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Morphological Characterization of Some Local Varieties of Fig (*Ficus carica* L.) Cultivated in Southern Italy

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Abstract: Figs (*Ficus carica* L.) are ancient fruits of the Mediterranean basin. In Southern Italy, they are particularly important in the traditional course of local cuisine. In Southern Italy, fig trees are rarely cultivated in specialized orchards but are present in association with other fruit trees (for example, olive, almond, pear, pomegranate, and grapevine). These mixed orchards are particularly important in the traditional agroecosystems of the south of Italy. This study reports preliminary results on the local fig variety's leaf morphological characterization, aiming to elucidate the presence of synonymousness or homonymy for in situ and ex situ conservation and further exploitation. A field survey was carried out during the summer of 2018 in some areas of the Basilicata district. Thirty local putative varieties were collected, and each of them was identified by GPS coordinates and recorded photographically. Moreover, they were cataloged with the name of the Municipality of origin, year, details of growing location (main crop, mixed orchard, gardens, and single plants), approximate age, and the local name supplied by the donor. All relevant information was included in the accession code. Leaf samples were collected from each accession from medium-length shoots. A digital image of each leaf sample was captured using a digital camera. Leaf morphometric traits were recorded using ImageJ and statistically analyzed using the software PAST 4.11 to discriminate among fig accessions. The multivariate morphometric approach applied correctly classified more than 90% of the leaves and helped to discriminate among accession. Moreover, linear discriminant analysis helped to recognize the presence of different synonymousness and homonymy of different accessions. The results revealed that measured leaf morphometric aided by image analysis could be a simple and inexpensive accessions classification tool.

Keywords: leaf morphometric analysis; digital image analysis; principal component analysis; linear discriminant analysis; synonymous; homonymous

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1. Introduction

Ficus carica L., a member of the *Moraceae* family, is a cropped (and wild) species largely diffused along the Mediterranean basin, where it has been cultivated for millennia [1]. Recent statistics show that fig is cultivated worldwide on 281,522 hectares; Mediterranean countries (Morocco, Turkey, Algeria, and Egypt) share about 65% of the cropped area [2]. In Italy, figs are cultivated on 2117 hectares almost entirely (96%) located in the south of Italy [3].

In most areas of Southern Italy, figs are typically cultivated in gardens or small rain-fed orchards in association with other fruit tree species (e.g., olive, almond, apricot, pear, pomegranate, and grapevine). These traditional agroecosystems and related indigenous cultures are relevant for biodiversity conservation purposes, securing food production, the diversification of agricultural systems and diet, and adaptation of agriculture to changing climatic conditions. These principles are summarized in part 2 of the convention on biological diversity (CBD), which defines sustainability as "the use of components of biological diversity in a way and at a rate that does not lead to the long-term decline of biological diversity, thereby maintaining its potential to meet the needs and aspirations of present and future generations".

Few surveys have been conducted to explore, characterize and identify the fig local varieties of Basilicata hampering adequate conservation or developing programs.

Considering that fig has limited chilling unit needs and that it is drought-tolerant [4], an improved knowledge of the fig germplasm variability might support sustainable agriculture by the replacement of high chilling temperatures and water-demanding species triggered by changing climatic scenario with increasing drought and mild winter occurrence [5].

Condit [6] gave an inclusive description of fig varieties in 1955, reporting more than 600 named fruit-producing varieties, but only a few were comprehensively described. For the majority, it was impossible to identify the cultivated variety or cultivar. Based on a Condit's early work [7], the International Plant Genetic Resources Institute (IPGRI) supplied an official descriptors list for *Ficus carica* L. accessions or cultivars [8], allowing the scientific communities to carry out characterization and identification work using the standardized method. Studies on fig germplasm characterization have been carried out by different authors using morphological traits [9–13], and their combination with chemical compounds [14,15], or with gene marker-based approaches [16–19].

Despite the excellent discrimination of DNA-based markers [18], their use could be expensive in time and economic resources and, in some circumstances, could also be difficult to access. The use of morphological plant traits has been suggested as a simple and inexpensive method for the first screening of genetic resources [15] or to integrate the genetic analysis because some DNA-based markers are not suitable for clone identifications (see Ref. [20] and literature cited therein). Taking all these together, additional (low-cost and fast) methods for varieties discrimination would be desirable.

In the last few decades, measurements of leaf traits (i.e., length, width of leaf parts, the distance between biologically homologous points, angles, and ratio) have evolved from manual to digital acquisition technologies, such as photocopiers, digital cameras, and image analysis software [21]. The measurement of morphological plant traits in combination with multivariate statistical analysis is named 'multivariate morphometrics analysis' [22]. Multivariate morphometrics analysis was used to discriminate among cultivars of fig [11,15,16,19], mulberry [23] grapevine [20,24] apple [25], and other species, making the multivariate morphometric approach an essential step for inventory and conservation [26].

To the best of our knowledge, morphometric characterizations have not been adequately implemented in Southern Italy on fig genotypes [17]. Therefore, this study aimed (i) to characterize fig accessions sampled in Basilicata district through multivariate morphometric analysis *sensu* [22]; (ii) to discriminate among synonymousness and homonymy of sampled fig accessions.

2. Materials and Methods

2.1. Plant Material

Cultivated fig trees located in various districts of Basilicata region (Figure 1) were selected with the support of local biodiversity associations who also provided the local denomination of figs. The six districts were located from the Ionian sea coast to Basilicata's hilly and internal areas (Figure 1). The climate of these municipalities ranged from a hot summer temperate climate (Bernalda, Nova Siri, and Tursi) to a warm summer temperate climate (Chiaromonte, San Mauro Forte and Tolve) [27]. In particular, the long-term average (1989–2021) of the Ionian coast districts shows 568.6 mm of annual rainfall, 22.0 °C of annual average maximum temperature, with July and August being the hottest months of the year (around 33 °C) and 10.8 °C the annual average minimum temperature, with January being the coolest month of the year (average monthly temperature 3.44 °C). The hilly and internal districts have a higher average annual rainfall (801.0 mm) and lower maximum and minimum temperatures (average annual maximum and minimum temperatures, respectively, of 19.1 and 9.2 °C).

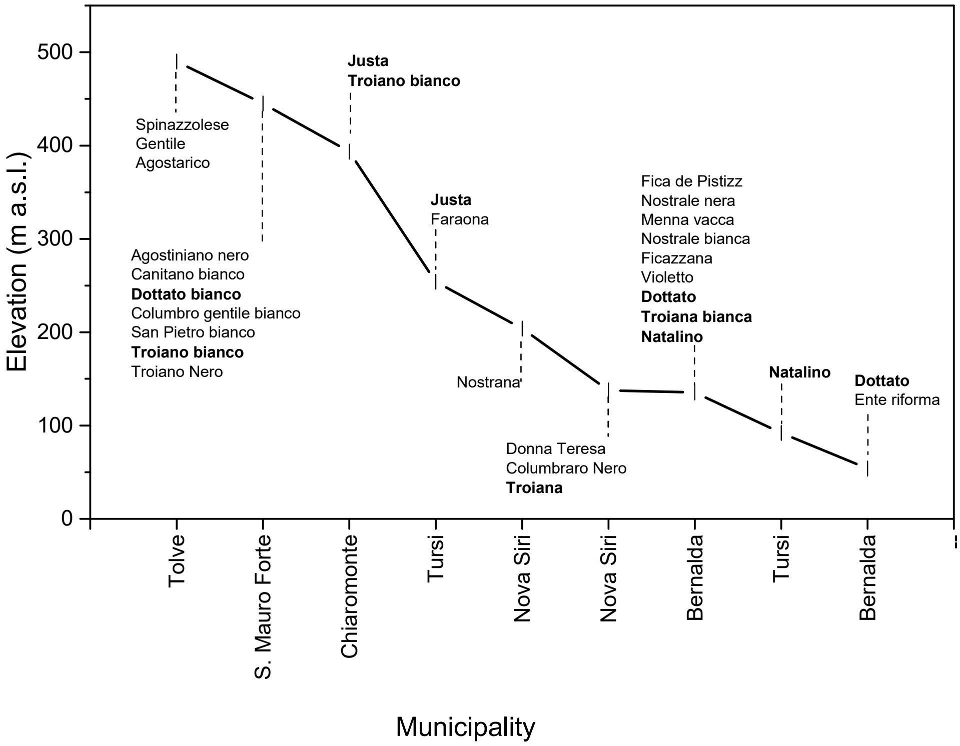

Figure 1. Distribution of the studied fig local varieties according to the elevation of the sampling site and municipality. Note that **bold** font indicates that the same name of a local variety was found in different municipalities. In grouped local varieties, the average elevation was used; for details, see Table S1.

A skin ground color was assigned according to point 7.4.26 of the freely accessible IPGRI fig descriptors list [8]. For the subsequent statistical analysis, each accession was labeled as reported in Table 1.

Table 1. Site, local variety name, accession code, skin color and shape of the base of the leaf.

Place	Name	Accession Code [(a)]	Skin Color [(b)]	Shape of Leaf Base [(c)]
Bernalda	Fica de Pistizz	BRDPSTCC	Greyed-Purple	Cordate
	Nostrale nera	BRDNSTRN	Black	Cordate
	Menna vacca	BRDMNNVC	Greyed-Purple	Cordate
	Nostrale bianca	BRDNSTRL	Yellow-Green	Cordate
	Ficazzana	BRNFCZZN	Black	Decurrent
	Violetto	BRFVLTT0	Greyed-Purple	Calcarate
	Dottato	BRNDTTT0	Green	Cordate
	Troiana bianca	BRNTRNB0	Yellow-Green	Calcarate
	Natalino	BRRNTLN0	Black	Cordate
	Dottato	BRXDTTT0	Green	Cordate
	Ente Riforma	BRXNTRFR	Greyed-Purple	Cordate
Chiaromonte	Justa	CHIJST00	Green	Cordate
	Troiano bianco	CHIFCTRN	Yellow-Green	Cordate
Nova Siri	Nostrana	NVTNSTRN	Green	Cordate
	Donna Teresa	NVPDNNTR	Green	Cordate
	Columbraro nero	NVPCLMBN	Black	Truncate
	Troiana	NVPTRNBN	Yellow-Green	Decurrent

Table 1. *Cont.*

Place	Name	Accession Code [(a)]	Skin Color [(b)]	Shape of Leaf Base [(c)]
San Mauro Forte	Agostiniano Nero	SMSAGSTN	Brown	Decurrent
	Canitano bianco	SMSCNTNB	Green	Truncate
	Dottato bianco	SMSDTTTB	Green	Cordate
	Columbro bianco gentile	SMSCLMBG	Green	Decurrent
	San Pietro bianco	SMSSPTRB	Green	Decurrent
	Troiano bianco	SMSTRNB0	Yellow-Green	Decurrent
	Troiano nero	SMSTRNN0	Black	Decurrent
Tolve	Spinazzolese	TLNSPNZZ	Black	Cordate
	Gentile	TLNGNTLB	Yellow-Green	Cordate
	Agostarico	TLNAGSTR	Black	Cordate
Tursi	Justa	TRVJST00	Green	Decurrent
	Natalino	TRPNTLNN	Black	Truncate
	Faraona	TRVFRN00	Green	Decurrent

[(a)] The code is formed by the first two consonants of the name of the Municipality (BR = Bernalda, CH = Chiaromonte; NV = Nova Siri; SM = San Mauro Forte; TL = Tolve; TR = Tursi). One character showing the name of the site or the name of the owner and five consonants for the short name of the accession. For example, BRDPSTCC is decoded as: BR = Bernalda, D = D'Ambrosio (the owner of the field), PSTCC = fica de Pistizz. When the letters of the name of the accession are less than five, one or more 0 were added (see, for example, CIJST00). [(b,c)] The description of skin color [(b)] and shape of leaf base [(c)] were based, respectively, on the morphological descriptors n. 7.4.26 and n. 7.3.7 of the freely accessible IPGRI fig descriptors list [8].

2.2. Leaf Sampling and Image Acquisition

The sampling unit consisted of a medium-length shoot (10–20 cm) with 8–10 leaves per shoot. On each tree, 20–25 sampling units were selected at eye level in the outer part of the crown. Starting from the base of the shoot, the fourth or the fifth mature, healthy and sunny leaf was collected for each sampling unit in the second half of July—the beginning of August 2018.

Leaves including petiole were detached from the shoots, labeled, and pressed between newspaper sheets for about 2–3 days. Thereafter, the abaxial leaf surface was pictured, along with a ruler as metric reference, using a NIKON D5100 camera equipped with an AF-P Nikkor 18–55 mm 1:3.5–5.6 G lens.

2.3. Image Analysis

Ten images (=10 leaves) per accession were used to manually configure, in 2D, 17 well-defined and morphologically relevant landmarks (LM), starting from the basal end of petiole (LM 0) to the leaf lamina–petiole junction point (LM 1), and the tip of the central vein (LM 2). Other landmarks (LM 3–LM 16) were placed on the left and right sides of the lamina margin (Figure 2A).

After scaling the images from pixels to the international M.K.S. unit system, landmarks were digitized using the multi-point tool [28,29]. Landmarks were converted to 2D Cartesian coordinates of each leaf, and data were stored using the ROY manager tool of ImageJ. Leaf angles were also measured using the angle tool of ImageJ. The petiolar sinus angle (SP) was defined as the angle between the left and right lines obtained by joining the LM 1 with LM 3 and LM 16, respectively (Figure 2D). Other angles are defined in Table 2 and shown in Figure 2D.

After digitizing the landmarks and the angles measurements, the leaf was segmented, the lamina and petiole were separated with the pencil tool, then the area and perimeter of the leaf lamina were automatically measured.

Cartesian coordinates of each LM were used to calculate the length of the elements reported in Table 2 and visually depicted in Figure 2B,C.

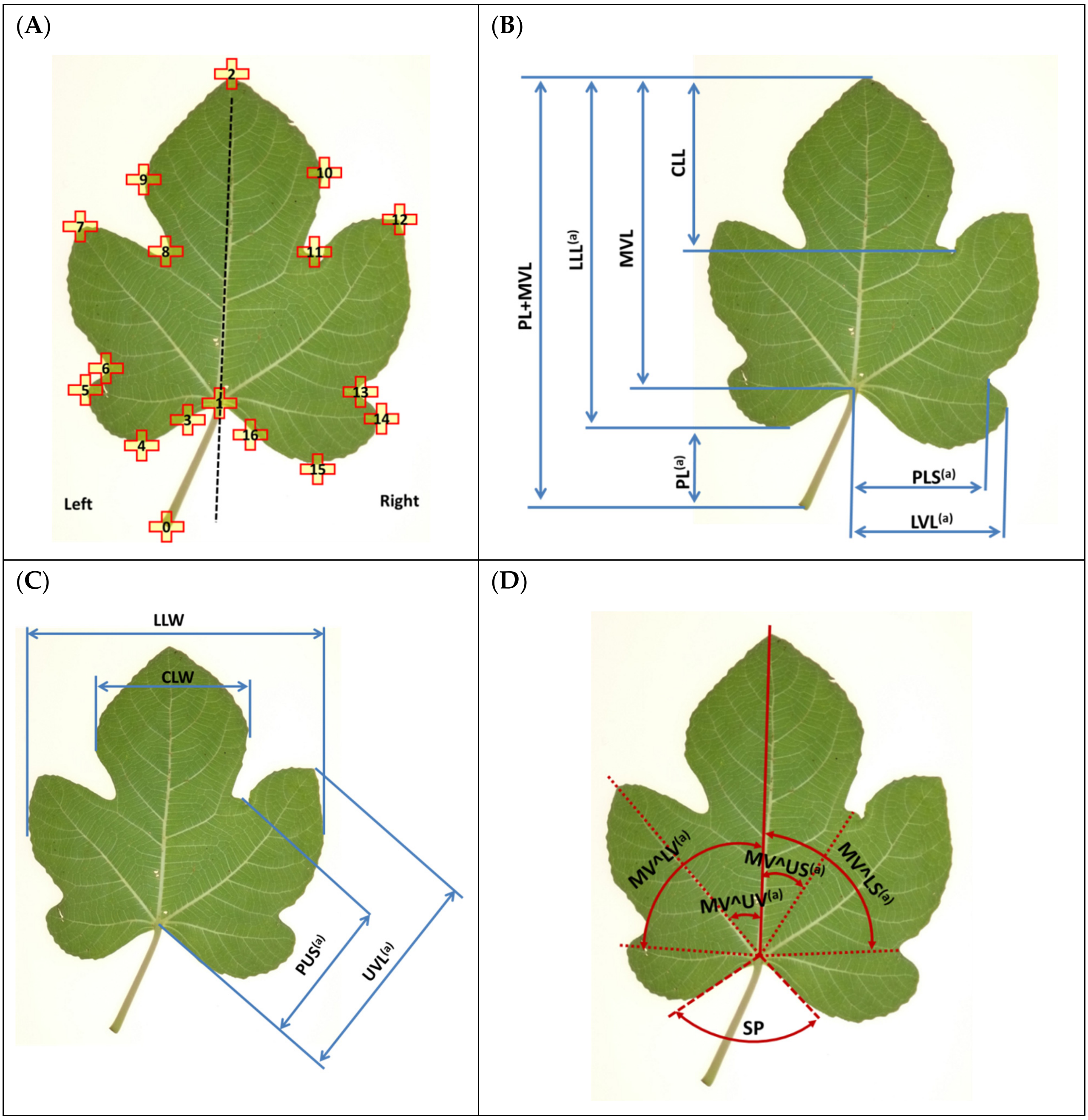

Figure 2. Visual maps of landmarks configuration and leaf lamina measurements used for morphometric analysis and in *Ficus carica* L. accessions. [a] Paired points measured separately from left and right side of the leaf and averaged.

Table 2. Leaf measurements used for morphometric analysis of the accessions of *Ficus carica* L.

Leaf Trait	Abbreviation	Units
Petiole length	PL	mm
Main vein length	MVL	mm
Upper vein length [a]	UVL	mm
Lower vein length [a]	LVL	mm
Total veins length	TVL	mm

Table 2. *Cont.*

Leaf Trait	Abbreviation	Units
Leaf lamina length [(a)]	LLL	mm
Leaf lamina width	LLW	mm
Leaf lamina area	LLA	mm^2
Leaf lamina perimeter	LLP	mm
Veins density	VD	mm mm^{-2}
Central lobe length	CLL	mm
Central lobe width	CLW	mm
Petiole to upper sinus distance [(a)]	PUS	mm
Petiole to lower sinus distance [(a)]	PLS	mm
Petiole sinus depth	PSD = LLL − MVL	mm
Leaf lamina area to leaf lamina perimeter ratio	LLA/LLP	mm
Leaf lamina length to leaf lamina width ratio	LLL/LLW	
Central lobe length to leaf lamina length	CLL/LLL	
Central lobe length to central lobe width	CLL/CLW	
Circularity	CRC	
Petiole sinus angle (angle between the left and right lines that connect LM 1 to LM 3 and LM 1 to LM 16)	SP	degree
Main vein to lower vein angle [(a)]	MVˆLV	degree
Main vein to lower sinus angle [(a)]	MVˆLS	degree
Main vein to upper vein angle [(a)]	MVˆUV	degree
Main vein to upper sinus angle [(a)]	MVˆUS	degree

[(a)] paired points measured separately from left and right side of the leaf and averaged.

2.4. Leaf Size Measurements

Leaf size was characterized by measuring all distances reported in Table 2. These measurements include also the descriptors listed in the IPGRI guidelines for fig leaves [8].

Cartesian coordinates of each LM were used to calculate the length of most of the elements reported in Table 2 and are visually depicted in Figure 2B,C.

Fig leaves, like many other plant species, show a symmetric pattern along the middle axis [30,31]. Structures (i.e., petiole, main vein, and central lobe length) positioned along this axis and landmark (LM 0, LM 1, LM 2) are defined as "unpaired", while all other are "paired" [32]. The distance between the jth and ith landmarks was obtained from the following equation:

$$D_{(x_i y_i, x_j y_j)} = \sqrt{(x_j - x_i)^2 + (y_j - y_i)^2}$$

where x and y are the coordinates of the jth and ith landmark. For paired LM, the distances measured on the left (landmarks 3:9) and right (landmarks 16:10) side of the lamina were averaged (Figure 2A), i.e.,

$$\overline{D} = \frac{D_{left} + D_{right}}{2}$$

where $\overline{D}$ is the average of the two distances calculated on the left (D_{left}) and right (D_{right}) side of the lamina.

The sum of the first-order veins (mid or central vein—MVL, and upper—UVL, and lower lateral veins—LVL) was used to calculate the total length of the first-order veins (TVL), and then the relative vein density (VD) was calculated from the ratio between the TVL and LLA [33]:

$$\text{TVL} = \text{MVL} + 2*\text{UVL} + 2*\text{LVL} \qquad [\text{mm}]$$

$$\text{VD} = 10 * \frac{\text{TVL}}{\text{LLA}} \qquad [\text{cm cm}^{-2}]$$

the circularity parameter was calculated as described in [28,29], i.e.,

$$\text{CRC} = \frac{4\pi(\text{LLA})}{\text{LLP}^2}$$

As suggested by the IPGRI descriptors list, the degree of leaf lobation was measured by the ratio between the length of the central lobe to the length of the leaf (CLL/LLL). Another estimation of lamina incision was made by the ratio between leaf area to leaf perimeter (LLA/LLP, mm). Moreover, the CLL/CLW and LLL/LLW ratios were also calculated.

2.5. Morphometric Data Analysis

Each leaf trait was expressed as the mean of 10 measurements performed on 10 leaf per accession and statistically analyzed using PAST 4.11 [34]. One-way ANOVA was performed for TVL, MVL, UVL, LLA/LLP, LLL, LLW, CLW and PUS variables after the check for normality of data distribution (Shapiro–Wilk test) and homogeneity of variance (Levené's test) (followed by Tukey's pairwise post-hoc tests for means separation. For all other traits analysis of variance was carried out by the non-parametric Kruskal–Wallis test followed by Dunn's post hoc multiple comparison test. Regression analysis was performed on all leaves using linear models for LLP vs. TVL and DP vs. SP, while non-linear models were using for LLP vs. LLA and LLA vs. VD. Lower and upper limits for 95% confidence intervals were calculated using the default bootstrap function available in PAST 4.11 statistical software.

Multivariate relationships, among accessions, were calculated by a principal component analysis (PCA) procedure on a variance-covariance matrix of the 24 morphological descriptors reported in Table 2. The LLA variable was not included in the multivariate analysis because has a different unit of measurements compared to other variables [35]. Since multivariate analysis is scale sensitive, before analysis all variables were standardized to zero mean per unit of standard deviation, according with the equation

$$z = \frac{x - \overline{x}}{SD}$$

where z is the standardised value, x is the specific value for which the standardised value is calculated, $\overline{x}$ and SD are, respectively, the mean and the standard deviation of the given variable. The analysis of differences in leaf traits among accessions was carried by a linear discriminant analysis (LDA).

The accessions were grouped based on the skin color in (i) dark skin (all accession with black, brown, and purple-green fruit skin) and (ii) green skin (all accessions with green or yellow-green skin) and the LDA applied separately in each group. The supervised LDA was also employed for accession classification purpose of the sampled leaves and the accuracy of classification was measured per each group as the ratio between the number of "correct classification" and the total number of leaves under classification (i.e., 10).

3. Results

A total of 30 putatively different local varieties were found across the 6 sites of the Basilicata district; 13 had fruit dark skin color, and 17 had fruit green skin color (Table 1). There were five local varieties named 'Troiana/o' (one with dark skin), three named 'Nostrana' or 'Nostrale' (one with dark skin), three 'Dottato/a', two 'Natalino' (both with dark skin), and two 'Justa'. 'Troiana' was found in four sites with different elevation (Figure 1). As for the 'Troiana', other names were present in at least two sites: 'Dottato' or 'Dottato bianco' (Bernalda and San Mauro Forte); 'Natalino' (Bernalda and Tursi); and 'Justa' (Chiaromonte and Tursi). To all these local varieties, a unique accession code was assigned and used in the text.

The morphometric traits of the leaves showed a significant variability among the accessions. For example, PSD, SP, and LLA showed the highest coefficients of variation, respectively, of 52.8%, 42,3%, and 33.4%. LLL/LLW and CLL/LLL showed the lowest coefficients of variation, of 7.5% and 9.2% (the coefficient of variation for the remaining variables was not shown).

In Basilicata, 16 accessions had a cordate base of the leaf, nine decurrent, three truncate, and two had a calcarate base.

The PSD was higher in the leaf with calcarate and cordate bases concerning the leaf with decurrent or truncate bases (Tables 1 and 3). BRNTRNB0, BRFVLTT0, and BRXNTRFR, the first two with calcarate base and the last one with cordate base, showed values of PSD significantly higher than those other leaves (Table 3). On the other hand, the SP was higher in the leaf with a truncate or decurrent base with respect to the other (Table 3).

The relationship between PSD and SP was statistically significant, with a coefficient of determination (R^2) of 0.42 (Figure 3A). As expected, the accessions BRXNTRFR, BRNTRNB0, and BRFVLTT0 are in the lower and right part of the graph (high PSD and lower SP), while, among others, SMSCNTNB, BRDNSTRL, and SMSDTTTB are in the higher and left part of the graph (low PSD and high SP).

The size of the leaves is mainly described by the length, width, surface, and perimeter. The leaf area (LLA) varies from 38,869.51 mm^2 to 9249.23 mm^2. In particular, there were four accessions with a leaf area higher than 30,000 mm^2 and seven with a leaf area lower than 20,000 mm^2 (Table 3). The leaf perimeter depends not only on the leaf length and width, but also on the number of lobes and the degree of the incision. The accessions reported in this study typically had three lobes, but there were some accessions with five lobes and different degrees of incision of the different lobes. The location effects can be appreciated by comparing the value of LLA reported in Table 3 with that obtained from the multiplication of LLL by leaf width LLW. Among the accessions, when the leaf area was not statistically different, the number of lobes and the degree of incision made for statistically different results in the perimeter of the leaf (Table 3). It is interesting to note that eight accessions had average perimeter values greater than 1 m. There were significant relationships between LLP and LLA and between LLP and the TVL of the leaf (Figure 3B,C). When the perimeter increases, the leaf area, and total veins length increase.

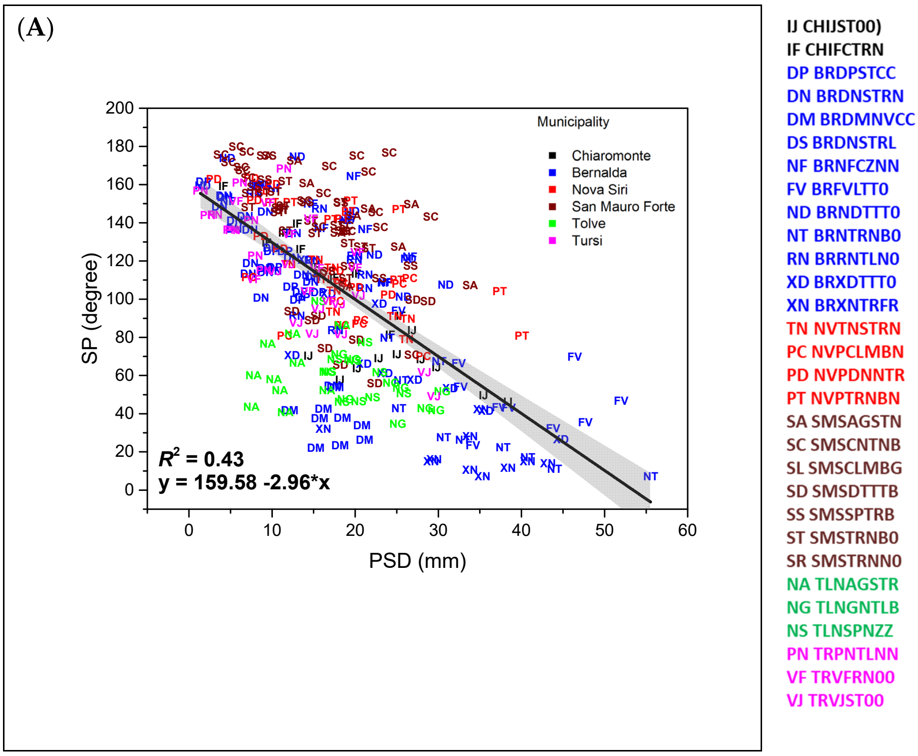

Figure 3. *Cont.*

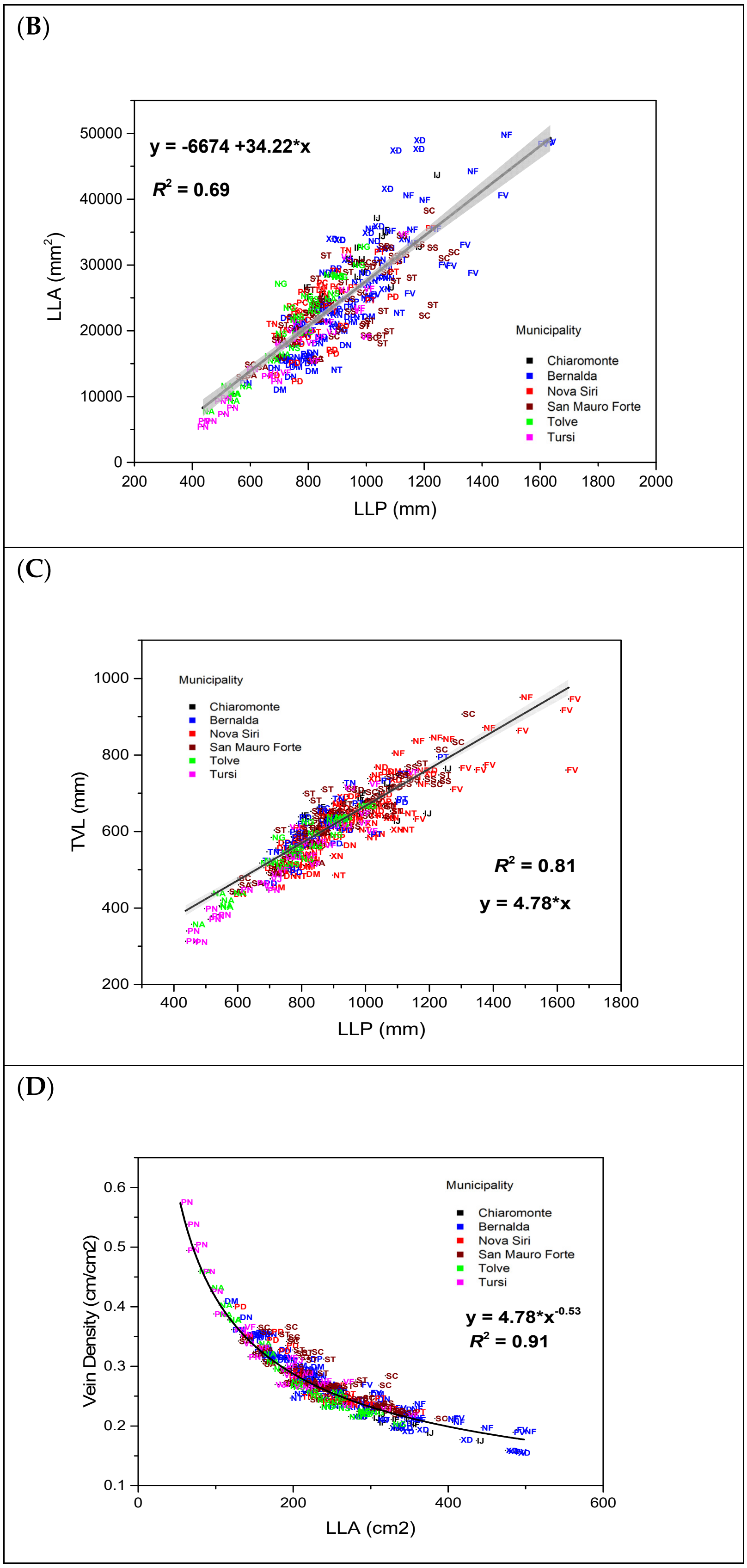

Figure 3. Linear regressions between (**A**) PSD versus SP; (**B**) LLP versus LLA; (**C**) LLP versus TVL, and (**D**) LLA versus VD. The regression lines are calculated on 300 leaves of *Ficus carica* L. accessions.

Table 3. Leaf traits of fig accessions characterized in six municipalities of Basilicata District [(a)].

Accession Code	PL (mm)		MVL (mm)		UVL (mm)		LVL (mm)		TLV (mm)		LLL (mm)		LLW (mm)	
BNDTTT0	80.43	bcd	193.79	bcdef	147.02	cdefg	73.28	defghi	805.57	a	210.65	defgh	184.59	cdefg
BXDTTT0	97.78	ab	243.53	a	174.09	ab	58.47	hijk	777.38	ab	268.51	a	222.33	a
BNFCZZN	102.72	a	241.84	a	186.90	a	94.96	abcd	708.66	abc	259.78	ab	213.28	abc
BDMNNVC	62.43	defgh	173.00	efghi	134.89	efghi	70.67	efghi	707.61	abc	190.28	fghi	150.81	hij
BDNSTRL	66.91	def	183.84	defgh	140.16	cdefgh	51.99	ijk	702.70	abcd	190.93	fghi	154.95	ghij
BRDNSTRN	65.24	defg	154.96	hi	124.25	ghi	65.21	efghij	690.21	bcde	165.20	ij	140.42	jk
BRXNTRFR	75.18	cdef	203.20	bcd	153.00	bcdef	62.27	fghijk	677.22	bcdef	236.72	bcde	190.11	bcdef
BRRNTLN0	59.57	fgh	171.71	efghi	135.63	efghi	74.62	defgh	666.20	bcdefg	187.86	ghij	155.64	ghij
BRDPSTCC	76.70	cdef	195.82	bcdef	149.96	bcdefg	66.99	efghij	663.42	bcdefg	206.87	efgh	173.80	efghi
BRNTRNB0	80.14	bcd	167.02	fghi	129.63	fghi	72.29	efghi	652.61	cdefg	201.66	fgh	172.07	efghi
BRFVLTT0	102.89	a	214.59	abc	174.65	ab	106.74	a	634.40	cdefgh	253.60	abc	215.48	ab
CHIFCTRN	90.21	abc	197.17	bcdef	153.32	bcdef	79.80	cdefgh	633.74	cdefgh	212.56	defgh	195.16	abcdef
CHIJST00	75.34	cdef	218.86	ab	162.25	abcd	66.93	efghij	629.72	cdefgh	244.76	abcd	201.54	abcde
NVPCLMBN	62.14	defgh	191.58	bcdef	142.99	cdefg	62.00	fghijk	616.91	cdefgh	211.03	defgh	174.16	efghi
NVPDNNTR	65.00	defg	168.78	fghi	132.92	efghi	68.66	efghij	611.88	cdefgh	180.64	hij	154.58	ghij
NVTNSTRN	72.11	cdef	199.18	bcde	144.10	cdefg	62.25	fghijk	610.89	cdefgh	218.23	defg	176.66	defgh
NVPTRNBN	61.86	defgh	187.40	cdefg	146.28	cdefg	86.32	abcde	603.94	cdefgh	209.80	efgh	184.18	cdefg
SMSAGSTN	71.86	cdef	160.30	ghi	116.24	hi	66.19	efghij	601.57	cdefgh	180.26	hij	156.12	ghij
SMSCLMBG	73.65	cdef	184.94	cdefgh	132.45	efghi	80.52	cdefg	599.82	cdefgh	206.12	efgh	176.61	defgh
SMSCNTNB	97.97	ab	196.72	bcdef	157.57	bcde	97.87	abc	592.23	defgh	206.48	efgh	165.48	fghij
SMSDTTTB	67.03	def	191.03	bcdef	143.07	cdefg	61.33	ghijk	584.11	efgh	210.14	efgh	171.25	fghi
SMSSPTRB	76.92	cdef	207.11	bcd	164.47	abc	83.33	bcdef	571.95	fghi	223.41	cdef	204.77	abcd
SMSTRNB0	78.50	cde	190.33	bcdefg	154.68	bcdef	83.26	bcdef	570.87	fghi	205.71	efgh	189.72	bcdef
SMTRNN0	72.66	cdef	181.86	defgh	151.16	bcdef	103.01	ab	568.13	fghi	194.16	fghi	171.92	efghi
TLNAGSTR	44.23	h	143.07	Ij	109.39	ij	51.60	ijk	561.76	ghi	154.85	jk	144.50	ij
TLNGNTLB	76.49	cdef	214.40	abc	141.85	cdefgh	59.41	ghijk	560.31	ghi	238.08	bcde	183.80	cdefg
TLNSPNZZ	71.30	def	189.04	bcdefg	137.25	defgh	48.38	jk	533.87	hi	209.16	efgh	175.98	defgh
TRFRN00	62.10	defgh	185.44	cdefg	141.79	cdefgh	67.46	efghij	525.16	hi	199.12	fghi	172.12	efghi
TRPJST00	60.92	efgh	177.78	defgh	133.94	efghi	58.05	hijk	465.06	ij	195.78	Fghi	166.37	fghij
TRPNTLNN	47.60	gh	123.33	J	88.932	j	42.05	k	385.29	j	129.24	K	113.65	k
Accession Code	**LLA (mm^2)**		**LLP (mm)**		**VD (mm/mm^2)**		**CLL (mm)**		**CLW (mm)**		**PUS (mm)**		**PLS (mm)**	
BNDTTT0	24,896.90	cdefghi	894.11	cdefg	0.0258	fghijk	105.03	cdefghi	75.78	efghij	95.098	bcdefg	72.30	cdefghi
BXDTTT0	38,869.51	a	1022.62	bcde	0.0186	l	134.73	ab	104.89	a	121.30	a	71.01	cdefghi
BNFCZZN	37,850.28	a	1175.20	b	0.0215	jkl	144.53	a	90.80	bcde	106.02	bc	94.50	a
BDMNNVC	19,075.72	hijk	879.04	cdefg	0.0318	bcde	96.23	fghij	67.60	hijkl	82.13	efghij	59.61	ghijk

Table 3. *Cont.*

Accession Code	LLA (mm^2)		LLP (mm)		VD (mm/mm^2)		CLL (mm)		CLW (mm)		PUS (mm)		PLS (mm)	
BDNSTRL	19,840.07	ghijk	782.42	fgh	0.0294	cdefgh	95.13	ghij	71.43	fghijk	97.22	bcdef	56.06	ijk
BRDNSTRN	15,718.37	jkl	779.85	fgh	0.0341	bc	86.27	ijk	62.88	jkl	76.33	hijk	55.99	ijk
BRXNTRFR	28,175.07	bcdef	1037.90	bcd	0.0227	ijkl	123.21	abc	81.40	cdefghi	85.06	defghij	65.87	efghij
BRRNTLN0	20,013.26	fghijk	825.27	efg	0.0299	cdefg	100.77	defghij	75.64	efghij	81.42	fghij	63.61	efghijk
BRDPSTCC	23,653.82	defghij	881.20	cdefg	0.0269	efghij	104.01	cdefghi	77.08	defghij	102.76	bcd	69.66	defghij
BRNTRNB0	22,565.61	defghij	925.65	cdef	0.0259	fghijk	117.88	bcdef	87.66	bcdef	55.07	l	60.07	ghijk
BRFVLTT0	35,938.39	ab	1379.74	a	0.0224	ijkl	145.00	a	76.54	defghij	76.32	hijk	75.34	bcdefg
CHIFCTRN	28,346.79	bcde	914.79	cdefg	0.0237	hijkl	108.78	cdefgh	103.71	ab	104.41	bc	84.16	abcd
CHIJST00	32,754.14	abc	1045.23	bcd	0.0209	kl	122.10	bcd	94.74	abc	107.62	bc	70.73	defghij
NVPCLMBN	23,846.44	defghij	800.92	fgh	0.0253	ghijk	101.29	defghij	81.89	cdefgh	102.19	bcd	67.62	efghij
NVPDNNTR	17,740.28	ijk	832.48	efg	0.0329	bcd	107.12	cdefghi	68.68	ghijkl	67.67	jkl	60.48	ghijk
NVTNSTRN	24,803.63	cdefghi	809.60	efg	0.0249	ghijk	105.79	cdefghi	84.52	cdefg	108.23	abc	67.42	efghij
NVPTRNBN	25,918.93	cdefghi	968.02	cdef	0.0257	fghijk	108.24	cdefgh	86.67	cdef	86.24	defghi	75.05	bcdefg
SMSAGSTN	18,240.32	hijk	730.39	ghi	0.0296	cdefg	89.24	hijk	83.30	cdefgh	83.70	efghij	59.45	ghijk
SMSCLMBG	23,719.15	defghij	858.09	defg	0.0262	efghijk	104.14	cdefghi	82.40	cdefgh	90.55	cdefgh	78.66	abcde
SMSCNTNB	23,238.54	defghij	1046.52	bcd	0.0319	bcde	119.13	bcde	69.91	ghijk	80.73	fghij	77.30	bcdef
SMSDTTTB	23,376.00	defghij	836.65	efg	0.0262	efghijk	100.79	defghij	75.77	efghij	99.34	bcde	67.65	efghij
SMSSPTRB	29,427.05	bcd	1061.29	bc	0.0243	ghijkl	119.83	bcde	88.03	bcde	93.82	bcdefgh	88.73	abc
SMSTRNB0	26,302.39	cdefgh	855.36	defg	0.0256	fghijk	98.92	efghij	93.40	bc	106.66	bc	91.30	ab
SMTRNN0	22,348.32	defghij	1073.94	bc	0.0312	bcdef	117.29	cdef	65.48	ijkl	69.04	ijkl	69.52	defghij
TLNAGSTR	13,416.85	kl	613.64	hi	0.0366	b	79.85	jk	55.60	kl	70.01	ijkl	56.68	hijk
TLNGNTLB	27,493.85	cdefg	861.82	defg	0.0225	ijkl	119.86	bcde	92.27	bcd	108.54	ab	64.56	efghijk
TLNSPNZZ	22,321.37	defghij	797.21	fgh	0.0254	ghijk	106.79	cdefghi	80.33	cdefghi	95.04	bcdefg	55.68	jk
TRFRN00	21,436.81	defghijk	916.02	cdefg	0.0291	cdefgh	111.76	cdefg	75.38	efghij	78.19	ghij	69.55	defghij
TRPJST00	20,663.83	efghijk	788.76	fgh	0.0278	defghi	93.57	ghij	77.01	defghij	91.01	bcdefgh	61.44	fghijk
TRPNTLNN	9249.23	l	535.99	i	0.0442	a	70.65	k	52.92	l	59.55	kl	49.05	k

Accession Code	PSD (mm)		LLA/LLP (mm^2 mm^{-1})		LLL/LLW		CLL/LLL		CLL/CLW		CRC	
BNDTTT0	16.86	efghij	27.77	bcdefgh	1.14	cde	0.50	gh	1.39	cdef	0.485	a
BXDTTT0	24.98	bcde	37.83	a	1.21	abc	0.50	fgh	1.28	efgh	0.474	ab
BNFCZZN	17.94	defghij	32.23	b	1.22	abc	0.56	abcdefg	1.59	bc	0.469	abc
BDMNNVC	17.28	efghij	21.32	jklm	1.27	ab	0.50	fgh	1.43	cdef	0.468	abc
BDNSTRL	7.09	jk	25.03	ghijkl	1.23	abc	0.50	gh	1.33	cdefg	0.456	abcd
BRDNSTRN	10.24	hijk	20.18	lm	1.18	abcde	0.52	defgh	1.38	cdef	0.441	abcde
BRXNTRFR	33.52	abc	27.06	cdefgh	1.25	abc	0.52	defgh	1.52	bcde	0.440	abcde
BRRNTLN0	16.15	efghijk	24.19	hijkl	1.21	abcde	0.54	bcdefgh	1.34	cdefg	0.431	abcdef

Table 3. *Cont.*

Accession Code	PSD (mm)		LLA/LLP (mm^2 mm^{-1})		LLL/LLW		CLL/LLL		CLL/CLW		CRC	
BRDPSTCC	11.06	hijk	26.89	defghi	1.19	abcde	0.50	fgh	1.36	cdef	0.428	abcdef
BRNTRNB0	34.63	ab	24.45	hijkl	1.18	abcde	0.59	abc	1.36	cdef	0.419	abcdefg
BRFVLTT0	39.01	a	25.69	fghijk	1.19	abcde	0.57	abcde	1.93	a	0.415	abcdefg
CHIFCTRN	15.39	efghijk	30.94	bcde	1.09	de	0.51	fgh	1.05	h	0.411	abcdefgh
CHIJST00	25.89	bcde	31.35	bcd	1.22	abcd	0.50	gh	1.29	efgh	0.405	abcdefghi
NVPCLMBN	19.44	defghi	29.78	bcdefg	1.21	abcde	0.48	h	1.24	fgh	0.398	abcdefghij
NVPDNNTR	11.86	ghijk	21.23	jklm	1.17	bcde	0.59	ab	1.57	bcd	0.393	bcdefghij
NVTNSTRN	19.05	defghi	30.50	bcdef	1.24	abc	0.48	h	1.25	efgh	0.388	cdefghijk
NVPTRNBN	22.40	defg	26.55	defghi	1.14	cde	0.52	efgh	1.25	efgh	0.381	defghijk
SMSAGSTN	19.96	defghi	24.78	ghijkl	1.16	bcde	0.50	gh	1.08	gh	0.369	efghijk
SMSCLMBG	21.17	defgh	27.52	bcdefgh	1.17	bcde	0.50	fgh	1.27	efgh	0.352	fghijk
SMSCNTNB	9.77	ijk	21.83	ijklm	1.25	abc	0.58	abcd	1.73	ab	0.350	fghijkl
SMSDTTTB	19.12	defghi	27.63	bcdefgh	1.25	abc	0.48	h	1.34	cdefg	0.339	ghijkl
SMSSPTRB	16.30	efghijk	27.60	bcdefgh	1.09	de	0.53	cdefgh	1.36	cdef	0.330	hijkl
SMSTRNB0	15.39	efghijk	30.75	bcdef	1.09	e	0.48	h	1.06	h	0.328	hijklm
SMTRNN0	12.30	ghijk	20.79	klm	1.13	cde	0.60	a	1.87	a	0.328	hijklm
TLNAGSTR	11.78	ghijk	21.30	jklm	1.09	de	0.52	efgh	1.44	cdef	0.325	ijklm
TLNGNTLB	23.68	cdef	32.01	bc	1.30	a	0.50	fgh	1.30	defgh	0.318	jklmn
TLNSPNZZ	20.07	defghi	27.90	bcdefgh	1.19	abcde	0.51	fgh	1.33	cdefg	0.304	klmn
TRFRN00	13.68	fghijk	23.08	hijkl	1.16	bcde	0.56	abcdef	1.49	bcdef	0.265	lmn
TRPJST00	18.00	defghij	25.97	efghij	1.18	abcde	0.49	h	1.22	fgh	0.244	mn
TRPNTLNN	5.91	k	16.91	m	1.14	cde	0.55	abcdefg	1.37	cdef	0.236	n

Accession Code	SP (Degree)		MV^LV (Degree)		MV^LS (Degree)		MV^UV (Degree)		MV^US (Degree)	
BNDTTT0	140.96	ab	79.71	efg	72.63	efghijk	32.22	abcd	16.22	cdef
BXDTTT0	69.40	ghij	105.08	a	92.93	a	30.46	bcd	18.46	abcdef
BNFCZZN	138.02	ab	77.11	efgh	64.14	ijkl	28.37	d	15.11	ef
BDMNNVC	37.53	kl	75.95	efghi	61.82	jkl	31.66	abcd	14.53	f
BDNSTRL	128.67	abcd	81.79	efg	72.99	efghij	29.83	cd	16.73	bcdef
BRDNSTRN	135.91	abc	78.02	efgh	63.50	ijkl	30.08	cd	19.16	abcde
BRXNTRFR	19.27	l	104.37	a	89.46	abc	31.32	bcd	14.92	ef
BRRNTLN0	117.61	bcdef	78.85	efg	64.38	ijkl	29.68	cd	19.87	abcd
BRDPSTCC	116.06	bcdef	79.21	efg	71.78	efghijk	29.87	cd	15.86	def
BRNTRNB0	36.08	kl	96.00	abc	80.40	bcdef	37.10	a	20.70	abc
BRFVLTT0	50.96	jkl	85.31	cde	65.49	hijkl	33.00	abcd	19.10	abcde
CHIFCTRN	120.39	bcde	78.59	efgh	74.12	defghij	37.26	a	21.10	ab

Table 3. *Cont.*

Accession Code	SP (Degree)		MV^LV (Degree)		MV^LS (Degree)		MV^UV (Degree)		MV^US (Degree)	
CHIJST00	64.57	hijk	98.13	ab	91.79	ab	31.867	abcd	18.44	abcdef
NVPCLMBN	95.26	defgh	84.95	cdef	78.37	cdefg	32.24	abcd	18.51	abcdef
NVPDNNTR	137.88	ab	72.73	fghi	59.94	kl	32.10	abcd	16.60	bcdef
NVTNSTRN	103.30	cdefg	85.01	cdef	78.71	cdefg	32.93	abcd	19.14	abcde
NVPTRNBN	132.66	abc	79.31	efg	66.82	ghijk	33.84	abcd	17.47	abcdef
SMSAGSTN	142.68	ab	81.57	efg	70.48	fghijk	36.20	ab	21.80	a
SMSCLMBG	154.55	a	82.82	defg	71.80	efghijk	36.32	ab	17.68	abcdef
SMSCNTNB	156.66	a	63.75	i	52.72	l	31.66	abcd	14.49	f
SMSDTTTB	86.22	fghi	86.36	bcde	77.50	cdefgh	31.33	bcd	17.13	bcdef
SMSSPTRB	139.55	ab	78.15	efgh	68.97	fghijk	35.08	abc	16.61	bcdef
SMSTRNB0	142.56	ab	72.26	ghi	66.28	ghijk	34.08	abc	19.17	abcde
SMTRNN0	142.12	ab	66.223	hi	53.34	l	30.71	bcd	16.03	def
TLNAGSTR	61.98	hijk	80.83	efg	71.65	fghijk	32.84	abcd	17.55	abcdef
TLNGNTLB	53.15	ijkl	94.35	abcd	84.53	abcde	33.27	abcd	18.63	abcdef
TLNSPNZZ	62.97	hijk	96.54	abc	85.99	abcd	32.23	abcd	18.38	abcdef
TRFRN00	124.98	abcd	80.68	efg	70.73	fghijk	34.50	abc	15.85	def
TRPJST00	89.03	efgh	85.76	bcde	75.40	defghi	32.80	abcd	17.43	abcdef
TRPNTLNN	142.76	ab	75.31	efghi	68.17	fghijk	34.31	abc	18.39	abcdef

[(a)] Values are the mean of ten leaf (different letters represents significant differences at $p < 0.05$). Explanation of accession code and leaf traits are given in Tables 1 and 2, respectively.

In Figure 3D is reported the regression between the LLA and VD. The power type relationship between LLA and VD had a high coefficient of determination (R^2 = 0.91) and showed that small leaves had about 27% more veins per unit of leaf area than big leaves.

The PCA performed on the entire data set (300 leaves) showed that only part of the accessions was separated in different clusters. For example, in the space formed by PC1 and PC2, BRFVLTT0 partially overlapped BRNFCZNN, which was clustered in different groups when compared to TRPNTLNN, TLNAGSTR, BRXDTTT0, and BRDNSTRN (Figure 4). In Figure S1A, BRNTRNB0 was separated by other varieties. PC1 vs. PC3 and PC1 vs. PC4 did not show any other cluster among varieties (Figure S1A,B), while PC2 vs. PC3 showed that SMSCNTNB and SMSTRNN0 were well-separated from all other accessions (Figure S1C).

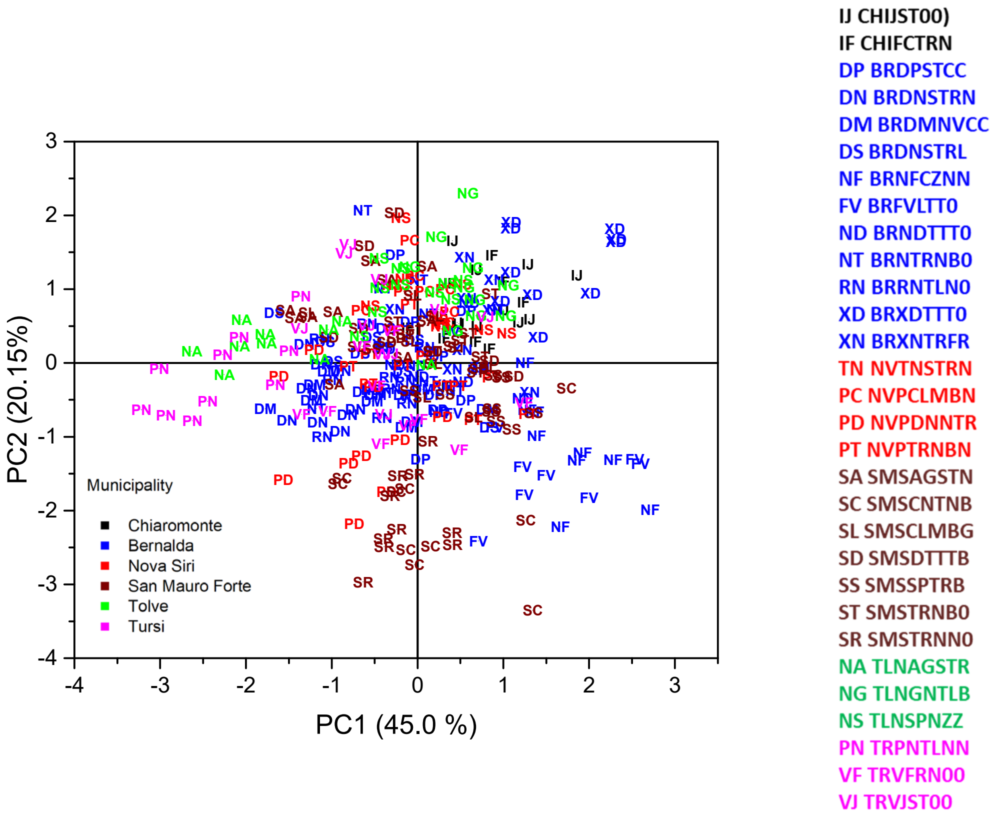

Figure 4. First two components of PCA analysis scores of 300 leaves of *Ficus carica* L. accessions.

The cumulated variance of the first, second, third, and fourth PCs account for 83.67% of the total variance (45.04%, 20.15%, 10.63%, and 8.08%, respectively, for PC1, PC2, PC3, and PC4) (Table 4). In Table 4, the loadings values above 0.20 or less than −0.20 are reported in bold. PC1 correlates positively with the size traits of leaves as, among others: LLP, LLL, LLW, and others. PC1 negatively correlates with vein density (VD). PC2 has an eigenvalue of 5.04, and proceeding from negative to positive loadings values correlates with CLL/LLL, CLW/CLL, LVL, and other traits related to MVL, UVL, LVL, PLS, CRC, and LLA/LLP (Table 4). PC3 and PC4 have eigenvalues of 2.66 and 2.02, respectively. PC3 showed loadings, above 0.20 or under −0.20, with traits mainly related to the shape of the base of the leaves, such for example PLS, PL, PUS, PSD, and SP (Table 4). PC4 correlates with the angle between the central vein and upper veins and LLL/LLW (Table 4).

Table 4. Loadings of the principal component axes from PCA of figs leaf accessions. For each PC, the eigenvalue and their contribution to total variance are reported. Loadings higher than 0.20 or lower than −0.20 are in bold.

Traits	PC1	PC2	PC3	PC4
LLP (mm)	**0.251**	−0.182	0.160	0.036
Vein Density (mm/mm^{-2})	**−0.268**	−0.151	−0.026	−0.020
MVL + LVL + UVL	**0.276**	−0.150	−0.067	0.024
LLL (mm)	**0.292**	0.036	0.051	−0.084
LLW (mm)	**0.273**	0.050	0.039	0.174
PL (mm)	**0.226**	−0.096	−0.008	0.056
MVL (mm)	**0.286**	0.003	−0.056	−0.118
UVL (mm)	**0.277**	−0.105	−0.068	−0.049
LVL (mm)	0.175	**−0.273**	−0.052	**0.216**
LLA/LLP (mm)	**0.237**	**0.222**	−0.161	−0.024
PSD (mm)	0.163	0.122	**0.361**	0.064
SP (degree)	−0.060	−0.178	**−0.391**	**0.218**
MV^LV (degree)	0.069	**0.322**	**0.340**	−0.114
MV^LS (degree)	0.050	**0.370**	**0.220**	−0.100
MV^UV (degree)	−0.022	0.140	0.176	**0.516**
MV^US (degree)	−0.026	**0.246**	0.126	**0.374**
PL + MVL (mm)	**0.287**	−0.036	−0.042	−0.059
CLL (mm)	**0.259**	−0.106	0.190	−0.025
CLW (mm)	**0.232**	0.194	−0.057	0.154
PUS (mm)	0.197	0.174	**−0.334**	−0.129
PLS (mm)	**0.218**	−0.010	**−0.222**	**0.207**
LLL/LLW	0.062	−0.027	0.031	−0.537
Circularity	−0.024	**0.352**	**−0.275**	−0.053
CLL/CLW	0.024	**−0.335**	**0.264**	−0.150
CLL/LLL	−0.014	**−0.282**	**0.294**	0.107
Eigenvalue	11.25	5.04	2.66	2.02
Contribution to total variance (%)	45.00	20.15	10.63	8.08

The correctness of LDA-based classification reached 92.31% and 91.18% for dark and green fruit skin, respectively (Tables 5 and 6). On a total of 30 fig accessions investigated, 7 with dark fruit skin and 10 with green fruit skin were all correctly classified (100%) (Table 5). In dark fruit skin, the accessions BRNFCZZN had the lowest percentage of correct classification (60%), one leaf was classified as BRDNSTRN, two leaves were classified as TLNAGSTR and another as TRPNTLNN.

Table 5. Given groups and percentage of correct classification using Linear Discriminant Analysis of fig accessions with dark skin.

Given Group	Number of Leaves	Correct Classification (n)	Correct Classification (%)	Predicted Group(s)		
BRDPSTCC	10	8	80	1 BRNFCZZN	1 NVPCLMBN	
BRDNSTRN	10	10	100			
BRDMNNVC	10	10	100			
BRNFCZZN	10	6	60	1 BRNFCZZN	2 TLNAGSTR	1 TRPNTLNN
BRFVLTT0	10	9	90	1 BRNFCZZN		
BRRNTLN0	10	10	100			
BRXNTRFR	10	9	90	1 TLNSPNZZ		
NVPCLMBN	10	9	90	1 BRDPSTCC		
SMSAGSTN	10	10	100			
SMSTRNN0	10	10	100			
TLNAGSTR	10	10	100			
TLNSPNZZ	10	10	100			
TRPNTLNN	10	9	90	1 BRRNTLN0		
Total Average	130	120	92.31			

Table 6. Given groups and percentage of correct classification using linear discriminant analysis of fig accessions with green skin.

Given Group	Number of Leaves	Correct Classification (n)	Correct Classification (%)	Predicted Groups	
CHIJST00	10	10	100		
CHIFCTRN	10	10	100		
BRDNSTRL	10	10	100		
BRNDTTT0	10	10	100		
BRNTRNB0	10	10	100		
BRXDTTT0	10	10	100		
NVTNSTRN	10	9	90	1 SMSDTTTB	
NVPDNNTR	10	8	80	2 TRVFRN00	
NVPTRNBN	10	10	100		
SMSCNTNB	10	10	100		
SMSCLMBG	10	9	90	1 NVPTRNBN	
SMSDTTTB	10	6	60	2 NVTNSTRN	2 TRVJST00
SMSSPTRB	10	10	100		
SMSTRNB0	10	10	100		
TLNGNTLB	10	9	90	1 CHIJST00	
TRVFRN00	10	7	70	1 NVPDNNTR	2 SMSSPTRB
TRVJST00	10	7	70	2 BRDNSTRL	1 NVTNSTRN
Total Average	170	155	91.18		

In green fruit skin, six leaves of SMSDTTTB from San Mauro Forte, and seven leaves of TRVFRN00 and TLVJST00 from Tursi, were correctly classified to the given groups (Table 6). For example, two leaves of SMSDTTTB were classified as TRVJUST00 and two as NVTNSTRN.

In Figure 5 and Figure S2A (axis one vs. axis three), S2B (axis one vs. axis four), and S2C (axis two vs. axis three), the accessions SMSTRNN0, BRFVLTT0, BRXNTRFR, TRPNTLNN, SMSAGSTN, were well-separated from other groups that showed some overlap.

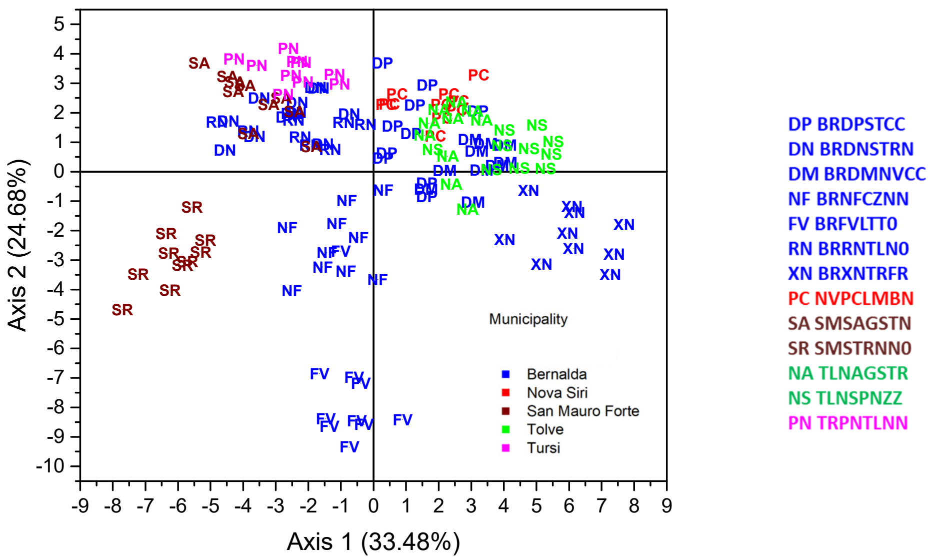

Figure 5. First two component of the linear discriminant analysis, scores 130 leaves of *Ficus carica* L. of the accessions with dark skin fruit.

Additionally, accessions with green fruit skin showed a reasonable separation and a certain degree of overlap for some accessions (Figure 6 and Figure S3A–C). In this set of accessions, the name 'Troiana/o' is present in four accessions (BRNTRNB0, CHIFCTRN, NVPTRNBN, SMSTRNB0) located at different altitudes. Three local varieties had the name 'Dottato' and were from Bernalda (BRNDTTT0, BRXDTTT0), and San Mauro Forte (SMSDTTTB). The name of 'Justa' was reported for two local varieties of two different sites, Chiaromonte (CHIJST00) and Tursi (TRVJST00).

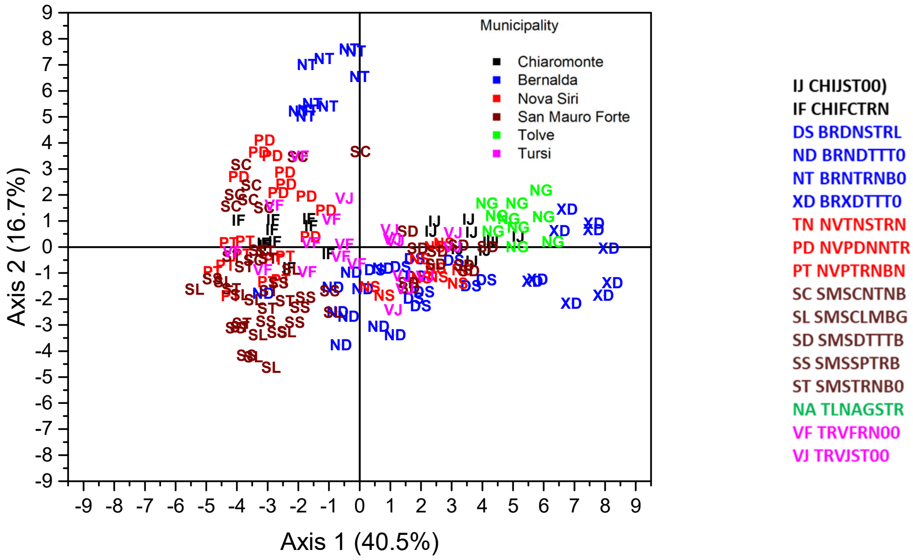

Figure 6. Linear discriminant analysis scores 170 leaves of *Ficus carica* L. of the accessions with green skin fruit.

LDA discriminated the local varieties named 'Troiana/o' indicating that the four accessions could be synonymous (i.e., four different genotypes with the same name) (Figure 7A). The LDA showed that the two local varieties of 'Dottato' from Bernalda (BRNDTTT0, BRXDTTT0), growing in the same environment, could be synonymous, while accessions SMSDTTTB from San Mauro Forte could be homonymous (i.e., same accessions called with different name) of the accession TRVJST00 from Tursi (Figure 7B).

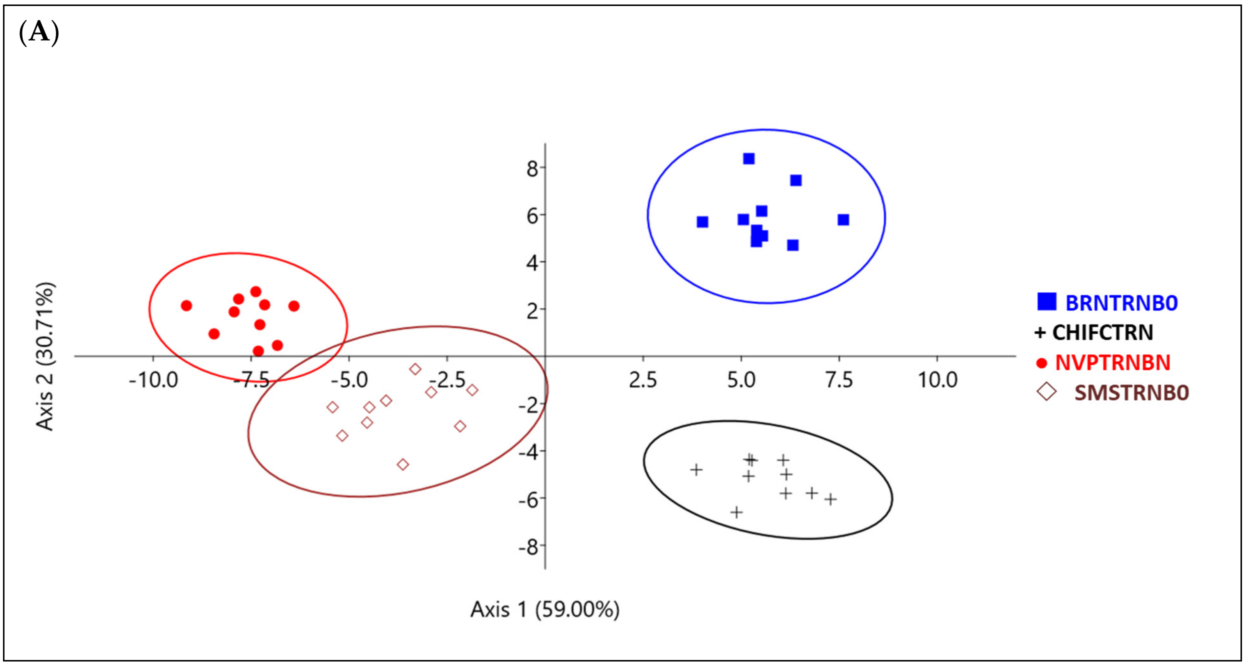

Figure 7. *Cont.*

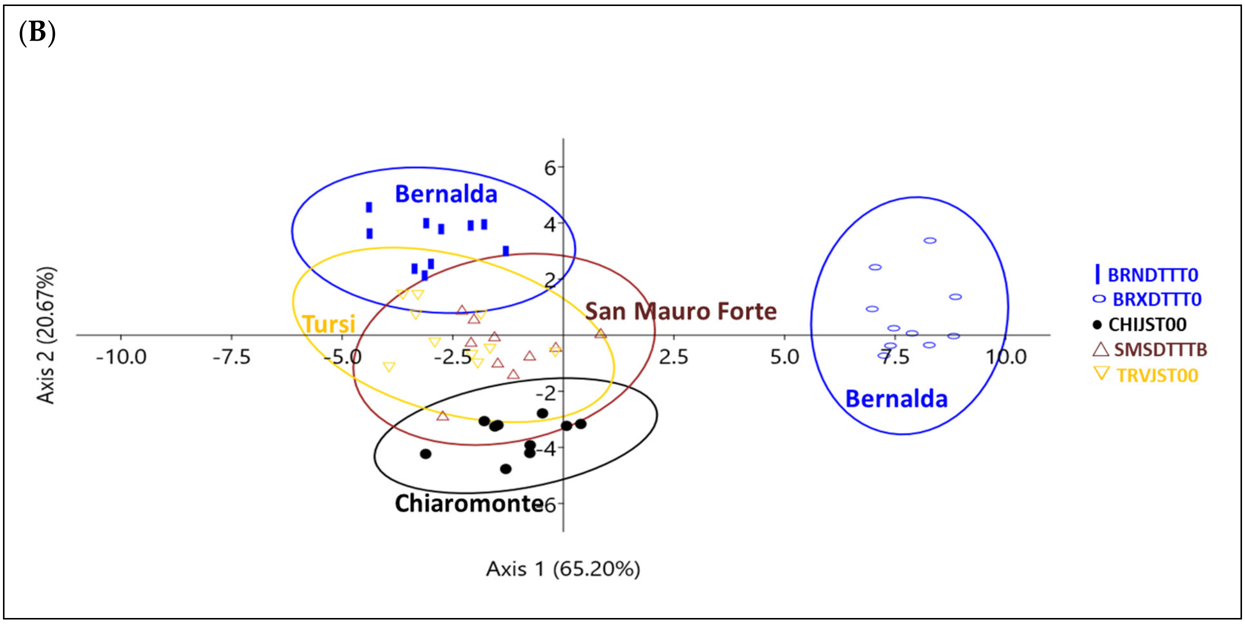

Figure 7. Linear discriminant analysis applied on selected synonymous of accessions figs. (**A**) Scores of BRNTRNB0, CHIFCTRN, NVPTRNBN, and SMSTRNB0 accessions. (**B**) BRNDTTT0, BRXDTTT0, SMSDTTTB, CHJST00, and TRVJST00.

4. Discussions

This study shows that the use of leaf morphometry aided by image analysis might help accessions classification. The LDA performed on the measured leaf traits extracted through imaging had an accuracy higher than 90% in both black and green skin. For BRNDTTT0, BRXDTTT0 with the same name (e.g., 'Dottato') sampled at similar sites (Bernalda) the LDA highlighted that they were morphometrically different, suggesting a putative genetic divergence (Figure 7A). On the contrary, the TRVJST00 ('Justa' of Tursi) shared the same LDA space of SMSDTTTB ('Dottato Bianco' of San Mauro Forte), suggesting that the genetic component is more relevant than environmental one.

Some of the local names (Troiana/o, Dottata/o, San Pietro, Gentile, Ficazzana/o, and Columbro or Columbraro) used in Basilicata have already been reported in the literature since 1583 [6]. However, the descriptions reported by [7,8] do not provide sufficient details to unravel the classification of the local varieties examined in the present study. Because the LDA maximizes the between-groups differences, while the within-group are minimized [34], it was possible to discriminate among accessions (Figure 7A,B).

Leaf morphological traits may be influenced by genetics, development, and biotic or abiotic factors [36]. For example, the leaf venation length and distribution and lamina leaf area may represent the response to water availability, light exposure, and other environmental factors [37]. Moreover, viruses or fungal infections may cause leaf deformation [24]. Thus, it is mandatory to consider biotic and abiotic conditions when comparing inter-individual variation of leaf morphological traits of different accessions, choosing appropriate experimental units. Size is also a critical morphological and physiological trait of the leaf because it determines the photosynthetic and the transpiration capacity of the leaf (= exchange of CO_2, H_2O and O_2) [38,39]. The size of the leaf is influenced by genetic and environmental factors (and their interaction) [36], such as temperature [40], soil fertility [41], water availability [42], and solar radiation [39]. Moreover, some size-related traits have been used to differentiate species and varieties [20,21,24,30]. Remarkably, the IPGRI fig descriptor list [8] recommends using LLL, LLW, CLL, PD, LLA, and PL to estimate leaf size.

The average values of LLL, LLW, and PL of the Basilicata accessions were similar to the figs accessions or local varieties characterized by [11,14,16,43] in India, Iran, Brazil, and Tunisia, respectively.

According to [31,44] the relationship between leaf area and leaf perimeter seems to be independent of environmental stress, even if environmental factors can be responsible for a change in leaf shape [45]. The accessions reported in this paper have shown a determination coefficient between LLP and LLA of (R^2 = 0.82) suggesting that these two parameters may reliably be used to characterize fig germplasm. To the best of our knowledge, the relationships between perimeter versus leaf area have not been explored. Further investigation is required to understand if the relationship between leaf perimeter and area can be correlated to environmental constraints.

Leaf veins provide physical support for leaf lamina and provide an efficient transport system to supply water and assimilate to nourish photosynthesis and transpiration [37]. In dicotyledons, the vein system has a hierarchical organization, the first three-order veins form the major veins system, and the higher order of veins forms the minor veins system. The length of the veins per unit of leaf area is defined as vein density [33,37]. It has been reported [33,37,46] that high major vein density may improve leaf functions, including leaf hydraulic conductance and photosynthesis and tolerance to drought.

Fig leaves have multiple first-order veins, the accessions reported in this work have a wide range of first-order veins length (TVL ranged from about 300 to 900 mm) and a first-order vein density (VD) of ≈0.55 cm cm^{-2} in small leaves of TRPNTLNN and TLNAGSTR, while big leaves of BRXDTTT0, BRFVLTTO and BRNFCZNN have a significantly lower value of VD (≈0.15 cm cm^{-2}) (Figure 3D). The values measured in figs of these accessions were higher than values reported by [33] for different species. Fig VD should be further investigated, including other major veins (2nd- and 3rd-order) and minor veins system, to understand if accessions with high vein density are more resistant to more stressful environmental conditions.

In *Ficus carica* L., the aspect of the base of the leaf is one of the primary descriptors of the leaf shape [7] and was also one primary source of variation observed by [16], which compared different Persian varieties of fig, most of them with a calcarate or cordate leaf base.

The importance of this region to differentiate among varieties is well-known in figs [6,7] and grapevine [47,48] and recently used by [49] in morphometric analysis of different *Vitis* specie. In this paper, the base of the leaf was described by measuring the petiolar sinus angle (SP) and the petiole sinus depth (PLS) (Figure 3D).

The principal component analysis performed on all data sets suggests that the genetic structure is the principal source of variance. PC1 has high loadings for all traits related to leaf size and vein density, according to classical morphometric studies [35,50]. The PC2 has high loadings with leaf lobations traits, and PC3 and PC4 with traits related to leaf width (PUS and UVL) and the shape of the leaf base (Figure 5). When measured, the same leaf traits were also found in PCs of different fig accessions of Iran [12,13,16], Tunisia [11], Libya and Egypt [51], Morocco [13,15], in Mulberry genotypes [23], and in grapevine [52].

As highlighted by Chitwood and coauthors [52], leaf shape differences due to genetic and environmental factors are largely independent and additive, giving the opportunity to identify leaves arising from different varieties or, on the other hand, to recognize the effects of the environmental factor on leaf development (see for example [52,53]).

Using linear discriminant analysis (LDA) on traditional morphometric leaf traits correctly classifies more than 90% of the leaves. In our data set, there were the same accessions collected in different sites with a similar name, such as 'Nostrana nera', 'Nostrana' or 'Nostrale bianca', 'Justa', 'Natalino', 'Dottato' or 'Dottato bianco', and 'Troiana bianca', 'Troiano bianco', 'Troiano nero' and 'Troiana'.

In some cases, the name is adjectivized with the fruit skin color, 'nera' or 'nero' (black), and 'bianca' or 'bianco' (white). The presence of the adjective is important to discriminate among varieties, but in our data set, there were four 'Troiana/o' and three 'Dottato' with similar fruit skin color. LDA identified the group of 'Troiana' (BRNTRNB0, CHIFCTRN, NVPTRNBN, and SMSTRNB0), the two 'Dottato' of Bernalda (BRNDTTT0, BRXDTTT0) and the two 'Justa' (CHIJST00, TRVJST00) as synonymous (Figure 7A,B), and SMSDTTTB homonymous of TRVJUST00.

In the group of accessions with dark skin fruit, genetic differences in size and shape among BRRNTLN0 and BRDNSTRN from Bernalda, and TRPNTLNN from Tursi may be masked by environmental factors (Figure 5 and Figure S2A–C).

The application of analytical methods able to separate shape from size may solve some uncertainties intrinsic to multivariate morphometric analysis [54]. Ultimately, the morphological traits measured and analyzed in this study were effective in identifying some fig genotypes and, as proposed in other studies [11–13,15,16,23,51,54], may help in identification and first evaluation of fig germplasm.

5. Conclusions

The analysis of morphological traits, even if they may be affected by environmental conditions and management practices, effectively supported characterization and group separation of the accessions. This study hypothesized that some easy-to-recognize and measurable leaf morphological traits could help to discriminate among fig accessions in in situ conditions. The sampling unit and the leaf morphological traits selected, combined with multivariate data analysis procedures, supported the identification of some accessions solving for homonymies and synonyms. This may be especially important for implementing biodiversity conservation programs based on an ex situ core collection or optimizing the sample size to be analyzed by, for example, molecular markers.

Finally, this study proposes using digital image and open-source image analysis software as accurate, simple, and time-saving procedures for extracting many leaf morphological traits.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su142315970/s1, Figure S1: Principal components scores of 300 leaves of Ficus carica L. accessions (A) PC1 versus PC3; (B) PC1 versus PC4; and (C) PC2 versus PC3; Figure S2: Linear discriminant analysis, scores 130 leaves of Ficus carica L. of the accessions with dark skin fruit (A) PC1 versus PC3; (B) PC1 versus PC4; and (C) PC2 versus PC3; Figure S3: Linear discriminant analysis, scores 170 leaves of Ficus carica L. of the accessions with green skin fruit. (A) Ax1 versus Ax3; (B) Ax1 versus Ax4, and (C) Ax2 versus Ax3; Table S1: Site, accession name and geographical origin of the genotype under investigation.

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References

1. Stover, E.; Aradhya, M.; Fergunson, L.; Crisosto, C.H. The fig: Overview of an ancient fruit. *Hortscience* **2007**, *42*, 1083–1087. [CrossRef]
2. Food and Agriculture Organization. Data Archives. FAOSTAT. 2022. Available online: https://www.fao.org/faostat/en/#data (accessed on 13 October 2022).
3. Istituto Italiano di Statistica. StatBase, Coltivazioni. 2022. Available online: http://dati.istat.it/viewhtml.aspx?il=blank&vh=0000&vf=0&vcq=1100&graph=0&view-metadata=1&lang=it&QueryId=33654&metadata=DCSP_COLTIVAZIONI# (accessed on 13 October 2022).
4. Flaishman, M.A.; Rodov, V.; Stover, E. Fig: Botany, horticulture, and breeding. *Hortic. Rev.* **2008**, *34*, 113–197.
5. Richardson, A.D.; Keenana, T.F.; Migliavacca, M.; Ryua, Y.; Sonnentaga, O.; Toomey, M. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agric. For. Meteorol.* **2013**, *169*, 156–173. [CrossRef]
6. Condit, I.J. Fig varieties: A monograph. *Hilgardia* **1955**, *23*, 323–538. [CrossRef]
7. Condit, I.J. Fig characteristics useful in the identification of varieties. *Hilgardia* **1941**, *14*, 1–69. [CrossRef]
8. *Descriptors for Fig. International Plant Genetic Resources Institute, Rome, Italy, and International Centre for Advanced Mediterranean Agronomic Studies*; IPGRI and CIHEAM: Paris, France, 2003.
9. Saddoud, O.; Baraket, G.; Chatti, K.; Trifi, M.; Marrakchi, M.; Salhi-Hannachi, A.; Mars, M. Morphological variability of fig (*Ficus carica* L.) cultivars. *Int. J. Fruit Sci.* **2008**, *8*, 35–51. [CrossRef]
10. Giraldo, E.; Lopez Corrales, M.; Hormaza, J.I. Selection of the most discriminating morphological qualitative variables for characterization of fig germplasm. *J. Am. Soc. Hortic. Sci.* **2010**, *135*, 240–249. [CrossRef]
11. Gaaliche, B.; Saddoud, O.; Mars, M. Morphological and pomological diversity of Fig (*Ficus carica* L.) cultivars in Northwest of Tunisia. International Scholarly Research Network. *Agronomy* **2012**, *2012*, 326461.
12. Khadivi-Khub, A.; Anjam, K. Characterization and evaluation of male fig (caprifig) accessions in Iran. *Plant Syst. Evol.* **2014**, *300*, 2177–2189. [CrossRef]
13. Khadivi, A.; Mirheidari, F. Selection of the promising fig (*Ficus carica* L.) accessions using fruit-related characters. *Food Sci. Nutr.* **2021**, *10*, 2911–2921. [CrossRef]
14. Mir, M.M.; Kumar, A.; Iqbal, U.; Mir, S.A.; Rehman, M.U.; Banday, S.A.; Rather, G.H.; Fayaz, S. Characterization of fig (*Ficus carica* L.) germplasm in central Kashmir of north western Himalayan region. *Indian J. Plant Genet. Resour.* **2018**, *31*, 57–63. [CrossRef]
15. Hssaini, L.; Hanine, H.; Razouk, R.; Ennahli, S.; Mekaoui, A.; Ejjilani, A.; Charafi, J. Assessment of genetic diversity in Moroccan fig (*Ficus carica* L.) collection by combining morphological and physicochemical descriptors. *Genet. Resour Crop Evol.* **2019**, *67*, 457–474. [CrossRef]
16. Baziar, G.; Jafari, M.; Noori, M.S.S.; Samarfard, S. Persian Fig Cultivars by Morphological Traits and RAPD Markers. *Hortscience* **2018**, *53*, 613–619. [CrossRef]
17. Chessa, I.; Nieddu, G. Analysis of diversity in the fruit tree genetic resources from a Mediterranean island. *Genet. Resour. Crop Evol.* **2005**, *52*, 267–276. [CrossRef]
18. Aradhya, M.K.; Stover, E.; Velasco, D.; Koehmstedt, A. Genetic structure and differentiation in cultivated fig (*Ficus carica* L.). *Genetica* **2010**, *138*, 681–694. [CrossRef] [PubMed]
19. Achtak, H.; Oukabli, A.; Ater, M.; Santoni, S.; Kjellberg, F.; Khadari, B. Microsatellite markers as reliable tools for fig cultivar identification. *J. Am. Soc. Hortic. Sci.* **2009**, *134*, 624–631. [CrossRef]
20. Garcia-Muñoz, S.; Muñoz-Organero, G.; de Andrés, M.T.; Cabello, F. Ampelography—An old technique with future uses: The case of minor varieties of Vitis vinifera L. from the Balearic Islands. *OENO One* **2011**, *45*, 125–137. [CrossRef]
21. Cope, J.S.; Corney, D.; Clark, J.Y.; Remagnino, P.; Wilkin, P. Plant species identification using digital morphometrics: A review. *Expert Syst. Appl.* **2012**, *39*, 7562–7573. [CrossRef]
22. Rohlf, F.J.; Marcus, L.F. A revolutions in morphometrics. *Tree* **1993**, *8*, 129–132.
23. Lo Bianco, R.; Mirabella, F. Use of leaf and fruit morphometric analysis to identify and classify white mulberry (*Morus alba* L.) genotypes. *Agriculture* **2018**, *8*, 157. [CrossRef]
24. Klein, L.L.; Caito, M.; Chapnick, C.; Kitchen, C.; O'Hanlon, R.; Chitwood, D.H.; Miller, A.J. Digital morphometrics of two north american arapevines (*Vitis: Vitaceae*) quantifies leaf variation between species, within species, and among individuals. *Front. Plant Sci.* **2017**, *8*, 373. [CrossRef] [PubMed]
25. Migicovsky, Z.; Li, M.; Chitwood, D.H.; Myles, S. Morphometrics Reveals Complex and Heritable Apple Leaf Shapes. *Front. Plant Sci.* **2018**, *8*, 2185. [CrossRef] [PubMed]
26. Podgornik, M.; Vuk, I.; Vrhovnik, I.; Bandelj, D. A survey and morphological evaluation of fig (*Ficus carica* L.) genetic resources from Slovenia. *Sci. Hortic.* **2010**, *125*, 380–389. [CrossRef]
27. Peel, M.C.; Finlayson, B.L.; McMahon, T.A. Updated world map of the Koppen-Geiger climate classification. *Hydrol. Earth Syst. Sci.* **2007**, *11*, 1633–1644. [CrossRef]
28. Rasband, W.S. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, 1997–2018. Available online: https://imagej.nih.gov/ij/ (accessed on 13 October 2022).

29. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671–675. [CrossRef]
30. Oso, O.A.; Jayeola, A.A. Digital Morphometrics: Application of Morpholeaf in shape visualization and species delimitation, using Cucurbitaceae leaves as a model. *Appl. Plant Sci.* **2021**, *9*, e11448. [CrossRef]
31. Shi, P.; Niinemets, Ü.; Hui, C.; Niklas, K.J.; Yu, X.; Hölscher, D. Leaf bilateral symmetry and the scaling of the perimeter vs. the surface area in 15 vine species. *Forests* **2020**, *11*, 246. [CrossRef]
32. Viscosi, V. Geometric morphometrics and leaf phenotypic plasticity: Assessing fluctuating asymmetry and allometry in European white oaks (Quercus). *Bot. J. Linn. Soc.* **2015**, *179*, 335–348. [CrossRef]
33. Scoffoni, C.; Rawls, M.; McKown, A.; Cochard, H.; Sack, L. Decline of leaf hydraulic conductance with dehydration: Relationship to leaf size and venation architecture. *Plant Physiol.* **2011**, *156*, 832–843. [CrossRef]
34. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Paleontol. Electron.* **2001**, *4*, 9.
35. Iezzoni, A.F.; Pritts, N.P. Applications of principal component analysis to Horticultural Research. *Hortscience* **1991**, *26*, 334–338. [CrossRef]
36. Fleming, A.J. The control of leaf development. *New Phytol.* **2005**, *166*, 9–20. [CrossRef] [PubMed]
37. Sack, L.; Scoffoni, C. Leaf venation: Structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytol.* **2013**, *198*, 983–1000. [CrossRef] [PubMed]
38. Smith, W.K.; Vogelmann, T.C.; DeLucia, E.H.; Bell, D.T.; Shepherd, K.A. Leaf form and phtosynthesis. *BioScience* **1997**, *47*, 785–793. [CrossRef]
39. Ren, T.; He, N.; Liu, Z.; Li, M.; Zhang, J.; Li, A.; Wei, C.; Lü, X.; Han, X. Environmental filtering rather than phylogeny determines plant leaf size in three floristically distinctive plateaus. *Ecol. Indic.* **2021**, *130*, 108049. [CrossRef]
40. Moles, A.T. Being John Harper: Using evolutionary ideas to improve understanding of global patterns in plant traits. *J. Ecol.* **2018**, *106*, 1–18. [CrossRef]
41. Reich, P.B.; Oleksyn, J. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11001–11006. [CrossRef]
42. Moles, A.T.; Perkins, S.E.; Laffan, S.W.; Flores-Moreno, H.; Awasthy, M.; Tindall, M.L.; Sack, L.; Pitman, A.; Kattge, J.; Aarssen, L.W.; et al. Which is a better predictor of plant traits: Temperature or precipitation? *J. Veg. Sci.* **2014**, *25*, 1167–1180. [CrossRef]
43. Rodrigues, M.G.F.; dos Santos, T.P.; Ferreira, A.F.A.; Monteiro, L.N.H.; Nakanishi, E.S.S.; Boliani, A.C. Morphological characterization of active germoplasm bank fig tree accessions. *Rev. Bras. Frutic. Jaboticabal* **2019**, *41*, e-074. [CrossRef]
44. Yu, X.; Shi, P.; Hui, C.; Miao, L.; Liu, C.; Zhang, Q.; Feng, C. Effects of salt stress on the leaf shape and scaling of *Pyrus betulifolia* Bunge. *Symmetry* **2019**, *11*, 991. [CrossRef]
45. Viscosi, V.; Lepais, O.; Gerber, S.; Fortini, P. Leaf morphological analyses in four European oak species (Quercus) and their hybrids: A comparison of traditional and geometric morphometric method. *Plant Biosys.* **2009**, *143*, 564–574. [CrossRef]
46. Sack, L.; Scoffoni, C.; John, G.P.; Poorter, H.; Mason, C.M.; Mendez-Alonzo, R.; Donovan, L.A. How do leaf veins influence the worldwide leaf economic spectrum? Review and synthesis. *J. Exp. Bot.* **2013**, *64*, 4053–4080. [CrossRef] [PubMed]
47. Viala, P.; Vermorel, V. *Ampelographié: Traité General de Viticulture*; Tome I. Masson and Cie: Paris, France, 1909.
48. Galet, P. *Précis d'Ampélographie Pratique*; Impr. P. Déhan: Montpellier, France, 1952.
49. Chitwood, D.H.; Ranjan, A.; Martinez, C.C.; Headland, L.R.; Thiem, T.; Kumar, R.; Covington, M.F.; Hatcher, T.; Taylor, D.T.; Zimmerman, S.; et al. A modern ampelography: A genetic basis for leaf shape and venation patterning in Grape. *Plant Physiol.* **2014**, *164*, 259–272. [CrossRef] [PubMed]
50. Bookstein, F.L. "size and shape": A comment on semantic. *Syst. Zool.* **1989**, *38*, 173–180. [CrossRef]
51. Abdelsalam, N.R.; Awad, R.M.; Ali, H.M.; Salem, M.Z.M.; Abdellatif, K.F.; Elshikh, M.S. Morphological, pomological, and specific molecular marker resources for genetic diversity analyses in Fig (*Ficus carica* L.). *Hortscience* **2019**, *54*, 1299–1309. [CrossRef]
52. Chitwood, D.H.; Rundell, S.M.; Li, D.Y.; Woodford, Q.L.; Yu, T.T.; Lopez, J.R.; Greenblatt, D.; Kang, J.; Londo, J.P. Climate and developmental plasticity: Interannual variability in grapevine leaf morphology. *Plant Physiol.* **2016**, *170*, 1480–1491. [CrossRef]
53. Viscosi, V.; Cardini, A. Leaf Morphology, Taxonomy and Geometric Morphometrics: A Simplified Protocol for Beginners. *PLoS ONE* **2011**, *6*, e25630. [CrossRef]
54. Milijković, D.; Stefanović, M.; Orlović, S.; Neđić, M.S.; Kesić, L.; Stojnić, S. Wild cherry (*Prunus avium* (L.) leaf shape and size variations in natural populations at different elevations. *Alp. Bot.* **2019**, *129*, 163–174. [CrossRef]