

# Construction of a genetic linkage map and identification of molecular markers in peach rootstocks for response to peach tree short life syndrome

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Received: 20 September 2005 / Accepted: 30 October 2006 / Published online: 16 January 2007  
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**Abstract** Peach tree short life (PTSL) is a devastating disease syndrome of peach [*Prunus persica* (L.) Batsch] caused by multiple factors; the molecular biology of its tolerance/susceptibility is unknown. The difficulty of studying PTSL is that tree survival or death is not obvious until 3 to 5 years after planting when the symptoms of PTSL first appear. Tolerance to PTSL was unknown in *Prunus* until the rootstock Guardian® ‘BY520-9’ was introduced into commercial orchards in 1994. To study the genetics of the response to PTSL, a controlled F<sub>2</sub> cross was made between Guardian® ‘BY520-9’ selection 3-17-7 (PTSL-tolerant) and Nemaguard (PTSL-susceptible). An F<sub>1</sub> hybrid was then selfed to generate an F<sub>2</sub> population expected to segregate for PTSL response. One hundred fifty-one AFLPs and 21 SSRs, including anchor loci from the *Prunus* reference genetic map, were used to construct a molecular genetic map based on 100 F<sub>2</sub> seedlings. This map covers a genetic distance of

737 cM with an average marker spacing of 4.7 cM and will be used as a framework to construct a highly saturated molecular genetic map. Of the 140 mapped AFLP markers, 38 were associated with PTSL response, as identified previously by bulked segregant analysis. The distribution of the markers associated with PTSL response on the newly constructed genetic map was compared with the recently published *Prunus* resistance map. This comparison revealed that some resistance gene analogs and several PTSL-associated AFLP markers were located in the same regions in several *Prunus* linkage groups: G1, G2, G4, G5, and G6. This peach rootstock map can also be viewed and compared with other *Prunus* maps in comparative map viewer CMap in the Genome Database for Rosaceae (GDR) at <http://www.rosaceae.org>

**Keywords** Disease susceptibility · Amplified fragment length polymorphism (AFLP) · Simple sequence repeat (SSR) · Indirect mapping · Ring nematode · *Prunus persica* · Resistance gene analog (RGA)

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Communicated by Peré Arús

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## Introduction

With the development of improved DNA-based molecular markers, the construction of high-resolution linkage maps can be accomplished in short periods of time, allowing fruit tree breeders to select tagged traits of interest at the earliest stages of growth. Significant progress has been made in the generation of *Prunus* molecular genetic linkage maps for

use in marker-assisted selection (Arus et al. 1994; Baird et al. 1996; Abbott et al. 1998; Joobeur et al. 1998; Aranzana et al. 2003; Dirlwanger et al. 2004b) with the eventual goal of identifying and isolating genes of interest, including those that confer resistance to important diseases.

In some cases, disease resistance, such as root-knot nematode resistance, was found to be determined by one or just a few dominant genes in *Prunus* (Claverie et al. 2004; Dirlwanger et al. 2004a; Esmenjaud et al. 1996; Gillen and Bliss 2005; Lu et al. 2000). In most cases though, agronomically important traits, including resistance and tolerance to various diseases and syndromes, show continuous phenotypic variation indicating more complex, polygenic control (Tanksley 1997). Quantitative trait loci (QTLs) controlling leaf curl resistance (Viruel et al. 1998) and powdery mildew resistance (Dirlwanger et al. 1996; Foulongne et al. 2003b; Verde et al. 2002) have been located on peach genetic linkage maps. Recently, QTLs for plum pox potyvirus (or sharka) resistance were detected in a cross between peach and *Prunus davidiana* (Carr.) Franch. (Decroocq et al. 2005).

In contrast to root-knot nematode resistance, peach tree short life (PTSL) is a nematode-related disease syndrome of peach caused by a complex of biotic, climatic, and edaphic factors (Okie et al. 1987); the molecular biology of its tolerance/susceptibility is virtually unknown, and no QTL (s) have been previously reported for PTSL response.

Overall, plant damage occurs due to extreme physiological stress in the presence of very high population densities of ring nematode [*Criconeoides xenoplax* Raski synonymous to *Mesocriconeuma xenoplax* (Raski) Loof and de Grisse; Nyczepir et al. 1988]. The resulting injury and physiological response to nematode feeding is thought to increase the vulnerability of peach trees to bacterial canker (*Pseudomonas syringae* pv. *syringae* van Hall) and/or to cold injury by the stimulation of indole-3-acetic acid production that interferes with dormancy (Nyczepir et al. 1983). Symptoms of PTSL appear in early spring as a wilting or sudden collapse of new growth. The first symptoms of PTSL usually appear during the third or even the fifth year of peach tree life after planting, but can appear as early as the second year. This problem affects more than 70% of the peach acreage in the southeastern USA with multi-million dollar losses for peach growers (Miller 1992).

We report the construction of the first genetic map for the identification of QTLs or major loci for PTSL-response in *Prunus* rootstocks. This map is also anchored to the *Prunus* reference map. In addition, amplified fragment length polymorphism (AFLP) markers associated with PTSL response, previously identified by bulked segregant analysis (BSA; Blenda et al. 2006), were positioned on the genetic map by using an indirect mapping approach. BSA is a detection strategy used to isolate molecular markers linked to a trait of

interest. Individuals from a segregating population are usually combined (bulked) into two classes of distinct phenotypes/traits, and the bulks are then screened with molecular markers (Giovannoni et al. 1991; Michelmore et al. 1991). In this work, we report the first attempt in *Prunus* to map BSA-based AFLP markers that were not derived from the studied mapping population to overcome the limitation of no phenotypic data in the analyzed progeny.

The distribution of the mapped PTSL-response-associated AFLP markers was evaluated in comparison with resistance gene analogs (RGAs) from the *Prunus* resistance map (Lalli et al. 2005). The ultimate goal of this research is to use the map to identify and isolate the genes responsible for the tolerance to PTSL and to facilitate the introgression of PTSL tolerance through marker-assisted breeding.

## Materials and methods

### Plant material and DNA extraction

PTSL-tolerant Guardian® selection 3-17-7 (*P. persica*) was used as the maternal parent in a controlled F<sub>2</sub> cross with the highly PTSL-susceptible seedling rootstock, Nemaguard (putative *P. persica* × *P. davidiana*). Guardian® ‘BY520-9’ has been released as a bulked seedlot for industry use as a rootstock for peach and nectarine. It consists of open-pollinated seeds of approximately five selections that trace several open-pollinated generations back to the PTSL-susceptible peach rootstocks S-37 and Nemaguard (Okie et al. 1994). A Guardian® open-pollinated selection 3-17-7 is being used as a core genotype in the Guardian® ‘BY520-9’ bulked seedlot and exhibits superior survival on PTSL-infected sites compared with other Guardian® selections (Reighard, unpublished). Fifteen F<sub>1</sub> hybrids were produced at the Clemson University Musser Fruit Research Center near Clemson, SC, USA. F<sub>1</sub> trees were selfed to obtain F<sub>2</sub> progeny expected to segregate for tolerance to PTSL. The F<sub>2</sub> family produced from F<sub>1</sub> hybrid no. 11 consisted of 100 seedlings and was labeled as family 11-100. After stratification at 4°C for 1.5–2 months, the germinating F<sub>2</sub> seeds from the family 11-100 were planted in 12-cm diameter plastic pots filled with 1,200 cm<sup>3</sup> sand–vermiculite medium (1:3 v/v) and grown in the greenhouse.

Young fresh leaves were collected in February and March from greenhouse plants and in April from orchard plants. All leaf samples were refrigerated during transportation before being stored at –80°C. Leaf genomic DNA was extracted using a modified 2× CTAB method (Eldredge et al. 1992) and measured using a minifluorometer (Hoefler Scientific, San Francisco, CA). DNA from several samples was extracted at least twice to test and theoretically confirm the reproducibility of the whole DNA sample set.

**Table 1** AFLP markers present in the F<sub>2</sub> peach rootstock family 11-100 (Guardian® selection 3-17-7×Nemaguard)

Primer combination	No. of detectable fragments	No. of polymorphic bands	No. of mapped markers
EAA/MCAA	68	5	5
EAA/MCAC	55	5	4
EAA/MCAT	73	5	4
EAA/MCTA	106	5	4
EAA/MCTC	66	5	1
EAA/MCTG	49	7	7
EAA/MCTT	83	7	3
EAA/MCCA	32	1	1
EAA/MCCC	64	3	3
EAC/MCAA	80	10	8
EAC/MCAC	36	5	3
EAC/MCAT	73	6	6
EAC/MCTA	52	9	8
EAC/MCTC	35	5	4
EAC/MCTG	46	4	1
EAC/MCTT	70	7	7
EAG/MCAA	54	6	4
EAG/MCAC	30	2	2
EAG/MCTA	38	4	4
EAG/MCTG	41	5	4
EAG/MCTT	61	6	3
EAT/MCAA	85	4	2
EAT/MCAC	53	2	2
EAT/MCAG	51	5	3
EAT/MCAT	85	1	1
EAT/MCTA	78	4	3
EAT/MCTC	82	8	7
EAT/MCTG	50	1	1
EAT/MCTT	90	12	9
ETA/MCAC	38	2	2
ETA/MCAG	49	10	9
ETA/MCTA	59	9	6
ETA/MCTC	47	4	3
ETA/MCCA	52	4	3
ETA/MCGT	23	1	1
ETA/MCGC	32	2	2
Total	2086	181	140
Average	57.9	5.0	3.9

### AFLP and SSR analyses

AFLP reactions were performed using the procedure of Vos et al. (1995) with modifications (Westman et al. 2002). Selective amplifications were performed using various combinations of *EcoRI* primers with two selective nucleotides (E+2) and *MseI* primers with three selective nucleotides (M+3). Thirty-six primer combinations (Table 1) were used for AFLP analysis to determine the level of polymorphisms in the parents (Guardian® selection 3-17-7 and Nemaguard), F<sub>1</sub> hybrid tree no. 11 and the F<sub>2</sub> family 11-100.

The choice of (E+2)/(M+3) primer combinations was based on their high information content for peach as described previously (Dirlewanger et al. 1998; Lu et al. 1998; Verde et al. 2005). Individual AFLP marker designations were based on the primer pairs used. Three classes of AFLP markers were scored: (1) dominant markers derived from Guardian selection 3-17-7 (DB), (2) dominant markers derived from Nemaguard (AC), and (3) co-dominant markers (ABH). All markers were scored at least three times from the same gel; in unclear cases, some markers were scored at least three times from different AFLP reactions and different gels. A set of AFLP markers putatively associated with PTSL response, previously obtained by Blenda et al. (2006) following BSA methodology, was included as part of the 151 AFLPs used for mapping in this work.

SSR analysis was performed according to the methods described in Sosinski et al. (2000). Thirty-two microsatellite primers previously developed for peach (Georgi et al. 2002a; Aranzana et al. 2002; Cipriani et al. 1999; Dirlewanger et al. 2002; Georgi et al. 2002b; Sosinski et al. 2000; Testolin et al. 2000; Wang et al. 2002) were also used and scored the same way as AFLP markers. To align the map with the *Prunus* reference map (Aranzana et al. 2003), 24 of the 32 SSRs were represented by a specific set of SSRs from the *Prunus* reference map. These 24 SSRs were suggested as anchor sites for merging different *Prunus* maps (Aranzana et al. 2003). Total, 21 of the 32 SSRs were found to be polymorphic and were used for mapping in the family 11-100 (Table 2).

### Linkage analysis

A genetic linkage map for the family 11-100 was constructed with the scored AFLP and SSR markers using Mapmaker EXP 3.0 (Lander et al. 1987). Departures from a Mendelian ratio were tested by using the chi-square goodness-of-fit test ( $P < 0.05$ ), available in JoinMap® 3.0 (Van Ooijen and Voorrips 2001). Linkage groups were established by using a 3.5 minimum log of odds (LOD) score and 0.30 maximum recombination frequency. Initially, linkage groups were established and ordered using only markers following Mendelian expectation with known linkage phase. Segregating AFLP bands present in both parents and F<sub>1</sub> hybrids were added to the previous data set with some precautions to avoid wrong phase attribution. These markers were scored in both coupling and repulsion phases (A, C and B, D) and added to the data set after linkage groups were established and ordered. Distorted markers were also added to the data set at this stage. Only the markers showing linkage with the groups that were originally established without them, were included in the groups with the “try” command. The map distances were computed using Kosambi’s mapping function.

## Southern hybridization

Southern hybridization was performed in a search for polymorphisms between the tolerant parent Guardian® selection 3-17-7 and the highly susceptible parent Nemaguard. Genomic DNA (10 µg) from Nemaguard and Guardian® selection 3-17-7 was digested with *EcoRI*, size fractionated, and transferred to Hybond N+ (Amersham Biosciences) following the manufacturer's standard protocol. The filter was hybridized as previously described (Georgi et al. 2002a) with a peach genomic clone consisting of an end fragment of peach bacterial artificial chromosome (BAC) PpN54C18 that was significantly similar to the TIR-LRR class of candidate resistance genes (Georgi, unpublished results).

## Results and discussion

The 36 AFLP primer combinations yielded 181 bands that were scored as polymorphic (Table 1). Of these AFLP products, 151 were used for mapping based on signal strength and robustness for scoring purposes. Thirteen SSRs (54%) out of the 21 of the anchoring set proposed by Aranzana et al. (2003) were polymorphic in the family 11-100 and were used for mapping (Table 2). Additionally, eight SSRs developed for peach and identified as polymorphic in the 3-17-7×Nemaguard F<sub>2</sub> cross were included in the study. Out of 32 microsatellite primer pairs tested in total, 21 were polymorphic in the family 11-100 (65%; Table 2).

**Table 2** Characterization of SSR markers tested and used for the construction of the genetic map for F<sub>2</sub> cross Guardian® selection 3-17-7×Nemaguard

Mapped in family 11-100			Not mapped in family 11-100	
On <i>Prunus</i> reference map	On other maps	New	Unlinked	Monomorphic/unclear
pchgms3	pchgms11	pchgms31	pchgms6	CPPCT016*
CPPCT002*	pchgms26	pchgms32	BPPCT28*	UDP-005*
UDP-024*	UDP-406		pchcms2*	BPPCT020*
BPPCT015*				UDP-013*
BPPCT017*				BPPCT007*
UDP-001*				UDP-003*
BPPCT008*				BPPCT014*
pchcms5				CPPCT022*
BPPCT025*				CPPCT033*
UDP-412*				CPPCT017*
PS1h3*				BPPCT006*
UDP-409*				
UDP-025*				

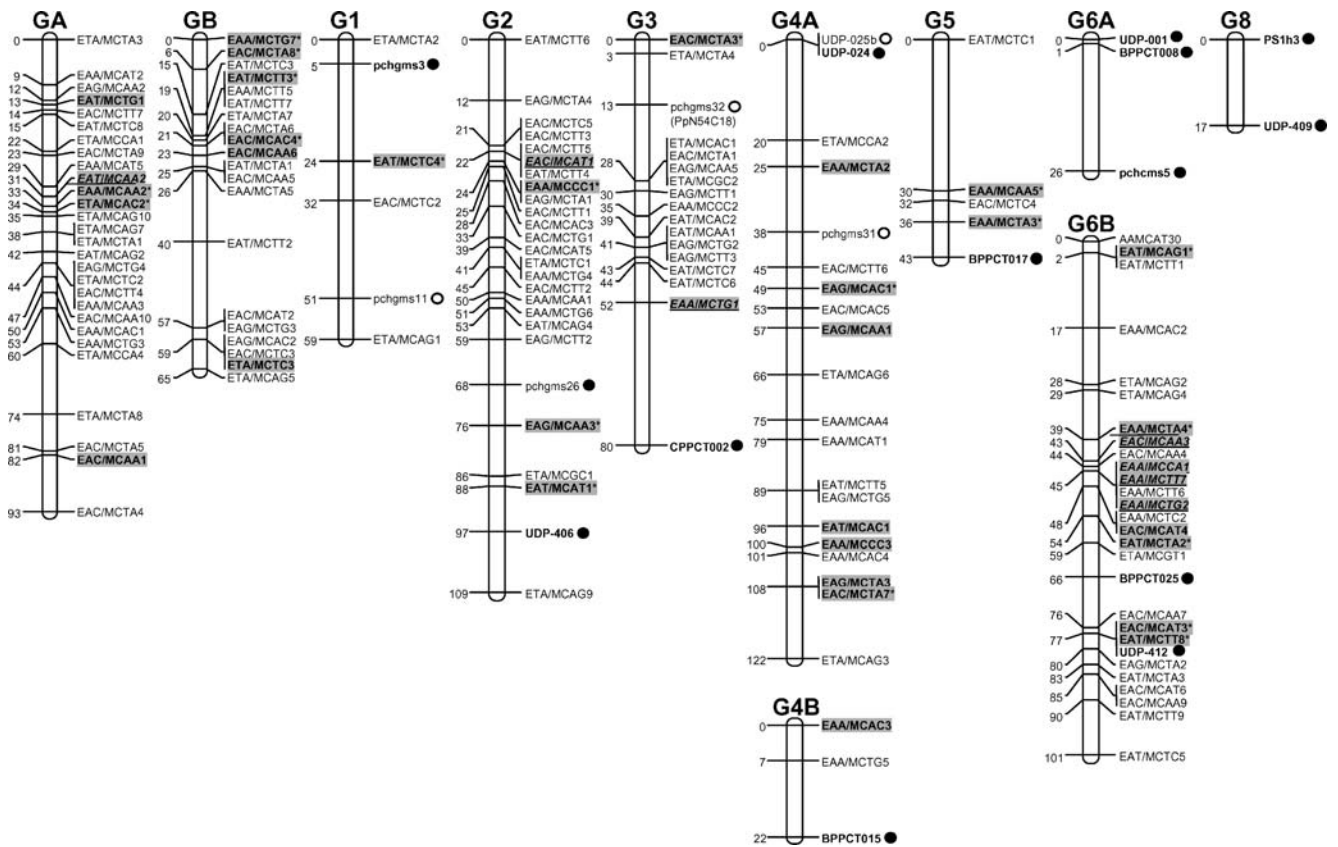
SSRs that serve as anchor points on the *Prunus* general map are indicated by an asterisk.

A total of 172 markers (AFLP and SSR) were polymorphic. Of these, 29 (17%) were designated as class ABH (co-dominant), 55 (32%) of the markers were scored as class DB, and the remaining 88 (51%) were class AC. Nineteen (14 AFLPs and 5 SSRs) of the 172 markers (11%) deviated from the expected segregation ratios (1:2:1 for co-dominant or 3:1 for dominant markers). The proportion of skewed markers in this progeny is higher than that typically observed within intraspecific peach progenies (e.g., Dirlewanger et al. 1998; Rajapakse et al. 1995) but is lower than that obtained with interspecific *Prunus* progenies (e.g., Aranzana et al. 2003; Foulongne et al. 2003a; Joobeur et al. 1998; Verde et al. 2005). This result is consistent with the proposed pedigree of the cross. The possibility that both parents (Guardian® selection 3-17-7 and Nemaguard) have *P. davidiana* in their backgrounds is not excluded (Okie 1998). Foulongne et al. (2003a), studying an F<sub>2</sub> progeny between peach and *P. davidiana*, observed a value of skewed segregation three times as high as that observed in this F<sub>2</sub> cross. Observed distortion of segregation ratios was found to be roughly similar for AC (14.7%) and ABH (20.7%), but much lower for DB (0%) type class markers, being that AC markers were derived from Nemaguard. This result supports the hypothesis of Okie (1998) that Nemaguard likely has *P. davidiana* as a parent.

A total of one hundred fifty-eight (92%) out of 172 polymorphic markers were used to construct the molecular genetic linkage map. These markers (140 AFLPs and 18 SSRs) were organized into 11 linkage groups (Fig. 1). The markers covered a total of 737 centimorgans (cM) of the peach nuclear genome, with an average interval of 4.7 cM between adjacent markers. The total distance covered in the current map is appreciably longer than that observed in the *Prunus* reference map, suggesting in general a different rate of recombination in the two crosses. These results are expected because the parental species used for the generation of the *Prunus* reference map between peach and almond, as reported in the literature (Foulongne et al. 2003a; Martínez-Gómez et al. 2003), are more distant than the species involved in the present F<sub>2</sub> cross (peach and, to a lesser extent, *P. davidiana*).

Linkage groups were named based on their correspondence (through common anchoring markers) to the *Prunus* reference map (Aranzana et al. 2003). Nonetheless, two linkage groups (i.e., G4 and G6) remained incompletely resolved, and each consisted of two unlinked segments. Thirteen SSRs from the *Prunus* reference map (Table 2) were positioned onto the map for the F<sub>2</sub> cross Guardian® selection 3-17-7×Nemaguard (Fig. 1). Of those 13 SSRs, eleven were from the anchoring set of 24 (Table 2). Five additional SSRs (pchgms11, pchgms26, pchgms31, pchgms32, and UDP-406) that were not included in the *Prunus* reference map were positioned onto the map as





**Fig. 1** Linkage map of the peach Guardian® selection 3-17-7 × Nemaguard F<sub>2</sub> family 11-100. Linkage groups containing SSRs are named according to the *Prunus* reference map and other anchored genetic maps. Microsatellite loci are marked as filled circles

(previously mapped) and open circles (newly mapped). Highlighted AFLP markers are associated with PTSL response: category A is shown in underlined italics and bold; category B is marked with an asterisk and in bold; category C is shown in bold

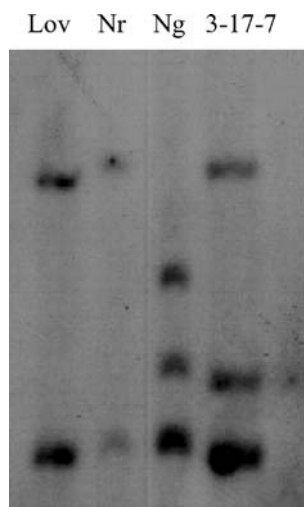
well. The SSR pchgms11 was developed by Wang et al. (2002b) from BAC clones of the evergrowing (*evg*) gene region. SSR markers from that region were recently mapped to the distal part of G1 in the *Prunus* reference map (Silva et al. 2005) and in a peach × *P. ferganensis* map (Verde et al. 2005). This marker, together with pchgms3 previously mapped in G1 (Aranzana et al. 2003), helped to correctly name and orient the group. The SSR UDP-406 was located in G2 in several *Prunus* maps anchored with the T × E map (Verde et al. 2005), allowing the identification of this group as G2. Moreover, pchgms26 belonging to the same group was also mapped on G2 in an unpublished peach map (Verde, unpublished results). The study reported in this paper was the first attempt to use the subset of SSRs proposed by Aranzana et al. (2003) to align a peach map with the *Prunus* reference map. Clearly, the limited number of mappable SSRs (that is, almost half were not polymorphic in the family 11-100) was insufficient to reach that goal. Additional SSRs were needed to align the two maps.

A BLASTX search of the end sequence of peach BAC PpN54C18 identified a significant similarity to leucine-rich repeat-Toll/interleukin receptor-like class candidate resis-

tance genes (Georgi, unpublished results). A Southern blot probed with this BAC end fragment revealed a polymorphism between the parents of family 11-100 (Fig. 2). This BAC was found to contain a simple sequence repeat, pchgms32 (Georgi et al. 2002b), which was polymorphic in the 11-110 family and was positioned near one end of the linkage group G3 (Fig. 1).

All but one of the previously mapped SSRs were located on the same linkage groups. This exceptional marker, UDP-025, was mapped to G2 of the *Prunus* reference map, whereas in family 11-100, it detected a second locus on G4 that was designated as UDP-025b. Only in G6A was the group of three SSR markers (UDP-001, BPPCT008, pchcms5) in common with the *Prunus* reference map. This allowed us to compare the order of the loci for this linkage group between the two maps that turned out to be the same in both maps. Moreover, four intervals were also in common: two in G6A (UDP-001–BPPCT008 and BPPCT008–pchcms5), one in G6B (BPPCT025–UDP-412), and one in G8 (Ps1h3–UDP-409). These intervals cover 54 cM in the rootstock map, whereas in the T × E map, they cover 70 cM. Three intervals were longer in the T × E map, whereas one (BPPCT008–pchcms5) was longer in the current rootstock map. This

**Fig. 2** Southern blot probed with the peach BAC PpN54C18 end fragment onto *Eco*RI-digested genomic DNA from Lovell (*Lov*), Nemared (*Nr*), Nemaguard (*Ng*), and Guardian® selection 3-17-7. Peach BAC PpN54C18 is associated with SSR pchgms32 mapped in linkage group G3



result is in contrast with those observed for other peach maps (Foulongne et al. 2003a; Verde et al. 2005) where suppression of recombination is more common in the reference map compared to peach maps.

Unanchored linkage group GA showed loose linkage (LOD 1.8) with G1. This is consistent with the length and with the number of loci in G1 in the majority of the *Prunus* maps. In fact, G1 is usually the longest and, in many cases, is the linkage group most populated with molecular markers (Dirlewanger et al. 2004a,b; Foulongne et al. 2003a,b; Joobeur et al. 1998; Lambert et al. 2004; Verde et al. 2005). Therefore, GA may represent the top segment of G1 of the reference map. Similarly, GB was loosely linked with G5 (LOD 1.7). None of the rootstock linkage groups could be identified with G7. In fact, two SSR markers (pchgms2 and pchgms6) located on G7 in the T × E cross were unlinked (showing no linkage with GA or GB; LOD < 0.1)

The difficulty of developing molecular markers linked to the PTSL syndrome and positioning them onto the genetic map is that the actual trait (i.e., survival or death from PTSL) in F<sub>2</sub> progeny is not an easily scored phenotype until 3 to 5 years after planting when characteristic symptoms of PTSL start to appear. Until then, it is not possible to unambiguously determine the trait's location on the genetic map or to determine whether a potential molecular marker is linked to the PTSL response. To approach this problem, BSA in combination with AFLP technology was applied to identify diagnostic markers for the PTSL syndrome in peach (Blenda et al. 2006).

Based on the combined results of BSA and selected tolerant and susceptible genotype screening, 44 AFLP markers were previously chosen as potentially associated with PTSL response (Blenda et al. 2006). Of those 44 potential PTSL markers, 38 mapped onto the family 11-100 genetic map. Based on their inheritance characteristics

**Table 3** Thirty-eight AFLP markers associated with the PTSL syndrome and mapped onto the genetic map for F<sub>2</sub> cross Guardian® selection 3-17-7 × Nemaguard

Category A <sup>a</sup>	Category B	Category C
EAT/MCAA2 (DB) <sup>b</sup>	EAA/MCAA2 (DB)	EAA/MCCC3 (DB)
EAA/MCTG1 (AC)	EAA/MCTG7 (DB)	EAC/MCAA1 (DB)
EAA/MCTG2 (AC)	EAC/MCAC4 (DB)	EAC/MCAA6 (DB)
EAA/MCCA1 (AC)	EAC/MCTA8 (DB)	EAG/MCAA1 (DB)
EAC/MCAA3 (AC)	EAG/MCAA3 (DB)	EAG/MCTA3 (DB)
EAC/MCAT1 (AC)	EAG/MCAC1 (DB)	EAT/MCAC1 (DB)
EAA/MCTT7 (AC)	EAT/MCTT3 (DB)	EAT/MCTG1 (DB)
	ETA/MCAC2 (DB)	EAC/MCAT4 (AC)
	EAA/MCTA3 (AC)	ETA/MCTC3 (AC)
	EAA/MCTA4 (AC)	EAA/MCAC3 (AC)
	EAA/MCAA5 (AC)	EAA/MCTA2 (ABH)
	EAA/MCCC1 (AC)	
	EAC/MCTA3 (AC)	
	EAC/MCTA7 (AC)	
	EAT/MCAG1 (AC)	
	EAT/MCAT1 (AC)	
	EAT/MCTA2 (AC)	
	EAT/MCTT8 (AC)	
	EAC/MCAT3 (ABH)	
	EAT/MCTC4 (ABH)	

<sup>a</sup> Category A: markers detecting polymorphisms between the susceptible and tolerant bulks. Category B: Intermediately PTSL-susceptible rootstock Lovell displayed or lacked the AFLP polymorphic fragment as did PTSL-tolerant parent Guardian® selection 3-17-7; the bulk of dead trees showed results opposite to the ones found for the susceptible parent Nemaguard. Category C: Intermediately PTSL-susceptible Lovell and highly PTSL-susceptible parent Nemaguard always display the same phenotype; highly susceptible rootstock Nemared had mostly the same phenotype as intermediately susceptible Lovell.

<sup>b</sup> DB Dominant markers contributed by a tolerant female parent Guardian® selection 3-17-7, AC dominant markers contributed by a susceptible male parent Nemaguard, and ABH co-dominant markers contributed by both parents.

(Blenda et al. 2006), these 38 mapped markers were divided into three categories (A, B, and C, Table 3).

The polymorphisms between the tolerant and susceptible bulks were observed for only seven of the 38 PTSL-associated markers (Table 3, category A). Another characteristic of these seven markers is that the ten tested tolerant Guardian® selections always displayed the same AFLP phenotype (presence or lack of the amplification fragment) as did the tolerant parent Guardian® selection 3-17-7 and the bulk of tolerant trees, as described by Blenda et al. (2006).

Another group of 20 markers (Table 3, category B) revealed that intermediately PTSL-susceptible rootstock Lovell displayed or lacked the AFLP fragment as did tolerant parent Guardian® selection 3-17-7. Furthermore, the bulk of dead trees showed results opposite to the ones found for the susceptible parent Nemaguard. Only one

**Table 4** Regions of co-localization of the PTSL-associated AFLP markers and the resistance gene analogs mapped onto the *Prunus* resistance map

Linkage group	PTSL-associated markers from the same linkage group	Mapped SSR markers flanking the regions of co-localization	Marker <sup>a</sup>	Source species <sup>b</sup>	ID <sup>c</sup>
G1	EAT/MCTC4	pchgms3, pchgms11	AC7A, FG5	Apricot	NBS <sup>d</sup> -like putative gene resistance homologue [ <i>Rosa roxburghii</i> ]/2e-14
G2	EAG/MCAA3, EAT/MCAT1	pchgms26, UDP-406	AG113 AC19, PP_LEa0030M24	<i>P. davidiana</i> Apricot	Resistance protein MG13 [ <i>Glycine max</i> ]/5e-50 Transcriptional activator RF2a [ <i>Arabidopsis thaliana</i> ]/2e-37 Eukaryotic translation initiation factor 4E [ <i>Pisum sativum</i> ]/1e-31
G4	EAT/MCAC1, EAA/MCCC3, EAG/MCTA3, EAC/MCTA7	UDP-024	AG8A	Apricot <i>P. davidiana</i> Apricot	NBS-kinase protein Z2 [ <i>Solanum tuberosum</i> ]/9e-36 Resistance protein MG13 [ <i>Glycine max</i> ]/5e-50 NBS-like putative gene resistance homologue [ <i>Rosa roxburghii</i> ]/2e-14
G5	EAA/MCAA5, EAA/MCTA3	BPPCT017	AG8B	Plum <i>P. davidiana</i> Plum Apricot Apricot	MRGH63 resistance gene [ <i>Cucumis melo</i> ]/9e-18 Resistance protein MG13 [ <i>Glycine max</i> ]/5e-50 MRGH63 resistance gene [ <i>Cucumis melo</i> ]/9e-18 NBS-kinase protein Z2 [ <i>Solanum tuberosum</i> ]/9e-36 NBS-like putative gene resistance homologue [ <i>Rosa roxburghii</i> ]/2e-14
G6	EAC/MCAT3, EAT/MCTT8	BPPCT025, UDP-412	AC55B, PP_LEa0009L15, PP_LEa0012A23, PP_LEa0012K18 AC55B, PP_LEa0010M17 LF11, PP_LEa0009L15, PP_LEa0012A23, PP_LEa0012K18 LF11, PP_LEa0010M17 PC29A, FG53, FG78	Peach Apricot Peach Apricot Peach	NBS-LRR putative resistance gene analog [ <i>Malus prunifolia</i> ]/1e-42 myb-related protein M4 [ <i>Arabidopsis thaliana</i> ]/2e-20 NBS-LRR putative resistance gene analog [ <i>Malus prunifolia</i> ]/1e-42 myb-related protein M4 [ <i>Arabidopsis thaliana</i> ]/2e-20 NBS-LRR putative resistance gene analog [ <i>Malus prunifolia</i> ]/1e-42, NBS-like putative resistance gene [ <i>Phaseolus vulgaris</i> ]/6e-40
	EAA/MCTA4, EAC/MCAA3, EAA/MCCA1, EAA/MCTT7, EAA/MCTG2, EAC/MCAT4, EAT/MCTA2	BPPCT008, BPPCT025	PC29A	Apricot	Resistance protein analog [ <i>Phaseolus vulgaris</i> ]/7e-39

<sup>a</sup> Genetic markers and ESTs mapped onto the *Prunus* resistance map (Lalli et al. 2005).<sup>b</sup> Origin of selected probes (source species)<sup>c</sup> Closest similarity with an accession of GenBank database (ID)<sup>d</sup> NBS = Nucleotide-Binding Site

marker from this group, EAA/MCCC1, detected the amplification product both in the susceptible parent Nemaguard and in the bulk of dead trees (Blenda et al. 2006).

In the remaining group of 11 markers (Table 3, category C), the intermediately susceptible rootstock Lovell and highly susceptible parent Nemaguard always displayed the same phenotype, and in the majority of markers in this group, highly susceptible rootstock Nemared also had the same phenotype as intermediate Lovell (Blenda et al. 2006).

The 38 mapped AFLP markers associated with PTSL-response were present in the linkage groups GA, GB, G1, G2, G3, G4A, G4B, G5, and G6B. Their number varied from one PTSL-response-associated marker in group G1 to ten PTSL-associated markers in linkage group G6B (Fig. 1). The largest number of phenotype-associated markers were those present in groups G6B (ten) and G4A + G4B (eight). Clustering of these markers was observed in four linkage groups: G6B (seven markers over 13 cM), G4A (four markers over 12 cM), GA (four markers over 19 cM), and GB (five markers over 23 cM; Fig. 1).

The comparison of the distribution of RGAs on the *Prunus* resistance map (Lalli et al. 2005) with the distribution of the PTSL-associated markers mapped in the rootstock F<sub>2</sub> cross Guardian<sup>®</sup> selection 3-17-7 × Nemaguard revealed that some RGAs and several PTSL-associated markers were located in the same regions in linkage groups G1, G2, G4, G5, and G6 (Table 4).

At 24 cM in G1, a PTSL-associated AFLP (EAT/MCTC4) was located approximately in a region homologous to that of the *Prunus* reference map where two RGAs were co-localized (Table 4). A common SSR, pchgms3, was placed 19 cM apart from the AFLP in the rootstock map and 7 cM apart from the RGAs (co-localized with markers AC7A, FG5, and AG113) in the *Prunus* reference map (Lalli et al. 2005). In this region, a severe suppression of recombination was observed in the *Prunus* reference map (Verde et al. 2005), indicating that the two regions may be homologous. Considering another common marker, pchgms11 located at the base of G1, its distance from both the associated AFLP in the current map and the mapped RGAs in T × E was similar (about 35 cM).

In G2, two PTSL-associated AFLPs were localized in the basal part of the linkage group at 9 and 21 cM from the anchor marker UDP-406. This marker in two *Prunus* maps (Verde et al. 2005; Lambert et al. 2004) was positioned at the very end of G2 approximately 10–20 cM from the AC19 region that co-localizes with RGAs in the *Prunus* resistance map (Table 4).

In G4, a cluster of four PTSL-associated AFLPs was localized in the basal part of G4A, in a region spanning 12 cM. The only anchor marker (UDP-024) was mapped far away

from the cluster, and the total length of G4 in the current map was twice as much as that of the T × E map. It is possible that this cluster is localized in a region homologous to that of the *Prunus* resistance map containing RGAs (marker AG8A).

In G5, two PTSL-associated AFLPs were mapped at 7 and 13 cM from the anchor marker BPPCT017. RGAs (co-mapping with AC55B and AG8B) were found in the same regions in the *Prunus* resistance map (7 and 14 cM from BPPCT017).

In G6, two regions containing PTSL-associated AFLPs were identified. A cluster of seven AFLPs mapped to a 12 to 27 cM region above the anchor SSR BPPCT025 (Table 4). This marker in the resistance map (Lalli et al. 2005) was localized at 15 cM from regions rich with RGAs (co-localized with markers PC29A, FG53, FG78, and LF98). Two additional PTSL-associated AFLPs co-segregated with the anchor SSR UDP-412. The marker LF11 was also mapped in that region (1.2 cM from UDP-412) in a *Prunus* syntenic map (Dirlewanger et al. 2004a). This marker hybridized with BACs containing RGAs, indicating a possible homology of the two regions (Lalli et al. 2005).

Additionally, for direct mapping of the PTSL-response trait on the genetic map, about 5,000 vegetative propagules from the four F<sub>2</sub> families were planted in January 2003 on severe PTSL orchard sites at both the Clemson University Sandhill Research and Education Center near Pontiac, SC, and in a grower's orchard in Ridge Spring, SC. These four families represent four different F<sub>2</sub> crosses expected to segregate for the trait of tolerance to PTSL (four PTSL-tolerant Guardian<sup>®</sup> selections 3-17-7, 4-26-2, 6-39-7, and SL1089 crossed with the highly PTSL-susceptible Nemaguard). Of those four F<sub>2</sub> crosses, the family 11-100 (cross Guardian<sup>®</sup> selection 3-17-7 × Nemaguard) was used in the present study. The markers described in this paper will be further evaluated on these cloned F<sub>2</sub> seedlings, allowing genetic analysis of the PTSL-response trait after its phenotypic appearance.

In this study, we developed a genetic linkage map for a peach rootstock using a population expected to segregate for tolerance/susceptibility to the PTSL syndrome. The current map will be used as a framework to construct a highly saturated molecular genetic map that, together with other genomic resources available for peach, can be used to positionally clone the gene(s) responsible for tolerance to PTSL and to facilitate introgression of PTSL tolerance through marker-assisted selection. Additionally, the application of AFLP markers in combination with microsatellites as anchor sites facilitated the merging of this rootstock map with other peach and *Prunus* maps, thus contributing potentially important markers and gene loci to the growing *Prunus* reference map. This eventually will provide the opportunity to utilize many marked agriculturally important characters, including tolerance to PTSL, in the development of superior *Prunus* rootstocks.



**Acknowledgment** The authors thank Dr. Bryon Sosinski (NC State University, USA) and Dr. Zhen-Xiang Lu (Lethbridge Research Centre, Agriculture and Agri-Food Canada) who made the original four F<sub>2</sub> crosses designed to segregate for PTSL response (PTSL-tolerant Guardian® selections 3-17-7, 4-26-2, 6-39-7, and SL1089 crossed with the highly PTSL-susceptible Nemaguard). Dr. Marisa Badenes (Instituto Valenciano de Investigaciones Agrarias, Spain), Dr. Renate Horn (University of Rostock, Germany), Dr. Alexander Kozik (UC Davis, USA), and Dr. Tatyana Zhebentyayeva (Clemson University, USA) are acknowledged for reviewing the manuscript and/or useful comments. This article is technical contribution no. 5098 of the Clemson University Agricultural Experiment Station. The work was supported by the Cooperative State Research, Education, and Extension Service (CSREES)/USDA under project SC-1700005, CSREES special research grant “Peach Tree Short Life in South Carolina”, and Natural Resources Institute grant 98-35311-6719 “Molecular Genetic Studies of Ring Nematode Tolerance/Peach Tree Short Life Tolerance.”

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