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PHENOTYPIC DIVERSITY OF WALNUT (*Juglans regia* L.) IN ROMANIA – OPPORTUNITY FOR GENETIC IMPROVEMENT

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ABSTRACT. *Romania has significant walnut (Juglans regia L.) trees, most of which are seedling-grown trees. Due to the extensive variability of biological material, the result of cross pollination and generative propagation prevailed about by natural dissemination or anthropogenic, Oltenia region of Romania has rich phenotypic diversity in walnut genotypes. In this study, phenotypic diversity of 80 walnut genotypes was investigated. Walnut genotypes with promising fruits characteristics were identified. Among the selections studied, the ranges of 6.61-20.66 g nut weight, 2.95-9.07 g kernel weight and 35.87-63.76% kernel ratio were observed. The most promising genotypes will be used to the benefit of conservation research, breeding and production.*

KEYWORDS*: Juglans regia L.; pomological analysis; populations; diversity*

INTRODUCTION

Walnut is one of the most widespread tree species in Romania. With its 2,000 hectares of walnut orchards and over 5 million walnut trees on own roots, Romania has achieved in the last decade a production of 32 to 34 thousand tons of walnuts (Botu et al. 2010a). There is a high genetic variability in walnut populations which exist in Romania, due to the seed

propagation, high heterozygosis and dichogamy (Cosmulescu and Botu 2012). Wide genetic variability gives great opportunities in creating varieties with valuable characters. Walnut biodiversity has been used in selection studies for producing the superior walnut cultivars in Romania (Godeanu et al. 1997, Deaconu and Vasilescu 1997, Botu et al. 2001, Draganescu et al. 2001, Botu et al. 2010b, Cosmulescu et al. 2010). The research of walnut in different location indicated great phenotypic diversity among the walnut trees within populations. Cosmulescu and Botu (2012) identified promising walnut genotypes in walnut populations located in South Western part of Romania. High variability in nut traits has been reported in walnut trees in different countries. In many countries, selection of walnut was carried out by method of simple selection out of natural seedling populations with high quality walnuts. A great range of variability was observed for various nut and kernel characters on 23 bearing seedling trees in Ladakh region of India by Sharma et al. (2010). The coefficient of variability observed was maximum (46.63%) for kernel weight. Variability was found in nut weight, by Zeneli et al. (2005) in native walnut populations of Northern Albania. The promising selected genotypes were identified in central Iran by Arzany et al. (2008) for their tree and nut characteristics. Diaz et al. (2005) found a highly significant difference in seed traits of common walnut among twenty populations from the western part of Spain. Mamadjanov (2005) studied the diversity of forest walnut in Kyrgyzstan and the best walnut forms are recommended to use as a seed material for leshozes (state forest farms), stakeholders for creation of high-yielding cultures and plantations in the walnut-fruit forest zone of Kyrgyzstan. Rezaei et al. (2008) studied the morphological features of seedling genotypes presented in Kahriz-Orumia in order to commercially select and horticultural genotypes. The majority of Serbian walnut seedlings had inferior traits, but a few genotypes with excellent traits were also found and described by Cerović et al. (2010).

In Oltenia Region of south-western Romania, there are rich walnut populations. Most walnut trees in this region have been propagated by seed thus showing high variability in tree and nut characteristics (Cosmulescu & Botu 2012). Given the quite large and so far unexploited variability within the *J. regia* in this region, the aim of this paper is to identify

superior and promising walnut genotypes within the walnut population of this region that will be further exploited through walnut breeding programmes for their tree and nut characteristics or for direct cultivation.

MATERIALS AND METHODS

In the areas of Oltenia region (Romania) two main walnut populations were investigated. 53 walnut trees were collected from the Galicea population (44°05′41″N 23°18′33″E) and 27 collected from Malaiesti population (44°30′17″N 23°45′02″ E). All trees under the study were of seedlings origin and are growing naturally. The selected genotypes were named based on their location, and these names were supplemented with numerical characters. The pomological analyses encompassed 100 nuts per tree in the period of 3 years. The nuts samples were collected from each tree over the 3 years in the time of maturity. The nut weight, kernel weight, kernel percentage, length and width of nut were measured. Features were recorded by considering the UPOV and IPGRI descriptors. For statistical analysis, Microsoft Excel and XLSTAT-Pro was used. All data were expressed as means ± standard deviations for each selection and populations. Data were subjected to Pearson correlations. Frequency distribution table for nut weight and kernel percentage characters were also prepared in order to identify the dominance in particular characters and most prevalent ranges.

RESULTS AND DISCUSSION

Nut characters diversity

The data about the extent of variation among selected walnut genotypes for nut characters are given in Table 1. Among the 53 genotypes (accessions) studied in the G location, the length of nuts varied from 28.28 mm to 46.11 mm, while in M location, among 27 genotypes, the length varied from 29.98 mm to 45.86 mm. Nuts height was found to be variable from one area selection, but the average population is very close to selection between areas (35.03 mm in M population and 35.65 mm in G location). The mean length of nuts in all 80 genotypes is 35.44 mm. The large variability in

Table1. Mean, minimum and maximum and coefficient of variation for nut characters of 80 trees from different walnut populations

Traits		Group*	
		M (n = 27)	G (n=53)
Nuts length (mm)	Mean	35.03	35.65
	Min	28.28	29.98
	Max	46.11	45.86
	SD	4.00	3.46
	CV (%)	11.42	9.7
Nuts diameter (mm)	Mean	29.30	31.55
	Min	25.39	25.70
	Max	38.61	45.32
	SD	2.79	3.18
	CV (%)	9.53	10.1
Nuts weight (g)	Mean	10.21	10.53
	Min	6.9	6.61
	Max	16.27	20.66
	SD	1.95	2.37
	CV (%)	19.18	22.5
Kernel weight (g)	Mean	5.15	5.08
	Min	2.95	3.51
	Max	8.59	9.07
	SD	1.15	2.37
	CV (%)	22.34	23.32
Kernel percentage (%)	Mean	50.97	48.51
	Min	41.66	35.87
	Max	63.17	63.76
	SD	6.21	6.16
	CV (%)	12.19	12.7

*G= Galicea population; M= Malaiesti population

height appears in the fruit of the tree the M location (CV%= 11.42). Nuts diameter ranged from 25.39 mm to 38.61 mm in location G, and from 25.70 mm to 45.32 mm in location M. The mean length of nuts in all 80 genotypes is 29.59 mm. The lowest coefficient of variation (CV %) was observed in nuts diameter (9.53 in location G and 10.1 in location M).

Nut weight and kernel percentage are the most common important parameters influencing the nut quality. Fruit size is the determining factor for the market. In this study, nuts weight varied from 6.9 g to 16.27 g in location G, while in location M the nuts weight ranged from 6.61 g to 20.66 g. The mean weight, in all 80 genotypes of fruits of all trees, is 10.41 g (Table 1). The lightest fruits (6.61 g) are on the tree No 12 from the G population, and the heaviest fruits (20.66 g) are on the tree No 36 from the G population. The highest variability in fruit weight is determined in the trees from the G population (CV 22.5%). Forty-one percent of all the fruits analyzed in the G location weigh from 8.61 to 10.62 g, thirty-two percent of nuts are weight from 10.62 to 12.63 g and only 2% of the trees exhibits nuts heavier then 16.64 g (Table 2). In the M population (Table 3) area forty-eight of fruits belong to the third size class (8.77-10.64 g), and twenty-nine of fruits belong to the fourth size class (10.64-12.52 g). Seven percent of the M population trees exhibit fruits heavier than 14.39 g. Almost fourteen percent of the trees from the two populations (G and M) have light fruits: 6.61-8.77 g. Selections from M population have medium fruit weight variability and the variability of the population of G has high. The mean nut weight in this study was 10.41g.The maximum nut weight (20.66 g) in M population was similar to the one reported by Zeneli et al. (2005) (21.1 g), but higher than those found by Arzany et al (2008) (15.2 g) and Cosmulescu and Botu (2012) (18.4g). Previous research (Cosmulescu and Botu 2012) in 109 walnut genotypes of seedling origin growing naturally in Oltenia region of Romania showed the diversity of the fruit: nut length (2.82-4.97cm), nut diameter (2.57-4.06cm) and nut weight (6.8-18.4g).

Percentage of kernel is a feature of great importance in setting the amount of selections and an important character for improvement. The higher the percentage of kernel, the lower the shell weight, while the ratio shell / kernel is lower and increases the value of the fruit. This character

varied from 41.66% to 63.17% in location G and between 35.87% and 63.76% in location M. The mean in all 80 genotypes is 49.34%. Among the nuts characters, maximum range was observed in kernel percentage, of 27.89 in location G followed by 21.51 in location G.

Table 2. Frequency distribution of walnut trees from the G population with regard to nut weight and kernel percentage of fruits

Nut weight (g)			Kernel percentage (%)		
Class	Frequency	%	Class	Frequency	%
6.61	1	1,89%	35,87	1	1,89%
8.61	6	13,21%	39.85	4	9.43%
10.62	22	54.72%	43.83	7	22.64%
12.63	17	86.79%	47.82	14	49.06%
14.63	4	94.34%	51.80	11	69.81%
16.64	2	98.11%	55.79	9	86.79%
18.65	0	98.11%	59.77	6	98.11%
More	1	100.00%	More	1	100.00%

Table 3. Frequency distribution of walnut trees from the M population with regard to nut weight and kernel percentage of fruits

Nut weight (g)			Kernel percentage (%)		
Class	Frequency	%	Class	Frequency	%
6.90	1	3.70%	41.66	1	3.70%
8.77	3	14.81%	45.96	6	25.93%
10.64	13	62.96%	50.26	6	48.15%
12.52	8	92.59%	54.56	8	77.78%
14.39	0	92.59%	58.86	3	88.89%
More	2	100.00%	More	3	100.00%

Following highest coefficient of variation (CV%) was observed in kernel weight (23.32 in location M and 22.34 in location G) followed by nuts weight (22.5 and 19.18 respectively). The maximum kernel percentage (63%) was similar to the data reported by Zeneli et al. (2005) (63.8%) and lower than the 79.6% reported by Arzany et al. (2008) and Cosmulescu and Botu (2012) (71.7%). Higher values for kernel percentage were found in China's cultivars (51-70% kernel ratio) by Baojun et al. (2010) and in Turkey's cultivars (45.66-67.14% kernel ratio) by Aslantaş (2006).

The frequency distribution was prepared to identify the distribution of export related to these characteristics. Kernel percentage in the M population (Table 3) ranged from 14.66 to 63.17%, with most accessions (29.63%) falling within the 50.26-54.56% range. Six accessions (22.23%) had a kernel percentage ranging from 41.66-45.96% and from 45.96-50.26%. Three accessions namely M7, M8 and M25 proved to be promising for new cultivars owing to higher kernel percentage i.e.>54.56%. Particularly M4 tended to show the highest kernel recovery (63.1%) among all the walnut selection. In the G population, most accessions (26.42%) had a kernel percentage ranging from 43.7-47.8 and from 47.8-51.8 (20.7%). Six accessions (11.32%) are considered the perspective of kernel percentage, content should be greater than 55.7% (Table 2).

Correlation

The correlation coefficients calculated (Table 4) among nut characters revealed significant positive correlation of nut weight with nut diameter (0.86 and 0.89) and nut length (0.76 and 0.72). Highly significant coefficient of correlation (0.68-0.66) was also recorded among nut length and nut diameter. A significant positive correlation (0.863) between nut weight and kernel weight was observed by Ghasemi et al.(2012). The highest correlation involving the kernel characteristics was found between fruit weight and kernel weight (r = 0.601) and large diameter (r = 0.567) by Cosmulescu & Botu (2012). 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).

Table 4. Correlation coefficient of different nut characters among selected walnut genotypes

Characters	Nut weight (g)		Nut length (mm)		Nut diameter (mm)		Kernel percentage (%)	
	G*	M*	G*	M*	G*	M*	G*	M*
Nut weight (g)	1.0	1.0						
Nut length (cm)	0.76	0.72	1.0	1.0				
Nut diameter (cm)	0.86	0.89	0.68	0.66	1.0	1.0		
Kernel percentage (%)	0.11	0.13	0.07	0.27	0.15	0.13	1.0	1.0

*G= Galicea population; M= Malaiesti population

CONCLUSION

Evaluation of 80 walnut seedling genotypes shows that there are valuable genotypes in Oltenia region. The best selections will be listed in collections in Romania for conservation to the benefit of research and commercial farms, avoiding their loss caused by genetic erosion. With the research which started and is supposed to continue in the future, the aim is to further explore and gain excellent domestic varieties for marketing production of high quality walnut in Romania.

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