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Pomological and Phenological Identification of Some Walnut (*Juglans regia* L.) Genotypes and Cultivars

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A R T I C L E I N F O

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A B S T R A C T

The Persian walnut (*Juglans regia* L.) is one of the most valuable crops in Iran. The present study was conducted in The rabor agriculture research station (RARS) in Kerman province to investigate 21 important pomological and phenological variables among 37 walnut native genotypes and commercial cultivars in the year 2020. In general, results showed that walnuts from RARS were phenotypically diverse with the significant variation found across most traits examined. The highest and lowest coefficient of variation (CV) was for kernel percentage (CV= 29.48%), and time of male flowering (CV= 14.77%), respectively. Results also showed a strong positive correlation between nut weight and kernel weight (r= 0.727). The date of nut maturity had the lowest correlation with kernel intensity of the ground color (r= -0.476). Principal component analysis of variables revealed that the first eight principal components (PCs) accounted for 79.43% of the total variation. Cluster analysis based on variables produced a dendrogram with five main clusters. The grouping of genotypes was not following their geographical origin. This study revealed the presence of high phenotypic diversity in the walnut genotypes of Rabor Agricultural Research Station (RARS), supporting their breeding value for possible use in the future.

Introduction

Persian walnut (*Juglans regia* L.) is one of the major nut crops in the world with a total worldwide production of 965.4000 tones. China, United States, and European Union contributed 47, 27.87, and 6.26 % of the total production, respectively (FAOSTAT, 2019). This nut crop is the most economically important member of its genus is which cultivated for its timber and edible nuts throughout temperate regions of the world (Vahdati, 2000). Because walnuts have been sexually propagated in Iran for many years, there is a high genetic diversity in the walnut population of Iran (Arab *et al.,* 2019; Vahdati *et al.,* 2019). Evaluation of genetic diversity and identification of superior genotypes is a fundamental step in breeding programs in walnut (Sarikhani *et al.,* 2020). Besides, information on morphological properties of superior genotypes can help breeders to release commercial varieties with high kernel quality (Thompson *et al.,* 1996). Estimating and determining relationships among morphological variables in the walnut germplasm can enhance the efficiency of its management and support effective genetic improvement efforts (Noor shah *et al.,* 2018). Moreover, characterization of walnut germplasm is important for ongoing walnut breeding programs around the world especially where the climates and elevations are similar (Akca *et al.,* 2020). Multivariate analysis (MA) methods, such as cluster analysis (CA) and principal component analysis (PCA) are useful approaches within this context (Mohammadi and Prasanna, 2003). These methods have been used frequently for genetic diversity analysis in walnut, *Juglans regia* L. (Alinia-Ahandani *et al.,* 2014;

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Pourkhaloee *et al.*, 2017; Ashrafi *et al.*, 2018; Hassanzadeh Khankahdani *et al.*, 2019; Mahmoodi *et al.*, 2019; Farrokhi Toolir and Mozzaffari, 2020). Iran is reported to be a center of diversity for (*Juglans regia* L.) and wild walnut trees are found in virtually every corner of the country (Vahdati *et al.*, 2015). Thus, Iran is considered a rich natural pool of walnut gene pool for developing improved genotypes. Kerman province is the leading area for walnut production in Iran. Most of the walnut orchards of the province are fruiting (13500 ha) and the rest are non-fruiting (3500 ha) (Anonymous, 2018). In addition to the extensive cultivated area in this province, native genotypes scattered in mountainous areas and valleys. Hence, some measures have begun regardings to collect, evaluate compatibility, and morphological characteristics of promising genotypes and compare their yield with several well-known commercial cultivars in some recent years. An example of these measures included the collection and cultivation of native genotypes and commercial cultivars from all over the country at the Rabor Agricultural Research Station (RARS). These actions have been done synchronously with National Walnut Program (NWP) in Iran. The output of this study is important for the next fruit breeding programs in Iran to make introgression resistance to winter frost, late flowering, and adaptation to different ecological conditions, as well as early harvest date, high yield, and fruit quality.

Materials and Methods

Rabor agriculture research station (RARS) is located in an area of 70000 m2 between 57° 04' to 57° 16' E and 29° 27' to 33° 39' N and altitude 2330 m above the sea. Forty-four genotypes and cultivars of the same age have been planted in a completely randomized block design in RARS that in this study, only 28 Iranian native genotypes and 9 commercial cultivars were evaluated in the year 2020. Kerman series (15, 72, 77, 95, 96, 111, 118, and 154) and also 88-1 belonged to Kerman province (Table 1). The basis of the selection of native genotypes was random. Trees planted at RARS are irrigated by drip irrigation with a debit 0.5 (l/s per ha) and their fertilizer program is adjusted based on leaf and soil analysis. Accordingly, the fertilizer composition included: urea, and boric-acid (5×10^{-3}) in fruit set stage, triple superphosphate (200 kg), potassium sulfate (400 kg), iron sulfate (70 kg), copper sulfate (20 kg) was given per (ha) in the year of the experiment. Pruning of trees was partial and limited to the removal of suckers, offsets, and dried shoots.

Table 1. Measured 21 variables of 37 studied genotypes and cultivars. Scale based on walnut descriptor UPOV (1999) (for abbreviation see below)

No.	Genotype/Cultivar	NS	NSLSS	NW	NSC	SBPS	NPAT	PoPS	DGPS	NPPS	SSS	TS	ATHS	KER	KIGC	KS	KW	KP	DNM	TLBB	TMF	TFF
1	Chandler	5	4	3	2	2	3	2	3	3	3	5	5	3	1	5	3	3	6	6	7	9
2	Fernor	3	6	3	1	2	7	1	5	5	2	5	7	5	1	3	3	1	6	7	8	9
3	Franquette	7	6	5	2	2	7	3	5	7	2	5	7	5	3	7	3	1	6	2	9	9
4	G3	7	1	7	1	3	3	2	3	3	2	5	5	3	3	5	7	3	6	4	6	8
5	Hartley	5	4	5	2	4	7	2	5	7	3	5	5	5	3	7	7	5	5	3	5	8
6	Kd23	5	4	1	3	2	7	5	5	7	3	3	5	3	5	5	5	9	4	7	7	6
7	Kh33	5	4	7	2	2	7	2	5	7	2	5	7	5	5	7	7	3	4	5	7	8
8	Kerman 77	5	8	5	3	3	3	1	7	5	2	3	5	5	7	5	5	3	3	2	7	5
9	Kerman 15	5	4	5	1	1	2	1	5	3	1	3	5	3	5	3	5	3	3	3	6	5
10	Kerman 72	5	5	9	2	3	3	2	7	7	3	7	7	5	7	5	7	1	7	4	6	8
11	Kerman 88-1	5	8	3	3	2	5	2	3	3	2	3	5	3	3	5	5	5	6	6	9	8
12	Kerman 95	7	8	3	1	2	5	2	5	3	3	3	5	3	5	5	5	3	5	4	6	8
13	Kerman 96	7	4	5	2	2	5	2	5	5	3	5	3	1	5	7	5	3	3	3	7	6
14	Kerman 111	5	7	3	1	3	5	1	3	3	1	3	7	5	5	3	5	5	3	2	7	5
15	Kerman 118	7	1	3	1	3	3	3	5	7	1	3	7	5	5	3	5	3	4	2	7	5
16	Kerman 154	7	6	7	1	4	3	3	3	3	1	3	3	3	7	7	7	3	3	2	7	6
17	Kz6	5	1	3	1	4	1	2	3	3	2	5	5	3	7	1	3	1	5	3	6	8
18	Lara	5	8	5	1	4	3	1	5	7	2	3	7	7	5	3	3	3	4	6	7	9
19	Or2	5	1	1	1	3	3	3	5	5	2	5	5	5	5	5	1	1	5	3	6	8
20	Or24	5	1	3	2	3	3	2	5	5	2	3	3	1	5	7	5	3	4	2	7	5
21	Or64	5	5	3	1	3	3	2	5	5	3	7	8	5	3	5	3	1	6	2	5	7
22	Or126	7	1	9	2	4	1	5	7	3	1	3	3	5	5	7	9	3	3	1	7	5
23	Pedro	5	5	5	1	4	3	1	5	3	5	5	7	7	3	3	5	1	5	6	7	9
24	R.D.M	3	5	3	1	2	5	3	5	3	3	3	5	3	5	3	5	1	4	5	8	6
25	Serr	5	6	5	1	4	7	3	7	5	3	5	7	5	5	3	5	3	5	7	5	8
26	Sh1	3	7	3	1	2	3	1	5	5	1	1	5	5	5	5	5	3	3	2	7	7
27	Sh14	5	3	3	2	3	3	3	5	3	3	3	5	3	5	7	7	5	5	2	5	8
28	Vina	7	6	7	2	4	5	3	5	7	3	5	3	5	3	7	5	1	6	5	8	9
29	Yazd	5	5	7	1	4	3	1	5	3	2	3	5	7	5	5	9	5	5	5	7	8
30	Z4	5	8	5	1	2	5	2	3	3	2	3	3	3	5	5	7	5	3	3	7	5
31	Z8	7	1	9	1	4	7	2	5	7	2	5	9	7	3	7	7	1	6	3	5	8
32	Z12	7	6	9	1	3	1	1	5	7	2	5	5	3	3	7	9	5	4	2	6	6
33	Z16	5	8	3	2	1	3	1	5	7	3	3	5	3	7	3	3	3	4	2	7	5
34	Z30 (Karaj)	9	6	3	3	1	3	2	3	3	3	3	5	3	3	8	5	5	3	2	7	8
35	Z60	7	6	9	1	4	7	3	5	7	2	5	7	5	5	7	9	3	4	5	7	9
36	Z63 (Jamal)	7	5	5	3	2	7	1	5	3	4	5	5	3	5	5	5	3	5	4	6	8
37	Zia 36	5	5	3	1	4	1	2	5	5	2	7	7	7	5	1	5	3	5	5	7	9

Nut size: (NS); Nut shape in longitudinal section through suture: (NSLSS); Nut weight: (NW); Nut shape in cross section: (NSC); Shape of base perpendicular to suture: (SBPS); Nut prominence of apical tip: (NPAT); Position of pad on suture: (PoPS); Depth of groove along pad on suture: (DGPS); Nut prominence of pad on stature : (NPPS); Structure of surface of shell: (SSS); Thickness of shell: (TS); Adherence of two halves of shell: (ATHS); Kernel ease of removal: (KER); Kernel intensity of ground color : (KIGC); Kernel size: (KS); Kernel weight: (KW); Kernel percentage: (KP); Date of nut maturity: (DNM); Time of leaf bud burst: (TLBB); Time of male flowering: (TMF); Time of female flowering: (TFF).

Variable measurement

The present study was done to investigate and document 21 phenological and pomological variables, by MA methods in 37 individuals (19 native genotypes from several provinces + 9 from Kerman province + 9 commercial cultivars) planted at RARS in one year. Based on standard phenotypic characteristics described for walnut in UPOV (1999), 21 pomological and morphological variables measured for all individuals. The scales of measurement for each variable listed in Table 2. For evaluation of pomological traits, 25 nuts per tree were hand-harvested randomly across the canopy at maturity between 10^{th} September and 11^{th} October, and were transferred to Kerman Agriculture and Natural Resources Research and Education Center laboratory for further analysis. The nuts collected from selected genotypes were dried and their humidity was reduced to 8%. Nut width, nut length, and shell thickness were measured with a 0.01 mm sensitive caliper. Kernel weight and nut weight of the genotypes were weighed with a 0.01 g sensitive electronic scale. The kernel ratio was calculated using the formula KR= (KW/NW) ×100 (Akça *et al.,* 2021). Walnut color was classified according to the California walnut color chart. The empty kernel and kernel shrivel rates in walnuts were also calculated.

Table 2. Measured variables and scales used for variables based on walnut descriptor UPOV (1999) (for abbreviation see below)

Traits	The qualitative measurement scale
NS	Very small (1), Small (3), Medium (5), Large (7), Very large (9).
NSLSS	Circular (1), Triangular (2), Broad ovate (3), Ovate (4), Short Trapezoid (5), Long Trapezoid (6), Broad Elliptic (7), Elliptic (8).
NW	< 8.5 g, Very low (1), 8.51-11.5 g, Low (3), 11.51- 13.5 g, Medium (5), 13.51-17.5 g, High (7), > 17.51 g, Very high (9).
NSC	Oblate (1), Circular (2), Elliptic (3).
SBPS	Cuneate (1), Rounded (2), Truncate (3), Emarginate (4).
NPAT	Weak (3), Medium (5), Strong (7).
PoPS	On upper half (1), On upper 2/3 (2), On whole length (3).
DGPS	Narrow (3), Medium (5), Broad (7).
NPPS	Weak (3), Medium (5), Strong (7).
SSS	Slightly grooved (1), Moderately grooved (2), Strongly grooved (3), Embossed (4).
TS	Very thin (1), Thin (3), Medium (5), Strong (7).
ATHS	Very weak (1), Weak (3), Medium (5), Strong (7), Very strong (9).
KER	Very easy (1), Easy (2), Medium (3), Difficult (4).
KIGC	Very light (1), Light (2), Medium (3), Dark (4).
KS	Very small (1), Small (3), Medium (5), Large (7), Very large (9).
KW	< 3.5 g, Very low (1), 3.51-4.5 g, Low (3), 4.51- 6.5 g, Medium (5), 6.51-7.5 g, High (7), > 7.51 g, very high (9).
KP	30- 40.99% Very low (1), 41 -50.99% Low (3), 51-60.99 % Medium (5), 61 -70.99 % High (7), 71 -80.99 % Very high (9).
DNM	Early (3), Early to medium (4), Medium (5), Medium to late (6), Late (7).
TLBB	Very early (1), Very early to early (2), Early (3), Early to medium (4), Medium (5), Medium to late (6), Late (7), Late to very late (8),Very late (9).
TMF	Very early (1), Very early to early (2), Early (3), Early to medium (4), Medium (5), Medium to late (6), Late (7), Late to very late (8),Very late (9).
TFF	Very early (1), Very early to early (2), Early (3), Early to medium (4), Medium (5), Medium to late (6), Late (7), Late to very late (8),Very late (9).

Nut size: (NS); Nut shape in longitudinal section through suture: (NSLSS); Nut weight: (NW); Nut shape in cross section: (NSC); Shape of base perpendicular to suture: (SBPS); Nut prominence of apical tip: (NPAT); Position of pad on suture: (PoPS); Depth of groove along pad on suture: (DGPS); Nut prominence of pad on stature : (NPPS); Structure of surface of shell: (SSS); Thickness of shell: (TS); Adherence of two halves of shell: (ATHS); Kernel ease of removal: (KER); Kernel intensity of ground color : (KIGC); Kernel size: (KS); Kernel weight: (KW); Kernel percentage: (KP); Date of nut maturity: (DNM); Time of leaf bud burst: (TLBB); Time of male flowering: (TMF); Time of female flowering: (TFF).

Statistical analysis

Kolmogorov–Smirnov (KS) test, a non-parametric statistical method, was used for the evaluation of descriptive statistics based on the type of scoring for qualitative traits (Vrbik, 2018). By this method, descriptive statistics including mean, minimum, maximum, standard deviation, and coefficient of variation (CV) were calculated for each variable per individual. Correlation between variables was determined using Spearman correlation coefficients. (Hauke and Kossowski, 2011). To identify patterns of morphological variations, principal component analysis (PCA) was conducted through a correlation matrix of variables. The clustering of genotypes into similar groups was performed using the Ward method based on squared Euclidean distances for variables. The cluster cut-off point was $\sqrt{\frac{n}{2}}$ where (n) equal the number of genotypes (Manning *et al.*, 2009). 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 high for kernel percentage (CV= 29.48%), kernel weight (CV= 28.57%), kernel size (CV= 28.26%), and adherence of two halves of a shell (CV= 28.07%), Whiles, it was the low for the shape of base perpendicular to suture (CV= 18.72%), and time of male flowering (CV= 14.77%) (Table 3). In our study, the highest nut weight was obtained in Kerman 72, Or126, Z8, Z12, and Z60. The highest kernel weights showed in Or126, Z60, Z12, and Yazd genotypes. (Table 1). The mean of nut weight, kernel weight, and kernel percentage were reported very low, medium, and low, respectively. The highest and the lowest mean belonged to the time of male flowering and nut shape in cross-section traits, respectively (Table 3). In the viewpoint of date of nut maturity, Chandler, G3, 88-1, Or64, Franquette, Vernor, Vina, and Z8 were medium to late, but Or126, Z30, Z4, and Kerman series (77, 96, 111, and 154) were early. About the time of leaf budburst, Or126 was the earliest, but 88-1, Chandler, Lara, Pedro, and Serr were medium to late and late. Or24 showed the medium time of female flowering, but Vernor, Vina, Lara, Z60, Zia 36, Pedro, Franquette blossomed later than everyone else. None of the studied genotypes and cultivars had simultaneous flowering.

Table 3. The descriptive analysis of 21 variables

Variable	Minimum	Maximum	Mean	Std. Deviation	C.V %
Nut size	3.00	9.00	5.59	1.32	23.61
Nut shape in longitudinal section through suture	1.00	9.00	4.86	1.34	27.57
Nut weight	1.00	9.00	4.72	1.31	27.75
Nut shape in cross section	1.00	3.00	2.56	0.58	22.65
Shape of base perpendicular to suture	1.00	4.00	2.83	0.53	18.72
Nut prominence of apical tip	1.00	7.00	4.10	0.99	24.14
Position of pad on suture	1.00	5.00	3.90	1.02	26.15
Depth of groove along pad on suture	3.00	7.00	4.78	1.13	23.60
Nut prominence of pad on stature	3.00	7.00	5.78	1.45	25.08
Structure of surface of shell	1.00	5.00	3.32	0.88	26.50
Thickness of shell	1.00	7.00	4.13	0.97	23.48
Adherence of two halves of shell	3.00	9.00	5.45	1.53	28.07
Kernel ease of removal	1.00	7.00	4.20	1.06	25.23
Kernel intensity of ground color	1.00	7.00	4.51	1.16	25.72
Kernel size	1.00	8.00	5.52	1.56	28.26
Kernel weight	1.00	9.00	5.32	1.52	28.57
Kernel percentage	1.00	9.00	4.24	1.25	29.48
Date of nut maturity	3.00	7.00	4.54	1.16	25.55
Time of leaf bud burst	1.00	7.00	4.70	1.04	22.12
Time of male flowering	5.00	9.00	6.70	0.99	14.77
Time of female flowering	5.00	9.00	7.24	1.49	20.58

Relationships between variables

Simple correlations among 21 variables were calculated and presented in Table 4. Significant correlations were found between some of the variables. The highest positive correlations were shown between nut weight and kernel weight (r= 0.727, P<0.01). There was a positive correlation between the date of nut maturity and time of female flowering (r= 0.664, P<0.01), and thickness of the shell (r= 0.637, P<0.01). Also, a positive correlation was observed among adherence of two halves of shell and kernel ease of removal (r= 0.648, P<0.01), time of leaf bud burst and time of female flowering (r= 0.606, P<0.01), the shape of base perpendicular to suture and kernel ease of removal (r= 0.569, P<0.01), nut shape and kernel shape (r= 0.558, P<0.01), and thickness of shell and time of female flowering (r= 0.551, P<0.01). Date of nut maturity was negatively correlated with kernel intensity of ground color (r= -0.476, P<0.01) and kernel percentage (r= -0.415, P<0.05). There was a negative correlation between the thickness of the shell and kernel percentage (r= -0.449, P<0.01).

Table 4. Correlation matrix of 21 variables using Spearman method in 37 walnut genotypes (for abbreviation see below)

Variable	NS	NSLSS	NW	NSC	SBPS	NPAT	PoPS	DGPS	NPPS	SSS	TS	ATHS	KER	KIGC	KS	KW	KP	DNM	TLBB	TMF	TFF
NS	1.000																				
NSLSS	-.109	1.000																			
NW	.460**	-.054	1.000																		
NSC	.158	-.009	-.035	1.000																	
SBPS	.120	-.201	.377*	-.353*	1.000																
NPAT	.029	.182	.072	.201	-.163	1.000															
PoPS	.289	-.373*	.001	.125	.196	.192	1.000														
DGPS	-.102	-.059	.258	.113	.209	.043	.078	1.000													
NPPS	.069	.033	.153	.101	.140	.284	.113	.453**	1.000												
SSS	-.004	.032	-.117	.380*	-.136	.297	.043	.147	.020	1.000											
TS	. 125	-.280	.296	-.079	.292	.131	.069	.148	.314	.357*	1.000										
ATHS	-.157	.054	-.027	-.320	.166	.229	-.151	.206	.374*	.016	.429**	1.000									
KER	-.148	.083	.209	-.326*	.569**	.059	-.111	.398*	.353*	-.172	.271	.648**	1.000								
KIGC	-.141	.068	-.021	-.012	.071	-.247	.035	.247	-.024	-.197	-.323	-.200	-.119	1.000							
KS	.558**	-.139	.445**	.444**	.010	.211	.334*	.009	.186	.052	.020	-.340*	-.195	-.243	1.000						
KW	.316	-.153	.727**	-.014	.304	.026	.144	.109	-.038	-.170	-.061	-.184	.024	.076	.494**	1.000					
KP	.093	.122	-.043	.296	-.208	.023	-.045	-.269	-.213	-.084	-.449**	-.351*	-.353*	.032	.248	.468**	1.000				
DNM	-.043	-.177	.032	.009	.184	.209	.098	.030	.147	.395*	.637**	.394*	.244	-.476**	-.074	-.183	-.415*	1.000			
TLBB	-.302	.096	-.060	-.077	.074	.404*	-.010	.000	.032	.369*	.299	.253	.195	-.238	-.354*	-.131	-.146	.435**	1.000		
TMF	-.099	.372*	-.133	.208	-.243	.142	.046	-.174	.012	-.195	-.299	-.162	.016	-.100	-.031	-.199	-.047	-.163	.171	1.000	
TFF	0.036	.065	.051	-.053	.272	.244	.039	-.053	.131	.338*	.551**	.405*	.419**	-.466**	.009	-.184	-.375*	.664**	.606**	.081	1.000

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

Nut size: (NS); Nut shape in longitudinal section through suture: (NSLSS); Nut weight: (NW); Nut shape in cross section: (NSC); Shape of base perpendicular to suture: (SBPS); Nut prominence of apical tip: (NPAT); Position of pad on suture: (PoPS); Depth of groove along pad on suture: (DGPS); Nut prominence of pad on stature : (NPPS); Structure of surface of shell: (SSS); Thickness of shell: (TS); Adherence of two halves of shell: (ATHS); Kernel ease of removal: (KER); Kernel intensity of ground color : (KIGC); Kernel size: (KS); Kernel weight: (KW); Kernel percentage: (KP); Date of nut maturity: (DNM); Time of leaf bud burst: (TLBB); Time of male flowering: (TMF); Time of female flowering: (TFF).

Principal Component Analysis (PCA)

The PCA showed that the first 8 components among variables (λ_1= 4.29, λ_2= 3.08, λ_3= 2.58, λ_4= 1.75, λ_5= 1.48, λ_6= 1.25, λ_7= 1.21, λ_8= 1.02,) explained 79.43% of the total variation. The first and second components (PC1, PC2) explaining 20.45%, and 14.67% of the total variation (Table 5).

Table 5. Eigenvalues, proportion of total variability as well as eigenvector and correlation between 21 variables and the 8 first principal components (PCs) in 37 walnut genotypes (for abbreviation see below)

Item				PC axis				
Eigenvalue	4.294	3.082	2.582	1.750	1.485	1.252	1.217	1.020
Proportion	20.450	14.674	12.293	8.333	7.072	5.960	5.794	4.858
Cumulative	20.450	35.124	47.417	55.750	62.822	68.782	74.576	79.434
				Eigenvector				
Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
NS	-0.171	0.579	.0323	-0.237	0.129	-0.279	-0.150	0.044
NSLSS	0.028	-0.418	.0137	0.550	0.362	-0.291	0.247	0.203
NW	0.121	0.810	-0.030	0.123	0.327	-0.156	0.130	0.198
NSC	-0.282	0.001	0.687	0.176	-0.329	-0.111	0.042	0.180
SBPS	0.407	0.509	-0.392	-0.048	0.106	0.352	0.055	0.213
NPAT	0.281	0.005	0.571	0.414	0.025	0.117	-0.038	0-.317
PoPS	-0.136	0.390	0.233	-0.007	-0.353	0.608	-0.375	0.071
DGPS	0.242	0.344	-0.189	0.523	-0.514	-0.046	0.112	0.197
NPPS	0.350	0.295	0.070	0.542	-0.252	-0.206	-0.333	-0.206
SSS	0.382	-0.134	0.496	-0.129	-0.329	-0.105	0.502	0.193
TS	0.722	0.254	0.088	-0.270	-0.192	-0.167	0.053	0.032
ATHS	0.726	-0.046	-0.185	0.210	0.041	-0.131	-0.056	-0.434
KER	0.656	0.139	-0.370	0.339	0.237	0.088	-0.063	0.015
KIGC	-0.394	0.083	-0.484	0.289	-0.366	0.003	0.192	0.243
KS	-0.259	0.630	0.543	0.016	0.096	-0.194	-0.131	-0.032
KW	-0.232	0.735	0.040	0.122	0.350	0.186	0.356	-0.024
KP	-0.545	0.037	0.343	0.182	0.104	0.356	0.328	-0.365
DNM	0.768	0.016	0.263	-0.284	-0.084	0.012	-0.033	0.057
TLBB	0.558	-0.343	0.311	0.181	0.115	0.479	0.195	0.127
TMF	-0.150	-0.362	0.271	0.288	0.338	0.072	-0.507	0.457
TFF	0.778	-0.039	0.314	-0.166	0.209	0.055	-0.001	0.156

Nut size: (NS); Nut shape in longitudinal section through suture: (NSLSS); Nut weight: (NW); Nut shape in cross section: (NSC); Shape of base perpendicular to suture: (SBPS); Nut prominence of apical tip: (NPAT); Position of pad on suture: (PoPS); Depth of groove along pad on suture: (DGPS); Nut prominence of pad on stature : (NPPS); Structure of surface of shell: (SSS); Thickness of shell: (TS); Adherence of two halves of shell: (ATHS); Kernel ease of removal: (KER); Kernel intensity of ground color : (KIGC); Kernel size: (KS); Kernel weight: (KW); Kernel percentage: (KP); Date of nut maturity: (DNM); Time of leaf bud burst: (TLBB); Time of male flowering: (TMF); Time of female flowering: (TFF).

Cluster analysis (CA)

According to a dendrogram generated by Ward methods based on squared Euclidean distance, 37 walnut individuals were classified into five separate groups based on variables. The first, second, third, fourth, and fifth clusters included 7, 9, 6, 6, and 9 individuals, respectively (Fig 1). None of the observed

clusters showed any clear separation between internal genotypes and foreign cultivars.

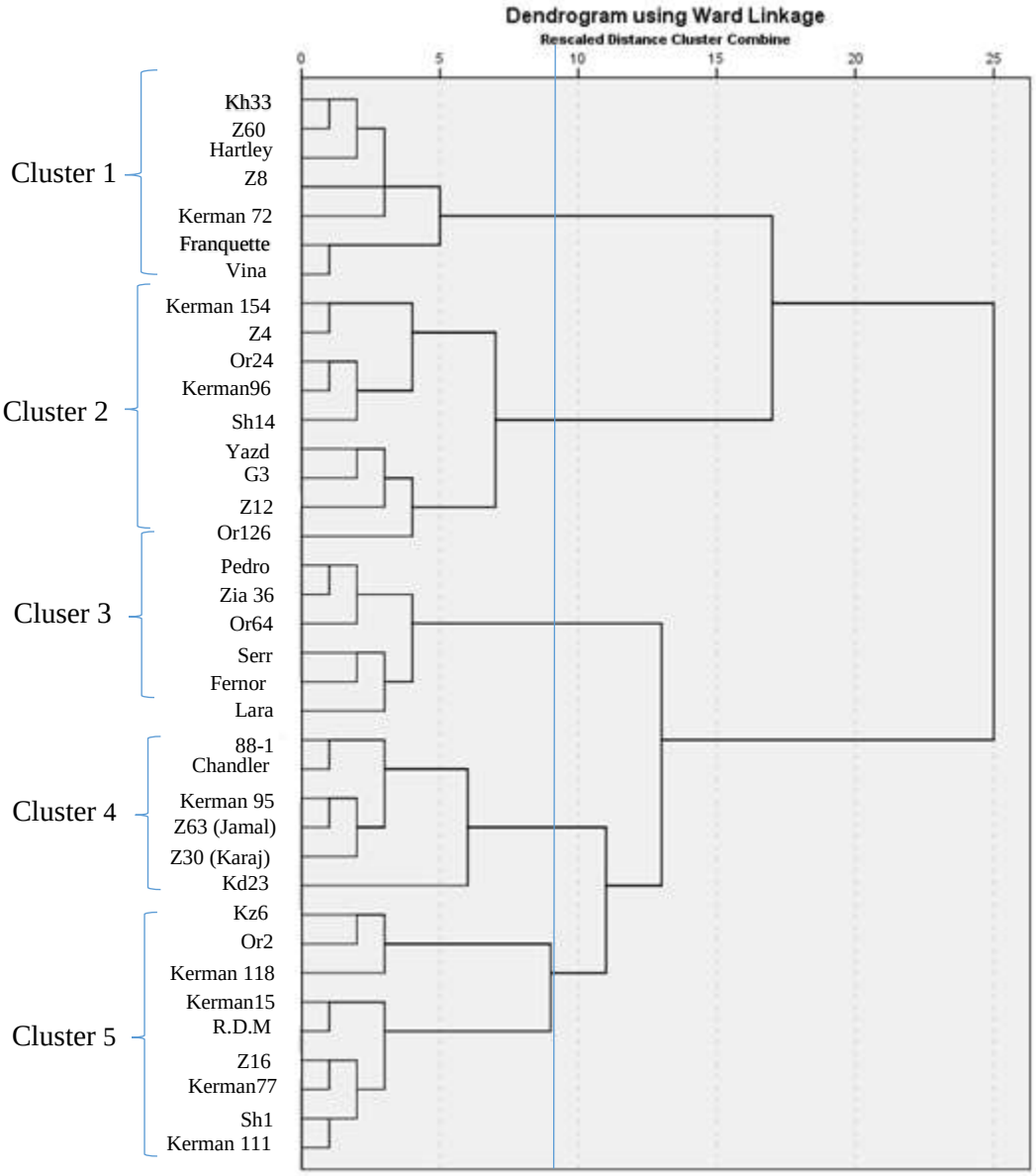

Fig 1. Dendrogram for the 37 walnut collected from RARS produced by Ward's cluster analysis; based on 21 variables (scale.Squared Euclidean distance)

Discussion

The data revealed significant variations across multiple traits, which indicated a high level of phenotypic diversity in the walnut germplasm of RARS. In all 37 studied individuals, the time of leaf budburst was early to medium and focused on 23 March to 3 April. The date of nut maturity occurred from 16 September to 25 September. In total, female flowers bloomed about 5 days after male flowering from 16 April to 22 April. Previous studies on RARS walnuts, revealed the flowering time has changed from one year

to the other (Mozzaffari *et al.,* 2009). In a study on walnuts in Ardabil province, 68% and 32% of genotypes were reported as synchronous and asynchronous, respectively (Ghanbari *et al.,* 2018). The walnut phenology depends greatly on the weather conditions in the year and observations made over two calendar years have shown that the flowering period was influenced by environmental factors, especially temperature (Črepinšek *et al,* 2009). Low temperature is a major limiting factor in the distribution of woody plants and freezes injury is a leading cause of horticultural yield losses. High temperatures in spring accelerate the evolution of the male flowers, but the female flowers were not influenced significantly. In walnut, the cognition of the type and timing of flowering is very important for pollination, because homogamy types are quite rare in the analyzed population (Marian and Niculina, 2017). In this study nut weight ranged (7.2-19.3g), kernel weight (3.4- 10.35g), and kernel percentage (33.48-80.55%) (Data not showed). In a study on Persian walnut with 18 important traits during 2014 and 2015, a wide variation was observed for nut weight (6.6- 15.33g), kernel weight (2.67– 8.21 g), and kernel percentage (35.39- 71.09%) (Mahmoodi *et al.*, 2019). The survey of superior Persian walnut genotypes originated from the southwest of Iran showed wide variation in kernel weight (7.2-10.2g), nut weight (13.2-18.4g), and kernel percentage (48.7 - 56.5%) (Sarikhani et al., 2020). In a study on native Persian walnut of Ardabil province of the northwest of Iran, the results showed variation in kernel weight (3.63 - 7.03g), nut weight (7.05 - 12.65g), and kernel percentage (42.7 - 63.73%) (Ghanbari et al., 2018). In a study on walnut genetic resources in Kazakhstan the average nut weight of the grafted genotypes varied from (8.87-15.48g), kernel weight (6.01-7.00g), and kernel percentages (47.76% - 70.87 %) (Akça et al., 2020). In our study, the highest nut weight obtained 19.35 g was quite similar to 20.28g reported by Khadivi-Khub et al. (2015). In several studies, the average nut weight reported as follows: 14.42g for genotypes from Qazvin, Iran, (Ebrahimi *et al.* 2015), 15.20g for Anatolia, Turkey (Akça and Ozongun ,2004), 14.60g for Romania genotypes (Pop *et al.,* 2013), and 13.62g for Karaj, Iran (Hassani *et al.,* 2014). The highest kernel weights showed in Or126, Z60, Z12, and Yazd genotypes (Table 1). The percentage of the kernel is another prominent character that is of great importance to walnut breeders. The mean of kernel percentage (46.41%) was lower than what reported by Zeneli *et al.* (2005) for walnuts in Albania, Aslantas (2006) for Turkish walnuts, Pop *et al.* (2013) for Romanian national collection of walnut, Ebrahimi *et al.* (2015) for walnuts genotypes in Neiriz region of Iran and Rezaei *et al.* (2018) for the Malayer county of Iran. Kd23 showed the highest kernel percentage (80.55%), with nut and kernel weights 7.2g and 5.8g, respectively. Despite its small size, it showed a fuller kernel compared to the rest (Tables 1, 2). The range of nut and kernel weights varied in commercial cultivars and reported as follows: Serr (8.5-11.5g, 4.51-6.5g), Franquette (11.51-13.5g, 3.5-4.5g), Pedro (11.5-13.5g, 4.5-6.5g), Hartley (11.5-13.5g, 13.5-17.5g), Lara (8.5-11.5g, 3.5-4.5g), Fernor (8.5-11.5g, 3.5-4.5g), Vina (13.5-17.5g, 4.5-6.5g), R.D.M (11.5-13.5g, 4.5-6.5g), and Chandler (8.5-11.5g, 3) (Tables 1, 2). Comparison of these data with what has been reported in other studies, as well as native genotypes, showed some of them, had less nut and kernel characteristics (Amiri *et al.,* 2010; Mosivand *et al.,* 2012; Hassani *et al.,* 2014; Mahmoodi *et al.,* 2019). It seems that several environmental factors such as the unique climate and soil conditions of the RARS location were the main reasons to step down as efficiency of foreign cultivars. Aslani Aslamarz *et al.* (2010) showed Lara, was hardy resistant to supercooling and cold hardiness. According to the above-mentioned reasons, it seems that its low yield was not related to the effect of low temperatures. In other words, unfavorable soil conditions of RARS

location, such as inappropriate soil texture and structure, and also the existence of thick hardpan in half a meter above the soil was more effective than the weather conditions in reducing the performance of Lara (Mozzaffari *et al.,* 2009). The coefficient of variation (CV) is one of the important statistics using to describe the traits. The highest and lowest CV belonged to kernel percentage and time of male flowering (Table 3). In our study CV of the shell, the thickness was 23.48 %. In other studies on Iranian walnut genotypes, CV of shell thickness was reported 29% and 31%, respectively by Arzani *et al.* (2008) and Khadivi-Khub *et al.* (2015). Also, high CV values in some traits were confirmed in a previous study on walnut genotypes and cultivars in Kerman province by Farrokhi Toolir and Mozzaffari. (2020). There was a significant correlation among pomological nut traits. Positive correlations were shown between nut weight and kernel weight (r= 0.727, P<0.01) and nut size (r= 0.460, P<0.01), kernel size (r= 0.445, P<0.01) (Table 4). Such correlations were observed among nut and kernel dimensions in previous studies of walnut genotypes (Amiri *et al.,* 2010; Mosivand *et al.,* 2012; Poggetti *et al.,* 2017). A highly significant correlation was obtained between nut weight, nut length, nut width, nut thickness, and kernel weight in Iranian walnut genotypes (Ghasemi *et al.,* 2012; Mahmoodi *et al.,* 2019). A positive correlation was found between nut length, nut width, nut thickness, nut weight and kernel weight. The highest correlation coefficient was calculated between nut width, and nut thickness (r= 0.857 P<0.01) in Kazakhstan (Akça *et al.,* 2020). Date of nut maturity was positively correlated with time of female flowering (r= 0.664, P<0.01), and time of leaf burst date (r= 0.435, P<0.01). Other results showed that heat accumulation had a significant effect *on* bud break and flowering date. In the other words, early flowering and leafing increase with high temperatures (Hassankhah *et al.,* 2017). Late-leafing cultivars are suitable to cultivate in mountain areas where the late spring frosts are frequent (Akca and Ozongun. 2004; Aslamarz *et al.,* 2009). Also, the date of nut maturity was positively correlated with the thickness of the shell (r= 0.664, P<0.01) which was consistent with the study of Mosivand *et al.* (2012). Similarly, in the study of Ghasemi et al. (2012) in our results, a negative correlation was found between kernel percentage and the thickness of the shell. Analyzing the coefficients of correlation between different variables provides informative data about the relative effect of every trait on yield. Response to direct selection for these variables may be unpredictable, unless there is 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 such as principal component analysis (PCA). Therefore, PCA was successfully utilized to comprehend the patterned variation in a set of variables, based on structural relationships among variables (Tadesse and Bekele, 2001). Because of the diversity of study walnut individuals, the analysis into main components was carried out to determine the contribution and effective rate of each trait 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 may be due to existing low correlation among study variables (Abdi and Williams, 2010). The PCA showed that the first 8 components among variables explained 79.43% of the total variation (Table 5). Variables loaded on PC1 were as for nut and phenological-related traits, but PC2, in addition to the previous traits, also included items related to the kernel traits (Table 5). 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. In another evaluation of some Iranian

native walnut genotypes, 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). Hierarchical cluster analysis was applied to investigate the similarities and dissimilarities among walnut genotypes based on 21 variables. The high number of generating clusters of variables showed that there was a high in-population diversity of walnuts in RARS, which was desirable potential for use in the next breeding programs. Regarding the dendrogram generated based on variables, cluster 2 and 5 were the largest ones with 9 individuals (Fig 1). The results of research on several Persian walnut species and interspecific hybrids showed were divided into three groups: black walnut, interspecific hybrids, and Persian walnut. Z63, Hartley, Serr, Z30, K72, Ronde, Pedro, Chandler, and B21 were located in a separate group (Mosivand *et al.,* 2012). In our study, three cultivars including Chandler, Serr, and Hartley were grouped separately. Pedro and Serr were located in group 3 (Fig 1). Thirty-one Iranian native walnut genotypes were clustered into three groups using the UPGMA cluster method (Ghanbari *et al.,* 2018). Cluster analysis was separated 70 genotypes into six groups (Ghasemi *et al.,* 2012). None of the observed clusters showed any clear separation between internal genotypes and foreign cultivars. Selection of parents of clusters showing the highest inter-cluster distance may be used for the 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).

Conclusions

Our study, as a part of an ongoing project for the breeding of the Persian walnut in Iran, has been done to characterize the 37 walnut genotypes and cultivars by 21 traits in the RARS collection. According to PCA, high diversity in morphological and phenological characteristics in individuals was revealed, supporting the presence of a high level of genetic diversity. In this study, the clustering pattern was independent of geographical distances. Although at first glance, it seems that the genotypes and cultivars in RARS were common as short shrubs with lower kernel traits than ones cultivated in other places, but because of having a native genetic basis, they may be useful in the breeding of improved plants. In summary, the analysis of multiple phenological and pomological traits of the walnut genotypes documents the significant variation present in the *in situ* found in the region and supports the need 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.

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