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Mapping QTLs for resistance to the cyst nematode *Globodera pallida* derived from the wild potato species *Solanum vernei*

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Abstract Resistance to the potato cyst nematode (PCN) species *Globodera pallida*, derived from the wild diploid potato species *Solanum vernei*, has been investigated. This source of resistance, which is effective against all of the major pathotypes of *G. pallida* and *Globodera rostochiensis*, has been assumed to be due to several genetic factors, but it has proved difficult to deploy effectively in breeding strategies for potato cultivars. Diploid and tetraploid potato populations segregating for 'vernei' resistance were analysed. At the tetraploid level, a bulk segregant analysis (BSA) approach was employed and detected AFLP markers linked to a resistance QTL on potato linkage group V. Conventional linkage analysis of a diploid population identified QTL on linkage groups V and IX. A marker linked to a QTL on linkage group V has been converted to a single-locus PCR-based marker, which can be used to detect the presence of the QTL in diploid and tetraploid potato germplasm. Moreover, there is evidence that one of the AFLPs detected by BSA appears to be specific to an introgressed segment of DNA from *S. vernei*. These results are compared with those obtained from other studies on resistance to the PCN species *G. pallida*.

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Introduction

Potato cyst nematodes (PCN) have become a major problem for potato producers in many countries, particularly those in Northern Europe. They are known to have spread to Europe following importation of potato germplasm from South America. There are two predominant species of PCN that represent a major threat to potato crops, *Globodera rostochiensis* and *Globodera pallida*. It has been possible to incorporate major resistance genes against *G. rostochiensis* into potato cultivars; for example the H1 gene, introgressed into cultivated potato from *Solanum tuberosum* ssp. *andigena* accession CPC1673. The use of cultivars with resistance to *G. rostochiensis* has decreased the populations of this species of nematode. However, a consequence of this decrease has been a rapid increase in population sizes of *G. pallida*. *G. pallida* populations are thought to be extremely diverse (Schnick et al. 1990), which has hampered efforts to incorporate effective resistance into potato cultivars. Several different sources of resistance to *G. pallida* have been described, including a major gene resistance, such as H2, which remains unmapped and which originates from the wild species *Solanum multidissectum*, as well as other, more complex sources, such as those from *S. tuberosum* ssp. *andigena* and *Solanum vernei*. The H2 gene confers resistance to only a single *G. pallida* pathotype, Pa1, which only occurs in a very restricted range within Europe and the UK in particular (Dunnett 1961). Another major gene, *Gpa2*, also from *S. tuberosum* ssp. *andigena* accession CPC1673, confers resistance to Pa2, and has recently been isolated by map-based cloning. *Gpa2* is a member of a complex cluster of resistance genes conferring resistance to viruses as well as nematodes (Kanyuka et al. 1999; Van der Vossen 1999).

More complex sources of resistance, derived from *S. tuberosum* ssp. *andigena* and from *S. vernei*, confer

high levels of resistance to the pathotypes of *G. pallida* (i.e. Pa2/3) commonly found within the UK and Europe. Broad-spectrum resistance to *G. pallida* introgressed into the breeding line 12601ab1 from *S. tuberosum* ssp. *andigena* has been previously analysed genetically, using a tetraploid mapping population (Bradshaw et al. 1998), and a large-effect QTL was localised to linkage group IV. Resistance derived from the wild diploid species *S. vernei* is thought to be even more genetically complex (Dale and Phillips 1982). This source of resistance has proved difficult to deploy in potato cultivars, and to date only partial resistance has been incorporated (Bradshaw et al. 1995). For example, the cultivars Sante, Nadine and Spey, which show NIAB scores of 6, 3 and 3 respectively (on a 1–9 scale with 9 being fully resistant), are thought to contain components of resistance from this source.

In this study, QTLs contributing to 'vernei'-derived resistance to PCN were mapped. The results were compared with those obtained from other recent, related studies, notably those of Rouppe van der Voort et al. (2000).

Materials and methods

Plant material

Segregating populations: two populations segregating for *S. vernei*-derived PCN resistance were used: Stirling × 12288af23 (consisting of 384 individuals) and PDH538 × IVP48 (consisting of 138 individuals) were employed for linkage analysis. Clone 12288af23 is a sister of the cultivar Glenna, released in 1987, and has a complex pedigree (Bradshaw et al. 1995). Diploid accessions of *S. vernei* (CPC 2487 and 2488), were inter-crossed and the F_1s treated with colchicine to derive a tetraploid '*S. vernei*', and an unknown *S. vernei* accession which was also colchicine-treated to give the tetraploid clone C3399. These tetraploid *S. vernei* clones were then crossed to *S. tuberosum* material. PDH538 is a primary dihaploid clone originating from the tetraploid clone 11198cf(15), descended from the same tetraploid *S. vernei* × *S. tuberosum* hybrids as Glenna and 12288af23. 11198cf(15) and PDH538 contain levels of resistance to *G. pallida* comparable to that of the original *S. vernei* sources used as introgression parents.

The G87D2.4.1 × I88.55.6 mapping population, comprising 89 individuals (Collins et al. 1999), hereafter referred to as G87 × I88, was used to identify the genetic location of markers detected using bulk segregant analysis in the Stirling × 12288af23 tetraploid population.

Three types of potato germplasm were screened with the PCR-based markers developed in this study to ascertain whether their presence was correlated with *S. vernei*-derived PCN resistance. These samples are as follows:

Potato breeding lines: 12288af23, 12385ab6, 14969ac9, 15119ac5, 12601ab1, 12636a2, 12674ab1, 14069a4 (Eden), 8204a(4), 11396ab12 (Teena), 11704ae26 (Shelagh), 13353ab2 (Torridon), 13740(4) (Cramond), 14697a1, 13121ab2 (Stirling), 14897ad17, G7707(2), G8107(1), G8743ac(7), G8776(4), G8866(11), G8884(2) and 15144a3.

Potato cultivars: Maris Piper, Estima, Cara, Pentland Dell, Nadine, Record, Marfona, Maris Bard, Wilja, Saturna, Romano, Pentland Squire, Russet, Fianna, Maris Piper, King Edward, Sante, Pentland Javelin, Morene, Shepody, Premiere, Lumpers, Yam, Fortyfold, Skerry Blue, Myatt's Ashleaf, Pink Fir Apple, Mr Bresee, Asparges, Early Rose, America, Magnum Bonum, Champion, International Kidney, Witchhill, Adirondack, Sutton Early Regent, Rural New Yorker, Satisfaction, Flourball, Puritan, British Queen and Up-to-Date.

Accessions of wild and primitive cultivated species: *Solanum brevidens* (CPC 7135), *Solanum ocranthum* (CPC 1289), *Solanum trifidum* (CPC7125), *Solanum tuquerrense* (CPC7322), *Solanum circaeifolium* ssp. *quimense* (CPC 7088), *Solanum chacoense* (CPC 3872), *Solanum tuberosum* ssp. *andigena* (CPC 395), *Solanum paucijugum* (CPC 7069), *Solanum microdontum* (CPC 4054), *Solanum acaule* ssp. *aemulans* (CPC 7004), *Solanum stoloniferum* (CPC 7201), *Solanum polyadenium* (CPC 7190), *Solanum commersonii* ssp. *malmeanum* (CPC 7058), *Solanum chomatophilum* (CPC 7145), *Solanum megistacrolobum* (CPC 3759), *Solanum bulbocastanum*, *Solanum demissum* (CPC 7101), *Solanum vernei* (CPC4078, a hybrid accession between CPC 2487 × CPC2488), *Solanum pinnatisectum* (CPC 3234), *Solanum papita* (CPC 7081) and *Solanum phureja* (84/2/P75).

DNA isolation from plants

DNA was extracted from frozen plant leaf tissue using the DNeasy Plant DNA Extraction kit (Qiagen, cat no. 69106).

Testing for *G. pallida* cyst numbers

Clear plastic pots (0.35 l) were placed in peat, and half-filled with sand, before being inoculated with 10 ± 1 cysts (*G. pallida*, Pa2/3 population Lindley). Cysts were pre-selected on size, with only those that passed through a 500-µm sieve being used. The pot was then completely filled with sand (total dry weight 450 g) and planted with a tuber piece having a single sprout. There were four replicates for each genotype and the experiment was laid out in a randomised complete block. After 10 weeks, the female nematodes visible through the clear walls of the pot were counted. The variance-stabilising square-root transformation was performed on the female counts prior to carrying out more detailed statistical analyses on the resulting PCN scores.

Simple sequence repeats (SSRs)

The SSRs deployed have been described previously by Milbourne et al. (1998). Sufficient forward primer for 75 PCRs (approximately 50 µmol) was 5′ end-labelled with γ-^{33}P ATP in a 20-µl reaction consisting of 0.3 µmol of primer, 30 uCi of 4500 Ci/mmol γ-^{33}P ATP (ICN), 30 U of T4 polynucleotide kinase (Gibco) and 1 × Gibco Forward Reaction Buffer (70 mM Tris-HCl pH 7.6, 10 mM $MgCl_2$, 100 mM KCl, 1 mM 2-mercaptoethanol), which was incubated at 37 °C for 1 h, then terminated by heating to 95 °C for 10 min.

SSR-PCR reactions (20 µl) consisted of 40 ng of genomic DNA, 1 × PCR buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl pH 8.3), 0.6 U of *Taq* polymerase (Gibco BRL), 0.6 µmol of forward and reverse primers and 400 µmol of dNTPs. The cycling conditions for PCR on a PE9700 thermocycler were as follows: 94 °C for 3 min, followed by 25 cycles of 94 °C for 30 s, annealing temperature for 30 s, 72 °C for 30 s, followed by 72 °C for 5 min. Equal volumes of electrophoresis loading buffer (95% formamide, 10 mM EDTA, 0.5 mg/ml bromophenol blue and xylene cyanol) were added to the samples, which were then denatured at 95 °C for 5 min, snap-cooled on ice, and subjected to polyacrylamide gel-electrophoresis on 5% acrylamide, 7 M Urea in 1 × TBE (89 mM Tris-Borate, 2 mM EDTA). Gels were prepared by adding 250 µl of 10% APS and 100 µl of TEMED to 100 ml of acrylamide solution. Electrophoresis was carried out on the Biorad Sequi-Gen GT system using 1 × TBE buffer. A Promega *fmol* DNA Cycle Sequencing System (Promega Q4100) marker (prepared according to the protocol, but only using 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).

Amplified fragment length polymorphism (AFLP)

AFLP assays were performed using a modification of the protocol of Vos et al. (1995). The 6-bp cutting enzyme *Pst*1 was obtained

from Boehringer Mannheim and the 4-bp cutting enzyme *Mse*1 from New England Biolabs. T4 DNA ligase, T4 polynucleotide kinase, *Taq* DNA polymerase, AFLP primers and adapters were obtained from Gibco BRL. Gel electrophoresis was performed as for SSRs, except that 0.5 × TBE buffer was used.

AFLP fragment nomenclature is as follows: $P_{AB}M_{CDE}xyz$, where 'AB' and 'CDE' are the selective base extensions on the *Pst*I and *Mse*I primers respectively and 'xyz' is the size of AFLP fragment in basepairs.

Bulk construction

Bulks were constructed by selecting the 20 most-resistant and susceptible clones (averaged over replicates) from the Stirling × 12288af23 tetraploid F_1 population. DNA samples, in the form of AFLP secondary template from each selected progeny clone, were pooled in equimolar amounts.

Isolation and cloning of AFLP fragments

The products of the selective AFLP amplification were run on acrylamide gels and the band of interest located by autoradiography. A segment of acrylamide corresponding to the fragment of interest was cut out of the gel and transferred to an Eppendorf tube containing 50 µl of 1 × TE. AFLP fragments were re-amplified non-radioactively using the original AFLP primer combination. PCR products were ligated into pGEM-T Easy (Promega Incorporated) and transformed into *Escherichia coli* strains DH5α or DH10B by electroporation. Electro-competent cells were prepared by standard procedures. Recombinant clones were verified by performing AFLP and running the products against the original targeted AFLP fragment.

DNA sequencing

Sequencing reactions were performed using an Applied Biosytems Perkin Elmer Big Dye Terminator cycle sequencing reaction kit (PE Biosystems, Warrington, UK), M13 universal forward and reverse primers, and an ABI377 automated sequencer (PE Biosystems, LaJolla, Calif., USA).

Sequence alignment

DNA sequences of cloned AFLP or PCR products were aligned using the programme CLUSTALW (Thompson et al. 1994).

PCR

Genomic sequences were routinely amplified in 20–50 µl PCR reactions containing 25–50 ng of template DNA, in the presence of 20 mM Tris-HCl pH 8.4, 1.5–2.5 mM of $MgCl_2$, 50 mM of KCl, 0.05% (v/v) W1, 200 nM of each primer, 100 µM of each dNTP, and 0.4–1 units of *Taq* DNA polymerase. Standard cycling conditions were as follows: initial denaturation step of 3 min at 94 °C, followed by 30 cycles of 30 s denaturation at 94 °C, 1 min annealing at the appropriate T_m, and 1 min extension at 72 °C.

Touchdown-method cycling conditions used for SPUD1636 were as follows: initial denaturation step of 3 min at 94 °C, 1 min at 65 °C, 1 min at 72 °C; followed by five cycles of 30 s at 94 °C, 30 s at 65 °C, 30 s at 72 °C, decreasing 1 degree per cycle; followed by 24 cycles of 30 s at 94 °C, 30 s at 60 °C, 30 s at 72 °C; followed by 5 min at 72 °C.

After cycling, PCR reactions were incubated for 5 min at 72 °C. PCR products were analysed by electrophoresis on agarose gels in 1 × TAE or TBE buffer and visualised by ethidium bromide staining.

PCR primers against cloned AFLP fragments and RFLP probes

AFLP fragment sequences were aligned (at least two sequences per fragment) and PCR primers were designed using PRIMER3 (Rozen and Skaletsky 1998).

Primers designed against sequenced AFLP fragments are as follows:

SPUD1636A:	GTGCGCACAGGGTAAAACC,
SPUD1636B:	ACCTTAGCGGATGAAAGCC,
SPUD1837A:	ATTCCCAGTCTGTTGTTTAC,
SPUD1837B:	GGAATATTGATGCTAATTTGG.

Primers designed against the potato RFLP probe, GP268, were:

GP268F:	AACCCGAAGTAACGCCTAAG,
GP268R:	CCATTACCCCAAATCAACAT.

Data analysis and genetic mapping

Autoradiograms were scored independently by two people using the CrossChecker software package (Buntjer 1999). Map construction was performed using Joinmap (Stam 1993) and MapQTL (Van Ooijen and Maliepaard 1996a, 1996b). Maps were drawn using the program MAPCHART v1.43 (Voorrips 2000). Genstat 5 (version 4.1) was also used for statistical analysis of mapping data. Broad-sense heritability (h^2_b) of the PCN score was estimated with the following formula:

$$h^2_b = \sigma^2_g / [\sigma^2_g + (\sigma^2_e / r)]$$

where σ^2_g = genetic variance, σ^2_e = error variance, and r = number of replicates.

Results

Bulk segregant analysis in the Stirling × 12288af23 population

The Stirling × 12288af23 population, comprising 384 F_1 individuals, was subjected to bulk segregant analysis (Michelmore et al. 1991) in order to identify AFLP markers that are linked to components of resistance to *G. pallida*. The population was scored for female cyst number using a replicated pot test and ranked on the basis of average (over replicates) PCN scores (square root of female counts). Figure 1a shows the distribution of scores in the F_1 population used. Bulks were constructed from the 20-most resistant (PCN scores: 1.82–3.51, mean = 2.77) and 20 most-susceptible (PCN scores 7.04–8.46, mean = 7.58) clones from this progeny. These bulks were screened with 96 PstI/MseI '+2/+3' AFLP primer combinations (i.e. *Pst* + 2 bp, *Mse* + 3 bp), three of which generated AFLP markers present only in the resistant bulk (or showed a very strong intensity difference between the two bulks), suggesting linkage to components of PCN resistance. The AFLP primer combinations and product sizes were: $P_{CC}M_{ACC}285$, $P_{CT}M_{ACG}172$, $P_{AG}M_{ATT}142$. Band presence/absence from individuals within the bulks was used to confirm linkage to components of resistance. In all three cases the AFLP primers were applied to 231 of the individual plants within the Stirling × 12288af23 population and the presence or absence of the 'test' fragments scored. The marker scores were used as the independent variable in a

Table 1 Segregation data of AFLP markers identified by bulk segregant analysis of the Stirling × 12288af23 population. The table shows segregation data for R and S bulks and for the entire populations, as well as the proportion of phenotypic variance accounted for by the presence/absence of each marker (i.e. R^2 values). All population-level segregation ratios conform to a 1:1 ratio (all chi-square values NS at the 5% level). All segregation ratios in bulks are highly significantly different from 1:1

AFLP marker	Segregation ratio in bulks (+/–)		Segregation ratio in population (+/–)	% Phenotypic variance R^2
	R	S		
$P_{CC}M_{ACC}285$	17/3	1/19	93/119	18%
$P_{CT}M_{ACG}172$	18/2	1/19	103/126	15%
$P_{AG}M_{ATT}142$	15/5	3/17	102/125	15%

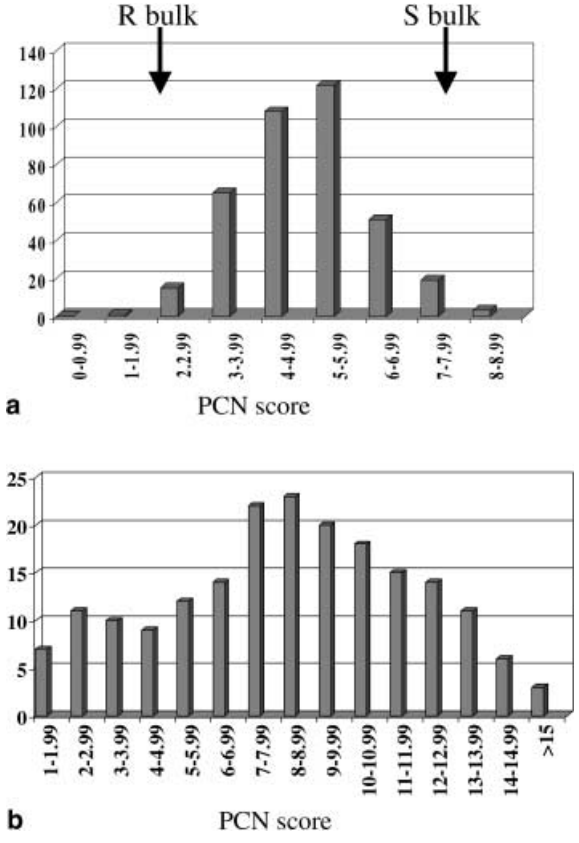

Fig. 1a, b Histograms of PCN scores (square root of female counts) from two potato populations. **a** and **b** show frequency distributions of PCN data for Stirling × 12288af23 (384 progeny) and PDH538 × IVP48 (195 progeny) populations respectively

simple linear regression analysis to determine the proportion of the variability in PCN scores explained by each of the three markers. The markers showed virtually identical patterns of inheritance across the population and so were most likely linked to a single QTL in the resistant parent of the cross. Table 1 shows the number of plants in each bulk in the population, the presence/absence of the three 'test' fragments, and the R^2 values. These markers segregate in an approximately 1:1 ratio, from which we infer that they, as well as the QTL to which they are linked, are present in the simplex (i.e. a single dose) condition in the resistant parent of the cross.

Conversion of AFLPs to PCR-based markers

Each of the three AFLP fragments was excised from an AFLP gel and ligated into pGEM-T Easy and sequenced. PCR primers were designed to each of the sequenced AFLP fragments and used to amplify the corresponding locus from the parents of both the G87 × I88 and Stirling × 12288af23 populations. Sequenced AFLP fragments were searched against sequence databases using BLAST software. Part of the $P_{CC}M_{ACC}285$ sequence shows very high levels (>90%) of homology (approximately 100 bp) to a number of potato/tomato genes of tomato and potato sequences (e.g. tomato Zn superoxide dismutase, tomato farnesyl-protein transferase, and the potato STAC1 gene for amino cyclopropane carboxylate synthase). Further investigation has revealed that the homologies are restricted to introns and other non-coding regions of these genes, suggesting that they represent short repetitive sequences in the tomato and potato genomes. The other two AFLP fragments detected no significant similarities to sequences in the databases.

Primers designed from $P_{AG}M_{ATT}142$ failed to produce the expected PCR product using genomic DNA. Primers designed from the $P_{CC}M_{ACC}285$ sequence (SPUD1636A and SPUD1636B) and from $P_{CT}M_{ACG}172$ (SPUD1837A and SPUD1837B) amplified products of the expected size [226-bp (see Fig. 2) and 129-bp respectively] from each of the four parental lines and from *S. vernei* accession CPC4078. These primers were also used to test the AFLP template from the population to confirm that they produced an identical amplification profile as the AFLP fragments from which they are derived. SPUD1636 and SPUD1837 PCR products from the parental lines G87 and I88 were cloned and sequenced, and aligned with the original AFLP fragment using CLUSTALW. Our objective was to discover DNA sequence polymorphism [e.g. a single nucleotide polymorphism (SNP) or a small insertion/deletion event] that could be exploited to locate the fragments onto the potato genetic linkage map and, albeit indirectly, the chromosomal location of the resistance QTL detected by BSA. A comparison of sequences from SPUD1837 for these genotypes showed a 3-bp length polymorphism, which was heterozygous in G87 and provided the basis for mapping the SPUD1837 locus.

The segregation data placed SPUD1837 on linkage group V, close to the tomato SSR marker LERNALX

Table 2 PCN data for SCRI breeding lines. All values are means of six replicate tests

Basis for use in breeding programme	Clone name	PCN score
S. vernei-derived PCN resistance	12288af23	2.36
	12385ab6	1.53
	14969ac9	1.24
	15119ac5	2.82
S. tuberosum ssp. *andigena* 'H3'-derived PCN resistance	12601ab1	1.02
	12636a2	2.34
	12674ab1	1.54
	14069a4 (Eden)	3.89
S. demissum-derived late blight resistance	8204a(4)	10.12
	11396ab12 (Teena)	10.56
	11704ae26 (Shelagh)	9.33
	13353ab2 (Torridon)	7.81
	13740(4) (Cramond)	8.73
	14697a1	10.91
	13121ab2 (Stirling)	9.74
	14897ad17	13.16
Virus resisters with complex pedigrees	G7707(2)	8.71
	G8107(1)	8.43
	G8743ac(7)	9.93
	G8776(4)	9.39
	G8866(11)	7.98
	G8884(2)	9.74
	15144a3	10.31

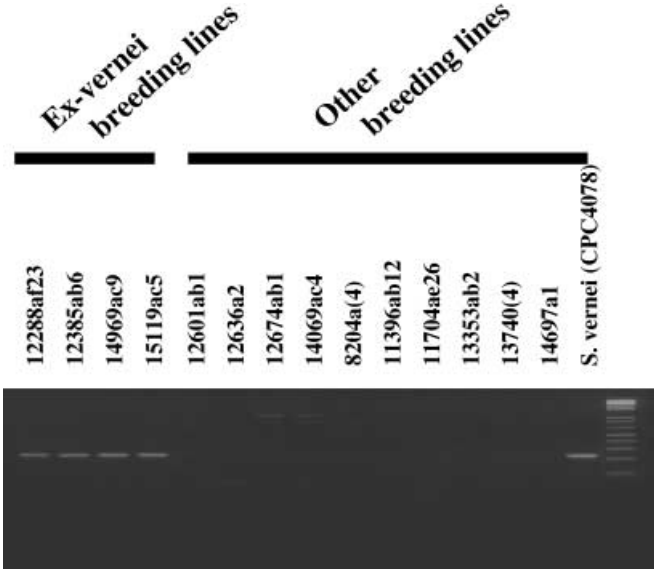

Fig. 2 Agarose gel showing PCR-based assay for the component of *S. vernei* resistance in tetraploid potato germplasm using SPUD1636 primers, which detect a 226-bp PCR product

(Smulders et al. 1997; Milbourne et al. 1998) (data not shown). This SSR was then retrospectively applied to the entire Stirling × 12288af23 population and showed virtually the same segregation pattern as SPUD1837 (only five recombinants) confirming tight linkage of the two markers, and supporting our conclusion that the PCN-QTL maps to linkage group V. SPUD1636 generally failed to amplify, or only poorly amplified, a product from material other than 12288af23, PDH538 and various *S. vernei* accessions.

Application of PCR markers to germplasm carrying *S.vernei*-derived resistance

As SPUD1636 only poorly amplified a product from samples other than 12288af23, PDH538 and *S. vernei* accessions, we further increased the specificity of this reaction by developing a stringent touchdown PCR assay. This touchdown PCR assay was then applied to the 45 most-resistant and 45 most-susceptible clones from the Stirling × 12288af23 population. Thirty three (73%) and five (11%) of the most-resistant and most-susceptible plants respectively generated this fragment, confirming linkage between the marker and a component of resistance (both X^2 values highly significant). Twenty three tetraploid potato breeding lines were then also tested to establish whether or not they supported the amplification of SPUD1636. Four genotypes supported the amplification of this fragment (12288af23, 12385ab6, 14969ac9 and 15119ac5) and these are all known to have been descended from crosses involving PCN-resistant *S. vernei* accessions (see Materials and methods) and to contain high-levels of resistance to *G. pallida.* From Table 2, it can be seen that the four breeding lines which support amplification of SPUD1636 show low PCN scores (1.24–2.82). Lines selected on the basis of late blight and virus resistance, and which do not support amplification of SPUD1636, show much higher PCN scores (7.81–13.16). The four lines selected as donors of 'H3' resistance (from *S. tuberosum* ssp. *andigena*) show low PCN scores (1.02–3.89), despite not amplifying the SPUD1636 marker. These results support the notion that the SPUD1636 marker can be deployed in an effective manner to select for a component of PCN resistance derived from *S. vernei*. The SPUD1636 touchdown assay

was then applied to 43 potato cultivars, including Sante, Spey and Nadine (all thought to contain resistance to *G. pallida* derived from *S. vernei*) and 21 wild and primitive cultivated species of potato, including *S. vernei*. PCN scores are not available for all of these cultivars or accessions. SPUD1636 failed to generate the specific 226-bp PCR fragment from any of these cultivars or accessions, other than *S. vernei* and the cultivar Sante. Hence, under very specific PCR conditions this marker is only detectable in *S. vernei* and *S. tuberosum* clones containing high-levels of *S.vernei*-derived resistance to *G. pallida*. These results are consistent with the conclusion that SPUD1636 is derived from a segment of chromosome V that has been introgressed into *S. tuberosum* from *S. vernei*. To rule out the possibility of failed PCR reactions, all assays were repeated in the presence of primers to the RFLP probe GP268. The expected GP268 product was generated in all assays (data not shown).

Linkage mapping in the diploid population PDH538 × IVP48

A population generated from a cross between the resistant *S. tuberosum* dihaploid PDH538 and the susceptible *S. phureja* clone IVP48, segregated for *S. vernei*-derived resistance to *G. pallida* (Fig. 1b). This population was used to analyse vernei-derived resistance at the diploid level. The male parent of this cross was the *S. phureja* clone IVP48, which is thought to be relatively highly inbred and very susceptible to *G. pallida* Pa2/3. This population, comprising 138 individuals, was scored for cyst number using a pot test with four replicates, and in parallel was analysed with 20 AFLP primer combinations and the following 18 potato SSRs: STM0003, STM0019, STM0024, STM0037, STM0038, STM1008, STM1020, STM1049, STM1051, STM1069, STM1104, STM2005, STM2012, STM2013, STM2020, STM3016 (Milbourne et al. 1998) and STM_GP179 (developed from a potato BAC clone identified using PCR primers to the linkage group V RFLP marker GP179), and a tomato SSR, LERNALX, previously mapped in potato to linkage group V (Smulders et al. 1997; Milbourne et al. 1998). On preliminary examination of the data it was apparent that a very high proportion of segregating markers (>80%) had originated from the PDH538 parent, presumably owing to the highly homozygous state of the susceptible *S. phureja* parent. We, therefore, decided to remove the IVP48 specific markers (i.e. ⟨aa × ab⟩) from the dataset and analyse only markers segregating from PDH538 (i.e. ⟨ab × aa⟩, ⟨ab × ab⟩ etc), the source of the PCN resistance. Four additional markers were added to the dataset: SPUD1837 (which was also analysed on the Stirling × 12288af23 population) and CAPS markers derived from chromosome V RFLP probes (GP21, GP179 and TG432) and a chromosome IX probe (CT220) (Meksem et al. 1995). The CAPS markers were analysed using the same enzyme digestion conditions as reported by Rouppe van der Voort et al. (2000). Unfortunately, CT220 and TG432 did not segregate in the PDH538 parent and were discounted from linkage analysis. However, the segregation pattern of CT220 in IVP48 relative to AFLP markers segregating from both parents did help in orientating the map of linkage group IX from PDH538 and in comparing our map with that of Rouppe van der Voort et al. (2000).

JOINMAP analysis of the marker data from 384 loci, identified 12 linkage groups at a LOD threshold of 5.5. Chromosomes were identified by use of SSRs and CAPS previously mapped on other potato populations (Milbourne et al. 1998; Collins et al. 1999; Rouppe van der Voort 2000). At this LOD threshold, linkage group XII remained split into two groups. As each contained only AFLP markers, it is impossible to say unequivocally that these actually do represent linkage group XII. However, as all of the other potato chromosomes were assigned using SSRs of known map location, it is likely that they represent segments of linkage group XII. Attempts to map linkage group XII SSRs were not successful. A linkage map of PDH538 is shown in Fig. 3. The map spans a total of 1,086 cM, with the longest linkage group being 131 cM (LG III) and the shortest 41 cM (LG V).

QTL mapping of the PDH538 × IVP48 population

The PDH538 × IVP48 linkage map data were used to perform QTL analysis using the PCN cyst count data. Results from the non-parametric Kruskal-Wallis test suggested the existence of QTLs for PCN resistance on linkage groups V and IX. A *P* value of 0.001 was used as a threshold-criterion for QTL detection. The QTL on linkage group V appears to be spread across a large number of markers, occupying approximately 60% of the length of the map. The range of *P* values suggests the possibility of two QTLs on this linkage group, one centred near the CAPS marker GP179/*Rsa*I and one further down near SPUD1837 and LERNALX, both shown above to be linked to a QTL in the Stirling × 12288af23 population. For chromosome IX the situation is much simpler with a single QTL centred around AFLP marker $P_{CT}M_{ATC}$ 146.9. A more rigorous QTL analysis was then carried out using the 'interval mapping' option of the MapQTL software. It gave similar results to the Kruskal-Wallis test. The maps of linkage groups V and IX and the approximate positions of the QTLs, denoted by LOD score plots, are shown in Fig. 4. If it is assumed that a single QTL is present on each linkage group, the marker showing the highest LOD scores, STM_GP179 on linkage group V and $P_{AT}M_{AAC}$ 184.0 on linkage group IX, explain 24% and 17.6% of the phenotypic variation in PCN scores. The interval mapping data for linkage group V hint at the possibility of a second QTL, close to marker $P_{AG}M_{ACT\_}$ 149.5, which, if present, would explain a further 16% of the phenotypic variation. However, the situation is complicated by the high degree of segregation distortion shown for markers on linkage group V in this cross. For example, markers near the top of the chro-

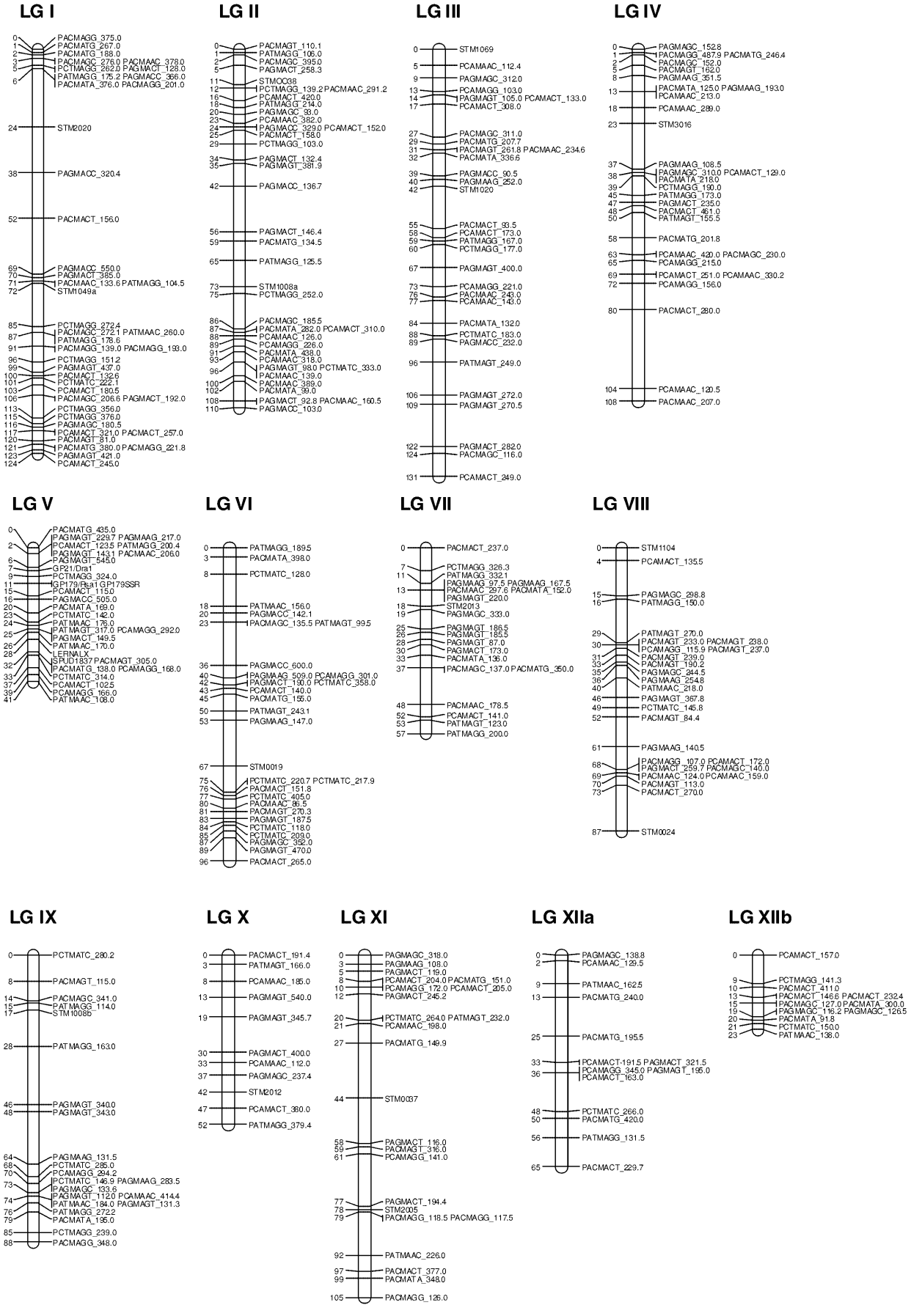

Fig. 3 Map of PDH538 (female parent of the PDH538 × IVP48 population). The total map length is 1,086 cM

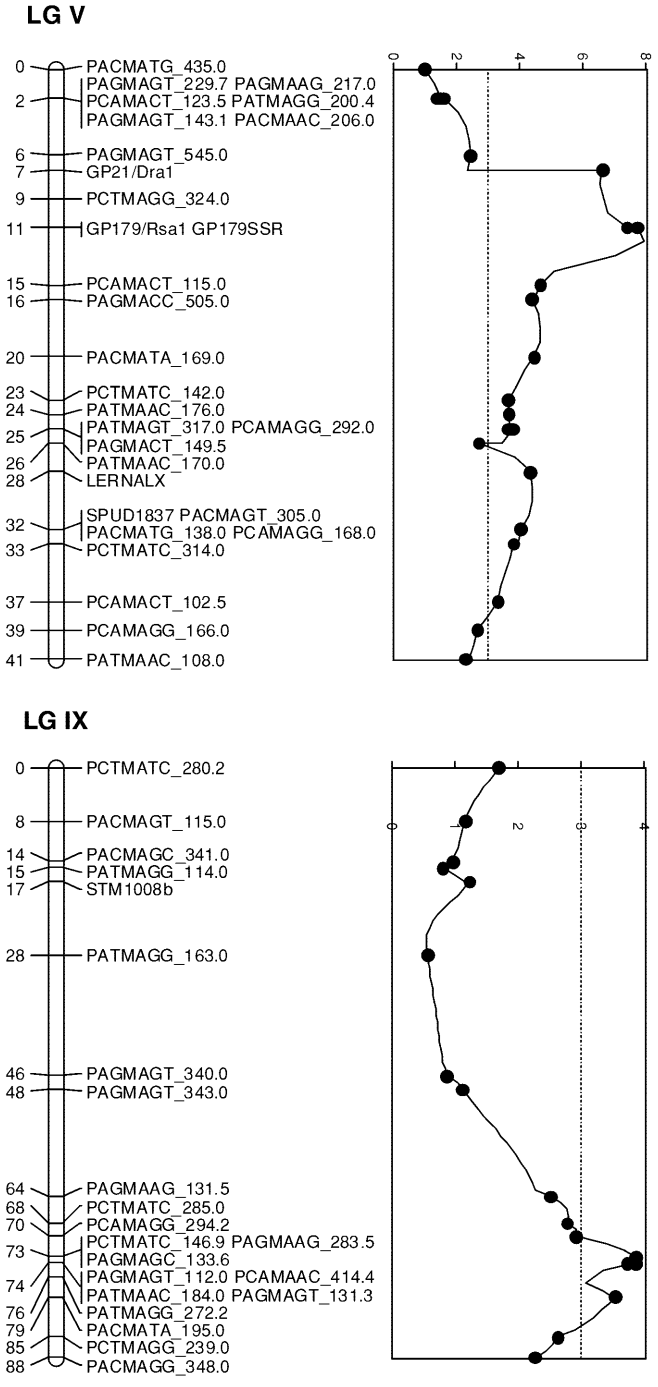

Fig. 4 LOD plots of linkage groups V and IX from interval mapping using MapQTL. The LOD threshold has been set at 3.0

mosome are showing segregation ratios approaching 10:1 in favour of the alleles for susceptibility. From the top, this level of segregation distortion drops sharply to a ratio of approximately 2:1 from marker $P_{AT}M_{AAC}$ 170.0, which is roughly 26 cM down the chromosome, then remains roughly constant. It is not clear to what extent this skewing affects QTL detection in this cross.

On the assumption that there are three QTLs detected, we have performed an ANOVA using three of the most-tightly linked markers (GP179, SPUD1837, PACMATA_195.0). The ANOVA suggests that these loci are acting in a simple additive fashion and all interaction mean squares are not significant (data not shown). The ANOVA also suggests that the small QTL near SPUD1837 is at the borderline of significance (P = 0.06), which is in approximate agreement with the MapQTL results. The overall mean PCN score is 7.92 and clones lacking any of the markers linked to the resistant QTL, have a mean score of 9.65 (n = 46), whereas clones with all three markers show a mean score of 3.08 (n = 10). The broad-sense heritability of PCN scores (square-root of cyst number) was estimated as 0.88 from the components of variance for F_1 clones (σ^2_g) and replicate × F_1 clones interaction (σ^2_e).

Discussion

In this paper we analyse the genetic architecture of resistance to the PCN species *G. pallida* pathotypes Pa2/3 derived from the wild diploid potato species, *S. vernei*. The analysis of two potato populations segregating for PCN resistance has indicated the contribution of at least two, but more-likely three, QTLs on chromosomes V and IX. *S. vernei*-derived resistance has also been studied by Rouppe van der Voort et al. (2000), who also localised QTLs for resistance to *G. pallida* on linkage groups V and IX. The resistant parent used in their study (JP, also known as 3704–76) is a diploid derived by prickle-pollination from a tetraploid clone (AM78-3704), which contains *S. vernei* "24/20", as well as other *S. vernei* and wild potato species, in its pedigree. "24/20" also features in the pedigree of PDH538. This raises the question of whether the QTLs detected in the two different studies are allelic, or whether they originate from different but linked loci. Prior to the analysis with the CAPS markers, GP21, GP179 and CT220, a complete lack of common markers between the studies precluded any such comparisons. As a result, the overall correspondence between the datasets suggests that the QTL identified in both studies are the same, especially when the similarity in the origin of the material is taken into consideration. That the CAPS markers were mapped using the same restriction enzymes in the two studies, further supports a common origin of the chromosomal segments involved. Further analyses are required to fully resolve these questions.

Our adoption of a bulking strategy for the identification of molecular markers linked to QTLs for PCN resistance in a tetraploid progeny was effective, and three AFLPs linked to a single resistance QTL were identified. These AFLPs explain similar proportions of the variance in the trait under study (approximately 15–20%), and show almost identical segregation patterns in the population. One of the detected AFLP markers, following conversion to a single-locus PCR-based marker (SPUD1837), was successfully mapped on a diploid reference population to linkage group V. This result was confirmed by mapping a linked SSR back onto the

tetraploid potato population. A second AFLP was converted to a PCR-based marker (SPUD 1636) which, in all cases tested to-date, can detect the chromosomal segment carrying the *S.vernei*-derived resistance QTL, which has been introgressed into linkage group V. Further work is required to verify this observation and to ascertain the extent of the introgressed segment. Nevertheless, our data suggest that the segment may be quite large, which has implications for potato breeding, where large amounts of linkage 'drag' are undesirable as they may have deleterious effects on the agronomic qualities of the breeding material under selection. It is possible that, by using species-specific PCR-based markers or cytological methods such as GISH, the extent of introgressed segments can be determined.

The use of BSA for QTL detection is becoming increasingly popular and strategies for using this approach have been reviewed in Wang and Paterson (1994) and more recently by Mackay and Caligari (2000). It is generally accepted that only QTLs of relatively large effect can be detected using this approach and this is consistent with our findings. However, we were surprised that only a single QTL was discovered in our analysis of chromosome V and that we did not detect markers linked to a QTL on linkage group IX. Of course this QTL may not be present or segregating in the tetraploid population under study. Disappointingly, the bulking strategy only accounted for a small proportion of the phenotypic variance in PCN score. This could partly be due to 'loose' linkage between marker and QTL.

Analysis of the PDH538 × *S. phureja* diploid population revealed QTLs contributing to PCN resistance on linkage groups V and IX. Heritability estimates using the replicate data for this population suggest that high proportions of the phenotypic variance (approximately 88%) can be explained by genetic factors. In contrast, the QTLs detected explain only 42% of the phenotypic variation in PCN score. This compares with a total proportion of approximately 85% accounted for by the QTLs detected on linkage groups V and IX by Rouppe van der Voort et al. (2000). The reasons for this difference are unclear. It may be that the very high level of skewing in favour of alleles for susceptibility on linkage group V have affected our ability to detect this QTL or are reducing its apparent effect on the trait under study. It is also possible that there are two or more linked QTLs segregating on linkage group V and that we are unable to separate them. The LOD plots shown in Fig. 4 support this hypothesis to some extent.

It is highly likely that the same resistance QTL on linkage group V was detected in both the diploid and tetraploid populations. PDH538 may be segregating for two QTLs on this chromosome. SPUD1636, a *vernei*-specific PCR marker developed in this study, detects the same QTL in the resistant parents of both populations. As the source of resistance in the pedigrees of both resistant parents is the same, and linkage to common markers is detected, it is probable that the QTLs are identical by descent. The QTLs on linkage groups V and IX, reported by Rouppe van der Voort et al. (2000), have been designated *Gpa5* and *Gpa6* respectively. It seems likely that these are the same or similar QTLs as those reported here. Thus, we have refrained from giving the QTLs discovered here new names.

Analysis of potato germplasm using SPUD1636 supports the conclusion that it resides on an introgressed segment of linkage group V from *S. vernei*. SPUD1636 is only detectable in germplasm known to carry the *S. vernei*-derived PCN resistance QTL on chromosome V and accessions of *S. vernei*. Furthermore, it is interesting that of the three potato cultivars stated to contain components of resistance to *G. pallida* derived from *S. vernei*, only Sante and Spey have been shown to contain this marker, whereas Nadine does not. This very easily deployable marker offers an obvious route to the use of marker-assisted selection or introgression for this component of resistance. It will be interesting to pursue this matter further and to verify whether some of the markers detected are on an introgressed segment from *S. vernei*. If they are, it may help explain the high level of segregation distortion observed and its direction (i.e. against the introgressed resistance alleles). Similar levels of skewed segregation have been observed previously for potato linkage group V. For example, the G87 × I88 population used in this study shows segregation ratios of approximately 5:1 in the region of linkage group V containing markers GP21 and GP179 (Collins et al. 1999). This region is known to harbour numerous disease resistance and other important genes, many of which have been introgressed from wild species of potato. Potential effects of these introgressed segments on viability may help explain the aberrant segregation ratios obtained in crosses. To what extent introgressed genetic material is affecting potato genetics and breeding through aberrant segregation ratios is not clear. However, the use of sequence-characterised markers and cytological techniques on material known to contain large segments of introgressed chromosomal material may cast further light on these and related phenomena.

A large number of recent genetical studies has documented the presence of resistance genes, as well as genes influencing maturity and vigour, in the GP21–GP179 region of chromosome V (Kreike et al. 1994; Leonards-Schippers et al. 1994; De Jong et al. 1997; Collins et al. 1999). In this study, and in that by Rouppe van der Voort et al. (2000), the largest QTL detected also maps to this region. It is becoming increasingly clear that a detailed molecular and genetic characterisation of this important chromosomal interval is required. This will yield information of great utility for studies aimed at the isolation and functional analysis of individual genes. Moreover, as a considerable number of resistance alleles have been introgressed into cultivated potato from wild or primitive cultivated Solanum species, a structural analysis of this region will provide a platform for the study of its evolutionary biology. It is likely that efforts are underway to isolate individual genes (e.g. R1, Nb) in this region (Marano et al. 2001). It is vitally important that resourc-

es (e.g. BAC clones, markers etc.) arising from such projects are used to perform comparative studies of this important chromosomal region.

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