

# *GpaXI<sup>tar</sup>* originating from *Solanum tarijense* is a major resistance locus to *Globodera pallida* and is localised on chromosome 11 of potato

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**Abstract** Resistance to *Globodera pallida* Rookmaker (Pa3), originating from wild species *Solanum tarijense* was identified by QTL analysis and can be largely ascribed to one major QTL. *GpaXI<sup>tar</sup>* explained 81.3% of the phenotypic variance in the disease test. *GpaXI<sup>tar</sup>* is mapped to the long arm of chromosome 11. Another minor QTL explained 5.3% of the phenotypic variance and mapped to the long arm of chromosome 9. Clones containing both QTL showed no lower cyst counts than clones with only *GpaXI<sup>tar</sup>*. After Mendelising the phenotypic data, *GpaXI<sup>tar</sup>* could be more precisely mapped near markers GP163 and FEN427, thus anchoring *GpaXI<sup>tar</sup>* to a region with a known *R*-gene cluster containing virus and nematode resistance genes.

## Introduction

The white potato cyst nematode (PCN) *Globodera pallida* (Stone) is a major pest in potato. Early work on resistance

breeding has been concentrated on the golden potato cyst nematode *G. rostochiensis*, although the distinction between *G. rostochiensis* and *G. pallida* has been introduced only since 1974 (Stone 1972). Breeders succeeded in producing potato cultivars resistant to *G. rostochiensis*, predominantly by incorporating the *H1* gene from *S. tuberosum* ssp. *andigena* CPC 1673 (Ellenby 1952; Huijsman 1957; Ross 1979). Since the discovery of *H1*, many more resistance genes against *G. rostochiensis* have been identified (reviewed by Gebhardt and Valkonen 2001). Due to the extensive use of *G. rostochiensis* resistant cultivars, *G. pallida* populations, free of competition from *G. rostochiensis*, have become the majority of the cyst nematode pest population in Europe. Great emphasis is now placed on producing varieties with resistance against a broad spectrum of populations (Hancock 1996; Turner 1989). Breeding for resistance to *G. pallida* was initially hampered by the seemingly quantitative inheritance of the resistances first identified in *S. vernei* and *S. tuberosum* ssp. *andigena* (Dale and Phillips 1982; Ross 1986). The resistance to pathotypes Pa2 and Pa3 from *S. tuberosum* ssp. *andigena* CPC 2802 was first thought to be monogenic (the *H3* gene) (Howard et al. 1970), but was later proven to be polygenic (Dale and Phillips 1982). Genetic analysis of a SCRI progenitor clone with resistance against *G. pallida*, and descending from CPC 2802, resulted in the identification of a major QTL on linkage group 4 (probably the *H3* gene), as well as a minor QTL on linkage group 11 (Bryan et al. 2004). The higher diversity of the *G. pallida* populations compared to *G. rostochiensis* populations also hampered incorporation of effective resistance (Folkertsma 1994; Schnick et al. 1990).

Monogenic resistance to *G. pallida* has been identified. Some appear to be only effective against specific *G. pallida* populations, such as the *H2* locus from

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*S. multidissectum*, conferring resistance to *G. pallida* populations of pathotype Pa1 (Dunnett 1961), and the *Gpa2* gene. *Gpa2*, derived from *S. tuberosum* spp. *andigena* CPC 1673 confers resistance to a small, distinct population of *G. pallida* Pa2. *Gpa2* maps on chromosome 12 of potato in a single cluster of virus and nematode resistance genes and has been cloned. The protein encoded by *Gpa2* turned out to be of the class of LZ–NBS–LRR type plant resistance genes (Arntzen et al. 1994; Rouppe van der Voort et al. 1999; van der Vossen et al. 2000).

Rouppe van der Voort et al. (2000) identified two QTL that are likely derived from *S. vernei*. One of the two loci, *Gpa5*, explained 61% of the total variation and the resistance conferred by this locus appears to work against a range of *Globodera* populations. The other locus, *Gpa6*, explained 24% of the total variation and acts in a pathotype-specific way. Interestingly, both QTL combined give additive resistance levels. The same holds true for potato clones carrying both QTL *GpaV<sub>spl</sub>* and *GpaXI<sub>spl</sub>* (Caromel et al. 2005). These *S. sparsipilum* derived QTL were mapped on chromosome 5 and 11, respectively. When combined they do not only show an additive effect on sex ratio of *G. pallida*, but also give a necrotic reaction in roots infected by nematodes; an effect not seen with the individual QTL. Another locus, *Grp1*, with resistance against both PCN species (Rouppe van der Voort et al. 1998a) showed resistance to *G. rostochiensis* line Ro<sub>5</sub>-22 and *G. pallida* populations Pa<sub>2</sub>-D383 and Pa<sub>3</sub>-Rookmaker. *Grp1* was mapped on chromosome 5 in the same region as *Gpa* and *Gpa5*. However, it has not been described in literature that any of the wild *Solanum* sources used to introgress *Grp1* showed resistance against both *G. rostochiensis* and *G. pallida*. It is not excluded that the combined resistance against *G. rostochiensis* and *G. pallida* of *Grp1* is caused by two, or possibly three, tightly linked *R*-genes.

For most *G. pallida* resistance loci described above, the phenotypic segregation data indicated quantitative inheritance. DNA markers offered the potential to trace the Mendelian loci underlying quantitatively inherited nematode resistance and in many cases the resistance appeared to be controlled by single major genes explaining a large proportion of the phenotypic variance. Moreover, the fact that most PCN resistances have been mapped in well-known resistance clusters indicates the likelihood that these major loci are NBS-LRR genes and operate on a gene-for-gene basis (Gebhardt and Valkonen 2001).

In this study, we report a major-effect locus involved in resistance against *G. pallida* Pa3-Rookmaker originating from *S. tarijense*. Qualitative interpretation of the resistance data allowed mapping of the major-effect locus to a known cluster of resistance genes on the long arm of chromosome 11.

## Materials and methods

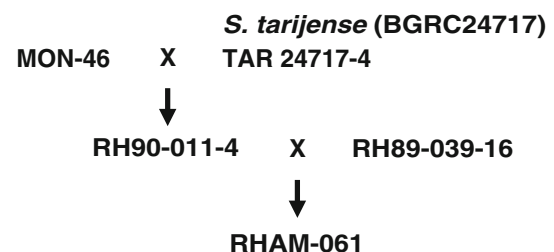
### Plant material

The diploid BC1 mapping population RHAM-061 was obtained from a cross of resistant parent RH90-011-4 and susceptible *S. tuberosum* parent RH89-039-16, as shown in Fig. 1. RH90-011-4 was obtained from an interspecific cross between *S. tarijense* (BGRC 24717/CGN 18107) and MON-46, a dihaploid clone of the susceptible cultivar Mondial.

### Nematode resistance assays

A closed container test (Phillips et al. 1980) with *G. rostochiensis* populations ASCRI (Ro1), C262 (Ro2\_3), G 1510 (Ro5) was performed on the *S. tarijense* parent RH90-011-4. After 8 weeks in the dark at 18°C, the newly formed cysts that were visible from the outside were counted.

All further *G. pallida* resistance assays were arranged in randomised complete block designs, including parents. As much as 188 offspring genotypes of RHAM-061 were multiplied in vitro and transplanted in 500-ml clay pots to the greenhouse in 3 replicates. Eight replications of the parents as well as the susceptible standard Bintje were included. *G. pallida* population Rookmaker (Pa3), originally sampled from a heavily infested site in Valthe, the Netherlands, was used for the inoculation. Population Rookmaker is one of the most virulent *G. pallida* populations in the Netherlands (Bakker et al. 1992). Four weeks after transplanting, the plants were inoculated. Inoculum of nematodes was prepared as described by Rouppe van der Voort et al. (1997a). Cysts were soaked overnight in tap water before crushing to obtain eggs and second-stage juveniles (J2). The egg/J2 suspension was then sieved through a 100-µm sieve to remove cell walls and debris. The suspension was inoculated to the plants to a final density of 6 eggs/J2 per ml soil. Two and a half months after inoculation, the cysts were elutriated with a Fenwick can and counted (Fenwick 1940).



**Fig. 1** Pedigree of the interspecific *GpaXI<sub>tar</sub>* mapping population RHAM-061

## DNA extraction

Genomic DNA was isolated from young leaf material as described by van der Beek et al. (1992). Tissue was ground with steel balls using a Retsch machine (Retsch Inc., Haan, Germany) in the STE-buffer, with volumes adapted to 2-ml deep 96-wells Costar plates (Corning Inc., Corning, NY, USA). DNA was visualised on agarose gel to check the integrity.

## *EcoRI/MseI* markers

AFLP was performed according to (Vos et al. 1995). Fourteen *EcoRI/MseI* primer-combinations eAACmCCA, eAACmCCT, eAACmCGA, eAACmCTG, eAAGmCCT, eAAGmCGA, eACAmCAA, eACAmCCA, eACAmCTA, eACTmCAA, eACTmCAG, eAGAmCAG, eAGAmCAT and eAGAmCTG were used. AFLP fragments were visualised on radioactive gels and scored visually for absence/presence.

## PCR markers

Two chromosome 11 specific markers, GP163 (Brigneti et al. 1997) and FEN427, were used. CAPS marker GP163 is a previously converted RFLP marker, previously used to map potato virus Y resistance gene *Ry<sub>sto</sub>* on chromosome 11 (Brigneti et al. 1997). CAPS marker FEN427 was developed on the basis of AFLP marker pATmCAC\_427 which was previously used to map *R<sub>Mc1-fen</sub>* on chromosome 11 (Draaistra 2006). AFLP band pATmCAC\_427 was excised from gel and dissolved in 50 µl water. Supernatant was re-amplified with P + 0 and M + C primers and purified with a G50 column before sequencing. PCR primers for FEN427 were designed on the sequence of AFLP marker pATmCAC\_427 with Primer3 software (Rozen and Skaletsky 2000). PCR-products of GP163 and FEN427 were digested with 27 restriction enzymes to identify polymorphic sites (Brugmans et al. 2003).

## Map construction

The grouping of markers in linkage groups and the marker order were calculated using the software package JoinMap (Stam 1993). Only AFLP markers with LOD scores >3.0 were included in mapping. Initially the maternal and paternal linkage groups were constructed separately, by taking only the 1:1 segregating AFLP loci (Aa × aa and aa × Aa for the maternal and paternal maps, respectively). The paternal linkage groups could be assigned to potato chromosomes using a reference mapping population that has the susceptible parent RH89-039-16 in common. This reference map SHxRH (Roupe van der Voort et al. 1997a;

van Os et al. 2006) was aligned along with other maps, and resulted in an online catalogue of AFLP markers covering the potato genome (Roupe van der Voort et al. 1998b). Once the chromosome numbers were known of the paternal linkage groups, subsequently the maternal linkage groups could be aligned using the so called ‘bridge markers’. Bridge markers are a small subset of the segregating AFLP loci, which are heterozygous in both parents (Aa × Aa and thus segregating in a 3:1 ratio).

The maternal linkage group, corresponding to potato chromosome 11 including the locus involved in PCN resistance gene was recalculated with RECORD (van Os et al. 2005a), to assess putative ambiguities in marker order. With the information on marker order, the raw data were inspected for spurious data points (singletons). Singletons are easily recognised in graphical genotypes that are generated from the raw data in MS-Excel, where cell colour is formatted conditional to marker observation and linkage phase.

## Data analysis

QTL analysis of the resistance data was performed with MapQTL 5.0 (van Ooijen and Maliepaard 1996). Data were transformed by taking the natural logarithm of the average cyst number (+1) per genotype, to obtain a uniform distribution of the variance. The heritability was estimated as follows:  $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2/n)$ , where  $n$  is the number of replications,  $\sigma_e^2$  is the expected mean square of the residuals and  $\sigma_g^2$  is retrieved from the ANOVA table as follows:  $E(\text{MS})_{\text{genotypes}} = n\sigma_g^2 + \sigma_e^2$ .

Two parametric methods: interval mapping and multiple-QTL mapping (MQM) were applied. The threshold for assigning a QTL was determined by a permutation test, as implemented in MapQTL 5.0.

## Classification of cyst counts into resistant/susceptible phenotypes

For the qualitative mapping of the resistance locus, data of the first nematode resistance assay were used. All genotypes up to a maximum mean of 8 newly formed cysts were assigned as resistant and genotypes with at least 25 newly formed cysts were assigned as susceptible.

## Results

### The inheritance of PCN resistance

In an evaluation of tuber bearing *Solanum* species by The Centre for Genetic Resources (CGN), it was detected that *S. tarijense* accession BGRC 24717 contained resistance



**Fig. 2** Swollen females (appearing as *white cysts*) and *brown cysts* of *G. pallida* formed on roots of a susceptible potato plant

against *G. pallida* pathotype Pa3 (<http://www.cgn.wur.nl>). The inheritance of *G. pallida* resistance was analysed by testing a segregating BC1 population RHAM-061 of *S. tarijense* (BGRC 24717). Plant material inoculated with *G. pallida* population Rookmaker clearly showed a continuous distribution of the average number of cysts developed per genotype, ranging from 1 to 419. The resistant F1 hybrid parent RH90-011-4 developed on average 2 cysts while the susceptible *S. tuberosum* parent RH89-039-16 developed on average 132 cysts. An example of cysts on a susceptible plant is shown in Fig. 2. Fourteen genotypes of RHAM-061 with highly variable number of cysts in the different replications were excluded from further analysis. The variance in average number of cysts per genotype greatly differed between resistant and susceptible genotypes. A uniform distribution of the variance was obtained by taking the natural logarithm of the average cyst number per genotype. Analysis of the variance within and between genotypes showed that the genotypes differed significantly ( $P < 0.001$ ). Broad sense heritability was estimated to be 0.82.

To test if the working spectrum of the *G. pallida* resistance also included *G. rostochiensis* resistance, the *S. tarijense* derived F1 parent RH90-011-4 was inoculated with *G. rostochiensis* in a container test. With all pathotypes, however, (Ro1, Ro2\_3 and Ro5), large number of cysts were developed (between 20 and 60 cysts). The resistance originating from *S. tarijense* against *G. pallida* Rookmaker is therefore not also functional against *G. rostochiensis*.

#### Map construction

A linkage map was constructed using the segregating markers of 14 *EcoRI/MseI* primer combinations. In total 518 segregating markers were identified. As much as 298 AFLP markers segregated from maternal genotype

RH90-011-4, 155 markers from paternal genotype RH89-039-16. There were 65 bridge-markers which are heterozygous in both parents, which were used to connect maternal and paternal linkage groups as identified on the basis of the 1:1 segregating maternal or paternal marker loci. The map of maternal clone RH90-011-4 comprised 12 linkage groups with a total map length of 830.3 cM. The markers of paternal clone RH89-039-16 (RH) were assigned into 12 linkage groups with a total length of 561.1 cM. As much as 19 AFLP markers remained unassigned at  $\text{LOD} = 3$ . Chromosome numbers of RH were identified by aligning the RH linkage groups from this mapping population with the RH linkage groups of reference maps in our lab (Roupe van der Voort et al. 1997a, 1998b; van Os et al. 2006). The 65 bridge-markers were sufficiently equally distributed over the 24 linkage groups and allowed us to pair all 12 maternal linkage groups to their homologous paternal (RH) linkage groups, hereby also identifying the chromosome numbers and orientations of the maternal linkage groups.

#### QTL mapping

The distribution of the ln-normalised cyst counts of the disease test of RHAM-061 was clearly bimodal which could be indicative of a large-effect QTL involved in quantitative resistance. QTL analysis was applied on all maternal and paternal linkage groups to identify all possible resistance factors. With the interval mapping method of MapQTL, one large-effect QTL was detected on maternal chromosome 11 ( $\text{LOD} = 57$ ), which explained 81.3% of the phenotypic variance. No other marker—trait associations exceeded the LOD thresholds of the individual linkage groups as determined by the permutation test. To enhance the power to detect minor-effect QTLs, the MQM mapping method was applied with the QTL on chromosome 11 as a cofactor. This resulted in the identification of a QTL ( $\text{LOD} = 4.4$ ) on the long arm of chromosome 9. The percentage of explained phenotypic variance of this QTL was only 5.3%. Because of the small effect we assume that the resistance is essentially monogenically inherited and located on chromosome 11.

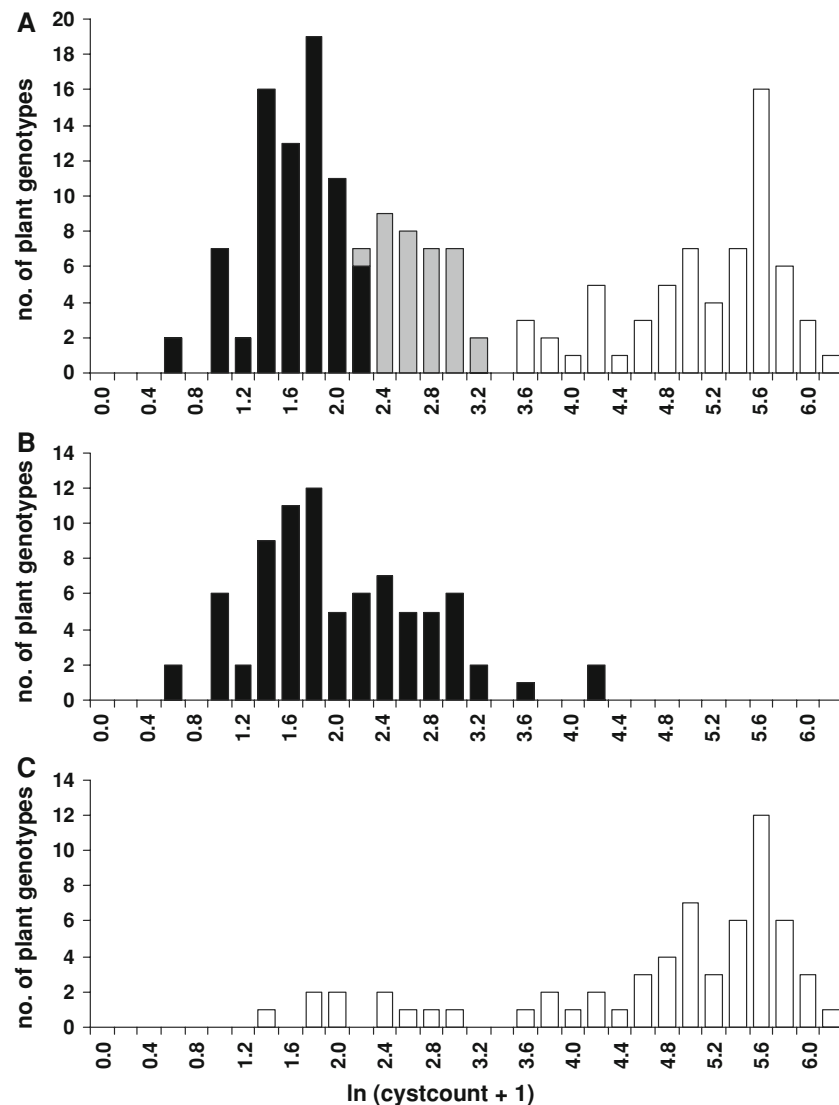
#### Qualitative mapping of resistance

In view of the single locus involved in PCN resistance, as identified with QTL analysis, we proceeded with a qualitative genetic approach. The disease test phenotypes were used for a tentative classification of the offspring, where 76 genotypes containing on average of less than 8 cysts ( $\ln 8 + 1 = 2.2$ ) were assigned as resistant and 64 genotypes with an average of more than 25 ( $\ln 25 + 1 = 3.3$ ) were assigned as susceptible. The remaining 34 genotypes

of intermediate phenotype were excluded from further analysis (Fig. 3a). The transmission of the resistance from the wild species to the BC1 mapping population, and the observed segregation ratio from the resistant parent, which is consistent with a 1:1 distribution ( $\chi^2 = 0.067$ ,  $P = 0.80$ ) allowed us to conclude that the effects can be explained by a single dominant gene.

The qualitatively segregating resistance data were added to the maternal marker dataset. According to

JoinMap analysis, the resistance locus showed linkage with 10 markers on chromosome *11* in the same region, where the resistance QTL was previously mapped. The marker order of chromosome *11* including the resistance locus was verified with RECORD. The resistance locus, which we propose to name *GpaXl<sup>tar</sup>*, is localised on a distal position on the long arm of chromosome *11* between AFLP markers eACTmCAA\_174 and eAGAmCTG\_222.



**Fig. 3** Histograms depicting the distributions of the average natural logarithms of cyst counts (+1) of the individuals of mapping population RHAM-061 after inoculation with *G. pallida* population Rookmaker (Pa3). **a** Histogram of all the individuals of mapping population RHAM-061. *Black* genotypes tentatively assigned as resistant; on average of less than 8 cysts. *Grey* genotypes not assigned; on average between 9 and 24 cysts. *White* genotypes tentatively assigned as susceptible; on average more than 25 cysts. This classification of genotypes into the classes resistant/susceptible allowed mapping of the locus between flanking AFLP markers.

**b** Histogram depicting the distribution of RHAM-061 genotypes where both flanking AFLP markers eACTmCAA\_174 and eAGAmCTG\_222 suggest the presence of resistance. Clones with recombination events between the markers were excluded from the figure. **c** Histogram depicting the distribution of RHAM-061 genotypes where both flanking AFLP markers eACTmCAA\_174 and eAGAmCTG\_222 suggest the absence of resistance. Clones with recombination events between the markers were excluded from the figure

In order to validate the classification and the resulting map location, the flanking AFLP markers can be used to predict in retrospect the absence or presence of the resistance gene. When classifying on the basis of the flanking AFLP markers eACTmCAA\_174 and eAGAmCTG\_222 linked with resistance, the resistant group had on average 9.0 cysts, while the susceptible group had on average 148.9 cysts. The expected resistance, based on the prediction, was compared with the observed phenotypes (Fig. 3b, c). Judging on the basis of the flanking AFLP markers, 8 genotypes out of a total of 140 genotypes showed cyst counts that were incongruent with the expected presence/absence of the resistance gene, and were putatively misclassified. Four of these misclassifications concerned genotypes with no recombination event between the markers on the long arm of maternal chromosome 11. The resistance phenotype of these descendants should be considered as false positives or negatives, without consequences for the position of the *R*-gene. The other four descendants can be best explained by assuming misclassification of the absence/presence of the flanking AFLP marker eAGAmCTG\_222.

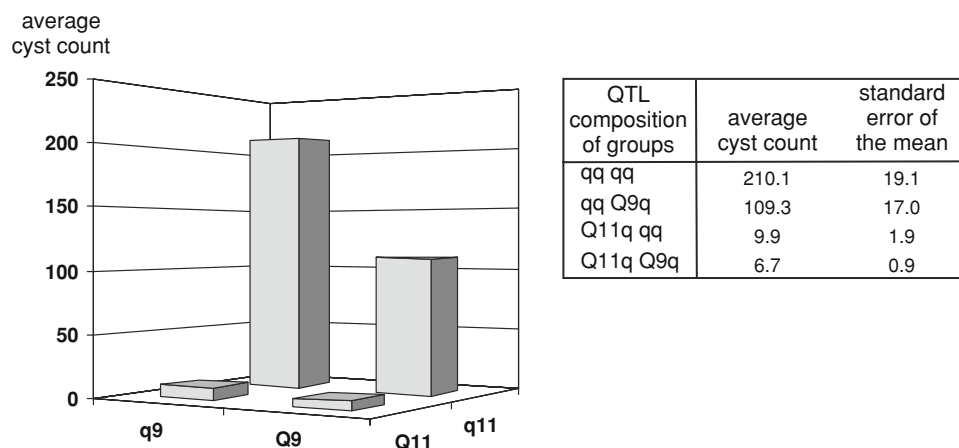
#### Interaction between *GpaXI<sub>tar</sub>*<sup>l</sup> and the QTL on chromosome 9

After identifying the two resistance QTL on chromosomes 9 and 11, the magnitude of the individual effects and the possible interaction between the two QTL was analysed using ANOVA. The flanking AFLP markers eACTmCAA\_174 and eAGAmCTG\_222 linked with the

*GpaXI<sub>tar</sub>*<sup>l</sup> locus were used as explaining variable, indicative for the *R*-gene on chromosome 11. AFLP marker eACAmCCA\_26 showed the closest linkage with the QTL on chromosome 9 and was used as explaining variable indicative for the minor-effect QTL. Analysis of variance demonstrated (as expected) significant main effects for both QTL, but also a significant interaction effect between the QTL of chromosomes 9 and 11. This interaction effect is best explored by illustrating the 2 × 2 interaction of both QTL alleles in Fig. 4, where the cyst counts of these four groups are shown. The group with the susceptible alleles for both *GpaXI<sub>tar</sub>*<sup>l</sup> and the QTL on chromosome 9 showed an average cyst count of 210 cysts ( $n = 29$ ). The group with the resistant allele of the QTL on chromosome 9 but the susceptible allele of *GpaXI<sub>tar</sub>*<sup>l</sup> had an average cyst count of 109 ( $n = 32$ ). This represents a significant decrease of 48% in the development of cyst numbers (2-sided *t*-test on  $\ln(\text{cyst count} + 1)$ ,  $df = 43$ ,  $P = 0.00012$ ). In the presence of *GpaXI<sub>tar</sub>*<sup>l</sup> the minor QTL had no statistically significant effect (2-sided *t*-test on  $\ln(\text{cyst count} + 1)$ ,  $df = 75$ ,  $P = 0.14$ ). If the combination of the two QTL would have resulted in an additive effect, the expected average cyst count should be 5. Therefore, it is concluded that *GpaXI<sub>tar</sub>*<sup>l</sup> displays a dominant epistatic interaction over the minor QTL.

#### Anchoring of *GpaXI<sub>tar</sub>*<sup>l</sup> to a well-known *R*-gene cluster

The long arm of potato chromosome 11 is known to harbour several resistance genes. In order to determine the position of *GpaXI<sub>tar</sub>*<sup>l</sup> relative to these genes, an attempt was



**Fig. 4** The reproduction of *G. pallida* Rookmaker (Pa3) in relation to the genetic composition of potato genotypes of the RHAM-061 mapping population. The height of the bars represent the average number of *G. pallida* Rookmaker (Pa3) cysts in the four QTL offspring classes. The presence or absence of *GpaXI<sub>tar</sub>*<sup>l</sup> is determined by flanking AFLP markers eACTmCAA\_174 and eAGAmCTG\_222.

Presence/absence of the QTL on chromosome 9 was identified by AFLP marker eACAmCCA\_26. Q9 = resistant allele of the QTL on chromosome 9. q9 = susceptible allele of the QTL on chromosome 9. Q11 = resistant allele of *GpaXI<sub>tar</sub>*<sup>l</sup>. q11 = susceptible allele of *GpaXI<sub>tar</sub>*<sup>l</sup>.

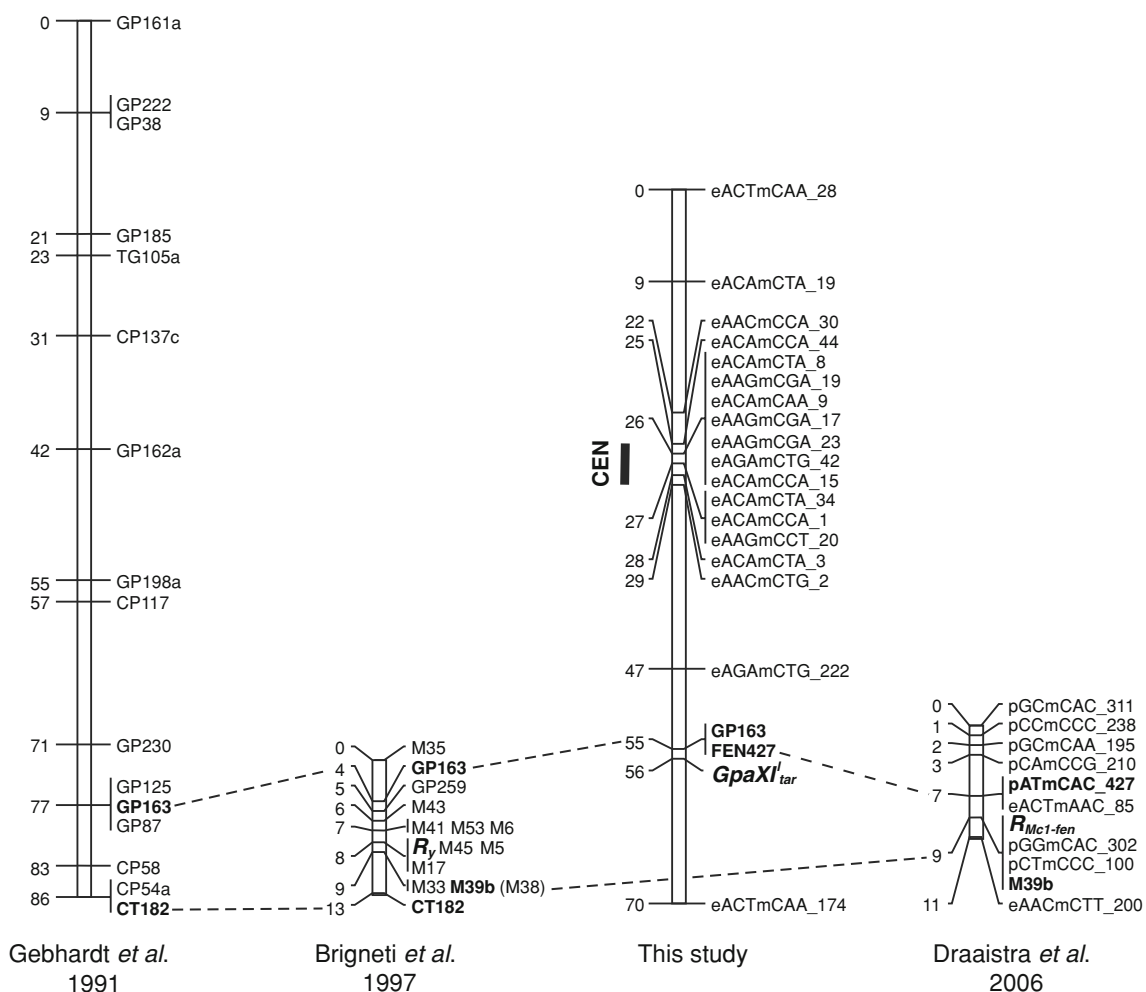
made to generate a better resolution and to add reference markers, which are known to reside close to the *R*-gene cluster.

To screen for recombinants, DNA of 324 newly sown genotypes from RHAM-061 was genotyped with the flanking AFLP markers eACTmCAA\_174 and eAGAmCTG\_222. Recombinant genotypes were identified and subjected to a disease test. For 32 genotypes we obtained phenotypic data allowing identification of the position of *GpaXI<sup>tar</sup>* relative to markers GP163 and FEN427. In the complete population of 324, no recombination events were detected between markers GP163 and FEN427. Both markers mapped 1.3 cM proximal of *GpaXI<sup>tar</sup>*. The position of GP163 and FEN427 relative to *GpaXI<sup>tar</sup>*, confirms that *GpaXI<sup>tar</sup>* maps to a well-known *R*-gene cluster on the long arm of chromosome 11. The resulting genetic map is presented in Fig. 5, also showing reference maps from literature used for anchoring purposes. As much as 17 of the 324 offspring clones (=5%) were excluded from map

construction. In these plants we observed two recombination events at close distance, suggesting data point(s) that are in conflict with both their flanking markers (singletons). In view of chiasma interference these events are more likely to represent data error than true recombination events and can be removed safely without influencing marker order (van Os et al. 2005b). Even if these were real recombination events, the map distances would be hardly affected in view of the total population size of 324 offsprings.

## Discussion

The inheritance of resistance to the white potato cyst nematode *G. pallida* has initially been regarded as complex (Dale and Phillips 1982; Ross 1986). With the aid of molecular markers it has been demonstrated that the allegedly complex resistance to *G. pallida* is simpler,



**Fig. 5** The genetic map of maternal chromosome 11 of mapping population RHAM-061 showing the *GpaXI<sup>tar</sup>* locus in connection to chromosome 11 reference maps from literature used for anchoring purposes. Chromosome orientation is according to Dong et al. (2000)

because in many cases large-effect QTL have been identified. For example, the QTL *Grp1*, *Gpa*, *Gpa5* and *GpaV<sup>spl</sup>* (Caromel et al. 2005; Kreike et al. 1994; Rouppe van der Voort et al. 1998a, 2000) invariably accounted for more than 45% of the phenotypic variance. In this study two loci have been mapped: a large-effect QTL derived from the wild *Solanum* species *S. tarijense* BGRC 24717, as well as a minor-effect QTL on potato chromosome 9.

#### Identification of the large-effect resistance locus *GpaXI<sup>tar</sup>*

A large-effect QTL has been identified on potato chromosome 11 explaining 81.3% of the phenotypic variance of the disease test. In view of the magnitude of the explained variance, the QTL was Mendelised. This allowed us to estimate the genetic distances between the locus and the flanking molecular markers and to place the locus name on the genetic map. Usually, a broad QTL interval does not offer precise ordering information of markers relative to the QTL, but in our case the order of the *R*-gene and the markers is regarded as a stable order, well supported by recombination events.

In the studied mapping population RHAM-016, the clones with the resistance allele *GpaXI<sup>tar</sup>* (as predicted by the flanking markers) developed on average 9.9 cysts, whereas clones without any resistance allele developed on average of 210 cysts. This constitutes a relative decrease of 95% in cyst count. The level of resistance obtained with *GpaXI<sup>tar</sup>* is therefore partial and not absolute. This non-absolute level of resistance seems to be common for most of the identified major loci involved in *G. pallida*. As discussed by Rouppe van der Voort et al. (1998a, 2000), the number of newly formed cysts in spite of the presence of a major *R*-gene, is likely due to heterogeneity in the *G. pallida* population Rookmaker (Pa3) at the respective (a) virulence gene. A single *R*-gene which operates on a gene-for-gene basis will confer partial resistance against the population as a whole, whereas on the level of the individual, the *R*-gene will confer absolute resistance against the matching avirulent genotype. Alternatively, it is also possible that the resistance mechanism of the *R*-gene in itself is not absolute. This has been demonstrated for the *H1* gene, where always a small number of cysts are formed on *H1* resistant plants after inoculation with a homozygous avirulent line of *G. rostochiensis* (Janssen et al. 1990). These cysts that are formed by a non-absolute resistance mechanism of a major *R*-gene are known as “escapers” and do not imply any kind of evolution of the nematode population towards increasing virulence. Whether the newly formed cysts on *GpaXI<sup>tar</sup>* resistant plants have been caused by the heterogeneity of the test population or by the strength of the *R*-gene itself can only be determined by

testing the *R*-gene with a homozygous avirulent nematode population.

The resistant *S. tarijense* hybrid, which was used as parent of the mapping population showed an average cyst count of 2. Even when both resistance factors were present, the major locus *GpaXI<sup>tar</sup>* and the minor QTL on chromosome 9, offspring clones did not achieve the same resistance level as their resistant parent. Apparently, there is an interaction between the *R*-gene(s) and the genetic background in which an *R*-gene is introgressed.

#### Identification of a small-effect resistance locus on chromosome 9

Besides the major QTL on chromosome 11, a minor QTL was detected on chromosome 9. At this moment we can only speculate on the function of this QTL.

The minor QTL could represent a classic NBS-LLR gene, but also a factor in non-necrogenic resistance, for instance in the formation of hatching substances. Finally, the QTL could represent a locus involved in the size of the root system. As demonstrated by Kreike et al. (1994) a root size QTL will initially appear as a QTL for resistance. The dominant epistatic interaction of *GpaXI<sup>tar</sup>* over the minor QTL does not exclude one of the three possible explanations.

#### Both loci map to well-known *R*-gene clusters

Two CAPS markers GP163 and FEN427 co-localised and mapped 1.3 cM proximal of *GpaXI<sup>tar</sup>*. Marker GP163 was previously positioned at a distance of 3.7 cM proximal to potato virus Y resistance gene *Ry<sub>sto</sub>* on chromosome 11 (Brigneti et al. 1997). AFLP marker pATmCAC\_427, on which CAPS marker FEN427 was based, was previously used to map nematode resistance *R<sub>Mc1-fen</sub>* on chromosome 11 and mapped 2 cM proximal of *R<sub>Mc1-fen</sub>* (Draaistra 2006). Therefore, we postulate that *GpaXI<sup>tar</sup>* is located in one and the same *R*-gene cluster, which is already comprising three nematode resistance genes *R<sub>Mc1-blb</sub>*, *R<sub>Mc1-fen</sub>*, *R<sub>Mc1-hou</sub>* (Draaistra 2006), as well as several more resistance genes against viruses and fungi (Gebhardt and Valkonen 2001). From this *R*-gene cluster only *N* (Whitham et al. 1994) has been cloned, suggesting that *GpaXI<sup>tar</sup>* could represent a TIR-NBS-LRR gene with homology to *N*. Future research may demonstrate the relation between *GpaXI<sup>tar</sup>* and the other nematode resistance genes in this cluster. Each of these nematode resistance genes could be involved in recognition of distinct effector molecules, but might also represent a casus similar to the *Mi* gene, recognising the nematode *Meloidogyne incognita* and the aphid *Macrosiphum euphorbiae* (Rossi et al. 1998; Vos et al. 1998).



Likewise, the minor QTL described in this study maps in close proximity to a known *R*-gene cluster. This cluster on chromosome 9 comprises the *Sw-5* resistance gene (Brommonschenkel et al. 2000), a homologue of *Mi*. Interestingly, this cluster is already implicated in *G. pallida* resistance, as the small-effect *Gpa6* QTL was mapped here (Roupe van der Voort et al. 2000). The coincidence of finding the minor QTL at a position indistinguishable from the *Gpa6* locus prompted us to verify the pedigree of our mapping population for the putative introgression segments of *S. vernei*. In Fig. 1, a crossing parent MON-46 is shown, a dihaploid of cv. Mondial. This parent is susceptible for *G. pallida*, but it has *S. vernei* in its pedigree. Therefore, it remains inconclusive if the minor QTL originates from *S. tarijense* or represents a gene identical by descent to *Gpa6* from *S. vernei*. Thus, we have refrained from giving the minor-effect QTL discovered on chromosome 9 a new name.

### Mapping strategy

The identification of the genetic locus involved in a phenotypic trait can be achieved with various mapping strategies, but a minimal requirement is the ability to align the newly mapped locus with previous linkage studies. This can be achieved with single copy locus specific markers, but in this study the high multiplex efficiency of AFLP fingerprinting was used. With 14 *EcoRI/MseI* AFLP primer combinations, 518 marker loci were obtained, which was sufficient to cover all 12 maternal and paternal linkage groups, as well as markers bridging the homologous parental linkage groups. Our strategy specifically exploited the principle that AFLP fragments of the same mobility on gel represent the same genetic map position, because of DNA sequence homology of the underlying DNA fragment captured in the AFLP fingerprint (Roupe van der Voort et al. 1997b). This approach has been successful not only in our own hands with proper control samples in the same AFLP gel image (Roupe van der Voort et al. 1997a), but also across labs (Bradshaw et al. 2006). The approach followed in this study, not only used the well-known reference genotype RH89-039-16 as a reference sample in AFLP fingerprinting, but it also served as susceptible crossing partner. The resistance locus, however, segregated from a wild species donor. The validity of locus-specificity of AFLP markers rapidly disappears with increasing taxonomic distance; as increasing taxonomic distance results in increasing AFLP fingerprint dissimilarity, where only insignificant coincidental co-migration remains. The obvious success to align the introgression of *S. tarijense* with the potato reference maps is due to the BC1 structure of the mapping population. For mapping purposes one could also use the F1, as both parents *S. tuberosum* and *S. tarijense* are non-inbred. A

mapping population of F1 RH90-011 siblings would result in a maternal and paternal map, but the paternal *S. tarijense* linkage groups would be comprised of markers which might not bear resemblance to known markers in our database. The next generation (BC1) results in linkage groups, which are comprised of known marker alleles linked in repulsion to the resistance gene. Hence, mapping of the *R*-gene locus can be achieved irrespective of genetic dissimilarity between the wild species and potato.

### Nomenclature of nematode resistance genes

At this moment the nomenclature of genes involved in nematode resistance is without consensus (Gebhardt and Valkonen 2001). On the one hand there is a series of major and minor-effect QTL against *G. pallida* with names ranging from *Gpa*, *Gpa2* until *Gpa6*, which are numbered successively following its order of identification. Likewise a series of *R*-genes and QTL against *G. rostochiensis* are known: *H1*, *Gro1*, *Gro1.2*, *Gro1.3*, *Gro1.4* and *GroV1* (Gebhardt and Valkonen 2001). Although such names nicely reflect history of science, it is hardly informative on the wild species origin, the resistance spectrum to various pathotypes, or the genetic location of the gene.

At this moment we wish to follow the syntax of Caromel et al. (2005) [pathogen species, potato linkage group (roman), long/short arm (superscript), source species (subscript)], and propose to name this *G. pallida* resistance  $GpaXI_{tar}^l$ . At the ‘Symposium on the Molecular Biology of the Potato’ held in 1998 at Bogensee, Germany, two large-effect QTLs were mentioned. An abstract by Wolters et al. (1998), describes *Gpa3* on chromosome 11 derived from *S. tarijense* and *Gpa4* on potato chromosome 5 derived from *S. sparsipilum*. We propose that *Gpa3* and *Gpa4* will no longer be used in scientific literature. *Gpa3* was mapped in an F1 segregating population different from our population. But since the same BGRC accession has been used as a source, it is very plausible that  $GpaXI_{tar}^l$  is identical to *Gpa3*. Whereas *Gpa3* and *Gpa4* have not yet entered the peer-reviewed literature, *Gpa4* has already caused some confusion as it appeared in the review by Gebhardt and Valkonen (2001), where *Gpa4* was used for the unnamed QTL on potato chromosome 4 segregating from SCRI clone 12601ab1 (Bradshaw et al. 1998). The most plausible name for this QTL is the *H3* gene from *Solanum tuberosum* spp. *andigena* CPC2802 (p. 115 [http://www.scri.ac.uk/scri/file/fullannualreports/annual\\_report\\_2001.pdf](http://www.scri.ac.uk/scri/file/fullannualreports/annual_report_2001.pdf)).

Practical value of the *G. pallida* (Pa3) resistance gene  $GpaXI_{tar}^l$  in potato breeding

The resistance level of  $GpaXI_{tar}^l$  (a relative decrease of 95% in cyst count) is comparable with the level conferred

by *Grp1* (Roupe van der Voort et al. 1998a) and *Gpa5* (Roupe van der Voort et al. 2000); both resistance genes that are presently used in commercially grown PCN resistant cultivars. We therefore state that *GpaXI<sup>tar</sup>* will be an equally valuable gene for the development of PCN resistant cultivars. A first demonstration of the value of the *H3* gene is cv. Vales Everest, which is currently the best PCN resistant cultivar on the UK National List.

In contrast to the limited durability of *R*-genes in many other plant–pathogen interactions, several aspects of the potato–PCN interaction raise the support of a relatively longer durability of the major resistance genes. The multiplication rate and the spread of PCN are limited, and the time between generations can range from 3 to 5 years depending on crop rotation schemes. Changes towards new virulent PCN types are therefore likely to be slow. Furthermore, the positive selection of virulent factors will be countered by the obligate sexual reproduction with genetically heterogeneous males.

Nowadays PCN resistant cultivars are available. However, because of the partial effect of the used *R*-genes a wide crop rotation is still required. As discussed before, the partial effect of the resistance genes can be explained by the genetic heterogeneity of the *G. pallida* field populations and by “escapers” that are caused by an ineffective resistance mechanism. The ideal situation for potato growers would be an absolute resistance in one cultivar. Possibly such an absolute resistance level could be achieved by combining or “pyramiding” of different *G. pallida* resistance genes. The development of breeding material with more than one allele at a given locus seems ineffective (Brodie and Plaisted 1992). Clones with a combination of major-effect QTL *GpaV<sup>spl</sup>* and minor-effect QTL *GpaXI<sup>spl</sup>* showed additively lower cyst counts than with the individual QTL (Caromel et al. 2005). The same was shown with a combination of major-effect QTL *Gpa5* with minor-effect QTL *Gpa6* (Roupe van der Voort et al. 2000). Both studies showed lower cyst counts when combining a major and a minor QTL, but no absolute resistance. It would be interesting whether combining major QTL could result in absolute resistance. It seems that a combination of *R*-genes with different resistance spectra should at least make it possible to efficiently tackle the problem of heterogeneity of the *G. pallida* populations.

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