Morphological Study of some Persian Walnut Genotypes and Commercial Cultivars Cultured in Kerman Region in Southeast of Iran

Javad FARROKHI TOOLIR (✉)
Mohammadreza MOZAFFARI

Summary

The Rabor Agriculture Research Station (RARS), located in the Kerman province of southeastern Iran, contains one of the largest *in situ* germplasm collections of Persian walnut (*Juglans regia* L.) in the country. In this study, the phenotypic diversity of 10 wild walnut genotypes originating in Iran and 6 foreign cultivars was examined. In 2018-2019, 13 quantitative and 18 qualitative variables were recorded for each individual, including traits related to nut and kernel morphology, kernel phytochemical composition, and tree architecture and phenology. In general, results showed that walnuts from RARS were phenotypically diverse with significant variation found across most traits examined. Results showed that the highest and lowest coefficient of variation (CV) was found for nut shape in perpendicular to suture (CV = 80.46%), and kernel height (CV = 2.73%), respectively. Results also showed a strong positive correlation between nut weight and width (r = 0.794), kernel weight and nut height (r = 0.776), nut weight and height (r = 0.770) kernel size and nut shape in longitudinal to suture (r = 0.701), date of leaf opening and color of annual shoot (r = 0.692), and prominence of pad on suture and shape of apex perpendicular to suture (r = 0.682). Principal component analysis of quantitative variables revealed that the first five principal components (PCs) accounted for 83.61% of the total variation. Regarding qualitative variables, the first seven PCs accounted for 85.95% of the total variation. Cluster analysis based on quantitative and qualitative variables produced a dendrogram with five and eight main clusters, respectively. Grouping of genotypes was not in accordance with their geographical location. Classification of genotypes was different based on qualitative and quantitative traits. This study revealed the presence of high phenotypic diversity in the walnut genotypes from RARS supporting their value for conserving genetic resources and possible use in breeding improved cultivars.

Key words

walnut, correlation analysis, principal component analysis, cluster analysis

[1] Kerman Agriculture and Natural Resources Research and Education Center, Agriculture Research Education and Extension (AREEO), Kerman, Iran

✉ Corresponding author: j.farrokhi@areeo.ac.ir

Received: January 4, 2020 | Accepted: February 4, 2020

Introduction

The Persian walnut (*Juglans regia* L.) is one of the world's major nut crops. Besides providing desirable flavor and texture to various foods, walnuts play an important role in human nutrition and health due to its high oil, protein, vitamin, and mineral contents (Mir et al., 2016). Its native range extends from the Carpathian Mountains of eastern Europe to the southern Caucasus, northern Turkey, Iran, to the Tien Shan province of western China to the Himalayan states of India, Sikkim, and Bhutan (Aradhya et al., 2010). In the sixteenth century, the conquest of the New World enabled the spread of Persian walnut into South America, particularly Chile, before its introduction to California in the nineteenth century (Bernard et al., 2018). Walnut production in the world is 3.8 million tonnes, with China contributing 51% of the total production. Other major producers were the United States and Iran with a production of 0.57 and 0.35 million tonnes, respectively (FAOSTAT, 2017). Based on the obtained maps, the suitable areas for walnut cultivation in Iran are northern strip and northwestern and western provinces of Iran. Also, parts of Fars, Isfahan, Yazd and Kerman provinces are considered suitable to walnut cultivation in Iran (Vahdati et al., 2018). Additionally, a number of walnut collections have been established to study traits and assess morphological and pomological diversity in recent years. The walnut collection at Rabor agriculture research station (RARS) is one of the collections established in 1997. The RARS with 70000 m^2 area is located in Kerman province of southeastern Iran. It is located at 57° 04' to 57° 16' E and 29° 27' to 33° 39' N and its elevation is 2330 m above the sea. Walnut genotypes cultivated in RARS express significant diversity in plant size, growth habit, nut size, nut shape, and many other morphological traits. For germplasm management and better utilization of its genetic potential for breeding programs, it is important to identify, describe, and classify the existing genetic resources in the region. Morphological evaluation is a useful first step to help achieve the goals of characterizing genetic resources (Thompson et al., 1996). Estimating diversity and determining relationships among variables in walnut germplasm can enhance efficiency of its management and support effective genetic improvement efforts (Noor shah et al., 2018). Multivariate analysis, such as cluster analysis (CA) and principal component analysis (PCA) is a useful approach within this context (Mohammadi and Prasanna, 2003) and has been used frequently for genetic diversity analysis in many horticultural crops such as apricot, *Prunus armeniaca* L., (Gurrieri et al., 2001); vineyard peach, *Prunus persica* (L.) Batsch, (Nikolic et al., 2010); apple, *Malus sp*. (Farrokhi et al., 2013); and walnut, *Juglans regia* L. (Alinia-Ahandani et al; 2014; Mahmoodi et al., 2019). The aim of our study was to investigate and document the phenotypic diversity of 9 native genotypes and 7 foreign cultivars in the RARS, using quantitative and qualitative morphological, phonological and pomological traits.

Materials and methods

Plant material

Forty-four coeval genotypes and cultivars were planted in a completely randomized block design in RARS. Among them, 9 promising native genotypes from north: Z series (Z12, Z30) from Qazvin, and Z63 (Jamal) from Karaj, southeast: (Zia8, Kr77, Kr72, Krr111, Kr1-25) from Kerman, and northwest of Iran: (Or-64) from Urmia were evaluated for quantitative and qualitative traits between 2018 and 2019 years, along with 7 commercial cultivars ('Vina', 'Serr', 'Chandler', 'Pedro', 'Franquette', 'Hartly', 'R.D.M'). The basis of selection of native genotypes was random, and attempts were made to select samples from different walnut areas of the country. Although, Kr77 and Kr1-25 were introduced as promising genomes of Kerman in previous studies.

Variable measurement

Based on standard phenotypic characteristics described for walnut in IPGRI (2008), 13 quantitative and 18 qualitative variables were measured for all individuals (Tables 1, 2). The scales of measurement for each qualitative variable are listed in Table 3. Ten fruits (including nut and involucre) per tree were hand harvested randomly across the canopy of the plant at maturity, from mid-September through mid-October, and were transferred to the lab for further analysis. Nut and kernel traits were measured per genotype. The amount of kernel oil and protein was measured by the method A.O.A.C. (1990).

Statistical analysis

Descriptive statistics including mean, minimum, maximum, standard deviation, and coefficient of variation (CV) were calculated for each variable per individual. To avoid effects due to scaling differences, the mean of each variable was normalized prior to cluster analysis using Z-scores. Correlations between quantitative and qualitative variables were determined separately using Pearson and Spearman correlation coefficients, respectively. In order to identify patterns of morphological variations, principal component analysis (PCA) was conducted through a correlation matrix of both quantitative and qualitative variables, separately. The proportion of variance that each eigenvector represents was calculated by dividing the eigenvalue corresponding to each eigenvector by the sum of all eigenvalues. The clustering of genotypes into similar groups was performed using the Ward method based on squared Euclidean distances for quantitative and qualitative variables, separately. All of the calculations were processed using SPSS® software version 20 (SPSS Inc., Chicago, IL, USA, Norusis, 1998).

Results

Descriptive statistics

Results showed that the coefficient of variation (CV) of examined variables was the highest for nut shape in perpendicular to suture (80.46%), followed by nut shape in longitudinal to suture (60.78%), shape of apex perpendicular to suture (59.55%), kernel color (47.76%), thickness of shell (44.01%), tree growth vigor (38.85%), shell color (38.60%), prominence of pad on suture (37.98%), while the lowest CV was found for kernel height (2.73%), kernel width (3.37%), tree height (3.62%), and kernel protein (3.73%) (Table 4). The mean of kernel oil and protein, kernel height, kernel width, nut height, nut width, and TCSA were 27.87%, 16.34%, 2.56 cm, 2.37 cm, 3.91 cm, 3.41 cm, 6.94 cm, respectively (Table 4). For instance, kernel oil content ranged from 16% in 'Vina' to 43% in Kr77. TCSA varied from 5 cm in Z30 to 8.70 cm in Kr72 (Table 1).

Table 1. Measured 13 quantitative variables of 16 studied genotypes and cultivars

Genotype/ Cultivar	Quantitative variable												
	TS	TH	TCSA	KPr	KO	KM	KP	KWE	KH	KWI	NWE	NH	NWI
Zia 8	0.60	200	7.12	14.31	32.00	2.00	42.00	8.41	3.21	2.70	20.00	5.00	5.40
Kr111	0.20	240	5.31	16.19	27.00	0.51	55.75	6.32	2.62	2.53	11.30	3.66	3.74
Z30	0.90	221	5.00	15.25	40.00	0.50	39.65	4.61	2.32	2.10	11.61	3.62	3.10
Vina	0.40	237	7.00	15.94	16.00	1.99	36.60	4.12	2.45	2.13	11.25	3.90	3.22
Serr	0.20	234	7.32	16.00	21.00	0.51	50.66	3.83	2.23	2.42	7.51	3.30	2.90
Chandler	0.40	202	6.50	15.31	17.00	1.48	41.83	4.12	2.82	2.23	9.82	3.80	2.80
Kr1-25	0.40	261	7.00	19.87	39.00	1.00	67.74	4.21	2.84	2.51	6.24	3.74	3.25
Kr77	1.10	203	5.65	16.94	43.00	0.52	44.20	6.12	2.32	1. 72	13.81	5.45	3.51
Or-64	0.20	208	5.64	13.37	23.00	1.00	39.09	4.31	2.71	2.50	11.00	3.54	3.40
Z63 (Jamal)	0.30	285	7.60	17.37	28.00	1.00	44.79	4.34	2.72	2.11	9.63	4.10	3.20
Pedro	0.40	252	8.00	20.25	18.00	1.00	39.94	2.81	2.13	2.81	8.51	3.51	3.12
Z12	0.20	278	8.50	12.75	33.00	1.99	52.27	6.92	2.81	3.00	13.21	4.53	3.20
R.D.M	0.30	287	6.40	12.69	32.00	0.49	52.17	4.80	2.32	2.12	9.21	3.62	3.90
Kr72	0.20	295	8.70	16.75	28.00	2.00	50.57	4.42	2.64	2. 72	8.71	3.65	3.10
Franquette	0.90	311	7.10	20.19	25.00	2.00	30.55	2.23	2.33	2.10	7.25	3.12	2.90
Hartly	0.50	241	8.20	18.31	24.00	0.99	45.25	6.21	2.52	2.31	13.72	4.12	3.90

TS: Thickness of shell (mm); TH: Tree height (cm); TCSA: Trunk cross sectional area; KPr: Kernel protein (%); KO: Kernel Oil (%); KM: Kernel moisture (%); KP: Kernel Percentage (%); KWE: Kernel weight (g); KH: Kernel height (cm); KWI: Kernel width (cm); NWE: Nut weight (g); NH: Nut height (cm); NWI: Nut width (cm)

The lowest tree height was found in 'Chandler' and Kr77, with 292 and 203 cm, respectively. 'Pedro' and R.D.M showed the highest tree growth vigor among the studied individuals. In the present study, both kernel size and kernel ease of removal was high in Z63, and Z30 genotypes, but kernel ease of removal was low in Z12. Date of nut maturity were reported as moderately late in all cultivars and genotypes. The start of male flower shedding was late in R.D.M, Kr77, and 'Franquette', while the start of female flower reception was early in Kr111, Kr1-25, Kr77, Z12, Z30 genotypes. In the present study, date of leaf bud break ranged from 19th March to 28th April across the studied genotypes and cultivars. It was medium for 'Vina', 'Chandler', and 'Pedro', but late for 'Franquette' and early for the rest of individuals (Table 2). Further, in our study kernel percent (ratio) ranged from 39.65% in Z30 to 67.74% in Kr1-25, with a mean of 45.81%.

Relationships between the variables

Simple correlations among 13 quantitative and 18 qualitative variables were calculated and presented in Tables 5-6. Significant correlations were found between some of the quantitative variables. Nut weight had positive a correlation with nut width (r = 0.794, $P < 0.01$) and nut height (r = 0.770, $P < 0.01$). Kernel weight was positively correlated with nut height (r = 0.776, $P<0.01$) and kernel oil (r = 0.544, $P < 0.01$). Kernel moisture was correlated with TCSA (r = 0.564, $P < 0.05$) (Table 5). Kernel size was positively correlated with nut shape in longitudinal to suture (r = 0.701, $P < 0.01$). Date of leaf opening showed positive correlation with color of annual shoot (r = 0.692, $P < 0.01$) and prominence of pad on suture was correlated with shape of apex perpendicular to suture (r = 0.682, $P < 0.01$). Dichogamy was negatively correlated with shell color (r = - 0.772, $P < 0.01$) and tree growth habit (r = - 0.707, $P < 0.01$) (Table 6).

Principal Component Analysis (PCA)

The PCA showed that the first five components among quantitative variables (λ_1 = 3.82, λ_2 = 2.70, λ_3 = 1.65, λ_4 = 1.46, λ_5 = 1.21) and the first seven components of qualitative variables (λ1= 4.11, λ_2 = 2.92, λ_3 = 2.67, λ_4 = 2.13, λ_5 = 1.36, λ_6 = 1.19, λ_7 = 1.06) explained 83.61% and 85.95% of the total variation, respectively (Tables 7-8). In quantitative traits, the first component (PC1) explained 29.39% of the total variation. Variables that positively loaded on PC2 explained 20.83% of the total variation (Table 7). With respect to qualitative characters, PC1 explained 22.87% of the total variation, and variables that positively loaded on PC2 explained 16.23% of the total variation (Table 8).

Table 2. Measured 18 qualitative variables of 16 studied genotypes and cultivars. Scale based on walnut descriptor IPGRI (2008)

Genotype/ Cultivar	Quantitative variable																	
	KER	KS	KC	SC	PPS	SAP	NSP	NSL	DNM	DLS	DI	SMS	SFR	DLO	CAS	DB	TGH	TGV
Zia 8	5	5	4	5	3	1	1	1	5	4	1	2	3	3	7	5	3	3
Kr111	3	5	2	5	7	7	1	5	3	4	1	4	2	3	5	5	2	3
Z30	7	7	2	1	3	3	1	8	4	3	2	4	2	3	5	5	2	3
Vina	5	5	1	7	7	7	7	6	5	4	1	4	3	4	7	7	3	3
Serr	5	7	1	3	7	7	5	7	4	4	1	2	3	3	5	5	3	3
Chandler	3	5	1	5	3	1	5	1	4	5	1	2	3	4	7	3	3	3
Kr1-25	7	3	4	3	3	3	4	1	4	3	2	4	2	3	5	5	2	3
Kr77	5	3	3	3	5	7	1	1	5	3	2	5	2	3	5	5	2	3
Or-64	7	5	2	7	3	1	1	1	4	3	1	2	3	3	5	3	3	3
Z63 (Jamal)	7	7	1	5	5	7	4	8	5	3	1	4	3	3	5	3	3	3
Pedro	5	5	3	7	7	1	1	6	5	4	1	4	3	4	7	3	3	7
Z12	1	7	3	7	5	7	5	4	5	3	1	4	2	3	5	5	2	3
R.D.M	5	7	2	3	3	3	1	4	5	3	2	5	3	3	7	5	1	7
Kr72	5	7	1	7	3	3	1	7	4	3	1	4	3	3	5	3	3	3
Franquette	5	5	2	7	7	7	2	4	5	3	1	5	4	5	7	5	2	3
Hartly	5	7	3	5	7	7	1	5	4	5	1	2	3	3	5	7	3	3

KER: Kernel ease of removal; KS: Kernel size; KC: Kernel color; SC: Shell color; PPS: Prominence of pad on suture; SAP: Shape of apex perpendicular to suture; NSP: Nut shape in perpendicular to suture; NSL: Nut shape in longitudinal to suture; DNM: Date of nut maturity; DLS: Date of leaf shedding; DI: Dichogamy; SMS: Start of male flower shedding; SFR: Start of female flower reception; DLO: Date of leaf opening; CAS: Color of annual shoot; DB: Density of branch; TGH: Tree growth habit; TGV: Tree growth vigor

Table 3. Measured variables and scales used for qualitative traits based on walnut descriptor (IPGRI)

Variable	The qualitative measurement scale																	
TGV					(3)	Weak			(5)	Medium			(7)	Strong				
TGH	(1)	Upright	(2)	Semi Upright	(3)	Spreading												
DB					(3)	Sparse			(5)	Medium			(7)	Dense				
CAS					(3)	Green Brown			(5)	Light Brown			(7)	Tend to black				
DLO					(3)	Early	(4)	Medium	(5)	Late								
SFR			(2)	Early	(3)	Semi early	(4)	Medium	(5)	Late								
SMS			(2)	Early	(3)	Semi early	(4)	Medium	(5)	Late								
DI	(1)	Protanderous	(2)	Protogenious	(3)	Homogame												
DLS					(3)	Early	(4)	Medium	(5)	Late								
DNM					(3)	Early	(4)	Medium	(5)	Late								
NSL	(1)	Rounded	(2)	Triangular	(3)	Broad ovate	(4)	Ovate	(5)	Short Trapezoid	(6)	Long Trapezoid	(7)	Broad Elliptic	(8)	Elliptic	(9)	Cordate
NSP	(1)	Rounded	(2)	Triangular	(3)	Broad ovate	(4)	Ovate	(5)	Short Trapezoid	(6)	Long Trapezoid	(7)	Broad Elliptic	(8)	Elliptic	(9)	Cordate
SAP	(1)	Rounded			(3)	Truncate			(5)	Emarginate			(7)	Pointed				
PPS					(3)	Weak			(5)	Medium			(7)	Strong				
SC	(1)	Very light			(3)	Light			(5)	Medium			(7)	Dark				
KC	(1)	Very light	(2)	Light	(3)	Light Amber	(4)	Amber										
KS					(3)	Weak			(5)	Medium			(7)	Well				
KER	(1)	Very easy			(3)	Easy			(5)	Medium			(7)	Difficult				

KER: Kernel ease of removal; KS: Kernel size; KC: Kernel color; SC: Shell color; PPS: Prominence of pad on suture; SAP: Shape of apex perpendicular to suture; NSP: Nut shape in perpendicular to suture; NSL: Nut shape in longitudinal to suture; DNM: Date of nut maturity; DLS: Date of leaf shedding; DI: Dichogamy; SMS: Start of male flower shedding; SFR: Start of female flower reception; DLO: Date of leaf opening; CAS: Color of annual shoot; DB: Density of branch; TGH: Tree growth habit; TGV: Tree growth vigor

Table 4. The descriptive analysis of 31 variable

Quantitative variable	Minimum	Maximum	Mean	Std. Deviation	C.V %
TS	0.20	40.00	9.11	4.01	44.01
TH	200.00	311.00	247.18	8.97	3.62
TCSA	5.00	8.70	6.94	0.28	4.03
KPr	12.69	20.25	16.34	0.61	3.73
KO	16.00	43.00	27.87	2.05	7.35
KM	0.49	2.00	1.18	0.15	12.71
KP	30.55	67.74	45.81	2.21	4.82
KWE	2.23	6.12	6.73	1.99	29.56
KH	2.13	3.21	2.56	0.07	2.73
KWI	1. 72	3.00	2.37	0.08	3.37
NWE	6.24	20.00	10.79	0.83	7.69
NH	3.12	5.45	3.91	0.15	3.83
NWI	2.80	5.40	3.41	0.156	4.58
Quantitative variable	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**	**C.V %**
KER	1.00	7.00	5.00	1.63	32.60
KS	3.00	7.00	5.62	1.41	25.08
KC	1.00	4.00	2.18	1.04	47.70
SC	1.00	7.00	5.00	1.93	38.60
PPS	3.00	7.00	4.87	1.85	37.98
SAP	1.00	7.00	4.50	2.68	59.55
NSP	1.00	7.00	2.56	2.06	80.46
NSL	1.00	8.00	4.31	2.62	60.78
DNM	3.00	5.00	4.43	0.629	14.19
DLS	3.00	5.00	3.56	0.73	20.50
DI	1.00	2.00	1.25	0.44	35.20
SMS	2.00	5.00	3.56	1.15	32.30
SFR	2.00	4.00	2.75	0.57	20.Kr72
DLO	3.00	5.00	3.31	0.60	18.12
CAS	5.00	7.00	5.75	1.00	17.39
DB	3.00	7.00	4.62	1.31	28.35
TGH	1.00	3.00	2.50	0.63	25.20
TGV	3.00	7.00	3.50	1.36	38.85

TS: Thickness of shell (mm); TH: Tree height (cm); TCSA: Trunk cross sectional area; KPr: Kernel protein (%); KO: Kernel oil (%); KM: Kernel moisture (%); KP: Kernel percentage (%); KWE: Kernel weight (g); KH: Kernel height (cm); KWI: Kernel width (cm); NWE: Nut weight (g); NH: Nut height (cm); NWI: Nut width (cm), KER: Kernel ease of removal; KS: Kernel size; KC: Kernel color; SC: Shell color; PPS: Prominence of pad on suture; SAP: Shape of apex perpendicular to suture; NSP: Nut shape in perpendicular to suture; NSL: Nut shape in longitudinal to suture; DNM: Date of nut maturity; DLS: Date of leaf shedding; DI: Dichogamy; SMS: Start of male flower shedding; SFR: Start of female flower reception; DLO: Date of leaf opening; CAS: Color of annual shoot; DB: Density of branch; TGH: Tree growth habit; TGV: Tree growth vigor.

Table 5. Correlation matrix of 13 qualitative variables using Pearson method in 16 walnut genotypes

Variable	TS	TH	TCSA	KPr	KO	KM	KP	KWE	KH	KWI	NWE	NH	NWI
TS	1.000												
TH	-0.224	1.000											
TCSA	-0.025	0.526*	1.000										
KPr	0.134	0.284	0.256	1.000									
KO	-0.293	0.000	-0.303	-0.103	1.000								
KM	0.150	0.261	0.564*	0.005	-0.289	1.000							
KP	0.199	0.159	0.101	-0.039	0.400	-0.308	1.000						
KWE	-0.179	-0.384	-0.294	-0.038	0.544*	-0.263	0.008	1.000					
KH	0.144	-0.201	0.123	-0.310	0.131	0.467	0.260	-0.096	1.000				
KWI	-0.127	0.143	0.527*	-0.112	-0.196	0.381	0.321	-0.449	0.390	1.000			
NWE	-0.326	-0.537*	-0.090	-0.443	0.233	0.197	-0.213	0.396	0.485	0.087	1.000		
NH	-0.159	-0.412	-0.003	-0.239	0.493	0.091	0.049	0. 776**	0.397	-0. 111	0. 770**	1.000	
NWI	-0.322	-0.311	-0.065	-0.299	0.255	0.069	0.065	0.190	0.501*	0.154	0.794**	0.543	1.000

*, ** Correlations significant at P<0.05 and P<0.01, respectively.

TS: Thickness of shell (mm); TH: Tree height (cm); TCSA: Trunk cross sectional area; KPr: Kernel protein (%); KO: Kernel oil (%); KM: Kernel moisture (%); KP: Kernel percentage (%); KWE: Kernel weight (g); KH: Kernel height (cm); KWI: Kernel width (cm); NWE: Nut weight (g); NH: Nut height (cm); NWI: Nut width (cm)

Table 6. Correlation matrix of 18 qualitative variables using Spearman method in 16 walnut genotypes

Variable	KER	KS	KC	SC	PPS	SAP	NSP	NSL	DNM	DLS	DI	SMS	SFR	DLO	CAS	DB	TGH	TGV
KER	1.000																	
KS	-0.116	1.000																
KC	0.000	-0.447	1.000															
SC	-0.338	0.000	-0.132	1.000														
PPS	-0.264	0.032	-0.124	0. 297	1.000													
SAP	-0.243	0.159	-0.202	0.000	0.682**	1.000												
NSP	-0.237	0.009	-0.361	0.134	0.193	0.319	1.000											
NSL	0.124	0.701**	-0.532*	-0.026	0.391	0.364	0.088	1.000										
DNM	0.000	-0.028	0.171	0.219	0.050	0.059	0.157	-0.048	1.000									
DLS	-0.337	0.024	-0.060	0.095	0.352	-0.051	0.175	-0.063	-0.282	1.000								
DI	0.365	-0.265	0.320	-0.720**	-0.441	-0. 11	-0.235	-0.184	0.059	-0.461	1.000							
SMS	0.000	-0.149	0.017	-0.060	0.097	0.312	-0.114	0.202	0.373	-0.641**	0.485	1.000						
SFR	0.141	0.205	-0.358	0.478	0.218	-0.086	0.014	0.099	0.321	0.198	-0.516*	-0.175	1.000					
DLO	-0.136	-0.246	-0.205	0.458	0.395	0.021	0.225	-0.024	0.319	0.181	-0.309	0.210	0.623**	1.000				
CAS	-0.163	-0.166	-0.016	0.276	0.054	-0.348	0.105	-0.197	0.503*	0.298	-.0149	0.0Kr72	0.577*	0.692**	1.000			
DB	-0.125	-0.009	0.249	-0.211	0.418	0.550*	0.182	0.036	0.051	0.236	0.171	0.061	-0.132	-0.011	0.025	1.000		
TGH	0.129	0.075	-0.252	0.436	0.170	-0.157	0.230	0.140	-0.084	0.507*	-0.707**	-0.686**	0.365	0.088	0.000	-0.241	1.000	
TGV	0.000	0.104	0.117	0.000	0.026	-0.364	-0.295	0.102	0.349	-0.034	0.218	0.317	0.169	0.122	0.488	-0.186	-0.309	1.000

*, ** Correlations significant at P<0.05 and P<0.01, respectively.

KER: Kernel ease of removal; KS: Kernel size; KC: Kernel color; SC: Shell color; PPS: Prominence of pad on suture; SAP: Shape of apex perpendicular to suture; NSP: Nut shape in perpendicular to suture; NSL: Nut shape in longitudinal to suture; DNM: Date of nut maturity; DLS: Date of leaf shedding; DI: Dichogamy; SMS: Start of male flower shedding; SFR: Start of female flower reception; DLO: Date of leaf opening; CAS: Color of annual shoot; DB: Density of branch; TGH: Tree growth habit; TGV: Tree growth vigor

Table 7. Eigenvalues, proportion of total variability as well as eigenvector and correlation between 13 quantitative variables and the 5 first principal components (PCs) in 16 walnut genotypes

Item			**PC axis**		
Eigenvalue	3.822	2.708	1.658	1.468	1.215
Proportion	29.398	20.832	12.754	11.290	9.343
Cumulative	29.398	50.229	62.983	74.274	83.617
			Eigenvector		
Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
TS	-0.280	-0.016	-0.235	-0.697	0.574
TH	-0.603	0.210	0.485	0.424	-0.039
TCSA	-0.351	0.683	0.214	0.344	0.282
KPr	-0.467	-0.168	0.138	0.293	0.498
KO	0.547	-0.278	0.644	0.065	0.059
KM	-0.101	0. 770	-0.278	0.214	0.346
KP	0.022	0.080	0.817	-0.527	0.047
KWE	0.674	-0.452	0.133	0.242	0.389
KH	0.438	0.673	0.048	-0.382	0.132
KWI	-0.128	0. 774	0.240	-0.129	-0.255
NWE	0.878	0.318	-0.239	0.146	-0.071
NH	0.869	0.093	0.092	0.165	0.384
NWI	0.734	0.355	0.023	0.043	-0.209

TS: Thickness of shell (mm); TH: Tree height (cm); TCSA: Trunk cross sectional area; KPr: Kernel protein (%); KO: Kernel oil (%); KM: Kernel moisture (%); KP: Kernel percentage (%); KWE: Kernel weight (g); KH: Kernel height (cm); KWI: Kernel width (cm); NWE: Nut weight (g); NH: Nut height (cm); NWI: Nut width (cm)

Table 8. Eigenvalues, proportion of total variability as well as eigenvector and correlation between 18 qualitative variables and the 7 first principal components (PCs) in 16 walnut genotypes

Item				**PC axis**			
Eigenvalue	4.117	2.921	2.672	2.134	1.368	1.199	1.068
Proportion	22.873	16.230	14.846	11.856	7.599	6.660	5.931
Cumulative	22.873	39.103	53.949	65.805	73.404	80.064	85.955
				Eigenvector			
Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**
KER	-.0315	0.040	-0.218	0.410	-0.171	0.696	0.366
KS	.0238	-0.384	0.175	0.628	0.387	-0.164	-0.152
KC	-0.462	0.265	-0.156	-0.551	0.156	-0.139	0.384
SC	0.697	0.247	-0.033	-0.016	-0.325	-0.438	0.226
PPS	0.543	-0.164	0.579	-0.247	0.160	0.025	0.379
SAP	0.151	-0.470	0.781	-0.207	-0.147	0.025	0.131
NSP	0.398	-0.191	0.233	-0.217	-0.326	0.219	-0.625
NSL	0.245	-0.389	0.463	0.637	0.201	0.074	0.080
DNM	0.100	0.624	0.362	0.054	-0.140	0.073	-0.027
DLS	0.570	-0.168	-0.286	-0.414	0.539	0.130	-0.103
DI	-0.899	0.164	0.166	-0.004	0.084	0.275	-0.151
SMS	-0.389	0.369	0.726	0.170	-0.218	-0.145	0.046
SFR	0.687	0.411	-0.035	0.290	0.015	0.274	0.157
DLO	0.573	0.571	0.249	-0.112	-0.161	0.185	-0.014
CAS	0.403	0.796	0.026	-0.097	0.246	0.161	-0.240
DB	-0.034	-0.217	0.507	-0.548	0.320	0.327	0.068
TGH	0.678	-0.251	-0.501	0.057	-0.133	0.181	0.174
TGV	-0.092	0.622	0.125	0.315	0.543	-0.168	0.008

KER: Kernel ease of removal; KS: Kernel size; KC: Kernel color; SC: Shell color; PPS: Prominence of pad on suture; SAP: Shape of apex perpendicular to suture; NSP: Nut shape in perpendicular to suture; NSL: Nut shape in longitudinal to suture; DNM: Date of nut maturity; DLS: Date of leaf shedding; DI: Dichogamy; SMS: Start of male flower shedding; SFR: Start of female flower reception; DLO: Date of leaf opening; CAS: Color of annual shoot; DB: Density of branch; TGH: Tree growth habit; TGV: Tree growth vigor

Cluster analysis (CA)

According to a dendrogram generated by Ward methods based on squared Euclidean distance, 16 walnut individuals were classified into five and eight separate groups on the basis of quantitative and qualitative variables, respectively. The cluster cut-off point was $\sqrt{(n/2)}$ where n equals to the number of genotypes (Manning et al., 2009). None of the observed clusters showed any clear separation between internal genotypes and foreign cultivars.

Discussion

The data showed significant variations across multiple traits, indicating a high level of phenotypic diversity present in the walnut germplasm of RARS. Based on walnut descriptor IPGRI (2008), the quantitative and qualitative traits were measured among 16 individuals via nominal and ordinal scales, respectively (Tables 1-3). Therefore, determining the coefficients of correlation, PCA, and clustering of both trait sets were carried out in a way different from each other. In this study, Pearson's and Spearman's coefficient were used for quantitative and qualitative traits, respectively. Pearson's correlation coefficient is a measure of the strength of the linear relationship between two such variables. In 1904 Spearman adopted Pearson's correlation coefficient as a measure of the strength of the relationship between two variables that cannot be measured quantitatively (Hauke and Kossowski, 2011). Generally, the genotypes and cultivars located in RARS had short shrubs with lower kernel traits compared to the ones located in other places. In this study, nut weight ranged from 6.24 to 20 g, kernel weight from 2.23 to 6.12 g, and kernel percentage from 30.55 to 67.74%). Among the analyzed characters, nut shape in perpendicular to suture showed the highest coefficient of variation (80.46%), and the lowest CV was found in kernel height (2.73%). In our study, CV of shell thickness was reported 44.01%. In a study of 18 important traits of Persian walnut from walnut collection of Karaj, Iran, during 2014 and 2015, a wide variation was observed for nut weight (6.60 – 15.33 g), kernel weight (2.67 – 8.21 g), and kernel percentage (35.39 – 71.09%). The thickness of membrane showed the highest coefficient of variation (134.03%), while the lowest CV was found in nut width (8.09%) and the nut roundness index (8.15%) (Mahmoodi et al., 2019). In a study on native Persian walnut of Ardabil province of northwest of Iran, the results showed wide variation for kernel weight (3.63 - 7.03 g), nut weight (7.05 - 12.65 g), and kernel percentage (42.7 - 63.73%) (Ghanbari et al., 2018). Arzani et al. (2008) and Khadivi-Khub et al. (2015) reported the highest CV for shell thickness (29% and 31%) and the lowest CV for the nut width (10% and 8%), respectively. Obviously, diversity in average nut weight was described in other reports too. The highest nut weight obtained in this study (20 g) was quite similar to 20.28 g that was reported by Khadivi-Khub et al. (2015), while the lowest (15.02 g) was similar to Arzani et al. (2008). In another study, Ebrahimi et al. (2017) reported an average nut weight of 14.42 g for walnuts from Qazvin, Iran, while Akca and Ozongun (2004) reported 15.20 g from Anatolia, Turkey. Pop et al. (2013) reported 14.60 g from Romania and Hassani et al. (2014) reported 13.62 g from Karaj, Iran. In this study, the highest value for kernel weight among the studied accessions (6.12 g) was lower than the kernel weight reported by Hassani et al. (2014) for walnuts in Karaj (7.55 g) and Zeneli et al. (2005) for walnuts in Albania (7.20 g). The percentage of kernel is another prominent character which is of great importance to walnut breeders. The kernel percentage is influenced by the weight of nut and kernel, and there are significant correlations between these traits (Cosmulescu and Botu, 2012). Genotypes with a kernel ratio above 50% are preferred (Germain, 1997; Korac et al., 1997). Data obtained in this study revealed that kernel percentage varied from 30.55 to 67.74% (Table 2). The mean of kernel percentage observed (45.81%) was lower than the data reported by Zeneli et al. (2005) (63.80%) for walnuts in Albania, Aslantas (2006) (67.14%) for walnuts in Turkey, Pop et al. (2013) (51.60%) for Romanian National Collection of Walnut, Ebrahimi et al. (2015) (62.18%) for walnuts genotypes in Neiriz region of Iran and Rezaei et al. (2018) (66.29) in Malayer County of Iran. In this study, kernel percentage (ratio) was reported 45.25, 36.6, 50.66, and 41.83% in 'Hartley', 'Vina', 'Serr', and 'Chandler', respectively. These values were lower than the kernel percentages reported for these cultivars (Serr and Ford, 1994). One of the reasons is the incompatibility of foreign cultivars with cold mountainous conditions of Iran. On the other hand, native genotypes in the soil and climatic conditions of RARS performed better than foreign cultivars.

Generally, the most desirable range of shell thickness in walnut was reported 0.70 – 1.50 mm (Zhadan and Strukov, 1977). The shell thickness range in our genotypes varied from 0.20 to 1.10 mm. The Kr111, 'Serr', Or-64, Z12, Kr72 had the lowest (0.20 mm) and the Kr77 had the highest shell thickness (1.10 mm). Rezaei et al. (2018) have reported a range of 0.72 – 2.46 mm for shell thickness. In another study, the shell thickness was reported from 1.00 to 2.70 mm (Ebrahimi et al., 2015). Mahmoodi et al. (2019) reported the shell thickness ranging from 0.64 to 2.02 mm. In the present study, nut width ranged from 2.8 cm in 'Chandler' to 5.4 cm in Zia8. This is higher than the values observed by Mahmoodi et al. (2019) and Cosmulescu et al. (2017), (2.79 – 3.97cm and 2.48 – 3.74 cm respectively). In our study, the nut height varied between 3.12 cm in 'Franquette' and 5.00 in Kr77. A previous research by (Cosmulescu and Botu, 2012) from Romania showed a nut height variation ranging from 2.82 cm to 4.97 cm. Furthermore, Ebrahimi et al. (2015) reported a range of 3.61 – 4.37 cm for nut height in walnuts from Iran. Our research showed that kernel protein ranged from 12.69 to 20.25%, and oil from 16 to 43%. The highest and lowest kernel oil was found in Kr77 and 'Vina', respectively. Kernel protein content was high among all genotypes and cultivars, but kernel oil was lower than in previous studies. The mean of kernel and oil protein was reported 9.62 and 58.04, respectively, by Ghanbari et al., 2017. Another study found walnut kernels containing 52 to 72% oil (Martinez et al., 2006). A study conducted in Turkey, Caglarirmak (2003) reported 63% as the average oil value of the studied genotypes. However, because of the economic value of the oil, these kernels could be used as potential sources of oils. The mean value for protein percentage among our evaluated nuts (16.34%) was higher than the corresponding data reported by Golzari et al. (2013). In our study, the majority of studied individuals broke their leaves early in the season, but leafing time was reported late for 'Franquette' and medium for 'Vina', 'Chandler' and 'Pedro'. None of the examined accessions were homogamous, but Kr1-25, Kr77, Z30, and R.D.M were protogenious. Kernel ease of removal was assessed as difficult in Z30, Or-64, Z63, Kr1-25, while it was very easy in Z12. 'Pedro' and R.D.M showed higher vigor compared to the rest of cultivars (Table 2).

Ghanbari et al. (2018) reported 68% and 32% of genotypes as synchronous and asynchronous, respectively. There was 94% protandrous and 6% protogynous. The removal of the kernel from its shell in the most genotypes was moderately difficult. Significant correlations were found among nut weight, nut width, and nut height. Kernel weight was positively correlated with nut weight and kernel oil, while kernel moisture was correlated with TCSA (Table 5). Kernel size was positively correlated with nut shape in longitudinal to suture. Date of leaf opening showed positive correlation with color of annual shoot (Table 6). Less correlations were found with qualitative traits compared to quantitative ones. Positive correlations were observed among nut and kernel dimensions in previous studies of walnut genotypes. A highly significant correlation was obtained between nut weight, nut length, nut width, nut thickness and kernel weight in the study of Mahmoodi et al., 2019. Similar correlations between these traits have been previously reported by other authors (Amiri et al., 2010; Mosivand et al., 2012; Poggetti et al., 2017). Ghanbari et al. (2018) reported significant correlations among the different traits such as kernel weight and nut diameter, kernel weight and leaf length, dry weight and nut diameter, and dry weight, nut length, kernel weight and nut length and dry weight and kernel weight. Analyzing the coefficients of correlation between different traits involved in walnut production provides informative data about the relative effect of every character on yield. Correlation coefficients between the phenological traits could determine whether selection for one trait may affect the other traits. Response to direct selection for these variables may be unpredictable, unless there is a good control of environmental variables. These complex relationships between traits certainly lead to difficulties in performing studies and analyses, however this issue should be overcome by utilizing multivariate statistical methods. This method is often described as a flexible approach by which numerous correlated variables are reduced to few main factors. Therefore, this analytic procedure could be successfully utilized to comprehend the patterned variation in a set of variables, based on structural relationships among variables (Tadesse and Bekele, 2001). In view of the diversity of walnut accessions under study, the analysis into main components was carried out to determine the contribution and effect rate of each trait under study on the present diversity. As a criterion to extract the main principal components, eigenvalues greater than one were taken into account. Low variance in eigenvectors among qualitative variables may be due to existing low correlation among studied characters (Abdi and Williams, 2010). To determine which PC scores accounted for the greatest variation, the eigenvalues of these components were compared for each character. The PCA showed that the first five components among quantitative variables and first seven components of qualitative variables explained 83.61% and 85.95% of the total variation, respectively (Tables 7 and 8). With respect to quantitative variables, kernel oil, kernel weight, kernel height, nut weight, nut height, and nut width were found influential in the first component (PC1), explaining 29.39% of the total variation, and this component was involved in nut and kernel traits. Variables that positively loaded on PC2, explaining 20.83% of the total variation, were kernel width, kernel moisture and TSCA (Table 7). With respect to qualitative characters, PC1 explaining 22.87% of the total variation, was positively associated with kernel size, shell color, prominence of pad on suture, nut shape in perpendicular to suture, nut shape in longitudinal to suture, date of leaf shedding, start of female flower reception, date of leaf opening, color of annual shoot, and tree growth habit. Variables that positively loaded on PC2, explaining 16.23% of the total variation, were date of nut maturity, start of male flower shedding, and tree growth vigor (Table 8). Mahmoodi et al., (2019) divided eighteen traits into six groups by using factor analysis. Their result indicated that six factors accounted for 79.95% of the total variance. The first and second PCs accounted for 19.99% and 13.93% of the variation. PC1 was strongly associated with nut weight, kernel weight, nut thickness and nut width. In an evaluation of morphological diversity among some Persian walnut accessions in Guilan, northern Iran, PC1 and PC2 explained 49.9%, and 20.5%, respectively, overall 84.4% of the total variance. The coefficient of eigenvectors in the first component indicated that nut yield, nut weight, kernel weight, and nut length had the greatest contribution to this component development (Alinia-Ahandani et al., 2014), and these results were in agreement with ours. Morphological characteristics are able to appropriately differentiate walnut species and genotypes into separate groups using multivariate statistical methods. Hierarchical cluster analysis was used to investigate the similarities and dissimilarities among the genotypes with respect to seed and nut variables. The high number of generated clusters for both sets of variables showed that there is high in-population diversity of walnuts in RARS, which is desirable potential for using in the next breeding programs. The clustering pattern of individuals was different due to differences in scoring and measurement between quantitative and qualitative traits. Regarding the dendrogram generated based on quantitative variables, cluster I was the largest, including 5 genotypes, followed by cluster II with 4 genotypes while each of the clusters III and IV included 3 genotypes. 'Vina', 'Serr', and 'Chandler' were grouped in cluster IV, while only one genotype was in cluster V (Figure 1). According to qualitative traits, cluster I was the largest with 3 genotypes, followed by clusters II, III, IV, V, VI, and VII, each with 2 genotypes and. cluster VIII with 1 genotype (Figure 2). None of the observed clusters showed any clear separation between internal genotypes and foreign cultivars. Selection of parents from clusters showing the highest inter-cluster distance may be used for hybridization program. In contrast, lower inter-cluster distance indicates close relationship and similarity among genotypes and selecting parents from these clusters should be avoided (Sirvastava et al., 2010). The results of a research on several Persian walnut species and inter-specific hybrids showed that Z63, 'Hartley', 'Serr', Z30, K72, 'Ronde', 'Pedro', 'Chandler', and B21 were located in a separate group (Mosivand et al., 2012).

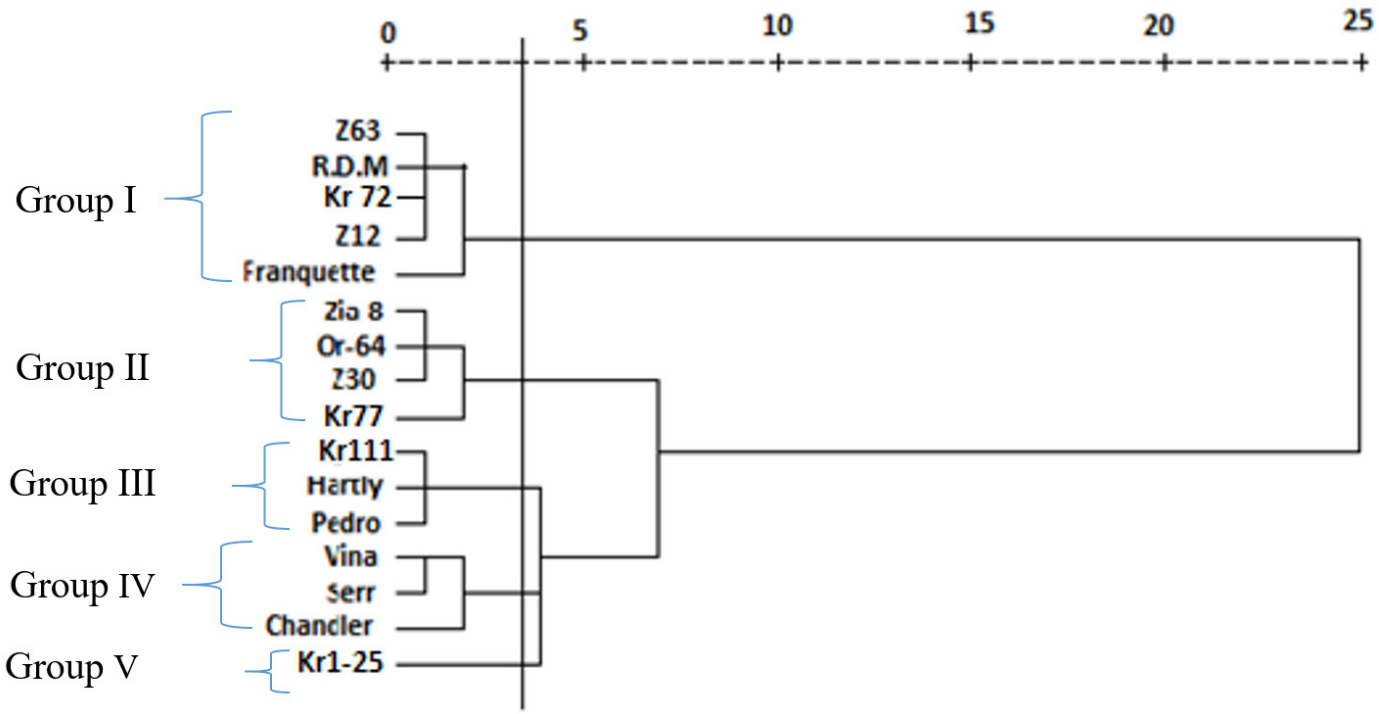

Figure 1. Dendrogram for the 16 walnuts collected from RARS produced by Ward's cluster analysis; based on 13 quantitative characters (scale: Squared Euclidean distance)

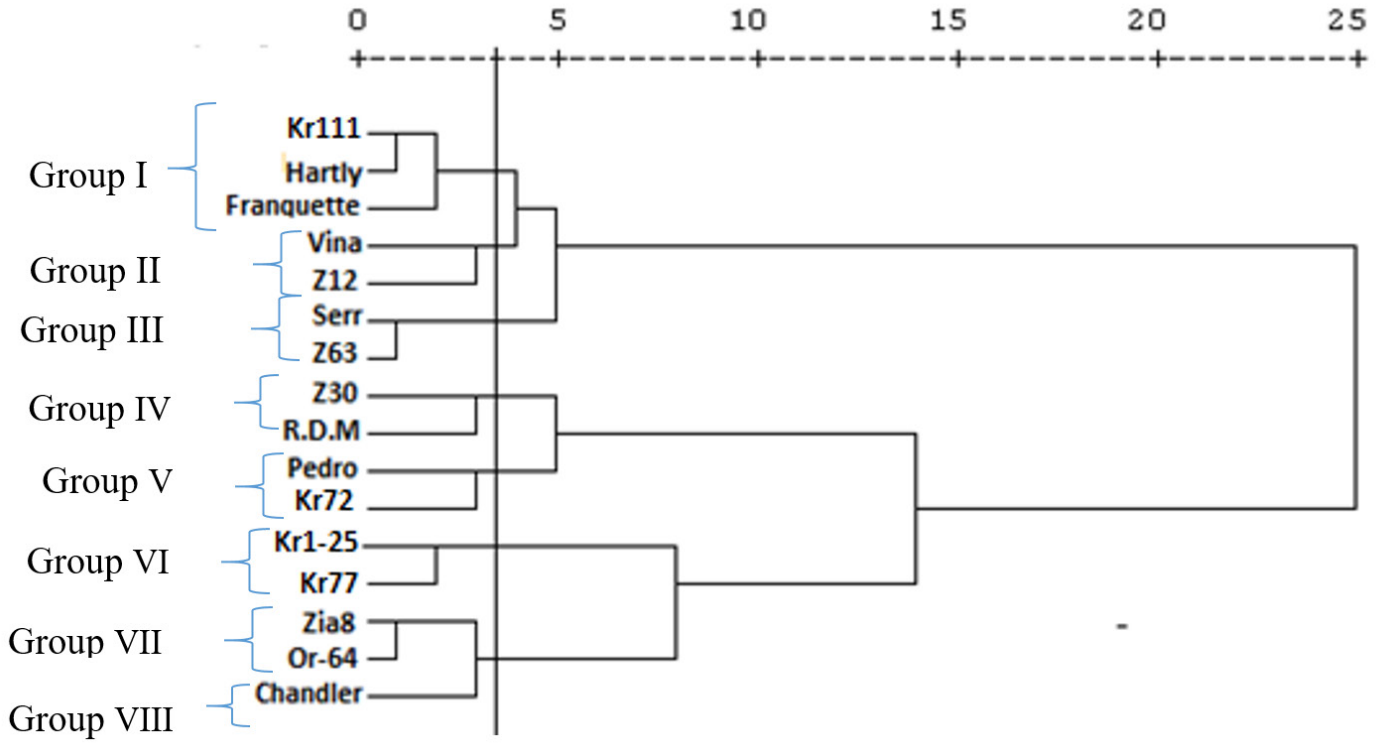

Figure 2. Dendrogram for the 16 walnut collected from RARS produced by Ward's cluster analysis; based on 18 qualitative characters (scale. Squared Euclidean distance)

Conclusion

Our study, as a part of an ongoing project for breeding of the Persian walnut in Iran, was carried out to characterize the 16 walnut genotypes and cultivars in RARS collection. The comparison of phenotypic diversity of 31 traits was analyzed using the SPSS software. The results revealed a high diversity in morphological and phenological characteristics in studied individuals, supporting the presence of a high level of genetic diversity. According to the PCA, the first component in quantitative traits was associated with nut and kernel traits, whereas for qualitative variables, it included kernel size, shell color, prominence of pad on suture, nut shape in perpendicular to suture, nut shape in longitudinal to suture, date of leaf shedding, start of female flower reception, date of leaf opening, color of annual shoot, and tree growth habit. Interestingly, while on average they had a smaller nut and kernel size and weight, a lower kernel oil content was also found when compared to some walnut germplasm pools previously studied around the world. These native genotypes may be useful in the breeding of improved plants exhibiting enhanced adaptation to harsh (cold/semi-dry) environments. Additionally, the genotype clustering pattern was independent of geographical distances among them. In summary, the analysis of multiple phenological and pomological traits of the walnut genotypes documents the significant variation present *in situ* found in the region, supporting the needs to conserve this valuable resource. The results may also help breeders choose the appropriate individual genotypes and utilize them as parents in a breeding program for improving future generations of commercial cultivars with improved adaptation to harsh climatic conditions.

Acknowledgement

The authors wish to thank the AREEO, Kerman, Iran, Kerman Agriculture and Natural Resources Research and Education Center for providing financial support to this study. The authors also thank Dr. Darab Hasani, Faculty member of Horticultural Science Research Institute (HSRI), Karaj, Iran for generously sharing his knowledge and experiences.

Refrences

A.O.A.C. (1990). Official methods of analysis. 15th Edition Association of Official Analytical Chemists INC, USA.

Abdi H., Williams L.J. (2010). Principal component analysis. Wiley Interdisciplinary Reviews Computational Staisticst 2: 433–459.

Akca Y., Ozongun S. (2004). Selection of late leafing, late flowering, laterally fruitful walnut (*Juglans regia*) types in Turkey. N. Z. J. Crop Hortic Sci 337–342.

Aliniaahandani E., Darzi Ramandi H., Sarmad J., Asadi Samani M., Alimohammad Yavari A., Alinia ahandani R. (2014). Evaluation of morphological diversity among some Persian Walnut accessions (*Juglans regia* L.) in Guilan, Northern Iran Int J Plant Biol Res 2(3): 1016–1024.

Amiri R., Vahdati K., Mohsenipoor S., Mozaffari, M. R., Leslie C. (2010). Correlations between some horticultural traits in walnut. Hortic Sci 45: 1690–1694.

Aradhya M., Woeste K., Velasco D. (2010). Genetic Diversity, Structure and Differentiation in Cultivated Walnut (*Juglans regia* L.). Proc. VIth Walnut Symposium Ed.: D.L. McNeil Acta Hortic ISHS, pp. 861.

Arzani K., Mansouri-Ardakan H., Vezvaei A., Roozban, M. (2008). Morphological variation among Persian walnut (*Juglans regia* L.) genotypes from central Iran. N. Z. J.Crop Hortic Sci 36: 159–168.

Aslantas R. (2006). Identification of superior walnut (*Juglans regia* L.) genotypes in north eastern Anatolia, Turkey. N. Z. J. Crop Hortic Sci 34: 231–237.

Bernard A., heureux F.L., Dirlewanger E. (2018). Walnut: past and future of genetic improvement Tree Genet & Genomes 14: 1–28.

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

Cosmulescu S., Botu M. (2012). Walnut biodiversity in southwestern Romania resource for perspective cultivars. Pak J Bot 44: 307–311.

Cosmulescu S., Stefanescu D., Birsanu Ionescu M. (2017). Genetic diversity among *Juglans regia* genotypes based on morphological characters of nut. Erwerbs-Obstbau. https://doi.org/10.1007/ s10341-017-0347-5.

Ebrahimi A., Khadivi-Khub A., Nosrati Z., Karimi R. (2015). Identification of superior walnut (*Juglans regia*) genotypes with late leafing and high kernel quality in Iran. Sci Hortic 193: 195–201.

Ebrahimi A., Zarei A., Zamani M., Lawson S. (2017). Evaluation of genetic variability among early mature *Juglans regia* using microsatellite markers and morphological traits. https://doi.org/10.7717/ peerj. 3834.

Farrokhi J., Darvishzadeh R., Hatami Maleki H., Naseri L. (2013). Evaluation of Iranian native apple (*Malus* × *domestica* Borkh.) germplasm using biochemical and morphological characteristics. Acs 78 (4): 307–313.

Food and Agriculture Organization of the United Nations. (2017). Production crops. At: http:// faostat. fao. Org /site /567 /Desktop Default. aspxPage ID=567.

Ghanbari A., Faraji M., Shokouhian A., Pyrayesh A. (2018). Evaluation of quantitative and qualitative characteristics of Persian walnut (*Juglans regia* L.) genotypes in the west of Meshkin-Shahr. J Nuts 9 (1): 57–65.

Golzari M., Rahemi M., Hassani D., Vahdati K., Mohammadi N. (2013). Protein content, fat and fatty acids of kernel in some Persian walnut (*Juglans regia* L.) cultivars affected by kind of pollen. J Food Sci & Tech 38(10): 21–31.

Gurrieri F., Audergon J.M., Albagnac G., Reich M. (2001). Soluble sugars and carboxylic acids in ripe apricot fruit as parameters for distinguishing different cultivars. Euphytica 117: 183–189.

Hassani D., Mozaffari M.R., Dehghan Shoraki Y., Soleimani A., looni A. (2014). Vegetative and reproductive traits of some iranian local and foreign cultivars and genotypes of walnut (*Juglans regia* L.). Seed Plant Improv J 4: 839–855.

Hauke J., Kossowski T. (2011). Comparison of values of Pearson's and Spearman's correlation Coefficients on the same sets of Data. Queastions geographic 30 (2):40–48.

Manning C.D., Raghavan P., Schütze H. (2009). Hierarchical clustering. Cambridge University Press. Feedback welcome. Chapter 17: 377-401.

I.P.G.R.I. (2008). Descriptor for walnut (*Juglans.* Spp.). International Plant Genetic Resources Institute Rome, Italy, pp. 57.

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

Mahmoodia R., Dadpour M.R., Hassani H., Zeinalabedini M., Vendramin E., Micali S., Zaare Nahandi F. (2019). Development of a core collection in Iranian walnut (*Juglans regia* L.) germplasm using the phenotypic diversity. Sci Hortic 249: 439–448.

Martinez M. L., Mattea M.A., Maestri D.M. (2006). Varietal and crop year effects on lipid composition of walnut (*Juglans regia* L.) genotypes. J American Oil Chem Soc 83(9): 791–796.

Mir G.M., Nisar O., Igbal U. (2016). Scientific Processing of Walnuts Necessary for Amazing Health Benefits. J Chem & Chemical Sci 6 (8): 783–793.

Mohammadi S.A., Prasanna B.M. (2003). Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Sci 43: 1235–1248.

Mosivand M., Hassani D., Payamnour V., JafarAghaei M. (2012). Comparison of tree, nut, and kernel characteristics in several walnut species and inter-specific hybrids. Crop Breed J 3(1): 25–29.

Nikolic D., Rakonjac V., Milatovic V., Fotiric M. (2010). Multivariate analysis of vineyard peach (*Prunus persica* (L.) Batsch.) germplasm collection. Euphytica 171: 227–234.

Noor Shah U., Mir J.I., Ahmed N., Fazli K.M. (2018). Assessment of germplasm diversity and genetic relationships among walnut (*Juglans regia* L.) genotypes through microsatellite markers. J Saudi Soc Agri Sci 17: 339–350.

Norusis M. (1993). SPSS for windows; advanced statistics. Release 6, SPSS Inc, Chicago. Illinois, USA, pp. 828.

Poggetti L., Ermacora P., Cipriani G., Pavan F., Testolin R. (2017). Morphological and carpological variability of walnut germplasm (*Juglans regia* L.) collected in North- Eastern Italy and selection of superior genotypes. Hortic Sci 225: 615–619.

Pop I.L., Vicol A.C., Botu M., Raica P.A., Vahdati K., Pamfi D. (2013). Relationships of walnut cultivars in a germplasm collection: comparative analysis of phenotypic and molecular data. Sci Hortic 153: 124–135.

Rezaei Z., Khadivi A., ValizadehKaji B., Abbasifar A. (2018). The selection of superior walnut (*Juglans regia* L.) genotypes as revealed by morphological characterization. Euphytica https:// doi.org/10.1007/s10681-018-2153-z.

Serr E.F., Forde H.I. (1994). The walnut germplasm collection of the University of California, Davis. Gene Res Conser Program 13: 1-37.

Sirvastava K., Khursheed K.A., Zargar A., Shyma R.S. (2010). Genetic divergence among *Corylus colurna* genotypes based on morphological characters of hazelnut. Biod Res & Conserv 17: 13–17.

Tadesse W., Bekele E. (2001). Factor analysis of components of yield in grass pea (*Lathyrus sativus* L.). Lathyrus Lathyrism News lett 2: 91–93.

Thompson M.M., Lagerstedt H.B., Mehlenbacher S.A. (1996) Hazelnuts Janick J., Moore J.N, (Ed.). Fruit Breeding.Wageningen Press, the Netherlands, pp. 125-184.

Vahdati K., Massah Bavani A., Khosh-khui M., Fakor P., Sarikhani S. (2018.) Land Suitability Classification of Persian Walnut Cultivation in Iran Using Geographic Information System (GIS). Iranian J Horti Sci & Tech 19 (3): 403 – 418.

Zeneli G., Kola H., Dida M. (2005). Phenotypic variation in native walnut populations of Northern Albania. Sci Hortic 105: 91–100.

Zhadan V.M., Strukov M.V. (1977). Breeding walnut for fruit size. Plant Breed 47: 918–925.

aCS85_14