Tree Genetics & Genomes (2013) 9:659–668
DOI 10.1007/s11295-012-0578-4

ORIGINAL PAPER

Identification of QTLs controlling seed dormancy in peach (*Prunus persica*)

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Received: 27 May 2011 / Revised: 26 September 2012 / Accepted: 11 October 2012 / Published online: 3 January 2013

Abstract Dormancy is a condition that delays or inhibits growth in seed, vegetative buds, and floral buds. In peach, seed germination occurs when seed accumulate sufficient stratification and growing degree hours to break dormancy and begin growing. Correlations have been reported between mean seed stratification requirements and mean bud chilling requirements among *Prunus* families, but an individual seed's germination date and subsequent vegetative and floral bud break date are not correlated. Prior to this study, the genetic factors involved in regulating seed dormancy and their location on the peach genomic map were unknown. Segregating F_2 seed were collected from a high×low chill F_1 peach hybrid in 2005, 2006, and 2008. Germination date and growth habit was measured after the stratification requirement of the 2005 seed was fully met. The seed collected in 2006 and 2008 received varying amounts of stratification, which enabled data on stratification requirement, heat requirement, and growth habit to be collected. Genomic DNA was extracted from seedling leaf tissue and screened with SSR markers selected from the *Prunus* reference map at an average resolution of 20 cM. Seed dormancy quantitative trait loci (QTLs) were detected on G1, G4, G6/8, and G7. The QTLs detected on G6/8 and G7 were discovered in the same region as QTLs associated with floral bud chilling requirement and bloom time in peach.

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Keywords Peach · Seed dormancy · Quantitative trait loci · Stratification · Germination

Abbreviations

AG	Abnormal growth
CU	Chilling unit
G	Linkage group
GD	Germination date
GDH	Growing degree hour
HR	Heat requirement
QTL	Quantitative trait loci
SR	Stratification requirement

Introduction

Dormancy is a condition of the meristem in which growth is unable to occur despite favorable environmental conditions (Rohde and Bhalerao 2007). In order to break dormancy and germinate, peach seed require stratification followed by warmer temperatures that enable growth to occur (Kester et al. 1977). Seed stratification requirements (SR) can be met between −2 and 15 °C in most temperate fruit crops; however, optimal temperatures in peach are between 4 and 6 °C (Seeley and Damavandy 1985). The temperature range at which dormancy is broken is initially narrow, but widens as seed are gradually released from dormancy (Dennis 1987). SR temperature durations can range from approximately 250 to 1,000 chill units (CU), where 1 CU is equal to 1 h at temperatures between 2.5 °C and 9.1 °C (Richardson et al. 1974).

The heat requirement (HR) necessary for germination to occur can be measured as growing degree hours (GDH), such that one GDH is calculated by subtracting 4.5 °C from each hourly temperature between 4.5 °C and 25 °C (Richardson et al. 1975). Embryo growth occurs slowly at temperatures just above the freezing point and at an

increased rate as temperatures rise (Seeley et al. 1998; Gianfanga and Rachmiel 1986; Thèvenot et al. 1983). Thus, the HR necessary for seedling growth to result in germination can eventually be met at the same temperature for which SR is met to break dormancy (Couvillon and Erez 1985). Growth cannot occur before dormancy release, but it remains unknown whether HR can begin to accumulate prior to dormancy release.

Germination, as evidenced by radicle protrusion (root perforation of the seed coat) or seedling emergence from the soil, is often misused to determine the release of seed dormancy (Frisby and Seeley 1993a). Dormancy release and subsequent embryo growth together determines a seed's germination date (GD). Germination can occur before an optimum SR is met, however, as evidenced by subsequent abnormal growth (AG). AG is characterized by physiological dwarfing and abnormal leaf development such that leaves form a rosette structure and fail to develop chlorophyll along the midrib (Frisby and Seeley 1993b; Pollock 1962; Wang and Beardow 1968). Seed given minimal amounts of stratification, or stratification at suboptimal temperatures, often demonstrate this abnormal growth habit; however, additional stratification can restore AG plants to a normal growth habit (Frisby and Seeley 1993b; Martinez-Gomez and Dicenta 2001; Pollock 1962; Seeley et al. 1998).

The ability to predict a cultivar's bloom date is agronomically important to ensure that floral buds will receive sufficient chilling to break dormancy for a full harvest yield, but are not released from dormancy before the risk of spring frost damage is past. Because chilling requirement in peach cannot be determined accurately until the third or fourth year of growth, seed dormancy has been suggested to predict bud dormancy requirements for making earlier breeding selections. There is a relationship between a parent's chilling requirement and the mean SR of its seed, but a high degree of variability is observed within that seed lot, and the correlation between a seed's SR and subsequent chilling requirement is low (Kester et al. 1977; Garcia-Gusano et al. 2003, 2005; Dicenta et al. 2005; Perez-Gonzalez 1990). This suggests that a loose genetic relationship between seed and bud dormancy exists, and that genetic regulation is controlled by multiple loci (Kester et al. 1977; Dennis 1994). Quantitative trait loci (QTL) for floral bud chilling requirement have been detected on linkage groups 1, 4, 5, and 7 in an F_2 family of peach (Fan et al. 2010). The genetic component of seed dormancy remains uncharacterized, but the use of molecular markers in QTL mapping could help elucidate the genetic relationship between seed and bud dormancy.

The use of molecular markers for mapping QTLs has become a powerful tool in plant breeding for genetic analysis, early selection, and fingerprinting (Cipriani et al. 1999). In the last 16 years, several maps have been constructed in peach (x=n=8) using AFLP, RFLP, RAPD, isozyme, and SSR markers (Chaparro et al. 1994; Dirlewanger et al. 2004b). Microsatellite markers, or simple sequence repeat (SSR) markers, are the molecular markers of choice in peach because they are codominant, highly polymorphic, frequent across the genome, and informative across populations, cultivars, and species (Powell 1987, Cipriani et al. 1999).

The objective of this study was to construct linkage maps for three F_2 families of peach using SSR markers and to identify QTLs associated with different seed dormancy traits including germination date (GD), stratification requirement (SR), heat requirement (HR), and abnormal growth (AG).

Materials and methods

Plant material

Three F_2 peach populations were developed in 2005, 2006, and 2008 at the Southeastern Fruit and Tree Nut Research Laboratory (Byron, GA) from two open pollinated F_1 hybrids selected from parents with contrasting chilling requirements for vegetative and floral bud break. The F_1 hybrid selection, 'R01528.3', resulting from a cross of 'Flordaguard' (FG) (approximately 250 CU) on 'Late Arkansas' (LA) (1,000+ CU), was open pollinated in the spring of 2005 and 2006, and an F_2 seed population was collected each year. The F_1 hybrid selection, 'R98625.01', resulting from selections 'SL0736' (SL) (approximately 250 CU) and 'PI091459' (PI) (1,000+ CU), was open pollinated in the spring of 2008 and a third F_2 population was collected that year. The F_2 seed were imbibed in water (changed at 24-h intervals) for 4 days, soaked in 0.4 % Captan fungicide solution, placed in a bag of moist perlite, and placed in a cold chamber at 7 °C. All seed were planted into cone containers of sphagnum peat and perlite (1:1) containing 6 kg/m^3 of 15-9-12 controlled release fertilizer. Seedlings were grown in a greenhouse at approximately 27 °C, except the 2008 seed population was transferred to a growth chamber at 18 °C due to inconsistent temperatures in the greenhouse that year.

Phenotypic evaluation

Germination date (GD) Seed from the 2005 LA×FG F_2 population was placed in the cold chamber on the same day. The duration of stratification time prior to radicle protrusion was observed and recorded twice per week as the GD. Only seed showing radicle protrusion were planted at weekly intervals.

Stratification requirement (SR) In 2006, stratification treatments of 50 seed from the 2006 LA×FG F_2 population were

initiated at 4-day intervals equivalent to 0, 100, 200, 300, 400, 500, 600, and 700 h of stratification and placed in the cooling chamber at 4-day intervals. In 2008, stratification treatments of 50 seed from the 2008 PI×SL F_2 population were initiated at intervals equivalent to 0, 200, 300, 400, 600, 800, 1,000, 1,500, 2,000, and 2,500 h of stratification and placed in the cooling chamber on different days according to stratification intervals. SR was measured based on duration of stratification treatments and the presence or absence of germination after the initial planting. The percent germination from each stratification treatment represent seed whose stratification requirement was met.

Heat requirement (HR) In 2006 and 2008, all seed were removed from stratification and planted on the same day. Germination was recorded at 2-day intervals based on the emergence of seedlings breaking the soil. HR was measured based on germination time and was defined as the time interval between the initial planting and recorded germination date. Seed that failed to germinate before 33 and 34 days in 2006 and 2008, respectively, were recovered and given additional stratification at 7 °C to break dormancy, and were replanted. HR and AG were not measured in these recovered seedlings.

Abnormal growth (AG) One of the morphological symptoms of insufficient stratification is AG, and is characterized by abnormal leaf expansion and rosetting of the apical meristem. All seedlings that germinated after the initial planting in 2005, 2006, and 2008 were given a score of 1–4 based on the degree of rosetting in their leaves and meristem. Seedlings with no rosetted leaves were given a score of 1, seedlings with 1–2 rosetted leaves were given a score of 2, seedlings with >2 rosetted leaves were given a score of 3, and seedlings with rosetting of the apical meristem were given a score of 4.

Red leaf/green leaf (Gr/gr) All F_2 populations segregated for red leaf color and this served as an additional marker for mapping purposes. Phenotypic expression of the red leaf trait (*Gr*) was recorded for all F_2 seedlings based on their leaf color. Seedlings with green leaves were scored homozygous for both alleles from LA or PI, seedlings with intermediate red leaves were scored as heterozygotes, and seedlings with dark red leaves were scored homozygous for both alleles from FG or SL.

Genotypic evaluation

Genomic DNA was extracted from leaf tissue using the Qiagen DNeasy 96 Plant Kit and amplified using fluorescently labeled SSR markers selected from the TxE *Prunus* genomic map (Dirlewanger et al., 2004b) (Table 1). Any seedlings that resulted from outcrossing were identified and discarded. Markers segregating for alleles that differed by more than 5 bp were visualized by size fractionation on a 3 % agarose gel and those that differed by less than 4 bp were detected by capillary electrophoresis on an ABI 3730 Automated Sequencer at the ICBR Genetics Analysis Laboratory, University of Florida. Allelic segregation was visualized using the Soft Genetics analysis program GeneMarker v.1.6.

Statistical analysis

A linkage map was constructed with Mapmaker Macintosh 2.0 (Lander et al. 1987) using the Kosambi map function with a minimum LOD of 3.0 and a maximum recombination fraction of 0.4. Linkage groups were formed and the order of markers was determined by relative LOD score using the "Compare" and "Ripple" functions. QTL analysis of GD, SR, HR, and AG in germinated seedlings was performed with QGene 4.3.7 (Joehanes and Nelson 2008) using the Single-trait Composite Interval Mapping and Least-Squares (CIM LS) function. GD data collected from the 2005 LAxFG population was transformed using $\log_{10}$ function prior to QTL analysis. Background markers, or cofactors, and intermarker positions were selected by the software program to reduce residual genetic variation from unlinked QTL. Threshold LOD scores significant at $\alpha_{0.05}$ and $\alpha_{0.01}$ were chosen to detect putative QTL based on significance levels determined by a permutation test (Churchill and Doerge 1994). ANOVA and Tukey's test were performed using SAS v.9.1.

Results

Phenotypic evaluation

Germination date (GD) A broad distribution of GDs was observed in 379 seed of the 2005 F_2 population with a mean GD of 89±28 days and range of 51 to 134 days (Fig. 1). GD was initially observed at 51 days, equivalent to approximately 1,200 h of stratification at 7 °C. GD was no longer measured after 133 days, which was equivalent to approximately 3,000 h of stratification. Seed that did not demonstrate radicle protrusion on or before 133 days were planted and assigned a GD of 134 days to be used in QTL analysis. Because these seed germinated after they were planted in the greenhouse, it can be assumed that their SR was sufficiently met in order for germination to occur. Their failure to demonstrate radicle protrusion prior to planting could be due to a lack of GDH necessary to fulfill HR.

Stratification requirement (SR) After the initial planting, germination was observed in 49 % (195/400) of the total

Table 1 Microsatellite (SSR) and morphological markers mapped in the LAxFG and PIxSL F2 populations

Linkage group	Marker	Locus[a] (cM)	T_a[b] (°C)	Reference
1	cppct026	33.9	55	Aranzana et al. 2002
1	pms67	45.9	55	Cantini et al. 2001
1	cppct029	65.1	55	Aranzana et al. 2002
1	bppct028	77.4	57	Dirlewanger et al. 2002
2	udp98025	9.6	57	Testolin et al. 2000
2	pchgms1	35.1	50-60	Sosinski et al. 2000
2	bppct030	38	57	Dirlewanger et al. 2002
2	cpsct034	48.6	62	Mnejja et al. 2004
3	bppct007	11.2	57	Dirlewanger et al. 2002
3	eppisf011	13.5	57	unpublished
3	bppct039	18	57	Dirlewanger et al. 2002
3	cpdct025	36.4	62	Mnejja et al. 2005
3	udp96008	36.4	57	Cipriani et al. 1999
4	cpsct039	1.8	62	Mnejja et al. 2004
4	cppct005	10.4	52	Aranzana et al. 2002
4	bppct040	18.4	57	Dirlewanger et al. 2002
4	udp96003	28.3	57	Cipriani et al. 1999
4	epdc3832	34.1	57	Sook et al. 2008
4	bppct023	45.4	57	Dirlewanger et al. 2002
5	bppct026	5.2	57	Dirlewanger et al. 2002
5	bppct017	20.1	57	Dirlewanger et al. 2002
5	bppct038	32.9	57	Dirlewanger et al. 2002
5	bppct014	44	57	Dirlewanger et al. 2002
6	cppct008	8.7	59	Aranzana et al., 2002
6	bppct008	30.1	57	Dirlewanger et al. 2002
6	*Gr*[d]	36.2	N/A	Dirlewanger et al. 2004a
6	bppct025	56.4	57	Dirlewanger et al. 2002
6	udp98412	72	57	Testolin et al. 2000
7	ampa107	0[c]	51	Dirlewanger et al. 1998
7	cppct022	18.6	50	Aranzana et al. 2002
7	bppct029	29.6	57	Dirlewanger et al. 2002
7	cppct033	38.9	50	Aranzana et al. 2002
7	pms2	47.8	55	Cantini et al. 2001
7	cppct017	61.8	60	Aranzana et al. 2002
8	cpsct018	0	52	Mnejja et al. 2004
8	udp96-019	20.8	57	Cipriani et al. 1999
8	epdcu3117	54.7	57	Sook et al. 2008

[a] Map locus on the TxE *Prunus* reference map (Dirlewanger et al. 2004b)

[b] T_a annealing temperature(°C)

[c] Map locus on the JxF=peach reference map (Dirlewanger et al. 1998)

[d] *Gr* leaf color (red/green) morphological marker

seed of the 2006 LAxFG F_2 population. Eighteen percent of seed (72/400) decayed after they were initially planted, and 33 % (133/400) were recovered after 33 days to receive additional stratification because they did not germinate. Eighteen percent of the recovered seed (24/133) did not germinate after receiving additional stratification. Because seed were given unlimited GDH to fulfill HR in the greenhouse, these results suggest that seed which began to decay after they were initially planted and seed that were recovered for additional stratification did not initially germinate because their SR had not been met. Disease could have also been a factor resulting in seed decay. The percentage of seed that decayed after planting decreased as stratification treatments increased. Fifty percent (25/50) of the seed that were planted without receiving any stratification (0 h treatment) decayed and could not be recovered for additional stratification. As a result, DNA could not be extracted from the genotypes that decayed, and this class of understratified and/or diseased seed was underrepresented in the QTL analysis.

The percent of seed germination increased in the 2006 LAxFG population as stratification time increased (Fig. 2). There was no significant difference between treatments 0, 100, and 200, nor between treatments 500, 600, and 700. Treatments 300 and 400 were significantly different from one another and from the 0, 100, 500, and 600 h treatments. Thirty-six percent emergence was observed in seed given 300 h of stratification and 60 % emergence was observed in seed given 400 h of stratification (Fig. 2). Approximately 80–90 % emergence was observed in seed given ≥500 h of stratification indicating that 500 h fulfilled the SR for germination to occur in most seed.

After the initial planting, germination was observed in only 23 % (114/500) of the total seed of the 2008 PIxSL F_2 population. When treatments were removed from stratification, 13 % (66/500) had already demonstrated radicle protrusion. These seed were all from the 1,500–2,500 h treatments and were considered to have fulfilled their SR and HR before planting. Five percent of seed (27/500) decomposed before they could be planted. Many of the decomposed seed had already demonstrated radical protrusion and were also considered to have met their SR and HR. Fifty-nine percent (293/500) of seed did not germinate after they were planted and were recovered after 34 days to receive additional stratification at 7 °C. These seed were considered to still be dormant because their SR was not fulfilled. Five percent of seed (27/500) decomposed before they could be recovered to receive additional stratification, and 9 % of the recovered seed (25/293) did not germinate after receiving additional stratification.

The low percentage of germinations (23 %) after the initial planting could be due to the wide range of stratification treatments applied in this study, such that the SR of low stratification treatments was insufficient to break dormancy, while the SR and HR of high stratification treatments were both sufficient for germination to occur prior to planting. This ultimately decreased the percent of seed that were given only enough stratification to break dormancy, but an

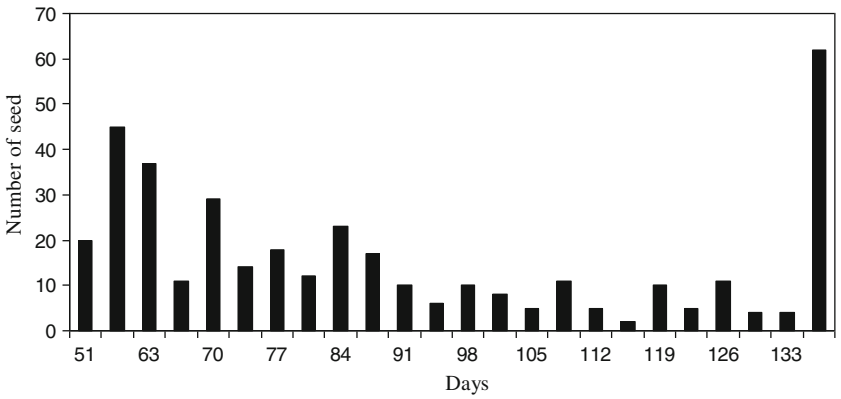

Fig. 1 Distribution of germination dates (GD) in the 2005 LAxFG F_2 seed lot after stratification at 7 °C

insufficient amount of GDH to germinate, and as a result diminished power of detection in the QTL analysis.

The percent of seed germination in the 2008 PIxSL population increased as stratification time increased (Fig. 2). A low percent of germination in the 2,500 h stratification treatment was the result of seed decay during stratification (data not shown). Seventy-four percent of seed in the 2,500 h treatment demonstrated radicle protrusion prior to planting; however, 38 % of those seed decomposed and could not be planted. Treatments 600–2,000 were significantly different from treatments 0–400, such that ≥48 % emergence was observed in seed given more than 600 h of stratification, and ≤12 % emergence was observed in seed given less than 400 h of stratification.

Heat requirement (HR) The first seedlings of the 2006 LAxFG population germinated 9 days after planting and seed continued to germinate until 33 days after planting. The mean days between planting and germination were significantly longer in seed given 0–200 h of stratification than in seed given 300–700 h of stratification as determined by Tukey's test (α=0.05) (Fig. 2). This supports the work of Seeley et al. (1998), which concluded that seed can acquire GDH to fulfill HR at stratification temperatures just above freezing. Therefore, seed given longer stratification treatments in this experiment were able to germinate earlier because they were able to accumulate more GDH during stratification and fulfill their HR sooner than seed from the 0–200 h stratification treatments.

The first seedlings of the 2008 PIxSL population germinated 14 days after planting and seed continued to germinate until 34 days after planting. The mean germination time decreased as stratification increased (Fig. 2). The mean germination time was significantly longer in the 200 h treatment than in treatments with ≥600 h of stratification as determined by Tukey's test (α=0.05). There was no significant difference in the mean germination time among seed given 300–1,000 h of stratification. Seed given longer stratification treatments germinated earlier than seed from shorter stratification treatments because they were able to accumulate more GDH during stratification (Seeley et al. 1998).

Abnormal growth (AG) A normal growth habit was observed in 80 % (133/166) of seedlings from the 2005 LAxFG population. The unlimited amount of stratification received by each seed prior to germination explains this high frequency of normal leaf and apical meristem development (Seeley et al. 1998). Though only 58 % (17/29) of

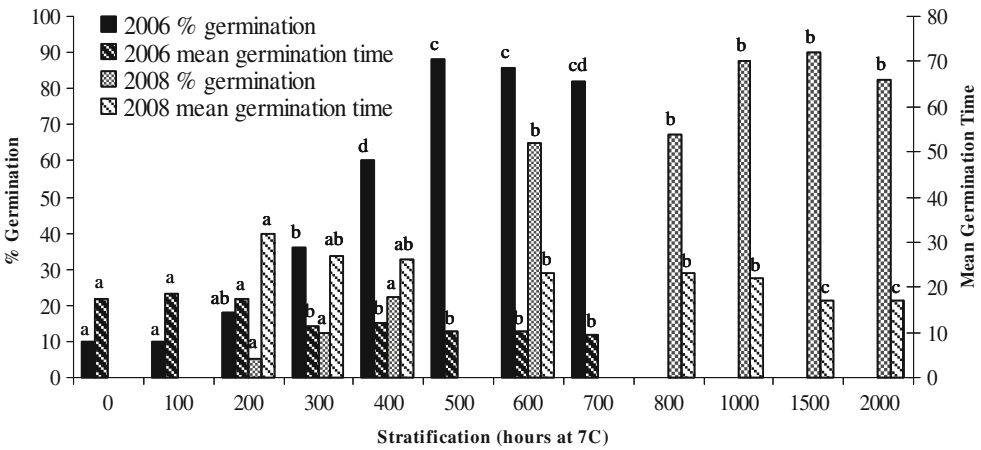

Fig. 2 Percent germination and mean germination time for each stratification treatment of the 2006 LAxFG and 2008 PIxSL F_2 seed populations. Different *letters* indicate significant differences between stratification treatments within each patterned series using Tukey's test ($P \leq 0.05$)

seedlings that did not demonstrate radical protrusion by 133 days demonstrated a normal growth habit, there was no significant difference in the frequency of AG between seedlings that demonstrated radical protrusion and those that were planted prior to showing radical protrusion (P=0.09). Seed that did not germinate until they were planted in the greenhouse were considered to have fulfilled their SR, but failed to demonstrate radical protrusion during stratification due to insufficient GDH.

AG was observed in 95 % (173/182) of seedlings from the 2006 LAxFG population. Even though 88 % of seed that received ≥500 h of stratification germinated, only 7 % demonstrated a normal growth habit. These results indicate that stratification was sufficient to break dormancy, but insufficient for normal meristem and leaf development. There was no significant difference in the degree of AG between treatments as determined by Tukey's test (α=0.05), which suggests that 0 to 700 h of stratification was equally insufficient for normal meristem and leaf development.

Fifty-nine percent (108/183) of seedlings from the 2008 PIxSL F2 population grew normally and 41 % (75/183) demonstrated some degree of AG. There was a significant difference in the degree of AG between treatments 300–600 and 1,000–2,500 as determined by Tukey's test (α=0.05). The frequency of AG decreased as stratification increased indicating that there is a negative correlation (R^2=0.8471) between stratification duration and AG of germinated seedlings. The correlation between AG and GDH (R^2=0.0439) was low. This supports previous observations that AG is the result of seed that have received minimal amounts of stratification, or stratification at suboptimal temperatures (Frisby and Seeley 1993b; Martinez-Gomez and Dicenta 2001; Pollock 1962).

Linkage map

LA, FG, and 'R01528.3' were screened with 123 SSR markers of which 42 were polymorphic and 35 were informative. A total of 29 SSR markers from the TxE *Prunus* reference map (Aranzana et al. 2002; Cantini et al. 2001; Cipriani et al. 1999; Dirlewanger et al. 2002, 2004b; Mnejja et al. 2004; Sook et al. 2008; Sosinski et al. 2000; Testolin et al. 2000) and JxF peach reference map (Dirlewanger et al. 1998), and one phenotypic marker (Dirlewanger et al. 2004a) were selected for use in the 2005 and 2006 LAxFG F_2 populations (Table 1). The markers selected from the TxE map spanned eight linkage groups (G) that covered 507.5 cM and were spaced at an average distance of 14.4 cM resulting in 93 % coverage of the TxE map. There were five regions in which no heterozygous markers were identified within the target spacing of 20 cM. Due to the low level of polymorphism in peach, and specifically to the low level of polymorphism (approximately 0.29) in these particular populations, no heterozygous markers were detected in the distal regions of G1, G3, and G6 (Fig. 3) (Sosinski et al. 2000).

The LAxFG F_2 linkage map consisted of 30 markers with a total map distance of 390.5 cM and an average distance of 16.98 cM between markers, with no interval exceeding 33.7 cM (Fig. 3). Seven linkage groups were detected with a single linkage group formed from markers on G8 (epdcu3117 and udp96-019) and G6 (bppct025, bppct008, and cppct008) of the TxE reference map. A single linkage group from markers on G6 and G8 was also detected in the 'Garfi' almond × 'Nemared' peach map constructed by Jáuregui et al. (2001) and the 'Akame' peach × 'Juseito' peach map constructed by Yamamoto et al. (2005). The fusion of G6 and G8 has been detected only in germplasm carrying the dominant *Gr* allele for red leaf color and is attributed to a reciprocal translocation near the *Gr* locus.

PI, SL, and 'R98625.01' were screened with 78 SSR markers of which 36 were polymorphic and 34 were informative. Twenty-five SSR markers from the TxE *Prunus* reference map (Aranzana et al. 2002; Cantini et al. 2001; Cipriani et al. 1999; Dirlewanger et al. 2002, 2004b; Mnejja et al. 2004, 2005; Sook et al. 2008; Sosinski et al. 2000; Testolin et al. 2000) and one phenotypic marker (Dirlewanger et al. 2004a) were selected for use in the 2008 PIxSL F_2 population (Table 1). The markers selected from the TxE map spanned eight linkage groups that covered 507.5 cM and were spaced at an average distance of 20.5 cM resulting in 78 % coverage of the TxE map. There were eight regions in which no heterozygous markers were identified within the target spacing of 20 cM on G1, G2, G3, G4, G6, and G8 (Fig. 4).

The PIxSL F_2 linkage map consisted of 25 markers with a total map distance of 292.3 cM and an average distance of 16.24 cM between markers, with no interval exceeding 43.4 cM (Fig. 4). Because this population also segregated for red leaf color, a single linkage group was formed from markers on G6 and G8 of the TxE reference map and a total of seven linkage groups were detected in the PIxSL F_2 linkage map.

More than half of the markers used in the LAxFG (63 %) and PIxSL (60 %) F_2 populations followed expected 1:2:1 segregation ratios (P<0.05). Five of the skewed markers were common to both linkage maps on G4, G5, and G6/8. The other distorted markers were located on G2 and G3 of the LAxFG linkage map and G1 and G7 of the PIxSL linkage map.

QTL analysis

QTLs associated with GD Five GD-associated QTLs were detected in the 2005 LAxFG F_2 population on linkage

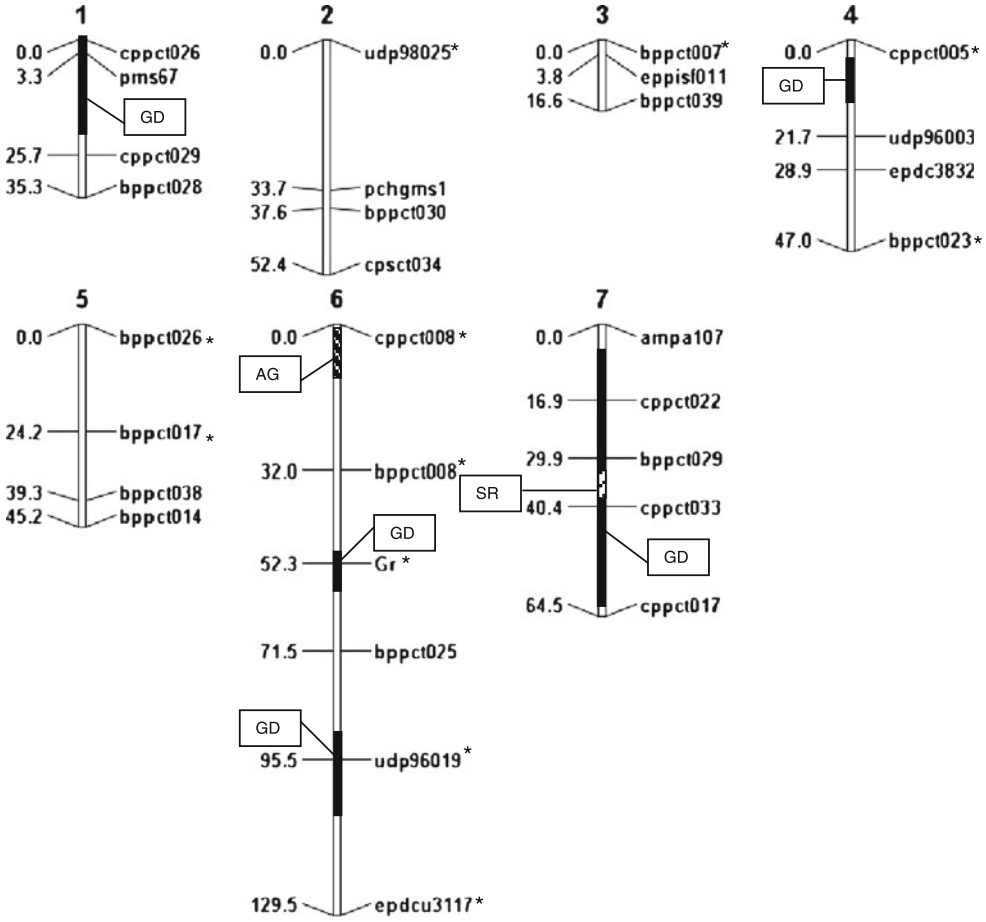

Fig. 3 Location of QTLs associated with GD (LOD 2.930=$\alpha_{0.05}$) and AG (LOD 3.105=$\alpha_{0.05}$) in the 2005 LAxFG F_2 population and QTL associated with SR (LOD 2.867=$\alpha_{0.05}$) in the 2006 LAxFG F_2 population. Trait associated QTLs are differentiated by *bar pattern*: *solid black* for GD, *black/white stripe* for AG, and *white/black stripe* for SR. Markers with an *asterisk* deviated from 1:2:1 segregation ratios ($P<0.05$)

groups G1, G4, G6/8, and G7 (Table 2, Fig. 3). The QTL on G7 demonstrated the greatest average effect on GD, such that seed homozygous for the LA allele at locus cppct033 demonstrated radical protrusion 19 days later than seed homozygous for the FG allele at the same locus. The average effect of the LA allele at two out of the five QTLs detected (Gr on G6/8 and cppct033 on G7) resulted in a delayed GD. The average effect of the LA allele at the remaining three QTLs (pms67 on G1, cppct005 on G4, and udp96-019 on G6/8) resulted in an earlier GD.

QTLs associated with SR A QTL associated with SR was detected in the 2006 LAxFG F_2 population on G7 with a LOD of 3.87 (α=0.05) (Table 2, Fig. 3). A QTL associated with SR was also detected in the 2008 PIxSL F_2 population on G7 with a LOD score of 3.73 (α=0.01) (Table 2, Fig. 4).

QTLs associated with HR An HR-associated QTL meeting the LOD threshold of 2.85 and 2.723 (α=0.05) was not detected in the 2006 LAxFG or 2008 PIxSL F_2 populations, respectively. Because HR could only be measured in seed that germinated after the initial planting, it is possible that there was not a sufficient sample size by which a QTL for germination time could be detected at a significant threshold using QGene.

QTLs associated with AG A QTL associated with AG was detected in the 2005 LAxFG F_2 population near marker cppct008 on G6/8 with a LOD score of 4.49 (α=0.01) (Table 2, Fig. 3). An AG-associated QTL meeting the LOD threshold of 3.012 (α=0.05) was not detected in the 2006 LAxFG F_2 population. Because AG was observed in 95 % (173/182) of all seedlings that germinated after the initial planting, a QTL for AG may not have been detected at a significant threshold using QGene due to this skewed distribution of growth habits. Additionally, marker cppct008 showed significant (α=0.001) segregation deviation in the 2006 LAxFG F_2 population, and for that reason may have been unable to detect a QTL at the LOD threshold. A LOD score of 2.996 (α>0.05) was detected at 0 cM on G6/8 flanking marker cppct008 at 0 cM. Among seed that demonstrated AG in the 2006 population, 49 % were homozygous for the LA allele and 10 % were homozygous for the FG allele at locus cppct008 (0 cM). A χ^2 test of independence found this distorted segregation ratio to be significant at α=0.001. These findings suggest that an AG-associated QTL may be present at the cppct008 locus on G6/8.

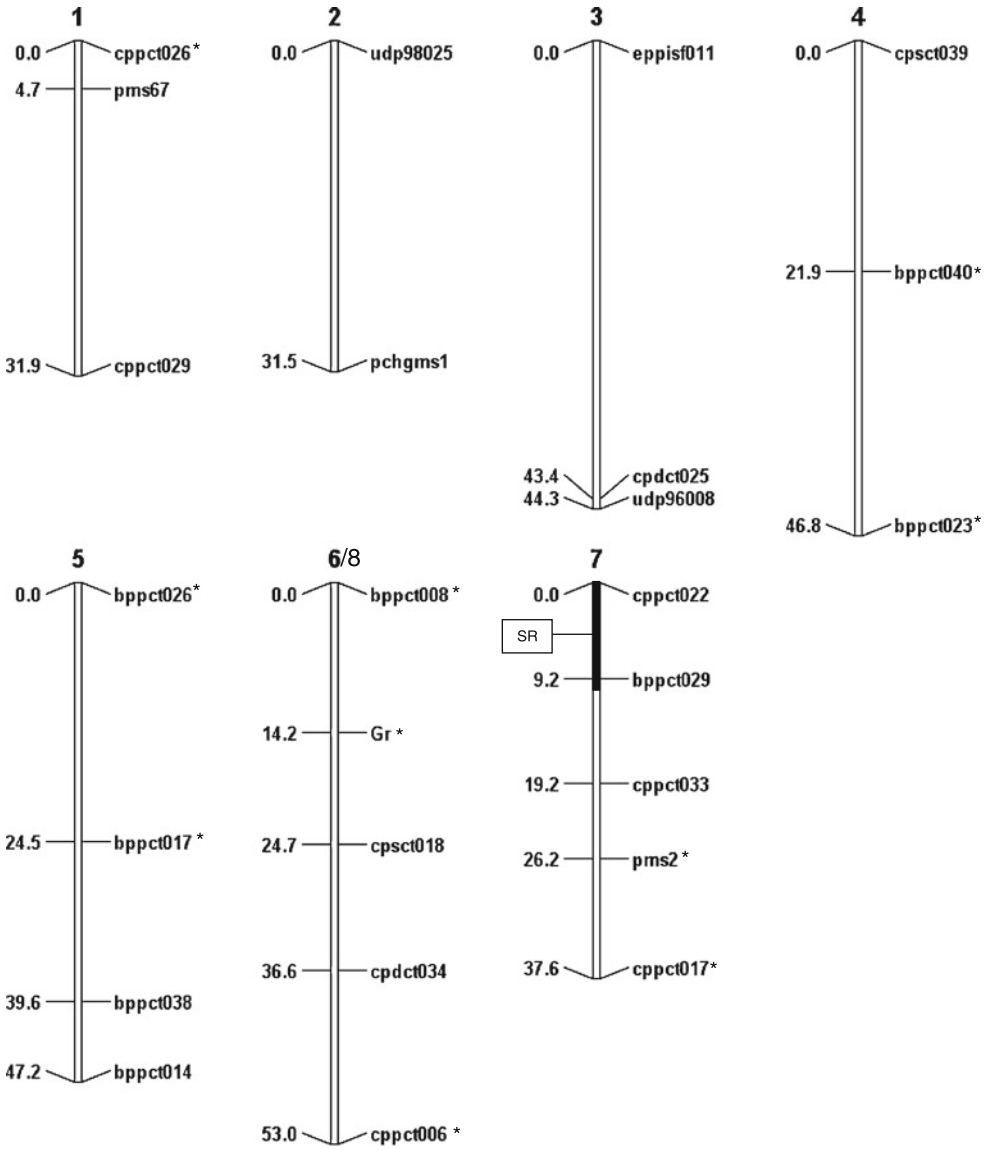

Fig. 4 Location of SR-associated QTLs (LOD 2.829=$\alpha_{0.01}$) detected in the 2008 PIxSL F_2 population and represented by the *solid black bar*. Markers with an *asterisk* deviated from 1:2:1 segregation ratios ($P<0.05$)

An AG-associated QTL meeting the LOD threshold of 2.92 ($\alpha=0.05$) was not detected in the 2008 PIxSL F_2 population. The AG-associated QTL detected in the 2005 and 2006 populations at marker cppct008 was not detected in the 2008 population most likely due to marker cppct008 being uninformative in this population and the lack of marker coverage at the top of G6/8.

Table 2 Map position of QTLs detected for germination date (GD), abnormal growth (AG), and stratification requirement (SR) in the LAxFG (2005, 2006) F_2 populations, and the PIxSL (2008) F_2 population

Year	Population	Trait	G	LOD peak (cM)	Nearest marker	Marker position (cM)	Max. LOD	R^2 (%)
2005	LA×FG	GD	1	6	pms67	3.3	4.15**	4.9
			4	8	cppct005	0	3.042*	3.6
			6/8	52	Gr	52.3	4.189**	5
			6/8	96	udp96-019	95.5	3.432*	4.1
			7	36	cppct033	40.4	5.801**	6.8
		AG	6/8	0	cppct008	0	4.494**	5.6
2006	LA×FG	SR	7	30.9	bppct029	29.9	3.874*	5.7
2008	PI×SL	SR	7	8	bppct029	9.2	3.725**	3.7

*$\alpha_{0.05}$, **$\alpha_{0.01}$

Discussion

The germination percentages observed after the initial planting were lower in the 2008 population than in the 2006 population among common stratification treatments (0, 200, 300, 400, and 600). A high frequency of germination was observed in the 2008 seed from those treatments after they were given additional stratification. Different parents were selected based on chilling requirement for the 2006 and 2008 populations, but the mean SR of the families were not previously known, and differences between SR genetic factors in these parents may account for the differences in the germination behavior observed after the initial planting as well as differences in the QTLs that could be detected.

Two linkage maps were constructed from F_2 populations of two peach families segregating for chilling requirement, and seed dormancy associated QTLs were mapped to G1, G4, G6/8, and G7. The GD-associated QTL detected on G6/8 at marker udp96-019 co-localized with a QTL described by Yamamoto et al. (2001) as being linked to flowering time in peach. The GD-associated QTL detected in the 2005 LAxFG population on G7 at marker bppct029 and cppct033 co-localized with SR-associated QTLs detected in the 2006 LAxFG and 2008 PIxSL populations at marker bppct029, and a QTL at marker cppct033 described by Fan et al. (2010) as being linked with chilling requirement and bloom date in peach. These QTLs that are common to GD, SR, bloom date, and chilling requirement support previously observed correlations between a cultivar's chilling requirement/bloom date and the mean SR/GD of its offspring (Perez-Gonzalez 1990; Powell 1987; Seeley and Damavandy 1985). A potential co-localization might exist between the GD-associated QTLs detected on G1 and G4 in the 2005 LAxFG population and the bloom date associated QTLs detected by Fan et al. (2010) on G1 and G4, but would need to be confirmed with further fine mapping. Chilling requirement and bloom date associated QTLs have been detected at loci other than those associated with SR and GD, which also explains the low correlation observed between a seedling's SR/GD and subsequent chilling requirement/bloom date (Geneve 2003; Garcia-Gusano et al. 2003; Perez-Gonzalez 1990; Dicenta et al. 2005; Fan et al. 2010).

The AG-associated QTL detected on G6/8 at marker cppct008 (0 cM) in the 2005 LAxFG population co-localized with the putative AG-associated QTL detected in the 2006 LAxFG population. This QTL could not be validated in the 2008 PIxSL population, however, due to lack of marker coverage on the distal end of G6/8 for that linkage map. Marker cppct008 was described by Sánchez-Pérez et al. (2012) as a marker linked to blooming time in almond.

No HR-associated QTLs were detected, and further studies should be conducted to determine whether genetic variability for HR exists in peach seed. HR was measured by the number of days from planting to germination and normalized across all treatments. All seedlings within a treatment for which HR was recorded had their SR fulfilled as evidenced by the fact that they germinated once they were planted, but the seedlings within a treatment likely had their SR fulfilled at different points in time and therefore began accumulating HR at different points as well. Therefore, our measure of HR from the time of planting to germination may in fact be a measure from the time of SR fulfillment to germination and therefore be a measure of SR rather than HR. To confirm whether there is in fact genetic variability for HR among seed, it would be necessary to fix all SR genetic loci and observe germination between various GDH treatments.

Conclusions

Together, these experiments suggest that GD, SR, and AG are genetically regulated, and that some of the QTLs associated with GD and SR are common with QTLs associated with bloom date and chilling requirement in peach. These results do not support the practice of early selection of chilling requirement based on germination dates in breeding programs; however, marker assisted selection (MAS) of chilling requirement may provide a means of early selection in the future.

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