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Identification and Genetic Relationship of Persian Walnut Genotypes Using Isozyme Markers

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Abstract

Horizontal starch gel electrophoresis was used to identify 108 walnut genotypes and to assess their genetic relationship. The DIA, 6-PGD, PGM, SDH, MDH and MPI enzyme systems were encoded for 12 loci, 8 of which were polymorphic. With just three systems (DIA, 6-PGD, PGM), nearly 70% of the studied genotypes were identified. Four systems (DIA, 6-PGD, PGM, SDH) allowed for more than 80% to be identified and all 6 systems studied made it possible to distinguish over 90%. Eight loci were found to be more than sufficient to identify 91 of the 108 genotypes studied.

The Nei and Li index suggests that walnut germplasm is very closely related, with an average value among all genotypes of 0.775. Cultivars from Romania ('Oprean', 'Maria Mamma' and 'Sibisel-39') showed markedly lower similarity indices than the other cultivars.

The UPGMA dendrogram based on the similarity matrix separated the walnut genotypes included in this study into two clear groups. The first group consisted of two sub-clusters, one of which mainly comprised genotypes from Spanish, French, Chilean and other European cultivars. The other sub-cluster consisted primarily of Californian cultivars closely related to the 'Payne' cultivar. The second main group, though much smaller in size, mainly comprised genotypes from Iran and Romania. Isozyme markers have proven to be useful tools for identifying and fingerprinting walnut cultivars.

Introduction

Walnut (*Juglans regia* L) is a deciduous monoecious nut tree of Central Asian origin (12, 14) that is currently widely cultivated. There are a great many cultivars specifically developed through breeding programs such as those at the University of California (Davis, CA, USA), INRA (Bordeaux, France) and the Fruit and Ornamental Research and Development Institute (Budapest, Hungary) (16). Local genotypes now used as cultivars have also been found in explorations of wild walnut populations in several areas of the world (2, 3, 17).

Cultivar verification is usually carried out based on morphological and pomological traits, but a long time is needed for successful identification and the cost is high. Trees represent a major investment in new orchards, and it is therefore very important for nurserymen, farmers and breeders to have a reliable tool for cultivar verification as soon as possible. Isozyme analysis offers a possible alternative method for cultivar identification if sufficient polymorphism exists (28). Isozyme markers are cheap, simple and easy to use. Isozyme banding patterns are generally not affected by the environment and have a codominant expression (9), which is not the case of the other markers (ISSR, RAPD and AFLP), which are dominant. Isozyme markers have been used to identify cultivars in many tree crops (11, 20, 25, 29, 31, 33, 36). The zymogram of each cultivar can be rapidly and easily determined with a small sample of plant tissue, even in winter during dormancy (1). Isozymes can be used effectively as markers at any stage of tissue development because no tissue-specific differences exist (6, 8, 36).

Previous studies on several isozyme systems have shown that polymorphism exists in walnut. The PGM, EST, 6-PGD, MDH, SDH, DIA, MPI, PX systems are polymorphic and GPI, AAT, LAP, ACO, GOT, ADH, IDH, AADH2, SOD, AAH are monomorphic in walnut (4, 6, 8, 15, 22, 32).

The objectives of this study were to evaluate the effectiveness of isozymes in culti-

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var identification in walnut and to assess the genetic relationship of these cultivars.

Materials and Methods

Plant material. The genotypes used in this study were part of the extensive walnut germplasm collection at the Centre Mas Bovè (IRTA) in Reus, Spain, except for 'Tulare' and 'Cisco', which were kindly provided by Sudoeste Recursos S.L., Spain. A total of 108 cultivars and selections were analyzed, forty-nine of which were of Spanish origin, twenty-one from California, fourteen from France, five from Romania, four from Oregon and Chile, two each from Portugal, Germany, Iran and Hungary, and one each from Greece, Italy, and Yugoslavia. Three kinds of tissue (young leaves, dormant and swelling buds) were used.

Isozyme analysis. Healthy, young, actively growing leaves of mature trees from selected cultivars were collected in the morning. Samples were used immediately for horizontal starch gel electrophoresis. Leaves were used to analyze 4 enzyme systems: malate dehydrogenase (MDH; EC. 1.1.1.37), phosphoglucomutase (PGM; EC. 2.7.5.1), shikimate dehydrogenase (SKDH; EC. 1.1.1.25), and 6-phosphogluconate dehydrogenase (6-PGD; EC. 1.1.1.44). The extraction buffer, gel and electrode buffers used followed Aletà et al. (4). The staining procedures were described by Vallejos (35). Bud-sticks with dormant buds were taken in winter and kept at 2-4°C in a refrigerator until enzyme extraction was performed. Dormant buds were used to analyze two enzyme systems: diaphorase (DIA; EC. 1.6.4.3) and mannose phosphate isomerase (MPI; EC. 5.3.1.8). Bud-sticks were forced in a climatic chamber and the swelling buds were also used for the DIA system. The extraction buffer was described by Fornari et al. (15), the staining procedures by Malvolti et al. (23), and the general isozyme analysis procedures by Arulsekar and Parfitt (7).

The inheritance of 6 isozyme systems (DIA, MDH, MPI, PGM, SDH, 6PGD) in walnut has been established in previous works (5, 6, 10, 22), thus allowing us to associate the zymogram pattern to alleles.

At least two replicate gels were made for each walnut genotype and enzyme system.

Data analysis. The Nei and Li index (similarity index) (26) between pairs of individual genotypes was computed. An unweighted pair-group method with arithmetic averages (UPGMA) was constructed from the similarity matrix using the Proc Cluster of SAS.

Results and Discussion

Cultivar fingerprinting. Repeated sampling of cultivars showed uniform zymogram expression regardless of tissue origin and development. Different tissues used showed good resolution for enzymatic systems, swelling buds produced the best zymogram resolution for a *Dia-1* and dormant buds performed likewise for a *Dia-2*. The six enzyme systems studied were encoded for 12 loci, 8 of which were polymorphic. Four enzyme systems (DIA, PGM, SDH, 6-PGD) were encoded for two loci, whereas MDH revealed three loci and MPI revealed only one locus. Most loci showed monomer patterns (*Dia-1, Mpi-1, Pgm-1, Sdh-1, Sdh-2,* and *6-Pgd-2*) as suggested by different authors (5, 6, 22). *Mdh-1* and *Mdh-3* showed dimer patterns (5), while *Dia-2* was tetrameric (21). Loci *Dia-1, Dia-2, Mdh-1, Mdh-3, Sdh-1* and *Sdh-2* were biallelic. *Pgm-1* and *Mpi-1* were triallelic loci and locus *6-Pgd-2* showed 4 alleles. Only four genotypes (two clones from Iran and the 'Serr' and 'Tulare' cultivars) contained the allele *d* in *6-Pgd-2*. The allele *a* in *Mpi-1* was found in only two cultivars ('Chico' and 'Chase D-9'). The *Mdh-1ab* genotype was only detected in an autochthonous Spanish clone ('MBT-119'); therefore, *the Mdh-1* locus was not considered polymorphic.

Most of the 108 genotypes included in this study (91) could be unequivocally identified when all 6 enzyme systems were used (Table 1). The 17 undistinguishable genotypes were distributed in 8 different groups. As expected, 'Payne' and 'Ashley' were joined in one group. 'Ashley' is known to be a bud-sport of 'Payne' (34) and both had the same pattern, as reported by Fjellstrom et al. (13) using restriction

Table 1. Isozyme genotypes of 9 loci in 108 *J. regia* cultivars of diverse origin.

	Loci										
Cultivar	Dia-1	Dia-2	6Pgd-2	Pgm-1	Sdh-1	Sdh-2	Mdh-1[X]	Mdh-3	Mpi-1	Origin[Z]	1[Y]
Adams-10	aa	bb	ab	ac	aa	aa	bb	ab	bb	Oregon[3]	*
Alcalde-2	ab	ab	bc	aa	aa	aa	bb	bb	bb	Spain	*
Algaida-1	bb	aa	ab	bb	aa	aa	bb	bb	bb	Spain	*
Algaida-2	aa	ab	bb	aa	aa	aa	bb	bb	bb	Spain	*
Amigo	aa	aa	cc	ac	ab	aa	bb	bb	bb	California	*
Arco	bb	ab	ab	bb	bb	aa	bb	ab	bb	Portugal	*
AS-0	ab	ab	bc	ab	ab	aa	bb	bb	bb	Chile	*
AS-1	ab	ab	cc	aa	ab	ab	bb	bb	bb	Chile	a
AS-5	aa	aa	bc	bb	ab	aa	bb	bb	bb	Chile	*
AS-7	ab	ab	bc	ab	ab	ab	bb	bb	bb	Chile	*
Ashley	ab	aa	cc	aa	aa	aa	bb	bb	bb	California	b
Chandler	ab	aa	bc	aa	bb	aa	bb	bb	bb	California[1]	*
Chase D-9	ab	ab	ab	ac	ab	aa	bb	bb	ab	Oregon	*
Chase D-12	ab	aa	ab	ac	aa	aa	bb	bb	bb	Oregon	*
Chico	aa	aa	ac	ac	ab	aa	bb	bb	ab	California	*
Cisco	bb	ab	bc	ab	aa	aa	bb	aa	bb	California[1]	*
Corne	bb	ab	bc	ab	ab	aa	bb	bb	bb	France	c
Eureka	ab	aa	cc	ab	bb	aa	bb	bb	bb	California[3]	*
Ferjean	bb	ab	bc	ab	ab	aa	bb	bb	bb	France[2]	c
Fernette	ab	bb	bb	aa	ab	aa	bb	bb	bb	France[2]	*
Fernor	ab	ab	bb	aa	aa	aa	bb	ab	bb	France[2]	*
FM-6	ab	bb	cc	cc	ab	aa	bb	bb	bc	Greece	*
Franquette	ab	ab	bb	aa	ab	aa	bb	ab	bb	France	d
Geisenheim-139	bb	ab	ab	ab	aa	aa	bb	ab	bb	Germany	*
Geisenheim-1239	aa	ab	cc	ab	aa	aa	bb	bb	bb	Germany	*
Germisara	ab	bb	cc	ab	bb	aa	bb	bb	bc	Romania	*
Gran Jefe	bb	ab	bb	ab	ab	aa	bb	ab	bb	Spain	*
Grandjean	ab	ab	bb	aa	ab	aa	bb	ab	bb	France	d
Grosvert	bb	bb	bb	ab	ab	aa	bb	bb	bb	France	*
Gustine	aa	ab	cc	aa	ab	aa	bb	bb	bb	California	*
Hartley	bb	aa	bb	aa	aa	aa	bb	aa	bb	California[1]	*
Howard	ab	aa	cc	ac	bb	aa	bb	bb	bb	California[1]	*
Iran A-10	aa	bb	ac	ab	aa	ab	bb	ab	bc	Iran	*
Iran B-9	aa	bb	bd	ab	bb	ab	bb	bb	bc	Iran	*
Lara	ab	aa	bc	aa	ab	aa	bb	ab	bb	France[2]	e
Marbot	bb	bb	bc	aa	aa	aa	bb	bb	bb	France	*
Maria Mamma	ab	bb	cc	bb	bb	ab	bb	bb	bc	Romania	*
Mayette	ab	ab	bb	aa	aa	aa	bb	aa	bb	France	*
MBC-31	bb	ab	bc	aa	ab	aa	bb	ab	bb	Spain	f
MBC-32	ab	ab	bb	ab	bb	aa	bb	ab	bb	Spain	*
MBC-33	bb	ab	ac	ab	ab	aa	bb	bb	bb	Spain	*
MBC-34	ab	aa	bc	ab	ab	aa	bb	bb	bb	Spain	*
MBC-36	ab	bb	bc	bb	ab	aa	bb	ab	bb	Spain	*
MBC-37	aa	ab	bb	bb	aa	aa	bb	bb	bb	Spain	*
MBC-39	ab	ab	bb	bb	bb	aa	bb	ab	bc	Spain	*
MBC-41-1	ab	bb	bc	aa	ab	aa	bb	ab	bb	Spain	*
MBC-41-2	ab	bb	bb	ab	bb	aa	bb	ab	bb	Spain	*
MBC-42	bb	bb	bb	bb	aa	aa	bb	bb	bb	Spain	*
MBC-43	bb	ab	bb	bb	aa	aa	bb	bb	bb	Spain	g

Table 1. (con't).

	Loci										
Cultivar	Dia-1	Dia-2	6Pgd-2	Pgm-1	Sdh-1	Sdh-2	Mdh-1[X]	Mdh-3	Mpi-1	Origin[Z]	1[Y]
MBC-62	ab	ab	bb	ab	ab	aa	bb	bb	bb	Spain	*
MBC-63	ab	ab	ac	ab	ab	aa	bb	ab	bb	Spain	*
MBC-64	aa	bb	bc	aa	ab	aa	bb	bb	bc	Spain	*
MBC-66	bb	ab	bc	aa	ab	aa	bb	ab	bb	Spain	f
MBC-67	ab	ab	bc	ab	ab	aa	bb	ab	bb	Spain	*
MBC-68	ab	ab	bc	ab	bb	aa	bb	bb	bb	Spain	*
MBC-303	ab	ab	bc	bb	aa	aa	bb	bb	bb	Spain	h
MBL-5	bb	ab	bc	bb	aa	aa	bb	bb	bb	Spain	*
MBLu-15	bb	ab	bb	ab	aa	aa	bb	ab	bb	Spain	*
MBLu-17	ab	bb	bb	aa	aa	aa	bb	ab	bb	Spain	*
MBLu-21	bb	aa	bb	ab	aa	aa	bb	ab	bb	Spain	*
MBLu-29	bb	aa	bc	aa	ab	aa	bb	bb	bb	Spain	*
MBLu-30	bb	ab	bb	aa	ab	aa	bb	bb	bc	Spain	*
MBLu-56	bb	aa	cc	ab	aa	aa	bb	bb	bb	Spain	*
MBLu-126	bb	ab	bb	bb	ab	aa	bb	bb	bb	Spain	*
MBO-57	ab	aa	ac	bb	aa	ab	bb	bb	bb	Spain	*
MBO-58	aa	bb	cc	bb	aa	ab	bb	bb	bc	Spain	*
MBO-59	bb	aa	bb	ab	ab	aa	bb	bb	bb	Spain	*
MBPo-6	ab	ab	ac	bb	ab	aa	bb	bb	bc	Spain	*
MBPo-10	ab	ab	bc	ab	aa	aa	bb	ab	bb	Spain	*
MBPo-11	aa	bb	cc	bb	ab	aa	bb	bb	bc	Spain	*
MBPo-26	ab	ab	bb	ab	ab	aa	bb	bb	bc	Spain	*
MBPo-27	ab	ab	bc	bb	aa	aa	bb	bb	bb	Spain	h
MBT-19	aa	aa	ab	bb	ab	aa	bb	bb	bb	Spain	*
MBT-31	bb	ab	bc	ab	aa	aa	bb	bb	bb	Spain	*
MBT-38	aa	bb	bb	aa	bb	aa	bb	bb	bb	Spain	*
MBT-49	ab	bb	ac	aa	aa	aa	bb	bb	bc	Spain	*
MBT-119	ab	aa	bb	bb	ab	aa	ab	bb	bb	Spain	*
MBT-122	bb	ab	bb	bb	aa	aa	bb	bb	bb	Spain	g
MBT-136	ab	ab	bb	bb	aa	aa	bb	aa	bc	Spain	*
MBT-159	bb	bb	bc	bc	aa	aa	bb	bb	bb	Spain	*
MBT-301	ab	bb	bc	ab	ab	aa	bb	bb	bb	Spain	*
MBT-348	bb	bb	bc	bb	ab	aa	bb	bb	bb	Spain	*
MBT-353	bb	ab	bb	ab	aa	aa	bb	bb	bb	Spain	*
Meylannaise	bb	bb	bb	ab	aa	aa	bb	aa	bb	France	*
Midland	ab	ab	bc	aa	ab	aa	bb	bb	bb	California[1]	*
Milotai-10	bb	ab	cc	aa	aa	aa	bb	bb	bb	Hungary	*
Nugget	bb	ab	bc	aa	ab	aa	bb	bb	bb	California	*
Oprean	aa	bb	ac	bb	bb	ab	bb	bb	bc	Romaina	*
Parisienne	bb	bb	bb	aa	aa	aa	bb	aa	bb	France	*
Payne	ab	aa	cc	aa	aa	aa	bb	bb	bb	California	b
Pedro	ab	aa	bc	aa	ab	aa	bb	ab	bb	California[1]	e
Rego	ab	aa	ab	bb	ab	aa	bb	bb	bb	Portugal	*
Ronde de Montignac	bb	bb	bc	ab	ab	aa	bb	bb	bb	France	*
Sampion	aa	bb	cc	bb	aa	ab	bb	ab	bb	Yugoslavia	*
Serr	aa	ab	cd	ac	ab	aa	bb	ab	bb	California[3]	*
Sharsch Franquette	ab	ab	bb	aa	ab	aa	bb	ab	bb	California[1]	d

Table 1. (con't).

	Loci										
Cultivar	Dia-1	Dia-2	6Pgd-2	Pgm-1	Sdh-1	Sdh-2	Mdh-1[X]	Mdh-3	Mpi-1	Origin[Z]	1[Y]
Sibisel-39	bb	bb	ac	bb	ab	bb	bb	bb	bb	Romaina	*
Simion	ab	ab	cc	bb	ab	ab	bb	bb	bb	Romaina	*
Soleze	bb	bb	cc	ab	bb	aa	bb	bb	bb	France	*
Sorrento	ab	bb	bc	ac	ab	aa	bb	bb	bb	Italy	*
Spurgeon	ab	aa	bb	ab	ab	aa	bb	ab	bb	Oregon[1]	*
Sunland	aa	ab	bc	ac	ab	aa	bb	ab	bc	California[3]	*
Tehama	aa	ab	bc	aa	ab	aa	bb	bb	bb	California	*
Tiszacsecsi-83	aa	ab	bc	bb	aa	aa	bb	bb	bb	Hungary	*
Trinta	ab	ab	cc	aa	ab	ab	bb	bb	bb	California	a
Tulare	aa	bb	bd	ac	aa	aa	bb	bb	bb	California[3]	*
Vina	ab	ab	bc	aa	ab	aa	bb	ab	bb	California[1]	*
Waterloo	aa	bb	bc	aa	bb	aa	bb	ab	bb	California	*

Z: 1: French germplasm ancestor or suspected; 2: Californian germplasm ancestor or suspected; 3: Iran or Afghanistan germplasm ancestor or suspected.
Y: * Unique isozyme phenotype; letters a-h: cultivars with the same letter have identical isozyme genotypes.
X: Not considered a polymorphic locus (the frequency of the less abundant allele is not greater that 5%).

fragment length polymorphisms (RFLPs). Nevertheless, these two cultivars appeared separately, though very close together, when intersimple sequence repeat (ISSR) markers were used, thus supporting the idea that 'Ashley' is a seedling of 'Payne' (30). In addition, 'Lara', which was suspected of being a seedling of 'Payne' (18), was undistinguishable from 'Pedro' using 4 enzymatic systems (4). Results did not change when two more enzyme systems were added.

A third group included two genotypes with different origins: 'AS-1' (Chile) and 'Trinta' (California). Another group included two traditional French cultivars, 'Franquette' and 'Grandjean', with 'Sharsch Franquette' (considered to be a 'Franquette' seedling (34)). Two other French cultivars, 'Corne' and 'Ferjean', were grouped together, both coming from a neighbouring geographic origin. Three groups of two Spanish clones were also grouped with them.

With just 3 systems (DIA, 6-PGD, PGM), nearly 70% of the genotypes studied could be identified. Four systems (DIA, 6-PGD, PGM, SDH) allowed for identification of more than 80% and all 6 systems studied made it possible to distinguish over 90%. DIA and 6-PGD were very useful systems for identification because of their high polymorphism. Cultivars were separated into 41 sets based only on diaphorase and 6-phosphogluconate dehydrogenase activities.

Isozyme genotypes are consistent with known pedigrees of studied cultivars (16, 34) except in 'Fernette' ('Franquette' x 'Lara'). The *Dia-2* 'Fernette' locus showed homozygosity for the *b* allele and its male parent did not have this allele.

Four walnut genotypes of European origin or having European ancestors were homozygous for all the systems studied ('Hartley', 'Parisienne', 'MBC-42' and 'MBT-38'). It is known that the California walnut cultivar 'Hartley' is probably a cross between two French cultivars (30, 34).

Genetic relationship among cultivars. The walnut tree genotypes analyzed in this study represented a wide range of *J. regia* germplasm from an extensive geographic area. The Nei and Li index among genotypes ranged from 1 to 0.259 between 'Hartley' and 'Oprean', with an average value among all genotypes of 0.775. Cultivars from Romania ('Oprean', 'Maria Mamma' and 'Sibisel-39') showed a markedly lower Nei and Li index in relation to the other cultivars (data not shown).

One of the highest similarity indices calculated (0.963) was found between many cultivars that were clearly related: 'Franquette' by 'Fernor' and 'Vina', 'Fran-

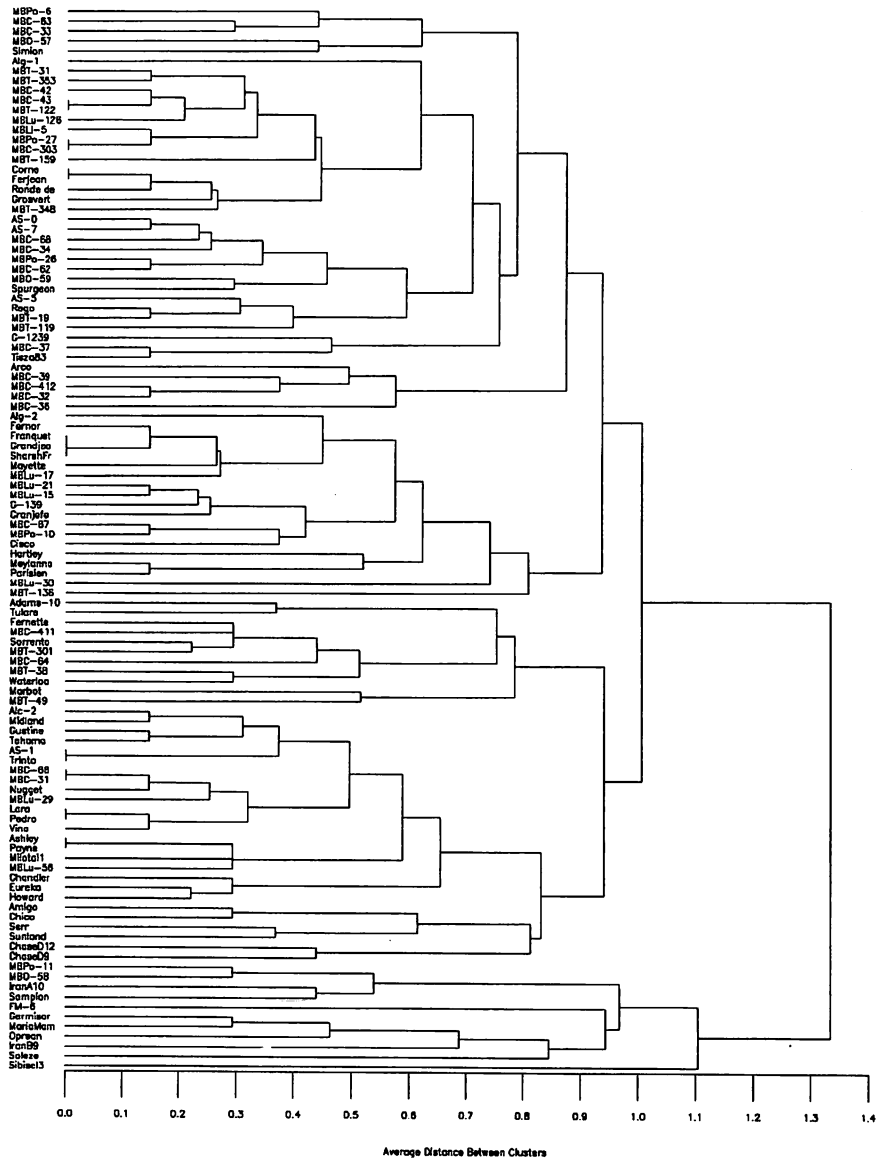

Fig. 1. Dendogram of UPGMA cluster analysis on Nei and Li index (26) among genotypes.

quette' being the parent of both (16). High genetic similarity between 'Midland' and 'Vina' and between 'Gustine' and 'Tehama' (0.963) revealed their close genetic relatedness (34).

The UPGMA dendogram based on the Nei and Li index is shown in Fig. 1. Two clear clusters were obtained: one large cluster with the French, Californian and nearly all the Spanish genotypes, and the

other, much smaller cluster (11 genotypes), consisting of four of the five genotypes from Romania, the two clones studied from Iran, the two from Spain and one each from France, Greece and Yugoslavia.

The first large cluster included two subclusters, the first comprising 60 genotypes: 37 Spanish, ten French, three Chilean, two Portuguese, two German and two old European cultivars from Romania and Hungary. The American cultivars 'Cisco', 'Hartley', 'Spurgeon' and 'Sharsch Franquette', directly related to French germplasm (34), were also present in this sub-cluster. In accordance with Potter et al. (30), the 'Cisco' cultivar was clustered with 'Franquette' and joined with 'Meylannaise', its female genitor (24). The 'Spurgeon' cultivar from Oregon was found next to its direct ancestor 'Franquette' (19). The new cultivars 'Fernor' and 'Ferjean' appeared very close to their female parents 'Franquette' and 'Grosvert', respectively. Four French cultivars ('Corne', 'Ferjean', 'Ronde de Montignac' and 'Grosvert') were clustered with a Spanish clone ('MBT-348'), thus showing the high level of relatedness among them.

The second sub-cluster consisted of 18 Californian cultivars and three from Oregon, ten from Spain, three from France, and one each from Italy, Chile and Hungary. 'Ashley', 'Pedro', 'Serr' and 'Sunland' appeared in the 'Payne' cluster as found by Potter et al. (30). As expected, 'Amigo' and 'Chico' were joined together, as were 'Chandler' and 'Howard'. The 'Howard' cultivar appeared very close to 'Eureka' (its great-grandfather) (34). The close relationship between 'Serr' and 'Sunland' agreed with Potter et al. (30). Our study revealed close relatedness between 'Gustine' and 'Tehama', both progenies from the same cross. 'Pedro' and 'Vina' (two cultivars sharing one common ancestor) also showed close relatedness. In general, as expected, cultivars sharing common parents tended to be grouped together, showing that the coefficient of similarity was accurate. The results of this first cluster agreed with the opinions of Forde (14), Nicese et al. (27) and Potter et al. (30) that many of the Californian cultivars contain genetic contributions of germplasm from diverse geographic regions, especially French cultivars.

The second cluster grouped germplasm from Iran and selections from the Balkans region (Greece, Yugoslavia and Romania). This gathered material agreed with the theory that the walnut survived in some glacial refugia in Europe during Pleistocene glaciations (15). Two genotypes from the northwest part of the Iberian Peninsula were clustered in this second group with the Iranian germplasm (walnut origin). Therefore, the possibility exists that some European populations could come from ancestral material.

The results obtained raised doubts as to whether the 'Soleze' cultivar was a chance seedling of 'Marbot', as supposed by Germain et al. (18), given that these two cultivars were found in different clusters.

The variability observed in the 6 enzyme systems was enough to conveniently and unambiguously identify 91 of the 108 walnut cultivars and selections. The results of this study clearly indicate the usefulness of isozyme markers for identifying walnut cultivars.

The results presented are very similar to those found by Potter et al. (30) using the ISSR technique. This study helps clarify relationships among walnut germplasm. It would be interesting to pool all the data found using different markers to expand our knowledge even further regarding the origin of walnut germplasm.

Acknowledgements

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Performance of 'Williams' Pear on Five Rootstocks

The performance of 'Williams' pear growing on OHF-333, BA-29, BA-29 with interstock B. Hardy, Kirschensaller, and the own-rooted 'Williams' rootstocks were compared for seven years. The trial was in Lleida, Spain on a well drained loamy calcareous soil with a pH of 8.5. Own-rooted 'Williams' trees had the largest trunks at 7 years, but there were no differences in tree spread. Lower uniformity and greater chlorosis occurred with quince (BA-29, BA-29 with Buerre Hardy interstock). The greatest yield was on OHF-333 rooted trees; other rootstocks did not differ. From: Urbina, V., J. Dalmases, M. Pascual, and R. Dalman. 2003. Performance of 'Williams' pear on five rootstocks. J. Hort. Sci. Biotech. 78:193-196.

Photoperiod Affects Blueberry Growth and Fruiting

Experiments with V. *darrowi* and two cultivars of southern highbush blueberry, 'Sharpblue' and 'Misty', tested whether V. *darrowi* and cultivars derived from it are photoperiodic with respect to flower bud initiation (FBI). Plants were grown under three light treatments: [long days (LD): 16 hour photoperiod; short days (SD): 8 hour photoperiod; and short days + night interruption (SD+NI): 8 hour photoperiod with 1 hour night interruption] at constant 21°C for 8 weeks. Vegetative growth was greater in LD plants for both cultivars. FBI occurred only in SD and the lack of FBI in the SD-NI treated plants indicates that FBI is a phytochrome mediated response in *Vaccinium*. The data indicate that FBI in both V. *darrowi* and southern highbush blueberry is photoperiodically sensitive, and is promoted by short days, while flower bud development is enhanced under long days. From: Spann, T.M., J.G. Williamson, and R.L. Darnell. Photoperiodic effects on vegetative and reproductive growth of *Vaccinium darrowi* and *V. corymbosum* interspecific hybrids. HortScience 38: 192-195.