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ORIGINAL PAPER

Mapping genetic loci for tolerance to lime-induced iron deficiency chlorosis in grapevine rootstocks (*Vitis* sp.)

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Abstract Iron is essential to plants for chlorophyll formation as well as for the functioning of various iron-containing enzymes. Iron deficiency chlorosis is a widespread disorder of plants, in particular, of those growing on calcareous soils. Among the different ways to control iron deficiency problems for crops, plant material and especially rootstock breeding is a suitable and reliable method, especially for fruit trees and grapes. The aim of the experiment was to characterize the genetic basis of grapevine chlorosis tolerance under lime stress conditions. A segregating population of 138 F1 genotypes issued from an inter-specific cross between *Vitis vinifera* Cabernet Sauvignon (tolerant) × *V. riparia* Gloire de Montpellier (sensitive) was developed and phenotyped both as cuttings and as rootstock grafted with Cabernet Sauvignon scions in pots containing non-chlorosing and chlorosing soils. Tolerance was evaluated by chlorosis score, leaf chlorophyll content and growth parameters of the shoots and roots. The experiments were performed in 2001, 2003 and 2006. The plants analysed in 2006 were reassessed in 2007. The most significant findings of the trial were: (a) the soil properties strongly affect plant development, (b) there are differences in tolerance among segregating genotypes when grown as cuttings or as rootstocks on calcareous soil, (c) calcareous conditions induced chlorosis and revealed quantitative trait loci (QTLs) implicated in polygenic control of tolerance, (d) rootstock strongly contributes to lime-induced chlorosis response, and (e) a QTL with strong effect (from 10 to 25 % of the chlorotic symptom variance) was identified on chromosome 13. This QTL colocalized with a QTL for chlorophyll content ($R^2 = 22$ %) and a major QTL for plant development that explains about 50 % of both aerial and root system biomass variation. These findings were supported by stable results among the different years of experiment. These results open new insights into the genetic control of chlorosis tolerance and could aid the development of iron chlorosis-tolerant rootstocks.

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Introduction

Iron deficiency chlorosis is a worldwide problem in crop production on calcareous soils (Álvarez-Fernández et al. 2005; Abadia et al. 2011). Lime-induced iron deficiency chlorosis is the main constraint for successful production of several fruit tree crops cultivated in many production areas worldwide (Rombolà and Tagliavini 2006), and it strongly impacts fruit industry because it affects yield and fruit quality, and on the other hand the fertilizers used for controlling and preventing iron chlorosis are often expensive, not very effective in the long term, and some are considered as not environmentally friendly (Abadia et al. 2011). Symptoms of iron chlorosis consist of a yellowing of leaf laminae, while the veins remain green in general. Yellowing, which is associated with the lack of chlorophyll, starts usually from the shoot tips, extends downwards, ending with necroses and fall of leaves in the more serious situations (Tagliavini and Rombolà 2001). Chlorotic symptoms exhibit temporal and spatial variability (Rombolà and Tagliavini 2006).

Iron is essential for chlorophyll biosynthesis (Jeong and Connolly 2009) and for many physiological processes

related to plant growth and development. Although iron is one of the most abundant metals in the earth's crust, its availability for plant roots is very low (Morrissey and Guerinot 2009), because it is mainly present as insoluble ferric oxides in the soil (Lindsay 1984). In addition, many environmental conditions, including high pH, high bicarbonate and water content can result in a further reduction of Fe^{2+} availability ("lime-induced chlorosis"; Mengel 1994). Plants use two mechanisms for Fe uptake: a reduction-based strategy (Strategy I) and a chelation-based one (Strategy II), the latter restricted to grasses (Römheld and Marschner 1986). Dicot species, including grapevine, utilize the Strategy I mechanism to absorb the Fe^{2+} ion (Varanini and Maggioni 1982; Jimenez et al. 2007). Roots of Strategy I plants utilize an ATPase to excrete fvprotons, which acidifies the rhizosphere and favours the release of Fe from chelating agents in the soil. A root membrane reductase reduces the prevalent Fe^{3+}ion to the biologically usable Fe^{2+} ion, which can then be absorbed by the roots and transported into the plant where it is available for use in various cellular processes. For Strategy I plants, iron reduction is the rate-limiting step which results in iron deficiency (Vert et al. 2003; Schmidt 2006). Strategy II plants, such as monocot species, release phytosiderophores from the roots that chelate Fe^{3+} ions. The entire phytosiderophore iron complex is then transported into the root system of the plant. Many of the molecular components of both strategies have been elucidated in the last two decades (Vert et al. 2003; Bauer et al. 2004; Schmidt 2006; Jeong and Connolly 2009; Morrissey and Guerinot 2009).

In practice, growers may take different steps in order to prevent and control the development of lime-induced Fe chlorosis. The use of synthetic chelates (EDTA, EDDHA and DTPA chelates), alternative fertilizers or soil management practices represent adequate strategies to solve the problem, but they may be costly and not always very efficient (Rombolà and Tagliavini 2006). The choice of adapted plant material, and especially tolerant rootstocks in orchards and vineyards, appears to be a sustainable and reliable method (Sociasi y Company et al. 1995; Bavaresco et al. 2010). Breeding and selecting tolerant plant material in perennial crops is time-consuming. It can be accelerated using molecular markers linked to a specific trait and explaining a major part of its phenotypic variance. So far, knowledge about the genetic determinism of resistance to lime-induced iron chlorosis is limited to few annual crops and one perennial (Srinives et al. 2010, Gonzalo et al. 2012). Quantitative trait loci (QTL) analyses were performed in soybean (Lin et al. 1997, 2000), maize (Nourse et al. 1999), mungbean (Srinives et al. 2010), *Phaseolus vulgaris* (Blair et al. 2010) and *Lotus japonicus* (Klein et al. 2012). Molecular markers associated with lime-induced chlorosis were identified for soybean (Charlson et al. 2003; Wang et al. 2008) and mungbean (Srinives et al. 2010). A single QTL was detected for ferric reductase activity in both studies on *P. vulgaris* and *L. japonicus*, but none of these QTLs co-localized with a ferric reductase gene FRO (Blair et al. 2010; Klein et al. 2012). Recently, Gonzalo et al. (2012) detected several QTLs for leaf chlorophyll content of *Prunus* trees growing under calcareous conditions. A candidate gene PFIT, associated with the Fe deficiency-induced transcription factor (Bauer et al. 2007) was localized within the confidence interval of one major QTL.

Grapes (*Vitis* spp.) are the most important fruit crop in terms of area; in 2011, world vineyards reached a total surface of 7.59 Mha with a global production of 691.7 Mqx (http://www.oiv.int/oiv/files/0-Actualites/EN/Report.pdf). *Vitis vinifera* is the most cultivated grape species throughout the world. However, since the second half of the nineteenth century, most vineyards worldwide are grafted. *V. vinifera* is highly sensitive to the soil born pest *Daktulosphaera vitifoliae* (phylloxera) originated from North America and introduced to most of the vine growing areas since then (Pouget 1987). Grapevine rootstocks are pure *Vitis* species or hybrids, mainly between *Vitis riparia, Vitis rupestris* and *Vitis berlandieri* which differ in their responses when grown in calcareous soil (Bavaresco et al. 1994). In addition to phylloxera tolerance, these rootstocks provide varying degrees of protection to biotic and abiotic problems including nematodes, drought, salt and iron deficiency chlorosis (Anwar et al. 2002; May 1994; Bavaresco et al. 1991).

As for other woody perennial fruit plants, breeding grapevine is not an easy task. In addition to long cycles between generations, grape genomes are highly heterozygous, and the majority of traits are not strongly heritable and under polygenic control. Therefore, the development of genetic maps based on molecular markers is interesting to identify and locate genes and QTL for agronomic traits of interest. This will help assist breeding by early seedling selection. For instance, previously published maps for grapevine (Troggio et al. 2007; Vezzulli et al. 2008) allowed the identification of QTLs for fruit quality (Doligez et al. 2006; Fanizza et al. 2005; Fournier-Level et al. 2009), phenology-related traits (Costantini et al. 2008), or disease resistance (e. g. Blasi et al. 2011; Fischer et al. 2004; Lowe and Walker 2006; Riaz et al. 2006, 2008, 2011; Xu et al. 2008; Marguerit et al. 2009; Salmaso et al. 2008; Zhang et al. 2009; Welter et al. 2007). Few analyses of abiotic stress tolerance were released (Mandl et al. 2006), with only one dealing with rootstocks (Marguerit et al. 2012). Rootstock brings an additional complexity since the behaviour of ungrafted rootstock may be different from that of a scion grafted onto the rootstock (Pouget and Ottenwælter 1973; Sociasi y Company et al. 1995). Grafting must be taken into account when testing for

rootstock resistance to chlorosis (Pouget and Ottenwælter 1973; Bavaresco et al. 1993; Bavaresco and Lovisolo 2000). Tomato is the only species so far for which the same population was studied ungrafted and as rootstocks in grafted plants in order to study rootstock effects on yield and salt tolerance (Villalta et al. 2007; Estan et al. 2009; Asins et al. 2010).

The objective of the present study was to analyse the genetic determinism of iron chlorosis tolerance in grapevine. We report the identification and analysis of QTLs using an F1 SSR map derived from an inter-specific cross of traits associated with lime-induced chlorosis and plant growth. Ungrafted and grafted plants with the progeny studied as rootstocks were compared. Chlorosing and non-chlorosing soils were used to grow the plants. The results recorded during the first growth cycle on grafted plants growing in chlorosing soils were repeated in three independent experiments performed over 3 years. In one case, phenotyping was performed on the same plants over two consecutive growing seasons. The results presented herein provide valuable information for future marker-assisted selection for grapevine rootstocks.

Materials and methods

Plant material

The segregating population used for genetic mapping consisted of 138 F1 individuals issued from an inter-specific cross between *V. vinifera* Cabernet Sauvignon (CS) × *V. riparia* Gloire de Montpellier (RGM). RGM is sensitive to iron chlorosis whereas Cabernet Sauvignon is more resistant (Ollat et al. 2003). This F1 population, named CS × RGM1995-1, was developed at INRA, Bordeaux (France).

Plant growth conditions

One hundred and thirty-eight full sib individuals and the parents of the progeny CS × RGM1995 were tested in different experiments run over one growth cycle in 2001 and 2003 and over two-growth cycles in 2006–2007.

In 2001, the progeny was phenotyped on the one hand as cuttings and on the other hand as rootstocks grafted with Cabernet Sauvignon scions (clone 191) in two different soil types. Three plants were distributed in a randomized block design of 3 and 2 blocks, respectively, for chlorosing soil (pH H_2O 8.4, pH KCl 7.5, total $CaCO_3$ 295 g/kg, easily extractible Fe 76 mg/kg and active lime 14 %) and non-chlorosing soil (pH H_2O 7.8, pH KCl 7.3, total $CaCO_3 < 5$ g/kg, easily extractible Fe 590 mg/kg and active lime < 3 %). Plants were grown in 4 L pots, trained to a single stem and axillary buds were removed every week. Plants were sub-irrigated once a day with a nutrient solution without iron. The composition of the nutrient solution was: 2.5 mM KNO_3, 0.25 mM $MgSO_4$, 0.62 mM NH_4NO_3, 1 mM $(NH_4)H_2PO_4$, 9.1 mM $MnCl_2$, 46.3 mM H_3BO_3, 2.4 mM $ZnSO_4$, 0.5 mM $CuSO_4$ and 0.013 mM $(NH_4)_6Mo_7O_{24}$. High level of humidity in the soil was used to maintain a high concentration of bicarbonates in the soil solution (Brand et al. 2000).

In the years 2003 and 2006–2007, the experiments were performed only on grafted plants with the progeny used as a rootstock with Cabernet Sauvignon (clone 191) as a scion. Plants were grown in chlorosing soil with a four-block random design, and trained under the same conditions as described for 2001. In 2007, the same plants grown in 2006 were phenotyped for a second growth cycle.

Recorded traits

Plant responses to chlorosing conditions were quantified by recording leaf chlorotic symptoms and plant growth parameters each year at two dates, first when symptoms were highly pronounced (named t1), and then 15–30 days later when some plants started to recover (named t2).

Leaf chlorotic symptoms were estimated visually on young apical leaves by the Pouget index (Pouget and Ottenwælter 1978), ranging from 0 (no symptoms, deep green leaves) to 5 (severe symptoms, yellow leaves, with more than 10 % of the blade necrotic). Chlorophyll content on corresponding leaves was evaluated by SPAD measurements (Bavaresco 1995) based on the optical density difference at two wavelengths (650 nm red and 940 nm infrared) using a SPAD 502 (Soil and Plant Analyzer Development; Minolta Corp., Ramsey, NJ, USA) portable leaf greenness chlorophyll meter (Peryea and Kammereck 1997). The SPAD-502 meter non-destructively measures leaf greenness. A positive linear relationship has been demonstrated between SPAD measurements and total extracted chlorophyll for a range of plant species (Yadava 1986). Expected values ranged from 0 for necrotic leaves to almost 40 for healthy leaves.

For the 2001 experiment, the Pouget index was noted on cuttings on the last expanding leaves, i.e. for leaf numbers 4–6 on August 2nd and on leaf numbers 6 and 7 on August 14th. Chlorophyll content was measured on leaf numbers 3 and 5 on July 30th and on leaf numbers 6 and 7 on August 14th. For grafted plants, the Pouget index and chlorophyll content were recorded on leaf numbers 3 and 5 from apex on July 27th, and numbers 5 and 6 on August 3rd. In 2003, trait notation was performed for the Pouget index (June 19th and July 4th) and chlorophyll content (June 26th and July 7th). In 2006, the Pouget index was the only recorded trait (July 27th and August 23th). In 2007, the Pouget index

and chlorophyll content were measured on June 19th and July 4th.

Growth and plant development were evaluated by stem length measurements performed at the same dates than leaf chlorotic symptoms in 2001, 2003, 2006 and 2007. Dry weight of stems and leaves were recorded at the end of the growth cycle in 2001, 2003 and 2007. As the plants were kept at the end of 2006 for a second growth cycle, dry matter of the overwintering shoots was determined when pruning was performed in February 2007. In 2003 and 2007, root dry weight was also determined in 2003 and 2007. Dry weights were determined after drying the plant parts in an oven at 80 °C for 48 h.

Genetic mapping

A version of the CS × RGM genetic map was published by Marguerit et al. (2009) using a F1 pseudo-testcross mapping strategy (Grattapaglia and Sederoff 1994). This map was built with 212 genetic markers and covered 1,249 cM. Genotyping was completed for some individuals of the progeny that had partially missing data for some SSR markers. Information on molecular markers characteristics, genotyping conditions and map construction parameters were the same as those described previously (Marguerit et al. 2009). Added small letters to the SSR marker names correspond to different loci on the genetic map associated with the same SSR primers. Joinmap 3.0 (Van Ooijen and Voorrips 2001) was used to construct the genetic linkage map with Kosambi mapping function (Kosambi 1944) and a step-wise decrease of the LOD score from 7 to 3 with a maximum recombination rate of 50 % to assign markers to linkage groups. Linkage groups are in the same order and orientation according to IGGP (http://www.vitaceae.org/index.php/Maps_and_Markers; Adam-Blondon et al. 2004) and the assembly of the chromosomes of PN40024.

In addition, genetic markers were developed from functional candidate gene corresponding to putative metal transporter genes: IRT1f (GSVIVT00030117001), IRT1d (GSVIVT00037540001), IRT1a and IRT1h2 (GSVIVT00032208001), IRT1i (GSVIVT00024060001), IRT1c (GSVIVT00024285001), IRT1c (GSVIVT00024285001), IRT1e (GSVIVT00033352001) and ferric reductase genes: FROb (GSVIVT00026930001), FROc (GSVIVT00015996001), FROd (GSVIVT00011355001).

QTL analysis

Broad sense heritability (H^2) was estimated for each trait as a ratio between genotypic ($\sigma^2 g$) and residual ($\sigma^2 e$) variances: $H^2 = \sigma g^2/\sigma p^2 = \sigma f^2/(\sigma f^2 + \sigma e^2/r)$, where the variance components are: σg^2, genetic variance; σp^2, phenotypic variance; σf^2, family variance; σe^2, error variance; and r, number of replications (Holland et al. 2003).

QTL analysis was performed on consensus map using MapQTL 4.0 (Van Ooijen 2000). Data for each experiment and for each trait were analysed separately. The Kruskal–Wallis test (a non-parametric equivalent of the one-way ANOVA) was used in a preliminary analysis to detect associations between markers and individual traits. Markers showing significant association at the most stringent 0.005 significance level (suggested by the authors of the software, Van Ooijen 2000) or higher in at least one experiment were considered putative. Putative QTL were confirmed by interval mapping (IM) to identify the major QTL. Automatic cofactor selection was used to fit the multiple QTL model (MQM) (backward elimination; $P < 0.02$) and to detect significantly associated markers as cofactors (6 maximum). For each trait, a permutation test was performed to identify the LOD threshold corresponding to a genome-wide false discovery rate of 5 %. Based on the permutation tests (1,000 permutations), a threshold LOD value was used to declare the presence of QTL in the interval MQM analyses. QTL identified in more than one trial which mapped closely to one another on the same linkage group within the same confidence interval were considered to represent the same QTL. Graphical representation of linkage groups and QTL was carried out using MapChart 2.2 software (Voorrips 2002). The abbreviations used for the QTLs correspond to the trait initials followed by the year, the conditions of the experiment and the linkage group number where the QTL mapped, e.g. QTL "pi01_ng_c-t1_9" means QTL identified for the Pouget index on 2001 experiment using non-grafted plants, under chlorosing condition at t1 date that located on LG9.

Results

Genetic map

The fact that we completed genotypic data for some previous missing data in SSR genotyping had very little impact on map organization. In this new and complete version, minor modifications were observed on LG3, LG4, LG6, LG9, LG10, LG13, LG16, LG18 and LG19 where local ordering for some closely linked markers had changed (LG3, LG4, LG9 and LG18). Newly linked markers were placed on LG3 (VMC9F4cs), LG6 (VVIP37), LG9 (VMC9F4y), LG10 (VRZAG67), LG13 (VMC8E6), LG16 (VVIN52), LG18 (VMC2A3 and VMC7F2) and LG19 (VMC9F4rgm). Previously mapped markers were removed from LG3 (VVHO2 and VVIB59), LG4 (VMC4D4), LG6 (VMC2G2), LG10 (VVIN85 and VMC3E11-2), LG13

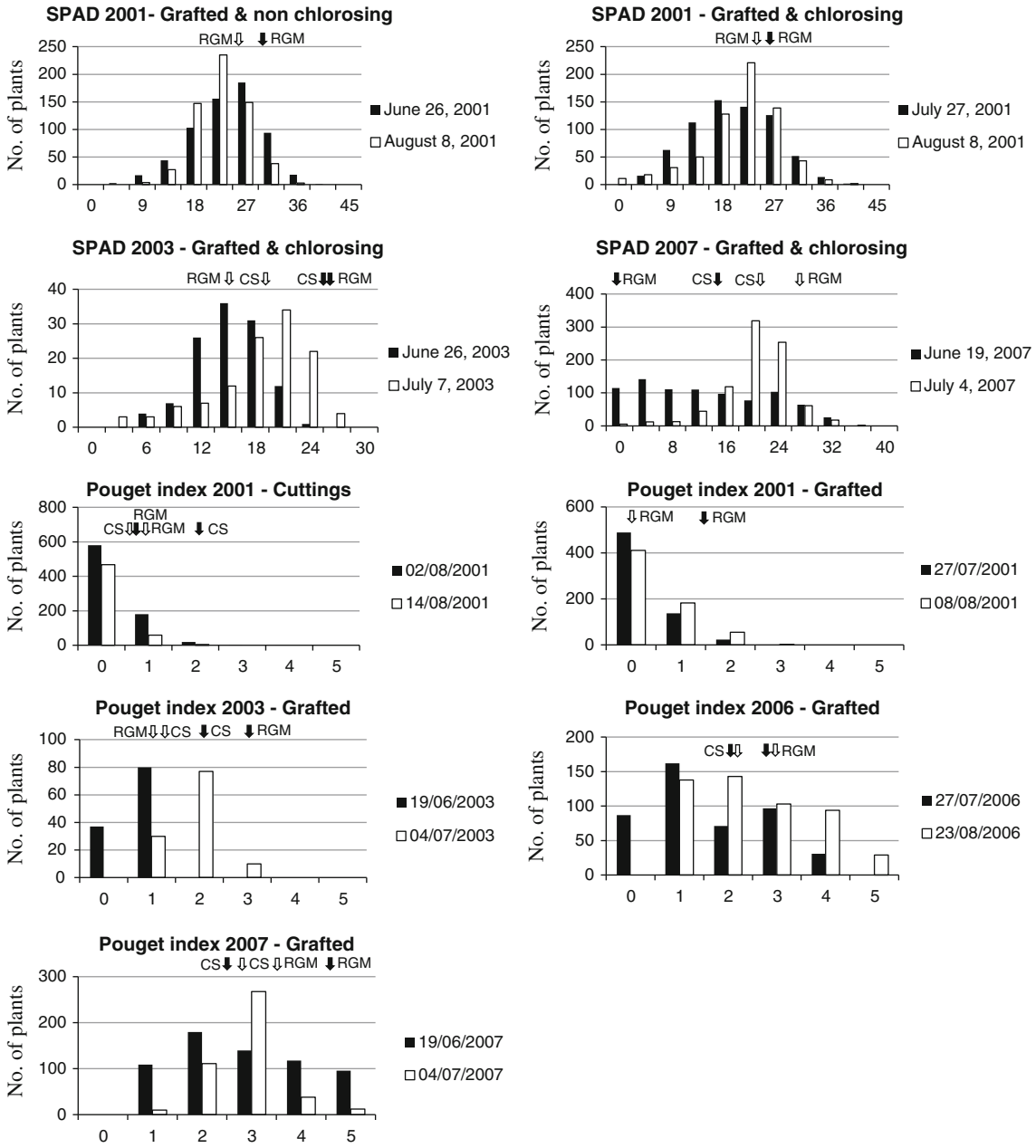

Fig. 1 Distributions of visual scores (Pouget index), chlorophyll concentrations (SPAD) and growth parameters during 2001, 2003, 2006 and 2007 in the CS × RGM F1 population. Parental values are indicated by *arrows*. The number of plants, varying between the different experimentations, corresponds to the number of plants phenotyped for trait evaluations

(VVIC51 and VMC3B12), LG16 (VVMD37), LG18 VMC7F2) and LG19 (VVIP17a and VVIP34), leading to few size variations in these linkage groups and to a more reliable assignation of markers on the map. However, consistency of markers localizations was checked according to other SSR genetic maps (Doligez et al. 2006; Lowe and Walker 2006) and physical map of the genome sequence (Jaillon et al. 2007; http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/). Finally, the mean density of one marker each 7 cM was well suitable for QTL analysis.

Phenotypic variation and trait correlations

Frequency distributions of all traits segregating among the F1 population are presented in Fig. 1 including parental values for CS and RGM. All traits showed approximately Gaussian continuous distributions, and transgressive segregants (Tables 1, 2; Fig. 1) indicating a polygenic inheritance.

Heritability, range, mean value, and standard deviation for all traits among segregating population are summarized in Table 1 (chlorosing parameters) and Table 2 (growth

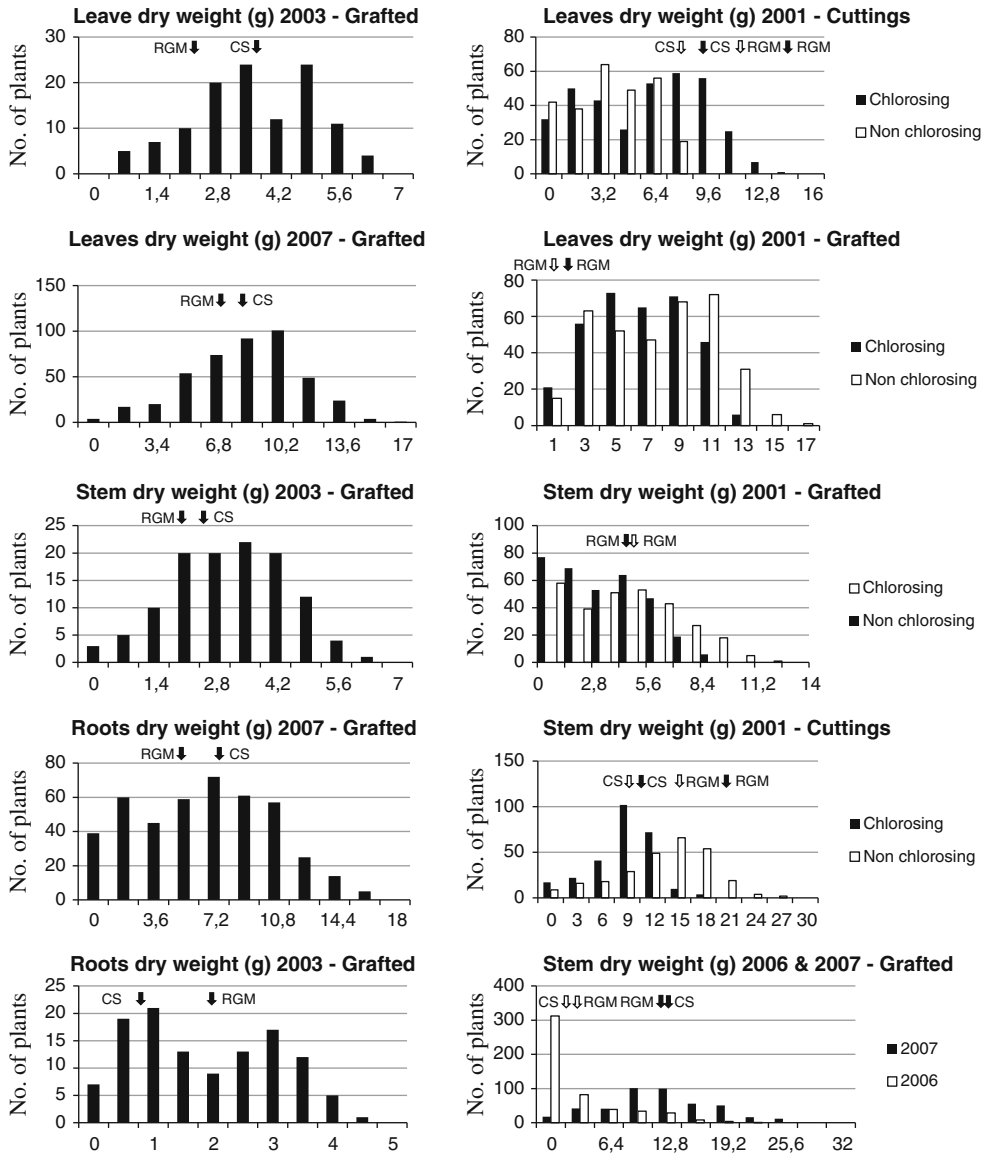

Fig. 1 continued

parameters) together with mean values for CS and RGM. Parental lines had similar trait values in all 4 years. Heritabilities for plant growth traits were high for all years and greater in 2001, which ranged from 0.48 to 0.86, compared to 2003 (0.37–0.55), 2006 (0.26–0.59) and 2007 (0.51–0.65). Chlorosis tolerance traits were more influenced by the environment, ranging from high values in 2001 for the Pouget index (0.46–0.68) to lower values in 2003 (0.11–0.12), 2006 (0.14–0.40) and 2007 (0.08–0.29). For SPADs, values ranged from 0.18 to 0.54 in 2001 and were lower in 2003 (0.07–0.10) and 2007 (0.16–0.33).

Correlations between the Pouget index and chlorophyll content evaluated by SPAD values were informative when recorded on the same leaves and at the same date. These two traits were negatively correlated ($-0.37 < r < -0.88$), confirming the observation than the more chlorosis symptoms, the less chlorophyll content in leaves. For each year of test, growth parameters (i.e. stem length, leaf number, root, stem and leaf weight) showed highly significant correlations between each other suggesting that the global plant development program was affected instead of local and particular plant organ modifications induced by chlorosing environment.

In 2001, stem length for plants growing under chlorosing and non-chlorosing soils were significantly correlated ($r = 0.65$ for cuttings and $r = 0.91$ for grafted plants; Table 3). No significant correlations were observed for

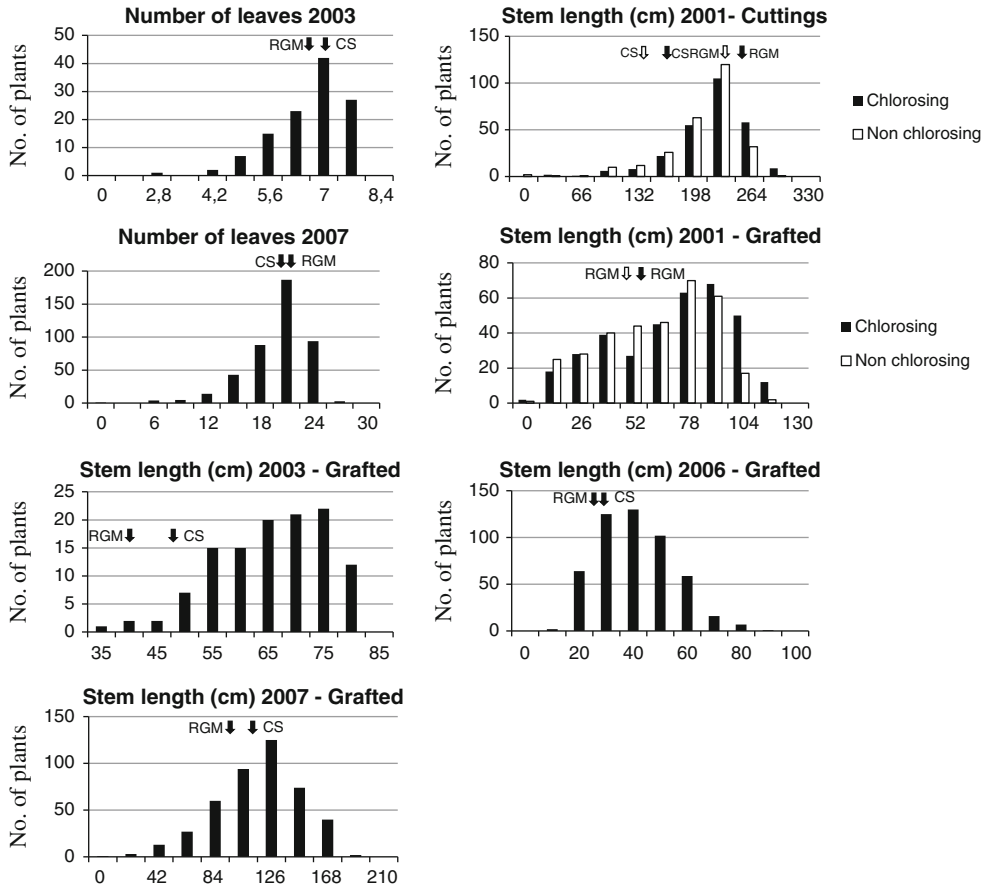

Fig. 1 continued

stem length between plants grown as cuttings and grafted plants whether cultivated under chlorosing and non-chlorosing ground suggesting different mechanisms of response between these two plant systems (data not shown).

Finally, significant correlations were detected among all years of experiments. For instance, SPAD were significantly correlated between 2001 and 2003 ($r = 0.27$) and between 2001 and 2007 ($r = 0.26$) and the Pouget index was also significantly correlated between 2001 and 2006 ($r = 0.18$), 2003 and 2006 ($r = 0.19$), and 2006 and 2007 ($r = 0.34$). For growth parameters, stem length was significantly correlated for all years of experiments, ranging from $r = 0.48$ between 2003 and 2006 to $r = 0.77$ between 2006 and 2007. The same general observations were made for leaf dry weight ($r = 0.44$ between 2003 and 2007; $r = 0.71$ between 2001 and 2007), stem dry weight ($r = 0.45$ between 2003 and 2007; $r = 0.73$ between 2001 and 2007), root dry weight ($r = 0.58$ between 2003 and 2007) and number of leaves ($r = 0.39$ between 2001 and 2003; $r = 0.43$ between 2001 and 2007).

QTL detection in the CS × RGM population

QTL analysis for leaf chlorotic symptoms and plant development on grafted plants versus cuttings, under non-chlorosing and chlorosing conditions

The Pouget index was recorded under chlorosing conditions only, since no symptoms were observable when plants were grown in control soil (Table 4; Fig. 2). In 2001, four QTLs ($7.7\ \% < R^2 < 17.5\ \%$) were detected on linkage groups 5, 9, 18 and 19 for cuttings at the first date of symptoms notation (t1), whereas only one ($R^2 = 8.2\ \%$) was detected on another location on LG5 on the second date of symptom survey (t2). For grafted plants, two QTLs were detected on LG13 ($R^2 = 13.2\ \%$) and 14 ($R^2 = 11.5\ \%$) at t1. The QTL on LG13 appeared also at t2 with a higher magnitude ($R^2 = 24.9\ \%$) and was accompanied by two other minor QTLs on LG2 ($R^2 = 12.7\ \%$) and LG17 ($R^2 = 10.5\ \%$). No common QTLs were detected for cuttings and grafted plants.

Chlorophyll content estimated with the SPAD measurements (Table 4) revealed for cutting plants six loci

Table 1 Broad sense heritability (H^2), range, mean value, standard deviation (SD) of chlorosis tolerance traits evaluated using the Pouget index and SPAD measurements on cuttings and grafted plants under chlorosing and non-chlorosing environments in 2001, 2003, 2006 and 2007 for F1 population and mean value for parental lines CS and RGM

Trait	Soil	Plant	Years	Date	H^2	F1 population ($n = 138$)				Parental lines	
						Min	Max	Mean	SD	CS	RGM
Pouget index	Calcareous	Cuttings	2001	August-02	0.46	0.00	2.67	0.86	0.71	1.94	0.47
				August-14	0.53	0.00	2.50	0.51	0.58	0.50	0.80
		Grafted	2001	July-27	0.46	0.00	3.00	1.15	0.74	–	1.33
				August-03	0.68	0.00	4.83	0.66	1.18	–	0.00
			2003	June-19	0.11	1.20	3.80	2.46	0.50	1.92	2.92
				July-04	0.12	0.50	2.30	1.29	0.39	1.25	1.08
			2006	July-27	0.14	1.00	4.50	2.27	0.61	2.17	2.92
				August-23	0.40	1.00	5.00	2.27	0.91	2.08	2.92
			2007	June-19	0.29	1.00	5.00	2.84	0.91	2.50	4.58
				July-04	0.08	2.00	4.00	2.85	0.39	2.83	3.33
SPAD	Non-calcareous	Cuttings	2001	July-30	0.35	17.63	32.65	24.77	2.58	22.58	22.14
				August-14	0.39	19.83	31.23	25.49	2.12	28.33	24.21
		Grafted	2001	July-26	0.28	11.00	32.00	26.03	3.61	–	28.08
				August-08	0.37	19.00	29.00	24.50	3.99	–	26.32
	Calcareous	Cuttings	2001	July-30	0.45	5.55	29.60	18.15	4.91	12.60	21.25
				August-14	0.48	12.23	31.95	22.66	4.06	23.30	19.96
		Grafted	2001	July-26	0.18	13.00	31.00	22.36	3.50	–	26.10
				August-10	0.54	7.78	29.10	20.68	4.58	–	23.90
			2003	June-26	0.07	12.50	30.70	23.49	3.63	25.64	25.68
				July-07	0.10	6.70	25.70	16.79	3.81	20.78	15.26
			2007	June-19	0.33	0.11	28.55	11.65	6.06	12.61	1.11
				July-04	0.16	13.40	28.10	22.59	2.66	20.11	26.25

($6.5\ \% < R^2 < 14.0\ \%$) at t1 and five different QTLs ($10.2\ \% < R^2 < 24.4\ \%$) at t2 under non-chlorosing conditions. Under chlorosing conditions (Table 4), QTL analysis detected four QTLs ($7.6\ \% < R^2 < 12.1\ \%$) at t1 and two QTLs ($R^2 = 16.0$ and 31.7 %) at t2. For grafted plants under non-chlorosing conditions, three QTLs ($6.3\ \% < R^2 < 14.4\ \%$) were identified at t1, and four QTLs ($7.6\ \% < R^2 < 45.2\ \%$) at t2. Under chlorosing conditions six QTLs were localized for grafted plants ($7.1\ \% < R^2 < 27.0\ \%$) at t1 and two QTLs ($R^2 = 10.1$ and 10.9 %) at t2. A major QTL on LG13, already detected for Pouget index, was identified for grafted plants under non-chlorosing conditions at t2 ($R^2 = 45.2\ \%$) and under chlorosing conditions at t1 ($R^2 = 27.0\ \%$). No common QTLs were detected for cuttings and grafted plants under both conditions. Three QTLs, *spad01-t1_5* and *spad01-t1_1* and *spad01-t1_18*, were surprisingly detected on cuttings under non-chlorosing conditions and on grafted plants under chlorosing conditions.

Scan for QTLs for plant development traits (Table 5; Fig. 3) revealed one common QTL for stem length, leaf dry weight and stem dry weight on LG2 ($13.6\ \% < R^2 < 14.3\ \%$) for cuttings grown under non-chlorosing conditions. A different QTL was detected for the same traits on LG7 ($7.8\ \% < R^2 < 9.5\ \%$) for cuttings grown under chlorosing conditions. For stem length three additional QTLs ($12.8\ \% < R^2 < 16.0\ \%$) were identified for cuttings grown under non-chlorosing conditions, and one different QTL explaining 14.0 % of the variation, under chlorosing conditions. For leaf and stem dry weights, additional QTLs were detected only under chlorosing conditions, explaining from 6.5 to 13.9 % of the variance. There were no common QTLs detected for cuttings under both growing conditions.

For grafted plants, a major QTL was detected on LG13 ($36.0\ \% < R^2 < 53.2\ \%$) under non-chlorosing and chlorosing conditions for all the recorded traits. QTLs with minor effects ($5.0\ \% < R^2 < 17.0\ \%$) were also detected. Five and four additional QTLs were detected for stem length under non-chlorosing and chlorosing conditions, respectively. Two minor QTLs for each trait were detected for leaf and stem dry weight under chlorosing conditions. Among these minor QTLs, only *sl01_7* was common to several traits and both soil conditions (Table 5). No common QTLs were detected on cuttings and grafted plants for plant development traits.

Table 2 Broad sense heritability (H^2), mean value, standard deviation (SD), and range of growth traits evaluated on cuttings and grafted plants under chlorosing and non-chlorosing environments in 2001, 2003, 2006 and 2007 for F1 population and parental lines CS and RGM

Trait	Soil	Plant	Years	H^2	F1 population ($n = 138$)				Parental lines	
					Min	Max	Mean	SD	CS	RGM
Stem length	Non-calcareous	Cuttings	2001	0.48	24.5	265.5	207.2	33.1	137.5	231.8
		Grafted	2001	0.84	13.5	103.0	66.0	23.7	–	50.3
	Calcareous	Cuttings	2001	0.60	91.0	303.5	237.6	37.3	155.0	255.8
		Grafted	2001	0.86	19.5	118.0	75.9	27.0	–	56.0
			2003	0.51	23.3	68.7	53.6	10.4	47.7	39.0
			2006	0.49	10.0	59.8	33.9	10.5	27.3	24.2
			2007	0.59	46.0	167.0	109.9	27.4	109.5	93.5
Leaves dry weight	Non-calcareous	Cuttings	2001	0.56	0.8	17.6	11.6	3.1	7.4	11.3
		Grafted	2001	0.78	0.4	11.9	5.5	2.8	–	1.9
	Calcareous	Cuttings	2001	0.59	1.7	25.1	15.6	4.5	9.6	14.2
		Grafted	2001	0.83	0.5	15.9	6.6	3.4	–	2.7
			2003	0.55	1.0	7.1	4.1	1.4	3.8	2.5
			2007	0.65	2.5	14.2	8.9	2.6	8.3	7.7
Stems dry weight	Non-calcareous	Cuttings	2001	0.52	0.6	16.5	10.2	3.2	10.7	14.1
		Grafted	2001	0.77	0.2	7.7	3.5	2.1	–	4.2
	Calcareous	Cuttings	2001	0.59	1.5	25.5	14.7	4.9	11.4	18.7
		Grafted	2001	0.81	0.4	10.1	4.8	2.9	–	4.4
			2003	0.55	0.5	6.6	3.5	1.4	2.6	1.7
			2006	0.26	0.4	15.9	4.7	3.4	1.8	0.9
			2007	0.61	3.3	24.0	13.1	5.1	12.3	12.0
Roots dry weight	Calcareous	Grafted	2003	0.37	0.4	5.7	2.2	1.3	2.0	0.8
			2007	0.63	0.3	15.9	7.1	3.6	7.5	5.0
Total dry weight	Calcareous	Grafted	2007	0.51	6.2	54.1	29.3	11.0	28.1	24.7
Number of leaves	Calcareous	Grafted	2003	0.40	3.4	8.3	7.0	0.9	7.0	6.5
			2007	0.55	12.0	26.0	21.6	2.9	21.0	22.2

QTL analysis for leaf chlorotic symptoms and plant development on grafted plants under chlorosing conditions in separate experiments

In 2003, genome-wide scan of QTL for leaf chlorotic symptoms revealed three QTLs on LG1, LG13 and LG18 ($9.8\ \% < R^2 < 18.8\ \%$) for Pouget index at t1, and two QTLs on LG11 ($R^2 = 14.6\ \%$) and LG18 ($R^2 = 10.7\ \%$) at t2 (Table 6). The two QTLs on LG18 were located at different positions. In 2006, QTLs were detected on LG5 ($R^2 = 7.4\ \%$) and LG7 ($R^2 = 7.2\ \%$) at t1, and on LG7 ($R^2 = 12.0\ \%$) and LG13 ($R^2 = 13.0\ \%$) at t2 (Table 6). Finally, in 2007, two QTLs were detected on LG13 ($R^2 = 19.9\ \%$) and LG18 ($R^2 = 15.4\ \%$) at t1, and on LG3 ($R^2 = 6.8\ \%$) and LG8 ($R^2 = 16.4\ \%$) at t2 (Table 6). QTL for the Pouget index on LG13 was found for all years of experiments for grafted plants under chlorosing conditions. In 2001, the QTL on LG13 was found for the two dates of record. It was the same for one QTL on LG18 in 2003. All other QTLs were specific of a single year and a single date (Table 6; Fig. 2).

Chlorophyll content was measured in 2001, 2003 and 2007 (Tables 4, 5). At t1, five and two QTLs were identified in 2003 and 2007, respectively. At t2, only one QTL was detected in 2007. For this trait, there was no common QTL detected for both dates of measurement within a year. *QTL spad_18* ($7.1\ \% < R^2 < 29.8\ \%$) was the only QTL detected for these 3 years of experiment under chlorosing conditions.

For plant development (Table 7), the major QTL on LG13 detected in 2001 for many parameters under chlorosing environment explained also a large part of the variation in 2003 for stem length ($R^2 = 39.4\ \%$), in 2006 ($R^2 = 28.6\ \%$) and in 2007 ($R^2 = 52.9\ \%$). It was also detected for leaf dry weight in 2003 ($R^2 = 30.1\ \%$) and in 2007 ($R^2 = 31.8\ \%$), for stem dry weight with $R^2 = 30.3\ \%$ in 2003, $R^2 = 11.5\ \%$ in 2006 and $R^2 = 41.1\ \%$ in 2007, for root dry weight in 2003 ($R^2 = 36.8\ \%$) in 2007 ($R^2 = 41.0\ \%$).and for leaf

Table 3 Phenotypic correlation coefficients among growth traits based on trait average values of the F1 population as cuttings and grafted plants under chlorosing and non-chlorosing conditions in 2001

	Non-chlorosing soil			Chlorosing soil		
	Stem length	Stem dry weight	Leaves dry weight	Stem length	Stem dry weight	Leaves dry weight
Cuttings plants						
Non-chlorosing soil						
Stem length	–					
Stem dry weight	0.87	–				
Leaves dry weight	0.79	0.90	–			
Chlorosing soil						
Stem length	0.65	0.57	0.56	–		
Stem dry weight	0.51	0.55	0.54	0.89	–	
Leaves dry weight	0.44	0.49	0.58	0.83	0.93	–
Grafted plants						
Non-chlorosing soil						
Stem length	–					
Stem dry weight	0.95	–				
Leaves dry weight	0.96	0.97	–			
Chlorosing soil						
Stem length	0.91	0.87	0.88	–		
Stem dry weight	0.89	0.88	0.88	0.97	–	
Leaves dry weight	0.90	0.89	0.89	0.98	0.98	–
Number of leaves	0.62	0.60	0.61	0.66	0.67	0.66

number with 35.2 % of variation explained in 2003 and 41.3 % in 2007.

According to the year, additional QTLs explaining less than 20 % of variance were detected for each parameter. Five minor QTLs affected stem length in 2003, one in 2006, and two in 2007. For leaf dry weight, four QTLs were detected in 2003 on LG1, LG2, LG9, LG16 ($7.4\ \% < R^2 < 21.5\ \%$), and one in 2007 on LG17 ($R^2 = 7.3\ \%$). Analysis for root dry weight identified two QTLs in 2003 on LG4 ($R^2 = 6.8\ \%$), and LG17 ($R^2 = 6.2\ \%$). Two additional QTLs for leaf number mapped on LG4 ($R^2 = 11.6\ \%$) and LG9 ($R^2 = 7.3\ \%$) in 2003. Among these minor QTLs, *sl_4* was detected in 2001 and 2003, and co-located with *rdw_4* and *nl_4* in 2003. One another QTL, *sl_12*, was detected in 2001 and 2006.

Discussion

Using an F1 SSR map derived from an inter-specific cross (*V.vinifera* × *V riparia*), we report the identification of QTLs for lime-induced chlorosis symptoms and plant growth parameters for cuttings and grafted plants, under chlorosing and non-chlorosing situations. So far the genetic determinism of plant response to iron deficiency has been mainly analysed on herbaceous plants, with a single report on *Prunus*. Our study is the first published one which reports QTL analyses performed on a progeny grown either as cuttings or as rootstocks in grafted plants under non-chlorosing and chlorosing conditions. Our work supports the findings that plant responses to chlorosing conditions in terms of leaf symptoms and plant development display specific genetic control in comparison to non-chlorosing conditions. Similarly, grafted plants display different genetic bases than ungrafted plants. Many QTLs were identified for every trait, most of them explaining a low part of the phenotypic variance. This result supports a polygenic control.

Calcareous conditions induced chlorosis symptoms and revealed specific QTLs implicated in plant responses

The experiments performed in 2001 displayed large differences for chlorosis symptoms and growth parameters between the two types of soil. The Pouget index recorded on plants, either cuttings or grafted plants, clearly indicated the development of chlorotic symptoms on young leaves when these plants were grown in chlorosing soil. Chlorophyll content estimated from SPAD measurements on young leaves confirmed the results in a quantitative way with lower mean values recorded on plants growing in chlorosing soil. There was one common QTL for leaf chlorophyll content detected on grafted plants for both

Table 4 QTLs identified for iron chlorosis tolerance from CS × RGM F1 population as cuttings and as rootstock grafted with CS under chlorosing and non-chlorosing conditions in 2001

Trait	Cuttings					Grafted				
	QTL name	LG	LOD score	QTL CI	Vp (%)	QTL name	LG	LOD score	QTL CI	Vp (%)
Pouget index on chlorosing soil										
t1 August-02	*pi01_ng_c-t1_5*	5	3.0	0–12	8.1	*pi01_g_c-t1_13*	13	3.5	34–48	13.2
	pi01_ng_c-t1_9	9	4.5	16–27	15.7	*pi01_g_c-t1_14*	14	3.2	45–58	11.5
	pi01_ng_c-t1_18	18	3.9	77–91	17.5					
	pi01_ng_c-t1_19	19	3.1	25–35	7.7					
t2 August-14	*pi01_ng_c-t2_5*	5	2.5	20–43	8.2	*pi01_g_c-t2_2*	2	3.1	11–13	12.7
						pi01_g_c-t2_13	13	8.0	34–48	24.9
						pi01_g_c-t2_17	17	3.4	30–47	10.5
SPAD on non-chlorosing soil										
t1 July-30	*spad01_ng_nc-t1_1*	1	2.7	21–36	7.6	*spad01_g_nc-t1_1*	1	4.6	72–89	14.4
	spad01_ng_nc-t1_5	5	4.9	2–9	14.0	*spad01_g_nc-t1_9*	9	5.6	45–51	6.3
	spad01_ng_nc-t1_8a	8	3.9	63–76	6.9	*spad01_g_nc-t1_17*	17	4.1	16–33	14.3
	spad01_ng_nc-t1_8b	8	3.1	13–40	10.0					
	spad01_ng_nc-t1_9	9	3.2	28–42	9.9					
	spad01_ng_nc-t1_18	18	3.2	0–5	6.5					
t2 August-14	*spad01_ng_nc-t2_1*	1	4.6	12–21	24.4	*spad01_g_nc-t2_1*	1	3.5	0–13	8.2
	spad01_ng_nc-t2_4	4	5.2	35–47	10.2	*spad01_g_nc-t2_6*	6	3.1	20–25	7.6
	spad01_ng_nc-t2_8	8	3.5	52–59	18.7	*spad01_g_nc-t2_13*	13	18.6	34–47	45.2
	spad01_ng_nc-t2_11	11	3.9	32–39	20.3	*spad01_g_nc-t2_18*	18	3.7	80–91	14.9
	spad01_ng_nc-t2_12	12	4.2	31–44	19.8					
SPAD on chlorosing soil										
t1 July-30	*spad01_ng_c-t1_2*	2	3.7	47–58	8.8	*spad01_g_c-t1_1*	1	3.8	24–45	11.9
	spad01_ng_c-t1_6	6	2.8	0–13	7.6	*spad01_g_c-t1_5*	5	3.7	3–9	9.5
	spad01_ng_c-t1_9	9	3.9	17–30	11.1	*spad01_g_c-t1_6*	6	4.1	0–6	26.5
	spad01_ng_c-t1_18	18	2.8	85–91	12.1	*spad01_g_c-t1_10*	10	4.4	21–25	12.6
						spad01_g_c-t1_13	13	7.0	28–45	27.0
						spad01_g_c-t1_18	18	5.5	0–5	7.1
t2 August-14	*spad01_ng_c-t2_10*	10	3.4	0–16	16.0	*spad01_g_c-t2_2*	2	2.7	0–18	10.1
	spad01_ng_c-t2_19	19	5.5	35–45	31.7	*spad01_g_c-t2_17*	17	3.7	37–47	10.9

QTL name, *trait and year of experiment_grafted or non-grafted_chlorosing or non-chlorosing soil_linkage group*; *LG*, linkage group; *LOD score*, LOD score max; *QTL CI*, QTL confidence interval in cM; *Vp*, proportion of phenotypic variance explained by QTL

control and chlorotic conditions in 2001 on LG13 but this QTL did not appeared in 2003 and 2007 (Tables 4, 6). On the contrary, plant growth parameters showed highly significant correlations in these two contrasting growing conditions. However, only two common QTLs were detected for growth parameters under control and chlorotic conditions on LG7 and LG13 for grafted plants, with QTL on LG13 explaining between 36.0 and 53.2 % of the phenotypic variance.

The Pouget index and SPAD measurements are commonly used in QTL detection analyses to phenotype plants submitted to iron chlorosis (Charlson et al. 2003; Gonzalo et al. 2012; Klein et al. 2012). In our study, broad sense heritability varied from rather low values up to 0.68 and 0.54 for the Pouget index and chlorophyll content, respectively, indicating that the environment (year, date of records) has a non-negligible effect on these traits. Consequently, controlled conditions of evaluation and multi-year experiments are required to analyse accurately the genetic determinism of these traits. As shown in other studies (Gonzalo et al. 2012), they were negatively correlated and it is interesting to identify which part is genetically common between them, and which loci are specific of each trait. Visual scoring is fast and convenient to evaluate chlorotic symptoms, but a five-score scale may not be precise enough to evaluate the phenotypic segregation (Lin et al. 1997). On the other hand, SPAD meter provides a quantitative measure of the severity of leaf chlorosis associated with iron deficiency, and is a quick and non-destructive estimation of the

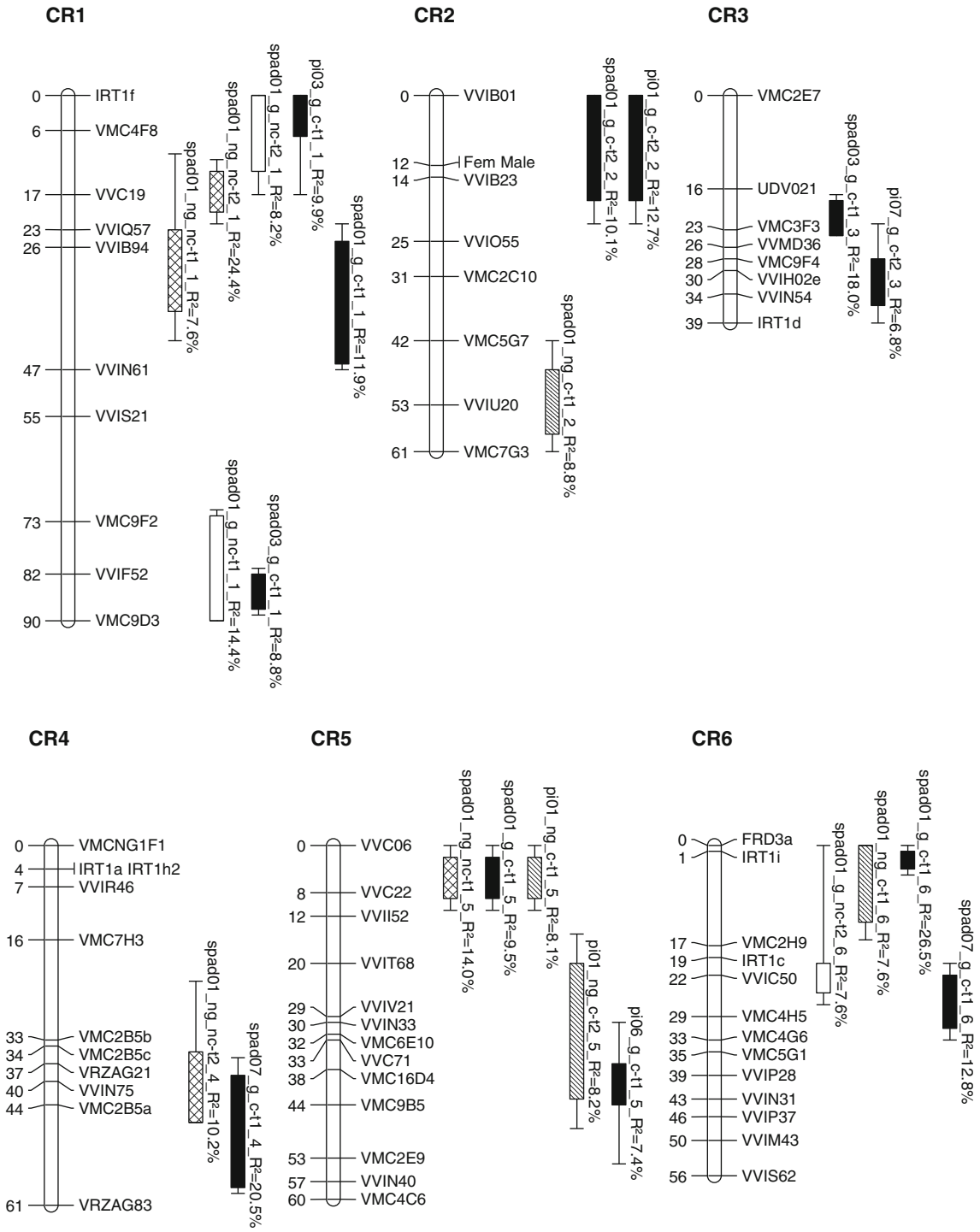

Fig. 2 Linkage map for chromosomes 1–19 of the CS × RGM F1 population showing the locations of QTL identified for lime-induced chlorosis. Linkage groups are named according to international consensus map. For each linkage group, the consensus map is labelled CR [i.e. "Cabernet Sauvignon × Riparia Gloire de Montpellier (CR) F1 population"]. Distances are in cM Kosambi

chlorophyll concentration (Peryea and Kammereck 1997; Yadava 1986). The number of QTLs detected for the Pouget index was in general lower than for chlorophyll content estimated from SPAD measurements. Two chromosomal regions are associated with the variation of visual chlorotic symptoms and the variation of chlorophyll concentrations, on LG18 for cuttings and four regions on LG2, LG13, LG17 and LG18 for grafted plants (Fig. 2). Therefore, visual score and chlorophyll concentration evaluations apparently share some common

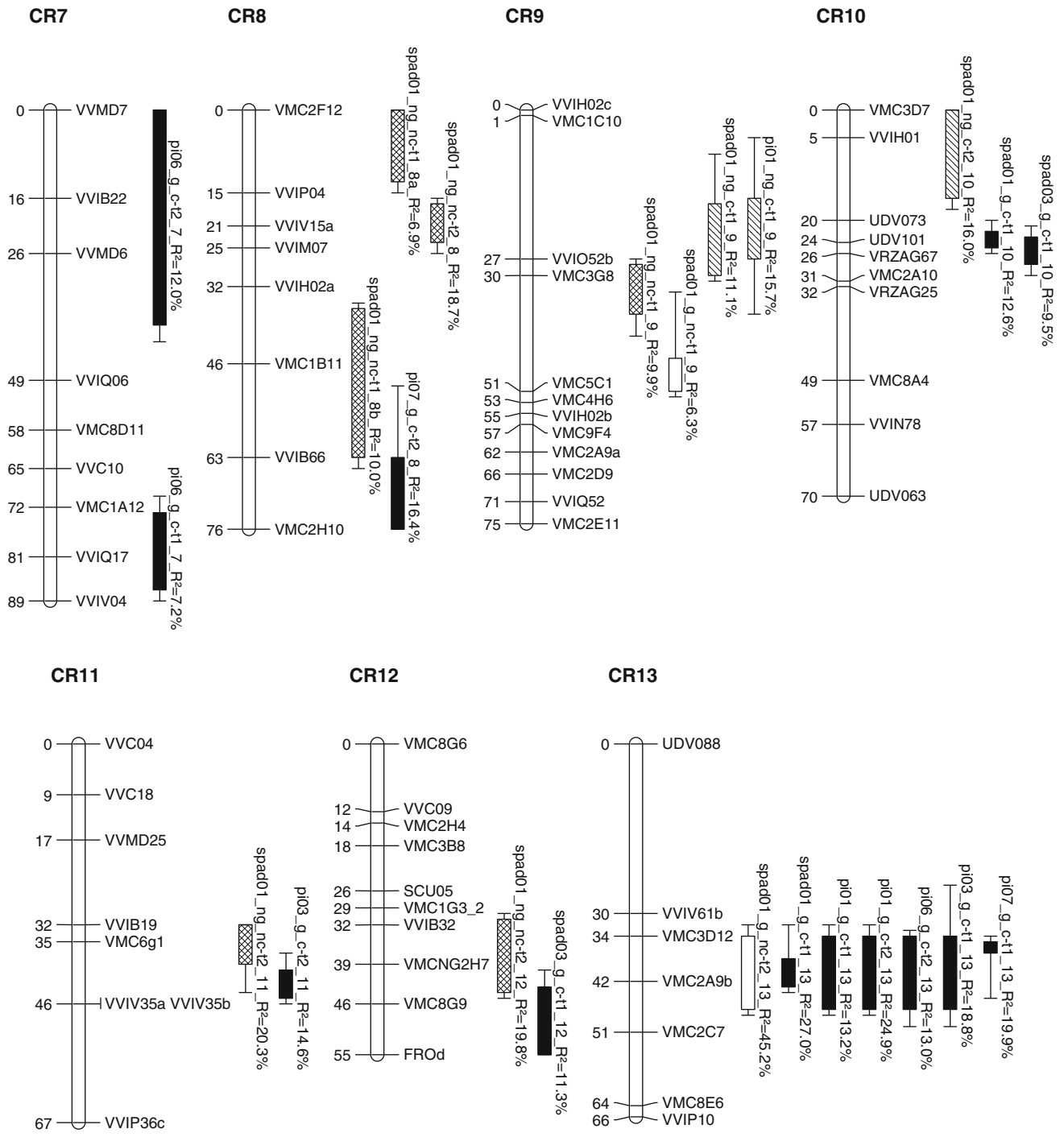

Fig. 2 continued

genetic mechanisms affecting iron deficiency in grapevine, but QTLs located apart from each other also indicated specificities for each parameter. In grapevine, the genetic control of symptom appearance under chlorotic conditions appears to be more complex, even for cuttings than in the case of Prunus (Gonzalo et al. 2012) where only one to three QTLs were detected for both traits.

Common QTLs for growth and chlorotic symptoms or common QTLs for growth under non-chlorosing and chlorosing conditions for both cuttings and grafted plants were identified. The QTL on LG13, explaining a large percentage of phenotyping variance for most traits recorded on grafted plants is of particular interest. For this locus, there was a negative correlation between most plant

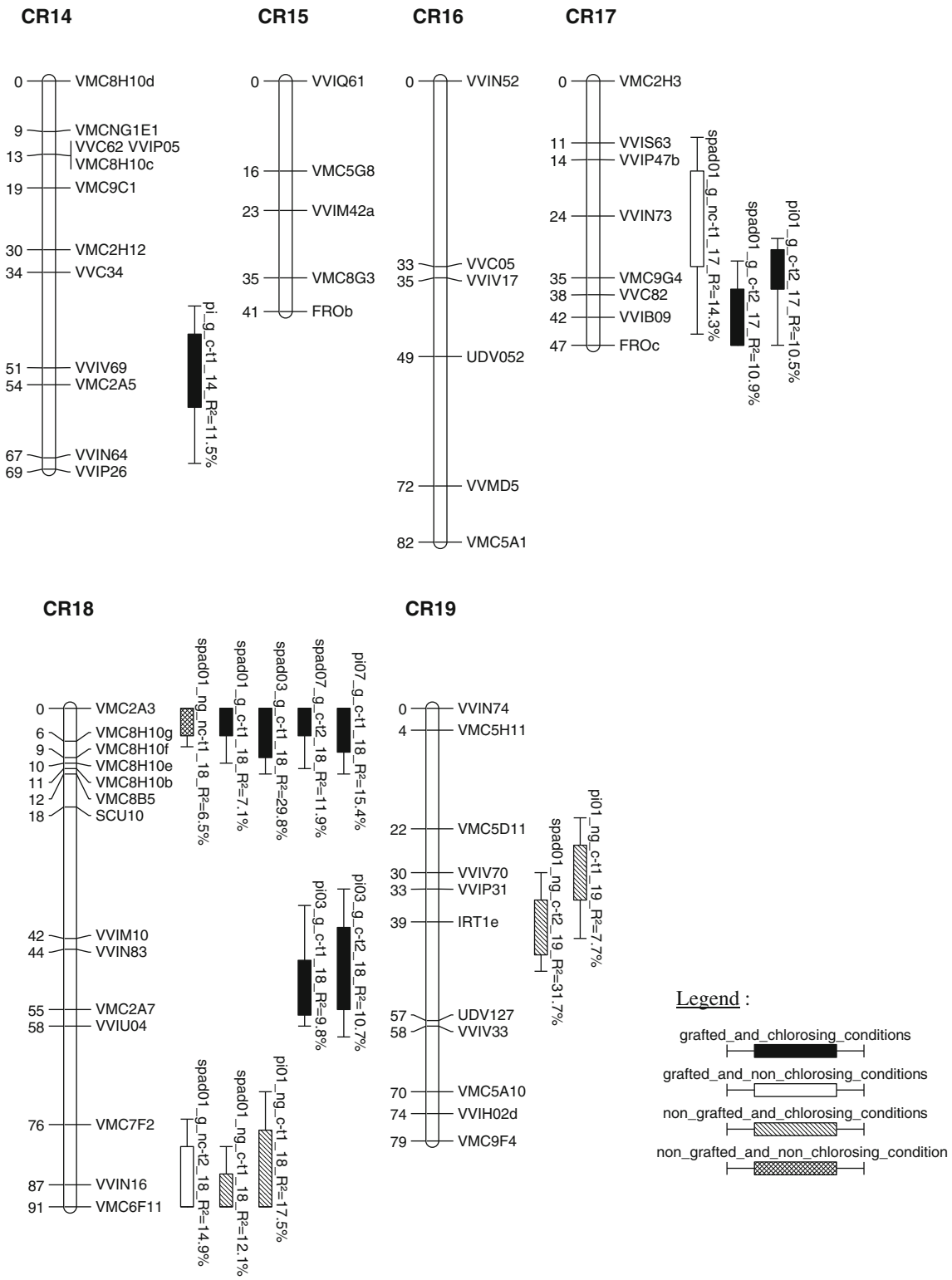

Fig. 2 continued

growth parameters and chlorotic symptom trait parameters, with the alleles coming from the male parent (RGM) being the source of variance. The link between grapevine growth and iron nutrition was already reported (Gruber and Kosegarten 2002; Ksouri et al. 2005). Bavaresco et al. (1994) described two mechanisms of response to lime-induced chlorosis: an adaptive one, typical of *V. berlandieri* and *Vitis cinerea*, characterized

Table 5 QTLs identified for plant growth parameters from CS × RGM F1 population as cuttings under chlorosing and non-chlorosing conditions and grafted with CS under non-chlorosing conditions in 2001

Soil	Trait	Cuttings					Grafted				
		QTL name	LG	LOD score	QTL CI (cM)	Vp (%)	QTL name	LG	LOD score	QTL CI (cM)	Vp (%)
Non-chlorosing	Stem length	*sl01_ng_nc_2*	2	4.6	31–52	14.3	*sl01_g_nc_2*	2	4.9	35–53	8.3
		sl01_ng_nc_10	10	4.8	0–13	15.7	*sl01_g_nc_4*	4	5.0	48–61	8.6
		sl01_ng_nc_18	18	3.4	75–91	12.8	*sl01_g_nc_7*	7	4.5	41–55	7.7
		sl01_ng_nc_19	19	5.1	1–15	16.0	*sl01_g_nc_9*	9	4.1	64–68	7.7
							sl01_g_nc_13	13	23.8	38–44	44.9
							sl01_g_nc_19	19	4.5	76–79	8.2
	Leaves dry weight	*ldw01_ng_nc_2*	2	3.4	52–61	13.6	*ldw01_g_nc_13*	13	22.3	37–44	44.4
	Stems dry weight	*sdw01_ng_nc_2*	2	3.4	53–61	13.9	*sdw01_g_nc_13*	13	20.3	38–46	42.7
Chlorosing	Stem length	*sl01_ng_c_7*	7	3.3	21–45	8.6	*sl01_g_c_5*	5	3.3	0–17	5.0
		sl01_ng_c_18	18	5.3	55–63	14.0	*sl01_g_c_7*	7	4.1	37–55	7.8
							sl01_g_c_12	12	3.4	42–53	7.1
							sl01_g_c_13	13	23.0	37–40	36.0
							sl01_g_c_18	18	4.9	78–91	9.2
	Leaves dry weight	*ldw01_ng_c_6*	6	3.7	18–28	6.5	*ldw01_g_c_7*	7	4.6	37–52	7.9
		ldw01_ng_c_7	7	5.4	21–37	9.5	*ldw01_g_c_13*	13	22.5	41–47	38.2
		ldw01_ng_c_9	9	4.1	26–36	7.4	*ldw01_g_c_18*	18	4.7	81–91	7.1
		ldw01_ng_c_10	10	4.3	25–28	7.5					
		ldw01_ng_c_11	11	4.6	21–41	9.2					
		ldw01_ng_c_15	15	5.1	11–22	9.2					
	Stems dry weight	*sdw01_ng_c_7*	7	3.5	21–44	7.8	*sdw01_g_c_1*	1	3.6	25–33	13.0
		sdw01_ng_c_11	11	3.0	31–46	6.5	*sdw01_g_c_7*	7	3.3	35–55	17.0
		sdw01_ng_c_13	13	3.4	28–35	7.3	*sdw01_g_c_13*	13	20.3	38–43	53.2
		sdw01_ng_c_18	18	6.0	49–56	13.9					

QTL name, *trait and year of experiment_grafted or non-grafted_chlorosing or non-chlorosing soil_linkage group*; *LG*, linkage group; *LOD score*, LOD score max; *QTL CI*, QTL confidence interval in cM; *Vp*, proportion of phenotypic variance explained by QTL

by high growth rate, large root system and high iron uptake; a protective one, typical of *Vitis champini*, characterized by a slow growth rate, weak root system and low iron uptake. According to our results, *V. riparia*, although described as sensitive, would be characterized by alleles leading to the adaptive responses. In general, plant growth tightly depends on mineral uptake. Séguéla et al. (2008) showed that root growth inhibitory conditions such as abiotic stresses and hormonal treatments repress the iron starvation response genes in *Arabidopsis*. It was suggested that cytokinins control the root iron uptake machinery through a root growth dependant pathway in order to adapt nutrient uptake to the demand of the plant. A QTL analysis performed on *Arabidopsis* demonstrated also that 85 % of growth variation can be explained by variation in iron content. Co-localization between root growth and iron content was described (Prinzenberg et al. 2010), supporting the close link between growth and iron uptake mechanisms.

Grafted plants display different genetic bases than ungrafted plants

A rootstock and a scion might be resistant when grown on their own roots and become sensitive once grafted together or vice versa (Pouget and Ottenwælter 1973). Studies on watermelon and tomato (Rivero et al. 2004) show that the good capacities for Fe uptake of a rootstock are not always related to an improved Fe status in the aerial parts of the scion. As such, scion-rootstock interactions must be taken into account when testing for grape resistance to chlorosis (Bavaresco et al. 1993; Bavaresco and Lovisolo 2000). At a phenotypical level, our study shows that there was no correlation between traits recorded on genotypes grown as

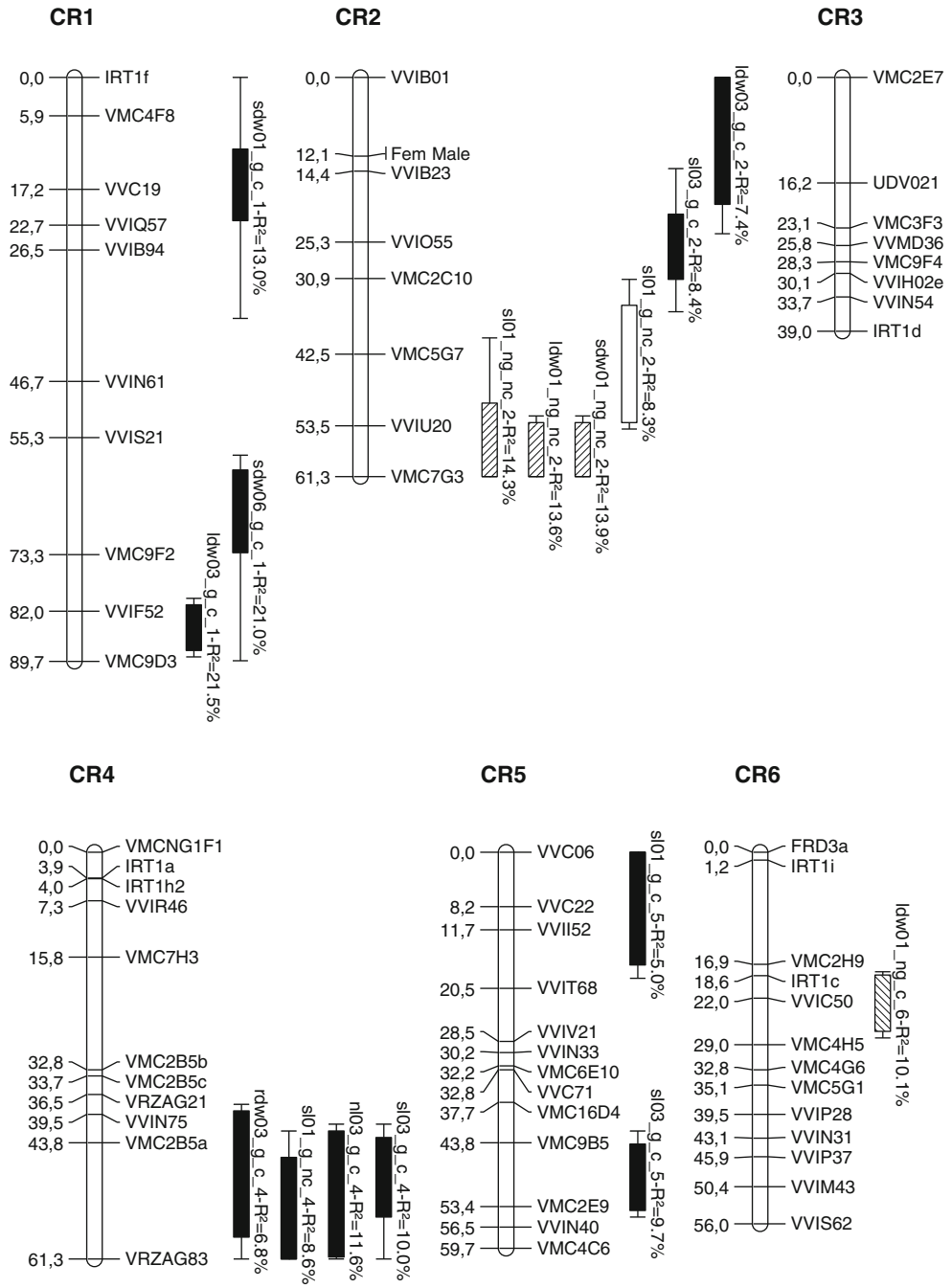

Fig. 3 Linkage map for chromosomes 1–19 of the CS × RGM F1 population showing the locations of QTL identified for plant growth. Linkage groups are named according to international consensus map. For each linkage group, the consensus map is labelled CR [i.e. "Cabernet Sauvignon × Riparia Gloire de Montpellier (CR) F1 population"]. Distances are in cM Kosambi

cuttings and as rootstocks with CS as a scion, regardless of the growing conditions. At a genetic level, QTL analyses revealed distinct chromosomal regions implicated in trait variations between cuttings and grafted plants.

The iron uptake system is known to respond to long-distance shoot-to-root signals. Molecular regulation at the root level has been extensively studied but the mechanism through which shoot-to root signalling is achieved remains a mystery (Ivanov et al. 2012). The metal chelator nicotianamine is a graft-transmissible substance which plays an important role in whole plant iron status signalling (Curie et al. 2009). It was observed that grafting wild-type rootstocks with mutants as tomato *chloronerva* characterized by low levels of nicotianamine allowed to get a normal

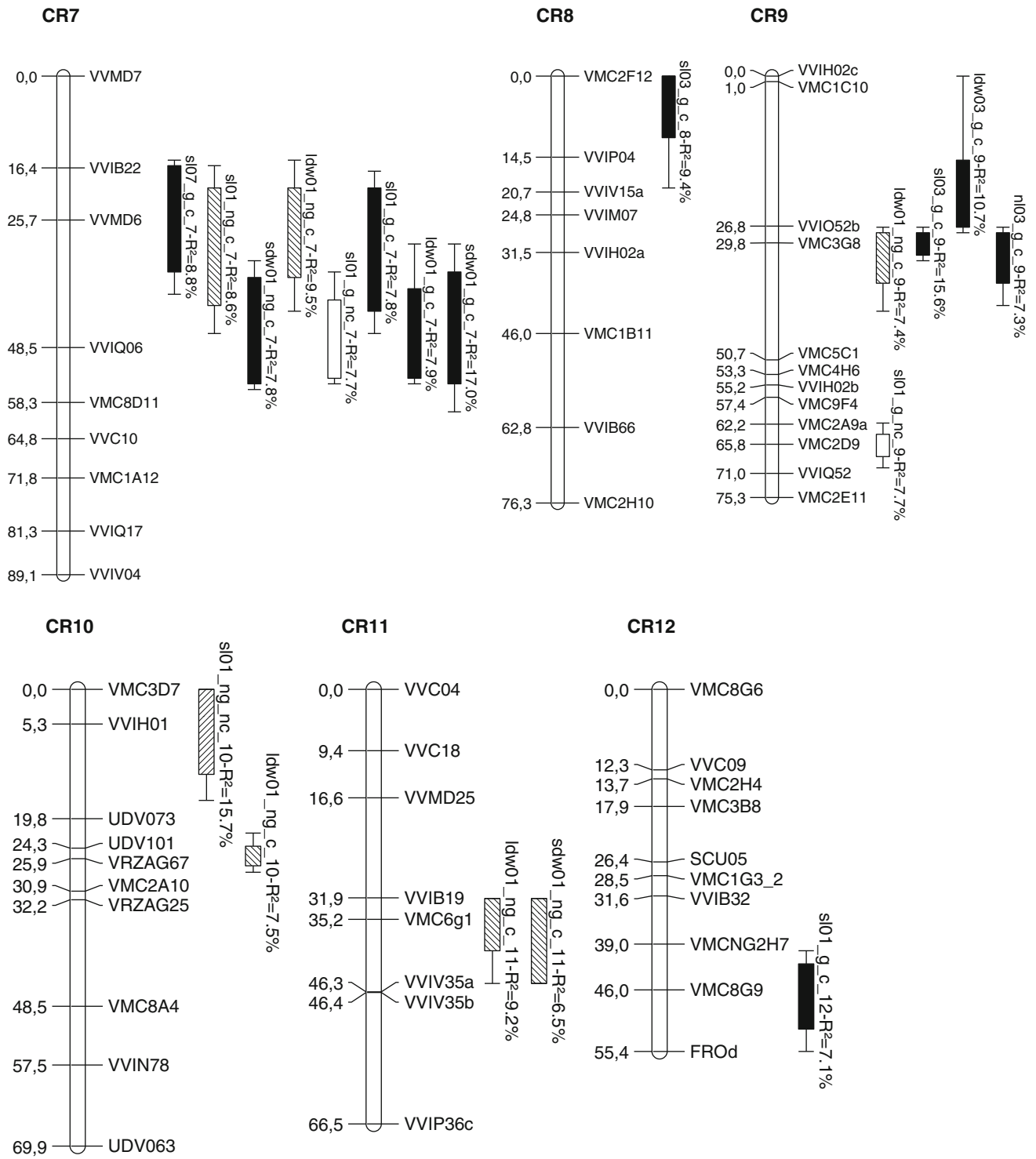

Fig. 3 continued

phenotype at the scion level (Ling et al. 1999). It was also shown in tobacco that the expression of ferric chelate reductase gene and of an iron transporter in roots decreased by removing the leaves of plant grown under iron-deficient conditions (Enomoto and Goto 2008). Moreover, it was reported that chloroplast integrity is essential for the induction of the iron reductase activity in the root under iron deficiency (Durret et al. 2006). Small RNAs trafficking could also be involved in signalling (Buhtz et al. 2010). Nitric oxide and several hormones as auxin, ethylene and

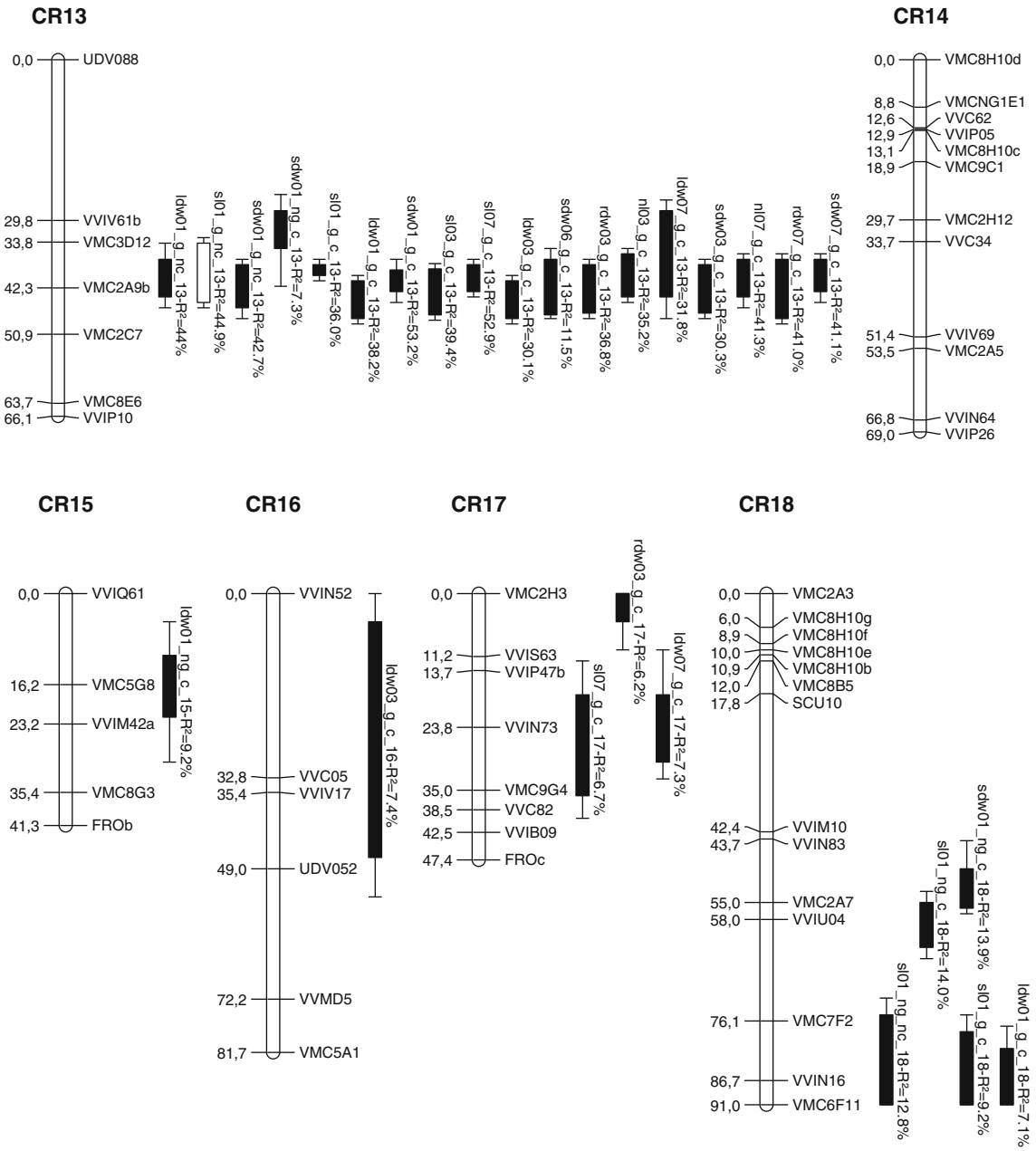

Fig. 3 continued

cytokinins have been demonstrated to participate to iron regulation responses, but their role in shoot-to-root signalling is not clear (Ivanov et al. 2012). A grafted plant is made from two genotypes which interact to give a new phenotype different from the phenotype of each genotype grown as cutting. Allelic variability for all the genes involved in iron deficiency responses at the root level and signalling and sensing at the shoot level is likely to explain that cuttings and grafted plants with the same genotype as rootstock display different phenotypes.

The fact that grafted plants display different genetic bases than ungrafted plant is not limited to lime-induced chlorosis responses. Indeed, Estan et al. (2009) reported only two potential common QTLs for fruit yield between non-grafted tomato lines and the corresponding lines studied as rootstocks. All together, these results clearly highlight the fact that the genetic dissection of rootstock effects on scion properties should be undergone with grafted material. Results obtained on cuttings, as those reported by Gonzalo et al. (2012), are surely not suffisant to select iron chlorosis-tolerant rootstocks. This specificity of rootstocks makes genetic analyses and breeding more complex. The few published works (Estan et al. 2009; Asins et al. 2010; Marguerit et al. 2012) showed that

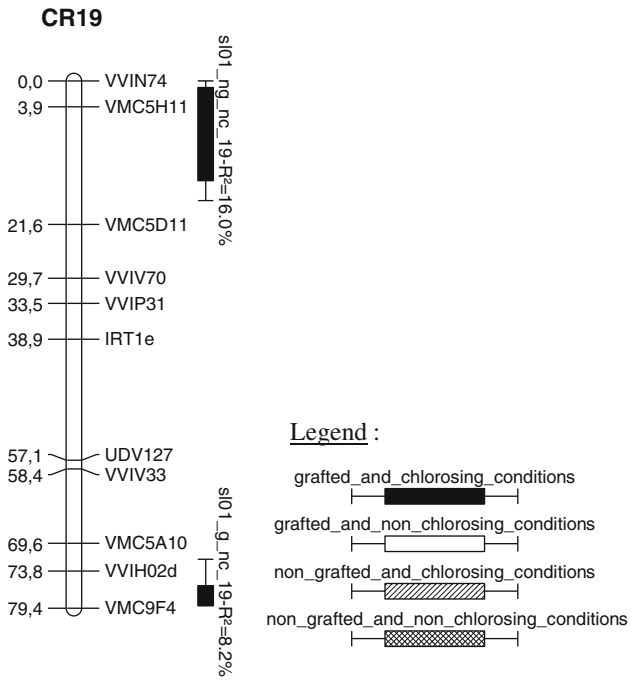

Fig. 3 continued

rootstock effects display usually a medium heritability up to 0.3–0.4. Most frequently, traits are controlled by several QTLs, each of them explaining a percentage of variance below 20. Our results support these observations. Despite these difficulties, some QTLs related to rootstock effect can be detected repetitively over separate experiments as shown by Marguerit et al. (2012) and in our study on LG13 and LG18. Here, the common zone detected on LG13, explaining a percentage of variance up to 45 for SPAD and up to 53 for growth parameters, is of particular interest and should be further investigated.

QTL analysis reveals polygenic control for lime-induced chlorosis tolerance in grapevine

Complex genetic and environmental interactions have made iron-induced chlorosis an extremely difficult trait to study in field trials. Today, the mechanisms of tolerance remain poorly understood. Moreover, the number of genes, gene action, and the magnitude of gene effect controlling chlorosis is unknown in grapevine. Despite economical repercussions of lime-induced chlorosis, there are very

Table 6 QTLs identified for iron chlorosis tolerance from CS x RGM F1 population grafted with CS under chlorosing conditions in 2003, 2006 and 2007

Trait	Year (date)	QTL name	LG	LOD score	QTL CI	Vp (%)
Pouget index	2003					
	t1 June-19	*pi03_g_c-t1_1*	1	3.9	0–7	9.9
		pi03_g_c-t1_13	13	6.1	25–30	18.8
		pi03_g_c-t1_18	18	3.4	46–56	9.8
	t2 July-04	*pi03_g_c-t2_11*	11	4.0	33–45	14.6
		pi03_g_c-t2_18	18	3.6	40–55	10.7
	2006					
	t1 July-27	*pi06_g_c-t1_5*	5	4.3	37–44	7.4
		pi06_g_c-t1_7	7	3.7	73–87	7.2
	t2 August-23	*pi06_g_c-t2_7*	7	3.8	0–39	12.0
		pi06_g_c-t2_13	13	5.8	33–50	13.0
	2007					
	t1 June-19	*pi07_g_c-t1_13*	13	5.7	35–45	19.9
		pi07_g_c-t1_18	18	2.7	0–8	15.4
	t2 July-04	*pi07_g_c-t2_3*	3	3.0	25–38	6.8
		pi07_g_c-t2_8	8	3.1	0–13	16.4
SPAD	2003					
	t1 June-26	*spad03_g_c-t1_1*	1	3.6	82–89	8.8
		spad03_g_c-t1_3	3	7.2	18–23	18.0
		spad03_g_c-t1_10	10	3.9	22–26	9.5
		spad03_g_c-t1_12	12	5.4	42–55	11.3
		spad03_g_c-t1_18	18	9.0	0–9	29.8
	2007					
	t1 June-19	*spad07_g_c-t1_4*	4	6.0	36–59	20.5
		spad07_g_c-t1_6	6	3.9		12.8
	t2 July-04	*spad07_g_c-t2_18*	18	3.4	0–5	11.9

QTL name, *trait and year of experiment_grafted or non-grafted_chlorosing or non-chlorosing soil_linkage group*; *LG*, linkage group; *LOD score*, LOD score max; *QTL CI*, QTL confidence interval in cM; *Vp*, proportion of phenotypic variance explained by QTL

Table 7 QTLs identified for plant growth parameters from CS × RGM F1 population grafted with CS under chlorosing conditions in 2003, 2006 and 2007

Trait	Years	QTL name	LG	LOD score	QTL CI (cM)	Vp (%)
Stem length	2003	*sl03_g_c_2*	2	3.4	20–29	8.4
		sl03_g_c_4	4	6.1	44–54	10.0
		sl03_g_c_5	5	5.5	43–54	9.7
		sl03_g_c_8	8	4.9	64–76	9.4
		sl03_g_c_9	9	7.0	27–32	15.6
		sl03_g_c_13	13	20	38–47	39.4
	2006	*sl06_g_c_12*	12	3.2	26–52	10.2
		sl06_g_c_13	13	9.7	36–46	28.6
	2007	*sl07_g_c_7*	7	4.6	17–34	8.8
		sl07_g_c_13	13	20.0	37–42	52.9
		sl07_g_c_17	17	3.5	18–36	6.7
Leaves dry weight	2003	*ldw03_g_c_1*	1	6.5	81–90	21.5
		ldw03_g_c_2	2	3.0	0–21	7.4
		ldw03_g_c_9	9	3.5	13–27	10.7
		ldw03_g_c_13	13	11.4	41–47	30.1
		ldw03_g_c_16	16	3.1	5–47	7.4
	2007	*ldw07_g_c_13*	13	12.1	27–43	31.8
		ldw07_g_c_17	17	3.2	18–30	7.3
Stems dry weight	2003	*sdw03_g_c_13*	13	9.8	37–47	30.3
	2006	*sdw06_g_c_1*	1	7.7	38–48	21.0
		sdw06_g_c_13	13	3.0	30–51	11.5
	2007	*sdw07_g_c_13*	13	16.7	38–43	41.1
Roots dry weight	2003	*rdw03_g_c_4*	4	3.2	38–59	6.8
		rdw03_g_c_13	13	15.2	38–42	36.8
		rdw03_g_c_17	17	3.2	0–5	6.2
	2007	*rdw07_g_c_13*	13	15.4	37–43	41.0
Number of leaves	2003	*nl03_g_c_4*	4	3.6	42–61	11.6
		nl03_g_c_9	9	3.1	27–35	7.3
		nl03_g_c_13	13	10.8	38–47	35.2
	2007	*nl07_g_c_13*	13	13.4	36–43	41.3

QTL name, *trait and year of experiment_grafted or non-grafted_chlorosing or non-chlorosing soil-linkage group*; *LG*, linkage group; *LOD score*, LOD score max; *QTL CI*, QTL confidence interval in cM; *Vp*, proportion of phenotypic variance explained by QTL

little experiments reported in the literature on the understanding of genetic control of tolerance in other plants.

In the present study, a gradation in symptoms severity and the fact that up to six QTLs per trait could be detected support the idea of a regulation by more than one gene. In other species, using association mapping approach, Wang et al. (2008) detected three markers associated with iron deficiency chlorosis in two independent soybean populations. Simko et al. (2008) reported polygenic control with a major QTL associated with chlorotic symptoms and a 50 % reduction in the chlorophyll *b* content from lighter green/yellowish blotches when compared to the green parts of the chlorotic leaves or to the asymptomatic leaves in potato. In tomato, it is also suggested that iron chlorosis tolerance is controlled by polygenic loci (Dasgan et al. 2004).

Among the ten candidate genes involved in iron uptake mapped in this work, no one mapped within the confidence interval of detected QTLs. QTLs stable over years for leaf chlorophyll content on LG18 and the major QTL for phenotypic variation of leaf chlorosis symptoms and all traits describing plant development on LG13 should be further investigated using all the genomic resources and genetic information available so far for grapevine (Jaillon et al. 2007). Due to confidence interval length observed with classical QTL analyses with limited population size, we cannot conclude whether tightly linked genes act independently on each trait or whether a pleiotropic effect act between chlorosis and plant development.

However, even when single major QTLs were detected for chlorotic symptoms and ferric reductase activity in *P. vulgaris* (Blair et al. 2010) and *L. japonicus* (Klein et al. 2012), genes encoding ferric reductase homologues did not map with these QTLs. Gonzalo et al. (2012) were the first authors to report the location of a transcription factor regulating iron deficiency responses pFIT within the confident interval of a QTL for leaf chlorophyll content of *Prunus* trees growing under calcareous conditions. This supports strongly that more than one gene contributes to

the plant's ability to minimize iron deficiency-associated leaf chlorosis. A huge number of genes underlying QTL are described as transcription factors (Fournier-Level et al. 2009). Such regulatory genes should be further studied in genetic analyses of iron deficiency responses. Finally, several genes involved in iron deficiency responses appear to be post-transcriptionally regulated, but nothing is known about these mechanisms (Ivanov et al. 2012).

Marker-assisted selection for grapevine rootstock breeding appears to be a great challenge, especially when adaptation to abiotic stress is concerned. Scion–rootstock interactions have to be taken into account and genetic analyses have to be performed on grafted material. Two QTLs, stable over several experiments could be of interest. One seems to be involved in the control of leaf chlorophyll content under chlorosing conditions only, whereas the other one appears to be involved in both growth control under any conditions and leaf chlorosis symptoms. This indicates that one part of the grapevine response to chlorosis is associated with growth properties and one part is independent. Further work is required to check the contribution of these two zones, first with a different scion, then in a different genetic background. Genes within the confidence interval of these two QTLs should be investigated with a special interest on regulatory genes.

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