

Morphological and Pomological Variability Analysis of Walnut (*Juglans regia* L.) Genetic Resources from the Middle and High Atlas of Morocco

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Received: October 12, 2018 / Accepted: December 1, 2018

Abstract

The walnut (*Juglans regia* L.) is conducted yet as a traditional fruit crop in different environments of Moroccan mountains. Besides, little data is available on its genetic diversity. Thus, twenty-one morphological and pomological characters of 11 Moroccan accessions were used to assess phenotypic variation of this species. Significant differences were found between accessions for the most examined traits, indicating a high phenotypic diversity. Multivariate analyses lead to identify three groups of accessions. The first group composed by the accessions of both Middle and High Atlas Mountain with high weight and percentage of kernel and low thickness of shell. The second group is made of accessions from the High Atlas Mountain characterised by large leaf, high nut dimension and large nut size. The third group is composed of two accessions; one belongs to the Middle Atlas Mountain and the other originating from High Atlas Mountain and characterized by small leaf and nut. Then, the structuration of accessions in three groups was not correlated to the mountain range type. So, this study showed a significant phenotypic heterogeneity between the eleven studied accessions which opens the way for the strategies for their conservation and the selection of efficient genotypes with the desired traits.

Keywords: Walnut; *Juglans regia* L; Morocco's accessions; Leaf variability; Fruit variability.

Introduction

The walnut species (*Juglans regia* L.) is one of the most widely cultivated and economically important species of edible nuts in the temperate regions of the world (McGranahan and Leslie, 1990). This species is originated from areas of Central Asia (Amiri et al., 2010) and its cultivation and production were centered in USA and China (Angmo et al., 2013). Throughout history, the nuts have been a staple food providing energy, protein, essential fatty acids, vitamins and minerals. They are also being investigated for their potential health benefits (Dreher et al., 1996). The walnut tree has a great nutritional value and the noble wood. Moreover, walnuts have significant economic value and medicinal importance for human health, because of their biochemical composition of polyunsaturated fatty acids, especially linoleic acid and alpha-linolenic and protein value (Savage et al., 2001).

The first introduction of this species, as a traditional fruit crop, to the great Maghreb was attributed to the Romans (Germain, 1992). In Morocco, walnut trees cover an area of 7600 Ha and are cultivated in different environments (Lansari et al., 2001). The trees can be found in humid and warm conditions, in the Rif and Atlas Mountains (High and Middle Atlas) and in arid regions in Southeastern Morocco. More than half of the plantings resulted from the prevailing way of seed propagation practiced by farmers, since grafting is less adopted (Lansari et al., 2001). Genetic research on Moroccan *Juglans regia* has received little attention and few studies have concerned its genetic diversity. Only one study of phenotypic variability of few South-Eastern populations was carried out by Kodad et al. (2014). Consequently, it would be very interesting to find more

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discriminating criteria that could provide additional information on genetic diversity of Moroccan walnut populations. Thus, the present study was conducted to characterize and evaluate the genetic variability of walnut accessions using some morphological and pomological traits and to look for any space structuring of such accessions according to their distribution on the Atlas Mountains in Morocco.

Materials and Methods

Plant Material and Phenotyping Traits

During September 2014, fresh leaves and mature nuts were collected from eleven Moroccan accessions representing the main cropping area of walnut. Table 1 and Figure 1 present the accessions investigated as well the characteristics of their geographical and ecological origins. For each accession, 10 trees were randomly chosen and sampled. From each tree, ten developed leaves and 20 nuts were collected from different sites and elevation of the tree to be subject of observations and measurements considered in the present study.

According to the IPGRI and UPOV descriptors (IPGRI, 1994; UPOV, 1999), 31 parameters related to leaves and fruits (Table 2) were considered, following instructions given by the IPGRI and UPOV descriptors. All traits on the kernel have been measured one month after harvest when the water content was below 8% (UPOV, 1999).

Statistical Analyses

The obtained data was submitted to Analysis of variance (ANOVA) to search for any significant differences among accessions for the parameters analyzed. When significant differences occurred among accessions, a LSD test was used to determine if there were significant differences between individual accessions. Association between traits was established using Pearson correlation coefficient. All these analyses were performed by using Statistical Analysis System program (SAS, 1999). Accessions ordination and classification were performed using the principal component analysis (PCA) (XLSTAT, 2015) and the hierarchical cluster analysis (Statistica StatSoft, 1997). The PCA and the cluster analyses were carried out on the matrix of mean values of measured characters.

Table 1. Geographic origin and ecological characteristics of the 11 walnut (*Juglan regia* L.) accessions.

Accessions	Code	Geographic Origin	Altitude (m)	Latitude	Longitude	Zone	Rainfall Average (mm)
				N	W		
Aghbala	AGH	32 Km North East of Aghbala	1673	32°32'	5°39'	Middle Atlas	450
Naour	NAO	Central Naour	1300	32°29'	5°58'	Middle Atlas	600
Taghzirte	TAG	12 Km East of Tagzirt	650	32 26	6° 12'	Middle Atlas	700
Ait Bougamez	ABZ	Ait Bougamez Centre	1996	31°38'	6° 28'	High Atlas	580
Ait M'hamed	AMD	20 Km South East of Azilal	1728	31° 25'	2° 28'	High Atlas	450
Demnate	DEM	3 km South East of Demnate	932	31° 43'	6° 58'	High Atlas	350
Imlil	IML	17 km South of Asni	1763	31° 8'	7° 55'	High Atlas	459
Anougal	ANG	40 km South of Amzmiz	1569	31° 9'	8° 15'	High Atlas	681
Beram	BER	5 km South of Midelt	1521	32° 40'	4° 44'	High Atlas	210
Amouguer	AMG	40 km West of Rich	1569	32° 12'	5° 8'	High Atlas	250
Tabrijjate	TBR	70 km East of Imilchil	1831	32° 16'	4° 56'	High Atlas	319

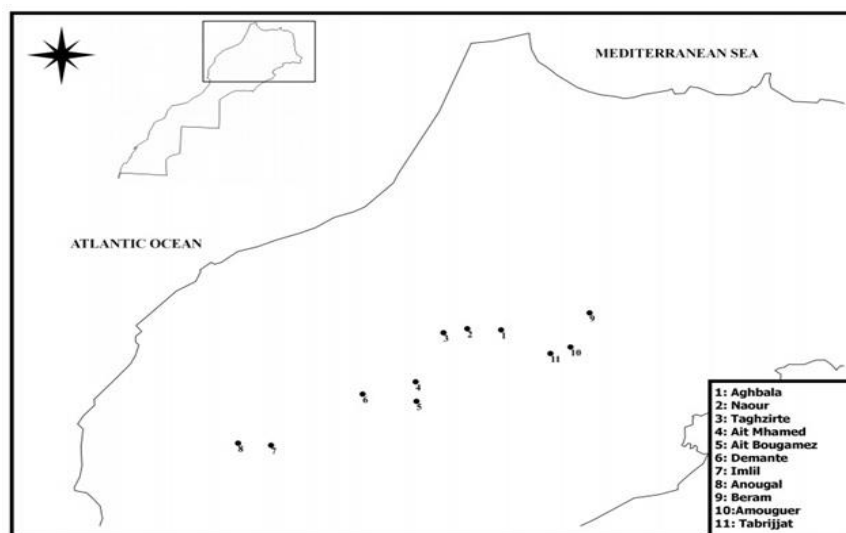


Figure 1. Morocco map showing sampling sites of the analyzed walnut accessions.

Table 2. Morphological and pomological traits analyzed in the 11 walnut accessions.

-Leaf traits		Width of Pad on Suture	WPS
Leaf Length	LL	Prominence of Pad on Suture	PPS
Leaf Width	LW	Shape of Base Perpendicular to Suture	SBPS
Number of Leaflet	Nlet	Shape of Apex Perpendicular to Suture	SAPS
Leaflet Shape	LetS	Prominence of Apical Tip	PAT
Leaflet Width	LetW	Structure of Surface of Shell	SSSh
Leaflet Length	LetL	Shell Colour	ShC
Leaflet Margin	LetM	Shell Strength	ShS
Leaf Colour	LC	Adherence of Two Halves of Shell	ATHSh
Rachis Colour	RC	Thickness of Shell	TSh
-Nut traits		-Kernel traits	
Nut Shape in Longitudinal section through Suture	NSLS	Ease of Removal of Kernel	ERK
Nut Shape in Longitudinal section Perpendicular to Suture	NSLPS	Kernel Weight	KW
Nut Width	NW _{th}	Kernel Percentage	KP*
Nut Length	NL	Kernel Fill	KF
Nut Weight	NW	Kernel Colour	KC
Nut: Position of Pad on Suture	NPPS	Kernel Flavor	KFV

Kernel percentage = Kernel weigh/nut weight*100

Results

Mean values of morphological and pomological characters measured and their standard deviations are presented in Table 3. Results of analysis of variance indicated significant differences between accessions for all parameters except number of leaflets and leaflet margin.

Leaf Morphological Traits

Morphological traits related to leaf show large variability between accessions. In fact, leaf length varied among accessions from 37.98 cm for Aghbala to 44.25 cm for Anougal with an average of 41.52cm, the leaf width, with a general mean of 25.85cm, ranged from 24.46 cm for Taghzirte to 27.17 cm for Beram and the number of leaflets scaled from 7.38 for Naour to 8.75 for Taghzirte with an average of 8.24. In addition, leaflet length, having an average of 10.75 cm, varied from 9.37 cm for Taghzirte to 11.16 cm for Imlil. With a general mean of 5.48 cm, the highest value of leaflet width was recorded in Tabrijjate (6.22 cm) and the lowest was observed in Taghzirte (4.98 cm). However, the number of leaflets per leaf has varied narrowly among accessions, within a range of 7.3 for Naour to 8.75 for Taghzirte, and averaged 8.24. Furthermore, with an entire leaflet margin for almost all, the accessions did not show any difference for this trait. In general, the color of leaf was green with a rachis color varied between green, yellow to red.

Fruit Pomological Traits

The nut weight for the accessions tested varies within the range of 8.26 g for Aghbala to 10.20 g for Tabrijjate, with an

average of 9.48 g. The average nut length was 35.46 mm and ranged from 32.08 mm for Demnate to 38.11 mm for Imlil. For nut width, the lowest value was obtained for Demnate (27.39 mm) and the highest for Tabrijjate (31.50 mm) with a mean of 30.10 mm. The shell thickness of the studied accessions in this investigation varied between 1.33 mm for Amouguer and 1.83 mm for Ait M'hamed with a value of 1.60 mm as average. Regarding the kernel weight, the highest value was observed in Amouguer (4.13 g), while the least was obtained for Aghbala (2.86 g), with a mean of 3.71g. The kernel percentage ranged from 33.65% in Demnate to 43.76% in Amouguer and averaged 38.28%. For nut shape, in general, it was elliptic board with a medium shell color and intermediate shell strength. Concerning kernel color, 9% of the walnut accessions were revealed with light kernel, 36% with amber kernel and 55% with light amber kernel. For kernel flavor, it was revealed satisfactory for all walnut accessions. For the remaining analyzed traits, they have varied widely among accessions indicating the existence of high pomological variability in Moroccan walnut germplasm.

Association Between Parameters Studied

Correlation among all morphological and pomological traits is summarized in table 4. The strongest negative correlations were observed between, on one hand, kernel weight (KW) and, on the other hand, leaf length, (LL), leaf width (LW), leaflet length (LetL), nut length (NL), nut weight (NW), shape of base perpendicular to suture (SBPS) and kernel colour (KC), with respective coefficients of -0.95, -0.93, -0.93, -0.92, -0.91, -0.95 and -0.96. However, strong positive correlations were found between kernel weight (KW) and number of leaflet (Nlet) ($r=0.97$), nut shape (NSLPS) ($r=0.97$), prominence of apical

Table 3. Mean values and standard deviation for leaf, nut and kernel traits measured in 11 Moroccan walnut accessions.

Traits	Taghzirte	Tabrijjate	Amouguer	Anougal	Ait Mhamed	Imilil	Beram	Aghbala	Naour	Ait bougamez	Demnate	Mean	F
LL (cm)	39.14±3.19a	42.14±3.76b	39.39±4.14a	44.25±6.51b	43.35±5.54b	44.14±5.88b	42.80±4.19b	37.98±3.85a	39.28±4.06a	40.17±4.75a	44.08±3.12a	41.52	4.68***
LW (cm)	24.46±2.20ab	25.91±4.29a	27.11±3.06c	25.47±3.95a	24.90±2.99a	27.03±3.67c	27.17±3.09c	24.74±4.11a	25.50±6.42b	25.46±3.20a	26.65±4.09c	25.85	6.23***
Nlet	8.75±0.62b	7.5±1.06 a	8.05±1.03ac	8.34±0.90bc	8.62±0.68b	8.56±0.98b	8.67±0.94b	7.6±1.03a	7.38±1.66a	8.37±0.83bc	8.60±0.77b	8.24	1.77
LetS	2.46±0.76ab	2.95±0.20c	2.52±0.64b	2.42±0.65ab	2.77±0.42bc	2.93±0.24c	2.52±0.66b	2.20±0.84a	2.3±0.59ab	2.67±0.70bc	2.93±0.36c	2.61	3.33***
LetW (cm)	4.98±0.77a	6.22±0.96c	5.31±0.67ab	5.75±1.51bc	5.59±0.78b	5.49±0.86b	5.34±0.80ab	5.35±0.95ab	5.56±1.02b	5.27±0.81ab	5.40±1.25ab	5.48	4.33***
LetL (cm)	9.37±1.42a	11.02±1.68b	10.78±1.37b	10.72±1.84b	10.51±1.14b	11.16±1.57b	10.53±1.56b	10.48±1.74b	10.65±2.01b	10.59±1.40b	10.49±1.65b	10.57	3.09**
LetM	1±00a	1±00a	1±00a	1±00a	1±00a	1±00a	1±00a	1±00a	1±00a	1±00a	1±00a	1.00	1.22
LC	5.68±1.09b	5.66±0.96b	4.78±1.01a	5.45±1.29b	5±00a	4.81±1.55a	5±00a	5.33±1.06b	5±00a	5.12±0.49a	5±00a	5.17	3.75***
RC	3.68±1.30a	3.33±0.96a	4.63±0.78c	3±00a	4.18±1.00bc	4.43±1.84bc	4.11±1.00b	4±1.36b	6.86±0.50d	4.06±1.01b	3.06±0.36a	4.12	4.14***
NSLS	6.32±1.74cd	4.97 ±1.97a	6.43±2.02c	5.27±1.96a	5.78±2.13c	5.34±2.58b	5.86±1.92c	6.57±1.13c	5.57±2.05a	6.23±1.70c	6.97±1.65d	5.95	10.34***
NSLPS	6.65±0.72d	4.77 ±2.39a	6.70±1.30c	5.94±1.56c	5.54±2.11c	5.01±2.44b	6.09±1.71c	6.65±1.10c	5.54±2.25a	6.35±1.87dc	6.26±1.81d	5.97	6.67***
NWth (mm)	29.67±2.47b	31.50±3.17a	29.18±2.69b	31.24±2.74c	31.36±4.60a	31.39±2.55c	29.77±3.29ab	29.50±1.95b	30.13±2.87b	30.30±1.94a	27.39±8.81a	30.10	19.87***
NL (mm)	34.13±2.81b	35.07±3.62bc	35.11±3.53c	37.02±3.36d	36.45±4.63a	38.11±3.27c	34.84±4.36ac	35.23±2.12ac	36.08±3.77a	36.74±3.13d	32.08±10.82a	35.46	17.27***
NW (g)	9.24±1.61c	10.20±2.11b	9.42±2.83c	9.84±2.14b	10.14±4.00b	9.25±2.12c	9.71±2.64b	8.26±2.05b	9.79±2.94b	9.54±1.55b	8.91±3.51b	9.48	7.61***
NPPS	2.15±0.75d	1.58±0.75b	1.61±0.91b	1.22±0.48a	1.73±0.68b	1.74±0.76b	1.86±0.77c	1.96±0.93c	2.15±0.65d	2.44±0.79e	2.03±1.04c	1.86	13.12***
WPS	3.94±0.99b	4.68±0.80ef	4.84±0.73f	5.24±0.86g	4.60±1.53e	5.04±0.94g	4.36±0.97cd	4.34±0.94d	4.12±1.31c	3.59±0.93a	3.70±0.95a	4.40	8.95***
PPS	4.32±1.10a	4.94±0.58c	4.60±1.13b	4.86±0.86c	4.64±0.76b	5.02±0.92cd	5.26±1.12d	4.98±0.77c	4.19±0.99a	4.48±0.96b	4.51±1.37b	4.71	6.2***
SBPS	1.77±0.50b	2.15±0.62d	1.45±0.55a	2.19±0.51d	2.02±0.47c	2.20±0.63d	1.78±0.71b	1.54±0.73a	2.06±0.93c	1.72±0.69b	1.82±1.20a	1.88	9.25***
SAPS	3.06±0.64d	2.84±0.58bc	2.68±0.46b	2.69±0.54b	2.41±0.55a	2.24±0.80a	2.85±0.85c	2.25±0.53a	2.17±0.87a	2.62±0.73b	3.03±0.94c	2.62	11.3***
PAT	3.05±0.64a	3.87±0.99b	3.54±0.95a	3.94±1.09b	3.72±1.03ab	4.9±0.77d	3.99±1.20b	4.4±1.16c	3.58±0.92a	4.02±1.10b	3.89±1.09b	3.90	3.05***
SSSh	2±00bc	2.38±0.49d	1.76±0.65a	2.35±0.64d	2.62±0.86ef	2.51±0.64e	2.70±0.81f	2.65±0.55ef	2.16±0.36c	2.14±0.37c	2.10±0.30bc	2.31	18.32***
ShC	6.42±1.48f	4.40±2.48cd	3.96±1.71bc	4.15±1.19c	5.15±2.20e	5.25±1.45e	3.86±1.45b	3.88±1.66b	4.40±1.99d	3.37±1.65a	4.97±0.61e	4.53	40.79***
ShS	4.70±0.77b	5.48±1.15d	4.68±0.93b	5.53±1.35d	5.27±0.91d	4.34±1.10a	5.32±0.88cd	5.1±0.99c	5.18±0.70cd	4.33±1.00a	5.71±1.83d	5.06	7.19***
ATHSh	4.65±0.78b	5.24±0.73d	4.47±0.88b	4.18±1.30a	5.21±1.02d	4.30±1.25ab	5.15±1.15cd	5.12±1.01cd	5.5±0.99e	4.35±1.00ab	4.96±0.84c	4.83	9.84***
TSh (mm)	1.57±0.32c	1.71±0.30d	1.33±0.40a	1.69±0.46d	1.83±0.51e	1.55±0.38c	1.66±0.41d	1.50±2.88bc	1.61±0.36cd	1.44±0.38b	1.72±0.34d	1.60	4.07***
DRK	4.3±1.01a	5.7±1.22a	4.56±0.98b	5.28±1.18d	4.87±1.36c	4.7±1.34bc	4.66±1.41bc	4.3±1.16a	4.79±1.14c	4.25±0.99a	5.24±1.32d	4.79	8.58***
KW (g)	3.71±1.12bc	4.02±1.15cc	4.13±1.48*	3.62±1.38bc	3.61±1.36bc	3.87±1.36c	3.78±1.27bc	2.86±1.11a	3.77±1.69bc	4.01±1.12ce	3.45±1.25b	3.71	5.56***
KP (%)	39.45±8.94cd	38.72±7.23c	43.76±9.74e	35.49±9.40ab	36.71±11.7b	41.16±10.23d	38.8±8.14c	33.61±10.03a	37.73±9.67c	42.01±8.42de	33.65±9.90ab	38.28	5.3***
KF	6.39±1.17d	6.45±1.24d	5.82±1.50bc	5.26±1.58a	6.31±1.33cd	5.60±1.62b	6±1.37c	5.56±1.68b	6.27±1.12d	6.45±1.15d	6.24±1.34cd	6.03	9.55***
KC	3.6±0.58	2.72±0.96	3.32±0.66	3.21±0.76	2.94±0.78	3.36±0.89	3.22±0.69	2.57±0.99	3.25±0.82	2.73±0.78	3.25±0.51	3.11	26.07***
KFV	1.06±0.25	1.04±0.20	1.05±0.22	1.30±0.46	1.06±0.25	1.15±0.36	1.07±0.26	1.31±0.46	1.02±0.14	1.02±0.14	1.08±0.28	1.11	7.83***

Significance level: ***: P<0.001; **: P<0.01; *: P<0.05. Different letters note significant differences (LSD at 0.05 level). In bold are minimum and maximum values

Table 4. Correlation coefficients among leaf, nut and kernel traits in the studied accessions.

	LL	LW	Nlet	LetS	LetW	LetL	LetM	LC	RC	NSLS	NSLPS	NW _{in}	NL	NW	NPSP	WPS	PPS	SBPS	SAPS	PAT	SSh	SHC	SIS	ATHSh	TSh	DRK	KW	KP	KF	KC		
LW	0.97***																															
Nlet	-0.95***	-0.93***																														
LetS	-0.95***	-0.93***	0.98***																													
LetW	-0.59***	-0.58***	0.732***	0.71***																												
LetL	0.96***	0.95***	-0.93***	-0.93***	-0.56***																											
LetM	-0.83***	-0.82***	0.85***	0.84***	0.62***	-0.82***																										
LC	0.15***	0.14***	-0.10***	-0.13***	-0.02	0.17***	-0.07																									
RC	-0.36***	-0.32***	0.38***	0.42***	0.29***	-0.37***	0.24***	-0.24***																								
NSLS	-0.04	0.01	0.03	0.05	0.05	-0.03	-0.06	0.48***																								
NSLPS	-0.95***	-0.93***	0.97***	0.97***	0.69***	-0.93***	0.84***	-0.13***	0.38***	0.05																						
NW _{in}	-0.59***	-0.57***	0.68***	0.7***	0.64***	-0.56***	0.56***	0.001	0.36***	0.06	0.68***																					
NL	0.95***	0.93***	-0.91***	-0.93***	-0.51***	0.93***	-0.80***	0.19***	-0.38***	-0.01	-0.91***	-0.47***																				
NW	0.92***	0.90***	-0.90***	-0.91***	-0.58***	0.92***	-0.81***	0.17***	-0.37***	-0.02	-0.91***	-0.49***	0.94***																			
NPSP	-0.72***	-0.72***	0.73***	0.73***	0.52***	-0.71***	0.83***	-0.06	0.15***	-0.08*	0.74***	0.52***	-0.69***	-0.71***																		
WPS	-0.23***	-0.21***	0.25***	0.24***	0.20***	-0.17***	0.21***	0.41***	-0.05	-0.13*	0.25***	0.29***	-0.17***	-0.13***	0.22***																	
PPS	-0.27***	-0.23***	0.30***	0.34***	0.28***	-0.29***	0.17***	0.62***	0.33***	0.30***	0.30***	0.30***	-0.29***	-0.28***	0.09*	-0.09*																
SBPS	0.98***	0.95***	-0.95***	-0.95***	-0.60***	0.95***	-0.83***	0.16***	-0.38***	-0.005	-0.95***	-0.59***	0.95***	0.92***	-0.72***	0.25***	0.11**	-0.44***														
SAPS	-0.47***	-0.45***	0.59***	0.56***	0.57***	-0.45***	0.50***	0.01	0.17***	-0.05	0.57***	0.56***	-0.42***	-0.45***	0.43***	0.25***	0.11**	-0.44***														
PAT	-0.94***	-0.93***	0.96***	0.98***	0.67***	-0.92***	0.83***	-0.15***	0.43	0.06	0.96***	0.70***	-0.92***	-0.90***	0.72***	0.23***	0.36***	-0.94***	0.59***													
SSh	-0.79***	-0.77***	0.84***	0.84***	0.74***	-0.78***	0.70***	-0.15***	0.38***	0.07	0.84***	0.67***	-0.72***	-0.76***	0.61***	0.17***	0.31***	-0.79***	0.66***	0.87***												
SHC	0.87***	0.85***	-0.84***	-0.84***	-0.53***	0.89***	-0.76***	0.17***	-0.33***	-0.002	-0.84***	-0.47***	0.85***	0.86***	-0.68***	-0.14**	-0.24***	0.88***	-0.34***	-0.84***	-0.69***											
SHS	0.89***	0.87***	-0.85***	-0.87***	-0.51***	0.87***	-0.65***	0.19***	-0.42***	-0.09*	-0.86***	-0.50***	0.88***	0.85***	-0.52***	-0.14**	-0.34***	0.90***	-0.28***	-0.86***	-0.70***	0.82***										
ATHSh	-0.87***	-0.85***	0.87***	0.87***	0.54***	-0.84***	0.76***	0.1*	0.30***	-0.007	0.87***	0.59***	-0.85***	-0.81***	0.67***	0.43***	0.21***	-0.86***	0.51***	0.88***	0.72***	-0.74***	-0.76***									
TSh	-0.11**	-0.07	0.15***	0.19***	0.15***	-0.16***	0.03	-0.08*	0.54***	0.37***	0.15***	0.22**	-0.15***	-0.15***	-0.01	-0.16***	0.60***	-0.13**	0.07	0.20***	0.19***	-0.13**	-0.21***	0.075								
DRK	-0.38***	-0.31***	0.37***	0.40***	0.23***	-0.38***	0.23***	-0.15***	0.52***	0.31***	0.37***	0.27***	-0.39***	-0.35***	0.14***	0.05	0.43***	-0.38***	0.13**	0.40***	0.33***	-0.34***	-0.43***	0.33***	0.55***							
KW	-0.95***	-0.93***	0.97***	0.97***	0.69***	-0.93***	0.84***	-0.13**	0.38***	0.05	0.97***	0.67***	-0.92***	-0.91***	0.74***	0.24***	0.29***	-0.95***	0.57***	0.97***	0.84***	-0.84***	-0.86***	0.88***	0.15***	0.37***						
KP	-0.95***	-0.93***	0.96***	0.98***	0.68***	-0.93***	0.83***	-0.14***	0.41***	0.06	0.97***	0.70***	-0.92***	-0.91***	0.73***	0.23***	0.34***	-0.95***	0.55***	0.98***	0.84***	-0.84***	-0.86***	0.87***	0.20***	0.41***	0.98***					
KF	0.94***	0.92***	-0.91***	-0.92***	-0.50***	0.92***	-0.79***	0.19***	-0.37***	-0.03	-0.91***	-0.48***	0.97***	0.93***	-0.68***	-0.17***	-0.30***	0.94***	-0.42***	-0.91***	-0.71***	0.84***	0.88***	-0.84***	-0.16***	-0.38***	-0.90***	0.91***				
KC	0.97***	0.95***	-0.96***	-0.97***	-0.62***	0.96***	-0.83***	0.17***	-0.40***	-0.03	-0.96***	-0.58***	0.97***	0.95***	-0.72***	-0.21***	-0.32***	0.97***	-0.49***	-0.96***	-0.80***	-0.88***	0.89***	-0.87***	-0.17***	-0.40***	-0.96***	0.97***				
KFV	0.81***	0.79***	-0.79***	-0.81***	-0.48***	0.79***	-0.58***	0.15***	-0.37***	-0.09*	-0.80***	-0.41***	0.84***	0.81***	-0.47***	-0.11*	-0.35***	0.81***	-0.35***	-0.81***	-0.67***	0.71***	0.85***	-0.72***	-0.22***	-0.40***	-0.79***	-0.80***	0.87***	0.86***		

Significance level: **P<0.001; *P<0.01; *: P<0.05. abbreviations as in table 1.

tip (PAT) ($r=0.97$) and kernel percentage (KP) ($r=0.98$). Furthermore, nut weight (NW) was revealed to be positively and strongly correlated with leaf length (LL) ($r=0.92$), leaf width (LW) ($r=0.90$), leaflet length (LetL) ($r=0.92$), nut length (NL) ($r=0.94$), shape of base perpendicular to suture (SBPS) ($r=0.92$), kernel fill (KF) ($r=0.93$) and kernel colour (KC) ($r=0.95$), while it has a negative correlation with number of leaflet (Nlet) ($r=-0.90$), shape of nut (NSLPS) ($r=-0.91$), prominence of apical tip (PAT) ($r=-0.90$), kernel weight (KW) ($r=-0.91$) and kernel percentage (KP) ($r=-0.91$).

Multivariate Analysis

The eigenvalues obtained by principal component analysis (PCA) indicate that the first two components provide a good summary of the data. They explained 71.29% of the total variation with each component explaining respectively 45.88% and 25.41%. The first component is defined negatively by weight and length of nut, color and fill of kernel and by length and width of leaf; and positively by weight and percentage of kernel and by shape and number of leaflet. The second component is positively correlated to thickness of shell, ease of removal the kernel and the color of rachis. Figure 2 illustrated accessions on

the plot of the first two axis spaces, shows that accessions may constitute three groups. The first one is composed of two accessions of Middle Atlas Mountain: Naour (NAO) and Taghzirte (TAG) and two other accessions of High Atlas: Ait Bougamaz (ABZ) and Amouguer (AMG), characterized by high weight and percentage of kernel and low thickness of shell. The second group is made of five accessions of High Atlas Mountain namely Ait M'hamed (AMD), Imlil (IML), Beram (BER), Tabrijjate (TAB) and Anougal (ANG), characterized with large leaf, high nut dimension and large nut size. The third group is composed of two accessions; one belongs to Middle Atlas (Aghbala, (AGH)) and the other arising from High Atlas (Demnate (DEM)) which are characterized by small leaf and nut.

Hierarchical cluster analysis identified three distinct groups (Figure 3) confirming the PCA results. The first group is composed of Taghzirte (TAG) and Naour (NAO) accessions originating from Middle Atlas Mountain and Amouguer (AMG) and Ait Bougamaz (ABZ) coming from High Atlas Mountain. The second group includes Ait M'hamed (AMD), Imlil (IML), Barrem (BAR), Tabrijjate (TBR) and Anougal (ANG) accessions, all belonging to High Atlas Mountain. The Demnate (DEM) and Aghbala (AGH) accessions represented the third group and seem to diverge significantly from the others.

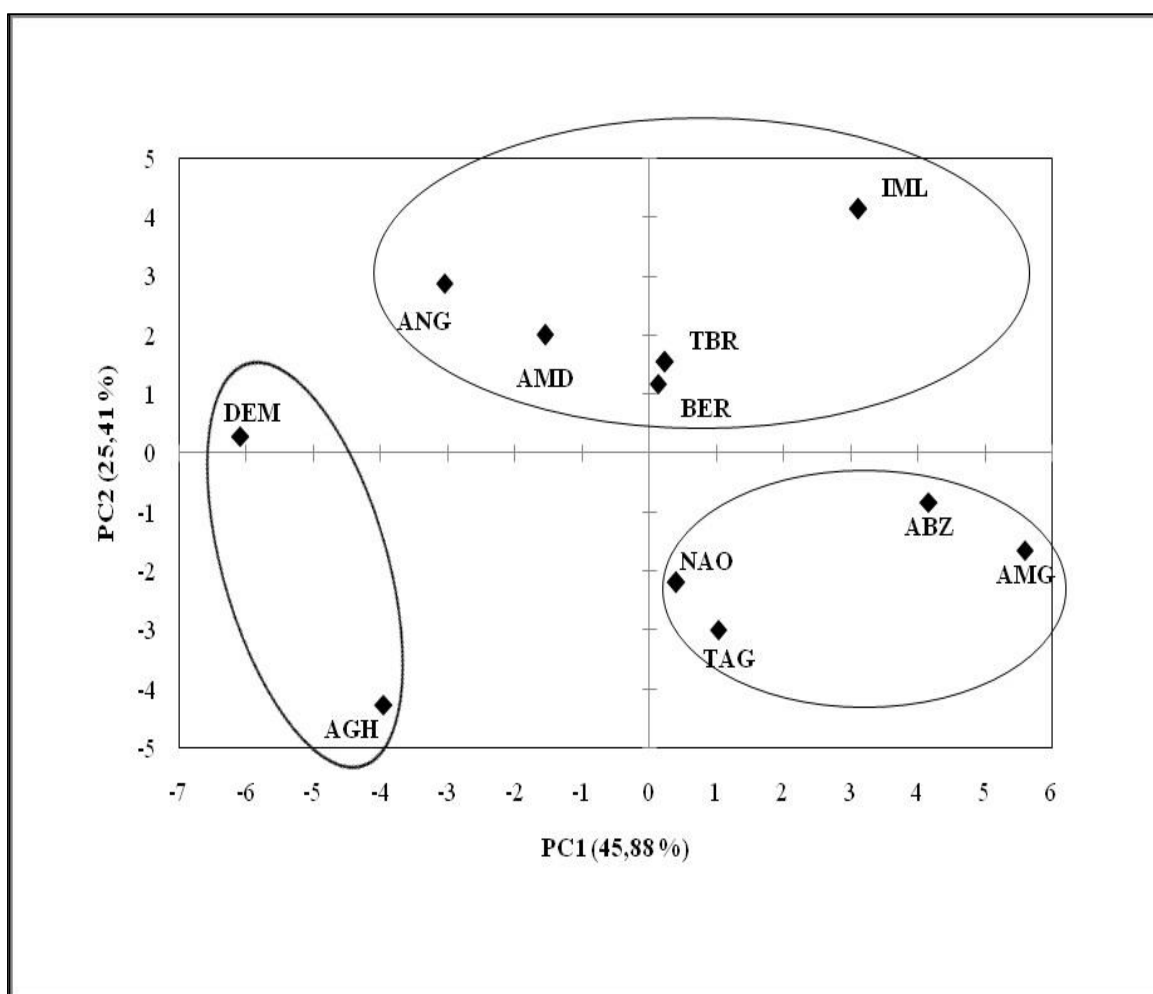


Figure 2. Plot on the two first principal components of 11 walnut accessions. Abbreviations as in table 1.

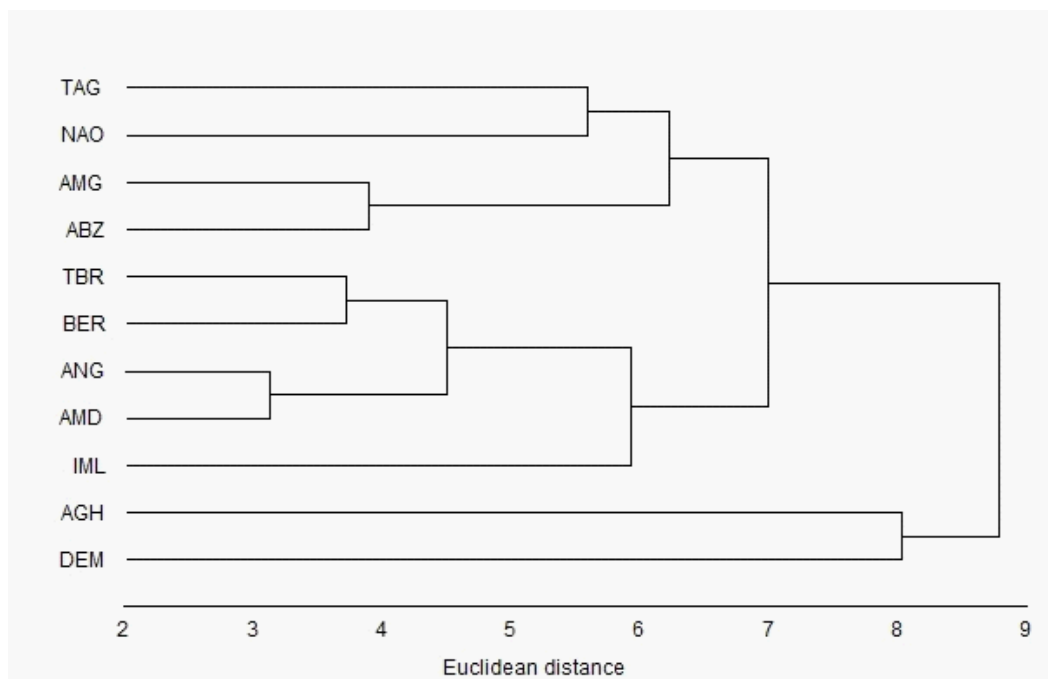


Figure 3. Dendrogram of 11 Moroccan walnut accessions based on morphological and pomological traits. Abbreviations as in table 1.

Discussion

The results of this study show that the accessions studied could be a very interesting source of walnut genetic diversity. The majority of morphological traits of leaves showed a great difference among walnut accessions, especially for the length and width of leaf. Furthermore, the pomological traits exhibited the highest variability among accessions, which was established for most of nut and kernel traits.

According to consulted literature, nut weight, kernel percentage and color of kernel have been considered as the most important indices of quality and economic yield in walnut trees, which should be further investigated. In the Moroccan accessions studied, the nut weight varied from 8.26 to 10.20 g. Values that are within the consulted literature are found between 6 and 16.89 g in Iranian genotypes (Arzani et al., 2008; Ghasemi et al., 2012; Ahandani et al., 2014), 7.46 and 15.21 g in Turkish genotypes (Karadag and Akça, 2011) and from 6.9 to 16.27 g in Romanian walnut (Cosmulescu, 2013). In addition, Moroccan accessions have an average kernel percentage between 33.65% and 43.76%. These values are lower than those obtained for some walnut genotypes from Turkey (46.15 to 63.16%) (Karadag and Akça, 2011) and Iran (48 to 59%) (Ahandani et al., 2014). According to color of kernel, our result showed that 55% of the walnut accessions investigated were revealed with light amber kernel. This result is in accordance with that reported in Iranian genotypes by Arzani et al. (2008), finding 52% of genotypes with light amber kernel. In fact, the light kernel color is a primary breeding objective for walnut. On other hand, the shell thickness of the Moroccan accessions analysed in this work varied from 1.33 to 1.83 mm, which is

in agreement with that obtained by Karadag and Akça (2011) (0.95 to 1.60 mm) and Akça et al. (2015) (1.11 to 2.33 mm) in Turkish walnut, but greater than that reported by Sharma et al. (2014) in Indian walnut (1.24 mm).

These results showed a considerable phenotypic diversity in the Moroccan walnut germplasm in comparison to that of other countries. This variability may be due, first, to genotypic variation or environmental conditions (Ghasemi et al., 2012). Second, walnuts species are monoecious and heterodichogamous, favoring outcrossing over selfing (Ebrahimi et al., 2016). Third, reproduce by seeds of this species causes a very important genetic variability that appears at the flowering period for pomological characters, vigor of the tree and type of fructification, which allowed each geographic region to maintain a diverse population (Lansari et al., 2001). Besides, Diaz et al. (2005) stated that the geographic location and the growing conditions affect fruit and kernel traits of walnut populations from western Spain.

Conclusion

These results show that Moroccan walnut presents a high variability in comparison to that of others countries with similar ecological conditions. This investigation showed significant differences among accessions, permitting discrimination between them according to morphological traits related to leaf, nut and kernel. These findings should be exploited to select the best walnut accession with the wanted traits to be multiplied in order to encourage the agriculture and raise the production of walnut. Taking into account the relevance of high kernel weight, kernel percentage and low thickness of shell, the wal-

nut accessions from Amouguer and Imlil should be considered as the first accessions to be useful as seed sources or multiplied vegetatively for walnut propagation in Morocco. It is obvious that survey of genetic diversity in Moroccan walnut using biochemical and molecular markers are projected and would be a complement for this study.

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