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B. Caromel · D. Mugniéry · V. Lefebvre · S. Andrzejewski · D. Ellissèche · M. C. Kerlan · P. Rousselle · F. Rousselle-Bourgeois

Mapping QTLs for resistance against *Globodera pallida* (Stone) Pa2/3 in a diploid potato progeny originating from *Solanum spegazzinii*

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Abstract A "F1" diploid population between *Solanum tuberosum* 2*x* and the wild *Solanum spegazzinii* was studied. It segregated for resistance against the potato cyst nematode *Globodera pallida* derived from the wild species. The inheritance had a quantitative nature. Linkage maps of AFLP and RFLP markers were constructed for both parents. Three QTLs were identified on the map of the resistant parent on chromosomes V, VI and XII, respectively. The first one had a major effect and explained more than 50% of the total variance of resistance. It is located in a cluster of resistance genes and may be the same locus as *Gpa* which has been described formerly. The two others explained about 20% of the total variance each. The QTL on chromosome XII is also in a cluster of resistance genes, and in an orthologous position with resistance genes against nematodes in tomato and pepper.

Keywords *Solanum tuberosum* · Potato cyst nematode · QTL · Resistance · Genetic mapping

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B. Caromel · V. Lefebvre · S. Andrzejewski · P. Rousselle · F. Rousselle-Bourgeois (✉)
Institut National de la Recherche Agronomique (INRA), Unité de Génétique et d'Amélioration des Fruits et Légumes, BP 94, 84143 Montfavet cedex, France,
e-mail: Francoise.Rousselle-Bourgeois@avignon.inra.fr
Fax: 33-490-32-72-27-02

D. Mugniéry
Institut National de la Recherche Agronomique (INRA), Unité de Biologie des Organismes et des Populations Appliquée à la Protection des Plantes, BP 35327, 35653 Le Rheu cedex, France

D. Ellissèche · M. C. Kerlan
Institut National de la Recherche Agronomique (INRA), Station d'Amélioration de la Pomme de terre et des Plantes à bulbes, Kéraiber, 29260 Ploudaniel, France

Introduction

Potato cyst nematodes (PCN), *Globodera rostochiensis* Wollenberg and *Globodera pallida* Stone, are major pests of the potato crop in European countries together with late blight (*Phytophthora infestans*). Control can be obtained by crop rotation, application of nematicides or resistant cultivars. The first solution is difficult to apply strictly due to economical considerations, the second is very undesirable from an environmental point of view. The latter solution is the most attractive method. Most cultivated potato varieties do not possess enough resistance to *G. pallida*. Breeding for resistance against this PCN is therefore a major aim of all research laboratories and private companies involved in potato breeding. Several sources of PCN resistance have been described in related species (e.g. Ross 1986; Dellaert et al. 1988; Phillips 1994; Rousselle-Bourgeois and Mugniéry 1995; Bradshaw et al. 1996; Rouppe van der Voort et al. 1998). Some resistances are monogenic, others are quantitatively inherited. In all cases, several backcrosses and intercrosses are necessary to introduce these resistance factors in an agronomically valuable background. On an other hand, as PCN are quarantine pests, screening tests are difficult to handle. Breeding for this trait would then be more effective with the help of marker-assisted selection.

Several genes or QTLs of resistance against PCN have already been mapped on potato: a gene *Gro1* from *Solanum spegazzinii* against *G. rostochiensis* on chromosome VII (Barone et al. 1990); a major gene *H1* from *Solanum tuberosum* ssp. *andigena* CPC 1673 against *G. rostochiensis* on the long arm of chromosome V (Gebhardt et al. 1993; Pineda et al. 1993); three QTLs *Gro1.2*, *Gro1.3* and *Gro1.4* from *S. spegazzinii* against *G. rostochiensis* on chromosomes X, XI and III, respectively (Kreike et al. 1993, 1996); a QTL *Gpa* from *S. spegazzinii* against *G. pallida* on chromosome V (Kreike et al. 1994); a gene *Gro V1* from *Solanum vernei* against *G. rostochiensis* on the long arm of chromosome V (Jacobs et al. 1996); a major gene *Gpa2* from *S. tuberosum* ssp. *andigena* CPC 1673 against *G. pallida* Pa2 on chromo-

some XII (Rouppe van der Voort et al. 1997); a QTL *Grp1* from a resistant genotype with several related species in its pedigree against *G. rostochiensis* and *G. pallida* on the short arm of chromosome V (Rouppe van der Voort et al. 1998); a QTL from *S. tuberosum* ssp. *andigena* against *G. pallida* on chromosome IV (Bradshaw et al. 1998); two QTLs *Gpa* 5 and *Gpa* 6 from a complex origin (mainly *S. vernei*) against *G. pallida* on chromosomes V and IX, respectively (Rouppe van der Voort et al. 2000); two QTLs from *S. vernei* on chromosomes V and IX which may be the same as the former ones (Bryan et al. 2002).

In the present paper we describe a study on a diploid potato progeny segregating for resistance against *G. pallida* originating from *S. spegazzinii*. The aim was to describe the inheritance of this quantitative resistance and to map involved QTLs on the linkage map. Besides the direct interest for the breeding programme run by our group, the work contributes new information on genetic factors involved in resistance against this very important pest.

Materials and methods

Plant material

The diploid (2n = 2x = 24) progeny 96D.32 (Rosa H1 ♀ * spg 88S.334.19 ♂) was studied. The female parent was a susceptible dihaploid of *S. tuberosum* cv Rosa. It developed nearly as many cysts as the susceptible control cv Désirée in other tests (data not shown). The male parent was a clone of *S. spegazzinii* PI 208876 of the Sturgeon Bay collection. It was resistant to *G. pallida* and did not develop any cysts in previous tests (Rousselle-Bourgeois and Mugniéry 1995). It is referred to as spg 334.19, hereafter. Three triploid individuals of the progeny 96D.32 were discarded after ploidy control by flow cytometry. Clones were maintained through tubers in pots in the open air. The progeny consisted of 117 individuals.

Resistance assessment

Four tubers per clone were planted each in a plastic pot filled with 400 cm^3 of a mixture of soil, sand and compost. Ten cysts of *G. pallida* were added into the pots in order to achieve an infestation of 5–10 juveniles per gram of soil. Pathotype Pa2/3 (Chavornay population) was used. This population is one of the most aggressive (Mugniéry and Balandras 1987). It has been chosen for official registration tests in Europe (Mugniéry et al. 1989) and for screening by private breeders. Results can then be directly applied in practical breeding schemes.

The susceptible control was cv Désirée. Plants were grown in the greenhouse for 4 months. Cyst number was assessed after the substrate was analysed with Kort's elutriator and cysts extracted by centrifugation. Raw data was transformed as log10 (cyst number +1). Data were then submitted to analysis of variance (Proc GLM, SAS Institute 1989) and to normality assessment (Proc UNIVARIATE, SAS Institute 1989). Broad-sense heritability was estimated with the following formula $h^2_b = \sigma^2_g/(\sigma^2_g + \sigma^2_e/n)$, where σ^2_g = genetic variance, σ^2_e = error variance and n = number of replicates.

DNA preparation

DNA was extracted from 10 g of fresh leaves of 2-month-old plants. The method was adapted from Bernatzky and Tanskley (1986) and Lefebvre et al. (1993).

RFLP analysis

Genomic DNA was digested using the restriction enzymes *Dra*I, *Eco*RI, *Eco*RV, *Hind*III and *Xba*I (Invitrogen). Restriction fragments were separated on 1% agarose gels and then blotted on a Hybond N+ membrane (Amersham Biosciences). DNA probes were labelled with α^{32}P-dCTP either by the random primer hexamer method (Feinberg and Vogelstein 1984) or by the Polymerase Chain Reaction (Saiki et al. 1988). Hybridisation and washing of the membranes were performed according to Lefebvre et al. (1993). X-Ray films (Kodak X-OMAT LS or BIOMAX MS) were exposed for 1–15 days at –80 °C with an intensifying screen (DuPont Lighning Plus or Kodak BioMax). Autoradiograms were scored visually by two persons, independently. Alleles from each parent were scored separately and the expected segregation ratio at a locus was thus 1:1 for each parent.

Tomato genomic DNA clones (TG) or cDNA clones (CD and CT) were provided by Dr. S.D. Tanksley, Cornell University, Ithaca, N.Y., USA. Potato genomic DNA clones (GP) and cDNA clones (CP) were provided by Dr. C. Gebhardt, Max Plank Institute, Cologne, Germany. Their location has been published in Tanksley et al. (1992) and Gebhardt et al. (1989, 1991), respectively. One clone (hom*Rx*) was obtained by PCR on spg 334.19 DNA with the 77L primers which amplify a DNA sequence physically linked to the resistance gene *Rx* (Kanyuka et al. 1999).

AFLP assays

The AFLP method was performed as described in Vos et al. (1995). We used the restriction enzymes *Sac*I (C), as a rare cutter, and *Mse*I (M), as a frequent cutter. Three primer combinations were used: C+AAG/M+CAA, C+ATA/M+CAA and C+ATA/M+CTT. Autoradiograms were scored visually by two persons, independently. Markers heterozygous for only one of the parents were considered (expected segregation ratio 1:1). Sizes of amplification products were not determined, so markers were designated by the primer combination followed by their serial number on the film.

Linkage-map construction

An independent map was constructed for each parent following the double pseudo-test-cross design (Grattapaglia and Sederoff 1994). Deviation from the 1:1 ratio was tested with the χ^2 goodness-of-fit test with a significance level of 1%. Software MapMaker/Exp 3.0 was employed (Lincoln et al. 1992). Linkage groups were determined with a minimum LOD of 5 and a maximum recombination rate of 0.3.

The most-likely marker order in each linkage group was determined with "compare" and "try" commands of MapMaker. They were controlled with the "ripple" command (LOD = 2). Recombination frequencies were converted into mapping distances (cM) using Kosambi's (1944) function.

QTL analysis

Software QTL Cartographer 1.12 was used (Basten et al. 1997) with interval mapping (Zmapqtl, model 3). The threshold for QTL detection was a LOD score of 3.0 confirmed after the permutation test (data not shown). The coefficient of determination (R^2) describes the percentage of the total variation explained by the QTL, and is computed at the maximum of the LOD score plot. Confidence interval was assessed from the LOD curve with limits

where the LOD decreased by 1.5 units from the maximum on the curve.

Results

Linkage maps

Two hundred and seventy RFLP probes (247 tomato probes, 22 potato probes and hom*Rx*) were hybridised on DNA of the parents after digestion with five enzymes. A choice was done depending on the quality of hybridisation, the assumed heterozygosity and the probability of genome coverage according to the Tanksley et al. (1992) tomato map. Finally, 76 RFLP probes were chosen to construct the maps. They were hybridised on DNA of the whole progeny. They detected 89 loci, 34 of which were common to both parents. Three AFLP primer combinations were added to fill possible gaps between RFLP loci. They produced 98 readable markers. As a whole, about 25% of the markers had a segregation significantly different from the expected 1:1 ratio ($P<0.01$).

The map of female parent Rosa H1 included 65 RFLP and 70 AFLP loci. It was constituted by 16 linkage groups covering a total length of 594 cM. All the groups were assigned to one chromosome or arm of one chromosome. The map of male parent spg 334.19 included 57 RFLP and 28 AFLP loci. It was constituted by 12 linkage groups covering a total length of 596 cM. Each group corresponded to a chromosome. Taking the tomato map as a reference (Tanksley et al. 1992), the evaluation of the percentage of genome saturation gave an average ratio of 70% for Rosa H1 (40% for the less-covered chromosome to 95% for the best-covered) and 80% for spg 334.19 (60% to 95%).

Resistance assessment

Cyst counts on individual plants of 96D.32 offspring ranged from 0 to 502. The average number of cysts per genotype was 0 to 345. The average number on the tetraploid control cv Désirée was 621 cysts. Figure 1a shows distribution of the 117 clones for the log (cyst number +1) classes. This trait showed a continuous distribution indicating its quantitative nature. The genetic variance was highly significant ($P=0.0001$). The heritability of the genotypic means was estimated to be 0.97. The estimation of the genotypic values by the phenotypic values was thus highly reliable. Based on the Shapiro and Wilk test, residual values for the transformed data showed a normal distribution ($Pr<W=0.40$).

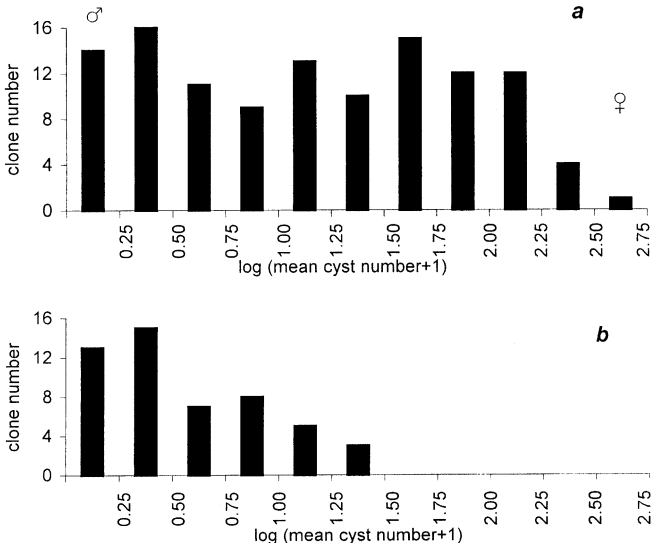

Fig. 1a, b Frequency distribution of the *G. pallida* Pa2/3 resistance score expressed by log (mean cyst number +1) in the offspring 96D.32. **a** On the 117 clones of the whole offspring. The symbols ♂ and ♀ indicate the class to which spg 334.19 and Rosa H1 belong. **b** On the 51 clones with the allele of spg 334.19 for marker CT242 at QTL *GpaM1*

QTL mapping

No QTL was detected on the Rosa H1 map. Three QTLs were detected by interval mapping on chromosomes V, VI and XII of the spg 334.19 map. They were provisionally named *GpaM1*, *GpaM2* and *GpaM3*, respectively (*Gpa* for *G. pallida* and *M* for Montfavet). Table 1 gives their main characteristics and Fig. 2 illustrates their position.

According to a three-factor ANOVA, the three QTLs explained about 72% of the total variation altogether. *GpaM1* explained more than 50% of the total variation and is located within an interval of 4.3 cM around CT242. The genotypes with this QTL had 21 cysts as a maximum average resistance score [log (x+1) = 1.34] (Fig. 1b). Effects of the other two QTLs were 19% and 15% each, and their confidence intervals were 31 and 48 cM. Linked markers were TG365 and TG581 for *GpaM2*, and hom*Rx*, CT079, CT019_A, TG618, CD064_B, C+ATA/M+CAA_03, CT080_B for *GpaM3*. The large confidence interval for *GpaM3* may be due to skewed segregation

Table 1 Characteristics of the three QTLs of resistance against *G. pallida* Pa2/3 detected in spg 334.19 by interval mapping

QTL	Chromosome	Linked marker(s)	Maximum LOD score	R^2 (%)	Confidence interval (cM)
GpaM1	V	GP021-TG569	20.9	56	4.3
GpaM2	VI	TG365-TG581	3.7	19	31
GpaM3	XII	CT019_A-CT080_B	3.5	15	48

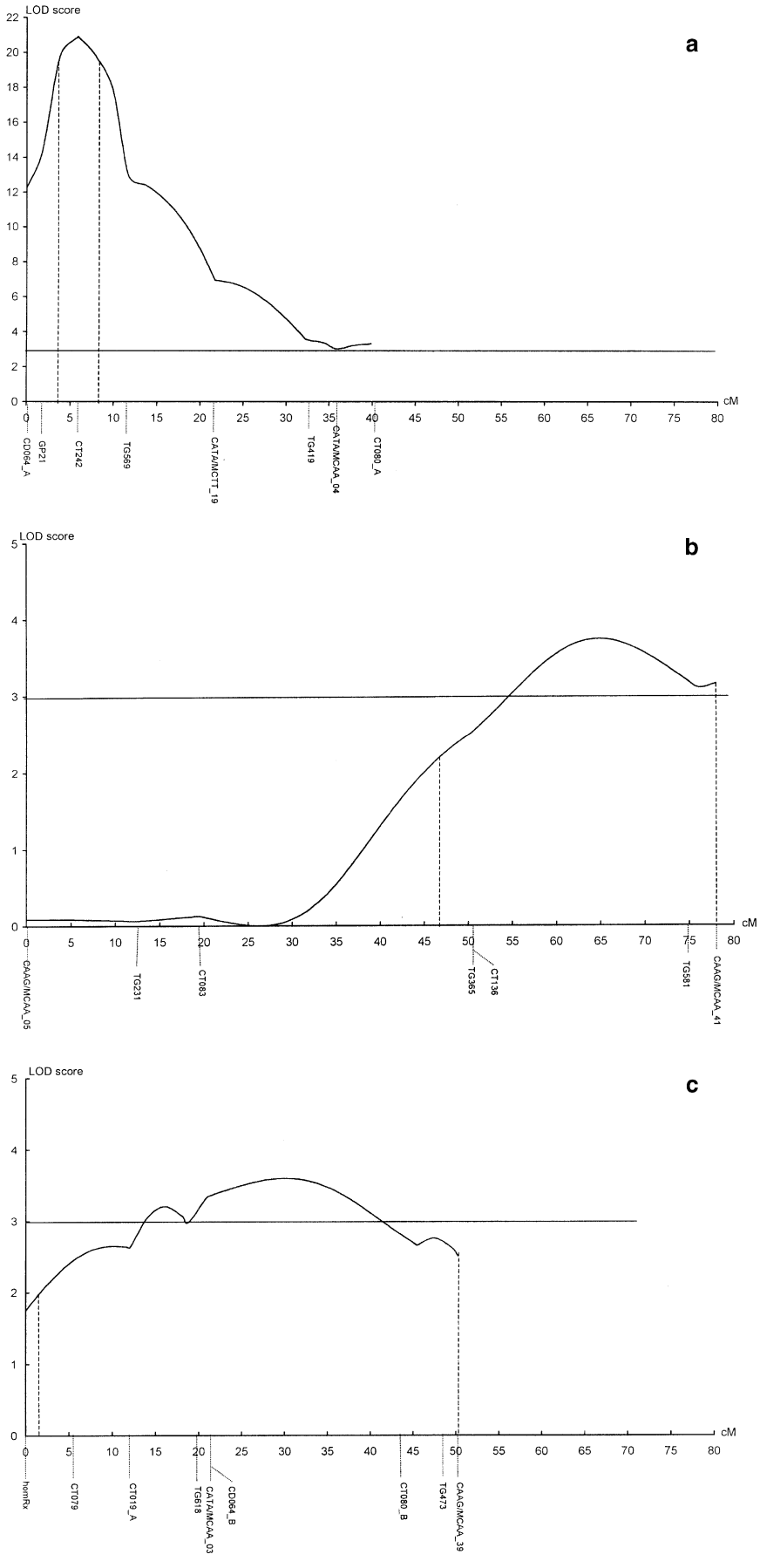

Fig. 2a–c LOD score plots for the *G. pallida* Pa2/3 resistance score for the three QTLs detected by interval mapping on the spg 334.19 map. The *solid line* shows the threshold at LOD = 3. *Dotted lines* are limits of the confidence interval. Markers are given on the bottom. **a** *GpaM1* on chromosome V. **b** *GpaM2* on chromosome VI. **c** *GpaM3* on chromosome XII

ratios in the concerned area of chromosome XII and the interval size between CD064_B and CT080_B.

Discussion

Potato (*S. tuberosum*) is known to be a highly polymorph species (Gebhardt et al. 1989). However, the male parent *S. spegazzinii* spg 334.19 appeared less heterozygous than Rosa H1: less RFLP probes giving two bands, less loci segregating in the progeny. Spg 334.19 was probably obtained from the original accession after an unknown number of selfing generations which can explain its relative homozygosity. Similar results were found by Kreike et al. (1993). On the other hand, di-haploid Rosa H1 is assumed to be highly heterozygous as it was able to be maintained over many years and as it had a good female fertility.

The resistant clone spg 334.19 issued from *S. spegazzinii* PI 208876 which originated from the same donor accession as BGRC 8218 (CGN 2000). This latter accession was evaluated by Dellaert et al. (1988) for resistance against *G. pallida* Pa3 (now considered as Pa2/3) and two clones were studied for inheritance of this trait. Two or three major genes were assumed to be involved and the presence of minor genes was predicted. One of these clones (8218-15) was used by Kreike et al. (1994) to map resistance factors. Characteristics of their progeny were similar to ours: heritability of genotypic mean values for resistance was estimated to be 0.88–0.89 and the range for the number of cysts from 1 to 300 per individual. They found one major locus on chromosome V, *Gpa*, which explained about 50% of the total variation of resistance. They used no more than 29 RFLP markers. This could be the reason why they did not find other QTLs. On our more dense map, we found three QTLs which could correspond to the three major genes mentioned by Dellaert et al. (1988). The clone from *S. spegazzinii* that we used is not the same as 8218-15, despite the fact they both come from the same accession. Both genetic structures may be slightly different because of recombination during the production of true seeds. Figure 3 shows the estimated relative position of markers and QTLs on chromosome V for 8218-15 and spg 334.19, compared with the core map drawn by Jacobs (1995). One flanking marker (Ssp37) of *Gpa* is in the interval above GP21 and the other (Ssp72) is between GP21 and Ssp88. Two markers linked to *GpaM1* (CD064 and TG569) are in the interval from the top of the linkage group to TG379, and GP21 is a common marker. Intervals of both QTLs overlap. It is thus obvious that *GpaM1* and *Gpa* are in the same region of the genome. They are likely to be the same gene but further studies are required to resolve this question.

As frequently observed (Grube et al. 2000), the three QTLs of spg 334.19 are located in resistance gene clusters. *GpaM1* on the short arm of chromosome V is in a region of the potato genome where several genetic factors for resistance have been detected: *Rx2* (Ritter et al. 1991) and *Nb* (De Jong et al. 1997) against Potato Virus X; *R1* (Leonards-Schippers et al. 1992), and QTLs against *Phytophthora infestans* (Leonards-Schippers et al. 1994; Collins et al. 1999; Oberhagemann et al. 1999); *Gpa* (Kreike et al. 1994), *Grp1* (Rouppe van der Voort et al. 1998), *Gpa5* (Rouppe van der Voort et al. 2000) and a not yet named QTL described by Bryan et al. (2002) which are four QTLs against cyst nematodes. Two major genes of resistance against *G. rostochiensis* have been mapped to the other arm of chromosome V: *H1* (Gebhardt et al. 1993; Pineda et al. 1993) and *GroV1* (Jacobs et al. 1996). *GpaM2* on the long arm of chromosome VI is in a region where QTLs for resistance against *P. infestans* have been detected (Leonards-Schippers et al. 1994; Collins et al. 1999; Oberhagemann et al. 1999). No other gene of resistance against PCN has been found in this region. *GpaM3* is on the short arm of chromosome XII. This part of the genome of potato and its orthologous regions in tomato and pepper contain several genes for resistance against nematodes and other pathogens: *Gpa2* against *G. pallida* (Rouppe van der Voort 1997), *Rx* against Potato Virus X (Ritter et al. 1991), QTL for resistance against *P. infestans* (Leonards-Schippers et al. 1994) in potato; *Mi-3* (Yaghoobi et al. 1995) and probably *Me3* (Djian-Caporalino et al. 2001) against root knot nematodes in tomato and pepper, respectively.

In conclusion, resistance against *G. pallida* originating from *S. spegazzinii* is known to be quantitatively inherited and indeed three QTLs were determined in our resistant progenitor. Identity with other published genes or QTLs has to be assessed. The effect of each QTL on the resistance mechanism is being analysed. The most relevant markers for each QTL are also being transformed into PCR markers to be used in a marker-assisted selection scheme of our diploid and tetraploid material derived from *S. spegazzinii*. On a more general point of

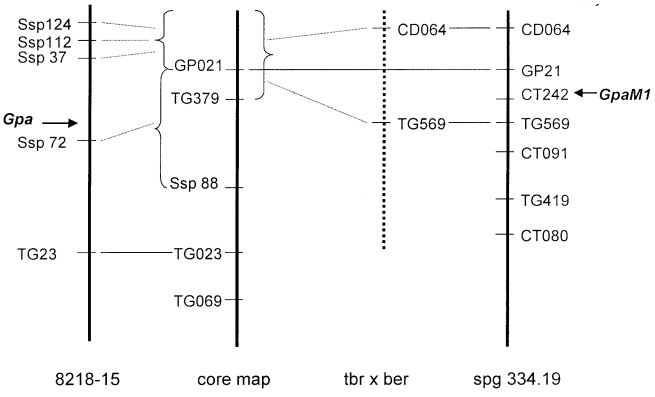

Fig. 3 Estimated relative position on chromosome V of linked markers, and *Gpa* and *GpaM1* from resistant genitors 8218-15 and spg 334.19, compared with the core map drawn by Jacobs (1995). From left to right: map of 8218-15 (Kreike et al. 1994), core map (Jacobs 1995), map of hybrid (*S. tuberosum* × *S. berthaultii*) (Tanksley et al. 1992), map of spg 334.19. *Dotted lines* join markers to intervals on the core map (Jacobs 1995), *solid lines* join common markers. Orders of markers are the same as in the original maps but distances are not proportional

view, the work presented in this paper adds new information to the list of known genetic factors for resistance against PCN, which is an important concern for potato breeders. This will contribute to studies on resistance genes and QTLs such as synteny and evolution, the activity spectrum against different pathotypes and the effect of association on more durable resistance.

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