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ORIGINAL PAPER

Genetic and QTL analysis of resistance to *Xiphinema index* in a grapevine cross

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Abstract Resistance to the dagger nematode *Xiphinema index* has been an important objective in grape rootstock breeding programs. This nematode not only causes severe feeding damage to the root system, but it also vectors grapevine fanleaf virus (GFLV), the causal agent of fanleaf degeneration and one of the most severe viral diseases of grape. The established screening procedures for dagger nematode resistance are time consuming and can produce inconsistent results. A fast and reliable greenhouse-based system for screening resistance to *X. index* that is suitable for genetic studies and capable of evaluating breeding populations is needed. In this report, the dynamics of nematode numbers, gall formation, and root weight loss were investigated using a variety of soil mixes and pot sizes over a 52-week period. Results indicated that the number of galls formed was correlated with the size of the nematode population and with the degree of root weight loss. After inoculation with 100 nematodes, gall formation could be reliably evaluated in 4–8 weeks in most plant growth conditions and results were obtained 6 months more rapidly than past evaluation methods. This modified *X. index* resistance screening method was successfully applied to 185 of the 188 F_1 progeny from a cross of D8909-15 × F8909-17 (the 9621 population), which segregates for a form of *X. index* resistance originally derived from *Vitis arizonica*. Quantitative trait loci (QTL) analysis was carried out on both parental genetic maps of 255 markers using MapQTL 4.0. Results revealed that *X. index* resistance is controlled by a major QTL, designated *Xiphinema index Resistance 1* (*XiR1*), near marker VMC5a10 on chromosome 19. The *XiR1* QTL was supported by a LOD score of 36.9 and explained 59.9% of the resistance variance in the mapping population.

Keywords Dagger nematode · Quantitative trait loci · *Vitis arizonica* · *Xiphinema index*

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Introduction

The dagger nematode *Xiphinema index* can cause severe damage to the root system of grapevines. Since the first identification of *X. index* in California (Thorne and Allen 1950), its occurrence has been reported across California vineyards (McKenry et al. 2004), and around the world (Jawhar et al. 2006; Leopold et al. 2007; Tzortzakakis et al. 2006). More significantly, *X. index* is recognized as the primary vector for transmission of grapevine fanleaf virus (GFLV), the causal agent of the fanleaf degeneration disease (Hewitt et al. 1958), which is considered to be one of the major threats to the grapevine industry (Andret-Link et al. 2004). Vines infected with this disease often exhibit

misshapen leaves with blotchy yellow or vein limited chlorosis, double nodes and shortened internodes on canes, and poor fruit set leading to yield reductions of up to 80% (Martelli and Savino 1988).

Xiphinema index can survive in vineyard soils and retain GFLV for many years with or without host plants (Demangeat et al. 2005). A fallow period of at least 10 years is necessary to ensure the elimination of *X. index* populations (http://www.ipm.ucdavis.edu/PMG/r302200111.html). The use of nematicides and fumigants to control *X. index* has not been successful because of the nematode's ability to exist on detached grape roots deep in the soil profile and because of the relatively poor penetration of fumigants (Raski and Goheen 1988). Breeding resistant rootstocks has long been considered to be an effective approach to controlling *X. index*.

Resistance to *X. index* has been identified in several *Vitis* species including *V. arizonica*, *V. candicans*, *V. rufotomentosa*, *V. smalliana*, and *V. solonis* (Kunde et al. 1968), and a preliminary investigation into the inheritance of *X. index* resistance has been conducted (Meredith et al. 1982). The lack of a rapid and reproducible resistance screen has been a major limitation to further study of *X. index* resistance. Greenhouse-based *X. index* resistance screening techniques have been described (Harris 1983; Kunde et al. 1968; McKenry and Anwar 2006; McKenry et al. 2001; Meredith et al. 1982). In these studies, the nematodes were collected from infested greenhouse or vineyard soils and resistance was assessed by changes in *X. index* populations and ratings of root damage 8–18 months after inoculation. These time consuming and labor intensive screening techniques have limited genetic studies and rootstock breeding programs, which require testing of large numbers of progeny.

The objective of this investigation was to understand the genetic basis of *X. index* resistance derived from *V. arizonica*. Here we report on, (1) the development of a rapid system to reliably evaluate *X. index* resistance; (2) the evaluation of *X. index* resistance in an existing mapping population using an optimized screening system; and (3) the identification of a major QTL largely responsible for the *V. arizonica*-derived resistance to *X. index*.

Materials and methods

Plant materials, rooting method and experimental arrangements

To optimize the evaluation of resistance to *X. index*, three grapevine genotypes from the University of California, Davis vineyards were selected: *V. rupestris* St George (susceptible to *X. index*), *Muscadinia rotundifolia* Trayshed and *V. rufotomentosa* DVIT 1416 (both resistant to *X. index*). For genetic and QTL analysis, the 9621 F_1 population of 188 siblings segregating for resistance to *X. index* was used. This population had previously been used to map resistance to Pierce's Disease (Riaz et al. 2006). The population was derived from a cross between two half-siblings D8909-15 (female) and F8909-17 (male) that share a common maternal parent *V. rupestris* A. de Serres, but have different paternal parents, *V. arizonica* b42-26 and *V. arizonica* b43-17, respectively. D8909-15 was tested and found to be resistant to *X. index* and F8909-17 was found to be susceptible (Walker and Jin 1998).

To optimize the resistance evaluation, herbaceous two-node cuttings (about 20 cm in length) were used to propagate St George, Trayshed and DVIT 1416. The cuttings were dipped in 17% Wood's Rooting Compound (Earth Science Production Corp., Wilsonville, OR) for 5 s before being inserted into small cellulose sponges (Grow-tech, Boothbay, ME), and placed on an intermittent mist propagation bed with 30°C bottom heat. After roots had emerged from the sponges, the St George cuttings were planted in both small and large pots. Trayshed and DVIT 1416 were planted only in small pots. The large pots held 500 cm^3 (6 by 24 cm in diameter and depth, respectively) of soil and the small pots held 140 cm^3 (4 cm in diameter and 8 cm in depth). Two types of soil were used for each pot size: washed coarse 12 mesh sand (1.7 mm sieve); and a 1:1 mix of Yolo clay loam and washed coarse sand. Both soil types were steam sterilized before use.

In order to screen the large number of plants, the 9621 population was divided into groups of 40–50 genotypes, each with four inoculated plants. These plants were also rooted from herbaceous cuttings. Each group contained inoculated and uninoculated plants of the following control vines, which were propagated in the same way: *V. rupestris* A. de Serres, D8909-15, F8909-17, and *V. rupestris* St George. These parent, progeny and control plants were tested in the small pots using the 1:1 coarse sand/Yolo clay loam soil mix. Greenhouse, watering and fertilization conditions were the same as presented below. Prior to inoculation, the shoots and roots of the 9621 progeny and the control plants were trimmed back. These plants were then completely randomized within the group blocks. A single row of potted grapevines was placed around the perimeter of each bench to reduce edge effects. Additional St George (susceptible control) plants were used to determine the appropriate time to evaluate feeding symptoms.

Xiphinema index collection and inoculation

The nematodes were collected from two vineyard sites known to have high populations of *X. index*. Large amounts of soil were collected and nematodes were extracted using the Baermann funnel and sieving procedure described in

Agrios (1997). The concentration of nematodes was estimated by using a microscope to count the number in 5% of the clean suspension on a Petri-dish with an etched grid. About 1 week after root trimming and repotting, each potted vine was inoculated by dispensing about 100 juvenile and adult nematodes in four 2.5-ml aliquots suspensions into four evenly spaced 2-cm deep holes. The uninoculated plants had the same amount of water similarly dispensed into the soil.

Optimizing the protocol for the identification of resistance to *X. index*

Once rooted, uniform sized St George, Trayshed and DVIT 1416 plants were used to optimize the *X. index* resistance screen. The St George plants were planted into each of the four pot size/soil mix treatments (three replicates for the inoculation treatment and three uninoculated controls) for each of 14 evaluation dates (1, 2, 4, 8, 11, 16, 21, 25, 30, 35, 39, 43, 47 and 52 weeks after inoculation) resulting in 336 potted plants. The Trayshed and DVIT 1416 plants were planted and sampled over the same dates, but there were only two plants of each (one inoculated and one uninoculated) and they were tested in small pots with the two soil mixes resulting in 56 plants of each genotype. The potted plants were positioned on benches in the greenhouse in a complete randomized block design. The uninoculated blocks were separated from the inoculated blocks to avoid cross contamination of *X. index*. The greenhouse was set to maintain a soil temperature of 22–30°C and daylengths of 13–15 h. All plants were automatically watered twice a day with overhead sprinklers, supplemented with hand watering as needed, and fertilized biweekly with Miracle-Gro™ for Roses (10N–20P–10K, Marysville, OH). About a week before nematode inoculation, plants were removed from the pots and roots were trimmed to stimulate new root growth for nematode feeding. To optimize the evaluation of *X. index* resistance, the nematode number, fresh root weight and number of galls from St George, Trayshed and DVIT 1416 were recorded 1, 2, 4, 8, 11, 16, 21, 25, 30, 35, 39, 43, 47 and 52 weeks after inoculation. Nematodes were extracted and counted following the procedures described above. Data analyses were carried out using the SAS program (SAS Ver. 8 for Windows).

Genetic analysis of resistance in the cross

Evaluation of *X. index* resistance in the 9621 population was based on the number of galls observed. To identify the appropriate time to evaluate gall numbers, the roots of two St George plants within each inoculated group of 9621 progeny were examined 4 weeks after inoculation, and weekly thereafter until more than ten galls were observed on the root system of each of the St George plants. The time period between *X. index* inoculation and evaluation ranged from 4 to 8 weeks. At the evaluation time for each group, four inoculated and four uninoculated D8909-15 and F8907-17 plants were also examined as highly resistant and susceptible standards, respectively. Resistance was analyzed within the 9621 population in two ways. First, the mean number of galls was obtained from the four replicated plants of each genotype. The second method assigned a categorical value to the number of galls observed and focused on the lower range of observed gall numbers: I = 0 galls, II = 0.1–1.0; III = 1.1 to 2.0; IV = 2.1 to 3.0; and V = >3 galls. Both the mean number of galls and these categorized values were used in the QTL analysis described below.

QTL analysis and mapping

Genome coverage of 19 chromosomes for both parental lines was obtained from linkage analysis involving 237 primarily simple sequence repeat (SSR) markers reported in Riaz et al. (2006). An additional 18 markers were added to the previous D8909-15 map of 159 markers. Seventeen of these were SSR markers adopted from published *Vitis* genetic maps (Doligez et al. 2006; Lamoureux et al. 2006). The last one, M4F3F, was a cleaved amplified polymorphic sequence (CAPS) marker developed from the DNA sequence of a genomic clone of D8909-15 by targeting a SNP (single nucleotide polymorphism) between D8909-15 and F8909-17 as well as between the two homologous chromosomes of D8909-15 in the recognition site of *Afl* II. The M4F3F marker was PCR amplified (30 cycles with annealing temperature of 56°C) with the two primers listed below: 5′TTGCCACACCATATCGATGCCTACTC and 5′GGACAATAATCTTGTGGCGAGGTTGG. Restriction digestion was performed with *Afl* II according to the manufacturer's instruction (New England Biolabs, Beverly, MA). QTL analyses of *X. index* resistance were performed using interval mapping and multiple-QTL model (MQM) mapping in MapQTL 4.0 (Van Ooijen et al. 2002) with the mean gall numbers, and the categorized gall scores from I through V. The LOD threshold scores for significant QTL were obtained with permutation tests of 1,000 at both genome and chromosomal levels (Van Ooijen et al. 2002).

Results

Optimizing evaluation of grapevine resistance to *X. index*

To optimize the greenhouse-based *X. index* resistance screen, root weights, the number of nematodes, and the number of galls were recorded after inoculations of St George growing in four soil mix, pot size combinations

(Supplementary Fig. 1). Gall formation on the root tips began 1 week after inoculation and could be reliably evaluated after 4–8 weeks in most plant growth conditions; a dramatic improvement over the time required with previous evaluation methods. Regression analyses showed that gall numbers were highly correlated not only with nematode population levels in all four pot/soil combinations ($R^2 = 0.5937^{**}$ to 0.9652^{**}, Supplementary Fig. 1), but also with root weight loss ($R^2 = 0.3752^{*}$ to 0.8920^{**}) in all but the small pot/sand combination, suggesting that gall number is a valid indicator of grape resistance to *X. index*. When large pots were used the soil treatment did not have an impact, however, when small pots were used the sand/soil mix was best. Thus, gall numbers were used as a quick and easy method of evaluating *X. index* resistance in the 9621 population.

Xiphinema index resistance screening in the 9621 population

Out of 188 F1 genotypes from the 9621 mapping population, 185 were successfully evaluated for *X. index* resistance. There were 72 genotypes with zero galls, and 27, 6, 5 and 75 genotypes with 0.1–1.0, 1.1–2.0, 2.1–3.0 and >3 galls, respectively (Fig. 1). The average number of galls for the 75 genotypes with more than three galls was 20.4 ± 16.4. The two parents, D8909-15 (resistant) and F8909-17 (susceptible), had 0 and 30.8 ± 10.1 galls, respectively. Analysis of variance revealed that there was a highly significant difference among genotypes ($P < 0.0001$) as well as among replicates ($P = 0.0049$), suggesting that the 9621 population segregates for resistance to *X. index*

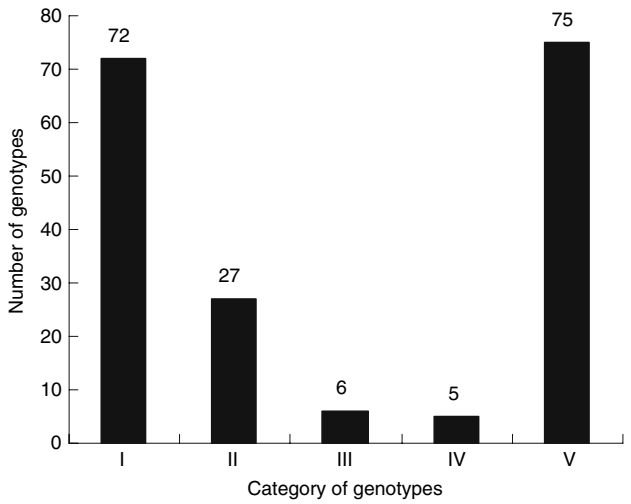

Fig. 1 Distribution of F_1 genotypes in the 9621 population based on gall numbers induced by *X. index* feeding. Categories I through V represent 0, 0.1–1.0, 1.1–2.0, 2.1–3.0 and >3 galls, respectively. The numbers of F_1 individuals observed in each category are shown on top of the columns

and that replication of tested genotypes was effective in reducing experimental errors.

In this experiment, the mean gall numbers of genotypes with more than zero galls had wide range of coefficient of variation (CV) values, 1.5–200%. A detailed data examination revealed that out of 28 genotypes with large CVs (180–200%), 24 had a mean gall number less than two, and often had a few galls observed in one replicate and none in the other three. These results could have been caused by variation in pot moisture, temperature and other unknown factors. In the QTL analysis presented below, these genotypes were included without any exclusion as outliers despite the observed large variation in mean gall numbers. However, to emphasize the differences among the genotypes with fewer than three galls per replicate and not to exaggerate the difference among genotypes with more than three galls per replicate, we converted the mean number of galls in the following ranges, 0, 0.1–1.0, 1.1–2.0, 2.1–3.0 and >3.1, into five resistance categories: I (most resistant), II, III, IV and V (most susceptible), respectively. This categorical data set was also used in QTL analysis.

QTL analysis of resistance to *X. index* in the 9621 population

The genetic map of D8909-15, the *X. index* resistant female parent, was primarily established on the basis of previous work (Riaz et al 2006) with the addition of 18 more markers. M4F3F was one of the additional markers (Fig. 2) developed from the DNA sequence of a genomic clone from D8909-15. It amplifies a region that carries a SNP in the recognition site of *Afl* II that distinguishes the homologous chromosomes of D8909-15 and F8909-17, and can be detected using agarose gel electrophoresis (Fig. 2). The current D8909-15 genome map consists of 177 markers with an average density of 5.7 cM per marker, covering the genome with 1013.7 cM. The male parent F8909-17 map, constructed with 158 markers covered 1055.0 cM was the same as previously reported (Riaz et al. 2006). QTL analysis was independently conducted on the five categorical resistance scores and the mean gall numbers. When the categorical data was evaluated, the analysis uncovered a strong QTL that peaked near marker VMC5a10 on chromosome 19 (Fig. 3.). This QTL was designated *X. index Resistance 1* (*XiR1*), and it was supported by a LOD score of 36.9 explaining 59.9% of the phenotypic variance in population 9621. The effect of the *XiR1* QTL, compounded with additive and dominance effects, was estimated to be 2.84 ± 1.16 for the resistant allele. Based on this effect, 99 of the 185 F_1 genotypes in categories I (0 galls) and II (0.1–1.0 galls) were considered to be resistant, 80 in categories IV (2.1–3.0 galls) and V (>3.0 galls) as susceptible and 6 in category III (1.1–2.0 galls) were undetermined. The segre-

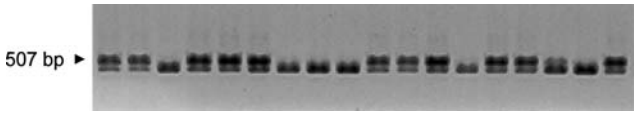

Fig. 2 Agarose gel profile of marker M4F3F assayed in the 9621 population. The *arrow* indicates the 507 bp band associated with resistance

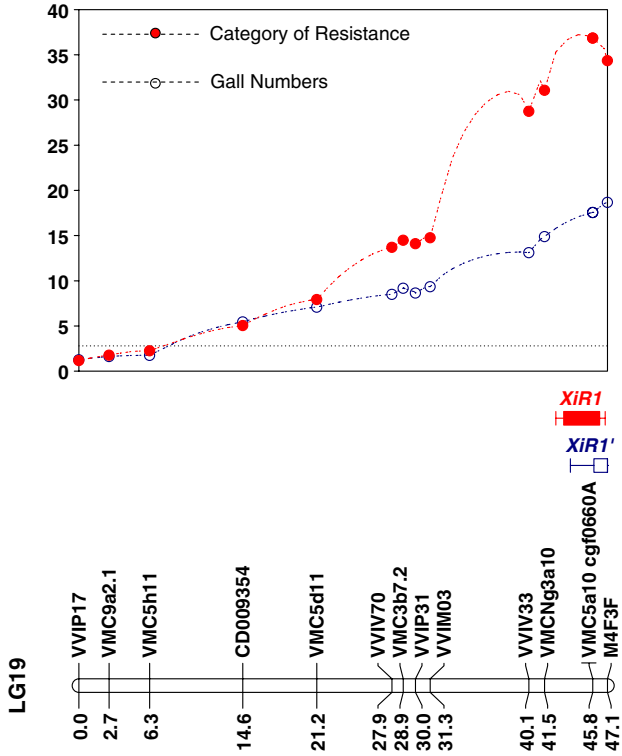

Fig. 3 Placement of the *XiR1* QTL on chromosome 19. The *XiR1* and *XiR1'* QTL were detected using five-categorical resistance scores and mean gall numbers, respectively. The putative QTL regions were calculated using thresholds of 1-LOD (inner) and 2-LOD (outer) from the peak, respectively. The numbers along the chromosome are genetic distance in centi-Morgans

gation ratio of 99 (R):80 (S) fit the expected ratio of 1:1 ($\chi^2 = 2.017$, $0.5 > P > 0.1$), indicating that *XiR1* is a heterozygous resistance QTL with a dominant resistant allele in D8909-15. When the mean gall number data set was used another strong QTL was detected, designated *XiR1'*, near marker M4F3F on chromosome 19, which was 1.3 cM away from *XiR1* and supported by a LOD score of 18.7 (Fig. 3). However, the *XiR1'* QTL only explained 37.0% of the resistance variance and had an effect estimated to be 17.5 ± 11.3 galls for the resistant allele. Since *XiR1* and *XiR1'* were so closely linked on chromosome 19 and the effect of *XiR1* was stronger, the two were considered to be the same QTL and *XiR1* was considered to be more representative.

In addition to the major QTL *XiR1*, a minor QTL was identified near marker VMC9g4 on chromosome 17 using the categorical data set. This minor QTL was supported by a LOD score of 2.5 and accounted for 5.9% of the phenotypic variance. A genome wide permutation test showed that a LOD score of 2.8 was required to have a 95% confidence of detecting a putative QTL. However, a chromosome wide permutation test suggested that the LOD of 2.5 was higher than the threshold LOD value of 2.1 at the 99% confidence level. No other significant QTL for *X. index* resistance were detected in either parental genome using both interval mapping and MQM mapping when the *XiR1* linked markers were used as cofactors.

Discussion

Gall number as a measure of resistance to *X. index*

The quest for resistance to *X. index* has been underway since the 1960s (Kunde et al. 1968), with the goal of preventing severe root damage and the vectoring of GFLV. A number of systems have been used to evaluate *X. index* resistance (Harris 1983; Kunde et al. 1968; McKenry and Anwar 2006; McKenry et al. 2001; Meredith et al. 1982). However, the relatively lengthy 8- to 18-month evaluation procedures used in these studies, limited comprehensive genetic studies and breeding programs involving a large number of progeny.

In an attempt to develop a more efficient and rapid screening system for evaluating *X. index* resistance, the dynamics of nematode reproduction, gall formation and root damage were investigated using the highly susceptible *V. rupestris* St George grown in four pot size/soil mix treatments under greenhouse conditions over the course of 1 year. There were significant increases in both nematode numbers and gall formation in all four growing conditions. Root weights declined under nematode pressure when big pots were used, although root weight was not significantly reduced when the small pots were used. Regression analyses revealed that the number of galls formed was highly correlated with the nematode population increases as well as with the degree of root weight loss in all conditions except when small pots with sand were used. These results suggested that in addition to nematode numbers and root damage (root weight loss), both commonly used in past studies, gall number is also a rational and reliable measurement for plant resistance to *X. index*. Root galling was detectable 1 week after inoculation and a large number of galls (11–27) were formed within 8 weeks in all conditions except for the small pot/sand treatment. This experiment found that gall numbers could be used as a reliable indicator of *X. index* resistance over a 1- to 2-month period.

The number of galls formed in response to root-knot nematode feeding (galling index) has also been recently used to evaluate resistance of a large number of cotton plants (Zhang et al. 2006).

Xiphinema index resistance screening in the 9621 mapping population

A total of 185 of the 9621 mapping population progeny were successfully screened for resistance to *X. index*. Because of limited greenhouse space and personnel time, the replicated progeny were divided into groups and screening experiments within each group were conducted independently. Two plants of the susceptible St George were included in each group to standardize the time at which evaluations were made; when these plants had ten or more galls the group was evaluated. This practice resulted in a 4- to 8- week difference in the time progeny groups were evaluated. However, the use of a susceptible genotype to standardize the time to evaluation proved to work better than a pre-determined evaluation time that resulted in highly variable numbers of galls.

Over the course of the evaluation, it was observed that *X. index* collected on different days using the same test procedures often developed galls at rates that varied by weeks. Possible reasons for this variability could be: slight variations in water temperature; the degree of agitation during nematode inoculation; or root quality and plant vigor at the time of inoculation. Screening the 9621 mapping population in larger pots over a longer period might be a better alternative. However, there were no significant differences in gall numbers produced among the pot/soil combinations in the first 4–8 weeks. The small pot/soil sand treatment was chosen because the impact on gall production was limited, while it reduced time and space required for evaluation. Another benefit of scoring the galls in a short time period was the avoidance of problems associated with longer term pot culture, such as gall decay, blackening of the roots, and root tip swelling or death due to restricted rooting area, all of which interfere with successful scoring of *X. index* galls.

The gall numbers on the susceptible progeny (genotypes with more than three galls) averaged 20.4 ± 16.4 galls per replicate, indicating that the screening conditions were suitable and the nematode inoculum was effective. However, there were large standard deviations for the mean number of galls for many genotypes. A number of factors could have contributed to this variation. Foremost, is the feeding quality of the root tips at the point of inoculation; however, galls were observed at all four of the inoculation sites in most of the tested plants. Similar large variations among replicates in feeding symptoms were encountered in other experiments despite the use of longer evaluation periods (Harris 1983; Kunde et al. 1968).

QTL for resistance to *X. index*

MapQTL successfully detected two strong QTL using data on mean gall numbers and the five categorical resistance scores. These two QTL, *XiR1* and *XiR1'*, were located near markers VMC5a10 and M4F3F on chromosome 19, which are 1.3 cM apart. However, *XiR1* was supported with larger LOD score than *XiR1'* (36.9 vs. 18.7) and it explained a higher percentage (59.9 vs. 37.0%) of the resistance variance in the 9621 population. These results suggest that *XiR1* and *XiR1'* are probably the same QTL and that *XiR1* is a better indication of *X. index* resistance derived from *V. arizonica* b42-26 through its progeny D8909-15.

The discovery of *XiR1* will have a large impact on efforts to breed *X. index* resistant rootstocks and to characterize the gene(s) involved. This major QTL is a suitable target for map-based cloning approaches to isolate the underlying gene(s) responsible for *X. index* resistance from *V. arizonica* b42-26. This approach has effectively isolated a number of agronomically important plant genes, including root-knot nematode resistance genes (Williamson and Kumar 2006). Map-based positional cloning has also been used in efforts to clone the *Run1* locus, derived from *M. rotundifolia*, which confers resistance to grapevine powdery mildew, *Uncinula necator* (Barker et al. 2005). In addition, the ongoing French–Italian collaborative project to sequence the *V. vinifera* genome has already resulted in release of shotgun sequences of 7× genome equivalents (http://www.genoscope.cns.fr/cgi-bin/blast_server/projet_ML/blast.pl). These sequences have been made available in public databases such GenBank and will greatly facilitate genomic studies and positional cloning of grape genes.

Cloning of *XiR1* will enhance our understanding of the genetic and pathological mechanisms involved in *XiR1*-mediated resistance. It may also lead to the enhancement of *X. index* resistance through genetic engineering. There may be other forms of *X. index* resistance in other grape species, which are not mediated by *XiR1*. Earlier studies on *X. index* resistance in *Vitis* species were done with relatively small population sizes, but they suggest that resistance from some sources could be controlled by two genes (Meredith et al. 1982). The resistance source derived from *M. rotundifolia*, such as observed from rootstock O39-16 (Walker et al. 1994), may also be very different from *XiR1* given the distant taxonomic and genetic status of this species.

The identification of *XiR1* provides an ideal means to introgress *X. index* resistance into rootstocks through marker-assisted selection (MAS). Three markers M4F3F, VMCNG 3a10 and VMC 5a10 are tightly linked to *XiR1* and have been used in marker-assisted screening in our rootstock breeding program to integrate dagger nematode and Pierce's disease resistance into a single rootstock. Many studies have demonstrated that resistant grapevines

can dramatically decrease *X. index* populations in greenhouse (Harris 1983; Kunde et al. 1968; McKenry and Anwar 2006; McKenry et al. 2001; Meredith et al. 1982) and vineyard situations (McKenry et al. 2004). However, combining resistance to *X. index* with tolerance to the fruit set disrupting effects of GFLV is necessary to produce a fanleaf degeneration resistant rootstock (Walker et al. 1989, 1994). Other efforts to control fanleaf have focused on genetically modifying rootstocks with anti-sense constructs of GFLV's coat protein (Gambino et al. 2005; Maghuly et al. 2006; Valat et al. 2006). For this strategy to be completely successful, candidate rootstocks must also include *X. index* resistance, which could be incorporated either through MAS or genetic engineering if the *XiR1* locus can be cloned.

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