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Evaluation of morphological diversity pattern in the Albanian barley (*Hordeum vulgare* L.) base collection

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Abstract

Barley (*Hordeum vulgare* L.) is an important winter cereal crop in Albania. A total of 20 accessions of barley part of base collection of Albanian Gene Bank, were object of the study for agro/morphological characteristics in Experimental field of Agriculture University in Tirana. Data on 16 traits (five quantitative and 11 qualitative) were recorded to assess diversity among the accessions. A range of variability was observed among the barley varieties for plant height trait (with an average of 102.2 cm), AGB 2326 resulted with the lowest value for spike length (±6.1 cm), AGB 2328 was the accession with the higher number of spikelet per spike recorded (±34.1). Morphological variation was observed for spike density, rachilla hair length, kernel covering, lemma color, 1000-grain weight trait, etcAbstract section.

Keywords: *Hordeum vulgare*, trait, diversity, morphological variation.

1. Introduction

Albania is an area rich in crop biodiversity, it is characterized by a high diversity of climatic and agronomic conditions. Barley (*Hordeum vulgare* L.) is one of the old winter cereals crop in our country, utilized for various purposes such as food, beverages and livestock feed. Based on the ability to tolerate cold climate the crop serves as an indicator of well-being on the community of remote area.

Barley is considered as one of the cereals with the most diversified genetic basis [4]. Morphological characterization is the foundation of genetic diversity research at any taxonomic level [6]. Genetic variability evaluation based on morphological characters of economic interest might be used to choose suitable materials in breeding programs for crop improvement. Also, in comparison to biochemical traits, morphological traits had crucial role in genetic study due to the ease of their identification, and their simple mode of inheritance in comparison to quantitative traits [12]. For that reason, markers to describe genetic diversity are morphological and agronomic traits celebrated as descriptors and presented by the International Plant Genetic Resources Institute (IPGRI) [9].

Agro-morphological characterization is still an important tool for the management of crop germplasm collections [2, 3], having been used to identify duplicates, to establish core collections, to investigate relationships between landraces and their wild, and to prioritize material for use in breeding programs. The characterization of accessions allows quantification and structuring of the genetic variability in the germplasm which is highly important for improvement programs and for the conservation and preservation of genetic diversity. To characterize a germplasm basically means to identify and describe differences between the accessions. Besides the information on the origin of the material (passport data) differences related to the agricultural performance of the accessions are normally also considered as well as botanical differences related to normally taxon-specific descriptors [14].Therefore a total of 20 barley accessions were planted under field condition and their agro morphological characters (5 quantitative traits) such us, plant height, spike length, 1000 kernel weight, days to maturity were recorded to evaluate morphological diversity.

2. Material and Methods

Plant Materials: Our study is based on the characterization of the diversity of 20 barley accession (*Hordeum vulgare* L.) part of the base collection of Albanian Gene Bank (Agriculture University of Tirana, Table 1).
Experimental site: The study was conducted in the Experimental Station of Institute of Plant Genetic Resources, Valias, Agriculture University of Tirana. It lies at an altitude of 40 m above sea level and at Latitude 41°24'6.14"N and Longitude 19°44'9.93"E.
Methods (Experimental Design): The plant material is characterized by a survey on land and laboratory, during the year 2016-2017 in the experimental field, Valias of the Agricultural University of Tirana. Each accession was planted in 1 m long plot with a between-row spacing of 25 cm. Fertilizer was broadcast on the plots at the rate of 400 kg ha-1, N.P.K. 8:16:20. At physiological maturity, seeds were harvested and after they were cleaned for analysis. During the crop year, the accessions were evaluated using 5 characters of quantitative type. These characters were: plant height (PH), Tillers per Plant (TP), Spike Length (SL), Number of Spikelet per Spike (NSpkSp) and Weight of 1000 Seeds (WS). Part of the field survey were phenotypic traits as: number of days from sowing to germination (SG), number of days from germination to spikelet (GSpk), days from germination to flowering (GF), number of days from germination to maturity (GM). Morphological characterization of the accessions was conducted according to international standards, particularly those of the International Plant Genetic Resources Institute [7].
Statistical analyses: After transferring data over a matrix, statistical tests were carried out by the Statistical Package for Social Sciences (version 21) and JPM. Analysis of variance (ANOVA) was used to calculate variation among accessions, using the JPM software. Hierarchical Ascendance Classification (HAC) or cluster analysis, was used to calculate the mean data of accessions using the SPSS software to better classify the 20 accessions of barley. Principal components analysis (PCA) was carried out on the correlation matrix calculating the mean data of the accessions.

Table 1. List of barley accessions, part of base collection of Gene Bank regenerated during 2016-2017

Nr.	***Accession code***	***Taxonomy name***	***Year of storage in Gene Bank***	***Year of regeneration***	***Experimental site***
1	AGB2273	*Hordeum vulgare* L.	31.03.00	2017	Valias,Tiranë
2	AGB2274	*Hordeum vulgare* L.	31.03.00	2017	Valias,Tiranë
3	AGB2275	*Hordeum vulgare* L.	31.03.00	2017	Valias,Tiranë
4	AGB2276	*Hordeum vulgare* L.	31.03.00	2017	Valias,Tiranë
5	AGB2277	*Hordeum vulgare* L.	31.03.00	2017	Valias,Tiranë
6	AGB2278	*Hordeum vulgare* L.	31.03.00	2017	Valias,Tiranë
7	AGB2279	*Hordeum vulgare* L.	31.03.00	2017	Valias,Tiranë
8	AGB2280	*Hordeum vulgare* L.	31.03.00	2017	Valias,Tiranë
9	AGB2281	*Hordeum vulgare* L.	31.03.00	2017	Valias,Tiranë
10	AGB2282	*Hordeum vulgare* L.	31.03.00	2017	Valias,Tiranë
11	AGB2283	*Hordeum vulgare* L.	31.03.00	2017	Valias,Tiranë
12	AGB2295	*Hordeum vulgare* L.	25.02.01	2017	Valias,Tiranë
13	AGB2296	*Hordeum vulgare* L.	25.02.01	2017	Valias,Tiranë
14	AGB2297	*Hordeum vulgare* L.	25.02.01	2017	Valias,Tiranë
15	AGB2298	*Hordeum vulgare* L.	25.02.01	2017	Valias,Tiranë
16	AGB2325	*Hordeum vulgare* L.	13.12.02	2017	Valias,Tiranë
17	AGB2326	*Hordeum vulgare* L.	13.12.02	2017	Valias,Tiranë
18	AGB2327	*Hordeum vulgare* L.	13.12.02	2017	Valias,Tiranë
19	AGB2328	*Hordeum vulgare* L.	13.12.02	2017	Valias,Tiranë
20	AGB2329	*Hordeum vulgare* L.	13.12.02	2017	Valias,Tiranë

3. Results and Discussion

Simple statistical analysis of the observed data showed a range of variability among the accessions of barley. The differences with high range and diversity indices were observed in Plant Height trait (82.6-117.9 cm), spike characters such as Spike Length (6.1-13.73 cm), and 50% of flowering days (135-153) in quantitative traits. The 20 accession of barley also showed variability in Number of Spikelets per Spike and Weight of 1000 grain (40.5-54.3g) traits (Table 2). Among the accessions AGB 2273 and AGB 2278 (Table 4) resulted with the lowest value for days to maturity (185). We observed the lowest value for days to germination (58) and days to spiklet formation (81) at AGB 2274. Barley genotypes AGB2274, AGB2275, AGB2276, AGB2277, AGB2297, AGB2325, AGB2326, AGB2327 presented all the same number of days to flowering (153 days).

In the present sudy we found that 10% of the plants have green stem pigmentation (Table 3) and 45% of them were purple (basal only), spike density was 5% for dense, 45% intermediate in agreement other authors [1,6]. Almost all barley genotypes were awned (90%), and 10% have smooth lemma bar.

Table 2. Data of quantitative traits characterization in twenty barley accessions

Accession code	***Quantitative traits***								
	PH/cm	TP	SL/cm	NSpS	WS/g	SG	GSp	GF	GM
AGB2273	97.4	3.3	8.27	31.9	40.76	61	86	142	185
AGB2274	99.9	2.6	8.35	25.9	50.43	58	81	153	188
AGB2275	98.9	2.9	8.15	28	49.3	61	93	153	188
AGB2276	105	2.9	10.52	27.4	50.1	61	86	153	188
AGB2277	107.8	2.8	10.71	32.3	50.4	61	86	153	188
AGB2278	107.4	2.6	11.87	32.6	40.9	61	94	142	185
AGB2279	112	2.5	13.73	31.8	50.4	61	94	142	188
AGB2280	111.8	2.8	10.59	32.6	40.8	61	94	142	193
AGB2281	82.6	2.8	8.56	27.3	40.5	61	86	135	186
AGB2282	98	2.6	9.18	28	44.7	61	94	135	186
AGB2283	116.5	3.7	9	28.1	52	61	94	135	186
AGB2295	106.5	3.5	9.8	29.6	46	61	94	142	188
AGB2296	88.3	3.6	9.4	26.6	50	61	86	142	188
AGB2297	109	3.7	11	28.4	53	61	86	153	188
AGB2298	117.9	3.8	12	31.4	54.1	58	81	142	188
AGB2325	93.5	3.8	7.25	17.1	50	58	81	153	193
AGB2326	89	3.7	6.1	17.2	53.7	61	81	153	193
AGB2327	96.3	3.8	7.65	18.2	54.3	61	86	153	193
AGB2328	114	4	11.9	34.1	53	61	88	135	188
AGB2329	92.2	3.3	7.6	29.4	50	61	86	142	196

Table 3. Percentage of Phenotypic classes of the qualitative characters used for diversity

Characters	*No. Class*	*Code*	*Classes*	*Frequency distribution*
Growth habit	3	3	Prostrate	0
		5	Intermediate	0
		7	Erect	100%
Stem pigmentation	3	1	Green	10%
		2	Purple(basal only)	45%
		3	Purple(half or more)	45%

Spike density	3	1	Lax	50%
		2	Intermediate	45%
		3	Dense	5%
Hoodedness/ awnedness	5	1	Awn less	0
		2	Awnleted	10%
		3	Awned	90%
		4	Sessile hoods	0
		5	Elevated hoods	0
Lemma awn bar	3	3	Smooth	10%
		5	Intermediate	30%
		7	Rough	60%
Glume and glume awn	5	1	Length of glume &awn shorter than kernel	45%
		2	Length of glume &awn as long as kernel	55%
		3	Glume plus awn longer than kernel	0
		4	Glume plus awn nearly twice as long as kernel	0
		5	Lemma like	0
Glume color	4	1	White	0
		2	Yellow	90%
		3	Brown	10%
		4	Black	0
Length of rachilla hairs	2	1	Short	70%
		2	Length	30%
Kernel covering	3	1	Naked grains	0
		2	Semi-covered grain	0
		3	Covered grains	100%
Grain color	3	1	white	0
		2	Tan/red	80%
		3	Purple	20%
Aleurone color	2	1	White	95%
		2	Blue	5

Table 4. Statistical parameters for 9 quantitative traits of barley

Characters	***Minimum***	***Maximum***	***Mean±SD***
Phenological			
SG days	58	61	60.55±1.09
GSp days	81	94	87.85±4.94
GF days	135	153	145±7.01
GM days	185	196	188.8±3.80
Morphological			
PH	82.6	117.9	102.2±10.07
TP	2.5	4	3.235±0.49
SL	6.1	13.73	9.5815±1.9
NSpS	17.1	34.1	27.895±5.03
WS	40.5	54.3	48.7195±4.73

Hierarchal clustering data: A pairwise association among accessions of barley was measured from the observed agro-morphological traits using Ward and Euclidean distance and revealed a clear clustering into different morphological groups (Table 5). The resulting dendrogram depicted in Figures 2 show the relationship between barley genotypes and grouped all 20 accessions into four distinct clusters. The number of the accessions per cluster varied from 3 in cluster IV to 8 accessions in cluster I. Accessions grouped in cluster I (8 acc.) are similar for trait as TP and WS. Barley 10 genotypes grouped in cluster II are similar for PH, TP and WS. Main text paragraph.

Table 5. Composition and characteristics of clusters based on five quantitative observed

Cluster	*Nr. of accessions*	*Cumulative characteristics of accessions in clusters*
I	8	Tillers per Plant and weight of 1000 seeds
II	5	Plant Height, Number of Spikelet per Spike & Weight of 1000 seeds
III	4	Tillers per Plant, Spike Length & Number of Spikelet per Spike
IV	3	Tillers per Plant, Number of Spikelet per Spike

The nearest neighbors clustered together with the lowest dissimilarity level (0.54) between them are AGB 2277 and AGB 2275. These two genotypes are fully similar for trait as TP and WS. This group is joined by two other accessions AGB 2296 and AGB 2329 similar in trait of Weight of 1000 Seeds (50 g). Accessions AGB 2280 and AGB 2278 part of 5 accessions grouped in cluster II, are considered as neighbors in trait as NSpS and WS but different in other traits. Joined to these two genotypes for similarity in Plant Height trait are AGB 2276 and AGB 2277. AGB 2298 and AGB 2328 are nearest grouped in cluster IV for having similarity in TP and SL traits. Accession AGB 2328 is identified as the most different from all the other accessions of barley. The dissimilarities with the other genotypes are based in the highest values for quantitative descriptors as TP (±4), NSpS (±43.1).

Accessions of the last cluster AGB 2325 and AGB 2327 have short distances in similarity basically on TP trait. AGB 2327 accession presented the highest value among the 20 barley genotypes part of the study, with the highest value of WS trait (±54.3g). The longest distance of dissimilarity (5.5) exist between AGB 2273 and AGB 2325 based on NSpS trait where the last accession present the lowest value (17.1) observed among 20 barley genotypes.

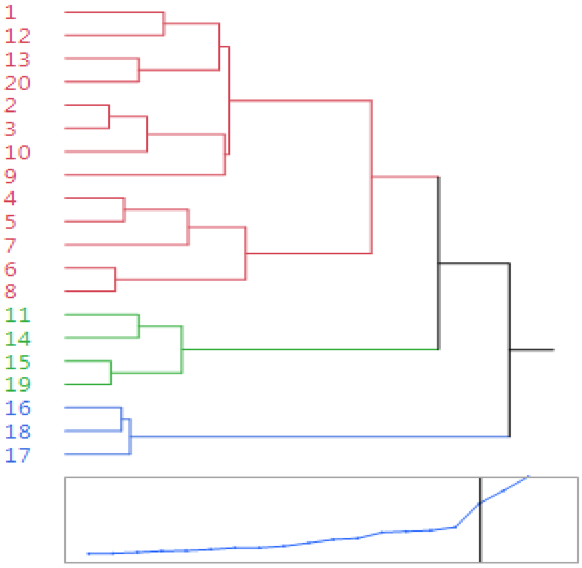

Figure 2. Dendrogram showing the patterns of relationships among 20 barley accessions based on five traits.

Principal components analysis (PCA) on correlations of quantitative morphological and agronomical traits identified the variances of the principal components (PC) and the proportion of the total variance accounted for by each factor (Table 6).

Table 6. Eigenvalues matrix of principal components analyzed

Nr.	Eigenvalue	Percent	Cum Percent	ChiSquare	DF	Prob>ChiSq
1	2.5029	50.057	50.057	45.841	9.250	<.0001*
2	1.6405	32.810	82.867	27.814	8.108	0.0006*
3	0.4630	9.261	92.128	4.396	5.489	<.0001*
4	0.2173	4.346	96.474	0.169	1.961	<.0001*
5	0.1763	3.526	100.000	0.000	0.008	<.0001*

χ^2 – Chi Square, DF – degree of freedom; Prob. – probability; **significance level 0.01 of probability

Comparing the eigenvalues for each factor using the minimum eigenvalue criterion [11]. The maximum information for analysis of the relationships among 20 barley accession and morphological traits was received using ordination methods (principal coordinate's analysis) in combination with cluster analyses [10]. PCA on correlations identified the total variance of the principal components (PC) and the proportion of the variances explained by each factor. All quantitative variables contribute to 100% of total variation. The first two PCs explain 82.8% > 65.0% and about equal to 70% of the total variation, acceptable for evaluation and characterization of ex situ collections in gene bank [8, 10]. The traits with more significant weighting on respective PC variance can be utilized successfully as quantitative markers for evaluation, characterization and classification of the cereal germplasm [6, 8] stored in gene bank, and in plant breeding programs [6, 8, 10].

As it is shown in Table 7, there exist positive correlations between Spike Length trait and Plant Height or SL and Number of spikelet/Spike too. Also positive relationship is found among Tillers per Plant and Weight of 1000 Seeds. The first two principal components with eigen value greater than unity explained 82.8% of the total variation among the studied barley accessions for the quantitative traits (Table 6). The first and second principal components accounted for 50.05% and 32.8% of the total variation. Plant height and Tiller per Plant were the most important traits contributing to the first principal components. Spike Length and grain weight were the important traits contributing to the second and third principal component. Scattered plot (Figure 3) of these genotypes clearly indicated that there is presence of variability.

Table 7. Principal components on correlations

	PH	TP	SL	NSpS	WS
PH	1.0000	0.0423	0.7415	0.5854	0.2010
TP	0.0423	1.0000	-0.2405	-0.3560	0.5698
SL	0.7415	-0.2405	1.0000	0.7353	-0.0320
NSpS	0.5854	-0.3560	0.7353	1.0000	-0.3723
WS	0.2010	0.5698	-0.0320	-0.3723	1.0000

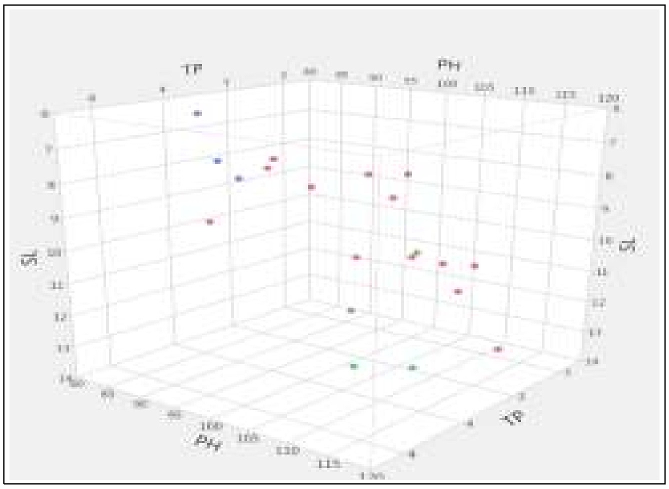

Figure 3. Scattered plot 3D of 20 barley genotypes

The studies on crop genotypes have shown the value and importance of agro-morphological characters with direct relevance to local farmers and breeders for conservation, and in estimating diversity of germplasm and in describing the level of discrimination of the varieties [5, 6, 7] have also shown how farmers select preferred maize types from their agro-morphological characters. Similarly [13] have shown how combining ethno-botanical methods with genetic analysis can give insights into how crop genetic diversity is maintained and managed. These descriptive values of morphological characters are genetically heritable and therefore worthy for the genetic diversity analysis.

From the analysis of the groups, the clustering pattern obtained showed an association with the agronomic performance of the accessions. This indicates that the classification can assist in discriminating the groups of material, which can be of future use for plant breeding programs.

This work has been a contribution to increase the knowledge about the barley germplasm conservation at Albanian Gene Bank (Agriculture University of Tirana). This better understanding should allow a better conservation and use of the collection in breeding programs. The research will also assist in the conservation of valuable germplasm, as is the case of local varieties, which hold important local adaptation and are of widespread use by farmers throughout the entire country.

4. Conclusions

Among barley (*Hordeum vulgare* L) genotypes object of this study, differences exist in phenotype and this suggest differences in genetic makeup. The differences with high range and diversity indices were observed in trait as Plant Height (82.6-117.9 cm), Spike Length (6.1-13.73 cm), and 50% of flowering days (135-153) in quantitative traits. The 20 accession of barley also showed variability in number of spikelet per spike and weight of 1000 seeds (40.5-54.3g) traits. Among the accessions AGB 2273 and AGB 2278 resulted with the lowest value for days to maturity (185). We observed the lowest value for days to germination (58) and days to spike formation (81) at AGB 2274.

The resulting dendrogram show the relationship between barley genotypes and grouped all 20 accessions into four distinct clusters. The number of the accessions per cluster varied from 3 in cluster IV to 8 accessions in cluster I. From the analysis of the groups, the clustering pattern obtained showed an association with the agronomic performance of the accessions. This indicates that the classification can assist in discriminating the groups of material, which can be of future use for plant breeding programs.

The study identified the agronomical traits with more significant weighting on PC variance (PH & SL significant at the probability F < P0.01). The significant differences found in the present study show the existence of a high genetic variability among the barley genotypes and quantitative traits analysed, sufficient for selection of desirable traits, and creation of new favourable gene combinations. This work has been a contribution to increase the knowledge about the barley germplasm conservation Albanian Gene Bank (Agriculture University of Tirana). This better understanding should allow a better conservation and use of the collection in breeding programs. The research will also assist in the conservation of valuable germplasm, as is the case of local varieties, which hold important local adaptation and are of widespread use by farmers throughout the entire country.

6. References

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