AgroLife Scientific Journal - Volume 7, Number 2, 2018
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

CHARACTERIZATION OF SOME WALNUT (*Juglans regia* L.) BIOTYPES BASED ON THE BIOMETRICAL AND BIOCHEMICAL PARAMETERS OF NUTS

Olimpia IORDĂNESCU, Maria BĂLA, Florin SALA, Daniela SCEDEI, Melinda TOTH

Banat University of Agricultural Sciences and Veterinary Medicine „King Michael I of Romania" from Timişoara, 119 Calea Aradului Street, 300645, Timișoara, Romania;
Phone: +40 256 277091; Emails: olimpia.iordanescu@yahoo.com; florin_sala@usab-tm.ro; mariabalamonicabala@yahoo.com; dana_olaru78@yahoo.com; toth_luisa@yahoo.com

Corresponding author email: florin_sala@usab-tm.ro

Abstract

The walnut (Juglans regia L.) is an important species cultivated worldwide for both fruit and its quality wood. There are very many biotypes that are studied in relation to pedoclimatic conditions and fruit quality to identify valuable genotypes. The present study aimed at the comparative evaluation of seven walnut genotypes (B501 - B507) based on the biometric parameters of fruits, the shape and size indices and the core quality indices (% of total fruit and biochemical indices). In terms of fruit size, genotypes B502 and B501 have been revealed with very large and large nuts. The fruit mass differentiated the studied genotypes and the B502 genotype had the highest values, while on the basis of the core mass parameter, the genotypes B502 and B506 were the ones with highest values. In terms of biochemical indices, B501 and B503 genotypes had the highest content of extraction protein - fat and B501 biotype had also the highest total carbohydrate content. Clustering analysis, based on Euclidean distances, has grouped the studied genotypes as statistically safe, Coph. corr. = 0.918. From PCA analysis, PC1 explained 94.692% and PC2 4.2127% of the existing variance between the genotypes in relation to the quality index, protein, carbohydrates and fats.

Key words: *biometric parameter, biochemical indices, Euclidian distances, PCA, walnut.*

INTRODUCTION

The walnut, *Juglans regia* L. is a species originating from Central Asia (Iran, Afghanistan, Turkey) as well as from some countries in the Balkan Peninsula and is being cultivated worldwide for its fruits and quality wood (Prasad, 2003; Rottoli and Castiglioni, 2009; Pollegioni et al., 2014, 2017).

The walnut has many genotypes that are studied in relation to pedoclimatic conditions and fruit quality (Khadivi-Khub et al., 2015).

The analysis and characterization of genotypes was based on morphological, fructification, stress tolerance parameters, both by classical methods and on genetic markers (Eskandari et al., 2005; Arzani et al., 2008; Ebrahimi et al., 2011; Cosmulescu and Botu, 2012), leaf study and characterization based on specific physiological indices such as foliar surface, leaf indexes, chlorophyll content, photosynthetic rate, and other pomologie studies (Jivan and Sala, 2014; Sala et al., 2015; Drienovski et al., 2017a, b; Kumar et al., 2017; Sala et al., 2017).

As a result of the walnut's major interest in fruit production and food consumption, many studies have analyzed and characterized the fruits and kernells in terms of specific biometric parameters and core quality (Sharma and Sharma, 2001; Solar et al., 2003; Arzani et al., 2008; Beyhan et al., 2016). The quality of nuts is dependent on genotype, but it also varies with the cultivation area, ecological factors such as soil, altitude, climatic conditions but also technological factors (Koyuncu et al., 2005; Ercisli et al., 2012; Yarilgaç et al., 2013). The walnuts core is highly appreciated for the nutritional value given by the content of minerals, fatty acids, proteins, carbohydrates, etc., these nutritional principles being evaluated in relation to genotype, vegetation and technological conditions (Amaral et al., 2003; Ozkan and Koyuncu, 2005; Martinez and Maestri, 2008).

The present study aimed at the evaluation and characterization of some walnut genotypes based on biometric parameters and specific and quality indices of fruits.

MATERIALS AND METHODS

For the characterization of the 7 walnut genotypes that were studied, the fruit dimensions, specific and quality indices of the fruits were evaluated.

The biological material was represented by seven walnut genotypes, marked B501-B507, from Timisoara area, respectively from a private family orchard, which is why the notation was done with the B sign followed by the number that represented the trees from which the samples were taken.

Biometric measurements

The fruit dimensions were determined by measuring with an electronic calliper, having an accuracy of ± 0.05 mm. Fruit weight was determined by weighing with a precision balance of ± 0.002 g. The size and shape indices were calculated based on the fruit size, and the percentage of kernel was calculated reporting to the fruit weight.

Biochemical determinations

The gross protein was determined by the Kjeldahl method of total nitrogen determination. Calculation of the total protein quantity was performed following the mineralization of the sample and determination of the total nitrogen. Gross fat was determined by Soxhlet method for total fat determination. Measurement of total lipids was achieved by applying the Soxhlet method and total carbohydrate content was determined by the Fehling method.

Statistical calculation of experimental data

The experimental data were analyzed by vari-ance analysis, correlation analysis, cluster analysis based on Euclidean distances and PCA method, using PAST software (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

The results obtained on fruit biometry, namely the large diameter, the small diameter and the height of the fruits, are presented in Tables 1-3. Walnuts' large diameter ranged from 24.67 mm for B 503 biotype that was statistically assured, being very significant negative compared to the control and 44.00 mm for B 502 biotype that was in turn statistically assured, being very significant positive compared to the control, the value of the control variant being 32.57 mm.

Table 1. Big diameter of walnuts

No.	Biotype	Big diameter (mm)	Relative value (%)	Difference to the control	Significance
1	Average value of the experiment	32.57	100.00	0.00	Control
2	B 501	35.00	107.47	2.43	-
3	B 502	44.00	135.11	11.43	xxx
4	B 503	24.67	75.74	-7.90	000
5	B 504	29.67	91.10	-2.90	-
6	B 505	31.33	96.21	-1.23	-
7	B 506	32.33	99.28	-0.23	-
8	B 507	31.00	95.19	-1.57	-
	LSD 5%=3.02 mm LSD 1%=4.08 mm LSD 0.1%=5.43 mm				

Table 2. Small diameter of walnuts

No.	Biotype	Small diameter (mm)	Relative value (%)	Difference to the control	Significance
1	Average value of the experiment	31.23	100.00	0.00	Control
2	B 501	33.33	106.72	2.10	x
3	B 502	39.67	127.00	8.43	xxx
4	B 503	24.67	78.98	-6.57	000
5	B 504	29.33	93.92	-1.90	-
6	B 505	30.67	98.19	-0.57	-
7	B 506	31.67	101.39	0.43	-
8	B 507	29.33	93.92	-1.90	-
	LSD 5%=2.10 mm LSD 1%=2.84 mm LSD 0.1%=3.78 mm				

Table 3. Height of walnuts

No.	Biotype	Height (mm)	Relative value (%)	Difference to the control	Significance
1	Average value of the experiment	37.30	100.00	0.00	Control
2	B 501	39.67	106.34	2.36	-
3	B 502	49.33	132.25	12.03	xxx
4	B 503	27.33	73.27	-9.97	000
5	B 504	37.33	100.08	0.03	-
6	B 505	38.00	101.87	0.70	-
7	B 506	36.33	97.40	-0.97	-
8	B 507	33.67	90.25	-3.64	0
	LSD 5%=3.08 mm		LSD 1%=4.16 mm	LSD 0.1%=5.54 mm	

The other biotypes recorded close values and also lose to the value of the control variant, which is why no significance was recorded. Along with B 503 biotype is also noted B501 biotype that exceeded the average value of the experiment, all the other biotypes having values below the average.

The small diameter of walnuts had values ranging from 24.67 mm for B 503 biotype and 39.67 mm for B 502 biotype, with an average value of the experiment of 31.23 mm. The biotypes that exceeded the value of the control variant are: B 502 - very significant positive, B 501 - significant positive and B 506 which was not statistically assured. Values below that of the control were recorded in biotypes: B 503 - very significant negative, B 504, B 507 and B 505, none of the three was statistically assured.

The height of walnuts belonging to the studied biotypes had values between 27.33 mm for B 503 biotype and 49.33 mm for B 502 biotype, with an average value of the experiment of 37.30 mm. Higher values than the control variant were recorded in biotypes: B 502, which was the only one statistically assured, being very significant positive, but also in the biotypes: B 501, B 505 and B 504, which due to the values close to that of the control variant did not register any significance.

On the opposite side there was B 503 biotype that was statistically assured, being very significant negative compared to the control, followed by B 507 biotype that was significant negative than the control and B 506 biotype that was not statistically assured.

Based on the walnuts' size, the size and shape indices were determined for the studied genotypes, and they are presented in Tables 4 and 5. According to the classification of walnuts in relation to the size index proposed by Cosmulescu and Baciu (2003) the fruits of the studied genotypes were classified into: very large walnuts, genotype B 502; large walnuts, genotype B 501 and medium walnuts, genotypes B 506, B 505, B 504, B 507 and B 503.

Table 4. Size index of walnuts

No.	Biotype	Size index (mm)	Relative value (%)	Difference to the control	Significance
1	Average value of the experiment	33.72	100.00	0.00	Control
2	B 501	36.00	106.75	2.28	-
3	B 502	44.33	131.47	10.61	xxx
4	B 503	25.55	75.78	-8.17	000
5	B 504	32.11	95.23	-1.61	-
6	B 505	33.33	98.84	-0.39	-
7	B 506	33.44	99.17	-0.28	-
8	B 507	31.33	92.91	-2.39	0
	LSD 5%=2.35 mm		DL 1%=3.18 mm	DL 0.1%=4.23 mm	

Table 5. Form index of walnuts

No.	Biotype	Form index (mm)	Walnut form
1	B 501	115.23	ovoidal
2	B 502	117.91	ovoidal
3	B 503	110.91	ovoidal
4	B 504	126.71	ellipsoidal
5	B 505	122.53	ovoidal
6	B 506	113.54	ovoidal
7	B 507	111.56	ovoidal

According to the biometric parameters of walnuts belonging to the studied biotypes, the values calculated for the shape index ranged from 110.91 for biotype B 503 and 126.71 for biotype B 504, therefore the form found in the genotypes studied was ovoidal for: B 501, B 502, B 503, B 505, B 506 and B 507; and ellipsoidal for B 504.

The weight of the walnuts was between 6.8 grams for biotype B 503 and 23.20 grams for biotype B 502 with an average value of the experiment of 12.54 grams. According to the classification proposed by Cosmulescu and Baciu (2003), the studied biotypes, in terms of the character of weight, are as follows: very large walnuts, biotype B 502; large walnuts, biotypes B 501, B 505 and B 506; medium walnuts: B 504 and B 507; small walnuts: B 503. The results on the fruit mass and the core quality are presented in Tables 6 and 7. Higher values than the one of the control were obtained from the B 502 biotype, which was also significant positive in relation to it, but also by biotypes B 506 and B 501, which were not statistically assured.

Lower values than the control were obtained in B 503 and B 504 biotypes, both of which were very significant negative, followed by B 505 biotype that was significant negative and B 507 biotype, which was not statistically assured.

Clustering analysis, based on Euclidean distances, grouped the studied genotypes as statistically safe, Coph. corr. = 0.918, Figure 1.

The core weight ranged from 2.26 g for biotype B 503 and 7.27 g for biotype B 507, with an average value of the experiment of 4.71 g (Table 7).

The highest core weight values were recorded in biotypes B 502 and B 506, both of which were very significant positive than the control variant, followed by B 505 and B 501 biotypes that were not statistically assured.

The lowest core weight values were recorded in biotypes B 503 and B 504, both of which were very significant negative compared to the control variant, followed by B 507 biotype that was distinctly significant negative to the control.

In the improvement of walnut species the main objectives were: productivity; large and quality nuts; lateral fructification; late flowering; tolerance or resistance to bacteriosis; lesser growth size etc. As regards the size of the fruit, weighing more than 15 g and the minimum percentage of the kernel to be of 40-42%.

Table 6. Walnuts weight

No.	Biotype	Weight (g)	Relative value (%)	Difference to the control	Significance
1	Average value of the experiment	12.54	100.00	0.00	Control
2	B 501	13.70	109.22	1.16	-
3	B 502	23.20	184.96	10.66	xxx
4	B 503	6.80	54.21	-5.74	000
5	B 504	8.21	65.45	-4.33	000
6	B 505	10.20	81.32	-2.34	00
7	B 506	13.93	111.08	1.39	-
8	B 507	11.70	93.28	-0.84	-
	LSD 5%=1.67 g	LSD 1%=2.25 g		LSD 0.1%=3.00 g	

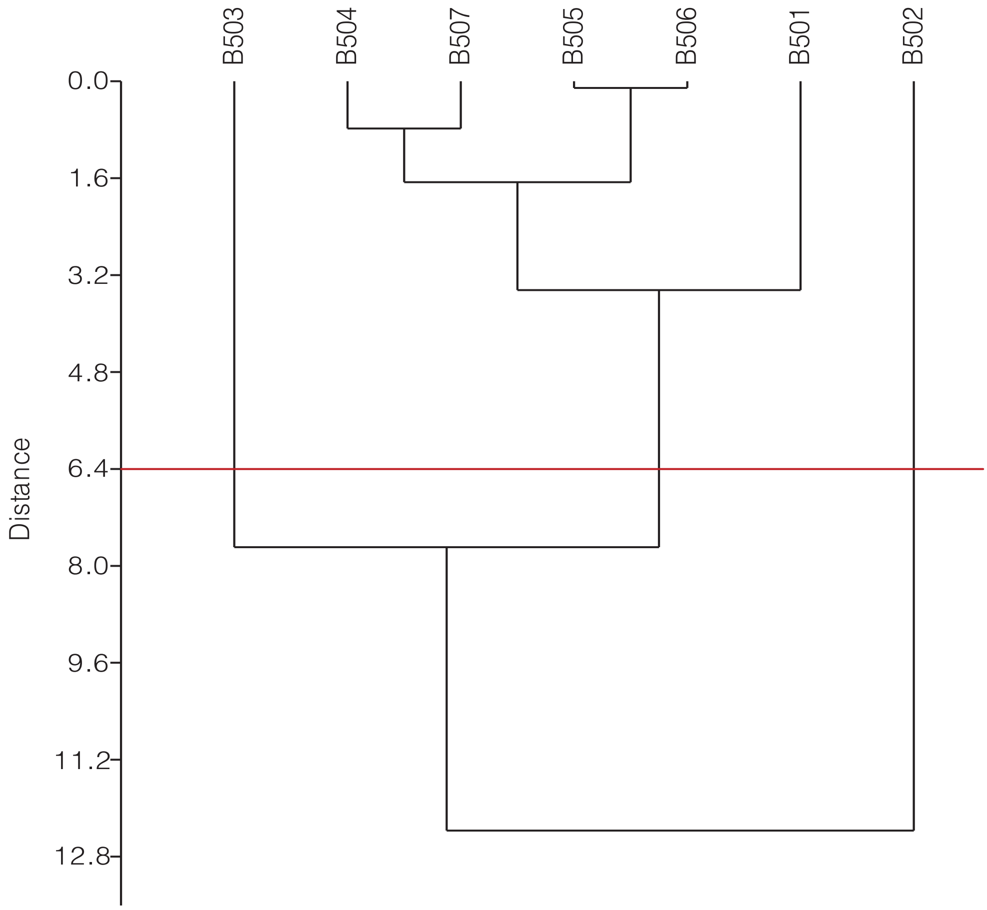

Figure 1. Clustering grouping of the studied biotypes based on Euclidean distances

Table 7. Core (kernel)'s weight

No.	Biotype	Core weight (g)	Relative value (%)	Difference to the control	Significance
1	Average value of the experiment	4.71	100.00	0.00	Control
2	B 501	4.90	103.89	0.18	-
3	B 502	6.50	137.84	1.78	xxx
4	B 503	2.26	48.02	-2.45	000
5	B 504	3.46	73.34	-1.26	000
6	B 505	4.91	104.10	0.19	-
7	B 506	7.27	154.17	2.55	xxx
8	B 507	3.74	79.35	-0.97	00
	LSD 5%=0.59 g	LSD 1%=0.80 g		LSD 0.1%=1.07 g	

The percentage of walnut kernel in the studied biotypes ranged from 28.01% for B 502 biotype and 52.19% for B 506 biotype (Table 8). From the analysis of the recorded data, the valuable biotypes regarding the core percentage of the fruit are: B 506, B 505, B 504 - all of which exceed 42% of the total fruit. Of the biochemical determinants of the studied biotypes, the extracted protein was between 5.5% (B 506) and 7.3% (B 503), the gross fat had values between 46.5% (B 507) and 55.3% (B 501), and the total carbohydrates had values that ranged from 0.7% (B 504) and 2.2% (B 501), all the values being presented in Table 9. From PCA analysis, PC1 explains 94.692% and PC2 explains 4.2127% of the existing variance within the studied genotypes, in relation to the quality index, the protein, carbohydrates and fats, Figure 2.

Table 8. Characteristics of the walnut core

No.	Biotype	The release of the core	Core's extraction	Core's color	Shell's thickness/walnut's weight	% core
1	B 501	easy	halves	dark yellow	8.8/13.7	36.34
2	B 502	easy	halves	brown	16.7/23.2	28.01
3	B 503	moderate	quarters	brown	3.7/6.3	37.76
4	B 504	moderate	quarters	dark yellow	4.8/8.21	41.41
5	B 505	easy	halves	brown	5.3/10.21	48.09
6	B 506	easy	halves	dark yellow	7.0/14.6	52.19
7	B 507	hard	broken	brown	8.7/12.7	31.49

Table 9. Chemical composition of walnut's core

No.	Biotype	Extracted protein %	Gross fat %	Total carbohydrates %
1	B 501	7.2	55.3	2.2
2	B 502	5.8	49.1	1.2
3	B 503	7.3	51.3	0.84
4	B 504	6.8	50.1	0.7
5	B 505	5.8	47.8	0.9
6	B 506	5.5	51.2	1.2
7	B 507	6.2	46.5	1.0

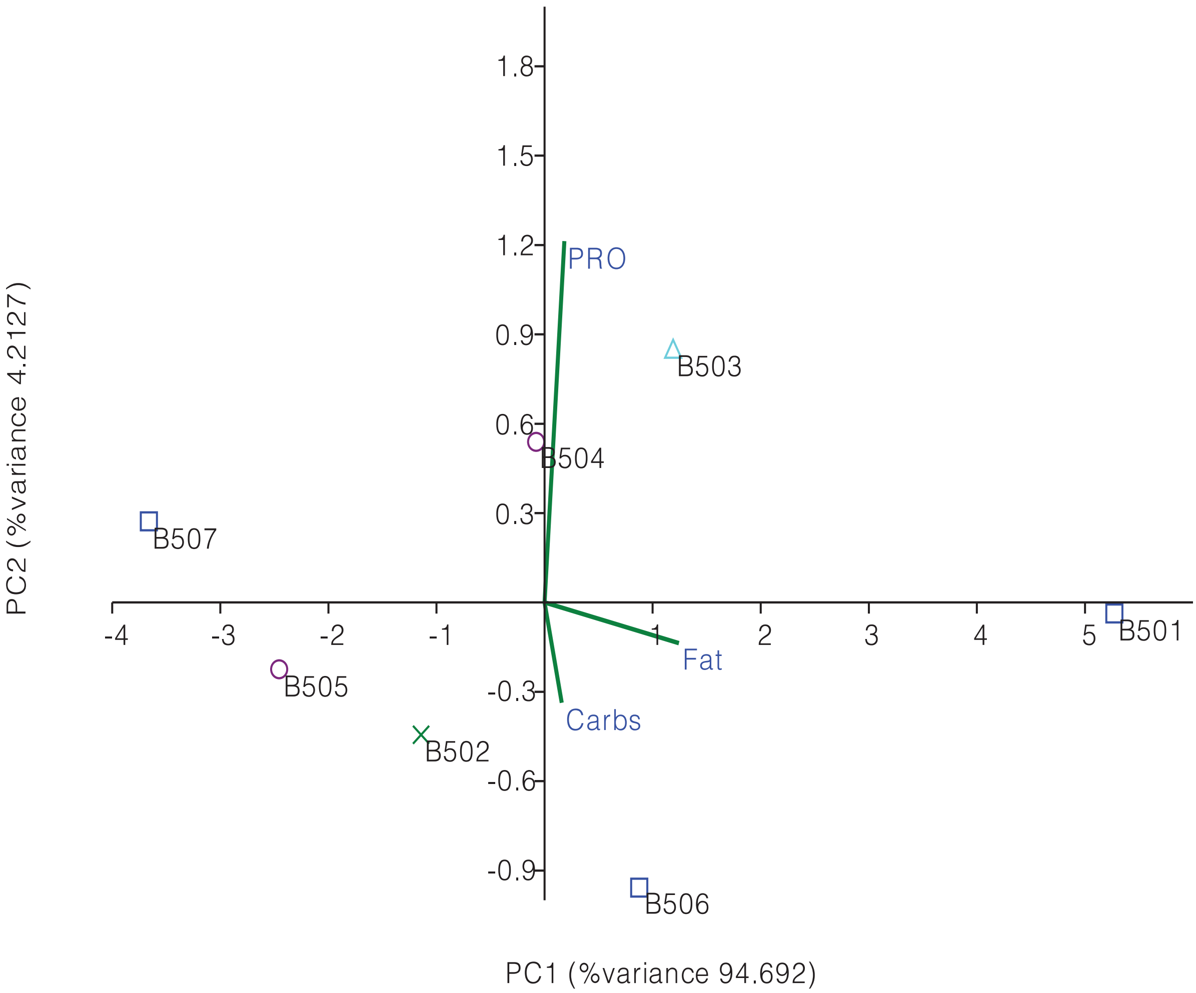

Figure 2. Principal Component Analysis for the studied walnut genotypes

The fruit shape and size indices have been studied in other species because they facilitate the classification of fruit by quality classes for both industrialization, market and seed material (Clark et al., 2008; Anghel et al., 2017). The variation in fruit size is determined by genotype, but also by soil conditions, mineral elements available for tree nutrition, climatic conditions and technological factors (Coggeshall, 2011; Sala, 2011). The quality indices of walnuts kernel are influenced by tree planting conditions, walnut formation conditions, harvest moment (Khir et al., 2011). Altuntas and Erkol (2010) found the variation in the physical properties of walnuts relative to the moisture content. Water regime and water stress also had a variable influence on the size of the walnuts and the quality of the walnut kernel (Ramos et al., 1978; Ogunsina and Bamgboye, 2014).

CONCLUSIONS

The analysis of the studied walnut biotypes (B 501-B 507) based on the biometric parameters of fruits, the shape and size indices and the core quality indices (% of the total fruit and biochemical indices) revealed some valuable biotypes both for the production of nuts, and also for germoplasm selection.

Among the biotypes, B 502 and B 501 genotypes remarked with very large and large walnuts, B 502 genotype with the largest fruit weight, B 502 and B 506 genotypes with the largest core weight, B 501 and B 503 biotypes with the highest extractable protein and fats content, and B 501 biotype with the highest total carbohydrate content.

REFERENCES

Altuntas E., Erkol M., 2010. Physical properties of shelled and kernel walnuts as affected by the moisture content. Czech Journal of Food Sciences 28 (6): p. 547-556.

Amaral J.S., Casal S., Pereira J.A., Seabra R.M., Oliveira B.P.P., 2003. Determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Portugal. Journal of Agricultural and Food Chemistry 51 (26): p. .7698-7702.

Anghel A., Sala F., Nicolin L.A., 2017. Variation of some biometric parameters in oak fruits. Journal of Horticulture, Forestrey and Biotechnology 21 (2): p. 26-32.

Arzani K., Mansouri Ardakan H., Vezvaei A., Reza Roozban M., 2008. Morphological variation among Persian walnut (*Juglans regia*) genotypes from central Iran. New Zealand Journal of Crop and Horticultural Science 36: p. 159-168.

Beyhan O., GozleS., Gundogdu M., Ercisli S., 2016. Physico-chemical and antioxidant characteristics in fruits of walnut (*Juglans regia* L.) genotypes from inner Anatolia. 2016. Notulae Botanicae Horti Agrobotanici 44 (2): p. 586-592.

Clark J.R., Hemery G.E., Savill P.S., 2008.Early growth and form of common walnut (*Juglans regia* L.) in mixture with tree and shrub nurse species in southern England. Forestry: An International Journal of Forest Research 81 (5): p. 631-644.

Coggeshall M.V., 2011. Black walnut: A nut crop for the Midwestern United States. Hort Science 46 (3): p. 340-342.

Cosmulescu S., Botu M., 2012. Walnut biodiversity in south-western Romania resource for perspective cultivars. Pakistan Journal of Botany 44: p. 307-311.

Drienovsky R., Nicolin A.L., Rujescu C., Sala F., 2017a. Scan Leaf Area – A software application used in the determination of the foliar surface of plants. Research Journal of Agricultural Science 49 (4): p. 215-224.

Drienovsky R., Nicolin A.L., Rujescu C., Sala F., 2017b. Scan Sick & Healthy Leaf - A software application for the determination of the degree of the leaves attack. Research Journal of Agricultural Science 49 (4): p. 225-233.

Ebrahimi A., Fatahi R., Zamani Z., 2011. Analysis of genetic diversity among some Persian walnut genotypes (*Juglans regia* L.) using morphological traits and SSRs markers. Scientia Horticulturae 130: p. 146-151.

Ercisli S., Sayinci B., Kara M., Yildiz C., Ozturk I., 2012. Determination of size and shape features of walnut (*Juglans regia* L.) cultivars using image processing. Scientia Horticulturae 133 (6): p. 47-55.

Eskandari S., Hassani D., Abdi A., 2005. Investigation on genetic diversity of Persian walnut and evaluation of promising genotypes. Acta Horticulturae 705: p. 159-166.

Hammer Ø., Harper D.A.T., Ryan P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4 (1): p. 1-9.

Jivan C., Sala F., 2014. Relationship between tree nutritional status and apple quality, Horticultural Science 41 (1): p. 1-9.

Khadivi-Khub Ebrahimi A., Mohammadi A., Kari A. 2015. Characterization and selection of walnut (*Juglans regia* L.) genotypes from seedling origin trees. Tree Genetics & Genomes 11:54.

Khir R., Pan Z., Atungulu G.G., Thompson J.F., Shao D., 2011. Size and moisture distribution characteristics of walnuts and their components. Food and Bioproces Biotechnology 6: p. 771-782.

Koyuncu M.A., Ekinci K., Gun A., 2005. The effects of altitude on fruit quality and compression load for cracking of walnuts (*Juglans regia* L.). Journal of Food Quality 27: p. 407-417.

Kumar, K., Kumar, S., Sankar, V., Sakthivel, T., Karunakaran, G., Tripathi, P.C., 2017. Non-destructive estimation of leaf area of durian (*Durio zibethinus*) - An artificial neural network approach. Scientia Horticulturae (219): p. 319-325.

Martinez M.L., Maestri D.M., 2008. Oil chemical variation in walnut (*Juglans regia* L.) genotypes grown in Argentina. European Journal of Lipid Science and Technology 110 (12): p. 1183-1189.

Ogunsina B.S., Bamgboye A.I., 2014. Pre-shelling parameters and conditions that influence the whole kernel out-turn of steam-boiled cashew nuts. Journal of the Saudy Society of Agricultural Sciences 13 (1): p. 29-34.

Ozkan G., Koyuncu M.A., 2005. Physical and chemical composition of some walnut (*Juglans regia* L.) genotypes grown in Turkey. Grasas y Aceites, 56 (2): p. 141-146.

Pollegioni P., Woeste K., Chiocchini F., Olimpieri I., Tortolano V., Clark J., Hemery G.E., Mapelli S., Malvolti M.E., 2014. Landscape genetics of Persian walnut (*Juglans regia* L.) across its Asian range. Tree Genetics & Genomes 10: p. 1027-1043.

Pollegioni P., Woeste K., Chiocchini F., Del Lungo S., Ciolfi M., Olimpieri I., Tortolano V., Clark J., Hemery G.E., Mapelli S., Malvolti M.E., 2017. Rethinking the history of common walnut (*Juglans regia* L.) in Europe: Its origins and human interactions. PLoS ONE 12 (3): e0172541.

Prasad R.B.N., 2003. Walnuts and Pecans. Encyclopedia of Food Sciences and Nutrition. Second Edition, p. 6071-6079.

Ramos D.E., Brown L.C., Uriu K., Marangoni B., 1978. Water stress affects size and quality of walnuts. California Agriculture, 5-6.

Rottoli M., Castiglioni E., 2009. Prehistory of plant growing and collecting in northern Italy, based on seed remains from the early Neolithic to the Chalcolithic (c.5600-2100 cal B.C.). Veg Hist Archaebot. 18: p. 91-103.

Sala F., 2011. Agrochimie. Editura Eurobit, Timișoara, 534 pp.

Sala F., Arsene G.-G., Iordanescu O., Boldea M., 2015. Leaf area constant model in optimizing foliar area measurement in plants: A case study in apple tree. Scientia Horticulturae, 193: p. 218-224.

Sala F., Iordanescu O., Dobrei A., 2017. Fractal analysis as a tool for pomology studies: case study in apple. AgroLife Scientific Journal 6 (1): p. 224-233.

Sharma O.C., Sharma S.D., 2001. Genetic divergence in seedling trees of Persian walnut *(Juglans regia* L.) for various metric nut and kernel characters in Himachal Pradesh. Scientia Horticulturae 88: p. 163-171.

Solar A.A., Ivančič A., Štampar F., 2003. Morphometric characteristics of fruit bearing shoots in Persian Walnut (*Juglans regia* L.) - Potential selection criteria for breeding. European Journal of Horticultural Science, 68 (2): p. 86-92.

Yarilgaç T., Balta M.F., Borasan L., Bülbül C., 2013. Fruit characteristics of natural walnut (*Juglans regia* L.) genotypes of Catak and Taspinar Villages (Corum). Acta Horticulturae, 981: p. 135-140.