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# Identification of rare traditional grapevine cultivars using SSR markers and their geographical location within the Czech Republic

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**Abstract:** The designation of traditional varieties of grapevine is usually based on verbal information or very dated records. Old rare cultivars found in the Czech Republic were identified by Simple Sequence Repeat (SSR) analysis, a generally accepted method for cultivar identification. These cultivars are primarily maintained in a national collection of genetic resources. Finally a total of 102 candidate genotypes was selected where 49 traditional varieties were identified on the base of nine SSR loci compared with the European Vitis Database. Thirty-six items were registered under the correct designation. The remaining genotypes included four clones, and two genotypes could be described as synonyms. Seven genotypes were found to be incorrectly marked. For three of them, the correct name was found in the database under their SSR profile and four items were considered to be unique as no identical profile was found.

**Keywords:** DNA markers; genetic resources; *Vitis*

Grapevine is one of the oldest cultural plants that has been grown since ancient times. The beginning of viticulture in Moravia, which is the main wine-growing region of the Czech Republic, dates back to second and third century with the gradual penetration of the Roman legions of Caesar Marc Aurelius Antonio (161-180 AD) into the then Pannonia and Germania regions. Viticulture in the Czech Republic has experienced several ups and downs, but it has never completely disappeared from the country (Kraus 2009). Currently, the production potential of the Czech Republic is 19 200 ha. Ninety percent of vineyard area is planted with twelve white varieties (63% of the area) and eight red varieties (27% of the

area). Another 42 registered grape and table varieties are planted in an area covering 1486 ha. All other varieties, rootstock nurseries, and breeding and experimental areas take up the remaining 234 ha (Bublíková 2016). There are also unregistered rare traditional varieties used in the past, which have been forgotten with the discovery of modern varieties. The latter group is especially at risk as their existence in vineyards will gradually decrease. This data shows the need to preserve gene diversity and maintain the gene pool at least at the current level. Traditional varieties are well adapted to local conditions and are therefore valuable for the breeding of modern varieties, but they are also interesting from

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## MATERIAL AND METHODS

**Rare traditional cultivars screening.** For the purpose of this work it was necessary to find the vineyard estates, especially in South Moravia, with established vineyards (35 years and older) that still produce fruit and are mainly planted with historical varieties (source of information – ÚKZÚZ 2017). On the base of available documentation and approximate determination by morphological traits, it was finally found 102 candidate genotypes for genetic analysis representing 49 cultivars from eleven different localities.

**Plant material and DNA extraction.** Young leaves were collected for DNA isolation from five plants of each genotype (Table 1). In the first phase, one DNA sample was analysed from each genotype. The profiles obtained were compared with the European Vitis Database and, if a difference was found, DNA from another four plants was isolated to confirm or disprove the obtained result.

**SSR analysis.** DNA was isolated from leaves using a DNA Plant Mini Kit (Qiagen, Netherlands). The PCR mix was prepared in total volume of 25 µl containing 1× NEB buffer, 0.2 mM dNTP (Promega, USA), 0.2 µM of each primer, 1 U of *Taq* Polymerase (NEB, Great Britain) and 10 ng of DNA. Nine SSR primer pairs were used: VVS 2 (Thomas et al. 1993), VVMD5, VVMD 7, VVMD 28 (Bowers et al. 1996), VVMD 25, VVMD 27, VVMD 32 (Bowers et al. 1999), VrZAG 62, VrZAG 79 (Sefc et al. 1999b). The PCR started with DNA denaturation at 95 °C for three min. During 35 cycles of PCR, DNA was denatured for 30 min at 95 °C, primers were hybridised for 45 s using different temperatures for each primer pair (44 °C to 53 °C) and elongation of DNA was done for 45 s at 68 °C. At the end of the PCR program ten minutes at 68 °C were added to fully complete elongation. PCR products were separated using a genetic analyser (ABI Prism 310; Applied Biosystems, Carlsbad, USA), and the size of the alleles was determined by relevant GeneScan software (Ver. 3.7, Applied Biosystems, USA).

**Cultivar identification.** Allele sizes were converted from base pairs to codes according to This *et al.* (2004) methodology to compare the results obtained by other authors. For each cultivar analysed the prime name and other synonyms were first searched according to the VIVC (Vitis International Variety Catalogue 2019). Subsequently, the SSR profiles of all of the synonyms were searched (European Vitis

Database and other sources) and compared with the profile of the analysed genotype.

## RESULTS

In 2017 and 2018, vineyards and gardens in South Moravia were visited, where ancient traditional varieties were sought. There were 182 vineyard estates (approximately 3 000 ha.) on the land register of 90 wine-growing villages. It has often been found that in contrast to documentation the old traditional varieties can be found in very few vineyards. In most cases the vineyards have been restored with new plants of modern varieties (Figure 1). Twelve of the older genotypes that are not yet in the national collection were found in the old vineyards among other collections of rare traditional cultivars (Alphonse Lavallee, AL Volský, Cabernet franc, Čabaňská perla, Damascenka, Malingrovo ranné, Ortliebšké, Prachtraube, Bzenecká lipka, Tramín modrý, Trolínské modré, Veritas). Individual locations are marked on the presented map (Figure 1).

For most of the items the original variety designation was confirmed by SSR analysis. However, as expected, items that had been named incorrectly have also been found and based on the results obtained, the names required correction. A total of 49 genotypes represented by 102 samples were analysed. Based on the SSR analysis and their comparison with the data in the European Vitis Database, 36 items were classified under the correct designation, four of which can be classified as clones rather than separate varieties (Bzenecká lipka, Modrý Janek, Alphonse Lavallee Volský, Ryzlink buketový). One mutation was found, which is typical for an analysed clone (Alphonse Lavallee Volský – loci VVMD5, allele size is 236 bp instead of 240 bp). Two genotypes can be considered synonymous from the SSR's perspective (Sylváňské ranné, Tramín modrý). Seven items were found to be incorrectly marked, three were matched with an SSR profile and the item was then marked with the correct name (marked in green, Table 1). Four items were not found, and therefore could be considered to be unique. These can be an interesting source for additional characterisation and breeding.

The aim of the presented work was also to try to determine the genotypes found in old vineyards, where information about possible origin and identity has disappeared. Of the four unknown samples, three were identified and one failed to match an SSR profile in the European Vitis Database (Table 1). The

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Table 1. List of analysed old traditional cultivars, their location and true identity according to SSR markers

The name used in the Czech Rep.	Prime name according VIVC	Location	Sample origin	Amount	Compared with	Identity by SSR markers
Alphonse Lavalle	Alphonse Lavallee	2	2	2	FRA 139, AUT024	Alphonse Lavalle
Alphonse Lavalle volský	Alphonse Lavallee	2	2	5	FRA 139, AUT024	Alphonse Lavalle
Augusta Luise	Augusta Luise	2, 10	2	5	VIVC	Augusta Luise
Bouvierův hrozen	Bouvier	2, 6, 7, 8, 10	2	1	DEU	Bouvier
Cabernet Franc	Cabernet franc	2, 6, 7, 8	2	2	AUT024, ESP080, DEU098	Cabernet franc
Čabaňská perla	Csaba Gyoengye	1, 2, 5, 6, 7	2	2	DEU098, FRA139	Csaba Gyoengye
Dívčí hrozen/ Leánka	Feteasca alba	1, 2, 11	2	1	HUN045	Feteasca alba
Damascenka	Chaouch blanc	2, 5, 6, 7	2	2	DEU098	Chaouch blanc
Favorit	Favorit	1, 2, 5, 11	2	1	ITA362	Favorit
Hedvábné žluté	Luglienga bianca	2, 11	2	1	FRA 139, AUT024, DEU098	Luglienga bianca
Chrupka Jalbertova	Chasselas blanc	1, 2, 7, 11	2	1	AUT024, FRA139, DEU098, HUN045	Chasselas blanc
Chrupka bílá	Chasselas blanc	1, 2, 3, 4, 5, 6, 9, 11	2	4	AUT024, FRA139, DEU098, HUN045	Chasselas blanc
Chrupka červená	Chasselas rose	1, 2, 4, 5, 7, 11	2	1	DEU098, FRA139	Chasselas rose
Chrupka petrželolistá	Chasselas cioutat	2, 7, 11	2	1	FRA139	Chasselas cioutat
Jakubské	Pinot noir precoce	6, 10, 11	10	1	ITA388	Pinot noir precoce
Kadarka	Kadarka Kek	2, 6, 10	2	1	AUT024, HUN045, DEU098	Kadarka Kek
Kamenorůžák	Koevidinka Feher	2, 6, 7, 10	2	5	DEU098	Prachtraube
Madlenka královská	Madeleine royal	2, 11	2, 11	5	AUT024, DEU098, FRA139	Madeleine angevine
Madlenka ranná	Madeleine angevine	2, 6, 7, 11	2, 11	2	HUN045	Madeleine angevine
Malingrovo ranné	Malingre precoce	2, 6, 7	2	1	DEU098	Malingre precoce
Modrý Janek	Hans	6, 7, 11	11	5	DEU098, FRA139	Veltliner gruen
Mlynářka	Pinot meunier	2, 3, 4, 11	2	1	AUT024, DEU098, FRA139	Pinot meunier
Muškat Ottonel	Muscat Ottonel	2, 3, 5, 8, 11	2	2	DEU098	Muscat Ottonel
Muškat žlutý	Muscat a petits grains	2, 3, 4, 6, 9, 10, 11	2	2	AUT024, FRA274, DEU098	Muscat a petits grains
Modrý Portugal	Portugieser blau	2, 9, 11	2	1	FRA139, AUT024	Portugieser blau
Portugalské bílé	Portugieser gruen	2, 6, 11	2	1	DEU098	Portugieser gruen

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Table 1. to be continued

The name used in the Czech Rep.	Prime name according VIVC	Location	Sample origin	Amount	Compared with	Identity by SSR markers
Portugalské šedé	Portugieser grau	2, 6, 7, 8, 9, 11	2	1	CZE041	Portugieser grau
Ortliebské	Knipperle	2, 6, 7	2	5	FRA139, DEU098	no match
Ortliebské ranné	Knipperle	11	11	5	FRA139, DEU098	no match
Ortliebské žluté	Knipperle	11	11	5	FRA139, DEU098	no match
Prachtraube	Prachtraube	2, 6, 7	2	1	AUT024, DEU098	Prachtraube
Ryzlink aromatický	Aromriesling	2, 11	2	1	FRA139	Aromriesling
Ryzlink buketový	Aromriesling	2, 10	2	1	FRA139	Aromriesling
Ryzlink červený	Riesling rot	2, 11	2, 11	2	AUT024, DEU098	Riesling rot
Bzenecká Lipka	Riesling weiss	1	1	1	FRA274, ITA362	Riesling weiss
Sylvánské modré	Silvaner blau	1, 2, 8, 9	2	1	DEU098	Silvaner blau
Sylvánské ranné	Silvaner gruen	2, 11	2	1	AUT024, DEU098	Silvaner gruen
Tramín bílý	Savagnin blanc	2, 8	2	1	FRA139	Savagnin blanc
Tramín červený	Savagnin rose	1, 2, 3, 5, 9, 11	2	3	AUT024, FRA139, ITA388	Savagnin rose
Tramín modrý	Savagnin rose	9	9	1	AUT024, FRA139, ITA388	Savagnin rose
Trolínské modré	Schiava Grossa	2	2	1	DEU098,	Schiava Grossa
Veltlínské červené ranné	Veltliner fruechrot	1, 3, 8, 9, 11	11	1	AUT024, DEU098	Veltliner fruechrot
Veltlínské červené ranné	Veltliner fruechrot	2	2	5	AUT024, DEU098	no match
Veltlínské červenobílé	Kadarka Kek	2, 6, 7, 10	2	5	AUT024	Veltliner braun
Veritas	Veritas	2, 3	2	1	CZE041	Veritas
Unknown sample 1		8	8	1	AUT024	Chatus
Unknown sample 2		8	8	1	AUT024	Saint Laurent
Unknown sample 3		8	8	1		no match
Unknown sample 4		11	11	1	DEU098	Chasselas blanc

Locations – all the places where the variety has been found; sample origin – places where samples used for DNA extraction were taken; numbers in columns Location and Sample origin corresponding with numbers of localities used in the map (Figure 1); items that are not kept in the National Collection of Genetic Resources (GRIN 2018) are marked in orange in the Location column; SSR profiles were compared with data from the European Vitis Database (2019); the presented tables have used the same codes of the institutions that provided their results in the database (<http://www.eu-vitis.de/index.php>, or Table S3 in ESM); identification was completed using SSR markers and interesting results are marked green for misnamed samples, blue for synonyms, and yellow for clones

SSR profiles of the analysed genotypes are shown in Table S1 in Electronic Supplementary Material (ESM).

## DISCUSSION

At present, we can find old varieties mainly in national collections of grapevine genetic resources and outdoor historical expositions, which have become the main source of material for this study. Evidence of old varieties in gene pools and other collections is often based on oral administration, possibly supported by a comparison of genotypes with old ampelographic books. Information is outdated and frequently unverified. It was one of the important reasons for subjecting these items to SSR analysis.

Most interesting results with emphasis on real local varieties and their scientific background are discussed in subsequent paragraphs.

**Clones.** The Alphonse Lavalée Volský variety has virtually the same SSR profile as Alphonse Lavalée. They differ in a single allele of the *VVMD 5* locus, which is smaller by four bp (Table S1 in ESM). The other alleles are the same with the standard variety, the similarity between the two genotypes is 0.945 ( $\geq 0.9$ ) and from this point of view, according to Cervera et al. (1998), it is possible to consider the AL Volský genotype to be a clone of Alphonse Lavalée. The variety simply named Volský is grown in various European countries and is synonymous with Dodrelyabi. The SSR profile is very similar to the Dodrelyabi profile (Sefc et al. 1999a), however, it is demonstrably different at the three loci. This corresponds to the documented crossing of Alphonse Lavalée, where Dodrelyabi is one of her parents (Ibanez et al. 2009; Lacombe et al. 2013).

Bzenecká Lipka (a small lime tree from Bzenec) is a local clone selection of Riesling, which is distinguished by its distinctive scent of lime and is protected by a trademark (Chateau Bzenec 2019) due to its unique character. The SSR profile is the same as other Riesling weiss clones (Table S1 in ESM). The specific character of this clone is probably determined by the terroir, or minor differences in genome, that are not within analysed SSR loci.

In the past, two aromatic varieties of Riesling were cultivated in Moravia called Ryzlink aromatický and Ryzlink buketový. In an old Czech ampelographic book, Blaha (1961) stated that Ryzlink buketový is closer to Riesling in appearance but had a delicate aroma of Muscat. Blaha (1961) also mentioned parents of Ryzlink buketový known at that time as Riesling

weiss and Muscat Précoce de Courtillier, while he noted that nothing more was known of the origin of Ryzlink aromatický. The two varieties are compared in the table using the grape characteristics in the same source, where the differences in the size and weight of the grapes are particularly evident. Greater and heavier grapes and the number of berries in the grape had Ryzlink aromatický compared with Ryzlink buketový (Blaha 1961). Both varieties were compared in Lednice in 2010. The biggest differences were found in the shape and size of the grapes, where those of Ryzlink buketový were small and faster to rot, and those of Ryzlink aromatický were cylindrical with a pendant and much larger (Sotolář 2010). Despite the obvious differences in morphology, SSR analysis has shown that they have identical profiles (Table S1 in ESM), which is the same as Aromriesling (Lacombe et al. 2013). Rather than being different varieties, they are probably different clones of the Aromriesling. Later, interestingly, SSR markers confirmed the parents of Aromriesling known in the sixties (Lacombe et al. 2013).

Modrý Janek is a variety that is characterised by shoots with blue to violet tips and blue unripe grapes in summer. At the time of ripening, the grapes fade, and at full maturity they are green or yellowish (Blaha 1961). This is also the Czech name of the variety coming out – “modrý” means blue, “Janek” is the diminutive name of “Jan” (John, Hans), and refers to a man who is defiant, unpredictable, or wild. According to the VIVC, the prime name of Modrý Janek should have its designated cultivar as Hans. An old Czech ampelographic book states that Modrý Janek with the synonym of Blauer Hans is a mutation of Grüner Veltliner (Blaha 1961). The obtained SSR profile suggests this, whereas the SSR genotype profiles, designated Hans (Vitis database, DEU098, Laucou et al. 2011) are similar, but demonstrably different (Table S2 in ESM). It is clear that Modrý Janek is a mutation of Grüner Veltliner and also that it is not a synonym of Hans.

**Misnamed cultivars.** Among the analysed varieties several genotypes were also found where the SSR profile did not match their original designation. These included all Madeleine royal plants. Their profile is the same as Madeleine Angevine (Galbacs et al. 2009).

Quite a problematic situation was revealed in the group of genotypes designated as Ortlieber. The prime name of this variety is Knipperle (VIVC). In the European Vitis Database we can trace seven genotypes from different European countries. These

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have the same SSR profile as the Ortliebské žluté and Ortliebské ranné (Vitis database, CZE 041- analysed by Skala from CRI Ruzyně), which was confirmed in this work (Table S1 in ESM), where the leaves of these varieties were analysed again after many years. The same profile (but different from Czech genotypes) is also available for Ortlieber from France (Lacombe et al. 2013) and Knipperle (Vitis database, DEU098). All other items in the Vitis database for Ortlieber spaet from France (Vitis database, DEU098) and Knipperle from France (Vitis database, ITA362) are different from each other. The genotype marked as Ortliebské has also been found in Lednice (analysed in presented work), its genotype is unique as well – it was not possible to find a similar genotype in the database. It follows from the above that there are several genotypes designated as Ortlieber or Knipperle in Europe and this raises the question of which one is true to the Knipperle type.

The genotype mistakenly designated as Kamenorůžák (PN Koevidinka Feher) should have been Prachtraube according the SSR profile (Lacombe et al. 2013, Vitis database DEU098).

Veltlínské červené ranné (PN Veltliner fruehrot) taken in Lednice has an SSR profile that does not match any profile from the database. Only alleles corresponding to the Veltliner varieties were detected in the SSR profile. It can therefore be assumed that this is an unknown hybrid of Veltliner varieties. In contrast, the profile of the Veltliner fruehrot from Karlštejn corresponds to the genotypes analysed in Germany and Austria (Vitis database, DEU098; Sefc et al. 1998).

The SSR profile for the Veltlínské červenobílé variety (PN Veltliner rotweiss) perfectly matches the Veltliner braun profile (Sefc et al. 1998, Vitis database DEU098).

Unknown samples as part of the mapping of vineyards in South Moravia, several samples of leaves from old plants were taken that were not named and could not be clearly determined by morphological traits. These unknown genotypes were classified as Chasselas blanc (compared with Vitis database DEU098), Saint Laurent (Sefc et al. 1997) and Chatus (Sefc et al. 2000). The last genotype specifically was a big surprise. It is an old French variety that was grown on the sandy terraces of the Bas-Vivarais, Drôme and Isère before the onset of the Phylloxera aphids. After its suppression it was almost forgotten and today it is grown only in small areas in the Ardèche region (Schneider 2012). How Chatus got to Moravia is

not clear. One sample with sharp cutleaves failed to determine this. Its SSR profile did not even closely match any variety in the Vitis database, so it is not only a mutation of a known cultivar, but probably a spontaneous hybrid that is particularly interesting from an aesthetic point of view and could be used, for example, on pergolas.

The purpose of this work was to find and identify by SSR markers, and subsequently preserve, as many old varieties as possible that have been cultivated in the Czech Republic and especially in South Moravia. SSR analyses have made it possible to find erroneously named genotypes in the collections of genetic resources and replace them with the correct ones. Moreover, some interesting genotypes with unique SSR profiles have been found that can be further utilised to extend the gene spectrum.

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