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Genetic analysis of grape berries and raisins using microsatellite markers

by

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S u m m a r y : Microsatellite markers have been used recently for the identification and pedigree analysis of grapevines with leaves and wood as sources of vine DNA. To identify grapes after harvest and their products, we applied DNA extraction protocols to grape berries and raisins. DNA was obtained from both sources, but that of raisins was highly degraded. The suitability of DNA for PCR amplification of single genetic loci was shown by amplification of 11 microsatellite markers. 18 commercially available table grape samples were genotyped, and 11 (61 %) matched the corresponding genetic profile in our reference database. Four samples were shown to be defined incorrectly and 4 samples did not match any of the genetic profiles present in the database. The investigated raisins were found to be cv. Sultanina. The results demonstrate that DNA-based cultivar identification methods can be applied to harvested grapes and raisins.

K e y w o r d s : microsatellites, simple sequence repeats, table grapes, raisins.

Introduction

Recently, methods to identify vine cultivars using molecular markers have been established (BOURQUIN *et al.* 1993; THOMAS *et al.* 1994; BOWERS and MEREDITH 1997; SEFC *et al.* 1998 a). In these studies, DNA was extracted mainly from leaves. BOURQUIN *et al.* (1992) isolated DNA for RFLP analyses from wood. However, in some cases it may be necessary to use tissue other than leaves or wood as a source of vine DNA, e.g. if harvested berries are to be examined. In this study, we extracted DNA from grape berries and raisins for genotyping on the basis of microsatellite analysis. In this paper we offer a proper method to identify table grapes, according to the rules established by the EU for trade and commerce of table grapevines (reg. 1730/87, EU n. 163, 23/6/87).

Material and methods

Table grapes were collected at various supermarkets and market places in Austria. The grape cultivar names and the origin of the grapes presented in the Table are those found at the market places. DNA extraction basically followed the protocol of THOMAS *et al.* (1993). 2-4 berries (ca. 4 g) with seeds removed were used. For DNA extraction from commercially available raisins a protocol of DOYLE and DOYLE (1990) was slightly modified: 200 mg raisins (i.e. half a raisin) were frozen and homogenized using a mixer mill (MM 2000, Retsch). The powder was suspended in 1080 µl CTAB buffer (2 % CTAB, 100 mM Tris/HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, 1 % w/v polyvinylpyrrolidone, 0.1 % v/v β-mercaptoethanol) and incubated at 65 °C for 90 min. Then 540 µl dichloromethane was added and incubated for 10 min at room temperature. The phases were separated by centrifugation (10 min, 13000 rpm). The aqueous layer was collected and the dichloromethane wash step was repeated. DNA was precipitated from the aqueous phase with 1 volume of isopropanol.

Berries and raisins were analysed at the following 11 microsatellite loci: VVS1, VVS 2 (THOMAS and SCOTT 1993), VVMD 5, VVMD 7 (BOWERS *et al.* 1996), VVMD 28, VVMD 32, VVMD 36 (BOWERS and MEREDITH, Department of Viticulture and Enology, UC Davis, CA, USA, pers. comm.), ssrVrZAG 21, ssrVrZAG 47, ssrVrZAG 79 and ssrVrZAG 83 (SEFC *et al.*, submitted). PCR reactions and electrophoresis were performed as described previously (SEFC *et al.* 1997).

Results and Discussion

Microsatellite markers have been used recently for the identification and pedigree analysis of grapevines (THOMAS *et al.* 1994; BOWERS *et al.* 1997; SEFC *et al.* 1997; SEFC *et al.* 1998 b). Data obtained from 120 grapevine and rootstock cultivars have been combined in a database (SEFC *et al.* 1997 and unpublished). In the present study, we investigated the applicability of this method to vine products such as grape berries and raisins. Figure, A, shows that non-degraded DNA was extracted from fresh berries (0.3-1.5 µg DNA·g^{-1}). Samples of 18 different commercially available table grapes were investigated using 11 microsatellite primers. The resulting genetic profiles (Table) were compared with our reference data set of 120 grapevine cultivars. In 11 cases (61 %), the genetic profiles of the samples matched the genetic profile of the corresponding cultivar. Fruit labeled simply as „Austrian table grapes" (No. 6 in the Table) were shown to match the cv. Portugieser blau and Hungarian grapes labeled as „Plattenseer" (No. 13) displayed the microsatellite profile of cv. Chasselas. One variety, Cardinal (No. 16), did not match

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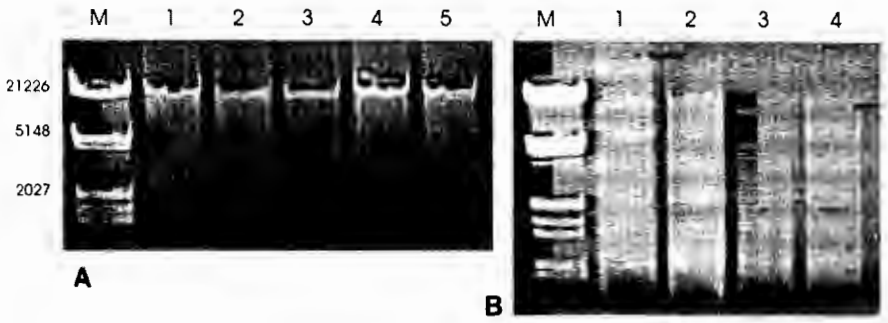

Figure: **A**. DNA extracted from grape berries. M: marker; lane (1) sample no. 8 a; (2) sample no. 5; (3) sample no. 6; (4) sample no. 9; (5) sample no. 2. **B**. DNA extracted from raisins (lanes 1-4).

the corresponding reference in our database, nor did it match any other genetic profile in our database. So far, we are not able to identify this cultivar by our limited reference set. Two mixtures of white and blue grapes were sold as Italia (No. 4 a and b) and Muskat (No. 8 a and b). In both cases, the white grapes were shown to be cv. Italia. The genotypes of the blue grapes were identical to sample No. 16, which could not be assigned to any of the genotypes in our reference database. A second sample of white Muskat grapes (No. 17), again, displayed the microsatellite profile of the cultivar Italia, while the genotype of a third Muskat sample (No. 5) did not match any of our reference profiles. However, the cultivar used for the production of Muskat grapes is not included in our reference set, and therefore the identity of this Muskat sample remains unclear. The sample designated as Greek Rosaki (No. 11), which is a synonym of Regina, matched the genetic profile of cv. Regina in the database.

In the second part of our study, we extracted DNA from commercially available raisins for PCR amplification. Since we were not able to extract DNA using the method described by THOMAS *et al.* (1993), we used a modified CTAB-based extraction procedure described by DOYLE and DOYLE (1990); ca. 3 µg DNA per raisin (ca. 400 mg) were obtained. Although extracted DNA was highly degraded, it was still suitable for PCR amplification (Figure, B). Four raisins each of two independent samples were analysed. The genetic profiles were identical in all cases and matched the genetic profile of cv. Sultanina in our database (No. 19 and 20, Table).

Our results demonstrate that the extraction of DNA and PCR amplification of single genetic loci, e.g. SSR markers, from grape berries and raisins are feasible, i.e. a genetic marker-based cultivar identification of table grapes, of grapes prior to vinification and of raisins is practicable. This allows to check table grapes according to the EU rules for the trade and commerce of grapevines.

Furthermore, the possibility to amplify single genetic loci from DNA of berries and raisins opens the potentiality of future monitoring the possible presence of transgenic sequences in grape products.

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Table

Table grapes and raisins investigated in this study. The first two columns show the sample identification number and the name of the table grapes and raisins at the market including the origin, if known. In case of 4 a and b and 8 a and b, blue and white grapes were mixed, and the berry color is given in parentheses. The third column indicates the results after matching the genotypes with our database. The following columns indicate the lengths of the alleles in base pairs (bp) at 11 microsatellite loci

Sample No.	Description	Cultivar	Genotypes of investigated table grapes and raisins (allele length in bp) at loci																					
			VVS1		VVS2		VVMD 5		VVMD 7		VVMD 28		VVMD 32		VVMD 36		ssrVrZAG 21		ssrVrZAG 47		ssrVrZAG 79		ssrVrZAG 83	
Table grapes																								
1	Chasselas (white, Hungary)	Chasselas	182	189	132	142	226	234	236	244	218	268	239	239	262	262	200	206	163	167	250	258	190	200
2	Chasselas (red, Hungary)	Chasselas	182	189	132	142	226	234	236	244	218	268	239	239	262	262	200	206	163	167	250	258	190	200
3	Italia (Italy)	Italia	161	189	132	148	230	236	240	244	234	244	251	271	252	274	190	200	157	172	254	256	188	194
4a	Italia (white)	Italia	161	189	132	148	230	236	240	244	234	244	251	271	252	274	190	200	157	172	254	256	188	194
4b	Italia (blue)	n.i.	180	180	134	136	236	236	240	252	234	244	251	271	262	286	202	206	163	172	250	256	194	194
5	Muskat	n.i.	180	186	150	150	236	236	244	248	246	248	254	271	242	262	190	206	159	172	250	256	188	194
6	Table grape (Austria)	Portugieser blau	179	180	142	150	224	230	240	252	228	260	251	271	262	274	200	206	159	172	248	258	190	194
7	Chasselas	Chasselas	182	189	132	142	226	234	236	244	218	268	239	239	262	262	200	206	163	167	250	258	190	200
8a	Muskat (white, Italy)	Italia	161	189	132	148	230	236	240	244	234	244	251	271	252	274	190	200	157	172	254	256	188	194
8b	Muskat (blue, Italy)	n.i.	180	180	134	136	236	236	240	252	234	244	251	271	262	286	202	206	163	172	250	256	194	194
9	Sultanina (Turkey)	Sultanina	180	187	144	150	232	232	236	250	218	244	249	249	248	266	190	202	159	172	246	258	188	194
10	Red Globe	Red Globe	180	180	134	150	234	236	236	246	258	258	251	171	262	262	206	206	159	159	246	258	190	200
11	Greek Rosaki	Regina	180	187	132	134	224	230	236	246	234	258	257	271	274	274	190	214	163	163	242	250	188	194
12	Regina	Regina	180	187	132	134	224	230	236	246	234	258	257	271	274	274	190	214	163	163	242	250	188	194
13	Plattenseer (Hungary)	Chasselas	182	189	132	142	226	234	236	244	218	268	239	239	262	262	200	206	163	167	250	258	190	200
14	Lavalle	Lavalle	180	180	132	134	224	236	246	252	244	244	251	271	252	262	190	202	163	163	238	250	194	200
15	Sultanina	Sultanina	180	187	144	150	232	232	236	250	218	244	249	249	248	266	190	202	159	172	246	258	188	194
16	Cardinal (Italy)	n.i.	180	180	134	136	236	236	240	252	234	244	251	271	262	286	202	206	163	172	250	256	194	194
17	Muskat (Italy)	Italia	161	189	132	148	230	236	240	244	234	244	251	271	252	274	190	200	157	172	254	256	188	194
18	Zweigelt (Austria, Wachau)	Zweigelt	189	189	136	142	224	226	236	236	234	246	249	261	252	262	202	206	157	163	236	238	194	194
Raisins																								
19	Raisins (Turkey)	Sultanina	180	187	144	150	232	232	236	250	218	244	249	249	248	266	190	202	159	172	246	258	188	194
20	Raisins (Turkey)	Sultanina	180	187	144	150	232	232	236	250	218	244	249	249	248	266	190	202	159	172	246	258	188	194

n.i.: not identified. The genotype of this grape does not correspond to any of our references.