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STUDY ON GENETIC VARIABILITY OF COMMON WALNUT (*Juglans regia* L.) FROM NORTHERN OLTENIA AND EPIRUS

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ABSTRACT. *There are common walnut populations comprising over 166,797 trees scattered all over the area in the northern part of Oltenia (Gorj and Vâlcea counties) in Romania. In Epirus region of Greece there are 156,493 registered walnut trees. All of these are natural hybrids, grown on their own roots, and exhibit important genetic variability for most of their characteristics. Natural hybrids have a share of over 95% of the walnut trees from Northern Oltenia and 62% from Epirus. The two areas studied are 1000 km away (northern Oltenia is located on the latitude of 45°N and Epirus on the latitude of 39°N), and are distinguished by geographic relief, climatic and soil conditions. Northern Oltenia has a temperate continental climate, with light Mediterranean influences (annual average temperature of 11.9°C) and Epirus has a typical Mediterranean climate in the coastal areas (annual average temperature of 17.9°C), but colder at 600-800 m elevations. In both regions, the ecological conditions are favorable to the continuity of this species and to the formation of many genotypes different from genetic and phenotypic point of view. The 53 genotypes studied (32 in Epirus and 21 in northern Oltenia) showed differences in terms of the tree vigor, type of growth, fruiting type, type and time of flowering, fruit morphological and biochemical properties.*

KEYWORDS: *Juglans regia, walnut, diversity, selections*

INTRODUCTION

Romania and Greece are two European countries holding rich common walnut (*Juglans regia* L.). The common walnut known also as European, English, Persian or Carpathian walnut was naturalized and it is widely grown in various areas of the two countries, contributing with 43.2% of the total EU production and over 1.89% of the world production (FAO State Database, 2013).

Walnut fruits are particularly demanded on various markets due to their nutritional value and the walnut timber is valued for furniture. *Juglans regia* species has a vast habitat, but is more and more affected by genetic erosion and vulnerability and for this reason the necessity of saving its genetic variability is very important. In order to preserve the walnut biodiversity for future generations several programs for the conservation and utilization of these genetic resources worldwide have been launched in the last decades. Worldwide there are several important walnut collections: National Clonal Germplasm Repository in Davis - California (USA) holding 210 accessions (Stebbins, 1993); National Clonal Germplasm Repository from Corvallis - Oregon (USA), where 25 accessions of *Juglans cinerea* L. are conserved; National Repository of Shandong – China (Wu et.al. 2010) ; Yalova Institute - Turkey (Kazankaya et.al. 2001, Ayanoglu et al. 2001), with a collections of 250 walnut genotypes (Akça and Sen 2001), etc.

In Romania, identification and preservation of walnut germplasm began after 1955-1960 period (Draganescu et al. 2001). Over 200 walnut genotypes are present nowadays in the national collection, most of them at the University of Craiova - SCDP Vâlcea. During last decades, more than 550 walnut genotypes have been identified and evaluated (Botu et. al. 2001, Botu et al. 2010, Cosmulescu et al. 2010, Cosmulescu and Botu 2012). In Greece, there have been implemented programs to detect walnut genetic resources since 1986. The collection of genetic resources from Lamia Station includes over 70 domestic selections; out of them 29 genotypes have lateral fruiting habits (Rouskas et al. 1997, Rouskas and Zakynthinos 2001). Present study has the aim to identify some of the common walnut genetic resources from North Oltenia - Romania and

Epirus - Greece, both areas being rich in walnut populations and individuals valuable for genetic conservation and for direct use in commercial farms.

MATERIALS AND METHODS

The biological material, which has been identified and evaluated during present study, consists of *Juglans regia* individuals located in different regions of North Oltenia (Romania) and Epirus (Greece), areas situated about 1000 km apart. The walnut genotypes identified were aged between 10 and 65 years. Selected genotypes are located between 210 to 546 m elevation in Northern Oltenia and from 3 to 967m elevation in Epirus. Research methods used were related to the study areas, walnut tree selection location, determining of the tree vigor of growth, growth and fruiting phases, fruiting characteristics, characteristics and chemical composition of the fruits, the degree of adaptability to climatic conditions, disease susceptibility, etc. IPGRI walnut descriptors have been used (IPGRI, 1994).

RESULTS AND DISCUSSION

In Northern Oltenia and Epirus, there are 166,797 walnut trees and 156,493 trees respectively. These trees are natural hybrids on own roots in different populations. Out of these populations, 21 biotypes have been located with a GPS device and selected in North Oltenia in different localities situated on 45^0N latitude and 23^0 and 24^0E longitudes. From Epirus 32 genotypes were selected between 39^0N latitude and 21^0 and 22^0 E longitude. The geographic, orographic and climatic conditions are different in the two study areas. In case of North Oltenia, the average annual temperature was 11.7^0C during present study. The maximum temperature recorded was 40.4^0C and the minimum temperature -22^0C. The annual rainfall level reached 720.7 mm. These characteristics are of a continental climate with mild Mediterranean influences. The Epirus region has a typical Mediterranean climate, slightly more temperate in the mountains at elevations over 800m. The average annual temperature is 17.8^0C in the field area and 15.5^0C in the mountains. The minimum temperatures drop to

-11^{0}C in the mountains and rainfall level reach 1376 mm annually. Most of the precipitations are distributed mainly from October till March. The evaluations conducted for all the 53 walnut selections show that 31 of them are vigorous in tree size, 19 selections have medium vigor and only three selections (only from Epirus) have low vigor. A number of 6 selections (4 from Epirus and 2 from Oltenia) are presented in this paper. The two selections from Oltenia have high vigor, in case of those of Epirus, two are vigorous and the other two have medium vigor (Table 1). Phenological

Table 1. Tree characteristics of walnut selections from Epirus and Northern Oltenia

No.	Area (Country)	Selection	Tree vigor	Fruit bearing type	Fruit yield	Resistance to blight
1.	Epirus (Greece)	GR. Epirus 12	Medium	Terminal	High	Less susceptible
		GR. Epirus 25	Medium	Terminal	High	Less susceptible
		GR. Epirus 7	High	Lateral	High	Less susceptible
		GR. Epirus 3	High	Terminal	High	Less susceptible
2.	Northern Oltenia (Romania)	Cernişoara P7	High	Terminal	High	Less susceptible
		Păuşeşti M. R1	High	Terminal	Medium	Less susceptible

stages of the walnut selections have been recorded during the study period. Blooming of the female flowers occurred between April 15 (GR. Epirus 6) and May 5 (GR. Epirus 25) in Epirus and from 15 April (Troianu 4) and April 30 (Dăeşti P3) in Northern Oltenia. The male flowers started blooming in Epirus from April 5 (GR. Epirus 15) and April 27 (GR. Epirus 3),

while in North Oltenia male flowers bloomed between April 20 (Troianu 8) and May 5 (Cernişoara T5).

Walnut blooming is characterized by dichogamy. Protandrous selections proved dominant in Epirus (71.8 %), the rest being protogynous (28.2 %). In North Oltenia protogynous genotypes are prevailing (61.9 %), the rest being protandrous (28.6 %) and homogamous (9.5%). Protandrous dichogamy proved dominant in warmer climatic areas in case of walnut.

Ripening time of walnut fruits vary in the two different areas but also within each region. Walnut selections from Epirus mature their fruits from the beginning of September till beginning of October. The selections from Northern Oltenia are maturing their fruits in September (Table 2). The selections from the two areas have high and constant productivity from one year to another (10-25kg/tree). All the 21 walnut selections from North Oltenia have terminal bearing type, while those of Epirus have terminal bearing (19 selections), intermediate bearing (5 of them) and lateral bearing (8 selections). The fruit characteristics exhibit variability in both of the selection areas due to a range of morphological and biochemical elements. Fruit size and weight varies very much from one selection to another in the both selection areas.

Walnut fruit size is one of the main determinants for international trade. Of the 32 Epirus selections, 14 selections (44%) have large fruits, 7 selections (22%) have large fruits, 6 selections (19%) have medium size fruits and 5 selections (15%) have small fruits. Out of the 21 walnut selections from North Oltenia, 10 of them (47.6%) have very large nuts, 8 selections (38.1%) have large fruits, 2 selections (9.5%) have average sized fruits and one selection (4.8%) has small fruits (Table 3). Fruit Size Index (F.S.I.) as an average of the widest fruit width (D), narrow width (d) and height (h) have been used (Table 3). The good quality walnut fruits of 9.0 to 10.0 g weight can be used for shelled market while those exceeding 11 grams can be used also for in-shell market

The walnuts of the Epirus selections have different weight or mass; 9 selections (28%) have less than 9.0 g weight, the rest of them (72%) weight between 9.5 to 16.99 g in average. In North Oltenia, 16 selections have

Table 2. Phenological characteristics of walnut selections from Epirus and Oltenia

No.	Area (Country)	Selection	Bud break	Male flower blooming time	Female flower blooming time	Dichogamy type	Fruit ripening time
1.	Epirus (Greece)	GR. Epirus 12	Medium	15-25 April	8-19 April	Protogynous	10-20 September
		GR. Epirus 25	Medium	7-15 April	10-18 April	Protandrous	20-30 September
		GR. Epirus 7	Very early	14-21 April	9-18 April	Protogynous	20-30 September
		GR. Epirus 3	Very early	27 April - 3 May	21April - 5 May	Protogynous	20-30 September
2.	Northern Oltenia (Romania)	Cernişoara P7	Medium	1-10 May	28 April - 6 May	Protogynous	20-30 September
		Păuşeşti M.R1	Medium	28 April - 3 May	24-30 April	Protogynous	1-10 September

fruits weighting from 9.0 to 16.2 g, the remaining five selections have average fruit weights less than 9.0 g. Another important element for defining the walnut quality is the percentage of kernel. In case of walnut selections from Epirus, the percentage of kernel varied between 39.6% (GR. Epirus 5) and 53% (GR. Epirus 2). A number of nine selections (28%) have 50% kernel ratio. In case of selections from Northern Oltenia, the percentage of kernel ranged from 45% (Troianu 1 and Cernişoara 7) to 55% (Mihăeşti T1). Of the 21 subjects selections, only 5 (23.8%) yields more than 50% kernel (Mihăeşti T1, Oteşani 1, Păuşeşti Otăsău 2, Cernişoara P7 and Vaideeni P1).

Nut shell of the walnut selections of Epirus and Northern Oltenia is thin or medium (0.8 to 1.2 mm in thickness). Walnut selections of Epirus have high content in fat, which varies between 60.0% (GR Epirus 20) to 65.8% (GR Epirus 32); proteins, which oscillate from 15.7% (GR. Epirus 37) to

21.3% (GR. Epirus 20) and carbohydrates from 12.2% (GR. Epirus 32) to 13.8% (GR. Epirus 7). The fat content of the selections from Northern Oltenia varies from 58.7 to 66.2% (Troianu 10), the protein content varied from 15.7% to 21.4% and the carbohydrates from 12.1% (Runcu 1) to 13.8% (Oteşani 1).

Table 3. Fruit characteristics of the walnut selections from Epirus and Oltenia

No.	Area / Country	Selection	Fruit Size Index (mm)	Fruit weight (g)	Kernel ratio (%)
1.	Epirus (Greece)	GR. Epirus 12	34.8	13.6	52.9
		GR. Epirus 25	34.3	11.3	52.2
		GR. Epirus 7	31.1	10.2	51.0
		GR. Epirus 3	33.2	11.3	49.6
2.	Northern Oltenia (Romania)	Cernişoara P7	40.1	18.5	50.0
		Păuşeşti M.R1	29.6	13.2	48.0

Analyses of climatic elements of the two regions, based on correlation and regression coefficients, showed certain differences in the growing and fruiting phases. It is also obvious that the selections of the North Oltenia area have mostly protogynous type of dichogamy, while the Epirus selections are dominant of protandrous type of dichogamy. The walnut selections of North Oltenia have terminal fruit bearing, while those of Epirus have terminal, mixed and lateral fruiting bearing habits. The two major diseases of walnuts: walnut blight or bacteriosis (caused by *Xanthomonas campestris* pv. *juglandis*) and anthracnose (caused by *Gnomonia leptostyla*) are affecting some selections from Epirus and Northern Oltenia. The walnut blight attack frequency and intensity of all selections in Epirus was between 5.5% (for GR. Epirus 22) and 28.6% (GR. Epirus 27) on fruits, while on leaves varied from 2.8% (GR. Epirus 5) and 19.6% (GR. Epirus 6).

The most affected selections by walnut blight proved those with lateral bearing fruits habit (5.8 to 28.6 %).

Walnut anthracnose attack also manifested in all selections of Epirus. Frequency ranged from 3.3% (GR. Epirus 10) and 14.6 % (GR. Epirus 21) on fruits and from 4.3% (GR. Epirus 17) to 16.2% (GR. Epirus 21) on leaves. Lateral fruiting selections proved also more susceptible than the others.

In case of walnut selections from Northern Oltenia the blight frequency attack on fruits ranged from 3.6% (Troianu 10) to 10.4% (Troianu 7) and on leaves between 3.9% (Troianu 6) and 11.2% (Troianu 3). The presence of anthracnose attack was recorded for all selection having variable levels on fruits between 3.2% (Dăeşti P3) and 4.9% (Cernişoara P7) while on leaves between 5.7% (Mihăeşti T1) and 9 6% (Troianu 3).

Taking into account the growth and fruiting characteristics of the walnut genotypes 1998 – Rapport de la Quatrieme Session Extraordinaire de la Commision des Resources Genetique pour l'Alimentation el l'Agriculture. CGRFA – Ex 4/97/Rapport FAO, Roma have been selected (4 of Epirus and 2 from Northern Oltenia) for further evaluation and direct use in the walnut farms, but only after clonal propagation.

CONCLUSIONS

The areas of Northern Oltenia (Romania) and Epirus (Greece) have important biodiversity of the *Juglans regia* L. species, wide variability, but increasingly subject to genetic erosion and genetic vulnerability pressure. The two areas have geographical and ecological characteristics differentiated between them and they have made their mark on the adaptability of walnut populations and selections. 32 walnut genotypes have been selected in Epirus and 21 in the North of Oltenia, these genotypes are highly differentiated between them on the basis of growth, fruiting, adaptation to climatic conditions and resistance to certain diseases. The 53 genotypes of common walnut will be introduced into the germplasm collections in Romania and Greece, each one according to origin and will

be used in the walnut breeding programs. A total number of six selections (GR. Epirus 3, GR. Epirus 7, GR. Epirus 12, GR. Epirus 25, Cernişoara P7 and Păuşeşti M.R1) have greater agro biological value similar to commercial cultivars and can be introduced, after clonal propagation, into the new walnut orchards established in the two areas.

REFERENCES

Akça, Y. and Sen, S.M. (2001): A study on the genetic variability and selection of superior walnut *(Juglans regia L.)* trees within seedling population of around of Van Lake. Acta Horticulturae 544: 119-124.

Ayanoglu, H. and Bayazit, S. (2001): Selection of walnut genotypes in Hatay Province of Turkey. Acta Horticulturae 544: 109-113.

Botu, I., Botu, M. and Achim, G. (2001): Cultura nucului în exploataţii nucicole moderne. Editura Phoenix, Braşov.

Botu, M., Tudor, M., Botu, I. Cosmulescu, S. and Papachatzis, A (2010): Evaluation of walnut cultivars in the conditions of the Oltenia's hill area regarding functioning potential. Analele Universitatii din Craiova, Biologie, Horticultura, Tehnologia prelucrarii produselor agricole, Ingineria mediului 15: 94-103.

Cosmulescu, S., Botu, M. and Trandafir, I. (2010): Mineral composition and physical characteristics of walnut (*Juglans regia* L.) cultivars originating in Romania. Selçuk Tarım ve Gıda Bilimleri Dergisi 24(4): 33-37.

Cosmulescu, S. and Botu M. (2012): Walnut biodiversity in South-Western Romania-resource for perspective cultivars. Pakistan Journal of Botany 44(1): 307-31.

Draganescu, E., Nedelea, G., Mihut, E. and Blidariu, A. (2001): Researches concerning the germplasm variability of walnut *(Juglans regia)* existing in Banat, Romania. Acta Horticulturae 544: 133-140.

FAO Stat Database. (2013): www.fao.org

IPGRI (1994): Descriptors for walnut (*Juglans* spp.) International Plant Genetic Resources Institute.

Kazankaya, A., Koyuncu, M.A., Koyuncu, F., Yarilgac, T. and Sen, S.M. (2001): Some nut properties of walnuts (*Juglans regia* L.) of Edremit Country. Acta Horticulturae 544: 97-100.

Rouskas, D., Katranis, N., Zakynthinos, G. and Isaakidis, R. (1997): Walnuts (*Juglans regia* L.) seedlings selection in Greece. Acta Horticulturae 442: 109-116.

Rouskas, D. and Zakynthinos, G. (2001): Preliminary evaluation of seventy walnut (*Juglans regia* L.) seedlings selections in Greece. Acta Horticulturae 544:61-72.

Stebbins, G. L. (1993): Cooperation in conservation of California's rare habitats and species. *In* J. E. Keeley ed. Interface between ecology and Land Development in California, pp. 1115. Los Angeles, Southern California Academy of Sciences.

Wu, G.L., Meng, H.J., Hao, Y.Y., Liu, Q.L., Wang, D. and Tian, J.B. (2010): Thirty years of breeding walnut in China. Acta Horticulturae 861: 109-118.