Journal of Nuts 8(1):21-30, 2017
ISSN: 2383 – 319X

Evaluation of some Phenological and Pomological Characteristics of Selected Walnut Genotypes from Shahroud-Iran

*Sara Akhiani[1], Hossein Afshari[*1], Tahereh Parvaneh[2]*

[1]Department of Horticulture, Damghan Branch, Islamic Azad University, Damghan, Iran

[2]Agriculture Research Center, Shahroud, Iran

Received: 20 October 2016 Accepted: 21 January 2017

Abstract

The first step in walnut breeding programs is to identify and evaluate superior genotypes of fruit trees. Hence, there are various walnut breeding programs in various areas of Iran. A study aimed to evaluate the morphological and chemical characteristics of selected superior genotypes of walnut was conducted in the Shahroud region in 2011-2012. The following genotypes were selected in this study as the best walnut genotypes: X-18 homogamous genotypes due to desirable late leafing; genotype X-11 for its high percentage of kernel production, easily removal of shell, thin shell; genotype X-52 due to its kernel plumpness compared to other genotypes, thin shell and high percentage of kernel and genotype X-70 for its kernel brightness, easily kernel extracting and high percentage of kernels. The X-49 and X-5 genotypes had the highest amount of linoleic and linolenic fatty acids and higher nutritional quality compared to other genotypes. Three genotypes, X-3, X-11 and X-22, had the highest amount of oil. Genotypes X-9 and X-45 had the highest amount of protein. The difference between oil content and fatty acid compositions was presumably due to genetic diversity and ecological conditions of the studied genotypes cultivation.

Keywords: *Juglans regia* L., Morphological traits, Superior genotypes, Walnut.

Introduction

The walnut genus has twenty-one species, and the most important species is *Juglans regia*, also known as the Persian walnut. It is grown commercially in many countries. However, other species of walnuts are grown for their fruit and wood regionally. The most important species in terms of wood belong to the East America "black walnut." The Persian walnut (*Juglans regia* L.) is considered the most important dried fruit, which is widely distributed and cultivated around the world.

One of the main objectives of breeding walnuts is to achieve regular production with high quality in order to compete in global markets. Achieving this objective requires of the use of diverse genetic resources in order to identify and introduce superior genotypes and cultivars of walnut. Identifying the genetic diversity and genetic potential of each plant species is necessary before breeding. Generally, genetic diversity in breeding is considered superiority (Ghasemi *et al*., 2008). Walnut has a very rich genetic diversity due to proliferation by seed. There are valuable traits in genotypes that can be used after identification. It is determined that there are wild genotypes of walnut in northern Iran forests and Kape Dagh, and these regions are the main source of Walnut (Ghasemi *et al*., 2008). Germplasm evaluation and superior genotypes of walnut collection have been carried out in many countries such as Turkey and Spain. The main and common objective of all programs is late leafing and lateral bearing (Aleta &

[*]**Corresponding author**: Email: h_afshari@ymail.com

Ninot, 1993). Studying morphological and genetic traits of jungle walnut trees in our country is the basis of native genetic resource identification. Their conservation and management is of great importance in order to be used in breeding programs (Banaeiann Zangeneh, 2011). Thus, many studies have been done in order to select superior genotypes of walnut and study the genetic diversity of native walnuts in different parts of Iran.

Walnut acreages are about 169 thousand hectares in Iran. Shahroud is one of the main areas of walnut production in Iran.

Materials and Methods

This study aimed to evaluate the morphological and chemical traits of genotypes of walnut fruit and trees in the Shahroud region and introduce the selected superior genotypes to producers of walnut. We attempted to study different walnut genotypes in the Shahroud region in terms of their quantity and quality characteristics so that their similarities and distance were genetically determined firstly. Then, if sufficient genetic variation in traits were found, we considered using the results in future breeding programs. This experiment was conducted in 2011-2012 in Shahroud and its geographical location is as follow: latitude of 25 minutes and 36 degrees, longitude of 58 minutes and 54 degree and longitudinal and height of 1380 meters above sea level.

According to previous surveys, 30 superior genotypes of walnut were selected in Shahroud in order to examine their different traits. The studied traits included oil, protein, ash and fatty acids of fruits, as well as other traits such as tree habit, leafing date, early leafing or late leafing compared to mean region tress, dicogamy status, protandreous or protogynous, male floribondity production frequency, productivity, susceptibility to cold, fruit weight, fruit shape, shell texture, shell seal, shell thickness, kernel weight, shell removal, kernel plumpness, kernel color, and percentage of kernel. The number of fruits was estimated by an average of fruit per cluster of each tree (at least 10 clusters). The selected trees yield was estimated by measuring the approximate number of fruits per tree. In addition, the trunk circumference was measured at a height of about 30-50 cm from soil. The chemical characteristics, such as fat percentage (Soxhlet, 1879) and protein (kjeldahl method) were measured. Firstly, the total lipids of seeds powder were extracted from by chloroform liquidation procedure in order to measure the fatty acids. For this purpose, seed powder was mixed with chloroform for 30 minutes and then centrifuged in 1000 g at 4°C. Supernatant was removed and precipitate was extracted by chloroform. The extracted oil contained fatty acids, which were isolated by chromatography gas with capillary columns of 30 meters in length and 0.5 mm internal diameter. They were identified and measured based on the extraction peak that had standard samples of fatty acids (Primomo *et al*., 2002).

Statistical analysis was done after data collection using SPSS software and comparison of means was determined by Duncan's multiple range tests at 5%.

Results

According to the results of variance analysis table, it was found that trunk circumference among the studied genotypes had a statistically significant difference at levels of 1% and 5%. According to the obtained results, the tree trunk diameter of studied genotypes was variable between 27 cm and 135 cm. The maximum trunk circumference belonged to X-17 and X-68 genotypes, and the minimum trunk circumference belonged to X-22 genotype (Tables 1 and 2).

Table 1.variance analysis of trunk circumference, number of fruits, of fruits per cluster, mean weight of fruits and mean weight of kernels, in 30 superior genotypes of walnut in Shahroud region

Variation resources	Mean squares					
	Degrees of freedom	Trunk circumference (cm)	Number of fruits	Number of fruits per cluster	Mean weight of 20 fruits (gr)	Mean weight of 20 piths (gr)
Genotypes	29	1217.30**	61154.41^{ns}	0.03^{ns}	3860.61^{ns}	933.90*
Error	**30**	**0.57**	**92795.13**	**0.03**	**2698.67**	**633.40**
Total	59					

Table 2. Study the fruit and kernel traits, 30 superior genotypes of walnut in Shahroud city

Genotypes	Trunk circumference (cm)	Tree growth habit	Tree growth Vigour	Type of Bearing	Leafing date
X-22	27	Direct	Strong	Intermediate	Early
X-17	135	Semi direct	Strong	Intermediate	Moderate
X-36	80	Semi direct	Moderate	Intermediate	Moderate
X-42	80	Semi direct	Moderate	Intermediate	Moderate
X-67	107	spread	Strong	Intermediate	Moderate
X-41	73	Semi direct	Moderate	Intermediate	Moderate
X-18	78	Semi direct	Moderate	Terminal	Late
X-3	59	Direct	Weak	Intermediate	Moderate
X-11	90	Semi direct	Strong	Intermediate	Moderate
X-5	100	Direct	Weak	Intermediate	Moderate
X-9	82	Semi direct	Strong	Intermediate	Moderate
X-52	30	Direct	Weak	Intermediate	Moderate
X-51	35	Direct	Weak	Intermediate	Early
X-68	135	Semi direct	Strong	Intermediate	Moderate
X-44	88	Semi direct	Moderate	Intermediate	Moderate
X-70	72	Direct	Strong	Intermediate	Moderate
X-15	80	Semi direct	Strong	Intermediate	Moderate
X-33	72	Semi direct	Moderate	Intermediate	Moderate
X-31	69	Semi direct	Moderate	Termianl	Moderate
X-7	95	Semi direct	Moderate	Intermediate	Moderate
X-55	100	Direct	Weak	Intermediate	Moderate
X-69	100	Direct	Weak	Intermediate	Moderate
X-20	76	Semi direct	Strong	Intermediate	Moderate
X-23	78	Semi direct	Moderate	Intermediate	Moderate
X-8	73	Semi direct	Moderate	Intermediate	Moderate
X-19	106	Semi spread	Strong	Side below 50%	Moderate
X-12	80	Semi direct	Moderate	Intermediate	Moderate
X-45	61	Semi direct	Moderate	Intermediate	Moderate
X-49	71	Semi direct	Strong	Intermediate	Moderate
X-57	75	Majnun	Strong	Intermediate	Moderate

In a study that identified and selected twelve promising and superior walnut genotypes in the Fars province, the trunk diameter was reported between 20 cm to 40 cm in superior genotypes (Sarikhani *et al*., 2012), This is not consistent with results of this study due to lack of compliance between plants age.

Among the studied genotypes in terms of tree growth habit, eight genotypes were upright including

X-22, X-3, X-52, X-5, X-51, X-70, X-55 and X-69, 19 genotypes were moderately upright including X -49, X-45, X-12, X-8 and X-23, X-20, X-7, X-31, X-33, X-15, X-44, X-68, X-9 , X-11, X-18, X-41, X-42, X-36 and X-17, X-19 genotype was semi-spread, X- 67genotype was spread and X-57 was "Hanging branches." Most of tree growth habits were observed as semi-direct.

Six genotypes were weak in vigor, including X-52, X-5, X-51, X-3, X-55 and X-69. Twelve genotypes were moderate including X-36, X-42, X-41, X -18, X-44, X-33, X-31, X-7, X-23, X-8, X-12, X-45 and 12 genotypes were high including X-22, X-17, X-67, X-11, X-6, X-68, X-70, X-15, X-20, X-19, X-49, X-57 in terms of tree growth power (Table 2). Thus, most of the studied genotypes have moderate to strong growth. Flowering habit in most of samples were intermediate, and 27 genotypes had intermediate flowering. Genotype X-19 had lateral bearing less than 50%, and X-18 and X-31 genotypes had terminal bearing flowering among 30 genotypes. Most of the genotypes were in mid-range in terms of leafing date. Three genotypes were early leafing, and two genotypes of X-7 and X-18 were late leafing (Table 2). According to the results of variance analysis table, there was no significant difference between evaluated genotypes in terms of fruits number and number of fruits per cluster, other than the X-51 genotype which had low Catkin abundance, and X-67, X-11 and X-57, which had high Catkin abundance. Other genotypes were moderate in terms of male inflorescence. The highest amount of fruits was observed in X-17 and X-57 genotypes as 1250, and the lowest amount was observed as 300 in X-3 genotype (Table 1 and 3).

Table 3. Phenological traits of 30 superior genotypes of walnut in Shahroud.

Genotypes	Dicogamy status	Catkin abundance	Estimated number of fruits	Number of fruits per cluster
X-22	PR	Moderate	550	2
X-17	PR	Moderate	1250	2
X-36	PR	Moderate	350	2
X-42	PR	Moderate	350	2
X-67	H	High	600	2
X-41	H	Moderate	500	2
X-18	H	Moderate	423	2
X-3	PR	Moderate	300	2
X-11	H	High	750	2
X-5	PG	Moderate	700	2
X-9	H	Moderate	650	2
X-52	H	Moderate	300	2
X-51	H	Low	350	2
X-68	PR	Moderate	1250	2
X-44	PR	Moderate	350	2
X-70	PR	Moderate	1000	2
X-15	H	Moderate	750	2
X-33	H	Moderate	450	2
X-31	H	Moderate	400	2
X-7	H	Moderate	500	2
X-55	PG	Moderate	700	2
X-69	PG	Moderate	700	2
X-20	H	Moderate	950	1
X-23	H	Moderate	550	2
X-19	H	Moderate	415	2

Table 3. Continued

X-12	H	Moderate	550	2
X-45	H	Moderate	450	2
X-49	PG	Moderate	450	2
X-57	PG	High	1250	2

Genotypes had an average of two numbers of fruits per cluster, except for the X-20 genotype. In addition, checking the results of one particular year showed that 17 genotypes were, including X-67, X-18, X-41, X-9, X-11, X-52, X-51, X-15, X-33, X-31, X-7, X-20, X-23, X-8, X-12, X-19 and X-46. Eight genotypes were protandreous including X-22, X-17, X-36, X-42, X- 3, X-68, X-44 and X-70, and five genotypes were protogynous including X-49, X-57, X-69, X-55 and X-5 among the 30 genotypes in Shahroud (Table 2). The results of this study showed that fruit shape of 30 genotypes in Shahroud city was mostly trapezoidal (9 genotypes), oval (8 genotypes) and elliptic shaped (7 genotypes).

Four genotypes of X-67, X-68, X-15 and X-70 had triangular-shaped fruits, and X-8 and X-33 genotypes had a circular shape (Table 4). The maximum oil content was observed in X- 3 genotype as 67.30%, X-11 genotype as 67.05%, X-22 genotype as 66.90%, respectively. The minimum content was observed in X-67 genotype as 56.53% (Table 4). The maximum amount of protein in X-9 and X-45 genotypes was 20.45%, and the minimum protein content of X-70 genotype was 12.25%. In the case of ash amount, the maximum amount belonged to X-19 genotype as 3.75, and the minimum amount belonged to X-57 genotype as 2.34% (Table 4).

Table 4. Comparison of oil, ash, protein percentage and fatty acids , 30 superior genotypes of walnut in Shahroud city.

Genotype	Oil	Protein	Ash	Linoleic	Linolenic	Oleic	Palmitic
X-22	59.30 ^{abcd}	14.50 ^{abcd}	2.78 ^{abcdef}	53 ^{bcde}	19.10 ^{ab}	15.20 ^{abcd}	4.10 ^{bc}
X-17	66.90 ^{a}	16.50 ^{abcd}	3.44 ^{abcdef}	58 ^{abc}	13.15 ^{bc}	14.40 ^{bcde}	4.10 ^{c}
X-36	57.50 ^{cd}	13.50 ^{cd}	^{f}2.95^{abcde}	49 ^{cde}	15.20 ^{abc}	21 ^{ab}	4.80 ^{abc}
X-42	65.80 ^{bcd}	12.80 ^{cd}	3.15 ^{abcdef}	51 ^{bcde}	14.20 ^{abc}	13 ^{cde}	5.15 ^{ab}
X-67	60 ^{abcd}	13.30 ^{cd}	2.68 ^{cdef}	48 ^{cde}	16.30 ^{abc}	18.90 ^{abcd}	4.60 ^{abc}
X-41	58.20 ^{bcd}	18.50 ^{abcd}	3.34 ^{abcdef}	59 ^{ab}	18.10 ^{abc}	15 ^{abcd}	4.80 ^{abc}
X-18	59.50 ^{abcd}	13.65 ^{bcd}	2.56 ^{def}	49 ^{cde}	15.10 ^{abc}	13.30 ^{cde}	4.10 ^{bc}
X-3	64.5 ^{abcd}	14.50 ^{abcd}	3.03 ^{abcdef}	53 ^{bcde}	12.30 ^{bc}	12.40 ^{de}	5.20 ^{ab}
X-11	56.53 ^{d}	15.10 ^{abcd}	2.81 ^{abcdef}	47 ^{cd}	16.40 ^{abc}	15.20 ^{abcd}	5.50 ^{ab}
X-5	60.65 ^{abcd}	18.55 ^{abcd}	2.71 ^{bcdef}	51 ^{bcde}	21.10 ^{a}	11.20^{e}	4.95 ^{abc}
X-9	59.40 ^{abcd}	20.60 ^{a}	3.31 ^{abcdef}	54 ^{bcde}	11.25 ^{c}	18.35 ^{bcde}	4.60 ^{abc}
X-52	57.65 ^{cd}	14.80 ^{abcd}	2.51 ^{def}	46 ^{d}	15.70 ^{abc}	14.40 ^{bcd}	5.20 ^{ab}
X-51	66.40 ^{ab}	12.85 ^{cd}	2.41 ^{ef}	49 ^{cde}	16.20 ^{abc}	21.95 ^{a}	5.60 ^{ab}
X-68	58.20 ^{bcd}	15 ^{abcd}	3.15 ^{abcdef}	45 ^{d}	16.35 ^{abc}	14.20 ^{bcde}	4.95 ^{abc}
X-44	60.40 ^{abcd}	13.15 ^{cd}	2.70 ^{bcdef}	51 ^{bcde}	13.20 ^{bc}	13 ^{cde}	4.60 ^{abc}
X-70	67.30 ^{a}	12.25 ^{d}	2.52 ^{def}	58 ^{abc}	11.20 ^{c}	19.85 ^{abc}	4.10 ^{bc}
X-15	61.50 ^{abcd}	20.35 ^{ab}	3.51 ^{abcd}	46 ^{d}	13 ^{bc}	15 ^{abcd}	5.50 ^{ab}
X-33	67.05 ^{a}	15.55 ^{abcd}	2.97 ^{abcdef}	44 ^{d}	15.30 ^{abc}	21.15 ^{ab}	5.65 ^{a}
X-31	65.25 ^{abc}	14.20 ^{abcd}	3.14 ^{abcdef}	59 ^{ab}	18.10 ^{abc}	18.55 ^{abcd}	4.30 ^{abc}

Table 4. Continued

X-7	60.55 abcd	13.50 cd	2.45 ef	48 cde	abc16.55	abcd15.20	ab5.50
X-55	57.40 cd	19.35 abc	3.71 ab	44 d	13.30 bc	19.30 abcd	4.85 abc
X-69	66.55 ab	13.50 cd	2.57 def	52 bcde	13.30 bc	13.10 cde	4.40 abc
X-20	61.25 abcd	18.25 abcd	2.76 abcdef	55 bcde	11.20 c	14.20 bcde	4.35 abc
X-23	59.60 abcd	14.45 abcd	3 abcdef	45 d	16 abc	19.65 abc	5.70 abc
X-8	62.10 abcd	12.85 cd	3.22 abcdef	49 cde	15.10 abc	17.10 abcd	5.80 a
X-19	59.36 abcd	16.20 abcd	3.75 a	46 d	12.1 bc	14.30 bcde	5.40 ab
X-12	61.10 abcd	15.05 abcd	2.65 def	51 bcde	17.30 abc	21.30 ab	4.90 abc
X-45	57.80 cd	20.45 a	3.70 abc	47 de	15.30 abc	16.25 abcd	5.80 a
X-49	62.15 abcd	15.35 abcd	2.97 abcdef	61 a	19.10 ab	12.10 de	4.95 abc
X-57	60.65 abcd	16.65 abcd	2.34 f	52 bcde	11.25 C	14.30 bcde	4.70 abc

Means with common letters in each column are significantly different at 5% level.

In the case of linoleic acid that includes the highest fatty acids, variations of 46-61 were observed in X-15 and X-49 genotypes (Table 4). The highest amount of linolenic acid was observed in X-5 genotype as 21.10. In the case of oleic acid, the variation was 11.50 in X-5 genotype and 21.95 in X-51 genotype. In the case of palmitic acid which has the lowest amount among the fatty acids of this study the variations were 3.65 and 5.80 in X-17 and X-45 genotypes (Table 4).

Discussion

Late leafing, early maturity, high yield and product quality are aims of walnut breeding program (Ebrahimi *et al*., 2009). Later emergence of leaves in spring (even for a few days) can play an important role in reducing the risk of spring frost damages. Late leafing walnut cultivars can be grown in mountainous regions as well as cold regions (Akca & Ozongun, 2004). The results of Sarikhani *et al.*, studies in 2012 showed that two genotypes of A92 and A63 in the Fars province were quite late leafing and were acceptable in terms of fruit traits. According to the severe spring frost damage \ and the necessity of considering late leafing genotypes, researchers have tried to improve the pomologic traits of two genotypes and transmit late leafing trait of two genotypes to other genotypes in future breeding programs. In addition, given that rootstock can induce resistance to cold (Hartmann *et al*., 2001), we can use late leafing genotypes as the rootstock of superior genotypes in the revival of traditional walnut orchards and the establishment of commercial orchards. As mentioned earlier, one of the solutions for protecting walnuts against cold is to select late leafing genotypes.

Flowering habit of walnut trees is important in the management of walnut orchards due to overlap of pollination and acceptance periods of female flowers (Akca *et al*., 2004). Therefore, the best cultivars of walnuts are (Polito & Pinney, 1997). It is also known in some walnut breeding programs that genotypes must be chosen so that protandreous and protogynous genotypes are together in order to overlap at flowering time (Arzani *et al*., 2008). It is important to note that flowering and other phenological traits are greatly influenced by environmental conditions (Polito *et al*., 1997).

The superior genotypes that were introduced by Arzani *et al*., (2008) had bright kernels, easy removal and thin septum. The major superior genotypes of walnut that are introduced in other studies have bright kernels and a thin septum which can be easily removed from the shell. The results of this study were consistent with Arzani *et al***.** (2008) Studies, except for kernel colour that was amber.

Numerous studies were done around the world in order to morphologically evaluate the walnut. Studies on walnut genotypes in Guvas region of East Antalya in Turkey showed that fruit weight varied between 10.38-17.04g, kernel weight between 5.85-7.88g, hard shell thickness between 0.75-1.76 mm and kernel percentage between 45.09-59.27% among superior genotypes (Arzani *et al*., 2008). Studying the morphological diversity of native walnut varieties of Golestan province of Iran showed that kernel percent was between 19.95 to 50.19% (Yarilgac *et al*., 2001).

A study conducted by Haqjuyan *et al.,* (2005) in the Shahroud region showed that walnut trees had the largest length, seed diameter, seed weight and higher seed shell, and they were superior statistically compared to other genotypes. In their review, the higher mean seed weight and higher shell hardness in the Shahroud region were due to length and diameter of larger seeds compared to other genotypes. These genotypes could be used as parents in future breeding programs (Haqjuyan *et al*., 2005; Dogan & Akgul, 2005).

Kernel percent of walnut trees is considered as an indicator of economic performance. This trait must be focused on and effective factors must be studied on kernel quantity and quality.

Therefore, using late leafing and X-18 genotypes can be considered as the basis of superior genotypes in the revival of traditional walnut orchards and business orchards because one of the most important solutions is to select late leafing genotypes in order to protect walnuts against the cold. The trait of this genotype is useful because the flowering habit of walnut trees is important in the management of walnut orchards due to overlapped pollination and acceptance period of female flowers. Therefore, the best cultivars of walnuts are. This genotype has trees with strong growth, full kernels and thin shells.

The changes of oil content between studied genotypes were 53.90-63.90, which was consistent with Dogan and Akgul (2005) and Simsek (2010) studies. Dogan and Akgul recognized that the oil content of different genotypes vary between 55% and 65%. Oguz and Askin determined superior genotypes of walnut oil as 67.63-54.07. Simsek found similar results, and the oil content of studied genotypes varied between 57.87-64.61% (Tables 5 and 6).

Table 5. Analysis of oil variance, ash amount, protein of fatty acid composition, 30 superior genotypes of walnut of Shahroud city

Variation resources	Mean squares							
	Degrees of freedom	Oil %	Protein %	Ash %	Linoleic acid %	Linoleic acid %	Oleic acid%	Palmitic acid%
Genotypes	29	22.16*	0.228*	12.53**	46.64*	13.40*	19.50*	0.68*
Error	**30**	**11.79**	**0.177**	**7.66**	**22.5**	**9.20**	**8.70**	**0.37**
Total	59							

Table 6. The mean, minimum, maximum, variation range and standard error of mean oil, ash, protein and fatty acid composition, 30 superior genotypes of walnut in Shahroud city

Matter percent	Mean	The maximum	The minimum	Mean standard error	Variation range	Number of sample
Oil %	61.34	63.90	53.90	0.53	16	60
Protein %	15.50	23.40	10.20	0.41	13.20	60
Ash %	2.96	3.98	2.02	0.07	1.91	60
Linoleic acid %	50.70	66.00	40.00	0.80	26	60
Linolenic acid %	14.98	24.00	9.40	0.43	14.60	60
Oleic acid%	16.10	24.10	10.00	0.48	14.10	60
Palmitic acid%	4.94	6.10	3.00	0.09	3.10	60

acids is important for their nutritional and economic value. For example, lower oil linoleic and linolenic leads to more durability of oil, while higher levels of these fatty acids are more important in terms of nutrition. Mean linoleic acid was 50.70% and the minimum and maximum were 40% and 66%. In the case of oleic acid, the mean was 16.10% and the minimum and maximum were 10% and 24.10%. Mean linolenic acid was 14.98%, and the minimum and maximum were 9.40% and 24%. In the case of palmitic acids, which have the lowest rate of fatty acids, the mean was 94.4 and the minimum and maximum were 3 and 6.10.

The results of this study showed that linoleic acid was the predominant fatty acid of walnut oil, followed by oleic, linoleic and palmitic acid. In a study conducted by Amaral *et al.*, in Portugal on six walnut cultivars, including Mayette, Marbot, Franquette, Lara, Mellanaise and Parisienne, it was found that linoleic acid is the predominant fatty acid among cultivars. The results of this experiment corresponded with Ghasemi *et al*., 2008. (Ghasemi *et al.,* 2008) Their study showed that the highest percentage of fatty acids in some walnut cultivars in the central province different areas (2007) belonged to linoleic acid at 49.8% and then oleic, linoleic, palmitic and stearic acid, respectively, with percentages of 29.04%, 11.04%, 6.97% and 3.04% (Table 4).

The mean amount of protein in the above experiment was 15.50, and the minimum and maximum were 10.20% and 23.40%. The mean amount of ash was 2.96%, and the minimum and maximum were 2.02% and 3.98% (Table 1-3), which was consistent with Dogan and Akgul study results (Haqjuyan *et al*., 2005). Simsek study results showed that protein can vary between a minimum of 13.76% to 20.5%, depending on the genotype type (Yarilgac *et al*., 2001). Oguz and Askin identified protein and ash amounts of walnut superior genotypes as 12.11-20.75, 1-2.22. The amount of oil, protein and ash can is variable due to environmental conditions and ecological conditions (Haqjuyan *et al*., 2005).

According to the assessments on studied genotypes, essential fatty acids of walnut oil included linoleic, linolenic and oleic acids. The results were consistent with studies conducted by Ghasemi *et al*., (2008). Linolenic acid variation of X -70, X-20 and X-57 and X-5 genotypes was 11.20 (minimum) and of X-5 genotype was 21.10 (maximum), which represented the highest amount of omega-3 in the above genotype. It must be noted that kernel of genotypes such as X-5 can be regarded as a valuable source of food with a considerable amount of linolenic acid (omega 3). Also, genotypes such as X-49 can be incorporated in the food industry with a significant amount of linoleic acid (omega-6). In another study conducted by Dogan and Akgul (2005), linoleic, linolenic and palmitic acid were high in oil genotypes, and oleic acid was lower in walnuts obtained from East Anatolia in Turkey. The amount of linoleic, linolenic and oleic acid of examined genotypes were higher than reported amounts, and the palmitic acid was less than the reported amount by Ozkan *et al*., 2005.

In addition, superior genotypes of Shahroud had higher proportion of linoleic and linolenic acid amounts and lower amounts of oleic and palmitic acid compared to fatty acids in other superior genotypes. In this study, it was found that walnut oil genotypes are rich in unsaturated fatty acids, and the percentage of saturated fatty acids is low. These traits and the presence of other useful compounds in the walnut kernel such as vitamins, plant sterols, polyphenols, etc. walnut importance in preventing heart attacks (Ozkan & Koyuncu, 2005).

Acknowledgements

We acknowledge Mr. Ebadi for his technical assistance in this project. The authors are also grateful to the curator of the Herbarium of the Shahroud Agriculture Research Station for helping to remove pollen material from herbarium specimens**.**

References

Akca Y, Ozongun S (2004) Selection of late leafing, late flowering, laterally fruitful walnut (*Juglans regia*) types in turkey. New Zealand Journal of Crop and Horticultural Science. 32(4), 337-342.

Aleta N, Ninot (1993) A Exploration and evaluation of Spanish native walnut (*Juglans regia* L.) populations from Catalonia and Galicia. Acta Horticulturae. 311, 17-23

Arzani K, Mansouri-ArdakanH, Vezvaei A, Roozban MR (2008) Morphological variation among Persian walnut (*Juglans regia*) genotypes from central Iran. New Zealand Journal of Crop and Horticultural Science. 36(3), 159-168.

Atefi J (1993) Evaluation of walnut genotypes in Iran. Acta Horticulturae. 311, 24-33.

Banaeian Zangeneh M (2011) Estimating Production Function of Walnut Production in Iran Using Cobb-Douglas Method. Agricultura Tropica et Subtropica. 44(4), 189-201.

Caglarirmak N (2003) Biochemical and physical properties of some walnut genotypes (*Juglans regia* L.). Nahrung. 1, 28-32.

Dogan M, Akgul A (2005) Fatty Acid Composition of Some Walnut (*Juglans regia* L.) Cultivars from East Anatolia. Grasasy Aceites. 56, 328-331.

Ebrahimi MR, Fatahi ZA, Zamani Vahdati K (2009) An investigation on genetic diversity of 608 Persian walnut accessions for screening of some genotypes of superior traits. Iranian Journal of Horticultural Sciences. 40(4), 83-94.

Ghasemi M, Arzani K, Hasani D, Ghasemi Sh (2008) Fatty acid composition of some selected walnut genotypes (*J. regia* L.) in Markazi province. Iranian journal of food science and Technology. 7(1), 31-37. [In Persian]

Haqjuyan R, QaraYazi B, Sanei ShariatPanahi M, Khalighi A (2005) Study the genetic variation of walnut genotypes in different regions of country using quantity morphological markers. Research and development. 18(4), 22-30.

Hartmann HT, DE Kester, FT Davies, Geneve R (2001) Hartmann and Kester's plant propagation: principles and practices. 7th edition. Prentice Hall, New Jersey. pp. 880.

Khadivi- khub A (2014) Genetic diversity in seedling trees of Persian walnuts for morphological characters in markazi province from Iran. Brazilian journal of Botany. 37(3), 273-281.

Oguz HA, Askın A (2007) Ermenek region of walnut (*Juglans regia* L.) selection through breeding on the birarastir. Centenary university. Journal of Agriculture science. 17, 21–28.

Ozkan G, Koyuncu (2005) MA Physical and chemical composition of some walnut (*Juglans regia* L.) genotypes grown in Turkey. Grasasy Aceites (Sevilla). (2), 141-146.

Piccirillo P, Fasano P, Mita G, Paolis A. de, Santino A (2006) Exploring the role of lipoxygenases on walnut quality and shelf-life. Acta Horticulturae. 705, 543-545.

Polito VS, Pinney K (1997) The relationship between phenology of pistillate flower organogenesis and mode of hetero dichogamy in *Juglans regia* L. (Juglandaceae). Sex Plant Reprod. 10, 36–39

Primomo VS, Falk ED, Albert GR (2002) Inheritance and interaction of low palmitic and low linoleic soybean. Crop Sciences. 42, 31-36.

Sarikhani S, Arzani K, Roozban MR (2012) Identification and Selection of Twelve Walnut Superior and Promising Genotypes in Fars Province, Iran. Journal of Plant and Seed Breeding. 1-28(2), 277-296.

Simsek M (2010) Determination of Walnut Genotypes with High Fruit Bearing and Quality in Dicle, Hani, Egil and Kocaköy Townships. GOI. Journal of Agriculture. 27, 85-93.

Soxhlet F (1879) Die gewichtsanalytische Bestimmung des Milchfettes. Dingler's Poly technisches Journal. 232, 461-465.

Wang H, Pei D (2008) Genetic Diversity and Structure of Walnut Populations in Central and Southwestern China Revealed by Microsatellite Markers. Journal of the American Society for Horticultural Science. 133(2), 197–203.

Yarilgac T, Koyuncu F, Koyuncu MA, A. Kazankaya A, Sen SM (2001) Some promising walnut selections (*Juglans regia* L.). Acta Horticulturae. 544, 93-96.