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Mapping of the cyst nematode resistance locus *Gpa2* in potato using a strategy based on comigrating AFLP markers

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Abstract The nematode resistance locus *Gpa2* was mapped on chromosome 12 of potato using information on the genomic positions of 733 known AFLP markers. The minimum number of AFLP primer combinations required to map *Gpa2* was three. This demonstrates that a reference collection of potato AFLP markers may be a valuable tool for mapping studies in potato. By use of RFLP probes, *Gpa2* was more precisely mapped at the distal end of chromosome 12. *Gpa2* confers resistance to a distinct group of populations of the potato cyst nematode *Globodera pallida* and originates from the same potato accession as locus *H1*, conferring resistance to pathotype Ro_1 of *G. rostochiensis*. This study shows that these two nematode resistance loci are unlinked and that *Gpa2* is linked to the *Rx1* locus conferring resistance to potato virus X. The efficiency of AFLPs for genetic mapping of a highly heterozygous crop like potato is discussed and compared with the RFLP technique.

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Introduction

The potato cyst nematodes *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida* Stone are severe pests of potato that cause worldwide an average production loss estimated to be 10–30% of the annual yield (Oerke et al. 1994). Crop rotation and nematicides are used to control potato cyst nematode populations, but these are undesirable from an economical as well as an environmental point of view. Consequently, the growth of resistant cultivars seems to be the most effective and durable means by which to control potato cyst nematodes.

Classical genetic analyses of wild *Solanum* species revealed a number of accessions with simply inherited resistance, some of which are still used to introgress nematode resistance into cultivars. Monogenic resistance has been identified in, for example, *Solanum tuberosum* ssp. *andigena* Hawkes CPC 1673 [locus *H1*; resistance to *G. rostochiensis* pathotypes Ro_1 and Ro_4 (Huijsman 1955) and an undefined locus conferring resistance to a distinct group of populations of *G. pallida* (Arntzen et al. 1994)], *S. kurtzianum* Bitt. et Wittm. [locus *A*, *B*; resistance to *G. rostochiensis* pathotypes Ro_1 and Ro_2 (Huijsman 1960)], *S. multidissectum* Hawkes [locus *H2*; resistance to *G. pallida* pathotype Pa_1 (Dunnett 1961)] and *S. spegazzinnii* Bitt. [locus *Fa*, *Fb*; resistance to *G. rostochiensis* pathotypes Ro_1 and Ro_5 (Ross 1962, 1986)]. A more complex inherited resistance against several populations of *G. pallida* has been found, among others, in various accessions of *S. vernei* Bitt. et Wittm. (e.g. Goffard and Ross 1954; Ross 1986) and in *S. tuberosum* ssp. *andigena* CPC 2802 (Dale and Phillips 1982).

Molecular markers have been used to place several of these nematode resistance loci on the genetic map of potato. Interestingly, resistance loci in potato have been mapped to a limited number of genomic regions. For example, locus *GroV*1, conferring resistance to *G. rostochiensis* pathotype Ro_1, was localized in a *S. vernei* accession (Jacobs et al. 1996) in a similar region of chromosome 5 where locus *H1* (Gebhardt et al. 1993; Pineda et al. 1993) mapped. At the other arm of chromosome 5, locus *Gpa*, conferring resistance to several populations of *G. pallida* (Kreike et al. 1994), was located and shown to be linked to restriction fragment length polymorphism (RFLP) markers which are in other potato genotypes linked to locus *R1* and *Rx2* [resistance to *P. infestans* and potato virus X (PVX) respectively (Leonards-Schippers et al. 1992; Ritter et al. 1991)].

So far, the majority of mapping studies in plants is still based on laborious RFLP markers. A novel DNA marker technique, called amplified fragment length polymorphism (AFLP) (Vos et al. 1995) has filled the need for a more efficient tool to construct dense linkage maps (e.g. Becker et al. 1995; Van Eck et al. 1995). Using the latter method, we developed a simple procedure to place any gene of interest on the genetic map of *S. tuberosum*. In this procedure the chromosomal localization of a gene is assessed by use of comigrating AFLP markers (Rouppe van der Voort et al. 1997).

In this paper the feasibility of this strategy is demonstrated by mapping monogenic resistance against *G. pallida* identified in *S. tuberosum* ssp. *andigena* CPC 1673. In addition, the genomic position of locus *H1* is confirmed.

Materials and methods

Plant material

A mapping population of diploid potato ($2n = 2x = 24$) was obtained from a cross between the diploid clones SH82-93-488 × RH89-039-16. The female parental clone SH82-93-488 originates from a cross between the diploid *S. tuberosum* clone SH76-128-1857 and a dihaploid of clone Y66-13-628 which has cv 'Amaryl', derived from *S. tuberosum* ssp. *andigena* CPC1673, in its pedigree. Clone SH82-93-488 is the resistance donor in our population and is referred to as SH. The susceptible male parental clone RH89-039-16 will be referred to as RH.

The mapping population F_1SH × RH consisted of 194 F_1 genotypes. Seeds were germinated on moist filter papers. After germination seedlings were transferred to flats containing potting compost. Well-rooted seedlings were transplanted into plastic pots 11 cm in diameter containing a peaty soil. After 6–8 weeks the top of each plant was taken for DNA extraction. In addition, 3 and 6 weeks later, stem cuttings of the potato genotypes were made for the two different nematode resistance assays. The initial seedling plants were maintained for tuber production.

Nematodes

G. pallida population D383 was originally sampled from heavily infested spot in a field at Anlo, the Netherlands. Initially, this population was encoded D234. The virulence characteristics as well as the molecular data of this population are very similar to those of the *G. pallida* population D372 (Bakker et al. 1992; Folkertsma et al. 1996) whose original code is D236. According to the scheme of Kort et al. (1977), the pathotype designation of these populations is Pa_2.

G. rostochiensis line Ro_1-19 was selected from a cross between one female and one male nematode as described previously (Janssen et al. 1990). Line Ro_1-19 is not able to reproduce on plants which contain the *H1* locus. Since avirulence to the *H1* locus is dominantly inherited at a single locus (Janssen et al. 1990), juveniles from line Ro_1-19 have the predicted genotype *AvrH1*/*AvrH1*.

Nematode populations were multiplied on the susceptible cultivar *S. tuberosum* ssp. *tuberosum* cv 'Eigenheimer', inoculated with approximately 200 cysts and placed in a growth chamber at 18°C and 16-h daylength.

Nematode resistance assays

Eggs and second-stage juveniles (J_2) were obtained by crushing cysts which had been soaked in tap water for 1 week. The egg/J_2 suspension was poured through a 100-µm sieve to remove debris and cyst walls. Before inoculation 3- to 4-week old stem cuttings were transferred from a peat mixture to 900-g pots containing a mixture of silversand and a sandy loam fertilized with Osmocote™ (N-P-K granulates). The plants were subsequently inoculated with nematodes to a final density of 5 egg/J_2 per gram soil. Of each plant genotype, three replicates per nematode source were inoculated. Six replicates of the parental clones as well as resistant and susceptible standards were included for resistance tests with each nematode source. Resistant standards were cv 'Multa' (resistant to D383), *S. vernei* hybrid 58.1642/4 (reistant to line Ro_1-19) and *S. vernei* hybrid 62-33-3 (resistant to D383 and Ro_1-19). The susceptible standard was cv 'Maritta'. In addition, 30 tubers of cv 'Saturna' were inoculated with line Ro_1-19 to assess the frequency of juveniles able to develop into females on plants which contain the *H1* locus (Janssen et al. 1990). Plants were arranged in a randomized block design and grown in a greenhouse with 15°C and 25°C as the minimum and maximum temperature, respectively.

After 3 months, cysts were recovered from the soil with a Fenwick can (Fenwick 1940), and the size of the root systems was judged on a scale of 0 to 3. Resistance data of a genotype were only recorded when at least 2 well-rooted plants of that genotype were available. The mean cyst numbers developed per genotype were standardized using a $\log_{10}(x + 1)$ transformation and then subjected to SAS Ward's minimum variance cluster analysis (SAS 1989). The data were separated into a resistant, unassigned and susceptible class. A chi-square test was applied to assess goodness of fit with appropriate genetic ratios.

DNA preparation and AFLP markers

DNA isolation was performed on frozen leaf tissue (Van der Beek et al. 1992) or tubers. The AFLP™ procedure was essentially as described by Vos et al. (1995). Template DNA was prepared using the restriction enzymes *Eco*RI and *Mse*I and pre-amplified using the "*Eco*RI-primer" E + 0 and the "*Mse*I-primer" M + 0 (Vos et al. 1995). For selective amplification the following combinations of primers were used: E + AAA/M + ACG, E + AAC/M + CAC, E + AAC/M + CAG, E + ATG/M + CTA, E + ATG/M + CTC. AFLP data were recorded as described (Van Eck et al. 1995). AFLP markers were designated by the primer combinations used followed by the approximate sizes of the amplification products expressed in nucleotides.

RFLP markers

To screen for polymorphisms we made restriction enzyme digests of genomic DNA using the enzymes *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I,

*Hae*III and *Cla*I (Gibco BRL, Gathersburg, Md.) at 5 units per μg DNA. Restriction fragements were separated on an 0.8% TAE buffered agarose gel (Sambrook et al. 1989) and transferred on a Hybond N+ membrane (Amersham, Little Chalfont, UK). DNA probes were radiolabeled with $\alpha[^{32}P]$ dATP by the random prime hexamer method (Feinberg and Vogelstein 1983). Blots were prehybridized, hybridized with radiolabeled inserts from DNA clones and washed according to Bernatzky and Tanksley (1986) using 0.5 *M* phosphate (NaH_2PO_4/Na_2HPO_4; M 7.5), 7% SDS and 1 m*M* EDTA as the hybridization buffer and 0.25 *M* phosphate and 1% SDS as the washing buffer. X-Ray films (Konica, Tokyo, Japan) were exposed for 1–10 days at −80°C using one intensifying screen.

Tomato genomic DNA clones (TG-clones) and cDNA clones (CT-clones) were provided by Dr. S. D. Tanksley, Cornell University, Ithaca N.Y. Potato CDNA probe CP113 was kindly supplied by Dr. C. Gebhardt, Max-Planck-Institute für Züchtungsforschung, Cologne (Germany). The chromosomal positions of the TG, CT and CP markers have been published in Tanksley et al. 1992 and Gebhardt et al. (1991).

RFLP clone GP34 (kindly provided by Dr. C. Gebhardt) was sequenced, and primers GP34F (CGTTGCTAGGTAAGCATGAAGAAG) and GP34R (GTTATCGTTGATTTCTCGTTCCG) were designed to screen for GP34 alleles by means of the polymerase chain reaction PCR) (temperature file: 30 s 94°C, 30 s 62°C and 1 min 72°C). One of the amplified alleles of GP34 cosegregated with *Gpa2* resistance. Marker NR14, which is closely linked to locus *Mi-3* in tomato, was obtained from Dr. V. Williamson, University of California, Davis Calif. The marker was generated from tomato by PCR amplification using 0.5 μ*M* of each of primer NR14A/R and NR14A/U. The sequences of the primers and amplification conditions were as described by Yaghoobi et al. (1995). Following amplification, the NR14 fragment was excised from the agarose gel, purified with a Glass Max Isolation Matrix System (Gibco BRL) as recommended by the manufacturer and hybridized to blots containing *Eco*RV-digested DNA as described above.

Mapping

The AFLP primer combinations used in this study had been previously applied to analyse two potato mapping populations in order to localize AFLP markers relative to chromosome-specific RFLP loci (Van Eck et al. 1995; Rouppe van der Voort et al. 1997). The AFLP profiles of the parental clones of our reference populations were compared with clone SH, and AFLP products of equal electrophoretic mobility which segregated in both populations were identified. Homology between a SH linkage group and one of the chromosomes identified in the reference populations was assumed when at least 3 comigrating AFLP markers were identified (Rouppe van der Voort et al. 1997). In this way, AFLP markers identified in the reference populations which comigrated with AFLP markers segregating from the resistant clone SH allowed identification of the chromosomes and regions thereon. The identities of the regions of interest on the SH linkage groups were confirmed by the use of RFLP markers.

Linkage analyses of pairwise recombination frequencies between markers and resistance loci were performed using JOINMAP 1.4 (Stam 1993). JOINMAP 1.4 tests linkage between all possible pairs of markers by calculating the logarithm of odds (LOD) score. At a LOD = 3 threshold level the test may be too conservative in assigning linkage when high recombination rates exist between two loci (Gerber and Radolphe 1994). A test for independence was therefore also applied to test for linkage between the two resistance loci.

For map construction, recombination frequencies were converted into map units (cM) by use of the Kosambi function. A graphic representation of a map was made by the computer program DRAWMAP (Van Ooijen 1994).

Results

Resistance testing

The inheritance of both the *Gpa2* and the *H1* gene was followed in the segregating population F_1SH × RH. For *G. pallida* population D383, the average number of cysts developed was 6 on clone SH and 286 on clone RH. Cyst counts on individual F_1 plants ranged from 0 to 681. On the basis of Ward's minimum variance cluster analysis (SAS institute 1989) a class containing genotypes on which an average of fewer than 23 cysts developed was considered as resistant and that on which an average (of more than) 51 cysts developed was taken as susceptible. Genotypes containing means of 23–51 cysts were assigned into an intermediate class and were not used for linkage analysis.

To assess the frequency of avirulent juveniles able to develop into females on *H1* resistant plants (i.e. "escapers"; Janssen et al. 1990), we tested, the parental clone SH and the cultivar 'Saturna', both containing the *H1* locus, with *G. rostochiensis* line Ro_1-19 (*AvrH1*/*AvrH1*). From the mean number of cysts of 0.3 (SD = 0.58) on clone SH and 2.6 (SD = 3.5) on cv 'Saturna' per 5000 inoculated eggs/J_2 juvenile we assumed the maximum number of cysts due to "escapers" to be 3. The mean number of cysts developed on clone RH was 80. With an inoculation density of 5000 inoculated eggs/J_2 juvenile per pot we chose the criterion of 3 or fewer cysts as resistant and more than 3 cysts as susceptible. Cyst counts on individual F_1 plants ranged from 0 to 447.

Segregation of resistance loci

The resistance in parent SH to *G. pallida* population D383 resulted from a cross between a diploid susceptible *S. tuberosum* clone and a diploid resistant clone which has *S. tuberosum* ssp. *andigena* CPC 1673 in its pedigree. This dominantly inherited monogenic resistance to *G. pallida* (Arntzen et al. 1994) was therefore expected to segregate in a 1:1 ratio after crossing SH with the susceptible clone RH. A resistance test was carried out on 3 stem cuttings per seedling. A total of 181 F_1 genotypes were tested and, based on the criteria described above, 78 genotypes were scored as susceptible and 77 as resistant (Table 1). The resistance or susceptibility of 11 genotypes could not be assessed based on Ward's minimum variance cluster analysis. From 15 genotypes, only poorly rooted plants were obtained. The segregation ratio is consistent with the assumption that a single dominant gene confers resistance to *G. pallida* population D383. We propose to name this locus *Gpa2*, in analogy to locus *Gpa* from *S. spegazzinii* (Kreike et al. 1994) which segregates with resistance to several *G. pallida* populations.

Table 1 Segregation ratios of the *Gpa2* and *H1* locus in population $F_1SH \times RH$ (*n.s.* not significant)

Locus	Resistance : susceptible F_1 plants	X^2 for 1 : 1	Significant at:
Gpa2	77 : 78	0.01	$P > 0.95$ (n.s.)
H1	55 : 75	4.17	$0.05 > P > 0.01$

Screening for *H1* resistance was performed on a second series of 3 stem cuttings from 120 seedlings previously tested for *Gpa2* resistance. Segregation analysis revealed a distortion from a 1 : 1 segregation ratio at locus *H1* (Table 1). Comparison between segregation data obtained for locus *Gpa2* and locus *H1* by means of a 2 × 2 contingency table showed that both loci segregated independently ($\chi^2 = 1.73$; $P > 0.05$).

Mapping by comigrating AFLP markers

Using five AFLP primer combinations, we were able to identify 322 segregating amplification products in population $F_1SH \times RH$. Out of 196 AFLP markers which segregated from clone SH, locus *Gpa2* showed linkage with 8 markers (LOD scores between 3.3 and 8.2). When AFLP profiles of clone SH were compared with AFLP patterns obtained from other potato genotypes (Rouppe van der Voort et al. 1997), 3 AFLP markers linked with *Gpa2* comigrated with AFLP markers previously mapped on chromosome 12 (Van Eck et al. 1995). Linkage of 1 of these chromosome-specific AFLP markers to *Gpa2* is presented in Fig. 1.

One AFLP marker was found to be linked with locus *H1* (LOD = 9.9). This AFLP marker showed linkage with 2 markers (with LOD scores of 9.2 and 10.6) which comigrated with chromosome 5-specific AFLP markers (Rouppe van der Voort et al. 1997).

Mapping *Gpa2* and *H1*

AFLP analysis positioned locus *Gpa2* on chromosome 12. This was confirmed by testing chromosome 12-specific RFLP probes on population $F_1SH \times RH$. Labeled DNA of the RFLP clones CT99, TG360, CT100, CT79, CT129, TG68, TG180 and CT19 (Tanksley et al. 1992) and the tomato PCR fragment NR14 (Yaghoobi et al. 1995) were used to probe blots containing digested DNA of the two parental clones and eight F_1 genotypes. Using the restriction enzyme *Eco*RV, we could show that RFLP markers CT100, CT129, CT79, TG68 and NR14 are linked to *Gpa2*. The other 4 probes, CT99, TG360, TG180 and CT19, revealed no polymorphisms between the two parental clones with the six restriction enzymes tested. Unfortunately, the segregating band identified with probe CT129 could not be scored reliably. In addition,

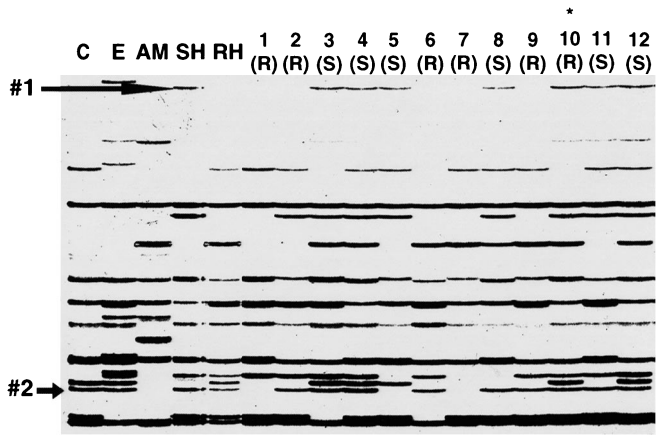

Fig. 1 Part of an autoradiogram showing AFLP markers generated from the parental genotypes SH and RH and a subset of the population $F_1SH \times RH$. Resistance or susceptibility to *G. pallida* is indicated. Comigrating AFLP markers were identified by comparison with the profiles generated from genotypes C, E and AM (Rouppe van der Voort et al. 1997). 1 Marker E + AAA/M + ACG-397; linked to *Gpa2* (in repulsion). The genotype marked by an *asterisk* shows a recombination event between marker 1 and the *Gpa2* resistance. 2 Marker E + AAA/M + ACG-278; linked to *Gpa2* (in repulsion) and mapped on chromosome 12 in clones *C*, *E*, *SH* and *RH*. Note that for marker E + AAA/M + ACG-278, the underlying genetic model of which is *Aa* × *Aa*, the heterozygous configuration is not informative. For this marker, only segregation data from lanes 1, 3, 4, 5 and 8 were used. *Lane* 5 represents a recombinant genotype for marker *2* and *Gpa2*

marker GP34 was mapped by segregation analysis of an allele-specific DNA fragment obtained by PCR. In this way, markers CT100, GP34, CT79, TG68 and NR14 were placed on the chromosome 12 map of clone SH.

The *Gpa2* locus was mapped between GP34 and CT79 (Fig. 2) on the distal end of chromosome 12. Five recombinants were identified between *Gpa2* and marker GP34, which corresponds to a map distance of 0.8 cM (LOD = 34.8). At the other side of *Gpa2*, locus CT79 mapped at a distance of 7.0 cM with a LOD score of 20.1. Marker CT100, closely linked to *Gpa2*, of which four segregating alleles could be identified, is shown in Fig. 3.

For both parents, significant deviations from the expected Mendelian ratios were observed for most chromosome 12 alleles. Mendelian ratios were observed only for alleles in the *Gpa2* region segregating from clone SH. For example, at locus CT100 (Fig. 3), the segregation of SH alleles *a* and *b* was as expected ($\chi^2 = 3.23$; $P > 0.05$). The segregation ratio of the RH alleles *c* and *b′* was strongly skewed ($\chi^2 = 97.2$; $P < 0.0001$) with allele *c* being the preferentially transmitted allele. Also at locus NR14, a distorted segregation ratio of the two RH alleles was observed ($\chi^2 = 21.7$; $P < 0.0001$), whereas the two SH alleles were equally distributed in the population ($\chi^2 = 0.93$; $P > 0.05$). Abnormal segregation affected all RH alleles identified on chromosome 12. In clone SH, segregation

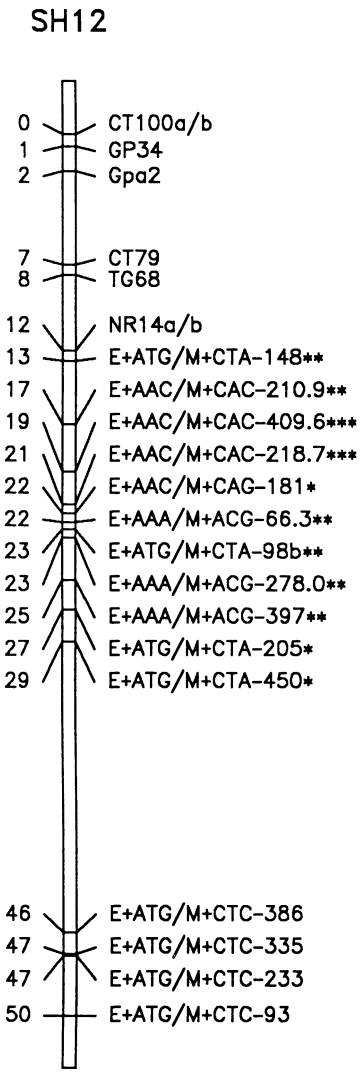

Fig. 2 Position of the *Gpa2* locus on the linkage map of chromosome 12 of clone SH. Markers which showed significant deviations from the expected segregation ratios are marked by one, two or three asterisks (at $0.05 > P > 0.01$; $0.01 > P > 0.001$ and $P < 0.001$, respectively)

distortion could be ascribed to a specific region, which was bracketed by markers exhibiting normal segregation ratios (Fig. 2).

The map position of the *H1* locus at chromosome 5 (Gebhardt et al. 1993) was confirmed by linkage with RFLP probe CP113 (LOD = 8.6).

Discussion

The map location of *Gpa2* could be assessed readily using AFLP markers comigrating with markers which had been, previously placed on the genetic map of potato. It has been demonstrated that between two

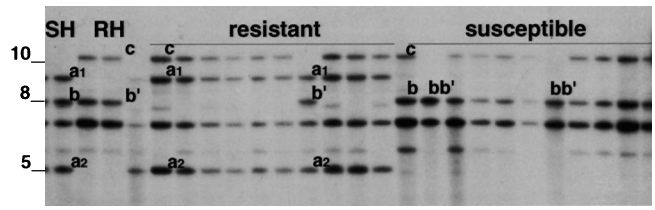

Fig. 3 Linkage of locus *Gpa2* to RFLP marker CT100. Four RFLP alleles segregate in the F_1 progeny: alleles *a* and *b* derive from clone SH; alleles *c* and *b*' derive from the susceptible clone RH. Allele *a* is linked to the resistance. Segregation of this allele can be followed by two bands (indicated by *a1* and *a2*). Size markers are indicated on the *left* in kilobasepairs

relatively unrelated potato clones, the majority of the AFLP fragments which have the same electrophoretic mobility are indeed identical alleles (Rouppe van der Voort et al. 1997). In retrospect, only three of the five primer combinations generated sufficient comigrating AFLP markers to map the *Gpa2* locus. This demonstrates that the 733 AFLP markers mapped so far are a valuable reference collection for other mapping studies in potato (Rouppe van der Voort et al. 1997).

The origin of the *Gpa2* resistance locus can be traced back to *S. tuberosum* ssp. *andigena* clone CPC 1673 (Arntzen et al. 1994). Out of 1200 wild *Solanum* clones collected in the Andean region in the early fifties (Ellenby 1952), this genotype was one of the five potato clones harbouring potato cyst nematode resistance. Although *Gpa2* is unlinked to *H1* and has never been used as a selective trait in breeding programmes, *Gpa2* has been introgressed apart from *H1* into a number of commercial cultivars. The chromosome 12 region between RFLP markers CT100 and CT79 also harbours locus *Rx1*, which confers extreme resistance to PVX (Bendahmane et al. 1997). *Rx*1 also originates most likely from *S. tuberosum* ssp. *andigena* CPC 1673 (Ritter et al. 1991; Bendahmane et al. 1997). It is therefore hypothesized that *Gpa2* has been introgressed from *S. tuberosum* ssp. *andigena* CPC 1673 by selection for resistance to PVX in breeding programmes.

The *Gpa2* locus confers specific resistance to the *G. pallida* populations D383 and D372 [Arntzen et al. (1994); herein, these populations are referred to as D234 and D236, respectively]. These *G. pallida* populations are at the molecular level very similar (Bakker et al. 1992; Folkertsma et al. 1996). Both populations form a distinct group that can be clearly differentiated from other *G. pallida* populations found in The Netherlands. It appears that potato cyst nematode populations which extensively diverge from other populations on the basis of their molecular characteristics can also be differentiated on the basis of their virulence characteristics (Bakker et al. 1993). This congruence between virulence and molecular characteristics holds for populations which are pathotyped Ro_1 of the species *G. rostochiensis* and Pa_1 of *G. pallida*. Ro_1 populations can

be differentiated from $Ro_2/Ro_3/Ro_5$ populations on the basis of low multiplication rates on plants containing the *H1* locus, whereas Pa_1 populations are discriminated from Pa_2/Pa_3 by multiplication rates on plants harbouring the *H2* gene. From co-evolution theory, it has been argued that in these cases the plant-nematode interaction may follow a gene-for-gene relationship (Parrot 1981; Bakker et al. 1993; Thompson 1994). Currently, a Mendelian proof for a gene-for-gene relationship has only been provided for the interaction between *G. rostochiensis* line Ro_1-19 and the *H1* gene (Janssen et al. 1991). In line with these examples, it may be possible that a gene-for-gene relationship exists between a dominant avirulence gene present in populations D383 and D372 and the *Gpa2* gene. This hypothesis is currently under, investigation in our laboratory by performing controlled single matings with juveniles derived from population D383.

With population D383, a maximum of 36 cysts and a mean number of 23 cysts were found on resistant genotypes. We propose two explanations for the occurrence of cysts on resistant plants. Populations D383 and D372 are probably not fixed for avirulence alleles. Hence, some virulent genotypes could be present in these populations, and these virulent nematodes are able to develop into females on *Gpa2* resistant plants. On the other hand, successful colonization of a pathogen is likely to be caused by a delayed plant defence expression rather than by complete absence of a defence mechanism (Dixon et al. 1994). Delayed plant response may result in incomplete resistance. Also with the homozygous avirulent line Ro_1-19, upto 3 cysts were recovered from *H1*-resistant plants. The small number of newly developed Ro_1 cysts cannot be explained by *H1* gene dosage (Brodie and Plaisted 1992) nor by an exceptional high mutation frequency at the *AvrH1* locus (unpublished results). It is therefore postulated that the exceptional cases in which a syncytium matures on a resistant plant may result from differences in the timing of the resistant reaction. As the speed of a resistance response determines largely the outcome of a plant-pathogen interaction (Goodman and Novacky 1994), the time span in which the plant defence mechanism is activated may vary between the different initiated feeding cells within a plant. Consequently, in a small fraction of cells, elicitation occurs relatively late, resulting in a sufficiently matured syncytium to enable female development.

As shown in this paper, RFLP markers are for potato still the more accurate means by which to identify a specific chromosomal interval. For potato and tomato, more than 1000 RFLP markers are available. Although we have mapped 733 AFLP markers on the genetic map of potato, the number of informative markers for targeting a specific genomic interval is less. Unlike RFLP markers, AFLPs are mapped as alleles rather than loci. This has important consequences for the alignment of genetic maps of different genotypes, especially for plant species like *S. tuberosum* having high levels of intraspecific variation. The ability to identify the map location of a gene with AFLPs depends on the number of comigrating AFLP markers (shared alleles) with reference genotypes. Currently, a total number of 195 comigrating AFLP markers have been mapped within *S. tuberosum* by analysing five genotypes. To increase the map resolution by use of a collection of comigrating AFLP markers, we have to analyse additional potato genotypes and primer combinations. Such reference collection makes the AFLP marker technique highly efficient for mapping studies within cultivated potato.

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