# Quantitative trait loci for the response to gibberellic acid of berry size and seed mass in tablegrape (*Vitis vinifera* L.)

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## Abstract

**Background and Aims:** Bioactive gibberellins are commonly applied to increase the size of berries in seedless tablegrapes. The genetic determinants for the response of berry size to gibberellic acid ($GA_3$) were investigated in a progeny (n = 137) of Ruby Seedless × Sultanina.

**Methods and Results:** Seed and berry size-related traits were measured at harvest (18°Brix). Heritability was high (~79%) for all traits under study, especially seed dry mass (>90%). All these traits responded to $GA_3$ and showed interaction between genotype and treatment ($g \times GA_3$). Based on quantitative trait loci (QTL) analysis and the grapevine reference genome, we identified about 200 annotated genes located in the corresponding confidence intervals in linkage groups 2 and 18. Nineteen of these genes were selected for further characterisation because of their possible participation in $g \times GA_3$.

**Conclusions:** The response to $GA_3$ has an important genetic basis which is given by QTLs localised on linkage groups 2 and 18. Under $GA_3$ treatment, the seed loses its genetic control on berry size given by its major QTLs, indicating a possible interaction between this genetic determinant and $GA_3$. Despite the masking effect, *VvAGL11* is associated with the intensity of the response to $GA_3$.

**Significance of the Study:** These results indicate the complex role of $g \times GA_3$ at the genetic level in the control of berry size.

***Keywords:*** *candidate gene, exogenous hormone, genetic mapping, heritability, mixed linear model, QTL*

## Introduction

Volume change of grape berries follows a double sigmoid pattern in which two periods of growth (stages I and III) are separated by a lag phase (stage II) (Coombe 1989). The transition from stage II to stage III is rapid and occurs within 1 or 2 days (Coombe 1976). Stage I starts with flowering and lasts for about 60 days, depending on the cultivar. During the first week of this stage, rapid cell division occurs and the final number of cells within the berry is formed. These newly formed cells expand and accumulate solutes during the remainder of the stage (Matthews and Shackel 2005). Many solutes increase their concentration as veraison (the onset of ripening) approaches; malic acid is the solute that accumulates most (Coombe and McCarthy 2000). The beginning of stage III coincides with veraison and is characterised by the initiation of the softening and coloration of the berries (Coombe and McCarthy 2000). The onset of veraison triggers several other events, such as an increase in the concentration of sugars and a decrease in acidity, while the berries continue to enlarge their volume (Coombe 1992). The regulation of berry growth at stage III is not well understood, although it is well established that stage III coincides roughly with an increase in sugar transport into the berry (Coombe and Bishop 1980) and with the continuous expansion of the berry skin tissues (Matthews et al. 1987). In seeded cultivars, the seeds complete their formation as the berry develops.

Seedless berries develop either through parthenocarpy (in a few known cases) or more commonly through stenospermocarpy. In this latter case, ovules abort at an early stage of development (Stout 1936, Pratt 1971). The most accepted hypothesis for seedlessness proposes the presence of a dominant allele at the Seed development inhibitor (*Sdi*) locus, which inhibits seed development by regulating operator genes, which in turn block the transcription of structural genes for seed development (Bouquet and Danglot 1996). The locus *Sdi* has been confirmed by mapping quantitative trait loci (QTLs) in linkage group (LG) 18 (Doligez et al. 2002, Cabezas et al. 2006, Mejía et al. 2007, Costantini et al. 2008), and is responsible for 50–90% of the total phenotypic variation related to seed-related traits. Mejía et al. (2011) confirmed that *VvAGL11* is the major functional candidate gene for seedlessness and that it corresponds to the *Sdi* locus. It has thus been proposed that the correlation between berry size and seed content observed both at the phenotypic and genetic level might be due to the pleiotropic effect of this genetic factor rather than to a tight linkage between these traits (Costantini et al. 2008, Mejía et al. 2011). Part of the phenotypic variation for berry size or seed content is not explained by the *VvAGL11*; therefore, another unidentified locus or loci may be involved (Doligez et al. 2002, 2013, Cabezas et al. 2006, Mejía et al. 2007). Recently, other loci on several LGs have been postulated to be participating in these traits (Doligez et al. 2013).

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In many fruits, there is a marked positive correlation between the content of seeds present and the final fruit size, and grapes are no exception, since normally large berry size correlates with fully developed seeds (Coombe 1960, Crane 1969). It is known that developing seeds stimulate growth of the fruit tissues surrounding them via hormones (Coombe 1960, Crane 1969, Pérez and Gómez 2000, Pérez et al. 2000) and that gibberellin production by grape seeds could contribute to berry development and growth (Iwahori et al. 1968, Pérez and Gómez 2000, Pérez et al. 2000). This correlation depends on the genetic background of the population. For instance, Sultanina-derived progenies have a higher correlation than seeded progenies (Doligez et al. 2002, 2013). Also, this correlation is stronger when the seed content is expressed as seed fresh mass than as seed number (Doligez et al. 2013). In addition, Doligez et al. (2013) have found several new QTLs for seed content and berry size that did not co-localise, providing new antecedents about the complex relation between these traits.

In seedless tablegrape cultivars, bioactive gibberellins are commonly applied to increase the size of berries by exogenous application. Since the best price for tablegrapes is always obtained for large berries in the international market, growers need to apply this hormone several times during the season, starting immediately after fruitset (2–4 mm in equatorial diameter) until veraison (Zoffoli et al. 2009). For example, in the case of Sultanina (= Thompson Seedless), one of the main tablegrape cultivars, 30–40 mg/L of gibberellic acid ($GA_3$) is applied two to three times to increase the size of the berries to achieve commercial standards.

Despite the economic importance of $GA_3$ treatment and the abundant literature on seedlessness, studies at the genetic level of the response to $GA_3$ treatment in relation to seed content and/or berry size are scarce. This study explored this interaction to identify and analyse the genetic determinants of the response of berry size to $GA_3$ and the influence of seed mass on this response. To do this, the interaction between genotype and $GA_3$ in a segregant progeny (n = 137) was investigated during three consecutive seasons. We confirmed the presence of the main QTLs related to seed mass and berry size in $LG_{18}$ (previously reported), but we also consistently found other minor QTLs in $LG_2$ and in different loci of $LG_{18}$ related to the effect of $GA_3$ on berry enlargement.

## Materials and methods

### *Plant material and treatments*

During three consecutive growing seasons (2009/10–2011/12) at La Platina Research Center of the Chilean Institute of Agriculture Research (33°34′20″S; 70°37′32″W; 630 m elevation), the plant material was an $F_1$ progeny (n = 137 segregants) of a controlled cross of Ruby Seedless × Sultanina (R × S) obtained through embryo rescue. Note that about 82% of the genotypes correspond to a seedless phenotype or have small-sized seed traces, and that each season begins approximately in September of year 1 and ends in May of year 2. This progeny is the same as in Mejía et al. (2007, 2011) and Correa et al. (2014). More details about the population are found in Correa et al. (2014).

For each genotype, there are three plants in the same plot. Three bunches, one of each plant, were first treated by immersion with 10 mg/L of $GA_3$ (GibGro 20%, effervescent tablets, Nufarm Americas Inc., Alsip, IL, USA), plus 0.1 mL/L siliconate coadjuvant (Break SL, soluble concentrate, BASF, Florham Park, NJ, USA), in order to loosen the bunches. This application was performed before anthesis, when the panicle reached 12–15 cm in length. To enlarge the berries, two doses of a solution of 20 mg/L of $GA_3$ prepared as previously described were applied when the berries reached 2–4 mm and 6–8 mm equatorial diameter. In addition, three bunches from the same three plants per genotype were treated with water plus coadjuvant and used as control.

In order to avoid any spatial impediment caused by the tight contact of growing berries causing limitations to their growth potential, every bunch was thinned by hand between the first and second spraying with $GA_3$. Berry thinning consisted of removing the terminal portion of the main axis and some shoulders, leaving three levels of four, three and three branches each; between each group of the remaining branches, we removed three intermediate branches. On average, this practice gave bunches with about 120–150 berries. After veraison, the total soluble solids (TSS) content of berries was monitored weekly by refractometer (Atago, Tokyo, Japan) for each genotype, randomly choosing 15 berries per plant from different bunches and harvesting differentially as they reached 18°Brix.

### *Phenotypic evaluation*

For each plant, two bunches were harvested each season, one with and one without $GA_3$, totalling six bunches harvested per genotype. Fifty berries randomly chosen were removed from each bunch for phenotypic evaluation. Berry fresh mass (BFM) was measured in these 50 berries. To simulate the post harvest conditions required for the fruit to reach the final destination market (15–45 days of travel by ship), these berries were conserved for 20 days under refrigeration at 0°C in a perforated plastic liner containing a sulfur dioxide ($SO_2$) generator pad.

After cold storage, berry equatorial diameter (BED) and berry polar diameter (BPD) were determined on 10 berries with a hand caliper. To determine seed dry mass (SDM), seeds and seed rudiments were carefully removed from 20 berries, cleaned from residual pulp and placed in an oven at 60°C to dry until constant mass, before weighing in an electronic balance.

Before carrying out statistical analysis, the assumptions of normality and homoscedasticity of variances were evaluated by the Anderson–Darling and Levene tests, respectively. Variables that failed to meet these assumptions were transformed to the natural logarithm [$ln(x + 1)$] or to the rank transformation using average ranks in case of ties (Conover and Iman 1981). The latter was specifically applied to SDM.

The relationships among traits were analysed based on Pearson's correlation coefficient ($r$). The normalised trait means over seasons for each genotype and treatment were used in order to calculate $r$. The relative response to $GA_3$ of berry mass was related to seed mass (SDM) by a non-linear regression analysis. The model selection criterion was based on the adjusted coefficient of determination or $R^2$; higher $R^2$ indicates a better fit.

To test the effect of each genotype on each trait given by genotypic variance (random genotypic effect) and its possible interaction with the $GA_3$ treatment (fixed effect) in each season, the following mixed linear models were used: $y_{ijk} = \mu + g_i + t_j + (g \times t)_{ij} + \varepsilon_{ijk}$, where $y_{ijk}$ is the phenotypic value measured for the trait $y$ on the bunch $k$ of the genotype $i$ with treatment $j$; $\mu$ corresponds to overall mean; $g_i$ is the random effect of genotype $i$ representing the effect of each genotype or genotypic effect on trait $y$; $t_j$ is the fixed effect of $GA_3$ treatment with two levels: control and treated; $(g \times t)_{ij}$ is the random interaction between genotype $i$ and treatment $j$; and $\varepsilon_{ijk}$ is the random residual error per bunch $k$ of the genotype $i$ with treatment $j$.

Broad sense heritability ($h_b^2$) was estimated according to restricted maximum likelihood variance components according

to the following expression (Holland et al. 2003, Kawamura et al. 2011, Duchêne et al. 2012): $h_b^2 = \sigma_g^2 \Big/ \left( \sigma_g^2 + \frac{\sigma_{gt}^2}{T} + \frac{\sigma_\varepsilon^2}{TR} \right)$, where $\sigma_g^2$ is the genotypic variance; $\sigma_{gt}^2$ is the variance of interaction between season and genotype; $\sigma_\varepsilon^2$ is the residual variance; $T$ is the number of treatment levels; and $R$ is the number of replicate bunches per genotype and treatment. The interaction between genotype and treatment of $GA_3$ (genotype × $GA_3$), expressed as relative response to $GA_3$, corresponded to the ratio between the $GA_3$ treated and control values for each season, trait, genotype and bunch.

The previous approaches were applied to relative response in order to calculate the variance components and broad sense heritability values. The only difference was the replacement of the fixed effect of $GA_3$ treatment by the fixed effect of season with three levels (2009/10, 2010/11 and 2011/12); within-subject effect given by plants effect was considered in the model as a repeated measurement analysis. The complete set of analyses and graphics were performed using the R statistical programming language (R Core Team 2011) and its *nortest* (Gross 2012) and *lme4* (Bates and Maechler 2009) packages for normality tests and linear mixed models, respectively.

### *Genetic evaluations*

Genetic evaluations were based on a linkage map using the R × S population. The consensus genetic map was based on 272 markers [simple sequence repeats (SSRs) or microsatellites, amplified fragment length polymorphisms, gene-based single-nucleotide polymorphisms, phenotypic markers and sequence characterised amplified regions]; previously mapped SSRs in other crossings were the most abundant. A genetic linkage map was built using JoinMap v3.0 (Van Ooijen and Voorrips 2001) and its join-combine groups for map integration function. In addition, a double haploid population approach was applied for the parental maps and a cross-pollination-type for the consensus map. Distances among markers were calculated using the Kosambi function; to define each LG, we used a logarithm of the odds ratio (LOD) threshold of 3.0. More information about the construction and characteristics of the genetic/linkage map is detailed in Correa et al. (2014).

For each trait, QTLs based on the phenotypic means of each genotype (segregant) under control and $GA_3$ treatment, as well as based on the relative response to $GA_3$ of each genotype, were identified using the consensus map via the non-parametric Kruskal–Wallis test, interval mapping and the multiple-QTL-model (MQM) procedure using the MAP QTL 4.0 software (Van Ooijen et al. 2002). To declare the presence of a QTL (significance of 0.05), LOD genome-wide error thresholds were determined using the same software, with 1000 permutations. More statistical details of these procedures are described by Mejía et al. (2007) and Correa et al. (2014). Each significant QTL was characterised by its LOD score, its proportion of explained variation and its confidence interval in centiMorgan (cM) corresponding to the maximum LOD (peak) score minus 1 and minus 2 units on either side of the LOD peak. The location of each QTL detected in each LG in the consensus map was plotted using the program MapChart (Wageningen UR, Wageningen, The Netherlands) (Voorrips 2002). Allelic effects of QTLs were calculated according to Segura et al. (2007).

### *Search for candidate genes*

A search for candidate genes for QTLs associated with $GA_3$ response was performed based on the genomic regions within the confidence interval calculated on the consensus map for each QTL. In order to identify candidate genes, the annotated reference genome (Genoscope 12×) of the quasi-homozygous line 40024 derived from Pinot Noir (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/; Jaillon et al. 2007) was used. The function of each gene was predicted using information obtained by http://blast.ncbi.nlm.nih.gov/Blast.cgi. Genes labelled as 'unknown function' or equivalent were not considered for further analyses.

## Results

### *Phenotyping and heritability*

All traits responded to $GA_3$ in every season evaluated. Treatment with $GA_3$ stimulated the growth of berry size considering the various associated subtraits and decreased SDM (Table 1). On average, the traits were determined by an important effect of genotype (>50%), being more relevant for SDM (~74%) than for the remaining studied traits. The effect of genotype × $GA_3$ interaction was lower (~4%) on SDM compared with berry size-associated traits (~18%). The heritability ($h_b^2$) of these traits was high, especially SDM with an estimated value of over 90%. On average, $h_b^2$ of berry size-associated traits was ca. 70% (Table 1). Under $GA_3$ treatment, the heritability was slightly lower than under control condition (Table 2). As expected, the relative response to $GA_3$ of berry size-associated traits was over 1.0, while it was much lower in SDM (~0.5) (Table 3). On average, BFM had the highest response to $GA_3$ treatment, ranging from 1.3 to 1.4. Response to $GA_3$ depended on the season; that is, there was an interaction between season and genotype; sometimes, depending on trait and season, it was affected by the plant (within-subject effect). The $h_b^2$ was high for the response of berry size-associated traits with $GA_3$ treatment (~75%) and was medium to low for that of SDM (~40%) (Table 3). Parental values and phenotypic distribution are shown in Table S1 and Figure S1, respectively.

### *Relations among traits*

Without $GA_3$ treatment (corresponding in this study to the control condition; Table 4), all traits were highly correlated ($r$ ranging from 64 to 93%). Berry size-associated traits (BED, BPD and BFM) had $r > 90\%$, while SDM had medium to high correlation (60–70%) with these traits. Although under $GA_3$ treatment (Table 4) the correlations among traits were lower than without $GA_3$, berry size-associated traits were still highly correlated ($r > 70\%$). In contrast, SDM lost the correlation with these latter traits (Table 4). In the case of the relative response to $GA_3$, berry size-associated traits were highly correlated ($r \sim 90\%$), whereas the response of SDM was not correlated to any other trait response (Table 4). In addition, the same pattern was found when the correlation between control and response was observed (Table 4) and in Table 5 the relationship among traits under control and treatment conditions are presented. The relationship between SDM and berry size was represented by $BFM = 0.413 \times \ln(SDM) + 4.4128$, while the relationship between SDM and the relative response of berry size to gibberellic acid was represented by $BFM = 0.5928 \times SDM^{-0.155}$ (Figure 1). The coefficients of determination ($R^2$) were for BFM 52.6% and for relative response to $GA_3$ 51.5%.

### *Quantitative trait loci analysis*

According to non-parametric analysis (Kruskal–Wallis rank sum test per marker) and the MQM procedure, 40 QTLs were detected on $LG_2$ and $LG_{18}$, with 10 and 30 QTLs, respectively. All the traits presented QTLs in every season. Significant QTLs were harboured by $LG_2$ only for the relative response to $GA_3$ for berry

**Table 1.** Descriptive statistics, heritability and proportion of restricted maximum likelihood variance in the total phenotypic variance for traits during 2009/10, 2010/11 and 2011/12 seasons.

| Trait | Season | $-GA_3$ | | | $+GA_3$ | | | REML variance (%)‡ | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | μ† | σ | Range | μ | σ | Range | $\sigma^2_g$ | $\sigma^2_{g\times t}$ | $h^2_b$ |
| BFM (g/berry) | 2009/10 | 1.99 | 0.89 | 0.41–5.56 | 2.69 | 0.98 | 0.83–5.64 | 31.8*** | 22.6*** | 62.71 |
| | 2010/11 | 2.51 | 1.05 | 0.76–6.73 | 3.30 | 1.21 | 1.03–7.56 | 44.1*** | 17.7*** | 74.36 |
| | 2011/12 | 2.00 | 0.93 | 0.24–6.66 | 2.59 | 0.93 | 0.2–6.52 | 40.7*** | 19.6*** | 71.25 |
| BFV (mL/berry) | 2009/10 | 2.05 | 0.93 | 0.2–5.2 | 2.74 | 1 | 0.2–6 | 39.1*** | 21.3*** | 69.4 |
| | 2010/11 | 2.56 | 1.11 | 0.9–7.8 | 3.27 | 1.17 | 0.9–7.5 | 47.5*** | 11*** | 79.27 |
| | 2011/12 | 2.14 | 0.96 | 0.3–6.5 | 2.69 | 0.97 | 0.3–6.8 | 41.3*** | 18.6*** | 72.1 |
| BED (mm) | 2009/10 | 12.7 | 2.2 | 6.2–27 | 14.2 | 2.4 | 5.4–35.6 | 36.8*** | 16.5*** | 69.68 |
| | 2010/11 | 14.4 | 2 | 10.4–23.3 | 15.4 | 1.9 | 10.5–22 | 48.0*** | 10.4*** | 79.82 |
| | 2011/12 | 13.9 | 2.1 | 8.3–21.7 | 15.1 | 1.9 | 7.5–20.8 | 43.9*** | 17.9*** | 74.15 |
| BPD (mm) | 2009/10 | 15.3 | 3.2 | 7.3–32.6 | 18.1 | 3.5 | 6.0–42.8 | 38.3*** | 20.7*** | 69 |
| | 2010/11 | 16.4 | 3.3 | 7.3–32.5 | 18.9 | 3.3 | 5.9–30.4 | 44.3*** | 14*** | 76.1 |
| | 2011/12 | 16.3 | 3.1 | 7.6–25.0 | 18.4 | 2.5 | 7.3–27.2 | 43.9*** | 19.4*** | 73.5 |
| SDM (g/berry) | 2009/10 | 0.009 | 0.012 | 0.000–0.069 | 0.005 | 0.011 | 0.000–0.069 | 72.7*** | 4.9*** | 92.21 |
| | 2010/11 | 0.013 | 0.020 | 0.000–0.089 | 0.008 | 0.015 | 0.000–0.083 | 73.0*** | 3.5* | 92.79 |
| | 2011/12 | 0.011 | 0.017 | 0.000–0.088 | 0.006 | 0.011 | 0.000–0.072 | 75.5*** | 5.2*** | 92.86 |

Significance codes according to likelihood ratio test of the variance components (*P* value): ***, 0–0.001; **, 0.001–0.01; *, 0.01–0.05. †μ, mean; σ, standard deviation and range for bunch without ($-GA_3$) or with gibberellic acid treatment ($+GA_3$) of each trait. ‡REML, variance components of genotype effect ($\sigma^2_g$), genotype × $GA_3$ interaction effect ($\sigma^2_{g\times t}$) and broad-sense heritability ($h^2_b$). BED, berry equatorial diameter; BFM, berry fresh mass; BFV, berry fresh volume; BPD, berry polar diameter; $GA_3$, gibberellic acid; SDM, seed dry mass.

**Table 2.** Heritability and proportion of restricted maximum likelihood variance in the total phenotypic variance for seed and berry traits under control conditions and gibberellic acid treatment during 2009/10, 2010/11 and 2011/12 seasons.

| Trait | Treatment† | REML variance (%)‡ | | | Season effect§ |
|---|---|---|---|---|---|
| | | $\sigma^2_g$ | $\sigma^2_{g\times s}$ | $h^2_b$ | |
| BFM | $-GA_3$ | 60.243*** | 5.974*** | 91.294 | *** |
| | $+GA_3$ | 38.657*** | 9.445*** | 81.261 | *** |
| BFV | $-GA_3$ | 59.172*** | 6.926*** | 90.688 | *** |
| | $+GA_3$ | 42.536*** | 6.76** | 84.358 | *** |
| BED | $-GA_3$ | 56.081*** | 9.725*** | 88.845 | *** |
| | $+GA_3$ | 34.155*** | 12.655*** | 77.128 | *** |
| BPD | $-GA_3$ | 56.862*** | 11.206*** | 88.645 | *** |
| | $+GA_3$ | 42.237*** | 8.056*** | 83.729 | *** |
| SDM | $-GA_3$ | 77.611*** | 1.786* | 96.416 | *** |
| | $+GA_3$ | 61.765*** | 8.47*** | 90.971 | *** |

Significance codes according to likelihood ratio test (*P* value): ***, 0–0.001; **, 0.001–0.01; *, 0.01–0.05. †$-GA_3$, control; $+GA_3$, treatment with gibberellic acid. ‡REML, variance components of genotype effect ($\sigma^2_g$), genotype × season interaction effect ($\sigma^2_{g\times s}$) and broad-sense heritability ($h^2_b$). §Significance of fixed effect of season. BED, berry equatorial diameter; BFM, berry fresh mass; BFV, berry fresh volume; BPD, berry polar diameter; $GA_3$, gibberellic acid; SDM, seed dry mass.

size-associated traits and only for the 2009/10 and 2010/11 seasons. Each QTL was closely linked with one marker or cofactor (Tables 6–8). On average, these QTLs had LOD values of ~12.6, ranging from 4 to 34. Their genomic LOD threshold was approximately 4.8.

The traits exhibiting the largest number of QTLs were BED and BPD, each with nine QTLs in total. In berry size-associated traits (BED, BPD and BFM), QTLs were found for both control condition and relative response to $GA_3$, but no QTL was found for any trait under $GA_3$ treatment (Tables 6–8). In contrast, SDM had significant QTLs for both control and $GA_3$-treated samples, but not for relative response to $GA_3$ (Tables 6–8).

On average and considering the variance explained ($R^2$), these QTLs had a significant effect, explaining approximately 31% of the total variance, ranging from 10 to 70% (coefficient of variation, CV ~ 53%). The main QTLs corresponded to SDM and were found on $LG_{18}$, close to the p3_VvAGL11 and VMC7F2 loci, with $R^2$ ~ 65%, ranging from 55 to 70%. The berry-associated traits had on average $R^2$ ~ 25%.

The majority of these QTLs were determined mainly by the male additive effect. There was also a visible difference between the additive and dominance effects, the additive being more relevant for the expression of these traits (Tables 6–8). All the QTLs detected in $LG_2$ and some of the ones detected in $LG_{18}$ by

**Table 3.** Descriptive statistics, heritability and proportion of restricted maximum likelihood variance in the total phenotypic variance for relative response to gibberellic acid treatment of the seed and berry traits during 2009/10, 2010/11 and 2011/12 seasons.

| Traits | Season | Mean | Standard deviation | Range | REML variance (%)† | | | | Season effect‡ |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | $\sigma^2_g$ | $\sigma^2_{g\times s}$ | $\sigma^2_p$ | $h^2_b$ | |
| BFM | 2009/10 | 1.42 | 0.6 | 0.08–4.70 | 36.07*** | 17.22*** | 6.73** | 76.98 | |
| | 2010/11 | 1.4 | 0.54 | 0.26–4.70 | | | | | n.s. |
| | 2011/12 | 1.41 | 0.6 | 0.22–5.70 | | | | | |
| BFV | 2009/10 | 1.32 | 0.45 | 0.29–2.60 | 32.67*** | 16.72*** | 6.2** | 74.39 | |
| | 2010/11 | 1.35 | 0.43 | 0.34–2.64 | | | | | n.s. |
| | 2011/12 | 1.33 | 0.47 | 0.18–2.63 | | | | | |
| BED | 2009/10 | 1.09 | 0.14 | 0.50–1.45 | 30.59*** | 16.43*** | n.s. | 71.47 | |
| | 2010/11 | 1.08 | 0.12 | 0.69–1.45 | | | | | ** |
| | 2011/12 | 1.09 | 0.14 | 0.63–1.42 | | | | | |
| BPD | 2009/10 | 1.17 | 0.19 | 0.46–1.68 | 37.24*** | 17.6*** | 4.21* | 77.09 | |
| | 2010/11 | 1.17 | 0.18 | 0.59–1.66 | | | | | * |
| | 2011/12 | 1.14 | 0.18 | 0.56–1.66 | | | | | |
| SDM | 2009/10 | 0.47 | 0.35 | 0.00–1.58 | 10.84*** | 22.43*** | n.s. | 40.35 | |
| | 2010/11 | 0.6 | 0.34 | 0.01–1.57 | | | | | *** |
| | 2011/12 | 0.56 | 0.35 | 0.00–1.60 | | | | | |

Significance codes according to likelihood ratio test (*P* value): ***, 0–0.001; **, 0.001–0.01; *, 0.01–0.05; n.s., not significant ($P > 0.05$). †REML, variance components of genotype effect ( $\sigma^2_g$), genotype × season interaction effect ( $\sigma^2_{g\times s}$), plant effect ( $\sigma^2_p$) and broad-sense heritability ( $h^2_b$). ‡Significance of fixed effect of season. BED, berry equatorial diameter; BFM, berry fresh mass; BFV, berry fresh volume; BPD, berry polar diameter; SDM, seed dry mass.

**Table 4.** Pearson's correlation coefficient among traits based on the means of each genotype during 2009/10, 2010/11 and 2011/12 seasons.

| Treatment† | Trait | BFM | BED | BPD |
|---|---|---|---|---|
| $-GA_3$ | BED | 0.932*** | – | – |
| | BPD | 0.923*** | 0.886*** | – |
| | SDM | 0.706*** | 0.645*** | 0.638*** |
| $+GA_3$ | BED | 0.864*** | – | – |
| | BPD | 0.907*** | 0.926*** | – |
| | SDM | −0.069n.s. | −0.123n.s. | −0.16n.s. |
| $+GA_3/-GA_3$ | BED | 0.851*** | – | – |
| | BPD | 0.872*** | 0.704*** | – |
| | SDM | 0.162n.s. | 0.145n.s. | 0.09n.s. |

Significance codes (*P* value): ***, 0–0.001; n.s., not significant ($P > 0.05$). †$-GA_3$: without gibberellic acid treatment; $+GA_3$: under gibberellic acid treatment; $+GA_3/-GA_3$: relative response to gibberellic acid treatment. BED, berry equatorial diameter; BFM, berry fresh mass; BPD, berry polar diameter; SDM, seed dry mass.

this procedure and their confidence intervals are shown in Figure 2.

### *Candidate genes*

Considering the QTL confidence intervals on $LG_2$, we found two regions: (i) *$LG_2$ – I*: flanked by the VvP02G3 and VMCF61 markers; and (ii) *$LG_2$ – II*: flanked by VvP02G5 and VMC2C10-1 (Tables 6–8, Tables S2–S4). We found 95 and 67 annotated genes, respectively, for these regions (Table S3). The genomic regions showed different gene densities (considering both number of genes or total codifying length). Some of these genes were related to gibberellin metabolism and signal transduction, cell size control, sugar transport, proteins related to the interaction between hormones, cell wall (pectinesterase 68), auxin-induced calcium-binding proteins (PBP1), cellular transport (such as SNARE proteins) and transcription factors (WRKY-type). In addition to *VvAGL11*, on $LG_{18}$ we identified some other putative genes related to $GA_3$ response, including MADS-box transcription factor, cytochrome P450 716B2 and tubulin β-1 chain-like (Table 9, Tables S2–S4). Details about the complete list of genes may be found in Table S4. Finally, a set of 19 genes underlying these QTLs were proposed as possibly related to response of berry size to $GA_3$ (Table 9).

## Discussion

### *Genotypic effect and heritability*

**Gibberellic acid effect on berry size.** The results shown in the current study are consistent with what traditionally has been known for post-bloom application of gibberellin in tablegrapes in order to stimulate the growth of berries (Weaver and McCune 1959, Casanova et al. 2009, Zoffoli et al. 2009, Wang et al. 2012). Several mechanisms have been proposed to explain the sizing effect of $GA_3$ on berries. Pérez and Gómez (2000) observed that $GA_3$ increased the activity of soluble invertase with subsequent increase in hexose concentration, which suggests that this enzyme has a role in berry growth by changing the water potential of the berries. The presence of solutes in the berry apoplast would increase the osmotic potential, which finally results in the increase of water transport via phloem (Matthews and Shackel 2005, Keller et al. 2006). Since the increase in size of berries by $GA_3$ treatment is well correlated with water content of berries, it has been suggested that aquaporins are involved in this response. Aquaporin expression genes may be regulated temporally and spatially during berry development (Kjellbom et al. 1999, Maurel and Chrispeels 2001) via hormones such as $GA_3$ (Maurel 1997, Kjellbom et al. 1999). The increase in berry volume may be limited by the

**Table 5.** Pearson's correlation coefficient between the means of each genotype without gibberellic acid (rows) and relative response to gibberellic acid treatment (columns) across the 2009/10, 2010/11 and 2011/12 seasons.

| | Relative response to $GA_3$ | | | | |
|---|---|---|---|---|---|
| **Control** | **BFM** | **BFV** | **BED** | **BPD** | **SDM** |
| BFM | −0.63*** | −0.599*** | −0.654*** | −0.611*** | −0.106 n.s. |
| BFV | −0.585*** | −0.614*** | −0.663*** | −0.614*** | −0.125 n.s. |
| BED | −0.594*** | −0.583*** | −0.661*** | −0.608*** | −0.152 n.s. |
| BPD | −0.619*** | −0.629*** | −0.694*** | −0.637*** | −0.129 n.s. |
| SDM | −0.517*** | −0.565*** | −0.64*** | −0.596*** | −0.067 n.s. |

Significance codes (*P* value): ***, 0–0.001; n.s., not significant ($P > 0.05$). BED, berry equatorial diameter; BFM, berry fresh mass; BFV, berry fresh volume; BPD, berry polar diameter; $GA_3$, gibberellic acid; SDM, seed dry mass.

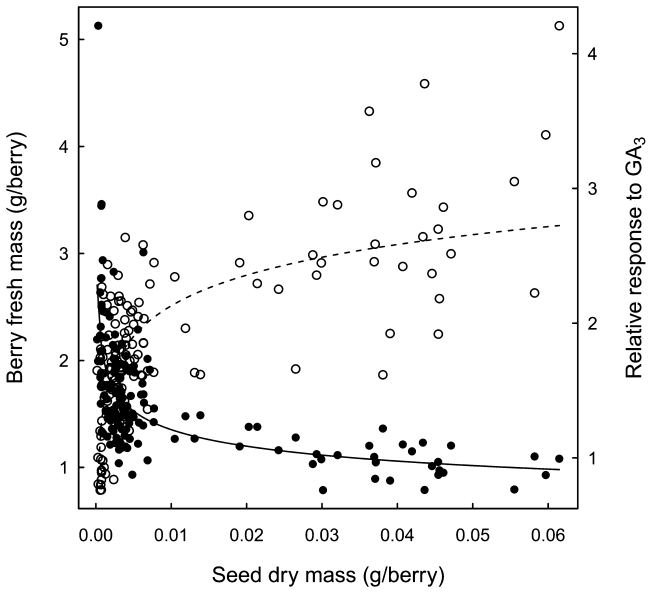


**Figure 1.** Relationship among seed dry mass (SDM), berry fresh mass (BFM) and relative response in berry growth to gibberellic acid ($GA_3$). Non-linear regression between BFM and SDM (○), coefficient of determination ($R^2$): 52.6%; non-linear regression between relative response in BFM and SDM (●), $R^2$: 51.5.

extent of cell expansion in the exocarp (skin). In agreement with this, Wang et al. (2012) observed at a proteomic level that $GA_3$ affected the expression of actins, tubulins and cell wall modification proteins in berries, which would result in an increase in berry size.

**Gibberellic acid effect on seed dry mass.** It is known that post-bloom application of $GA_3$ reduces vestigial seed formation in stenospermocarpic grapes, reducing the number and mass of seeds with developed (brown, hard) seed coats or testae, while increasing the number of undeveloped (green, soft) seeds (Reynolds and De Savigny 2004). This effect was observed here by the decrease of SDM under $GA_3$ versus the control condition (Tables 1–3). It should be noted, however, that the response in SDM was more pronounced in genotypes with large rather than smaller seeds. Furthermore, it should be considered that most of the genotypes belonging to the RxS population have small seeds; about 82% of the genotypes had SDM per berry below 0.5 g, which according to Bouquet and Danglot (1996) corresponds to a seedless phenotype or has small-sized seed traces.

*Genotype × gibberellic acid interaction*

Phenotypic description and correlation among traits revealed a strong influence of $GA_3$ on each trait. The results regarding possible interactions of genotype with $GA_3$ were determined by linear mixed models in the different seasons (Tables 1–3). The significant effect of genotype × $GA_3$ represented by its variance ($\sigma^2_{g\times t}$) demonstrated the existence of genetic diversity within the tested population that segregates in its response to $GA_3$, mainly represented by BFM. Genetic diversity in the response to $GA_3$ in berry size is well known, and so the optimal doses of gibberellins and the phenological stage for its application vary among cultivars (Weaver 1958, Weaver and McCune 1959, 1960). These factors may be involved in the effect of genotype × $GA_3$ interaction observed, which would be shown by the differences in response (some genotypes respond constitutively to $GA_3$, while others do not) and differences in the degree of this response. Furthermore, the effect of genotype × $GA_3$ interaction on the performance of these traits was greater in berry size-associated traits than on SDM (4 vs 18%, respectively). This result gave the first insight about the different degree or susceptibility to $GA_3$ of the different traits under study.

*Heritability*

We have estimated a heritability ($h^2_b$) of ~92% for SDM, which indicates that this trait is a good candidate for marker-assisted selection, due to this high $h^2_b$ and its inclusion in a major QTL. According to the latter, Wei et al. (2002) estimated a sensu stricto heritability of about 52–61%, and Doligez et al. (2013) found estimates of $h^2_b$ of about 57–94% depending on the progeny. Also, the $h^2_b$ for relative response to $GA_3$ was high for berry size-associated traits (~75%), while it was medium to low for SDM (~40%) (Table 2). In addition, $h^2_b$ was about 10% lower under $GA_3$ treatment than under control condition, indicating a lower genetic control on the expression of these traits under treatment (data not shown). These results gave insights on the relevance of the genotype regarding the extent of the response to this growth regulator.

*Seed dry mass and berry size relationship*

The relationship between SDM and berry size was positive in untreated berries (Figure 1 and Table 4). In contrast, the relationship between SDM without application and the response of the berry size to $GA_3$ was negative (Figure 1, Table 4). These relationships were known from earlier studies; according to Weaver and McCune (1959), the susceptibility of a berry to increase in size in response to the application of $GA_3$ is highly dependent on the degree of development of the seeds; that is, less seed development produces greater response in berry size.

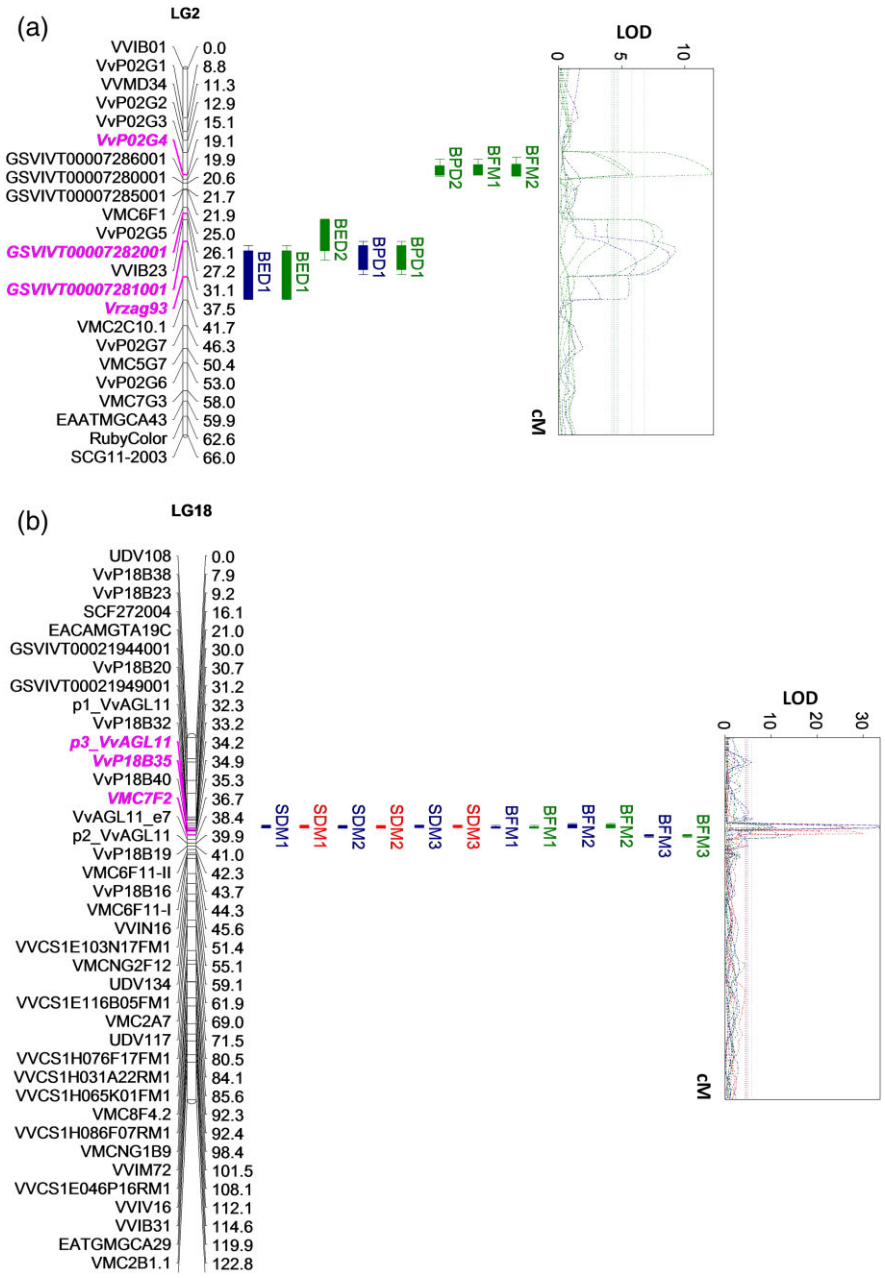


**Figure 2.** Genetic map of linkage groups (LG) 2 and 18 and their profile of logarithm of the odds ratio (LOD) values for detected quantitative trait loci (QTLs) of the Ruby Seedless × Sultanina progeny ($n = 137$) over the 2009/10, 2010/11 and 2011/12 seasons (1, 2 and 3, respectively) for BED, berry equatorial diameter; BFM, berry fresh mass; BPD, berry polar diameter; and SDM, seed dry mass. The coloured boxes and lines represent the confidence intervals of QTLs at LOD ± 1 and ± 2, respectively, for berry size-related traits and SDM. (a) QTLs detected on $LG_2$. (b) Some QTLs detected on $LG_{18}$ (only QTLs for SDM and BFM are shown). QTLs for traits expressed without gibberellic acid treatment (GA–) (■); QTLs for $GA_3$ response (■); and QTLs for relative response to $GA_3$ (■). Markers (■), bolded and italic are the co-factors associated to each QTL according to multiple QTL mapping procedure. The LOD curves (profile at the right of each LG) shows the value of each interval (1 cM). The dotted vertical lines correspond to genomic LOD thresholds at 5% for each trait. More detailed information is given in Tables 6–8.

According to the correlations among traits (Tables 4,5), the decrease of *r* values among traits under $GA_3$ treatment was more accentuated in SDM than in the other traits, which could indicate an effect of this growth regulator on the relation between SDM and berry size (Figure 1), and would indicate the loss of control or a masking of the response on the effect of SDM under $GA_3$ treatment on the phenotypic expression of berry size-related traits. Moreover, according to the relationships among relative response to $GA_3$ (Figure 1), the response of berry size (Tables 4,5) could be independent of response of SDM.

### *Genetic analysis*

**Genetic determinants estimated without gibberellic acid treatment.** As in previous studies (Doligez et al. 2002, Cabezas et al. 2006, Mejía et al. 2007, Costantini et al. 2008), QTLs showed the importance of $LG_{18}$ on these traits, giving a co-localised major QTL with relatively high LOD and $R^2$ values for SDM and berry size-associated traits in control conditions (Table 6). The QTLs found for SDM under the control condition were relatively stable across seasons and had a strong effect on the phenotype of this trait, with high $R^2$ of about 66% and a narrow 1-LOD confidence interval of QTL position, about 1 cM on average (Table 6, Table S2).

At the genetic level, the strong correlation found among some traits ($r > 90\%$, Figure 1) could be given either by a pleiotropic effect, that is a locus that affects the expression of two or more different traits. According to the QTL analysis, the co-localised QTLs found on $LG_{18}$ for SDM and berry size-related traits were associated with the VMC7F2 (Cabezas et al. 2006) and p3_VvAGL11 (Mejía et al. 2011) SSR markers (Table 6), which are part of the 5′ upstream sequence of the *VvAGL11* gene (Mejía et al. 2011, Karaagac et al. 2012). This could confirm that the correlation observed for berry size and SDM might be due to a pleiotropic effect of *VvAGL11* rather than to tight linkage with a second unidentified gene (Doligez et al. 2002, Fanizza et al. 2005, Cabezas et al. 2006, Costantini et al. 2008, Mejía et al. 2011). Furthermore, a physiological basis has been proposed in favour of pleiotropy because the production of gibberellins

**Table 6.** Quantitative trait loci for traits related to berry size and seed mass detected via multiple quantitative trait loci mapping estimated from genotypic means of Ruby Seedless × Sultanina progeny without gibberellic acid treatment.

| Traits | Season | Position | | LOD† | $R^2$ (%) | Co-factor‡ | K–W | Allelic effect | |
|---|---|---|---|---|---|---|---|---|---|
| | | LG | cM | | | | | $A_f/A_m$ | D/A |
| BED | 2009/10 | 2 | 35.1 | 6.2 (4.3) | 22 | *Vrzag93* | ** | 0.55 | 0.59 |
| | | 18 | 37.7 | 9.5 (4.3) | 24.6 | *VMC7F2* | *** | 1.13 | 0.11 |
| | 2010/11 | 18 | 34.2 | 12.2 (4.2) | 34.2 | *p3_VvAGL11* | *** | 0.96 | 0.16 |
| | 2011/12 | 18 | 36.7 | 13.5 (4.3) | 37.3 | *VMC7F2* | *** | 1.04 | 0.19 |
| BFV | 2009/10 | 18 | 36.3 | 12.2 (4.5) | 30 | *VMC7F2* | *** | 1.36 | 0.17 |
| | 2010/11 | 18 | 34.2 | 13.0 (4.4) | 32.3 | *p3_VvAGL11* | *** | 1.13 | 0.27 |
| | 2011/12 | 18 | 36.3 | 16.3 (4.3) | 45 | *VMC7F2* | *** | 1.15 | 0.35 |
| BFM | 2009/10 | 18 | 34.2 | 11.2 (4.3) | 24.4 | *p3_VvAGL11* | *** | 1.7 | 0.16 |
| | 2010/11 | 18 | 34.2 | 10.2 (4.3) | 26.9 | *p3_VvAGL11* | *** | 1.24 | 0.03 |
| | 2011/12 | 18 | 36.7 | 14.7 (4.6) | 39.7 | *VMC7F2* | *** | 1.28 | 0.06 |
| BPD | 2009/10 | 2 | 34.1 | 8.8 (4.4) | 28.8 | *GSVIVT00007281001* | *** | 0.7 | 0.88 |
| | | 18 | 36.3 | 8.8 (4.4) | 19.9 | *VvP18B40* | *** | 1.5 | 0 |
| | 2010/11 | 18 | 34.2 | 10.2 (4.4) | 29.7 | *p3_VvAGL11* | *** | 1.1 | 0.07 |
| | 2011/12 | 18 | 36.3 | 14.6 (4.6) | 35.1 | *VMC7F2* | *** | 1.21 | 0.1 |
| SDM | 2009/10 | 18 | 34.2 | 33.6 (4.5) | 69.6 | *p3_VvAGL11* | *** | 1.18 | 0.08 |
| | 2010/11 | 18 | 34.2 | 25.9 (4.6) | 59.9 | *p3_VvAGL11* | *** | 1.16 | 0.11 |
| | 2011/12 | 18 | 33.2 | 33.7 (5) | 69.8 | *p3_VvAGL11* | *** | 0.99 | 0.01 |

K–W, Kruskal–Wallis significance level (*P* value): **, 0.0005; ***, 0.0001. †Maximum LOD score with threshold in parentheses detected via multiple QTL mapping (MQM) procedure. ‡Markers used as co-factors for MQM procedure. $A_f/A_m$, relative additive effect of maternal to paternal parent; BED, berry equatorial diameter; BFM, berry fresh mass; BFV, berry fresh volume; BPD, berry polar diameter; cM, centiMorgan; D/A, relative allelic effect of dominance to total additive effect; LG, linkage group; QTL, quantitative trait loci; $R^2$, proportion of variance explained by QTL; SDM, seed dry mass.

by the seeds would affect berry growth (Doligez et al. 2002, Cabezas et al. 2006), as has been previously shown (Coombe 1960, Pérez and Gómez 2000, Pérez et al. 2000).

**Genetic determinants for the response to gibberellic acid.** Under $GA_3$ treatment, only QTLs for SDM were detected. These QTLs were associated with p3_VvAGL11 and VMC7F2 on $LG_{18}$. On average, the confidence interval was about 0.33 cM for 1 + LOD interval with $R^2 \sim 63\%$ (Table S2). This behaviour gave insight on a possible effect of $GA_3$ treatment on the relationship of the *Sdi* locus or *VvAGL11* gene to the phenotype of SDM and berry size. Another difference between the genetics of SDM and berry size related to $GA_3$ was given by the lack of QTLs for the response of SDM and by the association of p3_VvAGL11, VMC7F2 and other markers located on $LG_{18}$ with the response to $GA_3$ for all the berry size-associated traits over all seasons. Furthermore, the $LG_2$ had QTLs associated with the response of berry traits to $GA_3$ detected in two seasons (Table 8). In this study, QTLs were found on $LG_2$ only for berry diameters (BED and BPD, Figure 2, Tables 6,8), under control conditions and for the response to $GA_3$ of all berry size-related traits (except in the third season evaluated). The latter would indicate a pleiotropic effect of these QTLs on the response to $GA_3$ in berry size. Part of the phenotypic variation for berry size, about 11%, is not explained by the co-localised QTL on $LG_{18}$ for SDM; therefore, another unidentified locus or loci may be involved (Doligez et al. 2002, 2013, Cabezas et al. 2006, Mejía et al. 2007). Recently, other loci have been postulated to be participating on these traits; Doligez et al. (2013) found five new QTLs for berry mass, located on LGs 1, 8, 11, 17 and 18, and two QTLs for seed mass and number of seeds on $LG_2$ during two seasons in different populations. Another good candidate for this locus or loci might be localised on $LG_2$. It may be seen in Figure 2 that in $LG_2$, two groups of overlapping QTLs were found. The smaller one was associated with VvP02G4 marker and BFM and BPD located upstream to the remaining QTLs. This can be seen as a break in the LOD profile (25), which in turn could have been produced by a mapping error in the order of the markers. In contrast, no QTL for SDM was found on this LG, contrasting to the results presented by Costantini et al. (2008) and Doligez et al. (2013).

As QTLs were not detected for berry size-associated traits (BED, BFM and BPD) under $GA_3$ treatment, and the correlation between berry and seeds traits disappeared under $GA_3$ treatment, which would indicate that the berry size is no more limited by the gibberellins produced by seeds, the hypothesis for a pleiotropic effect of the QTL on $LG_{18}$ on berry and seeds, with a masking effect on the *Sdi* (*VvAGL11*) of exogenous application of gibberellins, might be proposed. Since a masking effect would imply a homogenising effect – without differences among genotypes – on the expression of phenotype rather than a differential response of each genotype, a decrease in phenotypic variation among genotypes had been expected. The latter was not statistically confirmed, although for berry size there was a non-significant tendency to find lower variation (expressed by the CV and analysed by Levene test for equality of variance) under $GA_3$ (Table S5), which could have had a negative impact on the statistical resolution of a more stringent methodology, such as QTL mapping and analysis. According to these antecedents, there was evidence that $GA_3$ has a genetic effect on the *VvAGL11* locus. This effect is given by a masking effect on *VvAGL11* under $GA_3$ treatment for berry size, which could explain in part why no QTL was found for berry size-related traits under $GA_3$ (Table 7). In contrast, there was an increase in SDM variation

**Table 7.** Quantitative trait loci for traits related to berry size and seed mass detected via multiple quantitative trait loci mapping estimated from genotypic means of Ruby Seedless × Sultanina progeny under gibberellic acid treatment.

| Trait | Season | Position | | LOD† | $R^2$ (%) | Co-factor‡ | K–W | Allelic effect | |
|---|---|---|---|---|---|---|---|---|---|
| | | LG | cM | | | | | $A_f/A_m$ | D/A |
| SDM | 2009/10 | 18 | 34.2 | 29.21 (4.8) | 64.2 | *p3_VvAGL11* | *** | 1.14 | 0.33 |
| | 2010/11 | 18 | 36.3 | 30.05 (4.6) | 70.2 | *VMC7F2* | *** | 0.94 | 0.33 |
| | 2011/12 | 18 | 36.3 | 21.12 (4.5) | 54.5 | *VMC7F2* | *** | 1.11 | 0.18 |

K–W, Kruskal–Wallis significance level (*P* value): ***, 0.0001. †Maximum LOD score with threshold in parentheses detected via multiple QTL mapping (MQM) procedure. ‡Markers used as co-factors for MQM procedure. $A_f/A_m$, relative additive effect of maternal to paternal parent; cM, centiMorgan; D/A, relative allelic effect of dominance to total additive effect; LG, linkage group; MQM, multiple QTL mapping; QTL, quantitative trait loci; $R^2$, proportion of variance explained by QTL; SDM, seed dry mass.

**Table 8.** Quantitative trait loci for the relative response to gibberellic acid treatment of traits related to berry size detected via multiple quantitative trait loci mapping estimated from genotypic means of Ruby Seedless × Sultanina progeny.

| Traits | Season | Position | | LOD† | $R^2$ (%) | Co-factor‡ | K–W | Allelic effect | |
|---|---|---|---|---|---|---|---|---|---|
| | | LG | cM | | | | | $A_f/A_m$ | D/A |
| BED | 2009/10 | 2 | 33.1 | 9.25 (4.7) | 23.7 | *GSVIVT00007281001* | *** | 2.09 | 0.73 |
| | | 18 | 36.7 | 12.69 (4.7) | 28.3 | *VMC7F2* | *** | 1.14 | 0.20 |
| | 2010/11 | 2 | 31.1 | 3.97 (4.2) | 10.1 | *GSVIVT00007281001* | ** | 0.69 | 0.83 |
| | | 18 | 33.2 | 6.39 (4.2) | 17 | *VvP18B32* | *** | 0.91 | 0.02 |
| | 2011/12 | 18 | 36.7 | 9.83 (4.4) | 28.9 | *VMC7F2* | *** | 0.89 | 0.03 |
| BFV | 2009/10 | 2 | 30.2 | 9.4 (6) | 21.6 | *GSVIVT00007281001* | *** | 2.28 | 0.84 |
| | | 18 | 36.7 | 10.69 (6) | 23.2 | *VMC7F2* | *** | 1.35 | 0.19 |
| | 2010/11 | 2 | 26.1 | 5.11 (4.3) | 12.7 | *GSVIVT00007282001* | *** | 1.50 | 0.77 |
| | | 18 | 34.9 | 6.6 (4.3) | 16.7 | *VvP18B35* | *** | 0.98 | 0.19 |
| | 2011/12 | 18 | 34.9 | 5.94 (4.4) | 18.5 | *VvP18B35* | *** | 0.75 | 0.38 |
| BFM | 2009/10 | 2 | 30.2 | 14.01 (5.8) | 30.2 | *GSVIVT00007281001* | *** | 2.18 | 0.67 |
| | | 18 | 36.7 | 11.66 (5.8) | 22 | *VMC7F2* | *** | 1.36 | 0.09 |
| | 2010/11 | 2 | 19.1 | 5.88 (4.6) | 13.7 | *VvP02G4* | ** | 0.93 | 0.70 |
| | | 18 | 33.2 | 8.5 (4.6) | 20.5 | *VvP18B32* | *** | 0.95 | 0.14 |
| | 2011/12 | 18 | 34.2 | 9.52 (5.8) | 27.9 | *p3_VvAGL11* | *** | 1.14 | 0.15 |
| BPD | 2009/10 | 2 | 32.1 | 7.84 (6.8) | 20.1 | *GSVIVT00007281001* | *** | 2.13 | 0.93 |
| | | 18 | 36.3 | 10.7 (6.8) | 24.7 | *VMC7F2* | *** | 1.03 | 0.12 |
| | 2010/11 | 2 | 19.1 | 5.62 (4.3) | 13.6 | *VvP02G4* | *** | 1.49 | 0.86 |
| | | 18 | 34.9 | 6.92 (4.3) | 16.6 | *VvP18B35* | *** | 0.77 | 0.25 |
| | 2011/12 | 18 | 34.9 | 10.07 (6) | 29.3 | *VvP18B35* | *** | 1.19 | 0.13 |

K–W, Kruskal–Wallis significance level (*P* value): **, 0.0005; ***, 0.0001. †Maximum LOD score with threshold in parentheses detected via multiple QTL mapping (MQM) procedure. ‡Markers used as co-factors for MQM procedure. $A_f/A_m$, relative additive effect of maternal to paternal parent; BED, berry equatorial diameter; BFM, berry fresh mass; BFV, berry fresh volume; BPD, berry polar diameter; D/A, relative allelic effect of dominance to total additive effect; cM, centiMorgan; LG, linkage group; QTL, quantitative trait loci; $R^2$, proportion of variance explained by QTL.

under $GA_3$ treatment that did not affect the QTL detection for this trait ($R^2$ of QTL from ~66 to ~63% for control condition and under $GA_3$, respectively). This could be given by the strong effect of the putative *Sdi* locus on the phenotype of SDM and/or by the low response of SDM to $GA_3$ observed above (Tables 1–3).

The genotype × $GA_3$ interaction given by $\sigma^2_{g\times t}$ may indicate that the effect of $GA_3$ treatment is given by one or several interacting genetic factors that would be involved in the loss of control or in the possible masking effect of the *Sdi* locus on berry size. The QTLs for interactions expressed as relative response to $GA_3$ (Table 8) may give insight about the possible effect of $GA_3$ on *Sdi* and its subsequent effect on the expression of berry size. Therefore and despite the masking effect, *VvAGL11* was associated with the intensity of the response to $GA_3$ with other genetic factors located on $LG_2$. The interaction with $GA_3$ could be directly on the *Sdi* locus, affecting it transcriptionally or post-transcriptionally or indirectly on putative operator genes, which are affected by this locus and block the transcription of structural genes of seed development. Possible epigenetic effects, however, should not be ruled out. In order to prove these hypotheses, more studies are necessary at different ~omic levels.

*Season effect*

The comparison among conditions according to phenotype and estimated heritability and mainly to genotype × season interaction for relative response to $GA_3$ showed an important effect of season (Tables 1–3). Specifically, the QTLs for relative response to $GA_3$ detected in $LG_2$ during the first two seasons evaluated were missing in the third season (Figure 2, Table 8). Also, QTLs in $LG_2$ for BED and BPD without $GA_3$ treatment were found only in the first season. Although these results are unwanted, they are not uncommon in woody species, in which case the environment strongly influences both the detection and the amount of variance of QTLs (Korol et al. 1998, Asíns 2002, Collard et al. 2005).

*Candidate genes*

We identified about 160 genes in the confidence intervals for the QTLs found on $LG_2$. Interestingly, this region exhibits quite high gene density, if we consider that 37.9% of the genomic sequence harbouring both QTLs corresponds to coding sequences. Among the genes found in these genomic regions, the most striking ones are those related to gibberellin (GA) metabolism and signal transduction, including *KNOX7*. The *KNOX* class of genes has been described as participating in the regulation of the metabolism of GAs and cytokinins both in meristems and stems (Jasinski et al. 2005). When expressed ectopically in tobacco, this gene showed inhibitory activity against GA synthesis (Kusaba et al. 1998) by repressing the expression of a GA-20 oxidase, a key gene in the GA metabolic pathway (Tanaka-Ueguchi et al. 1998).

Regarding cell size control, two genes were tagged in this region of the $LG_2$, an isoform of expansin 7 (*EXPA7*) and a gene of the *YABBY* family. Expansins are cell wall non-hydrolytic proteins supposedly in charge of cell expansion under low-pH condition. But these proteins have been associated with several physiological processes related to cell growth, such as fast-growing pollen tubes and fruit softening. In *Arabidopsis*, it has been shown that the inhibition of *EXPA7* in the root reduces root hair growth (Lin et al. 2011). The *YABBY* genes also play a key role in all plants propagated through seeds by controlling the abaxial growth of different organs (Siegfried et al. 1999). At the same time, the *YABBY* genes play a role in the regulation of the *KNOX* genes, resulting in the regulation of GA biosynthesis in the shoot in rice (Dai et al. 2007).

In the second QTL found on $LG_2$, there is also a gene coding for a cell wall modifying-enzyme, pectinerase-68. This type of enzyme catalyses the demethylation of pectin residues, which results in the aggregation of new pectin residues intercalated with calcium, producing a strengthening of the cell wall (Kumaran et al. 2002). Similarly, this demethylation implies the softening of the cell wall because pectin residues are more susceptible to be degraded by polygalacturonases (Grant et al. 1973).

Another gene of interest is the protein PBP1 (*PID-binding protein*). This protein interacts with the PINOID (PID) proteins, which in turn play a key role in the auxin transduction signal pathway (Carpita and Gibeaut 1993).

The identification of candidate genes on the $LG_{18}$ QTL presented particular difficulties. For instance, in spite of being one of the most saturated LGs of our mapping population, the QTL detected was not properly flanked by any marker used to build the map, rendering physically too large or too short confidence intervals. Because of this, we considered a region of ca. 1.0 Mb (500 kb upstream and downstream) surrounding the marker VMC7F2, located just in the centre of this QTL. This latter marker co-localises with the gene *VvAGL11*. This gene belongs to the *AGAMOUS* family, which has been proposed as the main gene responsible for stenospermocarpy-type seedlessness in

**Table 9.** Candidate genes selected for each quantitative trait loci region that could be participating in gibberellic acid treatment response.

| Region | Gene ID | Position (bp) | Annotation | Gene symbol | Reference |
|---|---|---|---|---|---|
| $LG_2$ – I | GSVIVT01019859001 | 3 898 374–3 904 052 | Sugar carrier protein C-like | – | N.A. |
| | GSVIVT01019880001 | 4 164 201–4 164 201 | Homeobox protein knotted-1-like 7 | *KNOX7* | Jasinski et al. (2005) |
| | GSVIVT01019883001 | 4 197 374–4 209 047 | MADS-box protein AGL19-like | *AGL19* | Schönrock et al. (2006) |
| | GSVIVT01019889001 | 4 249 304–4 250 807 | Expansin-A7 | *EXPA7* | Lin et al. (2011) |
| | GSVIVT01019914001 | 4 424 613–4 427 223 | Sugar phosphate/phosphate translocator | *SPT* | Pastore et al. (2011) |
| | GSVIVT01001263001 | 4 822 703–4 823 194 | Auxin-induced protein X10A | *X10A* | Dong et al. (2014) |
| | GSVIVT01001269001 | 4 861 965–4 864 774 | Axial regulator YABBY 1 | *YABBY1* | Siegfried et al. (1999) |
| | GSVIVT01001275001 | 4 898 034–4 901 145 | Trehalose-phosphate phosphatase | *TPP* | N.A. |
| | GSVIVT01001286001 | 4 974 657–4 978 139 | WRKY transcription factor | *WRKY* | Guo et al. (2014) |
| $LG_2$ – II | GSVIVT01001316001 | 5 255 210–5 256 154 | 23.6 kDa Heat shock protein | – | N.A. |
| | GSVIVT01001327001 | 5 378 526–5 380 976 | Pectinesterase 68 | *PE68* | Kumaran et al. (2002) |
| | GSVIVT01001327001 | 5 378 526–5 380 976 | Novel plant SNARE 11 | *SNARE11* | Mortimer et al. (2008) |
| | GSVIVT01013268001 | 5 915 695–5 997 069 | Vacuolar protein sorting-associated protein 13A | *VPS13A* | N.A. |
| | GSVIVT01013226001 | 6 526 981–6 534 000 | Calcium-binding protein PBP1 | *PBP1* | Carpita and Gibeaut (1993) |
| $LG_{18}$ | GSVIVT01025916001 | 27 268 865–27 284 577 | MADS-box transcription factor 26 | *MADS26* | De Folter et al. (2005) |
| | GSVIVT01025941001 | 26 935 654–26 938 124 | Elongation factor 1-gamma-like | *EEF1G* | Sasikumar et al. (2012) |
| | GSVIVT01025945001 | 26 888 677–26 896 544 | MADS-box protein 5 - AGL11 | *AGL11* | Mejía et al. (2011) |
| | GSVIVT01025953001 | 26 733 761–26 735 477 | Cytochrome P450 716B2 | *716B2* | Nelson (2009) |
| | GSVIVT01025978001 | 26 388 226–26 396 921 | Tubulin beta-1 chain-like | *TUB1* | N.A. |

LG, linkage group; N.A., no available references.

tablegrapes (Mejía et al. 2011), but also it is part of the main QTL described previously for berry size, explaining 30% or more of the phenotypic variance (Mejía et al. 2007). Other candidate genes located in the vicinity of *VvAGL11* are the gene for transcription elongation factor-1 and another *AGAMOUS* gene (MADS-box transcription factor 26). This later is a transcription factor with scarce information; members of this large and well-conserved gene family (over 100 *MADS* genes have been described in many species, including *Arabidopsis thaliana*, rice and petunia) are characterised by numerous interactions with different gene groups in plants under different physiological conditions or developmental stages (De Folter et al. 2005). Finally, a set of 19 genes underlying these QTLs on $LG_2$ and $LG_{18}$ were proposed as possibly related to response of berry size to $GA_3$ (Table 9).

## Conclusions

- Gibberellic acid treatments applied on Ruby Seedless × Sultanina progeny increase the size of berries and decrease their SDM at ripening (18°Brix), with each segregant exhibiting a differential response to $GA_3$.
- While there is a positive correlation between SDM and berry size, there is a negative correlation between the SDM and the response of berries to $GA_3$.
- Gibberellic acid affects the relationship between SDM and berry size, and is manifested by a loss of the correlation between SDM and berry size.
- Season has an important effect on the variation and the degree of the response to $GA_3$.
- The response to $GA_3$ has an important genetic basis that is given by QTLs localised on LGs 2 and 18. These genetic factors could have a pleiotropic effect on the phenotype.
- A set of 19 genes underlying these QTLs are proposed as possibly related to the response of berry size to $GA_3$.

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## Supporting information

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**Figure S1.** Phenotypic distribution of the traits evaluated based on genotypic means of each segregants. Under control condition (□) and under gibberellic treatment (■).

**Table S1.** Phenotypic values of parental.

**Table S2.** Confidence interval for the quantitative trait loci location across the linkage groups.

**Table S3.** Number of genes identified in each quantitative trait loci region for gibberellic acid treatment response, based on the grapevine reference genome PN40024.

**Table S4.** List of total genes identified in each quantitative trait loci region for gibberellic acid treatment response, based on the grapevine reference genome PN40024.

**Table S5.** Difference between control and treatment conditions in terms of phenotypic variance. The variance is shown as the coefficient of variation.