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Phenotypic Evaluation and Identification of Superior Persian Walnut (*Juglans regia* L.) Genotypes in Mazandaran Province, Iran

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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

Huge genetic diversity of Persian walnut in Iran provides a great opportunity for walnut breeders to use it for introducing varieties with desirable traits. In this regard, evaluating the genetic diversity and introducing superior genotypes is the foundation step of a walnut breeding program. This study was conducted to exploit the genetic diversity in Savadkuh county, Mazandaran province, Iran to identify some superior genotypes during 2013-2016. In the first step, 91 seed-originated genotypes were selected based on questionnaire. In the second stage, the morphological characteristics of the selected genotypes were studied using two IPGRI and UPOV descriptors in 2015. The results showed that nut and kernel weight ranged between 7.3-16.7 g and 3.2-8.6 g, respectively. Also, kernel percentage and shell thickness varied from 36 to 60.74% and from 0.8 to 2.8 mm, respectively. According to the morphological evaluation, 9 out of 91 genotypes (SR7, SR8, SR14, SR23, SR24, SR33, SR52, SR60 and SR83) were selected as superior genotypes. The selected superior genotypes were morphologically evaluated for two consecutive years (2015-2016). These superior walnut genotypes had high yield, lateral bearing habit (45-60%), heavy (13.4-16.7 g) and large nuts, high kernel percentage (48.6-56.6%), thin to moderate shell (1.1-1.6 mm) with light kernel color. Due to distinct and desirable characteristics, the selected superior genotypes can not only be used as parents in the further breeding program, but also some of them have the potential to release as commercial varieties.

Introduction

Persian walnut (*Juglans regia* L.) is a monoecious species with dichogamous habit which is grown in a wide range of temperate zone (Ebrahimi *et al.*, 2009 and Bernard *et al.*, 2020). Due to high amounts of unsaturated fatty acids, proteins and antioxidants compound in walnut kernel, demand for consumption and consequently demand for its production is rapidly rising (Ros and Mataix, 2006 and Sarikhani *et al.*, 2021). According to FAO data, the world walnut production and area harvest were 4498442 tones and 1305349 hectares which has increased by 62.5% and 29.7% over the prior 10 years, respectively. China, USA

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and Iran are leading the world walnut production with shares of 56%, 13% and 7% in 2018, respectively (FAO, 2019).

Due to the sexual propagation as well as dichogamous habit, walnut trees in Iran is highly variable in terms of the nut characteristics (Vahdati *et al.,* 2014 and Hassani *et al.,* 2020b). Since this genetic diversity reduces uniformity among the walnuts being produced and consequently reduces the walnut export capacity of Iran (Vahdati *et al.,* 2018); but it provides an opportunity to run walnut breeding programs based on genetic diversity utilization. Evaluating genetic diversity and introducing promising genotypes is an essential step in exploiting genetic diversity. In this way, native trees are considered more by breeders because, in addition to adaptation, their diversity is profound (Aslantaş, 2006). Walnut breeders then use the promising genotypes in various breeding programs, thereby increasing the economic output of this tree species (Arzani *et al.,* 2008). The most important breeding goals of walnut include high yield and quality with light kernel color, thin shell, large nut, high kernel percentage, lateral fruitfulness and resistance to biotic and abiotic stresses (Cosmulescu and Botu, 2012; Vahdati *et al.,* 2019 and Hassani *et al.,* 2020). In addition, in countries like Iran, where the risk of late-spring frosts is a threat, late leafing is a very important breeding goal in countering frost damage (Mehlenbacher, 2003).

For the first time, evaluation of Iran's walnut germplasm occurred by Jamal Atefi in 1984 (Atefi, 1997) and then continued with other researchers in different parts of the country (Eskandari *et al.,* 2005; Arzani *et al.,* 2008; Ebrahimi *et al.,* 2015; Vahdati *et al.,* 2015; Sarikhani *et al.,* 2017; Ghanbari *et al.,* 2019; Mahmoodi *et al.,* 2019 and Rezaei *et al.,* 2020). Arzani *et al*. (2008) morphologically evaluated walnut population in the Taft region of Yazd province and reported that nut and kernel weight and kernel percentage ranged 6.0–15.2 g, 2.6–9.1 g and 38.4–79.6%, respectively. Mahmoodi *et al*. (2016) selected 5 walnut superior genotypes out of 250 genotypes in Karaj and compared them with the world commercial cultivars in terms of phenological and pomological characteristics. Nut weight and kernel percentage of the selected superior genotypes were from 10.1g (H1/1) to 12/8g (B10) and 42.5% (H2/1) to 58.4% (H2-12), respectively (Mahmoudi *et al.,* 2016). Walnut population in southwest of Iran (Fars province) was morphologically evaluated based on UPOV and IPGRI descriptors during 2012-2015. After primary evaluation, 25 superior walnut genotypes were selected. They had high yield, lateral bearing habit, thin shell heavy nut and light to extra light kernel color. Among them, FaBaAg1 and FaBaAv2 were late-leafing genotypes (Sarikhani Khorami *et al.,* 2018). Evaluation of genetic diversity of walnut in Iran has so far led to introduce 6 commercial cultivars including 'Damavand', 'Jamal', 'Persia', 'Caspian', 'Chaldoran' and 'Alvand' (Hassani *et al.,* 2020a and Hassani *et al.,* 2020b).

In addition to Iran, several studies have been conducted on genetic diversity assessment and identification of superior genotypes in countries where are primary and secondary centers of walnut genetic diversity. There is a high genetic diversity in the walnut population of Kazakhstan which is provided the opportunity for joint breeding programs with other countries. A part of walnut population in this country was morphologically evaluated during 2015-2018 which led to introduce 10 superior genotypes. Due to high lateral bearing habit, these selected genotypes were valuable genetic resources for further breeding programs (Akça *et al.,* 2020). Physical and biochemical evaluation of some selected walnut genotypes in the Isparta region, Turkey showed nut and kernel weight ranged 8.43-11.09 g and 4.35-6.32 g, respectively (Ozkan and Koyuncu, 2005). Zeneli *et al.* (2005) evaluated 65 out of 253 native walnut genotypes in Northern Albania and reported that nut and kernel weight and kernel

percentage ranged between 3.8-21.1 g, 1.8-9.8 g and 32.6-63.8%, respectively. They reported that there is a high variability in the evaluated population in term of nut and forestry characteristics and therefore can serve for establishing a core collection (Zeneli *et al.*, 2005).

A suitable climate, rich lands and sufficient water resources provide desirable conditions for growing a variety of fruit trees especially walnuts in Mazandaran province, Iran. Alborz mountain range has caused a great variety in terms of climate and topographic characteristics in this province. These diverse climatic-topographic conditions along with sexual reproduction have led to huge genetic diversity in walnut population of Mazandaran province. Considering the fact that Savadkuh is an important center of walnut growing in the province, this study was conducted to evaluate a part of this walnut genetic resource in order to identify superior and promising genotypes in terms of nut and phenological characteristics.

Materials and Methods

The studied location and plant material

The present study was carried out in Savadkuh (35° 59’ 40” N; 52° 41’ 57” E), which is located in north of Iran during 2013-2016 (Fig. 1). A part of walnut population in Savadkuh county was labeled based on the design of questionnaire. Yield, leafing and harvest dates, kernel color, ease of kernel removal, lateral fruitfulness and the absence of blight symptoms were some of characteristics mentioned in questionnaire. Accordingly, 205 seed-originated trees were labeled in the first year and finally due to observe late-spring frost damage and lack of lateral bearing habit, 91 genotypes were selected for further study.

Fig. 1. The geographical location of Savadkuh, Mazandaran province, Iran

Phenotypic evaluation of the selected walnut genotypes

The 91 selected walnut genotypes were morphologically evaluated based on two international walnut descriptors, i.e., IPGRI and UPOV. The phenotypic traits included phenological traits (namely bud break date, pollen shedding date, female receptivity date, harvest date, dichogamy) and pomological traits (namely nut and kernel weight, kernel percentage, bearing habit, nut length, width and diameter, nut shape index, nut size index, shell thickness, packing tissue thickness, shell strength, shell seal, shell color, shell texture, ease of kernel removal and kernel color). Phenological traits are highly influenced by environmental conditions and are usually evaluated in comparison with the reference standard (days after

reference standard; DARS). In this study, the early leafing genotype was considered as the reference standard and phenological traits of other genotypes were recorded in comparison with this genotype (Sarikhani Khorami *et al.,* 2014). Bud break date was considered when more than 50% of terminal buds started to grow and the green leaf became visible (IPGRI, 1994). Harvest date was considered when almost all of the green hull (95%) could be easily removed from the hard shell. Walnut genotypes which pollen shedding and pollen receptivity by female flower overlapped for more than 6 days were considered as homogamous genotypes (Arzani *et al.,* 2008). To evaluate pomological traits, 20 nuts were harvested from each genotype (IPGRI, 1994) and after removal of the hull, the nuts were kept in shade at room temperature for one month (Zeneli *et al.,* 2005). Nut and kernel weights were measured using a digital balance. Kernel percentage was also calculated from the ratio of kernel weight to nut weight (Yarilgac *et al.,* 2001). The length (L), width (W) and diameter (D) of each nut as well as the shell thickness were measured using a digital caliper. The nut shape (FI) and size index (SI) were calculated based on formulas 1 and 2, respectively. Accordingly, if the FI is less than or equal to 110, the nut shape is spherical, but if it is between 111 and 125 or greater than 125, the nut shape is oval and elliptical, respectively (Arzani *et al.,* 2008). Table 1 shows some nut characteristics are scored based on IPGRI descriptor.

Formula 1: $FI = 200L/(W+D)$ Formula 2: $SI = (L+W+D)/3$

Table 1- Scoring the studied nut characteristics based on IPGRI descriptor

Trait	Score
Nut shape	1: Round 2: Triangular 3: Broad ovate 4: Ovate 5: Short trapezoid 6: Long trapezoid 7: Broad elliptic 8: Elliptic 9: Cordate
Ease of kernel removal	1: Very easy; 5: Moderate; 9: Very difficult
Kernel color	1: Extra light; 2: Light 3: Light amber; 4: Amber
Shell texture	1: Very smooth; 5: Medium; 9: Very rough
Packing tissue thickness	1: Very thin and sparse; 5: Medium; 9: Very thick
Shell strength	1: Paper; 5: Intermediate; 7: Strong
Kernel fill	3: Poor; 5: Moderate; 7; Well
Kernel plumpness	3: Thin 5: Moderate 7: Plump
Shell seal	1: Open or very weak; 5: Intermediate; 9: Very strong
Yield	3: Low; 5: intermediate; 7: High

Selection of Superior genotypes

The superior genotypes were selected after one-years evaluation and were evaluated for two consecutive years. The superior genotypes were characterized with nut weight≥13 g, kernel weight≥ 6.5 g; kernel percentage≥47%, 0.7≤shell thickness≤1.5 mm, lateral bearing habit, light to extra light kernel color which are easily removed from shell (Khorami *et al.,* 2012; Akca and Ozongun, 2004 and Arzani *et al.,* 2008). Nonetheless, walnut genotypes were only superior in some traits (not all mentioned traits) are also considered as superior genotypes because they can be important in future breeding programs.

Statistical analysis

The obtained data was analyzed using SPSS statistical software (ver.18). The high heritable and important breeding traits i.e., bud break and harvest date, pollen shedding and female receptivity date, nut

and kernel weight, kernel percentage, nut size and shell thickness were used to multivariate analysis (cluster (CA) and principal component (PCA) analysis) of the superior genotypes which was done using MetaboAnalyst 5.0 online software. Cluster analysis was conducted using Ward method (Ghanbari *et al.*, 2019).

Results

Phenotypic evaluation of the selected walnut genotypes

The leafing and harvest date of the selected genotypes varied from 6-March (SR41) to 2-April (SR58) and 21-August (SR9) to 3-October 2014 (SR13, SR21, SR55, SR56, SR58, SR61, SR66, SR85, SR86), respectively (Table 2). Based on the results, 79.12% of the selected genotypes were protandrous and homogamy was not observed in the selected genotypes. Study of nut characteristics of the selected genotypes showed that the average nut and kernel weight were 11.9 and 5.6 g, respectively. Nut and kernel weight ranged from 7.3 (SR47) to 16.7 g (SR8) and from 3.2 (SR7) to 8.6 g (SR14), respectively. The average kernel percentage was 46.8%, while the minimum and maximum kernel percentages were observed in SR10 (36.0%) and SR21 (60.7%), respectively. Lateral bearing is an important walnut breeding objective which is determined final yield. Lateral bearing of 91 selected genotypes in Savadkuh county varied between 10 and 85%. The highest lateral bearing (85%) was observed in SR21, whereas SR42 had the lowest percentage of lateral bearing. The shell thickness varied from 0.8 mm (SR47) to 2.8 mm (SR10), respectively. In general, shell thickness of 48.4% of the studied walnut genotypes were less than 1.5 mm. Also, the average nut length, width and diameter were 36.7, 31.3 and 31.8 mm, respectively (Table 2).

Based on IPGRI descriptor, some pomological traits were measured as a scale of 1 to 9. Based on the results, 24.2% of the studied genotypes had spherical nuts. Also, 8.8% and 15.4% of the genotypes had weak to paper shell and light shell, respectively, whereas 7.7% of the genotypes had strong to very strong nut shell. Light to extra light kernel color is a desirable trait for walnut consumers. Our results showed that 16 out of 91 studied genotypes had light to extra light kernel color. Also, the kernel of 13.2% of the studied genotypes is easily removed from shell. In terms of the nut shell texture, 15.4% of the genotypes had smooth to very smooth shell texture. Also, most of the studied genotypes were moderate in term of kernel fill (Table 3).

Table 2. Diversity in nut traits of 91 selected walnut genotypes in Savadkuh, Mazandaran province, Iran in 2014

Traits	Min	Max	Mean	SD.	CV (%)
Nut weight (g)	7.3	16.7	11.9	19.2	16.1
Kernel weight (g)	3.2	8.6	5.6	10.3	18.3
Kernel percentage (%)	36.0	60.7	46.8	48.7	10.4
Lateral bearing (%)	10.0	85.0	41.9	12.7	30.3
Shell thickness (mm)	0.8	2.8	1.6	0.3	20.3
Nut length (mm)	28.5	45.1	36.8	3.3	9.0
Nut width (mm)	27.1	35.4	31.3	2.1	6.8
Nut thickness (mm)	26.7	35.8	31.2	2.1	6.6

Table 3. Diversity in nut quality traits of 91 selected walnut genotypes in Savadkuh, Mazandaran province, Iran in 2014

Traits	Scoring* (%)								
	1	2	3	4	5	6	7	8	9
Nut shape	24.2	2.2	4.4	6.6	23.1	13.2	13.2	13.2	0.0
Shell thickness	0.0	1.1	7.7	28.6	35.2	19.8	5.5	1.1	1.1
Shell color	0.0	0.0	15.4	46.2	31.9	6.6	0.0	0.0	0.0
Packing tissue thickness	0.0	0.0	13.2	19.8	20.9	13.2	15.4	12.1	5.5
Shell texture	0.0	2.2	13.2	24.2	33.0	18.7	8.8	0.0	0.0
Kernel color	1.1	5.5	11.0	38.5	31.9	11.0	1.1	0.0	0.0
Ease of kernel removal	0.0	0.0	18.7	46.2	24.2	5.5	2.2	1.1	2.2
Kernel fill	0.0	0.0	2.2	7.7	36.3	40.7	11.0	2.2	0.0

* Numbers 1 to 9 are scoring of each trait based on Table 1. The data show the share of each trait (% of total) in the studied walnut population (91 genotypes)

Superior genotypes

High-heritability traits consider as main traits in walnut breeding programs to evaluate genetic diversity (Ramos, 1997). There are some important horticultural traits with high heritability which is used to select superior genotypes including phenological traits, nut weight, kernel weight, kernel percentage and shell thickness. In this study, 9 out of 91 studied genotypes (SR24, SR23, SR14, SR8, SR7, SR60, SR52, SR33 and SR83) were considered as superior genotypes in the Savadkuh county, Mazandaran, Iran (Fig. 2, Table 4 and 5). Among these, SR60, SR83 and SR52 were late-leafing compared to other studied genotypes with 23, 25 and 26 days delay compared to the reference standard, respectively. Among the superior genotypes, SR14 and SR52 had the shortest and longest growing season, respectively (Fig. 2). Nut and kernel weights and kernel percentage ranged between 13.4-16.7 g, 6.8-8.6 g and 47.8-56.6%, respectively. Lateral bearing of the studied superior genotypes was between 45% (SR23 and SR52 to 60% (SR83). The selected superior genotypes had high yield, large nut with thin to moderate shell thickness. So that, the average of nut size index was 34.5. Also, the thinnest and strongest shell were observed in SR23 (1.1 mm) and SR52 (1.6 mm) genotypes, respectively (Table 4). The nut shape and some nut quality-related traits of superior genotypes are presented in table 5. The selected superior genotypes had smooth to medium shell texture, thin and sparse packing tissue and light and plump kernel (Table 5).

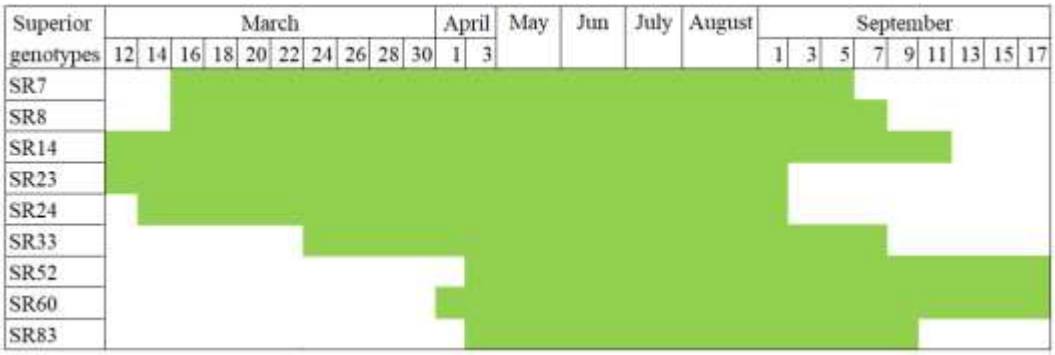

Fig. 2. The average length of growing season of walnut superior genotypes originated from Savadkuh region in the north of Iran (2014-2016)

Table 4. Pomological traits of superior walnut genotypes originated from Savadkuh county, Mazandaran province, Iran during 2014-2016

Walnut superior Genotype	Nut weight (g)	Kernel weight (g)	Kernel percentage (%)	Lateral bearing (%)	Nut length (mm)	Nut width (mm)	Nut thickness (mm)	Nut shape index	Nut size index	Shell Strength (mm)
SR7	13.6±0.4 e	7.0±0.3 cde	51.6±1.2 d	55.0 ±0.1 b	36.4±0.4 e	30.5±0.2 f	32.6±0.4 d	115.2±0.7 de	33.2±0.4 f	1.4±0.08 cd
SR8	16.7±0.1 a	8.2±0.2 ab	48.8±0.7 e	50.0±0.2 c	40.1±0.5 bcd	35.4±0.2 a	35.8±0.3 a	112.7±1.1 e	37.1±0.3 ab	1.5±0.04 ab
SR14	15.9±0.3 ab	8.6±0.1 a	53.8±0.3 ab	47.5±0.2 d	42.4±0.5 a	34.2±0.5 abc	35.5±0.4 ab	121.6±1.2 b	37.4±0.4 a	1.3±0.03 d
SR23	13.5±0.4 e	7.7±0.3 bc	56.6±1 a	45.0±0.2 e	39.1±0.3 cd	32.8±0.3 e	33.8±0.3 c	117.3±0.9 bcd	35.2±0.3 de	1.1±0.03 e
SR24	15.6±0.8 bc	7.4±0.2 cde	48.6±1.9 e	50.0±0.3 c	40.7±0.7 b	33±0.4 de	34.5±0.3 bc	120.5±1.2 bc	36.1±0.4 bcs	1.5±0.07 abc
SR33	14.4±0.2 de	7.6±0.1 bcd	52.8±0.6 bcd	52.5±0.3 c	38.7±0.1 d	34±0.5 abc	34.3±0.6 c	113.5±1.8 de	35.7±0.4 cd	1.5±0.04 a
SR52	14.3±0.3 de	6.8±0.2 e	47.8±1.3 e	45.0±0.5 e	42.9±1.1 a	33.4±0.4 cde	33.9±0.4 c	127.6±2.9 a	36.7±0.5 ab	1.6±0.04 a
SR60	14.8±0.1 cd	8.1±0.1 ab	54.3±0.7 abc	45.5±0.7 e	40.6±0.2 bc	34±0.3 bcd	35.5±0.1 ab	117±1.2 cde	36.7±0.1 abc	1.5±0.01 a
SR83	13.4±0.2 e	7±0.1 de	51.9±0.5 cd	60.0±0.4 a	34.2±0.3 f	34.9±0.2 ab	34.6±0.2 bc	98.3±0.9 f	34.6±0.2 e	1.4±0.03 bcd

- Genotypes with same letter within a column do not show significantly differences.

Table 5. Yield and nut quality-related traits of superior walnut genotypes originated from Savadkuh county, Mazandaran province, Iran during 2014-2016

Genotype	Yield	Nut shape	Shell texture	Packing tissue thickness	Kernel color	Ease of kernel removal	Kernel fill	Kernel plumpness
SR7	High	Elliptic	Smooth	Thin and sparse	Light	Easy	Well	Plump
SR8	High	Round	Smooth	Thin and sparse	Light	Moderate	Well	Moderate
SR14	High	Ovate	Smooth	Very thin and sparse	Light	Easy	Well	Plump
SR23	High	Round	Smooth	Medium	Light	Very easy	Well	Moderate
SR24	High	Ovate	Smooth	Thin and sparse	Light	Easy	Well	Moderate
SR33	High	Round	Medium	Medium	Light	Moderate	Well	Moderate
SR52	High	Ovate	Medium	Thin and sparse	Light	Easy	Well	Plump
SR60	High	Round	Smooth	Thin and sparse	Light	Very easy	Well	Moderate
SR83	High	Elliptic	Medium	Thin and sparse	Light	Easy	Well	Plump

Multivariate analysis of the selected superior genotypes

High heritability traits were used to group superior genotypes (Arzani *et al.*, 2008). The selected superior genotypes were classified into 3 groups. Based on results, the SR52 and SR60 genotypes which are late-leafing and moderate to late harvest, were clustered in a same group. Also, SR83 genotype had a genetic distance from other genotypes and was clustered in a separate group. The origin of this genotype is Lord region which is different from other superior genotypes. The third cluster includes SR24, SR7, SR8, SR33, SR14 and SR23. The results of principal component grouping and cluster analysis were somewhat consistent. According to the results, PC1, PC2 and PC3 explained 93.7% of the total variation. The PC1 accounted 64.1% of the total variation which is consist of bearing habit and bud break date. Bud break date and bearing habit were the main component of group II which included SR83 genotype. The main nut-related traits such as nut and kernel weight, kernel percentage and shell thickness were contributed to the third component (PC3) (Fig. 3).

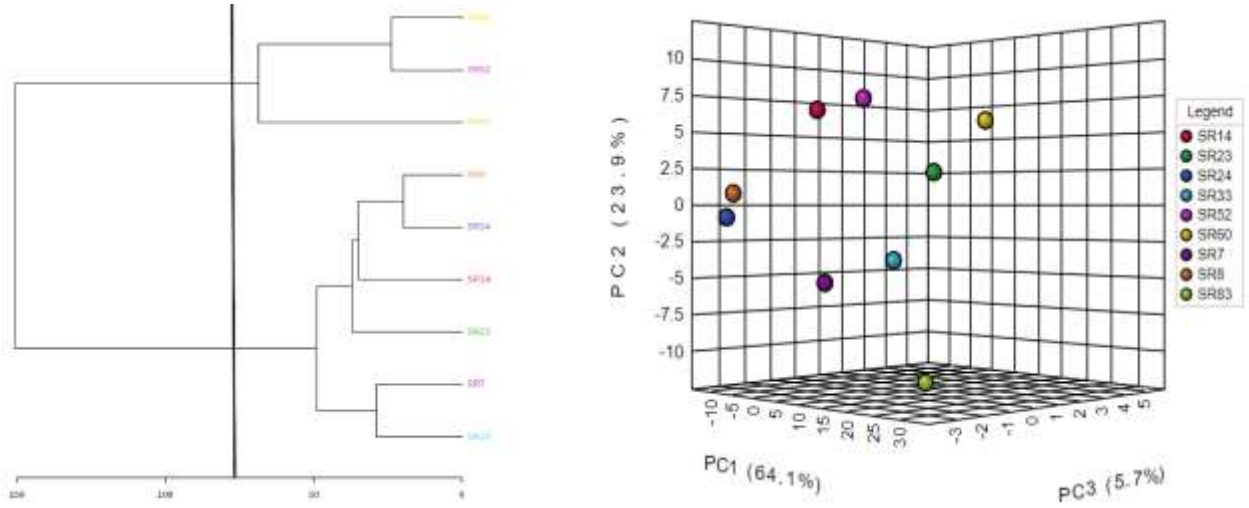

Fig. 3. Cluster (left) and PCA (right) analysis of the superior walnut genotypes originated from Savadkuh, Mazandaran province, Iran

Discussion

Based on the results, a high genetic diversity was observed among walnut genotypes originated from Savadkuh county, Mazandaran, Iran. 9 out of 91 walnut genotypes were selected as superior genotypes. The selected superior genotypes had a set of important walnut breeding traits including high yield and kernel quality, lateral bearing habit, late leafing, heavy and large nut with high kernel percentage and light kernel color (Ebrahimi *et al.*, 2009 and Ebrahimi *et al.*, 2015). Late-leafing walnut trees can be grown even in mountainous areas with persistent cold weather (Akca and Ozongun, 2004). Of course, late leafing is not only determined by high chilling requirement, and chilling and heat requirement together determine the leafing date of walnut trees (Hassankhah *et al.*, 2017). However, the late leafing varieties significantly reduces the damage of late-spring frosts, which is an important challenge in a wide range of walnut growing areas. In addition, late-leafing genotypes are resistance to bacterial blight disease (Akca and Ozongun, 2004 and Vahdati *et al.*, 2019). Among the selected superior genotypes in this study, SR60, SR83 and SR52 were late-leafing genotypes that the harvest date of SR83 genotype were earlier than other late-leafing genotypes. The flowering

pattern of walnut trees is of particular importance in the management of walnut orchards. This can be seen in terms of overlapping pollination dates with the period of female flower receptivity. Thus, the best walnut genotypes are homogamous (Forde and McGranahan, 1996). In this study, about 79.1% of the selected genotypes had protandrous and others had protogynous flowering habit. Accordingly, no homogamy was observed among the genotypes. These results correspond with those reported previously by Parsa *et al*. (2001).

The most important traits in walnut breeding programs are pomological traits because these are less affected by environmental conditions (Sharma and Sharma, 1998). Based on our results, the average nut and kernel weight of the studied genotypes were 16.72 and 8.57 g, respectively, which are higher than the results reported in the previous studies (Akca and Ozongun, 2004; Aslantaş, 2006; Arzani *et al.,* 2008; Ipek *et al.,* 2018). The result of the current experiment showed that the kernel percentage of the selected genotypes in Savadkuh county varied between 34.95 and 60.74% which is consistent with the results of previous studies (Sharma and Sharma, 2001; Khadivi-Khub and Ebrahimi, 2015). Evaluation of genetic divergence in Persian walnut population in Himachal Pradesh, India showed that kernel percentage ranged between 11.02-62.50% (Sharma and Sharma, 2001). Khadivi-Khub and Ebrahimi (2015) reported that the kernel percentage of some walnut genotypes originated from different regions of Iran was between 37.0 to 67.8%. Although the lateral bearing of the studied superior genotypes is less than the 'Chandler' cultivar, but the lateral bearing if the superior genotypes ranged between 6-45%which can be improved through better orchard management as well as further walnut breeding programs. Shell thickness is an important walnut breeding trait with high heritability (more than 0.8) (Hansche *et al.,* 1972; Koyuncu *et al.,* 2004 and Aslantaş, 2006). Shell thickness of superior genotypes should be between 0.7 and 1.5 mm (Akca and Ozongun, 2004). The results of this study showed that the shell thickness of superior genotypes varied between 1.05 to 1.58 mm. Light kernel color and other nut quality characteristics are important traits to selected superior genotypes (McGranahan and Leslie, 1990). In this study, all selected superior genotypes had light kernel color which was easily removed from shell.

PCA is routinely use to establish genetic relationships among genotypes and to detect and quantify the population genetic structure (Ma and Amos, 2012 and Ebrahimi *et al.,* 2015). In this study, PCA and cluster analysis was performed based on high-heritability phenotypic traits. The results of PCA and cluster analysis were somewhat consistent. Based on these analyses, genotypes were classified into three groups. In general, genotypes from the same region were classified into the same group which were somewhat consistent with some other studies on Iranian walnut germplasm (Khadivi-Khub and Ebrahimi, 2015and Ebrahimi *et al.,* 2015). Although high-heritability traits were used to group genotypes, it is better to use molecular data to validate this grouping and the relationship between walnut genotypes.

Conclusions

In conclusion, the 9 superior walnut genotypes were selected based on distinct and desirable phenotypic traits. The selected superior genotypes characterized by high yield, heavy and large nut with high kernel percentage, thin to moderate shell thickness with light kernel color. In addition, SR60, SR83 and SR52 genotypes were late-leafing compared to other studied genotypes which can be exploited in walnut breeding programs for late-spring frosts resistance. The selected superior genotypes can not only be used as parents in the further breeding program, but also some of them have the potential to introduce as commercial varieties.

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