DOI: http://dx.doi.org/10.22092/cbj.2013.100447

Comparison of tree, nut, and kernel characteristics in several walnut species and inter-specific hybrids

M. Mosivand[a], D. Hassani[b], V. Payamnour[c], and M. Jafar Aghaei[d]

[a] M.Sc. student, Agricultural and Natural Sciences University of Gorgan, Gorgan, Iran.
[b,d] Seed and Plant Improvement Institute, Karaj, Iran.
[c] Agricultural and Natural Sciences University of Gorgan, Gorgan, Iran.
* Corresponding author's E-mail address: Hassanida@spii.ir

Received: August 2012
Accepted: November 2012

ABSTRACT

Mosivand, M., Hassani, D., Payamnour, V., and Jafar Aghaei, M. 2013. Comparison of tree, nut, and kernel characteristics in several walnut species and inter-specific hybrids. **Crop Breeding Journal 3(1): 25-30.**

This study was carried out to investigate the important characteristics in some cultivars/genotypes of Persian walnut (*Juglans regia* L.), black walnut (*J. nigra* L.), and walnut inter-specific hybrids: Paradox (*J. hindsii* × *J. regia*) and Royal *(J. hindsii* × *J. nigra)* in Kamalshahr Research Station in Karaj in 2011-2012. In the study, vegetative vigor was evaluated based on trunk cross-sectional area (TCSA), canopy diameter, nut related traits such as nut weight and size (thickness, width, height), kernel weight, kernel percentage, kernel color, ease of removal, shell thickness, and oil percent. Results showed very high diversity among the species and interspecific hybrids, so that Paradox with 506 cm^2 had the highest TCSA, which was 214% more than that of *J. nigra*. Based on canopy diameter, Paradox showed the most vigorous growth, with an average of 7.95 m, while *J. regia* with 5.05 had about 37% less vigor relative to Paradox. Regarding nut height, it varied from 3.02 cm to 1.48 cm in *J. regia* and Paradox, respectively. Nut weight varied from 11.5 g in *J. nigra* to 3.62 g in Paradox. Principal coordinates (PCO) and cluster analysis were used to classify walnut cultivars/genotypes and interspecific hybrids. According to multivariate statistical analysis, the cultivars/genotypes were divided into three groups: black walnut, interspecific hybrids, and Persian walnut.

Keywords: *Juglans* species, morphological characteristics, walnut

INTRODUCTION

Walnut belongs to the family Juglandaceae that includes 60 species, 21 of which belong to the genus *Juglans* (Mitra *et al.*, 1991). Paleontology studies have shown that walnut genotypes have long been grown in areas of Asia, Europe, and North America (Forde *et al.*, 1975). One of these valuable trees, Persian walnut, is found in several parts of world, including Iran. Iran is a center of diversity for several species, including Persian walnut (Forde *et al.*, 1975). This species grows well in a very wide range of latitudes, longitudes, and altitudes, so that it is grown from Mazandaran Province at sea level to the foothills of Alborz and Zagros at altitudes above 2500 meters (Darvishian, 2003).

Walnut is a monoecious species that is pollinated by the wind (Westwood, 1993). Most wild walnuts have small nuts with hard shells. Despite sexual reproduction, over the centuries, superior walnut trees have been selected through a type of genetic improvement. In Iran, propagation of walnut using seedlings has produced huge variation and formed a large gene pool that can be utilized for breeding purposes. Germain (1993) reported that since Iran is a center of origin for walnut, there is considerable genetic diversity in native walnut populations.

Many researchers have selected superior genotypes from walnut populations. Solar and Stampar (2004) evaluated walnut genotypes in Slovenia and identified genotypes based on specific traits. Diaz *et al.* (2004) found a highly significant difference in the studied traits of Spain's walnut population. Mamadjanov (2001) studied the diversity of forest walnut in Kyrgyzstan and eventually selected three genotypes. Studying quantitative morphological traits, Sharma and Sharma (2001) determined the correlation between various nut traits. In recent decades, studies conducted in many countries, including France, India, Bulgaria, Albania, Yugoslavia, Turkey, China, Spain, Russia, and Poland, have focused on evaluating walnut populations and selecting or introducing superior genotypes (Zeneli *et al.*, 2005).

In Iran, Gholami (1990) screened walnut genotypes native to Hamadan Province for superior genotypes and identified 17. Moreover, Arzani *et al.*

(2008) evaluated superior walnut genotypes in Taft-Yazd based on morphological characters. In another study, Saadat and Zandi (2001) tried to identify and evaluate superior walnut trees in Fars Province and selected 101 genotypes with the desired characteristics. Jaffari-Sayadi (2006) studied the genetic diversity of native walnut populations in the forests of northern Iran. Rezaie *et al.* (2008) studied the morphological features of a few selected walnut genotypes in Kahriz-Urmia. Ehteshamnia *et al.* (2009) studied the morphological diversity of native walnut populations in different areas of Golestan Province. Hassani *et al.* (2012a,b) evaluated seven promising walnut genotypes with the objective of releasing new cultivars, Jamal and Damavand among them.

The main goal of genetic studies is to contribute to the improvement of new cultivars, and using walnut species as rootstock makes it necessary to compare the variation among and between walnut species and interspecific hybrids. The present research was conducted to study and compare the vegetative characteristics of different walnut species and interspecific hybrids that are used as rootstock for walnut cultivars.

MATERIALS AND METHODS

This study was conducted in 2011-2012 at the Kamalshahr Horticultural Research Station of the Horticultural Research Department of the Seed and Plant Improvement Institute, Karaj. The station is at an altitude of 1300 meters above sea level, with an average annual rainfall of 245.5 mm and an average annual temperature of 13.17° C; it has warm, arid summers and mild, wet winters.

Among walnut genotypes/cultivars and hybrids available in a walnut collection that was planted in 1994, 24 were selected for evaluation: six black walnut *(J. nigra)* genotypes (Nigra1, Nigra2, Nigra3, Nigra4, Nigra7, Nigra8); nine Persian walnut *(J. regia)* cultivars/genotypes including Serr, Damavand, K72, Ron de Montignac, B21, Pedro, Hartley, Chandler, and Jamal; five Royal interspecific hybrids (*J. hindsii* × *J. nigra*) and four Paradox interspecific hybrids (*J. hindsii* × *J. regia*). For nut and kernel evaluation, 15 healthy nuts were selected from each tree. The evaluated characteristics and units are shown in Table 1.

Statistical parameters such as mean, variance, and coefficient of variation were obtained for quantitative traits, and the Kruskal-Wallis test was used to compare the differences among means of qualitative traits (Steel and Torrie, 1980). Genotypes were classified using the first two principal factors of principal coordinate (PCO) analysis. Later genotypes were also clustered by the studied factors using the WARD method (Manly, 1994).

Table 1. Qualitative and quantitative traits and their units of measurement.

The studied traits	Unit of measurement
Nut weight	Gram
Kernel weight	Gram
Kernel percent	Percent (kernel weight: nut weight ratio)
Oil percent	Percent (oil weight: kernel weight ratio)
Nut height	Millimeter
Nut thickness	Millimeter
Nut width	Millimeter
Date of maturity	Very early to late (1-4)
Kernel color	Very light to dark (1-4)
Kernel size	Very small to very large (1-9)
Nut size	Very small to very large (1-5)
Roundness index	Too little-too much (see guidelines)
Shell thickness	Too thin-too thick(1-4)
Structure of shell surface	Too slight-too embossed (1-4)
Adherence of two shell halves	Very weak-very strong (1-9)
Ease of kernel removal	Very easy to difficult (1-7)
Trunk cross-sectional area	Square centimeter
Canopy diameter	Meter

RESULTS AND DISCUSSION

The descriptive trait statistics are shown in Table 2. As can be seen in Table 2, canopy diameter varied from a minimum of 3 m to more than 10 m after about 18 years. Variation in trunk cross-sectional area (TCSA) was also notable, ranging from 134 cm^2 to 963 cm^2. Mean comparisons for TCSA and canopy diameter using least square (LS) means are included in Table 3.

The average TCSA of Paradox and *J. nigra* was 506 and 237 cm^{-2}, respectively. Based on this result, *J. nigra* had very low vigor with a TCSA of about 47% compared to Paradox, while *J. regia* and Royal had 76% and 98% of Paradox TCSA. The average canopy diameter ranged from 5.05 m in *J. regia* to 7.95 m in Paradox. *Juglans regia* and *J. nigra* produced trees with average canopy diameters that were 64% and 66% of the average canopy diameter of Paradox, while Royal produced trees with 93% of Paradox.

Variation in nuts and kernel-related characteristics

Table 2. Basic statistics of selected quantitative traits of walnut genotypes.

Statistical parameters	Trunk cross sectional area	Canopy diameter	Nut thickness	Nut width	Nut height	Nut weight	Kernel percent	Kernel weight	Oil percent
Mean	382	6.10	2.69	2.55	3.02	9.38	40.26	3.37	66.66
Variance	150	0.03	0.52	5.42	0.60	3.21	9.98	1.51	2.55
Maximum	963	10.10	3.51	3.54	4.19	14.87	55.30	6.68	70.74
Minimum	134	3.00	1.68	1.48	1.97	3.62	20.98	1.50	60.99

Table 3. Mean comparison of trunk cross-sectional area and canopy diameter using least square means.

Average trunk cross-sectional area		Royal	*Juglans regia*	Paradox	*Juglans nigra*	Average canopy diameter
		Trunk cross-sectional area differences (cm^2)				
236.90	*J. nigra*	296.15* *	122.4 *	111.20 *		5.27
506.05	Paradox	146.7* *	11.25 ^{ns}		2.68 *	7.95
383.60	*J. regia*	257.9**		2.90 ^{ns}	0.22 ^{ns}	5.05
494.80	Royal		2.34 ^{ns}	0.56^{ns}	2.12 *	7.39
(cm^2)		Canopy diameter differences (m)				(m)

* and **: Significant at the 5% and 1% of probability levels, respectively
ns: Not significant

among walnut species, cultivars, and genotypes showed very broad diversity. Of course, considerable variation in *J. regia* has been reported by many researchers (Solar, 1990; Malvolti *et al.*, 1994; Balci *et al.*, 2001; Caglariymak, 2003; Eskandari *et al.*, 2006; Arzani *et al.*, 2008; Hagh-Jooyan, 2003; Jaffari-Sayadi, 2006).

Tables 4 and 5 show nut and kernel characteristics. *Juglans regia* had the maximum nut width, with 3.02 cm, while Royal had the minimum, 1.48 cm. Nut height variation fluctuated from 3.56 cm in *J. regia* to 2.13 cm in Paradox (Table 4). Nut and kernel weights are compared in Table 5. *Juglans nigra* produced the biggest nut, with 11.5 g, compared to Royal, with 3.56 g. Kernel weight was highest in *J. regia* (4.7 g), while Royal had the lowest (1.5 g). As can be seen in Table 5, kernel percent was 28, 41, 48 and 53 for *J. nigra*, Royal, *J. regia*, and Paradox, respectively. Ehteshamnia *et al.* (2009) reported nut weight ranging from 5.64 to 25.91 g, kernel weight from 2.14 to 7.5 g, and kernel percent from 19.95 to 50.19. In addition, Rezaei *et al.* (2008) reported nut weights of 10.3-16.2 g, kernel weights of 5.5-6.4 g, and the highest kernel percent (71%).

Minimum and maximum oil percentages among

Table 4. Comparison of average height and width means in different walnut species and interspecific hybrids using least square means.

Average nut width		Royal	*Juglans regia*	Paradox	*Juglans nigra*	Average nut height
		Nut width differences				
2.72	*J. nigra*	1.24 * *	1.54 * *	079 **		2.77
1.48	Royal	0.30 * *	0.75 * *		0.33 *	2.44
3.02	*J. regia*	0.49 **		1.12 **	0.79 **	3.56
2.23	Paradox		1.43 **	0.31^{ns}	0.64 * *	2.13
(cm)		Nut height differences				(cm)

* and **: Significant at the 5% and 1% of probability levels, respectively
ns: Not significant

Table 5. Average nut and kernel weight comparisons using least square means.

Average nut weight		Royal	*Juglans regia*	Paradox	*Juglans nigra*	Average kernel weight
		Kernel weight difference				
11.50	*J. nigra*	7.88 **	6.10 **	6.16 **		3.24
3.62	Royal	1.78 **	0.06^{ns}		1.74 **	1.50
9.72	*J. regia*	7.94 **		3.20 **	1.46 **	4.70
3.56	Paradox		2.83 *	0.37 ^{ns}	1.37 **	1.87
(g)		Nut weight differences				(g)

* and **: Significant at the 5% and 1% of probability levels, respectively
ns: Not significant

the samples were 60.99% and 70.74% (Table 2). 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 show more variation than those found in this study. Another study found walnut kernels containing from 52 to 72% oil (Martinez *et al.*, 2006). In a study conducted in Turkey, Caglariymak (2003) reported 63% as the average oil value of the studied genotypes.

The Kruskal-Wallis method was used to examine the differences in several qualitative traits among species and interspecific hybrids; they were found to be significant at the 1% statistical level, indicating broad diversity among genotypes, species, and hybrids (Table 6).

Correlations between pairs of characters (Table 7) indicated that there were highly significant correlations between characters such as nut weight and kernel size, with a roundness index of r = 0.921** and r = 0.754**, respectively, and kernel weight and time of maturity (r = 0.797**).

Principal coordinate analysis was used to classify the genotypes using qualitative data. The first two principal coordinates were used to create an outline of the differences and relationships between the samples (Fig. 1). According to Fig. 1, the studied species were clearly distinguishable from one other, with black walnut (*J. nigra*) genotypes at the bottom right of diagram and Persian walnut *(J. regia)* cultivars at the top left of the diagram. Royal interspecific hybrid shows a closer relationship with black walnut genotypes. Furthermore, in the figure, Paradox hybrid appears next to the black walnuts (Fig. 1). This classification and separation were also clearly observed in cluster analysis (Fig. 2).

Table 6. Evaluation of the differences in qualitative traits of genotypes using the Kruskal-Wallis method.

Df	Chi-Square	Attributes
3.	70.68**	Roundness index
3.	363.32**	Shape of base perpendicular to suture
3.	183.91**	Shape of apex perpendicular to suture
3.	385.42**	Prominence of apical tip
3.	263.40**	Prominence of pad on suture
3.	285.06**	Structure of shell surface
3.	405.15**	Adherence of two shell halves
3.	274.28**	Kernel: ease of removal
3.	44.78**	Intensity of ground color
3.	148.85**	Kernel size
3.	99.48**	Nut size
3.	226.60**	Time of maturity
3.	251.01**	Thickness of primary and secondary dividing membranes
3.	541.41**	Shell thickness

**: Significant at the 5% probability level

Table 7. Correlation coefficients between pairs of characters.

	Roundness index	Kernel size	Time of maturity	Shell thickness	Ease of kernel removal	Nut weightt	Kernel weight
Roundness index	1.000						
Kernel size	0.754 **	1.000					
Time of maturity	0.276 **	0.08 ns	1.000				
Shell thickness	0.416 **	0.085 ns	0.307 **	1.000			
Ease of removal	0.524 **	0.136 ns	0.005 ns	0.509 **	1.000		
Nut weight	0.921 **	0.700**	0.203 **	0.462 **	0.638 **	1.000	
Kernel weight	0.310 ns	0.061ns	0.797 **	0.025 ns	0.265 **	0.001 ns	1.000

* and **: Significant at the 5% and 1% of probability levels, respectively
ns: Not significant

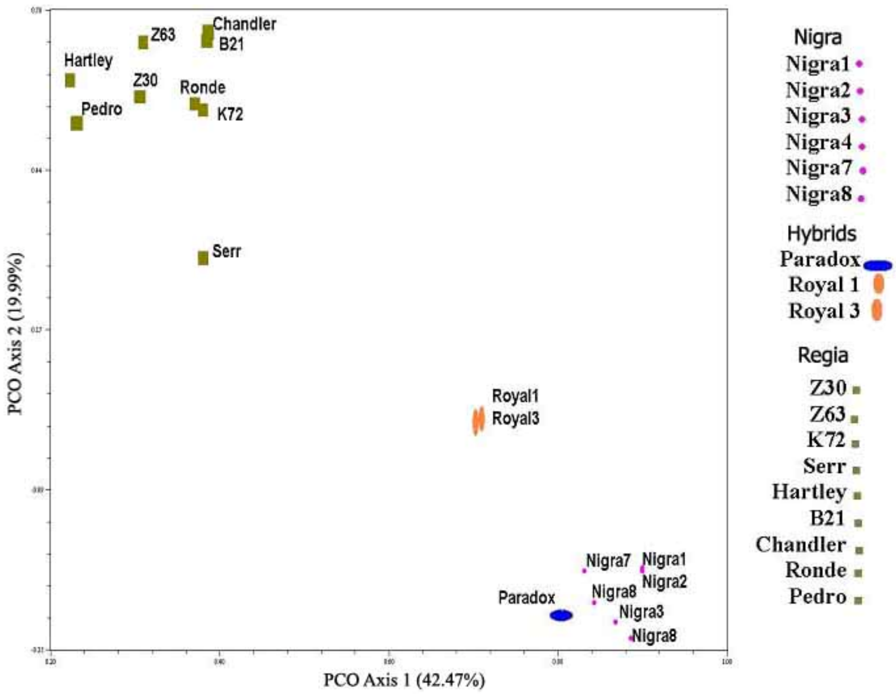

Fig. 1. Biplot for classifying genotypes using the first two principal coordinates (PCOs).

Meanwhile, the genotypes were classified using cluster analysis and the Ward method (Fig. 2). Based on cluster analysis, the genotypes were divided into three distinct groups. The first cluster consisted of six different black walnut genotypes (Nigra1, Nigra8, Nigra7, Nigra4, Nigra3, Nigra2,); the second cluster included Royal interspecific hybrids (Royal1, Royal3) and Paradox; and the third cluster consisted of Persian walnut cultivars/genotypes (Z63, Hartley, Seer, Z30, K72, Ronde, Pedro, Chandler, and B21) (Fig. 2). So morphological characteristics are able to appropriately differentiate walnut species and genotypes into separate groups using multivariate statistical methods.

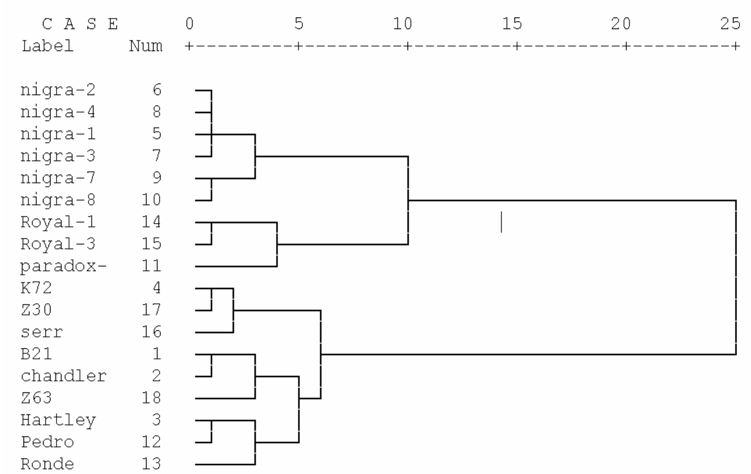

Fig. 2. Classification of genotypes using cluster analysis and the WARD method.

CONCLUSIONS

In conclusion, this study compared different characteristics (especially vegetative characteristics such as trunk cross-sectional area and canopy diameter) that could be very important when using the studied materials as rootstock for *J. regia* cultivars/genotypes. Results suggest that interspecific hybrids such as Paradox and Royal could grow much more vigorously and could be used as vigorous rootstock for walnut species. On the other hand, the differences among the species and interspecific hybrids in several of the evaluated characters were so pronounced that we were able to classify them in separate groups.

REFERENCES

Arzani, K., H. Mansouri Ardakan, and A. Vezvaei. 2008. Morphological variation among Persian walnut (*Juglans regia* L.) genotypes from central Iran. New Zealand J. Crop Hort. Sci. 36: 159-168.

Balci, I., F. Balta, A. Kazankaya, and S. M. Sen. 2001. Promising native walnut genotypes (*Juglans regia* L.) of the East Black Sea region of Turkey. J. Am. Pomol. Soc. 55(4): 204-208.

Caglarirmak, N. 2003. Biochemical and physical properties of some walnut genotypes (*Juglans regia* L.). Nahrung/Food 47(1): 28-32.

Darvishian, M. 2003. Walnut development new methods. Compilation Group of Engineers, Institute for Agricultural Research, Southwestern France. Tech. Publ. in Iran 36.

Diaz, R., E. Alonso, and J. Fernandez-Lopez. 2004. Genetic and geographic variation in seed traits of common walnut among twenty populations from the west of Spain. Acta Hort. 705-.

Ehteshamnia, A., M. Sharifani, K. Vahdati, V. Erfani, S. J. Musavizadeh, and S. Mohsenipoor. 2009. Investigation on morphological diversity among native populations of walnut (*Juglans regia*) in Golestan Province. J. Plant Production 16(3): 29-48 (In Persian).

Eskandari, S., D. Hassani, and A. Abdi. 2006. Investigation on genetic diversity of Persian walnut and evaluation of promising genotypes. Acta Hort. 705: 159-166.

Forde, H. I. 1975. Walnuts. Pp. 439-455. In. Janick, I. and Moore, J. N. (eds.). Advances in fruit breeding. Prudu University Press, West Lafayette.

Germain, E. 1993. The Persian walnut in Iran. NUCIS Newsletter No. S-6.

Ghasemi, M., K. Arzani, D. Hassani, and Sh. Ghasemi. 2010**.** Fatty acid composition of some selected walnut (*Juglans regia* L.) genotypes in Markazi province. JFST 7(1): 31-37.

Gholami, M. 1990. The study of walnut genotypes for cultivar selection in hamedan province. Pp. 4. In Proceedings of the First National Walnut Congress (In Persian).

Hagh-Jooyan, R. 2003. Investigation genetic diversity of tuyserkan walnut population and four walnut collections of country by morphologic and RAPD markers. Ph. D. Thesis in Horticulture Science, Research Sciences Unit of Tehran, Islamic Azad University,.

Hassani, D., J. Atefi, R. Haghjooyan, R. Dastjerdi, M. Keshavarzi, M. R. Mozaffari, A. Soleimani, A. R. Rahmanian, F. Nematzadeh, and A. Malmir. 2012a. Jamal, a new Persian walnut cultivar for moderate-cold areas of Iran. Seed and Plant Improvement J. 28(3): 523-525.

Hassani, D., J. Atefi, R. Haghjooyan, R. Dastjerdi, M. Keshavarzi, M. R. Mozaffari, A. Soleimani, A. R. Rahmanian, F. Nematzadeh, and A. Malmir. 2012b. Damavand, a new walnut cultivar as a pollinizer for Iranian walnut cultivars and genotypes. Seed and Plant Improvement J. 28(3): 529-531.

Jafari-Sayadi, M. H. 2006. Genetic diversity of iranian native walnut population of northern forests and morphological comparation them with walnut other region of country. Ph. D. Thesis in Forestry, Tehran University. (In Persian). Pp.

Malvolti, M. E., S. Fineschi, and M. Pigliucci. 1994. Morphological integration and genetic variability in *Juglans regia* L. J. Herdity 85: 389-394.

Mamadjanov, D. K. 2001. Walnut fruit forests and diversity of walnut trees in Kyrgyzstan. Acta Hort. 705 p.

Manly, B. F. J. 1994. Multivariate statistical methods. Chapman & Hall. 315 pp.

Martinez, M. L., M. A. Mattea, and D. M. Maestri. 2006. Varietal and crop year effects on lipid composition of walnut (*Juglans regia*) genotypes. JAOCS 83(9): 791-796.

Mitra, S. K., D. S. Rathor, and T. K. Bose. 1991. Temperate Fruit Horticulture and Allied Pub. India. 646 p.

Rezaei, R., G. H. Hassani, D. Hassani, and K. Vahdati. 2008. Morphobiological characteristics of some newly selected walnut genotypes from seedling collection of Kahriz-Orumia. J. Hort. Sci. 9(3): 205-214.

Saadat, Y. A., and P. Zandi. 2001. Identification and evaluation of Persian walnut elite trees in Fars Province. Pajoohesh va Sazandegi 52: 14-18. (In Persian)

Sharma, S. D., and O. C. Sharma. 2001. Studies on variation in nut and kernel characters and selection of superior walnut seedling (*Juglans regia* L.) from Garsa and Jogindernagar areas of Himachal Pradesh. Acta Hort. 544: 47-50.

Solar, A. 1990. Phenological and pomological characteristics of walnut cultivars in northeastern Slovenia. Acta Hort. 284: 167-174.

Solar, A., and F. Stampar. 2004. Genotypic differences in branching and fruiting habit in common walnut (*J. regia* L.). Ann. Bot. 92: 317-325.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics, a biometrical approach. 3rd edition McGraw-Hill Book Co. 633 pp.

Westwood, M. N. 1993. Temperate zone pomology: physiology and culture. 3rd edition. Timber Press, Portland. 553 pp.

Zeneli, G., H. Kola, and D. Maxhum. 2005. Phenotypic variation in native walnut populations of northern Albania. Sci. Hort. 105: 91-100.