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Morphological, Phenological, and Pomological Diversity Among 130 Seed-Propagated Walnut (*Juglans regia* L.) Trees and Apomixis Study in Some Selected Genotypes

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Abstract

About 130 seed-propagated walnut trees (*Juglans regia* L.) grown in the same location were evaluated for 2 years based on 37 quantitative and qualitative traits. The late-leafing trait ranged between 1 April and 14 April (13–27 days after Payne). The first female and male flowering dates ranged from 8 April to 5 May and from 7 to 29 April, respectively. The protandrous, protogynous, and homogamous percentages were 46.72%, 35.24%, and 18.03%, respectively. The harvest time changed from 7 September to 27 September. The bearing type rates were 3.5% for lateral, 13.4% for terminal, and 83.1% for both. The severity of blight and anthracnose disease varied from very low to very severe. The annual growth varied from 3.9 cm to 45.7 cm, while the leaf area ranged from 24.3 cm^2 to 48.01 cm^2. All types of tree growth habit were observed among the studied genotypes, with the spreading type predominating. The ranges of the fruit attributes were 1.4 mm –5.7 mm for husk thickness, 0.7 mm –2.3 mm for shell thickness, 25.4 mm –41.5 mm for nut length, 25.7 mm –38.5 mm for nut width, 7.27 g –17.73 g for nut weight, 3.68 g –9.49 g for kernel weight, 45.48%–63.86% for kernel percentage, and extra light to amber for kernel color. The highest recorded apomixis rate of the 30 genotypes was 25.55%, whereas this rate was low or even zero in some genotypes. In conclusion, the high diversity found in the studied germplasm resulted in the selection of some superior genotypes that can be considered promising plant materials for future walnut breeding programs.

Keywords Juglandaceae · Kernel weight · Superior genotypes · Selection · Nut traits

Introduction

The Juglandaceae family consists of 60 species within seven genera, including the genus *Juglans*, which contains 20 species and of which Persian walnut (*Juglans regia* L.) is one of the most important economic species. *Juglans regia* is cultivated in temperate regions for its edible nuts (McGranahan and Leslie 2009) and its multiple uses as an ornamental plant and in the wood industry and medicinal sector (Hayes et al. 2016; Salejda et al. 2016). Walnut species are native to the mountainous regions of Central Asia, and now they are the most widely distributed nut fruit tree in the world (Bayazit et al. 2007; Chen et al. 2014).

Iran is the third highest walnut producer globally after China and the United States, with a total annual production of 356,666 tons (FAO 2020). Walnut is a monoecious tree with male and female flowers. Since ancient times, the dichogamous pollination nature of walnut and its propagation by seeds has helped preserve the phenological, morphological, and pomological variance, even in orchards planted for productive purposes. This practice has enriched the genetic pool of Iranian walnut, with millions of seed-propagated trees (Rezaei et al. 2008).

This variability has led to extensive investigation of germplasm native to the main origin centers. Such investigations have resulted in the selection and introduction of some superior genotypes in Turkey (Akça et al. 2015; Aysen et al. 2019; Büküçü et al. 2020; Sutyemez 2016;

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Table 1 Quantitative and qualitative traits evaluated related to walnut genotype

Characteristic	Abbreviation	Unit	Measurement method
Habit of tree growth	HTG	Code	1 = vertical, 2 = semivertical, 3 = spreading
Annual growth	AG	cm	Average length of 10 annual branches
Average leaf area	ALA	mm^2	Leaf area meter
Leafing date	LD	Date	Since 5 March
First female flowering date	FFD	Date	Since 5 March
Peak of female flowers	PFF	Date	Since 5 March
Last female flowering date	LFD	Date	Since 5 March
Female flowering period	FFP	Date	LFD – FFD
First male flowering date	FMD	Date	Since 5 March
Peak of male flowering	PMF	Date	Since 5 March
Last male flowering date	LMD	Date	Since 5 March
Pollination period	PP	Date	LMD – FMD
Number of male flowers	NMF	NO	Average number of male flowers per 2 m of annual branches
Harvest date	HD	Date	Since peak of female flowers
Flowering type	FT	Code	1 = lateral, 2 = medium, 3 = terminal
Severity of leaf blight disease	SLB	Code	1 = very low, 3 = low, 5 = intermediate, 7 = high, 9 = very high
Severity of tree blight disease	STB	Code	1 = very low, 3 = low, 5 = intermediate, 7 = high, 9 = very high
Severity of anthracnose disease	SAD	Code	1 = very low, 3 = low, 5 = intermediate, 7 = high, 9 = very high
Husk thickness	HT	mm	Caliper
Nut shape	NSh	Code	1 = round, 2 = triangular, 3 = broad ovate, 4 = ovate, 5 = short trapezoid, 6 = long trapezoid, 7 = broad elliptic, 8 = elliptic, 9 = cordate
Nut length	NLE	mm	Caliper
Nut diameter	NDI	mm	Caliper
Nut weight	NW	g	Scale
Shell roughness	ShR	Code	1 = very smooth, 3 = smooth, 5 = medium, 7 = rough, 9 = very rough
Shell color	ShC	Code	1 = very light, 3 = light, 5 = medium, 7 = dark, 9 = very dark
Shell seal	ShS	Code	1 = open, 3 = poorly sealed, 5 = well sealed, 7 = very well sealed, 9 = fully sealed
Shell thickness	ST	mm	Caliper
Shell hardness	ShH	Code	1 = paper shell, 3 = weak, 5 = intermediate, 7 = strong
Shell integrity	ShI	Code	1 = low, 2 = intermediate, 3 = high
Middle blade thickness	MBT	Code	1 = very thin, 3 = thin, 5 = medium, 7 = thick, 9 = very thick
Ease of kernel extraction	EKE	Code	1 = very easy, 3 = easy, 5 = medium, 7 = hard, 9 = very hard
Kernel weight	KW	g	Average of 20 sound kernels
Kernel percentage	KP	%	(Kernel weight per nut weight) × 100
Kernel fullness	KF	Code	3 = weak, 5 = medium, 7 = good
Kernel plumpness	KP	Code	1 = thin, 2 = medium, 3 = plump
Kernel shrivel	KSh	Code	1 = no shrivel, 2 = < 50%, 3 = > 50%, 4 = kernel blank
Kernel color	KC	Code	1 = extra light, 2 = light, 3 = light amber, 4 = amber

Severity of leaf anthracnose and blight disease: 1 (very low severity) = < 5% of the leaflet was covered with scattered or merged spots; 3 (low severity) = 5%–15% of the leaflet was covered with scattered or merged spots; 5 (intermediate severity) = 15%–25%; 7 (high severity) = 25%–50%; 9 (very high severity) = > 50%

Sutyemez et al. 2019, 2021, 2022), Romania (Cosmulescu and Stefanescu 2018), Iran (Fatahi et al. 2010; Hassani et al. 2012, 2020), California (McGranahan and Leslie 2004), China (Liu et al. 2020), and India (Sharma and Sharma 2001; Sharma et al. 2014).

The selection of superior walnut genotypes is mostly based on suitable traits such as late leafing, lateral bearing, lack of pistillate flower abortion, high yield (> 6 t/h), large and relatively smooth nut, sealed suture, high kernel percentage (> 50%) light kernel color, and moderate to high resistance against pests and diseases (Botu et al. 2010; Cosmulescu et al. 2010). Other desirable characteristics in walnut selection include bacterial and anthracnose tolerance, homogamy and protogynous, apomixis, short dormancy in areas with mild winters, winter cold resistance in areas with hard winters, and high-quality fruits and kernels (plump-

Fig. 1 **a** Isolation stage of female flowers (the *black circle* indicates the female flowers before isolation and after removing all catkins). **b, c** Flowers after isolation using double-walled Glassine paper bags (15 × 25 cm). **d** Apomictic fruit after bag removal (two weeks after isolation)

ness, light color, high kernel ratio, easy kernel extraction [Botu et al. 2010; Cosmulescu et al. 2010; Jacimovic et al. 2020; McGranahan and Leslie 2012]).

The main objective of this study was to inventory the morphological, phenological, and pomological diversity among seedling genotypes and to select high-quality walnut genotypes for direct cultivation in preparation for studying the possibility of registering them as new cultivars or forming a primary core collection that will allow breeders to have more options in breeding programs.

Material and Methods

Experimental Location and Plant Materials

This research was conducted at the Research Station of the Department of Horticultural Sciences, University of Tehran, Iran, with an altitude of 1320 meters above sea level. In this study, 130 seed-propagated walnut trees (>15 years) were coded from G1 to G130 and evaluated for 2 years (2016–2017) in terms of some pomological, quantitative, and qualitative traits (Table 1). Nut and kernel traits were measured based on walnut descriptors (IPGRI 1994) with slight changes. Measurements were taken of at least 45 nuts of each genotype (15 nuts in three replications). Thirty leaves were randomly selected from each genotype (10 leaves in three replications) and were used to measure leaf traits in the laboratory. The leaf area was measured using a leaf area meter. Finally, after the information was collected, the data were analyzed using Microsoft Excel and IBM SPSS statistics version 22.0.0 software (SPSS Inc., Chicago, IL, USA).

Apomixis Investigation

Apomixis was studied in 30 walnut genotypes. For this purpose, about 100 female flowers on each genotype were randomly selected. All the catkins on each selected shoot were removed at the differentiation stage. Then, flowers were isolated using double-walled glassine paper bags (15 cm × 25 cm). After 15 days of isolation, and when the stigma had completely dried, the bags were removed, and the percentage of apomixis after 8 weeks of fruit set was determined (Fig. 1). The apomixis rate was calculated as follows:

$$\text{Apomixis rate} = \text{number of remaining fruits} / \text{number of isolated flowers} \times 100$$

Results and Discussion

The results showed significant differences among the studied genotypes for all the traits. Therefore, it was possible to select the genotypes for different values of each trait.

Growth and Phenology Characterization

In this research, the coefficient of variation (CV%) of the late leafing trait was 13.94%, and the leafing time ranged from 1 April to 14 April, (i.e., at 27–40 days after 5 March and 14–27 days after Payne). Walnut production is limited not only by cold winters but also by autumn and spring frosts, which cause quantitative and qualitative differences in walnut production (Akça and Ozongun 2004). The most important way to increase cold avoidance in walnuts is to select for late leafing (Haghjooyan et al. 2005). In addition, late-leafing cultivars/genotypes show less infection by anthracnose (Hassani et al. 2011) and blight disease (Forde 1975) because of opening buds in the late dry part of the spring season. Hakan and Akça (2011) reported that the leafing date of te selected genotypes in Turkey ranged from 25 April to 28 April, but in Simsek's research (2010) it varied from 1 April to 12 April. The heritability of this trait in walnuts is high (greater than 96%) (Hansche et al. 1972), so it can be easily transferred from parents to the next generations by selecting the late-leafing seedlings.

The first female and male flowering dates among the studied genotypes ranged from 8 April to 5 May and from 7 pril to 29 April, respectively (Table 2). The most prolonged female and male flowering periods were 17 days and 16 days, respectively. Akça and Ozongun (2004) showed that the flowering time of promising genotypes was limited from 15 April to 10 May in the Karaman region of Turkey. Simsek (2010) showed that the female and male flowering dates of selected genotypes in the Diyarbakir province of Turkey ranged from 7 April to 18 April and from 11 April to 18 April, respectively, while the results of Oguz and Aşkın (2007) in Ermenek, Turkey, were similar to those of Simsek (2010).

In the present study, the percentages of protandrous, protogynous, and homogamous genotypes were 46.72%, 35.24%, and 18.03%, respectively, i.e., the protandrous type was dominant, which is consistent with the results of Akça and Sen (1995). One of the main goals of walnut breeding programs is selecting homogamous types or obtaining cultivars with high overlap. In this study, about 43.07% of the studied genotypes had suitable homogamy (>50%), 6.15% had acceptable homogamy (25%–50%), 12.31% had low homogamy (<25%), and 38.47% had no homogamy.

The harvest time is an essential trait due to its impact on the nutrient content and chemical composition of the nut (Özcan and Lemiasheuski 2020; Matthäus et al. 2018). The harvest time in the current study ranged from 7 September to 27 September, with CV of 3.58%, while it ranged from 15 September to 10 October in the work by Simsik (2010).

Yield production is determined by the fruiting bearing type in walnut trees, which was evaluated based on the ratio of lateral and terminal bearing buds. The flowers usually appear terminally at the ends of the branches shortly after leaves appear. In some cultivars, female flowers also appear on the lateral buds. This type of flowering in walnut is called "lateral bearing" and is associated with high yield in young trees, but the bearing that includes both bearing types (terminal and lateral) is the choice of interest. Among the 130 studied genotypes, about 3.5% had a lateral-bearing

Table 2 Descriptive statistics for the phenology and growth characteristics in the studied walnut genotypes

Characteristic	Unit	Minimum	Maximum	Mean	SD	CV (%)
Habit of tree growth	Code	1	3	2.24	0.83	37.21
Annual growth	cm	3.9	45.7	22.78	7.58	33.29
Average leaf area	mm^2	24307.5	48017.4	33378.4	4815.8	14.42
Leafing date	Days	27	40	32.92	4.59	13.94
First female flowering date	Days	34	51	39.81	4.5	11.3
Peak of female flowers	Days	37	56	45.02	5.06	11.23
Last female flowering date	Days	46	61	52.37	3.56	6.79
Female flowering period	Days	9	17	12.54	1.98	15.78
First male flowering date	Days	33	46	38.33	3.28	8.55
Peak of male flowering	Days	35	49	41.88	3.83	9.14
Last male flowering date	Days	37	55	45.67	4.62	10.11
Pollination period	Days	4	13	7.56	1.85	24.47
Number of catkins	Number	0	192	43.05	37.45	86.99
Harvest day	Days	131	157	145.75	5.22	3.58
Flowering type	Code	1	3	2.1	0.39	18.57
Severity of leaf blight disease	Code	1	9	3.52	1.56	44.31
Severity of tree blight disease	Code	1	9	2.46	1.61	65.44
Severity of anthracnose disease	Code	1	9	1.56	1.54	98.71

CV coefficient of variation, *SD* standard deviation

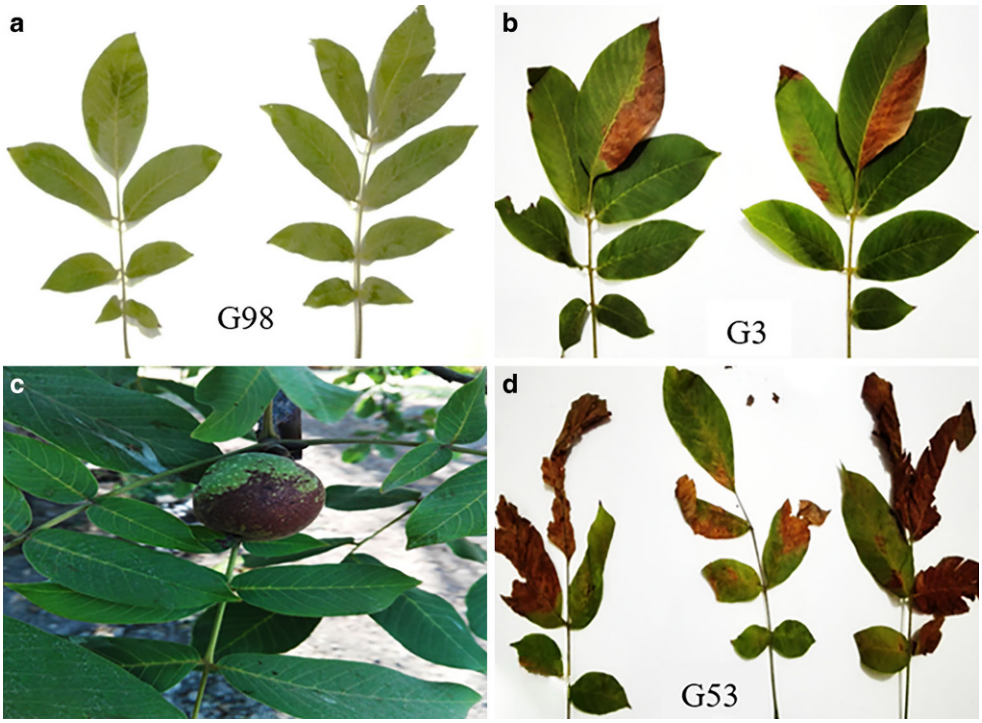

Fig. 2 Intensity of blight and anthracnose on some samples. **a** Very low blight and anthracnose intensity (tolerant genotypes, infection rate <5%). **b** Moderate anthracnose intensity (infection rate 5 to 25%) . **c** Very severe blight intensity (infection rate >25%). **d** Anthracnose on fruit

type, 13.4% had a terminal-bearing type, and 83.1% had both bearing types, with a CV of 18.57%. However, the ratio of lateral and terminal bearing differed among genotypes, and this was accompanied by a decrease in the percentage of lateral buds over time in old trees (Pinney et al. 1998; Polito 1998).

About four genotypes at the first year and 20 genotypes at the second year had meager fruit set because of the severe abscission of female flowers in the spring, which was probably caused by the pistillate flower abortion phenomenon that is usual in almost all walnut cultivars (McGranahan et al. 1994). Walnut also has an alternate bearing habit that reduces the "off" year's yield after a productive "on" year.

In the present research, the blight and anthracnose disease severity varied from very low to very severe, with CV of 65.44% and 98.71%, respectively (Fig. 2). Genotypes G95, G96, G97, and G98 were the most resistant to bacterial disease.

Other traits were also studied in this research, including the annual growth, which varied from 3.9 cm to 45.7 cm with a CV of 33.29%. The average leaf area ranged from 24.3 cm^2 to 48.01 cm^2 with a CV of 14.42%. Keramatlou et al. (2015) reported a close relationship between the leaf area and the nut size and fullness in walnuts. Among the studied genotypes, all types of tree growth habit (vertical, semivertical, and spreading) were observed. A recently introduced genotype is called BD6, which has a semivertical growth habit and semidense branching (Bujdosó et al. 2020).

Nut and Kernel Characterization

The husk thickness in dry and sunny areas is vital to prevent sunburn of the fruit. In this study it ranged between 1.4 mm and 5.7 mm, with a CV of 27.56%.

Nut shape had a high diversity and reached 70.23% (Fig. 3; Table 3). Hence, about 46, 30, and 25 genotypes had round, trapezoidal, and broad elliptical nut shapes, respectively. Trapezoid nuts are easily broken and their kernels easily extracted, which is marketable (Ebrahimi et al. 2010).

The greatest nut length in this study (41.5 mm) is less than the lengths reported by Simsek et al. (2017) (45.78 mm) and Cosmulescu et al. (2017) (53.6 mm), but it is close to the findings of Keles et al. (2014) (42.8 mm) and higher than that reported by Akça et al. (2015) (36.62 mm). The maximum nut diameter obtained in this study (38.5 mm) is higher than that reported by Simsek (2010) (36.71 mm), Keles et al. (2014) (34.77 mm), Akça et al. (2015) (36.15 mm), and Cosmulescu et al. (2017) (37.48 mm), while it is lower than that reported by Sharma et al. (2014) (42.07 g).

In the current study, the nut weight varied from 7.27 g to 17.73 g, and the CV was 19.41%. The optimal nut weight should be 12 g to 18 g (Cosmulescu 2013). The highest nut weight in this study is greater than that found in Turkey by Akça and Sen (2001) (13.93 g), Akça et al. (2015) (15.2 g), Keles et al. (2014) (13.92 g), and Özcan (2009) (10.5 g); lower than that found in the northwestern Himalaya (22.66 g; Shah et al. 2021), India (23.61 g; Sharma

Fig. 3 **a** Different shapes and sizes of nut. **b** Different thicknesses of the middle blade. **c** Shell seal types. **d** Different kernel colors among the studied genotypes

Table 3 Descriptive statistics for the nut and kernel characteristics in the studied walnut genotypes

Characteristic	Unit	Minimum	Maximum	Mean	SD	CV (%)
Husk thickness	mm	1.4	5.7	3.1	0.86	27.74
Nut shape	Code	1	9	3.83	2.69	70.23
Nut length	mm	25.4	41.5	31.7	3.44	10.85
Nut diameter	mm	25.7	38.5	30.7	2.52	8.2
Nut weight	g	7.27	17.73	11.02	2.14	19.41
Shell roughness	Code	1	7	4.2	2.9	69.04
Shell color	Code	1	8	4.66	1.97	42.27
Shell seal	Code	1	9	4.97	2.42	48.69
Shell thickness	mm	0.7	2.3	1.51	0.35	23.17
Shell hardness	Code	1	8	4.54	1.86	40.96
Shell integrity	Code	1	3	2.20	0.75	34.09
Middle blade thickness	Code	1	9	4.31	1.89	43.85
Ease of kernel extraction	Code	1	8	4.22	1.82	43.12
Kernel weight	g	3.68	9.49	6.08	1.31	21.54
Kernel percentage	%	45.48	63.86	55.1	5.25	9.52
Kernel fullness	Code	5	7	6.31	0.7	11.09
Kernel plumpness	Code	1.00	3.00	2.74	0.40	14.59
Kernel shrivel	Code	1.00	3.00	1.21	0.42	34.71
Kernel color	Code	1.00	4.00	2.19	0.85	42.27

CV coefficient of variation, *SD* standard deviation

and Sharma 2001), and Romania (20.9 g; Cosmulescu et al. 2017); and close to that of Turkish walnut (17.5 g; Asma 2012).

The tightly sealed shell suture is crucial for nut storage (McGranahan and Leslie 1990). During storage, nuts with open seals are exposed to fungal and insect infestation (Forde 1975). The diversity index obtained in this trait was 48.69%, indicating a significant difference among the studied genotypes for this trait (Fig. 3; Table 3).

Shell thickness is a determinant factor in the kernel percentage in walnuts. In the present study, the CV of shell thickness was 23.17% and ranged from 0.7 mm to 2.3 mm. This result is less than the results of Shah et al. (2021) (0.98–2.83 mm) and of Sharma and Sharma

Fig. 4 Nuts and kernels of some of the selected walnut genotypes

(2001) (0.48–2.6 mm) and close to the Turkish genotypes (1.11–2.33 mm [Akça et al. 2015] and 0.23–2.32 mm [Akça and Ozongun 2004]) and Iranian walnut (0.64–2.02 mm [Mahmoodi et al. 2019]), while it is higher than for Turkish walnut (0.95–1.75 mm [Asma 2012], 0.8–1.77 mm [Keles et al. 2014], 0.66–1.4 mm [Simsek et al. 2017]) and Iranian walnut (0.4–1.4 mm [Arzani et al. 2008]).

The kernel weight at an average of 2 years varied from 3.68 g to 9.49 g, with a CV of 21.54%. A kernel weight of 6 g to 10 g is suitable for commercial walnut production, and the kernel percentage should be at least 50% (Cosmulescu 2013; McGranahan and Leslie 1990). The highest kernel weight in the current study (9.49 g) is more than that found in the studies of Akça et al. (2015) (8 g), Asma (2012) (9.1 g), Cosmulescu (2013) (9.07 g), Jacimovic et al. (2020) (6.54 g), Karadağ and Akça (2011) (7.44 g), Keles et al. (2014) (7.36 g), and Simsek et al. (2017) (8.54 g); is close to that obtained in the research of Zeneli et al. (2005) (9.8 g) and Fatahi et al. (2010) (9.83 g); and is less than that reported by Shah et al. (2021) (14.00 g).

The kernel-to-nut ratio is considered an indicator of the yield's economic performance. According to Hansche et al. (1972), the kernel percentage has a very low heritability of 0.08. In breeding programs, the kernel rate of promising genotypes should be >48%–50% (Korac et al. 1997; Arzani et al. 2008). In the present study, the kernel percentage as an average of both years varied between 45.48% and 63.86%, with a CV of 9.52%. This result is higher than in the studies by Asma (2012) (60.8%), Jacimovic et al. (2020) (52.25%), and Simsek (2010) (58.04%), and is close to that reported by Karadağ and Akça (2011) (63.16%). The kernel rate in the present study was more than 48% for 73.84% of the evaluated genotypes. Factors that affect this trait include shell thickness, shell hardness, kernel weight, and nut weight. Genotypes with a thin shell and high nut weight have the highest kernel percentage.

In this study, the mean kernel color rank was 2.19 out of 4, with a CV of 38.81%, indicating that most genotypes had a light to light amber kernel color. About 21 and 39 genotypes had extra light and light kernel colors, respectively (Fig. 3; Table 3). Rezaei et al. (2018) reported that about 51.72%, 22.41%, and 25.86% of evaluated genotypes had light, very light, and amber color kernels, respectively. A clear and uniform kernel is an important trait in commercial cultivars (McGranahan and Leslie 1990).

Shell roughness varied from very smooth to very rough, with a CV of 69.04%, which indicates high diversity among the studied genotypes. The shell color ranged from very light to very dark, and the CV of this trait was 42.27%. A high diversity was recorded in the shell hardness trait (40.96%), which affects the ease of kernel extraction. Fruit with a hard shell that is smooth and thin, with a closed aperture seal and light kernel color is desirable in breeding programs (McGranahan and Leslie 1990). The high diversity of fruit characteristics indicates the high potential of genotypes in the region to select superior genotypes based on the goals of the breeding program (Cosmulescu and Botu 2012).

Table 4 Mean of some important traits measured in superior walnut genotypes

Trait	G21	G22	G23	G25	G31	G46	G51	G108	G126
Habit of tree growth	3	3	3	3	3	2	3	2	3
Annual growth	221	129	39	80	175	290	456	203	225
Leafing date	30	30	40	28	29	40	29	35	40
Female flowering period	12	13	12	11	16	15	12	10	12
Pollination period	8	11	12	8	12	8	12	4	5
Flowering type	2	2	1	2	2	3	2	2	2
Nut length	3.51	4.1	3.9	3.78	3.98	3.21	3.55	3.6	3.31
Nut diameter	3.20	3.36	3.2	3.45	3.63	3.3	3.1	3.6	2.88
Shell seal	7	3	3	9	1	5	7	3	7
Husk thickness	4.43	2.93	3.05	4.59	3.9	2.95	4.46	3.1	4.6
Nut weight	14.5	12.28	10.61	14.15	15.68	15.19	14	15.1	11.5
Shell hardness	7	7	7	5	5	5	5	3	5
Middle blade thickness	5	5	7	5	5	7	5	3	9
Ease of kernel extraction	5	5	3	5	3	5	5	3	3
Kernel weight	8.78	7.25	5.37	8.62	9.1	7.21	8.92	8.74	5.88
Kernel percentage	60.4	55.74	50.23	60.84	58.02	49.26	63.5	57.9	50.9
Kernel fullness	7	7	7	7	7	7	7	7	7
Kernel plumpness	3	3	3	3	3	3	3	3	3
Kernel shrivel	1	1	1	1	1	1	1	1	1
Kernel color	1	3	1	1	4	2	1	3	1
Severity of blight disease	15	10	8	15	7	10	5	20	10
Severity of anthracnose disease	0	0	0	0	0	0	0	0	0

As a result, genotypes G23, G46, G56, and G126 seem to be among the most promising genotypes. These genotypes were late-leafing (13 to 15 April, i.e., about 10 days after Chandler [4 April] and 27 days after Payne [18 March]), with a high lateral-bearing rate, high yield, and ease of kernel extraction. But one of the disadvantages of G56 was its poor shell sealing, although its kernel percentage was high (62%). Genotypes G9, G22, G25, G31, G51, G108 had high yield, high lateral-bearing, high kernel weight (>7 g), and high kernel percentage (>58.5%). Genotype G51 had a light kernel color, well-sealed shell, high kernel percentage (63.72%), high kernel weight (8.93 g), shell thickness of 1.2 mm, and easy kernel extraction. Genotype G31 had very good traits (kernel weight 9.1 g, nut weight 15.68 g, kernel rate 58.02%); however, it was characterized by an amber kernel color and medium plumpness. Genotypes G108, G22, G126, and G9 were superior in terms of kernel weight, nut weight, kernel color, and kernel percentage (Fig. 4; Table 4).

In the case of late-leafing genotypes, it was observed that genotypes G23, G46, and G76 were homogeneous, but genotype G23 had the best overlap with other late-leafing genotypes as a donor of pollen grains. Some selected genotypes in this study were homogeneous (G9, G46, G23), some genotypes were protogynous (G22, G21, G51, G25, and G51), and some others were protandrous (G108, G126). It was found that homogamy was extremely low in some

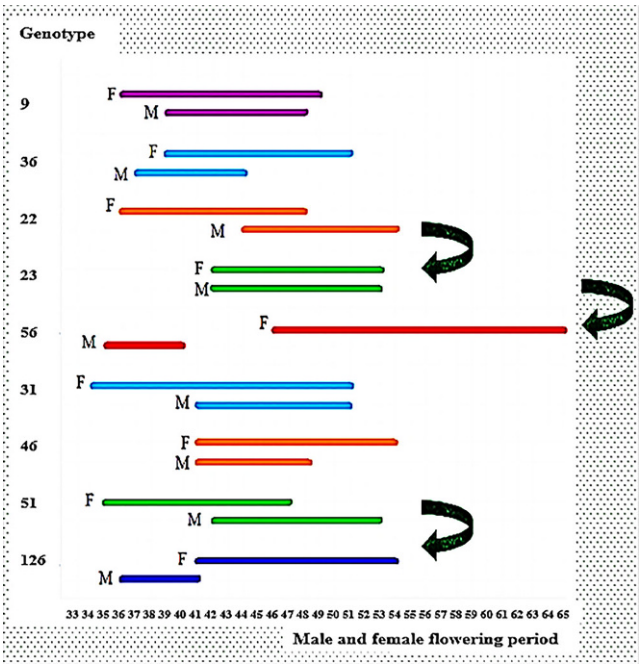

Fig. 5 Overlap of flowering period between some of the superior genotypes

Table 5 Total values, variance, and cumulative percentages of variances for eight main factors for 130 walnut genotypes

Principal component	Amount of variance	Variance percentage	Cumulative percentage of variance
1	5.96	22.92	22.92
2	3.47	13.36	36.29
3	2.41	9.28	45.58
4	2.06	7.92	53.51
5	1.69	6.5	60.01
6	1.46	5.63	65.64
7	1.44	5.54	71.19
8	1.08	4.18	75.37

superior genotypes (G22), or there was no homogamy at all (G56). Therefore, it is possible to plant the genotypes in overlapping pairs to ensure a high fruit set (Fig. 5).

Principal Component Analysis

Using principal component analysis (PCA), different attributes can be discussed in the form of components, each of which includes several attributes. This analysis can show the main differentiating factors between the studied genotypes. In this study, PCA was able to express 26 evaluated traits as eight main components, among which the first, second, and third components had the largest share in justifying total variance. A total of eight main and independent components whose variance values were greater than 1 were able to explain 75.37% of the total variance (Table 5).

The first component, PC1, consisted of four significantly contributed traits, including nut length, width, weight, and kernel weight, which explained 22.92% of the variance contribution. The second component, PC2, included the traits of leafing date, first female and male flowering, and harvest date, which accounted for 13.36% of the variance (Fig. 6). Traits including shell color, roughness, integrity, kernel fill, and middle blade thickness were in PC3, with a variance of 9.28%. The PC4 included the shell thickness, ease of kernel extraction, and kernel rate, which explained 7.92% of the variance. In PC5, kernel fullness, plumpness, shrivel, and color were included and explained 6.50% of the variance. These components played a major role in distinguishing the genotypes studied. The PC6 explained 5.63% of the variance and correlated with the severity of leaf and tree blight disease. The shell seal and nut shape were found in PC7

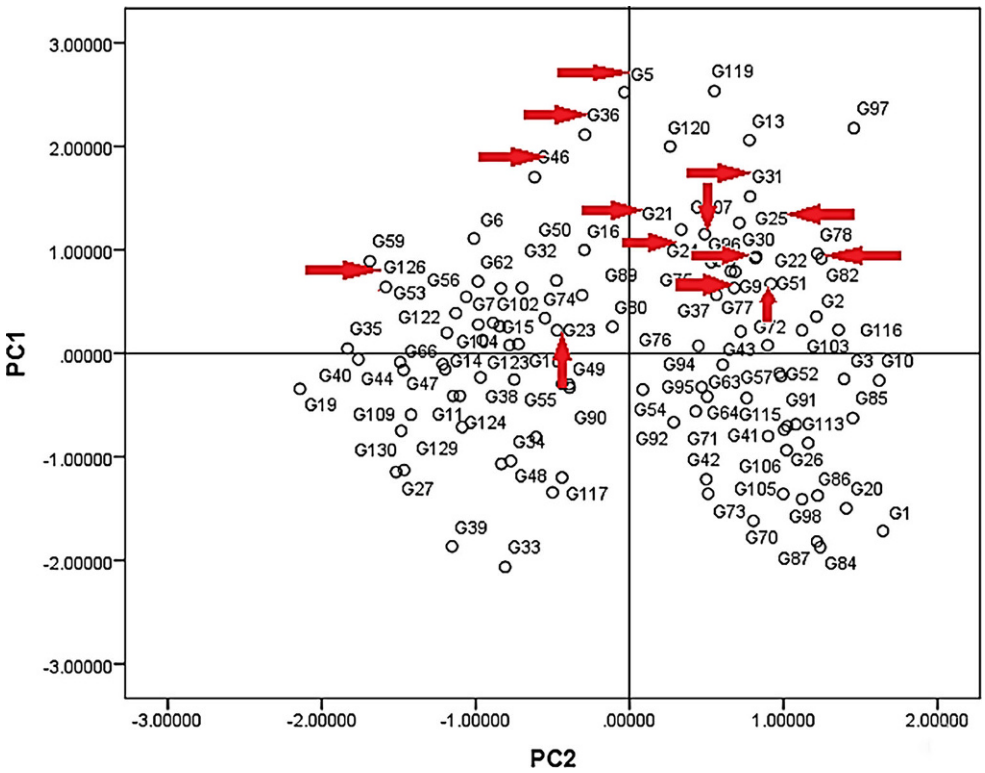

Fig. 6 Scatter plot results using the first and second factors for 130 walnut genotypes

Table 6 Eigenvectors of principal component axes from principal component analysis for the morphological characters in the studied walnut genotypes

Characteristic	Component							
	1	2	3	4	5	6	7	8
Nut length	**0.77**	0.02	0.20	–0.009	–0.008	0.01	0.45	0.09
Nut diameter	**0.85**	0.008	0.18	–0.07	–0.11	–0.14	–0.23	–0.12
Kernel weight	**0.88**	0.08	0.13	–0.17	0.29	–0.02	0.11	0.02
Nut weight	**0.91**	0.08	0.21	0.15	0.14	–0.04	0.07	0.07
Kernel percentage	0.13	–0.01	–0.17	**–0.76**	0.38	0.04	0.05	–0.07
Kernel fullness	0.08	0.09	–0.008	–0.21	**0.75**	0.10	0.10	–0.05
Kernel plumpness	–0.001	–0.06	–0.05	–0.08	**0.74**	–0.18	–0.09	0.02
Kernel shrivel	–0.14	0.13	0.02	–0.04	**–0.51**	–0.25	–0.23	–0.06
Kernel color	–0.02	–0.03	–0.13	–0.25	**–0.60**	–0.19	0.15	0.11
Middle blade thickness	–0.04	0.04	0.09	–0.20	0.04	–0.16	**0.79**	–0.07
Shell hardness	–0.03	0.19	0.10	**0.83**	0.07	0.006	0.28	0.01
Ease of kernel extraction	–0.17	0.26	0.14	**0.68**	0.01	0.05	0.11	–0.14
Thickness of shell	0.28	0.12	0.19	**0.75**	0.03	–0.04	0.05	0.10
Nut shape	0.19	0.24	0.14	0.10	–0.01	0.15	**0.75**	0.008
Shell color	0.08	0.15	**0.77**	–0.03	–0.08	0.04	0.14	–0.01
Shell roughness	0.21	0.23	**0.86**	0.2	0.05	0.04	0.08	0.02
Shell integrity	0.29	0.18	**0.84**	0.15	0.02	0.02	0.05	0.02
Shell seal	0.06	0.01	**0.72**	0.33	0.06	–0.12	0.006	0.09
Leafing date	–0.06	**–0.90**	–0.15	–0.03	0.09	0.04	–0.01	0.13
First female flowering date	–0.06	**–0.93**	–0.13	–0.10	0.05	0.08	–0.09	0.10
First male flowering date	0.05	**0.62**	0.005	0.34	0.24	0.04	0.12	0.11
Harvest date	0.01	**0.79**	0.27	0.18	–0.04	–0.001	–0.12	0.07
Flowering type	0.26	0.01	–0.02	0.11	0.05	0.20	–0.12	**0.75**
Severity of leaf blight disease	–0.09	–0.05	–0.03	–0.04	0.03	**0.88**	–0.03	0.01
Severity of tree blight disease	–0.05	–0.008	0.03	0.02	0.15	**0.84**	0.006	0.10
Severity of anthracnose disease	–0.44	–0.13	0.18	–0.10	0.03	–0.06	0.01	**0.70**
Percentage of variance	22.92	13.36	9.28	7.92	6.50	5.63	5.54	4.18
Cumulative percentage	22.92	36.29	45.58	53.51	60.01	65.64	71.19	75.37

The numbers in boldface indicate to be highly signifucant in each corresponding principal component (1 to 8)

and explained 5.54% of the variance, and PC8 explained 4.18% of the variance and correlated with severity of anthracnose disease and bearing type (Table 6). Haghjooyan et al. (2005) showed that the first two components, cumulatively, explained 99.87% of the changes in the initial data and included nut length, width, weight, shell percentage, and kernel rate. In the study of Pop et al. (2013) of 20 walnut cultivars in Romania, PC1 and PC2 explained 29.2% and 17.53% of the total variance, respectively. In research by Mousavi et al. (2015), a total of seven main components (with 21 traits) were able to explain 73.78% of the total variance: PC1 (nut weight, length, width, and diameter) was 22.04%, PC2 (kernel weight, rate and size, shell weight, and percentage) was 14.17%, PC3 (adhesion of shell to kernel and maturity heterogeneity) was 10.42%, PC4 (flowering habit, fruit ripening time and yield) was 9.11%, PC5 (shell thickness and tree shape) was 7.94%, PC6 (nut shape) was 6.75%, and PC7 (leafing time) was 3.35%. The first and second components in the study by Ahandani et al. (2014) explained about 69.6% of all the variances. In the studies by Pop et al. (2013) and Mousavi (2015), nut and kernel traits were the effective traits in the first factor.

Investigation of Apomictic Genotypes

Apomixis is a natural phenomenon of asexual reproduction in plants in which the embryo is formed without the fusion of male and female gametes. Apomict seeds are genetically similar to the female parent (Ulukan 2009). The apomixis percentage in this study varied between 0 and 25.75%. Genotypes G2, G11, and G9 recorded the highest apomixis rate (25.75%, 20%, and 10.44%, respectively), while it was the lowest in G87, G1, G22, and G53 (5.55%, 5.19%, 5%, and 3.75%, respectively). Many researchers have reported a low apomixis rate in walnuts (Laiko 1990; Şan and Du-

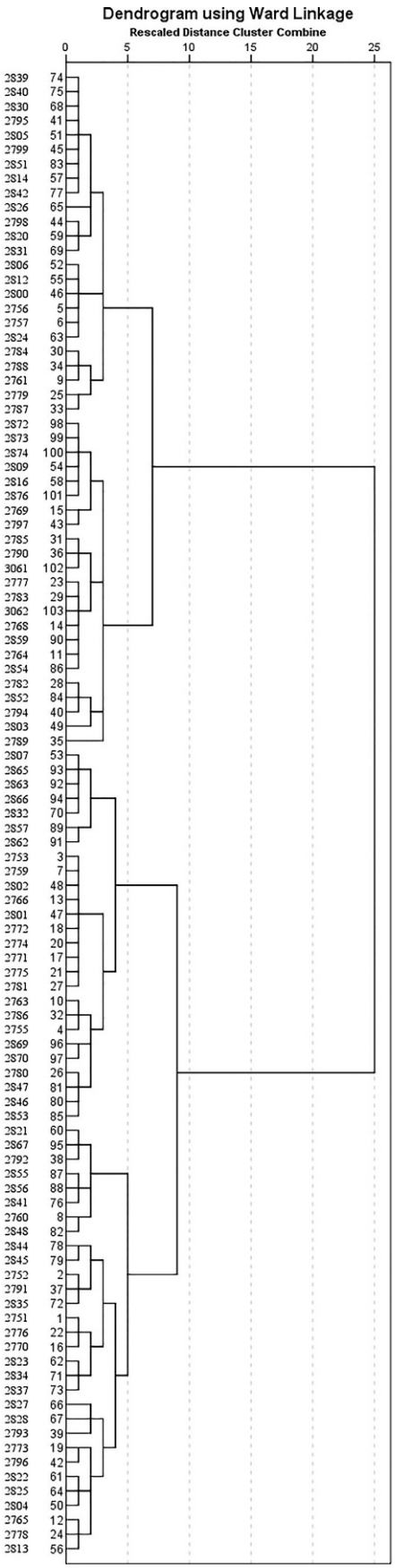

Fig. 7 Cluster analysis of 130 walnut genotypes based on morphological traits

manoğlu 2006; Valdiviesso 1990), which in some cases was almost nonexistent. Laiko (1990), Guo-liang et al. (2007), and Solar et al. (1995) reported a high apomixis rate in some genotypes (23.5% to 81.2%). The apomixis rate may vary from year to year depending on the environmental conditions (Guo-liang et al. 2007). Therefore, the rate of apomixis cannot be accurately predicted. Guo-liang et al. (2010) introduced a new walnut cultivar called 'Qinquan 1' that was selected from genotypes in northern China with an apomixis rate of 24.7%.

Cluster Analysis

Based on the Euclidean distance, the genotypes were divided into four main groups, and each group was divided into smaller clusters with more shared characteristics (Fig. 7).

The first group included three subgroups that included traits such as high annual growth, early pollination, intermediate fruit ripening, both flowering types, high kernel percentage, very thin to thin middle blade, ease of kernel extraction, thin shell to papery, kernel weight from 5 g to 7.81 g, plump kernel, and medium to good kernel fullness.

The second group included three subgroups with traits such as high annual growth, late leafing, short female flowering period (9–12 days), early pollination, both flowering types, and medium to good kernel fullness.

Three subgroups were found in the third group, which included traits such as high annual growth, early leafing, long female flowering period (12–14 days), both flowering types, zero anthracnose percentage, seed length and diameter greater than 29 mm, high nut weight, rough to very rough shell, and high shell integrity.

The fourth group consisted of three subgroups that had traits such as a very long female flowering period (12–17 days), nut diameter less than 33.5 mm, and nut length less than 37.5 mm.

Conclusions

Walnut propagation by seeds has long been standard in Iran. The long juvenile period of walnuts and their large size rendered the genetic evaluation difficult. Therefore, identifying, collecting, and evaluating superior genotypes from native walnut landraces and seed-propagated orchards or nurseries are essential for walnut breeding (McGranahan et al. 1998).

Although it is difficult, due to environmental influences, to distinguish different genotypes based on morphologi-

cal characteristics, these studies are necessary for evaluation, selection, and breeding programs. In the current study, about 130 seed genotypes were evaluated as part of an integrated project for walnut breeding in Iran to improve local cultivars or to develop new cultivars.

At the end of this research, several promising genotypes were determined to carry some important morphological, phenological, and morphological traits that are important plant materials that can be used in walnut breeding programs. A core collection of superior genotypes was formed, and a comprehensive study is currently being conducted on them, including their chemical composition (crude protein, free fatty acids, nutritional content, etc.), with an in-depth study of the phenomenon of apomixes.

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Author Contribution Experiments, methodology, data curation, data analysis, writing of original draft, and writing review and editing were performed by Adnan Sallom. Design and experiments, supervision, investigation, data analysis, and writing review and editing were performed by Dr. Reza Fatahi, and Dr. Zabihollah Zamani. All authors read and approved the final manuscript.

Conflict of interest A. Sallom, R. Fatahi, and Z. Zamani declare that they have no competing interests.

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