The Major Origin of Seedless Grapes Is Associated with a Missense Mutation in the MADS-Box GeneVviAGL11

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Running title: A *VviAGL11* point mutation led to seedless grapes

The major origin of seedless grapes is associated with a missense mutation in the MADS-box gene *VviAGL11*

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One-sentence summary: Integrative genetics and genomics approaches revealed a missense substitution in the MADS-box gene *VviAGL11* as the mutation leading to seed abortion in most seedless table grape varieties.

Author contributions

J.M-Z. and P.C-B. conceived the research. P.C-B., J.M-Z. and C.R designed experiments. C.R. and P.C-B. performed experiments. C.R., P.C-B., R.T-P., N.M., N.D. and J.A.C. analyzed data. J.C., M.T., C.R., J.I., C.M. and T.L. provided materials and contributed to phenotyping tasks. P.C-B., C.R. and J.M-Z. wrote the manuscript with input and comments from all the other authors.

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Abstract

Seedlessness is greatly prized by consumers of fresh grapes. While stenospermocarpic seed abortion determined by the *SEED DEVELOPMENT INHIBITOR* (*SDI*) locus is the usual source of seedlessness in commercial grapevine (*Vitis vinifera*) cultivars, the underlying *sdi* mutation remains unknown. Here, we undertook an integrative approach to identify the causal mutation. Quantitative genetics and fine mapping in two 'Crimson Seedless' (CS)-derived F_1 mapping populations confirmed the major effect of the *SDI* locus and delimited the *sdi* mutation to a 323-kb region on chromosome 18. RNA-seq comparing seed traces of seedless and seeds of seeded F_1 individuals identified processes triggered during *sdi*-determined seed abortion, including activation of salicylic acid-dependent defenses. The RNA-seq dataset was investigated for candidate genes and, while no evidence for causal *cis*-acting regulatory mutations was detected, deleterious nucleotide changes in coding sequences of the seedless haplotype were predicted in two genes within the *sdi* fine mapping interval. Targeted re-sequencing of the two genes in a collection of 124 grapevine cultivars showed that only the point variation causing the Arg197Leu substitution in the seed morphogenesis regulator gene AGAMOUS-LIKE 11 (*VviAGL11*) was fully linked with stenospermocarpy. The concurrent post-zygotic variation identified for this missense polymorphism and seedlessness phenotype in seeded somatic variants of the original stenospermocarpic cultivar supports a causal effect. We postulate that seed abortion caused by this amino acid substitution in VviAGL11 is the major cause of seedlessness in cultivated grapevine. This information can be exploited to boost seedless grape breeding.

Introduction

The reduction of seed content without altering fruit size is a major breeding goal in many fruit crops because it eases fruit eating, which increases attractiveness for consumers and improves the suitability of fruits for the food processing industry (Varoquaux et al., 2000). For centuries, seedlessness has been one of the most prized quality traits in grapevine (*Vitis vinifera*) berries intended for direct consumption either as fresh fruit or raisins (Ledbetter and Ramming, 1989). World demand for seedless grapes is rising and vine-growers are increasingly asking for seedless cultivars (FAO and OIV, 2016). Understanding the genetic control of seedlessness is therefore paramount to boost the success of table grape breeding and fulfill such demands.

Although seeds are the sexual propagules of angiosperm plants, they are not required for the propagation of many woody crops such as grapevine that are vegetatively multiplied. This propagation method enables the establishment of new cultivars of seedless somatic variants that appeared spontaneously along the history of grapevine domestication (This et al., 2006). Seedless grape variants can be classified into two major groups depending on the type of seedlessness (Stout, 1936; Pratt, 1971): (i) Stenospermocarpy, in which fertilization and embryo development take place, but seed development is prematurely aborted (Stout, 1936; Ledbetter and Ramming, 1989; Kovaleva et al., 1997); (ii) Parthenocarpy, in which fruits develop in the absence of fertilization yielding small berries that completely lack seeds, which has recently been related with impaired meiosis (Royo et al., 2016). Stenospermocarpy is widely used in the production of seedless table grape cultivars because berry

size is less compromised, likely due to the presence of seminal rudiments or seed traces that promote fruit growth (Stout, 1936; Nitsch et al., 1960; Pratt, 1971).

A stable stenospermocarpy phenotype is shown by a few ancient oriental grapevine cultivars known as 'Kishmish' and derived varieties. They include the white-berried 'Kishmish', also known as 'Sultanina' or 'Thompson Seedless' (Dangl et al., 2001), which has been the major source of seedlessness in table grape breeding programs (Adam-Blondon et al., 2001; Ibáñez et al., 2009; Ibáñez et al., 2015). Studies of stenospermocarpy have therefore focused on 'Sultanina' and 'Sultanina'-derived cultivars, which produce soft and often imperceptible seed traces generally lacking seed coat lignification. While the embryo usually remains viable, the degree of endosperm degeneration detected from 3-4 weeks after flowering (WAF) and the final size of seed traces are variable, depending on the genetic background (Pearson, 1932; Stout, 1936; Barritt, 1970; Pratt, 1971; Striem et al., 1992; Wang et al., 2015; Wang et al., 2016). Stenospermocarpy in 'Sultanina' has been associated with defects in the development of maternal seed coat tissues (Malabarba et al., 2017). Specifically, endotesta growth and lignification does not take place in seed traces. Abnormal development of the precursor inner ovule integument has also been reported in 'Sultanina' at earlier developmental stages (Pearson, 1932).

Concerning the genetic control of 'Sultanina'-derived seedlessness, different hypotheses were initially proposed depending on the approaches used to measure the trait and the genetic backgrounds analyzed (Bouquet and Danglot, 1996). However, a systematic analysis in several F_1 cross progenies shows that a model involving three independent recessive loci regulated by a dominant

locus could explain most segregations (Bouquet and Danglot, 1996). This dominant locus was later named *SEED DEVELOPMENT INHIBITOR* (*SDI*) (Lahogue et al., 1998). Different quantitative genetic studies located the *SDI* quantitative trait locus (QTL) on linkage group (LG) 18, explaining up to 70% of the phenotypic variance in seed content parameters (Cabezas et al., 2006; Mejia et al., 2007; Costantini et al., 2008; Mejia et al., 2011; Doligez et al., 2013).

Based on genetic linkage and putative homology, grapevine *AGAMOUS-LIKE 11* (*VviAGL11= AGAMOUS-LIKE 3*, *VviAG3= MINICHROMOSOME MAINTENANCE1, AGAMOUS, DEFICIENS and SERUMRESPONSE FACTOR 5*, *VvMADS5*) was proposed as the *SDI* candidate gene in the absence of information for other genes in the region (Costantini et al., 2008; Mejia et al., 2011). This assumption was done considering its homology to the MADS-box gene *AGL11*, also known as *SEEDSTICK* (*STK,* At4g09960), which controls ovule morphogenesis and seed coat differentiation in Arabidopsis (*Arabidopsis thaliana*) (Pinyopich et al., 2003; Mizzotti et al., 2014). A role in seed morphogenesis was also shown for grapevine VviAGL11 homolog proteins (Malabarba et al., 2017). Molecular analyses identified two non-silent single nucleotide polymorphisms (SNPs) in the seedless mutant haplotype (*sdi+*) of *VviAGL11* (Mejia et al., 2011; Malabarba et al., 2017). However, both amino acid substitutions were detected in homozygosity in the seeded wine cultivar 'Asyl Kara', a result that excluded them as functional dominant polymorphisms causing seedlessness (Mejía et al., 2011). Alternatively, the reduced expression of *VviAGL11* in fruits of seedless *sdi+* individuals compared to fruits or seeds of seeded individuals was related with the origin of seedlessness (Mejia et al.,

2011; Ocarez and Mejia, 2016; Malabarba et al., 2017). Following this hypothesis, sequence polymorphisms observed in non-coding regions of the seedless allele of *VviAGL11* were proposed as putative mutations causing misexpression and seedlessness (Mejia et al., 2011; Di Genova et al., 2014). In a later effort to confirm the misexpression hypothesis, Ocarez and Mejia (2016) related several putative gene conversion events in the promoter region of *VviAGL11* with reversion of stenospermocarpy in somatic variants of 'Sultanina' that produce regular seeds. However, these studies did not assess the question on whether the observed expression differences in *VviAGL11* are causes or consequences of the seedless syndrome. The misexpression hypothesis also has limitations in explaining the dominant nature of the *sdi* mutation. In addition, the fact that *VviAGL11* expression is decreased in seed tissues has not consistently been proven (Mejia et al., 2011; Ocarez and Mejia, 2016; Malabarba et al., 2017).

Here we have reassessed the genetic and molecular origin of the 'Sultanina'-derived seedlessness in an independent unbiased study. We used large F_1 crosses of table grape cultivars segregating for stenospermocarpy to delimit the location of the causal mutation through crossover mapping. To identify putative misexpression or coding mutations within the delimited interval, we followed a strategy that combined RNA-seq comparisons in F_1 hybrids and targeted sequencing in a large collection of seeded and stenospermocarpic grapevine cultivars. The results clearly point to a single nucleotide missense mutation in *VviAGL11* as the origin of the dominant seedless phenotype.

Results

QTL mapping of seed content variation

Since we used the bred cultivar CS as the seedlessness donor (Ramming et al., 1995) in our study, we wanted to confirm the effect of the major *SDI* QTL in this genetic background. For this purpose we used a F_1 mapping population derived from a RG ('Red Globe') by CS cross hybridization. As an indicator of seed lignification that is useful to discriminate stenospermocarpy (Bouquet and Danglot, 1996), we analyzed the variation in seed dry weight per berry (SDW). Both progenitors showed extreme divergence in their seed content (Fig. 1). RG berries had an average of 3.2±0.2 fully developed seeds with a mean SDW of 33.2±9.3 mg, while CS berries had an average of 2.2±0.7 seed traces with a mean SDW of 0.43±0.2 mg (Supplemental Table 1). SDW distribution in F_1 progeny was asymmetric and bimodal and did not fit a normal distribution in any of the analyzed years (Fig. 1 and Supplemental Table 1). Values for SDW were highly correlated over the three seasons analyzed ($0.86<r<0.93$) in agreement with a high broad sense heritability (0.80-0.91) of the trait.

To map the QTLs responsible for SDW in RG×CS progeny, we built a total of three linkage maps corresponding to each progenitor as well as a consensus map for the cross. Linkage maps comprised both simple sequence repeat (SSR) and SNP markers up to a total of 191 markers in the case of RG, 227 for CS and 290 for the consensus map. QTLs for SDW were analyzed in every linkage map and every season. The results confirmed the detection of a major QTL for seedlessness in LG 18 linked to the microsatellite marker VMC7F2 both in the CS and the consensus maps (Table 1). This QTL was consistently identified in the three years with a very high Logarithm of Odds (LOD) value and

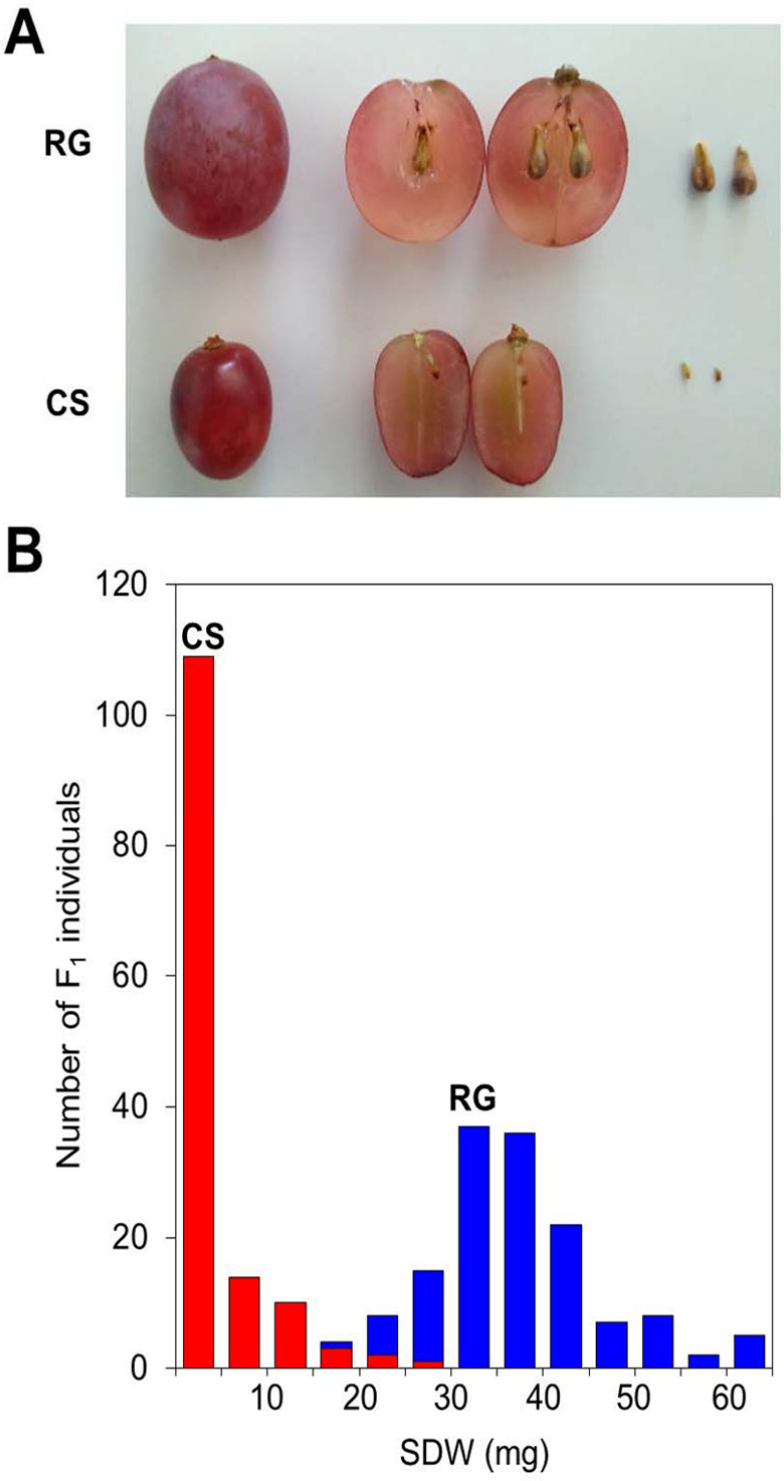


Figure 1. Stenospermocarpic phenotype and segregation in a RG×CS F1 population.

192 explained between 71 and 83% of the total variance for SDW depending on the

193 year and the linkage map. It co-localized with the *SDI* QTL detected previously
194 in different genetic backgrounds related with stenospermocarpic seedlessness
195 (Doligez et al., 2002; Cabezas et al., 2006; Mejia et al., 2007; Costantini et al.,
196 2008; Mejia et al., 2011; Doligez et al., 2013). Another QTL detected in all the
197 analyzed seasons for SDW was located on LG 2 (Table 1). Rather than
198 contributing to stenospermocarpy, the genotype in this QTL marker (VVIB23)
199 correlates with the variation for seed number per berry (Supplemental Table 1)
200 and likely co-localizes with the sex locus as has been described previously
201 (Costantini et al., 2008; Battilana et al., 2013; Doligez et al., 2013). Finally, two
202 other minor QTLs for SDW were located on LGs 5 and 14, but they showed
203 environmental interactions since they were not detected in every season (Table
204 1).
205
206 **Fine mapping of the *sdi* mutation**
207 A genetic fine mapping strategy was developed to delimit the location of the
208 mutation that underlies the *SDI* QTL. For every RG×CS F_1 individual, the
209 seedlessness phenotype *de visu*-determined during at least three years was
210 fully linked to the genotype in the VMC7F2 marker, with no recombinant
211 individuals or false detection observed (Supplemental Table 1). Every F_1
212 individual inheriting the *sdi*+ seedless allele (198-bp) or the *sdi*- seeded allele
213 (200-bp) from the CS progenitor showed a seedless or seeded phenotype,
214 respectively, confirming the dominant nature of the *sdi* mutation (Lahogue et al.,
215 1998; Cabezas et al., 2006; Karaagac et al., 2012; Bergamini et al., 2013). This
216 F_1 population was therefore screened for meiotic recombinants by studying the
217 VVIN16 SSR marker and a newly developed Cleaved Amplified Polymorphic

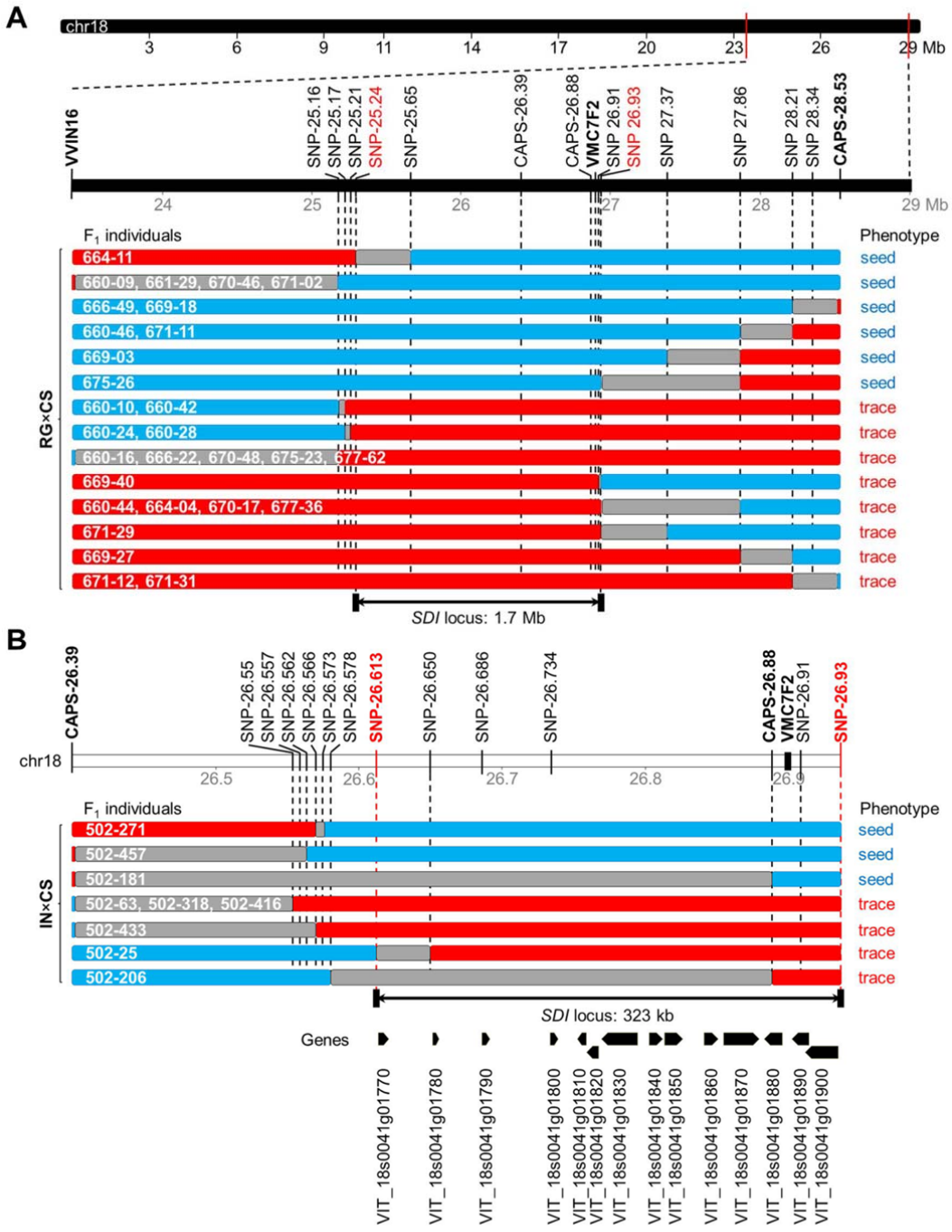


Figure 2. Fine mapping of the *sdi* mutation in two seedless × seeded F_1 populations

Sequence (CAPS) marker (CAPS-28.53) mapping at either side of the VMC7F2 marker, respectively (Fig. 2). This interval comprises a 5.1 Mb physical distance in the 12X.0 grapevine reference genome assembly (Fig. 2), which is equivalent to 5.5 Mb in the recently published 12X.v2 assembly as one small scaffold has

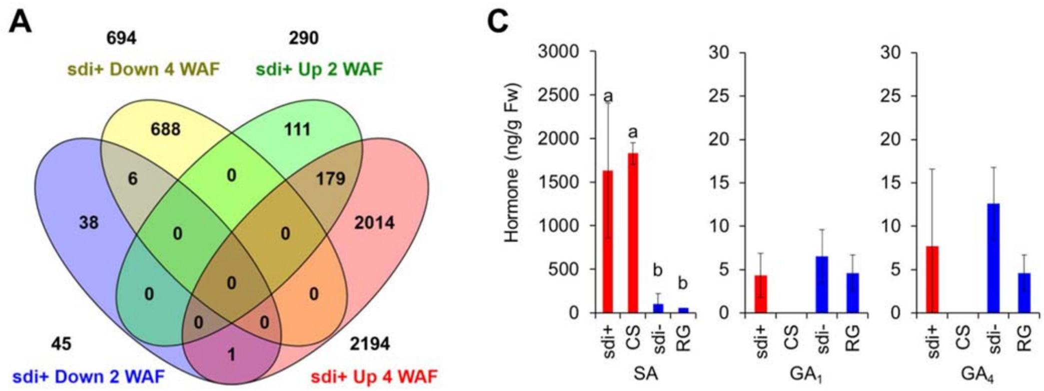
been remapped between the VVIN16 and SNP-25.16 markers (Canaguier et al., 2017). A total of 29 recombinants were detected, 14 and 15 of them corresponding to recombination in upstream and downstream sequences relative to VMC7F2, respectively (Fig. 2). Additional inner markers were genotyped to map the meiotic recombination breakpoint in these individuals. In this manner, the *sdi* mutation was delimited to an interval of 1.69 Mb (chr18:25,246,979- chr18:26,936,376 positions in the 12X.0 reference genome) flanked by markers SNP-25.24 and SNP-26.93 (Fig. 2 and Supplemental Table 2). Supporting its continuity in the grapevine reference genome, the delimited mapping interval remained intact in the re-assembled 12X.v2 version (Canaguier et al., 2017).

To further delimit the location of the *sdi* mutation, additional recombinants within the interval delimited in RG×CS progeny were searched for in another seeded × seedless F_1 mapping population derived from an 'Imperial Napoleon' (IN) by CS cross. Inheritance of seedlessness was fully linked to the presence of the *sdi+* allele for the CAPS-26.88 marker in this mapping population, which co-localizes with *VviAGL11* (*VIT_18s0041g01880*), in close proximity to the VMC7F2 marker (Fig. 2). The IN×CS progeny was screened using CAPS-26.39 and CAPS-26.88 markers (Fig. 2 and Supplemental Table 2) and nine recombinant F_1 individuals were detected. Additional genotyping for crossover mapping delimited the *sdi* mutation to an interval of 323 kb (chr18:26,613,101-26,936,376) flanked by markers SNP-26.613 and SNP-26.93. Unfortunately, recombination breakpoints for individuals 502-181 and 502-206 could not be more precisely mapped to further restrict the interval length because material for these recombinants is no longer available. According to grapevine 12X V1 gene annotations

(http://genomes.cribi.unipd.it/), the delimited *SDI* locus comprises 14 genes, although the two genes at each extreme were only partially included (Fig. 2). These 14 genes were considered as *SDI* QTL candidates in subsequent analyses.

Transcriptome analysis reveals the activation of salicylic acid (SA)-dependent defenses along with the repression of seed morphogenesis during stenospermocarpic seed abortion

Transcriptome comparisons were addressed to understand the processes involved in stenospermocarpic seed abortion and to search for candidate mutations underlying the *SDI* locus. To these aims, RNA-seq was used to compare seedless and seeded RG×CS F_1 individuals. To minimize genetic background effects, different F_1 individuals were analyzed as independent biological replicates. Individuals were selected to contrast in the allele of the *SDI* locus inherited from CS (*sdi*+, seedless or *sdi*-, seeded; Supplemental Table 1). From pea size fruits collected at 4 WAF, seeds or seed traces were extracted to specifically analyze gene expression in the affected organ. In this manner, we identified 2,888 differentially expressed genes (DEGs) with adjusted *p*-value ≤0.05 in edgeR and ≥2-fold change, most of which (76%) were upregulated in seed traces of seedless F_1 progeny (Fig. 3A, and Supplemental Table 3). To understand the biological meaning underlying these differences, a functional enrichment analysis was carried out. Remarkably, several pathogen response-related functions were over-represented among genes upregulated in seed traces, which coincided with the activation of senescence/catabolism processes (Fig. 3B and Supplemental Table 4). WRKY, Homeobox and NAC



B Functional enrichment summary in DEGs between seedles (*sdi+*) and seeded (*sdi-*) individuals

sdi+ Up		*sdi+* Down	
2 WAF	4 WAF	2 WAF	4 WAF
• Biotic stress response • Protein kinase • WRKY TFs • SA signaling • JA signaling • Phenylpropanoid biosynthesis • Stilbenoid biosynthesis • Ascorbate and aldarate metabolism	• Biotic stress response • NBS-LRR superfamily • Protein kinase • WRKY TFs • Homeobox TFs • NAC TFs • Ethylene signaling • SA signaling • JA signaling • Glycerolipid catabolism • Tyrosine catabolism • Auxin inactivation by conjugation • Glutathione metabolism • Photosynthesis • Senescence	• Lignin metabolism • Aromatic amino acid catabolism • PLATZ TFs	• Cell growth • Phenylpropanoid metabolism • Monosaccharide metabolism • Glycolysis/ Gluconeogenesis • Nitrogen and sulfur metabolism • Amino acid biosynthesis • S1Fa-like TFs

Figure 3. Transcriptome and phytohormone levels comparisons between seedless (*sdi+*) and seeded (*sdi-*) RG×CS F_1 individuals.

transcription factors (TFs) may regulate these processes during seed abortion as these TF families were over-represented in seed trace-upregulated genes as well. Likely connected with lack of seed differentiation, genes upregulated in seed traces were also over-represented in photosynthesis-related categories. As expected, genes downregulated in seed traces were over-represented in functional categories related with seed morphogenesis such as 'phenylpropanoid metabolism' (Fig. 3B and Supplemental Table 4). Collectively, these results are in line with general trends described by Wang et al. (2016) following a similar approach.

To assess for transcriptome differences at earlier stages, a similar comparison between *sdi*+ and *sdi*- RG×CS F_1 individuals was carried out at fruit set stage (2

283 WAF) using NimbleGen microarrays. Although we used whole setting fruits
284 because the extraction of seed content was difficult at this stage, this strategy
285 eased the analysis of a higher number of replicates. In this case, only 335
286 DEGs were detected (adjusted *p*-value ≤0.05 in limma and ≥2-fold change).
287 Nevertheless, 55% of these DEGs coincided with 4 WAF DEGs and seedless-
288 upregulated genes predominated this dataset as well (86.6% of DEGs identified
289 at 2 WAF), showing a consistency between the two experiments in spite of
290 ontogeny, tissue and inter-annual differences (Fig. 3A and Supplemental Table
291 3). Also in agreement with 4 WAF results, genes downregulated in seedless
292 fruits at 2 WAF were over-represented in seed differentiation related functions
293 such as 'lignin metabolism' (Fig. 3B and Supplemental Table 4). Immune-like
294 responses were activated in whole seedless fruits at 2 WAT, indicating that at 4
295 WAT these responses are not an artifact due to sample manipulation during the
296 extraction of seed traces.

297 Immune-like responses activated in stenospermocarpic offspring comprised SA
298 signaling pathway homologs such as *ENHANCED DISEASE SUSCEPTIBILITY*
299 *1* (*EDS1*, *VIT_17s0000g07370*, *VIT_17s0000g07400*, *VIT_17s0000g07420*,
300 *VIT_17s0000g07560*), a gene annotated as putative *SYSTEMIC ACQUIRED*
301 *RESISTANCE DEFICIENT 1* (*SARD1*, *VIT_17s0000g03370*, NCBI annotated
302 locus LOC100259493) and *PATHOGEN RESPONSE 1* (*PR1*,
303 *VIT_00s0207g00130*, *VIT_00s0207g00160*, *VIT_03s0088g00710*,
304 *VIT_03s0088g00780*, *VIT_03s0088g00810*; Supplemental Table 3).
305 Considering that EDS1 and SARD1 activate SA production and defense
306 responses in Arabidopsis (Zhang et al., 2010; Rietz et al., 2011), levels of SA
307 were compared between seeds and seed traces at 4 WAF. A prominent 16-fold

308 increase in SA levels was detected in seed traces when *sdi*+ and *sdi*- RG×CS
309 F_1 individuals were compared (Fig. 3C), suggesting a role of SA in *sdi*-mediated
310 seed abortion. A greater difference (33-fold) was observed between the
311 seedless progenitor CS, with small seed traces, and RG, with big seeds. When
312 the active gibberellins GA_1 and GA_4 were measured, no significant difference
313 was observed in F_1 progeny (Fig. 3C), suggesting a minor contribution of this
314 phytohormone to stenospermocarpy, at least in the studied developmental
315 stage.
316
317 **Stenospermocarpy does not associate with misexpression mutations**
318 To identify candidate misexpression mutations, we inspected the transcriptome
319 dataset for the presence of DEGs within the *SDI* fine mapping interval.
320 Supporting the continuity of this interval in the grapevine reference genome
321 (Jaillon et al., 2007), we found that it is almost fully covered by one contig
322 (000279F) in the genome assembly that was independently produced in the
323 'Cabernet-Sauvignon' cultivar (Chin et al., 2016). In addition, the same gene
324 models are annotated in that interval of the reference genome according to V1
325 and V2 gene predictions (Vitulo et al., 2014) and no additional missing gene
326 was supported by our RNA-seq read alignments (PRJNA418130 in NCBI
327 Sequence Read Archive). While no DEG was detected in the RNA-seq assay,
328 *VviAGL11* was the only one out of the 14 genes within the interval that was
329 differentially expressed in the microarrays of whole berries 2 WAF, showing a
330 3.4-fold repression in *sdi*+ fruits (Supplemental Table 3). When seed tissues
331 were specifically studied in the RNA-seq experiment at 4 WAF, *VviAGL11* did
332 not exceed the significance thresholds (adjusted *p*-value=0.23 and 2.0-fold

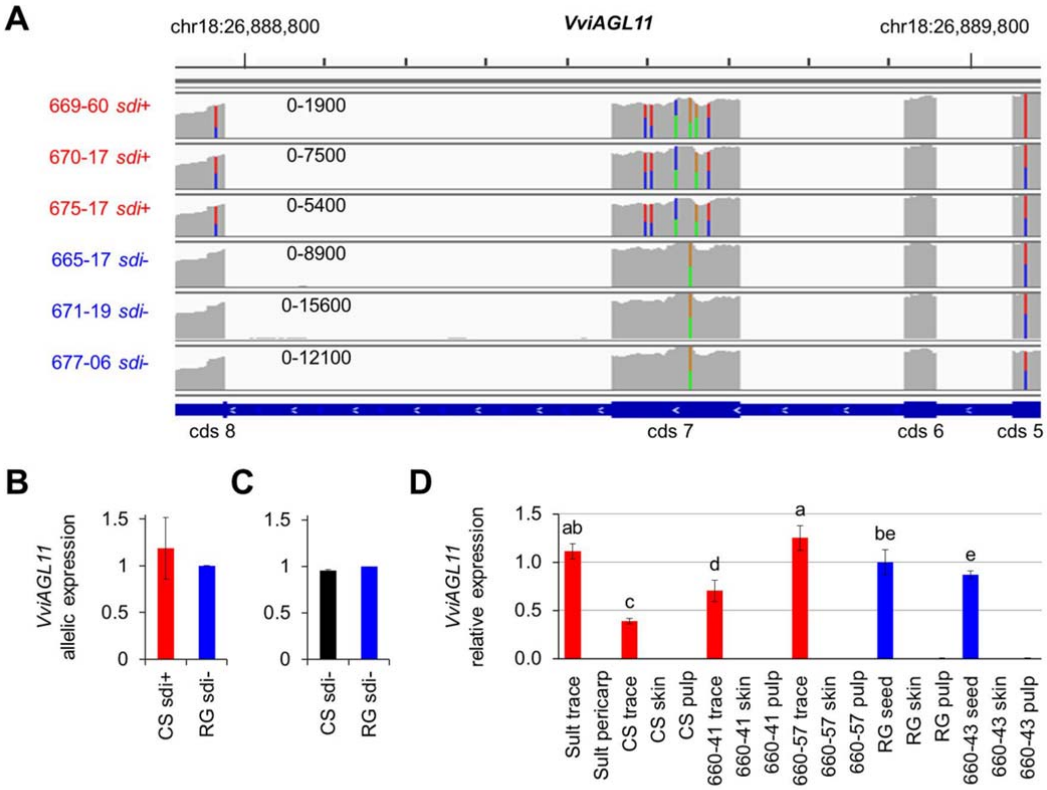


Figure 4. VviAGL11 transcript levels.

repression in *sdi+* individuals). Regardless, any potential allelic imbalance in *VviAGL11* transcript levels was assessed from the RNA-seq dataset. Considering the mean allelic frequency at heterozygous SNP positions (Fig. 4A), the expression between *sdi+* and *sdi-* alleles was balanced in the seedless RG×CS F_1 individuals analyzed (Fig. 4B). In fact, the seedless individual 669-60 showed *ca* 50% more counts for the *sdi+* allele (Fig. 4A), which indicates that no *cis*-acting regulatory mutation inhibiting the expression of *VviAGL11* was present in the *sdi+* haplotype. Similar balanced expression between the two *sdi-VviAGL11* alleles was observed in seeded F_1 individuals (Fig. 4C). In agreement with RNA-seq results, the absence of allelic imbalance in *VviAGL11* was confirmed when allele-specific primers were used to compare the expression between the two alleles in 'Sultanina' seed traces at fruit set stage

by reverse transcription quantitative PCR (RT-qPCR) (*sdi*+/*sdi*- allelic expression ratio = 0.91±0.1 SD). According to RNA-seq data, allelic imbalance was also absent in all other expressed genes located within the *SDI* interval (Supplemental Table 5 and GSE107014 entry of Gene Expression Omnibus (GEO) database).

We also compared absolute expression of *VviAGL11* in additional seedless and seeded accessions and different fruit tissues using RT-qPCR. This assay showed that at fruit set and pea size stages, the expression of *VviAGL11* was restricted to seed or seed traces and was not present in fruit pericarp (Fig. 4D). Remarkably, when *sdi*+ seed traces and *sdi*- developing seeds were directly compared, *VviAGL11* transcript levels were similar or even higher in several seedless accessions. Altogether, the balanced allelic expression of *VviAGL11* in seedless *sdi*+/*sdi*- heterozygous accessions, along with the lack of correlation of *VviAGL11* absolute expression in seed organs and seedlessness phenotype, reject the hypothesis of misexpression mutations in this gene as the origin of stenospermocarpy.

Transcriptome sequence analysis identifies *sdi* candidate coding mutations

Once the presence of causal misexpression mutations was ruled out, we investigated the RNA-seq dataset for candidate *sdi* mutations in coding sequences. Variant calling of RNA-seq data detected 68 variants specific to the *sdi*+ haplotype within the fine mapping interval and all of them were single nucleotide variants (SNVs). While these SNVs affected the sequence of six genes within the interval, no high effect (loss of start or gain of stop codon) was

predicted for any of them (Supplemental Table 5). Nonetheless, four of these genes collectively harbored six missense substitutions specific to the *sdi+* haplotype: two located in the putative pantetheine-phosphate adenylyltransferase *VviPPAT2* (*VIT_18s0041g01870*) gene, two in *VviAGL11* (*VIT_18s0041g01880*), one in an unknown protein (*VIT_18s0041g01890*) gene, and one in a translation elongation factor (*VIT_18s0041g01900*) gene (Table 2). An analysis using PROVEAN software predicted that three of these missense substitutions impact the biological function of the affected proteins: Arg151Cys (chr18:26,859,228) and Gln195Leu (chr18:26,871,891) in *VviPPAT2*, and Arg197Leu (chr18:26,889,437) in its consecutive downstream gene *VviAGL11* (Table 2). These three missense SNVs were thereby tested as candidate *sdi* mutations in subsequent phenotype-association genotyping analyses.

Candidate missense substitutions in *VviPPAT2* do not associate with seedlessness

VviPPAT2 is homolog to a gene coding for an enzyme involved in coenzyme A biosynthesis and lipid storage in Arabidopsis seeds (Rubio et al., 2008). We named this gene in that manner because a *VviPPAT1* paralog is present in the grapevine reference genome (*VIT_04s0023g01990*). To assess for any possible connection between the two predicted deleterious amino acid substitutions in *VviPPAT2* and seedlessness determination, amplicons containing each SNV were sequenced in 20 and 73 *sdi+* seedless and *sdi-* seeded cultivars, respectively (Table 3 and Supplemental Table 6). While both SNVs were validated by PCR in the CS progenitor, no genotype-phenotype association was confirmed. For the SNV at position chr18:26,859,228 (C:T), the T nucleotide

allele inferred the *sdi+* haplotype of CS from the RNA-seq analysis was present in ten seeded cultivars ('Afus Ali', 'Alphonse Lavallee', 'Aramon', 'Aubun', 'Morio Muskat', 'Naparo', 'Planta Nova', 'Pedro Ximenes', 'Semillon' and 'Verdil'); and similarly, for the SNV at position chr18:26,871,891 (A:T), the T nucleotide allele of the *sdi+* haplotype in CS was present in two seeded cultivars ('Cornichon Blanc' and 'Verdil', Table 3 and Supplemental Table 6). These results do not support a role for candidate variants in *VviPPAT2* as major seedlessness-responsible dominant mutations.

Stenospermocarpic seedlessness is specifically linked to one missense substitution in *VviAGL11*

Genotyping was also conducted to assess the role in stenospermocarpy for the candidate missense SNV detected in *VviAGL11*. Given that previous studies proposed several *sdi* candidate mutations in this gene (Mejia et al., 2011; Di Genova et al., 2014; Ocarez and Mejia, 2016; Malabarba et al., 2017), the whole gene plus the 2-kb upstream sequence were targeted for re-sequencing in a collection of 110 grapevine accessions using Illumina next-generation sequencing (NGS). Variant calling from NGS data identified 537 polymorphic sites in the 110 accessions, which included 448 SNPs and 89 insertion-deletions (INDELs) (Supplemental Table 7). For 146 polymorphic sites (124 SNPs and 22 INDELs), the variant allele compared to the reference genome was shared in all five 'Sultanina'-derived seedless accessions studied (Fig. 5A). These variants were considered to be linked in the *sdi+* haplotype because the analysis included two *sdi+*/*sdi+* homozygous seedless 'Ruby Seedless' × 'Moscatuel' (RS×MO) F_1 individuals obtained from a seedless by seedless

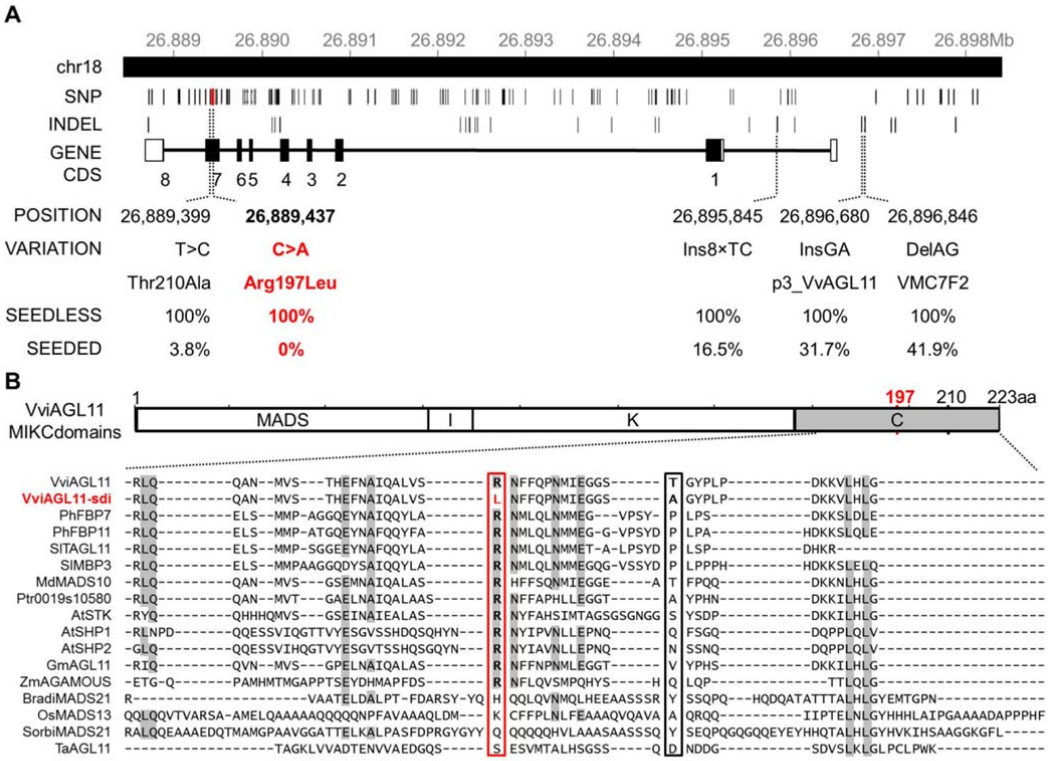


Figure 5. Sequence variation in *AGL11* and association with seedlessness.

cross. From all these positions, only the genotype for the SNP at position chr18:26,889,437 (A:C, according to the sequence in the plus strand) was fully associated with the seedlessness phenotype. Remarkably, this SNP causes the Arg197Leu substitution with the predicted deleterious effect already detected in the RNA-seq variant calling (Table 2). The A nucleotide allele was present and absent, respectively, in every stenospermocarpic and seeded accession (Fig. 5A and Supplemental Table 7). This amino acid substitution is located in the C-terminal domain characteristic of MIKC-type MADS-box genes (Fig. 5B). Although this domain is the most variable in MIKC genes (Kaufmann et al., 2005), the Arg residue at this position is generally conserved in AGL11 homologs of dicotyledonous species as well as in the related AGAMOUS-lineage SHATTERPROOF 1 (SHP1) and SHP2 proteins, (Fig. 5B and Pabon-

Mora et al. (2014)), which suggests that this residue could be functionally relevant.

Other candidate variants in *VviAGL11* were discarded in the re-sequencing analysis. For the SNP at position chr18:26,889,399 (C:T) corresponding to the second amino acid substitution in the *sdi*+ haplotype (Thr210Ala), the C nucleotide detected in the *sdi*+ haplotype was also present in 'Aubun', 'Cornichon Blanc' and 'Verdil' seeded cultivars (Fig. 5A and Supplemental Table 7). Similarly, the poly-GA insertion candidate allele (8×TC according to the sequence in the plus strand) proposed in intron 1 (Di Genova et al., 2014) was detected in 17 seeded cultivars. In the putative promoter, the AG and GA *sdi*+ alleles of VMC7F2 and p3_VvAGL11 markers, respectively, were detected in 44 and 33 seeded cultivars (Fig. 5A and Supplemental Table 7), which does not support the role in seedlessness determination proposed by Mejía et al., 2011 .

To validate the association of the Arg197Leu substitution in VviAGL11 with stenospermocarpic seedlessness, 16 stenospermocarpic and one seeded cultivars were specifically genotyped following PCR and Sanger sequencing. The full association for position chr18:26,889,437 (A:C) was again confirmed in these accessions (Supplemental Table 6). This study included 'Asyl Kara', a seeded cultivar that in our analysis was homozygous for the seeded allele (C:C) in that position despite that it was described as A:A homozygous in a previous study (Mejia et al., 2011). 'Asyl Kara' was independently genotyped several times for this position, confirming the C:C genotype. The accession of 'Asyl Kara' used at the Instituto de Ciencias de la Vid y del Vino (ICVV) collection comes from the Vassal-Montpellier collection and, following microsatellite marker genotyping, we confirmed in the same DNA used for *VviAGL11*

sequencing its true-to-typeness with the genetic profile indicated for 'Asyl Kara' in the *Vitis* International Variety Catalogue (VIVC) database (Supplemental Table 8 and www.vivc.de). 'Asyl Kara' was also homozygous for the seeded allele (T:T) in position chr18:26,889,399 (Supplemental Table 6). Again, this genotype contrasts with that reported by Mejia et al. (2011), suggesting a likely sample mistaking by these authors. In summary, the putative deleterious Arg197Leu substitution in *VviAGL11* is the only detected mutation within the *SDI* fine mapping interval that is fully linked to the stenospermocarpy phenotype with no false detection in our comprehensive approach, which strongly suggests that it could be the *sdi* causal mutation.

Concurrent somatic variation in the SNV resulting in the VviAGL11 Arg197Leu substitution and seedlessness trait supports a causal effect

Somatic or post-zygotic mutants occasionally appear during the characteristic vegetative propagation of grapevine cultivars (Torregrosa et al., 2011). In that manner, seeded somatic variants of the seedless cultivar 'Sultanina' have been reported previously and are known as 'Sultanina Monococco' (Adam-Blondon et al., 2001; Torregrosa et al., 2011; Ocarez and Mejia, 2016). We compared post-zygotic variants of the 'Sultanina' cultivar differing in their ability to develop seeds to evaluate the dominant effect of candidate *sdi* mutations.

We confirmed the seeded variant phenotype in two 'Sultanina Monococco' accessions (Fig. 6A). Mean SDW increased by more than 30 mg compared to a reference stenospermocarpic 'Sultanina' (Fig 6B), reaching values comparable to seeded individuals in other genetic backgrounds (Fig. 1 and Supplemental Table 1). Seeds produced by 'Sultanina Monococco' were generally filled and

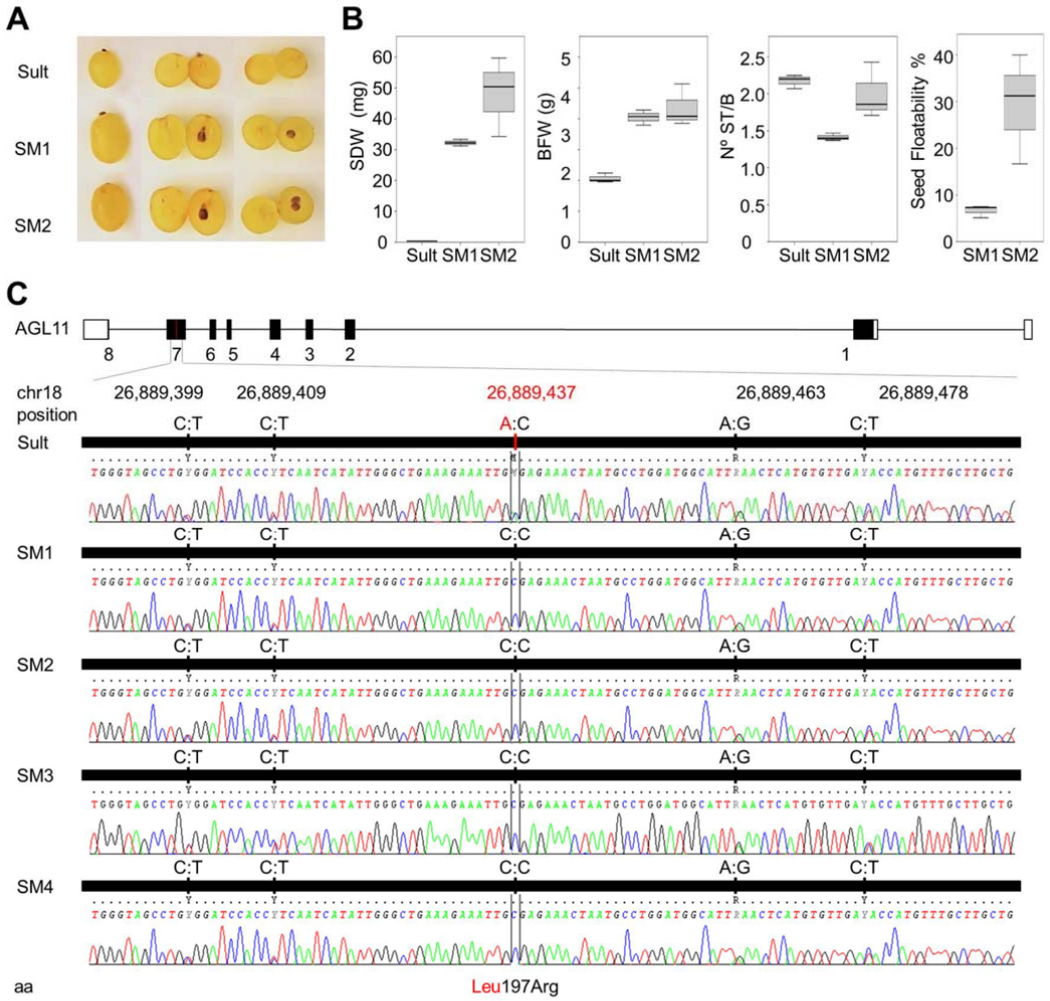


Figure 6. Concurrent somatic variation of the sdi mutation and seedlessness trait in Sultanina Monococco.

most of them sank in water (Fig. 6B). Moreover, 59.9% germination success under regular soil conditions was obtained for 'Sultanina Monococco' (2085Mpt1 accession) self-cross filled seeds, confirming that they are functional. A similar seeded variant phenotype was visually confirmed in two other available accessions of 'Sultanina Monococco'. Genotyping of 13 microsatellite markers confirmed the identity of the four studied 'Sultanina Monococco' accessions as post-zygotic variants of 'Sultanina' (Supplemental Table 8).

Amplicons containing the three candidate deleterious SNVs in *VviAGL11* and *VviPPAT2* were sequenced both in seedless and seeded post-zygotic variants of 'Sultanina' (Supplemental Table 6). Remarkably, the genotype obtained for the four 'Sultanina Monococco' accessions was identical to that in 'Sultanina' with the exception of the *VviAGL11* SNV at position chr18:26,889,437 (Fig. 6C and Supplemental Table 6), which in fact is the only polymorphism that was fully associated with seedlessness in a large collection of cultivars (Fig. 5A). At this position, the A:C genotype characteristic of 'Sultanina' was changed to C:C in all 'Sultanina Monococco' accessions. Altogether, given the unlikely casual coincidence in somatic variants, the concurrent post-zygotic variation in both the *VviAGL11* missense SNV and seedlessness phenotype that we identified in 'Sultanina Monococco' is consistent genetic proof for the causal effect of this mutation. Accordingly, we postulate that the Arg197Leu missense substitution in *VviAGL11* is the mutation responsible for stenospermocarpic seedlessness.

Discussion

Multiple lines of evidence converge on a *VviAGL11* missense mutation as the cause of stenospermocarpy

In this study we carried out genome-wide approaches, which were devised independently of previous hypotheses, in an unbiased attempt to identify the mutation responsible for stenospermocarpic seedlessness in grapevine. Our data converge on a missense SNV resulting in an Arg197Leu substitution in *VviAGL11* as the *sdi* mutation that is exploited worldwide for the production of commercial seedless grapes.

In the first place, crossover mapping in F_1 recombinants allowed us to delimit the *sdi* mutation to a 323-kb segment (Fig. 2). These recombinants involve physical/genetic distance ratios between 166 and 555 kb/cM in this chromosomal region depending on the mapping population (Fig. 2). These results are in line with average ratios between 198-390 kb/cM estimated in genetic maps produced in other grapevine crosses (Doligez et al., 2006; Houel et al., 2015; Teh et al., 2017) and therefore, they do not support the recombination hotspot proposed by Mejia et al. (2011) in the vicinity of the *SDI* locus. Crossover mapping would be required to validate the smaller 92-kb confidence interval considered by these authors for the screening of the *SDI* gene.

In the second place, RNA-seq, allele-specific RT-qPCR and genotyping studies conducted here do not support the presence of *cis*-acting misexpression mutations within the *sdi* fine mapping interval (Fig. 4, Fig. 5, Supplemental Table 3, Supplemental Table 5 and Supplemental Table 7). Specifically for *VviAGL11* we show that rather than misexpression, which was related by other

authors to the cause of stenospermocarpy, the lower proportion of seed-related tissues in developing stenospermocarpic fruits is probably the origin of the differential expression that is detected for this gene when whole seeded and seedless fruits are compared (Mejia et al., 2011; Ocarez and Mejia, 2016; Malabarba et al., 2017). The relatively high expression of *VviAGL11* that we detected in seed traces of 'Sultanina' (Fig. 4D) is also contradictory with the absence of expression that Malabarba et al. (2017) reported in sections of developing flowers and fruits of this cultivar following *in situ* hybridization. In agreement with our results, some degree of *VviAGL11* expression has repeatedly been detected in developing flowers and fruits of *sdi*+ accessions using diverse approaches (Mejia et al., 2011; Wang et al., 2015; Ocarez and Mejia, 2016; Wang et al., 2016; Malabarba et al., 2017). Considering the fact that 80% of the probe used by Malabarba et al. (2017) for the *in situ* hybridization does not match with *VviAGL11* transcripts synthesized by 'Sultanina' but with a specific allele of 'Pinot Noir', deficient probe-transcript hybridization instead of lack of expression would be a likely cause for the absence of signal in 'Sultanina' on that assay.

In line with the inconsistent difference in *VviAGL11* expression between seeds and seed traces (Fig. 4 and Supplemental Table 3), candidate *sdi* polymorphisms that were previously proposed in regulatory regions of *VviAGL11* (Mejia et al., 2011; Di Genova et al., 2014; Ocarez and Mejia, 2016) can be specifically discarded according to the dominant nature of the *sdi* mutation and the presence in seeded accessions of the alleles linked in the *sdi*+ haplotype (Fig. 5A and Supplemental Table 7; Mejia et al., 2011; Karaagac et al., 2012; Bergamini et al., 2013). Importantly, irrespective of the

556 presence of polymorphisms in regulatory regions of *VviAGL11*, we found lack
557 of allelic expression imbalance in seed traces of *sdi*+/*sdi*- heterozygous
558 seedless individuals (Fig. 4), which rules out the presence of *cis*-acting
559 misexpression mutations in the *sdi*+ haplotype of this gene. In the absence of
560 allelic imbalance, mitotic recombination between the two alleles of 'Sultanina'
561 could not recover *VviAGL11* expression as it was suggested previously for the
562 origin of ‘Sultanina Monococco’ somatic variants (Ocarez and Mejia, 2016).

563 Finally, after RNA-seq screening of coding sequences within the *sdi* mapping
564 interval (Table 2), the SNV causing the Arg197Leu substitution in *VvAGL11*
565 was the only predicted deleterious variant in which a full linkage with
566 seedlessness phenotype was confirmed in a collection of grapevine cultivars
567 (Fig. 5A). Although this missense variation was previously discarded according
568 to its homozygous presence in the seeded cultivar ‘Asyl Kara’ (Mejia et al.,
569 2011), in our study this cultivar was homozygous for the Arg codon similar to all
570 other 106 seeded cultivars analyzed (Supplemental Table 6 and Supplemental
571 Table 7). Remarkably, as consistent genetic proof, this polymorphism is the
572 only candidate mutation that is not detected in ‘Sultanina Monococco’ seeded
573 somatic variants of the original seedless cultivar ‘Sultanina’ (Fig. 6). Given that
574 unlikely coincidence in near-isogenic somatic variants, it can be assumed that
575 somatic variations (either genetic or cellular) that altered the genotype of the
576 missense SNV are the cause of the polymorphic seed development phenotype
577 displayed by post-zygotic variants of ‘Sultanina’. This causality proof, together
578 with all other genetic and molecular evidence described above, led us to
579 postulate that *VviAGL11* is the major dominant regulator gene and the SNV
580 resulting in the Arg197Leu substitution is the monogenic *sdi* mutation that two

decades ago were proposed for the main origin of seedlessness in grapevine (Bouquet and Danglot, 1996; Lahogue et al., 1998).

Molecular determination of stenospermocarpy by the *sdi* missense mutation

In agreement with the absence of the *VviAGL11* missense mutation in seeded somatic variants of 'Sultanina' (Fig. 6C), *AGL11* homologs participate in the development of maternal seed tissues that is triggered after fertilization in other plant species (Colombo et al., 1997; Mizzotti et al., 2014; Malabarba et al., 2017). Resembling the phenotype of grape stenospermocarpy (Pearson, 1932; Barritt, 1970; Pratt, 1971; Striem et al., 1992; Malabarba et al., 2017), co-suppression of *FLORAL BINDING PROTEIN7* (*FBP7*) and *FBP11*, two *AGL11* homologs present in petunia (*Petunia hybrida*), caused the development of shrunken seeds that lack seed coat sclerification with a consequent degeneration of the endosperm, while the embryo frequently remained viable (Colombo et al., 1997). As reported for *FBP7* and *FBP11* transcripts in wild-type petunia or AGL11/STK protein in Arabidopsis (Colombo et al., 1997; Mizzotti et al., 2014), *VviAGL11* is specifically transcribed in the developing seed coat in 'Pinot Noir' seeded grape cultivar (Malabarba et al., 2017). These coincidences suggest that *AGL11* function is impaired in grapevines carrying the Arg197Leu substitution.

Resembling the Arg197Leu substitution identified in grapevine (Table 2, Fig. 5 and Fig. 6), variation of endocarp lignification in different accessions of oil palm (*Elaeis guineensis*) is associated with five independent missense substitutions in the MADS-box domain of *SHELL*, an oil palm homolog of *AGL11* (Singh et al., 2013; Ooi et al., 2016). The Arg197Leu substitution in VviAGL11 is located

606 in the C domain characteristic of MIKC-type MADS-domain proteins (Fig. 5B).
607 This domain participates in binding activity of MIKC tetramers to DNA and it
608 may also determine protein-protein interaction specificity (Honma and Goto,
609 2001; Melzer et al., 2009; van Dijk et al., 2010). According to this function, and
610 considering also that VviAGL11-SEPALLATA heterodimers interact with
611 additional VviAGL11 units (Mellway and Lund, 2013), the Arg197Leu
612 substitution might cause dominant effects by disrupting the action of VviAGL11-
613 containing multiprotein complexes, even in the presence of the seeded allele in
614 heterozygous individuals as postulated for mutant alleles of SHELL (Singh et
615 al., 2013; Singh et al., 2015). This mechanism would be compatible with the
616 incomplete dominance of the *sdi* mutation that can be inferred in view of the
617 more extreme seedless phenotype that is often displayed by *sdi+*/*sdi+*
618 homozygous individuals (Mejia et al., 2011; Ocarez and Mejia, 2016).

619 In spite of genetic proofs leading to the missense substitution in *VviAGL11*
620 (Table 2, Fig. 5 and Fig. 6) and all other indirect evidence mentioned above, a
621 heterologous assay in Arabidopsis was inconclusive about the role in
622 seedlessness determination of the VviAGL11 protein encoded by the *sdi+*
623 haplotype (Malabarba et al., 2017). However, species-specific functions of
624 AGL11 homologs are conceivable since, contrasting with co-suppressed
625 petunia lines and *sdi* stenospermocarpy, the Arabidopsis *stk* loss-of-function
626 mutant over-accumulates polyphenols in the seed coat in the absence of
627 endosperm degeneration or embryo arrest (Mizzotti et al., 2014). In fact, while
628 28 DEGs between *sdi+* and *sdi-* RG×CS offspring were the closest grapevine
629 homologs to any out of the 248 de-regulated genes in the *stk* mutant (Mizzotti
630 et al., 2014), 71% of these DEGs showed inverted expression response

compared to the experiment in Arabidopsis (Supplemental Table 3). Genes involved in flavonoid accumulation were upregulated in seeds of the *stk* mutant, including *DIHYDROFLAVONOL 4-REDUCTASE/TRANSPARENT TESTA 3* (*DFR*/*TT3*), *BANYULS*/*ANTHOCYANIDIN REDUCTASE* (*BAN*/*ANR*) and *TT12*. In contrast, the grapevine homologs *VviDFR1* (*VIT_18s0001g12800*), *VviANR* (*VIT_00s0361g00040*) and *VviTT12* (*VIT_12s0028g01150*), respectively, together with the master regulator of proanthocyanidin accumulation in grape *MYB PROANTHOCYANIDIN 1* (*VviMYBPA1*, *VIT_15s0046g00170*) (Bogs et al., 2007), were down-regulated in *sdi*+ seed traces (Supplemental Table 3), which in turn accumulate less polyphenols than *sdi*- grapevine seeds (Merin et al., 1983). In line with these inverted effects, a reversible role of AGL11/STK in the control of tissue lignification has been shown in Arabidopsis depending on the presence of transcriptional co-suppressor partners (Balanza et al., 2016). We thereby assume that functional studies in genetically engineered vines are required to ensure the presence of grapevine-specific protein partners. While functional studies of fruit traits takes a long time in species with long juvenile phases, such as grapevine, we are conducting additional research in this direction to demonstrate the consequences of the Arg197Leu substitution for VviAGL11 protein function and the determination of seed development abortion.

At this point, according to all evidence described above, we suggest a working model in which the *sdi* missense substitution identified here disrupts the function of multimeric complexes containing VviAGL11 proteins (Fig. 7). This would prevent proper seed coat development and lignification (Malabarba et al., 2017), which is required for nutrient and signaling flux between maternal

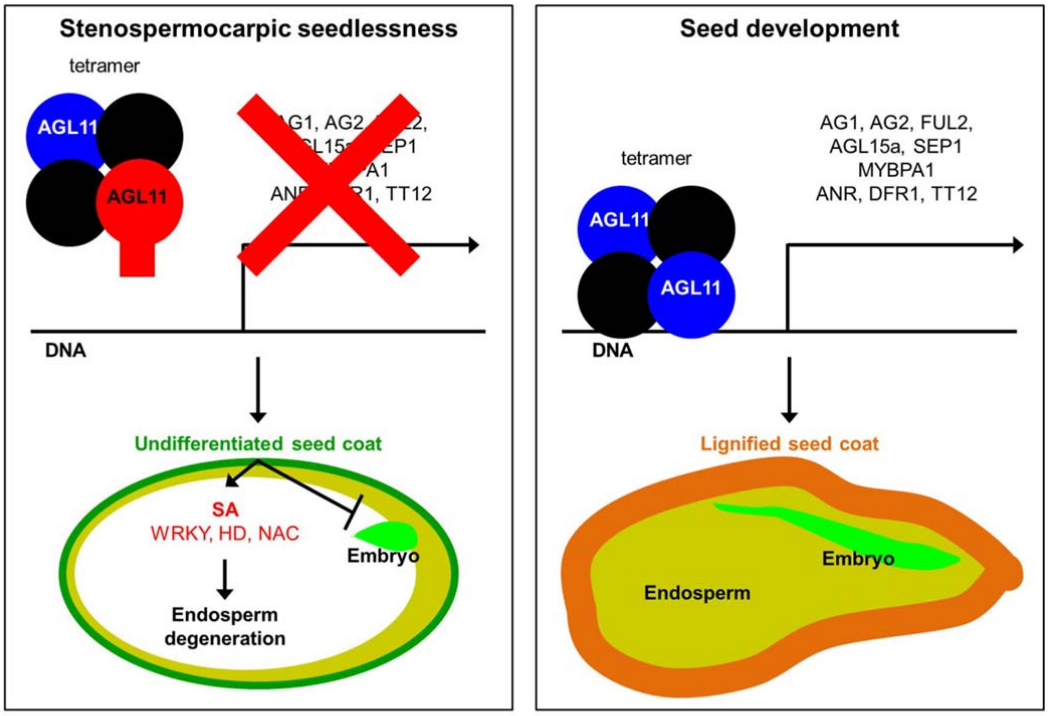


Figure 7. Model of grape seedlessness determined by the sdi locus.

tissues and fertilization products (Mizzotti et al., 2012; Figueiredo et al., 2016). Apparently, this impaired flux in developing seeds of stenospermocarpic grapes leads to SA-dependent autoimmune responses (Fig. 3), and to endosperm degeneration and embryo development arrest (Pratt, 1971), which at the transcriptome level associate with the repression of secondary metabolism and MADS-box genes and the activation of photosynthesis-related genes (Fig. 3, Supplemental Table 3, Supplemental Table 4). These molecular responses are very similar to those reported during SA-dependent seed abortion in incompatible Arabidopsis inter-specific hybrids that, like *sdi*-determined stenospermocarpy, proceed with abnormal seed coat development (Burkart-Waco et al., 2013).

Conclusions

Diverse approaches converge on the SNV causing the Arg197Leu substitution in *VviAGL11* as the seedlessness responsible mutation underlying the grapevine *SDI* locus. While molecular and expression assays rejected the hypothesis of *cis*-acting misexpression mutations, the deleterious effect predicted for this amino acid change, the full association between the variant allele and stenospermocarpy in a collection of grapevine cultivars, and the concurrent post-zygotic variation of both the missense polymorphism and seedlessness phenotype in somatic variants of the 'Sultanina' cultivar consistently point to this SNV as the causal mutation. This finding, together with previous discoveries in oil palm (Singh et al., 2013), show that variation in the amino acid sequence of AGL11 homologs has been selected in parallel during the domestication of distant monocot and dicot crops to control the proportion between pericarp and seed tissues of fruits as well as their level of sclerification. While seedlessness is the most relevant fruit feature for the greatly active table grape breeding field, our discovery enables the development of the most efficient marker-assisted selection to track the major locus controlling this trait. The simultaneous tracking of the *sdi* mutation and of minor *SDI*-independent loci controlling the degree of lignification and the size and number of seed traces will optimize the generation of new seedless cultivars in future table grape breeding programs. Remarkably, knowledge of the *sdi* mutation can also be exploited to introduce stenospermocarpy into other genetic backgrounds through controlled genome editing.

Materials and Methods

Plant material

Two table grape (*Vitis vinifera*) F_1 mapping populations, (RG×CS, *n* = 292 and IN×CS, *n* = 299) were generated from controlled crosses carried out in 2003 and 2008, respectively. For these crosses, emasculated flowers of RG and IN seeded cultivars were pollinated with pollen collected from CS that in both populations was the donor of the *sdi* mutation. One plant for each F_1 individual and three plants for each progenitor were grown in the same experimental field belonging to the Sociedad Murciana de Investigación y Tecnología de Uva de Mesa (ITUM) located in Blanca, Murcia, Spain. Plants were grafted onto 1103 Paulsen rootstock conducted under parral trellis, ferti-irrigated and cultivated under the same management practices.

For the study of sequence variation at selected loci, 124 grapevine cultivars and two RS×MO F_1 individuals were used (Supplemental Table 6 and Supplemental Table 7). Samples of 120 cultivars were obtained from the Grapevine Germplasm Collection of the Instituto de Ciencias de la Vid y del Vino (ICVV, ESP-217). At ICVV, ten plants per cultivar were maintained under the same agronomical conditions in 'Finca La Grajera' located in Logroño, La Rioja, Spain. These plants were grafted in 2010 onto 110 Richter rootstock, and their true-to-typeness was verified by genotyping SNP markers and comparing the results with the ICVV-SNP database (Cabezas et al. 2011). RG and CS samples were obtained from ITUM, whereas RS×MO F_1 individuals and the MO cultivar were obtained and cultivated as described elsewhere (Carreño et al., 2015). Material for 'Chasselas Doré' was obtained from plants, grafted onto 110 Richter in 2003, in the '*Vitis* Germplasm Bank' (ESP-080) of the Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA) located in 'Finca El Encín' in Alcalá de Henares (Madrid, Spain). In addition to

718 the previous 126 accessions, materials for four 'Sultanina Monococco'
719 accessions (2085Mpt1, 2594Mtp2, 2594Mtp1 and 2777Mtp1; SM1 to SM4,
720 respectively) and for a second reference accession of 'Sultanina' (1566Mtp2)
721 that all were confirmed previously as clonal lines (Laucou et al., 2011), were
722 obtained from the collection of the Institut National de la Recherche
723 Agronomique Centre de Ressources Biologiques de la Vigne at Vassal-
724 Montpellier (FRA-139), Marseillan-Plage, France. SM1 to SM4 accessions
725 entered the Vassal-Montpellier repository in 1966, 1959, 1966 and 1975 from
726 Cyprus, Turkey, Iran and Bulgaria, respectively
727 (https://bioweb.supagro.inra.fr/collections_vigne).

728 **Seedlessness trait assessment**

729 For RG×CS F_1 progeny and progenitors, seed content was evaluated at harvest
730 during three years (2007-2009). For each individual and year, 20 fruits randomly
731 collected from all the clusters produced by the plant were analyzed to estimate
732 seed or seed trace number per berry and dry weight of seed content per berry
733 (SDW). SDW was measured after 48 h of incubation of seeds and seed traces
734 in an 80ºC oven.

735 The seedlessness phenotype was also *de visu* registered as a qualitative trait
736 for every RG×CS F_1 individual in the same three seasons. For recombinants in
737 the *SDI* mapping interval, *de visu* determination of seedlessness was conducted
738 in 2012 and 2013 as well. To this aim, seeded and seedless individuals were
739 classified according to the presence of hard seeds with totally sclerified
740 integuments or soft seed traces with unsclerified or partially sclerified
741 integuments, respectively, as previously described (Costantini et al., 2008).
742 Similar *de visu* classification of the seedlessness trait was carried out at least

twice (between 2013 and 2016 seasons) for the IN×CS F_1 mapping population and for the 120 cultivars from the ICVV collection used for genotyping of candidate genes (Supplemental Table 1, Supplemental Table 6 and Supplemental Table 7). At least 20 berries from 2-3 representative clusters from the same plant were inspected each time. Furthermore seed content phenotypes in ‘Sultanina’ variant lines from the Vassal-Montpellier collection were quantitatively measured in the 2017 season following the same procedures described for RG×CS offspring.

DNA extraction

DNA was extracted from young leaves. For RG×CS progeny, DNA was extracted using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) and modified by adding 1% PVP40 to the AP1 buffer. The BioSprint 96 DNA Plant Kit (Qiagen) was used for IN×CS progeny following the manufacturer’s instruction. For all other accessions, the DNeasy Plant Mini kit (Qiagen, Hilden, Germany) was used without modifications.

QTL analysis

Marker genotyping. RG, CS and RG×CS F_1 individuals were genotyped for a set of 223 SSR markers previously mapped on the grapevine genome (Doligez et al., 2002; Cabezas et al., 2006; Costantini et al., 2008), 178 of which showed segregation types suitable for genetic mapping. Primers were modified at the 3’ end with a fluorochrome (NED, 6-FAM, VIC or PET) by Applied Biosystems. PCR amplifications were performed with Ecogen Taq polymerase. PCR products were analyzed with ABI Prism 3730 (Applied Biosystems) at the Unidad de Genómica-Campus Moncloa del Parque Científico de Madrid. Detection of amplified fragments and allele assignment was carried out using

768 GeneMapper™ v3.7 (Applied Biosystems). Additionally, F_1 individuals and
769 progenitors were genotyped for 335 SNP markers described in Lijavetzky et al.
770 (2007) and Cabezas et al. (2011), 112 of which showed segregation types
771 suitable for genetic mapping and were analyzed as described previously
772 (Cabezas et al., 2011).

773 **Genetic map construction.** Genetic maps were built according to a 2-way
774 pseudo-testcross strategy (Grattapaglia and Sederoff, 1994) using JoinMap 3.0
775 (van Ooijen and Voorrips, 2001). DH (*Doubled Haploid*) and CP (*Cross*
776 *Pollinators*) models were assumed for the construction of parental and
777 consensus maps, respectively. The global segregation of each marker was
778 tested for the expected Mendelian segregation ratio using a χ^2 goodness-of-fit
779 test (P<0.01). Markers were grouped in the same LG according to the threshold
780 LOD≥5.0 and ordered by paired markers with LOD≥3.0 and recombination
781 frequency (REC) ≤0.35. These parameters were reduced to LOD≥1.0 and
782 REC≤0.45 when required to adapt linkage groups to current information on the
783 physical genome map. Mapping distances were calculated (in cM) using the
784 Kosambi function (Kosambi, 1944).

785 **QTL detection.** QTL analyses were carried out on RG×CS mapping progeny
786 independently for each of the three SDW evaluations (seasons 2007, 2008 and
787 2009) using the framework maps of each progenitor and the consensus
788 framework map for the cross. QTL mapping was performed using MapQTL 4.0
789 (van Ooijen et al., 2002). Two parametric methods, Interval Mapping (IM)
790 (Lander and Botstein, 1989; van Ooijen, 1992) and Multiple QTL Mapping
791 (MQM) (Jansen and Stam, 1994) were used. LOD score thresholds of 0.99
792 significance for each LG and 0.95 for the whole genome were established

through 1000 permutations for each trait (Churchill and Doerge, 1994). QTL position was estimated from the location of the maximum LOD value and a minimum confidence interval (minimum overlapping interval for 1 LOD intervals independently detected for each year of analysis on each QTL and map).

Fine mapping of the *SDI* QTL

RG×CS F_1 individuals were screened for meiotic recombination around the *SDI* QTL according to the genotype at the microsatellite locus VVIN16 (Merdinoglu et al., 2005), and CAPS-28.53. This CAPS was designed for a BstBI (New England Biolabs, Inc., MA, USA) restriction enzyme target specific of the *sdi*+ haplotype in this progeny (Supplemental Table 2). Primer pairs used to obtain this and other target amplicons are described in Supplemental Table 9. PCR primers were designed with the NCBI Primer BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) using the grapevine 12X.0 reference genome assembly sequence as a template (https://urgi.versailles.inra.fr/Species/Vitis/Data-Sequences/Genome-sequences). Similarly, recombinant individuals within the fine mapping interval delimited in RG×CS F_1 progeny were searched for in IN×CS F_1 progeny by genotyping the CAPS-26.39 and CAPS-26.88 markers. These markers were respectively designed taking advantage of the presence of BlpI and MseI (New England Biolabs, Inc., Ipswich, MA, USA) restriction enzyme targets that are specific to the *sdi*+ haplotype in this F_1 mapping population. Crossovers were mapped in recombinant F_1 individuals by genotyping SNP markers indicated in Supplemental Table 2, which were developed and genotyped through PCR amplification and Sanger capillary electrophoresis sequencing. In most cases, primers were designed to align in coding sequences according to grapevine

12X V1 gene annotations (http://genomes.cribi.unipd.it/). For PCR, KAPA2G Fast DNA Polymerase (KAPA Biosystems, Cape Town, South Africa) was used for amplification from ~50 ng of gDNA. Amplification products were purified with ExoSAP-IT (USB Products Affymetrix, Cleveland, OH, USA) following the manufacturer's instructions and then sequenced by Sanger capillary electrophoresis using the same primers as in PCR. For every marker, the allele present in the *sdi*+ haplotype was inferred according to the genotype observed in cross progenitors, F_1 individuals with extreme seed/seedless phenotype and the two RS×MO *sdi* homozygous F_1 individuals (Supplemental Table 2).

Transcriptome analysis

RNA extraction. Total RNA was extracted from frozen tissue powder using the Spectrum™ Plant Total RNA kit (Sigma-Aldrich) with an added on-column DNase digestion step with the RNase-Free DNase Set (Qiagen).

Microarray analysis. Developing fruits at fruit set stage (~2 WAF, flowering time 20 May 2010) were collected on 3 June 2010, around midday, from four seeded (660-43, 660-50, 669-45, 671-19) and five seedless (660-40, 660-41, 660-57, 669-19, 671-34) RG×CS F_1 individuals and immediately frozen in liquid nitrogen. Individuals were selected according to contrasting genotypes in the VMC7F2 marker (Pellerone et al., 2001), which co-localizes with the *SDI* QTL (Table 1), and to extreme phenotypes for SDW in the corresponding progeny distribution tail (Supplemental Table 1). Each individual was analyzed in separate as independent biological replicates. At least 12 developing fruits collected from ≥2 different clusters were ground for each RNA extraction. Analysis of RNA integrity as well as synthesis, labeling, and hybridization of cDNA to NimbleGen microarray 090818 Vitus exp HX12 (NimbleGen-Roche)

and robust multi-array average (RMA) normalization were performed as indicated elsewhere (Carbonell-Bejerano et al., 2014). Linear models for microarray data (limma) were run in Babelomics (Medina et al., 2010) to search for DEGs between seedless and seeded individuals. DEGs were identified considering a Benjamini-Hochberg adjusted P-value ≤0.05 and ≥2-fold change as significance thresholds.

RNA-seq and differential gene expression analysis. RNA was obtained from seed traces of three seedless (669-60, 670-17, 675-17) and seeds from three seeded (665-17, 671-19, 677-06) RG×CS F_1 individuals. These individuals were selected according to contrasting genotypes for the *SDI* QTL marker VMC7F2 (Supplemental Table 1). Selected seeded individuals belonged to the higher tail of the distribution for SDW phenotype in the progeny, whereas seedless individuals without extremely low SDW were selected to ease the extraction of seed traces. Berries at pea size (10-12 mm) developmental stage were collected on 12 June 2012 (~4 WAF, flowering time 18 May 2012) around midday and immediately frozen in liquid nitrogen. Berries were allowed to briefly thaw in the laboratory, opened by cutting with a scalpel and then, seed content was rapidly recovered with tweezers and refrozen in nitrogen. Each individual was analyzed in separate as independent biological replicates. Seed content from 12-50 fruits collected from ≥2 different clusters was used for each RNA extraction. RNA-seq was performed in the Centre for Genomic Regulation (CRG) (Barcelona, Spain). The six corresponding cDNA libraries were prepared using the Illumina TruSeq Stranded mRNA Sample Prep kit starting from 1 µg of total RNA as described previously (Royo et al., 2016). A mean fragment size of 302 bp was obtained. Library sequencing was performed on an Illumina HiSeq

2000 using v4 chemistry (flow cells and sequencing reagents). Paired-end strand-specific reads of 125 nt were produced. Gapped alignment of reads to the PN40024 12X.0 grapevine reference genome assembly (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/) was carried out using TopHat2 v.2.0.13 (Kim et al., 2013). TopHat2 was run under default parameters with the exception of gap length ≤8, mismatch ≤8 and edit distance ≤8 that were allowed in 125 nt reads. These values were adjusted to compensate for the relatively high genetic distance between the table grape genotypes studied here and the reference genome of the wine grape-related PN40024 reference genome. As post-filtering, only uniquely mapped single copy reads with quality ≥20, aligned in the same chromosome and paired in the expected orientation were kept for further analysis. After filtering, an average of more than 15 million reads per replicate was considered for subsequent analysis (Supplemental Table 10). The htseq-count tool (v.0.5.4p5) from HTSeq (Anders et al., 2015) was used to estimate unambiguous read count for each 12X V1 annotated transcript. Normalization following the trimmed mean of M-values (TMM) method (Robinson and Oshlack, 2010), as well as seed trace *versus* seed DEGs search (adjusted Benjamini-Hochberg P-value ≤0.05 and ≥2-fold change) were performed in edgeR v.2.2.6 (Robinson et al., 2010). Finally, reads per kb of exon per million fragments mapped (RPKM) was calculated using edgeR and low expressed transcripts were filtered out if average RPKM was <1 in both seed traces and seeds.

Functional analysis of differentially expressed genes. Lists of DEGs identified from microarray and RNA-seq analyses were compared using Venny (http://bioinfogp.cnb.csic.es/tools/venny/). Gene lists were further analyzed for

functional enrichment compared to the whole set of transcripts predicted in the 12X V1 annotation of the grapevine reference genome following a grapevine-specific functional classification (Grimplet et al., 2012). The analysis was carried out in FatiGO as described elsewhere (Carbonell-Bejerano et al., 2014).

Search of RNA-seq data for candidate allelic imbalance. Aligned reads within the *sdi* fine-mapped interval were visually inspected for allele-specific expression with the Integrative Genomics Viewer (IGV) software (Thorvaldsdottir et al., 2013). For *VviAGL11*, the allelic ratio in each sample was estimated by comparing the frequency of each allele at heterozygous SNP positions in filtered Binary Alignment Map (BAM) files. A mean value was estimated for all the SNPs detected along the gene according to the parameters indicated in the following paragraph. The *sdi+* haplotype was inferred from the comparison of two seedless RG×CS F_1 individuals that shared the same allele inherited from RG for the *SDI* locus with the three seeded individuals analyzed by RNA-seq (Supplemental Table 1).

Search of RNA-seq data for candidate sequence variation. To detect sequence variation specific to the *sdi+* haplotype in expressed transcripts, RNA-seq alignments used for differential expression analysis were also analyzed for the presence of SNPs and INDELs, basically following a similar pipeline to that described previously (Royo et al., 2016), but adjusted to boost variant calling in both low expressed and allelic imbalanced genes. For variant calling, three tools implemented in SAMtools package v.1.5 (Li et al., 2009; Li, 2011) were used to compare each sample to the PN40024 reference genome: *samtools mpileup* for genotype probability estimation, *bcftools* for variant calling, and finally, strand bias and baseQ bias filters were applied using *varfilter*. Subsequently, using *ad*

hoc Bash shell and Perl scripts (Royo et al., 2016), we selected polymorphisms within the *SDI* fine-mapping interval specific to the three seedless individuals according to the following filters: average depth of contrasting alleles (variant or reference) per F_1 individual ≥5 counts, frequency of variant allele in *sdi*+ individuals ≥20%, frequency of variant allele in seeded individuals <2.5% and frequency of spurious alleles (alleles other than the reference and the first variant) <2.5% in both samples. In this case, reads corresponding to each transcription strand direction were independently computed. The effect of detected candidate *sdi* mutations considering grapevine 12X V1 gene annotations was estimated using SnpEff v.2.0.3 (Cingolani et al., 2012), whereas the effect of *sdi* candidate amino acid substitutions on protein function was predicted using PROVEAN (Choi et al., 2012).

Hormone analysis

To compare phytohormone levels between developing seeds and seed traces, five seedless (665-16, 669-60, 670-17, 675-17, 660-57) and five seeded (660-43, 660-50, 665-17, 671-19, 677-06) RG×CS F_1 individuals were analyzed (Supplemental Table 1). Additionally, two independent biological replicates of RG and CS, each from a different plant, were analyzed as well. Fruits at pea size stage (~4 WAF) were collected simultaneously to RNA-seq samples on 12 June 2012 and immediately frozen in liquid nitrogen. For each replicate, seed content from ≥20 fruits was extracted. SA and active GA (GA_1 and GA_4) levels were measured at the Plant Hormone Quantification service of the Institute for Plant Molecular and Cell Biology (IBMCP), Valencia, Spain. In summary, 200 mg fresh weight of seeds or seed traces was extracted for each replicate as indicated above for RNA-seq analysis. Frozen ground tissue was then analyzed

using an ultra performance liquid chromatography - tandem mass spectrometer (UHPLC-MS) system (Q-Exactive, ThermoFisher Scientific). The statistical treatment of results was carried out using SPSS software (v.24.0 for Windows; IBM Corp., Somers, NY, USA).

Reverse transcription quantitative PCR (RT-qPCR)

RNA was obtained from either berry skin, flesh, pericarp, seeds or seed traces. Pea size fruits collected from the ITUM experimental field on 26 June 2011 were used for three RG×CS F_1 individuals (660-41, 660-43, 660-57) and RG and CS progenitor accessions. ‘Sultanina’ samples from ESP217-5186 accession were collected at fruit set (6 June 2011) in the ICVV collection. At least 8 fruits were used for each RNA extraction. For reverse transcription from total RNA (1 µg), SuperScript™ III First Strand (Invitrogen, USA) and oligo (dT) were used following manufacturer’s instructions. Transcript levels were determined by RT-qPCR using a 7500 Real-Time PCR System (Applied Biosystems) and SYBR Green PCR Master Mix (Applied Biosystems). Reactions were performed in a final volume of 20 µl with 5 µl of a 1:10 dilution of cDNA. Gene-specific primers (Supplemental Table 9) were designed using the Oligo Explorer 1.2 software (Gene Link) and the gene sequences from the grapevine 12X.0 genome assembly (Jaillon et al., 2007) as design templates. For allele-specific RT-qPCR, the 3’ end of forward and reverse primers coincided with SNP positions in a way that each primer was specific for one allele. No-template controls were included for each primer pair, and each PCR was performed in triplicate. Amplification data were analyzed using the 7500 SDS software 1.3 (Applied Biosystems). Relative transcript levels were calculated after normalization to the grapevine *UBIQUITIN*

(*VIT_16s0098g01190*), *ELONGATION FACTOR 1-α* (*EF1-α* , *VIT_06s0004g03220*) and *GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE* (*GPDH* (*VIT_17s0000g10430*) using the ΔΔCt method. For allele-specific RT-qPCR, two independent biological replicates from different stenospermocarpic 'Sultanina' plants were analyzed and gDNA from the same accession was used for allelic ratio normalization assuming a balanced ratio in the genome. Graphical representations of relative transcript levels with standard deviations were performed using the Microsoft Excel software. The statistical treatment of results was carried out using SPSS software (v.24.0 for Windows; IBM Corp., Somers, NY, USA).

Genotyping of candidate mutations in *VviPPAT2*

For validation of candidate SNVs detected from RNA-seq data, the gene *VviPPAT2* (*VIT_18s0041g01870*) was partially sequenced in a collection of 93 grapevine accessions (Supplemental Table 6). Primer pairs PPAT2-R151C and PPAT2-Q195L were used for PCR and Sanger sequencing to this end (Supplemental Table 9).

Targeted re-sequencing of *VviAGL11*

The gene *VviAGL11* (*VIT_18s0041g01880*) plus a 2-kb upstream sequence (9,849-bp in total corresponding to positions chr18:26,888,672-26,898,521 in the PN40024 12X.0 grapevine reference genome) were sequenced in a collection of 110 accessions (Supplemental Table 7) by BGI company (http://www.genomics.cn/en) as described in Tello et al. (2016). Briefly, Agilent SureSelect (http://www.genomics.agilent.com) was used to target the *VviAGL11* locus that was then sequenced in 90 nt paired-end reads using Illumina HiSeq 2000. Sequencing data was analyzed as described by Tello et al. (2015): reads

were aligned to the PN40024 12X.0 reference genome using Bowtie 2 (Langmead and Salzberg, 2012). Then, the variant calling tool implemented in SAMtools was used to detect SNPs and small INDELs by comparing the sequence of aligned reads in each of the 110 accessions to the 12X.0 PN40024 reference genome. Polymorphisms were initially filtered by means of *ad hoc* Perl scripts as previously described (Tello et al., 2015). Significant polymorphisms present and absent in every seedless and seeded accession, respectively, were selected as candidate *sdi* mutations.

Validation by PCR and amplicon sequencing of the *sdi* candidate mutation identified in *VviAGL11* was carried out using the primer pair CAPS-26.88 from Supplemental Table 9 on gDNA of 15 additional seedless cultivars as well as in the seeded cultivar 'Asyl Kara' and in two 'Sultanina Monococco' accessions (Supplemental Table 6). Genotyping of 13 microsatellite markers in the same DNA sample used to genotype *sdi* candidate variants by PCR and Sanger sequencing was carried out to verify the identity of 'Asyl Kara' and 'Sultanina' seedless and seeded accessions (Supplemental Table 8). Microsatellite marker genotyping was performed as described elsewhere (Ibáñez et al., 2009).

Accession numbers

Microarray hybridization and RNA-seq data have been deposited in the NCBI GEO database under GSE106668 and GSE107014 accession numbers, respectively. BAM files for read alignments of *VviAGL11*-targeted NGS data were deposited under BioProject ID PRJNA418130 (SRP124845) of the NCBI Sequence Read Archive.

Supplemental data

Supplemental Table 1. Phenotype and genotype of offspring in F_1 mapping populations.

Supplemental Table 2. Genotyping for fine mapping in seeded × seedless F_1 mapping populations.

Supplemental Table 3. List of differentially-expressed genes detected between seedless and seeded RG×CS progeny.

Supplemental Table 4. Functional enrichment analysis of differentially-expressed genes detected between seedless and seeded RG×CS progeny.

Supplemental Table 5. List of single nucleotide variants linked to the *sdi* haplotype detected from RNA-seq data.

Supplemental Table 6. Genotype of candidate missense substitutions in *VviPPAT2* and *VviAGL11* and seedlessness phenotype in a collection of grapevine cultivars.

Supplemental Table 7. Polymorphisms in *VviAGL11* detected by next generation sequencing and seedlessness phenotype in a collection of grapevine cultivars.

Supplemental Table 8. Microsatellite genotyping identification for 'Sultanina' and 'Asyl Kara' accessions.

Supplemental Table 9. List of primer pairs used for PCR amplification and sequencing.

Supplemental Table 10. RNA-seq library size and normalization factors.

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We thank J.L. Pérez-Sotés and Servicio de Investigación Vitivinícola (SIVV, Gobierno de La Rioja) at ICVV collection and Rosa M. Arnau and María J. Candel at IMIDA and ITUM for plant maintenance and technical support in phenotyping tasks. We are very grateful to Nachi Montemayor, Rufino Aguirrezabal, Miguel Angulo and Silvia Hernáiz for technical assistance. We also thank the Genomics service of the CNB-CSIC for running microarray hybridizations and Dr. Iván Carreño for sharing information regarding RS×MO F_1 progeny.

Abbreviations

AGL = Agamous-like

Arg = Arginine

CAPS = Cleaved amplified polymorphic sequence

CS = Crimson Seedless

DEG = Differentially expressed gene

GEO: Gene expression omnibus

IN = Imperial Napoleon

INDEL = Insertion-deletion

Leu = Leucine

LG = Linkage group

LOD = Logarithm of odds

QTL = Quantitative trait locus

MO = Moscatuel

NGS = Next generation sequencing

PPAT = Pantetheine-phosphate adenylyltransferase

REC = Recombination frequency

RG = Red Globe

RS = Ruby Seedless

SA = Salicylic acid

SDI = Seed Development Inhibitor

SDW = Seed dry weight

SNP = Single nucleotide polymorphism

SNV = Single nucleotide variation

SSR = Simple sequence repeat

VIVC = *Vitis* International Variety Catalogue

WAF = Weeks after flowering

Tables

Table 1. Quantitative trait loci (QTLs) identified for seed dry weight per berry (SDW) in the 'Red Globe' (RG) × 'Crimson Seedless' (CS) F_1 mapping population. QTLs detected in the CS map and in the consensus map (c) are indicated. QTLs were not detected in the RG map. LG, linkage group; LOD, $\log_{10}$ of odds; variance, percentage of SDW variation explained by the QTL. Ranges of LOD and variance are shown if the QTL was detected in more than one year.

QTL-linked marker	Map	LG	Year	Position (cM)	Confidence interval (cM)	LOD	Variance (%)
VMC6F1-CS	CS	2	07, 08, 09	15.6	0.0-36.6	1.4-4.7	0.7-1.6
SNP1053_81-c	CS	5	08	7.9	0.0-19.9	2.7	0.8
VMC2H5-c	CS	14	08	21.3	0.0-42.0	1.6	0.4
VMC7F2-CS	CS	18	07, 08, 09	66.1	64.7-66.1	67.4-109.7	71.0-83.0
VVIB23-c	c	2	07, 08, 09	23.2	16.2-31.2	3.0-9.9	1.6-2.3
SNP1053_81-c	c	5	08	9.3	0.0-36.3	4.4	1.0
VMCNG1E1-CS	c	14	08, 09	0.0	0.0-9.0	2.7-2.8	0.7-1.1
VMC7F2-CS	c	18	07, 08, 09	73.3	72.1-73.3	69.6-116.2	71.8-80.9

Table 2. Nucleotide variation in the *sdi*+ haplotype generating protein sequence variation. Single nucleotide variants (SNVs) specific to the *sdi*+ haplotype located within the *sdi* fine mapping interval that were detected in the RNA-seq dataset (Supplemental Table 5) with an impact on protein coding sequence are presented here. For each SNV, the position and gene annotation in the 12X V1 grapevine reference genome, the nucleotide change in the coding sequence of the *sdi*+ haplotype, the amino acid change, the PROVEAN score and the predicted effect of the change in the function of the protein are indicated. PROVEAN score ≤ -2.5 and > -2.5 are considered 'deleterious' and 'neutral', respectively (Choi et al., 2012). Predicted deleterious changes are in bold.

Genome position	Gene ID	Functional annotation	Coding sequence change	Amino acid change	Score	Predicted effect
chr18:26,859,228	**VIT_18s0041g01870**	**VviPPAT2**	**451 C>T**	**Arg151Cys**	**-6.44**	**Deleterious**
chr18:26,871,891	**VIT_18s0041g01870**	**VviPPAT2**	**584 A>T**	**Gln195Leu**	**-4.27**	**Deleterious**
chr18:26,889,399	VIT_18s0041g01880	VviAGL11	628 A>G	Thr210Ala	-0.21	Neutral
chr18:26,889,437	**VIT_18s0041g01880**	**VviAGL11**	**590 G>T**	**Arg197Leu**	**-5.40**	**Deleterious**
chr18:26,914,516	VIT_18s0041g01890	Unknown protein	148 G>A	Asp50Asn	0.45	Neutral
chr18:26,918,160	VIT_18s0041g01900	Translation elongation factor	997 A>T	Thr333Ser	1.01	Neutral

Table 3. Genotype of the two *VviPPAT2* candidate single nucleotide variants (SNVs) in a collection of 93 accessions. The number of seedless and seeded accessions identified for each genotype are specified. For each position, the allele encoding candidate deleterious substitutions is denoted in bold. Accessions excluding these candidate SNVs are described in the footnote. The full list of genotypes for each accession can be found in Supplemental Table 6.

Genotype	VviPPAT2 Arg151Cys	VviPPAT2 Gln195Leu	Seedless accessions	Seeded accessions
1	**T**:**T**	**T**:**T**	1	0
2	**T**:**T**	A:**T**	1	0
3	C:**T**	A:**T**	14	1[a]
4	C:**T**	A:A	0	1[b]
5	C:C	A:A	0	28
6	**T**:T	nd	0	1[c]
7	nd	**T**:T	2	0
8	C:**T**	nd	0	7[d]
9	nd	A:**T**	2	1[e]
10	C:C	nd	0	1
11	nd	A:A	0	33

Footnote: [a]'Verdil', [b]'Pedro Ximenes', [c]'Planta Nova', [d]'Afus Ali', 'Alphonse Lavallee', 'Aramon', 'Aubun', 'Morio Muskat', 'Naparo', 'Semillon' and [e]'Cornichon Blanc'. nd, no data was obtained.

Figure legends

Figure 1. Stenospermocarpic phenotype and segregation in a RG×CS F_1 population. A, Berries of 'Red Globe' (RG) and 'Crimson Seedless' (CS) cross progenitors. Representative berries, seeds (RG) and stenospermocarpic seed traces (CS) at maturity are shown. **B,** Histogram showing the distribution of seed dry weight per berry (SDW) trait in RG×CS F_1 progeny. SDW values

correspond to the average of three seasons. The number of F_1 individuals is denoted in different colors depending on the genotype in the *SDI* QTL-linked marker VMC7F2: *sdi*+ (198:200) and *sdi*- (200:200) individuals are represented in red and blue, respectively. RG and CS indicate the phenotypic class coincident with the phenotype of each progenitor.

Figure 2. Fine mapping of the *sdi* mutation in two seedless × seeded F_1 populations. Scheme of crossover mapping for recombinants around the *SDI* locus in **A,** 'Red Globe' × 'Crimson Seedless' (RG×CS) and **B,** 'Imperial Napoleon' × 'Crimson Seedless' (IN×CS) F_1 mapping populations. Each class of recombinant F_1 individuals is depicted in different lines, where red, blue and grey colors denote chromosome fragments corresponding to the *sdi*+ seedless, *sdi*- seeded, or undetermined haplotypes, respectively. F_1 individuals considered and their phenotype (seed, seeded; trace, seedless) are indicated for each class in the left and right sides of the corresponding line, respectively. Markers studied in each F_1 mapping population, the *sdi* fine-mapped interval and the genes included on it according to distances in the PN40024 12X.0 reference genome and 12X V1 gene annotations are represented as well.

Figure 3. Transcriptome and phytohormone level comparisons between seedless (*sdi*+) and seeded (*sdi*-) RG×CS F_1 individuals. A, Venn diagram comparison of differentially expressed genes (DEGs) identified in 2 weeks after flowering (WAF) fruits (NimbleGen microarray) and 4 WAF aborting/developing seeds (RNA-seq). The number of up- and down-regulated DEGs in seedless (*sdi*+) *versus* seeded (*sdi*-) individuals in each experiment is shown. **B,** Summary of functional categories over-represented in up- and down-regulated DEGs in each experiment. This panel corresponds to a summary of the full list

of functional enrichment results presented in Supplemental Table 4. **C,** Levels of salicylic acid (SA) and bioactive gibberellins (GA_1 and GA_4) in RG×CS *sdi*+ and *sdi*- F_1 individuals and in the cross progenitors, 'Crimson Seedless' (CS) and 'Red Globe' (RG). Hormones were measured in seed traces or in seeds at 4 WAF. Average and SD concentration values of five replicates for *sdi*+ and *sdi*- F_1 individuals and two replicates for CS and RG are shown. Letters denote significant differences at *p*-value <0.01 using Duncan's post-hoc test.

Figure 4. *VviAGL11* transcript levels. A, Representation of RNA-seq reads aligned in a region of *VviAGL11* with a high density of heterozygous single nucleotide polymorphisms (SNPs). Image obtained from IGV viewer. Read depth is shown separately for each studied seedless (*sdi*+) and seeded (*sdi*-) RG×CS F_1 individual. The frequency of each allele at single nucleotide variant (SNV) positions compared to the reference genome is denoted in colors. The read depth range depicted for each individual is indicated as well. A scheme of the portion of *VviAGL11* 12X V1 gene model annotated in this region of the PN40024 12X.0 reference genome is shown in blue at the bottom of the image with coding sequence exons (cds) numbered from the start codon. **B,** Average *VviAGL11* allelic frequency in seedless RG×CS F_1 individuals estimated from RNA-seq data. **C,** Average *VviAGL11* allelic frequency in seeded RG×CS F_1 individuals estimated from RNA-seq. In B and C, frequencies were normalized to the *sdi*- allele inherited from RG, for which expression levels are depicted in blue. Only positions with a heterozygous genotype in the three replicates (six and three SNPs in seedless and seeded individuals, respectively) were considered to estimate average frequencies and SD (represented by error bars) in the three seeded or seedless replicates. **D,** *VviAGL11* expression estimated

by RT-qPCR. Expression in different pericarp and seed or seed trace (trace) tissues is shown for 'Sultanina' (Sult) at fruit set as well as for 'Crimson Seedless' (CS), 'Red Globe' (RG) and RG×CS F_1 individuals at 4 weeks after flowering (WAF). Expression levels were normalized relative to the *GPDH* house-keeping gene and to RG seed tissue. Similar results were obtained when two other control genes were used for normalization (see Methods). Data represent the mean and SD of three technical replicates. Different letters denote significant differences between seed-related tissue samples with *p*-value <0.01 using Duncan's post-hoc test. Red and blue colors denote expression in stenospermocarpic and seeded individuals, respectively.

Figure 5. Sequence variation in *AGL11* and association with seedlessness. A, Nucleotide sequence variation in the *sdi*+ haplotype of *VviAGL11* and association of the *sdi*-linked variants with seedlessness in a collection of grapevine cultivars. *VviAGL11* (exons + introns) plus the 2-kb upstream sequence were targeted for Illumina paired-end sequencing in 105 seeded and 5 seedless accessions. The position of single nucleotide polymorphisms (SNPs) and insertion-deletions (INDELs) identified in the *sdi* haplotype compared to the PN40024 reference genome is depicted on a scheme of the gene (encoded in the minus strand) with coding sequences (CDS) numbered from the start codon of the gene. The frequency of the variant allele in stenospermocarpic seedless and seeded accessions is indicated for specific candidate polymorphisms selected from the full list of polymorphisms available in Supplemental Table 7. Genotype-phenotype correlation of 100% was only identified for the SNP causing the Arg197Leu substitution in the *sdi* allele, which is highlighted in red. **B,** VviAGL11 protein domain model and

alignment of the C-terminal domain from homologous AGL11 and related Arabidopsis AGAMOUS-lineage MIKC-type proteins. Grapevine proteins encoded by the seeded (VviAGL11) and the seedless (VviAGL11-sdi) alleles, AGL11 homologs from dicotyledonous (*Petunia hybrida*, FBP7 CAA57311.1 and FBP11 CAA57445.1; *Solanum lycopersicum*, SlAGL11 AY098736.2 and SlMBP3 XM_010324479.2; *Malus domestica*, MdMADS10 CAA04324.1; *Populus trichocarpa*, Ptr_0019s10580 U5FIJ5; Arabidopsis AtSTK AT4G09960.3; *Glycine max*, GmAGL11 B9MSS8) and monocotyledonous (*Zea mays*, ZmAGAMOUS NP_001105946.1; *Brachypodium distachyum*, BradiMADS21 G9BIK9; *Oryza sativa*, OsMADS13 Q2QW53; *Sorghum bicolor*, SorbiMADS21 C5XEN4; *Triticum aestivum*, TaAGL11 ABF57916.1) species as well as related AGAMOUS-lineage SHATTERPROOF Arabidopsis homologs (AtSHP1, AT3G58780.3 and AtSHP2, AT2G42830.2) were compared using the Conserved Domain Database tool available from NCBI (http://www.ncbi.nlm.nih.gov/cdd/). The alignment was performed with ClustalW in Mega7 software (Kumar et al., 2016). The position of the two amino acid substitutions detected in the VviAGL11-sdi allele are highlighted in boxes, and the seedlessness-associated Arg197Leu substitution is shown in red.

Figure 6. Concurrent somatic variation of the *sdi* mutation and seedlessness trait in 'Sultanina Monococco'. A, Images of representative fruits in the stenospermocarpic cultivar 'Sultanina' (Sult) and in two seeded post-zygotic variants of 'Sultanina' that are known as 'Sultanina Monococco' (SM1 and SM2). **B,** Phenotype characterization of 'Sultanina' somatic variants. Seed dry weight per berry (SDW), berry fresh weight (BFW), number of seeds or traces per berry (Nº ST/B) and seed floatability are presented for Sult, SM1

and SM2 accessions. **C,** Post-zygotic variation in the single nucleotide variants determining the Arg197Leu *sdi* missense substitution in four 'Sultanina Monococco' accessions (SM1 to SM4). Data were obtained after capillary electrophoresis Sanger sequencing of a specific amplicon.

Figure 7. Model of grape seedlessness determined by the *sdi* locus. The left panel represents a heterozygous *sdi*+/*sdi*- individual in which the *sdi* Arg197Leu substitution prevents the activation of gene expression by multiprotein complexes containing the mutant VviAGL11 protein (depicted in red). This lack of gene expression activation precludes seed coat differentiation, which triggers salicylic acid (SA) production along with the over-expression of WRKY, Homeobox-domain (HD), and NAC transcription factors, and finally leads to endosperm degeneration and embryo developmental arrest in seed traces. The right panel depicts the activation of seed morphogenesis that takes place in seeded *sdi*-/*sdi*- individuals. Complexes containing the wild-type VviAGL11 protein (depicted in blue) directly or indirectly activate the expression of genes involved in seed coat sclerification, which enables endosperm maintenance and embryo development.

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Parsed Citations

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Abbreviations

AGL = Agamous-like

Arg = Arginine

CAPS = Cleaved amplified polymorphic sequence

CS = Crimson Seedless

DEG = Differentially expressed gene

GEO: Gene expression omnibus

IN = Imperial Napoleon

INDEL = Insertion-deletion

Leu = Leucine

LG = Linkage group

LOD = Logarithm of odds

QTL = Quantitative trait locus

MO = Moscatuel

NGS = Next generation sequencing

PPAT = Pantetheine-phosphate adenylyltransferase

REC = Recombination frequency

RG = Red Globe

RS = Ruby Seedless

SA = Salicylic acid

SDI = Seed Development Inhibitor

SDW = Seed dry weight

SNP = Single nucleotide polymorphism

SNV = Single nucleotide variation

SSR = Simple sequence repeat

VIVC = Vitis International Variety Catalogue

WAF = Weeks after flowering

Adam-Blondon AF, Lahogue-Esnault F, Bouquet A, Boursiquot JM, This P (2001) Usefulness of two SCAR markers for marker-assisted selection of seedless grapevine cultivars. Vitis 40: 147-155

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Anders S, Pyl PT, Huber W (2015) HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics 31: 166-169

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Balanza V, Roig-Villanova I, Di Marzo M, Masiero S, Colombo L (2016) Seed abscission and fruit dehiscence required for seed dispersal rely on similar genetic networks. Development 143: 3372-3381

Pubmed: Author and Title

Google Scholar: Author Only Title Only Author and Title

Barritt BH (1970) Ovule development in seeded and seedless grapes. Vitis 9: 7-14
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Battilana J, Lorenzi S, Moreira FM, Moreno-Sanz P, Failla O, Emanuelli F, Grando MS (2013) Linkage mapping and molecular diversity at the flower sex locus in wild and cultivated grapevine reveal a prominent SSR haplotype in hermaphrodite plants. Mol Biotechnol 54: 1031-1037
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bergamini C, Cardone MF, Anaclerio A, Perniola R, Pichierri A, Genghi R, Alba V, Forleo LR, Caputo AR, Montemurro C, Blanco A, Antonacci D (2013) Validation assay of p3_VvAGL11 marker in a wide range of genetic background for early selection of stenospermocarpy in *Vitis vinifera* L. Molecular Biotechnology 54: 1021-1030
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bogs J, Jaffe FW, Takos AM, Walker AR, Robinson SP (2007) The grapevine transcription factor VvMYBPA1 regulates proanthocyanidin synthesis during fruit development. Plant Physiol 143: 1347-1361
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bouquet A, Danglot Y (1996) Inheritance of seedlessness in grapevine, Vitis vinifera L. Vitis 35: 35-42
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Burkart-Waco D, Ngo K, Dilkes B, Josefsson C, Comai L (2013) Early disruption of maternal-zygotic interaction and activation of defense-like responses in Arabidopsis interspecific crosses. Plant Cell 25: 2037-2055
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Cabezas JA, Cervera MT, Ruiz-Garcia L, Carreno J, Martinez-Zapater JM (2006) A genetic analysis of seed and berry weight in grapevine. Genome 49: 1572-1585
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Cabezas JA, Ibanez J, Lijavetzky D, Velez D, Bravo G, Rodriguez V, Carreno I, Jermakow AM, Carreno J, Ruiz-Garcia L, Thomas MR, Martinez-Zapater JM (2011) A 48 SNP set for grapevine cultivar identification. BMC Plant Biol 11: 153
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Canaguier A, Grimplet J, Di Gaspero G, Scalabrin S, Duchene E, Choisne N, Mohellibi N, Guichard C, Rombauts S, Le Clainche I, Berard A, Chauveau A, Bounon R, Rustenholz C, Morgante M, Le Paslier MC, Brunel D, Adam-Blondon AF (2017) A new version of the grapevine reference genome assembly (12X.v2) and of its annotation (VCost.v3). Genom Data 14: 56-62
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Carbonell-Bejerano P, Rodriguez V, Royo C, Hernaiz S, Moro-Gonzalez LC, Torres-Vinals M, Martinez-Zapater JM (2014) Circadian oscillatory transcriptional programs in grapevine ripening fruits. BMC Plant Biol 14: 78
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Carreño I, Cabezas JA, Martínez-Mora C, Arroyo-García R, Cenis JL, Martínez-Zapater JM, Carreño J, Ruiz-García L (2015) Quantitative genetic analysis of berry firmness in table grape (Vitis vinifera L.). Tree Genetics & Genomes 11: 818
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 6: 80-92
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Colombo L, Franken J, Van der Krol AR, Wittich PE, Dons HJ, Angenent GC (1997) Downregulation of ovule-specific MADS box genes from petunia results in maternally controlled defects in seed development. Plant Cell 9: 703-715
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Costantini L, Battilana J, Lamaj F, Fanizza G, Grando MS (2008) Berry and phenology-related traits in grapevine (Vitis vinifera L.): from quantitative trait loci to underlying genes. BMC Plant Biology 8: 38
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Costantini L, Battilana J, Lamaj F, Fanizza G, Grando MS (2008) Berry and phenology-related traits in grapevine (Vitis vinifera L.): from

quantitative trait loci to underlying genes. BMC Plant Biol 8: 38
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chin CS, Peluso P, Sedlazeck FJ, Nattestad M, Concepcion GT, Clum A, Dunn C, O'Malley R, Figueroa-Balderas R, Morales-Cruz A, Cramer GR, Delledonne M, Luo C, Ecker JR, Cantu D, Rank DR, Schatz MC (2016) Phased diploid genome assembly with single-molecule real-time sequencing. Nat Methods 13: 1050-1054
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Choi Y, Sims GE, Murphy S, Miller JR, Chan AP (2012) Predicting the functional effect of amino acid substitutions and indels. PLoS One 7: e46688
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138: 963-971
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Dangl GS, Mendum ML, Prins BH, Walker MA, Meredith CP, Simon CJ (2001) Simple sequence repeat analysis of a clonally propagated species: a tool for managing a grape germplasm collection. Genome 44: 432-438
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Di Genova A, Almeida AM, Munoz-Espinoza C, Vizoso P, Travisany D, Moraga C, Pinto M, Hinrichsen P, Orellana A, Maass A (2014) Whole genome comparison between table and wine grapes reveals a comprehensive catalog of structural variants. BMC Plant Biology 14: 7
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Doligez A, Adam-Blondon AF, Cipriani G, Di Gaspero G, Laucou V, Merdinoglu D, Meredith CP, Riaz S, Roux C, This P (2006) An integrated SSR map of grapevine based on five mapping populations. Theor Appl Genet 113: 369-382
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Doligez A, Bertrand Y, Farnos M, Grolier M, Romieu C, Esnault F, Dias S, Berger G, Francois P, Pons T, Ortigosa P, Roux C, Houel C, Laucou V, Bacilieri R, Peros JP, This P (2013) New stable QTLs for berry weight do not colocalize with QTLs for seed traits in cultivated grapevine (Vitis vinifera L.). BMC Plant Biol 13: 217
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Doligez A, Bouquet A, Danglot Y, Lahogue F, Riaz S, Meredith P, Edwards J, This P (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
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

FAO, OIV (2016) FAO-OIV Focus 2016 Table and Dried Grapes. In FAO, ed, Non-alcoholic products of the vitivinicultural sector intended for human consumption, Vol I7042. FAO, p 64
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Figueiredo DD, Batista RA, Roszak PJ, Hennig L, Kohler C (2016) Auxin production in the endosperm drives seed coat development in Arabidopsis. Elife 5
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of Eucalyptus grandis and Eucalyptus urophylla using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137: 1121-1137
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Grimplet J, Van Hemert J, Carbonell-Bejerano P, Diaz-Riquelme J, Dickerson J, Fennell A, Pezzotti M, Martinez-Zapater JM (2012) Comparative analysis of grapevine whole-genome gene predictions, functional annotation, categorization and integration of the predicted gene sequences. BMC Res Notes 5: 213
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Honma T, Goto K (2001) Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature 409: 525-529
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Houel C, Chatbanyong R, Doligez A, Rienth M, Foria S, Luchaire N, Roux C, Adiveze A, Lopez G, Farnos M, Pellegrino A, This P, Romieu C, Torregrosa L (2015) Identification of stable QTLs for vegetative and reproductive traits in the microvine (Vitis vinifera L.) using the 18 K Infinium chip. BMC Plant Biol 15: 205

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ibáñez J, Carreño J, Yuste J, Martínez-Zapater JM (2015) Grapevine breeding and clonal selection programmes in Spain. In AG Reynolds, ed, Grapevine Breeding Programs for the Wine Industry. Woodhead Publishing, Oxford, pp 183-209
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ibáñez J, Vargas AM, Palancar M, Borrego J, de Andrés MT (2009) Genetic Relationships among Table-Grape Varieties. American Journal of Enology and Viticulture 60: 35-42
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ibáñez J, Vélez MD, de Andrés MT, Borrego J (2009) Molecular markers for establishing distinctness in vegetatively propagated crops: a case study in grapevine. Theoretical and Applied Genetics 119: 1213-1222
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C, Vezzi A, Legeai F, Hugueney P, Dasilva C, Horner D, Mica E, Jublot D, Poulain J, Bruyere C, Billault A, Segurens B, Gouyvenoux M, Ugarte E, Cattonaro F, Anthouard V, Vico V, Del Fabbro C, Alaux M, Di Gaspero G, Dumas V, Felice N, Paillard S, Juman I, Moroldo M, Scalabrin S, Canaguier A, Le Clainche I, Malacrida G, Durand E, Pesole G, Laucou V, Chatelet P, Merdinoglu D, Delledonne M, Pezzotti M, Lecharny A, Scarpelli C, Artiguenave F, Pe ME, Valle G, Morgante M, Caboche M, Adam-Blondon AF, Weissenbach J, Quetier F, Wincker P, French-Italian Public Consortium for Grapevine Genome C (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449: 463-467
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136: 1447-1455
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Karaagac E, Vargas AM, Andres MTd, Carreno I, Ibanez J, Carreno J, Martinez-Zapater JM, Cabezas JA (2012) Marker assisted selection for seedlessness in table grape breeding. Tree Genetics and Genomes 8: 1003-1015
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kaufmann K, Melzer R, Theissen G (2005) MIKC-type MADS-domain proteins: structural modularity, protein interactions and network evolution in land plants. Gene 347: 183-198
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol 14: R36
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kosambi DD (1944) The estimation of map distances from recombination values. Annals of Eugenics 12: 172-175
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kovaleva LV, Smirnova NK, Milyaeva EL (1997) Seedlessness: Structure and metabolic activity of Vitis vinifera L female gametophyte (cv Kishmish chernyi). Russian Journal of Plant Physiology 44: 368-373
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 33: 1870-1874
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lahogue F, This P, Bouquet A (1998) Identification of a codominant scar marker linked to the seedlessness character in grapevine. Theoretical and Applied Genetics 97: 950-959
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185-199
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9: 357-359
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Laucou V, Lacombe T, Dechesne F, Siret R, Bruno JP, Dessup M, Dessup T, Ortigosa P, Parra P, Roux C, Santoni S, Vares D, Peros JP,

Boursiquot JM, This P (2011) High throughput analysis of grape genetic diversity as a tool for germplasm collection management. Theoretical and Applied Genetics 122: 1233-1245
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ledbetter CA, Ramming DW (1989) Seedlessness in Grapes. In Horticultural Reviews. John Wiley & Sons, Inc., pp 159-184
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li H (2011) A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics 27: 2987-2993
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Genome Project Data Processing S (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics 25: 2078-2079
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lijavetzky D, Cabezas JA, Ibanez A, Rodriguez V, Martinez-Zapater JM (2007) High throughput SNP discovery and genotyping in grapevine (Vitis vinifera L.) by combining a re-sequencing approach and SNPlex technology. BMC Genomics 8: 424
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Malabarba J, Buffon V, Mariath JEA, Gaeta ML, Dornelas MC, Margis-Pinheiro M, Pasquali G, Revers LF (2017) The MADS-box gene Agamous-like 11 is essential for seed morphogenesis in grapevine. J Exp Bot 68: 1493-1506
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Medina I, Carbonell J, Pulido L, Madeira SC, Goetz S, Conesa A, Tarraga J, Pascual-Montano A, Nogales-Cadenas R, Santoyo J, Garcia F, Marba M, Montaner D, Dopazo J (2010) Babelomics: an integrative platform for the analysis of transcriptomics, proteomics and genomic data with advanced functional profiling. Nucleic Acids Res 38: W210-213
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mejia N, Gebauer M, Munoz L, Hewstone N, Munoz C, Hinrichsen P (2007) Identification of QTLs for seedlessness, berry size, and ripening date in a seedless x seedless table grape progeny. American Journal of Enology and Viticulture 58: 499-507
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mejia N, Soto B, Guerrero M, Casanueva X, Houel C, Miccono Mde L, Ramos R, Le Cunff L, Boursiquot JM, Hinrichsen P, Adam-Blondon AF (2011) Molecular, genetic and transcriptional evidence for a role of VvAGL11 in stenospermocarpic seedlessness in grapevine. BMC Plant Biol 11: 57
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Melzer R, Verelst W, Theissen G (2009) The class E floral homeotic protein SEPALLATA3 is sufficient to loop DNA in 'floral quartet'-like complexes in vitro. Nucleic Acids Res 37: 144-157
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mellway RD, Lund ST (2013) Interaction analysis of grapevine MIKC(c)-type MADS transcription factors and heterologous expression of putative veraison regulators in tomato. J Plant Physiol 170: 1424-1433
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Merdinoglu D, Butterlin G, Bevilacqua L, Chiquet V, Adam-Blondon A-F, Decroocq S (2005) Development and characterization of a large set of microsatellite markers in grapevine (Vitis vinifera L.) suitable for multiplex PCR. Molecular Breeding 15: 349-366
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Merin U, Rosenthal I, Lavi U (1983) A chemical method for the assessment of grapes according to their seed content. Vitis 22: 306-310
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mizzotti C, Ezquer I, Paolo D, Rueda-Romero P, Guerra RF, Battaglia R, Rogachev I, Aharoni A, Kater MM, Caporali E, Colombo L (2014) SEEDSTICK is a master regulator of development and metabolism in the Arabidopsis seed coat. PLoS Genet 10: e1004856
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mizzotti C, Mendes MA, Caporali E, Schnittger A, Kater MM, Battaglia R, Colombo L (2012) The MADS box genes SEEDSTICK and ARABIDOPSIS Bsister play a maternal role in fertilization and seed development. Plant J 70: 409-420
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nitsch JP, Pratt C, Nitsch C, Shaulis NJ (1960) Natural Growth Substances in Concord and Concord Seedless grapes in Relation to Berry Development. American Journal of Botany 47: 566-576
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ocarez N, Mejia N (2016) Suppression of the D-class MADS-box AGL11 gene triggers seedlessness in fleshy fruits. Plant Cell Rep 35: 239-254
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ooi LC, Low ET, Abdullah MO, Nookiah R, Ting NC, Nagappan J, Manaf MA, Chan KL, Halim MA, Azizi N, Omar W, Murad AJ, Lakey N, Ordway JM, Favello A, Budiman MA, Van Brunt A, Beil M, Leininger MT, Jiang N, Smith SW, Brown CR, Kuek AC, Bahrain S, Hoynes-O'Connor A, Nguyen AY, Chaudhari HG, Shah SA, Choo YM, Sambanthamurthi R, Singh R (2016) Non-tenera Contamination and the Economic Impact of SHELL Genetic Testing in the Malaysian Independent Oil Palm Industry. Front Plant Sci 7: 771
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Pabon-Mora N, Wong GK, Ambrose BA (2014) Evolution of fruit development genes in flowering plants. Front Plant Sci 5: 300
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Pearson HM (1932) Parthenocarpy and Seedlessness in Vitis vinifera. Science 76: 594
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Pellerone FI, Edwards KJ, Thomas MR (2001) Grapevine microsatellite repeats: Isolation, characterisation and use for genotyping of grape germplasm from Southern Italy. Vitis 40: 179-186
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Pinyopich A, Ditta GS, Savidge B, Liljegren SJ, Baumann E, Wisman E, Yanofsky MF (2003) Assessing the redundancy of MADS-box genes during carpel and ovule development. Nature 424: 85-88
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Pratt C (1971) Reproductive Anatomy in Cultivated Grapes - A Review. American Journal of Enology and Viticulture 22: 92-109
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ramming DW, Tarailo R, Badr SA (1995) `Crimson Seedless': A New Late-maturing, Red Seedless Grape. HortScience 30: 1473-1474
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Rietz S, Stamm A, Malonek S, Wagner S, Becker D, Medina-Escobar N, Vlot AC, Feys BJ, Niefind K, Parker JE (2011) Different roles of Enhanced Disease Susceptibility1 (EDS1) bound to and dissociated from Phytoalexin Deficient4 (PAD4) in Arabidopsis immunity. New Phytol 191: 107-119
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139-140
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Robinson MD, Oshlack A (2010) A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biology 11: R25-R25
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Royo C, Carbonell-Bejerano P, Torres-Perez R, Nebish A, Martinez O, Rey M, Aroutiounian R, Ibanez J, Martinez-Zapater JM (2016) Developmental, transcriptome, and genetic alterations associated with parthenocarpy in the grapevine seedless somatic variant Corinto bianco. J Exp Bot 67: 259-273
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Rubio S, Whitehead L, Larson TR, Graham IA, Rodriguez PL (2008) The coenzyme a biosynthetic enzyme phosphopantetheine adenylyltransferase plays a crucial role in plant growth, salt/osmotic stress resistance, and seed lipid storage. Plant Physiol 148: 546-556
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Singh R, Low ET, Ooi LC, Ong-Abdullah M, Ting NC, Nagappan J, Nookiah R, Amiruddin MD, Rosli R, Manaf MA, Chan KL, Halim MA, Azizi N, Lakey N, Smith SW, Budiman MA, Hogan M, Bacher B, Van Brunt A, Wang C, Ordway JM, Sambanthamurthi R, Martienssen RA (2013) The oil palm SHELL gene controls oil yield and encodes a homologue of SEEDSTICK. Nature 500: 340-344

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Singh R, Ti LLE, Li LOC, Abdullah MO, Nookiah R, Sambanthamurthi R, Ordway J, Lakey ND, Smith SW, Martienssen R, Hogan M (2015) Expression of SEP-like Genes for Identifying and Controlling Palm Plant Shell Phenotypes. In. MALAYSIAN PALM OIL BOARD, United States
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Stout AB (1936) Seedlessness in Grapes. In New York State Agricultural Experiment Station Technical Bulletin, Vol 238. New York State Agricultural Experiment Station, Geneva, NY, p 68
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Striem MJ, Spiegel-Roy P, Baron I, Sahar N (1992) The degrees of development of the seed-coat and the endosperm as separate subtraits of stenospermocarpic seedlessness in grapes. Vitis
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Teh SL, Fresnedo-Ramirez J, Clark MD, Gadoury DM, Sun Q, Cadle-Davidson L, Luby JJ (2017) Genetic dissection of powdery mildew resistance in interspecific half-sib grapevine families using SNP-based maps. Mol Breed 37: 1
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Tello J, Torres-Perez R, Grimplet J, Carbonell-Bejerano P, Martinez-Zapater JM, Ibanez J (2015) Polymorphisms and minihaplotypes in the VvNAC26 gene associate with berry size variation in grapevine. BMC Plant Biol 15: 253
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Tello J, Torres-Pérez R, Grimplet J, Ibáñez J (2016) Association analysis of grapevine bunch traits using a comprehensive approach. Theoretical and Applied Genetics 129: 227-242
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

This P, Lacombe T, Thomas MR (2006) Historical origins and genetic diversity of wine grapes. Trends Genet 22: 511-519
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Thorvaldsdottir H, Robinson JT, Mesirov JP (2013) Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief Bioinform 14: 178-192
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Torregrosa L, Fernandez L, Bouquet A, Boursiquot JM, Pelsy F, Martínez-Zapater JM (2011) Origins and Consequences of Somatic Variation in Grapevine. In A-F Adam-Blondon, J-M Martinez-Zapater, C Kole, eds, Genetics, Genomics, and Breeding of Grapes, Ed 1st. Science Publishers, Jersey, British Isles, pp 68-92
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

van Dijk AD, Morabito G, Fiers M, van Ham RC, Angenent GC, Immink RG (2010) Sequence motifs in MADS transcription factors responsible for specificity and diversification of protein-protein interaction. PLoS Comput Biol 6: e1001017
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

van Ooijen JW (1992) Accuracy of mapping quantitative trait loci in autogamous species. Theor Appl Genet 84: 803-811
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

van Ooijen JW, Boer MP, Jansen RC, Maliepaard C (2002) MapQTL 4.0, Software for the Calculation of QTL Positions on Genetic Maps. Wageningen: Plant Research International.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

van Ooijen JW, Voorrips RE (2001) JoinMap® version 3.0: software for the calculation of genetic linkage maps. Wageningen: Plant Research International.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Varoquaux F, Blanvillain R, Delseny M, Gallois P (2000) Less is better: new approaches for seedless fruit production. Trends in Biotechnology 18: 233-242
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Vitulo N, Forcato C, Carpinelli EC, Telatin A, Campagna D, D'Angelo M, Zimbello R, Corso M, Vannozzi A, Bonghi C, Lucchin M, Valle G (2014) A deep survey of alternative splicing in grape reveals changes in the splicing machinery related to tissue, stress condition and

genotype. BMC Plant Biol 14: 99
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang L, Hu X, Jiao C, Li Z, Fei Z, Yan X, Liu C, Wang Y, Wang X (2016) Transcriptome analyses of seed development in grape hybrids reveals a possible mechanism influencing seed size. BMC Genomics 17: 898
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang L, Yin X, Cheng C, Wang H, Guo R, Xu X, Zhao J, Zheng Y, Wang X (2015) Evolutionary and expression analysis of a MADS-box gene superfamily involved in ovule development of seeded and seedless grapevines. Mol Genet Genomics 290: 825-846
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhang Y, Xu S, Ding P, Wang D, Cheng YT, He J, Gao M, Xu F, Li Y, Zhu Z, Li X (2010) Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. Proc Natl Acad Sci U S A 107: 18220-18225
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title