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# Usefulness of two SCAR markers for marker-assisted selection of seedless grapevine cultivars

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## Summary

**A PCR-specific marker, SCP18, was developed from a RAPD marker linked to a major locus involved in seedlessness, *sdI*. A preliminary study of the usefulness of SCP18 and SCC8 (a marker linked to *sdI* previously developed by Lahogue *et al.* 1998) for the marker assisted selection of seedless varieties was realized using various strategies: *a posteriori* test in seedless x seedless and seeded x seeded progenies and test of their allelic diversity in a set of 81 seedless and seeded varieties. In contrast to SCP18, SSC8 was found to be a useful marker at least in the seedless x seedless progenies and to show a good linkage disequilibrium with seedlessness in our set of varieties.**

K e y w o r d s : marker-assisted selection, seedlessness, stenospermocarpic, *Vitis vinifera.*

## Introduction

Since consumers prefer more and more seedless grapes, seedlessness is becoming an important criterium in the selection of new table grape cultivars due to higher prices. Presently, the *V. vinifera* cv. Sultana or Thompson Seedless, accounts for most of the seedless grape production in the world. It has a stenospermocarpic seedlessness which is characterized by the abortion of the seeds soon after fertilization and is thus completely different from the parthenocarpic seedlessness of Black Corinth (Stout 1936). Sultana is also the source of seedlessness in most breeding programs for new seedless cultivars as can be inferred from the genealogy of major seedless cultivars (Ledbetter and Ramming 1989; Branas and Truel 1996).

Since seedlessness is a lately expressed trait, a better understanding of its genetic determinism together with the identification of molecular markers linked to the genes involved would provide tools for more efficient selection, such as marker-assisted selection (MAS) methods. Indeed, molecular markers are powerful for dissection of the inheritance of polygenic traits (Paterson *et al.* 1988, Tanksley 1993). They may be useful to improve the selection of such traits (Stuber 1989, Dudley 1993, Tanksley *et al.* 1993) and in perennial crops, they may help to dramatically decrease the intervals between two selection steps especially if late characters such as fruit characters are concerned (Weeden *et al.* 1994, Crouzillat *et al.* 1996, Grattapaglia *et al.* 1996, Striem *et al.* 1996). In the particular case of seedlessness in grapes, MAS may help to avoid the use of seedless x seedless progenies as a mean to increase the frequency of seedless individuals (Spiegel-Roy *et al.* 1990, Bouquet and Danglot 1996) as such crosses require an *in vitro* embryo rescue step. Many studies have been carried out concerning the genetics of seedlessness (Bouquet and Danglot 1996, Roytchev 1998) and most of them concluded that several genes are involved in its expression. Bouquet and Danglot (1996) proposed a model in which seedlessness might be controlled by three complementary recessive genes independently inherited and regulated by a dominant gene, the *sdI* (**s**eed **d**evelopment **I**nhibitor) gene. RAPD markers genetically linked to the *sdI* gene were searched for by the bulked segregant analysis of a progeny derived from the cross of two Sultana-derived partially seedless genotypes (Lahogue *et al.* 1998). Two RAPD markers (opC08-1020 and opP18-530) were found to be specifically present in seedless genotypes and absent in seeded genotypes and confirmed the existence of a major locus involved in this trait. A co-dominant sequence-characterised amplified region (SCAR) named SCC8 was developed from opC08-1020. Amplification of SCC8 produced a single band, both in the seedless and seeded individuals of the progeny, but a polymorphism was recovered after digestion of the amplification product with the restriction enzyme *Bgl*II (Lahogue *et al.* 1998). Two alleles could be distinguished: $SCC8^{+}$, in coupling with the allele favourable to seedlessness of the locus *sdI* (*sdI*+) and $scc8^{-}$. The genetic distance between *sdI* and *SCC8* was estimated to be 0.7 cM and between *sdI* and opP18-530 at 3.5 cM (Lahogue *et al.* 1998). Once markers linked to a trait of interest are identified, their stability in various genetic backgrounds has to be tested in order to check their interest for MAS. Such studies may reveal differences in the estimation of genetic distances between markers if scored in different progenies (Causse *et al.* 1996), will allow to assess the degree of linkage disequilibrium between the markers and the trait (e.g. Cheng *et al.* 1996, Fang *et al.* 1997, Schachermayr *et al.* 1997, Lawson *et al.* 1998) and thus confirm or not the usefulness of markers for the prediction of the trait (Cheng *et al.* 1996).

The aim of the present work was to transform opP18-530 into a SCAR marker (SCP18) and then to assess the usefulness of both SCC8 and SCP18 for MAS of seedless grape

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cultivars. For this purpose, they were analysed in three other full sib families segregating for seedlessness and their efficiency for selection was tested *a posteriori*. They were also scored in various seedless individuals as well as in seeded cultivars which can be used as genitors in table grape breeding programs and in parthenocarpic seedless cultivars. This allowed to detect the various alleles existing for each marker and to test their linkage disequilibrium with the seedless character.

## Material and Methods

Plant material: Forty-three seedless genotypes and 38 seeded genotypes were analysed (Tab. 1). Two progenies segregating for seedlessness were studied: Mtp3140 (95 individuals): 2223-27 x 2121-30 (LAHOGUE et al. 1998) and MtpDMV2: Danuta x Madina (61 individuals). Danuta and Madina are both seedless cultivars obtained from INRA. Plants of the progeny were thus recovered by *in vitro* em-

Table 1

Parentage (if known) and genotypes at *SCC8* and *SCP18* loci of several seeded and seedless varieties. Asterisks indicate the genotypes for which the presence or absence of a null allele was deduced either from a segregation observed in Mtp3140, MtpDMV2, Mtp3039 or Mtp3040 or from the genotype of its parents

| Name | Parentage | SCC8[a] | SCP18[b] |
|---|---|---|---|
| Stenospermocarpic seedless genotypes | | | |
| *1992-9 | Dattier de Beyrouth x Sultana Moscata | +/0 | b/c |
| *2121-30[c] | Alphonse Lavallée x Sultana | +/- | b/0 |
| 2121-61 | Alphonse Lavallée x Sultana | +/- | b/c |
| 2212-30 | Alphonse Lavallée x Sultana Moscata | +/- | b/c |
| *2223-27[c] | Dattier de Beyrouth x Sultana Moscata | +/- | b/0 |
| *2223-32 | Dattier de Beyrouth x Sultana Moscata | +/0 | b/c |
| 2711-6 | Madeleine angevine x Canner seedless | +/? | b/? |
| 2715-23 | Bicane x Sultana | +/- | b/? |
| *3041-153 | Muscat of Alexandria x Perlette | -/0 | b/? |
| *3046-20 | Italia x Canner seedless | +/0 | b/? |
| *3047-1 | Italia x Sultana | +/0 | b/? |
| 3048-30 | Italia x (Gros Colman x Sultana) | -/? | b/? |
| 3049-166 | Italia x (Dattier de Beyrouth x Sultana Moscata) | +/- | b/? |
| Askeri | | +/? | b/? |
| Bayad[c] | | +/- | a/b |
| Bidaneh ghelmez | | +/? | b/? |
| *Black monukka[c] | | +/+ | b/b |
| Blush seedless | Emperor x (Reine des Vignes x (Alphonse Lavallée x Sultana moscata) | +/? | nt |
| Canner seedless[c] | Hunisa x Sultana | +/- | b/? |
| Centennial seedless | Cultivar of complex origine | +/? | b/? |
| Chasselas seedless | Mutant of Chasselas | -/? | b/c |
| *Danuta | Dattier de Beyrouth x Sultana Moscata | +/0 | b/c |
| Emperor seedless | Mutant of Emperor | -/? | b/? |
| Flame seedless | Cultivar of complex origine | +/? | b/? |
| Khalili piskakes | | +/? | a/b |
| Kichmich chichraou | | +/? | b/? |
| Kichmich rond | | +/? | b/? |
| *Madina | Cardinal x Sultana | +/0 | b/b |
| Naosé | | +/? | a/b |
| Ozaan daii | | +/? | a/b |
| *Perlette | Reine des vignes x Sultana | +/0 | b/c |
| *Pink Sultana | Mutant of Sultana | +/? | b/0 |
| *Red Sultana | Mutant of Sultana | +/? | b/0 |
| Rouchaki | | +/? | a/b |
| Sugraone | | -/? | b/? |
| *Sultana moscata[c] | Muscat of Alexandria x Sultana | +/- | b/0 |
| *Sultana[c] | | +/? | b/0 |
| Tarnaou | Nimrang x Kichmich chernyi | +/? | b/? |
| Yaghasti | | -/? | a/b |

Tab. 1 continued:

| Name | Parentage | SCC8[a] | SCP18[b] |
|---|---|---|---|
| Parthenocarpic seedless genotypes | | | |
| Cape currant | Mutant of Muscat à petits grains | -/? | b/? |
| Corinthe blanc[d] | | -/? | b/d |
| Black Corinth | | -/? | a/b |
| Corinto bianco[d] | | 0/0 | a/b |
| Seeded genotypes | | | |
| Aetonychi | | 0/0 | a/b |
| Aledo | | -/? | b/? |
| Seeded genotypes | | | |
| *Alphonse Lavallée[c] | | -/- | b/c |
| Bicane | | -/? | a/b |
| Bouchouka | | 0/0 | a/b |
| *Cardinal | | -/0 | b/c |
| Carla | Cardinal x Alphonse Lavallée | -/? | b/c |
| Chaouch blanc | | +/- | b/? |
| Chasselas blanc | | -/? | b/c |
| Danlas | Dabouki x Chasselas | -/? | a/b |
| Dastachine | Putative ancestor or offspring of Sultana | +/? | b/? |
| *Dattier de Beyrouth[c] | | -/0 | b/c |
| Emperor | | -/? | b/? |
| *Frankentahl | | nt | b/0 |
| Gora Chirine[e] | Putative mutant of Sultana | +/? | b/? |
| Gros vert | | -/? | a/b |
| *Italia | Bicane x Muscat de Hambourg | -/0 | b/0 |
| Khoussaïne blanc | | 0/0 | a/b |
| Lival | Alphonse Lavallée x Lignan | -/? | a/b |
| Madeleine de Céline | Alphonse Lavallée x Molinera gorda | -/? | b/c |
| Matilde | Cardinal x Italia | -/? | b/c |
| Michele Palieri | | -/? | b/? |
| *Muscat of Alexandria | | -/- | b/0 |
| Muscat de Hambourg | Muscat of Alexandria x Frankenthal | -/? | 0/0 |
| Nehelescol | | -/? | b/? |
| Olivette noire | | -/? | a/b |
| Opsimos edessis | | -/? | b/? |
| Ora | (Cinsaut x Perle de Csaba) x Cardinal | -/? | a/b |
| Ouroum Uzumu[e] | Putative mutant of Sultana | +/? | b/? |
| Perle de Csaba | | nt | b/? |
| Phakri | | -/? | b/? |
| Pizzutello nero | | +/? | a/b |
| Prima | Lival x Cardinal | 0/0 | a/b |
| Red Globe | Emperor x Hunisa x Nocera | -/? | b/? |
| Sabalskankoï | | -/? | b/c |
| Santa Paula | | +/? | b/? |
| Servant | | -/? | b/? |
| *Sultana monococco[e] | Mutant of Sultana | +/? | b/0 |

[a] +,- represent the two codominant alleles at *SCC8*, respectively *SSC8*$^{+}$ and *scc8*$^{-}$, as defined by Lahogue *et al.* (1998). 0 represent a null allele. +/? is for +/+ or +/0 and -/? for -/- or -/0.

[b] a, b, c and d represent the four codominant alleles (234, 237, 240, 243 bp respectively) that were detected at the *SCP18* locus. 0 represents a null allele and b/? is for b/b or b/0.

[c] Data at the *SCC8* locus were obtained by Lahogue *et al.* (1998).

[d] Corinto bianco and Corinthe blanc are distinct varieties. Both are not white mutants of Black Corinth.

[e] It is unknown if Sultana is a mutant of these varieties or reciprocally.

bryo rescue. Two full sib families both obtained from a seeded x seedless cross, were also analysed: Mtp3039 (Alphonse Lavallée x Black Monukka, 19 individuals) and Mtp3040 (Muscat of Alexandria x Bayad, 14 individuals). Black Monukka and Bayad are two naturally occurring seedless varieties. The individuals of MtpDMV2, Mtp3039 and Mtp3040 have been qualitatively distributed into two phenotypic classes: seeded, if berries contained normally developed seeds with totally sclerified integument and seedless otherwise.

All these plants were grown at the viticultural experimental stations of Chapitre and Vassal (INRA), near Montpellier (France). Young leaves were collected during the growing season, frozen in liquid nitrogen and kept at -20 °C until DNA extraction.

DNA extraction: DNA extractions were performed from 0.2 g of leaves according either to Lahogue *et al.* (1998) or to Bowers *et al.* (1993). The DNA was quantified on a 0.8 % agarose gel by comparison with known amounts of lambda DNA (Boehringer Mannheim, Germany).

Conversion of the RAPD marker into a SCAR marker and SCAR analysis: The procedure followed for the conversion of opP18-530 into a SCAR marker has been described by Lahogue *et al.* (1998). Two specific primers were designed from the sequence of the marker using the PRIME procedure of the GCG package version 8.1-UNIX (Wisconsin package 1995), SCP18-S2 (GAG CTT GTC ATC ATC AAT G) and SCP18-AS2 (GTG AAA CTT TCC CTT TCC).

SCP18 was amplified using a standard PCR mix (Lahogue *et al.* 1998) and a Biometra uno thermocycler programmed as follows: 1 step of 4 min at 94 °C, 25 cycles of 1 min at 94 °C, 1 min at 53 °C, 1 min at 72 °C and a last step of 5 min at 72 °C. An equal volume of sequence loading buffer was added to the amplification product (Sambrook *et al.* 1989), denatured 5 min at 95 °C and 1 µl was loaded on a 6 % 19:1 (acrylamide/bis-acrylamide) denaturing polyacrylamide gel. Fragments were separated at 60 W for 3 h and stained with silver nitrate using the Silver Sequence Staining System (Promega Corporation, Madison, WI, USA). Their size was approximately deduced by comparison to a 30-330 ladder (GIBCO BRL, Life Technologies Inc, Gaithersburg, MD, USA).

The SCC8 fragment was amplified and analysed according to Lahogue *et al.* (1998) except that 10 % v/v glycerol was added to the PCR reaction mix.

SCC8 was scored on two independent extractions of each genotype, whereas for SCP18, only null alleles or ambiguous results were checked twice.

Statistical analysis: Goodness-of-fit between observed and expected segregation ratios at the different loci as well as the likelihood of independence between *SCC8* or *SCP18* and *sdI* were tested using a Chi-square analysis.

Map distances were estimated using the software JoinMap version 2.0 (Stam 1993). A minimum LOD Score of 3.0 was used to determine linkage groups. Recombination fractions were converted into centiMorgans (cM) by applying the Haldane function (Haldane 1919).

## Results

Development of a SCAR marker derived from the RAPD OpP18-530: A 540 bp insert corresponding to the opP18-530 fragment was sequenced (Genebank accession n° AY033647) and two primers were designed, allowing the amplification of a 237 bp fragment (*SCP18*). The SCAR primers are both internal in the opP18-530 fragment and thus do not overlap the RAPD primer. The 237 bp SCAR fragment contains a short imperfect microsatellite motif $(GAA)_6(GCA)_3(GAA)_1$ at 105 and 65 bp from the forward and the reverse primers respectively. Therefore, the *SCP18* fragment was analysed on denaturing polyacrylamide gels, in order to be able to detect the occurrence of different alleles corresponding to different numbers of repeats of the micro-satellite motif.

The genotypes of the parents and grandparents and the offspring individuals of the Mtp3140 population are summarized in Tab. 2. The analysis of the Mtp3140 population revealed a null allele at the *SCP18* locus which was thus analysed as a dominant marker (Tab. 2). A perfect correspondence between the segregations of *opP18* and *SCP18* was observed in the Mtp3140 population (data not shown): all individuals which showed the opP18-530 fragment also showed the 237 bp fragment at the *SCP18* marker and the individuals scored 0 for the RAPD markers, presented a null allele at the SCAR marker. This confirmed the correspondence between the RAPD and the SCAR marker. The two parents of the population (2121-30 and 2223-27) and the seedless grandparents (Sultana moscata and Sultana) presented only one band of 237 bp called allele "b". Because of the null allele segregating in the Mtp3140 population, 2121-30 and 2223-27 must be heterozygous for the null allele (Tab. 2). The seeded grand parents (Dattier de Beyrouth and Alphonse Lavallée) showed the "b" allele and an additional 240 bp band (called allele "c"). This allowed us to infer that both, Sultana and Sultana moscata are heterozygous for the null allele and must have transmitted it to the parents of the Mtp3140 population (Tab. 2). These results suggest also that the "b" allele is in coupling with the $sdI^+$ allele whereas the "c" and the null alleles are in coupling with the $sdI^-$ allele.

Analysis of *SCC8* and *SCP18* in the MtpDMV2, Mtp3039 and Mtp3040 full sib families: The MtpDMV2 progeny contained 52 seedless or partially seedless individuals and 9 seeded genotypes. According to the $\chi^2$ test, this phenotypic distribution of the trait was in agreement with the existence of a major gene fitting to a 3:1 segregation ratio (Tab. 2). When the SCC8 marker was scored, 11 individuals of the progeny did not show any amplification product. The other 50 individuals presented the allele $SCC8^+$. Such a distribution at the marker locus did not deviate significantly from a 3:1 segregation ratio (Tab. 2) and is in agreement with the existence of a null allele present in both Danuta and Madina. This null allele would have been transmitted by the seeded grandparents of the MtpDMV2 full sib family and is thus in coupling with the $sdI^-$ allele (Tab. 2). The genetic distance between *SCC8* and *sdI* is 4.0 cM (two recombinants were detected between the two loci, Tab. 3). At the *SCP18* locus, Danuta presented

Table 2

Genotypes and segregation of the SCAR markers and the RAPD markers from which they were derived in the grandparents, parents and offspring of the 4 analysed full sib families

| Parentage | Individual | Seedlessness[a] | opC08-1020 | SCC8[b] | opP18-530 | SCP18[c] |
|---|---|---|---|---|---|---|
| Mtp3140 | | | | | | |
| Maternal grandmother | Dattier de Beyrouth [d] | $S^-$ | 0 | -/0 | 1 | b/c |
| Maternal grandfather | Sultana moscata [d] | $S^+$ | 1 | +/- | 1 | b/0 |
| Mother | 2223-27[d] | $S^+$ | 1 | +/- | 1 | b/0 |
| Paternal grandmother | Alphonse Lavallée [d] | $S^-$ | 0 | -/- | 0 | b/c |
| Paternal grandfather | Sultana [d] | $S^+$ | 1 | +/? | 1 | b/0 |
| Father | 2121-30[d] | $S^+$ | 1 | +/- | 1 | b/0 |
| Offspring | Genotypic classes[e] | $S^+$: $S^-$ (64:31) | 1:0 (64:31) | +/+: +/-: -/- | 1:0 (64:31) | b/?: 0/0 (64:31) |
| (95 individuals) [a] | Segregation | 3:1 | 3:1 | (20:42:31) | 3:1 | 3:1 |
| | Chi2 of goodness of fit | 2.95 | 2.95 | 1:2:1 3.47 | 2.95 | 2.95 |
| MtpDMV2 | | | | | | |
| Maternal grandmother | Dattier de Beyrouth[d] | $S^-$ | | -/0 | | b/c |
| Maternal grandfather | Sultana moscata[d] | $S^+$ | | +/- | | b/0 |
| Mother | Danuta | $S^+$ | | +/0 | | b/c |
| Paternal grandmother | Cardinal | $S^-$ | | -/0 | | b/c |
| Paternal grandfather | Sultana[d] | $S^+$ | | +/? | | b/0 |
| Father | Madina | $S^+$ | | +/0 | | b/b |
| Offspring | Genotypic classes[e] | $S^+$: $S^-$ (52:9) | | +/?: 0/0 (50:11) | | b/b: b/c (23:35) |
| (61 individuals) | Segregation | 3:1 | | 3:1 | | 1:1 |
| | Chi2 of goodness of fit | 3.42 | | 1.58 | | 2.48 |
| Mtp3039 | | | | | | |
| Mother | Alphonse Lavallée[d] | $S^-$ | | -/- | | b/c |
| Father | Black Monukka[d] | $S^+$ | | +/+ | | b/b |
| Offspring | Genotypic classes[e] | $S^-$: $S^+$ (18:1) | | +/- | | b/c: b/b (8:11) |
| (19 individuals) | | | | | | |
| Mtp3040 | | | | | | |
| Mother | Muscat of Alexandria | $S^-$ | | -/- | | b/? |
| Father | Bayad[d] | $S^+$ | | +/- | | b/? |
| Offspring | Genotypic classes[e] | $S^-$ | | +/-: -/- (6:8) | | b/? |
| (14 individuals) | | | | | | |

[a] $S^-$,$S^+$ represent the phenotype for seedlessness: seeded and seedless.
[b] +,- represent the two codominant alleles at *SCC8*, *SSC8*$^+$ and *scc8*$^-$, as defined by Lahogue *et al*. (1998). 0 represents a null allele. +/? is for +/+ or +/0 and -/? for -/- or -/0
[c] a, b, c and d represent the 4 codominant alleles (234, 237, 240, 243 bp) that were detected at the *SCP18* locus. 0 represents a null allele and b/? is for b/b or b/0.
[d] Genotypes at *opC08-1020*, *SCC8* and *opP18-530* obtained by Lahogue *et al.* 1998).
[e] Numbers in brackets correspond to the size of the genotypic class. The 95 individuals of the Mtp3140 population represent a subset of this population (Lahogue *et al.* 1998)

the two-allele profile "bc", the "b" allele being in coupling with the *sdI*$^+$ allele whereas, Madina presented only the "b" allele (Tab. 2). The segregation of 23 "bc" individuals and 35 "b" individuals, fitting to a 1:1 ratio confirmed the "b/b" genotype of Madina (Tab. 2). It was inferred from the genotypes of the parents of Danuta that the ”c” allele is in coupling with the *scc8*$^-$ and the *sdI*$^-$ alleles. *SCP18* was found to be at 14.9 cM from the *sdI* locus and the *SCP18* and *SCC8* loci were inferred to be on the same side of the *sdI* locus (Figure).

Table 3

Genotype occurrence in the MtpDMV2 progeny

| Loci[a] | Genotypic classes | | | |
|---|---|---|---|---|
| *sdI* ; *SCC8* | [$sdI^+$/?] ; [$SCC8^+$/?] | [$sdI^+$/?] ; [0/0] | [$sdI^-$/$sdI^-$] ; [$SCC8^+$/?] | [$sdI^-$/$sdI^-$] ; [0/0] |
| | 50 | 2 | 0 | 9 |
| *sdI* ; *SCP18* | [$sdI^+$/?] ; [b/b] | [$sdI^+$/?] ; [b/c] | [$sdI^-$/$sdI^-$] ; [b/b] | [$sdI^-$/$sdI^-$] ; [b/c] |
| | 24 | 26 | 0 | 9 |
| *SCP18* ; *SCC8* | [$SCC8^+$/?] ; [b/b] | [$SCC8^+$/?] ; [b/c] | [0/0] ; [b/b] | [0/0] ; [b/c] |
| | 23 | 26 | 0 | 10 |

[a] According to the model of BOUQUET and DANGLOT (1996)

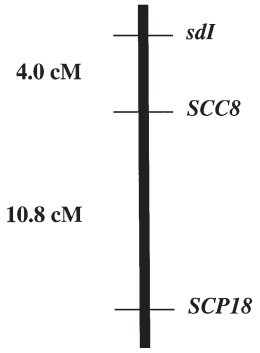


Figure: Local genetic map around the *sdI* locus calculated from segregation in MtpDMV2. Distances are in cM.

Individuals of two additional full sib families Mtp3039 (one partially seedless individual and 18 seeded individuals) and Mtp3040 (14 seeded individuals) both obtained from a seeded x seedless cross were scored at the *SCC8* locus. In the Mtp3039 progeny, the 19 individuals were scored $SCC8^+$/$scc8^-$ (Tab. 2). Alphonse Lavallée was thus deduced to be homozygous for $scc8^-$ and Black Monukka was deduced to be homozygous for $SCC8^+$ (Tab. 2). Indeed, the probability to observe at least either one homozygous null individual or one heterozygous individual for the null allele if both parents were heterozygous with a null allele is $1-(0.75)^{19} = 0.996$. In the Mtp3040 progeny, 6 individuals were scored $SCC8^+$/$scc8^-$ and 8 were scored $scc8^-$/$scc8^-$, fitting to a 1:1 ratio. In the Mtp3039 population, *SCP18* segregated in a 1:1 ratio for two genotypic classes (b/b and b/c) and presented only the "b" allele in the Mtp3040 population (Tab. 2).

Linkage disequilibrium between the seedless phenotype and SCC8: In order to determine to what extent the SCC8 marker would be useful in marker-assisted selection, it was scored in a set of table grapes which can be classified into three groups (Tab. 1): the first two groups contained nearly all the stenospermocarpic and parthenocarpic seedless genotypes available in the INRA germplasm collection at Domain of Vassal and the third group contained seeded genotypes from the most widely used table grape varieties to represent the genetic variability of table grapes as described by CUISSET (1998). Four genotypes did not show any amplification product, confirming the existence of a null allele. Based on the previous analysis of the segregation of the SCC8 marker in the 4 progenies, it was possible to infer the presence of this null allele at a heterozygous state in Cardinal and Dattier de Beyrouth and its absence in Black Monukka, Alphonse Lavallée and Muscat of Alexandria (Tabs 1 and 2). This information was further used to deduce the presence of a null allele in 1992-9 and 2223-32 (Tab. 1). For 3041-153, 3046-20, 3047-1, Perlette and Italia the same inference was made but their common parentage was also taken into account (Tab. 1). For instance, Muscat of Alexandria being $scc8^-$/$scc8^-$ and Perlette $SCC8^+$/?, the fact that their offspring 3041-153 is $scc8^-$/? allowed to infer that both, Perlette and 3041-153, have a null allele (Tab. 1). In the same manner, the $SCC8^+$ allele observed in 3046-20 and 3047-1 were necessarily given by Canner seedless and Sultana respectively and Italia must have given to both of its offspring a null allele (Tab. 1).

Most of the stenospermocarpic seedless genotypes harboured at least one $SCC8^+$ allele, whereas the parthenocarpic seedless genotypes and most of the seeded genotypes did not (Tabs 1 and 4). However, several exceptions can be noticed. Among the seedless genotypes, 3041-153, 3048-30, Chasselas seedless, Emperor seedless, Sugraone and Yaghasti showed no $SSC8^+$ allele (Tab. 1). Among the seeded genotypes, Chaouch blanc, Dastachine, Gora Chirine, Ouroum Uzumu, Pizzutello nero, Santa Paula and Sultana monococco presented at least one $SCC8^+$ allele (Tab. 1). The $\chi^2$-square test of independence showed that in the subset of 39 naturally stenospermocarpic seedless varieties analysed, the distribution of the different alleles at the *SCC8* locus is highly skewed towards the presence of at least one $SCC8^+$ allele whereas in the subset of seeded varieties, the distribution of the three alleles is skewed in favour of the absence of the $SCC8^+$ allele (Tab. 4). The distributions of genotypes are significantly different between the two subsets.

Linkage disequilibrium between the seedless phenotype and SCP18: The SCP18 marker was also scored in the same set of genotypes (Tab. 1). Five alleles were observed and labelled "a" (234 bp), "b" (237 bp), "c" (240 bp), "d" (243 bp) and "0" (null allele). Again, using information on the segregation of the *SCP18* loci in the 4 previously analysed populations, the presence of a null allele at heterozygous state was deduced in Sultana moscata, Sultana and Muscat of Alexandria and its absence was inferred for Black Monukka and Madina (Tabs 1 and 2).

Table 4

Distribution of the genotypic classes at the *SCC8* locus in the subsets of stenospermocarpic seedless and seeded varieties described in Tab. 1, $\chi^2$ test of goodness-of-fit to the expected 3:3:2:1 distribution in a panmictic population ($\chi^2_D$) and $\chi^2$ test of the difference of genotypic distribution between the two subsets ($\chi^2_R$)

| | *SCC8*$^+$/? | *scc8*$^-$/? | *SCC8*$^+$/*scc8*$^-$ | 0/0 | Total | $\chi^{2\,(a)}$ |
|---|---|---|---|---|---|---|
| Seedless | 24 | 6 | 9 | 0 | 39 | $\chi^2_D$=13.3 |
| Seeded | 6 | 25 | 1 | 4 | 36 | $\chi^2_D$=19.01 |
| Total | 30 | 31 | 10 | 4 | 75 | $\chi^2_R$=31.9 |

(a) For the $\chi^2$ calculation, classes were merged so that the presence (*SCC8*$^+$/?, *SCC8*$^+$/*scc8*$^-$) *versus* the absence (*scc8*$^-$/?, 0/0) of *SCC8*$^+$ was tested with 1 degree of freedom.

These results and, if known, the parentage of the varieties were then used to infer the genotype of Pink Sultana, Red Sultana, Sultana monococco, Frankenthal and Italia (Tab. 1).

The "b" allele is nearly always present (except for the double null genotype observed in Muscat of Hamburg) in either seedless or seeded varieties, at a homozygous state or in combination with the other alleles (Tabs 1 and 5). ”a”, ”c” and ”d” alleles were thus always observed in combination with the ”b” allele (Tab. 1). There was no significant difference in the genotypic distributions between the stenospermocarpic seedless and the seeded genotypes (Tab. 5).

Table 5

Distribution of the genotypic classes at the *SCP18* locus in the subsets of stenospermocarpic seedless and seeded varieties described in Tab. 1 and $\chi^2$ test of the difference of genotypic distribution between the two subsets ($\chi^2_R$)

| | b/? | a/b | c/b | 0/0 | Total | $\chi^{2\,a}$ |
|---|---|---|---|---|---|---|
| Seedless | 25 | 6 | 7 | 0 | 38 | |
| Seeded | 18 | 11 | 8 | 1 | 38 | |
| Total | 43 | 17 | 15 | 1 | 76 | $\chi^2_R$=2.9 |

[a] For the $\chi^2$ calculation, c/b and 0/0 classes were merged. The test was realized with 2 degrees of freedom.

## Discussion

The aim of the work presented here was to determine whether two RAPD markers, opC8-1020 and opP18-530, linked to a major gene involved in seedlessness (Lahogue *et al.* 1998) would be valuable for MAS for the seedless trait in a wide range of genetic backgrounds. Markers used for MAS purposes must be easy to handle and to score and stable across different genetic backgrounds. For this purpose, opC8-1020 was cloned and transformed into a co-dominant SCAR marker, SCC8 (Lahogue *et al.* 1998). The same procedure was successfully applied to opP18-530 in this work. The derived SCAR marker, SCP18, was shown to be dominant in the Mtp3140 population, due to the presence of a null allele. The polymorphism at the origin of this null allele is different from the polymorphism at the origin of the RAPD null allele since they may not always be scored on the same individuals. For instance, Alphonse Lavallée was scored ”0” at opP18-530 and ”bc” at SCP18. This may be due to the fact that the SCAR primer sequences do not overlap the RAPD primer sequences. This marker can be analysed as a micro- satellite marker due to the presence of a $(GAA)_6(GCA)_3(GAA)_1$ motif and the polymorphism was thus observed directly after amplification.

Those two PCR-specific markers have then been analysed in two seedless x seedless full sib families, Mtp3140 and MtpDMV2, in order to confirm their linkage to the *sdI* gene and to test *a posteriori* their relevance for MAS purposes. The genetic distances between the *sdI* gene and the two SCARs were larger if estimated with the MtpDMV2 population (14.7cM between *SCP18* and *sdI* for instance, Figure). The grandparents of both populations have similar genetic distances according to Cervera *et al.* (2000): Cardinal and Sultana (Madina); Alphonse Lavallée and Sultana (2121-30) and Dattier de Beyrouth and Sultana moscata for both 2223-27 and Danuta which are full sib individuals. No global difference in recombination abilities of those 4 individuals was thus expected. Such differences may reflect either sampling effects or differences in the ability to recombine in this specific region of the genome. While the SCC8 marker was co-dominant in the Mtp3140 population (Lahogue *et al.* 1998), a null allele, in coupling with the *sdI*$^-$ allele, was detected in the MtpDMV2 population. SCC8 was therefore less informative in the later cross than in the former. However the use of SCC8 would have led to the elimination of 11/61 individuals (0/0), 9 of which were seeded and two were seedless recombinant (Tab. 3). Therefore this marker would have allowed to save space with a relative efficiency if used before plantation in both Mtp3140 (Lahogue *et al.* 1998) and MtpDMV2 population. However, due to the recessive status of the null allele, it was impossible to test in the MtpDMV2 population if the genotypes which are homozygous for *SCC8*$^+$ express a more complete seedless phenotype than those which are heterozygous as could be done in the Mtp3140 population (Lahogue *et al.* 1998). The SCP18 marker was as informative as the opP18-530 marker in the

Mtp3140 progeny; in the MtpDMV2 population, only Danuta was heterozygous at the *SCP18* locus (Tab. 2). SCP18 was not informative for the selection of seedless genotypes in this population (Tab. 3) whereas it would have been useful in the Mtp3140 progeny (LAHOGUE *et al.* 1998). For both populations derived from seedless x seedless crosses, at least one of the two markers would have been useful to select seedless individuals in the progeny but also to make a choice of the parents.

This latter point was further examined by the analysis of a wide range of unrelated genotypes, characterized for the seedlessness. The linkage disequilibrium between the two markers and seedlessness was thus further examined by the analysis of a set of 81 seedless or seeded accessions of the INRA germplasm collection. The existence of null alleles was confirmed for both markers and two new alleles were detected at the *SCP18* locus, a 243 bp allele ("d") and a 234 bp allele ("a").

The size of the 4 alleles at *SCP18* locus was compatible with a variation of the number of repeats of a short microsatellite motif within the sequence. The "b" allele was found in all genotypes except in Muscat of Hamburg which was homozygous null. The small variability of the SCP18 microsatellite marker may be due to the small number of replicates, the fact that it is an imperfect motif but also to homoplasy (JARNE and LAGODA 1996). This latter phenomenon together with a long distance between SCP18 and *sdI* (Figure) may also partly explain the absence of linkage disequilibrium between *SCP18* and seedlessness (Tab. 5). The occurrence of homoplasy could be tested through the sequencing of various "b" alleles and the fact that the microsatellite motif is imperfect would be helpful (JARNE and LAGODA 1996). Nevertheless, these results confirm that the SCP18 marker is not useful. A contrasting result was obtained after the analysis of the *SCC8* locus: 33/39 stenospermocarpic seedless individuals harboured at least one $SCC8^+$ allele and 29/36 seeded individuals presented no $SCC8^+$ allele. Moreover, none of the parthenocarpic seedless genotypes harboured the $SCC8^+$ allele. The morphology and the physiology of this kind of seedlessness is completely different from the stenospermocarpic seedlessness (STOUT 1936) and is therefore most probably not under the control of the *sdI* locus. Some of the other exceptions were predictable. Chasselas seedless and Emperor seedless do not present the $SCC8^+$ allele. Both appeared recently by clonal variation in sports of Chasselas (BRANAS and TRUEL 1965) and Emperor (OLMO 1940), respectively.

The mutations involved in the appearance of those seedless varieties may thus have affected either punctually the *sdI* gene (*SCC8* is not affected) or another locus involved in the genetic control of the seedless trait. A comparative dissection of the genetic factors involved in seedlessness in Chasselas seedless, Emperor seedless or Sultana-derived seedless genotypes would allow to assume differences in the genetic factors involved. Indeed, most of the seedless genotypes analysed have Sultana in their parentage (Tab. 1) and many of the others have the geographic origin of Sultana, *i.e.* Middle East (BRANAS and TRUEL 1965) and may thus be genetically related to Sultana. The global linkage disequilibrium observed between seedlessness and the $SCC8^+$ allele, supports the hypothesis of a common origin of seedlessness in those varieties. However, it remains to be confirmed using more markers closer to the *sdI* gene as well as markers scattered throughout the genome to better evaluate genetic relationships between seedless cultivars. Such information together with more information on genetic factors involved in seedlessness would be valuable to select genetically distant parents and to avoid inbreeding along the construction of new seedless cultivars.

Dastachine, Gora Chirine, Ouroum Uzumu and Sultana monococco are seeded genotypes that show the $SCC8^+$ allele. This observation is in agreement with the hypothesis of Gora Chirine, Ouroum Uzumu and Sultana monococco being putative mutants of Sultana or reciprocally (KRIMBA 1933, BOUBALS and NAZEMILLE 1966) and Dastachine being a putative seeded ancestor of Sultana or reciprocally (NEDELCHEV and GUÉORGUIEFF 1930, KRIMBA 1933, BOUBALS and NAZEMILLE 1966). This hypothetical parentage of those varieties with Sultana need to be further investigated using other molecular markers such as microsatellite markers (BOWERS *et al.* 1999). Finally, the case of 3041-153 and 3048-30 which have no $SCC8^+$ allele whereas they originated from seedless varieties harbouring the $SCC8^+$ allele (respectively Perlette and Sultana) indicates that recombination has occurred between the two loci during the breeding process. Interestingly, two seeded varieties scored $SCC8^+$, Pizzutello nero and Santa Paula have arch-shaped berries, in which half of the seeds abort (BRANAS and TRUEL 1965). It is thus possible that in this case, the *sdI* gene is present and may have epistatic relationships with genes like homeostatic genes. All these results taken together confirm the interest of SCC8 as a marker of a main genomic region involved in seedlessness. It should also be possible to select parents of new crosses, knowing their genotype at *SCC8* so that this marker will be helpful to discard seeded individuals in progenies.

Progenies Mtp3039 and Mtp3040 are full sib families obtained from a seeded x seedless cross. In both cases, the seeded phenotype was independent of the genotype at the *SCC8* locus (Tab. 2). In Mtp3039, the two parents were inferred to be homozygous at the *SCC8* locus, thus no segregation was observed at this locus. In Mtp3140, the seeded parent was inferred to be homozygous ($scc8^-/scc8^-$) whereas the seedless parent was heterozygous ($SCC8^+/scc8^-$). However, out of 14 seeded individuals, 8 were $scc8^-/scc8^-$ and 6 were $SCC8^+/scc8^-$. This may reflect both the fact that *SCC8* is not close enough to the *sdI* gene and that it is specific of the genetic background of the Mtp3140 and MtpDMV2 populations as Danuta and 2223-27 are full sib individuals. Alternatively, other genes may be involved in seedlessness. This explanation agrees with the model of BOUQUET and DANGLOT (1996) and the fact that the *SCC8* locus did not explain the whole variability of quantitative subtraits of seedlessness in the Mtp3140 population (LAHOGUE *et al.* 1998). The whole set of genes involved in seedlessness are thus currently being searched for by QTL analysis and sets of markers linked to each of them will have to be developed for efficient selection for this trait in different genetic backgrounds. Moreover, in this study, *SCC8* seems to be more efficient in partially seedless x partially seedless crosses

(Mtp3140 and MtpDMV2 progenies) than in seeded x seedless progenies. Indeed, if seedlessness is taken as a qualitative character, the ratio of seeded to seedless individuals obtained in seeded x seedless crosses is most of the time close to 3:1 (Bouquet and Danglot 1996, Roytchev 1998) whereas in seedless x seedless it is close to 1:3 (Bouquet and Danglot 1996). The search for QTLs for seedlessness in different table grape cultivars and the study of their allelic variations will thus be of high value.

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