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Optimized identification of grapevine cultivars using multiplex microsatellite markers

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Abstract

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A database of grapevine cultivars, including old Swiss and generally known varieties, was developed using microsatellite markers. We started using the traditional silver-staining gel method, but later changed to a much faster, more reproducible, reliable and cheaper method. For this purpose, a method originally developed for marker assisted selection in apple was successfully adapted to grape cultivar identification, using multiplex PCR and analysis with a 16-capillary genetic analyzer. The results of both methods were similar and the new method could confirm results from the older one at already analyzed and new marker sites. So far, the database includes 147 *Vitis vinifera* and interspecific cultivars, including historical varieties, and four other species belonging to the Vitaceae family. The database will be further enlarged to establish a reliable tool for grapevine cultivar identification, and possible relatedness studies. Here, we show some examples of identification and non-identification of unknown cultivars.

The new method enables rapid, reliable confirmation in plant breeding, genetic profiling of new breeds, the identification of rootstocks, and is helpful most of all in cases where identification based on morphologic data are inconsistent.

Key words: Grape cultivar, rootstocks, multiplex PCR, microsatellite markers, *Vitis vinifera*.

Introduction

Microsatellite markers were shown to be an ideal tool for the identification of grape cultivars. The high polymorphism of microsatellite (or SSR) loci allows cultivar identification (Thomas et al. 1994) and their co-dominant Mendelian inheritance allows the reconstruction of crosses (Sefc et al. 1998b). Thus, plants can be identified based on genetic characteristics, that are not influenced by external factors such as climate or soil quality. Additionally, a person identifying a cultivar will not be biased or

influenced by the morphological appearance of the plant e.g. grape color or form of the leaves. In general, microsatellite-based grape cultivar identification should be used to confirm, rather than to substitute the classical ampelographic methods. In many cases, molecular methods may be used to validate a speculation with regard to cultivar status, because they are in general fast, relatively cheap and reliable. Today, more and more breeders, nursery managers and grape growers use this tool to confirm cultivar nomenclature. Even well-known cultivars sometimes need to be identified on a molecular level to confirm their identity (Regner 2003).

Identification of a grape cultivar may sometimes be very difficult. Depending on the regions, different synonyms exist; old, rarely grown specimen may be confused, or similar "sister" cultivars (e.g. Léon Millot and Maréchal Foch) may be mixed up. For example, in many ampelographies the old cultivar Completer from the Canton of Graubünden is mentioned as a synonym to Lafnetscha from the Canton of Valais. However, this was found to be incorrect (Vouillamoz et al. 2004 and this study). Further, two cultivars named Cornalin, one from the Canton of Valais, Switzerland, and one from the Aosta Valley, Italy, that are clearly morphologically different, only share the same name. These results were also confirmed using microsatellite markers (Maigre et al. 2000). Moreover, two plants of the same cultivar might be given different names. Often, differences between cultivars such as hairiness at the lower surface of the leaves may just be a mutation (Regner 2003). Often, cultivars that are hard to differentiate phenotypically using ampelographic techniques may be distinguished by DNA typing (Thomas et al. 1994).

This study started a few years ago using a common method for DNA extraction, PCR amplification and visualization on silverstained polyacrylamid gels (Bowers et al. 1996). With this method a number of marker sites in about 350 cultivars were analyzed. Our new method saves a lot of time and improves the reliability of the results. DNA extraction is easier (less plant material needed, less chemicals needed, less preparation steps), faster and cheaper. PCR amplification is also made considerably faster, using multiplex reactions (six primer pairs per reaction). Finally, data analysis was improved using a ABI3100 Genetic Analyzer, substantially reducing hands-on time, analyzing six primer pairs at a time and running the analysis overnight. Analyzing data using the GeneMapper Software is much more reliable than reading silver gels. Internal standards make it easier to compare results from different laboratories.

Material and Methods

Plant material

Grapevine leaves (*Vitis vinifera* L., interspecific crosses and a few other species from the Vitaceae family: *Ampelopsis latenophylla*, *Ampelopsis megalophylla*, *Parthenocissus quinquefolia* and *Parthenocissus tricuspidata*) were collected at several sites in Switzerland and other countries (Tab. 1). Leaves were harvested and then frozen until further use. Generally the plants collected at the Sortengarten Halbinsel Au were regarded to be the "true to type" cultivars. For this database, a total of 151 plant samples were used, representing the grape cultivars collected in our group so far.

DNA extraction and multiplex PCR

Two different DNA extraction methods were used. Originally, DNA was extracted according to Thomas et al. (1993) with some modifications (only one step with extrac-

Tab. 1. Collection sites of grapevine leaf samples for microsatellite analysis.

No of plants	Collection site	
54	Sortengarten Halbinsel Au	Zürich, Switzerland
37	Agroscope FAW Wädenswil	Wädenswil, Switzerland
1	Agroscope RAC Changins	Pully, Switzerland
26	Private Collections	Western Switzerland
12	Private Collections	Eastern Switzerland
15	Private Collection	Berne, Switzerland
5	Versuchsanstalt Geilweilerhof	Germany
1	Bundeslehranstalt Klosterneuburg	Austria

tion buffer: 0.5 M NaCl, 0.2 M TrisHCl, 50 mM EDTA, 1% v/v 2-mercaptoethanol, 2.5% w/v PVP, 3% sarkosyl, 20% ethanol, pH 8). Such DNA was used in both the older and the new analysis method. Additionally, a DNA extraction kit was used for some samples (Extract-N-AmpTM Plant PCR Kit, Sigma). This DNA extract was diluted 1:10 with water for use in the PCR reaction. Results were identical for both DNA extraction methods used (data not shown).

In the older method, PCR amplification and analysis of allele-lengths was done on silverstained gels as described by Bowers et al. (1996). In the new method, multiplex PCR and allele analysis by a 3100 Genetic Analyzer was done adapting a method used for apple leaves before (Frey et al. in press). Two six-plex PCR reactions were performed. To this end, a 10× primer mix was made containing 2 mM of each, forward and reverse primer. The forward primers were labeled with FAM, HEX or NED, respectively. Mix 1 contained the primers VVS2 (Thomas and Scott 1993), VVMD5, VVMD7 (Bowers et al. 1996), VVMD27 (Bowers et al. 1999), VrZAG62 and VrZAG79 (Sefc et al. 1999), and Mix 2 contained the primers VVS4 (Thomas and Scott 1993), VVMD6, VVMD8 (Bowers et al. 1996), VrZAG21, VrZAG67 and VrZAG112 (Sefc et al. 1999). The six primers of Mix 1 were used in the European GenRes081 Project and were chosen because they show a very high potential for differentiation that is strong enough to discriminate between grape cultivars (Regner 2003). We decided to use six more primers to render the method even more specific.

PCR reaction was performed with a Qiagen Multiplex PCR kit, but using a final PCR volume of 10 µl (5 µl of 2× Qiagen multiplex PCR mastermix, 1 µl of 5× Q-Solution, 1 µl of 10× primer mix containing 2 µM of each F and R primer (six-plex), 1 µl of pure H_2O and 2 µl of template DNA). PCR was done on a Techne Genius thermocycler (Witec AG, Littau, Switzerland) under the following cycling conditions: 2 min at 40 °C (for preheating the lid) and 15min at 95 °C (for initial denaturation), followed by 40 cycles of 40s at 94 °C, 90s at 57 °C and 90s at 72 °C; then 30 min at 60 °C and final hold at 10 °C (Frey et al. in press).

Allele measuring, data analysis and database construction

The silverstaining gel method gave first results. Since it was too time consuming and reading the allele pattern proved sometimes difficult, we changed to a faster, more reproducible and cheaper method. With this new method we were able to fill in missing data and to complete the table.

Tab. 2. Numbers of grape cultivars analyzed with both microsatellite methods (see text) at several marker sites and percent matches.

	Marker							
	VVS 2	VVS 4	VVMD 5	VVMD 6	VrZAG 21	VrZAG 62	VrZAG 79	VrZAG 112
Number of cultivars analyzed with both methods	108	88	95	38	106	100	24	24
Number of matches	106	88	91	37	105	96	24	23
Percent matches (%)	98.1	100.0	95.8	97.4	99.1	96.0	100.0	95.8

PCR products were diluted 1:10 with water. A mastermix containing 15 µl formamide and 0.5 µl of fluorescent GeneScan™-500 ROX™ standard dye per sample was mixed and 15 µl were pipetted in each well of a 96 well plate. Then, 1 µl of the diluted PCR product was added. After centrifugation, a 2 min at 96 °C heat denaturation and rapid cooling in the freezer followed. The plates were then transferred to a 3100 Genetic Analyzer (Applied Biosystems) and run on 50 cm capillaries with a high resolution polymer (POP-6; Applied Biosystems) and with the following modifications of the default settings for genotyping (GeneScan36_POP4DefaultModule parameters): Run temperature 60 °C, injection time of 30 sec and a run time of 5000 sec.

The data produced by the Genetic Analyzer were imported in the software Gene Mapper™ version 3.0 (Applied Biosystems) and automatically sized and analyzed using the default values. Bins were created based on the first results and autobinnig was performed with the rest of the data. The values were rounded to the nearest base and then exported into the spreadsheet program Excel (Microsoft). This program was used to construct a database of grapevine cultivars that had been DNA typed by PCR at 12 microsatellite loci.

Results

A complete database table was constructed including allele lengths of 151 grape cultivars at 12 microsatellite marker sites. This will be enlarged to a reliable collection of data for comparison with unknown grapevines. The data are the result of the new method, results from the older one were used for confirmation and will not be used for further analysis. Comparison of the results obtained by both methods resulted in 95.8% – 100% consistency at several markers – a weighed average of 97.8% – as shown in Table 2.

In most cases, the DNA showed good amplification in the expected range. Cultivars generally were diploid, only one sample showed tri- or tetraploidy at some marker sites (Clinton at VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, VrZAG79). VVMD 8 was the primer that did not amplify in a few cultivars (Soleil blanc, Seyval blanc and Concord). The four samples not belonging to the *Vitis* genus (*Ampelopsis* and *Parthenocissus*) did not amplify well; some primers did not amplify at all and some allele-lengths were difficult to identify according to the peak-pattern. Results were compared to grapevine databases available on the internet, as well as with the grape micro-

satellite collection GMC obtained from IASMA analysis and literature (http://217.222.71.209/genetica/gmc.html), and with data from publications (Vouillamoz et al. 2003; Vouillamoz et al. 2004; Sefc et al. 1998a).

Based on our comparatively small grapevine database, we could already solve some questions of grape cultivar identification. If all 12 loci show identical allele-lengths, we assume that we have two identical grape cultivars. As mentioned above, the results obtained with the old method could be confirmed with the new one, where more markers sites were used. Due to certain shifts in absolute allele-lengths depending on the method used they were named with letters in Table 3, A being the smallest allele at each marker site.

Grobe aus Österreich

A private collector of grape plants sent us a grape named "Grobe aus Österreich", a large fruited grape from Austria with greenish berries. It was speculated to be a Heunisch or an Elbling, based on the synonym "Weissgrobe" (Ambrosi et al. 1994; Hillebrand et al. 1998). According to Freiherr von Babo and Mach (1909), Heunisch is cultivated in Niederösterreich under the name "Grobe". Nevertheless, the grape shows many morphological characters that are similar to Elbling, but a clear identification was so far missing. The "Grobe" could be identified with our molecular methods as Elbling, with both Red and White Elbling showing the same pattern (Tab. 3). The reference samples were from different collection sites, such as Sortengarten Au, Sortengarten Pully and from a private collection. We found that Heunisch and Elbling are two different cultivars, but may be related (Tab. 3). According to Regner et al. (1999), Heunisch is one of the parents of Elbling.

Alte Rebe

Another case was an old grape from the Canton of Valais, named "Alte Rebe" (Old Grape). Ampelographic analysis and sensoric data mediates that the plant may have some relatedness with Cornalin. Our microsatellite identification confirmed that it really is a Cornalin (Tab. 3). However, it is a Cornalin du Valais and should not be confused with the Cornalin d'Aoste, which according to Vouillamoz et al. (2003) is a different cultivar and is the same as Humagne Rouge. An analysis for possible relationship with other cultivars showed that Cornalin (du Valais) is neither closely related to Eyholzer, Gwäss, Himbertscha, Heida, Amigne, Petite Arvine, Lafnetscha nor Ermitage, i.e., it does not share at least one allele at each of the 12 microsatellite loci (Tab. 3). Cornalin (du Valais) shares one allele at each marker site with Humagne Rouge and with Rèze (Tab. 3), both plants collected from the Sortengarten Au. This is suggesting a parent-offspring relationship, but based on our data it is not possible to determine which plant belongs to the parent and which to the offspring generation.

Findling von Muhen (Gibeler)

Some years ago, an unknown grapevine was found in Muhen, Canton of Aargau. In reference to the site of finding, it was called "Findling von Muhen" or "Gibeler". It was suggested to be a Red Chasselas (Roter Gutedel), although the berries are black. Because of the shape of the leafs, it was further speculated to be *Vitis silvestris* or a cross between Blauer Heunisch and Chasselas (M. Aeberhard, pers. comm.). None of the genotypes screened so far matched with this variety. However, we can exclude an identity with Chasselas (Gutedel) and with *Vitis silvestris* of our database (Tab. 3). We can also exclude a parent-offspring relationship with Chasselas since there are missing

Tab. 3. Results of several cultivar identifications using microsatellites: Allele-Lengths at 12 markers, indicated by letters, A being the smallest. Letters in **bold** are results confirmed with 2 methods (see text). Letters in *italic* are not consistant with the other method. Missing data means not analyzed (by older method).

			Microsatellite marker																							
DNA	**Sorte**	**Collection site**	**VVS2**		**VVS4**		**VVMD5**		**VVMD6**		**VVMD7**		**VVMD8**		**VVMD27**		**VrZAG21**		**VrZAG62**		**VrZAG67**		**VrZAG79**		**VrZAG112**	
230	Grobe aus A	Private Collection Bern	**C**	**D**	**B**	**E**	**F**	**G**	B	D	E	G	A	C	A	D	**C**	**E**	**B**	**E**	C	D	A	D	D	D
231	Elbling blanc[1]	Agroscope RAC Pully	C	D	B	E	F	G									C	E	B	E						
232	Elbling blanc[1]	Agroscope RAC Pully	C	D	B	E	F	G									C	E	B	E						
89	Elbling weiss	Au 41,7	**C**	**D**	**B**	**E**	**F**	**G**	B	D	E	G	A	C	A	D	**C**	**E**	**B**	**E**	C	D	A	D	D	D
88	Elbling weiss[1]	Au 42,7	C	D	B	E	F	G									C	E	B	E					D	D
101	Elbling weiss[1]	Au Histo	C	D	B	E	F	G									C	E	B	E					D	D
313	Elbling weiss	Private Collection Zürich	C	D	B	E	F	G	B	D	E	G	A	C	A	D	C	E	B	E	C	D	A	D	D	D
110	Elbling rot	Au Histo	**C**	**D**	**B**	**E**	**F**	**G**	B	D	E	G	A	C	A	D	**C**	**E**	**B**	**E**	C	D	A	D	**D**	**D**
229	Heunisch weiss	Private Collection Bern	**A**	**C**	**B**	**B**	**D**	**G**	A	D	B	E	A	C	A	B	**D**	**E**	**C**	**E**	C	C	A	C	D	E
214	Alte Rebe	Vitis antiqua, Leuk-Stadt	**A**	**B**	**C**	**E**	**A**	**B**	**C**	**C**	D	H	A	B	C	D	**C**	**D**	**B**	**B**	A	D	**B**	**E**	E	F
206	Cornalin	Vitis antiqua, Leuk-Stadt	**A**	**B**	C	E	**A**	**B**	**C**	**C**	D	H	A	B	C	D	**C**	**D**	**B**	**B**	A	D	**B**	**E**	E	F
82	Cornalin	Au 47,5	**A**	**B**	**C**	**E**	**A**	**B**	C	C	D	H	A	B	C	D	**C**	**D**	**B**	**B**	A	D	B	E	**E**	**F**
208	Amigne	Private Collection Visp	**A**	**A**	D	E	**A**	**D**	**B**	**C**	C	G	B	D	A	D	**C**	**E**	**A**	**B**	A	C	**F**	**G**	A	E
263	Ermitage	Au 44,8	**A**	**A**	**B**	**E**	**A**	**C**	C	D	B	F	B	B	D	E	**A**	**C**	**C**	**D**	C	E	D	G	A	C
261	Eyholz	Au 46,8	**A**	**C**	**B**	**D**	B	F	B	C	C	D	B	C	B	D	**C**	**C**	A	E	E	E	C	I	D	D
220	Gwäss	Au 45,4	**A**	**C**	**B**	**B**	**D**	**G**	**A**	**D**	B	E	A	C	A	B	**D**	**E**	**C**	**E**	C	C	**A**	**C**	*D*	**E**
221	Heida	Au 45,7	**D**	**D**	**B**	**E**	**C**	**F**	**B**	**C**	C	G	A	B	D	D	**C**	**E**	**A**	**B**	A	B	**D**	**G**	B	D
245	Himbertscha	Private Collection Bern	**A**	**A**	**D**	**E**	**A**	**E**	C	C	A	C	A	B	C	D	**A**	**C**	**A**	**C**	A	C	B	G	A	D
166	Humagne blanc	Private Collection Visp	**A**	**C**	**E**	**E**	**B**	**G**	**C**	**C**	B	E	A	B	A	A	**A**	**E**	**A**	**A**	A	C	**F**	**H**	B	E
329	Humagne rouge	Au 47,2	A	B	A	C	A	B	B	C	D	G	A	D	C	D	C	F	B	B	B	D	B	D	B	F
117	Lafnetscha	Au 46,4	**A**	**E**	**B**	**E**	**B**	**E**	B	C	B	F	B	B	A	C	**A**	**C**	**B**	**D**	A	D	B	I	C	E
169	Petite Arvine	Private Collection Visp	**A**	**D**	**B**	**D**	**F**	**F**	**C**	**D**	G	H	A	A	C	D	**A**	**C**	**B**	**B**	A	C	**B**	**G**	A	E
268	Rèze	Au 45,1	**A**	**A**	C	E	A	B	C	D	D	E	B	B	C	D	**A**	**D**	**B**	**D**	A	E	E	G	D	F
338	Gibeler	Private Collection	**A**	**D**	**C**	**C**	**B**	**D**	A	C	B	B	A	C	B	D	D	E	**B**	**C**	B	C	C	G	**A**	**D**
167	Chasselas	Private Collection Visp	**A**	**C**	**B**	**B**	**B**	**E**	**B**	**C**	B	D	B	B	C	D	**C**	**E**	**B**	**E**	A	E	**G**	**I**	D	E
97	Gutedel 14-33-4	Au 36,3	**A**	**C**	**B**	**B**	**B**	**E**	B	C	B	D	B	B	C	D	**C**	**E**	**B**	**E**	A	E	G	I	**D**	**E**
98	Gutedel 7-42	Au 36,1	**A**	**C**	**B**	**B**	**B**	**E**	B	C	B	D	B	B	C	D	**C**	**E**	**B**	**E**	A	E	G	I	**D**	**E**
92	Petersilien Gutedel	Agroscope FAW	**A**	**C**	**B**	**B**	B	E	B	C	B	D	B	B	C	D	**C**	**E**	**B**	**E**	A	E	G	I	D	E
199	Vitis silvestris weiblich	Au 4,1	**D**	**D**	**C**	**D**	**B**	**F**	**B**	**C**	G	G	A	A	D	D	**A**	**B**	**B**	**B**	E	E	E	E	E	E
200	Vitis silvestris männlich	Au 4,4	**D**	**D**	**C**	**D**	**C**	**C**	**B**	**C**	H	H	A	B	B	D	**A**	**B**	**B**	**B**	C	E	C	E	A	A
197	Vitis silvestris Turcovic	Au 4,5	**D**	**E**	**C**	**C**	**B**	**B**	**C**	**C**	B	H	A	A	D	D	**A**	**D**	**B**	**C**	C	F	G	G	A	E

[1] only analyzed with Method 1

common alleles at several marker sites (only 9 of 12 sites share an allele, Tab. 3). The possible relationship with Blauer Heunisch can not be dismissed on the basis of our data because true to type references are missing. A relation to *Vitis silvestris* "Turcovic" is possible as the samples share at least one allele at each locus (Tab. 3), but more data are required to confirm this.

Grape color and other phenotypic differences

We found that in some cases microsatellites were able to differentiate between grapes of different colors but not in others. For example, White and Red Elbling showed the same allele-pattern (Tab. 3) because they are two forms of the same cultivar (Hillebrand et al. 1998). In contrast, Humagne Blanc and Humagne Rouge showed different patterns (Tab. 3). These results make sense because the latter two names denominate two cultivars which are not related to each other.

Chasselas persilé (Petersilien Gutedel) shows clear phenotypic differences to the common Chasselas (Gutedel). The leaves of the former are slitted and look like parsley leaves (Fig. 1). However, they belong to the same cultivar and we found the same allele pattern despite of the obvious morphological differences (Tab. 3).

Our results further confirm that Completer and Lafnetscha are two different cultivars, as already proposed by Vouillamoz et al. (2004). While all Completer samples showed the same allele pattern, we found some variation among the Lafnetscha samples indicating that confusion exists with respect to the true to type status of the cultivar. We found a specific allele pattern in Lafnetscha from the Sortengarten Au and some other plants, but in three cases, putative Lafnetscha samples were found to be identical to other existing cultivars. These samples must be reanalyzed for confirmation. One of them was identical to Completer, a similar case was already reported by Vouillamoz et al. (2004). A labeling mistake cannot be dismissed since these cultivars are morphologically very similar.

Our results confirm that based on the allele pattern at 12 marker sites, Gouais blanc, Gwäss and Weisser Heunisch are the same cultivar and hence, these names are synonyms. Gewürztraminer, Savagnin blanc (Païen) and Heida, are red and white-berried forms of the same cultivar.

Discussion

We have successfully adapted highly multiplexed microsatellite analysis (originally developed for marker assisted selection in apples) to grape cultivar identification. The high level of matching results found between the new and the classical method shows that the former is at least as reliable, with the advantage that it is much faster. The quick and simple DNA extraction procedure used does not cause problems, because for microsatellite PCR amplification, purification of DNA is not necessary. The crude DNA-extract can even be stored frozen for several months without interfering with PCR reactions (B. Frey, pers. comm.). With this method we were able to analyze 48 samples at 12 marker sites in less than 3 days.

Except for the "Clinton" cultivar, all grapes were diploid. The there observed tetraploidy is not surprising, since it represents a natural hybrid between *Vitis riparia* and *Vitis labrusca* (Eggenberger and Lennert 1996). Some cultivars proved difficult to amplify with some of the markers, particularly interspecific varieties such as Concord, Seyval blanc and Soleil blanc. Since most of the grape microsatellites are designed on

Fig. 1. Chasselas persilé (Petersilien Gutedel). This variety and Chasselas are an example for clear morphological differences while having an identical genomic profile; they are two forms of the same cultivar.

the basis of *Vitis vinifera* it does not surprise that the DNA of other *Vitis* species and more so other genus of the *Vitaceae* family, do not amplify well on some marker loci. The primers VVS2, VVS4 and VVMD5 – VVMD8 were designed on *Vitis vinifera* (Thomas and Scott 1993; Bowers et al. 1996), while all VrZAG primers base on *Vitis riparia* (Sefc et al. 1999).

Identity at six marker loci (Mix 1) was reported to allow correct cultivar identification (Regner 2003). Using 12 loci as we did, considerably increased the confidence level in cultivar identification. Still, the probability of a false-positive identification exists, until all the potential parent vines are genotyped. Therefore, a non-identity (exclusion) when different alleles are present at several marker loci, is always more reliable.

With our microsatellite primers, clones cannot be distinguished. It seems possible to differ between certain grape clones, e.g. by designing primers for hypervariable genome sequences (Regner 2003), however, this was not the subject of this study.

Our results confirm that some confusion concerning the names and pedigree of certain grape cultivars exists, including many synonymous local naming. Probably the most prominent example, where microsatellite marker technique could clarify doubts, is the pedigree of the well known Müller-Thurgau. With our method, we confirmed the known fact that the Müller-Thurgau cultivar cannot be the cross between Riesling and Silvaner, but originates from a cross of Riesling x Madeleine Royale (Dettweiler et al. 2000). For this analysis we used the original Müller-Thurgau plant No 58 in our vineyard (Agroscope FAW Wädenswil) as well as a Madeleine Royale and a Riesling plant from the Sortengarten Au (data not shown).

Other identifications, such as Humagne Blanc and Humagne Rouge, both ancient Swiss varieties from the Canton of Valais, are quite confusing because they are not of the same cultivar and a relationship can be excluded as reported by Hillebrand et al. (1998). This fact was confirmed by our results (Tab. 3).

Elbling is a white grape variety that is today still found in Germany (Obermosel), however, some plants are still cultivated in the Canton of Aargau (Switzerland) or in certain grapevine cultivar collections (Eggenberger and Lennert 1996). This cultivar seems also still present in Austria under the name of "Grobe". Our analysis showed a 100% matching profile. White Elbling has large, dense clusters. Red Elbling represents a form (Spielart) with red berries and similar characteristics as White Elbling (Ambrosi et al. 1994). This fact that was confirmed by our analyses which showed the same pattern for both forms (Tab. 3).

Our results also support the relation between Humagne Rouge and Cornalin (du Valais) as suggested by Vouillamoz et al. (2003).

So far we were not able to confirm the status of "Findling aus Muhen".

A considerable problem with the identification of grape cultivars by microsatellites seems to be the low comparability between different laboratories and the concomitant shift of allele-lengths as mentioned by Regner (2003). The advantage of our system is the use of an internal standard. Thus, analyzing allele-lengths is highly reproducible and therefore provides a better basis for comparisons between laboratories.

In conclusion, the adaptation allows faster, cheaper and more reproducible microsatellite analysis and provides a reliable tool - together with ampelographic information – for identifying grape cultivars. We strive to collect more samples to improve the usefulness of our method in order to solve other cases of uncertain grape cultivars. The method has the potential to become a part of any cultivar identification program and can be adapted for other plants or animals.

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Zusammenfassung

Eine Datenbank von Rebsorten wurde aufgrund einer Analyse mit Mikrosatelliten Markern zusammengestellt. Die Datenbank enthält allgemein bekannte sowie alte Schweizer Sorten. Zu Beginn wurde die traditionelle Silbergelfärbungsmethode angewandt, später wurde diese durch eine schnellere, besser reproduzierbare und billigere Methode ersetzt. Dazu wurde eine Methode, die zuvor für die markergestützte Selektion von Apfelsorten entwickelt wurde, für die Identifizierung von Rebsorten angepasst. Diese Methode basiert auf multiplex PCR und der Analyse mit einem 16-Kapillaren Sequenziersystem. Die Resultate beider Methoden waren ähnlich und die neue Methode konnte die Sortenidentifikationen der älteren Methode sowohl an bekannten wie auch an neuen Markerabschnitten bestätigen. Die Datenbank besteht bis anhin aus 147 *Vitis vinifera* und interspezifischen Sorten, inklusive einige historische Rebsorten und vier Sorten, die zur Vitaceae Familie gehören. In Zukunft soll diese Datenbank erweitert werden, um ein geeignetes Verfahren zur Identifikation von Rebsorten zu haben oder Verwandtschaftsverhältnisse zu bestimmen. In diesem Artikel zeigen wir einige Beispiele für die Identifizierung unbekannter Rebsorten, sowie eine „Nicht-Identifizierung". Diese neue Methode kann angewandt werden zur raschen und zuverlässigen Bestätigung einer Sortenzugehörigkeit im Gebiet der Pflanzenzüchtung, der Definition und Erstellung eines genetischen Profils einer neuen Sorte, wie auch zur Bestimmung von Unterlagsreben. Sie ist besonders aufschlussreich in Fällen, wo eine morphologische Identifikation nicht eindeutig ist.

References

Ambrosi H., Dettweiler-Münch E., Rühl E.H., Schmid J. und Schumann F. 1994. Farbatlas Rebsorten. Ulmer, Stuttgart, Germany.

Babo A. Frhr. von, und Mach E. 1909. Handbuch des Weinbaus und der Kellerwirtschaft, 3.Aufl., Verlagsbuchhandlung P. Parey, Berlin.

Bowers J.E., Dangl G.S., Vignani R. and Meredith C.P. 1996. Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome 39: 628–633.

Bowers J.E., Dangl G.S. and Meredith C.P. 1999. Development and Characterization of Additional Microsatellite DNA Markers for Grape. Am. J. Enol. Vitic. 3: 243–246.

Dettweiler E., Jung A., Zyprian E. and Töpfe, R. 2000. Grapevine cultivar Müller-Thurgau and its true to type descent. Vitis 39(2): 63–65.

Eggenberger W. und Lennert J. 1996. Lexikon des Schweizer Weins. AT Verlag, Aarau, Schweiz.

Frey J.E., Frey B., Sauer C. and Kellerhals M. Efficient low-cost DNA extraction and multiplex fluorescent PCR method for marker-assisted selection in breeding. Plant Breeding, in press.

Hillebrand W., Lott H. und Pfaff F. 1998. Taschenbuch der Rebsorten. 12. Aufl. Fachverlag Dr. Fraund GmbH, Mainz, Germany.

Maigre D., Brugger J.-J., Gugerli P. and Pont M. 2000. Safeguarding, conserving and valorizing the genetic diversity in Valais (Switzerland). Acta Hort. (ISHS) 528: 59–66.

Regner F. 2003. Genetische Identifizierung von Rebsorten und deren Standardisierung. Dtsch. Weinbau-Jahrb. 2003: 65–69.

Regner F., Stadlbauer A. und Eisenheld C. 1999. Heunisch x Fränkisch, ein wichtiger Genpool europäischer Rebsorten. Vitic. Enol. Sci. 53(3): 114–118.
Sefc K.M., Regner F., Gloessl J. and Steinkellner H. 1998a. Genotyping of grapevine and rootstock cultivars using microsatellite markers. Vitis 37(1): 15–20.
Sefc K.M., Steinkellner H., Gloessl J., Kampfer S. and Regner F. 1998b. Reconstruction of a grapevine pedigree by microsatellite analysis. Theor. Appl. Genet. 97: 227–231.
Sefc K.M., Regner F., Turetschek E., Glössl J. and Steinkellner H. 1999. Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genome 42: 367–373.
Thomas M.R. and Scott N.S. 1993. Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theor. Appl. Genet. 86: 985–990.
Thomas M.R., Matsumoto S., Cain P. and Scott N.S. 1993. Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. Theor. Appl. Genet. 86: 173–180.
Thomas M.R., Cain P. and Scott N.S. 1994. DNA typing of grapevines: A universal methodology and database for describing cultivars and evaluating genetic relatedness. Plant Mol. Biol. 25: 939–949.
Vouillamoz J., Maigre D. and Meredith C.P. 2003. Microsatellite analysis of ancient alpine grape cultivars: pedigree reconstruction of *Vitis vinifera* L. 'Cornalin du Valais'. Theor. Appl. Genet. 107(3): 448–454.
Vouillamoz J.F., Maigre D. and Meredith C.P. 2004. Identity and parentage of two alpine grape cultivars from Switzerland (*Vitis vinifera* L. Lafnetscha and Himbertscha). Vitis 43(2): 81–87.