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ORIGINAL PAPER

Powdery mildew resistance in roses: QTL mapping in different environments using selective genotyping

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Abstract *Podosphaera pannosa*, the causal agent of rose powdery mildew, hampers the production of cut roses throughout the world. A major tool to control this disease is the use of resistant plant material. Single resistance genes, like *Rpp1*, may be overcome within a few years by high risk pathogens like powdery mildews. Durable resistance could be achieved using quantitative resistances. Here we describe mapping of QTLs for resistance to *P. pannosa* in six different environments (artificial and natural infections in the greenhouse over 3 years and natural infections in the field over 2 years). AFLPs, RGAs and other marker types were used to construct an integrated linkage map for the diploid population 97/7 containing 233 markers. In a selective genotyping procedure, marker segregation was analysed for 170 of the up to 270 phenotyped individuals. We identified seven linkage groups with an average length of 60 cM, corresponding to seven rose chromosomes in the haploid set. Using an LOD significance threshold of 3.9 we detected a total of 28 QTLs for the nine powdery mildew disease scores under analysis. Using the data from artificial inoculations with powdery mildew race 9, three resistance QTLs explaining about 84% of the variability were mapped. Twelve and 15 QTLs were detected for resistance to naturally occurring infections in the greenhouse and in the field, respectively, over several years.

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Introduction

Roses are thought to be cultivated for at least 5,000 years and are currently the most economically important ornamental crops (Gudin 2000). Unfortunately, their production and cultivation is complicated by the occurrence of several diseases including black spot, powdery and downy mildews and rust. While black spot, caused by *Diplocarpon rosae* Wolf, is recognized as the major disease of roses grown in the field, powdery mildew, caused by *Podosphaera pannosa* (Wallr.:Fr.) de Bary, is regarded as one of the most severe diseases of roses cultivated in greenhouses. With *Rdr1* against black spot (Von Malek et al. 2000) and *Rpp1* against powdery mildew (Linde et al. 2004), two major resistance genes have been located in rose.

The construction of genetic linkage maps and the mapping of QTLs has been an effective approach for the study and breeding of complex forms of disease resistance in cereals, tomatoes, soybeans, potatoes and other major crops for years (Overview in Young 1996). This is because single resistance genes tend to be overcome by new pathogenic races within few years of widespread crop cultivation, whereas resistance provided by quantitative resistance genes is regarded as more durable (McDonald and Linde 2002). Monogenic resistance to pathogenic fungi specializing on certain

plant species is often of the hypersensitive response type, where mutations in the avirulence gene could easily lead to non-recognition by the corresponding R-gene of the host, restoring the pathogenicity of the fungus (Parlevliet 2002). For ornamental plants only a few linkage maps have been constructed, e.g. *Antirrhinum*, *Lilium*, *Petunia*, *Rhododendron* and *Rosa* (Linde et al. 2006). For roses, being the most important ornamental, five genetic linkage maps have been established from different groups up to now. These maps differ highly in their total length, reaching from 238 cM (Crespel et al. 2002) up to 902 cM (Rajapakse et al. 2001) for a single parental map, and the number of markers covering the linkage groups (LGs). The most dense map was constructed by Yan et al. (2005) with about 270 molecular markers covering each parental map and an average distance between the markers of 1.9 and 2.0 cM. What is common to all these linkage maps is the relatively small size of the mapping populations comprising about 90 or fewer individuals. This could partly be explained by the large amount of work required to cultivate roses in the greenhouse over long periods as well as variable seed set and germination. Other often more restricting factors for large populations include genotyping costs such as DNA isolation and marker analysis. Yet, a sufficiently large mapping population is by far the most important factor in estimating accurate QTL positions, whereas there is only little gain in precision by increasing the marker density beyond 10–15 cM (Darvasi et al. 1993; Kearsey 1998; Kearsey and Farquhar 1998). Further more, effective population size is often decreased due to missing data and skewed allele frequencies (McCouch and Doerge 1995).

The aim of our analysis was to map QTLs for resistance to powdery mildew in different environments by measuring natural infections and artificial inoculations using a single spore isolate. In order to reduce the genotyping effort we used a selective genotyping strategy (Darvasi and Soller 1992) collecting disease resistance data from up to 270 individuals of the diploid population 97/7 ($2n = 2x = 14$) while performing marker analysis only with the 170 most extreme genotypes for resistance. Besides AFLPs we used resistance gene analogues (NBS-RGAs) in a candidate gene approach to enrich the linkage map with markers in chromosomal regions containing putative resistance genes and to find anchor points for the comparison with the map of Yan et al. (2005). The only known function of NBS-RGAs in plants is their involvement in disease resistance. Based on their predicted protein structures RGAs can be divided into several classes, with about 75% falling into the class of NBS-LRR RGAs (Dangl and Jones 2001). These can be subdivided into members sharing motifs of the toll and interleukin-1 receptor like (TIR) at the N terminus and the non-TIR classes (Meyers et al. 1999) An association to plant developmental genes involved in cell-to-cell communication like *CLAVATA1* and *ERECTA* can also be seen by the homology of their extracytoplasmic kinase domains with R genes like *Xa21* and *Pto* (Baker et al. 1997). In the present study we used specific primers amplifying TIR and non-TIR NBS-LRR RGAs developed from a rose RGA library and analysed them using a modified SSCP technique (A. Hattendorf and T. Debener, submitted for publication).

Materials and methods

Plant material

The rose genotypes used in this study originated from a cross of the diploid line 95/13-39 (resistant against powdery mildew isolate 9) and the susceptible diploid male parent Sp3 (82/78-1). Both genotypes are open pollinated seedlings from a breeding program aimed at the introgression of genes from tetraploid garden roses into *Rosa multiflora* (Reimann-Philipp 1981). The resulting population of 270 plants was used for the current investigation. Two copies of the plant population were grown in the greenhouse in 3- or 5-l plastic pots in rose substrate with pH 5.5 [70 vol% white peat, 30 vol% coco fibre, 1.2 kg/m^3 PG-Mix NPK fertilizer, 100 g/m^3 trace element fertilizer (Jost, Iserlohn, Germany)] and in a field plot of the Institute for Ornamental Plant Breeding (IZZ) in Ahrensburg, Germany.

Fungal isolates, inoculation assay and disease assessment

Powdery mildew resistance tests with race 9

The monoconidial powdery mildew isolate 9 was established by transferring single conidia, originating from the host genotype 97/7-13 grown in Ahrensburg (Germany), to in vitro shoots of the susceptible genotype 94/103-02 as described previously by Linde and Debener (2003). The isolate was multiplied for the experiments by inoculation of in vitro shoots in glass vessels. Leaves from powdery mildew free plants were sampled from November 2002 to March 2003 in the greenhouse. Leaflets from three rose genotypes each were placed in glass Petri dishes on water-agar (0.5% agar) containing 0.03% benzimidazole to prevent fungal contamination of the agar surface. From each genotype six to nine leaflets (third–fifth unfolded leaves from the shoot tip) were used. The leaflets were infected with about

2 conidia/mm^2 leaf surface as described by Linde and Debener (2003). Ten days after inoculation, the percentage of the leaf area covered with conidiophores was estimated in 10% steps (from 0 to 100%) with a stereomicroscope (8–50-fold magnification). The inoculations were repeated 5–7 times for 245 of the 270 individuals. The minimum and maximum values were excluded and the mean was calculated and taken as a disease index (DI) as described in Linde et al. (2004). QTLs for this disease score were named "*Isol 9*."

Powdery mildew resistance tests in the greenhouse

During the years 2001–2003 infection of the 270 plants of the population 97/7 with naturally occurring powdery mildew populations was recorded twice a year in July and September in the greenhouse. Heavily infected plants of this population, clonally propagated and grown in a separate greenhouse with a high infection pressure, were shaken in front of a large ventilator installed at the roof at one end of the glasshouse distributing the conidia over the whole population. About 6 weeks after the repeated inoculations the plants were visually evaluated for their powdery mildew resistance using a five class scale: 0 = resistant, no symptoms; 1 = susceptible, single young leaves infected; 2 = some sprouts infected; 3 = less than 50% of the leaves infected; 4 = 50% or more infected. The mean of two observations per year was recorded. We also calculated the average over the years 2001 and 2002 and for the period 2001–2003. QTLs detected for these disease scores were designated "*PM In 01*, *PM In 02*, *PM In 03*, *PM In 01 + 02*, *PM In 01-03*," respectively.

Powdery mildew resistance tests in the field plots

In the years 2001 and 2002 population 97/7 was scored during August and October for naturally occurring infections with *P. pannosa* utilizing the same disease score as in the greenhouse evaluation. Additionally the mean over both years was calculated. QTLs were named "*PM Out 01*," "*PM Out 02*" and "*PM Out 01 + 02*."

Black spot resistance test

The single conidial isolate Dort E4 was used for the black spot resistance tests which were performed from Kaufmann et al. (2003). The already in the population 94/1 localized gene *Rdr1* confers resistance to this isolate (Von Malek et al. 2000). Inoculum was produced by washing newly formed conidia from inoculated leaves with sterile tap water and adjusting the concentration to 10^5 conidia/ml. Three to five leaves were cut off at their base, washed briefly in tap water, and placed in moist, translucent plastic containers. Eight to 20 droplets (10 µl) of conidial suspension were pipetted onto the upper surface of the leaflets. The scoring of the interaction phenotype of the tested plants (resistant or susceptible) was done at 10 and 14 dpi. Genotypes showing mycelial growth beyond the area of inoculation and where formation of acervuli could be observed were considered to be susceptible. The resistance tests were repeated three to four times for each genotype (T. Debener, personal communication). Resistance data were kindly provided by Kaufmann et al. (2003).

Morphological characteristics

In addition to the above-mentioned resistance-related measurements we scored three horticulturally important morphological traits in a qualitative manner.

The presence/absence of *prickles* was recorded as a qualitative trait. The occurrence of prickles on the whole plant was evaluated. For the differentiation between *double flowers* (filled flowers) and non-filled flowers with only five petals the mean petal number of five flowers per plant was recorded in July 2002. This trait was mapped as a morphological marker in a qualitative way as the presence or absence of *double flowers*. Each of these traits was scored in the field-planted copy of the population 97/7 at the IZZ.

We also recorded the presence/absence of *white stripes* on the petals as an additional qualitative floral trait and mapped it together with the molecular markers.

Marker analysis

DNA extraction

Genomic DNA was extracted from 100 mg of frozen leaf tissue according to the method of Kobayashi et al. (1998) with the following modifications. After resuspending the DNA pellet in 500 µl TE, 10 µg RNase A (Roche, Ingelheim, Germany) was added, followed by incubation for 30 min at 37°C and an extraction with 500 µl phenol/chloroform. The DNA was precipitated with 1/10 volume 3 M NaAc and 0.75 volumes of isopropanol, washed with 70% ethanol twice, dried briefly and resuspended in H_2O.

AFLP analysis

A selective genotypic strategy was used for the construction of the genetic linkage map. From the 270

available genotypes of the population 97/7, 170 individuals were chosen for the marker analysis. We only used clearly resistant and highly susceptible genotypes with a DI < 3.0 or > 30 based on the resistance tests with 245 of 270 genotypes using powdery mildew isolate 9. AFLPs were performed as described by Von Malek et al. (2000) using the restriction enzymes *Mse*I and *Hin*dIII and specific primers with three selective bases each. The *Hin*dIII primers were fluorescently labelled with IRD700 and IRD800 dyes at their 5′ end (MWG Biotech, Ebersberg, Germany). DNA fragments were separated and visualized with LICOR Gene ReadIR 4200 automated sequencers (MWG Biotech, Ebersberg, Germany) on 6% denaturing polyacrylamide gels of 25 cm length. The following primer combinations were used: AAA-ATC, ACg-Agg, CAC-AAg, CAC-ATC, CAg-AAC, CAg-Agg, CAg-ATC, CAg-ATg, CAg-gCA and CAg-ATT. Data for three AFLP markers ATT-CAC, ATT-ACC and AAg-ACA were kindly provided by Kaufmann et al. (2003).

Analysis of SCAR, CAPS, RGAs and BAC end-derived markers

Two SCAR markers with close linkage to the powdery mildew resistance gene *Rpp1* were analysed according to Linde et al. (2004). Marker data for one CAPS (Rd1) and one BAC end-derived marker were used from Kaufmann et al. (2003).

Genomic rose RGAs were amplified using specific primers and analysed on 0.5 × MDE gels using the SSCP technique as described in A. Hattendorf and T. Debener (submitted for publication).

Segregation of markers

Marker segregation was analysed in MS Excel. Only molecular markers segregating 1:1 for uni-parental or 3:1 for bi-parental markers in a χ^2-test ($\alpha = 0.05$) were used for map construction.

Map construction

Version 3.0 of JoinMap (Stam 1993; Van Ooijen and Voorrips 2001) was used for the construction of the genetic linkage map. Independent maps for the parental plants were calculated using the different 1:1 segregating uni-parental markers and the common 3:1 distributed bi-parental markers. LOD groupings were calculated using LOD thresholds from 2.0 to 10.0 and LGs were determined using an LOD threshold of 7.0 with the mapping function of Kosambi (1944). Only linkages with a recombination frequency smaller than 0.3 and an LOD larger than 2.0 were used. The other mapping parameters were: Jump = 5, ripple after adding one locus, a third round was performed. Markers with insufficient linkages or with conflicts to other markers were removed.

The combined map was built by joining the homologous parental LGs according to their 3:1 segregating bi-parental markers (indicated in Fig. 3 with a "K" for the RGA- or a "3_1" for the other markers) under the above-mentioned mapping parameters. Markers clearly disturbing the orders in comparison to the parental maps were excluded. For LGs 5 and 6, the arrangement of the bi-parental markers were taken as fixed order. Due to their skewed distribution the morphological markers were integrated into the combined map in a final step after ordering all other markers.

QTL analyses

As the basis for the QTL analyses an LOD significance threshold was determined using the Monte Carlo simulations of Van Ooijen (1999). For a genome-wide false-positive rate of 5% in diploid roses ($n = 7$) and an average chromosome length of about 50 cM, an LOD threshold of 3.9 was determined for a cross between two non-inbred genotypes of an out crossing species (full-sib family). The computer software package, MapQTL version 4.0 (Van Ooijen et al. 2002), was used for interval mapping (Lander and Botstein 1989). Test statistic values were calculated in 1 cM intervals. In the region of the putative QTLs (> LOD 3.9), the markers with the highest LOD values were taken as co-factors for running a multiple-QTL mapping programme, the MQM method or restricted MQM (Jansen 1993; Jansen and Stam 1994). When LOD values of some markers on other regions reached the significance level of LOD 3.9, the MQM was repeated by adding those new markers as co-factors until the LOD profile stabilized. Map regions with an LOD ≥ 3.9 were considered as a QTL. Peak values of the test statistic were taken as the QTL position. Independent QTLs for the same trait were assumed when there was a minimal distance of 10 cM between the peaks. One LOD support intervals were determined as 90% confidence intervals (Lander and Botstein 1989; Van Ooijen 1992). Estimates for the percentage explained phenotypic variance by each QTL were always taken from the interval mapping output of MapQTL, as were estimates for QTL only detected with MQM mapping, because the values in the MQM output could be biased due to selective genotyping (Van Ooijen et al. 2002).

Results

Phenotypic traits

The traits *prickles*, *white stripes*, *double flowers* and *black spot* resistance were recorded in a qualitative manner as presence or absence of this character. With the exception of the character *double flowers* ($\chi^2 = 0.3$, not significant) all other traits showed significant deviations from a 1:1 segregation (*prickles* $\chi^2 = 11.2$, *white stripes* $\chi^2 = 8.5$, *black spot* $\chi^2 = 4.9$) and were therefore integrated into the established parental maps in a final step. The presence of *double flowers*, with a mean value of 16.8 petals, and the occurrence of *prickles* were inherited from the parental plant 95/13-39.

The population 97/7 exhibits a 1:2 segregation ($\chi^2 = 0.13$, not significant) for resistance to the powdery mildew race 9 with 79 resistant to 166 susceptible individuals (Fig. 1). The resistant parent 95/13-39 showed no production of conidiophores (DI = 0) whereas the susceptible parent displayed a DI of 20%. The progeny showed transgressive segregation for this disease score indicating regulation by more than one gene, putatively with effects from both parental plants.

Using a Chi square test for the disease scores for powdery mildew resistance in the greenhouse and in the field, the null hypothesis of independence between the observations in the different years and environments must be rejected with P values ≤ 0.005 at 8 degrees of freedom (data not shown). The disease score for powdery mildew indoors in 2002 displayed Cramers V values from 0.75 to 0.84 with *PM Out 01*, *PM Out 02* and *PM In 01* and *PM In 02* indicating a significant correlation between these characters. Significant correlations were also detected between the disease scores *PM In 03* and *PM In 01*, *PM In 03* and *PM Out 02* and also between *PM Out 02* and *PM In 01* with Cramers V values of 0.76, 0.84 and 0.77, respectively. The infection levels in the field plot were lower in 2002 than in 2001, with 108 powdery mildew free genotypes and only 4 progenies with the highest DI of 4. In 2001 only 108 plants were scored with a DI of 0 and 24 with a DI of 4. The infection level in the greenhouse was lowest in 2003 with 98 disease free genotypes of 215 evaluated plants and only 3 plants with a DI of 4 (Fig. 2). In the years 2001 and 2002 the mean infection levels in the greenhouse were quite equal, with more disease free plants in 2001 (70 compared to 56) and many more genotypes with a DI of 4 (47 compared to 8).

Marker analysis

The two parental plants Sp3 and 95/13-39 and their segregating progeny (97/7) were screened for polymorphisms using 24 AFLP, 18 RGA, 6 (simple sequence repeat) SSR and 1 SCAR primer combination. A deviation from the expected ratios of about 33% of all segregating markers was recorded for all marker types using a χ^2-test at $P = 0.05$. The 13 chosen AFLP primer combinations resulted in 277 markers from which 86 did not segregate in the expected ratios. From the 93 RGA markers 31 showed deviation and were discarded, as well as 2 of the 6 SSR markers. This adds up to 257 polymorphic DNA markers showing no deviations from the expected segregation.

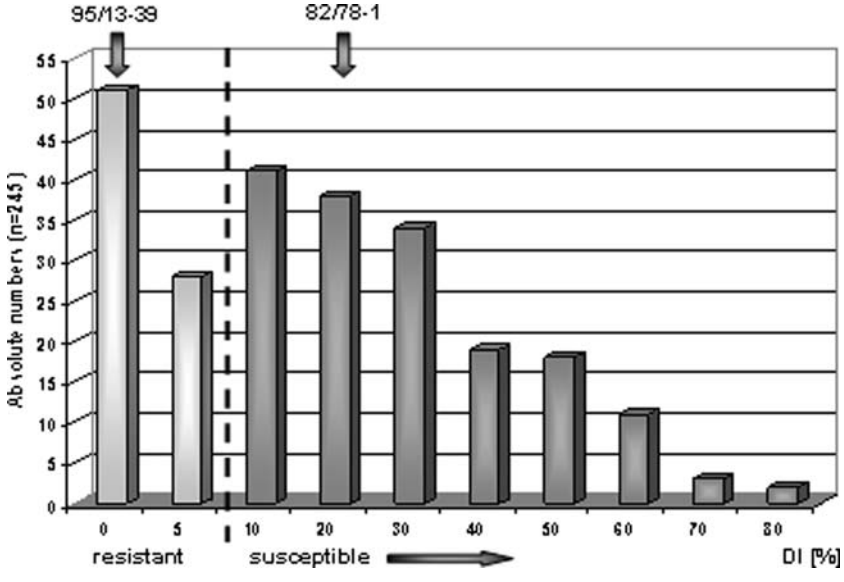

Fig. 1 Frequency distribution of the DI in the population 97/7 inoculated with the powdery mildew race 9. *DI*—leaf area covered with conidiophores at 10 dpi (mean of 5–8 replications)

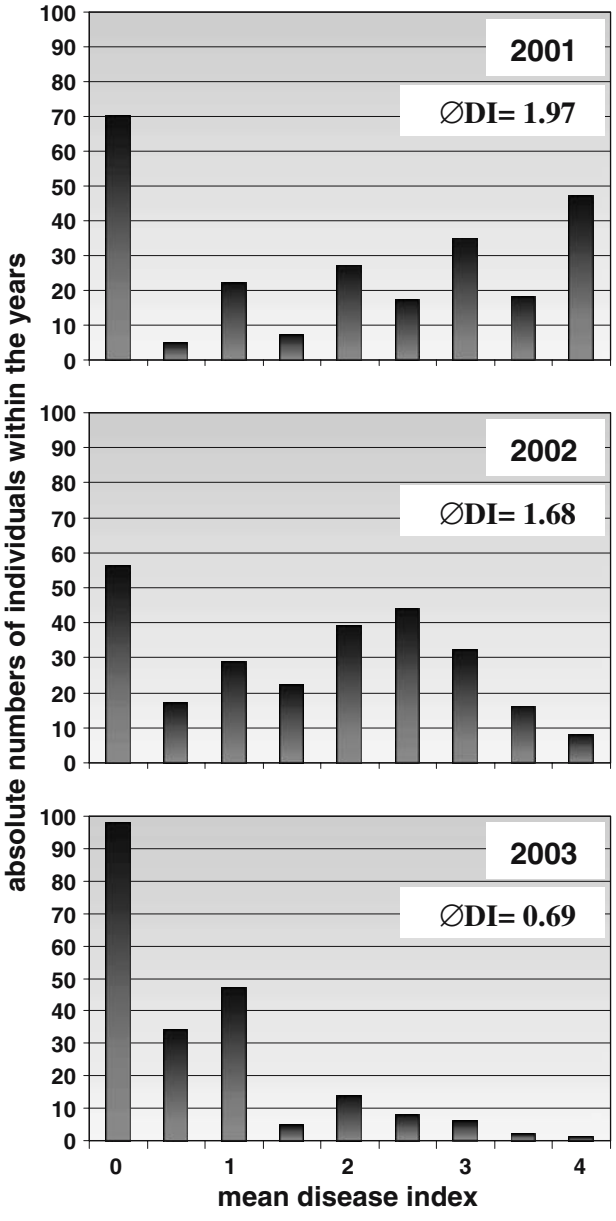

Fig. 2 Infection of the population 97/7 with powdery mildew during the years 2001–2003 in the greenhouse. Mean DI from two separate observations per year, each 6 weeks past inoculation with naturally occurring powdery mildew populations: *0* resistant, no symptoms; *1* susceptible, single young leaves infected; *2* some sprouts infected; *3* less than 50% of the leaves infected; *4* more than 50% infected

Parental maps

From the 257 DNA markers 249 could be integrated into the two parental linkage maps. Four RGA markers showing 100% identity and five additional markers causing distortions in the third round of mapping were excluded. After inspection of the LGs constructed with LODs from 2 to 10, the parental maps were built using an LOD threshold of 7. Each map consists of seven LGs corresponding to the seven rose chromosomes. The numbers of LGs for each parental map were quite stable from LOD 5 to 8, with some additional LGs containing only two markers appearing at LOD 8. The maps covered a total length of 370 and 354 cM for the parental plants 95/13-39 and Sp3 containing 141 (from 156 polymorphic markers) and 147 markers (from 165 polymorphic markers), respectively, from which 72 were bi-parental markers in each map. The mean marker distances were 2.6 and 2.4 cM and the largest gaps were 22 cM in the 95/13-39 map and 13 cM in the Sp3 map. The numbers of the LGs 1, 3, 4, 5 and 7 were assigned according to the maps of Debener and Mattiesch (1999) and Yan et al. (2005) by the common position of RGA markers and the locus for double flowers.

Integrated map

The integrated map was built by combining the homologous parental LGs according to the 72 bi-parental markers. Generally the marker order from the parental maps was recovered. For LGs 5 and 6, the arrangement of the bi-parental markers were taken as the fixed order because of some conflicting marker orders. The integrated map consisted of 233 markers [172 AFLPs, 50 RGAs, 4 SSRs, 4 morphological markers, 1 CAPS (Rd1), 1 SCAR and 1 BAC end-derived marker (Pr1)] spanning 418 cM over the seven LGs (Fig. 3). The average length of each group was ~60 cM, varying between 49 and 81 cM, with a mean marker distance of 1.8 cM. LG 3 contains the largest gap with 21 cM. The four morphological markers were added to the integrated map in a final step. They were mapped at the ends of the LGs 1 (*black spot*) and 3 (*prickles*, *double flowers* and *white stripes*). The 50 RGAs showed an uneven distribution over the seven LGs. Thirty-seven of them were mapped on LG 7 and only three to five on LGs 1, 4 and 5.

QTL analysis

Using a significance threshold of 3.9 for the mean LG length of 60 cM (Van Ooijen 1999) altogether 28 QTLs (Table 1) could be detected for the nine disease scores under analysis (Table 1; Fig. 3). Four of these (14%) could only be detected using MQM or restricted MQM mapping. Whereas five putative QTLs could be detected for *PM Out 01 + 02* only two QTLs could be resolved for the disease scores *PM In 02* and *PM In 03*.

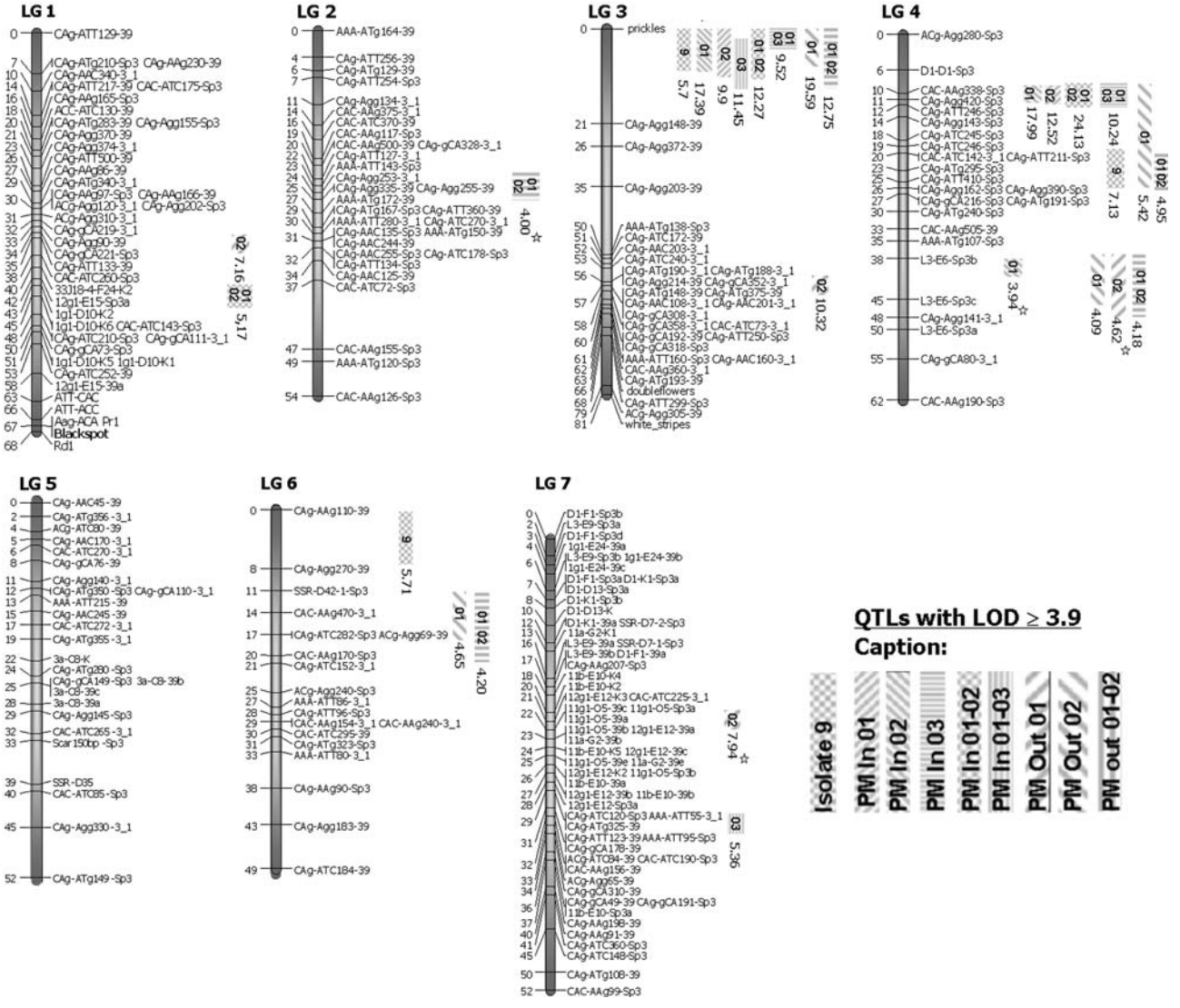

Fig. 3 Integrated linkage map of the diploid population 97/7. Genetical LGs are numbered from *1* to *7*. Marker names are indicated at the *right* of each LG. Distances are given in Kosambi cM at the *left*. The positions of the QTLs with an LOD > 3.9 are given by *bars* representing 1 LOD support intervals to the *right* of the markers. LOD scores for the QTLs are noted *below* the *bars*. QTLs marked with (*asterisk*) were only detected using multiple-QTL mapping

Powdery mildew resistance

Two regions controlling a high percentage of the explained phenotypic variance for nearly all evaluations concerning powdery mildew resistance were detected on LGs 4 and 3 (Fig. 3). The cluster around the prickles locus on LG 3 contains QTLs explaining about 65–80% of the variability for all resistance measurements excluding *PM Out 02* for which a QTL explaining 50% of the variability was detected at a distance of about 50 cM on LG 3. A second resistance cluster explaining a high amount of variability was detected on LG 4 spanning about 10 cM. Also in this cluster no QTL was detected for *PM Out 02*. However, a QTL with an LOD score of 4.6 for *PM Out 02* was detected about 30 cM apart.

No QTL was found within this cluster on LG 4 for *PM In 03* either, whereas for the resistance to powdery mildew in the greenhouse during the years 2001 and 2002 (*PM In 01* and *PM In 02*) strong QTLs with LOD scores of 18 and 12.5 could be located in this region (Figs. 3, 4). No significant QTL for powdery mildew resistance could be detected on LG 5.

For the resistance to the PM isolate 9 we could detect three significant QTLs. Two of these are located in the clusters on LGs 4 and 3, and one with an LOD of 5.7 is located on the top of LG 6, 9 cM from a QTL for *PM Out 01*. All PM-related QTLs, with the exception

Table 1 QTLs detected using SIM (simple interval mapping) and MQM (multiple QTL model) mapping with an LOD significance threshold > 3.9

Disease score	LG[a]	QTL position	cM	LOD	% var[b]	±1 1 LOD confidence interval	Length[c]
Isol 9	3	Prickles	0.0	5.7	64.6	Off–prickles+8	–3;-[d]
	4	CAg-ATg295-Sp3	20.2	7.13	9.4	CAg-ATC246-Sp3–CAg-ATT410-Sp3	5.2
	6	CAg-AAg110-39+5	5.0	5.71	10.3	CAg-AAg110-39+2 CAg-Agg270-39+2	8.0
PM In 01	3	Prickles	0.0	17.39	78.8	Off–prickles+7	7.0
	4	CAC-AAg338-Sp3	9.9	17.99	78.7	D1-D1-Sp3+4–CAg-Agg420-Sp3	1.9
		L3-E6-Sp3b+5[e]	49.9	3.94	3.9	AAA-ATg107-Sp3–CAg-Agg141-3_1	13.2
PM In 02	3	Prickles	0.0	9.90	72.5	Off–prickles+10	10.0
	4	CAC-AAg338-Sp3	9.9	12.52	71.5	D1-D1-Sp3+4–CAg-Agg420-Sp3	1.9
PM In 03	3	Prickles+10	10.0	11.45	64.3	Prickles+5–prickles+13	8.0
	7	CAg-ATg325-39	29.3	5.36	26.6	AAA-ATT55-3_1–CAg-ATT123-39	4.6
PM In 01+02	1	CAC-ATC260-Sp3	31.0	5.17	4.2	CAg-Agg90-39+2–1g2-D10-K2	9.0
	3	Prickles	0.0	12.27	74.2	Off–prickles+8	–-
	4	CAC-AAg338-Sp3	9.9	24.13	77.6	D1-D1-Sp3+4–CAg-Agg420-Sp3	1.0
PM In 01-03	3	Prickles	0.0	9.52	71.6	Off–prickles+2	2.0
	4	CAC-AAg338-Sp3	9.9	10.24	68.1	D1-D1-Sp3+4–CAg-Agg420-Sp3	5.4
PM Out 01	3	Prickles	0.0	19.59	80.6	Off–prickles+6	6.0
	4	CAC-AAg338-Sp3	9.9	5.42	25.0	D1-D1-Sp3+3–CAg-ATT410-Sp3	16.0
		L3-E6-Sp3c	44.9	4.09	12.1	L3-E6-Sp3b–L3-E6-Sp3c+1	7.7
	6	CAC-AAg470-3_1	14.0	4.65	13.6	SSR-D42-1-Sp3–ACg-Agg69-39	5.9
PM Out 02	1	CAg-Agg90_39	32.9	7.16	42.8	CAg-gCA219_3_1–Cag-gCA221-Sp3	1.7
	3	CAg-ATg148-39	56.8	10.32	48.1	CAg-gCA352_3_1–CAg-ATg375-39	0.7
	4	L3-E6-Sp3b+5[e]	43.2	4.62	35.9	L3-E6-Sp3b–CAg-Agg141-3_1	10.2
	7	11g1-O5-39a[e]	22.4	7.94	28.0	11g1-O5-39c–11g1-O5-39b	1.0
PM Out 01+02	2	CAg-Agg335-39[e]	24.8	4.0	3.4	CAg-Agg253-3_1–AAA-ATg172-39	3.4
	3	Prickles	0.0	12.75	76.6	Off–prickles+10	10.0
	4	CAg-ATg295-Sp3	22.6	4.95	15.6	CAg-ATT211-Sp3–CAg-ATT410-Sp3	4.4
		L3-E6-Sp3c	44.9	4.18	13.1	L3-E6-Sp3b–CAg-Agg141-3_1	10.2
	6	CAC-AAg470-3_1	14.0	4.20	13.4	SSR-D42-1-Sp3–CAC-AAg170-Sp3	9.8

[a] Linkage group

[b] Percentage explained variability by the particular QTL, values taken only from SIM

[c] Length of the confidence interval in cM

[d] No length value if the confidence interval starts or ends at the end of a LG

[e] QTLs only with MQM mapping detected

of *PM In 03*, showed effects coming from both parental plants. The alleles of the susceptible parent Sp3 contributed on average 1/3 to the explained variance for the QTLs. Looking at resistance to isolate 9, the alleles of the resistant parent 95/13-39 were responsible for 75 of the total 84% explained variability.

Powdery mildew QTLs and RGAs

Only a few QTLs for powdery mildew resistance could be detected in regions containing RGAs. No RGAs could be mapped close to the two large clusters for resistance QTL (Fig. 3). One QTL explaining about 43% of the phenotypic variability for *PM Out 02* was mapped on LG 1 near to five RGAs. A second one was mapped in a large cluster of RGAs on LG 7. One minor QTL was detected on LG 1 near to the RGA marker 33J18-4-F24-K2 for *PM In 01 + 02* and another one on LG 4 for *PM Out 01* and *PM Out 01 + 02* near L3-E6-Sp3c which may be the same QTL because it was calculated using partly the same data (from *PM Out 01*). About 4 cM apart from the large resistance QTL cluster on LG 4 one single RGA could be mapped. No RGAs mapped in the vicinity of the QTLs for the resistance to isolate 9.

Discussion

A significant portion of the cost and time in QTL analyses of newly established mapping populations is spent for DNA isolation and marker genotyping of the large populations are required. Selective genotyping is almost as or sometimes even more effective and reliable than the standard marker-based strategy for detecting linkage between marker loci and QTLs,

while considerably reducing genotyping costs. This is because those phenotypes with extreme values carry large numbers of either positive or negative QTL alleles and thus provide more linkage information than the other individuals (Ayoub and Mather 2002; Johnson et al. 1999; Lander and Botstein 1989; Zhang et al. 2003). From the 270 available genotypes in population 97/7 only the 170 individuals with a DI < 3.0 or > 30 from the resistance test using race 9 were genotyped. This saved 3800 PCR reactions for the 38 selected AFLP, RGA, SSR and SCAR primers while possibly retaining the same statistical power to detect QTLs. Biased estimates of QTL effects were prevented by including the phenotypic values of the 100 remaining non-extreme individuals into the analysis with their genotypes entered as missing values (Lander and Botstein 1989; Xu and Vogl 2000). The possible bias should be small due to the close marker spacing around the putative QTL positions (Darvasi and Soller 1992).

Linkage map and qualitative markers

With a total length of 418 cM and LG sizes between 49 and 81 cM the integrated map is about 20% shorter than the assumed length of around 500 cM for diploid roses by Yan et al. (2005) using the mean chiasma frequency and the average nuclear DNA content. The sizes of the five other rose linkage maps vary between 238 and 902 cM for single parental maps with mapping population sizes between 52 F_2 plants for the largest map (Rajapakse et al. 2001), and 91 F_1 plants for the shortest map from a pseudo-double-testcross (Crespel et al. 2002). The linkage maps of Debener and Mattiesch (1999) and Dugo et al. (2005) calculated from 60 and 96 F_1 plants, respectively, were of intermediate sizes. Therefore, according to its size, this map is within the range of the other rose linkage maps and near to the estimated length from Yan et al. (2005), yet it is derived from a larger mapping population of 170 genotyped individuals and up to 270 plants for QTL analyses.

The *Rdr1* locus conferring resistance to the black spot isolate DortE4 was mapped together with the RGA markers 12-g1-E15-39a and 1g2-D10, which is in accordance with the map of the rose population 94/1 where the *Rdr1* marker was also mapped together with these RGAs (Yan et al. 2005). The other three morphological markers for the occurrence of *prickles*, *double flowers* and *white stripes* were mapped on both ends of LG 3 (Fig. 3). At the *prickles* marker one of the two clusters of powdery mildew resistance QTL was observed. Interestingly, Yan et al. (2005) could also map a qualitative locus for powdery mildew resistance on the same LG with a locus for *double flowers* called Blfo-2 using the data observed by Debener and Mattiesch (1999) and Debener et al. (2001). Unlike the linkage map of Crespel et al. (2002) we located the loci for *prickles* and *double flowers* on one LG whereas in their map, based on a progeny of 91 genotypes from a cross of diploid *Rosa wichuriana* and a dihaploid rose, the locus *d6* for *double flowers* was mapped on LG A6 and the major and minor QTLs for the number of prickels *t4* and *t4b* were located on LG B4.

RGA clustering

A large cluster of 37 NBS-LRR RGAs was observed on LG 7 and a minor one with 6 RGAs on LG 1 (Fig. 3) amplified by the same or different specific primer pairs. Four RGAs each were located on LGs 4 and 5. Clustering to a minor extent was also obtained analysing a smaller set of these RGAs in the population 94/1 by Yan et al. (2005). Also in various other plant species such as *Prunus* species (Lalli et al. 2005), *Lycopersicon esculentum* (Zhang et al. 2003), *Oryza sativa* (Ramalingam et al. 2003), *Glycine max* (Kanazin et al. 1996) and *Arabidopsis thaliana* (Aarts et al. 1998) clustering of RGAs or resistance genes was demonstrated. The clustering of NBS-LRR resistance genes is thought to originate from tandem and segmental duplications of these genes in the genome of flowering plants as reviewed by Leister (2004).

Co-localization of RGA and QTLs

From the 28 resistance-related QTLs only 4 were detected in regions with RGA markers. This may be due to the strong clustering of RGAs on LGs 1 and 7 and the low coverage of the other LGs with RGAs. Half of the resistance-related QTLs were concentrated in two regions on LGs 3 and 4 with no RGAs. On the other hand, we only mapped RGAs from the NBS-LRR class in this population, which is indeed the most abundant class of resistance genes but is only one of five (Lalli et al. 2005). Since degenerated primers from TIR-NBS-LRR RGAs were used, it is likely that other RGAs are overlooked and therefore it is reasonable to find only a subset of QTLs co-located with the mapped NBS-LRR RGAs. In the *Blumeria graminis–Hordeum vulgare* interaction, only two out of eight powdery mildew resistance QTLs could be detected near NBS-LRR RGAs on LG 2Ha and 3H (Backes et al. 2003). Also in *Arabidopsis RPW10* the major QTL detected for powdery mildew resistance, accounting for 45% of the explained variability, is not a member of the class of NBS-LRR RGAs. By fine mapping it was shown that *RPW10* is allelic to the cloned *RPW8* locus which

encodes two non-NBS-LRR small novel proteins (Wilson et al. 2001; Xiao et al. 2001). Beside these results another cloned gene regulating powdery resistance was shown to be a MAPKK kinase (Frye et al. 2001). A similarly low co-localization for powdery mildew resistance QTLs and RGAs was also detected in rice, where only one out of six bacterial blight QTLs was located near an RGA on LG 3 using simple regression (Ramalingam et al. 2003).

QTLs for resistance to powdery mildew

The distribution of the 28 significant QTLs for powdery mildew resistance showed the contribution of alleles from both parents. The susceptible parent Sp3 contributed on average about 31% of the explained variability for all resistance-related QTLs (Table 1; Fig. 3). The presence of favourable QTL alleles in both parents is also suggested by the transgressive segregation for resistance against race 9 and the medium degree of susceptibility of the genotype Sp3. Similar results were observed for resistance to *Erysiphe graminis* in wheat (Keller et al. 2005), where ten QTLs showed effects from the alleles of the resistant parent Forno and eight resistance QTLs originated from the medium susceptible parent Oberkulmer. Such results were also reported for *Cercospora zeae-maydis* on maize (Bubeck et al. 1993).

Although we mapped 28 QTLs for powdery mildew resistance it is clear that we surely failed to detect some minor QTLs, since the population size of 270 individuals is still too small for high-resolution QTL mapping with the use of mainly dominant markers. In addition, only resistance QTLs could be identified which are induced by the limited spectrum of pathogen races used in the experiment. In a review of 85 QTL studies on the architecture on disease resistance, mainly against fungal pathogens, Kover and Caicedo (2001) reported that when a single pathogen strain was used only 32.5% of the LGs showed a resistance QTL. In eight of the ten studies dealing with different pathogen strains on the same mapping population strain specific resistance QTLs were located. This could also be proposed for this study, where we detected an additional QTL for resistance to race 9 on the top of LG 6 explaining about 10% of the genetic variation in a region were no other resistance QTL could be detected. Different QTLs for PM resistance from field observations and from tests with detached leaves could be explained by the different population structures of the inoculum: a mixture of different strains on one hand and a defined isolate on the other, as was demonstrated for barley (Backes et al. 2003).

Overall, a strong clustering of QTLs for powdery mildew resistance over all environments was observed on LGs 3 and 4 which was also predicted by the correlations using the Cramers *V* test. Such clustering indicate a general function of PM disease resistance, with these regions acting on different PM strains in different environments. For the resistance against natural infection with PM in the greenhouse we located highly significant QTLs in the years 2001 and 2002 with LODs of 18 and 12.5 near the RGA D1-D1-Sp3 on LG 4 (Fig. 4). With the data observed in the year 2003 this QTL could not be detected, but a new QTL on LG 7 with an LOD of 5.4 appeared in a region were no significant gene action could be located in the 2 years before. This could be due to at least two points:

(1) In comparison to the years 2001 and 2002 the mean DI in the year 2003 (0.69, Fig. 2) was much lower than in the years before with values of 1.97 and 1.68. This lower mean DI in 2003 could have resulted from environmental conditions unfavourable for *P. pannosa* in that year in the greenhouse or by a generally lower infection pressure. Therefore the effects of the QTL alleles on LG 4 may have been much lower in the year 2003 and consequently could not be detected.
(2) There could have been a change in the population structure of *P. pannosa* from the years 2001 and 2002 to the year 2003, with the disappearance of one or several pathogenic strains interacting with the particular QTL alleles on LG 4 in 2003, which then would not have been detected any more. Another indication of a shift in the pathogen population structure could be the appearance of an additional QTL with an LOD of 5.4 on LG 1, which could only be detected in 2003.

A shift in the population structure of *B. graminis* on barley was also proposed as one possible reason for the differences in early and late powdery mildew observations within 1 year by Backes et al. (2003). Varying QTL patterns were also detected by Keller et al. (1999) assessing powdery mildew resistance in bread wheat over 2 years in five environments. They detected 18 QTLs from which only two major loci were stable over all five environments, whereas 5 QTLs were only detected in the year 1995 or 1996 in a single test field.

Our research in the rose population 97/7 provides further insight into the nature of resistance against powdery mildew in roses, above the level of mapping single dominant resistance genes like *Rpp1* (Linde et al. 2004) or QTL analyses in a single environment (Lalli et al. 2005). We were able to detect resistance QTLs stable over different environments and pathogen

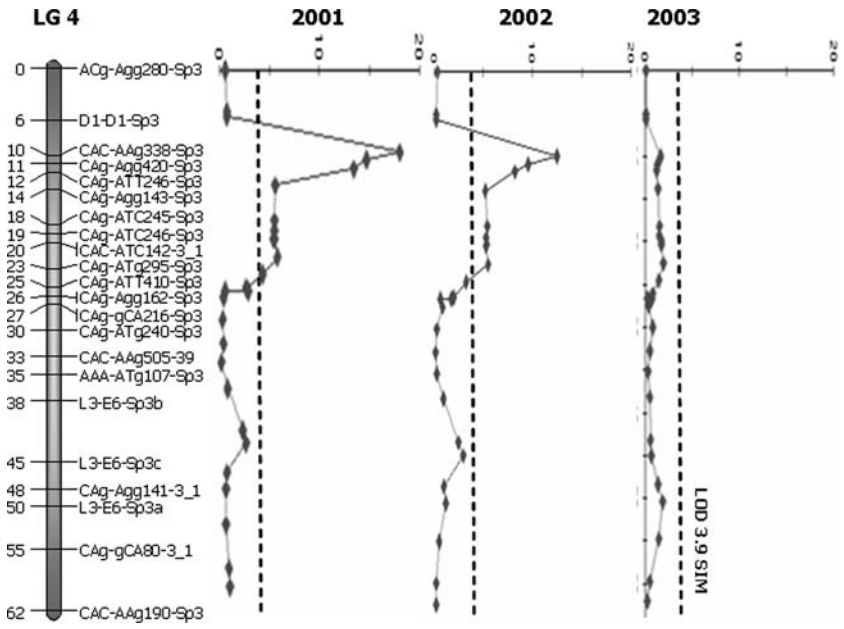

Fig. 4 LOD score curves on LG 4 obtained for the resistance to powdery mildew in the greenhouse in the years 2001–2003. The LOD significance threshold of 3.9 is shown as a *dashed line*

races and also race and environment specific QTLs. A closer connection of this map to the one from Yan et al. (2005) could make our results more powerful than they already are. The most suitable marker type for this goal would be SSRs which were used by Yan et al. (2005). These common markers could also serve as anchor points for future rose linkage maps.

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