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Deepening inside the homonyms of 'Wildbacher' by means of SSR markers

S. Meneghetti[1)], A. Costacurta[1)], M. Crespan[1)], E. Maul[2)], R. Hack[3)], F. Regner[3)]

[1)] CRA-VIT Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Centro di Ricerca per la Viticoltura, Conegliano, Italy
[2)] Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen, Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Germany
[3)] Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau, Klosterneuburg, Austria

Summary

Sixteen accessions of the grapevine cultivar 'Wildbacher' coming from different European repositories and from Styrian and Italian private vineyards were analysed by genetic fingerprinting with SSR markers. Five different molecular profiles were found, confirming that the name 'Wildbacher' is commonly used as a homonym. Several new genotypes could be linked to the previous list of the 'Wildbacher' family. In particular, in Italian commercial vineyards two main 'Wildbacher' varieties defined by A and B genetic profiles were found. They correspond to the two reference genotypes from Styria, 'Wildbacher Blau' ('Blauer Wildbacher)' and 'Wildbacher Spätblau'. In both countries 'Wildbacher Blau' represents the most spread and better performing type and it is earlier ripening compared to 'Wildbacher Spätblau'. They were morphologically and genetically very similar to each other and they shared at least one allele at each of the 11 SSR loci analyzed for cultivar identification. Three different other 'Wildbacher' genotypes from a German collection were identified as individual cultivars. While 'Rotblättriger' (genotype C) and 'Frühblau' from Germany (genotype D) showed some genetic similarity with A and B genotypes, E 'Wildbacher', coming from Hungary, proved to have a distinct genetic profile. Close relationship to the key variety (for the development of European diversity) 'Heunisch' is indicated by sharing one allele at all loci investigated so far. There exist some indications that the second parent is an extinct wild vine. Evaluation of morphological parameters resulted in some differences among all five genotypes.

K e y w o r d s : 'Blauer Wildbacher', 'Wildbacher blau', 'Schilcher', grapevine identification, genetic relationships.

Introduction

The Austrian traditional cultivar 'Wildbacher Blau' ('Blauer Wildbacher') is named after the small village Wildbach in Styria and its origin is not documented. Goethe (1887) was convinced that 'Wildbacher' was selected from wild vines and spread due to vigour and fruitfulness independent from soil, terroir and canopy management; also Babo and Mach (1881) favour this hypothesis. They supposed that the name 'Wildbacher' is related to the wild vines or the unusual type of wine. In fact 'Wildbacher Spätblau' shows morphological relationship to the wild vines (Renner *et al*. 2006 a). Actually, 'Wildbacher' is appreciated in other regions due to the high stability in growing under humid conditions on poor soils, the vines vigour and the high yields.

'Wildbacher Blau' is cultivated on about 1 % of total Austrian vineyard area, mainly in western Styria wine region (Documentation Austrian Wine 2003, www.winesfromaustria.com). Here, despite dark blue berries, the wines are processed to reach only rose colour, keep high acidity and reflect unripeness and fruityness. Nowadays the production of this style of wine is limited to a small region and the product with name 'Schilcher' is protected (Keppl 1990). However in the west Styrian plantages more than 450 ha of 'Wildbacher' are still cultivated (Ambrosi *et al*. 1998). Under a world wide aspect 'Wildbacher' is a rare and threatened variety. Especially some types (Trummer 1841) of this variety seem to be already extinct. Therefore the need of identifying, conserving and describing ancient and neglected varieties is of high priority. In several European countries there are still unexplored varities which will be lost without defining them. Current activities in the frame of European Agri Gen Res projects are on the way to assist survive of rare autochtonous cultivars. One of the steps is the documentation of these varieties in an European Database (www.genres.de/eccdb/vitis) by their morphological and genetic profiles.

In Italy 'Wildbacher Blau' is registered in the National Catalogue since 1980 (code no. 303) for the cultivation only in the Treviso's province (Veneto Region, North East Italy), where its presence is documented at least since the end of 19th century (Morten 1895). Nowadays the small Italian production of 'Wildbacher' wines has some commercial interest, prevalently for export to Austria, Germany, England and also Brazil. As a consequence of milder climate the Wildbacher wines produced in Italy are different from the Styrian ones, being ruby red in colour and less acidic.

Single vines named 'Wildbacher' could easily be found also in other European countries as Slovenia, Hungary and Germany and they are supposed to derive from original Styrian material (Renner *et al*. 2006 a).

In 1841 Trummer mentioned different types of 'Wildbacher' such as 'Frühblauer', 'Schlehenblauer', 'Später Blauer' and 'Rotblättriger' as original from Styria, giving their first detailed ampelographic description. Also Di Rovasenda (1877) has listed a series of 'Wildbacher' accessions and considered them as poor quality sub-varieties of

Correspondence to: Dr. F. Regner, Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau (HBLAuBA), Wienerstraße 74, 3400 Klosterneuburg. Fax: +43-2244-29554. E-mail: Ferdinand.Regner@hblawo.bmlfuw.gv.at

'Blauer Wildbacher'. Nevertheless, the small morphological differences of the types were not sufficient in the past to recognize them without doubts. Renner *et al*. (2006 a) using molecular markers and ampelographic descriptions showed that the name 'Blauer Wildbacher' can be regarded as a homonym and that the most spread in Austria was the so called "Frühblau type", covering more than 90 % of planted 'Wildbacher' vines: it was indicated as reference for 'Wildbacher Blau' ('Blauer Wildbacher') true-to-type.

Recent observations revealed that two morphological types are cultivated in commercial 'Wildbacher' vineyards in the Treviso province, sometimes mixed together in the same field. Therefore, the purpose of this study was to clarify the identity of 'Wildbacher' cultivated in Italy, by comparison with Austrian materials and by extension of the investigation to 'Wildbacher' of different European repositories in Germany, Italy and Switzerland, by means of SSR markers. Already the broader spectrum of 'Wildbacher' names within collections indicated that the variability in practical viticulture was in former times higher than nowadays.

Actually microsatellite markers became the most suitable and reliable tool for cultivar identification and clearing up of synonyms or homonyms (Regner *et al*. 2000, Fossati *et al*. 2001, Labra *et al*. 2001, Imazio *et al*. 2002, This *et al*. 2004). Due to their codominant heritage these markers could be used for parentage analysis (Bowers *et al*. 1996, Sefc *et al*. 1998) and mapping (Adam-Blondon *et al*. 2004, Fischer *et al*. 2004).

Material and Methods

Plant material: Sixteen 'Wildbacher' accessions from different countries were analyzed using SSR (Simple Sequence Repeat) markers. Particularly, we checked nine accessions from Italy (CRA-VIT collections and others), two from Austria (Styria), one from Switzerland (collection Changin) and four from Germany (collection Geilweilerhof).

DNA extraction: The DNA was extracted from young leaves according to the method described by Crespan *et al*. 1999. Genomic DNA was stored undiluted in TE (10 mM Tris, 1 mM EDTA) buffer pH 8.0 at -20 ºC. DNA quality was evaluated on standard agarose gels stained with Gel Red (Società Italiana Chimici, Roma, Italy) and DNA quantity with a DyNA Quant 200 fluorimeter (Pharmacia, Milan, Italy).

SSR analysis: Eleven microsatellite loci were analysed for the cultivars identification: the 6 core loci selected within GenRes081 European Project VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79 (This *et al.* 2004), plus VVMD28 (Bowers *et al*. 1996), ISV2, ISV3 and ISV4 (Crespan 2003). Cy™5 amidite fluorochrome was used for labeling the SSR forward primers.

The PCR reaction mixture contained: 5 ng total DNA, 7 µl Hotmaster mix (Eppendorf, Milan, Italy), 0.20 µl forward primer and 0.20 µl reverse primer, both at 20 pmol/µl, and sterile water to 15 µl final volume.

The PCR was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Milan, Italy) as follows: 95 °C for 1:30 min, 35 cycles at 94 °C for 30 sec, 55 °C for 30 sec and 65 °C for 30 sec, a final extension at 65 °C for 7 min and a last step at 8 °C to stop the reaction. 5 µl of the PCR product were tested on a 2 % agarose gel.

The SSR alleles were resolved on ReproGel™ High Resolution pre-made acrylamide-bisacrylamide solutions (8 % w/v) in modified TBE buffer (0.1 M Tris-HCl, 83 mM boric acid and 1 mM EDTA) and detected on a semi-automated DNA sequencer, ALFexpress-II (Amersham Biosciences, Milan, Italy). Separation was done at 1200 V, 60 mA, and 35 W for 400 min. at 55 ºC constant temperature. Fluorescence signals were collected every second and stored in a computer. A fluorescence labelled molecular marker size (Cy5 Sizer 50-500; Amersham Biosciences, Milan, Italy) comprising 10 fragments in the size range of 50 to 500 bp was used as an internal size marker. Allele sizes were determined using the software package ALFwin™ Fragment Analyser 1.04. Also amplification products of cultivars carrying alleles of known molecular size were used as a reference for allele sizing. According to This *et al.* (2004) SSR data are comparable with database SSR profiles as soon as they become standardized. Gained values were compared with alleles developed either in Conegliano or in Klosterneuburg.

The genetic relationships between 'Wildbacher' genotypes and Heunisch cultivar were performed using 34 SSR markers which the 6 core loci selected within GenRes081 (This *et al*. 2004), European Project VVS1 -29 (Thomas and Scott 1993), VVMD5 - VVMD8, (Bowers *et al*. 1996), VrZAG7 - VrZAG112 (Sefc *et al.* 1999), plus VVMD14 - VVMD28 (Bowers *et al*. 1999 a).

Data analysis: Genetic dissimilarity estimates between 'Wildbacher' accessions were calculated using the following formula: GDij = -Ln (PS), where PS is the percentage of common alleles within the i and j genotypes, according to Dangl *et al.* (2001). Thus, GDij = 0 indicates identity between i and j genotypes, whereas GDij > 0 indicates diversity. This method was suggested for its ability to find correlations without preliminary assumptions about the population under study or the frequency of alleles within that population; it is well suited for use with high variable SSR loci and unnatural populations such as grape (Dangl *et al.* 2001). A dendrogram was produced elaborating these data by means of the unweighted pair-group arithmetic average method (UPGMA) clustering algorithm and the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) Version 2.10 (Rohlf 2002). Nineteen outgroup varieties and Austrian cultivars were added for better comparison of genotypes relations, using SSR profiles from CRA-VIT molecular database.

Genetic similarity (GS) values between the same genotypes as above were calculated in all possible pair-wise comparisons using Dice's similarity coefficients (1945). They estimate the ratio between common alleles by total number of alleles detected in the two compared genotypes and were calculated using the following formula: GSij = 2a/(2a+b+c), where a represents the number of shared SSR alleles scored between the genotypes pairs (i and j) consid-

ered, b is the number of SSR alleles present in i but absent in j, c is the number of SSR alleles present in j but absent in i. Thus, GSij = 1 indicates identity between i and j genotypes, whereas GSij < 1 indicates diversity. Therefore, accessions showing the same SSR profile had DGij = 0 and GSij = 1.

Centroids of 'Wildbacher' varieties and 'Heunisch' ('Gouais') were bi-dimensionally plotted according to the principal coordinates extracted from the Dice's Genetic Similarity matrix estimated by SSR molecular markers using the Ordination\Eigen options of NTSYS-pc software (Rohlf 2002).

Results and Discussion

The 16 'Wildbacher' accessions showed 5 different microsatellite molecular profiles (Tab. 1) called A, B, C, D and E; allele lengths were reported in Tab. 2 with codes for the six GenRes081 loci. These findings reveal that the designation 'Wildbacher' functions as a homonym.

In particular, the 7 accessions named 'Wildbacher Blau' from Austria, 'Wildbacher Blau' N. 1 (Bersò), 'Wildbacher Blau' N. 2 (Capitello), 'Wildbacher' "Old Austrian Vineyard" and 'Wildbacher Blau' N. 6 (Collalto) from Italy, 'Wildbacher Blau' from Germany and 'Wildbacher Blauer' from Switzerland showed the A profile. The 6 accessions named 'Wildbacher Spätblau' from Austria, 'Wildbacher Blau' N. 3 (Sottoriva), 'Wildbacher Blau' N. 4 (Cuc), 'Wildbacher Blau' N. 5 (Collalto), 'Wildbacher Blau' N. 7 (Collalto) and 'Wildbacher Blau' CRA-VIT from Italy shared the B profile.

'Wildbacher Frühblau' (Germany), 'W. Rotblättriger' (Germany), 'Wildbacher Blau' from Hungary (Germany) showed the C, D, and E profiles, respectively.

Starting from Renner *et al.* (2006 a) results, it was no surprise to find many genetic profiles for the different 'Wildbacher' accessions. Therefore we could confirm that the name 'Wildbacher' is commonly used as a homonym and we were able to demonstrate other 'Wildbacher' genotypes besides Renner's list (2006 a). However, we could determine the two main 'Wildbacher' varieties defined by A and B SSR profile corresponding to the two reference genotypes from Styria, 'Wildbacher Blau' and 'Wildbacher Spätblau', respectively. Genetic analysis confirmed the visual observation that both genotypes are present and will be cultivated in Italian private vineyards. In some cases both types are mixed together in the same field (Teot and Collalto vineyards). In both countries 'Wildbacher Blau' is the most spread and better performing type and also ripens earlier compared to 'Wildbacher Spätblau'.

In the Swiss collection Changins 'Wildbacher' genotype A was detected; finally, at Geilweilerhof (BAZ, Germany) collection, C ('Rotblättriger'), D ('Frühblau') and E (from Hungary) genotypes were evaluated besides A. While in former studies (Renner *et al*. 2006 a) the additional 'Wildbacher' types could be verified as not 'Wildbacher' related, we were able to define 'Rotblättriger' and 'Frühblauer' as individual varieties. Only the Hungarian sample of 'Wildbacher' (E) results very different from the others ones (Tab. 1, Figs 1 and 2).

'Wildbacher Spätblau' and 'Frühblau' are rare to find in Austria, while 'Schlehenblauer' and 'Rotblättriger' disappeared from the vineyards. In Germany and Hungary there did not exist any tradition in cultivating 'Wildbacher', only few vines were imported from Austria in former times. Other 'Wildbacher' types (Bergstraße) in Germany could be identified as not true to type (Renner *et al.* 2006 a).

Genetic correlations among all 'Wildbacher' accessions plus 19 additional varieties were shown in the dendrogram of Fig. 1. All genotypes were divided into three distinct groups: the first one encompassed the individuals of 'Wildbacher' showing A or B profiles, being very close each other, plus C and D 'Wildbacher' genotypes; the remaining 'Wildbacher' coming from Hungary and showing E profile is included in the second group and represents a

Table 1

'Wildbacher accession' names and corresponding molecular profile (A, B, C, D, E)

SSR profile	Wildbacher name	Provenance	Reference	Identification number
A	Wildbacher Blau	Austria	1	Aut 024 Kl 404
A	Wildbacher Blau	Italy	3	Bersò Vineyard N. 1
A	Wildbacher Blau	Italy	3	Capitello Vineyard N. 2
A	Wildbacher	Italy	3	Old Austrian Vineyard
A	Wildbacher Blau	Italy	4	Collalto farm N. 6
A	Wildbacher Blau	Germany	2	DEU98-1993-185
A	Wildbacher Blauer	Switzerland	6	CRA-VIT 91.08 ex2008
B	Wildbacher Spätblau	Austria	1	Aut 024 Kl 630
B	Wildbacher Blau	Italy	3	Sottoriva Vineyard N. 3
B	Wildbacher Blau	Italy	3	Cuc Vineyard N. 4
B	Wildbacher Blau	Italy	4	Collalto farm N. 5
B	Wildbacher Blau	Italy	4	Collalto farm N. 7
B	Wildbacher Blau	Italy	5	Susegana (NCC, F33-P3-C3)
C	Wildbacher Rotblättriger	Germany	2	DEU98-1994-051
D	Wildbacher Frühblau	Germany	2	DEU98-1980-371
E	Wildbacher Blau from Hungary	Germany	2	DEU98-2001-092

Table 2

The 5 molecular profiles of the 16 'Wildbacher' accessions. Alleles are expressed in bp and also with codes for the six GenRes081 loci (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, VrZAG79)

Locus SSR	profile A		profile B		profile C		profile D		profile E	
VVS2	143	151	143	151	143	143	137	143	135	145
	CH2	SI1	CH2	SI1	CH2	CH2	CH1	CH2	BA2	SU1
VVMD5	228	240	228	240	226	240	228	238	226	246
	MU1	CF2	CF1	CF1	CF1	CF2	MU1	CH2	CF1	VE2
VVMD7	239	239	239	263	239	239	239	243	249	255
	CF1	CF1	CF1	CF2	CF1	CF1	CF1	TR1	MU2	PO2
VVMD27	181	191	181	189	179	194	179	189	185	194
	CF1	ME2	MU1	MU1	MU1	MU2	MU1	CS2	PI1	MU2
VrZAG62	193	195	193	195	187	195	187	187	193	203
	CF1	CH2	CH1	CH1	CH1	CH2	CH1	CH1	CF1	CF2
VrZAG79	242	250	242	246	236	250	244	250	248	248
	CH1	TR2	RO1	RO1	RO1	TR2	CH2	TR2	SI1	SI1
VVMD28	239	249	231	239	249	263	221	239	231	251
ISV2 (VMC6E1)	151	165	147	165	165	165	151	165	141	141
ISV3 (VMC6F1)	131	139	139	141	133	139	133	145	133	139
ISV4 (VMC 6G1)	169	197	169	197	187	187	177	177	193	197
VMCNG4b9	158	178	158	164	138	158	162	172	138	138

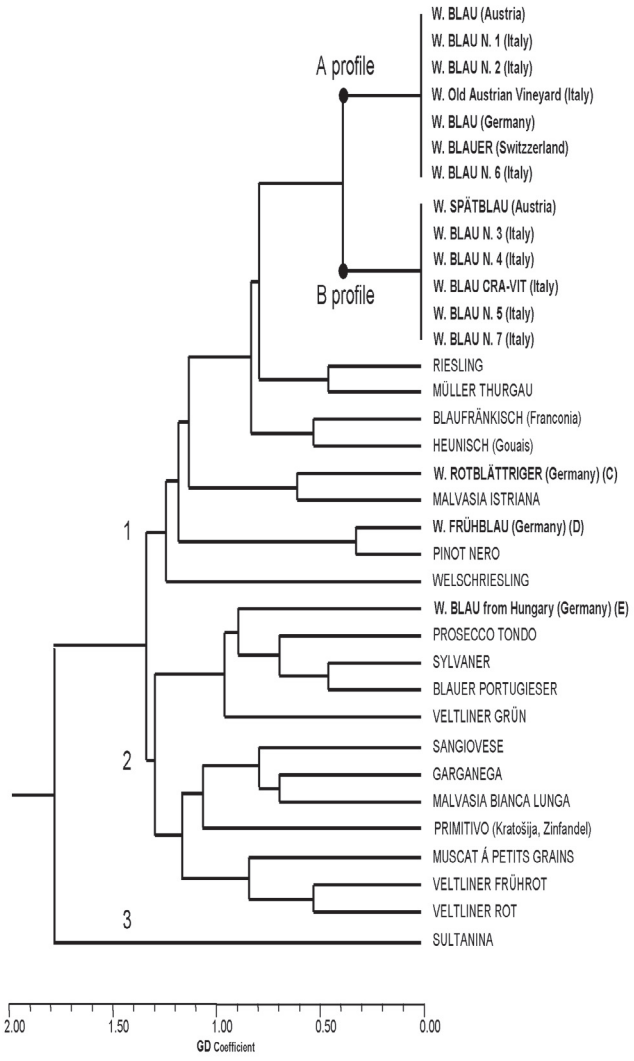

Fig. 1: Dendrogram of 16 'Wildbacher' accessions plus 19 outgroup varieties and Austrian cultivars (cophenetic correlation value: 0.8485), obtained with genetic dissimilarity indices.

well separated gene pool (A-B-C-D *vs*. E genotypes). Many other cultivars were selected mainly with some potential to be related to the 'Wildbacher' ('Riesling', 'Blaufränkisch', 'Heunisch', 'Malvasia' a.s.o.) or old traditional varieties from the same region or completely different phenotypes as 'Sultanina' or 'Müller-Thurgau'.

'Wildbacher Blau' and 'W. Spätblau' showed the closest relationship when the genetic similarity index was estimated. They reached the highest GS value (0.6818), as reported in Tab. 3 and represented as centroids in Fig. 2. Relationship of both cultivars is also supported from morphology (Tab. 5). The hypothesis that type A appeared as a mutation or a selfing from type B can be excluded because their SSR alleles are too different, in particular they shared at least one allele at each of the 11 microsatellite loci using for the cultivars identification: considering the CRA-VIT molecular database we can probably exclude the first degree relationship between A and B 'Wildbacher' types.

One of the key cultivars for the development of European diversity is the cultivar 'Heunisch' ('Gouais'). It could easily be that several seedlings from 'Heunisch' with local vines (or one local vine) represent the genetic base for the variability found in 'Wildbacher' (A, B and C types, Tab. 3). However the parentage of 'Heunisch' can be excluded for the types D and E in Tab. 3. 'Heunisch' was identified as a parent of a long list of cultivars (Bowers *et al*. 1999), some of them of great viticultural importance, such as 'Chardonnay', 'Riesling' or 'Blaufränkisch'. Therefore we added this ancient variety into the comparison studies with 'Wildbacher' genotypes. We could illuminate that it shares at the 34 investigated SSRs (Tab. 4) at least one allele per locus with A genotype. Therefore a first degree relationship of 'Heunisch' with this A genotype can be hypothesized.

A very brief description of some characteristics of shoot, mature leaf, cluster and phenology of the five 'Wildbacher' genotypes is reported in Tab. 5. It should allow to roughly define 'Wildbacher' types without assistance of ge-

Table 3

Genetic similarity calculated according to Dice's coefficients computed for the six SSR 'Wildbacher' genotypes and 'Heunisch' ('Gouais') cultivar

Genetic Similarity	A profile	B profile	C profile	D profile	E profile
B profile	0.6818				
C profile	0.4545	0.3182			
D profile	0.3182	0.2727	0.3182		
E profile	0.1364	0.1818	0.2273	0.0455	
Heunisch (Gouais)	0.5000	0.5000	0.5000	0.1818	0.2727

Table 4

'Wildbacher' type A and 'Heunisch' cultivar molecular profiles at 34 SSR loci

Locus SSR	Wildbacher A		Heunisch		Locus SSR	Wildbacher A		Heunisch	
VVS 1	189	189	189	189	VVMD31	205	213	211	213
VVS 2	143	151	133	143	VVMD32	249	271	251	271
VVS 3	212	218	218	218	VVMD36	252	262	262	274
VVS 4	167	172	167	168	VrZag7	155	155	155	155
VVS 29	168	176	168	176	VrZag15	165	191	165	165
VVMD5	228	240	234	240	VrZag21	194	206	202	206
VVMD6	199	209	189	209	VrZag25	225	225	225	238
VVMD7	239	239	239	249	VrZag29	112	116	112	116
VVMD8	138	144	138	144	VrZag30	149	151	147	149
VVMD14	222	232	222	234	VrZag62	193	195	195	203
VVMD17	221	222	220	222	VrZag64	137	159	159	159
VVMD21	248	248	248	248	VrZag67	139	152	139	139
VVMD24	207	215	207	215	VrZag79	242	250	236	242
VVMD25	256	256	240	256	VrZag82	251	277	251	263
VVMD26	249	255	249	251	VrZag83	188	190	188	194
VVMD27	181	191	179	181	VrZag93	188	188	188	188
VVMD28	239	249	231	249	VrZag112	240	242	240	242

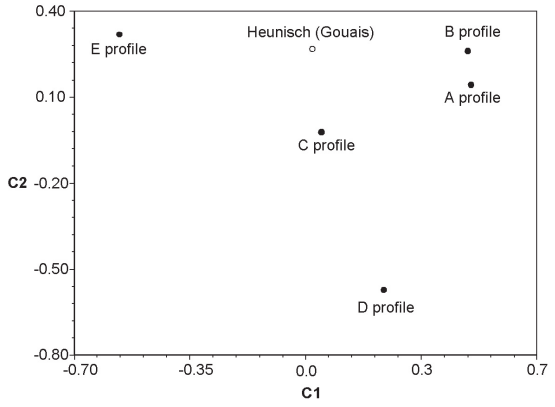

Fig. 2: Centroids of 5 'Wildbacher' genotypes and the 'Heunisch' ('Gouais') cultivar obtained with genetic similarity values.

netic markers but by following these morphological traits (OIV descriptors). Due to the age of the cultivar it is supposed that several 'Wildbacher' types still wait to become identified.

Considering leaf shape (OIV 67), profile of mature leaf (OIV 74), density of prostrate hairs (OIV 84) and size of bunches (OIV 202) the 'Wildbacher Rotblättriger' and the Hungarian 'Wildbacher' type are clearly different from the two main 'Wildbacher' varieties. 'Wildbacher Frühblau' can be recognized and differentiated by his overlapping lobes (OIV 79), while 'W. Spätblau' showed very small bunches (OIV 502) and late ripening behaviour (OIV 304). These morphological differences are also supported by the differing genetic profiles. Moreover 'Wildbacher Blau' and 'W. Spätblau' showed to be highly similar at morphological and genetic level (Figs 1 and 2, Tab. 3). Even from the morphological point of view 'W. Spätblau' resembles in some characters (bunch, berry, seeds) to wild vines. However, as the local wild vines were destroyed by phylloxera there is no opportunity to compare them nowadays directly with the wild vine parent. The second parent for at least type A and B (also possible for C) could be the variety 'Heunisch'. Due to their genetic differences it could not be defined if they derived from one sibling or from several ones.

Conclusions

Our work confirmed that several different varieties are grouped under the name 'Wildbacher' and the already by Renner and colleagues defined plurality could be enriched. By analyzing accessions from private vineyards and European collections, we identified 5 different genotypes. In those two countries where 'Wildbacher' is grown for commercial aims, Austria and Italy, the two different genotypes 'Wildbacher Blau' and 'Wildbacher Spätblau' are present in the fields. They are morphologically and genetically very similar and the main difference is that 'Wildbacher Blau'

Table 5

Short ampelographic description of the different types of 'Wildbacher' according to OIV descriptor list for grapevine varieties and *Vitis* species

Wildbacher Type	Wildbacher Blau	Wildbacher Spätblau	Wildbacher Rotblättriger	Wildbacher Frühblau	Wildbacher Blau from Hungary
SSR profile	A	B	C	D	E
OIV-065	5	5	5	5	5
OIV-067	3	3	2	3	2
OIV-068	3	2	2	3	1|2
OIV-074	2	3	1	2|4	3
OIV-075	5	3	2	5	4
OIV-078	5	5	5	5	5
OIV-079	4	3	3	7	3
OIV-084	5	3	1	3	7
OIV-202	5	5	7	3	5
OIV-204	7	5	7	7	9
OIV-221	5	3	5	5	4
OIV-225	6	6	6	6	6
OIV-303	5	7	5	5	5
OIV-304	5	7	5	5	5
OIV-502	3	1	6	3	5

ripens earlier with larger bunches than 'W. Spätblau'. The types A , B and even C could have the same parents probably 'Heunisch' as the first parent. While 'Heunisch' alleles are present at all 34 loci the second parental vine could be an extinct wild vine. The more appreciated and propagated type in both countries is 'Wildbacher Blau'. In Italy this variety reaches complete berry ripening, giving a dark coloured, less acidic and a well balanced product while in Austria mainly fresh rose wines and only rarely red wines are gained. From 'W. Spätblau' only acidic rose wines are produced.

The case of 'Wildbacher' is emblematic to underline the importance of repositories for conservation of rare and/or neglected cultivars, particularly nowadays, when disappearing of old varieties in their origin area is an increasing phenomenon. Identifying homonyms help to keep the genetic diversity within a cultivar and is a necessity to protect them from becoming lost. As a further step to investigate 'Wildbacher' it would be interesting to prove the viticultural performance and wine quality of the rare types C, D and E.

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