Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 6 Issue 9 September 2022

Research Article

Study on Productivity and Antioxidant Activity of Goji Berry Varieties Grown in South Bulgaria

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DOI: 10.31080/ASAG.2022.06.1177

Received: July 11, 2022

Published: August 12, 2022

Abstract

The popularity of goji berry lycium barbarum plant is increasing in recent years. The study is focused on some growing, reproductive and quality characteistics of Bulgarian varieties goji berry JB1, JB2, JB4 and JB10 cultivated in South Bulgaria when formed as a tree. There have been found differences between the studied varieties in the growing and productivity parameters, as well in the quality of the fruit.

Keywords: Goji Berry; Tree; Productivity; Antioxidant; Variety

Introduction

"Goji berry" refers to the botanical species Lycium barbarum L. and Lycium chinense M., which taxonomically belong to the Solanaceae family. Because of the medicinal and nutritional properties they possess, the plants have been used for many years in East Asia, and already in many other places [1]. At the end of XX. century, the growing need for healthy products made their cultivation targeted first in Asia and then in other continents [2]. Due to the large number of nutrients and bioactive ingredients contained in the fruit, such as polysaccharides, carotenoids, organic acids, antioxidants, vitamins, as well as many macro and micro elements, the species is included in the group of so-called "super foods" [3].

Goji berry is known in many places around the world. Goji berry growth and productivity are related to climatic conditions. The species has been identified as adaptable to the climatic Romania [4]. In Georgia, it is recommended for cultivation in the region of Shida Kartli and other areas with similar soil and climate conditions [5]. The plant is also known in Bulgaria [6], reported the first results of the study of the growth and reproductive manifestations of 2 varieties of goji berries under the conditions of the Plovdiv region. Goji berry is also known in Iraq and Greece [7].

Nutritionists describe goji berries as an "exotic superfood" because of their high content of polysaccharides, vitamins and carotenoids. Balan., *et al*. (2012) found goji berries to be high in vitamin C, polyphenols and antioxidants. A relationship has been established between fruit quantity and quality and growing region. [8]. It is reported that Goji berry fruit are rich sources of micronutrients and phytochemicals, such as organic acids, sugars, and phenolic compound. Soluble sugars and organic acids play important roles in the nutritional quality and organoleptic characteristics of fruits, such as taste, flavor and texture [9].

Italian L. barbarum berry is a rich source of bioactive molecules with nutraceutical properties confirming that goji berry is a source of bioactive components, such as vitamin E, minerals and fiber [10]. In recent years, there has been a growing trend in introducing Goji cultivation to different pedoclimates in Europe (Italy, Romania, Bulgaria, Portugal), or even developing new cultivars [11].

Materials and Methods

As a test plant were used four invitro multiplicated varieties goji berry (JB1, JB2, JB4 and JB10.) grown in South Bulgaria. The experi-

mental plants are formed as trees with trunk high from 70-90 cm. and grow under drip irrigation on a supporting construction. The climate in Plovdiv is typical for the temperate-continental climate zone with 3900°C active temperature sum and with precipitation in the amount of about 515 mm. Lyophilized material of goji berry fruits collected In the technological maturity phase was used for the analysis of: Total polyphenol flavonoids compounds and antioxidant activity. The data is processed statisticaly.

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For the determination of the polyphenol and flavonoid contend and measurements of the antioxidant activity of the fruit, extraction with 2 g smashed goji berry fruit mixed with 40 mL extragent (80% aceton and 0,5% formic acid) was made. The mixture of a maded sample stayed for one hour in a room temperature. Next, it was centrifuged (6000 × g) and determination of the total polyphenols, flavonoids and the analysis of the antioxidant activity of the fruit with HPLC, mg/100g were made.

The extraction of the polyphenols: 0,1 mL extract was mixed for 3 min with 3,1 mL deionized water and 0,2 mL Folin – Ciocalteu phenol reagent. Than to the solution was added 0,6 mL of 20% sodium carbonate. Next the samples were leaved at 50 °C for 5 min. Their absorbance was measured at 765nm. As a calibration standard was used gallic acid and the results are presented as gallic acid and equivalents (GAE) per 100g fresh weight (FW).

For the analysis of the total flavonoids (mg/100g) a calibration curve with quercetin dihydrate (10-200 mg/L) was made. The results are expressed as mg quercetin equivalents (QE) per 100g FW.

For the ORAC measurments it was prepared a mixture of fluorescein, trolox and 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.

Results and Discussion

Growth characteristic

Information about the growth manifestations of the trees of the investigated goji berry varieties is presented in table 1. The stems of varieties JB1, JB2 and JB4 thicken evenly over the years. For JB1 and JB2 the thickening is about 3-4 mm, and for JB4 about 2 mm. Regard to this growth indicator, two groups are to be formed. In the one with thicker stems are the varieties JB1 and JB2, and in the other with thinner varieties JB4 and JB10. At the end of the study, variety JB2 had the thickest stem, and JB4 the thinnest. The dynamics of stem thickening reflects the growing conditions of the plants. The low stem thickening of cultivars JB4 and JB10 is an indication of poorer growth in these cultivars compare to JB1 and JB2. It could be summarized that the trees of all studied varieties, at the end of the eighth growing season, still do not have sufficiently strengthened stems and need the support structure.

Variety	2019	2020	2021
JB1	18,47	21,89	25,44
JB2	27,49	31,17	35,21
JB4	13,95	15,65	16,78
JB10	16,38	19,45	20,75
GD 5 %	2,91	3,21	4,71

Table 1: Stem diameter of four goji berry varieties in the period 2017-2021, mm.

Significant differences by $p < 0.05\%$.

Crown diameter is a growth indicator directly related to tree planting density. The optimal occupation of the area in the rows means its maximum use. Table 2 shows the values of this parameter. In each of the years cultivar JB 2 showed a tendency to form trees with the largest crown diameter, while at the opposite site was cultivar JB 4. At the end of the study (8 growing seasons), trees of neither cultivar had fully occupied the allocated their area in the plantation.

Variety	2019	2020	2021
JB1	134,00	120,00	120,83
JB2	144,00	136,25	172,50
JB4	118,00	81,75	112,50
JB10	132,00	116,25	141,25
GD 5 %	15,70	29,37	40,07

Table 2: Crown diameter of four goji berry varieties in the period 2019-2021, cm.

Significant differences by $p < 0.05\%$.

Productivity

Fruit yield, kg/tree

The results for the yield of fresh fruit from the tested goji berry varieties are presented in table 3. In the varieties JB1 and JB2, the yield was decreasing, while in the case of the other two varieties JB4 and JB10, the opposite trend was noticeable. JB1 was the most productive variety, followed by JB2, JB4 and JB10.

Variety	2019	2020	2021
JB1	134,00	120,00	120,83
JB2	144,00	136,25	172,50
JB4	118,00	81,75	112,50
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Table 2: Crown diameter of four goji berry varieties in the period 2019-2021, cm.

Significant differences by $p < 0.05\%$.

For the three years of the study (2019-2021), the total yield fresh fruit (kg/tree) for JB1, JB2, JB4 and JB10 was, respectively; 4.25; 2.08; 0.84 and 0.75. According the productivity, two groups were to be formed. In the one with the more productive varities were JB1 and JB2, and in the other JB4 and JB10. The potential yield varied from 41.50 kg/dka in JB10 to 235.16 kg/dka in JB1. The theoretical yield was calculated based on the diameter of the crowns in the last year of the study and the average yield from a tree for the period 2019-2021. The estimated yield of the most productive variety JB1 was 390.50 kg/dka, which exceeds several times the yields of the other varieties. Data on growth indicators showed that denser planting of the plants is possible.

Antioxidant activity. Biologically active substances

The nature and concentration of sugars and organic acids are important factors, which influence the sensory characteristics of fruits and their taste. Data on carbohydrates in fruits is presented in table 4. Fructose is most desirable in fruits because it is beneficial and determines the sweet taste. Fructose in the fruits of the studied goji berry cultivars ranged from (1.21 - 3.19) g/100gDW, respectively, in JB 1 and JB10. The glucose content is the highest in the fruits of variety JB2 and the lowest in JB1.

Varity	Fructose	Glucose	Total
JB1	1,21	2,85	6,51
JB2	2,52	9,95	12,47
JB4	2,51	5,25	9,80
JB10	3,19	6,83	10,02

Table 4: Content and composition of sugars in the fruits of four goji berry varieties (g/100gDW).

Antioxidant effects is attributed to phenolic compounds. It is based on the reducing the amount of free radicals, which are the cause of inflammatory processes in the body. Table 5 presents data on the total content of polyphenols and flavonoids, as well the antioxidant activity of the investigated goji berry varieties. From the results it is evident that the content of polyphenols (mg/100g) is in the range of 204.5 - 278.0, respectively, in the varieties JB 2 and JB 10.

Variety	Total Polyphenols, mg/100g	Total Flavonoids, mg/100g	ORAC,m/mol, Te/g
JB1	219.7 ± 1,6	1.09 ± 0,4	76.5 ± 3,2
JB2	204.5 ± 5,7	5.07 ± 0,6	72.0 ± 3,8
JB4	251.9 ± 5,9	2.06 ± 0,3	81.5 ± 6,3
JB10	278.0 ± 9,9	3.05 ± 0,5	89.7 ± 5,4

Table 5: Total polyphenols and flavonoids and antioxidant activity of four goji berry cultivars during 2019-2021, by ORAC.

Results are presented as means ± standard deviation.

The content of flavonoids varies from 1.09 mg/100g to 5.07 mg/100g, respectively for JB2 and JB1. Not only the total amount of antioxidants is important, but also their composition. Table 6 presents the data on the content and composition of flavonoids. Usually, the accumulation of flavonoids is related to the immune response

of plant organisms when adverse conditions occur during their development. Identified are: Qercetin, Qercetin glucoside, Qercetin glucoside, Rutin, Catchetin, Epicathetin. In the first, with higher values of the identified flavonoids are the varieties JB4 and JB10, and in the second with lower content are the other two varieties JB1 and JB2. The results indicates that compared to JB4 and JB10, the other varieties JB1 and JB2 were developed under more favorable conditions. Quercetin has the property of acting as an antioxidant. Catchetin and Epicatetin values were highest in JB10, followed by JB4. These flavonoids not only have a direct antioxidant effect but are also able to preserve other antioxidants such as vitamins C and E. Small amounts of them are sufficient to protect vitamins C and E from oxidation. Regarding the content of flavonoids, which express antioxidant activity, JB10 has the highest values, followed by JB4, JB1 and JB2.

Variety	Qercetin	Qercetin glucoside	Rutin	Catchetin	Epicathetin
JB1	6,54	7,53	8,58	8,09	7,94
JB2	2,95	3,21	3,57	3,42	3,27
JB4	15,02	17,57	19,8	19,02	17,81
JB10	18.43	21,73	24,17	23,46	21,31

Table 6: The Composition of the flavonoids and antioxidant activity of four goji berry cultivars during 2019-2021 by ORAC.

The content of phenolic acids determined by HPLC is presented in table 7. Chlorogenic, neochlorogenic, ferulic, vanillic, gallic and uronic acids are present in the fruits of all goji berry varieties. Chlorogenic acid strongly influences antioxidant activity due to its ability to capture and neutralize free radicals. In the fruits of the studied cultivars, higher levels of this acid were found in JB10 and JB4. Uronic acid in the samples varied between 1.01 μmol and 1.35 μmol. This acid indicates the presence of pectin polysaccharides in goji berries.

Pectin polysaccharides are widely used for their valuable thickening properties. Ferulic acid is also an antioxidant. Without a stabilizing ingredient like ferulic acid, vitamin C would be ineffective. In this study, ferulic acid showed significant variation in abundance between goji cultivars ranging from 1.55 μmol to 7.41 μmol, with higher abundances in the fruits of JB 4 and JB 10. 3-4 dihydroxy benzoic acid was not found in the fruits of varieties JB4 and JB 10.

The highest levels of the phenolic acid were found in the fruit of JB10, followed by JB4. In this study, we did not detect the presence of this phenolic acid in cultivars JB1 and JB2. The data on the content of organic acids are presented in table 8. Malic, quinic, ascorbic, citric, succinic and oxalic acids were found in different amounts in all studied varieties. We did not detect tartaric acid in the samples of goji berry varieties JB1 and JB2. Malic acid in higher levels is found in the fruits of variety JB2 – 159.6 mg/100g, followed by

Variety	Chlorogenic acid μmol	3-4 Dihidroxi benzoic μmol acid	Neochlorogenic acid μmol	Ferulic acid μmol	Vanilic acid μmol	p-Coumaric acid μmol	Gallic acid μmol	Uronic acid μmol
JB1	4,07	5,23	18,08	1,55	4,09	n.d	18,08	1,01
JB2	3,38	3,14	10,64	1,55	1,9	n.d	13,96	1,02
JB4	6,45	n.d	14,26	5,14	7,12	28,73	1,32	1.14
JB10	10,2	n.d	14,63	7,41	8,82	3,2	30,91	1.35

Table 7: Content of phenolic acids in four varieties of goji berries, determined by HPLC, (mg /100g).

n.d: Not Detected

those of variety JB 1 with 84.3 mg/100g. The other varieties JB4 and JB10 have respectively 62.1 mg/100g and 66.5 mg/100g of this acid. Regarding citric acid, 2 groups are formed. In the first one with higher levels are JB4 and JB10, respectively 1235.0 mg/100g and 1483.9 mg/100g and in the second one with less citric acid content – JB1 and JB2. Ascorbic acid is in the highest amounts in the fruits of JB 2. The results show that goji berry is a source of various types of antioxidants and vitamin C.

Variety	Malic acid	Quinic acid	Ascorbic acid	Tartaric acid	Citric acid	Succinic acid	Oxalic acid	Total acids mg/100g
JB1	84,3	78,9	17,7	n.d	466,2	4,8	56,6	1415,8
JB2	159,6	1006,6	27.00	n.d	484,8	99,5	106,2	1883,6
JB4	62,1	955,3	20,4	19,10	1235	8,6	64,3	2364,9
JB10	66,5	886	22,2	23,90	1483,9	13,7	54,8	2551

Table 8: Content and composition of organic acids in four goji berry varieties, mg/100g.

n.d: Not Detected

Conclusion

- The varieties JB1, JB2, JB4 and JB10 of the goji berry species (*Lycium barbarum* L.) are suitable for growing in the climatic conditions of southern Bulgaria.
- With a cumulative yield of 4.25 kg/tree for the period 2019-2021, the most productive variety was JB1.
- With the strongest growth expresses variety JB2
- Fruits of JB10 and JB4 have higher antioxidant activity compare to JB1 and JB2

Acknowledgements

This Work was supported by Centre of research, technology transfer, and protection of intellectual property rights at the Agricultural University of Plovdiv, Bulgaria (Projects 11-19).

Conflict of Interest

Declare if any financial interest or any conflict of interest exists.

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