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ORIGINAL PAPER

Conditional QTL underlying resistance to late blight in a diploid potato population

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Abstract A large number of quantitative trait loci (QTL) for resistance to late blight of potato have been reported with a "conventional" method in which each phenotypic trait reflects the cumulative genetic effects for the duration of the disease process. However, as genes controlling response to disease may have unique contributions with specific temporal features, it is important to consider the phenotype as dynamic. Here, using the net genetic effects evidenced at consecutive time points during disease development, we report the first conditional mapping of QTL underlying late blight resistance in potato under five environments in Peru. Six conditional QTL were mapped, one each on chromosome 2, 7 and 12 and three on chromosome 9. These QTL represent distinct contributions to the phenotypic variation at different stages of disease development. By comparison, when conventional mapping was conducted, only one QTL was detected on chromosome 9. This QTL was the same as one of the conditional QTL. The results imply that conditional QTL reflect genes that function at particular stages during the host–pathogen interaction. The dynamics revealed by conditional QTL mapping could contribute to the understanding of the molecular mechanism of late blight resistance and these QTL could be used to target genes for marker development or manipulation to improve resistance.

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Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is the most devastating disease of potato (*Solanum tuberosum* L.) worldwide. Resistance to late blight can be classified into two categories: qualitative and quantitative. Quantitative resistance is expected to be more durable than qualitative resistance since it is governed by multigenes and provides resistance to diverse pathogen races (Rauscher et al. 2010). To date, more than 20 research articles have focused on quantitative trait locus (QTL) analysis of potato late blight resistance (Danan et al. 2009; Leonards-Schippers et al. 1994; Mihovilovich et al. 2010; Rauscher et al. 2010; Ritter et al. 2009; Simko 2002). Each of these previously reported sets of QTL was detected on the basis of a phenotypic value that represents disease accumulated during the evaluation period. However, quantitative resistance to late blight is characterized by temporal changes in gene expression (Li et al. 2009; Lindqvist-Kreuze et al. 2010; Wang et al. 2005) and therefore, it is possible that different QTL become significant at different time points during disease development.

A model to evaluate the net genetic effect (i.e. the conditional genetic effect) of a quantitative trait at a specific developmental stage was defined by Zhu (1995). This genetic model was first applied in QTL analysis, and called

conditional QTL mapping, to dissect the dynamic gene expressions associated with developmental aspects of plant height (Yan et al. 1998a) and tiller number (Yan et al. 1998b) in rice. In the experiment of Yan et al. (1998a), plant heights were measured every 10 days. Nine different measurements were taken during the growth period and used to calculate net genetic effects for each 10-day interval using the model of Zhu (1995). The net genetic effects were used for conditional QTL mapping, which can reflect the actions of genes during development in real time (Yan et al. 1998a).

The conditional mapping method has been used to map QTL for plant height in maize (Yan et al. 2003), brown plant hopper resistance in rice (Ren et al. 2004), pod number and the main stem and plant height of soybean (Sun et al. 2006), seed weight of soybean (Teng et al. 2009), plant height of wheat (Cui et al. 2011; Wang et al. 2010) and linolenic acid content of soybean seed (Han et al. 2011). But, so far, there is no report on the use of conditional QTL mapping to reveal the dynamics of plant disease resistance.

To elucidate the dynamics of QTL controlling potato late blight resistance, conditional QTL mapping was conducted with a diploid potato population, for which, the resistance to *P. infestans* was tested in five environments. For comparison, conventional QTL mapping was conducted simultaneously.

Materials and methods

Plant materials

A diploid potato F1 population named B3C1HP was developed by the International Potato Center (CIP). B3C1HP consists of 100 genotypes derived from a cross between a resistant dihaploid maternal parent (CIP301071.3), developed by subjecting a resistant tetraploid B3C1 selection (CIP393046.7) to haploid extraction by pollination with IVP35 (Jacobsen 1978), and a susceptible paternal parent (*S. phureja* CIP703308). The tetraploid B3C1 resistance source (CIP393046.7) has a complex genetic background since it originates from CIP's population A, which combines late blight resistance from native Andean primitive cultivars *S. tuberosum* ssp *andigena*, *S. phureja* and *S. stenotomun*, and wild species *S. acaule* and *S. bulbocastanum* through 4-way hybrids (ABPT's) and the resulting long-day adapted *Andigena* germplasm termed Neotuberosum (Landeo1989).

Field assessments of late blight resistance

The field assessments were conducted at two locations in the central and eastern mountain ranges of the highlands of Peru selected for high endemic late blight pressure, Comas (Department of Junin, 11°46′0″S, 75°5′0″W) in 2006 and 2007 and Oxapampa (Department of Pasco, 10°34′0″S, 75°24′0″W) in 2005, 2006 and 2009. Each of the five environments is designated a Roman numeral listed in Table 1. Peru is located in the central Andes which is considered a ''hot spot'' for diversity of the genus *Phytophthora* (Gomez-Alpizar et al. 2007). The genetic diversity of the *P. infestans* populations at both sites has been studied previously (Perez et al. 2001). The isolates present in both sites belong to the EC-1 lineage that has been the established *P. infestans* lineage on Petota plants in Peru and Ecuador for over a decade and should be well adapted to sources of resistance that are present in the currently analyzed B3C1HP population. At Comas and Oxapampa, the environments during the rainy season (from December to March) are highly conducive to late blight (Andrade-Piedra et al. 2005). Therefore, the population B3C1HP was evaluated for late blight resistance during the rainy season without artificial inoculation. The experiment location in Oxapampa is in a valley which itself has no commercial potato production but has other hosts of *P. infestans* growing abundantly. Potato is grown in higher altitudes on the mountain slopes, which presumably serve as the source of inoculum for our experiment site. The other site Comas has plenty of potatoes grown in the nearby fields as well as on the mountain slopes. Our experiments were planted later in the season than local potato crops to ensure the build up of inoculation. Oxapampa and Comas are areas where disease can remain present all year such that inoculum in varying levels is available all the time (Pérez et al. 2009).

The progeny individuals, the parental clones and control varieties Kory (resistant), Amarilis (moderately resistant) and Yungay (susceptible) were planted in the field using a randomized complete block design with two to four repetitions of single row plots of five plants each. The plants were fertilized with NPK at the rate of 200–200–180 kg ha^{-1}; soil borne diseases were controlled by applying Pentacloronitrobenzen, insects and nematodes were controlled with Karathe. Mancozeb (Dithane M-45) was applied two times at 450 g/200 l of water, first when all plants had emerged and then 7 days later to allow plant establishment despite heavy late blight pressure. The first disease assessment was done 10 days after the second Mancozeb application by visually estimating the percentage of leaf area affected. The planting dates and the dates for first disease evaluation are given in Table 1. The evaluation was done by the same person throughout each experiment, with data taken at intervals of 7 days. In each environment, seven records were taken for each plot and the percent mean values of replicate plots were used to calculate the area under the disease progress curve

Table 1 The mean values and standard deviations of conditional and conventional AUDPCs in five environments across weeks

Environment	Location	Planting date	First disease evaluation date	1[a]	2	3	4	5	6	7	$\sum 7^{b}$
I	Comas	Dec 15, 2005	Jan 21, 2006	3.93 ± 6.17	25.00 ± 14.07	111.87 ± 42.87	300.18 ± 38.17	562.14 ± 58.45	875.23 ± 66.56	1244.84 ± 74.78	1244.84 ± 1069.20
II	Comas	Dec 12, 2006	Jan 24, 2007	13.72 ± 6.95	57.62 ± 12.26	129.28 ± 16.45	279.59 ± 54.29	518.51 ± 62.87	862.80 ± 73.06	1272.93 ± 82.02	1272.93 ± 565.18
III	Oxapampa	Sep 27, 2005	Nov 05, 2005	8.12 ± 8.31	23.02 ± 3.50	82.82 ± 15.79	242.00 ± 47.48	470.85 ± 36.62	764.65 ± 48.72	1113.21 ± 58.68	1113.21 ± 875.61
IV	Oxapampa	Sep 27, 2006	Nov 21, 2006	8.48 ± 9.22	41.45 ± 13.92	126.12 ± 26.40	293.43 ± 30.62	546.48 ± 50.48	875.18 ± 76.46	1274.32 ± 93.17	1274.32 ± 1007.68
V	Oxapampa	Sep 21, 2009	Nov 05, 2009	16.40 ± 17.73	107.44 ± 30.66	266.72 ± 27.11	478.67 ± 43.90	771.16 ± 67.29	1069.57 ± 67.34	1414.49 ± 68.10	1414.49 ± 1349.50

[a] 1–7 indicate the mean values and standard deviations of conditional AUDPCs from the first week to the seventh week

[b] The mean values and standard deviations of conventional AUDPCs

(AUDPC) (Shaner and Finney 1977) for each progeny genotype. The percent mean values of leaf areas affected at all evaluation time points of all the individuals were analyzed by ANOVA and clustered according to the Scott-Knott algorithm (Scott and Knott 1974) in all five environments. As a result, the experiment mean values were divided into homogeneous distinct groups using the significance level of $p = 0.05$.

Marker detection

DNA was extracted according to CIP's cetyltrimethylammonium bromide (CTAB) protocol (Ghislain 1999). STS primer pairs were derived from publications of Gebhardt et al. (2004), Niewöhner et al. (1995), Wickramasinghe et al. (2009) and Yamanaka et al. (2005). SSR primers were from Milbourne et al. (1998), Feingold et al. (2005), Ghislain et al. (2009) and Sandbrink et al. (2000). AFLP assays were performed as described by Vos et al. (1995) followed by silver staining according to Han et al. (2008). All 25 possible primer combinations between five *Eco*RI selective primers (5′-GACTGCGTACCAATTC-3′ followed by selective extension: AAA, AAC, ACA, AGA and ATG, respectively) and five *Mse*I selective primers (5′-GATGAGTCCTGAGTAA-3′ followed by selective extension: ACG, CAC, CAG, CAT and CTA, respectively) were used in the AFLP assays. COSII markers were selected from The SOL Genomics Network (http://solgenomics.net). Candidate genes found differentially regulated during late blight attack were selected from a previous study by Lindqvist-Kreuze et al. (2010). Other positional candidate markers were designed based on the potato genome sequence superscaffold and pseudomolecule information (The Potato Genome Sequencing Consortium, http://www.potatogenome.net/index.php/Main_Page). Finally, the gene sequence of the *Rpi-vnt1* gene (Foster et al. 2009) was used to design primers specific for this gene. All primer sequences except that of AFLP are detailed in online supplementary (Table S1).

Polymerase chain reaction (PCR) products were screened for polymorphism between the parents and among up to 20 progeny individuals. For AFLP, STS and SSR markers, PCR products were separated by 6% denatured (7 M urea) polyacrylamide (19:1) gel electrophoresis, followed by silver staining. For COSII and candidate gene markers, agarose gel electrophoresis was sufficient for polymorphism detection in some cases, but for most of the markers single strand conformation polymorphism (SSCP) or high resolution melting (HRM, Gundry et al. 2003) analysis was required to detect polymorphism. SSCP followed by silver staining was used for the PCR fragments longer than 600 bp, whereas the shorter fragments were analyzed by HRM. LC-Green Plus dye (Idaho Technology

Inc.) was used with the LightScanner instrument (Idaho Technology Inc.) following manufacturer's instructions. The segregation of informative markers was monitored by visualization of melting curves and peaks generated by the software. After determining the most suitable technique for polymorphism detection, each polymorphic marker was analyzed in the whole progeny.

Map construction

Except for HRM, polymorphic marker alleles were recorded considering presence and absence. Each recorded marker allele was given a name indicating the primers used and, for AFLP markers, the size of the allele. The sizes were estimated according to DNA standard ΦX174 *Hae*III digest (NEB) by point-to-point semi-log method of Quantity One® *Version 4.6.2* attached to Universal Hood II (Bio-Rad). For HRM markers, the differences in the melting temperature and melting curve shape caused by variations in the DNA sequence were detected to differentiate heterozygous individuals from homozygous. The number of alleles for each marker was resolved based on the grouping of the progeny individuals in relation to the parents.

The band and HRM records were compiled according to the genotype codes of population type CP described in the Joinmap® 4 manual (Van Ooijen 2006). A pseudo-testcross strategy (Grattapaglia and Sederoff 1994) was adopted and a maternal map was constructed with *Kosambi*'s mapping function following along Joinmap® 4 manual (Van Ooijen, 2006). The linkage groups determined by Joinmap® 4 were assigned to the potato chromosomes according to the previously mapped SSRs and STSs in the references mentioned above and maintaining the orientation as reported by Ghislain et al. (2009).

QTL analysis

There were two kinds of trait values in our research: AUDPCs for the conventional QTL mapping and AUDPCs for the conditional QTL mapping. The AUDPCs for the conventional QTL mapping were calculated from the first to the seventh week's percentage scores in each of the five environments, reflecting accumulated genetic effects during the whole period of the field assessment. There are five conventional AUDPCs, one for each of the five environments described in Table 1. For the conditional QTL mapping, AUDPC value for each week in each environment was calculated separately and each AUDPC value was conditionally adjusted by the AUDPC value obtained in the previous week with software QGAStation 1.0 (http://ibi.zju.edu.cn/software/qga) in which the genetic model of Zhu (1995) was embedded. The adjusted AUDPC values were designated as the conditional AUDPCs, which represent the net genetic effects within each week (Table 1). For example, conditional AUDPC in environment V at week 7, which represents the net genetic effect of the seventh week in 2009 at Oxapampa, was calculated from the seventh week's AUDPC conditioned on the sixth week's AUDPC. Because there was no genetic effect before the first week, the five first AUDPCs do not need adjustment and represent the net genetic effect within the first week. In total, there were 35 conditional AUDPCs consisting of the five environments described above and a conditional AUDPC value for each of the 7 weeks of evaluation (Table 1).

With the maternal linkage map of population B3C1HP and two kinds of trait values, the QTL analysis was done by MapQTL® 6 based on multiple-QTL models (MQM) (Van Ooijen 2009). After 1,000 permutations, the genome wide threshold ($p = 0.05$) for each trait was identified and the highest LOD (logarithm of the odds) threshold (LOD = 2.9) was chosen as the threshold for all "traits". When a peak LOD exceeded the threshold, a QTL was declared and was given a name indicating the mapping method used, as well as the chromosome location. For example, dPI02 indicated a QTL underlying resistance to *P. infestans* mapped on chromosome 2 by the conditional QTL mapping based on the conditional AUDPCs. If the two-LOD support intervals of two QTL overlapped each other, the QTL were considered as the same QTL. The linkage map and the positions of QTL were drawn by MapChart 2.2 (Voorrips 2002).

Results

Phenotypic data of the population B3C1HP

Field assessments of late blight resistance were conducted in five environments in Peru. The frequency histograms of the progeny based on the disease development within each environment are shown in Fig. 1. The mean values and standard deviations (mean ± SD) of five conventional AUDPCs are listed in Table 1. In four environments (I, III, IV and V), nearly half of the progeny individuals were highly resistant, while the remaining progeny showed a normal distribution ranging from moderately resistant to susceptible. ScottKnott clustering of the progeny individuals into groups with similar rates of disease development in each environment is shown in Fig. 2. Looking at the % leaf area affected values of the most resistant individuals there was a small increase in the disease progress up to week 7 as compared to the most susceptible individuals that became completely infected. In four environments (I, III, IV and V) the most resistant group shows a markedly slower disease progress as compared to the other

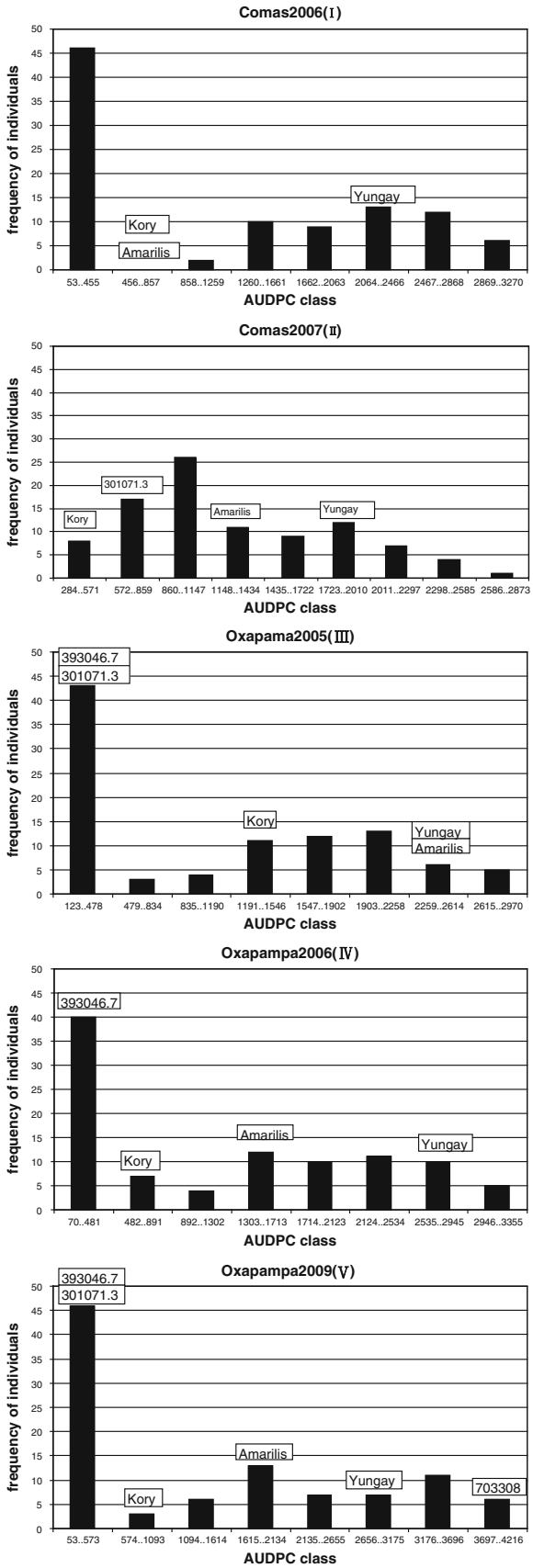


Fig. 1 *Histogram* showing the frequency distribution of conventional AUDPCs calculated from the first to seventh week's percentage scores in five environments. Comas2006, Comas2007, Oxapampa2005, Oxapampa2006 and Oxapampa2009 represent environment I, II, III, IV and V listed in Table 1, respectively. When the parent, grandparent and control varieties were recorded in a certain environment, they are indicated on the *top of the class* to which they belong

individuals. In total, 44 of the individuals in the most resistant group are the same in these four environments. In contrast, the most resistant group in environment II has a faster disease progress and consists of fewer individuals, of which only 18 are the same as in the most resistant groups in the other environments. However, there is still a significant proportion of progeny individuals with a slow disease progress and the AUDPC values show a normal distribution in this environment (Fig. 1).

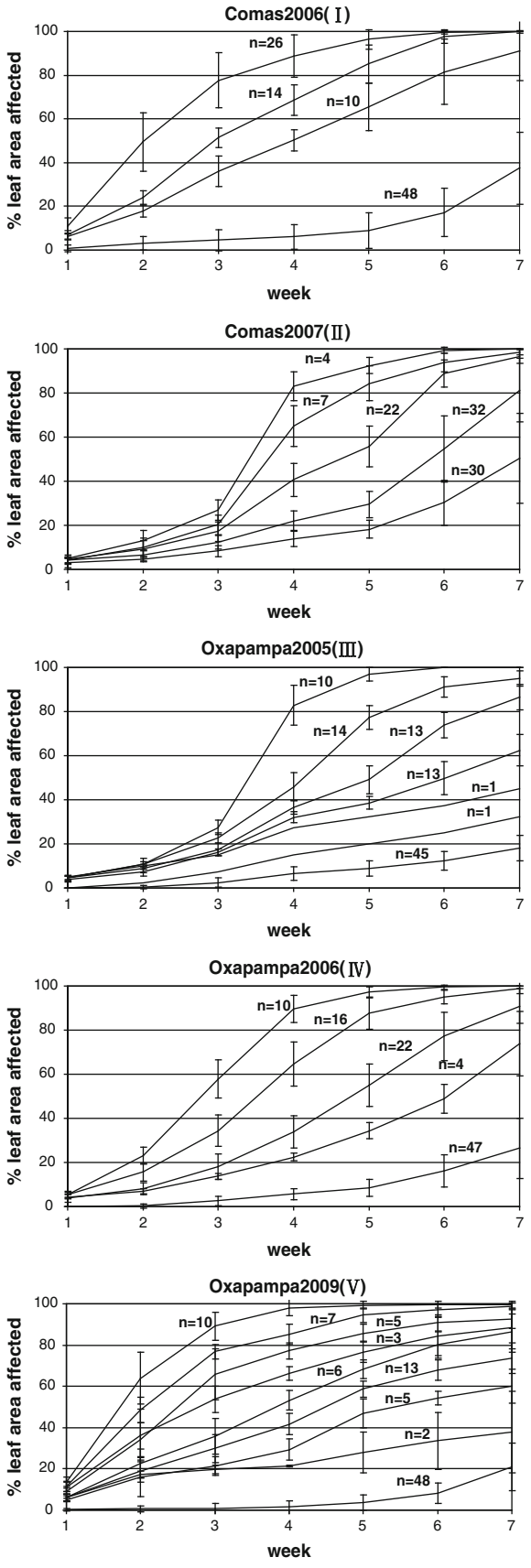
The mean ± SD of 35 conditional AUDPCs are listed in Table 1. The mean values of conditional AUDPCs of the seventh week in the five environments were approximately equal ranging from 1113.21 (environment III) to 1414.49 (environment V). The mean values increased from the first week to the seventh week and sharp increases were found in the last 4 weeks indicating an acceleration of disease progress toward the end of the experiment. In general, the standard deviation increased across the 7 weeks suggesting that the gap between resistant and susceptible individuals was getting larger toward the end of the experiment. Each pair of conventional AUDPC and conditional AUDPC of the seventh week has equal mean values but extraordinarily different SD.

Linkage map of population B3C1HP

The maternal linkage map of population B3C1HP consists of 145 markers (87 SSRs, 26 COSII, 24 AFLPs, 2 STSs and 6 candidate gene markers) assembled into 12 linkage groups. Chromosome and orientation of each linkage group were determined as mentioned in "Materials and methods". The total length of the B3C1HP maternal map was 810 cM, the average marker interval was 6.1 cM and the maximum interval was 28 cM on chromosome 1 (Fig. 3).

Conventional QTL mapping

By QTL mapping with the five conventional AUDPCs, one QTL, PI09, was detected with the peak LOD at 78.6 cM on chromosome 9 between marker DMC42152bf and DMC42144af, the two-LOD support intervals for which was 76.5–78.7 cM (Fig. 3). PI09 was detected in four environments (all but environment II). The percentage of phenotypic variation (AUDPC) explained by PI09 in



◀ **Fig. 2** Grouping of individuals according to their resistance level (the smaller the percentage of leaf area affected, the higher the level of resistance) based on ScottKnott clustering in five environments as described in Table 1. The number of individuals (*n*) within each group is shown above. *Each curve* represents a group

environments I, III, IV and V were 73.3, 73.9, 72.4 and 62.1%, respectively. PI09 always conferred negative additive effects on the traits (the higher value of AUDPC, the more susceptible to late blight), which implies that the allele of CIP301071.3 at this locus reduces disease (Table 2).

Conditional QTL mapping

QTL mapping with the 35 conditional AUDPCs uncovered six QTL (with prefix d- in their names, which indicates the dynamic aspect of the conditional QTL), dPI02, dPI07, dPI09a, dPI09b, dPI09c and dPI12. Three conditional QTL, dPI02, dPI07 and dPI12, were detected with the peak LOD at 53.2 cM on chromosome 2, 0.0 cM on chromosome 7 and 66.3 cM on chromosome 12. On chromosome 9, another three conditional QTL were detected with the peak LOD at 8.9 cM (dPI09a), 66.8 cM (dPI09b) and 77.2–78.6 cM (dPI09c) (Fig. 3). Across weeks and environments, subtle differences existed as far as the peak LOD positions of dPI09c were concerned. There were three different peak LOD positions of dPI09c, 77.2, 77.6 and 78.6 cM (Table 2). However, the two-LOD support intervals in all cases (75.5 to 77.7–78.7 cM) were almost the same, and therefore we interpret these as a single QTL at the interval of 75.5–78.7 cM. It is also likely that dPI09c and PI09 (two-LOD support interval: 76.5–78.7 cM) are the same QTL detected by the two mapping methods. The peak LOD positions of dPI09b and dPI09c are only 10.4 cM (or 11.8 cM) apart, but the two-LOD support intervals of dPI09b (64–68 cM) and dPI09c (75.5–78.7 cM) do not overlap and therefore we interpret these as separate QTL (Fig. 3).

All conditional QTL took temporal expression patterns across time intervals in each environment. dPI02, dPI07, dPI09a and dPI12 were only detected once across the environments and time intervals. dPI02, dPI07 and dPI12 were negative to the disease development, while dPI09a had a positive effect on disease development. dPI09b was detected with opposite effects in two environments: positive in the third week in environment IV and negative in the fourth week in environment V. dPI09c was expressed more dynamically than other conditional QTL. It was expressed during the first week in all five environments. In environment I, the expression of this QTL persisted to the second week. After 2 or 3 weeks of silence, dPI09c was detected again in the fifth week in all environments except environment III. Expression of dPI09c was maintained

Table 2 QTL detected by conditional and conventional QTL mapping

QTL	Week	Comas2006 (I)				Comas2007 (II)				Oxapampa2005 (III)				Oxapampa2006 (IV)				Oxapampa2009 (V)			
		P(cM)[a]	LOD[b]	A[c]	Expl.%[d]	P(cM)	LOD	A	Expl.%	P(cM)	LOD	A	Expl.%	P(cM)	LOD	A	Expl.%	P(cM)	LOD	A	Expl.%
dPI02[e]	3	53.2	2.9	−15.2	14.6																
dPI07	5					0.0	3.0	−21.9	11.8												
dPI09a	2	8.9	3.9	4.8	13.3																
dPI09b	3													66.8	3.5	8.4	14.9				
	4																	66.8	3.5	−16.6	17.1
dPI09c	1	78.6	7.0	−3.2	30.7	78.6	4.0	−3.2	20.2	78.6	32.7	−7.7	83.3	78.6	19.2	−7.5	64.2	78.6	18.5	−12.6	58.1
	2	77.6	8.7	−7.7	34.8																
	5	77.6	3.3	−22.3	15.7	78.6	6.4	33.7	27.4					77.6	4.1	−21.3	19.8	77.6	7.0	−32.0	28.7
	6	78.6	3.8	−24.7	17.1									77.6	7.3	−41.2	32.4	77.2	6.6	−33.0	27.5
	7	77.6	4.1	−26.8	18.5					77.6	3.5	−21.6	17.4	77.6	5.6	−45.5	25.7	77.2	3.0	−25.3	14.5
dPI12	1																	66.3	3.1	−4.2	6.6
PI09		78.6	25.0	−876.7	73.3					78.6	25.2	−703.0	73.9	78.6	25.2	−814.1	72.4	78.6	19.4	−996.5	62.1

[a] The position of the LOD peak, namely the distance in cM to the north end of the corresponding chromosome displayed in Fig. 3

[b] The significant peak LOD

[c] The additive effect of QTL

[d] The percentage of phenotypic variance explained by QTL

[e] QTL name "dPI02" means the conditional QTL detected on chromosome 2 underlying the resistance to *P. infestans*. "a", "b" and "c" are for three different conditional QTL on chromosome 9, respectively. PI09 without "d" is the conventional QTL

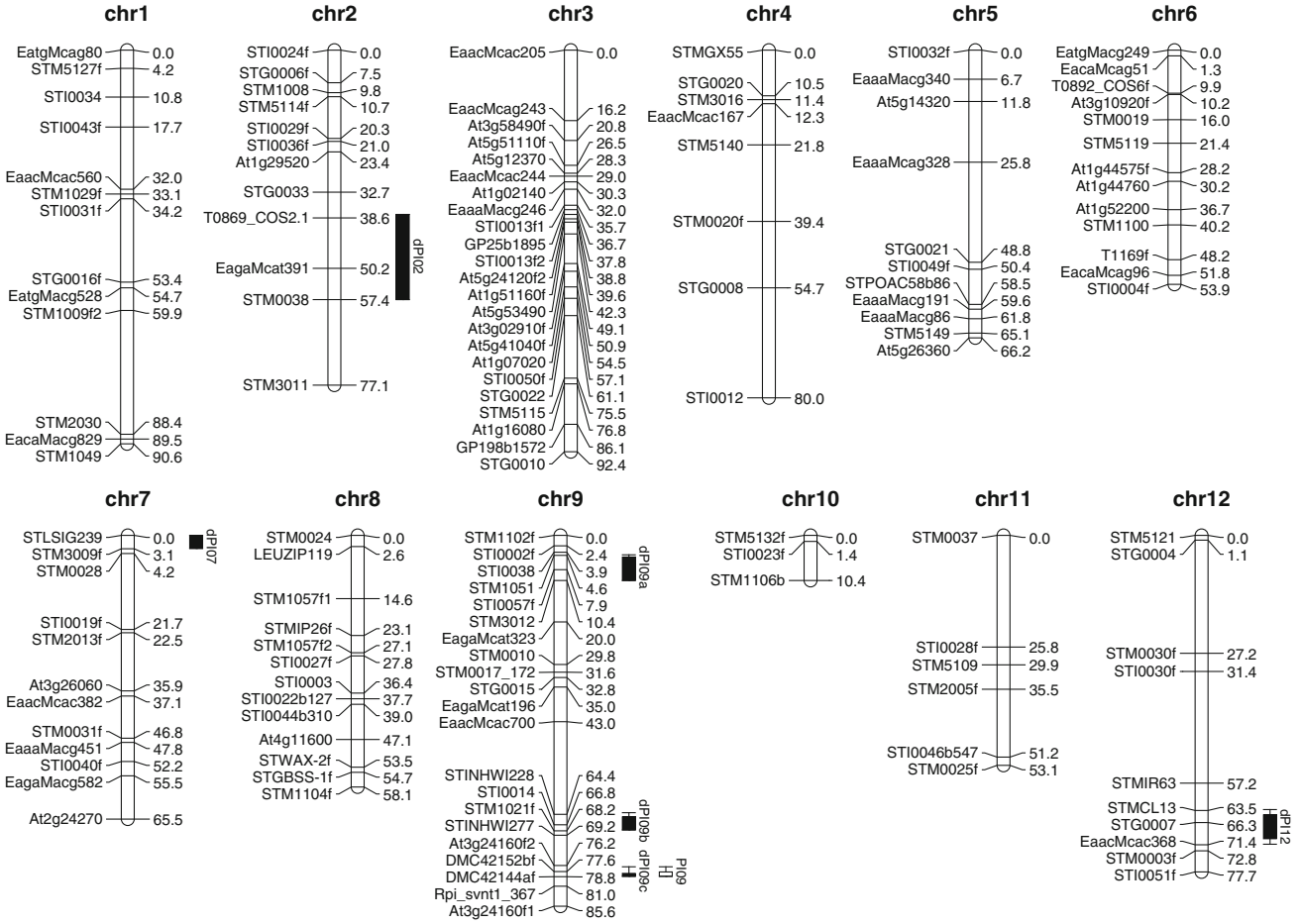


Fig. 3 Maternal linkage map of diploid potato population B3C1HP and locations of the QTL detected by the conventional and conditional QTL mapping. The denotation on the *top of each linkage group* indicates each of the 12 potato chromosomes. The markers are on the *left of the groups* and the cumulative distance in cM on the *right*. On the *far right*, locations of six conditional QTL (*black bars*) and one conventional QTL (*white bars*) are charted by *bars* (one-LOD support intervals) and the flanking lines (two-LOD support intervals). In QTL names beside the bars, "d" means conditional QTL (PI09 without "d" is the conventional QTL), "PI" means the QTL underlying the resistance to *P. infestans* and the *Arabic numerals* indicate on which chromosome the QTL were detected. "a", "b" and "c" are for three different conditional QTL on chromosome 9, respectively

across the final 3 weeks in three environments (I, IV and V). Only in 1 week was dPI09c detected in other two environments (the fifth week in environment II and the seventh week in environment III). The effects of dPI09c were negative to the disease development in all cases except that in the fifth week in environment II (Table 2).

The percentage of phenotypic variation that could be explained by the conditional QTL ranged from 6.6% (dPI12 in the first week in environment V) to 83.3% (dPI09c in the first week in environment III). In general, the percentage of phenotypic variation explained by dPI02, dPI07, dPI09a, dPI09b and dPI12 was smaller than that explained by dPI09c. The largest percentage explained by the former five conditional QTL was 17.1%, while dPI09c explained 14.5–83.3% of the disease variance across weeks and environments (Table 2). dPI09c explained on average 48.6% of the resistance variance in the first 2 weeks, which was much higher than that (22.2%) of the final 3 weeks. In the last 3 weeks of environment II, dPI09c was only detected in the fifth week explaining 27.4% of phenotypic variation which was in the opposite direction but of approximately the same magnitude of the effect as that (20.2%) detected in the first week. In environment III, dPI09c was detected again during the seventh week but with a much smaller effect (17.4%) (Table 2).

Discussion

Conditional QTL mapping in comparison with the conventional method

Conditional QTL mapping exploits the net effects of gene expression from time $t-1$ to t (Yan et al. 1998a).

Applying this approach, therefore, we were able to study the dynamics of genetic effects during late blight epidemics on a weekly basis and were able to detect more QTL which were not found by conventional QTL mapping. Dissecting the QTL expression in time effectively removes the interference of large genetic effects like that conferred by dPI09c in the first 2 weeks allowing for the detection of other QTL with relatively small effects (Zhao et al. 2006).

Each of the conditional QTL was detected temporally and the dynamics of conditional QTL can be related to the infection process during potato late blight epidemic. The induction and expression of defense pathways begin by recognition and therefore it is plausible that the QTL dPI09c showing strong effect in four environments during the first week of evaluation might harbor gene(s) encoding proteins that recognize the effectors of *P. infestans* and involve a hypersensitive response (HR).

The silence of dPI09c in week 3 and 4 might be explained by the epidemics caused by *P. infestans* populations and development of plants. It has been reported that the level of resistance to late blight is not constant during the development of the entire plant or of individual leaves (Struik 2010). Very young and old plants are generally more susceptible while plants of intermediate age are the most resistant (Visker et al. 2003). On one hand, potato plants in weeks 3 and 4, which are of intermediate age and are the most resistant during the development, might depress the late blight epidemics. On the other hand, in the field, the interaction is complex as it involves plants with varying levels of resistance and likely also a mixture of *P. infestans* isolates. Sharp increases of the mean values of AUDPCs in the last 4 weeks might indicate the explosions of *P. infestans* populations after several rounds of infection in the susceptible plants. It was the explosions that resulted in the re-detection of dPI09c.

In the last 3 weeks, variances of phenotype increased substantially, so the percentage of phenotypic variance explained by dPI09c decreased dramatically even if additive effects in the final 3 weeks are stronger than that of the first 2 weeks in all five environments. The magnitudes of effects of the QTL dPI09c in the first 2 weeks are dramatically different among environments, perhaps suggesting the presence of *P. infestans* races with varying virulence patterns. Previous study by Perez et al. (2001) and recent fingerprinting (W. Perez, unpublished results at CIP, personal communication) show that the populations of *P. infestans* at both experiment sites belong to the EC-1 lineage and have complex and high virulence diversity as shown by their ability to overcome the resistance provided by many of the known *R genes* derived from *S. demissum*. This could especially explain the difference of the magnitude of the effect of this QTL in environment II, where only a fraction of the progeny that was resistant in the other environments resisted the predominant pathogen race. A virulent race of *P. infestans* thrives on dead host tissue in its late necrotrophic phase, exploiting programed cell death in the host to acquire nutrients (Poland et al. 2009). Therefore, the HR mediated by dPI09c in environment II may increase susceptibility to *P. infestans* in this environment and account for the positive effect of this QTL for disease development at week 5. The other conditional QTL with positive effect on disease development may harbor genes that implement a different function from recognition of the pathogen and may facilitate the infection process.

The failure to detect QTL PI09 by the conventional method in environment II is likely because of the opposite additive effects of the same alleles during the first and fifth week. According to Yan et al. (1998a, b), opposite genetic effects in similar genomic positions expressed at different stages might counteract each other and result in failure to detect QTL for cumulative effects.

The conditional QTL discovered on chromosome 7 in environment II at week 5 is likely to contribute to the resistance in the presence of a virulent race that overcomes the resistance provided by dPI09c. The expression of the dPI07 coincides with the expression of the dPI09c that has a positive effect on the disease development in this environment. We are not aware of late blight resistance QTL having been mapped to this chromosome region previously, however, a maturity-related meta-QTL has been reported in this genome region, distal to the marker STM3009 (Danan et al. 2011). Conditional QTL dPI09a expressed in environment I at week 2 was mapped to the genomic region previously reported to have a late blight resistance QTL in the vicinity of the marker STM1051 (Danan et al. 2011). In addition, dPI02 between markers STG0033 and STM0038 and dPI12 close to marker STG0007 were in similar positions as late blight resistance QTL reported by the same authors (Danan et al. 2011). Each small effect QTL detected was specific to its environment suggesting again the temporal nature of the expression of the disease resistance pathways in quantitative resistance.

R genes on long arm of chromosome 9 and dPI09c (PI09)

The strong and early effects of the maternal alleles in dPI09c (PI09) in all environments and the opposite effects of the QTL in environment II suggest that the QTL region of dPI09c may contain an *R* gene. *R* genes conferring resistance to late blight, *Rpi-moc1*a.k.a. *Rpi-mcq1* (Smilde et al. 2005), *Rpi-vnt1.1* (Foster et al. 2009; Pel et al. 2009), *Rpi-phu1* (Śliwka et al. 2006), *Rpi-dlc1* (Golas et al. 2010) and *R8* (Jo et al. 2011) have been reported in a similar position as dPI09c on the long arm of chromosome 9 in

potato relatives. In the published sequence of the potato (DM) genome, nine *Rpi-vnt1*-like genes are present in the superscaffold PGSC0003DMB000000339, which in addition to the *Rpi-vnt1*-like genes contains other NBS-LRR-like genes. The molecular markers were designed on genes annotated as RNA recognition motif [PGSC0003DMC 200042144 (PGSC0003DMT200062593)] and Anthranilate *N*-transferonyltransferase protein [PGSC0003DMC 200042152 (PGSC0003DMT200062607)] in this superscaffold. Both genes are immediately adjacent to genes similar to *Rpi-vnt1* (http://solanaceae.plantbiology.msu.edu/cgi-bin/gbrowse/pgsc-potato-dm/#search) and, therefore, it is very likely that the resistance observed in our study is in fact *R* gene based. We mapped *Rpi-vnt1.1*-like gene but it fell out of the two-LOD interval (75.5–78.7 cM) of dPI09c (PI09). The *Rpi-svnt* marker originating from the susceptible phureja parent was segregating in the progeny but did not contribute to the resistance (result not shown). We made an attempt to map the putative *R* gene following the Mendelian classification as described by Tan et al. (2008) who mapped late light resistant gene *Rpi-mcd1*. In all environments except II, the tentative *R* gene was mapped on chromosome 9 near the location of dPI09c, but in different locations spanning over 7 cM. With QTL mapping we were able to achieve a narrower window for the loci associated with resistance since almost all of the LOD peaks of dPI09c (PI09) located within the marker interval DMC42152bf-DMC42144af and the widest two-LOD interval spanned only 3.2 cM (75.5–78.7 cM). Therefore, QTL mapping seems more suitable to localize factors underlying resistance to *P. infestans* in our experiments. Linkages between *R* genes and QTL underlying resistance to *P. infestans* have been reported (Danan et al. 2011; Gebhardt and Valkonen 2001; Rauscher et al. 2010) and we will not argue against the potential role of major genes in quantitative resistance. The resistant parent (CIP301071.3) of population B3C1HP from family CIP393046 was derived from the B3C1 population that was bred for horizontal late blight resistance in the absence of *R* genes from *S. demissum*, *R1-11* (Landeo 2001) and has been shown to withstand high pressure of late blight in different agro-ecological environments. Family CIP393046 has in its pedigree old European varieties such as Monalisa and Bornia as well as an Andigena variety Huagalina and thus may contain new *R* genes of South American origin. We consider dPI09c (PI09) as a new and valuable resistance resource against *P. infestans*. Research is proceeding toward the cloning of the gene(s) in the region of QTL dPI09c (PI09). Based on sequence information and functional analysis, it will be possible to know the molecular function of the QTL and to explain why it behaves differently facing different races of *P. infestans*, which might contribute to further understanding of the mechanism of late blight resistance.

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