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Full Length Research Paper

Study of Genetic Diversity in Different Genotypes of Barley (Hordeum vulgare L.) Based on Cluster and Principal Component Analyses

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ABSTRACT

Barley (*Hordeum vulgare L*.) belongs to the family Poaceae, tribe Triticaeae and genus Hordeum. An experiment was carried out to assess genetic diversity by cluster and principal component analysis (PCA) for yield and its contributing characters in Sixty four barley genotypes. The varieties were tested in 8x8 simple lattice design at Atsbi, Ofla and Quiha environments in Tigray region, in 2009/10. Cluster analysis revealed that the 64 genotypes were grouped in 5 distinct clusters for Atsbi and Quiha, while it was 6 for Ofla. Distance between clusters showed significant difference except between cluster I with III at Atsbi, between cluster II and III with IV at Quiha, and between cluster III and IV, and cluster II and IV at Ofla respectively, for most of studied characters. Principal component analysis showed that the first four principal components explained about 82.16% of the total variation, while the first three principal components with 69.27% and 72.04% at Ofla and Quiha environments respectively.

Keywords: Clustering, Genetic divergence, Principal component analysis

INTRODUCTION

Barley (*Hordeum vulgare* L.) belongs to the family Poaceae, tribe Triticaeae and genus *Hordeum*. The genus *Hordeum* consists of 32 species and 45 taxa including diploid (2*n* = 2*x* = 14), tetraploid (2*n* = 4*x* = 28) and hexaploid (2*n* = 6*x* = 42) cytotypes with a basic chromosome number x =7 (Bothmer *etal.* 1995). Cultivated barley (*H. vulgare* ssp. *vulgare* L.) and its wild progenitor (*H. vulgare* ssp. *spontaneum* C. Koch.) belong to a single biological species, which is an annual and is diploid. No crossing barriers have been developed between the wild and cultivated forms, therefore spontaneous and artificial crosses are easily obtained (zemede and Bothmer, 1990).

Barley is an important industrial crop providing raw material for malt, which is used for beer and whisky production. Barley grain contains 3 to 7% β-glucan, an important dietary fiber (Ullrich et al., 1986; Åman and Graham, 1987; Oscarsson et al., 1996) that has significant blood cholesterol lowering effects (Martinez et al., 1992). Barley plays an important role in ensuring food security, as it requires relatively low input. Its yield stability is far better than other cereals, making it a dependable source of food in bad seasons (Berhane et al., 1996).

In Ethiopia 1,019,477.93ha of land is covered by both food and malt barley and 1908262411q with an average yield about 1872 kg/ha are produced at national level (CSA, 2014).In Tigray, for 2013/14 meher season barley production was 246551795 kg, produced from 185,337.29 hectares of land (CSA, 2014).

Over 90% of the barley in Ethiopia, produced by subsistence farmers is landraces (Fekadu, 1997). Indeed, barley production represents about 20% of the total national cereal production. The national average yield for barley is still much below the world average. The mean yield for Ethiopia is only 1.37 ton per hectare FAOSTAT (2009) as compared to the world average yield of about 5.5 ton per hectare with 7 ton per hectare being achieved under optimum conditions (Taylor, 2009).

Cluster analysis is a process of identification and categorization of subsets of objects that are more often than not, continuously distributed or it refers to "a group of multivariate techniques whose primary purpose is to group individuals or objects based on the characteristics they possess, so that individuals with similar characteristics are mathematically gathered into the same cluster". The resulting clusters of individuals should then exhibit high internal (within cluster) homogeneity and high external (between clusters) heterogeneity. Thus, if the classification is successful, individuals within a cluster shall be closer when plotted geometrically and different clusters shall be farther apart (Hair *et al.,* 1995).

Principal component analysis is defined as "a method of data reduction to clarify the relationships between two or more characters and to divide the total variance of the original characters into a limited number of uncorrelated new variables" (Wiley, 1981). PCA can be used to drive a two dimensional scatter plot of individuals, such that the geometrical distance among individuals in the plot reflect the genetic distances among them with minimal distortion. Aggregates of individuals in such a plot will reveal sets of genetically similar individuals (Warburton and Crossa, 2000).

Although, it is easy to make analysis in a multivariable case, inference pertaining to their results is not an easy task. In cluster analysis, there are many distance measures and methods based on these measures. Depending on either distance measure or selected method, the results of cluster analysis could be different and this can lead researcher into an uncertainty. That is why, in recent years, in cluster analysis Principal Components is mostly used. By this way, on the one hand, the number of variables is reduced; on the other hand, the correlation pattern between variables, which is negatively affecting the multi variable analysis methods, can be removed. Furthermore, it is possible to derive detailed information from the plot of observations over the first two principal components. The resulting diagram can give the researcher an idea about the correctness and inference of cluster analysis results (Bensmail *et al*., 1997).

Most of the available genetic variation has been generated naturally and through crop improvement research. Advance in crop improvement for different characters more and over again the presence of germplasm with a very high genetic diversity. Therefore, the objective of this study was to assess the genetic diversity of barley accessions by cluster and principal component.

MATERIAL AND METHODS

Site description

The experiment was conducted at three locations of Tigray region, namely Atsbi, Ofla and Quiha where most of barley is grow on with an erratic rainfall where heavy rain alternate with dry periods resulting in alternating floods and dry periods. The region receives the least rainfall compared to other parts of Ethiopia. The average annual rainfall for the period from 1961 to 1987 was 571 mm, which was 38% less than the national average (921mm) for the same period (Webb and Braun, 1994). The mean annual rainfall ranges from 980 mm on the Central plateau to 450 mm on the Northeastern escarpments of the region (Solomon, 1999). The annual rainfall shows a high degree of variation ranging from 20% in the Western to 49% in the Eastern parts of Tigray (CoSAERT, 1994). The different characteristics of each location are presented in Table 1.

Table 1: Different characteristics of locations

Testing location	AEZ	Altitude (m.a.s.l)	Location		Annual Rainfall (mm)	Annual Temperature		Soil Type	Soil pH
			Latitude	Longitude		Min.	Max.		
Atsbi	SM2e	2630	$13^0$52'N	39^0 44'E	500 - 600	15^0c	35^0c	Sandy loam	6.1

Quiha	Not available	2247	$13^0$30'N	39^0 29 E	812.4	15.4^0c	20.4^0c	Clay loam	6.7
Ofla	SM2a	2539	12°30′N	39°31′E	450 - 800	6^0c	32^0 c	Clay loam	5.2

Plants Material

A total of 64 barley genotypes introduced from ICARDA and one local check named (Saesea) were considered in this study.

Implementation and Experimental Design

The experiments were conducted in 2009/10 in main cropping season using a 8x8 Lattice design with two replications at three locations. The varieties were planted in a plot consisted of a four rows with 2m long and 20 cm apart. The middle two rows were used for data collection. Planting was done by hand drilling using a seed rate of 80kg/ha for each variety. Nitrogen and phosphorous fertilizers were applied at the rate of 50kg/ha Urea and 100kg/ ha DAP at planting. All other management practices were uniformly applied to all plots at planting.

Data were collected for the following parameters like plant height, spike length and number of kernels per spike. The data were recorded on plant basis by randomly selecting 10 plants from each plot. Number of productive tillers/m^2 was recorded by counting the whole second row and then converted into $1m^2$ area, whereas days for heading, days for maturity, 1000-kernel weight, biological yield, grain yield, and germination test were estimated on plot basis (Table 3). The germination test was done by soaking 100 seeds of each genotype in water for 12 hours. Then the seeds were planted using top-dressing method on filter paper and two batches of fifty seeds of each genotype were germinated in a box, which were kept under its plastic cover to reduce evaporation. The germination boxes were placed on the laboratory bench at room temperature of 20^0c (± 0.5) and were watered every other day. Evaluation of germination test was done on the seventh day from sowing. A seed was considered to have germinated if the radicle exceeded 2mm in length (NSIA, 2001).

Statistical Analysis

Cluster analysis (CA)

Clustering of genotypes into different groups was carried out by average linkage method and the appropriate numbers of clusters were determined from the values of Pseudo F and Pseudo T^2 statistics using the procedures of SAS computer software version 9.2 facilities so as to group sets of genotypes into homogeneous clusters (SAS Institute, 2008).

Genetic divergence analysis

A measure of a group distance based on multiple characters was given by generalized Mahalanobis D^2 statistics (Mahalanobis, 1936) for 11 quantitative characters and was analyzed using the procedure Proc discrim of SAS version 9.2 facilities (SAS, 2008).

D^2 statistics is defined by the following formula:

$$D^2_{ij} = (Xi - Xj)\, S^{-1} (Xi - Xj)$$

Where, D^2_{ij} = the square distance between any two accessions i and j; X_i and X_j = the vectors for the values for accession i^{th} and j^{th} genotypes; and S^{-1} = the inverse of pooled variance covariance matrix within groups.

Testing the significance of the squared distance values obtained for a pair of clusters was taken as the calculated value of (^{2} (chi-square) and tested against the tabulated (^{2} values at p-1 degree of freedom at 1% and 5% probability level, where p = number of characters used for clustering genotypes.

Principal component analysis (PCA)

Principal components analysis is a multivariate technique for examining relationships among several quantitative variables. Data of quantitative characters were standardized for principal component analysis and cluster analysis to reduce the influence of outliers and differences in scale of measurements by subtracting the mean from each value and then divided by the standard deviation in order to scale to zero mean and unit variance.

Principal components analysis was performed using correlation matrix by employing Pastogram software of version 2.02 (Hammer *et al.,* 2001) in order to evaluate the relationships among characters that are correlated among each other by converting into uncorrelated characters called principal components. The contribution of each character in PCA is determined by eigenvector that is greater than half divided by the square root of the standard deviation of the eigenvalue of the respective PCA as suggested by (Johnson and Wichern, 1988).

RESULT AND DISCUSSION

Cluster Analysis

Clustering was made to categorize quantitative traits into components for the sake of understanding the share components contribute to major variation in the study (Tables 2, 3 and 4). The dendrogram obtained from the

cluster analysis grouped the 64 genotypes into five clusters (Fig 1, 2 and 3) for Atsbi and Quiha sites and six clusters for Ofla site based on the value of Pseduo F and Pseduo t-square value.

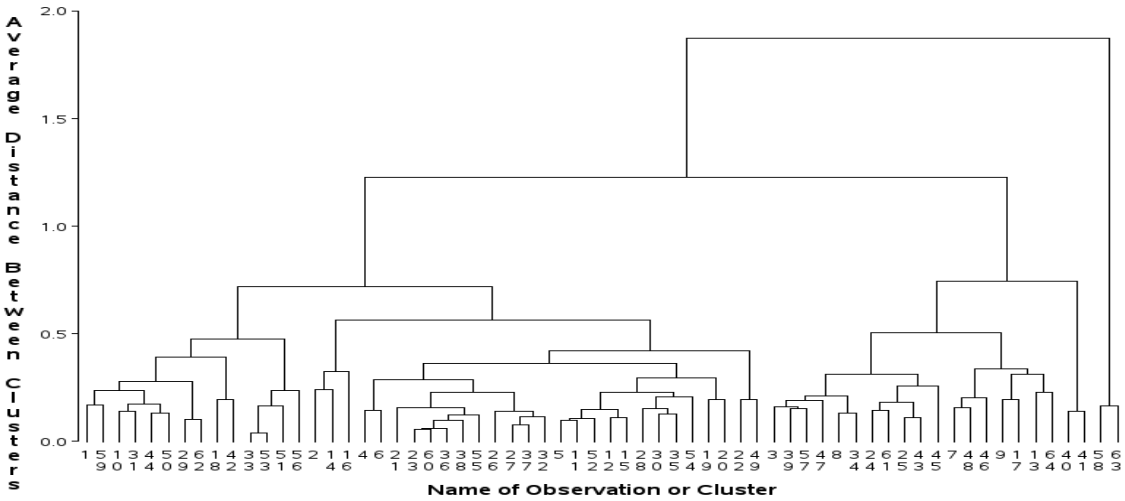

Fig. 1: Dendrogram showing the clusters of 64 ICARDA's barley genotypes tested at Atsbi2009

An attempt made to combine clusters over location remained unsuccessful, because most of the cluster members in each location resembled less than 80% (Girma, 2002). This could also because of the distinct variability of the environments, especially between Quiha and Ofla . Similarly, Dargicho *etal* (2015) grouped 68 bread wheat genotypes into six cluster groups. Therefore, at Atsbi site cluster II had the largest member of all clusters, included 28 (43.75%) genotypes, followed by cluster III that included 18 (28.13%) genotypes that comprised the local check (Saesea). These genotypes may be regarded as having the overall characteristics of this Saesea local check. Similarly, at Quiha site cluster IV constituted 17 (26.55%) genotypes; followed by cluster II and III had 15 (23.44%) genotypes each. In addition, at Ofla site cluster I consisted of the largest group 24 (37.5%) genotypes and cluster III was the second largest group, contained 13 (20.31%) genotypes.

In contrast, cluster IV and V at Atsbi, cluster VI at Ofla and cluster V at Quiha had the smallest component, constituted of 2(3.13%), 3(4.69%) and 1(1.56%) genotypes respectively. This cluster analysis revealed that ICARDA barley genotypes originated from different sources.

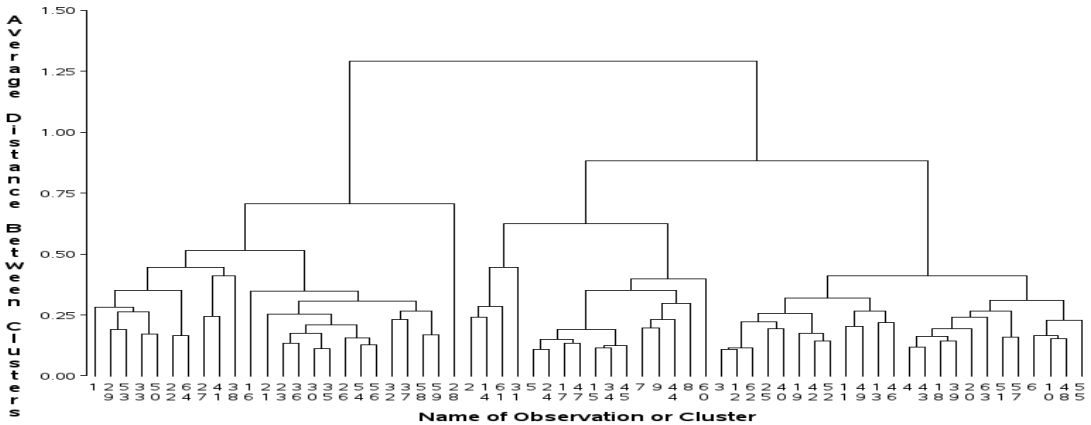

Fig. 2: Dendrogram showing the clusters of 64 ICARDA's barley genotypes tested at Ofla

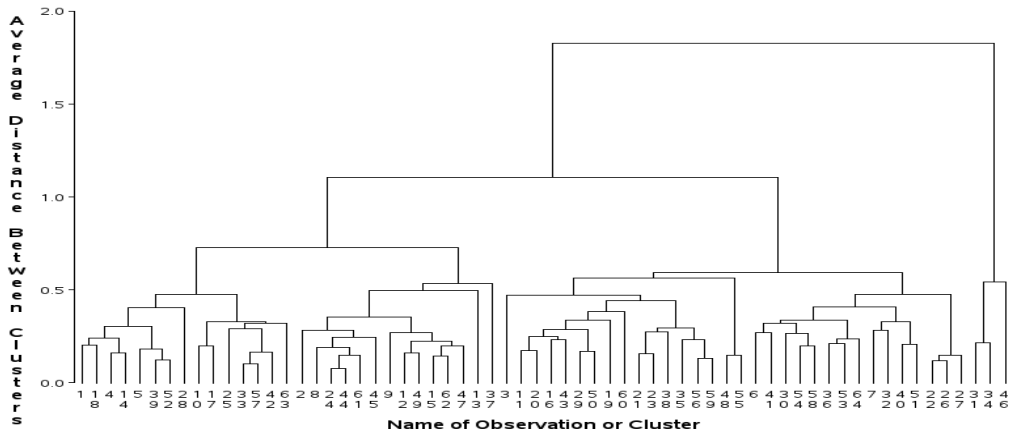

Fig. 3: Dendrogram showing the clusters of 64 ICARDA's barley genotypes tested at Quiha

Table 2: Distribution of 64 barley genotypes in different clusters groups at Atsbi

Cluster	Number of Genotypes	Accession Code
Cluster I	14	ERITREA07 6, ERITREA07 37, ERITREA07 81, ERETH07 64, ERETH07 47, ERETH07 2, ERETH07 87, ERETH07 39, ERETH07 13, ERETH07 25, ERETH07 82, ISEBON 93, ISEBON 36 and ISEBON 47
Cluster II	28	ERITREA07 27, ERITREA07 82, ERITREA07 73, ERITREA07 1, ERITREA07 62, ERITREA07 23, ERITREA07 9, ERITREA07 3, ERITREA07 34, ERITREA07 40, ERITREA07 74, ERITREA07 7, ERETH07 11, ERETH07 61, ERETH07 77, ISEBON 96, ISEBON 88, ISEBON 19, ISEBON 66, ISEBON 94, ISEBON 85, ISEBON 16, ISEBON 92, ISEBON 48, ISEBON 2, ISEBON 56, ISEBON 42 and ISEBON53
Cluster III	18	ERITREA07 64, ERITREA07 24, ERITREA07 72, ERITREA07 11, ERITREA07 43, ERETH07 65, ERETH07 54, ERETH07 79, ERETH07 5, ERETH07 89, ISEBON 90, ISEBON 14, ISEBON 9, ISEBON 71, ISEBON 59, ISEBON 69, ISEBON 7 and ISEBON 29
Cluster IV	2	ERETH07 29 and ERETH07 31
Cluster V	2	ERETH07 40 and ISEBON 18

Table 3: Distribution of 64 barley genotypes in different clusters groups at Ofla

Cluster	Number of Genotypes	Accession Code
Cluster I	24	ERITREA07 1, ERITREA07 64, ERITREA07 82, ERITREA07 37, ERITREA07 62, ERITREA07 23, ERITREA07 43, ERITREA07 81, ERITREA07 40, ERITREA07 74, ERETH07 29, ERETH07 87, ERETH07 54, ERETH07 61, ERETH07 25, ERETH07 77, ERETH07 5, ERETH07 82, ERETH07 40,ISEBON 14, ISEBON 71, ISEBON 59, ISEBON 7 and ISEBON 2
Cluster II	12	ERITREA07 72, ERITREA07 24, ERITREA07 7, ERITREA07 34, ERITREA07 73, ERITREA07 11, ERETH07 79, ERETH07 39, ERETH07 65, ISEBON 9, ISEBON 69 and ISEBON 90
Cluster III	13	ERITREA07 3, ERETH07 11, ISEBON 19, ISEBON 66, ISEBON 16, ISEBON 92, ISEBON 56, ISEBON 42, ISEBON 48, ISEBON 36, ISEBON 18, ISEBON 47 and ISEBON 96
Cluster IV	10	ERITREA07 6, ERETH07 64, ERETH07 2, ERETH07 31, ERETH07 13, ISEBON 88, ISEBON 94, ISEBON 53, ISEBON 29 and ISEBON 93
Cluster V	4	ERITREA07 9, ERITREA07 27, ERETH07 89 and ERETH07 47
Cluster VI	1	ISEBON 85

Table 4: Distribution of 64 barley genotypes in different clusters groups at Quiha

Cluster	Number of Genotypes	Accession Code
Cluster I	14	ERITREA07 72, ERITREA07 27, ERITREA07 11, ERITREA07 23, ERITREA07 43, ERITREA07 34, ERETH07 79, ERETH07 82, ERETH07 87, ERETH07 39, ERETH07 61, ERETH07 89, ISEBON 42 and ISEBON 90
Cluster II	15	ERITREA07 9, ERITREA07 37, ERITREA07 6, ERITREA07 82, ERITREA07 73, ERITREA07 81, ERETH07 65, ERETH07 2, ERETH07 87, ERETH07 77, ERETH07 5, ERETH07 40, ISEBON 14, ISEBON 85, and ISEBON 71
Cluster III	15	ERITREA07 1, ERITREA07 24, ERETH07 25, ERETH07 31, ERETH07 29, ISEBON 29, ISEBON 18, ISEBON 48, ISEBON 93, ISEBON 56, ISEBON 92, ISEBON 16, ISEBON 94, ISEBON 66 and ISEBON 88
Cluster IV	17	ERITREA07 64, ERITREA07 62, ERITREA07 3, ERITREA07 40, ERITREA07 74, ERITREA07 7, ERETH07 64, ERETH07 11, ERETH07 13, ERETH07 54, ISEBON 96, ISEBON 19, ISEBON 7, ISEBON 53, ISEBON 2, ISEBON 36 and ISEBON 47
Cluster V	3	ERETH07 47, ISEBON 9 and ISEBON 59

Mean cluster analysis for Atsbi site

The mean value of the 11 quantitative characters for each cluster group is presented in Table 5 for Atsbi location. Cluster I was characterized by all traits with a medium value that lied between the lowest and highest value. Cluster II was characterized by many number of kernels/spike, whereas with medium value for the rest characters. Cluster III is characterized by tallest plant height, earliest heading and maturing with fewest number of kernels/spike and lowest percentage of germinating seedlings. Cluster IV possessed desirable combinations of characters; namely, earliest maturing, highest 1000-kernel weight, longest spike length, many number of productive tillers/m^2, highest biological yield, highest grain yield highest harvest index and highest percentage of germinating seedlings. This cluster could serve as valuable parent for future crossing program for this specific location. In contrast to cluster IV and, Cluster V are characterized by shortest plant height, latest heading and maturing types with lowest values including, 1000-kernel weight, number of productive tillers/m^2, spike length, biological yield, grain yield and, harvest index.

Table 5: Cluster-wise mean values of characters in ICARDA barley genotypes at Atsbi

Cluster	PH	DM	DH	TKW	PT	SL	K/S	BY	GYLD	HI	GEM
I	63.48	80	49	43.58	265.50	6.03	22.63	82.89	38.10	0.46	93
II	59.91	81	50	38.02	190.85	5.59	30.82	72.55	34.45	0.46	94
III	66.06	78	46	43.67	344.73	6.12	21.02	81.11	37.09	0.45	91
IV	64.85	78	47	47.32	417.82	6.15	21.45	117.72	57.89	0.50	97
V	48.40	86	56	29.27	50.32	4.30	26.85	31.04	12.24	0.39	93

PH=Plant Height (cm), DM=Days to Maturity, DH=Days to Heading, TKW=1000-Kernel Weight (gm), PT=Number of Productive Tillers/m2, SL= Spike Length (cm), KS= Kernels/Spike, BY= Biological Yield (qt/ha), HI= Harvest Index, GEM= Germination Test and GYLD=Grain Yield (qt/ha).

Mean cluster analysis for Ofla site

Similarly, mean value of the 11 quantitative characters for each cluster group is presented in Table 6 for Ofla location. Cluster I was characterized by longest spike length, with the rest traits of medium value. Cluster II was characterized by highest 1000-kernel weight, fewest number of kernels/spike, and highest percentage of germinating seedlings. Cluster III was characterized by shortest plant height, highest harvest index and lowest percentage of germinating seedlings. Cluster IV was distinguished by tallest plant height and latest heading and maturing group. Cluster V distinguished with much of the desirable traits, where earliest heading and maturity combined with many number of productive tillers/m^2, highest biological and grain yield. Cluster VI possessed a combination of latest maturing, fewest number of productive tillers/m^2, shortest spike length, with lowest values of 1000-kernel weight, biological yield, grain yield, and harvest index but highest number of kernels/spike.

Table 6: Cluster-wise mean values of characters in ICARDA barley genotypes at Ofla

Cluster	PH	DM	DH	TKW	PT	SL	K/S	BY	GYLD	HI	GEM
I	73.27	96	62	20.95	175.73	6.17	20.77	84.38	37.87	0.45	91
II	72.17	94	61	21.12	249.17	5.99	19.31	90.78	40.38	0.45	92
III	66.65	97	63	17.69	67.21	5.25	39.58	59.93	29.56	0.49	85
IV	74.03	97	64	19.50	109.31	5.49	32.95	74.49	35.80	0.48	86
V	68.90	93	60	20.40	310.63	5.78	18.30	96.36	42.28	0.44	88
VI	69.65	101	63	14	30	4.85	35.75	26.79	7.84	0.29	89

PH=Plant Height (cm), DM=Days to Maturity, DH=Days to Heading, TKW=1000-Kernel Weight (gm), PT=Number of Productive Tillers/m2, SL= Spike Length (cm), KS= Kernels/Spike, BY= Biological Yield (qt/ha), HI= Harvest Index, GEM= Germination Test and GYLD=Grain Yield (qt/ha).

Mean cluster analysis for Quiha site

In addition, mean value of the 11 quantitative characters for each cluster group is presented in Table 7 for Quiha environment. Cluster I was characterized by where earliest heading and maturity, while the rest traits of medium value. This cluster could be used as parents to develop superior cultivars for dry-land areas, where terminal moisture-stress is a major problem. Cluster II was characterized by medium value that lied between the highest and lowest for all traits discussed. Cluster III was characterized by shortest plant height and spike length, latest heading and maturity with fewest number of productive tillers/m^2, with lowest values of 1000-kernel weight, biological yield, grain yield, harvest index and percentage of germinating seedlings however, many number of kernels/spike. Cluster IV was distinguished by latest heading and maturity with lowest harvest index value. Cluster V was identified as latest maturing group, with a combination of longest plant height and highest values of 1000-kernel weight, biological yield, grain yield, and harvest index and percentage of germinating seedlings but fewest number of kernels/spike. Besides, many number of productive tillers/m^2 and longest spike length were from Cluster V.

Table 7: Cluster-wise mean values of characters in ICARDA barley genotypes at Quiha.

Cluster	PH	DM	DH	TKW	PT	SL	K/S	BY	GYLD	HI	GEM
I	49.39	73	44	32.06	129.69	6.06	20.95	37.36	10.66	0.29	95
II	45.46	75	45	34.60	89.63	5.43	22.37	30.94	10.62	0.33	96
III	37.23	76	46	29.45	34.13	4.59	29.64	23.31	6.63	0.29	86
IV	46.34	76	46	31.31	58.13	5.60	26.56	32.25	8.78	0.27	94
V	52.53	75	46	35.93	186.67	6.15	18.68	46.03	16.91	0.37	99

PH=Plant Height (cm), DM=Days to Maturity, DH=Days to Heading, TKW=1000-Kernel Weight (gm), PT=Number of Productive Tillers/m^2, SL= Spike Length (cm), KS= Kernels/Spike, BY= Biological Yield (qt/ha), HI= Harvest Index, GEM= Germination Test and GYLD=Grain Yield (qt/ha).

Genetic Divergence (Distance)

Divergence analysis is usually performed by using D^2 techniques of Mahalanobis to classify the diverse genotypes for hybridization purpose (Mahalanobis, 1936). The genetic improvement through hybridization and selection depends upon the extent of genetic diversity between parents.

Estimation of mahalanobis distance (squared distance between groups)

The distance between clusters were assessed by the so called Mahalanobis distance such that the values calculated between pairs of clusters (Tables 8, 9 and 10) were considered as Chi-square values and were tested for significance using P-2 degrees of freedom, where 'P' is the number of characters used in the study (Singh and Chaudhary, 1985).

Table 8: Mahalanobis distance between groups of ICARDA's barley genotypes at Atsbi

Cluster	I	II	III	IV	V
I	_	20.85*	16.60	58.17***	143.31***
II		_	66.87***	138.35***	60.74***
III			_	23.84**	244.01***
IV				_	361.79***
V					_

x^2 = 18.31, 23.31 and 29.59 at 5%, 1% and 0.1% probability level respectively.

The highest inter-cluster distance were exhibited between cluster IV and V (D^2 = 361.79), cluster III and V (D^2 = 307.01) and cluster V and VI (D^2 = 415.80) at Atsbi, Quiha and Ofla sites respectively, this indicates wider genetic divergence among the clusters across locations. Maximum generalized squared distance between clusters obtained at Atsbi site was followed by cluster III and V (D^2 = 244.01) and cluster I and V (D^2 = 143.31). Similarly, the maximum generalized squared distance observed at Quiha site was followed by cluster IV and V (D^2 = 220.08) and cluster II and V (D^2= 130.06) and furthermore at Ofla between cluster II and VI (D^2=335.49) and cluster I and VI (D^2=263.43) respectively. Parents for hybridization could be selected on the basis of large inter-cluster distance for isolating useful recombinants in the segregating generations. Increasing parental distance implies a greater number of constraining alleles at the desired loci, and then to the extent that these loci recombine in the F_2 and F_3 generations following a cross of distantly related parents, the greater will be the opportunities for successful selection for any character of yield interest (Ghaderi *et al*., 1984).

Table 9: Mahalanobis distance between groups of ICARDA's barley genotypes at Quiha

Cluster	I	II	III	IV	V
I	_	22.93*	113.66***	63.14***	51.43***
II		_	41.19***	15.12	130.06***
III			_	10.78	307.01***
IV				_	220.08***
V					_

x^2 = 18.31, 23.31 and 29.59 at 5%, 1% and 0.1% probability level respectively.

The shortest squared distance was observed between cluster I and III (D^2 = 16.60) at Atsbi, between cluster III and IV (D^2 = 10.78) at Quiha and between cluster III and IV (D^2 = 11.16) at Ofla, indicated that these clusters were non-significant and genetically close. Thus, crossing of genotypes from these clusters may not produce a high amount of heterotic expression in the F_1's and broad-spectrum of variability in segregating (F_2) populations (Arega *et al*. 2007).

In short, divergence analysis showed the presence of high genetic divergence among ICARDA's barley genotypes evaluated in Tigray region. Hence, hybridization of these genetically divergent parents could lead to the development of desirable recombinants and transgresive segregants, that in turn, may lead to the development of better performing varieties. That is drought resistant types with better yield. Crossing genotypes belonging to distant clusters for wide Mahalanobis distance could maximize transgresive segregation (Amsalu and Endeshaw, 1999).

Table 10: Mahalanobis distance between groups of ICARDA's barley genotypes at Ofla

Cluster	I	II	III	IV	V	VI
I	_	19.15*	57.54***	20.90*	67.87***	263.43***
II		_	132.52***	74.12***	15.47	335.49***
III			_	11.16	226.40***	208.96***
IV				_	150.58***	202.47***
V					_	415.80***
VI						_

x^2 = 18.31, 23.31 and 29.59 at 5%, 1% and 0.1% probability level respectively.

Principal Component Analysis

Principal component analysis (PCA) is one of the multivariate statistical techniques which is a powerful tool for investigating and summarizing underlying trends in complex data structures (Legendre and Legendre, 1998). Principal component analysis reflects the importance of the largest contributor to the total variation at each axis for differentiation (Sharma, 1998). The first step in PCA was to calculate eigenvalues, which define the amount of total variation that is displayed on the PC axes. The PCs with eigenvalue > 1.0 were used as criteria to determine the number of PCs (Kaiser, 1960).

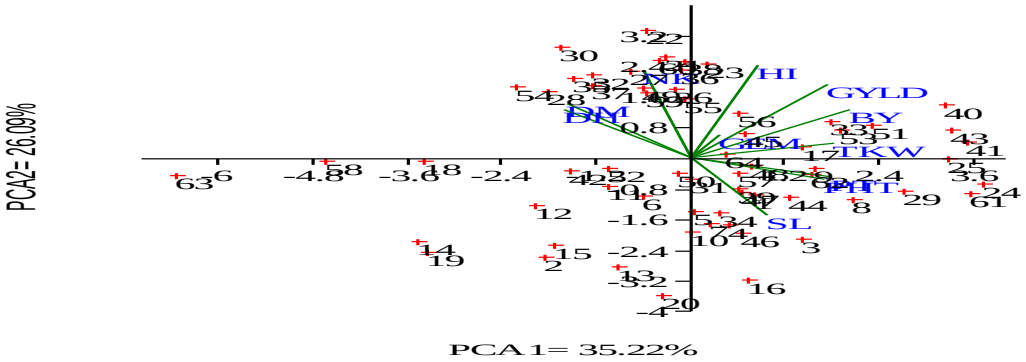

Fig. 4: PCA scatter diagram for 11 quantitative traits of ICARDA's barley genotype at Atsbi.

PH= Plant Height, DM= Days to maturity, DH= Days to heading, TKW= 1000- Kernel Weight, PT= Number of Productive Tillers/m^2, SL= Spike Length, NK= Kernels/spike, BY= Biological Yield, GYLD= Grain yield, HI= Harvest Index and GEM= Germination Test.

The principal component analysis revealed that four principal components PC1, PC2, PC3 and PC4 with eigenvalues 3.87, 2.87, 1.26 and 1.04, respectively have accounted for 82.16% of the total variation among genotypes for the 11 quantitative traits considered (Table 11). At Atsbi environment, the relative magnitude of eigenvectors from the first principal component was 35.22% showing that 1000-kernel weight, number of productive tillers/m^2 and biological yield were the most contributing traits. In the second principal component (PC2), which contributed 26.09% of the total variation, the most predominant characters were number of kernels/spike, grain yield and harvest index. Third principal component (PC3) explained 11.42% of the total variation contributed from plant height, days to heading and days to maturity. Quantitative characters such as spike length and germination test chiefly contributed to the fourth principal component 9.43%.

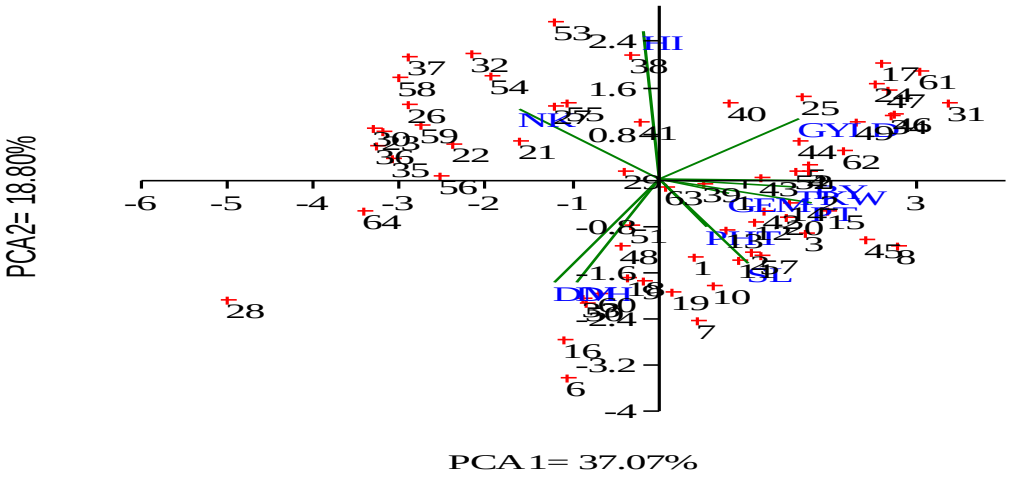

Fig. 5: PCA scatter diagram for 11 quantitative traits of ICARDA's barley genotype at Ofla

PH= Plant Height, DM= Days to maturity, DH= Days to heading, TKW= 1000- Kernel Weight, PT= Number of Productive Tillers/m2, SL= Spike Length, NK= Kernels/spike, BY= Biological Yield, GYLD= Grain yield, HI= Harvest Index and GEM= Germination Test.

This holds true for Ofla site, where the first three principal components PC1, PC2 and PC3 with eigenvalues of 4.08, 2.07 and 1.47 resulted in 69.27% total variation. The first principal component accounted for 37.07% of the variability among accessions were attributed to discriminatory traits such as 1000-kernel weight, number of productive tillers/m^2, number of kernels/spike and biological yield. Similarly, 18.80% of the total variability among the tested genotypes accounted for the second principal component (PC2) originated from variation in plant height, days to maturity, spike length and harvest index. The third principal component explained 13.40% of the total variation showed that days to heading grain yield and germination test were the major contributors.

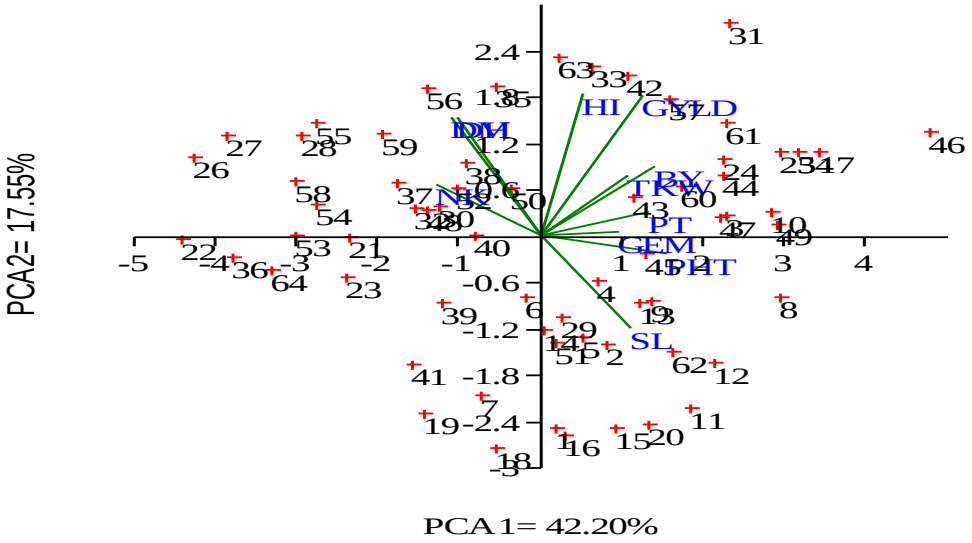

Fig. 6: PCA scatter diagram for 11 quantitative traits of ICARDA's barley genotype at Quiha site

PH= Plant Height, DM= Days to maturity, DH= Days to heading, TKW= 1000- Kernel Weight, PT= Number of Productive Tillers/m2, SL= Spike Length, NK= Kernels/spike, BY= Biological Yield, GYLD= Grain yield, HI= Harvest Index and GEM= Germination Test.

Moreover, the first three principal components PC1, PC2 and PC3 with eigenvalues 4.64, 1.93 and 1.35, respectively, have accounted for 72.04% of the total variation where the first two principal components PC1 and PC2 contributed values of 42.20% and 17.58%, respectively to the total variation at Quiha environment. The first principal component (PC1) explained 42.20% of the total variation. Characters that contributed more to the first principal component (characters with largest coefficients) were plant height, 1000-kernel weight number of productive tillers/ m^2, number of kernels/spike, biological yield and germination test. The second principal component contributed to 17.58% of the total variation showed that yield was the highest loading. Third principal component resulted in 12.26% of the total variation obtained from grain yield and harvest index. Quantitative characters such as days to heading, days to maturity and spike length chiefly contributed to the fourth principal component 12.26% of the total variation.

Generally the principal component analysis indicated diversity since the entire variation cannot be explained in terms of few PCs especially at Ofla and Quiha. This, in turn indicated the involvement of a number of traits in contributing towards the overall observed diversity. In line with the present findings, Demisse and Bjornstad (1996) also employed PCA for detecting variation in 49 barley populations in which the first four PCs contributed 63% of total variation. Similarly, Tiegist (2010) reported that the first three principal components (PCs) explained about 73% of the total variation among accessions for the nine quantitative traits in 130 accessions of barley.

Table 11: Eigenvectors and eigenvalues of the first four principal components (PCs) of barley genotypes evaluated at Atsbi, Ofla and Quiha

Characters	Eigenvectors											
	Atsbi				Ofla				Quiha			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
PH	0.347	-0.115	0.409	-0.356	0.129	-0.190	0.052	-0.895	0.396	-0.059	0.268	-0.032
DM	-0.324	0.295	0.443	0.000	-0.281	-0.411	0.393	0.184	-0.284	0.398	0.455	-0.086
DH	-0.335	0.262	0.466	-0.046	-0.224	-0.413	0.488	0.000	-0.265	0.398	0.486	-0.040
TKW	0.375	0.083	0.033	0.221	0.368	-0.030	0.269	-0.117	0.275	0.203	0.020	-0.434
PT	0.362	-0.098	0.167	0.340	0.406	-0.095	0.083	0.169	0.341	0.082	0.182	0.253
SL	0.202	-0.306	0.409	-0.474	0.241	-0.335	-0.334	-0.072	0.281	-0.311	0.319	0.082
K/S	-0.124	0.477	-0.021	-0.243	-0.381	0.287	0.105	-0.021	-0.333	0.174	-0.085	-0.094
BY	0.413	0.261	0.079	-0.004	0.418	-0.002	0.311	0.178	0.361	0.232	0.244	0.239
GYLD	0.358	0.397	0.034	0.010	0.374	0.243	0.379	0.075	0.323	0.471	-0.165	0.164
HI	0.178	0.503	-0.069	0.068	-0.040	0.598	0.230	-0.177	0.133	0.479	-0.507	0.077
GEM	0.071	0.124	-0.459	-0.646	0.186	-0.054	-0.331	0.221	0.243	0.015	-0.004	-0.795
Eigenvalue	3.87	2.87	1.26	1.04	4.08	2.07	1.47	0.96	4.64	1.93	1.35	0.90
Proportion	35.22	26.09	11.42	9.43	37.07	18.80	13.40	8.75	42.20	17.58	12.26	8.20
Cumulative	35.22	61.31	72.73	82.16	37.07	55.87	69.27	78.02	42.20	59.78	72.04	80.24

PH= Plant Height, DM= Days to maturity, DH= Days to heading, TKW= 1000- Kernel Weight, PT= Number of Productive Tillers/m2, SL= Spike Length, K/S = Kernels/spike, BY= Biological Yield, GYLD= Grain yield, HI= Harvest Index and GEM= Germination Test.

SUMMARY AND CONCLUSION

Clustering was made to categorize quantitative traits into components for the sake of understanding the share components contribute to major variation in the study. The dendrogram obtained from the cluster analysis grouped the 64 genotypes into five clusters for Atsbi and Quiha sites and six clusters for Ofla site. Each cluster had its own characteristic feature for its cluster formation. At Atsbi cluster II had the largest member of all clusters, included 28 (43.75%) genotypes, at Quiha cluster IV constituted 17 (26.55%) genotypes and at Ofla cluster I consisted of the largest group 24 (37.5%) genotypes. In contrast, cluster IV and V at Atsbi, cluster VI at Ofla and cluster V at Quiha had the smallest component, constituted of 2 (3.13%), 3 (4.69%) and 1(1.56%) genotypes respectively.

Genetic distance is very important for hybridization program to get better yield and best recombinant parents. Therefore, the highest inter-cluster distance were exhibited between cluster IV and V (D^2 = 361.79), cluster III and V (D^2 = 307.01) and cluster V and VI (D^2 = 415.80) at Atsbi, Quiha and Ofla locations, respectively, indicates wider genetic divergence among the clusters across locations. Whereas, the shortest squared distance was observed between cluster I and III (D^2 = 16.60) at Atsbi, between cluster III and IV (D^2 = 10.78) at Quiha and between cluster III and IV (D^2 = 11.16) at Ofla environments indicated that these clusters were non-significant and genetically close.

The principal component analysis revealed that four principal components PC1, PC2, PC3 and PC4 with eigenvalues 3.87, 2.87, 1.26 and 1.04 respectively, accounted for 82.16% of the total variation at Atsbi. Similarly, at Ofla the first three principal components PC1, PC2 and PC3 with eigenvalues of 4.08, 2.07 and 1.47 contributed for 69.27% total variation, and at Quiha, PC1, PC2 and PC3 with eigenvalues 4.64, 1.93 and 1.35 respectively, have accounted for 72.04% of the total variation existed among the genotypes with regard to the characters studied. This result further confirmed the presence of ample genetic diversity for use in improvement program.

Three types of seed color, as quality criteria observed were white, tan/red and black in the percentage of 96.87%, except the genotypes Eritrea07 1, and ISEBON 14 with a seed color of tan red and black respectively added to the variability of 1.56% each. From this study, 60.69 % found as two-row type, 31.26% six-row types and 7.81% irregular type across locations. It was observed that from these barley genotypes had a spike density of which 6.25% were lax, 64.06% intermediate and 29.69% dense. All the testing entries were awnletted. It was observed that the caryopsis or kernel covering with the percentage of the genotypes were, 21.88%, 10.94% and 65.64% stands for naked, semi-covered and covered types respectively across locations.

The information described above it can be used for the conservation of the studied germplasm and its future improvement.

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REFERENCES

Åman, P. and H. Graham (1987). Analysis of total and insoluble mixed-linked (1-3), (1-4)-ß-D-glucans in barley and oats. Journal of Agriculture and Food Chemistry, 35: 704-709.

Amsalu Ayana and Endeshaw Bekele, E. (1998). Geographical patterns of morphological variation in sorghum (*Sorghum bicolo*r (L.) Moench) germplasm from Ethiopia and Eritrea: qualitative characters. *Hereditas,* 129: 195-205.

Arega Gashaw, Hussein Mohammed and H. Singh (2007). Genetic divergence in selected durum wheat genotypes of Ethiopian plasm. *African Crop Science Journal,* 15(2): 67-72.

Bensmail. Halima., G. Celeux , A.E. Raftery, C.P. Robert (1997). Inference in model-based cluster analysis, Statistics and Computing, 7(1):1-10.

Berhane Lakew, Hailu Gebre and Fekedu Alemayehu (1996). Barley production and research. pp. 1-8. In: H. Gebre and J. van Leur, (eds). Proceedings, First Barley Research Review Workshop, Addis Ababa, Ethiopia, 16-19 October 1993, IAR/ICARDA.

Bothmer, R. Von, Seberg, O. and Jacobsen, N. (1992). Genetic resources in the Triticeae. *Hereditas,* 116:141-150.

CoSAERT, 1994. Sustainable agriculture and environmental rehabilitation in Tigray. Commission for Sustainable Agricultural and Environmental Rehabilitation in Tigray: Basic Text. Mekelle, Tigray.

CSA (2014). Central Statistical Agency: Report on area and production of crops. Agricultural Sample Survey. Addis Ababa, Ethiopia, statistical bulletin 532.

Dargicho Dutamo, Sentayehu Alamerew, Firdisa Eticha, Ermias Assefa (2015). Genetic variability in Bread Wheat (Triticum aestivum L.) Germplasm for Yield and Yield Component Traits. Journal of Biology, Agriculture and Healthcare, 5, (17): 140-147.

Demissie, A and Bjonstrand (1996). Phenotypic diversity of Ethiopian barley in relation to geographic regions, altitudinal range and agro-ecological zones: as an aid to germplasm collection and conservation strategy. Hereditas, 124:17-29

Ghaderi, A., M.W. Adams and A.M. Nassib (1984). Relationship between genetic distance and heterosis for yield and morphological traits in dry edible bean and faba bean. *Crop Science,* 24:37-24.

FAOSTAT (2009). Statistical Database of the Food and Agriculture of the United Nations. http://faostat.fao.org. Accessed on June, 2010.

Fekadu Alemayehu. and J.E. Parlevliet (1997). Variation between and within Ethiopian barley landraces. Euphytica, 94:183-189.

Girma Taye., Yohannis Tilahun. and Meaza Demisse (2002). Design and analysis of field experiments in agriculture. Technical Manual No. 15. EARO. Addis Ababa, Ethiopia.

Hair, J. F.; R. L. Tatham; R. E. Anderson and W. Black (1995). Multivariate data analysis. 5th Edition, Prentice-Hall. New Jersey. USA. 768 p.

Hammer, Ø., D.A.T. Harper and P. D. Ryan (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica,* 4 (1): 9.

Johnson, R. A. and D. W. Wichern (1992). Applied Multivariate Statistical Analysis. 3rd Edition, Prentice Hall Inc. Englewood Cliffs. New. Jersey, U.S.A. 642 p.

Kaiser, H. F. (1960). The application of electronic computers to factor analysis. *Educational and Psychological Measurement*, 20:141-151.

Legendre, P., and L. Legendre (1998). Numerical Ecology. 2nd edition. Amsterdam. Elsevier. 853p.

Mahalanobis, P. C. (1936). On the generalized distance in statistics. *Proc. Natl. Inst. Sc. India*., 2: 49-55.

Martinez, V.M., R.K. Newman and C.W. Newman (1992). Barley diets with different fat sources have hypo-cholesterolemic effects in chickens. Journal of Nutrition, 122: 1070-1076.

NSIA (2001). Seed testing laboratory mannual.pp.61-85. National Seed Industry Agency. Ethiopia, Addis Ababa.

Oscarsson, M., R. Andersson, A.C. Salomonsson and P. Åman (1996). Chemical composition of barley samples focusing on dietary fiber components. Journal of Cereal Science, 24: 161-170.

SAS Institute Inc, 2008. Statistical analysis Software version 9.2, Cary, NC: SAS Institute Inc. USA.

Sharma, J.R. 1998. Statistical and biometrical techniques in plant breeding. New Age International (P) limited, publishers. New Delhi. 432p.

Singh, R. K. and B.D. Chaundry (1985). Biometrical Methods in Quantitative Genetic Analysis. Kalayani Publishers, New Delhi-Ludhiana.78, 318p.

Solomon, H. (1999). Analysis of Irrigation Water Management Practices and Strategies in Betmera - Hiwane, Tigray, Ethiopia. An MSc Thesis presented to the school of Wageningen Agricultural University. Netherlands.

Taylor P. 2009. Arable Crops

Tiegist Dejene Abebe, A.M. Bauer and J. L É ON (20100. Morphological diversity of Ethiopian barleys (*Hordeum vulgare* L.) in relation to geographic regions and altitudes. *Hereditas*, 1–11.

Ullrich, S.E., J.A. Clancy, R.F. Eslick and R.C.M. Lance (1986). â-glucan content and viscosity of extracts from waxy barley. Journal of Cereal Science, 4: 279-285.

Warburton, M., and J. Crossa (2000). Data analysis in the CIMMYT Applied Biotechnology Center for Fingerprinting and Genetic Diversity Studies. CIMMYT, Mexico.

Webb P. and Braun J. V. (1994). Famine and Food Security in Ethiopia: Lessons for Africa. John Wiley and Sons Ltd. Chichetser, England.

Wiley, E.O. (1981). Phylogenetics: The Theory and Practice of Phylogenetics and Systematics. John Wiley, New York.

Zemede Asfaw, von Bothmer R., (1990). Hybridization between landrace varieties of Ethiopian barley (*Hordeum vulgare* ssp. *vulgare*) and the progenitor of barley (*H. vulgare* ssp. *spontaneum*) *Hereditas.*112:57–64.