

## Some Pomological Properties of Promising Seed Propagated Walnut Genotypes from Inner Turkey

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### Abstract

In this study, fruits from seed propagated walnut (*Juglans regia* L.) trees were collected two consecutive years in harvest seasons in Yozgat province in Turkey. Considering two years results, promising five genotypes were determined as cultivar candidate. In the promising genotypes, nut weight ranged from 12.55 (Y11) to 15.08 g (Y15), kernel weight ranged from 5.23 (Y11) to 7.34 g (Y15) and kernel ratio varied between 41.67 (Y11) to 50.84% (Y1), respectively. Linoleic acid was the only polyunsaturated fatty acids and oleic, palmitoleic and gondoic acids determined as major monounsaturated acids ranged from 30.36 to 48.43%, 0.05 to 0.14% and 0.22 to 0.29%, respectively. Propylparaben was the major phenolic acid among the determined phenolic acids in fruits of all five promising genotypes and Y16 had the highest amount of propylparaben (128.08 mg per kg) in its kernel. Malic and tartaric acid were the major organic acids in walnut kernels ranged from 47.88 to 78.51 mg per 100 g and 30.27 to 49.60 mg per 100 g, respectively. L-ascorbic acid was the another organic acids in walnut kernels ranged from 10.71 to 19.71 mg per 100 g. Citric acid was non-determined in kernels of Y1, Y14 and Y15 but determined at kernels of Y11 and Y16 as 4.51 and 7.55 mg per 100 g, respectively. It was determined that the oxalic, malonic, succinic, maleic and fumaric acid contents varied between 8.39-12.08 mg per 100 g, 6.02-9.19 mg per 100 g, 2.86-5.32 mg per 100 g, 0.26-3.00 mg per 100 g and 0.26-0.58 mg per 100 g, respectively.

**Keywords:** chemical; genotype; *Juglans regia* L.; morphological and pomological properties

### Introduction

Plant genetic resources are one of the most important natural richness of any country and they provide particularly food and medicine for people's livelihoods. Plant genetic resources are also an important source of some rare genetic traits such as resistance to some plant pest and diseases, resistance to some abiotic and biotic environmental conditions (Halasz *et al.*, 2010; Ersoy *et al.*, 2017; Guliyev *et al.*, 2018; Gunduz and Ozbay, 2018).

The overall aim of plant and animal production is to obtain high yield from both sources. Environmental and genetic background are strongly influence this trait. Thus plant genetic resources are accepted one of the ready breeding material to obtain high yield in different plant species (Sehirali and Ozgen, 1987; Serce *et al.*, 2010; Ersoy *et al.*, 2018).

*Juglans regia*, 'Persian' or 'British' walnut is the most valuable walnut species with great economic importance among eighteen walnut species around the world (Leslie and McGranahan, 1988). It is estimated that Turkey has

over 5 million native, obtained from seeds, walnut trees (Keles *et al.*, 2014). The country is also one of the most important diversity center of *Juglans regia* (Akca *et al.*, 2009). In terms of production amount, Turkey produce 210.000 tons walnut annually and ranked 4<sup>th</sup> after China (1,925,403 tons), USA (571,526 tons) and Iran (349,192 tons) (FAO, 2018).

In Turkey, commercial walnut orchards are available and new orchards are being established. However the majority of walnut trees propagated by seeds in most of the growing regions in Turkey and they can be used not only their fruits but also for timber production. Continuous seed propagation of walnut trees in different agro-climatic conditions in Turkey for a long time revealed promising walnut genetic resources for walnut breeding programs (Asma, 2012). To obtain new walnut cultivars, cross breeding in general has been used however selection among natural seed propagated seedlings could be used as effectively to determine superior genotypes (Keles *et al.*, 2014). Selection among native growing seed propagated genotypes is an easier and faster way to obtained new cultivar. In fact famous Turkish walnut cultivars such as 'Şebin', 'Bilecik',

'Kaman 1', 'Şen 1', 'Maraş 18', etc. have been selected among these natural populations (Akca *et al.* 2012).

Walnut kernels contain various beneficial contents such as fatty acids, mineral compounds, protein, carbohydrate, vitamin, phenolic acids and organic acids (Mitrovic *et al.*, 1997). Previously a wide number studies has been carried out on fat, fatty acids and tocopherol content of seed propagated walnut genotypes (Caglarirmak, 2003; Ozkan and Koyuncu, 2005; Gharibzahedi *et al.*, 2014; Keles *et al.*, 2014; Poggetti *et al.*, 2017). However limited number of study has been conducted on seed propagated genotypes related to phenolics and organic acid content. Thus more data are needed about the nutrient contents of walnut fruits.

## Materials and Methods

### *Plant material*

The study was carried out in 2017-2018 on naturally seed propagated genotypes found in the villages of Hisarbey and Akçakışla belonging to Yozgat province. Many walnut trees are naturally grown or cultivated in most of the region of Yozgat and Hisarbey and Akcakışla villages are very famous with seed propagated walnut trees. The aim of this study was to determine the promising genotypes selected from to the villages and provide more information about morphological, pomological and chemical properties of naturally grown walnut trees. At the beginning of the study, pre-selection was done according to the general condition of trees such as disease and pest situation, productivity and nut quality and totally 16 walnut genotypes were found suitable for research. Morphological and pomological properties were examined according to Keles *et al.* (2014). Promising genotypes among all genotypes were determined by considering weighted ranking method including the average nut weight, kernel ratio, ease of kernel's separate from the shell, kernel colour, anthracnose and codling moth situation of genotypes. The nut traits were measured on randomly selected 20 nuts (Keles *et al.*, 2014). After the use of weighted ranking method, 5 genotypes were determined as superior among all genotypes.

### *Extraction method for phenolic acids and organic acids*

The walnut fruit samples have been extracted according to the method described by Liu *et al.* (2010). Dried, walnut fruits milled into a fine powder. A 2.0 g of walnut fruit powder extracted three times with 20 ml of aqueous ethanol (80%) in an ultrasonicator bath at 40 °C, for 15 min for each extraction. The obtained extracts from three extractions have been combined. After the removal of ethanol using rotary evaporation, the extract dissolved in 10 ml methanol, followed by filtration through a 0.45 µm filter. After this, the clear solution placed in the glass vial of HPLC and the sample was analysed by HPLC-DAD.

### *HPLC analysis for phenolic acids*

Phenolic acids (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, syringic acid, and ferulic acid) separation was performed using HPLC autosampler system model LC-20AT, on a Inertsil C<sub>18</sub> ODS-3 column (5 µm particle size,

4.6 mm × 250 mm, Japan) at 25 °C. A binary solvent system employed consisting of methanol:water:formic acid (10:88:2, v/v) as solvent A and methanol:water:formic acid (90:8:2, v/v) as solvent B. The detection monitored at 280 nm (Ozturk and Tuncel, 2011) using an SPD-M20A photodiode arrays (PDA) detector (Shimadzu, Japan).

### *HPLC analysis for organic acids*

Organic acids (oxalic, tartaric, formic, malic, L-ascorbic, malonic, maleic, citric, succinic, fumaric) acids separation was performed using HPLC autosampler system model LC-20AT, on an Inertsil C<sub>18</sub> ODS-3 column (5µm particle size, 4.6 mm × 250 mm, Japan) at 40 °C. A 0.0125 M H<sub>2</sub>SO<sub>4</sub> was used as mobile phase. The detection monitored at 214 nm (Bhandari and Kawabata, 2004) using an SPD-M20A photodiode arrays (PDA) detector (Shimadzu, Japan).

### *Oil extraction*

Oil extraction was performed based on the method of Bligh and Dyer (1959). Oils of 20 g fruits were extracted using hexane solvent for 1 h using automatic Soxhlet equipment (Gerhardt Soxtherm) and triplicate analysis were reported for each genotype. The residue was dried until a constant weight was observed. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (FAMES) (AOAC, 1990).

### *Fatty acids analysis*

Fatty acid methyl esters (FAMES) contents were analyzed using gas chromatography, model GC-6890N coupled with the mass spectrometer, model MS-5973 MSD (mass selective detector) as described by Tariq *et al.* (2011). The separation was performed on a capillary column DB-5MS (30 m × 0.32 mm, 0.25 µm of film thickness). The conveyor gas was Helium with a flow rate of 1.5 mL/min. The column temperature was set to 120 to 300 °C at the rate of 10 °C/min. The temperature of both injector and detector was programmed at 250 °C. A sample volume of 0.1 µL RSOB in CHCl<sub>3</sub> was injected using a split mode, with the split ratio of 1:10. The mass spectrometer was set to scan in the range of m/z 50-550 with electron impact (EI) mode of ionization.

### *Statistical analysis*

All results were tested by SPSS 20.0 for Windows program. The differences between the means were compared using the Duncan test ( $p < 5\%$ ).

## Results and Discussion

Table 1 and 2 show some morphological and pomological characteristics and pests and disease situation of all genotypes. Promising genotypes are shown in Table 1 and 2 in bold.

### *Morphological characteristic*

As shown in Table 1, all genotypes showed strong or very strong tree vigor. Nine genotypes were determined as upright, five genotypes were spreading and two genotypes had semi-upright growth habit (Table 1).

Trunk circumferences were ranged from 90 to 263 cm among genotypes. Leaflet shape of genotypes showed some differences and eleven genotypes had elliptically, three genotypes had narrow elliptic and two genotypes had narrow elliptic leaflet shape (Table 1). Additionally, five genotypes were determined as anthracnose free and no codling moth was found in nuts of eleven genotypes (Table 1).

#### *Pomological characteristics*

Considering average nut weight, kernel ratio, ease of kernel's separate from the shell, kernel colour, anthracnose and codling moth situation of genotypes, five genotypes were determined as promising. In the selected genotypes, nut weight ranged from 12.55 g (Y11) to 15.08 g (Y15), kernel weight ranged from 5.23 g (Y11) to 7.34 g (Y15) and kernel ratio determined between 41.67% (Y11) to 50.84% (Y1) (Table 2). Shell thickness and nut weight were between 12-18 g, which ideal size for walnuts. Ideal walnut kernel weight should be 6-10 g and kernel should be light in colour (McGranahan and Leslie, 2012). In this study, all selected genotypes showed light kernel colour and two genotypes showed ease to separate kernel from shell, three genotypes showed very ease separation from shell. Moreover, shell thickness ranged from 1.20 mm to 1.56 mm, and an empty kernel percent range from 0 to 6%. In terms of some nut and kernel properties, selected genotypes can be considered as good quality. In previous selection studies carried on different region in Turkey, nut weight varied from 7.82 g to 18.74 g, kernel weight ranged from 4.04 g to 9.00 g, kernel ratio varied from 42.88% to 67.14% and shell thickness varied from 0.58 mm to 2.03 mm (Akca and Koroglu 2005; Keles *et al.*, 2014). The results of our study are similar to previous studies.

#### *Fatty acids*

Fatty acids analyses results are shown in Table 3. Linoleic acid (18:2) was the dominant fatty acid (between

41.48 and 58.35%) in all kernels of superior genotypes. This result is in accordance with the literature (Gharibzahedi *et al.*, 2014; Pogetti *et al.*, 2017). Linoleic acid was followed by oleic (C18:1), palmitic (C16:0), stearic (C18:0), gondoic (C20:1), arachidic (C20:0) and palmitoleic (C16:1) acids, respectively (Table 3). Some of these fatty acids are named as saturated fatty acids and palmitic acid (C16:0), stearic acid (C18:0) and arachidic acid (C20:0) are classified in this group and the others are named as unsaturated fatty acids. Walnut kernels are poor in saturated fatty acids while rich in unsaturated fatty acids that contain polyunsaturated and monounsaturated fatty acids which are valuable for human diet (Nettleton, 1995). In present study linoleic acid was the only polyunsaturated fatty acids and oleic, palmitoleic and gondoic were the monounsaturated acids ranged from 30.36 to 48.43%, 0.05 to 0.14% and 0.22 to 0.29%, respectively. In recent studies, it was stated that the amount of oleic, palmitoleic and gondoic acids ranged from 14.73 to 25.13%, 0.00 to 0.25 and 0.16 to 0.18% in walnut kernels, respectively (Pereira *et al.*, 2008; Gharibzahedi *et al.*, 2014).

Palmitic acid was the major saturated fatty acid in kernels ranged between 5.75 and 8.27% followed by stearic acid ranged from 3.20 to 3.86% and arachidic acid ranged from 0.11 to 0.13%, respectively. In previous studies, concentrations of palmitic acid ranged from 4.64 to 11.21, stearic acid ranged from 2.56 to 5.11% and arachidic acid ranged from 0 to 0.16%, respectively (Ozkan and Koyuncu, 2005; Gharibzahedi *et al.*, 2014; Pogetti *et al.*, 2018). The oil composition of kernels can be affected by some factors such as genotype, ecological conditions, location, harvest time etc. (Crews *et al.*, 2005).

#### *Phenolic acids*

Phenolic compounds are important for humans and many health-beneficial effects of walnut phenolics were reported previously (Anderson *et al.*, 2001; Fukuda *et al.*, 2003; Polonik *et al.*, 2003).

Table 1. Morphological characteristics, pests and disease situation of walnut genotypes

Genotypes	Vigor	Growth habit	Trunk circumference (cm)	Shape of leaflet	Anthracnose	Codling moth
Y1	Strong	Upright	169	Elliptic	Scale 1	+
Y2	Strong	Upright	120	Narrow Elliptic	Scale 1	-
Y3	Very Strong	Spreading	230	Elliptic	Scale 1	-
Y4	Strong	Spreading	90	Narrow Elliptic	Scale 2	-
Y5	Strong	Upright	160	Elliptic	Scale 1	-
Y6	Strong	Spreading	146	Elliptic	Scale 2	-
Y7	Strong	Upright	136	Broad Elliptic	Scale 1	-
Y8	Very Strong	Upright	132	Elliptic	Scale 2	-
Y9	Strong	Spreading	198	Elliptic	Scale 0	+
Y10	Strong	Upright	181	Elliptic	Scale 0	-
Y11	Strong	Upright	114	Elliptic	Scale 1	-
Y12	Strong	Semi-upright	154	Narrow Elliptic	Scale 0	-
Y13	Strong	Upright	133	Elliptic	Scale 2	+
Y14	Very Strong	Upright	263	Elliptic	Scale 0	-
Y15	Strong	Spreading	178	Elliptic	Scale 2	+
Y16	Strong	Semi-upright	163	Broad Elliptic	Scale 0	+

(+): shows the presence of codling moth, (-): shows the absence of codling moth and anthracnose

Table 2. Pomological characteristics of genotypes

Genotypes	Width (mm)	Length (mm)	Nut cheek (mm)	Nut weight (g)	Kernel weight (g)	Kernel ratio (%)	Kernel colour	Shell thickness (mm)	Ease of kernel's removal	Empty kernel percent (%)
Y1	33.84±1.87	39.23±4.56	35.06±2.52	14.32±1.87	7.28±0.83	50.84	Light	1.56±0.14	Easy	0
Y2	29.96±1.25	39.88±1.61	28.21±1.17	10.40±0.75	4.27±0.59	41.06	Very light	1.77±0.23	Difficult	0
Y3	30.63±1.94	41.50±2.67	30.27±2.66	9.96±1.95	4.25±0.56	42.67	Very light	1.40±0.21	Easy	0
Y4	30.55±1.38	32.97±0.86	33.92±1.54	8.16±0.77	4.32±1.37	53.00	Light	1.07±0.22	Very easy	8
Y5	30.45±1.62	38.21±2.09	31.09±2.07	8.98±1.21	4.70±0.70	52.34	Light	0.99±0.20	Very easy	0
Y6	28.37±1.92	31.80±2.05	32.57±1.91	8.01±2.07	3.99±1.77	49.81	Light	1.18±0.15	Medium	15
Y7	31.30±1.16	34.72±1.12	32.86±1.22	11.42±0.73	3.77±1.17	33.01	Light	1.90±0.17	Medium	8
Y8	29.45±1.91	37.77±3.29	30.95±1.75	9.16±2.27	3.97±1.57	43.23	Very light	1.48±0.22	Easy	6
Y9	29.81±1.07	34.56±1.21	31.99±1.55	10.92±1.95	5.35±2.13	48.99	Very light	1.31±0.26	Medium	8
Y10	32.73±1.85	37.31±2.08	36.95±2.05	10.24±1.06	4.06±0.96	39.59	Medium	1.48±0.23	Very easy	0
Y11	36.88±1.57	44.03±2.10	34.66±1.57	12.55±1.64	5.23±1.76	41.67	Light	1.35±0.21	Very easy	6
Y12	32.88±1.81	37.26±2.09	34.76±2.23	12.20±1.69	5.50±1.13	45.08	Light	1.42±0.19	medium	0
Y13	30.21±1.49	33.01±2.26	31.21±1.41	9.02±1.25	4.22±0.77	46.84	Very light	1.26±0.22	Easy	0
Y14	33.63±1.61	42.84±2.46	34.39±1.34	12.89±1.52	6.40±0.84	49.65	Light	1.27±0.15	Very easy	0
Y15	35.90±1.68	43.26±2.56	36.49±1.23	15.09±2.19	7.37±1.30	48.67	Light	1.39±0.13	Easy	0
Y16	36.39±2.14	49.37±2.01	38.26±1.32	14.10±1.98	6.08±1.53	43.15	Light	1.12±0.18	Very easy	0

Table 3. The fatty acid content of superior genotypes

Genotypes	Saturated fatty acids (%)			Unsaturated fatty acids (%)			
	C16:0	C18:0	C20:0	C16:1	C18:1	C20:1	C18:2
Y1	7.37ab*	3.70a	0.12 <sup>Ns</sup>	0.12ab	30.38b	0.29a	58.01a
Y11	8.27a	3.20b	0.13	0.14a	30.36b	0.22b	57.68a
Y14	6.27b	3.23b	0.11	0.05c	31.76b	0.23b	58.35a
Y15	7.28ab	3.75a	0.12	0.10b	30.63b	0.23b	57.98a
Y16	5.75b	3.86a	0.13	0.009b	48.43a	0.25ab	41.48b

\*Values within by the same letter are not significantly different at  $P < 0.05$  by Duncan

\*\*Ns: not significant

In previous studies some phenolic acids were determined in walnuts kernel, pellicle and leaves (Colaric *et al.*, 2005; Solar *et al.*, 2006; Pereira *et al.*, 2007; Slatnar *et al.*, 2015; Persic *et al.*, 2018). It is reported that, syringic, caffeic, ferulic, vanillic, gallic, protocatechuic and phenylacetic acid are found in walnut kernels (Prased, 2003). In addition, Colaric *et al.* (2005) reported that walnut kernels contained chlorogenic, *p*-coumaric, sinapic, ellagic, juglone and syringaldehyde acid in different quantities. In the present study, propyl-paraben, 4-hydroxybenzoic acid, gallic acid, syringic acid, chlorogenic acid, protocatechuic acid, vanillic acid, caffeic acid, and ferulic acid were identified in the walnut kernel (Table 3). Variations of phenolics in superior genotypes were determined and statistically significant differences were found with Duncan's test,  $p < 0.05$ ) among genotypes. Propylparaben was the major phenolic among the examined phenolic acids in all genotypes and Y16 had the highest amount of propylparaben (128.08 mg per kg) in kernel among all genotypes on the contrary other phenolics. Compared to Y1 and Y11, Y14 and Y15 had lowest contents of phenolics also Y16 poor in terms of phenolics except propylparaben. Y11 had the highest amount of 4-hydroxybenzoic acid, gallic acid, syringic acid, chlorogenic acid, protocatechuic acid, vanillic acid, caffeic acid, and followed by Y1. The analyses show that Y11 is the most valuable genotype in terms of phenolic acid contents among all superior genotypes.

#### Organic acids

Organic acids are found in fruits and vegetables as compounds such as salts, esters, and glycosides. Since the salt elements of these acids are in alkaline form, they are very important for human nutrition (Gundogdu *et al.*, 2014). In the present study, the contents of five superior genotypes were investigated in terms of some organic acids (oxalic, tartaric, formic, malic, malonic, maleic, citric, succinic and fumaric). In literature, there are not enough studies on the organic acid contents of walnuts. Organic acid contents in kernels of superior genotypes were examined in this study and there were statistically significant differences among the genotypes ( $p < 0.05$ ). Citric and malic acids are the most abundant organic acids in walnut kernels. While malic acid is usually found in pome fruit, citric acid is the dominant organic acid of citrus fruits and tartaric acid in grapes. In this research, it was determined that malic acid and tartaric acid were the major organic acids in walnut kernels ranged from 47.88 to 78.51 mg per 100 g and 30.27 to 49.60 mg per 100 g, respectively. L- ascorbic acid was the third in terms of the amount of organic acids in walnut kernels ranged from 10.71 to 19.71 mg per 100 g. Citric acid was non-determined in kernels of Y1, Y14 and Y15 but determined at kernels of Y11 and Y16 as 4.51 and 7.55 mg per 100 g, respectively. It was determined that the oxalic, malonic, succinic, maleic and fumaric acid contents ranged from 8.39 to 12.08 mg per 100 g, 6.02 to 9.19 mg per 100 g, 2.86 to 5.32 mg per 100 g, 0.26 to 3.00 mg per 100 g, 0.26 to 0.58 mg per 100 g, respectively (Table 5).

Table 4. Phenolic acid content of superior genotypes (mg/100 g)

	Propyl-paraben	4-Hydroxy benzoic	Gallic	Syringic	Chlorogenic	Proto-cathuric	Vanillic	Caffeic	Ferulic
Y1	117.40 c*	34.17 a	9.70 b	10.99 a	8.58 a	5.77 ab	4.26 ab	3.27 a	2.61 <sup>NS</sup>
Y11	122.05 b	36.04 a	12.42 a	10.50 a	10.33 a	6.61 a	6.39 a	4.02 a	2.32
Y14	112.17 d	15.14 b	4.17 c	2.33 b	3.64 b	Nd	0.96 c	0.36 b	2.17
Y15	111.00 d	10.12 c	5.07 c	3.06 b	5.28 b	3.23 bc	1.68 c	0.88 b	1.61
Y16	128.08 a	17.06 b	4.34 c	3.20 b	4.41 b	1.63 c	2.60 bc	Nd	2.09

\*Values within by the same letter are not significantly different at  $P < 0.05$  by Duncan

\*\*Nd: not detected

\*\*\*Ns: not significant, nd: non-detected

Table 5. Organic acid content of superior genotypes (mg/100 g)

	Malic	Tartaric	L-Ascorbic	Oxalic	Malonic	Succinic	Citric	Maleic	Fumaric
Y1	47.88 d*	30.27 c	15.30 b	8.39 b	6.72 b	2.86 c	Nd	0.26 b	0.26 b
Y11	62.26 c	49.20 a	10.71 c	12.08 a	6.42 b	4.36 ac	4.51 b	0.79 b	ND
Y14	78.51 a	35.08 b	14.88 b	9.62 ab	ND	5.32 a	ND	0.56 b	ND
Y15	67.21 b	47.44 a	19.37 a	11.51 a	6.02 b	3.31 bc	ND	1.46 ab	0.43 ab
Y16	69.31 b	49.60 a	19.71 a	10.53 ab	9.19 a	4.93 ab	7.55 a	3.00 a	0.58 a

\*Values within by the same letter are not significantly different at  $P < 0.05$  by Duncan

\*\*ND: non-detected

## Conclusions

In the study some morphological, pomological and phytochemical investigations were examined and significant differences were found among genotypes. As a result of weighted ranking methods, 5 genotypes were determined as superior. In order to make more objective evaluations related to plant characteristics, plants should be grown on the same location and same rootstocks to register them as cultivar.

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## Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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