



# EXPLORING THE DIVERSITY OF HORTICULTURE CROPS USING BIOCHEMICAL COMPONENT

**Mr. Raju Chaganrao Sarvade**

Assistant Professor, Department of Zoology, Pratap College, Amalner, Jalgaon Maharashtra

## Abstract

A resolution of the UN General Assembly has designated 2010 as the "International Year of Biodiversity" (IYB 2010). People all throughout the globe are making an effort to protect this priceless resource, which is critical to the well-being of present and future generations of humans. Availability and access to varied genetic sources will guarantee that the global food production network becomes more sustainable, since plant breeding research and cultivar improvement are essential components of boosting food production. Fruits of the black mulberry (*Morus nigra* L.) are well-known for their high anthocyanin content and delightful, sweet, slightly acidic taste. Twenty black mulberry genotypes, all native to Turkey's Artvin area, had their fruit analyzed for its unique phytochemical, sensory, and antioxidant properties. The sugars glucose (7.22-11.10 g/100 g fresh weight (fw)) and fructose (6.32-9.94 g/100 g fw) were measured chromatographically (HPLC/DAD), and malic acid (6.02-11.44 g/100 g fw) was found to be the most abundant organic acid in the fruits of the black mulberry genotypes studied, followed by citric acid. Ascorbic acid concentration was measured using titrative methods and found to be between 17.41 to 28.33 mg/100 g fresh weight. The goal of this study was to characterize the biochemical diversity of mulberry fruit from different genotypes growing in Muş Province, in eastern Anatolia, and to identify potential breeding material. Thirteen mulberry fruit genotypes, comprising five white (*Morus alba*) and eight black (*Morus nigra*), were analyzed for their morphological and biochemical features.

**Keywords:** - black mulberry, DPPH and FRAP; phenolic acids; HPLC, diversity, organic acids.

## Introduction

Horticulture is the study and practice of growing and caring for plants for human use, including edibles like fruits and vegetables as well as non-edibles such decorative plants, plantations, medicinal herbs, and fragrant spices and condiments. Planting horticultural crops, conducting intercultural operations, manipulating growth, harvesting, packing, selling, storing, and processing them all need meticulous attention to detail. India, behind China, is the world's second-largest provider of fresh food. About half to two-thirds of India's population relies on agriculture and related industries for their livelihood. Some of India's most valuable agricultural exports come from its horticultural sector. They are grown over a large area and account for around 28% of the country's GDP (GDP). Approximately 37% of India's total agricultural exports are these crops.

Horticulture is the practice of growing fruit, nuts, vegetables, mushrooms, roots and tubers, spices, aromatic and medicinal, and decorative plants in either rural or urban settings for the purpose of human use. However, this generalization does not do justice to the rich variety of horticultural crops and cultivars that exist in temperate, subtropical, and tropical zones due to their adaptability to distinct ecological and land-use conditions. Nature has given us the gift of biodiversity in plants, cultivated crops, and especially horticulture crops, which is crucial to long-term viability, food security, and health. Crop and food

diversification, as well as human well-being and survival, depend on horticultural biodiversity, which is essential along with other natural resources. Crop genetic variety has a crucial function in enhancing production levels and nutritional diversity over the complete spectrum of diverse agro ecological settings because genes for desirable characteristics are integrated in biodiversity. Also, farmers' access to a wide range of locally adapted types is essential, and natural pollination for horticultural crops continues to play a key role in this. To guarantee optimum pollination, it is essential that pollinators and horticulture crops be in sync with one another, and a variety of best practices may be used to sustain and improve pollination services. Protection of pollinator habitat, nectar-supply diversification, and decreased pesticide usage are only a few examples. Home gardens, which were historically tended by women, play an equally crucial role in ensuring a plentiful supply of fresh vegetables for relishes and sauces, as well as for personal and commercial use. These backyard plots serve as test gardens, where women may adopt and experiment with a wide variety of native and wild plant species. For instance, scientists have observed that female farmers in Thailand responded to dwindling natural resources by rescuing species from a nearby forest just before it was removed (Convention on Biological Diversity, 2008). As a consequence, modern-day mothers now cultivate a wide variety of traditional plants for use in sauces, which they harvest from their backyard gardens.

Plant breeders are able to create new and better cultivars with qualities that farmers and breeders value because to the diversity of PGR. These traits may be anything from increased yield to larger seeds to other characteristics that have been identified as important by farmers and breeders alike (pest and disease resistance and photosensitivity, etc.). Natural genetic variety within crop species has been harnessed from the beginning of agriculture, first to fulfill subsistence food requirements and today to provide excess food for expanding populations. Now, more than ever, it's crucial to see agriculture not only as a food-production mechanism, but as a significant contributor to the economy outside of farming as well. As a concept for future agriculture, preserving the reservoir for cultivated and cultivable crop species is analogous to preserving the cultural and spiritual expertise of different civilized individuals in different geographic locations as historical evidence. Increases in food production over the long term are often coupled with environmental damage, yet the former may play a crucial role by giving adaptable and prolific genes.

There has been a significant uptick in demand for fresh fruits in recent years, mostly as a result of shifts in production and marketing strategies, as well as extensive and successful social media promotion, both of which highlight the fruits' high concentrations of bioactive chemicals. Incorporating a variety of fruits into your daily diet is a great way to provide variety and health benefits. Fruits include high levels of provitamin A and vitamin C, as well as many other phytochemicals and bioactive components that have therapeutic promise in the treatment of cardiovascular disease and cancer prevention. For grown-ups, fruits are essential because of the fiber and nutrients they provide. These traits, together with the concentration of key bioactive substances in fruits, have been demonstrated to be strongly impacted by genotype and environmental growing circumstances.

## Literature review

**Mitra, Sisir. (2014).** Producing 81.2 million tons of fruit each year, India accounts for 12.6% of global fruit output, second only to China (21.2%). Recent years have seen a dramatic growth in the demand and need for fruits all over the world, including in India, as a result of rising populations and a greater understanding of their economic and nutritional merits. There has been consistent development in India's fruit-growing acreage and output since then. Even in 2012-2013, India had a 4.1-fold and 6.4-fold rise in fruit acreage and output, respectively, over 2011-2012. Mango, banana, papaya, citrus, guava, pineapple, litchi, sapota, and pomegranate all play significant roles, as do other tropical and subtropical fruits. There are numerous states in India where you may find jackfruit, bael, aonla, carambola, syzygiums, passion fruit, and tamarind, all of which are considered minor fruits since they take up less land to cultivate than the big fruits.

**Mishra, Ds (2018)** In order to identify the top guava genotypes with desired features in terms of form, size, pulp color, seed content, morphological, and qualitative qualities of fruits, a thorough study was conducted throughout the variety rich regions of Gujarat in 2016 and 2017. Quantitative and qualitative characteristics of 25 seedling-derived genotypes were analyzed. Most genotypes had fruit weights between 53.50 and 318.50 grams, with dimensions of between 4.09 and 9.53 centimeters and widths of between 4.30 and 7.90 centimeters; length to width ratios of 0.86 to 1.40; seed core lengths of 2.70 to 5.25 centimeters and pulp thicknesses of 1.00 to 2.25 centimeters; seed weights of 0.94 to 9.12 grams per fruit, with 100 seeds

weighing between 0.662 and 2. Similarly, there was a large range of variation in the chemical quality parameters across all of the genotypes. Mineral content of fruits showed wide variation, with readings ranging from 11.48 to 17.48 ppm (Po), 268.37 to 370.17 ppm (K), 16.31 to 23.18 ppm (Ca), and 12.62 to 24.66 ppm (FW), respectively, over a wide range of genotypes. Varieties of pink-fleshed guava have lycopene concentrations between 0.67 and 2.43 mg 100 g<sup>1</sup>. The study's findings, gathered from the rain-fed semiarid settings of Gujarat, showed that diverse genotypes displayed a broad range of variability with regard to quality parameters.

**Pereira, Fernando et.al. (2016)** Propagation of the guava (*Psidium guajava* L.) may be accomplished by budding, grafting, layering, air layering, cuttings (root or shoot), and tissue culture. Seed-based propagation is used in the early stages of breeding projects for the purposes of producing rootstocks and growing populations for screening purposes. Because it preserves all of the unique qualities of each cultivar, vegetative propagation techniques are employed to clone the best of the best from these initiatives and commercial orchards. In this article, we will examine the many techniques that may be used to propagate guava, as well as the commercially used techniques and the results that have been gained in recent years. Many distinct propagation methods exist; nevertheless, adoption rates vary greatly among major producing nations. The potential for enhancing guava tree output will be addressed. **SUMMARY OF ADVANCES IN GOIABIERA PROPAGATION** The goiabeira (*Psidium guajava* L.) may be grown from seeds, cuttings, rooted cuttings (mergulhia), rooted cuttings (alporquia), rooted cuttings (borbulhia or garfagem), rooted cuttings (estaquia), or rooted cuttings (ramia or rhizome). Seed propagation is used in the early stage of genetic improvement and in the creation of porta-enxertos. Vegetative propagation techniques are used to clone selected genotypes in advanced stages of genetic improvement programs and in commercial crops because they preserve all desirable traits indefinitely. The purpose of this literature review is to examine the many techniques that may be used to propagate goiabeira, as well as the commercially used techniques and the progress made in recent years. While several dissemination technologies exist, adoption rates vary greatly across different producing countries. Improvements in goiabeira mud formation are discussed as a need. *Psidium guajava*, semente, enxertia, estaquia, alporquia, and textile culture are all terms that may be used for indexing purposes.

**Kumar, Jitendra et.al. (2017)** Application of nutrients by foliar spray improves both utilization and response time. Spraying guava plants with mineral nutrients such boron (B), zinc (Zn), calcium (Ca), and potassium (K) at several development phases, including during fruit set and two weeks following fruit set, was the focus of this investigation (*Psidium guajava* L.). Zn (0.03%) sprayed at fruit set increased plant canopies and had the lowest seed index. Improvements in fruit size, sugar:acid ratio, and organoleptic score were seen in K (0.5%) sprayed at two weeks following fruit set. At two weeks following fruit set, Zn (0.01%) increased yield whereas B (0.03%) enhanced pectin. Fruits sprayed with Ca (0.5%) two weeks after fruit set saw the least amount of physiological loss in weight. An application of 0.5% potassium (K) spray two weeks after fruit set improved the quality of most desirable traits.

**Goswami, Dr Amit et.al. (2015)** Five-year-old guava cv. Pant Prabhat was used in an experiment to study the impact of organic (farm-yard-manure and vermicompost) and inorganic (macro- and micro-nutrients) fertilizers, bio-fertilizers (*Trichoderma*, *Azotobacter*, *Azospirillum*, *Pseudomonas fluorescens*, and *Aspergillus niger*), and organic mulches on For optimal vegetative development, fruit output, and fruit quality throughout the year, trees were shown to benefit most from a half dosage of required fertilizers (225 g N<sub>2</sub>O: 195 g P<sub>2</sub>O<sub>5</sub>: 150 g K<sub>2</sub>O) + 50 kg FYM inoculated with 250 g *Azospirillum* tree<sup>-1</sup> year<sup>-1</sup> in the aforementioned experiment. Applying 225g N<sub>2</sub>O, 195 g P<sub>2</sub>O<sub>5</sub>, and 150 g K<sub>2</sub>O in addition to 50 kg FYM supplemented with 250 g *Azospirillum* tree<sup>-1</sup> year<sup>-1</sup> resulted in maximum fruit set and yield during the rainy (83.33%, and 72.16 kg tree<sup>-1</sup>) and winter (34.32%, and 6.53 kg tree<sup>-1</sup>). Fruits from plants given 500 g: per plant had higher total soluble solids, ascorbic acid, percent reducing sugars, total sugar, TSS:acid ratio, and pectin content throughout both the wet and the cold seasons. Two applications (August and October) of a foliar spray containing 200 milligrams of nitrogen, 500 milligrams of phosphorus, and ten centimeters of zinc, boron, and manganese, together with a ten-centimeter layer of organic mulch, should be sufficient. For long-term, high-quality guava fruit production, it is advised to use half the necessary dosage of fertilizers in addition to bio-fertilizer-rich enhanced FYM.

## MATERIALS AND METHODS

### Fruit materials

For this research, we sought for and collected samples of fruit from natively growing mulberry trees in the Varto neighborhood of Muş Province. Thirteen different mulberry species (5 *Morus alba* and 8 *Morus nigra*) were utilized. The harvest occurred during the final week of June 2016. All of the genotypes under study had their fruits picked at their peak ripeness. Around 500 grams of fruit was obtained from each genotype. Uniformly sampled fruits were frozen at -80 degrees Celsius until laboratory tests could be run on them.

### Determination of agro morphological properties

A digital scale accurate to 0.01 gram was used to determine the weight of the fruit. A compass sensitive to 0.01 mm was used to measure the breadth and length of the fruit as well as the length of the peduncle. Twenty fruits were randomly sampled from each genotype, and their soluble solids content (SSC; measured with a hand refractometer), pH, and titratable acidity (TA; measured by the titration technique) were all calculated.

### Analysis of phenolic compounds

Phenolic compound analysis was performed by adapting the procedure developed by Rodriguez-Delgado et al (2001). We centrifuged the samples for 15 minutes at 15,000 rpm. After passing it through Millipore filters with a pore size of 0.45 m, the supernatant was fed into the HPLC machine. In order to separate substances chromatographically, an Agilent 1100 HPLC system equipped with a DAD detector and a 250 mm 4.6 mm, 4 m ODS column was used. The mobile phases were solvents A (methanol-acetic acid-water [10:2:88]) and B (methanol-acetic acid-water [90:2:8]). We used 254 and 280 nm for our separation, and an injection volume of 20 L.

### Analysis of organic acids

The technique developed by Bevilacqua and Califano (1989) was adapted for use in this organic acid analysis. The centrifuge tube contained a five-gram sample of the fruit. To ensure consistency, 20 mL of 0.009 N H<sub>2</sub> SO<sub>4</sub> was added to each sample. Each sample was centrifuged at 15,000 rpm for 15 minutes. To filter the supernatant, we used a SEP-PAK C18 cartridge. The HPLC system used a 300 mm 7.8 mm Aminex HPX-87H column and an Agilent packet software. The 214- and 280-nanometer wavelengths were used in the DAD detector analyses. The research used a mobile phase of 0.009 N H<sub>2</sub> SO<sub>4</sub> filtered over a 0.45 m membrane.

### Analysis of vitamin C

After weighing out five grams of fruit, we put it in a test tube and added five milliliters of a 2.5% M-phosphoric acid solution. After centrifuging the mixture at 6500 g for 10 minutes at 4 °C, 0.5 mL of the resulting supernatant solution was added to 9.5 mL of 2.5% M-phosphoric solution to finish the procedure. After passing it through a 0.45-m filter, the mixture was fed into the HPLC machine. For this study, we used a C18 column with a DAD detector (at 254 nm). The mobile phase (1 mL/min) was composed of sulfuric acid and ultrapure water (Cemerolu, 2007).

### Determination of Trolox equivalent antioxidant capacity (TEAC)

A solution of ABTS acetate in buffer and potassium persulfate were made for the TEAC test (Ozgen et al., 2006). 20 mM sodium acetate was diluted in an acidic medium (pH 4.5) in the buffer solution to yield 0.700 0.01 absorbance at 734 nm, ensuring the mixture's long-term stability. The absorbance readings at 734 nm were measured after incubating 3 mL of ABTS+ solution with 20 L of fruit extract for 10 minutes.

### Statistical analysis

Twenty fruits were tested across four independent duplicates. Both the mean and the standard deviation were used to describe the data. The research relied on a simple analysis of variance. Significant differences

between genotypes were identified using the Tukey multiple comparison test. The data was analyzed using the trial edition of MINITAB 17, a statistical tool.

## DATA ANALYSIS

### SSC, pH, Titratable Acidity, and Maturity Index

Twenty different black mulberry genotypes were tested for their SSC, pH, titratable acidity, and maturity indices. There were statistically significant ( $p < 0.05$ ) variations in SSC and pH levels across fruit genotypes. There were no statistically significant variations in the titratable acidity of the genotypes tested. The word "fruit quality" encompasses a wide range of concepts. Many different types of fruit place equal importance on weight, firmness, soluble solids, and titratable acidity.

Black mulberry fruits in this research had an SSC content of between 13.36% and 17.95%, with an average of 15.79%. The findings are consistent with those of a study conducted in Turkey including three different black mulberry genotypes (11.3-16.2%) by Elmaci and Altug. Okatan's research on 13 native black mulberry genotypes from the western area of Turkey showed comparable SSC data (14.23-19.43%) to studies by Koyuncu on 28 native black mulberry genotypes from the Mediterranean region of Turkey (SSC between 13.11 and 16.23%) and Ercisli et al. Okatan et al. evaluated eight black mulberry genotypes cultivated in eastern Anatolia, and they found an observed range from 15.6 to 22.1% in their SSC findings; similar results were seen in work performed by Caln-Sánchez et al., who studied four Spain genotypes, between 12.0 to 25.8%. Okatan et al. studied eight black mulberry varieties from eastern Anatolia and found that their fruits' pH ranged from 3.65 to 4.12. The assessed values of the fruits in the current research were within this range. Similar findings were reported by Elmaci and Altug (pH 3.60–3.80), Okatan (pH 3.66–4.42), and Koyuncu (3.22–3.47), all of whom studied native black mulberry genotypes from the Mediterranean area of Turkey. Although these numbers are lower than those Caln-Sánchez et al. reported for Spanish fruits, they are nevertheless worth noting (pH 5.95–7.39). Titratable acidity results varied from 1.38 to 1.87% citric acid equivalents; these values were similar to those reported by Elmaci and Altug [30] (1.51-1.79 %), Okatan (1.47-1.93%), Okatan et al., who analyzed eight black mulberry genotypes (1.45%-1.85%), Ercisli et al., (1.64-1.97%), and Koyuncu (1.42-1.86%). The values we found for SSC, pH, and titratable acidity were consistent with those found in the aforementioned investigations. Fruits evaluated had maturity indices (MI) ranging from 8.23 to 10.66. Okatan (8.06 - 12.69) and Koyuncu (8.06 - 12.69) both reported almost the same maturation indices (7.05–11.26). Our sensory study and the high maturity index (defined as the ratio of SSC to TA) both point to a sweet flavor. The use of these fresh fruits, with a higher MI, implies that high-quality fruit products may be made from them.

### Individual Sugars

Fruits' sugar content strongly affects their calorie density, as well as their taste and nutritional value. Photosynthesis' primary byproduct, sugars, are used to construct plant cell walls and supply energy, but they also play critical roles in communicating between cells and across tissues. Our research showed that the black mulberry genotypes differed significantly ( $p < 0.05$ ) in their levels of glucose and fructose, but not saccharose. Total sugar concentration is often lower in black and red mulberry fruits than in white mulberries. According to the analysis, black mulberry fruits have very little saccharose and a lot more reduced sugar. There is a direct correlation between the ratio of glucose to fructose and the resulting taste. We discovered that the glucose content of the black mulberry genotypes in this investigation varied from 7.22 to 11.10 g/100 g fw, with an average of 9.51 g/100 g. The glucose content observed in the study of Makhoul et al. which studied 11 black mulberry genotypes farmed in Syria, ranging from 2.21 to 14.69%, a larger scale, with an average 6.32%. Roussos et al. discovered that the glucose content of 11 Greek genotypes was lower, with values ranging from 1.83 to 5.85 g/100 g fw.

Fructose concentration in the twenty *M. nigra* fruit genotypes ranged from 6.32 to 9.94 g/100 g fw, with an average of 7.85 g/100 g. The average fructose level of the 11 Syrian genotypes studied by Makhoul et al. was 2.25%, which is lower than the average we found in our research (4.91%). Both our investigation and the aforementioned study found lower fructose levels than those found by Roussos et al. (1.88-6.25 g/100 g fw). Saccharose was found to contain the least amount of sugar (between 1.19 and 2.28 g/100 g fw), which is in line with the findings of the aforementioned studies by Makhoul et al., who found sucrose concentrations ranging from 0.03% to 1.47%, with an average value 1.2%, and by Roussos et al., who

reported values in the range of 0.03-0.16 g/100 g fw. Twenty different fruit genotypes had sweetness indices (SI) ranging from 23.7 to 35.3, with the higher numbers indicating a more favorable overall sweetness impression. This shows that there is a wide range of SI index values for black mulberry fruits. Fructose, the fruit sugar most responsible for the observed increase in SI values, was present in all of the examined fruits. Our findings are better than those of Roussos et al. for black mulberry (6.86-22.31), perhaps because of the variety of genotypes and environments under consideration.

### Individual Organic Acids

The organoleptic qualities of fruits are significantly affected by the presence and concentration of organic acids. Malic acid, citric acid, oxalic acid, and tartaric acid are the most prevalent organic acids in *M. nigra* fruit. Values for the concentration of these acids in the fruits of the 20 black mulberry genotypes studied. The results show that malic, citric, and oxalic acid content vary significantly ( $p < 0.05$ ) between genotypes, whereas tartaric acid level does not. Malic acid, followed by citric, oxalic, and tartaric acids, was found to be the most abundant organic acid in the fruits of all 20 black mulberry genotypes. Malic acid concentrations averaged 8.52 g/100 g fw, with a range of 6.02 to 11.44 g/100 g fw. Malic acid has been identified as the most common organic acid in black mulberry genotypes by many authors [14,17,20,31]. Malic acid was measured in a range of 12.3-21.8 g/100 g by Ercisli and Orhan, whereas Okatan measured levels between 6.65 and 13.65 g/100 g fw. In the current investigation, citric acid levels ranged from 2.41 to 4.02 g/100 g fw, which is only about one third of the concentration of malic acid. Ercisli and Orhan discovered very comparable citric acid data (2.1-4.1 g/100 g fw), but Okatan reported somewhat higher levels (2.12-7.02 g/100 g fw) in black mulberry fruits. Similar amounts of oxalic acid and tartaric acid (0.54-1.18 g/100 g fw and 0.37-1.05 g/100 g fw, respectively) may be found in different black mulberry varieties. It agrees with Okatan's findings about oxalic acid (0.45-1.25 g/100 g fw) and tartaric acid (0.22-0.86 g/100 g fw).

### Sensory Evaluation

Black mulberry genotypes were tested for sweetness, tartness, and fragrance. It was determined that four genotypes (VC1, VC10, VC11, and VC16) were mostly sour in flavor, four genotypes (VC2, VC8, VC17, and VC20) were primarily sweet, and the other twelve genotypes (12 total) were sweet-sour. Most black mulberry fruits demonstrated high juiciness (13 genotypes: VC1, VC3-6, VC9-11, VC14-16, VC18, and VC20), while the other 7 genotypes showed medium juiciness. Sixteen of the twenty genotypes had a strong odour, whereas the aroma was only slightly diminished in four genotypes (VC4-5, VC9, and VC16). Different *Morus nigra* genotypes were discovered to have varying degrees of juiciness, scent, and flavor, suggesting a genetically diverse population and the potential for selective breeding. Ascorbic acid is one of a class of powerful antioxidants that perform a wide range of critical tasks vital to maintaining human health. Anthocyanins and other phenolic chemicals are powerful antioxidants. Ascorbic acid (vitamin C) concentration, total phenolics, and total anthocyanins were analyzed for each of the 20 *M. nigra* fruit genotypes, and the findings are shown in Table 1. All reported parameters showed significant genotype-by-parameter differences ( $p < 0.05$ ).

**Table 1. Content of ascorbic and phenolic acids in fruits of *M. nigra* genotypes.**

Genotype	Ascorbic Acid		Chlorogenic Acid		Gallic Acid		Caffeic Acid		Ellagic Acid	
	[g/100 g fw]		[g/100 g fw]		[g/100 g fw]		[g/100 g fw]		[g/100 g fw]	
VC1	20.47	0.44 <sup>c,d</sup>	80.5	1.35 <sup>b</sup>	35.4	0.9 <sup>a,b</sup>	14.00	0.2 <sup>a,b</sup>	4.40	0.2 <sup>a,b</sup>
VC2		a,b		d,e		b,c		a,b		a,b
	25.48	0.77	59.6	1.44	24.2	0.8	8.45	0.3	3.89	0.1
VC3	28.3	1.10 <sup>a</sup>	60.3	1.04 <sup>d,e</sup>	28.4	0.8 <sup>c,d</sup>	11.08	0.1 <sup>a,b</sup>	6.32	0.2 <sup>a</sup>
VC4	25.40	0.80 <sup>b</sup>	85.4	1.84 <sup>a,b</sup>	37.2	1.0 <sup>a,b</sup>	14.84	0.5 <sup>a,b</sup>	3.55	0.1 <sup>a,b</sup>
VC5		b,c		c,d		a,b		b		a,b
	23.66	0.61	67.3	1.14	31.5	0.9	7.22	0.4	2.98	0.1
VC6	20.70	0.43 <sup>c,d</sup>	71.6	1.00 <sup>b,c</sup>	34.1	0.5 <sup>a,b</sup>	11.04	0.3 <sup>a,b</sup>	2.15	0.1 <sup>b</sup>
VC7		d,e		c,d		b,c		a,b		a,b
	19.36	0.37	62.4	0.78	30.5	0.5	12.06	0.3	4.70	0.2

VC8	21.90	0.42 <sup>c,d</sup>	82.6	3.11 <sup>a,b</sup>	38.5	0.8 <sup>a</sup>	13.90	0.3 <sup>a,b</sup>	5.20	0.2 <sup>a,b</sup>
VC9	26.40	0.80 <sup>a,b</sup>	70.7	2.44 <sup>c</sup>	30.6	0.4 <sup>b</sup>	14.50	0.4 <sup>a,b</sup>	5.80	0.2 <sup>a,b</sup>
VC10	17.41	0.35 <sup>e</sup>	69.5	1.60 <sup>c,d</sup>	31.2	0.6 <sup>a,b</sup>	10.65	0.3 <sup>a,b</sup>	4.78	0.1 <sup>a,b</sup>
VC11	22.56	0.58 <sup>c</sup>	66.6	1.54 <sup>c,d</sup>	29.0	0.5 <sup>b,c</sup>	12.00	0.5 <sup>a,b</sup>	4.00	0.1 <sup>a,b</sup>
VC12	19.02	0.50 <sup>d,e</sup>	74.3	1.76 <sup>b,c</sup>	35.3	1.1 <sup>a,b</sup>	15.30	0.2 <sup>a</sup>	5.10	0.2 <sup>a,b</sup>
VC13	24.80	0.43 <sup>b,c</sup>	51.3	1.20 <sup>e</sup>	22.4	0.4 <sup>c</sup>	14.10	0.2 <sup>a,b</sup>	4.90	0.2 <sup>a,b</sup>
VC14	19.50	0.62 <sup>d,e</sup>	90.8	2.67 <sup>a</sup>	37.9	1.2 <sup>a,b</sup>	13.40	0.4 <sup>a,b</sup>	3.03	0.1 <sup>a,b</sup>
VC15		d,e		d		b,c		a,b		a,b
	18.80	0.54	60.7	1.05	29.6	1.0	11.60	0.5	5.90	0.2
VC16	20.11	0.29 <sup>c,d</sup>	70.7	1.55 <sup>c</sup>	31.5	1.1 <sup>a,b</sup>	9.44	0.3 <sup>a,b</sup>	3.25	0.1 <sup>a,b</sup>
VC17		d,e		c,d		b,c		a,b		a,b
	18.30	0.66	65.4	1.62	26.5	0.7	12.44	0.7	5.40	0.2
VC18		d,e		b,c		a,b		a,b		a,b
	18.60	0.55	73.4	1.04	32.8	1.2	13.36	0.5	6.09	0.2
VC19	19.87	0.50 <sup>d</sup>	79.6	1.84 <sup>b,c</sup>	33.8	1.0 <sup>a,b</sup>	13.02	0.9 <sup>a,b</sup>	3.70	0.1 <sup>a,b</sup>
VC20		c,d		b,c		b,c		a,b		a,b
	21.27	0.63	72.5	1.42	28.2	0.7	11.00	0.8	4.90	0.1

Different letters in columns indicate significantly different values at  $p \leq 0.05$ .

There was an ascorbic acid range of 17.41 mg/100 mg fw to 28.33 mg/100 mg fw, with an average of 21.60 mg/100 mg fw (Table 1). These figures represent a moderate amount of ascorbic acid when compared to other berry fruits, such as blueberry or blackberry, which provide the daily consumption needed to avoid scurvy (10 mg/day). Studies by Eydurán et al. and Okatan et al. indicated lower vitamin C content values of black mulberry genotypes (10.12-16.29 mg/100 g fw and 18.40-23.67 mg/100 g fw, respectively), whereas our investigation found a comparable range. Several more studies on mulberry fruit species came to the same conclusion about the quantity of ascorbic acid present. Our research found an average ascorbic acid concentration of 21.27 mg/100 g fw in Syrian black mulberry fruits; a previous study by Makhoul et al. found a range of values from 3 to 42 mg/100 g fw. The vitamin C concentration of black mulberries is greater than that of white mulberries (which range from 2 to 16 mg/100 g fw, on average) by a wide margin. Figure 1 displays that the total phenolics in the studied fruits varied from 1951 g gallic acid equivalent/g fresh weight (fw) to 2733 g GAE/g fw, on average (SEM). Total phenolics of 13 black mulberry cultivars studied in the Aegean area of Turkey were discovered by Okatan to range from 1874 to 2977 g GAE/g fw, figures which are remarkably comparable to our own. Okayan et al. found total phenolics between 1920 and 2575 g GAE/g fw after analyzing eight black mulberry varieties from eastern Turkey. The average total phenolics was calculated by Ozgen et al. to be 2737 g GAE/g fw after they examined 14 different *Morus nigra* genotypes. Our findings are consistent with those from other regions of Turkey. Research conducted in Serbia by Kostic et al. presented values of total phenol compounds in fresh mulberry fruits that ranged from 902.6 to 1188.4 g GAE/g fw, which is approximately half the quantity of these compounds reported to be in Turkey fruits.



**Figure 1.** Content of total phenolics (TP) and total anthocyanins (TA) in fruits of 20 black mulberry

The fruit of *M. nigra* is high in the anthocyanins. Mulberry and other berry anthocyanin pigments perform very well as antioxidants. Additionally, black mulberries and other berry fruits high in anthocyanins are promising functional foods that might be eaten to prevent certain illnesses. Analyzed black mulberry genotypes showed total anthocyanin concentrations between 508 and 712 g cyanidin-3-glucoside equivalent/g fresh weight (Figure 1). Total anthocyanins in *M. nigra* fruits have been reported by Ozgen et al. to fall anywhere from 253 to 830 g C3GE/g fresh weight. Okatan et al. assessed the total anthocyanin content of eight black mulberry cultivars from eastern Turkey and found values between 643 and 826 g C3GE/g fresh weight. In a study of four different black mulberry genotypes, Ercisli et al. found that the amount of total anthocyanins varied from 674 g C3GE/g fw to 787 g C3GE/g fw. Researchers in Serbia found that some genotypes of black mulberry had anything from 1148.3 to 1286.8 milligrams of cyanidin-3-O-glucoside per gram of fresh weight.

### Determination of Phenolic Acids and Flavonoids

Bioactive chemicals, such as phenolic acids and flavonoids, may be found in abundance in berries (anthocyanins and flavonols). Certain beneficial health benefits, including stress protection and the lowering of risk for several conditions, may be attributed to these substances (inflammation, cardiovascular diseases, and lower risk of some cancers).

Table 2 displays the primary phenolic acid and flavonoid concentrations of 20 different black mulberry genotypes, showing significant variations among phenolic acids (chlorogenic, gallic, caffeic acid, and ellagic acid) and flavonoids (rutin, quercetin, catechin) at the p 0.05 level.

Chlorogenic acid was found to have concentrations between 51.3 and 90.80 milligrams per hundred grams fresh weight (mg/100 g fw) in the black mulberry fruit samples. Gallic acid analysis revealed less than half of the primary acid (22.40–38.56 mg/100 g fw), followed by caffeic acid (7.22–15.30 mg/100 g fw), and then ellagic acid (2.15–6.32 mg/100 g fw). According to Okatan [20], gallic acid (21.83–40.90 mg/100 g fw) and chlorogenic acid (43–97 mg/100 g fw) both have the maximum content in the fruits of the same species. It has been hypothesized by some writers that chlorogenic acid is a major phenolic component in black mulberry fruits.

Several studies defined rutin as the most important flavonoid in black mulberry fruits, and Okatan reported the highest concentration of rutin (92–133 mg/100 g) in the fruits of the same species. Here, we analyzed rutin, quercetin, and catechin to determine the relative abundance of these three flavonoids in black mulberry fruits (Table 2). Quercetin (3.11–9.78 mg/100 g fw) and catechin (3.15–9.40 mg/100 g fw) were found in lower concentrations in the fruits studied here.

**Table 2. Content of flavonoids in fruits of *M. nigra* genotypes.**

Genotype	Rutin		Quercetin		Catechin	
	[mg/100 g fw]		[mg/100 g fw]		[mg/100 g fw]	
VC1	85.4	1.74	5.50	0.1	7.10	0.1
VC2	67.7	1.45	4.40	0.2	4.10	0.2
VC3	62.1	0.95 <sup>cd</sup>	8.02	0.4 <sup>ab</sup>	9.40	0.3 <sup>a</sup>
VC4	97.2	3.31 <sup>a</sup>	3.88	0.3 <sup>ab</sup>	3.15	0.2 <sup>d</sup>
VC5	64.3	2.20	3.11	0.1	4.35	0.2
VC6	74.0	1.10 <sup>bc</sup>	4.90	0.2 <sup>ab</sup>	5.40	0.1 <sup>c</sup>
VC7	58.6	1.07	7.70	0.5	4.50	0.1
VC8	94.3	2.88	7.22	0.5	5.30	0.2
VC9	72.4	2.51	4.90	0.3	4.00	0.1
VC10						

VC11	73.8	3.12	6.23	0.3	4.80	0.2
VC12	71.4	2.56 <sup>c</sup>	6.80	0.2 <sup>ab</sup>	5.20	0.2 <sup>cd</sup>
VC13	75.6	1.89	5.89	0.2	4.65	0.1
VC14	47.1	0.77	6.80	0.1	6.40	0.3
VC15	96.7	2.45	4.90	0.2	4.00	0.1
VC16	69.6	1.88	7.02	0.4	8.07	0.4
VC17	77.3	2.05	7.56	0.2	6.80	0.2
VC18	69.3	1.20 <sup>cd</sup>	9.78	0.5 <sup>a</sup>	8.00	0.2 <sup>ab</sup>
VC19	79.6	3.13	7.70	0.2	5.32	0.1
VC20	88.6	2.90	8.08	0.3	8.20	0.3
	70.4	1.16 <sup>cd</sup>	4.08	0.1 <sup>ab</sup>	4.50	0.1 <sup>cd</sup>

Different letters in columns indicate significantly different values at  $p \leq 0.05$

Using the FRAP test, we discovered a robust positive correlation between chlorogenic acid, gallic acid, and rutin levels with antioxidant activity ( $r = 0.829, 0.762, \text{ and } 0.745$ , respectively).

## CONCLUSION

The agricultural community now understands that the world cannot afford to lose its rich variety of plant genetic resources. In order to sustain the world's rising population (expected to reach >9 billion in 2050), these resources will become more important. The capacity of breeders to enhance crops by creating new varieties and hybrids relies on the presence of genetic diversity. Characterizing PGR on a molecular and phenotypic level may help with this. Both consumers and farmers place a premium on fruit quality. Different black mulberry genotypes were found to have distinct variations in terms of their phytochemical and antioxidant profiles. The nutritional and phytomedicine value of black mulberry fruits is highlighted by their strong antioxidant quality. Several genotypes were shown to have very high levels of sweetness and antioxidant characteristics (phenolics, anthocyanins, ascorbic acid, chlorogenic acid, and rutin content) and antioxidant activity (VC4, VC8, VC12, VC14, VC16, and VC20). Since there is an increased demand for high-quality fruits of black mulberry (*Morus nigra*), production in Turkey was recommended for three *M. nigra* genotypes (VC4, VC8, and VC20) based on their best values of phytochemical and antioxidant characteristics and their lower ecological burden when grown without chemical products and in the absence of diseases and pests. These findings point to the possibility for using indigenous black mulberry genotypes in crop selection and breeding initiatives.

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