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Comparative Analysis of Physicochemical Traits in Some Steptoe × Morex DH Lines and Some Persian Varieties of Barely

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Abstract
This research was done to compare 15 double haploid lines and 15 Persian varieties by measuring physicochemical properties of barley seeds. Seven properties that were related to high quality malting, were studied in a Completely Randomized Design (CRD) using 3 replications, at the university of Tehran in 2012. There was negative correlation between protein content and malt extract, but the correlation α-amylase activity, diameter and length of grain were not significant. Principal components analysis classified seven physicochemical properties in to 2 new components. The first component explained 45.97% of total variations that mainly included diastatic power(°L), malt extract(%), α-amylase activity, protein content, kernel weight and grain diameter. The second component explained 18.56% of total variations that mainly included grain length. Analysis of these clusters showed that most of DH lines and some of persian varieties such as Dasht,Valfajr, Sahar and Kavir were suitable for production high quality malting processes.
Key words: α-amylase activity, Cluster analysis, Diastatic power, Malt extract, QTL.

Introduction
A major target for molecular breeding in barley (*Hordeum vulgare* L.) is malting quality, because just as grain yield is money to the farmers, malt extract is money to the maltsters & brewers. In the last 50 years, malt extract has steadily increased from around 75% to roughly 82% and we are now fast approaching the upper limit, estimated to be about 85% (Wright 2000). Today, the goal of barley breeding is to produce high grain yield with a good malting quality (Celus et al., 2006). Different pieces of research have used Quantitative Triat Louci (QTL) analysis for different criteria of Morex and Steptoe population such as malt quality (Ayoub et al., 2003), yield and its components (Peighambari et al., 2005), seed quality (Abdel-Haleem , 2004;Han et al, 2003; See et al, 2002), forage quality (Taleei et al, 2009; Siahsar and Narouei, 2010) and environmental adaptation (Mickelson et al, 2003; Kolatsou and Palmer, 2004; Malosetti,2004; Mayo, 2004). Malt is an intermediate product and is mainly used for making bear, pure alcohol, malt syrups and milk malt. Malt is also a rich enzyme-source for starch digestion specially α and β-amylase (Osman et al, 2002). In a freshly harvested barley caryopses and during storage, there was a correlation between high levels of a-amylase and subsequent loss of germination power (Schwarz et al, 2004; Izydorczyk, 2004; Lin et al, 2008). Electron microscope of endosperm showed in digestion process only the small granules (floury) of starch release from endosperm and large granules (horney) starch to with protein networks and remained on cell wall. Lower nitrogen leads to a better and uniform endosperm in germination phase (Agu, 2003). Starch and protein digestion of endosperm in germination phase, the amount of reserved protein decreases to lower half of primary amount (Celus et al; 2006). Twenty nine positions have been reported for α-amylase activity in proteomix analysis of barley seeds (Bak-Jensen et al, 2007). Amylose is digested more slowly and it has an important role in reducing the glycemic and insulin impact of foods (Behall and Scholfield, 2005). Rapid and economic measurement of amylose content in barley is important for genetic study and breeding improvement of this trait (Hu et al, 2010). By cDNA micro array studynig, germination genes expression of Morex and Steptoe population reported (Potokina et al, 2002; Watson and Henry, 2005). Unfortunately there is no sufficient data in Persian barley regarding high malting and low or free nitrogen barley. In this research the physicochemical characteristics different lines and Persian varieties were compared with each other to see their effects along with climate, location and genotypes on the malting quality.

Material and Methods
Plant materials

Fifteen double haploid lines (F_1 hybrids from Steptoe × Morex with modified method of *Hordeum bulbosum*,by North American Barley Genome Mapping Project (NABGMP) (**Chen and Hayes, 1989; Hayes, 1993**) and 15 Persian varieties (introduced by Seed and Plant Improvement Institute, Karaj, Iran) were selected to conduct the malting experiment (Table 1).

Percentage of Protein content

Barley seeds have a total protein content of around 10–15%.About 80% of the total protein is contributed by the storage proteins hordeins (**Görg et al, 1992; Jones, 2005**). Nitrogen in barley and malt was measured using Kjeldahl method which has three processes of digestion, distillation and titration. After titration, the amount of protein was calculated by using the following formula:

$$\%protein = \frac{titer \quad amount}{sample \quad weight} \times 14.008$$

Diastatic power, α-amylase activity and malt extract

Kernel weight calculation and seeds soaked for measuring Diastatic power, α amylase activity in 17-18 Cº water for 4 hours it was transferred to germinated chamber with 100% relative humidity for 5-7 days. The resulted seedlings were dried in 50-55 Cº in oven for 24 hours and finally radicals were separated. Some qualitative malt traits such as diastatic power, α-amylase and malt extract were measured in all genotypes (Schmitt and Budde, 2007).

Diastatic Power: Ten g milled malt was mixed with 12 ml amoniac 0.2 N in 200 ml final volume and incubated in 20 Cº. Every 30 minutes sample was stirred. After 3 hours based on enzyme power, 1-3 ml enzyme was mixed with 100 ml starch solution and incubated in 20 Cº for 1 hour. To stop enzyme activity, 30 ml NaOH 0.1 N was added to sample and the volume reached to 200 ml with distilled water (DW). To evaluate enzyme activity, A *Fehling's* solution (36.64 g crystalline copper sulfate in 500 ml DW) and B solution (173 g doubled sodium, potassium tartrate and 500 g NaOH in 500 ml DW) with 2.5:2.5 ratio were used to determine reducer sugar and diastatic power calculated with (L°) as follows Lintner :

$$Diastatic \quad power\left(L^{\circ}\right) = \frac{2000}{xy}$$

x: used malt extract (ml)

y: used starch in titration (ml)

α-amylase Activity: This enzyme used as catalyzer to hydrolize starch to sugar. 0.003 g used per 200 ml malt extract in every sample.

Malt Extract: Fifty mg malt was weighed and dissolved in about 100 ml 46℃ water, then its volume was increased until it reached 250ml and stored in a 70℃ heating chamber for one hour 250 Malt extract was filtered and water malt extract of efficiency was evaluated. Distinct volume from malt extract was put into measuring cylinder and weighed. After dividing this weight to volume, a special weight achieved and based on Malt Berix Charts, malt extract was measured for each genotype (**American Society of Brewing Chemists, 2004**). A complete randomized design (15 lines, 15 varieties) with 3 replications was performed and for data analysis we used SAS software. Mean comparison by Duncan's method in 5% confidence level was used. Correlation coefficient calculated for each treat group (lines, varieties, lines and varieties). Finally principal component analysis using SPSS and cluster analysis were performed by Past software.

Results and Discussion

While enzymes such as phosphatase, phytase, hemicellulase and protease are stimulated in early stages of germination, amylases were activated in late stages of this process. Malt extract has positive and negative correlations respectively with starch (related to seed size) and protein contents.If nitrogen of barely seed increases, the quality and quantity of resulting malt decreases (**Eneje et al, 2003; Canci et al, 2003**).When germination period (appearance of radical and coleoptile) of barely seed takes more time than usual, its respiration increases rapidly, and burning sugar and starch speed up very fast. In addition, more protein is released and suspended in barely kernel. However, protein content of malt extract should be low (~0.5%) and fixed for yeast feeding during fermentation. All traits except kernel weight and grain diameter in double haploid lines had significant differences in 1% confidence level (Table 2). α-amylase had an important role in germination and malting process. (Table 3) shows a high positive correlation (0.491**) between α-amylase and seed protein. Also there was a negative correlation (-0.664**) between malt extract and protein content of barely seed which is similar to what Peighambari et. al (3) and Jones and Budde (**Jones and Budde, 2005**) have found in their research. In order to determine the diversity of malt quality, PCA was performed (Table 4). Two primary components represented 64.54 % of total variation. First component with 45.97% of total variation represented diastatic power, malt extract, α-amylase activity, protein content, kernel weight and grain diameter. Second component with 18.56% of total variation represented grain length. To determine similarity and genetic distances between genotypes, cluster analysis by Ward method was used and all genotypes divided into 3 clusters with 0.3 similarity (Figure 1). First cluster included (11, 4, 9, 1, 10, 3, 7, 8, 5), second cluster (13, 12, Dasht, Valfajr, 2, 15, 14, Sahar, Kavir, 6) and third cluster included rest of varieties. So beside double haploid lines, Persian varieties such as Dasht,Valfajr, Sahar and Kavir are suitable for high quality malting processes (**Borràs-Gelonch et al, 2010**).

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Annexure

Table 1- Characteristics of DH lines and Persian varieties of barely

Persian varieties Seed and Plant Improvement Institute, Iran			Some of Steptoe/Morex DH lines (NABGMP)		
Name	Kernel weight	Protein cnotent%	Number	Kernel weight	Protein cnotent%
Sahra	37.99	12.83	1	36.01	11
Valfajr	40.95	12.8	2	35.73	11.93
Dasht	40.31	12.56	3	35.07	11.86
Kavir	37.81	12.11	4	37.19	12.2
Reyhan03	37.75	13.83	5	35.5	11.63
Torkaman	37.89	14.53	6	37.81	11.5
Zarjo	39.28	14.3	7	38.72	11.73
Star	40.46	13.43	8	35.87	11.4
Bahman	38.93	14.93	9	36.51	10.76
Aras	42.06	14.86	10	36.02	11.2
Eram	43.57	15.56	11	39.45	12.5
Gorgan	41.95	15.86	12	36.33	10.4
Goharjo	42.69	14.93	13	36.67	9.7
jnoob	41.25	14.66	14	36.45	11.03
Sahand	42.35	14.16	15	36.79	12.63

Table 2: Persian varieties and DH lines variance analysis

		Mean of Squares						
S.O.V	DF	Grain length	Grain diameter	Kernel weight	Protein (%)	Alpha amylase	Malt extract(%)	Diastatic power (L°)
Treat	29	1.23**	0.03ns	18.18**	8.24**	18.27**	8.56**	823.64**
GC	1	5.04**	0.28*	76.34**	10.88**	99.64**	0.03ns	38.49**
Error	60	0.21	0.04	6.02	0.05	4.04	0.92	3.95
C.V(%)		5.14	7.6	6.37	1.83	7.29	1.3	2.24

GC: Group Comparison (lines against varieties) ns: No Significant *: Significant at 5% level
**: Significant at 1% level

Table 3: Pearson correlation between traits in Persian varieties and DH lines

Traits	X1	X2	X3	X4	X5	X6	X7
X1: Diastatic power(L°)	1						
X2: Malt extract	0.713**	1					
X3: Alpha-amylase	0.228	0.279	1				
X4: Protein(%)	-0.538**	-0.664**	0.491**	1			
X5: Kernel weight	-0.357	0.39*	-0.531**	0.795**	1		
X6: Grain diameter	0.14	0.149	0.061	-0.255	-0.126	1	
X7: Grain length	-0.105	0.17	0.317	-0.384*	-0.316	0.243	1

*: Significant at 5% level **: Significant at 1% level

Table 4: Eigenvalues, cumulative variance and variance of 2 PCA

Traits	PCA 1	PCA 2
Diastatic power(L°)	0.665	-0.642
Malt extract	0.766	-0.417
Alpha-amylase	0.633	0.303
Protein(%)	-0.929	-0.029
Kernel weight	-0.805	-0.175
Grain diameter	0.31	0.244
Grain length	0.427	0.729
Eigenvalues	3.218	1.299
Variance	45.97	18.56
Cumulative variance	45.97	64.54

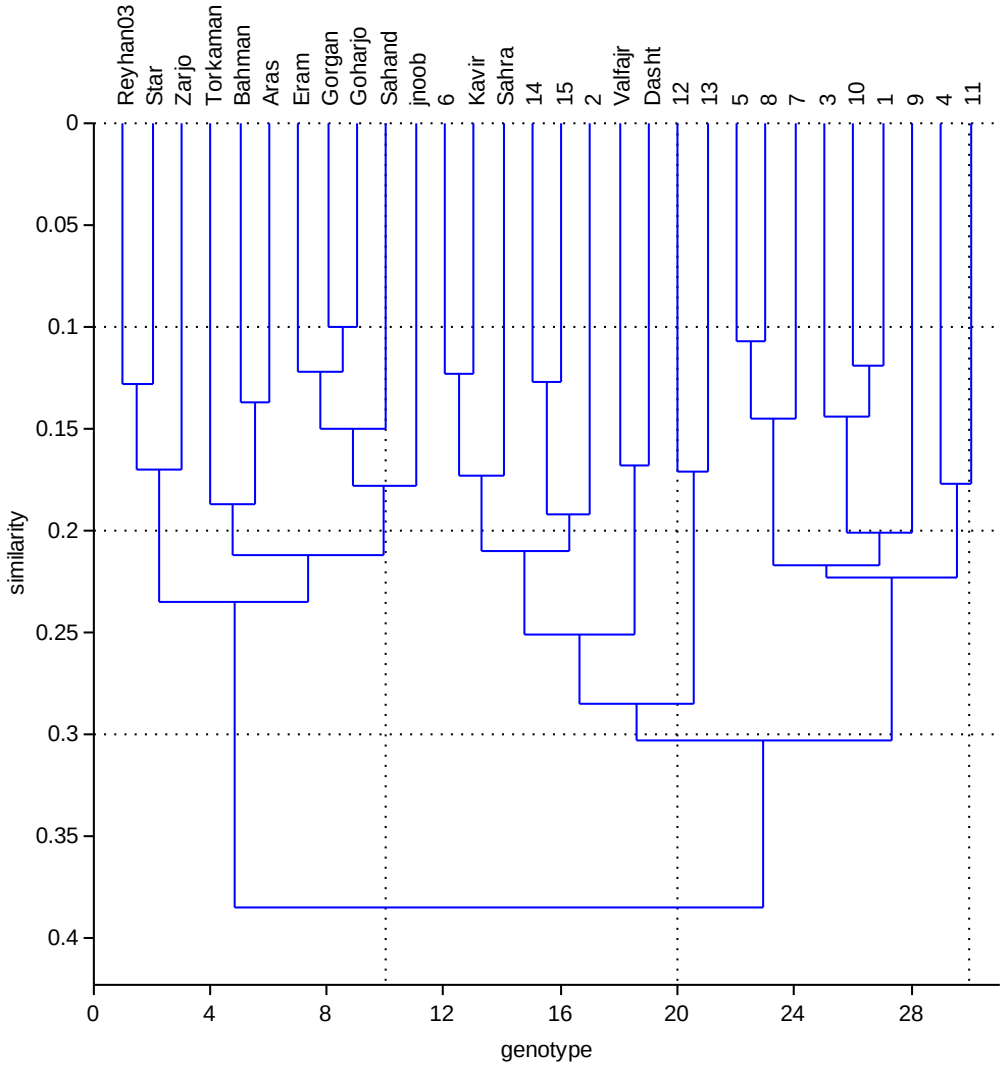

Fig1: Dendogram for malt quality traits in DH lines and Persian varieties