Behaviour of two SCAR markers for seedlessness within Central European varieties of grapevine

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Summary

Two working sequence-characterised amplified region markers, SCC8 and SCF27, linked to the seedless phenotype and particularly to the major locus involved in this trait, sdI, were identified in grapevine. Several seeded varieties also showed the alleles for seedlessness at these marker loci. Based on comparison of allelic distribution of two markers in a set of several seeded and seedless varieties, we provide evidence that 'Chaouch rose', an ancient seeded variety of unknown origin, has potential to promote seedlessness and most probably belongs to the group of seeded varieties that harbour the sdI allele. We conclude that using both SCC8 and SCF27 and, in special cases, even their null alleles can help to elucidate the seedlessness of individuals that lack the amplicon accountable for seedlessness at one marker locus. However, the presence of null alleles and the genetic distance of markers from the sdI locus involved in seedlessness may cause complications.

Key words: MAS, SCC8, SCF27, seedlessness, grapevine, 'Chaouch', female varieties.

Introduction

Seed development in angiosperms is a complex process involving many regulated steps. Recently, several genes affecting seed development have been identified in model species, such as Arabidopsis (RUUSKA et al. 2002, FAI S et al. 2006) and maize (CONSONNI et al. 2005). In grapevine (Vitis vinifera L.), stenospermocarpic seedless fruits are prized by consumers due to their better eating quality, but little is known about seed development at the molecular level.

Breeding of seedless varieties started in the 20th century and throughout the world a wide range has been produced. They mainly have mid-size berries and have replaced the mid-size-berried seeded ones. Indeed, only the seeded varieties producing loose clusters with very large berries (e.g. 'Red Globe', 'Afus Ali' and 'Italia') are still generally accepted and required in markets (KORPÁS 2006).

Parthenocarpy is described and well understood in grapevine (PRATT 1971), however, the original parthenocarpic group of Corinth varieties do not have large berries and so have not been used for creating varieties with large berries. Stenospermocarpic seedlessness is based on the abortion of endosperm or embryo, and subsequently of the whole seeds, soon after fertilization (STOUT 1936). This characteristic derives from the Sultana variety with its berry colour and shape variations (e.g. 'Kishmish rozovyi', 'Kishmish chornyi' and 'Thompson seedless'), and enables larger berry size and weight and so has been widely used in breeding (BRANAS and TRUEL 1965, KORPÁS 2006).

According to the recent model of BOUQUET and DAGLO T (1996), stenospermocarpic seedlessness might be controlled by three complementary recessive genes, independently inherited and regulated by a dominant gene, named sdI (seed development inhibitor). However, higher plant reproduction is characterized by five developmental phases: the diploid sporophyte, the haploid female gametophyte, the haploid male gametophyte, the developing diploid embryo, and the developing triploid endosperm. It is an important point that development of the embryo sac and seed are under both sporophytic and female gametophytic control (ZHANG et al. 2004). The paternal gametophytic and postfertilisation sporophytic controls are additional to complex genetic interactions governing seed development (EVANS and KERMICLE 2001). Very recently, it was shown that specific differences in gene expression during flower development between seeded and seedless grapevine varieties might be correlated with stenospermocarpic seedlessness (HANANIA et al. 2007).

Since seedlessness is a lately expressed trait in the life cycle of the plant, remarkable efforts have been made to identify molecular markers linked to the genes involved in seedlessness. Three RAPD-derived sequence-characterised amplified region (SCAR) markers, SCC8 (LAHOQUE et al. 1998), SCP18 (ADAM-BLONDON et al. 2001) and SCF27 (MEJIA and HINRICHS E 2003), linked to the putative major locus, sdI, have been published. These markers were derived from bulked segregant analyses (MICHELMORE et al. 1991) of progenies of crosses between two partially seedless genotypes. However, a broader genetic background represented by several world-renowned and newly bred varieties rendered SCP18 useless and, in contrast, confirmed the usefulness of SCC8 at least in seedless × seedless crosses (ADAM-BLONDON et al. 2001). SCF27 has not been tested in a broader genetic background.

Genetic studies for quantitative traits in grapevine have recently been greatly improved by the development of molecular markers and genetic maps. Preliminary results of quantitative trait loci (QTL) detection for berry

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size and seedlessness have been created (Doligez et al. 2002, Fischer et al. 2004, Fanizza et al. 2005, Cabezas et al. 2006, Meja et al. 2007, Costantini et al. 2008). All studies dealing with seedlessness (Doligez et al. 2002, Cabezas et al. 2006, Meja et al. 2007, Costantini et al. 2008) confirmed the existence of a major-effect QTL affecting both seed and berry weight on linkage group 18 (LG18) (defined by Adam-Blondon et al. 2004, Riaz et al. 2004) which coincides with the seedlessness gene sfd (Costantini et al. 2008). Cabezas et al. (2006) reported two microsatellite loci, VMC7f2 (Pellerone et al. 2001) and VMC6F11 (Arroyo-García and Martínez-Zapater 2004), closely linked to this major QTL. VMC7f2 was identified as a useful marker for selection of seedlessness. Very recently, Costantini et al. (2008) identified ‘Pinot noir’ genomic contigs (Velasco et al. 2007) that align with simple sequence repeat (SSR) markers underlying QTLs for berry- and phenotype-related traits. Two genes were predicted in the vicinity of VMC7f2, the closer one coding for MADS-box protein 5 (Vitis vinifera, AAM21345). Interestingly, this was the smallest protein identified in this study and consisted of only 85 amino acids. Additionally, in agreement with earlier studies (Doligez et al. 2002, Cabezas et al. 2006, Meja et al. 2007) and reinforcing the model of Bouquet and Danglot (1996), several other minor-effect QTLs for seedlessness subtraits have been reported (Costantini et al. 2008). Despite the fact that the identity of these minor QTLs is greatly influenced by reduced population sizes and the limitations posed by the two-way pseudo-testcross mapping strategy (Grattapaglia and Sederoff 1994), additional loci on LGs 2, 10 and 15 (defined by Adam-Blondon et al. 2004, Riaz et al. 2004) seem to be involved in seedlessness (Costantini et al. 2008).

Along with this encouraging progress in identification of seedlessness genes, there is a need to find seeded varieties with potential to promote seedlessness. Indeed, analyses of crosses between seeded and seedless varieties with SCAR markers (Adam-Blondon et al. 2001) have already identified seeded individuals that contain the SCC8 allele and probably the linked major locus involved in seedlessness. Particularly, the cross between seeded ‘Alphonse Lavallée’ and seedless ‘Black Monukka’ (the only known ancient homozygous variety SCC8/SCC8) yielded only one seeded individual out of 19 individuals (Adam-Blondon et al. 2001). In the same study, along with putative mutants and ancestors of ‘Sultana’ or reciprocally (‘Dastachine’, ‘Gora Chirine’, ‘Ouroum Uzumu’ and ‘Sultana monococco’), three seeded varieties, ‘Chauoch blanc’, ‘Pizzutello nero’ and ‘Santa Paula’, had at least one SCC8 allele. In addition, there may be seeded varieties with no sfd allele but with favourable operator genes, as defined by Bouquet and Danglot (1996).

‘Chauoch rose’, the rose form of ‘Chauoch blanc’, was used extensively in seedless table grape breeding in the former Czechoslovakia in the 1960s and 1970s and several improved varieties were selected (Pospíšilova and Korpás 1998). The aim of the present work was to study their allelic distribution at SCC8 and SCF27 loci using the corresponding SCAR markers, SCC8 and SCF27, respectively.

For the two seedless varieties, ‘Jupiter’ and ‘Neptuum’, full-sib families as well as individuals from various crosses, all represented by three-month-old juvenile seedlings, were analysed. The markers were also scored in various Central and Eastern European stenospermocarpic seedless and seeded varieties. This allowed comparison of the distribution of alleles responsible for seedlessness between the markers, as well as between the varieties, and determination of seeded varieties with increased potential to promote seedlessness.

### Material and Methods

**Plant material:** Twenty-four seedless genotypes (BV 16-16-3 (B3), BV 16-20-2 (B2), BV 47-1-6 (B6), ‘Carina’ (C), ‘Dawn seedless’ (D), ‘Edro bezseme’ (EB), ‘Elma’ (E), ‘Flame seedless’ (F), ‘Helios’ (H), ‘Jupiter’ (J), ‘Kish-mish luchysti’ (KL), ‘Kishnish moldavskii’ (KM), ‘Merku’ (M), ‘Neptuum’ (N), ‘Perlon’ (PN), ‘Picurka’ (PI), ‘Rosina’ (RA), ‘Rooziska’ (RO), ‘Ruby seedless’ (RY), ‘Rusalka 3’ (R3), ‘Slavianka’ (SA), ‘Sunred seedless’ (SD), ‘Urkim’ (UM) and ‘Venusia’ (V)), one genotype with hard seed traces but without endosperm (‘Mars’ (MS)) and nine seeded genotypes (‘Alphonse Lavallée’ (AL), ‘Chauoch rose’ (CR), ‘Heliotrop’ (HP), ‘Karneol’ (K), ‘Luna’ (L), ‘Olshava’ (O), ‘Queen of Vineyards’ (QV), ‘Uraan’ (U) and ‘Victoria’ (VI)) were analysed (Tab. 1).

Three progenies segregating for seedlessness were also studied (Tab. 3 A-C): NKL (31 individuals, N × KL), NSD (26 individuals, N × SD) and JKL (12 individuals, J × KL). All parental genotypes of analysed progenies were seedless, from crosses of seeded and seedless varieties (Tab. 1).

Plants of the progeny were produced using in vitro techniques: direct germination or embryo rescue. To elucidate parental allelic distribution, analysis was performed on additional 11 selected three-month-old juvenile seedlings from different crosses: JIA_90 (J × ‘Ilonka’ (IA)), JKM_85 (J × KM), JMO_97 (J × ‘Marrood seedless’ (MO)), JPA_102 (J × PA), JPN_87, JPN_88 (both J × PN), JRN_86 (J × RN), NCL_94 (N × CL), NMO_93 (N × MO), R3OP_92, R3OP_96 (both R3 open pollination (OP)), all obtained by in vitro techniques (Tab. 3 D).

All plants were grown in experimental vineyards or greenhouses in Lednice (Faculty of Horticulture, Mendel University of Agriculture and Forestry in Brno) and Strekov (PD Strekov Ltd.), Czech and Slovak Republic, respectively. Young leaves were collected during the growing season, frozen in liquid nitrogen and kept at -20 °C until DNA extraction.

From both NKL and NSD families, only two seedlings, NKL_32 and NKL_77, have reached maturity and only produced their first fruit very recently. Their berries were examined on field-grown plants at full maturity.

**DNA extraction:** DNA extractions were on 0.2 g of leaves by DNeasy Plant Mini Kit (Qiagen), according to manufacturer’s instructions. The DNA was quantified on the base of fluorometric determination with PicoGreen dye.
**SCAR analysis:** Two RAPD-derived SCAR markers were used: SCC8 (Lahogue et al. 1998) and SCF27 (Mejia and Hinchcliff 2003). Both markers were amplified using a standard PCR mix (Lahogue et al. 1998) and a TGradient thermocycler (Biometra) programmed as follows: A first step of 4 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 53 °C, 1 min at 72 °C and a last step of 7 min at 72 °C for SCC8; and for SCF27, a first step of 4 min at 94 °C, 35 cycles of 0.5 min at 95 °C, 1.5 min at 62 °C, 1 min at 72 °C and a last step of 7 min at 72 °C. Cutting of the SCC8 amplicons by Bgl II restriction endonuclease was performed in a final volume of 25 μL using 20 μL of the PCR reaction and 10 units of enzyme, according to the manufacturer’s instructions. SCF27 amplicons and SCC8 digestion products were divided electrophoretically in a 1.5 % agarose gel. The gels were stained with ethidium bromide. DNA was visualized by a UV transilluminator and photographed with a digital camera.

Both markers were scored on a single extraction of each genotype, only null alleles or ambiguous results were checked twice. In the case of samples with homozygous null alleles, their ability to amplify template DNA was successfully confirmed with SSR markers VVMD27 and VVMD7 under conditions described in Moravcova et al. (2006) and primers designed to amplify long PCR products in case of a malate dehydrogenase coding region (Nassuth et al. 2000).

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

**Results**

**Allelic distribution at SCC8 and SCF27 loci in a set of table grape varieties:** Both markers were scored in a set of table grape varieties (the SCC8 marker in a reduced number of the varieties used for the SCC8 marker), mainly bred in Central and Eastern Europe, divided into three groups (Tab. 1). The first group contained stenospermocarpic seedless genotypes, the second group was one genotype with hard seed traces but without endosperm, and the third contained seeded genotypes. According to information

**Table 1**

Parentage and genotypes at SCC8 and SCF27 loci of several stenospermocarpic seedless and seeded varieties

<table>
<thead>
<tr>
<th>Name</th>
<th>Parentage</th>
<th>SCC8&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SCF27&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Name</th>
<th>Parentage</th>
<th>SCC8&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SCF27&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>B2</td>
<td>BV 35-4-7 × B6 (+/-)</td>
<td>+/-</td>
<td>+/-</td>
<td>N&lt;sup&gt;c&lt;/sup&gt;</td>
<td>CR (+/+) × PA (+/+)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-/+&lt;sup&gt;0&lt;/sup&gt;</td>
<td>+/0&lt;sup&gt;0&lt;/sup&gt;</td>
</tr>
<tr>
<td>B3</td>
<td>BV 35-4-3 × B6 (+/-)</td>
<td>+/-</td>
<td>+/-</td>
<td>PI</td>
<td>CR (+/+) × DT (0/+)&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
<td>B6</td>
<td>AR × RS</td>
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<td>+/-</td>
<td>NA</td>
<td>RA</td>
<td>PL (-/0)* × JX (+/+)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>RO</td>
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<td>0/0</td>
<td>NA</td>
<td>SA</td>
<td>B (-?0&lt;sup&gt;c&lt;/sup&gt;) × S (+/0)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
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<td>CR (+/-) × V6 (+/+)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+/-</td>
<td>+/-</td>
<td>SD</td>
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<tr>
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<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>U (-/0)&lt;sup&gt;c&lt;/sup&gt; × KM (+/-)</td>
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<tr>
<td>KM</td>
<td>P (-/0)&lt;sup&gt;c&lt;/sup&gt; × KR (+/-)&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>V&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>C</td>
<td>MR × (CL (-/0)&lt;sup&gt;c&lt;/sup&gt; × S (+/0))</td>
<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>NA</td>
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<tr>
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<td>0/0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0/0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>M&lt;sup&gt;c&lt;/sup&gt;</td>
<td>CR (+/-) × QV (0/0) × AA (-/0)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
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**Stenospermocarpic genotype with hard seed traces but without endosperm**

<table>
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<th>Name</th>
<th>Parentage</th>
<th>SCC8&lt;sup&gt;a&lt;/sup&gt;</th>
<th>SCF27&lt;sup&gt;a&lt;/sup&gt;</th>
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<th>SCF27&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>AL</td>
<td>BO × LDS</td>
<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0/0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>O&lt;sup&gt;c&lt;/sup&gt;</td>
<td>KOL (0/+)* × BA (0/+)&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>unknown</td>
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<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>QV</td>
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<td>+/-&lt;sup&gt;c&lt;/sup&gt;</td>
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<sup>a</sup> Parentage with genotype at SCC8 locus if known.

<sup>b</sup> Genotype at SCC8 locus: +/- represents the two codominant alleles for seedless and seeded phenotype, respectively. 0 represents a null allele. +/- is for +/+ or +/0; +/- is for +/- or -/+0. At +/- and 0/0 is sure only that it contains the + and null allele, respectively.

<sup>c</sup> Genotype at SCF27 locus: +/ indicates the allele for seedlessness. 0 represents a null allele. +/- is for +/- or +/0.

<sup>d</sup> Presence or absence of a 0 allele was deduced from the analysed families or according to parentage.
from the developers (Lahogue et al. 1998), for the SCC8 marker we expected two different alleles, scc8− and SCC8+, (this linked to the seedless phenotype), and a null allele (Adam-Blondon et al. 2001). For the SCF27 marker, the presence or absence of the SCF27+ allele was expected, due to its association with the seedless phenotype (Meiša and Hinrichsen 2003).

Of 34 genotypes tested, four showed no amplification product at the SCC8 locus, confirming the existence of the null allele in a homozygous state. Two of these four, H and PN, belonged to the stenospermocarpic seedless group and the other two, O and QV, belonged to the group of seeded genotypes (Tab. 1).

Of twenty genotypes tested, six had no amplification product at the SCF27 locus. One of these genotypes, E, was from the stenospermocarpic seedless group and the other five genotypes, AL, K, O, QV and VI, belonged to the seeded genotypes group (Tab. 1). Contrary to O and QV, the other three genotypes, AL, K and VI, showed an amplification product at the SCC8 locus, representing the scc8+ allele. The stenospermocarpic seedless E also showed the scc8+ allele.

Of 24 stenospermocarpic seedless genotypes tested, twenty showed the SCC8+ allele. Of ten seeded genotypes tested (including MS), three had the SCC8− allele and seven did not (Tab. 1). Adding the present study varieties to those of Adam-Blondon et al. (2001), it is evident that the two subsets (i.e. stenospermocarpic seedless and seeded varieties at the SCC8 locus) showed different distribution of the genotypic classes ($\chi^2_D$), and both significantly differed from the expected 3:3:2:1 distribution (Adam-Blondon et al. 2001) in a panmictic population ($\chi^2_D$) (Tab. 2). Of 62 stenospermocarpic seedless genotypes from both studies, 52 (i.e. 84%) had at least one SCC8+ allele, and of 45 seeded genotypes tested, 35 (i.e. 78%) had no SCC8− allele.

Furthermore, parentage analysis enabled to elucidate complete or partial allelic distribution at the SCC8 locus. From the analysed varieties listed in the first column of Tab. 1, genotype at SCC8 locus of five varieties could be deduced. EB and M were SCC8+/0 instead of homozygous SCC8+, since the SCC8− allele was absent in their maternal (RE = 'Yantar' × 'Italia' where 'Yantar' = QV × AA) and paternal (QV × AA) grandparents, respectively.RY and UM were directly determined by the parents. UM being SCC8−? allowed the inference that both U and UM had a null allele, inherited from DT, since CR lacked the null allele. From the parental varieties listed in the second column of Tab. 1 (Twenty-seven seeded varieties ('Afus Ali' (AA), 'Arkadia' (AR), 'Bellino' (BO), 'Bicane' (B), 'Boskolenâ' (BA), BV 35-4-3, BV 35-4-7, 'Cardinal' (CL), 'Chaouch rose' (CR), 'Datil' (DL), 'Emperor' (ER), 'Gold' (G), 'Katta kurgan' (KK), 'Kossuth Lajos' (KOL), 'Lady Downes seedling' (LDS), 'Mirmii' (MI), 'Moscato rosa' (MR), 'Muscat of Alexandria' (MA), 'Pobeda' (P), 'Queen of Vineyards' (QV), 'Palatina' (PL), 'Pearl of Csaba' (PC), 'Red Malaga' (RM), 'Rusensko edro' (RE), 'Souvenir of Queen Elisabeth' (SQE), 'Tifafili Ahmer' (TA), 'Uranai' (U)) and twelve seedless varieties ('BV 47-1-6' (B6), 'Chibrid bezesmen V-6' (V6), 'Chibrid bezesmen VI-4' (VI4), 'Delight' (DT), 'Jupiter' (J), 'Kishmish moldavskii' (KM), 'Kishmish rozozyventing' ('Pink Sultana') (KR), 'Perletta' (PA), 'Remaily seedless' (RS), 'Ruby seedless' (RY), 'Sultana' (S), 'Sultana moscata' (SM)), together thirty-nine varieties, the genotype at SCC8 locus of 13 varieties, BA, DL, DT, ER, G, KK, KOL, P, PC, RE, SQE, V6 and VI4 could be completely or partially deduced.

Of ten stenospermocarpic seedless genotypes tested, nine (i.e. 90%) showed the SCF27+ allele. Of ten seeded genotypes tested (including MS), five (i.e. 50%) had no SCF27− allele (Tab. 1). However, taking into account the origin of these seeded varieties, those without the S variety in their parental varieties (AL, O, QV and VI) did not show the SCF27− allele. Five varieties (HP, K, L, MS and U) are direct descendants of seeded × seedless crosses, while the origin of CR is unknown.

Adam-Blondon et al. (2001) identified three seeded varieties carrying the SCC8− allele and probably the linked major locus involved in seedlessness, i.e. 'Chaouch blanc', 'Pizzutello nero' and 'Santa Paula'. We examined the rose form of the first one, CR, at both SCC8 and SCF27 loci. As expected, CR showed the SCC8+/scc8− genotype, simi-
larly to 'Chaouch blanc', and also the allele for seedlessness at the \textit{SCF27} locus', confirming that berry colour variants probably differ only in specific anthocyanin pathway genes.

To elucidate whether the \textit{SCC8} allele of CR is associated with the seedless phenotype, we tested its direct descendants, the stenospermocarpic seedless J, M, N and PI and the seeded U (Tab. 1). All showed the \textit{SCF27} allele (although PI was not tested at this locus); J, M and PI had the \textit{SCC8} allele, and N and U had the \textit{scc8} allele. Thus, there were inconsistencies in two genotypes: N showing no \textit{SCC8} allele and being seedless, and U showing the \textit{SCF27} allele and being seeded. Interestingly, M is a seedless offspring of two seeded varieties, and as expected had the alleles for seedlessness at both \textit{loci}.

\textbf{Analysis of \textit{SCC8} and \textit{SCF27} in the NKL, NSD and JKL full-sib families} as well as in individuals from other crosses: Produced by the CR \times PA cross, N was inferred to be heterozygous for a null allele at the \textit{SCC8} locus, due to the \textit{SCC8}/0 genotype of PA (ADAM-BLONDON et al. 2001). To determine which allele of N at the \textit{SCC8} locus was associated with its seedless phenotype, we examined the segregation of its alleles in two full-sib families, NKL and NSD. The JKL full-sib family and individuals from other crosses were also examined.

The genotypes of the parents and grandparents and the offspring individuals of the three families are summarized in Tab. 3. When the \textit{SCC8} marker was scored, the two stenospermocarpic seedless parents of the NKL family showed different alleles. The maternal parent, N, presented the allele \textit{scc8}, the paternal parent, KL, showed the allele \textit{SCC8}'s. For the \textit{SCF27} marker, both N and KL presented the \textit{SCF27} allele (Tabs 1 and 3A). Of 31 indi-

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{S} & \textbf{S'} & \textbf{NKL} & \textbf{NSD} & \textbf{JKL} \\
\hline
\textbf{CR} & \textbf{PA} & \textbf{CL} & \textbf{KR} & \textbf{CR} & \textbf{PA} & \textbf{DL} & \textbf{RY} \\
\hline
\textbf{S} & \textbf{S'} & \textbf{N} & \textbf{KL} & \textbf{S} & \textbf{S'} & \textbf{N} & \textbf{SD} \\
\hline
\textbf{SCC8} & \textbf{SCF27} & \textbf{S'} & \textbf{S'} & \textbf{S'} & \textbf{S'} & \textbf{S'} & \textbf{S'} \\
\hline
\end{tabular}
\caption{Parentage, segregation (S) of the SCAR markers and \textit{\chi} ² test of goodness-of-fit (\(a = 0.05\)) to the expected 1:1:1:1 distribution (A, B) in three analysed full-sib families (A, B, C) and several individuals from other different crosses (D), all obtained in vitro.}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{S} & \textbf{S'} & \textbf{S} & \textbf{S'} & \textbf{S} & \textbf{S'} & \textbf{S} & \textbf{S'} \\
\hline
\textbf{CR} & \textbf{PA} & \textbf{CL} & \textbf{KR} & \textbf{CR} & \textbf{PA} & \textbf{DL} & \textbf{RY} \\
\hline
\textbf{S} & \textbf{S'} & \textbf{N} & \textbf{KL} & \textbf{S} & \textbf{S'} & \textbf{N} & \textbf{SD} \\
\hline
\end{tabular}
\caption{Other different crosses: \textit{SCC8} and \textit{SCF27} alleles in other families.}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{JIA} \_90 & \textbf{+/?} & + \\
\textbf{JKM} \_85 & \textbf{+/?+} & + \\
\textbf{JMO} \_97 & \textbf{+/?} & + \\
\textbf{JPA} \_102 & \textbf{+/?} & + \\
\textbf{JPN} \_87 & \textbf{+/?} & + \\
\textbf{JPN} \_88 & \textbf{+/?} & + \\
\textbf{JR3} \_86 & \textbf{+/?} & + \\
\textbf{NCL} \_94 & \textbf{0/0} & 0 \\
\textbf{NMO} \_93 & \textbf{0/0} & + \\
\textbf{R3OP} \_92 & \textbf{0/0} & 0 \\
\textbf{R3OP} \_96 & \textbf{0/0} & + \\
\textbf{CR} & \textbf{+/?} & + \\
\textbf{V6} & \textbf{+/?} & + \\
\textbf{KL} & \textbf{+/?} & + \\
\textbf{JCL} & \textbf{+/?} & + \\
\textbf{KS} & \textbf{+/?} & + \\
\textbf{KL} & \textbf{+/?} & + \\
\end{tabular}
\caption{Legend: Phenotype\textsuperscript{a}: Genotype at \textit{SCF27} locus\textsuperscript{b}: Genotype at \textit{SCC8} locus\textsuperscript{c}: Parent name abbreviation}
\end{table}

\textsuperscript{a} \textit{S}, \textit{S'} represent the phenotype for seedlessness: seeded and seedless, respectively.
\textsuperscript{b} + indicates the allele for seedlessness. 0 represents a null allele. +/? is for +/+ or +/0. na means not analysed.
\textsuperscript{c} +, - represent the two codominant alleles, for seedless and seeded phenotype, respectively. 0 represents a null allele. +/? is for +/+ or +/0; +/? is for +/0 or -/0; +/? is for +/+ or +/0. For CL, KR and PA, the genotype was obtained by ADAM-BLONDON et al. (2001).

\textsuperscript{*} Presence or absence of a 0 allele was deduced from the analysed families or according to parentage.
individuals of the progeny, eight were heterozygous SCC8'/scc8' and seven showed no amplification product at the SCC8 locus. Therefore both parents must be heterozygous for a null allele, the presence of which clearly determined the remaining individuals being SCC8'/0 (seven individuals) and scc8'/0 (nine individuals). The distribution at the SCC8 marker locus did not deviate significantly from the expected 1:1:1:1 segregation ratio (Tab. 3 A). The segregation of 25 SCF27'/? individuals and six 0/0 individuals fitted a 3:1 ratio, indicating a null allele in both parents. All the SCC8'/scc8', SCC8'/0 and scc8'/0 individuals showed the SCF27' allele, whereas the 0/0 individuals at the SCC8 locus, with one exception, did not.

The NSD family had great similarities with the NKL family. When the SCC8 marker was scored, the two stenosperrmcarpic seedless parents of the NSD family showed different alleles. Similar to KL, the paternal parent, SD, showed the allele SCC8'. For the SCF27 marker, both N and SD presented the SCF27' allele (Tabs 1 and 3B). Of 26 individuals of the progeny, seven were heterozygous SCC8'/scc8' and four showed no amplification product at the SCC8 locus. Therefore both parents must be heterozygous for a null allele, the presence of which clearly determined the remaining individuals being SCC8'/0 (six individuals) and scc8'/0 (nine individuals). The distribution at the SCC8 marker locus did not deviate significantly from the expected 1:1:1:1 segregation ratio (Tab. 3 B). The segregation of 22 SCF27'/? individuals and four 0/0 individuals fitted a 3:1 ratio, indicating a null allele in both parents. All the SCC8'/scc8', SCC8'/0 and scc8'/0 individuals showed the SCF27' allele, whereas all the 0/0 individuals at the SCC8 locus did not.

In the NKL and NSD families, the SCF27' allele was mostly present, i.e. in 47 of the 57 individuals. This was in contrast to the SCC8 marker, which scored SCC8' in 28 of 57 individuals (Tab. 3 A and B). Detailed comparison of genotypic class distribution for both markers in the NKL and NSD families showed that all individuals without amplification products using the SCF27 marker were homozygous null genotypes at the SCC8 locus. Conversely, with one exception, the individuals without amplification products using the SCC8 marker were homozygous null genotypes at the SCF27 locus. Since the SCF27 marker, in contrast to SCC8, showed the expected allele for seedlessness in N, this marker was considered to show the correct genotype-phenotype association in this case. Thus, the scc8' allele should be associated with the sdi' allele in the scc8'/0 SCF27' individuals in the NKL and NSD families. Consequently, the NKL individual with homozygous null alleles at the SCC8 locus and containing the SCF27' allele (Tab. 3 A) should be a recombinant individual with recombination between the two marker loci and with a rate of recombination of 1 in 31 individuals, i.e. 3.23 %.

The two stenosperrmcarpic seedless parents of the JKL family, J and KL, both showed the alleles associated with the seedless phenotype at both loci, SCC8' and SCF27' (Tabs. 1 and 3 C). In the JKL progeny, the 12 individuals showed SCC8'/? and simultaneously SCF27' (Tab. 3 C). J was thus deduced to be homozygous for SCC8' and SCF27'. Indeed, the probability to observe at least one homozygous null individual between 12 individuals if both parents were heterozygous with a null allele is 1 - (0.75)^12 = 0.968. (Considering one individual, the probability to observe the SCC8' (or SCF27') allele is 0.75. Considering n individuals, the probability that all of them show the SCC8' (or SCF27') allele is (0.75)^n.)

Among individuals produced by other crosses, all descendants of J had SCC8' as well as SCF27' alleles, further confirming the homozygous status of J at both loci (Tab. 3 D). This information enabled the inference of the homozygous nature of JKM at least at the SCC8 locus. Consistent with the null allele of CL at the SCC8 locus, one descendant from the N × CL cross, NCL_94 was homozygous null at both loci. One individual from OP of R3, R3OP_92 revealed that R3 contains the null allele at both loci (Tabs 1 and 3D).

Veriﬁcation of the association of genotype with phenotype in two NKL seedlings: To further check the association of scc8' of N with sdi', we investigated the berries of two individuals from the NKL family. As expected, NKL_77, a SCC8'/scc8' SCF27' individual, was stenosperrmcarpic seedless. NKL_32, a scc8'/0 SCF27' individual, was seeded with 1.2 ± 0.4 (n = 5) seeds per berry. Along with the reduced seed number, these seeds were mostly underdeveloped, i.e. floaters.

Discussion

The presence of homozygous null alleles for both markers in several individuals can be an amplification problem. To check this, we veriﬁed the DNA quality with other PCR fragments, two SSR markers and primers designed to amplify long PCR products in case of a malate dehydrogenase coding region (Nassuth et al. 2000). As a result, all the questionable individuals produced the expected bands, conﬁrming the quality of DNA.

Thus, the presence of null alleles for the employed SCAR markers, SCC8 and SCF27, could be considered normal and, consequently, predicts the existence of homozygous null individuals at both loci simultaneously (Tabs. 1 and 3). Indeed, according to the developers' deﬁnition, all seeded individuals should be homozygous null for SCF27 (Meija and Hinchisen 2003) and in the case of SCC8 should contain the scc8' allele (LaRogue et al. 1998), which later turned out to be sometimes replaced by a null allele (Adam-Blondon et al. 2001). In fact, of 38 seeded genotypes tested by Adam-Blondon et al. (2001) only Al, 'Chaouch blanc' and MA did not contain this null allele with certainty. Therefore, the null allele (or pair of different null alleles) at the SCC8 locus, probably from a single nucleotide polymorphism in a site complementary to the primers (Dakin and Avise 2004), may be common in cultivated grapevine. All the scc8'/0 × scc8'/0, scc8'/0 × SCC8'/0 and SCC8'/0 × SCC8'/0 crosses would generate a majority of individuals with at least one null allele and 25 % would be homozygous null. The consequences of this phenomenon are summarized in Tab. 4. Of 25 stenosperrmcarpic genotypes tested, 21 had at least one SCC8'.
Table 4

Segregation, possibility of the occurrence of seedless phenotype and behaviour of null alleles in crosses between two seeded (A), seeded and seedless (B) and two seedless (C, D) individuals both heterozygous for a null allele at SCC8 locus

<table>
<thead>
<tr>
<th>Table 4</th>
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<tbody>
<tr>
<td>A. Cross between two seeded individuals both heterozygous for a 0 allele at SCC8 locus</td>
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<td>B. Cross between seeded and seedless individuals both heterozygous for a 0 allele at SCC8 locus</td>
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SCC8/? individuals can be seedless. All individuals 0/0 at SCC8 locus show a null allele at SCC8 locus.

Legend:
- +/- indicate the allele for seedless and seeded phenotype, respectively.
- a + indicates the allele for seedless phenotype.
- 0 represents a null allele.
- c +, - represent the two codominant alleles for seedless and seeded phenotype, respectively.
- 0 represents a null allele.

Individuals in the first two columns can be seedless. All individuals 0/0 at SCC8 locus show a null allele at SCC8 locus. However, scc8/? individuals also show a null allele at SCC8 locus.

Individuals in the first three columns can be seedless. All individuals 0/0 at SCC8 locus show a null allele at SCC8 locus. However, scc8/0 individuals do not show a null allele at SCC8 locus.

Considering that the genetic distance between the SCC8 and sfd loci was estimated at 0.7 and 4.0 cM (Lahogue et al. 1998, Adam-Blondon et al. 2001), respectively, the recombinations within these loci during breeding could explain seedlessness in genotypes without the SCC8+ allele. This may also be true for N, for which there are two possi-
bilities. Both parents of N had the SCC8 allele, and could have passed the linked sdI allele to N after recombination.

CR has no null allele, so the scc8 allele of N was inherited from this variety and the null allele from PA, enabling clear conclusions on inheritance of progeny. In the NKL and NSD families, the scc8/scc8 and scc8/0 individuals can be seedless because of the SCC8 allele inherited from paternal genotypes KL or SD. As we do not have the phenotypic data yet, it is difficult to determine whether the genotypes scc8/0 or 0/0 will be seedless. There is, however, some evidence of the right choice regarding seedlessness of scc8/0 individuals. First, the behaviour of the SCF27 marker, which showed the right genotype–phenotype association in the case of N, was considered. Based on the fact that in the NKL and NSD families, with one exception, all individuals 0/0 at the SCC8 locus showed a null allele at SCF27 locus, and scc8/0 individuals did not show a null allele at the SCF27 locus (Tab. 3A and B), the scc8 allele should be associated with sdI (Tab. 4 D1). This is due to recombination during ovule development in CR. Second, although the fruit-bearing NKL_32 was not seedless as expected, it showed reduced seed number per berry with a majority of the seeds being floaters. There are reports (Doligez et al. 2002, Cabezás et al. 2006) that the major QTL on LG18 can also influence seed number in the berry, thus NKL_32 could harbour the sdI allele along with unfavourable operator genes for seedlessness, as defined by Bouquet and Danglot (1996). Therefore, the phenotype of other individuals of the investigated families would be of great interest, although they could contain some recombinant individuals and also sdI individuals with unfavourable operator genes.

The scc8/0 individuals in the NKL and NSD families are expected to harbour the sdI allele due to recombination and not due to primer site misrecognition, since their scc8 allele was obtained after digestion of an SCC8 allele (Lahogue et al. 1998) and their null allele at the SCC8 locus was inherited from KL, hence from CL or from SD, hence from AA (DL being an AA × MA cross), and both null alleles were shown to be associated with sdI (Adam-Blondon et al. 2001).

Thus, the SCC8 allele of CR is coupled with the sdI allele. Along with the evidence for N, the most convincing argument for this is in M, a seedless variety with unfavourable operator genes.

Interestingly, CR and S belong to different geographical-ecological variety groups, CR is conv. pontica and S is conv. orientalis (Kozma 1991). Nevertheless, if we consider the glabrous leaves of S as a result of recessive genes, then CR with its hairy leaves (a dominant trait) can be a direct or distant offspring of S or its relatives, which is to be determined by SSR analysis in the future. Although considered as seeded, CR also has a huge amount of stenospermocarpic seeds with no endosperm and brown or black (precociously dead), but relatively soft testa (data not shown), as further evidence for the sdI allele. The case of CR is quite interesting and similar to those of ‘Pizzutello nero’ and ‘Santa Paula’ with arch-shaped berries as a result of stenospermocarpic affecting some seeds in the berry. The case of NKL_32 and the one-seeded (data not shown) L in our set of varieties also seems similar. The case of the seeded HP and U, both with the SCF27 allele, needs to be tested. Taking into account that U had some stenospermocarpic seeds while HP did not (data not shown), it is expected that U harbours the sdI allele (recombination between SCC8 and sdI) but HP does not (recombination between SCF27 and sdI).

Other seeded varieties that are now thought to be very useful in seedless table grape breeding because of promoting seedlessness are ‘Yantar’ (Perl et al. 2003) and its parents QV (Sz. Nagy, pers. comm.) and AA (Todorov 2000) as well as ‘Diamant’ (Bakonyi and Kocsis 2006) (resulting from the cross ‘Yulski biser’ × ‘Pannonia kinces’ (Pospíšilová and Korpás 1998), thus an indirect descendant of both AA and QV). The case of the seedless variety ‘Sáha királynője’ (‘Helikon’ × ‘Diamant’) seems similar to the case of M. Here, the putative sdI allele was probably inherited from seeded ‘Helikon’, a direct descendant of S. The mechanism of seedlessness from these varieties is not known, but most probably they contain no sdI allele at the sdI locus and harbour homozygous recessive operator genes responsible for seedlessness when they are crossed to seedless or even seeded varieties containing the sdI allele such as CR. Nevertheless, the present results indicating QV is homozygous null at the SCC8 locus (Tab. 1) and gaps in the parentage of QV leave open the hypothesis that QV harbours the sdI allele at the sdI locus. Interestingly, PA is the common paternal genotype for three putatively recombinant varieties in our set, H, N and PN; and the null allele of PA, which was inherited from QV, was passed to the progeny. However, the most probable explanation of seedlessness of these varieties is that both H and PN are seedless due to sdI and a recombination occurred between
the SCC8 and sdl loci during pollen development in PA; and N is seedless due to sdl" and a recombination occurred between the SCC8 and sdl loci during ovule development in CR.

Thus, we showed that null alleles at the SCC8 locus, along with being associated with the seeded phenotype, can also be coupled with sdl". Their use should be avoided, apart from special cases where the null allele at the SCC8 locus can be useful (when only one parent possesses the null allele, as in the cross CR × PA; or along with one null allele for each, the two seedless parents have different alleles, SCC8" and SCC8", respectively, as in the cross N × KL). Further, scoring is difficult and their presence in the homozygous state evokes additional verifying of DNA quality. Identifying the concrete major genomic region involved in seedlessness, probably VvMADS5, as well as other loci would therefore be of great promise in this field.

Conclusions

The present results show that both SCC8" and SCF27" are linked to sdl", a necessary but not sufficient locus for the seedless phenotype in grapevine. This supports the idea that along with the sdl locus there are probably other loci involved in seed development, a quite complex process.

It is evident from allelic distribution that there are seeded varieties with potential to promote seedlessness. These varieties can be divided into two groups. The first includes varieties that harbour the sdl" allele and can be selected using appropriate markers such as SCC8, SCF27 and VMC7f2 with great precision: 'Chaouch blanc', 'Chaouch rose', 'Luna' and probably 'Helikon' and 'Uraan'. The presence of stenospermocarpic seeds along with normal seeds in seedlessness may produce complications. Even the promising 'Jupiter' can be heterozygous at the sdl locus due to recombination.

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