Chapter 7

Genetic and Phenotypic Diversity and Relations Between Grapevine Varieties: Slovenian Germplasm

Denis Rusjan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/51773

1. Introduction

Slovenia is a small central European country situated between the Alps and the Adriatic Sea, at the crossing between Italy (to the West), Austria (to the North), Hungary (to the East) and Croatia (to the South), a historical place of significant grape and wine trading. Grapevines have been cultivated in Slovenia since ancient times, although the first literal proofs on grape growing and winemaking date back merely to the Austro-Hungarian Empire [1,2]. However, the oldest *Vitis vinifera* ssp. L. (grape) pips (seeds) found during the archaeological excavation of the late Neolithic (Copper Age) pile-dwelling settlement of Hočevarica at the Ljubljansko barje moor and date back to the 37th/36th century B.C. [3].

The geographical position of the country as well as the regarding climate conditions, and the socio-political development throughout the nation's history, have contributed to a diverse assortment of grapevine [1,2]. At least around 100 old and less known varieties are enumerated especially in the western part of Slovenia (Sub Mediterranean) and accompanied by the widespread European, allochthon, autochthon/landrace and local varieties, a large number of grapevines is cultivated in Slovenia nowadays [4,5].

The intense trade in grapes and wine, especially due to the Venetians (Venetian Republic from the end of 7th to the end of 18th Century; Figure 1), and the varying climatic and geological conditions contributed to a great diversity of *Vitis vinifera* L. varieties. Additionally, the multiculturalism and multilingualism of the area as well as the turbulent historical events have contributed to identical genotypes having different names [1,2].

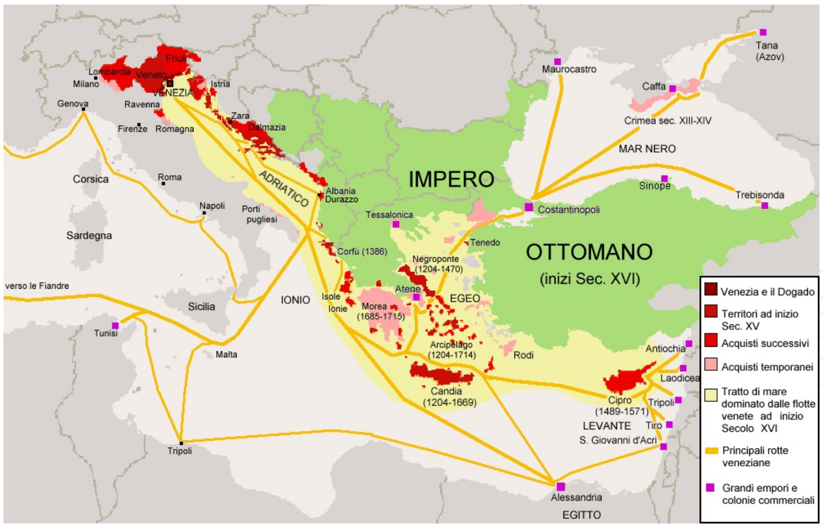

Figure 1. Territories of the Republic of Venice (697–1797). (http://en.wikipedia.org/wiki/File:Repubblica_di_Venezia.png)

Nowadays, Slovenian winegrowers (27.802) produce on 15.973 ha an annual of around 54.3 mio litres of wine; 62% whites and 38% reds. Fifty grapevine varieties (*Vitis vinifera* L.), international, allochthon, local and autochthon as well, are registered in the official Slovenian varietal list, but numerous accessions are still observed and non-descripted in many parts of Slovenia [1,6]. Although some of them form the basis of the renown regional varieties ('Rebula' in Goriška brda, 'Zelen' and 'Pinela' in Vipavska dolina, 'Šipon' in Ljutomer-Ormož etc.), many face extinction, since in some areas only a few plants are reported. However, Slovenia is facing the threat of the rapid erosion of the native/local germplasm on account of the introduction of foreign varieties, such as 'Merlot', 'Cabernet', 'Chardonnay' and 'Pinot'. Very often, however, the problems with the identification of these local, unknown varieties, occur, because of the presence of various synonyms of the same variety throughout the country [2,6,7].The gene pool of the varieties cultivated in Slovenia is composed of old allochthon, autochthonous, domestic/local varieties, which can all be additionally divided into two groups. The first group consists of commercially used varieties which are planted on more than 100 ha each, such as 'Rebula', 'Žametovka', 'Zelen', 'Pinela', 'Šipon', 'Radgonska Ranina', and 'Refošk'. The second group of varieties, such as 'Vitovska grganja' and 'Belina', are cultivated on less than 100 ha each and are known as local varieties. Moreover, around 50 well-known and rare varieties/accessions exist in Slovenia today; the majority of which have not yet been listed in the International List of Vine Varieties and Their Synonyms of the O.I.V. (International Organisation of Vine and Wine). Even though some morphological and agronomical descriptions related to these accessions exist, we are talking about mostly unexplored plant material and some of these varieties had survived only in less productive vineyards or owing to germplasm collections. The systematically collection of these endangered accessions started in 1980. Nowadays, there are five grapevine collections in the whole country: Slap at Vipava, Ampelografski vrt in Kromberk, the grapevine collection at Dobrovo in Goriška brda, Meranovo near Maribor and one near Ormož that include old varieties, clonal candidates and clones (Table1).

http://dx.doi.org/10.5772/51773

N	Variety	Synonym/Original name	Clone Code
1	'Barbera'		SI-36
2	'Beli pinot'	Pinot blanc	SI-19; SI-20
3	'Chardonnay'		SI-21; SI-39; SI-40
4	'Laški rizling'	Welschriesling, Graševina	SI-11; SI-12; SI-13; SI-41
5	'Istrska malvazija'	Malvasia d'Istria, Malvasia istriana	SI-37
6	'Pinela'	/	SI-28
7	'Ranina'	Bouvier Traube, Muscat de Saumur	SI-4; SI-5; SI-6; SI-7
8	'Ranfol'	Štajerska belina	SI-38
9	'Rebula'	Ribolla gialla	SI-30; SI-31; SI-32; SI-33; SI-34
10	'Refošk'	Refosco, Teran, Refosco d'Istria	SI-35
11	'Renski rizing'	Riesling, Rheinriesling	SI-22; SI-23; SI-24
12	'Sauvignon'	Sauvignon blanc	SI-1; SI-2; SI-3
13	'Šipon'	Furmint, Moslavac	SI-14; SI-15; SI-16; SI-17; SI-18
14	'Traminec'	Traminer	SI-8
15	'Zelen'	/	SI-26
16	'Žametovka'	Köllner blauer, Kavčina, Žametna črnina	SI-25

Table 1. List of varietal clones and clone candidates selected in Slovenia.

Nowadays, varieties can be characterized by several methods: (i) by means of a morphological description of plant parts at different phenological stages; (ii) morphometry based on the measurements of the parameters of the plant organs and (iii) quantitative or qualitative analysis of biochemical compounds. Furthermore, traditional methods of varietal description based on vegetative and reproductive (ampelography) parts of plants, contributed greatly to the full description of the identities and relationships among *V. vinifera* L. varieties; what also suggests that ampelographic characterization according to the characters put forward by the O.I.V. is the first step in the examination of grapevine varieties/accessions. Several authors described various analyses of primary and secondary metabolites and methods based on DNA polymorphism as outstandingly useful methods to complete the morphological identification of grapevine varieties [8,9].

2. Slovenian germplasm

One of the problems in the management of these germplasm collections is the use of synonymic and homonymic designations. The lack of order caused by synonyms and homonyms is caused by inadequate documentation and poor preservation of historical facts related to grape growing and trade. The identification and comparison of plant material by ampelographic methods often results in misinterpretations [10]. In contrast, DNA-based markers are independent of environmental factors and are therefore more appropriate for varietal identification [9,11]. In the last decade, more than 60 SSR primers from the genomic libraries of *Vitis vinifera* L. have been developed and used for identification purposes [12,13]. The genetic characterization of grapevine varieties (*Vitis vinifera* L.) using microsatellite markers and ampelometric methods has been done by many countries and regions already. The integration of the resulting molecular analyses and detection of synonymous grapevine varieties has already been performed among countries which share a common grapevine assortment, such as Croatia, North Italy, Austria, Germany, France, Spain, Portugal, Greece, etc. [5,9,14,15]. In the last decade many genetic studies of local varieties have been conducted in Slovenia [4,5,15-18] as well, and have consequently revealed the genetic biodiversity of grapevine varieties grown and cultivated in the country (Table 2).

Variety / Accession	Synonym	Homonym	Origin
Bela glera	/	Prosseco, Briška glera	G
Beli teran	/	Vitovska grganja, Vitouska	G
Belina	Heunish		L
Bianchera	Erbaluce, Albaluce, Albalucent, Bianco Rusti, Erbalus, Erbalucente, Uva Rustia		Al
Borgonja bela		Istrska malvazija	Al
Borgonja rdeča	Burgonja istarska, Gamay Beaujolais, Borgogna	Cipro	Al
Briška glera	Glera	Bela glera, Glera	G
Cipro	Muškat ruža Porečki, Muškat ruža omiški, Moscato rosa, Rosenmuskateller blauer, Likvor, Rdeča muškateljka	Borgonja rdeča	Al
Cividin	Cividino bianco		Al
Cohovka	/		A
Danijela	/		G
Dolga petlja	/		G
Drenik	/		G
Duranja	/		Al
Glera	Prosecco, Prosekar	/	Al
Grganc			G
Guštana	Auguštana		G
Istrijanka			
Istrska malvazija	Malvazija, Malvasia d'Istria, Istarska malvasia, Istrijanka, Malvasia Istra, Borgonja bela	/	L
Kanarjola		Canaiolo	Al
Klarnica	Klarnca, Klarna		A
Laščina			G
Maločrn	Piccola nera, Negra Tenera, Petite Raisin		Al

http://dx.doi.org/10.5772/51773

Variety / Accession	Synonym	Homonym	Origin
Medena glera	/	Glera	G
Pagadebiti	Curzola, Plavina		Al
Pergolin	/		Au
Pinela	/		Au
Planinka	/		G
Plavina	Brajdica, Curzola, Pagadebiti	Plavac mali	Al
Pokalca	Rdeča rebula, Pocalza, Ribolla Nera, Schioccoletto, Schiopetino, Schioppettino, Scoppiettino		Au
Pokov zelen	/		G
Poljšakica	/		Au
Pregarc	/		L
Prosecco	Ghera, Glera, Grappolo Spargolo, Prosecco Balbi, Prosecco Bianco, Prosecco Tondo, Proseko, Sciorina or Serprina	Briška Glera, Števerjena	Al
Racuk	/		G
Ranfol	Štajerska belina, Štajerka, Urbanka, Vrbanka, Sremska lipovina, Svetla belina, Heunisch		Al
Rebula	Garganja, Ribolla gialla		L
Rečigla	/		G
Refošk	Teran, Refosco, Teranovka, Terrano, Refosco peduncolo rosso		L
Rožica	Rožca		G
Sladkočrn	/		Au
Števerjana	/		G
Teran	Terrano, Refošk	/	G, L
Teran Istra	Terrano, Refošk		G, L
Trevolina			Al
Vitovska grganja	Vitovska, Grganja, Beli Teran	Vitouska, Garganja, Beli Refošk, Racuk	Au
Volovnik	Volovna		Al, G
Vrtovka	/		G
Zelen	/		Au
Zelenika	/		G
Zunek	Cunek		G

Table 2. Names, designations and classifications of the less known cultivated grapevine varieties/accessions (*Vitis vinifera* L.) in Slovenia. Legend: Al – allochthon; Au – autochthon (landrace); L – local (domestic); G – germplasm (grown only in certain gene banks).

2.1. Genetic diversity and relations among varieties

SSR markers offer some advantages over other molecular markers, including their co-dominant inheritance, hypervariability, and high cross-species transferability [8,13]. Consequently a large number of markers has been developed for characterisation of grapevine by many research groups [8,12,19] – these markers have provided a very useful and convenient tool for analysing genetic diversity of grapevine. In order to efficiently manage these conserved local germplasm resources and to understand the genetic relationships among them, it is necessary to characterize the genetic diversity existing in the Slovenian collection and production vineyards. However, a majority of cultivated and grown grapevine accessions in the Sub-Mediterranean part of Slovenia and a subset of 6 widespread European varieties taken as reference have been genotyped with microsatellite loci in order to: (1) identify and/or differentiate varieties, especially those of similar morphological characteristics; (2) assess genetic diversity and relationships among them; (3) and compare these varieties to their synonyms [5,15-18] (Figures 2 and 3).

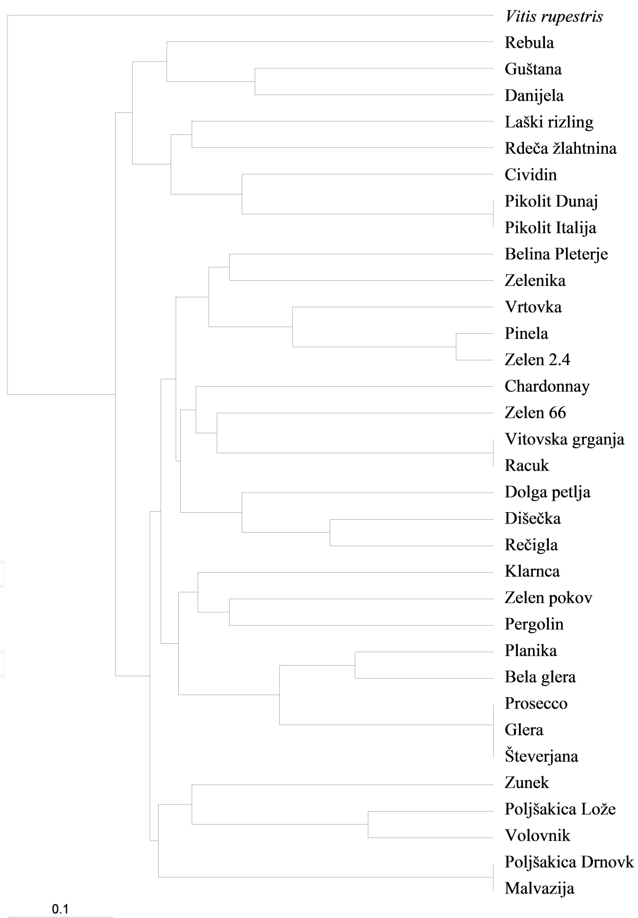

Figure 2. Dendrograms of genotyped grapevine accessions grown in Slovenia [5].

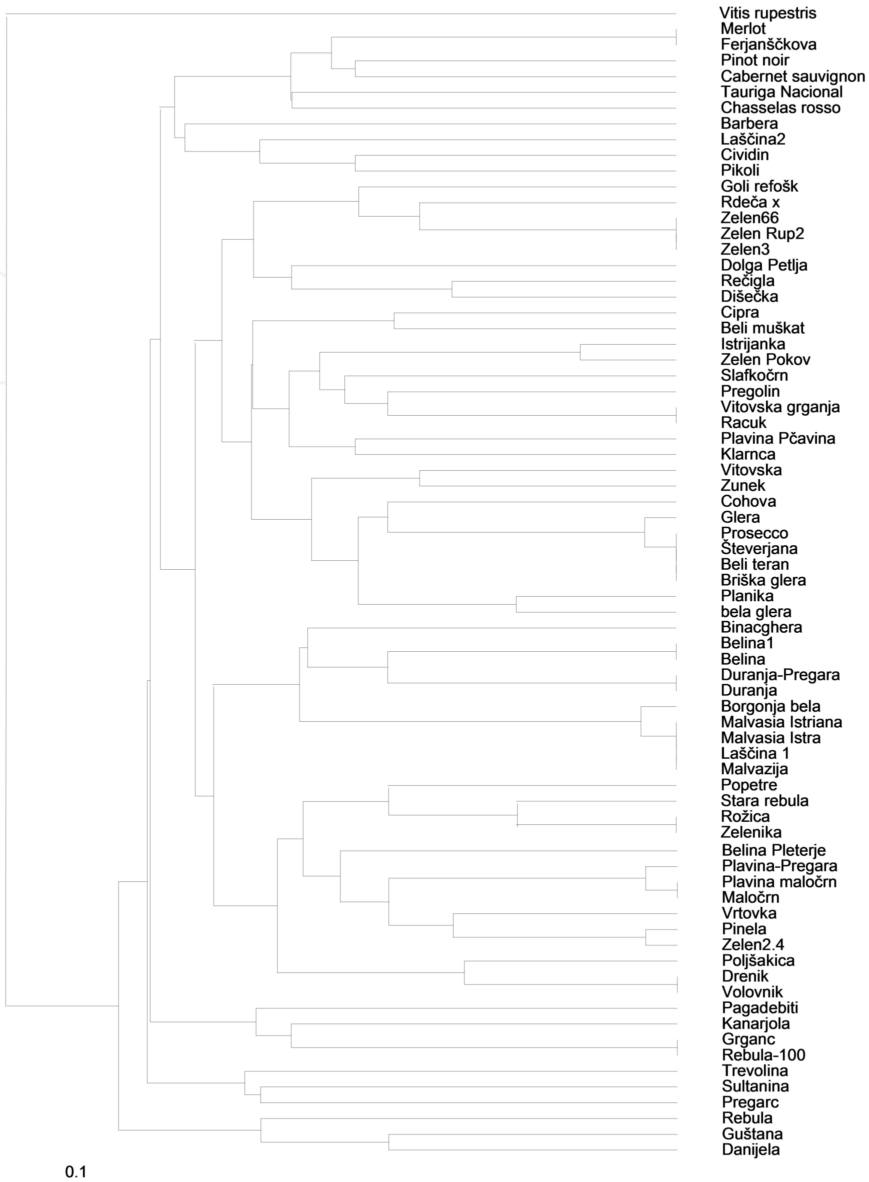

Figure 3. Dendrograms of genotyped grapevine accessions grown in Slovenia [17].

SSR profiles of groups of varieties with similar names but some different morphological characteristics were compared in order to assess their relationships and resolve existing doubts on their identity. In order to illustrate the population structure among Slovenian varieties/accessions dendrograms were constructed, which classify the varieties according to the proportion of shared alleles (Figures 2 and 3). The average similarity of all varieties is 34% of shared alleles (Figure 2, left), which is close to the average similarity observed for mid-European and Portuguese accessions [5]. Overall, two distinct clusters were obtained, with many sub-clusters. The accessions from the first cluster are related to 'Laški rizling' ('Welschriesling'), 'Rdeča žlahtnina' ('Roter Gutedel', 'Chasselas red') from the West European gene pool (*Proles occidentalis*), but also 'Rebula' ('Ribolla') known as *Proles pontica*. Another two varieties, 'Guštana' and 'Danijela', grouped with the first cluster, are both characterised by an early grape ripening. The name ‘Guštana’ may describe the ripening time of this variety, which is in August. The variety ‘Danijela’ is planted only in gene bank vineyards and its provenance is still uncertain. The variety ‘Picolit’ is cultivated in the western part of Slovenia (Northern Primorska) and in the north-east part of Italy – in Slovenia two distinct, morphologically different types of this variety/accession, ‘Picolit Italia’ and ‘Picolit Vienna’, are known. Microsatellite markers revealed no differences at 21 loci; matching also the same allelic profiles at all 7 SSR loci with a variety ‘Picolit’ from Italy [25]. In the second cluster, which includes 25 accessions, three groups of synonyms were discovered [5]: ‘Vitovska grganja’ = ‘Racuk’, ‘Poljšakica Drnovk’ = ‘Istrska Malvazija’ and ‘Prosecco’= ‘Briška Glera’ = ‘Števerjana’. ‘Vitovska grganja’ is an old grape variety cultivated in Slovenia in the winegrowing districts of the Vipavska dolina and Kras and also in the north-east part of Italy, where it played an important role in the past [1]. The synonymy found between ‘Vitovska grganja’ and ‘Racuk’ could not yet be confirmed despite obtaining identical allelic patterns, because an accurate morphological characterization of ‘Racuk’ is still lacking. The allelic profiles of our ‘Vitovska grganja’ have been further compared with the Italian variety ‘Vitouska’, recently published by [26] - dissimilarity was revealed at 14 out of 16 loci, and indicating that there is a compatible parent/progeny. The varieties ‘Prosecco’, ‘Briška Glera’ and ‘Števerjana’ form another group of synonyms. ‘Prosecco’ and ‘Glera’ have already been proved to be synonyms on the basis of morphological descriptors and isoenzyme analyses, while ‘Števerjana’ has not previously been considered to be a synonym. The variety ‘Prosecco’ takes its name from the village of Prosecco, in the Province of Trieste, where this variety is also known as ‘Glera’ [26]. The name for the variety ‘Števerjana’ may originate in Števerjan, a small village in North-East Italy (Collio), near the Slovenian border. The comparison of 16 SSR loci of our ‘Prosecco’ with Italian ‘Prosecco tondo’, which was recently analysed [26], revealed no differences - on the basis of this comparison ‘Prosecco’ = ‘Prosecco tondo’ = ‘Glera’ but according to [26] ‘Glera’ is related mainly to ‘Prosecco lungo’ and less frequently to ‘Prosecco tondo’ [5].

Comparison between the two accessions denominated ‘Glera’ (‘Briška Glera’ and ‘Bela Glera’) included in our analysis [5] revealed differences at 16 out of 21 loci, so they are considered homonyms. Two varieties, ‘Poljšakica Drnovk’ and ‘Poljšakica Lože’, which were expected to have the same genetic profile, were different at various analysed microsatellite loci. The synonymy of ‘Heunisch’ = ‘Ranfol’ = ‘Belina’, which was first mentioned by [2], was also analysed - discrepancies were discovered at 13 loci, but the two varieties share one common allele at all 13 loci [5]. Furthermore, the accession ‘Belina Pleterje’ was compared to the synonymic variety ‘Ranfol Bijeli’ from Croatia using microsatellite data [14] - 8 compared SSR loci data showed the same allelic profiles for all, except VVMD7, where a triallelic profile was observed. A mutation in the microsatellite sequence of locus VVMD7 was often responsible for identifying grapevine synonyms or related types [19].The SSR profiles of accessions linked to the designation ‘Zelen’ (‘Zelen Pokov’, ‘Zelen 66’ and ‘Zelen 2.4’) were compared as well. The differences at several microsatellite loci showed that ‘Zelen’ accessions are a heterogeneous group consisting of several genotypes. The differences were revealed also at the comparison of ‘Zelen’ to ‘Verduzzo’ and ‘Verdicchio’andcan therefore not be considered/used synonyms.In pairwise comparison, excluding *Vitis rupestris* L., the great-

http://dx.doi.org/10.5772/51773

est distances detected (86%) were between accesions 'Rebula' – 'Volovnik', 'Dolga petlja' – 'Guštana' and 'Klarnica' – 'Pikolit' (Figure 2).

Subsequent, more detailed study was done in 2011 [17], which included all accessions founded in the Sub-Mediterranean part of Slovenia. The observations are reported as follows [17] (Figure 3): The pairs/groups of vines denominated 'Duranja' and 'Duranja-Pregara', furthermore 'Belina' and 'Belina1', 'Zelen Rup2', Zelen66' and 'Zelen3' and 'Malvasia', 'Istrska Malvasia' and 'Malvasia Istriana' revealed identical genotypes in all SSR microsatellites analysed therefore they are regarded as synonyms. The designations related to 'Malvasia' very often comprise several types of grapevine varieties which are also morphologicaly heterogenous [20]. Very closely related to 'Malvasia Istra' was 'Borgonja bela', sharing 19 out of 20 alleles, what was also expected according to known ampelographic characteristics. Unexpectedly, vine denominated 'Laščina 1' also showed identical SSR profiles as 'Malvasia Istra' but completely different than accession 'Laščina 2', what is considered as a designation error or homonym. In the group of variety 'Plavina', the vines signed as 'Plavina maločrn' and 'Maločrn' showed same genotyping results and they are closely related to vine denominated 'Plavina Pregara', what cannot be affirmed for accession signed as 'Plavina-Pčavina'. 'Plavina-Pregara' in contrast to 'Plavina maločrn' and 'Maločrn' resulted in homozygous state (or heterozygous with a null allele) at locus VVMD7, but 'Plavina-Pčavina' was different from them in 8 loci out of 10 [17]. The difference in the stage of homozygosity/heterozygosity at locus VVMD7 was also obtained between accessions 'Glera' and 'Briška Glera' and these kind of mutations at locus VVMD7 have previously often been reported [5,9,21]. These results of close relatedness suggested that local varieties can be phenotypically partly different types or under-types and that one could have originated from the other through somatic mutations. Low relatedness (20% of similarity) was also found between Slovene 'Plavina' and Croatian 'Plavina' genotyped by [20]. 'Plavina' was reported the most common grape variety along the Dalmatian coast and was known as 'Pagadebiti' in 'Curzola', however in our study the vine denominated 'Pagadebiti' is more similar to variety 'Rebula' ('Ribolla') and 'Kanarjola' than to 'Plavina'; as pairwaise comparison revealed only 6% of genetic similarity. The obtained allelic profiles of 'Pagadebiti' are also different from the profiles of two 'Pagadebiti' accessions reported by [21] - 'Pagadebiti' translates as "pay the debt" and so the name may had been used for several very productive grapevine varieties, resulting in many homonyms.

To the group of synonyms related to the designation 'Glera' ('Glera', 'Prosecco', 'Briška Glera', 'Števerjana', 'Beli teran') the Croatian variety 'Teran bijeli' genotyped by [14] was compared. The comparison of data of 7 SSR loci resulted in identical SSR profile confirming their synonymy. A high genetic difference was revealed between the accessions denominated as 'Glera'/'Briška glera' and 'Bela glera' and could be explained with the fact that the term "Glera" was quite frequently used for white grapevine varieties in the Sub-Mediterranean part of Slovenia in the past [1,24], therefore the obtained homonymy is not a surprise. By comparing allele sizes of 7 SSR loci of the Croatian 'Muškat ruža Porečki' [14] with the Slovene 'Cipro', identical genotypes were found and therefore synonymy between these two varieties was confirmed. On the other hand, ‘Beli muškat’, which is clustered together with ‘Cipro’ in the dendrogram, is related to them with a similarity of 60%.

Unexpected the varieties ‘Rožca’ and ‘Zelenika’ showed the same genotype, what can be explained by the dissemination of scions of the same varieties throughout the winegrowing regions during the past, where the influence of dialects has also put its mark on the denomination. The variety ‘Rožca’ is found quite close to another white accession signed as ‘Rebula stara’; they share 77% of alleles. Vines denominated ‘Rebula stara’ and ‘Rebula-100’ were expected to be closely related but share only 55% of alleles; in common at least one allele at each locus, which could imply their parent/offspring relation.A genotype of ‘Rebula stara’ (=‘Stara Rebula’=‘Rebula old’) perfectly matched at 7 loci with variety ‘Gouais blanc’ (‘Heunisch’). Another interesting finding was the similarity between the vines ‘Grganc’ and ‘Rebula-100’, which showed similar genotypes with exception at locus VVMD5 at which ‘Rebula-100’ revealed a triallelic pattern [17].

2.1.1. Grapevine accessions designated ‘Rebula’ and ‘Vitovska’

2.1.1.1. Accessions related to designations ‘Rebula’ and ‘Ribolla’

‘Rebula’ (*Vitis vinifera* L.) has been one of the most important white grapevine varieties from the ancient times down to the present days and has been mostly cultivated in the area of Goriška brda and Collio where it is still gaining in importance. The first mentions of the name ‘Rebula’ date back to 1299, later 1376,to a deed of sale »Notariorum Joppi« in the area of the Slovenian Collio [27], whereas the first ampelographic descriptions of the variety are found in Vinoreja [1] and in Ampelographie [2]. Six different types of the variety ‘Rebula’ are enumerated and described (“green rebula”, “yellow rebula”, “less fertile rebula”, “unfertile rebula”, “crazy rebula” and “rebula with smaller and dissected leaf”) [1] what confirms its biodiversity which decreases with narrow clonal selection. For this variety many synonyms are used - ‘Garganja’, ‘Glera’, ‘Ribolla gialla’, ‘Rebolla’, ‘Ribolla’, ‘Ribolla Bianca’, ‘Ribuèle’, ‘Rabuèle’, ‘Rosazzo’, ‘Ribollat’, ‘Raibola’, ‘Ràbola’, ‘Ribuole’ and ‘Gargania’ [6,7,28]. DNA analyses regarding accessions of ‘Rebula’ have been already reported [15]. The variety 'Rebula' showed a very low similarity (16 %) with other analysed varieties, also with vines designed 'Rebula-100 years' and 'Rebula-old'. The accessions named ‘Prosecco', 'Števerjana', 'Beli teran' and 'Briška Glera', however, revealed identical genotypes in all 11 SSR microsatellites analysed, and are therefore regarded as synonyms. The varieties 'Rebula' and 'Ribolla gialla' revealed an identical SSR profile at 8 out of 9 SSR loci (Figure 4).

Moreover, the genetic identity and relationship among ‘Ribolla gialla’, ‘Rebula’ and ‘Robola’ that have been traditionally cultivated in other European Countries (Goriška brda in Slovenia and Kefalonia Island in Greece) grown in private vineyards and in grape collections were additionally studied (Table 3)[29]. For this purpose, 35 SSR loci were analysed to fingerprint 19 accessions uniformly grown in the three cultivation areas (Friulian Collio, Goriška brda and Ionian Islands). ‘Ribolla Gialla’ and ‘Rebula’ accessions revealed identical genotype in all 35 analysed SSR markers, therefore are regarded as synonymous. Data proved the existence of full-sibling relationships between ‘Ribolla gialla’ and ‘Robola’ (Figure 5) [29].

http://dx.doi.org/10.5772/51773

<table>
<tr><th>Accession</th><th>Origin</th></tr>
<tr><td>'Bela Glera 5/6'</td><td rowspan="5">Ampelographical collection - University of Ljubljana – Slovenia</td></tr>
<tr><td>'Bela Glera 8/1'</td></tr>
<tr><td>'Glera 1'</td></tr>
<tr><td>'Glera 2'</td></tr>
<tr><td>'Glera Medena'</td></tr>
<tr><td>'Rebula 1'(clone B5)</td><td>Collection NEBLO – Slovenia</td></tr>
<tr><td>'Rebula 2'</td><td>Ampelographical collection – University of Ljubljana – Slovenia</td></tr>
<tr><td>'Ribolla 1'</td><td rowspan="2">Breeding ground GENRRJEVO of RADIKON STANISLAO – Italy</td></tr>
<tr><td>'Ribolla 2'</td></tr>
<tr><td>'Ribolla 3'</td><td>Breeding ground AZ of RADIKON STANISLAO – Italy</td></tr>
<tr><td>'Ribolla 4'</td><td>Breeding ground CENO of RADIKON STANISLAO – Italy</td></tr>
<tr><td>'Ribolla 5'</td><td>Breeding ground of RADIKON STANISLAO – Italy</td></tr>
<tr><td>'Robola 1'</td><td>Ampelographical collection - University of Tessaloniki – Greece</td></tr>
<tr><td>'Robola 2'</td><td rowspan="6">Kefalonia – Greece</td></tr>
<tr><td>'Robola 3'</td></tr>
<tr><td>'Robola 4'</td></tr>
<tr><td>'Robola 5'</td></tr>
<tr><td>'Robola 6'</td></tr>
<tr><td>'Robola 7'</td></tr>
</table>

Table 3. List of analyzed accessions and origin area of plant materials related to the designations 'Rebula', 'Ribolla' [29].

At least 9 distinct genotypes resulted from the 19 analysed accessions [29], due to several cases of detected synonyms (Figure 5) – for example the same genotype was founded among vines designated 'Robola 1 and 'Robola 2', than between 'Robola 6' and 'Robola 7', moreover among five Italian vines designated 'Ribolla' accessions (1-5) and three Slovenian accessions ('Glera 2', 'Rebula 1' and 'Rebula 2'), 'Bela Glera 5/6' with 'Glera 1'. Besides, several cases of homonyms revealed, two Slovenian accessions 'Bela Glera 5/6' and 'Bela Glera 8/1' showed different allelic profiles, sharing 48 % of alleles, so did the accessions 'Glera 1' and 'Glera 2'(34% of shared alleles). Five cases of homonyms of the group of Greek accessions were revealed [29]: 'Robola 1', 'Robola 2' and 'Robola 6', 'Robola 7' accessions showed allelic profiles different from other 'Robola' accessions. Vine designated 'Robola 1' and 'Robola 2' shared 81 % of alleles with 'Ribolla Gialla', 'Rebula' and 'Glera 2' accessions. In addition[29] report the SSR profiles of five accessions resulted to be unique: 'Bela Glera 8/1', 'Glera Medena', 'Robola 3', 'Robola 4' and 'Robola 5'. After the accurate consideration of the obtained results, the studied accessions are clustered into three major groups, but 'Glera Medena' and 'Robola 3' are presented as isolated samples. The cluster 2 presents a major number of analysed vines, what reveals high homogeneity of the vines cultivated in Italy, as well as in the comparison with vines cultivated in Slovenia under the name 'Rebula' and those from Greece named 'Robola'. All these designations from cluster 2 can be perceived as synonyms;

therefore the original name of this variety is still uncertain. It is demonstrated, that the groups of Italian and Slovenian vine from cluster 2 share 90% of alleles, suggesting the existence of a common ancestor[29]. In the case of the other Greek accessions the situation seems to be more complicated and variability is higher – the largest number of accessions is grouped in cluster 1 ('Robola 4', 'Robola 5', 'Robola 6'/'Robola 7' accessions) as they share about 75% of alleles, what suggests very close relationships. Grouped in cluster 3, we find the accessions' Bela Glera 8/1', 'Glera 1' and 'Bela Glera 5/6', which show a similarity of 68% in comparison with cluster 2 (Figure 5). No case of parent-offspring (PO) relationships was revealed among analysed accessions. True-to-type 'Rebula' and 'Ribolla Gialla' (cluster 2) showed possible full-sibling (FS) relationships with 'Robola 1'/'Robola 2' accessions (same cluster), but shared only 55/70 alleles. Among the Greek accessions, 'Robola 4', 'Robola 5' and 'Robola 6'/'Robola 7', belonging to a different cluster are related by a FS relationship; 'Robola 4' and 'Robola 5' accessions sharing 68 % of alleles, 'Robola 4' and 'Robola 6'/'Robola 7' sharing 80 % of alleles and 'Robola 5' and 'Robola 6'/'Robola 7' sharing 78 % of alleles. Accessions 'Bela Glera 5/6', 'Bela Glera 8/1' and 'Glera 1' shared 48 % of alleles and show a probable HS relationship [29].

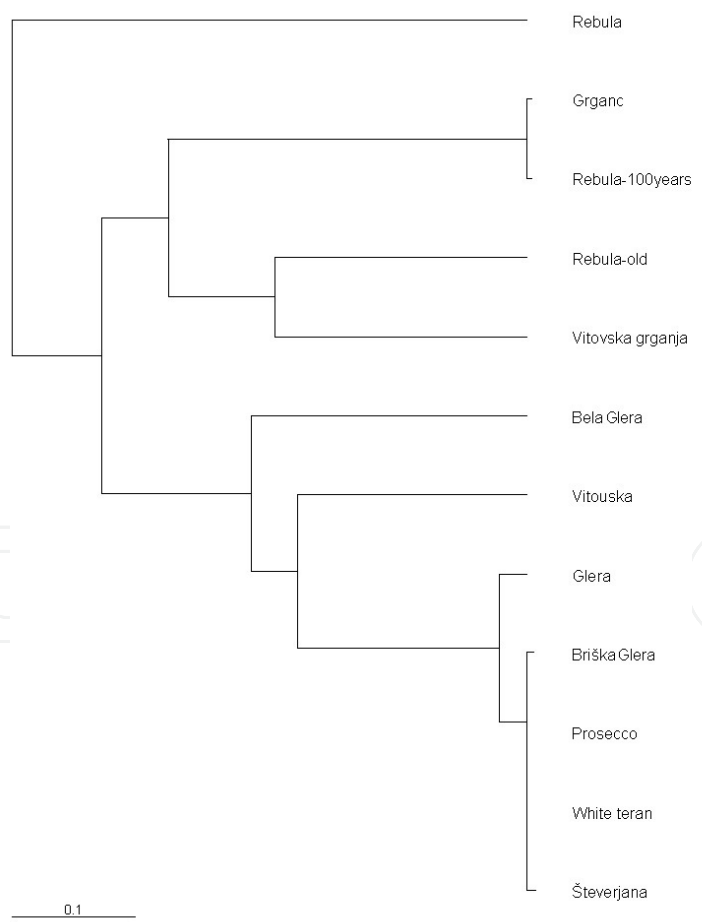

Figure 4. Dendrograms of grapevine accessions related to designations 'Rebula' and 'Ribolla' using D = 1-(proportion of shared alleles) as coefficient of distance and UPGMA as grouping method [15].

http://dx.doi.org/10.5772/51773

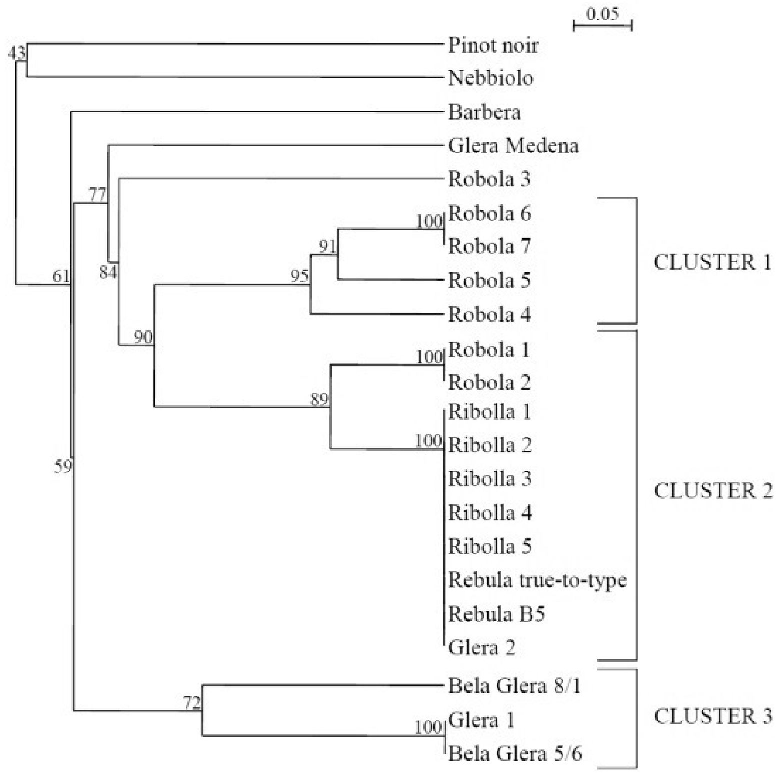

Figure 5. Dendrograms of grapevine accessions related to designations 'Rebula' and 'Ribolla' using D = 1-(proportion of shared alleles) as coefficient of distance and UPGMA as grouping method [29].

2.1.1.2. Accessions related to designations 'Vitovska' and 'Grganja'

The accessions related to 'Vitovska', 'Vitouska', 'Grganja' and 'Garganja' are cultivated in different winegrowing areas in Slovenia, Italy and Croatia; therefore their names (synonyms) still cause disorder, because they are often considered indigenous and ancient varieties in the area of cultivation. According to the accurate study [26], which included as much as 37 nuclear microsatellite locii confirmed the variety 'Vitouska' to be the progeny of 'Malvasia del Chianti' (syn. 'Malvasia bianca lunga', 'Malvasia lunga') and 'Prosecco tondo'. Following this, [30] found an original genotype of 'Malvasia del Chianti' with the varieties 'Pavlos' present in Greece and 'Maraština' cultivated in the south coastal region of Croatia (Dalmatia). Already in 1949 it was affirmed that 'Maraština' and 'Malvasia lunga' are one and the same variety [31]. In 2012 [18] reported genotyping and phenotyping of accessions (*Vitis vinifera* L.) cultivated in Slovenia, Croatia and Italia, mostly known as 'Vito(u/v)ska' and 'G(a)rgan(i)ja', while also referred as indigenous or landrace varieties [1,7,28]. The studied varieties, with an additional focus on variety 'Vitovska grganja', were mostly taken from germplasm collections: 'Vitovska grganja' from Slovenia, 'Vitouska', 'Ribolla gialla' and 'Prosecco' from Italy, 'Garganja' and 'Maraština' from Croatia.

Variety / Type / Vine / Abbreviation	Synonyms and homonyms (Translation)	Cultivation area and notes
'Vitovska grganja' 1 (VG)	Gerganja[1], Vitouska[2], Gargania[2], Ribolla gialla[2], Grganja[3], Garganja[3], Vitovška[3], Vitevška[3], Vitovka[3], Gorjanska[3], Malvazija s piko[3], Beli refošk[3], Vrbina[3], Vrbovna[3], Črna pika[3]	The vine is approx. 100 years old and grows in the village Sveto v (address Sveto 4), Kras (Slovenia) as an individual pergola-trained vine.
'Vitovska grganja' 2 (VG)	Gerganja[1], Vitouska[2], Gargania[2], Ribolla gialla[2], Grganja[3], Garganja[3], Vitovška[3], Vitevška[3], Vitovka[3], Gorjanska[3], Malvazija s piko[3], Beli refošk[3], Vrbina[3], Vrbovna[3], Črna pika[3]	The vine is over 110 years old and grows in the village Briščki (address Briščki 5), Carso, Italy cultivated as a pergola-trained individual vine.
'Vitouska' (V)	Vitovska grganja[2], Vitouska[2], Gargania[2], Ribolla gialla2	Sampled in the VCR (Rauscedo) selection of vines from vineyards planted near the Prosecco village, Italy. The variety is usually planted and cultivated in Carso region, north-east part of Italy.
'Prosecco' (P)	Gljera[6], Prosekar[6], Prosecco tondo[2], Prosecco bianco[2], Gargana[2], Brešanka[1,2] (Brescia)	Sampled in the VCR (Rauscedo) clone VCR101, Italy. The variety is usually planted and cultivated in Carso region, north-east part of Italy.
'Maraština' (M)	Pavlos[6], Malvasia del Chianti[6], Malvasia lunga[6], Rukatac[6], Marinkuša mala[6], Maraškin[6], Đerđevina[6], Kukuruz[6], Rukac[6], Krizol[6], Višana6	Sampled in the germplasm collection of the University of Zagreb, near Split (Dalmatia, Croatia). The variety is usually planted and cultivated in Dalmatia, coastal part of Croatia.
'Garganja' (G)	Rebula[6], Ribolla gialla[6]	Sampled in the germplasm collection of the Faculty of agriculture and tourism Poreč, near Poreč (Croatia). The variety is usually planted and cultivated in Croatian part of the Istria peninsula, near the village Buzet.
'Ribolla gialla' (RG)	Garganja[2], Glera[2], Refosco bianco[2], Teran bijeli[2], Ribola bijela[2], Erbula[2], Jerbula[2], Gorička ribola[2], Rebolla[2], Ribolla[2], Ribolla bianca[2], Ribuèle[2], Rabuèle[2], Rosazzo[2], Ribollat[2], Raibola[2], Ràbola[2], Ribuole[2], Gargania[2]	Sampled in the VCR (Rauscedo) clone VCR01, Italy. The variety is planted in Collio (Italy) presumably also in Goriška brda (Slovenian Collio), and Istria (Croatia). Sampled in the collection of the University of Ljubljana.

Table 4. List of the studied accessions related to the terms 'Vitovska', and 'Garganja' describing their already used synonyms, homonyms and cultivation areas[18]. Indexes: [1]-[1]; [2]-[28]; [3]-[32]; [4]-[7]; [5]-[30]; [6]-[31]

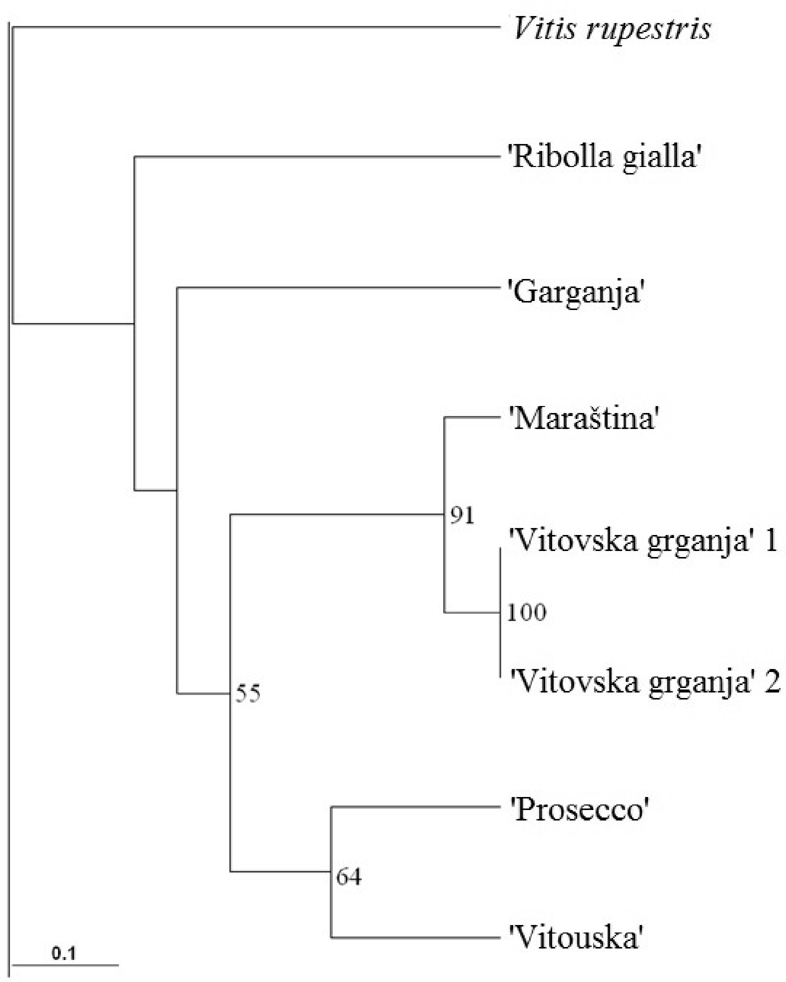

Figure 6. Dendrogram of studied grapevine (*Vitis vinifera* L.) accessions and of *Vitis rupestris* based on standard genetic distance [18].

Vines designated as 'Vitovska grganja 1' and 'Vitovska grganja 2', which have been grown for more than 100 years in the Slovenian Karst (Kras) revealed identical genotypes in all 11 analysed SSR microsatellites. The variety 'Maraština', nowadays known as an indigenous grapevine variety from Central and South Dalmatia (Croatia) surprisingly showed highly related SSR profile as both 'Vitovska grganja' cultivated in Slovenia. According to [30,31] the variety 'Maraština' coincides to the varieties 'Pavlos' and 'Malvasia del Chianti' (Malvasia lunga). In our study 'Maraština' coincided in seven of eleven SSR loci compared to 'Malvasia del Chianti' reported by [26], therefore we can conclude only that they are in strong relationship. Variety 'Vitouska' received from VCR Rauscedo (Italy), compared to 'Maraština' and 'Vitovska grganja' 1 and 2, differed in almost all used microsatellites, except at locus VVMD 5 and VVMD36, what additionally confirm the already reported affirmation of null (weak) relationship between both varieties [5,15,26]. Furthermore, considering that [26] affirmed the progeny of 'Vitouska' with 'Malvasia del Chianti' and 'Prosecco tondo', a quite weak match in their locii was found among them in our study.

2.2. Phenotyping experiences at grapevine varieties cultivated in Slovenia

2.2.1. Phyllometrical tolls for phenotyping

Morphological descriptions of different parts of the plant at different phenological stages, but also morphometry based on the measurements of parameters of plant organs are the oldest methods used in ampelography. Moreover, quantitative and qualitative analyses of biochemical compounds were also suggested at varietal description [33]. Despite the technical advances in biochemical and molecular approaches over the last decades, the descriptions of morphological characteristics remain an important methodology in the description and research of the diversity among species, varieties and clones. The most commune and used methodologies are descriptions according to the OIV codes [34] and ampelometry, especially phyllometry. The advances of morphological descriptions can be characterised as simple, cheap and applicable in the laboratory or directly in the field. Moreover, the present-day studies focus on finding new parameters in order to differentiate the varieties of vine precociously, quickly and efficiently [35]. The average vine leaves were reconstructed by measuring the proposed morphometrical parameters [36], furthermore a system for direct digitizing of phyllometric parameters from leaves was developed [37]. In grapevine collections approximately 5-10% of existing varieties are misnamed and even in commercial viticulture, misnaming and confusion related to synonyms and homonyms [38] is a frequent phenomena. We started to collect our allochthon, autochthone, local and endangered varieties systematically in 1980 and today we have few germplasm collections around Slovenia.

Additional attention has also been given to varietal phenotyping, especially at those varieties where some misunderstandings in nomination still cause disorders, for example at the varieties ‘Vitovska grganja’, ‘Refošk’, ‘Rebula’, ‘Glera’, ‘Vitouska’, ‘Garganja’ and others, which are frequently treated as an indigenous, autochthonous or landrace varieties in different countries [18]. Some new approaches to accelerate the phenotyping of leaves at different varieties were suggested; for example automatic measurements, as well as the possibility of typical-varietal mature leaf reconstruction [39,40].

2.2.1.1. Phyllometric measurements

Many studies in the last decades demonstrated the importance and contribution of phyllometry in grapevine description and distinction. The method provides around 82 criteria measurements of morphometrical characteristics on an individual leaf. According to a high variation in these characteristics among leaves of the same variety at least 10 mature, typically varietal leaves should be picked up, scanned or photocopied, placed in herbarium before being analysed. In our study we followed that procedure: Herbarium leaves were scanned (with the HP LaserJet M1005scanner) and analysed using AnalySIS image analysis program (Soft Imagining System GmbH). The quantitative base variables required the reconstruction of an average typical leaf of different varieties measured following the suggestions from [36] with some modifications. The following morphometrical parameters were measured in all leaves sampled per variety: leaf area (LA), length of nerves (L1, L2, L3, L4), distance from the petiole insertion to upper sinuses (OS), distance from the petiole insertion to lower sinuses (OI), total leaf height (H), total leaf width (W1), distance between the ends of left and right L2 (W2), distance between the basal tooth of the upper lobe (W3), angles between nerves measured from the petiole sinus to the first ramifications of vein (α, β, γ), angles between nerves from petiole sinus to apex of vein (α', β', τ), angle at the apex of upper lobe ($\delta 1$), angle between the apex of L1 and the apexes of L2 ($\delta 2$), angle between apex of L1 and apexes of L5 ($\delta 3$), distance between apex and basal tooth of upper lobes (D1), distance between the ends of L1 and L2 (D2), petiole length (lp), distance from the base of petiole sinus to the intersection of L3 vein to L4 vein (LO), tooth width at the end of L2 and L4 (b1, b2), tooth length at the end of L2 and L4 (h1, h2), distance between the ends of L5 (l), distance between the beginning of L5 (l′) suggested by [41]. Moreover, [42] suggested the following characterizing equations, in which both parts of the same leaf are used separately: Rel.1 = Lp/L, Rel.2 (left side) = L1/L, Rel.3 (right side) = L1/L, Rel.4 (left side) = $\alpha+\beta+\gamma$, Rel.5 (right side) = $\alpha+\beta+\gamma$, Rel.6 (left side) = $\alpha'+\beta'+\tau$, Rel.7 (right side) = $\alpha'+\beta'+\tau$, Rel.8 (left side) = (OS+OI)/(L1+L2), Rel.9 (right side) = (OS+OI)/(L1+L2), Rel.10 (left side) = OS/L1, Rel.11 (right side)= OS/L1, Rel.12 (left side) = OI/L2, Rel.13 (right side) = OI/L2 (Figure 7).

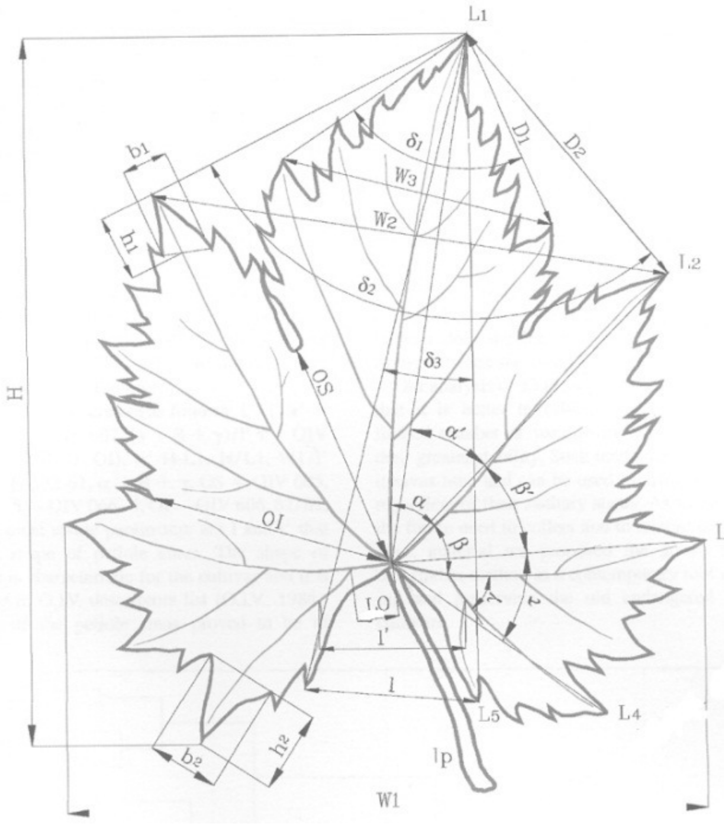

Figure 7. The morphometrical characteristics of mature leaves suggested by different authors [36,41,42].

The aim of the study was to investigate the efficiency of ampelographic and morphologic methods to evaluate the significance of O.I.V. code lists (Paragraph 2.2.2.2) and phyllometry regarding the varietal diversity. In addition, a graphic reconstruction method was applied and improved to form a typical varietal mature leaf (Figure 9). We evaluated 38 morphologic and 22 morphometric parameters per leaf. Statistical analysis was carried out, PCA (principal component analysis) was performed taking into account equations calculated from different leaf variables, and the qualitative variables proposed by the O.I.V. were preceded to Cluster Analysis. The combination of morphologic descriptors and phyllometric measurements proved to be complementary more than comparable methods. The results of the evaluation of O.I.V. descriptors showed a high level of varietal diversity, whereas accession 'Barbera type Bovcon' and 'Pokalca' as well as 'Barbera' and 'Syrah' showed some similarities. The most different variety in the group studied according to O.I.V. descriptors was 'Touriga national'. The PCA analysis showed the first three components responsible for more than 82% of the discriminating power. The reconstruction determinants were relations between the depth of the lateral sinuses and lateral veins (PC1), relation between first left lateral vein and central vein, relation between first right lateral vein and central vein (PC2), sum of angles between veins, relation between length of petiole and central vein and depth of the lateral sinuses (PC3). The enumerated relations enable and additionally improve the leaf reconstruction. At the varieties 'Sladkočrn' and 'Refošk' the shallowest lateral sinuses and at 'Tinta pinheira' the deepest ones were observed whereas 'Plovdina' and 'Touriga national'showed shorter lateral veins (L1d, L1g) in comparison with the main vein (L). The morphological descriptions and morphometric reconstructions of leaves gave a significant contribution to understanding the grapevine phenotypical biodiversity.

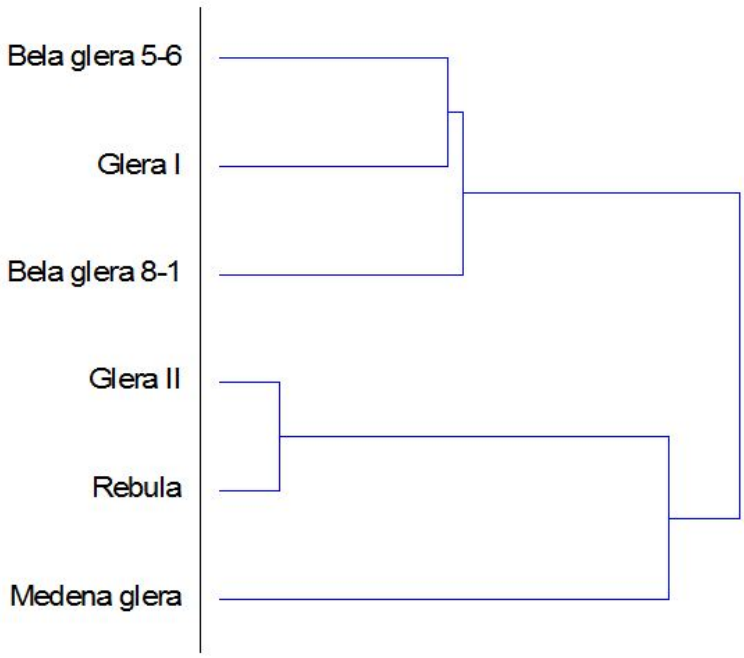

Figure 8. Dendrogram of grapevine accessions linked to the designations 'Glera' and 'Rebula' grouped on the basis of the 28 morphometric characteristics of mature leaves [40].

Furthermore, [40] studied 28 morphometric characteristics of leaves in detail and their contribution to the similarity among accessions with designations linked to 'Glera' and 'Rebula'. Phyllometric measurements affirmed the genotyping of same varieties studied by [29]. At the comparison of the groupings in Figure 5 and Figure 8, the measured 28 morphometric characteristics give suitable results what suggests that phyllometry grants an indispensable, cheap and credible methodology in the ampelography – varietal description and discrimination.

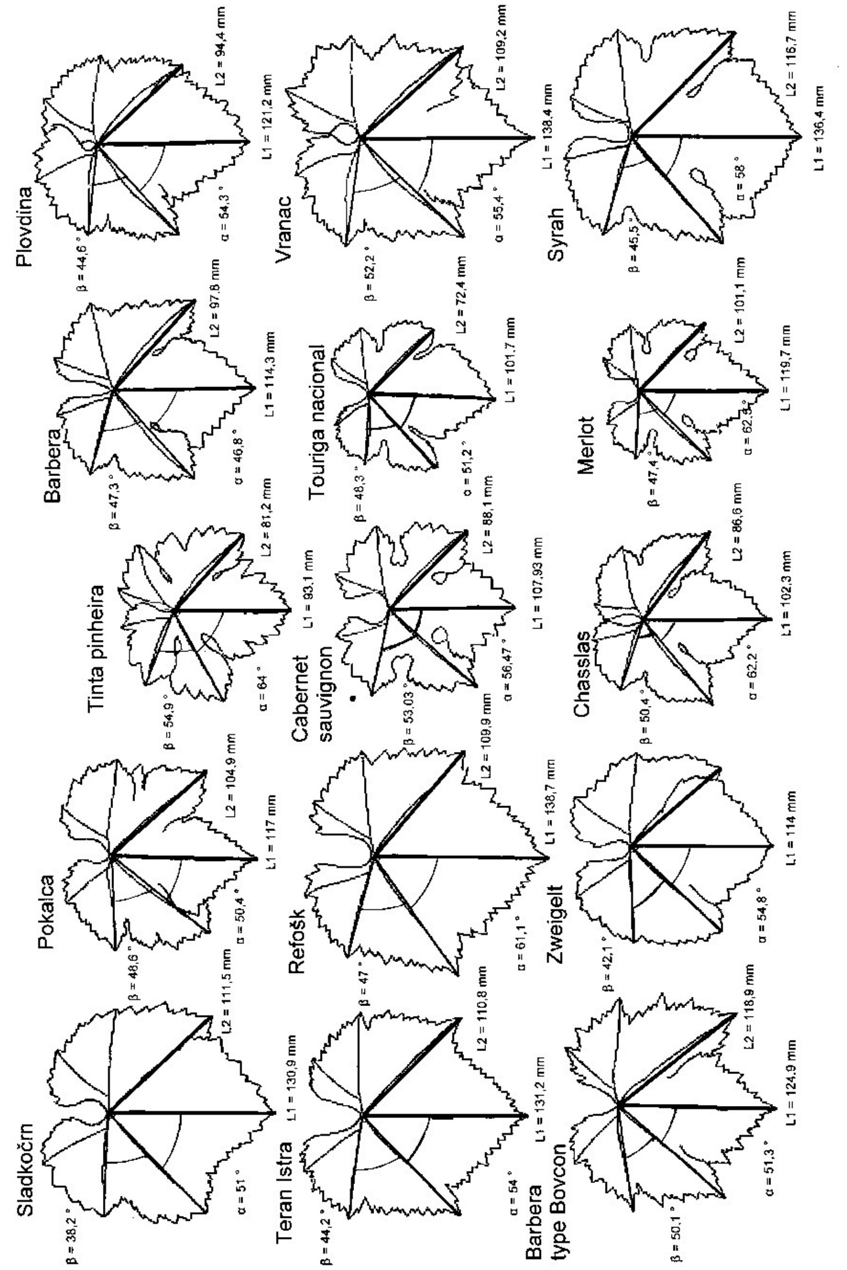

Figure 9. Graphical reconstruction of leaves of studied grapevine varieties according to determined phyllometric characteristics [39].

2.2.1.2. Morphological descriptions

The O.I.V. codes list still remains the most uses and suitable ampelographic tool in context of grapevine description [34]. There are approx. 147 descriptors; each regarding one significant characteristic, which offers a possibility of an accurate description of the main morphological parts of the vine, for example shoots tip, shoot, leaf, bunch, berry etc. [34]. For basic and relatively fast description of the varieties, the so called the Primary Descriptor list of OIV codes for Grapevine Varieties and Species *Vitis* was formed [34], which directs a description of 14 grapevine characteristics regarding shoots, leaves, bunches and berries. The OIV descriptors, for some leaf characteristics, overlap with phyllometric parameters: OIV 601 = L1, OIV 602 = L2, OIV 603 = L3, OIV 604 = L4, OIV 605 = OS, OIV 606 = OI, OIV 607 = α, OIV 608 = β, OIV 609 = γ, OIV 612 = h1, OIV 613 = b1, OIV 614 = h2, OIV 615 = b2, OIV 618 = l (Figure 7), therefore a collective use of both methodologies upgrades the varietal description. In few studies we used a different number of OIV codes, what depends especially on the condition of the grapevine, environmental conditions and the scope of the study.

The aim of the first study was the evaluation of selected OIV codes for grapevine description and discrimination – if the selected OIV code descriptorsare enough informative for a precise varietal discrimination? We sampled from 10 to 12 fully developed leaves from the sixth to ninth node of the shoot per certain variety. For the ampelographic description, shoot tips were sampled in the earlier phenological stages and berries were observed during the whole ripening period. The description and measurements of the particular parts of the vine were conducted according to the instructions of the individual descriptive codes.

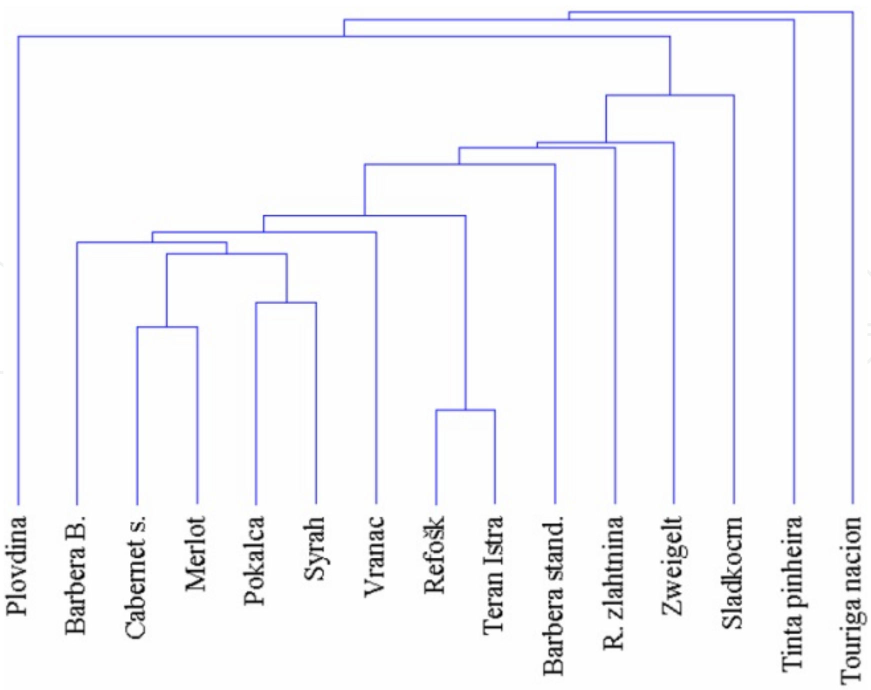

Figure 10. Dendrogram of grapevine varieties grouped according to the morphological evaluation, OIV codes [43].

According to the obtained data (Figure 10) and to the already acquired knowledge of the studied varieties some expected results revealed – for example similar OIV codes between accessions 'Refošk' and 'Teran Istra', frequently used as synonyms; or 'Pokalca' and 'Syrah' especially because of shape of the leaves berries. On the other hand the applied OIV codes revealed some imprecisions as well, for example similarities between varieties 'Merlot' and 'Cabernet sauvignon'. Therefore we suggest that the grouping according to the OIV code descriptors should be done and interpreted carefully, as many misunderstandingscould be provoked. For a comparison and interpretation of the studied accessions we suggest that the reference grapevine varieties should be included always, and as many as possible (also with greater differences in characteristics).

The further studies was focused on the variations, differences and possible mistakes linked to the subjective evaluations regarding OIV codes, where different descriptions of the same variety/accession were collected and compared. Therefore we selected accessions where many misunderstanding still provoke - accessions regarding the designations 'Vitovska' and 'Garganja' (Table 5) [18]. The misunderstandings in variety nomination are generally a result of false and misleading facts, which can partly be explained by neglect in observations and descriptions of variety characteristics, especially among those with a similar phenotype.

OIV code	Descriptor	VG	VGI*	V*	G*	M*,**
001	Young shoot: opening of the shoot tip	5	5	5	5	5
002	Young shoot: distribution of anthocyanin coloration on prostrate hairs of the shoot tip	2				2**
003	Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip	5	6	1	1	5
004	Young shoot: density of prostrate hairs on the shoot tip	7	7	7	3	9, (7**)
005	Young shoot: density of erect hairs on the shoot tip	1				
006	Shoot: attitude (before tying)	3	1	1	1	3
007	Shoot: colour of the dorsal side of internodes	2	2	2	2	2
008	Shoot: colour of the ventral side of internodes	1	1	2	2	2
009	Shoot: colour of the dorsal side of nodes	3				
010	Shoot: colour of the ventral side of nodes	1				
011	Shoot: density of erect hairs on nodes	1				
012	Shoot: density of erect hairs on internodes	1				
013	Shoot: density of prostrate hairs on nodes	1				
014	Shoot: density of prostrate hairs on internodes	1				
015-1	Shoot: distribution of anthocyanin coloration of the bud scale	2	3			
015-2	Shoot: intensity of anthocyanin coloration of the bud scale	1	4			
016	Shoot: number of consecutive tendrils	1	1	1	1	1

OIV code	Descriptor	VG	VGI*	V*	G*	M*,**
017	Shoot: length of tendrils	5				5
051	Young leaf: colour of upper side of blade (4th leaf)	2,4[1]	3	1	3	2-3, (2-3**)
053	Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf)	7	7	8	7	5-7
054	Young leaf: density of erect hairs between main veins on lower side of blade (4th leaf)	1				
055	Young leaf: density of prostrate hairs on main veins on lower side of blade (4th leaf)	7				
056	Young leaf: density of erect hairs on main veins on lower side of blade (4th leaf)	1				
065	Mature leaf: size of blade	7				7**
067	Mature leaf: shape of blade	3	3	4	4	4, (1,4**)
068	Mature leaf: number of lobes	3	2-3	3	2	5, (3**)
069	Mature leaf: colour of the upper side of blade	7				7**
070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade	2	1	2	2	1
071	Mature leaf: area of anthocyanin coloration of main veins on lower side of blade	2				
072	Mature leaf: goffering of blade	5	5	5	5	3, (7**)
073	Mature leaf: undulation of blade between main or lateral veins	1				
074	Mature leaf: profile of blade in cross section	4	5	5	5	1
075	Mature leaf: blistering of upper side of blade	5	3	7	3	3
076	Mature leaf: shape of teeth	3	2,4	4	5	3, (2**)
078	Mature leaf: length of teeth compared with their width	3				5**
079	Mature leaf: degree of opening / overlapping of petiole sinus	7	5	2	7	7
080	Mature leaf: shape of base of petiole sinus	1	3	1	3	1, (2**)
081-1	Mature leaf: teeth in the petiole sinus	1	1	1	1	1
081-2	Mature leaf: petiole sinus base limited by vein	1	1	1	1	3
082	Mature leaf: degree of opening/overlapping of upper lateral sinuses	2-3				
083-1	Mature leaf: shape of the base of upper lateral sinuses	1				
083-2	Mature leaf: teeth in the upper lateral sinus	1	1	1	1	1
084	Mature leaf: density of prostrate hairs between main veins on lower side of blade	7	1	6	5	7
086	Mature leaf: density of prostrate hairs on main veins on lower side of blade	7				

OIV code	Descriptor	VG	VGI*	V*	G*	M*,**
087	Mature leaf: density of erected hairs on main veins on lower side of blade	7	3	2	1	5
093	Mature leaf: length of petiole compared to length of middle vein	3				5**
094	Mature leaf: depth of upper lateral sinuses	5-7		4		3-5, (5**)
101	Woody shoot: cross section	1-2				2**
102	Woody shoot: structure of surface	1				1-2**
103	Woody shoot: main colour	1,3[2]				1,4**
104	Woody shoot: lenticels	1		1		1
105	Woody shoot: erect hair on nodes	1				
106	Woody shoot: erect hair on internodes	1				
151	Flower: sexual organs	3	3		3	3, (3**)
152	Inflorescence: insertion of 1st inflorescence	2				
153	Inflorescence: number of inflorescence per shoot	1				2**
155	Shoot: fertility of basal buds (buds 1-3)	5	1		5	5, (1**)
202	Bunch: length (peduncle excluded)	5-7	5	7	1	7, (7**)
203	Bunch: width	5-7				5**
204	Bunch: density	7	6	7	5	3, (5-7**)
206	Bunch: length of peduncle of primary bunch	3	4	5		5, (7**)
207	Bunch: lignifications of peduncle	5				5-7**
208	Bunch: shape	2	1	2	1	1-2, (1-2**)
209	Bunch: number of wings of the primary bunch	2	2-3	2	2	3, (2**)
220	Berry: length	3	5	5		3, (3**)
221	Berry: width	3	5	5		3, (3**)
222	Berry: uniformity of size	1				
223	Berry: shape	1,2	2	1	2	2, (2**)
225	Berry: colour of skin	1	1	1	1	1, (1**)
226	Berry: uniformity of skin colour	2				
227	Berry: bloom	5				7**
228	Berry: thickness of skin	3				7**
229	Berry: hilum	2				2**
231	Berry: intensity of flesh anthocyanin coloration	1	1	1	1	1
232	Berry: juiciness of flesh	2				2**
233	Berry: must yield	7				5**
235	Berry: firmness of flesh	2	1-2	1	2	3
236	Berry: particular flavour	1	5	1	1	1, (1**)
238	Berry: length of pedicel	3				7**
240	Berry: ease of detachment from pedicle	3				

OIV code	Descriptor	VG	VGI*	V*	G*	M*,**
241	Berry: formation of seed	3	3	3	3	3, (3**)
242	Berry: length of seeds	5,7				5,7**
243	Berry: weight of seeds	3				3-5**
244	Berry: transversal ridges on dorsal side of seeds	1		1		1
301	Time of bud burst	7	3			3
302	Time of full bloom	5-7				
303	Time of beginning of berry ripening (vérasion)	5	5			5
304	Time of full physiological maturity of the berry	5				7**
305	Time of beginning of wood maturity	7				
306	Autumn coloration of leaves	1				
351	Vigour of shoot growth	7	8			5, (5-7**)
352	Growth of lateral shoot	5				
353	Length of internodes	3-5				3**
354	Diameter of internodes	3				3**
401	Resistance to iron chlorosis	1				
402	Resistance to chlorides (salt)	1				
403	Resistance to drought	7				
452	Leaf: degree of resistance to *Plasmopara*	5				
453	Cluster: degree of resistance to *Plasmopara*	5				
455	Leaf: degree of resistance to *Oidium*	1,3				
456	Cluster: degree of resistance to *Oidium*	1,3				
458	Leaf: degree of resistance to *Botrytis*	5				
459	Cluster: degree of resistance to *Botrytis*	5				
501	Percentage of berry set	5				
502	Bunch: single bunch weight (g)	3	3			5, (3**)
503	Berry: single berry weight (g)	1-3	3			1, (1-3**)
504	Yield per m^2 (kg)	3-5[3]				5, (3**)
505	Sugar content of must	3	3		5	5, (5**)
506	Total acidity of must	3-5[4]	3		5	5, (5**)
508	Must specific pH	3,5[5]	3			7
601	Mature leaf: length of vein N1 (mm) = L_1***	5	7			5**
602	Mature leaf: length of vein N2 (mm) = L_2***	5	9			5**
603	Mature leaf: length of vein N3 (mm) = L_3***	5-7	7			5-7**
604	Mature leaf: length of vein N4 (mm) = L_4***	9	9			9**
607	Mature leaf: angle between N1 and N2 measured at the first ramification (°) = α***	5	7			7**
608	Mature leaf: angle between N2 and N3 measured at the first ramification (°) = β***	7	7			5**

OIV code	Descriptor	VG	VGI*	V*	G*	M*,**
609	Mature leaf: angle between N3 and N4 measured at the first ramification (°) = γ***	5	7			5**
612	Mature leaf: length of tooth of N2 = h1***	3	7			
613	Mature leaf: width of tooth of N2 = b1***	5	9			
614	Mature leaf: length of tooth of N4 = h2***	3	3-5			
615	Mature leaf: width of tooth of N4 = b2***	5	7			

Table 5. Ampelographic description according to O.I.V. descriptor codes and morphometric characteristics of 'Vitovska grganja' (VG), 'Vitovska grganija' (VGI), 'Vitouska' (V), 'Grganja' (G) and 'Maraština' (M) varieties [18]. *-[44]; **-[45]; ***-parameters of phyllometry [18]; 1-copper-reddish between veins; yellow around veins; 2-yellow and reddish; 3-0.8-1 kg m^{-2}; 4-total acidity 5.6 - 8.2 g L^{-1}; 5-pH 3.10 - 3.45.

The study was carried out on grapevine (*Vitis vinifera* L.) varieties cultivated in Slovenia, Croatia and Italia, mostly known as 'Vitovska', 'Vitouska', 'Grganja' and 'Garganija' but also referred to as indigenous or landrace varieties from the mentioned countries [1,7,28]. The studied varieties, with an additional focus on the 'Vitovska grganja' variety, were mostly taken from germplasm collections: 'Vitovska grganja' from Slovenia, 'Vitouska', 'Ribolla gialla' and 'Prosecco' from Italy, 'Garganja' and 'Maraština' from Croatia.

The comparison of the ampelographic descriptions, morphometric and phyllometric characteristics among the 'Vitovska grganja' (VG), 'Vitovska grganija' (VGI), 'Vitouska' (V), 'Grganja' (G) and 'Maraština' (M) varieties is presented in Table 5. The ampelographic descriptions of the VGI, V and G varieties highly differed from the VG and M varieties [18]. Similarities between VG and M varieties were observed in the characteristics of young and mature shoots, the opening shoot tips showed similar anthocyanin coloration and a similar intensity and coloration of prostrate hairs. Similarities between VGI and M varieties have to be underlined, especially in the size of young and mature leaves, dark green coloured upper side of the blade, both convex shaped sides of the teeth. The latter descriptor was in contrast to the report of [45], where both sides were described as straight. Moreover, [45] described the shape of the petiole base sinus of the M variety as 'U'-shaped whereas in [44] it is shown brace-shaped, what is also in accordance with our observations on VG variety. Both M and VG varieties were characterized by high density prostrate hairiness between the main veins on the lower side of the blade; however, hair density differed at other blades/vein parts. Our observations regarding the depth of the upper lateral sinuses of VG variety coincided with those mentioned by [45] for the M variety. In our study, M variety had a lower fertility of basal buds (1-3) compared to VG variety and also to the results reported by [45].

A comparison of bunch and berry characteristics among the studied varieties were also reported [18], what partly explains the reasons of the misuse of the variety nominations due to the rather similar phenotypes. All studied varieties have a cylindrical or conical shape of the bunches, obloid and globose berries, and a bunch length ranging between 160 and 200 mm with the exception of M and VG varieties, where berries are distinctively shorter and narrower. The description of single bunch and berry weight, as well as yield of the M variety reported by [45], coincided with our description for the VG variety. On the other hand, many significant differences were observed among the studied varieties, also between VG and M variety. Compared to M variety, the VG variety blooms later, has thinner berry skin, higher must yield, less firm flesh, shorter length of pedicle, lower sugar and total acidity content and lower specific pH level of the must. Moreover VG also has later bud bursting, but reaches full physiological maturity of the berry earlier. [18] found also some similarities among the studied varieties, specifically in morphometrical characteristics of mature leaves. Significant similarities were observed between our measurements of the VG variety and data cited by [45] for the M variety, which only differed in ‘α’ and ‘β’ angles. The comparison of phenotypic characteristics of the studied varieties showed many similarities, which suggests that a consistent attention to the ampelographic distinguishing among varieties, especially among those with the same designations or synonyms, has to be emphasized in the future.

3. Conclusions

The grapevine genepool is particularly vulnerable in the marginal areas of its distribution range. Many grapevine varieties have been already described and their genotypes determined; but many local grapevine accessions remain unidentified and their ampelographic characteristics overlooked. Accurate ampelographic description of variety should be done with combined methodologies which involve morphological description and DNA analyses. In the last decade a great effort was given to the DNA analyses – genotyping of the grapevine varieties, and quite soon revealed that DNA analyses is simply not enough to answer all questions about varietal discrimination and description. Parallel to the development of genetic techniques, the morphological and morphometric evaluations have fall into oblivion. Although, microsatellites are very powerful means of identifying synonyms in germplasm collections, they have to be supported with morphological descriptions, what was also demonstrated in our researches. Only such works will allow a characterization of a large number of varieties/accessions and will contribute to improve the organization of grapevine collections and the possibility of exchanging true-to-type material. The presented works are a first step towards true-to-type identification, which will facilitate the registration of varieties and allow growers to be sure of the value of their products. Starting true-to-type variety identification by comparing results with neighbouring, historically linked areas, will significantly clarify the confusion in nomenclature and help determine the origin and relationships among varieties over the whole area. In the future all the obtained results from different studies should be upgraded to the same database for grapevine varieties, to obtain a total overview of World germplasm, what may be the key to stop the erosion of the many varieties.

http://dx.doi.org/10.5772/51773

Author details

Denis Rusjan*

Address all correspondence to: denis.rusjan@bf.uni-lj.si

Biotechnical Faculty, University of Ljubljana, Slovenia

References

[1] Vertovec, M. (1845). Vinoreja za Slovence. *Ajdovščina: Agroind Vipava*.

[2] Goethe, H. (1887). Berlin: Handbuch der Ampelographie. *Beschreibung und Klassifikation der bis jetzt kultivierten Rebenarten und Trauben-varietäten mit Angabe ihrer Synonyme*, Kulturverhältnisse und Verwendungsart. Berlin: P. Parey.

[3] Tolar, T., Jakše, J., & Korošec-Koruza, Z. (2008). The oldest macroremains of Vitis from Slovenia. *Vegetation history archaeobotany*, 17(1), 93-102.

[4] Tomažič, I., & Korošec, Koruza. Z. (2003). Validity of phyllometric parameters used to differentiate local Vitis vinifera L. cultivars. *Genetic resources and crop evolution*, 50(7), 779-787.

[5] Štajner, N., Korošec-Koruza, Z., Rusjan, D., & Javornik, B. (2008). Microsatellite genotyping of old Slovenian grapevine varieties (Vitis vinifera L.) of the Primorje (coastal) winegrowing region. *Vitis*, 47(4), 201-204.

[6] Plahuta, P., & Korošec-Koruza, Z. (2009). x sto vinskih trt na Slovenskem. Ljubljana: Prešernova družba

[7] Hrček, L., & Korošec-Koruza, Z. (1996). Sorte in podlage vinske trte: ilustrirani prikaz trsnega izbora za Slovenijo. Ptuj: Slovenska akademija Veritas

[8] Thomas, M. R., & Scott, N. S. (1993). Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). *Theoretical and Applied Genetics*, 86(8), 985-990.

[9] Crespan, M. (2004). Evidence on the evolution of polymorphism of microsatellite markers in varieties of Vitis vinifera L. *Theoretical and Applied Genetics*, 108(7), 231-237.

[10] Dettweiller, E. (1993). Evaluation of breeding characteristics in Vitis. Influence of climate on morphologic characteristics of grapevines. *Vitis*, 32, 249-252.

[11] Cipriani, G., Frazza, G., Peterlunger, E., & Testolin, R. (1994). Grapevine fingerprinting using microsatellite repeats. *Vitis*, 33, 211-215.

[12] Bowers, J. E., Dangl, G. S., Vignani, R., & Meredith, C. P. (1996). Isolation and characterization of new polymorphic simple sequence repeat loci in grape (Vitis vinifera L). *Genome*, 39(4), 628-633.

[13] Sefc, K. M., Lefort, F., Grando, M. S., Scott, K., Steinkellner, H., & Thomas, M. (2001). Microsatellite markers for grapevine: A state of the art. In: Roubelakis-Angelakis KA. (ed.) Molecular Biology & Biotechnology of the Grapevine. Dordrecht: Kluwer Academic Publishers , 433-466.

[14] Maletić, E., Sefc, M. K., Steinkellner, H., Karoglan, Kontić. J., & Pejić, I. (1999). Genetic characterization of Croatian grapevine cultivars and detection of synonymous cultivars in neighboring regions. *Vitis*, 38(2), 79-83.

[15] Rusjan, D., Jug, T., & Štajner, N. (2010). Evaluation of genetic diversity: which of the varieties can be named 'Rebula' (Vitis vinifera L.)? *Vitis*, 49(4), 189-192.

[16] Kozjak, P., Korošec-Koruza, Z., & Javornik, B. (2003). Characterisation of cv. Refošk (Vitis vinifera L.) by SSR markers. *Vitis*, 42(2), 83-86.

[17] Štajner, N., Rusjan, D., Korošec-Koruza, Z., & Javornik, B. (2011). Genetic characterization of old Slovenian grapevine varieties of Vitis vinifera L. by microsatellite genotyping. *American Journal of Enology and Viticulture*, 62(2), 250-255.

[18] Rusjan, D., Pipan, B., Pelengić, R., & Meglič, V. (2012). Genotypic and phenotypic discrimination of grapevine (Vitis vinifera L.) varieties of the 'Vitovska' and 'Garganja' Denominations. *European Journal of Horticultural Science*, 77(2), 84-94.

[19] Lefort, F., & Roubelakis-Angelakis, K. A. (2001). Genetic comparison of Greek cultivars of Vitis vinifera L. by nuclear microsatellite profiling. *American Journal of Enology and Viticulture*, 52(2), 101-108.

[20] Lacombe, T., Boursiquot, J. M., Laucou, V., Dechesne, F., Varès, D., & This, P. (2007). Relationships and Genetic Diversity within the Accessions Related to Malvasia Held in the Domaine de Vassal Grape Germplasm Repository. *American Journal of Enology and Viticulture*, 58(1), 124-131.

[21] Riaz, S., Garrison, K. E., Dangl, G. S., Boursiquot, J. M., & Meredith, C.P. (2002). Genetic divergence and chimerism within ancient asexually propagated winegrape cultivars. *Journal of the American Society for Horticultural Science*, 127(4), 508-514.

[22] Caló, A., Costacurta, A., Maras, V., Meneghetti, S., & Crespan, M. (2008). Molecular correlation of Zinfandel (Primitivo) with Austrian, Croatian, and Hungarian cultivars and Kratošija, an additional synonym. *American Journal of Enology and Viticulture*, 59(2), 205-209.

[23] Muganu, M., Dangl, G., Aradhya, M., Frediani, M., Scossa, A., & Stover, E. (2009). Ampelographic and DNA characterization of local grapevine accessions of the Tuscia area (Latium, Italy). *American Journal of Enology and Viticulture*, 60(1), 110-115.

[24] Zulini, L., Fabro, E., & Peterlunger, E. (2005). Characterisation of the grapevine cultivar Picolit by means of morphological descriptors and molecular markers. *Vitis*, 44(1), 35-38.

[25] Crespan, M., Crespan, G., Giannetto, S., Meneghetti, S., & Costacurta, A. (2007). Vitouska' is the progeny of 'Prosecco tondo' and 'Malvasia Bianca lunga'. *Vitis*, 46(4), 192-194.

[26] Crespan, M., Cancellier, S., Chies, R., Giannetto, S., Meneghetti, S., & Costacurta, A. (2009). Molecular contribution to the knowledge of two ancient varietal populations: 'Rabosi' and 'Glere'. *Acta Horticulturae ISHS*, 827(1), 217-220.

[27] Cosmo, I., & Polsinelli, M. (1957). Ribolla gialla. Roma: Annali della sperimentazione agraria.

[28] Caló, A., Scienza, A., & Costacurta, A. (2006). Vitigni d'Italia. Bolognia: Edagricole-Edizioni Agricole Srl.

[29] De Lorenzis, G., Failla, O., Scienza, A., Rusjan, D., Nikolaou, N., Vouillamoz, J., & Imazio, S. (2012). Study of genetic differences among 'Ribolla Gialla', 'Rebula' and 'Robola' (Vitis vinifera L.) respectively from North Eastern Italy, Northern Balkans and Ionian Islands (under review).

[30] Šimon, S., Maletić, E., Karoglan Kontić, J., Crespan, M., Schneider, A., & Pejić, I. (2007). Cv. Maraština a new member of the Malvasia group Mediterranean Malvasias: conference proceedings. October 3-6, 2007, Salina, Italija.

[31] Bulić, S. (1949). Dalmatinska ampelografija., *Zagreb: Poljoprivredni nakladni zavod*.

[32] Blažina, I., Štolfa, D., Korošec-Koruza, Z., & Cerkvenik, D. (1990). Vitovska grganja' (Vitis vinifera L. 'Vitovska grganja') in domača 'Grganja' (Vitis vinifera L. 'Domača grganja'). *Zbornik Biotehniške fakultete*, 1, 10-14.

[33] Di Stefano, R. (1996). Metodi chimici nella caratterizzazione varietale. *Rivista di Viticoltura é Enologia*, 49(1), 51-56.

[34] OIV. (2008). 2nd Edition of the OIV descriptor list for grape varieties and Vitis species. *Paris: Organisation Internationale de la Vigne at du Vin*.

[35] Schneider, A. (1996). Grape variety identification by means of ampelographic and biometric descriptors. *Rivista di Viticoltura é Enologia*, 49(1), 11-16.

[36] Martínez, M. C., & Grenan, S. (1999). A graphic reconstruction method of an average leaf of vine. *Agronomie*, 19, 491-507.

[37] Alessandri, S., Vignozzi, N., & Vignini, A. M. (1996). AmpeloCADs (Ampelographic Computer-Aided Digitizing system). An integrated system to digitize, file and process biometrical data from Vitis spp. Leaves. *American Journal of Enology and Viticulture*, 47(3), 257-267.

[38] Weihl, T., & Dettweiler, E. (2000). Differentiation and identification of 500 grapevine (Vitis vinifera L.) cultivars using notations and measured leaf parameters. *Acta Horticulturae*, 528, 35-41.

[39] Pelengić, R., & Rusjan, D. (2010). Efficacy of ampelographic and phyllometric tools for the validation of grapevine Vitis vinifera L. biodiversity in Slovenia. *International Journal Of Food, Agriculture & Environment*, 8(3&4), 563-568.

[40] Pelengić, R. (2012). Ampelografski model za identifikacijo sort žlahtne vinske trte (Vitis vinifera L.). PhD thesis. *University of Ljubljana; (under review).*

[41] Alleweldt, G., & Dettweiler, E. (1989). A model to differentiate grapevine cultivars with the aid of morphological characteristics. *Rivista di Viticoltura é Enologia*, 42(1), 59-63.

[42] Santiago, J. L., Boso, S., Martínez, M., Pinto-Carnide, O., & Ortiz, J. M. (2005). Ampelographic comparison of grape cultivars in Northwestern Spain and Northern Portugal. *American Journal for Enology and Viticulture*, 56(3), 287-290.

[43] Pelengić, R. (2006). Ampelographic and ampelometric description of red grape varieties (Vitis vinifera L.) from collection Ampelografski vrt. *Graduation thesis.University of Ljubljana*.

[44] The European Vitis Database. (2011). *Institute for Grapevine Breeding: Geilweilerhof.*, http://www.eu-vitis.de/index.php, accessed 10 November 2011.

[45] Maletić, E. (1993). Ampelographic investigation of cvs Maraština, Bogdanuša, Vugava and Pošip (Vitis vinifera L.) in conditions of Ravni Kotari, region Coastal Croatia. *MSc thesis.University of Ljubljana*.