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C. M. Kreike · A. A. Kok-Westeneng · J. H. Vinke
W. J. Stiekema

Mapping of QTLs involved in nematode resistance, tuber yield and root development in *Solanum* sp.

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Abstract A backcross population, derived from the cross (*S. tuberosum*×*S. spegazzinii*)×*S. tuberosum* was used to map QTLs involved in nematode resistance, tuber yield and root development. Complete linkage maps were available for the interspecific hybrid parent as well as the *S. tuberosum* parent, and interval mapping for all traits was performed for both. Additionally, the intra- and inter-locus interactions of the QTLs were examined. The *Gro1.2* locus, involved in resistance to *G. rostochiensis* pathotype Ro1, that was previously mapped in the *S. tuberosum*×*S. spegazzinii* F_1 population, was located more precisely on chromosome 10. A new resistance locus, *Gro1.4*, also conferring resistance to *G. rostochiensis* pathotype Ro1, was found on chromosome 3. Different alleles of this locus originating from both parents contributed to the resistant phenotype, indicating multiallelism at this locus. No interlocus interactions were observed between these two resistance loci. For resistance to *G. pallida* no QTLs were detected. One minor QTL involved in tuber yield was located on chromosome 4. Two QTLs involved in root development and having large effects were mapped on chromosomes 2 and 6 and an epistatic interaction was found between these two loci.

Key words RFLPs · *Solanum spegazzinii* · *Globodera* sp. · Interlocus interaction · Intralocus interaction

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C. M. Kreike · A. A. Kok-Westeneng · W. J. Stiekema (✉)
Department of Molecular Biology, DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, The Netherlands

J. H. Vinke
Department of Arable and Fodder Crops, CPRO-DLO, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Introduction

Quantitative traits are characterised by a continuous distribution of the phenotypic value. This continuous variation is generally assumed to be due to the effects of both multiple genes, each of them indicated as a quantitative trait locus (QTL), and environmental effects (Johanssen 1909; East 1915; Nilsson-Ehle 1919). Early attempts to link a QTL to a qualitative gene, in this case a morphological marker, was described by Sax (1923) and in greater detail by Thoday (1961). However, for most organisms only a few neutral qualitative monogenic markers are available and only since the development of molecular markers, and the construction of molecular marker linkage maps (Botstein et al. 1980), have more extensive attempts been made to identify and localise QTLs.

If no complete linkage map is available, linkage between the markers and the QTLs can be detected with single-point analysis (Keim et al. 1990; Kreike et al. 1993, 1994). However, the availability of a complete linkage map allows the use of interval mapping which is more powerful (Lander and Botstein 1989). Several quantitative traits such as solids content, fruit pH and -weight and seed weight in tomato, as well as resistance to *Phytophthora infestans* in potato, have been mapped with this method (Paterson et al. 1988, 1991; De Vicente and Tanksley 1993; Leonards-Schippers et al. 1994).

QTLs for cyst nematode resistance in potato have been mapped previously by Kreike et al. (1993, 1994). These QTLs were segregating in an F_1 population of a cross between a di(ha)ploid *Solanum tuberosum* with the wild potato species *S. spegazzinii*. Two QTLs, *Gro1.2* and *Gro1.3*, involved in resistance to *Globodera rostochiensis* pathotype Ro1 were found on chromosomes 10 and 11 (Kreike et al. 1993) and a major QTL, *Gpa*, involved in resistance to *G. pallida* pathotype Pa2 and Pa3 was found on chromosome 5 (Kreike et al. 1994).

In the present paper we describe the mapping of nematode resistance loci in a backcross (BC) population derived from F_1 genotype F1-38, obtained from the above men-

tioned *S. tuberosum*×*S. spegazzinii* cross, and *S. tuberosum*. From RFLP analyses of the F_1 population, it was known that genotype F1-38 was heterozygous at the *Gro1.2* and *Gro1.3* loci but homozygous susceptible at the major *Gpa* locus. The BC population, therefore, would enable us to confirm and fine-map the *G. rostochiensis* QTLs and locate minor QTLs involved in *G. pallida* resistance. This BC population enabled us also to map QTLs from the *S. spegazzinii* parent involved in nematode resistance that were not segregating in the F_1 population. In addition to nematode resistance, QTLs for tuber yield and root development were mapped.

Another objective of this research was to determine the intra- and inter-locus interactions of the mapped QTLs. In a mapping analysis of inbreeding species, the segregation of only two alleles can be followed, whereas in a heterozygous species like di(ha)ploid *Solanum*, up to four different alleles, two from each parent, may be distinguished at a locus. With the exception of the work of Van Eck et al. (1994), intralocus interaction has not been studied in great detail. Stuber et al. (1992) described overdominant gene action at single QTLs in maize. In our BC population up to three different alleles could segregate at a QTL and the interaction between these alleles was analyzed.

Interaction between QTLs (interlocus interaction) was studied by Fatokun et al. (1992). He described epistasis between some of the QTLs, but mostly they displayed no interaction. In the present paper the interactions of the QTLs involved in nematode resistance, tuber yield and root development have also been examined.

Materials and methods

Plant material

Clone F1-38 is an interspecific hybrid between a di(ha)ploid *S. tuberosum* SH 78-88-1320 (Stub) and *S. spegazzinii* BGRC 8218-15 (Sspeg). F1-38 was used as a female parent in a backcross to Stub. The BC progeny of 80 plants was tested for resistance to *G. rostochiensis* pathotype Ro1 and *G. pallida* pathotypes Pa2 and Pa3 (see below).

RFLP analysis

The RFLP markers that were used in this study, as well as the RFLP analysis, have been described earlier by Kreike et al. (1993) and Jacobs et al. (1995). For the interspecific hybrid parent 68 markers were used and for the Stub parent 38 markers.

Analysis of the quantitative traits

The resistance tests with 80 BC plants was carried out in 1991 for *G. rostochiensis* pathotype Ro1 and *G. pallida* pathotypes Pa2 and Pa3 with nematode populations MOBA, P2-22 and Coll. 1077, respectively (Arntzen and Van Eeuwijk 1992). Each of the three resistance tests were performed in five replications in a randomized block design. Thirty cysts enclosed in a nylon net were used as the inoculum. After 5 months the newly formed cysts were collected and counted and the total cyst weight per plant was determined. A more detailed description of the resistance test is given by Kreike et al. (1993).

A normalizing transformation [^{10}log(x+1)] was performed on the number of cysts (=NC) and the total cyst weight (=TCW) to investigate these data with an analysis of variance (ANOVA). From these ANOVAs the heritabilities of NC and TCW under the different nematode infections was calculated as described by Kreike et al. (1993). For QTL mapping the data from the five replications were averaged. The frequency distribution is shown in Fig. 1.

After the resistance test the number of tubers, the total tuber weight and the development of the root system were determined. The latter was scored on an ordinal scale from 1 (poor root system) to 3 (good root system).

To obtain normality for the ANOVA, a square-root transformation was performed on the number of tubers (=NT) and total tuber weight (=TTW) data. No transformation was necessary for root development (RD). The three nematode infections had no significant effect on the NT, TTW and RD after ANOVA and therefore the heritability was determined using the 15 replications. For QTL mapping of NT, TTW and RD, the data of the 15 replications were averaged and their frequency distribution is shown in Fig. 1.

Correlation coefficients were calculated between the quantitative traits to detect any relationship between the various characters. The calculations were performed with the quantitative data that were used for QTL mapping, i.e. the [^{10}log(x+1)]-transformed and averaged (per nematode infection) NC and TCW data, the square-root transformed and averaged (over 15 replications) NT and TTW data, and the untransformed and averaged (over 15 replications) RD data.

QTL mapping

Due to heterozygosity in both parents, RFLP linkage maps could be constructed for F1-38 as well as Stub (Kreike et al. 1995). For most markers the segregation of the alleles per parent could be considered as a first-generation BC population with fully homozygous parents, which allowed the application of interval mapping, as described by Lander and Botstein (1989), but F_2-type segregating markers were also found. The parental linkage maps were made with JoinMap (Stam 1993), using BC- and F_2-type segregating markers. QTL mapping on the linkage maps of both parents was done using the computer program MapQTL (JW Van Ooijen, personal communication). This program has two options for mapping: single-point analysis using the non-parametric Kruskal-Wallis test and interval mapping. First, the Kruskal-Wallis test was used to search the chromosomes for markers significantly associated with the quantitative traits ($P<0.01$). This was done for both types of segregating markers. Then interval mapping was performed on only the BC-type segregating markers. A LOD value over 2 (Van Ooijen 1992) and a contribution to the total phenotypic variance (R^2) of >15% were taken as an indication that a segregating QTL was present.

Analysis of intra- and inter-locus interaction

For intralocus interactions three different alleles can be monitored in the segregation analysis; a *S. spegazzinii* allele from the hybrid F1-38 (speg-allele), a *S. tuberosum* allele derived from the hybrid and the *S. tuberosum* parent (tub1-allele), which is identical by descent, and a unique *S. tuberosum* allele derived from *S. tuberosum* (tub2-allele). In the case of linkage we presume that the tub1-allele is linked to the same QTL allele.

For the determination of the intra- and inter-locus interactions, the markers nearest to the QTL were taken to perform two- or three-way ANOVAs. From the two-way tables, which present the phenotypic values of the resulting marker genotype classes, the contribution of an allele to a trait could be deduced. A significant interaction term after ANOVA was taken to indicate epistasis.

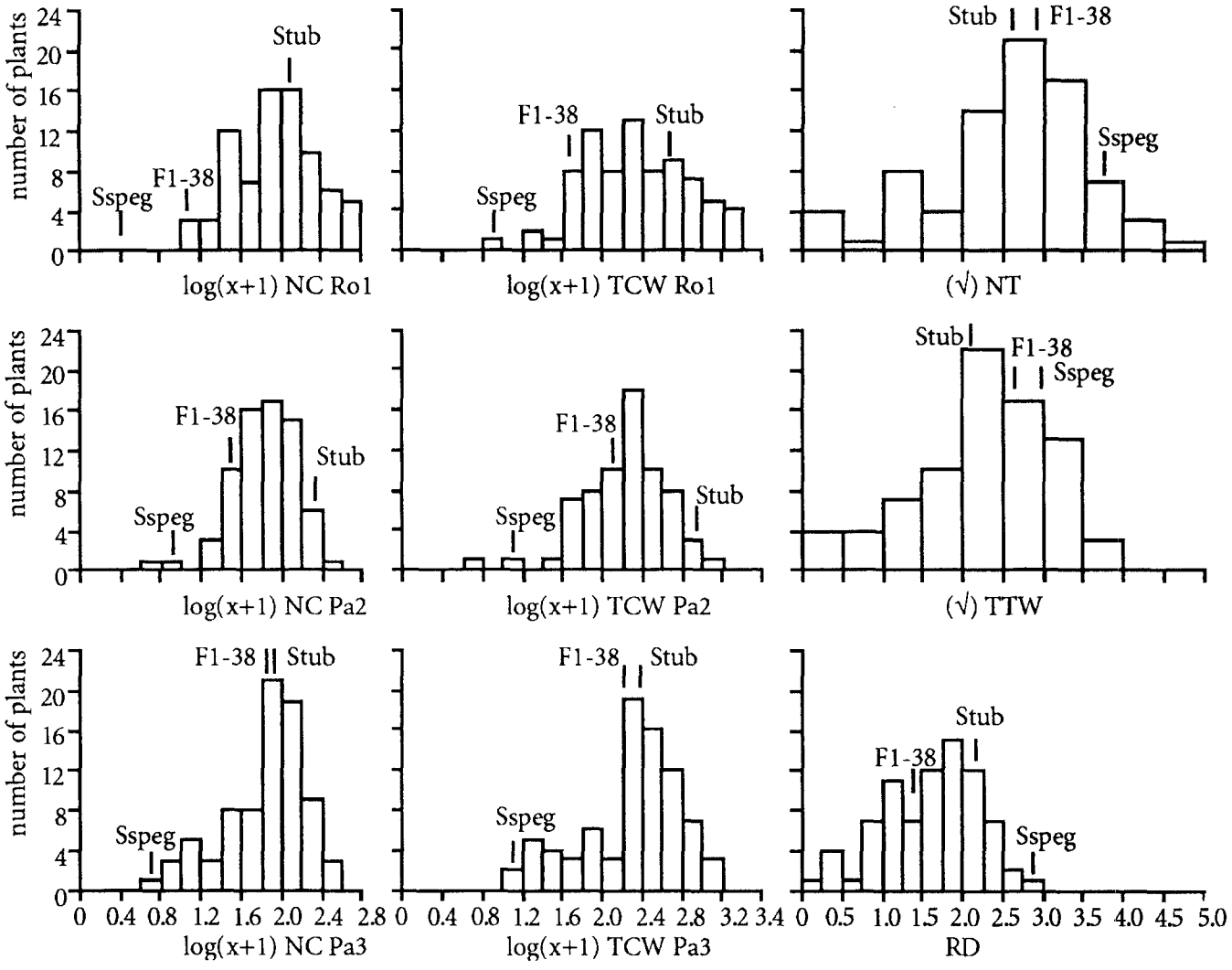

Fig. 1 Frequency distribution of the quantitative traits, *NC* [^{10}log(x+1) number of cysts], *TCW* [^{10}log(x+1) total cyst weight] under the different nematode infections, and *NT* (square root-transformed number of tubers), *TTW* (square root-transformed total tuber weight) and *RD* (root development) in the BC population. The phenotypic values of *S. spegazzinii* (Sspeg), *S. tuberosum* (Stub) and F1-38 are indicated

Results

Analysis of the quantitative traits

The frequency distribution for the measured traits, NC, TCW, NT, TTW and RD, in the BC population and the values of Sspeg, Stub and F1-38 are given in Fig. 1.

All traits show a continuous distribution indicating their quantitative nature. For some traits transgressive phenotypes were observed in the BC population. Correlation coefficients between the different quantitative traits were calculated to observe any relationships between the various characters studied (Table 1). As is to be expected the traits NC and TCW were highly correlated (r>0.97). Also NT was highly correlated with TTW (r=0.91). Subsequently only NC and NT will be described in this paper. The heritabilities for the quantitative traits are given in Table 2a and b and were very high for NT and RD.

RFLP map

Some differences in the map positions of several markers compared to the map described by Kreike et al. (1995) can be found on chromosomes 3 (TG134-TG42), 4 (Ssp61-Ssp118b-Ssp47), 5 (Ssp72-Ssp118a-GP21a) and 10 (Ssp106) of parent F1-38. Most differences can be attributed to the low recombination frequencies between these markers in the F1-38 parent which complicated their ordering with respect to more distant markers. The ambiguous position of marker Ssp106 on chromosome 10 is a result of its F_2-type segregation and the inaccuracy of determining the recombination frequency between the F_2-type and the flanking BC-type segregating markers.

Table 1 Correlation coefficients between the quantitative traits

Trait		NC Ro1	NC Pa2	NC Pa3	TCW Ro1	TCW Pa2	TCW Pa3	NT	TTW
NC	Ro1								
NC	Pa2	0.24							
NC	Pa3	0.28	0.59						
TCW	Ro1	0.97	0.31	0.28					
TCW	Pa2	0.25	0.99	0.56	0.34				
TCW	Pa3	0.31	0.59	0.99	0.32	0.58			
NT		0.25	0.27	0.39	0.25	0.29	0.40		
TTW		0.31	0.28	0.45	0.31	0.31	0.47	0.91	
RD		0.47	0.36	0.43	0.46	0.37	0.48	0.42	0.51

Table 2a Heritability of the quantitative traits and the map location of the QTLs with their nearest marker, segregating from parent F1-38. The LOD score and the R^2 at the QTL position are given. Abbreviations: h^2=heritability; Chr.=chromosome; R^2=variance explained by QTL; Marker=marker nearest to the QTL

Trait		h^2	Chr	QTL position (cM)	LOD score	R^2	Marker	Marker position (cM)
NC	Ro1	0.76	*3*	22.5	3	24	Ssp8	35
			10	51[a]	*P*=0.005	19.5	Ac15-7	51
NC	Pa2	0.58						
NC	Pa3	0.64						
NT		0.92	*4*	45	2.3	16	Ssp47	40
RD		0.91	*2*	30	2.6	32	TG34	47.5
			6	25	2.3	41	TG118	0

[a] The position of this QTL was not determined with interval mapping but with the Kruskal-Wallis test and therefore the *P*-value is given

Table 2b Heritability of the quantitative traits and the map location of the QTLs with their nearest marker, segregating from the Stub parent. The LOD score and the R^2 at the QTL position are given. Abbreviations: h^2=heritability; Chr.=chromosome; R^2=variance explained by QTL; Marker=marker nearest to QTL

Trait		h^2	Chr	QTL position (cM)	LOD score	R^2	Marker	Marker position (cM)
NC	Ro1	0.76	*3*	25	2.1	20	TG130	37.5
NC	Pa2	0.58						
NC	Pa3	0.64						
NT		0.92						
RD		0.91						

QTL mapping

The computer program MapQTL (JW Van Ooijen, personal communication) was used for mapping the quantitative traits. A Kruskal-Wallis test was performed on all markers individually. If significant associations ($P<0.01$) were found between a marker and a trait, interval mapping of the chromosomes containing these markers was performed. Interval mapping could only be performed for the BC-type segregating markers. The results of the interval mapping are given in Table 2a+b and Fig. 2.

For resistance against *G. rostochiensis* pathotype Ro1, determined as NC-Ro1, two QTLs were found, one on chromosome 3 and one on chromosome 10. The presence of a QTL on chromosome 10, *Gro1.2* segregating from *S. spegazzinii* BGRC 8218-15, conferring resistance to pathotype Ro1, was described earlier by Kreike et al. (1993). The position of this QTL, in the proximity of TG63, is more distal, and closer to marker Ac15-7 (Fig. 2). The exact location of this QTL could, however, not be determined with interval mapping since marker Ac15-7 had an F_2-type segregating pattern. The contribution of this locus to the total phenotypic variance was 19.5%. The other QTL involved in Ro1 resistance was observed on chromosome 3 and alleles from both parents contributed to the trait (Fig. 2). The phenotypic variance that could be explained by this locus was 24% for F1-38 alleles and 20% for Stub alleles. We propose to name this locus, *Gro1.4* in correspondence with the previously reported resistance loci to *G. rostochiensis* pathotype Ro1, derived from *S. spegazzinii* (Barone et al. 1990; Kreike et al. 1993). No QTL was found on chromosome 11, in the region of the *Gro1.3* locus. This locus was mapped in the F_1 population (Stub× Sspeg), and described by Kreike et al. (1993).

QTL mapping of NC-Pa2 and NC-Pa3 revealed no QTLs. Only on chromosome 5 was a possible QTL involved in resistance to *G. pallida* pathotype Pa3, and located near marker Ssp88, segregating from the Stub par-

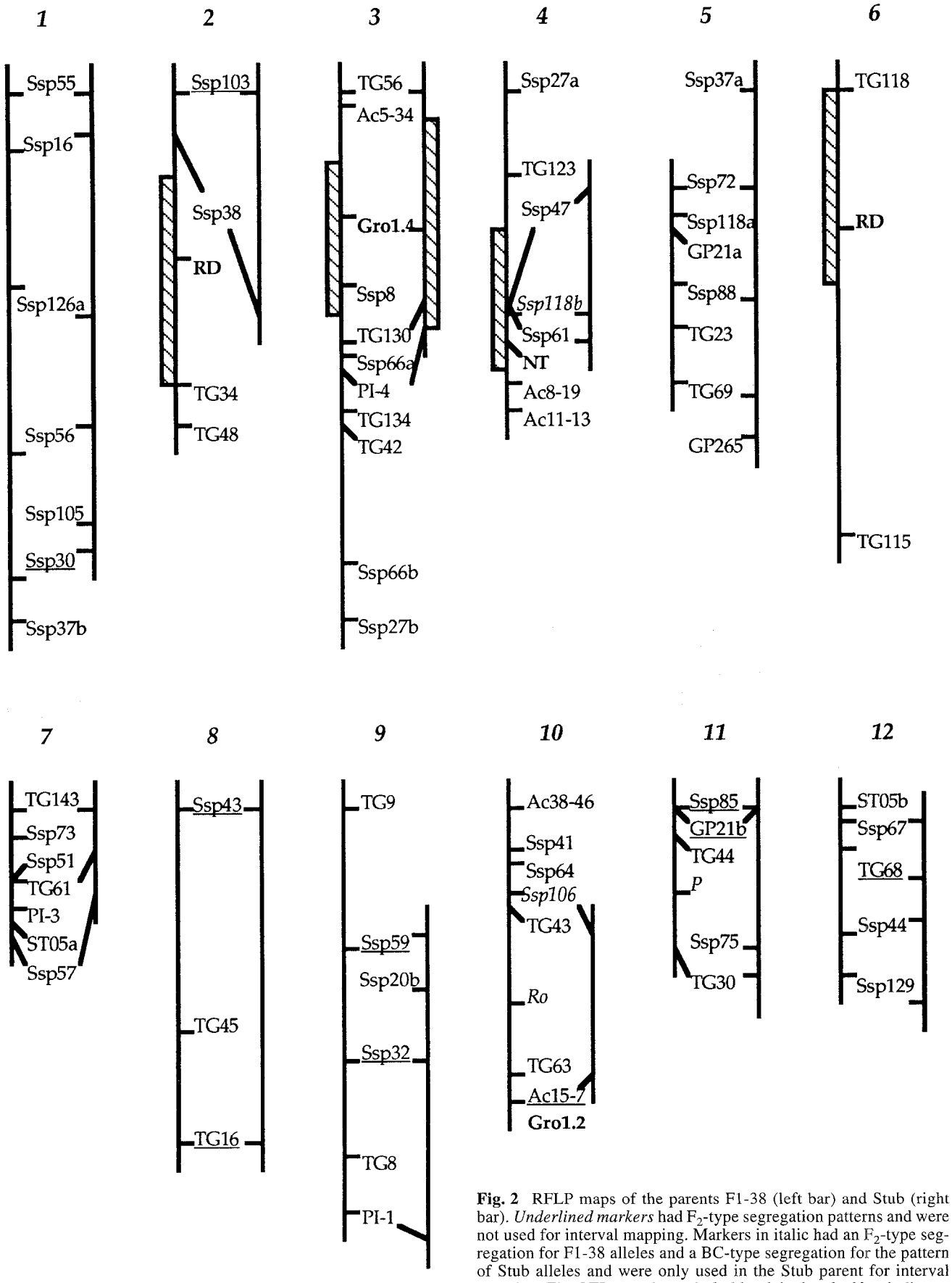

Fig. 2 RFLP maps of the parents F1-38 (left bar) and Stub (right bar). *Underlined markers* had F_2-type segregation patterns and were not used for interval mapping. Markers in italic had an F_2-type segregation for F1-38 alleles and a BC-type segregation for the pattern of Stub alleles and were only used in the Stub parent for interval mapping. The QTLs are shown in *bold* and the *hatched box* indicates the 1 LOD support interval

ent. The LOD score was just below the threshold level (LOD=1.8, R^2=15%). For NT, QTL analysis uncovered only one QTL that was located on chromosome 4, near marker Ssp47, derived from F1-38, with an effect of 16%. Analysis for RD yielded two QTLs from F1-38 with large effects. One locus on chromosome 2, near marker TG34, which explained 32% of the total phenotypic variation and another locus on chromosome 6, near marker TG118, which explained 41%.

Analysis of intra- and inter-locus interaction

Intralocus interactions at a QTL could be examined if both parents were heterozygous at the associated marker locus. For resistance to *G. rostochiensis* pathotype Ro1, QTLs on chromomoses 3 and 10 were found, respectively, at the *Gro1.4* and *Gro1.2* loci. The QTL on chromosome 3 is linked closest to marker Ssp8, segregating from F1-38 and TG130 segregating from Stub (Fig. 2, Table 2a, b). The segregation data for both markers were used to construct a two-way table (Table 3). The two-way ANOVA did not show a significant interaction; therefore, the effect of the speg-allele from F1-38 and the tub1-allele from Stub was additive.

Marker locus Ac15-7 was heterozygous in both parents, with two identical alleles yielding an F_2-type segregation. The three marker genotype classes are shown in Table 4. These results confirm that the speg-allele of marker Ac15-7 is linked to a QTL conferring resistance to *G. rostochiensis* pathotype Ro1. A three-way ANOVA was performed to determine the interlocus interaction of the two QTLs, including three alleles, involved in resistance to *G. rostochiensis* pathotype Ro1. In Table 5 the marker-genotype classes of the resistance-linked alleles of Ac15-7, TG130 and Ssp8 are shown and indicate that all effects between the alleles were additive as no significant interaction was found.

Mapping analysis of NT revealed only one QTL on chromosome 4. The two-way table showing the intralocus interactions at the Ssp47 marker, revealed that the speg-allele was associated with high tuber numbers (Table 6); again no interaction was evident after a two-way ANOVA.

Table 3 Intralocus interaction at the *Gro1.4* locus on chromosome 3 involved in resistance to *G. rostochiensis* pathotype Ro1. The two-way table is constructed with marker Ssp8 segregating from parent F1-38, and TG130 segregating from parent Stub. The ^{10}log(x+1)-transformed NC observed in each marker-genotype class is given together with the number of plants (in parenthesis). The speg-allele of Ssp8 and the tub1-allele of TG130 are associated with low cyst numbers. A two-way ANOVA showed no interaction (P=0.9)

Marker		Ssp8 tub1	speg	Total
TG130	tub1	2.07 (5)	1.76 (24)	1.81 (29)
	tub2	2.24 (19)	1.95 (29)	2.06 (48)
	Total	2.20 (24)	1.86 (53)	1.97 (77)

Table 4 Intralocus interaction at the Ac15-7 locus linked to the *Gro1.2* locus conferring resistance to *G. rostochiensis* pathotype Ro1 on chromosome 10. The ^{10}log(x+1)-transformed NC that was found in each marker-genotype class is given, together with the number of plants (in parenthesis). The speg-allele of Ac15-7 is known to be associated with low cyst numbers

Ac15-7	speg/tub2	speg/tub1+ tub2/tub1	tub1/tub1	Total
	1.74 (17)	1.94 (32)	2.12 (24)	1.95 (73)

Table 5 Three-way table of the markers Ac7, Ssp8 and TG130 linked to QTLs involved in resistance to *G. rostochiensis* pathotype Ro1. The ^{10}log(x+1)-transformed NC that was found in each marker-genotype class is given, together with the number of plants (in parenthesis). The speg-allele of Ac15-7, the speg-allele of Ssp8, and the tub1-allele of TG130 are associated with low cyst numbers. No interlocus interactions were found after ANOVA (*P* value>0.2 for all possible combinations)

Ssp8	TG130	Ac15-7 speg/tub2	speg/tub1+ tub2/tub1	tub1/tub1	Total
tub1	tub1	1.72 (2)	– (0)	2.31 (3)	2.07 (5)
tub1	tub2	2.00 (2)	2.11 (8)	2.45 (7)	2.24 (17)
speg	tub1	1.67 (7)	1.79 (10)	1.72 (6)	1.74 (23)
speg	tub2	1.74 (6)	1.94 (14)	2.06 (8)	1.93 (28)
	Total	1.74 (17)	1.94 (32)	2.12 (24)	1.95 (73)

Table 6 Intralocus interaction of marker Ssp47 linked to a QTL involved in NT. The square root transformed NT that was found in each marker-genotype class is given, together with the number of plants (in parenthesis). The speg-allele is associated with a high NT. A two-way ANOVA showed no interaction

Ssp47	tub1	speg	Total
tub1	1.02 (1)	2.90 (26)	2.83 (27)
tub2	2.26 (21)	2.97 (21)	2.62 (42)
Total	2.20 (22)	2.93 (47)	2.70 (69)

Table 7 Interlocus interactions of markers TG34 and TG118, segregating from parent F1-38, both linked to QTLs involved in RD. A two-way ANOVA yielded a significant interaction term (*P*=0.0015). The phenotypic value for RD that was found in each marker genotype class is given, together with the number of plants (in parenthesis)

Marker		TG34 tub	speg	Total
TG118	tub	1.81 (23)	1.79 (16)	1.80 (39)
	speg	1.14 (18)	1.93 (12)	1.46 (30)
	Total	1.52 (41)	1.85 (28)	1.65 (69)

QTLs with large effects were found for RD. For both loci on chromosomes 2 and 6, segregation of the RFLP alleles was only found for the *S. spegazzinii* parent, so the intralocus interactions could not be determined. Interlocus interaction was determined with a two-way ANOVA and the two-way table of TG34 and TG118 is shown in Table 7. A significant interaction was found, indicating epistasis.

Discussion

Analysis of the quantitative traits

The parental values are indicated in the frequency distributions shown in Fig. 1 . These values are very similar in four out of the nine quantitative traits. It can also be noted that the phenotypic values of the progeny go beyond the parental values in almost all the frequency distributions shown. This transgressive segregation implies that QTL alleles, with positive as well as negative effects, are to be expected from both parents.

QTL mapping

Qualitative resistance loci to *G. rostochiensis* pathotype Ro1 have been mapped on chromosome 5 and on chromosome 7, i.e., the *H1* locus (Gebhardt et al. 1993; Pineda et al. 1993) and the *Gro1* locus (Barone et al. 1990) respectively. QTLs to *G. pallida* and *G. rostochiensis* have been mapped on chromosomes 5, 10 and 11, respectively (Kreike et al. 1993, 1994). These QTLs have been determined in an F_1 population derived from a *S. tuberosum*×*S. spegazzinii* cross. The QTL analysis described in the present paper was performed in a BC population of *S. tuberosum* to confirm and fine map the *G. rostochiensis* QTLs and to locate minor QTLs involved in *G. pallida* resistance.

The *Gro1.2* locus was located more proximal on chromosome 10 near marker Ac15-7. The explained variance increased thereby from 7 (Kreike et al. 1993) to 19.5%. This increase can conceivably be attributed to a closer linkage between the QTL and the speg-allele of Ac15-7, the nearest marker. A decrease in the environmental variance can also give rise to a higher explained variance at marker Ac15-7; nevertheless, the estimation of the explained variance is ambiguous and variations are possible. Another option is that the tub2-allele of marker Ac15-7 is linked to a QTL involved in resistance, since it is identical to the speg-allele. The Kruskal-Wallis test, which was carried out to determine associations between RFLP markers and the quantitative trait, revealed in the F_1 as well as the BC population a *P*-value of 0.09 for marker Ssp106 on chromosome 10 from the Stub parent. This could point to the presence of a QTL at a large distance from Ssp106. If the Stub parent is also contributing to the resistance trait then the effects of the tub2-allele and the speg-allele are additive (Table 4).

The *Gro1.3* locus could not be identified in the BC population, although RFLP analysis of the F_1 progeny indicated its presence in the F1-38 genotype. A crossover can explain the absence of the resistance-conferring allele at the QTL when the speg-allele at the RFLP locus was present. However, a new QTL was located on chromosome 3, the *Gro*1.4 locus, that was not detected in the F_1 population. Most likely, the QTL-allele conferring resistance was present in a homozygous state in *S. spegazzinii* and could therefore only be revealed in a BC or F_2 population, but not in the F_1 population. However, the resistance allele from the Stub parent should have been detected in the F_1 population if more plants had been used for QTL mapping. Interestingly, the *Gro1.4* locus was associated with markers segregating from both parents, indicating the existence of multiple alleles at this locus. Multiple alleles have also been demonstrated at the *Ro* locus involved in tuber shape (Van Eck et al. 1994).

RFLP analyses of the F_1 population showed that genotype F1-38 was homozygous susceptible at the major *Gpa* locus. Therefore, we were able to attempt the mapping of minor QTLs, either from F1-38 or *S. tuberosum*, that were involved in resistance to *G. pallida*. Unfortunately, no such QTLs were found.

The amount of the total variation that can be explained by the mapped QTLs is 63.5% for NC-Ro1, 0% for NC-Pa2 and NC-Pa3, 16% for NT, and 73% for RD. If these figures are compared to the heritabilities of the quantitative traits (Table 1a, b) it can be noted that for NC-Ro1 and RD almost all genetic variation is characterised with the detected QTLs; consequently, additional major QTLs involved in either of these traits are not expected. For NC-Pa2, NC-Pa3 and NT only little of the genetic variation has been explained with the mapped QTLs. Perhaps a large number of minor QTLs are involved in these traits which could not be detected with the experimental design applied, e.g. the number of plants or the measurement of the quantitative data. Another explanation might be that the Stub linkage map is not saturated enough, leaving QTLs undetected.

MapQTL (J. W. Van Ooijen, personal communication) was used in this study for the localisation of the QTLs. Due to heterozygosity in both parents, RFLP linkage maps could be constructed for F1-38 as well as Stub, but only BC-type segregating markers were used for interval mapping, since they outnumbered the F_2-type segregating markers (Fig. 2). Currently the program is being adjusted for the analysis of non-inbred parents which permits the analysis of the segregation of all alleles at one locus (e.g., from both parents) simultaneously (C. Maliepaard, personal communication).

Analysis of intra- and inter-locus interaction

Gene action, as studied by Edwards et al. (1987) and Stuber et al. (1992), refers to the gene (allele) dosage effect and has mainly been analyzed in F_2 populations of inbreeding species. In both cases, QTLs in maize were examined and overdominance was frequently found. In the present research a BC population of an outbreeding species is used. A gene dosage effect can only be studied for the allele shared by the two parents (tub1-allele), while the other alleles can also contribute to the trait and interact. Since these interactions are in most cases not based on gene (allele) dosage effects of identical alleles, we preferred to use the term intralocus interaction rather than gene action.

Interlocus interaction, or epistasis, is a kind of gene interaction whereby one gene interferes with the phenotypic expression of another non-allelic gene. Research into epistasis has also been performed primarily in F_2 populations of inbreeding species. Paterson et al. (1991) localised QTLs involved in fruit size, soluble-solids concentration, and fruit pH in tomato but did not find interlocus interactions, which indicated that the allele effects of the different QTLs were additive. Fatokun et al. (1992), however, found two QTLs involved in seed weight in cowpea that did show a significant interaction.

In the BC population, we did not detect interlocus interactions between the QTLs involved in resistance to *G. rostochiensis* pathotype Ro1. However, for the QTLs involved in RD on chromosomes 2 and 6 a significant interaction was found. Unfortunately, we were not able to determine the intralocus interactions at these QTLs because we did not find heterozygosity in the Stub parent at the TG34 and TG118 loci. Therefore we could not assess whether an allele had a positive or negative contribution to the trait. Furthermore, for an accurate description of the interactions between all QTL alleles that contribute to a trait, very large populations are needed.

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