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Morphological variability in leaves of Chinese wild *Vitis* species

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ABSTRACT

China is one of the major origination for *Vitis* species, especially Chinese wild *Vitis* species. However, the leaf shape diversity among Chinese wild *Vitis* species remains unclear. Digital morphometric analysis was used to study the morphological variability in leaves of 59 Chinese wild grape accessions. Leaves were scanned and images were analyzed using normalized Elliptic Fourier Descriptors (EFD) and principal component analysis. The ampelometric traits of these leaves revealed a high degree of diversity within Chinese wild *Vitis* species. Principal component analysis (PCA) showed that the first three principal components accounted for 71% of the variation. Elliptic Fourier Descriptors and Generalised Procruste Analysis (GPA) were used to provide a global analysis of leaf outlines and lobe positioning and to describe differences in relative vein positions, respectively. A dendrogram was constructed based on the shape traits, in which almost all of the individuals were clearly distinguished. These findings suggest that digital morphometrics is a powerful tool for assessing leaf shape variations among grape species and would be of great use for the conservation and utilization of Chinese wild *Vitis* species.

1. Introduction

Grape has been recognized as one of the most important fruit crops in the world because of their wide use as fresh fruits and sources of wine and raisins. They can be found in South Europe, Asia Minor, East Asia, and North and Central America (Wan et al., 2008), and their worldwide distribution may be due to their high level of biodiversity. Researches concerning biodiversity is of great help for the efficient evaluation, conservation, management, and utilization of grape germplasm resources (Jing et al., 2013). In recent years, relevant work on grapes has primarily involved the taxonomy and germplasm identification, which have been completed based on morphological (Alba et al., 2014; Klein et al., 2017; Ma et al., 2016), palynological (Najmaddin, 2014; Roytchev, 1997), anatomical (Ma et al., 2016), isoenzymatic (Altube et al., 1991; Calderon et al., 2015) and some molecular biological approaches (Crespan et al., 2015; Jing et al., 2013; Tantasawat et al., 2012). Among these approaches, morphological analysis is a traditional method, simple and intuitive, without the need for sophisticated equipment or laborious procedures (OIV 2009). Ampelography is a discipline originally developed to identify morphological variations in the leaf shape of cultivated *V. vinifera* L. varieties (Galet, 1952). Over the past several decades, the technique has advanced from manually acquired measurements of veins, sinuses, and teeth (Galet, 1979), to a more precise, automated digital approach utilizing scanned leaf images—namely the digital morphometrics (Chitwood et al., 2014). This allows researchers to operate indoors on the digitalized pictures (Chitwood et al., 2014) or even specimens (Tomaszewski and Górzkowska, 2016) of detached grape leaves, which is time-efficient. A series of new programs or software packages like the grape leaf analysis software SuperAmpelos (Soldavini et al., 2009), LAMINA (Bylesj et al., 2008) and SHAPE (Iwata and Ukai, 2002) have been successively developed, and the Elliptic Fourier Descriptors (EFDs) of leaf outlines and Generalised Procrustes analysis were identified to be especially useful in global analysis of leaf shape (Cope et al., 2012; Rumpunen and Bartish, 2002), all of which make leaf shape assessment easy, fast, objective and inexpensive. Owing to these advantages, digital morphometrics has been extensively employed to describe leaf shape and distinguish *V. vinifera* varieties (Alba et al., 2014; Chitwood et al., 2014), as well as *V. vinifera* hybrids, and among *Vitis* and Ampelopsis species (Chitwood et al., 2016b). Recently, grape leaf variations between

Abbreviations: PCA, principal component analysis; GPA, Generalised Procruste Analysis; SSR, Simple Sequence Repeat; AFLP, Amplified Fragment Length Polymorphism; SRAP, Sequence-Related Amplified Polymorphism; Circ, Circularity; AR, Aspect Ratio; UPGMA, Unweighted Pair Group Method with Arithmetic Average; EFDs, Elliptic Fourier Descriptors

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species, within species and among individuals were also quantified using this method (Klein et al., 2017). In short, this is a reliable method to quantify variations in leaf morphology and to identify different germplasm resources.

China is one of the primary origins of grape, with over 35 native *Vitis* species (Wan et al., 2008). Chinese wild *Vitis* species have many excellent characteristics, such as strong resistance to biotic (Khiavi et al., 2009) and abiotic stress (Wang et al., 2004; Zhang et al., 2015), high sugar content, moderate acidity (Jiang et al., 2017), etc. Over the past several years, grape breeders have aimed to select progenies with better characteristics. This has resulted in many intermediate and transitional types (Jing et al., 2013), which has caused some controversy and confusion for taxonomists. Researchers have clarified these relationships by using the classical Galet's ampelometric determination method (Song et al., 2004; Song et al., 2008) and the molecular markers (Guo et al., 2012; Jing et al., 2013) technology. However, the classification of Chinese wild *Vitis* species remains unclear and there is still no uniform standard for describing and identifying grape species.

In the present study, digital morphometric analysis was used to characterize the blade variations among 59 Chinese wild grapevine accessions collected from a same environmental condition, thus facilitating the description, identification, and utilization of these precious Chinese grape germplasm resources. To our knowledge, this is the first attempt to examine relationships among Chinese wild *Vitis* species using modern digital morphometrics.

2. Materials and methods

2.1. Plant material and photography

Leaf samples were harvested from the *Vitis* germplasm repository of the Fruit Research Institute, Chinese Academy of Agricultural Sciences (Zhengzhou, China). Approximately 600 mature leaves were collected from 59 accessions (10 blades from each) representing 19 Chinese native wild *Vitis* species (Table 1) on June 10, 2015. Mature and fully expanded leaves were randomly selected from the midpoint of several shoots of representative plants with reference to Chitwood et al. (2014). After collection, samples were placed into Ziploc bags and stored in a 4 °C cooler until they were photographed. Leaves were then placed on a small vertical studio with a scale beside them, and the camera was attached to a tripod. All photographs were taken within 24 h post-harvest.

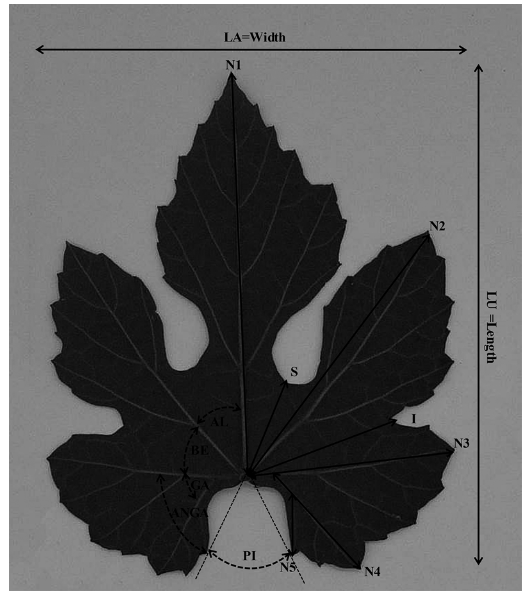

Fig. 1. Leaf quantitative traits recorded on mature leaf.

Table 1
Vitis materials used in this study.

Species (var.) name	Code No.	accessions	Origin	Species (var.) name	Code No.	accessions	Origin
V. davidii Foëx	A1	Fujian-4	R3		J31	Jiangxi-2	
	A2	Lueyang-4			J32	Liuba-1	
	A3	Tangwei			J33	Liuba-11(red)	
	A4	Xuefeng			J34	Liuba-7	
	A5	Ningqiang-6			J35	Pingli-7	
V. piasezkii Maxim	B6	Liu-6	R2	*V.* spp (Qiufuyie)	K36	Pingli-2	R2
	B7	Liu-9			K37	Meixian-6	
	B8	Nanzheng-2		*V. heyneana* Roem. & Schult subsp. *ficifolia* C. L. Li	L38	83-4-67	R2
V. pseudoreticulata W. T. Wang	C9	Baihe	R4		L39	Shandong	
	C10	Baihe-13			L40	Weinan-3	
	C11	Baihe-13-1		*V. amurensis* Rupr	M41	Huaxian-47	R1
	C12	Guangxi-1			M42	Shuangyou	
	C13	Hunan-1			M43	Taishan-11	
V. hancockii Hance	D14	Jiangxi-3	R3		M44	Tonghua-3	
V. davidii Foëx var. *cyanocarpa*	E15	Langao-5	R3		M45	Zuoshan-1	
V. bashanica He P.C	F16	Baihe-41	R2		M46	Zuoshan-2	
	F17	Baihe-42		*V. piasezkii* Maxim. Var. *Pagnuccii* Rehd.	N47	Baishui-40	R2
V. baihensis L. × . Niu	G18	Maihuang-1	R3		N48	Huaxian-1	
	G19	Baihe-40			N49	Tianshui-91	
V. heyneana Roem. & Schult	H20	83-4-49	R2	*V. adenoclada* Hand. -Mazz.	O50	Guangxining	R4
	H21	83-4-96		*V. tiubaensis* Niu L. × .	P51	Langao-2	R3
	H22	Dan-2			P52	Liuba-10	
	H23	Shang-24		*V. amurensis* var. *yanshanensis* D. Z. Lu et H. P. Liang	Q53	Yanshan-1	R2
	H24	Taishan-12		*V. bryoniifolia* Bge.	R54	Yingyu-1	R3
	H25	Nanzheng-1			R55	Anlin-28	
V. qinlingensis He P.C.	I26	Lueyang-8	R2		R56	Taishan-1	
	I27	Pingli-5		*V. zhejiang-adstricta* P. L. Qiu	S57	Zhejing-1	R3
V. romaneti Roman. du Caill. ex Planch	J28	Qiu-1	R3		S58	Anlin	
	J29	Baihe-22			S59	Anlin-18	
	J30	Jiangxi-1(green)					