

Research Article

Comparative analysis of morphological and biochemical properties of some Mulberry (*Morus spp.*) genotypes

Hosseinali Gharibi

Department of Agricultural Engineering, Aliabad Katoul Branch, Islamic Azad University, Aliabad Katoul, Iran

Alireza Ladan Moghadam

Department of Horticulture, Garmsar Branch, Islamic Azad University, Garmsar, Iran

Mahdi Alizadeh*

Department of Horticulture, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Postal address: Department of Horticulture, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources (GUASNR), P.O.Box 386, Golestan, Gorgan, I.R. Iran

Goni Baghdadi

Department of Agricultural Engineering, Aliabad Katoul Branch, Islamic Azad University, Aliabad Katoul, Iran

*Corresponding author. E-mail: mahdializadeh@gau.ac.ir

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Abstract

Mulberry is a multipurpose tree in horticulture. Besides nutritional importance, it is used for raising silkworms, fodder and landscape. There is quite a wide genetic diversity among different mulberry genotypes. The present study was undertaken to evaluate certain morphological and biochemical properties of 20 genotypes of *Morus alba*, *M. rubra*, and *M. nigra* species distributed in Golestan Province, northern Iran. The highest leaf length (15.51 cm), petiole length (5.37 cm), vitamin C (4.34 mg/100 g) and phenol (31.89 mg/g) were recorded in M12 genotype. The highest fruit length (5.52 cm), pedicle length (17 mm), soluble solids (18.26%) and sucrose (31.64 mg/g) were recorded in M10 genotype. The amount of glucose (288.82 mg/g) and fructose (121.97 mg/g) were also dominant in M17 genotype. The utmost fruit fresh and dry weights (0.3-67.80 g), fruit diameter (1.99 cm), leaf width (11.94 cm) and flavonoids (24.67 mg/g) were also observed in the M20 genotype. The M1 and M2 genotypes had higher anthocyanin (1.13 $\mu\text{mol/g}$) and total antioxidant activity (66.71%) compared to other genotypes. Furthermore, fruit fresh and dry weights were positively correlated with fruit length and diameter, pedicle length, and leaf and petiole length. The highest positive correlation was observed among anthocyanin, vitamin C and phenols. The morphological and biochemical traits explained 99 % of the total variance of four and three main components. The results of cluster analysis grouped these genotypes into 3 main groups. Therefore, the highest heritability and genetic advance were found for fruit weight, petiole length, leaf width, antioxidant activity, fructose and flavonoids. The valuable pomological traits recorded for some genotypes may be useful for future breeding programs.

Keywords: Biochemical, Cluster analysis, *Morus spp.*, Morphology, Genotype

INTRODUCTION

Morus spp. belongs to Moraceae family, which is widely found in temperate, subtropical, and tropical regions of the world (Hosseini *et al.*, 2018). Its three main species are *Morus alba*, *Morus rubra*, and *Morus nigra* (Venkatesh and Chouhan, 2008), which originate in southwest China, North America and Iran, respectively

(Yilmaz *et al.*, 2012). The fruit and leaf of *Morus* contain polyphenols whose biological effects include reduced weight and cholesterol, improved blood circulation and bone tissue, as well as reducing the aging process and prevention of some cancers are well known (Balik *et al.*, 2019). In most Asian countries, *Morus* tree is grown for silkworm nutrition, but today it is also grown for fresh, dried fruits, jams and juice (Barandoozi and Has-

sanpour, 2020).

Genetic diversity survives a species or population by adapting to environmental changes, so genetic diversity is essential for the long-term survival of a species and the first step of breeding programs. Investigating genetic diversity in plants, the existing genotypes can be identified and transferred to germplasm collections for better protection and maintenance (Aghapour *et al.*, 2019). Because *Morus*'s morphological and biochemical properties are highly dependent on genotypes and environmental factors (Krishna and Parashar, 2013), several studies have been conducted on the morphological and biochemical properties of different *Morus* species. In a diversity study, Jiang *et al.* (2015) investigated the biochemical properties of the species of *Morus* spp. in China. The results showed that the highest ascorbic acid, acidity, and Fe was observed in *Morus nigra*, and the highest ratio of essential amino acids to total amino acids was observed in Russian mulberry (44%), *Morus alba* (42%), and *M. nigra* (29%), respectively. Furthermore, the results of another study conducted on 34 selected species of *Morus* spp. in Turkey showed that high genetic diversity was observed among genotypes in fruit weight (0.66-3.07 g), titratable acidity (0.06-1.62%), soluble solids (17.33-30.67%) and pH (2.19-5.86) (Yilmaz *et al.*, 2012). The results of another experiment which was performed aimed to investigate nutrients of three species of *Morus alba*, *M. rubra*, and *M. nigra*, showed the highest phosphorus, calcium, magnesium, iron, copper, manganese and zinc were observed in *M. alba* and the highest potassium was observed in *M. nigra* (Gülser, 2019 and Cig). Furthermore, the study results of Balik *et al.* (2019) on the biochemical content of 13 genotypes of *M. alba* and *M. nigra* showed that there is high genotypic diversity among *Morus* species with the highest gluco-genic acid, rutin, and gallic acid were observed in *M. nigra*. The results of another study on morphological properties of 3 species of *M. alba*, *M. rubra*, and *M. nigra* fruits native to Tunisia showed a significant difference in fruit weight and width among *Morus* species, and the highest fruit weight (4.08 g) was obtained from *M. nigra* fruits (Aljane and Sdiri, 2016).

Regarding large populations of this fruit in Iran, the existence of morphological and biochemical diversity and knowledge of the genetic structure and inheritance of properties are the most valuable necessities to start fruit breeding. Therefore, the present study aimed to investigate the diversity and heritability of morphological and biochemical properties of different genotypes of *Morus* collected from different regions of Golestan Province, Northern Iran. Besides evaluating diversity and heritability patterns among these genotypes, the genotypes with superior pomological traits may be identified and further analyzed for commercial stocks in future fruit-growing programs.

MATERIALS AND METHODS

To evaluate heritability and genetic diversity based on morphological and biochemical properties of *Morus* genotypes in different regions of Golestan Province (geographical coordinates 48° 6' E and 36°47' N to 49° 22' E and 37° 13' N), 20 genotypes, including 7 *Morus alba*, 5 *M. rubra*, and 8 *M. nigra* genotypes were collected (Table 1). The natural habitats were first visited and the candidate mulberry trees were identified and properly labelled. The geographical coordinates of the sample collection sites are shown in Table 1. From each genotype, 15 fruits and 10 leaves were randomly harvested, and the samples were immediately transferred to the laboratory to measure morphological and biochemical properties.

To measure petiole and fruit length, leaf length and width, and fruit length and diameter, the digital caliper was used with an accuracy of 0.01 mm, and fresh and dry weights of fruits were measured by a digital scale with the accuracy of 0.01 g (Hassanpour and Alizadeh, 2017). Juice volume was calculated in ml, and titratable acidity was calculated in percentage (Shahi *et al.*, 2019). Soluble solids were measured by the method of Balik *et al.* (2019) using a refractometer in percent. The ratio of soluble solids to acidity was calculated as an index of taste (Varasteh *et al.*, 2008). The pH value was measured using a pH meter, and EC was measured using an electrical conductivity meter. Furthermore, vitamin C was measured by the titrimetric method in milligrams per 100 g of extract (Shahi *et al.*, 2019). Phenol, flavonoids, and antioxidant activity (DPPH) were calculated using the method of Fahmideh *et al.* (2019) in mg (gallic acid) per g of fruit fresh weight, mg (quercetin) per g of fruit fresh weight and percent, respectively. The anthocyanin was measured by the Acid methanol method in micromoles per g (Yavarpanah *et al.*, 2015). Glucose, fructose and sucrose were measured by the method of Rahimkhani *et al.* (2017) in mg per g.

The Analysis of Variance of data was performed in a completely randomized design with 5 replications for morphological properties and 3 replications for biochemical traits using SAS software (version 9.1). The mean comparison was undertaken by LSD method and the correlation among traits, factor analysis and cluster analysis were carried out by SAS software. Furthermore, the genetic parameters of heritability, coefficient of genetic and phenotypic variation and genetic progression were performed using the relevant formulas (Nzuve, 2014).

RESULTS AND DISCUSSION

Morphological properties

The results of the analysis of variance of data showed

Table 1. Geographical data related to growing sites of *Morus* genotypes

Genotype	<i>Morus</i> genotype	Location (UTM)
M1	<i>M. nigra</i>	309922.81, 409223.5
M2	<i>M. nigra</i>	4085147.53, 296400.41
M3	<i>M. nigra</i>	408736.72, 310432.32
M4	<i>M. alba</i>	4080327.9, 273062.37
M5	<i>M. nigra</i>	4080145.64, 272536.13
M6	<i>M. rubra</i>	4080151.7 272433.88
M7	<i>M. nigra</i>	4080405.02, 273076.72
M8	<i>M. nigra</i>	4099644.97, 310916.73
M9	<i>M. alba</i>	4080177.52, 272467.97
M10	<i>M. alba</i>	4091675.24, 309623.12
M11	<i>M. alba</i>	4080264.63, 271916.1
M12	<i>M. alba</i>	4080519.62, 272012.16
M13	<i>M. rubra</i>	4079176.06, 261294.55
M14	<i>M. rubra</i>	4079135.34, 261304.1
M15	<i>M. alba</i>	4080807.65, 274658.89
M16	<i>M. nigra</i>	4080236.7, 271916.79
M17	<i>M. rubra</i>	4115593.72, 348882.9
M18	<i>M. rubra</i>	4100566.7, 26962.64
M19	<i>M. alba</i>	4115739.3,
M20	<i>M. nigra</i>	4085217.37, 296341.42

that the effect of genotype was significant on all measured morphological properties ($p = 0.01$). According to the results of mean comparison data (Table 2), considerable morphological diversity was observed among different genotypes. Such diversity is already attributed to the genotype, cultivar, rootstock, and environmental and nutritional conditions (Jiang *et al.*, 2015). Furthermore, the greater this diversity in the studied population, the greater the possibility of selecting superior genotypes will be provided (Rasouli and Ershadi Qaraler, 2018). A combined photo of fruit diversity in 20 mulberry genotypes was depicted in Fig. 1. The fruit weight of mulberries is one of the most important properties in breeding programs (Aljane and Sdiri, 2016). In the pre-

sent study, studied genotypes showed significant differences in their fruit weight, and the highest fresh and dry weights of fruit (3.80 and 0.67 g, respectively) were observed in M20 genotype. In comparison, the lowest fruit fresh and dry weights (0.80 and 0.10 g, respectively) were obtained from M16 and M4 genotypes, respectively. In a previous study on the morphological properties of *Morus* trees in Turkey, a significant diversity was observed among morphological properties, especially in fruit weight (0.68-3.07 g) (Yilmaz *et al.*, 2012). Furthermore, the present study results were consistent with the results of Aljane and Sdiri (2016). According to the present results, the fruit length and diameter were also two characteristics with high diversity among studied genotypes. The highest fruit length and diameter (5.52 and 1.99 cm) were obtained from M10 and M20 genotypes, respectively, while the lowest fruit length and diameter (1.44 and 0.90 cm, respectively) were obtained from M18 genotype. Krishna *et al.* (2018) also reported fruit length of 1.50-5.80 cm and fruit diameter of 11.4-80 mm in some Indian mulberry genotypes. The recorded data showed that the highest pedicle length (17 mm) was observed in M10 genotype, and this genotype was significantly different from other studied genotypes with respect to this trait. However, the lowest pedicle length (1.86 mm) was observed in the M9 genotype. In a study by Balik *et al.* (2019) on 13 mulberry genotypes in Turkey, pedicle length varied from 7.68 to 11.90 mm.

The high level of diversity was also observed in the case of leaf morphology (Fig. 2). The leaf length and width (15.51 and 11.94 cm, respectively) in M12 and M20 genotypes were higher than other genotypes, respectively, and the lowest leaf length and width of 6.76 and 4.82 cm, respectively were recorded in M17 genotype. The results of the present study were consistent to Barandoozi and Hassanpour (2020) and Krishna *et al.* (2018). They all reported high leaf size diversity among studied genotypes. The results also showed that the petiole length in the M12 genotype (5.37 cm) was significantly higher than in other genotypes. The lowest petiole length (2.41 cm) was observed in the M3 genotype. The petiole length of 2.82-3.57 cm was already reported by Peris *et al.* (2014) during the evaluation of 5 *Morus* species. Moreover, in another study on *M. alba* genotypes, the petiole length was 2.5 to 8.97 cm (Barandoozi and Hassanpour, 2020).

Biochemical properties

The results of the analysis of variance of data showed that the effect of genotype was significant on all measured biochemical parameters ($p = 0.01$). The mean comparison data (Tables 3 and 4) showed significant diversity with respect to biochemical properties in studied *Morus* genotypes. It has already been demonstrat-

Table 2. Mean comparison of fruit and leaf morphological properties of 20 *Morus* genotypes

Genotype	Fruit fresh weight (g)	Fruit dry weight (g)	Fruit length (cm)	Fruit width (cm)	Fruit pedicle length (mm)	Leaf length (cm)	Leaf width (cm)	Leaf petiole length (cm)
M1	3.40 ^b	0.65 ^a	3.28 ^b	1.71 ^b	7.75 ^{gh}	14.38 ^{bc}	10.38 ^c	5.09 ^{ab}
M2	1.84 ^e	0.27 ^{de}	2.36 ^{de}	1.37 ^{efg}	14.12 ^b	7.57 ^k	4.27 ^h	2.50 ^{fg}
M3	0.97 ^{fg}	0.17 ^{fg}	1.54 ^{hi}	1.26 ^g	4.80 ^{mn}	8.55 ⁱ	5.02 ^{ik}	2.41 ^{gh}
M4	1.07 ^{fg}	0.10 ^g	1.86 ^{fgh}	1.38 ^{efg}	7.00 ^{ijk}	11.32 ^f	7.36 ^{fg}	2.86 ^f
M5	2.15 ^{de}	0.41 ^c	2.38 ^d	1.57 ^{bcd}	6.06 ^{klm}	11.51 ^{ef}	7.35 ^{fg}	3.58 ^d
M6	2.17 ^{de}	0.41 ^c	2.40 ^d	1.50 ^{cde}	7.66 ^{ghi}	7.29 ^{kl}	5.74 ^h	2.76 ^{fg}
M7	1.32 ^f	0.22 ^{ef}	1.90 ^{fg}	1.38 ^{efg}	7.13 ^{ijk}	9.38 ^{gh}	6.10 ^h	2.58 ^{fg}
M8	2.02 ^e	0.51 ^b	2.38 ^d	1.30 ^{fg}	9.80 ^{de}	13.87 ^c	8.03 ^{de}	3.33 ^{de}
M9	2.54 ^{cd}	0.48 ^{bc}	2.40 ^d	1.66 ^{bc}	1.86 ^o	11.70 ^{ef}	10.18 ^c	3.71 ^d
M10	3.58 ^{ab}	0.66 ^a	5.52 ^a	1.10 ^h	17.00 ^a	12.67 ^d	8.60 ^d	4.69 ^c
M11	1.32 ^f	0.17 ^{fg}	2.04 ^{ef}	1.51 ^{cde}	6.73 ^{ijk}	9.68 ^g	8.44 ^{de}	2.80 ^{fg}
M12	2.20 ^{de}	0.29 ^{de}	2.50 ^d	1.57 ^{bcd}	7.26 ^{ijk}	15.51 ^a	11.06 ^b	5.37 ^a
M13	2.20 ^{de}	0.43 ^c	2.28 ^d	1.44 ^{efg}	11.53 ^c	11.67 ^{ef}	7.92 ^{ef}	3.49 ^d
M14	1.85 ^e	0.31 ^d	2.80 ^c	1.45 ^{def}	5.13 ^{lmn}	14.95 ^{ab}	11.00 ^b	4.64 ^c
M15	3.71 ^{ab}	0.66 ^a	3.26 ^b	1.73 ^b	10.53 ^{cd}	11.64 ^{ef}	7.92 ^{ef}	5.30 ^a
M16	0.80 ^{gh}	0.15 ^{fg}	1.52 ⁱ	1.08 ^h	6.46 ^{kl}	7.59 ^k	5.84 ^h	2.92 ^{ef}
M17	0.81 ^{gh}	0.13 ^g	1.72 ^{ghi}	1.38 ^{efg}	6.46 ^{kl}	6.76 ^l	4.82 ^k	2.68 ^{fg}
M18	0.73 ^{gh}	0.11 ^g	1.44 ⁱ	0.90 ⁱ	8.71 ^{ef}	7.44 ^{kl}	7.11 ^g	3.70 ^d
M19	2.63 ^c	0.50 ^b	2.82 ^c	1.49 ^{cde}	8.06 ^{fg}	8.66 ^{hi}	5.55 ^{hi}	2.92 ^{ef}
M20	3.80 ^a	0.67 ^a	2.32 ^{de}	1.99 ^a	4.60 ⁿ	12.16 ^{de}	11.94 ^a	3.30 ^{de}

*Means that have at least one similar letter have no significant difference based on LSD test.

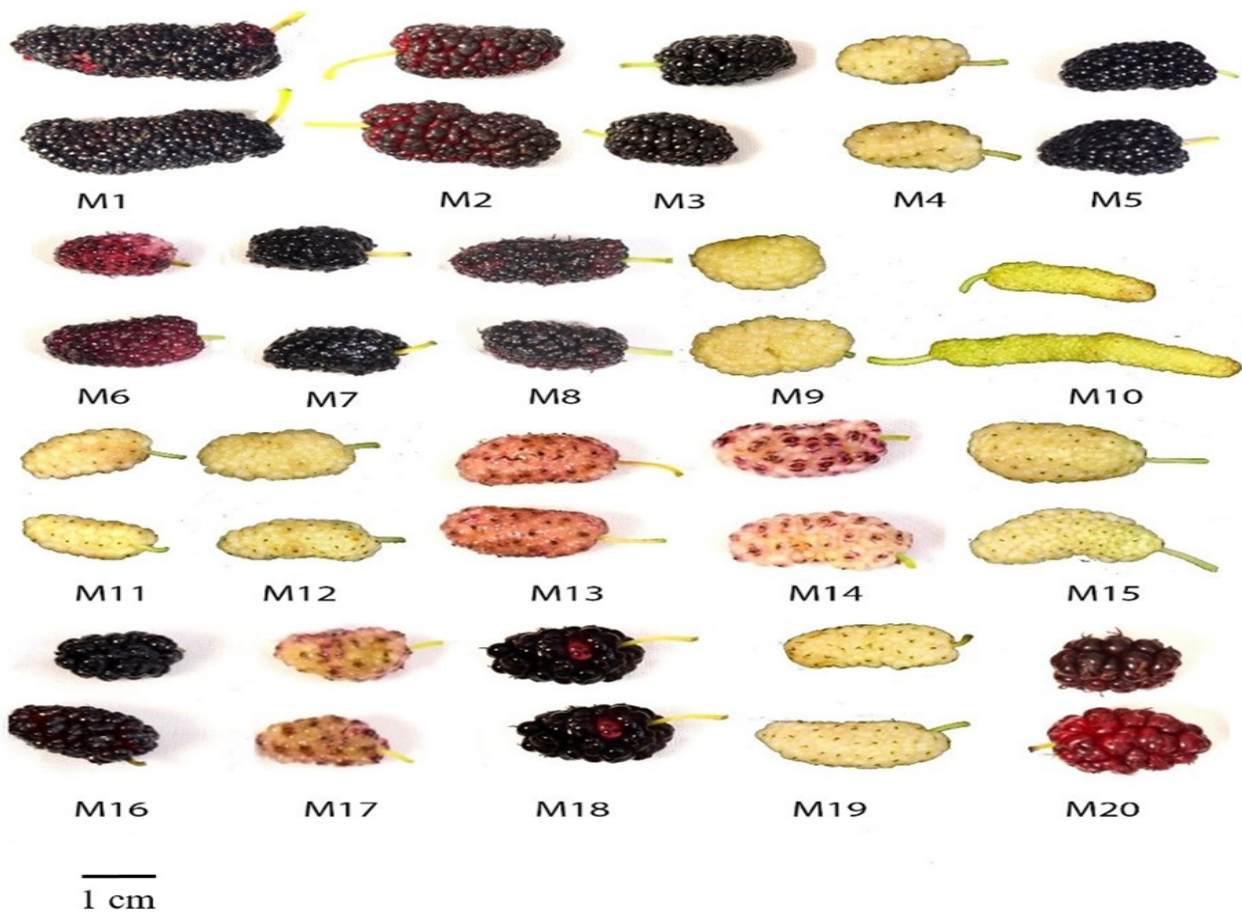


Fig. 1. A combined photo representing fruit diversity in studied *Morus* genotypes

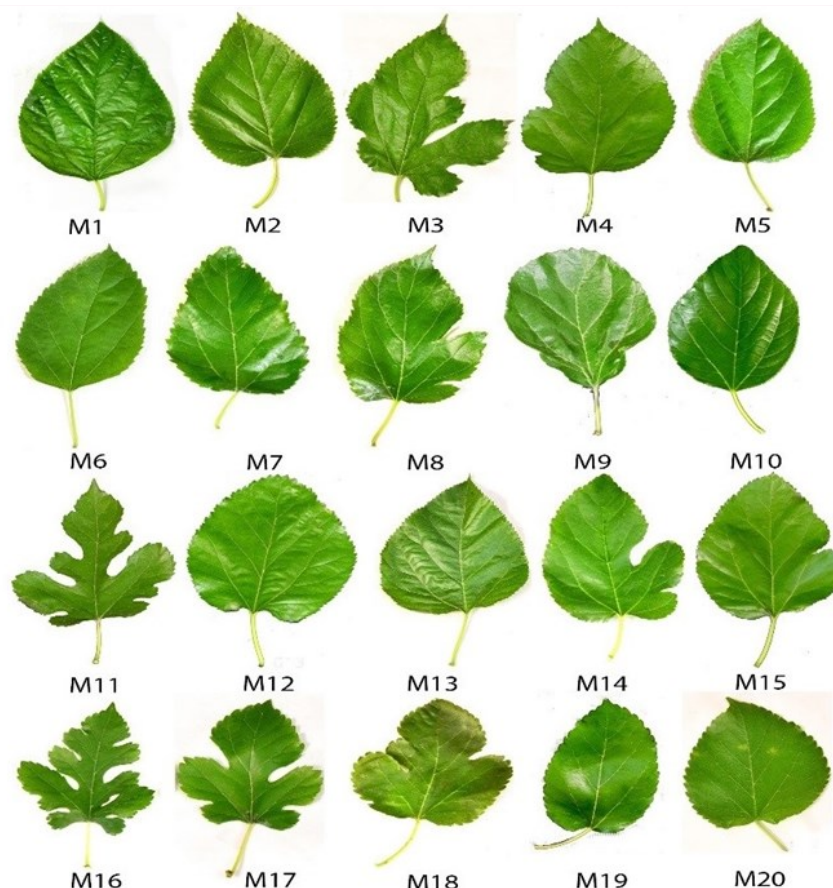


Fig. 2. A combined photo representing leaf morphological diversity in studied *Morus* genotypes.

ed that fruit biochemical properties and their morphological traits were affected by various factors such as genetics, climate, and soil (Gundogdu *et al.*, 2011). The fruit pH plays an important role to determine the flavor of mulberry fruits. In general, it has been cleared those fruits would have a sour taste at pH below 3.5 (Mikulic-Petkovsek *et al.*, 2012). In our samples, the pH of the fruit juice was 2.98-5.62, obtained from M9 and M20 genotypes, respectively. Aljane and Sdiri (2016) reported the pH of *M. alba* fruits as 5.98. Furthermore, in a study conducted by Yilmaz *et al.* (2012) on 34 *Morus* genotypes in Turkey, high diversity was observed among genotypes for pH (2.19 -5.51), consistent with the present experiment's results.

The EC of fruit juice depends on salt content of the juice. The more the salts, the higher the EC (Molaie *et al.*, 2018). The results revealed that EC in M20 genotype (6.25 ds / m) was higher than other genotypes and the M3 genotype had the lowest EC (3.01 ds / m). The reason for diversity in EC levels among genotypes can be partially attributed to environmental factors (Gundogdu *et al.*, 2011).

The soluble solids varied among genotypes of 4.76-18.26%, and the highest and lowest solids were related to M10 and M2 genotypes, respectively. Studies by Cekic and Gunes (2004) showed that soluble solids varied widely among *Morus* genotypes. In a study, Yil-

maz *et al.* (2012) also attributed diversity in soluble solids to genetic factors and reported the highest soluble solids (30.67%) in *M. alba*. In another study, Aljane and Sdiri (2016) investigated *Morus* genotypes and stated that soluble solids in *Morus* are 7.27-19.20, comparable with the present study results. The volume of fruit juice in the M19 genotype (69.93 ml) was more than other genotypes, and the lowest (32 ml) fruit juice was obtained from the M16 genotype. Yilmaz *et al.* (2012) reported fruit juice yield among *Morus* genotypes at the rate of 39-72%.

The fruit taste is a combination of the content and type of sugars, organic acids, and aromatic matter and is related to the ratio of sugar and acid (Barandoozi and Hassanpour, 2020). The taste of fruit juice was the highest in the M2 genotype and the lowest was in the M17 genotype.

Acidity is an important index for evaluating the quality of fresh fruits (Jiang and Nie, 2015). The species' differences in acidity may be caused by genetic and ecological factors (temperature, light, humidity, etc.) (Krishna *et al.*, 2018). The acidity of fruits in studied genotypes varied from 0.17 to 0.98% (M2 and M13 genotypes, respectively). Yilmaz *et al.* (2012) investigated the acidity of 34 *Morus* species. Their results showed that acidity varied from 0.06-1.62%.

Vitamin C content was also different among the evalu-

Table 3. Mean comparison of biochemical properties of fruit in 20 *Morus* genotypes.

Genotype	pH	(ds/m) EC	TSS (%)	Juice volume (cc)	Juice flavor (%)	Acidity (%)	Vitamin C (mg/g)
M1	3.33 ^k	4.30 ^l	8.56 ^m	61.10 ^{abc}	0.91 ^b	0.78 ^c	1.81 ^h
M2	4.05 ⁱ	3.14 ^p	4.76 ^s	45.86 ^{fgh}	2.07 ^a	0.98 ^a	0.90 ^l
M3	4.89 ^{fg}	3.01 ^r	6.03 ^q	38.46 ^{hi}	0.45 ^f	0.27 ^k	2.25 ^{fg}
M4	5.41 ^b	5.49 ^d	5.56 ^r	56.66 ^{cde}	0.53 ^e	0.29 ^k	3.08 ^d
M5	3.89 ^{ik}	4.69 ^f	10.13 ^j	55.80 ^{def}	0.39 ^g	0.40 ^{fg}	1.29 ^k
M6	5.29 ^{bc}	5.49 ^d	11.30 ^g	54.69 ^{def}	0.34 ⁿ	0.38 ^{fg}	3.28 ^c
M7	5.00 ^{ef}	4.60 ^g	7.36 ^o	48.60 ^{efg}	0.74 ^d	0.54 ^d	2.28 ^f
M8	3.72 ^k	5.55 ^c	9.70 ^l	36.13 ⁱ	0.86 ^c	0.83 ^b	1.73 ^h
M9	5.62 ^{gh}	3.64 ⁿ	17.83 ^b	53.23 ^{def}	0.12 ⁿ	0.22 ^l	2.22 ^{fg}
M10	4.77 ^{gh}	4.68 ^f	18.26 ^a	52.67 ^{def}	0.19 ^{lm}	0.36 ^{gh}	2.69 ^e
M11	5.61 ^a	4.56 ^h	9.90 ^k	66.03 ^{ab}	0.30 ^{ik}	0.30 ^{ik}	1.81 ^h
M12	5.26 ^{bcd}	3.80 ^l	12.36 ^f	55.20 ^{def}	0.15 ^{mn}	0.18 ^m	4.34 ^a
M13	5.07 ^{de}	4.26 ^k	14.56 ^e	58.96 ^{bcd}	0.12 ⁿ	0.17 ^m	1.46 ⁱ
M14	4.58 ^h	3.10 ^q	4.83 ^s	41.03 ^{ghi}	0.69 ^d	0.33 ^{hi}	1.70 ^h
M15	5.16 ^{cd}	6.02 ^b	8.46 ^m	52.83 ^{def}	0.37 ^{gh}	0.31 ^{lk}	2.96 ^d
M16	4.76 ^{gh}	3.67 ^m	10.30 ⁱ	32.00 ⁱ	0.45 ^f	0.46 ^e	3.63 ^b
M17	4.90 ^{fg}	4.37 ⁱ	16.86 ^c	58.96 ^{bcd}	0.11 ⁿ	0.19 ^{lm}	2.66 ^e
M18	4.41 ^h	5.53 ^c	8.20 ⁿ	55.26 ^{def}	0.50 ^e	0.41 ^f	2.17 ^{fg}
M19	4.85 ^{fg}	5.32 ^e	10.63 ^h	69.93 ^a	0.26 ^{ik}	0.27 ^k	2.75 ^e
M20	2.98 ^m	6.25 ^a	14.70 ^d	54.76 ^{def}	0.22 ^{kl}	0.32 ^{hi}	3.54 ^b

Means that have at least one similar letter have no significant difference based on the LSD test

and Sdiri (2016). Furthermore, the measured values of sucrose were similar to the report of Gundogdu *et al.* (2017).

Morus species are rich sources of phenolic compounds. Phenolic content of *Morus* trees can be affected by genetic and ecological factors such as humidity, light, temperature, and soil structure (Okatan, 2018). Among studied genotypes, the highest phenol (31.89 mg/g) was obtained from the fruits of M12 genotype and the lowest phenol (16.29 mg/g) was obtained from the M2 genotype. Aljane and Sdiri (2016) reported that phenol in *Morus* species was 13.51-30.45 mg/g, which is close to the values measured in the present experiment.

Flavonoids are among plant extracts' most important antioxidant agents (Taleb 6 *et al.*, 2020). The genotypes were located in different altitudes and grew under different light intensities, so flavonoid content differences were expected. The highest flavonoids (24.67 mg/g) were observed in the M20 genotype and the lowest level (1.08 mg/g) was obtained from the M5 genotype. Hassanpour and Alizadeh (2017) stated that flavonoid compounds can change depending on temperature, pH, and oxygen changes. Moreover, Aljane and Sdiri (2016) reported flavonoids in *Morus* species at the rate of 8.04-19.86 mg/g, which was consistent with the results of the present study.

The data showed that the highest total antioxidant ac-

tivity (66.71%) was observed in the M2 genotype, while the M12 genotype had the lowest total antioxidant activity (6.71%). The importance of mulberry fruits in healthy nutrition is related to their role in increasing antioxidant activity (Sanchez *et al.*, 2014). Several factors, including environmental factors, greatly affect the antioxidant activity and biochemical composition of the plant. Krishna *et al.* (2018) reported 61.3-83.2% antioxidant activity in their studied genotypes. They also stated increased antioxidant activity in those *Morus* genotypes with darker fruit color consistent with the above results.

Trait correlation

The correlation results for fruit morphological properties showed a positive and significant correlation between certain evaluated traits. Therefore, there was a significant correlation between fruit weight, fruit length, fruit diameter, leaf petiole length, pedicle and leaf dry weight. Moreover, a positive significant correlation was recorded between fruit length and pedicle length, leaf and petiole length. Similarly, a significant negative correlation was also recorded between pedicle length and diameter. Furthermore, fruit diameter had a positive and significant correlation with leaf and petiole length as well as fruit dry weight. The correlation between leaf and petiole length and dry weight was positive and significant (Table 5). Since all these traits are components

Table 4. Mean comparison of biochemical properties of fruit in 20 *Morus* genotypes

Genotype	Anthocyanin (µmol/gm)	Glucose (mg/g)	Sucrose (mg/g)	Fructose (mg/g)	Phenol (mg/g)	Flavonoid (mg/g)	DPPH (%)
M1	1.13 ^a	96.82 ^h	21.64 ^{cde}	44.17 ^l	20.42 ^h	7.68 ^d	50.82 ^d
M2	0.77 ^c	16.82 ^m	2.80 ⁿ	10.53 ⁿ	16.29 ^l	1.66 ^e	66.71 ^a
M3	0.7 ^d	12.37 ^l	7.15 ^{mn}	14.98 ⁿ	22.42 ^{fg}	15.14 ^c	43.12 ^{ef}
M4	0.08 ^j	13.08 ^m	2.92 ⁿ	11.54 ⁿ	26.16 ^d	19.58 ^b	28.76 ^h
M5	0.86 ^b	149.04 ^{fg}	29.46 ^a	91.94 ^h	18.02 ^k	1.08 ^e	60.05 ^b
M6	0.56 ^e	187.93 ^d	27.79 ^{ab}	127.30 ^f	27.09 ^c	15.65 ^c	28.17 ⁱ
M7	0.59 ^e	56.82 ⁱ	15.35 ^{ikl}	11.24 ⁿ	22.56 ^f	15.64 ^c	42.61 ^f
M8	0.89 ^b	134.60 ^g	20.35 ^{fgh}	78.81 ^k	20.02 ^h	6.18 ^d	52.35 ^d
M9	0.05 ^j	261.60 ^b	30.61 ^a	197.40 ^b	22.29 ^{fg}	14.64 ^c	43.64 ^{ef}
M10	0.22 ⁱ	281.27 ^a	31.64 ^a	190.54 ^c	24.42 ^e	19.19 ^b	35.43 ^g
M11	0.26 ⁱ	141.27 ^{fg}	26.61 ^{abc}	84.87 ⁱ	20.42 ^h	7.68 ^d	50.82 ^d
M12	0.23 ⁱ	213.27 ^c	20.55 ^{fgh}	149.22 ^e	31.89 ^a	20.42 ^b	6.71 ^l
M13	0.41 ^{gh}	264.60 ^b	19.73 ^{efg}	162.35 ^d	18.82 ⁱ	2.63 ^e	56.97 ^c
M14	0.43 ^f	46.37 ^{ik}	20.91 ^{efg}	11.54 ⁿ	19.89 ^h	5.69 ^d	52.87 ^d
M15	0.23 ⁱ	93.48 ^h	17.79 ^{hik}	41.44 ^l	25.62 ^d	20.69 ^b	30.82 ^h
M16	0.66 ^d	154.60 ^{ef}	11.26 ^{lm}	97.00 ^{gh}	28.69 ^b	23.55 ^a	19.02 ^k
M17	0.39 ^{gh}	288.82 ^a	24.09 ^{bcd}	121.97 ^a	24.29 ^e	21.12 ^b	35.94 ^g
M18	0.42 ^{gh}	84.60 ^h	7.53 ^{mn}	33.36 ^m	22.02 ^{fg}	13.65 ^c	44.66 ^{ef}
M19	0.26 ⁱ	165.71 ^e	13.17 ^{kl}	101.65 ^g	24.69 ^e	20.68 ^b	34.41 ^g
M20	0.57 ^e	272.28 ^{ab}	15.25 ^{ikl}	157.30 ^d	28.29 ^b	24.67 ^a	20.56 ^k

^{*}Means that have at least one similar letter have no significant difference based on the LSD test.

Table 5. Correlation between morphological properties of 20 *Morus* genotypes

	Fruit fresh weight	Fruit length	Fruit pedicle length	Fruit width	Leaf length	Leaf petiole length	Leaf width
Fruit fresh weight	1						
Fruit length	0.66 ^{**}	1					
Fruit pedicle length	0.24 [*]	0.49 ^{**}	1				
Fruit width	0.51 ^{**}	0.13	-0.21 [*]	1			
Leaf length	0.50 ^{**}	0.42 ^{**}	-0.01	0.41 ^{**}	1		
Leaf petiole length	0.47 ^{**}	0.48 ^{**}	0.08	0.25 [*]	0.71 ^{**}	1	
Leaf width	0.04	0.01	0.11	0.01	-0.01	0.01	1
Fruit dry weight	0.81 ^{**}	0.61 ^{**}	0.21 [*]	0.41 ^{**}	0.44 ^{**}	0.41 ^{**}	-0.01

^{*} Significant relationship at 5% level, ^{**} Significant relationship at 1% level

ated genotypes, so the lowest and highest vitamin C (0.4-90.34 mg/ 100g) were observed in M12 and M2 genotypes, respectively. The earlier citations reported different range of vitamin C in *Morus* genotypes. Balik *et al.* (2019) reported vitamin C 2.45-35.83 mg/g, and Orhan and Ercişli (2008) reported 8.9-18.14 mg/g, and Krishna *et al.* (2018) also reported 6.8-27.1 mg/g. This difference in vitamin C can be attributed to genetic factors and environmental conditions.

In addition to creating color, Anthocyanin in *Morus* fruits plays a role in inhibiting free radicals (Pham *et al.*, 2017). The results showed that anthocyanin in the M1 genotype (1.13 µmol/g) was significantly higher than in

other genotypes. While the lowest anthocyanin was obtained from the M9 genotype (0.05 µmol/g). Aljane and Sdiri (2016) reported that anthocyanin in *Morus* species was 1.35-10.05 mg/g. Krishna *et al.* (2018) reported the highest anthocyanins in *Morus nigra* species.

The highest glucose, sucrose and fructose were observed in M17, M10, and M17 (288.82, 31.64 and 121.97 mg/g, respectively) genotypes, while the lowest glucose, sucrose and fructose were in M3, M2 and M2 (12.37, 2.80, and 10.53 mg/g, respectively) genotypes. The measured values of fructose and glucose in present samples were consistent with the results of Aljane

of yield, the effective role of leaves as the main site of photosynthesis can be stated (Taiz and Zeiger, 2010). Hence, selection of genotypes with longer leaf length leads to the production of larger fruits with higher weight. Dirili *et al.* (2017) showed that leaf length had a positive and significant correlation with fruit weight and width in some hawthorn genotypes, and in hazelnut genotypes, a positive and significant relationship was observed between fruit weight and length (Marinoni *et al.*, 2013).

Regarding correlation among evaluated biochemical traits, it was found that pH had a significant negative correlation with fruit taste and acidity. The correlation between EC, juice volume, vitamin C, anthocyanin, glucose, phenol, and flavonoids was positive, while it was negatively and significantly correlated with antioxidant activity. The correlation of soluble solids with juice volume, juice taste, acidity, vitamin C, anthocyanin, glucose, sucrose, fructose, phenol, and flavonoids was significantly positive. The volume of juice had a positive and significant correlation with glucose, sucrose, and fructose and a negative and significant correlation with the taste of juice.

Furthermore, the juice taste was positively and significantly correlated with acidity and antioxidant activity and negatively and significantly correlated with vitamin C, anthocyanin, glucose, sucrose, fructose, phenol and flavonoids. Acidity was positively and significantly correlated with antioxidant activity while it was negatively and significantly correlated with vitamin C, anthocyanin, glucose, sucrose, fructose, phenol and flavonoids. The correlation between vitamin C and anthocyanin, glucose, fructose, phenol, flavonoids and antioxidant activity was significantly positive. Anthocyanin also had a positive and significant correlation with glucose, fructose, phenol and flavonoids and a negative and significant correlation with antioxidant activity. The correlation between glucose and sucrose, fructose, phenol and flavonoids was significantly positive and, while it was negatively and significantly correlated with antioxidant activity.

Moreover, a significant positive correlation was recorded between sucrose, and fructose. Fructose had a positive and significant correlation with phenol and flavonoid and a negative and significant correlation with antioxidant activity. Phenol was also positively and significantly correlated with flavonoids and negatively and significantly correlated with antioxidant activity (Table 6). The correlation among traits is useful to establish a logical relationship among traits that would be useful in breeding programs (Khadivi-Khub *et al.*, 2015). The environmental and climatic changes effectively affect the manifestation of *Morus* traits (Barandoozi and Hassanpour, 2020). Sánchez-Salcedo *et al.* (2015) also reported a positive and significant correlation between anthocyanin, phenol and flavonoids in *Morus* geno-

types. Eshghi *et al.* (2014) also reported a positive correlation between soluble solids and vitamin C and a negative correlation between pH and acidity in grape genotypes.

Cluster analysis

The results of cluster analysis based on morphological and biochemical properties (Fig.3) showed that the evaluated genotypes are classified into three groups. The genotypes in a same group shared the similarities in terms of morphological and biochemical properties in this experiment. The genotypes M5, M11, M8, M6, M19, M16, and M12 were located in first group, M17, M20, M9, M10 and M13 in the second group, and the M15, M18, M1, M7, M14, M3, M4 and M2 in third group. Cluster analysis would be useful for hybridization and breeding programs (Shrestha, 2016).

Factor analysis

According to the results obtained from principal component analysis of 8 morphological properties, it was found that the contribution of 4 principal components explained 71, 24, 0.3, and 0.1% of the changes, respectively, and 99% of the total variance. As shown, the value of the first component for justifying variance is much higher than other components, and this component could explain 71% of the total variance (Table 7).

The results obtained from Fig. 4 show that in 8 morphological properties and based on two principal components that explained 95% of the changes, the highest value was observed in the first component of M2, M17, and M6 genotypes and for the second component, M2, M13, and M15 genotypes were at a higher level compared to other genotypes. The results also showed that M2, M13, and M15 genotypes in both the first and second components had higher contributions than other treatments.

The results of the principal component analysis of biochemical traits showed that in this study, 14 components were studied, and three principal components explained 96, 0.3, 0.1% of the changes, respectively, and 99% of the total variance. As shown, the value of the first component in terms of justifying variance is much higher than other components, and this component has been able to explain 96% of the total variance (Table 8).

The results of Fig. 5 show that based on 14 biochemical properties tested and based on the two first and second principal components, M17, M10, M20, and M13 treatments showed the highest value of the first component compared to other treatments, and for the second component, M10, M11, and M1 treatments were at a higher level than other treatments. The results also showed that M17, M13, M12, and M6 treatments based on the first and second components have higher contributions than other treatments.

Table 6. Correlation between biochemical properties of 20 *Morus* genotypes.

	pH	EC	TSS	Juice volume	Juice flavor	Acidity	Vitamin C	Anthocyanin	Glucose	Sucrose	fructose	Phenol	Flavonoid
pH	1												
EC	0.27	1											
TSS	0.04	0.21	1										
Juice volume	0.05	0.41**	0.32**	1									
Juice flavor	-0.40**	-0.20	0.60**	-0.26*	1								
Acidity	-0.59**	0.02	0.37**	-0.23	0.88**	1							
Vitamin C	0.17	0.33**	0.30*	0.05	-0.48**	-0.40**	1						
Anthocyanin	0.16	0.32**	0.29*	0.04	-0.44**	-0.37**	0.99**	1					
Glucose	-0.03	0.27*	0.95**	0.35**	-0.58**	-0.36**	0.33**	0.32**	1				
Sucrose	0.04	0.13	0.64**	0.30*	-0.40**	-0.16	0.01	-0.02	0.65**	1			
fructose	0.04	0.19	0.97**	0.33**	-0.56**	-0.37**	0.35**	0.34**	0.97**	0.64**	1		
Phenol	0.17	0.33**	0.30*	0.05	-0.48**	-0.40**	0.99**	0.99**	0.3**	-0.01	0.35**	1	
Flavonoid	0.15	0.36**	0.33**	0.07	-0.45**	-0.38**	0.87**	0.87**	0.29*	-0.11	0.31**	0.87**	1
DPPH	-0.17	-0.33**	-0.30*	-0.05	0.48**	0.40*	0.99**	-0.99**	-0.33**	0.01	-0.35**	-0.99**	**0.87-

Table 7. Principal component analysis based on the studied morphological properties of *Morus* genotypes

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Standard deviation	9.56	3.21	3.03	0.85	0.53	0.35	0.11	0.03
Ratio of variance	0.71	0.24	0.03	0.01	0.00	0.001	0.009	0.001
Cumulative ratio	0.71	0.95	0.98	0.99	0.99	0.994	1.000	1.000

Table 8. Principal component analysis based on the studied biochemical properties of *Morus* genotypes.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
Standard deviation	111.56	16.12	15.04	9.01	5.94	2.80	0.98	0.59	0.54	0.27	0.13	0.05	0.005	0.001
Ratio of variance	0.96	0.03	0.01	0.006	0.003	0.0006	0.00007	0.00003	0.00002	0.00001	0.0000	0.0000	0.0000	0.0000
Cumulative ratio	0.96	0.98	0.99	0.996	0.999	0.9996	0.9996	0.9997	0.9997	1.0000	1.0000	1.0000	1.0000	1.0000

Coefficient of genotypic, phenotypic variation, heritability and genetic progression

The results presented in Table 9 showed that the highest coefficients of genotypic and phenotypic variation of morphological properties were related to pedicle length (16.16 and 24.71, respectively), and the lowest coefficients were related to fruit diameter (0.12 and 0.17, respectively). The highest coefficients of phenotypic and genotypic variation of morphological properties were related to leaf width (55.52 and 46.08), and the lowest coefficients were related to fruit diameter (5.15 and 4.33). The minor difference among phenotypic and genotypic variation coefficients for fruit diameter, dry weight and fruit weight indicates that genetic factors control these traits more. However, this difference had the highest value for leaf width, length, and pedicle, which can indicate the effect of environmental factors on controlling these traits. The highest heritability of morphological properties was related to fruit weight (83.22%), fruit length (72.38%), petiole length (71.27%), fruit diameter (70.84%), and leaf width (70.83%), respectively. The low genetic advance of fruit diameter and length is probably offset by their high heritability, indicating that high heritability is not always associated with large genetic progression. The results showed that the highest heritability and genetic progression were related to fruit weight, petiole length, and leaf width, and these morphological properties can be used as suitable indicators for parental selection in breeding programs.

The results presented in Table 10 showed that the highest values of genotypic and phenotypic variance were for fructose (879.587 and 1132.315, respectively), and the lowest values of genotypic and phenotypic variance were for acidity (0.027 and 0.035, respectively). In the study of biochemical traits, the highest coefficients of phenotypic and genotypic variation were for flavonoids (64.63 and 55.47), and the lowest was for glu-

cose (8.22 and 6.71). The high coefficient of phenotypic variation for traits indicates that the expression of these traits are greatly affected by the environment, and the high coefficient of genotypic and phenotypic variation for traits indicates a wide range of changes in these traits (Singh *et al.*, 2014). The similarity of phenotypic and genotypic coefficients of variation of some traits indicates the low effect of the environment on the expression of traits (Mekonnen *et al.*, 2014).

The slight difference between phenotypic and genotypic variation coefficients for pH, glucose, phenol, and antioxidant activity indicates that genetic factors control these traits more. However, this difference had the highest value for fruit flavor, anthocyanin, and flavonoids, which could indicate the effect of environmental factors in controlling these traits. Heritability is the most important parameter in genetic studies of quantitative traits and plays a vital role in the decision to select a particular trait (Lotfi Aghmioni *et al.*, 2015). In this study, the highest heritability in biochemical properties was for pH (95.49%), total antioxidant activity (84.24%), flavonoids (77.68%), acidity (77.14%), EC (76.39%), phenol (75.52%), flavonoids (73.66%) and juice volume (73.56%), respectively. High heritability indicates a low environmental effect on these traits. In fact, the effect of environment on traits with high heritability is negligible, and selection based on phenotype is effective on these traits.

Their high heritability probably offsets the low genetic progression. Regarding pH, acidity, EC, and phenol, it can be said that high heritability is not always associated with large genetic progression. High heritability and low genetic progression for some properties indicate the effects of dominance and epistasis of genes controlling these properties (Ogunniyan and Olakojo, 2014).

The results also showed the highest heritability and genetic progression for total antioxidant activity, fruc-

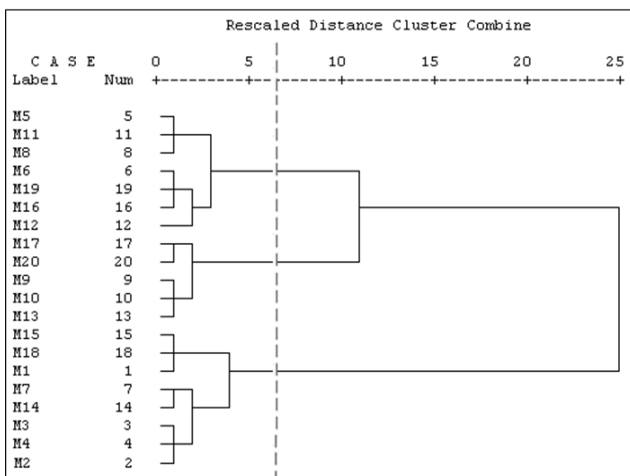


Fig. 3. Dendrogram related to cluster analysis of different *Morus* genotypes based on morphological and biochemical properties.

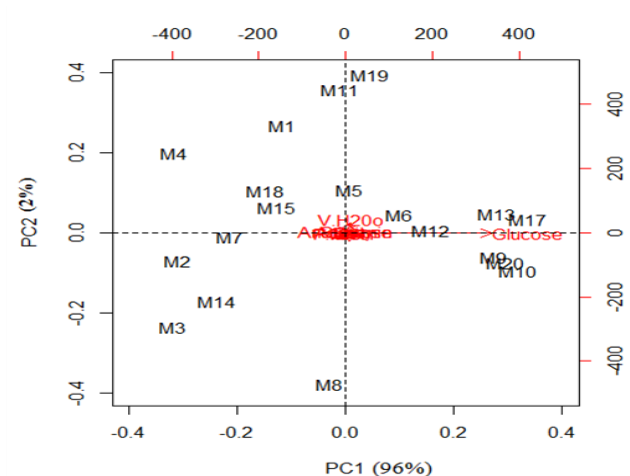


Fig. 4. Biplot depiction of the first and second main components of *Morus* genotypes based on biochemical properties

Table 9. Genetic parameters for morphological traits in *Morus* genotypes.

Plant trait	Components of variance			Coefficient of variation		Heritability	Genetic advance
	Environmental	Genetic	Phenotypic	Phenotypic	Genotypic		
Fruit fresh weight	0.424	2.104	2.528	15.14	13.81	83.22	2.72
Fruit length	0.117	0.893	1.01	48.64	45.73	72.38	1.50
Fruit pedicle length	8.553	16.158	24.711	24.32	19.66	65.38	6.71
Fruit width	0.049	0.119	0.168	5.15	4.33	70.84	0.59
Leaf length	3.988	8.302	12.29	30.64	25.18	67.55	4.88
Leaf petiole length	0.640	1.588	2.228	13.92	11.75	71.27	2.19
Leaf width	1.578	2.264	3.842	55.52	46.08	70.83	2.98
Fruit dry weight	0.08	0.168	0.248	6.37	5.24	67.74	0.69

Table 10. Genetic parameters for biochemical traits in 20 *Morus* genotypes

Plant trait	Components of variance			Coefficient of variation		Heritability	genetic advance
	Environmental	Genetic	Phenotypic	Phenotypic	Genotypic		
pH	0.085	1.80	1.885	29.33	28.66	95.49	2.70
EC	0.415	1.343	1.758	28.97	25.32	76.39	2.08
TSS	6.893	13.557	20.45	42.99	35.00	66.29	6.18
Juice volume	64.488	179.338	243.876	29.78	25.54	73.56	23.69
Juice flavor	0.023	0.036	0.059	49.36	38.56	61.01	0.305
Acidity	0.008	0.027	0.035	40.23	35.33	77.14	0.29
Vitamin C	0.238	0.482	0.720	28.53	20.05	66.94	1.17
Anthocyanin	0.028	0.075	0.103	65.17	55.46	72.81	0.48
Glucose	48.839	96.856	145.695	8.22	6.71	66.47	16.55
Sucrose	5.687	14.009	19.696	24.20	20.41	71.12	6.51
Fructose	252.728	879.587	1132.315	36.77	32.40	77.68	53.92
Phenol	3.113	9.298	12.311	15.11	13.13	75.52	5.46
Flavonoid	21.148	59.161	80.309	64.63	55.47	73.66	13.61
DPPH	20.72	110.804	131.524	28.61	26.26	84.24	19.93

tose, and flavonoids. Selecting traits with high heritability and genetic progression can be successful (Gul et al., 2013). Therefore, total antioxidant activity, fructose, and flavonoids can be used as suitable indicators for parental selection in breeding programs.

Conclusion

The study results showed that 20 *Morus* genotypes collected from different regions of Golestan Province, northern Iran, had a high level of diversity in terms of morphological and biochemical properties and significant differences in all evaluated traits. The studied genotypes were divided into three separate groups in terms of morphological and biochemical properties using cluster analysis. As per the results, samples can be found at long genetic distances from each other, and fruit weight, petiole length, fruit width, antioxidant activity,

fructose, and flavonoids due to high heritability and genetic progression can be used as suitable indicators for parental selection in hybridization programs.

Conflict of interest

The authors declare that they have no conflict of interest.

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