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Genetic divergence among barley accessions from Ethiopia

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ABSTRACT - *The study was done with the objective of assessing the genetic diversity existing among Ethiopian and ICARDA barley germplasm using multivariate data analyses. The experiment was conducted at Asasa and Ambo in Ethiopia, in 10 x 10 simple lattices with two replications. To quantify the differentiation among genotypes canonical discriminant analysis, clustering analysis and Mahanalobis (D^2) distance were used. The study indicated that the first two canonical variates explained 95% and 91% of the total variation at Asasa and Ambo, respectively. At both the locations, genotypes showed maximum differentiation on days to maturity, grain filling period, tiller per plant and spike per plant. Analysis of clustering grouped the 100 genotypes into four cluster groups at Asasa and six clusters at Ambo. Ethiopian landraces and genotypes from ICARDA grouped in the same cluster groups indicated the germplasm exchange between the Ethiopian and ICARDA barley breeding programs.*

Key words: *Hordeum vulgare* L., Ethiopian landraces, genetic variability, multivariate analysis.

INTRODUCTION

Barley (*Hordeum vulgare*. L) is an annual cereal crop which belongs to the grass family Poaceae of the tribe Triticeae. In Ethiopia, barley is the most important cereal crop used as a source of food and animal feed. It is grown with altitude range of 1,500 to 3,500 m asl, predominantly between 2,000 to 3,000 m asl (Lakew et al. 1996). According to Central Statistical Authority (CSA 2007), barley is the fourth most important cereal crop after maize, teff, and sorghum in area coverage and production. The total area covered by this crop is 1.019 million hectare with a total production of 1.352 million tones per year (CSA 2007). Ethiopia is considered a center of diversity for barley. To use this genetic diversity for the development of improved variety in the breeding program assessing the extent and pattern of genetic variability within accessions is absolutely essential.

The study of the genetic variability to select parent lines for the crossing program is a crucial step in any breeding program. So, analyzing the genetic diversity of the base population and establishing well defined groups considering a number of traits are important steps during the planning of the crossing program. Measuring this genetic diversity, considering a number of traits jointly, can be possible using the multivariate statistical method. The multivariate technique explains how genotypes differed when all measured traits are considered jointly.

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Different authors used multivariate analysis to study the genetic diversity of breeding populations. Eticha et al. (2006), Manjunatha et al. (2007) and Hailu et al. (2006) used multivariate statistical techniques to study genetic variability in Ethiopian wheat landraces, barley landraces in India, and tetraploid wheat from Ethiopia, respectively. Jaradat et al. (2004) also used the multivariate data analysis technique to study the genetic diversity of barley landraces from Oman. The multivariate technique has been used to assess genetic diversity among genotypes in barley (Chaundary and Singh 1975, Singh et al. 1980, Varma and Gulati 1982, Singh et al. 1996), pepper and bell pepper (Suderé et al. 2005), elephant grass (Shimoya et al. 2002) sesame (Arriel et al. 2007), cassava (Nick et al. 2008), the common bean (Chiorato et al. 2005) passion fruit (Viana et al. 2006) coffee (Fonseca et al. 2004) and tall fescue (Vaylay and van Santen 2002).

The use of the multivariate technique in combination with genetic distance measures will give a clear picture about the genetic differentiation among accessions under study. Several measurements of distance have been proposed over the past decades to suit various objectives. Among these the Mahanalobis's generalized distance (D^2 statistics) (Mahalanobis 1936, Rao 1952) in combination with canonical discriminant analysis is a powerful technique to assess the genetic variability among populations. In the Canonical discriminant analysis all independent variables (traits) are considered simultaneously in differentiation of cultivars (Vayalay and van Santen 2002). The canonical discriminant analysis can separate among-population effects by maximizing discrimination among populations when tested against determination within populations (Riggs 1973). After determining the among-population variability, the Mahalanobis distance (D^2) statistics can be used as an indicator of the difference between populations (Loos 1993). Loos (1993) also used canonical discriminate analysis for extracting variability among populations and Mahanalobis' D^2 statistics to measure genetic differentiation between populations. Kebebew et al. (2001) used canonical discriminant analysis to study the genetic diversity among farmer's varieties in Ethiopia. Canonical discriminant analysis with Mahanalobis D^2 distance used to study the genetic variation among accessions of hairy vetch (*Vicia villosa* Roth) by Yeater et al. (2004).

Selecting parental materials based on the analysis of genetic diversity using multivariate techniques will increase the likelihood of getting potential parental material to produce segregating populations. This work was done to study the genetic diversity existent among Ethiopian and ICARDA barley germplasms using multivariate data analysis techniques.

MATERIAL AND METHODS

Plant materials and experimental design

One hundred barley genotypes (65 from the ICARDA barley breeding program and 35 land race lines developed by the National Barley Improvement program of the country) were included and planted at two locations, Ambo and Asasa. The Ambo Plant Protection Research Center lies at lat 08º 57' N, long 38º 7' E and alt 2200 m asl. The total rainfall during the cropping season was 996.1 mm. The mean minimum and maximum temperature was 9.37 ºC and 25.26 ºC, respectively. Asasa lies at lat 7º 07' N, long 39º 11' E and alt 2300 m asl. The total rainfall during the cropping season was 544.5 mm. The mean minimum and maximum temperature were 5.35 ºC and 23.67 ºC, respectively.

The experiment was laid in a 10x10 simple lattice design with two replications and a plot size of 1.2 m wide and 2.5 m length with a row to row spacing of 20 cm. Seed and fertilizer rates were used as recommended for the experimental sites. At Asasa 18 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5 were applied while at Ambo 20 kg ha^{-1} N and 55 kg ha^{-1} P_2O_5 were applied during planting. The seed rate used was 85 kg ha^{-1}.

Data collected and analysis

For grain yield and other characters studied, measurements were taken from the four central rows of each plot. Data on grain yield $plant^{-1}$ (gm) and other traits studied [plant height (cm), number of tillers $plant^{-1}$, number of spikes $plant^{-1}$, spike length (cm), number of spikelets $spike^{-1}$ and number of kernels $spike^{-1}$] were recorded on 10 randomly taken plants from each plot, whereas kernel weight $spike^{-1}$ (g) and spike weight (g) were determined from five randomly taken plants. The traits 1000-grain weight (g), hectoliter weight (kg hL^{-1}), days to heading and days to maturity, grain yield per plot (g m^{-2}) and biomass yield (kg m^{-2}) were determined on a whole plot basis.

Each variable was subjected to an analyses of variance and the test of comparison between mean was done using the Scott-Knott test (Scott and Knott 1974). The analysis of variance and mean separation test among genotypes were done using Genes statistical analysis software (Cruz 2006).

After the analysis of variance, the significance traits were used to discriminate the accessions. Then canonical variables were determined by the Canonical Discriminant Analysis (CDA) to aggregate the accessions into groups in meaningful ways using the Ward minimum- variance method (Ward 1963) of proc cluster (SAS Institute Inc. 2002). First, the proc aceclus was used to transform the original data set (seventeen variables) into two canonical variable scores for each accession. Then proc discrim was used to discriminate cluster groups formed in cluster analysis and estimate the Mahanalobis D^2 distance among them. The mean values of the canonical variables are referred to as the group of centroid. The difference between the centroid values of two groups (D^2 distance) is calculated as: $D^2 = (\bar{X}_1 - \bar{X}_2)'S^{-1}(\bar{X}_1 - \bar{X}_2)$ where $\bar{X}_1$ and $\bar{X}_2$ are the estimated mean vectors in respective groups, and is S^{-1} the inverse of the pooled sample error variance covariance matrix (Dillon and Goldstein 1984).

RESULTS AND DISCUSSION

Analysis of variance and Scott-Knott test

The analysis of variance showed significant difference among genotypes for all traits at Asasa and Ambo (Table 1). The range of variability was very wide for tillers/plant, spikes/plant, kernel weight spike^{-1}, spike weight and yield plant^{-1} (Table 2). At Ambo, the mean of the highest accession for tillers plant^{-1}, kernel weight spike^{-1} and yield plant^{-1} was more than six times the entry having the lowest observed mean for these traits (Table 2). Similarly, at Asasa, the accession recorded the highest means for kernel weight spike^{-1} and yield plant^{-1} had more than six times greater the mean than the entry with the lowest recorded mean. The over all mean for the entire measured trait showed the genotypes performed better at Asasa than Ambo (Table 2). Most of the accessions matured early at Ambo than Asasa (Table 2). This is due to forced maturation caused by a high incidence of net blotch disease in the region. At Ambo, the genotypes had shorter mean plant heights, resultant due to the vertisol nature of the soil which caused a slow growth rate at the beginning due to the water logging problem and consequently affected plant growth in general.

The long vegetative period was recorded by six row barleys rather than two rows due to the fact that most of the six row barleys originated from high land areas which received high rainfall. Demissie and Bjørnstad (1996) also confirmed the predominant distribution of six row barleys in the high lands of Ethiopia. High grain yield and yield components (1000 kernel weight, spike per plant, hectoliter weight) were observed by two row barley which has a lower number of kernels per spike helping uniform grain fill produce plump grain. Accessions from ICARDA performed better in compression to landraces for grain yield and grain character (kernel weight, hectoliter weight and 1000-kernel weight) supported by long grain filling period. This may be due to the impact of the breeding program which made directional selection towards high yielding and good kernel quality (Table 2). The results also showed the breeding program at ICARDA developed high yielding varieties by maximizing the agronomic traits contributing to high productivity and quality seed.

Clustering and genetic diversity

At Ambo the first two canonical variates explained 91% of the total variation where as Asasa explained 95% of the total variation (Table 3). The canonical loading measures the simple linear correlation between the original independent variable (traits) and the canonical variate and it reflects the variance that observed variables share with the canonical variate and can be interpreted in assessing the relative contribution of each variable to the canonical function. At Ambo, days to heading, days to maturity and grain filling period have high canonical loading in the first canonical variate followed by kernel weight per spike and yield per plant (Table 3). The second canonical discriminant function is dominated by a large loading from plant height, tiller per plant, spike per plant and spike length, indicating these traits contributed to the genetic differentiation among accessions at Ambo. At Asasa, days to maturity, grain filling period, tiller plant^{-1}, spike plant^{-1} and biomass have high loading in the first canonical discriminant function which gave evidence the genetic composition of the accessions differed in relation to these traits at Asasa. The high canonical loading of kernel weight, 1000 kernel weight, hectoliter weight, grain yield per plant and grain yield per plot on second canonical discriminant function proved the importance of these traits in discriminating the barley accessions (Table 3).

The same result also found by Manjunatha et al. (2007) on barley landraces from Uttaranchal Himalaya of India. Jaradat et al. (2004) also found high loading for number of seeds spike^{-1}, spiklets spike^{-1}, 1000-kernel weight and spike length which are important for the genetic differentiation between landraces from Oman. At Ambo canonical correlation between the first canonical variate

Table 1. Analysis of variance for 17 traits evaluated at Ambo and Asasa

Ambo		**Means Square**															
Source of variation	**df**	**Days to heading**	**Days to maturity**	**Grain filling period**	**Plant height**	**Tillers plant^{-1}**	**Spikes plant^{-1}**	**Spike length**	**Spikelet spike^{-1}**	**Kernel No spike^{-1}**	**kernel wt spike^{-1}**	**Spike weight**	**1000-seed weight**	**HLW**	**Grain yield plant^{-1}**	**Grain yield plot^{-1}**	**Biological yield**
Replication	1	1.62	4.80	12.00	0.01	0.09	0.04	0.04	64.72	4.39	0.00	0.01	0.51	44.37	0.01	6306.77	1.18
Genotypes	99	63.20**	78.40**	43.05**	481.50**	1.30**	1.30**	1.20**	316.30**	170.80**	0.14**	0.16**	118.00**	47.70**	1.20**	11284.40**	0.11**
Error	99	2.61	11.98	12.41	19.67	0.30	0.30	0.26	18.08	10.89	0.03	0.03	8.22	5.81	0.40	1895.75	0.04
CV (%)		2.64	3.59		4.95	20.72	16.00	7.55	10.97	10.24	21.59	18.59	8.44	4.36	22.17	12.62	14.93
Mean		61.39	96.35	34.96	90.01	3.50	2.74	6.74	37.40	31.25	0.80	0.98	35.15	55.32	2.82	341.81	1.35
Asasa		**Means Square**															
Replication	1	49.00	8.40	16.86	292.82	3.92	4.15	0.35	129.25	62.05	0.46	0.76	62.72	3.48	12.10	39443.70	5.77
Genotypes	99	85.30**	69.50**	60.69**	239.90**	4.40**	3.90**	1.40**	551.70**	378.40**	0.35**	0.37**	139.48**	44.28**	6.93**	17283.80**	0.44**
Error	99	6.48	20.26	23.17	24.50	1.81	1.58	0.28	18.35	15.47	0.07	0.07	11.97	3.89	3.47	6497.01	0.21
CV (%)		3.42	3.85		4.92	21.49	18.97	7.06	9.28	10.00	19.85	17.41	9.21	3.49	23.39	15.68	19.27
Mean		74.62	116.97	42.35	101.15	6.74	6.39	7.53	44.12	37.58	1.28	1.46	37.75	56.52	7.84	507.48	2.40

Table 2. Range and mean separation test between accessions from ICARDA and Landraces using Scott-Knott (p = 0.05) at Ambo and Asasa

Variables	Ambo			Asasa			Mean	
	Range	ICARDA (65)*	Landraces (35)	Range	ICARDA (65)	Landraces (35)	Ambo (100)	Asasa (100)
Days to heading	49-78	58.43b	66.03a	57-97	72.10b	78.54a	61.09b	74.35a
Days to maturity	91-125	95.49b	98.28a	81-136	117.38a	115.96a	96.27b	116.88a
Grain filling period	25-52	37.06a	31.7b	23.5-56	45.28a	37.41b	35.18b	42.53a
Plant height	62-119	81.17b	103.87a	73-125	95.12b	110.84a	89.12b	100.62a
Tiller per plant	1.7-6.9	3.47a	3.35a	3.1-12.3	6.66a	6.56a	2.66b	6.26a
Spike per plant	0.7-6	2.64a	2.71a	2.8-12.1	6.21a	6.34a	3.43b	6.63a
Spike length	4.9-9.8	6.79a	6.60a	5.5-10.4	7.57a	7.43a	6.72b	7.52a
Spikelet per spike	18.2-65.3	37.47a	41.15a	20.6-78.2	43.14b	50.73a	38.75b	45.79a
Kernel per spike	16.4-51.3	31.48a	33.65a	17.4-68.8	37.21b	42.32a	32.24b	38.99a
Kernel weight per spike	0.2-1.6	0.90a	0.70b	0.4-2.9	1.37a	1.27a	0.83b	1.33a
Kernel weight	9.1-41.1	29.90a	20.89b	14.9-59.2	38.36a	30.30a	26.75b	35.54a
Spike weight	0.4-1.8	1.08a	0.88b	0.6-3	1.55a	1.44a	1.01b	1.51a
1000 kernel weight	16-52	37.25a	27.83b	18-60	40.91a	31.34b	33.95b	37.56a
Hectoliter weight	42.7-66.7	57.82a	50.71b	37-64.8	58.81a	52.46b	55.33b	56.59a
Yield per plant	0.9-6.2	3.14a	2.30b	2.5-15.9	8.39a	7.18b	2.84b	7.97a
Yield per plot	139.6-539.6	377.03a	285.48b	252.8-816.9	551.14a	445.25b	344.98b	514.08a
Biomass	0.7-2.2	1.36a	1.33a	1.0-4.3	2.42a	2.30a	1.35b	2.38a

Table 3. Canonical loadings of the 17 measured traits on the first two canonical variables of barley accessions at Ambo and Asasa

Traits	Ambo		Asasa	
	Can1	Can2	Can1	Can2
Days to heading	0.5658	0.1939	-0.0721	-0.2385
Days to maturity	0.8871	0.1123	-0.3686	0.0331
Grain filling period	0.5113	-0.0836	-0.3092	0.3183
Plant height	0.0805	0.3944	-0.0139	-0.3479
Tiller plant^{-1}	0.0434	0.6182	-0.2146	0.0766
Spike plant^{-1}	-0.0113	0.5431	-0.2685	0.1442
Spike length	0.2837	0.4658	-0.0273	-0.2872
Spikelet spike^{-1}	0.1815	-0.1608	-0.1136	-0.1707
Kernel spike^{-1}	0.1847	-0.1839	-0.0797	-0.1373
Kernel weight spike^{-1}	0.3354	-0.2590	-0.0382	0.4235
Kernel weight	0.1735	-0.1004	0.0647	0.7144
Spike weight	0.3201	-0.2338	-0.0298	0.4209
1000 kernel weight	0.0493	-0.0541	0.1353	0.6031
Hectoliter weight	-0.1138	-0.2967	-0.0695	0.8247
Yield plant^{-1}	0.3259	-0.1095	-0.1335	0.4888
Yield plot^{-1}	0.0758	-0.1736	0.1452	0.4587
Biomass	0.1497	-0.0020	0.2295	0.2130
Canonical correlation	0.94	0.86	0.88	0.807
P level of significance	0.01	0.01	0.01	0.01
Variance accounted (%)	0.68	0.23	0.62	0.33

and second canonical variate with the accessions were $r_c = 0.94$ and $r_c = 0.86$, respectively (Table 3), which showed the first and the second canonical variates explained the differentiation of the accessions studied. At Asasa the canonical correlation between the first and the second canonical variates were significant $r_c = 0.88$ and $r_c = 0.80$, respectively (Table 3) which also indicates the first and the second canonical variates explained the differentiation of the accessions.

The proc cluster method of the SAS program was used to cluster the genotypes at each location using the Ward minimum-variance method (Ward 1963). The analysis grouped the 100 accessions into six and four clusters at Ambo and Asasa, respectively. The difference in the grouping pattern at Ambo and Asasa resulted due to the difference in the response of the accession at these locations (Table 2). The distribution of accessions at both locations showed that materials from ICARDA and landraces grouped in the same group which indicates the existence of genotype exchange programs between the Ethiopian barley breeding program and ICARDA. ICARDA is an international center for the barley research programs in the world. One of its objectives is to introduce barley accessions from the center of diversity in different parts of the world and develop new crosses for redistribution to countries in different parts of the world. Since Ethiopia is the center of diversity for barley and work in collaboration with ICARDA barley breeding program, the Ethiopian barley breeding program exchanges accessions with ICARDA. The cluster analysis also grouped six rowed with two rowed barleys. Even if only 35 accessions out of 100 are landraces from Ethiopia, the landraces distributed in all of the clusters formed which indicates the existence of high genetic variability among them. The high genetic variability within landrace lines of Ethiopia were also reported by Negassa (1985), Demissie and Bjørnstad (1996) and Allemayehu and Labuschagne (2004).

The Mahalanobis distance measures (D^2) which indicate the difference between the centroid values of two groups showed significant genetic distance between clusters at both locations (Table 4). At Ambo, high genetic divergence was observed between Cluster III, Cluster I and Cluster VI (Table 4, Figure 1). The genotypes in cluster VI had the highest mean value for all characters except plant height which is the medium plant height that contributes to the tolerance of lodging. At Asasa the highest genetic distance was observed between Cluster II, III and Cluster IV (Table 4, Figure 2).

Canonical discriminant analysis (CDA), a multivariate technique employed here, was efficient to study the genetic diversity and differentiation among barley genotypes. It also helped to identify traits contributing to the genetic differentiation among the genotypes studied. In addition this result showed the importance of a genetic diversity study before planning any crossing program. Since genotypes originating from different geographic locations may not always be genetically divergent and produce high potential segregate populations.

Table 4. Pairwise squared distance between cluster groups, as calculated by Mahalanobis D^2 Distance, at Ambo (above the diagonal) and Asasa (below the diagonal)

Cluster*	I	II	III	IV	V	VI
I	-	23.37	17.62	46.91	131.30	195.34
II	13.66	-	51.14	39.74	68.47	97.24
III	32.05	36.37	-	37.75	164.58	262.98
IV	30.99	74.10	52.30	-	73.38	182.43
V					-	60.82

*All the distance between cluster groups are significant ($P < 0.01$) at both locations.

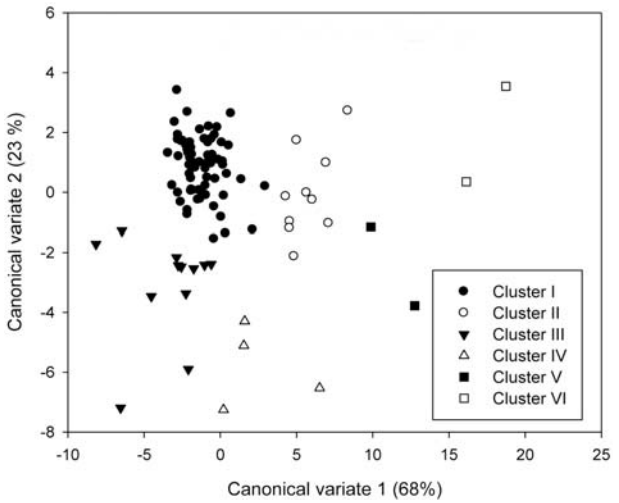

Figure 1. Scatterplot of the six clusters on the two canonical variates at Ambo.

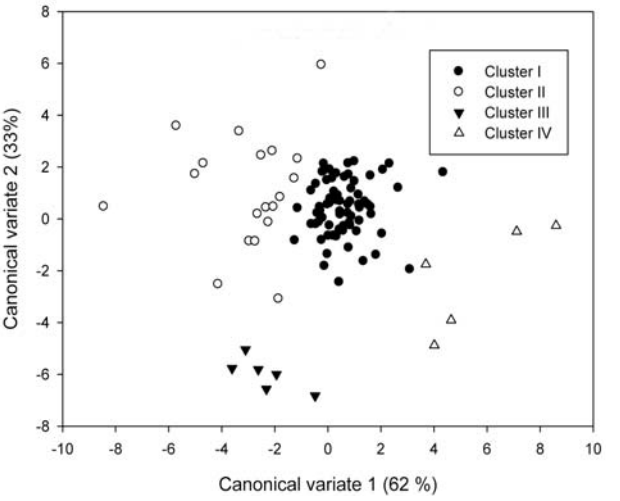

Figure 2. Scatterplot of the four clusters on the two canonical variates at Asasa.

Divergência genética entre acessos de cevada da Etiópia

RESUMO *– O objetivo deste trabalho foi acessar a diversidade genética existente entre os germoplasmas de cevada da Etiópia e ICARDA, usando análise multivariada. O experimento foi conduzido em Asasa e Ambo na Etiópia, no delineamento em látice simples 10x10, com duas repetições. Para quantificar a diferenciação entre os acessos foram utilizadas a análise canônica, análise de agrupamento e a distância de Mahalanobis (D^2). O estudo indicou que as duas primeiras variáveis canônicas explicaram 95% e 91% da variação total em Asasa e Ambo, respectivamente. Em ambos os locais, os acessos mostraram o máximo de diferenciação em dias para maturidade, período de enchimento de grãos, perfilhos por planta e espigas por planta. A análise de agrupamento agrupou os acessos em quatro grupos em Asasa e seis grupos em Ambo. Variedades crioulas da Etiópia e os acessos do ICARDA foram agrupados no mesmo grupo, indicando a troca de germoplasma entre os programas de melhoramento de cevada da Etiópia e ICARDA.*

Palavras chave: *Hordeum vulgare* L., variedades crioulas, variabilidade genética, análise multivariada.

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