
Sidamo	25.89**	0.31**	1.30**	8.79*	0.26*	11.46**	58.05**	0.00	30.05**	54.18**
Tigray	53.59**	0.04^{ns}	0.81*	5.84^{ns}	0.25*	7.66**	91.18**	0.12**	35.47**	44.66**
Wellega	29.10**	0.54**	4.02**	9.32*	0.46**	9.29**	75.45**	0.06^{ns}	28.03**	36.29**
Wello	29.10**	0.05^{ns}	1.24**	10.44*	0.55**	7.77**	50.95**	0.00	18.85**	39.11**
Altitude group (m.a.s.l)										
Group I	31.18**	0.30**	1.50**	4.10^{ns}	0.13^{ns}	5.37**	54.89**	0.07*	33.32**	46.53**
Group II	19.63**	0.08*	1.56**	6.85*	0.27*	8.83**	59.28**	0.05^{ns}	31.08**	40.49**
Group III	26.52**	0.08*	1.17**	7.48**	0.35*	5.96**	48.93**	0.09*	15.69**	35.39**
Group IV	31.67**	0.10*	0.78**	5.73^{ns}	0.28*	11.31**	37.72**	0.03^{ns}	10.88**	22.58**

*,**,ns: Indicates significant at p = 0.05, p = 0.01 and non significant, respectively. PLH: Plant height, AWL: Awn length, PDER: Peduncle extrusion, PDL: Peduncle length, SPL: Spike length, TSW: Thousand seed weight, NSPS: No. of seed per spike, NFTPP: No. of fertile tiller per plant, DFL: Days to 50% flowering, DMA: Days to maturity; m.a.s.l: Meter above sea level, group I (1500-2000), group II (2001-2500), group III (2501-3000) and group IV (3001-3500)

Genotypic variation within altitudinal gradients: Most of the morphological characters showed significant variation among altitude groups except peduncle length in altitude group I (1500-2000) and IV (3001-3500), spike length in altitude group I and number of fertile tiller per plant in altitude group II (2001-2500) and IV (Table 5). The altitude group III (2501-3000) showed significant genotypic variation for all characters measured. In general, high genotypic variation was observed in an altitude groups II and III which comprised the major barley growing areas in the country.

Correlation among quantitative characters: The correlation coefficient for 10 quantitative characters and altitudes were presented in Table 7, days to maturity had positive and significant correlation with plant height (r = 0.31), number of seeds per spike (r = 0.30) and days to 50% flowering (r = 0.81). Days to 50% flowering had positive and significant correlation with number

Table 7: Correlation coefficients among ten quantitative characters and altitude

Characters	PLH	AWL	PDER	PDL	SPL	TSW	NSPS	NFTPP	DFL	DMA	ALT
PLH	1	-0.073	0.042	0.033	0.016	0.041	0.138	-0.245**	0.243**	0.306**	0.085
AWL		1	0.257**	0.196*	0.198*	-0.056	0.073	-0.070	-0.102	-0.193*	0.165
PDER			1	0.436**	0.286**	0.327**	-0.228*	0.051	-0.405**	-0.276**	-0.280**
PDL				1	0.088	0.052	-0.182	-0.104	-0.378**	-0.315**	-0.211*
SPL					1	0.380**	-0.255**	0.112	-0.056	-0.003	-0.092
TSW						1	-0.556**	0.232**	-0.045	0.050	-0.156
NSPS							1	-0.448**	0.432**	0.303**	0.393**
NFTPP								1	-0.273 **	-0.201*	-0.248**
DFL									1	0.809**	0.469**
DMA										1	0.439**
ALT											1

*,**: Indicates significant at p = 0.05 and p = 0.01 levels, respectively; PLH: Plant height, AWL: Awn length, PDER: Peduncle extrusion, PDL: Peduncle length, SPL: Spike length, TSW: Thousand seed weight, NSPS: No. of seed per spike, NFTPP: No. of fertile tiller per plant, DFL: Days to 50% flowering, DMA: Days to maturity and ALT: Altitude

of seeds per spike (r = 0.43) but negative and significant correlation with peduncle extrusion (r = -0.41) and peduncle length (r = -0.38). Number of fertile tiller per plant had negative and significant correlation with number of seed per spike (r = -0.45). In contrast, number of seed per spike showed negative and significant correlation with thousand seed weight (r = -0.56). Altitude had positive and significant correlation with number of seeds per spike (r = 0.39), days to 50% flowering (r = 0.47) and days to maturity (r = 0.44). Altitude had positive and significant correlation with number of seeds per spike (r = 0.39), days to 50% flowering (r = 0.47) and days to maturity (r = 0.44) (Table 7). This correlation indicates that at higher altitude, there is high rainfall and low temperature which allow longer growing season, longer plant height, high number of seeds per spike but short peduncle extrusion due to high growth of flag leaf. Negative and significant correlation between number of fertile tillers per plant and number of seeds per spike is because plants compete for food assimilation during growth and development period in which it decrease the number of productive tillering per plant. This correlation pattern indicated the phenotypic diversity in Ethiopian barley which was highly linked to variation in altitudinal gradients.

Cluster analysis

Cluster analysis for accessions: Cluster analysis grouped the 102 accessions in to five distinct groups (Table 8). Numbers of accessions per cluster varied from 44 accessions in cluster I-4 accessions in cluster V. Cluster means and percent of populations (accessions) in each cluster are presented in Table 9 and 10. Forty four accessions were found in cluster I which was 43.1% of the total experimental materials. This cluster has been characterized by intermediate plant height, relatively the heaviest thousand seed weight, relatively higher number of fertile tillers per plant, early flowering and early maturity. Accessions grouped under cluster I were scattered along all regions and more at altitude group I (1500-2000) and II (2501-3000). Cluster II accounts 22.6% of the population and included 23 accessions and had shorter peduncle extrusion, longer days to 50% flowering and longer days to maturity. Majority of these accessions were collected at altitude group III (2501-3000) from all regions except Shewa and Tigray. Relatively accessions with shorter plant height, earlier days to 50% flowering, earlier maturity and smaller thousand seed weight were grouped under cluster III which contribute 17.7% to the population (18 accessions).

Table 8: Distribution of 102 barley accessions over four clusters by nine regions of origin and four altitude groups based on ten quantitative characters

	Clusters					
Regions	I	II	III	IV	V	No. of accessions
Arsi	4	4	3	-	1	12
Bale	5	3	2	-	1	11
Gojam	5	6	-	-	-	11
Gonder	2	7	2	-	-	11
Shewa	2	-	4	6	-	12
Sidamo	6	1	3	1	-	11
Tigray	9	-	-	3	-	12
Wellega	7	1	1	-	2	11
Wello	4	1	3	3	-	11
Total	44	23	18	13	4	102
Altitude groups						
Group I	20	4	-	1	-	25
Group II	13	1	8	4	1	27
Group III	9	11	7	5	1	33
Group IV	2	7	3	3	2	17
Total	44	23	18	13	4	102

Table 9: Summary of cluster mean of 102 barley accessions for ten quantitative characters

	Cluster means				
Characters	I	II	III	IV	V
Plant height	99.8	108.4	100.8	113.1	115.5
Awn length	11.5	11.0	11.8	11.8	11.7
Peduncle extrusion	14.9	12.5	13.1	14.4	17.1
Peduncle length	40.0	32.7	37.9	37.6	46.7
Spike length	8.2	8.3	7.6	7.5	8.6
Thousand seed weight	47.4	45.6	39.1	42.2	50.3
Number of seed per spike	25.1	40.7	44.6	58.5	25.5
Number of fertile tiller per plant	4.1	3.7	3.4	3.4	3.3
Days to 50% flowering	70.3	88.9	75.9	78.5	83.0
Days to maturity	116.0	138.2	117.6	125.1	135.5
Number of accessions	44.0	23.0	18.0	13.0	4.0

Cluster IV consisted of thirteen accessions, 12.8% of the population characterized by high number of seeds per spike and moderate in days to 50% flowering and days to maturity which includes more accessions collected from Shewa and from all altitude groups. This cluster, cluster IV contains accessions which have high number of seeds per spike and early maturity, especially accession number 4879, 243571, 235068 and 242093. Cluster V included four accessions (3.9% of the population) and characterized by taller plant height, longer awn length, peduncle extrusion, peduncle length, spike length and heavier thousand seed weight, fewer number of seeds per spike, lower number of fertile tillers per plant, relatively late days to 50% flowering and days to maturity, in which accessions were collected from Arsi, Bale and Wellega from altitude groups II (2001-2500), III (2501-3000) and IV (3001-3500).

Cluster analysis for regions: Regional cluster analysis grouped the nine regions of barley accessions in to four groups based on 10 quantitative characters (Fig. 1). Arsi, Bale, Gojam and Wellega grouped in to cluster I characterized with the longest spike length and earlier flowering. Cluster II was characterized with the longest plant height, awn length, peduncle extrusion, peduncle length and number of seeds per spike in which Shewa, Wello and Sidamo were grouped in this cluster. The shortest plant height, awn length, peduncle length, spike length, the heaviest thousand seed weight, the lowest number of seeds per spike and the highest number of fertile tillers

Table 10: Clustering pattern of 102 Barley accessions based on ten quantitative characters

Clusters	Barley accessions	No. of accessions	Percentage out of populations
I	64194,3663, 4513, 208918, 237021, 230651, 230618, 64195, 3597, 4534, 4420, 229997, 3634, 4538, 237340, 235238, 242087, 230626, 4554, 3589, 222934, 235292, 235256, 1690, 4202, 223188, 3713, 231225, 1673, 243562, 231223, 3630, 212969,3599, 239524, 237366, 3592, 4376, 221710, 223166, 243573, 3236, 235061, 208859	44	43.14
II	64076, 235730, 225996, 64174, 235883, 4434, 3712, 208816, 3389, 3369, 216975, 242096, 4396, 3470, 4393, 243211, 3736, 3744, 64089, 219777, 4552, 64141, 239527	23	22.55
III	3276, 3980, 64055, 4204, 212955, 3372, 64219, 64185, 243600, 4515, 243409, 204685, 3877, 243254, 3721, 235099, 235108, 219040,	18	17.65
IV	225786, 4881, 3987, 243571, 4879, 4871, 235068, 243408, 234307, 242093, 219573, 223187, 243576	13	12.75
V	4532, 243204, 64145, 4545	4	3.92
Total accessions	102		

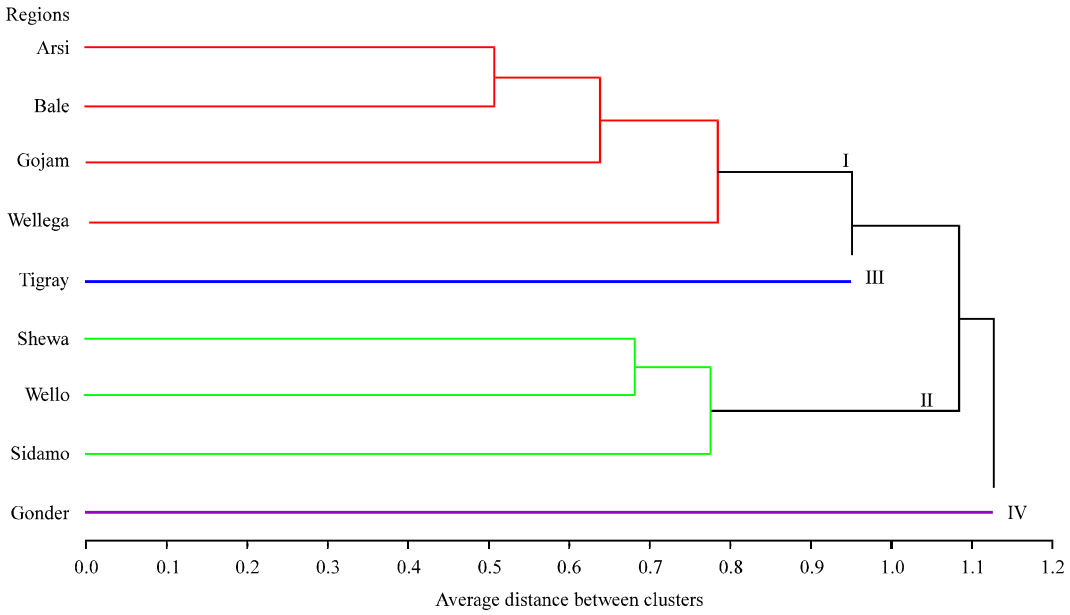

Fig. 1: Dendrogram of nine regions of barley accessions revealed by UPGMA cluster analysis based on 10 quantitative characters, depicting genotypic relationships among regions (average linkage clustering methods)

per plant were clustered under cluster III in which Tigray is the source of collection for this cluster. Cluster IV comprised one region (Gonder) which was characterized with shorter peduncle extrusion, longer spike length, smaller number of fertile tiller per plant, delayed flowering and maturity.

PRINCIPAL COMPONENT ANALYSIS

Principal component analysis for accessions: The principal component analysis exhibited variances of 30, 17, 15 and 10% were extracted for the first four principal components and accounts about 72% of total variation (Table 11). Days to 50% flowering, days to maturity, number of seeds per spike and peduncle extrusion showed greater loading for the variation in the first principal components. Similarly, thousand seed weight, days to maturity, spike length and number of seeds per spike contributed major variation in the second principal component. The variation in the third principal component were mainly due to number of fertile tiller per plant, peduncle extrusion, plant height, awn length and peduncle length while the fourth principal component showed 10% of total variation with greater loading from awn length, plant height and spike length.

Principal component analysis for regions: Principal component analysis showed that 83% of total variation among regions was extracted for the first three principal components having eigenvalue greater than one (Table 11). Peduncle extrusion, peduncle length, days to flowering, days to maturity and plant height gave the most loading contribution for the variation in first principal component which contributed 34% of the variation. The second principal component contributed 31% of the variation in which thousand seed weight, number of seed per spike, awn length and number of fertile tillers per plant contributed greater variation. Similarly, days to maturity, days to flowering, spike length and plant height were the most loading contributors for the third principal component.

Table 11: Eigenvectors, total variance, cumulative variance and eigenvalues for ten quantitative characters of 102 barley landrace in Ethiopia for first four, three and two principal components for accessions, regions and altitude groups, respectively

	Eigen vectors for accessions				Eigen vectors for regions			Eigen vectors for altitude groups	
Characters	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC1	PC2
PLH	-0.18	0.16	0.39	-0.51	0.30	0.25	0.32	0.32	0.23
AWL	0.14	-0.23	0.38	0.62	0.29	0.43	-0.01	0.24	-0.54
PDER	0.36	0.01	0.42	-0.11	0.49	0.05	0.10	-0.34	-0.24
PDL	0.29	-0.24	0.35	-0.28	0.42	0.09	0.21	-0.32	-0.27
SPL	0.21	0.35	0.31	0.42	0.27	-0.23	0.45	-0.22	0.56
TSW	0.24	0.57	0.12	-0.08	0.22	-0.51	0.01	-0.31	0.26
NSPS	-0.40	-0.34	0.19	0.16	-0.20	0.47	-0.02	0.35	-0.16
NFTPP	0.25	0.25	-0.45	0.17	0.08	-0.38	-0.23	-0.33	-0.03
DFL	-0.47	0.27	0.14	0.18	-0.35	0.06	0.52	0.33	0.24
DMA	-0.41	0.37	0.18	0.04	-0.31	-0.19	0.54	0.32	0.18
Eigen value	2.96	1.70	1.52	1.02	3.40	3.08	1.80	7.47	1.86
Total variance (%)	30.00	17.00	15.00	10.00	34.00	31.00	18.00	75.00	19.00
Cumulative variance (%)	30.00	47.00	62.00	72.00	34.00	65.00	83.00	75.00	93.00

PLH: Plant height, AWL: Awn length, PDER: Peduncle extrusion, PDL: Peduncle length, SPL: Spike length, TSW: Thousand seed weight, NSPS: No. of seed per spike, NFTPP: No. of fertile tiller per plant, DFL 50%: Days to 50% flowering, DMA: Days to maturity, PC: Principal component

Table 12: Discriminant analysis of 102 Ethiopian barley accessions for region of origin and altitude based on ten quantitative characters

Regions	Original accession No.	Arsi	Bale	Gojam	Gonder	Shewa	Sidamo	Tigray	Wellega	Wello	Accessions classified under their regions of origin (%)
Arsi	12	**4**	0	1	1	2	0	0	2	2	33.33
Bale	11	2	**2**	1	1	0	1	2	2	0	18.18
Gojam	11	3	1	**4**	1	0	0	1	1	0	36.36
Gonder	11	1	0	0	**8**	0	0	1	0	1	72.73
Shewa	12	0	0	0	0	**8**	0	0	2	2	66.67
Sidamo	11	0	0	0	1	0	**5**	1	1	3	45.45
Tigray	12	0	1	0	1	1	0	**8**	0	1	66.67
Wellega	11	0	1	0	0	0	2	0	**8**	0	72.73
Wello	11	1	1	0	1	1	2	1	1	**3**	27.27

Altitude groups	Original accession No.	G I (1500-2000)	G II (2001-2500)	G III (2501-3000)	G IV (3001-3500)	Accessions classified under their altitude group (%)
G I (1500-2000)	25	**12**	7	5	1	48.00
G II (2001-2500)	27	7	**14**	5	1	51.85
G III (2501-3000)	33	4	6	**19**	4	57.58
G IV (3001-3500)	17	1	2	5	**9**	52.94

Diagonal bold number indicates No. of accession classified under their region of origin and altitude group

Principal component analysis for altitude groups: The first two principal components extracted 93% of total variation among altitude groups having eigenvalue greater than one (Table 11). Number of seed per spike, peduncle extrusion, number of fertile tiller per plant and days to 50% flowering were the most loading contributors in the first principal component. Similarly, spike length, awn length and peduncle length were showed greater loading in the second principal component.

Discriminant analysis: Discriminant analysis of accessions to using the region of origin as a classification variable showed that 49% (50 of the 102) of the accessions were correctly classified to their respective regions of origin (Table 12). The percentage of accessions correctly classified under their regions of origin was high for accessions from Gonder (72.7%), Shewa (66. 7%), Tigray (66.7%) and Wellega (72.7%). While, Bale (18.2%) was the region with smallest percentage of accessions in their respective region of origin. Most of the accessions from the rest regions were scattered all over the regions. Discriminant analysis over altitude groups showed that 53% (54 of 102) of the accessions were correctly placed in to their respective altitudinal groups (Table 12). The percentage of accessions correctly classified was relatively high for the third (2501-3000 m.a.s.l) and fourth altitudinal groups (3001-3500 m.a.s.l).

Based on discriminant analysis, around 51 and 47% of studied accessions were misclassified from their respective regions of origin and altitude groups, respectively. Misclassified accessions of each region revealed that most of the accessions were either placed separately in different regions or in the adjacent regions or in regions having similar climatic conditions or seed exchange and seed flow among regions (seed exchange can be carried out through informal seed exchange rather formal within and among regions in which farmers were the source of seed).

DISCUSSION

The ultimate goal of plant breeding program is to improve the plant characters for agronomic and economic superiority. The knowledge of nature and extent of genetic variation and diversity available in the germplasm or breeding material helps the breeder for planning sound breeding program. Hence, this study was used 102 Ethiopian food barley accessions those collected from nine regions of origin and four altitude groups to evaluate; genetic diversity and interrelationship among characters.

Pattern of genotypic diversity in Ethiopia barley landraces: The wider ranges of variation for several characters observed among barley populations in their regions of origin and altitude groups which are essential for effective collection conservation and suitable improvement of barley by combining the desirable characters together. The variation for days to 50% flowering and days to maturity offers greater flexibility for developing improved varieties suitable for various agro-ecologies of the countries or regions which have variable length of growing period and also to use in various systems. It also guide breeder to develop a variety which escape late season drought by improving traits which correlate to days to maturity in required direction.

This study detected high genotypic variation among accessions in regions of origin and different altitude groups based quantitative characters which suggested that the structure of morphological variation in Ethiopian barley landraces strongly influenced by environmental factors; so that, the degree of variation of characters differ with regions and altitudes from where the accessions collected. Morphological diversity in Ethiopia barley was also reported by different authors (Hadado *et al*., 2009; Abay *et al*., 2009; Firdissa *et al*., 2010; Abebe *et al*., 2010; Hadado *et al*., 2010; Muhe and Alemayehu, 2011; Jalata *et al*., 2011).

Regional diversity: Analysis of diversity pattern among accessions from different regions for quantitative characters revealed existence of morphological diversity within regions; indicating different agro-ecological condition of Ethiopia across regions contributed for morphological diversity of barley accessions. The same results were reported by Negassa (1985), Demissie and Bjornstad (1997) and Hadado *et al*. (2010).

Altitudinal diversity: High level of diversity in respect to different altitude classes in Ethiopian barley landraces and high broad sense heritability indicated presence of substantial variation in the germplasm and possibility of selection response in these traits. Generally, high heritability values was observed for all studied traits except number of tiller per plant which showed relative ease with which selection can be made based on phenotype. High genotypic variation was observed in an altitude class II and III which included the major barley growing areas in the country. Similar result was reported by Demissie and Bjornstad (1997) and Abebe *et al*. (2010) where they found high variation concentration in areas between 2000-3000 and 2400 and 3000 m.a.s.l., respectively. This high variation attributed to mixed farming system which is typically found in areas of higher elevation usually above 2000 m.a.s.l. (Hadado *et al*., 2010) also reported the reduction of area of cultivation for barley as altitude decreased which indicated that barley is cool climate crop.

Inter-relationships of characters: It is commonly known that grain yield is the result of many characters which are interdependent and breeders always look for genetic variation among these

characters to select desirable types that are highly associated among them and with grain yield. The analysis of the inter-relationship among these characters and their association with grain yield is also essential to establish selection criteria (Singh *et al*., 1990). The variation in plant height, peduncle extrusion and peduncle length indicates the possibility to combat lodging problem. Variation in spike length, number of seed per spike, thousand seed weight and number of fertile tiller per plant implies the possibility to create a variety with higher grain yield.

This study revealed that days to maturity had positive and significant correlation with plant height, number of seeds per spike and days to 50% flowering. Indicating interrelationship of characters with days to maturity determine the performance of maturity dates at different regions in different altitude. Abera (2009) found that the positive and significant correlation of days to maturity with days to heading and thousand seed weight with productive tillering. Kebebew (2001) reported positive and significant correlation among days to maturity, days to heading and plant height. Wolie and Dessalegn (2011) found that positive and significant correlation of maturity date with days to heading for Ethiopian finger millet. Days to 50% flowering had positive and significant correlation with plant height and number of seeds per spike. Similarly, Abebe *et al*. (2010) found that days to flowering was positive and significant correlation with plant height.

Altitude had positive and significant correlation with number of seeds per spike, days to 50% flowering and days to maturity but negative correlation with peduncle extrusion length, peduncle length and number of fertile tillering per plant. This study indicate that, morphology diversity in Ethiopia barley were lined with difference in altitude across the country. In line with this study, (Demissie and Bjornstad, 1996; Woldeab *et al*., 2007; Firdissa *et al*., 2010; Hadado *et al*., 2010; Muhe and Alemayehu, 2011) reported morphological diversity of Ethiopian barley landraces were due to difference in geographical and agro-ecological nature of the country at which different characters found at different altitude level. Kebebew (2001) found that positive and significant correlation of altitude with number of seeds per spike, days to 50% flowering and days to maturity in Ethiopia barley. Alemayehu (1995) reported significant delayed maturity and an increased resistance to scald (*Rhynchosporium secalis*) with altitude. His hypothesis was that the increased rainfall in the cooler highlands allowed a longer growing season but also better conditions for foliar diseases. Demissie and Bjornstad (1996) found that six row and short rachilla correlated with high altitude and two-row and long rachilla correlated with low altitude. High correlation between and among characters may show that the characters share some common genetic and geographical information as well as pleiotropic and linkge of genes governing the traits. Ayana *et al*. (2000) reported that correlations among characters are of interest to plant breeders because they help in the identification of easily measured characters that could be used as indicators of more important (but more complex to score) characters.

Principal component: The principal component analysis was used as a data reduction tool to summarize the information from the data set so, that the influence of noise and outliers on the results is reduced. PCA also decreases the number of descriptors responsible for the highest percentage of total variance of the experimental data. It allows the relationship between variables and observations to be studied as well as recognizing the data structure.

Principal component analysis in this study confirmed the existence of high genotypic diversity in barley landraces since, all characters have their own contribution in a variance degree for the variance observed in every component and since, the character variation was explained in several

PCs. The data revealed that first four principal components were extracted having eigenvalues greater than 1 contributed 72% of a total variation among 102 barley accessions based on ten quantitative characters. The first principal component accounted 30%, the second a 17%, the third 15% and the fourth 10% of the total variance. In line with the present finding, Demissie and Bjornstad (1996) also employed principal component analysis for detecting variation in 49 barley populations in which the first four PCs contributed 63% of total variation. Ayalew *et al.* (2011) extracted first four PCs contributed 81% of total variation for tef lanraces. Similarly, the first four PCs explained 93.9% of regional variation in tef germplasm (Assefa *et al.*, 2003). Based on the mean of the first Eigenvector for the first PC of accessions, regions and altitude: Peduncle extrusion length, date to 50% flowering and days to maturity were the most important characters contributing for the overall variability observed among accessions geographical location and agro-ecologies.

Cluster analysis: Hierarchical cluster analysis using UWPGMA grouped 102 Ethiopia barley accessions in to five clusters and also accessions have been grouped in to a particular cluster on the basis of morphological character similarities. Although, cluster analysis grouped the barley accessions with greater morphological similarity, the cluster did not necessarily included all accessions from the same or adjacent sites. This result is in agreement with the study of Abebe *et al.* (2010); it was reported that clustering of accessions based on the agronomic characters revealed no distinct regional grouping patterns in which accessions from same or adjacent regions appeared in different clusters. The same results were reported by Kebebew *et al.* (2001) for ten germplasm. Clustering indicates that the environment has an impact on the performance of barley, specifically altitude has got a great contribution for the performance of characters. The same result was reported by (Hailu *et al.*, 2006) for tetraploid wheat germplasm from Ethiopia.

The result of cluster analysis at regional level indicated that neighboring regions shared some morphological similarities which expressed by accessions. The similarity could be either due to the fact that farmer's selection criteria for a given characters might be similar, particularly based on the adaptive role of characters for the environment, source of seed and seed exchange among regions. Even, if the geographical location of Arsi and Bale are at distance to Gojam and Wellega, from those regions showed some similarities (Fig. 1). Although the regions shared some morphological similarities, dendrogram for regions showed high genotypic diversity among regions. The same results were reported by Abebe *et al.* (2010) and Demissie and Bjornstad (1996).

Characterization of accessions and clustering them on the basis of their morphological traits and genetic similarity will help in identification and selection of the best parents for hybridization (Souza and Sorrells, 1991). Therefore, grouping of accessions by using multivariate methods of analysis based on their similarity in the present study would be valuable for barley breeders in that the most important accessions in the population may be selected from different clusters for improvement programmes.

Discriminant analysis: Based on discriminant analysis around 51 and 47% of studied accessions are misclassified from their respective regions and altitude groups, respectively. Misclassified accessions of each region revealed that most of such accessions were either placed separately in different regions or in the adjacent regions or in regions having similar climatic conditions or seed exchange among regions.

CONCLUSION

A total of 102 barley accessions were evaluated for ten quantitative characters to analyze genotypic diversity and interrelationships of characters in Ethiopian food barley landraces. Analysis of variance indicated the existence of genotypic diversity and interrelationships among characters in Ethiopia food barley landraces. Correlations among quantitative characters revealed that days to maturity had positive and significant correlation with days to 50% flowering (r = 0.81). While days to 50% flowering had positive and significant correlation with number of seeds per spike (r = 0.432). On the other hand, number of fertile tillers per plant had negative and significant correlation with number of seed per spike (r = -0.448). In contrast, number of seed per spike showed negative and significant correlation with thousand seed weight (r = -0.556). Altitude had positive and significant correlation with days to 50% flowering (r = 0.469) and days to maturity (r = 0.439).

Analysis of quantitative characters for their regions of origin and altitude groups indicated significant morphological variation among accessions. This indicates that difference in agro-ecological performance of the country was the most influencing factor for barley morphological variations. Greater genotypic diversity index was observed in Arsi, Wellega and Wello and also high genotypic diversity was observed in altitude groups II (2001-2500) and III (2501-3000) which comprised the major barley growing areas in the country.

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