Journal of Nuts 7(2):101-108, 2016
ISSN: 2383 – 319X

Xenia and Metaxenia in Persian Walnut (*Juglans regia* L.)

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Received: 14 May 2016 Accepted: 5 September 2016

Abstract

In 2007 and 2008, the influence of pollen source on the shell and kernel traits of resulting seeds and on fruit set was examined to evaluate the potential for Xenia or Metaxenia in Persian walnut. Pistillate flowers of 'Jamal', 'Chandler', 'Hartley' and 'Pedro' were crossed with the pollen of 'Serr', 'Z60' and 'Damavand'. The pistillate flowers on each female parent were covered about one week before starting the reception period of the flowers, and the bags remained 7-10 days after pollination. The catkins of the male parents were collected just prior to pollen shedding, and they were kept in room temperature for 24- 48 hours. Collected pollen was refrigerated until use. Pollination was done when the stigmas were expanded and were pinkish in color. Data recording were started after removing the bags and measuring the percent of fruit set and recording the fruit growth and time of ripening. The fruits, nut and kernel weight, length, diameter, length to diameter, shell thickness, shell weight and kernel percentage were evaluated after harvest. The result showed that nut and kernel diameter, kernel weight, length to diameter, shell-thickness and shell weight were affected by pollen source. There were also significant differences in the fruit set of the different pollen parents. Pollens of 'Damavand' and 'Z60' significantly decreased the nut diameter and weight in some female parents. Total fat and protein content of kernels were significantly affected by the type of pollen parent.

Keywords: Catkin, Pistillate flower, Pollen, Pollination.

Introduction

Persian walnut (*Juglans regia* L.) is native to an area extending from the Balkans in southeastern Europe through southwest and central Asia to the eastern Himalayas and western China. It is grown widely for its nutritious nuts, used as a landscape tree and its valuable wood. Major walnut producers include France, Romania, Ukrain, Serbia, Italy, Germany and Greece in Europe; China, Turkey and, Iran in Asia; United State of America (California) in North America; Chile in South America; New Zealand and southeast of Australia in Oceania (FAO Stat). In Iran, wild walnut trees are grown mainly in the northern forests at altitudes ranging from Caspian Sea level to more than 2000 m (Vahdati, 2000).

Walnut is mainly cultivated for its edible kernels, and numerous walnut cultivars have been selected for their production traits. Walnut cultivation and orchard management can also benefit from inclusion of pollinizers cultivars. Although walnuts are self-fertile, they are heterodichogamous, and cultivars usually do not completely cover their female blooming period with their own pollen. Therefore, orchard yields generally

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benefit from a pollinizer. However, excessive amounts of pollen can cause pistillate flower abscission (Por and Por 1990, McGranahan *et al*., 1994) and thus, pollinizers reduce yield at times (Polito *et al*., 1994). Effects of a particular pollen parent on seed (xenia) or fruit (metaxenia) characteristics are known to occur in several nut crops. Xenia and metaxenia have been observed in chestnut (Pontikis, 1977; Crane and Iwakiri, 1980), pecan (Marquard, 1988), and almond (Vezvaie and Jackson, 1995; Kumar and Das, 1996). In hazelnut, cross pollination increased nut and kernel weight and decreased blank percentage (Rahemi and Javadi, 2001). In fig. (what figure?) (*Ficus carica* L. cv. Sabz), fruit length, total soluble solids, fruit eye diameter, time of fruit ripening and skin color were significantly influenced by the pollen source (Rahemi and Jafari, 2008). Since the effect of pollen source on nut and kernel characteristics of walnut has not been studied yet, the objective of this study was to evaluate the existence of xenia and metaxenia in walnut.

Materials and Methods

Location

The experiments were conducted during 2007-2008 at the Kamalshahr Station of Horticulture Department of the Seed and Plant Improvement Institute located in Karaj, Iran. In 2006, temperatures ranged from -17 to 37 °C and relative humidity from 6% to 97%. Temperatures in 2007 varied between -7 and 40 °C and humidity ranged from 8% to 100%.

Pollen collection and viability test

Catkins of 'Serr', 'Z_{60}' and 'Damavand' were collected just before pollen shedding. Catkins were placed on Wattman® paper. After one night in the laboratory, pollen was collected and dehydrated for four hours, put into air-tight tubes and kept at 4ºC until used for pollinations (Germain, 1997). Viability of pollen was determined by growth on a germination medium containing 0.65 % agar, 20% sucrose, 1.0 mM $CaCl_2$ and 0.161mM boric acid (Luza and Polito, 1985). Germination percentage for all pollen sources was more than 50% at the time of collection.

Controlled pollination

In April 2007 and 2008, all catkins were removed from four selected branches in each of four selected maternal cultivars 'Jamal', Chandler, 'Hartley' and 'Pedro' in tree replication which was then enclosed with double cheese cloth bags to exclude unwanted pollination. The pistillate flowers on the selected branches were pollinated at the stage of maximum receptivity, i.e., when the stigmas were well expanded and their two lobes were visibly separated at an angle of 45ºand pollen applied on stigma by hand (Polito *et al*., 1998).

Data analysis

Fruit set

The bags were removed 14 to 18 days after pollination, and fruit set was recorded after one month, in June and at harvest time.

Nut and kernel characteristics

The nuts from each of the crosses were harvested separately and dried at room temperature. Nut length and diameter, shell and kernel weight, kernel percentage, kernel length, shell diameter and thickness were measured for 10 nut samples in each replication.

Chemical analysis

Crude fat content was determined using a Soxhlet extractor (Horwitz, 2000). Total protein was determined using the semi-micro Kjeldahl method (Horwitz, 2000).

Statistical analyses

Treatment means and standard error were obtained using basic statistics in SPSS software (Ver.16).

Results

Pollen effect on fruit set. The effect of pollen source on fruit set of walnut cultivars are shown in Table 1. Fruit set in different female cultivars pollinated with three pollinizers vs. open pollination showed significant differences. Chandler's initial set decreased by 15.33% using the pollen of 'Serr' compared to open pollination. The harvest time set of 'Chandler' pollinated with 'Serr', 'Z_{60}' and 'Damavand' was 18.64, 11.91 and 13.21% less than open pollination, respectively (Table 1). 'Hartley' pollinated with 'Serr', 'Z_{60}'and 'Damavand', showed 15.04%, 16.42%, and 10.73% less initial fruit set and 17.29%, 11.36%, and 12.38%, less final fruit set to open pollination, respectively (Table. 1). In 'Hartley', pollination with 'Serr', 'Z_{60}' and 'Damavand' reduced the final set by 24.05, 24.01 and 18.82% compared to open pollination, respectively.

'Hartley' pollinated with 'Serr', 'Z_{60}' and 'Damavand', gave 18.44, 17.99 and 14.9%, less fruit set than open pollination (Table 1). In 'Pedro', pollination with 'Z_{60}' and 'Damavand' produced 16.87% and 20.07% less fruit set than open pollinated flowers (Table 1).

Table 1. Effect of pollen source on fruit set of walnut cultivars.

Female Cultivar	Fruit setting[1] %	Cultivar used as Pollinizer			
		'Serr'♂	'Z_{60}'♂	'Damavand'♂	Open pollinated
'Jamal' ♀	Initial set	59.31± 16.89	59.15 ± 17.51	56.66 ± 15.91	58.97 ± 15.66
	Final set	32.18 ± 10.96	39.01 ± 12.78	35.12 ± 12.92	48.87 ± 13.14
	Harvest time	29.65 ± 11.32	34.82 ± 12.87	29.43 ± 11.57	43.5 ± 11.64
'Chandler' ♀	Initial set	42.09 ± 6.97	48.42 ± 10.11	47.98 ± 8.36	57.32 ± 2.04
	Final set	36.52 ± 8.70	42.45 ± 5.49	40.98 ± 8.02	53.81 ± 4.28
	Harvest time	29.53 ± 7.84	36.08 ± 10.99	34.87 ± 8.55	47.99 ± 4.83
'Hartley' ♀	Initial set	41.66 ± 6.05	40.28 ± 4.55	45.97 ± 6.00	56.70 ± 4.54
	June set	26.05 ± 11.17	26.00 ± 9.04	31.28 ± 9.82	50.10 ± 3.17
	Harvest time	22.14 ± 10.39	22.68 ± 6.54	25.68 ± 6.06	40.58 ± 5.48
'Pedro' ♀	Initial set	50.84 ± 10.14	38.57 ± 9.03	45.81 ± 11.24	55.56 ± 7.80
	Final set	32.18 ± 4.99	39.01 ± 4.68	35.12 ± 10.46	48.87 ± 6.49
	Harvest time	29.65 ± 7.82	34.82 ± 6.93	29.43 ± 9.96	43.50 ± 6.86

[1] Fruit set was recorded one month after pollination , in June and at harvest time

Pollen effect on nut and kernel traits

The pollen parent had a significant effect on the nut length. In 'Chandler', using 'Z_{60}' and 'Damavand' as a pollinator reduced the nut length by 3.05 mm and 1.77 mm, respectively (Table 2). No reduction in nut length was seen in 'Hartley', 'Pedro' or 'Jamal', while using 'Damavand' pollen reduced 'Chandler' kernel diameter (0.91 mm) compared to open pollination. Pollen parent did not affect kernel diameter in 'Hartley', 'Pedro', or 'Jamal'. Pollen parent did not affect 'Chandler' nut or kernel weight (Table 2), while reductions in nut weight were seen on 'Chandler' pollinated with 'Z_{60}' and 'Damavand'. Pollen parents 'Z_{60}', 'Damavand' and

'Serr' reduced nut weight by 1.98, 1.89 and 1.33 g, respectively (Table 2).

Kernel weight was affected in 'Chandler' and 'Pedro', in that 'Chandler' pollinated with 'Z_{60}' and 'Damavand', reduced the kernel weight by 0.62 and 0.72 g, vs. open pollination, respectively. In 'Pedro,' the same trend was observed, and kernel weight was reduced by 0.91 and 0.73 g, respectively (Table 2).

Kernel percentage was also significantly ($P \leq 0.05$) affected by the type of pollen. 'Jamal' pollinated with 'Z_{60}' produced the highest kernel percentage with 2.92% higher kernel percentage respect to other treatments and open pollination treatment (Table 2).

A decrease was observed in shell thickness in 'Chandler' pollinated with 'Serr', 'Damavand' and 'Z_{60}', by 0.25, 0.27 and 0.30 mm in comparison with open

Pollination treatment, respectively. In 'Jamal' pollinated with 'Z_{60}', shell thickness decreased 0.18 mm compared to open pollination treatment, and the difference was significant. 'Chandler' and 'Jamal' pollinated with 'Z_{60}' significantly produced lower shell weight than other pollen sources (Table 2). Pollen parents 'Z_{60}', 'Damavand' and 'Serr' reduced shell weight by 1.08, 1.01 and 0.63 g, respectively (Table 2). In 'Jamal' genotype, pollen of 'Damavand' significantly reduced shell weight (0.72 g) more than 'Z_{60}' (0.38 g) and 'Serr' (0.26 g) pollen sources.

Table 2. Effect of pollen sources on nut and kernel characteristics of different walnut cultivars.

Female Cultivar	Cultivar used as Pollinizer				
	trait	'Serr'♂	'Z_{60}'♂	'Damavand'♂	Open pollinated
'Jamal' ♀	Nut length (mm)	40.52 ± 0.19	40.13 ± 0.30	40.55 ± 0.33	40.52 ± 0.63
	Nut diameter (mm)	28.80 ± 0.13	28.15 ± 0.12	29.06 ± 0.17	29.12 ± 0. 27
	Nut weight (g)	10.60 ± 0.18	10.07 ± 0.05	10.74 ± 0.24	11.27 ± 0.24
	Kernel length (mm)	31.90 ± 0.26	31.44 ± 0.24	31.23 ± 0.33	31.89 ± 0.21
	Kernel diameter (mm)	19.79 ± 0.23	19.77 ± 0.26	19.41 ± 0.30	20.25 0.26
	Kernel weight (g)	5.26 ± 0.14	5.16 ± 0.11	5.25 ± 0.16	5.44 ± 0.13
	Shell thickness (mm)	1.49 ± 0.01	1.43 ± 0.02	1.52 ± 0.03	1.61 ± 0.02
	Shell weight (g)	5.28 ± 0.07	5.44 ± 0.10	5.10 ± 0.12	5.56 ± 0.14
	Kernel percentage (%)	49.37 ± 0.59	51.28 ± 0.25	48.45 ± 0.61	48.36 ± 0.40
'Chandler' ♀	Nut length (mm)	34.98 ± 0.17	33.27 ± 0.15	34.55 ± 0.23	36.32 ± 0.18
	Nut diameter (mm)	30.83 ± 0.19	35.66 ± 0.11	30.21 ± 0.22	32.66 ± 0.17
	Nut weight (g)	9.33 ± 0.13	8.62 ± 0.22	8.71 ± 0.19	10.60 ± 0.17
	Kernel length (mm)	20.70 ± 0.26	27.00 ± 0.13	22.81 ± 1.91	28.67 ± 0.24
	Kernel diameter (mm)	19.61 ± 0.06	19.43 ± 0.14	18.91 ± 0.12	19.82 ± 0.15
	Kernel weight (g)	4.76 ± 0.06	4.55 ± 0.05	4.44 ± 0.10	5.71 ± 0.07
	Shell thickness (mm)	1.38 ± 0.01	1.33 ± 0.02	1.36 ± 0.03	1.60 ± 0.03
	Shell weight (g)	5.32 ± 0.08	4.69 ± 0.07	4.24 ± 0.09	4.31 ± 0.09
	Kernel percentage (%)	50.75 ± 0.33	51.86 ± 0.33	49.54 ± 0.31	48.66 ± 0.64
'Hartley' ♀	Nut Length (mm)	36.97 ± 0.35	36.05 ± 0.16	36.03 ± 0.25	37.29 ± 0.14
	Nut Diameter (mm)	31.65 ± 0.21	30.23 ± 0.13	30.37 ± 0. 18	30.68 0.18
	Nut Weight (g)	9.87 ± 0.14	9.72 ± 0.14	9.31 ± 0.13	9.92 ± 0.11
	Kernel Length (mm)	28.68 ± 0.20	27.76 ± 0.34	27.68 ± 0.29	29.07 ± 0.24
	Kernel Diameter (mm)	19.72 ± 0.12	19.09 ± 0.14	19.90 ± 0.12	20.85 ± 0.34
	Kernel Weight (g)	4.82 ± 0.06	4.21 ± 0.12	4.52 ± 0.07	4.82 ± 0.08

Table 2. Continued

	Shell Thickness (mm)	1.43 ± 0.02	1.33 ± 0.01	1.35 ± 0.02	1.39 ± 0.02
	Shell Weight (g)	5.11 ± 0.08	4.76 ± 0.14	4.90 ± 0.11	5.20 ± 0.07
	Kernel Percentage (%)	48.50 ± 0.42	48.01 ± 1.10	48.58 ± 0.61	48.13 ± 0.44
Pedro ♀	Nut Length (mm)	37.43 ± 0.33	37.04 ± 0.36	37.29 ± 0.21	38.03 ± 0.43
	Nut Diameter (mm)	33.58 ± 0.28	32.78 ± 0.16	32.43 ± 0.16	31.99 ± 0.11
	Nut Weight (g)	11.08 ± 0.27	10.85 ± 0.08	9.37 ± 0.13	8.99 ± 0.09
	Kernel Length (mm)	38.03 ± 0.31	37.43 ± 0.32	37.04 ± 0.19	37.29 ± 0.30
	Kernel Diameter (mm)	33.58 ± 0.76	33.78 ± 0.14	32.43 ± 0.12	31.99 ± 0.17
	Kernel Weight (g)	5.12 ± 0.20	5.26 ± 0.05	4.21 ± 0.10	4.39 ± 0.12
	Shell Thickness (mm)	1.56 ± 0.03	1.51 ± 0.02	1.49 ± 0.01	1.49 ± 0.02
	Shell Weight (g)	5.95 ± 0.14	5.59 ± 0.12	5.35 ± 0.04	5.18 ± 0.04
	Kernel Percentage (%)	48.21 ± 0.89	43.53 ± 0.63	43.48 ± 0.92	45.89 ± 0.49

Effect of pollen on chemical composition

In 'Pedro', pollen of 'Damavand' significantly increased the protein content of the kernel (Fig. 1). Pollen parents of 'Serr', 'Damavand' and 'Z_{60}' had no significant fect on the kernel protein content of 'Chandler', 'Hartley' and 'Jamal' (Fig. 1).

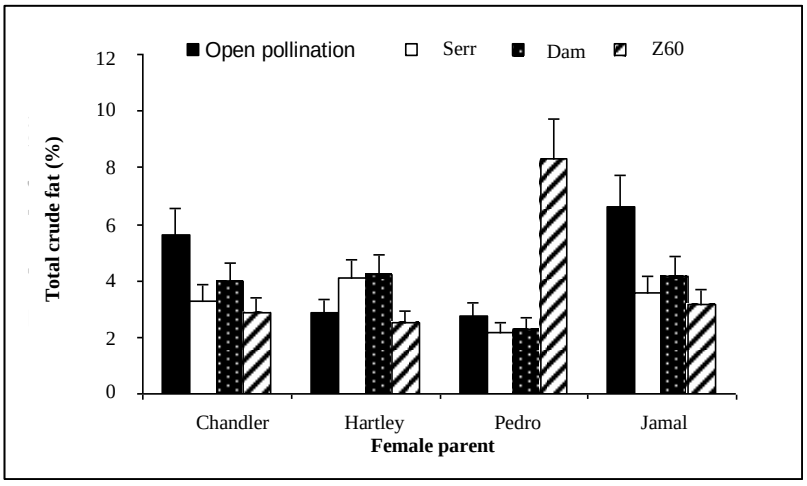

Fig.1. Effect of pollen source on kernel oil content of walnut genotypes.

Oil content was significantly affected by type of pollen (Fig. 2). In 'Hartley', pollen of 'Z_{60}' and 'Serr' reduced the oil content of the kernel (Fig. 2). Pollen of 'Z_{60}' and 'Serr' compared with open pollination treatment reduced the kernel oil content by 6.25% and 6.02%, respectively. No effect was observed on kernel oil content of the other cultivars or genotypes.

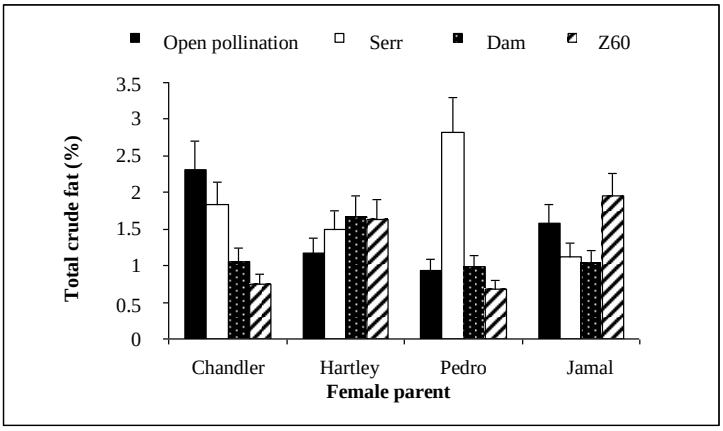

Fig. 2. Effect of pollen sources on kernel protein content.

Discussion

'Serr' pollen reduced fruit set more than other the pollinators. The result was in agreement with McGranahan *et al*. (1994), who reported 'Serr' pollen at high and low pollen load resulted in more pistillate flower abscission (PFA) than 'Tehama'. It has also been found that fruit set of artificially pollinated flowers sets were always lower than open pollinated flowers. It was reported that when 'Serr' pollen was applied to unbagged flowers of 'Serr' and other Persian walnut cultivars, pistillate flower abscission was significantly higher than open pollinated flowers (McGranahan *et al*., 1994).

The effect of pollen type on nut and kernel characteristics was in agreement with results previously reported in pistachio (Crane and Iwakiri, 1980), hazelnut (Mehlenbacher and Smith, 1993; Javadi and Gheshlaghi, 2006) and date (Osman *et al*., 1974).

In macadamia, mean fruit yield from mixed orchards of two or more cultivars was reported to be 14% heavier than mono-cultivar orchards (Ito and Hamilton, 1980). Riazi *et al*., (1996) reported that open pollination produced heavier nuts and kernel weights on three pistachio cultivars. In almond, open pollination produced heavier nuts and kernels compared to self-pollination treatments (Vargas *et al*., 2005; Kodad and Socias, 2008). Bahmani *et al*., (2003) reported that pollination of almond with pollen of the cultivars with higher kernel percentage significantly increased kernel percentage.

The effect of pollen on kernel protein and oil content in walnut has not been previously studied. However, in species such as corn, higher protein content has been reported in seeds after self-pollination compared to cross pollination (East and Jones, 1920). In almond, self-pollination decreased the oil content and percentage of linolenic acid but increased the percentage of oleic acid in almond (Kodad and Socias, 2008).

In conclusion, the effects of pollen source on nut and kernel characteristics were known in several nut crops. In walnut, our results confirmed the effect of pollen on the physical characteristics of the nut and kernel, while further research could clarify the effect of pollen on the chemical composition of the nut.

Acknowledgements

The authors acknowledge the University of Tehran, and Seed and Plant Improvement Institute (SPII) for financial support of this research.

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