

Using SCC8, SCF27 and VMC7f2 markers in grapevine breeding for seedlessness via marker assisted selection

M. Akkurt¹, A. Çakır¹, M. Shidfar¹, B.P. Çelikkol² and G. Söylemezoğlu¹

¹Department of Horticulture, Faculty of Agriculture, Ankara University, Ankara, Turkey

²Biotechnology Institute, Ankara University, Ankara, Turkey

Corresponding author: M. Akkurt
E-mail: akkurt@agri.ankara.edu.tr

Genet. Mol. Res. 11 (3): 2288-2294 (2012)

Received December 3, 2011

Accepted March 8, 2012

Published August 13, 2012

DOI <http://dx.doi.org/10.4238/2012.August.13.2>

ABSTRACT. We used molecular markers associated with seedlessness in grapes, namely SCC8, SCF27 and VMC7f2, to improve the efficiency of seedless grapevine breeding via marker assisted selection (MAS). DNA from 372 F₁ hybrid progeny from the cross between seeded “Alphonse Lavallée” and seedless “Sultani” was amplified by PCR using three markers. After digestion of SCC8 marker amplification products by restriction enzyme *Bgl*III, 40 individuals showed homozygous SCC8+/SCC8+ alleles at the seed development inhibitor (*Sd1*) locus. DNA from 80 of the progeny amplified with the SCF27 marker produced bands; 174 individuals had 198-bp alleles of the VMC7f2 marker associated with seedlessness. In the second year, based on MAS, 183 F₁ hybrids were designated as seedless grapevine candidates because they were positive for a minimum of one marker. Twenty individuals were selected as genetic resources for future studies on seedless grapevine breeding because they carried alleles for the three markers associated with seedlessness. The VMC7f2 SSR marker was identified as the marker most associated with seedlessness.

Key words: Grapevine breeding; Marker assisted selection; Seedlessness; Molecular marker

INTRODUCTION

Seedless grapes, which are consumed fresh or dried, are among the most widely produced grape cultivars in the world. There are 2 types of seedlessness in grapevines, namely parthenocarpic seedlessness, in which the ovule develops without fertilization, and stenospermocarpic seedlessness, which is characterized by the abortion of the seeds soon after fertilization. The majority of seedless grapes have the latter type of seedlessness.

The conventional breeding method used for production of new seedless grape cultivars is based on seeded x seedless hybridizations (Bouquet and Danglot, 1996; Lahogue et al., 1998; This et al., 2000). However, the use of conventional hybridization methods in grapevines has significant disadvantages. Due to the heterozygous genetic structure of grapevine, the F_1 hybrids display wide variability, leading to low proportions of the desired individuals among the progeny. In order to eliminate this disadvantage and to increase the frequency of seedless individuals in the progeny, seedless x seedless grape hybridization via *in vitro* embryo rescue is now employed (Cain et al., 1983; Emershard and Ramming, 1984; Spiegel-Roy et al., 1985; Tangolar et al., 1999; Mejia and Hinrichsen, 2003). Despite intensive and time-consuming laboratory studies on *in vitro* embryo culture techniques, the number of F_1 hybrids acquired by this method remains inadequate for breeding studies on seedlessness (This et al., 2000). Another important disadvantage of hybridization breeding is the delay in the detection of seedless individuals in F_1 hybrids until the vines are 4-5 years of age, because of the long-lasting juvenile sterility period. Hence, the production of new cultivars in woody perennials, such as grapevines, by conventional hybridization methods is costly, labor-intensive, as well as time- and space-consuming.

In recent years, based on the advantages associated with molecular markers, the biotechnological marker assisted selection (MAS) technique has been employed in conventional breeding studies with markers linked to the seedlessness trait (Lahogue et al., 1998; Adam-Blondon et al., 2001; Mejia and Hinrichsen, 2003; Fatahi et al., 2004; Cabezas et al., 2006).

Molecular marker techniques have also been used in genetic mapping studies conducted to identify the genetic mechanism of seedlessness. In genetic mapping based on progeny obtained from seedless grape parents, chromosomes and loci linked to seedlessness were identified. Researchers have identified a major quantitative trait locus (QTL) linked to seedlessness on chromosome 18 (Doligez et al., 2002; Fanizza et al., 2005; Cabezas et al., 2006; Constantini et al., 2008; Mejia et al., 2007).

In this study, DNA from F_1 hybrids obtained by hybridization of “Alphonse Lavallée” with “Sultani” was amplified with primers for the molecular markers linked to seedlessness by PCR. The purpose of the study was to achieve higher frequencies of seedless individuals as well as early selection by MAS. For this purpose, three markers linked to seedlessness were used to improve the efficiency of breeding practices for future studies on seedlessness.

MATERIAL AND METHODS

Plant material and DNA extraction

A total of 372 F_1 hybrids obtained from a cross between “Alphonse Lavallée” and “Sultani” were used in this study. F_1 plants were grown in greenhouses of Department of Hor-

ticulture, Faculty of Agriculture, Ankara University, Ankara. When the hybrids produced just about 4-5 true leaves, young leaf samples were taken for DNA analyses and stored at -80°C until DNA isolation.

DNA was isolated from young leaves by using the Wizard Genomic DNA Purification Kit (Promega, Madison, USA) according to the protocol described by the manufacturer.

Molecular markers

Primers for markers previously identified as linked to seedlessness in grapevines, namely, the primers for the sequence characterized amplified region (SCAR) markers SCC8 (Lahogue et al., 1998) and SCF27 (Mejia and Hinrichsen, 2003) and simple sequence repeat (SSR) marker VMC7f2 (Cabezas et al., 2006), were selected for genotypic analyses of seedlessness. Genomic DNA from F₁ hybrids was amplified by PCR using the selected primers.

PCRs for SCAR primers were performed in a total volume of 20 µL containing 0.25 mM of each dNTP, 0.25 µM of each primer, 0.5 U Taq DNA polymerase, 1.5 mM MgCl₂, and 20-40 ng template DNA. Temperature profiles were run in Biometra T-1 Thermoblock (Biometra, Göttingen, Germany) and consisted of an initial denaturation step at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing for 1 min, a synthesis step at 72°C for 2 min, and final extension at 72°C for 10 min. A touch-down PCR amplification was programmed for the SCF27 primer, consisting of 30 cycles of denaturation (3 min at 94°C), annealing (1 min), and extension (2 min, at 72°C). The annealing temperature was 55°C for the first cycle, was after which it was reduced by 1°C for the next 5 cycles and maintained at 51°C for the last 25 cycles. PCR amplifications were performed for the VMC7f2 SSR primers in a reaction volume of 10 µL containing 15-ng template DNA, 0.25 µM of each primer, 0.25 mM of each dNTP, 0.5 U Taq DNA polymerase, and 1.5 mM MgCl₂. Forward primers were labeled with WellRED fluorescent dyes D2 (black), D3 (green), and D4 (blue) (Prologo, Paris, France). The PCR conditions were as follows: an initial cycle of 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 51°C, 2 min at 72°C, and a final extension at 72°C for 10 min. The PCR products were diluted with a sample-loading solution (SLS) in certain proportions according to the fluorescent dyes used in labeling, followed by the addition of Genomelab DNA Standard Kit-400 and electrophoresed in the CEQ 8800XL capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA). Allele sizes were determined using a Beckman CEQ fragment analysis software.

In each PCR run, "Sultani" was included as the reference cultivar because it provides amplification products with all 3 markers. These analyses were repeated at least twice to ensure reproducibility of the results.

Amplification products obtained using SCC8 primers were first digested with the restriction enzyme *Bgl*III, according to the protocol described by Lahogue et al. (1998). Digestion products of SCC8, and amplification products obtained using SCF27 SCAR primers were resolved by electrophoresis on 2% agarose gels, visualized under UV light, and documented using a Bio Imaging System (Syngene, Cambridge, UK).

Data analysis

Markers were analyzed for all 372 F₁ sibling progeny from this cross. Alleles for the

SCAR marker SCF27 were scored as dominant markers, with a designation of “1” indicating the presence of an amplification product, and “0”, indicating the absence of an amplification product. Alleles for the SCAR marker SCC8 were scored as *SCC8+/SCC8+*, *SCC8+/scc8-*, or *scc8-/scc8-*, according to the method used by Lahogue et al. (1998). The marker VMC7f2 was scored according to the allelic distribution of 198/200, 198/206, 200/200, or 200/206 bp. Goodness-of-fit between the observed and expected segregation ratios at the marker loci was tested by chi-square analysis.

RESULTS AND DISCUSSION

Molecular marker analysis

Of 372 F₁ hybrids amplified using the SCC8 primer, 350 generated products typical of those obtained using SCAR primers. After the digestion of the amplification products by *Bg*/II, the allelic distribution occurred as *SCC8+/SCC8+* (single band), *SCC8+/scc8-* (3 bands), and *scc8-/scc8-* (2 bands), as previously observed by Lahogue et al. (1998). The allelic distribution was identified as 1:2:1, in agreement with the findings of Lahogue et al. (1998) and was statistically non-significant, as indicated by the chi-square test ($\chi^2 = 35.39$; $P < 0.01$; Table 1). Lahogue et al. (1998) determined the allelic distribution of the paternal parent (“Sultani”) as +/? and the maternal parent (“Alphonse Lavallée”) as -/- by the SCC8 marker (Table 2). Therefore, the genotypic distribution of 1:2:1 in our progeny is thought to be the effect of a null allele. Since F₁ hybrids are young, phenotypic evaluation was not possible, and therefore, the effect of the null allele could not be tested. Adam-Blodon et al. (2001) reported that SCC8 mostly amplifies a null allele. For this reason, SCC8 was not useful in all the evaluated progeny.

Table 1. Allelic frequencies of F₁ hybrids detected by amplification with SCC8, SCF27, and VMC7f2 markers, and results of chi-square analysis.

Marker	Genotype	Number of F ₁ progeny	Chi-square test of allelic frequencies
SCC8	<i>SCC8+/SCC8+</i>	40	1:2:1 non-significant ($\chi^2 = 35.39$; $P < 0.01$)
	<i>SCC8+/scc8-</i>	199	
	<i>scc8-/scc8-</i>	111	
SCF27	Present (1)	80	1:3 significant ($\chi^2 = 2.42$; $P \geq 0.05$)
	Absent (0)	292	
VMC7f2	198/200	80	1:1:1:1 non-significant ($\chi^2 = 11.34$; $P < 0.01$)
	198/206	94	
	200/200	60	
	200/206	100	

Table 2. Genotypes of the parents and allelic distributions of the three markers in F₁ hybrids.

Parent	SCC8	SCF27	VMC7f2 (bp)
Sultani	<i>SCC8+/?^a</i>	+/?	198/200
Alphonse Lavallée	<i>scc8-/scc8-^a</i>	0/0 ^{b,c}	200/206
Offspring	<i>SCC8+/SCC8+</i> ; <i>SCC8+/scc8-</i> ; <i>scc8-/scc8-</i>	+/-	198/200; 198/206; 200/200; 200/206

^aSCC8 locus obtained by Lahogue et al. (1998) or Adam Blondon et al. (2001). ^bSCF27 locus obtained by Korpás et al. (2009). ^c0 represents a null allele.

Fatahi et al. (2004) reported that the SCC8 marker showed a distribution of 1:1 in the progenies they studied, and they proposed that this marker be tested in progenies from different crosses. Mejia and Hinrichsen (2003) hybridized 2 seedless grape varieties (“Ruby Seedless” x “Sultanina”); of the obtained F₁ hybrids, only 42% amplified a band. On the basis of this finding, they stated that the SCC8 marker was inadequate for early selection of seedless grapes in the lines they studied. Korpás et al. (2009) used SCC8 markers in 3 different seedless x seedless crosses [NKL (“Neptuun” x “Kishmish luchistyi”), 31 siblings; NSD (“Neptuun” x “Sunred seedless”), 26 siblings; and JKL (“Jupiter” x “Kishmish luchistyi”), 12 siblings]. They found that SCC8 alleles segregated 1:1:1:1 in the NKL and NSD progenies, which was statistically non-significant. While only 28 out of 57 individuals from the NKL and NSD progenies amplified a band, all 12 of the JKL progeny were identified as SCC8+. A genetic mapping study by Constantini et al. (2008) revealed that SCC8 alleles segregated in a ratio of 1:1.

On the other hand, the SCC8 marker was useful for MAS in our cross between “Alphonse Lavallée” x “Sultani”. In our progeny, 94% of F₁ hybrids generated amplification products with the SCC8 marker. Forty individuals showed an allelic distribution of SCC8+/SCC8+, typical of *Sd1*. These genotypes were designated as seedless cultivar candidates in the second year of the study by early selection. Homozygous recessives, which included 111 F₁ hybrids carrying the *scc8-/scc8-* alleles, were discarded from the study.

Eighty of the F₁ hybrids amplified with the SCF27 marker produced bands consistent with the 2.0-kb size of the marker. Of all of the plants analyzed, 292 (79%) did not show amplification (Table 1). The distribution of genotypic frequencies for this marker in seeded x seedless crosses was consistent with the expected ratio of 1:3 (seedless/seeded), which was statistically significant ($\chi^2 = 2.42$; $P \geq 0.05$; Table 1). Mejia and Hinrichsen (2003), who developed the marker, reported a 3:1 ratio of seedless/seeded in the progeny. They found a correlation of 81% between the seedlessness trait and amplification of a band in hybrid progeny from the seedless x seedless cross. They suggested that if F₁ hybrids heterozygous for the marker could be identified at the *in vitro* stage, breeding costs could be reduced by 25%, whereby the marker for MAS is rendered very cost-effective. Nevertheless, the authors added that the use of the marker for MAS needs to be tested with higher numbers of F₁ progeny and with other crosses between seeded x seedless lines. Korpás et al. (2009) have also analyzed the SCF27 marker in 3 different seedless x seedless hybridizations. Most of the individuals in the NKL (31 siblings) and NSD (26 siblings) progenies afforded PCR products on amplification (47 of the 57 individuals), and genotypic frequencies fit an expected ratio of 3:1. All 12 of the JKL progeny afforded the expected PCR product on amplification. The findings of Korpás et al. (2009) conflict with those of our study. This may be attributable to the use of the progenies of seedless x seedless crosses in their study. No previous studies have been conducted on the use of this marker in seeded x seedless crosses. In our progeny derived from a seeded x seedless cross, the SCF27 marker was not chosen for MAS because only 21% of a total of 371 F₁ hybrids tested yielded the expected PCR product on amplification.

The genotypes and allelic distributions observed in F₁ hybrids amplified with the VMC7f2 marker are presented in Table 1. The male and female parents of the “Alphonse Lavallée” x “Sultani” cross had genotypes of 200/206 and 198/200 bp, respectively, and the F₁ hybrids displayed the genotypes of 198/200, 198/206, 200/200, and 200/206 bp (Table 2). Chi-square tests indicated a Mendelian distribution of 1:1:1:1, which is statistically non-

significant ($\chi^2 = 11.34$; $P < 0.01$) (Table 1). Cabezas et al. (2006) identified a strong correlation between the individuals carrying the 198-bp allele and seedlessness and found that this marker can be effectively used for MAS. Among the progeny investigated in this study, 174 (48%) F_1 individuals carried the 198-bp allele. These individuals were designated as seedless candidates, assuming that they will be either seedless or have variable seedlessness. The 160 (52%) individuals that do not carry the 198-bp allele will be discarded from future breeding studies. Considering these results, VMC7f2 was found to be an appropriate marker for MAS of seedlessness in this cross.

In earlier genetic mapping studies on seedlessness and berry weight of grapevines, the VMC7f2 marker was found to be closely linked to the seedlessness-associated major QTL identified on grape chromosome 18 (Cabezas et al., 2006; Costantini et al., 2008; Mejia et al., 2007, 2011). For this reason, the VMC7f2 SSR marker was recommended for early screening of F_1 hybrids via MAS. Mejia et al. (2011) suggested that this marker and p3 VvAGL11 were the most useful markers for MAS. They stated that these markers need to be tested for their robustness in larger genetic backgrounds segregating for seedlessness.

The VMC7f2 marker has shown similar genotypic results in the preliminary results obtained in another study on seedlessness, which was conducted in progeny from a different cross grown at the Department of Horticulture, Faculty of Agriculture, Ankara University (data not shown).

From the PCR analyses, a total of 183 out of 372 offspring generated amplification products with at least 1 of the 3 markers linked to seedlessness used here. After early MAS, 183 of 372 individuals were designated as seedless cultivar candidates to carry forward for the second stage of breeding studies. Seventy-one individuals tested positive for at least 2 markers; these individuals were expected to have a high possibility of seedlessness. Twenty of the 71 individuals were selected as progenitors to be used in seedless grapevine breeding studies because they were found to be positive for all markers linked to seedlessness (SCC8+/SCC8+; “1” or “present” for SCF27, and the 198-bp allele VMC7F2) after MAS (Figure 1).

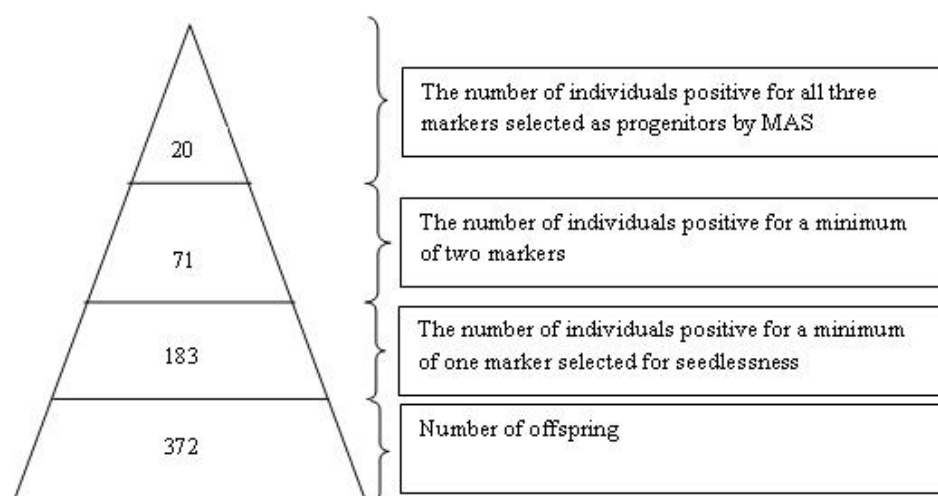


Figure 1. Pyramid based on marker assisted selection (MAS) of markers linked to *Sd1* in F_1 progeny.

ACKNOWLEDGMENTS

Research supported by the Scientific and Technological Research Council of Turkey (TUBITAK; #TOVAG-107O066). The authors would like to thank Kamil Karataş and Çağrı Baral for the assistance with laboratory work.

REFERENCES

- Adam-Blondon AF, Lahogue-Esnault F, Bouquet A and Boursiquot JM (2001). Usefulness of two SCAR markers for marker-assisted selection of seedless grapevine cultivars. *Vitis* 40: 147-155.
- Bouquet A and Danglot Y (1996). Inheritance of seedlessness in grapevine (*Vitis vinifera* L.). *Vitis* 35: 35-42.
- Cabezas JA, Cervera MT, Ruiz-Garcia L, Carreno J, et al. (2006). A genetic analysis of seed and berry weight in grapevine. *Genome* 49: 1572-1585.
- Cain DW, Emershad RL and Tarailo RE (1983). *In-ovulo* embryo culture and seedling development of seeded and seedless grapes (*Vitis vinifera* L.). *Vitis* 22: 9-14.
- Costantini L, Battilana J, Lamaj F and Fanizza G (2008). Berry and phenology-related traits in grapevine (*Vitis vinifera* L.): from quantitative trait loci to underlying genes. *BMC Plant Biol.* 8: 38.
- Doligez A, Bouquet A, Danglot Y, Lahogue F, et al. (2002). Genetic mapping of grapevine (*Vitis vinifera* L.) applied to the detection of QTLs for seedlessness and berry weight. *Theor. Appl. Genet.* 105: 780-795.
- Emershad RL and Ramming DW (1984). *In ovulo* embryo culture of *Vitis vinifera* L. cv Thompson Seedless. *Am. J. Bot.* 71: 873-877.
- Fanizza G, Lamaj F, Costantini L, Chaabane R, et al. (2005). QTL analysis for fruit yield components in table grapes (*Vitis vinifera*). *Theor. Appl. Genet.* 111: 658-664.
- Fatahi R, Zamani Z, Ebadi A and Mehlenbacher SA (2004). The inheritance of seedless SCC8-SCAR and SSR Loci alleles in progeny of 'Muscat Hamburg' x 'Bidane Qermez' grapes. *Acta Hort.* 652: 329-335.
- Korpás A, Baránek M, Pidra M and Hradilík J (2009). Behaviour of two SCAR markers for seedlessness within Central European varieties of grapevine. *Vitis* 48: 33-42.
- Lahogue F, This P and Bouquet A (1998). Identification of a codominant SCAR marker linked to the seedlessness character in grapevine. *Theor. Appl. Genet.* 97: 950-959.
- Mejia N and Hinrichsen P (2003). A new, highly asservative SCAR Marker potentially useful to assist selection for seedlessness in table grape breeding. *Acta Hort.* 603: 559-564.
- Mejia N, Gebauer M, Muñoz L and Hewstone N (2007). Identification of QTLs for seedlessness, berry size, and ripening date in a seedless x seedless table grape progeny. *Am. J. Enol. Vitic.* 58: 499-507.
- Mejia N, Soto B, Guerrero M, Casanueva X, et al. (2011). Molecular, genetic and transcriptional evidence for a role of VvAGL11 in stenopericarpic seedlessness in grapevine. *BMC Plant Biol.* 11: 57.
- Spiegel-Roy P, Sahar N, Baron J and Lavi U (1985). *In vitro* culture and plant formation from grape cultivars with abortive ovules and seeds. *J. Am. Soc. Hort. Sci.* 110: 109-112.
- Tangolar S, Eti S, Gök S and Ergenoglu F (1999). Obtaining plants from seedless x seedless grape crosses using embryo culture. *Turk. J. Agr. For.* 23: 935-942.
- This P, Lahogue F, Adam-Blondon AF and Doligez A (2000). Towards marker-assisted selection for seedlessness in grapevine. *Acta Hort.* 528: 221-229.