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Investigations into the genetic variability of Traminer clones

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The phenotypical appearance of different Traminer clones encouraged us to investigate the genetic base of these individual grapevine genotypes. Significant differences of morphological parameters indicated a broad variability within the clones. By applying RAPD and SSR markers it was feasible to find several genetic differences within the variety. RAPD polymorphism was used to estimate the heterozygosity. The microsatellite profiles offer a potential for the identification of a single individuum. Stable SSR loci usually used for the identification of cultivars are not suited for clonal differentiation. SSR loci located on hypervariable regions provide sufficient polymorphic alleles for identification of clonal material. Hence clonal grapevine material could now be defined by its genetic profile and can be identified out of other individuals of the same variety.
The supposed variability of a cultivar can be confirmed with these data. Clonal selection will be regarded again as a genetic selection process with some phytopathological aspects. Each individual possesses its own genetic fingerprint and even vegetatively propagated material tends to differ from the mother plant. These are the first steps for any kind of clonal protection.
Keywords: *Vitis vinifera,* Gewürztraminer, clonal selection, clone identification

Untersuchungen über die genetische Variabilität von Traminer-Klonen. *Das phänotypische Erscheinungsbild verschiedener Traminer-Klone forderte uns auf, die unterschiedliche Genetik innerhalb einer Sorte zu studieren. Signifikant abgesicherte Unterschiede in einigen Parametern zeigten die große genetische Breite schon vorher an. Mit der Anwendung von RAPD- und SSR-Markern wurde es möglich, einige der genetischen Unterschiede innerhalb einer Sorte aufzuspüren. Die genetischen Polymorphismen aus der RAPD-Analyse wurden verwendet, um den Grad der Heterozygotie abzuschätzen. Die Profile aus der Mikrosatellitenanalyse ermöglichen die Identifizierung eines einzelnen Individuums. Dabei haben sich stabile SSR-Loci, die für die Identifizierung von Sorten verwendet werden, als nicht geeignet herausgestellt. Genorte, die auf Grund ihrer Hypervariabilität für die Sortenidentifizierung ausgeschlossen wurden, sind für die Klonenunterscheidung geeignet. Folglich können mit dieser Methodik Klone einer Rebsorte definiert und von anderen unterschieden werden. Diese Daten bekräftigen die schon immer vermutete genetische Variabilität innerhalb einer Sorte. Die Klonenzüchtung kann wiederum als eine genetische Selektion unter Berücksichtigung von phytopathologischen Aspekten betrachtet werden. Jedes Individuum besitzt seinen individuellen genetischen Fingerabdruck, und auch vegetativ vermehrtes Material tendiert dazu, sich von der Ausgangspflanze zu unterscheiden. Die genetische Identifizierung von Klonen ist der erste Schritt hin zur Möglichkeit eines Klonenschutzes.*
Schlagwörter: *Vitis vinifera,* Traminer, Roter Traminer (Gewürztraminer), Klonenselektion, Klonenidentifizierung

Recherches sur la variabilité génétique de clones Traminer. *L'apparence phénotypique des divers clones Traminer nous a amené à étudier la génétique différente au sein d'un cépage. Les différences significatives, basées sur des données scientifiques, de quelques paramètres indiquaient déjà à l'avance la grande diversité génétique. L'utilisation de marqueurs RAPD et SSR a permis de détecter quelques-unes des différences génétiques au sein d'un cépage. Les polymorphismes génétiques de l'analyse RAPD ont été utilisés pour estimer le degré d'hétérozygotie. Les profils tirés de l'analyse microsatellite permettent l'identification d'un individu unique. Dans ce contexte, les loci SSR stables*

utilisés pour l'identification de variétés se sont avérés inadéquates. Les loci génétiques qui ont été exclus pour l'identification de variétés en raison de leur hypervariabilité conviennent à la distinction de clones. Cette méthodique permet par conséquent de définir les clones d'un cépage et de les distinguer des autres. Ces données confirment la variabilité génétique toujours supposée au sein d'un cépage. La culture de clones, elle, peut être considérée comme une sélection génétique en tenant compte d'aspects phytopathologiques. Chaque individu possède son empreinte génétique individuelle, et le matériel multiplié par voie végétative, lui aussi, a tendance à se distinguer de la plante de départ. L'identification génétique de clones est le premier pas vers la possibilité de protéger les clones.
Mots clés : *Vitis vinifera,* Traminer, Roter Traminer (Gewürztraminer), sélection de clones, identification de clones

In the areas of moderate climate the cultivar 'Traminer' is still of economic importance (AMBROSI et al., 1994). 'Traminer' is one of the oldest traditional grapevine cultivars (BASSERMANN-JORDAN, 1975). As usual for traditional cultivars no precise information about the origin is available. From the morphological point of view closely related genotypes could be detected among the wild vines growing in forests (KIRCHHEIMER, 1944; SCHUMANN, 1971). When these genotypes were involved in genetic studies the close relationship of 'Traminer' to the wild vines named 'Gmelin' could be confirmed (REGNER et al., 2000). According to its first appearance the name of the cultivar is identical to the village Tramin in Southern Tyrol. In 1349 its name was mentioned for the first time and this document about 'Traminer' still exists (SCHUMANN, 1968). It is supposed that this variety was already used by the Romans. The historical spread of the variety to all wine districts in Europe and intensive propagation may have happened during the time of the Roman Empire and its effort to improve wine quality (BASSERMANN-JORDAN, 1975). Vegetative propagation over the centuries allows us to find intervarietal variations nowadays. As we already could observe types and clones with specific traits the genetic base should offer some genetic differences. As we can find different berry colour, leaf shapes and aroma profiles we suppose a different genetic background. Even clonal differences were attested in nutrients uptake to the same five clones also involved in our studies (FARDOSSI et al., 1993). Sometimes the name of the specific trait was added to the cultivars name, for instance as used for spicy types called 'Gewürztraminer' or for Muscat flavoured types with the name 'Muskat-Traminer'. Nowadays specific markers for specific traits are still not available. Nevertheless a lot of effort is made worldwide to find the markers linked to specific traits such as Muscat aroma (EIBACH et al., 2002).

Due to the heritage of quality relevant traits 'Traminer' is even appreciated as a crossing partner. On the one hand it could be detected that many grapevine cultivars used today derived from a crossing with 'Traminer' (REGNER et al., 2000), on the other hand new crossings with 'Traminer' convinced by their sensory impression (HAJDU, 2000). Therefore 'Traminer' is an interesting variety for clonal selection as well as for cross-breeding. For the comparison of obtained data it would be useful if types or clones of 'Traminer' could be differentiated. The genetic markers used nowadays for characterization and identification of cultivars are SSR markers. This kind of marker is defined by the core region of a repeat sequence, namely the microsatellite. The loci were chosen according to their behaviour in many different varieties. Therefore most useful SSR loci are located on very stable regions in the genome. Hence most markers are useful for stable amplification with the aim of identification of cultivars but do not cover gene coding regions. On the other hand there are hypervariable SSR markers not very useful for identification as they lack stability and seem to be located on hypervariable loci.

One can suppose that even on these loci differences for intervarietal differentiation will be found. The advantage of the SSR markers is the high reproducibility and the knowledge of the sequence, at least the sequence of the annealing side (REGNER et al., 2000). These markers, however, will guarantee the possibility to identify a specific genotype or clone.

The second class of markers we used for this study were RAPD. As this method is very cheap and is productive for creating polymorphism the utility is given. The stability of RAPD is not sufficient to reproduce the result neither in other laboratories nor in the same lab under different conditions (BÜSCHER et al., 1993). But the method is favoured to show very quickly how heterozygous individual grapevines can be (REGNER et al., 2000). Considering the limits of theses markers we tried to get an answer if there exist different genotypes of 'Traminer' and how far they differ from others.

The aim of this work was to get the tool for identification of clonal material and to evaluate its heterozygosity.

Material and methods

The plant material of the Traminer clones is kept at the Department for Grapevine Breeding at the Federal College and Institute for Viticulture and Pomology in Klosterneuburg. The material originated from different locations of Middle Europe and the specific origin is shown in Table 1.

Table 1:
Definition of the Traminer material used in this study

Number	Designation	Berry colour	Origin	Spicy flavour
1 / A	Tinn 1	RG	Steiermark	+
2 / B	Tinn 2	B	Steiermark	
3 / C	Reg 1	G	Steiermark	
4 / D	Reg 2	R	Steiermark	
5 / E	Kl 47	RG	Nieder-österr.	
6 / F	Kl 48	RG	Nieder-österr.	
7 / G	643	RG	Elsass	+
8 / H	AF6	RG	Elsass	+
9 / I	Wonisch	RG	Steiermark	+
10 / J	Tatt SG 2	RG	Nieder-österr.	
11 / K	Traminer weiß	B	Rhein-land-Pfalz	
12 / L	Kl 42	G	Nieder-österr.	

The grapevines were evaluated according to the O.I.V descriptors (O.I.V., 1983) and the data were compared for their pronounced morphology. The leaves were measured with the help of a digitizer tablet and the calculation of the parameters was performed with a self-written software and Excel (MS Office).
DNA from the Traminer clones was extracted from young leaves by following the protocol described by Thomas et al. (1993) and modified by Regner et al. (1998). The clones were analysed with 45 SSR markers.

The VVS markers were developed by Thomas and Scott (1993) and the VVMD markers by Bowers et al. (1996) as well as by Bowers et al. (1999). The VRZAG markers (Sefc et al., 1999) and VRG (Regner et al., paper in preparation) were obtained from investigations into simple sequence repeats of *Vitis riparia*.
RAPD analyses were carried out with 180 decamer oligonucleotides. They were obtained from Operon Technologies, Alameda, USA (kit B, C, D, E) and Metabion GmbH, Martinsried, Germany (kit G, M, N) as well as MWG GesmbH, Ebersberg, Germany (kit A, F, H, V).
Amplification was performed in 20 µl of the buffer solution, which consisted of 16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl pH = 8.8, 1.5 mM $MgCl_2$, 0.01 % Tween 20, 0.1 mM each dNTP (GenXpress, Maria Wörth, Austria), 0.2 µM primer, 1 Unit Biotherm Taq DNA polymerase (GenXpress, Maria Wörth, Austria), and 20 ng genomic DNA of grapevine.
An Omnigene (Hybaid, Teddington, Great Britain) thermocycler processed for RAPD 40 cycles of 30 sec. at 92 °C, 90 sec. at 38 °C and 60 sec. at 72 °C. The arbitrarily amplified fragments were separated on a 2 % agarose gel and detected by staining with ethidium bromide. Documentation was done by taking Polaroid photographs.
The amplification of the SSR loci was performed by following our general protocol but by applying specific annealing conditions. The general PCR protocol applied for these studies was 2 min. denaturation at 94 °C and 35 cycles with annealing phase for 30 sec. (temperature between 45 °C and 55 °C) and denaturation for 15 sec. at 92 °C. The annealing temperature for each locus was set according to the original protocol. A final extension of the fragments was performed at 72 °C for 5 min. Due to the different size range of the involved loci multiplex PCR was feasible. At least the alleles of three loci were separated on one sequencing gel.
Yield of DNA fragments was estimated by running an aliquot of the sample on a 2 % agarose gel stained with ethidium bromide. The samples were denaturated by heating up with formamide and loaded together with a size standard (Genescan 350 Tamra, Appl. Biosystems, Warrington, Great Britain) to a 6 % polyacrylamid gel. Detection of the SSR fragments labelled with the fluorescent dyes 6FAM, TET and HEX was carried out by an automated sequencer (ABI 373, Perkin-Elmer, Vienna). Labelling with these different fluorescent colouring agents facilitated the application of multiplex PCR.
The calculation of the RAPD based heterozygosity index was performed by using the software program SSPS.

Results and Discussion

Morphological differences were gained by comparing seven of the twelve involved Traminer clones by application of O.I.V. descriptors (Table 2). Significant differences are distributed over all tissues. On the shoot we detected various distribution patterns and different intensity of the anthocyanin colouration. The colour of

Table 2:
Stable SSR profile of 'Traminer' within the 12 genotypes used for genetic analysis

OIV Nr	Characteristic	Wonisch	Kl 47	Kl 42	AF 6	643	Kl 48	Weißer Tr.
015-1	Shoot: distribution of the anthocyanin colouration on the bud scales	up to the middle	up to the middle	up to the middle	absent	absent	absent	absent
015-2	Shoot: intensity of anthocyanin colouration on the bud scale base	weak	weak	weak	absent	absent	absent	absent
083-2	Mature leaf: presence of teeth at the base of the upper leaf sinuses	none	frequently	frequently	frequently	none	none	frequently
007	Shoot: colour of dorsal side of internodes	green with red stripes	green with red stripes	green	green and green with red stripes	green and green with red stripes	green and green with red stripes	green and green with red stripes
074	Mature leaf: profile	revolute	V-shaped	ondulate	V-shaped	V-shaped	V-shaped and ondulate	
206	Bunch: length of peduncle	3 - 5 cm	3 - 5 cm	5 - 7 cm	<3 u. 3-5cm	<3 u. 3-5cm	<3 u. 3-5cm	<3 u. 3-5cm
225	Berry: colour of skin	rose-red	rose-red	rose-red	rose-red	rose-red	rose-red	green-yellow
616	Mature leaf: number of teeth between N2 and sec. vein	5,10	5,50	4,60	5,10	5,20	5,40	5,10
617	Mature leaf: length between tooth tip N2 - sec. vein N2	51,21	56,66	53,22	50,51	57,23	55,19	51,77
066-5	Mature leaf: vein N3, length petiole sinus to vein N4	7,24	8,71	8,52	7,03	9,31	8,37	8,03
066-4	Mature leaf: length of vein N5	16,81	15,97	21,55	18,51	16,41	15,71	17,35

Table 3:

Polymorphic SSR profile for identification of Traminer genotypes

Locus	Traminer 1-12	
VVS 1	162	190
VVS 2	151	151
VVS 3	212	218
VVS 4	168	175
VVS 29	171	171
VVMD 5	232	238
VVMD 6	200	207
VVMD 7	243	257
VVMD 8	140	142
VVMD17	220	220
VVMD 21	249	249
VVMD 24	212	216
VVMD 25	253	253
VVMD 26	251	253
VVMD 27	189	189
VVMD 28	237	239
VVMD 31	204	216
VVMD 32	241	273
VVMD 34	254	264
VVMD 36	254	264
VRZAG 7	157	159
VRZAG 12	153	
VRZAG 15	167	167
VRZAG 21	202	208
VRZAG 25	236	245
VRZAG 29	114	118
VRZAG 30	149	151
VRZAG 62	189	195
VRZAG 64	140	164
VRZAG 67	126	132
VRZAG 79	246	252
VRZAG 82	251	271
VRZAG 83	190	202
VRZAG 93	188	188
VRZAG 112	236	242

the dorsal side of the internodes differs as well as the profile of the mature leaf and teeth at the base of the upper leaf sinuses. Numerous deviations were found in the distances of specific points on the mature leaf. As already designated with the clone the berry colour is rose-red or green-yellow. These findings show that the variability in Traminer clones seems to be very high.

All twelve clones of 'Traminer' (Table 1) were also analysed by genetic markers for detecting genetic polymorphism. As the reproducibility is best with SSR markers we used 45 loci to genotype the clonal material. The VVS, VVMD and VRZAG (Table 3) markers often used in varietal identification of grapevines could not proliferate any polymorphism. All twelve clones of 'Traminer' showed the same profile by using these standard SSR loci. Nevertheless with these data the trueness to type is confirmed. But other SSR loci as some of the VRG markers resulted in different allelic profiles (Table 4). The polymorphic alleles could be reproduced in a second trial. The genetic differences are not surprising, however, the possibility to find some deviations located in the range of an SSR marker enabled us to use them for identification. The occurrence of null alleles is one main source of polymorphic SSR loci. Mutations at the annealing side easily can inhibit the amplification of the allele. A null allele was accepted if the second assay resulted in the same way. But we did not try to change the PCR protocol for easier annealing conditions. The formation of new alleles could be observed. If the size is out of the frame of the locus a larger rearrangement in the genome has taken place. Few of them represent a third allele and could be created by crossing over.

All these deviations render possible an identification system of clones within the variety. Each genotype differs from all others at least by combining several SSR loci.

112 polymorphic fragments (Table 5) were gained by applying 180 RAPD markers. Only 26 oligonucleotides show any kind of different DNA fragments. As the stability of the RAPD polymorphism is limited we decided not to use them for identification but for differentiation and estimation of heterozygosity. To avoid the integration of false fragments for calculation we limited the fragment size from 100 to 3000 bp .

Bands of different sizes out of this range were not involved for the calculation. Each of the clone could be differentiated from all the others at least by combining several of the polymorphic markers.

Polymorphism was used to calculate proximity (Table 6) and to form clusters of more closely related clones

Table 4:
RAPD polymorphism used for estimating heterozygosity of 12 Traminer individuals

Geno-typ locus	1/A	2/B	3/C	4/D	5/E	6/F	7/G	8/H	9/I	10/J	11/K	12/L
VRG 1	200:206	200:206	200:206	200:206	200:206	200:206	200:206	200:206	200:206	200:206	***92:112***	200:206
												92
VRG 2	***158***	158:164	158:164	158:164	158:164	158:164	158:164	158:164	158:164	158:164	158:164	158:164
				106	***106***				***292***	***292***		
VRG 3	185:214/40	185:214/40	185:214/40	185:214/40	185:214/40	185:214/40	185:214/40	185:214/40	185:214/40	185:214/40	214: 224	185:214/40
					283		***220***			***220***	***140***	
VRG 4	152	152	152	***150***	152	152	152	***108:152***	***108:152***	152	152	152
VRG 7	191	191	***191:208***	191	191	191	191	191	191	-	191	191
VRG 9	***220:246***	***220:246***	246	246	246	246	246	246	246	***220***	246	246
VRG 10	117	117	117	117	117	117	117	117	117	117	117	117
						276	***276***	***276***	***276***	***276***		
VRG 11	304:312	304:312	304:312	304:312	***312***	***312***	***300:312***	***300:312***	304:312	-	304:312	304:312
VRG 13	128:152	128:152	128:152	128:152	128:152	128:152	128:152	128:152	128:152	128:152	128:152	128:152
VRG 15	150	150	***150:158***	150	***114:150***	150	150	150	150	***128:136***	***128***	***152***
VRG 16	248	248	248	248	248	248	248	248	248	248	248	248
VMC 62	230/232	230/232	230/232	230/232	230/232	230/232	230/232	230/232	230/232	230/232	230/232	230/232

(Table 7). The genetic relationship of the clones was expressed in the dendrogram (Table 8). As the genetic polymorphism represents a deeper insight into the genome than the differences in morphology it is not surprising that the outcome is not identical. The RAPD profiles, however, summarise all these differences seen as single deviation from the morphology. As the groups contain either local individuals, spicy types or colour types the range of 'Traminer' can be confirmed as a broad one. The most individual genotype was the 'Traminer white' from Germany, followed from a type selected in the south of Vienna from an ancient mixed population vineyard. It was interesting to see that the spicy types 'Gewürztraminer' are closely related despite their different selection area. But it can be supposed that these individuals were dispersed all over Europe.

The tool of genetic analysis to verify clonal variation is a perfect method for the breeding process, as most growers prefer to cultivate not the variety but clonal material of a traditional cultivar. In France and other countries with controlled production systems (appellation d' origine controllée) it is obligatory to use specific clones to get the wine accepted for the common A.O.C. labelling. Therefore identification of clonal material is highly appreciated for clonal breeding.
The possibility of identification of a clone raises up the question of any kind of clonal protection. Comparing the costs of transgenic plants and classically bred clones it is not logical why genetic modified (GVO) grapevines should be patented whereas clones do not get any kind of protection. In practical viticulture it would be very helpful if private wineries would offer their clonal material to the public. But they will not do it without the prospect of any benefit. Therefore interesting genetic material is kept away from the wine community due to the lack of any financial advantage for clonal breeding.
Hence if the genetic analysis reveals that genetic base of an individual clone differs from all other registrated clones, this is one of the prerequisites, that the novelity can be demonstrated. For any kind of protection additionally the uniformity and the stability have to be considered. In the past these criteria could not be evaluated for clones. Despite morphological differences the differentiation of clones was neglected by international law for plant protection.

Table 5:
Proximity matrix based on the RAPD polymorphism

Primer	Tinn. RG	Tinn. B	Regele G	Regele B	Kl 47 G	Kl 48 G	Els. 643 G	Els. AF6 G	Woni. G	Tatten. G	Neust. B	K42
A 7	1	1	1	1	1	1	1	1	1	1	0	1
	1	1	1	1	1	1	1	1	1	1	0	1
	1	1	1	1	0	1	1	1	1	0	0	0
	1	1	1	1	1	1	1	1	1	0	1	1
	1	1	1	1	1	1	1	1	1	0	0	1
	0	0	0	0	0	0	0	0	0	0	1	0
A 10	1	1	1	1	1	1	1	1	1	1	0	1
A 18	1	1	1	1	0	1	1	1	1	0	0	0
	0	0	0	0	0	0	1	0	0	0	0	1
	1	1	1	1	0	1	1	1	1	1	0	0
	1	1	1	1	0	0	1	1	1	1	0	0
	0	0	0	0	0	0	0	0	0	0	1	0
	0	1	0	0	0	0	0	0	0	1	0	0
	1	1	1	1	1	1	1	1	1	1	0	1
	0	0	0	0	0	0	0	0	0	0	1	0
AP 12	1	1	1	1	1	1	0	0	1	0	1	1
	1	1	1	1	1	1	1	1	1	0	1	1
	0	1	1	1	1	1	0	0	1	1	1	1
	0	1	1	1	1	1	1	1	1	1	0	1
B 3	1	1	1	1	1	1	0	0	0	1	1	1
	1	1	1	1	1	0	0	0	0	1	1	1
	0	0	0	0	0	0	0	0	0	0	1	0
B 302	0	1	0	0	1	0	0	0	0	0	0	0
	0	0	0	0	0	0	1	0	0	0	0	0
	1	1	1	1	1	1	1	0	0	0	1	1
	0	0	0	0	0	0	0	1	1	1	0	0
	1	1	1	1	1	1	1	0	0	0	0	1
	0	0	0	0	0	0	0	0	0	1	0	0
	0	0	0	0	0	0	0	0	0	0	1	0
C 5	1	1	0	1	0	1	1	1	1	1	1	1
C 6	1	1	1	1	1	1	1	0	0	0	0	1
	0	0	0	0	0	0	0	0	0	0	1	0
	1	1	0	1	1	1	1	1	1	1	1	1
	0	0	0	0	0	0	0	0	0	0	1	0

Primer	Tinn. RG	Tinn. B	Regele G	Regele B	Kl 47 G	Kl 48 G	Els. 643 G	Els. AF6 G	Woni. G	Tatten. G	Neust. B	K42
D 1	0	0	0	0	0	0	0	0	0	0	1	1
	1	1	1	1	1	1	1	1	1	0	0	0
	1	1	1	1	1	1	1	1	1	1	0	0
	0	0	1	0	0	0	0	0	0	0	0	0
	0	1	0	1	1	1	1	0	0	1	0	0
	1	1	0	1	1	1	1	1	1	0	1	1
	0	0	0	0	0	0	1	0	0	0	0	0
	1	0	0	1	0	1	1	1	1	0	1	0
	0	0	1	1	0	1	0	0	0	0	1	1
	0	1	0	0	1	1	1	1	0	0	1	1
	1	0	1	0	0	0	0	0	0	0	1	1
	1	1	1	1	1	0	1	0	1	1	1	1
	0	1	0	0	1	1	1	0	0	0	0	0
GTO3	0	0	0	0	0	0	0	0	0	0	1	0
	0	0	0	0	0	0	0	0	0	0	1	0
	1	1	1	1	1	1	1	1	1	1	0	1
	0	0	0	0	0	0	0	0	0	0	1	0
	1	1	1	1	1	1	1	1	1	0	0	0
	1	1	1	1	1	1	1	1	1	0	0	0
	1	1	1	1	1	1	1	0	0	1	1	1
	1	1	1	1	1	1	1	1	1	0	0	1
E 13	1	1	1	0	1	1	1	1	1	1	1	0
	1	1	0	1	1	1	0	0	0	0	0	1
	0	1	1	0	0	0	0	1	1	1	1	1
	0	0	0	0	1	0	0	0	0	0	0	0
	0	0	1	0	0	1	0	0	0	0	1	0
E 14	1	1	0	0	1	1	1	1	1	1	1	1
	1	0	0	1	0	0	0	1	1	0	0	0
	0	1	1	0	1	1	1	0	1	1	1	1
	0	1	1	1	1	1	0	0	1	1	1	0
	0	1	1	1	1	1	1	0	1	1	1	1
	0	1	1	1	0	0	0	0	0	0	0	0
	0	1	1	1	1	1	0	0	0	1	1	0
K 16	0	0	0	0	0	1	0	0	0	0	0	0

Table 5 (continued):
Proximity matrix based on the RAPD polymorphism

Primer	Tinn. RG	Tinn. B	Regele G	Regele B	Kl 47 G	Kl 48 G	Els. 643 G	Els. AF6 G	Woni. G	Tatten. G	Neust. B	K42
P 166	0	0	0	1	0	1	1	1	1	0	0	0
	0	0	0	0	0	0	0	0	0	0	1	0
	1	1	1	1	1	1	1	1	1	1	0	1
	0	0	0	0	1	0	1	0	0	0	0	0
	1	1	1	1	1	1	1	1	1	1	0	1
	0	0	0	1	0	1	1	1	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	1	0
V 6	1	0	0	0	0	1	0	1	1	1	1	1
	1	1	1	1	1	1	1	1	1	1	0	1
	0	1	0	0	0	1	0	0	0	0	1	0
	0	1	1	1	1	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	1	1	0
	0	0	0	0	0	0	0	0	0	0	1	0

Primer	Tinn. RG	Tinn. B	Regele G	Regele B	Kl 47 G	Kl 48 G	Els. 643 G	Els. AF6 G	Woni. G	Tatten. G	Neust. B	
V 9	1	1	1	1	1	1	1	1	1	1	0	1
	0	0	1	0	0	0	0	0	0	0	1	0
	1	0	0	1	0	1	1	1	1	0	0	0
	0	0	0	1	1	1	1	1	1	1	1	1
V 10	1	1	1	1	1	1	1	1	1	0	1	1
	1	1	1	1	1	1	1	0	1	0	1	0
Q 5	1	1	1	1	0	1	1	1	1	0	0	1
V 15	1	1	0	0	1	1	1	1	1	1	1	1
	1	1	0	0	0	0	0	1	1	1	1	0
	0	0	0	0	0	0	0	0	0	1	0	0
	0	0	0	0	0	0	0	0	0	0	1	0
	1	1	1	1	1	1	1	1	1	1	0	1
V 5	0	0	0	0	0	0	0	0	0	0	1	0
	1	1	1	1	0	1	1	1	1	1	1	1
	1	1	1	1	1	1	1	1	1	1	0	1

Table 6:
Cluster analysis with method of WARD performed with SSPS

Case	Matrix file input											
	A	B	C	D	E	F	G	H	I	J	K	L
A		23,000	29,000	24,000	35,000	28,000	25,000	23,000	20,000	42,000	63,000	29,000
B	23,000		20,000	21,000	20,000	21,000	26,000	32,000	27,000	37,000	62,000	28,000
C	29,000	20,000		19,000	28,000	29,000	36,000	38,000	33,000	45,000	64,000	32,000
D	24,000	21,000	19,000		25,000	20,000	27,000	29,000	28,000	48,000	71,000	33,000
E	35,000	20,000	28,000	25,000		23,000	32,000	42,000	39,000	41,000	62,000	26,000
F	28,000	21,000	29,000	20,000	23,000		23,000	27,000	28,000	44,000	59,000	27,000
G	25,000	26,000	36,000	27,000	32,000	23,000		22,000	21,000	45,000	74,000	34,000
H	23,000	32,000	38,000	29,000	42,000	27,000	22,000		13,000	41,000	70,000	36,000
J	20,000	27,000	33,000	28,000	39,000	28,000	21,000	13,000		32,000	65,000	35,000
I	42,000	37,000	45,000	48,000	41,000	44,000	45,000	41,000	32,000		59,000	35,000
K	63,000	62,000	64,000	71,000	62,000	59,000	74,000	70,000	65,000	59,000		48,000
L	29,000	28,000	32,000	33,000	26,000	27,000	34,000	36,000	35,000	35,000	48,000	

Table 7:
Dendrogram of Traminer individuals using average linkage group

Case	5 Cluster
A	1
B	2
C	2
D	2
E	2
F	2
G	1
H	1
I	1
J	3
K	4
L	5

The genetic markers, however, would enable us to observe these parameters during propagation. If the criteria of distinctness, uniformity and stability can be fulfilled there is no reason why clonal protection should not be established. At the moment the UPOV (International Union for Protection of Varieties) does not accept genetic differences as the only criteria for distinctness. The main argument is that the DNA sequence is not considered as long as it is not linked to a specific trait for the morphology. Concerning the proceedings in genetic mapping and definition of specific clones hopefully we will reach in some years the situation that the mutations resulting in a new clone can be linked to the DNA. The DUS (distinctness, uniformity, stability) test as criterion for varietal protection is easily applicable even to clonal material by using hypervariable SSR markers. Moreover these genetic information about a clone allows to avoid duplicates in clonal collections and renders possible to control the clonal identity. Whatever the grapevine community decides to do with the clonal identification, thanks to this work, it was never easier to document clonal selection.

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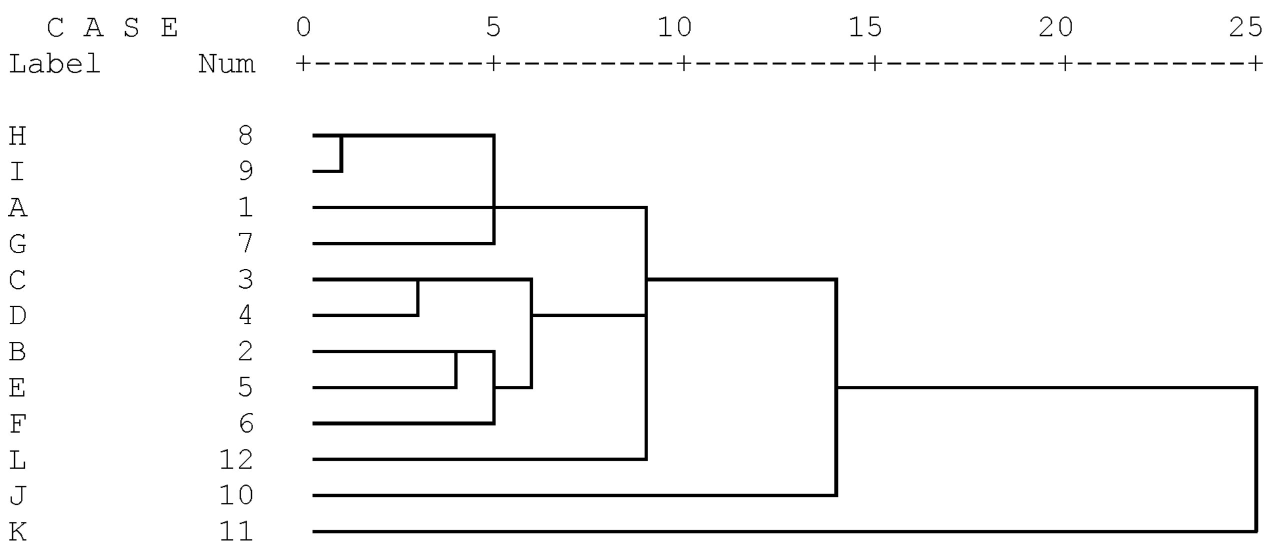
Table 8:
Some OIV descriptors with differences within the variety



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