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Two additive QTLs conferring broad-spectrum resistance in potato to *Globodera pallida* are localized on resistance gene clusters

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Abstract Broad-spectrum resistance in potato to the potato cyst nematode (PCN) is commonly regarded as a complex inherited trait. Yet, in this paper we show that, by use of a selected set of PCN test populations, broad-spectrum resistance to the species *Globodera pallida* can be fully ascribed to the action of two loci: *Gpa5* and *Gpa6*. These loci were readily mapped by means of a strategy based on two steps. Firstly, the chromosomal localization of both loci was assessed by use of an online catalogue of AFLP markers covering a substantial part of the potato genome (http://www.spg.wau.nl/pv/aflp/catalog.htm). Subsequently the chromosomal regions of both loci were identified by means of CAPS markers based on RFLP insert sequences. Locus *Gpa5* explains at least 61% of the genetic variation. This locus maps to chromosome *5* on a region which has previously been shown to harbor resistance factors to viral (*Nb*, *Rx2*), fungal (*R1*) and nematodal (*Gpa*, *Grp1*) pathogens. The *Gpa6* locus exhibits a minor effect on the resistance (24%) and acts additively to *Gpa5*. Interestingly, the *Gpa6* locus maps to a region on chromosome *9* where, in the homoeologous tomato genome, the virus resistance gene *Sw-5* resides as part of a resistance gene cluster. In potato, resistance to potato virus X has been reported in the vicinity of this region. The map location of *Gpa6* indicates the presence of a resistance gene cluster at the end of the long arm of chromosome *9* of potato.

Key words AFLP · CAPS · *G. rostochiensis* · *Solanum tuberosum* · QTL mapping

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Introduction

Quantitative resistance calls up the image of complex, polygenic inheritance patterns with multiple genes having small additive effects on the resistant phenotypes. According to Van der Plank (1982) these minor genes may be equally effective to different pathogen isolates (pathotypes) and may be relatively durable in the field. However, quantitative resistance based on several minor genes is less desirable for breeding purposes given its complexity to introgress this type of resistance in existing cultivars. Breeders rely therefore on major genes for resistance. Monogenic resistance is usually referred to as resistance which complies with a gene-for-gene interaction. It contrasts to quantitative resistance by its pathotype specificity; for each major gene in the plant host there exists an avirulence gene in the pathogen (Flor 1956, 1971). Major resistance (*R*-) genes are often clustered in the genome and molecular analyses have shown that the gene-for-gene model holds true for several plant-pathogen systems (reviewed in Hammond-Kosack and Jones 1997). In these systems, the cloned *R*-genes are thought to encode receptor molecules which perceive a pathogen (avirulence) signal, whereupon a resistance response is triggered (Baker et al. 1997). As a result of the strong selective force of monogenic field resistance, the site of recognition in the pathogen may be altered in a short time span. Qualitative resistance is therefore considered to be less-durable than quantitative resistance.

The image of multiple minor genes underlying quantitative resistance is inconsistent with the present outcomes of most quantitative trait locus (QTL) mapping studies in different plant-pathogen combinations. Although some examples exist where quantitative resistance is explained by more than five QTLs (Geiger and Heun 1989; Bubeck et al. 1993; Leonards-Schippers et al. 1994; Wang et al. 1994; Qi et al. 1998) it is more common to find two to five loci (summarized in Young et al. 1996) underlying complex disease resistance. In these cases, quantitative resistance is polygenic in the sense that a few genes have large effects on the phenotype. Evidence is accumulating that a wide variety of molecular and genetic mechanisms may underly quantitative resistance. For example, insect resistance in potato is mediated by the glandular secretions of two types of leaf trichromes. QTLs associated with the resistance are co-localized with loci linked with phenol oxidase, trichrome density and sucrose ester production (Bonierbale et al. 1994). The expression of a resistance phenotype may also be developmentally regulated; e.g. while a single gene was found to control resistance to the spot blotch pathogen in the seedling stage, two QTLs were detected for resistance in the adult plant stage (Steffenson et al. 1996). Quantitative resistance may also fit in a classical gene-for-gene relationship. A major *R*-gene which triggers a specific HR response upon recognition of the appropriate avirulence gene product (Baker et al. 1997) may have a reduced effect once a mutated version of this avirulence gene product is encountered. This phenomenon has been observed for the rice resistance gene *Xa4* which acts as a QTL against a virulent strain of the fungus *Xanthomonas oryzae* pv *oryzae* (Li et al. 1999).

In the present study, quantitative resistance in potato to the potato cyst nematode *Globodera pallida* can be fully ascribed to two additive QTLs, which are likely to be explained by a gene-for-gene mechanism in which two distinct avirulence determinants in the nematode population interact with two *R*-genes. The potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are serious pests in potato and especially *G. pallida* is a threat for West-European potato culture. Broad-spectrum monogenic resistance to this species is not available and the supposed heterogeneity of the species would obstruct resistance breeding efforts (Schnick et al. 1990; Folkertsma et al. 1996a, b). By use of a selected set of PCN test populations (Folkertsma 1997), in the present study quantitative resistance to *G. pallida* is dissected into the resistance loci *Gpa5* and *Gpa6*. These resistance loci map to two *R*-gene clusters in potato and one of these clusters appears to be novel for the potato genome. Although it is likely that these *R* loci act in a pathotype-specific way, it is argued that, as a result of the population genetics of potato cyst nematodes, the combination of *Gpa5* and *Gpa6* may confer durable resistance to *G. pallida*.

Materials and methods

Plant material

The broadness of PCN resistance was tested in potato clones AM78–3704, 3704–76 and 3704–27. The tetraploid clone AM78–3704 contains resistance to both PCN species and is an interspecific hybrid between *Solanum tuberosum* and several wild potato species including *Solanum vernei* 24/20, *S. vernei* ssp. *ballsii* 2/1, *S. vernei* LGU 8, *Solanum oplocense* EBS 1786, *S. tuberosum* ssp. *andigena* CPC 1673 and *Solanum spegazzinii* 440. Clones 3704–76 and 3704–27 are di-haploids (2n = 2*x* = 24) produced by prickle-pollination of clone AM78–3704 with haploid inducer *Solanum phureja* clones (Hutten et al. 1994).

A mapping population of diploid potato was obtained from a cross between the clones 3704–76×RH89–039–16. Clone 3704–76 is the resistant parent in our population and is referred to as JP. The susceptible male parent, clone RH89–039–16, will be referred to as RH. The mapping population F_1JP × RH consisted of 103 vigorous F_1 genotypes. Leaf material for DNA isolation was collected in the greenhouse at the seedling stage. Tubers for nematode tests were produced by first year clones on the field.

The origin of the resistance segregating from genotype JP was compared with the resistance segregating from genotype 3778–16. With the exception of *S. spegazzinii* 440, genotype 3778–16 has a similar wild-species background. Locus *Grp1* conferring resistance to *G. rostochiensis* and *G. pallida* has been identified by analysing a population derived from a cross between clones 3778–16 and RH (Rouppe van der Voort et al. 1998b).

Nematodes

The nematode populations used in this study are listed in Table 1. *G. rostochiensis* and *G. pallida* populations are indicated by a ''Ro'' and ''Pa'' prefix respectively, followed by the pathotype designation and the code of the population. Resistance screening of the segregating potato population was carried out by use of the populations ''Pa_2-D383'' and ''Rookmaker''. Population Pa_2-D383 is unrelated to population Rookmaker (which is referred to as population Pa_3-Rook). Population Pa_3-Rook is selected out of a set of 226 *G. pallida* populations found in the Netherlands as being a representative test population for the majority of these populations (Bakker et al. 1992; Folkertsma et al. 1996a, b). This population appeared to contain the highest number of virulent genotypes when tested with a range of resistant clones and cultivars.

Table 1 Level of PCN resistance in the tetraploid genotype AM78–3704 and the AM78–3704 derived di-haploid clones. Presented are average numbers of cysts recovered from closed container tests

Potato clone	Ro1-19	Ro5–22	Pa2-D383	Pa2-D350	Pa2-HPL1	Pa3–1097	Pa2-Rook	Pa3–74.768.20
AM78—3704	1	86	0	1	0	2	4	2
3704–76	6	42	0	2	0	1	2	2
3704–27	n.d.[a]	n.d.	8	4	3	130	109	52
RH89–039–16	189	98	101	132	146	240	153	166

[a] n.d.: not determined

Resistance testing and data collection

Preparation of the PCN inoculum was as described by Rouppe van der Voort et al. (1997a). The resistance spectrum assay was performed in a closed-container test (Phillips et al. 1980) using 125 cc plastic beakers filled with silver sand using one tuber per assay and inoculated with nematodes to a final density of five eggs/J_2 per g of soil. The containers were maintained in the dark at approximately 20°C for at least 3 months.

The inheritance of the resistance to populations Pa_2-D383 and Pa_3-Rook was analysed in mapping population $F_1JP \times RH$ in three replications. Resistant standards were cv Multa (resistant to Pa_2-D383), *S. vernei* hybrid cv Nika (resistant to Pa_2-D383) and cvs Florijn and Seresta (resistant to Pa_2-D383 and Pa_3-Rook). As a susceptible standard cv Eigenheimer was used. Plant growth conditions and the design of the experiment were as in Rouppe van der Voort et al. (1997a). Resistance data were only recorded when at least three well-rooted plants of a single plant genotype were available.

DNA-marker analysis and linkage-map construction

The isolation of genomic DNA, the generation of AFLP markers and data analysis were done as described previously (Van Eck et al. 1995; Vos et al. 1995). A genetic map was constructed only from the resistant genotype JP. This map was constructed on the basis of segregating AFLP markers which were generated by use of the AFLP primer combinations E+AAA/M+ACG, E+AAC/M+CAC, E+AAC/M+CAG, E+ACA/M+CAC, E+AGA/M+CAT and E+ATG/M+CTA (the restriction enzymes *Eco*RI and *Mse*I, abbreviated ''E'' and ''M'' in the primer designation were used for template preparation). These primer combinations have previously been used to build up a catalogue of chromosome-specific AFLP markers for a set of different potato genotypes (Rouppe van der Voort et al. 1997b, 1998a). Alignment of the JP map was achieved by the identification of common AFLP markers between any of the potato genotypes included in the catalogue. The genetic reference maps are available from URL: http://www.spg.wau.nl/pv/aflp/catalog.htm.

CAPS markers (cleaved amplified polymorphic sequences; Konieczny and Ausubel 1993) for potato RFLP loci GP21 and GP179 (Meksem et al. 1995), GP186 and TG432 (De Jong et al. 1997), and CT220 (Ganal et al. 1998) were applied to determine the chromosomal regions of the QTLs. Segregating JP alleles were detected after digestion of the amplification products using the restriction endonucleases *Dra*I for marker GP21, *Rsa*I for marker GP179 and *Nci*I for GP186 and TG432. The primer sequences for locus CT220 were derived from Folkertsma et al. (1999) and were designed from the published tomato cDNA sequence of tomato RFLP probe CT220 [Ganal et al. 1998; sequences can be retrieved via the Solgenes database (Paul et al. 1994) which can be accessed via http://probe.nalusda.gov:8300/cgi-bin/browse/solgenes]. Primers CT220F (AAGCGAATTATCTGTCAAC) and CT220R (GTTCCTGACCATTACAAAAGTAC) were used to amplify a genomic DNA fragment of approximately 220 bp from potato DNA by applying the following temperature cycle file: 3 min 93°C followed by 30 s 93°C, 45 s 60°C, 90 s 72°C for 35 cycles and finished by 10-min elongation at 72°C. The amplification product was digested with *Mse*I for identification of a segregating JP allele

The grouping of markers and the determination of marker orders were calculated using the software package JoinMap 1.4 (Stam 1993). Maps were drawn by means of the graphical package Drawmap (Van Ooijen 1994).

Statistical analysis

Analysis of variance (ANOVA) were carried out on $\log^{10}(x+1)$-transformed average cyst counts per plant genotype according to the model: $\sigma^2_{tot}=\sigma^2_{plant}+\sigma^2_{rep}$. The phenotypic variance (σ^2_{tot}), the genetic variance among the plant genotypes (σ^2_{plant}) and the environmental variance among replications (σ^2_{rep}) were estimated using Genstat 5 release 3.2 (Payne et al. 1987), and the broad-sense heritabilities were estimated based on the appropriate mean squares from the ANOVA. QTL analyses were performed by using the program MapQTL (Van Ooijen and Maliepaard 1996a, b). Both the non-parametric rank-sum test of Kruskal-Wallis (see e.g. Sokal and Rohlf 1995) and two parametric methods, interval mapping (Lander and Botstein 1989) and multiple-QTL mapping (MQM) (Jansen 1993; Jansen and Stam 1994) were applied. These procedures are implemented in the MapQTL software. Threshold values for assigning a QTL to a map position are P <0.001 for the Kruskal Wallis test and a LOD score of 3.0 for interval and MQM mapping. The percentage of the total variance explained by a marker genotype (R^2) was calculated by $(\sigma^2_m-\sigma^2_{res})/\sigma^2_m$ where σ^2_m represents the variance between the two marker-genotype classes (i.e. presence or absence of a particular marker) and σ^2_{res} the residual variance.

Results

Broadness of PCN resistance from AM78–3704

The level of PCN resistance present in the tetraploid genotype AM78–3704 and two AM78–3704 derived di-haploid clones 3704–76 and 3704–27 was assessed by a resistance screen with a diverse set of PCN populations. These PCN populations are known to harbour differences in molecular as well as virulence characteristics (Folkertsma et al. 1996b; Folkertsma 1997). Comparison of the tetraploid genotype with the di-haploid clones showed that resistance against *G. pallida* has been retained in clone 3704–76 (Table 1). However, loss of resistance to the Pa_3 populations was observed in clone 3704–27. No resistance to *G. rostochiensis* Ro_5-22 was found. The diploid clone RH89–039–16 was susceptible to all populations tested. From these data it was decided to analyse a segregating population derived from the cross 3704–76 × RH89–039–16 ($F_1JP \times RH$). Since large differences exist in the molecular and virulence characteristics of *G. pallida* populations Pa_2-D383 and Pa_3-Rook, these populations were used for further resistance testing.

Inheritance of resistance

The average numbers of cysts developed on the parental genotypes as assessed in pot tests for *G. pallida* populations Pa_2-D383 and Pa_3-Rook are shown in Table 2. The average number of newly developed cysts in the progeny ranged from 0 to 1361 for population Pa_2-D383 and from 9 to 1047 for population Pa_3-Rook. For population Pa2-D383 susceptible plants could be clearly distinguished from plants assigned to the resistant class. The numbers of cysts developed on resistant plants varied from 0 to 42, whereas these numbers ranged from 202 to 1361 on the susceptible plants.

The Pa_3-Rook cyst numbers showed a continuous distribution among the plant genotypes; no clear distinction between a resistant and susceptible class could be made. Analysis of variance on normalized cyst counts showed

Table 2 Results of the quantitative analysis of PCN resistance as measured by counted cyst numbers of the respective PCN populations. Presented are the heritability, the most flanking marker to the resistance loci, the test values indicating the appropriate map position, and the R^2 at the QTL position

Trait	#Cysts[a] JP	# Cysts RH	h^{2}[b]	Marker[c]	Chrom. location[d]	*P* value[e]	R^{2}[f]	LOD[g]
Pa2-D383	1	856	–	TG432	Chr. 5	< 0.0001	–	21
Pa3-Rook	23	635	0.91	TG432	Chr. 5	< 0.0001	61%	12
				CT220	Chr. 9	< 0.001	24%	3

[a] # Cysts: average number of cysts,
[b] h^2 : heritability,
[c] Marker : nearest marker to resistance locus,
[d] Chrom. location: chromosomal localization of resistance locus,
[e] *P* value of the nearest marker in a Kruskal Wallis test,
[f] R^2 : percentage of the total variance explained by the marker genotypes,
[g] LOD: logarithm of odds score which indicates the likelihood that two loci are linked (Lander and Botstein 1989)

significant differences in the level of resistance among the plant genotypes ($0.001 < P < 0.0001$). No significant differences in cyst numbers were found among the blocks of replicates. The broad-sense heritability, listed in Table 2, indicated that the variation in the numbers of cysts developed was genetically determined. Skewness and kurtosis values were – 0.68 and 0.07 respectively showing that the assumption on normally distributed $\log^{10}(x+1)$-transformed cyst numbers was justified (Snedecor and Cochran 1967).

AFLP mapping

A genetic map of genotype JP was produced in order to the find the association between molecular markers and the resistance trait(s). The AFLP map of genotype JP consisted of 149 AFLP markers and spanned a total map length of 603 cM. The sizes of the linkage groups varied between 13 cM (for chromosome *4*) and 69 cM (for chromosome *5*). Comparison of the positions of common AFLP markers present on the JP map and the maps of five other potato genotypes revealed that parts of the maps of chromosomes *1, 4, 8, 10* and *11* were not fully covered with AFLP markers. It was therefore anticipated that QTLs may be unresolved as a result of the relative incompleteness of the JP map.

Mapping of Pa_2-D383 resistance

Linkage analysis showed a significant association between the resistance to Pa_2-D383 and AFLP markers which were localized at the top of chromosome *5*. This map position is well known for its presence of a complex *R*-gene cluster in potato harboring resistance to different pathogen species (summarized in Leister et al. 1996). Therefore CAPS markers GP186, GP21, TG432 and GP179, residing on the short arm of potato chromosome *5*, were tested in population F_1JP×RH for a more precise mapping of this locus. Three (GP186, GP21 and TG432) of the four markers segregated in F_1JP×RH. Marker TG432 showed the highest linkage with the resistance locus (recombination fraction=3/102; LOD=21). As two different resistance loci to *G. pallida* have previously been assigned to this map position (*Gpa*, Kreike et al. 1994 and *Grp1*, Rouppe van der Voort et al. 1998b) the origin of the resistance was further investigated.

Mapping of Pa_3-Rook resistance

The presence of the TG432 marker allele in the plant genotypes also appeared to be associated with a reduced number of Pa_3-Rook cysts. A total of 32 out of 42 plant genotypes which harbored the TG432 allele contained <100 cysts, whereas among the remaining 61 genotypes (without the TG432 allele) 58 genotypes were found which contained >100 cysts (Fig. 1). Subsequent analysis using MapQTL software revealed a significant effect of marker TG432 on the resistance, both in the Kruskal Wallis test and by interval mapping; the highest significance levels were found at marker locus TG432 (Table 2, Fig. 2). This QTL explained about 61% of the total variance which is in the same order of magnitude as the amount of variation in Pa_3-Rook resistance explained by locus *Grp1* (Rouppe van der Voort et al. 1998b). Interestingly, the LOD profiles for Pa_3-Rook resistance of *Grp1* and the present QTL coincide, indicating that a similar gene(s) underlies *G. pallida* resistance in genotypes 3778–16 and JP. However, *Grp1* differs from the JP QTL at chromosome *5* as it confers resistance to both species of the potato cyst nematode. By analogy with the nomenclature of other *G. pallida* resistance loci in potato (Kreike et al. 1994; Rouppe van der Voort et al. 1997a) we propose to name the QTL on chromosome *5*, *Gpa5*.

An additional effect on Pa_3-Rook resistance was found at AFLP markers on the long arm of chromosome *9*. The position of this locus could be more precisely defined by application of the most-distal RFLP marker CT220 at this chromosomal region as a CAPS marker. Locus CT220 yielded the highest significance level for Pa_3-Rook resistance (Fig. 3). Since CT220 is localized at the end of a linkage group only one neighboring marker was used in the interval mapping. The addition of more neighboring markers (to a maximum of 5) in the analysis showed an increase in the LOD score to a maximum of 9.1 at this end of chromosome *9* but resulted in a less-precise localization of the QTL.

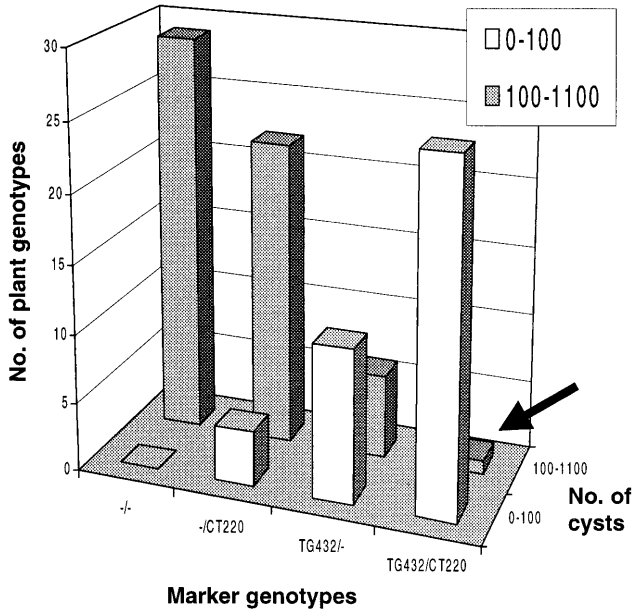

Fig. 1 Resistance to Pa_3-Rook is conferred by the combined action of *Gpa5* and *Gpa6*. Shown is the distribution of the number of *G. pallida* Pa_3-Rook cysts recovered within the TG432/CT220 offspring classes TG432/CT220 (presence of both marker alleles), TG432/- (presence of the TG432 allele, absence of the CT220 allele), -/CT220 (absence of the TG432 allele, presence of the CT220 allele) and -/- (absence of both marker alleles). The genotype indicated by an *arrow* most likely harbours a recombination event between TG432 and *Gpa5* as this genotype lacks all subsequent distal marker alleles in coupling with the TG432 allele

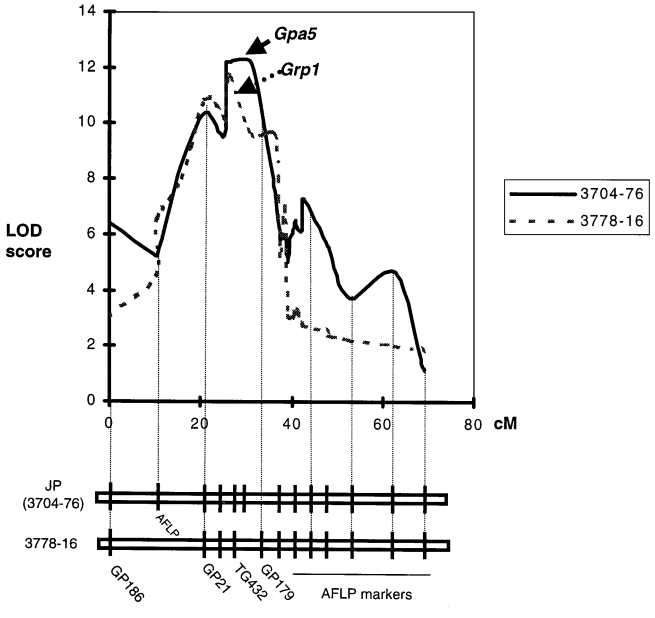

Fig. 2 LOD plots for *G. pallida* Pa_3-Rook resistance on chromosome *5* in genotypes JP and 3778–16 which show that the effect on Pa_3-Rook resistance is localized on the same map region. The Pa_3-Rook resistance locus on chromosome *5* segregating from clone JP is referred to as *Gpa5* (see text). The PCN resistance in 3778–16 is ascribed to the action of locus *Grp1*. For reasons of simplicity, only schematic maps are drawn. More details on the AFLP markers mapped on chromosome *5* are presented in Fig. 4

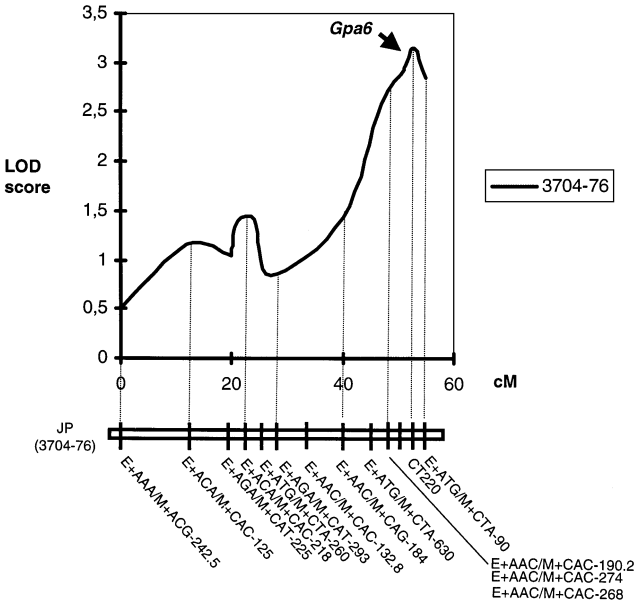

Fig. 3 LOD plot for *G. pallida* Pa_3-Rook resistance on chromosome *9*. This resistance locus is referred to as *Gpa6* (see text)

The QTL at chromosome *9* explained 24% of the total variance (Table 2) and acts addititively with *Gpa5*. The additive effect of the chromosome-*5* and chromosome-*9* loci is shown in Fig. 1. A significant reduction in the numbers of Pa_3-Rook cysts was observed in the class of plants harboring the TG432 and CT220 alleles as compared to the three other marker genotype classes. In the TG432/CT220 class, one plant genotype was found with an average number of 550 cysts. This plant most-likely lacks *Gpa5* as a result of a recombination event in the TG432 – E+AAA/M+ACG-244.0 region. The respective plant genotype contains all JP marker alleles in the GP186 – TG432 region and lacks all subsequent JP alleles onwards which are in coupling with *Gpa5* resistance. Since no resistance specificity to PCN species has been assigned to chromosome *9*, we propose to name the QTL at chromosome *9* *Gpa6*.

Gpa5 may be part of the *Grp1* resistance locus

To address the question whether the same gene(s) underlies the *G. pallida* resistance conferred by *Gpa5* and *Grp1*, we compared the occurrence of common marker alleles linked in coupling with the QTLs at chromosome 5 of clones JP and 3778–16. The result of this analysis is shown in Fig. 4. At the short arm of chromosome *5*, identical marker alleles were identified for CAPS loci GP186, GP21 and TG432, and AFLP loci E+ATG/M+CTA-350, E+AGA/M+CAT-88.9. This similarity is lost from marker GP179 onwards. Marker GP179 and all subsequent AFLP markers which were identified in clone 3778–16 were either absent in JP or present in repulsion with *Gpa5*. The occurrence of two sets of common marker alleles (intervals GP21 –TG432 and

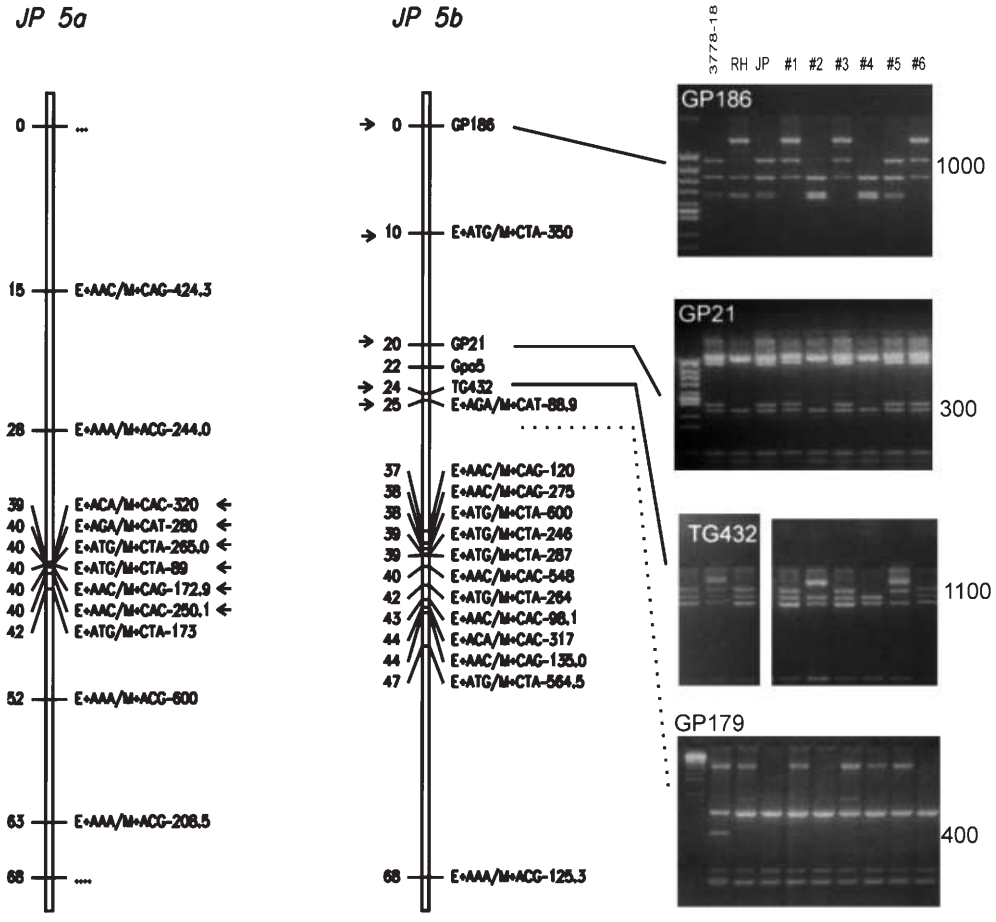

Fig. 4 Comparison of the presence of common marker alleles between clones JP and 3778–16 indicates that *Gpa5* is likely to be introgressed by a common ancestor of both parental clones. Shown are the maps of the two homologous chromosomes 5 of clone JP which harbors linkage blocks of marker alleles which are also present in 3778–16 (indicated by arrow heads). The comparative analysis is exemplified by the profiles of the CAPS markers localized on top of chromosome 5. Next to the marker lane, amplification products of clones 3778–16, RH, JP and a series of six progeny clones are loaded. Markers GP186, GP21 and TG432 show the similar profiles of clones 3778–16 and JP. For marker GP179 (shown is the profile which contains the mapped 3778–16 allele which is identified after *Rsa*I digestion) no segregating JP allele is identified even after the use of a series of 20 restriction enzymes. The corresponding map position of locus GP179 on the potato map is indicated by a *dashed line*. The *numbers* to the right indicate the molecular weights (in bp) of the segregating alleles

E+ACA/M+CAC-320 – E+AAC/M+CAC-250.1) indicate the presence of similar introgression segments in clones JP and 3778–16. Within these intervals, the DNA sequences between both clones may be highly similar. Since *G. pallida* resistance has been an important trait for selection to generate both breeding lines, we argue that the same gene(s) underlies the *G. pallida* resistance. However, the linkage between TG432 and GP179 and all subsequent marker alleles on the 3778–16 chromosome harboring *Grp1* may have been lost during the course of the breeding process of clone JP. We therefore hypothesize that the recombination between loci TG432 and GP179 in a progenitor of clone JP has been associated with loss of *G. rostochiensis* resistance in clone JP.

Discussion

This study has shown that the map position of the two resistance loci *Gpa5* and *Gpa6* coincides with two resistance gene clusters in potato. *Gpa5* maps close to marker TG432 in a region where resistance to potato virus X (*Rx2*, Ritter et al. 1991; *Nb*, De Jong et al. 1997) and to the fungus *Phytophthora infestans* (*R1*; Leonards-Schippers et al. 1992; Pi-QTL Leonards-Schippers et al. 1994) have been mapped. In addition, two resistance loci to PCN are located in this region. *Gpa* which acts against *G. pallida* (Kreike et al. 1994), and *Grp1* which confers resistance to both PCN species (Rouppe van der Voort et al. 1998b), reside within the GP21 – GP179 marker interval. This prompted us to the question whether the resistance mapped in clone JP could be ascribed to either of these loci. However, combining various data revealed that *Gpa5* is different from *Gpa* and *Grp1*. Locus *Gpa* has been mapped in an intraspecific population of the wild potato species *S. spegazzinni*, and this wild species is not included in the pedigree of the resistant genotype JP. Moreover, the specificity of *Gpa* extends to that of *Gpa5*; *Gpa* confers complete resistance to the population Pa_3-Rook (Wolters, personal communication) whereas 61% of the Pa_3-Rook resistance is explained by *Gpa5*. Locus *Gpa5* can also be distinguished from *Grp1* as it confers only partial resistance to *G. pallida*. Since the level of resistance of both loci is in the same order of magnitude, it may be possible that the *G. pallida* resistance is mediated by the same gene(s). This hypothesis is corroborated by the fact that the pedigrees of the parental clones JP and 3778–16 share the major part of the progenitors which have been used to introgress wild species resistance. In addition, it has previously been argued that *Grp1* is a compound locus since *G. rostochiensis* and *G. pallida* diverged millions of years ago (Bakker et al. 1992) and the multiple specificity of *Grp1* is unlikely to be the result of the action of a single gene (Rouppe

van der Voort et al. 1998b). Circumstantial evidence for the occurrence of *Gpa5* in the genome of 3778–16 was found by a comparative analysis of the maps of both genotypes. On the basis of common marker alleles within a 5-cM interval on the short arm of chromosome *5* (Fig. 4), it can be argued that the same gene(s) underlies the *G. pallida* resistance. Recombination in the TG432 – GP179 interval during the process of generating clone JP may thus be associated with loss of *G. rostochiensis* resistance in clone JP.

Locus *Gpa6* explains only a minor part of the genetic variation of the resistance to population Pa_3-Rook. However, the finding of *Gpa6* is significant because the combined action of *Gpa5* and *Gpa6* results in the same level of resistance as has been transmitted by clone JP to the progeny. *Gpa6* is not able to resist the population Pa_2-D383, which indicates that the resistance at *Gpa6* acts in a pathotype-specific way. The pathotype specificity of *Gpa6* indeed suggests that a major gene(s) underlies the resistance of *Gpa6*. The involvement of a major gene(s) in *Gpa6* resistance is further corroborated by the map location of this locus. *Gpa6* maps close to marker CT220, an RFLP marker locus which targets a resistance-gene cluster in tomato containing the *Sw-5* resistance gene (Brommonschenkel and Tanksley 1997; Folkertsma et al. 1999). The *Sw-5* gene confers broad-spectrum resistance to tomato spotted wilt virus and belongs to the class of resistance genes which encodes a putative gene-product harboring a leucine zipper (LZ), a nucleotide binding site (NBS) and a leucine-rich region (LRR) (Brommonschenkel et al. 1998; Folkertsma et al. 1999). The gene cluster to which the *Sw-5* gene belongs is composed of at least five resistance gene analogues (Folkertsma et al. 1999; Folkertsma and Prins, personal communication) and the location of *Gpa6* suggests the presence of a similar cluster on the homoeologous potato genome.

On the potato genome, no additional resistance specificities have been mapped in the vicinity of marker CT220. The closest resistance locus to marker CT220 is the Nx_{phu} locus which confers resistance to potato virus X (Tommiska et al. 1999). This locus maps at 11 cM from marker CT220 and the map positions of these loci should therefore be considered different. The question of whether any molecular relationship exists between *Gpa6*, Nx_{phu} and *Sw-5* can only be resolved when more information is available on the genes which underly *Gpa6* and Nx_{phu} resistance.

The partial resistance phenotype of *Gpa5* and *Gpa6* does not conflict with the hypothesis that major genes are responsible for both resistance specificities, considering that the *G. pallida* test population Pa_3-Rook is likely to be heterogeneous at the respective (a)virulence loci. In case avirulence alleles are not fixed in a population, a single *R* gene which operates on the basis of a gene-for-gene relationship, will confer partial resistance against the population as a whole whereas, at the level of the individual, the *R* gene will confer absolute resistance against the matching avirulent genotype. The potato cyst nematode is an obligate outbreeding species and field populations, such as Pa_3-Rook, are often composed of a mixture of virulent and avirulent genotypes (Bakker et al. 1993). Formal proof for the occurrence of a gene-for-gene relationship has so far only been demonstrated for the interaction between *G. rostochiensis* and the *H1* gene from *S. tuberosum* ssp. *andigena* CPC1673 (Janssen et al. 1991), but evidence is accumulating that various other PCN resistance genes operate on the same basis. For example, the *Gpa2* gene which is part of a resistance gene cluster on chromosome *12* of potato (Kanyuka et al. 1999), and which encodes a LZ-NBS-LRR type of protein (Van der Vossen et al. 1999), confers specific resistance against population Pa_2-D383. At the molecular level this population is clearly separated from all other *G. pallida* populations which are found in the Netherlands (Folkertsma et al. 1996b). Also the *H2* gene (Dunnet 1961), whose map location is presently unknown, confers resistance against a set of *G. pallida* populations pathotyped ''Pa_1'' which are well-defined at the molecular level (Blok et al. 1997; Folkertsma 1997).

The availability of broad-spectrum resistance against *G. pallida* is of practical importance. For example, combining *Gpa6* with *Grp1* brings complete resistance against both PCN species into focus. Compared to major gene resistance against various other pathogen species, it is expected that resistance against potato cyst nematodes may be relatively durable. PCN species are endemic in the Andean region of South-America and are thought to have been introduced into Europe last century. Only a limited part of the genetic variation (and thus the variation at virulence loci also) present in their centre of origin has been introduced. Because the multiplication rate of PCN is low, their active spread is limited and the time between generations is often 2–4 years in a normal crop rotation, it seems highly unlikely that the broad-spectrum resistance obtained by combining *Gpa5* and *Grp1* is broken down rapidly.

In summary, this study reports on the QTL mapping of the resistance loci *Gpa5* and *Gpa6* in potato. These QTLs coincide with the map location of other resistance factors which are known to be involved in gene-for-gene relationships. On the basis of the occurrence of common motifs in previously cloned *R*-genes, it is hypothesized that *R*-genes are evolutionary related components of a recognition system and that the mechanisms generating variation are entailed with a clustered distribution on the genome (Baker et al. 1997). Recent advances in the identification and cloning of resistance-gene candidates (RGCs) has shown that these types of sequences occur abundantly in plant genomes (Botella et al. 1997; Shen et al. 1998). With the aid of an increasing amount of sequence information, different RGCs in the genome will be discriminated and resistance specificities will be assigned to the genes. This may eventually lead to a detailed dissection of the resistance gene clusters harbouring *Gpa5* and *Gpa6* and may confirm our hypothesis that these two additive QTLs operate on the basis of a classical gene-for-gene relationship.

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