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Fine mapping of two major QTLs conferring resistance to powdery mildew in tomato

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Abstract Tomato (*Solanum lycopersicum*) is the most cultivated crop in the Solanaceae family and is a host for *Oidium neolycopersici*, the cause agent of powdery mildew disease. In wild species of tomato, genes (*Ol-1*–*Ol-6*) for monogenic resistance have been identified. Moreover, three quantitative resistance loci (QRLs), namely *Ol-qtl1*, *Ol-qtl2* and *Ol-qtl3*, have been mapped in *Solanum neorickii* G1.1601. In this work, we developed several advanced backcross populations in order to fine-map these *Ol-qtls.* Resistant lines harboring individual *Ol-qtl* were produced and used in recombinant screening. Ten recombinants were identified in chromosomal regions carrying *Ol-qtl1s.* The recombinant individuals were used to produce recombinant families (RFs). By screening these RFs with molecular markers and testing them with *O. neolycopersici*, we could localize *Ol-qtl1* in a region of about 2.3 Mbp on the long arm of chromosome 6 and *Ol-qtl2* in a region of 2.5 Mbp on the short arm of chromosome 12. On the other hand, the presence of *Ol-qtl3* locus was not confirmed in this study. The fine-mapping results further demonstrated the co-localization between *Ol-qtls* and genes for monogenic resistance; the *Ol-qtl1* interval contains the *Ol-1* gene and the *Ol-qtl2* interval harbors the *Lv* gene that confers monogenic resistance to *Leveillula taurica*, another species of tomato powdery mildew.

Keywords Quantitative resistance loci (QRLs) · Powdery mildew · Recombinant families · Fine mapping

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Introduction

The cultivated tomato (*Solanum lycopersicum*) is a very important vegetable worldwide (Foolad et al. 2008). Tomato hosts more than 200 species of a wide variety of pests and pathogens that can cause significant economic losses (Bai and Lindhout 2007). Since the 1980s, powdery mildew epidemics caused by the biotrophic fungus, *Oidium neolycopersici*, have become a problem in tomato production worldwide (Jankovics et al. 2008). Symptoms of tomato powdery mildew infection are white circular pustules that

appear predominantly on the upper sides of leaves, stems and petioles (Mieslerova et al. 2004). Although fungicide can be used to control the disease, such treatments are undesirable in relation to both their costs and their failure to achieve sustainable productivity. Thus, breeding of resistant cultivars is desired to control this disease.

Cultivated tomato has limited variability, largely because of natural and artificial selection that occurred during domestication and development of modern cultivars. To improve disease resistance and agronomic traits, tomato wild germplasm has been demonstrated to be a useful resource (Bai and Lindhout 2007). Resistance to *O. neolycopersici* has been detected among related *Solanum* species (Bai et al. 2005). Based on mechanisms and genetics, resistance to *O. neolycopersici* in tomato can be divided into three categories. The first category is monogenic resistance that is controlled by dominant genes (*Ol-1*, *Ol-3–Ol-6*) and associated with a hypersensitive response (HR) (Bai et al. 2005; Li et al. 2007). These dominant genes originated from different accessions of *S. habrochaites* and *S. peruvianum*. The second category is monogenic resistance that is controlled by the recessive *ol-2* gene and associated with papilla formation (Bai et al. 2008). The *ol-2* gene is found in *S. lycopersicum* var. *cerasiforme* (cherry tomato) and belongs to a natural loss-of-function in the *SlMlo* gene (Bai et al. 2008). The third category is polygenic resistance that is governed by three quantitative trait loci for resistance (QRL) identified in *S. neorickii* G1.1601 (Bai et al. 2003) and associated with both HR and papilla formation (Li et al. 2011).

In the last decades, considerable progress has been achieved in our understanding about the interaction of plants with pathogens. Most knowledge has been documented in complete resistance which is controlled by single dominant resistance (*R*) genes. According to the current hypothesis about the plant immune system, *R*-genes encode proteins that recognize specific pathogen effectors. This recognition triggers a cascade of defense responses and the resistance is manifested as localized HR at the site of infection (Bruce and Pickett 2007; Chisholm et al. 2006; Jones and Dangl 2006; Robert-Seilaniantz et al. 2007). Many *R*-genes have been cloned and most of them encode proteins with an N-terminal nucleotide-binding site (NBS) and C-terminal leucine-rich repeats (LRRs) (Takken et al. 2006). Protein structural similarities of the cloned *R*-genes have allowed isolation and mapping of structurally related sequences referred to as resistance gene analogues (RGAs).

In contrast, only a few QRLs have been characterized and the molecular basis underlying resistance conferred by QRLs is limited. By studying the organization of QRLs in the potato genome, Gebhardt and Valkonen (2001) proposed that the molecular basis of quantitative resistance in potato can be based on genes having structural similarity with cloned *R*-genes. An example of this hypothesis is the *Rpi-mcd1* QRL, which confers partial resistance to *Phytophthora infestans* in potato and is located in a cluster of NBS-LRR genes on chromosome 4 (Tan et al. 2008). Moreover, Gebhardt and Valkonen (2001) described that genes involved in the cascade of the defense response can have a role in QRL-based resistance. The difficulty in cloning QRLs is mainly due to the small effect on disease resistance that each QRL can explain. Recently, three QRLs have been cloned: the recessive *pi21* gene conferring resistance to blast disease in rice and coding a proline-rich protein (Fukuoka et al. 2009); the *Yr36* gene giving resistance to wheat stripe rust and coding a kinase-START protein (Fu et al. 2009); and the *Lr34* gene coding for an ABC transporter protein and involved in resistance against fungal pathogens in wheat (leaf rust, stripe rust, and powdery mildew) (Krattinger et al. 2009). All the QRLs isolated until now are structurally different from *R*-genes and do not have a typical *R*-gene motif (St Clair 2010). Even though none of the cloned QRLs has a similarity with known *R*-genes, there is evidence of co-localizations of QRLs with *R*-genes and/or RGAs (e.g. (Bai et al. 2003; Geffroy et al. 2000; Grube et al. 2000).

Our research aims to isolate QRLs for resistance to *O. neolycopersici* via map-based cloning. Previously, we reported that map positions of the three QRLs identified in *S. neorickii* G1.1601 co-localize with dominant genes for resistance to powdery mildew in tomato (Bai et al. 2005). The *Ol-qtl1* interval overlaps with *Ol-1*, *Ol-3* and *Ol-5*, while the other two linked *Ol-qtls* are located on chromosome 12 in the vicinity of the *Lv* locus that confers resistance to another powdery mildew species, *Leveillula taurica*. Though co-localization of QRLs with *R*-genes and RGAs has been identified in many cases (Geffroy et al. 2000; Tan et al. 2008), only a few functional studies support that QRLs are weak alleles of *R*-genes. Thus, cloning of

Ol-qtls will help to elucidate the structural relationships between the co-localized *R*-genes (*Ol*-genes and *Lv*) and *Ol-qtls*. In this study, we fine mapped the *Ol-qtl1* and *Ol-qtl2* regions on the short arm of chromosome 6 and 12 respectively.

Materials and methods

Plant materials

An F_2 population, advanced backcrosses and their selfing populations (BC_2, BC_2S_1 and BC_2S_2) were used in this study, which are derived from an inter specific cross between the susceptible *S. lycopersicum* cv. Moneymaker (MM) and resistant accession *S. neorickii* G1.1601 (SN). MM was used as recurrent parent in the backcrossing. For fine mapping O*l-qtl1* on chromosome 6, we used one BC_2 family and one BC_2S_1 population, which were derived from different BC_1 plants. Both populations were segregating for the resistant *Ol-qtl1* allele (SN allele) on chromosome 6 and lacking the SN alleles of *Ol-qtl2* and *Ol-qtl3* on chromosome 12 (Bai et al. 2003). For fine-mapping *Ol-qtl2*, we used one BC_2S_1 population, in which the SN alleles of both *Ol-qtl2* and *Ol-qtl3* are present and the SN allele of *Ol-qtl1* is absent (Bai et al. 2003). The selected recombinants were maintained to produce selfing progenies, which were named as recombinant families (RFs).

Fungal material

Oidium neolycopersici (The *On*-Ne isolate, Bai et al. 2005) was maintained and propagated on susceptible MM plants in a growth chamber at 20 ± 2°C with 70% relative humidity (RH) and 16 h of light/day. The inoculum preparation and the inoculation were performed as described in Bai et al. (2003) by washing conidial spores from freshly sporulating leaves of heavily infected MM plants in tap water. Then the inoculum concentration was adjusted to 2×10^4 conidia/ml.

Disease tests

One month-old tomato plants (growing stage: three to four true leaves) were inoculated by spraying the fungal inoculum. Inoculated plants were grown in a greenhouse compartment at 20 ± 3°C with 70% RH for symptom development. Fungal growth was evaluated at 11, 14 and 19 days post inoculation according to the following disease index (DI): 0 = no visible sporulation, 1 = very few fungal spots surrounded by necrosis and less than 5% foliar area affected (weak sporulation), 2 = moderate number of fungal spots, 5–30% foliar area affected (moderate sporulation), and 3 = very high number of fungal spots, more than 30% foliar area affected (heavy sporulation). An average DI was calculated over three evaluation times for each plant. As applied in our previous studies on monogenic resistance to *O. neolycopersici* (Bai et al. 2003), plants were considered as resistant with a DI $\leq$ 1, intermediated resistant with a DI 1 $<$ DI $\leq$ 2 and susceptible with a DI $>$ 2.

Marker generation

Markers were designed from sequences in the SGN database (Appendix 1). Primers were designed by using Primer3 software (http://redb.croplab.org/modules/redbtools/primer3.php) and polymorphism detection were performed according to the method described by Bai et al. (2003).

Linkage map construction and QTL mapping

Joinmap 4.0 (Van Ooijen 2006) was used to generate a genetic map applying the Kosambi mapping function. QTL mapping was performed according to MapQTL®5 software (Van Ooijen 2004). A LOD threshold value of 4 was set for declaring a QTL in interval mapping (IM). A one-LOD support interval was taken as a confidence interval for a putative QTL (Van Ooijen 2004).

Results

Previously, three *Ol-qtls* were detected by using dominant AFLP markers in an F_2 population (Bai et al. 2003). To increase the power in QTL detection, co-dominant markers were generated in this study. Sequences of tomato chromosome 6 and 12 were selected and converted to co-dominant CAPS makers (Appendix 1). In total, 37 markers were generated and mapped in the F_2 population. For map comparison,

some AFLP markers (Bai et al. 2003) were included to generate linkage groups. Then, QTL analysis was performed resulting in the detection of the three *Ol-qtls* in the chromosomal regions as previously defined (Figs. 1a, 3a). However, the QTL intervals could not be narrowed down with co-dominant markers, which is probably due to the small size of the F_2 population.

Ol-qtl2 is confirmed to be co-localized with the *Lv* gene

In order to confirm the presence of two closely linked QRLs on chromosome 12, we used one BC_2S_1 population (named P-222; $n = 164$) which carries SN introgressions for *Ol-qtl2* and *Ol-qtl3,* but lack the *Ol-qtl1* SN introgression on chromosome 6. In P-222, DI was segregating in a range between 0.6 and 3, showing a large effect of these two QRLs on resistance to *O. neolycopersici*. Twenty-one co-dominant markers were used to genotype the P-222 population and to produce a genetic map for QTL mapping (Fig. 1b). A large chromosomal region between markers TG180 and CD22 covering both *Ol-qtl2* and *Ol-qtl3* showed a significant LOD value. The one-LOD confidence interval spans 1.3 cM between markers CT121 and T1263 (Fig. 1b), which overlaps with the chromosomal region where *Ol-qtl2* was previously mapped in the F_2 population (Fig. 1a, b). This QTL showed a LOD value of 12 and explained nearly 30% of the phenotypic variation in the analyzed population. Though only one peak was observed on the short

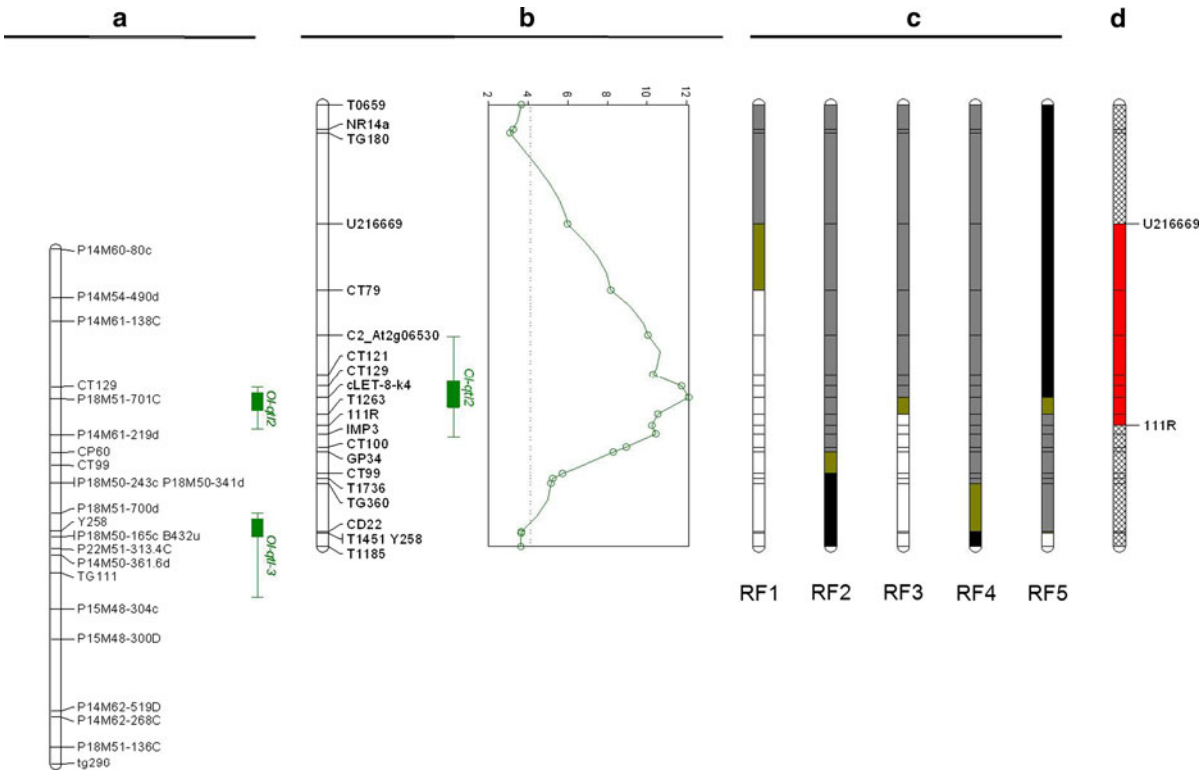

Fig. 1 The map positions of *Ol-qtl2* and *Ol-qtl3* shown on linkage groups of the short arm of tomato chromosome 12, which are constructed by using an F_2 and BC_2S_1 population, respectively, derived from a cross between *Solanum lycopersicum* cv. MM and SN G1.1601. **a** *Ol-qtl2* and *Ol-qtl3* mapped in the F_2 population and with CAPS markers. **b** *Ol-qtl2* mapped in the BC_2S_1 population with CAPS markers. The graph shows the QTL likelihood profiles of interval mapping. The *dotted line* represents the LOD threshold value. In both **a** and **b**, the *green bars* indicate the QTL intervals of which the *inner bar* shows a 1-LOD interval and the *outer bar* shows a 2-LOD interval. **c** Genotypes of five recombinants identified from the BC_2S_1 population. The *black bars* on the linkage groups indicate the presence of homozygous MM alleles, *white bars* for homozygous SN alleles and *grey bars* indicate heterozygous alleles. The *yellow bars* show regions where crossing-over occurred. **d** The fine-mapped position of *Ol-qtl2* indicated by the *red bar*. (Color figure online)

arm of chromosome 12, the chromosomal region above the LOD threshold covers also the *Ol-qtl3* locus (Fig. 1b). Thus, the presence of the *Ol-qtl3* locus could not be excluded.

In order to pinpoint *Ol-qtl2*, five recombinants with contrasting genotypes between T0659 and T1185 were selected to produce BC_2S_1 populations which were named as RF (RF-1, RF-2, RF-3, RF-4 and RF-5; Fig. 1c; Table 1). For each RF, 40 individuals were genotyped and tested with *O. neolycopersici*. DI was scored at three time points and a mean DI was calculated for each plant. All susceptible control MM plants were scored as DI = 3 (Fig. 2a). According to the DI values of plants of these RFs, three groups could be discerned. The first group is the resistant group represented by RF-1, of which all the individuals except two showed a DI $\leq$ 2 (Table 2). Since all tested plants of this RF showed complete or intermediate levels of resistance, it is expected that *Ol-qtl2* is homozygous in a chromosomal region below the marker U216669 towards centromere, where all markers were homozygous for resistant SN alleles as in the parental BC_2S_1 plant (Fig. 1c).

The second group is the susceptible group represented by RF-5. In this RF, 35 plants showed a DI > 2 and 5 plants with a DI between 1 and 2. Since no plants could be considered as fully resistant, we regarded this RF as susceptible (Table 1). Thus, it is likely that the SN allele of *Ol-qtl2* is not present in this RF, suggesting that *Ol-qtl2* is located above marker 111R towards the telomere since the parental BC_2S_1 plant of this RF was homozygous for the MM allele (Fig. 1c) in the chromosomal region above marker 111R.

The third group is segregating for resistance with DI from 0.7 to 3. Three RFs, RF-2, RF-3 and RF-4, were segregating for resistance (Fig. 2b, c), indicating that their parental BC_2S_1 plants were heterozygous for *Ol-qtl2*. According to marker genotypes of these plants, the chromosomal region between markers T0659 and 111R was heterozygous. In agreement with RF-5, this group pointed *Ol-qtl2* to be located above 111R.

In sum, the results of the five RFs indicated that *Ol-qtl2* is located between markers U216669 and 111R, in a chromosome region corresponding to the 2-LOD interval of 12.2 cM in this BC_2S_1 population (Fig. 1d) with a physical distance of about 2.5 Mbp. In the *Ol-qtl2* region 311 genes are predicted by using the ITAG2 annotation of the Solanaceae Genome Network (http://www.solgenomics.net). Out of 311 genes, about 50 are annotated as genes that can be involved in plant pathogen interaction with 13 genes containing a leucine reach repeat motif.

Ol-qtl1 is confirmed to be co-localized with the *Ol-1* gene

In order to confirm the presence of *Ol-qtl1* on chromosome 6, we used a population that carries a SN introgression for *Ol-qtl1*, but lacks the SN introgression for *Ol-qtl2* and *Ol-qtl3* on chromosome 12. Sixteen CAPS markers were developed and used on the F_2 population. By using the markers dCT21 and dCT136 which flank *Ol-qtl1*, two rounds of recombinant screening were conducted. In the first round, seven recombinants between the two markers were obtained by screening 220 BC_2 plants. In the second

Table 1 Results of disease test on recombinants identified in the fine-mapping of *Ol-qtl2*

Recombinant family Name generation	Disease index (DI) (Mean ± SD, $n = 40$)	DI range	Tukey's test[a]
RF-1 BC_2S_2	1.4 ± 0.58	0.5–2[b]	a
RF-2 BC_2S_2	1.9 ± 0.50	0.7–2.8	b
RF-3 BC_2S_2	2.2 ± 0.56	0.8–3	bc
RF-4 BC_2S_2	2.2 ± 0.41	1–3	bc
RF-5 BC_2S_2	2.5 ± 0.36	2–3[c]	c

SD standard deviation

[a] Means with different letters are significantly different at 5% level ($P < 0.05$)

[b] Indicates that two out of 40 plants showed a DI > 2

[c] Indicates that 5 out of 40 plants showed a DI < 2

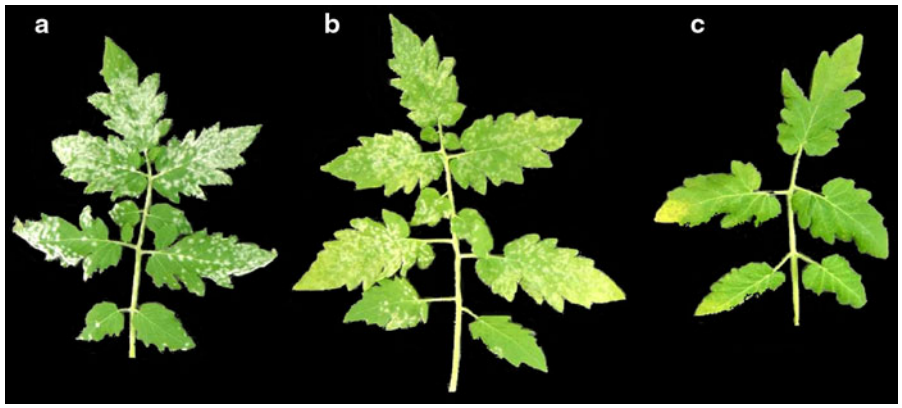

Fig. 2 Tomato leave infected by *O. neolycopersici*. **a** Leaflet of susceptible MM plants scored as DI = 3, **b** leaflet of susceptible genotype (DI = 3) and **c** leaflet of resistant genotype (DI = 1) from the RF-3 population

Table 2 Results of disease test on recombinants identified in the fine-mapping of *Ol-qtl1*

Recombinant family Name generation	Genotype	Disease index (DI) (Mean ± SD, $n = 10$)	Turkey's test[a]
RF-6 BC_2S_2	MM	2.97 ± 0.08	a
	SN	2.92 ± 0.18	a
RF-7 BC_2S_1	MM	1.89 ± 0.74	a
	SN	2.25 ± 0.71	a
RF-8 BC_2S_1	MM	2.72 ± 0.23	a
	SN	1.63 ± 0.44	b
RF-9 BC_2S_2	MM	2.10 ± 0.47	a
	SN	1.19 ± 0.34	b
RF-10 BC_2S_2	SN	0.38 ± 0.48	

SD standard deviation

[a] Means with different letters are significantly different at 5% level ($P < 0.05$)

round, 35 recombinants were found from 1100 BC_2S_1 plants. These recombinants were further genotyped with all the 16 CAPS markers to define crossing-over events (Fig. 3b).

Most recombinants carried a crossing-over event in the chromosomal region nearby dCT21, which is the upper maker flanking *Ol-qtl1* (Fig. 1a; Bai et al. 2003). Only five recombinants had a crossing-over event close to TG25 that showed the highest LOD value for *Ol-qtl1* in the F2 population (Bai et al. 2003). The later five recombinants were selected to produce selfing progenies, which were named as RF-6, RF-7, RF-8, RF-9 and RF-10 (Fig. 1b). Disease tests were performed on these RFs (Table 2). Since the resistance level of *Ol-qtl1* is low, we selected plants that were homozygous for either SN or MM alleles of markers in the *Ol-qtl1* interval in order to visualize contrasting phenotypes (Fig. 3b). DI was scored at three time points and a mean DI was calculated for each plant (Table 2). All susceptible control MM plants were scored as DI = 3 (Table 2; Fig. 4a), while the resistant control RF-10 had a DI of 0.43 (Fig. 4b). Results of disease tests showed that, in RF-8 and RF-9, plants homozygous for SN alleles (Fig. 4c) had significant lower DI than plants homozygous for MM alleles (Fig. 4d), suggesting that the resistance is associated with the presence of SN alleles for markers in the interval between P21M47 and dct136 (Table 2; Fig. 3b). In this interval, the populations RF-6 and RF-7 showed a homozygous MM genotype. As expected, the RF-6 and RF-7 displayed no significant difference of DI between plants homozygous for SN alleles and plants for MM alleles (Table 2). Thus, we could conclude that *Ol-qtl1* is located between marker P21M47 and dct136 (Fig. 3b) in a 2.3 Mbp region, corresponding to a genetic distance of 0.9 cM in the BC_2 population and 0.14 cM in the BC_2S_1 population. In the *Ol-qtl1* region about 300 genes are predicted by using the ITAG2 annotation (http://www.solgenomics.net). Out of 300 genes, about 40 are annotated as genes that can be

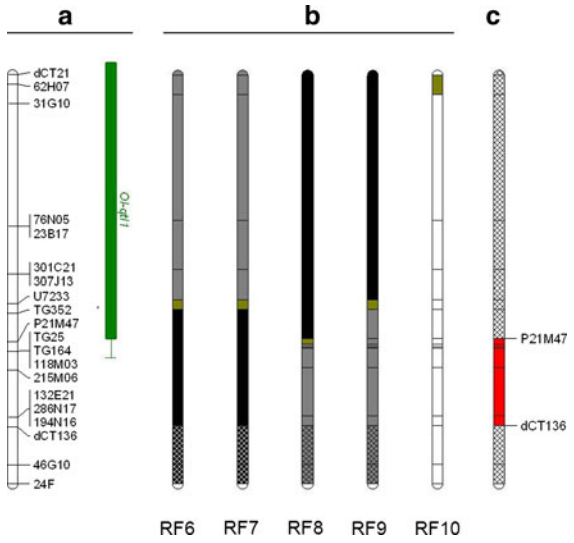

Fig. 3 The map position of *Ol-qtl1* shown on linkage groups of the long arm of tomato chromosome 6, which are constructed by using an F_2 and BC_2S_1 population, respectively, derived from a cross between *Solanum lycopersicum* cv. MM and SN G1.1601. **a** *Ol-qtl1* mapped in the F_2 population with CAPS markers. The *green bars* indicate the QTL intervals of which the *inner bar* shows a 1-LOD interval and the outer bar shows a 2-LOD interval. **b** Genotypes of five recombinants identified from the BC_2S_1 population. The *black bars* on the linkage groups indicate the presence of homozygous MM alleles, *white bars* for homozygous SN alleles and *grey bars* indicate heterozygous alleles. *Yellow* marked area shows regions where crossing-over occurred. **c** The fine-mapped position of *Ol-qtl1* indicated by the *red bar*. (Color figure online)

involved in plant pathogen interaction and 7 of them are predicted to have NBS-like or LRR-like motifs, which are typical of *R*-gene.

Discussion

In this study we developed co-dominant CAPS markers based on tomato sequences and used to fine map the three QRLs that have been identified in *S. neorickii* G1.1601 conferring resistance to *O. neolycopersici*. By using advanced backcross populations, we fine mapped *Ol-qtl1* in a chromosomal interval of 2.3 Mbp on chromosome 6 and *Ol-qtl2* in a region of about 2.5 Mbp on chromosome 12. *Ol-qtl3* could not be detected unambiguously in this study though we could not exclude its presence. Our results showed an effective approach in fine-mapping of QTLs, a large-scale recombinant screening in combination with phenotyping progenies of the recombinants.

Factors influencing QTL mapping

Lander and Botstein (1989) proposed the first statistical tractable algorithm for dissecting a quantitative trait into individual genetic loci. Since then, several papers have been published to describe statistical models for QTL mapping (Wu and Lin 2006). Currently, the QTL mapping usually allows to assign a QTL in a region of about 10–20 cM in F_2 populations (Peleman et al. 2005). With the availability of the next generation of sequencing, new approaches have been proposed (Schneeberger et al. 2009). In general, several factors have been tested in order to reduce the confidence interval of a QTL such as the nature of the population used (e.g. F2 of BCnSn) (Darvasi and Soller 1995), the population size, the gene effect on the phenotype (Darvasi et al. 1993) and the phenotypic evaluation (Price 2006). In this study several types of populations and markers were used. Our results showed that, in the F_2 population, two marker types (AFLP and CAPS) resulted in the same resolution for QTL detection in the F_2 population (Figs. 1a, 3a), suggesting that marker types has not much impact when population size is too limited. With advanced backcross populations and enlarged population size, we could narrow down the *Ol-qtl2* on chromosome 12 in a small interval of 1.3 cM (one-LOD interval).

Although the QTL mapping has been used already for 20 years and evolved rapidly due to the development of an almost unlimited number of markers from available genome sequences, very few genes underlying the quantitative trait have been cloned. In tomato, the two QTLs, *fw2.2* and *Ovate*, have been cloned by using map-based cloning approaches (Frary et al. 2000; Liu et al. 2002a; Liu et al. 2002b). A crucial factor in QTL fine-mapping and cloning is the phenotype. Two aspects of the phenotyping are important: 1) the reliability of a single plant phenotype for a quantitative trait and 2) the QTL effect on the phenotype (Peleman et al. 2005). The first aspect can be solved by using selfing progenies of an individual plant to increase the statistical power in QTL mapping (Darvasi and Soller 1995; Peleman et al. 2005; Ronin et al. 2003). The second aspect involves mainly minor QTLs with small effect, which can be possibly overcome by using homozygous plants for each allele for phenotyping. As we did in fine-mapping of *Ol-qtl1*, the relatively small effect of *Ol-qtl1* was evaluated in

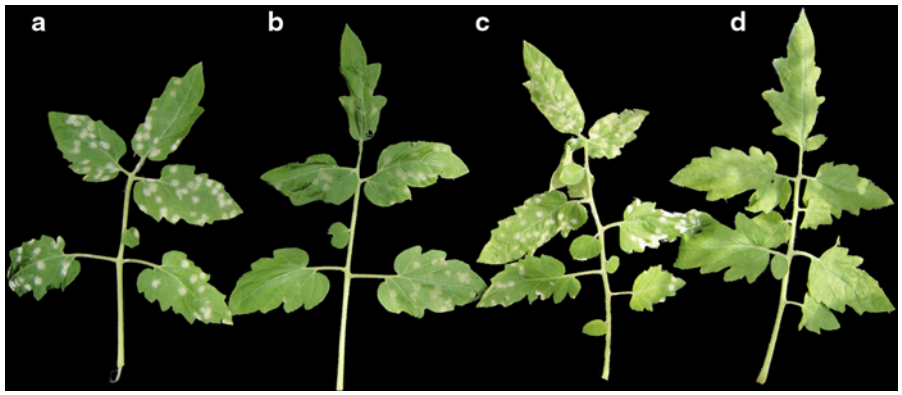

Fig. 4 Tomato leave infected by *O. neolycopersici*. **a** Leaflet of susceptible MM plants scored as DI = 3, **b** leaflet of the resistant control (DI = 1.5). **c** leaflet from susceptible phenotype (**c**) of the RF-8 population showing that disease symptom is comparable to the MM phenotype (**a**). **d** Leaflet from resistant genotype of the RF-8 population showing that disease symptom is comparable to that of the resistant control (**b**)

selfing progenies of recombinants, which were homozygous for two different alleles of the same QRL.

Transgressive segregation in QTL mapping

In quantitative trait studies it has been reported that an introgression line can have a different phenotype compared to the parental lines (Lippman et al. 2007). This phenomena is called transgressive segregation and it was reviewed by Rieseberg et al. (1999). Complementary genes are the primary cause of transgression in plants (Rieseberg et al. 1999). However, epistasis and overdominance play a role, too. Complementary genes and overdominance, involved in transgressive segregation, were described in a cross between *S. lycopersicum* × *S. pennelii*. In the IL6-3 and IL12-2 the color of the fruit is dark orange while in the cultivated tomato it is red and in the wild species of tomato it is green (Lippman et al. 2007). The tomato fruit yield is another example in which complementary genes and overdominance are involved (Gur and Zamir 2004).

In our study, transgressive segregation was observed in the RF-10 which showed a high level of resistance compared with other populations having the same genotype. On average, a homozygous plant carrying the *Ol-qtl1* locus has a DI of about 1.5 which is comparable to the homozygous plants carrying *Ol-qtl2*. The population RF-10 showed a DI of 0.34. RF-10 is a BC_2S_2 population which was subjected only to positive selection for the *Ol-qtl1* region. For all the tested populations there is no further information about other loci in the genome and it is possible that complementary loci/genes can have a role in the extreme phenotype of this population. These results suggest that undetected loci in *S. neorickii* with a minor effect are involved in the resistance.

Ol-qtls are co-localized with dominant resistance genes

Two recent reviews discussed the function of the gene(s) which can be involved in the QRL-based resistance (St Clair 2010; Poland et al. 2009). One hypothesis is that weak alleles of *R*-genes can play an important role (Grube et al. 2000; Gebhardt and Valkonen 2001; Brouwer et al. 2004; Tan et al. 2008). It has shown that RGAs in *R*-gene clusters conferring partial resistance to several pathogens (Simons et al. 1998; Andaya and Ronald 2003; Parniske et al. 1997). The *I2C-1* is an homologue of the *I2* gene, which confers resistance to *Fusarium oxysoporum* f. sp. *lycoperisicy* race 2. The *I2C-1* belong to the same cluster as *I2* but confers only partial resistance (Simons et al. 1998). The same phenomena has been described for three *Cf9* homologues, *Hcr9A*, *Hcr9B* and *Hcr9E*. The *Cf-9* gene confers complete resistance to *Cladosporium fulvum* race 9 in tomato, while, all the *Hcrs* show partial resistance against the some pathogen (Parniske et al. 1997). The partial resistance conferred by a defeated or homologues *R*-gene was illustrated in rice for the *Xa21* gene cluster, too. The homologue *Xa21D*, which is a member of the *Xa21* cluster, confers only partial resistance to the *Xanthomonas oryzae* pv *oryzae* (Andaya and Ronald 2003).

Moreover, in *Arabidopsis* the genes *BRI1* and *BRF1*, which are involved in the perception of flagellin peptide (FLG22) through *FLS2*, were mapped as QRLs (Forsyth et al. 2010) showing that genes targeting PAMPs contribute to QRL-based resistance.

Leucine-rich repeats-profile and NBS-profile (van der Linden et al. 2004) have been use to map *R*-genes and *R*-gene analogues (RGAs) on genetic maps of many crops. In tomato, two different populations of *S. lycopersicum* with wild relative tomatoes were used to produce RGA maps (Zhang et al. 2002; Sharma et al. 2008). Based on the two RGA(s) maps and on the tomato annotation for the region of interest, several *R*-gene like are mapped in both the *Ol-qtl1* and *Ol-qtl2* regions. Further functional analysis is needed to verify whether these RGAs are involved in the resistance conferred by *Ol-qtls*.

The *Ol-qtl2* is a major QRL, while, the *Ol-qtl1* is a minor QTL based on the definition of Price (2006). The chromosomal region of the *Ol-qtl2* overlaps with the *Lv* locus (Chunwongse et al. 1997). The *Lv* gene confers resistance to *L. taurica*, another powdery mildew (Chunwongse et al. 1997). This gene has been mapped in the chromosomal region between the CT121 and the CT129 (1 cM) in which genes with an LRR motif are predicted. The mechanism of resistance conferred by *Lv* is based on a HR, a fast and strong form of cell death upon pathogen invasion (data not shown). Though cell death is also the main mechanism associated with resistance conferred by *Ol-qtl2*, it is delayed and not fast and effective enough to stop the fungal growth at early infection stages (Li et al. 2011). The *Ol-qtl1* interval contains the *Ol-1* gene. Resistance mediated by both *Ol-qtl1* and *Ol-1* is associated with delayed cell death (Li et al. 2007). The co-localization and similarity in resistance mechanisms between *Ol-qtl1* and *Ol-1*, as well as between *Ol-qtl2* and *Lv,* lead to the hypothesis that *Ol-qtl1* and *Ol-qtl2* could be allelic variants or homologues of *Ol-1* and *Lv*, respectively. Currently, cloning of the *Ol-1* and *Lv* genes are in progressing and candidate genes identified will be included in functional analysis for their effect on resistance conferred by *Ol-qtls*.

Appendix 1

See Table 3.

Table 3 CAPS markers showing polymorphism between *Solanum lycopersicum* cv. Moneymaker and *S. neorickii* G1.1601

Name	Sequence primers	Tm	Product size	Restriction enzyme
CT100	TCAACCACAACAACGACAAAA	50	362	*BstXI*
	CCAAGGAGGCAGAGTGAAAA			
TG360	GACCCGGACATTTAATGGAT	50	364	*XmnI*
	TTTCGCTCTTAGCATCATGG			
T1263	CAGGGCCACTTAAGCTCTTG	50	364	*TaaI*
	GAATACCCAGTCCGAACCAA			
T1211	GCTTGGATCGTCACCCTAAA	50	356	*MnlI*
	TGCACTTGGAAATGAAGCTG			
T1736	ATTCTCGATCAACGGACCAC	55	1200	*TaqI*
	ACACTGAGCAATGCGAATCA			
cLET-8-k4	CACTTTGTGGCAATCGACAT	55	1100	*BsedI*
	TGCCTTATGCCAAACAGAAA			
T1451	GGCCGTCTCAATCTCTCTTG	55	288	*MboII*
	GTGCCCTTAACGGGTTCTTT			
T1093	TGCATCTCCCGGTGTAACG	55	339	*AluI*
	TCTTTCGCGGTAGGCTGATAA			

Table 3 continued

Name	Sequence primers	Tm	Product size	Restriction enzyme
T1185	GATCCTCGTGAGCAAGAAGC	55	365	*SsiI*
	GGAATGGGCCCTGTGAAC			
CT189	TGTGCGTGCAATATCTGACTG	55	397	*Cfr13I*
	CAAATGGTAATGCGGCTGTGC			
CD22	ATTCTGAAAAGGCAAAACATCCAC	55	257	*AluI*
	GCACGCCACATTCTTCAACAG			
CT79	GGACCGGGTGGTATTACTAT	55	470	*MspI*
	CAAATTTTGTGCAAAGTGAA			
TG180	CCGTGTAATATCCAACGAGCTT	55	300	*XapI*
	CTGCATTGGCAGAAGATTCA			
T0659	GGGCAAATGAACTTGTTCTCA	55	1400	*HhaI,PsuI*
	AATGGTCATGGAATGGGAAA			
U216669	CATACCATTTTTCCCCGATG	55	2000	*AluI*
	TAGGCGTTATGGATGGCTTC			
c2_At2g06530	ATCTGTCCCATTGCCTTTGTAAC	55	900	*RsaI*
	AAGGTGTCTCCCTCAGAATTCAG			
CT121	ATTGAAACTTGGGGCTTGTG	55	270	*HaeIII*
	GTGTAGCCAGGTGGTGGACT			
NR14A	GTGGCAGGTATCTCATGGAA	55	700	*PsuI*
	GGGAACCTCCATATACAAG			
GP34	GTTATCGTTGATTTCTCGTTCCG	55	700	*HpyCH4IV*
	CGTTGCTAGGTAAGCATGAAGAAG			
IPM3	CAACATCACTTGATCAGAC	55	700	*DdEI*
	AGTAGTTTCAGGCTAGTG			
111R	AGATGAAGATTTTCTTGTCTGATGG	55	750	*RsaI*
	CACTGTGTAAGGGTCAACTATAGTC			
76N05	GGACATAGGTTGAGGGGCTA	58	866	
	GTCACAGTTCCGCTCCAGAT			
23B17	AAGGTGCATCGAGAATGTCC	60	1000	*Dde I*
	CACACCCACACCATATCCAA			
307J13	TCCAACCATCAGACCATTCA	60	500+800	*Dde I*
	AAGCAATCCGAGAAGGTTCA			
62H07	ATTCGGTACGAGGCAGTTGA	58	853	*DdeI*
	AAATGGCAAGCCAACGTAGT			
286N17	TCCAATTGCACTCTCACCAA	58	861	*ApoI*
	AGAAATGTGGGCTCCAACTG			
132E23	TCTCATGCTATTGCGTGCTC	58	900	*Dde I*
	ATGCCCTTTTGGTGTTCTTG			
46G10	CCGCTTTGAAACTAGGTGGA	60	900	*AluI*
	TAATCGAGGTGCACAAGCTG			
118M03	AAGAAGACGCCTTCACTCCA	60	904	*MboI*
	CAGGCAAGTCCCTTTGAGAC			
194N16	TCAGGATCCGTTTGATCTCC	60	887	*Apo I*
	GCTTTTGCTCCATCAACACA			

Table 3 continued

Name	Sequence primers	Tm	Product size	Restriction enzyme
TG164	AAGCTGCCCTGGTTCATTA	50	400	*Hinf I*
	CATTCACGTTGGTATAGTTTCAT			
24F	TGAGGGGAGGATTGAATGTT	58	1400	*Hind II*
	TGACCAGCCAACTGTTTGAA			
215M16	GTTTTAGGCCCCTGATCGTT	58	1000	*Alu I*
	GGCGTTAATCTCCGTCTTGA			
TG352	CAGTGGAGGGCTTTTGATGT	58	350	*Tsa I*
	TGACTTATGCGGTTGTGCTG			
U7233	AGGCATAGCAATTCTATGGATGGG	55	1500	*HpyCH4IV*
	TTGGAACGTGCAGCAGATTGTC			
301C21	CCTTAGTCGAGCCCTTTTCA	60	1000	*Dde I*
	GATCAACGCTGAGAGCACTG			
31G10	TGTTTTGAAAAGCGAATCGT	55	450	*HpyCH IV*
	GGTCGTTACGTTATCCACCA			

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