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Identification and mapping of resistance gene analogs (RGAs) in *Prunus*: a resistance map for *Prunus*

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Abstract The genetically anchored physical map of peach is a valuable tool for identifying loci controlling economically important traits in *Prunus*. Breeding for disease resistance is a key component of most breeding programs. The identification of loci for pathogen resistance in peach provides information about resistance loci, the organization of resistance genes throughout the genome, and permits comparison of resistance regions among other genomes in the Rosaceae. This information will facilitate the breeding of resistant species of *Prunus*. A candidate gene approach was implemented for locating resistance loci in the genome of peach. Candidate genes representing NBS-LRR, kinase, transmembrane domain classes, as well as, pathogen response (PR) proteins and resistance-associated transcription factors were hybridized to a peach BAC library and mapped by using the peach physical map database and the Genome Database for Rosaceae (GDR). A resistance map for *Prunus* was generated and currently contains 42 map locations for putative resistance regions distributed among 7 of the 8 linkage groups.

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Introduction

Breeding for disease resistance is one of the most important objectives in any breeding program and is particularly relevant in fruit tree crops where generation time and population size hamper rapid breeding response to pathogens and pests. Augmenting traditional breeding practices with more modern molecular mapping technologies better equips the breeder to meet the challenge of breeding sustainable resistance. The development of genetic maps and molecular markers enables the mapping of quantitative trait loci (QTL) and assists breeders in selecting desirable traits early on in the breeding program (Foulongne et al. 2003). The identification of loci for pathogen resistance in peach: provides information about genomic structure of individual resistance loci; elucidates the genomic distribution of various classes of resistance genes; and permits structural and functional comparisons of resistance regions among other genomes in *Prunus*. Previous mapping and comparison of resistance loci in the family Solanaceae revealed that there is some conserved order of resistance genes, but the individual gene functions may vary across species (Grube et al. 2000). The development of a resistance map for *Prunus* is an important first step in examining resistance gene order and gene function in *Prunus* and potentially in the Rosaceae as well.

Advances in genomics, such as the creation of complete BAC physical maps for genomes, allow the mapping of cloned sequences without the need for

segregating populations (Michelmore 2000). In this regard, a BAC library for peach was previously developed by Georgi et al. (2002). Wang et al. (2002a, b) used this library for high-throughput simple sequence repeats (SSR) development in peach and for mapping the peach evergrowing region that controls an economically important trait. It is also the basis, together with a haploid peach BAC library (L.L. Georgi et al., unpublished), for the development of an integrated physical/genetic map of peach (Horn et al. 2005). This peach physical map is anchored genetically to the widely used general map for *Prunus* (Joobeur et al. 1998; Aranzana et al. 2003) and is a valuable resource for identification and cloning of genes conferring pathogen and pest resistance (Horn et al. 2005). Other laboratories have illustrated the value of BAC library resources for identifying genes associated with disease resistance in apple (Vinatzer et al. 2001; Xu et al. 2002), chick pea (Rajesh et al. 2004), citrus (Deng and Gmitter 2003), *Arabidopsis thaliana* (Aarts et al. 1998), Myrobalan plum (Claverie et al. 2004a) and cocoa (Clément et al. 2004).

There are five classes of resistance genes (R-genes), with the most abundant class encoding proteins containing the nucleotide binding site-leucine rich repeat (NBS-LRR) domain. The NBS-LRR class is further divided into two subclasses: the TIR-NBS-LRR (Drosophila Toll and mammalian interleukin like receptors) and the non-TIR NBS-LRR. The other four classes encode R-gene proteins with domains as follows: the extracellular LRR with transmembrane receptor and intracellular protein kinase domain; membrane spanning proteins with large extracellular LRRs; membrane proteins with a coil–coil domain; and those with cytoplasmic ser/thr kinase domains (Ellis et al. 2000; Dangl and Jones 2001).

In this paper, we present the use of a peach BAC library and a genetically anchored peach physical map for creating a resistance gene map for *Prunus*. Candidate genes representing analogs of major resistance genes (NBS-LRR, kinase, and transmembrane domain classes), translation initiation factors (eIF4E) known to be involved in recessive resistance to plant viruses (Rodriguez et al. 1998; Duprat et al. 2002; Nicaise et al. 2003) and defence response genes were hybridized to a peach BAC library. Resistance regions were mapped by using the peach physical map database and the Genome Database for Rosaceae (GDR) (Jung et al. 2004). A resistance map for *Prunus* was generated and currently contains 42 map locations for putative resistance regions distributed among 7 of the 8 linkage groups. The development of a resistance map for *Prunus* provides marked resistance loci for designing breeding strategies to pyramid resistance genes for broad sustainable resistance. This map also serves as a tool for comparing resistance regions and determining the relationship between gene order and function of resistance genes across the Rosaceae, ultimately, identifying particular species that can be used as valuable donors of resistance in breeding programs.

Materials and methods

Identification of resistance and defence-related gene fragments in *Prunus* species

Resistance and defence gene analogs were identified and cloned as described in Decroocq et al. (2002, 2005). In brief, a large set of degenerate primers was designed based on conserved motifs in the aligned amino acid sequences derived from known resistance and defence-related genes (Table S1, available online). DNA templates for the polymerase chain reactions originated from various *Prunus* species: *P. armeniaca* cv. Stark Early Orange, *P. persica* cv. Summergrand and rootstock Guardian^{TM} selection 3-17-7, *P. domestica* cv. Jojo. PCR products were separated on a 1.5% agarose gel and DNA fragments equal or larger than the expected sizes were cloned in the pGEM-T vector (Promega) and sequenced. Nucleotide sequences of *Prunus* resistance and defence gene analogs have been deposited in the GenBank database under accession numbers CZ445405–CZ445433. Other candidate genes were identified by screening the peach cv. Nemared EST (expressed sequence tags) database (http://www.genome.clemson.edu/gdr/)

Sequence analysis

Similarity of the PCR products and peach ESTs was confirmed by comparison of translated sequences with the non-redundant GenBank database, using the Advanced BLASTX program at the National Center for Biotechnology Information (Bethesda, MD) (http://www.ncbi.nlm.nih.gov).

Genetic similarity analyses were performed on the putative *Prunus* RGA nucleotide sequences as well as the deduced protein sequences. Pairwise comparisons and multiple alignments were performed using the ClustalX (http://www.infobiogen.fr/), and neighbor-joining trees were generated from sequence alignments with the Treeview package (Win32 version, http://taxonomy.zoology.gla.ac.uk/rod/rod.html). The bootstrap method was employed to evaluate the reliability of tree branching. NBS sequences along with R-genes from other plant species were included in the genetic similarity analysis: N (U15605), L6 (U27081), LM6 (AAG09951) representing the TIR NBS-LRR class of R-genes, and RPM1 (Q39214), HRT/RPP8 (AAF36987), GPA2 (AAF04603), RSP2 (Q42484), Xa1 (T00020), and RCa7 (AA38218) of the non-TIR NBS-LRR R-gene class.

Identification of BAC clones containing the resistance and defence gene analogs

The PCR fragments described above were re-amplified directly from bacterial stocks using the T7 and SP6

primers, purified on Qiaquick PCR purification columns (Qiagen) and labeled with [$\alpha-^{32}$ P]dCTP (Amersham) by random priming method (Feinberg and Vogelstein 1983). These labeled probes were hybridized onto a peach BAC library as described in Wang et al. (2002a). This BAC library was constructed from DNA of the cv. Nemared, and contains 44,000 pBeloBAC11 clones arrayed on two and one-half 22 cm^2 Hybond-N+ filters and is one of the libraries currently being used to construct a physical map of the peach genome (Georgi et al. 2002) (http://www.genome.clemson/edu/GDR).

BAC clones identified in the first screening were inoculated into 100 µl of LB/chloramphenicol, and incubated at 37°C overnight. BAC clones were then stamped onto Hybond-N+ filters (Amersham, Piscataway, NJ) placed on LB/chloramphenicol agar plates, and incubated overnight at 37°C. The filters were removed from the agar plates and treated with a denaturing solution (1.5 M NaCl, 0.5 M NaOH) for 7 min followed by a neutralizing solution (1.5 M NaCl, 0.5 M Tris, pH 7.2, 1.0 mM EDTA) for 7 min, rinsed with 2× SSC, and the filters were baked at 80°C for 2 h in order to fix the DNA to the filters. Probes were labeled as mentioned above. Prehybridization, hybridization, and detection of positive BAC clones on these filters were carried out as previously described, with the exception of hybridization temperature and washing of the filters (Wang et al. 2002a). Filters were prehybridized for 1 to 2 h and hybridized overnight at 60°C, and filters were washed twice with 2× SSC, 0.1% SDS and once with 1× SSC, 0.1% SDS.

Mapping of peach BACs containing resistance and defence gene analogs

The peach physical map database and GDR (http://www.genome.clemson.edu/gdr/) were used to determine the map location of the confirmed positive BACs.

Results

RGA probe identification, sequence analysis, and alignment

Resistance genes have highly conserved amino acid domains that allow the use of degenerate primers and PCR to amplify resistance gene analogs (RGAs) from genomic DNA (Michelmore 2000). In order to map resistance genes of different classes, we used degenerate primers representing the NBS-like, Cf-like, and the receptor kinase domains. Additional degenerate primers representing translation initiation factors, kinases and MYB, and b-Zip transcription factors as well were used due to their potential roles in the host defence mechanism and recessive resistance (Yin et al. 1997; van der Fits et al. 2000; Park et al. 2001; Asai et al. 2002; Duprat et al. 2002; Lellis et al. 2002; Nicaise et al. 2003; Ruffel et al. 2002; Gao et al. 2004; Sato et al. 2005; Xiao et al. 2005). The cloned sequences of the amplified putative RGA products were aligned. Redundant sequences were eliminated and clones were selected for hybridization to the peach BAC library based on their sequence similarity to certain classes of R-genes or PR proteins.

Identification of BAC clones and mapping resistance and/or defence analog loci with the peach physical map database and the GDR

From the above-mentioned analyses, a total of 58 PCR fragments representing putative RGAs and/or genes involved in host resistance or defence were hybridized to a peach BAC library. Positively hybridizing BACs were re-screened and a total of 161 BACs were confirmed as containing putative resistance and/or defence gene analogs. A search of the physical map database revealed the presence of 120 of these BACs. The remaining 41 BACs were absent from the database presumably because they had not previously been analyzed. Currently, of these 120 BACs, 93 are present within 73 contigs and 27 are present as singletons.

The peach physical map database also provides information about contig assembly, EST hybridization, and genetic marker hybridization data. Of the 58 resistance gene and/or defence analog PCR fragments, 10 hybridized to BACS positive for 7 genetic markers (indicated by an asterisk) (Table 1); thus, 7 resistance regions were directly placed on the *Prunus* general genetic map (Joobeur et al. 1998; Aranzana et al. 2003; Dirlewanger et al. 2004b). Twenty of the 58 probes hybridized to BACs located within contigs containing genetic markers, thus, indirectly mapping 24 additional regions of resistance (Table 1).

In the situation when a probe hybridized to BACs located within a contig that did not contain any *Prunus* genetic markers, they could be mapped through their co-localization with a mapped EST. A search of the GDR at http://www.genome.clemson.edu/gdr/ was performed for the ESTs which hybridized to the same BACs to which candidate genes hybridized. If a map location has been determined for a particular EST, the GDR will provide this information through an EST search in the GDR. Since the peach physical map is anchored on the *Prunus* general genetic map, EST hybridization data to BACs identified as containing putative RGAs or defence-related genes also allows mapping of putative resistance regions. Nine of the 58 probes co-hybridized with mapped ESTs (Table 2) and these resolved into five more mapped putative regions of resistance. Three regions of resistance were determined simply by searching the EST database for any resistance-like genes. In this case, one particular EST PP_LE0026O013 has been mapped to three locations on the peach physical map (Table 2).

Table 1 Resistance gene probes that co-localize with mapped markers of the *Prunus* general genetic map to BAC contigs of the peach physical map

Probe (GenBank accession numbers)	Source species[a]	ID[b]	Marker[c]	Linkage group
A12 (CZ445423)	Peach	TIR-NBS-LRR R protein 7 [*Malus baccata*]/6e-35	CC63*	G7
C5 (CZ445424)	Peach	NBS-LRR putative resistance gene analog [*Malus prunifolia*]/2e-44	CC63*	G7
D2 (CZ445425)	Peach	RCa4 [*Manihot esculenta*] TIR-NBS-LRR/3e-42	FG81A, AG53	G1, G1
D5 (CZ445426)	Peach	NBS-LRR putative resistance gene analog [*Malus prunifolia*]/1e-42	PC29A	G6
D12 (CZ445427)	Peach	NBS-like putative resistance gene [*Phaseolus vulgaris*]/6e-40	FG53, FG78, CC63*, FG28*	G6, G6, G7, G1
E5 (CZ445428)	Peach	Resistance protein candidate [*Vitis amurensis*]/3e-37	CC63*	G7
F4 (CZ445429)	Peach	NBS-LRR disease resistance like protein [*Mentha longifolia*]/2e-30	AG17A, 5-E2* SCAL-19-MYRO*	G7, G7, G7
Cd76 (CZ445405)	Apricot	Putative disease resistance protein (TIR-NBS-LRR class) [*Arabidopsis thaliana*]/3e-37	FG28*	G1
Cd77 (CZ445406)	Apricot	Resistance protein analog [*Phaseolus vulgaris*]/7e-39	PC29A	G6
Cd78 (CZ445407)	Apricot	NBS-kinase protein Z2 [*Solanum tuberosum*]/9e-36	AG8 A, B, C	G4, 5, 1
Cd81 (CZ445432)	*P. davidiana*	NBS-kinase protein Z2 [*Solanum tuberosum*]/6e-58	AG104	G7
Cd84 (CZ445408)	Apricot	CC-NBS-LRR protein [*Solanum tuberosum*]/2e-06	AC31	G2
Cd131 (CZ445409)	Apricot	NBS-like putative gene resistance homolog [*Rosa roxburghii*]/2e-14	AG8 A, B, C AC7A, FG5*	G 4, 5, 1 G1, G1
Cd134 (CZ445410)	Apricot	NBS-like putative resistance protein [*Phaseolus vulgaris*]/2e-39	FG28	G1
Cd136 (CZ445433)	*P. davidiana*	Resistance protein MG13 [*Glycine max*]/5[e] −50	AC3, FG36 AG8 A, B, C AG113, B6H11	G1, G1 G 4, 5, 1 G1, G1
Cd140 (CZ445431)	Plum	MRGH63 resistance gene [*Cucumis melo*]/9e-18	AG8 A, B, C	G 4, 5, 1
Cd38 (CZ445411)	Apricot	Wall-associated kinase (Wak4) [*Arabidopsis thaliana*]/4e-40	AG14A, PRU1	G8, G8
Cd39 (CZ445412)	Apricot	Wall-associated kinase (Wak2) [*Arabidopsis thaliana*]/5e-35	AG14A, PRU1	G8, G8
Cd113 (CZ445413)	Apricot	Receptor-like protein kinase [*Arabidopsis thaliana*]/9e-32	LF98	G6
Cd82 (CZ445422)	Peach	myb-related transcription factor [*Arabidopsis thaliana*]/4e-08	AG108	G5
Cd195[d] (CZ445415)	Apricot	Eukaryotic translation initiation factor 4E [*Pisum sativum*]/1e-31	AG4A	G8
Cd207 (CZ445416)	Apricot	Eukaryotic translation initiation factor 4A [*Arabidopsis thaliana*]/2e-76	LF573_PP2C*	G1
Cd210[d] CO370600	Apricot	Eukaryotic translation initiation factor 4E [*Pisum sativum*]/7e-40	AG4A	G8
Cd213 (CZ445417)	Apricot	Potyvirus VPg interacting protein [*Pisum sativum*]/2e-76	SCAL-19-MYRO*	G7
Cd47 (CZ445418)	Apricot	Calcium-binding transporter-like protein [*Arabidopsis thaliana*]/3e-57	AG25 A, B, AG29A	G1, G1
Cd68 (CZ445419)	Apricot	Short chain alcohol dehydrogenase [*Nicotiana tabacum*]/7e-19	AG58, FG49*	G7, G7

[a]Origin of selected probes (source species)
[b]Closest similarity with a member of GenBank database (ID)
[c]Genetic marker mapping on the same BAC clone(*) or contig
[d]Cd210 was cloned from cDNA as described in Decroocq et al. (2005). Cd195 is a PCR fragment obtained from apricot genomic DNA

Further analysis of co-hybridization of ESTs and resistance-associated sequences to mapped BAC contigs revealed the presence of EST-derived SSRs that could have utility as markers for resistance. The ESTs containing SSR sequences are PP_LEa0026L09f (G2), PP_LEa0009B03f (G5), and PP_LEa0025O02f (G7) and can be found at http://www.genome.clemson.edu/gdr/. These three localized putative resistance gene containing

Table 2 Resistance gene probes that co-localize with peach ESTs to BAC contigs of the peach physical map

Probe (GenBank accession numbers)	Source species[a]	ID[b]	EST[c]	Marker[c]	Linkage group
D5 (CZ445426)	Peach	NBS-LRR putative resistance gene analog [*Malus prunifolia*]/1e-42	PP_LEa0009L15 PP_LEa0012A23 PP_LEa0012K18	AC41A	G4 (TXE, Joobeur et al. 1998) G4 (JXF, Dirlewanger et al. 1998) G4F (FXT, Joobeur et al. 2000)
				AC55A,B LF11	G4, G5 (TXE, Joobeur et al. 1998) GN6 (P2175XGN, Dirlewanger et al. 2004a)
D9 (CZ445430)	Peach	Putative NBS-LRR type resistance gene [*Prunus persica*]/4e-60	PP_LEa0030M23 PP_LEa0030N07	AC10	G2 (TXE, Joobeur et al. 1998) G2T (FXT, Joobeur et al. 2000) G2F (FXT, Joobeur et al. 2000)
D12 (CZ445427)	Peach	NBS-like putative resistance gene [*Phaseolus vulgaris*]/6e-40	PP_LEa0009L15 PP_LEa0012A23 PP_LEa0012K18	AC41A	G4 (TXE, Joobeur et al. 1998) G4 (JXF, Dirlewanger et al. 1998) G4F (FXT, Joobeur et al. 2000)
E5 (CZ445428)	Peach	Resistance protein candidate [*Vitis amurensis*]/3e-37	PP_LEa0009L15 PP_LEa0012A23 PP_LEa0012K18	AC41A	G4 (TXE, Joobeur et al. 1998) G4 (JXF, Dirlewanger et al. 1998) G4F (FXT, Joobeur et al. 2000)
Cd68 (CZ445419)	Apricot	Short chain alcohol dehydrogenase [*Nicotiana tabacum*]/7e-19	PP_LEa0009J20	FG49a	G7 (PXF, Dettori et al. 2001)
Cd99 (CZ445420)	Apricot	Transcriptional activator RF2a [*Arabidopsis thaliana*]/2e-37	PP_LEa0030M24	AC19	G2 (TXE, Joobeur et al. 1998) G2 (GXN, Jauregui et al. 2001) G2T (FXT, Joobeur et al. 2000) G2F (FXT, Joobeur et al. 2000) G2F (FXB, Ballester et al. 1998)
				EAT/CAG7	G7 (SCXB, Sosinski et al. 1998)
Cd107 (CZ445421)	Apricot	UDP-glucose:salicylic acid glucosyltransferase [*Nicotiana tabacum*]/4e-19	PP_LEa0011F03	FG94	G8 (PXF, Dettori et al. 2001)
Cd161 (CZ445414)	Apricot	myb-related protein M4 [*Arabidopsis thaliana*]/2e-20	PP_LEa0010M17	AC55A,B LF11	G4, G5 (TXE, Joobeur et al. 1998) GN6 (P2175XGN, Dirlewanger et al. 2004a)
Cd195 (CZ445415)	Apricot	Eukaryotic translation initiation factor 4E [*Pisum sativum*]/1e-31	PP_LEa0030M24	AC19	G2 (TXE, Joobeur et al. 1998) G2 (GXN, Jauregui et al. 2001) G2T (FXT, Joobeur et al. 2000) G2F (FXT, Joobeur et al. 2000) G2F (FXB, Ballester et al. 1998)
				EAT/CAG7	G7 (SCXB, Sosinski et al. 1998)
EST search		Mlo-like resistance gene	PP_LEa0026013 [1]	AC33A	G2 (TXE, Joobeur et al. 1998)
				AC37A	G2 (TXE, Joobeur et al. 1998)
				AG6	G4 (TXE, Joobeur et al. 1998)

[a]Indicates origin of selected probe (source species)
[b]Closest similarity with a member of the GenBank database (ID)
[c]The EST and genetic marker information for the same BAC clone

regions map to locations previously identified as containing QTLs for powdery mildew resistance in linkage groups G2 and G5 (Foulongne et al. 2003; Dettori et al. 2001) and a nematode resistance gene, Ma1, in linkage group G7 (Dirlewanger et al. 2004a; Claverie et al. 2004b) (Fig. 1).

In addition to the 39 putative resistance regions positioned in the general *Prunus* map, an additional three were identified in other *Prunus* maps as they co-localize to contigs identified by the markers LF11, FG94, and AT/CAG7 that are mapped in P2175 × GN (Myrobalan plum × almond peach) (Dirlewanger et al. 2004a), P × F (peach × (peach × *Prunus ferganensis*)) (Dettori et al. 2001), and SC × B (peach)(Sosinski et al. 1998), respectively. In summary, we identified a total of 42 regions of resistance in the *Prunus* genome, 39 mapped on the peach physical/*Prunus* general genetic map (Fig. 1) and 3 in linkage maps derived from the other mapping populations of *Prunus* mentioned above. The putative regions of resistance span 7 of the 8 linkage groups on the *Prunus* general genetic map.

Genetic similarity analysis

Genetic similarity analyses of the mapped *Prunus* RGA sequences along with known R-genes from other plant species N, LM6, RPM1, HRT/RPP8, L6, GPA2, RPS2, Xa1, and RCa7 were performed at the nucleotide and the deduced amino acid sequence levels (Figs. 2, 3). The PCR fragments obtained with the degenerate primers representing translation initiation factors, transcription factors, and kinases were not included in these analyses since they shared no homology with the R-genes or with each other. Among the R-genes, L6 and N are representative of the TIR-NBS-

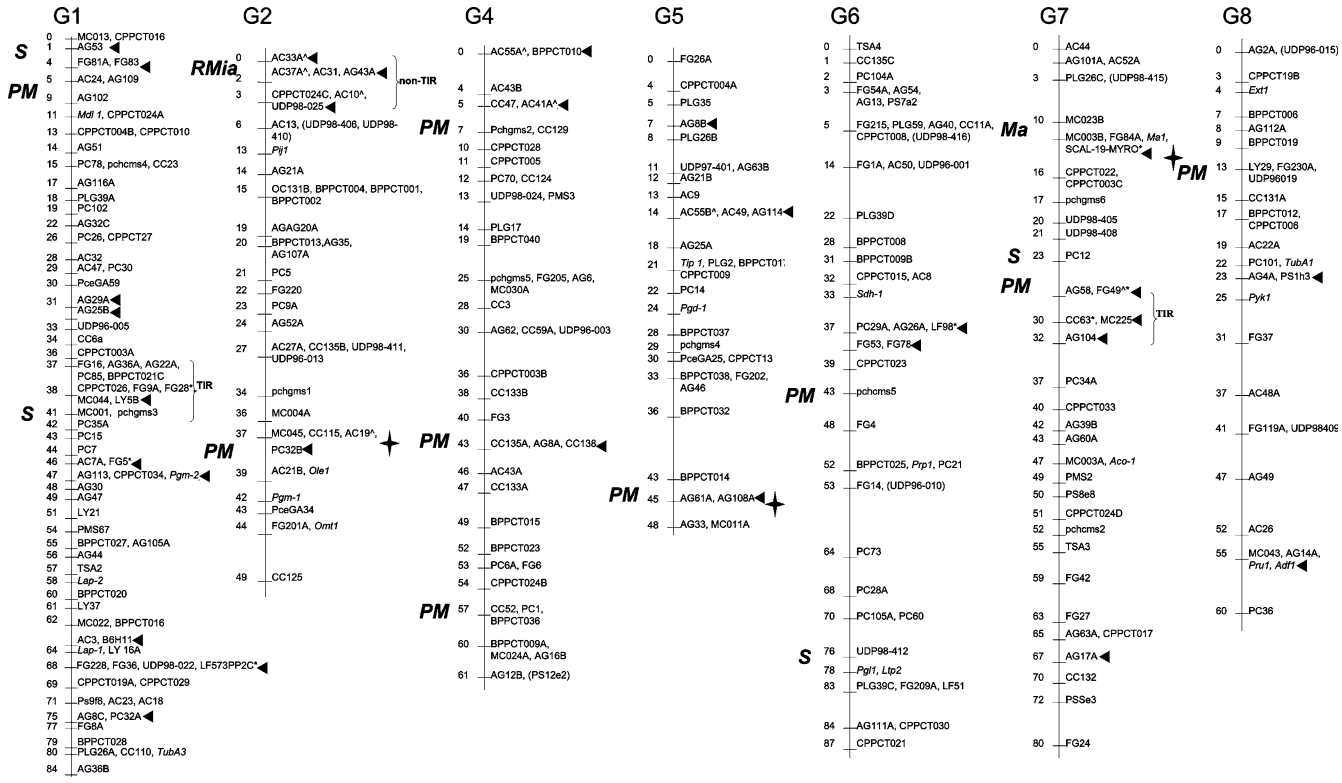

Fig. 1 A resistance map for *Prunus:* resistance loci and QTLs are indicated by *large bold letters* placed to the left of the linkage groups, QTLs for powdery mildew (*PM*) (Dettori et al. 2001; Foulongne et al. 2003; Dirlewanger et al. 2004b; I. Verde, personal communication) and Sharka (*S*) (Vilanova et al. 2003; Decroocq et al. 2005) as well as loci for nematode resistance (*Ma* and *RMia*) (Claverie et al. 2004a; Dirlewanger et al. 2004a). Markers in *bold* with (*asterisk*) show resistance regions mapped directly: RGA/defence-related probes positive for BACs were also positive for genetic markers (see Table 1). Markers in *bold* with (∧) indicate resistance regions mapped by EST hybridization data (see Table 2). ✦ indicates an SSR identified from EST sequence data (http://www.genome.clemson.edu/gdr/). The map is based on the Aranzana et al. (2003) consensus map for *Prunus* and the peach physical map (Horn et al. 2005). *Curly brackets* indicate clusters of non-TIR (non-TIR NBS-LRR gene class) or TIR (TIR NBS-LRR gene class). Note: Linkage group 3 is not shown here because no RGA locations were discovered

LRR class (Lawrence et al. 1995; Michelmore 2000); RPM1 (McDowell 2004) and HRT/RPP8 (Cooley et al. 2000) represent the non-TIR-NBS-LRR class. On the basis of the genetic similarity analyses, the *Prunus* RGAs separate into 2 distinct clusters. One of the clusters consists of the non-TIR NBS-LRR class and the second cluster consists of the TIR-NBS-LRR class of resistance genes (Fig. 2).

Analogs of Cf-like, kinase and other classes of R-genes were hybridized to the BAC library but did not map in the initial peach physical/*Prunus* general genetic map since this map database is not yet complete. Few co-localizations of *Prunus* RGA probes were observed. This is presumably due to our preselection of non-redundant RGA sequences for probe development. However, we noticed that two distinct non-TIR-NBS-LRR probes, D9 and Cd84, mapped to the same distal region of linkage group G2 (Fig. 1). Similarly, four TIR-NBS-LRR analogs (A12, D12, C5 and E5) mapped to the same region in linkage group G7 (CC63 marker) and three other TIR-NBS-LRR analogs (CD134, CD76, and D12) in linkage group G1 (FG28 marker), (Table 1, Fig 1).

Discussion

Identifying and mapping RGAs with the use of the peach BAC library and physical map have allowed us to locate putative regions of resistance in *Prunus* without the use of segregating populations. In this study, we have mapped a total of 42 regions of resistance based on hybridization data obtained from 30 of 58 probes. As the peach physical map is not yet complete, many of the BACs identified as containing RGAs or putative defence-related genes with the other 28 probes have not been mapped. This is for several reasons: (1) not all BACs identified by hybridization to RGAs have been fingerprinted, (2) some BACs are present as singletons, and (3) BACs belong to contigs that are not yet anchored on the genetic map. In the future, additional RGAs will be mapped as the assembly of the peach physical map comes to completion. Similar limitations with physical mapping of RGAs in soybean have been described by Peñuela et al. (2002).

Of the 30 probes which proved to be informative for mapping, only 17 returned BLAST results with sequence

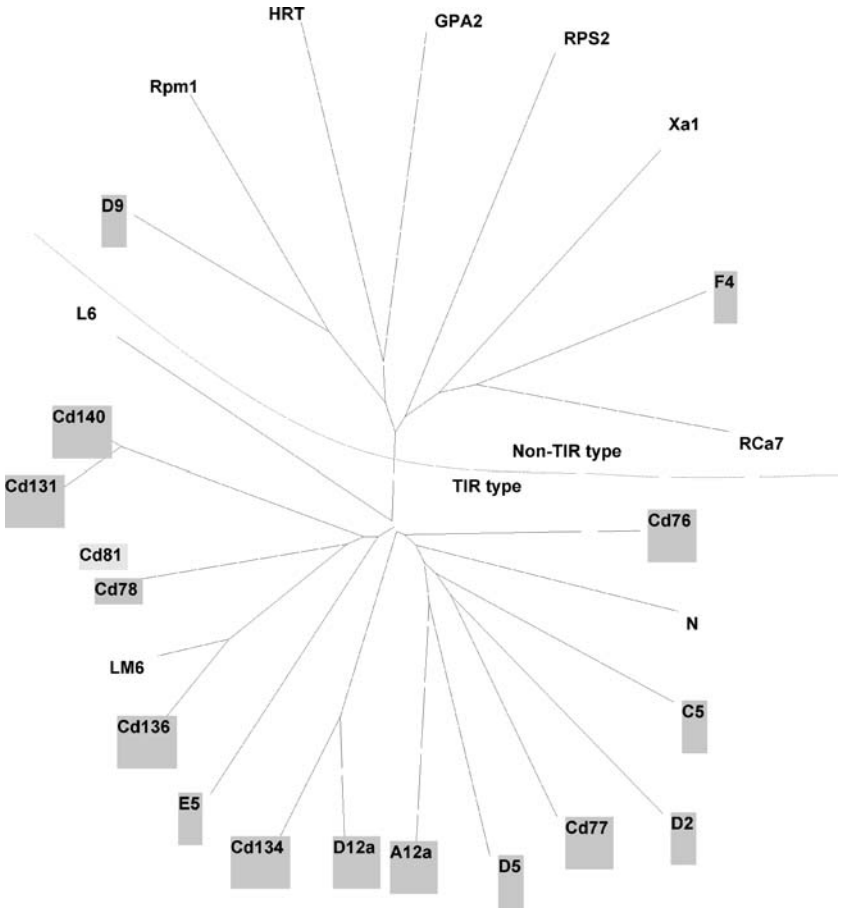

Fig. 2 Genetic similarity analyses of the mapped *Prunus* RGA sequences along with known R-genes from other plant species. Pairwise comparisons and multiple alignments were performed using the ClustalX, and neighbor-joining trees were generated with the Treeview package. The bootstrap method was employed to evaluate the reliability of the tree branching. The following NBS sequences from other plant species were added in the genetic similarity analyses: N (U15605), L6 (U27081), LM6 (AAG09951), RPM1 (Q39214), HRT/RPP8 (AAF36987), GPA2 (AAF04603), RSP2 (Q42484), Xa1 (T00020), and RCa7 (AA38218). *Grey shading* indicates RGAs cloned from *Prunus* genomic DNA and located on the peach physical map

similarity to resistance genes of the NBS-LRR class; however, these 17 probes accounted for more than half of the total map locations identified. More interestingly, several of these probes mapped to locations where QTLs for resistance mapped for traits such as, powdery mildew (Dettori et al. 2001; Foulongne et al. 2003; Dirlewanger et al. 2004b; I. Verde, personal communication) and Sharka resistance (Decroocq et al. 2005). Moreover, 3 amplified RGAs mapped to the region of G7 that is known to contain the *Ma* gene or on G2 close to the *RMai* gene, both of which control resistance to root-knot nematodes (Claverie et al. 2004a; Dirlewanger et al. 2004a). Foulongne et al. (2003) described the QTLs, with the strongest effect for powdery mildew caused by *Sphaerotheca pannosa*, as being located in G6 and G8 in the three related crosses: SD (*Prunus persica* cv Summergrand and *Prunus davidiana* clone P1908), SD40[2] (a selected genotype from SD selfed), and SD40 backcrossed with Summergrand®. We mapped RGAs in the region of the QTL for powdery mildew in G6 but not in G8. In the case of QTLs for Sharka, although we did not map in the same location as the strongest effect for Sharka in linkage group G6, we did map RGAs in regions associated with QTLs for Sharka in G1 and G7 (Decroocq et al. 2005). Additionally, we mapped multiple RGA clones in the same region of G1 as Vilanova et al. (2003) mapped Sharka resistance. RGAs were mapped in all of the linkage groups except linkage group G3. Bliss et al. (2002) also failed to map any RGAs or resistance-related sequences in G3 of the *Prunus dulcis* × *Prunus persica* L. Batsch cross.

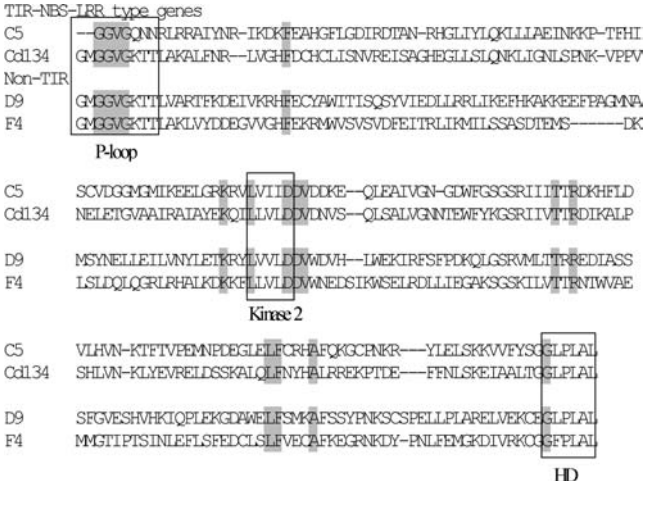

Fig. 3 Comparison of the predicted amino acid sequences of four *Prunus* resistance gene-like fragments. Two *Prunus* representatives of the TIR-type and two of the non-TIR type NBS-LRR genes were selected and aligned using the ClustalX software. These sequences correspond to the NBS domain. Three motifs characteristic of this domain (P-loop, kinase 2 and HD = hydrophobic domain) have been *circled*

Sequence comparison revealed two distinct clusters of RGAs, with majority of the RGAs belonging to the TIR-NBS-LRR class. At this juncture, it is not possible to determine if this is due to a greater abundance of this class of RGA in the *Prunus* genome or a bias in the amplification of RGAs due to limitations of primer design. Mapping of RGAs indicated separate clustering of TIR and non-TIR NBS-LRRs in the *Prunus* genome (Fig. 1) as has been noted in *Medicago truncatula* (Zhu et al. 2002) and soybean (Kanazin et al. 1996); however, this is in contrast with findings in *Arabidopsis* and cassava, where clustering together of TIR NBS-LRR and non-TIR NBS-LRR was observed (Meyers et al. 1999; Lopez et al. 2003). In future, further RGA mapping or genomic sequencing in peach should help to resolve this issue as well as to map other classes of RGAs (e.g. Cf-like, Pto- like, Xa21-like, and RPW8-like).

Probe 210 with sequence similarity to eIF4E (a translation initiation factor involved in recessive resistance to plant viruses) was mapped by RFLP in linkage group G4 of the previously mentioned SD40[2] population (Decroocq et al. 2005). There are four reported eIF4E and iso4E in *Arabidopsis thaliana* (http://www.arabidopsis.org). On the basis of this information, it is anticipated that additional locations of eIF4E exist in the *Prunus* genome. Indeed, we identified an additional location for the same probe in linkage group G8 near AG4A on the peach physical map. These results illustrate how physical mapping of genes complements mapping by molecular marker techniques that are labor intensive, time consuming and limited to genes displaying detectable polymorphism.

Our research identifies regions of the *Prunus* genome that contain resistance-related gene sequences and correlates them with previously mapped phenotypic resistance traits to different pathogens. This information will be extremely useful to the *Prunus* community, as breeding for resistance is time consuming due to the lengthy maturation time of most *Prunus* species. The use of a peach BAC library in conjunction with the peach physical map database and GDR provides BAC library and EST information which can facilitate map-based cloning of genes involved in resistance to many pests and pathogens, as well as the development of molecular markers useful in marker-assisted selection (MAS) of resistant species. In this study, three SSRs were detected in putative regions of resistance and may prove to be useful in MAS. The development of a resistance map for *Prunus* based on the peach physical map, which is anchored to the general *Prunus* map, creates a framework for these endeavors. The creation of such a map is also an important initiative in determining resistance gene order and gene function between other genomes of the *Prunoideae*, as well as in the Rosaceae.

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