

## SELECTION OF SUPERIOR PERSIAN WALNUT (*Juglans regia* L.) FROM SEEDLING ORIGIN IN TURKEY

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**Abstract.** There are seed propagated walnut (*Juglans regia* L.) populations with the vast genetic variation in different part of Turkey. There are also lots of monoecious and dichogamous genotypes in Turkey due to continuing sexual propagation. This study was conducted to determine genetic variability and to select superior walnut genotypes within seedling population grown in Kemah district in Eastern Anatolia during 2009–2012. In the study over 25.000 walnut trees were examined with high heterozygosis from point of yield (lateral fruitfulness), tolerance to anthracnose, bacterial blight and nut quality parameters. The ratio of lateral fruitfulness of selected genotypes was ranged from 50 to 80%. Leafing time in selected genotypes are found medium group according to international well-known 'Franquette' cultivar that is late leafing characteristic. Nut weight, length, diameter (at suture) and shell thickness varied from 11.18 to 15.20 g, 32.55 to 36.62 mm, 31.58 to 36.15 mm and 1.11 to 2.33 mm, respectively. Kernel weight and percentage varied from 6.14 g to 8.00 g and 47.08 to 58.57%. Kernel fat and protein content were between 55.18 to 65.70% and 14.70 to 20.10%, respectively.

**Key words:** Walnut, selection, late leafing, lateral fruitfulness, fruit quality

### INTRODUCTION

The English or Persian walnut (*Juglans regia* L.) is the most horticulturally developed and widely cultivated among walnut species [Ercisli 2004, Akça et al. 2012]. The Persian walnut is native to central Asia and grows as a wild or semi-cultivated tree in a wide area from southeastern Europe and the Caucasus to Turkey and Iran, through southern portions of the former Soviet Union into China and the eastern Himalayas [Ercisli et al. 2011]. High variability in the shape and size of fruit, color and thickness of shell and kernel, and shape and size of crown, stem and leaves have been reported for

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walnut trees from different region in the world [Sharma and Sharma 2001, Solar et al. 2002, Mitrovic et al. 2003, Zeneli et al. 2005, Norouzi et al. 2013].

Turkey is reported to be one of the genetic origins of *Juglans regia* and un-grafted wild trees of walnut are found in every corner of the country and trees exhibit considerable variation in respect of vegetative growth and fruit characters [Akça et al. 2012, Ercisli et al. 2012]. Use of this variability in the selection of superior seedlings with desirable traits will be of paramount importance in the expansion of walnut cultivation in this country. These seedling selections will not only produce nuts of desirable quality, but are also well adapted to the local climatic and soil conditions [Ercisli 2004, Asma 2012].

Modern objectives in plant breeding may be achieved by the evaluation of traits amongst genetic resources and combination of those in one cultivar. Although, molecular indexes like molecular markers are used already, but these methods are expensive. More over in walnut, because of sufficient varieties and easy application, morphological indexes might be appropriate for classification [Asadian and Pieber 2005, Arzani et al. 2008, Karimi et al. 2009, Norouzi et al. 2013]. Morphological characters must be recorded for selection of parents and are also the first choice used for describing and classifying the germplasm [Koc and Bilgener 2013, Radivojevic et al. 2014]. Statistical methods including principle components or cluster analysis can be used as useful tools for screening the accessions. In addition, morphological characteristics sometimes have correlation or are associated with characteristics that are difficult to evaluate such as disease susceptibility. Therefore, they may be useful as markers in breeding programs [Karimi et al. 2009].

Turkish walnut cultivars have to a too early leafing out and most of them are susceptible to bacterial blight, and they are bear fruit on terminal buds. The most widely used breeding method in walnut breeding programs in Turkey is by selection. Selection studies have been made in the populations that it has mostly been made propagated from seed in Turkey. More than 200 promising genotypes were selected among these trees as cultivar candidate [Akça and Ozogun 2004, Aslantas 2006, Beyhan and Demir 2006, Simsek 2010, Asma 2012, Keles et al. 2014]. These selections studies aimed to find new walnut genotypes with high nut quality and yield.

The aim o this study was to select new selections combining lateral fruitfulness, late leaf break, resistance to pathogens (*Xanthomonas juglandis* and *Gnomonia juglandis*), regular yield, and high fruit quality. The best selections were propagated and will be used as parents in variety breeding programs in the future.

## MATERIALS AND METHODS

It has been estimated that the research region has around 25000 walnut genotypes propagated naturally by seeds, and the region has more than 2298 km<sup>2</sup> situated between 39°44'–39°01' N latitude and 38°05'–39°03' E longitude in eastarn Anatolia region, at altitude from 980 to 1900 meters above sea level. In the research area, average annual temperature and precipitation is 10°C, and 350 mm, respectively.

Pre-selection was done according to leafing time in population. The types with early leafing were eliminated. Out of the total population of seedling walnut trees, 48 geno-

types were selected according to leafing time and lateral bud fruitfulness. The trees with both late leafing and lateral bud fruitfulness also were selected as superior selections. The most important identifying characteristics of superior selections were the date of leaf bud break, lateral bud fruitfulness, and pomological characteristics, tolerance to diseases and pests. The late leafing, lateral bud fruitfulness, nut quality and tolerance to diseases and pests were evaluated by modified weighted-rankit method (tab. 1). As a result, 9 superior selections were selected according to by weighted-rankit method. Nine selected superior selections were grafted on seedling rootstocks (*Juglans regia L.*), and the leafing time of grafted selections were compared to 'Franquette' cultivar in breeding parcel at same conditions. Leafing times were grouped according to the standard guidelines [Anonymous 1994].

Table 1. The scores of the characteristics and their relative values

Characteristics	Relative Scores			Class of the characteristics and their scores			
	A*	B*	C*	days differences according to leafing date of 'Franquette' (2011–2012)			
Late leafing	30	5	20	late (-02) to (+03)	10	medium-early (-15) to (-20)	4
				medium-late (-03) to (-08)	8		
				medium (-09) to (-14)	6		
Lateral bud fruitfulness (%)	5	25	30	>78	10	46–61	6
				62–77	8	31–45	3
				>14.51	10	8.6–10.5	3
Nut weight (g)	20	20	15	12.6–14.50	8	<8.5	1
				10.6–12.5	6	–	–
				>55.00	10	40.10–44.00	3
Kernel ratio (%)	15	15	10	49.01–55.00	9	<40	1
				44.01–49.00	6	–	–
				very easy	10	medium	4
Shell removal	10	5	5	easy	6	hard	2
				extra light	10	dark yellow	4
Kernel color	10	10	10	light	6	dark	2
				no	10	–	–
Damage of codling	5	10	5	few	6	–	–
				middle	2	–	–
				tolerant	10	sensitive	6
Damage of bacterial blight	5	10	5	medium tolerant	4	very sensitive	2

A\* – for late leafing, nut quality, tolerance to diseases and pests

B\* – for lateral bud fruitfulness, nut quality, tolerance to diseases and pests

C\* – for late leafing, lateral bud fruitfulness, nut quality, and tolerance to diseases

**Physical analysis.** Thirty nuts were selected randomly from the trees. Physical analysis include nut fruit properties (i.e. nut diameter, nut length, nut thickness, nut shape, nut size, nut weight, shell thickness, shell roughness and etc.) and kernel proper-

ties (kernel weight, kernel ratio and kernel color and etc.) were determined according to the UPOV standards [Anonymous 1994].

**Chemical analysis.** For the chemical analysis AOAC methods were used. Fat contents were determined by AOAC [1995].

## RESULTS AND DISCUSSION

Breeding of new walnut cultivars is characterized by earlier fruiting, higher yield, lateral bearing, good adaptability to different ecological conditions, good fruit quality and tolerant to pests and diseases. The ideal walnut cultivar would be relatively late leafing to escape frost and the rains that spread walnut blight (*Xanthomonas campestris* pv. *juglandis*), precocious and vegetative vigorous with bearing on both terminal and lateral shoots. The nutshell would be relatively smooth, well sealed, and make up no more than 50% of the nut weight. The nuts would fit the category of large or jumbo. The kernel would be plump and light colored, weighing about 8–9 g, and come out easily in halves. The tree would be at least moderately resistant to pests and diseases [Germain 1999, Akça 2012]

Table 2. Total scores of the selected genotypes according to weighted-rankit method (2009–2012 average)

Genotypes	A*	B*	C*	Genotypes	A*	B*	C*
24 KMH 01	540	610	560	25	695	665	670
24 KMH 02	805	825	760	26	625	645	675
24 KMH 03	615	665	660	27	775	585	640
24 KMH 04	615	605	600	28	700	600	660
24 KMH 05	515	515	525	29	750	620	690
24 KMH 06	740	890	820	30	950	910	910
24 KMH 07	660	570	570	31	855	835	840
24 KMH 08	480	410	445	32	600	490	520
24 KMH 09	600	510	520	33	815	825	820
24 KMH 10	685	615	590	34	505	520	470
24 KMH 11	705	585	590	35	895	885	870
24 KMH 12	735	565	600	36	410	300	385
24 KMH 13	620	450	525	37	560	570	570
24 KMH 14	660	500	555	38	660	680	660
24 KMH 15	760	530	580	39	480	420	395
24 KMH 16	665	595	640	40	740	740	710
24 KMH 17	705	575	580	41	570	660	645
24 KMH 18	600	550	595	42	600	670	620
24 KMH 19	645	465	530	43	815	875	820
24 KMH 20	565	395	475	44	585	615	595
24 KMH 21	500	400	445	45	435	515	485
24 KMH 22	670	570	570	46	635	880	760
24 KMH 23	725	715	705	47	455	540	430
24 KMH 24	575	555	585	48	535	600	480

A\* – for late leafing, nut quality, tolerance to diseases and pests

B\* – for lateral bud fruitfulness, nut quality, tolerance to diseases and pests

C\* – for late leafing, lateral bud fruitfulness, nut quality, tolerance to diseases

The results of yield, pomological and morphological characteristic, and tolerance to diseases and pests status and total weighted-rankit scores were determined for 48 walnut genotypes. The highest score for late leafing, lateral bud fruitfulness, nut quality, tolerance to diseases was observed in genotypes KMH 02, KMH 06, KMH 30, KMH 31, KMH 33, KMH 35, KMH 40, KMH 43, KMH 46 (tab. 2).

Late leafing walnut cultivars are also known tolerant to bacterial blight [Forde 1975]. Late leafing is a particularly important character in Turkey where many areas have late spring frosts. Leafing time in selected selections are found medium group according to 'Franquette' cv. (tab. 3). But, leafing time in selected genotypes were later than Turkish walnut cultivars including 'Sebin', 'Sen 1' and 'Sen 2'. 'Sen 1' and 'Sen 2' walnut cultivars were selected same region by Sen [1980].

Table 3. The leafing time and the percentage of lateral bud fruitfulness of selected superior selections

Genotypes/Cultivars	Lateral bud fruitfulness (%)	Leafing times		Leafing group (According to 'Franquette')
24 KMH 02	50	27.04.2011	18.04.2012	medium
24 KMH 06	65	26.04.2011	19.04.2012	medium
24 KMH 30	65	25.04.2011	17.04.2012	medium
24 KMH 31	70	26.04.2012	19.04.2012	medium
24 KMH 33	75	24.04.2012	17.04.2012	medium
24 KMH 35	65	25.04.2011	20.04.2012	medium
24 KMH 40	50	25.04.2011	20.04.2012	medium
24 KMH 43	70	28.04.2011	21.04.2012	medium
24 KMH 46	80	25.04.2011	20.04.2012	medium
'Franquette'	0	07.05.2011	30.04.2012	–
'Sebin'	40	16.04.2011	08.04.2012	early
'Sen 1'	5	20.04.2011	10.04.2012	early
'Sen 2'	5	18.04.2012	12.04.2012	early

The lateral bud fruitfulness characteristics is the important factor that determined the potential yield in *J. regia* L. Lateral fruitfulness is also associated with precocity [Gemain 1999]. The percentage of lateral bud fruitfulness should be at 50 to 70% in new promising types that is late leafing. The percentage of lateral bud fruitfulness of Turkish walnut cultivars is very low than Californian and French walnut cultivars. The percentage of lateral bud fruitfulness varied from 50 (KMH 02) to 80% (KMH 46) in selected superior selections. The average of percentage of lateral bud fruitfulness Turkish walnut cultivars, 'Sebin', 'Bilecik', 'Kaman 1', 'Sen 1', 'Yalova 1', 'Yalova 3', 'Yalova 4' and 'Bursa 95' were determined between 20 and 40% [Akça 2012]. The percentage of lateral fruitfulness ranged between 40 and 63% in the selected types in Çoruh valley [Aslantaş 2006], 10 and 70% in Middle of Karadeniz region [Akça and Ozongun 2004]. Our results are better than that shown by Aslantaş [2006] and Akça and Ozongun [2004].

Table 4. The morphological characteristics of selected superior walnut selections

Selections	Tree age (years)	Tree circumferences (cm)	Vigor	Growth habit	Density of branch	One year old shoot color	Time of maturity	Time of male flowering compared to female flowering	Flower number of male catkins	Leaf shape of lateral leaflet	Predominant location of fruit buds
24 KMH 02	12	90	strong	upright	dense	dark yellow	15–20 September	protandry	many	elliptic	fruiting on cluster
24 KMH 06	25	108	strong	spreading	dense	green brown	15–20 September	protogyny	medium	broad elliptic	fruiting on cluster
24 KMH 30	45	100	strong	semi-upright	very dense	green brown	20–25 September	protogyny	medium	narrow elliptic	fruiting on cluster
24 KMH 31	12	66	strong	semi-upright	very dense	green brown	20–25 September	protandry	few	elliptic	fruiting on lateral brindles
24 KMH 33	20	85	strong	semi-upright	very dense	dark yellow	15–20 September	homogamy	few	broad elliptic	fruiting on lateral brindles
24 KMH 35	70	185	strong	spreading	very dense	light brown	15–20 September	protogyny	medium	narrow elliptic	fruiting on cluster
24 KMH 40	25	80	medium	upright	very sparse	green brown	20–25 September	homogamy	medium	broad elliptic	fruiting on cluster
24 KMH 43	25	135	strong	semi-upright	very sparse	green brown	15–20 September	protandry	few	broad elliptic	fruiting on lateral brindles
24 KMH 46	65	180	strong	spreading	dense	green brown	15–20 September	homogamy	medium	elliptic	fruiting on lateral brindles

Table 5. The pomological characteristics of selected superior walnut selections

Selections	Nut width (mm)	Nut thickness (mm)	Nut length (mm)	Nut weight (g)	Kernel weight (g)	Thickness of shell (mm)	Kernel percentage (%)	Protein content (%)	Fat content (%)	Ash content (%)	Nut size	Kernel percentage of weight relative to total weight of nut
24 KMH 02	34.22	34.36	38.44	13.86	7.60	1.29	54.87	16.10	65.46	1.79	large	high
24 KMH 06	31.62	32.55	38.81	11.18	6.55	1.45	58.57	20.00	61.43	1.85	medium	very high
24 KMH 30	31.58	32.65	40.89	14.48	8.00	1.45	55.26	14.70	65.70	2.19	large	very high
24 KMH 31	35.03	35.31	39.97	12.94	6.72	1.64	51.96	17.10	59.21	2.34	large	high
24 KMH 33	33.74	35.04	38.54	13.22	6.90	1.55	52.20	17.50	59.87	1.50	large	high
24 KMH 35	34.56	34.87	38.17	15.20	7.52	1.58	51.60	16.10	55.18	2.27	very large	high
24 KMH 40	34.06	34.08	42.29	13.06	6.14	1.28	47.08	18.80	57.28	1.67	large	medium
24 KMH 43	35.39	36.62	37.73	13.88	7.20	1.51	51.75	19.70	58.22	2.04	large	high
24 KMH 46	36.15	35.86	38.01	13.99	7.08	1.56	50.98	20.10	62.12	1.80	large	high

Table 6. The pomological characteristics of selected superior walnut selections

Selections	Nut shape in longitudinal section perpendicular to suture	Nut shape in cross section	Nut shape of base perpendicular to suture	Nut shape of apex perpendicular to suture	Nut prominence of apical tip	Structure of surface of shell	Kernel ease of removal two halves of shell	Kernel intensity of ground color
24 KMH 02	circular	oblate	rounded	truncate	medium	slightly grooved	medium	light
24 KMH 06	elliptic	elliptic	cuneate	cuneate	strong	embossed	medium	light
24 KMH 30	elliptic	oblate	cuneate	rounded	weak	strongly grooved	easy	medium
24 KMH 31	ovate	circular	emarginated	rounded	strong	embossed	easy	dark
24 KMH 33	trapezium	circular	emarginated	emarginated	strong	strongly grooved	medium	light
24 KMH 35	trapezium	oblate	emarginated	emarginated	medium	embossed	easy	dark
24 KMH 40	triangular	elliptic	cuneate	cuneate	strong	embossed	very easy	medium
24 KMH 43	circular	oblate	rounded	emarginated	weak	slightly grooved	very easy	medium
24 KMH 46	circular	oblate	emarginated	emarginated	weak	strongly grooved	very easy	medium



In our study we observed that 3 out of walnut selections were protandrous, 3 selections were protogynous and also 3 genotypes were homogenous flowering behaviour (tab. 4). Akça and Ozongun [2004] reported that among the selected 17 walnut genotypes, 11 genotypes were protogynous and 6 selections were protandrous in inner Anatolia.

The results regarding the pomological characteristics of the 9 superior walnut selections are shown in Table 5 and 6. The maximum nut length was obtained as 42.29 mm for KMH 43 while the minimum (42.29 mm) was recorded for the KMH 40 genotype. The highest nut diameter was 36.15 mm in KMH 46 and the lowest nut diameter was 31.58 mm in KMH 30. Nut size was determined extra for all the genotypes according to Turkish standards. Nut shape was determined as oval for all the genotypes (tab. 5).

The lowest nut weight was recorded as 11.18 g in KMH 06 and the highest fruit weight was 15.20 g in KMH 35. Kernel weight was in the range of 6.55 (KMH 06) g to 8.00 g (KMH 30), kernel percentage ranged from 58.57 (KMH 06) to 47.08 (KMH 40) (tab. 5). Bigger nuts (> 14.5 g) is found in selection KMH 35. Kernel percentage of selected genotypes to be found high (> 55%) in selection KMH 06 and KMH 30. We did not find very low (< 40%) and low (40–44%) kernel ratio in selected genotypes. The shell thickness was in the range of 1.28 (KMH 40) to 1.64 mm (KMH 31) (tab. 5). Akça and Ozongun [2004] commented that kernel weight, kernel percentage and shell thickness should be between 6.0 to 8.0 g, 50 to 55%, and 0.7 to 1.5 mm in promising new walnut cultivars and selections. Akça and Sen [2001] reported nut weight (7.49–13.93 g), kernel weight (2.61–5.73), shell thickness (1.32–2.45 mm) and nut diameter (22.30–32.26 mm) in walnut selections from southeastern parts of Turkey. Ali et al. [2010] found nut weight as 19.22 g ('Chitral-3') and 10.3 g ('Swat-1') of the promising walnut cultivars. Simsek [2010] selected eleven new walnut genotypes in Sanliurfa province located southern Anatolia. In this study, average nut weight and kernel weight varied between 14.31 to 9.63 g and 6.99 to 5.38 g respectively (tab. 5). In the selected genotypes in Amasya Province in inner Anatolia, nut weight ranged from 7.46 to 15.21 g, kernel weight ranged from 3.73 to 7.44 g and kernel percent varied from 46.15 to 63.16% [Karadag and Akça 2011]. Asma [2012] determined the nut weight (12.6–17.5 g), kernel weight (6.9–9.1 g) and kernel ratio (47.3–60.8%), shell thickness (0.95–17.5 mm) for the 16 walnut selections in Anatolia. Our values higher than that shown by Karadag and Akça [2011], Yartilgac et al. [2001], Balci et al. [2001], Aslantaş [2006] and Simsek [2010].

Ash, fat and protein percentage of 9 superior walnut selections were between 1.50 (KMH 33) to 2.27% (KMH 35), 55.18 (KMH 35) to 65.70% (KMH 30) and 14.70 (KMH 30) to 20.10% (KMH 46) (tab. 5). Simsek [2010] reported ash, fat and protein percentage of the selected walnut genotypes in Turkey were between 1.88 to 2.89%, 58.88 to 65.64% and 13.70 to 20.18%, respectively. Yerlikaya et al [2012] reported ash, fat and protein content between 1.53 to 1.99%, 61.32 to 69.35% and 10.58 to 18.19% among 11 walnut genotypes and 3 cultivars in western Anatolia. Sahin and Akbas [2001] have reported that 35 walnut genotypes from Turkey contained 1.66–2.8% ash, 56.38–70.59% fat and 13.59–22.99% protein. Pereira et al. [2008] have evaluated the chemical composition of six walnut cultivars in Portugal. They found that the total fat content of the nuts ranged from 68.83 to 72.14% and protein content ranged from 14.38

to 18.03%. The results of the ash, fat and protein content of our selected walnut genotypes are in agreement with above studies.

Fruit shape was determined as round, cuneate and emarginated; kernel color was light yellow, yellow and dark. Kernel removal was easy in the majority of the selected genotypes (tab. 6).

Under the field observations, we have not observed any diseases and pest symptoms on selected superior walnut selections. But a few symptoms and very light infection were observed on some Turkish walnut cultivars such as 'Kaman 1', 'Sebin', 'Maras 18' and 'Mara 12'.

## CONCLUSION

The study imply that the richness of walnut germplasm in Turkey. Our study showed that 7 out of 9 superior walnut selections has late leafing, lateral bud fruitfulness and good nut quality. These selections are particularly important in Turkey where many areas have late spring frost. The results described here provide a solid foundation upon which working towards a morphological marker-based breeding programme for germplasm improvement in Turkey and underscore the need for including new sources of germplasm into current breeding efforts. The selected superior walnut genotypes have nutritionally promising levels of fat and protein. The data reported in this paper also confirmed that walnuts were a rich source of important nutrients that would be very beneficial to human health.

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### SELEKCJA NAJLEPSZYCH GENOTYPÓW ORZECHA WŁOSKIEGO (*Juglans regia* L.) Z SIEWEK POCHODZĄCYCH Z TURCJI

**Streszczenie.** W różnych częściach Turcji istnieją populacje orzecha włoskiego (*Juglans regia* L.) rozmnażane z nasion o dużej różnorodności genetycznej oraz wiele monoecyjnych i dychotomicznych genotypów, co jest efektem ciągłego rozmnażania płciowego. Badanie przeprowadzono w celu określenia różnorodności genetycznej oraz wyselekcjonowania genotypów orzecha włoskiego w populacji siewek hodowanych w rejonie Ke-mach we Wschodniej Antolii w latach 2009–2012. Zbadano ponad 25 000 drzew orzecha o wysokiej heterozji pod kątem wydawania plonu (boczne owocowanie), tolerancji na antraknozę, bakteryjną plamistość oraz parametrów jakości. Wskaźnik bocznego owocowania u wybranych genotypów wynosił od 50 do 80%. Czas tworzenia liści w wybranych genotypach jest średni w stosunku do dobrze znanej odmiany ‘Franquette’. Masa, długość i średnica (na spojeniu) orzecha wahała się odpowiednio od 11,18 do 15,20 g, od 32,55 do 36,62 mm, od 31,58 do 36,15 mm oraz od 1,11 do 2,33 mm. Masa i proporcje jądra wynosiły od 6,14 g do 8,00 g oraz od 47,08 do 58,57%. Zawartość tłuszczu i białka w jądrze wynosiły odpowiednio od 55,18 do 65,70% oraz od 14,70 do 20,10%.

**Słowa kluczowe:** orzech włoski, selekcja, późne tworzenie liści, boczne owocowanie, jakość owoców

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