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ORIGINAL PAPER

A high-resolution map of the *Grp1* locus on chromosome V of potato harbouring broad-spectrum resistance to the cyst nematode species *Globodera pallida* and *Globodera rostochiensis*

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Abstract The *Grp1* locus confers broad-spectrum resistance to the potato cyst nematode species *Globodera pallida* and *Globodera rostochiensis* and is located in the GP21-GP179 interval on the short arm of chromosome V of potato. A high-resolution map has been developed using the diploid mapping population RHAM026, comprising 1,536 genotypes. The flanking markers GP21 and GP179 have been used to screen the 1,536 genotypes for recombination events. Interval mapping of the resistances to *G. pallida* Pa2 and *G. rostochiensis* Ro5 resulted in two nearly identical LOD graphs with the highest LOD score just north of marker TG432. Detailed analysis of the 44 recombinant genotypes showed that *G. pallida* and *G. rostochiensis* resistance could not be separated and map to the same location between marker SPUD838 and TG432. It is suggested that the quantitative resistance to both nematode species at the *Grp1* locus is mediated by one or more tightly linked *R* genes that might belong to the NBS-LRR class.

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Introduction

The potato cyst nematode (PCN) species *Globodera pallida* and *Globodera rostochiensis* cause serious yield losses in potato crops worldwide (Oerke et al. 1994). PCN can be controlled by crop rotation, chemical soil disinfestations and the use of resistant cultivars. However, due to the formation of cysts, PCN can survive in the soil for many years in the absence of a host, making crop rotation unattractive for potato farmers. Chemical control of PCN involves very unspecific and extremely harmful pesticides. Due to increasing concern about environmental issues and governmental regulations, this method has been practically abandoned in many countries. Therefore, resistant cultivars are becoming increasingly important and, hence, scientific studies on the underlying genes and resistance mechanisms are of great interest.

A total of 14 PCN resistance loci have been mapped in potato on chromosomes III, IV, V, VII, IX, X, XI and XII (reviewed by Gebhardt and Valkonen 2001; Caromel et al. 2003, 2005. Ten resistance traits confer partial resistance (*Gro1.4, Gpa4, Gpa, Gpa5, Grp1, Gpa6, Gro1.2, Gro1.3* and *GpaM1*), while four of them (*H1, GroVI, GroI* and *Gpa2*) and the combination of $GpaV^{s}_{spl}$ and $GpaXI^{s}_{spl}$ confer nearly absolute resistance to one or more pathotypes. Many

of these PCN resistance loci are mapped in regions of the potato genome where clusters of resistance gene homologues are located. This is not only true for the single dominantly inherited PCN resistance (*R*) genes *Gpa2* and *Gro1* (Barone et al. 1990; Rouppe van der Voort et al. 1997), but also for quantitative trait loci (QTL) such as *Grp1, Gpa, GpaV*$_{spl}$, *GpaM1* and *Gpa5* (Caromel et al. 2003, 2005; Kreike et al. 1994; Rouppe van der Voort et al. 1998, 2000). So far, the only nematode *R* genes that have been characterised at the molecular level in potato are *Gpa2* and *Gro1* (Paal et al. 2004; Van der Vossen et al. 2000).

Resistance conferred by the *Grp1 locus* was discovered in 1998 in the tetraploid clone AM78-3778, an interspecific hybrid between *S. tuberosum* and several wild potato species including *S. vernei, S. oplocense* and *S. tuberosum* ssp. *andigena* (Rouppe van der Voort et al. 1998). This locus is particularly interesting since it confers major resistance to the potato cyst nematode (PCN) pathotypes Ro5 (*G. rostochiensis* line 22) and Pa2 (*G. pallida* population D383), as well as partial resistance to pathotype Pa3 (*G. pallida* population Rookmaker). The *Grp1* locus has been mapped in the GP21-GP179 interval on the short arm of chromosome V of the diploid potato clone 3778-16, which is derived from AM78-3778 (Rouppe van der Voort et al. 1998). This region is known to harbour resistance loci with specificities to different plant pathogens, including *R1* (Leonards-Schippers et al. 1992) and a major QTL (Leonards-Schippers et al. 1994) to *Phytophthora infestans*, *Rx2* (Ritter et al. 1991) and *Nb* (De Jong et al. 1997) to potato virus X, as well as *Gpa5* (Rouppe van der Voort et al. 2000), *Gpa* (Kreike et al. 1994), *GpaM1* (Caromel et al. 2003) and *GpaV*$^{s}_{spl}$ (Caromel et al. 2005) to *G. pallida*. A QTL involved in trichome-mediated insect resistance has also been detected in this region (Bonierbale et al. 1994). *R1* and *Rx2* have been shown to belong to the nucleotide binding site leucine-rich repeat (NBS-LRR) superfamily of *R* genes (Ballvora et al. 2002; Bendahmane et al. 2000).

The broad-spectrum resistance of *Grp1* together with its location in a hotspot of resistance led to the hypothesis that *Grp1* is a compound locus containing different *R* genes for PCN resistance (Rouppe van der Voort et al. 1998). This is strengthened by the fact that AM78-3778 is the result of many generations of breeding, involving different wild *Solanum* sources (Dellaert and Vinke 1987) and by the detection of the *G. pallida* resistance locus *Gpa5* in clone 3704-76, which is very closely related to 3778-16 (Rouppe van der Voort et al. 2000). The use of common markers for the mapping of the QTL for both *Grp1* and *Gpa5* showed that the two QTLs perfectly superimpose on each other, which indicates the presence of similar introgression segments. Since *G. pallida* resistance has been an important trait for the selection of both breeding lines, it is argued that the same gene(s) underlie(s) the *G. pallida* resistance. It is assumed that the *G. rostochiensis* resistance may have been lost during the course of the breeding process due to a recombination between loci TG432 and GP179 in a progenitor of clone 3704-76.

In this study, a high-resolution map of the *Grp1* locus has been constructed, using QTL analysis. The resulting high resolution map shows similar QTLs for both *G. rostochiensis* and *G. pallida* resistance close to marker TG432. After classification of the genotypes for resistance to *G. pallida* Pa2 and *G. rostochiensis* Ro5, *Grp1* resistance behaved as a monogenic *R* gene and could be mapped between the markers SPUD839 and TG432. The segregating alleles of the two flanking markers are in coupling phase with and tightly linked to the *Grp1* locus (0.9 and 0.2 cM, respectively). Together with information on candidate genes in the area, this will form the basis for the identification of the gene(s) underlying *Grp1* resistance to populations of both *G. pallida* and *G. rostochiensis*.

Materials and methods

Plant material

A population (F1AMRH) of 1,536 F1 genotypes from the cross between the diploid potato clones 3778-16 (AM) × RH89-039-16 (RH) were used (Park et al. 2005). The female parent (AM) harbours the *Grp1* locus that confers major resistance to the PCN pathotypes Ro5 (*G. rostochiensis* line 22) and Pa2 (*G. pallida* population D383), as well as partial resistance to pathotype Pa3 (*G. pallida* population Rookmaker) and was produced by prickle pollination of the tetraploid potato clone AM 78-3778 with haploid *S. phureja* inducer clones (Rouppe van der Voort et al. 1998). AM78-3778 is an interspecific hybrid between *S. tuberosum* and several wild potato species including *S. vernei* 24/20, *S. vernei* ssp. *ballsii* 2/1, *S. vernei* LGU 8, *S. oplocense* EBS 1786 and *S. tuberosum* ssp. *andigena* CPC 1673. The male parent (RH) is fully susceptible to all potato cyst nematode populations tested.

Marker analysis

Genomic DNA from AM, RH and progeny was available (Park et al. 2005). CAPS markers GP21 and GP179 were used as described (Rouppe van der Voort et al. 1998). Thirteen PCR markers were designed based on sequence information derived from chromosome V of *S. demissum* or from the GABI database (Riano-Pachon et al. 2009). The DNA sequences of the PCR primers, their background and the corresponding thermal cycling conditions used for each are presented in Table 1. Five primer combinations resulted in AM allele specific PCR markers. The remaining

Table 1 Details for markers mapped in the GP21–GP179 interval of the diploid potato clone AM

Marker	Type	Enzyme	Origin/source	Primer sequences (forward/reverse)	Fragment size (bp)	PCR conditions
GP21	CAPS	DraI	Meksem et al. (1995)	GGTTGGTGGCCTATTAGCCA/ GCTCCAACACGGAAGGTTTTC	850	93°C, 45 s; 55°C, 45 s; 72°C, 2 min 30 s; 40×
SPUD839	CAPS	Tsp509I	De Jong et al. (1997)	GACATGAGTTTTAGCAACAGTG/ AAGTAGCTGATATTGGGGATTC	420	94°C, 1 min; 50°C, 1 min; 72°C, 2 min; 30×
TG432	CAPS	RsaI	De Jong et al. (1997)	GGACAGTCATCAGATTGTGG/ GTACTCCTGCTTGAGCCATT	1,900	94°C, 30 s; 66°C, 45 s; 72°C, 2 min 30 s; 35×
Ba47f2	CAPS	AluI	http://gabi.rzpd.de	GAAGATGTTAACGTGCGGGG/ CGCTACAGTGTCCCTAGTAG	449	94°C, 1 min; 60°C, 1 min; 72°C, 2 min; 30×
SD1	PCR marker	–	Genbank AC146506	CATAGCGGGATACATAGCG/ CGTTTGCGTCTCATTTGGTC	593	94°C, 30 s; 55°C, 1 min; 72°C, 1 min 30 s; 35×
SD2	PCR marker	–	Genbank AC149487	TCAGTTGGGATGTGATGGAG/ GTGTCCAACACCTTAGTCAG	430	94°C 1 min; 60°C, 1 min; 72°C 2 min; 35×
Ba213c14	CAPS	NlaIII	http://gabi.rzpd.de	TTAACAAGAACGCCGAAGAC/ GGCTGGAAGAGCTTGCAAG	434	94°C 1 min; 58°C, 1 min; 72°C, 2 min; 35×
Ba87d17	CAPS	DraI	http://gabi.rzpd.de	CCAGCCACAACTAGAACATG/ TCAATCGCCTTACTTCCCTG	334	94°C 1 min; 60°C, 1 min; 72°C, 2 min; 35×
Ba76o11	CAPS	MseI	http://gabi.rzpd.de	ACAACCACACAACCATTGCG/ TTTGCCTACTTGGAAGGAGG	375	94°C 1 min; 60°C, 1 min; 72°C, 2 min; 30×
p3f8	PCR marker		http://gabi.rzpd.de	GCTGTGAGGGCCTTGAGG/ CGACTCAGCTGAGCCCTG	260	94°C 1 min; 66.5°C, 1 min; 72°C, 2 min; 40×
Ba43a11	CAPS	NlaIII	http://gabi.rzpd.de	GGATCAACTGATCACAAGGG/ CTGGAGCACAAGTTCTATCG	593	94°C, 1 min; 60C, 1 min; 72°C, 2 min; 30×
SD3	CAPS	NlaIII	Genbank AC151815	ACGTGGGAGTTGTGGGAAG/ GCAACAACATAGACGTGACG	760	94°C, 1 min; 60°C 1 min; 72°C, 2 min; 35×
SD4	CAPS	NlaIII	Genbank AC144791	AAGACCGGGCACAGGGAG/ GTCCTCTAGTGTAGGTATGG	900	94°C 1 min; 50°C 1 min; 72°C, 2 min; 40×
SD5	PCR marker		Genbank AC149301	AGTATGACGGAGGTATCTGC/ CAACGTCCTGATACATCGTG	1,209	94°C, 30 s; 55°C, 1 min; 72°C, 1 min 30 s; 35×
SD6	CAPS	AluI	Genbank AC135288	GTGGATACTTGAGCGATGTG/ CTTGGAGATGTGGGATTTCG	829	94°C, 30 s; 60°C, 1 min; 72°C, 1min30 s; 35×
SPUD1636	PCR marker		Bryan et al. (2002)	GTGCGCACAGGGTAAAACC/ ACCTTAGCGGATGAAAGCC	210	94°C, 1 min; 60°C, 1 min; 72°C, 2 min; 35×

Table 1 continued

Marker	Type	Enzyme	Origin/source	Primer sequences (forward/reverse)	Fragment size (bp)	PCR conditions
SD7	PCR marker		Genbank AC139840	TGGATGTTACTGTGTGGAGG/ TTCCTTCTCAGTAGCAGCAG	827	94°C, 30 s; 63°C, 1 min; 72°C, 1 min 30 s; 35×
GP179	CAPS	RsaI	Meksem et al. (1995)	GGTTTTAGTGATTGTGCTGC/ AATTTCAGACGAGTAGGCACT	450	93°C, 45 s; 55°C, 45 s; 72°C, 1min 20 s; 40×

PCR markers segregate from the resistant haplotype without additional digestion with a restriction enzyme

eight primer combinations resulted in segregating AM alleles after digestion of the amplification products using indicated restriction endonuclease (Table 1). Primer sequences of markers SPUD839, TG432 and SPUD1636 were as described (Bryan et al. 2002; De Jong et al. 1997).

Resistance assays

PCN resistance assays were performed on plants derived from tubers as described (Rouppe van der Voort et al. 1997). The inheritance of resistance to PCN populations Ro5-22 and Pa2-D383 was analysed in population F1AMRH using three replicates of genotypes that showed a recombination event between markers GP21 and GP179. Cv Eigenheimer was included as a susceptible standard.

Data analysis and QTL mapping

Analysis of variance was carried out on $\log_{10}(x + 1)$ transformed average cyst counts per plant genotype according to the model:

$$\sigma^2_{\text{tot}} = \sigma^2_{\text{gen}} + \sigma^2_{\text{rep}}$$

where σ^2_{tot} is the phenotypic variance, σ^2_{gen} is the genetic variance among the plant genotypes and σ^2_{rep} is the environmental variance among replications.

Broad-sense heritability was estimated according to the formula:

$$h^2 = \sigma^2_{\text{gen}} / \left(\sigma^2_{\text{gen}} + \sigma^2_e / n \right)$$

where σ^2_{gen} is the genetic variance among the plant genotypes, σ^2_e is the error variance and n is the number of replicates.

The data on marker segregation of the resistant parent AM was included for QTL analysis using the program MapQTL5 (Van Ooijen 2004). An LOD value of 2.5 was chosen as a threshold value (Lander and Botstein 1989).

Results

Markers closely linked to the Grp1 locus

The nematode resistance locus *Grp1* has previously been mapped in a 3-cM interval on chromosome V of the diploid potato clone AM flanked by CAPS markers GP21 and GP179 (Rouppe van der Voort et al. 1998). To increase the resolution in this interval, 1,536 progeny of the mapping population RHAM026 were screened for the presence or absence of the markers GP21 and GP179 (Table 1). A total

of 61 genotypes showed a recombination event between these two markers, resulting in an interval of 3.97 cM.

To identify markers closely linked to the *Grp1* locus, primers were designed on sequences derived from this locus in other potato genotypes that were retrieved from Genbank and the GABI database. Thirteen primer combinations resulted in a segregating the AM allele. Table 1 presents the markers in more detail. Five markers were AM allele specific, showing the presence or absence of the amplification product. Eight markers were polymorphic after digestion with an appropriate enzyme (CAPS markers). In addition, polymorphisms were found for markers SPUD839 and TG432 (De Jong et al. 1997) and for SPUD1636 (Bryan et al. 2002). The 16 markers that revealed a polymorphism between AM and RH were subsequently tested on a subset of 44 genotypes that showed a recombination event in the GP21–GP179 interval. All markers were placed between GP21 and GP179 (Fig. 1). Some markers were not separated by recombination events and were grouped together.

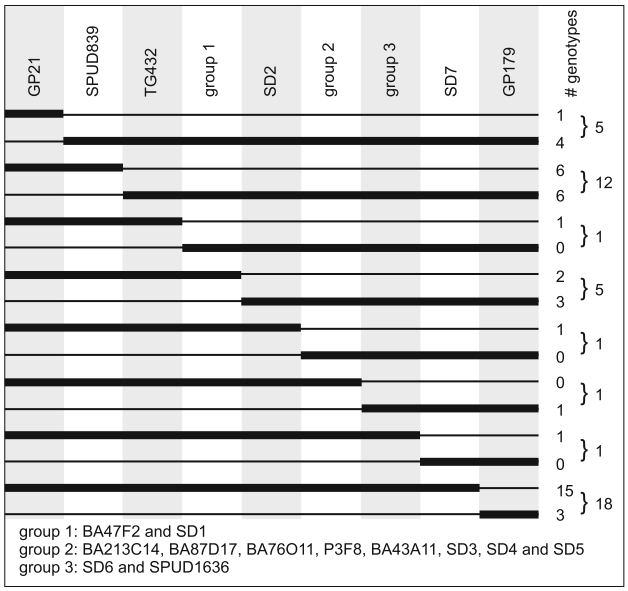

Fig. 1 Fine mapping of the GP21–GP179 interval on chromosome V of the diploid potato clone AM. Markers are presented in the correct genetic order. SPUD839, TG432, BA47F2, SD1, BA87D17, P3F8, BA43A11, SD4, SD5, SPUD1636, SD7 and GP179 are in coupling with GP21, and SD2, BA213C14, BA76O11, SD3 and SD6 are in repulsion with GP21. *Bold horizontal lines* represent chromosomal regions derived from the haplotype harbouring GP21 and *thin horizontal lines* represent chromosomal regions derived from the other haplotype. In the *column* on the *right*, the number of genotypes for each recombination event is given

High-resolution map of the Grp1 locus

The *Grp1* locus harbours resistance to two potato cyst nematode species viz. a major resistance to *Globodera pallida* pathotype Pa2 and a major resistance to *G. rostochiensis* Ro5 (Rouppe van der Voort et al. 1998). In addition, a minor resistance to *G. pallida* Pa3 was detected (Rouppe van der Voort et al. 1998). It was shown that the QTLs for Pa3 coincided with the QTL for Pa2. Therefore, the 44 recombinants were tested for resistance to nematode population D383 (Pa2) and line 22 (Ro5).

Resistance was evaluated by counting the number of newly formed cysts on a subset of 54 progenies with recombination events between the markers GP21 and GP179. The progenies were split into two identical sets that were separately, but simultaneously, assessed for Ro5 and Pa2 resistances. The average number of cysts developed per plant genotype for each nematode population is presented in Suplementary Table 1. The resistant parent showed an average of 2 cysts per plant for Ro5 and 1 for Pa2, while on the susceptible parent 103 and 70 cysts per plant developed for Ro5 and Pa2, respectively.

Analysis of variance on normalised cyst counts revealed that the genetic variance for both *G. pallida* and *G. rostochiensis* resistance was significant ($P < 0.0001$). The log-transformed values of the averaged number of cysts per genotype showed a continuous distribution skewed towards susceptibility for both nematode populations. The broad-sense heritability was estimated to be 0.95 for *G. rostochiensis* and 0.93 for *G. pallida*. Interval mapping in the GP21–GP179 region was applied on the average number of cysts per genotype. For both *G. pallida* and *G. rostochiensis*, a significant QTL was detected with a maximum LOD score of 9.03 and 7.90, respectively. The QTL for *G. pallida* resistance explains 67% of total *G. pallida* resistance and the QTL for *G. rostochiensis* resistance explains 62% of total *G. rostochiensis* resistance. LOD score graphs for *G. rostochiensis* and *G. pallida* resistance are presented in Fig. 2.

Grp1 resistance is located in an interval that is known to harbour single dominant *R* genes in other potato genotypes (Kuang et al. 2005; Ballvora et al. 2007). In addition, because PCN are obligatory outbreeding nematode species, the nematode populations used to test for resistance are most likely a mixture of virulent and avirulent genotypes (Janssen et al. 1990). Therefore, it is possible that the quantitative effect of the *Grp1* resistance is caused by the genetic diversity of the nematodes and that resistance is monogenic. To test this, the plant genotypes were first sorted on the presence or absence of the closest marker, which is TG432 for both *G. rostochiensis* (LOD 7.3) and *G. pallida* (LOD 7.9) resistance, and then according to ascending cyst numbers (Fig. 3). For the majority of the genotypes, the presence of marker TG432 was indicative of the degree of resistance (Fig. 3). A small number of genotypes with an intermediate level of resistance lacked a correlation with marker TG432, indicating that also other

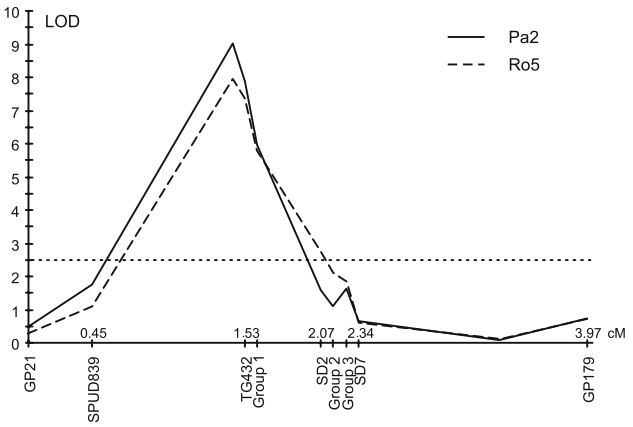

Fig. 2 LOD graphs of *G. rostochiensis* Ro5 resistance (*dashed line*) and *G. pallida* Pa2 resistance (*continuous line*). The *vertical axis* represents the LOD score and the *horizontal axis* represents the genetic map. The threshold LOD of 2.5 is indicated by the *dotted horizontal line*

regions in the potato genome affect the degree of resistance.

The *G. pallida* and *G. rostochiensis* resistance conferred by the *Grp1* locus showed tight linkage in the test population of 1,536 potato genotypes. Both resistances could be mapped between markers SPUD839 (with nine recombination events) and TG432 (with one recombination event; Fig. 4). Eight susceptible recombinants clearly indicated that resistance conferred by *Grp1* is located south of SPUD839 and one recombinant between marker TG432 and *Grp1* (4A7) gives a clear resistant phenotype indicating that *Grp1* resistance is located north of TG432. Two potato genotypes (3H1 and 1B12) have a recombination event between markers SPUD839 and TG432, but have intermediate levels of resistance for *G. pallida* and *G. rostochiensis*. It is, therefore, not clear where these recombination events take place exactly. *Grp1* resistance behaves as a single gene; none of the recombinants between SPUD839 and TG432 showed a clear difference in resistance or susceptibility to either one of the two nematode species. Only the recombinant genotype 2H8 showed some, but inconclusive, difference in resistance level. This genotype was classified as susceptible for *G. rostochiensis* (90 cysts), but had an intermediate level of resistance for *G. pallida* (36 cysts; Fig. 3). One genotype (12C6) showed a marked difference in average cyst number to both PCN species (Fig. 3). However, this double recombination event results in a singleton. This genotype has a recombination event between group1 and marker SD2 (Fig. 1) and the four other genotypes that have a recombination event between group1 and SD2 show resistance and susceptibility as expected. Therefore, it is suspected that the *G. rostochiensis* resistance phenotype of genotype 12C6 is the result of a mistake, which is also corroborated by a large standard deviation for the cyst numbers of *G. rostochiensis* (Table 2), and this genotype was omitted from further analysis. Markers flanking the *Grp1* locus define an interval of 1.08 cM. From these results, it can be concluded that PCN resistance conferred by the *Grp1* locus may be attributed to a single gene or to two or more tightly linked genes.

Discussion

In this study, a high-resolution map of the broad-spectrum potato cyst nematode resistance locus *Grp1* was constructed. Sixteen markers were identified within the 4 cM interval that harbours this locus. A recombination analysis

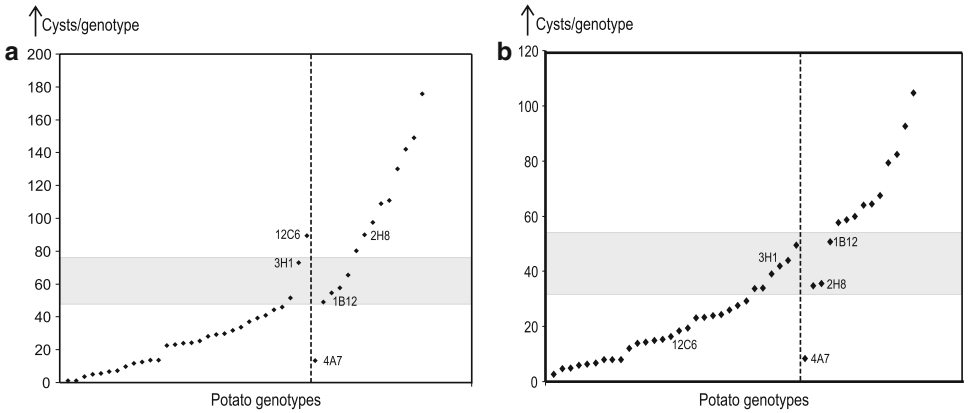

Fig. 3 Distribution graphs for the number of *G. rostochiensis* cysts (**a**) and *G. pallida* cysts (**b**). The *Y* axes represent the number of cysts per plant. The *X* axes represent the potato genotypes. In the potato genotypes to the left of the *dashed vertical line*, marker TG432 is present and in the potato genotypes to the *right* of this *line*, marker TG432 is absent. Potato genotypes in the *grey areas* are classified as intermediate, while those *above* the *grey areas* are classified as susceptible and those *below* as resistant. Potato genotypes that are discussed in the text are indicated with a code

followed by phenotyping for resistance resulted in the identification of significant QTLs for both *G. rostochiensis* and *G. pallida* resistance. The graphs of *G. pallida* and *G. rostochiensis* resistances are almost identical, both with the highest LOD score close to marker TG432. In this region, several other PCN resistance loci have been mapped. *Gpa* and *GpaM1* in *S. spegazzinii* (Caromel et al. 2003; Kreike et al. 1994), *GpaV^{s}spl* in *S. sparsipilum* (Caromel et al. 2005) and *Gpa5* (Rouppe van der Voort et al. 2000). In addition another *G. pallida* resistance gene was also mapped in this region, which resembles the *Gpa5* locus so much that it is suggested to be the same locus in *S. vernei* (Bryan et al. 2002; Rouppe van der Voort et al. 2000). All these loci confer resistance to *G. pallida* only and at least some of them have been introgressed in commercial potato varieties (Sattarzadeh et al. 2006). In contrast, *Grp1* confers resistance to both *G. pallida* and *G. rostochiensis* (Rouppe van der Voort et al. 1998).

The *Grp1* locus explains 62% of the total *G. rostochiensis* resistance and 68% of the total *G. pallida* resistance. This suggests that other places in the genome harbour minor resistance effects, which could be an explanation for the occurrence of the intermediate genotypes after the ordering based on the presence and absence of marker TG432 (Fig. 3). Remarkably, no significant QTLs have been detected elsewhere in the genome (Rouppe van der Voort et al. 1998). Interestingly, all *G. pallida* resistance loci that map in the GP21-GP179 interval on chromosome V are found in genotypes that harbour other QTLs for resistance. Kreike et al. (1994) and Caromel et al. (2003) found two other minor *G. pallida* resistance loci on chromosomes IV and VII, and VI and XII, respectively. For *GpaV^{s}spl* and *Gpa5* additive *G. pallida* resistance loci were identified on chromosomes XI and IX, respectively (Bryan et al. 2002; Caromel et al. 2005; Rouppe van der Voort et al. 2000).

Genome-wide sequence analysis and genetic mapping of *R* gene candidates have shown that *R* genes are often located in clusters spread throughout the plant genome (reviewed by Gebhardt and Valkonen 2001). Notably, QTLs conferring resistance to potato cyst nematodes often co-localise with clusters of *R* gene homologues. A *Phytophthora infestans* resistance locus, explaining 50% of the field resistance, resides in a region comprising *R* gene homologues of the NBS-LRR class (Tan et al. 2008). In addition, this resistance is associated with a hypersensitive response, normally resulting from dominant *R* genes of the NBS-LRR class. This indicates that *R* genes of the NBS-LRR class may contribute to partial resistance to *P. infestans,* a situation that may also apply to the quantitative resistance conferred by *Grp1*.

It is also noted that potato cyst nematodes reproduce by obligate outcrossing, and that PCN populations often consist of a mixture of virulent and avirulent genotypes (Bakker et al. 1993). As a consequence, quantitative resistance to PCN may be conferred by dominant *R* genes operating on the basis of a classical gene-for-gene relationship. Indeed, after dividing the recombinant genotypes used to fine map the *Grp1* locus into classes of resistant, intermediate and susceptible genotypes for *G. pallida* and *G. rostochiensis*, both resistances behaved as single dominant *R* genes. The resistances to *G. pallida* and *G. rostochiensis* could not be separated by a recombination event suggesting that *Grp1* resistance is conferred by a single *R* gene or tightly linked *R* genes. Preliminary comparative mapping (data not shown) indicates that *Grp1* resistance is positioned in the same area as the homologues of *R1, Bs4* and *Prf*. *R1* and *Bs4* mediate resistance to *P. infestans* in potato (Ballvora et al. 2002) and *Xanthomonas campestris* pv. *vesicatoria* in tomato (Schornack et al. 2004), respectively. *Prf* interacts with *AvrPto* (Salmeron et al. 1996). All three genes belong to the NBS-LRR class of resistance genes and could be used as candidate genes for *Grp1*.

Because so many *G. pallida* resistances have been mapped to the same region on chromosome V in various potato species, it can be suggested that several of these resistances have a common evolutionary background. This suggestion is strengthened by the fact that all these loci confer resistance to the pathotypes Pa2/3. In case of a common origin, the resistance specificity must be relatively old and arisen before the speciation of *S. vernei, S. sparsipilum* and *S. spegazzinii*. However, the resistance locus *Grp1* confers not only resistance to *G. pallida* Pa2/3, but also to *G. rostochiensis* Ro5. The potato genotype that harbours *Gpa5* is closely related to the genotype that harbours *Grp1* and many common markers have been identified (Rouppe van der Voort et al. 2000). Because breeding was only focussed on *G. pallida* resistance, it was speculated that *G. rostochiensis* resistance was lost during selection (Rouppe van der Voort et al. 2000). Based on the comparison of common markers at the *Gpa5* locus and the *Grp1* locus, it was suggested that *G. pallida* resistance was conferred by a gene north of TG432 and *G. rostochiensis* resistance by a gene south of marker TG432. This is not confirmed by the high-resolution map of the *Grp1* locus produced in this study, which suggests that both Pa2/3 and Ro5 resistances are conferred by a single gene, or two or more tightly linked genes just north of TG432.

If *Grp1* resistance is conferred by a single gene, this gene has a dual specificity. So far, multiple specificities have been shown for *Mi-1* and *RPM1* (Grant et al. 1995; Nombela et al. 2003; Rossi et al. 1998; Vos et al. 1998). *Mi-1* confers resistance to several species of root knot nematodes as well as to aphids and whiteflies (Milligan et al. 1998; Nombela et al. 2003; Rossi et al. 1998; Vos

et al. 1998). It is unlikely that *Mi-1* interacts with identical ligands from all these pests. Even within these pest species, variability in virulence occurs, indicating that ligand conservation among these taxonomically unrelated species is not very likely. Although *G. pallida* and *G. rostochiensis* are sibling species, it has been shown that they are extremely distinct at the molecular level (Bakker and Bouwman-Smits 1988). Like *Mi-1* resistance, *Grp1* resistance also shows within-species variability in virulence. Therefore, we assume that *Grp1* does not recognise identical ligands from *G. pallida* and *G. rostochiensis.* It is more likely that *Grp1*, like RPM1 (Mackey et al. 2002) recognises indirectly ("guard model") two unrelated effectors that modify the same host protein. Proof for such a dual specificity of *Grp1* can only be given after the identification of the gene underlying this resistance locus, for which this study will form the basis.

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