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## Evaluation of genetic diversity: Which of the varieties can be named 'Rebula' (*Vitis vinifera* L.)?

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### Summary

The variety 'Rebula' is an indigenous but also ancient variety in the Slovenian and Italian world-renowned winegrowing district Collio dating back to the ages of the Roman Empire. The DNA analyses of the variety 'Rebula' and its potential related varieties were conducted to evaluate its biodiversity. The variety 'Rebula' showed a very low similarity (16 %) with other analysed varieties, also with 'Rebula briška' and 'Rebula-old'. The varieties called 'Prosecco', 'Števerjana', 'Beli teran' and 'Briška Glera' revealed identical genotypes in all 11 SSR microsatellites analysed, therefore are regarded as synonyms. The varieties 'Rebula' and 'Ribolla gialla' revealed the identical SSR profile at 8 out of 9 SSR loci. 'Vitovska Grganja' share with 'Vitovska' only 55 % of analysed alleles but show their first degree relationship. The DNA analysis still showed existing deviations in synonyms and homonyms linked to the variety 'Rebula' what impedes its standardisation and revitalization.

**Key words:** *Vitis vinifera* L., grapevine, 'Ribolla', 'Rebula', microsatellite, synonyms.

### Introduction

'Rebula' (*Vitis vinifera* L.) has been one of the most important white grapevine varieties from ancient times and is still mostly cultivated in the area of Goriška brda and Collio where it is steadily gaining in importance. The first references of the name Rebula date back in 1299, later in 1376 as part of the deed of sale "Notariorum Joppi" in the area of the Slovenian Collio (COSMO and POLSINELLI 1957), however the first ampelographic descriptions of the variety are found in Vinoreja (VERTOVEC 1844) and in Handbuch der Ampelographie (GOETHE 1887). Some relations were also presumed to be with the varietal descriptions mentioned in "De re Rustica" by Lucius Janius Moderatus Colomella in 60-65-*rubellana alubelis*, *rabuncula* and *rabucula* according to COSMO and POLSINELLI (1957). VERTOVEC (1844) enumerated and described six different types of the variety 'Rebula' confirming its biodiversity which decreases with narrow clonal selection. VITOLOVIĆ (1960), HRČEK and KOROŠEC-KORUZA (1996), furthermore CALÓ *et al.* (2006) mention different synonyms for 'Rebula'. Many nurseries have selected some clones of this variety, but its biodiver-

sity is still not exploited in total, what is also observed in old vineyards and consequently in cellars in Goriška brda (Slovenian Collio) where the grape and wine quality of 'Rebula' has not been standardized yet.

The objective of our study was a determination of the variety's biodiversity through a confirmation of potential synonyms and homonyms of variety 'Rebula' used in the sub-Mediterranean part of Slovenia. The preliminary results could serve as a basis for further clonal selections of variety 'Rebula' produced in Slovenia.

### Material and Methods

**Plant material for microsatellite analysis:** According to the citations of related grapevine varieties and to the synonyms and homonyms of 'Rebula' (VERTOVEC 1844, GOETHE 1887, HRČEK and KOROŠEC-KORUZA 1996, CALÓ *et al.* 2006) the grapevine types and varieties listed in Tab. 1 were studied. Other varieties were used as standard/reference.

**DNA isolation:** Total genomic DNA was extracted from about 200 mg of young leaves from shoot tips of individual vines using the modified CTAB extraction method (KUMP *et al.* 1996).

**Microsatellite analysis:** Eleven previously described microsatellite loci were used for genotyping (Tab. 2).

**PCR conditions:** PCR reactions were performed in a final volume of 10 µl, containing 20 ng of genomic DNA, 1 x PCR buffer (Fermentas), 0.2 mM of each dNTP's (Sigma), 2 mM MgCl<sub>2</sub> (Fermentas), 0.5 µM of each primer and 0.25 U of *Taq* DNA polymerase (Fermentas). One of the primers for each loci was labelled with fluorescent Cy-5 dye for fluorescent detection (IDT Inc., BioScineces). The amplification of microsatellite loci was performed according to).

**Statistical analysis of microsatellites:** Expected heterozygosity (He), observed heterozygosity (Ho) and probability of identity (PI) were calculated using Identity software (1.0 version; Centre for Applied Genetics, University of Agricultural Sciences, Vienna) and polymorphism information content was calculated with Cervus program (2.0 versions, the University of Edinburgh 1998, written by Tristan Marshall). The Identity software was also used to detect identical genotypes. Genetic distances ( $D = 1 - \text{proportion of shared alleles}$ ) were

Table 1

List of the studied types and varieties related to the variety 'Rebula' belonging to their synonyms, homonyms and cultivation area

Variety / Type / Vine	Synonyms and homonyms (Translation)	Cultivation area and notes
Rebula	Garganja <sup>1</sup> , Ribolla gialla <sup>1</sup> , Glera <sup>1</sup> , Refosco bianco <sup>2</sup> , Teran bijeli <sup>2</sup> , Ribola bijela <sup>2</sup> , Erbula <sup>2</sup> , Jerbula <sup>2</sup> , Goriška ribola <sup>2</sup> , Rebolla <sup>3</sup> , Ribolla <sup>3</sup> , Ribolla bianca <sup>3</sup> , Ribuèle <sup>3</sup> , Rabuèle <sup>3</sup> , Rosazzo <sup>3</sup> , Ribollat <sup>3</sup> , Raibola <sup>3</sup> , Ràbola <sup>3</sup> , Ribuole <sup>3</sup> , Gargania <sup>3</sup>	Goriška brda (Slovenian Collio), Collio (Italy) and Istria (Croatia). Sampled in the collection of University of Ljubljana
Rebula briška		The variety (last trunk) sampled in Stomaž (Vipavska dolina). It is more than 100 years old
Rebula stara	Rebula old	Vipavska dolina, Goriška brda (Slovenian Collio), Collio (Italy)
Grganc		The variety (last trunk) sampled in Stomaž (Vipavska dolina)
Briška Glera	Glera Collio <sup>2</sup> , Glera <sup>1</sup> , Gljera <sup>1</sup>	Vipavska dolina (Slovenia). Sampled in collection of University of Ljubljana
Bela Glera	White Glera <sup>1</sup>	Vipavska dolina (Slovenia), Goriška brda. Sampled in collection of University of Ljubljana
Glera		Vipavska dolina (Slovenia), Goriška brda, Carso (Slovenia and Italy)
Prosecco	Gljera <sup>1</sup> , Prosekar <sup>1</sup> , Prosecco tondo <sup>3</sup> , Prosecco bianco <sup>3</sup> , Gargana <sup>3</sup> , Brešanka <sup>1,3</sup> (Brescia)	Vipavska dolina (Slovenia), Goriška brda, Carso (Slovenia and Italy)
Vitouska	Grganja <sup>1,3</sup> , Vitouska <sup>3</sup> , Gargania <sup>3</sup> , Ribolla gialla <sup>3</sup>	Carso (Slovenia and Italy), Vipavska dolina (Slovenia). The variety (last trunk) sampled in Stomaž (Vipavska dolina)
Vitovska grganja	Garganja <sup>1</sup> , Vitouska <sup>3</sup> , Gargania <sup>3</sup> , Ribolla gialla <sup>3</sup>	Carso (Slovenia and Italy), Vipavska dolina (Slovenia). Sampled in collection of University of Ljubljana
Števerjana	Name related to a village San Floriano (Števerjan) in Collio (Italy).	Goriška brda (Slovenian Collio), Collio (Italy). Sampled in collection of University of Ljubljana
Beli teran (Bijeli teran)	Teranovka <sup>4</sup> , Terano <sup>4</sup> , Beli refošk <sup>4</sup> , Refosko bianco <sup>4</sup>	Carso (Slovenia and Italy), Vipava Valley (Slovenia). Sampled in collection of University of Ljubljana

<sup>1</sup>HRČEK and KOROŠEC-KORUZA 1996; <sup>2</sup>VITOLOVIĆ 1960; <sup>3</sup>CALÓ *et al* 2006; <sup>4</sup>GOETHE 1887.

calculated by the Microsat program (Version 1.5b. Stanford University Medical Center, Stanford) and were applied to draw a phenogram by using the Neighbor program (UPGMA algorithm) from the PHYLIP software package (version 3.6b; University of Washington, Seattle) and Treeview in order to visualize the obtained phenogram.

## Results and Discussion

SSR profiles of groups of varieties with similar phenotypic characteristics as variety 'Rebula' according to ampelographic descriptions and affirmations (VERTOVEC 1884, GOETHE 1887, COSMO and POLSINELLI 1957, VITOLOVIĆ 1960, HRČEK and KOROŠEC-KORUZA 1996) were compared in order to assess their relationships and resolve existing doubts on their identity. The general data about the selected varie-

ties is given in Tab. 1. The group of varieties named 'Prosecco', 'Števerjana', 'Beli teran' and 'Briška Glera' revealed identical genotypes in all 11 SSR microsatellites analysed and are therefore regarded as synonyms (Tab. 2), but variety 'Glera' differed at one locus compared to the previous group. For varieties called 'Grganc' and 'Rebula briška' the identical genotypes in all 11 SSR were observed, where 'Grganc' can be a dialect derivative from known synonym for 'Rebula', namely 'Grganja' (Tab. 1). The most unexpected results happened to be with the variety 'Rebula' which was chosen as a standard but showed a difference at most loci. Variety 'Rebula' is classified in the group of varieties *Proles occidentalis*, although HRČEK in KOROŠEC-KORUZA (1996) presumed it according to phenotypical characteristics as *Proles pontica*.

**Genetic relationships:** The proportion of shared alleles was used as basis for distance measure-

Table 2

Microsatellite profiles of 12 potential varieties at 11 microsatellite loci; in addition the profiles of six reference varieties are listed. Allele sizes are given in base pairs

Name of variety	VVS2	SsrVrZAG62	SsrVrZAG79	VVMD5	VVMD7	VVMD25	VVMD27	VVMD32	VVMD36	SsrVrZAG21	ssrVrZAG47
Griganc	141	149	196	200	238	252	ND <sup>a</sup>	238	244	244	252
Rebula stara	131	141	196	204	238	244	232	238	239	249	241
Rebula briška	141	149	196	200	238	252	230	232	239	249	257
Vitouska	131	143	196	204	244	250	224	238	239	247	241
Beli Teran	131	141	188	204	250	260	224	244	239	247	241
Glera	131	141	188	204	250	260	224	244	239	239	241
Vitovska grganja	131	141	194	204	244	252	224	238	239	255	241
Števerjana	131	141	188	204	250	260	224	244	239	247	241
Prosecco	131	141	188	204	250	260	224	244	239	247	241
Rebula	143	149	188	200	240	252	226	226	243	249	243
Briška Glera	131	141	188	204	250	260	224	244	239	247	241
Bela Glera	141	143	188	196	252	260	224	244	239	239	241
Reference varieties:											
Merlot	137	149	194	194	260	260	224	234	239	247	241
Pinot Noir	135	149	188	194	240	246	226	236	239	243	241
Cabernet Sauvignon	137	149	188	194	248	248	230	238	239	239	241
Sultanne	143	149	188	188	248	260	232	232	239	253	241
Touriga Nacional	141	149	188	194	246	246	224	234	239	239	251
Barbera	131	133	192	200	244	260	224	224	249	253	241

<sup>a</sup> ND = not determined.

ment among grapevine varieties. The average proportion of shared alleles among analysed set of varieties was 45 % (data not showed).

The varieties 'Grganc', 'Rebula briška', 'Rebula'-old and 'Vitovska grganja' are grouped together in the phenogram and the average proportion of shared alleles among them is 54 %. 'Rebula' old and 'Rebula briška' which were expected to be very closely related, share only 55 %. 'Rebula briška' revealed identical SSR profile as 'Grganc', with the only difference of triallelic pattern at locus VVMD5 obtained by 'Grganc'.

An interesting conclusion was that the variety 'Rebula' which was set as a standard in the collection vineyard was not drawn in the phenogram (data not shown). It resulted in very low similarity (16 %) to other analysed varieties and also to 'Rebula briška' and 'Rebula'-old, where the proportion of shared alleles is only 20 %. In pairwise comparisons, the highest genetic dissimilarity (91 %) was found between the group of synonyms ('Prosecco', 'Briška Glera', 'Števerjana', 'Beli teran') and the variety 'Rebula'. Regarding the genotyping results obtained by SSR analysis the varieties designated as 'Rebula' ('Rebula', 'Rebula briška' and 'Rebula-old') are homonyms. The comparison of genotyping results of 'Rebula' varieties with results of variety 'Ribolla gialla' (COSTACURTA *et al.* 2006) revealed the identical SSR profile at 8 out of 9 SSR loci between 'Ribolla gialla' and 'Rebula briška'.

The second group of the phenogram resulted in 77 % similarity among varieties and comprises a group of synonyms ('Prosecco', 'Briška Glera', 'Števerjana', 'Beli teran'). These synonyms are very closely related to the variety 'Glera'. The only difference between synonymic varieties and 'Glera' was in the stage of homozygosity/heterozygosity at locus VVMD7 and such mutations at this locus have often previously been reported (ŠTAJNER *et al.* 2008). The high genetic difference revealed between 'Glera'/'Briška Glera' and 'Bela Glera' can be explained by the fact that the name 'Glera' was frequently used for white grapevine vari-

eties in the sub-Mediterranean part of Slovenia (VERTOVEC 1844, CRESPIAN *et al.* 2009) and the obtained homonymy is therefore not surprising.

The comparison of Slovenian 'Vitovska Grganja' with 'Vitouska' from Trieste, Italy resulted in identical SSR profiles over all 10 SSR loci compared. 'Vitovska Grganja' shares only 55 % of analysed alleles with 'Vitouska' but shows their first degree relationship.

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## Corrigendum

On page 192 in the first sentence of the last paragraph of the manuscript:

### **Evaluation of genetic diversity: Which of the varieties can be named ,Rebula‘ (*Vitis vinifera* L.)?**

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the correct sentence is: The comparison of Slovenian 'Vitovska' with 'Vitouska' from Trieste, Italy resulted in identical SSR profiles over all 10 SSR loci compared.

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