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Patrick Lambert, Thierry Pascal

Version définitive du manuscrit publié dans / Final version of the manuscript published in : Tree Genetics & Genomes, 2011, vol., no., pp , DOI 10.1007/s11295-011-0394-2

Mapping *Rm2* gene conferring resistance to the green peach aphid (*Myzus persicae* Sulzer) in the peach cultivar "Rubira®"

P. Lambert and T. Pascal

[1]*INRA, Unité de Génétique et d'Amélioration des Fruits et Légumes, Domaine Saint Maurice, BP 94, 84143 Montfavet Cedex, France*

Corresponding author
Patrick Lambert
Unité de Génétique et d'Amélioration des Fruits et Légumes
INRA
Domaine Saint Maurice
BP 94
84143 Montfavet Cedex
Phone: 33 (0) 4 32 72 28 15
Fax: 33 (0) 4 32 72 27 02
E-mail: patrick.lambert@avignon.inra.fr

Abstract

The green peach aphid (GPA), *Myzus persicae* (Sulzer), is a widespread pest insect that significantly reduces yield in peach orchards [*Prunus persica* (L.) Batsch]. Chemical control of the GPA population in the orchards showed little efficiency because of the development of resistance to most classes of insecticides. Biological control partially gave convincing results. Breeding for resistant peach cultivars is therefore a serious option to take into account for the development of sustainable pest management. Among the few available resistance cultivars, the rootstock peach "Rubira®" shows a strong induced antixenosis-type GPA resistance. This was demonstrated segregating as a single dominant gene. In order to investigate the genetic basis of resistance and develop molecular tools useful in breeding programs, a F_2 population derived from "Rubira®" also segregating for leaf color was grown and scored for GPA resistance under contrasted environmental conditions. A SSR-based genetic linkage map composed of 120 SSR loci spanned over a distance of 497.8 cM was then established. The GPA resistance mapped to a single locus at the bottom end of linkage group 1. We propose to name *Rm2* the dominant allele of the underlying gene. Additionally, a reciprocal translocation was identified near the *Gr* gene controlling leaf color. The red-leaf parent "Rubira®" was demonstrated responsible for the translocation. This study provides the basis for future molecular analysis for the use of *Rm2* in peach breeding programs against GPA in peach orchards.

Introduction

Challenging breeding programs have been developed since several years at INRA-Avignon to select new peach cultivars [*Prunus persica* (L.) Batsch] introgressed with resistance factors against several important pests and diseases (Kervella et al. 1998; Foulongne et al. 2003b; Decroocq et al. 2005; Rubio et al. 2010), such as powdery mildew (*Sphaerotheca pannosa* var. *persicae*), peach leaf curl (*Taphrina deformans*), sharka disease and the green peach aphid (GPA) [*Myzus persicae* (Sulzer)]. GPA is a European native aphid with a worldwide distribution. This generalist insect pest infests hundreds of species from 40 plant families (Blackman and Eastop 2000) and is commonly found in peach and nectarine orchards throughout Southern Europe and North America. In spring and summer, GPA reproduces parthenogenetically on peach tree, a primary host, and sexually in autumn. Heavy infestation causes direct damage to the trees due to penetration of stylet by founders into flowers and to development of colonies which are responsible for leaf curling, heavy breakdown in shoot growth by sucking the phloem-sap and reduced fruit quality due to aphid punctures (Sauge et al. 1998a; Pascal et al. 2002). Besides direct injuries to leaves, shoots and fruits, GPA may act as a vector of the *plum pox potyvirus* (PPV), the causative agent of sharka disease in *Prunoideae* (Decroocq et al. 2005). This is one of the most serious diseases affecting *Prunus* species in the whole world. GPA has a well-documented history of resistance to most classes of insecticides due to their continuous use to control infestation (Devonshire et al. 1998; Mazzoni and Cravedi 2002; Foster et al. 2007). GPA insecticide resistance involves multiple mechanisms. Thus, chemical control of the GPA population has little efficiency in the peach orchard management (Cravedi and Cervato 1997). In addition, the use of effective systemic insecticides such as imidacloprid has raised concerns in an increasing number of countries because of their possible non-targeted impact on beneficial insects (Decourtye et al. 2004; Peck 2009). Natural enemies as biological control means or novel approaches such as kaolin treatments has been alternatively proposed (Barker et al. 2007; Gentz et al. 2010). Host-plant resistance seems to be more promising as a long-term solution since it could contribute to a more sustainable integrated pest management. However, peach germplasm has been rarely tested for resistance to this insect pest. Some studies were performed on this topic at the INRA Bordeaux, France (Massonié et al. 1982; Monet and Massonié, 1994; Kfoury et al. 1995). Additional studies were carried out on the probing and settling behaviors of the GPA on selected cultivars at the INRA Avignon (Sauge et al. 1998a, 1998b, 2002, 2006). Five resistant genotypes were identified among which two peach cultivars, "Weeping Flower Peach" (S2678) and "Rubira®" (S2605), showing strong antixenosis resistance (Massonié et al. 1982; Monet and Massonié 1994). This mechanism of resistance prevents plant colonization (Sauge et al. 1998b). It was linked with hypersensitive-like necrotic reactions that appeared on the apices within 2-3 days after the infestation in the kind of reddish or yellowish spots generally located at the puncture point. In addition, induced systemic resistance was demonstrated for "Rubira®" (Kfoury and Massonié 1995; Sauge et al. 2002, 2006) and the possible involvement of phenolic compounds was suggested (Poëssel et al. 2002). A simple dominant genetic determinism was established for both "Weeping Flower Peach" (Monet and Massonié 1994) and "Rubira®" (Pascal et al. 2002). The putative resistance gene in "Weeping Flower Peach" was named *Rm1* (*Rm* for resistance to *Myzus persicae*) by Monet and Massonié (1994). However differences in the aphid behaviour suggested that the underlying resistance mechanism might be different in "Rubira®" (Sauge et al. 1998b, 2002, 2006).

Following these studies, "Rubira®" was selected as preferential GPA resistance source because of its higher agronomical potential and lower level of heterozygosity compared to the botanical cultivar ''Weeping Flower Peach''. This might therefore limit variability in derived

hybrid genotypes. An intra-specific F_2 population, obtained from a cross between the susceptible rootstock peach "Pamirskij 5" and "Rubira®" as pollen source, was grown and scored for GPA resistance. The aim of the present study was to build a SSR-based F_2 map anchored to the "Texas" × "Earlygold" general map for *Prunus* referred to as T × E map (Genome Database for Rosaceae, http://www.rosaceae.org) and to map the induced GPA resistance carried by "Rubira®". The overall goal of our work is to subsequently develop reliable markers linked to the resistance locus in order to use "Rubira®" as a genitor for introgressing resistance to GPA into peach cultivars of high agronomical value.

Materials and methods

Plant material

The F_2 mapping population (n = 187) was obtained from the selfing of a single individual derived from the controlled pollination of "Pamirskij 5" (clone S6146) by Rubira® (clone S2605). "Pamirskij 5" is a green-leaf rootstock peach (*Prunus persica* L. Batsch) derived from seeds obtained from the Nikita Botanical Garden of Yalta (Crimea, Ukraine); it is resistant to peach powdery mildew (*Sphaerotheca pannosa)* and susceptible to GPA. Rubira® is a red-leaf rootstock peach selected from peach seedlings grown at the INRA from a Californian seed lot imported in 1960; it is resistant to GPA and susceptible to powdery mildew. Resistance to powdery mildew and GPA are both dominant to susceptibility. The "Pamirskij 5" × "Rubira®" F_2 population (hereafter referred to as PR^2) also segregates for leaf color. This phenotypic trait is controlled by a single gene (*Gr*), red being dominant and green recessive (Blake 1937). "Rubira®" is homozygous for both red color and GPA resistance.

Assessment of aphid-plant interactions

Two phenotypic trials were carried out. The first one (Trial 1) was performed in a greenhouse under controlled conditions using an aphid colony established from a single GPA female (Mp03 clone) collected from a peach tree in 1997 in Avignon, Southern France. Mp03 apterous aphids were continuously reared on susceptible GF305 peach seedlings under parthenogenesis-inducing conditions (19 ± 1°C; long-day photo period of L16:D8) in a growth chamber (Sauge et al. 1998b; 2002). Procedure and scoring method of trial 1 were similar to Pascal et al. (2002). After a 3-month stratification and radical emergence, 187 seeds derived from the selfing of the F_1 parent were individually potted, placed in a greenhouse and maintained at 23 ± 5°C. After 3 months, once seedlings were 30-35 cm high, controlled infestations were achieved in April by placing two 5- to 7-day-old apterous adults of *M. persicae* on the terminal apex of each seedling. First plants were observed four times during 2 weeks (referred to Trial 1 stage 1). The last scoring dataset was kept for further analyses. Then a final control assessment was performed one month later (i.e. in May, referred to Trial 1 control). For both assessments, each plant was visually rated for (i) aphid colony development and (ii) leaf curling responses, These two parameters were scored separately using a well-tried linear ordinal scale from 0 (no aphid, no curling respectively) to 4 (all apices colonized by numerous aphid, all apices curled respectively). In addition the presence/absence of reddish necrotic spots was recorded using a binary score (i.e. 1/0).

The second trial (Trial 2) was conducted on the whole population, under reinforced natural conditions of infestation in the experimental field of "les Garrigues" at the INRA Avignon. Seedlings had been planted three years before on their own roots in rows 4.0 m apart and with a tree-to-tree distance of 0.5m. Trees were about 2 m high, blooming for the first time during

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the year of evaluation. Before and during Trial 2, orchard was treated with fertilizers but no insecticides and fungicides were applied. Naturally occurring aphids were allowed to infest the trees over the grooving season (April to June). Initial aphid infestation was assessed at least on two branches of each genotype by visual check. For peach trees showing little or no GPA infestation, aphids were collected from nearby peach trees and placed onto the apical part of the main shoots to reinforce natural conditions of infestation. Peach trees were assessed at the end of the grooving season. They were scored as resistant (no aphid colony and a small number of leaves weakly to moderately curled) or susceptible for the other cases. Necrotic spots were scored by using the same method as for Trial 1.

DNA isolation

Samples of young expanded terminal leaves from the parents and the 187 seedlings were collected in May 2006 and kept at -80 ºC until DNA isolation. Genomic DNA isolation was then performed following the protocol of Bernatzky and Tanksley (1986). DNA concentrations were measured using a spectrophotometer Thermo Scientific NanoDrop™. DNA quality was assessed by electrophoresis on 1% agarose gel.

SSR analysis

Publicly available *Prunus* SSR primer pairs (see Table 1) were tested for their polymorphism by using the protocol of Rubio et al. (2010). They were first screened by using the two parents, then a set of eight individuals of the population when it was necessary for establishing segregation patterns. Segregating SSRs with easily readable profiles were then selected from their position in the T × E map or in other published *Prunus* maps, and mapped in the whole population using the multiplex protocol**.**

Multiplex protocol and genotyping

The whole population was amplified by using the QIAGEN® Multiplex PCR Kit (Qiagen Inc. Valencia, Ca) with 2 to 6 primer pairs simultaneously (0.2 and 0.4 µM concentration for each IRD700 and IRD800 labeled primers respectively) with 2× QIAGEN multiplex PCR master mix (final concentration 1×) and 5× of Q-Solution (final concentration 0.5×). The same concentration was used for the complementary primers. Ten ng of genomic DNA were used in a final reaction volume of 10 µl. DNA amplifications were carried out in a Mastercycler®ep gradient thermal cycler (Eppendorf GmbH, Germany) using the universal multiplex cycling protocol (QIAGEN Multiplex PCR kit; QIAGEN): 15 min at 95 °C (initial denaturation step) followed by 35 cycles consisting of 94 °C for 30 s, 57 °C for 90 s, and 72 °C for 60 s, with a final extension of 10 min at 72 ºC. The PCR products were then diluted in formamide blue in a 1:40 ratio and denatured at 95 ºC for 3 min. Then, 0.5-0.8 µl of each sample was loaded on a 6% polyacrylamide sequencing gel and run at constant power (1,500 W) for 1-2 hours using a LI-COR (IR2) sequencer (Model 4200, *LI-COR*; Nebraska).

Segregation analysis and map bulding

Departures from the 1:2:1 or 3:1 ratios expected for a F_2 population were tested by using chi-square goodness-of-fit on segregation data. Linkage analysis was performed using MAPMAKER/EXP V3.0 software (Lincoln et al. 1992). Linkage groups (G) were initially established by using a critical logarithm of the odds (LOD) threshold of 8.0 and a recombination fraction of 0.30. Marker distances were calculated using the Kosambi mapping function (Kosambi 1944). After mapping, the “error detection” option of Mapmaker was used

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to detect possible errors. Map figures were obtained by using MapChart software (Voorrips et al. 2002).

QTL analysis and mapping of GPA resistance, necrotic reactions and Gr gene

In first approach, a single-gene inheritance model was applied for GPA resistance. The quantitative Trial 1 control dataset was transformed according to a binary distribution (resistant/susceptible). Seedlings scored 0 to 1 for both colony development and leaf curling were considered resistant whereas others were considered susceptible. Plants scored up to 2 for leaf curling were nevertheless considered resistant when no aphid was observed and necrosis spots were clearly visible. Three binary datasets were then used for mapping GPA resistance: (i) the transformed Trial 1 control dataset (ii) Trial 2 dataset. (iii) Final dataset (thereafter referred to as FD) which corresponds to the genotypes common to both trials. These datasets were added to the marker dataset used for map construction after coding as defined in MAPMAKER/EXP V3.0 for dominant markers. The presence of necrotic spots was accordingly mapped.

Then, in order to ascertain the single-gene inheritance of the resistance, QTL detection was performed by composite interval mapping (CIM) (Zeng 1994) with MapQTL 5.0 software (Van Ooijen et al. 2004) using the quantitative datasets of Trial 1, independently for colony development and leaf curling. Moreover, since distributions heavily departed from normality and particularly for colony development, a non-parametric test based on the Kruskal-Wallis (KW) methodology (Kruglyak and Lander 1995) was performed using the same software; as recommended by Van Ooijen et al. (2004) a stringent association level of 0.005 (P-value) was chosen. For CIM, the most appropriate LOD threshold to declare a QTL putative (type-I error $\alpha = 0.05$) was estimated by using the 1000-permutation test. The percentage of phenotypic variation explained was estimated for each QTL.
In addition, each seedling was scored for leaf color (red/green) according to a binary distribution (1/0 respectively). The dominant *Gr* gene responsible for leaf color was then mapped with the same way for GPA resistance.

Results

Linkage map construction

One hundred eighty-seven progenies were used for map construction. The 120 SSR loci and the two phenotypic traits, *Rm2* and *Gr*, mapped to seven linkage groups instead of the eight expected in *Prunus*, at a LOD score of 19.0. Six of the groups (Fig. 1) were homologous to six groups of the T × E map (G1, G2, G3, G4, G5 and G7). The remaining one was composed of the dominant *Gr* gene and thirty-five SSR markers (Fig. 2) among which 17 located to G6 and 14 to G8 in published *Prunus* maps. This pseudo-linkage between G6 and G8 suggested a reciprocal translocation between the corresponding chromosomes in one of the parents of the PR^2 population. In order to separate the two groups, two sets of markers which unambiguously mapped to G6 or G8 in published *Prunus* maps were selected and independently mapped. Among the four remaining markers, three mapped to different groups in *Prunus* maps (BPPCT042C, EPPCU4962B and EPPB4213B) and one (MA023a) at two possible loci (MA023aA in G6 or MA023aB in G8 in the T × E bin-map). Three among them (EPPCU4962B, EPPB4213B, MA023aA/B) mapped with similar LOD to G6 and G8. They

were finally assigned to one of the group (Fig. 2) by blasting the respective primer pair sequences onto the peach genome sequence v1.0 (http://www.rosaceae.org/peach/genome). The most likely position of the translocation was estimated by testing each marker of G6 against G8 framework and reciprocally by using the "try" command (Fig. 2).

The PR² map covered a total distance of 497.8 cM (Fig. 1). Three among the 116 SSR primer pairs used for mapping revealed several loci: BPPCT019 amplified three loci (on G1, G7 and G8) whereas EPPCU4962 and UDP96-015 amplified two loci (on G5 and G8, G7 and G8 respectively). They were named according to the general nomenclature. Seven loci were not previously mapped: BPPCT019C (G1), UDAp-471 (G2), BPPCT042C (G6), EPPB4213B and EPPCU4962B (G8), UDAp-444 and UDP96-015B (G7). Seven SSR markers (BPPCT019A, B and C, AMPA109, BPPCT013, ECU4962A and UDAp-444) and the two phenotypic markers were scored dominant. Sixteen SSRs in total (13.3 %) deviated significantly from their chi-square expectations for the 1:2:1 ratio ($P < 0.1$): 5 were in G2, 11 were in G6 or G8 in the interval comprising or flanking the translocation region (Fig. 1). In G6 and G8, the deviation was due to an excess of heterozygous individuals (61.4% and 59.8% on average respectively) linked to a lack of homozygous individuals for the "Rubira®" allele (13.7% and 15% on average respectively). In G2, it was the opposite: the excess of heterozygous individuals (58.7%) was linked to a lack of homozygous individuals for the "Pamirskij 5" allele (14.1%). Forty-four SSR loci (37%) were common with the T × E map. The map coverage was estimated to 87% of the T × E map by using the information derived from published *Prunus* maps with some disparities (from nearly 100% for G1 and G6 to 48% for G3). The number of loci mapped to each linkage group ranged from 10 (G5) to 19 (G1, G6) with an average of 15. The length of each linkage group was comprised between 98.7 cM (G1) and 35.4 cM (G3) The average distance between loci ranged from 3.2 cM (G3) to 5.8 cM (G1) with an overall average distance of 4.1 cM. Three gaps longer than 15 cM were observed in three of the eight linkage groups (G1, G3 and G5).

Phenotypic assessment of resistance to GPA

One hundred sixty-six plants were tested in greenhouse conditions (Trial 1). The remaining ones (21) were not subjected to GPA infestation owing to insufficient development. Two weeks after infestation (Trial 1 stage 1), aphids had left 120 seedlings (72.3%) and one GPA remained on each of 5 additional ones. All of them showed clearly visible reddish necrotic spots. GPA colonization score was 2.4 on average for the 41 remaining ones (range 1.5-3). One hundred and seventeen seedlings (70.5%) showed various levels of leaf curling (score range 0.5 to 3.75). The average score was 0.61 for the 120 seedlings showing no GPA and 2.32 for the others. One month later (Trial 1 control), the 125 seedlings showing necrotic spots housed no GPA (75.3%). The 41 remaining seedlings showed important colony development (average score 3.74) and leaf curling (average score 3.72 comparatively to 0.31 for those considered resistant). As expected, data were on the whole not normally distributed and particularly for aphid colonization (Fig 3).

In Trial 2, the whole population (187) was assessed in orchard conditions: 136 (75.8%) were scored resistant, 43 susceptible (24.2%) and 8 missing data. In the latter no GPA was observed as well as no necrotic spot whereas very light leaf curling was detected. This suggests escape from GPA infestation. Altogether, 165 genotypes were common to both trials and thus assessed in both conditions: 123 were scored resistant (74.5%) and 42 susceptible (25.5%). The associated dataset is thereafter referred to as FD (for final dataset). Complete association was observed between resistance and presence of necrotic reactions in both trials.

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These results agree with the 3:1 segregation ratio expected in a F_2 population for a dominant trait (χ^2=0.008, 0.018 and 0.09 for Trial 1, Trial 2 and FD respectively) and are thus in agreement with a single-gene model of inheritance of the GPA resistance for "Rubira®".

Mapping of GPA resistance and QTL analysis

The putative resistance gene was mapped as a dominant marker using the binary datasets issued from Trial 1, Trial 2 and FD. The gene positions computed for each of them were distributed over a 2.5 cM interval between pchgms29 and UDAp-467 markers at the bottom end of G1 as well as for the "necrotic spot" trait. The consensus position in the PR² map was determined by using FD (Fig. 1).
With KW test (Table 2) the strongest association was detected with UDAp-467 for colonization (K= 134.2; $P<10^{-9}$) as well as for leaf curling (K= 80.1; $P<10^{-9}$). An additional marker at the bottom of G7, CPPCT017, was found significantly associated with leaf curling (K=13.8; $P=2x10^{-3}$).
With CIM, a major QTL was detected in the same region as for KW for colonization as well as for leaf curling (Table 2) and was co-located with the dominant locus. The phenotypic variations explained were 80.9% and 74% respectively. We therefore propose to name *Rm2* both QTL and dominant locus in reference to *Rm1* gene. An additional QTL was detected for leaf curling in the same region of G7 as for KW. The additive effects showed that "Rubira®" carried the unfavorable allele (Table 2). However, as the computed LOD threshold was 2.62, it was only significant for KW. This putative QTL (*Curl-PR²-7*.1) was named according to the trait name, the mapping population, the linkage group and a number.

Mapping of the Gr gene

Forty-height seedlings had green foliage in the mapping population (25.7%) which is in accordance with the 3:1 segregation ratio expected for a dominant character (χ^2=0.088). The 139 other ones were homozygous for the red color of the leaves or heterozygous. The *Gr* gene mapped in the middle of G6 slightly above CPSCT012 (Fig 1).

Discussion

PR² genetic map

Peach is genetically the best characterized species in the genus *Prunus*. However, only few genetic maps derived from modern peach cultivars are available owing to the low degree of polymorphism generally observed due to the narrow genetic base (Rajapakse et al. 1995; Dirlewanger et al. 2006). Most of the published maps were built by using rootstock peach cultivars or cultivars having divergent breeding histories (Yamamoto et al. 2005; Ogundwin et al. 2009). The other maps involving a peach cultivar were derived from interspecific crosses (Jáuregui et al. 2001; Aranzana et al. 2003; Foulongne et al. 2003a; Verde et al. 2005; Blenda et al. 2007; Marandel et al. 2009). In this study, we constructed a F_2 SSR-based genetic map derived from two rootstock peach cultivars of different origins in order to take advantage of the genetic backgrounds from which they were issued. Since a pattern of complete synteny was demonstrated for all studied *Prunus* species (Arús et al. 2005) the use of SSRs enabled for easy cross-referencing of marker and trait locus positions with the T × E map (Genome database for rosaceae**:** http://www.rosaceae.org), numerous published *Prunus*

maps (Dirlewanger et al. 2004 and 2006; Yamamoto et al. 2005; Verde et al. 2005; Howad et al. 2005; Dondini et al. 2007; Rubio et al. 2010) and the peach genome sequence (http://www.rosaceae.org/peach/genome). This allowed for identifying of a reciprocal translocation between the chromosomes corresponding to G6 and G8 in the PR² map and to correctly assign markers to their respective groups. Reciprocal translocations have already been reported in previous studies for populations derived from parents contrasting for leaf-color: the interspecific "Garfi" almond × "Nemared" peach population (Jauregui et al. 2001; Dirlewanger et al. 2004) and the intraspecific peach "Akame" × "Juseitsou" population (Yamamoto et al. 2005) in which "Nemared" and "Akame" are the red-leaf cultivars. They were identified in similar map positions close to the *Gr* gene (Jauregui et al. 2001; Dirlewanger et al. 2004). Jauregui et al. (2001) suggested that "Nemared" might be a more-probable candidate for the translocation. In "Nemared", the red-leaf character was reported to come from "Bound Brook" which derives from Tennessee naturals, one of the first peach germplasm introduced in the USA, and genetically different from the most-wide spread peach germplasm (Hesse 1975). Based on the results of the current study and on those obtained with crosses between "Pamirskij 5" and another green-leaf peach cultivar (data not published) we confirm that "Rubira®" carries the rearrangement. Also, we suggest that the latter could probably have the same origin for the three red-leaf cultivars.

Only few differences in locus order were observed between the PR² map and the *Prunus* maps. In G5, AMP105, PacD30 and BPPCT026 co-located in the GN22 map (Dirlewanger et al. 2004) whereas they were comprised in a 26.6 cM interval in the PR² map. This is probably due to the important degree of segregation distortion observed in the GN22 map (41.5%). In G3, UDP-403 and BPPCT007 mapped at inverted position in the T × E map, probably due to the difference in population size. Again, this confirms synteny in *Prunus* species (Arús et al. 2005). Seven SSRs mapped at new loci compared to the other maps. The positions of all the other markers mapped in the T × E bin-map (Howad et al. 2005) were consistent with their locations in the PR² map. This confirms the relevance of the bin-mapping method for new markers, although the peach genome sequence is now a more practical tool.

Resistance to GPA

The experimental device used in this study was established to assess the PR² population in contrasted conditions and thus clearly ascertain the resistant *vs* susceptible status of each progeny. Seedlings were thereby planted on their own roots to prevent genotype-rootstock interactions. They were observed in two different environments and resistance was assessed both at the young and grown-up stage. In addition, two unrelated GPA populations were used. In resistant-scored genotypes, most aphids (i.e. GPA) left the plants within the first week following infestation and for half of them within the first two days, as previously reported by Sauge et al. (1998b) for "Rubira®". The latter indeed exhibits antixenosis causing a dissuasive effect of GPA settlement for adult and nymph stadium. This dissuasive effect enables the identification of genotypes carrying resistance in the PR² population. GPA resistance was also shown to be linked with hypersensitive-like necrotic reactions. This suggests that they might be governed by the same mechanism, but also allows confirming resistance. Consequently, a simple rating scale with two classes, resistant and susceptible, could have been used to score the plants, as reported in other aphid resistance studies (Klingler et al. 2005; Hill et al.2006; Bus et al. 2008; Evans et al. 2008), because only the two distinctive parental phenotypes were observed in the segregating population. This was confirmed with the QTL analysis as the qualitative resistance locus and the QTL peak mapped to the same region of the PR² map whatever the method and the environmental conditions. For leaf curling, despite data distributions suggested that several QTLs could have been involved, the same QTL as for resistance to colonization was identified suggesting that no specific genomic region was

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involved. Leaf curl injuries are indeed a consequence of aphid punctures, sap ingestion and interaction of salivary products with cell wall elements of the leaves. They are therefore linked to the number of aphids feeding on the plant. This explains why the same major QTL region was identified for both traits. An additional QTL was nevertheless identified in G7 but its effect and significance level were too low to draw a firm conclusion.

These findings demonstrate consistency of the results and the single-gene inheritance previously demonstrated by Pascal et al. (2002) for resistance to colonization in "Rubira®". A similar GPA resistance gene (*Rm1*) was reported by Monet and Massonié (1994) in "Weeping Flower Peach", but to date, its genomic position as well as its nature is unknown. Sauge et al. (2002, 2006) demonstrated significant differences in the aphid behavior between these two cultivars. In "Rubira®", plant resistance is induced by the feeding punctures and evolves even in the absence of aphids, after a short initiation stage followed by a latency period. It is systemic and disappears 48h after aphid removal as demonstrated by Kfoury and Massonié (1995). Moreover, previous infestation dramatically increases the expression of antixenotic host plant resistance as indicated by its strong negative impact on the GPA settlement (Sauge et al. 2002). In contrast, no induced resistance was observed in "Weeping Flower Peach" as well as no modification in the level of resistance (Sauge et al. 2006). We have consequently hypothesized that these genes might be different and proposed naming *Rm2* the dominant allele responsible for GPA resistance in "Rubira®". However, this point would have to be settled in future studies.

Contrary to peach for which only two studies are available on the genetic determinism of GPA resistance (Monet and Massonié 1994; Pascal et al. 2002), there are many examples of dominant genes conferring monogenic resistance to aphids. These genes were identified in a wide range of annual crops such as the model-legume *Medicago truncatula* Gaert (Klingler et al. 2005, 2007; Gao et al. 2008), soybean (Hill et al. 2006; Li et al. 2007), tomato (Rossi et al. 1998; Goggin et al. 2004), melon (Dogimond et al. 2004), lettuce (Wroblewski et al. 2007) or wheat (Liu et al. 2005). In the *Rosaceae* family, several genes were identified in apple (Cevik et al. 2002; Bus et al. 2008; 2010) or pear (Evans et al. 2008). Analysis of those that were characterized has shown strong homology or tight linkage with genes which encode members of the large nucleotide-binding site leucine-rich repeat (NBS-LRR) resistance protein family (Milligan et al. 1998; Cevik et al. 2002; Dogimond et al. 2004; Klingler et al. 2005, 2007; Gao et al. 2008; Tagu et al. 2008). Moreover, studies about the non-persistent transmission of *plum pox potyvirus* demonstrated that *M. persicae* was a poor vector in "Rubira®" (Fos and Massonié 1993). Similar difficulties to transmit non-persistent virus were reported for the aphid *Aphis gossypii* in melon accessions carrying the *Vat* gene, a member of the CC-NBS-LRR gene family which confers resistance to this aphid (Pitrat and Lecoq 1980). Without prejudice on mechanisms involved in GPA resistance, these findings strongly suggest that *Rm2* is also a member of the NBS-LRR family and give clues for gene discovery. Candidate gene searches on this family would be a valuable option in first approach using published resistance genes analogs (RGAs) sequences and the peach genome sequence (http://www.rosaceae.org/peach/genome) like primary resources. In peach, several authors reported the identification of loci conferring pest or pathogen resistance (Yamamoto et al. 2002b; Foulongne et al. 2003b; Dirlewanger et al. 2004; Decroocq et al. 2005; Lalli et al. 2005; Marandel et al. 2009) and few of these identified and mapped RGAs for the most part focused on PPV resistance. Among these authors, Lalli et al. (2005) generated a resistance map for *Prunus* based on candidate genes representing various classes of resistant genes; three of them, Cd77, C5 and D5 (GenBank accession N° CZ445406, CZ445424 and CZ445426 respectively) hit close to the GPA resistance region. The first one, a NBS-like RGA, has already been mapped in the same region of apricot by Lambert et al. (2007); the others developed from a peach "Nemared" BAC library belong to the TIR-NBS-LRR class.

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They were initially mapped in other groups by Lalli et al. (2005), but the comparison of their sequences with the peach genome sequence v1.0 demonstrated a main assignment to the bottom end of scaffold 1 (G1) in the interval between EMPA011 and UDP-022. However, a rough analysis of the genes that were annotated in the GPA resistance region has revealed the presence of numerous additional genes that belong to the NBS-LRR family or coding for various R proteins. They could equally be considered candidates for GPA resistance.

The use of single-gene based resistance has often been controversial since it has been shown to be an ineffective approach to achieving durable resistance. Indeed, most of the resistance genes have been overcome such as the *Mi-1* gene for aphid and nematode resistance in tomato (Rossi et al 1998) or several genes involved in apple scab, powdery mildew, fire blight, and woolly apple resistance in apple (Bus et al. 2010). This concern has been taken into account at the INRA Avignon and several strategies have been straightaway considered (Lambert et al. 2008). For instance, the combination with other single-gene resistance such as that from "Weeping Flower Peach" if demonstrated different, or with quantitative antibiosis-based resistance such as that derived from *P. davidiana* P1908 (Massonié et al. 1982; Sauge et al. 1998b) for which QTLs for resistance have been identified (Sauge et al. 2004). The combination of both antixenosis and antibiosis in improved genotypes would be more difficult to overcome and thus would increase durable resistance in a context of sustainable aphid management programs.

Conclusion

In this study we have mapped for the first time a gene (*Rm2*) conferring dominant resistance to GPA in peach. *Rm2* will be very useful in the development of new peach cultivars combining several types of aphid resistance in improved genotypes with the aim of durable resistance or with gene pyramids for multiple resistances. The identification of DNA markers tightly linked with *Rm2* would increase the efficiency of selection for resistant plants in segregating populations through marker-assisted breeding methods (MAB). This would be facilitated by the use of the peach genome sequence (Genome Database for Rosaceae http://www.rosaceae.org).

Acknowledgements

The authors acknowledge A. Bachellez, E. Lecerf and X. Titeca for technical assistance and the International Peach Initiative Consortium (IPGI) for the early release of the peach genome sequence v1.0. All experiments described in this paper comply with the current laws in the European Union.

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Table 1 Origin and number of *Prunus* simple sequence repeat markers tested for amplification and polymorphism and used in the development of the 'Pamirskij5' x 'Rubira' F_2 genetic linkage map

SSR name	*Prunus* species	Origin	Tested	Amplified	Polymorphic	Mapped	Reference
AMPA	*P. armeniaca*	Genomic	16	15	8	4	Hagen et al. (2004)
AMPA	*P. armeniaca*	cDNA	10	9	4	2	Hagen et al. (2004)
BPPCT	*P. persica*	Genomic	39	39	22	19	Dirlewanger et al. (2002)
Cd	*P. persica*	Genomic	3	3	2	1	Sicard et al. (2008)
CPDCT	*P. dulcis*	Genomic	33	30	7	4	Mnejja et al. (2005)
CPPCT	*P. persica*	Genomic	27	27	11	11	Aranzana et al. (2002)
CPSCT	*P. salicina*	Genomic	26	24	7	4	Mnejja et al. (2004)
EMPA	*P. avium*	Genomic	4	4	1	1	Clarke and Tobutt (2003)
EPDCU	*P. dulcis*	cDNA	3	3	3	1	GDR
EPPB	*P. persica*	cDNA	20	20	3	2	GDR
EPPCU	*P. persica*	cDNA	74	71	27	7	GDR
G	*P. persica*	Genomic	4	4	3	1	Marandel et al. (2009)
M	*P. persica*	cDNA	6	4	3	3	Yamamoto et al. (2002)
MA	*P. persica*	Genomic	21	19	13	10	Yamamoto et al. (2002)
Pac	*P. armeniaca*	cDNA	11	11	2	2	Decroocq et al. (2003)
PceGA	*P. cerasus*	Genomic	1	1	1	1	Downey and Iezzoni (2000)
pchcms	*P. persica*	cDNA	5	5	3	1	Sosinski et al. (2000)
pchgms	*P. persica*	Genomic	10	10	6	5	Sosinski et al. (2000) Verde et al. (2005)
PdavW	*P. davidiana*	Genomic	1	1	0	0	Lambert et al. (2004)
pms	*P. avium*	Genomic	5	5	1	1	Cantini et al. (2001)
PS	*P. avium*	Genomic	5	4	1	1	Sosinski et al. (2000) Joobeur et al. (2000)
ssrPaCITA	*P. armeniaca*	Genomic	21	17	5	2	Lopes et al. (2002)
UDA	*P. dulcis*	Genomic	4	4	2	1	Testolin et al. (2004)
UDAp	*P. armeniaca*	Genomic	63	59	26	14	Messina et al. (2004)
UDP	*P. persica*	Genomic	25	25	19	17	Cipriani et al. (1999) Testolin et al. (2000)
		Total	437	414	180	116	

GDR : Genome Database for Rosacea (http://www.rosaceae.org/)

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Table 2 Summary of the QTLS detected by Kruskal-Wallis test (KW) and Composite Interval Mapping (CIM) for Trial 1. LOD scores below the significant threshold are in italics.

				KW			CIM				
Traits[a]	**QTL/locus**	**G**[b]	**Closest Marker**	**Posit.**[c]	**K**	***P*-value**[d]	**Posit.**[c]	**LOD**[e]	**Add.**[f]	**Dom.**[g]	**Effect (%)**[h]
Colonization S1	*Rm2*	1	UDAp-467	98.7	120.8	$<10^{-9}$	96.3	54.6	1.064	0.965	76.5
Colonization C	*Rm2*	1	UDAp-467	98.7	134.2	$<10^{-9}$	96.3	59.1	1.732	1.639	80.9
Leaf curling S1	*Rm2*	1	UDAp-467	98.7	45.8	$<10^{-9}$	96.3	17.8	0.799	0.559	39.5
	Curl-PR²-7.1	7	CPPCT017	63.3	7.5	$2x10^{-2}$	61.4	*1.52*	-0.078	-0.401	4.2
Leaf curling C	*Rm2*	1	UDAp-467	98.7	80.1	$<10^{-9}$	96.3	47.9	1.598	1.390	74.0
	Curl-PR²-7.1	7	CPPCT017	63.3	13.8	10^{-3}	61.4	*1.74*	-0.223	-0.606	4.8

[a]S1=stage 1; C= control
[b]Linkage group
[c]Position of the QTL peak/locus from the upper part of the linkage group in cM
[d]Probability of association between the marker and the trait according to Kruskal-Wallis test
[e]Logarithm of odds score under Composite interval mapping
[f]Additive effect
[g]Dominance effect
[h]Part of the phenotypic variance explained by the QTL (%)

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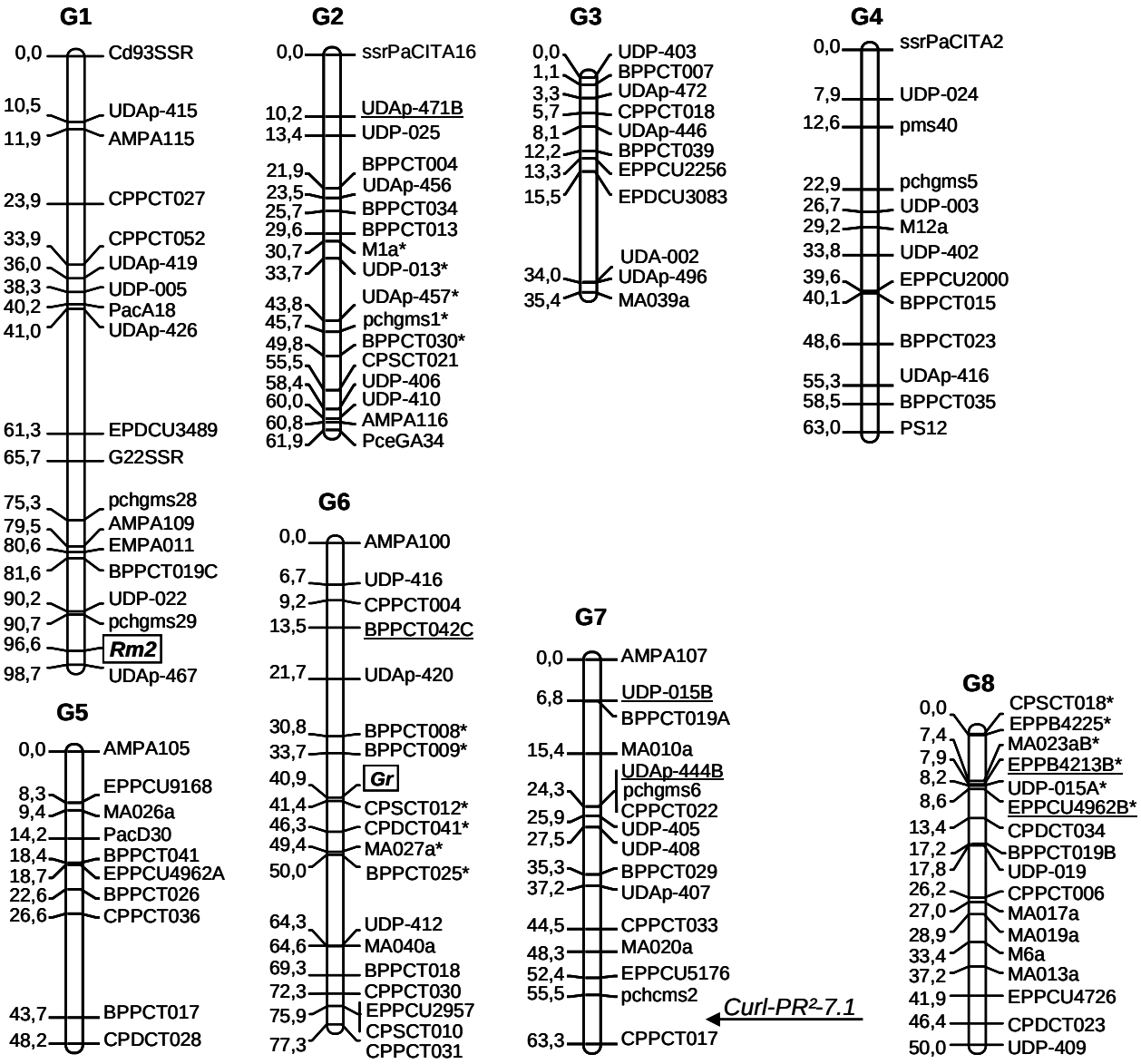

Fig. 1 Linkage map derived from the "Pamirskij 5" × "Rubira®" F_2 population. The different loci mapped with a SSR primer pair are shown with a capital letter (A, B, C) following the locus name. Loci not mapped in other published maps are underlined. Loci followed by an asterisk after the locus name have distorted segregations (P<0.1). Framed loci in *italics* are the morphological markers. The putative QTL in G7 is figured with an arrow on the right of the linkage group.

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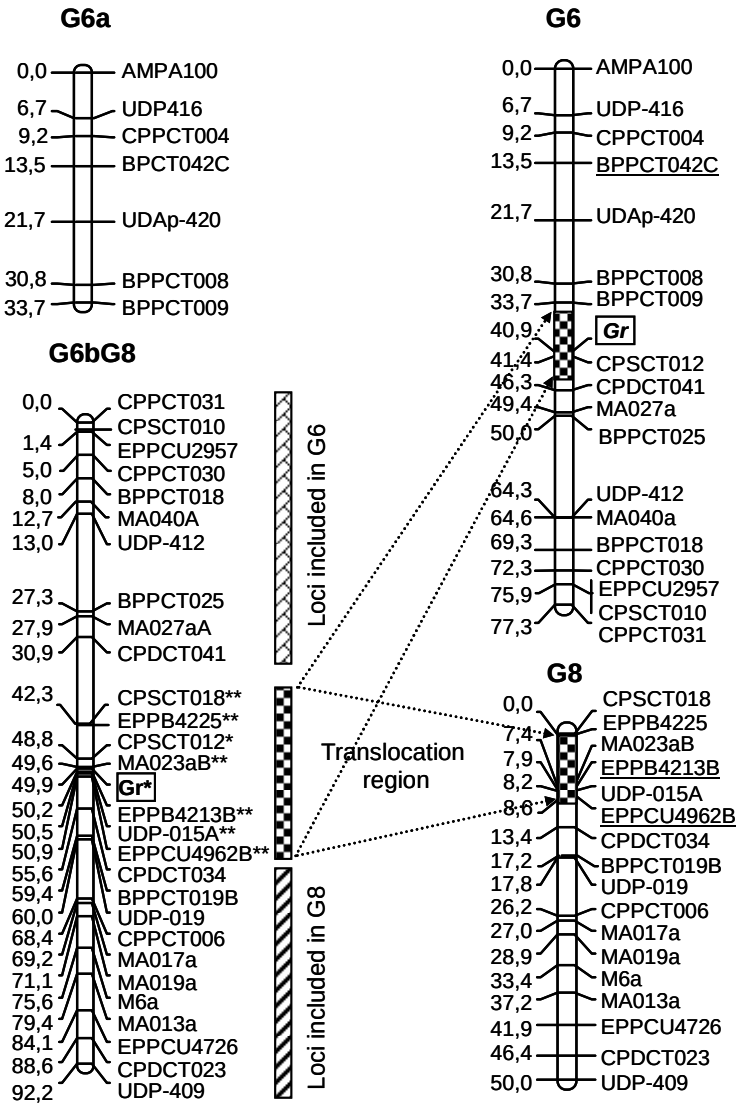

Fig. 2 Linkage groups 6 and 8 of the PR² genetic map showing the position of the translocation region. On the left of the figure: linkage group obtained with a LOD >19.0; it is composed of all the markers of G6 and G8 grouped by pseudo-linkage. Loci followed by an asterisk in the translocation region belong to G6; those followed by two asterisks belong to G8. Bars on the right of the pseudo linkage group indicate the linkage group to which the loci belong to.

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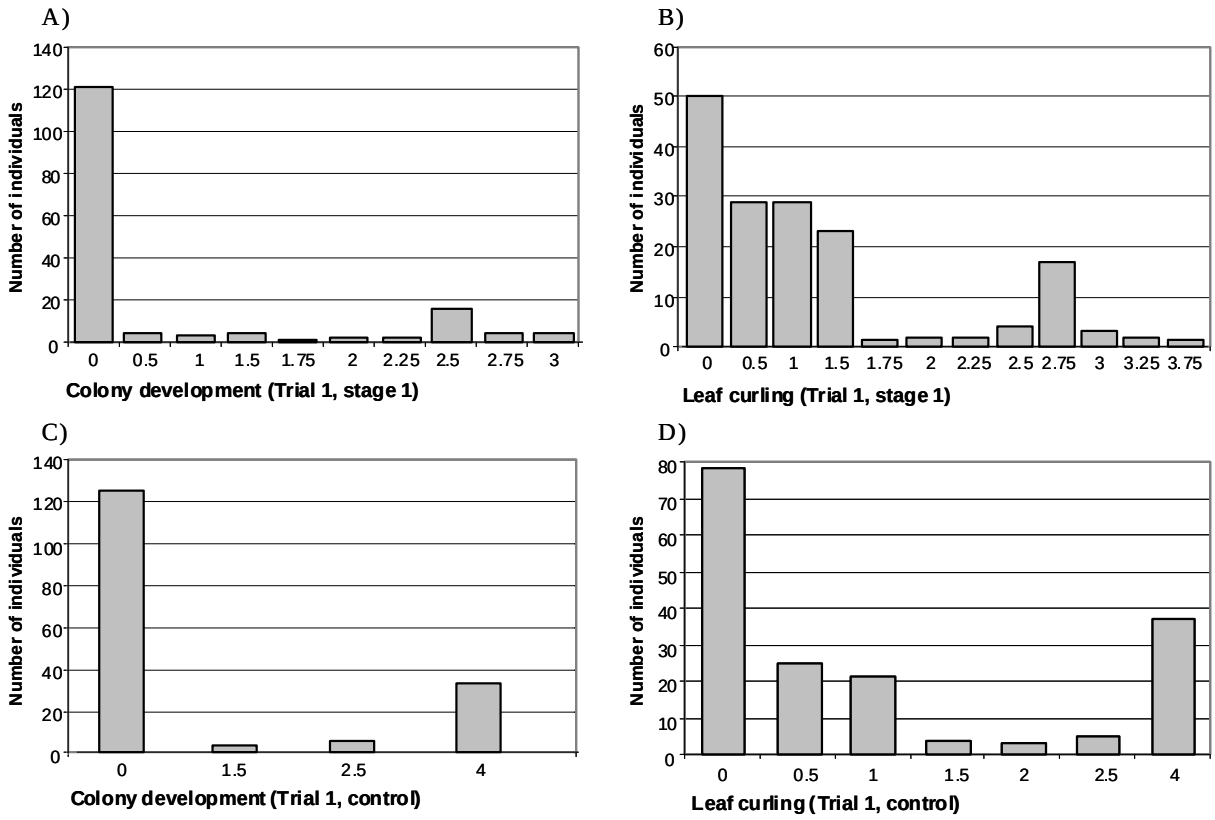

Fig. 3 GPA colonization and leaf curling score distributions for Trial 1 dataset at stage 1 (A, B) and control (C, D). On the ordinate: number of seedlings; on the abscissa: degree of colony development or leaf curling according to the ordinal scale used.

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