

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.

Key words : 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 cen-

trifugation (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, VVS2 (THOMAS and SCOTT 1993), VVMD5, VVMD7 (BOWERS *et al.* 1996), VVMD28, VVMD32, VVMD36 (BOWERS and MEREDITH, Department of Viticulture and Enology, UC Davis, CA, USA, pers. comm.), ssrVrZAG21, ssrVrZAG47, ssrVrZAG79 and ssrVrZAG83 (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

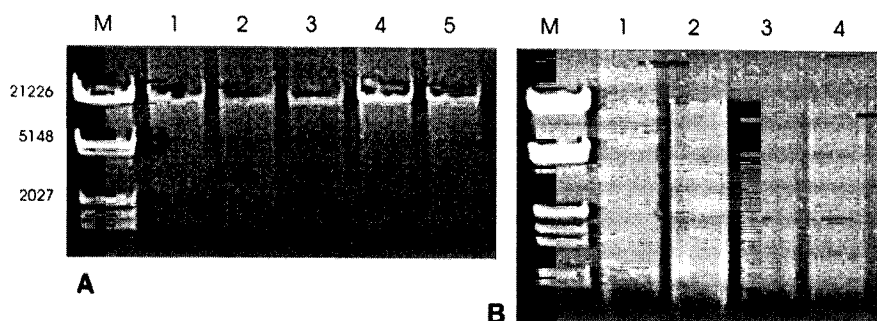


Figure: **A.** DNA extracted from grape berries. M: marker; lane (1) sample no. 8 a; (2) sample no. 5; (3) sample no. 6; (4) sample no. 9; (5) sample no. 2. **B.** DNA extracted from raisins (lanes 1-4).

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.

Acknowledgements

We thank C. MEREDITH and J. BOWERS (UC Davis, California) for providing unpublished primer sequences of *Vitis vinifera*. This work was supported in part by the Austrian Federal Ministry of Agriculture and Forestry.

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Received April 20, 1998

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