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**J. Rouppe van der Voort · W. Lindeman
R. Folkertsma · R. Hutten · H. Overmars
E. van der Vossen · E. Jacobsen · J. Bakker**

A QTL for broad-spectrum resistance to cyst nematode species (*Globodera* spp.) maps to a resistance gene cluster in potato

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Abstract Broad-spectrum resistance in potato to the potato cyst nematode (PCN) species *Globodera rostochiensis* and *G. pallida* is commonly regarded as a polygenically inherited trait. Yet, by use of QTL analysis and a selected set of PCN populations, resistance to both PCN species could be ascribed to the action of locus *Grp1*. *Grp1* confers major resistance to *G. rostochiensis* line Ro_5-22 and *G. pallida* population Pa_2-D383 and partial resistance to *G. pallida* population Pa_3-Rookmaker. *Grp1* was mapped on chromosome 5 using previously characterized AFLP markers. Cleaved amplified polymorphic sequence (CAPS) markers available for RFLP loci GP21 and GP179 revealed that *Grp1* maps on a genomic region harboring other resistance factors to viral, fungal and nematodal pathogens. The present data indicate that *Grp1* is a compound locus which contains multiple genes involved in PCN resistance.

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J. Rouppe van der Voort (✉) · W. Lindeman
R. Folkertsma[1] · H. Overmars · J. Bakker
The Graduate School of Experimental Plant Sciences,
Wageningen Agricultural University, Department of Nematology,
P.O. Box 8123, 6700 ES Wageningen, The Netherlands
Fax: 31-317-484254
E-mail: jeroen.rouppevandervoort@medew.nema.wau.nl

W. Lindeman · R. Hutten · E. Jacobsen
The Graduate School of Experimental Plant Sciences,
Wageningen Agricultural University,
Department of Plant Breeding,
P.O. Box 386, 6700 AJ Wageningen, The Netherlands

E. van der Vossen
The Graduate School of Experimental Plant Sciences,
Wageningen Agricultural University, Centre for Plant Breeding
and Reproduction Research, P.O. Box 16, 6700 AA Wageningen,
The Netherlands

Present address:
[1] Department of Virology, P.O. Box 8045, 6700 EM Wageningen,
The Netherlands

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Introduction

Characterization of complex genetic factors has been greatly facilitated by recent advances in DNA marker technology. Polymerase chain reaction (PCR)-based markers like amplified fragment length polymorphisms (AFLP™, Vos et al. 1995) are efficient tools to generate dense linkage maps. With these linkage maps, genomic regions containing quantitative trait loci (QTL) can be identified and accurately analyzed with the aid of statistical procedures which are applicable through the use of publicly available computer packages (Lander and Botstein 1989; Lincoln et al. 1992; Van Ooijen and Maliepaard 1996a,b). The ability to trace Mendelian loci of quantitatively inherited characters through their association with DNA markers has been used to study both complex agronomic traits like fruit mass, contents of soluble solids and fruit pH in tomato (Paterson et al. 1988), and polygenic disease resistances to, for example, bacterial wilt in tomato (Danesh et al. 1994), potato late blight (Leonards-Schippers et al. 1994) and rice blast (Wang et al. 1994).

The present study focuses on resistance to potato cyst nematodes (PCN: *Globodera rostochiensis* and *G. pallida*) which are severe pests in potato (*Solanum tuberosum* ssp. *tuberosum*). Several sources of monogenic resistance are available to potato breeders, either to *G. rostochiensis* [among others, genes *H1* (Huijsman 1955), *Gro1* (Barone et al. 1990) and *GroV1* (Jacobs et al. 1996)] or to *G. pallida* [*Gpa* (Kreike et al. 1994) and *Gpa2* (Rouppe van der Voort et al. 1997b)]. Interestingly, these resistance genes are clustered with loci involved in resistance to other plant pathogens (summarized in Leister et al. 1996). Quantitatively inherited resistance to *G. pallida* has also been found (e.g.

Goffard and Ross 1954; Ross 1986; Dale and Phillips 1982; Mugniéry et al. 1989). However, the aforementioned sources of resistance are only effective against a limited number of PCN populations.

To obtain broad-spectrum resistance, breeders have accumulated an unknown number of genes in breeding lines, which are derived from a range of wild *Solanum* species (Dellaert and Vinke 1987). Although these breeding lines confer complete resistance against a large number of PCN populations, the identification of resistant offspring clones is obscured by a continuous distribution in resistance levels; no distinct classes of resistant and susceptible clones are observed. This phenomenon is typical for various sources of PCN resistance. In addition, assessment of the level of quantitatively inherited resistance against PCN is complicated by the variation in multiplication rates of the nematodes in different tests. It has been shown that variation between tests is largely due to genotype-environment interactions (Mugniéry et al. 1989). Environmental factors, such as temperature and moisture, affect the infection capabilities and the sex ratio of the nematode (Mugniéry and Fayet 1984). Another difficulty in breeding for resistance against PCN is the choice of a proper test population. As with many other soil-borne pathogens, PCN have mosaic distribution patterns, and even in a small area populations may have distinct genetic structures (Folkertsma 1997). The current international pathotype scheme (Kort et al. 1977) reflects only a limited part of this variation (Bakker et al. 1993). To obtain insight in the genetic diversity of potato cyst nematodes, Folkertsma (1997), used molecular techniques to analyze more than 300 populations sampled all over Europe. At present, a diverse set of representatives of the PCN metapopulation is available to evaluate the resistance spectra of potato clones.

In this paper, we report on the genetic analysis of broad-spectrum resistance against PCN. Following screening with diverse representants of the PCN metapopulation, we selected the tetraploid breeding line AM78-3778 as it confers broad-spectrum resistance and subjected it to a detailed genetic study. QTL analysis revealed one major locus which is part of a resistance gene cluster on chromosome 5. Contrary to the spectra of previously identified PCN resistance genes, this locus confers resistance to both PCN species.

Materials and methods

Plant material

The broadness of PCN resistance was tested in potato clones AM78-3778, 3778-14 and 3778-16. The tetraploid clone AM78-3778 contains resistance to both PCN species and 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. Clones 3778-14 and 3778-16 are dihaploids ($2n = 2x = 24$) produced by prickle pollination of clone AM78-3778 with haploid inducer *S. phureja* clones (Hutten et al. 1994).

A mapping population of diploid potato was obtained from a cross between the clones 3778-16 × RH89-039-16. Clone 3778-16 is the resistant parent in our population and is referred to as AM. The susceptible male parent, clone RH89-039-16, will be referred to as RH. The mapping population F_1AM × RH consisted of 122 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 in the field.

Nematodes

The nematode populations used in this study are listed in Table 1. The code of the population is preceded by its pathotype designation. *G. rostochiensis* lines Ro_1-19 and Ro_5-22 are heterogeneous populations except that they are fixed for the alleles at the avirulence and virulence locus to the *H1* resistance gene, respectively. Lines Ro_1-19 and Ro_5-22 were selected from controlled single nematode matings as described (Janssen et al. 1990). Pathotype Ro_1 belongs to the most avirulent and Ro_5 to the most virulent category of *G. rostochiensis* populations (Kort et al. 1977). The *G. pallida* populations were originally sampled from heavily infested spots in a field. Population Pa_2-D383 is a relatively avirulent *G. pallida* population. Population Rookmaker, termed Pa_3-Rook, is one of the most virulent *G. pallida* populations found in the Netherlands and is currently used as test-inoculum for *G. pallida* resistance in several commercial breeding programs. The virulence characteristics as well as the molecular data of these *G. pallida* populations are extensively described in Bakker et al. (1992). Nematode populations were multiplied on susceptible cv 'Eigenheimer', inoculated with approximately 200 cysts and placed in a growth chamber at 18°C and 16-h daylength. PCN populations were stored at −80°C until use (Folkertsma et al. 1997).

Resistance testing and data collection

Preparation of the PCN inoculum was as described by Rouppe van der Voort et al. (1997b). The resistance spectrum assay was carried out in a closed container (Phillips et al. 1980) using 125-cc plastic containers filled with silversand. Per container, one tuber was added

Table 1 Broadness of resistance in AM78-3778-derived clones. The average numbers of cysts recovered in a closed container test are presented (*n.d.* not determined)

Potato clone	Ro1-19	Ro5-22	Pa2-D383	Pa2-D350	Pa2-HPL1	Pa3-1097	Pa3-Rook	Pa3-74.768.20
AM78-3778	2	10	0	0	2	2	2	4
3778-16	2	7	0	0	1	4	4	13
3778-14	n.d.	n.d.	1	9	7	135	108	73
RH89-039-16	189	98	101	132	146	240	153	166

and inoculated with nematodes to a final density of 5 eggs/J_2 per gram soil. The containers were maintained in the dark at approximately 20°C for at least 3 months.

The inheritance of the resistance to populations Ro_5-22, Pa_2-D383 and Pa_3-Rook was analyzed in mapping population F_1AMRH in three replications. Resistant standards were cv 'Multa' (resistant to Pa_2-D383), *S. vernei* hybrid cv 'Santé' (resistant to Pa_2-D383 and Ro_5-22) and AM78-3778 (resistant to Pa_2-D383, Pa_3-Rook and Ro_5-22). As susceptible standards cv 'Eigenheimer' and cv 'Maritta' were used. The tubers were inoculated with nematodes (final density of 5 eggs/J_2 per gram soil) in 900-g pots containing a mixture of silversand and a sandy loam fertilized with Osmocote (N-P-K granulates). Plants were arranged in a randomized block design and grown in a greenhouse with 15°C and 25°C as minimum and maximum temperatures, respectively.

After 3 months, cysts were recovered from the soil by elutriation and counted. In addition, the size of the root systems was classified on a scale of 0 to 3. Resistance data of a genotype were only recorded when at least 3 well-rooted plants of this genotype were available.

DNA marker analysis and linkage map construction

DNA isolation, AFLP analysis and data recording were done as described previously (Vos et al. 1995; Van Eck et al. 1995). From the segregation of 408 AFLP markers, generated by use of 11 primer combinations, separate genetic maps of the parental clones were constructed (Rouppe van der Voort et al. 1997a). The separate maternal AM and paternal RH maps consisted of 242 and 220 AFLP markers, respectively. These maps were aligned with the genetic map of potato by means of common AFLP markers which have been mapped relative to restriction fragment length polymorphism (RFLP) markers in a reference population (Van Eck et al. 1995). Common AFLP markers were visually recognized on autoradiogram images as co-migrating bands in fingerprints generated from different genotypes using the primer combinations E + AAA/M + ACG, E + AAC/M + CAC, E + AAC/M + CAG, E + ACA/M + CGT, E + AGA/M + CAT and E + ATG/M + CTA. Genetics maps are available from URL: http://www.spg.wau.nl/pv/aflp/catalog.htm.

The PCR primer sequences and the temperature cycle files of the CAPS markers (cleaved amplified polymorphic sequences; Konieczny and Ausubel 1993) for loci GP21 and GP179 were obtained from Meksem et al. (1995). Segregating AM alleles were detected after digestion of the amplification products using the restriction endonucleases *Dra*I for marker GP21 and *Rsa*I for marker GP179. The marker order for CAPS markers GP21 and GP179 was calculated using the software package JOINMAP 1.4 (Stam 1993). The primer sequences for locus CP113; CP113-5′1, CP113-3′1 and CP113-3′3* were obtained from Niewöhner et al. (1995). The following temperature cycle files were applied for these markers: CP113-5′1/CP113-3′1: 3 min at 93°C, followed by 35 cycles of 30 s at 93°C, 45 s at 60°C, 90 s at 72°C and finished by a 10 min-elongation at 72°C; CP113-5′1/CP113-3′3*: 3 min at 93°C, followed by 5 cycles of 30 s at 93°C, 45 s at 50°C and 90 s at 72°C, after which the annealing temperature was decreased to 48°C. This file was also completed by an elongation step at 72°C for 10 min.

Statistical analysis

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

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

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

The broad-sense heritability was calculated according to the formulas:

$$\sigma^2_{gen} = (MS_{tot} - MS_{rep})/3$$

$$h^2 = \sigma^2_{gen}/\sigma^2_{gen} + \sigma^2_{rep}$$

where σ^2_{gen} is the genetic variance among the genotypes.

The data on marker segregation of both parents were included for QTL analysis using the program MAPQTL 3.0 (Van Ooijen and Maliepaard 1996a,b). The markers were transferred into a biallelic code according to the manual. Three different mapping methods were applied for QTL detection. The first method was a non-parametric rank-sum test of Kruskal-Wallis (see e.g. Sokal and Rohlf 1995) in which the non-transformed, average cyst counts were analyzed. A threshold value of $P < 0.0001$ was used for the individual marker tests. In the second option of MAPQTL, interval mapping for cross-pollinating species (CP) was applied. The likelihood that a QTL is present between two flanking marker loci is indicated by the LOD score (Lander and Botstein 1989). A LOD value of 3.0 was chosen as threshold value (Lander and Botstein 1989). As a third method, MQM mapping (Jansen 1993; Jansen et al. 1995) was applied. The QTL with the largest effect on the trait examined was used as covariate to enhance the power in the detection of putative other QTLs. The magnitude of the marker-associated phenotypic effect is presented by the coefficient of determination (R^2), which describes the percentage of the total variance explained for by the marker genotypes in the interval mapping procedure.

Results

Broadness of PCN resistance from AM78-3778

The broadness of PCN resistance present in clone AM78-3778 was assessed relative to the resistance present in the dihaploids 3778-14 and 3778-16 (AM). AM78-3778 as well as clone AM appeared to be resistant to all of the PCN populations tested (Table 1). Loss of resistance to the Pa_3 populations was observed in clone 3778-14. The diploid clone RH89-039-16 (RH) was susceptible to all of the populations tested.

Inheritance of the resistance

The average numbers of cysts developed on the parental genotypes are shown in Table 2. Analysis of variance on normalized cyst counts revealed that the genetic variance for both *G. pallida* and *G. rostochiensis* resistance was significant ($P < 0.0001$). No significant differences in cyst numbers were found among the blocks of replicates. The broad-sense heritabilities, listed in Table 2, indicate that the variation in cyst counts was barely affected by environmental factors and root-system development. It has been noted that the values for skewness and kurtosis sometimes deviate from the test criteria on normally distributed $^{10}\log(x + 1)$ transformed cyst numbers (Snedecor and Cochran 1967). The analyses on these data may therefore be slightly biased.

Figure 1A shows that in the progeny, the resistance to population Pa_2-D383 was correlated with resistance

Table 2 Results of the quantitative analysis of PCN resistance as measured by the number of cysts counted in the respective PCN populations. Skewness and kurtosis of $^{10}\log(x + 1)$ transformed cyst numbers, heritability of the specific resistance, the map location of the QTLs with their nearest marker, the *P* value of the nearest marker in a Kruskal-Wallis test, the LOD score and the R^2 at the QTL position are given

Trait	Average no. of cysts AM	Average no. of cysts RH	Skewness	Kurtosis	h^{2a}	Marker[b]	R^{2c}	*P* value	LOD
Pa2-D383	5	662	− 0.45	− 0.87	0.86	GP179	66%	< 0.0001	16.8
Pa3-Rook	30	1067	− 0.70	0.19	0.83	GP179	45%	< 0.0001	11.7
Ro5-22	5	3961	− 0.41	− 1.32	0.83	GP21	77%	< 0.0001	17.9
Root system	–	–	–	–	0.086	–	–	–	–

[a] h^2 = Heritability
[b] Marker nearest to QTL
[c] R^2 = Percentage of the total variance explained by the marker genotypes

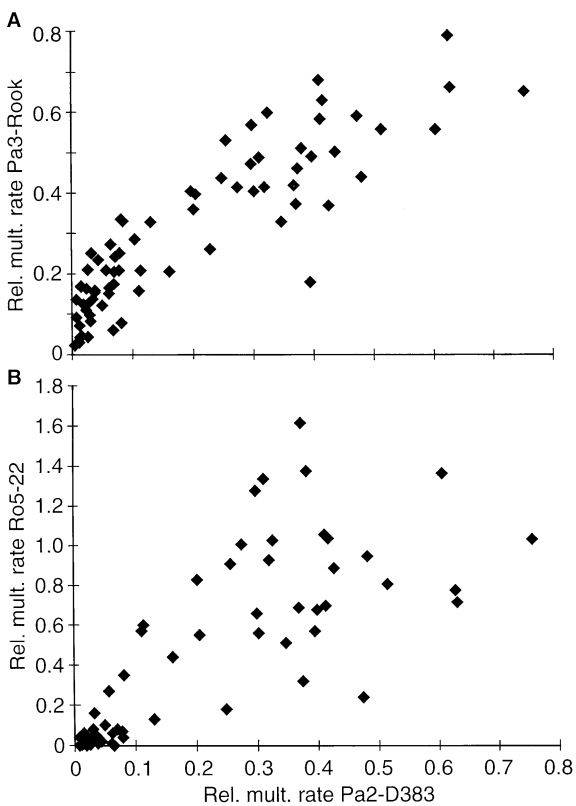

Fig. 1A, B Comparison of relative multiplication rates (rel. mult. rates) of different PCN inocula on F_1 genotypes. The relative multiplication rates on F_1 genotypes are expressed by the number of newly developed cysts divided by the number of cysts developed on the susceptible parent RH. **A** Comparison of rel. mult. rates on F_1 genotypes between populations Pa_2-D383 and Pa_3-Rook, **B** Comparison of rel. mult. rates between populations Pa_2-D383 and Ro_5-22

to population Pa_3-Rook. A decrease in the relative multiplication rate of population Pa_2-D383 on a F_1 genotype is associated with a decrease in the relative multiplication rate of population Pa_3-Rook. For example, the set of F_1 genotypes for which relative multiplication rates between 0 and 0.08 (actual cyst numbers between 0 and 56) for population Pa_2-D383 were found showed relative multiplication rates between 0 and 0.2 (cyst numbers between 0 and 140) for population Pa_3-Rook. Similarly, it is shown that the resistance to *G. pallida* Pa_2-D383 was correlated with resistance to *G. rostochiensis* line Ro_5-22 (Fig. 1B).

AFLP markers

By scoring resistance to Pa_2-D383 as a monogenic trait using the arbitrary criterion of genotypes containing fewer than 56 cysts as being resistant and genotypes containing more than 56 as being susceptible, we observed linkage (at LOD > 3.0) with 6 AFLP markers localized on the map of genotype AM. These AFLP markers reside on chromosome 5, as determined by their linkage with previously mapped common AFLP markers. Common AFLP markers can be visually recognized as co-migrating bands in fingerprints of potato genotypes which have also been analyzed with chromosome-specific RFLP markers (Rouppe van der Voort et al. 1997a). Pa_2-D383 resistance could not be precisely mapped on chromosome 5, apparently because the variance was too high to be explained by a monogenic analysis. A more profound analysis of the data was therefore performed by a QTL approach (see below). In addition, resistance to both Pa_3-Rook and Ro_5-22 could only be mapped on chromosome 5 by means of Pa_2-D383 resistance as the bridging marker. Nevertheless, monogenic analysis of the datasets indicates the presence of genetic factor(s) allocated on chromosome 5. A detailed description of the AFLP markers, used for alignment of potato maps are given at URL: http://www.spg.wau.nl/pv/aflp/catalog.htm.

CAPS markers

CAPS markers were tested to identify additional markers on both arms of chromosome 5. The loci GP21

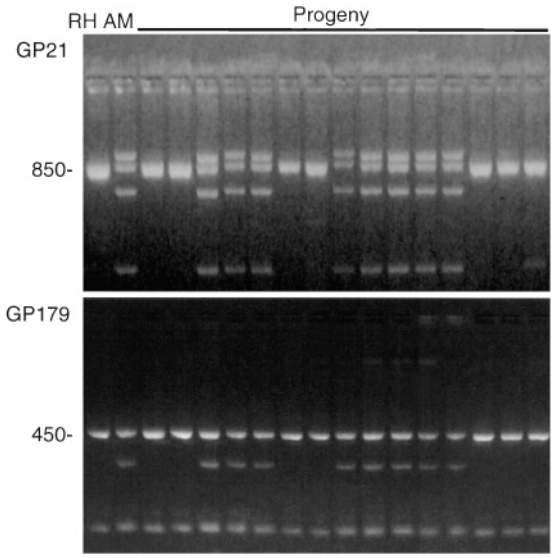

Fig. 2 CAPS analysis of RFLP markers GP21 and GP179. Linkage of both CAPS markers is shown by the profiles generated from the susceptible parent RH, the resistant parent AM and a subset of their progeny. The segregating AM alleles are linked in coupling phase with an allele conferring PCN resistance. Molecular weights of the DNA fragments are given on the *left*

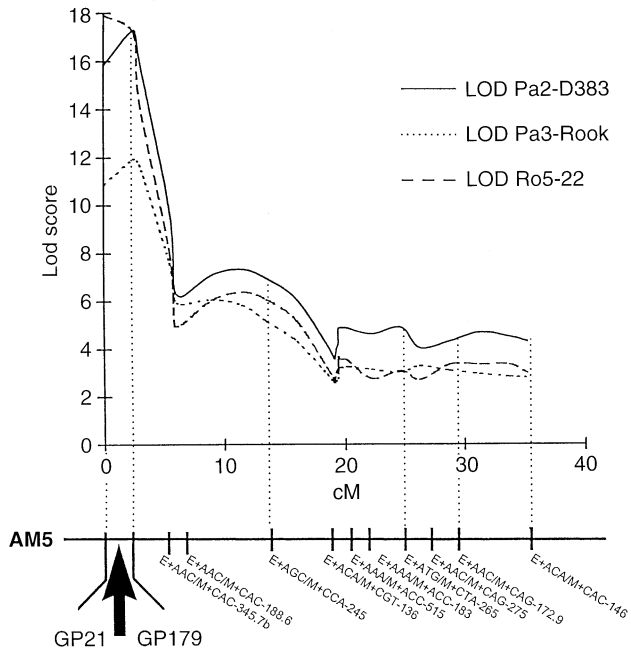

Fig. 3 LOD plot for PCN resistance on chromosome 5 of genotype AM. The position of the QTL on the map is indicated by an *arrow*

and GP179 (Gebhardt et al. 1991; Meksem et al. 1995) were detected after digestion of the amplification products using restriction endonucleases *Dra*I for marker GP21 and *Rsa*I for marker GP179 (Fig. 2). In agreement with the *S. tuberosum* map of Gebhardt et al. (1991), GP21 and GP179 mapped on chromosome 5 at 3 cM from each other. Unfortunately, locus CP113 could not be mapped as a CAPS marker in population F_1AM × RH. Both primer combinations (Niewöhner et al. 1995) produced a monomorphic amplification product even after digestion using a series of 12 four basepairs recognizing restriction endonucleases.

QTL mapping

The computer program MAPQTL (Van Ooijen and Maliepaard 1996a,b) was used to analyze both the resistant and the susceptible parental dataset. The Kruskal-Wallis test revealed significant associations between resistance against the *G. pallida* and *G. rostochiensis* populations and chromosome-5 markers segregating from clone AM. The highest significance levels were found at markers GP21 and GP179 for the three inoculum treatments (Table 2). Further on this chromosome, a second gradient in the Kruskal-Wallis test statistic was observed, presumably due to missing values for markers mapped on this region.

The LOD profiles of the interval mapping are presented in Figure 3. This figure shows high LOD values for markers GP21 and GP179 (3 cM), indicating a large effect on resistance for the three PCN populations tested (see also Table 2). From the interval mapping, it was unclear whether the high LOD values for other chromosome-5 intervals were the result of their linkage with the GP21-GP179 interval or a second QTL. However, the MQM mapping method revealed no additional statistically significant effects for PCN resistance at other genomic intervals.

Discussion

The results of the QTL mapping show that a locus with large effects on the resistance to both PCN species is localized on the genomic region possessing loci GP21 and GP179. This locus confers major resistance to *G. rostochiensis* line Ro_5-22 and *G. pallida* population Pa_2-D383 as well as partial resistance to *G. pallida* population Pa_3-Rookmaker. On the potato map, the GP21-GP179 region is known to contain a cluster of resistance genes encoding specificities to many different plant pathogens, for example, to the fungus *Phytophthora infestans*, (gene *R*1 and a major QTL; (Leonards-Schippers et al. 1992, 1994)), to potato virus X (extreme resistance *Rx*2, Ritter et al. 1991; hypersensitive resistance *Nb*, De Jong et al. 1997) as well as to *G. pallida* (locus *Gpa*, Kreike et al. 1994). Although mechanistically considered to be a different class of resistance, a QTL involved in trichome-mediated insect resistance (Bonierbale et al. 1994) resides also in this region.

The presence of both quantitative and qualitative resistance on the same chromosome region opens the possibility that resistance genes and the genes underlying QTLs are alleles of the same genetic locus. This assumption fits with the hypothesis that qualitative phenotypes are extreme, mutated allelic variants at a quantitative trait locus (Robertson 1985). Moreover, major resistance (*R*) genes cloned from several plant species share striking structural similarities despite their intimate interaction with a diversity of pathogen species. These major genes seem to be members of large multigene families that are arranged in large arrays of complex, evolutionary related but different loci having different specificities (reviewed in Baker et al. 1997).

In this context it seems likely that the QTL for PCN resistance mapped in this study is a compound locus containing different but related *R* genes for PCN resistance. The diploid clone AM used to map PCN resistance is derived from AM78-3778, a tetraploid clone which combines the resistance introgressed from many separate wild *Solanum* sources (Dellaert and Vinke 1987). As neither of these wild species contain resistance to both *G. rostochiensis* and *G. pallida*, the underlying genetic model for the locus identified in AM78-3778 probably includes more than one gene. Since these genes have been separately introgressed, the in-coupling linkage phase of these genes should be explained by the many generations involved in breeding clone AM78-3778, which may have resulted in fortuitous recombination events producing multiple *R* genes on the same homologous chromosome. We propose to name the PCN resistance locus *Grp1* (for *G. rostochiensis* and *G. pallida* resistance) until future research will enable us to ascribe the resistance of *Grp1* to the action of different genes.

The resistance to Pa_3-Rook seems to involve additional loci given the fact that 7 offspring clones harbor resistance levels comparable to that of parent AM. In addition, *R* genes which confer complete resistance at the level of the individual nematode may have the appearance of quantitatively inherited resistance genes when heterogeneous pathogen populations are used in the resistance test. The occurrence of the latter genetic model is not inconceivable given that resistance tests rely predominantly on screening with field populations. These field populations are often not homogeneous for virulence traits but are mixtures of virulent and avirulent genotypes. In case a PCN population is not homogeneous for the virulence trait examined, a single *R* gene operating on the basis of a classical 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. Formal proof for a gene-for-gene relationship has so far only been obtained for the interaction between *G. rostochiensis* and the *H1* gene from *S. tuberosum* ssp. *andigena* CPC1673 (Janssen et al. 1991), but it is likely that various other PCN resistance genes act in a similar way. It is therefore hypothesized that for complete resistance to Pa_3-Rook as observed in AM78-3778 only a few loci are involved. A precedent consistent with this hypothesis is that in the dihaploid genotype 3778-14, Pa_3 resistance was lost whereas the Pa_2 resistance was maintained. Thus, complete resistance can be obtained by accumulating *R* genes with specificities which match with the different avirulent genotypes in a heterogeneous field population.

The question of whether the resistance to Pa_2-D383 and Pa_3-Rook is mediated by the same *Grp1* allele remains unanswered by the results of this study. No recombination was found between Pa_2-D383 and Pa_3-Rook resistance among the three GP21/GP179 recombinant genotypes identified in population $F_1AM \times RH$. Kreike et al. (1994) found recombination between Pa_2 and Pa_3 resistance as well as between markers in the same genomic interval. This indicates that *Gpa* may indeed be a compound locus. Partial overlap has been found in the resistance spectra of *Grp1* and *Gpa*; *Grp1* confers resistance to populations of both PCN species and only partial resistance to Pa_3-Rook, whereas *Gpa* confers resistance to all *G. pallida* populations tested so far but not to *G. rostochiensis* (Kreike et al. 1994; P. Wolters, personal communication). Therefore, *Grp1* should be considered as being different from *Gpa* while possibly having allele(s) in common with it.

In this report it is shown that by testing with a diverse set of representants of the PCN metapopulation, broad-spectrum PCN resistance in AM78-3778 can be determined by the action of different genes at a compound locus. The *Grp1* locus harbors resistance to both PCN species. Although it confers incomplete resistance to *G. pallida*, it is expected that complete resistance can be achieved by the introgression of *R* genes with complementary specificities. Current breeding strategies for PCN resistance rely on trial and error approaches. However, the availability of a representative set of pathotype populations and the development of MAS assays will allow a more directed approach towards achievement of complete PCN resistance in commercial cultivars by combining genes with complementary specificities.

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