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Agrobiological Evaluation of 'Lara' Walnut Cultivar under the Climatic Conditions of South Bulgaria

S. Gandev¹, V. Arnaudov¹, V. Dzhuvinov¹, M. Perifanova-Nemska² and K. Koumanov¹

¹ Fruitgrowing Institute, 12 Ostromila Str., 4004 Plovdiv, Bulgaria
² University of Food Technologies, 26 Maritza Str., 4002 Plovdiv, Bulgaria

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Abstract

One of the first steps to walnut improvement in Bulgaria is the introduction of cultivars with high productivity and better fruit quality. In this context, there is an increasing interest in cultivars of lateral bearing habits. The objectives of the present study include a complex evaluation of 'Lara' walnut cultivar and assessment of the possibility for growing it in Bulgaria. Subjects of investigations are: growth, yield, fruit quality, kernel oil content, spring-frost tolerance, susceptibility to walnut anthracnose (Gnomonia leptostyla) and walnut bacterial blight (Xanthomonas arboricola pv. Juglandis). The experimental orchard was established in the spring of 2003 on the territory of the Fruitgrowing Institute in Ploydiv. Presented results are for the period 2008-2010, i.e. sixth-eighth vegetations of the walnut trees. The most widely grown Bulgarian cultivars, 'Sheynovo' and 'Izvor 10', were used as controls. All experimental trees were grafted on English walnut (Juglans regia L.) rootstock. After the eighth vegetation, 'Lara' and 'Sheynovo' cultivars had relatively similar canopy volumes, 24.5 m³ and 18.8 m³, respectively. In contrast, the 9.1 m³ canopy volume of 'Izvor 10' is significantly lower. Both the vegetation onset and the blossom of 'Lara' occurred later compared to the control cultivars. Because of that, there were no spring-frost injuries found on 'Lara' trees. The mean weight of 'Lara' fruits was 13.4 g, which was close to that of 'Sheynovo' - 12.5 g, and significantly higher than the 10.6 g mean weight of 'Izvor 10'. 'Lara' was less susceptible to anthracnose compared to 'Sheynovo' and 'Izvor 10'. All the three cultivars had low susceptibility to walnut bacterial blight and showed no differences regarding the degree of infestation. The kernel oil content of 'Izvor 10', 'Sheynovo' and 'Lara' was 69.02%, 66.35% and 62.26%, respectively. According to the obtained results, the 'Lara' walnut cultivar is both economically valuable and biologically adaptable to the local soil and climatic conditions, which makes it attractive for the Bulgarian fruit growers.

INTRODUCTION

The diversity of walnut cultivars in Bulgaria has not been changed since the 70s of 20 c. and at present the local cultivars 'Izvor 10' and 'Sheynovo' are the most widely spread (Manolova and Gandev, 2004). One of the steps to walnut improvement in the country is the diversification of walnut cultivars by introducing new ones that comply with modern requirements. The cultivars, which are envisaged to be grown in Bulgaria, should have the following characteristics: suitability to be grown under the climatic conditions of the country (Gandev et al., 2009); good resistance to anthracnose (*Gnomonia leptostyla*) and walnut bacterial blight (*Xanthomonas arboricola* pv. *Juglandis*), (Dzhuvinov et. al., 2010); balanced mineral and fatty acid content (Perifanova-Nemska et al., 2001) and, last but not least, lateral bearing habit of the cultivar, that guarantees obtaining of `high yields (Hendricks et al., 1985; Germain and Prunet, 1999).

The aim of the study was to carry out an agrobiological evaluation of the introduced walnut cultivar 'Lara' and to assess the possibilities of its growing in South Bulgaria.

MATERIALS AND METHODS

The experimental plot was established at the Fruit-Growing Institute – Plovdiv in the spring of 2003 and the study covered the period 2008-2010, i.e. sixth, seventh and eighth vegetations of the walnut trees. The biological characteristics and economic parameters of the fruit produce obtained from the introduced cultivar 'Lara' were studied and compared to those of the standard Bulgarian cultivars 'Izvor 10' and 'Sheynovo'. All the three walnut cultivars were grafted on English walnut rootstock (*Juglans regia* L.).

The vegetative and reproductive habits of the cultivars were reported following the methods of studying fruit tree resources (Nedev et al., 1979) and the adopted international standard for description of walnut genetic resources (Germain, 2004).

Susceptibility to the economically important diseases anthracnose (*Gnomonia leptostyla*) and walnut bacterial blight (*Xanthomonas arboricola* pv. *juglandis*) was evaluated according to the infection index calculated following the formula of McKinney (1923), using collected walnut leaves.

The chemical composition of the walnut kernel oil obtained from 'Lara' cultivar, was studied and compared to the control cultivars 'Izvor 10' and 'Sheynovo'. The kernel oil was extracted with petroleum ether using the Soxhlet apparatus (Hadzhiiski and Perifanova-Nemska, 1994). The tocopherol content of the obtained oil was determined by liquid chromatography and the individual fatty acid content in the triacylglycerols – by gas chromatography, investigating the obtained pure methyl esters by gas chromatography (Metcalfe, Wang 1981; Kyriakidis, Katsiloulis, 2000).

Data were statistically processed by Duncan's test (Steele and Torrie, 1980).

RESULTS AND DISCUSSION

1. Growth habits

Data presented in Table 1 showed that at the end of the eighth vegetation, the trunk cross-section area of the trees of the studied cultivars 'Izvor 10', 'Sheynovo' and 'Lara' did not differ considerably. However, the data about the canopy volume did not show the same tendency. While the canopy volumes of 'Lara' and 'Sheynovo' had similar values – 24.5 m³ of the former and 18.8 m³ of the latter, the canopy volume of 'Izvor 10' was 9.1 m³, i.e. significantly lower compared to the other two cultivars. The poorer growth of 'Izvor 10' cultivar in comparison with the growth habit of 'Sheynovo' confirmed the results obtained in a previous study (Nedev et. al., 2002).

2. Phenological observations

The phenological observations showed that in the two experimental years, the beginning of vegetation and blooming of 'Lara' cultivar started later compared to the cultivars 'Izvor 10' and 'Sheynovo' (Table 2). All the three cultivars showed dichogamy in flowering, the dichogamy being incomplete, i.e. there was a partial overlapping period of coming out of the male and female flowers. It should be noted that the period of overlapping in 'Lara' cultivar was within 9-11 days, which is a precondition of successful self-pollination of the cultivar. Table 2 also showed that blooming of 'Sheynovo' is protandrous and for 'Izvor 10' and 'Lara' is protogynous. The results obtained about the flowering type of 'Lara' cultivar did not confirm the results of Germain et al. (1999) that blooming of 'Lara' cultivar is of a protandrous type. According to Nedev et al. (1983) the direction of dichogamy in some cultivars depended on the temperature factor, i.e. on the climatic conditions under which the cultivar was grown.

3. Biometric characteristics of fruits, yields and harvest time.

The results of the fruit biometry presented in Table 3 showed that the largest mean weight was reported for the 'Lara' cultivar – 13.4 g. The nuts of the control cultivars 'Izvor 10' and 'Sheynovo' had a mean weight of 10.6 g and 12.5 g, respectively. Concerning the kernel percentage, it should be noted that the Bulgarian cultivars 'Izvor 10' and 'Sheynovo' had higher values of that characteristic compared to 'Lara'. The kernel percentage of 'Lara' was 44.5% versus 53.5% of 'Izvor 10' and 54.5% of 'Sheynovo'.

The data presented in Table 4 showed that in 2009 and 2010 the yields per tree for the lateral bearing cultivars 'Izvor 10' and 'Lara' were similar. 20.4 kg of fruit per tree were harvested from 'Izvor 10' and 18.4 kg from 'Lara', in average for the two-year period. The yields obtained from the 'Sheynovo' cultivar were lower compared to the other two cultivars in the two reported years, which was due to the intermediate type of fruit-bearing. The average yield of the cultivar was 12.5 kg per tree.

The period of harvest time was different for the three cultivars. 'Izvor 10' has a medium early ripening period. In the separate years its fruits ripened between 6^{th} and 15^{th} of September. 'Sheynovo' has a medium late period of harvest time – between 16^{th} and 25^{th} of September and 'Lara' is a late cultivar, its fruits ripening from the end of September till the first decade of October.

4. Resistance to late spring frosts.

A temperature drop down to -4,4 °C on 27 March 2008 provided the opportunity to study the resistance of 'Lara' trees to low spring temperatures and to compare the resistance to the 'Sheynovo' cultivar, which was adopted as a standard for frost resistance under the Bulgarian conditions (Nedev et al., 1979). In Sheynovo 41% of the young shoots were damaged by the frost, while in 'Lara' frost damages were not established. The different frost susceptibility of the two cultivars could be attributed to their phenological development. At the time of the spring frost, the trees of 'Sheynovo' were at the phenological stage Df (leaf differentiation) and those of 'Lara' at Af (winter dormancy) that protected them from the negative effect of the late spring frost.

5. Disease resistance.

The results of the carried out investigations on establishing the susceptibility of the studied walnut cultivars to the causative agents of the two economically most important diseases – anthracnose (*Gnomonia leptostyla*) and walnut bacteria blight (*Xamthomonas arboricola pv. Juglandis*) – were presented in Tables 5 and 6. Data showed that 'Lara' cultivar is slightly susceptible to the attacks of *G. leptostyla* in contrast to 'Izvor 10' and 'Sheynovo' cvs., which are susceptible. Regarding the degree of infestation by *X. arboricola pv. juglandis*, all the three cultivars showed slight susceptibility. In 'Lara' cultivar the reported average infection index was 23.0. It was close to the index reported in 'Izvor 10' – 24.3 and significantly higher that 'Sheynovo' one – 14.3.

6. Chemical composition of walnut oils.

The major characteristics of the walnut kernel oils showed that the highest fat content was reported in 'Izvor 10' – 69.02% and the lowest in 'Lara' – 62.26%. 'Sheynovo' occupied the intermediate position between the two cultivars with content of 66.35%. The individual content of the oils obtained from the studied cultivars showed that the content of tocopherols in 'Lara' was 225 mg/kg and it was higher compared to 'Sheynovo' (136 mg/kg) and lower than of 'Izvor 10' (242 mg/kg). The ratio of saturated to unsaturated fatty acids was the

following: 'Lara' -21.7: 78.3; 'Izvor 10' -24.7: 75.3 and 'Sheynovo' -8.86: 91.16, respectively.

Similar contents of linoleic, linolenic and arachinic fatty acids were established in the three walnut cultivar oils. It confirmed that the studied cultivar 'Lara' showed good vitamin activity like the widely grown in the country cultivars 'Izvor 10' and 'Sheynovo'. In 'Lara' the content of linoleic acid was 45,8%, linolenic -4,1% and arachinic -0,2%. Other fatty acids were also established in "Lara' cultivar, such as palmitic acid (13,2%), oleic (24,3%), laurinic (1.7%), etc.

CONCLUSIONS

Under the conditions of South Bulgaria, the 'Lara' cultivar is characterized by moderate to vigorous growth, a late blooming period, high fertility, good resistance to late spring frosts and low susceptibility to anthracnose (*G. leptostyla*) and walnut bacterial blight (*X. arboricola pv. juglandis*) attacks. The mean weight is 13.4 g and the kernel percentage is 44.5%. The kernel oil content of the cultivar is 62.26%. Under the climatic conditions of South Bulgaria, fruits ripen from the end of September till the first decade of October.

The economic results obtained about the 'Lara' cultivar and its good adaptability under the climatic conditions of South Bulgaria determined it as attractive to be grown in the country.

Literature Cited

- Dzhuvinov, V., Gandev, S., Koumanov, K. and Arnaudov, V. 2010. Walnut. Biofruit, Plovdiv.
- Gandev, S., Dzhuvinov, V. and Arnaudov V. 2009. Susceptibility of introduced walnut cultivars to late spring frost. Plant Science, 46: 75-77.
- Germain E., Prunet, Y-P., Garcin, A. 1999. Le Noyer. CTIFL, Paris.
- Germain, E. 2004. Inventory of walnut research, germplasm and references. Food and Agriculture Organization of the United Nations, Rome
- Hadjiski Cv., Perifanova-Nemska, M. 1994. Deriving of vegetable oils(Laboratory practis book), University of food technology, Plovdiv, 1994.
- Hendricks, L., McGranahan, G., Ramos, D., Iwakiri, B. and Forde, H. 1985. Selection of varieties, p. 46-51. In: Ramos, D. (Ed.), Walnut orchard management. Publication 21410, University of California, USA.
- Kyriakidis N., Katsiloulis T., 2000. Calculation of Iodine Value from Measurements of Fatty Acid methyl esters of some Oils:Comparison with the relevant American Oil Chemists Society Method, Journal of the American Oil Chemists' Society, 77: 1232 – 1238.
- Manolova.V., Gandev, S. 2004. Survey on walnut production in Bulgaria. Agricultural economics and management, 49: 3-5.
- McKinney, H. H. 1923. A new system of grading plant diseases. Journal of Agricultural Research, 26: 195-218.
- Metcalfe L., Wang, C. 1981. Rapid preparation of fatty acid methyl esters using organic base catalized transesterification, Journal of Chromatography Science, 19: 530 533.
- Nedev, N., Grigorov, J., Baev, H., Serafimov, S., Stranjev, A., Kavardjikov, L., Lazarov, K., Nikolov, N., Dzhuvinov, V., Popova, L., Slavov, N., Iliev, P., Stoianov, D., Kanev, I., Krinkov, H., Vishanska, Y., Topchiiska, M., Petrova, L. 1979. Methodology for studying of plant resources among fruit species. Plovdiv.

- Nedev, N., Serafimov, S., Anadoliev, G., Kavardjikov, L., Krinkov, H., Radev, R., Dochev, D., Stamatov, I., Slavov, N., Vishanska, Y., Rusalimov, J., Iovchev, I., Djeneva, A., Lalev, N., Iliev, I., Slavcheva, R. 1983. Fruit Nuts. H. G. Danov, Plovdiv.
- Nedev, N., Kavardjikov, L. and Gandev, S. 2002. Izvor 10 a promising walnut cultivar. Plant Science, 39: 54-56.
- Perifanova-Nemska, M., Gandev, S., Zlatanov, M. and Antova G. 2001. Chemical composition of walnuts. Agricultural University-Plovdiv, Bulgaria, Scientific Works, vol. XLVI, 5: 195-200.
- Steele, R. and Torrie, J. 1980. Principles and procedures of statistics. McGraw-Hill, New York.

<u>Tables</u>

Cultivar	Trunk cross-section area (cm ²)	Canopy volume (m ³)
Izvor 10	278.3 a	9.1 b
Sheynovo	316.4 a	18.8 a
Lara	278.0 a	24.5 a

Table 1. Trunk cross-section area and canopy volume of the 'Izvor 10', 'Sheynovo' and 'Lara' walnut cultivars at the end of the eighth vegetation period.

Table 2. Beginning of vegetation and blooming of the 'Izvor 10', 'Sheynovo' and	l 'Lara'
walnut cultivars in 2009 and 2010.	

Cultivar	Year	Time of bud	Female	e bloomin	g date	Male blooming date		
	break	Begi- nning	Mass	End	Begi- nning	Mass	End	
Izvor 10	2009	02.04.	16.04.	22.04.	02.05.	30.04.	02.05.	06.05.
	2010	30.03.	08.04.	10.04.	16.04.	26.04.	30.04.	02.05.
Sheynovo	2009	04.04.	28.04.	02.05.	04.05.	18.04.	22.04.	30.04.
	2010	06.04.	26.04.	30.04.	02.05.	16.04.	20.04.	28.04.
Lara	2009	28.04.	07.05.	10.05.	20.05.	10.05.	14.05.	18.05.
	2010	23.04.	26.04	02.05.	14.05.	04.05.	10.05.	14.05.

Cultivar	Nut shape (mm)	Nut length (mm)	Nut width (mm)	Mean weight (g)	Kernel colour	Kernel percentage (%)
Izvor 10	Oblong	40.6 a	30.7 b	10.6 b	light	53.5 a
Sheynovo	Oblong	42.3 a	31.7 b	12.5 ab	amber	54.3 a
Lara	Oval	38.4 a	36.2 a	13.4 a	light	44.5 b

Table 3. Fruit characteristics of the 'Izvor 10', 'Sheynovo' and 'Lara' cultivars for the period of study (2008-2010).

Table 4. Average yields obtained from the 'Izvor 10', 'Sheynovo' and 'Lara' cultivars in 2009 and 2010.

Cultivar	Yield per tree (kg)					
	2009	2010	2009-2010			
Izvor 10	17.8 a	23.0 a	20.4 a			
Sheynovo	9.1 b	15.8 b	12.5 b			
Lara	16.8 a	20.0 a	18.4 a			

	Leaf infestation index, by McKinney							
Cultivar		June October						
	2005	2006	2007	Average	2005	2006	2007	Average
Izvor 10	16.9	26.8	20.8	21.5	32.0	50.8	39.3	$40.7a^4$
Sheynovo	13.6	21.8	14.2	16.5	29.7	47.5	30.9	36.0a ⁴
Lara	10.9	15.0	13.4	11.1	15.8	27.1	20.0	$21.0b^{3}$

Table 5. Response of walnut leaves to *G. leptostyla* attacks in the period 2005-2007.

Cultivar susceptibility: (1) Highly resistant (up to 1 % infected area; (2) Resistant (1–5 % infected area); (3) Slightly susceptible (5–25 % infected area); (4) Susceptible (25-50 % infected area); (5) Highly susceptible (50 - 100 % infected area).

Table 6. Response of walnut leaves to *X. arboricola pv. juglandis* attacks in the period 2006-2008.

	Leaf infestation index, by McKinney							
Cultivar		June				June October		
	2006	2007	2008	Average	2006	2007	2008	Average
Izvor 10	3.9	8.2	7.9	6.67	16.4	28.7	27.8	$24.30a^{3}$
Sheynovo	2.1	4.9	5.2	4.07	9.5	14.7	18.6	$14.27b^{3}$
Lara	4.3	7.8	10.4	7.50	12.1	24.4	32.50	$23.00a^{3}$

Cultivar susceptibility: (1) Highly resistant (0-3 % infected area); (2) Resistant (3-10 % infected area); (3) Slightly susceptible (10-25 % infected area); (4) Susceptible (25-50 % infected area); (5) Highly susceptible (50- 100 % infected area)