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Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes

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Summary

- The stomatal control of transpiration is one of the major strategies by which plants cope with water stress. Here, we investigated the genetic architecture of the rootstock control of scion transpiration-related traits over a period of 3 yr.
- The rootstocks studied were full sibs from a controlled interspecific cross (*Vitis vinifera* cv. Cabernet Sauvignon × *Vitis riparia* cv. Gloire de Montpellier), onto which we grafted a single scion genotype. After 10 d without stress, the water supply was progressively limited over a period of 10 d, and a stable water deficit was then applied for 15 d. Transpiration rate was estimated daily and a mathematical curve was fitted to its response to water deficit intensity. We also determined $\delta^{13}C$ values in leaves, transpiration efficiency and water extraction capacity. These traits were then analysed in a multienvironment (year and water status) quantitative trait locus (QTL) analysis.
- Quantitative trait loci, independent of year and water status, were detected for each trait. One genomic region was specifically implicated in the acclimation of scion transpiration induced by the rootstock. The QTLs identified colocalized with genes involved in water deficit responses, such as those relating to ABA and hydraulic regulation.
- Scion transpiration rate and its acclimation to water deficit are thus controlled genetically by the rootstock, through different genetic architectures.

Introduction

Grapevines are generally grown as a scion grafted onto a rootstock, because of the devastating effect of phylloxera (*Daktulosphaira vitifoliae*), a soilborne aphid pest introduced into Europe in the middle of the 19th century. Cultivated grapevines thus have two genotypes, those of the scion and the rootstock, which interact together to create the final phenotype. Water availability is one of the major factors limiting plant productivity (Boyer, 1982) and the breeding of crop varieties that use water more efficiently is a key strategy for the improvement of agrosystems (Condon *et al.*, 2004). Improvement of the genetic material of grapevines is essential in the context of predicted changes in global climate. Rootstock selection is the most promising method for achieving this goal, because the scion genotype controls the typical features of the grape, raisin and wine (Vivier & Pretorius, 2002). It would therefore be difficult to modify the genotype of the scion without also altering the characteristics of the final product. Grapevine rootstocks have been shown to play an important role in adaptation to water deficit in the vineyard (Samson & Casteran, 1971; Soar *et al.*, 2006) and in pots (Carbonneau, 1985). However, little is known about the genetic determinism of rootstock effects on water deficit responses in the scion.

Plants display complex drought responses, depending on the duration and intensity of the water deficit (Bray, 1997). They may respond at many levels of organization, from the molecular and physiological levels to the level of the whole plant (reviewed by Chaves *et al.*, 2003). Stomata play a critical role in regulating water loss (Buckley, 2005), and stomatal closure is one of the earliest responses to water deficit (Hsiao, 1973; Damour *et al.*, 2010). The selection of plants that partially close their stomata in response to water deficit can be used to increase water-use efficiency (WUE, i.e. biomass gain as a function of water use), but only at the expense of carbon accumulation and, potentially, yield (Boyer, 1982; Flexas *et al.*, 2010). The maintenance of yield in conditions of water shortage has also been identified as an important target in grapevine breeding programmes (Flexas *et al.*, 2010).

The genetic determinism of drought tolerance has been widely studied in annual plants (Kamoshita *et al.*, 2008; Fleury *et al.*, 2010; Hao *et al.*, 2010). In perennial plants, QTLs for WUE (estimated by $\delta^{13}C$ analyses; Farquhar & Richards, 1984) have been identified by classic quantitative genetics and QTL

detection approaches (Brendel *et al.*, 2002, 2008; Rönnberg-Wästljung *et al.*, 2005; Street *et al.*, 2006). In general, the QTLs identified for drought responses have accounted for only a small proportion of phenotypic variation (< 10%), with very few accounting for > 20% of the variance. Moreover, transpiration-related traits interact with environmental factors, such as the duration and intensity of water deficit and the climatic conditions in which the plants are grown (Collins *et al.*, 2008). Genotype × environment interactions therefore complicate genetic dissection (Reymond *et al.*, 2004; Chenu *et al.*, 2009).

Two approaches for taking genotype × environment interactions into account in QTL studies have been developed. In the first, phenotypic responses are fitted to environmental changes for each genotype and QTLs are detected for fitted response curve parameters (Reymond *et al.*, 2004). The response curve parameters describe acclimation (defined as a special type of plasticity): the phenotypic variation generated by a genotype in response to an environmental variable (Bradshaw, 1965). The QTL mapping of response curves has been described as 'functional mapping' (Wu & Lin, 2006). Functional mapping has been successfully used in analyses of the effects of light on flowering time in *Arabidopsis* (Stratton, 1998) and to characterize the response of leaf growth to temperature and water status in maize (Reymond *et al.*, 2003). The alternative approach combines data from different conditions in a multienvironment QTL model, including data for different environmental conditions (Korol *et al.*, 1998), over different years (Brendel *et al.*, 2008) or between different treatments (Parelle *et al.*, 2007).

In grapevines, QTL analyses have been carried out on quantitative traits relating to fruit yield components, berry colour, seedlessness, aroma and disease or pest resistance (as reviewed by Martinez-Zapater *et al.*, 2010). Only two studies have dealt with responses to environmental conditions: magnesium deficiency (Mandl *et al.*, 2006) and photoperiod-induced growth cessation (Garris *et al.*, 2009). We are not aware of any published studies on the genetic determinism of grapevine responses to abiotic stresses or using both functional mapping and multi-year analysis of QTL in a woody plant species.

In this study, we aimed to dissect the genetic architecture of the control of transpiration and its acclimation to water deficit by the rootstock. We used an interspecific F1 rootstock progeny, onto which we grafted a *V. vinifera* cv Cabernet Sauvignon scion, to detect QTLs for transpiration-related traits. We used two methods for QTL detection: functional mapping, in which we quantified the acclimation of transpiration rate to water deficit by means of response curves, with the detection of QTL based on the fitted curve parameters; and multienvironment QTL mapping.

Materials and Methods

Plant material and growing conditions

A mapping pedigree of 138 F1 genotypes derived from a *Vitis vinifera* cv. Cabernet Sauvignon (CS, female parent) × *Vitis riparia* cv. Gloire de Montpellier (RGM, male parent) interspecific cross was studied over a period of 3 yr. This family, named CS×RGM1995-1, was produced at INRA Bordeaux (France) and mapped with microsatellite markers. Details of the mapping methodology used were provided by Marguerit *et al.* (2009). Linkage groups were numbered according to their parental maps (CS for the female vs RGM for the male) and a serial number from 1 to 19. The rootstock behaviour of the members of this family was characterized independently in three consecutive years: 2007, 2008 and 2009 (i.e. grafting onto a new rootstock in each year). The year before each experiment, the *V. vinifera* cv. Cabernet Sauvignon (CS) scion was grafted onto the rootstock. Plants were pruned to one stem and trained, with side branches removed three times per wk. For the three sets of experiments, plants were grown in 7 l pots filled with 5 kg of silty/clayey soil (20% clay, 18% silt and 62% sand), under semicontrolled conditions in a glasshouse equipped with a cooling system. Each experiment started when the plants had 10 unfolded leaves. Global radiation, air temperature and humidity were recorded at two different locations in the glasshouse with pyranometers (LI-Q23022, Li-Cor, Lincoln, NE, USA) and thermohygrometers connected to a data logger (21X; Campbell Scientific Ltd, Shepshed, Leicestershire, UK) (Supporting Information, Table S1).

Water deficit treatment

The water retention capacity of the soil was determined by weighing soil samples at field capacity and then after drying to constant weight. The amount of soil per pot was determined at potting, so the maximum water content per pot was known. Before bud burst, we determined the weight of each pot at field capacity by fully irrigating each pot and then allowing the water to drain for period sufficiently long for a stable weight to be attained. Pots were covered with a plastic bag to prevent water losses by evapotranspiration. At field capacity, the water content of the soil was, on average, 45% of its DW.

Throughout the experiments, the amount of water in the soil was determined by weighing the pot daily in an experimental setup of 150 balances (CH15R11, OHAUS type CHAMP, Nänikon, Switzerland, precision 1 g) (Sadok *et al.*, 2007). Pots were irrigated by manually watering mid-morning, to achieve the target water content (Fig. 1).

In 2007, a water deficit (a daily fixed decrease) was gradually established over a period of 29 d. Soil water content was decreased by a mean of 3% of the retention capacity per d until transpiration ceased. On average, the amount of water remaining in the pots at the end of the drought cycle corresponded to 11% of soil DW. For the control plants, soil water content was maintained close to field capacity. Each genotype was represented by a single control plant and one plant subjected to water deficit. In 2008 and 2009, after 10 d of measurements at field capacity, a water deficit was gradually established over a period of 10 d (decreasing by 6% of retention capacity per day) and a stable water deficit corresponding to 50% of the soil water content determined at field capacity was then applied for 15 d (Fig. 1). Each genotype was represented by two replicates, and no separate

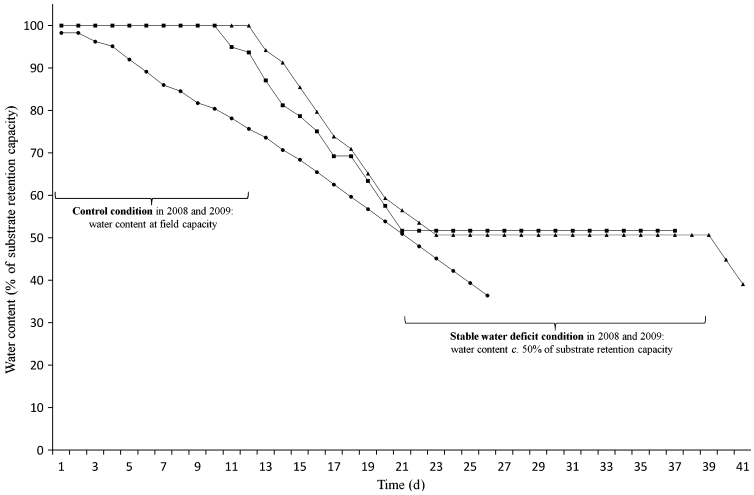

Fig. 1 Duration of deficit and targeted water content during the 3 yr experiment. The symbols indicate the targeted water content: circles, 2007; squares, 2008; triangles, 2009. The two periods of stable water content (at field capacity and at 50% of substrate retention capacity) are specified. For each of these periods, we calculated mean transpiration rate, $\delta^{13}C$ values and transpiration efficiency.

control plants were studied. However, measurements before the establishment of the water deficit made it possible to characterize field capacity traits for each genotype.

Traits studied

We recorded transpiration daily and leaf area weekly, for each plant in each experiment. Transpiration rate per leaf area unit (Tr) was then assessed daily. Whole-plant transpiration was calculated as the daily change in weight of each pot (including watering). For the determination of leaf area, we measured the length of the main vein of each leaf on each plant weekly. The allometric relationship between the length of the main vein and leaf area (Carbonneau, 1976) was established yearly with an area meter (Li-Cor 3100), on control plants, in each experiment (Fig. S1).

In 2008 and 2009, intrinsic WUE was estimated by carbon isotope discrimination analysis of bulk leaf matter (Farquhar & Richards, 1984) for the two periods of stable water content (field capacity and 50% water retention capacity). A preliminary experiment demonstrated that water supply was the main factor affecting the carbon isotope composition ($\delta^{13}C$) of bulk leaf dry matter in developing leaves, with no significant effect of leaf age (data not shown). Consequently, sampling for WUE analysis was performed at the end of the experiments. Three leaves that had grown mostly in control conditions (field capacity) and three leaves that had grown during the stable water deficit period (50% water retention capacity) were identified and collected from each plant. These leaves were dried in an oven at 80°C. For each leaf, we obtained four discs of 15 mm in diameter, which we pooled and ground to a fine powder in a ball mill (Retsch, MM200, Haan, Germany). We added 1 mg of powder to a 4 × 6 mm tin capsule and subjected it to combustion in an elemental analyser (Carlo Erba NA1500 Serie 2, Milan, Italy). Carbon isotope composition ($\delta^{13}C$) was analysed with a continuous flow isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany) and is expressed relative to the Pee Dee Belemnite standard, as described by Craig (1957):

$$\delta^{13}C = (R_{sa} - R_{sd}) \times 1000/(R_{sd})[‰] \quad \text{Eqn 1}$$

where R_{sa} and R_{sd} are the $^{13}C : {}^{12}C$ ratios of the sample and the standard, respectively.

Transpiration efficiency (TE), defined as biomass gain divided by transpiration water loss (Farquhar & Richards, 1984), was estimated in 2008 and 2009, for the two periods of stable water content (field capacity and 50% water retention capacity). In this study, biomass accumulation actually corresponded to shoot dry matter accumulation and was estimated from the relationship between shoot dry biomass and shoot length established for a subset of plants (Fig. S2). The shoot length was measured three times per wk allowing the previous estimation. The calibration equation thus obtained was used in 2008 and 2009.

Fitting of the curves for the response of transpiration rate to soil water availability

For each experiment, the total transpirable soil water (TTSW) content was calculated for each plant as the difference between soil water content at field capacity (measured at the beginning of the experiment) and soil water content at 10% of maximal transpiration rate (Sinclair & Ludlow, 1986). The fraction of transpirable soil water (FTSW) remaining in the soil at a given date was then calculated for each plant, by dividing the TTSW by total soil water content.

The acclimation of transpiration rate per unit of leaf area to FTSW was assessed for each genotype. In 2007, the normalized transpiration rate was calculated as described by Sinclair & Ludlow (1986). This procedure was slightly modified in 2008 and 2009, because there were no control plants for individual genotypes. Transpiration rate was normalized with the mean transpiration rate measured on a panel of 15 genotypes grown in

control conditions for the total duration of the experiment (two replicates of each genotype in 2008 and three in 2009). R software was used for curve-fitting (R Development Core Team, 2005), with the following equation: $NTR = 1/(1 + 9 \times e^{(-\mu \times FTSW)})$, where NTR is the normalized transpiration rate, μ is the curve parameter and FTSW is the fraction of transpirable soil water expressed as a percentage. The constant nine is the intercept with the *y*-axis at FTSW = 0 (related to NTR = 0.1). Higher values of the coefficient μ were associated with an earlier down-regulation (in terms of FTSW or water stress intensity) of transpiration. These curves were used to calculate NTR when FTSW reached 60% ($NTR_{FTSW60\%}$), 40% ($NTR_{FTSW40\%}$) and 20% ($NTR_{FTSW20\%}$).

The traits used for QTL mapping (Table 1) were classified into four categories: transpiration rates, including the mean transpiration rate under control condition (Tr_C) and under stable water deficit (Tr_WD); WUE, estimated by determining carbon isotope composition under control condition (δ^{13}C_C) and under a stable water deficit (δ^{13}C_WD) or transpiration efficiency under control condition (TE_C) and under a stable water deficit (TE_WD); water extraction capacity, evaluated by calculating TTSW; and the acclimation of transpiration rate to water deficit, calculated with μ (Coefμ) and values of NTR when FTSW reached 60% ($NTR_{FTSW60\%}$), 40% ($NTR_{FTSW40\%}$) and 20% ($NTR_{FTSW20\%}$).

Statistical and QTL analyses

The broad-sense heritability of phenotypic means was defined as $\sigma_G^2/[\sigma_G^2 + (\sigma_e^2/n)]$, where σ_G^2 and σ_e^2 are the genotypic and residual variances, and n is the mean number of replicates. It was derived from the *F*-value in the one-way ANOVA as $1 - (1/F)$ (Gallais, 1990).

Quantitative trait locus analyses were carried out for each quantitative trait. Data were not transformed, despite slight deviations from normality (Shapiro–Wilkinson test), because the interval mapping method is robust to deviations from this assumption (Rebaï, 1997). Moreover, permutation analysis provides valid thresholds for nonstandard situations, such as nonnormal distributions (Members of the Complex Trait Consortium, 2003). We used MultiQTL V2.6 software for QTL detection (http://www.multiqtl.com). This software can be used for single-environment analysis for each trait and for multienvironment analyses in which phenotypic data for different years or different water status conditions are considered together (Korol *et al.*, 1998). Moreover, permutation tests are available (bootstrap analyses) for estimating the precision of each QTL position.

The multiple interval mapping (MIM) procedure was used to reduce the uncontrolled variation, by taking into account QTL effects from other chromosomes (Jansen & Stam, 1994). A permutation test (1000 permutations) on the data set was carried out to determine statistical thresholds (*P*) for estimating significance levels for QTLs at the chromosome level ($P_{chr} < 0.05$) (Doerge & Churchill, 1996). The significance for all detected QTLs was recalculated at the genome-wide level, as a function of the total number of markers in the parental map and the number of markers in each linkage group (Brendel *et al.*, 2002; Scotti-Saintagne *et al.*, 2004). QTLs were qualified as significant only at the whole-genome level ($P_G < 0.05$) or as 'suggestive' ($P_{chr} < 0.05$) (Members of the Complex Trait Consortium, 2003).

Quantitative trait locus detection was performed separately for each parental map, by MIM, using a two-QTL model per linkage group followed by a model assuming one-QTL per linkage group if the two-QTL model was not significant. The number of linkage groups with two QTLs progressively decreased, leading to the retention of a model with two linked QTLs only for linkage groups in which this model was significant. The advantage of this method is that it reduces the number of models to be tested (Parelle, 2006). We used the QTL detection described by Parelle *et al.* (2007) and Brendel *et al.* (2008).

The 'position range' of a QTL was defined as the range between the positions calculated from the original dataset (L) and from bootstrap analysis (L_{BS}). The confidence interval of the QTL position was calculated relative to L_{BS}. Colocalization between QTLs was reported when the L–L_{BS} intervals of two QTLs overlapped. Colocalization of a candidate gene and a QTL was reported when the candidate gene was located within the confidence interval for the QTL. The allelic effect of the QTL and the percentage explained variance (PEV) were calculated with the MIM procedure (Jansen *et al.*, 1995).

Table 1 Trait abbreviations and description (measurement units and years of measurement)

Trait	Description	2007	2008	2009
Tr_C	Transpiration rate (g. cm^{-2}) during the 10 d of control conditions		X	X
Tr_WD	Transpiration rate (g. cm^{-2}) during the 15 d of stable water deficit		X	X
δ^{13}C_C	Carbon isotopic discrimination during the 10 d of control conditions		X	X
δ^{13}C_WD	Carbon isotopic discrimination during the 15 d of stable water deficit		X	X
TE_C	Transpiration efficiency calculated as the ratio of aerial dry mass (g) to water used (g) for the control period		X	X
TE_WD	Transpiration efficiency calculated as the ratio of aerial dry mass (g) to water used (g) for the period of stable water deficit		X	X
TTSW	Total transpirable soil water	X	X	X
Coefμ	Coefficient μ for the curve of transpiration rate response to changes in FTSW	X	X	X
$NTR_{FTSW60\%}$	Threshold for the curve of the transpiration rate response to FTSW: NTR when FTSW = 60%	X	X	X
$NTR_{FTSW40\%}$	Threshold for the curve of the transpiration rate response to FTSW: NTR when FTSW = 40%	X	X	X
$NTR_{FTSW20\%}$	Threshold for the curve of the transpiration rate response to FTSW: NTR when FTSW = 20%	X	X	X

*FTSW, fraction of transpirable soil water; NTR, normalized transpiration rate.

Traits measured in several years or for different water conditions were also analysed with the multienvironment QTL detection module available in MultiQTL, with data for control and water deficit conditions (multi-water status analysis) or data from several years of measurements (multi-year analysis). By reducing environmental variance, this method enhances the detection power of QTLs with weak effects (Jansen *et al.*, 1995). A two-QTL model was used in the multienvironment analysis, with the same requirements for significance as described earlier, except that allelic effects needed to be significant for at least one environment (water status or year) for a QTL to be considered significant. When the two-QTL model was not significant, it was replaced by a one-QTL model. Finally, we carried out statistical tests on the difference in the allelic effects of a QTL between years or water status conditions, and the significance of the QTL × environment effect (Distelfeld *et al.*, 2008).

Results

Phenotypic variability within the progeny and correlations between traits

The coefficients of phenotypic variation were low in the family tested (2.5–18.9%), except for five traits: TE_C in 2009, TE_WD in 2008 and 2009, Coefμ and $NTR_{FTSW20\%}$ in 2007 (Table 2). Transpiration efficiency displayed the highest amount of variation in all years and for all water status conditions studied. Variation greater than that for parental values was observed, consistent with a transgression phenomenon (Fig. S3). Plants grafted onto CS parental rootstocks generally had higher transpiration rates than those grafted onto RGM rootstocks, regardless of water status.

Broad-sense heritability in the mapping population – the proportion of the genetic variance relating to the phenotypic variance of a trait – was calculated for transpiration rate and WUE-related traits (Table 2). It exceeded 0.3 for most traits. The highest values were obtained for TTSW and the lowest for TE under water deficit conditions. As the response curves were based on pooled data for the two replicates, it was not possible to calculate broad-sense heritability for acclimation-related traits. The effect of the year on genetic variance was significant for all traits (Student's *t*-test, *P*-value = 0.007).

Principal component analysis (PCA) was performed each year, for all traits phenotyped in the progeny (Fig. S4). The first two axes of the PCA (F1 × F2) accounted for 66, 47 and 40% of the phenotypic variability in 2007, 2008 and 2009, respectively. For each year, the first axis corresponded essentially to traits relating to the acclimation of transpiration rate to water deficit. The second axis related to transpiration rate, WUE-related traits and water extraction capacity. No clear grouping of the individuals of the progeny was observed (data not shown). Correlations between the $\delta^{13}C$ values measured in control and water deficit

Table 2 Descriptive statistics for measured and estimated traits for transpiration rate response curves

Trait	Year	n_0	h^2	*N*	Mean	Minimum	Maximum	SD	CPV
Tr_C	2008	2	0.59	124	0.23	0.18	0.33	0.01	6.3
Tr_C	2009	2	0.43	128	0.21	0.20	0.34	0.02	10.2
Tr_WD	2008	2	0.61	124	0.11	0.04	0.17	0.02	18.9
Tr_WD	2009	2	0.36	128	0.09	0.07	0.16	0.02	17.8
$\delta^{13}C$_C	2008	2	0.65	124	−29.48	−30.82	−27.22	0.63	–
$\delta^{13}C$_C	2009	2	0.33	128	−28.31	−30.18	−27.28	0.52	–
$\delta^{13}C$_WD	2008	2	0.64	124	−26.16	−27.94	−23.57	0.95	–
$\delta^{13}C$_WD	2009	2	0.49	128	−24.32	−28.01	−21.80	1.19	–
TE_C	2008	2	0.48	124	0.0024	0.0011	0.005	0.00039	15.4
TE_C	2009	2	0.54	128	0.0055	0.0029	0.009	0.00108	21.1
TE_WD	2008	2	0.36	124	0.0002	0.00003	0.001	0.00015	72.4
TE_WD	2009	2	0.21	128	0.0020	0.00022	0.003	0.00057	27.9
TTSW	2007	1	–	83	1230	985	1494	108	8.8
TTSW	2008	2	0.70	124	1209	903	1472	99	8.2
TTSW	2009	2	0.47	128	1370	1168	1904	122	8.9
Coefμ	2007	1	–	83	0.14	0.06	0.31	0.04	28.5
Coefμ	2008	2	–	124	0.07	0.05	0.09	0.008	10.5
Coefμ	2009	2	–	128	0.08	0.07	0.11	0.007	8.2
$NTR_{FTSW60\%}$	2007	1	–	83	0.99	0.76	1.0	0.03	3.0
$NTR_{FTSW60\%}$	2008	2	–	124	0.90	0.74	0.97	0.04	4.8
$NTR_{FTSW60\%}$	2009	2	–	128	0.93	0.86	0.99	0.02	2.5
$NTR_{FTSW40\%}$	2007	1	–	83	0.93	0.51	1.0	0.08	8.6
$NTR_{FTSW40\%}$	2008	2	–	124	0.68	0.49	0.83	0.07	9.8
$NTR_{FTSW40\%}$	2009	2	–	128	0.74	0.61	0.90	0.05	6.6
$NTR_{FTSW20\%}$	2007	1	–	83	0.62	0.25	0.98	0.15	23.8
$NTR_{FTSW20\%}$	2008	2	–	124	0.33	0.25	0.42	0.03	10.4
$NTR_{FTSW20\%}$	2009	2	–	128	0.36	0.30	0.49	0.03	8.6

n_0, adjusted mean number of replicates per genotype subjected to water deficit; h^2, broad-sense heritability; *N*, the number of genotypes taken into account; SD, standard deviation; CPV, coefficient of phenotypic variation expressed as a % (SD/mean × 100), which cannot be calculated for $\delta^{13}C$ because this trait is calculated relative to a standard.

conditions were significant in both 2008 (r^2 = 0.69) and 2009 (r^2 = 0.34). Within a given year, a few significant correlations were observed between traits. In 2008, the rate of transpiration in water deficit condition was correlated with $\delta^{13}C$ values (whatever the water status) and transpiration rate acclimation-related traits (r^2 ranging from 0.36 with Coefμ to 0.63 with $\delta^{13}C$_WD). In 2009, the transpiration rate in water deficit condition was correlated with $\delta^{13}C$ values in water deficit condition and water extraction capacity (r^2 ranging from 0.33 with δ13C_WD to 0.60 with TTSW). There was no significant correlation between traits measured in different years.

Multi-year QTL analysis

A QTL analysis of combined data for all 3 yr was carried out for each water status condition (Table 3), with the aim of identifying stable regions controlling the trait studied in all years. Eight of the 23 QTLs detected for all traits were significant for transpiration rate on CS1 and CS17, for transpiration efficiency on CS6 and RGM11 and for water extraction capacity on RGM 3, RGM5 and RGM11. We identified one to six QTLs per trait, accounting for between < 1 and 15% of phenotypic variance. A few colocalizations between different QTLs were observed: for Tr_C and TTSW on CS17; for TE_C and TE_WD on CS6; for Tr_WD and TTSW on RGM3 (Fig. 2). Two regions were identified in the same area of both parental maps, on linkage groups 3 and 12.

Of the 23 QTLs detected in the multi-year analysis, 14 displayed a significant QTL × year interaction. For QTLs on CS17 and RGM3, the significance of the interaction with year varied with the trait studied (significant for TTSW but not for Tr_C or Tr_WD).

Multi-water status QTL analysis

A QTL analysis was carried out for transpiration rate, $\delta^{13}C$ values and transpiration efficiency each year, on combined data for control and water deficit conditions, with a view to detecting stable regions controlling the trait regardless of water status (Table S2). Six of the 11 QTLs detected were significant. QTLs were identified for transpiration efficiency in 2008 and for transpiration rate, $\delta^{13}C$ values and transpiration efficiency in 2009. We detected significant QTLs for $\delta^{13}C$ values in this analysis, whereas no such QTLs were detected in either water status condition with the multi-year analysis. No colocalization was detected with this analysis. By comparing multi-water status and multi-year analyses, we were able to identify constitutive QTLs for atmospheric (multi-year analysis) and soil water status conditions (multi-water status analysis) for transpiration rate on CS1, RGM3 and RGM16 and for transpiration efficiency on RGM5 and CS6 (Fig. 2).

The water status effect was significant for five QTLs (Table S2); for $\delta^{13}C$, the QTLs detected under conditions of water deficit accounted for a greater proportion of the variance, whereas those for TE detected under conditions of water deficit accounted for a weaker proportion of variance.

Comparison of QTL detection in multi-environment analysis with that in a single-year and single-water status analysis

Single-year and single-water status QTL analyses were also carried out. We detected 55 QTLs in these analyses, 27 of which were significant (Table S3), twice the number detected with the multi-year and multi-water status analyses. On average, we detected one to seven QTLs per trait, accounting for 3–15% of the phenotypic variance. No QTL for transpiration rate was detected with the single-trait QTL analysis, whereas significant QTLs were identified with the multienvironment analyses. Only QTLs for water extraction capacity were colocalized in all 3 yr of study; they were localized at one end of RGM3, close to the microsatellite marker VVMD36. Eighteen of the 27 significant QTLs identified with the single-year and single-water status approach were detected with the multi-environment analysis. Conversely, four of the 13 significant QTLs detected with the multi-environment analysis were not identified in the single-trait analysis. The various QTL analyses resulted in the identification of genomic regions of particular interest, on linkage group 6 and on CS1, CS17, CS19, RGM3, RGM5, RGM11, RGM13, RGM 16 and RGM18 (Fig. 2).

Colocalization of QTLs with genes potentially involved in water deficit responses

Several genes involved in responses to water deficit have been identified in herbaceous plants (e.g. Nilson & Assmann, 2007). We used the *V. vinifera* Pinot noir genome sequence ((Jaillon *et al.*, 2007); http://www.genoscope.cns.fr/spip/Vitis-vinifera-sequencage.html) to investigate the colocalization of genes potentially involved in water deficit responses with the QTLs identified in this work (Table 4).

Discussion

By strictly controlling water deficit through the daily weighing of pots, the use of response curves and a multienvironment approach, we were able to determine the genetic architecture of the regulation of transpiration in the scion by the rootstock in both well watered and water deficit conditions. We found that scion transpiration was controlled by a small number of loci, each accounting for < 10% of the phenotypic variance. We were also able to demonstrate that the genetic control of transpiration rate and water extraction capacity was completely independent of the genetic control of transpiration rate acclimation, as no colocalization of QTLs was observed for these traits.

QTL detection for traits relating to the acclimation of transpiration rate to water deficit

This work clearly demonstrates the power of traits relating to transpiration rate acclimation for QTL analysis. No QTLs were detected for mean transpiration rate in the single-year analysis. An analysis of the traits relating to the acclimation of

Table 3 Significant quantitative trait loci (QTLs) detected in the multi-year analysis of rootstock control of water deficit responses in grafted grapevine (in bold); suggestive QTL (P_{chr} > 0.05; in normal typeface) are given only when colocalized with a significant QTL or another suggestive QTL

									PEV %			*d*			
Trait	Year	Map	LG	LOD	P_G	L cM	L_{BS} cM	[–]at 0.05%	07	08	09	07	08	09	Year effect (*P*)
Tr_C	2008	F	CS1	**4.2**	**0.02**	**59.2**	**56.8 ± 10.4**	**36.3–75.5**	–	**7.0**	**7.1**	–	**−0.008***	**−0.011***	0.66
	2009	F	CS17	**4.1**	**0.0009**	**15.9**	**19.2 ± 11.1**	**0–40.9**		**8.7**	**3.4**		**0.008***	**0.006**	0.83
Tr_WD	2008	M	RGM3	2.1	0.63	22.9	20.1 ± 7.1	6.2–22.9	–	1.4	7.1	–	−0.0004	−0.0083*	0.12
	2009	M	RGM16	2.7	0.44	32.2	36.3 ± 16.1	4.7–67.9		2.6	8.2		0.0036	−0.0083	0.004
δ^{13}C_C	2008	M	RGM12	2.3	0.50	41.2	34.3 ± 24.4	0–82.2	–	2.7	13.8	–	0.12	−0.38*	0.03
	2009														
δ^{13}C_WD	2008	F	CS3	2.8	0.15	6.3	16.0 ± 14.5	0–44.5	–	5.5	8.3	–	0.39	−0.64*	<0.00001
	2009														
TE_C	2008	F	CS6	**4.8**	**0.0007**	**24.7**	**29.7 ± 9.8**	**10.5–48.9**	–	**2.5**	**10.7**	–	**1.1×10^{-4}**	**7×10^{-4} ***	0.001
	2009	F	CS12	2.3	0.39	41.8	34.6 ± 16.1	3.1–64.3		1.4	6.1		7×10^{-5}	5.3×10^{-4}*	0.06
		M	RGM11	**3.3**	**0.05**	**0**	**7.0 ± 16.1**	**0–38.6**		**4.7**	**5.4**		**-1.8×10^{-4} ***	**-4.2×10^{-4} ***	0.12
TE_WD	2008	F	CS6	2.3	0.25	34.3	33.6 ± 13.2	7.8–59.4	–	1.5	8.1	–	-5×10^{-6}	3.0×10^{-4}	0.006
	2009	M	RGM5	2.3	0.40	70.5	57.4 ± 22.8	12.7–71.1		6.5	4.3		-7×10^{-5}	1.1×10^{-4}	0.06
TTSW	2007	F	CS17	3.5	0.14	10.5	14.0 ± 12.3	0–38.2	0.7	2	9.6	2	−13	−74*	0.01
	2008	M	RGM2	3.6	0.18	3.7	6.7 ± 10.1	0–26.5	9.6	4.4	0.8	63*	39*	2	0.11
	2009	M	RGM3	**8.0**	**0.003[1]**	**5.9**	**5.8 ± 4.1**	**0–13.8**	**7.4**	**20.9**	**9.7**	**−26**	**−100***	**25**	0.03
		M	RGM3		**0.05[2]**	**22.9**	**22.5 ± 1.0**	**20.4–22.9**				**−37***	**58***	**−79***	0.002
		M	RGM5	**5.3**	**0.0009**	**68.8**	**67.5 ± 8.2**	**51.5–71.0**	**12.2**	**1.6**	**4.9**	**−72***	**−19**	**50***	<0.00001
		M	RGM8	3.3	0.21	55.4	47.4 ± 18.3	11.5–71.5	7.5	8.1	2.2	−47	−56*	−20	0.63
		M	RGM11	**4.3**	**0.02**	**57.2**	**54.6 ± 11.3**	**32.4–71.5**	**15.4**	**1.4**	**2.1**	**−86***	**−15**	**−24**	0.03
Coefμ	2007	M	RGM13	3.3	0.09	48.6	50.1 ± 18.6	13.6–86.5	8.3	7.2	0.6	0.024*	0.004*	-4.2×10^{-4}	0.005
	2008														
	2009														
NTR_{FTSW60}	2007	M	RGM13	2.9	0.36	45.9	55.4 ± 14.6	26.8–84.1	5.9	6.3	0.8	0.012	0.018	5×10^{-4}	0.009
	2008														
	2009														
NTR_{FTSW40}	2007	M	RGM13	3.1	0.28	56.4	54.5 ± 20.8	13.8–87.7	13.8	4.7	<0.1	0.056*	0.031	9×10^{-4}	0.04
	2008	M	RGM18	2.8	0.38	64.7	61.7 ± 25.1	12.6–110.8	2.9	7.2	<0.1	0.022	0.027	−0.007	0.06
	2009														
NTR_{FTSW20}	2007	M	RGM13	3.4	0.21	52.5	55.2 ± 18.8	18.3–87.7	11.0	7.6	0.2	0.11*	0.018	−0.001	0.009
	2008														
	2009														

Year, the year in which the trait was measured; Map: M, the male map; F, the female map; LG, linkage group; P_G, the level of significance at the genomic level, for a valid two-QTL model. [1]the test of H_2 vs H_0 and [2] of H_2 vs H_1; L, position of the QTL on the LG in cM estimated from the original data set; L_{BS}, position of the QTL, as calculated from bootstrap analysis ± SD; [–], confidence interval for $P = 0.05$; PEV, percentage of explained variance; *d*, effect of allelic substitution, effects marked with * are significantly different from zero; year effect was characterized by determining the probability of significant differences between the model in which each year has a different *d* effect and the model in which the *d* effect is similar between years.

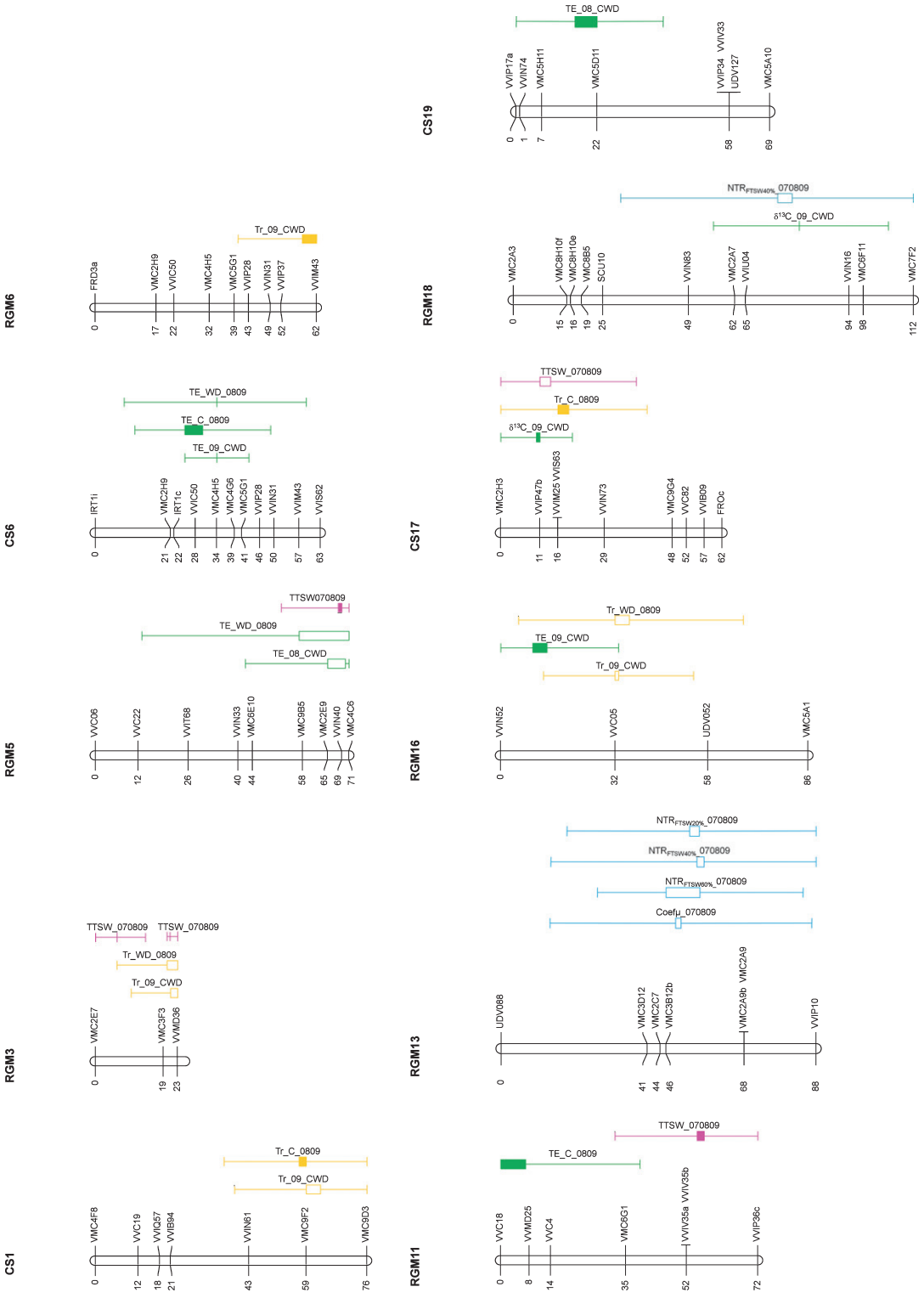

Fig. 2 Localization of the most important quantitative trait loci (QTLs) involved in the genetic determinism of transpiration and water-use efficiency responses to water deficit controlled by the rootstock. '070809' or '0809' is added after the trait abbreviation to indicate the year for multi-year analysis. 'CWD' is added after the trait abbreviation for multi-water status analysis. Bars indicate the range of mean positions from L and L_{BS} (see Table 3 and Table S2); black bars indicate significance QTL; white bars are suggestive QTL; whiskers are the 95% confidence interval around L_{BS}.

Table 4 List of genes involved in water-deficit responses localized in the confidence intervals of the quantitative trait loci (QTLs) detected in the multi-year analysis

Gene name	Linkage group of the QTL detected	Traits linked to the QTL detected	Grapevine locus	Function/homologue and its putative function	Reference
HOS10	RGM3	Tr; TTSW	GSVIVT00024036001	AT1G35515, *HIGH EXPRESSION OF OSMOTICALLY*	Zhu *et al.* (2005)
	CS17	Tr; $\delta^{13}C$; TTSW	GSVIVT00008402001	*RESPONSIVE GENES*, R2R3-MYB transcription	
	RGM18	$\delta^{13}C$; $NTR_{FTSW40\%}$	GSVIVT00012777001	factor that responds to osmotic stress and induced *NCED3*	
ABF3/AREB3	RGM3	Tr; TTSW	GSVIVT00031730001	AT4G34000, *ABRE BINDING FACTOR3*, an ABRE-binding bZip transcription factor, is a positive regulator of ABA responses	Kang *et al.* (2002)
OST1	RGM3	Tr; TTSW	GSVIVT00031806001	AT4G33950, *OPENSTOMATA1*, calcium-independent ABA-activated protein kinase involved in stomatal closure	Mustilli *et al.* (2002)
VvPIP2	RGM3	Tr; TTSW	GSVIVT00024010001	*PLASMA MEMBRANE INTRINSIC PROTEIN2*, aquaporin functioning as a water channel and inducing a higher water permeability under water deficit conditions	Galmés *et al.* (2007) Vandeleur *et al.* (2009)
NAC1	RGM3	Tr; TTSW	GSVIVT01023921001	AT1G56010, *NAC1* is a member of genes specific	Xie *et al.* (2000)
	RGM18	$\delta^{13}C$; $NTR_{FTSW40\%}$	GSVIVT01034485001	to plants involved in diverse set of developmental processes, one of which auxin-mediated lateral root development	
ABI1	6	Tr; TE	GSVIVT00024875001	AT4G26080, *ABA INSENSITIVE1*, a protein	Meyer *et al.* (1994)
	RGM16	Tr; TE	GSVIVT00018464001	phosphatase 2C, is a negative regulator of ABA responses	Tähtiharju & Palva (2001)
VvTIP1; VvTIP2	RGM16	Tr; TE	GSVIVT00024235001	*TONOPLAST INTRINSIC PROTEIN1* and *2*,	Chrispeels & Maurel (1994)
			GSVIVT00018678001	aquaporin	Galmés *et al.* (2007)
ERECTALIKE 2	RGM16	Tr; TE	GSVIVT00018490001	AT5G07180, *ERECTA LIKE2*, leucine-rich repeat receptor-like serine/threonine kinases regulates transpiration efficiency	Masle *et al.* (2005)
VvNCED	RGM13	Coefμ; $NTR_{FTSW60\%40\%20\%}$	GSVIVT00032103001	9-cis-epoxycarotenoid dioxygenase enzyme,	Soar *et al.* (2004)
	RGM16	Tr; TE	GSVIVT00024318001	catalyses the cleavage of the epoxy-carotenoids violaxanthin and neoxanthin to form xanthoxin a precursor of ABA	
AAO3	6	Tr; TE	GSVIVT00037549001	AT2G27150, *ABSCISIC ALDEHYDE OXIDASE 3*,	Seo *et al.* (2000)
	RGM18	$\delta^{13}C$; $NTR_{FTSW40\%}$	GSVIVT00025879001	catalyses the final step in ABA biosynthesis	
ACC	CS6	Tr; TE	GSVIVT01019414001	ATU26542, 1-aminocyclopropane-1-carboxylate	Young *et al.* (2004)
	RGM11	TE	GSVIVT01015239001	synthase-like protein catalyses the first step of	
	RGM16	Tr; TE	GSVIVT01018494001	ethylene biosynthesis; its mutation causes delayed leaf senescence and is linked to low ethylene leaf content	
ABH1/CP80	6	Tr; TE	GSVIVT00037230001	AT2G13540, nuclear mRNA cap-binding protein, is a negative regulator of ABA signalling; its mutation induces ABA hypersensitivity	Hugouvieux *et al.* (2001)
VvZEP	RGM13	Coefμ; $NTR_{FTSW60\%40\%20\%}$	GSVIVT00001372001	*ZEAXANTHIN EPOXIDASE*, catalyses the first step of ABA synthesis	Marin *et al.* (1996) Soar *et al.* (2004)
ERF7	RGM16	Tr; TE	GSVIVT0001393500	*ETHYLENE RESPONSE FACTOR 7*, has a role in ABA responses and which mutation reduced sensitivity to ABA	Song *et al.* (2005)

Table 4 (Continued)

Gene name	Linkage group of the QTL detected	Traits linked to the QTL detected	Grapevine locus	Function/homologue and its putative function	Reference
FRIGIDALIKE1	CS17	Tr; $\delta^{13}C$; TTSW	GSVIVT00008593001	AT5G16320, protein binding, control of flowering time, is thought to affect $\delta^{13}C$	Michaels *et al.* (2004) McKay *et al.* (2003)
MIZ1	CS17	Tr; $\delta^{13}C$; TTSW	GSVIVT01008216001	AT2641660, has an essential role for hydrotropism in roots	Kobayashi *et al.* (2007)

NTR, normalized transpiration rate; Tr, Transpiration rate per leaf area unit; TE, Transpiration efficiency; TTSW, total transpirable soil water.
A Blast analysis of the protein sequence against the grapevine proteome was used to obtain the position on the grapevine sequence genome.

transpiration rate (e.g. Coefμ, NTR at an FTSW of 60, 40 and 20%) led to the identification of one QTL on RGM13 common to all traits and years of study. This QTL was different from all those detected for the other three trait categories (i.e. transpiration rate, WUE and water extraction capacity traits).

Our results demonstrate that the effects of the rootstock on transpiration rate and its acclimation to soil water deficit are subject to independent genetic controls, because no QTL common to these effects was identified. Our results are consistent with the 'epistatic model' proposed by Via *et al.* (1995), as the QTLs for the acclimation of transpiration rate to water deficit did not colocalize with the QTLs for transpiration rate. Given current predictions about climate change, the selection of genotypes with a large capacity to respond to changes in their environment is of particular interest (Nicotra & Davidson, 2010) for perennial plants, as this response may be considered adaptive. This study is the first to use functional mapping to describe the genetic basis of the acclimation to soil water deficit induced by the root system.

At the multienvironment scale, NTR traits at three levels of FTSW allowed us to determine whether the genetic architecture of the acclimation of transpiration rate was dependent on the intensity of water deficit. All these traits were highly correlated, but they were nonetheless retained for the analysis. The QTLs detected for NTR at FTSW values of 60 and 40% were not different from those detected at an FTSW of 20%. This result suggested that the genes involved in the acclimation of transpiration rate were not specific to increasing water deficit.

Multi-year and multi-water status QTL analysis

The single-year QTL detection analysis was greatly improved by the use of a multienvironment approach, which identified stable QTLs independently of amount of soil water, atmospheric variation and grafting success. This approach was particularly successful for the detection of transpiration rate QTLs (Tr_C and Tr_WD), for which the single-year analysis yielded no QTLs. Multi-year analysis had a higher power for QTL detection than single-year analysis. Indeed, the QTLs detected generally accounted for a smaller proportion of the variance than those detected in single-year analysis (Brendel *et al.*, 2008). The QTL accounting for the smallest proportion of the variance accounted for 5% of the variance in single-year analysis, but < 1% of the variance in the multi-year analysis. Despite having a greater power for QTL detection, the multienvironment analysis identified fewer QTLs than the single-year analysis, which is unusual (Parelle *et al.*, 2007; Brendel *et al.*, 2008). Muranty (1996) established that QTL detection power depends on trait heritability, explained phenotypic variance and pedigree size. Based on the work of Muranty (1996), we estimate a QTL detection power of 28–57% in this study. Consequently, the real number of QTLs involved is underestimated. The lack of a strong correlation for a given trait between different years of measurement may account for the inability of multi-environment QTL analysis to decrease the confidence intervals in our study.

The multienvironment analysis also made it possible to dissect the year and water status effects on each QTL. In previous studies

on the genetic determinism of WUE, stable QTLs were detected reproducibly at different experimental sites (Price *et al.*, 2002) and over 3 yr in a multi-year QTL analysis (Brendel *et al.*, 2008), but QTL effect × environment interactions were not extensively investigated. We identified three types of QTL with multi-year and multi-water status analysis: QTLs with effects not significantly influenced by year and water status (CS1 for Tr and RGM5 for TE); QTLs with effects influenced significantly by either year or water status (RGM3 for Tr, which was unaffected by year but subject to a significant water status effect, the reverse being true for RGM16 for Tr); and QTLs the effect of which was significantly affected by both year and water status (CS6 for TE). These environmental interactions highlight the difficulties involved in understanding the genetic determinism of transpiration and WUE (Van Eeuwijk *et al.*, 2010): several of the QTLs identified explain < 10% of the phenotypic variance and have effects significantly influenced by the environment. The traits related to drought response are composite (several mechanisms involved); consequently, it was quite predictable that their genetic architecture is based on the Fisher's infinitesimal model.

WUE-related traits

Our work provides new insight into the genetic architecture of TE, which, unlike $\delta^{13}C$, is rarely studied at the whole-plant level. The broad-sense heritability calculated here was lower than that reported by Brendel *et al.* (2008) for oak (0.8). In our work, WUE-related traits were mediated exclusively by rootstock genetic variability, rendering the model more complex. No QTLs common to TE and $\delta^{13}C$ were identified, supporting the hypothesis of independent genetic architectures. This suggests that $\delta^{13}C$ is not an 'easy' trait to study when trying to select plants with a high WUE (Condon *et al.*, 2004), particularly in grapevine rootstock breeding programmes (Flexas *et al.*, 2010). The QTLs detected for TE in multi-year and multi-water status analyses were colocalized, suggesting that the genetic architecture for TE is stable in all environments and thus potentially easier to use for breeding purposes.

TTSW can be used to study extraction capacity and to fit NTR acclimation

In this study, we characterized the acclimation of plant transpiration rate over the course of the water deficit regime by expressing the available soil water as the FTSW. The relationship shown here between FTSW and predawn leaf water potential demonstrates that FTSW accurately describes the intensity of the water deficit (Fig. S5; FTSW = 85.4 $\exp(2.03\Psi p)$; $r^2 = 0.73$). The three thresholds of 60, 40 and 20% of FTSW correspond to an absence of water deficit (> −0.2 MPa), a weak water deficit (> −0.4 MPa) and a moderate to severe water deficit (< −0.7 MPa), respectively (Van Leeuwen *et al.*, 2009).

Fraction of transpirable soil water is a widely used parameter in water deficit experiments (Lecoeur & Sinclair, 1996; Sadras & Milroy, 1996; Lacape *et al.*, 1998; Lebon *et al.*, 2003; Bindi *et al.*, 2005) and has been used to characterize the transpiration and growth responses to water deficit of various sunflower genotypes (Casadebaig *et al.*, 2008) and transgenic lines of chickpea (Bhatnagar-Mathur *et al.*, 2009). Total transpirable soil water provides information about water extraction capacity in relation to the total water pool (Sinclair & Ludlow, 1986), making it possible to compare phenotypic responses between genotypes for similar proportions of water.

Physiological characterization of the rootstock response to water deficit

Use of the concept of TTSW made it possible to study the genetic control of the water extraction capacity of the rootstocks. The occurrence of transgression for this trait may be linked to the presence of complementary QTL alleles in the two parental species (Rieseberg *et al.*, 1999). The broad-sense heritability was highest for TTSW and similar to the values reported by Asins *et al.* (2010) for traits related to rootstock-induced salinity resistance in *Solanum* spp. However, as for the other traits studied, broad-sense heritability values differed considerably between the experimental years 2008 and 2009. A genomic region for this trait was identified on linkage group 3, for all 3 yr. This suggests that rootstocks may differ in their ability to extract water from the soil. This hypothesis was also proposed by Carbonneau (1985) and Soar *et al.* (2006). Given the behaviour of the male parent of the *V. riparia* cv. Gloire de Montpellier pedigree (Fig. S3A, 3C), which is known to be drought-sensitive, the sensitive rootstock may induce low rates of scion transpiration under drought conditions and an early decrease in transpiration (low Coefμ) when the soil is drying. Similarly, Alsina *et al.* (2011) showed that scion stomatal conductance was more strongly down-regulated with drought-sensitive than with drought-tolerant rootstocks. According to Chaves & Davies (2010), the relative importance of the hydraulic and chemical signals sent from drying roots to the shoot for the regulation of stomatal aperture remains a matter of debate. It has been suggested, but not unequivocably demonstrated, that rootstock effects are mediated principally by chemical signalling (Soar *et al.*, 2006; Alsina *et al.*, 2011). However, differences have been reported between rootstocks, in terms of the hydraulic characteristics of roots and the root/scion interface (Peterlunger *et al.*, 1990; De Herralde *et al.*, 2006; Lovisolo *et al.*, 2008). A role for root aquaporins in stomatal regulation through hydraulic signalling has also recently been demonstrated (Vandeleur *et al.*, 2009). The QTLs identified and the genes included in the QTL confidence intervals, despite their large size, are consistent with these hypotheses.

Hypothesis concerning candidate genes involved in ABA and hydraulic signalling

This study identified eight principal genomic regions associated with scion transpiration-related traits. The confidence intervals of these QTLs encompassed a number of genes potentially involved in water deficit responses. First, these coincidences suggest that the QTLs detected are valid. Second, they suggest that hormonal (particularly for ABA) and hydraulic (aquaporins) signalling

between the scion and rootstock plays an important role in responses to water deficit (Soar *et al.*, 2006; Vandeleur *et al.*, 2009). It is not easy to identify the allelic variation underlying a QTL (from 350 to 800 genes lying in the QTL confidence interval, data not shown and obtained with Flagdb software), but this has been successfully achieved in a number of cases (e.g. Fournier-Level *et al.*, 2009). It has been suggested that the position of the QTL peak accurately indicates the position of the genes or gene clusters responsible (Price, 2006). The density of microsatellites on the map was not sufficient for accurate positioning on the genome sequence, but the list of candidate genes provided here could be used as a starting point for further studies based on forward (association mapping) or reverse genetic approaches.

Conclusions

This is the first quantitative genetic study of the acclimation of transpiration rate to water deficit in higher plants. In addition, the use of a grafted plant model made it possible to identify specifically QTLs for the control of transpiration responses to water deficit by the roots. The multienvironment QTL analysis demonstrated the need to take environmental variations into account when trying to detect QTLs for transpiration rate. The differences in the QTLs detected for transpiration rate *per se* and transpiration rate acclimation to water deficit suggest that genetic control is independent for these two aspects. This work highlights the value of studying drought responses and their relationship to drought intensity, and the role of the rootstock in controlling scion transpiration.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Leaf area in relation to main vein length for each experiment, for each year: 2007, 2008 and 2009.

Fig. S2 Shoot biomass plotted as a function of shoot length.

Fig. S3 Phenotypic distributions of the traits of interest of the mapping population CS×RGM1995-1.

Fig. S4 Correlation circle in the main plane (F1 × F2) of the principal component analysis, (a) for the five variables in 2007, (b) for the 11 variables in 2008, and (c) for the 11 variables in 2009.

Fig. S5 Fraction of transpirable soil water (FTSW) plotted as a function of predawn leaf water potential (ψ_p).

Table S1 Characterization of the climate of the glasshouse for each experiment

Table S2 Significant QTLs detected in the multi-water status analysis of the rootstock control of water deficit responses in grafted grapevine

Table S3 Significant QTLs detected in the single-year analysis of rootstock-controlled water deficit responses in grafted grapevine

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