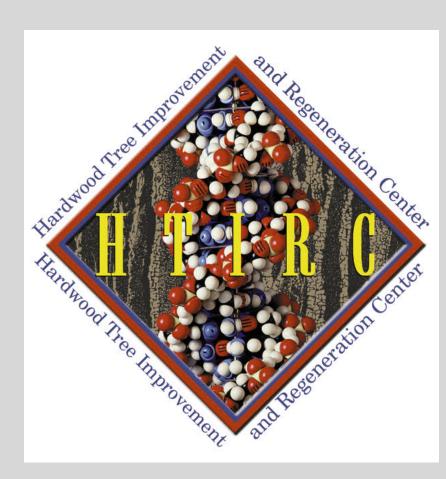


Genetic Diversity of Persian Walnut from the Midwestern USA Determined by Microsatellite Markers

Aziz Ebrahimi^{*}, James McKenna, and Keith Woeste

USDA-Forest Service, Department of Forestry and natural Resources, Purdue University



Abstract

Midwestern U.S. nutgrowers desire locally adapted Persian walnut (Juglans regia L.) varieties as a source of valuable edible nuts and for high-value timber production. Despite a century of effort, however, few healthy and productive Persian walnut cultivars or reliable seed sources have been identified in the Midwestern USA. Common problems with Persian walnut in the Midwest include low cold-hardiness, spring frost damage, and poor growth. The genetic diversity of Midwestern Persian walnut remains to be characterized. We used 14 microsatellite (SSR) markers developed for black walnut (J. nigra L.) to evaluate 48 Persian walnut genotypes and cultivars from naturalized seedlings and cultivars grown in Indiana. Many of the evaluated genotypes were previously screened for cold-hardiness. Genotypes were derived from three different geographic regions: Indiana and the Midwest, California, and Europe. All of the markers were polymorphic and revealed a total of 70 alleles ranging from 4 to 8 alleles per locus with an average of 5. Using this information, dissimilarities and similarities between regions and genotypes were estimated. Midwestern and California cultivars formed two distinct Our analysis revealed links between the groups. Midwestern USA and European walnut genotypes. Midwestern genotypes were closely related amongst themselves. We concluded the genetic diversity of Indiana Persian walnut germplasm can be improved by the additional of new germplasm to develop better adapted Persian walnut stock for the Midwestern USA environment.

Material and Methods

Genomic DNA extraction, PCR amplification and PCR product analysis

- DNA was extracted from the phloem of one year old twigs using CTAB buffer.
- Pairs (14) of SSR primers first described by Dangl et al. (2005) were used for PCR.

Results and Discussion

Cluster Analysis:

Walnut genotypes clustered according to their geographic origin (Fig. 1). Two general groups emerged:

1) The Mediterranean cultivars of California and France sorted together.

1) A cluster of genotypes from the U.S. and Europe constituted

Conclusion

The genetic diversity of Persian walnut in Indiana and the Midwestern USA was comparable to that of Eastern Europe where it originated. Persian walnut accessions from Indiana separated into two distinct groups based similarity analysis of 14 SSRs. Based on available evidence, we cannot say if the two groups

Keywords: Persian Walnut; Cold hardiness; Breeding; Genetic diversity; SSR Markers; *Juglans regia*; *Juglans* species and hybrids.

Introduction

Persian walnut (J. regia) is not well adapted to the eastern U.S. because of its cold winter and generally wet and humid climate. The effort to identify germplasm of Persian walnut suitable for the eastern U.S began when German immigrants brought Persian walnut to Pennsylvania and other States in the 1700s (Devitt, 1953; Grimo, 1979). In the late 1920s, a Presbyterian missionary in eastern Carpathia (modern eastern Europe), Reverend Paul C. Crath, searched out the best selections and shipped large quantities of nuts to Canada and United States. Methods of germplasm evaluation based on morphology often do not provide enough information to evaluate genetic diversity. Thus, DNA markers such as SSR marker are used (Pollegioni et al., 2012; Ebrahimi et al., 2011). The aim of this study was to evaluate genetic relationships among Persian walnut genotypes growing in Indiana, USA, utilizing established SSR markers. In addition, we present data on the relative growth and cold hardiness of some of these accessions in comparison to native Juglans and a set of F1 hybrids comprised of native cold hardy \times exotic non-cold hardy species.

• Gene mapper software version 3.7 and NTsys 2.2 (Rohlf, 2000) were used to analyze the output.

Evaluation of Cold Hardiness - Winter Kill

The winter of 2013-14 was unusually cold in Indiana. Record lows were recorded two consecutive days in early February. (-20°F, -29 °C).

Winter kill damage was assessed using a 5 point rating scale: 1) No damage 2) slight damage (5-20%) 3) Moderate damage (20-50%) 4) Heavy damage (50-90%) 5) Tree killed (90-100%).

Table 1. Characteristics of walnuts evaluated for winter injury.

Fernette 12 1.5 5.0.21 Grift J.rogio France Laszenne 11 1.7 4.8.21 Grift J.rogio France Idbb 12 1.3 3.0.21 Grift J.rogio M # 69 11 2.9 2.0.21 Grift J.rogio M # 694 11 3.0 2.0.21 Grift J.rogio M # 694 11 1.9 3.0.11 Sdig. J.rogio M # 694 11 1.9 3.0.11 Sdig. J.rogio M # 602-695-0 11 1.5 2.0.21 Sdig. J.rogio M # 603-695-0 11 1.9 2.0.11 Grift J.rogio M # 603-695-0 10 1.7 2.0.11 Grift J.rogio M # 600 1.1 2.0.21 Grift J.rogio M # 600 2.0 2.0.11 Sdig. J.rogi	Cultivar / Genotype	Age ^z	DBH (in) ^y	Avg. Winter Kill ^x	Stock Type	Species	Origin
Instruct Interime Interim Interime Interime	Franquette	12	1.5	5.0 ±1		J.regia	France
Index Index <th< td=""><td>Fernette</td><td>12</td><td>1.5</td><td>5.0 ±1</td><td>Graft</td><td>J.regia</td><td>Franc</td></th<>	Fernette	12	1.5	5.0 ±1	Graft	J.regia	Franc
Junio In John John <thj< td=""><td>Lozeronne</td><td>11</td><td>1.7</td><td>4.8 ±1</td><td>Graft</td><td>J.regia</td><td>Franc</td></thj<>	Lozeronne	11	1.7	4.8 ±1	Graft	J.regia	Franc
mage 11 2.3 2.011 Graft i.regia III # 695 11 2.3 2.011 Graft j.regia IN # 695 11 2.5 2.011 Graft j.regia IN # 695 11 2.5 2.011 Solg. j.regia IN # 033 649_0 11 2.5 2.011 Solg. j.regia IN # 033 649_0 11 1.5 2.911 Solg. j.regia N # 033 649_0 11 1.5 2.911 Solg. j.regia N # 034 649_0 10 1.4 2.311 Graft j.regia N Summer's Solg 10 1.4 2.311 Graft j.regia N Meridian St. 40 16 2.011 Solg. j.regia N Meridian St. 40 15 2.511 Solg. j.regia N Merifarm - N. 50 20	Idaho	12	1.9	3.0 ±1	Graft	J.regia	ID
$s \ organ beta beta beta beta beta beta beta beta$		11	3	2.5 ±1	Graft		IN
# 694 11 3 2.0 ±1 Graft J.regia M.N. # 695 11 2.6 2.0 ±1 Graft J.regia M.N. # 695 11 2.0 ±1 Solg. J.regia M.N. # 693 5.11 2.0 ±1 Solg. J.regia M.N. # 693 5.11 1.5 2.9 ±1 Solg. J.regia N.N. # 693 6559-4 11 1.5 2.9 ±1 Solg. J.regia N.N. # 693 6559-4 11 1.5 2.9 ±1 Solg. J.regia N.N. # 693 6 1.2 2.1 ±1 Graft J.regia M.N. Hansen Sdig 6 1.2 2.1 ±1 Solg. J.regia M.N. Meridan St. 40 1.6 2.0 ±1 Solg. J.regia M.N. Meridan St. 40 1.6 2.0 ±1 Solg. J.regia M.N. Meridan St. 40 1.			2.9		Graft		
# 695 11 2.6 2.0 ±1 Graft J.regin NN #03-639-1 11 2 3.0 ±1 Solg. J.regin NN #03-639-2 11 2.8 2.0 ±1 Solg. J.regin NN #03-639-4 11 1.5 2.9 ±1 Solg. J.regin NN #03-639-4 11 1.5 2.9 ±1 Solg. J.regin NN #03-639-4 11 1.5 2.9 ±1 Solg. J.regin NN #03-639-4 10 1.4 2.3 ±1 Graft J.regin NN Montan 8 1.2 2.1 ±1 Graft J.regin MO M02-33 8 1.2 2.1 ± Solg. J.regin MO Montan St. 40 15 2.0 ±1 Solg. J.regin NN Merian St. 40 15 2.5 ±1 Solg. J.regin NN Merian Tree 7 3.5 ±2.5 ±1					Graft	J.regia	
B03-00 International State Internate Internation					Graft	J.regia	
Index Style Index Index <thindex< th=""> Index Index</thindex<>							
International and the set of the							
International and the set of the							
International and the set of the	#03-694p-2						
Incompo Inc	#03-695p-4	11	1.5	2.9 ±1	Sdlg.		IN
Summer's sign 10 1.4 2.3 ± Graft J.regin Numer's sign	#03-695p-8	11	1.9	2.0 ±1	Sdlg.	J.regia	IN
Summer's Sdig 10 1.4 2.3 ±1 Graft Lregia Initial Hannen Sdig 8 1.3 2.2 ±1 Sdig. Lregia Reside Uale 8 1.3 2.2 ±1 Sdig. Laregia MO Behr 60 22 2.2 ±1 Sdig. Lregia MO Behr 60 22 2.0 ±1 Sdig. Lregia N SW Hort Farm – N. 50 20 2.0 ±1 Sdig. Lregia N Univ. Farms 28 13 2.0 ±1 Sdig. Lregia N Uapz Tree 75 34 2.0 ±1 Sdig. Lregia N Besque-2 15 7 2.0 ±1 Sdig. Lregia N King-1 25 10 2.5 ±1 Sdig. Lregia N King-2 25 13 2.5 ±1 Sdig. Lregia N King-4 25 10 2.0 ±1	Κοςογο	10	1.7	2.0 +1	Graft	J.regia	Serbi
Jones Jog B L2 L11 Graft Jregia In #02-3-3 8 1.3 2.5 ±1 Selig. Jregia Ressi Lake 8 1 1.9 ±1 Graft Jregia MO Behr 60 22 2.2 ±1 Selig. Jregia MN Merdian St. 40 16 2.0 ±1 Selig. Jregia NN SW Hort Farm – N. 50 20 2.0 ±1 Selig. Jregia NN Yeager Road 40 18 2.2 ±1 Selig. Jregia NN Univ Farm 7 3.44 2.0 ±1 Selig. Jregia NN Besque-2 15 7 2.0 ±1 Selig. Jregia NN King-1 25 11 2.5 ±1 Selig. Jregia NN King-2 25 11 2.5 ±1 Selig. Jregia NN King-1 25 10 2.0 ±1							
International of the second							
Lake 8 1 1.9.1 Graft J.regia Mon Behr 60 22 2.2.21 Solg. J.regia N Meridian St. 40 16 2.0.11 Solg. J.regia N SW Hort Farm - N. 50 20 2.0.11 Solg. J.regia N Timber Type 40 15 2.5.11 Graft J.regia N Univ. Farm 28 13 2.0.11 Solg. J.regia N Besque-1 15 7 1.5.11 Solg. J.regia N Besque-2 15 7 2.0.21 Solg. J.regia N Besque-3 40 25 2.0.21 Solg. J.regia N King-2 25 11 2.5.21 Solg. J.regia N King-3 25 13 2.5.21 Solg. J.regia N Obsen tree -1 45 19 2.2.2.1							
Behr60222.2 ±1Sdig.J.regiaINMeridian St.40162.0 ±1Sdig.J.regiaNSW Hort Farm – N.50202.0 ±1Sdig.J.regiaNTimber Type40152.5 ±1GraftJ.regiaNUniv. Farms28132.0 ±1Sdig.J.regiaNVager Road40182.2 ±1Sdig.J.regiaNBesque-11571.5 ±1Sdig.J.regiaNBesque-21572.0 ±1Sdig.J.regiaNBesque-21572.0 ±1Sdig.J.regiaNBesque-21572.0 ±1Sdig.J.regiaNNeger 340252.0 ±1Sdig.J.regiaNNing 125102.5 ±1Sdig.J.regiaNKing 225112.5 ±1Sdig.J.regiaNNing 425102.0 ±1Sdig.J.regiaNOlsen tree -145222.0 ±1Sdig.J.regiaNNing 425102.0 ±1Sdig.J.regiaNNing 425102.0 ±1Sdig.J.regiaNNing 425102.0 ±1Sdig.J.regiaNNing 425102.0 ±1Sdig.J.regiaNNing 41562.0 ±1Sdig.J.re	#02-3-3	8	1.3				
Merkilan St.40152.0 ±1Sdig.LægiaInSW Hort Farm – N.502.0 ±1Sdig.LægiaInTimber Type40152.5 ±1GraftLægiaInUhk Farms28132.0 ±1Sdig.LægiaLægiaMNVæger God40182.2 ±1Sdig.LægiaMRMRUhger Fade75342.0 ±1Sdig.LægiaMRMRBesque-11571.5 ±1Sdig.LægiaMRMRBesque-21572.0 ±1Sdig.LægiaMNBesque-3402.52.0 ±1Sdig.LægiaMNKing-125102.5 ±1Sdig.LægiaMNKing-225102.5 ±1Sdig.LægiaMNKing-325102.0 ±1Sdig.LægiaMNOlsen tree-1452.0 ±1Sdig.LægiaMNOlsen tree-1452.0 ±1Sdig.LægiaMNOlsen tree-1452.0 ±1Sdig.LægiaMNOlsen tree-1452.0 ±1Sdig.LægiaMNOlsen tree-1452.0 ±1Sdig.LægiaMNOran Barton-21562.0 ±1Sdig.LægiaMNOran Barton-31572.0 ±1GraftLægiaMNAsier Persian1562.0 ±1Graft <t< td=""><td>Lake</td><td>8</td><td>1</td><td>1.9 ±1</td><td>Graft</td><td>J.regia</td><td>MO</td></t<>	Lake	8	1	1.9 ±1	Graft	J.regia	MO
SW Hort Farm N 50 20 2.0 ±1 Sdig. j.regia IN Timber Type 40 15 2.5 ±1 Graft j.regia IN Univ. Farms 28 13 2.0 ±1 Sdig. j.regia IN Univ. Farms 28 13 2.0 ±1 Sdig. j.regia IN Lapaz Tree 75 34 2.0 ±1 Sdig. j.regia IN Besque-1 15 7 2.0 ±1 Sdig. j.regia IN Besque-2 15 7 2.0 ±1 Sdig. j.regia IN King-1 25 10 2.5 ±1 Sdig. j.regia IN King-3 25 13 2.5 ±1 Sdig. j.regia IN King-4 25 10 2.0 ±1 Sdig. j.regia IN Olsen tree-1 45 19 2.2 ±1 Sdig. j.regia IN Obsen tree-2 45	Behr	60	22	2.2 ±1	Sdlg.	J.regia	IN
Timber Type 40 15 2.5 ±1 Graft Linegia IN Venk Farms 28 13 2.0 ±1 Sdlg. J.regia NN Venger Road 40 18 2.0 ±1 Sdlg. J.regia N Lapaz Tree 75 34 2.0 ±1 Sdlg. J.regia N Besque-1 15 7 2.0 ±1 Sdlg. J.regia N Mesque-2 15 7 2.0 ±1 Sdlg. J.regia N King-1 25 10 2.5 ±1 Sdlg. J.regia N King-3 25 13 2.5 ±1 Sdlg. J.regia N Ning-4 25 10 2.0 ±1 Sdlg. J.regia N Olsen tree-2 45 21 2.5 ±1 Sdlg. J.regia N Olsen tree-3 45 2 2.0 ±1 Sdlg. J.regia N Oran Barton-1 95 36 2.0	Meridian St.	40	16	2.0 ±1	Sdlg.	J.regia	IN
Timber Type 40 15 2.5 ±1 Graft Linegia IN Venk Farms 28 13 2.0 ±1 Sdlg. J.regia NN Venger Road 40 18 2.0 ±1 Sdlg. J.regia N Lapaz Tree 75 34 2.0 ±1 Sdlg. J.regia N Besque-1 15 7 2.0 ±1 Sdlg. J.regia N Mesque-2 15 7 2.0 ±1 Sdlg. J.regia N King-1 25 10 2.5 ±1 Sdlg. J.regia N King-3 25 13 2.5 ±1 Sdlg. J.regia N Ning-4 25 10 2.0 ±1 Sdlg. J.regia N Olsen tree-2 45 21 2.5 ±1 Sdlg. J.regia N Olsen tree-3 45 2 2.0 ±1 Sdlg. J.regia N Oran Barton-1 95 36 2.0	SW/ Hort Form - N	50	20	2.0.+1	Sdla	l regia	IN
Univ. Farms 28 13 2.0 ±1 Sdig. J.regia IN Veager Road 40 18 2.2 ±1 Sdig. J.regia IN Lapaz Tree 75 34 2.0 ±1 Sdig. J.regia IN Besque-1 15 7 2.0 ±1 Sdig. J.regia IN Besque-2 15 7 2.0 ±1 Sdig. J.regia IN Resque-3 40 25 2.0 ±1 Sdig. J.regia IN King-1 25 10 2.5 ±1 Sdig. J.regia IN King-3 25 13 2.5 ±1 Sdig. J.regia IN Olsen tree-2 45 21 2.5 ±1 Sdig. J.regia IN Olsen tree-2 45 2.2 2.0 ±1 Sdig. J.regia IN Ovan Barton-2 15 6 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7							
Own Name Jos Jo							
Trongen House To To <thto< th=""> To To</thto<>							
Besque-1 15 7 1.5 ±1 Sdig. J.regia IN Besque-2 15 7 2.0 ±1 Sdig. J.regia IN Besque-3 40 25 2.0 ±1 Sdig. J.regia IN King-1 25 10 2.5 ±1 Sdig. J.regia IN King-2 25 11 2.5 ±1 Sdig. J.regia IN King-4 25 10 2.0 ±1 Sdig. J.regia IN Olsen tree-1 45 19 2.2 ±1 Sdig. J.regia IN Olsen tree-2 45 2.2 2.0 ±1 Sdig. J.regia IN Olsen tree-2 45 2.2 2.0 ±1 Sdig. J.regia IN Oran Barton-1 95 36 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Caraft treresian 15 8	Yeager Road						
Besque-2 15 7 2.0 ±1 Sdig. L.regia IN Besque-3 40 25 2.0 ±1 Sdig. J.regia IN King-1 25 10 2.5 ±1 Sdig. J.regia IN King-2 25 11 2.5 ±1 Sdig. J.regia IN King-3 25 13 2.5 ±1 Sdig. J.regia IN King-4 25 10 2.0 ±1 Sdig. J.regia IN Obsen tree-1 45 19 2.2 ±1 Sdig. J.regia IN ID0 wtree 45 22 2.0 ±1 Sdig. J.regia IN Oran Barton-1 95 36 2.0 ±1 Sdig. J.regia IN Oran Barton-2 15 6 2.0 ±1 Graft J.regia IN Kaiser Persian 15 8 2.0 ±1 Graft J.regia IN Kaiser Persian 15 6	Lapaz Tree	75	34	2.0 ±1	Sdlg.	J.regia	IN
Besque-3 40 25 2.0 ±1 Sdlg. J.regia IN King-1 25 10 2.5 ±1 Sdlg. J.regia IN King-2 25 11 2.5 ±1 Sdlg. J.regia IN King-4 25 10 2.0 ±1 Sdlg. J.regia IN Olsen tree-1 45 19 2.2 ±1 Sdlg. J.regia IN Olsen tree-2 45 2.1 2.5 ±1 Sdlg. J.regia IN Ubgert tree 25 10 2.0 ±1 Sdlg. J.regia IN Oran Barton-1 95 36 2.0 ±1 Sdlg. J.regia IN Oran Barton-2 15 6 2.0 ±1 Graft J.regia IN Cara Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 6 2.0 ±1 Graft J.regia IN Kaiser Persian 15	Besque-1	15	7	1.5 ±1	Sdlg.	J.regia	IN
King-1 25 10 2.5 ±1 Sdlg. J.regia IN King-2 25 11 2.5 ±1 Sdlg. J.regia IN King-3 25 13 2.5 ±1 Sdlg. J.regia IN King-4 25 10 2.0 ±1 Sdlg. J.regia IN Olsen tree-1 45 19 2.2 ±1 Sdlg. J.regia IN Olsen tree-2 45 21 2.5 ±1 Sdlg. J.regia IN Olsen tree-2 45 21 2.5 ±1 Sdlg. J.regia IN Olsen tree-2 45 22 2.0 ±1 Sdlg. J.regia IN Oran Barton-1 95 36 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 6 2.0 ±1 Graft J.regia × J. hindsii CA Royal 11 <t< td=""><td>Besque-2</td><td>15</td><td>7</td><td>2.0 ±1</td><td>Sdlg.</td><td>J.regia</td><td>IN</td></t<>	Besque-2	15	7	2.0 ±1	Sdlg.	J.regia	IN
King-2 25 11 2.5 ±1 Sdlg. J.regia IN King-3 25 13 2.5 ±1 Sdlg. J.regia IN King-4 25 10 2.0 ±1 Sdlg. J.regia IN Olsen tree-1 45 19 2.2 ±1 Sdlg. J.regia IN Olsen tree-2 45 21 2.5 ±1 Sdlg. J.regia IN Lhuppert tree 25 10 2.0 ±1 Sdlg. J.regia IN 100 wtree 45 22 2.0 ±1 Sdlg. J.regia IN Oran Barton-1 95 36 2.0 ±1 Graft J.regia IN Oran Barton-1 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 8 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia × J. hindsii CA Royal 11	Besque-3	40	25	2.0 ±1	Sdlg.	J.regia	IN
King-3 25 13 2.5 ±1 Sdig. J.regia IN King-4 25 10 2.0 ±1 Sdig. J.regia IN Olsen tree-1 45 19 2.2 ±1 Sdig. J.regia IN Olsen tree-2 45 21 2.5 ±1 Sdig. J.regia IN Lhuppert tree 25 10 2.0 ±1 Sdig. J.regia IN 100 w tree 45 22 2.0 ±1 Sdig. J.regia IN Oran Barton-1 95 36 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 6 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Hybrids / Species Age DBH (in) WK Type Species Origi Royal 11	King-1	25	10	2.5 ±1	Sdlg.	J.regia	IN
King-3 25 13 2.5 ±1 Sdig. J.regia IN King-4 25 10 2.0 ±1 Sdig. J.regia IN Olsen tree-1 45 19 2.2 ±1 Sdig. J.regia IN Olsen tree-2 45 21 2.5 ±1 Sdig. J.regia IN Lhuppert tree 25 10 2.0 ±1 Sdig. J.regia IN 100 w tree 45 22 2.0 ±1 Sdig. J.regia IN Oran Barton-1 95 36 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 6 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Hybrids / Species Age DBH (in) WK Type Species Origi Royal 11	King-2	25	11	2.5 ±1	Sdlg.	J.regia	IN
King-4 25 10 2.0 ±1 Sdig. J.regia IN Olsen tree-1 45 19 2.2 ±1 Sdig. J.regia IN Olsen tree-2 45 21 2.5 ±1 Sdig. J.regia IN Lhuppert tree 25 10 2.0 ±1 Sdig. J.regia IN 100 w tree 45 22 2.0 ±1 Sdig. J.regia IN Oran Barton-2 15 6 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 8 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Buart 11 3.9 1.0 ±1 Grafts J. cinerea × J. ailantifolia IN Persian Backcross F1 </td <td></td> <td>25</td> <td>13</td> <td></td> <td></td> <td></td> <td>IN</td>		25	13				IN
Olsen tree-1 45 19 2.2 ±1 Sdg. J.regia IN Olsen tree-2 45 21 2.5 ±1 Sdig. J.regia IN Lhuppert tree 25 10 2.0 ±1 Sdig. J.regia IN 100 w tree 45 22 2.0 ±1 Sdig. J.regia IN Oran Barton-1 95 36 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 6 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Hybrids / Species Age DBH (in) WK Type Species Origi Royal 11 4.1 1.0 ±1 Grafts J. cinerea × J. ailantifolia IN Persia							
Olsen tree-2 45 21 2.5 ±1 Sdlg. J.regia IN Lhuppert tree 25 10 2.0 ±1 Sdlg. J.regia IN 100 w tree 45 22 2.0 ±1 Sdlg. J.regia IN Oran Barton-1 95 36 2.0 ±1 Sdlg. J.regia IN Oran Barton-2 15 6 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 6 2.0 ±1 Graft J.regia IN Lake Hansen 15 6 2.0 ±1 Graft J.regia IN Hybrids / Species Age DBH (in) WK Type Species Origi Royal 11 4.1 1.0 ±1 Grafts J. enerea × J. ailantifolia IN Persian Backcross F, 10 3.2 2.0 ±1 Grafts J. enerea × J. ailantifolia IN							
Lhuppert tree 25 10 2.0 ±1 Sdlg. J.regia IN 100 w tree 45 22 2.0 ±1 Sdlg. J.regia IN Oran Barton-1 95 36 2.0 ±1 Sdlg. J.regia IN Oran Barton-2 15 6 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 8 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Hybrids / Species Age DBH (in) WK Type Species Origi Royal 11 4.1 1.0 ±1 Grafts J. ergia × J. hindsii CA Persian Backcross F, 10 3.2 2.0 ±1 Grafts J. ergia × J. cinerea × J. ailantifolia IN							
100 wree 45 22 2.0 ±1 Sdlg. J.regia IN Oran Barton-1 95 36 2.0 ±1 Sdlg. J.regia IN Oran Barton-2 15 6 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 8 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Hybrids / Species Age DBH (in) WK Type Species Origi Royal 11 4.1 1.0 ±1 Grafts J. nigra × J. hindsii CA Buart 11 3.2 2.0 ±1 Grafts J. regia × J. nigra IN Persian							
Oran Barton-1 95 36 2.0 ±1 Sdlg. J.regia IN Oran Barton-2 15 6 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 8 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Hybrids / Species Age DBH (in) WK Type Species Origi Royal 11 4.1 1.0 ±1 Grafts J. regia × J. hindsii CA Buart 11 3.9 1.0 ±1 Grafts J. regia × J. nigra] IN Persian Backcross F, 10 3.2 2.0 ±1 J. regia × J. cinerea IN Hybrid Butternut 11 3.1 1.0 ±1 Grafts J. regia × J. ailantifolia IN							
Oran Barton-2 15 6 2.0 ±1 Graft J.regia IN Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 8 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Hybrids / Species Age DBH (in) WK Type Species Origi Royal 11 4.1 1.0 ±1 Grafts J. rigra × J. hindsii CA Buart 11 3.9 1.0 ±1 Grafts J. rigra × J. hindsii CA Persian Backcross F1 10 3.2 2.0 ±1 J. regia × J. cinerea × J. ailantifolia IN Hybrid Butternut 9 3.1 1.0 ±1 Grafts J. regia × J. cinerea × J. ailantifolia IN Black X Japanese 10 2.7 1.0 ±1 Grafts J. ailantifolia × J. nigra IN Black Walnut 11 3.4 1.0 ±1 Grafts <td>100 w tree</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	100 w tree						
Oran Barton-3 15 7 2.0 ±1 Graft J.regia IN Kaiser Persian 15 8 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Hybrids / Species Age DBH (in) WK Type Species Orrigi Royal 11 4.1 1.0 ±1 Grafts J. nigra × J. hindsii CA Buart 11 3.9 1.0 ±1 Grafts J. cinerea × J. ailantifolia IN Persian Backcross F, 10 3.2 2.0 ±1 Grafts J. regia × J. nigra IN Persian x Butternut 9 3.1 1.0 ±1 Grafts J. cinerea × [J. cinerea × J. ailantifolia] IN Black x Japanese 10 2.7 1.0 ±1 Grafts J. regia × J. nigra IN Black Walnut 11 3.4 1.0 ±1 <	Oran Barton-1	95	36	2.0 ±1	Sdlg.	J.regia	IN
Kaiser Persian 15 8 2.0 ±1 Graft J.regia IN Lake × Hansen 15 6 2.0 ±1 Graft J.regia IN Hybrids / Species Age DBH (in) WK Type Species Origi Royal 11 4.1 1.0 ±1 Grafts J. nigra × J. hindsii CA Buart 11 3.9 1.0 ±1 Grafts J. cinerea × J. ailantifolia IN Persian Backcross F, 10 3.2 2.0 ±1 Grafts J. regia × J. nigra] IN Persian x Butternut 9 3.1 1.0 ±1 Grafts J. regia × J. nigra] IN Black x Japanese 10 2.7 1.0 ±1 Grafts J. regia × J. nigra IN Black X Versian 9 1.1 1.0 ±1 Grafts J. regia × J. nigra IN Black Walnut 11 3.4 1.0 ±1 Grafts J. nigra USA Arizona 10 2.8 1.0 ±1 Graft	Oran Barton-2	15	6	2.0 ±1	Graft	J.regia	IN
Lake × Hansen1562.0 ±1GraftJ.regiaINHybrids / SpeciesAgeDBH (in)WKTypeSpeciesOrigiRoyal114.11.0 ±1GraftsJ. nigra × J. hindsiiCABuart113.91.0 ±1GraftsJ. cinerea × J. ailantifoliaINPersian Backcross F,103.22.0 ±1J. regia × J. lingraJ. rigra × J. hindsiiCAPersian x Butternut93.11.0 ±1GraftsJ. regia × J. nigra]INBlack x Japanese102.71.0 ±1GraftsJ. cinerea × J. ailantifolia × J. nigraINBlack x Valpanese102.71.0 ±1GraftsJ. ailantifolia × J. nigraINBlack walnut113.41.0 ±1GraftsJ. regia × J. nigraINBlack walnut102.81.0 ±1GraftsJ. nigraUSAArizona102.24.8 ±1Graft / SdlgsJ. majorAZPersian101.83.1 ±1Graft / SdlgsJ. regiaJ. regiaIn	Oran Barton-3	15	7	2.0 ±1	Graft	J.regia	IN
Hybrids / SpeciesAgeDBH (in)WKTypeSpeciesOrigiRoyal114.11.0 ±1GraftsJ. nigra × J. hindsiiCABuart113.91.0 ±1GraftsJ. nigra × J. hindsiiCABuart113.91.0 ±1GraftsJ. cinerea × J. ailantifoliaINPersian Backcross F1103.22.0 ±1J. regia × J. regia × J. nigra]INPersian x Butternut93.11.0 ±1GraftsJ. regia × J. cinereaINBlack x Japanese102.71.0 ±1GraftsJ. cinerea × J. ailantifolia × J. nigraINBlack x Valpanese102.71.0 ±1GraftsJ. regia × J. nigraINBlack Walnut113.41.0 ±1GraftsJ. nigraUSABlack Walnut113.41.0 ±1GraftsJ. nigraUSABlack Walnut102.81.0 ±1GraftsJ. nigraUSABlack Tapanesi102.24.8 ±1Graft / SdlgsJ. majorAZPersian101.83.1 ±1Graft / SdlgsJ. regiaI. nigraIN, CA	Kaiser Persian	15	8	2.0 ±1	Graft	J.regia	IN
Royal114.11.0 ±1GraftsJ. nigra × J. hindsiiCABuart113.91.0 ±1GraftsJ. cinerea × J. ailantifoliaINPersian Backcross F1103.22.0 ±1GraftsJ. regia × [J. regia × J. nigra]INPersian x Butternut93.11.0 ±1GraftsJ. regia × J. cinereaINPersian x Butternut93.11.0 ±1J. regia × J. cinereaINBlack x Japanese102.71.0 ±1GraftsJ. cinerea × J. ailantifoliaINBlack x Persian91.11.0 ±1GraftsJ. ailantifolia × J. nigraINBlack Walnut113.41.0 ±1GraftsJ. regia × J. nigraINBlack Walnut102.81.0 ±1GraftsJ. nigraUSAArizona102.24.8 ±1Graft / SdlgsJ. majorAZPersian101.83.1 ±1Graft / SdlgsJ. regiaI. nigraIN, C/	Lake × Hansen	15	6	2.0 ±1	Graft	J.regia	IN
Royal114.11.0 ±1GraftsJ. nigra × J. hindsiiCABuart113.91.0 ±1GraftsJ. cinerea × J. ailantifoliaINPersian Backcross F1103.22.0 ±1GraftsJ. regia × [J. regia × J. nigra]INPersian x Butternut93.11.0 ±1GraftsJ. regia × J. cinereaINPersian x Butternut93.11.0 ±1J. regia × J. cinereaINBlack x Japanese102.71.0 ±1GraftsJ. cinerea × J. ailantifoliaINBlack x Persian91.11.0 ±1GraftsJ. ailantifolia × J. nigraINBlack Walnut113.41.0 ±1GraftsJ. regia × J. nigraINBlack Walnut102.81.0 ±1GraftsJ. nigraUSAArizona102.24.8 ±1Graft / SdlgsJ. majorAZPersian101.83.1 ±1Graft / SdlgsJ. regiaI. nigraIN, C/		A			T	Current and	0
Buart113.9 1.0 ± 1 GraftsJ. cinerea × J. ailantifoliaINPersian Backcross F110 3.2 2.0 ± 1 GraftsJ. regia × J. nigra]INPersian Backcross F110 3.2 2.0 ± 1 GraftsJ. regia × J. nigra]INPersian x Butternut9 3.1 1.0 ± 1 GraftsJ. regia × J. cinereaINPersian x Butternut9 3.1 1.0 ± 1 GraftsJ. cinerea × J. ailantifolia]INHybrid Butternut11 3.1 1.0 ± 1 GraftsJ. cinerea × J. ailantifolia × J. nigraINBlack x Japanese10 2.7 1.0 ± 1 GraftsJ. ailantifolia × J. nigraINBlack x Persian9 1.1 1.0 ± 1 GraftsJ. regia × J. nigraINBlack Walnut11 3.4 1.0 ± 1 GraftsJ. nigraUSAButternut10 2.8 1.0 ± 1 GraftsJ. cinereaJ. nigraUSAArizona10 2.2 4.8 ± 1 Graft / SdlgsJ. majorAZPersian10 1.8 3.1 ± 1 Graft / SdlgsJ. regiaIN, C/	Hyprids / Species	Age	DBH (IN)	VVK	туре	Species	Origi
Buart113.9 1.0 ± 1 GraftsJ. cinerea × J. ailantifoliaINPersian Backcross F110 3.2 2.0 ± 1 GraftsJ. regia × J. nigra]INPersian Backcross F110 3.2 2.0 ± 1 GraftsJ. regia × J. nigra]INPersian x Butternut9 3.1 1.0 ± 1 GraftsJ. regia × J. cinereaINPersian x Butternut9 3.1 1.0 ± 1 GraftsJ. cinerea × J. ailantifolia]INHybrid Butternut11 3.1 1.0 ± 1 GraftsJ. cinerea × J. ailantifolia × J. nigraINBlack x Japanese10 2.7 1.0 ± 1 GraftsJ. ailantifolia × J. nigraINBlack x Persian9 1.1 1.0 ± 1 GraftsJ. regia × J. nigraINBlack Walnut11 3.4 1.0 ± 1 GraftsJ. nigraUSAButternut10 2.8 1.0 ± 1 GraftsJ. cinereaJ. nigraUSAArizona10 2.2 4.8 ± 1 Graft / SdlgsJ. majorAZPersian10 1.8 3.1 ± 1 Graft / SdlgsJ. regiaIN, C/	Royal	11	4 1	10+1	Grafts	I. nigra x. I. hindsii	CA.
DuartIfIfIfIfIfIfIfPersian Backcross F110 3.2 2.0 ± 1 GraftsJ. regia × [J. regia × J. nigra]INPersian x Butternut9 3.1 1.0 ± 1 GraftsJ. regia × J. cinereaINPersian x Butternut9 3.1 1.0 ± 1 GraftsJ. cinerea × [J. cinerea × J. ailantifolia]Hybrid Butternut11 3.1 1.0 ± 1 GraftsJ. cinerea × [J. cinerea × J. ailantifolia]Black x Japanese10 2.7 1.0 ± 1 GraftsJ. ailantifolia × J. nigraINBlack x Persian9 1.1 1.0 ± 1 GraftsJ. regia × J. nigraINBlack Walnut11 3.4 1.0 ± 1 GraftsJ. nigraUSAButternut10 2.8 1.0 ± 1 GraftsJ. nigraUSAArizona10 2.2 4.8 ± 1 Graft / SdlgsJ. majorAZPersian10 1.8 3.1 ± 1 Graft / SdlgsJ. regiaI. nigraIN, C/							
Persian Backcross F1103.22.0 ±1J. regia × [J. regia × J. nigra]INPersian x Butternut93.11.0 ±1GraftsJ. regia × J. cinereaINPersian x Butternut93.11.0 ±1GraftsJ. cinerea × [J. cinerea × J. ailantifolia]INHybrid Butternut113.11.0 ±1GraftsJ. cinerea × [J. cinerea × J. ailantifolia]INBlack x Japanese102.71.0 ±1GraftsJ. ailantifolia × J. nigraINBlack x Persian91.11.0 ±1GraftsJ. regia × J. nigraINBlack Walnut113.41.0 ±1GraftsJ. regia × J. nigraUSABlack Walnut113.41.0 ±1GraftsJ. nigraUSAArizona102.24.8 ±1Graft / SdlgsJ. majorAZPersian101.83.1 ±1Graft / SdlgsJ. regiaJ. regia	Buart	11	5.9	1.0 11		J. CITETER × J. ANANTITONA	IN
Persian x Butternut93.11.0 ±1GraftsJ. regia × J. cinereaINHybrid Butternut113.11.0 ±1GraftsJ. cinerea × [J. cinerea × J. ailantifolia]Black x Japanese102.71.0 ±1GraftsJ. ailantifolia × J. nigraINBlack x Persian91.11.0 ±1GraftsJ. regia × J. nigraINBlack Walnut113.41.0 ±1GraftsJ. regia × J. nigraUSABlack Walnut113.41.0 ±1GraftsJ. nigraUSAArizona102.81.0 ±1GraftsJ. nigraUSAArizona102.24.8 ±1Graft / SdlgsJ. majorAZPersian101.83.1 ±1Graft / SdlgsJ. regia × J. nigraIN	Persian Backcross F ₁	10	3.2	2.0 ±1		J. regia × [J. regia × J. nigra]	IN
Hybrid Butternut113.11.0 ±1GraftsJ. cinerea × J. ailantifolia]Black x Japanese102.71.0 ±1GraftsJ. ailantifolia × J. nigraINBlack x Persian91.11.0 ±1GraftsJ. regia × J. nigraINBlack Walnut113.41.0 ±1GraftsJ. nigraUSABlack Walnut113.41.0 ±1GraftsJ. nigraUSABlack Walnut102.81.0 ±1GraftsJ. nigraUSAArizona102.24.8 ±1Graft / SdlgsJ. majorAZPersian101.83.1 ±1Graft / SdlgsJ. regiaI. nigraIN, C/					Grafts		
Hybrid Butternut113.1 1.0 ± 1 GraftsJ. ailantifolia × J. nigraINBlack x Japanese10 2.7 1.0 ± 1 GraftsJ. ailantifolia × J. nigraINBlack x Persian9 1.1 1.0 ± 1 GraftsJ. regia × J. nigraINBlack Walnut11 3.4 1.0 ± 1 GraftsJ. nigraUSABlack Walnut10 2.8 1.0 ± 1 GraftsJ. nigraUSAArizona10 2.2 4.8 ± 1 Graft / SdlgsJ. majorAzPersian10 1.8 3.1 ± 1 Graft / SdlgsJ. regiaJ. regia	Persian x Butternut	9	3.1	1.0 ±1	Crofts	-	IN
Black x Japanese102.71.0 ±1GraftsJ. ailantifolia × J. nigraINBlack x Persian91.11.0 ±1GraftsJ. regia × J. nigraINBlack Walnut113.41.0 ±1GraftsJ. nigraUSAButternut102.81.0 ±1GraftsJ. cinereaUSAArizona102.24.8 ±1Graft / SdlgsJ. majorAZPersian101.83.1 ±1Graft / SdlgsJ. regiaIN, CA					Grants	J. Cherea × [J. Cherea × J. allantifolia]	
Diack X Sapanese10101.0					Cuefts		
Black X Persian J Int	Black x Japanese						
Butternut 10 2.8 1.0 ±1 Grafts J. cinerea USA Arizona 10 2.2 4.8 ±1 Graft / Sdlgs J. major AZ Persian 10 1.8 3.1 ±1 Graft / Sdlgs J. regia IN, C/	Black x Persian	9	1.1	1.0 ±1	Grafts	J. regia × J. nigra	IN
Butternut 10 2.8 1.0 ±1 Grafts J. cinerea USA Arizona 10 2.2 4.8 ±1 Graft / Sdlgs J. major AZ Persian 10 1.8 3.1 ±1 Graft / Sdlgs J. regia IN, C/		11	2.4	10+1	Crofts	L pique	LICA
Arizona 10 2.2 4.8 ±1 Graft / Sdlgs J. major AZ Persian 10 1.8 3.1 ±1 Graft / Sdlgs J. regia IN, C/							
Persian 10 1.8 3.1 ±1 Graft / Sdlgs J. regia IN, C/	Butternut	10	2.8	1.0 ±1	Grafts	J. cinerea	USA
	Arizona	10	2.2	4.8 ±1	Graft / Sdlgs	J. major	AZ
Japanese81.91.0 ±1GraftsJ. ailantifoliaCA	Persian	10					IN, CA
	Japanese	8	1.9	1.0 ±1	Grafts	J. ailantifolia	CA

group 2. Midwestern and Eastern European germplasm were similar, but they both differ from the California and French germplasm. In this respect, genotypes cluster reflected cold tolerance. It also appears that the Midwestern germplasm is a sample of Eastern European germplasm.

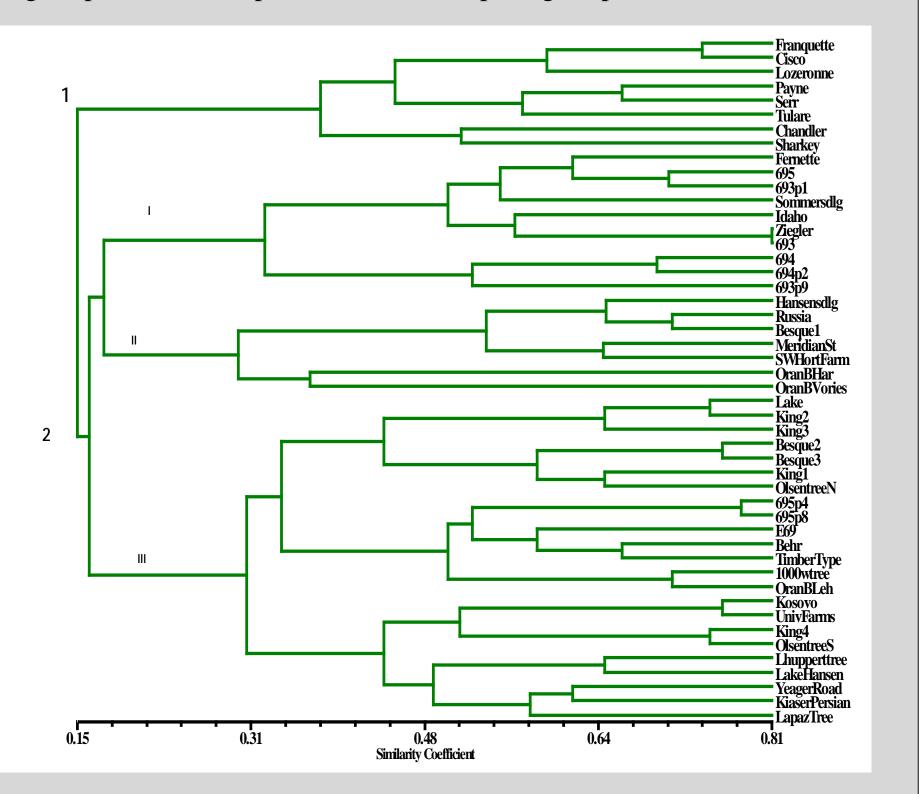


Fig. 1. UPGMA Dendrogram of the 48 walnut genotype assayed in this study based on the similarity matrix obtained using Neighbor joining genetic distance.

Genetic variation in cold hardiness

All Persian walnuts showed some winter kill ranging from slight damage to being killed to the ground. Persian walnuts originating in California and the Mediterranean were most sensitive and the Carpathian types from the Midwestern U.S. and Eastern European genotypes most tolerant (Figure 2). reflect two different introduction events, i.e., German sources in the 1700's and Polish sources in the 1900's.
Now that nut growers and breeders have tools to verify that they have correct copies of clones, they may be able to improve the diversity and cold-hardiness of Midwestern *J. regia* by crossing between dissimilar groups and avoiding crosses between similar genotypes. For example, the cross 'Ziegler' × #693 would not take advantage of differences in gene pools, but by crossing trees of different SSR groups, greater variation in the progeny should aid in selection of new individuals with better growth, form, yield, and cold hardiness.

Acknowledgement

We are grateful to the Indiana Nut and Fruit Growers Association and the Fred M. van Eck Foundation of the HTIRC at Purdue University, for funding this work. We thank Mallikarjuna K. Aradhya of the USDA National Clonal Germplasm Repository, Wolfskill, CA, for sharing SSR data. We thank the Burchell Nursery, Oakdale, CA, for providing 'Fernette' and 'Fernor' and the UC Davis walnut improvement program for providing scion wood of California and French cultivars. Of the INFGA, Ken Dooley, Bill Heiman, Jerry Lehman, John Harrell, and Bill Deeter all provided scion wood and knowledge of the history and growth of Persian walnut across Indiana. Additional material was provided by the Forest Glenn Conservation Preserve, Vermillion County, Illinois, and by Stark Brothers Nursery Inc., Vera, Missouri.

Material and Methods

Plant Material

Forty eight walnut genotypes originating from three different regions were evaluated (Table 1).

One year old twigs were collected in the germplasm orchard of:

1) Hardwood Tree Improvement and Regeneration Center (HTIRC), Purdue University.

2) Indiana Nut and Fruit Growers Association (INFGA) in Indiana.

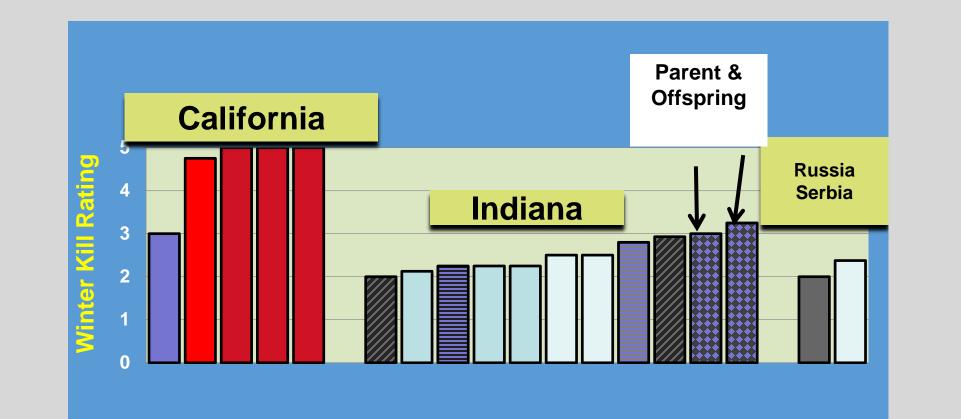


Fig. 2. Winter kill of Persian walnut (J. regia) genotypes by region of origin scored following the winter of 2013-14 (-20.9 °F for two consecutive days in February).

Juglans Species Hybrids and Cold Hardiness

The (*J. regia* \times [*J. regia* \times *J. nigra*]) BC1 hybrid walnut was much more cold hardy than pure *J. regia*, with the BC1 showing only slight damage (2.0) compared to the moderate damage *J. regia* (Table 1). The cold hardiness of interspecific F1 hybrids is considerable when *J. nigra* or *J. cinerea* (the two native northern species) were a parent. When cold sensitive *J. regia*, *J. hindsii*, or *J. major* form interspecific hybrids with *J. nigra* or *J. cinerea*, the F1 hybrids were as cold tolerant as the

References

- Dangl, G. S., Woeste, K., Aradhya, M. K., Koehmstedt, A., Simon, C., Potter, D., and McGranahan, G. (2005). Characterization of 14 microsatellite markers for genetic analysis and cultivar identification of walnut. *Journal of the American Society for Horticultural Science*, *130*(3), 348-354.
- Devitt, 1953. "Late Rev. Paul C. Crath" 44 Annual Report of Northern nut grower Association. 80-84.
- Ebrahimi A, Fatahi R, Zamani Z (2011). Analysis of genetic diversity among some Persian walnut genotypes (*Juglans regia* L.) using morphological traits and SSRs markers. Sci Hortic 130:146 151.
- Grimo, E. (1979). In: Nut tree culture in North America. Jaynes, R. A. editor. Northern Nut Growers Association. p. 74-83.
- Pollegioni P, Olimpieri I, Woeste KE, Simoni G, Gras M, Malvolti

3) Accessions from California and Europe (Table 1).For each type of accession, there were 1 or 3 grafted ramets or 1 to 6 half-sib seedlings.

N. Calif. Black^U 8 1.2 Graft J. hindsii IL

3.0 ±1



^Z Age was based on records and estimates
^Y Diameter at breast height or 4.5-feet above ground..
^X Rating scale was 1 = no damage; 2 = slight damage; 3 = moderate damage; 4 = heavy damage; and 5 = killed to graft union or ground.
^W Country or USA State where the accession originated to the best of our knowledge.
^U Based on a single grafted tree – winter kill occurs every year.

ME (2012) Barriers to interspecific hybridization between *Juglans nigra* L. and *J. regia* L species. Tree Genetics Genomes 9:291 305.
Rohlf, F.J. 2000. NTsys-pc Numerical Taxonomy and Multivariate Analysis System, Version 2.2. Exeter Publications, Setauket, NY.