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ORIGINAL PAPER

**Erin Bakker · Ute Achenbach · Jeroen Bakker · Joke van Vliet · Johan Peleman · Bart Segers · Stefan van der Heijden · Piet van der Linde · Robert Graveland · Ronald Hutten · Herman van Eck · Eric Coppoolse · Edwin van der Vossen · Jaap Bakker · Aska Goverse**

# A high-resolution map of the *H1* locus harbouring resistance to the potato cyst nematode *Globodera rostochiensis*

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**Abstract** The resistance gene *H1* confers resistance to the potato cyst nematode *Globodera rostochiensis* and is located at the distal end of the long arm of chromosome V of potato. For marker enrichment of the *H1* locus, a bulked segregant analysis (BSA) was carried out using 704 AFLP primer combinations. A second source of markers tightly linked to *H1* is the ultra-high-density (UHD) genetic map of the potato cross SH × RH. This map has been produced with 387 AFLP primer combinations and consists of 10,365 AFLP markers in 1,118 bins (http://www.dpw.wageningen-ur.nl/uhd/). Comparing these two methods revealed that BSA resulted in one marker/cM and the UHD map in four markers/cM in the *H1* interval. Subsequently, a high-resolution genetic map of the *H1* locus has been developed using a segregating $F_1$ SH × RH population consisting of 1,209 genotypes. Two PCR-based markers were designed at either side of the *H1* gene to screen the 1,209 genotypes for recombination events. In the high-resolution genetic map, two of the four co-segregating AFLP markers could be separated from the *H1* gene. Marker EM1 is located at a distance of 0.2 cM, and marker EM14 is located at a distance of 0.8 cM. The other two co-segregating markers CM1 (in coupling) and EM15 (in repulsion) could not be separated from the *H1* gene.

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E. Bakker (✉) · U. Achenbach · J. Bakker · J. van Vliet · J. Bakker · A. Goverse
Laboratory of Nematology,
The Graduate School of Experimental Plant Sciences,
Wageningen University,
P.O. Box 8123, 6700 ES Wageningen, The Netherlands
e-mail: erin.bakker@wur.nl
Tel.: +31-317-485086
Fax: +31-317-484254

R. Hutten · H. van Eck
Laboratory of Plant Breeding,
The Graduate School of Experimental Plant Sciences,
Wageningen University,
P.O. Box 8123, 6700 ES Wageningen, The Netherlands

J. Peleman · B. Segers
Keygene NV,
P.O. Box 216, 6700 AE Wageningen, The Netherlands

S. van der Heijden
Cebeco Seeds,
P.O. Box 139, 8200 AC Lelystad, The Netherlands

P. van der Linde · R. Graveland
HZPC R and D,
P.O. Box 2, 9123 ZR Metslawier, The Netherlands

E. Coppoolse · E. van der Vossen
Plant Research International,
P.O. Box 16, 6700 AA Wageningen, The Netherlands

## Introduction

The potato cyst nematode (PCN) species *Globodera rostochiensis* and *G. pallida* cause serious yield losses in potato crops worldwide (Ross 1986). An effective and durable way to control PCN is the use of resistant potato cultivars. Twelve PCN resistance loci have been mapped in potato on chromosomes III, IV, V, VII, IX, X, XI and XII (reviewed by Gebhardt and Valkonen 2001). Eight resistance traits (*Gro1.4*, *Gpa4*, *Gpa*, *Gpa5*, *Grp1*, *Gpa6*, *Gro1.2* and *Gro1.3*) confer partial resistance, while four of them (*H1*, *GroV1*, *Gro1* and *Gpa2*) confer nearly absolute resistance to one or more pathotypes. Interestingly, many PCN resistance loci map to a region where at least one other (and often more) single, dominantly inherited resistance gene is present (so-called 'hot spots' for resistance). This holds not only for the single, dominantly inherited PCN resistance genes *Gpa2*, *Gro1* and *H1*, but also for quantitative trait loci (QTL) like *Grp1*, *Gpa3*, *Gpa5* and *Gpa6*. So far, the only nematode resistance genes that have been characterised at the

molecular level in potato are *Gpa2* and *Gro1* (Van der Vossen et al. 2000; Gebhardt, personal communication).

*H1* resistance was discovered in 1952 in *Solanum tuberosum* ssp. *andigena* in the Commonwealth Potato Collection (Ellenby 1952). Since then, the *H1* gene has been introgressed in many commercially available cultivars. Even today, after many decades of use, the gene is very effective against *G. rostochiensis* in the United Kingdom. This makes it one of the most durable resistance genes known (Evans 1993). The *H1* gene is the only nematode resistance gene for which formal genetic proof for a gene-for-gene interaction (Flor 1971) has been demonstrated (Janssen et al. 1991). The *H1* gene confers resistance to *G. rostochiensis* pathotypes $Ro_1$ and $Ro_4$ (reviewed by Jones et al. 1981) by the activation of an HR. The feeding site that is initiated by the nematode becomes encapsulated by a layer of necrotic cells and degenerates in the course of a week (Rice et al. 1985). For cyst nematodes, sex is epigenetically determined in this first week of feeding site development and depends on the amount of food available (Trudgill 1967). Therefore, the *H1* resistance response results in starvation of the nematodes, while a limited number of individuals will develop into males.

The *H1* locus has been mapped in potato on the distal end of the long arm of chromosome V and is closely linked to RFLP markers CP113 and CD78 (Gebhardt et al. 1993; Pineda et al. 1993). In the same chromosomal region of *S. vernei*, another PCN resistance gene has been mapped (*GroV1*) that might be allelic to the *H1* gene (Jacobs et al. 1996). In this study, the ultra-dense genetic map of potato (http://www.dpw.wageningen-ur.nl/uhd/) and bulked segregant analysis have been used to identify markers closely linked to the *H1* gene. CAPS markers $CT51_{CAPS}$ and $239E4left_{CAPS}$ have subsequently been used to screen for recombination events in a population of 1,209 genotypes segregating for the *H1* gene. Finally, AFLP markers and nematode resistance assays have been used to construct a high-resolution map of the *H1* locus. The markers EM1 and CM1 are in coupling with and tightly linked to the *H1* gene (0.2 cM and 0 cM, respectively) and will form the basis for a positional cloning strategy.

## Materials and methods

### Plant material

A mapping population of 136 $F_1$ genotypes from the cross between the diploid potato clones SH83-92-488 (SH) × RH89-039-16 (RH) was available (Rouppe van der Voort et al. 1997). Additional offspring extended the population to a total of 1,209 genotypes. The female parent (SH) harbours the *H1* locus introgressed from *S. tuberosum* ssp. *andigena* CPC 1673, and the male parent (RH) is fully susceptible to all *G. rostochiensis* populations tested. A second population was produced comprising 120 $F_1$ progeny from the cross between the diploid potato clones DH84-25-2389 (DH, resistant) and KW84-16-2396 (KW, susceptible). Forty-five potato cultivars were used to test markers for marker-assisted selection (MAS).

### DNA extraction

Genomic DNA from SH, RH and progeny was extracted from the plants as described (Stewart and Via 1993). The method was adjusted for 96-well format using 1-ml tubes (Micronic BV, Lelystad, The Netherlands), supplemented with two steel balls (ø 2 mm). Samples were homogenised using a Retsch 300-mm shaker at maximum speed (Retsch BV, Ochten, The Netherlands). The genomic DNA of 45 potato cultivars, DH, KW and progeny was isolated as described (Van der Beek et al. 1992).

### Bulked segregant and marker analyses

For bulked segregant analysis (BSA), 704 *Eco*RI/*Mse*I primer combinations with three selective nucleotides at each primer were performed on bulks containing DNA of ten susceptible $F_1$ genotypes and bulks containing DNA of ten resistant $F_1$ genotypes derived from the cross DH × KW. AFLP analyses for BSA and for the markers derived from the ultra-high-density (UHD) genetic map of SH (Table 1) was performed according to Vos et al. (1995). The UHD map and the AFLP primer combinations used to produce the map are available at http://www.dpw.wageningen-ur.nl/uhd/.

Two CAPS markers (Konieczny and Ausubel 1993) were designed. $CT51_{CAPS}$ has been derived from the sequenced RFLP

**Table 1** AFLP markers derived from the ultra-high-density (UHD) genetic map of potato. Marker names, UHD map marker names, restriction enzymes, selective nucleotides and band sizes are indicated

| Marker | UHD map | Restriction enzymes | | Selective nucleotides | | Band size |
|---|---|---|---|---|---|---|
| CM1 | Caga/Mcac_233S5 | *Sac*I | *Mse*I | aga | cac | 233 bp |
| EM1 | Eatg/Mgca_40S5 | *Eco*RI | *Mse*I | atg | gca | 152 bp |
| EM14 | Eacc/Maac_16 | *Eco*RI | *Mse*I | acc | aac | Not sized |
| EM15 | Eagt/Mcac_98S5 | *Eco*RI | *Mse*I | agt | cac | 98 bp |
| EM16 | Eacc/Maag_22 | *Eco*RI | *Mse*I | acc | aag | Not sized |

**Table 2** Primer sequences, thermal cycling conditions and the polymorphic restriction site for CAPS markers used to screen for recombination events in the *H1* region of potato

| Marker | Primers | PCR conditions | Restriction enzyme |
|---|---|---|---|
| $CT51_{CAPS}$ | 5′-GCAGGATTCCATTTGCTTGC<br>5′-GTTATTGTCTAACCACCTCGG | 94°C, 3 min; 94°C, 30 s; 51°C 30 s; 72°C, 120 s, 35 cycles; 72°C, 5 min | *Alu*I |
| $239E4left_{CAPS}$ | 5′-GGCCCCACAAACAAGAAAAC<br>5′-AGGTACCTCCATCTCCATTTTGTAAG | 94°C, 3 min; 94°C, 30 s; 51°C, 30 s; 72°C 90 s, 35 cycles; 72°C 5 min | *Alu*I |

probe CT51 (Tanksley et al. 1992). The sequence could be retrieved from the SolGenes database (Paul et al. 1994), which can be accessed via http://ukcrop.net/perl/ace/search/SolGenes. Marker 239E4left$_{CAPS}$ has been derived from the sequenced left border of SH BAC clone 239E4. The DNA sequences of the PCR primers and the corresponding thermal cycling conditions are presented in Table 2.

### Resistance assays

PCN resistance assays were performed on plants derived from stem cuttings, tubers or in vitro stocks. The assays were performed as described (Rouppe van der Voort et al. 1997, 1999). Two PCN lines, homozygous in their virulence character, have been used in the *H1* resistance assays in the SH × RH population. *G. rostochiensis* line 19 is avirulent on potato genotypes harbouring the *H1* gene, while line 22 is virulent (Janssen et al. 1990). For the resistance assay of the DH × KW population, the Ro$_1$ field population A57 was used. Plants with <5 cysts are considered to be resistant.

## Results

### Markers linked to *H1* generated by BSA

The 120 F$_1$ progeny of DH × KW were tested for resistance to *G. rostochiensis* pathotype Ro$_1$, resulting in 79 susceptible and 41 resistant genotypes. BSA was carried out on pools of ten susceptible and ten resistant genotypes. Testing 704 AFLP primer combinations resulted in 13 candidate markers, which were subsequently tested on the individual genotypes of the DH × KW population. This resulted in, respectively, six markers (EM1 to EM6) located at a distance of 0.95 cM, four markers (EM7 to EM10) at 1.9 cM, two markers (EM11 and EM12) at 2.9 cM and one marker (EM 13) at 6.3 cM from the *H1* locus. The 13 candidate markers, their extensions, the band sizes and their distance to *H1* are presented in Table 3.

In addition, 11 out of 13 candidate markers (EM1 to EM8 and EM10 to EM12) were tested on 45 potato cultivars. Table 4 shows the presence or absence of a marker and susceptibility or resistance of each potato cultivar for nematode resistance to *G. rostochiensis* pathotype Ro$_1$. Markers EM1 to EM6 have the same distance to the *H1* locus in the DH × KW population, but EM1 is the only marker that co-segregates with nematode resistance in all potato cultivars. This is an indication that marker EM1 is more closely linked to the *H1* gene.

**Table 3** Candidate markers derived from bulked segregant analysis (BSA) on pools of susceptible and resistant F$_1$ genotypes of the DH × KW population. The marker names, selective nucleotides, sizes and distances to the *H1* locus are indicated

| Marker | Selective nucleotides | Size (bp) | Distance to *H1* (cM) |
|---|---|---|---|
| EM1 | ATG/GCA | 152 | 0.95 |
| EM2 | ACT/CGT | 137 | 0.95 |
| EM3 | ATG/CAC | 132 | 0.95 |
| EM4 | AAG/GAG | 239 | 0.95 |
| EM5 | CCC/CAC | 98 | 0.95 |
| EM6 | CAA/CCT | 87 | 0.95 |
| EM7 | ACG/GGA | 53 | 1.9 |
| EM8 | AGA/CAC | 233 | 1.9 |
| EM9 | AGA/CGC | 273 | 1.9 |
| EM10 | CAA/CAA | 115 | 1.9 |
| EM11 | ATG/CCA | 234 | 2.9 |
| EM12 | AAC/CCA | 121 | 2.9 |
| EM13 | ACA/CAA | 132 | 6.3 |

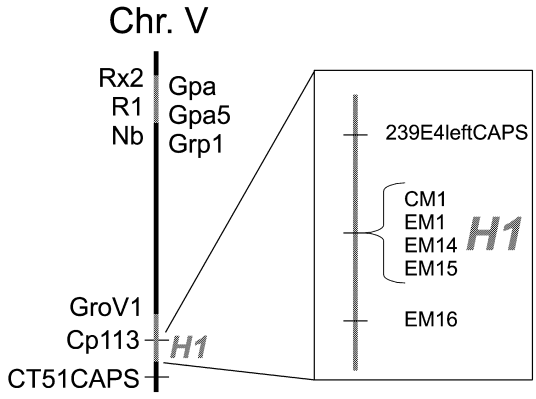


**Fig. 1** Schematic representation of chromosome V of potato, which contains two *R* gene regions. *Rx2* (Ritter et al. 1991), *R1* (Leonards-Schippers et al. 1992); *Nb* (De Jong et al. 1997); *Gpa* (Kreike et al. 1994), *Gpa5* (Rouppe van der Voort et al. 2000), and *Grp1* (Rouppe van der Voort et al. 1998) are located at the short arm of chromosome V, while *GroV1* (Jacobs et al. 1996) and *H1* (Gebhardt et al. 1993; Pineda et al. 1993) are located at the long arm of chromosome V. The *box at the right* contains a close-up of the *H1* locus in the UHD genetic map. The *H1* gene co-segregates with EM1, EM14, EM15 and CM1 and is flanked by EM16 and 239E4left$_{CAPS}$

### Chromosomal position of the *H1* gene in SH

Forty-seven F$_1$ genotypes, randomly selected from the original mapping population F$_1$ SH× RH, were screened for resistance to *G. rostochiensis* line 19. Twenty plants were resistant and twenty-seven were susceptible. The results of the resistance assay were integrated into the UHD genetic map (http://www.dpw.wageningen-ur.nl/uhd/), resulting in the mapping of the *H1* gene at the distal end of the long arm of chromosome V, where the markers CP113 and CD78 are also located. In the UHD mapping population, the *H1* gene co-segregates with four AFLP markers (Table 2). Markers EM1, EM14 and CM1 are in coupling with *H1*, whilst EM15 is in repulsion. EM16 flanks the *H1* locus at the telomeric side and is separated by one recombination event (Fig. 1).

### The high-resolution map

To fine-map the *H1* locus, a population of 1,073 plants was screened for recombination events using CAPS markers. At the telomeric side of the *H1* gene, CAPS marker CT51$_{CAPS}$ (Fig. 2a) was designed, which was located at a distance of 10 cM from the *H1* gene. Instead

**Table 4** Eleven candidate markers resulting from the BSA are tested on 45 potato cultivars for co-segregation with the *H1* locus

| Cultivar | Status[a] | EM1[b] | EM2 | EM3 | EM4 | EM5 | EM6 | EM7 | EM8 | EM10 | EM11 | EM12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bartina | S | – | – | – | – | – | – | – | – | – | – | – |
| Concurrent | S | – | – | – | + | – | – | n.t. | – | – | – | – |
| Lizen | S | – | – | – | + | + | n.t. | n.t. | – | – | – | – |
| Mansour | S | – | – | – | – | + | n.t. | – | – | – | – | – |
| Starletta | S | – | – | – | + | + | n.t. | – | – | n.t. | – | n.t. |
| Alpha | S | – | – | – | – | – | – | – | – | – | – | – |
| Bea | S | – | – | – | – | + | – | – | – | – | – | – |
| Blanka | S | – | – | – | – | – | – | – | – | – | – | – |
| Bintje | S | – | – | – | – | – | – | – | n.t. | – | – | – |
| Burmania | S | – | – | – | – | – | – | – | – | – | – | – |
| Cleopatra | S | – | – | – | – | + | n.t. | – | – | – | – | – |
| Eigenheimer | S | – | – | – | + | – | – | – | n.t. | – | – | n.t. |
| Furore | S | – | – | – | – | – | – | – | n.t. | – | – | – |
| Humalda | S | – | – | – | – | + | – | – | – | – | – | + |
| Saskia | S | – | – | – | – | + | – | – | – | – | – | + |
| Baraka | S | – | – | – | – | + | – | – | – | – | – | – |
| Desiree | S | – | – | – | + | – | – | – | – | – | – | – |
| Edzina | S | – | – | – | – | – | – | – | – | – | – | n.t. |
| Favorita | S | – | – | – | – | + | – | – | – | – | – | – |
| Joakla | S | – | – | – | + | + | – | – | – | – | – | n.t. |
| Manna | S | – | – | – | – | + | – | – | – | – | – | n.t. |
| Aphrodite | S | – | – | – | + | + | – | – | – | – | – | n.t. |
| Monalisa | S | – | – | – | – | + | n.t. | – | – | – | – | – |
| Sirtema | S | – | – | – | + | n.t. | – | – | – | – | – | + |
| Provita | R | + | + | + | – | – | n.t. | – | + | – | + | – |
| Fresco | R | + | + | + | – | – | – | – | – | – | + | – |
| Premiere | R | + | + | + | – | – | – | – | + | – | + | + |
| Revelino | R | + | + | + | + | + | + | + | + | + | + | + |
| Amigo | R | + | + | + | + | + | + | + | + | + | + | n.t. |
| Anosta | R | + | + | + | + | + | + | + | + | + | + | + |
| Prevalent | R | + | + | + | – | – | – | – | – | – | + | – |
| Prior | R | + | + | + | – | + | n.t. | – | – | – | + | – |
| Producent | R | + | + | + | – | + | – | – | – | – | + | – |
| Asterix | R | + | + | + | + | + | + | + | + | + | + | + |
| Cardinal | R | + | + | + | – | – | – | – | – | – | + | + |
| Lutetia | R | + | + | + | – | + | n.t. | – | – | – | + | + |
| Marijke | R | + | + | + | + | – | – | – | – | – | + | + |
| Obelix | R | + | + | + | – | – | – | – | – | – | + | + |
| Sante | R | + | + | + | + | + | + | + | + | + | + | + |
| Ukama | R | + | + | + | + | + | – | – | – | – | + | + |
| Van Gogh | R | + | + | + | + | + | + | + | + | – | + | + |
| Vebeca | R | + | – | – | + | + | + | + | – | – | – | + |
| Sinaeda | R | + | + | n.t. | + | + | + | + | + | + | n.t. | + |

[a] *S* Susceptible to *G. rostochiensis* pathotype $Ro_1$, *R* resistant to *G. rostochiensis* pathotype $Ro_1$
[b] For all candidate markers, + present, – absent, *n.t.* not tested

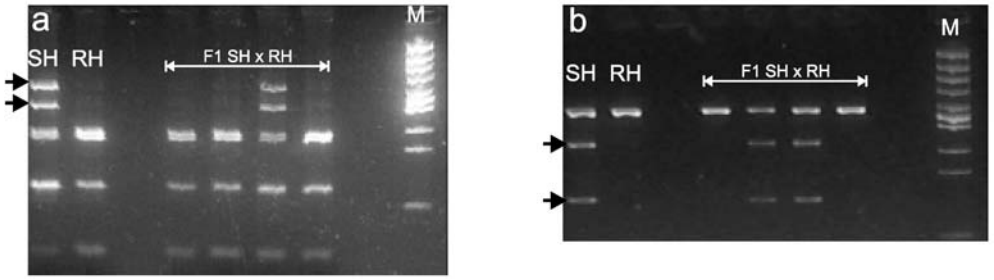


**Fig. 2** Performance of the CAPS markers $CT51_{CAPS}$ (**a**) and $239E4left_{CAPS}$ (**b**) on the resistant parent, SH83-92-488 (SH), the susceptible parent, RH89-039-16 (RH) and four descendants. Digestion of the PCR product with *Alu*I resulted for both markers in two polymorphic bands, segregating from the resistant parent as indicated with *arrows*

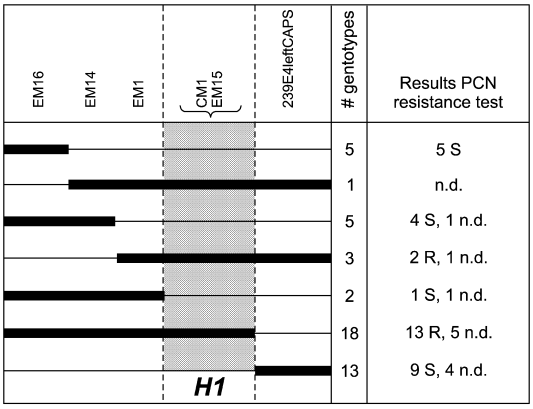


**Fig. 3** The high-resolution map of the *H1* locus in potato. Markers EM1, EM14, EM16, CM1 and 239E4left$_{CAPS}$ (in coupling) and EM15 (in repulsion) are presented in the correct genetic order. Markers EM14, EM1, CM1 and EM15 co-segregated in the population used to produce the UHD map. *Bolded horizontal lines* represent chromosomal regions derived from the resistant haplotype of SH; *thin horizontal lines* represent chromosomal regions derived from the susceptible haplotype of SH. The region were the *H1* gene is genetically located is indicated by a *grey column between dotted lines*. In the *second column*, the number of genotypes for each recombination event is given and in the *third column*, the results of the potato cyst nematode (PCN) resistance assays are listed. *n.d.* Not done, *S* susceptible, *R* resistant

of CAPS marker CP113 (Niewohner et al. 1995), an alternative CAPS marker 239E4left$_{CAPS}$ (Fig. 2b) was designed that mapped to a position 0.8 cM from the *H1* gene at the centromeric side.

Screening the 1,073 $F_1$ SH × RH genotypes resulted in 129 plants showing a recombination event between the PCR-based markers CT51$_{CAPS}$ and 239E4left$_{CAPS}$. Screening these 129 genotypes with EM16, which flanks the *H1* interval, resulted in 56 genotypes with a recombination event between this marker and 239E4left$_{CAPS}$. To map these recombination events more precisely, a third marker screening was carried out with the four AFLP markers that co-segregate with *H1* in the UHD population. For nine genotypes, AFLP analysis resulted in missing values and they were omitted from further analysis. Subsequently, a successful resistance assay on 40 genotypes resulted in the identification of 23 susceptible and 17 resistant plants. Integrating the results of the marker screening and the resistance assay resulted in a high-resolution map as shown in Fig. 3. In a total of 1,209 offspring, there are no recombination events between the *H1* gene and the markers CM1 (in coupling) and EM15 (in repulsion), demonstrating complete linkage of these two markers. Furthermore, the *H1* gene is separated from EM1, EM14 and 239E4left$_{CAPS}$ by 2, 8 and 31 recombination events respectively, equating to 0.2, 0.8 and 3.0 cM distance.

## Discussion

In this study, a high-resolution map of the *H1* locus has been made in potato linking the *H1* gene with the AFLP markers CM1 (coupling) and EM15 (repulsion). To identify markers closely linked to the *H1* gene, a BSA has been carried out using 704 AFLP primer combinations. In addition, markers closely linked to the *H1* locus have been selected from the UHD genetic map of potato that was produced with 387 AFLP primer combinations (http://www.dpw.wageningen-ur.nl/uhd/). Of the 387 AFLP primer combinations used for the UHD genetic map, 106 have also been used for the BSA. Of the markers identified in the BSA, only one marker could be mapped at a distance of less than 1 cM from the *H1* gene in the high-resolution map, while four markers derived from the UHD map are located at a distance of less than 1 cM from the *H1* gene. Apparently, with BSA some markers linked to the *H1* gene are not recognised. The UHD genetic map of potato is a globally saturated map and will therefore be suitable for mapping purposes of traits located anywhere in the potato genome. With the high-resolution map of *H1*, we have shown the usefulness of a globally saturated UHD genetic map for high-resolution mapping.

The majority of the 129 recombination events between the markers EM1 and 239E4left$_{CAPS}$ were observed between *H1* and the marker 239E4left$_{CAPS}$. This could be explained by a large physical distance, or by the presence of a so-called ‘hot spot’ for recombination. In case of a hot spot for recombination, a small physical distance results in a large genetic distance. This phenomenon has been reported for resistance-gene loci previously. For instance, the tomato resistance-gene loci *Asc* (Mesbah et al. 1999) and *I2* (Segal et al. 1992) measure 125 kb/cM and 43 kb/cM, respectively. These physical distances are much shorter than the average distance of 750 kb/cM for the tomato genome (Tanksley et al. 1992). A physical map of the *H1* locus is needed to draw further conclusions on this issue.

MAS can be very useful to select for interesting traits. In the case of resistance to PCN, the selection can already take place at the seedling stage and laborious nematode resistance tests can be avoided. Of course, for successful MAS, it is essential to have markers at a very short distance from the gene of interest. Marker EM1 has been tested in 45 potato cultivars and always co-segregated with resistance. This marker could be a suitable candidate for MAS. In the high-resolution genetic map, however, EM1 is not the closest marker. Marker CM1 co-segregates with resistance in a progeny of 1,209 genotypes and, in theory, should be a better candidate. Markers EM1 and CM1 are AFLP markers and, for MAS, cheaper and easy-to-handle CAPS markers are preferred. Therefore, these markers should first be converted to CAPS markers before they can be used in MAS.

In this study, six genotypes have been tested for nematode resistance in vitro as well as with tubers (data not shown). The results of both resistance assays were

identical for all genotypes. Testing for nematode resistance in vitro is quicker than testing with tubers. The resistance response of the *H1* triggers an HR already within the first week of nematode infection (Rice et al. 1985). In addition, a virulent (line 22) *G. rostochiensis* population and an avirulent (line 19) *G. rostochiensis* population were used to perform the nematode resistance tests (Janssen et al. 1990). Therefore, the results of the in vitro assay can be observed already at 3 weeks after inoculation, while the results of a tuber assay are ready at 10 to 12 weeks after inoculation. Furthermore, the in vitro assay can be performed on shoots derived from a seedling. For a reliable tuber assay at least four tubers are required, which takes at least 12 additional months before the experiment can be started.

The development of a high-resolution genetic map is a crucial step in the positional cloning of the *H1* gene. Marker CM1 is in coupling phase and located at a distance of less than 0.1 cM from the *H1* locus. Therefore, this is a suitable marker for screening an SH BAC library for the construction of a physical map and subsequent cloning of the *H1* gene. The gene-for-gene interaction between the *H1* gene and *G. rostochiensis* (Janssen et al. 1991) may provide us with a useful model system to study the molecular mechanisms underlying cyst nematode resistance, especially if the corresponding avirulence gene will be isolated from the nematode.

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