Evaluation of cultivated and wild barley cultivars affinities using micro and macro-morphological traits of grain, pollen, and stomata

Sayyedh Masomeh Hosseini [1]*, Mahlagh Ghorbanli [2] and Hossein Sabouri [3]

1. Department of Biology, Faculty of Science, Islamic Azad University, Gorgan Branch, Gorgan, Iran
2. Department of Biology, Faculty of Science, Islamic Azad University, Gorgan Branch, Gorgan, Iran
3. Department of Plant Production, Faculty of Agriculture, Gonbad Kavous University, Gonbad Kavous, Iran

Abstract

In an ongoing research, 24 cultivated and wild cultivars of barley were evaluated for morphological characteristics of grain, pollen, and stomata. Traits of interest included length, width, and area. Results of variance analysis showed that all samples were differed in traits of stomata, grain, and pollen at probability levels of 1 and 5%, suggesting remarkable genetic variation among studied samples. According to cluster analysis, morphological traits of pollen and stomata fell into three cluster and those for grain were categorized in two clusters. Genotypes for arid area like Izeh, Maravetapeh, Kavir, and Nimrooz showed higher levels of stomata densities and area compared to those for cold area like Bahman and A1C84-14. Here, affinity rate of 24 barley cultivars were evaluated using micro and macro-morphological traits as those rare-studied ones.

Keywords: cluster analysis; affinity; micro and macro morphology traits

Hosseini, S. M., M. Ghorbanli and **H. Sabouri.** 2014. 'Evaluation of cultivated and wild barley cultivars affinities using micro and macro-morphological traits of grain, pollen, and stomata'. *Iranian Journal of Plant Physiology* 4 (4), 1119-1127.

Introduction

Barley, a member of the grass family (Poeace), genus Hordeum and species Vulgare or Sativum, is a major cereal as a component of various healthy foods. Morphology of 3 species Desf. Q.dilatataRaf, Q. incanaRoxb, and Q. ballota were studied and these species were distinguished in pollen type, pollen size, and pollen decorations (Syed Tarigh et al., 2005). Classification of different cultivated and wild crops in terms of plant systematicity is found to be one of the earliest actions taken by botanists to ascertain affinities and association among them. Sheikh Akbari and Azizian (2006) evaluated 15 seeds of Epilobium L using TME and SME. In recent years, genetic variations in wide varieties of crops have been subject to great losses mostly due to exploitation on improved and uniform cultivars. Hence, evaluation of germplasm genetic variation is proven to be vital to maintain genetic variation in gene pools and germplasms as well as breeding programs and using them to gene incorporation (Omidbakhesh Fard, 2005). Estimating genetic variation among cultivated crops contributes substantially to breeding programs efficiency and genetic materials conservation. To this end, morphological traits

*Corresponding author
E-mail address: hosseinim@gmail.com
Received: November, 2013
Accepted: July, 2014

are suitable and satisfying. The increased crop production and higher yield quality as well as efficient gene resource utilization entails for collecting, conserving, describing, and eventually evaluating genetic materials. At the same time, developing genetic variation, ideotypes, and new germplasm in wheat breeding and improving yield traits have great deal of importance (Pearce et al., 2000). Akgul et al. (2008) studied seeds and pollens of 19 species of Marrubium L using TME and SME. they concluded that grains morphological traits are useful for classification. Similarly, grain number varies in planting dates and locations, fertilization, and number of wrinkled hollow grains. Number of grain per spike in barley varies 30-40 and 16-28 under typical and adverse conditions, respectively (Emam, 2004). Understanding ecological demands and genetic variation levels using appropriate methods is essential to conserve genetic resources as a suitable classification criterion to screen and to identify different species of the same genera. Stomata morphology has been utilized for many years (Prabhaker, 2004). Micro and morphological traits in seeds of Egyptian acacia were investigated to identify and distinguish different species and 12 species along with key traits were released (Al- Gohary and Al-Mohamed, 2007). Palynology is the study of pollens and spores. Indeed, pollen morphology is an important part of plant taxonomy, phylogeny, and paleontology. The fact that pollen must disperse to be efficient has made them to be similar architecturally (Zarafshar, 2009). Pollen has short longevity in grasses, with a life span varying from few minutes to several hours. Kermian et al. (2009) studied pollens in 20 species of Onobrychis and Heliobrychis in Iran. Eight pollen traits were evaluated using stolise and SME methods. Clustering and principle component analysis were used for data analysis. Chenki et al. (2007) used stomata type in taxonomical clustering of Camellia L. pollen and leaves traits applied to classify oak species using SME and TME (Panahi et al., 2011). Hamza et al. (2004) used 12 agronomy traits while evaluating variation among 26 Tunisian winter barley cultivars followed by principle component and cluster analysis on traits and genotypes. Cluster analysis is commonly used to classify plant varieties geographically and genetically and subsequently to determine parents in hybridization. Arzani, (2002) used cluster analysis to classify 450 varieties on which total germplasm was categorized into 17 different clusters. This study was aimed to evaluate barleys genetic variation and classify them on the basis of morphological data. In addition, it seeks to identify verities with more genetic distance and higher heterosis as results of their crosses. Classification and determination of genetic variation will be more facilitated and time-effective. Considering, wide varieties of barley uses as a main crop in Iran as well as importance of its different species taxonomy and variation, the present research was aimed to determine the affinities of barley varieties using grain, stomata, and pollen traits.

Materials and Methods

In the present study, affinity for 24 barley varieties (Table 1) was evaluated by means of micro and macro-morphological traits of grain, stomata, and pollen. The varieties were arranged in completely randomized block design in three replications in the research farm of Gonbad Kavoos University on November 2011. For this, first 10 barley plants were randomly selected followed by cutting each plant on peduncle. Peduncle length was measured by a ruler. Spike length was measured as the distance of peduncle node to top of the longest spike by electronic digital calipers. To measure grain length and width, first grains were separated from spike and then electronic digital calipers were used to continue measuring. Digital balance with precision 0.001 was used to weigh grains. To measure micro morphological traits of pollen grains, first pollens were dissected by means of a needle under loop and an amount of pollens were placed on white paper. Pollens immersed in stolise solution (sulphuric acid: anhydride acetic, 8:2). After taking images on specimens, pollen features such as length, width, area, and two layer thicknesses were measured using Axiovision 4 software. To evaluate stomata micro morphological traits, after boiling leaf specimens for 10 minutes, epidermis innermost layers were separated by a cutter to extract chlorophyll using

Table 1
Studied varieties pedigree

No	variteis	pedigree	Spike arrangement	Genera/species
1	Yusef Cultivar	Yousef	Six	*Hordeum vulgare*
2	Izeh Cultivar	Izeh	Six	*Hordeum vulgare*
3	EM81-12 line	Comp 89-9C r-79-07 / Atem // (Alpha/HC1905//Robur)/3/	Six	*Hordeum vulgare*
4	EC83-4line	L.131/Gerbel//Ager-Ceres / 3 / (Scotia/Wa...)	Six	*Hordeum vulgare*
5	Nimrooz cultivar	Nimrooz	Six	Hordeum vulgare
6	Kavir cultivar	Kavir	Two	*Hordeum vulgare*
7	Productivecultivar	Productive	Six	*Hordeum vulgare*
8	Bahman cultivar	Bahman	Six	*Hordeum vulgare*
9	MaravehtapehPupulation sample	Maravehtapeh	Six	*Hordeum spontaneum*
10	TillabadPupulation sample	Tillabad	Six	*Hordeum spontaneum*
11	Khorasan Razavi Pupulation sample	KhorasanRazavi	Six	*Hordeum spontaneum*
12	MB-82-12 line	MB-82-12	Two	*Hordeum vulgare*
13	EB-86-14line	(Bllu/Mja)	Six	*Hordeum vulgare*
14	EB-86-6 line	(Alanda/Hamra//Alanda-01)	Six	*Hordeum vulgare*
15	EB-86-4 line	(Arabian Barley/3/Aths//Md.AT1)	Six	*Hordeum vulgare*
16	EB-86-3line	(Productive//As46/Aths)	Six	*Hordeum vulgare*
17	KarajTN2173 population sample	TN2173	Two	*Hordeum bulbosum*
18	Karaj76063population sample	76063	Two	*Hordeum bulbosum*
19	KarajTN49402 population sample	TN49402	Six	*Hordeum spontaneum*
20	KarajTN55502 population sample	TN55502	Six	*Hordeum spontaneum*
21	FI CC 0598line	COPAL/cl14230/lignee527/4Delo/CARDO/4/CABUYA	Six	*Hordeum vulgare*
22	BF891M-584line	Rojo/3/Beecher//POST/3/ROBUST//COME/3/CIRU	Six	*Hordeum vulgare*
23	ALISOS/CI03909-2(F1)Cross	ALISOS/CI03909-2	Six	*Hordeum vulgare*
24	A1C84-14line	Narcis//k-281/skorobod	Six	*Hordeum vulgare*

distilled and NaClO treatments. Finally, suitable microscope slides and cover glass were applied for evaluation. Stomata attributes such as length, width, density, and area were measured using Axiovision 4 software when magnified through optical microscope. Variance analysis and mean separation were carried out using SAS software. NTSYSpc 2.25 and SPSS (16.0), 2007 were utilized for cluster and principle components analyses, respectively. In addition, to separate cluster analysis dendrogram, cutting line was determined by SAS software and CCC plot.

Results

Grains macro-morphological traits

Variance analysis on grain traits (Table 2) showed that grain perimeter and weight differed significantly ($p \leq 0.01$) whereas this was the case for grain width at probability level of 5%. It can be concluded that there is enough genetic variation in traits of grain perimeter, width, and weight in varieties of interest. Coefficient of correlation in traits showed that grain length correlated positively with its width (0.734) (Table 3) suggesting that those sample varieties with

Table 2
Variance analysis of the studied traits

Sources of variation	Degree of freedom	Mean squares			
		Grain Circumference	Grain length	Grain width	Grain weight
Block	2	13.31	0.6	0.47	0.0000008
Genotype	23	**48.22	2.14	**0.47	**0.000013
Experimental error	46	9.03	2.5	0.23	0.000002
Coefficient of variation		11.95	15.01	14.11	25.5

**,* represent significant at probability levels of 1% and 5%, respectively.

long grain had also wide grains. Grain length and width correlated with grain perimeter significantly. Grain parameter is affected by grain dimensions. Coefficient of variation showed that the highest and lowest coefficients were related to grain weight and perimeter, respectively.

Mean separation of the interested traits indicted that EC83-4 line had the lowest grain length and width while the highest grain length and perimeter were recorded in Tillabad population samples. The least and highest grain weight was attributed to Kavir and Nimrooz cultivars respectively. FICC0598 line had the lowest grain perimeter. The widest grains were found for Maraveh Tapeh varieties. Tillabad varieties did not varied compared to others except FICC0598 (Table 4).

Cluster analysis deals with the classification of varieties to discriminate genetic affinity and relationship. When dendrogram from 5 units distance (Fig. I) was separated, barley varieties fell into two clusters. The first cluster included 15 varieties of Nimrooz, Productive, Bahman, Izeh, Kavir, Yusef, ALISOS/CI03909-2(F2), EB-86-3, EB-86-6, MB-82-12, BF891M-584, EB-86-14, A1C84-14, and FICC0598. The second cluster included 9 varieties of Maraveh Tapeh, Karaj varieties 76063, Tillabad, Khorasan Razavi, Karaj varieties TN49402, TN2173TN2173, Karaj varieties TN55502, EM81-12, and EC83-4 lines.

Variance analysis of traits indicated that traits of stomata length, width, area, and density differed significantly among all samples at probability level of 1%. Therefore, it can be concluded that there is enough genetic variation among varieties. The highest and lowest coefficients were related to stomata width and length (Table 5). Coefficient of correlation in

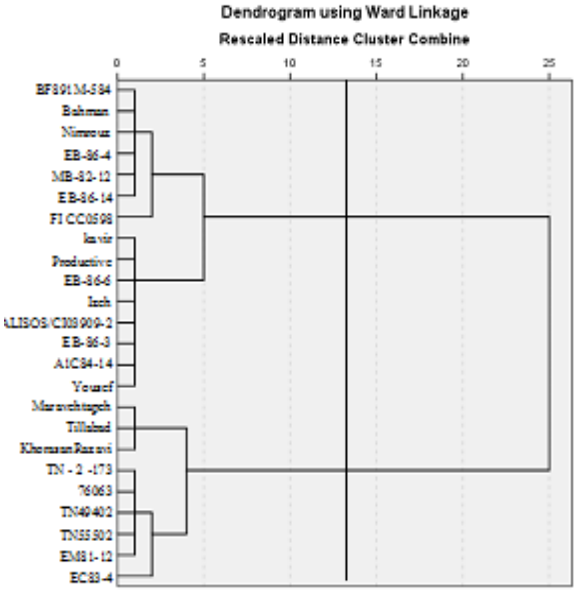

Fig. I. Dendrogram of cluster analysis based on the studied grain traits average

Table 3
Correlation of the studied traits

Traits	Grain Circumference	Grain Length	Grain Width	Grain Weight
Grain Circumference	1+			
Grain Length	0.568**	1+		
Grain Widtht	0.659**	0.734**	1+	
Grain Weight	-0.05	0.347	0.01	1+

** and * represent significant at $p \leq 0.01$ and $p \leq 0.05$, respectively.

traits showed that stomata width and area correlated significantly to its length. Stomata density also correlated with its area significantly

Table 4
Mean separation analysis of the interested traits

Samples	Grain Circumference	Grain Weight
Sample population Marave Tappe	abc 3.65	3.72abc
Yousef Cultiver	24.24defghi	3.46abcd
population TN2173 karaje sample	23.62efghi	3.46abcd
Cultiver Izeh	28.93abcd	3.91ab
Line EM81-12	21.24ghi	3.47abcd
Line A1C84-14	28.45bcde	3.92ab
ALISOS/CI03909-2(F1) cross(F1) ALISOS/CI03909-2	20.56ghi	3.02cde
population 76063 karaje sample	19.48ij	3.20bcd
Line EB-86-6	25.09defgh	3.40abcd
Line EB-86-3	27.51bcdef	3.34abcd
sample population Til Abad	27.20bcdefg	3.56abcd
Line FI CC0598	27.08cdefg	2.33e
Line EB-86-4	24.03defghi	3.40abcd
Line EC83-4	32.11ab	4.09a
Cultiver Nimrouz	22.48ghi	3.21bcd
Cultiver Kavir	23.44fghi	3.44abcd
Line MB-82-12	24.06defghi	3.44abcd
Line EB-86-14	33.48a	4.01a
Line BF891M-584	28.50bcde	3.76abc
sample population Khorasan Razavi	22.08hi	2.88de
Cultiver Productive	25.05defgh	3.47abcd
Cultiver Bahman	24.74defgh	3.40abcd
sample population TN49402karaje	22.44ghi	3.19bcd
sample population TN55502 karaje	16.74j	2.87de

- Columns with common letters represent non-significance at probability level of 5% according to LSD test.

as stomata area is not affected by stomata dimension (Table 6).

Results of mean separation showed that Izeh and Bahman cultivars had the highest and lowest stomata width among others. The highest stomata density was recorded for EM81-12 line. Karaj population sample TN2173 and Izeh cultivar showed the longest and shortest stomata. Bahman cultivar and A1C84-14 line exhibited the lowest stomata density. Karaj population sample 76063 and EM81-12 line showed the highest and least stomata areas, respectively (Table 7).

Dendrogram analysis (Fig. II) categorized barley varieties into three different classes. The first cluster involved 8 varieties of Productive, Bahman, Nimrooz, EB-86-4, EM81-12, FICC0598, A1C84-14, and ALISOS/CI03909-2 (F1) cross. The second cluster included cultivars of Izeh, Yusef, Kavir, EB-86-3, EB-86-6, EC83-4, EB-86-14, BF891M-584, and MB-82-12 lines. Finally, the third cluster included seven varieties of Tillabad, Khorasan Razavi, Maraveh Tapeh, karaj population sample 76063, TN49402, TN55502, and TN2173.

Pollen micromorphological traits

Variance analysis of pollen traits showed that pollen length differed at probability levels of 1% and 5% and its width, area, and two layers

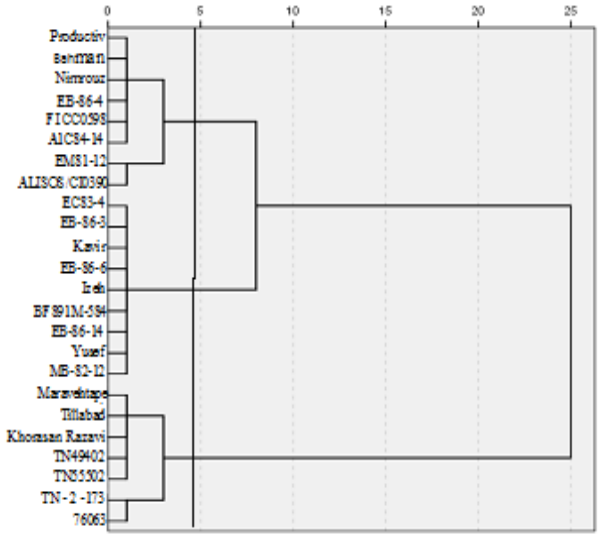

Fig. II. Dendrogram of cluster analysis based on the studied grain stomata traits

Table 5
Variance analysis of stomatal traits

Sources of variation	Degree of freedom	Mean squares			
		Stomata length	Stomata width	Stomata area	Stomata density
Block	2	13.06	1585	2703.22	*0.55
Genotype	23	**200.87	**74.46	**33832.79	**0.64
Experimental error	46	19.72	20.38	3547.69	0.14
Coefficient of variation		9	33.55	9.55	18.45

**,* represent significant at probability levels of 1% and 5%, respectively.

thickness varied at probability level of 5%. Coefficients of variation indicated that the highest and lowest values belonged to two pollen layers thickness and length (Table 8). Pollen area correlated significantly with its length and width (0.851 and 0.837) (Table 9). As pollen length and width increased, so did its area. Pollen width related positively with its length (0.441).

Mean separation analysis of traits showed that FICC0598 line had the lowest pollen length, width, and area among others. Population sample of Maraveh Tapeh showed the longest pollen. Khorasan Razavi varieties had the highest pollen area and width. EC83-4 line showed the highest two pollen thickness while the least was for Izeh cultivar (Table 10).

Dendrogram analysis (Fig. III) categorized barley varieties into three different classes. The first cluster included twelve varieties of Yusef, Izeh, Nimrooz, Bahman, Productive as well as BF891M-584, EM81-12, A1C84-14, MB-82-12, EB-86-4, EC83-4, and EB-86-3 lines. The second cluster contained lines FICC0598, EB-86-6, EB-86-14, Kavir, and ALISOS/CI03909-2(F1) cross. The third one accounted for Karaj population samples of TN55502, 76063, TN2173, TN49402, Maraveh Tapeh, Tillabad, and Khorasan Razavi.

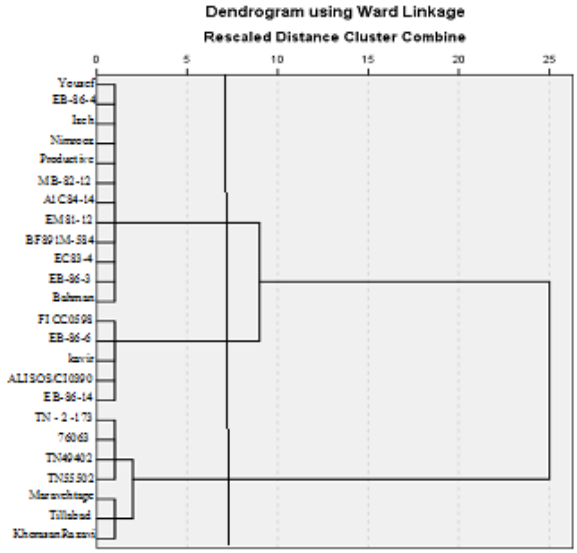

Fig. III. Dendrogram of cluster analysis based on the studied grain pollen traits

Table 6
Correlation of stomata traits

Traits	Stomata length	Stomata Width	Stomata Area	Stomata Density
Stomata length	1+			
Stomata width	0.611**-	1+		
Stomata area	0.633**	0.134	1+	
Stomata density	0.385-	0.001-	0.436*-	1+

**,* represent significant at probability levels of 1 and 5% respectively

Discussion

Stomata morphology can be used as criteria for classification of different types of a genus for years (Prabhaker, 2004). Warm season varieties of Izeh, Marave Tapeh, Kavir, and Nimrooz showed increased stomata density and area. When temperate varieties shorten their vegetative period due to water limitation, stomata density and area increase to terminate vegetative period. Cold season varieties of Bahman and A1C84-14 line exhibited low stomatal density and area. The varieties can prolong their vegetative period because of low

Table 7
Mean separation of stomata traits

Samples	Stomata Length	Stomata Area	Stomata Density
Yousef Cultiver	47.09fg	1.05f	661.21 cdefghi
Cultiver Izeh	23.86i	1.92 cde	606.18 hgiklj
Line EM81-12	36.85 h	3.04a	431.98n
Line EC83-4	48.85defg	2.33 bc	590.15 hikljm
Cultiver Nimrouz	42.94 gh	2.33 bc	549.32kljm
Cultiver Kavir	47.13fg	2.92 ab	613.48 fhgiklj
Sample population Marave Tappe	51.04 bcdef	1.62 def	740.83 bc
sample population TN55502 karaje	58.09 ab	1.97 cde	694.4 bcdefg
population TN2173 karaje sample	64.1a	1.48 ef	787.92 ab
Cultiver Productive	50.33 cdef	2.33 bc	570.14 ikljm
Cultiver Bahman	52.29bcdef	1cde	570.11 ikljm
Line FI CC0598	46.19 fg	2 cde	520.59 lmn
population 76063 karaje sample	54.45 bcde	1.58 def	871.43a
Line BF891M-584	52.71 bcdef	1.88 cde	636.64 defghij
sample population Til Abad	55.16 bcde	1.72 cde	733.47 bcd
cross(F1) ALISOS/CI03909-2	38.2h	3a	434.9 n
sample population Khorasan Razavi	56.82 abc	1.87 cde	717.43 bcde
sample population TN49402karaje	56.09 bcd	2 cde	708.26 bcdef
Line MB-82-12	54.57 bcde	2 cde	674.44 cdefgh
Line EB-86-14	54.92 bcde	2 cde	629.34 efghkij
Line EB-86-6	50.36 cdef	1.68 de	602.7 hgklij
Line EB-86-4	46.82 fg	2 cde	536.64 klm
Line EB-86-3	47.92 efg	2.12 cd	594.93 hklijm

Columns with common letters represent non-significance at probability level of 5% according to LSD test.

Table 8
Variance analysis of pollen traits

Sources of variation	Degree of freedom	Mean squares			
		Pollen length	Pollen width	Pollen area	Thickness of two layer of pollen
Block	2	29.27*	7.47	103532.80	0.06
Genotype	23	**27.11	*24.99	*107691.30	*0.08
Experimental error	46	11.04	13.53	48252	0.04
Coefficient of variation		6.77	8.39	12.77	14.59

**,* represent significant at probability levels of 1% and 5%, respectively

temperature and more moisture retention condition. Hence, stomata density is reduced. This means that plant can sequestrate more carbon.

Clustering analysis using grain associated traits showed that in the first cluster, all varieties in six-row arrangements of morphological traits were from H. vulgare. MB-82-12 and two- row arranged spike cultivars were from H. vulgare. The second cluster included six-row spike varieties of Maraveh Tapeh, Tillabad, Khorasan Razavi, and TN55502 Karaj sample population from *H. Spontaneum*. Two-row spiked varieties of Karaj sample populations TN2173 and 76063 were tracked to *H. spontaneum*. Also six-row spiked Karaj sample population was for *H. spontaneum*. *H. vulgare* involved six-row spiked lines EM81-12 and EC83-4. Clustering analysis using stomata associated traits showed that all six-row spiked varieties belonged to *H. vulgare*. The second one showed six-row spiked varieties from *H. vulgare*. The third one was from *H.*

spontaneum and *H. bulbosum.* In this cluster, six-row arranged spike population samples of Tillabad, Khorasan Razavi, Maraveh Tapeh and population samples TN55502 were from *H. spontaneum*. Tow-row spiked Karaj samples of TN2173 and 76063 were from *H. bulbosum* and six-rowed spike TN49402 was from *H. spontaneum.* Clustering analysis using pollen associated traits showed that all six-row spiked varieties in first class belonged to *H. vulgare* except MB-82-12 which was from *H. vulgare (*tow-row spiked). All six-rowed varieties in the second cluster were from *H. vulgare*. The third cluster belonged to wild species where varieties of Khorasan Razavi, Tillabad, Maraveh Tapeh, and six-row spiked TN55502 were tracked to *H. spontaneum*. Karaj population sample of six-row spiked TN49402 was from *H. spontaneum. H. Bulbosum* involved karaj population samples two-row spiked TN2173 and 76063.

Table 9
Correlation of pollen traits

Traits	Pollen Length	Pollen Width	Pollen Area	Thickness of Two Layers of Pollen
Pollen Length	1+			
Pollen Width	0.441*	1+		
Pollen Area	0.851**	0.837**	1+	
Thickness of Two Layers of Pollen	0.032	0.041	0.084	1+

**,* represent significant at probability levels of 1% and 5%, respectively.

Table 10
Mean separation of pollen traits

Samples	Pollen Length	Pollen Width	Pollen Area	Thickness of Two Layers of Pollen
Yousef Cultivar	51.35ab	42.43bcdefg	1696.8bcdefghi	1.16gh
Cultivar Izeh	48.67abcd	44.13abcdefg	1696.9bcdefghi	1.08h
Line EM81-12	48.65abcd	41.7cdefg	1606.9defghi	1.32bcdefgh
Sample Population Khorasan Razavi	53.51a	49.14a	2091.3a	1.22defgh
Line EC83-4	51.42ab	41.46defg	1757.3abcdefgh	1.66a
Cultivar Nimrouz	44.43d	47.55abc	1690.8bcdefghi	1.56abc
Line MB-82-12	48.23abcd	42.66bcdefg	cdefghi 1663.2	1.5abcde
Line EB-86-14	45.52cd	41.34defg	1471.5ghi	1.25 cdefgh
Sample Population Maraveh Tapeh	53.68a	47.58abc	2026.3ab	1.1gh
Cultivar Kavir	47.22bcd	40.86defg	1512.7ghi	1.26 cdefgh
Cultivar Productive	47.03bcd	45.38abc	bcdefghi 1685.9	1.4 abcdefg
Cultivar Bahman	48.44abcd	45.91abcde	1752abcdefgh	1.3bcdefgh
Line FI CC0598	44.1d	39.38g	1377.5i	1.18 fgh
Population TN2173 Karaj Sample	50.29abc	46.71abcd	1874.4abcde	1.24 defgh
Line BF891M-584	47.27bcd	bcdefg 42.38	1612defghi	1.52 abcd
Sample Population TN49402 Karaj	51.29ab	45.45abcdef	1852.9abcdef	1.61 ab
Line EB-86-6	44.4d	39.79g	1398.7hi	1.21 defgh
Line EB-86-4	52.05ab	40.33efg	1697.4bcdefghi	1.36 abcdefgh
Line EB-86-3	51.04ab	42.74bcdefg	1760.8abcdefg	1.3 bcdefgh
Population 76063 Karaj Sample	52.99a	44.45abcdefg	1893.4abcde	1.34 bcdefgh
ALISOS/CI03909-2(F1)	46.81bcd	41.27defg	1540.3efghi	1.2 efgh
Line A1C84-14	47.18bcd	44.98abcdefg	1671.2bcdefghi	1.39 abcdefgh
Sample Population Tillabad	53.47a	46.9abcd	2001.6abc	1.49 abcdef
Sample Population TN55502 Karaj	49.94abc	48.29ab	1938.6ab	1.21 defgh

Columns with common letters represent non-significance at probability level of 5% according to LSD test.

In the present research, three morphological traits of grain, pollen, and stomata in all wild species fell into the same group using cluster analysis. While in cluster analysis based on pollen and stomata related traits, all six-rowed spikelet samples fell into the same class except Kavir cultivar belonging to *H. vulgare* with two-rowed arrangement. It was found that pollen and stomata traits are promising to appropriate barley clustering. Panahi et al. (2011) confirmed importance of such traits to classify oak species in Iran. Kermian et al. (2009) while studying pollen traits of 20 species of *Onobrychis* and *Heliobrychis* concluded that pollens are useful for grouping this valuable genera.

References

Akgul, G., O. Ketenoglu, N. M. Pinar and **L. Kurt.** 2008. 'Pollen and Seed Morphology of the Genus Marrubium (Lamiaceae) in Turkey'. *Journal of Annales Botanici Fennici*, 45: 1-10.

Al- Gohary, I and **A. H. Al- Mohamed.** 2007. 'Seed morphology of *Acacia* in Egypt and its taxonomic significance'. *International Journal of Agriculture & Bioligy*, 9 (3): 435-438.

Arzani, A. 2002**.** 'Grain yield performance of durum wheat germplasm under Iranian dry land and irrigated Field conditions'.*Sabrao Journal of Breeding and Genetics,*34:9-18.

Chengqi, A., Y. Chuangxing and **Z. Hongda.** 2007. 'A systematic investigation of leaf epidermis in *Camellia* using light Microscopy'. *Biologia*, 62:157-162.

Emam, E. 2004. 'Cereal Grain Crops'. Shiraze university Publhshers. (In Farsi).

Hamza S., W. B. Hamida., A. Rebai and **M. Harrabi.** 2004'. SSR-based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits'. *Euphytica*, 135: 107-118.

Kermian, R. , **A. Moradi-bhjo** and **M. Ranjbar.** 2009**.** 'Pollen morphology in of *Onobrychis Miller* (Sect. Heliobrychis) species in Iran'. *Journal of Taxonomy and Biosystematics*, 1(1):63-72.

OmidbakhshFard, M. A. 2005. 'Staudy of genetic diversity in durum wheat using SSR marker'. MSc Thesis. Tehran University. Tehran, Iran.

Panahi, P., M. R. Pourmajidian., Z. Jamzad and **A. Fallah.** 2011. 'Importance of micromorphological characteristics of foliar and pollen grains for delimitation of Oak species in Iran'. *Iranian Journal of Forest and Poplar Research*, 19(1):163-179

Pearce, S. R., M. Knox., T. N. H. Ellis., A. J. Flavelland and **A. Kumar.** 2000. 'Pea Ty1-copia group retrotransposons: transitional activity and use as markers to study genetic diversity in *Pisum mulcular'. Gen Genetics* 263: 898–907.

Prabhaker, M. 2004. 'Structure, delimitation, nomenclature and classification of stomata'. *Acta Botanica Sinica* 46:242-252.

Sheikh Akbari, R and **D. Azizian.** 2006. 'Seed morphology and seed coat sculpturing of *Epilobium* L. species (*Onagraceae Juss*.) from Iran'. *Turkish Journal of Botany*, 30: 435-440.

Syed Tarigh, S., A. Habib and **Z. Roshan.** 2005. 'Pollen morphology of three species of *Quercus* (Family Fagaceae)'. *Journal of Agriculture Social Sciences*, 4:359-360.

Zarafshar, M., M. Akbarinia., H. Yosefzade and **A. Sattarian.** 2009. 'The survey of diversity in leaf and fruit morphological characters of *Celtisaustralis* in various geographical conditions. *Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research,* 17(1):123-134.