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GROWTH, REPRODUCTIVE CHARACTERISTICS AND ANTIOXIDANT ACTIVITY OF FOUR GOJI BERRY (*LYCIUM BARBARUM* L.) CULTIVARS

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Abstract

The aim of the study was to characterize the growth and reproductive characteristics of four in vitro propagated goji berry varieties (JB1, JB 2, JB 4, JB 10) of the species (Lycium barbarum L.) and antioxidant properties of their fruits.The experiment was set in 2014 in the experimental base of the Department of Fruit Growing at the Agricultural University - Plovdiv. The results are for the period 2017-2019. Growth and quality indicators were studied, as well as the amount of total polyphenols and total flavonoids responsible for the antioxidant activity of the fruit. On average for the study period, variety JB1 emerged as the most productive, and the lowest yields were obtained from JB10. The data were processed by the method of analysis of variance.

There are differences in the content of total polyphenols and flavonoids in the fruits of the studied varieties, and hence in their antioxidant activity. Variety JB 10 is characterized by the highest content of total polyphenols - 278 mg/100g and the highest ORAC value - 89.7 μmol TE/g.

Keywords*: goji berry, Lycium barbarum L., growth, yield, antioxidant activity.*

INTRODUCTION

Goji berry belongs to the species Lycium barbarum L from (Solanaceae). It has been grown for centuries in China. From the first decade of the XXI century, its spread began in many countries in Europe and America. Currently, there is an increase in consumption of its fruits, due to their healing properties. (Institute of Chinese Materia Medica, 1997). The interest in growing the plant is due to its fruits. They contain a huge amount of vitamins, trace elements, proteins, amino acids, polysaccharides, monosaccharides, which is why this species is classified in the group of super foods (Van Straten and Griggs, 2006). Natural phenols are common ingredients in plants. They affect a number of physiological and biochemical processes in the human body. Goji berry fruits have high levels of active ingredients that have a wide range of biological activity. Georgiev K., 2013. Polysaccharides and total sugars in goji berries are defined as very important quality characteristics. The taste and size of the fruit grown under different conditions are not the same. Guo.Qi et al 2010, studied the content of glucose, fructose, sucrose, polysaccharides and total sugars in the fruits of different varieties of goji berries of the species Lycium barbarum.L and Lycium chinense collected from different regions. The authors found differences in the content of polysaccharides. They found that the accumulation of sugars is influenced by environmental factors such as HCO3−, Na +, Ca2 +, Mg2 + and Cl−, pH, soil organic matter and available nitrogen. Yordanova et al., 2015 recommend fertilizer rates for good growth and fruiting of berries. Wang .Y., Et al. 2019 found that the composition of monosaccharides and polysaccharides in fruits is similar regardless of the different growing conditions. Lu, Y. et al 2019 investigate the content of carotenoids, phenols, polysaccharides and antioxidant activity of Lycium barbarum L fruits collected from 13 regions of China. The results showed that total carotenoid

contents ranged from 12.93 to 25.35 mg β-carotene equivalents/g DW. Zeaxanthin dipalmitate was the predominant carotenoid (4.260–10.07 mg/g DW) in FLB. The total phenolic, total flavonoid, and total polysaccharide contents ranged from 6.899 to 8.253 mg gallic acid equivalents/g DW, 3.177 to 6.144 mg rutin equivalents/g DW, and 23.62 to 42.45 mg/g DW, respectively. Rutin content ranged from 0.1812 to 0.4391 mg/g DW, and ferulic acid content ranged from 0.0994 to 0.1726 mg/g DW. Zhen-Yu, W. A. N. G. (2003) in a study of the active ingredients in the fruits of Lycium barbarum L. found significant differences in their amounts depending on the growing conditions. According to the authors, the accumulation of a large temperature amount under the conditions of slightly alkaline soil are prerequisites for the accumulation in the fruits of more polysaccharides and carotenoids. There is no data on the growth and reproductive characteristics of the presented varieties of goji berry formed as trees, as well as on the content of biologically active substances in the fruits when grown in Bulgaria.

MATERIAL AND METHODS

The study was conducted in the experimental base of the Department of Fruit Growing at the Agricultural University - Plovdiv with in vitro propagated plants of varieties JB 1, JB 2, JB 4, JB 10 (Fig.2) in the period 2017-2019. The experimental plants were planted at distances 3x2 m in June 2014. They were formed as trees with stem height of 70-90 cm, central axis, four main fruit shoulders and are on a supporting structure. Before the beginning of the vegetation, the branches above the central axis are thinned and the rest shortened to 10 cm. With the winter pruning (Fig. 1), the branches on the fruiting shoulders (upright, growing downwards and inwards towards the central axis) were removed from the bottom. The other branches from the previous year were shortened to 10 cm each year During the vegetation all shoots were shortened to a length of 30 cm. The plants are grown under drip irrigation. Fertilizer 300 g / tree NPK (14:10:12)is given twice a year, first after pruning and then before flowering. The climate in Plovdiv is typical for the temperate climate zone with an active temperature sum of 3900 ° and precipitation of about 515 mm. The following parameters were studied: plant height, cm; stem diameter (30 cm above the soil), mm; crown diameter, cm ; crown volume, m^3; yield of the fresh fruit, kg/tree; content of total polyphenols and flavonoids and antioxidant activity of the fruit of JB 1, JB 2, JB 4, JB 10.

- Extraction of polyphenols and flavonoids

Briefly, approximately 2 g homogenated goji berry fruits were weighted accurately, transferred to extraction tubes and mixed with 40 mL of the extragent (80% acetone solution in 0.5% formic acid). The extraction was conducted on an orbital shaker at room temperature for one hour. After that, the samples were centrifuged (6000 ×g) and supernatants were further used for antioxidant activity determination and analysis of total polyphenols and flavonoids.

- Total polyphenol compounds analysis

The total polyphenols were determined colorimetrically with the Folin-Ciocalteu's reagent according to the method of Singleton and Rossi. Briefly, 0.1 mL of the extracts were mixed with 3.1 mL deionized water and 0.2 mL of Folin–Ciocalteu phenol reagents. After 3 min, 0.6 mL of 20% sodium carbonate was added to the mixture. Samples were incubated for 5 min at 50 °C and their absorbance was measured at 765 nm. Gallic acid was employed as a calibration standard and the results were expressed as gallic acid equivalents (GAE) per 100 g fresh weight (FW).

- Total flavonoid content analysis

The total flavonoid content was determined with AlCl3 reagent according to Chang et al. The calibration curve was constructed with quercetin dihydrate (10-200 mg/L). The results are expressed as mg quercetin equivalents (QE) per 100 g FW.

- Oxygen Radical Absorbance Capacity (ORAC) assay

ORAC was measured according to the method of Ou et al. Solutions of AAPH, fluorescein and trolox were prepared in a phosphate buffer (75 mM, pH 7.4). Samples were diluted in the phosphate buffer as well. Reaction mixture (total volume 200 µL) contained FL – (170 µL, final concentration 5.36×10-8 mol/L), AAPH – (20 µL, final concentration 51.51 mM), and sample – 10 µL. The FL solution and sample were incubated at 37°C for 20 min directly in a microplate reader, and AAPH (dissolved in buffer at 37°C) was added. The mixture was incubated for 30 s before the initial fluorescence was measured. After that, the fluorescence readings were taken at the end of every cycle (1 min) after shaking. For the blank, 10 µL of phosphate buffer was used instead of the

extract. The antioxidant activity was expressed in micromole trolox equivalents (μmol TE) per liter of extract. Trolox solutions (6.25; 12.5; 25 and 50 μmol/L) were used for defining the standard curve. ORAC and HORAC analyses were carried out using a FLUOstar OPTIMA plate reader (BMG Labtech, Germany), excitation wavelength of 485 nm and emission wavelength of 520 nm were used. The results are expressed in micromole Trolox equivalents (μmol TE) per g FW.

The data for the growth, reproductive and quality characteristics were processed by the analysis of variance.

Fig.1. Goji berry plant after winter pruning

Fig. 2. Fruits of the JB1, JB2, JB4 and JB 10.

RESULTS AND DISSCUSION

The data on the height of the trees is presented in Table 1. In each year, the JB 4 and JB 10 trees are taller than the JB 1 and JB 2 trees. The results show that at the end of the sixth vegetation, the JB 4 and JB 10 varieties are taller than the JB 1 and JB 2 varieties. Fruit picking in JB 1 and JB 2 can be performed without the use of ladders, while in JB 4 and JB 10, if not limited, they will probably be needed.

Table.1. Height of the plants 2017-2019, cm

Variety	Height		
	2017	2018	2019
JB 1	161.00	162.00	182.50
JB 2	180.00	170.00	198.75
JB 4	208.00	200.00	247.50
JB 10	212.50	202.50	245.00

The differences are significant P< 0.05%

Information about the diameter of the stem gives Table 2. The smallest stem diameter in all years of the experiment had variety JB 4, followed by JB 10. In all varieties the values of the indicator

increase over the years. From the beginning to the end of the study period, the stem diameter in the variety JB 2 increased the most (8.61mm). For the other varieties JB1, JB4 and JB10 the thickening for the same period is 4.38 mm, 3.56 mm and 4.23 mm, respectively.

Table.2. Stem diameter for the period 2017-2019, mm

Variety	Stem diameter		
	2017	2018	2019
JB 1	14.09	17.24	18.47
JB 2	18.88	22.34	27.49
JB 4	10.39	12.47	13.95
JB 10	12.15	13.66	16.38

The differences are significant P< 0.05%

The analysis of the data for the diameter of the crowns in the period 2017-2019 is presented in Table 3. Of all the cultivars, only the cultivar JB10 showed an increase in crown diameter during all the years of the study. The other varieties in the second year of the study form narrower crowns compared to the beginning of the experiment, and in the last year they are the widest. This variation is probably due to the typical plant sagging of the crowns. During all the years of the study, the JB2 variety formed the widest crowns. At the end of the sixth vegetation with the narrowest crown is variety JB4, followed by JB10. The results show that at the selected planting distances of 2m / 3m the trees have not yet completely occupied their allotted area.

Table.3. Crown diameter for the period 2017-2019, cm

Variety		Crown diameter	
	2017	2018	2019
JB 1	108.80	91.00	134.00
JB 2	134.25	111.25	144.00
JB 4	77.00	73.00	118.00
JB 10	82.50	95.00	132.00

The differences are significant P< 0.05%

Information on the volume of the crown gives Table 4. As with crown diameter and with this indicator, a decrease in the values of varieties JB1, JB2 and JB 4 is observed in the second year of the study and an increase in the last. JB 10 variety had the most voluminous crown.

Table.4. Crown volume for the period 2017-2019, m^3

Variety	Crown volume		
	2017	2018	2019
JB 1	0.26	0.18	0,47
JB 2	0.47	0.30	0,41
JB 4	0.21	0.19	0,43
JB 10	0.22	0.30	0,53

The differences are significant P< 0.05%

Analysis of the results for the yields are in Table 5. The highest yield in each year and the average for the period of the study is obtained by variety JB 1. In varieties JB1 and JB2 the yields

increase during the years of the experiment, while in JB 10 and JB 4 it decreases. The average yield for the study period is from 0.17 kg to 1.11 kg. It is highest in variety JB1 and lowest in JB10, although its crown was smaller than the other varieties.

Table 5. . Yield for the period 2017-2019, kg/tree

Variety	Yield			
	2017	2018	2019	2017-2019
JB 1	0.96	0.66	1.71	1,11
JB 2	0.34	0.62	0.71	0,56
JB 4	0.34	0.15	0.09	0,19
JB 10	0.22	0.18	0.12	0,17

The differences are significant P< 0.05%

Table 6 presents data on the content of total polyphenols, total flavonoids and antioxidant activity measured by the ORAC method. As can be seen from the results, there are significant differences between the tested varieties. JB 10 was the cultivar with the highest content of phenolic compounds rendering the highest antioxidant activity. All studied cultivars have relatively low content of flavonoids, and the highest content of 5.7 mg/100g was detected in JB 2.

Table.6. Total polyphenol and flavonoid contents and antioxidant activity of goji berry cultivars

Variant	Total polyphenol content, mg/100g	content, mg/100g Total flavonoid	ORAC, μmol TE/g
JB 1	219.7±1.6	1.9±0.4	76.5±3.2
JB 2	204.5±5.7	5.7±0.6	72.0±3.8
JB 4	251.9±5.9	2.6±0.3	81.5±6.3
JB 10	278.0±9.9	3.5±0.5	89.7±5.4

Results are presented as mean values ± standard deviations

CONCLUSIONS

At the end of the sixth vegetation:

- ✓ The average yield (kg / tree) for the study period is from 0.17 kg to 1.11 kg. The most productive variety is JB1.
- ✓ In varieties JB1 and JB2 the yields increase during the years of the experiment, while in JB 10 and JB 4 they decrease.
- ✓ With the narrowest crown is variety JB4 and JB 10 had the most voluminous crown
- ✓ There are significant differences in the content of total polyphenols and flavonoids in the fruits of the studied varieties, and hence in their antioxidant activity.

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