Theor Appl Genet (2006) 113:943–951
DOI 10.1007/s00122-006-0353-8

ORIGINAL PAPER

Detection of a quantitative trait locus for both foliage and tuber resistance to late blight [*Phytophthora infestans* (Mont.) de Bary] on chromosome 4 of a dihaploid potato clone (*Solanum tuberosum* subsp. *tuberosum*)

John E. Bradshaw · Christine A. Hackett · Robert Lowe · Karen McLean · Helen E. Stewart · Irene Tierney · Marco D. R. Vilaro · Glenn J. Bryan

Received: 18 April 2006 / Accepted: 21 June 2006 / Published online: 15 July 2006

Abstract Linkage analysis, Kruskal–Wallis analysis, interval mapping and graphical genotyping were performed on a potato diploid backcross family comprising 120 clones segregating for resistance to late blight. A hybrid between the *Solanum tuberosum* dihaploid clone PDH247 and the long-day-adapted *S. phureja* clone DB226(70) had been crossed to DB226(70) to produce the backcross family. Eighteen AFLP primer combinations provided 186 and 123 informative maternal and paternal markers respectively, with 63 markers in common to both parents. Eleven microsatellite (SSR) markers proved useful for identifying chromosomes. Linkage maps of both backcross parents were constructed. The results of a Kruskal–Wallis analysis, interval mapping and graphical genotyping were all consistent with a QTL or QTLs for blight resistance between two AFLP markers 30 cM apart on chromosome 4, which was identified by a microsatellite marker. The simplest explanation of the results is a single QTL with an allele from the dihaploid parent conferring resistance to race 1, 4 of *P. infestans* in the foliage in the glasshouse and to race 1, 2, 3, 4, 6, 7 in the foliage in the field and in tubers from glasshouse raised plants. The QTL was of large effect, and explained 78 and 51% of the variation in phenotypic scores for foliage blight in the glasshouse and field respectively, as well as 27% of the variation in tuber blight. Graphical genotyping and the differences in blight scores between the parental clones showed that all of the foliage blight resistance is accounted for by chromosome 4, whereas undetected QTLs for tuber resistance probably exist on other chromosomes. Graphical genotyping also explained the lack of precision in mapping the QTL(s) in terms of lack of appropriate recombinant chromosomes.

Communicated by C. Gebhardt

J. E. Bradshaw (✉) · C. A. Hackett · R. Lowe · K. McLean · H. E. Stewart · I. Tierney · M. D. R. Vilaro · G. J. Bryan
Scottish Crop Research Institute, Invergowrie,
Dundee, DD2 5DA, UK
e-mail: john.bradshaw@scri.ac.uk

C. A. Hackett
Biomathematics and Statistics Scotland, Invergowrie,
Dundee, DD2 5DA, UK

Introduction

Late blight, caused by the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary, is the most serious disease in potato (*Solanum tuberosum*) worldwide, attacking both the foliage and tubers. The International Potato Center (CIP) estimated (CIP 1997) annual damage in developing countries at $3 billion, with poor farming communities in highland ecoregions disproportionately affected. In 1996, CIP launched a 10-year Global Initiative on Late Blight (GILB) to meet the threat to food security posed by the new populations of *P. infestans* which had been spreading from Mexico to the rest of the world since 1984 (Goodwin and Drenth 1997). These new populations comprised both the A1 and the A2 mating types and hence had the potential for sexual reproduction. They also contained strains with increased aggressiveness and strains insensitive to the widely used systemic fungicide metalaxyl (GILB 1999). Breeding for resistance intensified in many countries and was supported by projects such

as ECOPAPA which was funded under the EU-INCO scheme from 1999 to 2002. Its goal was 'the enrichment of potato breeding programmes in Latin America and Europe with resistance to late blight (*P. infestans*)'. Resistant germplasm (34 genotypes) for assessment was supplied by three countries in Latin America (Argentina, Bolivia and Uruguay), and three in Europe (France, The Netherlands and Scotland). The emphasis was on quantitative field resistance as the major dominant R-genes which had been introgressed from *S. demissum* had failed to provide durable resistance due to the evolution of new races of *P. infestans* (Malcolmson 1969). The 34 genotypes included two diploid clones from the Scottish Crop Research Institute which had been used as parents to study foliage resistance in the glasshouse. One was a dihaploid *S. tuberosum* clone whose foliage was confirmed to be highly resistant in the field in The Netherlands and Scotland, and the other was a long-day-adapted diploid *S. phureja* clone which was moderately susceptible in these countries. They were used to determine the inheritance of quantitative resistance in both the foliage and tubers in a diploid backcross mapping population, the results of which are presented in this paper. It was considered important to determine the resistance of the tubers as well as the foliage because there is a lack of information on the inheritance of the former and on the extent of any genetic correlation between the two aspects of resistance (Swiezynski and Zimnoch-Guzowska 2001).

Materials and methods

Plant material

PDH247 was a dihaploid *S. tuberosum* clone derived from the tetraploid clone 8318(4) (De Maine 1982). The parents of 8318(4) were at least six backcrosses removed from the blight resistant Mexican species *S. demissum*. Both the dihaploid and its tetraploid parent had very high levels of foliage blight resistance, scoring 8.7 and 7.7, respectively on a 1–9 scale of increasing resistance to a complex race of *P. infestans* capable of overcoming any *S. demissum* R-genes present in the germplasm (De Maine 1982). Clone 8318(4) had the highest General Combining Ability of 26 parents assessed by Malcolmson and Killick (1980) for foliage resistance in whole seedling tests. DB226(70) was the offspring of a pair cross between two diploid clones of *S. phureja* derived from a population which had been selected to tuber in long days (Carroll 1982).

All 162 F1 clones from the cross between PDH247 (female) and DB226(70) (male) proved resistant to isolate 23 (race 1,2,3,4,7) of *P. infestans* (late blight scores of 7.5–9.0) in a whole plant foliage test in which DB226(70) was susceptible (score 3.1). Hence an F1 clone HB171(13), which scored 9.0, was crossed with DB226(70) (male) in 1993 to produce a backcross population HB193 for studying the inheritance of resistance. Seedlings were raised in a glasshouse in 1994 and the population was subsequently maintained annually from tubers in the glasshouse and at a high-grade seed site in Scotland. All 120 clones used for mapping were assessed for their resistance to tuber blight whereas foliage resistance was assessed on a subset of 87 in a glasshouse test and a slightly different subset of 97 in a field test.

Glasshouse foliage blight test in 1996

The whole-plant glasshouse test developed by Malcolmson (1976) and Stewart et al. (1983) was used to assess foliage resistance to isolate FG17.3.2 (race 1,4) of *P. infestans.* This isolate was known to be aggressive on the parental clone DB226(70) which has some foliage resistance to late blight. The test had a randomized complete block design with two replicates. There was one plant of each backcross clone in each replicate and two of PDH247, three of DB226(70) and four of HB171(13). Replicate one was inoculated on 27 June and replicate two on 4 July. Each replicate was scored 6 days after inoculation on Malcolmson's 1–9 scale of increasing resistance which is related to the percentage of necrotic tissue as illustrated by Cruickshank et al. (1982). An analysis of variance was done using GENSTAT 5 Release 3 (GENSTAT 5 Committee 1993).

Field assessment of foliage blight resistance in 2000

The assessment was done at Yonderton Farm, Ayrshire in the west of Scotland where weather conditions are conducive to the development of blight. The trial was planted on 9 May and had a randomized complete block design with two replicates and two-plant plots along the drills. Every third drill was planted with the susceptible cultivar King Edward. There was one plot of each backcross clone in each replicate and three of PDH247, DB226(70) and HB171(13). Glasshouse-grown plants of cultivar King Edward were inoculated in the laboratory with a zoospore suspension of isolate 36.4.3 (race 1,2,3,4,6,7) of *P. infestans* and were placed in their pots at metre intervals along the 'spreader drills' of King Edward on 13 July. Despite dry weather, blight developed and by 29 August the 'spreaders'

were uniformly dead. The percentage of necrotic tissue was scored by eye using the illustrated assessment key of Cruickshank et al. (1982) for each plot in the trial on 4 September. This was 12 days after the rest of the breeding material being assessed at Yonderton because the backcross population proved more resistant. The data were converted to degrees by the variance-stabilising angular transformation (angle whose sine is square root of percentage) before doing an analysis of variance using GENSTAT 5 Release 3 (GENSTAT 5 Committee 1993).

Tuber blight test in 2001

Seed tubers were planted on 23 April in 10-cm pots of soil-based compost placed on a glasshouse bench in a two-replicate randomized complete block design. Within each replicate, each backcross clone was represented once, and PDH247, DB226(70) and HB171(13) three times, by plots of eight plants in adjacent pots. The plants were defoliated on 23 July, just as they were coming to flower and before the tubers were fully mature. Tubers were harvested the next day and the produce of the eight plants was bulked and the 20 largest tubers were selected and placed in a 12.5-cm pot. In practice, an average of 19 tubers was achieved, with three backcross clones as low as 9 and PDH247 failing to produce tubers of sufficient size to include in the test. The tuber samples were washed and inoculated immediately after harvest by dipping the pots momentarily in a suspension containing 2.5×10^4 zoospores/ml of isolate 99/23 (race 1,2,3,4,6,7) of *P. infestans* which had been produced by dipping infected leaflets in cold distilled water and adjusting the concentration. The pots were stored for 48 h in a constant environment chamber at 15°C and 100% relative humidity and then left to dry on the floor of a glasshouse header house at ambient temperature. The number of infected tubers in each sample was recorded on 6 August, excluding infections through wounds or the heel end scar. The percentage of infected tubers was calculated and converted to degrees by the variance-stabilising angular transformation before doing an analysis of variance using GENSTAT 5 Release 3 (GENSTAT 5 Committee 1993).

DNA Isolation from plants

Leaf material was collected from the parents and every clone of the HB193 population that had been assessed for tuber blight resistance. DNA was extracted from frozen plant leaf tissue using the Dneasy Plant DNA Extraction kit (Qiagen, Cat. No. 69106). Parents of a ultra high density (UHD) mapping population, SH and RH (http://www.dpw.wageningen-ur.nl/uhd), were also included in the marker assays to enable identification of alleles which co-migrated with previously mapped markers and hence to help confirm chromosomal identification of linkage groups, as done by Rouppe van der Voort et al. (1997).

Marker assays

Amplified fragment length polymorphism (AFLP) assays were performed using a modification of the protocol of Vos et al. (1995), as described in Bryan et al. (2002). The 6-bp cutting restriction enzyme *Pst*1 was used in combination with the 4-bp cutting restriction enzyme *Mse*I. AFLP fragment nomenclature is PABMCDE_XYZ where AB and CDE are the selective base extensions on the *Pst*1 and *Mse*I primers, respectively, and XYZ is the size of the AFLP fragment in basepairs. Eighteen primer combinations were used and a total of 246 bands were scored. Several of these AFLP primer combinations were known to generate informative and reproducible banding patterns in potato from their use in the UHD map (Isidore et al. 2003) as well as in other studies (Bryan et al. 2002).

The following 11 potato microsatellite (SSR) markers were used to identify linkage groups: STM0003, STM0024, STM0028, STM0038, STM1005, STM1024, STM1058, STM1041, STM1100, STM1106 (Milbourne et al. 1998) and STM5140 which Ghislain et al. (2004) had mapped to chromosome 4. The sequences for STM5140 (kindly provided by Ghislain) were as follows: forward primer, GCTATTGTTGCAGATAAT ACG and reverse primer, GCCATGCACTAATCT TTGA. The SSR assays were done as described by Bryan et al. (2002).

Electrophoresis was performed on a Biorad SequiGen GT system using 1× TBE Buffer. A Promega *fmol* DNA Cycle Sequencing System (Promega Q4100) marker (prepared according to the protocol, but using only a d/ddT Nucleotide Mix) was run to estimate the product size. Gels were dried onto paper and visualised by exposure to X-ray film (Kodak BIOMAX MR). Autoradiograms were scored independently by two people using the Cross Checker software package (Buntjer 1999). Error checking was also performed by constructing graphical genotypes of the individuals in the backcross population from the marker data for each linkage group. Where the number and distribution of crossovers seemed unlikely, the scoring was verified on the actual gel image.

Linkage map and QTL analyses

Linkage map construction and QTL analysis was performed using JoinMap 3.0 (Van Ooijen and Voorrips 2001) and MapQTL5 (Van Ooijen 2004), respectively. For the JoinMap analysis, the marker data were split into a maternal set containing 186 AFLP markers and a paternal set containing 123 markers. There were 63 AFLP markers common to both parents. Markers were removed from the data set if they caused problems such as an increase in the chi-square value for goodness of fit to significantly ($P < 0.001$) greater than the number of degrees of freedom (in practice often by a factor of 2 or more). However, this did not happen for chromosome 4. In addition, of the 11 SSR loci, six had a single allele which was present in the maternal parent, one had a single allele present in the paternal parent and four had alleles present in both parents which contributed a total of 13 alleles.

An initial rapid search for associations between markers and traits was done using nonparametric mapping (Kruskal–Wallis analysis) as no assumptions are made about the probability distribution of the quantitative trait. All of the statistically significant associations between markers and traits were confined to dominant markers on a single maternal linkage group. More detailed analyses of this group were performed on markers present in the maternal parent and absent from the paternal parent as these are linked in coupling. Interval mapping was done assuming the segregation of a resistant and susceptible allele at a QTL in the maternal parent. Visual examination of the phenotypic distributions for the three traits analysed had indicated that the underlying assumptions for interval mapping were reasonable. Graphical genotyping was also done to examine associations between trait data and recombinant chromosomes.

Results

Blight results

Analyses of variance revealed statistically significant differences ($P < 0.001$) between the clones in the backcross population for all three traits (Table 1). The residual degrees of freedom are less than those for clones due to a few missing values. Broad sense heritabilities were 0.896 for foliage blight in the glasshouse, 0.825 for foliage blight in the field and 0.874 for tuber blight. The frequency distributions for the three traits are shown in Fig. 1. The glasshouse foliage blight scores displayed a bimodal distribution whereas the field scores and tuber blight scores were approximately normally distributed after angular transformation. There were seven clones where the foliage blight scores for the two replicates in the glasshouse differed by three or more units and hence their mean scores may not be an accurate reflection of resistance. However, there was no a priori criterion for their omission. For tuber blight, five clones were completely susceptible and are shown as such, rather than including them in the 80–90° category. As expected, HB171(13) was resistant and DB226(70) was susceptible to blight in both foliage and tubers. PDH247 was slightly more resistant to foliage blight than HB171(13), but could not be assessed for tuber resistance as it failed to produce any tubers of sufficient size.

Table 1 Analyses of variance

Source of variation	Foliage blight glasshouse		Foliage blight field		Tuber blight	
	df	ms	df	ms	df	ms
Blocks	1	20.115*	1	38.70	1	3078*
Clones	86	8.369*	96	428.75*	119	893*
Residual	74	0.867	89	74.94	114	113

*$P < 0.001$

There were statistically significant ($P < 0.001$) correlations between the three traits (clone means), but the magnitudes of the correlations between foliage and tuber resistance ($r = 0.48$ and 0.47 for glasshouse and field, respectively) were less than that for the two foliage scores ($r = 0.68$).

Linkage mapping

Twelve linkage groups were created using 147 loci from the maternal data at a LOD threshold of 7.2, with one exception. At LOD 7.2, linkage group LGIV split into two distinct groups and therefore LOD 7.0 was used. Nine linkage groups were identified using SSR's previously mapped in other potato populations. The three remaining linkage groups, LGI, LGIX and LGXI were tentatively identified using co-migrating AFLP markers in the SH x RH UHD reference population (http://www.dpw.wageningen-ur.nl/uhd). The total maternal map length was 686.5 cM. Linkage group LGIV (chromosome 4) proved of most interest and comprised ten markers of which nine were in coupling, including one allele of microsatellite STM5140 known to be on chromosome 4, and one was in repulsion. The AFLP marker in repulsion was also present in the paternal parent and mapped to the end of the linkage group which was 61.0 cM long. It was excluded from the QTL and graphical genotyping analyses and this

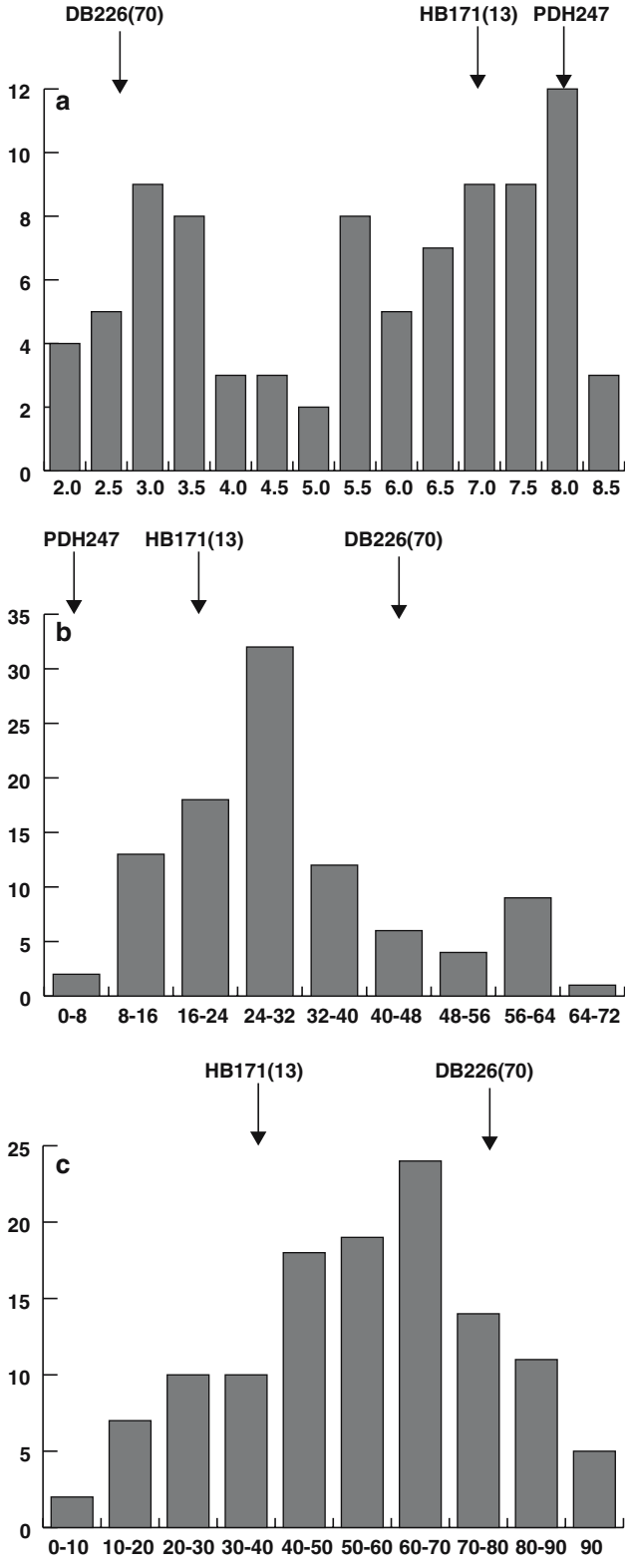

Fig. 1 Frequency distributions in backcross family for resistance to late blight: **a** glasshouse foliage blight on a 1 (susceptible) to 9 (resistant) scale, **b** field foliage blight as percentage of necrotic tissue in degrees, **c** tuber blight as percentage of infected tubers in degrees

altered the order of three closely linked markers to that shown in Tables 2 and 4. There was distorted segregation the whole way along the linkage group with an average ratio of 2.22 for presence/absence of marker.

Twelve linkage groups were created using 84 loci from the paternal data at a LOD threshold of 6.5 except for Linkage Group LGXI where a LOD of 6.0 was used to prevent it splitting into two groups. Two linkage groups were identified using SSRs, three were tentatively identified using AFLP markers which co-migrated with ones from the SH × RH population and the remainder through markers common to both maternal and paternal maps, although not all of the markers segregating in a 3:1 ratio could be fitted to the final maps. The total paternal map length was 510.7 cM.

QTL mapping

All of the associations between markers and the three traits revealed by the Kruskal–Wallis analysis at a stringent significance level of $P < 0.001$ were on maternal Linkage Group LGIV. There was a gradient in the test statistic along the linkage group with the highest values ($K^* > 21$) for the four markers between map positions 47 and 60 cM (Table 2). Presence of the marker was associated with resistance and the differences between the means of the backcross genotypes with and without the marker was large for all three traits (Table 2).

The interval mapping provided evidence for a QTL for all three traits between markers PAGMAGC_435 and PACMACT_267 at map position 39 cM (Table 3). This map position gave the largest QTL effect (difference between resistant and susceptible allele), and the percentage variation explained and the residual variance were either at or close to the maximum and minimum, respectively (Table 3). However, for the glasshouse foliage blight scores, very similar values occurred at map position 56 cM but at a higher LOD score (Fig. 2).

Graphical genotyping

There were nine markers present in the female parent and absent in the male parent which were linked in coupling on Linkage Group LGIV. This allowed haplotypes to be recognized in the offspring and these and their mean blight scores are shown in Table 4. Haplotypes 3 and 4 are mixtures of three and two haplotypes respectively which have been merged to test the hypothesis that there is a QTL for blight resistance

Table 2 Kruskal–Wallis analyses: differences between means of backcross genotypes with and without the marker allele associated with resistance at five loci, all differences significant at $P < 0.001$

Marker		Glasshouse foliage blight 1 (sus) to 9 (res)	Foliage blight leaf area infected (degrees)	Tuber blight infected tubers (degrees)
Position	Locus			
29.05	PAGMAGC_435	2.35	13.18	17.29
47.69	PACMACT_267	3.27	19.26	19.88
49.23	STM5140	3.19	19.08	21.72
51.71	PCCMACA_118	3.06	17.55	21.20
59.11	PCCMACA_256	3.38	18.44	19.35

Table 3 Interval mapping

	Quantitative trait locus			
	Position (cM)	LOD	Difference	Percentage variation (%)
Glasshouse foliage blight	39.05	13.71	3.75	77.3
	55.71	21.32	3.71	78.1
Field foliage blight	39.05	10.10	22.77	50.7
Tuber blight	39.05	6.30	23.74	27.2

between markers PAGMAGC_435 and PACMACT_267. Of the 120 offspring, 60 had non-recombinant parental chromosomes, but there was distorted segregation as 47 were haplotype 1 (all markers present) and 13 were haplotype 2 (no markers present), a statistically significant departure from a 1:1 ratio ($\chi^2 = 19.27$, $P < 0.01$). Forty three of the offspring were single recombinants (haplotypes 3–6 and 8 and 9). Again distorted segregation can be seen as the ratio of haplotypes 5 and 6 is a significant departure from a 1:1 ratio (4:15, $\chi^2 = 6.37$, $P < 0.05$). There appeared to be 17 double recombinants, but eight of them were singletons which remained after the gels had been checked for scoring errors. Five double recombinants of interest (haplotype 7) are shown in Table 4. Haplotype 10, which would have been of particular interest, did not occur. Haplotypes 1 and 3 were resistant to blight and haplotypes 2 and 4 were susceptible, although the former was slightly more susceptible than the latter. Haplotype 5 was more resistant than haplotype 6, but there were fewer clones of the former, as already noted. Haplotypes 7–9 gave variable results, but there were few clones of each.

Discussion

Kruskal–Wallis analysis, interval mapping and graphical genotyping

The results of the Kruskal–Wallis analysis, the interval mapping and the graphical genotyping are all consistent with a QTL or QTLs for blight resistance between markers PAGMAGC_435 and PCCMACA_256 on chromosome 4. Interval mapping placed a QTL at 39 cM for all three resistance traits, but graphical genotyping revealed a lack of sufficient recombinants in this region for precise mapping. The graphical genotyping also explained why position 56 cM had a higher LOD score than position 39 cM for the glasshouse foliage blight scores. Haplotypes 7 and 9 lacked marker PCCMACA_256 and were more susceptible than might have been expected, but for no obvious reason. Hence a separate QTL at 56 cM for glasshouse foliage resistance cannot be ruled out. Nevertheless, the simplest explanation of the results is a single QTL at 39 cM with an allele from dihaploid PDH247 conferring resistance to race 1, 4 of *P. infestans* in the foliage in the glasshouse and to race 1, 2, 3, 4, 6, 7 in the foliage in the field and in tubers from glasshouse raised plants. The QTL(s) is of large effect, as revealed from the estimates in the interval mapping, and explained 78% of the variation in phenotypic scores for foliage blight in the glasshouse, 51% of the variation for foliage blight in the field and 27% of the variation in tuber blight. Furthermore, it can be concluded from comparing the effect of the whole of chromosome 4 on resistance (difference between haplotypes 1 and 2 in Table 4) with the differences between the parents of the backcross, HB171(13) and DB226(70), that all of the foliage blight resistance can be accounted for by chromosome 4 (3.97 for chromosome 4 vs. 4.46 for the difference between parents in the glasshouse and 29.5 vs. 26.0 in the field) whereas undetected QTLs for tuber resistance probably exist on other chromosomes (29.5 vs. 39.0). This would explain the lower correlation between foliage and tuber resistance than between foliage resistance in the glasshouse and field. A backcross population does not allow estimates of dominance effects, but foliage blight scores were recorded for PDH247 (it did not produce tubers) and these revealed that it was slightly more resistant than HB171(13) so that dominance was high but incomplete.

Table 4 Graphical genotyping: number of clones and blight scores of each of ten haplotypes

Position (cM)	Locus	Haplotype									
		1	2	3	4	5	6	7	8	9	10
0.00	PAGMAGT_203	1	0	0	1	0	1	0	1	1	0
16.48	PAGMAGT_158	1	0	–	–	0	1	1	1	1	0
22.24	PAGMACC_181.5R4	1	0	–	–	0	1	1	1	1	0
22.97	PCCMACA_131	1	0	–	–	0	1	1	1	1	0
29.05	PAGMAGC_435	1	0	1	0	0	1	1	1	1	1
47.69	PACMACT_267	1	0	1	0	1	0	1	1	1	1
49.23	STM5140	1	0	1	0	1	0	1	1	1	0
51.71	PCCMACA_118	1	0	1	0	1	0	1	0	1	0
59.11	PCCMACA_256	1	0	1	0	1	0	0	0	0	0
Glasshouse foliage blight: number of clones		37	12	10	5	2	10	4	1	2	0
Score 1 (sus) to 9 (res)		6.87	2.90	6.67	3.50	6.49	3.73	4.38	5.46	4.50	–
Field foliage blight: number of clones		37	9	11	7	3	14	5	1	1	0
Leaf area infected (degrees)		22.8	52.3	23.1	42.7	22.5	38.7	35.9	36.8	9.5	–
Tuber blight: number of clones		47	13	13	7	4	15	5	1	3	0
Infected tubers (degrees)		44.2	73.7	46.8	70.0	50.2	65.2	47.5	68.2	51.2	–

Evidence for QTL being the same as the one found in cultivar Stirling

The QTL could be the same one that was found in the resistant cultivar Stirling from interval mapping in a tetraploid population (Bradshaw et al. 2004). The parent of PDH247 as well as one of the parents of cultivar Stirling came from the cross between clones 4517(1) and 3071ab1 which Black (1970) had selected for their high and moderate field resistance, respectively. Both QTLs conferred resistance in the tubers as well as the foliage and their map positions were consistent but not conclusive. The map position of the QTL was 24 cM from STM5140 on a map of total length 105 cM in Stirling compared with 10 cM from STM5140 on a map of length 61 cM in HB171(13). Simko (2002), in his comparative analysis of QTLs for foliage resistance, concluded that the QTL in Stirling was on the distal part of chromosome 4, in the same region as *R2* (see below), but we cannot be more precise as STM5140 has not been mapped in a population segregating for *R2*. What is not in doubt is that there is a QTL for blight resistance on chromosome 4 which was selected in breeding for quantitative field resistance and which continues to be of importance in potato breeding.

Other blight resistant genes on chromosome 4

Major dominant R genes and other QTLs for blight resistance have also been mapped to chromosome 4. Li et al. (1998) mapped the *R2* gene from *S. demissum* using AFLP markers in a bulked segregant analysis of a tetraploid population. Park et al. (2005a) then discovered that the *R2* locus is part of a major late blight R gene cluster that also contains *Rpi-abpt* and *Rpi-blb3* from *S. bulbocastanum* and *R2*-like which was found in a tetraploid breeding clone with a number of wild species in its ancestry. The isolates of *P. infestans* used could distinguish *Rpi-abpt* from *Rpi-blb3* but not *R2*-like from *R2*. Leonards-Schippers et al. (1994) discovered a number of QTLs for foliage resistance in their F1 population including one on chromosome 4 which segregated from the susceptible parent, a *S. tuberosum–S. spegazzinii* hybrid. One of the two *S. microdontum* genotypes used by Sandbrink et al. (2000) in their F1 populations had a major QTL on chromosome 4 which explained about 30% of the variation in foliage blight scores in two field trials. It mapped to the same region as *R2* and the QTL found by Leonards-Schippers et al. (1994). Interestingly, Park et al. (2005b) also found a QTL on chromosome 4 in a parent derived from *S. microdontum* and showed that it conferred resistance in the tubers as well as the foliage. In contrast, they demonstrated that the major gene *Rpi-abpt* was foliage specific. Hence this paper is the second report of a region of chromosome 4 (possibly a single QTL) that confers resistance in both the foliage and tubers and hence is especially valuable in potato breeding where both aspects of resistance are desirable. The QTL mapped in this paper is unlikely to be the same gene as the one found in *S. microdontum* as this species is from Bolivia and Argentina and has not been used in the SPBS/SCRI potato breeding programme for blight resistance which has concentrated on resistance from the Mexican species *S. demissum*. Furthermore, the resistance level found in PDH247 and HB171(13) with race 1,2,3,4,6,7 of *P. infestans* was too high to be due to

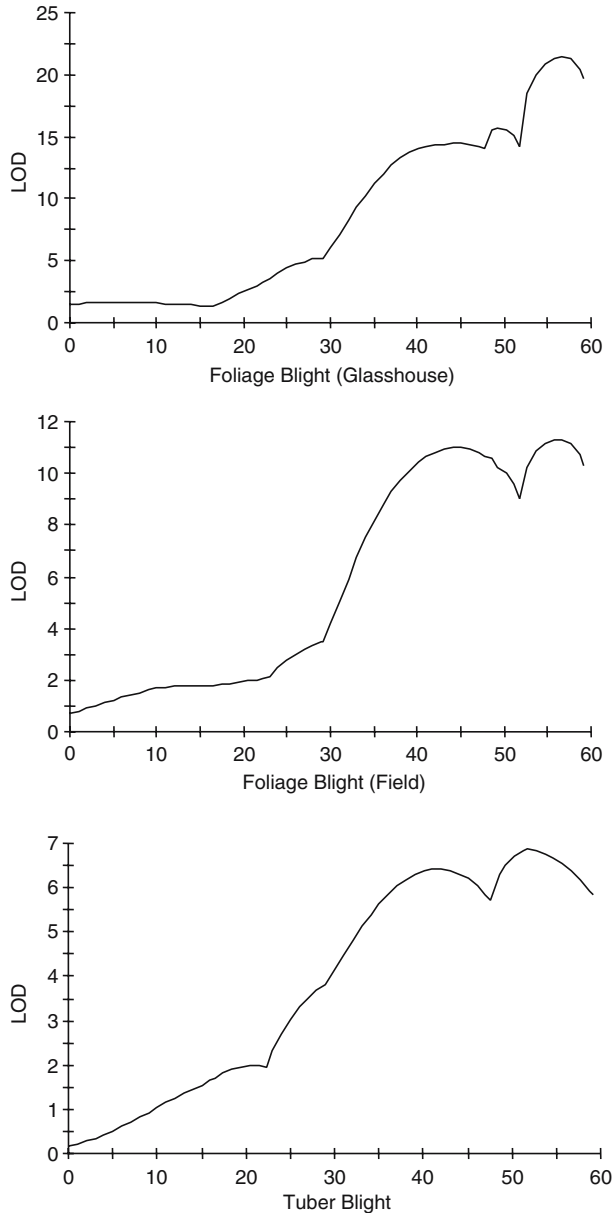

Fig. 2 Interval mapping: LOD profile of maternal Linkage Group IV (chromosome 4) from 0 to 60 cM

a residual effect of defeated *R2*. Hence, it is likely that the QTL mapped in this paper was introgressed by chance along with *R2* in the days before potato breeders started to select for quantitative field resistance.

Advantages and disadvantages of mapping in diploids and tetraploids

Finally it is instructive to compare the advantages and disadvantages of linkage analysis and QTL mapping in diploid and tetraploid populations. If the assumption is made that the dihaploid clone PDH247 and cultivar Stirling contain the same QTL, a comparison can be made between the diploid backcross population reported in this paper and the tetraploid F1 population analysed by Bradshaw et al. (2004). The advantages of the diploid analysis were that all 12 linkage groups were recognized with fewer AFLP primer combinations and a smaller population size, namely 18 primer combinations compared with 38 and a population size of 120 compared with 227. In addition, more phenotypic variation was explained in the diploid than the tetraploid population, for example, 27% compared with only 14% for tuber blight. The problem that arises and needs to be overcome in tetraploids is that of adequate genome coverage with appropriate and informative markers because coupling and repulsion linkages are not detected with equal precision. If there is a single copy of a QTL allele in one parent, 48 chromosomes need to be considered for a marker in coupling, and if there are two copies, they are on one possible pair of chromosomes out of six in one of 12 linkage groups. The potential advantages of the more difficult tetraploid analysis are that the results are directly applicable to the genetical aims of a tetraploid breeding programme and there is the possibility of assessing allelic diversity and interaction, but these may not be realized when analysing a single full-sib family. Whilst Bradshaw et al. (2004) were able to demonstrate partial dominance for blight resistance (QQqq superior to Qqqq), the same was true at the diploid level where the foliage resistance of PDH247 was superior to that of HB171(13). Hence tetraploid analyses will probably be confined to situations where suitable dihaploids are not available, as occurred with the H3 source of resistance to *G. pallida* (Bryan et al. 2004), or where sufficient dihaploids can be produced for them to be assessed and analysed as the gametes from a tetraploid parent, as done by Song (2004). In opting to work at the diploid level, however, researchers must remember that the analysis of diploid outbreeders is more complicated than that for diploid inbreeders. For example, whilst the population size of 120 was adequate for locating the QTL to a region of chromosome 4, a much larger population size of over 1,000 would be required for precise mapping in which sufficient rare double recombinants (e.g. haplotype 10 in Table 4) are produced to average out the effects of segregation in the background genotype of a heterozygous parent.

Acknowledgments Thanks are due to the many colleagues who contributed to the ECOPAPA project, especially Dr Jim Duncan, to the European Union for funding EU-INCO project ERBIC18 CT98 0318 and to the Scottish Executive Environment and Rural Affairs Department for additional funding.

References

Black W (1970) The nature and inheritance of field resistance to late blight (*Phytophthora infestans*) in potatoes. Am Potato J 47:279–288

Bradshaw JE, Pande B, Bryan GJ, Hackett CA, McLean K, Stewart HE, Waugh R (2004) Interval mapping of quantitative trait loci for resistance to late blight [*Phytophthora infestans* (Mont.) de Bary], height and maturity in a tetraploid population of potato (*Solanum tuberosum* subsp. *tuberosum*). Genetics 168:983–995

Bryan GJ, McLean K, Bradshaw JE, De Jong WS, Phillips M, Castelli L, Waugh R (2002) Mapping QTLs for resistance to the cyst nematode *Globodera pallida* derived from the wild potato species *Solanum vernei*. Theor Appl Genet 105:68–77

Bryan GJ, McLean K, Pande B, Purvis A, Hackett CA, Bradshaw JE, Waugh R (2004) Genetical dissection of H3-mediated polygenic PCN resistance in a heterozygous autotetraploid potato population. Mol Breed 14:105–116

Buntjer JB (1999) Cross Checker fingerprint analysis software v2.9. Wageningen University and Research Centre, The Netherlands

Carroll CP (1982) A mass-selection method for the acclimatization and improvement of edible diploid potatoes in the United Kingdom. J Agric Sci Camb 99:631–640

CIP (1997) Medium-term plan 1998–2000. International Potato Center, Lima, Peru

Cruickshank G, Stewart HE, Wastie RL (1982) An illustrated assessment key for foliage blight of potatoes. Potato Res 25:213–214

De Maine MJ (1982) An evaluation of the use of dihaploids and unreduced gametes in breeding for quantitative resistance to potato pathogens. J Agric Sci Camb 99:79–83

GENSTAT 5 Committee (1993) GENSTAT 5 release 3 reference manual. Clarendon Press, Oxford

Ghislain M, Spooner DM, Rodriguez F, Villamon F, Nunez J, Vasquez C, Waugh R, Bonierbale M (2004) Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. Theor Appl Genet 108:881–890

GILB (1999) Late blight: a threat to global food security. In: Proceedings of the global initiative on late blight conference, 16–19 March 1999, Quito, Ecuador

Goodwin SB, Drenth A (1997) Origin of the A2 mating type of *Phytophthora infestans* outside Mexico. Phytopathology 87:992–999

Isidore E, Van Os H, Andrzejewski S, Bakker J, Barrena I et al (2003) Toward a marker-dense meiotic map of the potato genome: lessons from linkage group 1. Genetics 165:2107–2116

Leonards-Schippers C, Gieffers W, Schafer-Pregl 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

Li X, van Eck HJ, Rouppe van der Voort JNAM, Huigen DJ, Stam P, Jacobsen E (1998) Autotetraploids and genetic mapping using common AFLP markers: the *R2* allele conferring resistance to *Phytophthora infestans* mapped on potato chromosome 4. Theor Appl Genet 96:1121–1128

Malcolmson JF (1969) Races of *Phytophthora infestans* occurring in Great Britain. Trans Br Mycol Soc 53:417–423

Malcolmson JF (1976) Assessment of field resistance to blight (*Phytophthora infestans*) in potatoes. Trans Br Mycol Soc 67:321–325

Malcolmson JF, Killick RJ (1980) The breeding values of potato parents for field resistance to late blight measured by whole seedlings. Euphytica 29:489–495

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C, Waugh R (1998) Isolation, characterisation and mapping of simple sequence repeat loci in potato. Mol Gen Genet 259:233–245

Park T-H, Gros J, Sikkema A, Vleeshouwers VGAA, Muskens M, Allefs S, Jacobsen E, Visser RGF, van der Vossen EAG (2005a) The late blight resistance locus *Rpi-blb3* from *Solanum bulbocastanum* belongs to a major late blight *R* gene cluster on chromosome 4 of potato. Mol Plant Microbe Interact 18:722–729

Park T-H, Vleeshouwers VGAA, Kim J-B, Hutten RCB, Visser RGF (2005b) Dissection of foliage and tuber late blight resistance in mapping populations of potato. Euphytica 143:75–83

Rouppe van der Voort JNAM, van Zandvoort P, van Eck HJ, Folkertsma RT, Hutten RCB, Draaistra J, Gommers FJ, Jacobsen E, Helder J, Bakker J (1997) Use of allele specificity of comigrating AFLP markers to align genetic maps from different potato genotypes. Mol Gen Genet 255:438–447

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

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

Song Y-S (2004) Genetic marker analysis in potato for extreme resistance (*Rysto*) to PVY and for chip quality after long term storage at 4°C. PhD Thesis, Technical University, Munich

Stewart HE, Flavelle PH, McCalmont DC, Wastie RL (1983) Correlation between glasshouse and field tests for resistance to foliage blight caused by *Phytophthora infestans*. Potato Res 26:41–48

Swiezynski KM, Zimnoch-Guzowska E (2001) Breeding potato cultivars with tubers resistant to *Phytophthora infestans*. Potato Res 44:97–117

Van Ooijen JW (2004) MapQTL5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma B.V., Wageningen, Netherlands

Van Ooijen JW, Voorrips RE (2001) JoinMap 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, the Netherlands

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414