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Quantitatively-inherited resistance to *Globodera pallida* is dominated by one major locus in *Solanum spegazzinii*

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Abstract A high level of resistance to *Globodera pallida* pathotypes Pa2 and Pa3 exists in *Solanum spegazzinii*, a wild relative of potato (*S. tuberosum* ssp. *tuberosum*). Here we report the mapping of loci involved in quantitatively-inherited nematode resistance with the use of RFLPs. One major locus, *Gpa*, was mapped on chromosome 5 and two minor loci on chromosomes 4 and 7 of *S. spegazzinii*. Additionally, the contribution of the susceptible parent to nematode resistance was determined. The *Gpa* locus was solely responsible for the high resistance level found in the segregating population. However, the RFLP marker closely linked to this resistance locus showed a distorted segregation, with a shortage of plants having the resistance linked allele. Our results indicate that a prediction of the genetic constitution of a quantitative trait based solely on phenotypic observations can lead to erroneous conclusions.

Key words Potato cyst-nematode · Quantitative trait loci RFLP · Distorted segregation · *Solanum spegazzinii*

Introduction

The potato cyst-nematode *Globodera pallida* (Jones et al. 1970; Stone 1972) can cause severe damage to the potato crop. Three pathotypes of *G. pallida*, Pa1 to Pa3, can be distinguished with a set of differentials which are derived from backcrosses of *Solanum tuberosum* with *S. multidissectum* and with *S. vernei* (Kort et al. 1977). Most cultivated potato varieties (*S. tuberosum* ssp. *tuberosum*) do not posses good resistance to this nematode but resistance against *G. pallida* has been found in wild *Solanum* species. *S. multidissectum* shows resistance to pathotype Pa1 due to the presence of one major gene, H2 (Dunnet 1962). A gene-for-gene relationship between this resistance gene and a *G. pallida* avirulence gene has been described by Parrott (1981). Resistance to pathotypes Pa2 and Pa3 was found in *S. tuberosum* ssp. *andigena* CPC 2802. This resistance was first thought to be monogenic (Howard et al. 1970), but was later proven to be polygenic (Dale and Phillips 1982). Another source of resistance to *G. pallida* is *S. vernei*, which has a quantitative, presumably polygenic, resistance (Ross 1986).

Recently, Arntzen et al. (1993) described a qualitative and monogenic resistance to Pa2 population D236. This resistance was derived from *S. tuberosum* ssp. *andigena* CPC1673. So far, this locus only gives resistance to one population of pathotype Pa2, namely D236, and not to other Pa2 or Pa3 populations tested (Arntzen et al. 1992).

New sources with a broad spectrum resistance to *G. pallida* as well as *G. rostochiensis* have been searched for in other wild *Solanum* species (Van Soest et al. 1983; Dellaert and Hoekstra 1987; Jackson et al. 1988; Turner 1989). Most resistances of these wild species showed a quantitative inheritance, suggesting the action of several genes (Dellaert et al. 1988). *S. spegazzinii* 8218–15 was one of the resistant accessions that had been studied in detail. Dellaert et al. (1988) assumed that the resistance to *G. pallida* pathotype Pa3 was dominated by two to three major genes, each with incomplete resistance and in addition they predicted the presence of minor genes.

Quantitative traits can be dissected into discrete genetic factors with the use of detailed RFLP linkage maps; all regions of the genome can be assayed and accurate estimates of phenotypic effects and genetic positions can be derived (Paterson et al. 1988). In the present paper we describe the identification and mapping of loci, involved in resistance to *G. pallida* pathotypes Pa2 and Pa3, derived from *S. spegazzinii* 8218–15. The aim of this study was to examine

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the assumptions about the genetic basis of resistance made by Dellaert et al. (1988). In addition we investigated the possibility of the presence of QTLs, contributing to the nematode resistance, from the susceptible parent *S. tuberosum*.

Materials and methods

Plant material

S. spegazzinii BGRC accession 8218 seedling number 15 was used as pollinator in a cross with the diploid susceptible *S. tuberosum* SH 78–88–1320. The F1 progeny were tested for resistance to *G. pallida* pathotypes Pa2 and Pa3 (see below). The *S. spegazzinii* parent appeared to be heterozygous at the resistance loci because resistance to pathotypes Pa2 and Pa3 was segregating in the F1 progeny. Mapping of the loci involved in resistance with RFLPs was, therefore, performed in this F1 population.

RFLP analysis

The RFLP markers that were used in this study and the RFLP analysis have both been described earlier by Kreike et al. (1993).

Resistance test

The resistance test for pathotype Pa2 was done in 1988 on 96 F1 plants (total F1 progeny) with nematode population P2-22 (Arntzen and Van Eeuwijk 1992) and for pathotype Pa3 in 1987 (44 plants) and 1989 (39 plants) with nematode population Coll. 1077 (Arntzen and Van Eeuwijk 1992). The F1 population was also tested for resistance against *G. rostochiensis* pathotype Ro1, which is described by Kreike et al. (1993). The resistance tests were performed in five replications in a randomized block design. Thirty cysts enclosed in a nylon net were used as inoculum. After 5 months the newly-formed cysts were collected and counted. A more detailed description of the resistance test is given by Kreike et al. (1993). The differentials $VT(N)^2$62–33–3 and AM78-3778 were used to discriminate between the *G. pallida* pathotypes Pa2 and Pa3 used in this study.

Statistical analysis

A normalizing transformation [^{10}log(x+1)] was performed on the counts of the newly-formed cysts per replicate. These transformed data of the resistance test were investigated with analysis of variance (ANOVA) for a randomized block design. From this ANOVA the heritability of the genotypic mean values for each pathotype was calculated as described by Kreike et al. (1993). The association of marker genotypes with resistance was assessed with one-way ANOVAs based on the averages over the five replicates of the log-transformed cyst counts. In these ANOVAs the data were classified with the alleles of each parent separately i.e., for each marker having two *S. spegazzinii* and two *S. tuberosum* alleles two separate ANOVAs were performed. A significance level of $P< 0.05$ was employed. The magnitude of the marker-associated phenotypic effect is described by the coefficient of determination (R^2), which represents the fraction of the total variance accounted for by the marker genotypes. Since the ANOVA assumes a normal distribution of the data within the classes, and this assumption might be violated, the ANOVA results were verified with a nonparametric test (Mann-Witney U-test).

An RFLP linkage map of chromosome 5 was constructed with the computer program JoinMap (Stam 1993). Interval mapping, as described by Lander and Botstein (1989), could be performed for chromosome 5 for the alleles from the *S. spegazzinii* parent, by treating the F1 as a first generation backcross progeny with fully homozygous genotypes. This analysis was done using the computer program MapQTL, written by Van Ooijen. Readers who are interested in receiving MapQTL should contact Van Ooijen fur further details [E-mail (internet): j.w.van.ooijen@cpro.agro.nl].

Results and discussion

Resistance test

The genetic variance in the F1 for both Pa2 and Pa3 resistance was very significant (for both $P< 0.0001$), which means that the different levels of Pa2 and Pa3 resistance found in the F1 population had a genetic basis. The heritability of the genotypic mean values for resistance to *G. pallida* was very high, 0.88 for Pa2 and 0.89 for Pa3 (both 1987 and 1989 experiments). A high correlation (r=0.65) was detected between Pa2 and Pa3 resistance. This indicated either genes with pleiotropic effects, or closely linked genes. No significant correlations (Pa2-Ro1: r=0.17 and Pa3-Ro1: r=0.10) were detected between resistances to *G. pallida* and *G. rostochiensis* (h^2=0.63) (Kreike et. al., 1993) and therefore different, unlinked loci are assumed to be involved in resistance for the two nematode species.

Figure 1 shows the frequency distribution of Pa2 and Pa3 resistance in the F1 population. A continuous range of cyst numbers was found in this population for both pathotypes. Hence, no qualitative inheritance can be detected. The average newly formed number of cysts for the parents, *S. spegazzinii* and *S. tuberosum*, were 1 and 300, respectively. The differential $VT(N)^2$ 62–33–3 was susceptible for Pa3 (on average 128 cysts) while AM78-3778 was resistant for Pa3 (on average 1,2 cysts) but susceptible for Pa2 (on average 63 cysts)

RFLP analysis

One hundred and seven RFLP markers were tested for heterozygosity within the parental genotypes using four restriction enzymes. This search yielded 29 RFLP markers heterozygous for *S. spegazzinii* and 43 RFLP markers for *S. tuberosum*. Table 1 shows the number of heterozygous markers per chromosome for each parent. The segregation of these markers was determined in the F1 population and

Table 1 Number of polymorphic markers used per chromosome to detect association with nematode resistance from *S. spegazzinii* or *S. tuberosum*

Chromosome	*S. spegazzinii*	*S. tuberosum*
1	1	8
2	1	5
3	2	2
4	2	1
5	5	4
6	2	0
7	4	3
8	0	3
9	2	5
10	2	3
11	2	2
12	5	4
Not mapped	1	4
Total	29	43

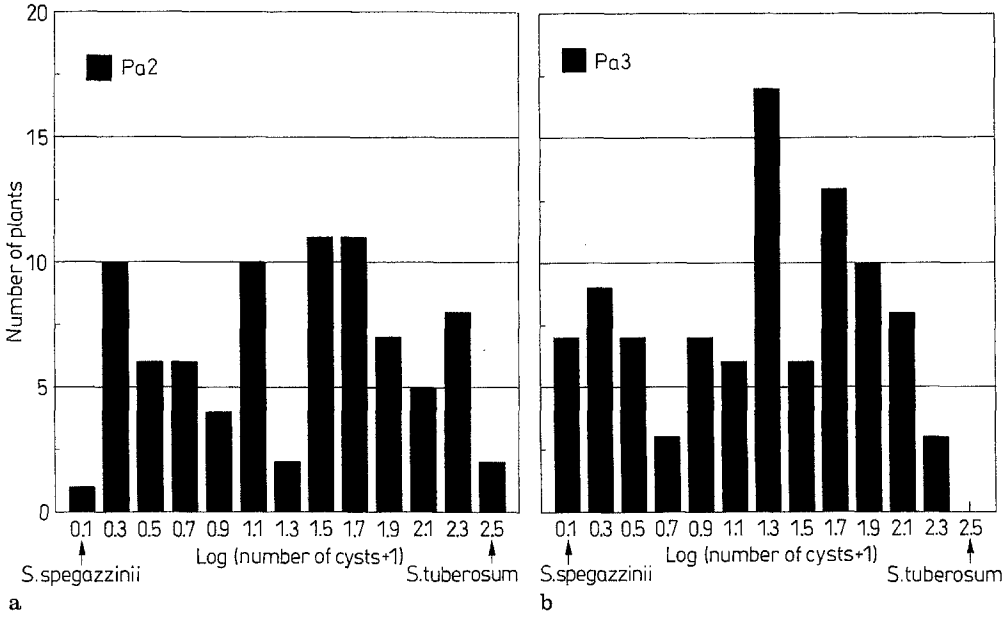

Fig. 1 Frequency distribution of the mean number of cysts found on the F1 plants with Pa2 (**a**) and Pa3 (**b**) resistance tests. The data of the resistance tests were transformed [^{10}log(x+1)]

Table 2 Association between RFLP markers and nematode resistance after ANOVA and explained variance per RFLP marker in % of the total phenotypic variance of clone means. *P*-values that are given in parenthesis are considered not significant. Abbreviations: Chr., chromosome; Segr., segregation; Expl. var., explained variance

Marker	Chr.	Segr. ratio	*P* value Pa2	*P* value Pa3	Expl. var. Pa2	Expl. var. Pa3
S. spegazzinii						
Ssp27	4	47:40	(0.50)	0.03	–	5
Ssp124	5	29:22	0.005	0.02	14	9
Ssp112	5	37:30	0.0008	0.002	15	13
Ssp37	5	49:41	0.0001	0.0001	27	22
Ssp72	5	56:24[a]	0.0001	0.0001	51	45
TG143	7	48:33	0.03	0.02	5	7
Ssp51	7	37:33	0.03	0.007	6	9
S. tuberosum						
Ssp56	1	10:32[a]	0.03	(0.11)	9	–
TG130	3	21:33	(0.06)	0.03	–	7
Ssp37	5	34:42	(0.09)	0.03	–	5
Ssp75	11	17:19	(0.22)	0.05	–	9
TG68	12	17:20	0.03	(0.27)	10	–
Total[b]					76	80

[a] Distorted segregation
[b] The highest explained variances for each chromosome are added up to give the total explained variance

used for linkage analysis, which is described by Kreike et al. (1993).

Mapping of the resistance loci

The association of marker genotypes with nematode resistance was assessed with one-way ANOVAs, of which the results are shown in Table 2. Significant associations ($P< 0.05$) indicated that the RFLP marker was linked to a resistance locus. As a verification, associations were also determined with a nonparametric test and these confirmed the results found with the one-way ANOVAs.

The RFLP markers, Ssp124, Ssp112, Ssp37 and Ssp72, with the highest significance for association with nematode resistance were all located on chromosome 5 (Table 2) and showed association with *G. pallida* pathotype Pa2, as well as pathotype Pa3, resistance. Also two markers on chromosome 7, TG143 and Ssp51, showed association with resistance to both pathotypes. On chromosome 4, however, marker Ssp27 was associated only with resistance to *G. pallida* pathotype Pa3.

In order to obtain an estimate of the map position of the resistance locus, interval mapping (Lander and Botstein 1989) was performed for chromosome 5 in which an RFLP map could be constructed. The F1 population was treated as a first generation backcross population by using the two *S. spegazzini* alleles for each marker only. In this way only the segregation of resistance loci heterozygous in *S. spegazzinii* is studied. The results from the interval mapping on chromosome 5 are presented in Fig. 2 a. The threshold was set at LOD 3.0 according to Van Ooijen (1992). These

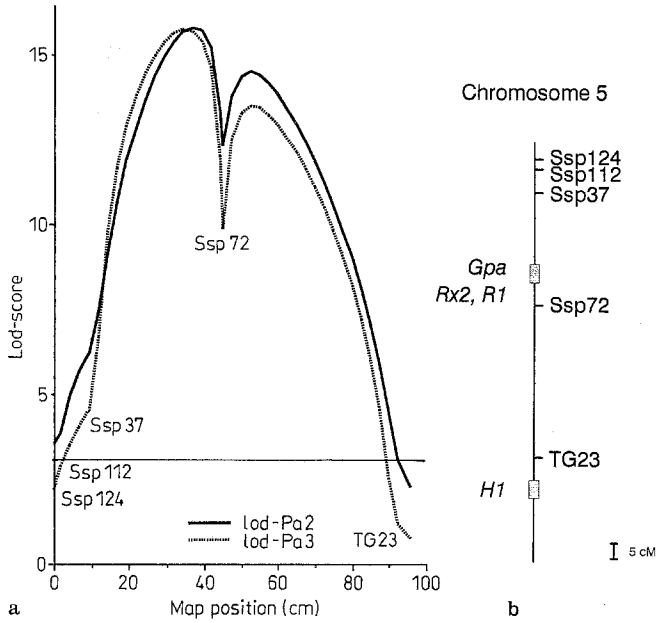

Fig. 2 a LOD scores for *G. pallida* pathotype Pa2 and Pa3 resistance on chromosome 5 of *S. spegazzinii*. **b** Position of the *Gpa* locus on chromosome 5 and the location of other resistance loci on this chromosome (Ritter et al. 1991; Leonards-Schippers et al. 1992; Gebhardt et al. 1993; Pineda et al. 1993)

results show that there is most probably one major resistance locus active against both Pa2 and Pa3, which is located between the markers Ssp37 and Ssp72, approximately 10 cM from Ssp72. We propose to name the major resistance locus on chromosome 5, *Gpa*, since it is involved in resistance to *G. pallida* pathotype Pa2 as well as Pa3 (Fig. 2b).

The existence of loci, heterozygous for alleles involved in nematode resistance, in the susceptible parent *S. tuberosum*, was also determined. Indications for the presence of QTLs were found on chromosomes 1, 3, 5, 11 and 12 (Table 2). Since the segregation of the corresponding RFLP alleles was determined on a limited number of plants only, these associations have to be seen as mere indications for the presence of loci contributing to nematode resistance. The existence of these loci, in the susceptible *S. tuberosum* ssp. *tuberosum* has been described previously. Dale and Phillips (1985) found variation in nematode resistance level within *S. tuberosum* ssp. *tuberosum* genotypes, ranging from fully susceptible to fully resistant.

It is possible that the *Gpa* locus does not consists of one locus that is responsible for resistance against both pathotypes but consists of two closely-linked loci, each conferring resistance to an individual pathotype. Four F1plants were found which were resistant [log (x+1)< 1.0] to one pathotype only and susceptible [log (x+1)>1.0] to the other (Table 3). The presence of the *Gpa* locus can be determined with the RFLP markers Ssp72 and Ssp37, which surround the resistance locus. For two plants the presence of this locus cannot be determined because the RFLP pattern of Ssp72, which is closely linked to the locus, is not known. Based on the RFLP markers, plant SP1 did not possess the

Table 3. Mean cyst numbers of F1 plants which are resistant to only one pathotype and susceptible to the other, and the RFLP data for markers Ssp72 and Ssp37 on chromosome 5. In brackets the mean log (x + 1) transformed cyst numbers are given with the standard error. 1, presence of resistance linked RFLP allele; 0, absence; nd, not determined

Plant	Cysts Pa2	Cysts Pa3	Ssp72	Ssp37
SP17	101.4 (2.01+0.19)	9.0 (1.00+0.19)	nd	0
V28	100.2 (2.01+0.21)	3.5 (0.65+0.21)	nd	1
SP1	5.6 (0.82+0.19)	85.3 (1.94+0.19)	0	0
V223	4.9 (0.77+0.21)	42.9 (1.61+0.21)	1	0

Gpa locus, therefore the high-resistance level to pathotype Pa2 could be caused by the action of several minor loci. Based on marker Ssp72, plant V223 most likely possessed the *Gpa* locus, but has a recombination between marker Ssp72 and Ssp37, for which it has the susceptibility-linked allele. The recombination event could have taken place at the *Gpa* locus, separating the Pa2 and the Pa3 pathotype-specific resistance loci.

A large part of the total phenotypic variance could be explained with the associated RFLP markers (Table 2). Marker Ssp72, which is linked to a major locus, explains 51% and 45% of the total variance of Pa2 and Pa3 resistance, respectively. The other markers on chromosome 5 all showed linkage with the same major resistance locus. The markers on chromosomes 4 and 7 from *S. spegazzinii* and on chromosomes 1, 3, 5, 11 and 12 from *S. tuberosum* explained 10% or less of the total variance and are therefore considered to be minor loci. Because we were not able to employ more segregating markers, we could not investigate whether the low explained variance of some markers was due to either a large distance between marker and resistance locus, or to a small genotypic effect of a closely-linked locus. On the other hand, adding up the explained variance of all significant and unlinked markers leads to a total of 76% and 80% of the explained variance for Pa2 and Pa3 respectively (Table 2). And because the heritability for Pa2 and Pa3 resistance was 0.88 and 0.90, respectively, it seems that most of the genetic variantion can be explained with these markers. Hence, we do not expect much larger genotypic effects of the loci linked to these markers.

Classical segregation analysis versus Genetic analysis with markers

Based on the research of Dellaert et al. (1988) we expected to find two to three major loci which are involved in resistance to *G. pallida*. However only one major locus was detected. There are several reasons for this discrepancy. First, Dellaert et al. performed segregation analysis on 44 plants only which is the population tested against Pa3 in 1987

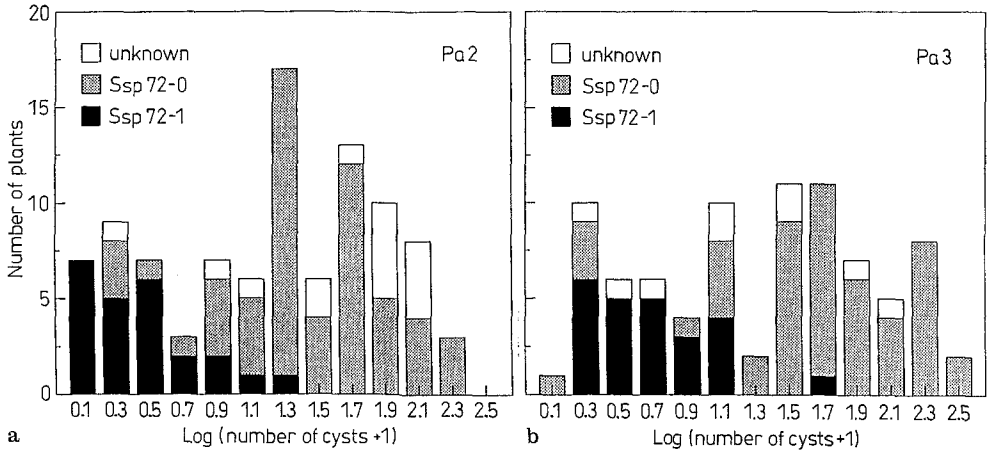

Fig. 3 Presence of the alleles of marker Ssp72, which is closely linked to the *Gpa* locus, in the frequency distribution of Pa2 (**a**) and Pa3 (**b**) resistance in the F1 population

also used in the present paper (see Materials and methods). A population size of only 44 plants is relatively small for segregation analysis on a quantitative trait such as the present nematode resistance. Secondly, segregation analysis of a continuously-distributed trait relies heavily on the choice of the threshold for genotype classification (e.g., resistant or susceptible). Genetic analysis based on (molecular) markers circumvents the choice of genotype classification. Thirdly, classical segregation analysis assumes segregation to be according to the Mendalian ratios. The estimation of the number of genes is based on the best fitting segregation ratio, given the chosen-genotype classification and genetic model. Minor deviations are often explained by adjusting the genetic model, for instance, by assuming incomplete dominance or linkage. When, however, genetic analysis with markers is performed, the realised segregation can be determined, and possible distortion can even be tested.

We found that the *Gpa* locus is situated in a region where segregation is distorted. Marker Ssp72, which is closely linked to the resistance locus, showed a skewed segregation with a shortage of the resistance-linked allele (Table 2, Fig. 3). The choice of the genotype classification threshold based on Fig. 1 is quite arbitrary; thus, our results demonstrate that classical segregation analysis can easily lead to erroneous conclusions.

Distorted segregation in this region of potato chromosome 5 has been observed previously (Ritter et al. 1991). Resistance to PVX also showed a segregation ratio that deviated significantly from 1:1 in the F1 progeny. Additionally in this case a marker was found on chromosome 5 which cosegregated with the resistance trait. Several mechanisms that cause distorted segregation are known, such as incompatibility, male /female gamete selection, or even sporophytic selection. The precise mechanism causing this disturbance at chromosome 5 remains to be elucidated.

Other resistance loci on chromosome 5

The *Gpa* locus on chromosome 5 is yet another nematode resistance locus on this chromosome (Fig. 2 b). The H1 gene from *S. tuberosum* ssp. *andigena* CPC 1673 which conferred resistance to *G. rostochiensis* pathotype Ro1 was also mapped on chromosome 5, near TG23 (Gebhardt et al. 1993; Pineda et al. 1993). Furthermore the locus determining resistance to the D236 population of *G. pallida* pathotype Pa2 is probably located on chromosome 5 too, because Arntzen et al. (1993) assumed linkage with the H1 locus. Resistance loci against other pathogens of potato have also been mapped on this chromosome, e.g., the *Rx*2 locus against PVX (Ritter et al. 1991), the *R*1 locus against *Phytophthora infestans* (Leonards-Schippers et al. 1992), and trichome-mediated insect resistance (Bonierbale et al. 1992)(Fig 2 b). The location of the latter could not be shown in Fig. 2 b, because other markers were used to map this resistance, but its location is most likely in the same region as the *Gpa* locus.

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References

Arntzen FK, Van Eeuwijk FA (1992) Variation in resistance level of potato genotype and virulence level of potato cyst nematode populations. Euphytica 62: 135–143

Arntzen FK, Vinke JH, Hoogendoorn J (1993) Inheritance, level and origin of resistance to *Globodera pallida* in the potato cultivar Multa, derived from *Solanum tuberosum* ssp. *andigena* CPC 1673. FundAppl Nematol 16: 155–162

Bonierbale MW, Plaisted R, Tanksley SD (1992) Genetic mapping and utilization of quantitative trichome-mediated insect resistance in potato. Neth J Pl Pathol Suppl 2: 211–214

Dale MFB, Phillips MS (1982) An investigation of resistance to the white potato cyst-nematode. J Agric Sci 99: 325–328

Dellaert LMW, Hoekstra R (1987) Resistance to potato cyst nematodes, *Globodera* spp., in wild and primitive *Solanum* species. Potato Res 30: 579–587

Dellaert LMW, Vinke H, Meyer K (1988) The inheritance of resistance to the potato cyst-nematode *Globodera pallida* Pa3 in wild Solanum species with broad spectrum resistance. Euphytica Suppl: 105–116

Dunnet JM (1962) Inheritance of resistance to potato root eelworm in a breeding line stemming from *Solanum multidissectum* Haw-

kes. In: Anonymous (eds), Ann Report Scottish Plant Breed Station 99161:39–46

Gebhardt C, Mugniery D, Ritter E, Salamini F, Bonnel E (1993) Identification of RFLP markers closely linked to the *H1* gene conferring resistance to *Globodera rostochiensis* in potato. Theor Appl Genet 85: 541–544

Howard HW, Cole CS, Fuller JM (1970) Further sources of resistance to *Heterodera rostochiensis* Woll. in the andigena potatoes. Euphytica 19: 210–216

Jackson MT, Hawkes JG, Male-Kayiwa BS, Wanyers NWM (1988) The importance of the Bolivian wild potato species in breeding for *Globodera pallida* resistance. Plant Breed 101: 261–268

Jones FGW, Carpenter JM, Parrott DM, Stone AR, Trudgill DL (1970) Potato cyst nematode: one species or two? Nature 227: 83–84

Kort J, Ross H, Rumpenhorst HJ, Stone AR (1977) An international scheme for identifying and classifying pathotypes of potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. Nematologica 23: 333–339

Kreike CM, de Koning JRA, Vinke JH, van Ooijen JW, Gebhardt C, Stiekema WJ (1993) Mapping of loci involved in quantitatively-inherited resistance to the potato cyst-nematode *Globodera rostochiensis* pathotype Ro1. Theor Appl Genet 87:464–470

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199

Leonards- Schippers C, Gieffen W, Salamini F, Gebhardt C (1992) The R1 gene conferring race-specific resistance to *Phytophthora infestans* on potato is located on chromosome V. Mol Gen Genet 233: 278–283

Parrott DM (1981) Evidence for a gene-for-gene relationship between resistance gene H1 from *Solanum tuberosum* ssp. *andigena* and a gene in *Globodera rostochiensis*, and between H2 from *S. multidissectum* and a gene in *G. pallida*. Nematologica 27: 372–384

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335: 721–726

Pineda O, MW Bonierbale, Plaisted RL, Brodie BB, Tanksley SD (1993). Identification of RFLP markers linked to the H1 gene conferring resistance to the potato cyst nematode *Globodera rostochiensis*. Genome 36: 152–156.

Ritter E, Debener T, Barone A, Salamini F, Gebhardt C (1991) RFLP mapping on potato chromosomes of two genes controlling extreme resistance to potato virus X (PVX). Mol Gen Genet 27: 81–85

Ross H (1986) Potato breeding – problems and perspectives. Advances in Plant Breeding 13

Stam P (1993) Construction of integrated linkage maps by means of a new computer package: JoinMap. Plant J 3: 739–744

Stone AR (1972) *Heterodera pallida* n sp. (Nematoda: Heteroderidae), a second species of potato cyst nematode. Nematologica 18: 591–606

Turner SJ (1989) New sources of resistance to potato cyst nematodes in the Commonwealth Potato Collection. Euphytica 42: 145–153

Van Soest LJM, Rumpenhorst HJ, Huijsman CA (1983) Resistance to potato cyst-nematodes in tuber-bearing *Solanum* species and its geographical distribution. Euphytica 32: 65–74

Van Ooijen JW (1992) Accuracy of mapping quantitative trait loci in autogamous species. Theor Appl Genet 84: 803–811