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E. Dirlewanger · T. Pascal · C. Zuger · J. Kervella

Analysis of molecular markers associated with powdery mildew resistance genes in peach (*Prunus persica* (L.) Batsch)×*Prunus davidiana* hybrids

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Abstract A progeny of 77 hybrids issued from a cross between two heterozygous *Prunus*, peach [*P. persica* (L.) Batsch] (variety 'Summergrand') and a related species, *P. davidiana* (clone 1908), was analysed for powdery mildew resistance in five independent experiments. This population was also analysed for its genotype with isoenzyme and RAPD markers in order to map the genes responsible for resistance. A genetic linkage map was generated for each parent. The 'Summergrand' linkage map is composed of only four linkage groups including 15 RAPD markers and covering 83.1 centiMorgans (cM) of the peach nuclear genome, whereas the *P. davidiana* linkage map contains 84 RAPD markers and one isoenzyme assigned to ten linkage groups and covering 536 cM. Significant associations between molecular markers and powdery mildew resistance were found in each parent. For *P. davidiana*, one major QTL with a very strong effect and five other QTLs with minor effects were located in different linkage groups. For 'Summergrand', three QTLs for powdery mildew resistance, with minor effects, were also detected. Consequently, evidence is given here that the powdery mildew resistance of *P. davidiana* clone 1908 and *P. persica* variety 'Summergrand' is not a monogenic character but is controlled by at least one major gene and several minor genes.

Key words RAPD markers · Linkage map · QTLs · Peach, *Prunus* · Powdery mildew

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E. Dirlewanger (✉) · C. Zuger
INRA, Unité de Recherches sur les Espèces Fruitières et la Vigne, Centre de Recherches de Bordeaux,
BP 81, 33883 Villenave d'Ornon Cedex, France

T. Pascal · J. Kervella
INRA, Station de Recherches Fruitières Méditerranéennes, Centre d'Avignon, Domaine de Saint Paul,
84140 Montfavet, France

Introduction

Peach (*Prunus persica* (L.) Batsch) breeding programmes generally focus on fruit improvement. However, several diseases are responsible for economic losses, and one of these, powdery mildew, is one of the most serious diseases in peach production areas throughout the world. In French orchards, powdery mildew caused by *Sphaerotheca pannosa* (Wallr.) var '*persicae*' is the fungal disease that requires the largest amount of chemical spraying. This fungus induces the formation of circular white spots on leaves, shoots and fruits. Seriously damaged leaves shrivel and may fall off, and contamination occurs early on the fruits, growth stops at the place of contamination, causing deformations and frequently a premature fall.

Resistances to powdery mildew have been studied in many species. Complete mono- or digenic resistances have been described, for example, in barley (Lundqvist et al. 1991), soybeans (Lohnes and Bernard 1992), muskmelon (McCreight et al. 1987), mungbeans (Reddy et al. 1994) and apple trees (Knight and Alston 1968). As powdery mildew pathogens are obligate parasites, for which isolates are difficult to maintain (Korban and Riemer 1990), corresponding virulent and avirulent strains have been identified in only a few cases including barley (Brown et al. 1993) and muskmelon (McCreight et al. 1987). Cases in which these monogenic resistances have been broken by new pathogen races have often been reported (Wolfe and Schwarzbach 1978 in Balkema-Boomstra and Mastebroek 1993; Ibrahim and Barrett 1991; Brown et al. 1993). Consequently, breeding programmes for the selection of polygenic partial resistance have been recently initiated for barley (Saghai-Maroof et al. 1994; Heun 1992) and rye (Miedaner et al. 1993).

Some major genes influencing the powdery mildew resistance of peach trees have been identified. Varieties without leaf glands, a monogenic character, are very susceptible (Rolfs 1907). For this reason, all cultivated varieties selected have leaf glands. In addition, a complete resistance, controlled by a single dominant gene, was detected

in the 'Ustoichivy Pozdni' variety (Tsukanova et al. 1982). Two loci were reported to be involved in the resistance of 'Ferganensis jaune'; the first one controls the presence and type of leaf glands and the second one has a epistatic effect upon the first one, with the dominant allele conferring complete resistance to powdery mildew (Dabov 1974; 1975; 1983).

This paper reports a first step in studying the genes involved in the resistance to powdery mildew of another possible donor parent for peach breeding: *Prunus davidiana* clone 1908. *P. davidiana* is a species closely related to *P. persica*, a native of the arid region west of Lanzhou in North-west China (Li 1984). Interspecific hybridisation between peach and related species has been carried out to provide resistance sources in rootstock and cultivar breeding because of the narrow base of cultivated peach germplasm (Scorza et al. 1985).

The establishement of a breeding strategy requires a knowledge of the mode of inheritance – mono-, oligo- or polygenic – of powdery mildew resistance. Oligo- or polygenic resistances are often reported as being more durable than monogenic resistances (Fischer et al. 1994; Wang et al. 1994). However, when several resistance genes are initially involved in a donor parent, some of them may be lost during the breeding programme (Parisi et al. 1993). The chance of losing resistance genes can be reduced if they are detected early. This is particularly useful when the breeding process is very long, e.g. when exotic germplasm is used as a resistant parent or when generations are long, as in fruit trees.

Our approach was to characterise a *P. persica* (L.) Batsch×*P. davidiana* hybrid progeny using isoenzymes and random amplified polymorphic DNA (RAPD) markers and to score the hybrids for powdery mildew resistance. Linkage maps were established for the two parents of the hybrids ('Summergrand' and *P. davidiana* clone 1908) and quantitative trait loci (QTLs) affecting powdery mildew resistance were localised.

Materials and methods

Plant material

A progeny of 77 hybrids was obtained from an interspecific cross between *P. persica* 'Summergrand' and *P. davidiana* clone 1908. Both species are diploid with 2n=2x=16. 'Summergrand' is a yellow nectarine cultivar with leaf glands that is susceptible to powdery mildew. *P. davidiana* clone 1908 is an accession used as a potential source of resistance to several pests and diseases.

Evaluation of powdery mildew resistance

To obtain a more reliable assessment of their resistance to powdery mildew, we evaluated the hybrids as seedlings and as grafted plants, both in the greenhouse and the nursery, in five independent trials (Table 1).

In the first two trials, hybrids were evaluated as seedlings in Avignon, first in the greenhouse, 2 months after sowing (trial S1), and then, after transplanting, in the nursery (trial S2). In the last three trials, hybrids were evaluated as grafted plants in Avignon, in two nurseries neighbouring the seedling nursery (trials G1 and G2), and in Gotheron (trial G3). Avignon and Gotheron are both located in the South-east of France. Each hybrid was present once in each trial. 'Summergrand' and *P. davidiana* were used as susceptible and resistant controls, respectively, in trap nurseries.

In the greenhouse, the plants were contaminated by repeated sprinkling from powdery mildew-infected organs. In trap nurseries, a variety without leaf glands (S3215), planted every six plants, pro-

Table 1 Observations for powdery mildew resistance

Type of hybrids	Location	Growth conditions	Trial	Date	Observer	Evaluation code
Seedlings	Avignon	Greenhouse	S1	2 October 1987	A	$S1_{1A}$
				16 October 1987	A	$S1_{2A}$
		Nursery	S2	21 July 1988	A	$S2_{1A}$
					B	$S2_{1B}$
				18 August 1988	A	$S2_{2A}$
				23 September 1988	A	$S2_{3A}$
					B	$S2_{3B}$
				13 July 1989	A	$S2_{4A}$
				12 July 1990	A	$S2_{5A}$
Grafted	Avignon	Nursery (block 1)	G1	7 July 1989	A	$G1_{1A}$
				5 August 1989	A	$G1_{2A}$
				22 September 1989	A	$G1_{3A}$
		Nursery (block 2)	G2	7 July 1989	A	$G2_{1A}$
				5 August 1989	A	$G2_{2A}$
				22 September 1989	A	$G2_{3A}$
	Gotheron	Nursery	G3	19 July 1990	A	$G3_{1A}$
					C	$G3_{1C}$
				2 August 1990	C	$G3_{2C}$
				16 August 1990	A	$G3_{3A}$
					C	$G3_{3C}$
				21 October 1990	C	$G3_{4C}$

Avignon and Gotheron are both located in the South-east of France

vided permanent inoculum and homogenous contamination throughout the growing season.

Hybrids were evaluated by the same observer (A) on several dates in all the trials (Table 1). In addition, two other observers (B and C) scored the hybrids independently, in trials S2 and G3, respectively, on two evaluation dates. Therefore, each hybrid was evaluated 21 times For all the evaluations, except the fourth and fifth ones in trial S2, the trees were young and had not yet flowered.

For each evaluation, hybrids were given a score ranging from 0 (most resistant) to 4 (most susceptible). The classes used were as follows: 0 (no damage), 1 (one or two spots on the whole plant), 2 (scattered spots, on several leaves), 3 (coalescent spots on many leaves), 4 (white spots completely covering leaves and young stems).

RAPD

DNA was extracted from young expanding leaves using a CTAB procedure (Saghai-Maroof et al. 1984). DNA was diluted to a working concentration of 15 ng/µl by fluorimetric measure. RAPD reactions were performed as described by Williams et al. (1990) using 30 ng of template, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 200 m*M* Tris, 100 µ*M* dNTP, 1 ng/µl of 10-base primers from Operon Technologies Kits A-AD (Operon Technologies, Alameda, Calif.) and 0.04 U/µl of *Taq* DNA polymerase. Reactions were performed in a final volume of 12 µl per sample in a 96-well Perkin Elmer GeneAmp PCR System 9600. The samples were held at 95°C for 1 min and were then subjected to 40 polymerase chain reaction (PCR) cycles of melting at 94°C for 10 s, annealing at 37°C for 15 s, extending at 72°C for 75 s. RAPD fragments were separated on 1.5 % agarose gels that were subsequently stained with ethidium bromide.

The primers were first screened on the parents. Whenever differences between the parents were found, 10 hybrids were then tested to see if the candidate markers segregated in the hybrid progeny. If no difference was found between the parents, the primer was not tested on the progeny. The reason is that if segregation occurred then in the progeny, marker mapping would not be very accurate since both parents would be heterozygous, and heterozygous and dominant homozygous genotypes, both expected in the progeny, can not be distinguished with RADP dominant markers (Ritter et al. 1990).

Isoenzymes

Three isoenzymes (α-amylase, alcohol dehydrogenase and malate dehydrogenase) were tested. Proteins were extracted from lyophilised young leaves using the procedure described by Monet and Gibault (1991). The extracts were subjected to a 7.5% acrylamide gel electrophoresis (16 V/cm) and examined by staining the gels for specific enzyme activity.

Linkage analysis

RAPD markers for genomic mapping were chosen on the basis of repeatability and inheritance. All RAPD markers used for the map were tested at least twice to confirm repeatability of the results. Segregation data were tested for deviation from the expected 1:1 Mendelian ratio for isoenzyme alleles and for presence/absence of RAPD marker bands using a Chi-square test. RAPD markers demonstrating significant deviation ($P<0.05$) were not used to construct the framework linkage map.

The linkage relationships of the markers were analysed with Mapmaker (Lander et al 1987) version 2.0 for the Macintosh. The genetic model of segregation data for hybrids from heterozygous parents is the 'F_2 backcross' model, which is analogous to a testcross with unknown parental linkage phase. Two maps were constructed according to the two-way pseudo testcross mapping strategy (Grattapaglia et al. 1994). The 'Summergrand' map is composed of loci heterozygous in 'Summergrand' and homozygous in *P. davidiana*, whereas the *P. davidiana* map is composed of loci heterozygous in *P. davidiana* and homozygous in 'Summergrand'. Linkage groups were obtained by choosing 0.3 as the maximum recombinant fraction and 6 as the minimum LOD score value. The Kosambi mapping function was used to convert recombination fractions into map distances.

QTL detection

Analyses were performed separately for each evaluation of powdery mildew resistance. Both the Maximum Likelihood (ML) interval mapping method (Lander and Botstein 1989) and the standard multiple regression of resistance on markers were used to declare significant marker-trait associations. The ML interval mapping method of QTL detection is more efficient for accurately mapping and estimating the effect of a single QTL, while linear multiple regression methods are more robust to departures from normality (discrete ordinal data in our case). The latter allow possible interfering effects between QTLs to be taken into account and thus may be more powerful in detecting chromosome regions with minor effects.

Mapmaker/QTL (Lincoln et al. 1992a, b) with a LOD score threshold of 2.00 was used to perform ML interval mapping. This threshold is intentionally not very high in order to reduce the chance of comitting type-II errors.

A stepwise procedure was first used for multiple regression analysis. The number of markers exceeded the number of hybrids in the progeny. Therefore, all of the markers could not be involved simultaneously. Three subsets of markers were considered sequentially. A stepwise procedure was applied to the first subset. The markers selected at this first stage were then considered together with the markers of the second subset in a new stepwise procedure. This provided a new set of selected markers, which were subsequently involved with the third subset of markers in a last stepwise procedure. The markers from *P. davidiana* were shared between two subsets: the other subset included the markers from 'Summergrand'. Several sequences of the three subsets were considered because the selection of a new regressor by a stepwise procedure may depend on the regressors already selected. For the same reason, the marker with the highest effect in the Mapmaker/QTL analysis (O20-0.98 marker from *P. davidiana* linkage group 3) was always included in the first subset considered.

A large number of markers were selected at least for one evaluation: those significant at the 0.05 level for only one evaluation were discarded at the end of the stepwise procedures.

A model including all of the markers retained at that stage was fitted to each set of evaluation data. When all of these markers were involved, some markers that were selected after the stepwise procedure were no longer significant at the 0.05 level for any of the evaluations. These were discarded to define the ultimate set of markers.

Results

Isoenzyme and RAPD analysis

Among the three isoenzymes used, only α-amylase revealed polymorphism. *P. davidiana* (clone 1908) was heterozygous with a slow and a fast allele and 'Summergrand' was homozygous for the slow allele. Segregation followed a 1:1 ratio ($\chi^2=0.18$) with 37 individuals homozygous for the slow allele and 40 heterozygous.

For RAPD, 279 primers out of the 500 studied (55.8%) revealed polymorphism between 'Summergrand' and *P. davidiana* and were tested on 10 hybrids. Among them, only 54 primers (19.2%) also revealed polymorphism within the progeny. With these 54 primers, a total of 111 RAPD markers were detected (2 markers per primer on

Table 2 Origin and segregation behaviour of RAPD markers segregating in 'Summergrand' × *P. davidiana* hybrids. Each RAPD marker is designated by its Operon primer code and its size in kilobase pairs. Chi-square values are given for 1:1 segregation if the band is present in 'Summergrand' (*S*) or in *P. davidiana* (*d*)

Operon primer code RAPD fragment size (kb)		Origin	χ^2
A02	1.91	d	0.12
A04	2.60	d	0.33
	1.75	d	1.57
	0.95	S	1.70
	0.78	S	0.33
	0.50	d	2.12
A07	1.39	d	4.69*
	0.80	d	1.95
	0.53	d	1.66
	0.47	d	1.95
A09	0.86	d	10.56**
A10	1.39	d	2 72
	0.52	d	0.12
	0.42	d	0.47
A11	2.17	d	1.57
A13	1.41	d	0.32
	0.61	d	0.12
A19	1.80	S	2.00
B17	0.89	d	0.01
	0.85	S	9.13**
B18	0.82	d	3.46
	0.25	S	1.05
I02	0.62	d	9.47**
I06	1.50	d	0.01
	0.90	d	0.05
	0.48	d	0 01
I07	0.54	d	4.26*
I11	0.76	d	1.35
	0.69	d	0.22
I13	1.99	d	4.63*
	1.34	d	0.01
	1.10	d	2.25
I14	0.57	d	11.21***
I16	2.06	d	1.66
	0.90	d	5.26*
	0.74	d	0.00
I18	1.81	S	1.89
	0.65	S	0.47
I20	1.53	d	2.25
	0.66	d	2.25
O06	1.50	d	1.61
	0.70	d	1.08
O07	1.66	d	3.76
O10	1.42	S	1.25
	0.76	d	0.69
O12	1.30	S	6.87**
	0 48	d	2 00
O20	1.50	d	0.12
	1.24	S	5.72*
	1.03	S	14.14***
	0.98	d	2.19
	0.87	S	3.45
	0.56	S	2.64
	0.36	d	0.17
	0.29	d	0.32
Q05	1.13	d	2.65
	0.75	d	0.67
	0 74	S	0.47
Q06	2.68	S	0.86
	0.85	d	2.25
	0.84	S	6 21*
	0.35	d	1.89
Q13	1.70	d	0.01
	1.54	d	0.05
	0.50	S	0.48
U10	1.03	d	0.63
	0.62	S	2.64
	0.49	d	3.75
	0.36	d	0.12
X15	1.44	d	4.69*
	1.20	d	1.61
Y07	0.81	d	3.75
Y16	1.55	d	1.05
Z06	0.97	d	0.37
Z07	1 28	d	0.21
	0.92	d	1.94
Z12	0.84	d	1.35
Z13	1.81	d	3.65
Z17	0.72	d	0.33
Z19	0.67	d	0.63
AA01	1.30	d	0.11
	1.04	d	2.92
AA03	0.58	d	4.69*
AA04	1.52	d	1.05
AA09	1.57	d	3.08
	1.10	S	2.00
	1.05	d	3.45
	0.90	d	0.00
	0 87	d	0.32
AA11	1.39	d	0.11
	1.10	d	14.14***
AA16	1.37	d	0.94
	1 11	d	1 05
	0.79	d	3.00
AA17	1.56	S	1.05
AA18	1.30	d	4.26*
AB02	2.19	d	1 14
AB04	1.14	d	1.65
AB11	1.10	d	0 94
AB18	1.92	d	0.65
AB19	1.81	d	3.85
AC02	1.98	d	1.14
	1.45	S	0.22
AC07	2.47	S	13.47***
	2.20	d	13.47***
	1.49	d	4.68*
	1.21	S	0.11
	0.64	d	7.45**
AD04	1.82	d	0.01
	0 53	d	1.57
Ad11	1.13	S	0.33

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

average). Four primers revealed heterozygous loci in 'Summergrand' exclusively, 37 in *P. davidiana* and 13 in both parents. Among the 111 RAPD loci identified, 87 were heterozygous in *P. davidiana* and 24 in 'Summergrand'. Polymorphic fragments ranged from 0.5 kbp to 2.5 kbp. At 20 of the 111 RAPD loci identified (14 *P. davidiana* and 6 in 'Summergrand'), significantly distorted segregations were observed (Table 2).

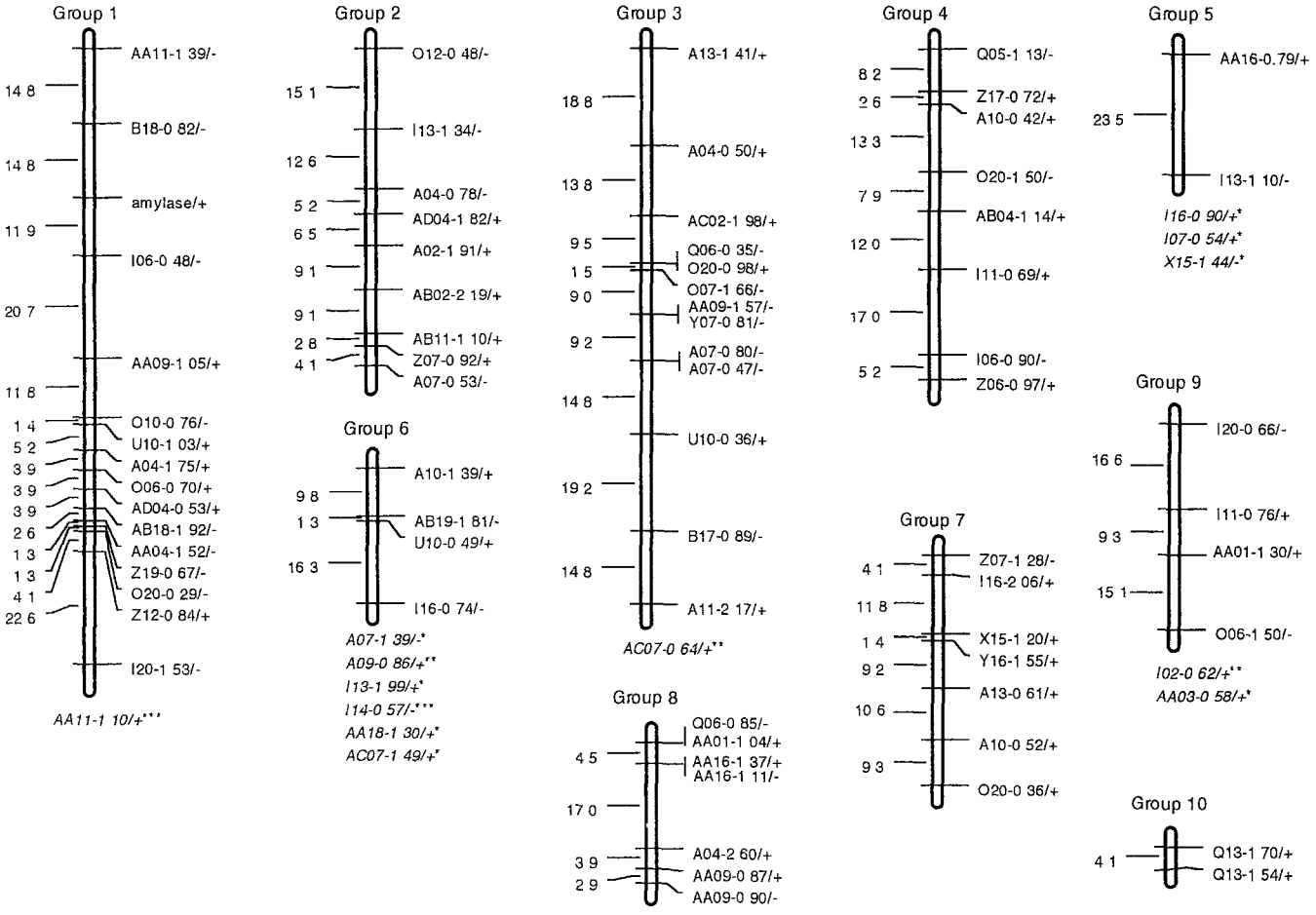


Fig. 1 Linkage map of *P. davidiana*. Loci are listed on the *right* and map distances (cM, Kosambi function) are listed on the *left* of each linkage group. Markers were grouped with a LOD>6 and recombinant fraction <0 30. For RAPD markers, the Operon code and the fragment size in base pairs are indicated. The locus phase is noted as + or –. Significant deviations from the 1·1 ratio are indicated with *asterisks*: * $P<0.05$, ** $P<0$ 001, *** $P<0.0001$ Markers in *italics*, indicated at the *bottom* of the groups, are linked to this group with LOD>6 and recombinant fraction <0.30 but are not located in the group because they present a deviation from the 1:1 ratio

Genetic map

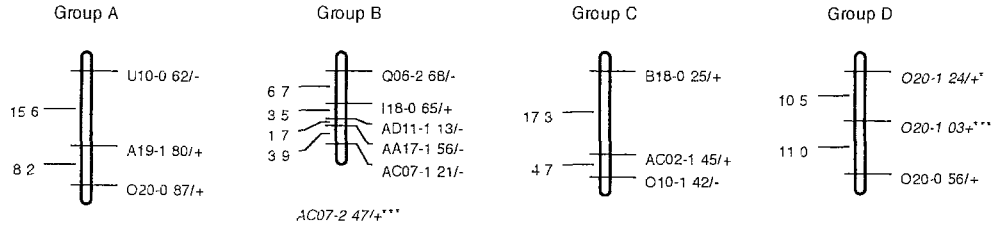
Unrelated genetics maps were obtained for *P. davidiana* clone 1908 (Fig. 1) and 'Summergrand (Fig. 2). In the linkage genetic map of *P. davidiana*, 84 RAPD markers and the isoenzyme were assigned to 10 linkage groups covering 536.2 cM with an average density of 6.4 cM between pairs of markers. Three RAPD markers remained unlinked. For 'Summergrand', only four linkage groups, composed of 15 RAPD markers covering 83.1 cM, were established.

Evaluation of resistance to powdery mildew

When present, 'Summergrand' was scored 4 and *P. davidiana* was scored 0 or 1 in all of the evaluations. For the hybrids, scores ranged from 1 to 3 in most cases (Table 3). If hybrids scored 0 and 1 were pooled in a resistant group, and hybrids scored 2, 3 and 4 pooled in a susceptible group, then a 1:1 (resistant: susceptible) segregation was observed for most evaluations. The categorisation of hybrids as resistant or susceptible was consistent from one evaluation to the other. This could suggest that powdery mildew resistance in *P. davidiana* is controlled by a single major gene. However, in the case of a monogenic resistance, the scores attributed within each group (susceptible or resistant) would not depend on genetic differences, and should therefore be independent for two independent evaluations. This hypothesis of independence was rejected by a statistical analysis for all pairs of evaluations, with a probability always lower than 2.10^{-4}. The control of powdery mildew resistance should therefore be polygenic.

With MAPMAKER/QTL, a QTL located on linkage group 3 of the *P. davidiana* map was detected for each of the 21 evaluations for powdery mildew resistance (Fig. 3). R^2 values (proportion of phenotypic variation explained by the marker) and LOD scores were high, ranging respectively from 21.9% to 78.4% and from 3.9 to 18.1 (Table 4). For most of the evaluations (18 out of 21), the highest probability for the location of this QTL was between the markers AC02-1.97 and O20-0.98. For three early evaluations however ($S2_{1A}$, $S2_{1B}$ and $G1_{1A}$), it was between markers A04-0.50 and AC02-1.97.

Three other QTLs, located on linkage groups 1, 4 and 9, were also detected for some powdery mildew evaluation



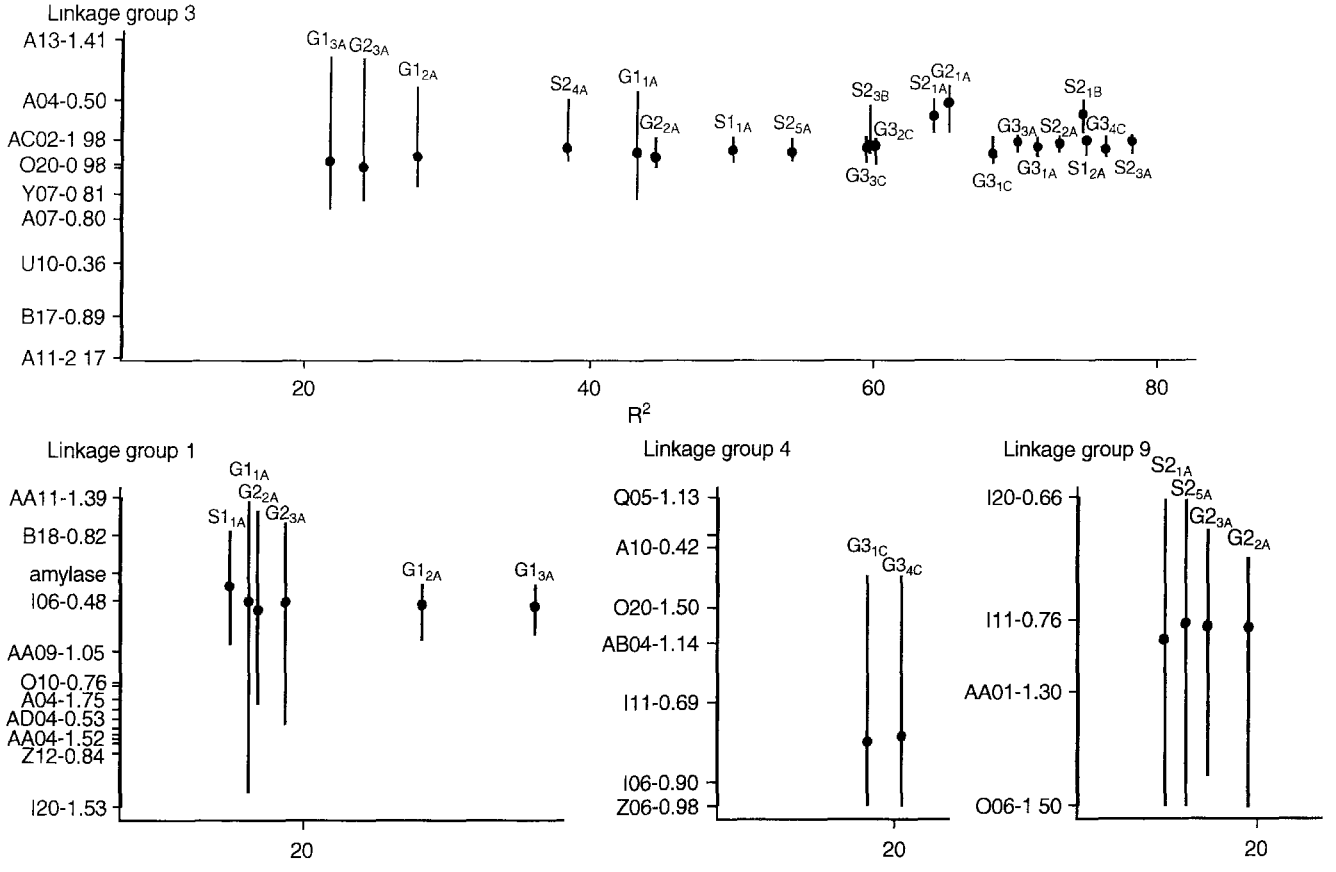


Fig. 3 QTLs associated with powdery mildew resistance detected with MAPMAKER/QTL using the *P. davidiana* linkage map. The R^2 value (*x-axis*) and the position (*y-axis*) for each QTL is given. The positions of the markers in the linkage groups are indicated. With respect to the location of a QTL, the highest probability is noted with a dot, and the confidence interval is indicated by a *vertical line*

(Fig. 3). The R^2 value ranged from 15.0% to 36.3%, 18.0% to 20.5% and 13.5% to 19.4% for QTLs located in linkage groups 1, 4 and 9, respectively. The highest probability for the locations of the QTLs was near marker I06-0.48 in linkage group 1, between I11-0.69 and I06-0.90 in linkage group 4 and near I11-0.76 in linkage group 9. When all of the QTLs detected were taken into account, 38.6% to 78.4% of the variation for powdery mildew resistance was explained, depending on the evaluations (Table 4).

With the stepwise regression procedure described in the materials and methods, markers significantly associated with powdery mildew resistance were found on all the linkage groups where QTLs were detected with MAPMAKER/QTL: markers O20-0.98, I06-0.48, I06-0.90 and I11-0.76, located in *P. davidiana* linkage groups 3, 1, 4 and 9 respectively, were selected (Table 5). Furthermore, stepwise regression revealed 2 other markers from *P. davidiana*, (A02-1.91 from linkage group 2 and AA09-0.90 from linkage group 8) as well as 3 markers from 'Summergrand' (AA17-1.56 and O20-1.03 located in linkage groups B and D, respectively, and Q05-0.74, an independent marker).

The markers selected in *P. davidiana* linkage groups 3, 1, 4 and 9 by the stepwise regression were located within the confidence intervals for the positions of QTLs obtained with MAPMAKER/QTL, close to their more probable location (Fig. 3).

As for the MAPMAKER/QTL analysis, the correlation of the marker O20-0.98 (*P. davidiana* group 3) with powdery mildew resistance was highly significant for all the evalu-

Table 3 Score distribution for the various evaluations for powdery mildew resistance and chi-square goodness-of-fit analysis for 1:1 segregation between resistant individuals (class 0 or 1) and susceptible ones (class 2, 3 or 4)

Evaluation code	Score for powdery mildew resistance					χ^2
	R		S			
	0	1	2	3	4	1:1 (R/S)
$S1_{1A}$	5	40	17	13	0	2.58
$S1_{2A}$	0	35	19	21	0	0.47
$S2_{1A}$	0	29	20	23	3	4.26*
$S2_{1B}$	0	29	14	21	11	4.26*
$S2_{2A}$	0	36	21	18	0	0.21
$S2_{3A}$	0	35	16	24	0	0.47
$S2_{3B}$	0	21	21	17	16	15.21***
$S2_{4A}$	0	28	35	12	0	5.26*
$S2_{5A}$	0	32	29	14	0	1.89
$G1_{1A}$	0	29	25	12	0	0.97
$G1_{2A}$	0	32	25	8	0	0.02
$G1_{3A}$	0	29	28	8	0	0.75
$G2_{1A}$	0	31	28	9	0	0.53
$G2_{2A}$	0	34	29	5	0	0.00
$G2_{3A}$	0	29	35	4	0	1.47
$G3_{1A}$	20	19	16	10	0	2.60
$G3_{1C}$	25	16	16	8	0	4.45*
$G3_{2C}$	11	26	23	5	0	1.25
$G3_{3A}$	15	22	19	9	0	1.25
$G3_{3C}$	19	22	13	11	0	4.45*
$G3_{4C}$	20	14	15	16	0	0.14

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

ations (P from 2.10^{-4} to 3.10^{-13}) (Table 5). When a QTL was detected with MAPMAKER/QTL in *P. davidiana* linkage groups 1, 4 or 9 for one evaluation, the coefficient of the marker retained on that linkage group differed significantly from 0 ($P<0.05$) for that evaluation, except for marker I06-0.90 for the evaluation $G3_{4C}$ ($P=0.06$).

The coefficients associated with 1 marker were consistent in most evaluations. They generally had the same sign and were of similar order of magnitude (Table 5). When it was not the case, the associated coefficient values were generally low and did not significantly differ from 0.

The effects associated with marker O20-0.98 (*P. davidiana* linkage group 3) were generally the highest. The markers selected on linkage groups 1 and 9 also had highly significant effects in several cases. The effects associated with the other markers were generally lower. Variations were, however, noted among the values and in the relative importance of the marker coefficients, depending on the evaluations. High coefficients for marker O20-0.98 were generally associated with high coefficients for marker I06-0.90 and low coefficients for marker I11-0.76. Higher coefficients were obtained in evaluations made by observer B (evaluations $S2_{1B}$ and $S2_{3B}$) than in evaluations made on the same days by observer A (evaluations $S2_{1A}$ and $S2_{3A}$). Similarly, coefficients for marker I06-0.90 were higher in evaluations made by observer C ($G3_{1C}$ and $G3_{3C}$) than in those made by observer A on the same days ($G3_{1A}$ and $G3_{1C}$). In a series of evaluations performed during the same growing season, the earliest ones (i.e. evaluation $S1_{1A}$ in 1987; $S2_{1A}$ and $S2_{1B}$ in 1988; $G1_{1A}$ and $G2_{1A}$ in

Table 4 R^2 and LOD score values for QTLs associated with powdery mildew resistance detected with MAPMAKER/QTL on *P. davidiana* linkage groups 3, 1, 4 and 9

Evaluation code	Linkage groups								Cumulative values	
	3		1		4		9			
	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD
$S1_{1A}$	50.1	9.4	15.0	2.0					71.3	16.3
$S1_{2A}$	75.1	16.2							75.1	16.2
$S2_{1A}$	64.4	13.0					13.5	2.2	66.4	14.1
$S2_{1B}$	74.9	15.9							74.9	15.9
$S2_{2A}$	73.2	18.1							73.2	18 1
$S2_{3A}$	78.4	15.9							78.4	15.9
$S2_{3B}$	60.0	11.8							60.0	11.8
$S2_{4A}$	38.6	6.7							38.6	6.7
$S2_{5A}$	54.3	10 9					15.0	2.7	56.2	12.0
$G1_{1A}$	43.4	6 5	16.7	2.6					60.6	10.8
$G1_{2A}$	28.0	3.9	28.3	4.7					53.2	9.7
$G1_{3A}$	21.9	4.7	36.3	6.4					55.6	10.7
$G2_{1A}$	65.5	12.5							65.5	12.5
$G2_{2A}$	44.8	7.8	16.7	2.5			19.4	3.2	63.7	14.2
$G2_{3A}$	24.2	4.0	18.6	2.8			16.4	2.6	52.9	10.3
$G3_{1A}$	71.7	13.2							71.7	13.2
$G3_{1C}$	68.5	13.2			18.0	2.2			75.5	14.9
$G3_{2C}$	60.3	11.4							60.3	11.4
$G3_{3A}$	70.3	14.6							70.3	14.6
$G3_{3C}$	59.7	10.9							59.7	10.9
$G3_{4C}$	76.5	15.6			20.5	2.6			79.9	17.3

Table 5 Markers retained by a multiple regression procedure to fit the evaluation data. Coefficient of the marker in the regression and the associated probability: * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Evaluation code	Markers located on the *P. davidiana* linkage map					
	O20-0.98 (group 3)	I06-0.48 (group 1)	I06-0.90 (group 4)	I11-0.76 (group 9)	A02-1.91 (group 2)	AA09-0.90 (group 8)
$S1_{1A}$	1.14***	0.68***	−0.12	−0.10	0.02	0.02
$S1_{2A}$	1.08***	0.40**	0.12	−0.39**	−0.01	0.27
$S2_{1A}$	1.15***	0.55***	0.18	−0.33*	−0.22	0.22
$S2_{1B}$	1.54***	0.43*	0.35*	−0.27	−0.25	0.05
$S2_{2A}$	1.07***	0.19	0.15	−0.28*	−0.08	0.25
$S2_{3A}$	1.12***	0.31	0.17	−0.27	−0.06	0.30
$S2_{3B}$	1.25***	0.45	0.01	−0.19	−0.16	0.24
$S2_{4A}$	0.72***	0.17	0.29*	−0.27*	−0.15	0.02
$S2_{5A}$	0.82***	0.28*	0.16	−0.44***	−0.09	0.26*
$G1_{1A}$	0.78***	0.37*	−0.04	−0.26	0.00	0.13
$G1_{2A}$	0.56***	0.66***	−0.16	−0.34**	−0.12	−0.01
$G1_{3A}$	0.55***	0.78***	−0.12	−0.43***	−0.14	−0.07
$G2_{1A}$	0.92***	0.33*	−0.05	−0.27*	0.06	0.00
$G2_{2A}$	0.78***	0.43***	−0.12	−0.37***	−0.04	−0.01
$G2_{3A}$	0.48***	0.37**	0.01	−0.30**	−0.12	0.28*
$G3_{1A}$	1.46***	0.67***	0.30	−0.21	−0.38*	−0.04
$G3_{1C}$	1.44***	0.56**	0.43*	−0.20	−0.31	0.21
$G3_{2C}$	1.10***	0.11	0.20	−0.24	−0.02	0.07
$G3_{3C}$	1.40***	0.36*	0.03	−0.29	−0.27	0.01
$G3_{3A}$	1.41***	0.39*	0.32	−0.30	−0.28	0.05
$G3_{4C}$	1.66***	0.35	0.36	−0.21	−0.20	0.14

Evaluation code	Markers located on the Summergrand linkage map		
	AA17-1.56 (group B)	O20-1.03 (group D)	Q05-0.74 (unlinked)
$S1_{1A}$	−0.21	−0.06	−0.29
$S1_{2A}$	−0.05	−0.27	−0.08
$S2_{1A}$	−0.38**	−0.14	−0.37**
$S2_{1B}$	−0.41**	−0.34	−0.65***
$S2_{2A}$	−0.26*	−0.37*	−0.20
$S2_{3A}$	−0.27	−0.31	−0.18
$S2_{3B}$	−0.55**	−0.08	−0.17
$S2_{4A}$	−0.09	−0.40**	−0.32
$S2_{5A}$	−0.07	−0.29	−0.09
$G1_{1A}$	−0.28*	−0.54**	−0.15
$G1_{2A}$	−0.12	−0.29	−0.08
$G1_{3A}$	−0.10	−0.21	−0.16
$G2_{1A}$	−0.16	−0.02	−0.26*
$G2_{2A}$	0.02	−0.06	−0.22*
$G2_{3A}$	−0.07	−0.28	−0.16
$G3_{1A}$	−0.09	−0.11	−0.27
$G3_{1C}$	0.13	−0.12	0.02
$G3_{2C}$	−0.45*	−0.26	−0.16
$G3_{3C}$	−0.41**	−0.28	−0.41**
$G3_{3A}$	−0.19	−0.45*	−0.25
$G3_{4C}$	−0.24	−0.19	−0.34

1989; $G3_{1A}$ and $G3_{1C}$ in 1990) generally corresponded to higher coefficients for markers O20-0.98 and I06-0.47, and lower ones for I11-0.76.

Discussion

Genetic map

Only 54 of the 500 RAPD primers tested revealed polymorphism both between 'Summergrand' and *P. davidiana* and in the hybrid progeny. This level of polymorphism is low for an interspecific cross. However, it must be remembered that the polymorphism was not fully exploited in this first study. Among the 500 primers tested, 279 revealed polymorphism between 'Summergrand' and *P. davidiana* and would therefore segregate in a further generation, when recombinations between 'Summergrand' and *P. davidiana* occur. In this first generation, however, only markers corresponding to heterozygous loci in one of the two parents could be used. Some of the primers that did not reveal polymorphism between 'Summergrand' and *P. davidiana* could correspond to heterozygous loci in both

parents and therefore reveal polymorphism in the hybrid progeny.

Among the 111 RAPD markers, 87 were heterozygous in *P. davidiana* and only 24 in 'Summergrand'. The low level of RAPD polymorphism observed in peach is in agreement with the low level of isoenzyme polymorphism (Mowrey et al. 1990) and of RAPD polymorphism (Chaparro et al. 1994) previously observed. This low level of polymorphism is due to the high level of inbreeding in the cultivated peach varieties (Scorza et al. 1985; Reynders and Monet 1987). Heterozygosity was much higher in *P. davidiana* (1908), probably due to its wild origin and to the allogamy of *P. davidiana*.

The map obtained for *P. davidiana* was composed of 10 linkage groups covering 536 cM. Several partial *Prunus* maps have previously been obtained: peach maps (Chaparro et al. 1994; Dirlewanger and Bodo 1994; Rajapakse et al. 1995), an almond map (Viruel et al. 1995) and a map based on an interspecific cross between peach and almond (Foolad et al. 1995). The total recombinational length of the *P. davidiana* genome, estimated from our data according to the Hulbert et al. (1988) method modified by Chakravarti et al. (1991), lies between 550 and 740 cM (is 645 cM±95 cM). Our present linkage map therefore covers at least 70% of the total nuclear genome, with an average spacing of less than 7 cM.

QTL analysis of powdery mildew resistance

Our study shows that powdery mildew resistance conferred by *P. davidiana* is not a monogenic character. The QTLs located on *P. davidiana* linkage groups 1, 3 and 9 were detected for all or most of the evaluations with a high significance level. They explain a high proportion of the phenotypic varaition for powdery mildew resistance. Other putative QTLs are located on *P. davidiana* linkage groups 2, 4 and 8, and on 'Summergrand' linkage groups B and D and next to markers Q05-0.74. Further studies of a larger progeny of the present cross or of other unrelated pedigrees will be useful for mapping them more securely. Moreover, some additional QTLs may have remained undetected. QTLs located on chromosomal segments not yet mapped cannot be identified. QTLs with minor effects are difficult to detect when the population size is limited, as it was in our study (Lander and Botstein 1989; Moreno-Gonzalez 1992; Darvasi et al. 1993). In addition, genes at homozygous loci in *P. davidiana* or 'Summergrand' do not segregate and therefore would not be detected in the progeny studied. Such cases of homozygous genes may not be uncommon. As seen above, a high proportion of primers which revealed polymorphism between the parents did not segregate in the progeny. However, from the observations reported here, we can conclude that if some of the genes involved in powdery mildew resistance are homozygous in *P. davidiana*, they are not major dominant genes conferring complete resistance. (If this was the case, all the hybrids would be as resistant as *P. davidiana*). Conversely, resistance genes homozygous in *P. davidiana* could bring a high level of resistance, but would then be recessive or co-dominant. They could also be dominant and bring only low partial resistance. The existence of co-dominant genes or dominant genes bringing partial resistance, homozygous in *P.davidiana*, would explain why none of the hybrids was as susceptible as 'Summergrand'. Further studies of progenies obtained by the selfing or backcrossing of the hybrids will be necessary to test these hypotheses.

The large number of independent evaluations and the use of multiple regression were determinant in confirming the presence of the QTLs in linkage groups 1 and 9. If some effects are detected in several independent evaluations, they are less likely to be random. The fitting of the same set of markers for all the evaluations was a good way of selecting regressors. Stepwise multiple regression is often criticised in the field of QTL detection because the genetic interpretability of the results is not straightforward. Indeed, for some evaluations, markers not selected by the stepwise procedure could be significant in the last step, when the selection of a subset of markers was based on the data from all the evaluations. And when these markers were involved in the regression model, other markers previously selected were no longer significant. Multiple regression also improved the efficiency of QTL detection by taking into account segregation for all the QTLs of interest. In several cases, and noticeably for the 'Summergrand' genome, QTLs not detected by the MAPMAKER/QTL procedure were highly significant in the multiple regression fitting.

QTLs located in *P. davidiana* linkage groups 1 and 9 were detected in several independent evaluations but not all of them. Some of the resistance genes might be expressed only under specific environmental conditions. The coefficients of the markers retained were often similar for evaluations with some common feature. For example, for most of the early evaluations, the QTL effects on linkage groups 3 and 1 were high, whereas the QTL effect on linkage group 9 was low. For evaluations made on grafted plants in Avignon (evaluations $G1_{1A}$, $G1_{2A}$, $G1_{3A}$, $G2_{1A}$, $G2_{2A}$ and $G2_{3A}$), the QTL effect on linkage group 1 was also high, but the QTL effect on linkage group 3 was much lower than in other evaluations. In their evaluations, observers B and C emphasised the effects of markers on linkage groups 3 and 4 respectively, compared to observer A. The observers probably weighted differently some of the components of powdery mildew resistance in their scoring. A study of resistance components could help in detecting QTLs more accurately. The evaluations on grafted hybrids in Gotheron are very different from those made on grafted hybrids in Avignon, and much more like the evaluations on self-rooted hybrids in Avignon. Further investigations will be necessary to understand the underlying phenomena. Genetic variations of the inoculum could also explain variations in the expression of resistance genes, but unfortunately these were not accessible in our study.

The results for 'Summergrand' show that cultivated peach varieties may segregate for some minor powdery mildew resistance genes. It may be interesting to use other varieties as agronomic parents to see if such genes are nu-

merous and to be used to reinforce the resistance brought by exotic germplasm.

The QTL linked to marker O20-0.98 of *P. davidiana* linkage group 3 is the most important QTL. Segregation at this QTL corresponds approximately to the 1:1 segregation observed when grouping the hybrids scored 0 or 1 on one hand, and the hybrids scored 2, 3 or 4 on the other hand. This example clearly illustrates the risk of focusing the breeding work on 1 or 2 of the genes initially involved in the parents. Without detailed analysis of the evaluation data and search for QTLs, the hypothesis of a monogenic control of the resistance conferred by *P. davidiana* could be accepted. As the genes situated at other QTLs are not expressed as consistently as the QTL linked to marker O20-0.98, they would not be submitted to a high selection pressure in every cycle of selection. They would probably be lost during the breeding process, especially if they were linked to undesirable agronomic characters.

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