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Using seedlessness-related molecular markers in grapevine breeding for seedlessness via marker-assisted selection into Muscat of Hamburg × Sultani progeny

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Abstract: The seedlessness-related molecular markers VMC7f2, SCC8, and SCF27 were used for the early selection of seedless individuals in the Muscat of Hamburg (seeded) × Sultani (seedless) progeny of 314 F1 hybrids. The DNA from F1 hybrids was amplified by polymerase chain reaction (PCR) using 3 markers. After digestion of the SCC8 marker PCR amplification products by restriction enzyme Bgl II, 72 individuals showed homozygous SCC8+/SCC8+ alleles at the seed development inhibitor locus, 76 individuals of the progeny amplified with the SCF27 marker produced 2.0-kb bands, and 118 individuals with seedlessness-related 198-bp alleles amplified with the VMC7f2 marker were selected as seedless grapevine candidates. Based on marker-assisted selection, 190 F1 hybrids were designated as seedless grapevine candidates since they carried a minimum of 1 seedlessness-related allele, and the 124 F1 hybrids were removed from the seedless grapevine breeding studies. A total of 13 individuals that carried alleles for the 3 markers associated with seedlessness were selected as genetic resources for future studies on seedless grapevine breeding.

Key words: Grapevine, marker-assisted selection, seedlessness

1. Introduction

Seedless grapes, consumed fresh or dried, are among the most widely produced grape cultivars in the world due to their high market value. Following the growing consumer demand, studies on the production of new and high quality seedless grape cultivars are ongoing around the world.

The conventional breeding method used for the production of new seedless grape cultivars is based on seeded × seedless progenies (1,2). Nevertheless, the use of conventional hybridization methods in grapevines has significant disadvantages. The genetically heterozygous structure of a grapevine leads to a wide diversity of F1 hybrids, which, in turn, results in low proportions of desired individuals among the progeny. In order to increase the frequency of seedless individuals in the progeny, seedless × seedless grape hybridization via in vitro embryo rescue techniques has been employed in recent studies (3–7). On the other hand, despite intensive and time-consuming laboratory studies on in vitro embryo culture techniques, the number of F1 hybrids acquired by this method remains inadequate for breeding studies on seedlessness (2). Another important disadvantage of hybridization breeding is the delay in selection of seedless individuals in F1 hybrids until the vines are 4–5 years of age due to the long juvenile sterility period and the difficulty in selection due to inbreeding depression in backcrossing cases (8). Hence, the production of new cultivars in woody perennials such as grapevines by conventional hybridization methods is costly, labor- and time-intensive, and space-consuming.

In a step towards overcoming the disadvantages associated with conventional breeding techniques, successful results have been obtained recently in studies of the inheritance of seedlessness in grapevines (1,9–13) and the development of seedlessness-related molecular markers in grapevines (7,11–18). The predominant genetic model on the inheritance of seedlessness in grapes assumes that this trait is controlled by 3 complementary recessive genes regulated by a dominant gene, termed the seed development inhibitor (SdI) (11,12,19).

In a study by Lahogue et al. (11) on the development of seedlessness-related molecular markers in grapevines, most SCC8+/SCC8+ single band individuals after the digestion of SCC8 (a sequence characterized amplified region [SCAR] marker derived from opC08-1020 RAPD marker) amplification products by restriction enzyme Bgl II were found to be seedless. In their study on Ruby (seedless) × Sultana (seedless) progeny, Mejia and Hinrichsen (7) found the WF27-2000 RAPD marker to be linked to

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seedlessness by the bulk segregant analysis technique and reported that the SCF27 SCAR marker derived from this marker can be used for the marker-assisted selection (MAS) of seedlessness. Cabezas et al. (13) performed a quantitative trait locus (QTL) mapping of Montana (seeded) × Autumn seedless (seedless) progeny and identified a major QTL linked to seedlessness. The microsatellite locus VMC7f2 was found to be closely linked to this major QTL, and the marker was found to be useful for the selection of seedlessness. Yang et al. (15) detected 2 seedlessness-related markers by RFLP analysis. Zhijian et al. (17) transformed these markers into SCAR markers, performed their molecular characterization, and concluded that these markers cannot be utilized for the selection of seeded and seedless genotypes.

This study aims for early selection of seedless individuals in the Muscat of Hamburg (seeded) × Sultani (seedless) progeny of 314 F1 hybrids by using seedlessness-related SCC8 (11), SCF27 (7), and VMC7f2 (13) markers, based on the MAS technique.

2. Materials and methods

2.1. Plant material and DNA extraction

A total of 314 F1 hybrids obtained from a cross between Muscat of Hamburg and Sultani were used in this study. The hybrids produced about 4–5 true leaves, and the young leaf samples were taken for DNA analyses and stored at −80 °C until DNA isolation. DNA was isolated from the young leaves following the protocol of the Promega Wizard Genomic DNA Purification Kit (Madison, WI, USA).

2.2. Molecular markers

The primers for markers previously identified as linked to seedlessness in grapevines, SCC8 (11) and SCF27 (7) SCAR primers, and VMC7f2 SSR (13) primers were selected for genotypic analyses of seedlessness. The genomic DNA from F1 hybrids was amplified by polymerase chain reaction (PCR) using the selected primers.

PCR reactions 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 of Taq DNA polymerase, 1.5 mM of $MgCl_2$, and 20–40 ng of template DNA. Temperature profiles were run in a Biometra T1 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, and a synthesis step at 72 °C for 2 min with a final extension at 72 °C for 10 min. A touch-down PCR amplification consisting of 30 cycles of denaturation (3 min at 94 °C), annealing (1 min), and an extension (2 min at 72 °C) was programmed for the SCF27 primer. The annealing temperature was 55 °C for the first cycle, was reduced by 1 °C for the next 5 cycles, and was 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 of template DNA, 0.25 µM of each primer, 0.25 mM of each dNTP, 0.5 U of Taq DNA polymerase, and 1.5 mM of $MgCl_2$. Forward primers were labeled with WellRED fluorescent dyes D2 (black), D3 (green), and D4 (blue) (Proligo, Paris, France). PCR conditions had an initial cycle of 3 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 51 °C, and 2 min at 72 °C with a final extension at 72 °C for 10 min. PCR products were diluted with sample loading solution in certain proportions according to the fluorescent dyes used in labeling followed by the addition of GenomeLab DNA Standard Kit-400, and were electrophoresed in the CEQ 8800XL capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA). The allele sizes were determined using Beckman CEQ fragment analysis software.

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

The amplification products obtained using SCC8 primers were first digested with the Bgl II restriction enzyme, according to the protocol of Lahogue et al. (11). The digestion products of SCC8 and the amplification products obtained using SCF27 SCAR primers were resolved by electrophoresis on 2% agarose gels, visualized under UV light, and documented using a bioimaging system (Syngene, Cambridge, UK).

2.3. Data analysis

The markers were analyzed for all 314 F1 sibling progeny from this cross. The alleles for SCAR marker SCF27 were scored as a dominant marker by a designation of “1” for the presence of an amplification product and “0” for the absence of an amplification product. The alleles for SCAR marker SCC8 were scored as *SCC8+/SCC8+*, *SCC8+/scc8-*, or *scc8-/scc8-*, according to the method of Lahogue et al. (11). The marker VMC7f2 was scored according to the following allelic distribution: 198/200 bp, 198/206 bp, 200/200 bp, or 200/206 bp. The goodness-of-fit between the observed and expected segregation ratios at marker loci was tested by chi-square analysis.

3. Results

The genomic DNA of the 314 F1 hybrids and both maternal and paternal parents was amplified by PCR using the VMC7f2 SSR primer selected for MAS of seedlessness. As a result of PCR analysis, the seeded maternal parent was found to carry 200/206 bp alleles, and the seedless paternal parent was found to carry 198/200 bp alleles. The genotypical distribution in F1 individuals was 198/206 bp (44 individuals), 200/206 bp (60 individuals), 198/200 bp (74 individuals), and 200/200 bp (45 individuals) (Table).

Table. Allelic frequencies of F1 hybrids amplified with SCC8, SCF27, and VMC7f2 markers.

Marker	Genotype	Number of F1 progeny	Chi-square test of allelic frequencies
SCC8	SCC8+/SCC8+	72	1:2:1 nonsignificant ($\chi^2 = 8.74$; $P < 0.05$)
	SCC8+/scc8-	133	
	scc8-/scc8-	42	
SCF27	Present (1)	76	1:3 significant ($\chi^2 = 0.106$; $P \geq 0.05$).
	Absent (0)	238	
VMC7f2	198/200	74	1:1:1:1 nonsignificant ($\chi^2 = 10.84$; $P < 0.05$)
	198/206	44	
	200/200	45	
	200/206	60	

Chi-square tests indicated a Mendelian distribution of 1:1:1:1, which is statistically nonsignificant ($\chi^2 = 10.84$; $P < 0.05$; Table).

Of the individuals amplified with the SCC8 primer, 247 produced bands consistent with the 988-bp size of the SCAR primer. After digestion of the amplification products by Bgl II, the allelic distribution was SCC8+/SCC8+ (single band) in 72 individuals, SCC8+/scc8- (3 bands) in 133 individuals, and scc8-/scc8- (2 bands) in 42 individuals, as observed by Lahogue et al. (11) (Table). While the maternal parent was found to have SCC8+/scc8- (heterozygous) alleles after digestion of the amplification products, the paternal parent was found to have no digestion site (SCC8+). Chi-square tests indicated that the distribution of the genotypic frequencies for the SCC8 marker were inconsistent with the expected Mendelian distribution of 1:2:1 for the 3 genotypes ($\chi^2 = 8.74$; $P < 0.05$; Table).

Of the F1 hybrids amplified with the SCF27 marker, 76 produced bands of 2.0 kb in size, identical to the marker, and no amplification occurred in 238 of the F1 hybrids (Table). The distribution of genotypic frequencies for this marker was consistent with the expected ratio of 1:3 (seedless/seeded), which was statistically significant ($\chi^2 = 0.106$; $P \geq 0.05$; Table).

4. Discussion

The study aimed for early selection of seedless individuals by DNA screening of a total of 314 F1 hybrids in Muscat of Hamburg × Sultani progeny using seedlessness-related molecular markers, based on PCR technique.

Using the VMC7f2 marker for MAS, 118 individuals were found to have 198-bp alleles. Cabezas et al. (13) detected a strong correlation between the individuals carrying 198-bp alleles and the seedlessness trait and found that this marker could be effectively used for MAS. They reported that in a collection of 46 seeded and seedless grape cultivars, all the seedless grapes carried 198-bp alleles, and among those seeded, 198-bp alleles were detected only in Muscat of Alexandria and Dona Maria grapes.

The VMC7f2 marker was closely linked to the seedlessness-associated major QTL identified in grape chromosome 18 in different genetic mapping studies of the seedlessness of grapevines (16,18,20). For this reason, the VMC7f2 SSR marker was recommended for the early screening of F1 hybrids via MAS. While 53% (118 of 223) of the F1 hybrids with alleles of 198 bp in size were designated as seedless grapevine candidates, 47% (105 of 223) were removed from the seedless grapevine breeding studies after MAS.

In their genetic mapping study, Mejia et al. (18) identified a major QTL linked to seedlessness in chromosome 18 and designated VvAGL11 in this QTL as the main positional candidate gene that determines seed and fruit development. Researchers developed markers from VvAGL11, a major functional candidate gene for seedlessness, and they suggested that the VMC7f2 and p3 VvAGL11 were the most useful markers for MAS. They explained that these markers need to be tested for their solidity in larger genetic backgrounds while segregating for seedlessness.

Of the 314 F1 hybrids amplified with the SCC8 primer, 247 produced bands consistent with the 988-kb size of the SCAR primer. Both seeded and seedless individuals produced amplification products with the SCC8 marker, and polymorphism occurred after the digestion of amplification products by the restriction enzyme Bgl II. The 2 alleles generated after the digestion were the seedlessness-related SCC8+ allele (SdI+) and the unrelated

scc8- allele (12). The allelic distribution identified in our study after the digestion of the amplification products by restriction enzyme Bgl II was in agreement with the findings of Lahogue et al. (11).

Mejia and Hinrichsen (7) studied seedless × seedless (Ruby Seedless × Sultanina) progeny and reported that the SCC8 marker proved to be useless in the early selection of seedless individuals since only 42% of the F1 hybrids amplified with the SCC8 marker produced an amplification. Korpas et al. (21) employed the SCC8 marker in 3 different seedless × seedless progenies (NKL:31 sibling, NSD:26 sibling, and JKL:12 sibling) and obtained a statistically nonsignificant Mendelian distribution of 1:1:1:1 in NKL and NSD progenies. Only 28 of 57 individuals among the NKL and NSD progenies showed amplification, and all of the 12 individuals in JKL were SCC8+. In our study, 79% (247 of 314) of the progeny generated amplification with the SCC8 marker. While 72 individuals (29%) with a SCC8+/SCC8+ allelic distribution specific to the SdI locus were designated as seedless grapevine candidates, 41 F1 hybrids (17%) with scc8-/scc8- allelic distribution were discarded from the seedless grapevine breeding studies. Therefore, the SCC8 marker was useful for MAS in our cross between Muscat of Hamburg and Sultani.

Chi-square tests indicated that the distribution of genotypic frequencies for the SCC8 marker was inconsistent with the expected Mendelian distribution of 1:2:1 for the 3 genotypes. In the genetic mapping of Italia (seeded) × Big Perlon (seedless) progeny performed by Constantini et al. (20), the SCC8 marker was segregated in a 1:1 ratio. Fatahi et al. (22) found that the SCC8 marker displayed a distribution of 1:1 in their progenies. Adam-Blondon et al. (12) used SCC8 to determine the availability of MAS in seedless × seedless and seeded × seeded progenies and tested their allelic diversity in a set of 81 seedless and seeded varieties. They found the SCC8 marker to be a useful marker, at least in the seedless × seedless progenies.

Of the individuals amplified with the SCF27 marker, 76 produced bands of a 2.0-kb size specific to the marker. Of the 314 F1 hybrids, 238 (75.8%) did not generate any amplification. Mejia and Hinrichsen (7) found an 81% correlation between individuals showing an amplification product and seedlessness trait in their Ruby Seedless × Sultanina progeny. 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%, rendering the marker for MAS very cost-effective. According to the findings of our study, 75.8% of the F1 hybrids in the seeded × seedless progeny did not show amplification with the SCF27 marker. Hence, this marker was not useful for MAS among our progeny. Korpas et al. (21) tested the SCF27 marker in 3 different seedless × seedless progenies. Most (47 of 57) of the individuals among NKL (31 siblings) and NSD (26 siblings) progenies showed amplification, and the genotypical distribution was 3:1. All of the 12 individuals among the JKL progeny showed amplification. Mejia and Hinrichsen (7) and Korpas et al. (21) tested the marker in seedless × seedless progenies. The discrepancy between the findings of previous researchers and our study can be attributed to the difference in crossing populations. No previous studies have been conducted on the use of this marker in seeded × seedless crosses.

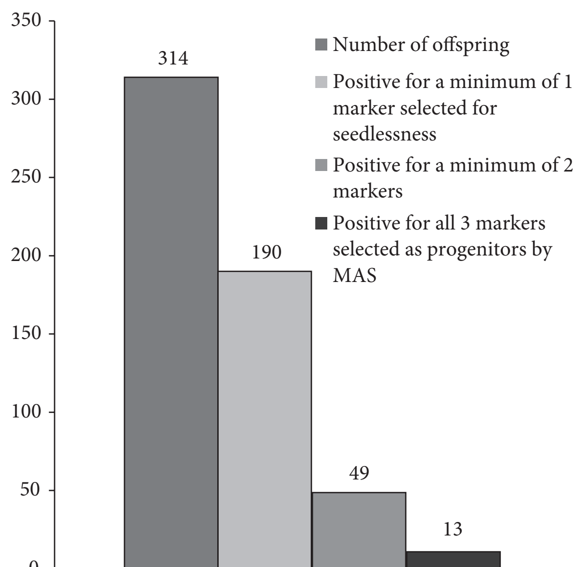

Figure. Number of F1 progeny linked to seedlessness as a result of MAS.

As a result of MAS, a total of 190 out of 314 offspring generated amplification products with at least 1 of the 3 markers linked to seedlessness used here. These individuals were designated as the seedless cultivar candidates to carry forward for the second stage of the breeding studies. Additionally, 49 individuals tested positive for at least 2 markers; these individuals were expected to have a high possibility of seedlessness. Of the 49 individuals, 13 were positive for all markers linked to seedlessness (SCC8+/SCC8+; “1” or “present” for SCF27; and the 198-bp allele VMC7F2). After MAS, these were selected as progenitors to be used in the grapevine breeding studies for seedlessness (Figure).

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References

1. Roytchev V. Inheritance of grape seedlessness in seeded and seedless hybrid combinations of grape cultivars with complex genealogy. Am J Enol Viticult 49: 302–305, 1998.
2. This P, Lahogue F, Adam-Blondon AF et al. Towards marker-assisted selection for seedlessness in grapevine. Acta Hortic 528: 221–229, 2000.
3. Cain DW, Emershad RL, Tarailo RE. *In-ovulo* embryo culture and seedling development of seeded and seedless grapes (*Vitis vinifera* L.). Vitis 22: 9–14, 1983.
4. Emershard RL, Ramming DW. In-ovulo embryo culture of *Vitis vinifera* L. cv 'Thompson Seedless'. Am J Bot 71: 873–877, 1984.
5. Spiegel-Roy P, Sahar N, Baron J et al. *In vitro* culture and plant formation from grape cultivars with abortive ovules and seeds. J Am Soc Hortic Sci 110: 109–112, 1985.
6. Tangolar S, Eti S, Gök S et al. Obtaining plants from seedless x seedless grape crosses using embryo culture. Turk J Agric For 23: 935–942, 1999.
7. Mejia N, Hinrichsen P. A new, highly assertive SCAR marker potentially useful to assist selection for seedlessness in table grape breeding. Acta Hortic 603: 559–564, 2003.
8. Perl A, Sahar N, Eliassi R et al. Breeding of new seedless table grapes in Israel: conventional and biotechnological approach. Acta Hortic 603: 185–187, 2003.
9. Ledbetter CA, Burgos L. Inheritance of stenospermocarpic seedlessness in *Vitis vinifera* L. J Hered 85: 157–160, 1994.
10. Bouquet A, Danglot Y. Inheritance of seedlessness in grapevine (*Vitis vinifera* L.). Vitis 35: 35–42, 1996.
11. Lahogue F, This P, Bouquet A. Identification of a codominant SCAR marker linked to the seedlessness character in grapevine. Theor Appl Genet 97: 950–959, 1998.
12. Adam-Blondon AF, Lahogue-Esnault F, Bouquet A et al. Usefulness of two SCAR markers for marker-assisted selection of seedless grapevine cultivars. Vitis 40: 147–155, 2001.
13. Cabezas JA, Cervera MT, Ruiz-Garcia L et al. A genetic analysis of seed and berry weight in grapevine. Genome 49: 1572–1585, 2006.
14. Striem MJ, Ben-Hayyim G, Spiegel-Roy P. Identifying molecular genetic markers associated with seedlessness in grape. J Am Soc Hortic Sci 121: 758–763, 1996.
15. Yang KQ, Wang YJ, Zhang JJ et al. Analysis of restriction sites and Southern blotting of two molecular markers linked to grape seedless gene. Chin J Agr Biotechnol 3: 13–17, 2006.
16. Meija N, Gebauer M, Muñoz L et al. Identification of QTLs for seedlessness, berry size, and ripening date in a seedless x seedless table grape progeny. Am J Enol Viticult 58: 499–507, 2007.
17. Zhijian TL, Dhekney SA, Gray DJ. Molecular characterization of a SCAR marker purportedly linked to seedlessness in grapevine (*Vitis*). Mol Breeding 25: 637–644, 2010.
18. Mejía N, Soto B, Guerrero M et al. Molecular genetic and transcriptional evidence for a role of VvAGL11 in stenospermocarpic seedlessness in grapevine. BMC Plant Biol 11: 57, 2011.
19. Doligez A, Bouquet A, Danglot Y et al. Genetic mapping of grapevine (*Vitis vinifera* L.) applied to the detection of QTLs for seedlessness and berry weight. Theor Appl Genet 105: 780–795, 2002.
20. Costantini L, Battilana J, Lamaj F et al. Berry and phenology-related traits in grapevine (*Vitis vinifera* L.): from quantitative trait loci to underlying genes. BMC Plant Biol 8: 38, 2008.
21. Korpas A, Baranek M, Pidra M et al. Behaviour of two SCAR markers for seedlessness within Central European varieties of grapevine. Vitis 48: 33–42, 2009.
22. Fatahi R, Zamani Z, Ebadi A et al. The inheritance of seedless SCC8-SCAR and SSR loci alleles in progeny of 'Muscat Hamburg' x 'Bidane Qermez' grapes. Acta Hort 652: 329–335, 2004.