quality is excellent. Therefore nuts reach an average Ø of 32 mm.

Some scientific papers show results on higher sensitivity of novel bred walnut varieties. The novel Hungarian bred walnut varieties are also a bit more sensitive to *Xaj* compared to standard varieties (Rozsnyay 2006, Rozsnyay-Szügyi, 2009). Growers agree that they have to spray the novel bred varieties a couple of times more, but they obtain higher yields using hybrid instead of landscape selected varieties.

'Alsoszentivani 117' and 'Milotai 10' are the most important and largely planted varieties. 'Alsoszentivani 117' has the earliest ripening time and therefore is very popular. 'Milotai 10' has the best shell and kernel quality and therefore is highly appreciated by growers. Usage of novel bred walnut varieties was started in the last decade.

When establishing a new walnut orchard, growers plant double tree rows on a one hectare orchard to obtain first yields as high as possible. Tree thinning is made at the 13th to 20th leafing out after planting, when canopies get really close to each other.

Today, irrigation is an important issue in walnut production, as average yearly precipitation (500 to 700 mm yearly) is not enough. Hungarian growers do not irrigate walnut orchards, but they are considering it. In order to achieve stable productions and good fruit quality, the Hungarian walnut orchards must be irrigated in the future. There is enough water available and growers have the right to use it.

The most important disease is *Xaj* (*Xanthomonas arbicola pv. juglandis*), which can cause great damage, mainly on novel bred hybrid varieties' fruits and leaves. The most sensitive variety is 'Milotai intenzív', while the less sensitive are 'Milotai kései', 'Alsoszentivani kesei' and 'Bonifac'. Phoma/Phomopsis is spreading in Hungarian walnut orchards (Vajna – Rozsnyay, 2005).

At present, the walnut husk fly (*Rhagoletis complete* Cresson) has not been isolated yet in Hungary. The most important pest is the codling moth (*Cydia pomonella* L.).

The Research Institute for Fruit growing and Ornamentals, Budapest-Erd, has made numerous innovations in the field of mechanization since the 1970s. Therefore, it is common to see the most suitable machines in the walnut orchards and processing plants. As mechanization also includes post-harvest technology, Dr. Andor has pieced together a special line which contains a husk removal machine, a washing and a drying machine, a sizer, as well as a manipulation line and/or cracking adapter.

There are 14 processing plants in the country, which capacity covers the whole Hungarian walnut processing capacity.

It is not easy to decide on the best way growers may sell their products. Walnut products can be commercialized in shelled or kernel form. Hungarian growers prefer shelled walnuts, because Hungarian bred walnut varieties ripe first on the Northern Hemisphere and their fruit size is larger than the competitor's varieties. Also, their shell colour and surface are excellent.

Unfortunately, Hungary has no field advisor system supported by the State. The Ministry of Rural Development used to stimulate the establishment of new walnut orchards. Today, growers or co-operatives have the possibility to apply for developing projects in agreement with the EU policy.

Thus, the Hungarian walnut industry is increasing year after year, due to a good and stable market situation. In order to increase the success of the Hungarian walnut industry, a Walnut Association will be founded by Industry members in the near future, with the hope that Hungary may stabilize its current success in the future.

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KERNEL QUALITY IN A LOCAL WALNUT *(JUGLANS REGIA)* POPULATION GROWN UNDER DIFFERENT ECOLOGICAL CONDITIONS IN MOROCCO

INTRODUCTION

Persian or English walnut (*Juglans regia* L.) is native to the mountain ranges of Central Asia (Leslie and McGranahan, 1998). It is a traditional fruit crop in North Africa and its first introduction into the Maghreb is attributed to the Romans (Germain, 1992). Walnut covers an area of 7,600 ha in Morocco, being considered by local farmers and populations as a forestry fruit tree. Walnuts are found in mountainous and remote areas between 800 and 1,800 m above sea level and under different environments (Lansari *et al*., 1999). Its nuts are easily stored and transported over long distances. Thus, the walnut tree can be found in humid and warm Mountains (north of Morocco), semi-arid and cooler Mountains (high Atlas Mountains) and arid regions in the southeast of Morocco. More than half of the trees are seedlings resulting from the prevailing form of propagation known by farmers, since grafting is unusual. The genetic variability of Moroccan walnut groups, defined as "populations" or "geographic provenances" and named by sampling site, was investigated using morphological traits (Kodad, 2000; Lansari *et al*., 2001). The results of these studies showed that the genetic variability of the local Moroccan walnut populations is assumed to be very large. Morocco, as well as other countries with forestry resources, is paying great attention in protecting seedling stands as valuable tools for biodiversity conservation, and as a source of high quality plant material. Moreover, the selection of productive and drought tolerant genotypes with high kernel quality could be essential for the conservation of walnut in different producer regions of Morocco. Taking into account the climate change scenarios from drought and heat stress, low rainfall and increase of biotic stresses, the selection of the seed source may be crucial for the success of future plantations (Hemery, 2008). Moreover, Callaham (1994) reported that the provenance research provides an excellent basis for the selection of seed sources and refers to the geographical origin of seeds or trees. In fact, several studies reported that the physical fruit traits (McGranaham and Leslie, 1990) and chemical kernel profile (Amaral *et al*., 2003; Crews *et al*., 2005; Martinez *et al*., 2006) depend on the genotypes, with a strong effect of the environmental conditions. The present study aims at the evaluation of genetic diversity and genotype

Walnut population in High Atlas Mountain in Morocco.

performance of the local walnut seedlings from different Moroccan eco-geographical provenances.

MATERIAL AND METHODS

Plant material. This study was carried out with walnut genotypes from four different regions rich in genetic resources: Bni Mtir in the Middle-East of the Atlas Mountains, Imlile and Oukaimeden in the high Atlas Mountains (Central-Southern Morocco), Midelt situated in the high valley of Moulouya in Central-Eastern Morocco, and Er-Rich in South-Eastern Morocco. A total of 25 local genotypes from different zones of each region were selected because of the general status of the plant (vigour, foliar density and appearance), a lateral fructification, and kernel physical quality appreciated by the local population. These genotypes were unique seedlings; therefore each genotype was a single tree. These genotypes were marked and fruits were collected in winter in 2010. The nuts were collected when the fruit mesocarp had split and peduncle abscission was complete. After cracking, the kernels were soaked in liquid nitrogen and then ground using an electrical grinder (IKA, Janke & Kunkel, Germany) to obtain fine flour.

Physical fruit traits. Nut thickness and width were measured at the midpoint of the length, perpendicular to each other, considering width the larger dimension. Length, width, and thickness were measured with a precision of 0.01 mm in all nuts with a digital caliper. After measurements, nuts were cracked to obtain the kernel and determine the shelling percentage by weight using an electronic balance. Length, width, and thickness were similarly measured in all nuts.

Kernel chemical determination. Oil was extracted from 5 grams of ground walnut kernel using a fat extractor Soxtec during 5 hours and using hexane as a solvent (AOCS Ce 2-66 modified). The oil content was expressed as the difference in weight of the dried kernel samples before and after extraction. The protein content was obtained indirectly by determining the total N content obtained by the Kjeldahl method (AOAC, 1995) and multiplying by nitrogen-protein conversion factor (Kc =6.25) (% Protein = Kc * % Total nitrogen).

Statistical analysis. All statistical analyses were performed with the SAS program. Analysis of variance was performed with a two random factors design. The factor genotype was hierarchical to the factor population because the trees were not repeated between sites. To draw a general conclusion from the four walnut locations, the population was considered as a random effect (Steel and Torrie, 1960). The Principal Component Analysis (PCA) was applied to describe the pattern of walnut diversity.

Table 1. Analysis of variance of physical traits and oil and protein contents of Moroccan walnut seedlings.

Variable	d.l	Mean squares	*F*-Value	*P*
Nut weight				
Population	4	86.09	221.34	<.0001
Genotype(population)	21	27.83	71.55	<.0001
Error	365	0.38		
Kernel weight				
Population	4	3.96	249.82	<.0001
Genotype(population)	21	0.72	45.64	<.0001
Error	365	1.015		
Shell weight				
Population	4	70.75	172.93	<.0001
Genotype(population)	21	22.75	55.62	<.0001
Error	365	0.41		
Kernel weight/Nut weight				
Population	4	0.32	194.44	<.0001
Genotype(population)	21	0.08	48.50	<.0001
Error	365	0.001		
Oil content				
Population	4	77.11	37.27	<.0001
Genotype(population)	20	34.99	16.91	<.0001
Error	25	2.06		
Protein content				
Population	4	25.59	24.06	<.0001
Genotype(population)	20	6.62	6.23	<.0001
Error	25	1.06		

RESULTS AND DISCUSSION

Genotype and location variability. The analysis of variance was carried out on some nut and kernel traits considered as important quality parameters in walnut. This analysis showed high variability between genotypes for nut and kernel weight, shell hardness, kernel ratio, protein and oil content (Table 1). The range of variability for oil content was between 51.59 and 69.91%, and between 9.21% and 13.77% for protein content (Table 2). The protein content agreed with previous reports (Amaral *et al.*, 2003), as well as

Table 2. Mean value of oil content, protein content and physical trait of nut and kernel of Moroccan walnut seedlings.

Genotype	Protein content (%DM)	Oil content (%DM)	Nut length (mm)	Nut width (mm)	Nut thickness (mm)	Nut weight(g)	Shell thickness (mm)	Kernel weight(g)	Partition weight(g)	Shell weight(g)	Kernel ratio
AM2	11.22	68.47	40.60	32.30	31.10	13.80	1.80	5.01	0.40	8.40	0.36
AM3	9.58	68.66	38.20	31.40	31.00	11.60	1.50	5.10	0.40	6.10	0.44
AM4	10.20	55.09	35.50	29.80	30.70	6.60	1.20	1.70	0.20	4.70	0.26
ZH1	9.62	61.94	36.10	31.50	31.50	10.70	1.30	4.30	0.30	6.10	0.40
ZH2	11.01	62.39	34.80	32.10	30.30	10.50	1.30	4.50	0.20	5.80	0.43
ZH3	11.63	58.74	34.70	32.10	31.30	11.70	1.50	4.60	0.20	6.90	0.39
Er-Rich	10.71	62.55	36.65	31.53	30.98	10.82	1.43	4.20	0.28	6.33	0.38
BM1	11.02	68.08	35.40	26.80	27.40	7.80	1.10	3.70	0.20	3.90	0.47
BM2	12.03	62.36	35.90	28.10	27.50	6.70	1.10	2.20	0.20	4.30	0.33
BM3	12.22	65.23	35.90	29.40	30.30	9.10	1.30	3.60	0.20	5.30	0.40
BM4	10.59	62.82	34.40	29.29	28.90	8.80	1.30	4.10	0.20	4.50	0.47
Bni Mtir	11.47	64.62	35.40	28.40	28.53	8.10	1.20	3.40	0.20	4.50	0.42
IM10	11.16	65.81	38.40	32.90	35.40	14.90	1.70	5.80	0.30	8.80	0.39
IM12	12.44	58.10	41.30	32.40	33.80	9.20	1.20	3.50	0.30	5.40	0.38
IM13	11.84	65.82	34.70	31.40	32.20	10.50	1.50	4.40	0.30	5.80	0.42
IM2	12.34	62.70	35.30	27.80	28.10	8.08	1.40	3.30	0.20	4.50	0.41
IM4	11.42	61.79	35.60	31.10	32.80	10.20	1.40	4.50	0.30	5.40	0.44
IM5	9.89	64.68	33.30	28.40	28.28	8.20	1.40	3.50	0.20	4.50	0.43
Imlile	11.68	63.15	36.43	30.67	31.76	10.18	1.43	4.17	0.27	5.73	0.41
O1	11.89	58.75	33.30	30.30	30.80	10.01	1.40	4.50	0.30	5.20	0.45
O2	11.90	52.34	39.20	35.90	36.40	12.80	1.60	5.10	0.30	7.40	0.40
O3	11.98	65.62	39.10	32.30	34.30	14.60	2.20	5.40	0.40	8.80	0.37
O5	11.08	53.97	37.90	30.70	28.90	9.60	1.50	3.70	0.20	5.70	0.39
O6	10.80	55.91	36.40	29.30	30.60	8.20	1.10	3.50	0.30	4.40	0.43
Oukaimeden	11.53	57.32	37.18	31.70	32.20	11.04	1.56	4.44	0.30	6.30	0.41
MT1	10.70	59.84	33.03	28.70	28.50	8.40	1.60	2.70	0.30	5.40	0.32
MT2	11.31	61.39	30.80	26.50	28.10	8.20	1.60	3.10	0.20	4.90	0.38
MT3	10.42	65.62	30.70	28.60	28.80	8.30	1.30	4.04	0.20	4.10	0.48
MT4	10.55	66.72	35.70	30.30	31.20	10.70	1.50	4.20	0.30	6.20	0.39
Midelt	10.74	63.39	32.56	28.53	29.15	8.90	1.50	3.51	0.25	5.15	0.39

the fat content, although the lowest value obtained was lower than any previous report (Amaral *et al.*, 2003; Bada *et al.*, 2010). The range of variability for nut weight was between 6.6 and 14.9 g; and 1.7 and 5.8 g for kernel weight; and between 25.75% and 48.19% for kernel ratio (Table 2). In general, the values for these fruit parameters are lower than those reported in other local populations of walnut (Iran: Arzani, 2008; Turkey: Aslantas, 2006; Albania: Zeneli *et al.*, 2005). The kernel weight should range from 6 to 8 g and the kernel ratio should range from 50 to 55% in promising walnut cultivars according to Akça (2009) and Nenjuhin (1971), but in the present study no genotypes satisfy these commercial criteria.

The population effect was significant for all studied traits (Table 2). For nut parameters, the Oukaimeden genotypes had the highest values of nut weight (Table 1). The lowest values for nut weight were obtained in the Er-Rcih gene pool (Table 1). For kernel weight and kernel ratio, the highest values were obtained in the Imlile and Oukaimeden gene pools (Table 1), and the lowest values in the Er-Rich gene pool (Table 1). The location and the growing conditions have been reported to affect fruit and kernel weight (Diaz *et al.*, 2005). For oil and protein content the growing conditions appear to affect these components in walnut (Amaral *et al.*, 2003; Crews *et al.*, 2005; Martinez *et al.*, 2006), where the same genotypes were tested in different locations. In our study, however, the genotypes are different in each population, showing that the geographical origin of the genotypes affects the physicochemical components of the kernels of local walnut populations, probably as a consequence of local adaptation of these genotypes. Almost all of the genotypes from humid and cooler regions (Imlile and Oukaimeden) show heavy and fat kernels (Table 2). These results could be explained in part by the differences in the growing and climatic conditions between geographical origins, as reported in shea butter, *Vitellaria paradoxa* C.F. Gaertn. (Maranz and Weisman, 2004) and almond, *Prunus amugdaus* Batsch (Kodad *et al.*, 2013). Furthermore, these results clearly show that nut and kernel weight and protein and oil content not only depend on the genotype (Amaral *et al.*, 2003; Diaz *et al.*, 2005), but also on the gene pool origin. Similar results have been reported in almond (Kodad *et al.*, 2010; 2011). The present results show a clear effect of geographical origin on the physical and chemical components of the walnut kernels placing the emphasis on selecting the promising genotypes in each cultivation area in Morocco.

Genetic diversity. Statistical methods such as principal component analysis and cluster analysis are useful tools for studying the genetic diversity and have been applied to tree nut species such as almond (Lansari *et al.*, 1994; Kodad *et al.*, 2011). The best model with the minimum number of dimensions explaining the data structure was selected by the exclusion rule, based on the amount of residual variability to be tolerated, retaining a sufficient number of PCs capable of explaining a percentage of variance > 80%. With this rule, the first three PCs were enough because they described 78.58% of the sample vari-

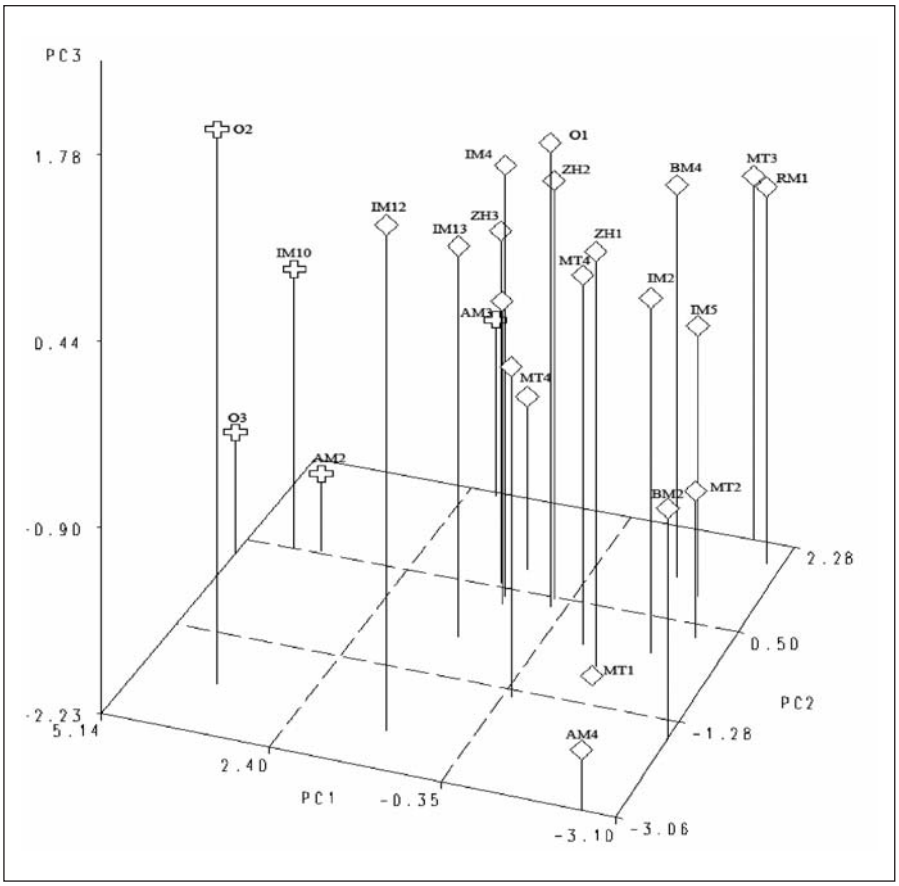
Table 3. Eigenvectors of the three principal components axes from PCA analysis of the Moroccan walnut seedlings.

Variable	Axe 1	Axe 2	Axe 3
Protein content (% DM)	0.07	-0.29	**0.37**
Oil content (%DM)	0.05	**0.58**	-0.27
Nut length (mm)	**0.29**	-0.26	0.05
Nut width (mm)	**0.36**	-0.19	0.25
Nut thickness (mm)	**0.36**	-0.18	0.23
Nut weight (g) (A)	**0.41**	0.13	-0.01
Shell thickness (mm)	**0.31**	0.09	**-0.38**
Kernel weight (g) (B)	**0.35**	0.14	0.26
Partition weight (g)	**0.32**	0.09	-0.23
Shell weight (g)	**0.40**	-0.03	-0.19
Kernel ratio (B/A)	-0.02	**0.54**	**0.61**

ability. The contribution of each PC to the total variance is shown in Table 3. Nut, kernel, shell and wall weight, and nut length and width were primarily responsible for the separation on the PC1. The second component is represented by oil content and kernel ratio and the third component is represented negatively by shell thickness and positively by kernel ratio. The present results confirm that nut and kernel physical traits are the most variable among walnut genotypes in local Moroccan seedlings (Lansari *et al*., 2001).

When means were plotted on the three principal axes (Fig. 1), more than 56% of the genotypes showed intermediate to low nut and kernel weight and dimension and oil content (Table 2). When the analysis focused on the origin of the genotypes, it appears that genotypes from Bni Mtir (Middle-East Atlas Moutains) and genotypes from Midelt (High Valley of Moulouya) showed the lowest values for nut and kernel weight and dimensions (Fig. 1; Table 2). However, some genotypes such as BM1 from Bni Mtir and MT3 from Midelt showed high oil content. In contrast, almost all genotypes from the high Atlas Mountains showed intermediate to high values for nut and kernel weight and dimensions and fat content. These results are in accordance with those found applying analysis of variance (Table 1).

Figure 1. Position of the first three principal components (PC) scores of the physical and chemical component of the Moroccan walnut seedlings.



At individual level, the genotypes AM2 from Er-Rich (South of Morocco), IM10 from Imlile and O3 from Oukaimeden (High Atlas) had a very high positive value on PC1. This showed the highest values for kernel and nut weight, nut length and width, and the highest shell hardness (Table 2). On the second component, these genotypes had slightly positive values showing an intermediate value of kernel ration and oil content (Table 2). Regarding the third component, these genotypes had a negative value indicating that these genotypes show low values for protein content (Table 2). Genotype AM2 from Er-Rich (Southern Morocco) is very interesting because of its high positive value on PC1 and PC2 (Fig. 1), indicating its heavy nut and kernel and high fatty content (Table 2). Furthermore, genotypes IM4, IM12, O1 and O2 (High Atlas mountains) showed a high value on PC3 (Fig 1), indicating that these genotypes had very high protein content.

The results of the multidimensional analysis clearly showed that walnut grown in Morocco is characterized by the high variability of physical andchemical traits of nut and kernel. This variability could be used to select the best genotypes with adapted traits, high productivity and good kernel in each region to be propagated vegetatively as new local cultivars or to select the genotypes with high productivity and fatty kernel to be used as source seed for extending the recovery of degraded walnut forests in Morocco. Taking into account the relevance of high lipid contents as a source of carbon and energy during germination and seedling growth (Chenvard *et al*., 1994), the genotypes IM5 from Imlile (high Atlas mountain), BM1 and BM3 from Bni Mtir (Middle-Eastern Atlas Mountain), AM3 from Er-Rich (Southern Morocco) and MT3 from Midelt (high valley of Moulouya) could be considered as seed sources for walnut propagation in each walnut productive region in Morocco as a tool to recover from forest degradation, since the choice of the seed source is considered crucial for the success of future plantations in silvicultural management (Hemery, 2008; Callaham, 1994).

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PISTACHIO RESEARCH IN TUNISIA: PAST, CURRENT AND FUTURE INVESTIGATIONS

INTRODUCTION

The cultivation of pistachio nut trees in Tunisia is probably very old. Archaeological studies bear witness to the presence of pistachio nuts during the Carthaginian period (Hurst and Stager, 1978). The need to develop pistachio culture in Tunisia increased since the Robert Willard Hodgson mission (1930-1931). Hodgson (1931) highlighted the potential economic and social gains of pistachio culture due to its adaptation to the extreme conditions of arid areas and its fruit quality. However, its expansion was very limited until the 1960s. In 1964, pistachio cultivation occupied only 30 ha mainly in the central and south part of the country. The two FAO-INRAT projects (1964-1972) greatly contributed to the extension of this crop. Many technical problems related to crop multiplication were resolved, new varieties and rootstocks were introduced and orchards were installed in different bioclimatic areas to study the behavior of local and foreign genotypes. Currently, pistachio cultivation occupies 37,000 to 43,000 ha with a total annual production of 2100 to 2700 tons (official national data, 2011; FAO, 2012). The FAO world classification of pistachio cultivation placed Tunisia in the 5th position regarding cultivated area, 9th for production and 17th for productivity (FAO, 2012). Despite the relatively large land occupation, the crop domestic productivity does not exceed 60 kg/ha on average. Tunisian pistachio research started in 1948 at the National Agronomic Research Institute of Tunis (INRAT) previously known as "Service Botanique et Agronomique de Tunis (SBAT)" and is still ongoing by different teams in a few other national research institutions such as the Olive Tree Institute (IO) and the National Agronomic Institute of Tunisia (INAT). This report overviews the main axes developed and related results.

PROPAGATION AND MICROPROPAGATION

Pistachio propagation was one of the earliest concerns of pistachio research in Tunisia. Seeding and budding techniques were studied since 1948 by Crossa-Raynaud and allowed for the production of hundreds of plants in Gafsa (south-eastern Tunisia). During the 1964-1972 years, several collections of varieties and rootstocks were installed in different areas of the country (Jacquy, 1972). Budding suc-