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ORIGINAL PAPER

**Kouassi Abou Bakari · Kerlan Marie-Claire
Caromel Bernard · Dantec Jean-Paul · Fouville Didier
Manzanares-Dauleux Maria · Ellissèche Daniel
Mugniéry Didier**

A major gene mapped on chromosome XII is the main factor of a quantitatively inherited resistance to *Meloidogyne fallax* in *Solanum sparsipilum*

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Abstract *Meloidogyne fallax* is an emerging pest in Europe and represents a threat for potato production. We report the mapping of genetic factors controlling a quantitative resistance against *M. fallax* identified in the *Solanum sparsipilum* genotype 88S.329.15. When infected, this genotype develops a necrotic reaction at the feeding site of the juveniles and totally prevents their development to the female stage. A "F1" diploid progeny consisting of 128 individuals was obtained using the potato (*S. tuberosum*) dihaploid genotype BF15 H1 as female progenitor. Sixty-eight hybrid genotypes displayed necrosis at the feeding site of the juveniles and 60 other genotypes showed no defence reaction. This suggested a monogenic control of the resistance. However, when considering the number of nematode females developed in their roots, a continuous distribution was observed for both "necrotic" and "non-necrotic" hybrid genotypes, indicating a polygenic control of the resistance. A linkage map of each parental genotype was constructed using AFLP markers. The necrotic reaction (NR) was mapped as a qualitative trait on chromosome XII of the resistant genotype 88S.329.15. Quantitative trait locus (QTL) analysis for the number of nematode females developed per "F1" plant genotype was performed using the QTL cartographer software. No QTL was detected on the linkage map of the susceptible parent. A QTL explaining 94.5% of the phenotypic variation was mapped on chromosome XII of the resistant progenitor. This QTL, named *MfaXIIspl*, was mapped in a genomic region collinear to the map position of the *Mi-3* gene conferring resistance to *Meloidogyne incognita* in tomato. It corresponds to the NR locus.

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K. A. Bakari · F. Didier · M. Didier (✉)
Biologie des Organismes et des Populations appliquée à la Protection des Plantes (Bio3P), Laboratoire de nématologie, Umr inra—Agrocampus Rennes, Domaine la Motte, BP 35327, 35653 Le Rheu Cedex, France
E-mail: didier.mugniery@rennes.inra.fr

K. Marie-Claire · D. Jean-Paul · E. Daniel
Station d'Amélioration de la Pomme de Terre et des Plantes à Bulbes, Umr inra—Agrocampus Rennes, Keraïber, 29260 Ploudaniel, France

C. Bernard
Inra—Unité de Génétique et d'Amélioration des Fruits et Légumes, BP 94, 84143 Montfavet cedex, France

M.-D. Maria
Amélioration des Plantes et Biotechnologies Végétales (APBV), Umr inra—Agrocampus Rennes, Domaine la Motte, BP 35327, 35653 Le Rheu Cedex, France

Introduction

Plant parasitic nematodes infest the cultivated potato *Solanum tuberosum* worldwide. In Europe, the cyst nematodes *Globodera pallida* and *G. rostochiensis* have been the most detrimental to potato for a long time. But more recently, two root-knot nematode species *Meloidogyne chitwoodi* and *M. fallax* have emerged as a serious threat for potato production (Karssen 1996). In contrast to cyst nematodes, root-knot nematodes are polyphagous parasites and classical control methods such as crop rotations are not effective. The use of chemicals is also restricted in sustainable agriculture due to their toxicity for plants, animals and human beings. Breeding potato for resistance to nematodes is a promising alternative control strategy (Boerma and Hussey 1992) and several resistances have been detected in wild tuber-bearing *Solanum* species (Janssen et al. 1996, 1997).

Reproduction of root-knot nematodes relies on the ability of juveniles to become females (Trudgill and Blok 2001). Phenotypic assessment of host plant resistance is therefore based on either the number of females or the

egg mass score and gall index recorded on infected roots (Cousins and Walker 2002). Based on these criteria, genetic analyses have revealed a monogenic or polygenic determinism of the resistances identified in wild plants. A necrotic reaction (NR) at the infection site also characterizes several resistance responses. So far, this trait is known to totally prevent the development of the juveniles into females and is generally under monogenic control (Gilbert and McGuire 1956; Choi et al. 1999; Berthou et al. 2003). Monogenic resistances are desirable for breeding purpose because of their simplicity to be introgressed. However, these resistances are pathotype or species specific and their effectiveness can be rapidly lost due to the evolution of the pathogen populations. In contrast, quantitative resistances are, in general, non-pathotype specific and they are more interesting in term of management because they may be relatively durable in field (Hammond-Kosack and Jones 1997).

Introgression of quantitatively inherited resistance through phenotypic selection is less effective than the use of molecular markers in marker-assisted selection (Thabuis et al. 2004). During the last decade, many mapping investigations have allowed the genetic dissection of quantitative resistances to potato cyst nematodes (Kreike et al. 1994; Rouppe Van der Voort et al. 1998, 2000; Bryan et al. 2002; Caromel et al. 2003, 2004). Resistance-linked markers have been identified from these studies and they can potentially be used for marker-assisted selection and simplify resistance introgression. The situation is very different for resistance to root-knot nematodes in potato. Only one resistance gene, originating from *Solanum bulbocastanum* and effective against *M. chitwoodi* has been mapped so far (Brown et al. 1996; Rouppe Van der Voort et al. 1999a, b).

In the present work, we report the study of a resistance against the root-knot nematode *M. fallax* identified in *S. sparsipilum*. Our objectives were (1) to analyze the genetic determinism of the resistance through its segregation in a pseudo test cross progeny, (2) to map genetic factors (major genes and quantitative trait loci) involved in the resistance on a AFLP molecular linkage map of the resistant progenitor, (3) to identify resistance-linked molecular markers which could be used for marker-assisted selection.

Materials and methods

Plant material

A "F1" diploid potato population ($2n = 2x = 24$) was obtained from an interspecific cross between the resistant diploid clone 88S.329.15 of *S. sparsipilum* (male progenitor) and the susceptible dihaploid clone BF15 H1 of cultivated potato *S. tuberosum* subsp. *tuberosum* (female progenitor). The resulting progeny, termed 00D50, consisted of 128 genotypes, which were maintained through tubers.

Nematodes

Nematode inoculum consisted of an isolate of *M. fallax* collected in Baexem in The Netherlands and provided by the Plant Research International (Wageningen). After multiplication on the susceptible potato cv. 'Désirée', the purity of this isolate was verified through PCR-RFLP mediated detection of *M. fallax* based on sequence analysis of the internal transcript spacer of rDNA (Zijlstra et al. 1995; Daher et al. 1996). Juveniles from checked egg masses were multiplied on potato cv. 'Désirée' in closed canisters in order to avoid any later contamination (Foot 1977). Second stage juveniles (J2s) were then collected for inoculation.

Nematode resistance assays

Four tubers per genotype were individually germinated on a 2-cm thick layer of moist sand in 250 cm^3 perforated plastic pots (7×36 cm^2) and inoculated each with 200 J2s. Two days after inoculation, pots were filled with organic substrate. Plants were arranged in greenhouse in a completely randomized design with four replicates and allowed to grow at 20 ± 2°C with regular watering.

Six weeks after inoculation, root systems were washed out, stained with acid fuchsin in a mixture of acetic acid with ethanol (1:1) and re-hydrated in a mixture of water with chloral hydrate (1:4) according to Daykin and Hussey (1985). Roots were gently squashed between two glass slides and examined with a light microscope. The number of nematode females and NRs were assessed in the roots of each plant genotype as described previously (Kouassi et al. 2004).

Analysis of female number

Plant genotypes in the mapping population were separated according to the presence or absence of cytological reaction (necrosis) in their roots. A Chi-square test was applied to assess goodness of fit with appropriate genetic ratios. The mean numbers of nematode females scored in the plant genotypes were standardized using a Logarithm transformation [Ln(number of developed females + 1)] and then subjected to one way analysis of variance (ANOVA). Test for normality was carried out on the distribution of the residuals according to the Wilk–Shapiro test (SAS General linear model procedure) (SAS institute 1989). The broad-sense heritability was estimated with the formula $h_b^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/n)$; σ_g^2 and σ_e^2 being, respectively, the genotypic variance and the error variance from the ANOVA and n the number of replicates.

Table 1 Primer combinations used for selective amplifications in AFLP assays

Primer combinations	C + AAG	C + AAG	C + ACA	C + ATA	C + ATA	C + ATA	C + ATC	C + ATG
	M + CAG	M + CAT	M + CAT	M + CAA	M + CAT	M + CTT	M + CCA	M + CTA
Primer code	C33	C33	C35	C43	C43	C43	C44	C45
	M49	M50	M50	M47	M50	M62	M51	M59
Primer combinations	E + AAC	E + AAC	E + ACA	E + ACA	E + ACA	E + ACA	E + ACA	E + ATG
	M + CAC	M + CCA	M + CAC	M + CAG	M + CCT	M + CGT	M + CTG	M + CAC
Primer code	E32	E32	E35	E35	E35	E35	E35	E45
	M48	M51	M48	M49	M54	M58	M61	M48

AFLP assays and marker nomenclature

DNA extraction was performed on fresh leaf tissue as described by Caromel et al. (2003). The AFLP^{TM} procedure was essentially as described by Vos et al. (1995). Genomic DNA was restricted using enzymes *Eco*RI (E) and *Sac*I (C) as rare cutters and *Mse*I (M) as a frequent cutter. Pre-amplifications were carried out with one-base pair (1 bp) extension primers combinations (*Eco*RI + A or *Sac*I + A/*Mse*I + C). Sixteen 3-bp extension primers combinations (*Eco*RI + 3-bp or *Sac*I + 3-bp/*Mse*I + 3-bp) were used for selective amplifications (Table 1) These primer combinations have been previously used to map quantitative trait locus (QTLs) for resistance to the potato cyst nematode *G. pallida* in a "F1" diploid population obtained from the cross between *S. sparsipilum* clone 88S.329.18 (a full sib of the resistant clone 88S.329.15 used in the present study) and the potato dihaploid clone Caspar H3 (Caromel et al. 2004). The mapping population used by Caromel et al. (2004) served as reference population. AFLP profiles of the resistant and susceptible parental clones of our mapping population were, respectively, compared to the profiles of the clones 88S.329.18 and Caspar H3. AFLP products of equal electrophoretic mobility (co-migrating markers) which segregated in both mapping populations were assumed to be identical (Rouppe Van der Voort et al. 1997a, b) and they were allotted the same serial numbers. Alphabetic letters were assigned to segregating AFLP bands specific to the parental clones 88S.329.15 and BF15 H1. For each parental clone, a mirror AFLP data set was produced by inverting the recorded normal data set. Each AFLP marker was designated by the symbol of the rare cutter restriction enzyme (C or E) followed by the numbers of selective primers used and then the allotted serial number or alphabetic letter preceded by the letter m or n which indicate the mirror or normal state of the marker, respectively (e.g. C3550mA, E4560n1).

Linkage map construction

Segregation of AFLP markers was tested for significant deviation from the expected Mendelian ratio 1:1 using a Chi-square test. A map was constructed separately for each parental genotype according to the double pseudo test cross design (Grattapaglia and Sederoff 1994). Both mirror and normal AFLP data sets were pooled for the construction of the linkage map in order to allow the detection of markers in coupling or repulsion phase (Yin et al. 2004). Linkage groups were established with the Mapmaker/Exp 3.0 software (Lincoln et al. 1992) using a LOD threshold of 5. In order to avoid pseudo linkages, a LOD threshold of 10 was set for linkage groups containing many distorted markers. Recombination frequency was converted into map units centi-Morgan (cM) using the Kosambi (1944) function. AFLP markers identified in the reference population (Caromel et al.

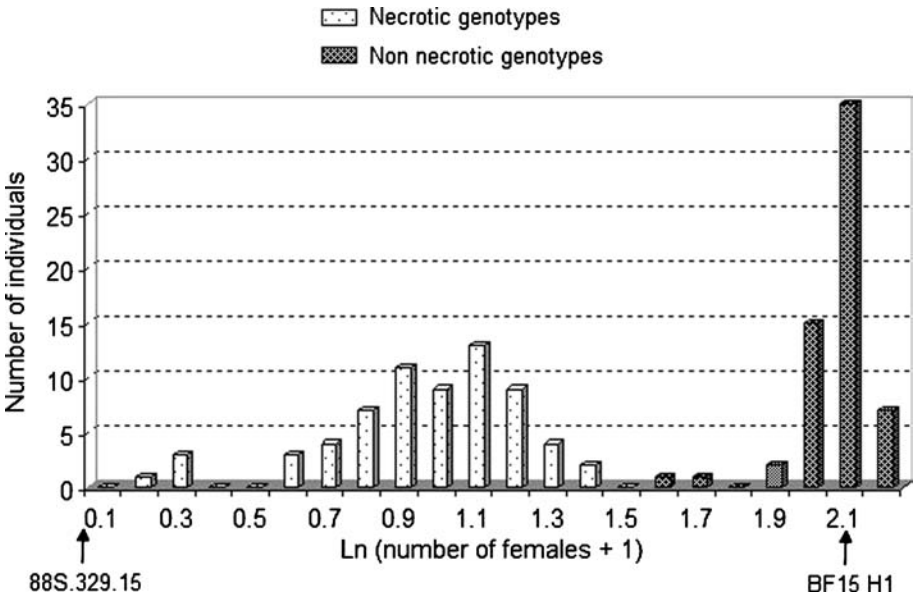

Fig. 1 Frequency distribution of the Ln (mean number of females + 1) transformed values in the "F1" progeny of the cross between the resistant genotype 88S.329.15 of *Solanum sparsipilum* and the susceptible genotype BF15 H1 of the cultivated potato, *S. tuberosum*. The positions of parental genotypes are indicated

2004) which co-migrated with AFLP markers segregating from the parental genotypes BF15 H1 and 88S.329.15 were used to assign the linkage groups to chromosomes (Rouppe Van der Voort et al. 1997a, b).

QTL mapping

The mean number of nematode females per plant genotype was used as a quantitative trait for the QTL analysis. QTL detection was performed with the QTL cartographer v 2.0 software (Basten et al. 2004). Interval mapping and composite interval mapping methods were first applied to map data of each parental genotype. Pooled map data from both parents were then analyzed by composite interval mapping. The LOD score threshold was set at 3.0 (type I error = 0.05) after a 1,000-permutation test.

Results

Inheritance of nematode resistance

Both NRs and the number of nematode females in the roots of inoculated plants were used to assess the resistance responses. The resistant parental genotype 88S.329.15 developed a NR at the feeding site of the juveniles and totally prevented their development into females. The susceptible parental genotype BF15 H1 showed no defence reaction and all the juveniles that infected its roots became females. Two distinct phenotypic classes were observed in the "F1" progeny: 68 genotypes showed NR and 60 genotypes showed no reaction. The observed segregation fitted to a 1:1 ratio ($\chi^2 = 0.47$, $P = 0.488$) and suggested a monogenic control of the NR.

Distribution of the "F1" progeny for the logarithm of the female number + 1 showed a continuous, but near asymmetric, bimodal distribution (Fig. 1). Two distinct distribution curves corresponding to "necrotic" and "non-necrotic" plant groups were observed. The number of females retrieved in "necrotic" genotypes varied from 0 to 49, with a mean number of 14. "Non-necrotic" genotypes contained 92–157 females, with a mean number of 127. Mean number of females in the "non-necrotic" genotypes did not significantly differ ($P < 0.001$) from that of the susceptible parental genotype BF15 H1 (109 females on average). These results suggested that continuous variation for the mean female number was mainly controlled by a gene with a major effect in a background of polygenic variation. The broad-sense heritability of the genotypic means for the female numbers was 0.997 and conferred a great reliability to the estimation of genotypic values by phenotypic values.

Marker analysis and linkage maps description

A total of 358 segregating markers were obtained from the 16 AFLP primer combinations used. One hundred and fifty markers (41.9%) originated from the resistant parental genotype 88S.329.15 and 208 markers (58.1%) originated from the susceptible progenitor BF15 H1.

One hundred and thirty two AFLP markers (88%) of the genotype 88S.329.15 mapped on 16 linkage groups and spanned a total length of 579.8 cM (Fig. 2). Eighteen markers (12%) remained unlinked. Fifty markers (37.88%) co-migrated with markers of the reference genotype 88S.329.18 (Caromel et al. 2004) and ranged from 1 to 8 per linkage group. Nine linkage groups with at least two co-migrating markers were assigned to chromosomes I–V and I–XII. Linkage groups consisted

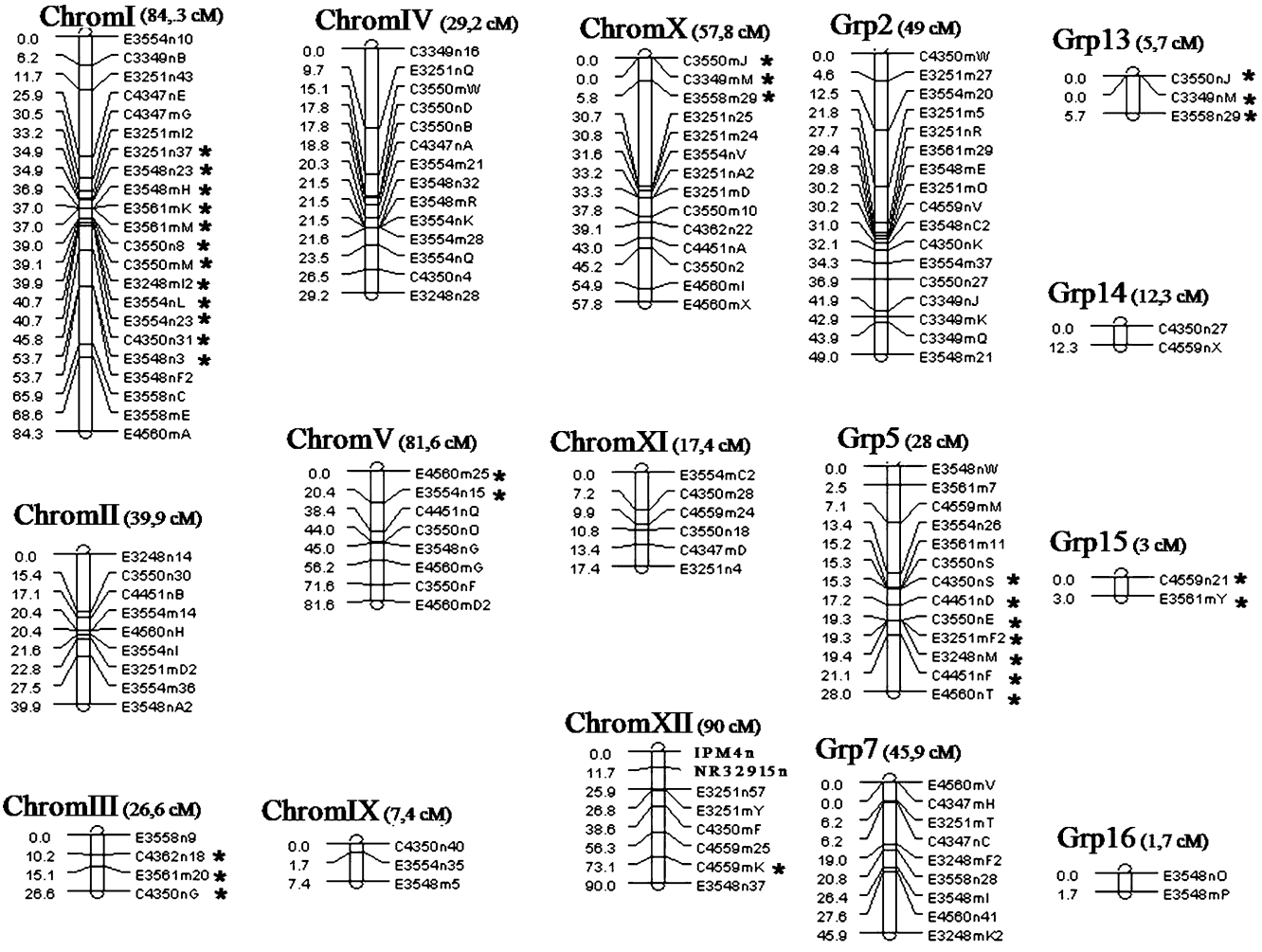

Fig. 2 Molecular linkage map of the resistant parental genotype 88S.329.15. Markers with a numerical serial number at the end of their name were common to the reference genotype 88S.329.18 (Caromel et al. 2004). They were used to assign the linkage groups to chromosomes. ChromI, chromII, etc. are linkage groups assigned to potato chromosomes and Grp2, Grp5, etc. are unassigned linkage groups. Distorted markers (*asterisks*) mapped mainly on chromosome I and linkage group 5. The phenotypic marker (NR32915) of the necrotic reaction mapped on chromosome XII, 11.7 cM (coupling phase) from the PCR marker IPM4 defined from the *Gpa2* gene

of 2 (linkage groups 14, 15 and 16) to 23 markers (chromosome I). Their sizes varied from 1.7 cM (linkage group 16) to 90 cM (chromosome XII).

Among the 208 segregating AFLP markers obtained from the susceptible parental genotype BF15 H1, 190 (91.35%) formed 16 linkage groups of 2–31 markers and 18 (8.65%) markers were unlinked (map not shown). The total map length was 951.6 cM and linkage groups ranged from 15.0 to 125.3 cM in size. Twenty markers (10.52%) co-migrated with markers of the reference genotype Caspar H3 (Caromel et al. 2004) and ranged from 1 to 4 per linkage group. Six linkage groups had at least two co-migrating markers and they were assigned to chromosomes I–IV, VI and VIII.

Ninety-six (26.74%) markers (46 and 50 for the resistant and susceptible parent, respectively) showed significant deviation from the expected Mendelian 1:1 ratio according to the pseudo test cross design ($P < 0.05$). Thirty-eight distorted markers mapped on seven linkage groups of the resistant genotype 88S.329.15, twenty-eight (73.68%) of them being located on chromosome I and linkage group 4. Ten linkage groups of the susceptible genotype BF15 H1 showed from 1 to 16 distorted markers. These markers mapped mainly (34 out of 45) on chromosome II, linkage groups 4, 8 and 9.

Mapping the necrotic reaction locus as a qualitative trait

Since the "F1" plant genotypes could be classified as "necrotic" and "non-necrotic", the NR was scored as a Mendelian trait which was coded NR32915. The recorded data were added to the AFLP data set originating from the resistant parental genotype 88S.329.15. So, the NR locus was mapped on the chromosome XII (Fig. 2).

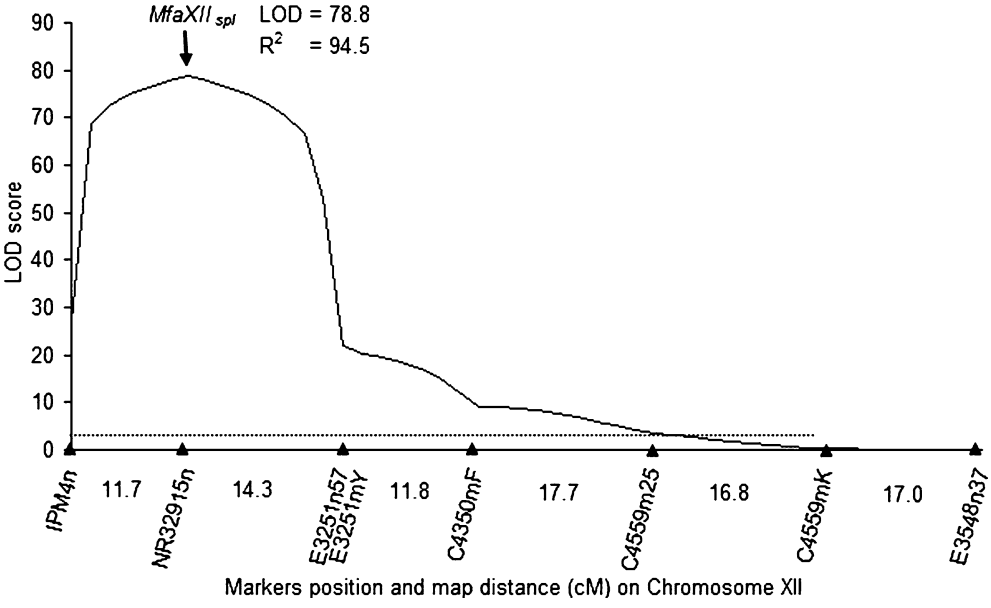

Fig. 3 LOD score plot for the QTL *MfaXII*$_{spl}$ detected by interval mapping on chromosome XII of the resistant progenitor 88S.329.15. The mean number of nematode females per plant genotype was used as a quantitative trait for the QTL analysis. The *horizontal dotted line* indicates the LOD threshold set to 3. The maximum logarithm of odds ratio (LOD score) for the QTL is observed at the position of the necrotic reaction marker NR32915, which explains 94.5% (R^2) of the variance of the number of nematode females developed in the different plant genotypes

Mapping QTLs controlling the inhibition of the development of juveniles into females

The number of nematode females in the necrotic hybrids ranges from 0 to 49. In contrast, no female developed from the 800 J2s (four replicates of 200 J2s each) used to inoculate the resistant parental genotype 88S.329.15. These results suggest the segregation of resistance factors in the hybrids rather than heterogeneity of the *M. fallax* population for the avirulence gene. Thus, the putative genetic factors controlling the resistance were mapped through QTL analysis using the mean number of nematode females as a quantitative trait. No QTL was observed on the map of the susceptible parent BF15 H1. A QTL was detected by interval mapping (Fig. 3) and composite interval mapping (data not shown) on a distal end of chromosome XII of the resistant parent 88S.329.15. It explained 94.5% of the phenotypic variability and its maximum LOD score (78.8) was observed at the position of the NR locus (Fig. 3). This QTL was named *MfaXII*$_{spl}$ as the first QTL of resistance to *M. fallax* mapped on chromosome XII of *S. sparsipilum*. Since the large effect of this QTL is expected to have an impact on the detection of other QTLs with minor effects, interval mapping was conducted using the full data set with *MfaXII*$_{spl}$ fixed in the model to control its effect. No other QTL was detected.

Several resistance genes to different parasites including virus and nematodes have been mapped on chromosome XII in potato and tomato (reviewed in Grube et al. 2000). In order to confirm the assignment of the linkage group harboring *MfaXII*$_{spl}$ to chromosome XII and to check the position of this QTL relatively to the other resistance genes, we used the PCR marker IPM4. This marker corresponds to the 3′ end of the sequence of the *Gpa2* gene conferring resistance to the potato cyst nematode *G. pallida* (Kanyuka et al. 1999; Van der Vossen et al. 2000). No segregating band was obtained from the susceptible parental genotype BF15 H1. A band originating from the resistant genotype 88S.329.15 segregated in the progeny (Fig. 4). It mapped on chromosome XII (coupling phase) 11.7 cM from the NR marker NR32915 (Fig. 2).

Discussion

This study is the first report on the genetic dissection of a quantitative resistance to *M. fallax* in a wild tuber-bearing *Solanum* species. We analyzed the genetic

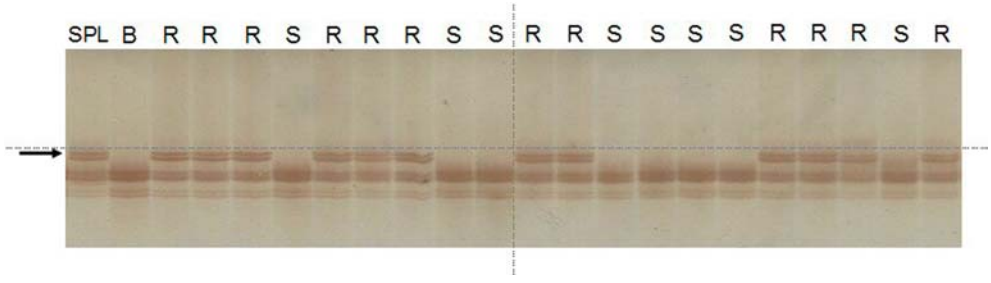

Fig. 4 Electrophoretic profile of PCR products obtained with the marker IPM4 on parental genotypes 88S.329.15 (*SPL*), BF15 H1 (*B*) and 20 necrotic (*R*) and non-necrotic (*S*), "F1" genotypes. The *arrow* indicates the segregating band originating from the resistant parent 88S.329.15

determinism of the resistance of the *S. sparsipilum* genotype 88S.329.15 against the root-knot nematode *M. fallax*. When infected, this genotype develops a NR at the feeding site of juveniles and totally prevents their development to the female stage. In a diploid "F1" progeny obtained from the cross between the genotype 88S.329.15 and the susceptible potato genotype BF15 H1, the segregation of the NR fitted to a 1:1 ratio. This result suggested a monogenic control of the resistance as it has been reported for hypersensitive resistances against root-knot nematodes in other Solanaceae species such as tomato and pepper (Gilbert and McGuire 1956; Hendy et al. 1985; Yaghoobi et al. 1995; Djian-Caporalino et al. 2001).

The number of nematode females developed in the roots exhibited a clear-cut bimodal feature but showed a continuous distribution in both necrotic and non-necrotic plant groups. We therefore concluded that the NR locus controls an important part of the resistance but is likely to be associated to other resistance loci. The broad-sense heritability of the resistance based on female number was estimated to be 0.997 indicating that it is fully under genetic control.

When mapping the NR as a Mendelian trait, the NR locus was located on the chromosome XII of the genotype 88S.329.15. Mapping of putative genetic factors involved in the inhibition of the development of the juveniles into females was performed through QTL analysis. No QTL was detected on the map of BF15 H1. This demonstrates that no allele from the susceptible parental genotype contributes to the resistance. A QTL explaining 94.5% of the variation of the development of nematode females in the roots was detected on the map of the resistant genotype 88S.329.15. The most likely position of this QTL corresponds to the position of the NR locus on chromosome XII. Moreover the value of its contribution (94.5%) to the resistance is close to the heritability estimated to 0.997. These results confirm that the NR locus is the main factor of the resistance.

The *Gpa2* gene is located on potato chromosome XII, 0.02 cM from the *Rx1* gene conferring resistance to the potato virus X (Rouppe van der Voort et al. 1999a, b). Genetic and physical mapping investigations plus southern blot analysis on genomic DNA identified resistance gene homologues only within a stretch of less than 200 kb in the *Gpa2*/*Rx1* cluster on chromosome XII (Bakker et al. 2003). On the other hand, the *Gpa2*/*Rx1* genes mapped 10 cM from the PCR-based marker NR14 (Rouppe van der Voort et al. 1997a, b), which is closely linked to the *Mi3*/*Mi5* genes on tomato chromosome 12 (Yaghoobi et al. 1995). The orthologous region in pepper also harbor, *Me3* and *Me4* (Djian-Caporalino et al. 2001) genes conferring resistances to *Meloidogyne* spp. In our study, the NR locus mapped 11.7 cM from the PCR-based marker IPM4 corresponding to the 3′ end of *Gpa2*, that is, in collinear position with the Mi3/Mi5 genes. In the light of these mapping data and considering the colinearity between the genomes of potato, tomato and pepper, our results suggest the presence of a second resistance genes cluster 10–11 cM from the *Gpa2*/*Rx1* cluster on potato chromosome XII.

Molecular analyses of many R-genes clusters have shown that most of them contain an array of related sequences, several of which can have differential pathogen recognition (Bent 1996; Botella et al. 1998). Van der Vossen et al. (2000), for instance, obtained high sequence homology for *Gpa2* and *Rx1* genes and both ranged to leucine-zipper, nucleotide-binding site, leucine reach repeat (LZ-NBS-LRR)-containing class of plant resistance genes. The QTL $MfaXII_{spl}$ and the R-genes (*Mi3, Mi5, Me3, Me4*) mapping in syntenic position on chromosome XII of potato and tomato and chromosome P9 of pepper consistently induce NRs of infected plants. These genes may therefore likely originate from a single ancestral gene in Solanaceae (Michelmore and Meyers 1998).

Major effect QTLs have been described in quantitative resistances against *G. pallida* in *S. spegazzinii* (Kreike et al. 1994; Rouppe van der Voort et al. 1998) and in *S. bulbocastanum* against *M. chitwoodi* (Brown et al. 1996). In the last two examples and in our case, minor effect QTLs were not detected. Indeed, as shown by Charcosset and Gallais (1996), the size of our mapping population (128 individuals) may not be high enough to enable detection of minor QTLs contributing to less than 5% of the resistance. On the other hand, these QTLs may exist on the genomic regions not yet covered by the constructed linkage map. Most distorted markers mapped on chromosome I and linkage group 4 and they may also affect the detection of such minor QTLs when they induce a shortage of plants having the resistance-linked alleles (Kreike et al. 1994; Bryan et al. 2002).

Phenotypic selection is not very effective for the transmission of minor effect QTLs and hence limits the creation of fully resistant hybrid lines in breeding programs dealing with quantitatively inherited resistance. The use of molecular markers in marker-assisted selection is a potentially powerful tool to overcome that difficulty (Thabuis et al. 2004). The QTL identified in the present study is closely linked to the phenotypic marker NR32915 which is only recorded by time-consuming resistance tests. The nearest molecular markers mapped 11.7 and 14.3 cM apart and they can reasonably be used for marker-assisted selection. However, additional molecular markers are needed in the interval in order to reduce as much as possible the chromosomal portion to be introgressed and thereby limit transmission of putative undesirable traits originating from *S. sparsipilum*. As observed by Caromel et al. (2004), minor effect QTLs should not be neglected as they enhance the effects of major ones to ensure a satisfying expression of quantitatively inherited resistances. Further analysis will therefore focus on the detection of these minor effect QTLs mainly by increasing the number of individuals of the mapping population and also by mapping additional molecular markers on the linkage map of the genotype

88S.329.15 in order to ensure a best covering of its genome.

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