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WALNUT BIODIVERSITY IN SOUTH-WESTERN ROMANIA-RESOURCE FOR PERSPECTIVE CULTIVARS

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Abstract

Juglans regia L. (*Juglandaceae*) germplasm from the Oltenia region, located in the South Western part of Romania, was evaluated to determine the variability in walnut germplasm and to identify the promising material. Important pomological and phenological characteristics were found in native trees which were of seedling origin. Variability found in nut weight was between 6.8-18.4g, in kernel weight between 1.7-8.79g, in weight kernel/weight nut ratio between 23.6-71.7%. Nut size, bud breaking time, nut maturity time and phenological characteristics were also evaluated. The data obtained have indicated that walnut trees studied in this region have recorded the occurrence of high variations in fruit characteristics that indicate the higher potential in selecting new genotypes of material under study.

Introduction

Carpathian, English or Persian walnut (Juglans regia L.) is growing well in temperate climate areas. There is a high genetic variability in walnut populations which exist in Romania. Oltenia, having various eco-geographical areas, is one of the major regions wellknown for walnut diversity. Oltenia region is located in southwestern Romania and has 5 counties (Dolj, Olt, Gorj, Mehedinți and Vâlcea). The climate is temperate, with Mediterranean influences owing to its South-Western position, with average yearly temperature of 10°-11.5°C. There are many open pollinated walnut seedlings on their own roots in Oltenia region, and these are an important source of genetic diversity for *J. regia*. As most of the trees occurred through open pollination, a very rich genetic material has been formed among these local walnut populations (Godeanu *et al*., 1997). Walnut germplasm has been extensively used in the selection studies for producing the superior walnut clones (Botu *et al*., 2010; Godeanu *et al*., 2004; Achim *et al*., 2007; Cosmulescu *et al*., 2010a).

High variability in nut traits e.g. nut sizes, shape, shell thickness, kernel procent, colour of kernels, and taste of kernels, etc. has been reported in walnut trees in different regions (Solar, 1990; Malvolti *et al.,* 1993; Balci *et al*., 2001; Diaz *et al*., 2005; Gazmend *et al*., 2005; Casal *et al*., 2005; Khan *et al*., 2010). The most promising genotypes are being kept in various national germplasm collections (Aleta & Ninot, 1997; Rouskas & Zakynthinos, 2001; Solar *et al*., 2002; Solar & Štampar, 2005; Miletic *et al*., 2010). Given the quite large and so far unexploited variability within the *J. regia* L. species, Germain (1997) considers that it would be useful to make selections among natural populations or to create new cultivars through hybridization by combining the characters of improved climate adaptation, early fruiting, high productivity, tolerance to disease, and quality fruit crop. Selection of walnut has a long history, and it was carried out by method of simple selection out of natural seed population of high-yielding plants with high quality walnuts to assure the sustainability of agricultural systems. And up to now this method is the basic one. In every selection programme, the optimal criterion should be identified as early as possible. The knowledge of genetic relationships among walnut genotypes and their pomological characteristics will be really useful in walnut cross-breeding programs.

The aim of this paper is to provide additional information regarding genetic diversity in walnut populations in Oltenia region, while the study of phenotypic correlation between traits for identified promising genotypes will be further exploited through walnut breeding programmes for their tree and nut characteristics.

Material and Method

One hundred and nine seed germplasm of *J. regia* were collected from different areas of Oltenia region, Romania. All trees under the study were of seedlings origin and are growing naturally.

Recorded data have included quantitative and qualitative traits. It was also followed up the reaction to *Xanthomonas arboricola* pv. *juglandis* and *Gnomonia leptostyla* pathogens; and many trees were excluded because of their symptoms showed. Phenological traits have followed the anthesis of flowers, flowering time, dichogamy and maturity time. Data were recorded over three successive years. Nut traits were analyzed for fruits i.e., nut weight, kernel weight, kernel weight/nut weight ratio, length and width of nut and size index (average of the three dimensions). Data collected from a sample of 100 randomly selected nuts per tree, and are reported as the average over the 3 years. Features were recorded by considering the UPOV and IPGRI descriptors. All measurements were repeated over three years.

For statistical analysis, Microsoft Excel and XLSTAT-Pro was used. All data were expressed as means ± standard deviations of triplicate measurements. Differences between means were first analyzed by ANOVA test and then by Least Significant Difference (LSD) test ($p<0.05$). Data were subjected to Pearson correlations and PCA (Principal component analysis) analysis.

Results and Discussion

Phenological traits: Walnut flowering in Oltenia area occurs during April-May. Depending on flowering time, walnut cultivars can be grouped into 4 classes, namely: the early class (April 20-30), semi-early class (May 1-5), semi-late class (May 6-10) and late class (after May 10) (Cosmulescu *et al*., 2010b). Flowering time classification was different from a year to another, with slight differences. Most of the researched trees had their flowering time between 20 and 30 of April (62.7%), while 35% of them between 1 to 10 May. Late flowering (after May 10th) was rare (2.3%). Botu *et al*., (2010) shows that flowering of walnut cultivars takes place between 4 of April and 21 of May over the five year study period, under the conditions of Oltenia's hilly area. Dichogamy of walnut genotypes is of protandrous type (59.7%), protogynous type (33.1%) and a few genotypes are of homogenous type (7,2%). Average temperature was influenced on flowering period and timing, and also on the degree of dichogamy (Cosmulescu *et al*., 2010b). Cerović *et al*. (2010), regarding dichogamy in walnut genotypes studies in Serbia, reported that most types (~ 80%) were protandrous, a small number (~ 12%) were protogynous, and only a few (~ 8%) were homogamous. With reference to walnut cultivars, regarding fruit ripening, the researched genotypes are ripening between September 1 and 15, and occurred in 47.5% of the genotype. Genotypes with ripening after 15 of September represented 39.4% of genotype. A small percentage (13.1%) are ripening before 1 of September or after 30 of September.

Morphological variability: Physical characteristics of fruits represent a quality feature of nuts. Table 1 presents the average values of fruit and kernel, standard deviations, minimum and maximum values of characteristics studied. Data indicate the high variations of fruit characteristics from a genotype to another, a large difference (2.7 times) for nut weight (6.8-18.4g), 5 times higher for kernel weight (1.7-8.79g), 3 times higher for kernel percentage (23.6-71.7%), and 1.5 times higher for size index. Occurrence of such high variations in fruit characteristics does indicate a high potential in selection of new genotypes of analysed material. Previous researches (Cosmulescu *et al*,. 2010a) showed that Romanian walnut cultivars are superior; their fruit characteristics have varied within small limits (56.54-59.64% kernel percentage, 14.0-16.65g nut weight, 7.80-9.92g kernel weight, 33.3-38.15mm fruit diameter, 38.2-42.2mm fruit height). Regarding fruit characteristics, Akca & Sen (2001) showed that nut diameter varied between 22.30-32.26mm, nut weight varied between 7.49-13.93g, while kernel weight varied between 2.61- 5.73g of the promising walnut genotypes.

Table 1. Descriptive statistics for walnut genotype.

Specification	H (mm)	D_1(mm)	D_2(mm)	FW (g)	KW (g)	KP (%)	Is (mm)
Mean	36.455	31.622	30.136	10.614	5.182	49.520	32.737
Standard Error	0.423	0.271	0.234	0.215	0.092	0.896	0.271
Standard Deviation	4.37	2.851	2.454	2.257	0.971305	9.400	2.845
Sample Variance	19.082	8.128	6.026	5.096	0.943	88.369	8.096
Range	21.5	14.4	10.5	11.6	7.09	48.1	14.066
Minimum	28.2	26.2	25.7	6.8	1.7	23.6	27.733
Maximum	49.7	40.6	36.2	18.4	8.79	71.7	41.8
Count	110	110	110	110	110	110	110
Confidence Level (95.0%)	0.825	0.538	0.463	0.426	0.183	1.776	0.762

H= Fruit length; D1= Diameter 1; D2= Diameter 2; FW = Fruit weight; KW=Kernel weight; KP = Kernel percentage; Is = Index size.

In this study, the fruit weight varied between 6.8-18.4g, while kernel weight varied between 1.7-8.79g, and average weight of fruit was 10.61g. Average weight of fruit in the same genotype ranged from a year to another with slight differences. Part of variation can be the result of fluctuations in environmental factors. Thus, ability to distinguish among superior walnut genotypes can be prevented by effects of yearly climatic variations on crop and fruit size. Large variations in these characteristics were found by other authors (Balci *et al.,* 2001; Casal *et al*., 2005; Diaz *et al*., 2005; Solar & Štampar, 2005; Aslantas, 2006; Beyhan & Demir, 2006; Arzani *et al*., 2008; Simsek *et al*., 2010; Ali *et al*., 2010; Hussain *et al*., 2011). By calculating the ratio between kernel weight and fruit weight, the variation limits were between 23.6% and 71.7% (Table 1). The previous research (Botu *et al.*, 2010; Cosmulescu *et al*., 2010a) made on assessment of some Romanian walnut cultivars has indicated that average kernel percentage has varied between 48.0% and 53.0%. Variation for nut weight, kernel weight, kernel ratio, and shell thickness, have been reported by Arzani *et al*., (2008) in the range of 6.0-15.2g, 2.6-9.1g, 38.4-79.6%, and 0.4-1.4mm, respectively. Higher values for kernel percentage were found in China's cultivars (51-70% kernel ratio) (Baojun *et al*., 2010) and in Turkey's cultivars (45.66-67.14% kernel ratio) (Aslantaş, 2006). As regards nut dimensions, nut length has varied between 28.2mm and 49.7mm, and diameter between 26.2mm and 40.6mm. Fruit shape is broad ovate, round and ovate. The index size (average of three dimensions) has varied between 27.73-41.8 mm.

Through the histogram analysis that enables us to visualize the distribution of frequency according to „fruit weight", it was found that most of genotypes fall into the size class 10.28-12.6 g (64.54%). Only fourteen genotypes showed higher values for fruit weight (over 12.6 g) (Table 2). For „kernel percentage", the percentage distribution of genotypes is as follows: 24.54% have kernel content between 47.65-52.46%. Percentage of genotypes whose kernel content exceeds 52.46%, is 33.64%. Most of genotypes are the ones whose kernel content varies within the range 42.84-57.27% (70% of genotypes). In terms of kernel characteristics, kernel content of over 50% are desirable in selection of walnut varieties. Selection for high nut quality should include increased kernel weight. In this study, most of the genotypes (58%) had a high content of kernel (over 47.65%), (Table 3). For “index size”, the histogram analysis shows a distribution of frequencies of 65.45% within the range 30.54-34.76mm; more than half of genotypes analysed fall into the class of medium-large sizeed.

Table 2. Frequency distribution of walnut genotype.

Fruit weight (g)			Kernel percent (%)			Index size (mm)		
Bin	Frequency	Cumulative %	Bin	Frequency	Cumulative %	Bin	Frequency	Cumulative %
006.8	1	00.91%	023.6	1	00.91%	27.73	1	00.91%
07.96	9	09.09%	28.41	0	00.91%	29.14	5	05.45%
09.12	15	22.73%	33.22	5	05.45%	30.54	17	20.91%
10.28	28	48.18%	38.03	6	10.91%	31.95	24	42.73%
11.44	25	70.91%	42.84	15	24.55%	33.36	21	61.82%
012.6	18	87.27%	47.65	19	41.82%	34.76	27	86.36%
13.76	5	91.82%	52.46	27	66.36%	36.17	8	93.64%
14.92	2	93.64%	57.27	16	80.91%	37.58	1	94.55%
16.08	3	96.36%	62.08	9	89.09%	38.98	0	94.55%
17.24	3	99.09%	66.89	6	94.55%	40.39	2	96.36%
More	1	100.00%	More	6	100.00%	More	4	100.00%

Table 3. Correlations between nut characteristics*.

	H (mm)	D_1 (mm)	D_2 (mm)	FW (g)	KW (g)	KP (%)	SW (g)	Is (mm)
H (mm)	1							
D_1 (mm)	0.766	1						
D_2 (mm)	0.555	0.614	1					
FW (g)	0.823	0.722	0.616	1				
KW (g)	0.344	0.416	0.567	0.601	1			
KP (%0	-0.628	-0.410	-0.126	-0.536	0.306	1		
SW (g)	0.825	0.664	0.455	0.907	0.209	-0.818	1	
Is (mm)	0.927	0.903	0.777	0.839	0.478	-0.495	0.775	1

* H= Fruit length , D1= Diameter 1, D2= Diameter 2; FW = Fruit weight, KW= Kernel weight, KP = Kernel percentage, SW = Walnut shell weight , Is = Index size

In the breeding program of walnut, increasing the amount of kernel is a priority. Kernel content is highly influenced by height, diameter and weight of fruit. For selection purposes it would be a useful method for quick estimation of kernel amount and it would get to knowing that there are correlations between these characters. The highest correlation involving the kernel characteristics (in the genotypes researched) was found between fruit weight and kernel weight (r = 0.601) and large diameter (r = 0.567). Although they were significantly correlated with other features of walnut kernel weight, all coefficients were lower than in fruit weight (Table 3). The high correlation involving fruit weight was of parameters of fruit size, height (r=0.823) and the two diameters (r=0.722 and r=0.617 respectively); large diameter and height of fruits were best correlated with other physical characteristics of fruit. The index size has been correlated with fruit weight (r=0.839).

Regarding correlations between nut characteristics, Sharma & Sharma (2001) show that the fruit weight was found significantly and positively correlated with nut width, nut length, kernel weight and nut width with the nut length, nut thickness, index of roundness, kernel weight, kernel width. In this case, of the three parameters of fruit size, diameter 2 show the highest potential for selection in terms of kernel weight (r=0.567) (Table 3). We assume that the negative correlation between kernel percentage and fruit weight (r =-0.536) and fruit size (r=-0.410, r=-0.126 and r=-0.628 respectively) would be a reflection of higher weight of endocarp. Negative correlation between kernel percentage and shell thickness was found by Sharma *et al*., (2001). Eskandari *et al*., (2005) found that there was also a positive correlation between kernel weight and fruit weight (r=0.837), and also between the kernel weight and shell thickness (r=0.299). In this case the correlation between kernel weight and fruit weight is also positive (r=0.601). A few significant correlations have been found by Arzani *et al*., (2008); they were found between nut weight and nut length (0.57), nut width (0.68), nut thickness (0.67), kernel weight (0.75), and shell thickness (0.32); whereas a negative correlation was detected between shell thickness and kernel ratio (-0.34).

The data correlation matrix was subject to principal component analysis, a technique that reduced the dimensionality of data set and revealed the predominating variables. The method was used to study diversity in other species too (Sultana *et al*., 2010; Farooq *et al*., 2011; Yousuf *et al*., 2011; Ghafoor & Arshad, 2011).

Analysis of PCA for characteristics “fruit weight” (FW), “kernel weight” (KW), “kernel percentage” (KP), “walnut shell weight” (SW) and “index size” (IS) has shown that the three characteristics (PC1- Fruit weight , PC2- Kernel weight , PC3- Kernel percentage) have represented 99.074% of the variant’s total (Table 4).

Table 4. Eigenvalues and component score coefficients (Eigenvectors).

Explained variance (Eigenvalues)					
Value	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**
Eigenvalue	3.349	1.385	0.220	0.046	0.000
% of Var.	66.970	27.697	4.406	0.926	0.000
Cum. %	66.970	94.667	99.074	100.000	100.000

Component score coefficients (Eigenvectors)		
Variable	**PC 1**	**PC 2**
FW	0.529	0.160
KW	0.237	0.754
KP	-0.382	0.592
SW	0.524	-0.194
Is	0.493	0.130

FW = Fruit weight, KW= Kernel weight, KP = Kernel percentage, SW = Walnut shell weight , Is = Index size

The plot established according to the first two PCA axes (Fig. 1) suggests the existence of four groups of cultivars. Group I, made of thirty genotypes with the lowest percentage of fruit and kernel weight (negative values for both components). Group II, comprised twenty-one genotypes grouped into positive values for PCA 1 and negative values for PCA 2. Group III, comprising thirty genotypes grouped into positive values for both components, and Group IV comprising twenty-eight genotypes grouped into positive values for PCA 2 (Fig. 1)

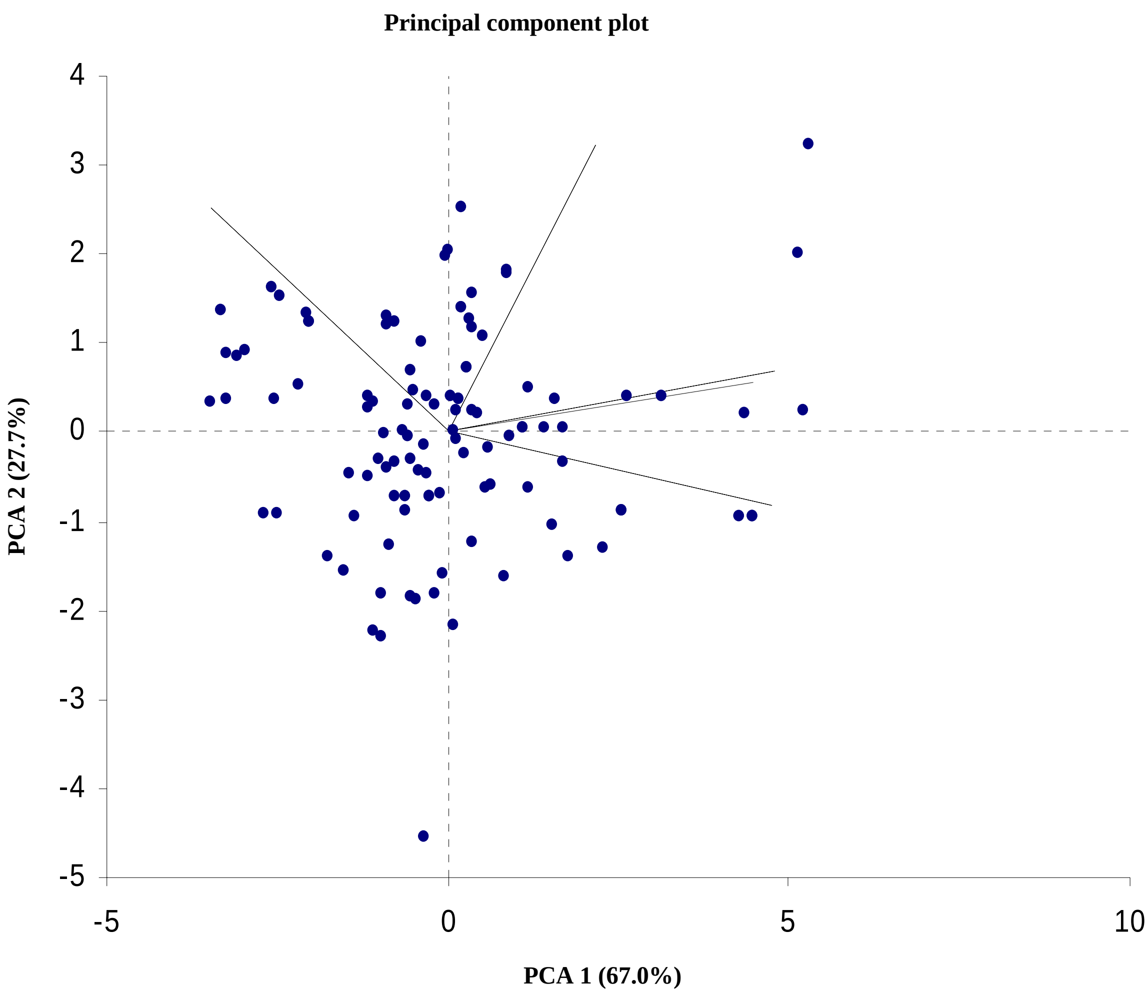

Fig. 1. Plot of the first two principal components (PCA1 and PCA2). Eigenvalues for each two principal component are listed in parentheses.

Conclusions

The data indicate that walnut fruits are highly variable in terms of weight, kernel weight, and kernel percentage. The variability observed in these characteristics is likely caused by agro-climate conditions and seed propagation. This large variation of fruit characteristics indicate a high potential in selection of new genotypes that have been studied in the material. The promising selected genotypes thus identified will be further exploited through walnut breeding programmes for their tree and nut characteristics.

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