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## Ampelographic characterisation of grapevine accessions denominated 'Refošk', 'Refosco', 'Teran' and 'Terrano' (*Vitis vinifera* L.) from Slovenia, Croatia and Italy

D. RUSJAN<sup>1</sup>), M. BUBOLA<sup>2</sup>), D. JANJANIN<sup>2</sup>), Z. UŽILA<sup>2</sup>), S. RADEKA<sup>2</sup>), D. POLJUHA<sup>2</sup>), R. PELENGIĆ<sup>3</sup>), B. JAVORNIK<sup>1</sup>) and N. ŠTAJNER<sup>1</sup>)

<sup>1</sup>) University of Ljubljana, Biotechnical Faculty, Agronomy Department, Ljubljana, Slovenia

<sup>2</sup>) Institute of Agriculture and Tourism Poreč, Croatia

<sup>3</sup>) Agricultural Institute of Slovenia, Slovenia

### Summary

Grapevines denominated 'Refošk', 'Refosco', 'Teran' and 'Terrano' have been cultivated in the area of western Slovenia, north-western Croatia and north-eastern Italy for centuries. Despite historical documents reporting the longstanding tradition of grapevine cultivation and winemaking, the denomination and origin of these varieties is still questionable. The aims of this work were to study the genetic identity and relationship of the grapevine accessions denominated 'Refošk', 'Refosco', 'Teran' and 'Terrano' that have been traditionally cultivated in Slovenia, Croatia and Italy. For this purpose, 9 SSR loci were analysed to fingerprint 53 accessions with denominations or similar true-to-type morphologies of 'Refošk' and 'Teran'. The grapevine variety 'Refošk' cultivated in Slovenia and most accessions denominated 'Teran' in Croatia showed identical genotypes in all analysed SSR markers, and can therefore be used as synonyms. Five accessions showed identical genotypes to 'Refosco dal peduncolo rosso' variety, however five other genotyped accessions suggested individual profiles, and can be characterized as clonal mutants of true-to-type 'Refošk'/'Teran'. Accessions 'Sladki Teran' and 'Ref5/31' shared 56 % and 61 % of the alleles with true-to-type profiles of 'Refošk'/'Teran' and their parentage analysis strongly suggested a full-siblings relationship. Obtained results contribute to the understanding of the genetic diversity of grapevine varieties cultivated in this part of Europe.

**Key words:** ampelography; microsatellite marker; OIV descriptor; phyllometry.

### Introduction

Slovenia, Croatia and Italy have undoubtedly a common and inter-related vitiviniculture from ancient times, but different ecological and socio-economical influences in last centuries have resulted in having a disorder in denominations of grapevine varieties, where CIPRIANI *et al.* (2010) and ŠTAJNER *et al.* (2013) reported different grape-

vine groups. An identification and separation of grapevines from "family" of 'Refošk', 'Refosco', 'Teran' and 'Terrano', still today causes many problems although many of them are treated as traditional, local and indigenous in many areas (VITOLOVIĆ 1960, VIVODA 1996, CALÒ 2004). 'Refošk' is the most important grapevine variety in Slovenska Istra, even more in Kras winegrowing district (Slovenia), where it is used for a production of an EU protected wine *teran* with recognized traditional denomination since 2007. On the other hand, the long lasting presence of presumably different varieties denominated as 'Teran' and 'Terrano' cultivated in Croatia and Italy for centuries, nowadays gives rise to a problem with wine denomination actualized in particular by recent Croatian EU accession. This problem is very complex and its roots go deep into the past. The first record of variety 'Teran' in Istria (Croatia) dates back to the early 19<sup>th</sup> century (STANCOVICH 1825 cited by VITOLOVIĆ 1960) and there are documents that recorded the importance and dominance of 'Teran' variety in that time. In the second part of 19<sup>th</sup> century 'Teran' was a prevalent variety in Istria (Croatia), with more than 80 % spread in total Istrian vineyard surfaces and *teran* wine was even exported. First reports about a denomination 'Refosco' supposedly dating back to the middle of 18<sup>th</sup> Century in the area of Friuli (CALÒ 2004). Moreover, the use of the term "terrano" for a wine originated from the time of the Venetian Republic, when all wines produced on the "continent" were denominated as "terra" (soil), contrary to those produced in foreign countries around the Adriatic and Mediterranean sea and exported to Italy. An abundant number of ampelographic descriptions regarding 'Refosco', 'Refošk' and 'Teran', 'Terrano' can be observed in the late 19<sup>th</sup> and beginning of 20<sup>th</sup> Century (VERTOVEC 1844, GOETHE 1887), where the complexity of "family" of 'Refošk' and 'Teran' varieties is reported. A confusion regarding the naming of vines 'Refošk' and 'Refosco' were also noticed in the European *Vitis* Database in 2013. At the reviewing of the list of the varieties denominated "refosco" and their morphological characteristics many shortcomings, mistakes and inaccurate data were observed. Although many corrections in the last decade have been included in the European *Vitis* Database, many doubts and uncertainties regarding the group of "Refošk" and 'Teran' remain, especially in the field of

morphological description. For further clarification of the biodiversity in the group of grapevines with denominations 'Refošk', 'Refosco', 'Teran' and 'Terrano', a representative number of grapevines from Slovenia and Croatia were sampled and genotyped, including also some plant material from Italian grapevine nurseries.

### Material and Methods

According to the grapevine denominations (VERTOVEC 1844, GOETHE 1887, CALÒ 2004) fifty three vines denominated 'Refošk', 'Teran', 'Refosco dal peduncolo rosso', 'Sladki teran' or 'Teranovka' were characterised – phenotyped and genotyped (a list of studied accessions available at the author). Representative vines were chosen according to their origin and age; however most vines originated from old Slovenian and Croatian vineyards and some also from Italian grapevine nurseries. Six reference varieties ('Barbera', 'Cabernet Sauvignon', 'Chardonnay', 'Merlot', 'Pinot Noir', 'Sultanine') were included in genotyping analysis to enable standardization and comparison of allele sizes. The true-to-type vines denominated 'Refošk' (Slovenia) and 'Teran' (Croatia) were also phenotyped according to the 66 O.I.V. descriptors codes and to phyllometry (PELENGIĆ and RUSJAN 2010), where at least 10 observations or measurements per individual characteristic were performed. The significance of the morphometrical characteristics between studied accessions was analysed with Statgraphics Plus 5.0 using Fisher's least significant difference (LSD) range test with a significance level of  $P \leq 0.05$  and statistical differences between same characteristic from different blades (accessions) are given with "\*". Samples were genotyped at nine microsatellite loci VVS2, VrZAG62, VrZAG79, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, and VVMD32. DNA isolation, PCR conditions and allel sizing were performed as reported in SCHUELKE (2000) and ŠTAJNER *et al.* (2013). Genetic similarity was calculated per each pair of samples using the Jaccard's coefficient of similarity. A dendrogram was constructed from the matrix of pairwise distances, using the Unweighted Pair Group Method (UPGMA) for clustering in the NTSYS-PC software package version 1.80 (ROHLF 1993).

### Results and Discussion

**Genetic characterization:** The microsatellite profiles of 53 samples plus 6 references are performed (all data available at the author). Microsatellite fingerprinting of 53 samples revealed 7 unique genotypes. The biggest group of associations (perfect matches of allele sizes) comprised 40 samples with identical SSR profiles for all analysed loci as true-to-type accessions denominated 'Refošk' and 'Teran' (Fig. 1). Another group is represented by 5 samples of 'Refosco dal peduncolo rosso' variety (Ter4, Ter13, Ter36, Ter39 and RefPR) sharing 44.4 % of alleles with true-to-type profiles of 'Refošk' and 'Teran'. The third group was composed of two associations (Ter11

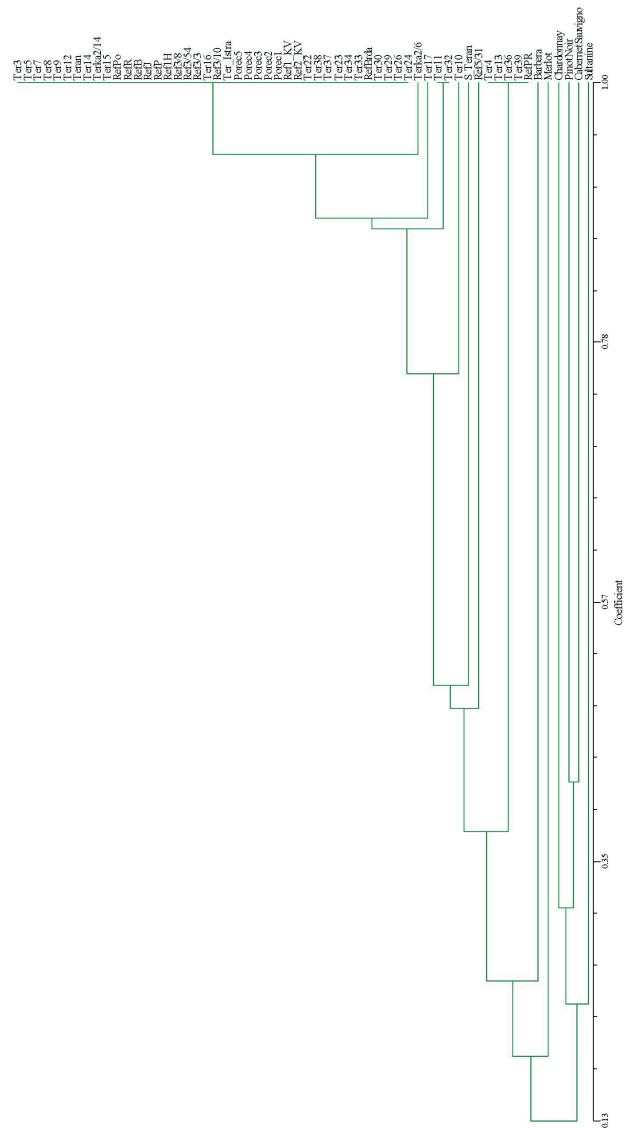


Fig. 1: Genetic similarity of studied grapevine accessions related to the denominations 'Refošk', 'Refosco', 'Teran' and 'Terrano' from Slovenia, Croatia and Italy based on the Jaccard's coefficient of similarity.

and Ter32), but the results suggested another 5 genotypes of individual profiles. Among those, five of them (Terka2/6, Ter17, Ter11, Ter32 and Ter10) can be characterized as clonal mutants of true-to-type 'Refošk'/'Teran', each resulting in only one mutation (difference at one allele) from true-to-type profiles. Only in the case of Terka2/6 the difference obtained was regarding homo/heterozygous stage at locus VrZAG62 but in other four accessions the difference was due to the shifting in allele length. On the other hand accessions 'Sladki Teran' and 'Ref5/31' shared 56 % and 61 % of the alleles with true-to-type profiles of 'Refošk' or 'Teran', respectively, showing with them first degree relationships.

The group of 40 accessions sampled in Slovenia, Croatia and plant material of 'Terrano' from Italian grapevine nurseries, which was planted in Croatia, represent the true-to-type of 'Refošk'/'Teran' and results so far show that there are no differences among these genotypes at SSR

level, what is in accordance with Maletić *et al.* (2014). Majority of genotyped accessions of 'Refošk' and 'Teran' from vineyards older than 60 years resulted in the same SSR profiles, what might be the supportive evidence for exchange of plant material between Slovenian and Croatian vine-growing areas through last centuries. Therefore, 'Refošk' from Slovenia and 'Teran' from Croatia should be recognised as the same variety and their denominations could be considered as synonym.

The reference profile of 'Refošk'/'Teran' group was further compared to the allelic profiles reported by COSTACURTA *et al.* (2004) and CRESPIAN *et al.* (2011). The first comparison resulted in some discrepancies at few loci, but the genotypes of 'Terrano' published by CRESPIAN *et al.* (2011) compared to our data, resulted in identical SSR profiles for all loci compared (7 out of 7).

The accession 'Sladki teran' was sampled many years ago in a very old vineyard near Lesiščina (Lupoglav, Istria, Croatia) and later introduced in an ampelographic vineyard in Kromberk (Nova Gorica, Slovenia). A name 'Sladki teran' was given to this accession because of its morphological similarities with variety 'Teran', and "sladki" means "sweet" because it produces grapes with a quite high content of sugar and less acidity in comparison to 'Teran'.

**Phenotyping:** According to the O.I.V. descriptors true-to-type vines 'Refošk' and 'Teran' showed identical characteristics in shoot tip; low density of erect, but high density of prostrate hairs. The length of the tendrils was same at both vines, between 25 and 30 cm, the young leaves were copper-reddish coloured, with high to very high density of prostrate but none to very low density of erect hairs between and on main veins on the lower side of a blade. 'Teran' and 'Refošk' have the same large, pentagonal, three lobed, dark green coloured, strong goffered blade, not limited and brace-shaped base of petiole and upper shallow depth lateral sinuses, petiole is slightly shorter compared to middle vein with high density of prostrate and low of erect hair. At 'Refošk' conical but at 'Teran' funnel shaped bunches were recorded; both with high bunch weight. 'Teran' and 'Refošk' gave same medium berry weight, broad ellipsoid shaped, uniform sized and blue dark coloured, with medium thickness of skin and hilum. 'Refošk' reached

medium length and narrow to medium width berries with high bloom, but 'Teran' short to medium length and narrow width berries with medium bloom. The observations with O.I.V. descriptor suggested the genotypically equal vines denominated 'Refošk' in Slovenia and 'Teran' in Croatia showed just few differences in morphological characteristics. On the other hand, the phyllometric measurements of the significant mature leaf characteristics of true-to-type 'Refošk' and 'Teran' (Fig. 2) unexpectedly showed significant differences in almost all measured leaf characteristics, what might be a reason that in the past they were quite frequently treated as different varieties and not as different biotype or even clone.

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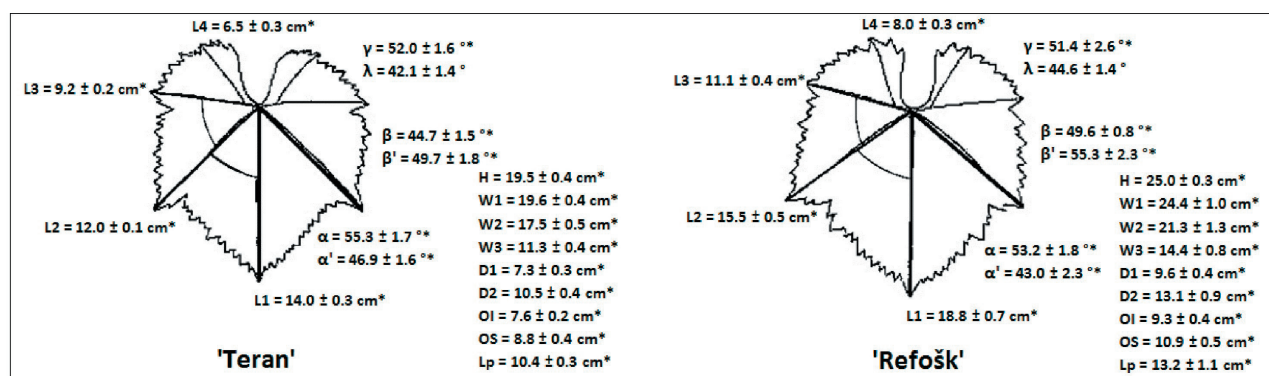


Fig. 2: Morphometrical characteristics of accessions denominated 'Teran' and 'Refošk', mostly cultivated in Slovenia and Croatia. Statistical differences between the same characteristics of different varietal blades are given with "\*" (LSD multiple range test,  $P \leq 0.05$ ).

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