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

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Summary

Two working sequence-characterised 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 seedless and seeded varieties, as well as three segregating progenies, 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.

K e y   w o r d s : 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, Fait *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 Danglot (1996), stenospermocarpic seedlessness might be controlled by three complementary recessive genes, independently inherited and regulated by a dominant gene, named *sdI* (**s**eed **d**evelopment **i**nhibitor). 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 (Lahogue *et al*. 1998), SCP18 (Adam-Blondon *et al*. 2001) and SCF27 (Mejía and Hinrichsen 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, MEJÍA *et al.* 2007, COSTANTINI *et al.* 2008). All studies dealing with seedlessness (DOLIGEZ *et al.* 2002, CA-BEZAS *et al.* 2006, MEJÍA *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 *sdI* (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 phenology-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, MEJÍA *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 seedless 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, 'Chaouch blanc', 'Pizzutello nero' and 'Santa Paula', had at least one $SCC8^+$ allele. In addition, there may be seeded varieties with no sdI^+ allele but with favourable operator genes, as defined by BOUQUET and DANGLOT (1996).

'Chaouch rose', the rose form of 'Chaouch blanc', was used extensively in seedless table grape breeding in the former Czechoslovakia in the 1960s and 1970s and several improved varieties were selected (POSPÍŠILOVÁ 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 'Neptuun', 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

P l a n t   m a t e r i a l : 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), 'Kishmish luchistyi' (KL), 'Kishmish moldavskii' (KM), 'Merkuur' (M), 'Neptuun' (N), 'Perlon' (PN), 'Picurka' (PI), 'Rosina' (RA), 'Roozsika' (RO), 'Ruby seedless' (RY), 'Rusalka 3' (R3), 'Slavianka' (SA), 'Sunred seedless' (SD), 'Urkim' (UM) and 'Venusha' (V)), one genotype with hard seed traces but without endosperm ('Mars' (MS)) and nine seeded genotypes ('Alphonse Lavallée' (AL), 'Chaouch 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 × 'Marroo seedless' (MO)), JPA_102 (J × PA), JPN_87, JPN_88 (both J × PN), JR3_86 (J × R3), 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.

D N A   e x t r a c t i o n : 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.

S C A R   a n a l y s i s : Two RAPD-derived SCAR markers were used: SCC8 (Lahogue *et al.* 1998) and SCF27 (Mejía and Hinrichsen 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 Moravcová *et al.* (2006) and primers designed to amplify long PCR products in case of a malate dehydrogenase coding region (Nassuth *et al.* 2000).

S t a t i s t i c a l   a n a l y s i s : Goodness-of-fit between observed and expected segregation ratios at the different *loci*, as well as the likelihood of independence between *SCC8* and *sdI* were tested using a Chi-square analysis.

Results

A l l e l i c   d i s t r i b u t i o n   a t   *S C C 8*   a n d   *S C F 2 7   l o c i*   i n   a   s e t   o f   t a b l e   g r a p e   v a r i e t i e s : Both markers were scored in a set of table grape varieties (the SCF27 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

T a b l e   1

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

Name	Parentage[a]	SCC8[b]	SCF27[c]	Name	Parentage[a]	SCC8[b]	SCF27[c]
Stenospermocarpic seedless genotypes				N[e]	CR (+/-) × PA (+/0)[d]	-/0*	+/0*
B2	BV 35-4-7 × B6 (+/-)	+/?	na	PI	CR (+/-) × DT (0/)*	+/?	na
B3	BV 35-4-3 × B6 (+/-)	+/?	na	PN	ER (-/0)* × PA (+/0)[d]	0/0	+/?
B6	AR × RS	+/-	na	RA	PL (-/0)* × J[e] (+/+)*	+/-	na
D	G (-/?)* × PA (+/0)[d]	+/-	+/?	RO	PL (-/0)* × J[e] (+/+)*	+/-	na
E	U (-/0)* × KM (+/-)	-/?	0/0	RY	ER (-/0)* × SM (+/-)[d]	+/0*	na
EB	RE (0/)* × VI4 (+/)*	+/0*	na	R3	MI × V6 (+/)*	+/0*	+/0*
H	KK (0/)* × PA (+/0)[d]	0/0	na	SA	B (-/?)[d] × S (+/?)[d]	+/-	na
J[e]	CR (+/-) × V6 (+/)*	+/+*	+/+*	SD	DL (-/0)* × RY (+/0)*	+/0*	+/0*
KL	CL (-/0)[d] × KR (+/?)[d]	+/0*	+/0*	UM	U (-/0)* × KM (+/-)	+/0*	na
KM	P (-/?)* × KR (+/?)[d]	+/-	na	V[e]	KK (0/)* × V6 (+/)*	+/?	+/?
C	MR × (CL (-/0)[d] × S (+/?)[d])					+/?	na
F	(CL (-/0)[d] × S (+/?)[d]) × ((RM × TA) × (MA (-/-)[d] × S (+/?)[d]))					+/?[d]	na
M	CR (+/-) × (QV (0/0) × AA (-/0)[d])					+/0*	+/?
Stenospermocarpic genotype with hard seed traces but without endosperm							
MS[e]	KK (0/)* × V6 (+/)*	+/-	+/?				
Seeded genotypes				L	KK (0/)* × PA (+/0)[d]	+/?	+/?
AL	BO × LDS	-/-[d]	0/0	O	KOL (0/)* × BA (0/)*	0/0	0/0
CR	unknown	+/-	+/?	QV	SQE (0/)* × PC (0/)*	0/0	0/0
HP	KK (0/)* × V6 (+/)*	-/?	+/?	U	CR (+/-) × DT (0/)*	-/0*	+/?
K	KK (0/)* × V6 (+/)*	-/?	0/0	VI	CL (-/0)[d] × AA (-/0)[d]	-/?	0/0

[a] Parentage with genotype at *SCC8 locus* if known.

[b] Genotype at *SCC8 locus*: +, - represent 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/ is sure only that it contains the + and null allele, respectively.

[c] Genotype at *SCF27 locus*: + indicates the allele for seedlessness. *0* represents a null allele. +/? is for +/+ or +/0. na means not analysed.

[d] Genotype at *SCC8 locus* obtained by Lahogue *et al.* (1998) or Adam-Blondon *et al.* (2001).

[e] The varieties indicated are pure *V. vinifera* varieties and are not to be confused with Arkansas varieties with similar names (see parentage).

* 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 (Mejía 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 (χ^2_R), and both significantly differed from the expected 3:3:2:1 distribution (Adam-Blondon *et al.* 2001) in a panmictic population (χ^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), 'Arkadiia' (AR), 'Bellino' (BO), 'Bicane' (B), 'Boskolena' (BA), BV 35-4-3, BV 35-4-7, 'Cardinal' (CL), 'Chaouch rose' (CR), 'Datal' (DL), 'Emperor' (ER), 'Gold' (G), 'Katta kurgan' (KK), 'Kossuth Lajos' (KOL), 'Lady Downes seedling' (LDS), 'Mirnii' (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), 'Tifafihi Ahmer' (TA), 'Uraan' (U)) and twelve seedless varieties ('BV 47-1-6' (B6), 'Chibrid bezsemen V-6' (V6), 'Chibrid bezsemen VI-4' (VI4), 'Delight' (DT), 'Jupiter' (J), 'Kishmish moldavskii' (KM), 'Kishmish rozovyi' (= '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 parentage (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-

Table 2

Distribution of the genotypic classes at the *SCC8 locus* in the subsets of stenospermocarpic seedless and seeded varieties described in Tab. 1 and by Adam-Blondon *et al.* (2001), χ^2 test of goodness-of-fit ($\alpha = 0.05$) to the expected 3:3:2:1 distribution (χ^2_D) and χ^2 test of the difference ($\alpha = 0.05$) of genotypic distribution between the two subsets (χ^2_R)

	SCC8⁺/?	*scc8⁻/?*	*SCC8⁺/scc8⁻*	*0/0*	Total	$\chi^{2\,a}$
Seedless	37	8	15	2	62	$\chi^2_D=20.13$
Seeded	7	29	3[b]	6	45	$\chi^2_D=20.25$
Total	44	37	18	8	107	$\chi^2_R=40.67$

[a] For the χ^2 calculation, classes were merged so that the presence (*SCC8⁺/?*, *SCC8⁺/scc8⁻*) *versus* the absence (*scc8⁻/?*, *0/0*) of *SCC8⁺*, *i.e.* the 5:4 ratio was tested with 1 degree of freedom.
[b] Including the stenospermocarpic genotype with hard seed traces but without endosperm, 'Mars'.

larly to 'Chaouch blanc', and also the allele for seedlessness at the *SCF27 locus*, confirming that berry colour variants probably differ only in specific anthocyanin pathway genes.

To elucidate whether the *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 *SCF27+* allele (although PI was not tested at this *locus*); J, M and PI had the *SCC8+* allele, and N and U had the *scc8−* allele. Thus, there were inconsistencies in two genotypes: N showing no *SCC8+* allele and being seedless, and U showing the *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 *loci*.

Analysis of SCC8 and SCF27 in the NKL, NSD and JKL full-sib families

as well as in individuals from other crosses: Produced by the CR × PA cross, N was inferred to be heterozygous for a null allele at the *SCC8 locus*, due to the *SCC8+/0* genotype of PA (ADAM-BLONDON *et al*. 2001). To determine which allele of N at the *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 SCC8 marker was scored, the two stenospermocarpic seedless parents of the NKL family showed different alleles. The maternal parent, N, presented the allele *scc8−*, the paternal parent, KL, showed the allele *SCC8+*. For the SCF27 marker, both N and KL presented the *SCF27+* allele (Tabs 1 and 3A). Of 31 indi-

T a b l e 3

Parentage, segregation (S) of the SCAR markers and χ^2 test of goodness-of-fit (α = 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*

A — NKL

Parents: CR (S−, +/?, +/−) × PA (S+, na, +/0) → N (S+, +/0, −/0); CL (S−, na, −/0) × KR (S+, na, +/?) → KL (S+, +/0, +/0); N × KL ►

NKL		SCC8						
		+/−	+/0	−/0	0/0	Σ	S	χ^2
SCF27	+	8	7	9	1	25	3	0.53
	0	0	0	0	6	6	1	
	Σ	8	7	9	7	31		
	S	1	1	1	1			
	χ^2		0.36					A

B — NSD

Parents: CR (S−, +/?, +/−) × PA (S+, na, +/0) → N (S+, +/0, −/0); DL (S−, na, −/0) × RY (S+, na, +/0) → SD (S+, +/0, +/0); N × SD ►

NSD		SCC8						
		+/−	+/0	−/0	0/0	Σ	S	χ^2
SCF27	+	7	6	9	0	22	3	1.28
	0	0	0	0	4	4	1	
	Σ	7	6	9	4	26		
	S	1	1	1	1			
	χ^2		2.00					B

D

Other different crosses	SCC8	SCF27
JIA_90	+/?	+
JKM_85	+/+*	+
JMO_97	+/−	+
JPA_102	+/?	+
JPN_87	+/0*	+
JPN_88	+/0*	+
JR3_86	+/?	+
NCL_94	0/0	0
NMO_93	−/?	+
R3OP_92	0/0	0
R3OP_96	+/?	+

C — JKL

Parents: CR (S−, +/?, +/−) × V6 (S+, na, +/) → J (S+, +/+, +/+); KL (S+, +/0, +/0); J × KL ►

JKL		SCC8
		+/?
SCF27	+/?	12
		C

Legend:
Phenotype[a] — S+
Genotype at *SCF27 locus*[b] — +/0
Genotype at *SCC8 locus*[c] — +/0
Parent name abbreviation — KL

[a] S−, S+ represent the phenotype for seedlessness: seeded and seedless, respectively.

[b] + indicates the allele for seedlessness. *0* represents a null allele. +/? is for +/+ or +/0. na means not analysed.

[c] +, − represent the two codominant alleles, for seedless and seeded phenotype, respectively. *0* represents a null allele. +/? is for +/+ or +/0; −/? is for −/− or −/0; +/ is for +/− or +/0. For CL, KR and PA, the genotype was obtained by ADAM-BLONDON *et al*. (2001).

* Presence or absence of a *0* allele was deduced from the analysed families or according to parentage.

viduals 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 stenospermocarpic 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 stenospermocarpic 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 ho-

mozygous 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_85 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 stenospermocarpic 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 (Mejía and Hinrichsen 2003) and in the case of SCC8 should contain the $scc8^-$ allele (Lahogue *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 stenospermocarpic genotypes tested, 21 had at least one $SCC8^+$

T a b l e   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*

A. Cross between two seeded individuals both heterozygous for a *0* allele at *SCC8 locus*

-/-		-/-
0	×	0
-/0		-/0

▼

-/-	:	-/-	:	-/-
0	:	0	:	0
-/-	:	-/0	:	0/0
1	:	2	:	1
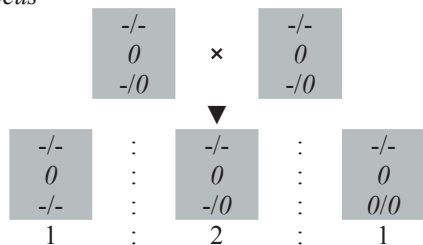
Only seeded individuals.
All individuals *0/0* at *SCC8 locus* show a null allele at *SCF27 locus*.
However, *scc8⁻/?* individuals also show a null allele at *SCF27 locus*.

B. Cross between seeded and seedless individuals both heterozygous for a *0* allele at *SCC8 locus*

-/-		+/-
0	×	+
-/0		+/0

▼

+/-	:	+/-	:	-/-	:	-/-
+	:	+	:	0	:	0
+/-	:	+/0	:	-/0	:	0/0
1	:	1	:	1	:	1
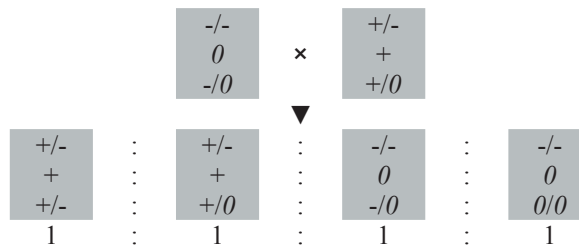
Individuals in the first two columns can be seedless.
All individuals *0/0* at *SCC8 locus* show a null allele at *SCF27 locus*.
However, *scc8⁻/0* individuals also show a null allele at *SCF27 locus*.

C. Cross between two seedless individuals both heterozygous for a *0* allele at *SCC8 locus*

+/-		+/-
+	×	+
+/0		+/0

▼

+/+	:	+/-	:	-/-
+	:	+	:	0
+/+	:	+/0	:	0/0
1	:	2	:	1
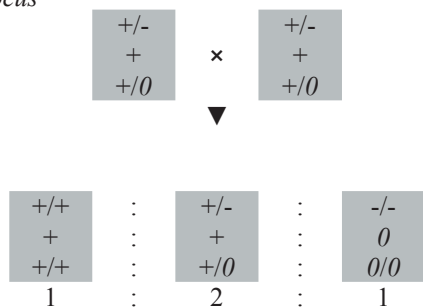
SCC8⁺/? individuals can be seedless.
All individuals *0/0* at *SCC8 locus* show a null allele at *SCF27 locus*.

D. Cross between two seedless individuals both heterozygous for a *0* allele at *SCC8 locus* and one showing no *SCC8⁺* allele

+/-		+/-
+	×	+
-/0		+/0

▼

D1: The mother is recombinant between *SCC8* and *sdI* and *scc8⁻* serves for *sdI⁺*
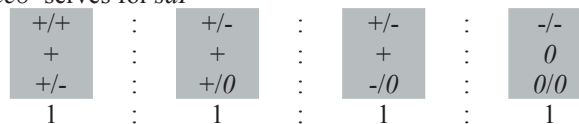
+/+	:	+/-	:	+/-	:	-/-
+	:	+	:	+	:	0
+/-	:	+/0	:	-/0	:	0/0
1	:	1	:	1	:	1

Individuals in the first three columns can be seedless.
All individuals *0/0* at *SCC8 locus* show a null allele at *SCF27 locus*.
However, *scc8⁻/0* individuals do not show a null allele at *SCF27 locus*.

D2: The mother is recombinant between *SCC8* and *sdI* (but not necessarily) and the null allele at *SCC8 locus* serves for *sdI⁺*

+/+	:	+/-	:	+/-	:	-/-
+	:	+	:	+	:	0
+/0	:	+/-	:	0/0	:	-/0
1	:	1	:	1	:	1

Individuals in the first three columns can be seedless.
Individuals *0/0* at *SCC8 locus* do not show a null allele at *SCF27 locus*.
However, *scc8⁻/0* individuals show a null allele at *SCF27 locus*.

Legend:

Genotype at *sdI locus*[a]	+/-
Genotype at *SCF27 locus*[b]	+
Genotype at *SCC8 locus*[c]	+/-
Segregation	1

[a] +, - indicate the allele for seedless and seeded phenotype, respectively.
[b] + 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.

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allele (Tab. 1). From the remaining four genotypes, H and PN were homozygous null, N was *scc8⁻/0* and E was *scc8⁻/?*. This raised the question of why E, H, N, PN and other genotypes, described by ADAM-BLONDON *et al.* (2001) (such as 3041-153, 3048-30, 'Sugraone' and 'Yaghasti') with no *SCC8⁺* allele were seedless.

Considering that the genetic distance between the *SCC8* and *sdI 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, Cabezas *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 from the cross between two seeded varieties, CR and a Bulgarian selection from the cross QV × AA, sister of or Yantar itself. Despite its unknown origin, CR was used extensively in breeding programs (Pospíšilová and Korpás 1998) as represented by our set of varieties, evidently due to its functionally female flowers that need no emasculation. The working hypothesis that genotypes with functionally female flowers promote seedlessness (Smirnov 1962, cit. in Pospíšilová and Korpás 1998) has to be refined. It seems that the large number of seedlings easily produced and the excessive vigour in connection with hormonal content rather than favourable genetic constitution at the *sdI locus* of genotypes with functionally female flowers favours the selection process. However, some of them harbour the *sdI⁺* allele, as for CR, and some do not, as for 'Bicane' (Adam-Blondon *et al.* 2001). Nevertheless, further tests are required in this field, involving other genotypes such as KK, 'Madeleine angevine', 'Nimrang' and others, and closer markers to the *sdI locus* such as VMC7f2. This would help determine whether the null allele at the *SCC8 locus* is associated with the *sdI⁺* allele in these varieties. However, the molecular pattern of S at the *VMC7f2 locus* is not specific, as for the seeded 'Cabernet Sauvignon', the most famous red-wine variety, and for the seeded botanical species *Vitis riparia* Michx., both showing the same alleles as S at this *locus* (Pellerone *et al.* 2001).

Interestingly, CR and S belong to different geographical-ecological variety groups, CR is *convarietas pontica* and S is *convarietas 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 stenospermocarpy 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' × 'Pannónia kincse' (Pospíšilová and Korpás 1998), thus an indirect descendant of both AA and QV). The case of the seedless variety 'Sába 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 *sdI loci* during pollen development in PA; and N is seedless due to *sdI⁺* and a recombination occurred between the *SCC8* and *sdI 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 *sdI⁺*. 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 *sdI⁺*, a necessary but not sufficient *locus* for the seedless phenotype in grapevine. This supports the idea that along with the *sdI 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 *sdI⁺* 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 these varieties could aid their identification. In fact, all seeded individuals from crosses between *sdI⁻/sdI⁻* (or *sdI⁺/sdI⁻*) and *sdI⁺/sdI⁺* individuals should belong to this group.

The second group includes varieties that do not harbour the *sdI⁺* allele but contain favourable operator genes as defined by Bouquet and Danglot (1996): 'Afus Ali', 'Diamant', 'Queen of Vineyards' and 'Yantar'. There is a need for appropriate markers linked to these *loci*.

As seen in the case of 'Merkuur', if crossed together these seeded varieties from different groups can produce seedless individuals.

However, the presence of null alleles and the genetic distance of markers from the *sdI locus* involved in seedlessness may produce complications. Even the promising *SCC8⁺/SCC8⁺ SCF27⁺/SCF27⁺* 'Jupiter' can be heterozygous at the *sdI locus* due to recombination.

Acknowledgements

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