# New Stable QTLs for Berry Firmness in Table Grapes

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**Abstract:** Berry firmness is one of the most important quality traits in table grape production and, consequently, a key aspect for table grape breeding programs. To identify the genes determining the berry firmness in grapes, a progeny of Ruby Seedless × Sultanina (n = 137) was evaluated during three consecutive seasons. Results showed that even though the heritability was ~90%, season had an important effect on this trait. Quantitative trait loci (QTL) analysis and genetic mapping showed that the determinants for this trait are distributed in linkage groups 8 and 18. This is the first time that a stable QTL for berry firmness across seasons has been identified on linkage group 8. This QTL is mainly given by a male allelic and additive effect. Together, these two QTLs explained ~27.6% of the phenotypic variance, with confidence intervals of up to 10 cM. Among the tens of genes found in these two QTLs, we highlight a cation/calcium exchanger, a xylosyltransferase, a probable cellulose synthase, and a putative invertase. This study shows that berry firmness has a clear genetic basis. These results could also be used for the development of markers to assist table grape breeding.

**Key words:** berry texture, grapevine, heritability, linear mixed models, quality traits, QTL analysis and mapping

Fruit quality is mainly determined by sensory traits, such as appearance, color, taste, aroma, flesh texture, and berry firmness. *Vitis vinifera* berries undergo several changes in developing and maturity stages. The curve of growth of table grapes corresponds to a double sigmoid with a typical intermediate lag phase (Dokoozlian 2000). The first stage is described as a fast growth based on cell division and enlargement, followed by a second stage that resembles a plateau called veraison, and a third stage of rapid growth mainly due to cell elongation (Harris et al 1968). On veraison, multiple physiological changes occur in table grape berries, rendering the final physical and sensorial properties at harvest. One of the most relevant modifications of the berry on veraison is the reduction of firmness (Nunan et al. 1998, Balic et al. 2014).

In the case of table grapes, berry firmness is defined by the interaction of numerous genes and pathways associated with degradation of the cell wall and cuticle properties. For instance, genes such as polygalacturonase (PG), pectin methylesterase, pectate lyase, galactanase, and galactosidase (Chapman et al. 2012, Longhi et al. 2013, Vargas et al. 2013), and those acting on the primary cell wall such as xyloglucanase, cellulase, and expansin (Deluc et al. 2007, Dal Santo et al. 2013, Longhi et al. 2013), have been associated with pectin modification. PG catalyzes the hydrolytic cleavage of α(1-4) galacturonan, and has been associated with fruit firmness and softening in tomato (Sheehy et al. 1988), apple (Costa et al. 2010, Longhi et al. 2012, 2013), and grape (Lijavetzky et al. 2012). In apples, via quantitative trait loci (QTL) analysis, *Md-PG1* has been associated with several parameters of fruit texture (Longhi et al. 2012, 2013). In grapes, the *VvPG1* transcript has been positively correlated with berry softening, as it is upregulated in berry flesh after veraison (Lijavetzky et al. 2012). Vargas et al. (2013) associated a grape pectate lyase (*VvPel*) with berry texture in a core collection of 96 table grape accessions. In another study using the cultivar Kyoho, Ishimaru and Kobayashi (2002) showed that xyloglucan endotransglycosylase gene expression is closely related to berry development and softening, and was markedly increased at veraison. Similar results have been reported in Cabernet Sauvignon (Schlosser et al. 2008) and in Muscat Hamburg (Lijavetzky et al. 2012). Xyloglucan endotransglycosylase was recently reported by Carreño et al. (2015) within a QTL for berry firmness. Gene expression of cellulases, such as endo-1,4-β-D-glucanase, has been associated with fruit ripening (Fischer and Bennett 1991, Sexton et al. 1997); in grapes, endo-1,4-β-D-glucanase gene expression increases during the first stages of ripening, during the onset of veraison, but decreases during the late stages of berry growth or stage III (Schlosser et al. 2008). β-galactosidases also have been suggested as responsible for firmness in grapes (Carreño et al. 2015). Ishimaru et al. (2007) found that expression of putative expansins in mature Kyoho berries was closely correlated with berry softening. Deluc et al. (2007) and Schlosser et al. (2008) found that the expression of some expansins was upregulated during the later phases of the slow period of growth before veraison or stage II, and during the transition from stage II to III. Mesocarp cell turgor has also been associated

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with berry firmness as a mechanistic cause of berry softening (Vicente et al. 2007, Thomas et al. 2008, Wada et al. 2009). Furthermore, accumulation of solutes, such as sugars, organics acids, and ions, in the apoplast would play an important role in berry softening (Vicente et al. 2007, Wada et al. 2008). Aquaporins have also been indicated to be related to firmness (Vicente et al. 2007).

Although the importance of berry firmness is evident, only a few studies have dealt with it in table grapes (Balic et al. 2014, Carreño et al. 2015). Considering the relevance of berry firmness, we carried out an evaluation of the heritability, genetic components, and determinants of berry firmness using a segregant population of Ruby Seedless × Sultanina during three consecutive seasons. The results revealed the presence of a new QTL, stable over seasons, and a group of underlying genes that could become markers used in assisted breeding after validating them in the proper genetic backgrounds.

## Materials and Methods

**Plant material.** This study was performed in central Chile during three consecutive growing seasons (2011-2012 to 2013-2014) at the Research Center La Platina (lat. 33°34′20″S; long. 70°37′32″W; 630 m elevation) that belongs to the Institute for Agriculture Research (INIA), Chile. The plant material corresponded to the $F_1$ progeny (n = 137 segregants) of the controlled cross of Ruby Seedless × Sultanina (R × S). Plants grafted on Sultanina rootstock were trained in an overhead horizontal trellis system. Three clusters per segregant, each from a different plant of each segregant, were used as replicates. Clusters used in the assay were thinned, leaving 120-150 berries per bunch.

**Maturity parameters.** After veraison, the soluble solid content (SSC) of berries was monitored weekly in each segregant until it reached maturity based on an SSC level close to 18 Brix. The SSC was measured with a manual temperature-compensated refractometer (ATC-1E, Atago) in a sample of juice from 20 berries collected within each cluster. Because of the different sugar accumulation rates among the segregants, maturation occurred differentially from February to the beginning of April each season. Then, 20 healthy and homogenous berries with their cap stems were randomly sampled from each cluster. The firmness of these intact berries (considering the berry skin and flesh) was determined as soon as possible on the same day that clusters were harvested. Measurements were performed using a firmness tester (Firmtech II, BioWorks), which returns the grams necessary to compress the berry to 1 mm. By using this device, firmness was expressed in g/mm. Heritability for berry firmness was estimated based on genotypic variance (restricted maximum likelihood variance) of a mixed linear model. This model was given by the effect of each genotype on berry firmness (random genotypic effect) and its possible random effect of interaction with the season effect (fixed effect). Genetic evaluations were based on a linkage map using the R × S population. The consensus genetic map was built using 272 markers (simple sequence repeats, amplified fragment length polymorphisms, gene-based single nucleotide polymorphisms, phenotypic markers, and sequence characterized amplified regions) distributed on 19 linkage groups (LG); this map had a total length of 1,334 centiMorgans (cM). QTL analysis was based on the phenotypic means of each genotype for each season. QTLs were identified using the consensus map by consecutively applying the non-parametric Kruskal-Wallis test (K-W), interval mapping, and the multiple-QTL model (MQM) analyses. Then, QTLs were characterized by their K-W results ($p$ values), logarithm of odds (LOD), proportion of explained variation, and confidence intervals in cM. More details of these procedures and information about the construction and characteristics of the genetic/linkage map are described by Correa et al. (2014). Finally, a search for candidate genes was conducted in the genomic region corresponding to the confidence interval for each QTL detected using the annotated reference genome (Genoscope 12×) of the Pinot noir-derived 40024 line (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/; Jaillon et al. [2007]). Before the analysis, the assumptions of normality and homoscedasticity of variances were evaluated by the Shapiro-Wilk and Levene tests, respectively. When the trait failed to meet these assumptions, the natural logarithm transformation was applied.

## Results and Discussion

The phenotype of firmness showed potential variation among seasons and a non-normal distribution (Figure 1). The trait was successfully normalized by a log transformation. Parents showed discordance in firmness across seasons, indicating a significant effect of environmental factor(s) on this trait.

Table 1 shows some phenotypic characteristics of berry firmness for R × S progeny. Some phenotypic differences can be observed among seasons. The 2011-2012 season was the more dissimilar one, having the lowest mean and variance values. In addition, this season had the minor genotypic contribution to the phenotypic expression of berry firmness ($\sigma_g^2$ and $h_b^2$). However, there was no effect of the variation in berry firmness among clones or plants ($\sigma_{plant}^2$). The latter indicates a homogenous phenotypic behavior of plants of the same genotype under the experimental conditions of R × S.

In general, the broad-sense heritability ($h_b^2$) of this trait was relatively high compared to others assessed in grapes, reaching values of ~88% (Table 1). For instance, for quality traits in grapes, $h_b^2$ has been estimated to be ~96% for SSC (Brix) of berries (Schneider and Staudt 1979), while narrow-sense heritability for this trait has been estimated to be 48 to 62% (Wei et al. 2002). In addition, Liu et al. (2007) estimated for glucose and fructose content and total sugars at 59 to 72, 61 to 77, and 61 to 74%, respectively. Broad-sense heritability and narrow-sense heritability for acidity of berries has been estimated to be 75% (Schneider and Staudt 1979) and ~30 to 42% (Wei et al. 2002), respectively. For malic and tartaric acids, $h_b^2$ has been estimated to be ~69 to 91 and 47 to 75%, respectively (Liu et al. 2007). These antecedents indicate that firmness in our progeny has a clear genetic basis, which could be useful for breeding the species.

Interaction between season and genotype was significant, in accordance with the results regarding phenotypic distribution and those found by Carreño et al. (2015), in which season had a significant effect on this trait. In our study, the season effect was as important as the effect of the individual QTLs (Tables 1 and 2). Several environmental factors have been shown to affect fruit texture, such as intensity of solar radiation, temperature, water availability, nitrogen, and calcium, which could be involved in the variation among seasons or locations (Sams 1999). The polygenic feature of firmness added to the environmental factors would make the assessment of this trait difficult in a breeding program (Carreño et al. 2015).

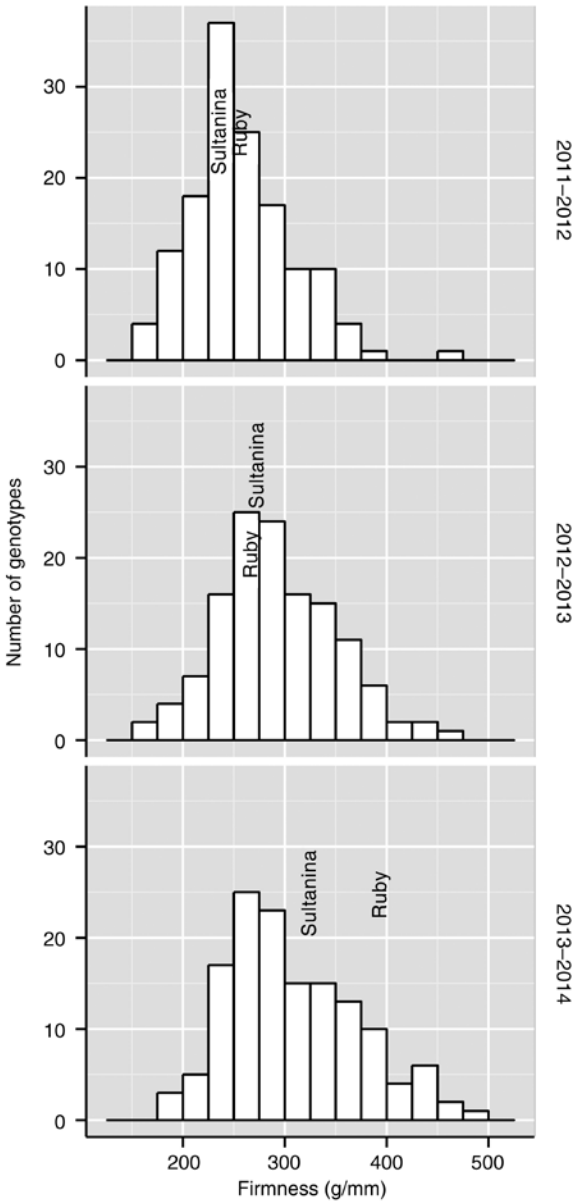


**Figure 1** Phenotypic distribution of berry firmness across seasons for Ruby Seedless × Sultanina (n = 137) progeny. The plot was based on mean values of each genotype. The parental mean values are indicated by their names.

The QTL analysis was performed using the consensus genetic map. As previously mentioned, this map consisted of 272 markers distributed on 19 LGs. Only two LGs harbored QTLs for berry firmness, located on LG8 and LG18 (Table 2). The position of QTL peaks was steady across the three seasons. However, the QTL LODs, $R^2$s, and allelic effects showed some changes, indicating possible environment or error effects. Cofactors for seasons 2012-2013 and 2013-2014 were the same and were significant according to K-W analysis. This result contrasts with the larger number of QTLs found previously for firmness on LGs 1, 4, 5, 9, 10, and 13 by Carreño et al. (2015); the QTL found on LG18 in both works (Carreño et al. 2015, and this work) were located in different positions on this LG. Overall, these results could be due to the more stringent thresholds used in the present work. In addition, we did not consider putative QTLs, as Carreño et al. (2015) did for most of their QTLs. However, the QTL described here on LG8 was consistent across seasons. On average, this QTL explained ~15.6% of the phenotypic variation. The QTL detected on LG18 explained ~12%. In total, these QTLs account for ~27.6% of the phenotypic variation of firmness (Table 2).

The QTL on LG8 was associated with the UDV125 and VMCNG2H2.2 markers (Table 2, Figure 2) in a region of 6.2 to 8.2 cM, depending on the season (Table 3). In this same linkage group, Doligez et al. (2013) found QTLs close to the VMC1B11 marker for seed fresh weight, berry weight, and for the residual of berry weight unexplained by seed number. In our genetic map, this locus is located ~30 cM from the markers associated with berry firmness. Also, this QTL is mainly given by a male allelic and additive effect (Table 2); this "paternal" effect is a common feature in this progeny due to the inbreeding of the parents (Correa et al. 2014). Fifty-seven genes were found in the QTL of LG8 highlighting two of them: a cation/calcium exchanger gene and a xylosyltransferase gene (Supplemental Table 1). The former could be involved in cell-wall

**Table 1** Descriptive statistics, heritabilities, and percentages of restricted maximum likelihood (REML) variance in the total phenotypic variance for berry firmness during the 2011 to 2012, 2012 to 2013, and 2013 to 2014 seasons for Ruby Seedless × Sultanina progeny.

| | Firmness phenotype (g/mm)[a] | | | | Genotypic effect (%)[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Per season | | Across seasons | | | |
| Season | Mean | SD | Min | Max | $\sigma^2_g$ | $h^2_b$ | $\sigma^2_g$ | $\sigma^2_{g\times season}$ | $\sigma^2_{plant}$ | $h^2_b$ |
| 2011-2012 | 258.7 | 53.4 | 146.5 | 458.9 | 68.748*** | 86.841 | 56.549*** | 16.248*** | ns | 87.751 |
| 2012-2013 | 295.6 | 63.2 | 166.2 | 497.8 | 72.773*** | 88.911 | | | | |
| 2013-2014 | 292.8 | 69.0 | 146.5 | 575.2 | 76.181*** | 94.843 | | | | |

[a]SD, standard deviation; Min, minimum; Max, maximum.
[b]REML variance components of genotype effect ($\sigma^2_g$), Genotype × Season interaction effect ($\sigma^2_{g\times season}$), plant effect ($\sigma^2_{plant}$), and broad-sense heritability ($h^2_b$). Significance codes according to likelihood ratio test ($p$ value): 0-0.001'***'; ns: not significant ($p > 0.05$).

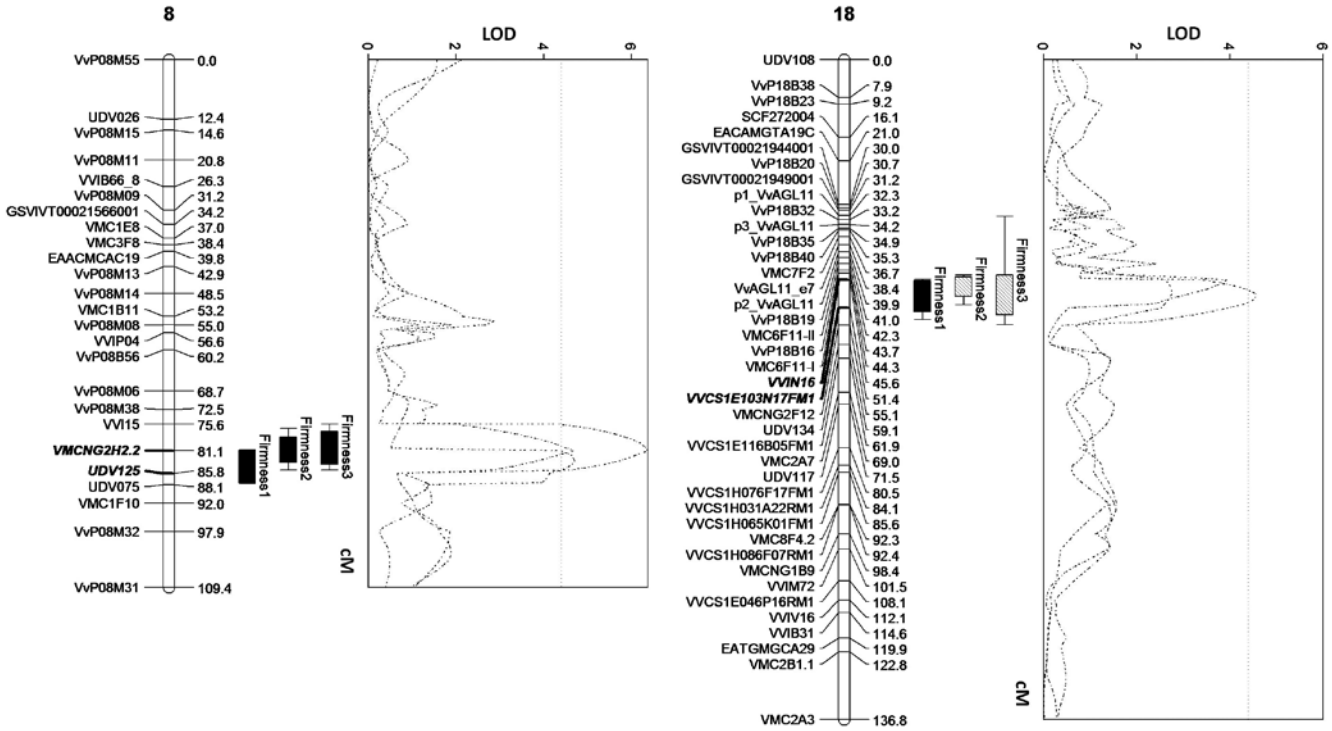


**Figure 2** Genetic map of linkage groups (LG) 8 and 18 and their profile of logarithm of odds (LOD) for detected quantitative trait loci (QTLs) for berry firmness of the Ruby Seedless × Sultanina progeny (n = 137) over the 2011 to 2012, 2012 to 2013, and 2013 to 2014 seasons: 1, 2 and 3, respectively. The boxes and lines represent the confidence intervals of QTLs at LOD ± 1 and ± 2, respectively. Black boxes are significant QTLs at the genome-wide LOD threshold, and boxes with hatch pattern are QTLs only significant at linkage-group–wide LOD threshold (putative QTL). Markers in bold and italics are the cofactors associated with each QTL according to a multiple QTL mapping procedure. The LOD curves (profile at the right of each LG) show the value of each interval in centiMorgans (cM). The dotted vertical lines correspond to genomic LOD thresholds at 5% for each trait. More detailed information is given in Tables 2 and 3.

**Table 2** Quantitative trait loci (QTLs) for traits related to berry firmness detected via multiple QTL mapping (MQM), estimated from genotypic means of Ruby Seedless × Sultanina progeny.

| | Position[a] | | LOD[b] | | | | | | Allelic effect[f] | |
|---|---|---|---|---|---|---|---|---|---|---|
| Season | LG | cM | Peak | GWT | LGWT | $R^2$ (%)[c] | Cofactor[d] | K-W[e] | $A_m/A_f$ | D/A |
| 2011-2012 | 8 | 83.1 | 4.71 | 4.4 | 3.3 | 13.8 | UDV125 | ** | 4.584 | 0.648 |
| | 18 | 48.6 | 4.56 | | 3.4 | 16.1 | VVCS1E103N17FM1 | ns | 0.165 | 0.423 |
| 2012-2013 | 8 | 81.1 | 4.68 | 4.4 | 3.1 | 13.4 | VMCNG2H2.2 | ***** | 690.905 | 0.522 |
| | 18 | 45.6 | 3.93 | | 3.4 | 11.5 | VVIN16 | **** | 0.358 | 1.836 |
| 2013-2014 | 8 | 81.1 | 6.38 | 4.4 | 3.3 | 19.6 | VMCNG2H2.2 | ***** | 14.065 | 0.226 |
| | 18 | 47.6 | 2.75 | | 3.2 | 8.5 | VVIN16 | * | 0.194 | 0.620 |

[a]LG, linkage group; cM, centiMorgan.
[b]Maximum logarithm of odds (LOD) score with genome-wide threshold (GWT) and linkage-group–wide threshold (LGWT) detected via MQM procedure.
[c]$R^2$, percentage of variance explained by QTL.
[d]Markers used as cofactors for MQM procedure.
[e]K-W, Kruskal-Wallis significance levels ($p$ value): *, 0.01; **, 0.005; ****, 0.0005; *****, 0.0001; ns, not significant ($p > 0.1$).
[f]$A_m/A_f$, relative additive effect of maternal to paternal parent; D/A, relative allelic effect of dominance to total additive effect.

**Table 3** Confidence interval and location across the linkage groups (LG) for the quantitative trait loci (QTLs) for berry firmness of Ruby Seedless × Sultanina progeny.

| | | Confidence interval (cM)[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Season | LG | LOD-2 | LOD-1 | Peak | LOD+1 | LOD+2 | Range+1 | Range+2 |
| 2011-2012 | 8 | 81.1 | 81.1 | 83.1 | 87.8 | 87.8 | 6.7 | 6.7 |
| | 18 | 45.6 | 45.6 | 48.6 | 52.4 | 53.4 | 6.8 | 7.8 |
| 2012-2013 | 8 | 76.6 | 78.6 | 81.1 | 83.1 | 85.1 | 4.5 | 8.5 |
| | 18 | 44.3 | 45.3 | 45.6 | 48.6 | 50.6 | 3.3 | 6.3 |
| 2013-2014 | 8 | 75.6 | 76.6 | 81.1 | 84.1 | 85.1 | 7.5 | 9.5 |
| | 18 | 32.3 | 44.3 | 47.6 | 53.4 | 55.1 | 9.1 | 22.8 |

[a]QTL confidence intervals in centiMorgans (cM) corresponding to the maximum (peak) logarithm of odds (LOD) score minus 1 and minus 2 units on either side of the LOD peak (upper limit at -1 and -2 LOD and lower limit at +1 and +2 LOD); Range±1 and Range±2: length of interval in cM at 1 and 2 LOD, respectively.

metabolism, transporting calcium from cytosol to apoplast and vice-versa. Calcium is an essential nutrient with structural roles in the cell wall, and its presence has important effects on fruit firmness in several fruit crops (White and Broadley 2003). Calcium forms cross-bridges of pectin chains in the cell wall, strengthening the structure (Fry 2004). Exogenous applications of this mineral stabilize the plant cell wall, reducing the action of cell-wall–degrading enzymes that have a relevant impact on firmness (White and Broadly 2003, Ciccarese et al. 2013). Accordingly, Balic et al. (2014) demonstrated that berries with higher calcium concentration in the cell wall are firmer at harvest than varieties with lower calcium content. Also, xylosyltransferases have been associated with cell wall polysaccharide biosynthesis, such as xyloglucans (Fry 2004, Cavalier et al. 2008). Xyloglucans are the main hemicellulose polysaccharide found in the primary cell walls (Cavalier et al. 2008), and their depolymerization has been associated with fruit ripening (Rose and Bennett 1999).

The QTL on LG18 was not as stable as the QTL on LG8 across seasons. In LG18 QTL, 158 genes were found (Table 2, Supplemental Table 2). LG18 QTL was genome-wide significant in only the first season evaluated (Table 2). In the second season, it was only significant at the linkage-group–wide LOD threshold (putative QTLs). Unfortunately, LG18 QTL was nonsignificant in the third season (Table 2). Apparently, this putative QTL is close to the QTL found by Carreño et al. (2015) on LG18. According to these authors, the peak of this QTL is relatively near the VMC7F2 and VMC6F11 markers; the latter are located 1-2 cM from the VVIN16 and VVCS1E103N17FM1 markers that we used as cofactors. In addition, Duchêne et al. (2012) presented QTLs for heat sums for flowering to veraison, colocalized on LG18 during several seasons. They proposed genes related to sugar transport and signaling (putative sucrose sensor and ABRE-binding factor) and genes related to abscisic acid (ABA) response (ASR, ABA stress- and ripening-induced protein). Taking into account that the evaluation of Duchêne et al. (2012) was based on berry softening, those genes could be participating in berry firmness, as berries treated with sucrose and ABA presented changes in softening given by a decrease in elasticity (Gambetta et al. 2010). Also, a probable cellulose synthase was found on this same QTL (Supplemental Table 2). Some of the textural changes resulting in softening of the fruit are due to enzyme-mediated alterations in the structure of cellulose (Prasanna et al. 2007). Therefore, we believe that variations in cellulose content could have the same effect as variations in gene expression and activity of cellulose-degrading enzymes. One of the genes found in the LG18 confidence interval is a putative invertase (Supplemental Table 2). The presence of solutes in the apoplast increases water outflow from the phloem to the apoplast in the fruit, resulting in an increase of the water transport via phloem into the fruit, which in turn increases cell turgor (Matthews and Shackel 2005) and consequently impacts fruit firmness (Vicente et al. 2007).

Expansins have been associated with changes related to fruit softening in some species, such as tomato (Brummell et al. 1999), peach (Hayama et al. 2003), and grape (Deluc et al. 2007, Ishimaru et al. 2007, Schlosser et al. 2008). These proteins are widely distributed in the grape genome (Dal Santo et al. 2013); based on the Genoscope 12× whole genome sequence of the PN40024 line, some of them are found in LG8 (position: 10.68, 14.73, and 18.62 megabase pairs) and LG18 (position: 1.78 megabase pairs). However, expansins are localized in different regions with respect to the QTL for firmness. For example, in LG8, we found an alpha-expansin 2 located at 10,683,552–10,683,967 base pairs (bp; 416 bp long), an alpha-expansin 4 positioned at 14,733,280–14,734,473 bp (1,194 bp), and an alpha-expansin 4 placed at 18,624,552–18,624,834 bp (283 bp), whereas the QTL location ranged between 1,900,037 and 3,385,686 bp. In addition, an alpha-expansin 2 on LG18 was located at 1,778,997–1,779,432 bp (436 bp); nevertheless, the QTL found on this chromosome was located at ~19,427,037–23,780,549 bp.

## Conclusions

The assessment of firmness in the R × S (n = 137) progeny during three consecutive seasons revealed that this trait has a significant genetic basis, which was given by a high value of heritability and the presence of one stable QTL. In addition, this trait showed important variation among seasons. We found a new, stable QTL for berry firmness located in LG8, explaining ~16% of the phenotypic variation of firmness in table grapes. Furthermore, we found a QTL previously associated with firmness in LG18. Based on their confidence intervals and the reference genome available, we propose a cation/calcium exchanger, a xylosyltransferase, a cellulose synthase, and a putative invertase as candidate genes participating in the control of berry firmness.

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