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ORIGINAL PAPER

Major-effect QTLs for stem and foliage resistance to late blight in the wild potato relatives *Solanum sparsipilum* and *S. spegazzinii* are mapped to chromosome X

Sarah Danan · Jean-Eric Chauvin · Bernard Caromel · Jean-Denis Moal · Roland Pellé · Véronique Lefebvre

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Abstract To find out new resistance sources to late blight in the wild germplasm for potato breeding, we examined the polygenic resistance of *Solanum sparsipilum* and *S. spegazzinii* by a quantitative trait locus (QTL) analysis. We performed stem and foliage tests under controlled conditions in two diploid mapping progenies. Four traits were selected for QTL detection. A total of 30 QTLs were mapped, with a large-effect QTL region on chromosome X detected in both potato relatives. The mapping of literature-derived markers highlighted colinearities with published late blight QTLs or R-genes. Results showed (a) the resistance potential of *S. sparsipilum* and *S. spegazzinii* for late blight control, and (b) the efficacy of the stem test as a complement to the foliage test to break down the complex late blight resistance into elementary components. The relationships of late blight resistance QTLs with R-genes and maturity QTLs are discussed.

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S. Danan · B. Caromel · V. Lefebvre (✉)
INRA, UR 1052 GAFL Génétique et Amélioration des Fruits et Légumes, BP 94, 84140 Montfavet, France
e-mail: veronique.lefebvre@avignon.inra.fr

J.-E. Chauvin · J.-D. Moal · R. Pellé
INRA, UMR 118 APBV Amélioration des Plantes et Biotechnologies Végétales, 29260 Ploudaniel, France

Present Address:
J.-D. Moal
Comité Nord, Station de recherche de Bretteville, 76110 Bretteville du Grand Caux, France

Abbreviations

AFLP	Amplified fragment length polymorphism
CAPS	Cleaved amplified polymorphic sequence
DPI	Days post-inoculation
PCA	Principal component analysis
QTL	Quantitative trait locus
rAUDPC	Time-relative area under the disease progress curve
RFLP	Restriction fragment length polymorphism
RGA	Resistance gene analogue
SCAR	Sequence characterized amplified region
SD	Standard deviation
SSR	Simple sequence repeat
STS	Sequence tagged site

Introduction

Strict environmental regulations on the application of chemicals for pest and disease control lead to promote plant breeding for disease resistance as the most environmentally friendly and effective way to limit pathogen epidemics. Two kinds of genetic resistances are often described in a dualistic way: monogenic resistance controlled by R-genes, and polygenic resistance (also called quantitative, partial or field resistance) controlled by quantitative trait loci (QTLs). Monogenic resistances are often rapidly overcome by new virulent strains. Deployment of new R-genes as soon as new virulent strains arise can thus become a very costly and endless cycle. One solution to increase both durability and level of resistance is pyramiding several R-genes by molecular-assisted breeding (Tan 2008) or by cisgenesis (Haverkort et al. 2008). Another promising solution is to exploit the polygenic resistance of the available gene pool,

either alone or in combination with R-genes (Solomon-Blackburn et al. 2007). Polygenic resistance, characterized by a quantitative phenotypic expression, has been suggested to be stable and durable (Van der Plank 1968; Parlevliet 1979; Lindhout 2002). However, the phenotypic assessment of polygenic resistance is complex and, to promote the use of this resistance in breeding programmes, as well as to better understand its molecular basis, one proposed method is to break down the general resistance into elementary phenotypic components which can be accurately evaluated in a reproducible manner. Two subdivision levels of the complex resistance are often considered: the plant organ and the disease stage. In potato, for example, the foliage and the tuber resistances were assessed in the same plant materials with two different tests (Bradshaw et al. 2006b). In cereals and in poplar, partial resistance to rust was subdivided in key-epidemiological components as infection frequency, rate of spore production, latent period and spore lesion size (Parlevliet and Ommeren 1975; Dowkiw et al. 2003). Most of the time, these elementary components are assessed under controlled environment. By having a low environmental variance, they may thus accurately subdivide the genetic variance. Chosen to be weakly correlated, these complementary elementary components would thus help to detect the controlling loci by highlighting the genomic regions which are specific to an organ resistance or to an infection stage. In addition, the break-down of complex resistances could help breeders to construct new ideotypes by associating organ- or stage-specific resistance QTLs, which would increase or stabilize the resistance level.

Potatoes (*Solanum tuberosum*) rank third in the world's most important crops. Up to 30% of total expected yield is lost every year due to late blight, the most devastating potato disease caused by the oomycete *Phytophthora infestans* (Haverkort et al. 2008). Symptoms of late blight are observable on the whole plant. Lesions first appear on the foliage. Then, in a humid environment, the pathogen produces spores on the underside of the leaflet and lesions develop on stems under the foliar canopy, which enables the pathogen to persist in the plant even during dry conditions. In addition, large amounts of inoculum develop in the stem and become sources of infection for potato tubers by water running down the stem. Stem lesions and infection of plant apex have increased with new strains of *P. infestans* and are especially dangerous because stems are difficult to protect with fungicides (Weingartner 1997; Roberts et al. 2007). Moreover, large amounts of fungicides have been spread over potato fields worldwide and this polluting and costly method tends to select strains resistant to chemicals.

Since the nineteenth century, many R-genes have been identified in wild potato relatives and introgressed into cultivated potato cultivars. Eleven race-specific dominant R-genes (*R1* to *R11*) were identified in *S. demissum* (Müller and Black 1952) but their extensive deployment resulted in the loss of their efficiency (Turkensteen 1993). Additional R-genes have been mapped in other wild potato-related species (reviewed in Wang et al. 2008) but they might also be rapidly overcome by fast-evolving *P. infestans* strains. In fact, the common *P. infestans* A1 mating type has progressively been replaced by a mixture of A1 and A2 mating types in most potato growing areas. As sexual reproduction can now occur, the recombination events have increased and new virulent strains appear in short time (Spielman et al. 1991; Fry et al. 1993).

Simultaneously, intensive research has been conducted on polygenic resistance in wild potato relatives (Leonards-Schippers et al. 1994; Meyer et al. 1998; Collins et al. 1999; Oberhagemann et al. 1999; Ewing et al. 2000; Sandbrink et al. 2000; Ghislain et al. 2001; Kuhl et al. 2001; Trognitz et al. 2002; Bormann et al. 2004; Bisognin et al. 2005; Costanzo et al. 2005; Villamon et al. 2005; Visker et al. 2005; Simko et al. 2006; Sorensen et al. 2006; Sliwka et al. 2006, 2007). QTLs have been mapped on all 12 chromosomes, with clusters on chromosomes IV, V and XI, colocalizing with already reported R-genes (Gebhardt and Valkonen 2001). The relationships between QTLs and R-genes are still unclear and further research is needed to decipher their possible links. Some QTLs for late blight resistance were also mapped in the same regions as QTLs for plant maturity, such as on chromosome V. In fact, it has been observed that the most resistant potato plants often mature the latest, which is an undesirable association for breeders. A few studies have attempted to find out the genetic relationship between polygenic resistance to late blight and maturity but their association is still not clearly understood (Van Eck and Jacobsen 1996; Collins et al. 1999; Visker et al. 2003, 2005; Bormann et al. 2004; Bradshaw et al. 2004; Simko et al. 2006; Sliwka et al. 2007).

To assess late blight polygenic resistance in potato, several methods have been used on different plant organs: detached leaflet test, field foliage test, whole tuber or tuber slice tests. The stem resistance has been overlooked in the past, and little is known on the actual resistance potential of this organ, neither on its genetic control. However, variability for the stem resistance to late blight was reported (Glendinning 1989; Dorrance and Inglis 1997; Chauvin and Pellé, unpublished data). To get insight in the stem resistance, the stem resistance assessment, as achieved on pepper for *Phytophthora capsici* (Lefebvre and Palloix 1996), may bring additional valuable information about potato polygenic resistance to late blight.

The present study reports the investigation of polygenic late blight resistance in the diploid *S. sparsipilum* and *S. spegazzinii*-derived progenies. Two independent resistance tests were performed under controlled environmental conditions, on stems and foliage. A selection of literature-derived markers made it possible the comparison of several significantly detected QTLs with published QTLs and R-genes for late blight resistance. Relationships with published maturity QTLs are also discussed.

Materials and methods

Plant material

The *S. sparsipilum* PI310984-derived clone (SPL) and *S. spegazzinii* PI208876-derived clone (SPG) are wild potato-related clones used as *P. infestans* resistant genitors in our study. Two potato diploid mapping progenies, 96D31 and 96D32, were obtained by crossing SPL and SPG with the susceptible dihaploid *S. tuberosum* clones Caspar H3 (CASP) and Rosa H1 (ROSA), respectively (Caromel et al. 2003, 2005). CASP might contain R-genes, especially *R1* and *R3*, but no information is available concerning ROSA (H. van Eck, personal communication). The phenotyping of late blight resistance was achieved on sub-groups of 93 and 116 clones for the stem test and on sub-groups of 96 and 114 clones for the foliage test, for the 96D31 and 96D32 progenies, respectively.

Molecular data

The parental genetic maps were generated using AFLP, CAPS, RFLP, STS and SSR markers as described in Caromel et al. (2003, 2005). The LG24 and LG26 linkage groups of SPL were not assigned to a known chromosome. Table 1 specifically reports the CAPS, RFLP, STS and SSR markers mapped in the vicinity of reported R-genes and in the confidence interval of literature QTLs for late blight resistance. We added SSR markers "STM", "SSR", "Sti" (Milbourne et al. 1998; Bryan et al. 2004; Feingold et al. 2005; Frary et al. 2005) and STPoAC58 (Drouin and Dover 1990), and the CAPS markers TG495 (Bradeen et al. 2003), CT11, CT214, CD5 (Rauscher et al. 2006), P1433 (Park et al. 2005a), TG313 (SGN database 2005), TPT, CP287, BA66k2 (Sliwka et al. 2007), TG303 (Yamanaka et al. 2005), Rca, Glo, Pk (Chen et al. 2001) and P8h11 (Gebhardt et al. 2003). Sets of 115, 106, 121 and 112 markers constituted the framework maps of the parents CASP, SPL, ROSA, and SPG, respectively, with an average inter-marker distance of 8.0 cM (SD ± 3.2), 8.0 cM (SD ± 2.3), 10.0 cM (SD ± 1.8) and 9.0 cM (SD ± 0.9), respectively.

Indoor stem test

Six tubers per clone were used for the stem test. The susceptible cultivar 'Bintje' and the partially resistant cultivars 'Robijn' and 'Möwe' were included as controls. The stem resistance test was performed in a greenhouse in controlled conditions. After a 4-week pre-germination phase, tubers were planted into 0.5 L pots (100% peat) during the spring and placed in a greenhouse with 20/15°C day/night temperatures. High humidity was maintained by artificial mist. Only one aerial stem per tuber was kept. After about 5 weeks and before flowering, the stem test protocol was applied as described in Lefebvre and Palloix (1996). As soon as the plant apex was cut, a plug of *P. infestans* mycelium was put on the fresh surface and covered with a piece of aluminium foil to ensure infection. The *P. infestans* NII72.10 isolate collected on *S. tuberosum* 'Naturella' (*R2* gene, Ploudaniel, France) was used. It was of A1 mating type and of complex race (1, 2, 4, 10, 11). Its mycelium was grown on pea juice-based agar medium for 10 days (Glais and Corbière 2005). Necrosis lengths were scored every week (at 5- to 9-day intervals) over a period of 6.5 weeks (dpi).

Six necrosis length traits were obtained (Ldpi in mm): L5, L12, L19, L26 for both progenies, L33 and L40 for 96D31, and L35 and L43 for 96D32. The speed of the necrosis progress at the different scoring dates were calculated according to the formula $\text{Sdpi} = [\text{L}(\text{dpi}') - \text{L}(\text{dpi})]/t_{i'} - t_i$ (in mm day^{-1}), where t_i and $t_{i'}$ are the number of days post-inoculation at two consecutive scoring dates. Inducibility (IND in mm day^{-2}) was calculated with the formula IND = [(S33 or S35) − S19]/(14 or 16, respectively), and stability was calculated with the formula STA = mean(S26, S33 or S35, S40 or S43) (in mm day^{-1}). Three traits actually represent the different stages of the plant defence mechanisms which are progressively activated: receptivity (REC), the plant's necrosis speed 5 days after inoculation (=S5), corresponds to the plant's capacity to be infected; inducibility (IND) corresponds to the plant's speed in triggering its defence mechanisms; stability and the final necrosis lengths correspond to the plant's capacity to maintain its resistance over time (Lefebvre and Palloix 1996).

Outdoor foliage test

Four tubers per clone were used for the foliage test. The standard controls r0 and R1–R11 differential hosts were included. The foliage test was performed outdoors where several environmental parameters were controlled. After a 4-week pre-germination phase, tubers were planted into 5 L containers (50% peat, 35% sand and 15% pine bark) and placed outdoors on a concrete surface in May 2004

Table 1 Literature-derived markers tagging late blight resistance loci that were mapped in the 96D31 and 96D32 parental maps

Marker	Reference or primer sequences	Type of polymorphism (restriction enzyme)	Parental map where the marker mapped	Literature colinear QTLs or *R*-genes
57t3	Bormann et al. (2004)	CAPS (*AluI*)	VIII ROSA, VIII CASP, VIII SPG	QTL (Bormann et al. 2004)
CT287	Tanksley et al. (1992)	RFLP	VIII ROSA, VIII SPG	QTL (Collins et al. 1999)
CT88-3	van der Vossen et al. (2003) F: GGCAGAAGAGCTAGGAAGAG; R: ATGGCGTGATACAATCCGAG	CAPS (*AluI*)	VIII ROSA, VIII SPL	*Rpi-blb1* (van der Vossen et al. 2003), *RB* (Naess et al. 2000)
CD5	Rauscher et al. (2006)	RFLP	X ROSA, X SPG	QTL (Villamon et al. 2005)
GP21	Gebhardt et al. (1989)	RFLP	V CASP, V SPL, V SPG	QTL (Leonards-Schippers et al. 1994; Collins et al. 1999; Oberhagemann et al. 1999; Ghislain et al. 2001; Visker et al. 2003; Visker et al. 2005)
GP179	Gebhardt et al. (1989)	RFLP	V SPL	QTL (Leonards-Schippers et al. 1994; Collins et al. 1999; Oberhagemann et al. 1999; Bormann et al. 2004)
Lernalx	Milbourne et al. (1998)	SSR	V SPL	QTL (Collins et al. 1999)
RB	Millett and Bradeen (2007) RB-f/ INDEL-r	CAPS (*DraI*)	VIII CASP, VIII ROSA	*RB* (Millett and Bradeen 2007)
STM1049	Milbourne et al. (1998)	SSR	I CASP, I SPL	QTL (Collins et al. 1999)
STM1057	Milbourne et al. (1998)	SSR	VIII CASP	QTL (Collins et al. 1999)
STM1105	Milbourne et al. (1998)	SSR	VIII ROSA, VIII SPG	QTL (Collins et al. 1999)
STM2030	Milbourne et al. (1998)	SSR	I ROSA, I SPG	QTL (Collins et al. 1999)
STM3015	Milbourne et al. (1998)	SSR	VIII CASP, VIII ROSA	QTL (Collins et al. 1999)
STM3016	Milbourne et al. (1998)	SSR	IV ROSA, IV SPG	QTL (Meyer et al. 1998; Collins et al. 1999; Bormann et al. 2004)
STM3160	Bradshaw et al. (2004)	SSR	IV SPG	QTL (Bradshaw et al. 2004)
STM5140	Bradshaw et al. (2004)	SSR	IV ROSA, IV CASP	QTL (Bradshaw et al. 2004; Bradshaw et al. 2006b)
STWAX-2	Ghislain et al. (2004)	SSR	VIII SPG	QTL (Oberhagemann et al. 1999)
TG63	Rauscher et al. (2006)	STS/CAPS (*MboI*)	X CASP, X SPG	QTL (Sliwka et al. 2007)
TPT	Sliwka et al. (2007)	STS	X CASP, X ROSA	QTL (Sliwka et al. 2007)
TG28	Tanksley et al. (1992)	RFLP	XII ROSA	QTL (Villamon et al. 2005)
TG497	Tanksley et al. (1992)	RFLP	XI ROSA, XI SPG	QTL (Ewing et al. 2000; Villamon et al. 2005)
TG123	Tanksley et al. (1992)	RFLP	IV ROSA, IV SPG	QTL (Leonards-Schippers et al. 1994; Oberhagemann et al. 1999)
TG45	Tanksley et al. (1992)	RFLP	VIII ROSA, VIII SPG	QTL (Collins et al. 1999)

(96D32) and May 2005 (96D31). The trial had a randomized block design with four blocks, one replicate per clone in each block. Each plant was separated from its neighbours by a 50 cm interval. Plants were naturally infected by a local aerial isolate. Thanks to r0 and R1–R11 differential hosts, the isolate nature was determined as a mixture of complex races with virulence genes 1, 2, 3, 4, 6, 7, 10, 11. The susceptible control 'Bintje' plants were placed one every two rows of tested clones throughout the trial to ensure a homogeneous *P. infestans* spread. The homogeneous behaviour of the differential hosts through the trial suggests that the mixture of races was homogeneous on the assay. Plants were regularly watered by sprinklers to maintain a local humid climate in the experimental area. Foliage scoring was done weekly on all the plants of the trial for 5 weeks. Scoring began as soon as the first late blight spot was detected on 'Bintje' and ended when all the plants were senescent. Disease scores corresponded to the proportion of infected leaves per plant, according to James (1971). Values of time-relative area under the disease progress curve (rAUDPC) were calculated by applying the formula $\Sigma[(y_i + y_{i'})/2] \times [(t_{i'} - t_i)]/(100 \times T_{\text{tot}})$, where y_i and $y_{i'}$ are the percentages of foliage destruction areas at the two consecutive scoring dates t_i and $t_{i'}$, respectively, and T_{tot} is the total vegetation time of the plant.

Data analysis

Statistical analyses were performed using the general statistical software package RGUI version 2.4.0 (R Development Core Team 2005). Concerning the foliage test, to take into account the effect of heterogeneity of pathogen dispersal on the scored values, an analysis of covariance (ANCOVA) was performed for rAUDPC with the values of the adjacent 'Bintje' plants taken as covariate. rAUDPC adjusted fitted values of each scored plant were extracted from the ANCOVA analysis and were used for subsequent analysis.

For all traits, the replicate median was calculated for each clone and used for QTL analyses. The median better represents the central tendency of a small number of observations and is less influenced by extreme values due to environmental factors than the arithmetic mean. The Mann–Whitney test was used to compare parental values (P value <0.05). Within each progeny, the Pearson correlation test was used for trait associations (P value <0.05). Broad-sense heritability was calculated for each trait after testing the "clone" effect by analysis of variance (ANOVA) for the stem traits and by ANCOVA for the foliage trait rAUDPC, by applying the formula $h^2_{\text{BS}} = \sigma^2_{\text{g}}/[\sigma^2_{\text{g}} + (\sigma^2_{\text{e}}/n)]$, where σ^2_{g} is genetic variance, σ^2_{e} is environmental variance and n the number of replicates per clone (6 replicates for the stem test and 4 for the foliage test). The normality of the distribution of the studentized residuals was checked with the Shapiro and Wilk test (P value <0.05). To determine the traits that explained most of the observed variation and which were as much complementary as possible, we performed a principal component analysis (PCA).

QTL mapping and epistatic interactions

The QTL detection was performed on the medians of the clones for all selected traits using interval mapping (IM) and composite interval mapping (CIM) as implemented in the QTLCartographer software (Basten et al. 1997). Markers showing high F statistics (F stat. >7) after the forward–backward stepwise regression analysis were taken as cofactors for the CIM procedure (P value ≤0.1). A maximum of ten cofactors were used for the CIM method, with a window size of 10 cM and a walking speed of 2 cM. Genome-wide significance levels were estimated by running 1,000-permutation tests for each trait. As the LOD thresholds of each trait were very close to each other (data not shown), we applied a mean CIM LOD threshold of 2.8 for all the traits in both progenies (for a type I error of 0.1). QTL confidence intervals were determined by the maximum LOD-1 projection of the IM LOD curve onto the chromosome length axis or the CIM LOD curve when QTLs were detected by the CIM method only. The graphic representation of the maps with the QTL confidence intervals was generated with MapChart 2.1 (Voorrips 2002). When the confidence intervals of several QTLs overlapped, we considered the whole region as a single QTL region, named "*Pi-*" for *P. infestans* followed by the potato chromosome in Roman numerals, and an Arabic numeral when there were several QTL regions per chromosome. The magnitude of the marker-associated phenotypic effect is given by the determination coefficient of the model (R^2). Global R^2 values were calculated for each selected trait by multiple-way ANOVA procedure using the markers which individually had the highest effects at each QTL.

For each progeny and each selected trait, digenic interactions of all marker pairs of the framework maps of both parents were tested using a two-way analysis of variance with an interaction component. Based on the number of tests, a signification threshold of $P < 10^{-5}$ was employed. Significant epistatic interactions were validated by comparing the number of clones for each genotype class to expected numbers (χ^2 test, $P < 0.05$). In the case of no significant skewing, the homogeneity of variances between classes was checked (Bartlett test, $P < 0.05$).

Table 2 Medians of the parental lines, mean of medians of the clones of the progeny, and broad-sense heritability values for the four stem and foliage traits used for QTL detection in 96D31 (a) and 96D32 (b) progenies

(a) Trait (96D31)	CASP median	SPL median	Parent diff.[a]	Progeny mean of clone medians	Broad-sense heritability (%)
REC	5.2	3.3	**	3.1	84
IND	0.0	0.4	*	0.0	72
L40	226.0	116.0	**	142.7	83
rAUDPC	0.3	NA[b]	NA	0.2	91
(b) Trait (96D32)	ROSA median	SPG median	Parent diff.[a]	Progeny mean of clone medians	Broad-sense heritability (%)
REC	3.3	3.2	NS	3.3	81
IND	−0.1	−0.3	**	−0.1	71
L35	244.5	107.5	**	172.6	84
rAUDPC	0.4	0.4	NS	0.4	78

NS non significant, *NA* no available data

[a] Significant difference between parental values, Mann–Whitney test significance levels: ** *P* value <1%, * *P* value <5%

[b] No value was available for SPL as the plants were too small to be assessed

Results

Trait selection and description

The 14 initial traits were reduced to four major traits: REC, IND, L40 for 96D31 and L35 for 96D32 for the stem test, and rAUDPC for the foliage test. First, these traits well represented the genetic variability as they had high heritability values in both progenies, ranging from 71 to 91% (Table 2). L35 and L40 particularly well explained the PCA first axis which itself explained about 43% of the overall phenotypic variability of the 14 initial traits. Second, these four traits were weakly correlated as it was shown by their quite well-distributed coordinates on the PCA correlation circle (Fig. 1). The limited redundancy between these traits was confirmed by the Pearson correlation values. Third, all four traits were normally distributed, except rAUDPC in 96D32 progeny which was slightly skewed towards susceptibility (Fig. 1). Parental clones displayed contrasting phenotypes and the means of progeny clones were generally flanked by parental means (Table 2). These four major traits were thus selected for QTL detection. REC, IND and L40/L35 notably corresponded to the three steps which represent the stages of the plant defence mechanisms, as described in "Materials and methods".

Anchor markers

On one hand, a total of 80 anchor markers, or up to 13 per chromosome, made it possible to align parental maps together (within or between progenies, Fig. 2). On the other hand, a total of 196 markers, or up to 31 per chromosome, also made it possible to anchor our maps to published potato maps and specifically in QTL regions (Table 1). No anchor marker was mapped on chromosomes VI of CASP, II of SPL, and on the LG26 linkage group of SPL, making it impossible for us to compare the locations of their QTLs with the ones described in literature.

QTL mapping and epistatic interactions

By using the four selected traits for QTL detection, 18 and 12 QTLs were detected with the CIM method in the 96D31 and 96D32 progenies, respectively (Table 3). Only the CIM QTLs were presented as, by taking cofactors into account in the analytical model, the CIM method made it possible the detection of several additional small-effect QTLs which were not detected with the IM method (Zeng 1994; Melchinger et al. 1998; Thabuis et al. 2003). Altogether, QTLs mapped on ten potato chromosomes and on the LG26 linkage group of SPL (Fig. 2). They individually explained between 4 and 29% of the phenotypic variance. No QTL was detected with the REC trait in the 96D32 progeny. The overlapping of QTL confidence intervals and the mapping of anchor markers in or around the QTL regions revealed 13 *Pi* QTL regions over the 4 parental maps. However, for certain chromosomes, alignments were relatively loose and additional anchor markers are needed to confirm apparent QTL colinearities.

Chromosome X displayed the most consistent QTL regions in terms of effect level and number of involved traits. *Pi-X* QTL regions are notably detected across the three parental maps, and especially in both resistant clones SPG and SPL. The SPG resistant clone comprised the largest-effect QTL, which was detected with the L35 stem

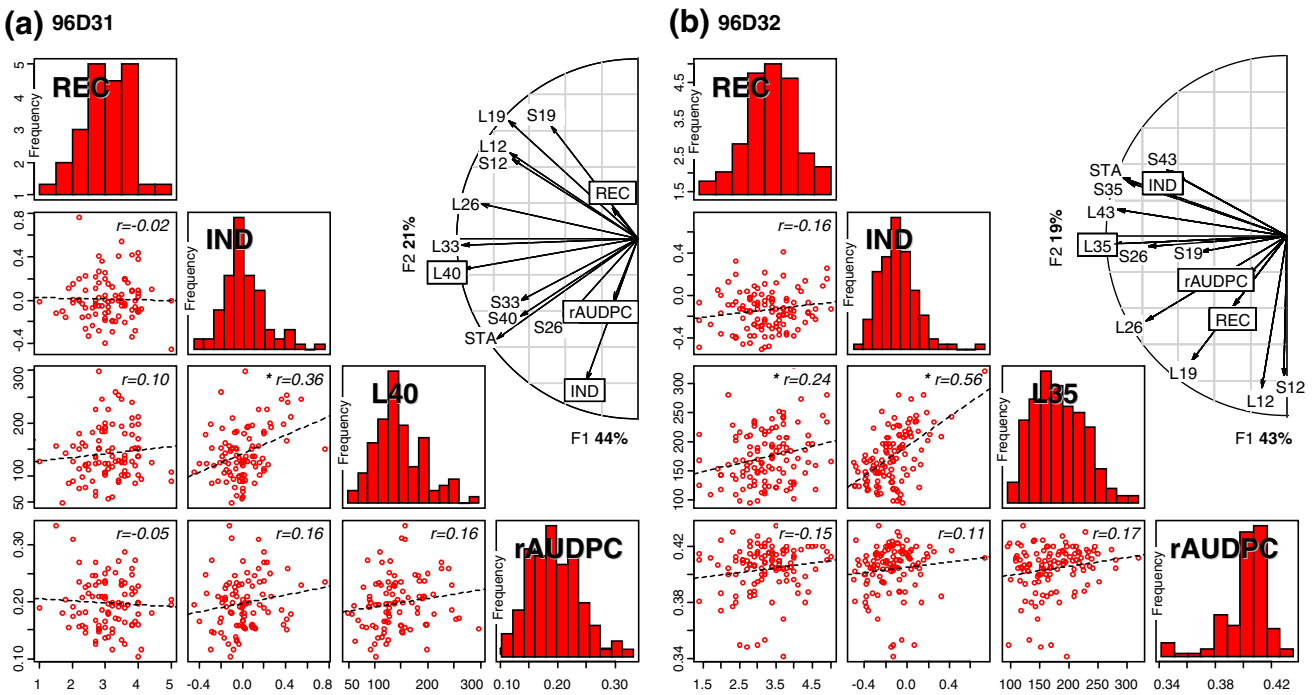

Fig. 1 Principal component analysis (*PCA*) correlation circles, frequency distribution histograms and scatter-plot matrix of stem and foliage traits for the 96D31 (**a**) and 96D32 (**b**) progenies. PCA *correlation circles* present the coordinates of the 14 initial traits. For setting reasons, only half of the circles were presented. The two main axis of the PCA circles explained altogether 65 and 62% of the phenotypic variation of the 14 traits in the 96D31 and 96D32 progenies, respectively. The four selected traits for QTL detection are framed. Frequency distribution histograms and scatter-plots are presented for the four selected traits only. Pearson correlation values between the four selected traits are indicated in the upper right-hand corner of the scatter-plots. An *asterisk* is added next to it in case of significant correlation

trait (R^2 = 29%, LOD = 14.7). Chromosome X of SPL comprised two distinct QTL regions: *Pi-X.1* detected with REC and *Pi-X.2* detected with IND and L40. In addition, these stem QTLs of both regions overlapped with distinct foliage QTLs in SPL. Smaller effect QTLs for IND and L35 stem traits were also detected in the colinear region of ROSA. The anchor marker CD5 shared by ROSA and SPG would suggest a nonalignment between *Pi-X* of ROSA and *Pi-X* of SPG. Conversely, the flanking SSR markers STM0051 and STM1040 shared by SPL and SPG would suggest the alignment of *Pi-X* of ROSA and *Pi-X* of SPG with *Pi-X.1* and *Pi-X.2* of SPL, respectively. Additional anchor markers are needed to make the QTL alignments clearer.

Chromosome I also displayed colinear QTL regions between the maps of both resistant parents, gathered under the *Pi-I.2* region. Whereas only stem QTLs were detected in SPG and SPL, the QTLs in CASP were detected with stem and foliage traits. Another QTL region, named *Pi-I.1* affecting stem traits, was mapped above *Pi-I.2* in CASP and SPL. For chromosome VIII, the *Pi-VIII* region comprised stem QTLs in the SPG resistant clone and foliage QTLs in both susceptible clones CASP and ROSA. That tight colinearity was notably deduced by the Sti027 and CT287 co-segregating anchor markers. For chromosome IX, the QTL regions of the three parental maps CASP, ROSA and SPG also appeared to be colinear but additional anchor markers are needed to confirm this assumption. All the other QTLs were detected on single parental maps of chromosomes II, III, IV, VI, XI and XII, with a single trait (either a stem trait or the foliage trait). All of them have relatively small effects. For the unassigned linkage group LG26 of SPL, a stem QTL of medium-effect colocalized with a foliage QTL.

Multiple regression (global R^2 calculation) revealed that the additive QTLs explained up to 81% (96D31) and 54% (96D32) of the phenotypic variance of the stem traits, and up to 73% (96D31) and 19% (96D32) of the phenotypic variance of the foliage trait (Table 3). No significant epistatic interaction was detected with any of the four traits selected for QTL detection.

Discussion

Two novel valuable sources of resistance to late blight

To our knowledge, it is the first time that polygenic resistance to late blight has been described in the wild relatives of cultivated potato *S. spegazzinii* and *S. sparsipilum*. These

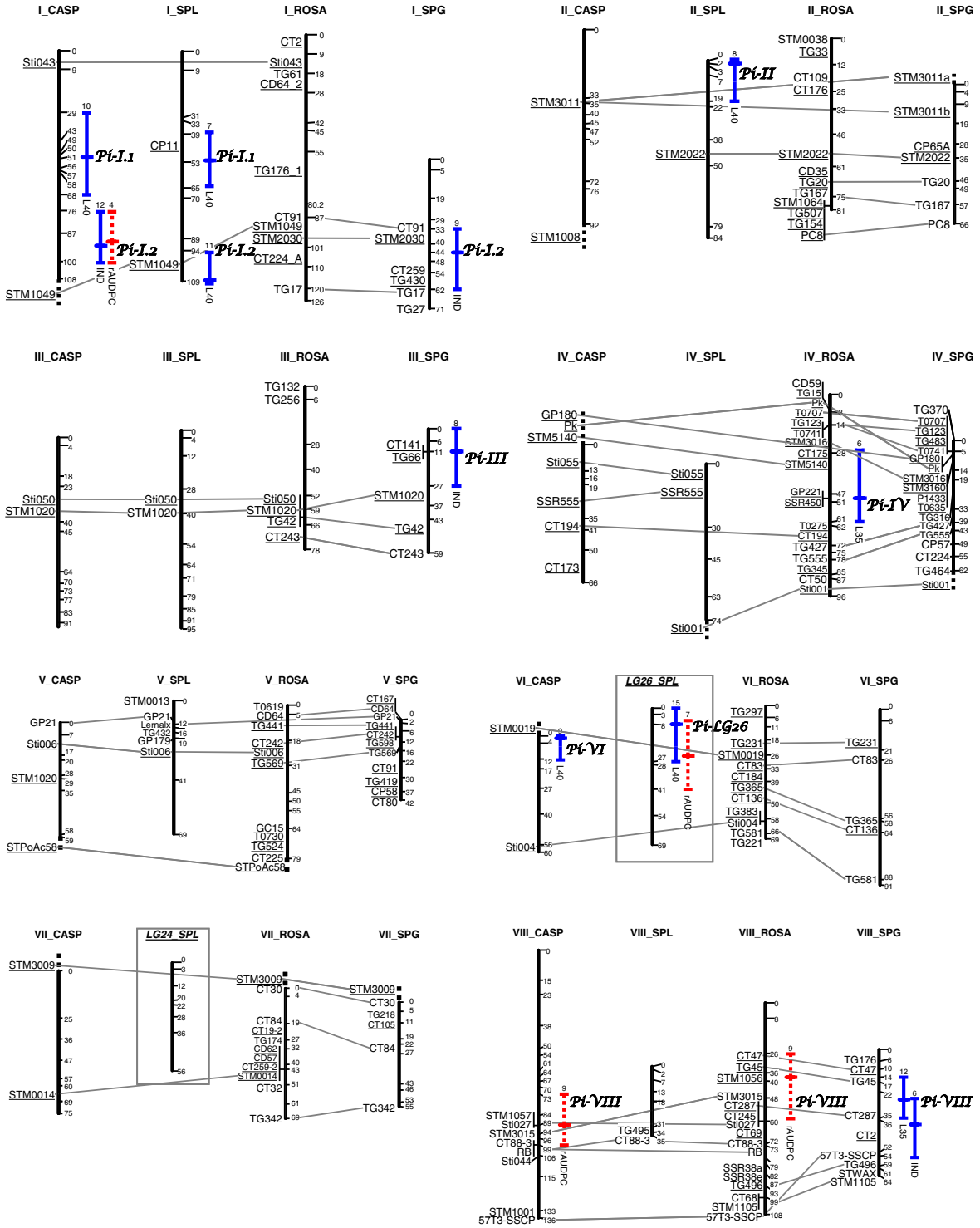

Fig. 2 Positions of quantitative trait loci (*QTLs*) on the parental maps of the 96D31 and 96D32 progenies detected with stem and foliage traits. Positions are given in cM (Kosambi units) to the right of the linkage groups. We indicated the literature-derived anchor markers to the left of the linkage groups. *Underlined markers* were not included in the framework maps used for QTL detection. Anchor markers shared by several chromosomes are linked with plain *grey lines*. The confidence intervals of QTLs are represented by *dotted lines* for foliage QTLs and by *plain lines* for stem QTLs. The position of the maximum LOD value is indicated with a *dash*. The R^2 value of each QTL and the related-trait are given at the top and the bottom of their confidence intervals, respectively. QTL regions are named "*Pi-*" for *P. infestans* followed by the potato chromosome concerned in Roman numerals, and an Arabic numeral when there are several QTL regions per chromosome. For setting reasons, the LG26 and LG24 linkage groups of SPL were arbitrary placed with chromosomes VI and chromosomes VII, respectively

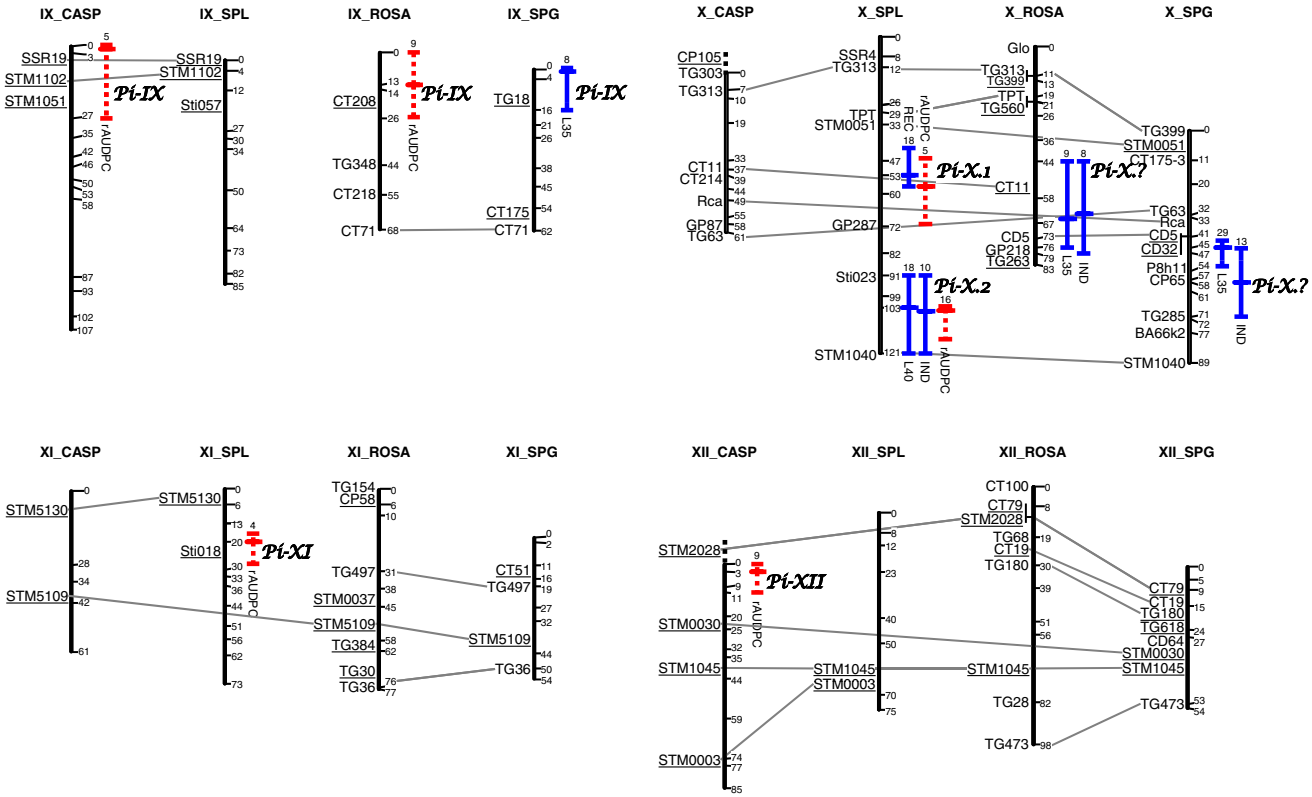

Fig. 2 continued

species confer valuable late blight resistance, especially for the stem resistance. QTLs were detected in both species, including a large-effect QTL region on chromosome X. Several genes and QTLs controlling resistance to the cyst nematodes *Globodera pallida* and *G. rostochiensis* have been mapped in *S. spegazzinii* (Kreike et al. 1993). Also, we used the same clones of *S. sparsipilum* and *S. spegazzinii* as the ones in which *G. pallida* resistance QTLs were detected (Kreike et al. 1994; Caromel et al. 2003; Caromel et al. 2005). These accessions can thus serve as valuable genitors in marker-assisted breeding programmes for multiple pathogen resistances.

The stem test: an innovative way to break down polygenic late blight resistance

Breeders noticed that potato plants were able to recover from completely late blight-damaged foliage thanks to their remaining healthy stems (Chauvin and Pellé, unpublished data). Despite the absence of data concerning the impact of the stem recovery on the yield, we assumed that stems play a central role in the infection process of potatoes and that the stem resistance component could have a significant potential in controlling late blight epidemics in the field. To further study the variability of the stem resistance to late blight, we applied the protocol designed for *P. capsici* resistance assessment in pepper (Lefebvre and Palloix 1996). As the stem test can be performed on young pot-plants, in a short time and under controlled conditions, it makes it possible to design large experiments and to yield accurate statistical data analysis.

In our study, stem QTLs were detected in 11 regions spread on 8 potato chromosomes and on the LG26 linkage group of SPL. Six out of the 11 QTL regions were also involved in foliage resistance. When foliage QTLs colocalized with stem QTLs (chromosomes I of CASP, X of SPL, and the LG26 linkage group of SPL), we observed equivalent LOD curves, either superimposed or slightly shifted. The stem test thus yields a reliable evaluation of the stem component of late blight resistance. In complement to already existing tests on tuber and leaves, the stem test can be considered as an efficient means of breaking down the complex resistance to *P. infestans* into organ-specific elementary components. The *Pi-X* QTL regions on chromosome X especially played an important role in stem resistance (R^2 up to 29%) and were also involved in foliage resistance. Comparisons with literature showed that the *Pi-X.2* QTL region, flanked by the Sti023 and STM1040 markers, also corresponds to QTLs involved in leaf, tuber, and to a lesser extent field resistance (Oberhagemann et al.

Table 3 QTL positions and magnitude for 96D31 (a) and 96D32 (b) progenies

	QTL name	Chrom.	Position (cM)	LOD Score	Locus R^2 (%)	Global R^2 (%)
(a) Trait (96D31)						
REC	Pi-X.1	X SPL	53	4.3	18	18
IND	Pi-I.2	I CASP	92	3.1	12	22
	Pi-X.2	X SPL	105	2.8	10	
L40	Pi-I.1	I CASP	50	4.7	10	81
	Pi-I.1	I SPL	52	3.5	7	
	Pi-I.2	I SPL	109	5.2	11	
	Pi-II	II SPL	2	4.0	8	
	Pi-VI	VI CASP	0	4.2	9	
	Pi-X.2	X SPL	103	8.1	18	
	Pi-LG26	LG26 SPL	8	6.8	15	
rAUDPC	Pi-I.2	I CASP	90	3.1	4	73
	Pi-VIII	VIII CASP	89	6.5	9	
	Pi-IX	IX CASP	0	4.1	5	
	Pi-X.1	X SPL	58	4.0	5	
	Pi-X.2	X SPL	103	10.9	16	
	Pi-XI	XI SPL	20	3.3	4	
	Pi-XII	XII CASP	3	6.8	9	
	Pi-LG26	LG26 SPL	24	4.9	7	
(b) Trait (96D32)						
IND	Pi-I.2	I SPG	44	4.7	9	29
	Pi-III	III SPG	11	4.3	8	
	Pi-VIII	VIII SPG	38	3.0	6	
	Pi-X[a]	X ROSA	64	3.6	8	
	Pi-X[a]	X SPG	58	5.9	13	
L35	Pi-IV	IV ROSA	51	3.7	6	54
	Pi-VIII	VIII SPG	26	6.6	12	
	Pi-IX	IX SPG	0	4.9	8	
	Pi-X[a]	X ROSA	66	5.4	9	
	Pi-X[a]	X SPG	45	14.7	29	
rAUDPC	Pi-VIII	VIII ROSA	38	2.9	9	19
	Pi-IX	IX ROSA	12	2.8	9	

[a] The assignment of *Pi-X* QTL region of ROSA and of SPG to one of the two QTL regions *Pi-X.1* or *Pi-X.2* of SPL is still uncertain because of a lack of anchor markers between the maps

1999; Villamon et al. 2005; Simko et al. 2006; Sliwka et al. 2007). Our results with the stem test thus confirmed that the *Pi-X* QTL regions are potential targets for marker-assisted introgression into breeding material.

The sole stem resistance does not reflect the overall resistance of potato to late blight, because the yield will be affected as soon as the foliage is damaged, irrespective of the stem resistance. The stem resistance test should rather be considered as a means (a) to dissect the polygenic resistance, and (b) to provide complementary genetic factors to associate to other specific-organ resistance factors, such as foliage resistance and tuber resistance ones.

Conservation of QTL regions across potato species

The comparison of QTLs of our study with published QTLs from diverse species was possible thanks to the literature-derived anchor markers mapped in the QTL confidence intervals. The QTL regions *Pi-I.2* (Collins et al. 1999; Oberhagemann et al. 1999), *Pi-IV* (Leonards-Schippers et al. 1994; Meyer et al. 1998; Collins et al. 1999; Oberhagemann et al. 1999; Sandbrink et al. 2000; Bormann et al. 2004; Bradshaw et al. 2004; Sliwka et al. 2007), *Pi-VI* (Collins et al. 1999; Oberhagemann et al. 1999), *Pi-VIII* (Collins et al. 1999; Bormann et al. 2004), *Pi-IX* (Collins

et al. 1999; Sorensen et al. 2006), and *Pi-X.2* (Oberhagemann et al. 1999; Villamon et al. 2005; Simko et al. 2006; Sliwka et al. 2007) were concerned. These QTL regions, originating from multiple germplasms, are thus conserved despite speciation and diversification processes. These loci could thus be priority targets to further explore new resistance sources and to investigate potential durability of late blight resistance.

Comparison of QTL and literature R-gene locations

The mapping of R-gene markers on our maps highlighted consistent colocations of some QTLs detected in our study with literature R-genes. The *Pi-IV* QTL region detected on ROSA is actually located in a pathogen resistance hotspot containing seven reported R-genes for resistance to late blight (*R2*, *R2-like*, *Rpi-blb3*, *Rpi-abpt*, *Rpi-demf1*, *Rpi-mcd* and *Rpi-mcd1*; Sandbrink et al. 2000; Park et al. 2005a, b; Hein et al. 2007; Tan et al. 2008). Among them, the *Rpi-mcd1* R-gene also confers partial resistance in the field (Tan et al. 2008). An R-gene could thus underlie *Pi-IV* of ROSA. For the *Pi-VIII* QTL region on chromosomes VIII of CASP, ROSA and SPG, the literature RB marker (for *RB* R-gene, Millett and Bradeen 2007) mapped on the edge of the QTL intervals on CASP and ROSA. The relationships between *RB* and the *Pi-VIII* QTL region are thus unclear. Likewise it is difficult to state whether the *Pi-X* QTL regions were alleles or paralogues of *Rpi-ber* clusters (Ewing et al. 2000; Rauscher et al. 2006; Park et al. 2009). It was reported that the markers TG63, and probably CD5 and CD32, separate *Rpi-ber1* and *Rpi-ber2* loci (Park et al. 2009). In our study, TG63 was located at the upper edge of the QTL confidence interval of the *Pi-X* QTL of SPG, suggesting that *Pi-X* of SPG could belong to the *Rpi-ber2* cluster. The CD5 marker mapped on the lower side of *Pi-X* QTL of ROSA, suggesting that *Pi-X* of ROSA could belong to the *Rpi-ber1* cluster. These suspected colocations need to be further investigated to state whether the detected QTLs could correspond to defeated R-genes or to paralogues of the characterized R-genes. Other QTL regions, which were mapped on the R-gene-bearing chromosomes VI, IX and XI, lacked anchor markers, which prevents from comparisons with reported R-genes.

As far as the relationships between R-genes/RGA and QTLs are concerned, two main scenarios were described in the plant–pathogen interaction field (Poland et al. 2009). In the first scenario, qualitative and quantitative resistances may be controlled by the same genes and the differences observed at the phenotypic levels may be the result of extreme allelic variation (Gebhardt 1994). The genes underlying QTLs could be defeated major genes controlling monogenic resistances and which have a weak effect (Stewart 2003). In different species, some QTLs have been shown to colocalize with mapped known R-genes (Rouppe van der Voort et al. 2000; Caromel et al. 2005), or with NBS-LRR-like genes (Pflieger et al. 1999; Calenge et al. 2005). It has also been demonstrated that the same locus could segregate either as an R-gene or as a QTL in the same material according to the scoring scale (Bradshaw et al. 2006a; Hein et al. 2007; Tan et al. 2008). The difference in the kinds and levels of the detected resistance effects can also be due to the genetic background (Dowkiw and Bastien 2004). In fact, QTL mapping results indicate that quantitative resistance is often controlled by one or two large-effect QTLs in association with a few minor-effect QTLs which can interact with the major QTLs (reviewed in Lefebvre and Chèvre 1995). Also, a few paralogues of R-genes were shown to express partial resistance (e.g. *Xa21D*, Wang et al. 1998). Conversely, the *pvr2*^{3} QTL was shown to be allelic of the *pvr2*^{2} recessive major gene (Ayme et al. 2006). The resistance gene cluster of potato chromosome V also supports the allelic or paralogous relationship hypothesis: a linkage disequilibrium mapping study revealed strong association of markers around GP179 locus with the foliage and tuber quantitative resistances to late blight (Ballvora et al. 2002), while GP179 marker precisely tags the *R1* late blight resistance gene and its homologues (Kuang et al. 2005; Ballvora et al. 2007). In the second scenario, resistance QTLs colocalize with genes encoding proteins with completely different functions as those of known R-genes and which are rather involved in defence mechanisms (Pflieger et al. 2001). This was especially demonstrated in the late blight–potato interaction, where an allene oxide synthase 2, that is essential for biosynthesis of the defence signalling molecule jasmonic acid, has been identified as a candidate gene for a QTL of chromosome XI (Pajerowska-Mukhtar et al. 2008). Another study in rice demonstrated, through differential expression and functional analysis, that a gene underlying a QTL of resistance to *Magnaporthe grisea* and *Xanthomonas oryzae* pv. *oryzae* was a WRKY gene encoding for a transcription factor involved in salicylic acid- and jasmonic acid-dependent signalling pathways (Qiu et al. 2007). It has also been shown that there was no similarity between the protein encoded by the rice *Pi21* gene controlling partial resistance to *M. grisea* and any known RGA or defence protein (Fukuoka and Okuno 2001).

Late blight resistance and maturity type

The well-documented relationship between plant maturity type and foliage resistance to late blight demonstrates that early-maturing genotypes are more susceptible to *P. infestans* infection than late-maturing genotypes. This association hinders the improvement of “early potatoes” for late

blight resistance. From the genetic point of view, genomic regions are known to be involved in both plant maturity and polygenic late blight resistance on chromosomes II, III, IV, V, VI, VIII, X, XI and XII (Van Eck and Jacobsen 1996; Collins et al. 1999; Visker et al. 2003; Bormann et al. 2004; Bradshaw et al. 2004; Visker et al. 2005; Simko et al. 2006). Because no data have been collected for plant maturity on our progenies, we were not able to analyse the association between the resistance QTLs to late blight and the plant maturity. We cannot exclude that the QTL we detected on chromosome VI of CASP was independent from maturity (Simko et al. 2006). Conversely, for the regions of chromosomes II, III, IV, VIII, X, XI, and XII where we detected QTLs, the literature gives several arguments in favour of the independence of maturity type and late blight resistance (Bormann et al. 2004; Bradshaw et al. 2004; Visker et al. 2005; Simko et al. 2006).

To conclude, the QTL mapping in the late blight resistant *S. sparsipilum* and *S. spegazzinii* derived-clones highlighted, on one hand, the resistance potential of these potato-related sources, especially using the innovative stem assessment, and, on the other hand, the importance of well-conserved QTLs of chromosomes I, VIII and X. These loci were shown to be conserved in the potato germplasm and would thus be interesting targets for breeding programmes. However, further study, and especially more precise molecular data, are needed to be able to determine the genetic relationships between *Pi* R-genes or maturity QTLs described in literature with QTLs of late blight resistance.

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