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ORIGINAL PAPER

Influence of day-length and isolates of *Phytophthora infestans* on field resistance to late blight of potato

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Abstract Main and interaction effects of day-length and pathogen isolate on the reaction and expression of field resistance to *Phytophthora infestans* were analyzed in a sample of standard clones for partial resistance to potato late blight, and in the BCT mapping population derived from a backcross of *Solanum berthaultii* to *Solanum tuberosum*. Detached leaves from plants grown in field plots exposed to short- and long day-length conditions were independently inoculated with two *P. infestans* isolates and incubated in chambers under short- and long photoperiods, respectively. Lesion growth rate (LGR) was used for resistance assessment. Analysis of variance revealed a significant contribution of genotype × isolate × day-length interaction to variation in LGR indicating that field resistance of genotypes to foliar late blight under a given day-length depended on the infecting isolate. An allele segregating from *S. berthaultii* with opposite effects on foliar resistance to late blight under long- and short day-lengths, respectively, was identified at a quantitative trait locus (QTL) that mapped on chromosome 1. This allele was associated with positive (decreased resistance) and negative (increased resistance) additive effects on LGR, under short- and long day-length conditions, respectively. Disease progress on whole plants inoculated with the same isolate under field conditions validated the direction of its effect in short day-length regimes. The present study suggests the occurrence of an isolate-specific QTL that displays interaction with isolate behavior under contrasting environments, such as those with different day-lengths. This study highlights the importance of exposing genotypes to a highly variable population of the pathogen under contrasting environments when stability to late blight resistance is to be assessed or marker-assisted selection is attempted for the manipulation of quantitative resistance to late blight.

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Introduction

Stability and predictability of performance are decisive factors in the success of crop varieties, but agronomically important traits are more often than not, influenced by environmental conditions that vary from year to year and across locations.

Field or non-race-specific resistance to potato late blight caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is considered durable, although its robustness in terms of stability can be strongly influenced by environmental conditions (Umaerus and Umaerus 1994). Some experiences have indicated a marked decrease of the resistance of temperate-adapted varieties grown under tropical conditions (Colon 1994; Trognitz et al. 1996), while others have demonstrated stability of the performance of potato lines across environments, including different day-length regimes (Haynes et al. 1998; Forbes 1999; Forbes et al. 2005). One of the most influential environmental distinctions between temperate and tropical potato-growing environments is day-length. In day-length-sensitive germplasm, tuberization is a short-day response, and tuber filling is associated with other components of maturity including senescence. Changing the photoperiod

from long to short day-length is known both to cause early onset of tuberization (Werner 1940), the main determinant of earliness, and to decrease resistance to late blight (Pohjakallio et al. 1957; Umaerus 1960). Thus, early tuberization and early physiological maturity, either genetically inherited or induced by short photoperiod, have both been negatively correlated with field resistance to late blight (Colon 1994; Oberhagemann et al. 1999). This system also illustrates the influence of tertiary components—beyond the host genotype and the environment per se—to pathogen population variability, or a combination of these (Forbes et al. 2005). Changes in ranking of field-resistant cultivars against different genotypes of the pathogen may reflect instability or erosion of field resistance (Nelson 1979). Strong evidence of differential interaction between potato cultivars and *P. infestans* isolates, not involving the presence of *R* genes, has been demonstrated in some studies (James and Fry 1983; Inglis et al. 1996), while others have considered this interaction to be of little importance (Latin et al. 1981; Flier et al. 2003).

Relevant information on the factors that affect the stability of late blight resistance has emerged from the use of molecular maps and suitable statistical methods to locate polygenes that contribute to quantitative resistance on genetic maps of segregating populations (Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000; Sandbrink et al. 2000). Thus, isolate-specific quantitative trait loci (QTL) have been suggested from the greater ratio of congruent QTLs detected when the same rather than different isolates of *Phytophthora infestans* are tested in genetically related mapping populations (Simko 2002). On the other hand, factors controlling maturity and tuberization have been found co-localized with QTL for late blight resistance (Collins et al. 1999; Visker et al. 2004). Taken together, studies in a number of progenies derived from different *Solanum* species have provided evidence for the effects of pathogen isolates and environmental conditions on the occurrence of resistance QTL (reviewed in Simko 2002). However, no attempts have yet been made to untangle the components that are confounded in the so-called environmental effect and determine their individual or combined influence on the effects of the genes that control this resistance. The degree to which individual factors, such as photoperiod, humidity, temperature, etc., affect both pathogen and potato biology, may determine the expression of field resistance, and thus its stability.

In this context, the present study examined the effect of photoperiod and pathogen isolates on foliage resistance to late blight in the segregating mapping population designated as BCT (Bonierbale et al. 1994) and in five standard clones reported to be free of known *R*-genes from *S. demissum*. BCT population was also used to measure the influence of day-length on the occurrence of resistance QTL.

Materials and methods

Plant material

The diploid segregating population BCT was obtained by backcrossing the interspecific F_1 hybrid M200-30 (a clone selected from a cross between the dihaploid *S. tuberosum*, USW2230, and a *S. berthaultii* clone selected from PI1473331, namely 'B11B', to a different dihaploid *S. tuberosum* clone, HH1-9, used as male parent (Bonierbale et al. 1994). This population of 150 progeny genotypes was formerly used to construct an RFLP-based map and to locate QTL for tuberization, dormancy, and late blight resistance (van den Berg et al. 1996a, b; Ewing et al. 2000). The two BCT-population parents, the original parents of the hybrid female parent M200-30, i.e., the *S. tuberosum* USW2230 and the S. *berthaultii* clone B11B, and 110 of the original progeny were used in this study. A set of standards for quantitative resistance to late blight, reported to be free of *R* resistance genes from *S. demissum* (Forbes and Tolstrup 1999; Forbes 1999; Wulff et al. 2007), was also included: 393280.64, (moderately resistant breeding line derived from CIP's Population B) (Landeo et al. 2000), Cruza-148 (resistant cv. from Mexico), Amarilis and Yungay (moderately resistant and susceptible cvs. from Peru, respectively), and Tomasa Condemayta (susceptible cv. from Peru).

Twenty stock plants of each progeny genotype, parent, and standard clone were established in the greenhouse from in vitro culture in La Molina (Lima, Peru) during March, 2000, and propagated once by stem cuttings, to produce uniform planting material for transplanting to the field in May. Fifteen transplants per entry were randomly allocated to either of two experiments planted in isolated fields within an interval of 1 week.

The two experiments were set in randomized complete block designs (RCBD) consisting of three replications of five-hill plots, and managed under short- and long day-length regimes, respectively, during the cool humid 'winter' season (May–July) of 2000 in La Molina (Lima, Peru). Outdoor incandescent lamps were used to extend the natural short day photoperiod (12H) for the long day-length (16H) regime. Transplants were set at 0.30 m between hills, and 0.9 m between rows, and subject to the same management practices other than day-length, in both experiments.

Detached leaf bioassays

Resistance of genotypes was measured against two complex isolates of *P. infestans*, 'PCO-002' (race 1.2.3.4.6.7.8.10.11), and 'PCO-055' (race 1.2.3.4.6.7.8. (9).10.11), taken at random from those historically collected from the

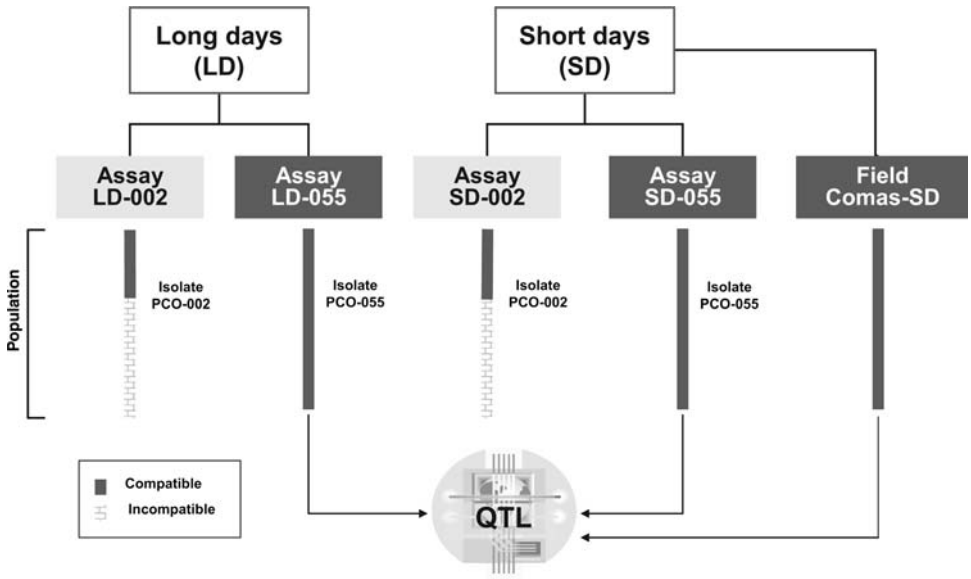

Fig. 1 Description of detached leaf (DLA) and field assays performed for classical and QTL analysis

same region of the Peruvian highlands (Mariscal Castilla, Comas, Junín, 2800 masl, 11°42′54″S latitude). The former was isolated from an *S. tuberosum* breeding line in 1997, while the latter was isolated in 1999 from a plant of the BCT population and maintained since then in CIP's pathogen collection. Both isolates belonged to the A1 mating type. PCO-055 was known from prior testing to be particularly aggressive and compatible with the whole population of BCT plants. Inoculum of both isolates was propagated on tubers of the susceptible cv. Ccompis.

Four series of detached leaf assays (DLA) were conducted and designated, respectively, as 'SD-002', 'SD-055', 'LD-002', and 'LD-055', in which SD and LD refer to short- and long-day conditions, and 002 and 005 refer to isolates 'PCO-002' and 'PCO-055' of *P. infestans*, respectively (Fig. 1). The former two were represented by leaves sampled from field plants grown under short day-length regime (SD), inoculated, respectively, with isolates 'PCO-002' and 'PCO-055'. The latter two, by leaves sampled from field plants grown under long day-length regime (LD), were inoculated, respectively, with the two mentioned isolates. Two compound leaves were cut from the 3rd node below the apex of one plant from each replication per field trial, and placed independently in 15-cm Petri-dishes containing 1.5% water agar, for inoculation with each isolate. Samples were taken from the plants grown under short-day conditions at 9–10 weeks after planting (WAP), and from those under the long-day regime, at 14–15 WAP, about a month before harvest in both cases, considering the differences in vegetative periods of plants growing under the different day-length regimes and trying to match up crop physiological stages. Each compound leaf was inoculated with one of the two isolates by placing a droplet of inoculum (3,500 sporangia/ml) on the abaxial side of each of its five leaflets using a micropippetor. Plates were incubated in growth chambers at 16 h/8 h and 12 h/12 h day/night photoperiods for samples from the long- and short-day field conditions, respectively, provided by means of fluorescent lights at an intensity of 29.4 W m^{-2}, and conditions of 18/15°C day/night and 100% RH.

Lesions were measured on three consecutive days from the 3rd day after inoculation using a digital caliper. The largest length and width of each lesion was measured, and the ellipse area ($A = ¼ \pi \times$ length $\times$ width) calculated. The radius was estimated as the square root of lesion area. Lesion growth rate (LGR) was estimated by linear regression of the lesion radius over time. Lesions not larger than the size of the inoculum droplet, i.e., cc. $A \leq 16$ mm^2 (Vleeshouwers et al. 1999) were regarded as unsuccessful infections where the pathogen was arrested by a hypersensitive reaction (HR) leading to a LGR of zero, and hence defined as an incompatible response (Flier et al. 2003).

The hybrid female parent M200-30, its original *S. berthaultii* parent B11B, and about 64% (69 genotypes) of BCT population resulted incompatible with isolate 'PCO-002'presenting LGR values of zero. Very small necrotic spots that at most reached the size of the inoculum droplet were observed in plants of these genotypes in DLA 'LD-002' and 'SD-002'. The population size compatible with this isolate was therefore reduced to less than 40 genotypes. Consequently, quantitative analysis involving these DLA in BCT population was reduced to those genotypes compatible with this isolate, and QTL analysis could not be performed (Fig. 1).

Average LGR values obtained from the five leaflets of each compound leaf per replication of each DLA was subjected to standard analysis of variance (ANOVA) using

Proc GLM (general linear model) of SAS Institute (2001). Standard clones and parents were not included in ANOVA. Variance components were estimated from expected mean squares. Mean LGR values calculated for each genotype by day-length regime and isolate, were used to estimate basic statistical parameters (standard deviation, population mean, and coefficient of skewness).

Combined ANOVA were performed independently for each isolate across day-length regimes. One of them used LGR data of 100 BCT genotypes common across assays 'LD-055' and 'SD-055', both inoculated with isolate 'PCO-055', whereas the other used LGR data of 30 BCT genotypes compatible with isolate 'PCO-002', and common across assays 'LD-002' and 'SD-002'. Loss of ten genotypes that did not grow well and died before sampling for DLA in the field trial under natural short day-length regime accounted for the reduced number of common genotypes across assays. A mixed model was assumed and the analysis was run using the Mixed Procedure of SAS Institute (2001) and the residual maximum likelihood (REML) procedure, (Littell et al. 1996).

Likewise, assuming a mixed model and performing a similar analysis as that described above (Mix Procedure of SAS), two combined ANOVA were performed over the four DLA, i.e., 'SD-002', 'SD-055', 'LD-002', and 'LD-055'. One used LGR data of 30 BCT genotypes common across the four DLA, and the other used data from the five standard clones, the two BCT-population parents, and the original parents of the BCT hybrid female parent M200-30.

Field assessment of late blight resistance in whole plants

Tubers harvested from the short day-length regime experiment in La Molina were stored and allowed to sprout to carry out a field assessment of foliar resistance in whole plants in the Peruvian highlands (Mariscal Castilla, Comas, 2800 masl, 11°42′54″ latitude) during the rainy season of 2000. The field assay, which represented a short day-length environment, was denoted 'Field Comas-SD' and was carried out to look for consistency of QTL from detached leaf assays under short day-length conditions against isolate 'PCO-055' isolate, for which the whole population was compatible. A RCBD consisting of three replications of five-hills per genotype was used and included the parents and standard clones. Plants were protected against *P. infestans* with a contact fungicide (Mancozeb 45%) for the first 45 days after planting, after which, plots were spray inoculated directly with a suspension of 3,500 sporangia/ml of isolate 'PCO-055'. Five readings were recorded on a plot basis as the percent foliage infected at approximately 5-day intervals from the inoculation date. These data were used to calculate area under the disease progress curve (AUDPC), (Shaner and Finney 1977). AUDPC values were subjected to ANOVA, without standard clones and parents. Variance components were calculated as indicated before. Mean AUDPC values calculated for each BCT population genotype were used to estimate basic statistical parameters (standard deviation, population mean, and coefficient of skewness) and for QTL analysis.

QTL analysis

The framework map of BCT based on RFLP marker alleles segregating from the inter-specific hybrid female parent, M200-30 (Bonierbale et al. 1994) was augmented with 57 microsatellite (SSR) markers in the present work. Since the framework RFLP map was built on marker alleles from the original *S. berthaultii* parent (B11B), segregating from the inter-specific hybrid female parent M200-30, the same approach was used to map the SSR, in order to identify linked QTLs segregating exclusively from *S. berthaultii*. Eight SSR markers analyzed on 120 of the 155 genotypes comprising this population were placed on the framework map using the "try" command of MapMaker software v 3.0, and verified using the "ripple" command of the same software (Lander et al. 1987). The remaining 49 SSR markers were analyzed on a most-informative mapping sample of 34 selected genotypes from the entire 155 used to construct the framework map, following the 'selective mapping' strategy developed by Vision et al. (2000). The strategy is based on the selection of an optimized sample of genotypes bearing complementary recombinational cross-over sites from the entire population, permitting the placement of new markers with almost the same precision obtained with the entire population. MapPop software v1.0 (Vision et al. 2000) was employed for sampling and bin mapping of SSR on BCT framework map. A 'Java' interface to MapPop v1.0 developed by Simon and Bonierbale (2003) called 'IntiMap' was used to provide a graphical map output.

Mean LGR values of BCT population genotypes for long- and short day-length regimes inoculated with isolate 'PCO-055', i.e., DLA, 'LD-055' and 'SD-055', were analyzed for marker–trait association (Fig. 1).

QTL analysis was also performed with mean AUDPC values calculated from disease assessment in the field experiment, 'Field Comas-SD'. This analysis looked for congruence of QTL detected in the field assay with those detected in DLA.

Composite interval mapping (CIM) (Zeng 1993) was performed using QTL Cartographer v2.5 (Wang et al. 2001). The following hypothesis was tested H_0: no QTL effect at the test position, i.e., $a = 0$, where 'a' denotes additive effect. The 5% empirical threshold values were

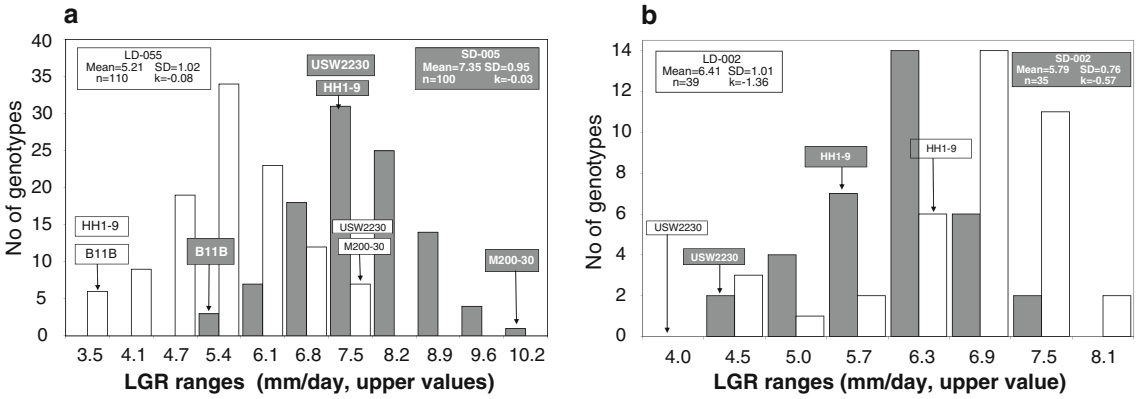

Fig. 2 Frequency distribution of lesion growth rate (LGR) values in detached leaf assays of BCT population **a**. 'LD-055' and 'SD-055' **b**. 'LD-002' and 'SD-002'

calculated by QTL Cartographer based on 1,000 permutations of the original data (Churchill and Doerge 1994).

Results

Detached leaf assays

Four DLA combining 2 day-lengths and two isolates, coded as 'LD-002', 'SD-002', 'LD-055', and 'SD-055' (see material and methods for details) were analyzed for lesion growth rate (LGR) in the diploid mapping population BCT, its parents and five standard clones free of *S. demissum*-derived *R* resistance genes.

Incompatibility (LGR = 0.0) of more than half of BCT population (64%) against one of the isolates, namely 'PCO-002,' precluded QTL mapping using data from DLA 'LD-002' and 'SD-002'. Hence, QTL mapping was performed with data from DLA 'LD-055' and 'SD-055', both tested against isolate 'PCO-055', for which the whole mapping population showed a compatible reaction (LGR > 0.0) (Fig. 1).

Means, standard deviations, and skewness coefficients for each DLA are shown in Fig. 2 (standard clones are not represented). LGR mean values varied considerably in the population and their parents. Phenotypic variability was similar and showed almost a perfect normal distribution in DLA 'LD-055' and 'SD-055'; however, population LGR mean value was higher (more susceptible) in assay 'SD-055' (Fig. 2a). On the other hand, the fraction of BCT genotypes compatible with isolate 'PCO-002' in assays, 'LD-002' and 'SD-002', showed a skew toward greater LGR values (more susceptible). Population LGR mean was slightly higher (more susceptible) in assay 'LD-002' (Fig. 2b).

Late blight resistance performance of BCT parents in DLA, LD-055 and SD-055 were as follows: The interspecific female parent, M200-30, was distributed with BCT population genotypes with greatest LGR values (most susceptible genotypes), contrasting with its original parents which generally performed as more resistant (Fig. 2a). Its *S. berthaultii* parent B11B, was distributed with a few BCT genotypes within the range of lowest LGR values (most resistant) in both assays, while its *S. tuberosum* parent USW2230, was much more resistant than M200-30 in assay 'SD-055' but as susceptible as, in DLA 'LD-055'. On the other hand, the *S. tuberosum* male parent, HH1-9, was within the group of the most resistant BCT genotypes in 'LD-055', while together with genotype USW22-30, within the group holding BCT population mean in assay 'SD-055' (moderately resistant) (Fig. 2b).

With regard to DLA, 'LD-002' and 'SD-002' (Fig. 2), the hybrid female parent M200-30 and its *S. berthaultii* parent B11B, were incompatible with isolate PCO-002 used to inoculate detached leaves in both assays (not represented). The *S. tuberosum* parent of M200-30, the genotype USW2230, was compatible; and in contrast to what was observed in DLA against isolate 'PCO-055', it showed the lowest LGR mean value in both assays. LGR of the *S. tuberosum* male parent, HH1-9, placed it within the group immediately to the left of the population mean, indicating a moderately resistant reaction

Simple analysis of variance of LGR per DLA showed highly significant genotypic variation; however, the variation due to the environment was larger than the genotypic variation in all assays, indicating the strong influence of non-controlled experimental factors over genetic ones (Table 1).

The combined ANOVA of LGR values over DLA 'SD-055' and 'LD-055' showed highly significant differences for genotype ($p < 0.001$), day-length ($p < 0.001$), and genotype × day-length interaction ($p = 0.003$) while that over DLA 'LD-002' and 'SD-002' showed high significance for genotype ($p = 0.002$), no significance for

Table 1 Variance components for BCT population estimated from expected mean squares of lesion growth rate (LGR, mm/day) in detached leaf assays (DLA) and of AUDPC values from a field assessment of whole plants conducted under short day-conditions in Peru

Source	Detached leaf assay (DLA)				'Field Comas-SD'
	LD055	SD055	LD002	SD002	
Block	0.05*	0.10**	0.00	0.26**	12,937**
Genotype	0.49**	0.28*	0.40**	0.31**	22,779**
Error	1.57	1.89	0.59	0.77	25,925
N° genotypes	110	100	39	37	100
Mean	5.21	7.35	6.41	5.79	657
CV (%)	24	19	12	15	24

**, * Significant at 0.01 and 0.05%

day-length ($p = 0.06$), and a barely significant genotype × day-length interaction ($p = 0.023$). Significance of the first-order interaction indicates that LGR variation of genotypes in BCT population depends on day-length regime. Simple effects showed about 50% (54 out of 100) of BCT population genotypes with significantly increased LGR values under short day-length conditions in DLA inoculated with isolate 'PCO-055', i.e., 'SD-055' (data not shown). However, opposed to this finding, an increase in LGR values under long day-length conditions of 4 out of 30 BCT population genotypes accounted for the significant differences of genotypes across day-lengths in DLA inoculated with isolate 'PCO-002', i.e., 'LD-002' and 'SD-002'.

Combined ANOVA over the four DLA using LGR data of 30 BCT population genotypes common across DLA revealed significance for genotype ($p = 0.039$) and genotype × isolate × day-length interaction ($p = 0.029$) and no contribution of any other effect involving the genotype. On the other hand, the combined ANOVA using LGR data from BCT parents and standard clones showed significance for genotype ($p < 0.0001$), genotype × isolate ($p < 0.0001$), and genotype × isolate × day-length interaction ($p = 0.006$).

The high significance of the genotypic variance in both analyses indicated that BCT population genotypes, standard clones, and BCT parents differed in their genetic potential for LGR.

Significance of genotype × isolate interaction encountered in the analysis involving BCT parents and standard clones showed a differential performance of these genotypes against isolates. Actually, the inclusion in this analysis of the hybrid female parent M200-30 and its original *S. berthaultii* parent B11B, both of which with an incompatibility reaction (LGR = 0.0) against isolate 'PCO-002', contributed to the significance of genotype × isolate interaction given their differential performances with regard to isolate 'PCO-055', for which both were compatible (LGR > 0) (Fig. 3). However, differential performance of genotypes across isolates were also encountered in genotypes compatible with both isolates, as found in the cases of the *S. tuberosum* parent USW2230, and the resistant standard clone Cruza-148 (Fig. 3). A cross-over interaction between LGR and isolate was also apparent between these genotypes.

Significance of the second-order interaction indicated that differences among genotypes across day-length depended on the isolate present. This interaction was independently illustrated only for parents and standard clones (Fig. 4a, b). Changes in the relative rankings of either parents or standard clones, as well as changes in the magnitudes of the differences between them across DLA accounted for this statistical interaction. Tomasa Condemayta and Yungay were the most susceptible clones, with overall mean LGR values of 7.3 and 6.4 mm/day, respectively, though the latter was slightly less susceptible in assay 'SD-002' (Fig. 4a). The moderately resistant breeding line 393280.64 had an overall mean LGR value of 4.6 mm/day, significantly greater than those of the two standard resistant clones Amarilis and Cruza-148 which were the most resistant ones, with overall LGR values of 3.7 and 3.4 mm/day, respectively. Remarkable differences in stability of resistance were observed for these three standard clones. Although Cruza-148 was the most resistant clone against isolate 'PCO-055' in assays 'LD-055' and 'SD-055', this clone showed a significantly greater LGR mean value (greater susceptibility) in the short day-length assay, i.e., 'SD-055'. The opposite was true against isolate 'PCO-002'; a significantly greater LGR mean value was found in the long day-length assay 'LD-002'. On the other hand, Amarilis and 393280.64 showed stability against isolate 'PCO-055' in assays

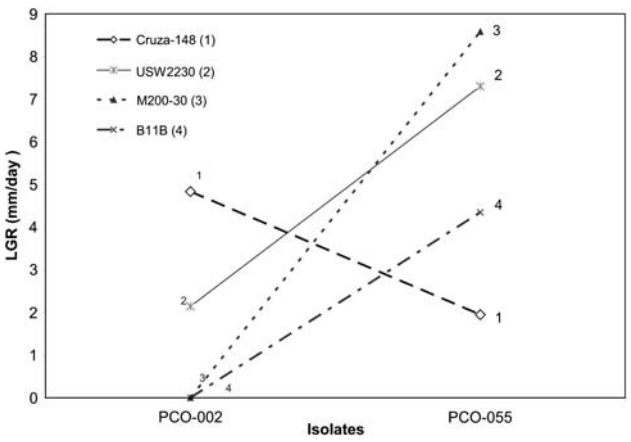

Fig. 3 The effect of genotype × *P. infestans* isolate interaction on lesion growth rate (LGR). $LSD_{(0.05)}$ to compare means with the same or different isolate is 1.28

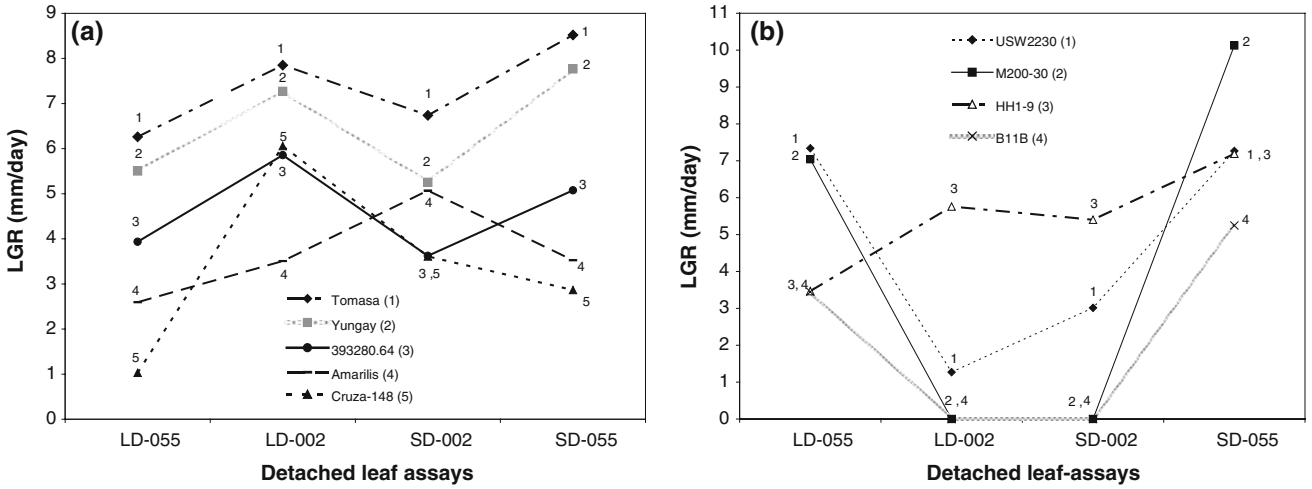

Fig. 4 The effect of genotype × day-length × *P. infestans* isolate interaction on LGR values. (**a**) Standard clones and (**b**) BCT parents. Combination of day-length and isolate are represented by the four DLA indicated in the axis 'LD-055' = long day-length regime + isolate 'PCO-055', 'LD-002' = long day-length regime + isolate 'PCO-002', 'SD-002' = short day-length regime + isolate 'PCO-002', and 'SD-055' = short day-length regime + isolate 'PCO-055'. $LSD_{(0.05)}$ to compare means with the same isolate and day-length combination is 1.79 $LSD_{(0.05)}$ to compare means at different isolate by day-length combination is 1.83

'LD-055' and 'SD-055'. However, this was not the case against isolate 'PCO-002' for which the former showed a significantly greater LGR value in assay 'SD-002' (greater susceptibility) and the latter did so in assay 'LD-002'. Disregarding the incompatibility reaction to one of the isolates in the *S. berthaultii* parent, B11B, and the interspecific female parent M200-30, parents also showed great differences in stability of resistance (Fig. 4b). The *S. tuberosum* parent USW22-30 showed significantly increased levels of susceptibility when exposed to isolate 'PCO-055' in assays 'LD-055' and 'SD-055'. On the other hand, BCT parents, M200-30 and HH1-9, showed higher levels of susceptibility in assay 'SD-055' compared with their performance in assay 'LD-055'. However, HH1-9 proved to be stable across assays 'LD-002' and 'SD-002'.

Combined ANOVA performed for LGR data of BCT population, and that for parents and standard clones also showed isolates to perform differently between day-length regimes ($p = 0.0002$ and $p < 0.001$, respectively). Isolate 'PCO-055' caused significantly more disease severity than isolate 'PCO-002' under short day-length regime. LGR mean values of 7.8 versus 5.9 mm/day, and of 6.4 versus 3.6 mm/day were obtained, respectively, for LGR data of BCT genotypes, and LGR data of BCT parents and standard clones. However, no statistical differences between isolates were found under long day-length regimes (LGR mean values of 5.4 vs. 6.8 mm/day and of 4.5 vs. 4.1 mm/day, respectively, as described above). A statistical difference in the level of severity across day-lengths was also encountered for isolate 'PCO-055'. Disease severity caused by this isolate was again significantly greater under short day-length regimes (LGR values of 7.8 vs. 5.40 mm/day, and 6.4 vs. 4.5 mm/day, respectively, as described above).

QTL analysis

QTL-marker analysis was performed following alleles of the original *S. berthaultii* parent, B11B, segregating from the hybrid parent M200-30, because BCT genetic map was built from markers present exclusively in this parent. BCT genetic map currently consists of 140 mapped markers, i.e., 82 RFLPs (Bonierbale et al. 1994), 1 *R* gene (R_{ber}, Ewing et al. 2000) and 57 SSRs added in the present study, with a total length of c.a. 648 cM (http://research.cip.cgiar.org/cmap/).

The major gene responsible for incompatibility against 'PCO-002' mapped on chromosome X between the RFLP marker CT214 and the *Rber* gene identified by Ewing et al. (2000) in this same population. It mapped at a distance of 2 cM from this R gene. Seven genotypes found incompatible here but compatible in Ewing et al. (2000) must have accounted for the lack of the expected co-segregation. We do not know if differences in compatibility of these genotypes could be the result of altered levels of resistance under certain conditions, as suggested for some weak *R* gene-*Avr* interactions (Vleeshouwers et al. 2000), or a mixture of genotypes between Ewing et al. (2000) and our study. Therefore, the major gene found in this study was assumed to be the R_{ber} gene and hence it was not displayed in the map again.

QTL analysis was realized only for assays 'LD-055' and 'SD-055' tested with isolate 'PCO-055' for which the

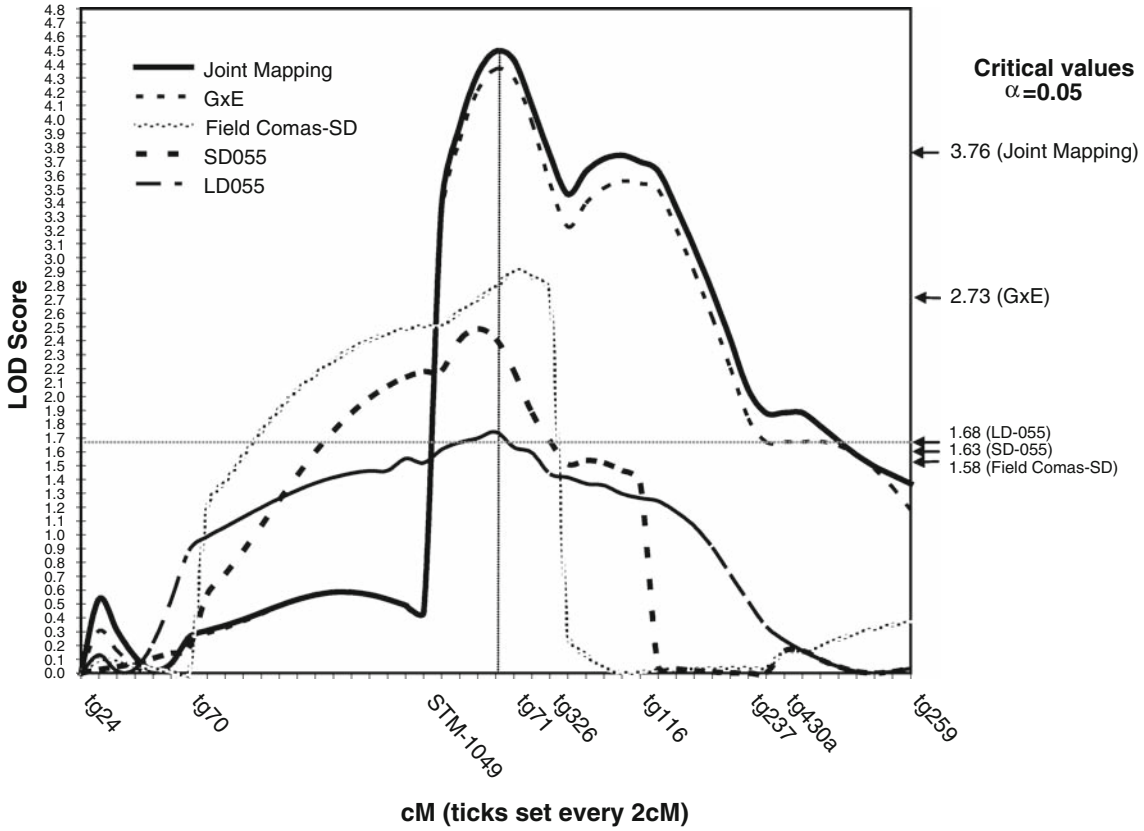

Fig. 5 Location and LOD-score plots of a common QTL for late blight resistance identified under long- and short day-length regimes in BCT population

whole population was compatible ($n > 100$). Two peaks rose to LOD > 1 on chromosomes 1, and 10 in 'SD-055' and, three on chromosomes 1, 5, and 11 in 'LD-055'. The QTL located on chromosome 1 in both DLA was the only significant QTL at a threshold value of 5% after 1,000 permutations of each data, and spans a genomic region of c.a. 38 cM length (confidence interval outside of which LOD falls by 1.0), comprising several marker intervals (TG 70-STM1049- TG 71- TG 326- TG 116). Its most likely position was between STM1049 and TG 71 in both assays as indicated by the highest log-likelihood (LOD) of CIM and joint mapping analysis performed using the Multiple Trait Analysis module of QTL Cartographer V2.5 (Wang et al. 2001) (Fig. 5).

The allele segregating from *S.berthaultii* at this QTL explained 11 and 8% of the total phenotypic variance for 'SD055' and 'LD055' assays, and was associated with a positive ($a = +0.62$ mm/day; decrease resistance) and negative ($a = -0.62$ mm/day, increase resistance) additive effect, respectively.

The differential expression of a QTL in different environments is usually attributed to a QTL by environment interaction (Jiang and Zeng 1995). The joint mapping module JZMapQtl of the Multiple Trait Analysis program of QTL Cartographer V2.5 (Wang et al. 2001), was employed to perform a QTL × day-length interaction test at the most likely position of this common QTL. The following hypothesis was tested H_0: $a_{SD055} = a_{LD055}$; H_1: $a_{SD055} \neq a_{LD055}$, where a = additive effect. Since this test is conditional on the significance of the joint mapping analysis (Jiang and Zeng 1995), the 5% empirical threshold values were calculated on 1,000 permutations of the original data by QTL Cartographer for the joint mapping and QTLxE analysis, respectively (Churchill and Doerge 1994). Both, joint mapping and QTL by environment interaction resulted significant at 5% indicating that this QTL effect was differential with respect to day-length (Fig. 5).

Field Assay ('Field Comas-SD')

ANOVA for AUDPC values of the BCT population showed highly significant genotypic variation ($p < 0.001$), (Table 1). BCT parents, HH1-9 (mean = 653) and M200-30 (mean = 623), and the *S. tuberosum* parent of M200-30, the genotype USW22-30 (mean = 697) were not statistically different and were within the group holding the population mean (mean = 657). On the other hand, the

S. berthaultii parent B11B (mean = 246) was the most resistant and not significantly different from the standard resistant cultivar, Cruza-148 (mean = 308).

Two peaks at LOD > 2 rose on chromosomes 1 and 10, respectively, though only that on chromosome 1 remained significant at a threshold value of 5% after 1,000 permutations of the data. The significant QTL on chromosome 1 spanned the same regions as those found in the DLA, though its most likely position was in the immediate next interval, flanked by markers TG71 and TG326, as indicated by the highest log-likelihood (LOD) of CIM (Fig. 5). The allele segregating from *S. berthaultii* at this QTL accounted for 12% of the total phenotypic variance and coincided in the direction of its effect (+130 AUDPC units) to that detected in DLA 'SD-055', indicating an increase of susceptibility.

Discussion

The effect of day-length regimes and variability of *P. infestans* population on stability of quantitative foliar resistance to late blight in potato have been treated individually in several studies (Tooley and Fry 1985; James and Fry 1983; Colon 1994; Inglis et al. 1996; Forbes 1999; Carlisle et al. 2002; Flier et al. 2003). To the best of our knowledge, this is the first time in which these two factors have been set together in a single study to gain insight into their main and combined effects on the expression of this type of resistance.

Classical statistic analysis and QTL mapping was performed using estimations of lesion growth rate (LGR) on detached leaf assays (DLA). Nevertheless, an incompatibility reaction observed against one of the two isolates in more than 50% of the BCT mapping population restricted the molecular analysis to examining the stability of QTL in DLA performed under short- and long day-length regimes using a single isolate, namely 'PCO-055', for which the whole population showed a compatible reaction. A field assessment of resistance in whole plants under the short photoperiods of Peru, using this same isolate was also conducted in order to look for consistency of QTL detected in DLA. On the other hand, data from 30 common BCT population genotypes across DLA, and those from BCT population parents, and standard clones with different levels of quantitative resistance to foliar late blight were used to accomplish our objective of analyzing the stability of field resistance against the combination of two isolates and photoperiods by means of quantitative genetics.

Significant genotypic variation for field resistance was found in BCT population under both, short- and long day-length regimes, with the two isolates 'PCO-055' and 'PCO-002', despite the high magnitude of environmental variance. It is difficult to point out experimental factors that may have brought about the high environmental variation, considering that this study comprised several procedures, from growing field plants to leaf sampling, inoculation, incubation, and assessment. Non-controlled factors at any of these steps due to human manipulation or otherwise, by microclimatic effects during field growing or detached leaf incubation, may account for the variation in the expression of resistance of a genotype.

Differences in quantitative resistance were observed between BCT parents. The interspecific female parent M200-30 showed significantly higher levels of susceptibility than its parental counterpart, the *S. tuberosum* clone HH1-9 in DLA inoculated with isolate 'PCO-055'. HH1-9 showed high and moderate levels of resistance under long- and short day-length regimes, respectively. On the other hand, the *S. berthaultii* parent of the interspecific female parent M200-30, the genotype B11B, showed the highest levels of resistance under both day-length regimes, in contrast to the *S. tuberosum* parent USW2230 that showed moderate and high levels of susceptibility under short- and long day-length, respectively, against this isolate. However, in contrast with what was observed in the performance of the *S. tuberosum* parent USW2230 against isolate 'PCO-055', this genotype showed high levels of resistance in DLA inoculated with isolate 'PCO-002', suggesting the presence of isolate-specific resistance genes controlling foliar resistance to late blight in this parental clone.

Except for the interspecific hybrid M200-30, parents' performance in detached leaf assay under short day-length regime adequately reflected that of whole plants in field conditions under the same day-length regime, and isolate, namely, isolate 'PCO-055'. This field assay was conducted to validate assessments made under the detached leaf assay. The two *S. tuberosum* parents, HH1-9 and USW22-30 showed moderate levels of resistance with mean values close to BCT population mean in both the field trial and DLA, while the *S. berthaultii* parent B11B showed the highest level of resistance, with LGR mean values within the range of lowest mean values. In contrast, the interspecific hybrid, M200-30, showed comparatively higher levels of susceptibility when assessed in detached leaf. On the other hand, this genotype showed similar levels of resistance to its parental counterpart, the genotype HH1-9 and its *S. tuberosum* parent USW22-30 in the field assay. A particular sensitivity of detached leaves of this parent might account for this difference. A greater susceptibility of detached leaves with regard to whole plants in wild genotypes was also observed by Vleeshouwers et al. (1999).

Stability of partial resistance to late blight by classical genetic analysis

Genotype by environment (G × E) interaction studies that involve both the environment and pathogen variability provide valuable information to outline optimum strategies for testing and selecting for stability to late blight resistance. The present work revealed that variability of *P. infestans* isolates and day-length are important sources of variation for quantitative resistance to foliar late blight.

A strong differential response of genotypes to day-length was found in BCT population and parents when exposed to isolate 'PCO-055'. The two BCT parents, M200-30 and HH1-9, and most of their offspring showed a greater susceptibility under short day-length regimes against this isolate. Most studies dealing with the influence of day-length on the expression of late blight resistance ascribe genotype × day-length variation to differences in the sensitivity of host genotypes to photoperiod. Sensitive genotypes have shown increased foliar susceptibility to late blight under short photoperiod (Grainger 1956; Umaerus 1960; Turkensteen 1993). However, this sensitive response under short photoperiod was not observed in DLA against isolate 'PCO-002'. Instead, the few genotypes that interacted with day-length showed a greater susceptibility under long day-length regimes than short. A more complex interaction effect was encountered, when in addition to the genotype and day-length, the presence of different isolates of the pathogen was included in the analysis. The analysis performed using the LGR values of a fraction of genotypes compatible with both isolates, the four BCT parents, and five standard clones with known levels of field resistance showed that variation in the expression of resistance to foliar late blight under a given day-length depended on the isolate present. This was illustrated for the standard clones and BCT parents. Thus, under long day-length regimes, the standard resistant clone Cruza-148 showed a high level of resistance against isolate 'PCO-055', but increased levels of susceptibility against isolate 'PCO-002'. However, no differences were found in the performance of this clone across isolates under short day-length regimes. Likewise, while the moderately resistant standard clones Amarilis and 393280.64 showed stability across day-length regimes against isolate 'PCO-055', the former showed an increased level of susceptibility under short-, and the latter under long day-length regimes when assayed with isolate 'PCO-002'. The interspecific hybrid M200-30 and its parental counterpart the *S. tuberosum* clone HH1-9 showed consistent high levels of susceptibility under short day-length regimes against isolate 'PCO-055', whereas the latter showed stable resistance to isolate 'PCO-002'. Hence, our results do not invoke sensitivity to short photoperiods as accounting for increased levels of susceptibility to foliar late blight, but rather the interaction involving the genotype, day-length, and pathogen isolate. If genotype × day-length interaction depends on the isolate present, it is very likely that isolate specific QTL may be present and their effects influenced by day-length or rather by an isolate × day-length interaction, as will be shown later. This would not be the case with major genes. As shown in this study, the incompatible reaction of the *S. berthaultii* (B11B), the hybrid parent M200-30, and the fraction of BCT genotypes presenting LGR of zero against isolate 'PCO-002', was stable across day-length regimes.

Genotype × isolate × day-length interaction might also account for the marked decrease of the resistance of temperate-adapted varieties when grown under tropical conditions found in some genotypes (Colon 1994; Trognitz et al. 1996), indicating that proper screening for assessing stability should involve exposure to a highly variable population of the pathogen under such contrasting environments.

It is worthy to discuss about the genotype by isolate interaction found in this study, in order to explain the likely presence of isolate specific QTL. This study clearly showed the case of a crossover interaction between the cv Cruza-148 and one of the *tuberosum* BCT parents, the clone USW-2230, that showed specific susceptibility to the isolates used in this study. The BCT *tuberosum* grandparent USW-2230 lost its resistance when exposed to isolate 'PCO-055', while the resistant standard clone Cruza-148 did so against isolate 'PCO-002'. Differential responses as revealed by specific susceptibility of cultivars suggest that genes for resistance to different genotypes of the pathogen exist in different genotypes of the host (Yan and Falk 2002). Presence of differential genotype by isolate interaction independent of *R*-gene based resistance in foliage has also been demonstrated in other studies (Peters et al. 1999; Latin et al. 1981; James and Fry 1983; Flier et al. 2003).

On the other hand, some studies have demonstrated robustness of partial resistance to *P. infestans* of old known resistant cultivars in international evaluation trials, indicating that the observed levels of instability do not represent a significant loss of the resistance. This was not the case of the two known resistant cultivars Cruza-148 and Amarilis, in this study. Even though none of them reach susceptibility levels as high as those of the susceptible standard clone Tomasa Condemayta, both showed a significantly increased level of susceptibility under a particular combination of isolate and day-length regime. However, this does not rule out that the presence of several resistance genes in a single cultivar, as found by QTL mapping (Leonards-Schippers et al. 1994), may counter the pathogenicity and aggressiveness of the pathogen and allow the host to better confront conditions that are

favorable for disease development. In fact, since the epidemics that destroyed potato crops in Europe in the 1840s which led to the knowledge of the vulnerability of major genes, breeding for horizontal resistance to late blight has aimed at accumulating partial resistance genes in order to achieve broad stability. However, the potential adaptive ability of *P. infestans* to partial resistance together with its ability to fit different environments call for increased efforts to identify novel sources of resistance in the wild and cultivated gene pool of potato for introgression and broadening of the genetic base in order to prevent erosion of field resistance.

Isolate × day-length interaction also found here, was expressed as differences in the disease severity caused by isolates across day-lengths. Isolate 'PCO-055' caused significantly greater disease severity under short day-length regimes, while isolate 'PCO-002' performed similarly across day-length regimes. Disease measures such as lesion area, assessed here to estimate lesion growth rate, have been successfully used in pathogen population assessment for quantifying fitness and in estimating parameters of aggressiveness (Tooley and Fry 1985; Carlisle et al. 2002). This has been possible because lesion area seems to reflect patterns of multiplication and inoculum potential of *P. infestans* (Rotem et al. 1983; Tooley and Fry 1985). Highly significant location by isolate interactions in pathogen fitness have been reported specifically for *Phytophthora infestans* indicating that the degree of aggressiveness to foliage of an isolate is a response to its fitness in a given environment (Tooley and Fry 1985). Hence, it is likely that the variation in aggressiveness shown by isolate 'PCO-055' in this study might be related to its level of fitness under different photoperiods. However, further work would need to be performed testing a greater number of isolates to gain understanding of the extent and frequency of this isolate by day-length variation. Whatever the causes of this isolate × day-length interaction may have been, it seems that in the presence of isolate-specific QTL, this interaction might affect the level of blight-resistance expression.

Analysis of stability of partial resistance to late blight by QTL approach

Incompatibility segregating from the interspecific hybrid parent M200-30 against one of the two isolates used in this study, namely 'PCO-002', was identified by genetic mapping to be the major *Rber* locus described by Ewing et al. (2000). Incompatibility was clearly manifested under both day-lengths with this isolate while a compatible reaction of the entire population was evident with isolate 'PCO-055', indicating the presence of the specific virulence gene in this isolate.

Since QTL mapping was performed on alleles segregating only from the *S. berthaultii* grandparent in the BCT parental hybrid M200-30, alleles rather than QTL effects were detected. An allele for partial resistance to foliar blight with opposite effects under different day-lengths was detected in the detached leaf assay with isolate 'PCO-055'. This allele segregates from a QTL located on chromosome 1 spanning several marker intervals with its most likely position between STM1049 and TG71. This lack of precision on the estimate of QTL position is typical of QTL that account for a small portion of the variance for a trait (van Ooijen 1992). The high contribution of uncontrolled environmental factors to the variation of LGR in DLA would account for the small effect of this allele and might have prevented the detection or significance of additional *S. berthaultii* alleles of smaller effects. Despite this, the finding of the same allele under field conditions in which whole plants were inoculated with the same isolate proved its consistency. Interestingly, though not surprising because of the short day-length latitude of the country in which the field trial was conducted, the allele effect was in the same direction as that from DLA under short day-length, i.e., decreasing resistance. Good agreement between disease development in detached leaves and in whole plants has been reported previously (Miller et al. 1998; Lebreton et al. 1999). Likewise, using rankings, levels of resistance of clones by detached leaf assay have been comparable with those from field trials (Stewart et al. 1983; Dorrance and Inglis 1997).

The fact that the map used was based on marker alleles segregating only from the original *S. berthaultii* parent may also account for the lack of detection of additional QTL, particularly those derived from the *S. tuberosum* parents. It seems that the wild parent B11B, which showed the highest levels of resistance in this study, did not pass its best alleles in terms of positive and high effects on foliar resistance to its offspring, the interspecific hybrid M200-30, unless they were of a recessive effect, as no additional alleles with higher effects at other QTL were detected segregating from this parent.

Significant opposite effects of the detected allele under different day-length regimes indicated a differential response to day-length. QTL × environment interaction is an important property of many QTL, and one of the causes of inconsistency of QTL detection across environments. Differential expression of a QTL for foliage resistance to late blight under different day-length regimes has been suggested to be associated with a physiological response (Swiezynski 1990). At the molecular level, this is supported by the common concurrence of QTL for late blight resistance and foliage maturity type in potatoes (van Eck and Jacobsen 1996; Collins et al. 1999; Oberhagemann et al. 1999). However, our finding of a genotype ×

isolate × day-length interaction suggests specificity of the allele to the isolate and its unstable response to day-length as the result of an interaction effect between the isolate and photoperiod. Contrary to the theory of the non-specificity of polygenes, isolate-specific QTL for field resistance to late blight have been identified (Leonards-Schippers et al. 1994). Even in other pathogen systems, like pepper/*Potyvirus* (Caranta et al. 1997), barley/*Pyrenophora graminea* (Arru et al. 2003), and sunflower/*Phoma macdonaldii* (Abou-Alfadil et al. 2007), clear isolate-specificity of QTLs has been observed.

Since quantitative relationships among potato genotype, *P. infestans,* and day-length in the expression of stability of partial resistance to late blight have not previously been analyzed in a single study, individual explanations for either genotype × isolate or genotype × day-length interactions have been proposed (Umaerus et al. 1983; Swiezynski 1990), most of which have been well supported and clarified later by molecular mapping (Visker et al. 2003; Oberhagemann et al. 1999; Simko 2002). The present results and those cited above corroborate previous hypotheses about the occurrence in the nature of several types of partial resistance. Bradshaw (2009) proposed the occurrence of three types of partial resistance: (1) Field resistance associated with late maturity; (2) Field resistance that displays resistance by isolate interactions; and (3) Field resistance that does not yet display resistance by isolate interactions. The partial resistance found in this study would belong to the second type as suggested by both the genotype × isolate × day-length interaction and the significant opposite effect of the detected allele against the differential behavior of the isolate under contrasting day-length regimes. Though most of QTL identified for resistance to *P. infestans* (reviewed in Simko 2002) can fit type of resistance number three, the QTL detected in this study and that reported in diploid potato lines by means of interval mapping in Leonards-Schippers et al. (1994) fit type of resistance number two. However, there is clear evidence of isolate-specific QTL in other pathosystems (Arru et al. 2003; Abou-Alfadil et al. 2007).

From the results of this study, it is concluded that differences in the isolate behavior under different photoperiods might also account for the instability of partial resistance to late blight. Similar studies as the present in which the genotype, isolate, and environment are analyzed together should be performed to corroborate this hypothesis. A primary concern of mapped QTLs that might become candidates for marker-assisted selection is the possible expression of environmental instability. Contrasting with major *R* genes, which are stable but not durable, isolate-specific QTL, though durable, would be unstable. The small contribution of genotype by isolate interaction to resistance variation found in most studies suggests that isolate-specific QTL are uncommon or otherwise display smaller effects than those without isolate-specificity. Hence, candidates for marker-assisted selection should focus on QTL common for partial resistance to different isolates.

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References

Abou-Alfadil T, Poormphammad K, Dechamp-Guillaume L, Gentzbittel L, Sarrafi A (2007) QTL mapping of partial resistance to Phoma basal stem and root necrosis in sunflower (*Helianthus annus* L.). Plant Sci 172(4):815–823

Arru L, Francia E, Pecchioni N (2003) Isolate-specific QTLs of resistance to leaf stripe (*Pyrenophora graminea*) in the'Steptoe' × 'Morex' spring barley cross. Theor Appl Genet 106:668–675

Bonierbale MW, Plaisted RL, Pineda O, Tanksley SD (1994) QTL analysis of thricome-mediated insect resistance in potato. Theor Appl Genet 87:973–987

Bradshaw JE (2009) Breeding for field resistance to late blight of potato at SCRI. Acta Hortic 834:87–100

Caranta C, Lefebvre V, Palloix A (1997) Polygenic resistance of pepper to potyviruses consists of a combination of isolate specific and broad-spectrum quantitative trait loci. Mol Plant Microbe Interact 10:828–878

Carlisle DJ, Cooke LR, Watson S, Brown AE (2002) Foliar aggressiveness of Northern Ireland isolates of *Phytophthora infestans* on detached leaflets of three potato cultivars. Plant Pathol 51:424–434

Churchill GA, Doerge RW (1994) Empirical threshold for quantitative mapping. Genetics 138:963–971

Collins A, Milbourne D, Ramsay L, Meyer R, Chatot-Balandras C, Oberhagemann P, de Jong W, Gerbhardt C, Bonnel E, Waugh R (1999) QTL for field resistance to late blight in potato are strongly correlated with maturity and vigour. Mol Breed 5:387–398

Colon L (1994) Resistance to *Phytophthora infestans* in *Solanum tuberosum* and wild *Solanum* species. PhD thesis. Wageningen Agricultural University, 159 p. Ponsen & Looijen, Wageningen, Netherlands. ISBN 90_5485_226_7

Dorrance AE, Inglis DA (1997) Assessment of greenhouse and laboratory screening methods for evaluating potato foliage for resistance to late blight. Plant Dis 81:1206–1213

Ewing EE, Simko I, Smart CD, Bonierbale MW, Mizubuti ESG, May GD, Fry WE (2000) Genetic mapping from field tests of qualitative and quantitatve resistance to *Phytophthora infestans* in a population derived from *Solanum tuberosum* and *Solanum berthaultii*. Mol Breed 6:25–36

Flier WG, van den Bosch GBM, Turkensteen LJ (2003) Stability of partial resistance in potato cultivars exposed to aggressive strains of *Phytphthora infestans*. Plant Pathol 52:326–337

Forbes GA (1999) Genotype by environment reaction of potato to the late blight pathogen. In: Impact on a changing world: Program report, 1997–98. International Potato Center (CIP), Lima, pp 57–66

Forbes GA, Tolstrup K (1999) Genotype × environment study. In: Crissman L, Lizarraga C (eds) Late blight: a threat to global food security, vol 1, Proceedings of the global initiative on late blight

(GILB) conference, vol 1. 16–19 March 1999, Quito, Ecuador. International Potato Center (CIP) Lima, Peru, pp 57–58
Forbes GA, Chacón G, Kirk HG, Huarte M, Damme MV, Distel S, Capezio S, Mackay G, Stewart H, Lowe R, Duncan J, Mayton H, Fry WE, Andrivon D, Ellisèche D, Pellé R, Platt H, MacKenzie G, Tarn R, Colon LT, Budding DJ, Lozoya-Saldaña H, Hernandez-Vilchis A (2005) Stability of resistance to *Phytophthora infestans* in potato: an international evaluation. Plant Pathol 54:364–372
Grainger J (1956) Host nutrition and attack by fungal parasites. Phytopathology 46:445–456
Haynes KG, Lambert DH, Christ BJ, Weingartner DP, Douches DS, Backlund JE, Secor G, Fry W, Stevenson W (1998) Phenotypic stability of resistance to late blight in potato clones evaluated at eight sites in the United States. Am J Potato Res 75:211–217
Inglis DA, Johnson DA, Legard DE, Fry WE, Hamm PB (1996) Relative resistances of potato clones in response to new and old populations of *Phytophthora infestans*. Plant Dis 80:575–578
James RV, Fry WE (1983) Potential for *Phytophthora infestans* populations to adapt to potato cultivars with rate-reducing resistance. Phytopathology 73:984–988
Jiang C, Zeng ZB (1995) Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics 140:1111–1127
Landeo JA, Gastelo M, Roncal E, Mendoza HA (2000) Phenotypic stability for horizontal resistance to potato late blight in population B. Am J Potato Res 77:406
Lander E, Green P, Abrahamson J, Barlow A, Daley M, Lincoln S, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181
Latin RX, Mackenzie DR, Cole Jr H (1981) The influence of host and pathogen genotypes on the apparent infection rates of potato late blight epidemics. Phytopathology 71:82–85
Lebreton L, Lucas JM, Andrivon D (1999) Aggressiveness and competitive fitness of *Phytophthora infestans* isolates collected from potato and tomato in France. Phytopathology 89:679–686
Leonards-Schippers C, Gieffers W, Schaferpregl R, Ritter E, Knapp SJ, Salamini F, Gebhardt C (1994) Quantitative resistance to *Phytophthora infestans* in potato: a case study for QTL mapping in an allogamous plant species. Genetics 137:67–77
Littell RC, Milliken GA, Stroup WN, Wolfinger RD (1996) SAS System for Mixed Models. SAS Institute Inc., Cary, p 633
Miller JS, Johnson DA, Hamm PB (1998) Aggressiveness of isolates of *Phytophthora infestans* from the Colombia Basin of Washington and Oregon. Phytopathology 88:190–197
Nelson RR (1979) The evolution of parasitic fitness. In: Horsfall JB, Cowling EB (eds) Plant disease: an advanced treatise, vol 4. Academic Press, New York, pp 23–46
Oberhagemann P, Chatot-Balandras C, Schäffer-Pregl R, Wegener D, Palomino C, Salamini F, Bonnel E, Gebhardt C (1999) A genetic analysis of quantitative resistance to late blight in potato. Mol Breed 5:399–415
Peters RD, Plant HW, Hall R, Medina M (1999) Variation in aggressiveness of Canadian isolates of *Phytophthora infestans* as indicated by their relative abilities to cause potato tuber rot. Plant Dis 83:173–178
Pohjakallio O, Salonen A, Antila S (1957) Analysis of earliness in potato. Acta Agric Scand 7:361–388
Rotem J, Kranz J, Bashi E (1983) Measurement of healthy and diseased haulm area for assessing late blight epidemics in potatoes. Plant Pathol 32:109–115
Sandbrink JM, Colon LT, Wolters PJCC, Stiekema WJ (2000) Two related genotypes of *Solanum microdontum* carry different segregating alleles for field resistance to *Phytophthora infestans*. Mol Breed 6:215–225
SAS Institute (2001) *SAS/STAT* user's guide, version 9.1. SAS Institute, Cary
Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. Phytopathology 67:1051–1056
Simko I (2002) Comparative analysis of quantitative trait loci for foliage resistance to *Phytophthora infestans* in tuber-bearing *Solanum* species. Am J Potato Res 79:125–132
Simon R, Bonierbale MW (2003) IntiMap—a program for comparing and publishing genomic maps. In: Plant and animal genome XI conference, San Diego, CA, USA, 11–15 January 2003. http://www.intl-pag.org/pag/11/abstracts/P10c_P894_XI.html
Stewart HE, Flavelle PH, Mc Calmont DC, Wastie RL (1983) Correlation between glasshouse and field tests for resistance to foliage blight caused by *Phytphthora infestans*. Potato Res 26:41–48
Swiezynski KM (1990) Resistance to *Phytphthora infestans* in potato cultivars and its relation to maturity. Genet Pol 31:99–106
Tooley PW, Fry WE (1985) Field assessment of fitness of isolates of *P. infestans*. Phytopathology 75:982–988
Trognitz B, Ghislain M, Crissman C, Hardy B (1996) Breeding potatoes with durable resistance to late blight. In: CIP circular 22(1):6–9. Lima, Peru
Turkensteen LJ (1993) Durable resistance of potatoes against *Phytophthora infestans*. In: Jacobs T, Parlevliet JE (eds) Durability of disease resistance. Kluwer, Dordrecht, pp 115–124
Umaerus V (1960) Some observations on field resistance to late blight. Sveriges Utsädesförenings Tidskrift (1–2):59–89
Umaerus V, Umaerus M, Erjefalt L, Nilsson BA (1983) Control of *Phytphthora infestans* by host resistance: problems and progress. In: Erwin DC, Bartnicki-Garcia S, Tsao PH (eds) Phytphthora: its biology, taxonomy, ecology and pathology. American Phytopathological Society, St Paul, pp 315–326
Umaerus V, Umaerus M (1994) Inheritance of resistance to late blight. In: Potato Genetics, JE Bradshaw and GR Mackay (eds) CAB International, UK, pp 365-401
van den Berg JH, Ewing EE, Plaisted RL, McMurry S, Bonierbale MW (1996a) QTL analysis of potato tuberization. Theor Appl Genet 93:307–316
van den Berg JH, Ewing EE, Plaisted RL, McMurry S, Bonierbale MW (1996b) QTL analysis of potato tuber dormancy. Theor Appl Genet 93:317–324
van Eck HJ, Jacobsen E (1996) Application of molecular markers in the genetic analysis of quantitative traits. In: Struik PC, Hoogendoorn J, Kouwenhoven JK, Mastenbtoek LJ, Turkensteen LJ, Veerman A Vos J (eds) Abstracts of conference papers of the EAPR. European Association for Potato Research, Wageningen, pp 130–131
van Ooijen JW (1992) Accuracy of mapping quantitative trait loci in autogamous species. Theor Appl Genet 84:803–811
Vision TJ, Brown DG, Shmoys DB, Durres RT, Tanksley SD (2000) Selective mapping: a strategy for optimizing the construction of high-density linkage maps. Genetics 155:407–420
Visker MHPW, Keizer LCP, van Eck HJ, Jacobsen E, Colon LT, Struik PC (2003) Can the QTL for late blight resistance on potato chromosome 5 be attributed to foliage maturity type? Theor App Genet 106:317–325
Visker MHPW, van Raaij HMG, Keizer LCP, Struik PC, Colon LT (2004) Correlation between late blight resistance and foliage maturity in potato. Euphytica 137:311–323
Vleeshouwers VG, van Dooijeweert W, Keizer LCP, Sijpkes L, Govers F, Colon LT (1999) A laboratory assay for *Phytophthora infestans* resistance in various *Solanum* species reflects the field situation. Eur J Plant Pathol 105:150–241
Vleeshouwers VG, van Dooijeweert W, Govers F, Kamoun S, Colon LT (2000) The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. Planta 210:853–864

Wang S, Basten CJ, Zeng ZB (2001) Windows QTL Cartographer v2.5. Department of Statistics, NCSU, Raleigh, NC. http://statgen.ncsu.edu/brcwebsite/home.php

Werner HO (1940) Response of two clonal strains of Triumph potatoes to various controlled environments. J Agric Res 61:761–790

Wulff EG, Pérez W, Nelson RJ, Bonierbale M, Landeo JA, Forbes GA (2007) Identification of stable resistance to *P. infestans* in potato genotypes evaluated in field experiments in Peru. Exp Agric 43:353–363

Yan W, Falk DE (2002) Biplot analysis of host-by–pathogen data. Plant Dis 86(12):1396–1401

Zeng ZB (1993) Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976