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Use of Leaf and Fruit Morphometric Analysis to Identify and Classify White Mulberry (*Morus alba* L.) Genotypes

Riccardo Lo Bianco * and Fabio Mirabella

Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, 90128 Palermo, Italy; fabmirabella@gmail.com

* Correspondence: riccardo.lobianco@unipa.it; Tel.: +39-091-238-96097

Received: 15 September 2018; Accepted: 3 October 2018; Published: 7 October 2018

Abstract: Digital image analysis and multivariate data analysis were used in this study to identify a set of leaf and fruit morphometric traits to discriminate white mulberry (*Morus alba* L.) cultivars. The trial was conducted using three- to five-year-old potted cuttings of several white mulberry cultivars. 32 leaf morphometric descriptors were recorded in 2011 and 2012 from 11 mulberry cultivars using image analysis of scanned leaves, whereas six fruit descriptors were recorded in 2011 from nine mulberry cultivars. Linear discriminant analysis (LDA) was used to identify a subset of measured variables that could discriminate the cultivars in trial. Biplot analysis, followed by cluster analysis, was performed on the discriminant variables to investigate any possible cultivar grouping based on similar morphometric traits. LDA was able to discriminate the 11 cultivars with a canonical function, which included 13 leaf descriptors. Using those 13 descriptors, the Biplot showed that over 84% of the variability could be explained by the first three factors. Clustering of standardized biplot coordinates recognized three groups: the first including 'Korinne' and 'Miura' with similar leaf angles and apical tooth size; the second including 'Cattaneo', 'Florio', 'Kokusò-21', 'Kokusò-27', and 'Kokusò Rosso' with similar leaf size and shape; and the third including 'Ichinose', 'Kayrio', 'Morettiana', and 'Restelli', with similar leaf margin. Fruit descriptors were fewer and measured on fewer cultivars, yielding smaller discriminatory power than leaf descriptors. Use of leaf morphometric descriptors, along with image and multivariate analysis, proved to be effective for discriminating mulberry cultivars and showed promise for the implementation of a simple and inexpensive characterization and classification tool.

Keywords: biplot; descriptor; digital image analysis; linear discriminant analysis; *Morus alba*; multivariate analysis

1. Introduction

Mulberry (*Morus* spp. L.) is a fast-growing, deciduous, woody perennial tree, which belongs to the Moraceae family and adapts to a variety of soils and climatic regions, spreading all across the Seric belt from the 50th parallel north to the 35th parallel south. Mulberry has been cultivated since the ancient times, and has tremendous economic importance today—especially in Asia—in regard to its use as feed to silk worms, as animal fodder, and also in regard to its fruit production.

Mulberry species tend to hybridize easily, which has led to its considerable genetic variability. Currently, there are more than 68 species of the more widely recognized mulberry [1], of which only a few (e.g., *Morus alba* L., *Morus indica* L., *Morus bombycis* Koidz., *Morus latifolia* L., *Morus multicaulis* Perr., and *Morus nigra* L.) are cultivated for either fruit or leaf production. Cytologically, mulberry exhibits different ploidy levels, ranging from $2n = 28$ all the way up to $22n = 308$ [2]. The frequent hybridization of mulberry species and cultivars has often generated confusion and difficulty with identification, and mulberry cultivars have been mistakenly classified as a subspecies of *Morus alba* or *Morus nigra*. New forms of improved and stress-tolerant mulberry cultivars are being strongly requested for the development of a modern sericulture with efficient silk productions [3]. For this reason, thousands of mulberry germplasm accessions have been collected in several countries, and because of the considerable phenotypic and genotypic variability among the accessions, huge effort is continuously needed to characterize and evaluate accessions for their cultivation and production potential [4].

Studies based on leaf morphology [5,6] and molecular biology [7,8] have been conducted to characterize mulberry cultivars; nevertheless, leaf morphology may be inadequate for discriminating cultivars with a similar genetic base, whereas molecular techniques may prove expensive and, in some cases, be difficult to access. Furthermore, limited progress has been made in developing linkage maps or obtaining haploids [9], and significant efforts are still needed to implement functional genomics for the development of improved cultivars.

In grapes, the description of plant traits along with morphometric techniques resulted in the development of ampelography (the science concerned with the identification and classification of grapevines), which is very useful and was the sole instrument for the identification and classification of grape cultivars before the development of molecular techniques. Ampelographic studies using OIV (Officine International de la Vigne et du Vin), UPOV (Union Internationale pour la Protection des Obtentions Végétales), and UPGRI (International Plant Genetic Resources Institute) official descriptors supplied insights into plant material identity [10]. Even today, ampelography continues to be recognized as a relatively simple and inexpensive method for the identification of grapevines [11], and an ampelographic description is required for subscribing new cultivars into the register of commercial cultivars [12,13]. In the last two decades, biometry of measurable plant traits has been introduced to make ampelography a more objective and reliable technique [14,15]. Studies on grapevines and olives have tested and validated the reliability of morphometric analysis, indicating the possibility of discriminating cultivars and even groups of clones at the subvarietal level [16,17]. In addition to grapevines and olives, digital morphometric systems using different plant organs have also been successfully developed for cultivar identification and classification in wheat [18], rice [19], wild pear [20], and hazelnut [21].

Despite their good discriminatory potential, fruit descriptors have only been successfully used in a few cases compared to leaves, probably because of their relatively short presence on the plant. For example, fruit descriptors have been used to characterize cultivars in fig (*Ficus carica* L.) [22], olive (*Olea europaea* L.) [23], sweet cherry (*Prunus avium* L.) [24], and *Sorbus domestica* L. [25]. In our experience, white mulberry fruits may vary greatly in color, size, and shape among cultivars, suggesting good discriminatory potential. For this reason, we decided to include fruit descriptors in our trial.

The objective of this study was to find a set of leaf and fruit morphometric traits for the purposes of discriminating between white mulberry cultivars. The same set of descriptors was also used to evaluate cultivar grouping by similar leaf and fruit traits. Digital image analysis and multivariate data analysis were used to pursue these objectives. Similar multivariate procedures have been successfully used to discriminate and classify apple, apricot, and olive genotypes by leaf morphometric traits, fruit sensory attributes, or primary and secondary fruit metabolites [23,26,27].

2. Materials and Methods

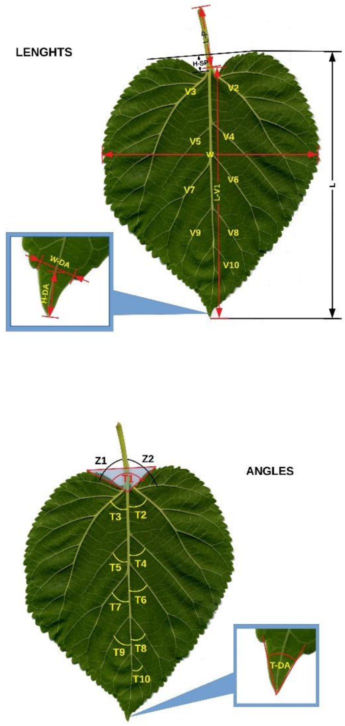
The morphometric analysis was conducted on leaf and fruit samples from white mulberry trees present in a private collection located near Giardinello (38°5′24″ N, 13°9′36″ E), Sicily, Italy. The trial was conducted in 2011 and 2012 using three- to five-year-old rooted cuttings of several white mulberry cultivars. All trees were grown outdoors in 30 L pots filled with 50% of mineral soil and 50% of organic mix (peat moss and pine bark), and were regularly fertilized and irrigated. Growing seasons in 2011 and 2012 had similar temperature trends.

2.1. Leaf Morphometric Determinations

Leaf morphometric determinations were carried out in 2011 and 2012 on 11 mulberry cultivars: Cattaneo, Florio, Ichinose, Kayrio, Kokusò-21, Kokusò-27, Kokusò Rosso, Korinne, Miura, Morettiana, and Restelli. Leaf descriptors were selected and measured on four mature leaves from each of four plants per cultivar, for a total of 16 replicates per cultivar and year. Leaves were sampled in late spring and early summer from the middle portion of four different extension shoots per plant. An image of each leaf was acquired using a digital scanner, along with a metric reference for subsequent measurement calibrations. Digital images were used to measure 29 different leaf descriptors (Table 1) using Image Tool 3.0 software (UTHSCSA, University of Texas, Austin, TX, USA) procedures. In addition, leaf blade width/length ratio, apical tooth width/height ratio, and an index of leaf margin serration were determined. Leaf margin was analyzed using an algorithm developed on MATLAB software (The MathWorks Inc., Natick, MA, USA), where firstly, the leaf margin was detected using the EDGEDETECTION function; next, the BOUNDARY/REGIONPROPS functions were used to determine the regularity of the margin by calculating a ratio between the smoothed perimeter (smoothed outline) and the real perimeter; and finally, the margin serration index was defined as a boundary index. The index ranged from 0 (highly serrated margin) to 1 (smooth margin).

Table 1. White mulberry leaf measurements used for morphometric analysis.

Leaf Trait	Abbreviation
Blade length	L
Blade width	W
Petiole length	L-P
Petiole sinus depth	H-SP
Length of main vein	L-V1
Length of vein 2 (left)	L-V2
Length of vein 3	L-V3
Length of vein 4	L-V4
Length of vein 5	L-V5
Length of vein 6	L-V6
Length of vein 7	L-V7
Length of vein 8	L-V8
Length of vein 9	L-V9
Length of vein 10	L-V10
Height of apical tooth	H-DA
Basal width of apical tooth	W-DA
Petiole sinus angle (concave up)	T1
Inner angle V2 (left)	T2
Inner angle V3	T3
Inner angle V4	T4
Inner angle V5	T5
Inner angle V6	T6
Inner angle V7	T7
Inner angle V8	T8
Inner angle V9	T9
Inner angle V10	T10
Apical tooth angle	T-DA
Petiole vein angle (3rd order, left)	Z1
Petiole vein angle (3rd order, right)	Z2
Ratio between blade width and length	W/L
Ratio between apical tooth basal width and height	W-DA/H-DA
Margin index (serrated = 0, smooth = 1)	BOUNDARY



2.2. Fruit Morphometric Determinations

Due to an insufficient amount of fruits, morphometric determinations were carried out only in 2011 on nine mulberry cultivars: Kokusò-21, Kokusò Rosso, Spagna Rosso, Cattaneo, Florio, Giazzola, Kayrio, Korinne, and Miura. Fruit descriptors were selected and measured on 10 mature fruits from each of four plants per cultivar (a total of 40 fruits per cultivar). Six different parameters were selected as fruit descriptors: height (H), width (W), width/height ratio (W/H), length of peduncle (L-ped), weight, and total soluble solids (TSS). Digital images of each fruit were acquired with a digital camera, along with a metric reference for subsequent measurement calibrations. Image Tool 3.0 software was used to record lengths and widths. Fruits were weighed using a Vibra SJ 620 precision scale (Shinko Denshi Co., Ltd., Tokyo, Japan), and a few drops of juice were used for soluble solid determinations, using an Atago Palette PR-32 digital refractometer (Atago Co. Ltd., Tokyo, Japan).

2.3. Data Analysis

Data of leaf and fruit descriptors were analyzed using Systat multivariate procedures (Systat Software Inc., Richmond, CA, USA). Specifically, linear discriminant analysis (LDA) with backward stepwise selection of variables was performed using all leaf or fruit replicates per cultivar to verify if there was a subset of measured variables that could significantly discriminate the cultivars in trial. Using the subset of variables identified with LDA, a biplot analysis (a distance-based class of principal component analysis) using the multidimensional scaling preferences procedure (MDPREF) [14] was performed to investigate the relationship between leaf or fruit descriptors and any possible cultivar groupings, based on similar properties. Average data per cultivar and year were standardized, and factor and object loadings were fit in a common space with the MDPREF procedure [14]. Finally, cluster analysis using the *k*-means technique was performed on standardized factor coordinates to individuate the grouping of descriptors and cultivars.

3. Results and Discussion

A fair amount of variation across cultivars was detected in all measured leaf and fruit descriptors (Tables 2 and 3), which proved the initial selection of descriptors to be effective for mulberry cultivar identification purposes. Leaf- and fruit-size-related descriptors were among the most variable parameters, with a coefficient of variation (c.v.) ranging from 9% to 49%. Even those descriptors that were not related to leaf size, such as T1, T2, T-DA, W/L, and boundary index, showed a significant amount of variation (c.v. 5%–26%) across cultivars (Table 2). Similarly, descriptors that were not related to fruit size, such as length of peduncle, TSS, and W/H, exhibited a significant amount of variation (c.v. 16%–30%) across cultivars despite the reduced number of cultivars tested in a single year (Table 3).

Using leaf descriptors, LDA was able to fully separate (Wilk's Lambda statistics, $p < 0.001$) the 11 cultivars with a canonical discriminant function after a backward step analysis, which included L, W, L-P, H-SP, L-V2, L-V3, H-DA, T3, T5, T6, T9, W/L, and BOUNDARY. Using these 13 leaf descriptors selected by LDA, the biplot analysis showed that over 84% of the variability observed was explained by the first three factors, and about 73% was explained by the first two factors (Figure 1). *k*-means clustering of standardized biplot coordinates from all three factors separated the leaf descriptors into three groups (different symbol shapes in the biplot): the first associating vein angles and H-DA; the second associating blade size, vein lengths, L-P, H-SP, and W/L; and the third including BOUNDARY alone (Figure 1). Based on this clustering, some expected relationships were shown by the biplot analysis, such as various vein angles in cluster 1 and leaf-size-related descriptors in cluster 2. Leaf shape was associated with leaf-size-related descriptors rather than with vein angles, whereas H-DA was associated with vein angles. Cluster 1 included 'Korinne' and 'Miura', grouped by similar leaf angles and H-DA; cluster 2 included 'Cattaneo', 'Florio', 'Kokusò-21', 'Kokusò-27' and 'Kokusò Rosso', grouped by similar leaf size and shape; and cluster 3 included 'Ichinose', 'Kayrio', 'Morettiana', and 'Restelli', grouped by similar leaf margin type (Figure 1).

Table 2. Leaf descriptors from white mulberry cultivars (averages of 2011 and 2012). Lengths are in cm, angles in degrees, and the coefficient of variation (c.v.) is expressed in %. Means ± standard errors.

Leaf Descriptors	Cattaneo	Florio	Ichinose	Kayrio	Kokusò-21	Kokusò-27	Kokusò-R	Korinne	Miura	Morettiana	Restelli	C.V.
L	18.0 ± 0.64	15.4 ± 0.57	8.10 ± 0.38	14.4 ± 0.39	19.4 ± 0.77	16.9 ± 0.69	16.7 ± 0.95	15.6 ± 0.37	19.0 ± 0.69	13.8 ± 0.54	15.5 ± 0.54	19.7
W	11.0 ± 0.32	11.8 ± 0.43	4.81 ± 0.19	9.40 ± 0.19	13.4 ± 0.56	11.6 ± 0.51	10.8 ± 0.36	10.2 ± 0.29	12.6 ± 0.77	10.3 ± 0.50	10.3 ± 0.35	21.1
L-P	4.87 ± 0.25	4.57 ± 0.18	1.82 ± 0.11	3.37 ± 0.10	4.35 ± 0.15	3.13 ± 0.23	4.41 ± 0.32	3.10 ± 0.08	4.17 ± 0.21	4.07 ± 0.15	3.06 ± 0.10	24.3
H-SP	1.64 ± 0.10	1.33 ± 0.09	0.35 ± 0.04	0.77 ± 0.06	1.46 ± 0.09	1.07 ± 0.05	0.97 ± 0.06	0.46 ± 0.04	0.59 ± 0.10	0.82 ± 0.09	0.43 ± 0.07	48.8
L-V1	16.7 ± 0.67	14.3 ± 0.52	7.73 ± 0.36	13.6 ± 0.40	17.6 ± 0.78	15.9 ± 0.69	15.9 ± 0.95	15.2 ± 0.37	18.1 ± 0.60	13.1 ± 0.49	15.3 ± 0.53	19.0
L-V2	10.8 ± 0.34	9.04 ± 0.36	4.41 ± 0.32	7.61 ± 0.21	10.3 ± 0.52	9.17 ± 0.44	9.49 ± 0.44	7.91 ± 0.23	10.1 ± 0.52	7.96 ± 0.38	9.12 ± 0.30	20.2
L-V3	10.4 ± 0.45	8.67 ± 0.29	4.14 ± 0.28	7.78 ± 0.25	10.2 ± 0.44	8.88 ± 0.43	9.38 ± 0.42	8.18 ± 0.28	9.21 ± 0.47	7.87 ± 0.31	9.30 ± 0.37	19.8
L-V4	8.24 ± 0.19	6.93 ± 0.26	3.22 ± 0.25	6.97 ± 0.16	8.55 ± 0.36	6.21 ± 0.37	7.08 ± 0.31	6.89 ± 0.36	8.04 ± 0.57	6.05 ± 0.33	7.00 ± 0.23	21.0
L-V5	7.34 ± 0.29	6.59 ± 0.28	3.22 ± 0.22	7.02 ± 0.17	8.58 ± 0.36	6.84 ± 0.37	6.68 ± 0.31	6.44 ± 0.25	7.66 ± 0.41	6.14 ± 0.28	6.73 ± 0.20	19.9
L-V6	6.10 ± 0.18	5.66 ± 0.22	2.50 ± 0.24	5.52 ± 0.18	7.53 ± 0.37	6.19 ± 0.31	5.75 ± 0.44	5.58 ± 0.28	6.85 ± 0.38	5.23 ± 0.22	5.61 ± 0.22	22.0
L-V7	5.17 ± 0.23	5.07 ± 0.23	2.01 ± 0.29	4.84 ± 0.23	6.46 ± 0.33	5.57 ± 0.32	4.96 ± 0.31	5.47 ± 0.28	6.36 ± 0.23	4.63 ± 0.20	4.95 ± 0.21	23.2
L-V8	3.96 ± 0.17	4.16 ± 0.17	1.88 ± 0.20	3.57 ± 0.19	5.26 ± 0.28	4.54 ± 0.29	3.92 ± 0.34	4.59 ± 0.23	5.11 ± 0.25	3.98 ± 0.15	4.02 ± 0.25	21.9
L-V9	3.21 ± 0.21	3.31 ± 0.18	1.48 ± 0.20	2.87 ± 0.23	4.25 ± 0.23	3.45 ± 0.23	3.00 ± 0.21	4.39 ± 0.18	4.63 ± 0.25	3.19 ± 0.24	3.13 ± 0.19	25.8
L-V10	2.28 ± 0.15	2.56 ± 0.17	0.95 ± 0.18	2.10 ± 0.11	3.39 ± 0.19	2.82 ± 0.21	2.36 ± 0.28	3.48 ± 0.20	3.60 ± 0.22	2.41 ± 0.15	2.21 ± 0.17	29.6
H-DA	1.24 ± 0.12	0.80 ± 0.05	0.74 ± 0.06	1.17 ± 0.09	1.24 ± 0.08	1.12 ± 0.05	1.46 ± 0.11	1.61 ± 0.09	1.65 ± 0.12	1.04 ± 0.05	0.92 ± 0.06	25.7
W-DA	0.92 ± 0.06	0.64 ± 0.04	0.44 ± 0.05	0.67 ± 0.04	0.75 ± 0.04	0.59 ± 0.03	0.76 ± 0.02	0.82 ± 0.04	0.88 ± 0.05	0.67 ± 0.03	0.62 ± 0.04	19.7
T1	117 ± 5.19	115 ± 3.65	150 ± 7.65	139 ± 3.97	115 ± 3.06	121 ± 3.09	125 ± 2.52	158 ± 1.67	97 ± 10.7	134 ± 4.27	135 ± 12.5	13.7
T2	40.1 ± 1.24	37.3 ± 1.30	41.5 ± 3.59	43.1 ± 1.61	48.7 ± 2.36	41.3 ± 1.94	36.3 ± 1.80	55.7 ± 2.02	46.8 ± 1.06	32.9 ± 1.29	45.0 ± 1.69	14.9
T3	42.6 ± 1.47	34.5 ± 1.35	44.3 ± 2.55	42.9 ± 1.70	41.8 ± 1.24	42.3 ± 1.82	35.5 ± 1.69	52.4 ± 1.95	47.5 ± 1.45	30.8 ± 1.44	46.4 ± 1.82	14.9
T4	40.7 ± 1.81	44.7 ± 1.24	44.0 ± 1.64	37.3 ± 1.26	44.9 ± 1.39	44.2 ± 1.15	32.0 ± 1.67	52.4 ± 1.88	50.1 ± 1.09	38.8 ± 1.62	38.2 ± 0.96	13.8
T5	42.7 ± 1.75	44.6 ± 1.31	45.5 ± 1.60	35.6 ± 0.97	39.5 ± 1.26	40.5 ± 1.36	30.4 ± 1.26	53.3 ± 1.24	49.5 ± 1.06	38.9 ± 1.09	38.2 ± 1.27	15.4
T6	40.4 ± 0.75	42.8 ± 1.09	41.6 ± 2.27	37.1 ± 1.24	42.3 ± 1.57	39.1 ± 1.20	31.9 ± 1.88	52.6 ± 1.36	48.7 ± 1.18	37.0 ± 1.02	37.2 ± 1.23	14.0
T7	42.2 ± 1.01	43.0 ± 1.41	40.6 ± 2.58	36.7 ± 1.39	40.1 ± 1.13	40.8 ± 1.26	35.2 ± 1.44	49.1 ± 1.72	49.2 ± 1.07	38.3 ± 1.10	38.0 ± 1.09	11.0
T8	43.3 ± 1.91	43.8 ± 1.12	42.6 ± 2.77	38.0 ± 1.49	42.5 ± 1.99	43.5 ± 1.27	35.8 ± 1.68	48.6 ± 1.34	51.0 ± 1.55	40.4 ± 2.05	37.5 ± 1.03	10.7
T9	43.4 ± 1.39	45.0 ± 1.23	43.6 ± 2.13	38.0 ± 1.80	42.0 ± 1.12	45.1 ± 1.36	36.8 ± 1.73	48.9 ± 1.21	49.3 ± 1.32	37.7 ± 0.82	38.6 ± 0.97	10.3
T10	45.6 ± 2.46	43.5 ± 1.21	40.5 ± 2.98	36.9 ± 1.08	43.0 ± 1.50	43.1 ± 1.41	39.0 ± 2.02	49.8 ± 1.15	49.0 ± 1.28	41.1 ± 1.65	41.6 ± 1.35	9.11
T-DA	52.0 ± 4.13	50.1 ± 3.80	33.1 ± 3.63	31.3 ± 1.93	37.9 ± 4.01	27.8 ± 2.32	38.7 ± 2.48	23.0 ± 0.88	24.9 ± 2.22	34.4 ± 3.46	42.1 ± 2.83	26.3
Z1	116 ± 3.17	128 ± 2.29	129 ± 3.89	125 ± 2.57	117 ± 1.68	124 ± 1.64	126 ± 3.15	128 ± 2.48	121 ± 5.12	128 ± 3.67	109 ± 2.07	5.29
Z2	120 ± 2.14	126 ± 1.69	129 ± 3.94	132 ±1.36	121 ± 1.50	123 ± 1.32	130 ± 2.44	129 ± 2.61	119 ± 4.54	132 ± 2.85	110 ± 3.04	5.38
W/L	0.62 ± 0.01	0.77 ± 0.01	0.60 ± 0.01	0.66 ± 0.02	0.69 ± 0.01	0.69 ± 0.01	0.66 ± 0.02	0.66 ± 0.01	0.65 ± 0.02	0.75 ± 0.01	0.67 ± 0.01	7.47
W-DA/H-DA	0.88 ± 0.09	0.88 ± 0.07	0.59 ± 0.05	0.59 ± 0.04	0.73 ± 0.09	0.57 ± 0.05	0.55 ± 0.03	0.52 ± 0.02	0.57 ± 0.04	0.67 ± 0.04	0.72 ± 0.05	19.5
BOUNDARY	0.90 ± 0.01	0.87 ± 0.01	0.94 ± 0.02	0.91 ± 0.01	0.85 ± 0.01	0.78 ± 0.01	0.88 ± 0.02	0.93 ± 0.01	0.86 ± 0.01	0.87 ± 0.01	0.94 ± 0.01	5.26

Table 3. Average fruit height (H), width (W), peduncle length (L-ped), total soluble solids (TSS), and width/height ratio (W/H) from white mulberry cultivars in 2011. Means ± standard errors. Coefficient of variation (c.v.) is expressed in %.

Cultivars	H (cm)	W (cm)	L-ped (cm)	TSS (°Brix)	Weight (g)	W/H (g/cm)
Cattaneo	2.68 ± 0.08	1.54 ± 0.04	0.90 ± 0.05	13.9 ± 1.13	3.13 ± 0.29	0.58 ± 0.02
Florio	2.28 ± 0.07	1.19 ± 0.03	1.01 ± 0.04	16.1 ± 0.50	1.38 ± 0.08	0.52 ± 0.01
Giazzola	1.21 ± 0.08	0.89 ± 0.06	0.76 ± 0.06	12.1 ± 0.16	0.42 ± 0.11	0.73 ± 0.02
Kayrio	1.55 ± 0.03	1.03 ± 0.02	0.67 ± 0.04	8.01 ± 0.32	0.80 ± 0.03	0.67 ± 0.01
Kokusò-21	2.66 ± 0.08	1.32 ± 0.03	0.99 ± 0.05	17.1 ± 0.86	2.27 ± 0.14	0.50 ± 0.01
Kokusò Rosso	1.50 ± 0.06	0.96 ± 0.03	0.56 ± 0.04	17.5 ± 1.73	0.71 ± 0.06	0.65 ± 0.03
Korinne	1.39 ± 0.06	1.11 ± 0.07	0.96 ± 0.12	20.4 ± 1.35	0.76 ± 0.12	0.80 ± 0.03
Miura	1.51 ± 0.05	1.13 ± 0.03	1.20 ± 0.07	25.7 ± 0.92	0.76 ± 0.07	0.76 ± 0.04
Spagna Rosso	2.57 ± 0.08	1.56 ± 0.03	0.73 ± 0.05	17.0 ± 0.57	3.11 ± 0.25	0.61 ± 0.01
C.V.	31.4	20.1	23.0	30.5	72.5	16.1

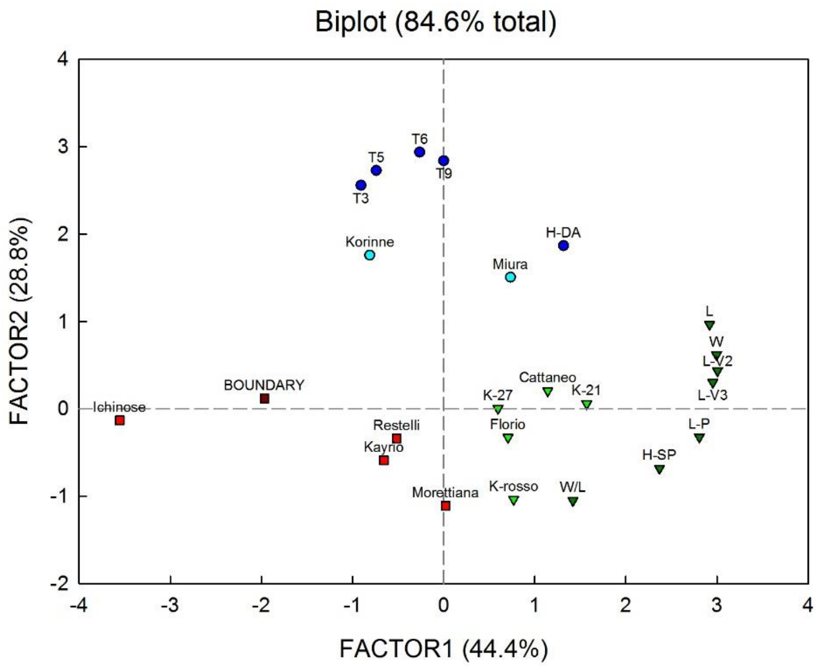


Figure 1. Grouping of 11 mulberry cultivars according to the 13 discriminant leaf descriptors (see Table 1 for abbreviations) determined by biplot analysis, followed by *k*-means clustering of standardized biplot coordinates. Different symbol shapes denote cluster groups, and colors distinguish cultivars and descriptors within clusters. 'Kokusò' genotypes are abbreviated with K.

As for fruit, LDA was able to fully separate (Wilk's Lambda statistics, $p < 0.001$) the nine cultivars with a canonical discriminant function after a backward step analysis including all six descriptors. In other words, all six measured descriptors were needed to get a significant discrimination of the cultivars, so no reduction of the number of descriptors could be achieved with this first analytical step. Using all six descriptors, the biplot analysis showed that about 86% of the variability observed was explained by the first two factors (Figure 2). *k*-means clustering of standardized biplot coordinates separated fruit descriptors into two groups; the first associating L-ped, TSS, and W/H, with the second associating W, H and weight (Figure 2). Based on this clustering, some expected relationships were shown by the biplot analysis, such as fruit-size-related descriptors, (i.e., W, H), and weight in cluster 2. Fruit shape was associated with juice sugars and peduncle length. Cluster 1 consisted of 'Korinne', 'Miura', 'Giazzola', 'Kokusò Rosso', and 'Kayrio', grouped by similar fruit shape, sweetness, and peduncle length; cluster 2 consisted of 'Cattaneo', 'Florio', 'Spagna Rosso', and 'Kokusò-21', grouped by similar fruit size (Figure 2).

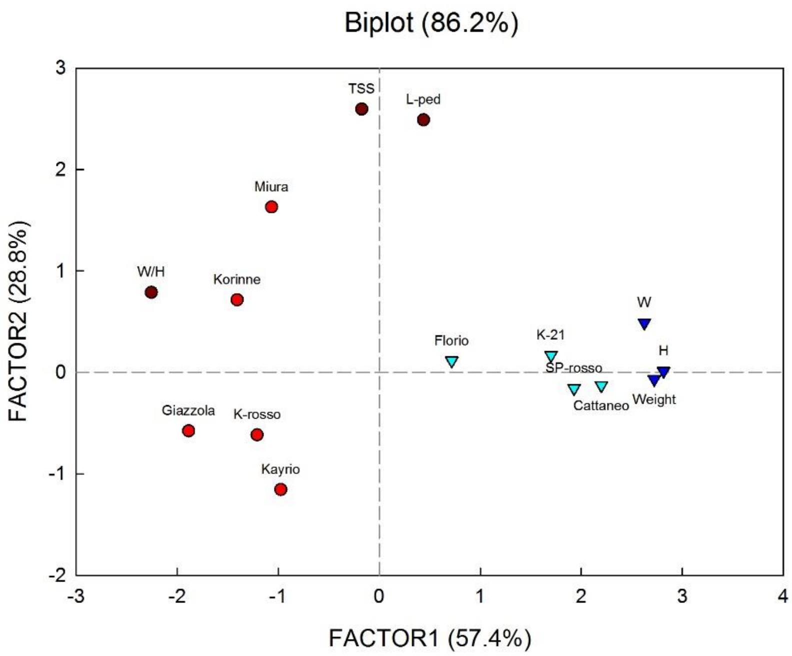


Figure 2. Grouping of nine mulberry cultivars according to fruit descriptors determined by biplot analysis, followed by *k*-means clustering of standardized biplot coordinates. Different symbol shapes denote cluster groups, and colors distinguish cultivars and descriptors within clusters. W—fruit width, H—fruit height, W/H—width/height, L-ped—length of fruit peduncle, TSS—total soluble solids. 'Kokusò' genotypes are abbreviated with K, and SP stands for Spagna.

The number of fruit descriptors investigated were definitely fewer than the leaf descriptors, and this may in part explain the greater discriminatory power of leaf descriptors, although a very high proportion of variance was explained by the first two factors with both leaf and fruit descriptors. This indicates that the leaf and fruit morphometric analysis performed in this trial may represent a simple and powerful tool to identify and classify white mulberry cultivars. Good discriminatory power could also be obtained using different approaches, such as circularity and elliptical Fourier descriptors [28], fruit chemometric descriptors [29], topological data analysis [30], or even machine learning methods [31] although those kind of measurements require more complex analytical approaches.

Interestingly, both sets of descriptors (leaf and fruit separately) were able to find similarities between 'Korinne' and 'Miura' in one group, and 'Florio', 'Cattaneo', and 'Kokusò-21' in another group. This was despite using different sets of cultivars for leaf and fruit data analyses. The information on cultivar similarities obtained using leaf and fruit morphometrics may prove useful for future classification work, and also suggests some degree of parentage within those two groups of cultivars that could be confirmed by molecular tests. On the other hand, the association between certain cultivars and some specific descriptors may prove useful for field identification work; for example, cultivars like Korinne and Miura are characterized by and can be recognized for having pronounced leaf apical tooth and wide insertion angles of leaf veins, as well as for having sweet fruit with long peduncles.

4. Conclusions

Overall, the results of this study pointed to a restricted set of descriptors for the identification of white mulberry genotypes. This shows great potential for using leaf (more than fruit) morphometric descriptors to discriminate mulberry cultivars. This is in agreement with recent studies showing the importance of agro-morphological traits for the selection and improvement of mulberry genotypes [32]. The use of digital image analysis, along with multivariate procedures, is also very promising for implementing a relatively simple, accurate, and inexpensive tool for the characterization and classification of white mulberry genotypes.

Author Contributions: Conceptualization, R.L.B.; Methodology, R.L.B. and F.M.; Formal Analysis, R.L.B.; Investigation, F.M.; Data Curation, F.M.; Writing-Original Draft Preparation, R.L.B.; Writing-Review & Editing, R.L.B.; Supervision, R.L.B.

Funding: This research received no external funding.

Acknowledgments: Sincere thanks go to Angela and Milko Sinacori for providing plant material and help with the experiment logistics.

Conflicts of Interest: The authors declare no conflict of interest.

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