GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genetic dissection of agronomic traits within a segregating population of breeding table grapes

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Genet. Mol. Res. 12 (2): 951-964 (2013)
Received October 18, 2012
Accepted January 16, 2013
Published April 2, 2013
DOI http://dx.doi.org/10.4238/2013.April.2.11

ABSTRACT. Grapes (*Vitis vinifera*) are of great economic importance worldwide. We genetically dissected a table grape breeding population, using hidden Markov models (HMM) applied to quantitative trait locus (QTL) analyses. We evaluated and dissected the following traits: total number of clusters, leaf score, peduncle length, cluster length, number of berries, weight of 10 berries, average seed number, nature of seeds, berry skin color, soluble solids, titratable acidity, and berry anthocyanin. A consensus map was developed with 255 SSR molecular markers, ordered into 19 linkage groups. The observed length of this map was 1871.4 cM, with 89.7% coverage. QTL were identified using interval mapping with HMM. The number of QTL detected for each trait varied between 1 and 8, reflecting the quantitative nature of these traits. The percentage of variation explained by these QTL was small, varying between 1.56 and 11.98%. We found QTL across linkage groups 2, 7, 12, 13, and 14 for berry anthocyanin.

Key words: *Vitis* sp; QTL analysis; Agronomic traits

INTRODUCTION

Agronomic traits are the most commonly measured but poorly understood characteristics in grapevine. It is an extremely complex quantitative character both because of the number of segregating loci controlling all of the traits involved in yield and of the influence of non-genetic factors (Fanizza et al., 2005).

A multitude of investigations have been conducted on the inheritance of yield and yield components in fruit tree species using the classic biometrical approach, and, while these studies have been useful for making predictions on the genetic progress occurring in plant breeding programs, they have not provided information on individual genes (or group of genes) influencing quantitative trait loci (QTL) (Fanizza et al., 2005).

However, genetic studies for quantitative traits have recently been greatly facilitated by the development of molecular markers. Modern strategies for the investigation of these genes controlling these quantitative traits are based on the construction of genetic linkage maps, using different DNA markers.

To date, a number of QTL analyses have been reported in fruit tree species (Conner et al., 1998; Dirlewanger et al., 1999; Garcìa et al., 2000; King et al., 2000; Wang et al., 2000; Ballester et al., 2001; Quilot et al., 2004).

Grapes (*Vitis vinifera*) are of great economic importance worldwide. There are about 60 *Vitis* species in the world, with greatest concentration in Asia and North America and 5000 known cultivated varieties within the species *V. vinifera* (http://www.ars-grin.gov/). All *vinifera*-based cultivars lack resistance to all known pests and diseases except recently reported 'Kishmish vatkana' and 'Dzhandzhal kara', which do not allow powdery mildew to proliferate (Coleman et al., 2009). All cultivated *vinifera*-based varieties have a wide range of differences in morphology and fruit characteristics owing to their extreme heterozygous nature, which makes it a challenging organism for breeding due to inbreeding depression and linkage drag.

There are many studies that have focused on the development of genetic population diversity in morphology with the aim to identify genomic regions that are linked to the traits of interest to isolate genes. The majority of these studies have concentrated on disease resistance introgressed from native North American species (Barker et al., 2005; Riaz et al., 2008; Marguerit et al., 2009; Coleman et al., 2009). Other studies focus on developing genetic maps and identifying QTL for agronomic traits such as seedlessness, berry size, fruit ripening, fruit quality, and cluster structure (Doligez et al., 2002; Fanizza et al., 2005; Mejía et al., 2007). Costantini et al. (2007) identified clusters of QTL for different berry and phenology traits associated with each other, suggesting that the involved genes are either linked or have pleiotropic relationships. Mejía et al. (2007) found that the existence of a major QTL for stenospermocarpic seedlessness was confirmed in a specific linkage group (LG) in a segregating population of table grapes, derived from a cross of Ruby Seedless x Thompson Seedless, where this QTL was associated with pleiotropic effect on berry size or weight and on ripening date, and it was not possible to dissociate seedlessness and small berry size.

Cabezas et al. (2006) conducted a genetic analysis of seed and berry weight in grapevine, and they detected 12 QTL responsible for the variation in seedlessness and berry weight in the 'Dominga' x 'Autumn Seedless' F_1 progeny. Among them, 2 linked regions on LG 18 showed effects on all 3 traits considered, namely berry weight, seed number and seed fresh weight, with one of them having a major effect on QTL. VMC7F2 microsatellite, close to this QTL, was shown to be a useful tool for seedlessness breeding.

Doligez et al. (2010) studied QTL for fertility in table grapes (*V. vinifera* L.), and they found a main QTL on LG 5 in the F_1 progeny studied, which was also the most stable one across years. It explained up to 18.5% of total intra-cross phenotypic variance. Three other QTL, one on LG 5 and two on LG 14, were repeated over years but were found in a single progeny, where the authors' results suggest that the QTL effect was mainly additive.

However, there is no published study using agronomical traits in QTL analysis of production and quality of fruits, to identify a suitable set of genotypes for use as potential parents in ongoing breeding program. It is essential to identify genomic regions that can affect these traits, which would provide important information to be effectively used as parents for future crosses in a successful breeding program, using marker-assisted selection.

In the use of hidden Markov models (HMM), an important aspect of the QTL mapping problem is the treatment of missing genotype data. If complete genotype data were available, QTL mapping could be reduced to the problem of model selection in linear regression. However, considering loci in the interval between the available genetic markers, genotype data are inherently missing. Even with typed genetic markers, genotype data are seldom complete, as a result of failures in the genotyping assays or because of economic limits (e.g., in the case of selective genotyping, where only individuals with extreme phenotypes are genotyped) (Broman and Sen, 2009).

In standard interval mapping, one deals with the missing QTL genotype data by performing maximum likelihood under a mixed of models, using a version of the EM algorithm. Central to this approach is the calculation of the distribution of QTL genotypes conditional on the observed multipoint marker data. In the multiple imputation approach to QTL mapping, one must be able to simulate from the joint distribution of the genotype at position on a grid along a chromosome, conditional on the observed marker data (Broman and Sen, 2009).

Here, we present the results of genetic dissection, using HMM applied to QTL analyses, in a table grape breeding population that was obtained by a cross between hybrid genotype D8909-15 (*V. rupestris* x *V. arizonica*/*girdiana*) resistant to the dagger nematode and Pierce's disease (PD) and 'B90-116', a susceptible *V. vinifera* cultivar with desirable fruit characteristics. Our objective was to extend our knowledge of the genetic determinism of the variation of agronomic traits in table grape population for future use in marker-assisted selection, by locating some of the genomic regions involved in this variation. The QTL region appears to have role in controlling trait expression.

MATERIAL AND METHODS

Mapping population

An F_1 mapping population "AT0023" of 203 individuals was developed from a cross between D8909-15 (female) and *V. vinifera* B90-116 (male; a seedless table grape cultivar), which were highly resistant and susceptible to PD, respectively. D8909-15 was derived from the cross *V. rupestris* A. de Serres x *V. arizonica* b42-26 (Figure 1). The maternal grandparent line was also highly susceptible to PD while the paternal grandparent was strongly resistant to PD. Of the 203 progenies, 111 were evaluated for agronomic traits and used for construction of genome maps and QTL analysis.

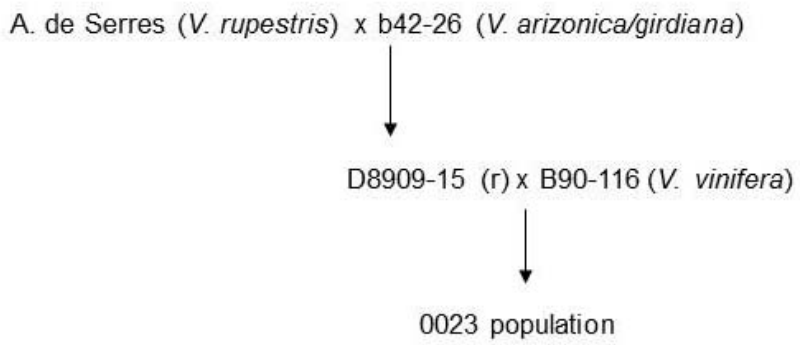

Figure 1. Description of the crosses and the relationships among the different parents that were used to develop the mapping population (r = Pierce diasease resistant).

Agronomic traits

In September 2009, 5 clusters were randomly chosen from each genotype, and the following qualitative and quantitative evaluations were made. A) Total number of clusters per genotype was determined. B) Leaf score was based on visual comparison of the attributes of each parent, giving scores of 1 to 5. The D8909-15 parent, which appeared more *V. rupestris*-like, was given a score of 1 (based on the depth and width of the petiolar sinus) and the B90-116 *V. vinifera* parent was given a score of 5 (also based on the shape of the petiolar sinus). The progenies were assigned to scores based on where they fell within this continuum. C) Peduncle length was obtained by measuring the peduncle on each of the collected clusters. D) Cluster length was obtained by measuring the collected clusters from the tip to the peduncle insertion. E) The number of berries was obtained from each of the collected clusters. F) The weight of 10 berries was obtained by weighing on an electronic scale. G) The average seed number was determined from 10 berry samples. H) The nature of seeds was scored on the basis of the IPGRI et al. (1997) classification, where 1 = seedless, 2 = rudimentary seeds, and 3 = well-developed seeds. I) Berry skin color was also evaluated and was based on IPGRI criteria: 1 = green/yellow; 2 = rose, 3 = red, 4 = red/gray, 5 = dark red-violet, and 6 = blue black. J) The soluble solids were determined by a portable refractometer and recorded as °Brix. Juice samples were also prepared from 25 berries collected in 3 different clusters, by squashing the berries and filtering the juice through a nylon mesh. The following measurements were taken. K) Juice pH was determined using a calibrated pH meter (Corning pH meter 430). L) Titratable acidity was determined in a 5-mL juice sample to which three drops of 1% phenolphthalein indicator were added, and then titrated, with mixing, using 0.1 N NaOH solution standardized beforehand with potassium biphthalate. M) Berry anthocyanin concentration was measured from skin discs (4 mm in diameter) removed from the center of 15 frozen berries. The discs were placed in polystyrene tubes containing 30 mL acidified methanol (1% HCl, v/v) and extracted in darkness. After 48 h, samples were mixed and anthocyanin content determined at 520 nm with a spectrophotometer (Amerine and Ough, 1980; Dokoozlian and Kliewer, 1996).

Construction of genome maps

Genomic DNA was extracted from 111 genotypes of the AT0023 population along with parental lines D8909-15, B90-116, A. de Serres, and b42-26 according to a published protocol (Riaz et al., 2004). The source of SSR markers was the same as described previously. PCR amplification of SSR markers and visual scoring were also similarly conducted as explained earlier (Riaz et al., 2006).

The double pseudo-testcross strategy (Grattapaglia and Sederoff, 1994) and the JoinMap 3.0 software (Plant Research International, Wageningen, Netherlands) were used to build the genetic maps. Markers with a high distortion or unexpected chi-square test results were discarded. LG were determined using the Kosambi function for the translation of recombinatorial units to genetic distance. The LOD score threshold for determination of LG was 3.5. The recombination fraction permitted was 0.45. Markers within the resulting groups were ordered relative to each other by automatic multipoint analyses using the default values of JoinMap 3.0 (mapping threshold LOD >1, recombination frequency threshold <0.4).

A consensus map was constructed using the parameters for a cross-pollinated derived population and the integrate map function of JoinMap 3.0. The LG were numbered according to the reference map of Riaz et al. (2004) and the international agreement achieved within IGGP (International Grape Genome Program; www.vitaceae.org).

QTL analyses

For the parametrical traits, we used HMM in the QTL analysis by multiple imputation as described by Broman and Sen (2009), where each genotype was randomly imputed, but conditional on the observed marker genotype data. We estimated the μ_i and σ by maximum likelihood; that is, we took as our estimates those values for which the observed data were most probable. The likelihood function was:

$$L(\mu\sigma) = \Pi_i \sum_j P_{ij} \Phi(y_i; \mu_j, \sigma^2)$$

where the sum is over the possible QTL genotypes. We used a form of the EM algorithm. We began with initial estimates $\hat{\mu}_j^0$ and $\sigma^{2(0)}$.

In the E-step at interaction *S*, we calculated the conditional probability that an individual is in the QTL genotype group *j* given its marker data, phenotype, and our current estimates of the $\hat{\mu}_j$ and σ.

$$w_{ij}^s = \Pr(g_i = j / M_i, y_i, \hat{\mu}_j^{(s-1)}, \hat{\sigma}^{(s-1)})$$

$$= \frac{p_{ij} \Phi(y_i; \hat{\mu}_j^{(s-1)}, \hat{\sigma}^{(s-1)})}{\sum_k p_{ik} \Phi(y_i; \hat{\mu}_k^{(s-1)}, \hat{\sigma}^{(s-1)})}$$

In the M-step, we updated our estimates of μ_j and σ, treating the $w_{ij}^{(s)}$ as weight.

$$\hat{\mu}_j^{(s)} = \sum_i w_{ij}^{(s)} y_i / \sum_i w_{ij}^{(s)}$$

$$\hat{\sigma}^{(s)} = \sqrt{\frac{\sum_{ij} w_{ij}^{(s)} (y_i - \hat{\mu}_j^{(s)})^2}{n}}$$

Iterations were repeated until the estimates converged (i.e., until the estimates stop changing). The EM algorithm has the advantage that the likelihood is nondecreasing across iterations. It may be that the algorithm converges to a local maximum, but with relatively dense markers and relatively complete marker genotype data, the likelihood is well behaved and the EM algorithm will converge to the global maximum.

Once the maximum likelihood estimates of the μ_j and σ have been obtained, an LOD score is calculated as follows.

$$LOD = \log_{10}\left(\frac{\prod_i \sum_j p_{ij} \Phi(y_i; \hat{\mu}_i, \hat{\sigma}^2)}{\prod_i \Phi(y_i; \hat{\mu}_0, \hat{\sigma}_0^2)}\right)$$

where $\hat{\mu}_0$ and $\hat{\sigma}_0$ are the average and SD of y_i, so that the denominator of the LOD score is the likelihood under the null hypothesis that there is no QTL anywhere in the genome.

In standard interval mapping, the EM algorithm is performed at each position on a grid of putative QTL locations along the genome, while the estimates and likelihood under the null hypothesis are calculated.

For nonparametric interval mapping, we used the Kruskal-Wallis test, where we considered some fixed position in the genome as the location of a putative QTL, and let $P_{ij} = \Pr(g_i = j / M_i)$, the QTL genotype probabilities given the available multipoint marker data, M_i. Whereas in the Kruskal-Wallis test statistic one considers the sum of the ranks within each group, here, the exact assignment of an individual to QTL genotype groups is not known, but rather, individual *i* has prior probability p_{ij} of belonging to group *j*. Thus, we considered the expected rank-sum.

We then determined the statistic: $S_i = \sum_i p_{ij} R_i$

$$H = \sum_i \left(\frac{n - \sum_i p_{ij}}{n}\right)\left[\frac{(S_j - E_{oj})^2}{V_{oj}}\right]$$

where E_{0j} and V_{0j} are the mean and variance of S_j under the null hypothesis of no linkage, considering the p_{ij} as fixed. That is, E_{0j} and V_{0j} are the average and variance of the S_j if we take the R_i to be random permutation of the integers *1*, ……., *n*. We sought loci for which the expected

rank sums, S_j, deviate from their average under the null hypothesis of no linkage. We used the following formula:

$$H = \frac{12}{n(n+1)} \sum_j \frac{(n - \sum_i p_{ij})(\sum_i p_{ij})^2}{n \sum_i p_{ij}^2 - (\sum_i p_{ij})^2} \left[\frac{\sum_i p_{ij} R_i}{\sum_i p_{ij}} - \frac{n+1}{2} \right]^2$$

In the case that the putative QTL is at a fully typed genetic marker, p_{ij} will all be 0 or 1, and the above statistic reduces to the Kruskal-Wallis test statistic.

We used the R program - "Project for Statistical Computing, 4 Mega, package QTL", to structure all the QTL analyses. For the descriptive statistics and correlation estimates we used the program Genes (Cruz, 2006).

RESULTS

Trait distribution and correlations

Descriptive statistics of the traits are presented in Table 1. The number of clusters was highly variable (maximum and minimum), which was expected due to the large differences between the parents. *V. rupestris* and *V. arizonica* are known to be highly fruitful and produce many small clusters, while *V. vinifera* cultivars typically produce few large clusters, soluble solids, numbers of berries, and berry weight, confirming the high variability for these characteristics in the study population. Although the cross was between a seeded female (D8909-15) and a seedless male (B90-116) and could have generated some seedless progenies, none were observed in this population. On a positive note, given the uniform presence of seeds in all of the genotypes, any genotype that has good combination of fruit traits and resistance could be used as male or female parents in the next generation of crosses.

Table 1. Mean, standard deviation, minimum, and maximum values in 111 genotypes derived from a (*Vitis rupestris* x *V. arizonica*/*girdiana*) x *V. vinifera* population.

Statistic	NC	LS	°Brix	pH	TA	PL	CLP	NB	WtB	SN	BC	BA
Mean	58.38	3.31	23.47	3.73	40.03	3.97	10.61	39.15	17.73	1.93	5.41	0.041
SD	39.55	0.92	3.36	0.21	9.97	0.94	3.20	17.15	5.09	0.67	0.88	0.025
Min	3.00	1.00	13.5	3.24	24.75	1.38	4.25	4.5	6.61	1.00	3.00	0.002
Max	150.00	5.00	31.5	4.25	82.5	5.95	21.25	77.75	30.13	4.00	6.00	0.075

NC = number of clusters; LS = leaf score; TA = titratable acidity; PL = peduncle length; CLP = cluster length to peduncle; NB = number of berries; WtB = weight of 10 berries; SN = seed number; BC = berry skin color; BA = berry anthocyanin.

Correlation of different traits was estimated and results are presented in Table 2. The number of clusters was positively correlated with cluster length to peduncle (0.647) and number of berries (0.537). In another study, Fanizza et al. (2005) detected that cluster weight was positively correlated to a larger extent with the number of berries per cluster (r ranging from 0.73 to 0.78) and to a lesser extent with the berry weight (r ranging from 0.36 to 0.52). In other words, number of berries is the most important characteristic that determines cluster weight and yield. The positive correlation observed in this study between cluster number and berry

number is significant help for the grape breeders as they have to count only the cluster number, which is a trait that is very easily studied as compared to counting the number of berries and weighing the clusters.

Table 2. Estimates of the phenotypic (r_f) correlation among 111 genotypes derived from a (*Vitis rupestris* x *V. arizonica*/*girdiana*) x *V. vinifera* population.

Traits	NC	LS	°Brix	pH	TA	PL	CLP	NB	WtB	SN	BC	BA
NC		0.002	-0.014	-0.145	-0.016	-0.087	0.647**	0.537**	0.347**	0.193*	0.046	0.200*
LS			-0.062	0.031	-0.043	0.172	0.069	0.089	0.100	-0.044	-0.105	-0.125
°Brix				0.760*	-0.647**	0.167	-0.244**	-0.159	0.172	-0.218*	0.210*	0.128
pH					-0.756**	0.152	-0.239*	-0.137	0.247**	-0.184*	0.254**	0.180
TA						-0.114	0.102	0.059	-0.258**	0.256**	-0.179	-0.250**
PL							-0.066	-0.070	-0.062	-0.144	0.159	0.142
CLP								0.664**	0.409**	0.262**	-0.072	0.125
NB									0.380**	0.416**	-0.191*	-0.014
WtB										0.153	0.074	0.157
SN											-0.025	-0.024
BC												0.548**
BA												

*,**Significantly different from zero at 0.05 and 0.01% probability levels for a *t*-test, respectively. For abbreviations, see legend to Table 1.

Fruit quality in terms of °Brix was positively correlated with pH (0.760) and negatively correlated with titratable acidity (-0.647) and seed number (-0.218). On the other hand, juice pH was negatively correlated with titratable acidity (-0.756). These correlations were expected and make sense as fruit juice with high sugars and high pH should have low titratable acidity. Calculation of °Brix is a very amenable trait for the breeder to access fruit quality in terms of pH and titratable acidity, which take longer time and involve sample preparation. The results from the correlation of different traits demonstrated that the selection of genotypes with high °Brix levels, moderate acidity, and fewer seeds is possible. Berry color had a high positive correlation with anthocyanin content (0.548), which was expected.

Construction of genome maps

The 111 individuals of the AT0023 population were genotyped with 255 markers, with 152 (59.3%) specific for D8909-15 and 103 (40.2%) specific for B90-116. The genotypic data of the 206 common markers were combined with the consensus marker data, respectively, to establish two data sets of combined markers with one for D8909-15 and the other for B90-116. Estimates of genome coverage for the parental and consensus maps are presented in Table 3.

The D8909-15 genome map consisted of 19 LG, corresponding to each of the 19 chromosomes as expected. The estimated length was 1804.2 cM, and the observed length was 1190.7 cM. For this parent we observed 66.4% map coverage (Table 3). There were 152 markers mapped, and on average, each chromosome was defined with an average of 62.6 cM. For the same parental, 75% genome overall coverage has been found in the D8909-15 map constructed in the "9621" population (Riaz et al., 2006).

The B90-116 genome map consisted of 19 LG, corresponding to each of the 19 chromosomes as expected (Table 3). The estimated length was 1629.30 cM, and the observed length was 912.20 cM. For this parent we observed 56.4% map coverage (Table 3). There were 103 markers mapped, and on average, each chromosome was defined with an average of 48.01 cM.

Table 3. Estimates of genome coverage for the parental and consensus maps.

Map	Observed length (cM)	Estimated length (cM)	Confidence interval (cM)	Calculated length (cM)	Expected coverage adjusted for chromosome ends (%)	Expected coverage for linear chromosomes (%)	Observed map coverage (%)
D8990-15	1190.7	1804.2	1045.7-1355.2	1428.8	79.6	83.3	66.4
Consensus	1871.4	2102.8	2562.8-2850.4	2245.6	96.7	99.8	89.7
B90-116	912.2	1629.3	947.0-1258.0	1094.6	75.7	78.6	56.4

Genetic distances are given in Kosambi centimorgans (cM).

The consensus map was developed with 255 molecular markers, ordered into 19 LGs. The estimated length was 2102.8 cM, and the observed length was 1871.4 cM. For this map, we observed 89.7% coverage (Table 3). There were 255 markers mapped, and on average, each chromosome was defined with an average of 98.49 cM and an average distance of 7.3 cM between markers (Table 3).

QTL description

QTL identified by interval mapping using HMM are presented in Table 4 and Figure 2. The number of QTL detected for each trait varied between 1 and 8, reflecting the quantitative nature of these traits. The percentage of variation explained by these QTL was small, varying between 1.23 and 11.98% (Table 4).

Table 4. Characteristics of the detected QTLs for each trait measured in segregant population of table grapes derived from a (*Vitis rupestris* x *V. arizonica*/*girdiana*) x *V. vinifera* population.

Trait	Linkage group[c]	Marker	Position (cM)	LOD score	LOD[d] threshold	% of variation
Number of cluster[a]	1	VMCNg1h7	0.00	2.039	4.29	2.34
	5	VMC9f4a	5.00	2.047	4.29	2.10
	6	VVMD21	0.00	2.029	4.29	2.36
	7	VMC16f3	35.00	3.041	4.29	4.45
	12	VMCNg2d11a	25.00	2.359	4.29	2.45
	13	VMC3d12	10.00	3.430	4.29	5.12
	14	Scu15	20.00	2.806	4.29	3.12
	19	VMCNg3a10	20.00	2.794	4.29	3.07
Leaf score[b]	3	VMC1g7	5.00	2.521	4.79	2.08
	5	Ctg6305	10.00	2.221	4.79	2.09
	14	VVMD24	15.00	2.178	4.79	3.78
°Brix per cluster[b]	3	VMC1a5	0.00	4.094	3.79	9.45
pH[a]	1	VMC7g5	15.00	2.992	4.82	3.45
	6	VVMD21	0.00	4.666	4.82	10.34
	11	VVMD25	0.00	2.110	4.82	3.09
	13	VMC3d12	10.00	2.115	4.82	2.12
	16	VMCNg2h7	30.00	2.085	4.82	2.09
Tartaric acid[a]	6	VVMD21	0.00	2.541	8.12	2.34
	13	VVMD29	5.00	3.871	8.12	1.56
	19	VMC5d11	0.00	2.645	8.12	1.23
Length of peduncle[a]	9	VMC6e4	5.00	3.123	4.08	5.45
	10	VMC3d7	10.00	2.202	4.08	2.76
	12	ctg382	40.00	2.120	4.08	2.98
Length of cluster to peduncle[a]	14	VVC34	90.00	3.750	4.23	4.09
Number of berries[b]	4	VMCNg2e1	35.00	2.474	3.79	2.76
	9	VMC2d9	25.00	2.342	3.79	2.09
	14	VVC34	90.00	2.448	3.79	2.87
Weight of 10 berries[a]	1	AF8125	15.00	2.097	3.95	2.09
	8	VMC2h10	0.00	2.296	3.95	2.13
	10	VMC3d7	10.00	2.858	3.95	3.09
	11	VVMD25	0.00	3.963	3.95	8.45
Seed number[a]	6	VMC2a9b	70.00	2.451	4.95	2.13
	13	VMC9h4.2	30.00	2.378	4.95	2.16
Berry color[b]	4	VRZAG83	10.00	2.184	4.62	2.34
	5	ctg6305	10.00	2.368	4.62	2.09
	13	VMC3d12	10.00	3.037	4.62	3.34
Berry anthocyanin[a]	2	VMC5g7	10.00	3.928	3.59	11.98
	7	VVC82	25.00	2.222	3.59	2.34
	12	VMC8g9	15.00	2.879	3.59	2.65
	13	VMC3d12	10.00	2.679	3.59	2.09
	14	VMC1e12	30.00	2.602	3.59	2.43

[a]Method of analysis interval mapping for the quantitative traits. [b]Method of analysis nonparametric interval mapping. [c]Linkage group as the International Genome Program (IGGP) and Riaz et al., 2004. [d]Determined by permutation test at 5% of probability.

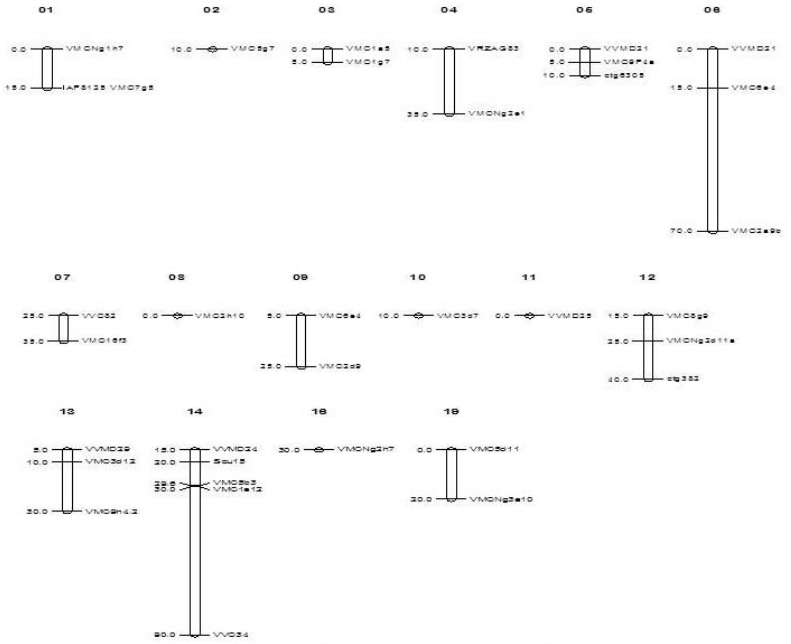

Figure 2. Relative position of QTLs detected by interval mapping using hidden Markov models in an AT0023 population.

Eight QTL were identified for the number of clusters across LG 1, 5, 6, 7, 12, 13, 14, and 19 (Table 4). Three QTL were detected for leaf score across LG 3, 5, and 14, and for °Brix per cluster we found QTL in LG 3. For pH, we found QTL in LG (1, 6, 11, 13, 16). For tartaric acid, we found QTL in LG 6, 13 and 19. Three QTL were detected for peduncle length across LG 9, 10, and 12 and number of berries (LG 4, 9, and 14), and one QTL was observed for cluster length to peduncle (LG 14). For weight of 10 berries we found four QTL (LG 1, 8, 10, and 11), seed number showed QTL across LG 6 and 13, and berry color showed QTL across LG 4, 5, and 13. Five QTL were detected for anthocyanin across LG 2, 7, 12, 13, and 14.

DISCUSSION

Genetic dissection

In this study, we identified several QTL affecting agronomic traits in table grapes. We observed clusters of QTL for closely related traits on several LG (Figure 2). QTL for the number of clusters were found in several LG (1, 5, 6, 7, 12, 13, 14, 19) and for weight of 10 berries, we found QTL in LG 1, 8, 10, and 11; for these 2 traits we found a significant correlation, with one marker explaining 8.45% of the total phenotypic variation (VVMD25). In another marker, we observed different and low variation for the number of clusters, in this case, reflecting the quantitative nature of this trait. These results differed in part from QTL previously published by Fanizza et al. (2005) and Doligez et al. (2010), who found QTL for similar traits in different LG. This discrepancy possibly resulted in part from genotype x years interactions and/or from

segregation differences between crosses. Part of this divergence may also be attributed to differences in trait measurement. Those authors measured number of inflorescences per shoot at anthesis, and number of clusters per vine at harvest, which is a composite trait integrating not only the number of inflorescences per shoot ant anthesis, but also the number of shoots per vine and the rate of full development of inflorescences into clusters. The range of fertility values was very similar in the 3 progenies, although one of them harbored alleles from different *vinifera* cultivars and species.

The range of fertility values obtained in the progeny of these table grapes was somewhat narrower than observed in the Vassal collection (0-3.5; Boursiquot et al., 1995), which includes both table and wine grape cultivars. This suggests that additional alleles and additional loci underlying fertility variation may be present in wine grapes (Doligez et al., 2010). The different distribution of fertility values in the 3 progenies might have resulted from a selection bias at overgrafting, favoring buds that were no longer in the juvenile stage.

We found QTL for peduncle length and cluster length to peduncle in different LG (9, 10, 12, 14), where in this study there was no correlation between them. For the trait length of cluster to peduncle we found significant correlation with number of berries (0.664); in the same LG we found QTL for the number of berries (4, 9, and 14). Fanizza et al. (2005) found that several QTL were detected for number of berries, but none of these were observed in successive years. The lack of QTL stability in different years for all traits analyzed might have been due to the presence of different genes or the differential expression of these genes as a result of differential genotype sensitivity to yearly climate variations. Yearly variations in temperature had a considerable effect on flowering and berry set in some of the genotype, affecting QTL stability of the number of berries per cluster and cluster weight (Fanizza et al., 2005).

If all these factors are taken into consideration, it is logical to imagine that a large number of genes and different physiological mechanisms may be involved in the determination of each fruit trait in response to yearly environmental variations. Thus, the detection of different QTL for the same trait should be expected in different years because QTL detection will depend on the environmental conditions of that specific year (Fanizza et al., 2005).

For the weight of 10 berries we found QTL across LG 1, 8, 10, and 11. Other investigations have detected stable QTL for berry weight in table grapes and wine grapes; however, the QTL detected in these investigations were all different. These different results may be due to the different progeny used (Doligez et al., 2002; Fischer et al., 2004; Fanizza et al., 2005). This variability in berry weight in the different progeny might have affected the detection of the QTL and, together with progeny size and heritability, may play an important part in explaining the different results. Small sample size and medium-to-low trait heritability might have biased QTL detection (Beavis, 1998; Melchinger et al., 2004).

For the seed number, we found only 2 QTL across LG 6 and 13. Costantini et al. (2008), for the same trait and seed content, found QTL on LG 2 and LG 13 of 3 maps in 2 years, which explained 19.6-22.9% of the total phenotypic variation, and, according to these authors, these QTL, along with those specific for seed content identified on LG 2 and 13, may allow dissociation of the unfavorable correlation between berry size and seedlessness in a breeding program. In our case, we found significant correlation between seed number and number of berries (0.416), and in the same study, they found a correlation between flowering time and seedlessness traits that we observed at the genetic level on LG 2 and could be due to the known effect of gibberellins on flowering. On the contrary, the observed phenotypic cor-

relation between véraison time and seedleeness traits was not supported at the molecular level, which may indicate that genes controlling the 2 traits function independently of each other, but further confirmation is needed.

Cabezas et al. (2006) and Mejía et al. (2007) found major QTL for seed number across LG 18 in different years. This QTL was associated with a pleiotropic effect on berry size or weight and ripening date, and it was not possible to dissociate seedlessness and small berry size. At least 4 independent minor QTL for seedlessness were identified in different LG. In our case, we used different populations for the QTL analysis. All the genotypes in the segregating population showed normal seed, and different results were observed for the parents used for this study.

For the fruit quality we found several QTL, explaining the moderate values of the phenotypic variation. For °Brix per cluster, we found QTL across LG 3; in LG 3 the SSR marker (VMC1a5) explained 9.45% of the total phenotypic variation. For this trait, we found high correlation with pH and negative correlation with titratable acidity (0.760 and -0.647). For pH, we found QTL across LG 1, 6, 11, 13, and 16. The SSR marker VVMD21 (LG 6) explained 10.34% of total phenotypic variation, and for the titratable acidity, we found QTL across LG 6, 13, and 19, and they explained low variation.

There are no reports of QTL analysis for traits related to fruit quality of grape (°Brix, pH, and titratable acidity). The location of QTL in different LG suggests that the genetic control of these characters are influenced by several genes involved in complex metabolic pathways, so a higher saturation of the LG obtained should be performed for the localization of QTL with major effect.

For anthocyanin, we found QTL across LG 2, 7, 12, 13, and 14. The SSR marker VMC5g7 (LG 2) explained 11.98% of the total phenotypic variation.

Doligez et al. (2002) found a 1:1 segregation for berry color in the progeny. In recoding of the 3 SSR markers for a 1:2:1 segregation with null allele, they used one cross where phenotypic segregation for this trait was not observed.

Fournier-Level et al. (2009) showed a single QTL located on LG 2 between the VMC6B11 and VVIU20 markers that was identified in all analyses with a 5.9-cM confidence interval at LOD 1 defined on the consensus map. This locus accounted for 48-62% of the total variation in anthocyanin content in the berry and repeated across all blocks and years.

ACKNOWLEDGMENTS

A.P. Viana is grateful to the National Council for Scientific and Technological Development (CNPq) for a fellowship that allowed training in the Department of Viticulture and Enology, University of California at Davis.

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