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# Chilling injury susceptibility in an intra-specific peach [*Prunus persica* (L.) Batsch] progeny

C.M. Cantín[a], C.H. Crisosto[b], E.A. Ogundiwin[b], T. Gradziel[b], J. Torrents[c], M.A. Moreno[a], Y. Gogorcena[a,*]

[a] Departamento de Pomología, Estación Experimental de Aula Dei-CSIC, Apdo. 13034, 50.080 Zaragoza, Spain
[b] Plant Sciences Department, University of California Davis, 1 Shields Ave., Davis, CA 95616, USA
[c] Agromillora Catalana S.A., El Rebato s/n, E-08739 T.M. Subirats, Barcelona, Spain

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ABSTRACT

Chilling injury (CI) is the collective term for various disorders that occur during prolonged cold storage and/or after subsequent ripening of stone fruit. Major symptoms of CI include mealiness, graininess, flesh browning, loss of flavor (off flavor), and red pigmentation (bleeding). These symptoms were evaluated over 2 years in an intra-specific progeny population derived from the cross of cultivars 'Venus' (freestone, melting and yellow-flesh nectarine) and 'BigTop' (clingstone, melting and yellow-flesh nectarine) after storage of fruit at 5 °C (CI inducing conditions) for 2 and 4 weeks. All the evaluated traits in the progeny showed continuous variation which is typical of quantitative or polygenic inheritance. Longer cold storage periods increased the incidence and severity of CI symptoms, except for bleeding and leatheriness, which were not affected by time of storage. CI symptoms showed high and significant heritability or genotype effect in the studied population, with no significant effect of harvesting year. Browning, mealiness and graininess were significantly correlated and were the main CI symptoms observed in this population. Mealiness and graininess were negatively correlated with stone adhesion which reflects the higher susceptibility to CI disorders of freestone fruit. A genetic linkage map of linkage group 4 (LG4) was constructed with SSR and candidate genes (CGs). Significant quantitative trait loci (QTLs) for mealiness, graininess, leatheriness and bleeding were found in this linkage group, validating QTLs for CI symptoms previously reported in this linkage group from an unrelated progeny population. In addition, QTLs controlling other agronomic and fruit quality traits were also localized in this linkage group.



## 1. Introduction

Peach [*Prunus persica* (L.) Batsch] is one of the most important fruit crops in the world in terms of production (approx. 18 million tons in 2008) with a cultivated area of around 1,608,000 ha (FAOSTAT, 2010), and it is the most important species of the genus *Prunus*. However, the rapid ripening of fruit during storage results in a short shelf-life of the commodity and represents a serious constraint for efficient handling and transportation. Ripening can be slowed by refrigeration, but extended storage of peaches, nectarines and other stone fruit at low temperatures between freezing point and 10 °C, or more severely in the range of 2.2–7.6 °C (killing temperature zone), can negatively affect fruit quality. Under these storage conditions, several physiological disorders collectively known as chilling injury are developed (Lill et al., 1989; Crisosto et al., 1999). These symptoms are of commercial importance since shipping of peaches to distant markets and storage before selling requires low temperature (Campos-Vargas et al., 2006). This disorder, also called internal breakdown, is the major reason for low consumption of this fruit when compared to other fresh fruit such as apples and bananas (Crisosto, 2006).

Susceptibility of stone fruit to chilling injury is highly influenced by the genetic background of the cultivar (Peace et al., 2006). CI in peaches and nectarines can induce different symptoms, including mealiness or lack of juice, flesh browning and impaired softening, which is referred to as leatheriness. The physiological basis of CI symptoms has been studied in detail in peach (reviewed in Lurie and Crisosto, 2005). However, the exact mechanism by which chilling injury affects a commodity is not fully understood. It has been shown to involve loss of membrane integrity and ion leakage from cells and changes in enzyme activity (Brummell et al., 2004), but exactly why some crops are susceptible and some resistant still remains unclear. Understanding the genetic control of these traits, in order to grow only cultivars free of chilling injury susceptibility, promises to greatly benefit producers, shippers and consumers in the peach industry.

* Corresponding author. Tel.: +34 976 716133; fax: +34 976 716145.
*E-mail address:* aoiz@eead.csic.es (Y. Gogorcena).

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In the last decade, several linkage maps, obtained by using molecular markers, have been constructed for peach (Abbott et al., 1998; Dirlewanger et al., 1998; Lu et al., 1998; Yamamoto et al., 2001; Aranzana et al., 2002; Dirlewanger et al., 2002, 2006; Etienne et al., 2002a; Dondini, 2007; Ogundiwin et al., 2009b). A consensus map from an inter-specific almond × peach $F_2$ population ('Texas' × 'Earlygold', T×E) is considered the reference map of the *Prunus* genus (Joobeur et al., 1998; Howad et al., 2005). However, many important agronomic characters of *Prunus* species have not yet been mapped, and very few of those already mapped (such as major genes for disease and pest resistances, self-incompatibility, and several fruit quality traits such as flesh color, endocarp staining, flesh adherence to stone, non-acid fruit, skin pubescence, skin color and fruit shape) are currently being used for marker assisted selection (MAS) (Dirlewanger et al., 2004; Shulaev et al., 2008).

The genetic control of CI in peach has been studied and it has been demonstrated that mealiness, browning and bleeding are probably controlled by major genes (Peace et al., 2006; Ogundiwin et al., 2007). Moreover, one major quantitative trait loci (QTL) has been detected for each of these symptoms of CI in linkage group (LG) 4 and 5, using a linkage map constructed from two segregating populations—Pop-DG ('Dr. Davis' × 'Georgia Belle') and Pop-G ('Georgia Belle' selfed) (Peace et al., 2006; Ogundiwin et al., 2007). A major QTL for mealiness and bleeding was found at the *F-M* locus at the bottom end of LG4. Other minor QTLs for mealiness were also found on LG4 and LG6. Besides, an expressed sequence tags (ESTs) database has been developed specifically to study chilling injury (Ogundiwin et al., 2008). Microarray analysis involving these ESTs has identified several cold-regulated peach genes some of which have been mapped close to CI QTLs on Pop-DG (Ogundiwin et al., 2009a). However, in these populations (Pop-DG and Pop-G), only clingstone non-melting flesh (CNMF) and freestone melting flesh (FMF) progeny were obtained. As CNMF progeny did not get mealy (Peace et al., 2006; Ogundiwin et al., 2007), and FMF and CMF (clingstone melting flesh) genotypes have the potential to develop this symptom depending on whether they carry further genes for susceptibility, an entirely melting segregating population is of interest for the study of CI susceptibility.

The main objectives of this work were (1) to quantify the expression of different CI symptoms after two different lengths of cold storage in an entirely melting nectarine segregating population from 'Venus' × 'BigTop' over a 2-year study and (2) to identify QTLs for quality traits and other traits mainly involved in the control of the main CI symptoms in the LG4 of this population, and validate the results previously obtained on unrelated populations.

## 2. Materials and methods

### 2.1. Plant material

The population assayed was a segregating $F_1$ population (75 seedlings) obtained from a controlled intra-specific cross made in 2000–2001 between *P. persica* cvs. 'Venus' (female parent) and 'BigTop' (male parent), in collaboration with Agromillora Catalana S.A. 'Venus' is a FMF (freestone melting flesh) nectarine cultivar whereas 'BigTop' is a CMF (clingstone melting flesh) nectarine cultivar. The segregating population is entirely melting flesh, either cling- or freestone. This population is referred to as V×BT throughout the paper.

Seedlings were budded on the same rootstock (GF-677) and established (one tree per genotype) in an experimental orchard at the Experimental Station of Aula Dei (northern Spain, Zaragoza) in 2002. Trees were trained to the standard open vase system and planted at a spacing of 4 m × 2.5 m. Hand-thinning was carried out to reduce fruit load when required. Trees were grown under standard conditions of irrigation, fertilization and pest and disease control. Since 2004, different agronomic and quality traits have been evaluated in this population (Cantín et al., 2009).

Preliminary SSR marker analysis (see below) identified five selfs of 'Venus'. These selfs contained only alleles present in the 'Venus' parent, and were lacking any 'BigTop' alleles of codominant SSR marker that were inherited by other progeny. Population size was, therefore, reduced to 70 seedlings. The five selfs remained in the field for selection purposes but were excluded for further molecular genetic analysis.

### 2.2. Agronomic and quality traits evaluation

Quantitative and qualitative traits were recorded over 3 years (2005, 2006 and 2007). Blooming date according to Fleckinger (1945), harvesting date and annual yield were evaluated in each independent progeny. When the fruit was ripe, yield (kg/tree) was measured and a representative fruit sample (approx. 20 fruit) was taken for the fruit quality evaluations (Cantín et al., 2009). Some pomological traits such as fruit weight, height, suture diameter (SD), cheek diameter (CD), skin blush, stone type (free or cling), endocarp staining, or flesh firmness were also scored. Flesh firmness measurements were performed by a hand penetrometer with an 8 mm flat probe in two opposite sides of the fruit that had previously been peeled to remove the epidermis. Data were expressed in Newtons. The soluble solids content (SSC) of the juice was measured with a temperature compensated refractometer (model ATC-1, Atago Co., Tokyo, Japan) and expressed as a percentage of soluble solids in 100 g of juice. The initial pH and TA (titratable acidity) was measured by titration with NaOH 0.1 N to pH 8.1. The TA was expressed as percentage of malic acid in 100 g of fresh weight. The ripening index (RI) was calculated as the ratio between SSC and TA.

### 2.3. Chilling injury symptoms evaluation

Chilling injury (CI) susceptibility was evaluated after cold storage at 5 °C and 95% RH (relative humidity) according to Crisosto et al. (1999). After different periods of 2 and 4 weeks of cold storage, a group of 10 fruit from each seedling was ripened at room temperature during 2–3 days until firmness reached between 10 and 18 N. Fruit were then evaluated for different symptoms of CI such as lack of juiciness (flesh mealiness), graininess, fail to ripening (leatheriness), flesh browning and flesh bleeding. Observations were made on the mesocarp and the area around the pit immediately after the fruit were cut into two halves through the suture plane. Fruit that had a dry appearance and little or no juice after hand squeezing were considered mealy. Fruit were also informally tasted for a feeling of graininess and/or off flavors to corroborate visual mealiness assessment. Mealiness, graininess and off flavor was scored as the proportion of fruit affected with these symptoms in the sample. Internal browning was visually scored on a scale of 1 (no browning) to 6 (severe browning). Bleeding was visually scored on a scale of 1 (no bleeding) to 3 (more than 50% of the flesh with bleeding). Then the percentage of progenies with each proportion/score was calculated for every CI symptom. Eventually, the degree of CI (CI index) was visually assessed according to the global fruit appearance of each genotype, from healthy fruit with no symptoms (1) to severe CI symptoms (6) when the fruit was extremely injured with mealiness/graininess, browning and bleeding symptoms.

### 2.4. DNA extraction and molecular analysis

DNA was extracted from young leaves of 'Venus', 'BigTop' and each tree of the progeny by using the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA) following the manufacturer's instructions. A

**Table 1**
SSR and CG markers used in this study. Marker type, reference and species in which they were developed are also reported.

| Marker | Marker type | Reference | Species |
|---|---|---|---|
| BPPCT009[a] | SSR | Dirlewanger et al. (2002) | Peach |
| BPPCT010 | SSR | Dirlewanger et al. (2002) | Peach |
| BPPCT015[a] | SSR | Dirlewanger et al. (2002) | Peach |
| BPPCT023[a] | SSR | Dirlewanger et al. (2002) | Peach |
| BPPCT036[a] | SSR | Dirlewanger et al. (2002) | Peach |
| BPPCT040 | SSR | Dirlewanger et al. (2002) | Peach |
| C0212[a] | SSR | Ogundiwin et al. (2009b) | Peach |
| C1077 | SSR | Ogundiwin et al. (2009b) | Peach |
| C-PPN18B09[a] | SSR | Ogundiwin et al. (2008) | Peach |
| C-PPN70A04[a] | SSR | Ogundiwin et al. (2008) | Peach |
| C-PPN52H08 | SSR | Ogundiwin et al. (2008) | Peach |
| CPPCT005[a] | SSR | Aranzana et al. (2002) | Peach |
| CPPCT024[a] | SSR | Aranzana et al. (2002) | Peach |
| CPPCT028[a] | SSR | Aranzana et al. (2002) | Peach |
| CPSCT005[a] | SSR | Mnejja et al. (2004) | Plum |
| EndoPG[a] | SSR | Peace et al. (2005) | Peach |
| EPPCU8503[a] | SSR | GDR | Almond & peach |
| GPPDE | CG | Ogundiwin et al. (2009b) | Peach |
| pchgmsO55[a] | SSR | Sosinski et al. (2000) | Peach |
| pchgms2 | SSR | Sosinski et al. (2000) | Peach |
| pchgms5 | SSR | Sosinski et al. (2000) | Peach |
| UDA003[a] | SSR | Testolin et al. (2004) | Almond |
| UDA027[a] | SSR | Testolin et al. (2004) | Almond |
| UDP96-003[a] | SSR | Testolin et al. (2000) | Almond |
| UDP97-402 | SSR | Testolin et al. (2000) | Almond |
| UDP98-024[a] | SSR | Testolin et al. (2000) | Peach |
| Unknown-12[a] | SSR | Unpublished (developed in UC Davis) | Peach |

*Abbreviations*: SSR, simple sequence repeat; GDR, genome database for Rosaceae; CG, candidate gene.
[a] Polymorphic markers in the 'Venus' × 'BigTop' (V×BT) population.

total of 27 SSR (simple sequence repeats) and candidate gene (CG) markers mainly from the LG4 were employed to provide marker profiles. Markers were nominated from published work and review *Prunus* articles as detailed in Table 1. The marker Unk12 is an unpublished SSR related with chilling injury susceptibility (Ogundiwin, personal communication). The SSR locus C0212 (Ogundiwin et al., 2008) identified five selfs in the population.

PCR were carried out according to Etienne et al. (2002a). Twenty nanograms of genomic DNA were amplified using PCR in a final volume of 10 μL containing 100 mM Tris–HCl, pH 8.3, 500 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM of each dNTP, 5 pmol each primer and 0.25 U Taq DNA polymerase (Sigma, St. Louis, MO). The PCR conditions for SSR markers were as follows: preliminary denaturation (94 °C, 3 min), followed by 30 cycles consisting of denaturation (94 °C, 45 s); annealing (50 or 57 °C, 45 s, depending on the marker to amplify) and extension (72 °C, 2 min); and a final extension (72 °C, 4 min). For the GPPDE (CG marker), different PCR conditions were used: preliminary denaturation (95 °C, 5 min), followed by 30 cycles consisting of denaturation (95 °C, 30 s); annealing (60 °C, 45 s) and extension (72 °C, 90 s); and a final extension (72 °C, 5 min). The PCR products were then denatured by adding one volume of 95% formamide/dye solution, heated at 94 °C for 5 min, and chilled on ice. Finally, 4.5 μL of the denatured preparation was loaded on a 4% polyacrylamide sequencing gel containing 7.5 M urea in 1× TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA). The gels were run at 80 W for 2.5 h. Following electrophoresis, the gel was silver-stained (Promega Corporation, Madison, WI) following the protocol described by Peace et al. (2005). Fragment sizes were estimated with the 100-bp ladder-DNA sizing markers (Promega Corporation, Madison, WI). For initial polymorphism testing of each primer, assays were performed on 'Venus', 'BigTop', and six progenies. Subsequent analyses were performed on all progeny, including selfs, only for the primers that were polymorphic on the preliminary assay (Table 1).

### 2.5. Mapping and QTL analysis

Genome mapping of LG4 from the segregating population developed using the cross 'Venus' × 'BigTop' was constructed with JoinMap® 4.0 software (Van Ooijen, 2006), using segregation data from SSR and CG markers. Linkage analyses involved all linked markers, setting the population type as cross-pollination (CP). The Kosambi mapping function (Kosambi, 1944) was used to convert recombination fraction to map distances in centimorgans (cM). QTL analysis was performed with MapQTL® 5.0 software (Van Ooijen, 2005). Maximum likelihood-based interval mapping of MapQTL® 5.0 software was used for QTL analysis. The likelihood value of the presence of a QTL was expressed as a LOD (log of odds) score, which is the 10-base logarithm of the quotient of the likelihood of the existence of a segregating QTL, and the likelihood for the situation when a locus with zero genetic effect would segregate (i.e. there is no segregating QTL). When the LOD score exceeds the predefined significance threshold somewhere on a LG, a segregating QTL is detected. Permutation test (1,000 linkage group-based), with which the significance threshold can be determined based on the actual data rather than on assumed normally distributed data, was used to determine LOD threshold for quality and other phenotypic traits. A LOD score of 3, which means that it is 1000:1 more likely that the alternate hypothesis in favor of linkage holds, was used an arbitrary threshold for CI symptoms.

### 2.6. Statistical analysis

Data were treated for multiple comparisons by analysis of variance (ANOVA), followed by Tukey's test with significance level $p < 0.05$. Significance of factors (genotype, year and storage duration) affecting chilling injury symptoms was determined by ANOVA ($p < 0.1$), considering genotype, year and storage duration as fixed factors. The contribution of genotype, year and storage duration to the phenotypic variance of chilling injury symptoms was estimated by the partial eta-squared statistic, which describes the proportion of total variability attributable to a factor. All the statistical analyses

**Table 2**
Basic statistics based on single plant observations in the 'Venus' × 'BigTop' population, for annual and cumulative yield and fruit traits. For each trait, minimum, maximum, mean value, mean standard error (MSE) and standard deviation (SD) for 3 years of study are presented.

| Traits | Minimum | Maximum | Mean | MSE | SD |
|---|---|---|---|---|---|
| Annual yield (kg) | 0.4 | 16.0 | 6.6 | 0.4 | 3.3 |
| Cumulative yield (kg) | 0.4 | 48.0 | 17.9 | 1.2 | 10.3 |
| Fruit weight (g) | 100.6 | 270.8 | 191.2 | 4.3 | 37.5 |
| Blush (%) | 53.3 | 100.0 | 82.7 | 1.2 | 10.4 |
| Hardness (1–10)[a] | 4.8 | 8.7 | 7.9 | 0.1 | 0.7 |
| Stone adhesion (1–10)[a] | 1.7 | 10.0 | 8.3 | 0.3 | 2.5 |
| Endocarp staining (1–10)[a] | 1.3 | 9.5 | 4.3 | 0.3 | 2.8 |
| Height (mm) | 51.5 | 86.7 | 75.5 | 0.7 | 5.8 |
| Suture diameter (mm) | 59.5 | 82.0 | 73.0 | 0.6 | 4.7 |
| Cheek diameter (mm) | 57.1 | 90.6 | 76.9 | 0.8 | 6.4 |
| SSC (%) | 10.3 | 19.7 | 14.7 | 0.2 | 2.1 |
| pH | 3.1 | 4.2 | 3.5 | 0.0 | 0.2 |
| TA (%) | 0.3 | 1.2 | 0.7 | 0.0 | 0.3 |
| Ripening index (RI)[b] | 11.2 | 59.6 | 25.7 | 1.3 | 11.1 |
| Firmness (N)[c] | 10.5 | 50.1 | 33.0 | 0.9 | 7.7 |

*Abbreviations*: SSC, soluble solids content; TA, titratable acidity; N, Newtons.
[a] Hardness, stone adhesion and endocarp staining were scored on a scale of 1 (not observed trait) to 10 (extremely high intensity).
[b] Ripening index (RI), calculated as the ratio between SSC and TA.
[c] Flesh firmness measured by a hand penetrometer with a 8-mm flat probe.

were performed using the statistical software SPSS 15.0 (SPSS Inc., Chicago, USA).

## 3. Results and discussion

### 3.1. Fruit quality traits

V×BT population showed variability for the vegetative and fruit quality traits recorded (Table 2). All these traits exhibited continuous variation, which is typical of quantitative or polygenic inheritance. Stone adhesion ranged from 1.7 (freestone) to 10.0 (clingstone) showing the variability of this trait in the population (FMF and CMF progenies). Regarding SSC, all the genotypes showed mean values over 10° Bx, which is considered the minimum value for consumer acceptance for yellow-flesh nectarines (Kader, 1999). The variability found in SSC among the progenies can be explained by the quantitative behavior of this quality trait (Dirlewanger et al., 1999; Quilot et al., 2004). In the progeny, there was a four-fold range in titratable acidity (TA), whereas pH varied from 3.1 to 4.2. A small change in pH represented a large change in TA because of different scales. Therefore, non-acid and acid fruit were found within the progeny, since fruit with a pH at maturity higher than 4.0 are considered as non-acid (Monet, 1979). 'Venus' is an acid nectarine, and 'BigTop' is a non-acid nectarine, which explains the segregation of these traits in the progeny. Fruit firmness, measured on both cheeks, was highly variable among the seedlings (from 10.5 to 50.1 N). Some of the progeny showed firmness values higher than 35 N, which has been defined as the threshold between mature and immature fruit (Valero et al., 2007), due to the variability of fruit softening within a tree. However, only mature fruit, with firmness lower than 35 N, were selected for the evaluations since fruit maturity has been reported to affect CI susceptibility (Lill et al., 1989; Infante et al., 2008).

### 3.2. CI susceptibility

The $F_1$ progeny also showed variability for all the evaluated chilling injury symptoms. The distribution of the different traits was studied using the mean of 2-year data (Fig. 1). Continuous distributions were shown for browning, bleeding, mealiness, graininess, off

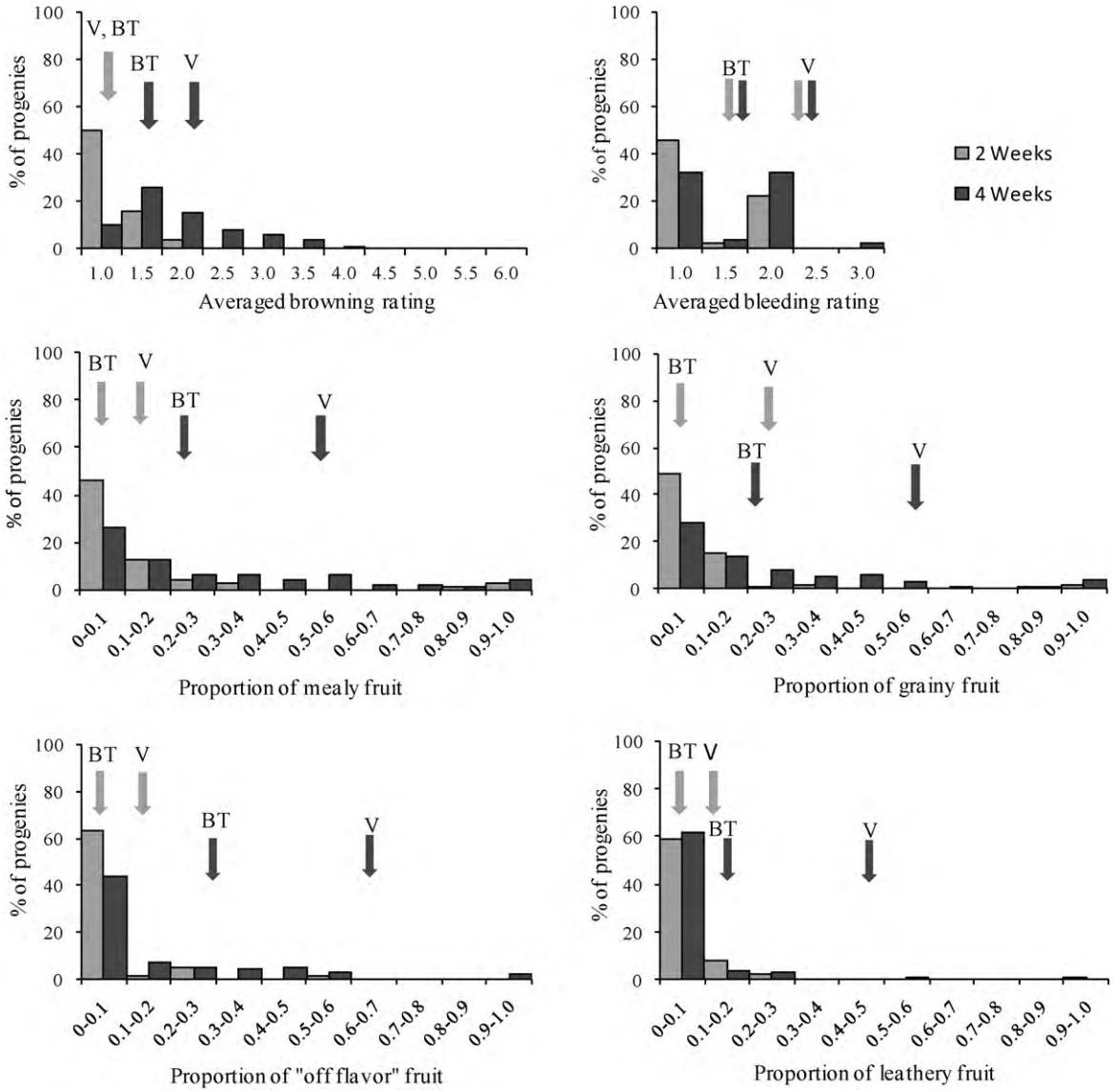


**Fig. 1.** Distribution of chilling injury symptoms in the 'Venus' (V) × 'BigTop' (BT) population averaged over 2 years of study after storage at 5 °C for 2 and 4 weeks and then ripened at 20 °C during 2 or 3 days. Intensity of browning was visually scored on a (1–6) scale and bleeding was scored on a (1–3) scale. Mealiness, graininess, off flavor and leatheriness were scored according to the percentage of progenies with a determined proportion (0–1) of injured fruit in the sample. The values for the chilling injury symptoms on the parents 'Venus' (V) and 'BigTop' (BT) after 2 and 4 weeks of cold storage at 5 °C are indicated by arrows.

**Table 3**
Comparison of chilling injury symptoms in the 'Venus' × 'BigTop' population after 2 and 4 weeks of cold storage at 5 °C. Averaged proportion of fruit affected by each CI symptom is shown. Data are mean of 2 years.

| Storage time | Browning[a] (1–6) | Bleeding[b] (1–3) | Mealiness | Graininess | Off flavor | Leatheriness | CI index[c] (1–6) |
|---|---|---|---|---|---|---|---|
| 2 weeks | 1.2 b | 1.4 a | 12.0 b | 9.1 b | 3.7 b | 5.5 a | 1.5 b |
| 4 weeks | 1.9 a | 1.5 a | 26.9 a | 24.0 a | 15.6 a | 3.2 a | 2.6 a |

In each column, values bearing the same letter are not significantly different.
[a] Browning scored on a scale of 1 (no browning) to 6 (severe browning).
[b] Bleeding scored on a scale of 1 (no bleeding) to 3 (more than 50% of the flesh with bleeding).
[c] CI index scored on a scale of 1 (no CI symptoms) to 6 (severe symptoms).

flavor and leatheriness, suggesting polygenic control of these symptoms as was earlier reported in other non-related peach progeny population (Peace et al., 2006). Variation from the Normal distribution was observed for these traits, which indicates that there may be only a few major genes controlling these traits (Peace et al., 2006). On the other hand, it is worth noting that browning scoring might be underestimated in the population since the visual scoring of this trait in the area surrounding the stone is more difficult to accomplish in the clingstone individuals due to the adhesion of the flesh tissue to the stone. The progeny distribution for mealiness, graininess, off flavor and leatheriness traits was skewed toward lower susceptibility to these symptoms than the parents after 4 weeks of cold storage.

The V×BT population showed lower susceptibility to CI symptoms than previous studied populations Pop-DG and Pop-G (Peace et al., 2006; Ogundiwin et al., 2007). However, similar results were found for bleeding when analyzed only within the FMF progeny and for mealiness when analyzed only within the CNMF progeny of those populations. These authors reported that mealiness was higher in FMF progeny whereas it was almost non-existent in CNMF progeny, while bleeding incidence was higher in CNMF and very low in FMF progeny (Peace et al., 2006). In contrast, mealiness was lower in V×BT progeny (averaging 27.4%) than in FMF progeny of Pop-DG (45%) and Pop-G (64%). Differences with other peach populations corroborate the reported genotype influence on the CI susceptibility (Peace et al., 2006).

The duration of the time of storage (2 or 4 weeks at 5 °C) modified the development and severity of CI symptoms. After 4 weeks of cold storage we found a significantly higher proportion of fruit that were significantly affected by CI symptoms (except for bleeding and leatheriness) (Table 3), suggesting that these disorders are triggered by the cold storage duration, as previously reported (Lill et al., 1989; Crisosto and Labavitch, 2002; Lurie and Crisosto, 2005; Campos-Vargas et al., 2006). However, no significant differences were found for bleeding after 2 and 4 weeks of cold storage. In some cases, flesh bleeding has been associated with fruit senescence and not with CI disorders (Lurie and Crisosto, 2005) which could be an explanation to the low impact of storage duration on this CI symptom. No significant differences were found for leatheriness among both durations of cold storage, maybe due to the low susceptibility of this germplasm to this symptom. Indeed, slightly lower leatheriness was observed after 4 weeks of cold storage, probably due to the softening that occurred during the ripening process.

Genotype was the main factor contributing to phenotypic variation for all the CI symptoms measured (Table 4), showing a contribution between 29% and 65%, corroborating the significant genetic component on the CI susceptibility (Crisosto et al., 1999; Peace et al., 2006). It is accepted that peach cultivars are more susceptible to CI than nectarine cultivars, and melting flesh cultivars are also more susceptible than the firmer non-melting flesh cultivars (Lester et al., 1996; Brovelli et al., 1999). Mealiness and graininess showed the higher proportion of phenotypic variance attributed to genotype, reflecting the high genetic control of these symptoms. This is an important result since mealiness is the most important CI symptom affecting peach postharvest quality. This heritability indicates that there is considerable genetic control that will allow the identification of QTLs in this population and the development of MAS for these CI symptoms. Similar heritability estimates for mealiness, browning and bleeding have been reported in other mapping populations (Peace et al., 2005).

Due to the reported variations between years that may occur in CI symptoms (Crisosto and Labavitch, 2002; Peace et al., 2005; Campos-Vargas et al., 2006) it is important to evaluate the CI susceptibility for several years. In our work, year did not show any significant effect on the CI symptoms, except for CI index (Table 4). CI symptoms were quite consistent over the 2 years at different storage durations. This result reflected the high heritability values obtained for all CI symptoms (Table 4).

### 3.3. Correlations between CI symptoms

All pairs of the CI symptoms were positively and significantly correlated except for leatheriness which was only positively correlated with the general CI index and not with any other symptom (Table 5). Mealiness and graininess were highly correlated ($r = 0.90$), probably because graininess is the sensorial feeling of visual mealiness. CI index is a global estimation of CI severity in the fruit, therefore, a significant positive correlation was observed between this value and all the CI symptoms evaluated. It is worthy to note that browning, mealiness and graininess were highly correlated and contributed the most to the general CI index, corroborating that these symptoms are the main CI disorders affecting peach quality (Crisosto et al., 1999; Brummell et al., 2004; Lurie and Crisosto, 2005). The fact that mealiness and graininess were negatively correlated with stone adhesion ($r = -0.25$ and $r = -0.26$, respectively) reflects a higher susceptibility of free stone fruit to CI disorders. The genetic locus for freestone appears to contain a cluster of *endo-PG* genes controlling these traits (Callahan et al., 2004; Peace et al., 2005), which can explain the correlation found between both traits. Off flavor was highly positively correlated with mealiness and graininess ($r = 0.63$ and $r = 0.68$, respectively) which confirms that these CI symptoms negatively affect fruit taste (Lurie and Crisosto, 2005). Moreover, mealiness and graininess were negatively correlated with flowering date ($r = -0.31$ and $r = -0.32$, respectively).

**Table 4**
Factors (genotype, year and storage duration) affecting chilling injury symptoms, observed for 2 years in the 'Venus' × 'BigTop' population. Significant factors ($p < 0.1$) and their contribution (%) to phenotypic variance are indicated, as determined by ANOVA.

| CI symptoms | Genotype[a] | Year | Storage duration |
|---|---|---|---|
| Browning | 29.5 | NS[b] | 26.7 |
| Bleeding | 46.2 | NS | NS |
| Mealiness | 63.5 | NS | 18.8 |
| Graininess | 64.9 | NS | 21.7 |
| Off flavor | 47.8 | NS | 19.8 |
| Leatheriness | 31.8 | NS | NS |
| CI index | 44.8 | 2.4 | 39.1 |

[a] This proportion of phenotypic variance attributed to Genotype is the broad sense heritability ($H_b$).
[b] Not significant.

**Table 5**
Phenotypic correlations (Spearman *r*-values) between chilling injury (CI) symptoms, observed for 2 years in the 'Venus' × 'BigTop' population.

| CI symptoms | Bleeding | Mealiness | Graininess | Off flavor | Leatheriness | CI index |
|---|---|---|---|---|---|---|
| Browning | NS[a] | 0.31** | 0.31** | 0.27** | NS | 0.62** |
| Bleeding | | 0.20** | 0.18** | 0.20** | NS | 0.29** |
| Mealiness | | | 0.90** | 0.63** | NS | 0.67** |
| Graininess | | | | 0.68** | NS | 0.67** |
| Off flavor | | | | | NS | 0.57** |
| Leatheriness | | | | | | 0.16** |

[a] Not significant.
** Correlation is significant at the 0.01 level (2-tailed).

These results suggest a tendency from earlier flowering genotypes to be more susceptible to suffer CI symptoms. On the contrary, different results were shown by Peace et al. (2006) who reported a positive correlation between flowering date and mealiness and bleeding. On the other hand, bleeding was negatively correlated with harvesting date ($r = -0.46$) in our population. The phenotypic correlations found between traits can be due to shared or linked controlling genes.

### 3.4. Linkage mapping

Linkage mapping and QTL analysis of LG4 were used to determine the location, number and effect of genomic sites contributing to the phenotypic variation in the V×BT population for the CI symptoms. The selected markers (Table 1) were known to be linked to important regions involved in the control of the main chilling injury symptoms (Ogundiwin et al., 2007, 2008, 2009b). Some of them were obtained from the ChillPeach EST database (Ogundiwin et al., 2008). From the 27 markers analyzed from the LG4, 19 were polymorphic (Table 1) in the V×BT population, and 14 (17 loci in total) were anchored to this group (Fig. 2). The high level of polymorphism found with these markers might be explained since the ChillPeach database is a specialized collection of ESTs from peach mesocarp tissue subjected to cold storage and ripening. Additional data from the same population showed that the polymorphism in this population was ~50% (Abidi et al., 2010), lower than the observed in T×E (~85%), but higher than the observed for Pop-DG population (~25%) (Ogundiwin et al., 2009b). The lower rate of polymorphism observed in V×BT and Pop-DG compared to T×E could be explained since T×E is a $F_2$ population from an inter-specific cross. In any case, V×BT showed a relatively high polymorphism for being an intra-specific population derived from modern commercial cultivars, which allowed the achievement of interesting results. Common markers with the published *Prunus* T×E reference map enabled the determination of LG orientation. The V×BT LG4 map is almost entirely co-linear with the *Prunus* consensus (T×E) map (GDR, http://www.bioinfo.wsu.edu/gdr/), and with Pop-DG map (Ogundiwin et al., 2009b), with the exception of an inversion between two adjacent loci (CPPCT028 and CPPCT005) on the upper end of the LG (Fig. 2). The proximity of CPPCT028 and CPPCT005 markers on the V×BT LG4 map (0.4 cM), suggests that the inversion is more probably to be due to errors in the assignment of markers order than to inversion of chromosome fragments (Dirlewanger et al., 2004). Interestingly, markers UDA027, EPPCU8503, pchgms055 and UDA003 were co-lineared in the bin 4:63 in the T×E *Prunus* reference map (Howad et al., 2005). Distances between markers varied slightly when compared our map with T×E and Pop-DG LG4 maps, probably due to differences in the rate of recombination in the two sets of parents. The genetic diversity of the individuals involved in the crosses may explain this phenomenon. It is also noticeable that different software was used to elaborate the two maps (JoinMap for V×BT and MAPMAKER for T×E) and differences in the genetic distances have been reported depending on which one is used (Van Ooijen et al., 1994). These results confirm the substantial co-linearity and the previously reported transferability of the molecular markers among different *Prunus* species (Sosinski et al., 2000; Testolin et al., 2000; Dirlewanger et al., 2004; Dondini, 2007; Sánchez-Pérez et al., 2007).

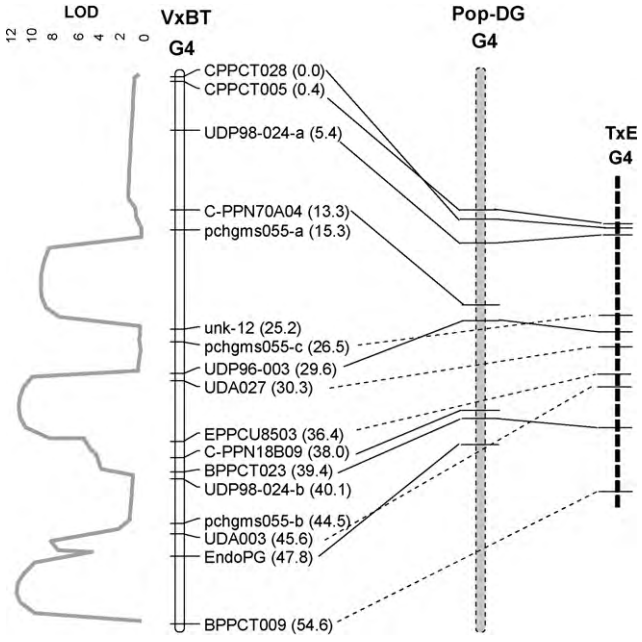


**Fig. 2.** Linkage group 4 map of 'Venus' × 'BigTop' (V×BT) $F_1$ progeny showing the position of DNA markers. Map distances (cM) of the markers are provided between parentheses. The QTLs detected for mealiness are shown on the left. A section of LG4 of T×E *Prunus* reference map (Dirlewanger et al., 2006) and a section of LG4 of the Pop-DG map (Ogundiwin et al., 2007, 2009b) are represented showing the position of common SSR markers connected with solid lines to LG4 of V×BT. Dashed lines represent common markers with the T×E map, but not with Pop-DG map.

### 3.5. QTL analysis

QTLs for several agronomic and quality traits and for CI symptoms were detected on LG4 of the V×BT map by interval mapping (Figs. 2 and 3) and accounted between the 26% and the 92% of the observed variation (Table 6). Significant QTLs for SSC, pH, TA, firmness, fruit height, harvesting date, endocarp staining, SD (fruit suture diameter), CD (fruit cheek diameter), fruit weight, and blush were detected on the LG4 (Table 6). Significant QTLs were also found on LG4 for CI symptoms such as mealiness, graininess, leatheriness and bleeding (Table 6, Fig. 3). Most of the QTLs found were consistent through the 2-year study showing that the expression of these genes could be independent of the environmental conditions as the phenotypic analysis showed (Table 4). QTLs for several traits were detected in the same region, what may correspond to link QTLs or to one QTL with pleiotropic effect. A high contribution QTL (87.2%) for harvesting date was detected near the UDP96-003 marker (Table 6). Dirlewanger et al. (1999) and Etienne et al. (2002b) also identified

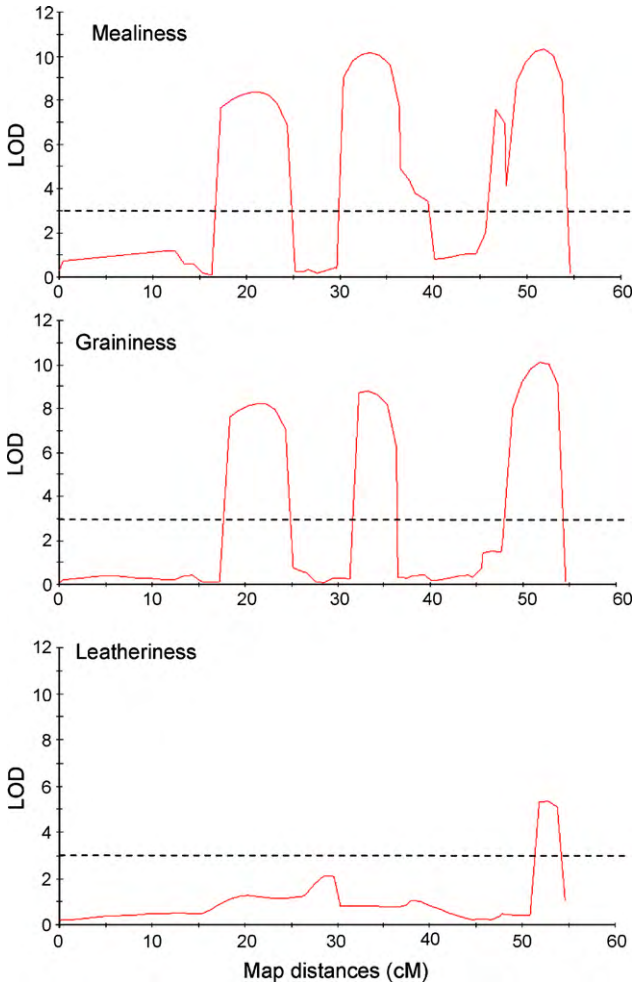


**Fig. 3.** LOD plots for mealiness, graininess, and leatheriness CI symptoms resulting from interval mapping of the 'Venus' × 'BigTop' data averaged for 2 years on linkage group 4.

QTLs for harvesting date at the top of LG4. QTLs for fruit dimensions (height, SD and CD) were found near the markers UDA027 and EPPCU8503. These results agreed with the location of major genes or QTLs on the LG4 controlling fruit dimensions described previously using different *Prunus* maps and different molecular markers (Quilot et al., 2004; Sánchez-Pérez et al., 2007). With respect to SSC, a QTL explaining 82.5% of the variation observed was found close to the pchgms055a marker. QTLs for SSC, sucrose and fructose have been previously mapped on LG4 by other authors (Dirlewanger et al., 1999; Etienne et al., 2002b; Quilot et al., 2004). QTLs for the pH and TA were also localized in the same region of LG4, accounting for the 91.8% and 30.9%, respectively, of the observed variance. These results agree with previous QTLs found by Quilot et al. (2004), who localized QTLs for citric acid, quinic acid, total acid, sorbitol and malic acid on LG4. These results are supported by the localization of some CGs related with organic acid metabolism, such as ACO3 and C0212, found on that region of LG4 by Ogundiwin et al. (2009b). However, the major locus controlling fruit acidity, *D*, have been reported to be localized on the proximal end of the LG5 (Quilot et al., 2004; Boudehri et al., 2009), which would explain the low percentage of variance explained by the QTL for TA and low LOD score found in our population on LG4 (Table 6).

QTLs for mealiness, graininess, leatheriness and bleeding were also localized on the LG4 of V×BT population (Table 6). Variation explained by these QTLs was 75.5%, 75.2%, 58.9%, and 75.6% for mealiness, graininess, leatheriness and bleeding, respectively (Table 6). It should be noted that the marker (EPPCU8503) linked with the QTL for bleeding (red pigmentation), was also related with a QTL controlling endocarp staining (Lurie and Crisosto, 2005). QTLs for mealiness and graininess co-localized possibly because graininess is the sensorial manifestation of visual mealiness, and both are texture problems. As previously reported on other no-related populations (Peace et al., 2006; Ogundiwin et al., 2007), co-localization of the CG *endoPG* and the major QTL for mealiness was validated in V×BT population (Fig. 2). Moreover, the three peaks for mealiness found in the LG4 of V×BT (Fig. 3) match with the additional minor QTLs reported for mealiness in the LG4 of Pop-DG by Ogundiwin et al. (2007). On the other hand, QTLs for browning were not found on LG4 on this work, in agreement with other authors (Peace et al., 2006; Ogundiwin et al., 2007), who reported one major QTL for browning and two minor QTLs on LG5 and LG2, respectively. On the other hand, some of the QTLs controlling some fruit quality traits co-localized with CI trait QTLs, being a possible explanation for the phenotypic correlations found between them.

The results found in this work showed that some genotypes have a better performance during cold storage than others, having, as a result, a better marketability. The elucidation of the inheritance mechanism of the chilling injury will provide a long-term solution of this problem and enable the breeding of new CI-tolerant cultivars. The application of MAS will enable the selection of those CI-tolerant cultivars, diminishing the global peach

**Table 6**
Nearest marker, peak position, maximum LOD score and percentage variance explained for QTLs identified on the linkage group 4 (LG4) by interval mapping in the $F_1$ progeny population of 'Venus' × 'BigTop'.

| Trait | Nearest marker | LOD peak position (cM) | Max. LOD score | % Variance explained |
|---|---|---|---|---|
| SSC | pchgmsO55-a | 17.3 | 14.6 | 82.5 |
| pH | pchgmsO55-a | 17.3 | 26.4 | 91.8 |
| TA | pchgmsO55-a | 17.3 | 3.2 | 30.9 |
| Firmness | pchgmsO55-a | 17.3 | 12.7 | 79.4 |
| Height | UDA027 | 31.3 | 4.2 | 26.5 |
| Harvesting date | EPPCU8503 | 36.4 | 25.9 | 87.2 |
| Endocarp staining | EPPCU8503 | 36.4 | 13.1 | 79.6 |
| Suture diameter | EPPCU8503 | 36.4 | 6.2 | 42.4 |
| Cheek diameter | EPPCU8503 | 36.4 | 6.7 | 41.5 |
| Bleeding | EPPCU8503 | 36.4 | 3.8 | 75.6 |
| Fruit weight | EPPCU8503 | 36.5 | 27.0 | 89.6 |
| Mealiness | BPPCT009 | 51.8 | 10.3 | 75.5 |
| Graininess | BPPCT009 | 51.8 | 10.1 | 75.2 |
| Leatheriness | BPPCT009 | 52.8 | 5.3 | 58.9 |
| Blush | BPPCT009 | 52.8 | 5.4 | 68.7 |

*Abbreviations*: SSC, soluble solids content; TA, titratable acidity.

industry losses due to this postharvest disorder. Our results supported and validated the markers mapping and QTLs positions related to CI susceptibility found in other unrelated peach progeny populations (Peace et al., 2006; Ogundiwin et al., 2007, 2009b), and contributed to a better understanding of the genetic control of this important disorder affecting peach and nectarine fruit.

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