

Albanian grapevine cultivars: preliminary results of molecular, phenolic and ampelometric profiles and relatedness

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Summary

'Shesh i Zi', 'Shesh i Bardhë', 'Kallmet' and 'Vlosh' are commercialised grapevine wine varieties which best represent the high variability of the Albanian autochthonous germplasm because of their different origin within the country and their great wine potential. We characterised these cultivars at molecular, phenolic and ampelometric level as well as assessing their relatedness. The leaves were scanned and images were analyzed using SuperAmpelo, which allows to measure leaves, clusters, berries, and seeds, and to record the main descriptive ampelographic characters, including carpometric indices. The content of total anthocyanins, total flavonoids, non-anthocyanic flavonoids, proanthocyanidins and total polyphenols were determined in the extracts of the grape skins and seeds of 10 berries from each of 10 representative clusters as well as in samples of one year old wines. For the molecular characterization, 10 SSR loci were used. The similarity of cultivars was studied by applying the Ward's method for hierarchical clustering along with other Albanian genotypes. This work has completed and made more accurate the existing ampelographic descriptions found in literature with the aim to valorize these autochthonous cultivars, which could represent a valuable instrument for improving the local economies.

Key words: ampelometry; polyphenols; genetic relatedness; microsatellites.

Introduction

Albania is characterized by a high variability of local grape populations (KULLAJ 2008, KULLAJ *et al.* 2011). The four grapevine cultivars 'Shesh i Zi' (SHZ), 'Shesh i Bardhë' (SHB), 'Kallmet' (KLL) and 'Vlosh' (VLO), evaluated in this study, represent the most important ones. They originated from different parts of Albania and are considered to have a great wine potential. The aim of this research is the valorization of these autochthonous cultivars, which could represent a valuable instrument for improving the local economies.

Material and Methods

For ampelometric characterization, ten mature leaves from 5 random plants of each cultivar were collected at the National Grapevine Collection at the Centre for Agricultural Technology Transfer of Vlora. The leaves were scanned and images were analyzed using SuperAmpelo, which allows to measure leaves, clusters, berries, and seeds, and to record the main descriptive ampelographic characters, including carpometric indices (SOLDAVINI *et al.* 2009). The content of total anthocyanins, total flavonoids, non-anthocyanic flavonoids, proanthocyanidins and total polyphenols were determined in the extracts of the grape skins and seeds of 10 berries from each of 10 representative clusters as well as in samples of one year old wines. Extraction was based on the method proposed by MATTIVI *et al.* (2002). Spectrophotometric determinations were carried out on the extracts according to the methods described by Di Stefano *et al.* (1989). For the molecular characterization, 10 SSR loci (VVMD27, VVMD7, VrZAG 67, VVMD 2732, VrZAG 62, VrZAG79, VVS2, VVMD 25, VVMD 32, VVMD 5) were used (THIS *et al.* 2004, LAUCOU *et al.* 2011).

Polymerase Chain Reactions (PCRs) were performed in a volume of 20 µL including 30 ng genomic DNA, 200 mM of each dNTPs, 10 pmol primers, 4 µL 5X MyTaq Reaction Buffer, and 1u MyTaq DNA Polymerase (Bioline, UK). PCR amplifications were performed in a 96-well Eppendorf Ep Mastercycler (Eppendorf, USA) as follows: 1 cycle [95 °C, 2 min], 35 cycles [95 °C, 15 s; 52 to 60 °C (depending on the primer), 15 s; 72 °C, 10 s], and 1 cycle [72 °C, 20 min]. The capillary electrophoresis of the amplified products was completed in a Genetic Analyzer 3130 (Applied Biosystems) with 16 capillaries. The binning set of the Institute of Applied Biosciences at CERTH, Greece, was used to compare the row data with molecular database for identification.

The similarity of cultivars was studied by applying the Ward's method for hierarchical clustering along with other Albanian genotypes, thus the distance between the clusters was calculated using the squared Euclidean distance. The JMP platform (JMP) was used to calculate similarities automatically.

Results and Discussions

Albania, located at the heart of the Mediterranean, has a millennial viticulture tradition and a wide germplasm to be valorized. In this study our preliminary results, on ampelometric, biochemical and genetic data confirm the distances between the cultivars (Table). From ampelometric

indices we report "Distances" (Table, A) for one variety, 'Shesh i Zi' but we have omitted "Angles", "Ratios" and O.I.V. codes (KULLAJ and ÇAKALLI 2013a). The Figure shows the standard leaf profile and the actual leaf of the cultivar. Regarding the metabolic characterization, we reported only the phenolic profiles for the skin and seeds for all the varieties (Table, B) but omitted those for flesh as

Table

A summary of ampelometric, phenolic and molecular profiles of 'Shesh i Zi' (SHZ), 'Shesh i Bardhë' (SHB), 'Kallmet' (KLL) and 'Vlosh' (VLO) in three sections respectively

A. Values (mm) of the ampelometric indices (distances) for cv. Shesh i Zi										
Indices	Value	Indices	Value	Indices	Value	Indices	Value	Indices	Value	
Length	88.4 ± 18.8	Width	88.3 ± 18.5	Lgth.+ peti.	121.5 ± 24.6	OP			65.6 ± 13.8	
ON1	65.5 ± 13.8	N2N2'	75.7 ± 15.2	N3N3'	88.8 ± 19.5	N4N4			53.0 ± 13.7	
SPSP'	10.4 ± 3.4	ON2	59.8 ± 12.5	ON2'	59.0 ± 12.4	ON3			44.9 ± 9.1	
ON3'	44.6 ± 10.7	ON4	34.7 ± 8.6	ON4'	35.1 ± 8.1	O3N4			30.1 ± 7.3	
O3'N4'	30.4 ± 7.0	O4N5	17.4 ± 4.0	O4'N5'	17.0 ± 4.0	OO3			6.4 ± 1.5	
OO3'	6.3 ± 1.6	OS'	40.9 ± 9.7	OS'	41.4 ± 10.0	OI			35.9 ± 9.0	
OI'	34.3 ± 9.0	HN2	5.2 ± 1.5	HN2'	5.0 ± 1.5	HN4			4.4 ± 1.2	
HN4'	4.5 ± 1.4	BN2	7.0 ± 1.8	BN2'	6.7 ± 1.7	BN4			7.2 ± 1.8	
BN4'	7.5 ± 2.0	FN2	28.2 ± 6.0	FN2'	25.9 ± 6.2					

B. Average values (mg·kg ⁻¹) and confidence interval (95 %) of phenolic profiles in the skin and seeds										
Indices	Skin									
	SHZ	SHB	KLL	VLO						
Total anthocyanins	1198.4 ± 63.7	0	818.7 ± 81.9	520.5 ± 32.9						
Total flavonoids	1829.3 ± 121.5	558.5 ± 60.9	1203.0 ± 89.4	632.6 ± 2.6						
Non-anthocyanic flavonoids	942.9 ± 70.5	771.0 ± 103.2	n.m.	395.0 ± 28.9						
Proanthocyanidins	1600.9 ± 161.5	1006.0 ± 58.2	1644.8 ± 31.4	1201.1 ± 78.5						
Total polyphenols	2651.1 ± 379.3	1373.3 ± 49.7	3274.8 ± 253.3	1631.6 ± 174.5						

Indices	Seeds			
	SHZ	SHB	KLL	VLO
Total anthocyanins	-	-	-	-
Total flavonoids	347.0 ± 70.8	613.4 ± 84.0	816.8 ± 80.1	346.9 ± 70.8
Non-anthocyanic flavonoids	-	-	-	-
Proanthocyanidins	1050.6 ± 49.6	701.8 ± 96.1	2228.9 ± 132.7	1330.9 ± 128.5
Total polyphenols	1327.7 ± 184.2	871.0 ± 107.9	4831.1 ± 310.2	1498.3 ± 110.4

C. Size of the alleles (A1 and A2, two for each marker) for ten microsatellite loci										
Locus	MD27		MD7		VVS2		ZAG79		MD25	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
Kallmet	177	189	238	248	142	144	240	252	246	254
Shesh i Bardhë	177	180	238	-1	132	134	234	256	254	238
Shesh i Zi	180	180	238	244	142	142	240	256	254	238
Vlosh	180	180	-1	-1	-1	240	240	256	254	238

Locus	MD5		ZAG62		MD28		MD32		ZAG67	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
Kallmet	228	234	186	192	244	256	-1	-1	146	146
Shesh i Bardhë	224	240	186	186	226	234	264	272	137	146
Shesh i Zi	230	230	186	186	244	256	250	272	137	146
Vlosh	230	234	-1	-1	-1	-1	-1	-1	137	146

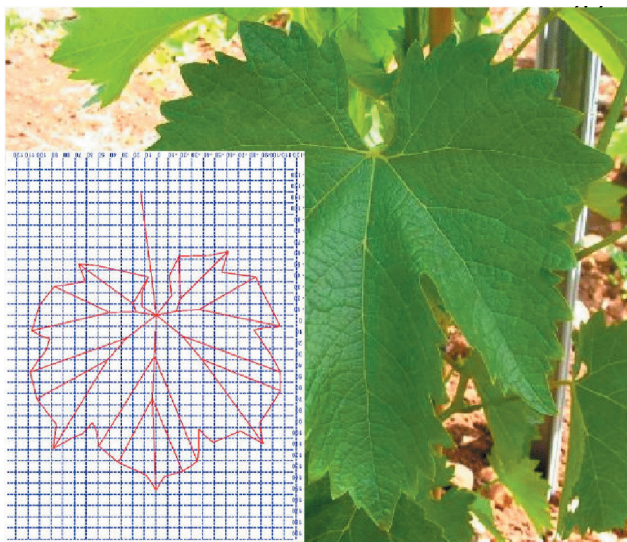


Figure: Leaf and standard leaf profile of 'Kallmet' processed with SuperAmpelo.

well as for the wines (KULLAJ and ÇAKALLI 2013b). The set of microsatellite loci used (Table, C), was polymorphic and informative enough to discriminate the analyzed cultivars.

Ampelometric results show a high inter- and intracultivar variation, attributable to the high leaf dimorphism which characterizes a grapevine. The high variation in polyphenolic content among cultivars is due to the grape composition or varietal characteristics whilst it is assumed that changes between wines of the same cultivar (not shown) could be related to the oenological practices. 'Kallmet' and 'Sheshi i Zi' produce wines suitable for ageing because they contain more phenolic compounds. Analysis of relatedness of cultivars, among other Albanian genotypes in a database, showed that the most distant is 'Vlosh', which in hierarchical clustering belongs to cluster 16; then 'Kallmet' at cluster 27, 'Shesh i Bardhë' at cluster 61 and 'Shesh i Zi' at cluster 66.

These characterizations complete and makes more accurate existing ampelographic descriptions found in literature.

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