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Abstract

In France, walnut crop is the second largest fruit crop after apple, with 36,000 tons of in-shell walnuts produced in 2016, making France the 9th producer in the world with almost 20,000 hectares devoted. The INRA's walnut germplasm collection includes 253 accessions from worldwide thanks to the prospecting work of Eric Germain from 1977 to 2007, the former head of breeding program at INRA of Bordeaux. Among them, 217 are *Juglans regia* accessions including 194 cultivars and 23 intraspecific hybrids, coming from the major growing areas such as North-America, Europe and Asia. The germplasm collection includes also 36 *Juglans* accessions of 14 related species from the two sections *Rhysocaryon* (*J. nigra*, *J. hindsii*, *J. microcarpa*, *J. californica*, *J. major*, *J. mollis*) and *Cardiocaryon* (*J. sieboldiana*, *J. cathayensis*, *J. mandshurica*, *J. cinerea*). In this study, 13 simple sequence repeat (SSR) markers, selected from the literature (10 genomic SSRs from *J. nigra* and 3 EST-SSRs from *J. regia*), were used to genotype the 253 accessions. All SSR loci were highly polymorphic with a range from 2 to 17 alleles/locus (mean: 8.92) considering all the *J. regia* accessions. The results indicate a high diversity among the genotypes which could be useful for the new French walnut improvement program. This analysis permitted to select 170 accessions that will be used for association genetics studies, using the 600K SNP Affymetrix® array, in order to identify the genetic determinism of agronomic traits of interest such as those related to phenology, fruit and kernel quality, and susceptibility to different diseases.

Keywords:

Juglans spp., walnut germplasm, genetic diversity, French walnut improvement program, SSR

INTRODUCTION

Thought to be domesticated in Central Asia (Zeven & Zhukovsky, 1975), more precisely in the foothills of the Western Himalayas from the Kashmir region to Tajikistan and Kyrgyzstan, Persian walnut (*Juglans regia* L.) is a monoecious and dichogamous tree species (Germain et al., 1999) widely disseminated in many temperate regions of northern hemisphere and South

America, South Africa, Australia and New-Zealand which has always fed people. The genus *Juglans* belongs to the order *Fagales* and includes more than 20 diploid species (Woodworth, 1930) divided into three sections: *Rhysocaryon*, including *J. nigra*, *Cardiocaryon* and *Dioscaryon*, containing *J. regia* (Fjellstrom & Parfitt, 1994).

Knowledge of the genetic diversity is crucial for an effective management and use of germplasm. In walnut, several studies reviewed recently have been conducted using a wide range of molecular markers (Bernard et al., 2018). In France till now, the 'Institut National de Recherche Agronomique' (INRA, in English: French National Institute for Agricultural Research) has driven two walnut breeding programs in Bordeaux. In the first program, crosses were conducted to obtain hybrids combining a late budbreak date and good fruit quality mainly from French female parents, and lateral bearing habit found in Californian male parents. Seven cultivars were released between 1995 and 2010, including 'Fernor', well established nowadays in French walnut orchards. In the second program, a diverse genetic material was used (from Mediterranean area, Middle-East and Asia) with the best hybrids from the first program (Germain, 1997). However, even if some hybrids were characterized and evaluated as promising, following INRA's strategic decisions, the INRA's walnut breeding program stopped in 2007.

In light of the important economic development of walnuts (Fig. 1), the global competition and the climate change context, the choice of cultivars in France seems to be insufficient nowadays. The study of INRA's walnut germplasm collection is a first step for the initiation of a new breeding program using marker-assisted selection. The 'Centre Technique Interprofessionnel des Fruits et Légumes' (Ctifl, in English: Fruit and Vegetable Interprofessional Technique Center) will be at the origin of this program, based on basic research (genetic diversity evaluation of germplasm and genetic determinism identification of traits of interest) and applied research (establishment of tools for marker-assisted selection achievement).

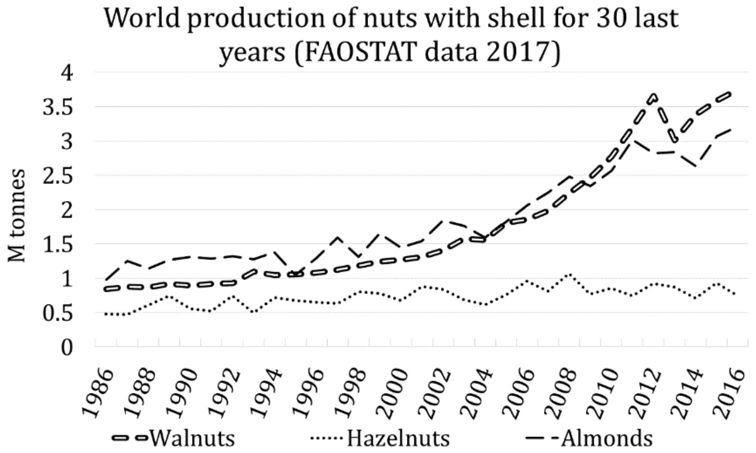

Fig. 1. World production of main nuts crops in-shell for three last decades (FAOSTAT data dec. 2017)

MATERIALS AND METHODS

Plant materials: 253 accessions from the INRA's walnut germplasm collection were analyzed (Table 1), including 217 *J. regia* accessions (194 cultivars and 23 intraspecific hybrids) and 36 accessions part of 14 related species: *J. ailantifolia* Carr. (syn: *J. sieboldiana* Maxim.), *J. californica* S. Wats., *J. cathayensis* Dode, *J. cinerea* L., *J. hindsii* Jeps. *J. major* Heller, *J. mandshurica* Maxim., *J. microcarpa* Berl., *J. mollis* Engelm., *J. nigra* L., *J. pitteursii* C. Morren (isotype of *J. nigra*), *J. rupestris* Engelm. ex Torr. (isotype of *J. major*), *J. sieboldiana* Maxim., *J. sieboldiana* var. *cordiformis*. These accessions come from different countries of Europe, America and Asia, thanks to the prospecting work of M. Eric Germain, former head of breeding program at INRA of Bordeaux from 1977 to 2007. All accessions are 20-30 years grafted trees located in the Arboriculture Experimental Unit of INRA in Toulenne (latitude 44°34'37.442''N – longitude 0°16'51.48''W), near Bordeaux (France).

SSR genotyping: Leafs were collected and sent to the BioGEVES laboratory in France to perform DNA extraction, using Macherey-Nagel NucleoSpin 96 Plant II Core kit. DNA concentration measurement was performed with spectrophotometry method (SPECTROstar Omega). All accessions were genotyped by the BioGEVES laboratory using 15 SSR markers chosen from the literature (Dang et al., 2016; Dangl et al., 2005; Woeste et al., 2002). The list of markers is reported in Table 2. The PCR reactions were carried out in 10 µL containing 4 µL of diluted DNA (2.5 ng/µL) and 6 µL of PCR mix. PCR mix consisted of 3.80 µL of ultra-pure water, 1 µL of 10X buffer, 0.2 µL of dNTP (10 mM), 0.60 µL of $MgCl_2$ (25 mM), 0.10 µL of Ampli Taq Gold polymerase, 0.10 µL of reverse (R) primers, 1 µM of forward (F) primers and 10 µM of 35 S oligo sequence. PCR steps were: 10 min – 94°C, (30 sec – 94°C, 1 min – 58°C, 40 sec – 72°C) x30 cycles (or x35 depending on the primers) and 10 min – 72°C. Amplification products were diluted and 5.8 µL of formamide, 0.2 µL of ladder and 4 µL of amplicon are added in each well. An Applied Biosystem 3730 DNA Analyzer was used and data were processed using GeneMapper software.

Table 2. List of the 15 SSR markers selected

SSR marker	SSR type and source species	Primers sequences 5'-3'	Repeat array	Reference
WGA 001	genomic SSR from *J. nigra*	F ATTGGAAGGGAAGGGAAATG R CGCGCACATACGTAAATCAC	$(GA)_5GCA(GA)_3GCA(GA)_3$	Dangl et al., 2005
WGA 004	genomic SSR from *J. nigra*	F TGTTGCATTGACCCACTTGT R TAAGCCAACATGGTATGCCA	$(GT)_5,(GA)_{15},(GA)_{11}$	Woeste et al., 2002
WGA 009	genomic SSR from *J. nigra*	F CATCAAAGCAAGCAATGGG R CCATTGCTCTGTGATTGGG	$(GA)_{16}$	Dangl et al., 2005
WGA 027	genomic SSR from *J. nigra*	F AACCCTACAACGCCTTGATG R TGCTCAGGCTCCACTTCC	$(GA)_{30}$	Woeste et al., 2002
WGA 069	genomic SSR from *J. nigra*	F TTAGTTAGCAAACCCACCCG R AGATGCACAGACCAACCCTC	$(GA)_4ATATAA(GA)_{16}$	Woeste et al., 2002
WGA 072	genomic SSR from *J. nigra*	F AAACCACCTAAACCCTGCA R ACCCATCCATGATCTTCCAA	$(CT)_{14}$	Woeste et al., 2002
WGA 202	genomic SSR from *J. nigra*	F CCCATCTACCGTTGCACTTT R GCTGGTGGTTCTATCATGGG	$(GA)_{11}$	Dangl et al., 2005
WGA 276	genomic SSR from *J. nigra*	F CTCACTTTCTCGGCTCTTCC R GGTCTTATGTGGGCAGTCGT	$(GA)_{14}$	Dangl et al., 2005
WGA 349	genomic SSR from *J. nigra*	F GTGGCGAAAGTTTATTTTTTGC R ACAAATGCACAGCAGCAAAC	$(CT)_{14}$	Dangl et al., 2005
WGA 376	genomic SSR from *J. nigra*	F GCCCTCAAAGTGATGAACGT R TCATCCATATTTACCCCTTTCG	$(AG)_2AA(AG)_6$	Dangl et al., 2005
JR 0160	EST-SSR from *J. regia*	F TCTCGGATTTGGGCTGTGAC R TCCGGGACCCTCGTCTAATT	$(TC)_{10}$	Dang et al., 2016
JR 1739	EST-SSR from *J. regia*	F GGATGTGGAGACGGCAAAGA R CGTCCACCCAAACCAAGAGA	$(GAGCCG)_8$	Dang et al., 2016
JR 1817	EST-SSR from *J. regia*	F CCTCAGAGCCAACCATCCTT R AGAACAGAACCAGCGTCACA	$(AC)_{11}$	Dang et al., 2016
JR 6160	EST-SSR from *J. regia*	F ACTTCAGGTTCCCAACGCAA R TAGAGGGAAGGTCTCCGGTG	$(GA)_{10}$	Dang et al., 2016
JR 6439	EST-SSR from *J. regia*	F TCGATGCGATCATCTCCGTG R CGGCACCAAAACAGAACTCG	$(TGCG)_5$	Dang et al., 2016

F: Forward, R: Reverse

Genetic diversity and population structure analyses: Diversity parameters were estimated for each locus with the “adegenet 2.1.0” R package (Jombart, 2008), for the 217 *J. regia* accessions only. A Principal Coordinate Analysis (PCoA) was performed to determine the relationships between *Juglans* accessions using DARwin 6.0.14 software (Perrier & Jacquemoud-Collet, 2006). Software STRUCTURE (Pritchard et al., 2000) was used to explore population structure. Twenty runs were done by setting the number of clusters (K) from 1 to 10 to identify the best K. Each run consisted of a length of burn-in period of 5,000 followed by 50,000 Markov Chain Monte Carlo (MCMC) replicates, assuming an admixture model and correlated allele frequencies. When K was estimated, 10 runs were done by setting the K from 1 to 5. Each run consisted of a length of burn-in period of 100,000 followed by 750,000 MCMC replicates. For the choice of the most likely K, the plateau criterion described by Pritchard et al. (2000) and the ΔK method described by Evanno et al. (2005) were used. Accessions with an estimated membership below 0.8 were assigned to the "admixed group".

RESULTS AND DISCUSSION

SSR genetic diversity: 13 SSR markers among the 15 studied were retained for the analysis after control of amplification and goodness of signal recorded. For some SSRs, PCR amplification was observed only for part of the *Juglans* species: the WGA 001 and WGA 276 SSRs amplify in *J. regia* and *Rhysocaryon* section species but do not in *Cardiocaryon* section, whereas the JR 1817 SSR does not amplify in *Cardiocaryon* section species and in *J. californica* of the *Rhysocaryon* section but amplifies in other species of this section and in *J. regia*. Furthermore, for some SSRs, specific alleles are observed among the different *Juglans* species: for WGA 001 SSR, the '184 bp' allele is found only in *J. mollis* whereas the '197 bp' and '205 bp' alleles are found only in *J. nigra*. Considering the 217 *J. regia* accessions, the number of alleles per locus (A) ranges from 2 to 17 with an average of 8.92 (Table 3). The expected heterozygosity H_e is higher than the observed heterozygosity H_o and with a mean value of 0.56 (p-value of *t*-test: 8.24E^{-04}), showing a deficiency of heterozygotes, due to the presence of modern cultivars and hybrids. JR 1817 and JR 6439, both SSRs derived from EST, have the lower values of H_o and H_e, but transcribed regions of the genome are known to be more conserved, as observed on other crops (Scott et al., 2000; Hu et al., 2011).

Table 3. Genetic diversity estimations of the 217 *J. regia* accessions

SSR markers	A	H_o	H_e	F_{IS}
WGA 001	8	0.65	0.71	0.08
WGA 004	7	0.46	0.50	0.08
WGA 009	7	0.59	0.66	0.11
WGA 027	2	0.43	0.46	0.07
WGA 069	9	0.57	0.76	0.25
WGA 072	6	0.32	0.42	0.24
WGA 202	17	0.68	0.78	0.13
WGA 276	16	0.63	0.76	0.17
WGA 349	11	0.49	0.78	0.37
WGA 376	13	0.61	0.66	0.08
JR 1817	5	0.10	0.15	0.33
JR 6160	10	0.43	0.49	0.12
JR 6439	5	0.14	0.17	0.18
Min	2	0.10	0.15	0.07
Mean	8.92	0.47	0.56	0.17
Max	17	0.68	0.78	0.37

A: number of alleles, H_o: observed heterozygosity, H_e: expected heterozigosity, F_{IS}: inbreeding coefficient

The PCoA performed with all the *Juglans* accessions reveal three well separated clusters: the biggest is formed by the 217 *J. regia* accessions and two additional clusters include on the one hand all the accessions of *Cardiocaryon* section, and on the other hand those of the *Rhysocaryon* section. These results are in agreement with the botanical classification and a recent work regarding the phylogenetic resolution in *Juglans* (Dong et al., 2017).

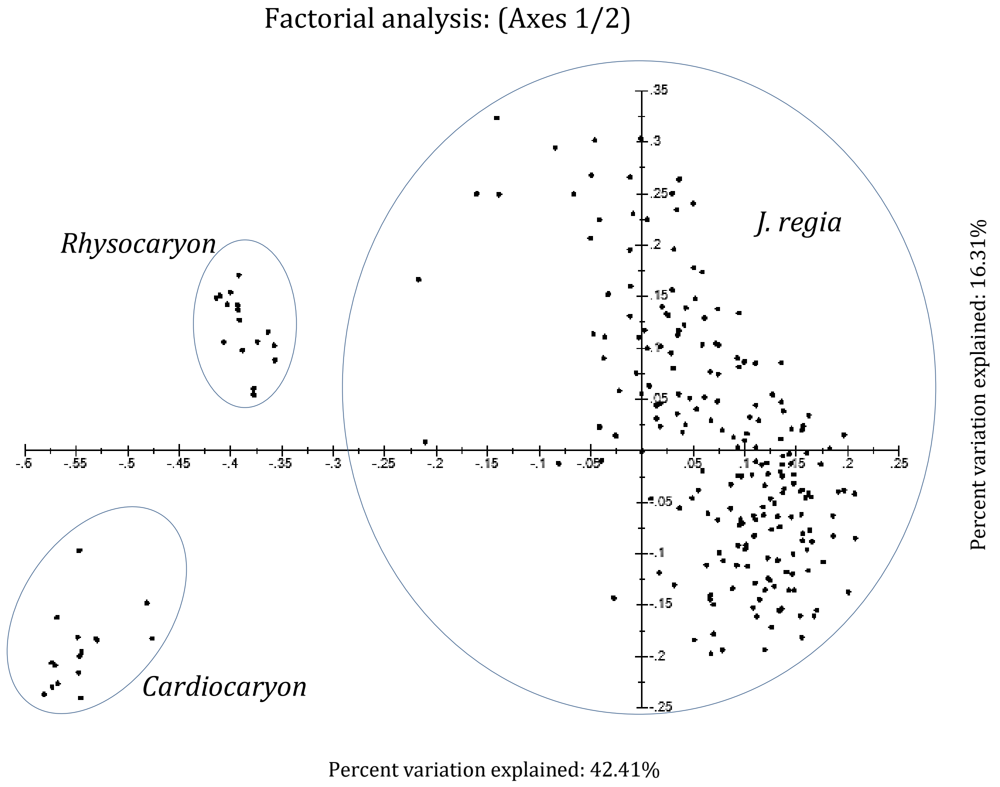

Fig. 2. Principal Coordinate Analysis (PCoA) plot of 13 SSRs with the set of 253 *Juglans* accessions

Population structure: Regarding population structure, the highest value was for K=2 (Fig. 3) with the identification of two ancestral populations: the first one contains 63 accessions mainly from Eastern Europe and Asia, whereas the second one comprises 127 accessions mainly from Western Europe and America. 27 accessions showed clear mixed ancestry with membership values lower than 80% in any of the two clusters. In this admixed cluster, there are INRA's hybrids and USA cultivars obtained from intraspecific crosses ('Feradam', 'Fernette', 'Serr', 'Chico', 'Amigo', 'Gillet', 'Forde' and 'Tulare'). As mentioned in Table 1, pedigree can explain this result. For examples, 'Gillet' and 'Forde', two cultivars released in California in 2004, have 'Chico' and 'UC 61-25' in their background. 'Chico' is a cross between 'Sharkey' and 'Marchetti' and 'Marchetti' is itself a cross between 'Eureka' and 'Payne'. 'Payne' for its part is a cross between a French cultivar and a Chinese seedling. UC 61-25 is a cross between 'Conway Mayette' and 'PI 18256', coming from China. So it is clear that 'Gillet', 'Forde' and 'Chico' show a complex pedigree. The first cluster contains accessions from different countries, such as: Bulgaria

('Sheinovo' and 'Izvor 10'), Romania ('Sibisel 39', 'Sibisel 44', 'VL4B' and 'Germisara'), Greece ('S 28 A Achille', 'S 4 B Thétis', 'S 34 B Pyrrus' and 'EAA 6'), Hungary ('Milotai n°10'), Ukraine ('UK-series'), India ('Sopore'), Iran ('IR-series' and 'Z 53'), China ('Jin Long 1' and 'Lu Guang'), Japan ('Shinrei'), and surprisingly, France (INRA's hybrids H 110-34 and H 119-13) and USA ('Sexton', 'Chase C7', 'Wepster W2', 'Adams 10' and 'PI series'). INRA's hybrid H 110-34 is a cross between 'Fernette' and 'Serr', 'Serr' issued from a cross between 'Payne' and 'PI 15 95 68', coming from Afghanistan. H 119-13 is a cross between 'Fernor' and 'EAA 6', coming from Greece. Consequently, the clustering of these two hybrids is in agreement with their origin. In the same way, the USA accessions 'Chase C7', 'Wepster W2' and 'Adams 10' are Manregian-type walnuts, coming from China. The second cluster contains old French cultivars, INRA's hybrids released recently such as 'Fernor', 'Ferjean', 'Ferbel', 'Ferouette', 'Fertignac', and accessions from Germany ('Allemagne 139' and 'Geisenheim 286'), England ('Northdown Clawnut 252'), Spain ('MB- and MBT-series', 'Del Carril' and 'Gran Jefe'), Greece ('S 1 A Diane' and 'S 1 B Ariane'), Portugal ('Rego'), Hungary ('M 10-37'), Chile ('AS 1') and USA (old cultivar 'Payne' and modern cultivars having the latter as genitors: 'Howe', 'Pedro', 'Vina' and 'Chandler'). The membership of the accessions for the two clusters is indicated in Table 1.

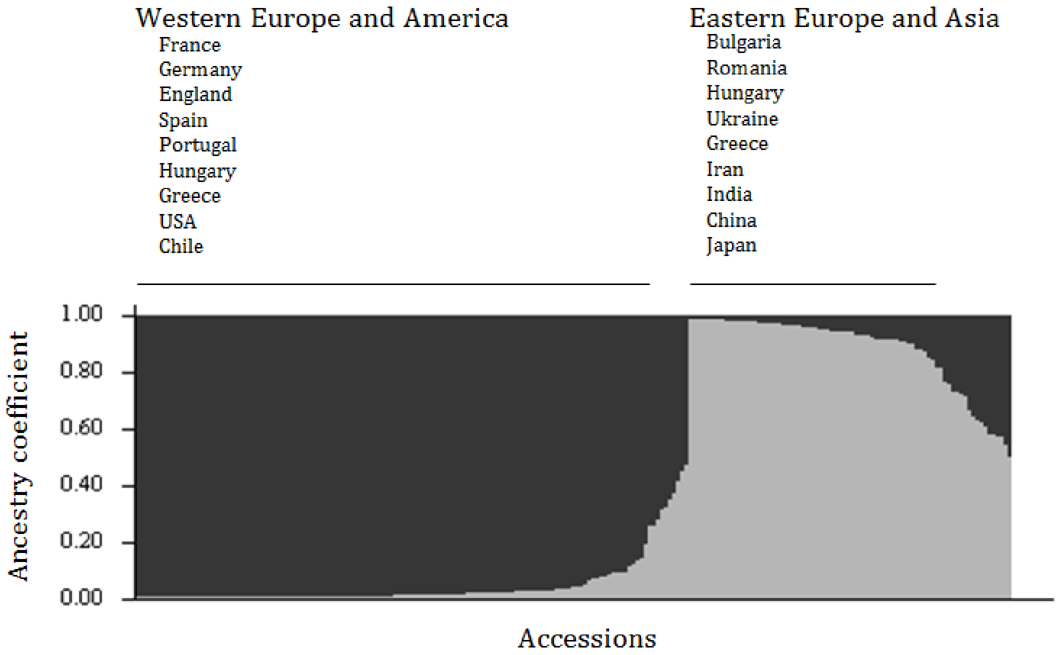

Fig. 3. Inferred population structure of the *J. regia* collection using STRUCTURE. Individual ancestry proportions (Q values) are sorted within each cluster

Selection of 170 accessions for further analysis in the frame of the new French walnut improvement program: This first genetic diversity analysis of the INRA's walnut germplasm collection permitted the choice of accessions for further genetic analysis. Thanks to the first

600K SNP Axiom® Affymetrix array available and developed in the University of Davis (Marrano, 2018), following the walnut genome sequencing (Martínez-García et al., 2016), investigation of genetic diversity will continue in the frame of the new French walnut improvement program, led by the Ctifl. Synonymous accessions or very close genetic ones based on this analysis have been set aside. The 170 *J. regia* remaining accessions have been characterized for main agronomical traits such as: phenology (budbreak date, peak male/female bloom date), fruit traits (weight, size, height, diameter), and pathology (tolerance to main walnut pests and diseases in France). A genome-wide association study is scheduled for last quarter 2018 in order to determine the main genetic determinisms of traits of interest.

CONCLUSION

The INRA's walnut germplasm collection is a very rich collection in terms of geographical origins and is the result of an important prospecting work carried out by E. Germain between 1988 and 2000 in many countries of the world. Aware of the importance of safeguarding biodiversity, E. Germain knew that creating a valuable reservoir of genetic diversity is crucial to a successful and fruitful breeding program. The genetic diversity found in the INRA's walnut collection has proved to be high and the population structure follows the geographical origins of the accessions. This preliminary work permitted to select the suitable accessions for a future genome-wide association study.

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Table 1. List of *J. regia* accessions analyzed

Accession	Name	Source country	Supposed origin	Breeding level	Pedigree	STRUCTURE K=2
RA 1100	-	France	France, Rhône-Alpes	Selection	-	Western Europe and America
RA 0248 AF5	-	France	France	Selection	Self-fertilization of RA 0248	Western Europe and America
RA 0550 AF4	-	France	France	Selection	Self-fertilization of RA 0550	Western Europe and America
RA 0639 AF1	-	France	France	Selection	Self-fertilization of RA 0639	Western Europe and America
RA 0665-29	-	France	France	Selection	-	Western Europe and America
RA 0248-8	-	France	France, Rhône-Alpes	Selection	-	Western Europe and America
RA 1136	-	Germany	Germany	Selection	-	Western Europe and America
RA 1044	-	France	France, Aquitaine	Selection	-	Western Europe and America
IR 13-1	-	Iran	Iran	Selection	-	Eastern Europe and Asia
IR 60-3	-	Iran	Iran	Selection	-	Eastern Europe and Asia
IR TA 1-1	-	Iran	Iran	Selection	-	Eastern Europe and Asia
UK 11-4	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 6-2	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 47-1	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 52C9	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 53-3	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 56-21	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 107C-D2-2	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 123-D1-14	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Admixed
UK 123-D7-8	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 56-10	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 56-2	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 47-10	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 21-4	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
IR 100-2	-	Iran	Iran	Selection	-	Eastern Europe and Asia
IR 60-1	-	Iran	Iran	Selection	-	Eastern Europe and Asia
UK 127AG11	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 56-12	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 118-23	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 55-40	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 52-12	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 127-D1-24	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 41-17	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 212AG5	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 214-D1-14	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 214-D7-15	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Admixed
UK 215AG12	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 216AG5	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 216AG18	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 216-D7-25	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia

UK 220-D5-3	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 223-1	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 224-6	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 234-5	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 239-10	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 239-13	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Eastern Europe and Asia
UK 239-23	-	Ukraine	Uzbekistan, Tajikistan, Kyrgyzstan	Selection	-	Western Europe and America
IR 21-7	-	Iran	Iran	Selection	-	Eastern Europe and Asia
RA 1041 AF6	-	France	France	Selection	Self-fertilization of RA 1041	Western Europe and America
RA 1041 AF9	-	France	France	Selection	Self-fertilization of RA 1041	Western Europe and America
RA 1088 AF17	-	France	France	Selection	Self-fertilization of RA 1088	Western Europe and America
RA 1223	-	Switzerland	-	Selection	-	Eastern Europe and Asia
RA 1195	-	France	France	Selection	-	Western Europe and America
RA 1196	-	France	France	Selection	-	Western Europe and America
RA 1217	-	Switzerland	-	Selection	-	Western Europe and America
RA 1218	-	Switzerland	-	Selection	-	Admixed
RA 1220	-	Switzerland	-	Selection	-	Western Europe and America
RA 1219	-	Switzerland	-	Selection	-	Western Europe and America
RA 1141	‘A 117-15’	Hungary	Hungary	Modern	‘Alsószentiváni 117’ × ‘Pedro’	Admixed
RA 0488	‘Adams 10’	U.S.A.	China	Selection	Open-pollination of Manregian walnut	Eastern Europe and Asia
RA 0399	‘Allemagne 139’ ('Geisenheim 139')	Germany	Germany	Selection	-	Western Europe and America
RA 0298	‘Amigo’	U.S.A.	U.S.A.	Modern	‘Sharkey’ × ‘Marchetti’	Admixed
RA 1135	‘AS 1’	Spain	Chile	Selection	-	Western Europe and America
RA 0982	‘Ashley’	U.S.A.	U.S.A., California	Selection	Chance seedling	Western Europe and America
RA 1176	‘Bijou’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 0065	‘Candelou’	France	France, Midi-Pyrénées	Landrace	-	Western Europe and America
RA 0381	‘Carmelo’	U.S.A.	U.S.A., California	Selection	Open-pollination of ‘Payne’	Western Europe and America
RA 0370	‘Chaberte’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 1036	‘Chandler’	U.S.A.	U.S.A.	Modern	‘Pedro’ × [‘Sharkey’ × ‘Marchetti’]	Western Europe and America
RA 0493	‘Chase C7’	U.S.A.	China	Selection	Open-pollination of Manregian walnut	Eastern Europe and Asia
RA 0223	‘Cheinovo’ ('Sheinovo', 'Sheynovo')	Bulgaria	Bulgaria	Landrace	-	Eastern Europe and Asia
RA 0430	‘Chico’	U.S.A.	U.S.A.	Modern	‘Sharkey’ × ‘Marchetti’	Admixed
RA 1060	‘Cisco’	U.S.A.	U.S.A.	Modern	‘Meylan’ × ‘Pedro’	Western Europe and America
RA 0095	‘Corne’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 0354	‘Corne’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 0066	‘Corne’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 0968	‘Culplat’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 1207	‘Del Carril’	Spain	Spain, Murcia	Landrace	-	Western Europe and America
RA 1106	‘EAA 6’	Greece	Greece, Central Greece	Selection	-	Eastern Europe and Asia
RA 1126	‘Early Ehrhardt’	U.S.A.	U.S.A.	Selection	Open-pollination or budsport of ‘Ehrhardt’	Western Europe and America
H 103-12	‘Feradam’	France	France	Modern	‘Adams 10’ × ‘Chandler’	Admixed
H 108-4	‘Ferbel’	France	France	Modern	‘Chandler’ × ‘Lara®’	Western Europe and America
H 102-3	‘Ferjean’	France	France	Modern	‘Grosvert’ × ‘Lara®’	Western Europe and America
H 94-11	‘Fernette’	France	France	Modern	‘Franquette’ × ‘Lara®’	Admixed
H 94-12	‘Fernor’	France	France	Modern	‘Franquette’ × ‘Lara®’	Western Europe and America
H 100-30	‘Ferouette’	France	France	Modern	‘Franquette’ × ‘Howard’	Western Europe and America

H 106-28	‘Fertignac’	France	France	Modern	‘Ronde de Montignac’ × ‘Chandler’	Western Europe and America
RA 1229	‘Forde’	U.S.A.	U.S.A.	Modern	UC 61-25 × ‘Chico’	Admixed
RA 0319	‘Franquette’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 0212	‘Franquette’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 0314	‘Franquette’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 0311	‘Franquette’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 0468	‘Geisenheim 286’	Germany	Germany	Selection	-	Western Europe and America
RA 1050	‘Germisara’	Romania	Romania	Selection	-	Eastern Europe and Asia
RA 1228	‘Gillet’	U.S.A.	U.S.A.	Modern	[‘Chandler’ × UC 61-25] × ‘Chico’	Admixed
RA 1101	‘Gran Jefe’	Spain	Spain, Murcia	Landrace	-	Western Europe and America
RA 0059	‘Grandjean’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 0289	‘Grosjean’	France	France, Midi-Pyrénées	Landrace	-	Western Europe and America
RA 0118	‘Grosvert’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 0439	‘Hartley’	U.S.A.	U.S.A.	Modern	‘Franquette’ × ‘Mayette’	Western Europe and America
RA 1037	‘Howard’	U.S.A.	U.S.A.	Modern	‘Pedro’ × [‘Sharkey’ × ‘Marchetti’]	Western Europe and America
RA 1098	‘Howe’	U.S.A.	U.S.A., Oregon	Selection	Chance seedling	Western Europe and America
H 95-87	Hybrid INRA	France	France	Modern	‘Meylannaise’ × ‘Payne’	Western Europe and America
H 91-88	Hybrid INRA	France	France	Modern	‘Franquette’ × ‘Payne’	Western Europe and America
H 110-34	Hybrid INRA	France	France	Modern	‘Fernette’ × ‘Serr’	Eastern Europe and Asia
H 109-3	Hybrid INRA	France	France	Modern	H 92-28 × ‘Chandler’	Western Europe and America
H 118-13	Hybrid INRA	France	France	Modern	‘Fernor’ × ‘Chandler’	Western Europe and America
H 99-104	Hybrid INRA	France	France	Modern	‘Franquette’ × ‘Chandler’	Western Europe and America
H 113-24	Hybrid INRA	France	France	Modern	‘Fernette’ × ‘Chandler’	Western Europe and America
H 117-12	Hybrid INRA	France	France	Modern	‘Fernor’ × H 101-2	Western Europe and America
H 119-13	Hybrid INRA	France	France	Modern	‘Fernor’ × RA 1106	Eastern Europe and Asia
H 122-49	Hybrid INRA	France	France	Modern	‘Fernor’ × UK 41-17	Admixed
H 122-27	Hybrid INRA	France	France	Modern	‘Fernor’ × UK 41-17	Admixed
H 122-25	Hybrid INRA	France	France	Modern	‘Fernor’ × UK 41-17	Admixed
H 122-4	Hybrid INRA	France	France	Modern	‘Fernor’ × UK 41-17	Admixed
H 121-8	Hybrid INRA	France	France	Modern	‘Fernor’ × UK 41-10	Admixed
H 120-3	Hybrid INRA	France	France	Modern	‘Fernor’ × UK 11-4	Admixed
H 122-22	Hybrid INRA	France	France	Modern	‘Fernor’ × UK 41-17	Admixed
H 122-33	Hybrid INRA	France	France	Modern	‘Fernor’ × UK 41-17	Admixed
H 92-28	Hybrid INRA	France	France	Modern	‘Franquette’ × ‘Ashley’	Western Europe and America
H 107-35	Hybrid INRA	France	France	Modern	‘Lara®’ × ‘Chandler’	Western Europe and America
H 93-63	Hybrid INRA	France	France	Modern	‘Franquette’ × ‘Pedro’	Western Europe and America
H 101-2	Hybrid INRA	France	France	Modern	‘Grosvert’ × ‘Chandler’	Western Europe and America
H 94-5	Hybrid INRA	France	France	Modern	‘Franquette’ × ‘Lara®’	Admixed
H 102-15	Hybrid INRA	France	France	Modern	‘Grosvert’ × ‘Lara®’	Western Europe and America
RA 1074	‘Izvor 10’	Bulgaria	Bulgaria	Landrace	-	Eastern Europe and Asia
RA 0983	J. regia A	France	France	Selection	-	Western Europe and America
RA 0984	J. regia B	France	France	Selection	-	Western Europe and America
RA 0996	J. regia E	France	France	Selection	-	Western Europe and America
RA 0998	J. regia IX	France	France	Selection	-	Western Europe and America
RA 0930	J. regia R41	France	France	Selection	-	Western Europe and America
RA 0932	J. regia R462	France	France	Selection	-	Western Europe and America

RA 1190	‘Jin Long 1	China	China	Selection	-	Eastern Europe and Asia
RA 1117	‘Kasni Rodni’	Serbia	Serbia	Selection	-	Western Europe and America
RA 1031	‘Kfar Hanania’	Israel	Israel	Selection	-	Admixed
RA 0102-3	‘Lalande’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 0480	‘Lara®’	France	U.S.A.	Selection	Open-pollination of ‘Payne’	Admixed
RA 0967	‘Le Bordelais’	France	France, Aquitaine	Landrace	-	Western Europe and America
RG 2	‘Liba’	France	France	Selection	-	Western Europe and America
RA 0391	‘Lieb Mayette’ ('Leib Mayette')	France	U.S.A.	Selection	-	Western Europe and America
RA 0248	‘Lozeronne’	France	France, Rhône-Alpes	Selection	-	Western Europe and America
RA 1187	‘Lu Guang’	China	China	Selection	-	Eastern Europe and Asia
RA 0611	‘Lub’	France	France	Selection	-	Western Europe and America
RA 1140	‘M 10-37’	Hungary	Hungary	Modern	‘Milotaï n°10’ × ‘Pedro’	Western Europe and America
RA 0306	‘Marbot’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 0459	‘Marbot’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 0097	‘Marbot’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 1103	‘Marchetti’	U.S.A.	U.S.A.	Selection	Maybe ‘Eureka’ × ‘Payne’	Western Europe and America
RA 1065	‘Maribor’	Slovenia	Slovenia	Selection	-	Western Europe and America
RA 0474	‘Mayette’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 0058	‘Mayette’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 1197	MB CO 45	Spain	Spain, Galicia	Selection	-	Western Europe and America
RA 1198	MB LU 21	Spain	Spain, Galicia	Selection	-	Western Europe and America
RA 1199	MB PO 2	Spain	Spain, Galicia	Selection	-	Western Europe and America
RA 1200	MB PO 3	Spain	Spain, Galicia	Selection	-	Western Europe and America
RA 1201	MB PO 55	Spain	Spain, Galicia	Selection	-	Western Europe and America
RA 1203	MBT 159	Spain	Spain, Catalonia	Selection	-	Western Europe and America
RA 1204	MBT 218	Spain	Spain, Catalonia	Selection	-	Western Europe and America
RA 1205	MBT 231	Spain	Spain, Catalonia	Selection	-	Western Europe and America
RA 1206	MBT 232	Spain	Spain, Catalonia	Selection	-	Western Europe and America
RA 1202	MBT 40	Spain	Spain, Catalonia	Selection	-	Western Europe and America
RA 1019	‘Meylannaise’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 0953	‘Midland’	U.S.A.	U.S.A.	Modern	‘Franquette’ × ‘Payne’	Western Europe and America
RA 0498	‘Milotai n°10’	Hungary	Hungary	Selection	-	Eastern Europe and Asia
RA 1168	‘Mire’	Slovenia	Slovenia	Selection	-	Western Europe and America
RA 0489	‘Moyer’	U.S.A.	U.S.A., Oregon	Selection	Chance seedling	Western Europe and America
RA 0394	‘Northdown Clawnut 252’	France	England, Northdown	Selection	-	Western Europe and America
RA 0475	‘Parisienne’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 0473	‘Parisienne’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 0216	‘Parisienne’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 0385	‘Payne’	U.S.A.	U.S.A., California	Selection	French cultivar × Chinese seedling	Western Europe and America
RA 1038	‘Pedro’	U.S.A.	U.S.A.	Modern	‘Conway Mayette’ × ‘Payne’	Western Europe and America
RA 1023	PI 14 23 23	U.S.A.	Poland, Lublin	Selection	-	Eastern Europe and Asia
RA 1104	PI 15 95 68	U.S.A.	Afghanistan	Selection	Open-pollination of PI 12 74 60 (Afghanistan)	Eastern Europe and Asia
RA 1014	PI 2 657 12	U.S.A.	Russia, Sochi	Selection	-	Eastern Europe and Asia
RA 0976	‘Plovdivski’	Bulgaria	Bulgaria	Landrace	-	Admixed
RA 1215	Pourpre Hollande	Netherlands	-	Selection	-	Admixed
RA 0061	‘Quenouille’ ('Moussine')	France	France, Aquitaine	Landrace	-	Western Europe and America

RA 1090	‘Rego’	Portugal	Portugal	Selection	-	Western Europe and America
RA 1221	‘Robert Livermore’	U.S.A.	U.S.A.	Modern	‘Howard’ × ‘Rouge de la Réole’	Western Europe and America
RA 0367-2	‘Romaine’	France	France, Rhône-Alpes	Landrace	-	Western Europe and America
RA 0038	‘Ronde de Montignac’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 1182	‘Rosée de Montmorin’	France	France, Provence-Alpes-Côte d'Azur	Landrace	-	Western Europe and America
RA 1041	‘Rouge de la Donau’ ('Geisenheim 1239')	Switzerland	Austria	Landrace	-	Western Europe and America
RA 1088	‘Rouge de la Réole’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 1089	‘Rouge de Laquenexy’ ('Geisenheim 509')	France	France, Moselle	Landrace	-	Western Europe and America
RA 1209	S 1 A Diane	Greece	Greece, Crete	Selection	-	Western Europe and America
RA 1212	S 1 B Ariane	Greece	Greece, Crete	Selection	-	Western Europe and America
RA 1211	S 14 B Sparte	Greece	Greece, Peloponnese	Selection	-	Admixed
RA 1208	S 28 A Achille	Greece	Greece, Central Greece	Selection	-	Eastern Europe and Asia
RA 1213	S 34 B Pyrrus	Greece	Macedonia	Selection	-	Eastern Europe and Asia
RA 1210	S 4 B Thétis	Greece	Greece, Crete	Selection	-	Eastern Europe and Asia
RA 0179	‘Saint Jean’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 0147	‘Saint Jean’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 0117	‘Saint Martial’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 0429	‘Scharsch – Franquette’	U.S.A.	France, Rhône-Alpes	Selection	-	Western Europe and America
RA 0828	Semence comité Dordogne	France	France, Aquitaine	Selection	-	Western Europe and America
RA 0499	‘Serr’	U.S.A.	U.S.A.	Modern	‘Payne’ × PI 15 95 68 (Afghanistan)	Admixed
RA 1230	‘Sexton’	U.S.A.	U.S.A.	Modern	‘Chandler’ × UC 85-8 (China)	Eastern Europe and Asia
RA 1032	‘Shinrei’	Japan	Japan	Landrace	-	Eastern Europe and Asia
RA 0948	‘Sibisel 39’	Romania	Romania	Selection	-	Eastern Europe and Asia
RA 0409	‘Sibisel 44’	Romania	Romania	Selection	-	Eastern Europe and Asia
RA 0137	‘Solèze’	France	France, Aquitaine	Landrace	-	Western Europe and America
RA 1029	‘Sopore’	India	India, Kashmir	Selection	-	Eastern Europe and Asia
RA 0962	Souvenir du congrès	France	France, Aquitaine	Selection	-	Western Europe and America
RA 0491	‘Spurgeon’	U.S.A.	U.S.A., Washington	Selection	Maybe open-pollination of 'Franquette'	Western Europe and America
RA 0447	‘Tehama’	U.S.A.	U.S.A.	Modern	‘Waterloo’ × ‘Payne’	Western Europe and America
RA 0472	‘Trinta’	U.S.A.	U.S.A., California	Selection	Open-pollination of ‘Waterloo’	Western Europe and America
RA 1099	‘Tulare’	U.S.A.	U.S.A.	Modern	‘Tehama’ × ‘Serr’	Admixed
RA 1161	UC77012	U.S.A.	U.S.A.	Selection	-	Western Europe and America
RA 0119	‘Verdelet’	France	France, Aquitaine	Landrace	Maybe open-pollination of 'Grandjean'	Western Europe and America
RA 0428	‘Vina’	U.S.A.	U.S.A.	Modern	‘Franquette’ × ‘Payne’	Western Europe and America
RA 1175	‘VL25B’	Romania	Romania	Selection	-	Admixed
RA 1174	‘VL4B’	Romania	Romania	Selection	-	Eastern Europe and Asia
RA 0386	‘Waterloo’	U.S.A.	U.S.A., California	Selection	Open-pollination of ‘Eureka’	Western Europe and America
RA 0492	‘Wepster W2’	U.S.A.	China	Selection	Open-pollination of Manregian walnut	Eastern Europe and Asia
RA 1166	‘Z 53’	Iran	Iran, Zabad	Selection	-	Eastern Europe and Asia