

Evaluation and identification of walnut (*Juglans regia* L.) genotypes in Markazi province of Iran

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ABSTRACT

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The Persian walnut (*Juglans regia* L.) is one of the most important horticultural crops grown in Iran. This research was conducted to determine the variation in walnut germplasm grown in the Arak region of Iran during the 2007-2008 growing seasons. All of the selected genotypes originated from seed, and their phenological and pomological characteristics were evaluated. Results indicated that there were promising genotypes within the walnut populations in the studied region. Genotypes MS26, MS6, MS34, MS45, MS70, MS49 and MS54 were late bearing leaves (21 to 25 April). Genotypes MS27 and MS29 were completely homogamous. Bearing habit was lateral and terminal in 38.57 and 61.43% genotypes, respectively. Genotypes MS8, MS10, MS48 and MS66 were early maturing and their nuts matured before 10 September. High variation for nut and kernel characteristics was found among the studied genotypes. Genotypes MS15, MS17, MS19, MS20, MS23, MS26, MS27 and MS29 were identified as promising, because their kernel weight, nut weight and kernel ratio were higher than 6 g, 12 g and 50%, respectively. These superior genotypes could be used for cultivation or as parents in future breeding programs.

Keywords: genetic variation, *Juglans regia* L., phenology, pomology, promising genotypes

INTRODUCTION

Juglans regia (Persian walnut), is a temperate nut crop and Iran is one of its centers of origin and diversity (Arzani *et al.*, 2008). According to the Food and Agriculture Organization, Iran ranks fourth (after China, the United States and Turkey) in global walnut production (FAO, 2009). Most walnut trees in Iran originated from seed, so there is considerable variability in their nut and kernel characteristics (Asadian and Pieber, 2005). The high genetic variation in walnut trees is due to their seed-based propagation, high heterozygosity and dichogamy (Aslantas, 2006; Germain, 1997). Local trees with high variation are important in breeding programs (Aslantas, 2006; Zeneli *et al.*, 2005), for they may show characters such as high quality nuts, late leafing, low chilling requirement and disease resistance (Germain, 1997). High variability in phenological and nut traits has been reported in walnut trees from different regions. Haghjooyan *et al.* (2005) evaluated morphological traits of 138 seedling walnut genotypes grown in different regions of Iran. Arzani *et al.* (2008) identified promising walnut genotypes in the Taft region of Yazd Province. Identification of promising walnut genotypes has also been reported in other countries such as India (Sharma and Sharma, 1998; Sharma

and Sharma, 2001), Turkey (Yarilgac *et al.*, 2001; Kazankaya *et al.*, 2001; Sutyemez and Kaska, 2005; Ozkan and Koyunca, 2005; Aslantas, 2006), Kyrgyzstan (Hemery, 1998) and Slovenia (Solar and Stampar, 2005; Zeneli *et al.*, 2005; Colaric *et al.*, 2006).

There is a rich walnut population in Markazi Province, western Iran. Most walnut trees in this region have been propagated by seed thus show high variability in phenological characters such as leafing, flowering date, date of maturity and nut characteristics. Because late spring frosts are sometimes a problem in this province, trees that leaf-out late enough to escape these frosts should be selected (Shreve, 1999). It has been reported that there are more than 3234 ha sown to walnut trees in Markazi Province; walnut production in this province is thus economically important in Iran (Anonymous, 2008). The aim of this research was to identify superior and promising walnut genotypes within the walnut population of Markazi Province for direct cultivation or for use as parents in future breeding programs.

MATERIAL AND METHODS

This research was carried out during the 2007-2008 growing seasons in some regions of Arak (Fig.

1), Markazi Province, in western Iran. The studied regions were Karahrood (latitude 34° 3' N, longitude 49° 38' E and altitude 1847 m), Senejan (latitude 34° 2' N, longitude 49° 37' E and altitude 1867 m),

Dehsad (latitude 34° 11' N, longitude 49° 21' E and altitude 1857 m) and Enaj (latitude 34° 13' N, longitude 49° 17' E and altitude 1852 m).

The climate in the evaluated regions features cold

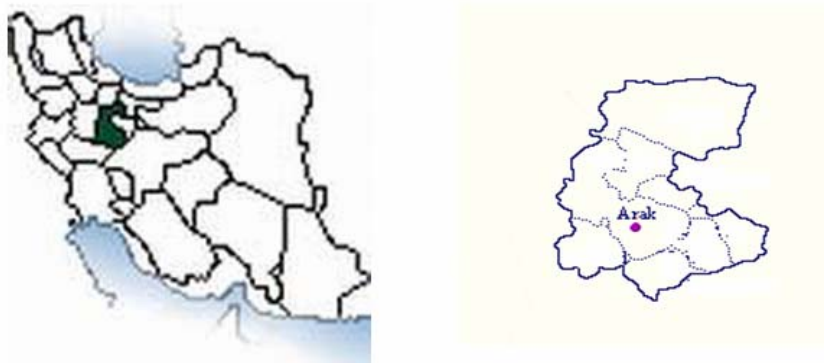


Fig. 1. Iran (left) and Markazi Province (right), where the study region (Arak) is located.

winters and warm, semi-arid summers (annual relative humidity of about 46%). The average annual temperature and rainfall in the study regions were 13.8 °C and 300 mm, respectively. To identify promising walnut genotypes, 70 mature walnut trees were selected (and labeled) based on interviews with growers; phenological and pomological characteristics were evaluated. All trees had been seed-propagated and thus had high variation in their phenotypical and pomological characteristics. The age of the trees ranged between 12 and 150 years.

Phenological traits

Characters such as leafing date, beginning and end of pollen shedding, beginning and end of pistillate receptivity, lateral bearing and time of nut maturity were evaluated based on IPGRI descriptors. According to IPGRI descriptors (1994), leafing date is when 50% of terminal buds are open and leaves are visible from inside the buds. The dates on which the first and last pollen shedding occurred is considered the beginning and end of pollen shedding. In addition, the start and end of flower receptivity was when the first and last female flowers became receptive (IPGRI, 1994). Homogamous characteristics and percentage were calculated as follows:

$$\frac{\text{No. of days that female blooms overlapped with staminate blooms (days)} \times 100}{\text{Duration of female blooms (days)}}$$

Nuts were harvested when the membrane between the kernel halves turned completely brown, which is when the kernels have the lightest color and highest quality (Ramos, 1998).

Pomological traits

To measure nut and kernel traits, 15 healthy fruits per tree were randomly harvested at maturity. These fruits were immediately shelled and then dried at room temperature until 8% moisture content was achieved. Then nut weight, length, width, thickness, shell thickness, kernel weight, kernel percentage, kernel color and removal of kernel from nut were measured. The nut form index was calculated by the following equation:

$$\text{Form Index} = 2(100H)/(L+E)$$

where H, E and L are height, thickness and width of nut, respectively, as shown in Fig. 2. Nuts with a form index less than or equal to 110, between 111 and 125, or more than 125 were considered as having a spheroid, ovoid and ellipsoidal-elongated form, respectively (Arzani *et al.*, 2008).

Oil extraction

For oil extraction, dried kernels were ground to a diameter less than 0.25 mm. Then 10 g of the ground sample was loaded into the soxhlet apparatus, and extraction was carried out using n-hexane as a solvent (AOAC, 1984). The solvent was separated from the oils by rotary evaporator at 40°C. The Statistical Package SPSS, version 16, was used for data analysis.

RESULTS AND DISCUSSION

The results showed that evaluated trees had high variation for phenological and pomological traits.

Phenological traits

Date of leafing among genotypes varied from 9

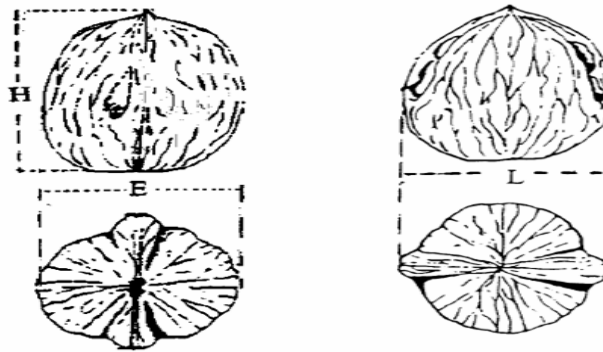


Fig. 2. Height (H), thickness (E) and width (L) of nuts in walnut.

to 25 April. Eighty-three percent of genotypes were early leafing (from 9 to 14 April), 7% were mid-leaf genotypes (15 to 20 April), and 10% showed late leaf bearing (21 to 25 April). Late leafing is a very important trait because late leafing genotypes can escape spring frost injury. Results showed that genotypes MS26, MS6, MS34, MS45, MS70, MS49 and MS54 were late leafing. Pollen shedding took place between 19 April and 18 May. Genotypes MS16 and MS59 had the shortest (4 days) and genotype MS46 the longest (13 days) pollen shedding period. Receptivity of pistillate flowers lasted from 20 April to 18 May. Genotypes MS18 and MS20 had the shortest (3 days) and the longest (13 days) pistillate receptivity periods, respectively. Percentage of homogamy varied from 0 to 100. Type of bearing habit was lateral and terminal in 38.57 and 61.43% of genotypes, respectively. Based on nut harvest date, genotypes were classified into three groups: early, intermediate and late maturing. Of the genotypes, 48.6% were late maturing (22 September to 9 October), 22.85% showed intermediate maturity

(11 to 21 September) and 28.6% were early maturing (before 10 September).

Nut and kernel traits

The study genotypes showed high variability for nut and kernel characteristics, some of which are shown in Table 1. The lowest and highest nut weight among genotypes were 6.34 and 16.89 g, respectively. Kernel weight varied between 2.69 and 8.1 g. Nut length, width and thickness among genotypes were 27.94-42.21 mm, 26.62-35.99 mm and 26.92-37.12 mm, respectively. The minimum and maximum kernel percentages were 31.75 (MS28) and 60.5% (MS43), respectively. The minimum and maximum shell thicknesses were 1.04 mm (MS9) and 2.07 mm (MS5 and MS38), respectively. Kernel removal from nut was very easy, easy, medium, hard and very hard in 2.85, 64.3, 15.7, 14.3 and 2.85% of genotypes, respectively. Oil content fluctuated between 51% (MS15) and 73.06% (MS43), with a mean of 63.62%.

Table 1. Fruit characteristics of 70 walnut genotypes grown in Markazi Province during the 2007-2008 growing season.

Trait	Mean± SD	Min.	Max.	Variation range
Nut length (mm)	34.59±3.13	27.94	42.20	14.26
Nut thickness (mm)	31.78±2.37	26.92	37.11	10.19
Nut width (mm)	30.94±2.03	26.62	35.98	9.36
Kernel weight (g)	4.91±1.23	2.69	8.10	5.40
Nut weight (g)	10.31±2.14	6.33	16.89	10.56
Shell thickness (mm)	1.43±0.23	1.03	2.06	1.03
Kernel percentage	49.95±6.14	31.76	60.51	28.75
Oil content (%)	63.62±5.75	51.00	73.06	22.06

Cluster analysis

Cluster analysis of all genotypes (70 genotypes) was carried out based on traits with high heritability (leafing date, duration of female flower receptivity, duration of pollen shedding, date of nut maturity, nut weight, kernel weight and nut size) because they are less affected by environmental factors (Aslantas, 2006; Sharma and Sharma, 1998). According to the cluster analysis, there was diversity among

genotypes, which were separated into six groups (Fig. 3). Genotype MS2 was placed separately in the last group, indicating that it showed the greatest difference from the others. Genotypes in the same group have the greatest similarity.

The results of this research indicate wide variation in phenological and pomological characteristics among the studied genotypes. The highest mean nut weight (16.89 g) in the evaluated

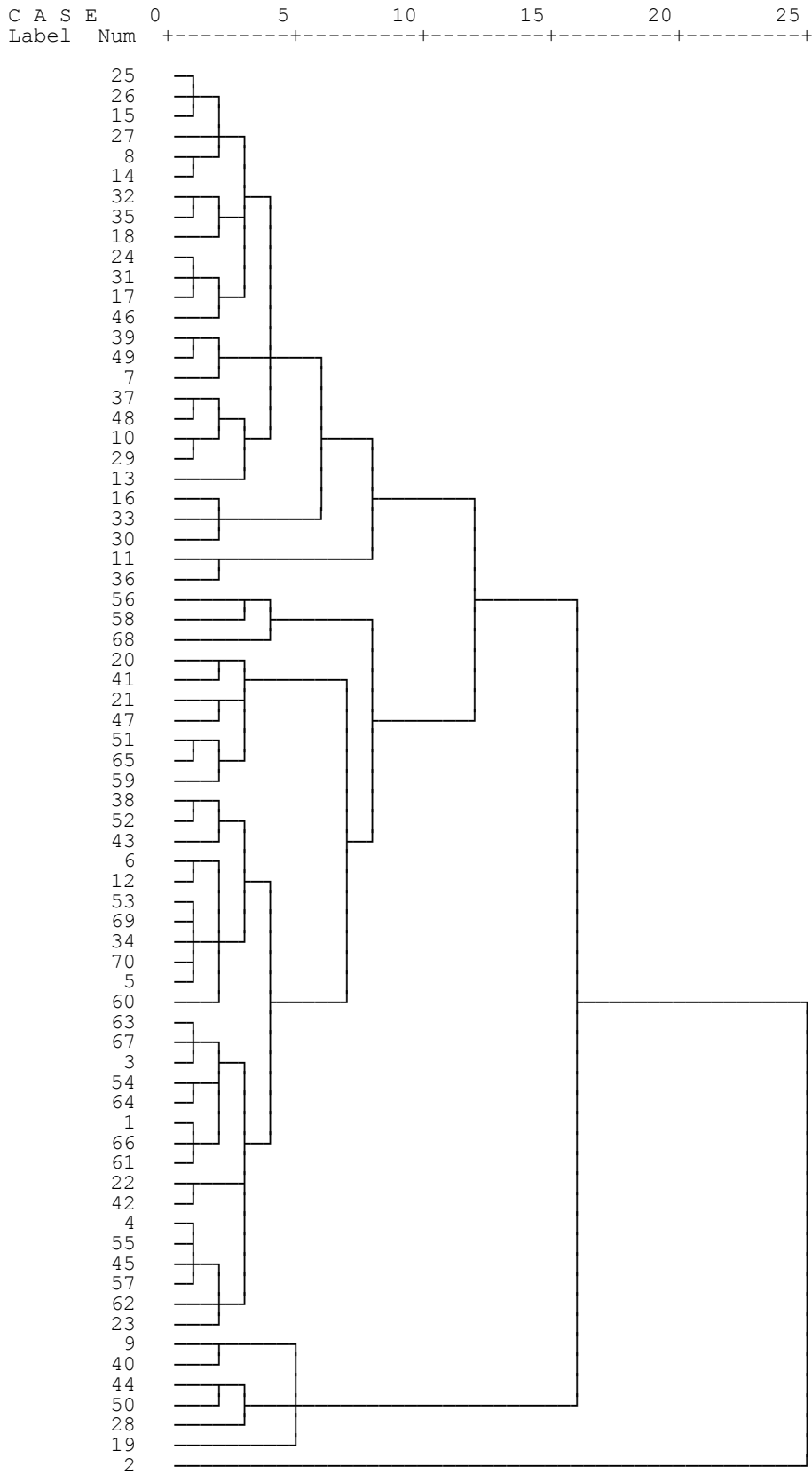


Fig. 3. Dendrogram for 70 selected genotypes based on traits with high heritability (leafing date, duration of female flower receptivity, duration of pollen shedding, date of nut maturity, nut weight, kernel weight and nut size).

Table 2. Correlation coefficients among nut and kernel characteristics of 70 genotypes evaluated during the 2007-2008 growing season.

	KW	ST	NL	NWi	NT	NW	FI	KR
ST	-0.021							
NL	0.395**	0.079						
NWi	0.551**	0.242*	0.537**					
NT	0.550**	0.297*	0.479**	0.881**				
NW	0.863**	0.375**	0.485**	0.706**	0.72**			
FI	-0.044	-0.134	0.691**	-0.206	-0.28*	-0.076		
KR	0.499**	-0.639**	-0.08	-0.125	-0.133	0.002	0.004	

KW: Kernel weight; ST: Shell thickness; NL: Nut length; NWi: Nut width;

NT: Nut thickness; NW: Nut weight; FI: Form index; KR: Kernel ratio

* and **: Significant at the 0.05 and 0.01 probability levels, respectively.

genotypes was higher than the data reported by Arzani *et al.* (2008) (15.2 g) and Aslantas (2006) (16.01 g), but lower than those reported by Sen and Tekintas (1992) (23.81 g), Atefi (1997) (20.9 g), Sharma and Sharma (1998) (18.6 g), Atefi (2001) (19.4 g), Yarilgac *et al.* (2001) (17.04 g), Sharma and Sharma (2001) (23.61 g), and Zeneli *et al.* (2005) (21.1 g). The highest mean kernel weight (8.1 g) was higher than the data reported by Yarilgac *et al.* (2001) (7.88 g), but lower than the 9.8 g reported by Zeneli *et al.* (2005). The maximum kernel ratio (60.51%) was higher than the data reported by Arandhya *et al.* (2006) (57.41%) and Yarilgac *et al.* (2001) (59.27%), but lower than those found by Zeneli *et al.* (2005) (63.8%).

As reported by other researchers, a significant positive correlation (0.863) between nut weight and kernel weight was observed in this study, but no relationship between nut weight and kernel ratio ($P \leq 0.01$) was found. Shell thickness showed a negative correlation (-0.639) with kernel ratio, which agrees with the results of Sharma and Sharma (2001) and Arzani *et al.* (2008); however, nut weight showed a positive correlation (+0.375) with shell thickness ($P \leq 0.01$). This means that heavier nuts have thicker, heavier shells. Correlation coefficients among other pomological traits are shown in Table 2.

In conclusion, evaluation of 70 walnut seedling genotypes shows that there are valuable genotypes in

Markazi Province. For example, genotypes MS6, MS26, MS34, MS45, MS49, MS54 and MS70 are late leafing and therefore suitable for areas with frequent spring frost, such as Markazi Province. These genotypes are able to escape late spring frost (Zeneli *et al.*, 2005). Late leafing genotypes can also be cultivated effectively in mountainous regions with frequent frosts (Akca and Ozongun, 2004). Solar and Stampar (2005) introduced cultivars Z-62, Krnc and Z-60 as late leafing. The flowering habit of genotypes was not similar and protandry was dominant. Homogamy is a useful trait and genotypes with higher homogamy such as M27 and M29 are highly valued. Early maturing genotypes are also desirable because of their short growing period, which allows them to escape early autumn frost injury (Korac *et al.*, 1997; Aslantas, 2006). Genotypes MS8, MS10, MS48 and MS66, whose nuts were harvested earlier than others, were found to be desirable. McGranahan and Leslie (1990) reported that in promising genotypes, kernel ratio should be more than 50%, and kernel and nut weight should be higher than 6 and 12 g, respectively. Genotypes MS15, MS17, MS19, MS20, MS23, MS26, MS27 and MS29 had higher quality nuts, and their nut weight, kernel weight and kernel ratio were also higher (12 g, 6 g, and 50%, respectively). Nut and kernel characteristics of promising genotypes are shown in Table 3.

Table 3. Characteristics of eight promising genotypes evaluated during the 2007-2008 growing season.

Genotype	Nut weight (g)	Kernel weight (g)	Kernel ratio	Kernel moval from nut	Form index	Oil (%)	Bearing habit	Homogamy (%)
MS26	16.89	8.04	47.61	M	S	67.53	T	75
MS15	15.25	8.10	52.17	E	S	51.00	L	10
MS17	14.67	7.59	51.71	M	O	70.27	L	50
MS19	13.25	7.30	53.25	E	O	53.00	L	0
MS20	12.73	6.56	51.60	E	S	71.47	T	46
MS23	12.28	7.19	58.59	E	S	66.07	T	0
MS27	13.50	7.81	57.86	E	O	59.07	T	100
MS29	12.38	6.70	54.12	E	O	60.00	T	100

M: Medium; E: Easy; S: Spheroid; O: Ovoid; T: Terminal; L: Lateral

As reported by Kazankaya *et al.* (2002) in Turkey, nut weight, kernel weight and kernel ratio of superior walnut genotypes were 11-12 g, 6-7 g and

more than 45%, respectively. It has been reported that fruit characteristics are not affected by tree age (Sharma and Sharma, 1998). Therefore diversity in

pomological traits among genotypes may be due to climatic or genetic differences. Aslantas (2006) found that the best genotypes have heavier nuts with thinner shells and more lateral fruitfulness. The present research showed that there are promising

walnut genotypes within the walnut population in Markazi Province. The selected superior genotypes could be used for cultivation or as parents in future breeding programs.

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