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Mature Leaf Features of Wild Grapevine: Populations grown in Three Different River Valleys of North Albania

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Abstract

Wild grapevine (*Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi) is one of the most ancient and disseminated species in the riverbanks, forests and villages of the Northern Albania. An ampelographic and ampelometric study of the mature leaf characters was carried out during 2009-2013 in three wild grapevines populations, located in three river basins. Individuals of the wild grapevines populations belongig to the Mati River Valley, the lower part of Drini Valley and Shkreli Valley, were compared by means of mature leaf characters, using IPGRI, OIV and UPOV ampelographic methods. There was found that from twentseven observed, measured and evaluated mature leaf characters, fifteen of them did not show any significant differences between three wild grapevine populations, while for twelve characters, such as size of blade (length of N_1 and length of petiole), number of lobes, length of tooth N_2, ratio length/width of tooth N_2, and leaf angle between N_1 and N_3 showed significant differences. The highest value for the size of blade was measured in Drini Valley wild grapevine population, followed by Mati Valley population, while Shkreli Valley wild population showed the lowest size of blade. The same results were observed for length of tooth N_2 and the ratio length/width of tooth N_2. There was observed an inverse correlation between the size of blade and the angle between N_1 and N_3 measured at the first ramification. The highest value for angle ($\alpha + \beta$) was measured for Shkreli Valley, while the lowest value was measured for Drini Valley wild grapevine population.

Keywords: wild grapevine, leaf characters, population, Drini Valley, Mati Valley, Shkreli Valley.

1. Introduction

The Euroasian grape, *Vitis vinifera* L is one of the most widely cultivated and economically important agricultural species in the world. Two forms still co-exist in Eurasia and in North Africa: the cultivated form, *Vitis vinifera* L. subsp. *vinifera* (or *sativa*), and the wild form, [*Vitis vinifera* L. subsp. *sylvestris* (or *sylvestris*) (Gmelin) Hegi] [20; 21; 42; 46]. This historical separation into subspecies was based on morphological differences [15; 42; 45]. The majority of cultivars widely cultivated for fruit, juice and wine, derive from the wild grapevine. The wild grapevine is distributed in a wide area from the Atlantic coast of central and southeastern Europe to the Trans-Caucasian zone and around the Mediterranean Basin, North Africa, Middle East and southern Caspian Belt, western Himalayas, and in the Assian regions between Black Sea and Hindukush mountains, between the 43rd and 49th northern parallels, from sea level up to an altitude of 1000 m [2; 3; 6; 23; 32]. The wild grapevine represents an indigenous plant in Albania, as well [14; 38; 39; 40]. It is a perennial, climbing, multi-stemmed woody tendril vine; wich can reach a height up to 15-17 m. The wild grapevine produces deeply lobed, small to medium size leaves; blackish, dark blue or purple hanging bunches; tiny greenish or white, insect pollinated, flowers in elongated clusters; and small sour berries [7; 14]. It grows in a broad range of habitats and soils, but its distribution has dramatically been reduced over the last 150 years. The present distribution of the wild grapevine is highly fragmented, in small isolated populations, with few individuals, at least in the western part of the Mediterranean Basin [15; 41; 43] along riverbanks [31], roadsides, fencerows, and forest edges, on periodically or permanently flooded areas, as well as in hardwood forests [3;, 4; 7; 13; 46] and in alluvial and colluvial deciduous and semi-deciduous forest, contributing to the unique environment of these species-rich ecosystems. Anthropogenic pressure on

their natural habitats and pathogens introduced from North America during the second part of the 19th century, such as *Downy mildew*, *Powdery mildew* and *Phylloxera* may explain the progressive decline of wild grape populations [3; 4; 5; 8; 22; 26]. In 1980s, it was added to the IUCN list of endangered European species and, since 1995, this species has been strictly protected in France [3]. However, in most European countries wild grapevine is still not protected [3]. The future of *Vitis vinifera* subsp. *sylvestris* represents a major stake in biodiversity conservation [41].

Wild-growing grapevine individuals have been identified in France [2; 3; 8; 24], Spain [13; 23; 24, 25], Italy [18], Germany, Switzerland [42], Portugal [9; 11; 25], Austria, Romania [31], Slovenia [29; 34] and Georgia [16; 30], as well as many other European countries [4; 5; 22], up to Puebla, Mexico [17], but they are real *silvestris* individuals, that have never undergone cultivation, or "escaped" individuals from vineyards or hybrids between wild and cultivated forms as described by Levadoux [20; 42]. The wild grapevine and cultivated forms that were derived from it have undergone through successive changes, therefore their identification and differentation has been very complicated. Ampelographic studies of the main ampelometric and ampelographic characters, according to several common international methodologies, serve as the right solution [13; 15; 33].

The wild ancestors and current cultivars show several similar morphological traits, but there are several distinct traits, as well. The main dinstict trait is that the wild grapevine is dioecious (gyno-dioecious) and cross pollination is very important for its dissemination, while cultivated forms are mainly hermaphrodite and selfpollinated [1; 9; 12].

Some other dinstict morphological traits of the plant, such as young shoot aperture of tip, mature leaf, flower sex (male, female and hermaphrodite for *sylvestris*), berry and bunch size, and seeds are very important for the characterization of true *sylvestris types* [12; 38; 39; 41, 42]. Wild grapevine individuals in Albania belong to *Vitis vinifera* ssp *sylvestris typica* [40].

Intensive grape cultivation is associated with the use of a limited number of cultivars and clones, which narrows or drastically reduces the genetic variation and germoplasm of the grapevine. At certain moments of the viticulture development, the only solution for a given situation may be wild grapevine genes and characters [35].

The present study was based on the comparison of the main mature leaf characters of three wild grapevine populations grown in three river valleys of Northen Albania, Drini Valley, Mati Valley and Shkreli Valley (Malësia e Madhe), in order to discriminate differences between wild forms in different geographical and environmental growing conditions.

Observations, measurements and assesment of the main ampelographic mature leaf, bunch and berries, must be carried out using IPGRI, OIV and UPOV Descriptors for Grapevine (*Vitis* sp) [19; 27; 44].

2. Material and Methods

The individuals belonging to three different wild grapevines populations *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi, located in three river basins of three geographic regions of Northern Albania, Shkreli, Drini and Mati Valleys were compared by means of the mature leaf characters, using usual ampelographic methods [19; 27; 44]. Drini Valley wild grapevine population was studied in Koman and Markaj villages, in a latitude of 42°5'25" to 42°18'00" N, longitude 19°48′44″ to 19°56′00″ E, and an altitude of 270-330 m. Mati Valley wild grapevine population was studied in Shkopet and Ulëz villages, in a latitude of 41°41'10" N to 41°42'22" N, longitude 19°49′30″ E to 19°53′40″ E, and an altitude of 112-149 m. Shkreli Valley wild grapevine population was studied through Shkrel, Vrith, and Dedaj villages, in a latitude of 42°14'6" N to 42°19'58" N, longitude 19°31′60″ to 19°32′28″ E, and an altitude of 526-550 m.

Study was carried out during the period 2009-2013, on samples constituted by 15 vines for each population. Observations, measurements and evaluations of the mature leaf characters were based on codes and levels of the International Descriptors of Grapevine [19; 27; 44] and the experience of the Albanian and foreigner researchers [11; 21; 23; 29; 36; 37]. Vines were marked with plastic labels placed on perennial branches from 01 to 15, which were unmoved during the study period. Measurements and evaluation of the ampelometric mature leaf characters, such as leaf size, number of lobes, length of petiole, length of the main veins (N_1, N_2, N_3, N_4, N_5), length and width of tooth N_2, length of upper and lower lateral sinuses, angle between N_1 and N_2 (angle

α), angle between N_2 and N_3 (β), etc., was performed in the period July 10-20, between berry set and veraison, each year, in representative samples of 10 mature leaves taken from the middle third of

several shoots/vine, according to IPGRI, OIV and UPOV Descriptors (Figure 1).

Observed and measured mean data of the ampelographic and ampelometric mature leaf characters of three wild grapevine populations were compared in order to find similarities and differences between them. Differences between three wild grapevine populations were tested using ANOVA tests, while the relationship between the main different mature leaf characters was tested using the correlation coefficient [28].

Observations, measurements and evaluation of the main characters of three samples of 15 vines of three populations of different locations (riverbanks) of the Northern Albania (Shkreli Valley, Drini Valley and Mati Valley) were carried out each year in the period July 10-20, between berry set and veraison period in respective representative samples of 10 mature leaves taken from the middle third of several shoots of each vine, according to international methods, as they are described by Descriptors for Grapevine (*Vitis ssp*.) of IPGRI [19], OIV [27], and UPOV [44].

3. Results and Discussion

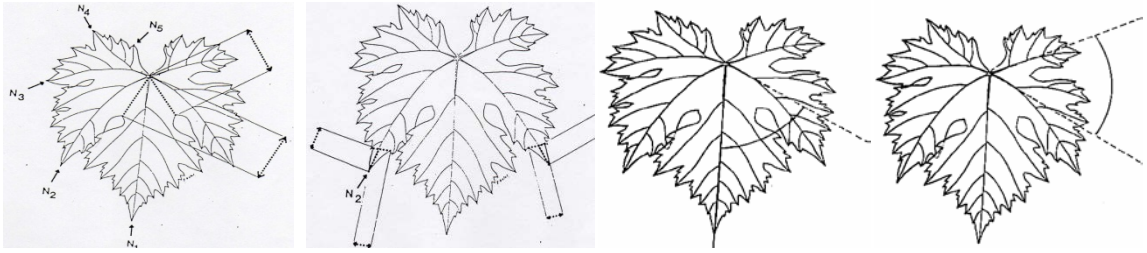

Figure 1. Methods of measurements of the main veins length, upper and lower sinuses, and angles between N_1 and N_2 (α) and between N_2 and N_3 (β) of the mature leaf [19; 27; 44].

3.1. Length of the main veins (N_1, N_2, N_3, N_4, and N_5) and length of petiole

Length of the main vein (N_1) (LN_1) (middle vein length or leaf length) (OIV 601). There was observed that in Drini Valley wild grapevine population 26.7% of the individuals showed a very short length (1) of N_1, 60% of the individuals showed a short length (3) of N_1, and 13.3% of the individuals showed a medium length (5) of N_1. In Mati Valley wild grapevine population, 86.7% of the showed a very short length (1) of N_1 and 13% of the individuals showed a short length (3) of N_1. In Shkreli Valley wild grapevine population, 100% of the individuals showed a very short length (1) of N_1 (Table 1 and Table 2).

Length of vein N_2 (OIV 602). Individuals of Drini Valley wild grapevine population showed very short (1) to short (3) length of N_2, individuals of Mati Valley wild grapevine population and Shkreli Valley population showed very short (1) length of N_2.

Length of vein N_3 (OIV 603) was evaluated short to medium (3, 5) for Drini Valley WGP, very short to short (1, 3) for Mati Valley WGP, and very short (1) for Shkreli Valley WGP (Table 1).

Length of vein N_4 (OIV 604) was evaluated medium (5) and long (7) for Drini Valley WGP, short to medium (3, 5) for Mati Valley WGP, and short (3) for Shkreli Valley WGP (Table 1).

Length of vein N_5 (OIV 611) was evaluated short (3) for Drini Valley WGP, and very short (1) for Mati Valley WGP and Shkreli Valley WGP (Table 1).

Length of petiole compared to length of middle vein (N_1) (OIV 093, UPOV 30, IPGRI 6.1.40) was slighlty shorter (3) for Drini Valley WGP and for Mati Valley WGP, and almost equal (5) for Shkreli Valley WGP (Table 1).

Number of lobes (OIV 068, UPOV 20, IPGRI 6.1.23). In Drini Valley WGP, 60% of the individuals had three lobes (2), and 40% of the individuals had five lobes (3). In Mati Valley WGP, 73.3% of the individuals had 3 lobes (2) and 26.7% of the individuals had five lobes (3), while in Shkreli Valley WGP, 46.7% of the individuals had three lobes (2) and 53.3% of the individuals had five lobes (3).

Table 1. Length of the main veins (N_1, N_2, N_3, N_4, and N_5) (mm) and length of petiole of the mature leaf of three representative samples of wild grapevine populations (mean values)

№	Wild grapevine population (WGP)	Length (mm)					
		N_1	Petiole	N_2	N_3	N_4	N_5
1	Drini Valley	105.6	93.53	87.7	66.46	44.26	23.86
2	Mati Valley	75.2	59.6	53.06	42.32	29.17	17.8
3	Shkreli Valley	58.2	56.86	38.16	28.73	21	14.96

Length and width of tooth N_2, length petiole sinus to upper lateral leaf sinus and lower lateral leaf sinus, and angle between N_1 and N_3 ($\alpha + \beta$)

Length of tooth N_2 (LTN_2) (OIV 612). There was found that in Drini Valley wild grapevine population 46.7% of the individuals showed a short length (3) of tooth N_2, 13.3% of the individuals showed a medium length (5) of tooth N_2, and 40% of the individuals showed a long length (7) of tooth N_2. In Mati Valley wild grapevine population, 46.7% of the individuals showed a very short length (1) of tooth N_2, 46.7% of the individuals showed a short length (3) of tooth N_2, and 6.6% of the individuals showed a medium length (5) of tooth N_2. In Shkreli Valley wild grapevine population, 100% of the individuals showed a very short length (1) of tooth N_2 (Table 2 and Table 3).

Width of tooth N_2 (WTN_2) (OIV 613). There was found that in Drini Valley wild grapevine population 6.7% of the individuals showed a very narrow width (1) of tooth N_2, 53.3% of the individuals showed a narrow width (3) of tooth N_2, and 40% of the individuals showed a medium width (5) of tooth N_2. In Mati Valley wild grapevine population, 46.7% of the individuals showed a very narrow width (1) of tooth N_2 and 53.3% of the individuals showed a narrow width (3) of tooth N_2. In Shkreli Valley wild grapevine population, 93.3% of the individuals showed a very narrow width (1) of tooth N_2 and 6.7% of the individuals showed a narrow width (3) of tooth N_2 (Table 2 and Table 3).

Ratio length/width of tooth N_2 (LTN_2/WTN_2) for individuals of Drini Valley WGP ranged from 1.07 to 1.33 (μ_D = 1.206 (3), for individuals of Mati Valley WGP ranged from 0.8 to 1.1 (μ_M = 1.06 (3), for individuals of Shkreli Valley WGP ranged from 0.66 to 1.0 (μ_S = 0.86) (Table 2 and Table 3).

Length petiole sinus to upper lateral leaf sinus (SULLS) (OIV 605) was short (3) for individuals of Drini Valley WGP and very short (1) for individuals of Mati and Shkreli Valleys WGPs (Table 3).

Length petiole sinus to lower lateral leaf sinus (SLLLS) (OIV 606) was short (3) for individuals of Drini Valley WGP and very short (1) for individuals of Mati and Shkreli Valleys WGPs (Table 3).

Table 2: Length of N1, and length and width of tooth N2 of three representative samples of wild grapevine populations

Vine	*Drini Valley WGP*				*Mati Valley WGP*				*Shkreli Valley WGP*			
№	LN_1	LTN_2	WTN_2	LTN_2/WTN_2	LN_1	LTN_2	WTN_2	LTN_2/WTN_2	LN_1	LTN_2	WTN_2	LTN_2/WTN_2
1	78	13	11	1.18	41	7	9	1.1	60	5	8	0.62
2	91	12	10	1.2	81	11	9	1	64	4	6	0.66
3	112	16	13	1.23	63	10	10	0.8	61	6	6	1
4	116	18	14	1.28	90	12	10	1	53	7	8	0.87
5	98	12	11	1.09	88	9	9	1	58	6	6	1
6	117	11	9	1.22	60	7	8	1	62	8	7	1.1
7	125	16	13	1.23	68	8	9	1	45	6	7	0.85
8	101	9	7	1.28	85	11	6	1	61	5	6	0.83
9	97	12	10	1.2	99	13	9	1	57	7	7	1
10	122	17	14	1.21	64	8	8	1	48	5	7	0.71
11	116	16	12	1.33	78	9	9	1	61	4	6	0.66
12	89	12	9	1.33	61	7	6	1	49	7	8	0.87
13	94	11	10	1.1	92	10	7	1.1	54	6	6	1
14	118	16	14	1.14	60	7	6	1.1	71	6	7	0.85
15	110	14	13	1.07	98	8	8	1	69	8	9	0.88
Mean	105.6	13.6	11.33	1.206	75.2	9.13	8.2	1.06	58.2	6	6.93	0.86

Table 3. Length and width of tooth N_2, length petiole sinus to upper lateral leaf sinus and lower lateral leaf sinus, and angle between N_1 and N_3 ($\alpha + \beta$) of three different representative samples of wild grapevine populations (mean values)

№	*WGP*	*LTN_2*	*WTN_2*	*LTN_2/WTN_2*	*SULLS*	*SLLLS*	*Angle ($\alpha + \beta$)*
1	Drini Valley	13.6	11.33	1.206	51.9	47.1	118.8
2	Mati Valley	9.13	8.2	1.06	17.3	20.8	122.5
3	Shkreli Valley	6.0	6.93	0.86	15.33	17	124.6

Table 4. Two Factorial Analysis of Variance (ANOVA – test) for the main mature leaf characters of three different WGPs

Source of Variation	SS	df	MS	F	P-value	F crit
Rows (populations)	4268.682	2	2134.341	**23.35689**	**1.79E-05**	**3.633723**
Columns (leaf characters)	26101.9	8	3262.737	**35.70535**	**8.95E-09**	**2.591096**
Error	1462.072	16	91.3795			
Total	31832.65	26				

Tabela 5. Correlation analysis of the main leaf characters of three wild grapevine populations

Characters	*Length of N_1*	*Length of petiole*	*Length of upper sinus*	*Length of lower sinus*	*Angle ($\alpha + \beta$)*
Length of N_1	1				
Length of petiole (LP)	0.956892813	1			
Length of upper sinus	0.951136169	0.999815428	1		
Length of lower sinus	0.969983047	0.998797156	0.997670771	1	
Angle ($\alpha + \beta$)	0.746862823	0.907804777	0.915694702	0.886148706	1

Table 6. Comparison and evaluation of the main mature leaf characters of three wild grapevine populations grown in three river valleys of the Northern Albania

Nr	*Mature leaf characters*	*OIV Code*	*Evaluation of WGPs*		
			Drin	Mat	Shkrel
1	Shape of blade	O-067	3	3	3
2	Number of lobes	O-068	2, 3	2, 3	2, 3
3	Anthocyanin coloration of main veins on upper side of blade	O-070	1	1	1
4	Profile of blade in cross section	O-074	1	1	1
5	Blistering of upper side of blade	O-075	1	1	1
6	Shape of teeth	O-076	5	5	5
7	Degree of opening/overlapping of petiole sinus	O-079	3	3	3
8	Shape of base of petiole sinus	O-080	1	1	1
9	Teeth in the petiole sinus	O-081-1	1	1	1
10	Degree of opening/overlapping of upper lateral sinuses	O-082	1	1	1
11	Density of prostrate hairs between main veins on lower side of blade	O-084	5	5	5
12	Density of erect hairs between main veins on lower side of blade	O-085	5	5	5
13	Density of prostrate hairs on main veins on lower side of blade	O-086	3	3	3
14	Density of erect hairs on main veins on lower side of blade	O-087	5	5	5
15	Prostrate hairs on main veins on upper side of blade	O-088	0	0	0
16	Length of petiole compared to N_1	O-093	2	2	2
17	Length of vein N_1	O-601	1, 3, 5	1, 3	1
18	Length of vein N_2	O-602	1, 3	1	1
19	Length of vein N_3	O-603	3, 5	3	1
20	Length of vein N_4	O-604	5, 7	3, 5	3
21	Length of vein N_5	O-611	3	1	1
22	Length of tooth N_2	O-612	3, 5, 7	1, 3, 5	1
23	Width of tooth N_2	O-613	1, 3, 5	1, 3	1, 3
24	Length petiole sinus to upper lateral leaf sinus	O-605	3	1	1
25	Length petiole sinus to lower lateral leaf sinus	O-606	3	1	1
26	Angle between N_1 and N_2 (α)	O-607	5, 7	5, 7	7
27	Angle between N_2 and N_3 (β)	O-608	5, 7	5, 7	7

Angle between N_1 and N_3 ($\alpha + \beta$) [Sum of angles α (between N_1 and N_2 - OIV 607) and β (between N_2 and N_3 - OIV 608), measured at the first ramification, was evaluated medium (5) to large (7) for Drini Valley WGP individuals ($\mu_M = 118.8^o$) and Mati Valley WGP individuals ($\mu_M = 122.5^o$), and large (7) for Shkreli Valley WGP individuals ($\mu_S = 124.6^o$). There was observed an inverse correlation between the size of blade and the angle between N_1 and N_3 measured at the first ramification (Table 3).

There were observed significant differences between individuals of three wild grapevine populations for the mature leaf characters, such as the angle between N_1 and N_3, length of upper and lower lateral leaf sinuses, length of petiole and length of the main veins (N_1, N_2, N_3, N_4, and N5). These differences were confirmed by ANOVA – tests (Two – Factorial Analysis of Variance) $p \leq 0.05$ (Table 4).

Sixteen other characters were expressed in the same way, which means that, the wild grapevine, expresses its unique and specific consolidated characters despite of the environment where it can be grown. These specific characters are easily distinguishable from other types of forest vines and cultivated forms. Correlation analysis confirmed that among main mature leaf characters of the wild grapevine populations grown in the riverbanks of Drini, Mati and Kiri rivers, such as length of main vein, length of petiole, length petiole sinus to upper and lower lateral leaf sinuses, and angle ($\alpha + \beta$) exists a strong positive linear relationship (r^2 = 0.746-0.999) (Table 5).

Comparison of expression levels and evaluation of the main mature leaf characters of three wild grapevine populations grown in three river valleys of the Northern Albania is given in Table 6.

4. Conclusions

Wild grapevine *[Vitis vinifera. ssp sylvestris* (Gmelin) Hegi] is a widespread and well preserved plant in riverbanks of Drin and Mati rivers, and in Shkreli Valley, as well. From twentyseven observed, measured and evaluated mature leaf characters, fifteen of them showed the same expression way for all populations, while, twelve characters showed significant differences between populations and between individuals of each population. Such characters distinguish the wild grapevine individuals, *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi, from other grapevine forms and cultivars. There was found a strong correlation relationship between main mature leaf characters (r^2 = 0.746-0.999). Individuals of Drini Valley WGP formed a larger leaf compared to the Mati Valley and Shkreli Valley WGPs. There was observed an inverse correlation between the size of blade and the angle between N_1 and N_3. The highest value was measured for Shkreli Valley, while the lowest value was measured for Drini Valley wild grapevine population.

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