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# Marker assisted selection for seedlessness in table grape breeding

**Eda Karaagac · Alba M. Vargas · María Teresa de Andrés · Iván Carreño · Javier Ibáñez · Juan Carreño · José Miguel Martínez-Zapater · José Antonio Cabezas**

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**Abstract** Seedlessness is one of the most appreciated traits in the table grape (*Vitis vinifera* L.). The development of new seedless varieties is expensive and time consuming, involving the generation and selection of thousands of hybrids each year. In seeded × seedless crosses, seedlessness commonly segregates 1:1, so molecular markers allowing for the early identification of plants that will produce seedless berries are very useful. This early selection can lead to savings in maintenance and evaluation costs, and allows additional space for larger effective progenies. The variety Sultanina has been the main source of stenospermocarpic seedlessness in table grape breeding. In a previous work, we showed a 198-bp allele at the VMC7F2 microsatellite locus as a potential marker for selection of seedless genotypes due to its close linkage to the major effect seedless QTL, *SDI*. In this study, we show that stenospermocarpic bred cultivars share a main haplotype around this locus not found in seeded cultivars, which facilitating the development and use of marker assisted selection (MAS) strategies for the selection of seedless plants. In this way, VMC7F2 on its own can be a very useful marker for selecting seedless individuals from segregating crosses. A MAS program based on the presence of the 198-bp allele at VMC7F2 allows the reduction of the progeny size to 54%, selecting most of the seedless individuals. In addition, our results show the existence of other possible sources of stenospermocarpic seedlessness that could provide alternative sources of genetic variation for breeding of this trait.

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E. Karaagac · J. M. Martínez-Zapater · J. A. Cabezas
Dpto. de Genética Molecular de Plantas,
Centro Nacional de Biotecnología (CNB–CSIC),
C/Darwin 3,
28049 Madrid, Spain

E. Karaagac
Crop and Soil Science Department,
Oregon State University,
3050 Campus Way,
Corvallis, OR 97331, USA

A. M. Vargas · M. T. de Andrés · J. Ibáñez · J. A. Cabezas
Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA),
Finca "El Encín", Ctra A2, Km 38.200,
28800 Alcalá de Henares, Madrid, Spain

J. Ibáñez · J. M. Martínez-Zapater
Instituto de Ciencias de la Vid y del Vino (CSIC, Gobierno de La Rioja, Universidad de La Rioja),
Complejo Científico Tecnológico, C/Madre de Dios 51,
26006 Logroño, Spain

I. Carreño · J. Carreño
Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Estación Sericícola, Área de Biotecnología,
C/Mayor s/n.,
30150 La Alberca, Murcia, Spain

J. A. Cabezas (✉)
Dpto. Ecología y Genética Forestal, Instituto Nacional de Investigación Agraria y Agroalimentaria - Centro de Investigaciones Forestales (INIA-CIFOR),
Ctra. de La Coruña Km 7,
28040 Madrid, Spain
e-mail: cabezas.joseantonio@inia.es

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## Introduction

Development and marketing of new table grape (*Vitis vinifera* L.) varieties is an expensive and time-consuming process. Table grape breeding involves the generation of thousands of F1 hybrids each year and testing them for multiple traits. These plants can take 2–4 years to yield fruit due to the length of their juvenile phase. Once they are productive, they must be evaluated during several seasons for the relevant fruit traits. The most promising individuals are selected for further trials or used as parents for additional crosses, until a plant combining all the desired features can be obtained. The availability of large populations increases the chances of identifying genotypes with all the desired traits. However, growing space in testing fields is one of the most important constraints for table grape breeders. Early identification of individuals carrying the desired allele combinations allows breeders to grow larger effective populations, which results in decreasing maintenance and evaluation costs. During the last two decades, there has been considerable progress in the development of molecular markers in grape. They have been used in quantitative trait loci (QTL) mapping for berry quality traits (Cabezas et al. 2006; Costantini et al. 2007, 2008; Doligez et al. 2002, 2006, 2010; Fanizza et al. 2005; Fischer et al. 2004; Fournier-Level et al. 2009; Mejía et al. 2007) or disease resistance (Akkurt et al. 2007; Lowe and Walker 2006; Marguerit et al. 2009; Pauquet et al. 2001; Riaz et al. 2010; Welter et al. 2007; Xu et al. 2008; Zhang et al. 2009). As a result, molecular markers closely linked to major loci responsible for important traits in table grape breeding are currently available. They provide an opportunity to increase the efficiency and effectiveness of conventional breeding by marker assisted selection (MAS).

Seedlessness is one of the most appreciated traits in table grapes. All seedless table grape cultivars with known pedigrees derive from the stenospermocarpic variety Sultanina (Ibáñez et al. 2009; Vargas et al. 2009; the *Vitis* international variety catalogue, VIVC, http://www.vivc.de/index.php), also known as Sultanine or Thompson Seedless. In stenospermocarpic cultivars, the ovule is fecundated but seed development is aborted 2–4 weeks after fertilization. As a result, stenospermocarpic seedless cultivars contain seed traces in different degrees of development. Although Sultanina has been the only source of seedlessness in table grape breeding (Bouquet and Danglot 1996; Loomis and Weinberger 1979; Stout 1936), a similar phenotype has spontaneously appeared in natural variants as result of somatic mutations, such as in the cultivar Chasselas apyrene. Several hypotheses have been proposed to explain the different segregation patterns observed in different crosses involving stenospermocarpic seedlessness (see Bouquet and Danglot 1996 for a review). One hypothesis postulated the presence of a dominant allele at a single locus, later named *SDI* for "seed development inhibitor" (Lahogue et al. 1998), inhibiting the development of the seed by regulating several recessive genes (Bouquet and Danglot 1996). The existence of this locus has been confirmed by QTL mapping (Cabezas et al. 2006; Costantini et al. 2008; Doligez et al. 2002; Mejía et al. 2007). These studies demonstrated that Sultanina-derived seedlessness is mainly regulated by *SDI*, which is responsible for between 50% and 90% of total phenotypic variance for this trait, depending on the mapping population and trait evaluation. In addition, several minor effect QTLs that could be modifying its action have also been described. The seedless phenotype is determined by the presence of a dominant allele at *SDI*, which has been located on chromosome 18 (Cabezas et al. 2006; Costantini et al. 2008; Mejía et al. 2007). In this region, the MADS-box gene *VvAGL11* has been proposed as a candidate for *SDI* (Costantini et al. 2008; Mejía et al. 2011) because of its homology with genes of known function that are involved in ovule and seed development and its female flower carpel-specific expression (Díaz-Riquelme et al. 2009).

In the last decades, the development of new seedless varieties has been accelerated by embryo rescue in seedless × seedless crosses (SLxSL), which produce very high ratios of seedless individuals (Cain et al. 1983). However, seeded × seedless crosses (SDxSL) are still very important, since many of the best table grape cultivars are seeded, and the recurrent use of SLxSL crosses reduces the genetic pool and can produce inbreeding depression. In addition, SDxSL crosses are essential when the objective is the introgression of new traits from related species, such as exotic flavors or disease resistances. Introgressions may require many backcrosses with *V. vinifera* and very large population sizes. In these crosses, seedlessness usually segregates in a 1:1 ratio. The availability of tools that allow for the early identification of plants that will produce seedless berries can be very useful since plants that will produce seeded berries can be discarded in an early stage, saving 2 to 4 years in the breeding process. The results represent important savings in maintenance and evaluation costs, and create larger effective populations, providing additional space for new hybrids.

MAS is especially effective when applied to major effect QTLs that have limited interaction with the environment and no epistatic interactions. In order to be efficient, MAS requires highly reproducible markers linked in coupling phase to the target locus (Kelly 1995). Stenospermocarpic seedlessness derived from Sultanina is a good candidate trait for MAS because it is determined mainly by the presence of a dominant allele at the *SDI* locus and its high heritability (narrow sense heritability estimated between 0.52 and 0.61;

Wei et al. 2002). To date, four molecular markers have been proposed for MAS for stenospermocarpic seedlessness. The first two were sequence-characterized amplified region (SCAR) markers derived from bulked segregant analyses (Michelmore et al. 1991): SCC8 (Lahogue et al. 1998) and SCF27 (Mejía and Hinrichsen 2003). Although these markers could help to predict seedlessness, the presence of null alleles and the weak linkage with the *SDI* locus compromises their use in many genetic backgrounds (Adam-Blondon et al. 2001; Korpas et al. 2009). The third one is the microsatellite VMC7F2 (Cabezas et al. 2006), a gene specific marker located 463 bp upstream of the predicted ORF for *VvAGL11*, proposed as the candidate gene responsible for this major effect QTL (Mejía et al. 2011). QTL analyses showed a strong association between the seedlessness donor allele at *SDI* and a 198-bp allele at this locus. However, the 198-bp allele has been found in several seeded cultivars as well (Cabezas et al. 2006). The fourth one, which has been recently identified, is the microsatellite p3_VvAGL11: also a gene specific marker in the promoter region of *VvAGL11*, but for which usefulness has been shown in seedless backgrounds, but not in seeded ones (Mejía et al. 2011).

The aim of this work was to assess the usefulness of the 198-bp allele at the microsatellite locus VMC7F2 for MAS of seedless genotypes in table grape breeding programs involving Sultanina-derived stenospermocarpy. We morphologically and genetically characterized a large collection of cultivars representing a large extent of the genetic diversity existing in table grapes in order to identify any spurious associations which could cause problems in the use of this molecular marker. With this purpose, we performed the following analyses: (1) study the previously reported presence of the 198-bp allele in seeded cultivars and (2) investigate the existence of possible null alleles for this locus. The results demonstrate that all seedless cultivars derived from Sultanina share a main haplotype for the region around *SDI* and that no seeded cultivars share this main haplotype. Based on those results, we propose MAS strategies for the selection of seedless plants using microsatellite markers that can be used in breeding programs involving Sultanina-derived seedlessness. Implications for the use of these strategies for stenospermocarpic seedlessness are discussed using *a posteriori* proof of concept of what their use would represent in a table grape breeding program.

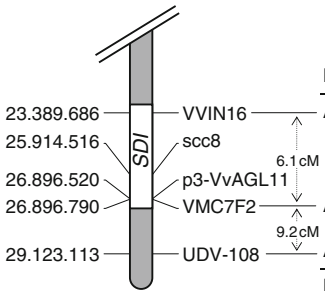
## Materials and methods

### Plant material

A total of 1,332 grapevine genotypes were analyzed in this study. The set comprises two distinct groups. The first one includes a collection of 311 table grape and mixed-use (table and wine production) grapevine cultivars, including 22 stenospermocarpic and four parthenocarpic seedless cultivars (Online resource 1). Sultanina, which has been the source of stenospermocarpic seedlessness in most table grape breeding programs, takes part in the pedigrees of 16 out of the 22 stenospermocarpic seedless accessions studied. The remaining six are as follows: a Chasselas somatic variant for seedlessness (Chasselas apyrene); the accessions of unknown origin Apirena di Velletri, Black monucca, Bayad, and Seleccion Bruni 1; and the bred cultivar Beauty seedless, in which seedlessness derives from Black seedless, also of unknown origin. This collection, which represents a large extent of the existing table grape genetic diversity, is made up by 309 unique genotypes and two somatic variants for seedlessness (Chasselas apyrene and the parthenocarpic Corinthe blanc) based on the analysis of 20 microsatellite loci (Ibáñez et al. 2009; Vargas et al. 2009) and is part of the *Vitis* Germplasm bank of El Encín (IMIDRA, Madrid). The second group consists of 1,012 hybrid plants from a breeding program, derived from eight different crosses involving nine cultivars as parents. Crimson seedless (Emperor × C33-199; *Vitis* International Variety Catalogue—http://www.vivc.de/index.php) was used as the male seedless parent for all of them, whereas the different female parents are Dominga (52 progeny individuals), Napoleon (27), Ohanez (39), Red Globe (224), and the bred hybrids 94-45-7 (Napoleon × Ruby seedless; 113 individuals), 96-55-19 (Dominga × Rutilia; 83), 96-71-11 (Napoleon × Ruby seedless; 348), and 96-71-7 (Ruby seedless × Flame seedless; 126). All female parents with the exception of 96-71-7 are seeded. These hybrids were developed and maintained at IMIDA (Murcia).

### DNA extraction, genotyping, and haplotype determination

Genomic DNA was extracted from young leaf tissue, collected and stored at −80°C, using the BioSprint 96 DNA Plant Kit (QIAGEN). All samples were genotyped for three nuclear microsatellite loci: VMC7F2 (Pellerone et al. 2001), VVIN16 (Merdinoglu et al. 2005), and UDV-108 (Di Gaspero et al. 2005). These markers were selected based on their positions around the *SDI* locus (Fig. 1) located on chromosome 18 (Cabezas et al. 2006; Costantini et al. 2008; Mejía et al. 2007, http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/). Recently, Mejía et al. (2011) have identified additional microsatellite markers closely linked to *SDI*, but this information was not available when the study was carried out, 5 years ago. Simultaneous genotyping for the three loci was performed using a multiplex PCR protocol with forward primers labeled with fluorochromes (6-FAM, VIC, and NED for VMC7F2, VVIN16, and UDV-108, respectively) and ABI-Prism 3130 sequencer (Applied Biosystems). PCR amplifications were carried out in a total volume of 20 μl with 0.2 mM dNTPs, 0.5 μM of each VVIN16 and UDV-108 primers and 0.25 μM




| Haplotype name | S1 | S2 | S1r | S2r | MA1 | MA2 | MA1r | MA2r |
|---|---|---|---|---|---|---|---|---|
| Allele size (bp) | **157** | 153 | **157** | 153 | 149 | 151 | 149 | 149 |
| Allele size (bp) | **198** | 200 | **198** | 200 | 198 | 205 | 198 | 198 |
| Allele size (bp) | **212** | 200 | 200 | **212** | 242 | 236 | 236 | 236 |
| Phenotype | seedless | seedless | seedless | seeded | seeded | seeded | seeded | seeded |
| *Number of accessions* | 17 | 1 | 2 | 31 | 14 | 35 | 9 | 8 |
| *% seedless* | 100% | 100% | 100% | 0% | 14% | 3% | 11% | 0% |

**Fig. 1** Sultanina and Muscat of Alexandria haplotypes for three markers around the *SDI* region, on the long arm of chromosome 18. The *left* part of the figure shows marker positions in base pairs, according to the 12× genomic sequence of *Vitis* (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/); the 1-LOD confidence interval for the seedlessness main effect QTL (*SDI*), according to Cabezas et al. 2006; and the genetic distance between the studied loci in centimorgans (cM), estimated using the 96-71-11 × Crimson Seedless progeny. The *right* part of the figure shows Sultanina (S1 and S2) and Muscat of Alexandria (MA1 and MA2) haplotypes, as well as putative recombinants (S1r, S2r, MA1r, and MA2r). For each one is shown the effect of the linked *SDI* allele (*Phenotype*), the *Number of accessions* in which the haplotype was found studying the collection of cultivars, and the percentage of them that are seedless (*% seedlessness*)

of VMC7F2 primers, 1× BSA buffer, 1.5 mM $MgCl_2$, 1× Taq Gold buffer, 0.05 U of Taq Gold DNA polymerase (Applied Biosystems), and 20 ng of DNA. PCR was performed with the following parameters: 95°C for 10 min; 10 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, in which the annealing temperature was decreasing 0.5°C/cycle; 15 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 30s; and then a final extension step of 72°C for 30 min. Allele binning was carried out using GeneMapper v3.1 software (Applied Biosystems).

The allelic and genetic frequencies at each locus and the frequencies of null alleles were estimated from the genotypes obtained for the 311 cultivars that were included in the diversity analysis using the software Identity (Wagner and Sefc 1999). The haplotypes of the 311 cultivars were estimated using the software PHASE v2.1 (Stephens et al. 2001) complemented with pedigree information when available (Ibáñez et al. 2009; Vargas et al. 2009; *Vitis* International Variety Catalogue, VIVC, http://www.vivc.de/index.php). Haplotype frequencies and their goodness of fit to an approximate coalescent model were compared across five different runs. Results showing the highest average value for the goodness of fit were selected. The observed allelic and phenotypic segregations of each breeding population were tested for goodness of fit to the expected ratios with chi-square ($\chi^2$) tests. The haplotypes of progenitors and hybrids were inferred from linkage analysis.

Genetic distances were estimated using as mapping population the largest progeny (96-71-11 × Crimson Seedless; 348 hybrids), the software Joinmap v3.0 (Van Ooijen and Voorrips 2001) with the CP population type, the regression mapping algorithm, and the Kosambi mapping function.

### Association analyses

All plants were tested for seedlessness and scored as seeded (completely developed or noticeable seeds) and seedless (with no seeds or only seed traces). Association between seedlessness and the presence of each allele, genotype, and haplotype was evaluated with chi-square ($\chi^2$) tests using 2× 2 contingency tables, with a significance threshold of $P$ <0.01 and the correction of Yates when the frequency of at least one of the classes was lower than 5%.

## Results and discussion

### Genetic diversity for stenospermocarpic seedlessness in table grape

In order to further study the presence of the 198-bp allele at VMC7F2 in seeded cultivars, we studied a large collection consisting of 311 accessions, mainly table grape and mixed use (table grape and wine production) cultivars and a few wine grapes, in which a large extent of the existing table grape diversity is represented (Online resource 1). This sample was genotyped for VMC7F2 and two flanking microsatellites (VVIN16 and UDV-108) and phenotyped for the presence of seeds. Genotypic analysis of the collection allowed us to identify six alleles at the VVIN16 locus, eight at VMC7F2 and 14 at UDV-108 (Table 1), including a null allele at UDV-108 detected initially in cultivars for which no amplification occurred at this locus (homozygotes for the null allele) and confirmed later by segregation patterns in the progenies. No significant association ($P$<0.01) between seedlessness and alleles at locus UDV-108 was found (Table 1). However, there was a close correlation between seedlessness and the presence of the 157-bp and the 198-bp allele at VVIN16 and VMC7F2 loci, respectively. Nineteen out of the 22 stenospermocarpic cultivars studied, including Sultanina, showed the 198-bp allele at VMC7F2 and a 157-bp allele at VVIN16. The only

**Table 1** Microsatellite allele distributions in relation to seedlessness within the cultivar collection

| | Allele | | Seed | | Association | | | Allele | | Seed | | Association | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | bp | Freq. | − | + | $\chi^2$ | *P* value | | bp | Freq. | − | + | $\chi^2$ | *P* value |
| VVIN16 | 149 | 0.076 | 8 | 39 | 5.56 | 0.0184 | UDV-108 | 186 | 0.002 | 0 | 1 | 2.39 | 0.1220$^{Y}$ |
| | 151 | 0.436 | 11 | 260 | 9.02 | 0.0027$^{Y}$* | | 200 | 0.014 | 3 | 6 | 4.82 | 0.0281$^{Y}$ |
| | 153 | 0.244 | 4 | 148 | 6.90 | 0.0086$^{Y}$ | | 211 | 0.003 | 0 | 2 | 0.78 | 0.3761$^{Y}$ |
| | 155 | 0.002 | | 1 | 2.39 | 0.1220$^{Y}$ | | **212** | 0.238 | 13 | 135 | 0.15 | 0.6988* |
| | **157** | 0.114 | 22 | 49 | 56.96 | 0.0000* | | 226 | 0.003 | 0 | 2 | 0.78 | 0.3761$^{Y}$ |
| | 159 | 0.129 | 7 | 73 | 0.07 | 0.7987 | | 230 | 0.019 | 0 | 12 | 0.25 | 0.6195$^{Y}$ |
| VMC7F2 | 194 | 0.002 | 0 | 1 | 2.39 | 0.1220$^{Y}$ | | 232 | 0.048 | 3 | 27 | 0.00 | 0.9492$^{Y}$ |
| | 196 | 0.003 | 0 | 2 | 0.78 | 0.3761$^{Y}$ | | 234 | 0.005 | 0 | 3 | 0.30 | 0.5080$^{Y}$ |
| | **198** | 0.138 | 26 | 60 | 62.84 | 0.0000$^{Y}$* | | 236 | 0.175 | 10 | 99 | 0.23 | 0.6281 |
| | 200 | 0.550 | 20 | 322 | 4.65 | 0.0311 | | 238 | 0.127 | 3 | 76 | 1.57 | 0.2095 |
| | 202 | 0.047 | 1 | 28 | 0.33 | 0.5629$^{Y}$ | | 242 | 0.061 | 4 | 34 | 0.34 | 0.5585 |
| | 204 | 0.133 | 1 | 82 | 4.99 | 0.0256$^{Y}$ | | 246 | 0.002 | 0 | 1 | 2.39 | 0.1220$^{Y}$ |
| | 206 | 0.124 | 4 | 73 | 0.95 | 0.3302 | | 250 | 0.011 | 0 | 7 | 0.01 | 0.9312$^{Y}$ |
| | 210 | 0.003 | 0 | 2 | 0.78 | 0.3761$^{Y}$ | | Null | 0.124 | | | | |

For every allele (bp) the table shows the frequency, the number of seedless (−) and seeded (+) cultivars in which it was detected, and the chi-square ($\chi^2$) and significance (*P*) values obtained for association between the presence of the allele and the absence of seeds ($^{Y}$ means data adjusted with the correction of Yates). Microsatellite alleles linked to the seedless *SDI* allele in cultivar Sultanina are indicated in bold

*Significant association ($P<0.01$)

stenospermocarpic cultivars not carrying these alleles are the somatic mutant Chasselas apyrene and the cultivars of natural origin Bayad and Seleccion Bruni 1 (Branas and Truel 1965). In spite of being significantly associated with seedlessness, the 198-bp allele at VMC7F2 was also present in 61 seeded cultivars, 21 of them with Muscat flavor (Online resource 1). In the same way, the 157-bp allele at VVIN16 was present in 52 seeded cultivars, nine of them having Muscat flavor. The significant associations observed for the 151-bp and 153-bp alleles at VVIN16 (Table 1) were not due to linkage between these alleles and stenospermocarpic seedlessness, but likely the result of a spurious genotypic effect: All four seedless accessions carrying the 153-bp allele also carry the 157-bp allele and ten out of the 11 with the 151-bp allele also carry the 157-bp allele.

When considering genotypes, the existence of a null allele at UDV-108 prevented the distinction between homozygous and heterozygous genotypes carrying the null allele at this locus. Under this consideration, alleles were distributed in 16, 19, and 37 genotypic combinations for VVIN16, VMC7F2, and UDV-108, respectively (Table 2, Online resource 1). Significant associations with seedlessness were found only for genotypes containing the 157-bp and the 198-bp allele at VVIN16 and VMC7F2, respectively (Table 2). With the exception of the three accessions of natural origin mentioned above, all stenospermocarpic cultivars analyzed carried the 157-bp and 198-bp alleles at these loci. However, many seeded cultivars also carried these alleles.

To identify the putative haplotype of origin of the mutation responsible for Sultanina seedlessness, we investigated the existing haplotypes around the *SDI* locus on chromosome 18 (Fig. 1) (Cabezas et al. 2006; Costantini et al. 2008; Mejía et al. 2007, 2011). Linkage analyses developed in different crosses (Cabezas et al. 2006 and the eight breeding progenies analyzed in this study) and pedigree information (Ibáñez et al. 2009; Vargas et al. 2009; *Vitis* International Variety Catalogue, http://www.vivc.de/index.php) allowed us to infer both Sultanina haplotypes for this region (Fig. 1). The Sultanina haplotype linked in coupling phase with seedlessness (coded as S1) carries the 157-bp, 198-bp, and 212-bp alleles at VVIN16, VMC7F2, and UDV-108, respectively, whereas the haplotype in repulsion phase with seedlessness (S2) carries the 153-bp, 200-bp, and 200-bp alleles at these loci. On the other hand, the reconstruction of the haplotypes of the 311 cultivars included in the table grape collection using the software PHASE v2.1 (Stephens et al. 2001) allowed the identification of 84 haplotypes in this region (Table 3, Online resource 1), with the 198-bp allele of locus VMC7F2 being present in 12 of them (121 and 21 considering null alleles). The high number of haplotypes found is likely related to the difficulty to establish the correct phase between UDV-108 alleles and VVIN16 and VMC7F2 alleles because of the presence of null alleles at UDV-108. In fact, most of the corrections introduced when using the pedigree information to complement the haplotype assignation involved this locus. With that in

**Table 2** Microsatellite genotype distributions in relation to seedlessness in the cultivar collection

| | Genotype | | Seed | | Association | | | Genotype | | Seed | | Association | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Alleles | Freq. | − | + | $\chi^2$ | *P* value | | Alleles | Freq. | − | + | $\chi^2$ | *P* value |
| VVIN16 | 149:149 | 0.010 | 1 | 2 | 0.27 | $0.6014^{Y}$ | UDV-108 | 186:**212** | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | 149:151 | 0.058 | 2 | 16 | 0.19 | 0.6640 | | 200:200 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | 149:153 | 0.042 | 1 | 12 | 0.18 | $0.6723^{Y}$ | | 200:**212** | 0.013 | 1 | 3 | 0.09 | $0.7634^{Y}$ |
| | 149:**157** | 0.023 | 3 | 4 | 6.99 | $0.0082^{Y}$* | | 200:232 | 0.003 | 1 | 0 | 2.27 | $0.1319^{Y}$ |
| | 149:159 | 0.010 | 0 | 3 | 0.27 | $0.6014^{Y}$ | | 200:238 | 0.006 | 0 | 2 | 0.73 | $0.3937^{Y}$ |
| | 151:151 | 0.183 | 0 | 57 | 5.10 | $0.0239^{Y}$ | | 200:242 | 0.003 | 1 | 0 | 2.27 | $0.1319^{Y}$ |
| | 151:153 | 0.225 | 1 | 69 | 4.56 | $0.0328^{Y}$ | | 211:232 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | 151:**157** | 0.100 | 7 | 24 | 9.09 | $0.0075^{Y}$* | | 211:236 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | 151:159 | 0.125 | 1 | 38 | 1.19 | $0.2761^{Y}$ | | **212:212** | 0.225 | 8 | 62 | 1.11 | 0.2921 |
| | 153:153 | 0.074 | 0 | 23 | 1.24 | $0.2653^{Y}$ | | **212**:230 | 0.016 | 0 | 5 | 0.02 | $0.8938^{Y}$ |
| | 153:**157** | 0.039 | 2 | 10 | 0.28 | $0.5972^{Y}$ | | **212**:232 | 0.023 | 2 | 5 | 1.60 | $0.2064^{Y}$ |
| | 153:159 | 0.035 | 0 | 11 | 0.22 | $0.6416^{Y}$ | | **212**:234 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | 155:159 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ | | **212**:236 | 0.125 | 5 | 34 | 1.16 | 0.2819 |
| | **157:157** | 0.016 | 3 | 2 | 11.50 | $0.0007^{Y}$* | | **212**:238 | 0.096 | 2 | 28 | 0.12 | 0.7244 |
| | **157**:159 | 0.035 | 4 | 7 | 8.19 | $0.0042^{Y}$* | | **212**:242 | 0.051 | 1 | 15 | 0.02 | $0.8803^{Y}$ |
| | 159:159 | 0.023 | 1 | 6 | 0.01 | $0.9063^{Y}$ | | **212**:250 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | | | | | | | | 226:236 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | | | | | | | | 226:250 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| VMC7F2 | 194:200 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ | | 230:230 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | 196:200 | 0.006 | 0 | 2 | 0.73 | $0.3937^{Y}$ | | 230:234 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | **198:198** | 0.039 | 5 | 7 | 13.83 | $0.0002^{Y}$* | | 230:236 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | **198**:200 | 0.135 | 13 | 29 | 29.03 | $0.0000^{Y}$* | | 230:238 | 0.006 | 0 | 2 | 0.73 | $0.3937^{Y}$ |
| | **198**:202 | 0.010 | 1 | 2 | 0.27 | $0.6014^{Y}$ | | 230:242 | 0.006 | 0 | 2 | 0.73 | $0.3937^{Y}$ |
| | **198**:204 | 0.039 | 0 | 12 | 0.29 | $0.5925^{Y}$ | | 232:232 | 0.023 | 0 | 7 | 0.01 | $0.9063^{Y}$ |
| | **198**:206 | 0.019 | 2 | 4 | 2.10 | $0.1370^{Y}$ | | 232:236 | 0.032 | 0 | 10 | 0.15 | $0.6964^{Y}$ |
| | 200:200 | 0.302 | 2 | 92 | 5.71 | $0.0168^{Y}$ | | 232:238 | 0.013 | 0 | 4 | 0.09 | $0.7634^{Y}$ |
| | 200:202 | 0.029 | 0 | 9 | 0.10 | $0.7577^{Y}$ | | 232:242 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | 200:204 | 0.145 | 1 | 44 | 1.74 | $0.1877^{Y}$ | | 236:236 | 0.106 | 2 | 31 | 0.25 | 0.6137 |
| | 200:206 | 0.167 | 2 | 50 | 1.03 | $0.3105^{Y}$ | | 236:238 | 0.042 | 1 | 12 | 0.02 | $0.6723^{Y}$ |
| | 200:210 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ | | 236:242 | 0.029 | 2 | 7 | 0.83 | $0.3609^{Y}$ |
| | 202:202 | 0.010 | 0 | 3 | 0.27 | $0.6014^{Y}$ | | 238:238 | 0.068 | 0 | 21 | 1.05 | $0.3053^{Y}$ |
| | 202:204 | 0.019 | 0 | 6 | 0.00 | $0.9981^{Y}$ | | 238:242 | 0.013 | 0 | 4 | 0.09 | $0.7634^{Y}$ |
| | 202:206 | 0.016 | 0 | 5 | 0.02 | $0.8938^{Y}$ | | 238:246 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ |
| | 202:210 | 0.003 | 0 | 1 | 2.27 | $0.1319^{Y}$ | | 238:250 | 0.010 | 0 | 3 | 0.27 | $0.6014^{Y}$ |
| | 204:204 | 0.019 | 0 | 6 | 0.00 | $0.9981^{Y}$ | | 242:242 | 0.016 | 0 | 5 | 0.02 | $0.8938^{Y}$ |
| | 204:206 | 0.026 | 0 | 8 | 0.05 | $0.8271^{Y}$ | | 250:250 | 0.006 | 0 | 2 | 0.73 | $0.3937^{Y}$ |
| | 206:206 | 0.010 | 0 | 3 | 0.27 | $0.6014^{Y}$ | | Null:null | 0.026 | 0 | 8 | 0.05 | $0.8271^{Y}$ |

For every genotype the table shows the frequency, the number of seedless (−) and seeded (+) cultivars in which it was detected, and the chi-square ($\chi^2$) and significance (*P*) values obtained for association between the presence of the allele and the absence of seeds ($^{Y}$ means data adjusted with the correction of Yates). Microsatellite alleles linked to the seedless allele at *SDI* locus in cultivar Sultanina are shown in bold

*Significant association ($P<0.01$)

mind, haplotype frequencies follow a decreasing exponential distribution, ranging between 0.001 and 0.094 with an average frequency of 0.008 and with 24% of the haplotypes being present in only one cultivar. Only two haplotypes show significant associations with seedlessness ($P<0.01$) (Table 3). Seventeen stenospermocarpic accessions carry the Sultanina haplotype linked in phase with seedlessness (S1). Two other (Basile logothetis and Beauty seedless) carry the haplotype S1r (Fig. 1), which is likely derived from independent recombination events between both

**Table 3** Association analysis between haplotypes and seedlessness in the cultivar collection

| Haplotype | | | Seed | | Association | | Haplotype | | | Seed | | Association | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alleles | Code | Freq. | − | + | $\chi^2$ | *P* value | Haplotype | Code | Freq. | − | + | $\chi^2$ | *P* value |
| 149-198-236 | MA1R | 0.015 | 2 | 7 | 0.82 | $0.3645^{Y}$ | 153-200-212 | S2r | 0.051 | 0 | 31 | 1.94 | $0.1638^{Y}$ |
| 149-198-238 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 153-200-212/null | | 0.034 | 0 | 21 | 1.01 | $0.3139^{Y}$ |
| 149-198-242 | MA1 | 0.023 | 3 | 11 | 1.69 | $0.1941^{Y}$ | 153-200-230 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 149-198-null | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 153-200-232/null | | 0.005 | 0 | 3 | 0.27 | $0.6023^{Y}$ |
| 149-200-212 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 153-200-236 | | 0.005 | 0 | 3 | 0.27 | $0.6023^{Y}$ |
| 149-200-230 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 153-200-236/null | | 0.015 | 1 | 8 | 0.09 | $0.7595^{Y}$ |
| 149-200-236 | | 0.020 | 2 | 10 | 0.27 | $0.6008^{Y}$ | 153-200-238 | | 0.011 | 0 | 7 | 0.01 | $0.9068^{Y}$ |
| 149-206-236 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 153-200-238/null | | 0.010 | 1 | 5 | 0.00 | $0.9981^{Y}$ |
| 149-210-234 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 153-200-242/null | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ |
| 151-196-236 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 153-200-250/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-198-212 | | 0.007 | 0 | 4 | 0.09 | $0.7641^{Y}$ | 153-200-null | | 0.008 | 0 | 5 | 0.02 | $0.0894^{Y}$ |
| 151-198-236 | | 0.005 | 0 | 3 | 0.27 | $0.6023^{Y}$ | 153-202-212 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ |
| 151-198-236/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 153-202-212/null | | 0.002 | 1 | 0 | 2.27 | $0.1322^{Y}$ |
| 151-198-242 | | 0.003 | 1 | 1 | 0.73 | $0.3945^{Y}$ | 153-202-230 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ |
| 151-198-242/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 153-202-232/null | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ |
| 151-200-200 | | 0.007 | 0 | 4 | 0.09 | $0.7641^{Y}$ | 153-202-236 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-200-212 | | 0.031 | 1 | 18 | 0.01 | $0.9407^{Y}$ | 153-202-236/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-200-212/null | | 0.043 | 2 | 24 | 0.06 | $0.8133^{Y}$ | 153-204-210 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-200-230 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 153-204-212 | | 0.008 | 0 | 5 | 0.02 | $0.0894^{Y}$ |
| 151-200-232 | | 0.020 | 2 | 10 | 0.27 | $0.6008^{Y}$ | 153-204-212/null | | 0.005 | 0 | 3 | 0.27 | $0.6023^{Y}$ |
| 151-200-232/null | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 153-204-232 | | 0.005 | 0 | 3 | 0.27 | $0.6023^{Y}$ |
| 151-200-236 | | 0.038 | 1 | 22 | 0.11 | $0.7455^{Y}$ | 153-204-234 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-200-236/null | | 0.015 | 0 | 9 | 0.09 | $0.7595^{Y}$ | 153-204-236 | | 0.013 | 0 | 8 | 0.05 | $0.8282^{Y}$ |
| 151-200-238 | | 0.029 | 0 | 18 | 0.75 | $0.3852^{Y}$ | 153-204-236/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-200-238/null | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 153-204-238 | | 0.008 | 0 | 5 | 0.02 | $0.0894^{Y}$ |
| 151-200-242 | | 0.005 | 0 | 3 | 0.27 | $0.6023^{Y}$ | 153-204-238/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-200-242/null | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 153-204-null | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ |
| 151-200-null | | 0.008 | 0 | 5 | 0.02 | $0.0894^{Y}$ | 153-206-212 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ |
| 151-202-232 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 155-200-238 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-202-236 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | **157-198-200** | **S1r** | **0.003** | **2** | **0** | **11.63** | $\mathbf{0.0006^{Y}}$***** |
| 151-202-236/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | **157-198-212** | **S1** | **0.018** | **17** | **0** | **179.48** | $\mathbf{0.0000^{Y}}$***** |
| 151-202-238 | | 0.007 | 0 | 4 | 0.09 | $0.7641^{Y}$ | 157-198-238 | | 0.005 | 1 | 2 | 0.27 | $0.6023^{Y}$ |
| 151-202-238/null | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 157-198-238/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-202-null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 157-200-186 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-204-212 | | 0.005 | 0 | 3 | 0.27 | $0.6023^{Y}$ | 157-200-210 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-204-212/null | | 0.007 | 0 | 4 | 0.09 | $0.7641^{Y}$ | 157-200-212 | | 0.054 | 0 | 33 | 2.13 | $0.1443^{Y}$ |
| 151-204-232 | | 0.021 | 0 | 13 | 0.35 | $0.5523^{Y}$ | 157-200-212/null | | 0.005 | 1 | 2 | 0.27 | $0.6023^{Y}$ |
| 151-204-236/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 157-200-226 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-204-238 | | 0.046 | 2 | 26 | 0.01 | $0.9115^{Y}$ | 157-200-230 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ |
| 151-204-238/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 157-200-232 | | 0.002 | 1 | 0 | 2.27 | $0.1322^{Y}$ |
| 151-204-250 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 157-206-236 | | 0.005 | 0 | 3 | 0.27 | $0.6023^{Y}$ |
| 151-204-250/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 157-200-238/null | | 0.005 | 0 | 3 | 0.27 | $0.6023^{Y}$ |
| 151-204-null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 159-194-246 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-206-212 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 159-196-212/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-206-212/null | | 0.013 | 0 | 8 | 0.05 | $0.8282^{Y}$ | 159-198-212/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-206-226 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 159-198-236/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 151-206-236/null | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ | 159-198-242 | | 0.005 | 0 | 3 | 0.27 | $0.6023^{Y}$ |

**Table 3** (continued)

| Haplotype | | | Seed | | Association | | Haplotype | | | Seed | | Association | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alleles | Code | Freq. | − | + | $\chi^2$ | *P* value | Haplotype | Code | Freq. | − | + | $\chi^2$ | *P* value |
| 151-206-230 | | 0.007 | 0 | 4 | 0.09 | $0.7641^{Y}$ | 159-200-212 | | 0.046 | 1 | 27 | 0.35 | $0.5569^{Y}$ |
| 151-206-236 | MA2 | 0.061 | 3 | 34 | 0.06 | $0.8033^{Y}$ | 159-200-212/null | | 0.029 | 4 | 14 | 2.97 | $0.0847^{Y}$ |
| 151-206-238 | | 0.007 | 0 | 4 | 0.09 | $0.7641^{Y}$ | 159-200-236 | | 0.005 | 1 | 2 | 0.27 | $0.6023^{Y}$ |
| 151-206-238/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 159-200-236/null | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ |
| 151-206-242 | MA2r | 0.013 | 0 | 8 | 0.05 | $0.8282^{Y}$ | 159-200-238 | | 0.011 | 1 | 6 | 0.01 | $0.9068^{Y}$ |
| 151-206-null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 159-200-238/null | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ |
| 151-210-234 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 159-200-242 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ |
| 153-198-212 | | 0.010 | 0 | 6 | 0.00 | $0.9981^{Y}$ | 159-200-null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 153-198-212/null | | 0.007 | 0 | 4 | 0.09 | $0.7641^{Y}$ | 159-202-200 | | 0.003 | 0 | 2 | 0.73 | $0.3945^{Y}$ |
| 153-198-236/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | 159-202-236/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 153-198-238/null | | 0.005 | 0 | 3 | 0.27 | $0.6023^{Y}$ | 159-202-238 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 153-198-242 | | 0.010 | 0 | 6 | 0.00 | $0.9981^{Y}$ | 159-202-250 | | 0.008 | 0 | 5 | 0.02 | $0.0894^{Y}$ |
| 153-200-200 | S2 | 0.002 | 1 | 0 | 2.27 | $0.1322^{Y}$ | 159-206-236 | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ |
| 153-200-200/null | | 0.002 | 0 | 1 | 2.27 | $0.1322^{Y}$ | | | | | | | |

For every haplotype the table shows the frequency, the number of seedless (−) and seeded (+) cultivars in which it was detected, and the chi-square ($\chi^2$) and significance (*P*) values obtained for association between the presence of the allele and the absence of seeds ($^{Y}$ means data adjusted with the correction of Yates). Sultanina and Muscat of Alexandria haplotypes, as well as putative recombinant haplotypes related with them, have a name assigned (code). Sultanina haplotype in coupling with the seedless allele at *SDI* locus is shown in bold

*Significant association ($P<0.01$)

Sultanina haplotypes since the genetic profiles of these two cultivars for 20 microsatellite loci rule out a parent–offspring relationship with Sultanina (Ibáñez et al. 2009). The 9.25% of recombination rate between VVIN16 and UDV-108 that these two accessions represents is lower than the 15.23% of frequency of recombination estimated using the mapping population Therefore, it should be expected to find a higher number of cultivars carrying recombinant Sultanina-related haplotypes. This discrepancy could be related with the different sample sizes (21 Sultanina-derived accessions in the diversity analysis versus 348 hybrids in the mapping population). Finally, the three seedless accessions of natural origin with no relationship to Sultanina haplotypes do not share any haplotypes among them. Unlike the 198-bp allele at VMC7F2, neither S1 nor S1r haplotypes were found in any seeded or parthenocarpic cultivar. The presence of the 198-bp allele in 61 seeded cultivars could be related with the use of Muscat of Alexandria or related cultivars to provide Muscat flavor in table grape breeding since more than half of these accessions (32 cultivars) show Muscat flavors and/or haplotypes that could be related with those of Muscat of Alexandria (Online resource 1).

The studied material represents a large extent of table grape existing diversity, but there are many table grape cultivars not included in this study. That does not imply major bias in the results. Similar results can be expected increasing the number of bred cultivars since Sultanina has been the only source of stenospermocarpic seedlessness in table grape breeding. Within the studied sample, three out of the six stenospermocarpic seedless accessions with unknown relationship to Sultanina also carry S1 or S1r haplotypes, which suggests a family relationship with this cultivar. In fact, further analyses of 20 additional microsatellite loci agreed with this hypothesis (Ibáñez et al. 2009; Vargas et al. 2009). The only stenospermocarpic accessions not carrying Sultanina-related haplotypes linked with seedlessness (S1 or S1r) are very likely independent natural variants: Chasselas apyrene, a somatic mutant; Bayad, an accession native from Yemen (Branas and Truel 1965); and Seleccion Bruni 1, selected in an Italian vineyard with a mixture of varieties (Branas and Truel 1965); and probably many others could be found in other germplasm banks among the maintained local or ancient material. In these cultivars, the phenotype could result from independent mutations on the *SDI* locus, or in different loci giving rise to a similar seedless phenotype. Genetic and phenotypic characterization of these additional sources of genetic variation could be interesting for future table grape breeding.

## Marker assisted selection for stenospermocarpic seedlessness

To date, Sultanina has been the unique source of stenospermocarpic seedlessness in table grape breeding. All seedless

cultivars with known pedigrees derive from this cultivar (Ibáñez et al. 2009; Vargas et al. 2009; the *Vitis* international variety catalogue, VIVC, http://www.vivc.de/index.php), which has been confirmed by means of haplotype analysis in this study. QTL and diversity analyses indicate that VMC7F2 is a potential candidate for marker assisted selection of Sultanina-derived seedlessness. However, the presence of the 198-bp allele in seeded cultivars precludes the detection of the seedless accessions in a varietal collection by using only this locus. For germplasm sets that include unrelated materials or have unknown kinship relationships, the estimation of allelic phases using haplotypes rather than genotypes is required. This can be achieved by the additional genotyping of one or a few linked microsatellite loci. However, in a table grape breeding program, the progenitors of each plant are known and the origin of each allele can be deduced from the genotypes of the progenitors. In most cases, the genotyping of the VMC7F2 locus is enough to identify the seedless genotypes, assuming identity by descent. Genotyping of additional loci could only be needed in specific crosses, such as those involving progenitors with the 198-bp allele in homozygosis at VMC7F2 or with null alleles at this locus.

In order to assess the real usefulness of VMC7F2 for MAS of the seedless genotypes in table grape breeding programs, we evaluated 1,012 progeny individuals derived from eight different crosses developed within the framework of a breeding program (Table 4). Seedlessness was provided by Crimson Seedless in all progenies, although in the cross 96-71-7×Crimson Seedless the female progenitor was also seedless. Seedlessness segregations fit the expected 1:1 ratio in all SDxSL crosses (*P* values ranging from 0.178 to 0.894) and 3:1 in the only SLxSL cross ($P=0.918$). The 198-bp allele at VMC7F2 showed similar segregation ratios (*P* values between 0.110 and 0.758 for the SDxSL crosses and 0.758 for the SLxSL cross) and was associated with seedlessness in all crosses ($P<0.005$), with 4.4% false positives (seeded individuals carrying the 198-bp allele) and 2.4% false negatives (seedless individuals not carrying the allele, Table 4). With these data, the utilization of MAS for the selection of seedless genotypes based on the presence of the 198-bp allele at VMC7F2 would have led to classifying as seeded and discarding 46% of individuals initially obtained. Among these 465 hybrids, 5.2% (2.4% of the total) would be seedless plants that would have been mistakenly discarded (false negatives). On the other hand, 8.2% of the 547 plants that would have been maintained since their genotypic classification as seedless would be seeded (false positives). Similar results (5% of false positives) were obtained by Mejía et al. (2011) when using this marker to study 146 hybrids derived from 14 crosses between 11 seedless cultivars. To summarize, if MAS based on VMC7F2 genotyping had been used in this breeding program, 4.6% less seedless individuals would have been obtained (from 526 to 502). However, instead of maintaining and evaluating 1,012 plants over many years, of which only the 52% would be seedless, they should have maintained 547 plants, of which 92% would be seedless.

The output of MAS for seedlessness based on VMC7F2, a gene specific marker located in the promoter region of the candidate gene proposed for SDI locus (Mejía et al. 2011) could represent an important improvement over traditional breeding, but also over most of MAS approaches for stenospermocarpic seedlessness proposed to date. For example, the use of the marker SCC8, located at 1 Mbp from VMC7F2, led to 22% false negatives in the SLxSL cross MtpDMV2 and 42% false positives in the SDxSL cross Mto3039 (Adam-Blondon et al. 2001). In any case, the presence of null alleles for this locus in many genetic backgrounds hampered its utilization in crosses involving these cultivars. Null alleles for VMC7F2 have not been observed in the segregating progenies analyzed in this study or in five additional QTL mapping populations (Cabezas et al. 2006; Costantini et al. 2008; Mejía et al. 2007; L. Ruiz-García, personal communication). Even if null alleles at VMC7F2 were found in larger germplasm collections, its estimated frequency is expected to be very low: based on the data of 311 cultivars analyzed in this study, the frequency of null alleles at VMC7F2 is 0.018, which is similar to the estimated for VVIN16 (0.013) and much lower than that of UDV-108 (0.124). Recently, Mejía et al. (2011) identified 11 additional microsatellite markers in the region around *SDI*, confirming VMC7F2 as one of the best markers for progeny screening, together with p3_VvAGL11, which is located only 270 bp further away. MAS of homozygous genotypes yielded the best results when using the marker p3_VvAGL11 since they did not find any false positives among 146 hybrids derived from 14 crosses, whereas they found 5% when using VMC7F2. Nevertheless, the usefulness of p3_VvAGL11 still needs to be tested in additional genetic backgrounds and especially in SDxSL crosses, in which the seedless individuals are heterozygous.

For other crosses in which VMC7F2 was not informative enough, because of the presence of both 198-bp alleles, the linked and the not linked with seedlessness (for example, in the crosses of Sultanina × Muscat Saint Laurent and Maria Pirovano × any other accession), or putative existing null alleles, a suitable strategy is the use of a multiplex PCR genotyping protocol such as the one used in this study. The existence of null alleles at UDV-108 significantly decreases the yield and discourages the use of this marker. However, the 157-bp allele at VVIN16 and the haplotype information based on the three microsatellite loci can be used. Their use decreases the number of selected individuals from 54% with VMC7F2 to 51% with VVIN16 and to 45% when using the whole haplotype. Even when the percentage of seedless

**Table 4** Marker assisted selection for seedlessness based on VMC7F2 genotyping in a proof of concept table grape breeding program

| Cross | | Seedlessness | | VMC7F2 genotyping | | | | | VMC7F2 based marker assisted selection | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 198 bp | | Significance | | To keep | SL | False positives | False negatives |
| Progenitors | VMC7F2 | Seed | *N* (%) | 198:198 | 198:200 | 200:200 | 198:204 | 200:204 | + | − | $\chi^2$ | *P* value | | | | |
| 94-45-7× Crimson S. | 200:200×198:200 | − | 58 (51%) | | 53 | 5 | | | 53 | 5 | 55.9 | <0.0001 | 65 (58%) | 53 (82%) | 12 (10.6%) | 5 (4.4%) |
| | | + | 55 (49%) | | 12 | 43 | | | 12 | 43 | | | | | | |
| | | Total | 113 | | 65 | 48 | | | 65 | 48 | | | | | | |
| 96-55-19× Crimson S. | 200:204×198:200 | − | 37 (45%) | | 24 | 1 | 11 | 1 | 35 | 2 | 51.4$^{Y}$ | <0.0001$^{Y}$ | 41 (49%) | 35 (85%) | 6 (7.2%) | 2 (2.4%) |
| | | + | 46 (55%) | | 1 | 21 | 5 | 19 | 6 | 40 | | | | | | |
| | | Total | 83 | | 25 | 22 | 16 | 20 | 41 | 42 | | | | | | |
| 96-71-11× Crimson S. | 200:200×198:200 | − | 176 (51%) | | 170 | 6 | | | 170 | 6 | 283.4$^{Y}$ | <0.0001$^{Y}$ | 180 (52%) | 170 (94%) | 10 (2.9%) | 6 (1.7%) |
| | | + | 172 (49%) | | 10 | 162 | | | 10 | 162 | | | | | | |
| | | Total | 348 | | 180 | 168 | | | 180 | 168 | | | | | | |
| Dominga × Crimson S. | 200:204×198:200 | − | 24 (46%) | | 10 | 3 | 10 | 1 | 20 | 4 | 30.7 | <0.0001 | 22 (42%) | 20 (91%) | 2 (3.8%) | 4 (7.7%) |
| | | + | 28 (54%) | | 0 | 11 | 2 | 15 | 2 | 26 | | | | | | |
| | | Total | 52 | | 10 | 14 | 12 | 16 | 22 | 30 | | | | | | |
| Napoleón × Crimson S. | 200:204×198:200 | − | 10 (37%) | | 6 | 0 | 4 | | 10 | 0 | 16.4$^{Y}$ | 0.0001$^{Y}$ | 12 (44%) | 10 (83%) | 2 (7.4%) | 0 (0%) |
| | | + | 17 (63%) | | 0 | 12 | 2 | 3 | 2 | 15 | | | | | | |
| | | Total | 27 | | 6 | 12 | 6 | 3 | 12 | 15 | | | | | | |
| Ohanez × Crimson S. | 200:204×198:200 | − | 15 (38%) | | 6 | 0 | 9 | | 15 | 0 | 22.43$^{Y}$ | <0.0001$^{Y}$ | 19 (49%) | 15 (79%) | 4 (10.3%) | 0 (0%) |
| | | + | 24 (62%) | | 2 | 9 | 2 | 11 | 4 | 20 | | | | | | |
| | | Total | 39 | | 8 | 9 | 11 | 11 | 19 | 20 | | | | | | |
| Red Globe × Crimson S. | 200:200×198:200 | − | 111 (50%) | | 106 | 5 | | | 106 | 5 | 178.6$^{Y}$ | <0.0001$^{Y}$ | 112 (50%) | 106 (95%) | 6 (2.7%) | 5 (2.2%) |
| | | + | 113 (50%) | | 6 | 107 | | | 6 | 107 | | | | | | |
| | | Total | 224 | | 112 | 112 | | | 112 | 112 | | | | | | |
| 96-71-7× Crimson S. | 198:204×198:200 | − | 95 (75%) | 30 | 32 | | 31 | 2 | 93 | 2 | 95.5$^{Y}$ | <0.0001$^{Y}$ | 96 (76%) | 93 (97%) | 3 (2.4%) | 2 (1.6%) |
| | | + | 31 (25%) | 1 | 1 | | 1 | 28 | 3 | 28 | | | | | | |
| | | Total | 126 | 31 | 33 | | 32 | 30 | 96 | 30 | | | | | | |
| Global | | − | 526 (52%) | | | | | | 502 | 24 | 751.9$^{Y}$ | <0.0005$^{Y}$ | 547 (54%) | 502 (92%) | 45 (4.4%) | 24 (2.4%) |
| | | + | 486 (48%) | | | | | | 45 | 441 | | | | | | |
| | | Total | 1,012 | | | | | | 547 | 465 | | | | | | |

For every breeding population the table shows the number and percentage of seeded (SD) and seedless (SL) hybrids analyzed, the genotype of both progenitors at VMC7F2 (cross) and the number of individuals with each genotype in each subset, as well as in which the 198-bp allele was present (+) or not (−). Association analyses between the presence of the allele and seedlessness are shown using chi-square ($\chi^2$) and significance (*P*) values seeds ($^{Y}$ means data adjusted with the correction of Yates). The results of applying MAS strategy based on the detection of the 198-bp allele are indicated with the number and percentages of plants that should be maintained in the field (to keep), the representation of seedless individuals among them (SL), and the number and percentages of false positives and negatives identified

genotypes in the selected hybrids is not significantly different for the three strategies, there is an important decrease in the final number of seedless hybrids selected (502, 468, and 421, respectively) due to the different rates of false negatives. The higher number of false negatives with VVIN16 seems to be caused by the larger distance between the marker and *SDI*. For haplotypes, the increase in false negatives is related with the exclusion of individuals that show recombination events between flanking markers, which prevents the determination of the linked allele at *SDI*. The alternative strategy, maintaining the individuals that show recombination events between flanking markers, might increase the seedless individuals selected by 6% but also the number of individuals to be maintained by 9%. In any case, the results obtained with the multiplex PCR used in this study could be improved with additional highly informative microsatellite loci closely linked to *SDI*, as the markers p3_VvAGL11 (Mejía et al. 2011).

There are three possible reasons for the false positives and false negatives found in the MAS study using VMC7F2. They could be recombinants between the marker and the gene, the result of phenotypic misclassifications, or their phenotype could be caused by a combination of minor effect QTLs. Several minor effect QTLs modifying the action of *SDI* have been identified in different genetic backgrounds on chromosomes 1, 3, 6, 8, 9, 10, 13, 14, 15, and 18 (Cabezas et al. 2006; Costantini et al. 2008; Doligez et al. 2002; Mejía et al. 2007). In this study, we have obtained similar results when using MAS in all Crimson Seedless crosses, which suggests that there was not an important effect from the minor effect QTLs. On the other side, stenospermocarpic seedlessness is a complex trait that can be scored using several descriptors (Ledbetter et al. 1994; Striem et al. 1992). For QTL mapping, the best results have been obtained using precise evaluations, such as the average fresh weight of the seeds collected from between 20 and 150 berries, or classifications based on the degree of development of the embryo and the hardiness of seed coat (Cabezas et al. 2006; Costantini et al. 2008; Doligez et al. 2002; Mejía et al. 2007, 2011). However, these time-consuming evaluations are not suitable in breeding programs involving thousands of hybrids in which seedlessness is evaluated by tasting. When considering the organoleptic point of view, stenospermocarpic seedlessness is influenced by many factors, such as seed hardiness, seed number, and even features of the berry flesh (Ledbetter et al. 1994; Striem et al. 1992). Given the close linkage between the marker and *SDI*, the complexity of trait evaluation and the minor effect of other QTLs on the phenotype, for stenospermocarpic seedlessness the genotypic selection seems to be more precise than phenotypic selection. In fact, when individuals were scored as seeded, seedless but with noticeable seed traces, or seedless, false negatives were much more frequent among the seedless plants with noticeable seed traces (9%) than among the seedless ones (3%). These possible phenotyping mistakes would decrease the recombination fraction between the marker VMC7F2 and *SDI* locus from 6.8% to the values that led Mejía et al. (2011) to propose that Sultanina seedlessness could be caused by variations in *VvAGL11* promoter region.

The different QTL analyses developed up until now have shown that Sultanina-derived seedlessness is regulated mainly by a dominant allele at *SDI*, both in SDxSL crosses [Dominga × Autumn seedless (Cabezas et al. 2006), Italia × Big Perlon (Costantini et al. 2008), and Muscat of Hamburg × Superior seedless and Moscatuel × Ruby seedless (L. Ruiz-García, personal communication)] and in SLxSL crosses [(Dattier of Beyrouth × 75 Pirovano) × (Alphonse Lavallée × Sultanine) (Doligez et al. 2002) and Ruby seedless × Thompson seedless (Mejía et al. 2007, 2011)]. Variations on the partial dominance effect of the seedless allele have been observed depending on the different seeded alleles (Mejía et al. 2011). The similar results obtained when using MAS based on VMC7F2 in all Crimson Seedless crosses suggest that there was not an important effect of the other alleles at *SDI* or from minor effect QTLs. Analogous results were also found in the cross Dominga × Autumn Seedless (data not shown), in which both parents carry alleles that increase and decrease seed content at minor effect QTLs (Cabezas et al. 2006), as well as in crosses between Muscat of Hamburg and Superior Seedless and Moscatuel and Ruby seedless (L. Ruiz-García, personal communication). Our results demonstrate the utility of the proposed strategies for MAS for seedlessness in crosses derived from Crimson Seedless, but also suggest that there should be a widespread use for Sultanina-derived seedlessness in table grape breeding. From the point of view of the breeder, MAS must be more efficient than methods already available, considering investments and returns (Hospital 2009). The utilization of multiplex PCR protocols and high-throughput genotyping assays (such as SNP determination technologies) will make MAS approaches cheaper and will allow the simultaneous selection for many traits, such as berry skin color, Muscat flavor, and disease resistances through genomic selection. This will accelerate the development of new table grape cultivars and will become an important advantage in a very competitive market that is continuously searching for new flavors, colors, and other features, in the context of climate change, and with limitations on the use of pesticides.

## Conclusions

In the last years, there have been many reports associating a trait of interest with molecular markers in grapes. However,

only in a few cases have these markers been applied in actual breeding programs (Dalbó et al. 2001; Eibach et al. 2007; Riaz et al. 2009; Molnár et al. 2007). In this study, we have shown the usefulness of MAS strategies for indirect selection of seedless plants by using microsatellite markers and quantifying the returns in a real table grape breeding program. We have shown that the 198-bp allele at the microsatellite locus VMC7F2 can be used as a marker for stenospermocarpic seedlessness derived from Sultanina. When used for the MAS of seedless genotypes in SDxSL or SLxSL crosses, the outcome was nearly the same number of seedless individuals by maintaining only 54% of the progeny plants initially obtained. However, the 198-bp allele at VMC7F2 is also present in several seeded cultivars, so it cannot be used on its own to predict the seedlessness trait in a collection of cultivars of unknown origin. In this situation, we have shown the usefulness of using haplotype information. By studying a large collection representing most table grape diversity, we found that seedless accessions derived from Sultanina, the source of stenospermocarpic seedlessness in table grape breeding, share a main haplotype for three microsatellite loci—VVIN16, VMC7F2, and UDV-108—in the region of the locus that is regulating this trait. Besides that, we have identified additional sources of genetic variation for this trait.

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