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Evaluation of Quantitative and Qualitative Characteristics of Persian Walnut (*Juglans regia* L.) Genotypes in the West of Meshkin-Shahr

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ABSTRACT

The Persian walnut (*Juglans regia* L.) is one of the most valuable resources in Iran because of its multi benefits. The present study was conducted in Meshkin-Shahr in Ardabil province to investigate the important quantitative and qualitative characteristics of 31 walnut genotypes. Classification of genotypes was analyzed using the cluster method. The results showed that among selected walnut genotypes, SS genotype had the highest kernel weight (7.03 g), and fruits weight (12.65 g), and RM3 had the best kernel percentage 63.73%. The walnut genotypes were clustered into three groups using UPGMA cluster method. This preliminary study demonstrated that quantitative and qualitative characteristics were effective in evaluating the genetic diversity of walnut genotypes.

Introduction

The walnut belongs to the Juglandaceae family includes 60 species, which 21 of them belong to the *Juglans* genus (Mitra *et al*., 1991). Paleontology studies have shown that walnut genotypes are grown in Asia, Europe, and North America (Forde *et al*., 1975).

According to the Food and Agriculture Organization of the United Nations (FAO, 2015), in 2012, China was the major world producer followed by Iran, the US and Turkey. Kerman province is the leading area for walnut production in Iran, with about 17,095 ha under cultivation. This province, with varied eco-geographical regions, is one of the major centers for Persian walnut diversity, and walnut populations are widely scattered in this region (Vahdati, 2000).

There are over 20 species of *Juglans*, which the Persian walnut (*Juglans regia* L.) widely grows in the majority of the world, especially in Iran because of its wide compatibility with respect to geographical latitudes longitude and height above sea levels. It is considered as one of the multi-purpose trees and is one of the most economical and commercial species. The kernel inside this species is used in food, cosmetics and pharmaceutical industries. It is planted in parks and green areas for its wonderful shade, beauty and pleasant morphology. It is especially valuable for its versatile and beautiful wood properties (Haj-Amiri, 2003).

The global average of walnut production in Asian regions was reported 68.4% during the last five years (2008-2012), followed by America (19.1%). The leading walnut producing countries are China, Islamic Republic of Iran, USA and Turkey (Anonymous, 2013). In Pakistan walnut production was reduced during the last many years. However, its production was increased by 11.5 thousand tones during the last two years (Anonymous, 2013). Genetic variability of

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walnut populations is very high and exists in various parts of the world (Sharma, 2001; Khan *et al*., 2010).Morphological characters are considered as an option for selection and classification of promising germplasm. Morphological variations in nut sizes, the thickness of the shell, and kernel percentageand yield of kernels in walnut trees have been reported from various geographical (Casal *et al*., 2005; Zeneli *et al*., 2005; Karimi *et al*., 2014).

Promising walnut genotypes were identified from various regions (Arzani *et al*., 2008). The high protein and oil contents of the kernels in *Juglans regia* L. makes this fruit in dispensable for human nutrition. Therefore, the walnut is classified as a strategic species for human nutrition in FAO List of priority plants (Gandev, 2007).

Morphological markers are visible plant traits controlled by Mendelian genes, which congregate with genes determining the expression of the trait of interest to allow selection for suitable individuals from a population. The main purpose of the present study was to identify and analyze the quantitative and qualitative special characteristics of walnut (*Juglans regia* L.) genotypes in the west of Meshkin-Shahr.

Materials and Methods

Plant Materials

In this study, 31 genotypes of walnut were evaluated using 12 quantitative and 9 qualitative traits. The samples were collected from non-grafted trees with the age of 25–50 years from Ghasabe (AH1, AH2, AH3, AH4, AH5, AH6, AH7, AH8, AH9, AH10, AH11, AH12, ES2, ES1, YS, NM1, RM1, RM2, RM3, UM, AB, RB, SS, AA, jM1, JM2), Majandeh (MZ3, MZ4) and Andzagh (AK1, AK2, AK4) region in the west of Meshkin-Shahr. Meshkin-Shahr is a region located in the central northern part of the Ardabil Province, northwest of Iran. It is situated at an altitude of 1490 m above sea level between longitudes 47° 190´and 48° 170´ East and latitudes 38° 570´ and 38° 130´ North.

These genotypes were selected based on a number of important quantitative and qualitative traits including fruit diameter, fruit length, dry weight, kernel weight, kernel percentage, leaf length, width leaf, branch length, protein percentage, oil percentage, leafing time, type of flowering, date of flowering, the thickness of the shell, ease of kernel removal, kernel color, fruit shape, full brain and tree vigor. The evaluated characteristics, units and measuring methods were shown in Table 1. Characters such as leafing time, thickness of shell, type of flowering, date of flowering,the thickness of the shell, ease of kernel removal, kernel color, fruit shape, full brain and tree vigorwere evaluated based on IPGRI descriptors (IPGRI, 1994). According to IPGRI descriptors (1994), when 50% of terminal buds are open and leaves are visible from inside the buds, it is known as leafing date. Homogenous characteristics and percentages were calculated as follows:

No. of days that female blooms overlapped with staminate blooms (days) × 100 / Duration of female blooms (days)

Table 1.Quantitativeandqualitative traits and their units of measurement

No.	The studied traits	Measuring methods
1	Fruit diameter	Caliper(mm)
2	Fruit length	Caliper(mm)
3	Dry weight	Digital scale(gram)
4	Kernel weight	Digital scale(gram)
5	Kernel percent	kernel weight: nut weight ratio(percent)
6	Leaf length	Caliper(mm)
7	Widthleaf	Caliper(mm)
8	Branch length	Caliper(mm)
9	Protein percent	Kjeldahl method

Table 1. Continued.

10	Oil percent	Oil weight: kernel weight ratio(percent)
11	Leafing time	Leafing more than 50%
12	Type of flowering	Homogamous, dichogamy, protogynous
13	Date of Flowering (Male, Female)	Little- much(1-3)
14	Thickness of shell	Too thin-too thick(1-4)
15	Ease of kernel removal	Very easy to difficult (1-7)
16	Kernel color	Very light to dark (1-4)
17	Fruit shape	Most desirable at least desirable(1-8)
18	Full brain	Weak- strong (1-3)
19	Tree vigor	Weak- strong(1-3)

Data analysis

The Statistical Package for the Social Sciences (SPSS), version 9.0 (SPSS Inc., Chicago, United States, Norusis, 1988) was used for data analysis. Cluster analysis was also performed based on the quantitative traits with coefficients of heritability more than 0.80 (Hansche *et al.* 1972) using the un-weighted pair-group mean average method (UPGMA).

Results

The results showed that the walnut trees had high variation for phenological and pomological traits. It was concluded that genotypes with average leafing could be planted in the majority of walnut producing regions, especially regions with a hot and dry climate. Besides, walnut cultivars with late leafing could be cultivated in mountain areas, where the late frosts were frequent. By studying the opening time of buds in the genotypes, ES1 and AH12 genotypes showed the earliest date for female-flower buds break, and the AH2genotype had the earliest date male-flower buds break. The latest date for female and male-flower buds break was recorded for JM2 and RM2 genotypes, respectively.

The early opening time for female and male flowers was observed in the AH5 and AH9 genotypes, respectively. Besides, 68% and 32% of genotypes were synchronous and asynchronous, respectively.

Fig. 1 shows the status of the flowering in the 31 walnut genotypes.

There was %94 protandrous, %6 protogynous. About 28 of the 31 genotypes had kernels with alight color, three individuals were very light, and one was amber. In addition, the removal of the kernel fromits shells in the more genotypes was moderate. The fruit shape in the more genotypes was ovoid, and full brain the more genotypes were moderate. The tree vigor meaning the more genotypes was much.

We detected highly significant positive correlations between the flowering peak to the end of the male flowerand flower beginning to flower peak(r= 0.356*), also between flower peak to the end of the male flower and bud break to female flower (0.454*) (Table 2).

The lateral bearing habit was analyzed on the basis of main quantitative traits. Mean values of the quantitative traits recorded among genotypes are presented in Table 3. Descriptive analysis of each quantitative trait including mean value, maximum, minimum, range, and coefficient of variation among genotypes showed a relatively high degree of variation (Table 3).

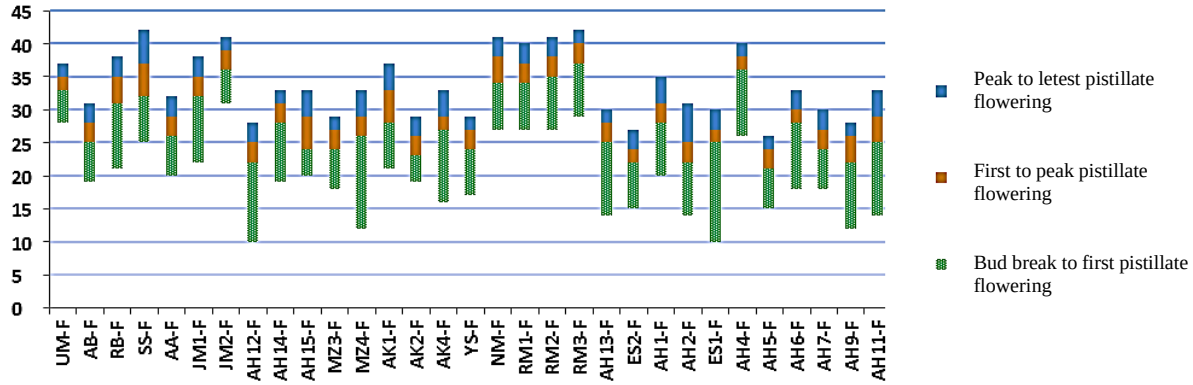

Fig 1. Flowering status,pistillate in the between 31 walnut genotypes.

Table 2. Correlation coefficients between pairs of flowering characters.

Traits	Bud break to male flowering	Male flower Beginning to male flower peak	Flower peak to the end of the male flower	Bud break to female flower	Pistillate flower Beginning to pistillate flower	Pistillate flower peak to the end of the pistillate flower
Bud break to male flowering	1					
Male flower Beginning to maleflower peak	-0.295	1				
Male flower peak to the end of the male flower	0.024	0.356*	1			
Bud break to female flower	0.255	-0.343	-0.189	1		
Pistillate flower Beginning to pistillate flower peak	-0.087	-0.124	-0.013	0.149	1	
Pistillate flower peak to the end of the pistillate flower	-0.051	-0.132	0.011	0.454*	0.107	1

The length of the branch in the season showed the highest variations coefficient (33.15%). The kernel percentage varied from 42.70% (AH8) to 63.73% (RM3). RM3 (63.72) and AA (63.27) genotypes had the highest kernel percentage. The dry weight was recorded between 7.05g (AH3) and 12.65g (SS) and kernel weight from 3.63g (AH6) to 7.03g (SS).

Among the samples minimum and maximum oil percentages were 43.97% (AH3) and 63.42%(JM1) and minimum and maximum protein percentages were 6.14% (JM2) and 13.99% (AK1) (Table 4). Among the evaluated trees, the highest kernel percentage was 63.73 %. The branch length of 31 selected genotypes varied between 7.82 and 28.25 mm. Thus, it was clear that the 33.15% coefficient of variations were suitable in terms of branch length.

Table 3.Minimum, maximum, mean values, standard deviation and coefficient of variations for importanttraits observed in 31 walnuts.

Traits	Min.	Max.	Mean	SD	CV (%)
Fruit diameter	25.46	37.03	29.98	2.47	6.108
Fruit length	27.82	39.21	33.33	2.53	6.44
Dry weight	7.05	12.65	9.65	1.47	2.18
Kernel weight	3.63	7.03	4.86	0.762	0.581
Kernel percent	42.70	63.73	50.68	5.07	25.80
Leaf length	8.96	18.85	12.87	1.2	4.41
Leaf width	5.10	8.25	6.17	0.79	0.625
Branch length	7.82	28.25	16.41	5.75	33.15
Protein percent	6.14	13.99	9.62	2.027	4.11
Oil percent	43.97	63.42	58.04	3.48	12.12

Table 4. Some fruit and nut characteristics.

Genotype	FD	FL	DW	KW	KP	LL	LW	BL	PP	OP
AH1	28.4	30.59	8.8	4.4	50	11.2	5.4	8.8	8.63	57.69
AH2	28.56	35.92	10.18	4.799	47.05	14.6	6.5	19.2	9.22	58.9
AH3	26.44	34.16	7.05	3.79	53.76	12.1	5.1	13.85	8.05	43.97
AH4	30.12	34.38	10.54	5.22	49.53	15.3	6.9	20.5	9.32	57.84
AH5	25.46	31.18	8.03	4.05	50.44	14.1	5.2	13.3	9.11	59.25
AH6	27.58	33.76	7.43	3.63	48.86	12.2	5.9	14.1	9.54	56.54
AH7	30.22	32.44	0.54	4.84	45.92	12.6	5.92	12.78	9.54	61.88
AH8	30.84	33.38	0.28	4.39	42.7	11.38	5.68	16.5	10.7	60.61
AH9	29.91	31.75	9.08	4.34	47.8	11.77	5.52	20.65	10.7	59.05
AH10	30.45	32.33	0.55	5.48	51.94	11	5.25	15	12.19	60.69
AH11	28.27	32.98	8.22	4.22	51.34	12.88	7.23	17.06	11.23	57.29
AH12	31.33	32.58	10.68	5.45	51.03	13.65	6.85	14.65	9.54	58.09
ES2	31.06	35.86	7.91	4.33	54.74	11.27	7.3	12.55	9.64	62.76
ES1	25.78	31.48	11.7	5.81	49.66	16.52	7.92	28.25	12.93	61.11
MZ3	30.88	33.39	10.13	4.77	47.09	9.85	5.12	13.6	6.36	57.53
MZ4	31.54	32.97	11.32	5.03	44.43	11.1	6.22	7.82	6.36	58.27
AK1	33.49	31.83	10.38	4.86	46.87	10.5	5.7	8.25	13.99	55.67
AK2	27.73	30.08	8.61	4.39	50.99	12.92	5.62	8.45	11.44	56.91
AK4	32.6	31.06	10.22	4.83	47.26	12.18	6.48	14.52	13.25	57.7
YS	29.15	32.97	8.8	5.07	57.61	11.82	6.4	20.22	6.99	59.35
NM1	30	30.24	9.27	5.36	57.82	11.42	5.25	26.75	9.59	54.2
RM1	83.29	36.5	9.68	4.7	48.55	13.51	6.76	8.1	10.54	59.05
RM2	32.61	37.9	11.34	5.42	47.8	15.35	6.13	21	11.44	57.37
RM3	33.47	36.61	10.42	6.64	63.73	16.41	6.07	20.37	10.54	60.13
UM	29.81	33.49	9.3	4.6	49.46	13.35	5.95	8.52	8.58	59.35
AB	31.69	39.21	12.14	5.39	44.4	12.05	6.11	18.57	10.49	56.28
RB	30.21	33.67	10.71	4.88	45.56	8.96	5.9	21.13	9.59	58.3
SS	37.03	37.66	12.65	7.03	55.57	18.85	8.25	15.55	7.42	54.45
AA	30.35	33.96	8.33	5.27	63.27	12.58	5.91	18.25	8. 05	54.46
jM1	28.2	27.82	7.46	3.77	50.54	14.16	6.78	23.55	6.99	63.42
JM2	26.5	31.30	7.51	4.16	55.39	13.4	6.15	27	6.14	61.25

FD(Fruit diameter); FL (Fruit length); DW (Dry weight); KW (Kernel weight);KP (Kernel percent);LL (Leaf length); LW (leaf Width); BL (Branch length); PP (Protein percent); OP (Oil percent).

In the study, correlation of coefficient between different traits of walnut genotypes revealed significant correlations among the different traits such as leaf width and leaf length ($r=0.623^{**}$), kernel weight and fruit diameter (r= 0.672**), kernel weight and leaf length ($r=0.502^{**}$), dry weight and fruit diameter ($r=0.647^{**}$), and dry weight and fruit length ($r=0.458^{**}$), kernel weight and fruit length ($r=0.458^{**}$). The highest and significant positive correlation was found between dry weight and kernel weight ($r=0.78^{**}$) (Table 5).

The data obtained from quantitative characteristics analyse were used to perform genetic similarity analysis among the 31 walnut genotypes. Cophenetic correlation between ultra-metric similarities of the tree and the similarity matrix was r=0.91 with $P < 0.0001$. Cluster analysis of the 31 selected genotypes based on of 12 traits was used to estimate the relationships between the selected genotypes in a dendrogram (Fig. 2). Based on this technique, genotypes were classified into three different cluster groups. The first cluster consisted of two different walnut genotypes such as::

RM3 and SS; the second cluster included JM1,JM2, ES1 and AH3 genotypes; and the third cluster consisted AH1, AH2, AH4, AH5, AH6, AH7, AH8, AH9, AH10, AH11, AH12, , UM, RM1, ES2, YS, MZ3, MZ4, NM1, AA, AK2, RM2, AB, AK1, AK4 and RB genotypes (Fig. 1).

Table 5. Correlation coefficients between pairs of characters

	Fruit diameter	Fruit length	Dry weight	Kernel weight	Kernel percent	Leaf length	Leaf width	Branch length	Protein percent	Oil percent
Fruit diameter	1									
Fruit length	0.492**	1								
Dry weight	0.647**	0.458**	1							
Kernel weight	0.672**	0.458**	0.78**	1						
Kernel percent	0.025	0.017	-0.334	0.325	1					
Leaf length	0.132	0.294	0.265	0.502**	0.314	1				
Leaf width	0.238	0.275	0.319	0.391*	0.09	0.623**	1			
Branch length	-0.183	-0.063	0.034	0.214	0.293	0.287	0.217	1		
Protein percent	0.136	0.013	0.285	0.139	-0.236	0.002	0.044	-0.057	1	
Oil percent	-0.031	-0.191	0.101	0.003	-0.165	0.066	0.268	0.151	0.056	1

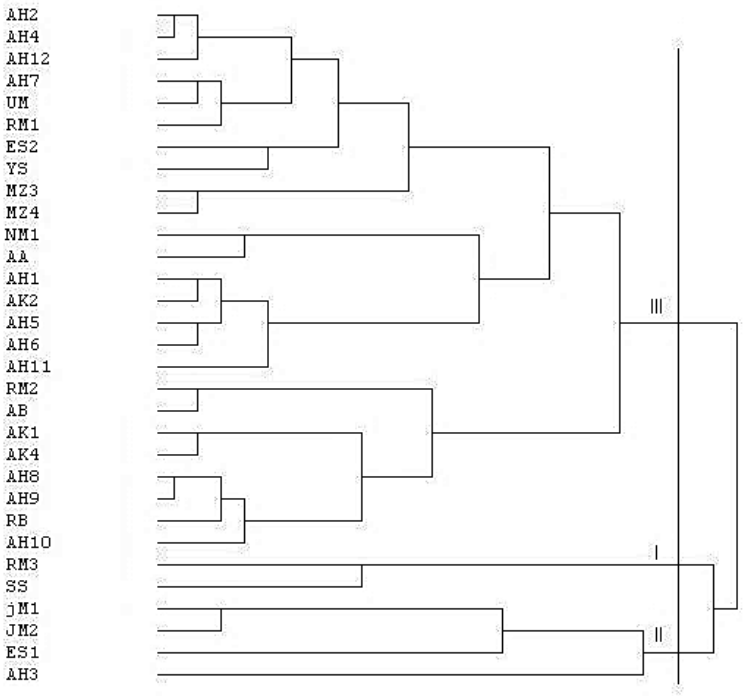

Fig.2. Classification of walnut genotypes (31) using cluster analysis (UPGMA)

Discussion

In the selected genotypes type of dochogamy was not all the same, and protandrous was the most predominant dichogamy. There was %94 protandrous and %6 protogynous. Protogyny is a useful characteristic in the selection of pollinizers for the main cultivated varieties that usually shed their pollen before their peak of pistillate receptivity (Germain, 1997).

Due to the high diversity in the measure of quantitative traits, it is more probable to use those from the feature breeding programs in order to obtain superior genotype. Desirable nut and kernel weight

should range from 12 to 18 g and 6 to 10 g, respectively, or kernel weight should be at least 50% of the entire nut weight, and the kernel should have a light color (McGranahan and Leslie., 1990).In our study, RM3 was the genotype that had these kernel traits.

In the present study, the oil values among the samples were ranged from 43.97% (AH3) to 63.42% (JM1) (Table 4). Ghasemi *et al*. (2010) examined the fatty acid composition of selected walnut genotypes in Arak province and reported oil values ranging from 48 to 75%, which showed more variation than those found in this study. Another study found walnut kernels containing 52 to 72% oil (Martinez *et al*., 2006). In a study conducted in Turkey, Caglar Irmak (2003) reported 63% as the average oil value of the studied genotypes. However, because of the economic value of the oil, these kernels could be used as potential sources of oils. The highest value for protein percentage among our evaluated nuts (13.99 %) was less than the corresponding data reported by Golzari *et al*. (2013).The highest value for kernel percentage among our evaluated trees (63.73 %) was less than the corresponding data reported by Ebrahimi *et al*.(2009) and Sarikhani Khorami *et al*. (2012). However, the highest value for kernel percentage among our evaluated trees was higher than reported values by Ehteshamnia *et al*. (2009) and Wang *et al*. (2015). Variation in nuts and kernel-related traits among walnut species, cultivars, and genotypes showed very broad diversity. Of course, considerable variation in walnut has been reported by many researchers (Balli *et al*., 2001; Caglariymak, 2003; Iskandari *et al*., 2006; Arzani *et al*., 2008; Jaffari-Sayadi, 2006).

In the classification of walnut genotypes (31) using cluster analysis (UPGMA), genotypes AH1 and AH2 were placed separately in the third group based on important traits. It showed that they had the greatest difference from the others. Genotypes in the same group have the greatest similarity. High among genotypes genetic differentiation can be closely related to various factors, such as the long term evolutionary history of species, genetic drift, breeding system, gene flow habitat fragmentation and population isolation. Artificial selection and cultivation may also partly explain the genotypes genetic differentiation (Li *et al*., 2011). A high variability in important traits has been reported for walnut trees from different studies (Malvolio *et al*., 1994; Balli *et al*., 2001). Mosivand *et al*. (2013) divided the walnut genotypes into three distinct groups.

Conclusions

The Persian walnut *(Juglans regia* L.) is a species with a very wide distribution range from east to west. In this study, the wide range of variation was observed in oil, protein and kernel characteristics as well as in other studied traits. The presence of broad genetic diversity encourages exploration, collection, conservation and utilization of genetically distinct genotypes for future use of germplasm in order to achieve desirable breeding objectives for walnut improvement.

To the best of our knowledge, this report is the first assessment on the genetic diversity of walnut genotypes in the west of Meshkin Shahr, which is sampled from diverse locations based on quantitative and qualitative traits. According to the present results, it is recommended that quantitative and qualitative traits be used in describing the diversity of the walnut genotypes. Further studies with wider sampling of locations, populations and individuals and with more advanced molecular techniques such as AFLP, SSRs and SNPs are needed to reveal comprehensively the genetic structure of different walnut populations and genotypes in the west of Meshkin-Shahr. On the other hand, the differences among the walnut genotypes in several of the evaluated characters were so pronounced that we were able to classify them into separate groups.

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