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Evaluation of genetic diversity in barley (*Hordeum vulgare* L.) from Wollo high land areas using agro-morphological traits and hordein

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This study aimed to determine the genetic diversity and relationships among barley varieties (*Hordeum vulgare* L.) growing at Wollo Highland areas by using hordein and agro-morphological traits. Twenty (20) varieties were laid down in randomized complete block design (RCBD) design with three replications; they were planted by irrigation at Wollo University, Dessie Campus from January to May 2014. The genetic analysis using hordein was done in the laboratory of Ethiopian Biodiversity Institute (EBI) in July 2014. Ten (10) competitive random plants from the rows of the experimental plots were taken for recording their agro-morphological characters. Electrophoretic separation of barley storage proteins or hordeins was done using acid polyacrylamide gel electrophoresis (A-PAGE). The traits: day of heading, day of maturity, grain yield (kg/ha), plant height, spike length, number of spiklet per spike, kernel number per spike, weight of seed per spike and biomass yield (g/plot) were highly significant for the diversity of barely, whereas thousand seed weight was less significant. The results reveal positive correlation between spike length and number of spiklet per spike (the highest correlations from the agro morphological traits); the next highly correlated traits were kernel weight per spike and thousand seed weight. The A-PAGE analysis showed limited variation among the analysed accessions. The Nei's genetic distance for all varieties of barely varied from 0.0000 to 1.6094. It is found that the 20 genotypes of barely investigated in this research were having a gene diversity (h) of overall populations (0.138) using hordein. The cluster analysis grouped the 20 barely genotypes into three different clusters using agro-morphological traits and into four clusters using hordein. This indicates the presence of wide diversity among the tested genotypes. From cluster mean values of agro-morphological traits, genotypes in cluster III deserve consideration for directly developing high yielding barely varieties. The result of the principal components analysis revealed that the first three principal components having greater than 1 eigenvalue contributed 84.22% of the total variation. From this study, it can be concluded that the presence of high morphological variation indicated the potential of Wollo Highland areas in contributing to barley improvement and conservation activities of land areas.

Key words: Acid polyacrylamide gel electrophoresis, agro-morphological traits, hordein, genetic distance, hordeum vulgare, variability.

INTRODUCTION

Evaluation and assessment of genetic diversity in crop species is fundamental for their improvement. The study of genetic diversity is the process by which variation among varieties or groups of individuals or populations is analysed by a specific method or a combination of methods. Genetic diversity assessment with different methods and their comparison could provide complementary information on improvement and conservation

programmes.

Criteria for the estimation of genetic diversity can be different: pedigree records, morphological traits, biochemical markers and molecular markers (Eshghi and Akhundova, 2010). In order to obtain a good insight into the available variation and into the structure of landraces, it is necessary to assess the level of variation between as well as within a representative set of landraces. This information is also important for the maintenance and future use of the varieties.

According to Muhie and Assefa (2011), knowledge of the genetic diversity and agronomic potential of barley landraces in variable environments is an important task to design strategic utilization, targeted collections and introductions of germplasms. In line with this, Tefera (2012) indicated that barley is cultivated by smallholders in every region of Ethiopia, since it is able to grow in all elevations; though it performs best at higher elevations in the northern and central regions of the country.

Among the cereal crops, barley is a species with the greatest adaptability to a wide range of environments. In terms of the area and production worldwide, barley is the fourth most important cereal after wheat, rice and maize (Abebe, 2010). At the global level, the most important cereal crops are maize (*Zea mays*), rice (*Oryza sativa*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare* spp. *vulgare*), with a total of 2.4 billion tons produced annually at a value of more than 446 billion Int. $ (FAOSTAT, 2012). All four of these crops are members of the Poaceae family.

Barley is cultivated from arctic latitudes to tropical areas, and it is grown at the highest altitudes. It is cultivated from 1,400 metre above sea level (m.a.s.l.) to over 4,000 m.a.s.l, and it has adapted to specific sets of agro-ecological areas (Alemayehu and Parlevliet, 1997). Barley is adapted to a broad range of agro-ecological environments and it is tolerant to soil salinity, drought and frost to a considerable level. The crop successfully grows in the arid climates of the Sahara, the Tibetan plateaus, the highlands of the Himalayas, and the Andean countries, the tropical plains of India and the mountains of Ethiopia (Abebe et al., 2010). As barley is one of the major cereals grown in wide agro-ecology of the country like Ethiopia, it has immense economic and social importance for Ethiopians.

In its ambitious five-year growth and transformation plan, the Government of Ethiopia aimed to double the production of grains by 2015 (Tefera, 2012). However, the land in the densely-populated highlands and semi-highlands is fully utilized; therefore, there is little chance for increased area planted with highland crops, especially wheat and barley (Tefera, 2012). It is the most important crop with total area coverage of 1,129,112 ha and total annual production of about 1.7 million tons in main season (CSA, 2010). As a highland crop, there is little to increase in the area planted with barley; the small increase in 2011/12 and forecast for 2012/13 are due to heightened interest by local breweries and local malt producer (Tefera, 2012). Therefore, based on this, there is a need to identify the variety of better traits leading to better production of Wollo Highland areas.

According to Alemayehu and Parlevliet (1997), landraces of such crops are expected to consist of more or less homozygous plants. The observed variation on and within landraces was very large for all traits and the magnitude of variation was so large that most, if not all, plants within a landrace had a different genotype. The landraces also varied from the degree of variation and they added that in order to obtain a good insight into the available variation and the structure of Ethiopian landraces, it is necessary to assess the level of variation between as well as within representatives of landraces. Variation between the Ethiopian barley landraces has been observed by Bekele (1983) and Asfaw (1988, 1990). The increase in barley yields over the recent period has largely affected the introduction of more productive cultivars into farming practice.

In determining the productive varieties analysis of storage protein, hordein and monomeric prolamins (having a great inter-genotypic variation) have been used as marker in cultivar identification, genetic diversity studies, and phylogeny origins in barley (Eshghi and Akhundova, 2009). Potential grain yield of a given cultivar depends on various characters including the plant growth habit. However, there is a lack of information on different responses of new barley cultivars to delayed sowing date as expressed by plant morphological characters and grain yield (Noworolnik, 2012).

In contrast to the genetic uniformity of modern cultivars, landraces show variation both between and within populations. Landraces represent a very interesting model for studying the processes of adaptation and identifying genes and genomic regions that have adaptive roles in a crop species (Hadado et al., 2010). Though there are studies on Ethiopian barley, such as phenotypic genetic diversity in relation to altitude (Engels, 1994), eco geographical distribution of isozyme, allozyme and hordein alleles (Bekele, 1983; Demissie and Bjornstad 1996), and the relationships between hordein and morphotype variation (Asfaw, 1989), the information obtained is still limited. The experiment is done mainly in the limited region of the country (Assefa and Labuschagne, 2004; Abay et al., 2009; Hadado et al., 2009) and out of the area where the farmers try to grow the crop. On the other hand, Engels (1994) reported that only a small fraction of the total phenotypic diversity was

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present among administrative regions, while almost all of the characters were considerably influenced by difference in altitude within regions.

Therefore, the objectives of this study are to assess and evaluate the barley accessions that are grown at the highlands of Wollo Province using agro-morphological and protein variation (hordeins) and to identify groups of accessions with desirable and major traits that contribute to the overall observed diversity as well as barley improvement and conservation in the study area.

MATERIALS AND METHODS

Experimental site

The field experiment was carried out at Wollo University, Dessie Campus from January to May 2014 using irrigation. The research station has 38°, 39´ E longitude and 8°, 11´N latitude with an elevation between 2470 and 2550 meters above sea level; the mean annual maximum and minimum temperatures are 24 and 8°C. The storage protein (hordein) analysis was done at Ethiopian Biodiversity Institute (EBI), Addis Ababa, Ethiopia, Laboratory in July 2014.

Plant material and design

A total of 20 barely genotypes, representing 15 landraces and five released varieties were used for the study. From the 15 landraces, eight were obtained from EBI (202802, 217126, 217125, 202793, 202813, 217119, 225241 and 202799) and the remaining seven (Arusigebs, Anbedat, Enatgebs, Workye, Temaj, Nechita, Wogera) were collected by the researcher; the five released varieties (Estayish, Trit, Yedogit, Shedeho and Agegnehu) were obtained from Sirinka Agricultural Research Centre.

The genotypes were planted in January 2014 in randomized complete block design (RCBD) with three replications. Seed rate of 85 kg/ha was used and sown by hand drilling. Each treatment was planted in a plot area of 2 m^2 consisting of five rows 2 m long, spaced 0.2 m apart between rows and 0.5 m between plots, respectively. An approximate distance of 10 cm was maintained between plant to plant by hand thinning. Ten (10) competitive random plants from the middle rows of the experimental plots were taken for recording the agro-morphological characters. The first weeding was carried out at 21 days after crop planting and the second weeding was performed 15 days after the first weeding. A total of four weeding was done. The experiment was regularly prevented from bird damage. Generally, maximum care was taken in the experiment to minimize the possible occurrence of yield limiting factors which could affect yield potential performance of the varieties. The DAP was applied during planting at the rate of 60 kg/ha. Total nitrogen was applied at rate of 60 kg/ha as urea in two splits: first split (½) and the second split (½) of the total dose during planting and mid-tillering stages, respectively.

Data collection

Agro-morphological data

Data were recorded for quantitative characters using barley descriptors (IPGRI, 1994). The descriptions used in the study were according to IPGRI (1994) including plant based characters in which 10 plants from each plot were selected randomly and then tagged with threads. Data were collected for spike length (SL), number of seeds per spike (NSpS), plant height (PH), number of spikelet per spike (NSKPS), and weight of seed per spike (WSPS). Whereas on plot bases, the traits that were recorded were days to heading (DH), days to maturity (DM), thousand seed weight (TSW), biomass yield (BY) (g/plot), and grain yield (GY) (g/plot).

Methods used in Hordein extraction and identification

Hordein representing major group of storage proteins was extracted from each dry seed in buffered alcohol according to Shewry et al. (1985), with some modifications. Identification of several proteins extracted with various solvents from barley grains was performed by acid polyacrylamide gel (acid-PAGE).

Sample preparation for gliadin (hordein) analysis of barely

For the analysed accessions, 16 seeds per accession and for the released varieties five seeds from each variety were used. Seeds were mechanically crushed into powder and transferred into a capped eppendorf tube. Propanol (55%) containing 2% B - mercaptoethanol was used for extraction and 250 micro litre of extraction buffer was added to each eppendorf tube containing barely powder and vortex and kept at room temperature overnight. 180 µl of loading buffer was added before electrophoresis and vortex, and kept at room temperature until use .

Single barley seed was crushed and ground individually to fine powders with a pestle in a mortar. The powders were extracted with 250 µl extracting solvent in test tubes by mixing vigorously on a vortex mixer at 15-min intervals over a 1-h period.

Gel preparation

Twenty micro litre of hydrogen per oxide (15%) was added to 150 ml gel solution (acrylamide gel); it was mixed well and poured in the gel cast. Combs were inserted and allowed to polymerize for about 10 min.

Sample loading and running

Extra gel was removed from the surface and 250 ml of aluminium lactate buffer was added. Then the combs were removed. Thirty five microliter of sample was transferred into each well with a micropipette. The upper gel cast was kept in the lower buffer tank and connected to power supply. Finally, it was run for about 2 and half hours at 50, 150 and 250 v for 10 min each, 350 v for 30 min and 550 v for one and half hour. At the end of the run, the power supply was switched off and the gel cast was taken out. The side clips were removed. The side of the gel was cut from the starting position to mark the orientation of the samples.

Staining and destaining

Gel was removed from the glass and kept in staining boxes after the addition of tri-chloro acetic acid (TCA) at room temperature for 20 min. The fixing solution was poured back into another material from staining boxes and staining solution was added and kept for one or two days. Finally, the gel was de-stained using tap water. Gel pictures were taken using digital camera and scoring was done by scoring band as present (1) and absent (0).

Analysis of data

Agro-morphological traits analysis

Each variable was subjected to cluster analyses and principal

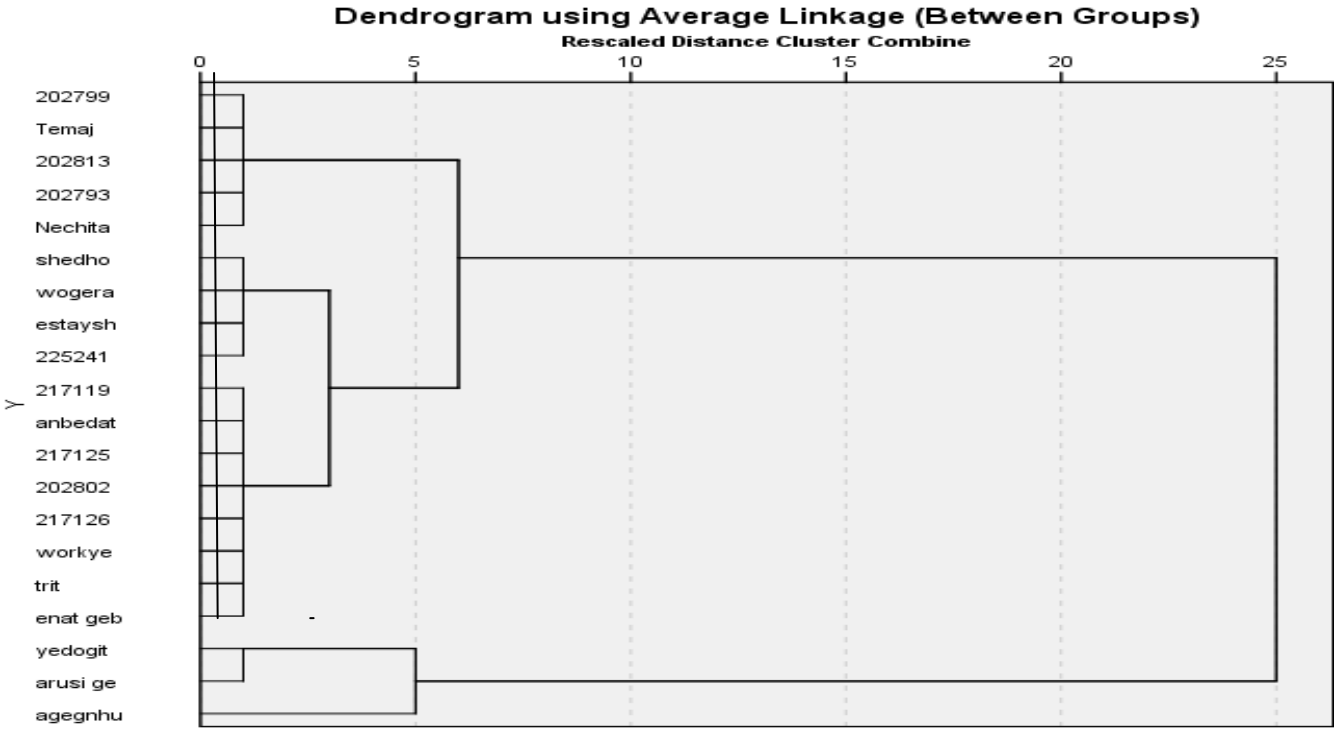

Figure 1. Dendrogram of barley genotypes constructed using agro-morphological traits.

component analysis, and the test of comparison between mean was done. For all traits, cluster mean analysis was used to compare and classify the observed variation in the varieties. For all the traits assessed on individual sample plant basis, the means of the sample plants from each row were used for analyses. The mean and variance of agro-morphological traits was done using GENStat (13th edition) and for the principal component, SAS software (SAS, 9.01) (SAS, 2004) was used. Dendrogram of 20 barley genotypes was done using hierarchical analysis, between–groups linkage methods of SPSS version 20 statistical software based on the agro-morphological traits (Figure 1).

Analysis of barley storage proteins

In order to obtain a grouping by quantitative estimate of variation based on data of the major hordein bands genetic distance matrix was done using Popgene 1.31 and cluster analysis was done using TFPGA 1.3. Hordein bands were scored as either present (1) or absent (0), and these bands were used to calculate genetic similarity and distance among all the twenty varieties. Nei (1978)'s Unbiased Measures of Genetic Identity and Genetic distance generated for the twenty barely varieties was employed to see the genetic distance and genetic identity using pop gene software 1.31.The genetic distance analysis was employed to assess the pattern of genetic diversity among the 20 barely varieties using the standard genetic distances.

RESULTS AND DISCUSSION

Analysis of variance

A wide range of values were observed in agro-morphological traits of the studied barley genotypes (Table 1). Grain yield exhibited the widest range (2258 to 6202 kg/ha) followed by biomass yield per plot (1483 to 2733 gm), plant height (82.9 to 118.1 cm) and days to maturity (110.3 to 137). Derbew et al. (2013) reported that grain yield exhibited the widest range (436 to 3752.5 kg/ ha) followed by plant height (44.95 to 94.1 cm), days to maturity (92 to 131) and heading (57 to 94). In this study, the genotypes had broad range of grain yield, narrow ranges in plant height and days to maturity compared with the work of Derbew et al. (2013). The early maturing genotypes reached physiological maturity in 110.3 days while the late maturing genotype took 137 days to mature. Anbedat and Temaj were early maturing genotypes and Nechita was late maturing genotype. With regard to grain yield, Arusi gebs was the highest yielding genotype and 202799 was low yielding genotype (Table 2).

With regard to days of heading and plant height, the barely varieties used in this research show narrow range of variation compared to the varieties used by Alemayehu and Parlevliet (1997) in which they reported 62 to 97 days and 70.5 to 112.2 cm for heading and plant height, respectively. Lakew et al. (1997) obtained a wide range of variation for days to heading (96 to 116), maturity (137 to 174), plant height (80 - 140 cm) and grain yield (4202 to 5705 kg/ha). In this study, wide range of variation was observed with grain yield and days of heading and narrow range of variation in days to maturity and plant height than the genotypes used by Lakew et al. (1997).

Table 1. Mean, minimum and maximum values of and range of agro morphological traits.

Agro morphological trait	Range (min to max)		Range unit	Mean
	Min	Max		
Days to heading	75 (Temaj)	100 (Nechita)	25	80.78
Days to maturity	110.3 (Temaj & Anbedat)	137(Nechita)	27	121.10
Thousand seed weight(g)	29.66 (202802)	40.05 (Anbedat)	10.41	35.2533
Plant height (cm)	82.9 (Temaj)	118.1(217119)	35.2	101.3197
Number of Spikelet per spike	14.06 (Nechita)	22.62(202793)	8.56	18.1938
Kernel number per spike	28.07 (Temaj)	59.54 (202793)	31.43	46.0983
Spike length (cm)	3.823 (Enat gebs)	9.38(202793)	5.557	6.9300
Biomass per plot (g)	1233 (Nechita)	2733 (Arusigebs)	1250	1786
Grain yield per plot kg/ha	2258 (202799)	6202 (Arusigebs)	3944	3493
Weight of kernel per spike	1.117 (Temaj)	1.94 (Yedogit)	0.823	1.586

Table 2. Average agro-morphological data for barely.

Genotype	BY	GY	KNPS	NSPS	PH	SL	TSW	DH	WSPS	DM
Shedho	1700^{cdef}	3820^{cde}	48.89^{abcd}	18.58^{bcd}	100.7^{bcde}	6.577^{efghi}	34.92^{abc}	79^{bcde}	1.62^{abcd}	121.3^{def}
Estaysh	1783^{cdef}	3998^{cd}	53.89^{abc}	19.6^{abc}	102.6^{bcde}	6.783^{cdefghi}	36.04^{abc}	78.33^{abcde}	1.87^{a}	121.7^{def}
Trit	2100^{bcd}	3403^{def}	37.62^{de}	15.33^{de}	100.7^{bcde}	6.453^{efghi}	37.5^{abc}	85^{f}	1.343^{bcde}	126.3^{ef}
Agegnhu	2133^{bc}	4851^{bc}	43.69^{cd}	17.52^{bcde}	106.5^{bc}	6.247^{fghi}	37.86^{abc}	77^{abcd}	1.69^{abc}	118.3^{d}
Yedogit	2000^{bcde}	5602^{ab}	50.86^{abcd}	18.86^{abcd}	101.1^{bcde}	6.707^{defghi}	39.11^{ab}	74.33^{a}	1.94^{a}	122.7^{def}
202802	1483^{ef}	2889^{def}	44.24^{bcd}	19.94^{abc}	96.1^{cde}	7.963^{abcde}	29.66^{c}	81^{def}	1.213^{de}	122^{def}
217126	1667^{cdef}	3371^{def}	52.83^{abc}	20.91^{ab}	104.8^{bc}	8.993^{ab}	34.66^{abc}	80.33^{cde}	1.727^{ab}	121.3^{def}
217125	1483^{def}	2995^{def}	47.39^{abcd}	19.44^{abc}	101.7^{bcde}	8.177^{abcd}	33.06^{abc}	81^{def}	1.52^{abcde}	119.7^{de}
202793	1967^{bcde}	2523^{ef}	59.54^{a}	22.62^{a}	93.3^{de}	9.38^{a}	30.96^{bc}	94.67^{g}	1.72^{ab}	128^{f}
202813	1700^{cdef}	2335^{f}	45.44^{bcd}	17.59^{bcde}	103.7^{bcd}	7.38^{cdefg}	31.45^{abc}	81^{def}	1.243^{cde}	121.7^{def}
217119	1733^{cdef}	3029^{def}	47.67^{abcd}	19.37^{abc}	118.1^{a}	7.63^{bcdef}	35.43^{abc}	79.67^{cde}	1.653^{abcd}	126^{ef}
225241	2433^{ab}	4124^{cd}	50.82^{abcd}	19.25^{abc}	109.9^{ab}	7.077^{cdefgh}	35.56^{abc}	80.67^{def}	1.783^{ab}	122^{def}
202799	1517^{def}	2258^{f}	43.31^{cd}	17.09^{bcde}	92.3^{e}	7.16^{cdefgh}	33.41^{abc}	79.33^{bcde}	1.54^{abcde}	121.7^{def}
Arusigebs	2733^{a}	6202^{a}	56.91^{ab}	20.82^{ab}	109.4^{ab}	8.313^{abc}	38.82^{ab}	82.67^{ef}	1.927^{a}	126.3^{ef}
Anbedat	1667^{cdef}	3039^{def}	39.49^{de}	16.74^{cde}	105.3^{bc}	5.623^{hi}	40.05^{a}	76^{abc}	1.623^{abcd}	110.3^{a}
Enatgebs	1617^{cdef}	3509^{def}	38.25^{de}	14.69^{e}	96.6^{cde}	3.823^{j}	39.65^{ab}	74.33^{a}	1.52^{abcde}	112^{abc}
Workye	1933^{bcde}	3316^{def}	49.36^{abcd}	19.05^{abcd}	98.6^{cde}	6.53^{efghi}	35.2^{abc}	79.67^{cde}	1.697^{abc}	117^{cd}
Temaj	1267^{f}	2259^{f}	28.07^{e}	14.68^{e}	82.9^{f}	5.327^{i}	31.41^{abc}	75^{ab}	1.117^{e}	110.3^{ab}
Nechita	1233^{f}	2457^{f}	39.42^{de}	14.06^{e}	102.7^{bcde}	6.01^{ghi}	36.3^{abc}	100^{h}	1.507^{abcde}	137^{g}
Wogera	1567^{cdef}	3871^{cd}	44.27^{bcd}	17.75^{bcde}	99.4^{bcde}	6.447^{efghi}	34.02^{abc}	76.67^{abcd}	1.467^{abcde}	116.3^{acd}
Mean	1786	3493	46.098	18.19	101.3	6.93	35.25	80.78	1.586	121.1
DMRT (5%)	***	***	***	***	***	***	NS	***	*	***
CV (%)	17.5	19.2	14.6	10.7	5.4	11.7	12.5	2.9	15.2	2.8
S.E	58.32	154.4	1.169	0.360	1.103	0.188	0.614	0.843	0.0399	3.45

*,**,***Significant at P=0.05, P= 0. 01and P= 0. 001 level and Non-significant at P>0.05, respectively; DH=days to head, DM=days to maturity, TSW=thousand seed weight; BY =biomass yield; GY=grain yield; SL=spike length; KNPS=kernel number per spike; PH=plant height, NSPS=number of spiklet per spike; WSPS = weight of seed per spike.

Among traits under this study, a considerable diversity was observed for grain yield per plot, biomass per plot, plant height, kernel number per spike, days of maturity, day of heading and 100 seed weight, respectively.

It is shown that Temaj and Yedogit show early heading (74.33 days) and Nechita shows late heading (100 days); and with regard to day of maturity, Anbedat and Temaj were early maturing varieties (110.33 days) and Nechita was late maturing variety (137 days). With regard to grain yield per plot in hectare Arusi gebs shows better perfor-

mance (6202 kg/ha) of all of the varieties used, and from the checked varieties, Yedogit had better yield (5602 kg/ha); from the accessions collected EBI 225241 was better in yield per plot, which was 4851 kg/ha. The variety 202799 shows the least yield from the observed varieties, which was 2258 kg/ha followed by Temaj 2259 kg/ha. Variety 202813 was 2335 kg/ha respectively. Regarding plant height, variety 217119 had the highest height (118.1 cm) followed by variety 225241 (109.9cm); Temaj had the lowest height (82.9 cm).

In the case of spike length, variety 202793 had higher length than the other varieties (9.38 cm), had the best number of spiklet per spike, which was 22.62 and contained 59.54 seeds per spike; whereas, Enat gebs had the smallest spike length (3.823 cm), which was farmers' new collection. In the case of biomass yield per plot, Arusi gebs had the highest value of 2733 g/plot and varieties Nechita and Temaj had the smallest values of 1233 g/plot and 1267 g/plot, respectively. With regard to kernel number per spike, variety 202793 had the highest value of 59.54 kernel per spike and Temaj had the least kernel number per spike (28.07). The variety with the highest number of spiklet per spike was 202793, which was 22.62 spiklets per spike. Nechita had the least number of spikelet per spike (14.06).

Concerning the weight of thousand seed, Anbedat variety had 40.05 g, and 202802 had least values of 29.66 g. In the case of kernel weight per spike, the released variety, Yedogit showed the highest measure of 1.94 g per spike and Temaj was the variety with 1.17 g of kernels per spike. Generally, from Table 2, it is observed that varieties Temaj and Nechita had the least results in the observed agro-morphological traits, which were new collections of the researcher and from varieties collected from farmers. Arusi gebs had better results in the observed agro-morphological traits. The released variety Yedogit was also a better variety in showing better results in the observed traits.

The traits day of heading, day of maturity, grain yield (kg/ha), plant height, spike length, number of spikelet per spike, kernel number per spike, weight of seed per spike and biomass yield (g/plot) were highly significant in showing the diversity of barely. Whereas seed weights per spike were significant and thousand seed weight was less significant.

Cluster analysis

If the cutting is done as shown with lines on the dendrogram with 90% similarities, the 20 varieties are divided into four hierarchical cluster groups and Agegnhu remains solitary. The four clusters observed were: cluster I containing the varieties 202793, 202813, 202799, Temaj and Nechita; cluster II containing the varieties 225241, Estaysh, Shedho and Wogera; cluster III containing 217119, Anbedat, Enatgegn, Workye, 217125, 217126 and 202802; and cluster IV contains Arusigebs and Yedogit. This clustering of varieties indicates that barley varieties included in those clusters are variable for the traits considered.

In cluster I, 40 and 60% of the genotypes were from farmers' collection and collections from EBI, respectively; they contain both the early maturing variety, Temaj and the late maturing variety, Nechita- both of which are collections from farmers. All of them were with higher altitudinal locations of more than 2800 based on the information of collected site, and this cluster also contains variety having maturation and day of heading less than the average. Early matured accessions and short plant height containing genotypes were clustered under cluster I. While cluster II included four genotypes in which two of them were released varieties of Sirinka Agricultural Research Institute which were Shedho and Estaysh (50%); the remaining 50% were landraces. All the cluster members from cluster II contain yield per hectare more than the total mean, and variety 225241 was a better yielding variety than the other three varieties, giving a yield of 4851 kg per hectare.

Cluster III contains eight genotypes, four obtained from EBI accounting for 50% of the cluster, three farmers' collections accounting for 37.5% of the cluster and 1 (12.5%) which was the released variety. All of the variety in this cluster contains a yield per hectare less than the total mean (3493 kg/ha) except Enat gebs which yields more than the average (3509 kg/ha), and this variety had the least spike length (3.823 cm). Cluster IV contains a total of two varieties in which 50% was released variety, that is, Yedogit and the other was farmers' collection, Arusi gebs (50%). This cluster contains the highest yielding varieties, Arusi gebs and and Yedogit that yield 6202 kg/ha 5602 kg/ha, respectively. The variety that remains ungrouped, Agegnehu, has got high yield of 4851 kg/ha which was above the mean. Therefore, it is recommended to use variety Arusi gebs from farmers' collection and Agegnehu and Yedogit which were released varieties having the highest yield and highest biomass per plot. So it is preferable both for its yield and biomass which increases the amount of straw and yield for both human and animal use. Significant variations were observed among genotypes in the agro-morphological traits investigated.

Correlation analysis

Based on the Pearson correlation, grain yield was positively correlated to kernel number per spike (0.858), biomass yield (0.656), plant height (0.846) and negatively correlated to thousand seed weight (-0.795), spike length (-0.769), kernel weight per spike (-0.814), number of spikelet per spike (-0.755), day of maturity (-0.760) and day of heading (-0.816). Thousand seed weight was highly positively correlated with spike length, kernel

Table 3. Correlation (Pearson correlation) between agro-morphological traits.

	GY	TSW	KNPS	SL	WSPS	NSPS	BY	PH	DM	DH
GY	1									
TSW	-0.795	1								
KNPS	0.858	-0.925	1							
SL	-0.769	0.939	-0.858	1						
WSPS	-0.814	0.973	-0.948	0.933	1					
NSPS	-0.755	0.955	-0.864	0.983	0.946	1				
BY	0.656	0.411	0.523	0.207	0.457	0.434	1			
PH	0.846	-0.953	0.959	-0.919	-0.980	-0.926	0.414	1		
DM	-0.760	0.963	-0.882	0.901	0.939	0.928	0.177	-0.911	1	
DH	-0.816	0.920	-0.889	0.928	0.933	0.914	0.016	-0.922	0.881	1

DH=Days to heading, DM=Days to maturity, TSW=Thousand seed weight, PH=Plant height, WSPS = Weight of seed per spike, SL=Spike length, NSPS=Number of spikelet per spike, KWPS= Kernel weight per spike, KNPS=Kernel number per spike.

weight per spike, number of spikelet per spike, day of maturity and day of heading, biomass yield, kernel weight per spike and negatively correlated with kernel number per spike (-0.925), grain yield and plant height (Table 3).

Kernel number per spike is positively correlated with grain yield, biomass yield and plant height, and negatively correlated with kernel weight per spike, number of spikelet per spike, day of maturity and day of heading and spike length. Biomass yield is positively correlated with all the traits investigated.

Thousand seed weight is highly positively correlated with spike length, kernel weight per spike, number of spikelet per spike, days of maturity and days of heading, respectively and negatively correlated with grain yield, kernel number per spike and plant height. Kernel number per spike is positively correlated with plant height and grain yield and negatively correlated with other traits. Spike length is highly correlated with thousand seed weight, kernel weight per spike, number of spikelet per spike and day of maturity and day of heading and negatively correlated with kernel number per spike, grain yield and plant height. Spike length and number of spikelet per spike were the traits showing the highest correlations from the agro morphological traits and the next highly correlated traits were kernel weight per spike and thousand seed weight from the ten agro morphological traits investigated. The highest diversity among the genotypes under study was those of grain yield per plot and thousand seed weight.

Similar results for grain yield and plant height in barley were reported by other studies (Bhutta et al., 2005; Kisana et al., 1999; Samarrai et al., 1987). Akdeniz et al. (2004) observed positive and significant correlations between grain yield and yield components such as plant height, spike length and spike number per m^2 but found negative and non-significant correlations between grain yield and kernel number per spike. Positive and significant correlations of grain yield with spike number per m^2 and 1000-kernel weight were reported by Ataei (2006).

Principal component analysis (PCA)

Principal component analysis was used to observe the general pattern for variation of traits. The first three principal components with eigenvalues greater than unity (1) together extracted about 84.22% of the total variation. According to Johnson and Wichern (2002), based on the Eigen values and vectors, it is possible to indicate which traits are mainly responsible to explain the variation. Accordingly, the first principal components which contributed about 42.03% of total variation were due to kernel number per spike, kernel weight per spike and number of spikelet per spike, respectively (Table 4). Similarly, about 26.54% of the variation, accounted for by the second principal component, was due to contributions of thousand seed weight, spike length, yield per plot and days to heading followed by days to maturity.

On the other hand, the third principal component which explained about 15.65% of the variation was mainly through days to heading, days to maturity followed by number of spikelet per spike, thousand seed weight and plant height. In the work of Abebe et al. (2010), the first three principal components (PCs), with eigenvalues greater than unity, explained about 73% of the total variation among accessions for the nine quantitative traits. Hence, even if the genotypes and the number of traits used vary, the value of the first three principal components greater than unity shows a better percentage in this study than the genotypes investigated by Abebe et al. (2010). According to the work of Zaheer et al. (2008), the variation studied through Principal Component Analysis revealed that five principal components having greater than 1 eigenvalues contributed 83.40% of the total variation. In this study, three principal components greater than 1 Eigen values contributed 84.22% of the total variation.

Table 4. Eigenvectors and eigenvalues of four principal components of quantitative traits of 20 barley genotypes.

Quantitative trait	Pc1	Pc2	Pc3	Pc4
Days to head	0.0996	-0.37607	0.5622	0.2585
Days to mature	0.2371	-0.2747	0.5586	0.0674
Plant height	0.3093	0.1644	0.2267	-0.865
Spike length	0.3148	-0.4119	-0.1496	-0.137
No of spikelet per spike	0.3734	-0.2425	-0.3886	-0.039
Kernel no per spike	0.4389	-0.1568	-0.1816	0.1009
Kernel weight per spike	0.4050	0.1991	-0.0201	0.2100
Thousand seed weight	0.1079	0.5136	0.3290	0.0429
Biomass yield per plot	0.3632	0.2318	0.0227	0.1831
Grain yield per plot	0.3154	0.3819	-0.0706	0.2647
Eigen value	4.2026	2.6539	1.56472	0.5858
Difference	1.54864	1.0892	0.9789	0.1147
Proportion	0.4203	0.2654	0.1565	0.0586
Cumulative	0.4203	0.6857	0.8421	0.9007

Storage protein analysis

Nei (1978) reported that the formula for obtaining unbiased estimates of average heterozygosity and genetic distance can be applied to any sample size and are superior to sample average heterozygosity and genetic distance, as long as many loci are used. However, the difference between the biased and unbiased estimators is very small when the number of individuals used is large, say more than 50.

In this study, most of the bands were common for all the analysed samples that show very limited variation among the analysed accession. Due to this, the gene diversity estimate was done for all populations. In this work, Nei's (1978) gene diversity was computed with pop gene software 1.31. This procedure was used by Hailu et al. (2005, 2010) using Nei's (1973) with popgene software 1.31 to see the genetic distances of tetraploid wheat germplasm (Table 5).

Regarding the genetic identity and genetic distance of the 20 genotypes of barely using hordein, it is found that the highest genetic diversity was observed in varieties 217119 and 202793 and 217125 with genetic distance of 1.6094; the second genetic distance observed was found between varieties 217119 and 225241, and 217126, 202813, Temaj, Nechita, Wogera, Shedho, Estaysh, Trit, Agegnehu and Yedogit with a genetic distance of 0.9163. The third genetic distance observed was between variety 217119 and Anbedat, Workye with a genetic distance of 0.8417. The fourth genetic distance found between the varieties was between varieties 217119 and 202799, Arusi gebs, 225241, Enat gebs, Temaj, with a genetic distance of 0.6931. In genetic identity found between the genotypes used, it is found that variety 202799, Arusi gebs, Enat gebs, Temaj were totally identical to each other and varieties 225241, 217126, 202813 were identical to each other. Finally, varieties Wogera, Shedho, Estaysh, Trit, Agegnehu and Yedogit, except Wogera which was new farmers' collection were genetically identical, using hordein.

It is found that the 20 genotypes of barely investigated in this research were having a gene diversity (h) overall populations (0.138). Eshgehi and Akundova (2010) found that the average of genetic diversity index for the proteins investigated was calculated as H = 0.856. With this regard, the average genetic diversity obtained in this study compared with the work of Eshgehi and Akundova (2010) shows a minimum genetic distance between the populations using hordein. However, in the genetic distance observed between individual accessions, 217119 and 202793 and 217125, with a genetic distance of 1.6094 showed higher genetic distance compared to the works of Eshgehi and Akundova (2010).

Variety 217119, a collection of EBI, had the highest genetic distance (1.6094) compared to the other investigated genotypes, using Hordein. According to Nei (1978), when a dendrogram for a group of species is constructed from genetic distance estimates, the reliability of the topology of the dendrogram depends on the differences in genetic distance among different pairs of species.

The 20 barely varieties were clustered into three distinct groups and two of the germplasm (Nechita and 217119) remain solitary based on hordein. The dendrogram shows the relation among the different genotypes used in the study based on Hordein analysis (Figure 2). Cluster I consisted of 11 genotypes whereas clusters two and three consisted of four and three genotypes, respectively. Workye, Ambedat, Wegera, Shedho, Stayish, Tirit, Agegnhu, Yedogit 225241, 217126 and 202813 were grouped together under cluster one. The second cluster consists of 202799, Arusi gebse, Enat

Table 5. Nei's Unbiased Measures of Genetic Identity and Genetic distance of 20 genotypes using hordein.

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	****																			
2	0.0000	****																		
3	0.1054	0.1054	****																	
4	0.3567	0.3567	0.2231	****																
5	0.3567	0.3567	0.2231	0.0000	****															
6	0.3567	0.3567	0.2231	0.0000	0.0000	****														
7	0.6931	0.6931	0.9163	1.6094	1.6094	1.6094	****													
8	0.1054	0.1054	0.0000	0.2231	0.2231	0.2231	0.9163	****												
9	0.1054	0.1054	0.0000	0.2231	0.2231	0.2231	0.9163	0.0000	****											
10	0.0608	0.0608	0.0030	0.2319	0.2319	0.2319	0.8417	0.0030	0.0030	****										
11	0.0000	0.0000	0.1054	0.3567	0.3567	0.3567	0.6931	0.1054	0.1054	0.0608	****									
12	0.0608	0.0608	0.0030	0.2319	0.2319	0.2319	0.8417	0.0030	0.0030	0.0040	0.0608	****								
13	0.0000	0.0000	0.1054	0.3567	0.3567	0.3567	0.6931	0.1054	0.1054	0.0608	0.0000	0.0608	****							
14	0.1054	0.1054	0.0000	0.2231	0.2231	0.2231	0.9163	0.0000	0.0000	0.0030	0.1054	0.0030	0.1054	****						
15	0.1054	0.1054	0.0000	0.2231	0.2231	0.2231	0.9163	0.0000	0.0000	0.0030	0.1054	0.0030	0.1054	0.0000	****					
16	0.1054	0.1054	0.0000	0.2231	0.2231	0.2231	0.9163	0.0000	0.0000	0.0030	0.1054	0.0030	0.1054	0.0000	0.0000	****				
17	0.1054	0.1054	0.0000	0.2231	0.2231	0.2231	0.9163	0.0000	0.0000	0.0030	0.1054	0.0030	0.1054	0.0000	0.0000	0.0000	****			
18	0.1054	0.1054	0.0000	0.2231	0.2231	0.2231	0.9163	0.0000	0.0000	0.0030	0.1054	0.0030	0.1054	0.0000	0.0000	0.0000	0.0000	****		
19	0.1054	0.1054	0.0000	0.2231	0.2231	0.2231	0.9163	0.0000	0.0000	0.0030	0.1054	0.0030	0.1054	0.0000	0.0000	0.0000	0.0000	0.0000	****	
20	0.1054	0.1054	0.0000	0.2231	0.2231	0.2231	0.9163	0.0000	0.0000	0.0030	0.1054	0.0030	0.1054	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	****

1, 202799; 2, Arusi gebs; 3, 225241; 4 , 202802; 5, 202793; 6, 217125; 7, 217119; 8, 217126; 9, 202813; 10, Anbedat; 11, Enat gebs; 12, Workye; 13, Temaj; 14, Nechita; 15, Wogera; 16, Shedho; 17, Estaysh; 18, Trit; 19, Agegnhu; 20, Yedogit.

gebs, and Temaj. And the third cluster consists of 202802, 202793 and 217125. The analysis of hodein helps to group the released varieties together under cluster I which was not possible to cluster together based on the agro-morphological criteria.

In general, the results of this study provide information about diversity of barley which should be of particular interest for the further collecting of genetic resources and show a wide agro morphological variability between the genotypes investigated. The results obtained have shown that sets of agro morphological data are very useful. The study of inter relationship between different variables showed that the yield is significantly and positively correlated with kernel number per spike, biomass yield and plant height. It can be concluded that genotypes, Arusi gebs of farmers' collection and local checks of Agegnehu and Yedogit, collection of EBI 225241 had overall good performance in the experimental fields. As indicated in the correlation analysis above, spike length had a strong positive correlation with number of spikelet per pike.

In the future, selection needs to be conducted, that is, each spike needs to be planted for comparison. The best performed materials need to be promoted to the next step and tested across locations in Wollo high land areas. Finally, after the varieties are selected and approved for the better yield and yield related traits on both research site and on local farmers land with full involvement of farmers living in the research areas proper collection, storage and usage of the varieties in the selected area should be the next activity to be investigated.

Conflict of interests

The author(s) did not declare any conflict of interest.

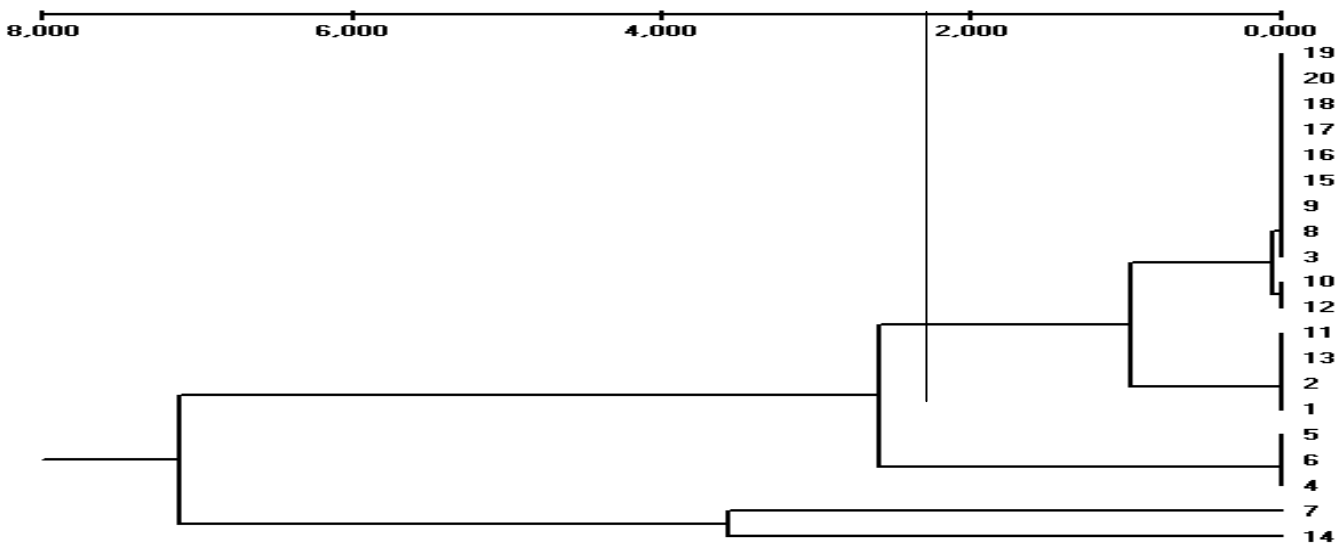

Figure 2. Dendrogram of 20 barley genotypes constructed based on Hordein bands. 1, 202799; 2, Arusigebs; 3, 225241; 4, 202802; 5, 202793; 6, 217125; 7, 217119; 8, 217126; 9, 202813; 10, Anbedat; 11, Enat gebs; 12, Workye; 13, Temaj; 14, Nechita; 15, Wogera; 16, Shedho; 17, Estaysh; 18, Trit; 19, Agegnhu; 20, Yedogit.

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