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Morphological Characterization of Several *Morus* Species from Turkey

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Abstract. Among edible mulberry (*Morus* spp.) species, *M. alba*, *M. nigra*, *M. rubra*, and *M. laevigata* grow in Turkey. Several regions of Turkey were surveyed during the 2007 and 2008 growing seasons. More than 200 promising mulberry accessions were visited and preliminary tree and fruit characteristics were investigated. Based on the first year results, a total of 22 superior accessions from four different species were morphologically characterized in the second year. Considerable variations were observed for most of the traits. For example, fruit weight ranged from 3.3 to 8.2 g for an average of 4.9 g. *M. nigra* accessions had much smaller mean values when compared to those of *M. rubra* and *M. alba*. The juice yield ranged from 56.1 to 67.8% for *M. nigra* accessions, 20.0 to 31.1% for *M. rubra* and 25.6 to 35.0% for *M. alba*. The mean total acidity of *M. nigra* was 2.7%, 3.1-fold higher than those of *M. rubra* and *M. alba* accessions. Soluble solids ranged from 14.1 to 27.1% for an average of 21.8%. These fruit traits were subjected to principle component analysis and the results indicated that *M. nigra* accessions were grouped together, while *M. laevigata* accession was distinct from other genotypes. The accessions of *M. rubra* and *M. alba* separated from *M. nigra* and *M. laevigata* accessions, although the two groups did not separate from each other.

***Additional key words*:** genetic diversity, germplasm collection, mulberry, principal component analysis

Introduction

The mulberry family (*Moraceae*) is distributed in a wide area of tropical, subtropical, and temperate zones in Asia, Europe, North and South America, and Africa with the majority of the species native to Asia (Berg, 2001). There are more than 20 species described (Koidzumi, 1917), and five of these species have edible fruit: *Morus alba*, *M. nigra*, *M. rubra*, *M. indica*, and *M. laevigata* (Awasthi et al., 2004).

Turkey is an important germplasm center for fruit genetic resources and most parts of Anatolia have wild and cultivated forms of mulberries such as *M. alba* (white or purple colored mulberry), *M. nigra* (black mulberry), *M. rubra* (red mulberry), and *M. laevigata* (long-fruited mulberry), while *M. alba* is the dominant species in cultivation (95%) (Ercisli, 2004). Indeed, mulberry species have been known and cultivated in Turkey for more than 400 years (Ercisli and Orhan, 2007).

Several mulberry accessions from Turkey have been characterized for different horticultural attributes. For example, Polat (2004) studied the horticultural characteristics of the four most commonly grown *M. alba* genotypes from Hatay Province. Mulberry fruit characteristics of different genotypes from different parts of Turkey were also studied by other researchers (Güneş and Çekiç, 2004; Koyuncu, 2004a). Gungor and Sengul (2008) determined the total phenolic content, antioxidant activity, mineral content, and selected physicochemical properties of three *M. alba* genotypes. Similar properties of *M. rubra* (Koca et al., 2008) and *M. nigra* genotypes (Elmac and Altuğ, 2000; Ercişli and Orhan, 2008) were also determined. Recently, characterization of all these accessions by molecular markers was attempted (Kafkas et al., 2008; Orhan et al., 2007).

Especially, *M. nigra* has gained an important position in fruit markets as well as in food industry due to its important phytonutrient constituents and unique flavor. Therefore, many researcher efforts were devoted to characterize *M. nigra* accessions from different parts of Turkey (Ercişli and Orhan, 2007; Güneş and Çekiç, 2004; Koyuncu, 2004b). *M. nigra* fruit has good source of several phytonutrients and contain high amounts of total phenolics, total flavonoids, and ascorbic acid (Ercişli and Orhan 2007; Güneş and Çekiç 2004; Koyuncu, 2004a). Also, the fruit has pleasant taste with slightly acidic flavor and attractive dark red color. It can be consumed fresh; however, it is very perishable and it is mostly used for making processed food such as pekmez, marmalades, juices, liquors, natural dyes, and frozen fruits for ice cream.

The objective of the present study was to evaluate *Morus* species from the diverse ecological regions of Turkey and to identify the accessions with superior horticultural characteristics.

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Materials and Methods

Several regions of Turkey were surveyed during the growing seasons of 2007 and 2008. In general, the mulberry trees were grown individually or grown from seed as a wild type. There were only few professional growers. More than 200 promising mulberry accessions were visited and preliminary tree and fruit characteristics were investigated during the summer of 2007. The same trees were revisited in 2008 and horticultural attributes of more than 50 accessions were determined. Based on the results from 2008, a total of 22 superior accessions were selected. Here, we report horticultural traits of the selected accessions only. The region, province as well as longitude, latitude, and altitude of each accession are presented in Table 1.

Approximately 0.5 kg fully matured fruits were randomly harvested from each accession. The fruits representing the typical characteristics of each accession were then transported to laboratory for analysis. The pomological analyses were conducted from ten replicates, each having ten fruits. Fruit weight was measured by using a digital balance with a sensitivity of 0.001 g. Linear dimensions, length and width of fruits were measured by using a digital caliper gauge with a sensitivity of 0.01 mm. Seed numbers were counted and to standardize the seeds, the numbers were divided by the fresh weight. Juice yield was determined by centrifugation of fruit slurry with 10,000 g and expressed as % juice yield. Fruit samples were used to determine total soluble solids content (SSC) by using a refractometer (model Pal-1, Atago, Tokyo, Japan) and titratable acidity (TA) using a standard methodology.

Mineral concentrations in ground samples were analyzed. For N values, the Kjeldahl distillation method (Lindsay and Norwell, 1978) was used. For determining P, K, Mg, Ca, Fe, Zn, Mn, and Cu, ground samples were burned using a mixture of H_2O_2 and HNO_3 in a closed system microwave oven (Milestone 1200 Mega, Rome, Italy). Element concentrations in filtrated samples were determined using inductively coupled plasma -atomic emission spectrometry (ICP-AES, Jobin Yuon JY 138 Ultrace, Horiba, Japan). Fe, Zn, Mn, Cu, N, P, K, Ca, and Mg

Table 1. Geographic coordinates and origins of accessions, species, codes, and fruit color of *Morus* accessions studied. The species consist of *M. nigra* (N), *M. rubra* (R), *M. alba* (A), and *M. laevigata* (P).

Accession	Province	Region	Longitude (N)	Latitude (E)	Altitude (m)
M. nigra (black mulberry)					
N9	Kahramanmaraş	Mediterranean	37° 34' 32"	36° 55' 08"	544
N21	Elazığ	Eastern Anatolia	38° 04' 36"	39° 12' 66"	1227
N22	Malatya	Eastern Anatolia	38° 20' 06"	38° 21' 07"	990
N24	Malatya	Eastern Anatolia	38° 24' 86"	38° 21' 91"	809
N35	Erzincan	Eastern Anatolia	39° 15' 79"	38° 29' 58"	890
N44	Tokat	Central Anatolia	40° 21' 13"	36° 31' 25"	571
N45	Tokat	Central Anatolia	40° 21' 17"	36° 31' 29"	606
N49	Tokat	Central Anatolia	40° 20' 80"	36° 30' 94"	589
N54	Tokat	Central Anatolia	40° 18' 57"	36° 30' 96"	691
N60	Tokat	Central Anatolia	40° 19' 43"	36° 32' 89"	606
N62	Amasya	Central Anatolia	40° 38' 82"	35° 50' 05"	385
N63	Amasya	Central Anatolia	40° 39' 01"	35° 48' 12"	399
N68	Amasya	Central Anatolia	40° 40' 13"	35° 50' 02"	421
N76	Giresun	Black Sea	40° 17' 12"	38° 26' 47"	1107
M. rubra (red mulberry)					
R2	Kahramanmaraş	Mediterranean	37° 34' 32"	36° 55' 08"	544
R6	Adana	Mediterranean	36° 58' 03"	35° 06' 43"	40
R7	Tokat	Central Anatolia	40° 32' 10"	36° 56' 23"	325
R8	Tokat	Central Anatolia	40° 23' 23"	36° 42' 15"	783
M. alba (purple-fruited mulberry)					
A1	Adana	Mediterranean	36° 58' 03"	35° 06' 43"	120
A2	Kahramanmaras	Mediterranean	37° 34' 54"	36° 55' 02"	544
A5	Tokat	Central Anatolia	40° 20' 39"	36° 32' 21"	626
M. alba var. laevigata (long-fruited mulberry)					
P1	Adana	Mediterranean	36° 52' 91"	35° 14' 73"	38

measurements were checked against certified reference samples obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). The measurements were made by three replications.

The statistical analyses were carried out using SAS (SAS Inst., 2005). For the descriptive statistics, TABULATE was employed. To reveal the overall pattern of variation among the accessions, pomological characteristics were subjected to principle component analysis (PCA) using the PRINCOMP procedure. In this analysis, the relationships were developed from a covariance matrix derived from standardized morphological fruit characteristics means and the output data sets consisted of eigenvalues, eigenvectors, and standardized principal component scores.

Results and Discussion

We determined several horticultural characteristics of *Morus* accessions from Turkey with emphasis on *M. nigra*. Considerable variations for both among and within the same species for most of the traits tested were found. The means and the standard deviations for fruit size traits, seed number, juice yield, dry matter contents, TA, and SSC are presented in Table 2. Coefficient of variation (CV), which is an indication of the variation among the accessions, ranged from 10 to 83% for fruit length and no. of seeds/g fresh weight. Fruit weight ranged from 3.3 (R2 and A1) to 8.2 g (A5) for an average of 4.9 g. Fruit width and length were less variable when compared to the fruit weight, although P1 had longer fruits. No. of seeds /g fresh weight is an indication of the seediness and smaller

Table 2. Several characteristics of mulberry fruits sampled from Turkey. The species consist of *M. nigra* (N), *M. rubra* (R), *M. alba* (A), and *M. laevigata* (P). Values represent means ± standard deviations and were calculated from ten replicates.

	Fruit			No. of seeds/	Juice	Dry matter		SSC
Accession	weight (g)	width (mm)	length (mm)	g fresh wt	content (%)	content (%)	TA (%)	(%)
M. nigra (black mulberry)								
N9	3.9 ± 0.8	17.7 ± 2.5	22.7 ± 4.0	3.0 ± 2.4	60.1 ± 0.2	20.8 ± 0.2	2.11 ± 0.01	21.6 ± 0.0
N21	4.5 ± 0.8	17.8 ± 3.5	23.8 ± 4.0	4.1 ± 2.8	62.2 ± 1.0	18.0 ± 0.2	2.04 ± 0.02	20.6 ± 0.1
N22	5.1 ± 1.0	19.2 ± 5.4	27.8 ± 4.5	3.3 ± 2.7	56.1 ± 1.0	20.7 ± 0.2	2.25 ± 0.02	23.9 ± 0.1
N24	5.4 ± 0.8	18.7 ± 4.0	26.1 ± 4.2	2.8 ± 1.5	62.8 ± 1.0	21.0 ± 0.1	2.41 ± 0.00	22.8 ± 0.1
N35	4.2 ± 0.9	19.2 ± 4.1	24.1 ± 3.1	3.6 ± 4.7	64.4 ± 1.0	18.9 ± 0.2	2.88 ± 0.01	20.5 ± 0.1
N44	4.7 ± 0.9	18.5 ± 4.1	26.0 ± 4.4	4.0 ± 2.0	63.9 ± 1.0	19.1 ± 0.3	1.83 ± 0.11	21.4 ± 0.2
N45	5.3 ± 0.8	17.9 ± 4.3	27.3 ± 7.0	3.9 ± 3.6	65.6 ± 1.0	17.2 ± 0.8	2.24 ± 0.04	19.8 ± 0.2
N49	4.7 ± 1.0	18.0 ± 4.9	25.9 ± 5.1	3.0 ± 4.9	68.9 ± 1.9	19.0 ± 0.4	1.59 ± 0.00	20.9 ± 0.1
N54	4.6 ± 0.5	18.3 ± 3.8	25.4 ± 4.4	2.7 ± 3.8	64.4 ± 1.0	16.6 ± 0.4	1.74 ± 0.00	18.5 ± 0.7
N60	6.8 ± 1.0	19.8 ± 5.3	30.7 ± 6.6	3.3 ± 4.1	67.8 ± 1.9	16.7 ± 0.1	2.13 ± 0.01	18.0 ± 0.6
N62	5.2 ± 1.5	19.4 ± 4.3	25.3 ± 4.4	4.1 ± 3.3	57.2 ± 1.0	19.4 ± 0.5	2.04 ± 0.01	21.9 ± 0.1
N63	4.8 ± 1.3	17.9 ± 5.0	26.9 ± 5.6	4.1 ± 3.4	59.4 ± 1.0	20.1 ± 0.1	1.62 ± 0.11	23.1 ± 0.1
N68	3.6 ± 0.9	18.1 ± 3.7	25.7 ± 6.2	5.7 ± 2.0	67.2 ± 1.0	18.3 ± 0.2	2.10 ± 0.03	20.0 ± 0.2
N76	3.4 ± 0.3	16.7 ± 4.0	23.6 ± 4.0	4.0 ± 3.6	65.6 ± 1.0	18.1 ± 0.2	2.10 ± 0.03	21.3 ± 0.2
M. rubra (red mulberry)								
R2	3.3 ± 0.7	16.8 ± 4.4	24.3 ± 3.9	15.5 ± 6.9	20.0 ± 0.8	13.3 ± 0.1	1.04 ± 0.03	31.9 ± 0.1
R6	5.8 ± 0.7	18.9 ± 6.2	30.4 ± 6.4	13.9 ± 8.4	29.7 ± 0.5	7.5 ± 0.1	0.36 ± 0.00	20.3 ± 0.2
R7	4.5 ± 1.5	17.0 ± 5.5	27.8 ± 7.3	21.6 ± 7.6	31.1 ± 1.0	11.1 ± 0.0	0.70 ± 0.02	27.1 ± 0.3
R8	3.4 ± 0.6	17.4 ± 4.7	25.0 ± 5.2	18.9 ± 5.1	27.2 ± 1.0	9.7 ± 0.1	1.01 ± 0.02	24.7 ± 1.1
M. alba (purple-fruited mulberry)								
A1	3.3 ± 0.6	16.1 ± 5.3	25.3 ± 4.8	21.4 ± 3.2	35.0 ± 0.6	32.9 ± 0.1	0.75 ± 0.00	24.3 ± 0.2
A2	4.2 ± 1.0	19.2 ± 6.4	26.2 ± 5.6	11.7 ± 4.8	40.0 ± 0.8	24.6 ± 0.2	0.60 ± 0.00	21.6 ± 0.4
A5	8.2 ± 1.2	21.1 ± 7.7	35.0 ± 7.6	10.7 ± 2.1	25.6 ± 0.5	21.7 ± 0.1	0.53 ± 0.00	24.6 ± 0.3
M. alba var. laevigata (long-fruited mulberry)								
P1	6.9 ± 0.6	16.5 ± 12.4	41.6 ± 12.4	5.4 ± 1.8	71.1 ± 1.0	4.9 ± 0.2	1.26 ± 0.01	14.1 ± 0.2
Mean	4.9	22.4	23.0	7.8	56.3	16.6	1.7	21.8
St. Dev.	1.3	2.5	2.3	6.4	15.3	4.8	0.7	3.6
CV (%)	26	11	10	83	27	29	40	17

Table 3. Coefficients and eigenvalues for the first three principle components (PC) of PCA for mulberries (*Morus* spp.) sampled from Turkey.

Variable	PC1	PC2	PC3
Fruit weight	0.22	0.55	0.24
Fruit width	0.20	0.24	0.68
Fruit length	0.09	0.60	-0.15
No. of seeds/g fresh weight	-0.52	0.11	-0.07
Juice content	0.51	-0.17	-0.20
Dry matter content	0.01	-0.32	0.57
TA	0.42	-0.34	0.01
SSC	-0.44	-0.16	0.28
Eigenvalue	3.22	2.37	1.29
Proportion	0.40	0.30	0.16

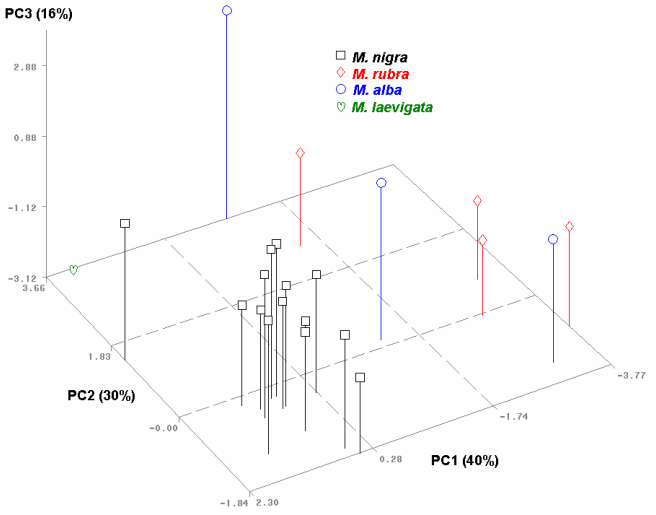

Fig. 1. PCA plot of the first three PCs depicting relationships among mulberry (*Morus* spp.) accessions sampled from Turkey.

values are desirable. In general, *M. nigra* accessions had much smaller mean values (mean = 3.7) compared to *M. rubra* (mean = 17.5) and *M. alba* (mean = 14.6). Juice yield showed a converse pattern. Juice content ranges were 56.1 to 67.8%, 20.0 to 31.1% and 25.6 to 35.0% for *M. nigra*, *M. rubra* and *M. alba* accessions, respectively. The lowest and greatest dry matter contents were recovered from R6 (7.5 %) and A1 (32.9%). *M. nigra* accessions had the highest TA at 2.7%, 3.1-fold higher than those of *M. rubra* and *M. alba* accessions. SSC ranged from 14.1 (P1) to 27.1% (R6) for an average of 21.8%.

When our pomological results were compared to previous studies, differences were detected for some of the traits; however, this is not surprising when possible genotypic and/or environment effects are considered. For example, Polat (2004) found fruit weight, SSC, and TA of four accessions of *M. alba* in the ranges of 1.13-4.25 g, 13.73-16.01%, and 0.06-1.00%, respectively. Although fruit weight and TA were comparable to our results, our *M. alba* accession had much higher SSC (21.6 to 24.6%). Ranges of the same traits by Gungor and Sengul (2008) were also comparable to those of our accessions. The ranges of *M. nigra* accessions of Koyuncu (2004a) for fruit weight, fruit diameter, fruit length, SSC, and TA were 3.11-4.49 g, 15.34-16.29 mm, 22.33-25.15 mm, 13.91-18.36%, and 1.24-1.64%, respectively, comparable to those of our ranges for *M. nigra* accessions.

All trait means from Table 2 were subjected to PCA. The results indicated that the first three components showed 40, 30, and 16 % of the phenotypic variations, for a total of 88% (Table 3). The important traits highly correlating with PC1 were number of seeds and SSC having negative correlations, and TA and juice yield having positive correlations. Fruit length and weight had high positive correlations with PC2 while TA and dry matter content had important negative correlations. Dry matter content and fruit width were important for PC3.

The accessions were plotted on three dimensions based on their PCA results (Fig. 1). Overall, *M. nigra* accessions were grouped together. The analysis also indicated that *M. laevigata* accession was distinct from other genotypes. The accessions of *M. rubra* and *M. alba* separated from *M. nigra* and *M. laevigata* accessions; however, the two groups did not separate from each other. Kafkas et al. (2008) studied the molecular relationship of a similar population by amplified fragment length polymorphism markers. In their study, they used groups of *M. nigra*, *M. rubra*, and white and dark-colored *M. alba* accessions. Their results indicated that *M. nigra* accessions formed a group by themselves and showed little genetic variation. Rest of the accessions, however, did not create distinct groups. In other words, those accessions did not group based on their species. Therefore, present results revealed that in general, morphological and molecular diversity patterns were similar among the accessions selected from Turkey. A variation was recovered among the *M. nigra* accessions, although they were found to be closely related molecularly. This variation may be attributed to differences in the environments.

We also determined several macro- and microelement concentrations of a smaller group with 13 accessions (8 *M. nigra*, 3 *M. rubra*, 1 *M. alba*, and 1 *M. laevigate* accessions) (Table 4). Our results revealed that the nutrient concentrations varied considerably as CV values were higher than 30% except for Mg (12%) and Si (27%) concentrations. The greatest N, P, and K concentrations were recovered from N9 (0.34%, 744 mg·kg^{-1}, and 3,744 mg·kg^{-1}, respectively), while the lowest concentrations were measured from P1 (0.10 %, 180 g·kg^{-1}, and 1,118 g·kg^{-1}, respectively). Ca concentrations ranged from 183 g·kg^{-1} (R6) to 976 mg·kg^{-1} (R2), while a narrower range was recovered for Mg (281 to 436 mg·kg^{-1}) and for Si (54 to 128 mg·kg^{-1}). Fe concentration ranged from 4.3 to 27.9 mg·kg^{-1} for an average of 13.8 mg·kg^{-1}. Zn, Cu, and Mn concentrations were detected in smaller amounts.

Table 4. Several macro- and microelement concentrations of mulberry fruits sampled from Turkey. The species consist of *M. nigra* (N), *M. rubra* (R), *M. alba* (A), and *M. laevigata* (P). Values represent means ± standard deviations and were calculated from three replicates. The concentrations were expressed in $mg \cdot kg^{-1}$ except for N.

Accession	N (%)	P	K	Ca	Mg	Si	Fe	Zn	Cu	Mn
M. nigra (black mulberry)										
N24	0.24 ± 0.01	416 ± 19	3342 ± 50	617 ± 32	365 ± 10	81 ± 0	7.6 ± 0.5	2.3 ± 0.3	0.7 ± 0.1	1.0 ± 0.0
N35	0.24 ± 0.01	469 ± 1	3425 ± 30	411 ± 15	325 ± 7	62 ± 13	7.6 ± 0.8	2.0 ± 0.1	0.7 ± 0.3	0.8 ± 0.1
N45	0.24 ± 0.02	403 ± 25	2993 ± 29	448 ± 29	335 ± 9	85 ± 27	14.6 ± 2.8	2.7 ± 0.2	1.0 ± 0.0	1.5 ± 0.0
N49	0.16 ± 0.01	429 ± 6	2543 ± 22	348 ± 15	323 ± 9	68 ± 17	11.4 ± 0.4	1.7 ± 0.1	0.5 ± 0.0	1.8 ± 0.1
N60	0.11 ± 0.01	538 ± 18	3165 ± 32	407 ± 14	329 ± 15	128 ± 18	27.9 ± 3.7	1.2 ± 0.1	0.7 ± 0.0	1.7 ± 0.1
N62	0.24 ± 0.01	461 ± 5	3402 ± 32	471 ± 41	383 ± 16	114 ± 15	11.4 ± 1.4	2.2 ± 0.4	0.8 ± 0.0	1.6 ± 0.1
N68	0.23 ± 0.01	401 ± 11	3299 ± 73	510 ± 7	311 ± 34	95 ± 12	17.0 ± 0.6	2.7 ± 1.6	0.9 ± 0.1	1.3 ± 0.1
N9	0.34 ± 0.01	744 ± 370	3744 ± 82	634 ± 26	379 ± 28	96 ± 15	27.7 ± 3.0	3.2 ± 0.4	0.8 ± 0.0	1.8 ± 0.0
M. rubra (red mulberry)										
R2	0.21 ± 0.01	296 ± 3	2096 ± 30	976 ± 76	386 ± 4	101 ± 15	15.7 ± 0.9	2.3 ± 0.1	0.5 ± 0.0	1.5 ± 0.1
R6	0.10 ± 0.01	182 ± 1	1222 ± 11	183 ± 11	281 ± 7	54 ± 2	4.3 ± 0.2	1.4 ± 0.0	0.1 ± 0.0	0.4 ± 0.1
R7	0.13 ± 0.01	208 ± 3	1822 ± 24	325 ± 34	339 ± 4	89 ± 15	5.8 ± 0.3	1.6 ± 0.1	0.3 ± 0.1	0.9 ± 0.0
M. alba (purple-fruited mulberry)										
A5	0.32 ± 0.02	422 ± 10	2968 ± 44	778 ± 82	436 ± 26	97 ± 13	21.0 ± 1.9	3.8 ± 0.3	0.9 ± 0.0	2.2 ± 0.1
M. alba var. laevigata (long-fruited mulberry)										
P1	0.10 ± 0.01	180 ± 1	1118 ± 18	490 ± 2	313 ± 16	74 ± 0.7	7.1 ± 0.2	1.7 ± 0.7	0.2 ± 0.0	1.1 ±0 .1
Mean	0.20	396	2703	508	347	88	13.8	2.2	0.6	1.4
St. Dev.	0.08	174	848	203	42	24	7.8	0.8	0.3	0.5
CV (%)	37	44	31	40	12	27	57	37	46	36

In conclusion, we compared and characterized the morphological variation among the selected mulberry species and accessions of Turkey. As the germplasm pool of these species is essentially unexploited, the use of wild germplasm may offer an opportunity to encorporate a greater array of superior horticultural or processing traits into new cultivars.

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