Construction of a Linkage Map and Identification of Resistance Gene Analog Markers for Root-knot Nematodes in Wild Peach, *Prunus kansuensis*

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ABSTRACT. The root-knot nematode (RKN) *Meloidogyne incognita* can cause severe crop loss in economically important *Prunus* species like peach (*P. persica*), almond (*P. communis*), plum (*P. salicina*), and apricot (*P. armeniaca*). Some peach rootstock, including Nemaguard (*P. persica*), Nemared (*P. persica*), and Myrobalan plum (*P. cerasifera*), display significant resistance to RKN. We present a genetic linkage map constructed by using simple sequence repeat (SSR) and sequence-related amplified polymorphism (SRAP) for a peach backcross population (190 individuals) of RKN-resistant ‘Honggengansutao’ (*P. kansuensis*) and susceptible ‘Bailey’ (*P. persica*). Degenerate primers designed from conserved motifs of known plant resistance gene (*R*) products were used to amplify genomic DNA sequences. Twenty-two resistance gene analog (RGA) sequences were selected from 48 RGAs with open-reading frames to design sequence-tagged site markers. The linkage map of ‘Honggengansutao’ is composed of 138 loci (30 SSRs, 102 SRAPs, five RGAs, and one morphological marker for RKN resistance) assigned to eight linkage groups. The map covers 616 cM of the peach genome with an average marker spacing of 4.9 cM. The five RGAs were mapped to Groups 2, 7, and 8. One gene (designated *PkMi*) involved in resistance to RKN was mapped to Group 2 (which also includes the known RKN-resistance *RMia* gene). BLASTN analysis mapped all RGAs to the peach genome sequence. The map constructed in the study will aid future rootstock breeding with marker-assisted selection to identify additional candidate RGA sequences.

Root-knot nematodes are damaging pests of fruit tree crops and numerous other perennial or annual plants. Several rootstock breeding programs using interspecific hybridization have introduced useful traits for size control, adaptation to the new environments, and pest resistance (Desmond and Daniele, 2008). Three major RKN species, *Meloidogyne incognita* (MI), *M. javanica* (MJ), and *M. arenaria* (MA), are present in most areas with tropical and Mediterranean climates (Lamberti, 1979; Sasser, 1977). Different levels of RKN resistance have been observed in *Prunus* species and the corresponding resistance (*R*) genes used for rootstock breeding (Esmenjaud et al., 1997). The Myrobalan plum clone P.2175 is highly resistant to all tested RKN species, including MI, MA, MJ, and *M. floridensis* (Lecouls et al., 1997; Rubio-Cabetas et al., 1999). Resistance is conferred by the major dominant gene *Ma*. The *Ma* gene, carried by myrobalan plum and almond, has been mapped to linkage Group 7 (G7) of the *Prunus* reference map (Claverie et al., 2004; Dirlewanger et al., 2004; Van Ghelder et al., 2010). The *RMia* gene also is a dominant gene carried by the peach cultivars Nemaguard and Nemared (Ramming and Tanner, 1983) and the almond × peach hybrid ‘GF.557’ (Claverie et al., 2004) confers resistance limited to MI and MA. The *RMia* gene maps to linkage Group 2 (G2) of both the *Prunus* reference map (Dirlewanger et al., 2004) and the ‘P.2175’ × ‘Felinem’ map (Claverie et al., 2004).

The use of degenerate *R* gene primers has proven very effective for identifying *R* genes from diverse species of plants (Aarts et al., 1998; Robert et al., 2008; Wang et al., 2010). Crop

scientists have benefited greatly from the identification and mapping of *R* genes linked to pathogen and pest resistance phenotypes. Over the last 15 years, over 40 *R* genes, conferring resistance to several pathogens, have been characterized in both model plants and crop species (Qiang et al., 2005). The most abundant class of *R* genes encodes proteins containing nucleotide-binding sites and leucine-rich repeat (NBS-LRR) domains (Dangl and Jones, 2001). Many NBS-LRR homologs, also known as RGAs, are phylogenetically related to known *R* genes, and a number of studies have found homologs mapping to *R* gene loci (Hinchliffe et al., 2005; Gaspero et al., 2007) providing further evidence that such genomic regions code for resistance.

In this report, we have characterized an *R* gene (*PkMi*) from *P. kansuensis* ‘Honggengansutao’. A genetic linkage map was constructed by using SSR, SRAP, and RGA-sequence-tagged site (STS) markers for mapping RKN resistance. This map is a valuable tool for locating the regions involved in RKN resistance and for the development of additional molecular markers within these regions.

Materials and Methods

PLANT MATERIAL. Seven *P. persica* rootstock cultivars and two *P. kansuensis* cultivars were used in this study. Resistance to RKN was evaluated in 1998 at Zhengzhou Fruit Institute, Zhengzhou, China. In 2000, ‘Honggengansutao’ (immune to *M. incognita*) was crossed as a female parent to ‘Bailey’ (susceptible to *M. incognita*) to obtain F₁ hybrids. A randomly selected F₁ hybrid was then crossed with ‘Bailey’ to generate a BC₁ population expected to segregate for RKN resistance. Seeds from BC₁ were stratified and the subsequent seedlings were planted in a greenhouse in 2005. One hundred ninety individuals were used to construct the genetic map.

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ROOT-KNOT NEMATODE ISOLATES AND EVALUATION OF PLANT MATERIAL. A single MI population was used to evaluate resistance to RKN. The MIs were isolates reared from a single egg mass and maintained on tomato (*Solanum lycopersicum*). The identity of the population, at the species level, was verified each year before inoculation by means of their morphological characteristics. For example, the female stylet of the MI population is unique; the anterior half of the stylet cone is cylindrical and distinctly curved dorsally. The shaft is only slightly wider posteriorly. The stylet knobs of MI are set off from the shaft.

All plant materials (parents, controls, F₁, and BC₁) were grown in 10-L containers with sterile soil in an open field. Thirty seedlings of nine rootstock cultivars represented their parents, and only one seedling of BC₁ hybrids represented itself. When the seedling stems were ≈5 mm in diameter, they were inoculated individually with 500 s-stage MI juveniles (J2s) in May. Four to 5 months after inoculation, the seedlings were washed and roots rated for gall severity as previously described (Barker, 1985) with 0 = no gall, 1 = 1% to 10% of root system galled, 2 = 11% to 30%, 3 = 31% to 70%, 4 = 71% to 90%, and 5 = greater than 90% galled. The F₁ populations were evaluated once, whereas the BC₁ evaluations were repeated for 3 years (consecutive). Each evaluation of RKN resistance included population parents and control plants (tomato).

DNA EXTRACTION. Genomic DNA was isolated from fresh leaf tissue using a DNA extraction kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The DNA concentrations were checked on 1% TAE agarose gels and working solutions of genomic DNA (100 ng·μL⁻¹) were kept in TE buffer (pH 8.0).

SIMPLE SEQUENCE REPEAT ANALYSIS. For SSR markers, the optimal annealing of 134 primer pairs was determined using Temperature Gradient PCR (Biometra, Goettingen, Germany). The polymerase chain reaction (PCR) products were run on 1.5% agarose gels to verify amplification and to determine optimal annealing temperatures. Successfully amplified SSRs were screened in both parents and a set of eight mapping progeny chosen to establish segregation patterns. Segregating SSRs were then studied in the whole mapping population. The PCR reaction mixture (10 μL) contained 0.6 μL dNTP (2.5 mM), 1 μL 10 × PCR buffer (with Mg²⁺), 0.35 μL (10 mM) forward and reverse primers, 10 ng of genomic DNA, 0.5 U Taq DNA polymerase (Promega, Beijing, China), and 6.6 μL double-distilled water. PCR reactions were performed in a Biometra T1 thermal cycler under the following conditions: an initial denaturation for 3.5 min at 94 °C followed by 30 cycles of 45 s at 94 °C, then 45 s at 49 to 57 °C, then 1 min at 72 °C, and a final extension of 5 min at 72 °C. The PCR products were visualized by electrophoresis on 8% polyacrylamide gels run for 1 h at 250 W with 1 × TBE.

SEQUENCE-RELATED AMPLIFIED POLYMORPHISM ANALYSIS. For SRAP analysis, 14 forward primers (me1 to me14) and 17 reverse primers (em1 to em17) were used in 238 primer combinations. Amplification was performed as described (Li and Quiros, 2001) and the PCR products were visualized using 1.5% agarose gels.

DEGENERATE PRIMER DESIGN AND POLYMERASE CHAIN REACTION CONDITIONS FOR THE RESISTANCE GENE ANALOG. A pair of degenerate primers was designed based on the following NBS-LRR encoding *R* genes *N* (GenBank AB120513) and *M* (GenBank U73916) from the TIR-NBS-LRR class and *RPS2* (GenBank NM_118742) and *RPM1* (GenBank NM_111584) from the non-TIR-NBS-LRR class. The PCR reaction mixture

and thermocycle protocols were the same as those used for SSR analysis.

Amplified products of degenerate RGA primer pairs were separated on 1.2% agarose gels in 1 × TAE buffer. Desired bands were purified using a Tiangel Midi PCR purification kit (Tiangen), cloned into the pGEM-T easy vector (Promega), and then transferred into *Escherichia coli* competent cells (DH5α). Sequencing of the DNA was performed using an ABI 3730 automated sequencer (BGI, Shenzhen, China). The DNA sequences obtained from 'Honggengansutao' were processed to remove vector sequences using the National Center of Biotechnology Information (NCBI) VecScreen program (NCBI, 2010a). The amino acid sequences of RGAs were deduced on the basis of sequence similarity using the software DNAMAN (Lynnon Corp., Pointe-Claire, Quebec, Canada). To convert the RGAs with uninterrupted open-reading frame (ORF) sequences into RGA-STS markers, gene-specific primers were designed by primer-blast software (NCBI, 2010b). The RGA-STS primers developed from the sequences of the RGAs produced polymorphic fragments in the BC₁ population. The specific PCR reactions with the RGA-STS primers were also performed in 10 μL and used the same program in RGA cloning. A cleaved amplified polymorphic (CAP) marker was also created by digesting the RGA-STS products with *Eco*RI, *Mse*I, *Sau*3AI, *Alu*I, or *Hae*III (Takara, Dalian, China). The whole BC₁ population was then amplified and digested by the optimum enzyme in 8 μL H₂O, 1 μL 10 × buffer tango (Fermentas, Shenzhen, China), and 0.25 μL enzyme (2.5 U) at 37 °C for 3 h.

SCORING OF MARKERS. There were four classes of markers scored in SSR, SRAP, and RGA-STS statistics: 1) heterozygous markers segregating for the female parent; 2) heterozygous markers segregating for the male parent; 3) dominant markers where the band was contributed by the female 'Honggengansutao' but was absent in the male parent 'Bailey'; and 4) dominant markers where the band was contributed by the male parent, 'Bailey', but was absent in the female parent, 'Honggengansutao'. The RKN resistance of peach is controlled by a dominant gene originating from 'Honggengansutao', so only markers (1) and (3) that segregated in a 1 present:1 absent ratio were included in the mapping files. Chi-square tests of goodness of fit were performed on segregation data for all markers to determine their agreement with the expected ratios.

LINKAGE MAP CONSTRUCTION. A female parent genetic linkage map for the BC₁ family was constructed using JoinMap 2.0 software (Stam and Van Ooijen, 1995). The mapping analysis was conducted using a minimum logarithm of odds threshold (LOD) score of three and a 0.40 maximum recombination frequency. The map distances were computed using the Kosambi mapping function.

PHYSICAL DISTRIBUTION OF RESISTANCE GENE ANALOGS ON CHROMOSOMES OF 'HONGGENGANSUTAO' PEACH. Twenty-two RGAs were mapped by linkage analysis. BLASTN analysis of RGA sequences against the Pp whole genome sequence (Version 1.0; Washington State University, 2010a) was used to infer their location on specific chromosomes. The RGAs with same alignment results were thought as a "false" RGA.

Results

ROOT-KNOT NEMATODE RESISTANCE TESTS OF THE CULTIVARS AND CHOICE OF THE PARENTS FOR F₁ AND BC₁ CROSSES. In the nine

cultivars tested, the high inoculum pressure allowed separation into immune (gall index = 0), resistant ($1.0 > \text{gall index} > 0$), and susceptible (gall index > 1.0) classes (Table 1). The peaches ‘Bailey’, ‘Shouxingtao 2’, and ‘Shouxingtao 3’ were susceptible to RKN, whereas ‘Maotao 1’, ‘Maotao 2’, and ‘Maotao 3’ were resistant. ‘Shouxingtao 1’, ‘Honggengansutao’, and ‘Baigengansutao’ were immune. Thus, ‘Bailey’ and ‘Honggengansutao’ were selected as parents for creation of BC₁ population.

RESISTANCE SEGREGATION ANALYSIS OF THE BC₁ POPULATION. One hundred ninety individuals from the (‘Honggengansutao’ × ‘Bailey’) × ‘Bailey’ population were screened for RKN resistance. Plants were classified as resistant or susceptible based on the gall index. Eighty-seven individuals were resistant and 103 susceptible. Resistant individuals were coded as heterozygous for the trait and those susceptible were coded as homozygous recessives. The segregation ratio 87R:103S was close to 1R:1S expected from the *PkMi* (chi-square = 1.35; threshold value = 3.84 for $P = 0.95$).

SIMPLE SEQUENCE REPEAT ANALYSIS. The 134 SSRs tested for their polymorphisms originated from several *Prunus* species: 102 were from peach, 21 from Japanese plum (*P. salicina*), six from almond, four from sweet cherry (*P. avium*), and one from sour cherry (*P. cerasus*) (Table 2). Differences in the rate of polymorphism were observed among the different sets of SSRs. The UDP set gave the highest rate of polymorphism (60.0%), whereas the BPPCT, CPPCT, and CPDCT

sets had rates of 27.0%, 37.5%, and 33.3%, respectively (Table 2).

Among the SSRs producing an amplification product, 20 had complex profiles on polyacrylamide gels, 71 revealed no polymorphisms, 30 revealed polymorphisms in ‘Honggengansutao’, five revealed polymorphisms in ‘Bailey’, and eight were polymorphic in both parents. Because the *R* gene originated from ‘Honggengansutao’, SSRs with easily readable polymorphism profiles in ‘Honggengansutao’ and in both parents that had an amplification product were used.

SEQUENCE-RELATED AMPLIFIED POLYMORPHISM ANALYSIS. Of 238 SRAP primer pairs tested, 53 primer pair combinations generated polymorphic bands that revealed 638 polymorphic loci (mean of 12.0 polymorphic bands per primer pair; range, 1 to 15). Each primer pair produced 10 to 20 clear bands ≈ 100 to 1000 bp in length. Two hundred eighty-six of the SRAP fragments in the BC₁ hybrid and 115 in the ‘Honggengansutao’ segregated in a 1:1 ratio.

CLONING OF NBS-TYPE RESISTANCE GENE ANALOGS AND RESISTANCE GENE ANALOG-SEQUENCE-TAGGED SITE ANALYSIS. The forward primer (5’-GGIGGIRTIGGIAAIAACIAC-3’) was designed on the basis of the NBS motif [GG(L/V/S/I) GKTT] in the sense direction, and the reverse primer (5’-AGIGYIA GIGGIAGICC-3’) was based on the membrane-spanning motif (GLPLAL) in the antisense direction. Amplification by PCR using a pair of degenerate primers resulted in a single DNA gel

band of ≈ 550 bp that was recovered and cloned. A total of 300 clones was generated from insert-containing recombinant plasmids, and 100 positive clones were retained for further sequencing analysis. After removing the vector and the repetitive sequences, 75 different sequences, NBS1 to NBS75, were identified. Forty-five (60%) sequences showed significant similarity to known *Arabidopsis thaliana* *R* genes and homologs (E-value 10^{-5} or less) based on searches using the BLASTX program.

Forty-eight of 75 RGAs with uninterrupted ORFs were deposited in GenBank (HM219232-HM219254,

Table 1. Evaluation of root-knot nematode resistance in nine *Prunus* rootstocks.

Cultivar	Origin	Gall index (0–5 scale) ^z	Evaluation ^y
Maotao 1 (<i>P. persica</i>)	Southwestern China	0.71	Resistant
Maotao 2 (<i>P. persica</i>)	Henan, China	0.18	Resistant
Maotao 3 (<i>P. persica</i>)	United States	0.36	Resistant
Bailey (<i>P. persica</i>)	Canada	4.11	Susceptible
Shouxingtao 1 (<i>P. persica</i>)	Shanghai, China	0.00	Immune
Shouxingtao 2 (<i>P. persica</i>)	Shanghai, China	3.75	Susceptible
Shouxingtao 3 (<i>P. persica</i>)	Shanghai, China	4.46	Susceptible
Honggengansutao (<i>P. kansuensis</i>)	Gansu, China	0.00	Immune
Baigengansutao (<i>P. kansuensis</i>)	Henan, China	0.00	Immune

^z0 = no gall, 1 = 1% to 10% of root system galled, 2 = 11% to 30%, 3 = 31% to 70%, 4 = 71% to 90%, and 5 greater than 90% galled; data are means of 30 replicates.

^yImmune (gall index = 0), resistant ($1.0 > \text{gall index} > 0$), and susceptible (gall index > 1.0).

Table 2. Origin of the simple sequence repeat (SSR) markers used for linkage map construction in the seedling of the *Prunus kansuensis* ‘Honggengansutao’ BC₁ population.

Source species	Origin	Designation of primer	SSRs tested (no.)	Loci segregating (no.)	Polymorphism of primer (%)	Anchor loci (no.)	Reference
Peach	Genomic	pchgms	4	1	25.0	0	Sosinski et al. (2000)
Peach	cDNA	pchcms	2	1	50.0	1	Sosinski et al. (2000)
Peach	Genomic	UDP	15	9	60.0	8	Cipriani et al. (1999)
Peach	Genomic	BPPCT	37	10	27.0	9	Dirlewanger et al. (2002)
Peach	Genomic	CPPCT	32	12	37.5	6	Aranzana et al. (2003)
Almond	Genomic	CPDCT	12	4	33.3	1	Mnejja et al. (2005)
Almond	cDNA	EPDCU	6	1	16.7	1	Washington State University (2010b)
Japanese plum	Genomic	CPSCT	21	5	23.8	4	Mnejja et al. (2004)
Sweet cherry	Genomic	Ps	4	0	0	0	Sosinski et al. (2000)
Sour cherry	Genomic	PceGA	1	0	0	0	Cantini et al. (2001)
Total			134	43	32.1	30	

Table 3. The sequence information of resistance gene analog (RGA) specific primers used for polymerase chain reactions in the seedling of the *Prunus kansuensis* 'Honggengansutao' BC₁ population.

RGA	Forward primer (5'-3')	Reverse primer (5'-3')	Amplification size (bp)
NBS1	GAAGATTGAGGAGCGCTTTG	GGGAGGCCCTTACACTTCTC	459
NBS3	TTTTGACAGCATCCGTGCTA	TCCACTCCAAATGTGCTCAG	320
NBS4	GGTGTGGGAAAGACGACAAT	TAGCGTATTGCACGTTCTGC	486
NBS5	GGGGTTTCTCAACGTGGTAA	GGAGGCCTCTGGCATAATTT	409
NBS9	GGGGGAGTGGGAAAGACTAC	TTTCTTAAAGGCATGTCGCC	420
NBS10	CTGAGAATGCTTTGCTGCTG	ATTCCACGAAGCAAACAAGG	310
NBS11	AGGAGGGAAAGACTTGGAGC	GTCTTTCTCTGGAAAGGCA	293
NBS15	GGAGCTCTTTAGTTGGCACGCT	GAGCCAGGGGAAGCCCTCCA	104
NBS16	AGGGATTGCGGATGACGAGGC	CCTTGAGGGCGsAGTGGAAGGC	135
NBS17	AGCTGAGAATGCTTTGCCGC	TGGGTGGGCTTGGGAAAACGA	435
NBS18	CTTGAGCGCCAGGGGCAGTC	TGGAGCTCTTCAGTTGGCACGC	110
NBS19	AGGGCGAGTGGGAGACCCTT	CGGGAAGACCACCCTTGCGG	504
NBS20	AGCTCCACGCATCACCCCT	CGTGTGGGATGTCCACCTTTGGG	171
NBS21	GGGATGGGTGGGCTGGGAAA	GCAAGTGGGAGGCCTTGAGCA	494
NBS22	TGGTTTGGTTCAGGCAGCAGA	CTAGGGGGAGCCCGTCAGCA	220
NBS23	GGTGCATTGGTTTGGTTTGGGC	GCAAGGGGCAGACCTCCAGC	230
NBS25	GGGGGAGTGGGAAAGACTACACT	AGAGCGAGGGGGAGGCCTTT	493
NBS26	ACGGGTTCCTTGTTCGATG	CGAGGGGGAGTCCTCCGCAA	287
NBS28	GGGATGGGCGGATTGGGCAAA	CGAGGGGTAGGCCTCTGGCA	510
NBS29	GCGCTAGGGGAAGGCCATCAG	GGTGTGGACCAATTGGGGCAGTT	262
NBS30	GGGAAAGACTTGGAGCTGGCCC	AGGGCTAGAGGTAGGCCTCG	360
NBS32	GGGAGGGGTGGGTAAGACGACC	AGGTCCCCGCTGTTTCATCCCA	194

HQ263295-HQ263322). Their deduced amino acid sequences contained the characteristic motifs of NBS-type proteins such as P-loop, motif kinase-2, kinase-3, and GLPL motifs. Analysis using RGA-STS was performed on 22 RGAs selected randomly with gene-specific primers designed by the primer-blast program (Table 3).

Of the 22 RGA-STS primer pairs used in the PCR reactions, eight had complex profiles between 'Honggengansutao' and 'Bailey', seven have no amplification bands, two revealed no polymorphisms, whereas only five amplified DNA fragments that were polymorphic and used to map the BC₁ population (Table 4). RGA-STS markers NBS1 and NBS16, which amplified bands but were not polymorphic, were converted to CAP markers using AluI and HaeIII, respectively.

LINKAGE MAP CONSTRUCTION. A total of 161 genetic markers (38 SSRs, 115 SRAPs, seven RGA-STSs, and one morphological marker for RKN resistance) was analyzed with chi-square tests of goodness of fit and used for the linkage map construction of 'Honggengansutao'. All but 23 loci were assigned to eight linkage groups. The linkage map comprised 138 loci (30 SSRs, 102 SRAPs, five RGA-STS, and *PkMi* gene) (Fig. 1). The map covered 616 cM of the peach genome with an average marker spacing of 4.9 cM. The average number of loci per linkage group was 17.25 and ranged from 10 (G5) to 24 (G1 or G2). The length of each linkage group ranged from 65 cM (G3 or G8) to 104 cM (G1). Linkage group G2 had the highest marker density with an average distance of 3.9 cM per marker

Table 4. Polymorphic status of 22 resistance gene analog (RGA)-sequence-tagged site (STS) amplified product in the seedling of the *Prunus kansuensis* 'Honggengansutao' BC₁ population.

Name	Amplification	Alternate marker type
NBS1, NBS16	No polymorphism	Cleaved amplified polymorphic (CAP)
NBS3, NBS5, NBS10, NBS11, NBS29	Polymorphism	Sequence characterized amplified region (SCAR)
NBS4, NBS17, NBS18, NBS19, NBS20, NBS21, NBS23	No bands	
NBS9, NBS15, NBS22, NBS25, NBS26, NBS28, NBS30, NBS32	Complex profiles	

resulting from the presence of several cosegregating markers, whereas the least saturated linkage group G5 averaged 8.2 cM per marker. Seven gaps (greater than 10 cM) ranging from 11 to 17 cM were observed in this map (Fig. 1).

COMPARATIVE ANALYSIS WITH THE *PRUNUS* REFERENCE MAP. Thirty SSR markers were common to the *Prunus* reference map T × E ['Texas' almond × 'Earlygold' peach (Joobeur et al., 1998)] allowing for comparative analysis. SSR markers in G4 were not syntenic. The distance between other markers was calculated based on colinear loci. In the 'Honggengansutao' × 'Bailey' (H × B) map, 233 cM (37.8% of the total length) was anchored by common SSRs and in the T × E map, 213 cM (40.7% of the total length). On average, H × B distances were 1.06-fold longer than the T × E distances. Among the linkage groups, this rate ranged from 0.49 for G6 to 2.58 for G1 (Table 5).

THE LOCATION OF THE ROOT-KNOT NEMATODE *R* GENE. The *PkMi* gene controlling the nematode resistance from 'Honggengansutao' peach was mapped to linkage group G2 between NBS29 (6 cM) and the SRAPs M4E11-100 (2.0 cM) (LOD

Fig. 1. Genetic linkage map generated with the mapping program JoinMap 2.0 (Stam and Van Ooijen, 1995) from *Prunus kansuensis* 'Honggengansutao' × *P. persica* 'Bailey' BC₁ population. Numbers identifying the linkage groups are shown on the top of each group. The names of the loci are shown on the right. Distance is shown on the left in centimorgans.

Table 5. Comparison distances covered by the common loci in every linkage group between *Prunus kansuensis* 'Honggengansutao' × *P. persica* 'Bailey' (H × B) and *P. communis* 'Texas' × *P. persica* 'Earlygold' (T × E) linkage maps.

Group	Segments	H × B distances (cM) ^a	T × E distances (cM) ^b	Difference (cM)	Ratio H × B/T × E
G1	CPPCT026-CPPCT034	17.0	6.6	10.4	2.58
G2	BPPCT004-UDP98-406	43.0	60.0	-17.0	0.72
G3	CPSC032-UDP96-008	34.0	17.5	16.5	1.94
G5	CPSC011-BPPCT032	49.0	29.5	19.5	1.66
G6	CPPCT023-UDP98-412	15.0	30.5	-15.5	0.49
G7	UDP98-405-CPPCT017	40.0	39.5	0.5	1.01
G8	CPDCT034-UDP98-409	25.0	27.7	-2.7	0.90
Total		223.0	211.3	11.7	1.06

^aVia JoinMap 2.0 (Stam and Van Ooijen, 1995).

^bVia MapMaker 3.0 (Lander et al., 1987).

greater than 3.0). The nearest RGA-STS was NBS3 (5 cM) and the nearest SSR marker was UDP98-025 (10 cM).

PHYSICAL DISTRIBUTION OF ALL RESISTANCE GENE ANALOGS IN CHROMOSOME OF 'HONGGENGANSUTAO' PEACH. Five of seven

RGA-STS markers were mapped to G2, G7, and G8 (Fig. 1) through linkage analysis. BLASTN analysis of the five RGAs on the peach genome sequence was used to determine chromosome location. Location of the RGA-STS markers within the physical map by BLASTN analysis was consistent with genetic linkage mapping results (Table 6), indicating that BLASTN analysis on the peach genome sequence may be a shortcut for RGA mapping. BLASTN analysis of all RGA-STS markers against the 'Lovell' peach was used to determine the distribution of all RGAs on peach chromosomes. The results showed that highly similar homologs for 46 of 48 RGA sequences within the peach genome sequence were found (Table 7), whereas an acceptable E-value (10^{-4}) homologs could not be found for only two RGA sequences (NBS62, NBS69). Different RGAs were sometimes

Table 6. Comparison of the distribution of the five resistance gene analog (RGA)-sequence-tagged site (STS) markers in the *Prunus kansuensis* ‘Honggengansutao’ × *P. persica* ‘Bailey’ (H × B) map by linkage analysis and peach genome by blast analysis, respectively.

RGAs	Length (bp)	H × B genetic map location	Peach genome location	Blastn identities	Blastn E-value
NBS1	528	G7 (57.0 cM)	scaffold_7 (17074521–17074700 bp)	480/482 (99%)	0
NBS3	516	G2 (27.0 cM)	scaffold_2 (5656463–5656882 bp)	457/467 (97%)	0
NBS5	522	G8 (19.0 cM)	scaffold_8 (3010706–3011170 bp)	464/500 (92%)	0
NBS11	528	G8 (37.0 cM)	scaffold_8 (13206393–13206890 bp)	487/498 (97%)	0
NBS29	504	G2 (16.0 cM)	scaffold_2 (8908157–8908632 bp)	466/476 (97%)	0

Table 7. Physical distribution of 48 resistance gene analog (RGA) sequences in the peach genome.

Location	RGA	RGAs and RGAs with the same loci	RGAs with different loci (no.)
Scaffold_1	NBS40, NBS48		2
Scaffold_2	NBS3, NBS16, NBS21, NBS29, NBS38, NBS47	NBS21 and NBS38; NBS29 and NBS47	4
Scaffold_3	NBS72		1
Scaffold_4	NBS18		1
Scaffold_5	NBS4, NBS57, NBS64	NBS4, NBS57 and NBS64	1
Scaffold_6	NBS20, NBS37, NBS43, NBS51, NBS63, NBS67	NBS37, NBS43, NBS51, and NBS67	3
Scaffold_7	NBS1, NBS15, NBS26		3
Scaffold_8	NBS5, NBS9, NBS10, NBS11, NBS17, NBS22, NBS23, NBS25, NBS28, NBS30, NBS32, NBS36, NBS39, NBS45, NBS49, NBS50, NBS53, NBS56, NBS60, NBS61, NBS68	NBS9 and NBS25; NBS28 and NBS32; NBS11 and NBS30; NBS39, NBS45, NBS49, NBS50 and NBS61; NBS60 and NBS68	13
Scaffold_10	NBS41		1
Scaffold_29	NBS19, NBS34	NBS19 and NBS34	1
No scaffold	NBS62, NBS69		
Total			30

most similar to the same locus within the RGA analysis. This could also occur as a result of PCR artifacts or if genes are duplicated in ‘Honggengansutao’ versus the peach reference genome. After filtering the “false” RGAs, 28 RGAs were placed on scaffold_1 to scaffold_8. Scaffold_8 had the highest RGA density, and Scaffolds_3, Scaffolds_4, and Scaffolds_5 had the lowest.

Discussion

Several intraspecific and interspecific *Prunus* maps, developed using different molecular markers (Dirlewanger et al., 1998, 2004; Jauregui et al., 2001; Lu et al., 1998), provide a framework for construction of a highly saturated molecular genetic map. The most recent linkage map was constructed by a controlled F₂ cross between ‘Guardian’ and ‘Nemaguard’ (Blenda et al., 2007). This map covers a genetic distance of 737 cM with an average marker spacing of 4.7 cM. In the present study, the H × B map covers 616 cM of the peach genome with an average marker spacing of 4.9 cM. The genetic distances were up to 1.19-fold longer in the ‘Guardian’ × ‘Nemaguard’ map than in the H × B map, possibly as a result of a decreased chiasmata frequency resulting from reduced homology between the DNA strands (Borts and Haber, 1987).

Considering all the 134 SSRs tested in our study, we observed a polymorphism rate of 32.1%, which is a value significantly lower than that reported in other *Prunus* progenies (Aranzana et al., 2003; Lambert et al., 2004). This rate increased to 36.7% (33 of 90) for peach primer pairs (pchgms,

pchcms, UDP, BPPCT, and CPPCT). These results demonstrated that SSRs derived from almond, plum, and cherry had a low polymorphism in peach.

Loci rearrangement was observed in the H × B map. Two SSR markers, CPPCT005 and UDP97-402, were mapped within the same linkage group (G4) in the present study. However, the CPPCT005 was mapped in G2 for the J × F map and in G4 for the T × E map, whereas UDP97-402 was positioned in G4 for the J × F map and G2 for the T × E map. The possibility maybe is that there are two or more alleles of these primers, for example UDP97-402a (G4) and UDP97-402b (G2) in the R × D linkage map (Sanchez-Perez et al., 2005). The rearrangement of CPPCT005 in different linkage groups could aid to a more precise definition of the location of it.

Limited segregation was observed for SSR markers, so the SRAP technique was used to increase the density of markers in our study. These SRAP markers have been shown to be reproducible, reliable, and locus-specific markers for rapid increase of the coverage of genetic maps (Li and Quiros, 2001). By adding SRAPs to the map developed by SSR markers, we were able to generate maps that are comparatively longer than the T × E map (519 cM) generated with SSRs and RFLPs alone (Dirlewanger et al., 2004). This increase in the total length was mainly caused by the addition of markers to regions near the edge of some groups where SSRs were poorly represented, including the top regions on G1, G2, G3, G6, G7, and G8 and the bottom of G1 and G4 in the H × B map.

A single gene, designated *RMia*, controlling resistance to both MA and MI, is mapped on linkage Group 2 of the *Prunus*

reference map and ≈ 10 cM with UDP98-025 (Dirlewanger et al., 2004). In our study, an *R* gene to MA, *PkMi*, was mapped onto the same region of Group 2 by using an intraspecific BC₁ population cross between *P. persica* and *P. kansuensis*, and for the nearest SSR marker, UDP98-025, the distance is also 10 cM. Our results suggest that the *R* gene in 'Honggengansutao' is an allele of *Rm1a*. This is the first report of an *R* gene for RKN in *P. kansuensis*.

Evaluation of RKN resistance is a time- and labor-intensive process. Therefore, molecular markers tightly linked to the nematode *R* genes are of special interest for breeding and improving peach rootstocks. Several SSR markers around the loci of the *R* gene were screened in the BC₁ population but most showed no polymorphism. The nearest SSR marker, UDP98-025 at 10 cM, had a greater genetic distance to resistance loci, and it may be of little use. Additional markers saturating the region around *PkMi* are necessary for marker-assisted selection (MAS).

Many sources of nematode resistance have been identified and major resistance loci have been described and mapped in diverse plant crops. These *R* genes include *Mi-1* (Milligan et al., 1998) and *Hero* (Karin et al., 2002) in tomato and *Cre3* (Lagudah et al., 1997) in wheat (*Triticum aestivum*). Map-based cloning has also defined an *R* gene in *Prunus* species. The other study proved that the RKN *R* gene (*Ma*) in Myrobalan plum (another resistance resource in *Prunus*) belongs to a cluster of NBS-LRR candidate genes (Claverie et al., 2006; Esmenjaud and Dirlewanger, 2007); however, *Ma* has not been sequenced. What about *Rm1a*? The report in our study may be helpful to analyze the sequence of the *Rm1a* gene.

In this article, we describe the homologous sequence cloning of partial NBS sequences from RGA using degenerate primers and anchored all cloned RGAs on peach genetic linkage map and genome for creating a *R* gene map to map the *PkMi* gene. In a previously constructed resistance map (Lalli et al., 2005), a total of 42 regions of resistance mapped onto seven of the eight linkage groups (RGAs were absent in Group 3) and the RGA in the *Rm1a* region of Group 2 belonged to a non-TIR type. In our study, a resistance genetic map for peach was generated through linkage analysis and currently contains five RGAs distributed to G2, G7, and G8 (Fig. 1). Twenty-eight RGAs, which placed on scaffold_1 to scaffold_8 by BLAST analysis with a peach genome sequence (Table 7), identified putative RGAs within the genome. The RGAs NBS3 and NBS29, mapped in the region of the RKN *R* gene, belong to the TIR type characterized by the presence of aspartate residues (D) instead of tryptophan residues (W) in the final position of kinase-2. The development of a resistance map for *Prunus* provides resistance loci for designing breeding strategies to yield broadly resistant crops (Lalli et al., 2005).

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