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Some phytochemicals and sugar contents of black mulberry (*Morus nigra* L.) genotypes from Simav District, Kütahya Province, Turkey

Volkan Okatan[1], Muhammet Ali Gündeşli[2], Anna Yu. Melyanovskaya[3], Olga V. Panfilova[3], Nina G. Krasova[3]

[1] *Eskisehir Osmangazi University, Eskişehir, Turkey*

[2] *Gaziantep University, Gaziantep, Turkey*

[3] *Russian Research Institute of Fruit Crop Breeding, Orel Province, Russia*

Corresponding authors: Volkan Okatan, okatan.volkan@gmail.com
and Anna Melyanovskaya, melyanovskaya@vniispk.ru

The purpose of this research was to determine the biochemical contents in black (*Morus nigra* L.) mulberry genotypes grown in Kütahya Province. Total soluble solids content, pH, titratable acidity, total phenolics, vitamin C, DPPH radical scavenging activity, and soluble sugars (fructose, glucose, and sucrose) of black mulberry genotypes were determined at the end of the study. The highest total phenolics value was detected in SIM02 (2995.16 mg GAE g-1). The SIM03 genotype had the highest vitamin C content of 31.34 mg 100 g-1. The study indicated that radical scavenging activity (DPPH) of 19.05 (SIM03) was the highest. In terms of the most valuable chemical composition, the SIM01, SIM02 and SIM03 genotypes can be suggested and used for future breeding reasons. It is desirable to take actions in Turkey to conduct an extensive conservation program for *Morus nigra* biodiversity.

Keywords: mulberry, phenolic compounds, organic acids, sugars

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Научная статья
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Некоторые фитохимические вещества и содержание сахара в генотипах шелковицы черной (*Morus nigra* L.) из района Симав провинции Кютахья (Турция)

В. Окатан[1], М. А. Гюндешли[2], А. Ю. Мельяновская[3], О. В. Панфилова[3], Н. Г. Красова[3]

[1] Эскишехирский университет Османгази, Эскишехир, Турция

[2] Газиантепский университет, Газиантеп, Турция

[3] Всероссийский научно-исследовательский институт селекции плодовых культур, Орловская область, Россия

Авторы, ответственные за переписку: Волкан Окатан, okatan.volkan@gmail.com
и Анна Юрьевна Мельяновская, melyanovskaya@vniispk.ru

Целью данного исследования было определение биохимического содержания генотипов шелковицы черной (*Morus nigra* L.), выращенных в провинции Кютахья. В конце исследования определяли общее содержание растворимых сухих веществ, pH, титруемую кислотность, общее количество фенольных соединений, содержание витамина С, активность по удалению радикалов DPPH и содержание растворимых сахаров (фруктоза, глюкоза, сахароза) у генотипов шелковицы черной.
Наибольшее общее количество фенольных соединений было обнаружено в SIM02 (2995,16 мг GAE g-1). Генотип SIM03 имел самое высокое содержание витамина С – 31,34 мг/100 г-1. Исследование показало, что активность поглощения радикалов (DPPH) 19,05 (SIM03) является самой высокой. С точки зрения наиболее ценного химического состава, генотипы SIM01, SIM02 и SIM03 могут быть предложены и использованы для целей будущей селекции. Желательно принять в Турции меры для проведения обширной программы сохранения биоразнообразия *Morus nigra*.

Ключевые слова: *Morus nigra* L., фенольные соединения, органические кислоты, сахара

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Introduction

Morus L. is the genus of a flowering plant in the Moraceae family that is generally known as mulberry. Its plants can be found in Asia's subtropical regions (such as India, China, Japan, and Korea), Africa, Europe, and North America. *Morus nigra* L., also known as black mulberry, originated in Southwestern Asia. For centuries, it has been cultivated all over the Mediterranean and Europe. Although *M. nigra*'s pharmacological and biological activities have been studied less extensively than those of *M. alba* L., a lot of bioactive compounds isolated from *M. nigra* have also been used as traditional drugs for humans and animals due to their anti-inflammatory and analgesic effects (Hussain et al., 2017). However, in most European countries mulberry is cultivated for fruit production, not for leaves (Pawlowska et al., 2010). Mulberry has been used for silk worm production in Asian countries because its leaves are a large and significant nutrition source for silk worms (*Bombyx mori* L.) (Vijayan et al., 1997). Meanwhile, mulberry fruits are commonly used to make marmalades, jams, wine, vinegars, juices, and cosmetic items in most European countries (Natić et al., 2014; Polat et al., 2020). Traditional herbal treatments have also utilized various components of mulberry bushes (Sánchez-Salcedo et al., 2016). The most characteristic bioactive chemicals found from Sang-Bai-Pi (Chinese name for root barks of *Morus* species) are stilbenes, flavonoids, Diels-Alder-type adducts, benzofurans and polyhydroxylated alkaloids (Wei et al., 2016). Biochemical composition of most berry crops depends on weather conditions (Şelale, Alben, 2013; Panfilova et al., 2021a; Panfilova et al., 2021b; Panfilova et al., 2022). Berry crops, including *Morus*, are a good source of natural antioxidants containing a number of various antioxidant components which provide protection from harmful free radicals and are connected with a lower level of diseases and death because of cancer and heart disorders, in addition to a number of other advantages for heath (Yang et al., 2014; Sánchez-Salcedo et al. 2015; Sánchez-Salcedo et al., 2016; Teker, Altindişli, 2021). An interesting complex ability of pectins from *M. nigra* berries has been revealed in respect to lead ions (Pb^{+2}). The degree of lead ion extraction by pectins is 67.7%, which is significantly higher that by water soluble polysaccharides (22.1%) (Vakhrusheva et al., 2014). In nature, black mulberry is extremely helpful for medicine and chemistry, and it is becoming increasingly important in illness prevention and therapy. The goal of this research was to identify the most essential biochemical features of seven mulberry (*M. nigra*) genotypes from Turkey and nominate the most beneficial for preservation, future research, and breeding.

Material and method

Study area

Simav District is included in the Aegean Region. The altitude is 830 meters. Its area is 1,557 km^2. The district also shows diversity as a river basin. Simav is the gateway area between the Aegean and Central Anatolian Regions and is located in the continental climate zone. The annual mean temperature is 11.4 degrees. Average precipitation is 854 mm.

Fruit material

Fruits of seven different native black mulberry genotypes were harvested in Simav, Turkey's Aegean Region. The trees were naturally developed and were close to being fifty years old. The numbers SIM01 to SIM07 were assigned to each genotype discovered. The harvesting took place in 2018 and 2019, when the fruits of the genotypes under investigation had achieved commercial maturity. Three replicates were employed, with each repeat including 30 uniform mulberry genotype fruits. The fruits collected from the genotypes were then taken to the lab for testing.

Total soluble solids content, pH, and titratable acidity

At room temperature, the total soluble solids content (TSS) was evaluated using a digital refractometer (Model HI-96801 Hanna, Germany). A Hanna-HI 98103 pH meter was used to measure the pH value, which was calibrated using pH 4.0 and 7.0 buffer solutions. Titratable acidity was determined by titrating the sample with 0.1 NaOH until the pH reached 8.01, and the result was stated as percent citric acid equivalent.

Determination of total phenolics

The Folin-Ciocalteu phenol reagent technique was used to calculate total phenolics in mulberry genotypes (Singleton, Rossi, 1965). A spectrophotometer (Jasco V-530) was used to measure absorbance at 765 nm. The total phenolic content of the extract was measured in milligrams of gallic acid equivalents (GAE) per liter.

Determination of vitamin C

Juice samples were obtained after mashing and sifting the fruit of mulberry genotypes. Vitamin C analysis was performed on the juice samples. The samples were homogenized by centrifugation, and the supernatant was treated with 400 mL oxalic acid (0.4 percent) and 4.5 mL 2,6-diclorofenolindofenol solution. The data were spectrophotometrically read against a blank at a wavelength of 520 nm.

Analysis of DPPH radical scavenging activity

The method of Brand-Williams et al. (1995) was used to determine DPPH radical scavenging activity. Before the analysis, the DPPH solution was newly produced. After that, 1 mL of 10-4 M DPPH in methanol was transferred to a glass tube coated with aluminum foil. To the DPPH solution, 3 mL samples of the produced 0, 3, 1.25, 6.25, 12.5, 25, 50, 100, 200, and 400 g mL^{-1} antioxidant solutions in methanol were added. In the control tubes, 3 mL of pure methanol was used instead of the antioxidant solution. After 30 minutes in the dark and at room temperature, the samples' absorbance was measured against methanol at 517 nm. As standards, ascorbic acid and Trolox were utilized (Somparn et al., 2007; Mishra et al., 2012). The following equation was used to compute the percentage of DPPH scavenging activity:

$$\% \text{ DPPH} = [(Ac - As)/Ac] \times 100,$$

where Ac is the absorbance of the negative control (containing the extraction solvent instead of the sample) and as is the absorbance of the samples.

The results were expressed as EC50 (μg mL^{-1}).

Determination of soluble sugars

Sugar (fructose, glucose, and sucrose) assays were performed using a modified Melgarejo et al. (2000) method. At a temperature of 4°C, 5 mL of fruit juice was centrifuged for 2 minutes at 15,000 rpm. A SEP-PAKC18 cartridge was used to filter the supernatants. With a Bondapak-NH2 column and a refractive index detector, HPLC measurements were obtained using 85 percent acetonitrile as the liquid phase (IR). For sugar estimations, fructose and glucose standards were utilized.

Statistical analysis

The mean SE was used to depict descriptive statistics of the investigated parameters. Analysis of variance (ANOVA) was used to examine the experimental data, and Duncan's multiple range test was used to determine significant differences between the means of three replicates (p 0.05) using the SPSS 20 for Windows.

Results and dıscussıon

All genotypes had statistically significant differences in total soluble solids content (TSS), pH, and titratable acidity ($p < 0.05$). (Table 1). The fruits of the SIM07 genotype (19.55°Brix) had the highest TSS among the genotypes evaluated. The SIM05 genotype had the greatest pH value of 4.44, while the SIM02 genotype had the lowest pH value of 4.18. The titratable acidity concentration was found to be between 1.81% (SIM05) and 1.98% percent (SIM07).

M. Polat (2020), in a study conducted in Hatay, Turkey, four identified different mulberry types in the region. Fruits harvested at different times were examined for TSS, pH, and titratable acid values. TSS was found between 13.73% and 16.01%, and titratable acid content between 0.06% and 1.00%. Akbulut et al. (2006) aimed to determine the chemical properties and mineral content in black mulberry, purple mulberry, white mulberry and seedless white mulberry cultivars. In the study, the TSS in a black mulberry variety was determined as 29.5%, pH as 5.41, and titratable acid as 0.27%.

At a statistically significant level, total phenolics, vitamin C content, and radical scavenging activity (DPPH) were significantly different in all genotypes, $p < 0.05$ (Table 2). In terms of total phenolic content, there were differences between the genotypes. The SIM02 genotype had the greatest total phenolic value of 2995.16 mg GAE g^{-1}, while the SIM04 genotype had the lowest total phenolics value of 2118.79 mg GAE g^{-1}. The SIM03 genotype had the highest vitamin C content of 31.34 mg 100 g^{-1}, and the SIM06 genotype had the lowest value of 22.22 mg 100 g^{-1}. The study indicated that radical scavenging activity (DPPH) ranged from 19.05 (SIM03) to 26.93% (SIM05).

The weather conditions of the growing season (2018/2019) did not differ much. Changes in the set of biochemical traits were more associated with genotypic features, therefore, the content of vitamin C, DPPH, and phenolic compounds in most of the studied samples did not differ much from the average values. The exception was in 3 genotypes (SIM01, SIM02, SIM03) which had statistically significant differences from the average indicators of biochemical traits for this region (Kütahya Province of Turkey).

Y. Huo (2004) researched ten species of mulberry (*M. atropurpurea* Roxb) from China's Guandong region and found that the mean vitamin C concentration was 10.02 mg 100 g^{-1}. The chemical, physicochemical and mineral salt distributions of four different mulberry species taken from Gaziantep, Konya and Malatya Districts were determined by Akbulut

Table 1. Total soluble solids content (TSS, Brix), pH and titratable acidity values (%) of mulberry genotypes

Таблица 1. Общее содержание растворимых сухих веществ (TSS, Brix), pH и показателей титруемой кислотности (%) у генотипов шелковицы

Genotypes	TSS	pH	Titratable acidity
SIM01	18.95 ± 1.16 c	4.13 ± 0.11 g	1.87 ± 0.14 d
SIM02	19.13 ± 1.18 b	4.18 ± 0.25 f*	1.90 ± 0.03 c
SIM03	117.67 ± 0.92 g	4.25 ± 0.43 e	1.94 ± 0.06 b
SIM04	17.97 ± 0.64 e	4.42 ± 0.12 b	1.77 ± 0.02 g
SIM05	18.32 ± 0.09 d	4.44 ± 0.06 a	1.81 ± 0.07 f*
SIM06	17.48 ± 0.17 f*	4.33 ± 0.04 d	1.83 ± 0.08 e
SIM07	19.55 ± 0.16 a	4.40 ± 0.18 c	1.98 ± 0.09 a

Note: difference between means designated with the same letter in the same column is not significant at 0.05 level

Примечание: различие между средними, обозначенными одной и той же буквой в одном столбце, недостоверно на уровне 0,05

Table 2. Total phenolics, vitamin C, and DPPH of mulberry genotypes

Таблица 2. Общее количество фенольных соединений, витамина C и DPPH у генотипов шелковицы

Genotypes	Total phenolics (mg GAE g^{-1})	Vitamin C (mg 100 g^{-1})	DPPH (%)
SIM01	2801.16 ± 4.22 b	21.44 ± 1.63 g	18.34 ± 0.35 g
SIM02	2995.16 ± 5.18 a	29.43 ± 0.98 b	25.73 ± 0.12 b
SIM03	2050.93 ± 15.24 g	31.47 ± 0.48 a	19.05 ± 0.16 f*
SIM04	2118.79 ± 12.39 f*	25.24 ± 0.19 d	21.60 ± 0.64 d
SIM05	2402.63 ± 13.85 d	26.37 ± 3.16 c	26.93 ± 0.22 a
SIM06	2509.67 ± 10.46 c	22.22 ± 2.88 f*	22.56 ± 0.18 c
SIM07	2156.06 ± 9.65 e	22.65 ± 0.91 e	19.39 ± 0.27 e

Note: difference between means designated with the same letter in the same column is not significant at 0.05 level

Примечание: различие между средними, обозначенными одной и той же буквой в одном столбце, недостоверно на уровне 0,05

Table 3. Sugar content of mulberry genotypes

Таблица 3. Содержание сахаров у генотипов шелковицы

Genotypes	Glucose (g 100 g^{-1})	Fructose (g 100 g^{-1})	Sucrose (g 100 g^{-1})
SIM01	10.39 ± 0.22 a	5.96 ± 0.11 f*	1.52 ± 0.04 c
SIM02	9.21 ± 0.19 b	8.89 ± 0.14 a	1.29 ± 0.03 d
SIM03	7.97 ± 0.17 e	7.99 ± 0.12 b	1.24 ± 0.04 e
SIM04	7.81 ± 0.08 f*	7.63 ± 0.11 c	1.09 ± 0.07 f*
SIM05	7.22 ± 0.14 g	6.87 ± 0.23 d	1.17 ± 0.02 e
SIM06	8.10 ± 0.28 d	6.60 ± 0.18 e	1.80 ± 0.08 b
SIM07	8.67 ± 0.19 c	5.79 ± 0.04 g	2.15 ± 0.06 a

Note: difference between means designated with the same letter in the same column is not significant at 0.05 level

Примечание: различие между средними, обозначенными одной и той же буквой в одном столбце, недостоверно на уровне 0,05

et al. (2006), V. Okatan (2018). As a consequence of the research, red mulberry fruits had the greatest ascorbic acid content (12.45 mg 100 g^{-1}). The total phenolic content ranged from 114.3 to 354.5 mg 100 g^{-1}. According to I. Karacali (2012), fruit species can be divided into three groups based on their vitamin C concentration: poor, medium, and rich, with the mulberry fruit falling into the middle group in terms of vitamin C content. According to S. Ercıslı, E. Orhan (2008), the vitamin C content in fruits of mulberry genotypes grown in Northeastern Turkey ranged from 14.9 to 18.8 mg 100 mL^{-1}. S. Ercıslı and E. Orhan (2007) determined the vitamin C content of white, red, and black mulberries to be 22.4, 19.4, and 21.8 mg 100 mL^{-1}, respectively. Antioxidant activity in *M. nigra* was previously reported to be 15.037–24.443 M TE g^{-1} (Ozkaya, 2015). In the report on the identification of phenolic acids and antioxidant activity in the extracts of three species (*M. nigra*, *M. alba* and *M. laevigata* Wall.) cultivated in Pakistan, the content of phenolics was in the range of 3.89 to 11.79 mmol/100 g of the gallic acid equivalent, while the antioxidantt activity by DPPH analysis was in the range of 22.85 to 76.88 mkmol/100 g of the quercentin equivalent. The sugar concentration in different samples fluctuated in the range of 36.56–82.15 mmol/100 g of the sucrose equivalent (Memon et al., 2010).

In *M. nigra* fruits, total phenolic content ranged within 1515–2570 GAE mg g^{-1} (Bae, Suh, 2007; Lin, Tang, 2007; Sánchez-Salcedo et al., 2015). The chemical makeup of mulberry varieties and cultivars is highly differentiated, as evidenced by the parallelism among current investigations and cited references.

There were statistically significant differences ($p < 0.05$) in sugar concentration among the genotypes of black mulberry (Table 3). The highest glucose value was 10.39 g 100 g^{-1} for the SIM01 genotype, whereas the lowest glucose value was 7.81 g 100 g^{-1} for the SIM04 genotype. The SIM02 genotype had the highest fructose content, at 8.89 g 100 g^{-1}, while the SIM01 genotype had the least, at 5.96 g 100 g^{-1}. According to the research, sucrose values ranged from 1.09 (SIM04) to 2.15 g 100 g^{-1} (SIM06).

Citric and malic acids are concentrated as the organic acids found in the fruits of *M. nigra* varieties are widely diverse, according to M. Özgen et al. (2009) and E. M. Sánchez et al. (2014). In the black mulberry types, M. Gundogdu et al. (2011) detected malic acid, tartaric acid, and citric acid as 1.323, 0.123, and 1.084 g 100 g^{-1}, respectively. According to S. P. Eyduran et al. (2015), malic acid was the most prevalent organic acid in all genotypes, with levels ranging from 1.13 to 3.04 g 100 g^{-1}. The maximum rates of malic acid content among organic acids of black and white mulberry were 3.07 and 2.13 g 100 g^{-1}, respectively, according to M. K. Geçer et al. (2016). Citric acid, tartaric acid, succinic acid and fumaric acid concentrations ranged from 0.48 to 1.03 g 100 g^{-1}, 0.15 to 0.43 g 100 g^{-1}, 0.12 to 0.44 g 100 g^{-1}, and 0.01 to 0.12 g 100 g^{-1}, respectively, in the same study. M. Gundogdu et al. (2017) found caffeic acid (21.09–2.44 mg 100 g^{-1}), syringic acid (11.91–1.16 mg 100 g^{-1}), and p-coumaric acid (5.67–0.70mg 100 g^{-1}).

Conclusıon

The genotypic specificity and the need for an ecological study of the composition of black mulberry (*Morus nigra*) fruits were determined to identify promising genotypes for cultivation in the region.

All the studied black mulberry samples have a high polyphenolic complex, which increases their importance for human nutrition.

Noticeable genotypic differences in the accumulation of polyphenols in mulberry fruits have been revealed. The range of variation in the quantitative characteristics of the biochemical composition of fruits in the years of the research is very significant, which indicates a pronounced dependence of the studied traits on abiotic factors.

Promising black mulberry genotypes (SIM01, SIM02 and SIM03) have been identified for breeding for improved biochemical composition of fruits.

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Information about the authors

Volkan Okatan, Dr. (Horticulture), Associate Professor, Department of Horticulture, Faculty of Agriculture, Eskisehir Osmangazi University, Büyükdere Meşelik Yerleşkesi 26160, Turkey, okatan.volkan@gmail.com, https://orcid.org/0000-0001-5787-7573

Muhammet Ali Gündeşli, Dr. (Horticulture), Associate Professor, Department of Plant and Animal Production, Nurdagı Vocational School, Gaziantep University, Osmangazi, Üniversite Blv., Gaziantep 27410, Turkey, maligun4646@gmail.com, https://orcid.org/0000-0002-7068-8248

Anna Yu. Melyanovskaya, Postgraduate Student, Associate Researcher, Russian Research Institute of Fruit Crop Breeding, Zhilina, Orel District, Orel Province 302530, Russia, melyanovskaya@vniispk.ru

Olga V. Panfilova, Cand. Sci. (Agriculture), Scientific Secretary, Russian Research Institute of Fruit Crop Breeding, Zhilina, Orel District, Orel Province 302530, Russia, us@vniispk.ru, https://orcid.org/ 0000-0003-4156-6919

Nina G. Krasova, Dr. Sci. (Agriculture), Chief Researcher, Russian Research Institute of Fruit Crop Breeding, Zhilina, Orel District, Orel Province 302530, Russia, krasova@vniispk.ru, https://orcid.org/0000-0001-7896-0149

Информация об авторах

Волкан Окатан, доктор садоводства, доцент, кафедра садоводства, факультет сельского хозяйства, Эскишехирский университет Османгази, 26160 Турция, Эскишехир, кампус Бююкдере Мешелик, okatan.volkan@gmail.com, https://orcid.org/0000-0001-5787-7573

Мухаммет Али Гюндешли, доктор садоводства, доцент, кафедра растениеводства и животноводства, профессиональное училище Нурдагы, Газиантепский университет, 27310 Турция, Газиантеп, бульв. Юниверсите, Османгази, maligun4646@gmail.com, https://orcid.org/0000-0002-7068-8248

Анна Юрьевна Мельяновская, аспирант, младший научный сотрудник, Всероссийский научно-исследовательский институт селекции плодовых культур, 302530 Россия, Орловская область, Орловский район, д. Жилина, melyanovskaya@vniispk.ru

Ольга Витальевна Панфилова, кандидат сельскохозяйственных наук, ученый секретарь, Всероссийский научно-исследовательский институт селекции плодовых культур, 302530 Россия, Орловская область, Орловский район, д. Жилина, us@vniispk.ru, https://orcid.org/ 0000-0003-4156-6919

Нина Глебовна Красова, доктор сельскохозяйственных наук, главный научный сотрудник, Всероссийский научно-исследовательский институт селекции плодовых культур, 302530 Россия, Орловская область, Орловский район, д. Жилина, krasova@vniispk.ru, https://orcid.org/0000-0001-7896-0149

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