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Study of the Morphological Fruit Quality, Total Polyphenol Content, and Antioxidant Activity in Four Mulberry Species (*Morus* spp.) in Malatya-Türkiye

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ABSTRACT

The present study evaluated the morphological and biochemical characteristics of fruits from four widely distributed mulberry species in Türkiye (*Morus spp.: M. alba*, *M. nigra*, *M. rubra*, and *M. laevigata*) in order to inform species selection and promote the utilisation of underexploited genotypes for food and nutraceutical applications. The fruits and juice were analysed for size, weight, juice yield, total soluble solids (TSS), titratable acidity (TA), pH, colour (CIELAB coordinates), total polyphenol content (TPC), and antioxidant activity (DPPH assay). Significant interspecific variation was observed for most traits. *M. rubra* and *M. laevigata* produced the largest and heaviest fruits, with *M. rubra* also presenting the highest juice yield (57.15%). Colour analysis separated pale-coloured *M. alba* from the anthocyanin-rich, dark-pigmented *M. nigra* and *M. rubra*. *M. alba* and *M. nigra* presented the highest levels of TSS at 19.93% and 19.64%, respectively. However, *M. alba* had the lowest levels of TA and TPC. In contrast, *M. nigra* had the highest TPC (1608.40 mg GAE 100 g^{-1} fw). The antioxidant activity (DPPH) of the three dark-pigmented mulberries (*M. nigra*, *M. rubra*, and *M. laevigata*) was found to be similar to each other but higher than that of the pale-coloured *M. alba*. These findings highlight the potential of the dark-pigmented mulberry species as valuable sources of bioactive compounds for the development of functional foods.

Keywords: Antioxidant Activity, Biochemical Composition, Fruit, Morphological Traits, *Morus ssp*., Türkiye.

INTRODUCTION

Mulberry (*Morus* spp.), a member of the Moraceae family, is a perennial deciduous tree cultivated across tropical, subtropical, and temperate regions (Sánchez-Salcedo et al., 2015; Chauhan et al., 2018). Nineteen species are currently recognized worldwide (Butt et al., 2008; Ercisli and Orhan, 2008). It is hypothesised that mulberries first originated in the Himalayan foothills, from which they have adapted to a wide range of ecological zones, from sea level to 4,000 m (Pel et al., 2017; Yuan et al., 2017). Within the geographical limits of Türkiye, *M. alba*

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is predominant, accounting for approximately 95% of the population; in contrast, *M. rubra* (3%) and *M. nigra* (2%) are observed to occur with comparatively lower frequency (Ercisli, 2004). *M. laevigata,* distinguished by its substantial, red-black fruits, has recently attracted interest for cultivation in select regions.

Mulberries represent a multipurpose crop of nutritional, medicinal, and economic importance. Beyond their role in sericulture and animal husbandry, the fruits are consumed fresh or processed into jams, juices, and desserts (Salih et al., 2022). Their popularity can be attributed to their nutritional and functional properties. Mulberries provide carbohydrates, dietary fiber, minerals, and vitamins, while being relatively low in calories (Jan et al., 2021). They are also rich in polyphenols, particularly anthocyanins, flavonols, and flavanols, which contribute to strong antioxidant capacity (Hassimotto et al., 2008; Natić et al., 2015). Dark-pigmented species such as *M. nigra* and *M. rubra* typically accumulate more anthocyanins and phenolic acids, whereas pale-coloured *M. alba* is characterised by glycosylated flavonoids (Chan et al., 2020). These compounds are associated with protection against oxidative stress, metabolic disorders, and cardiovascular risks (Ahmad et al., 2022; Batiha et al., 2023)

Mulberries also hold significant cultural and medicinal value. Traditional uses of fruits and extracts include treatment of oral wounds, anaemia, and respiratory conditions, while mulberry leaves are employed against diabetes, hypertension, and wounds (Abbasi et al., 2014; Younus et al., 2016; Thaipitakwong et al., 2018). Such practices reinforce their dual role as food and medicine across Asia and the Mediterranean.

Fruit quality traits, however, are influenced not only by genetics but also by environment. Studies worldwide show that warm, humid climates enhance sugar accumulation but reduce acidity, whereas cooler or drier conditions promote phenolic and anthocyanin synthesis as well as smaller fruit size (Aljane et al., 2016; Lou et al., 2024; Rong et al., 2024). These regional patterns underline the need to evaluate mulberries under defined climatic conditions. The continental climate of Malatya, Türkiye, thus provides a valuable setting for assessing interspecific differences while enabling comparisons with studies from other regions.

Despite increasing interest in mulberries, most prior studies have either focused on a single species or compared accessions grown under variable environments, making it difficult to separate genetic from environmental influences. The present study is novel in simultaneously evaluating four widely distributed *Morus* species (*M. alba, M. nigra, M. rubra, and M. laevigata*) cultivated under uniform environmental conditions in Türkiye. This design minimises climatic and soil-related confounding factors, allowing robust identification of species-specific differences. In addition, the use of multivariate analyses provides new insights into relationships among morphological, physicochemical, and antioxidant traits.

The objectives of this study were threefold: firstly, to characterise interspecific variation in fruit quality traits; secondly, to examine relationships among morphological, biochemical, and antioxidant properties; and thirdly, to provide guidance for the utilisation of mulberry fruits in food and nutraceutical applications.

MATERIALS AND METHODS

Climatic Conditions of the Study Area

The study area has a continental climate characterised by hot, dry summers and cold, snowy winters. The mean annual temperature is 13.6°C, while maximum temperatures reach 27 to 30°C in June and July. Annual precipitation averages 400–500 mm (Malatya İl Kültür ve Turizm Müdürlüğü, 2025).

Plant Material and Sampling

The study was conducted in the Battalgazi district of Malatya, Türkiye (38–39° N, 38–39° E; 900 m asl). Four mulberry species (*M. alba*, *M. nigra*, *M. rubra*, and *M. laevigata*) were evaluated. For each species, ten healthy, productive trees were selected. Approximately 1.5 kg of fully ripe fruits were harvested per tree, randomly sampled from all parts of the canopy. Samples were transported under refrigerated conditions (5°C) to the laboratory for analysis. All measurements were performed on 10 replicates per species, with each replicate consisting of individual fruits (for weight and size parameters) or pooled 50-g fruit samples (for juice, biochemical, and colour analyses).

Morphological Mulberry Fruit Characteristics

Fruit length and width were measured with a digital calliper, and fresh weight was recorded with a precision electronic balance. Dry weight was determined by drying the sample at 65°C (Cemeroğlu, 1992). Fruit skin colour was assessed using a handheld reflectometer, with CIELAB colour coordinates *L**, *a**, and *b** recorded.

Chemical Mulberry Fruit Juice Characters

Juice was extracted and filtered through cheesecloth. Juice yield was calculated as the ratio of the fresh fruit weight to juice weight. Total soluble solids (TSS) content was measured using a digital refractometer, while titratable acidity (TA) was determined by titrating with 0.1 N

NaOH to pH 8.2 and expressed as malic acid equivalent. The pH and colour of the juice were measured using a digital pH meter and the CIELAB scale (*L**, *a**, *b**), respectively.

Mulberry Fruit Total Polyphenol Composition

The quantification of total polyphenol content (TCP) was performed using employing Folin-Ciocalteu's reagent, as described by Slinkard and Singleton (1977), with a minor modification. The reaction mixture contained 0.02 ml of extract, 3.9 ml of distilled water, 0.25 ml of reagent, and 0.75 ml of Na_2CO_3. After a 2-h dark incubation, the absorbances were measured at 745 nm using a UV-vis spectrophotometer. Results were expressed as mg gallic acid equivalent (GAE) per 100 g fresh weight (fw).

Mulberry DPPH Activity

Antioxidant activity was determined using the DPPH radical-scavenging assay, as described by Blois (1958). An aliquot of 0.2 ml of extract was mixed with 2 ml of 0.1 mM DPPH solution and incubated at room temperature for 30 min in the dark. Absorbance was recorded at 517 nm. The inhibition (%) of DPPH radicals was calculated as: Inhibition percentage (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 and A_1 represent the absorbances of the control and sample, respectively. Results were expressed in %.

Statistical Analysis

Data analysis was performed using SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) and Duncan's multiple range test were applied to compare mean values across species at a significance level of $p \leq 0.05$. Pearson correlation coefficients were calculated to evaluate relationships among variables. Principal component analysis (PCA) and hierarchical cluster analysis were conducted based on Euclidean distances to explore multivariate patterns among species.

RESULTS AND DISCUSSION

Fruit Morphological Characteristics

Morphological traits such as fruit size, weight, and juice yield are key factors influencing consumer preference, marketability, and yield potential. Among the four *Morus* species evaluated, *M. rubra* exhibited the greatest fruit width (19.29 mm;), while *M. laevigata* produced the longest fruits (37.47 mm;) (Figure 1). In contrast, *M. alba* and *M. nigra* yielded more compact fruits with moderate widths and shorter lengths (15.09 and 24.83 mm in *M. alba*, and

16.45 and 23.49 mm in *M. nigra*), consistent with prior reports of size variability across *Morus* species (Ercisli, 2004; Orhan et al., 2020; Gnanesh et al., 2023).

Similar trends were observed for fruit weight. *M. laevigata* and *M. rubra* exhibited the highest fresh weights (6.19 g and 6.14 g, respectively), while *M. alba* produced the lightest fruits (3.36 g) (Figure 2). Dry weight followed comparable patterns. *M. rubra* had a significantly higher juice yield (57.15%) than *M. alba*; however, it did not differ statistically from *M. nigra* or *M. laevigata* These results suggest that the three dark-pigmented Morus species are particularly suitable for fresh consumption and juice production.

Fruit and Juice Colorimetric Attributes

Fruit and juice colour parameters (*L**, *a**, *b**) differed significantly among species (Figures 3 and 4). *M. alba* presented the highest fruit *L** value (66.47), indicative of its light skin. In contrast, the remaining samples demonstrated significantly lower *L** values, measuring less than 20, suggesting that the fruit skins were of a darker hue. Negative *a** values for *M. alba* (–3.00;) reflected its greenish hue, whereas positive *a** values in other species indicated red pigmentation linked to anthocyanins. Lower *b** values (0.375–0.94) in *M. rubra*, *M. nigra*, and *M. laevigata* also indicated darker, anthocyanin-rich pigmentation, in line with earlier findings (Özgen et al., 2009; Ercisli and Orhan, 2007, 2008).

Juice colour followed a similar pattern. *M. alba* exhibited the highest juice *L** value (39.12), a negative a* value (–2.39), and a higher b* value (5.76), resulting in a light, greenish-yellow colour. In contrast, juices from *M. rubra*, *M. nigra*, and *M. laevigata* were darker and reddish, reflecting their higher anthocyanin content. These colour differences further support the classification of *Morus* fruits based on pigment profiles and their potential functional food value.

Fruit Chemical Properties

Total soluble solids (TSS), titratable acidity (TA), and pH are important determinants of flavour and processing suitability. As shown in Figure 5, both *M. alba* and *M. nigra* presented the highest TSS values (19.93% and 19.64%, respectively), indicating higher sugar content, which is desirable for fresh consumption, drying and sweet products. In contrast, *M. rubra* (11.48%) and *M. laevigata* (6.54%) exhibited significantly lower TSS values, making them more suitable for processing.

Acidity profiles also varied markedly (Figure 5). *M. nigra* exhibited the highest TA (11.57%) and lowest pH (3.85), contributing to its pronounced tartness. Conversely, *M. alba* had the lowest TA (1.52%) and highest pH (6.10), consistent with its mild flavour. *M. rubra* and *M. laevigata* displayed intermediate acidity values. These species-specific acidity profiles align with previous studies (Özgen et al., 2009; Gundogdu et al., 2011) and provide guidance for cultivar selection based on target flavour attributes.

The ecology of mulberry plants has been demonstrated to exert a significant impact on the TSS and acidity present in the fruit. Mulberries from tropical regions of China, where warmer climates favoured higher sugar accumulation but lower acidity compared to cooler regions (Lou et al., 2022). In contrast, mulberries grown off-season, corresponding to shorter and cooler summers, typically present lower sugar content but especially higher acidity (Liu et al., 2024), aligning more closely with our observations in *M. nigra*.

Total Polyphenol Content and DPPH Antioxidant Activity

Total polyphenol content (TPC) and antioxidant activity (DPPH assay) exhibited significant interspecific differences (Figure 6). *M. nigra* had the highest TPC (1608.40 mg GAE 100 g^{-1} fw), followed by *M. rubra*, *M. laevigata*, and *M. alba*. Correspondingly, dark-pigmented mulberries (*M. nigra*, *M. rubra*, and *M. laevigata*) presented higher DPPH radical-scavenging activity compared to the pale-coloured mulberry (*M. alba*).

These results corroborate the strong association between dark pigmentation, polyphenol richness, and antioxidant capacity, as reported in previous studies (Imran et al., 2010; Ercisli et al., 2010). The findings highlight especially *M. nigra* and *M. rubra* as promising candidates for functional food and nutraceutical applications.

The high phenolic and antioxidant levels observed in *M. nigra* and *M. rubra* under the Malatya climate may result from protective mechanisms in the plants, which enhance anthocyanin production to serve as antioxidants and UV protectants (Li and Ahammed, 2023). Conversely, mulberries cultivated in tropical Asian regions often display higher sugar levels but comparatively lower phenolic concentrations (Lou et al., 2024), illustrating the strong role of climatic adaptation in shaping biochemical traits.

Correlation Parameters and Multivariate Analysis

Significant morphological, colorimetric and biochemical relationships among the species, about anticipated and unanticipated interrelations, were evident from the correlation matrix (Figure 7). Dry and fresh fruit weight exhibited strong positive correlations (r = 0.99), consistent with the findings of Ercisli and Orhan (2007), who reported significant correlations between water content and biomass accumulation in fleshy fruits. Notably, the fruit colour parameters

(L^*, a^*, b^*) are almost perfectly correlated (r > 0.95), which reflects the coordinated regulation of the anthocyanin biosynthesis pathways established by Gundogdu *et al.,* (2011). The correlation between TA and pH (r = -0.85) was consistent with previous studies on organic acid metabolism, which associate lower pH values with higher levels of malic and citric acid (Meng *et al.,* 2024). Contrary to expectations, the TSS exhibited a negligible correlation with TPC (r = 0.06). This phenomenon is likely attributable to interspecific contrasts: *M. alba* exhibits a combination of high TSS and low TPC, while dark-fruited species demonstrate the opposite, with higher TPC but lower TSS. Such cross-species patterns reduce the overall correlation.

Clustering and PCA Analysis

The hierarchical clustering heatmap analysis revealed distinct phenotypic and biochemical differences among the four mulberry species (Figure 8). The heatmap clearly delineated the species into two primary clusters. The first cluster consisted solely of *M. alba*, which was distinguished by its significantly higher fruit lightness (L^*) and lower a^*/b^* colour values, indicating a lighter fruit colour compared to the other species. Additionally, *M. alba* exhibited lower TPC and DPPH antioxidant activity, suggesting reduced biochemical potency relative to the other species. The second cluster was characterised by the aggregation of *M. nigra, M. rubra,* and *M. laevigata*, primarily due to the presence of shared characteristics, including higher TPC, greater fruit dimensions, and stronger antioxidant properties. Within this cluster, *M. nigra* and *M. rubra* were more closely related, likely due to their exceptionally high TPC levels and notable DPPH activity. *M. laevigata*, while still within this group, displayed intermediate traits, including moderate fruit size and slightly lower acidity (TA), which may explain its slight divergence from the other two species.

The 16 traits were grouped into three broad categories by the x-axis dendrogram, indicating common physiological or biochemical activities (Figure 8). Cluster I included juice colour L^*, a^*, b^*, fruit colour a^*, pH, and TSS, primarily associated with sensory quality, ripeness, and sugar metabolism. These traits were tightly clustered due to their co-regulation during fruit ripening and pigmentation processes, particularly in the biosynthesis of anthocyanins and flavonoids (Giovanelli and Buratti, 2009). Cluster II consisted of DPPH activity, juice yield, and fruit width, which indicate antioxidant capacity (DPPH) and processing characteristics. This cluster lends further support to the prevailing hypothesis that increased fruit size and juice content are concomitant with elevated levels of bioactive compounds (Özgen *et al.,* 2009). Cluster III included fruit length, fresh and dry fruit weight, and TPC, which are traits associated with biomass and nutritional value. These are the most significant traits for breeding programs aimed at improving yield and promote health (Mohammadi and Prasanna, 2003).

The first two principal components (PC1 and PC2) accounted for 83.2% of the total variance, with PC1 contributing 66.4% and PC2 contributing 16.8%. PC1 was positively correlated with TA, TPC, juice colour *a**, and fruit colour *a**, suggesting that this component reflects fruit acidity and antioxidant potential. Conversely, PC1 was negatively correlated with TSS, fruit colour *L**, and juice colour *L**, which are indicative of fruit sweetness and brightness. The strong association of *M. nigra* with TA and TPC suggests that this species has a superior antioxidant profile. This finding is consistent with previous reports emphasizing the higher phenolic and anthocyanin content in M. nigra fruits (Ercisli and Orhan, 2007; Özgen *et al.,* 2009).

PC2 was positively associated with fruit length, dry and fresh fruit weight, juice yield, and pH. These traits are primarily linked to fruit size and productivity. The vectors for *M. laevigata* and *M. rubra* were located in the positive quadrant of PC2, indicating their association with larger fruits and higher juice yield. This supports earlier findings that *M. laevigata* typically produces larger and juicier fruits compared to other *Morus* species (Koca *et al.,* 2021). On the other hand, *M. alba* was separated negatively along PC1 and PC2, indicating lower TPC, acidity, and fruit size. The fruit and juice colour (*L**, *b**) were also closely associated with *M. alba*, supporting the characterization of its fruits as lighter-coloured and less acidic (Redha *et al.,* 2023).

The biplot showed a clear separation among the four *Morus* species based on the PCA axes: *M. nigra* was strongly associated with TA, TPC, and darker colour attributes (juice/fruit colour *a**), highlighting its potential for functional food and nutraceutical applications. *M. rubra* and *M. laevigata* were positioned closely along PC1 and PC2, showing high juice yield, pH, and fruit dimensions, making them suitable for fresh consumption and juice production. *M. alba* was negatively correlated with most traits linked to fruit quality and bioactivity. It may be better suited for dried fruit or culinary use where lower acidity and mild flavour are desired.

CONCLUSIONS

This study demonstrated significant interspecific variation in fruit quality traits among the four mulberry species grown in Malatya, Türkiye. *M. rubra* and *M. nigra* showed the highest TPC and antioxidant activity, supporting their use in functional food and nutraceutical applications. *M. alba*, with its high sugar content and mild acidity, was better suited for fresh consumption, drying or culinary use, while *M. laevigata* offered potential for fresh consumption and juice production due to its large fruit size. These findings provide practical guidance for cultivar selection and highlight the combined influence of genetics and regional climate on mulberry fruit quality.

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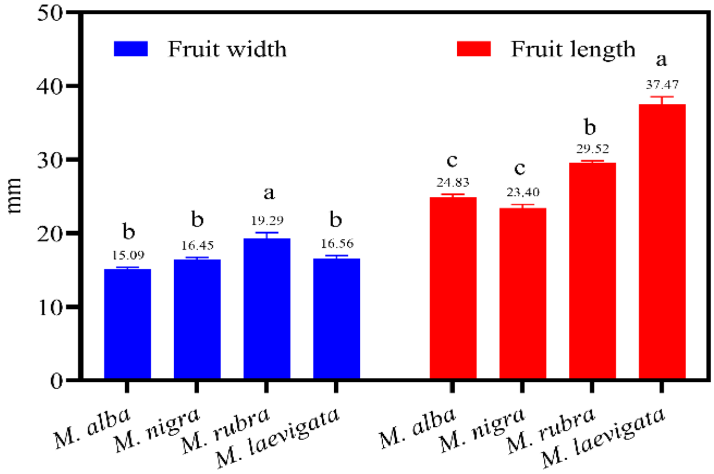

Fig. 1. Fruit width and length of the four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test (p ≤ 0.05).

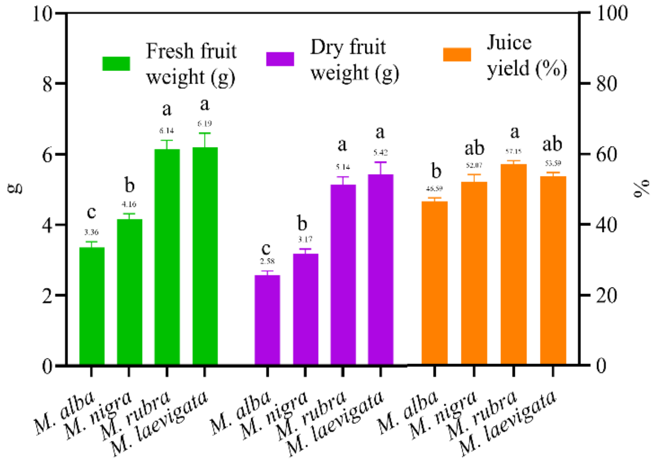

Fig. 2. Fresh, dry weight, and juice yield of the four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

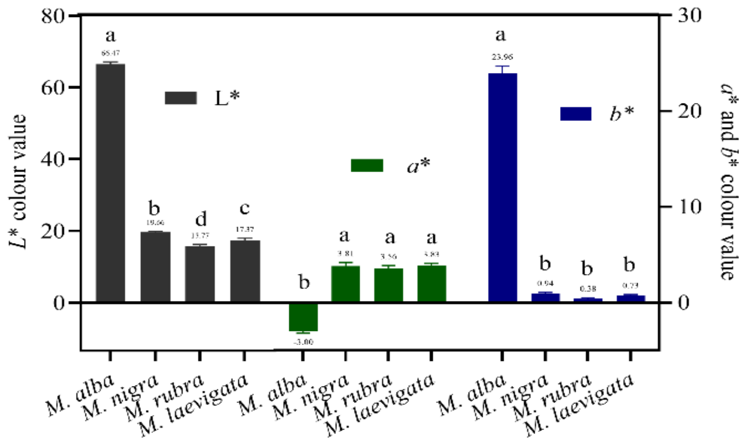

Fig. 3. CIE *L* a* b** fruit colour space values for the four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

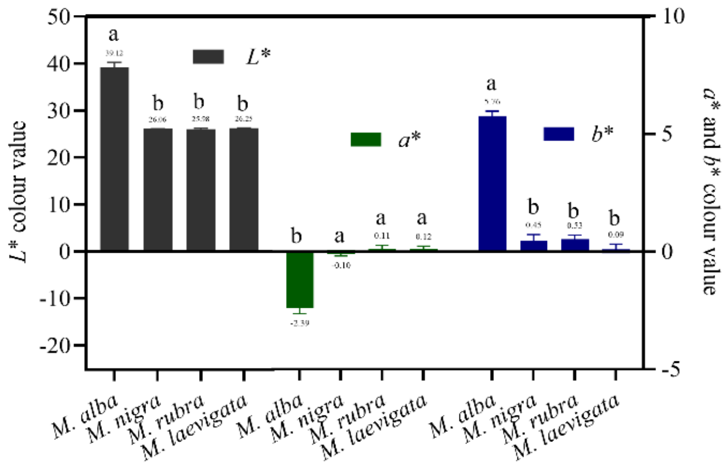

Fig. 4. CIE *L* a* b** juice colour space values for the four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

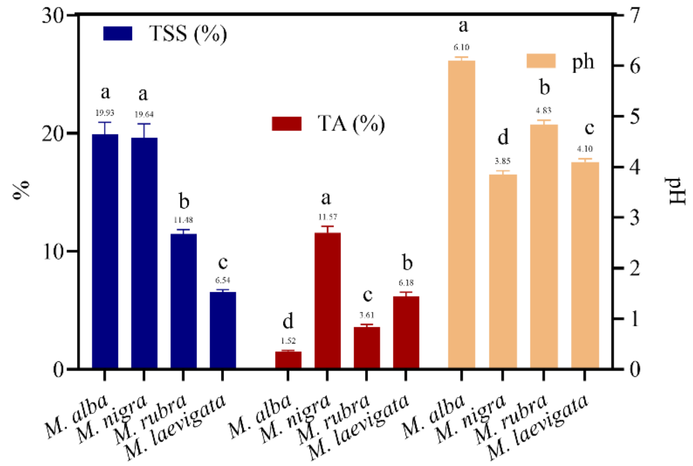

Fig. 5. Total soluble solids (TSS), titratable acidity (TA), and pH values of the for four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

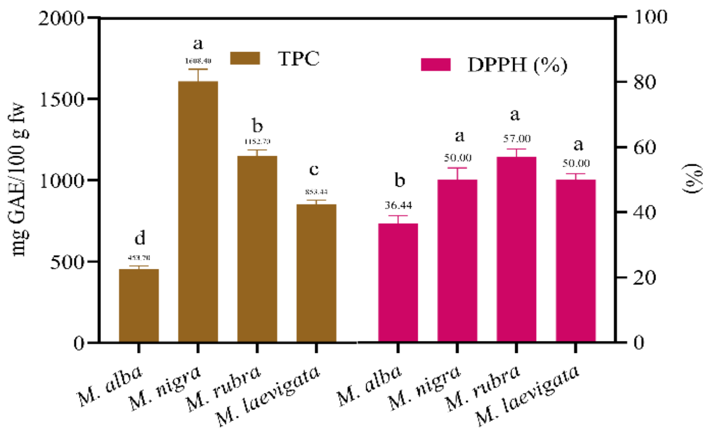

Fig. 6. Total polyphenol content (TCP) and DPPH radical-scavenging activity for the four mulberry species. Vertical bars represent standard error of the mean. Different letters above bars indicate significant differences according to Duncan's multiple range test ($p \leq 0.05$).

Fig. 7. Heatmap of Pearson correlation coefficients among morphological and biochemical traits of the four mulberry species.

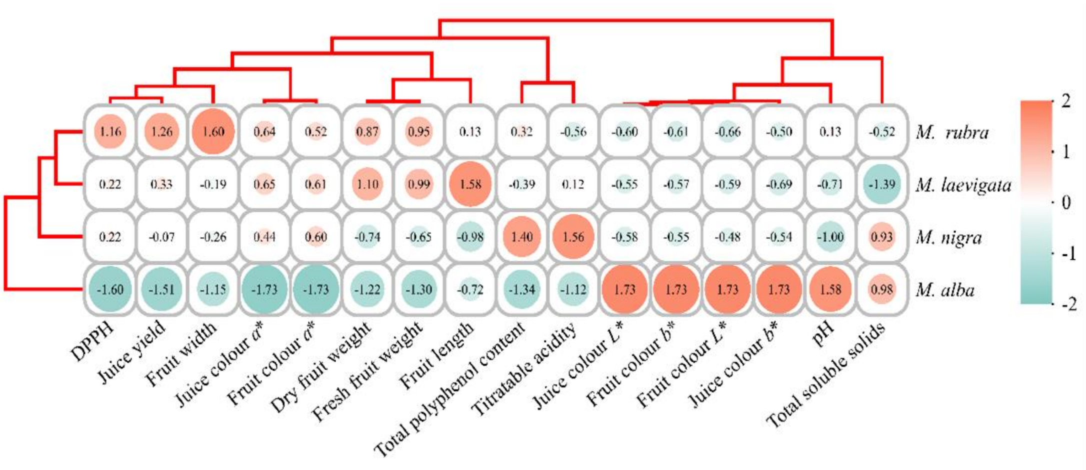

Fig. 8. Hierarchical clustering heatmap of the four mulberry species based on morphological and biochemical traits.

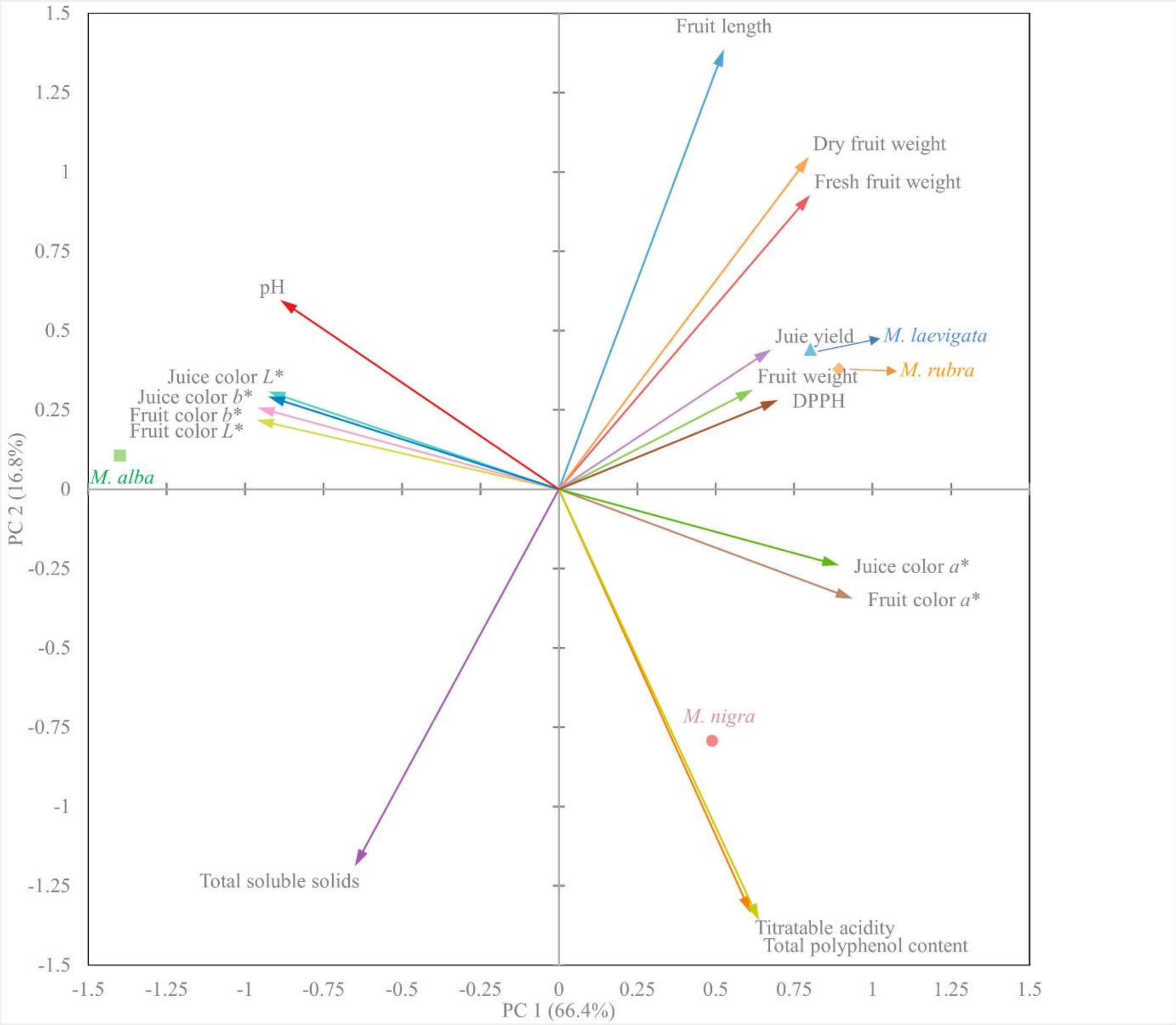

Fig. 9. Principal component analysis (PCA) biplot of the four mulberry species based on morphological and biochemical traits.