Genet Resour Crop Evol (2021) 68:1425–1437
https://doi.org/10.1007/s10722-020-01072-6

RESEARCH ARTICLE

Identification of genetic diversity among *Juglans regia* L. genotypes using molecular, morphological, and fatty acid data

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Received: 25 June 2020 / Accepted: 18 November 2020 / Published online: 24 November 2020

Abstract In this study, 40 walnut (*Juglans regia* L.) samples including international cultivars, national cultivars and local genotypes were analyzed in terms of detailed morphological traits, molecular characteristics, and biochemical (fatty acids) features. The results showed high morphological diversity among the genotypes, and the kernel ratio was found to have major contribution for the principal component analysis (PCA). Molecular results showed that the 19 SRAP primer combinations produced, on average, 171 bands in the accessions examined, of which 156 (90.6%) were polymorphic and DICE's similarity coefficient ranged from 0.49 to 0.94 indicating high genetic diversity. The polymorphism ratio of 11 primers was 100% while the lowest polymorphism was 66.7%. There were no significant differences in terms of correlation coefficient between the similarity matrices of the morphological traits and SRAP (r = 0.03). The main fatty acids of walnut kernel oils were found to be linoleic acid ($C_{18:2}$) followed by oleic acid ($C_{18:1}$) and linolenic acid ($C_{18:3}$). The polyunsaturated fatty acids constitute the main group of fatty acids in walnut kernel oils, and these ratios varied between 85.99–92.19%. It has been determined that the morphological and biochemical data may be insufficient alone in identification of genetic diversity and relativeness in walnut because they should be supported by molecular data.

Keywords *Juglans regia* · PCA · Fatty acid · SRAP · Polymorphism

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Introduction

Fruit species has numerous cultivars, genotypes, accessions etc. that grown mainly in temperate, subtropical and tropical regions. In particular open pollinated and dioecious fruit species has higher genetic diversity. Fruits have also been recognized for their human health benefits because the majority of fruits have high content of non-nutritive, nutritive, and bioactive compounds (Ercisli et al. 2011; Eyduran et al. 2015; Senica et al. 2019).

The *Juglans* genus contains about 20 species, however the most common and cultivated among them is known as *Juglans regia* L. (Zhao et al. 2017; Demir et al. 2018). In recent year, walnut production has gradually increased both in Turkey and worldwide (Yaman et al. 2017; Orhan et al. 2020). The main

reasons is due to increased interest to many health benefits of walnut nuts (50–75% oil, 12–15% protein, high minerals, and vitamins), and find buyers at higher prices compared to many other fruit species (Hayes et al. 2016). Nowadays, walnuts have an important place in terms of nutritional value due to its fatty acid contents, and which is consumed in the form of dried nuts. The world walnut production has reached 3.5 million tons. Turkey has an important position in walnut cultivation; it has the 4th place in world walnut production after China, USA, and Iran. With 215.000 tons annual production, Turkey provides 5.2% of world production (FAO 2018).

Walnut kernels have higher healthy omega-3 fats than any other common nuts, although they have different nutrients. Fat consumption is associated with health risks, but there is a general acceptance that the important matter is the fatty acid profile and distribution present in walnut oil. The amount and distribution of fatty acids in walnut genotypes is considered important for their economic and nutritional value (Zwarts et al. 1999). Walnut fat contains higher omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) than other nuts ones, and they have been associated with beneficial effects on serum lipids (Amaral et al. 2003). Human body cannot synthesize some essential PUFAs such as linoleic acid and α-linolenic acid, except many of these fatty acids (Garcia et al. 1994). The linoleic acid content in walnut oil is in range of 40–70%, and is higher level compared to other nut oils (Lopez et al. 1998; Ruggeri et al. 1998).

Turkey has a very special position for fruit genetic resources because of different geographical and ecological conditions (Ercisli et al. 2008; Pınar et al. 2019; Gecer et al. 2020). Walnut is widely grown in different ecological regions of Turkey including Anatolia, which has been called a walnut open air museum. There has been genetically rich walnut populations since it has been mostly grown from seeds for many years in Turkey (Orhan et al. 2020). In this regard, Turkey, which has very valuable walnut genotypes obtained from seed propagation, has an advantage that is not found in many other countries around the world in terms of the tree structure, production, and ready breeding material. This wide genetic diversity provides great benefits in the selection of individuals with superior characteristics especially in breeding studies (Uzun et al. 2017). It is very important for walnut cultivation is that the genetic material is original, and the genetic relationship of these materials is revealed. Morphological and biochemical traits in walnut are affected by environmental conditions (Iqbal 2019). Therefore, it is needed to use different methods to identify genotypes correctly. The use of molecular markers, which are not affected by environmental conditions, has one of the better choices and increased its importance in recent years (Ahmad et al. 2017; Guney et al. 2019). Deciphering walnut genetic diversity and structure is important for efficient management and use of genetic resources and knowledge of the genetic diversity of walnut germplasm is crucial for effective management and use germplasm.

Genetic diversity studies in walnut have been started since the mid-1990s, and in the first studies mostly on Restriction Fragment Length Polymorphism (RFLP) (Fjellstrom and Parfitt 1994,1995) and Random Amplified Polymorphic DNA (RAPD) (Woeste et al. 1996; Malvolti et al. 1997; Nicese et al. 1998) markers were employed. In recent years, many molecular marker techniques have been improved and these techniques are widely used in genetic studies. Molecular markers have been used commonly for a variety of applications such as genetic diversity and phylogenetic analysis in cultivated plants. As a result of the studies, it has been reported that RAPD and ISSR (Inter Simple Sequence Repeat) techniques are advantageous in terms of polymorphism and RFLP, SSR (Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism) and SRAP (Sequence-related Amplified Polymorphism) techniques are quite high repeatability (Powell et al. 1996). SRAP marker system, which is a method that can be repeated quite easily and applied easily, is generally used in fingerprint studies since it is a cheap and effective system (Uzun et al. 2017).

For germplasm management and crop improvement, morphological, biochemical and molecular markers have their own implication and validity and none is superior. The information on genetic variability in walnut has generated many economically important traits like late leafing, lateral bud nut fulness, nut weight, shell thickness, kernel ratio, kernel color, fatty acid profiles etc. being a complex trait expression of characteristics of walnut genotypes is controlled by many associated traits. With above views, current experiment was framed to find the morphological, biochemical and molecular genetic diversity in a large number of diverse walnut

germplasm Thus, the multidisciplinary data, which using together the molecular markers (SRAP), morphological and biochemical (fatty acids) properties of nuts was obtained, was evaluated for discrimination between walnut germplasm. In addition, determining the morphological and fatty acid characteristics of these walnut genotypes as well as genetic diversity may be guiding for breeders to accelerate the future breeding programs.

Materials and methods

Plant material

We used a total 40 walnut genotypes and 21 of them were walnut genotypes selected from Uşak province (29° 20′ E, 38° 32′ N; 900 m above the sea level) located in the Inner Aegean Region/Turkey. These genotypes were determined based on a number of important pomological and morphological traits such as nut weight, nut size, nut width, etc. (Yildiz et al. 2017). Additionally, 16 standard walnut cultivars and 3 local genotypes in the commercial walnut orchard established in Uşak province were used. Foreign walnut cultivars (Chandler, Pedro, Fernor, Fernette, Franquette, Cisco, Tulare, Lara and Howard), local cultivars (Şebin, Kaman-1, Bayrak, Yavuz, Sütyemez, Maraş-18, Oğuzlar-77),) and local genotypes (Balkal, Arslan-1 and Arslan-2) were also included in the study. The young leaf tissues were immediately frozen in liquid nitrogen and then stored at –80 °C until the analyses. Totally 40 plants were analyzed by SRAP markers.

DNA extraction

The leaf tissues were pulverized in a muller under liquid nitrogen, then genomic DNA was extracted with CTAB method according to Doyle and Doyle (1990) protocol. DNA concentrations were determined with a spectrophotometer (BioTek Instruments, Inc., Winooski, VT, United States). The DNA solutions in the samples was brought to a working concentration of 10 ng/µL, and stored at − 20 °C.

SRAP analysis

The 208 SRAP primer combinations were screened primarily on mix DNA of walnut genotypes to obtain the most polymorphic primers. Considering the screening results, 19 polymorphic primer combinations were selected to evaluate whole walnut genotypes (Table 1). Polymerase chain reaction (PCR) was done in 15 µL of reaction volume containing 2 µl DNA (20 ng), 1.5 µl 10xPCR Buffer, 0.2 µl Taq DNA polymerase (5 u/µL), 1 µl dNTP (2.5 mM), 1.5 µl $MgCl_2$ (25 mM), 2 µl 10 mM SRAP primer combination and 6.8 µl H_2O. Reactions were performed in according to the following procedure with the method specified by Uzun et al. (2017): initial denaturation at 94 °C for 3 min, 35 cycles with denaturation at 94 °C for 1 min, annealing at 38 °C for 45 s (depending on the primer), extension at 72 °C for 2 min, and a final extension step at 72 °C for 10 min. PCR products were run in 2% agarose gel at 100 V for 2–3 h. To determine band widths, 100 bp DNA ladder was used. Resultant bands were imaged under UV light.

Morphological data analysis

The nut characteristic such as weight, nut size, nut width, etc. reported in our previous studies (Yildiz et al. 2017; Yildiz and Sumer 2019) was used to perform statistical analysis of the twelve morphological characteristics, and three total fatty acids profile. The nut weight (g), nut dimensions (length, suture and cheek (mm)), nut shape index, shell traits (thickness (mm), color, roughness and breakage) and kernel traits (weight (g), ratio (%) and color) of all walnut genotypes was used as morphological characterization data. The minimum and maximum values in genotypes were as follows: The 9.11–15.28 g for nut weight, 31.09–43.62 mm for nut length, 25.08–37.45 mm for nut suture, 27.83–39.54 mm for nut cheek, 0.79–0.86 for nut shape index, 1.61–3.23 mm for shell thickness, 3.95–9.29 g for kernel weight, and 34.96–59.70% for kernel ratio. The shell and kernel colors were determined as light, brown and dark; shell roughness was determined as smooth, medium and rough; shell breakage was determined as easy, medium and hard according to the UPOV (1999) guidelines. In addition, total contents of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated

Table 1 SRAP primers used and polymorphism in 40 walnut genotypes

Primer		Primer combination	Number total bands	Polymor-phic bands (%)	Size range (bp)	Obs. h.zygosity	Exp. h.zygosity
Sequence (3′–5′)							
Me2	TGAGTCCAAACCGGAGC	Me2-Em7	8	100.0	150–800	0.256	0.252
Me3	TGAGTCCAAACCGGAAT	Me2-Em12	18	72.2	280–1400	0.232	0.229
Me5	TGAGTCCAAACCGGAAG	Me3-Em6	11	100.0	100–1500	0.345	0.340
Me7	TGAGTCCAAACCGGACG	Me3-Em7	8	75.0	200–700	0.064	0.063
Me8	TGAGTCCAAACCGGACT	Me3-Em10	6	100.0	400–1300	0.110	0.108
Me10	TGAGTCCAAACCGGAAA	Me5-Em1	7	100.0	200–1100	0.332	0.318
Sequence (5′–3′)							
Em1	GACTGCGTACGAATTAAT	Me5-Em4	7	100.0	225–1200	0.282	0.279
Em2	GACTGCGTACGAATTTGC	Me5-Em8	6	100.0	200–1200	0.276	0.272
Em3	GACTGCGTACGAATTGAC	Me5-Em11	2	100.0	900–1000	0.305	0.301
Em4	GACTGCGTACGAATTTGA	Me5-Em13	17	100.0	275–1400	0.248	0.245
Em6	GACTGCGTACGAATTGCA	Me5-Em14	3	100.0	325–700	0.270	0.267
Em7	GACTGCGTACGAATTGAG	Me7-Em2	16	93.8	200–1500	0.147	0.146
Em8	GACTGCGTACGAATTCAC	Me7-Em12	6	66.7	225–1200	0.129	0.127
Em10	GACTGCGTACGAATTCAT	Me7-Em13	9	88.9	225–1100	0.133	0.131
Em11	GACTGCGTACGAATTCTA	Me7-Em15	4	100.0	400–1000	0.086	0.085
Em12	GACTGCGTACGAATTTGC	Me8-Em3	12	91.7	175–1400	0.108	0.106
Em13	GACTGCGTACGAATTCGA	Me8-Em15	5	100.0	350–800	0.334	0.330
Em14	GACTGCGTACGAATTCTT	Me10-Em6	14	85.7	300–1300	0.179	0.177
Em15	GACTGCGTACGAATTAGC	Me10-Em7	12	83.3	300–1400	0.214	0.211
		Mean	9.0	90.6	–	0.213	0.210

(*Obs. h.zygosity* Observed heterozygosity *Exp. h.zygosity* Expected heterozygosity)

fatty acids (PUFAs) were used for morphological data analysis.

SRAP Data analysis

The SRAP amplified products were recorded either as 1 with a band and as 0 without a band. The observed heterozygosity (Ho) and the expected heterozygosity (He) were estimated using the GenAlEx (vers. 6.5 program) software (Peakall and Smouse 2012). Data were subjected to statistical analyses with NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.1, Exeter Software, Setauket, N.Y., USA) software (Rohlf 2004) and then SIMQUAL module was used to compute a DICE similarity coefficient (Dice 1945). DICE similarity coefficient was used to construct a dendrogram based on the UPGMA method using the SHAN module. Morphological data analysis was carried out using NTSYSpc 2.11 software (Rohlf 2004). Morphological data were standardized using the STAN module, and then SIMINT module was used to compute a distance matrix. The distance matrix was used to construct a dendrogram based on the UPGMA method using the SHAN module. The genetic similarity matrix and ultra-metric distance matrix produced from UPGMA-based dendrogram with COPH module nested in the same software was compared in the matrix comparison (MxCOMP) program. To determine the efficiency of clustering, the cophenetic correlation coefficient was calculated with the Mantel method (Mantel 1967).

Principal components analysis (PCA) was used to reduce dimension, and differences were measured among groups. A correlation matrix of the morphological data was calculated with the PAST (Paleontological Statistics-Version 4.20) software.

Comparison between morphological and SRAP data was performed by calculating the correlation between the two data sets using the mantel test with 1000 permutations in the matrix comparison (MxCOMP) program of NTSYS. The degree of fit can be interpreted subjectively as follows: $0.9 < r$, very good fit; $0.8 < r < 0.9$, good fit; $0.7 < r < 0.8$, poor fit; and $r < 0.7$, very poor fit (NTSYS PC 2.1 Manual). The result of this test is a cophenetic correlation coefficient, *r*, which indicates how well the dendrogram represents the similarity data.

Analysis of fatty acids

Nuts were collected at the harvest time of walnut genotypes. These samples were then kept in a flowing-air drying train of 43 °C for 24 h to provide a homogenous drying (Sen 1980). Then, they were deshelled, and the kernels were powdered in a porcelain mortar. For oil analyses, each sample was homogenized and subjected to extraction for 6 h with petroleum ether (boiling range 30–60 °C) in a Soxhlet apparatus in accordance with the method specified by AOAC (1990). After extraction procedure, fatty acid methyl esters (FAMEs) were prepared by hydrolysis with a 11 g/L methanolic potassium hydroxide solution, methyl esterification with BF3/MeOH, and extraction with n-hexane. The extract was then centrifuged at 10,000 rpm for 30 min and the upper-liquid collected. The FAME profile for a 0.6-µL sample at a split ratio of 1:50 was generated using a gas chromatograph (Schimadzu, GC 2010 plus) equipped with a flame ionization detector (Schimadzu, Kyoto, Japan), a 100-m fused silica capillary column (i.d. 0.25 mm) and H_2 as the carrier and fuel gas. The FAME was separated using a temperature gradient program (Chilliard et al. 2013) and the peaks were identified based on comparing retention times with authentic standard (Beyzi et al. 2019).

Since 21 genotypes selected from Uşak province is represented by a tree, the interpretation of findings was made the using mean, maximum and minimum values. In the 19 local and foreign walnut cultivars, the experimental design was a completely randomized one with three replicates and a single tree per plot. Trees were planted at 7 × 8 m spacing in 2007. Another part of the study material, as a result of the selection study from the province of Uşak, constitutes 21 genotypes that show superior characteristics and are represented by a tree in nature. The trees were at various ages, but not younger than 15 years old. These data were subjected to ANAOVA by using SAS Software (SAS Version V.8, SAS Institute, Cary, N.C.) and mean separation was performed by Tukey's test at $P = 0.05$ confidence level (SAS 2005).

Results and discussion

SRAP analysis

The genetic diversity of the 40 walnut genotypes was also revealed using 19 SRAP primer combinations. A total of 171 bands were obtained and 156 of them were polymorphic. It was determined that the mean number of primer and polymorphic bands were 9.0 and 8.2 respectively. Primer combinations did not produce monomeric bands. Me2-Em12 primer combination had the highest bands (18) whereas Me5-Em11 primer combination had the lowest bands (2). It was recorded a polymorphism ratio of 100% in 11 primers while the lowest polymorphism (66.7%) was obtained in Me7-Em12 primer combination. The lowest observed (Ho) and expected heterozygosity (He) were obtained from Me3-Em7 primer combination with 0.064 and 0.063, respectively. The Me3-Em6 primer combination had the highest Ho and He values with 0.345 and 0.340, respectively. The average Ho and He values for primer combination studied were 0.213 and 0.210, respectively (Table 1).

These SRAP marker systems was repeatability and highly polymorphic in *Juglans regia* L. (Akcan et al. 2008; Uzun et al. 2017). The polymorphism ratio, which is determined, was enough to discriminate all genotypes. The polymorphism rate results were higher than those reported by Akcan et al. (2008), who found that the polymorphism rate of 10 SRAP primer combinations in different walnut genotypes was 55.3%. On the other hand, the results agreed with the findings of Uzun et al. (2017) using SRAP technique in walnut genotypes, but both the total bands and the number of polymorphic bands per primary were found higher than those. The reason for this difference may be attributed to the different genetic background and primer combinations used. However, the average Ho and He values in this study were 0.213 and 0.210 which indicated a low level of genetic diversity compared to previous studies such as

in 20 walnut cultivars from five countries (Pop et al. 2013) and in local walnut genotypes from Iran (Ebrahimi et al. 2011), Tibet (Wang et al. 2015) and China (Li et al. 2019).

Morphological data principal component analyses

Principal components analysis (PCA) was performed based on morphological data of walnut genotypes with the PAST software. The large part of the total variation (88.6%) in the twelve nuts morphological and three fatty acids traits have occurred from the first three main components (with Eigen values > 1) according to correlation matrix analysis. The first (PC1), second (PC2) and third (PC3) principal components represented 41.8%, 29.5% and 17.3% of total variance, respectively. The major components were defined by variables and their relationship was reflected on PC scores (Table 2). Score plots that contribute to visualizing the position and corresponding correlations of walnut genotypes are shown in Fig. 1, considering the first two major components. In general, walnut genotypes showed distribution on the chart, but not clustered.

Table 2 Principal component (PC) coefficients of twelve nut and kernel traits in walnut genotypes

Trait (units)	PC 1	PC 2	PC 3
Nut weight (g)	− 0.0388	0.1943	0.1878
Nut length (mm)	0.0244	0.4591	0.4458
Nut suture (mm)	0.0876	0.2801	0.4358
Nut cheek (mm)	0.0334	0.3039	0.3439
Nut shape index	0.0015	− 0.0024	0.0015
Shell thickness (mm)	− 0.0291	− 0.0033	0.0005
Shell color	0.0294	− 0.0014	0.0417
Shell roughness	0.0041	0.0193	0.0441
Shell breakage	− 0.0457	0.0174	0.0228
Kernel weight (g)	0.1157	0.1090	0.0965
Kernel ratio (%)	0.9641	0.1064	− 0.1983
Kernel color	0.0223	− 0.0225	− 0.0144
SFAs	0.0072	− 0.0132	0.1408
MUFAs	− 0.1492	0.5315	− 0.5067
PUFAs	0.1398	− 0.5228	0.3583
Eigen value	36.78	25.99	15.22
Variance %	41.79	29.54	17.29
Cumulative variance %	41.79	71.33	88.62

Our finding, for the first two components, is similar with the values obtained (89%) by Cosmulescu et al. (2018) on 40 walnut genotypes from Romania, but higher than those found by Wang et al. (2015) on 86 walnut genotypes from Tibet, by Ebrahimi et al. (2015) on 61 walnut genotypes from Iran, and by Rojano-Hernández et al. (2017) on 30 walnut genotypes from Mexico. The high PC1 and PC2 variance values in the study prognosticate that the properties observed are extremely perfect for the classification of walnut genotypes. Principal components analysis in 40 walnut genotypes, made by Cosmulescu et al. (2018) revealed that traits related to nut weight and diameter, and kernel weight accounted for a large proportion of the observed variability. The kernel ratio and weight, and PUFAs provided positive effect, but MUFAs had a negative effect for the first principal component (PC1) in the PCA (Table 2). To differentiate the variance that occurs among walnut genotypes, the relationship between nut properties have been considered successfully used. (Ebrahimi et al. 2015; Wang et al. 2015; Poggetti et al. 2017; Rojano-Hernández et al. 2017; Cosmulescu et al. 2018).

Morphological and molecular cluster analysis

Genetic similarity among walnut genotypes was calculated using DIST coefficient based on the twelve morphological data (Fig. 2a). Genetic similarity coefficient ranged from 0.44 to 0.98. The UPGMA clustering divided 40 walnut genotypes into two main clusters, and they were further divided into two subclusters. Thirty-five of the 40 genotypes were included in the first cluster (I). There were eighteen genotypes in the 1st sub-cluster (I-A), and seventeen genotypes in the 2nd sub-cluster (I-B). Five genotypes were located in the second cluster (II), and the genotypes UŞAK-17 and UŞAK-34 were grouped separately from the others in the first sub-cluster (II-A). On the other hand, genotypes in the second sub-cluster (II-B) were grouped into two sub-groups with a similarity value more than 0.61.

The data showed that the SRAP primer combinations were useful for discriminate of genetic diversity and variations among the 40 walnut genotypes (Fig. 2b). UPGMA cluster analysis was applied to demonstrate the genetic relationships among the genotypes. The 40 walnut cultivars and genotypes were divided into 2 main clusters in the dendogram.

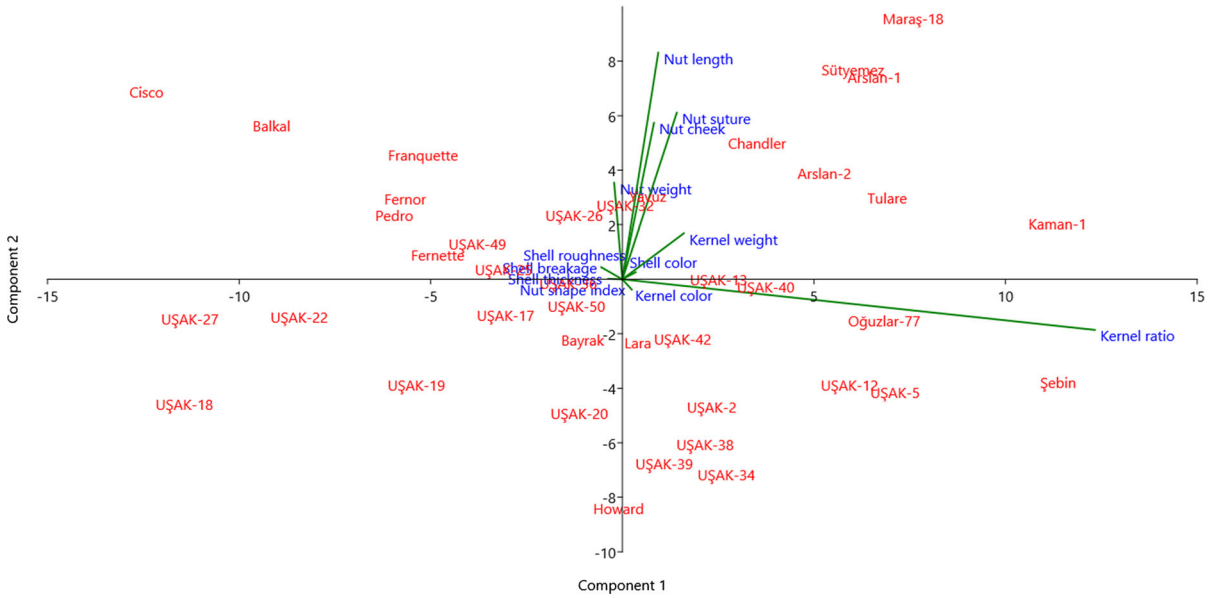

Fig. 1 Principal Component Analysis plot estimated from the correlation matrix of variables observed on walnut genotypes

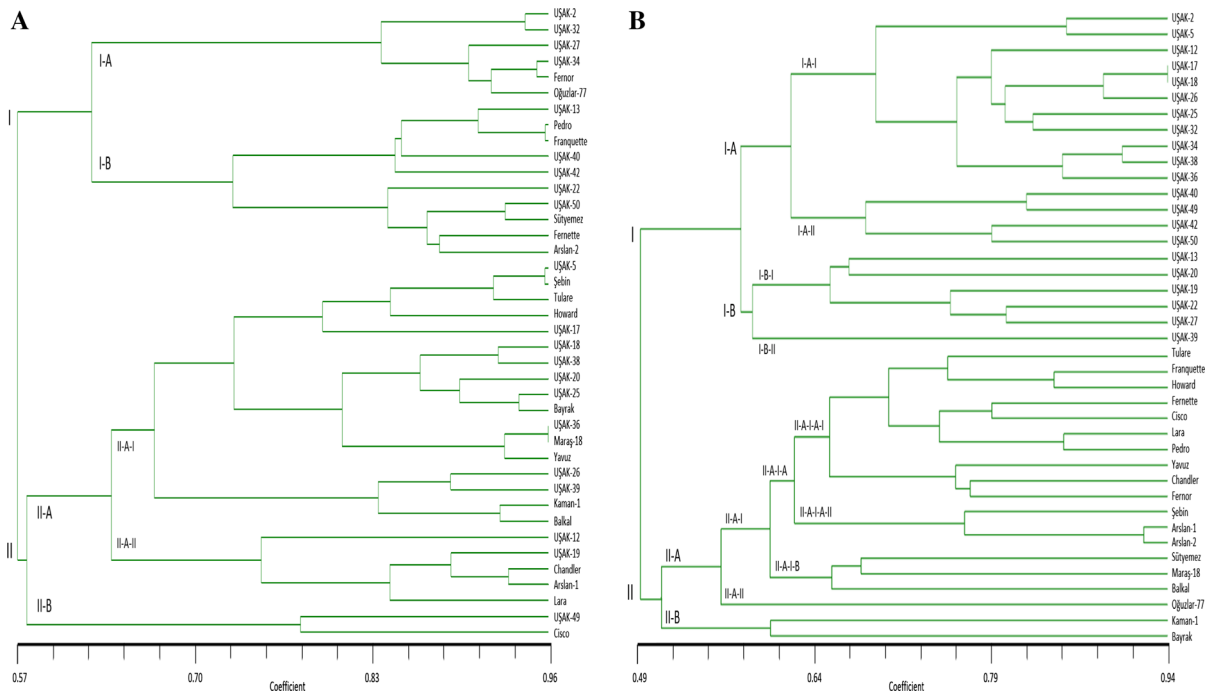

Fig. 2 Dendrogram of the 40 walnut genotypes based on **a** morphological traits using DIST coefficient and **b** SRAP markers using DICE's similarity coefficient

There were 21 genotypes selected from Uşak province in the 1st cluster (I), and 19 local and foreign walnut cultivars in the 2nd cluster (II). The second cluster was further divided into two sub-clusters, and the Kaman-1 and Bayrak genotypes were grouped (II-B) separately from the other genotypes (II-A). The foreign walnut cultivars were classified in the same sub-group of II-A

group with a similarity value more than 0.66, except for Yavuz local genotype.

Morphological traits are not as informative as DNA markers in determining genetic relationships (Ebrahimi et al. 2011; Pop et al. 2013; Wang et al. 2015). In accordance with this explanation, polymorphisms determined by SRAP in all genotypes in the study were determinant from morphological markers. The main clusters in the dendrogram grouped well with their geographic origin. Especially, it showed that genotypes in the Uşak province had experienced little gene flow than the other areas of Turkey because of geographical barriers.

Comparison between morphological and SRAP markers

The SRAP co-phenetic correlation coefficient, which represents the relationship between cophenetic matrices, was significant (r = 0.87). As a result of the test of morphological data, co-phenetic correlation coefficient was determined as r = 0.72. This value indicates that the dendogram represented weak similarity index. The mantel test showed quite low correlations between morphological and molecular dendrograms (r = 0.03). Therefore, there were no significant differences in terms of correlation coefficient between the similarity matrices of the morphological traits and SRAP. Morphological features can be affected by environmental conditions. In the walnut varieties and genotypes used in the study, it is possible that the resulting correlation is low because the SRAP marker system is connected to the regions related to the sequence of the marker rather than the whole genome and it will not always reflect the morphological features in these regions. Despite the poor correlation both techniques showed a high degree of variation among the walnut genotypes. The same result was observed in walnut genotypes in Iran (Ebrahimi et al. 2011) and Tibet (Wang et al. 2015). It has been demonstrated by studies in walnut and other fruit species that morphological properties are affected by many non-genetic factors. Thus, similar results regarding the low correlation coefficient of morphological and molecular data were registered in fruit species, such as grapevine (Martinez et al. 2003), olive (Belaj et al. 2011) and walnut (Ebrahimi et al. 2011; Wang et al. 2015). As a result, there will be significant differences in terms of genetic relationships between morphological variation and data obtained by SRAP.

Fatty acids

The data of fatty acids content in kernels were reported in Tables 3 and 4. The main saturated fatty acids (SFAs) of walnut kernel oils in all walnut varieties were palmitic acid ($C_{16:0}$), followed by stearic acid ($C_{18:0}$). Also, it was determined that there were traces of myristic acid ($C_{14:0}$). In the selected genotypes, palmitic acid contents varied from 5.57% (UŞAK-40) to 7.20% (UŞAK-13). The average palmitic acid ratio in local and foreign walnut cultivars was 7.13%, whereas the lowest and highest ratios were found as 5.72% (Arslan-1) and 8.90% (Balkal), respectively. Total SFAs content of walnut types and cultivars ranged from 7.81 to 10.45%, and from 7.89 to 11.87%, respectively. The walnut cultivars had no significant effects on total unsaturated fatty acid content of walnut kernel oils. Total MUFAs ratio varied between 11.02–24.59% for walnut types, and between 14.48–26.98% for walnut cultivars. The major MUFAs in kernel oil were oleic acid ($C_{18:1}$). In general, PUFAs constitute the main group of fatty acids in walnut kernel oils. The most PUFAs was found to be linoleic acid ($C_{18:2}$) followed by linolenic acid ($C_{18:3}$). The linoleic acid rates were determined to vary between 52.83% (UŞAK-36) and 64.71% (UŞAK-19) of select superior walnut types, between 55.22% (Cisco) and 63.53% (Fernette) of local and foreign walnut cultivars. The mean linoleic acid ratio identified as 13.75% for walnut types, and as 11.37% for walnut cultivars. Additionally, the total PUFAs/total MUFAs ratio of walnut types and cultivars was found as 4.13 and 3.98, respectively.

There was wide variation among the walnut genotypes and cultivars in terms of the fatty acids composition kind of the previous studies conducted in different areas of Turkey. In general, the obtained results in this study were in agreement with earlier data observed in other geographical origin such as Italy (Ruggeri et al. 1998), New Zealand (Zwarts et al. 1999), Portugal (Amaral et al. 2003; Pereira et al. 2008) and Canada (Li et al. 2007). In a previous research, it was determined that SFAs was less than both MUFAs and PUFAs in local walnut genotypes in Turkey (Muradoglu et al. 2010).

Table 3 The fatty acids profiles of superior walnut genotypes selected from Uşak/Turkey (%)

Genotype	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	SFAs	$C_{16:1}$	$C_{18:1}$	$C_{20:1}$	MUFAs	$C_{18:2}$	$C_{18:3}$	$C_{20:2}$	$C_{22:2}$	PUFAs
UŞAK-2	0.025	5.89	2.38	8.30	0.047	15.94	0.111	16.10	61.18	14.26	0.083	0.078	75.61
UŞAK-5	0.030	6.24	2.91	9.19	0.053	10.81	0.162	11.02	63.69	15.93	0.103	0.071	79.79
UŞAK-12	0.022	6.23	2.20	8.46	0.046	15.14	0.062	15.25	63.10	13.01	0.146	0.042	76.30
UŞAK-13	0.029	7.20	2.81	10.05	0.049	14.87	0.140	15.06	62.81	11.94	0.099	0.053	74.90
UŞAK-17	0.022	6.45	2.26	8.72	0.048	16.13	0.086	16.26	61.21	13.68	0.082	0.041	75.01
UŞAK-18	0.033	6.38	2.89	9.30	0.045	16.60	0.153	16.80	54.82	18.89	0.109	0.080	73.90
UŞAK-19	0.023	6.62	2.30	8.94	0.041	11.70	0.143	11.88	64.71	14.31	0.074	0.083	79.18
UŞAK-20	0.027	6.59	2.35	8.96	0.061	13.44	0.164	13.67	64.10	13.03	0.076	0.163	77.37
UŞAK-22	0.029	6.12	3.12	9.27	0.052	16.31	0.102	16.47	57.74	16.37	0.116	0.038	74.26
UŞAK-25	0.018	6.29	2.54	8.84	0.054	20.33	0.076	20.46	60.10	10.47	0.079	0.052	70.70
UŞAK-26	0.029	6.59	2.30	8.92	0.041	21.32	0.128	21.49	55.46	14.00	0.092	0.042	69.59
UŞAK-27	0.017	5.73	2.65	8.40	0.036	22.30	0.135	22.48	58.84	10.14	0.073	0.069	69.12
UŞAK-32	0.022	6.43	2.91	9.36	0.052	17.99	0.143	18.19	59.21	13.07	0.090	0.089	72.46
UŞAK-34	0.022	6.46	2.44	8.92	0.040	22.88	0.145	23.07	57.30	10.58	0.084	0.045	68.01
UŞAK-36	0.028	6.12	2.68	8.83	0.049	24.40	0.133	24.59	52.83	13.57	0.100	0.078	66.58
UŞAK-38	0.025	6.91	2.27	9.20	0.046	18.86	0.137	19.04	58.39	13.16	0.085	0.122	71.76
UŞAK-39	0.027	6.36	4.06	10.45	0.063	15.85	0.113	16.02	59.00	14.27	0.138	0.122	73.53
UŞAK-40	0.021	5.57	2.22	7.81	0.048	19.15	0.151	19.35	58.60	14.09	0.077	0.080	72.84
UŞAK-42	0.024	5.96	3.14	9.13	0.055	17.50	0.132	17.68	58.80	14.18	0.107	0.101	73.19
UŞAK-49	0.030	6.26	2.52	8.81	0.050	17.67	0.160	17.88	59.00	14.09	0.120	0.100	73.31
UŞAK-50	0.026	7.17	2.00	9.19	0.035	19.90	0.105	20.04	54.83	15.78	0.092	0.064	70.77
Minimum	0.017	5.57	2.00	7.81	0.035	10.81	0.062	11.02	52.83	10.14	0.073	0.038	66.58
Maxsimum	0.033	7.20	4.06	10.45	0.063	24.40	0.164	24.59	64.71	18.89	0.146	0.163	79.79
Mean	0.025	6.36	2.62	9.00	0.048	17.58	0.128	17.75	59.32	13.75	0.096	0.077	73.25

A previous study (Aslansoy 2012) showed that the fatty acid compositions of walnut genotypes was 7.05–8.97% SFAs, 16.67–18.98% MUFAs, and 73.02–75.04% PUFAs. Bilgin (2015) found that the fatty acids content of walnut genotypes was 10.14–11.28% SFAs, 15.23–23.65% MUFAs, and 65.91–74.39% PUFAs. Additionally, one report (Simsek 2016) found that the fatty acid composition was 4.00–7.86% for SFAs, 22.17–29.73% for MUFAs, and 62.73–71.43% for PUFAs. Another previous study (Yerlikaya et al. 2012) determined that the PUFA/MUFA ratio varied from 1.54 to 3.97. It has been stated that the walnut oils consist of MUFAs such as oleic acid and PUFAs such as linoleic and linolenic acid, and this status has positive effective to walnut consumption when considered in terms of health and nutrition (Ayaz 2008). In our study, it has been determined that unsaturated fatty acids constitute more than 90% of the fatty acid distribution of the oil belonging to walnut varieties. This indicates that walnut kernels are very sensitive to oxidation during processing and storage. The fatty acids of walnut kernels are affected by walnut genotype, fertilizers applied during growth, geographical location, and climatic and soil conditions. Additionally, oil composition is affected by the maturity of seed at harvest, seed position on the tree, and seed handling after harvest (Davis et al. 2007; Rabrenovic et al. 2008).

Conclusions

Among genotypes, some differences were observed in molecular and morphological data, and fatty acids profiles. The walnut fatty acid composition was greatly rich in terms of content the linoleic acid and

Table 4 The fatty acids profiles of some local genotypes and local, foreign walnut cultivars (%)

Genotip	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	SAFs	$C_{16:1}$	$C_{18:1}$	$C_{20:1}$	MUFAs	$C_{18:2}$	$C_{18:3}$	$C_{20:2}$	$C_{22:2}$	PUFAs
Chandler	0.016c	6.20gh	1.67i	7.89f	0.036b–e	20.43b–d	0.126a–c	20.60b–d	57.54a–c	13.81a	0.073i	0.089d–f	71.46ab
Pedro	0.025c	6.87d–g	1.83hi	8.73ef	0.048b–d	18.21d–f	0.093a–c	18.35d–f	59.99a–c	12.77a–c	0.080g–i	0.079ef	72.86ab
Fernor	0.027c	8.31ab	2.41fg	10.75a–c	0.042b–e	16.41e–g	0.094a–c	16.55e–g	62.98a	9.59jk	0.070i	0.069fg	72.66ab
Fernette	0.024c	8.00a–c	2.44fg	10.47a–d	0.075a	14.73g	0.091a–c	14.90g	63.53a	10.91e–i	0.078hi	0.105c–e	74.58a
Franquette	0.022c	7.20c–g	2.38fg	9.61c–e	0.041b–e	20.94bc	0.098a–c	21.08bc	58.74a–c	10.39g–j	0.076hi	0.110cd	69.26a–c
Cisco	0.024c	6.26gh	2.26gh	8.54ef	0.034c–e	26.80a	0.138a–c	26.98a	55.22c	9.08k	0.083g–i	0.097d–f	64.42c
Tulare	0.015c	6.31f–h	2.63e–g	8.95d–f	0.045b–d	21.43b	0.174a	21.65b	59.23a–c	9.89i–k	0.153b	0.133bc	69.27a–c
Lara	0.021c	6.41e–h	2.65e–g	9.08d–f	0.051a–d	18.59c–e	0.121a–c	18.77c–e	60.22a–c	11.73cf	0.120c–e	0.088d–f	72.06ab
Howard	0.026c	6.51e–h	2.67e–g	9.20c–f	0.057a–c	15.76fg	0.069bc	15.89fg	61.21a–c	13.56a	0.086g–i	0.044gh	74.85a
Şebin	0.120a	6.32f–h	2.51fg	8.94d–f	0.049a–d	18.54c–e	0.128a–c	18.71c–e	60.22a–c	11.95c–e	0.087f–i	0.089d–f	72.38ab
Kaman-1	0.064b	7.27b–g	1.73hi	9.06d–f	0.039b–e	24.39a	0.118a–c	24.54a	56.69bc	9.47jk	0.148bc	0.087d–f	66.31bc
Bayrak	0.036c	8.17a–c	3.66a–c	11.87a	0.043b–d	14.54g	0.139a–c	14.72g	60.74a–c	12.33b–d	0.203a	0.143b	73.24a
Yavuz	0.035c	7.61b–d	3.69ab	11.34ab	0.052a–d	14.65g	0.141a–c	14.85g	60.20a–c	13.24ab	0.199a	0.179a	73.65a
Sütyemez	0.033c	7.48b–e	3.25b–d	10.76a–c	0.032c–e	14.82g	0.153ab	15.00g	62.85a	11.01e–h	0.228a	0.152ab	74.04a
Maraş-18	0.032c	7.67b–d	4.09a	11.80a	0.045b–d	15.47g	0.133a–c	15.65g	62.20ab	10.16h–k	0.122cd	0.069fg	72.47ab
Oğuzlar-77	0.026c	6.87d–g	2.89d–f	9.78b–e	0.047a–d	15.34g	0.106a–c	15.49g	62.22ab	12.31b–d	0.110d–g	0.084d–f	74.64a
Balkal	0.030c	8.90a	2.61e–g	11.54a	0.016e	14.36g	0.107a–c	14.48g	62.98a	10.77f–i	0.090e–i	0.129bc	73.91a
Arslan-1	0.125a	5.72h	2.74d–g	8.58ef	0.030de	18.27c–f	0.059c	18.36d–f	61.27ab	11.38d–g	0.106d–h	0.032h	72.81ab
Arslan-2	0.034c	7.35b–f	3.14c–e	10.52a–d	0.063ab	16.44e–g	0.059c	16.56e–g	61.00a–c	11.76cf	0.118c–f	0.040gh	72.84ab
HSD (5%)	0.022	1.077	0.536	1.591	0.027	2.713	0.092	2.596	6.027	1.110	0.030	0.030	6.699
Mean	0.039	7.13	2.70	9.86	0.044	17.90	0.113	18.07	60.48	11.37	0.117	0.096	71.98

linolenic acid, which are beneficial to human health. The morphological and biochemical data may be insufficient alone in identification of genetic diversity and relativeness. Compared the morphological markers, the SRAP data was more credible to identify the different walnut genotypes. Dendrograms based on SRAP markers clustered genotypes according to their geographic origin, but not morphological descriptors. There were no differences in terms of the correlation coefficients between morphological properties and molecular (SRAP marker) diversity of the walnut genotypes. We could conclude that especially genotypes selected from Uşak province could be used in future breeding studies.

Acknowledgement This work has been supported by TUBITAK-119O018 and Erciyes University Scientific Research Projects Coordination Unit under Grant number of FHD-2019-8756.

Author contributions (Ercan YILDIZ 25%, Hasan PINAR 15%, Aydın UZUN 15%, Mehmet YAMAN 25%, Ahmet SUMBUL 10%, Sezai ERCISLI 10%).

Funding (This work has been supported by TUBITAK-119O018 and Erciyes University Scientific Research Projects Coordination Unit under Grant number of FHD-2019–8756).

Availability of data and material Available.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

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