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A major QTL is associated with berry grape texture characteristics

Manna Crespan[1*], Daniele Migliaro[1], Silvia Vezzulli[2], Sara Zenoni[3], Giovanni Battista Tornielli[3], Simone Giacosa[4], Maria Alessandra Paissoni[4], Susana Río Segade[4] and Luca Rolle[4]

[1]CREA Research Centre of Viticulture and Enology, Viale XXVIII Aprile, 26 - 31015 Conegliano, Treviso,, Italy.
[2]Research and Innovation Centre, Fondazione Edmund Mach, Via Mach, 1 - 38010 San Michele all'Adige, Trento, Italy.
[3]Department of Biotechnology, University of Verona, Cà Vignal 1, Strada Le Grazie 15, 37134 Verona, Italy.
[4]Department of Agricultural, Forest and Food Sciences, University of Torino, Largo Paolo Braccini, 2, 10095 Grugliasco, Torino, Italy.

*corresponding author: manna.crespan@crea.gov.it

ABSTRACT

Berry texture and berry skin mechanical properties are traits with high agronomic relevance because they are related to quality parameters and marketing requirements of wine, table, and raisin grapes. Searching for QTLs linked to berry texture, an F1 population of 152 individuals and their parents were used in this study. These F1 plants were obtained crossing Raboso Veronese, a seeded black wine grape cultivar, and Sultanina, a seedless white grape variety, especially used for raisins. Density flotation was applied for berry sorting improving the management of many and highly variable genotypes, irrespective of the quantification of specific molecule classes. Berries were evaluated for technological ripeness parameters and mechanical properties. Texture parameters were taken as raw data and as data normalised on berry dimensions, i.e., berry diameter or surface or volume. SSR molecular markers were used to produce a genetic map and a major QTL for berry texture was found on chromosome 18 with traits related to berry firmness showing a phenotypical explained variance higher than 60 %, and traits related to berry resilience, springiness and cohesiveness showing a variance higher than 50 %. Surprisingly, this QTL showed to be associated with SSR markers linked to *VviAGL11*, the main gene linked to seedlessness. *VviAGL11* expression and co-expression profiling during grape ripening was evaluated using available information; this data suggested a role for this gene on the texture of a ripe berry.

Abbreviations:

ABW, average berry weight
BR, berry resilience
BR_diam, berry resilience normalised on berry diameter
BR_sur, berry resilience normalised on berry surface
BR_vol, berry resilience normalised on berry volume
BS_ratio, berry springiness
BS_ratio_diam, berry springiness normalised on berry diameter
BS_ratio_sur, berry springiness normalised on berry surface
BS_ratio_vol, berry springiness normalised on berry volume
BCo, berry cohesiveness
BCo_diam, berry cohesiveness normalised on berry diameter
BCo_sur, berry cohesiveness normalised on berry surface
BCo_vol, berry cohesiveness normalised on berry volume
BH, berry hardness

BH_diam, berry hardness normalised on berry diameter
BH_sur, berry hardness normalised on berry surface
BH_vol, berry hardness normalised on berry volume
BG, berry gumminess
BG_diam, berry gumminess normalised on berry diameter
BG_sur, berry gumminess normalised on berry surface
BG_vol, berry gumminess normalised on berry volume
BCh_ratio, berry chewiness
BCh_ratio_diam, berry chewiness normalised on berry diameter
BCh_ratio_sur, berry chewiness normalised on berry surface
BCh_ratio_vol, berry chewiness normalised on berry volume
F_{sk}, berry skin break force
W_{sk}, berry skin break energy
E_{sk}, berry skin resistance to the axial deformation
Sp_{sk}, berry skin thickness

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/3994

INTRODUCTION

Nowadays, texture analysis is a well-established analytical technique in the food industry for evaluating the mechanical and physical characteristics of both raw ingredients and finished products. Several types and methods of texture tests (compression, penetration, traction, cutting, double compression) allow an objective measurement of mechanical variables/properties of vegetables and fruits. Specific studies on grape texture have been carried out since the 1980s (Bernstein and Lustig, 1981; Sato *et al.*, 1997). Berry texture and berry skin mechanical properties are traits with high agronomic relevance because they are related to quality parameters and marketing requirements of wine, table, and raisin grapes (Rolle *et al.*, 2012a). In particular, skin break force and thickness are important mechanical characteristics for wine grapes because they impact the kinetics and yield of anthocyanin release during the maceration process (Rolle *et al.*, 2008; Maury *et al.*, 2009; Río Segade *et al.*, 2011a) and on the speed of grape withering process (Rolle *et al.*, 2011a). In table grapes, hardness, cohesiveness, gumminess, springiness, chewiness, and resilience are typical berry mechanical parameters instrumentally measured to define the textural quality and consumer's acceptance of whole berry and pulp, being related with sensory firmness and crunchiness (Deng *et al.*, 2005; Giacosa *et al.*, 2015). In general, the "instrumental" berry firmness can be considered a characteristic of the whole berry, where skin and pulp physical properties can impact this trait (Mencarelli *et al.*, 1994), or of the pulp only (Giacosa *et al.*, 2014). Instead, crunchiness is preferably associated with the pulp (Giacosa *et al.*, 2015). Moreover, skin, pulp and whole berry mechanical properties can be used to characterise and to classify grape varieties also according to the OIV ampelographic descriptors (code 228 and 235) (Giacosa *et al.*, 2014; Río Segade *et al.*, 2013a).

A QTL (Quantitative Trait Locus) is a portion of DNA linked to the expression of a phenotypic trait showing a quantitative inheritance, because being under polygenic control and is obtained through genetic mapping and phenotyping of populations segregating for the trait. Detection of molecular markers associated with desired traits contributes to unveiling the genetic and physiological mechanisms underlying these traits and improves the efficiency of breeding through Marker-Assisted Selection (MAS). MAS is particularly useful for traits linked to fruit quality in perennial plants because it is time, space, and cost-saving while allowing an early selection of seedlings with the required traits. Some efforts were already done focused on searching QTLs and candidate genes linked to berry "firmness" (Carreño *et al.*, 2014; Ban *et al.*, 2016; Correa *et al.*, 2016; Guo *et al.*, 2019; Wang *et al.*, 2020). In these works, grape ripening status was established through sensory evaluation or measured according to berries soluble solid content or the ratio between sugar content and acidity. Different approaches were applied also to evaluate berry texture, using sensory evaluation or instrumental texture analyses, repeating the phenotypic evaluations at least for two years. Variable number of QTLs were found, spread on different LGs, showing low contribution rates to the trait and little consistent results. More QTLs on LG18 were found by Jiang *et al.* (2020) similar to Carreño *et al.* (2014) and Correa *et al.* (2016), but showing better support to the phenotypic variation explained. Part of the difficulties found in these studies to obtain significant results can be probably ascribed to a large intrasample heterogeneity of the berries, when picked randomly in the vineyard or on the cluster present on the market (Río Segade *et al.*, 2013a; Zouid *et al.*, 2013). This variability, issue not previously considered in the works already present in the scientific literature, can be attributed mainly to the different ripening stages reached from each berry even from the same cluster, which impact on the grape texture parameters measured (Río Segade *et al.*, 2011b; Río Segade *et al.*, 2013b; Zouid *et al.*, 2013). A useful method to reduce sample variability, thus decreasing its heterogeneity, is berries sorting through density flotation, which allows the selection of berries with similar ripeness level (i.e., sugar contents and organic acid composition) (Rolle *et al.*, 2015).

An F1 population was produced in 2006 at CREA Research Centre of Viticulture and Enology of Conegliano (Treviso, Italy), by crossing Raboso Veronese and Sultanina. Raboso Veronese is a black-berried wine grape cultivar autochthonous of the Veneto region (North East of Italy), characterised by late ripening, high acidity and significantly rich in polyphenols; Sultanina is a seedless, stenospermocarpic, white-berried variety of the Middle East, largely cultivated around the world especially for raisins. Beyond the initial goal for which the population was created, i.e., finding the molecular markers linked to seedlessness, the wide ampelographic and phenological variability displayed by this F1 population allowed to study many different traits, among them, grape

texture characteristics. This work focused on searching QTLs linked to grape whole berry texture parameters (hardness, cohesiveness, gumminess, springiness, chewiness, resilience) and grape berry skin mechanical properties (skin break force, skin break energy, skin resistance to the axial deformation, berry skin thickness) by phenotyping the grape berries selected at harvest through flotation and using 161 SSR molecular markers for genetic map construction. This study represents an effective contribution towards supporting the genetic improvement of table and wine grapes via MAS.

MATERIALS AND METHODS

1. Plant material and studied traits

An F1 population was obtained in 2006 crossing a wine cultivar, Raboso Veronese, and a seedless table grape, Sultanina. This progeny included 200 seedlings, selected after exclusion of plants derived from self-pollination or pollen donor other than the desired male parent, as checked with SSR markers. Seedlings were propagated (5 vines/genotype), grafted on Kober 5BB, and planted in 2012 in the CREA Viticulture and Enology experimental farm in Spresiano (Treviso, Italy, geographic coordinates 45°46'36"12 N, 12°15'38"16 E). Thirty traits related to the berry were studied in 152 F1 genotypes and their parents in the year 2017 and in 28 F1 genotypes in the year 2019: berry colour (anthocyanins presence/absence), average berry weight, berry mechanical properties, i.e., resilience, springiness, cohesiveness, hardness, gumminess, chewiness, and berry skin characteristics, i.e., break force, break energy, resistance to the axial deformation and thickness, as detailed in Table 1.

2. Cluster sampling and evaluation of berry chemical and mechanical properties

Clusters sampling was performed measuring soluble solid content of some single berries randomly picked up and showing values around 20°Brix. A digital refractometer HI 96811 (Hanna Instruments) was used.

Chemical and texture analyses were performed on the grapes of 152 F1 genotypes, and the two parents in 2017. The second round of analyses was repeated in 2019 on 28 genotypes randomly selected to evaluate possible seasonal effects on berry texture properties; 22 of them were analysed also in 2017, six were analysed in 2019 for the first time.

3. Berry density flotation study

At the respective harvest date (from 12 September to 15 October in 2017 and from 20 September to 20 October in 2019), about 3 kg of grapes for each genotype were randomly picked from different clusters from the five cloned plants per genotype. The pedicel of every single berry was cut in the proximity of the receptacle. For each genotype, all berries were then immediately sorted according to density through their flotation in five different saline solutions (110, 120, 130, 140 and 150 g L^{-1} NaCl), to reduce the possible impact of the different ripening levels. These saline solutions had densities ranging from 1069 to 1125 kg m^{-3} (Fournand *et al.*, 2006; Rolle *et al.*, 2015). Briefly, the berries were introduced into the less dense solution, considering that the floating berries have the same density as the solution. These berries were separated from those that sunk, washed, and weighed. Subsequently, the sunken berries were introduced into the following denser solution. Once the same process was repeated for all saline solutions, the berries belonging to the density class of 1088 kg m^{-3}, corresponding to the solution 130 g L^{-1} NaCl, were visually inspected and those with undamaged skins were selected for this study. This density class was selected because was the most representative class for each genotype (i.e., the maximum percentage of berries floated).

4. Berry size measurement

The length between top and bottom sides (L), as well as the length between both lateral sides at the middle of berry height (i.e., maximum of berry diameter, l), were measured with a calliper. Surface and volume were calculated considering the berry form as an ellipsoid, through the following equations:

$$\text{Volume (cm}^3\text{)} = 4 \pi a b c / 3$$

$$\text{Surface (cm}^2\text{)} = 4 \pi ((a^p b^p + a^p c^p + b^p c^p) / 3)^{1/p}$$

where a = b = l/2; c = L/2; for surface assessment, p = 1.6075 according to the Knud Thomsen formula (error maximum of 1.061 %)[3] (Río Segade *et al.*, 2011a). The berry weight (g) was measured by means of a technical balance (Gibertini E1700, Modena, Italy). Twenty berries per genotype were measured and weighted.

5. Technological ripeness evaluation

For each genotype, about 100 berries belonging to the density class selected were manually crushed and reducing sugars (g L^{-1}), pH, and titratable

acidity (g L^{-1} as tartaric acid) were determined in the obtained musts, according to the methods of the International Organization of Vine and Wine (OIV, 2008). Citric, tartaric, and malic acids (g L^{-1}) were determined using an Agilent 1260 Infinity (Agilent Technologies, Santa Clara, CA, USA) HPLC system equipped with a refractive index detector and a diode array detector (DAD) set to 210 nm. The chromatographic separation was performed isocratically using 0.7 mL min^{-1} flow-rate of 0.0065 N sulphuric acid at 65 °C temperature in a 300 × 7.8 mm i.d. cation exchange column (Aminex HPX-87H) coupled to a Cation H$^+$ Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA) (Río Segade *et al.*, 2013a).

6. Berry mechanical property evaluation

A Universal Testing Machine TA.XTplus texture analyser (Stable Micro Systems, Godalming, Surrey, UK), equipped with an HDP/90 platform and a 5 kg load cell, was used for grape berry texture analysis. All data acquisitions were made at 500 points per second. Typical deformation curves for all the different tests performed and the respective mechanical parameters measured were reported by (Rolle *et al.*, 2012a). The berry skin hardness was assessed by a specific puncture test using a P/2N needle probe (Stable Micro Systems), a test speed of 1 mm s^{-1}, and a penetration applied of 3 mm (Letaief *et al.*, 2008b). Each berry was individually punctured in the lateral face, matching the maximum diameter. The following parameters were measured: skin break force (N, as F$_{sk}$), skin break energy (mJ, as W$_{sk}$), and skin resistance to the axial deformation (N mm^{-1}, as E$_{sk}$). Berry skin thickness (μm, as Sp$_{sk}$) was determined by a compression test, which required the manual separation of a piece of skin (ca. 0.25 cm^2) from the lateral side of each berry using a razor blade. The test was carried out using a 2 mm P/2 flat cylindrical probe (Battista *et al.*, 2015) (Stable Micro Systems) and a test speed of 0.2 mm s^{-1}.

The mechanical properties of the whole berry were evaluated by a Texture Profile Analysis (TPA) test. Each whole berry was individually compressed in the equatorial position using a 35 mm P/35 flat cylindrical probe (Stable Micro Systems) under 25 % deformation, with a waiting time of 2 s between the two bites and a test speed of 1 mm s^{-1} as described by (Río Segade *et al.*, 2013b). The following typical berry TPA parameters were determined: hardness (N, as BH), cohesiveness (adimensional, as BCo), gumminess (N, as BG), springiness (adimensional, as BS), chewiness (N, as BCh), and resilience (adimensional, as

BR) (Río Segade *et al.*, 2013b). As described by Letaief *et al.* (2008a): berry hardness is the force necessary to achieve a given deformation, berry cohesiveness is the strength of internal bonds making up the berry body, berry gumminess is the force necessary to disintegrate a semisolid food until ready for swallowing, berry springiness is the distance recovered during the time between the end of the first bite and the beginning of second bite, i.e., elasticity, berry chewiness is the energy necessary to chew a solid food until ready for swallowing, and berry resilience is the berry ability to regain the original position.

Taking into account that some TPA parameters can be influenced by berry size, they were also normalised according to the respective berry diameter (_diam, expressed in cm) (Río Segade *et al.*, 2013b), berry volume (_vol, expressed in cm^3) (Río Segade *et al.*, 2011a), and berry surface (_sur, expressed in cm^2) (Río Segade *et al.*, 2013b). Each texture test was applied to fifteen floated berries for each genotype (Zouid *et al.*, 2013).

7. Statistical analyses on physical, chemical and texture parameters

Statistical analyses and data visualisation concerning physico-chemical and texture data were carried out using R software (version 3.6.2; R Foundation, Vienna, Austria). In brief, boxplots were obtained through the "ggplot2" package (Wickham, 2016) and used to highlight the variability of the assessed parameters. Principal Component Analysis (PCA) was performed on berry diameter and berry texture data using the package "factoextra" (Kassambara and Mundt, 2020). Furthermore, a hierarchical cluster of texture parameters, obtained by calculating the Euclidean distance and using an average linkage criterion, was prepared with the base R package ("stats") and clustering functions ("dist", "hclust", "plot"). The hierarchical cluster is available as supplementary material to highlight the different clustering of genotypes according to the normalisation applied.

8. Genotyping, genetic and QTL mapping

A total of 190 SSR (Simple Sequence Repeats) markers, around ten per chromosome, were first tested for polymorphism on the two parents and 161 (Table S1) were selected for genotyping the entire F1 population (200 individuals). Markers monomorphic (aa) or homozygous (aa × bb) in both parents were discarded. DNA was extracted from 100 mg young leaves using Qiagen
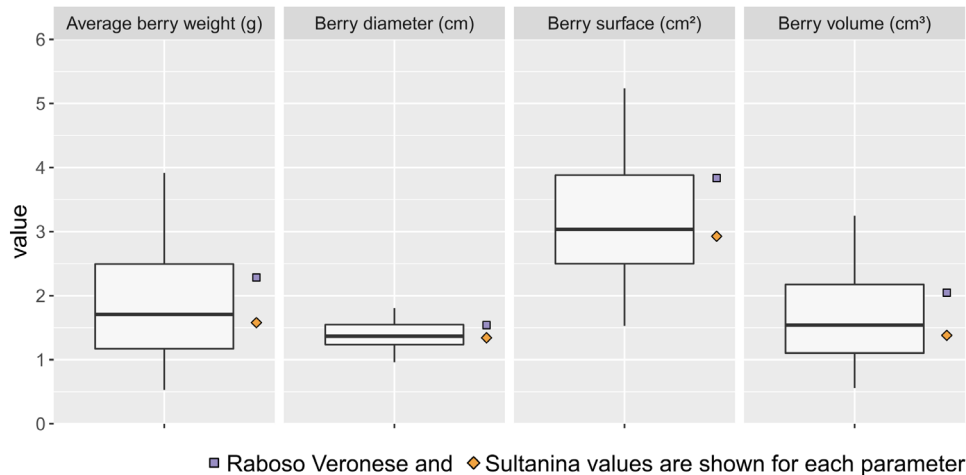
FIGURE 1. Distribution of the main physical parameters at harvest (average berry weight, berry diameter, berry surface, berry volume) of the 152 analysed samples belonging to Raboso Veronese × Sultanina F1 population.

DNeasy Plant mini-kit (Qiagen) following the manufacturer instructions. Amplifications were performed combining one, two, three or more SSR markers in the same PCR reaction, depending on the expected size of fragments and primers fluorescence. General amplification conditions were: 1x Taq buffer (Qiagen), dNTPs 200 µM, MgCl2 1.5 or 2.0 mM, Taq DNA polymerase (Qiagen) 0.5 U, primers and H_2O to 12.5 µl final volume; PCR protocol: 94 °C for 2'; then 30 cycles at 94 °C for 30", 58 °C for 30" and 72 °C for 30"; final extension 72 °C for 10'.

The genetic map was constructed using JoinMap® 4 (Van Ooijen, 2006), following the segregation of double pseudo-test cross. The independence LOD with threshold ranges between 5 and 10 was used for marker grouping and regression mapping algorithm with Kosambi's mapping function for marker ordering. LGs were numbered referring to the International Grape Genome Program (IGGP, www.vitaceae.org).

MapQTL® 6 (Van Ooijen, 2009) was used to identify QTLs linked to all skin and berry physical and mechanical characteristics previously described for both raw and normalised data. Skin colour (presence/absence of anthocyanins) was also considered as a check trait as its localisation on LG2 is well known (Walker *et al.*, 2006). Interval mapping (IM) computational method was used. Permutation tests using 1,000 permutations were done for each trait to calculate the significant genome-wide threshold of the LOD score, with a *P*-value of 0.01. The localisation of the QTLs detected was plotted with Results Charts function of MapQTL software. AGL11-

VMC7F2 SSR marker allelic combinations in the F1 population were tested for association with the measured phenotypes using the significance test of means computed with the analysis of variance (ANOVA) and multiple comparisons test (Tukey HSD; p = 0.05), using the "agricolae" package (de Mendiburu, 2020).

9. *In silico* evaluation of *VviAGL11* expression profiling and co-expression analysis during grape ripening

VviAGL11 gene expression was profiled using transcriptomic datasets previously released, i.e., the entire Corvina expression atlas (Fasoli *et al.*, 2012) and two berry specific expression surveys (Massonnet *et al.*, 2017; Fasoli *et al.*, 2018). *VviAGL11* co-expression analysis was based on Pearson's correlation coefficient and carried out using Cor.To software (Amato *et al.*, 2019). Four independent analyses were performed using two subsets of samples from the expression atlas (Fasoli *et al.*, 2012), i.e., seeds at four developmental stages and berry at three ripening stages, and two subsets of samples from the highly detailed berry transcriptomic map (Fasoli *et al.*, 2018), i.e., berry from veraison to the ripening of Cabernet-Sauvignon and berry from veraison to the ripening of Pinot noir.

RESULTS

1. Berry size characterisation

The results obtained for the parameters related to berry size (i.e., average berry weight, berry diameter, berry surface, berry volume) for floated

berries of the F1 genotypes and parents (i.e., Raboso Veronese and Sultanina cultivars) are reported in Figure 1.

F1 genotypes showed high variability in the evaluated physical and morphological characters, although the parameters studied are partly inter-dependent. The relationships among the mechanical parameters studied are widely demonstrated, as reported in Rolle *et al.*, 2012a. Thanks to the density-based berries selection, the chemical differences at technological maturity (reducing sugars, pH, acidic composition) are minimised, even if berries density is influenced by chemical composition, as demonstrated by Rolle *et al.*, 2012b. The most homogenous distribution of values corresponded to berry diameter (minimum average value 0.96 cm; maximum average value 1.80 cm). Average berry weight values (in grams) ranged from 0.53 to 3.92, while the surface ranged from 1.53 to 5.24 cm^2 and related volume ranged from 0.56 to 3.25 cm^3. For all the evaluated traits, Raboso Veronese showed higher values than Sultanina, belonging to the third and second quartile, respectively.

2. Variability of technological ripeness parameters

The parameters commonly used to assess the ripeness of grape berries were studied only for their variability. Compared to a random sampling of berries in the vineyard, density sorting allowed to reduce the heterogeneity in the ripening status naturally present inside a grape berries sample, as demonstrated by Río Segade *et al.* (2013a). Great variability was found in the F1 population concerning the technological parameters considered, as shown in Figure 2.

Many F1 genotypes were transgressive segregants for reducing sugars, pH, and titratable acidity (expressed as tartaric acid). Sultanina was characterised by higher average values of reducing sugar content (269 g/L) and pH value (3.48) than Raboso Veronese (210 g/L and 3.21 pH). In agreement with titratable acidity variability, high dispersion was also observed for the organic acid composition of grape must among F1 genotypes, with values ranging from 0.09 to 0.75 g/L for citric acid, from 4.37 to 10.73 g/L for tartaric acid and from 0.78 to 4.88 g/L for malic acid. Tartaric and malic acid contents were different in the two parents: Sultanina showed a higher tartaric acid content but much lower malic acid amount than Raboso Veronese. The amount of citric acid was similar: 0.32 and 0.34 g/L for Raboso Veronese and Sultanina, respectively.

3. Berry skin mechanical properties

The skin mechanical properties of the F1 genotypes and parents are shown in Figure 3.

Using the needle probe, the puncture test applied in this study permitted to evaluate specifically the skin characteristics, minimising the possible interference caused by the different pulp texture of F1 genotypes and parents. The main and more known parameter characterising the skin hardness, skin break force (F_{sk}), showed a high and quite regular dispersion of the values (from 0.31 to 0.89 N). Sultanina was characterised by soft skin (0.36 N, average value) whereas Raboso Veronese skin had medium hardness (0.59 N, average value), close to the median value (0.56 N). Similar distribution and parents' behaviour were detected for the skin break energy parameter (W_{sk}), which is also used to define skin hardness. The values of W_{sk} ranged from 0.21 to 0.87 mJ for F1 genotypes. Skin stiffness (E_{sk}) values ranged from 0.21 to 0.47 N mm^{-1}; both parents fell in the second quartile with values of 0.28 and 0.30 N mm^{-1} for Sultanina and Raboso Veronese, respectively. The berry skin thickness (Sp_{sk}) parameter varied between 191 and 396 µm for F1 genotypes. However, identical mean values for skin thickness were found for both parents (302 µm), classifying them in the fourth quartile.

4. Whole berry mechanical properties

The Texture Profile Analysis (TPA) test characterised the mechanical behaviour of the whole berry under a double compression using six codified parameters, as described by Letaief *et al.* (2008a). However, all these mechanical parameters are strongly influenced by berry dimensions (Río Segade *et al.*, 2013b). In the present study, berry size showed high variability among the F1 genotypes, as mentioned above. Therefore, the acquired TPA data were also normalised separately on diameter, surface, and volume. Figure 4 summarises the distribution of the genotypes for each parameter, being it parameter-dependent.

Sultanina (average berry weight of 1.58 g, volume of 1.38 cm^3) and Raboso Veronese (average berry weight of 2.28 g, volume of 2.05 cm^3) values for each parameter considered were influenced by the normalisation applied, as it can be evidenced by the different plotting in Figure 4.
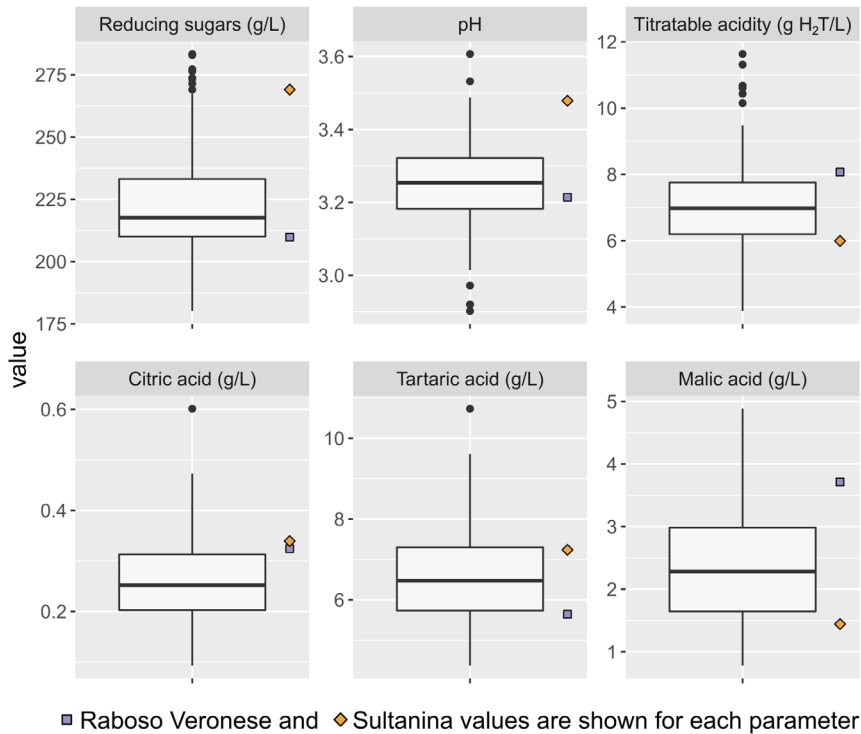
FIGURE 2. Distribution at harvest of the main grape must compositional parameters obtained from the 152 analysed samples belonging to Raboso Veronese × Sultanina F1 population.
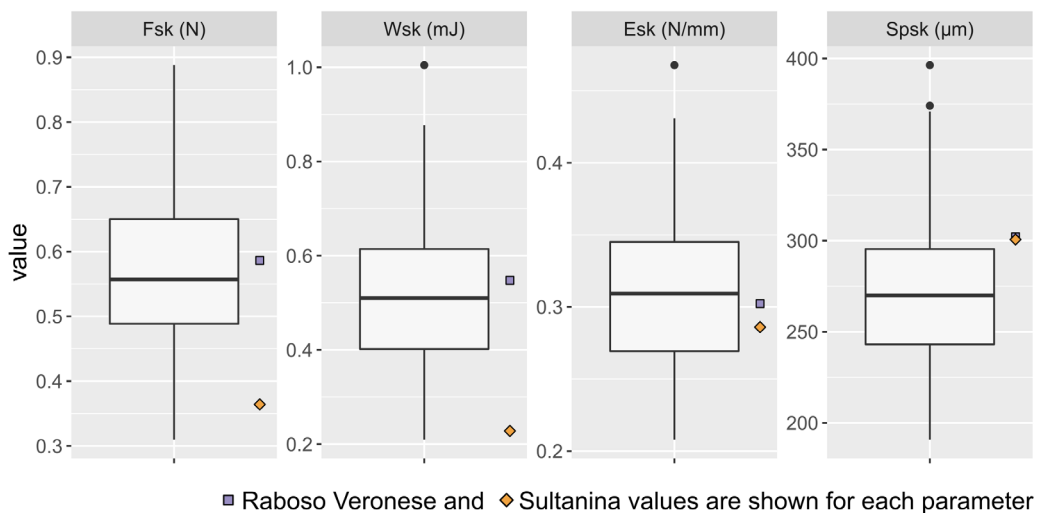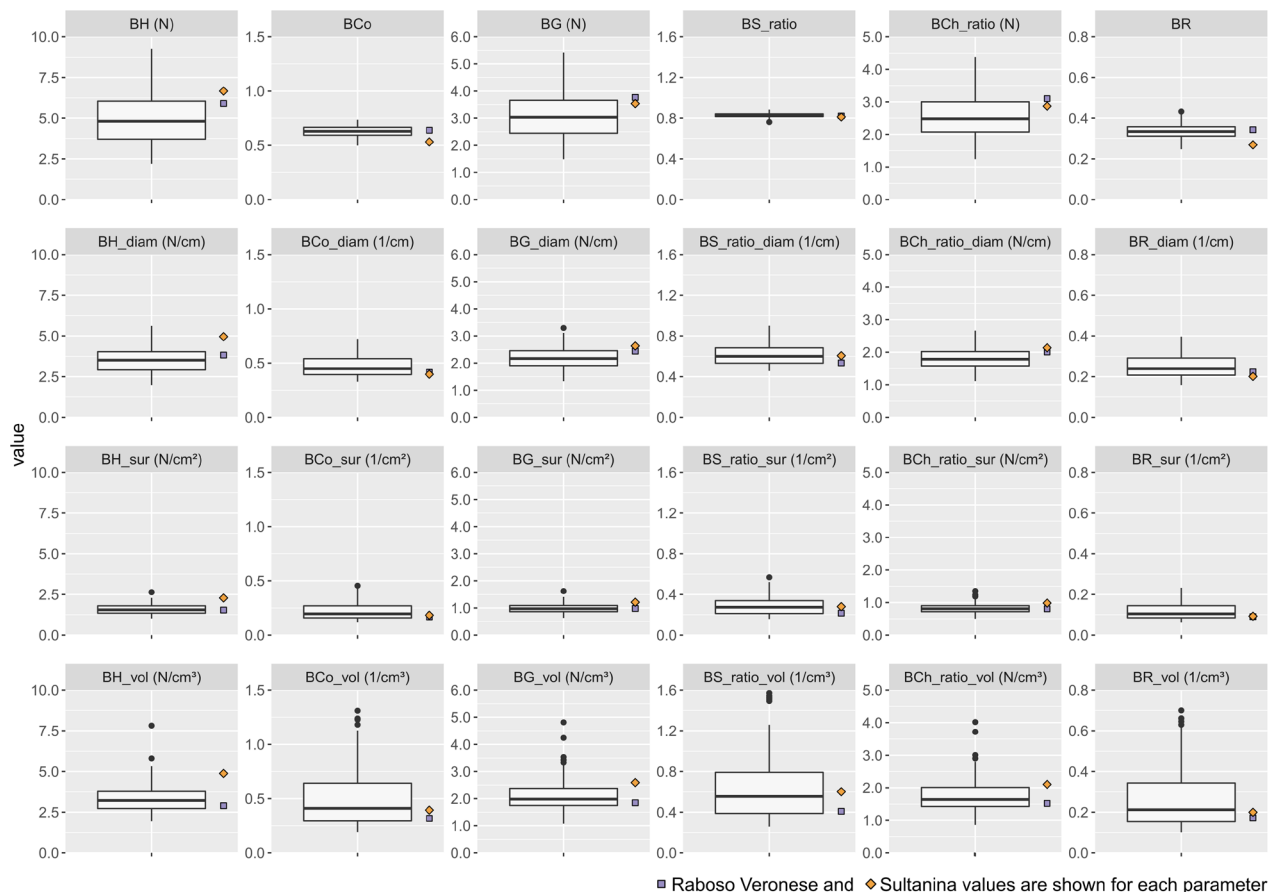

FIGURE 3. Berry skin mechanical parameters distribution at harvest in the 152 analysed samples belonging to Raboso Veronese × Sultanina F1 population.

FIGURE 4. Visualisation of whole berry TPA parameters at harvest in the 152 analysed samples belonging to Raboso Veronese × Sultanina F1 population, according to different normalisation criteria: raw data (first row), data normalised on berry diameter (second row), berry surface (third row), berry volume (fourth row).

Cluster analysis of the single data set of TPA parameters (Figure S1) also showed that different genotypes were differently grouped depending on the normalisation applied. Therefore, all four data sets were used for QTL detection on whole berry texture parameters.

5. Comparison of berry texture parameters and berry diameter

The average berry texture parameters and berry diameter for each genotype were analysed with PCA and the results are reported in Figure 5.

Two principal components were extracted (Dim1 and Dim2), explaining 54.2 % and 20.4 % of the total variance, respectively. Berry TPA parameters were separated into two main groups by Dim1, achieving absolute correlation values with this principal component greater than 0.7. In particular, berry hardness, gumminess, and chewiness parameters were characterised by correlation values with Dim1 above 0.89, coupled with low Dim2 correlation values (range from –0.03 to 0.15). Berry cohesiveness, springiness, and chewiness TPA parameters were grouped closely on the opposite side of the graph and negatively correlated with Dim1 (r < –0.7). Furthermore, berry diameter was also positively correlated with Dim1 (r = 0.75). TPA parameters confirm the link between berry hardness and its derived parameters (berry gumminess and chewiness). A close relation among berry cohesiveness, berry springiness, and berry resilience was also found, as shown at the left side of the graph.

A similar, opposing behaviour was found for berry skin break force and energy (F_{sk} and W_{sk}) parameters, and berry skin thickness (Sp_{sk}). In this case, Dim2 was the most correlated principal component, with coefficient values above 0.77 for F_{sk} and W_{sk}, and a negative value of r = –0.50 for Sp_{sk}.

6. Genetic map

The genetic map was obtained with 158 SSR markers; they were uniformly distributed, from six to eleven per chromosome, with 8.3 markers per chromosome on average (Figure 6).
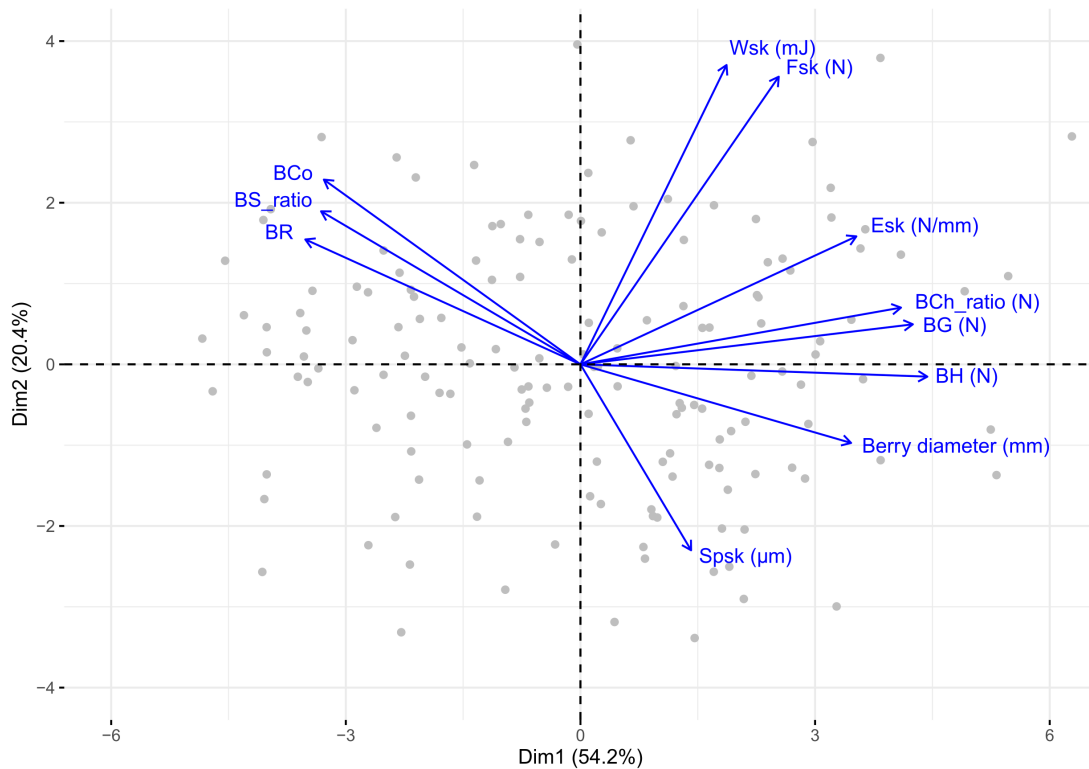
FIGURE 5. 2D-PCA loadings plot and score plot for berries TPA, berries diameter, berry skin thickness and break forces data obtained in the 152 analysed samples belonging to Raboso Veronese × Sultanina F1 population in 2017.
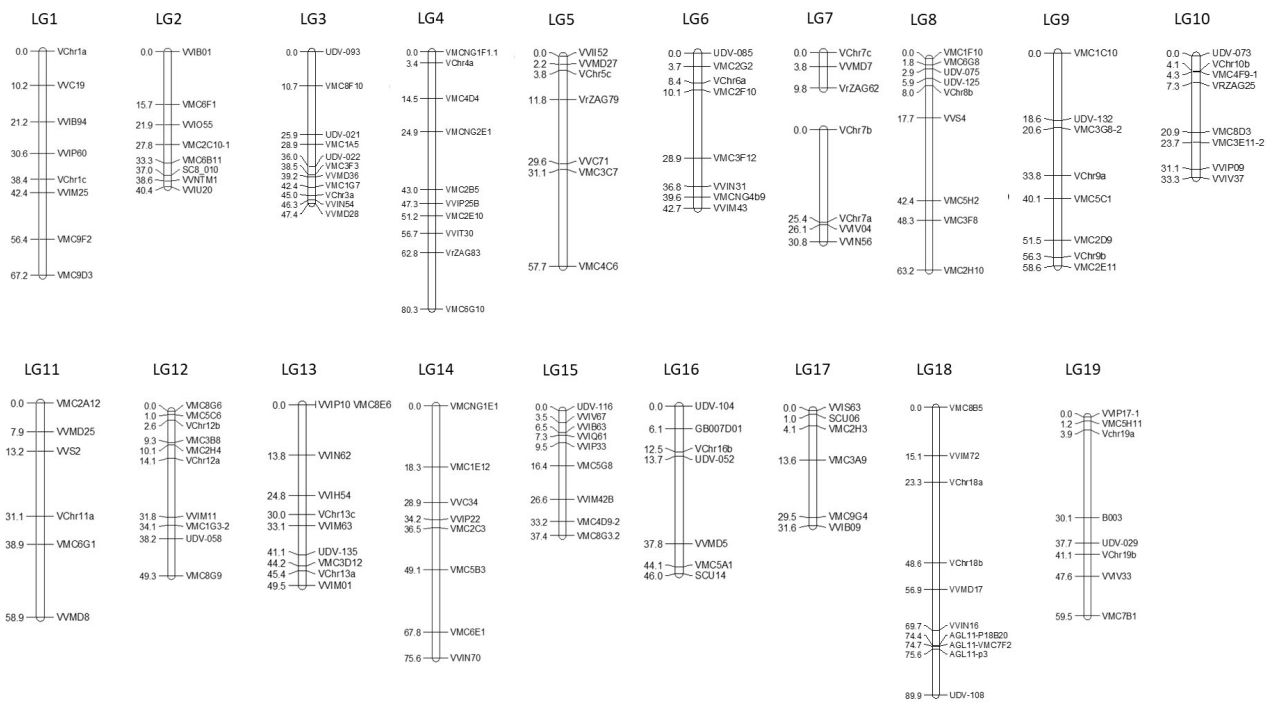

FIGURE 6. Genetic map obtained with JoinMap 4.0 software analysing the entire F1 population Raboso Veronese × Sultanina (200 individuals) with 161 SSR markers.

TABLE 1. QTL-associated SSR markers obtained with MapQTL6 software and Interval Mapping (IM) computational method for the 30 traits studied. The markers were selected over the significant genome-wide threshold of the LOD score for a P-value of 0.01. QTLs were ordered by descending LOD scores and percentages of variance explained.

Trait	LG	SSR	Significance LOD Threshold (P = 0.01)	LOD score	% of Variance Explained
Berry colour	2	VVNTM1	5	60.88	75.6
		VVIU20		60.83	75.5
		SC8_010		47.99	67.1
		VMC6B11		28.19	47.9
		VMC2C10-1		21.99	39.9
		VVIO55		13.33	26.5
		VMC6F1		11.68	23.7
		VVIB01		10.81	22.1
ABW	18	AGL11-VMC7F2	5	33.25	63.5
		AGL11-P18B20		33.17	63.4
		AGL11-p3		31.17	61.1
		VVIN16		23.8	51.4
		UDV-108		13.52	33.6
		VVMD17		10.66	27.6
BR	18	AGL11-VMC7F2	4.6	11.73	29.9
		AGL11-p3		11.7	29.8
		AGL11-P18B20		11.57	29.6
		VVIN16		6.9	18.9
	17	VMC3A9		5.03	14.1
BR_diam	18	AGL11-VMC7F2	4.8	32.29	62.4
		AGL11-P18B20		32	62.1
		AGL11-p3		31.12	61
		VVIN16		20.2	45.8
		UDV-108		11.99	30.5
		VVMD17		10.66	27.6
BR_sur	18	AGL11-VMC7F2	4.9	32.4	62.5
		AGL11-P18B20		32.16	62.3
		AGL11-p3		30.83	60.7
		VVIN16		21.07	47.2
		UDV-108		12.75	32
		VVMD17		11.22	28.8
BR_vol	18	AGL11-VMC7F2	4.7	31.2	61.1
		AGL11-P18B20		31.01	60.9
		AGL11-p3		29.74	59.4
		VVIN16		20.69	46.6
		UDV-108		12.67	31.9
		VVMD17		11.05	28.5

BS_ratio	18	AGL11-p3		8.34	23.1
		AGL11-VMC7F2	4.8	7.85	21.9
		AGL11-P18B20		7.77	21.7
		VVIN16		5.02	14.7
BS_ratio_diam	18	AGL11-VMC7F2		31.92	63.5
		AGL11-P18B20		31.77	63.3
		AGL11-p3	4.9	30.4	61.7
		VVIN16		21.62	49.4
		UDV-108		13.02	33.7
		VVMD17		11.06	29.4
BS_ratio_sur	18	AGL11-VMC7F2		29.97	61.1
		AGL11-P18B20		29.84	61
		AGL11-p3	4.9	28.29	59
		VVIN16		20.61	47.8
		UDV-108		12.77	33.1
		VVMD17		10.58	28.4
BS_ratio_vol	18	AGL11-VMC7F2		29.29	60.3
		AGL11-P18B20		29.17	60.1
		AGL11-p3	4.2	27.78	58.4
		VVIN16		20.12	47
		UDV-108		12.49	32.6
		VVMD17		10.25	27.6
BCo	18	AGL11-p3		10.35	26.9
		AGL11-VMC7F2		9.92	26
		AGL11-P18B20		9.84	25.8
	17	VMC3A9	4.8	7.07	19.3
	18	VVIN16		6.72	18.4
	17	VMC9G4		6.59	18.1
		VVIB09		6.44	17.7
BCo_diam	18	AGL11-VMC7F2		31.73	61.8
		AGL11-P18B20		31.54	61.5
		AGL11-p3	4.7	31.06	61
		VVIN16		21.2	47.4
		UDV-108		12.79	32.1
		VVMD17		10.58	27.4
BCo_sur	18	AGL11-VMC7F2		31.89	61.9
		AGL11-P18B20		31.73	61.8
		AGL11-p3	4.9	30.67	60.5
		VVIN16		21.69	48.2
		UDV-108		13.26	33.1
		VVMD17		10.99	28.3
BCo_vol	18	AGL11-VMC7F2		30.97	60.9
		AGL11-P18B20		30.82	60.7
		AGL11-p3	4.9	29.77	59.4
		VVIN16		21.16	47.3
		UDV-108		12.98	32.5
		VVMD17		10.76	27.8

				25.78	54.2
		AGL11-VMC7F2		25.78	54.2
		AGL11-P18B20		25.64	54
BH	18	AGL11-p3	4.9	25.35	53.6
		VVIN16		17.84	41.7
		UDV-108		10.66	27.6
		VVMD17		9.85	25.8
		AGL11-p3		13.33	33.2
	18	AGL11-VMC7F2		12.96	32.5
		AGL11-P18B20		12.89	32.3
		VVIN16		9.55	25.1
BH_diam		VMC3A9	4.8	7.55	20.4
	17	VMC9G4		7.34	19.9
		VVIB09		7.28	19.8
	18	VVMD17		5.95	16.5
		UDV-108		5.74	16
		VMC3A9		8.28	22.2
	17	VMC9G4		7.92	21.3
BH_sur		VVIB09	5.2	7.86	21.2
		VMC2H3		6.04	16.7
	16	GB007D01		5.76	16
		VChr16b		5.68	15.8
		AGL11-VMC7F2		8.29	22.2
BH_vol	18	AGL11-P18B20	4.9	8.29	22.2
		AGL11-p3		7.27	19.8
		VVIN16		6.99	19.1
		AGL11-VMC7F2		25.07	53.2
		AGL11-P18B20		24.95	53
BG	18	AGL11-p3	4.8	24.38	52.2
		VVIN16		17.66	41.4
		UDV-108		10.76	27.8
		VVMD17		9.74	25.6
		AGL11-p3		9.69	25.4
	18	AGL11-VMC7F2		9.52	25
		AGL11-P18B20		9.48	25
BG_diam		VVIN16	5.1	7.33	19.9
		VMC9G4		4.86	13.7
	17	VMC3A9		4.85	13.7
		VVIB09		4.83	13.6
	18	VVMD17		4.82	13.6
BG_sur		no association			
		AGL11-VMC7F2		13.57	33.7
		AGL11-P18B20		13.53	33.6
BG_vol	18	AGL11-p3	4.8	12.38	31.3
		VVIN16		10.31	26.8
		UDV-108		7.1	19.3
		VVMD17		6.3	17.4

Trait	LG	Marker		LOD	%
		AGL11-VMC7F2		24.13	53.3
		AGL11-P18B20		24.03	53.1
BCh_ratio	18	AGL11-p3	4.7	23.3	52
		VVIN16		17.05	41.6
		UDV-108		10.91	29.1
		VVMD17		9.02	24.8
		AGL11-p3		8.19	22.8
BCh_ratio_diam	18	AGL11-VMC7F2	4.9	8.15	22.7
		AGL11-P18B20		8.12	22.6
		VVIN16		6.42	18.3
BCh_ratio_sur		no association			
		AGL11-VMC7F2		14.3	36.3
		AGL11-P18B20		14.25	36.2
BCh_ratio_vol	18	AGL11-p3	4.8	13.17	34
		VVIN16		10.54	28.3
		UDV-108		7.26	20.5
		VVMD17		6.33	18.1
Fsk		no association			
Wsk		no association			
	18	AGL11-p3		5.03	14.1
Esk		GB007D01	5	5.03	14.1
	16	UDV-104		5.01	14.1
		VChr16b		5.12	14.4
		AGL11-p3		5.95	16.5
Spsk	18	AGL11-VMC7F2	5	5.84	16.2
		AGL11-P18B20		5.83	16.2

Three SSRs, namely VChr2a, UDV-011a and UDV-11b, were not located, even if belonging to the same LG2. Twenty LGs were found, because two sets of SSRs belonging to the same chromosome were kept separated, indicating low recombination information; from previous literature, we know that these SSRs map on LG7 (Doligez *et al.*, 2006). The consensus map length was 1,028.95 cM, therefore within the "reference" range between 1,000 and 1,500 cM for *Vitis* map length (Vezzulli *et al.*, 2019). SSR physical location was derived from Genoscope using the reference genome of PN40024 (Jaillon *et al.*, 2007); for SSRs missing in Genoscope, BLAT alignment analyses were performed against the same reference genome. After comparison, SSRs were in the same order in both genetic map and genome position in all LGs, except for one or few invertions found in LG5, LG9, LG12, LG13, LG16 and LG19.

7. Texture-related QTLs

QTLs obtained with IM method are shown in Table 1 and Figure 7. Significant values for QTLs were found in four LGs: 2, 16, 17 and 18 (Table 1). The significant, genome-wide threshold of the LOD scores at P-value of 0.01 spanned between 4.2 and 5.2 for the 30 traits analysed. The highest LOD score (60.88) was obtained for berry colour, strongly associated to the SSR markers close to the berry colour locus on LG2, with the highest variance explained, 75.6 %, as expected based on literature data (Walker *et al.*, 2006). Percentages of variance explaining more than 60 % were obtained for average berry weight, all normalised berry resilience data, all normalised berry springiness data, and all normalised berry cohesiveness data; LOD scores of the above-mentioned traits were also high, spanning from 29.29 to 33.25. Berry hardness, gumminess, and chewiness showed the highest variance explained
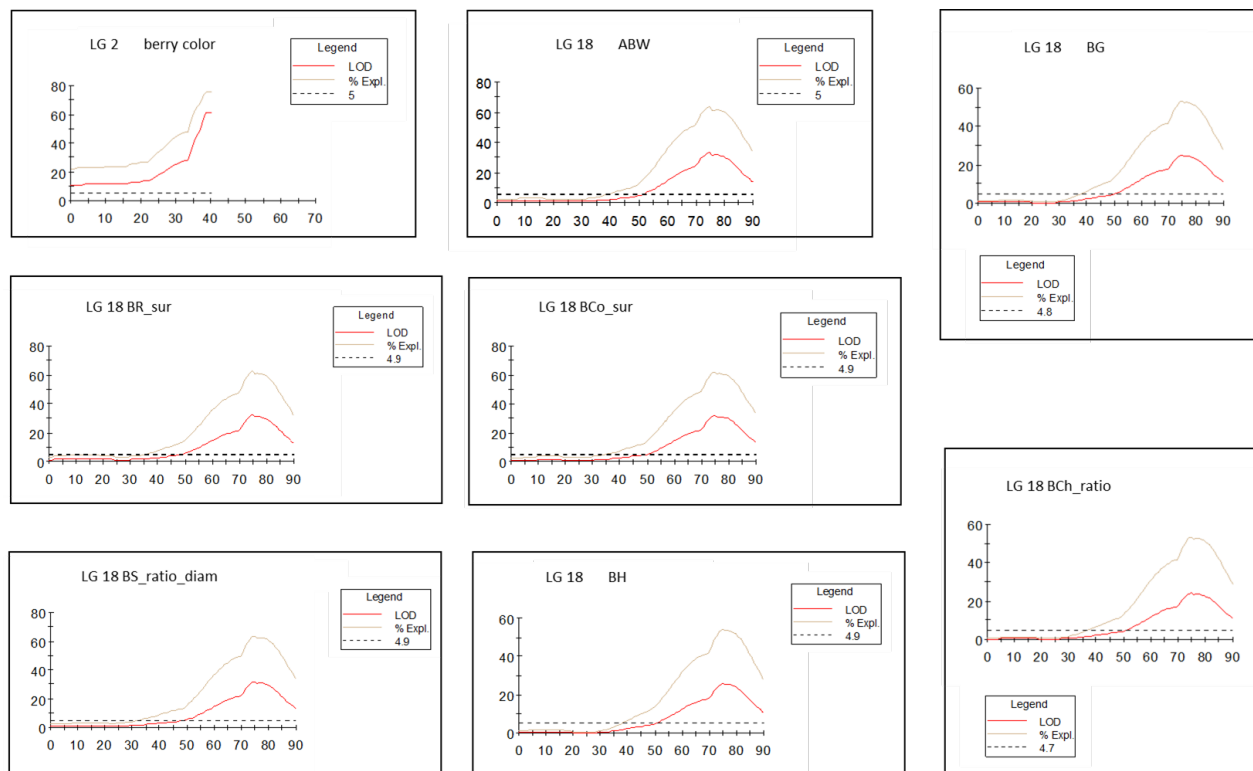
FIGURE 7. Main QTLs found for berry colour, ABW, BG, BR_sur, BCo_sur, BS_ratio_diam, BH and BCh_ratio using MapQTL6 software and Interval Mapping (IM) method. Dashed lines refer to the LOD significance threshold computed genome-wide (with P = 0.01)

on the raw data, showing still high values of 54.2, 53.2 and 53.3, respectively; LOD scores span from 24.13 to 25.78. No association was found for berry gumminess and chewiness normalised on the berry surface. All these traits co-localised in the same QTL on LG18, which is linked in turn to the SSR markers associated with *VviAGL11*, the major gene for seedlessness (Royo *et al.*, 2018). Additionally, less significant associations were found for berry resilience, cohesiveness, hardness (normalised both on the diameter and the surface), and gumminess (normalised on the diameter) on LG17, with LOD values from 4.83 to 8.28 and phenotypical variance explained from 13.6 to 22.2 %. Another minor QTL was found for berry hardness normalised on berry surface on LG 16 (LOD 5.76, variance explained 16.0 %).

Very weak associations were found for two traits linked to berry skin elasticity and thickness, with LOD scores a little bit higher than the significant threshold and phenotypic variance explained of 14.1 and 16.5 %, respectively. The QTL found for these traits is still that on LG18 associated with SSRs linked to seedlessness; another poorly supported QTL was found for skin elasticity on LG16. No association was found for skin break force and skin break energy.

8. Statistical analyses to validate the major QTL found with 2017 phenotypic data

Previous works indicate a wide variation for skin mechanical properties in the cultivated grapevine, being affected by seasonal conditions (Rolle *et al.*, 2011b). Nowadays, no study is available on our knowledge on the variability of whole berry texture characteristics (i.e., all TPA parameters) in different vintages. Thus, TPA test on floated berries belonging to 22 F1 genotypes was performed in the same experimental conditions in two different years, 2017 and 2019, while additional six genotypes were analysed only in 2019. The results obtained in both 2017 and 2019 years are reported in Figure 8 and showed that whole berry texture characteristics are highly correlated.

Concretely, although the absolute values of all parameters considered (raw or normalised) varied for each genotype depending on the year of harvest, a significant and very high correlation was found for each texture characteristic between the two years ($p < 0.001$), except for berry springiness, not normalised data, where the correlation is significant only at $p < 0.01$. R^2 values ranged from 0.42 for raw berry springiness to 0.88 for raw berry hardness. Moreover, genotype-phenotype
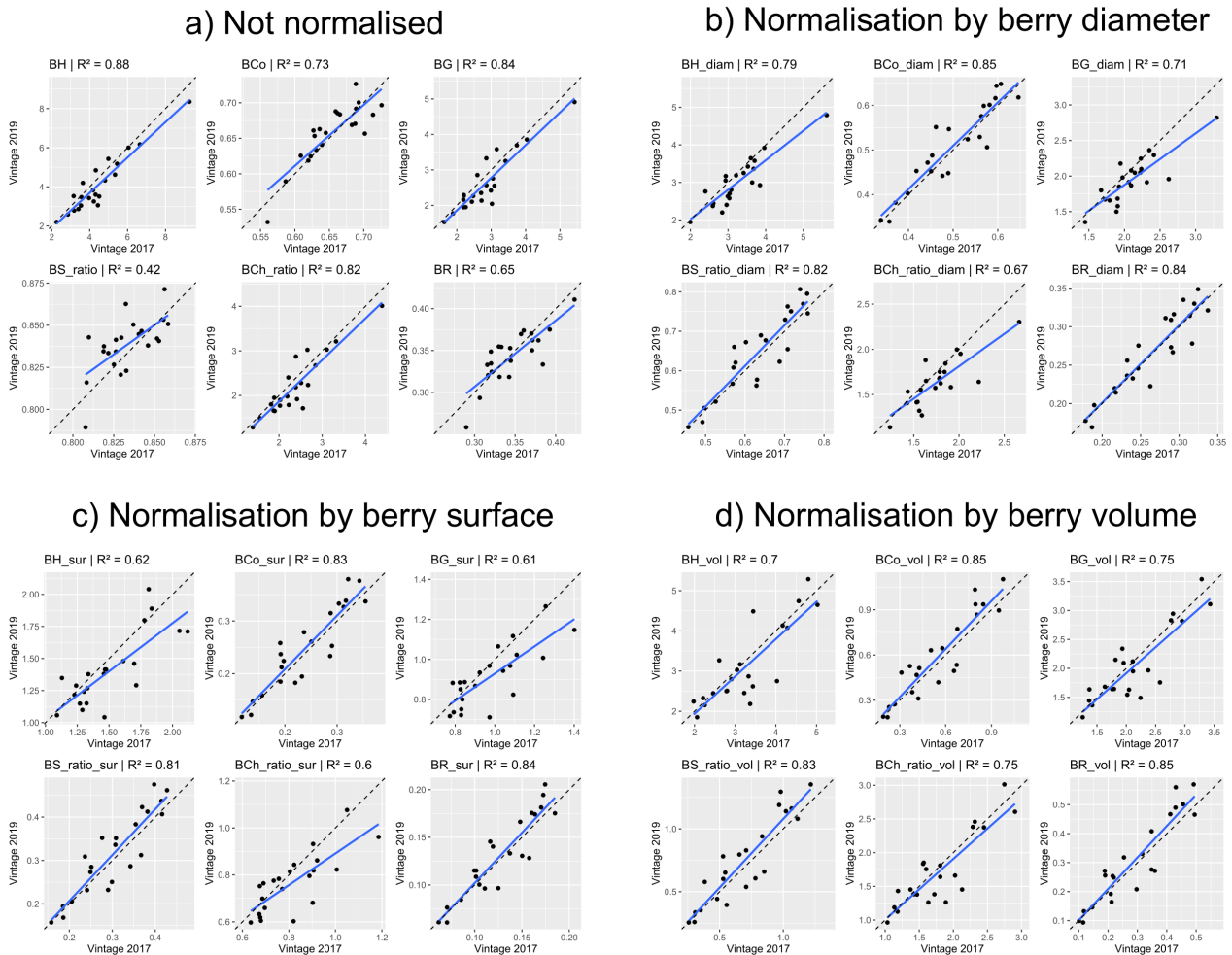
FIGURE 8. Correlation of raw or normalised TPA parameters for the 152 genotypes analysed in 2017 and the 28 genotypes analysed in 2019 of Raboso Veronese × Sultanina F1 population. Blue line: linear regression of data; dashed line: y = x.

associations were evaluated using the segregation classes obtained with AGL11-VMC7F2 SSR, the molecular marker most strictly associated to the main QTL found (Table 1). Comparisons were performed among mean TPA values of the 152 genotypes phenotyped in 2017 and mean TPA values of the subset of 28 genotypes phenotyped in 2019. The significance test showed that the differences in average data were highly significant, with $p < 0.001$, in the genotypes analysed in 2017 and also in those analysed in 2019 (Table 2). Noteworthy, the lowest values for average berry weight and berry "firmness" (BH, BG, BCh_ratio) and the highest values for berry resilience, springiness and cohesiveness normalised on the berry volume (BR_vol, Bsratio_vol, BCo_vol) showed to be associated to the segregation classes ac and bc of AGL11-VMC7F2, then to the genotypes that inherited the Sultanina allele c, linked to seedlessness (Mejía *et al.*, 2011).

All these data showed to be almost identical in the two years. Therefore, all performed statistical analyses on correlations between phenotypic data and associated genotypes using the most strictly linked marker, AGL11-VMC7F2 SSR, validate the major QTL found in 2017 with the data acquired on a subset of genotypes phenotyped in 2019.

9. *VviAGL11* expression profiling and co-expression analysis during grape ripening

To evaluate the possible involvement of *VviAGL11* in the determination of berry texture, we deeply inspected its expression profile during grapevine fruit development and in other plant organs taking advantage from previously published transcriptomic datasets. The analysis performed on the grapevine expression atlas, based on 54 samples representing different plant organs and tissues at different developmental stages

TABLE 2. Test of mean differences significance for genotype-phenotype association using aov and HSD test for average berry weight and six TPA parameters. Phenotypic data were obtained in 2017 vintage for 152 genotypes and in 2019 vintage for 28 genotypes of Raboso Veronese × Sultanina F1 population.

2017					
Segregation classes*	ac	ad	bc	bd	Sig.
No. of genotypes	41	44	30	37	
ABW	22.5 b	46.9 a	24.9 b	48.9 a	***
BH	3.86 b	6.10 a	3.64 b	5.91 a	***
BG	2.46 b	3.68 a	2.41 b	3.54 a	***
BCh_ratio	2.07 b	3.03 a	2.03 b	2.93 a	***
BR_vol	0.380 a	0.166 b	0.376 a	0.167 b	***
Bsratio_vol	0.888 a	0.425 b	0.882 a	0.423 b	***
BCo_vol	0.693 a	0.315 b	0.696 a	0.314 b	***
2019					
	ac	ad	bc	bd	Sig.
No. of genotypes	9	6	7	6	
ABW	30.5 bc	59.0 a	24.1 c	46.5 ab	***
BH	3.48 bc	5.99 a	3.07 c	4.83 ab	***
BG	2.33 bc	3.76 a	2.04 c	2.99 ab	***
BCh_ratio	1.96 bc	3.12 a	1.73 c	2.48 ab	***
BR_vol	0.333 ab	0.140 c	0.439 a	0.210 bc	***
Bsratio_vol	0.783 ab	0.345 c	1.059 a	0.536 bc	***
BCo_vol	0.629 ab	0.265 c	0.849 a	0.406 bc	***

* AGL11-VMC7F2 SSR marker genotypes allelic combinations from the two parents: Raboso Veronese (a, b) and Sultanina (c, d). Sultanina allele c is linked to seedlessness trait (Mejía *et al.*, 2011).

Sig. = all p < 0.001 (***). Post-hoc: Tukey HSD test (p = 0.05).

(Fasoli *et al.*, 2012), clearly showed that the highest expression of *VviAGL11* occurs in seed (Figure 9a).

In particular, *VviAGL11* is mainly expressed in seed at fruit set and the expression level declines throughout seed development, well supporting the major role of this MIKC-Type MADS-box factor in the control of ovule morphogenesis and seed coat differentiation (Royo *et al.*,

2018). Nevertheless, *VviAGL11* showed a high expression also during berry development with a very low expression around veraison followed by a steady increase during ripening, peaking at the second stage of postharvest berry withering (PHWII) (Figure 9a). Intriguingly, *VviAGL11* showed a higher expression in the skin than in the pulp during the last stages of berry ripening. A slight expression of *VviAGL11* was also found in flower organs and rachis after veraison, whereas the expression was very low or absent in all the other grapevine samples (Figure 9a).

The *VviAGL11* expression trend, retrieved from the berry transcriptomic dataset obtained in ten grapevine varieties (Massonnet *et al.*, 2017), confirmed the general higher expression level during early development and ripening stages, albeit with some differences among genotypes (Figure 9b). Also, thanks to the recent transcriptomic dataset consisting of a weekly collection of Cabernet-Sauvignon and Pinot noir berries from pea size to harvest during three consecutive years (Fasoli *et al.*, 2018), it was possible to ascertain with high precision that *VviAGL11* reached its minimum expression level at 10 days after veraison and featured an up-regulation about three weeks after veraison (Figure 9c).

To investigate the transcriptional relationships of *VviAGL11* in the seed and the berry during ripening, a co-expression analysis was performed separately on the four stages of seed development and the three ripening stages of the berry of the Corvina expression atlas (Supplementary Dataset S1 online). Genes that were highly co-expressed with *VviAGL11* in seed were characterised by very high Pearson correlation coefficients (216 genes; r > 0.9), whereas genes highly co-expressed with *VviAGL11* in berries during ripening were characterised by lower Pearson correlation coefficients (only 4 genes > 0.9). Interestingly, no genes were shared between the top-200 genes highly co-expressed with *VviAGL11* in seed and the top-200 genes highly co-expressed with *VviAGL11* in berry, strongly suggesting that different genes are targeted by *VviAGL11* and that a different role is exerted by this factor in the two organs.

The co-expression analysis of *VviAGL11* in berry during ripening was further performed using the post-veraison samples of Cabernet-Sauvignon and Pinot noir transcriptomic datasets (Fasoli *et al.*, 2018). The comparison of the list of the top-200 highest co-expressed genes in Corvina,

FIGURE 9. a) *VviAGL11* expression profile in the *V. vinifera* cv. Corvina atlas in 54 organs/tissues during development (Fasoli *et al.*, 2012). Y: Young, WD: Well developed, FB: Flowering begins, F: Flowering, FS: Fruit set, PFS: Post-fruit set; V: Veraison, MR: Mid-ripening, R: Ripening, PHWI (II-III): Post-harvest withering after 1 (2–3) months, S: Senescencing, G: Green, Stem-W: Woody stem, L: Latent, Bud-W: Winter bud, S: Swell, B: Burst, AB: After-burst. b) *VviAGL11* expression profile in berry pericarp during the development of ten varieties (Massonnet *et al.*, 2017). c) *VviAGL11* expression profile during the development of Cabernet-Sauvignon berry pericarp collected in 2012 (Fasoli *et al.*, 2018).
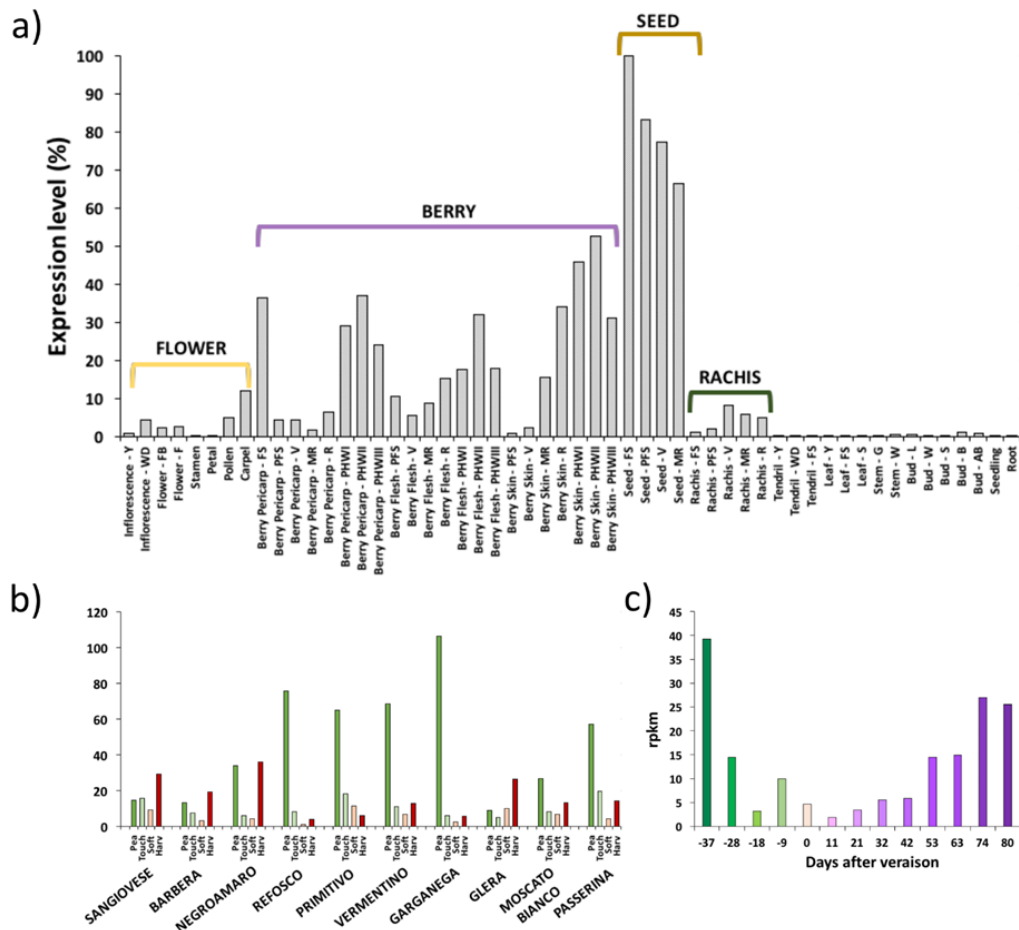
Cabernet-Sauvignon and Pinot noir, revealed some common putative metabolic partners of *VviAGL11* (Supplementary Dataset S1 online). By looking at the highest co-expressed genes (Pearson coefficient ≥ 0.8) we found 31 genes in Corvina, 41 in Pinot noir and only three in Cabernet-Sauvignon (Supplementary Dataset S1 online). The potassium-sodium symporter HKT2 was found among the top-ranked genes in all the three lists (Table 3).

A methyl-Jasmonate esterase (*VIT_00s0253g00080*) and a lipase GDSL (*VIT_09s0002g00640*) were found in the list of Corvina and Pinot noir, and the R2R3MYB transcription factor Myb 61 was highly co-expressed with *VviAGL11* commonly in Pinot noir and Cabernet-Sauvignon (Table 3).

DISCUSSION

Berry sorting by density flotation permits reducing the sample heterogeneity in terms of ripeness in both table and wine grape varieties (Río Segade *et al.*, 2013a; Zouid *et al.*, 2013). In particular, Fournand *et al.* (2006) reported for wine grapes that the difference in the total sugar content of Shiraz berries belonging to two consecutive density classes (i.e., classes differing from each other by 10 g/L NaCl concentration in the flotation solutions) was ~ 17 g/L (i.e., 1 % v/v potential alcohol). Similar differences were reported by Torchio *et al.* (2010) and Rolle *et al.* (2012a) on Barbera and Nebbiolo grapes, respectively, but only for some density classes. These authors found that the difference in berries sugar content throughout the density classes was not constant, but it varied

TABLE 3. Top-ranked (Pearson coefficient ≥ 0.8) genes commonly co-expressed with *VviAGL11* in at least two varieties.

Gene ID	Functional annotation	Corvina	Cabernet-Sauvignon	Pinot noir
VIT_11S0103G00010	*Potassium-sodium symporter HKT2*	*	*	*
VIT_09S0002G00580	*Lipase GDSL 3*	*		*
VIT_00S0253G00080	*Methyl jasmonate esterase*	*		*
VIT_14S0060G00240	*Myb domain protein 61*		*	*

and decreased by increasing the density of the floating solutions. This different trend, within the same variety, was linked not only to total sugar content but also to vineyard location (Rolle *et al.*, 2012b). The sugar content of berries belonging to different density classes varied similarly also when density flotation was applied to berries with larger volumes (i.e., on table grape varieties), as demonstrated on Italia (Río Segade *et al.*, 2013a), Muscat of Hamburg (Rolle *et al.*, 2015), and Red Globe and Crimson Seedless (Río Segade *et al.*, 2013b) grapes. However, when berries of different varieties even though belonging to the same density class were compared, the chemical composition in terms of technological parameters was different. This is in agreement with the results of this study, which clearly showed that density flotation, and subsequent selection of only one density class, separated F1 genotypes and their parents in different quartiles concerning chemical composition (reducing sugar content, pH, titratable acidity, organic acid composition), berry characteristics (weight, size, and seedless character) as well as skin mechanical properties. Therefore, berry sorting by density flotation has shown to be a strategic approach when managing many and highly variable genotypes because, irrespective to the quantification of specific molecules classes, the method introduces a common and more general criterion for berries selection of all genotypes. The wide variability present in our F1 population was confirmed by the transgressive segregation displayed also for highly similar traits in the two parents, like what found by Carreño *et al.* (2014) and Jiang *et al.* (2020) studying other F1 progenies.

Our results point to one major QTL for all texture traits; the confidence interval was difficult to define because this QTL is located at the end of LG18 and only a few SSRs were available per chromosome. Our data provide well-supported information on the co-localisation of average berry weight and all TPA traits on the same QTL on LG 18. The higher impact of whole berry mechanical properties can be due to the different response of each genotype under double compression test, as previously evidenced for grape cultivars (Río Segade *et al.*, 2011b). This study found a link also for berry skin thickness on this major QTL, with a LOD score a little bit higher than the significance threshold. Weak associations with lower percentages of explained variance were found for berry skin elasticity on the same major QTL on LG18 and along LG16.

The suspicion that seeds may affect berry hardness measurements, making the grapes harder, is diluted by our data: the seedless Sultanina variety showed berries harder than Raboso Veronese. This result is in agreement with Migicovsky *et al.* (2017), that grapes from the Middle East are firmer than those from West Europe, because of human selection for table and wine grapes, respectively. Moreover, TPA data normalisation on berry diameter, surface, and volume reduces the influence of berry dimensions. Berry resilience, springiness, and cohesiveness are highly correlated with each other, as demonstrated by PCA analysis, because they are linked to the ability of the berry to return to its original form after compression. For these parameters, normalised data found stronger associations than raw ones, independently on the type of normalisation adopted. LOD scores were around three-four times higher with normalised TPA parameters and percentages of explained variance were more than double compared to raw data. Consequently, berry dimensions affected raw TPA data in performing the associations with QTLs.

Berry hardness, gumminess, and chewiness assess berry consistency (i.e., firmness) and grouped together in PCA analysis. For these traits, raw TPA data showed two-three times higher LOD values and the percentage of phenotypic explained variance was also 1.5–2.5 times higher than the normalised ones. Even for berry gumminess and chewiness, no marker-trait association was found for TPA parameters normalised on the berry surface.

Therefore, berry consistency was independent of berry size. Our results are partially consistent with those obtained by Carreño *et al.* (2014) and Correa *et al.* (2016) and agree with Jiang *et al.* (2020). In particular, the methods developed by Carreño *et al.* (2014) and used also by Wang *et al.* (2020), define berry "firmness" as values expressed in N required for a 20 % deformation of the berry grape. This mechanical variable can be associated with berry hardness evaluated in the present study calculated using deformation of the berry equal to 25 %. Compared to previous studies, the novelty of the present one is the different approach to grape phenotyping because we used the berries density as the main factor for selecting the samples for subsequent analyses. Reducing sugars measurement was used in the vineyard only for a rough determination of the harvesting time, but hereafter berries selection was based on flotation and therefore on density, a concept that normalises the berry density characteristics by incorporating more chemical-physical parameters.

Furthermore, flotation is a non-destructive sorting method, which allows the same berries to be subjected to TPA avoiding the need to use an additional sample, presumably similar but not verified. In this way, the effect of different concentrations of the compounds present in the must, especially sugars and acids, is cumulated and balanced. In our F1 the variability in the chemical composition of the berries belonging to the same density class was high.

The average berry weight associates with the SSRs of *VviAGL11*, because the development of normal seeds stimulates berry growth, probably through growth regulators produced by seeds (Doligez *et al*., 2013), whereas seedlessness affects the formation of small or very small berries (Royo *et al*., 2018). Similar results were obtained working on F1 populations with a seedless background (Doligez *et al.*, 2002; Cabezas *et al.*, 2006; Costantini *et al.*, 2008; Mejía *et al.*, 2011). Interestingly, other QTLs, independent from seedlessness and located on LGs different from LG18, were proposed affecting berry dimensions (Ban *et al.*, 2016; Doligez *et al.*, 2013).

In previous studies, doubts about the reliability of some common QTLs identified for different berry traits were explained as possibly related to common ancestors in the parentage lineages analysed (Carreño *et al.*, 2014). This is not the case for our F1, because Raboso Veronese is a local wine grape cultivar of the Veneto region; its parents are Raboso Piave and Marzemina bianca

(Crespan *et al.*, 2006, Crespan *et al.*, 2015), two other local varieties having no genetic proximity with Sultanina. Therefore, the main QTL that we found to be related to texture traits is not affected by inbreeding effects and appears to be strong and solid.

All six TPA parameters listed in Table 2 were divided into two statistically different groups (Figure 5). The lowest values for average berry weight and berry firmness (BH, BG, BCh_ratio) and the highest values for berry resilience, springiness and cohesiveness (BR_vol, Bsratio_vol, BCo_vol) showed to be associated to segregation classes ac and bc of AGL11-VMC7F2 marker, then to the genotypes that inherited the Sultanina allele c, linked to seedlessness. While the association between average berry weight and AGL11-VMC7F2 was expected (Costantini *et al.*, 2008), it is a novelty for berry texture features.

Our major QTL is also the only one recurring in three previous works (Carreño *et al.*, 2014; Correa *et al.*, 2016; Jiang *et al.*, 2020), where instrumental quantitative analyses and not sensorial analyses were used for texture measurements. Having found, this time with clearer evidence, a main QTL for berry texture on LG18 on the same chromosomal interval using a different and innovative approach for phenotyping, it is the counter-proof that in that portion of the grapevine genome there is, with high probability, the main key gene to understand the texture characteristics of grapes.

About *AGL11*, the UNIPROT database (https://www.uniprot.org/uniprot/Q38836) suggests that the related protein, Agamous-like MADS-box protein AGL11, is a probable transcription factor. It is required, together with TT16/AGL32, for the maternal control of endothelium formation, which is essential for female gametophyte development and fertilisation, and seed formation. So, *VviAGL11* biological role involves the ovule development, with pleiotropic effects on other traits linked to the poor development of seeds, like berry dimensions. Studies on *VviAGL11* monitored its expression at fruit set and pea-size stages in seeds or seed traces (Royo *et al.*, 2018), but information is lacking if this gene plays a role on berry ripening at further fruit developmental stages. By inspecting transcriptomic databases, we could trace the expression of *VviAGL11* during the entire berry development and noticed that it raises after veraison in pericarp tissues with a steady up-regulation during ripening. Co-expression analyses highlighted possible transcriptional

relationships between *VviAGL11* and other genes, different in the seed and the berry, and strongly suggest that different biological functions are exerted by *VviAGL11* in the two grapevine organs.

To infer a role of *VviAGL11* in the definition of the berry texture characteristics at harvest, we looked at the function of the genes most highly co-expressed with *VviAGL11* in berry pericarp after veraison, representing either its potential targets or partners in the same metabolic/developmental process. Intriguingly, transcriptional analyses revealed high co-expression values of *VviAGL11* with the *VIT_14S0060G00240* gene encoding an R2R3MYB transcription factor functionally annotated as MYB61. This MYB member belongs to the previously defined S13 subgroup of the MYB family (Wong *et al.*, 2016) that, in addition to AtMYB61, includes AtMYB50, AtMYB55, and AtMYB86, all involved in secondary cell wall formation and lignin biosynthesis in *Arabidopsis* (Huang *et al.*, 2015; MacMillan *et al.*, 2017; Rao and Dixon, 2018; Taylor-Teeples *et al.*, 2015). AtMYB61, the best characterised member of this subgroup, was shown to have a role in the synthesis of the seed coat rhamnogalacturonan or the appropriate deposition of this extracellular polymer (Penfield *et al.*, 2001), and to be a positive regulator of secondary cell wall biosynthesis (Rao and Dixon, 2018). Target genes of AtMYB61, such as a secondary wall repressor, a pectin methylesterase and a caffeoyl CoA 3-O-methyltransferase of monolignol biosynthesis have been identified (Romano *et al.*, 2012). Consistently, in rice the closest homolog of *AtMYB61* targeted a *CAD2* (involved in lignin synthesis) and a *CESA* (involved in cellulose deposition) gene, supporting a role in the coordination of both cellulose and lignin biosynthesis in secondary wall formation (Huang *et al*., 2015). Overall, these pieces of evidence support the hypothesis that *MYB61* could be involved in texture changes during late ripening through the control of cell wall metabolism in berry pericarp.

The identification of the high-affinity K+ potassium transporter 1 (*VviHKT1;1*), as one of the most highly co-expressed with *VviAGL11* in berries, strongly suggests that *VviAGL11* could be involved in the maintenance of intracellular K+/Na+ homeostasis. The *VviHKT1;1* has been recently characterised by Henderson *et al.* (2018)

as a major gene involved in the control of Na+ exclusion in grapevine roots. We hypothesise that *VviHKT1;1* could also have a role in berry in the maintenance of the correct cytosolic ion ratio, that in turn may control aspects like osmoregulation, enzyme activation and cell expansion, all of crucial importance during ripening.

Noteworthy, *VviAGL11* resulted highly co-expressed with a MeJA (methyl Jasmonate) esterase, a hydrolase that converts the volatile MeJAs to JAs (Jasmonates), being well-recognised lipid-derived stress phytohormones involved in plant responses to biotic and abiotic stresses (Creelman and Mullet, 1995; Hettenhausen *et al.*, 2013; Huang *et al.*, 2017; Wang and Wu, 2013). As a methyl ester of JA, MeJA has been postulated to be the inactive or storage form of JA (Wasternack and Strnad, 2016; Westfall *et al.*, 2013), and the demethylation process would be, therefore, the first step to activate MeJA. Interestingly, it has been observed that JAs induce leaf senescence activating senescence-associated genes and chlorophyll catabolism-related genes (CCGs), and repressing photosynthesis-related genes (e.g., Chlorophyll A/B Binding Protein1) (Huang *et al.*, 2017; Qi *et al.*, 2015; Zhu *et al.*, 2015). Furthermore, JAs stimulate organ abscission by affecting the metabolism of cell wall polysaccharides (Miyamoto *et al.*, 1997). Thus, in the context of berry ripening, JA could be involved in senescence cellular processes associated with ripening, including cell wall polysaccharides rearrangement.

A strong co-expression between *VviAGL11* and a *GDSL lipase*, an esterase/lipase hydrolytic enzyme with important functions in plants such as the regulation of plant cell wall components (de la Torre *et al.*, 2002), was also found. Interestingly, the involvement of two GDSL-like lipases in the regulation of plant systemic resistance associated with ethylene and auxin signalling was reported (Kim *et al.*, 2014; Lee *et al.*, 2009). Although far to be conclusive, altogether this data strongly suggests that *VviAGL11* targets genes involved in lipid and hormone metabolism, transport and gene expression regulation, that in turn may impact cell wall polymer rearrangement and texture features of berry at ripening.

Concluding, previous studies showed how difficult it is to determine the genetic control of berry texture. Our results clearly underline the major role of a QTL located on LG18. This information contributes towards the development of additional tools supporting the early selection of desirable

traits in newly bred grapevine varieties. No QTLs strongly associated with berry skin hardness and thickness were found. Large gaps (>20 cM) in some LGs (LG7b, LG8, LG18, LG19) and map length (1028.946 cM) close to the inferior limit of the reference range for *Vitis*, indicate that the low number of markers used (158) was not enough to obtain a homogeneous saturation for wide QTL detection.

Berry sorting through density flotation showed to be a very advantageous and non-destructive method for highly heterogeneous genotypes population characterisation, in fact, it enables to apply TPA on berries belonging to the same density class, regardless of specific molecules classes quantity. Destructive methods were used in previous studies based on berries ripeness evaluation through crushing and must analysis, which supposes that the other berries used for the following TPA analyses, even though from the same clusters, reached more or less the same ripening stage. Samples heterogeneity, and therefore weak phenotyping data, can be regarded as one of the reasons for the low explained percentage of phenotypic variation associated to the QTLs found in previous studies. Our phenotypic method significantly improved berry texture studies intended be searching for QTLs associated with these traits.

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