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GENETIC ANALYSIS OF *VITIS* INTERSPECIFIC HYBRIDS OCCURRING IN VINEYARDS OF THE CZECH REPUBLIC

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Abstract

SSR analysis of 18 unknown uncultured *Vitis* genotypes planted in an area mostly dedicated to viticulture in the Czech Republic was performed in this work. The aim of this study was to identify analysed samples by comparing their SSR profiles with described standards and classify their mutual relationships based on their distribution in obtained dendrogram. Results show that 50% of unknown genotypes belongs to old American interspecific cultivar 'Noah' and 11% belong to another old American cultivar 'Isabella'. The rest of analysed genotypes remain unidentified, but three of them suggest relatedness with 'Noah' cultivar, one genotype shows relatednessto 'Isabella' cultivar. From practical point of view the most interesting ones are three genotypes, which were clearly clustered with the genotypes of cultural varities (botanically *V. vinifera* L.) used as standard. Based on this it is then possible to assume that those genotypes probably originated from crossing of non-*V. vinifera* genotype specimen with unknown cultural variety. Potential importance of analysed hybrids for further investigation and breeding, especialy in an "eco-friendly" viticulture, is also discussed.

Key words: *Vitis*, Interspecific hybrids, SSR, Identification.

Introduction

The grapevine was domesticated between the seventh and the fourth millennia BC, in a geographical area between the Black Sea and Iran (McGovern *et al.,* 1996; McGovern & Rudolph, 1996; Zohary, 1996; Zohary & Hopf, 2000). From this area, cultivated forms would have been spread by humans in the Near East, Middle East and Central Europe. As a result these areas may have made up secondary domestication centres (Grassi *et al.,* 2003; Arroyo-García *et al.,* 2006; Terral *et al.,* 2010).

Botanically grapevine (*Vitis vinifera*) belongs to the family *Vitaceae*, comprising of around 60 inter-fertile wild *Vitis* species distributed in Asia, North America and Europe under subtropical, Mediterranean and continental–temperate climatic conditions. It is the single *Vitis* species that acquired significant economic interest over time; a great majority of cultivars widely cultivated for fruit, juice and mainly for wine are classified as *Vitis vinifera* L. subsp. *vinifera* (or *sativa*) derived from wild forms [*Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi] (Rossetto *et al.,* 2002; Sefc *et al.,* 2003; Crespan, 2004; This *et al.,* 2004). Some other species, for example the North American *V. rupestris*, *V. riparia* or *V. berlandieri* are used as a rootstocks due to their resistance against grapevine pathogens such as *Phylloxera*, *Oidium* and mildews (Terral *et al*., 2010).

The woods of North America gave rise to large number of different species of vine. Among these species natural or artificial interspecific crossing can occur. On the other hand there is only one European indigenous species - *V. vinifera* subsp. *silvestris* (CC Gmel) HEGI.), then subsequent natural crossing between a forest vines and cultural varieties (V. vinifera subsp. sativa (DC) HEGI) happened within one species (Zohary, 1996; Zohary & Hopf, 2000; Kraus *et al.,* 2000). The first interspecific hybrids probably appeared in America. These most likely originated solely from the American indigenous vine species natural crossing. Later on, the European noble vine (*Vitis vinifera*), which was imported by settlers was brought into the crossing. In America, these genotypes are termed "primary hybrids", in Europe as American "primary hybrids" (APH). Nowadays the most frequently used term to describe such a hybrid is "interspecific variety".

The beginnings of the discovery of American interspecific hybrids date back to 1802, when the variety 'Catawba' with pink and purple colored berries was discovered, taken and spread by general Lévy (Kraus, 2004). In 1816, South California, Isabelle Gibbs described aromatic and interesting plant that bore large violet-blue berries with strawberry flavor. The plant also spread to Europe under the name 'Isabella' as an ornamental climbing vine (Prince, 1827). After that followed the discovery of naturally formed hybrid *V. labrusca* L. and *V. riparia* Michaux. named 'Clinton'. Later another variety called 'Delaware' was discovered in New Jersey in 1849 and together with newly bred varieties like 'Diana' and 'Concord' made up the new generation of hybrids (Kraus, 2004).

These interspecific genotypes were imported to Europe (primarily to France from North America) as ornamental plants for parks and gardens, then later as plant material for vineyards. Unfortunately, due to the import of foreign plant material European viticulture starts to suffer. The introduction of previously absent serious grapevine pests and diseases for which the native European vines (*Vitis vinifera*) had no resistance unlike the newly imported American species or hybrids. These diseases were of fungal origin (powdery mildew, downy mildew and black rot) or were part of the insect world, like the most dangerous pest until now - the grapevine root and leaf aphid phylloxera. The impact of these pests was devastating and thousands of acres of vineyards were destroyed during the second part of the 19th century. In fact this „phylloxera crisis" had a considerable impact on future utilisation of North American genotypes (Smartt & Simmonds, 1995; Arnold *et al.,* 1998; Arnold *et al.,*

2005), because various North American wild species are in fact resistant to phylloxera (*Dactylosphaera vitifolii* SHIM.) (Zohary, 2004). Therefore some of the first interspecific hybrids were bred for phyloxera resistance with the purpose of saving the European wine industry from this insect by using them as rootstocks for grafting (Alleweldt & Possingham, 1988). These were the first intentional interspecific *Vitis* hybrids and marked the beginning of efforts to introduce new traits from the diverse gene pool of wild species into commercially grown grapes (Smartt & Simmonds, 1995). During the sixties of the 19th century the first attempts on combining the positive traits of American grapevines (frost and fungal resistance) with qualitative characteristics of European *Vitis vinifera* L. varieties were made (Kraus *et al.,* 2000). Very famous example of such old variety is 'Othello' ('Clinton' (*V. riparia* x *V. labrusca*) x *Vitis vinifera* ('Black Hamburg) (http://www.eu-vitis.de), which was bred in 1859 by Charles Arnold.

Very important in the history of interspecific breeding is the period of the first quarter of the 20th century during which mainly in France the newly originated genotypes were called French first-generation hybrids (FFGH). Usually it means cross of American and European species with cultural French varieties. This kind of crossing is mainly associated with breeder named Adalbert Seibel, who has developed a wide and interesting base for the subsequent works of many contemporary breeders (Rombough, 2002). Other prominent breeders were Ganzin, Oberlin, Couderc and Baco. Some varieties made by them like 'Baco noir' is grown until today (Kraus, 2009). Due to the low low taste quality of wines prepared from these hybrids they were gradually restricted by legal regulations. In the first half of the 20th century it is possible to notice a new interest in breeding interspecific hybrids.These are usually referred to as the so-called French second-generation hybrids (FSGH). Most of breeders of these second-genertaion hybrids stem from work of A. Seibel. They very often crossed native genotypes among themselves or with cultural European varieties (Kraus *et al.,* 2000). With these hybrids is mainly associated the breeder Seyve - Villard (Kraus *et al.,* 2005).

From the genetic point of view the first generation hybrids contained less than half of the genome of the European varieties and is characterized by low quality of wines. Hybrids of the second generation already contain 55-68% of the genome of European varieties, thereby increasing the quality of the wine (Kraus, 2004).

Increasing popularity of interspecific hybrids caused that in 1955 they were grown on approximately one-third of the area of French vineyards to the detriment of the cultural European varieties. The success and their totally different and typical aromatic character, along with low-quality wine, began to be in famous wine regions such as Bordeaux and Burgundy, perceived as a threat (Jackson, 2008). Therefore a ban on the cultivation of interspecific varieties in France was declared and similar restrictions were later also held in other European countries. This situation was exacerbated by subsequent prohibition of interspecific wine and by setting penalties for their planting, enhanced by awarding bonuses for their grubbing (Jackson, 2008).

Despite that the FFGH and FSGH have been popular only for a short while and in fact caused the ban of the APH cultivation, it is not possible to consider the almost 100 years of effort on their breeding as unnecessary. They have become the base for breeding of modern resistant varieties called Piwi resistant interspecific varieties (from the German „pilzwiderstandsfähige rebsorten“ - vine varieties resistant to fungal diseases). The genome of these varieties already have a 85% match to European cultural varieties (Kraus, 2004), and in spite of European Commission Council Regulation No. 1493/1999 enabling to produce „quality wines“ only from varieties belonging to the botanical species *Vitis vinifera,* PIWI cultivars are frequently used in some northern European viticultural regions. For example the cultivars 'Hibernal', 'Regent', 'Solaris' are bred frequently in Germany and another interspecific hybrids widespread in the Czech Republic are 'Malverina', 'Savilon' or 'Laurot'. All these varieties meet the requirement of „quality wines“, show enhanced resistance to fungal diseases and therefore are quite popular, especially for organic vineyards.

In spite of recent law restrictions it is still possible to find in the wine-growing regions in the Czech Republic locations, where unknown varieties showing typical traits for first generation of interspecific cultivars are planted. These are usually plant materials that remained in the vineyards from the past. Laics call them generally as „Croat“, the more experienced viticulturist assume that genotypes producing dark red grapes belong to the cultivar 'Isabella' and yellow-green grapes belong to 'Noah' cultivar. But the occurrence of these varieties in vineyards is still only a hypothesis since no complex phenological or genetical comparison of this unique plant materials was performed till now.

Regarding cultivar identification and pedigree analysis SSR markers are usually used as the most suitable and reliable tool (Rabbani *et al*., 2010; Turi *et al*., 2012; Fayyaz *et al*, 2014; Kanwal *et al*, 2014; Polat *et al*., 2015; Shah *et al*., 2015). Main advantage of SSR markers is the high level of polymorphism in case of different cultivar comparison (This *et al.,* 2004) and conversely its high stability if different clones of one cultivar were analysed (Regner *et al.,* 2000; Imazio *et al*., 2002). Thus SSRs have been extensively exploited in a number of countries for identification of cultivars, characterization of grape genetic resources (Grando *et al*., 1998; Fatahi *et al.,* 2003; Hvarleva *et al.,* 2004; Moravcova *et al.,* 2006), verification of synonyms or homonyms (Fossati *et al.,* 2001; Labra *et al.,* 2001), parentage analysis (Bowers *et al.,* 1999a; Sefc *et al.,* 1998a; Sefc *et al.,*1998b) or mapping (Adam-Blondon *et al.,* 2004; Fisher *et al.,* 2004; Riaz *et al.,* 2004; Guo *et al*., 2015). The frequent usage of SSR markers for grapevine genotype studies finally made possible selection of 6 generally recommended SSR markers. Moreover, concept of coding of the allele lenghts described in This *et al.* (2004) allowed these markers to become one of the OIV descriptors and their usage as a base to establish worldwide database of SSR profiles usually found in individual cultivars (EUVITIS; www.euvitis.de).

Due to the above mentioned lack of information comprehensive overview of genetic background of interspecific genotypes naturally occurirng in southern Moravia (Czech Republic) was performed this work. For these purposes 6 SSR loci working as OIV decriptors were analyzed within collected genotypes and obtained results were compared with SSR profiles in the database EUVITIS. The obtained results were used to clarify the genetic background of observed genotypes and their mutual genetic relationships. On the basis of this information is also discussed further potential of analyzed genotypes for further breeding.

Materials and Methods

The screening of the genotypes locally called „Croat" was initially performed in the different regions of South Moravia. The traits typical to APH, FFGH and FSGH were detected - resistance to leaf fungal diseases; large leaves with minimal edging, strongly tomentose underneath, very firm skin of berries and solid gelatinous flesh that appears by pressing the berries. Afterwards 18 genotypes following above mentioned criteria were selected from different vineyards of South Moravia. Three cultural varieties ('Chardonnay', 'Cabernet Franc' and 'Blaufrankish') from germplasm collection of the Department of viticulture and enology (Faculty of Horticulture, Lednice, Czech Republic) were used as reference for subsequent comparison with data from Euvitis database. List of samples and their names based on the sampling sites (except names for reference cultivars) is presented in Table 1.

DNA extraction: DNA corresponding to 21 analysed genotypes was extracted from young frozen leaves (0.1 g) using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) in accordance with manufacturer instructions. The DNA concentration and quality was determined by means of an electrophoresis on a 1% agarose gel compared with lambda DNA standards and by a fluorometer using PicoGreen kit (Invitrogen) as manufacturer recomended.

SSR analysis: Six SSR primers previously described and internationally approved for the identification of grapevine varieties (This *et al.,* 2004) were used: VVMD 5 and VVMD 7 (Bowers *et al.,* 1996), VVMD 27 (Bowers *et al.,* 1999b), VrZAG 62 and VrZAG 79 (Sefc *et al.,* 1999), VVS 2 (Thomas *et al.,* 1993). The SSR amplification was performed according to the protocol described in Moravcova *et al*. (2006). The only difference was in the used *Taq* polymerase (originally Finnzymes company, newly from New England Biolab) and the consequent need to raise the annealing temperature for VrZAG 62 and VrZAG 79 by 2°C (for reasons see Results and Discussion).

The success of the SSR amplification was primarily controlled by electrophoresis on 1.5% agarose gel. Products of SSR amplification were subsequently analyzed by an ABI Prism 310 genetic analyzer (Applied Biosystems Inc., Forest City, USA). Exact determination of allele lenghts and mutual comparison was carried out using the GeneScan analysis software (Applied Biosystem, Forest City, USA).

Evaluation of obtained results: To simplify the comparison of obtained results with worldwide databases of SSR profiles (mainly EUVITIS; www.eu-vitis.de) obtained results were converted into the coding system developed within the Genres 081 project (This *et al.,* 2004). Allele lenghts detected for reference varieties were used as a standard to determine the codes obtained for unknown genotypes. Due to general lack of SSR profiles typical for interspecific APH, FFGH or FSGH genotypes in databases, codes for cultivars with most anticipated appearance in Moravian vineyards - 'Noah' and 'Isabella' were asked and obtained from French National Institute for Agricultural Research (INRA) in Montpellier, France, personally Valérie Laucou.

Another aim was to evaluate genetic similarity between analysed genotypes. For that, scoring of distribution of individual SSR alleles among analysed genotypes as 1 for their presence and 0 for absence was performed. Obtained binary matrix was then transferred into a FreeTree software package (Hampl *et al.,* 2001), whereas similarity among all cultivars was estimated according to the Nei and Li distances; using UPGMA analyses. The dendrogram was displayed using the Tree View 1.6.6 software (Bio-Soft Net, Glasgow, UK) (Page, 1996).

Table 1. List of samples and their names based on the sampling sites.

No. of sample	Location of sample	No. of sample	Location of sample
1.	Milovice	12.	Kyjov
2.	Boršice	13.	Velké Bílovice
3.	Velké Bílovice	14.	* Chardonnay
4.	Kostice	15.	*Cabernet Franc
5.	Hlohovec	16.	Ratíškovice
6.	Valtice	17.	Hlohovec
7.	Zaječí	18.	Hlohovec
8.	Zaječí	19.	Lednice
9.	Zaječí	20.	Lednice
10.	Charvatská Nová Ves	21.	*Blaufrankish
11.	Charvatská Nová Ves		

* Asterisk indicates the reference varieties collected from germplasm collection in Lednice

Results and Discussion

SSR analysis: As described above, conditions used for SSR amplification were nearly identical with protocols in Moravcova *et al.* (2006). The only exception was that *Taq* polymerase from another company was used in this study (originally Finnzymes, newly New England Biolab). By this exchange many non-specific amplicons at loci VrZAG 62 and VrZAG 79 were noticed on the control agarose gel. Their presence can be explained by the non-specific hybridization of the primer to the DNA template. Therefore the optimal annealing temperature was found by increasing of annealing temperature by 2 °C at both loci, resulting in significant reducing of amount of amplified products.

As a first, specific SSR allele lenghts expressed as DNA base pairs (bp) were recorded for individual genotypes (Table 1). Subsequently data obtained from reference varieties were used to establish the basic anchor codes for individual loci (N+x), using Euvitis database (www.eu-vitis.de) or descriptors on OIV home page (http://www.oiv.int/oiv/info/enplubicationoiv#grape; OIV descriptor list for grape varieties and *Vitis* species (2nd edition). Anchor codes were used for subsequent coding of the rest of unknown genotypes (Table 1).

One of the aims of this work was to compare unknown cultivars with the profiles typical for the two cultivars with most anticipated appearance in Moravian vineyards, 'Isabella' and 'Noah'. Unfortunately no SSR profiles of these two cultivars are available in the accessible databases, but thankfully standardised SSR profiles and coded SSR alleles were kindly offered by colleagues from INRA Montpeliere (France). Expected values of coded alleles of these two cultivars are described in the Table 2. These standardised SSR profiles were subsequently compared with profiles obtained for all unknown cultivars. The results are described within Table 3 with differently highlighted cells in the case of identity of the codes with one of the cultivar.

Nine samples were entirely identical according to their size of alleles (Tables 2 and 3, highlighted in yellow). These samples are almost identical in terms of the standardised SSR profile for variety 'Noah' with the exception of smaller allele of VrZAG 62 locus. Reference allele expect N +8, whereas all nine specimens appearing in Moravia region have shown allele N + 6. In all other alleles these 9 varieties show the same results as standard 'Noah' profile. Bearing the usual criteria in mind, such small difference in one allele is generally considered as a clone of respective variety, thus it is possible to brand this group of genotypes as 'Noah'. Sample No. 17 is different in both values for locus VrZag 62, therefore it is possible to count the sample as a clone of 'Noah' variety or its very close relative. Sample No. 19 differ also in the locus VVMD 27 and probably can be described as a hybrid between 'Noah' and other unknown parent. Samples 12 and 13 (highlighted in green) are possible to identify as a variety 'Isabella', because of the results fully consistent to the pattern typical for this cultivar (Table 2).

All other samples (No. 1, 8, 9, 18, 20) show low number or no alleles shared with 'Noah' or 'Isabella' standards. Therefore it will be better to discuse their genetic background on the base of their distribution within constructed dendrogram of genetic similarity (Fig. 1).

The dendrogram divide the samples into two main clusters, whereas the bottom cluster is subdivided in another two. Samples No. 2, 3, 4, 5, 6, 7, 10, 11, 16 in the upper part of dendrogram make up the most numerous cluster and are fully identical or highly similar with standard SSR profile for 'Noah' cultivar, which was artificially added to analysed data (similarly as 'Isabella' standard). This slight divergence from Noah cultivar is reasoned by above described difference in the size of the smaller allele at the locus VrZAG 62. Next neighbour group includes samples No. 17, 18 and 19, which were statistically evaluated as related to this cultivar on the base of few conjoint alleles with SSR standard profile for 'Noah'.

The bottom cluster is much more branched and divided into another subclusters. Samples No. 12 and No. 13 are consistent in size of their alleles with standard for cultivar 'Isabella' and sample No. 1 was evaluated as a relative to them because of 6 conjoint alleles. Another subcluster in the bottom part consists of reference varieties 'Blaufränkisch' and 'Cabernet Franc'. Lowermost subcluster consists of four samples. There are two identical samples No. 8 and No. 9 from Zaječí locality, whereas these samples show relatedness with sample No. 20 and very surprisingly with reference cultural variety 'Chardonnay' (*Vitis vinifera* L.). It is then possible to assume that in the case of samples No. 8, 9 and 20 there is an increase of portion of their genome originating from the cultural *Vitis vinifera* L., pointing them out as artificially or spontaneously bred with some cultural grape in the past.

One of the aims of presented work was to obtain an overview about genetic background of plants showing clear non *V.vinifera* traits and are planted in the region of South Moravia. Therefore number of candidate plants from various regions was collected to obtain as complex information as possible. On the base of performed analysis it is possible to sum up the largest proportion (50%) of all genotypes as a variety of 'Noah' (Fig. 2). Regarding 'Isabella' cultivar, 11.0% of analysed genotypes offer identical profile with corresponding standard. Four samples (22%) show relatedness with above mentioned cultivars. Remaining three genotypes (17%) probably originated as a progeny of old interspecific cultivar with some cultural variety belonging to *V. vinifera*. These three cultivars are especially interesting because of their potential for usage within breeding process. They can act as potential new source of resistance against important funghi diseases, whereas exhibit interestingly high similarity with the genotype of cultural grapes (botanically *V. vinifera*). Using these genotypes the breeding process can be shortened by one generation at least.

Table 2. Values of coded SSR alleles expected for 'Noah' and 'Isabella' cultivars from INRA Montpeliere (France).

	VVS 2	VVMD 5	VVMD 7	VVMD 27	VrZAG 62	VrZAG 79
Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+10:N+12	N+8:N+32	N+12:N+22
Isabella	N:N+28	N+16:N+16	N+4:N+18	N+4:N+8	N+28:N+30	N:N+10

Table 3. SSR geneotyping of analysed genotypes and their comparison with standards for 'Noah' and 'Isabella' cultivars.

	N=120	N=220	N=228	N=172	N=173	N=235
Sample	VVS 2	VVMD 5	VVMD 7	VVMD 27	VrZAG 62	VrZAG 79
1	146:148	228	232:246	176:176	185:201	245:251
rel. to Isabela	N+26:N+28	N+8	N+4:N+18	N+4:N+4	N+12:N+28	N+10:N+16
2	122:126	248:248	232:252	182:184	179:205	247:257
Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+10:N+12	N+6:N+32	N+12:N+22
3	122:126	248:248	232:252	182:184	179:205	247:257
Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+10:N+12	N+6:N+32	N+12:N+22
4	122:126	248:248	232:252	182:184	179:205	247:257
Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+10:N+12	N+6:N+32	N+12:N+22
5	122:126	248:248	232:252	182:184	179:205	247:257
Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+10:N+12	N+6:N+32	N+12:N+22
6	122:126	248:248	232:252	182:184	179:205	247:257
Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+10:N+12	N+6:N+32	N+12:N+22
7	122:126	248:248	232:252	182:184	179:205	247:257
Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+10:N+12	N+6:N+32	N+12:N+22
8	122:130	232:264	232:240	178:180	187:189	245:257
Unknown	N+2:N+10	N+12:N+44	N+4:N+12	N+6:N+8	N+14:N+16	N+10:N+22
9	122:130	232:264	232:240	178:180	187:189	245:257
Unknown	N+2:N+10	N+12:N+44	N+4:N+12	N+6:N+8	N+14:N+16	N+10:N+22
10	122:126	248:248	232:252	182:184	179:205	247:257
Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+10:N+12	N+6:N+32	N+12:N+22
11	122:126	248:248	232:252	182:184	179:205	247:257
Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+10:N+12	N+6:N+32	N+12:N+22
12	120:148	236:236	232:246	176:180	201:203	235:245
Isabella	N:N+28	N+16:N+16	N+4:N+18	N+4:N+8	N+28:N+30	N:N+10
13	120:148	236:236	232:246	176:180	201:203	235:245
Isabella	N:N+28	N+16:N+16	N+4:N+18	N+4:N+8	N+28:N+30	N:N+10
14	134:140	232:236	236:240	178:186	187:195	241:243
Chardonnay	N+14:N+20	N+12:N+16	N+8:N+12	N+6:N+14	N+14:N+22	N+6:N+8
15	136:144	224:238	236:260	178:186	193:203	245:257
Cabernet Franc	N+16:N+24	N+4:N+18	N+8:N+32	N+6:N+14	N+20:N+30	N+10:N+22
16	122:126	248:248	232:252	182:184	179:205	247:257
Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+10:N+12	N+6:N+32	N+12:N+22
17	122:126	248:248	232:252	182:184	181:207	247:257
related to Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+10:N+12	N+8:N+34	N+12:N+22
18	122:130	234:234	232:238	182:184	201:205	235:239
related to Noah	N+2:N+10	N+14:N+14	N+4:N+10	N+10:N+12	N+28:N+32	N:N+4
19	122:126	248:248	232:252	178:180	179:205	247:257
related to Noah	N+2:N+6	N+28:N+28	N+4:N+24	N+6:N+8	N+6:N+32	N+12:N+22
20	130:160	248:248	240:248	178:180	187:201	237:245
unknown	N+10:N+40	N+28:N+28	N+12:N+20	N+6:N+8	N+14:N+28	N+2:N+10
21	122:140	224:238	236:246	176:192	193:203	235:249
Blaufrankish	N+2:N+20	N+4:N+18	N+8:N+18	N+4:N+20	N+20:N+30	N:N+14

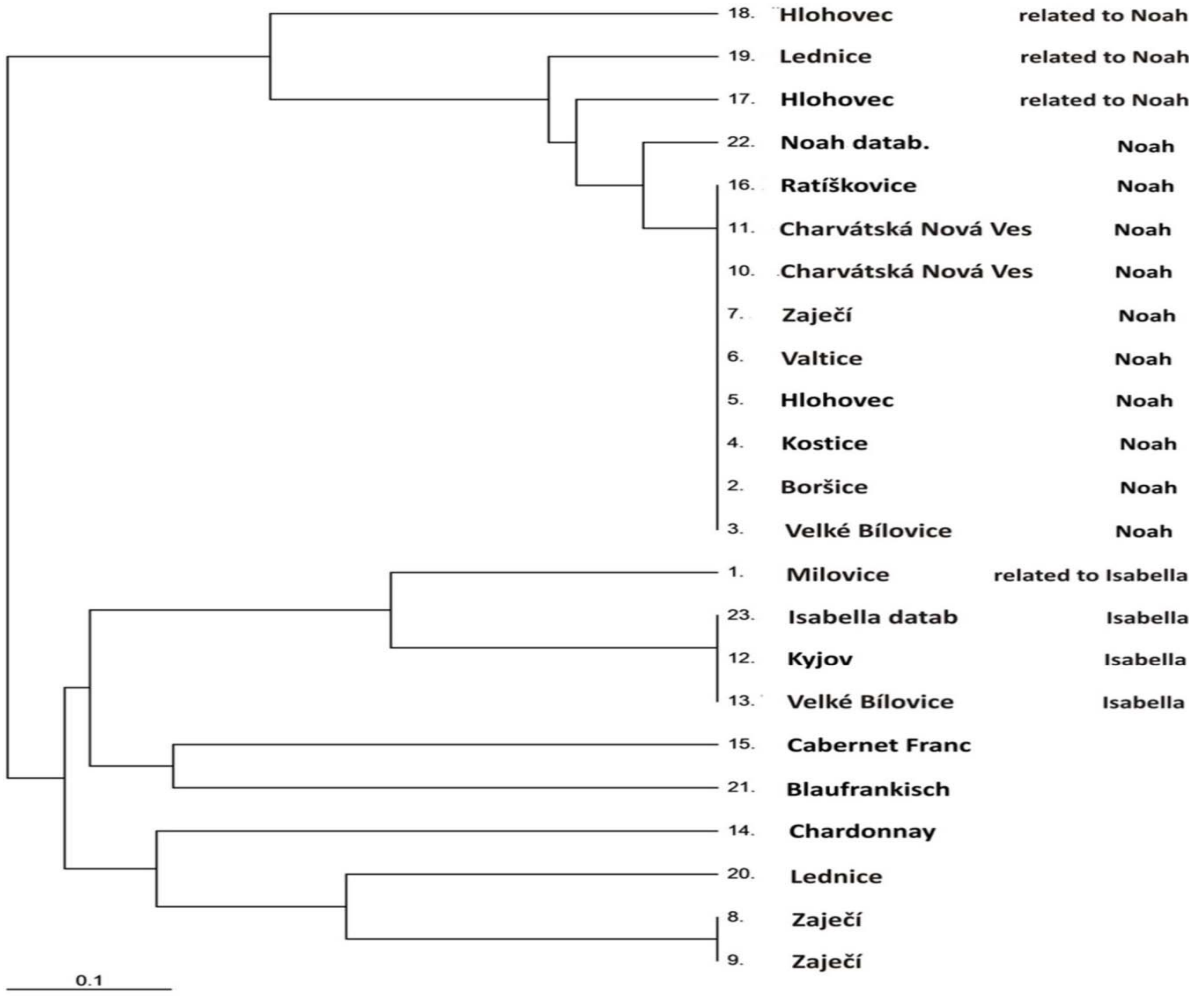

Fig. 1. Dendrogram of genetic similarity of the unknown genotypes of the genus Vitis based SSR analysis.

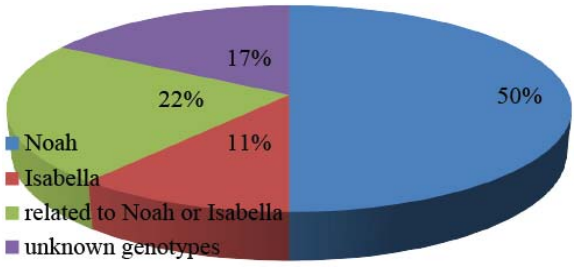

Fig. 2. Pie chart of the percentage of analysed samples results.

Conclusion

General aim of this work was to obtain more informations about genetic background of interspecific hybrids naturally occurring in the region of South Moravia (Czech Republic). Until now the current knowledge about these materials was rather sparse, or as small texts. To obtain required informations, six commonly used SSR primers were used for analysis 18 collected unknown genotypes and three reference cultivars, which were used as a base to establish coding system.

Coding system allows comparison of obtained results with world wide database, but unfortunately in most cases the only available data are for cultural varieties (*Vitis vinifera* L.); data for hybrids with other species of the genus *Vitis* are quite rare. Therefore it was necessary to compare our results with results from INRA Montpellier, where a similar analysis was carried out in the past.

On the basis of the comparison it was detected that majority of unknown genotypes really belongs to one of two cultivars, which were most supposed to be present in Czech vineyard. In fact 50% belongs to 'Noah' cultivar, 22% is related to 'Noah' or 'Isabella' cultivar and 11% belong to 'Isabella' cultivar. The rest of analysed genotypes (17%) remain unknown, as they could not be traced in the available databases because of the absence of similar SSR profiles. The only source of information about their genetic background or relationship with the rest of analysed cultivars was via created dendrogram. Most interesting from practical point of view are the three genotypes, clearly clustered with the genotypes of cultural varieties (*V. vinifera* L.).

This design of dendrogram imply that these genotypes probably originated from crossing of some non-*V. vinifera* genotype with cultural variety (*Vitis vinifera* L.). Therefore,

they could work as interesting pre-breeding materials because of their highly desirable resistance against fungal diseases. There are already examples where such breeding concept initiated the creation of popular and widely used cultivars ('Hibernal', 'Regent', 'Solaris', 'Laurot' and so on). Obtained results also show that the non-*V.vinifera* genotypes still have their place in the vineyards in South Moravia. Although they occur only locally, they can contribute to the increase of varietal or phenotypic diversity, especially in the light of increasing interest of customers in biological production.

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