

# Ampelographic and genetic studies into 'Teran'/'Refošk' grapes in Istria (Croatia) – one or two varieties?

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*'Teran' and 'Refošk' grapes are traditionally grown on the Istrian peninsula, a region encompassing three different national territories (Croatia, Slovenia and Italy). The question of identity of these grape varieties has therefore intrigued stakeholders in the wine industry for many years now. The confusion about their identity has also led to problems related to labeling planting material and protecting designation of origin for 'Teran' wines at the level of the EU. This paper details the history of this problem with an outline of past research. It then shows the results of ampelographic and genetic characterization of selected samples of 'Teran'/'Refošk' vines of different age and origin, all from Istrian vineyards. The results show that these are two different grape varieties (on the basis of 21 SSR loci), but also that these are phenotypically similar grape varieties (on the basis of OIV descriptors), which might have been the main reason for the confusion. Finally, the paper defines characteristic morphological differences which enable us to distinguish them on a practical level.*

**Keywords:** autochthonous grapevine varieties, *V. vinifera* L., ampelographic identification, genetic identification, SSR analysis, phyllometry

***Ampelographische und genetische Untersuchungen von Teran-/Refošk-Reben in Istrien (Kroatien) – eine Sorte oder zwei?*** Teran- und Refošk-Reben werden traditionell auf der istrischen Halbinsel angebaut, eine Region, die drei verschiedene nationale Territorien hat (Kroatien, Slowenien und Italien). Die Frage nach der Identität dieser Rebsorte(n) hat deshalb die Weinindustrie seit vielen Jahren fasziniert. Die Unsicherheit über ihre Identität hat auch zu Problemen bei der Kennzeichnung von Pflanzmaterial und bei der Geschützten Ursprungsbezeichnung für Teran-Weine auf EU-Ebene geführt. Diese Arbeit beschreibt die Geschichte dieses Problems mit einem Abriss früherer Forschung. Dann werden die Ergebnisse der ampelographischen und genetischen Charakterisierung ausgewählter Proben von Teran/Refošk-Reben unterschiedlichen Alters und Herkunft, die alle aus istrischen Weinbergen stammten, dargestellt. Die Ergebnisse zeigen, dass es sich um zwei verschiedene Sorten (auf Basis von 21 SSR-Loci) handelt, aber auch, dass sie sich phänotypisch ähnlich sind (auf Basis der OIV-Deskriptoren), was der Hauptgrund für die Unsicherheit gewesen sein könnte. Schließlich definiert die Arbeit charakteristische morphologische Unterschiede, die es ermöglichen, die zwei Sorten auf einer praktischen Ebene zu unterscheiden.

**Schlagwörter:** autochthone Rebsorten, *V. vinifera* L., ampelographische Identifizierung, genetische Identifizierung, SSR-Analyse, Phyllometrie

'Teran' is an old grape variety of the Istrian peninsula which has been grown in that region since antiquity. The first written trace of 'Teran' wine dates back to 1390 (MARESCALCHI and DALMASSO, 1937). The first description and other information on 'Teran' were provided by STANCOVICH (1824) which is also considered the first work dealing with the ampelography of Istria. For a long time it had been the leading variety in Istria (in 1880 with more than 90 % of all vineyards), but after World War II white varieties (especially 'Malvazija') took over, and today, according to the official data of the Croatian Paying Agency for Agriculture, Fisheries and Rural Development of the Republic of Croatia it is grown on a total of 242 ha, i.e. on 8 % of surface areas in the Istrian County only.

'Teran' has often been confused with 'Refošk', and throughout history there have been different opinions on the identity of that variety/varieties. The name 'Refošk' was mentioned for the first time in the Italian region of Friuli Venezia Giulia (MARESCALCHI and DALMASSO, 1937) in the letters written by the well-known agronomist ANTONIO ZANON, published in Venice in 1767. Many classical works of ampelography also mention and describe 'Teran' and/or 'Refošk', clearly showing that there were opposing opinions about their identity: some argued that 'Teran' and 'Refošk' were two different varieties, and others contended that they were synonyms for one and the same variety. GOETHE (1887) described them as different varieties stating that due to morphological similarities 'Teran' was often confused with 'Refošk'. He was also the first who reported the appearance of two "types" in the population of 'Refošk': one with red and the other with green peduncles. HUGUES (1889) mentioned 'Teran' from Kringa ('Terrano di Corridico'), 'Teran' from Kanfanar ('Terrano di Canfanaro') and 'Refošk' pasozobac ('Refosco magnacan'), but he did not state their mutual differences or similarities. LIBUTTI (1913) described them as one variety, stating that 'Teran' grown in karst conditions was used to produce wine known under the name of 'Karst Refošk' ('Refosco del Carso'). LIBUTTI thought that the appearance of 'Teran' with the green berry peduncle, or 'Teran' with red peduncles, was the result of environmental conditions. VITOLOVIĆ (1960) distinguished two different varieties, but added that they were mistaken for one another which commonly led to confusion. TURKOVIĆ (1952) mentioned the following synonyms for the 'Teran' variety: 'Refošk istarski', 'Istrijanac', 'Terrano' or 'Refosco d'Istria', 'Refosco del Carso' (a pedunculo e raspo verde), 'Teran noir', 'Teran blauer'. In addition

to the description of 'Teran', he mentioned two types/varieties, 'Refošk veliki' (Italian: 'Refoscone', 'R. Grosso', 'R. Di Faedis', 'R. Nostrano') and 'Refošk' or 'Teran', with red peduncles (Italian: 'Refosco a pedunculo e raspo rosso') warning that in that respect experts had differing opinions. On the basis of ampelographic and ampelometric research VIVODA (1996) concluded that 'Refošk' and 'Teran' were different varieties and that in 'Teran' population one could find types with green and red peduncles, noticing also that this feature was not stable. ČALO (2001) described 'Terrano', 'Refosco con pedunculo rosso' and 'Refosco nostrano' as different varieties.

More recently, the use of SSR markers has shed additional light on the identity of 'Teran' and 'Refošk'. KOZJAK et al. (2003) used microsatellite markers to analyse a number of 'Refošk' samples from Slovenia, a few samples from Italy, and three samples of 'Teran' from Croatia ('Teran', 'Teran Sladki' and 'Teran Beli'), but they did not provide precise data on the origin of their samples and their ampelographic characteristics. They concluded that most samples analysed had a unique (predominant) 'Refošk' genotype, among which they situated the 'Teran' sample from Croatia. However, they also distinguished several genetically very different samples which included two under the name of 'Refošk', originating from Slovenia, and two from Croatia ('Sladki Teran' and 'Beli Teran'). They did not consider these samples as 'Refošk' but as different (unknown) varieties.

COSTACURTA et al. (2005) conducted a systematic study of 14 samples from "Refosco group", including one sample under the name 'Terrano o Refosco d'Istria'. Using 16 SSR markers, samples were sorted into six groups which the authors considered distinct varieties. Five out of fourteen tested samples had an identical genotype and among them was the true-to-type sample of 'Refosco dal peduncolo rosso' variety. The authors thereby defined the SSR profile for that variety. The sample under the name 'Terrano o Refosco d'Istria' had a unique SSR profile which did not coincide with any other sample. This variety was entered in the List of Italian Grape Varieties under the same name. CIPRIANI et al. (2010) as well as CRESPIAN et al. (2011) worked on the molecular characterization of the autochthonous wine varieties of the region Friuli Venezia Giulia, among which they analysed several more varieties from the "Refosco group" as well as a sample under the name of 'Terrano', which in both studies showed a unique SSR profile, different from other samples under the name of 'Refosco'.

This long lasting confusion has affected wine production and production of propagation material of these varieties in the region which covers three national territories: Croatia, Slovenia and Italy. For instance, the official National List of Varieties in Slovenia (MKPG, 2011) mentions the 'Refošk' variety as synonymous with 'Refosco d'Istria'. On the other hand, the catalogues of great Italian producers of propagation material (VCR, 2005; VCR, 2007) consider 'Refosco d'Istria' as one of the synonyms for the 'Terrano' variety. These producers offer the grafts of 'Refosco dal peduncolo rosso' and 'Terrano' separately.

Recently this problem has created legal consequences for 'Teran' ('Terrano') wine producers in Croatia and Italy because they can no longer sell their wine on the European market under the name of the variety. This happened after Slovenia registered the name 'Teran' as Protected Designation of Origin for wines made of 'Refošk' in the Kras region.

A comprehensive list of grape varieties registered in 18 countries was published to support the implementation of regulations on propagation material in Europe (LACOMBE et al., 2011). That list states varieties 'Refosco dal peduncolo rosso' (with synonyms 'Refosco' and 'Malvoise'), 'Refosco' (with the synonym 'Refošk') and 'Terrano' (with synonyms 'Cagnina', 'Teran' and 'Lambrusco dal peduncolo rosso'). The first objective of this paper is therefore to determine if 'Teran' and 'Refošk' grown in the Croatian part of Istria are synonyms for the same variety, or if these are in fact different genotypes. We then want to compare the results obtained with similar studies conducted by other authors. This will contribute to a better understanding of regional and national grape varieties of Croatia, Italy and Slovenia, as well as contri-

bute to a solution of practical problems related to the system of protected designation of origin and precise labeling of propagation material. The aim of this paper is also to define characteristic phenotypic differences by using ampelographic and ampelometric methods, which will be helpful in their practical differentiation.

## Material and methods

Fourteen vineyards have been selected for this research on the basis of recommendations made by experts specializing in local varieties. These vineyards are of different age and origin of planting material, situated in different locations in Istria. In five vineyards a variety is declared as 'Teran', in two as 'Teran stari', in one as 'Teran mali', and in five as 'Refošk'. For each genotype we determined the colour of the berry peduncle, which most authors recognize as a significant morphological feature of this/these variety/varieties (Table 1). After selecting three representative vines, we initiated ampelographic evaluation with OIV descriptors (OIV, 2009) for two consecutive years. The evaluation based on five leaves and bunches per vine was conducted partly in situ and partly in an ampelographic laboratory.

## SSR analysis

Samples for DNA analysis were taken individually from all 42 vines. Genetic identification was carried out using microsatellite markers (SSR). All samples were first screened on 5 SSR loci (VVS-2, VVMD-7, VVMD-27, VrZAG-62 and VrZAG-79) according to the protocol described in ŽULJ-MIHALJEVIĆ et al.

Table 1: List of analyzed samples, description of their origin and berry peduncle color

Sample name	Sample No	Location	Plant material origin	Age of vineyard	Peduncle color
Teran	VV 551-553	Barban	unknown	> 70 years	green
Teran	VV 554-556	Velanov Brijeg	unknown	> 80 years	green
Teran	VV 557-559	Podmerišće	Croatia	10 years	red
Teran stari	VV 560-562	Podmerišće	unknown	> 100 years	green
Refošk	VV 563-565	Momjan	Slovenia	15 years	red
Refošk	VV 566-568	Momjan	Italy	10 years	green
Teran	VV 569-571	Motovun	Croatia	50 years	red
Teran mali	VV 572-574	Kaldir	Croatia	10 years	green
Teran	VV 575-577	Vižinada - I	Serbia	35 years	green
Refošk	VV 578-580	Vižinada - II	Slovenia	14 years	green
Refošk	VV 581-583	Vižinada - III	Italy	13 years	green
Teran stari	VV 584-586	Vižinada - IV	Croatia	46 years	green
Refošk	VV 587-589	Vižinada - V	Italy	> 20 years	red
Teran	VV 590-592	Koreniki	Italy	13 years	green

(2013). Two sets of four samples representing two different genotypes revealed in the first step were then used for extended analysis by additional 15 markers. Out of those, four loci (VVMD5, VVMD25, VVMD28 and VVMD32) were analysed by the protocol described by ŽULJ-MIHALJEVIĆ et al. (2013), while the remaining 11 loci (VVIq52, VVIIn16, VVIp60, VVIv67, VMC6B11, VVIb01, VVIh54, VVIp31, VVIIn73, VMC4f3, VVMD31 and VVMD24) were analysed by the protocol suggested by LAUCOU et al. (2011). The results were compared with the EU Vitis database (MAUL et al., 2012) and with the profiles of varieties from the Vasal collection in France (LACOMBE et al., 2012.)

### OIV description

To identify varieties, 20 descriptors were selected (mature leaf: OIV 067 - shape of blade; OIV 068 - number of lobes, OIV 070 - anthocyanin coloration of the main veins on the upper side of the blade; OIV 072 - goffering of blade; OIV 074 - profile; OIV 075 - blistering of upper side; OIV 076 - shape of teeth; OIV 079 - degree of petiole sinus opening; OIV 080 - shape of base of petiole sinus; OIV 081-1 - presence of teeth in the petiole sinus; OIV 081-2 - petiole sinus limited by veins; OIV 083-2 - presence of teeth at the base of the upper leaf sinuses; OIV 084 - density of prostrate hairs between the main veins (lower side); OIV 087 - density of erect hairs on the main veins (lower side); bunch: OIV 202 - length; OIV 208 - shape; OIV 209 - number of wings; berry: OIV 223 - shape; OIV 505 - must sugar content; OIV 506 - titratable acidity (Orv, 2009).

### Cluster and berry composition

Bunch and berry measurements were carried out on grape samples from vines which were genetically proven to belong to two different varieties. In order to be able to compare the obtained results, samples were taken from the same location paying special attention to uniform growing conditions within the location for both varieties. The following measurements were made on the average sample of ten bunches of each variety: length, width, weight of bunch, number and weight of berries per bunch. Calculation was made for the average weight of one berry (total berry weight/number of berries). Comparison of average values was tested by Bonferroni t-test.

### Phyllometry

Leaf characteristics were measured on samples representing two different SSR genotypes. The objective was to determine specific differences between these two varieties that might eventually be used for reliable determination at this level. For this purpose, a total of 79 phyllometric variables were used, 37 variables were obtained as a result of measurements using SuperAmpelo software (Superampelo 1.01.; Comunita Monastica; SS Pietro e Paulo, Germagno, Italy), while the remaining 42 parameters were calculated as ratios between the stated parameters as shown in PREINER et al. (in print).

Leaves were used from the medium third of the shoot in the phase of full maturity. Five leaves per vine representing all vines and locations were sampled for measurements. One-way ANOVA was employed to determine the statistical level of difference between two studied varieties. After that, canonical discriminant analysis was performed using statistical software SAS 9.1.3. Service Pack 4 (SAS Institute Inc.; Cary, NC, USA.). Using the stepwise discriminant analysis (PROC STEPDISC), a ranking of measured and calculated phyllometric parameters was made considering their efficiency in separating two studied varieties. This was followed by discriminant analysis (PROC DISCRIM) with the application of a minimal number of highest ranking phyllometric parameters from STEPDISC analysis to achieve the exact grouping of leaf samples into the respective variety. Using SuperAmpelo software a schematic image of leaves of both varieties was created on the basis of all leaf samples.

### Results and discussion

#### SSR analysis results

After the first part of SSR analysis with 5 markers in all 42 samples, only two different profiles were detected. The first profile was determined in 36 samples and the other in 6 samples only. Comparing the profiles with the baseline sample of the 'Teran' variety (HRV041-SA#11-63) from EU Vitis database (MAUL et al., 2012) it was found that the dominant profile corresponded to 'Teran' ('Terrano') variety, whereas the less represented profile, determined in VV 566-

568 and VV 587-589 samples, corresponded to 'Refosco dal peduncolo rosso' (COSTACURTA et al., 2005) variety, so that it is justified to use the Istrian name 'Refošk' for it.

Expanding the genetic analysis to additional 16 loci in eight samples (4 of each profile from the first phase), a consistent result was determined, i.e. two profiles were still different in all loci, while no differences were established between the samples of the same initial profile. The SSR analysis results are shown in Table 2. Proportion of shared alleles between 'Teran' and 'Refošk' found in this study is 45,3 % (varieties share one allele in 17 out of 20 loci). MALETIĆ et al. (1999) reported that average proportion of shared alleles among Croatian varieties was 37,2 % and RUSJAN (2013) found that average similarity of Slovenian germplasm was 34 % of shared alleles. High proportion of shared alleles between 'Teran' and 'Refošk' varieties indicates a certain degree of genetic relatedness.

### OIV description results

The results of OIV descriptors analysis (Table 3) do not indicate any significant differences in the most relevant morphological characteristics of fourteen selected samples of two different varieties. In particular, the expression of features defined by OIV descriptors (067, 074, 076, 079, 080, 81-1, 81-2, 83-2, 087, 202, 209 and 223) was identical in all genotypes. A higher variability was determined in the density of prostrate hairs between the main veins (lower side, OIV-084). In fact, even if all samples showed some hairiness of the lower side of the blade, the density of hairs was established in the range weak to medium up to dense. In the samples which were determined as 'Refošk' by means of genetic analysis (VV 566 - VV 568, and VV 587 - VV 589), medium density of hairiness of the lower side of the blade was noticed, while in other samples identified as 'Teran' this feature varied to a much higher degree. These differences can be explained by an intravarietal variability which regularly appears in the population of varieties which have been planted for a long time.

The color of the berry peduncle, which was determined in the first year of research in all samples, and which is often stated as a very important feature in distinguishing 'Teran' from 'Refošk', did not prove to be a stable feature. The red peduncle was noticed both in genotypes which were later determined as 'Teran' as well as in those of 'Refošk'. Similarly, this feature was

Table 2: SSR profiles of 'Teran' and 'Refošk' samples

SSR loci	Teran	Refošk
	VV571, VV573, VV579, VV580	VV566, VV587, VV588, VV589
VVMD5	222	222
	224	228
VVMD25	239	239
	253	253
VVMD28	216	232
	232	234
VVMD32	250	250
	272	262
VVIn16	152	157
	159	159
VVIp60	314	317
	320	320
VVIv67	362	356
	370	365
VMC6B11	85	85
	85	95
VVIb01	287	289
	293	293
VVIh54	165	159
	167	165
VVIp31	183	175
	193	183
VVIn73	258	263
	265	265
VMC4f3	164	166
	182	172
VVMD31	209	211
	211	215
VVMD24	212	208
	217	217
	Teran*	Refošk**
VVS2	VV551-565	VV566-568
	VV569-586	VV587-589
VVMD7	VV590-592	
	133	131
VVMD27	153	131
	245	237
VrZAG62	247	245
	185	177
VrZAG79	185	185
	193	191
	193	193
	236	248
	248	256

\* SSR profile identical to true to type sample of 'Teran' from EU Vitis database (ACCNUM: HRV041-SA#11-63)

\*\* profile corresponds to the sample of 'Refosco dal peduncolo rosso' from Costacurta et al. (2005)

not consistently repeated in the ampelographic evaluation of bunches in the second year of research. These observations are in agreement with the arguments made by LIBUTTI (1913) and VIVODA (1996).

The differences between 'Teran' and 'Refošk' genotypes are greater in some agronomic features such as characteristics susceptible to environmental conditions. For instance, the level of must sugar content (OIV 505) was medium in 'Teran' (note 5), and high in 'Refošk' (note 7). As was expected, 'Teran' exhibited a higher level of must titratable acidity than 'Refošk' (OIV 506). Table 4 shows the results of cluster and berry composition of samples determined as 'Teran' or 'Refošk', taken from the same vineyard. The statistical analysis showed that the characteristics of the bunch in these two varieties differed significantly. 'Teran' had significantly higher average weight of the bunch (312.5 vs. 248.1 g), a smaller number of berries in the bunch (114.2 vs. 154.7), but a greater weight of an individual berry (2.65 vs. 1.56 g) in relation to 'Refošk'. A significant difference was also determined in the width of the bunch ('Teran' had a wider bunch), but there was no difference in length.

COSTACURTA et al. (2005) also reported that these were morphologically similar varieties. They determined that the expression of most features of the two varieties did not vary.

## Phyllometric results

The ranking of phyllometric parameters by STEP-DISC analysis is shown in Table 5. The complete (100 % accurate) separation of individual leaves in the res-

pective variety was achieved by using only the first four parameters. It can therefore be concluded that the most significant differences between these two varieties were found in the R2 ratio. In particular, this ratio was higher in 'Refošk'. Then, the difference was found in the R5 ratio which was also higher in 'Refošk', which indicates that the leaf petiole in relation to the length of the N1 vein was significantly higher in 'Refošk' than in 'Teran'. The next parameter which significantly differentiated two varieties was PI angle (degree of petiole sinus opening), i.e. 'Teran' had a more open sinus than 'Refošk'. Similarly, the base of the top of the vein N2 (BN2) varied, being significantly higher in 'Teran' than in 'Refošk'. On the basis of all leaf measurements, an average leaf of both varieties was created as shown in Figure 1. In addition to the bigger size of 'Teran' leaves, it is clear that the ampelometric characteristics of 'Teran' and 'Refošk' leaves show many similarities. The evaluation of OIV descriptors (Table 3) reveals no significant difference in leaf characteristics, but measurements show that leaves of both varieties significantly differ in the above-mentioned characteristics and that phyllometric results can explain their differences with a high degree of reliability. COSTACURTA et al. (2005) discovered that on the basis of phyllometric characteristics 'Teranno' and 'Refosco dal peduncolo rosso' varieties were clustered very closely, but they also observed that the 'Teran' variety had a petiole sinus more opened than 'Refošk', which has been proven in our research as well as one of the major distinguishing characteristics. RUSJAN (2013) confirmed that phyllometry is a reliable method for distinguishing grapevine varieties.

Table 3: OIV descriptors showing differences between/within 'Teran' and 'Refošk' samples

Variety	Sample	OIV code							
		068 Leaf number of lobes on mature leaf	070 Leaf anthocyanin coloration of main vein on upper side	072 Leaf goffering of the blade	075 Leaf blistering of upper side of blade	084 Leaf density of prostrate hairs between main veins on lower side	208 Bunch shape	505 Must sugar content	506 Must titratable acidity
Teran	VV 551-553	2/3: three/five	3: up to the 1 <sup>st</sup> bifurcation	5: medium	5: medium	7: dense	2: conical	5: medium	5: medium
	VV 554-556	2: three	1: none	3-5: weak-medium	3-5: weak-medium	3-5: weak/medium			
	VV 557-559			5: medium		5-7: medium/dense			
	VV 560-562	2/3: three/five	3: up to the 1 <sup>st</sup> bifurcation	3: weak	5: medium	5: medium			
	VV 569-571					7: dense			
	VV 572-574					5: medium			
	VV 575-577					7: dense			
	VV 584-586					5: medium			
	VV 590-592					7: dense			
	VV 578-580					3/4: up to the 1 <sup>st</sup> /2 <sup>nd</sup> bifurcation			
VV 581-583	5: medium								
VV 563-565	3: up to the 1 <sup>st</sup> bifurcation	3-5: weak-medium	7: dense						
Refošk	VV 566-568	2: three	3: up to the 1 <sup>st</sup> bifurcation	3: weak	5: medium	5: medium	2-3: conical/funnel	7: high	3: low
	VV 5873-589						2: conical		

Table 4: Cluster and berry composition of 'Teran' and 'Refošk' (Means followed by the different letters are significantly different according to Bonferroni t-test,  $p < 0.05$ )

Variety	Cluster length (cm)	Cluster width (cm)	Cluster weight (g)	Berries (number/cluster)	Single berry weight (g)
Teran	17.5 a	13.5 a	312.5 a	114.2 b	2.65 a
Refošk	18.1 a	10.9 b	248.1 b	154.7 a	1.56 b

Table 5: Stepwise discriminant analysis: 10 leaf parameters showing the highest efficiency (discrimination power) in determining 'Teran' and 'Refošk' samples

Ranking	Variable		Wilks's Lambda	Pr < Lambda
1	R2	AL+BE+GA/OS+OI (ratio of sum of angles $\alpha + \beta + \gamma$ and sum of length petiole point to lower leaf sinus and length petiole point to upper leaf sinus)	0.551134	<0.0001
2	R5	OP/ON1 (ratio of petiole length and vein N1 length)	0.437084	<0.0001
3	PI	angle of opening/overlapping of petiole sinus with centre in petiole point	0.386569	<0.0001
4	BN2	width of teeth N2	0.349111	<0.0001
5	TA	angle between N3 and N4 measured at tooth tip	0.326943	<0.0001
6	R12	OO3/ON3 (ratio between vein N3 length from petiole sinus to vein N4 and length of vein N3)	0.309437	<0.0001
7	R24	GA/AL+BE+GA (ratio of angle $\gamma$ and sum of angles $\alpha + \beta + \gamma$ )	0.289881	<0.0001
8	ON1	length of vein N1	0.273379	<0.0001
9	LUPIC	length of leaf with petiole	0.237208	<0.0001
10	R34	O3N4/ON3 (ratio of vein N4 length and vein N3 length)	0.231192	<0.0001

## Conclusion

The results of genetic analysis show that 'Teran' and 'Refošk' are definitely two different varieties but ampelographic observation and measurements have determined that they are morphologically very similar, which creates the problem of their reliable distinction and correct identification in the vineyard. In Croatia, 'Teran' and 'Refošk' are still considered as two distinct varieties, which is confirmed by this research. It has been determined that all samples taken from vineyards grown with the planting material originating from Croatia, or from older plantations (more than 80 or 100 years old) established by grafting in situ, have the 'Teran' genotype. The planting material originating from Italy was mostly declared correctly, i.e. in vineyards planted with grafts declared as 'Teran', the study determined that these actually had the 'Teran' genotype, and in those planted with 'Refošk' grafts, the study determined the 'Refošk' genotype ('Refosco con peduncolo rosso'). In all the samples of 'Refošk' taken from two vineyards planted with grafts originating from Slovenian nurseries, it has been determined that their genotype is identical to the 'Teran' one. This confirms that there is a problem in declaring these varieties in Slovenia, and that the variety considered in

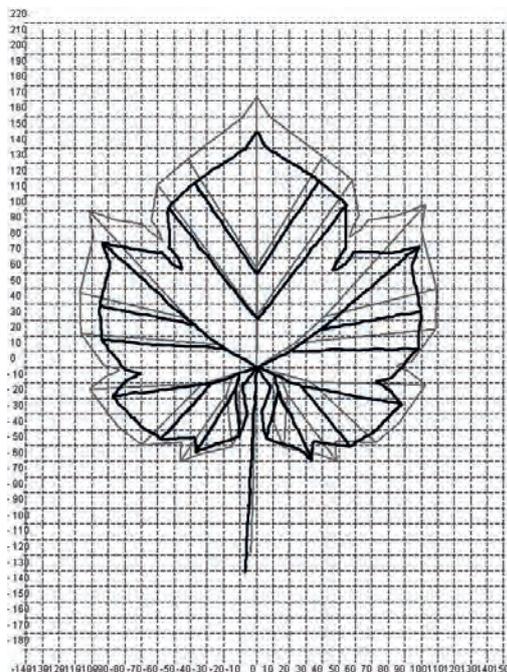


Figure 1: Average leaf shape image of 'Teran' and 'Refošk' (grey: 'Teran', black: 'Refošk')

Slovenia as 'Refošk' to produce "Teran of Kras" wine is actually the 'Teran' variety.

The detailed ampelometric analysis has shown that these two varieties differ significantly in the size of the grape bunch and berries, as well as in the content of must sugar and total must acidity. However, since these features can significantly be affected by the environment, they are not best suited for identifying varieties. Still, from the point of view of winemaking, they are highly important. At the same time, phyllometric results, or the comparison of selected leaf parameters, proved to be suitable for distinguishing these two grape varieties. In that respect, we have found that the accurate identification of samples as belonging to a certain variety can be achieved with only four parameters.

Since this is a simple and cheap method it could be useful for identifying grape varieties in the field, especially when it is not possible to conduct genetic analysis.

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## References

- CÂLO, A. (2001): Vitigni d'Italia. - Bologna Calderini edagricole, 2001
- CIPRIANI, G., MARRAZZO, M.T. AND PETERLUNGER, E. 2010: Molecular characterization of the autochthonous grape cultivars of the region Friuli Venezia Giulia – North-Eastern Italy. *Vitis* 49(1): 29-38
- COSTACURTA, A., CALÒ, A., CARRARO, R., GIUST, M., AGGIO, L., BORSA, D., DI STEFANO, R., DEL ZAN, F., FABBRO, A. E CRESPIAN, M. (2005): L'identificazione e la caratterizzazione dei Refoschi. In: Proc. Conference "dei Refoschi" (pp. 25-45). – Trieste: Ed. Lloyd of San Dorligo della Valle, 2005
- CRESPIAN, M., FABBRO, A., GIANNETTO, S., MENEGHETTI, S., PETRUSSI, C., DEL ZAN, F. AND SIVILOTTI, P. 2011: Recognition and genotyping of minor germplasm of Friuli Venezia Giulia revealed high diversity. *Vitis* 50(1): 21-28
- GOETHE, H. (1887): *Handbuch der Ampelographie*. – Berlin: Parey, 1887
- HUGUES, C. (1889): *Sull'adattamento dei vitigni o sul modo di determinarli*. - Parenzo, 1889
- KOZJAK, P., KOROSK-KORUZA, Z. AND JAVORNIK, B. 2003: Characterisation of cv. Refosk (*Vitis vinifera* L.) by SSR markers. *Vitis* 42(2): 83-86
- LACOMBE, T., AUDEGUIN, L., BOSELLI, M., BUCCHETTI, B., CABELLO, F., CHATELET, P., CRESPIAN, M., D'ONOFRIO, C., DIAS, J.E., ERCISLI, S., GARDIMAN, M., GRANDO, M.S., IMAZIO, S., JANDUROVA, O., JUNG, A., KISS, E., KOZMA, P., MAUL, E., MAGHRADZE, D., MARTINEZ, M.C., MUNOZ, G., PATKOVA, J.K., PEJIC, I., PETERLUNGER, E., PITSOLI, D., PREINER, D., RAIMONDI, S., REGNER, F., SAVIN, G., SAVVIDES, S., SCHNEIDER, A., SPRING, J.L., SZOKE, A., VERES, A., BOURSQUOT, J.M., BACILIERI, R. AND THIS, P. 2011: Grapevine European Catalogue: Towards a comprehensive list. *Vitis* 50(2): 65-68
- LACOMBE, T., BOURSQUOT J.M., LACOU, V., DI VECCHI-STARAZ, M., PEROS, J.-M. AND THIS, P. 2012: Large-scale parentage analysis in an extended set of grapevine cultivars (*V. vinifera* L.). *Theor. Appl. Genetics* 126(2): 401-414
- LAUCOU, V., LACOMBE, T., DECHESNE, F., SIRET, R., BRUNO, J.P., DESSUP, M., DESSUP, T., ORTIGA, P., PARRA, P., ROUX, C., SANTONI, S., VARES, D., PEROS, J.-P., BOURSQUOT, J.-M. AND THIS, P. 2011: High throughput analysis of grape genetic diversity as a tool for germplasm collection management. *Theor. Appl. Genetics* 122: 1233-1245
- LIBUTTI, D. (1913): Cenni su alcuni vitigni indigeni coltivati in Istria, *L'Istria Agricola* N° 8. - Poreč, 1913
- MALETIĆ, E., SEFC, K.M., STEINKELLNER, H., KAROGLAN KONTIĆ, J. UND PEJIC, I. 1999: Genetic characterisation of Croatian grapevine cultivars and detection of synonymous cultivars in neighboring regions. *Vitis* 38(2): 79-83
- MARESCALCHI, A. E DALMASSO, G. (1937): *Storia della vite e del vino in Italia*. -Milano: Ed. Unione Ital. Vini, 1979
- MAUL, E., SUDHARMA, K.N., KECKE, S., MARX, G., MULLER, C., AUDEGUIN, L., BOSELLI, M., BOURSQUOT, J.M., BUCCHETTI, B., CABELLO, F., CARRARO, R., CRESPIAN, M., DE ANDRES, M.T., ERAS DIAS, J., EKHAVALA, J., GAFORIO, L., GARDIMAN, M., GRANDO, S., GYROPOULOS, D., JANDUROVA, O., KISS, E., KAROGLAN KONTIĆ, J., KOZMA, P., LACOMBE, T., LAUCOU, V., LEGRAND, D., MAGHRADZE., MARINONI, D., MALETIĆ, E., MOREIRA, F., MUNOZ-ORGANERO, G., NAKHUTTSISHVILI, G., PEJIC, I., PETERLUNGER, E., PITSOLI, D., POSPISILOVA, D., PREINER, D., RAIMONDI, S., REGNER, F., SAVIN, G., SAVVIDES, S., SCHNEIDER, A., SERENO, C., ŠIMON, S., STARAZ, M., ZULINI, L., BACILIERI, R. AND THIS, P. 2012: The European Vitis Database ([www.eu-vitis.de](http://www.eu-vitis.de)) - a technical innovation through an online uploading and interaction modification system. *VITIS* 51(2): 79-85
- MKGP (2011): Ministrstvo za kmetijstvo, gozdarstvo in prehrano, Fitosanitarna uprava Republike Slovenije. National List of Varieties. – Ljubljana, 2011
- OIV (2009): OIV descriptor list for grape varieties and *Vitis* species. 2<sup>nd</sup> ed. – Paris: Dedon, 2009
- PREINER, D., SAFNER, T., KAROGLAN KONTIĆ, J., MARKOVIĆ, Z., ŠIMON, S. AND MALETIĆ, E. (in press): Analysis of phyllometric parameters efficiency in discrimination of Croatian native *V. vinifera* cultivars. *Vitis*
- RUSJAN, D. (2013): Genetic and phenotypic diversity and relations between grapevine varieties: Slovenian germplasm. In: POLJUHA, D. and SLADONJA, B. (eds.): *The Mediterranean Genetic Code - Grapevine and Olive*, chapter 7. - Rijeka, Croatia: In Tech, 2013 (Available from: <http://www.intechopen.com/books/the-mediterranean-genetic-code-grapevine-and-olive/genetic-and-phenotypic-diversity-and-relations-between-grapevine-varieties-slovenian-germplasm>, 1.12.2013)

- STANCOVICH, P. (1824): Classificazione di alcune varietà di viti delle vigne. In: GERA, F.: Nuovo dizionario universale di agricoltura. - Venezia, 1845
- TURKOVIĆ, Z. (1952): Ampelografski atlas. – Zagreb: Nakladni zavod Znanje, 1952
- VCR (2005): Vivai Cooperativi Rauscedo Catalogue (<http://www.vivairauscedo.com/it/catalogo.php>)
- VCR (2007): Vitis Rauscedo General Catalogue (<http://www.vitis-rauscedo.it/pdf/catalogue-en.pdf>)
- VITOLOVIĆ, V. (1960): Vinogradarstvo Istre, Biblioteka arhiva za poljoprivredne nauke. - Beograd, 1960
- VIVODA, V. (1996): Teran i Refošk u Istri, Zagreb, Hrvatsko agronomsko društvo, Grafo 900-Zagreb, University of Zagreb, Croatia, 1996
- ŽULJ-MIHALJEVIĆ, M., ŠIMON, S., PEJIĆ, I., ČARKA, F., KOJIĆ, A., GAŠI, F., TOMIĆ, L., JOVANOVIĆ CVETKOVIĆ, T., MALETIĆ, E., PREINER, D., BOŽINOVIĆ, S., CORNEA, V., MARAŠ, V., TOMIĆ MUGOŠA, M., BOTU, M., POPA, A. AND BELESKI, K. 2013: Molecular characterization of old local grapevine varieties from South East European countries. *Vitis* 52(2): 69-76

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