QTL MAPPING THROUGH PEDIGREE-BASED ANALYSIS FOR SIX PHENOLOGICAL AND QUALITY TRAITS IN PEACH

A Dissertation

by

ZENA RAWANDOOZI

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	David Byrne
Committee Members,	David Appel
	Amir Ibrahim
	Bhimanagouda Patil
Head of Department,	Dan Lineberger

May 2019

Major Subject: Plant Breeding

ABSTRACT

Three studies were conducted on peaches *Prunus persica* (L.) Batsch]. The first was to identify QTLs and SNP haplotypes linked to the predictive SNP marker(s) for six traits. The second was to assess peaches for postharvest durability, and the third was to screen peach seedlings for susceptibility to bacterial leaf spot (BLS).

To identify QTLs, seven F_1 peach families were phenotyped for bloom date (BD), ripe date (RD), fruit development period (FDP), blush (BL), soluble solids content (SSC), and titratable acidity (TA) over two years in Texas and California and genotyped with the 9K SNP Illumina array. One QTL for RD and FDP was co-localized at the central part of LG4 (40 – 44 cM) with ~35 % phenotypic variance explained (PVE). Three QTLs were discovered for DB on LG1 (88 – 92 cM), LG4 (48 – 50 cM), and LG7 (40 – 44 cM), with 17 - 94%, 11 - 55%, and 11 - 18% PVE, respectively. One QTL on the central part of LG4 was found for BL (42 – 44 cM) with ~20 % PVE. A major QTL for TA co-localized with the *D*-locus at the proximal end of LG5 (0 - 0 cM) with ~60 % PVE. A QTL at the distal end of LG5 (52 - 62 cM) was associated with both TA and SSC with ~15 % PVE. Unique SNP haplotypes associated with the predictive SNP marker(s) of desired QTL alleles along with their original sources were identified.

Thirty-five peach and nectarine genotypes grown in Texas were assessed for internal breakdown over three years (2016 - 2018) by measuring expressible juice, mealiness, and texture. Mealiness developed in TX3C394N, ‘Royal Zest Two’, ‘Texstar’, ‘Royal Zest Three’, TX3B376LWP and ‘Harvester’ fruits after storing them for two weeks at 5° C, whereas ‘Flavorrich’, ‘White Delight One’, and TX2D357LW genotypes performed well in storage. Expressible juice, mealiness, and texture were well correlated.

Bacterial leaf spot incidence assessed using field evaluation and lab assessments indicated that the seven peach families were susceptible to BLS. The disease severity in the field was greatest in years with more total and more frequent rains.

DEDICATION

I dedicate this dissertation to my parents who raised me, loved me, and taught me but could not see this work completed. Also, this work is dedicated to my beloved husband, Maad Rawandoozi, who has been a constant and unconditional source of encouragement and support during the challenges and stressful times. I am very thankful to have him in my life. To my beautiful daughters Dalia and Shams, and my son Zaid for their patience and support, I love you all.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor and mentor Dr. David Byrne for his support, patience, guidance, and inspiration throughout my time in his program. I will carry all his scientific, professional, and life lessons with me throughout my future career and life.

Appreciation is extended to Dr. David Appel, Dr. Amir Ibrahim Dr. Bhimu Patil for serving as a committee member, and for their valuable input and guidance. Special recognition is owed to Dr. David Appel for his help and support that made the bacterial leaf spot project possible, and for allowing and facilitating my work in his lab.

I am thankful to my colleague Tim Hartmann for collecting and providing the phenotyping data used in the QTL analysis. In addition, I am grateful for Dr. Patil for opening his lab for me, providing materials, and facilitating my work for measuring titratable acidity.

I would like to thank the RosBREED project and the Department of Horticultural Sciences for their provision through funding and all other support. Furthermore, many thanks to all colleagues and professionals I met and learned from through my participation in the RosBREED project. Thanks to Dr. Amy Iezzoni, Dr. Ksenija Gasic, Dr. Nahla Bassil, Dr. Cameron Peace, Lichun Cai, Cassia da Silva Linge, and Laima Antanaviciute.

Special thanks go out to Jonathan Motloch at the Department of Plant Pathology and Microbiology at Texas A&M University for preparing media, culture, and inoculum suspension. Moreover, I would like to thank the Texas Plant Disease Diagnostic Lab, particularly, Jake Ueckert who helped in bacterial identification.

My sincere thanks go out to all those who helped me perform the FlexQTL analysis. This includes Dr. Eric van de Weg, the co-developer of the software, without his guidance, the

successful FlexQTL and haplotype results in this dissertation would not have been achieved. Furthermore, my special thanks to Dr. Amir Ibrahim who guided me with the statistical analysis for this work.

I would like to thank all colleagues and friends at the Department of Horticultural Sciences, particularly Ellen Young, Jeekin Lau, Stella King, and Jade Wu, for their assistance, support, and encouragement.

Thanks to Natalie Anderson for providing me with all the materials that I needed to conduct the phenotyping. Special thanks to Pamela Hornby who maintained the peach seedlings over the years of this work.

Finally, many thanks to my family for giving me the strength and encouragement to succeed. I am especially indebted to my husband Maad.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Drs. David Byrne, Bhimanagouda Patil of the Department of Horticultural Sciences, Dr. Amir Ibrahim of the Department of Soil and Crop Sciences, and Dr. David Appel of the Department of Plant Pathology and Microbiology.

Phenotypic data in Chapters II & III was provided by Dr. David Byrne and Tim Hartmann of the Department of Horticultural Sciences, and the genotypic data was provided by the RosBREED project.

The Visual FlexQTL software and license were provided by Dr. Eric van de Weg of the Wageningen University & Research, Netherlands.

Funding Sources

This work was made possible in part by the USDA's National Institute of Food and Agriculture (NIFA) Specialty Crop Research Initiative projects, RosBREED".

CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The overall objective of this dissertation is to improve fruit quality and phenological traits across low-medium chill peach germplasm through 1) identifying quantitative trait loci (QTLs) of six important phenological and fruit quality traits including bloom date, ripe date, fruit development period, soluble solids content, titratable acidity, and blush, through pedigree-based analysis (PBA) on Texas peach/nectarine germplasm; 2) estimating QTL genotypes for important breeding parents of seven full-sib families; 3) identifying the haplotype alleles that are linked to the predictive SNP marker(s) of desired QTL alleles and their origin sources; 4) implementing a standardized phenotyping protocol for postharvest traits in peaches; and 5) assessing the sensitivity of seven F_1 peach populations to bacterial leaf spot disease.

Background

Peaches and nectarines [*Prunus persica* (L.) Batsch] are deciduous fruit trees belonging to the Rosaceae family and are native to China. Peach is the third-most important temperate tree fruit species after apple and pear (FAO, 2018). They can grow in a wide range of environments but must achieve a chilling requirement to fruit. Currently, the largest producers of peaches are China, Italy, and the United States. California followed by South Carolina, Georgia, and New Jersey are the main peach producing states in the United States. California is the dominant state for producing fresh and processing peaches, whereas the other states mostly produce the fresh market peaches (NASS, 2018). The estimated gross production value of peaches and nectarines in the United States was $653 million compared to $21,687 million in the world (FAO, 2013).

Peaches are clonally propagated by budding or grafting to regionally adapted rootstocks. A commercial peach tree generally begins producing after 2 – 3 years and produces for 10 – 25 years. For a tree crop, the peach has a relatively fast breeding cycle and small genomic size that has led to it being used as a model organism for the Rosaceae family (Carrasco et al., 2013).

Peaches are soft-fleshed fruits composed of about 87% water. Thus, peaches are highly perishable and have a limited market life. In addition, they are attractive to consumers, since fruits contain organic acids, pigments, carbohydrates, phenolics, antioxidants, vitamins, and very small amounts of lipids and proteins (Kader and Mitchell, 1989; USDA, 2003). Researches have shown that phytochemicals in peach fruits (carotenoids, anthocyanins, and phenolic compounds) have potential antioxidant properties for preventing various diseases such as atherosclerosis, inflammation, cancers, and others (Cevallos-Casals et al., 2006; Gil et al., 2002; Prior and Cao, 2000; Tomas-Barberan et al., 2001; Vizzotto et al., 2007; Wargovich, 2000). Phenolic compounds have a wide range of human health benefits such as reducing the incidence of some chronic diseases such as diabetes, cardiovascular disease, and cancers. Additionally, some of these compounds have a significant potency as anti-obesity and anti-inflammatory substances and reduce the risk of metabolic syndrome (Lin et al., 2016). Noratto et al. (2009) reported that phytochemicals in peaches and plums inhibited the cell proliferation for estrogen-receptor negative breast cancer. Byrne et al. (2009) also mentioned that peach, plum, and nectarine varieties have anticancer properties against colon and prostate cancer cell lines.

Peaches are commercially harvested before full ripeness and stored at cold temperatures until they go to market because they deteriorate quickly at ambient temperature. Low-temperature storage between 0 to 5° C is recommended to reduce the ripening processes and

decay development during storage and/or shipment (Crisosto et al., 1999; Lurie and Crisosto, 2005).

The main postharvest loss of peaches is due to decay and chilling injury (CI), also known as internal breakdown (IB) (Ceponis et al., 1987; Kader and Mitchell, 1989). CI develops more rapidly when fruits of susceptible cultivars are kept at temperatures between 2.2° C and 7.6° C (Kader and Mitchell, 1989). There are many symptoms of CI such as mealiness (the lack of juice in the fruit), flesh or pit cavity browning, flesh translucency radiating from the pit, and red pigment development in the flesh of some cultivars (Crisosto and Labavitch, 2002). Orchard management practices such as fertilization, irrigation, tree thinning, canopy architecture, and cultivar selection play a significant role in determining postharvest qualities, (Crisosto et al., 1997). Commercial practices such as calcium applications, controlled atmosphere (CA) storage (Anderson, 1979; Garner et al., 2001; Nanos and Mitchell, 1991), modified atmosphere packaging (MAP), plant growth regulators (PGRs), controlled delayed cooling, and intermittent warming (IW) (Zhou et al., 1999; 2000) have been employed to reduce postharvest deterioration.

Important Traits of Improvement in Breeding Programs

Throughout the world, there are hundreds of peach and nectarine cultivars used commercially (Brooks and Olmo, 1997; Okie, 1998; Wang, 2002). Peach breeding programs around the world work to accomplish objectives such as specific environmental adaptation, disease resistance, extended harvest periods to expand marketing season, enhanced fruit quality (shape, color, flavor, aroma, texture, and firmness), and improved postharvest life. However, all breeding programs have a common focus to produce new and improved cultivars (Infante et al., 2006). Byrne et al. (2012) suggested that the most significant achievements in peach breeding have been the expansion of its adaptation, the extension of the harvest period, and the

diversification of its market. The development of early ripe and low-chill peaches and nectarine cultivars, an active area of breeding, is driven by the desire to have year-round fruit available. Other work has resulted in cultivars that are resistant to bacterial leaf and fruit spot (Byrne, 2005; Byrne et al., 2000).

In the last decade, the rate of fresh peach consumption has decreased from 2.3 to 1.3 kg per capita per year in the U.S. (USDA, 2018). Low fruit quality is the main factor limiting fresh consumption (Crisosto et al., 1999; Lurie and Crisosto, 2005). According to consumer surveys, the primary complaints of peach consumers are poor firmness, lack of aroma and flavor, low sweetness, non-ripening fruit and the lack of consistent quality (Crisosto and Kader, 2000; Scorza et al., 2004).

Several reasons may be attributed to this issue including harvesting at immature stages for storage and shipping reasons (Crisosto, 2002; Crisosto and Valero, 2008; Fideghelli et al., 1998; Sansavini et al., 2006), deficiency of postharvest handling protocol, and the focus on yield rather than quality traits by growers, and the emphasis by breeders on external quality for developing new cultivars rather than the internal quality of fruits (Crisosto et al., 2006).

Agronomic practices can enhance postharvest qualities in the short term, but for long-term improvement, peach programs should focus on characterizing and improving the genetic basis of flavor, chilling injury, and antioxidant pathways (Crisosto, 2006). Selection for qualitative traits is complicated since these traits (firmness, fruit size, skin color, SSC and flavor) have polygenic control and are influenced by the environment. It is hard to determine the number of minor genes involved in the expression of these characters (Della Strada et al., 1996).

Traditional fruit breeding is a long-term process. Fruit trees often have long juvenile periods and require many years from planting trees to the first fruiting for traits to be evaluated.

Frequently it takes 15 to 20 years to develop a novel cultivar that satisfies consumer demands. Peach breeders need a minimum of three years from the first fruiting season until quality phenotypes can be assessed (Dirlewanger et al., 2006; Dirlewanger et al., 2004; Dirlewanger et al., 1998). Then they need between 10 - 20 years for data analysis, selection, and regional testing before releasing a new cultivar. Moreover, planting trees in the permanent orchard requires large areas of land and maintenance. Therefore, breeding perennial crops is a challenging task but marker-assisted breeding (MAB) in the form of marker-assisted parent selection (MAPS), would help in crossing decision by a quick genotypic screening of favorable alleles of selected parents. Later, marker-assisted seedling selection (MASS) can be used to screen for desired seedlings to be kept and grown in the field (Collard et al., 2005). That would provide great advantages to overcome the obstacles of traditional breeding methods.

Breeding programs can utilize molecular markers to identify superior parents, to improve the selection of elite alleles for essential traits at loci, and stack desired alleles at multiple loci (Bliss, 2010; Testolin and Cipriani, 2010).

Many molecular marker systems such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), simple sequence repeats (SSRs), and recently, single nucleotide polymorphisms (SNPs) have been used in peaches for the identification of markers linked to traits of interest (Chaparro et al., 1994; da Silva Linge et al., 2015; Dettori et al., 2001; Dirlewanger et al., 1998; Frett et al., 2014; Joobeur et al., 1998; Nuñez-Lillo et al., 2015; Quarta et al., 1998; Sosinski et al., 1998; Verde et al., 2005; Yang et al., 2013b).

DNA-based markers have commonly been used for linkage map construction, quantitative trait loci (QTL) identification, mapping, fine mapping, candidate gene identification,

and ultimately marker-assisted selection (MAS) (Bliss, 2010). In the last two decades, abundant genetic maps of important crops have been created, including peach (Cantín et al., 2009; Dirlewanger et al., 2006; Eduardo et al., 2011; Sosinski et al., 2009). Most recently, the International Peach Sequence Consortium (IPSC 9K) array (Verde et al., 2012) was used to develop high-quality genetic linkage maps (da Silva Linge et al., 2015; Frett, 2016; Nuñez-Lillo et al., 2015; Yang et al., 2013b).

QTLs of soluble solids content have been mapped to linkage groups (LGs) 2, 3, 4, 5, and 6 (Abbott et al., 1998; Cantín et al., 2010; Eduardo et al., 2011; Etienne et al., 2002; Hernández Mora et al., 2017; Quilot et al., 2004) and QTLs for organic acids have been mapped to LGs 1, 2, 4, 5, and 6 (Cantín et al., 2010; Dirlewanger et al., 1999; Etienne et al., 2002; Hernández Mora et al., 2017). QTLs associated with chilling injury and maturity date have discovered on different LGs with diverse levels of reliability (Cantín et al., 2010; Hernández Mora et al., 2017; Ogundiwin et al., 2009; Peace et al., 2005).

The USDA funded project RosBREED (http://www.rosbreed.org) involves the collaboration of various breeders and geneticists identifying QTLs and markers linked to fruit quality traits. Further QTLs and tighter linkages are uncovered through more markers being used in collaboration (de Pascual-Teresa et al., 2010; Dirlewanger et al., 1999), and allowing wider adaptation of seedling selection and marker-assisted parents, which helps breeders produce higher quality peaches that meet consumer needs. This method will save space, time, and money (Bliss, 2010).

Standardized Phenotyping for Peach Post-harvest Quality Traits

Most of the important quality traits in peaches are quantitatively inherited and genetically controlled. Phenotyping is essential for QTL analysis to connect genetic variation with biological functions (Iezzoni et al., 2010). Therefore, the lack of phenotypic documentation hampers the association between genotypic and phenotypic data, and thus negatively impacts QTL detection. The protocols for phenotyping quality traits should be designated and standardized across private and public breeding programs to overcome this obstacle (Iezzoni et al., 2010). Therefore, collaborations among peach breeding programs are needed to develop the standardized phenotypic protocol for quality traits (Volk, 2010).

The RosBREED project has created a standardized phenotyping protocol for commercially important peach traits and coordinated the phenotypic data collection across the four U.S. peach breeding programs in California, South Carolina, Texas, and Arkansas (Frett et al., 2012). This protocol facilitated PBA and the detection of molecular markers connected to QTLs that control complex fruit quality traits (Bink, 2005; Bink et al., 2008; 2014).

Bacterial Leaf Spot Disease

Bacterial leaf spot (BLS) caused by *Xanthomonas arboricola* pv. *pruni* (Smith), also called Xap, was first described in North America in 1902 on plum trees (Smith, 1903). It is a significant disease in peach and nectarine fruits and is widespread in warm and humid, and windy areas (Werner et al., 1986). The eastern region of the United States has more problems with this disease than does the western states (NASS, 2004).

The symptoms appear as small, circular or irregular, pale-green areas with a light-tan center on the lower surface of leaves (EPPO, 2006). As the spots enlarge, they become angular and darken to a brown, or black visible on the upper surface of the leaves. A yellow halo may

develop around the diseased tissue. Often these spots concentrate on leaf tips where water from rain and dew commonly accumulate (EPPO, 2006). Bacterial leaf spot can cause severe defoliation on susceptible cultivars and a decrease in fruit quality and production.

Bactericides such as copper-based compounds and the antibiotic oxytetracycline are the traditional method for controlling bacterial spot. However, peach foliage is very sensitive to copper compounds and severe phytotoxicity may occur if misused. Furthermore, it is difficult to control the disease when established in the orchard, particularly for susceptible varieties (Ritchie, 1995). Extensive screening of *Prunus* for resistance was done in North America (Scorza and Sherman, 1996; Yang et al., 2013b) and Brazil (Raseira and Nakasu, 1998). Peach cultivars such as 'Candor' and 'Clayton' are resistant to Xap (Keil and Fogle, 1974; Kretzschmar et al., 1998), although, leaves and fruits have shown variable range of resistance to bacterial leaf spot (Keil and Fogle, 1974; Rom and Moore, 1979; Werner et al., 1986). However, no cultivar is immune to the pathogen, it is possible that under high pathogen pressure, resistance will be overcome by the disease (Werner et al., 1986).

Several methods have been used for evaluating bacterial spot incidence. These include field evaluation (Hansche, 1983; Yang, 2012), greenhouse evaluation by immersing leaves in inoculum (Daines and Hough, 1951; Topp et al., 1993) or infiltrating by high pressure sprays (Civerolo and Keil, 1976; du Plessis, 1986), and laboratory evaluation (detached-leaf bioassay) by infiltrating with a needleless syringe (du Plessis, 1986; Frett, 2016; Hammerschlag, 1988; Randhawa and Civerolo, 1985; Topp et al., 1993).

Traditional breeding for resistant cultivars is challenging because it is difficult to phenotype the level of resistance in the field and resistance appears to be polygenic in nature

(Yang et al., 2010). Molecular marker tools have been employed for understanding the genetic control of complex traits and increasing the efficiency of traditional breeding.

A putative QTL associated to leaf resistance to Xap was detected on LG4 in peach, however, the low-density linkage map limited the discovery of other QTLs with major effects (Yang et al., 2013a; 2013b).

Mapping Techniques

In the last two decades, several methods of QTL mapping have been developed. These include least square (LS) or maximum likelihood (ML) estimation and, most recently, Bayesian methods (Soller et al., 1976). Single-marker analysis, also called single-point analysis, is the basic method for detecting QTLs associated with single markers because it does not require a linkage map and can be performed with a basic statistical test including t-test, analysis of variance (ANOVA), and linear regression (Collard et al., 2005). The simple interval mapping (SIM) method was first proposed by Lander and Botstein in 1989. It takes advantages of the linkage map and it evaluates intervals between a pair of adjacent marker loci (flanking) for the presence of a QTL (Lander and Botstein, 1989; Liu, 1997). The map position of a QTL is determined as the maximum likelihood from the distribution of likelihood values. Two problems may result from this approach; 1) sampling variance will be affected by each additional QTL, and 2) the combined effects of linked QTLs will result in biased estimates. Composite Interval Mapping (CIM) was developed by Jansen and Stam in 1994 and it combines interval mapping with multiple regression analysis that makes it the more precise and effective method because it reduces the bias in estimating the effect of multiple linked QTLs (Sehgal et al., 2016).

Pedigree-based analysis (PBA) using the Bayesian approach is a useful approach to identify and/or validate QTLs in breeding germplasm (Bink et al., 2014; Peace et al., 2014). This

method offers the capability to study multiple full-sib families with known pedigrees simultaneously (Bink et al., 2014; Bink et al., 2012). The use of multiple populations overcomes the issues that usually are present in classical QTL analysis by using a single population. A larger genetic background increases the ability to detect minor and major QTLs associated with a trait across breeding programs (Yu and Buckler, 2006). The Bayesian approach implemented in FlexQTL software (Bink et al., 2002), has been used on highly heterozygous clonally propagated crops including peach, apple, strawberry and sweet cherry (Fresnedo-Ramírez et al., 2015; Fresnedo-Ramirez et al., 2016; Roach et al., 2016; Rosyara et al., 2013). It calculates heritability, genetic variance parameters, and breeding values, and allows the tracing back of QTL alleles through several generations of the pedigree.

References

Abbott, A.G., S. Rajapakse, B. Sosinski, Z.X. Lu, K. Sossey-Alaoui, M. Gannavarapu, G. Reighard, R.E. Ballard, W.V. Baird, R. Scorza, and A. Callahan. 1998. Construction of saturated linkage maps of peach crosses segregating for characters controlling fruit quality, tree architecture and pest resistance. *Acta Hort.* 465:41-49.

Anderson, R.E. 1979. The influence of storage temperature and warming during storage on peach and nectarine fruit quality [chilling injury, internal breakdown]. *J. Amer. Soc. Hort. Sci.* 104:459-461.

Bink, M.C.A.M. 2005. FlexQTL software: Efficient estimation of identity by descent probabilities and QTL mapping in pedigreed populations, Plant and Animal Genomes XIII Conference, San Diego, CA, USA.

Bink, M.C.A.M., M.P. Boer, C.J.F. ter Braak, J. Jansen, R.E. Voorrips, and W.E. van de Weg. 2008. Bayesian analysis of complex traits in pedigreed plant populations. *Euphytica* 161:85-96.

Bink, M.C.A.M., J. Jansen, M. Madduri, R.E. Voorrips, C.E. Durel, A.B. Kouassi, F. Laurens, F. Mathis, C. Gessler, D. Gobbin, F. Rezzonico, A. Patocchi, M. Kellerhals, A. Boudichevskaia, F. Dunemann, A. Peil, A. Nowicka, B. Lata, M. Stankiewicz-Kosyl, K. Jeziorek, E. Pitera, A. Soska, K. Tomala, K.M. Evans, F. Fernandez-Fernandez, W. Guerra, M. Korbin, S. Keller, M. Lewandowski, W. Plocharski, K. Rutkowski, E. Zurawicz, F. Costa, S. Sansavini, S. Tartarini, M. Komjanc, D. Mott, A. Antofie, M. Lateur, A. Rondia, L. Gianfranceschi, and W.E. van de Weg. 2014. Bayesian QTL analyses using pedigreed families of an outcrossing species, with application to fruit firmness in apple. *Theor. Appl. Genet.* 127:1073-1090.

Bink, M.C.A.M., L.R. Totir, C.J.F. Braak, C.R. Winkler, M.P. Boer, and O.S. Smith. 2012. QTL linkage analysis of connected populations using ancestral marker and pedigree information. *Theor. Appl. Genet.* 124:1097-1113.

Bink, M.C.A.M., P. Uimari, M. Sillanpää, L. Janss, and R. Jansen. 2002. Multiple QTL mapping in related plant populations via a pedigree-analysis approach. *Theor. Appl. Genet.* 104:751-762.

Bliss, F.A. 2010. Marker-assisted breeding in horticultural crops. *Acta Hort.* 859:339-350.

Brooks, R.M. and H.P. Olmo. 1997. Register of fruit and nut varieties. 3rd ed. ASHS Press, Alexandria, Virginia, USA.

Byrne, D., G.D. Noratto, L. Cisneros-Zevallos, W. Porter, and M. Vizzotto. 2009. Health benefits of peach, nectarine and plums. *Acta Hort.* 841:267-273.

Byrne, D.H. 2005. Trends in stone fruit cultivar development. *HortTechnol.* 15:494-500.

Byrne, D.H., M.B. Raseira, D. Bassi, M.C. Piagnani, K. Gasic, G.L. Reighard, M.A. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: L.M. Badenes and H.D. Byrne (eds.), Fruit Breeding. Springer US, Boston, MA.

Byrne, D.H., W.B. Sherman, and T.A. Bacon. 2000. Stone Fruit Genetic Pool and Its Exploitation for Growing under Warm Winter Conditions, p. 157-230. In: A. Erez (ed.), Temperate Fruit Crops in Warm Climates. Springer Netherlands, Dordrecht.

Cantín, C.M., C.H. Crisosto, E.A. Ogundiwin, T. Gradziel, J. Torrents, M.A. Moreno, and Y. Gogorcena. 2010. Chilling injury susceptibility in an intra-specific peach [*Prunus persica* (L.) Batsch] progeny. *Postharvest Biol. Tec.* 58:79-87.

Cantín, C.M., Y. Gogorcena, and M.A. Moreno. 2009. Analysis of phenotypic variation of sugar profile in different peach and nectarine *Prunus persica* (L.) Batsch breeding progenies. *J. Sci. Food Agr.* 89:1909-1917.

Carrasco, B., L. Meisel, M. Gebauer, R. Garcia-Gonzales, and H. Silva. 2013. Breeding in peach, cherry and plum: from a tissue culture, genetic, transcriptomic and genomic perspective. *Biol. Res.* 46:219-230.

Ceponis, M.J., R.A. Cappellini, J.M. Wells, and G.W. Lightner. 1987. Disorders in plum, peach, and nectarine shipments to the new york market, 1972-1985. *Plant Dis.* 71:947-952.

Cevallos-Casals, B.A., D. Byrne, W.R. Okie, and L. Cisneros-Zevallos. 2006. Selecting new peach and plum genotypes rich in phenolic compounds and enhanced functional properties. *Food Chem.* 96:273-280.

Chaparro, J.X., D.J. Werner, D. Omalley, and R.R. Sederoff. 1994. Targeted mapping and linkage analysis of morphological isozyme, and RAPD markers in peach. *Theor. Appl. Genet.* 87:805-815.

Civerolo, E.L. and H.L. Keil. 1976. Evaluation of *Prunus* spp resistance to *Xanthomonas pruni* by artificial inoculation. *Fruit Var. J.* 30:17-18.

Collard, B.C.Y., M.Z.Z. Jahufer, J.B. Brouwer, and E.C.K. Pang. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* 142:169-196.

Crisosto, C.H. 2002. How do we increase peach consumption? *Acta Hort.* 592:601-605.

Crisosto, C.H. 2006. Peach quality and postharvest technology. Acta Hort. 713:479-488.

Crisosto, C.H., G.M. Crisosto, G. Echeverria, and J. Puy. 2006. Segregation of peach and nectarine (*Prunus persica* (L.) Batsch) cultivars according to their organoleptic characteristics. *Postharvest Biol. Tec.* 39:10-18.

Crisosto, C.H., D. Garner, L. Cid, and K. Day. 1999. Peach size affects storage, market life. *Calif. Agr.* 53:33-36.

Crisosto, C.H., R.S. Johnson, T. DeJong, and K.R. Day. 1997. Orchard factors affecting postharvest stone fruit quality. *HortScience* 32:820-823.

Crisosto, C.H. and A.A. Kader. 2000. Peach, Postharvest quality maintenance guidelines. *Department of Pomology. University of California. Davis*:1-10.

Crisosto, C.H. and J.M. Labavitch. 2002. Developing a quantitative method to evaluate peach (*Prunus persica*) flesh mealiness. *Postharvest Biol. Tec.* 25:151-158.

Crisosto, C.H. and D. Valero. 2008. Harvesting and postharvest handling of peaches for the fresh market, p. 575-596. In: D.R. Layne and D. Bassi (eds.), The Peach: Botany, Production and Uses. CABI, Wallingford, Oxfordshire, U.K.

da Silva Linge, C., D. Bassi, L. Bianco, I. Pacheco, R. Pirona, and L. Rossini. 2015. Genetic dissection of fruit weight and size in an F_2 peach (*Prunus persica* (L.) Batsch) progeny. *Mol. Breeding* 35.

Daines, R.H. and L.F. Hough. 1951. Artificial inoculation of peach seedlings with *Xanthomonas pruni*. *Phytopathol.* 41:8-9.

de Pascual-Teresa, S., D.A. Moreno, and C. Garcia-Viguera. 2010. Flavanols and anthocyanins in cardiovascular health: A review of current evidence. *Intl. J. Mol. Sci.* 11:1679-1703.

Della Strada, G., C. Fideghelli, and F. Grassi. 1996. Peach and nectarine cultivars introduced in the world from 1980 to 1992. *Acta Hort.* 374:43-51.

Dettori, M.T., R. Quarta, and I. Verde. 2001. A peach linkage map integrating RFLPs, SSRs, RAPDs, and morphological markers. *Genome* 44:783-790.

Dirlewanger, E., P. Cosson, K. Boudehri, C. Renaud, G. Capdeville, Y. Tauzin, F. Laigret, and A. Moing. 2006. Development of a second-generation genetic linkage map for peach [*Prunus persica* (L.) Batsch] and characterization of morphological traits affecting flower and fruit. *Tree Genet. & Genomes* 3:1-13.

Dirlewanger, E., E. Graziano, T. Joobeur, F. Garriga-Calderé, P. Cosson, W. Howad, and P. Arús. 2004. Comparative mapping and marker-assisted selection in Rosaceae fruit crops. *Proc. Natl. Acad. Sci. United States Amer.* 101:9891-9896.

Dirlewanger, E., A. Moing, C. Rothan, L. Svanella, V. Pronier, A. Guye, C. Plomion, and R. Monet. 1999. Mapping QTLs controlling fruit quality in peach (*Prunus persica* (L.) Batsch). *Theor. Appl. Genet.* 98:18-31.

Dirlewanger, E., V. Pronier, C. Parvery, C. Rothan, A. Guye, and R. Monet. 1998. Genetic linkage map of peach [*Prunus persica* (L.) Batsch] using morphological and molecular markers. *Theor. Appl. Genet.* 97:888-895.

du Plessis, H.J. 1986. Systemic migration and establishment of *Xanthomonas campestris* pv. *pruni* in plum leaves and twigs. *J. Phytopathol.* 116:221-227.

Eduardo, I., I. Pacheco, G. Chietera, D. Bassi, C. Pozzi, A. Vecchietti, and L. Rossini. 2011. QTL analysis of fruit quality traits in two peach intraspecific populations and importance of maturity date pleiotropic effect. *Tree Genet. & Genomes* 7:323-335.

EPPO. 2006. *Xanthomonas arboricola* pv. *pruni*. *EPPO Bulletin (European and Mediterranean Plant Protection Organization)* 36:129-133.

Etienne, C., C. Rothan, A. Moing, C. Plomion, C. Bodénès, L. Svanella-Dumas, P. Cosson, V. Pronier, R. Monet, and E. Dirlewanger. 2002. Candidate genes and QTLs for sugar and organic acid content in peach [*Prunus persica* (L.) Batsch]. *Theor. Appl. Genet.* 105:145-159.

FAO. 2013. The Statistics Division of the Food and Agriculture Organization of the United Nations (FAOSTAT). http://faostat3.fao.org/home/E. Apr. 15, 2018.

FAO. 2018. The Statistics Division of the Food and Agriculture Organization of the United Nations (FAOSTAT). http://www.fao.org/faostat/en/#data/QC. Apr. 15, 2018.

Fideghelli, C., G. Della Strada, F. Grassi, and G. Morico. 1998. The peach industry in the world: Present situation and trend. *Acta Hort.* 465:29-40.

Fresnedo-Ramírez, J., M.C.A.M. Bink, E. van de Weg, T.R. Famula, C.H. Crisosto, T.J. Frett, K. Gasic, C.P. Peace, and T.M. Gradziel. 2015. QTL mapping of pomological traits in peach and related species breeding germplasm. *Mol. Breeding* 35:1-19.

Fresnedo-Ramirez, J., T.J. Frett, P.J. Sandefur, A. Salgado-Rojas, J.R. Clark, K. Gasic, C.P. Peace, N. Anderson, T.P. Hartmann, D.H. Byrne, M. Bink, E. van de Weg, C.H. Crisosto, and T.M. Gradziel. 2016. QTL mapping and breeding value estimation through pedigree-based analysis of fruit size and weight in four diverse peach breeding programs. *Tree Genet. & Genomes* 12:25.

Frett, T.J. 2016. Genetic determinism of *Xanthomonas arboricola* pv. *pruni* (Xap) resistance, fruit quality, and phenological traits in peach and incorporation of marker-assisted selection (MAS) in the University of Arkansas peach and nectarine breeding program. University of Arkansas, Fayetteville, AK, Ph.D Dissertation.

Frett, T.J., K. Gasic, J.R. Clark, D. Byrne, T. Gradziel, and C. Crisosto. 2012. Standardized phenotyping for fruit quality in peach [*Prunus persica* (L.) Batsch]. *J. Amer. Pomol. Soc.* 66:214-219.

Frett, T.J., G.L. Reighard, W.R. Okie, and K. Gasic. 2014. Mapping quantitative trait loci associated with blush in peach [*Prunus persica* (L.) Batsch]. *Tree Genet. & Genomes* 10:367-381.

Garner, D., C.H. Crisosto, and E. Otieza. 2001. Controlled atmosphere storage and aminoethoxyvinyl- glycine postharvest dip delay post cold storage softening of 'Snow King' peach. *HortTechnol.* 11:598-602.

Gil, M.I., F.A. Tomas-Barberan, B. Hess-Pierce, and A.A. Kader. 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *J. Agr. Food Chem.* 50:4976-4982.

Hammerschlag, F.A. 1988. Selection of peach cells for insensitivity to culture filtrates of *Xanthomonas campestris* pv *pruni* and regeneration of resistant plants. *Theor. Appl. Genet.* 76:865-869.

Hansche, P.E. 1983. Response to selection, p. 154-171. In: J. J. and M.J. N. (eds.), Methods in Fruit Breeding. Purdue University Press, West Lafayette, USA.

Hernández Mora, J.R., D. Micheletti, M. Bink, E. Van de Weg, C. Cantín, N. Nazzicari, A. Caprera, M.T. Dettori, S. Micali, E. Banchi, J.A. Campoy, E. Dirlewanger, P. Lambert, T. Pascal, M. Troggio, D. Bassi, L. Rossini, I. Verde, B. Quilot-Turion, F. Laurens, P. Arús, and M.J. Aranzana. 2017. Integrated QTL detection for key breeding traits in multiple peach progenies. *BMC Genomics* 18:404.

Iezzoni, A., C. Weebadde, J. Luby, C.Y. Yue, E. van de Weg, G. Fazio, D. Main, C.P. Peace, N.V. Bassil, and J. McFerson. 2010. RosBREED: Enabling marker-assisted breeding in rosaceae. *Acta Hort.* 859:389-394.

Infante, R., C. Meneses, and D.H. Byrne. 2006. Present situation of peach breeding programs: Post harvest and fruit quality assessment. *Acta Hort.* 713:121-124.

Joobeur, T., M.A. Viruel, M.C. de Vicente, B. Jáuregui, J. Ballester, M.T. Dettori, I. Verde, M.J. Truco, R. Messeguer, I. Batlle, R. Quarta, E. Dirlewanger, and P. Arús. 1998. Construction of a saturated linkage map for *Prunus* using an almond×peach F_2 progeny. *Theor. Appl. Genet.* 97:1034-1041.

Kader, A.A. and F.G. Mitchell. 1989. Postharvest physiology, p. 158-164. In: J.H. LaRue and R.S. Johnson (eds.), Peaches, Plums, and Nectarines: Growing and Handling for Fresh Market. Cooperative Extension, University of California, Division of Agriculture and Natural Resources, Oakland, CA.

Keil, H. and H. Fogle. 1974. Orchard susceptibility of some apricot, peach, and plum cultivars and selections to *Xanthomonas pruni*. *Fruit Var. J.* 28:16-19.

Kretzschmar, A., E. Rossetto, and O. Martins. 1998. Resistance of some peach cultivars to *Xanthomonas campestris* pv. *pruni* inoculated by infiltration of detached leaves. *Revista Brasileira de Fruticultura (Brazil)*. 20:213-219

Lander, E.S. and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genet.* 121:185-199.

Lin, D.R., M.S. Xiao, J.J. Zhao, Z.H. Li, B.S. Xing, X.D. Li, M.Z. Kong, L.Y. Li, Q. Zhang, Y.W. Liu, H. Chen, W. Qin, H.J. Wu, and S.Y. Chen. 2016. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules* 21:1374.

Liu, B.H. 1997. Statistical Genomics: Linkage, Mapping, and QTL Analysis. CRC press, Boca Raton, FL.

Lurie, S. and C.H. Crisosto. 2005. Chilling injury in peach and nectarine. *Postharvest Biol. Tec.* 37:195-208.

Nanos, G.D. and F.G. Mitchell. 1991. High-temperature conditioning to delay internal breakdown development in peaches and nectarines. *HortScience* 26:882-885.

NASS. 2004. Crop Profile for Peaches in Georgia and South Carolina. https://ipmdata.ipmcenters.org/documents/cropprofiles/GASCpeaches.pdf. Oct. 20, 2018.

NASS. 2018. Crop Production. http://usda.mannlib.cornell.edu/usda/nass/CropProd//2010s/2018/CropProd-08-10-2018.pdf. Oct. 20, 2018.

Noratto, G., W. Porter, D. Byrne, and L. Cisneros-Zevallos. 2009. Identifying peach and plum polyphenols with chemopreventive potential against estrogen-independent breast cancer cells. *J. Agr. Food Chem.* 57:5219-5226.

Nuñez-Lillo, G., A. Cifuentes-Esquivel, M. Troggio, D. Micheletti, R. Infante, R. Campos-Vargas, A. Orellana, F. Blanco-Herrera, and C. Meneses. 2015. Identification of candidate genes associated with mealiness and maturity date in peach [*Prunus persica* (L.) Batsch] using QTL analysis and deep sequencing. *Tree Genet. & Genomes* 11:86.

Ogundiwin, E.A., C.P. Peace, T.M. Gradziel, D.E. Parfitt, F.A. Bliss, and C.H. Crisosto. 2009. A fruit quality gene map of *Prunus*. *BMC Genomics* 10:587.

Okie, W.R. 1998. Handbook of peach and nectarine varieties: performance in the southeastern United States and index of names. Agriculture handbook number 714. USDA/ARS, Springfield, VA.

Peace, C.P., C.H. Crisosto, and T.M. Gradziel. 2005. Endopolygalacturonase: A candidate gene for Freestone and Melting flesh in peach. *Mol. Breeding* 16:21-31.

Peace, C.P., J.J. Luby, W.E. van de Weg, M.C.A.M. Bink, and A.F. Iezzoni. 2014. A strategy for developing representative germplasm sets for systematic QTL validation, demonstrated for apple, peach, and sweet cherry. *Tree Genet. & Genomes* 10:1679-1694.

Prior, R.L. and G.H. Cao. 2000. Antioxidant phytochemicals in fruits and vegetables: Diet and health implications. *HortScience* 35:588-592.

Quarta, R., M.T. Dettori, I. Verde, A. Gentile, and Z. Broda. 1998. Genetic analysis of agronomic traits and genetic linkage mapping in a BC1 peach population using RFLPs and RAPDs. *Acta Hort.* 465:51-60.

Quilot, B., B.H. Wu, J. Kervella, M. Génard, M. Foulongne, and K. Moreau. 2004. QTL analysis of quality traits in an advanced backcross between *Prunus persica* cultivars and the wild relative species *P. davidiana*. *Theor. Appl. Genet.* 109:884-897.

Randhawa, P.S. and E.L. Civerolo. 1985. A detached-leaf bioassay for *Xanthomonas campestris* pv *pruni*. *Phytopathol.* 75:1060-1063.

Raseira, M.C.B. and B.H. Nakasu. 1998. Cultivares: descrição e recomendação. In: C.A.B. Medeiros and M.C.B. Raseira (eds.), A Cultura do Pessegueiro. Embrapa-CPACT, Brasilia, Brasil.

Ritchie, D.F. 1995. Bacterial spot, p. 50-52. In: J.M. Ogawa, E.I. Zehr, G.W. Bird, D.F. Ritchie, K. Uriu, and J.K. Uyemoto (eds.), Compendium of Stone Fruit Diseases. APS Press, St. Paul, MN.

Roach, J.A., S. Verma, N.A. Peres, A.R. Jamieson, W.E. van de Weg, M. Bink, N.V. Bassil, S. Lee, and V.M. Whitaker. 2016. FaRXf1: a locus conferring resistance to angular leaf spot caused by *Xanthomonas fragariae* in octoploid strawberry. *Theor. Appl. Genet.* 129:1191-1201.

Rom, R.C. and J.N. Moore. 1979. Peach cultivar situation in the midwest and central south. *Fruit Var. J.* 33:25-29.

Rosyara, U.R., M. Bink, E. van de Weg, G.R. Zhang, D.C. Wang, A. Sebolt, E. Dirlewanger, J. Quero-Garcia, M. Schuster, and A.F. Iezzoni. 2013. Fruit size QTL identification and the

prediction of parental QTL genotypes and breeding values in multiple pedigreed populations of sweet cherry. *Mol. Breeding* 32:875-887.

Sansavini, S., A. Gamberini, and D. Bassi. 2006. Peach breeding, genetics and new cultivar trends. *Acta Hort.* 713:23-48.

Scorza, R., W. Anger, D. Peterson, K. Bett, E. Champagne, J. Beaulieu, and D.A. Ingram. 2004. Non-destructive evaluation of post-harvest peach fruit softening. *Acta Hort*. 663:269-274.

Scorza, R. and W.B. Sherman. 1996. Peaches, p. 325-440. In: J. Janick and J.N. Moore (eds.). Wiley, New York, NY, USA.

Sehgal, D., R. Singh, and V.R. Rajpal. 2016. Quantitative trait loci mapping in plants: Concepts and approaches, p. 31-59. In: V.R. Rajpal, S.R. Rao, and S.N. Raina (eds.), Molecular Breeding for Sustainable Crop Improvement, Vol 2. Springer International, Cham, Switzerland.

Smith, E.F. 1903. Observations on a Hitherto Unreported Bacterial Disease the Cause of which Enters the Plant Through Ordinary Stomata. *Science* 17:456-457.

Soller, M., T. Brody, and A. Genizi. 1976. On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. *Theor. Appl. Genet.* 47:35-39.

Sosinski, B., V. Shulaev, A. Dhingra, A. Kalyanaraman, R. Bumgarner, D. Rokhsar, I. Verde, R. Velasco, and A.G. Abbott. 2009. Rosaceaous Genome Sequencing: Perspectives and Progress, p. 601-615. In: K.M. Folta and S.E. Gardiner (eds.), Genetics and Genomics of Rosaceae. Springer New York, New York, NY.

Sosinski, B., K. Sossey-Alaoui, S. Rajapakse, K. Glassmoyer, R.E. Ballard, A.G. Abbott, Z.X. Lu, W.V. Baird, G. Reighard, A. Tabb, and R. Scorza. 1998. Use of AFLP and RFLP

markers to create a combined linkage map in peach *Prunus persica* (L.) Batsch for use in marker assisted selection. *Acta Hort.* 465:61-68.

Testolin, R. and G. Cipriani. 2010. Molecular Markers for Germplasm Identification and Characterization. *Acta Hort.* 859:59-72.

Tomas-Barberan, F.A., M.I. Gil, P. Cremin, A.L. Waterhouse, B. Hess-Pierce, and A.A. Kader. 2001. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. *J Agric Food Chem* 49:4748-4760.

Topp, B.L., W.B. Sherman, R.E. Stall, G.V. Minsavage, and C.J. Wilcox. 1993. Comparison of Greenhouse Methods for Assessing Resistance to Bacterial Leaf Spot in Plum. *J. Amer. Soc. Hort. Sci.* 118:667-671.

USDA. 2003. Composition of Foods: Fruits and Fruit Juices – Raw, Processed, Prepared. USDA Agriculture Handbook No. 8–9, US Department of Agriculture, Washington, DC.

Crisosto, C.H. 2006. Peach quality and postharvest technology. Acta Hort. 713:479-488.

FAO. 2013. The Statistics Division of the Food and Agriculture Organization of the United Nations (FAOSTAT). http://faostat3.fao.org/home/E. Apr. 15, 2018

Kretzschmar, A., E. Rossetto, and O. Martins. 1998. Resistance of some peach cultivars to *Xanthomonas campestris* pv. *pruni* inoculated by infiltration of detached leaves. Revista Brasileira de Fruticultura (Brazil) 20:213-219.

NASS. 2004. Crop Profile for Peaches in Georgia and South Carolina. https://ipmdata.ipmcenters.org/documents/cropprofiles/GASCpeaches.pdf. Oct. 20, 2018

NASS. 2018. Crop Production. http://usda.mannlib.cornell.edu/usda/nass/CropProd//2010s/2018/CropProd-08-10-2018.pdf. Oct. 20, 2018

USDA. 2018. Fruit and Tree Nut Yearbook Tables. https://www.ers.usda.gov/data-products/fruit-and-tree-nut-data/fruit-and-tree-nut-yearbook-tables/,. Oct. 10, 2018.

Verde, I., N. Bassil, S. Scalabrin, B. Gilmore, C.T. Lawley, K. Gasic, D. Micheletti, U.R. Rosyara, F. Cattonaro, E. Vendramin, D. Main, V. Aramini, A.L. Blas, T.C. Mockler, D.W. Bryant, L. Wilhelm, M. Troggio, B. Sosinski, M.J. Aranzana, P. Arús, A. Iezzoni, M. Morgante, and C. Peace. 2012. Development and Evaluation of a 9K SNP Array for Peach by Internationally Coordinated SNP Detection and Validation in Breeding Germplasm. *PLOS ONE* 7:e35668.

Verde, I., M. Lauria, M.T. Dettori, E. Vendramin, C. Balconi, S. Micali, Y. Wang, M.T. Marrazzo, G. Cipriani, H. Hartings, R. Testolin, A.G. Abbott, M. Motto, and R. Quarta. 2005. Microsatellite and AFLP markers in the *Prunus persica* [L. (Batsch)]×*P. ferganensis* BC_1 linkage map: saturation and coverage improvement. *Theor. Appl. Genet.* 111:1013-1021.

Vizzotto, M., L. Cisneros-Zevallos, D.H. Byrne, D.W. Ramming, and W.R. Okie. 2007. Large variation found in the phytochemical and antioxidant activity of peach and plum germplasm. *J. Amer. Soc. Hort. Sci.* 132:334-340.

Volk, G.M. 2010. Advantages for the use of standardized phenotyping in databases. *HortScience* 45:1310-1313.

Wang, Y. 2002. Peach, p. 135 - 156. In: Y. Wang (ed.), Genetic Resources of Deciduous Fruit and Nut Crops in China. China Agricultural Science and Technology Press, Beijing, China.

Wargovich, M.J. 2000. Anticancer properties of fruits and vegetables. *HortScience* 35:573-575.

Werner, D.J., D.F. Ritchie, D.W. Cain, and E.I. Zehr. 1986. Susceptibility of peaches and nectarines, plant introductions, and other *Prunus* species to bacterial spot. *HortScience* 21:127-130.

Yang, N. 2012. Mapping quantitative trait loci associated with resistance to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in peach Clemson University, Clemson, SC, Ph.D. dissertation.

Yang, N., G. Reighard, K. Gasic, D. Ritchie, and W. Okie. 2013a. Development of a genetic linkage map for identification of molecular markers associated with resistance to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in peach. *Acta Hort.* 976:561-566.

Yang, N., G. Reighard, D. Ritchie, W. Okie, and K. Gasic. 2013b. Mapping quantitative trait loci associated with resistance to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in peach. *Tree Genet. & Genomes* 9:573-586.

Yang, N., G. Righard, D. Ritchie, W. Okie, and K. Gasic. 2010. Construction of a genetic linkage map for identification of molecular markers associated with resistance to *Xanthomonas arboricola* pv. *pruni* in peach [*Prunus persica* (L.) Batsch]. *HortScience* 45:S304.

Yu, J.M. and E.S. Buckler. 2006. Genetic association mapping and genome organization of maize. *Current Opinion Biotechnol.* 17:155-160.

Zhou, H.-W., L. Sonego, R. Ben-Arie, and S. Lurie. 1999. Analysis of cell wall components in juice of `Flavortop' nectarines during normal ripening and woolliness development. *J. Amer. Soc. Hort. Sci.* 124:424-429.

Zhou, H.-W., L. Sonego, A. Khalchitski, R. Ben-Arie, A. Lers, and S. Lurie. 2000. Cell wall enzymes and cell wall changes in `Flavortop' nectarines: mRNA abundance, enzyme

activity, and changes in pectic and neutral polymers during ripening and in woolly fruit. *J. Amer. Soc. Hort. Sci.* 125:630-637.

CHAPTER II

IDENTIFICATION OF QTLS FOR BLOOM DATE, FRUIT DEVELOPMENT PERIOD, AND RIPE DATE TRAITS IN PEACHES

Synopsis

Phenological traits of peaches [*Prunus persica* (L.) Batsch] are important for breeders to evaluate in various environments for determining cultivar adaptability, and for the grower to efficiently manage their commercial orchards. Pedigree-based analysis (PBA) using Visual FlexQTL software was conducted on 162 peach individuals (143 F_1 seedlings and 19 founders and parents) grown in four environments (CA 2011, CA 2012, TX 2012, and TX 2013). A 9K SNP Illumina array was used for the genotyping. The objectives of this study were to 1) identify QTL(s) of three phenological traits including bloom date (BD), ripe date (RD), and fruit development period (FDP); 2) estimate QTL genotypes for important breeding parents of seven full-sib families; and 3) identify the haplotype alleles that are linked to the predictive SNP marker(s) of desired QTL alleles. The QTL for RD and FDP was co-localized at the central part of LG4 (40 - 44 cM) and explained about 35 % of the phenotypic variance. Three QTLs were discovered for BD. These were found on LG1 (88 – 92 cM), LG4 (48 – 50 cM), and LG7 (40 – 44 cM), explaining between 17-94%, 11-55%, and 11-18% of the phenotypic variance respectively. Haplotype analyses for these QTLs revealed predictive SNP haplotypes of desired QTL alleles along with their original sources among the important breeding parents of seven full-sib families. Our results will help peach breeders in developing new predictive, DNA-based molecular marker tests for routine use in marker-assisted breeding (MAB).

Introduction

Peaches [*Prunus persica* (L.) Batsch] and nectarines [*P. persica* (L.) Batsch] are deciduous fruit trees belonging to the Rosaceae family. These are native to China and grown throughout the world in a wide range of environments. The gross production value of peaches and nectarines in 2014 was $795 million in the United States and $20,671 million globally (FAO, 2018).

Breeding perennial crops is a challenging task due to their long breeding cycles, large size, and highly heterozygous nature. The use of marker-assisted breeding (MAB) provides a tool to do an early selection of seedlings, to identify superior parents, to improve the selection of elite alleles for essential traits, and to stack desired alleles at multiple loci (Bliss, 2010; Testolin and Cipriani, 2010). A USDA funded project, RosBREED, involves the collaboration of various breeders and geneticists to identify QTLs and markers linked to fruit quality traits. Further QTLs and tighter linkages are uncovered through more markers being used in collaboration (de Pascual-Teresa et al., 2010; Dirlewanger et al., 1999), and allowing wider adaptation of marker-assisted parent selection (MAPS) and marker-assisted seedling selection (MASS), which helps breeders produce higher quality peaches that meet consumer needs. This method will save space, time, and money (Bliss, 2010).

QTL identification for peaches has been conducted in several studies (Zeballos, 2012), for traits such as acidity, total sugar content, organic acids, fruit weight, blooming and harvest dates (Dirlewanger et al., 1999; Etienne et al., 2002; Quilot et al., 2004), and chilling injury susceptibility (Cantín et al., 2010). However, most of these studies have been limited due to the low marker density of genetic maps and some of the linkage groups lacked markers (Eduardo et al., 2013). Recently, these issues have been overcome due to the availability of the peach

genome v1.0 and v2.0 (Arús et al., 2012; Verde et al., 2012) sequence and the development of the International Peach SNP Consortium peach 9K SNP array (Verde et al., 2012). Moreover, the Pedigree-Based Analysis (PBA) approach (Bink et al., 2014; Bink et al., 2012) of multi-families allows discovering more QTL or QTL-alleles per locus and detecting the performance of QTL(s) across a wide range of genetic backgrounds. Therefore, the above tools facilitated mapping numerous QTLs for various peach traits such as blush (Frett et al., 2014; Hernández Mora et al., 2017a; Hernández Mora et al., 2017b), ripe date (Fresnedo-Ramírez et al., 2015; Hernández Mora et al., 2017a; Nuñez-Lillo et al., 2015; Pirona et al., 2013) chilling and heat requirements (Romeu et al., 2014), soluble solids content (Fresnedo-Ramírez et al., 2015; Hernández Mora et al., 2017a; Zeballos et al., 2016), fruit weight, and titratable acidity (Fresnedo-Ramírez et al., 2015; Hernández Mora et al., 2017a; Zeballos et al., 2016).

Bloom date is an important trait for improving peach adaptation, especially for mild winter regions. Thus, knowledge of the genetic basis for bloom date would help breeders develop peach cultivars with better adaptation. In peaches, the bloom order is consistent across years and environments (Scorza and Sherman, 1996). Chilling requirement is the major factor that determines the bloom date (Alburquerque et al., 2008; Byrne et al., 2000; Citadin et al., 2001; 2003; Egea et al., 2003; Ruiz et al., 2007).

For numerous peach breeders, a lower chilling requirement is a priority trait for developing peach cultivars. In the USA, low-chill peach breeding programs started in the late 1940s and early 1950s and used ‘Peento’ and ‘Hawaiian’ peaches from south China as sources of the low chill trait (Byrne, 2003). Five decades ago, about 90% of peach cultivars required more than 800 chilling hours to break dormancy, while nowadays many of peach cultivars break dormancy with less than 200 chilling hours (Sansavini et al., 2006). The current breeding work

on low chill stone fruit focused on early ripe to allow a year-round supply of peach fruits (Byrne, 2005). Low chill peach trees bloom early and are susceptible to frost damage in areas/years that receive freezes during the spring season (Fan et al., 2010). In contrast, high chill peach cultivars can suffer from insufficient chilling in warm areas/years that leads to irregular leaf and floral bud break and poor production and quality.

Bloom date has been reported as a moderately to highly heritable trait (0.39- 0.92) (de Souza et al., 1998a; 2000; Hansche, 1990; Hansche et al., 1972; Hernández Mora et al., 2017a; Monet and Bastard, 1982; Mowrey and Sherman, 1986). Several QTLs were detected on different LGs for bloom date. These include QTLs reported at the end of LG1 (40-60% of phenotypic variance), on LG2 (27% of phenotypic variance), on LG4 (32-35% phenotypic variance) and on LG7 (21% of phenotypic variance). Not all the QTLs were found in all the studies indicating the population-specific nature of these QTLs (Dirlewanger et al., 1999; Fan et al., 2010; Fresnedo-Ramírez et al., 2015; Hernández Mora et al., 2017a; Romeu et al., 2014; Verde et al., 2002).

Ripe date in peach trees is a crucial element for extending the production season and ensuring a constant fruit supply for peach consumers. Besides, the ripening process is responsible for regulating several metabolic pathways such as blush, sugar/acid balance, and the softening level in peach fruits (Dirlewanger et al., 2012). Thus, genetic knowledge of this trait would help the peach breeder to develop cultivars with specific ripe dates to fill gaps in the ripening sequence.

Narrow sense heritability for ripe date ranges from high to very high (0.79 - 0.94) (de Souza et al., 1998b; Hansche, 1986; Hansche et al., 1972; Hernández Mora et al., 2017a). Genomic regions that have genes of controlling ripe date (RD) were reported in many peach

studies (Dirlewanger et al., 2012; Eduardo et al., 2011; Etienne et al., 2002; Verde et al., 2002). QTLs associated with RD have been mapped on many LGs with varying degrees of reliability (Cantín et al., 2010; Fresnedo-Ramírez et al., 2015; Frett, 2016; Hernández Mora et al., 2017b; Ogundiwin et al., 2009; Peace et al., 2005).

A major QTL was reported for RD on LG 4 at ~44 cM in the *Prunus* T×E reference map and a putative candidate gene was located at ~10.5 Mbp on the peach genome sequence v.1 (Dirlewanger et al., 2012). This QTL explained up to 80% of the genetic variation of ripe date. The RosBREED project has verified that this locus is significant in the U.S. breeding programs (Frett, 2016). This same QTL associated with the ripe date was also identified in several studies and explained ~ 50 to 98 % of the phenotypic variability (Eduardo et al., 2011; Nuñez-Lillo et al., 2015; Romeu et al., 2014). Likewise, a QTL for RD on chromosome 4 was detected in apricot, sweet cherry (Dirlewanger et al., 2012) and almond (Sánchez-Pérez et al., 2007).

Fruit development period (FDP) is defined as the period between bloom and ripe date (Blake, 1932), and it is influenced by both genetic and non-genetic factors (Weinberger, 1948). However, FDP has a strong positive correlation (r = 0.94) with the ripe date and negative correlation (r = -0.49) with bloom date (Hartmann, 2013). This trait was reported to be highly heritable (h^2= 0.73 - 0.98) (de Souza et al., 1998a; Hernández Mora et al., 2017a; Monet and Bastard, 1982; Vileila-Morales et al., 1981). Thus, rapid genetic gains can be achieved for FDP in breeding programs (Hansche et al., 1972). A few studies have mapped QTLs for fruit development period and compared them to other phenological traits such as bloom and ripe dates. QTLs for FDP were mapped on LGs 1, 2, 3, 4, 5, and 6 with decisive evidence. The QTLs on LGs 1 and 6 co-localized with ripe date QTLs (Hernández Mora et al., 2017a). Likewise,

Etienne et al. (2002) mapped only one major QTL for FDP on LG4 from two data sets with phenotypic variance explained (PVE) ~76 %, which co-localized ripe date QTL.

The objectives of this study were to:

1. Identify QTL(s) of three phenological traits including bloom date, ripe date, and fruit development period among low-medium chill peach/nectarine germplasm.
2. Estimate QTL genotypes for important breeding parents of seven full-sib families and to identify the haplotype alleles that are linked to the predictive SNP marker(s) of desired QTL alleles and their original sources.

We hypothesized that phenological trait related markers can be developed and employed by breeders in the development of peach cultivars with a target of extending the production season and environmental adaptation.

Materials and Methods

Plant materials

This study included 162 seedlings from seven related F_1 families derived from seven parents descending from 12 founders (Fig. II-1). Parents were medium to low chill selections from the Texas A&M University breeding program, and high chill selections from the USDA Stone Fruit Breeding Program in Parlier, CA. The number of seedlings in each family ranged from 8 to 36 with an average size of 20. These seedlings, along with parental genotypes, were budded onto 'Nemaguard' peach rootstocks and planted in College Station, TX, and Fowler, CA. Each site included one replicate of each seedling and three to four replicates of each parent.

Plot establishment and design

The College Station plot was randomized with one replicate of each seedling and four replicates of each parent, whereas planting at the Fowler site was organized by progeny with three replicates of each parent. Trees at College Station were planted in staggered double-rows, with 1.7 meters between rows, and 0.67 meter spacing within rows. Double rows were spaced five meters apart. All trees were trained as a central leader. Trees in the Fowler plot were trained as a two-scaffold 'Y' and spaced approximately one meter within rows spaced approximately 4 meters apart. At each location, irrigation, fertilization, pest and weed control, pruning, and fruit thinning were carried out according to typical commercial practice.

Seedlings and parents were evaluated at the two locations over two years: Fowler, CA for 2011 and 2012, and College Station, TX for 2012 and 2013. College Station is located in east central Texas with a sub-humid and warm temperate climate with mild winters and warm to hot, humid summers. Fowler is located in the San Joaquin Valley in central California and is ideal for peach production with a semi-arid Mediterranean climate. The locations have mean January / July temperatures 4.0° C / 36.5° C (Fowler) and 7.0° C / 35.0° C (College Station), however, College Station has greater rainfall (1022 mm; 248 mm for Fowler), higher humidity (67.5% for College Station; 55.1% for Fowler), more cloudy days (College Station receives 27% less sunlight per year), and warmer night temperatures during fruit development (15.8° C for College Station and 12.4° C for Fowler) (Weather Underground, 2013). In addition, College Station is more subject to late spring freezes, low chilling accumulation, and has a heavy textured soil. These environmental factors make College Station much less suitable for stone fruit production as compared to the California site.

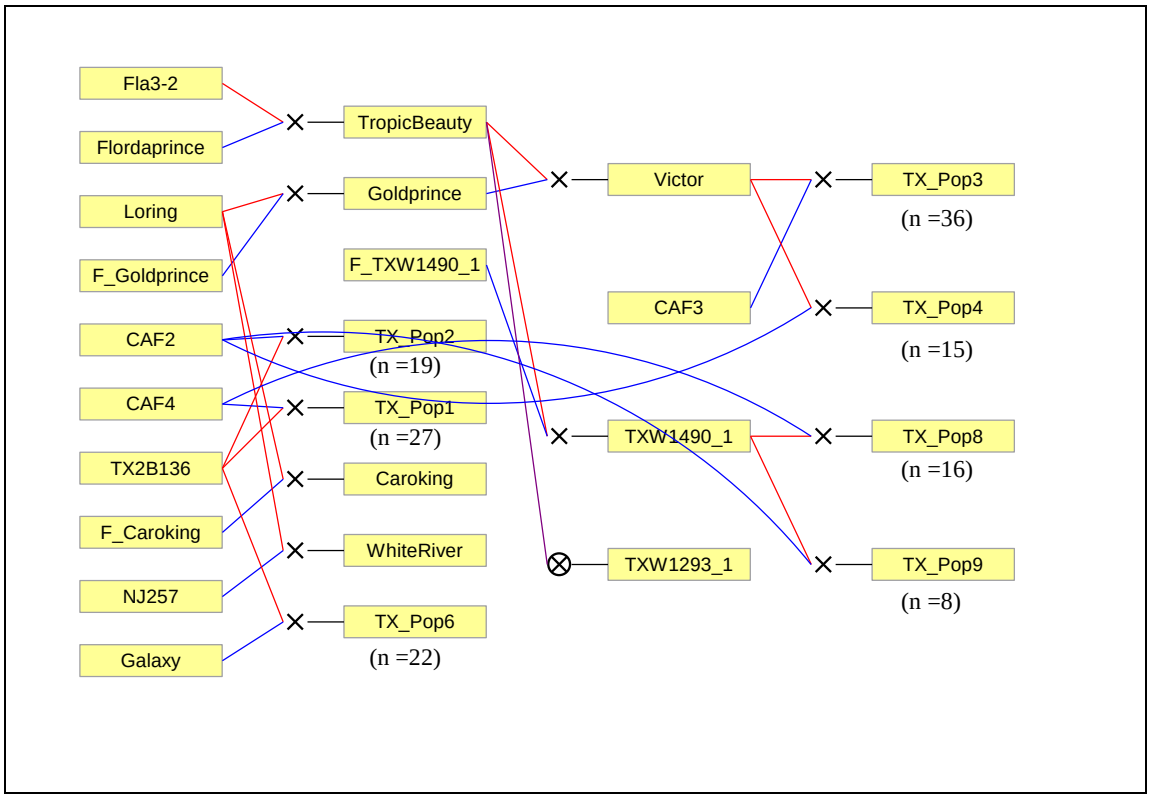

Fig. II-1. Pedigree of the seven peach populations and their progeny number. Red and blue lines link progeny to female and male parents, respectively.

Phenological traits evaluations

Phenotypic data was taken at both locations across two years (2011-2012 in CA, and 2012-2013 in TX) on individual trees for three phenological traits, bloom date (BD), ripe date (RD), and fruit development period (FDP). The date of first (10% blossoms open) and full bloom (60% to 80% of the blossoms open) were visually assessed in the field and recorded for each tree. Date of ripe was determined when 20% of fruits are pickable by visually inspecting the presence of a few soft fruits in the field for maturity two times per week. Both full bloom and ripe dates were converted to Julian days (0-365). FDP was calculated as the number of days between the date of full bloom and date of ripe.

SNP genotyping and genetic linkage map

Individuals were previously genotyped as part of the US Peach Crop Reference Set and Breeding Pedigree Set established in the RosBREED project (Peace et al., 2014) using the IPSC 9K SNP Array for Peach (Verde et al., 2012). The raw iScan data from 9K SNP array was initially processed into the GenomeStudio software v2010.3 (Illumina Inc., 2010) using the Genotyping Module with a Gen Call threshold of 0.15. Parentage records and SNP data curation was performed following the procedures described in the pipeline recently developed for apple, sweet cherry and peach (Vanderzande et al., 2018). A subset of 4,005 informative SNPs that showed no null alleles and no parentage conflicts were retained after the pipeline steps. Genetic positions of the selected SNPs were obtained using the peach consensus map (da Silva Linge et al., 2018) as a reference. In view of computing time, we decreased the number of markers by eliminating markers at identical map positions, which resulted in 1,487 informative SNP markers spread over the eight LGs.

QTL detection

Genotypic and phenotypic data for bloom date (BD), ripe date (RD), and fruit development period (FDP) traits for all seedlings and seven parents were combined and analyzed by applying FlexQTL software (Bink et al., 2014). The software utilizes a Bayesian approach to estimate the number of QTLs. FlexQTL analyses were conducted three times, on data from each location and the overall (mean of both locations) with different chain length, prior and maximum QTL number to reach an effective chain size (ECS) (Sorensen and Gianola, 2002) of at least 100 for phenotypic mean, residual variance and number of QTLs as needed to make sound inferences and conclusions. The length of Markov Chain Monte Carlo (MCMC) simulations varied between

100,000 and 3,600,000 iterations, from which thousands of simulations were sampled for statistical inference, thus storing one sample every hundred to three thousand iterations for subsequent inference. The analysis was carried out with an additive genetic model and QTLs were detected as twice the natural logarithm of the obtained of Bayes Factors (BF) [*2ln(Bf)*] as described by Kass and Raftery (1995). The evidence of QTLs presence can be interpreted as hardly any (BF < 2), positive (2 ≤ BF < 5), strong (5 ≤ BF < 10), and decisive (BF ≥ 10). QTL intervals were defined as a series of successive 2-cM bins with intensities corresponding to 2lnBF > 2. For inferences on the number of QTLs, we considered loci that had a 2lnBF greater than 5 for at least one data set, or that had a 2lnBF greater than 2 for at least two independent data sets and being co-localized within ± 4 cM for identified regions, with an effective chain size (ECS) of at least 100, and explaining at least 10% of phenotypic variation.

In addition, trace and intensity plots per trait were evaluated to determine QTLs reliability. Additive variance (σ_A^2) for each trait was calculated by subtracting the residual variance (σ_e^2) from the phenotypic variance (σ_P^2) and the narrow sense heritability (h^2) was calculated as follows:

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2}$$

The proportion of phenotypic variance explained (PVE) by each QTL was estimated from FlexQTL output by dividing the additive variance explained by the QTL region ('AVt1') by the total phenotypic variance of the trait ('variance') as follows:

$$PVE = \frac{\sigma_A^2}{\sigma_P^2} \times 100 \quad \text{where } \sigma_A^2 = additive\ variance\ (AVT1)\ of\ QTL$$

Mapped QTLs were denoted with two or three letters for the trait, two letters and two-digit number representing location and evaluation year, and the letter ‘G’ followed by the linkage group number. The three parts of denotation were separated by an underscore. If more than one QTL was present in the same genomic region, the letter a, b or c was added.

SNP haplotypes and QTL genotypes of important breeding parents

Considering the 1,487 informative SNP markers subset, SNPs within the interval of a significant QTL were chosen for haplotyping. Haplotypes were constructed across the germplasm using PediHaplotyper (Voorrips et al., 2016) as an R package (R Core Team, 2018) including, as input files, ‘mhaplotypes.csv’, ‘flexqtl.par’, ‘flexqtl.sort’, and ‘HaploBlocks.map’ files obtained from FlexQTL.

The non-parametric Kruskal-Wallis test with the Steele–Dwass nonparametric multiple comparison test ($P < 0.05$) using JMP Pro Version 13.2 (SAS Institute Inc., Cary, NC, 2016) was conducted to determine differences among haplotype and diplotype effects. Haplotype effects were deduced from combinations of diplotypes. For instance, the effects of haplotypes H1 and H2 can be obtained by comparing the effects of 3H|H1 and 3H|H2 diplotypes. Then, haplotypes were assigned to QTL allele categories (*Q* or *q*) based on the direction of their effects by increasing or decreasing of phenotypic values for each trait. Lastly, QTL genotypes were assigned to each individual based on its SNP haplotypes as *QQ*, *Qq*/*qQ*, and *qq*. Allele sequence of haplotypes and QTL genotypes along with pedigree records allowed the tracing back of favorable alleles to the original sources.

Results

Genome-wide QTL analysis

Bloom date (BD)

Narrow sense heritability (h^2) ranged between moderate (0.44) for CA11 to high (0.82) for overall analysis (Table II-1). Three putative QTLs were mapped on LG1, 4, and 7. The QTL on LG1 was at the distal end and was common to all environments and the overall analysis. The QTL on LG4 mapped in three environments and the overall analysis. The QTL on LG7 was seen in only two environments and the overall analysis (Fig. II-2). The proportion of phenotypic variation explained (PVE) by all loci ranged from 17 to 94%, 11 to 55%, 11 to 18% for LG1, LG4, and LG7, respectively (Table II-2). The highest posterior QTL intensity (0.93) showed in BD_overall_G1 and the lowest intensity (0.21) was found in TX12_G4. The highest additive effect (~14 days) was in CA11_G1 and the lowest (~2 days) showed in LGs 1, 4, and 7 for CA12. The QTL on LG1 was consistent overall data sets and ranged between 88 - 92 cM (peaks 88, 90, and 92 cM) and the physical position of this chromosomal region was 43,578,596 - 44,913,729 bp on the peach genome sequence, (Table II-2 and Table II-3; Fig. II-3). Likewise, peaks of QTL on LG4 of three data sets, except CA12, clustered at mode 48 and 50 cM, with an interval between 48 - 50 cM and physical chromosomal position between 11,956,738 – 13,442,233 bp. Regarding LG7, the peaks co-localized at 40 cM with an interval from 40 - 44 cM and physical chromosomal position between 15,513,277 – 17,226,623 bp on the peach genome sequence (Table II-2 and Table II-3; Fig. II-4).

Table II-1. QTLs mapped for the bloom date (BD), ripe date (RD), and fruit development period (FDP) traits evaluated in four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean for 162 peach (*Prunus persica*) seedlings.

									2ln(BF)		
Trait	***MCMC***	***Records***	μ	σ^2_p	σ^2_e	σ^2_A	h^2	***LG***	***1/0***	***2/1***	***3/2***
BD_CA11	150,000	82	42.28	15.22	8.52	6.70	0.44	1	6.6	0.1	0
BD_CA12	250,000	138	43.83	10.52	2.20	8.32	0.79	1	11.4	2.7	0.2
								4	10.4	0.3	-0.5
								7	29.5	1.0	-0.1
BD_TX12	150,000	114	49.29	76.33	23.48	52.85	0.69	1	5.1	1.3	0.7
								4	3.9	1.0	0.4
								7	15.6	1.3	0.6
BD_TX13	150,000	124	50.16	89.26	23.53	65.73	0.74	1	14.1	-0.4	-0.3
								4	29.6	-1.3	na
BD_overall	3,600,000	143	46.96	42.59	7.55	35.05	0.82	1	13.9	5.5	-1.2
								4	4.6	-2.0	na
								7	14.6	-0.9	na
RD_CA11	500,000	104	157.38	313.89	99.90	213.99	0.68	4	26.3	6.3	0.8
RD_CA12	750,000	138	147.32	238.99	42.49	196.51	0.82	4	na	13.7	1.3
RD_TX12	100,000	94	129.20	278.77	112.63	166.13	0.60	4	29.3	0.6	-0.4
								7	2.3	0.2	na
RD_TX13	500,000	114	141.80	293.67	119.84	173.83	0.59	4	27.6	4.5	0.7
RD_overall	750,000	143	144.82	269.25	76.68	192.57	0.72	4	na	14.9	1.5
FDP_CA11	1,500,000	59	115.33	285.19	92.29	192.90	0.68	4	27.0	5.3	1.4
FDP_CA12	150,000	138	103.48	249.88	43.79	206.09	0.83	4	na	30.3	2.8
FDP_TX12	250,000	94	81.22	286.48	91.64	194.84	0.68	4	29.0	1.8	1.0
								6	4.5	1.3	0.0
FDP_TX13	150,000	114	91.29	320.95	105.54	215.40	0.67	4	28.2	3.6	1.0
FDP_overall	100,000	141	96.67	288.78	74.42	214.36	0.74	4	na	11.7	3.2

Markov chain Monte Carlo (MCMC) run length, phenotypic mean (μ), phenotypic variance (σ^2_P), residual variance(σ^2_e), additive variance(σ^2_A), narrow-sense heritability (h^2), the linkage groups (LG) that QTLs were mapped on, and the QTL evidence [*2ln(BF)*] which is hardly any (0-2); positive (2-5); strong (5-10); and decisive (>10).

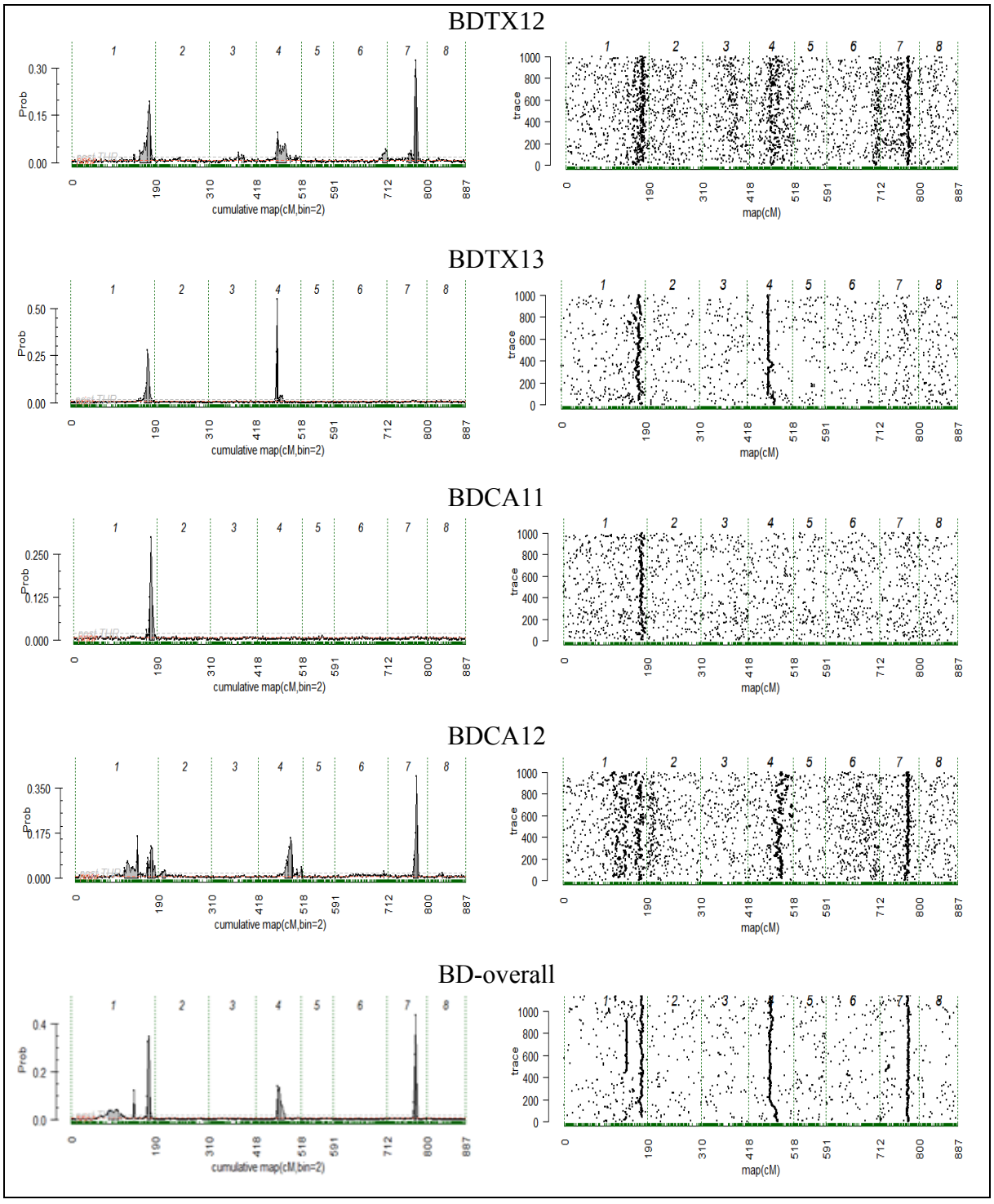

Fig. II-2. Posterior positions (left) and trace samples QTL positions (right) based on an additive model performed using Visual FlexQTL software (Bink et al., 2008) for the bloom date (BD) from four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean for 162 peach seedlings.

Ripe date (RD)

Narrow sense heritability (h^2) varied from moderate (0.59) for TX13 to high (0.82) for CA12 (Table II-1). FlexQTL software found one to two QTLs per environment; however, the QTL at the middle part of LG4 was consistently mapped with decisive evidence (BF ≥ 10) in each environment and in the overall analysis (Fig. II-5). The proportion of phenotypic variation explained (PVE) by this QTL ranged between 40 and 54% (Table II-2). The highest posterior QTL intensity (1.4) was for CA12 and the lowest (0.84) was for CA11. The highest additive effect was found in TX12 and TX13 by increasing ripe date ~17 days. This QTL had a mode of ~44cM, overlapping intervals from 40 to 44 cM across all data sets, and the physical chromosomal position between ~10,015,773 to 11,298,736 of the peach genome sequence v2.0 (Table II-2 and Table II-3; Fig. II-5).

Fruit development period (FDP)

Narrow sense heritability of this trait ranged between moderate (0.67) for TX13 to high (0.83) for CA12 (Table II-1). FlexQTL software found one to three QTLs per environment; however, the QTL at the middle part of LG4 was consistent over environments and showed decisive evidence (BF ≥ 10) (Table II-1; Fig. II-6).

The proportion of phenotypic variation explained (PVE) by this QTL ranged between 36 and 62 % (Table II-2). The highest posterior QTL intensity (1.07) was for CA12 and the lowest (0.79) was for TX12. The highest additive effect was found in TX13 by increasing FDP ~20 days. This QTL had a mode at 44 cM, overlapping intervals from 42 to 48 cM across all data sets, except TX12, and has a physical chromosomal position between ~10,582,092 to 11,956,738 bp of the peach genome sequence v2.0 (Table II-2 and Table II-3).

Table II-2. QTL name, linkage group, interval, mode peak, intensity, additive effect, and phenotypic variance explained (PVE) for the bloom date (BD), ripe date (RD), and fruit development period (FDP) traits evaluated in four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean for 162 peach seedlings.

QTL name	***Linkage Group***	***Interval (cM)***	***Mode peak (cM)***	***Intensity***	***Additive Effect (d)***	***PVE***
BD_CA11_G1	1	[88, 92]	92	0.61	14.29	94
BD_CA12_G1	1	[88, 92]	88	0.33	2.02	19
BD_TX12_G1	1	[88, 92]	90	0.58	5.15	17
BD_TX13_G1	1	[88, 92]	88	0.72	6.27	20
BD_overall_G1	1	[88, 92]	92	0.93	5.49	35
BD_CA12_G4	4	[58, 60]	60	0.52	2.02	18
BD_TX12_G4	4	[48, 50]	50	0.21	4.26	11
BD_TX13_G4	4	[48, 50]	48	0.85	10.01	55
BD_overall_G4	4	[48, 50]	50	0.42	3.89	14
BD_CA12_G7	7	[40, 44]	40	0.87	1.97	17
BD_TX12_G7	7	[40, 44]	40	0.81	5.35	18
BD_overall_G7	7	[40, 42]	40	0.91	3.02	11
RD_CA11_G4	4	[42, 44]	44	0.84	15.88	40
RD_CA12_G4	4	[42, 44]	44	1.06	14.38	42
RD_TX12_G4	4	[42, 44]	44	0.85	17.67	54
RD_TX13_G4	4	[40, 44]	44	1.20	17.46	52
RD_overall_G4	4	[42, 44]	44	1.13	14.831	41
FDP_CA11_G4	4	[42, 44]	44	0.83	15.15	40
FDP_CA12_G4	4	[44, 48]	44	1.07	13.466	36
FDP_TX12_G4	4	[48, 50]	48	0.79	18.1	56
FDP_TX13_G4	4	[42, 44]	44	0.94	20.04	62
FDP_overall_G4	4	[42, 44]	44	0.83	14.57	37

For each QTL reported, the evidence [*2ln(BF)*] is either positive (2-5), strong (5-10) or decisive (>10).

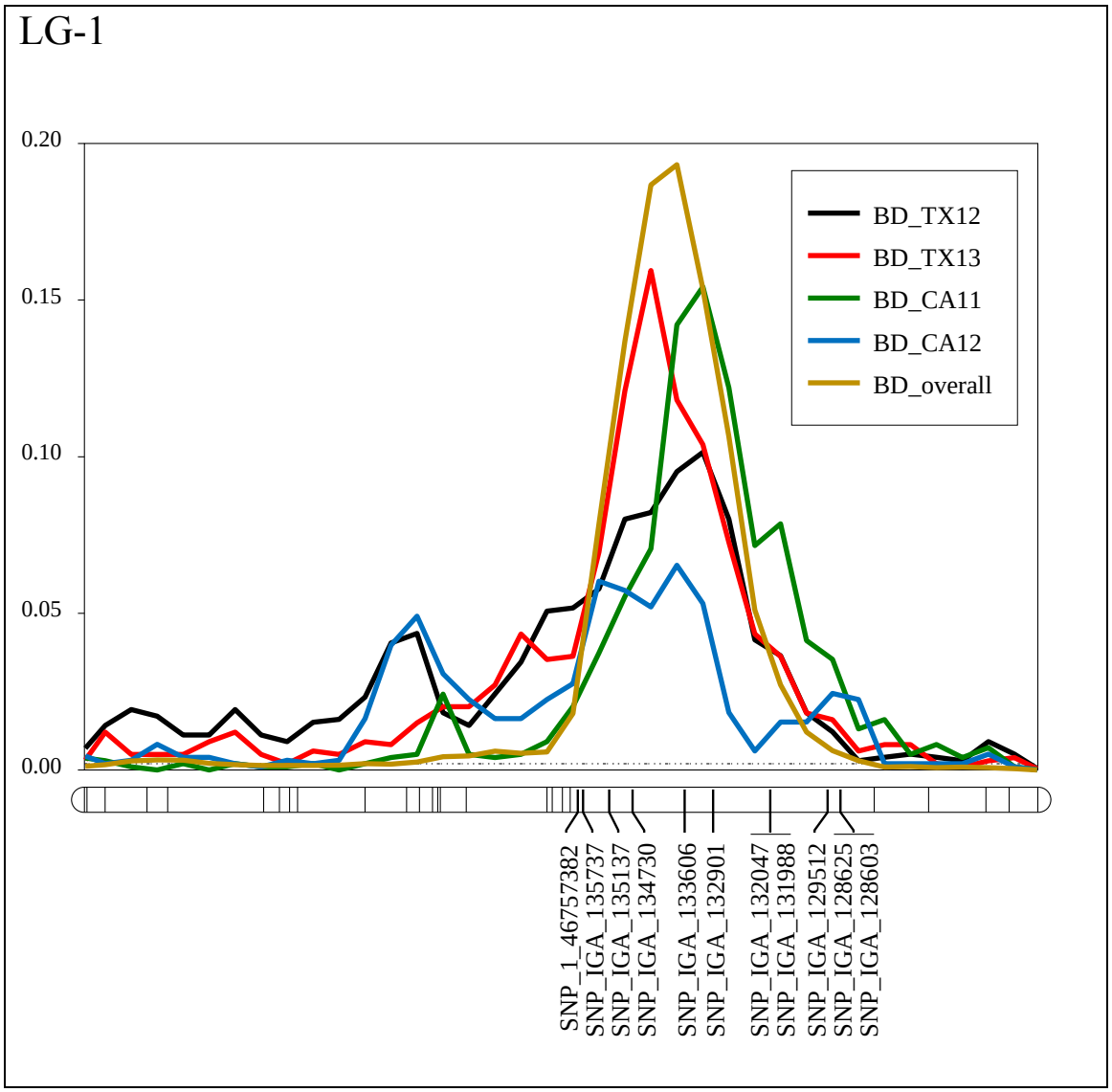

Fig. II-3. The position of putative QTLs controlling the bloom date (BD) at linkage group 1 (LG-1) from four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean generated using MapChart software (Voorrips, 2002).

Table II-3. QTL name, linkage group, along with SNP name, genetic position, and physical location of flanking markers and nearest marker to the center of the mode for the bloom date (BD), ripe date (RD), and fruit development period (FDP) traits evaluated in four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean for 162 peach seedlings.

		Flanking markers			***Nearest marker***		
QTL name	***Linkage group***	***Name***	***Genetic position (cM)***	***Physical location***	***Name***	***Genetic position (cM)***	***Physical location***
BD_CA11_G1	1	SNP_IGA_134730	88.96	43,578,596	SNP_IGA_132901	91.05	44,350,825
		SNP_IGA_131988	92.05	44,913,729			
BD_CA12_G1	1	SNP_1_46757382	88.02	43,058,300	SNP_IGA_134730	88.96	43,578,596
		SNP_IGA_132901	91.05	44,350,825			
BD_TX12_G1	1	SNP_1_46757382	88.02	43,058,300	SNP_IGA_132901	91.05	44,350,825
		SNP_IGA_131988	92.05	44,913,729			
BD_TX13_G1	1	SNP_1_46757382	88.02	43,058,300	SNP_IGA_134730	88.96	43,578,596
		SNP_IGA_131988	92.05	44,913,729			
BD_overall_G1	1	SNP_1_46757382	88.02	43,058,300	SNP_IGA_132901	91.05	44,350,825
		SNP_IGA_131988	92.05	44,913,729			
BD_CA12_G4	4	SNP_IGA_446745	58.54	17,587,156	SNP_IGA_465473	59.40	18,845,078
		SNP_IGA_469044	59.40	19,206,580			
BD_TX12_G4	4	SNP_IGA_413934	47.90	11,956,738	SNP_IGA_415301	49.20	12,523,245
		SNP_IGA_417094	50.00	12,971,285			
BD_TX13_G4	4	SNP_IGA_413934	47.90	11,956,738	SNP_IGA_414387	48.40	12,107,191
		SNP_IGA_417094	50.00	12,971,285			
BD-overall_G4	4	SNP_IGA_413934	47.90	11,956,738	SNP_IGA_415301	49.20	12,523,245
		SNP_IGA_418890	50.80	13,442,233			
BD_CA12_G7	7	SNP_IGA_778568	40.05	15,513,277	SNP_IGA_780816	40.32	16,365,104
		SNP_7_17628094	44.69	17,226,623			
BD_TX12_G7	7	SNP_IGA_778568	40.05	15,513,277	SNP_IGA_780816	40.32	16,365,104
		SNP_7_17628094	44.69	17,226,623			

Table II-3 Continued

QTL name	*Linkage group*	*Flanking markers*			*Nearest marker*		
		Name	*Genetic position (cM)*	*Physical location*	*Name*	*Genetic position (cM)*	*Physical location*
BD-overall_G7	7	SNP_IGA_778568	40.05	15,513,277	SNP_IGA_780816	40.32	16,365,104
		SNP_IGA_781455	42.92	16,567,648			
RD_CA11_G4	4	SNP_IGA_409901	41.98	10,582,092	SNP_IGA_410794	43.31	10,890,653
		SNP_IGA_412662	44.97	11,298,736			
RD_CA12_G4	4	SNP_IGA_409901	41.98	10,582,092	SNP_IGA_412662	44.97	11,298,736
		SNP_IGA_412662	44.97	11,298,736			
RD_TX12_G4	4	SNP_IGA_409901	41.98	10,582,092	SNP_IGA_410794	43.31	10,890,653
		SNP_IGA_412662	44.97	11,298,736			
RD_TX13_G4	4	SNP_IGA_407364	39.21	10,015,773	SNP_IGA_410794	43.31	10,890,653
		SNP_IGA_412662	44.97	11,298,736			
RD_overall_G4	4	SNP_IGA_409901	41.98	10,582,092	SNP_IGA_410794	43.31	10,890,653
		SNP_IGA_412662	44.97	11,298,736			
FDP_CA11_G4	4	SNP_IGA_409901	41.98	10,582,092	SNP_IGA_412662	44.97	11,298,736
		SNP_IGA_412662	44.97	11,298,736			
FDP_CA12_G4	4	SNP_IGA_412338	44.97	11,208,347	SNP_IGA_412662	44.97	11,298,736
		SNP_IGA_413934	47.90	11,956,738			
FDP_TX12_G4	4	SNP_IGA_413115	47.47	11,593,768	SNP_IGA_414387	48.40	12,107,191
		SNP_IGA_417094	50.00	12,971,285			
FDP_TX13_G4	4	SNP_IGA_409901	41.98	10,582,092	SNP_IGA_410794	43.31	10,890,653
		SNP_IGA_412662	44.97	11,298,736			
FDP_overall_G4	4	SNP_IGA_409901	41.98	10,582,092	SNP_IGA_410794	43.31	10,890,653
		SNP_IGA_412662	44.97	11,298,736			

For each QTL reported, the evidence [*2ln(BF)*] is either positive (2-5), strong (5-10) or decisive (>10).

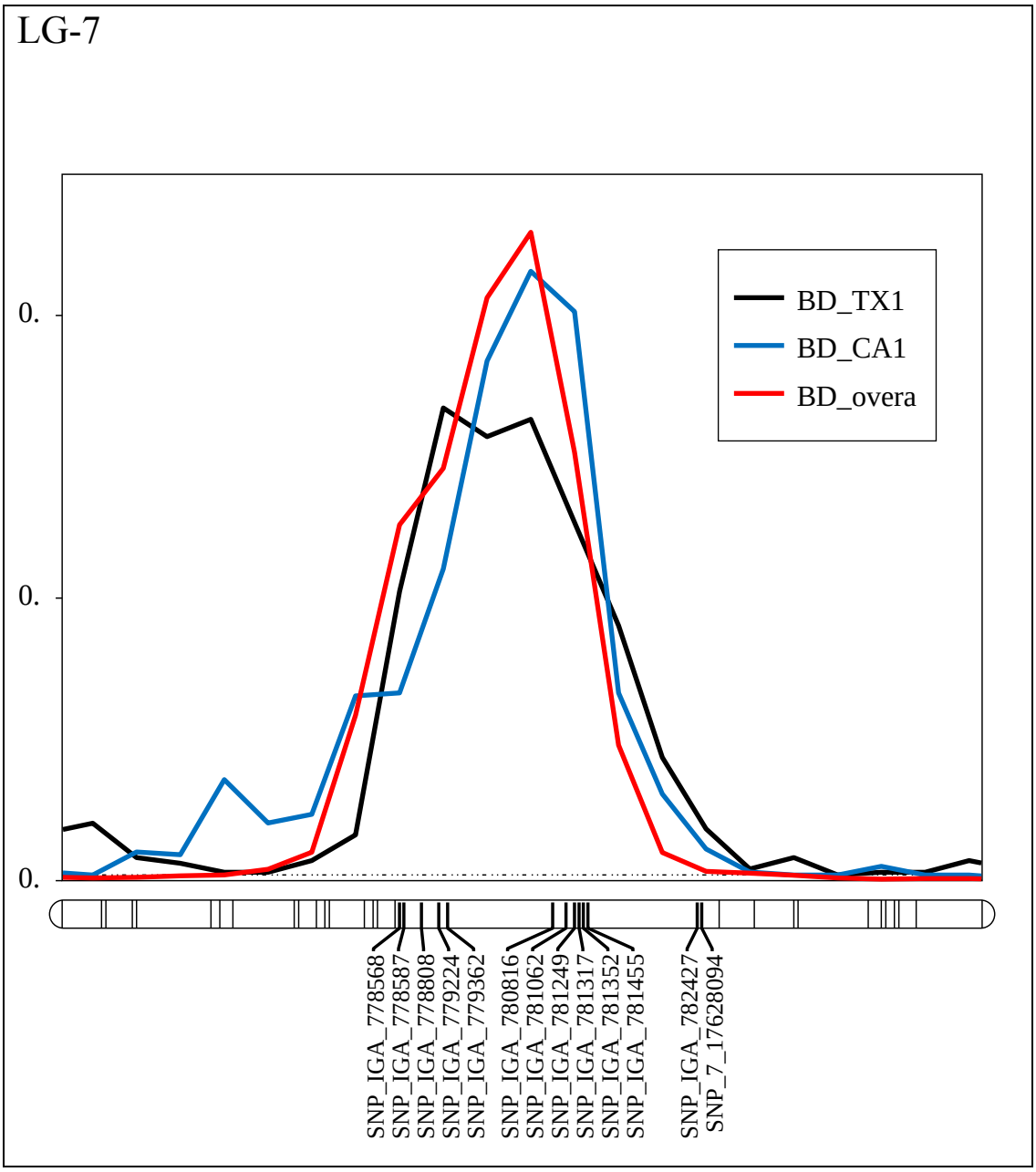

Fig. II-4. The position of putative QTLs controlling the bloom date (BD) at linkage group 7 (LG-7) from CA 2012, TX 2012, and the overall combined mean generated using MapChart software (Voorrips, 2002).

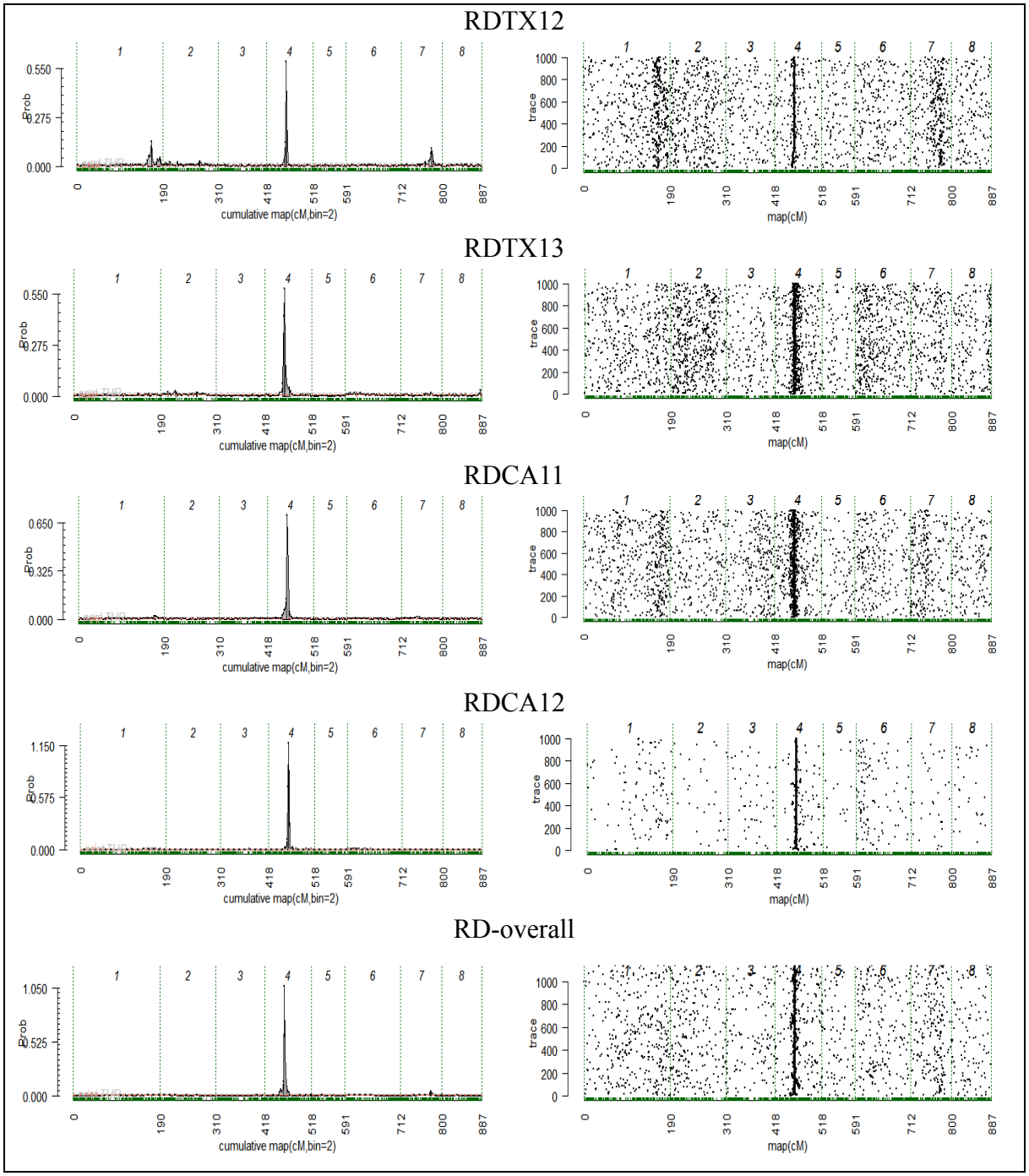

Fig. II-5. Posterior positions (left) and trace samples QTL positions (right) based on an additive model performed using Visual FlexQTL software (Bink et al., 2008) for the ripe date (RD) from four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean for 162 peach seedlings.

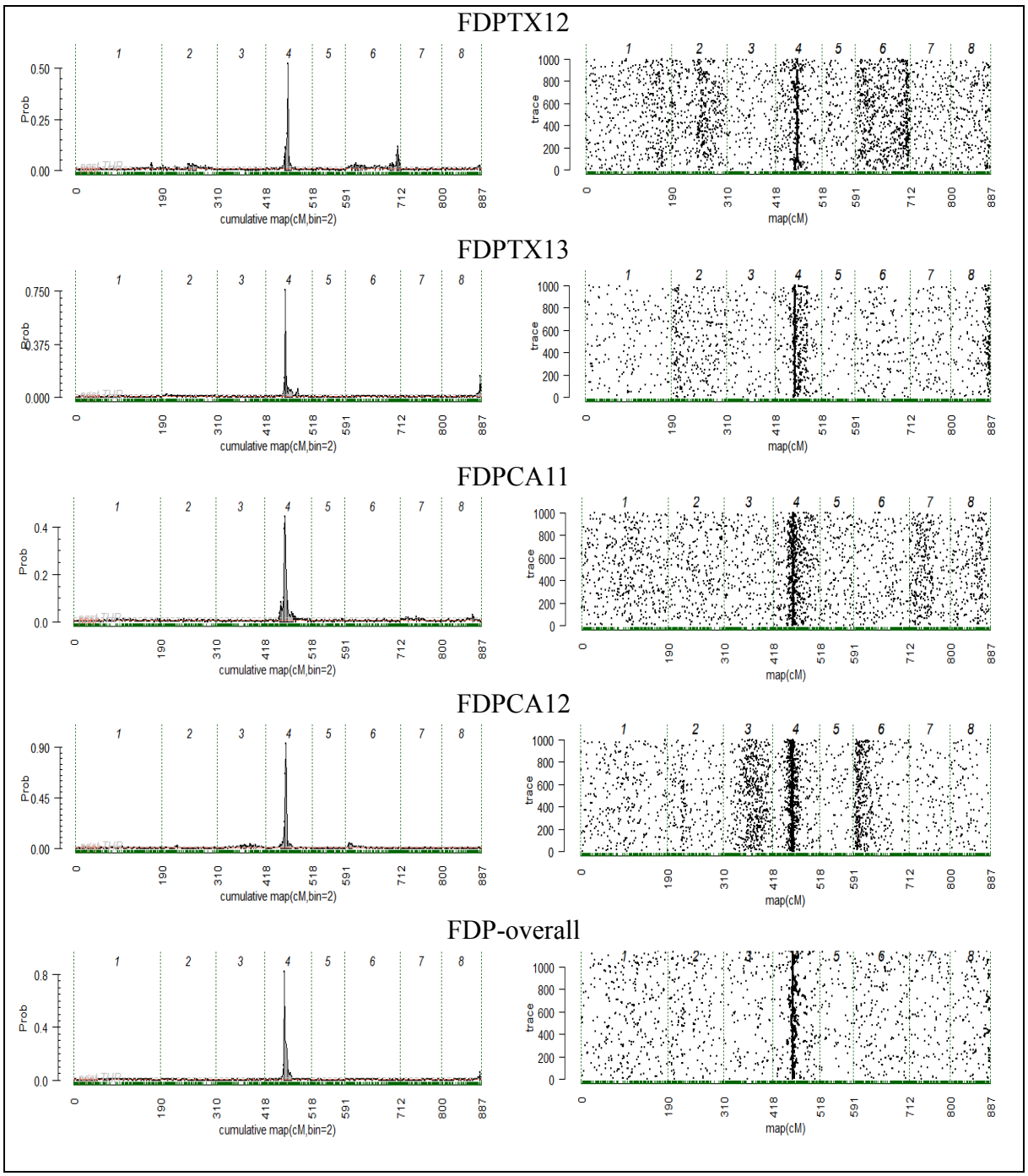

Fig. II-6. Posterior positions (left) and trace samples QTL positions (right) based on an additive model performed using Visual FlexQTL software (Bink et al., 2008) for the fruit development period (FDP) from four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean for 162 peach seedlings.

Haplotype characterization, predictive markers, and validation of QTL effects

Bloom date (BD)

The haplotyping analysis was conducted on the three QTLs at LG1, 4, and 7 for bloom date. Eleven SNPs in the predicted QTL region on LG1 (88.02- 93.48 cM) chosen for haplotyping, revealed eight unique SNP haplotypes across the seven parents in which H8 was a common haplotype (Table II-4). The estimation of diplotypes effect showed that the lower bloom date mean values were associated with individuals had diplotypes containing H8, suggesting that these haplotypes may be associated with *q*-alleles for decreasing bloom date. This haplotype was present in TXW1490_1 and TX2B136 as homozygous *qq* alleles with a diplotype mean of 38 d for H8H8 (Fig. II-7), and in 'CAF4' and 'Victor' as a heterozygous condition. On the other hand, the rest of haplotypes (H1 to H7) were associated with high bloom date values and assigned to *Q*-allele and were found in 'CAF4' and 'Victor', and as homozygous *QQ* alleles in 'CAF2', 'CAF3', and 'Galaxy' with mean BD ~55 d for the diplotypes H5H7 and H5H1. The mean value of heterozygous *Q*-alleles ranged between ~44 to 52 d for the diplotypes H8H2 and H8H1, respectively. All of these haplotypes could be differentiated from H8 by either AB- or BA-alleles at the two adjacent SNP markers SNP_1_46757382 (88.02 cM, ~43 Mb) and SNP_IGA_135737 (88.06 cM, ~43.1 Mb) (Table II-5). Breeding parents 'Galaxy', 'CAF3', 'CAF2' and 'CAF4' were considered as founders in this study and the source of these SNPs because their ancestors were not available for genotyping. On the other hand, the *Q*-allele of 'Victor' was traced back to the unknown paternal parent of 'Goldprince'. However, H8 was distinguished by the two consecutive BB-alleles at SNP_1_46757382 and SNP_IGA_135737 and the three consecutive AAA-alleles at the end of the allele sequence (Table II-5). We were unable to trace sources of homozygous *q*-allele of TX2B136 and the 'CAF4'. The *q*-allele of both 'Victor' and TXW1490-

1 was inherited from the maternal parent ‘TropicBeauty’ and derived from ‘Fla3-2’, and the second *q*-allele of TXW1490-1 was traced back to the unknown paternal parents of TXW1490-1.

Regarding the QTL at LG4, 13 SNP markers in this QTL region (47.90 to 50.80 cM) were selected for haplotyping (Table II-4). Haplotype analyses found five SNP haplotypes associated with BD among the seven parents in which H1 and H3 were the most common haplotypes. Diplotype effects revealed that H2 and H3 were associated with high BD values and were assigned to the *Q*-allele. The homozygous *Q*-allele was found in TX2B136 and TXW1490-1 with BD ~54 d for the diplotype H3H3, however, H3H2 mean value was less consistent with average ~47 d that might be due to different effects size of the haplotypes or smaller sample sizes (Fig. II-7). The heterozygous *Q*-allele was in ‘CAF2’, ‘CAF3’, ‘Galaxy’, and ‘Victor’ with diplotype mean between 41 to 50 d for H3H4 and H5H3, respectively. Low BD values were consistent in individuals with diplotypes containing H1, H4, and H5. These haplotypes were present in ‘CAF4’ as homozygous *q*-alleles, and as a single *q*-allele in ‘CAF2’, ‘CAF3’, ‘Galaxy’, and ‘Victor’. We were unable to estimate the diplotype effect of *qq*-alleles because of the few numbers of individuals containing the combinations of these diplotypes.

Table II-4. SNP name, genetic position (cM), and physical location for SNPs in each allele sequence of haplotypes identified for QTL genotypes for bloom date (BD), ripe date (RD), and fruit development period (FDP) traits of seven important breeding parents.

SNP name	Genetic position (cM)	Physical location	Haplotype							
	BD_G1		**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**
SNP_1_46757382	88.02	43,058,300	A	A	A	A	A	B	B	B
SNP_IGA_135737	88.06	43,109,980	B	B	B	B	B	A	A	B
SNP_IGA_135137	88.45	43,337,658	A	A	A	A	B	A	B	A
SNP_IGA_134730	88.96	43,578,596	B	B	B	B	B	B	B	B
SNP_IGA_133606	90.25	44,069,867	B	B	B	B	B	B	B	B
SNP_IGA_132901	91.05	44,350,825	B	B	B	B	B	B	B	B
SNP_IGA_132047	92.05	44,904,968	A	A	A	B	A	B	A	B
SNP_IGA_131988	92.05	44,913,729	A	A	B	B	B	B	A	B
SNP_IGA_129512	93.05	45,448,596	A	A	B	A	B	A	A	A
SNP_IGA_128625	93.46	45,581,205	A	B	B	A	B	A	B	A
SNP_IGA_128603	93.48	45,586,061	B	B	A	B	A	B	B	A
	BD_G4		**H1**	**H2**	**H3**	**H4**	**H5**			
SNP_IGA_413934	47.90	11,956,738	B	B	A	A	A			
SNP_IGA_414387	48.40	12,107,191	B	A	B	B	B			
SNP_IGA_415301	49.20	12,523,245	B	B	A	B	B			
SNP_IGA_417094	50.00	12,971,285	B	B	A	B	A			
SNP_IGA_417310	50.60	13,034,674	B	B	A	B	B			
SNP_IGA_417637	50.60	13,078,233	B	B	A	B	A			
SNP_IGA_417666	50.60	13,091,850	B	A	B	B	B			
SNP_IGA_417715	50.60	13,108,512	A	A	B	A	A			
SNP_IGA_417840	50.60	13,123,061	B	B	A	B	B			
SNP_IGA_418024	50.60	13,227,341	A	B	A	A	B			
SNP_IGA_418108	50.60	13,271,987	A	A	B	A	B			
SNP_IGA_418890	50.80	13,442,233	A	B	A	A	A			
SNP_IGA_419614	50.80	13,633,831	A	A	B	A	B			
	BD_G7		**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	
SNP_IGA_778568	40.05	15,513,277	B	B	A	A	A	B	A	
SNP_IGA_778587	40.32	15,535,610	B	B	B	A	A	B	A	
SNP_IGA_778808	40.32	15,620,500	A	A	A	B	B	A	B	
SNP_IGA_779224	40.32	15,717,070	B	B	A	A	A	B	B	
SNP_IGA_779362	40.32	15,784,304	B	B	A	B	A	A	B	
SNP_IGA_780816	40.32	16,365,104	A	A	B	B	A	B	A	
SNP_IGA_781062	42.52	16,439,849	A	A	A	B	A	B	A	
SNP_IGA_781249	42.92	16,511,121	A	A	A	B	A	A	A	
SNP_IGA_781317	42.92	16,526,406	A	A	B	B	A	B	A	
SNP_IGA_781352	42.92	16,541,302	B	B	B	A	B	A	B	
SNP_IGA_781455	42.92	16,567,648	A	A	A	B	A	B	A	
SNP_IGA_782427	44.69	17,205,367	A	B	B	A	B	B	B	
SNP_7_17628094	44.69	17,226,623	B	A	B	B	A	A	A	

Table II-4 Continued

SNP name	Genetic position (cM)	Physical location	Haplotype			
RD_G4			**H1**	**H2**	**H3**	**H4**
SNP_IGA_409901	41.98	10,582,092	A	B	B	A
SNP_IGA_410134	42.41	10,626,874	B	B	A	A
SNP_IGA_410165	42.41	10,641,209	B	B	A	A
SNP_IGA_410336	42.41	10,676,008	B	B	A	B
SNP_IGA_410398	42.82	10,696,489	B	B	A	A
SNP_IGA_410478	43.31	10,760,086	B	B	A	B
SNP_IGA_410794	43.31	10,890,653	B	B	A	A
SNP_IGA_410955	43.31	10,904,526	B	B	A	A
SNP_IGA_411147	43.31	10,921,604	B	B	A	B
SNP_IGA_411188	43.31	10,923,251	A	A	B	A
SNP_IGA_411196	43.31	10,923,464	B	B	A	A
SNP_IGA_411601	44.11	10,976,364	B	B	A	B
SNP_IGA_411637	44.11	10,981,971	B	B	A	B
SNP_IGA_412338	44.97	11,208,347	B	B	A	B
SNP_IGA_412662	44.97	11,298,736	A	A	B	B
FDP_G4			**H1**	**H2**	**H3**	**H4**
SNP_IGA_409901	41.98	10,582,092	A	B	B	A
SNP_IGA_410134	42.41	10,626,874	B	B	A	A
SNP_IGA_410165	42.41	10,641,209	B	B	A	A
SNP_IGA_410336	42.41	10,676,008	B	B	A	B
SNP_IGA_410398	42.82	10,696,489	B	B	A	A
SNP_IGA_410478	43.31	10,760,086	B	B	A	B
SNP_IGA_410794	43.31	10,890,653	B	B	A	A
SNP_IGA_410955	43.31	10,904,526	B	B	A	A
SNP_IGA_411147	43.31	10,921,604	B	B	A	B
SNP_IGA_411188	43.31	10,923,251	A	A	B	A
SNP_IGA_411196	43.31	10,923,464	B	B	A	A
SNP_IGA_411601	44.11	10,976,364	B	B	A	B
SNP_IGA_411637	44.11	10,981,971	B	B	A	B
SNP_IGA_412338	44.97	11,208,347	B	B	A	B
SNP_IGA_412662	44.97	11,298,736	A	A	B	B

Table II-5. QTL genotypes for bloom date (BD), ripe date (RD), and fruit development period (FDP) traits of seven important breeding parents, with associated linkage groups, haplotype names, SNP haplotypes, and original sources. QTL alleles for each parent cultivar are presented with ♀ and ♂ for maternal and paternal parent sources, respectively. Allele(s) for predictive SNP marker(s) associated with *Q* or *q*-alleles for increasing or decreasing a given trait, respectively, are shown in **underscored bold**.

Trait/LG	Parents	QTL allele	Hap.	SNP haplotype Allele sequence	Successive ancestors (founders in bold)
BD/LG1	Galaxy	*Q* ♀	H4	**AB**ABBBBBAAB	**Galaxy**
	Galaxy	*Q* ♂	H4	**AB**ABBBBBAAB	**Galaxy**
	CAF3	*Q* ♀	H1	**AB**ABBBAAAAB	**CAF3**
	CAF3	*Q* ♂	H7	**BA**BBBBAAABB	**CAF3**
	CAF4	*Q* ♂	H2	**AB**ABBBAAABB	**CAF4**
	Victor	*Q* ♂	H5	**AB**BBBBABBBA	Goldprince > **F_Goldprince**
	CAF2	*Q* ♀	H6	**BA**ABBBBBAAB	**CAF2**
	CAF2	*Q* ♂	H3	**AB**ABBBABBBA	**CAF2**
	CAF4	*q* ♀	H8	**BB**ABBBBB**AAA**	**CAF4**
	Victor	*q* ♀	H8	**BB**ABBBBB**AAA**	TropicBeauty > **Fla3-2**
	TX2B136	*q* ♀	H8	**BB**ABBBBB**AAA**	**TX2B136**
	TX2B136	*q* ♂	H8	**BB**ABBBBB**AAA**	**TX2B136**
	TXW1490_1	*q* ♀	H8	**BB**ABBBBB**AAA**	TropicBeauty > **Fla3-2**
	TXW1490_1	*q* ♂	H8	**BB**ABBBBB**AAA**	**F_TXW1490_1**
BD/LG4	TX2B136	*Q* ♀	H3	AB**A**A**A**AB**BA**ABAB	**TX2B136**
	TX2B136	*Q* ♂	H3	AB**A**A**A**AB**BA**ABAB	**TX2B136**
	TXW1490_1	*Q* ♀	H3	AB**A**A**A**AB**BA**ABAB	TropicBeauty > **Flordaprince**
	TXW1490_1	*Q* ♂	H3	AB**A**A**A**AB**BA**ABAB	**F_TXW1490_1**
	CAF3	*Q* ♀	H3	AB**A**A**A**AB**BA**ABAB	**CAF3**
	Victor	*Q* ♂	H3	AB**A**A**A**AB**BA**ABAB	Goldprince > **F_Goldprince**
	CAF2	*Q* ♂	H2	B**A**BBBB**A**ABBA**B**A	**CAF2**
	Galaxy	*Q* ♂	H2	B**A**BBBB**A**ABBA**B**A	**Galaxy**
	CAF2	*q* ♀	H1	B**BB**BBBBABAAAA	**CAF2**
	CAF3	*q* ♂	H1	B**BB**BBBBABAAAA	**CAF3**
	Galaxy	*q* ♀	H1	B**BB**BBBBABAAAA	**Galaxy**
	CAF4	*q* ♂	H1	B**BB**BBBBABAAAA	**CAF4**
	CAF4	*q* ♀	H4	A**BB**BBBBABAAAA	**CAF4**
	Victor	*q* ♀	H5	A**BB**ABABABBBAB	TropicBeauty > **Fla3-2**

Table II-5 Continued

Trait/LG	Parents	QTL allele	Hap.	SNP haplotype Allele sequence	Successive ancestors (founders in bold)
BD/LG7	CAF2	*Q* ♂	H3	AB**A**AABAABBABB	**CAF2**
	Galaxy	*Q* ♀	H6	BB**A**BABBABABBA	**Galaxy**
	Victor	*Q* ♂	H6	BB**A**BABBABABBA	Goldprince > **F_Goldprince**
	TX2B136	*Q* ♀	H1	BB**A**BBAAAABAAB	**TX2B136**
	CAF3	*Q* ♂	H2	BB**A**BBAAAABABA	**CAF3**
	CAF2	*Q* ♀	H2	BB**A**BBAAAABABA	**CAF2**
	CAF4	*Q* ♀	H2	BB**A**BBAAAABABA	**CAF4**
	Galaxy	*q* ♂	H4	AA**B**ABBBBBABAB	**Galaxy**
	CAF3	*q* ♀	H4	AA**B**ABBBBBABAB	**CAF3**
	CAF4	*q* ♂	H5	AA**B**AAAAAABABA	**CAF4**
	Victor	*q* ♀	H7	AA**B**BBAAAABABA	TropicBeauty > **Flordaprince**
	TX2B136	*q* ♂	H7	AA**B**BBAAAABABA	**TX2B136**
	TXW1490_1	*q* ♀	H7	AA**B**BBAAAABABA	TropicBeauty > **Flordaprince**
	TXW1490_1	*q* ♂	H7	AA**B**BBAAAABABA	**F_TXW1490_1**
RD/LG4	CAF3	*Q* ♂	H3	B**AA**A**A**A**AA**AB**A**AAA**B**	**CAF3**
	CAF4	*Q* ♂	H3	B**AA**A**A**A**AA**AB**A**AAA**B**	**CAF4**
	Galaxy	*Q* ♀	H3	B**AA**A**A**A**AA**AB**A**AAA**B**	**Galaxy**
	Victor	*Q* ♀	H4	A**AA**B**A**B**AA**BA**A**BBB**B**	TropicBeauty > **Fla3-2**
	TXW1490_1	*Q* ♀	H4	A**AA**B**A**B**AA**BA**A**BBB**B**	TropicBeauty > **Fla3-2**
	CAF2	*q* ♀	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**CAF2**
	CAF2	*q* ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**CAF2**
	CAF4	*q* ♀	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**CAF4**
	Galaxy	*q* ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**Galaxy**
	Victor	*q* ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	Goldprince > **F_Goldprince**
	TX2B136	*q* ♀	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**TX2B136**
	TX2B136	*q* ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**TX2B136**
	TXW1490_1	*q* ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**F_TXW1490_1**
	CAF3	*q* ♀	H2	B**BB**B**B**B**BB**BA**B**BBB**A**	**CAF3**
FDP/LG4	CAF3	*Q* ♂	H3	B**AA**A**A**A**AA**AB**A**AAA**B**	**CAF3**
	CAF4	*Q* ♂	H3	B**AA**A**A**A**AA**AB**A**AAA**B**	**CAF4**
	Galaxy	*Q* ♀	H3	B**AA**A**A**A**AA**AB**A**AAA**B**	**Galaxy**
	Victor	*Q* ♀	H4	A**AA**B**A**B**AA**BA**A**BBB**B**	TropicBeauty > **Fla3-2**
	TXW1490_1	*Q* ♀	H4	A**AA**B**A**B**AA**BA**A**BBB**B**	TropicBeauty > **Fla3-2**
	CAF2	*q* ♀	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**CAF2**
	CAF2	*q* ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**CAF2**
	CAF4	*q* ♀	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**CAF4**
	Galaxy	*q* ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**Galaxy**
	Victor	*q* ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	Goldprince > **F_Goldprince**
	TX2B136	*q* ♀	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**TX2B136**
	TX2B136	*q* ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**TX2B136**
	TXW1490_1	*q* ♂	H1	A**BB**B**B**B**BB**BA**B**BBB**A**	**F_TXW1490_1**
	CAF3	*q* ♀	H2	B**BB**B**B**B**BB**BA**B**BBB**A**	**CAF3**

More than one predictive SNP marker associated with BD differentiated H2 and H3 from the other haplotypes (Table II-5). A-allele at SNP_IGA_415301 (49.2 cM) along with three more SNP markers distinguished H3, whereas, an A-allele at SNP_IGA_414387 (48.4 cM) and other two SNP markers were unique for H2. These predictive SNPs were always associated with a *Q*-allele for increasing BD. The haplotypes H1, H4, and H5 were distinguished from other haplotypes by two adjacent SNP markers, BB-alleles at SNP_IGA_414387 and SNP_IGA_415301 and were associated with *q*-allele.

The original source of both *Q* and *q*-alleles of 'CAF2', 'CAF3', 'CAF4', TX2B136, and 'Galaxy' were unknown as their ancestors were not available for genotyping. The *Q*-allele in TXW1490-1 was inherited from the maternal parent 'TropicBeauty' that came from 'Flordaprince', whereas the second *Q*-allele was traced back to the unknown paternal parents of TXW1490-1. Same was for the *Q*-allele for 'Victor' which was traced back to the unknown paternal parent of 'Goldprince', meanwhile, the *q*-allele was traced back to the maternal parent 'TropicBeauty' which derived from the founder Fla3-2.

As for the QTL at LG7, 13 SNPs (40.05- 44.69 cM) in the QTL region were chosen for haplotyping (Table II-4). There are seven unique SNP haplotypes associated with BD across the seven parents. Estimation of diplotypes effect revealed that the higher BD values were for individuals with diplotypes containing H1, H2, H3, and H6. These were assigned to the *Q*-allele. Homozygous *Q*-alleles were present in 'CAF2', and as a heterozygous *Q*-allele in 'CAF3', 'CAF4', 'Victor', 'Galaxy' and TX2B136. In contrast, haplotypes H4, H5, and H7 were associated with low BD values and were assigned to *q*-allele. The breeding parent TXW1490-1 was homozygous *q*-allele, while 'CAF3', 'CAF4', 'Victor', 'Galaxy' and TX2B136 were heterozygous *q*-allele. The mean value of diplotype H6H2 (*QQ*) was 54 d, while the value of

diplotypes containing *Qq*-alleles ranged between 41 to 54 d in H1H5 and H6H4, respectively. Lowest BD (39 d) was associated with the diplotype H7H5 (Fig. II-7).

A predictive SNP marker was associated with H1, H2, H3, and H6 at the genetic position of 40.32 (~15.6 Mb) of LG7 (Table II-5). An A-allele at the SNP marker SNP_IGA_778808 was associated with *Q*-alleles for increasing BD in peach. This SNP allele inherited from the parents 'Galaxy', 'CAF3', 'CAF2', 'CAF4' and TX2B136 that were considered as founders in this study. The *Q*-allele of 'Victor' was traced back to the unknown paternal parent of 'Goldprince'. In addition, H4, H5, and H7 were distinguished by a single predictive SNP marker, a B-allele at SNP_IGA_778808. We were unable to trace the original source of this SNP allele as most studied parents were treated as founders. However, the *q*-allele of both 'Victor' and TXW1490-1 were inherited from the maternal parent 'TropicBeauty' and derived from 'Flordaprince', and the second *q*-allele of TXW1490-1 was traced back to its unknown paternal parent.

Ripe date (RD) and fruit development period (FDP)

The haplotype analyses for RD and FDP indicated that they shared the same specific haplotypes and were associated with the same QTL alleles for increasing or decreasing the trait values. Furthermore, favorable SNP alleles associated with these traits and their original sources coincided. This finding was expected as they were highly correlated (r=0.87).

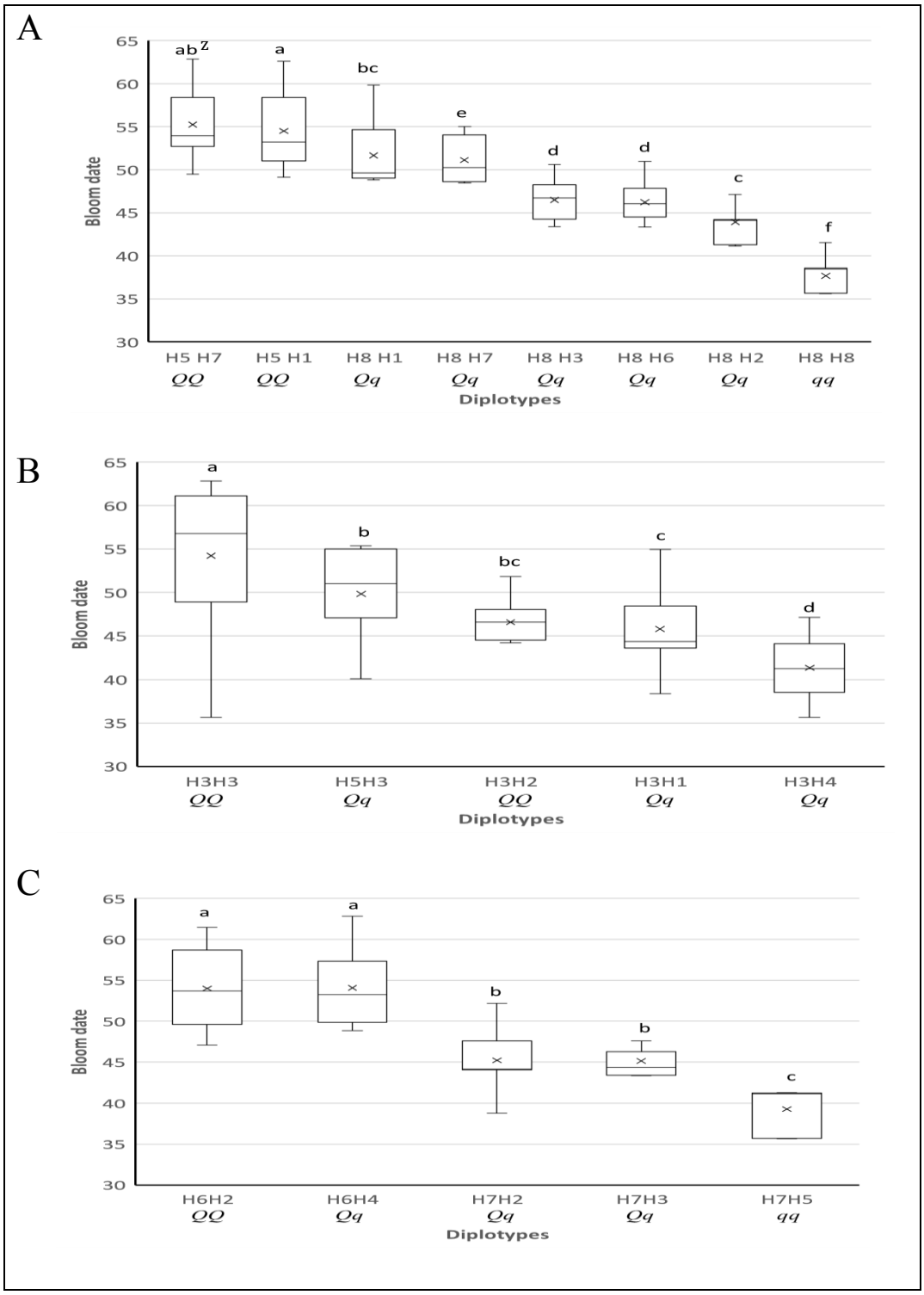

Fig. II-7. Diplotype effect of the most common haplotypes associated with bloom date (BD) for the three QTLs mapped on LG1 (A), LG4 (B), and LG7 (C).
[z] Means not connected by the same letter are significantly different ($P<0.05$) within each linkage group using HSD test.

Fifteen SNP markers in the predictive QTL region for both traits (41.98 to 44.97cM) were selected for haplotyping (Table II-4). Haplotype analyses discovered four unique SNP haplotypes associated with RD and FDP among the seven parents in which H1 was common. Diplotype effect revealed that H3 and H4 were associated with high RD and FDP values and were assigned to *Q*-allele with mean values 170 and 124 d for the diplotype H4H3, respectively (Fig. II-8). Heterozygous *Q*-allele was found in 'CAF3', 'CAF4', 'Galaxy', 'Victor', and TXW1490-1. In contrast, the lower RD and FDP values were for individuals with diplotypes containing H1 and H2, and the lowest values for RD (126 d) and FDP (64 d) were for the diplotype H1H2. These haplotypes were present in 'CAF2' and TX2B136 as homozygous *q*-alleles and as heterozygous in the other parents ('CAF3', 'CAF4', 'Galaxy', 'Victor', and TXW1490-1). More than one predictive SNP marker was identified which distinguished H3 and H4 from the other two haplotypes. The two AA-alleles at SNP markers SNP_IGA_410134 (10.6 Mb) and SNP_IGA_410165 (10.6 Mb), and several other predictive SNP markers were constantly associated with *Q*-alleles (Table II-5). There were two consecutive predictive SNP markers (BB-alleles) that were always associated with *q*-alleles at the same genetic positions above.

The original sources for Q and *q*-alleles could not be traced back to the breeding parents 'CAF2', 'CAF3', 'Galaxy' 'CAF4', and TX2B136. The *Q*-allele of both 'Victor' and TXW1490-1 was inherited from the maternal parent 'TropicBeauty' that came from 'Fla3-2'. Whereas the *q*-allele of their paternal parents was traced back to the unknown paternal parents of 'Goldprince' and TXW1490-1, respectively.

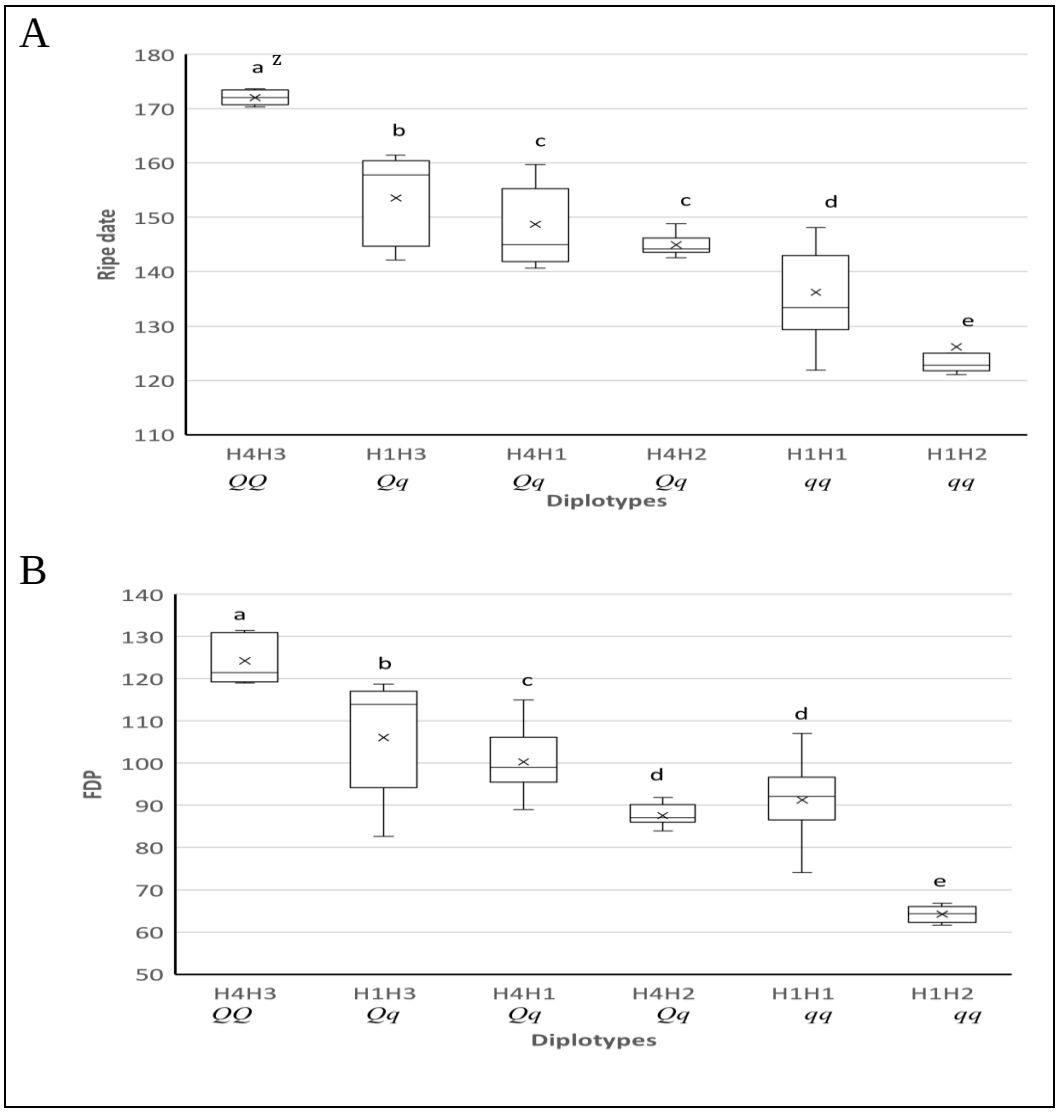

Fig. II-8. Diplotype effect of the most common haplotypes associated with ripe date (RD) (A) and fruit development period (FDP) (B) for the QTLs mapped on LG4.

[z] Means not connected by the same letter are significantly different ($P<0.05$) within each linkage group using HSD test.

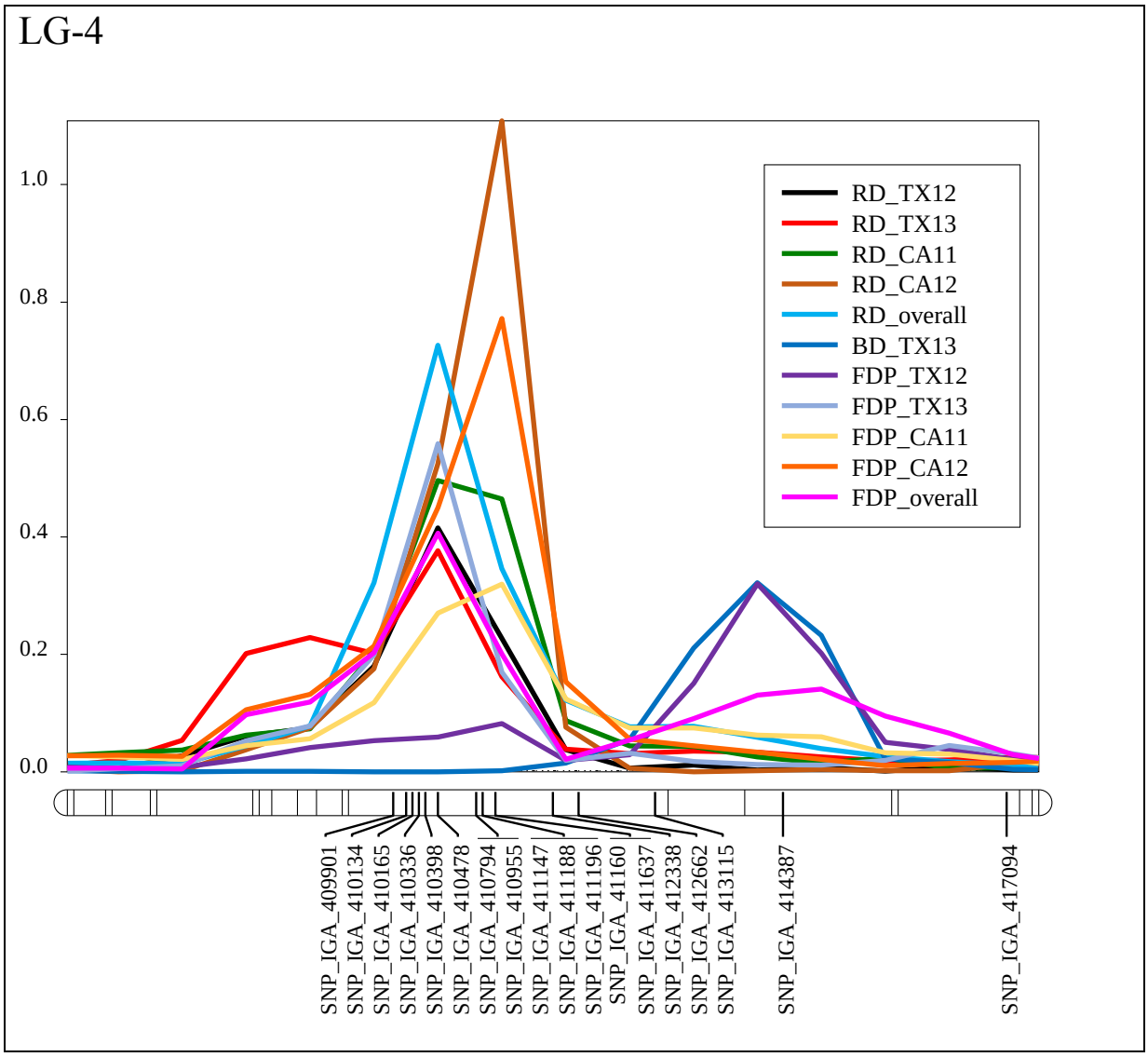

Fig. II-9. Position of putative QTLs controlling the ripe date (RD), bloom date (BD), and fruit development period (FDP) at linkage group 4 (LG-4) from four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean generated using MapChart software (Voorrips, 2002).

Discussion

Bloom date (BD)

In this study, bloom date was moderately (0.44) to highly (0.82) heritable (Table II-1). These results correspond with several studies (de Souza et al., 1998a; de Souza et al., 2000; Hansche, 1990; Hansche et al., 1972; Hernández Mora et al., 2017a; Mowrey and Sherman, 1986), indicating that expression of bloom date is not heavily influenced by environmental effects.

This study mapped QTLs for BD on LG1, 4, and 7 (Table II-1; Fig. II-2). The QTL at the end of LG1 was flanked by SNP_1_46757382 and SNP_IGA_128603, and it spanned the region from ~43.1 – 45.6 Mb and had a PVE from ~17 to 94 % (Table II-2 and 3). Our QTL mapped close to the BD QTL reported by Romeu et al. (2014) who mapped a major QTL for BD close to SNP_IGA_ 122057 marker (~41.2 Mb) at the end of LG1 (PVE ~60 %) and Fan et al. (2010) who mapped the major QTL located close to the marker BPPCT028, at ~45.6 Mb on the peach genome sequence (PVE ~40%).

The QTL at the middle region of LG4 mapped between SNP_IGA_413934 and SNP_IGA_419614, in the interval between ~12 – 13.6 Mb, and had a PVE from 11 to 55 %. The QTL we have detected overlaps with the QTL related to the bloom date on LG4 (qFD4.2) between ~13.1 and 16.0 Mb with nearest markers SNP_IGA_417840 and SNP_IGA_440116 reported by Hernández Mora et al. (2017a).

Lastly, the QTL at the distal end of LG7 was flanked by SNP_IGA_778568 and SNP_7_17628094, spanned the region from ~15.5 to 17.2 Mb and explained ~11 to 18 % of BD phenotypic variation. This finding agreed with Romeu et al. (2014) who found a QTL for BD on

LG7 at the nearest marker SNP_IGA_779224 (~15.7 Mb), which was close to our QTL peak SNP_IGA_780816 (~16.4 Mb).

However, the total PVE of all mapped QTLs (G1, G4, and G7) per environment ranged 46 % (sum of 17, 11, and 18 %) in TX12 to 94 % in CA11, indicating this trait is highly heritable and that key components of the genetic variation were explained in this study.

Overall, the three QTLs mapped on LGs 1, 4, and 7 for BD in this study, were previously mapped at either the same or different genomic regions using different germplasm. Joobeur et al., (1998) mapped four QTLs on LGs 1, 4, 6, and 7 using an almond × peach F_2 population, while Dirlewanger et al., (1999) found QTLs on LG2 and LG7 in a peach F_2 population. A single QTL on LG4 was mapped by Verde et al. (2002) in a peach backcross (BC1) population. Lastly, Romeu et al. (2014) reported two major QTLs on LG1 and LG7 in the 'V6' × 'Granada' progeny. This provides more evidence that there are several genomic regions controlling bloom date in peach. However, the biggest deviation was noticed in CA11 as a single QTL was segregating in LG1 that could be attributed to this environment had several rainy days during the blooming period which hampered the blooming data collection and, subsequently, to a fewer number of records (82).

Ripe date (RD)

Fruit ripe date is of economic importance and is a potential candidate for applying marker-assisted breeding. Several studies were conducted to understand and map the genetic control of this trait.

In this study, the narrow sense heritability was moderate (0.59) to high (0.82) for this trait (Table II-1), suggesting the proportion of variation in this trait within our populations was attributed mainly to genetic component rather than environmental effects. This finding agreed with previous studies (Bailey and Hough, 1959; de Souza et al., 1998a; French, 1951; Frett, 2016; Hernández Mora et al., 2017a; Yu et al., 1997). FlexQTL software mapped a single QTL at the middle part of LG4 for RD that explained 40 to 54 % of the phenotypic variation (Table II-2). This QTL was flanked by SNP _IGA_409901 and SNP _IGA_412662 at ~10.6 and 11.3 Mb. The nearest marker was SNP _IGA_410794, ~10.8 Mb. Our observations are in agreement with Nuñez-Lillo et al. (2015) and Romeu et al. (2014) who also reported QTLs for the ripe date at the SNP_IGA_411147 (~10.9 Mb) and at the SNP_IGA_410398 (~10.7 Mb), respectively, and explained between 52 to 77% of phenotypic variance. In addition, Frett (2016) mapped a major QTL of maturity date (G4MD.1) across four data sets that explained on average 61% of the phenotypic variance. This QTL was located between 10.7 to 11.3 Mbp, and the four SNPs, SNP_IGA_410398, SNP_IGA_411601, SNP_IGA_411637, and SNP_IGA_412662 spanned this QTL that was within our QTL interval (Table II-4; Fig. II-9).

Moreover, Eduardo et al. (2011) and Hernández Mora et al. (2017a) mapped RD QTLs on LG4 ~11.0 - 11.2 Mb and ~11.2 - 14.1 Mb, respectively. A candidate gene (ppa008301m) for a major locus controlling ripe date was identified (Eduardo et al., 2013).

In summary, the QTL controlling RD in this study was at the same genomic region that was reported on LG4 in previous studies and was stable over different genetic backgrounds. This held true using either a single bi-parental (Nuñez-Lillo et al., 2015; Romeu et al., 2014) or multiple populations mapping methods (Frett, 2016; Hernández Mora et al., 2017a; Hernández Mora et al., 2017b).

Fruit development period (FDP)

FDP is often associated with RD and is the trait that has been taking less attention from researchers compared to the blooming and ripe dates (Donoso et al., 2016; Etienne et al., 2002). Fresnedo-Ramírez et al. (2015) and Hernández Mora et al. (2017a) were reported QTLs for FDP on LGs 1, 2, 3, 4, 6, and 8 by applying pedigree-based analysis (PBA).

In our study, the estimated narrow sense heritability for FDP ranged from high (0.67) to very high (0.83) (Table II-1), which agrees with previous studies (de Souza et al., 1998a; 2000; Hansche, 1990; Hansche et al., 1972; Hernández Mora et al., 2017a; Mowrey and Sherman, 1986), suggesting an important additive genetic component for this trait. We found one significant QTLs for FDP on LG4 that passed our threshold criteria, between flanking markers SNP_IGA_409901 and SNP_IGA_412662, spans ~10.6 – 11.3 Mb and explained between 36 – 62 % of the phenotypic variance (Table II-2 and Table II-3). Our results support and validate the QTL positions for FDP previously reported using different peach germplasm by Hernández Mora et al. (2017a) who identified a QTL for FDP at the nearest marker SNP_IGA_412338 (~11.2 Mb) on LG4. Etienne et al. (2002), also mapped the major QTL for FDP ~11.3 Mb and was explained ~76 % of the variation in FDP.

Interestingly, in this study, nine mapped QTLs of RD and FDP traits were clustered at the middle part of LG4 at either the SNP marker SNP_IGA_410794 or SNP_IGA_412662 (Fig.

II-9). This specific genomic region, which has been identified in several previous studies, is associated with RD trait (Dirlewanger et al., 2012; Eduardo et al., 2011; Frett, 2016; Hernández Mora et al., 2017a; Nuñez-Lillo et al., 2015; Romeu et al., 2014). This synchrony between QTLs for RD and FDP was supported by the strong correlation (r=0.87) between these traits which was also supported by previous researches to be co-localized (Etienne et al., 2002; Hernández Mora et al., 2017a). Furthermore, RD QTL on LG4 was previously reported to co-localize with several traits such as soluble solids content (SSC) and blush (Dirlewanger et al., 1999; Eduardo et al., 2011; Hernández Mora et al., 2017a; 2017b). Thus, this cluster could illustrate the presence of a single QTL with pleiotropic effects or a strong link between QTLs of these various traits (Dirlewanger et al., 1999; Kenis et al., 2008). The relationship between RD and SSC can be explained by the fact that the longer the ripe date, the longer there is for the fruit to develop quality and high SSC (Eduardo et al., 2011).

Conclusions

In this study, we identified QTLs associated with three important phenological traits on different linkage groups across four environments and overall analysis for low- medium-chill peach/nectarine germplasm. A single QTL on the central part of LG4 explained the variation for both RD and FDP, which both measure the time to fruit ripe. Furthermore, three QTLs for BD were mapped on LG1, 4, and 7. These results are supported by previous studies, despite a few differences in their genomic positions in some cases.

Pedigree-Based Analysis (PBA) has been successfully used as a statistical method for discovering and validating QTLs by using multiple segregating populations of various sizes connected in a pedigree, which increases the genetic background explored, improves statistical power, and allows the simultaneous detection and validation of QTLs.

Haplotypes associated with BD would help breeders make crossing decisions to pick the combination of parents that have SNP haplotypes associated with lowering BD to produce progeny with better adaptation to subtropical environments like Texas. Regarding the haplotypes associated with RD and FDP, breeders can utilize them for developing peach cultivars with a wide range of ripe dates to extend peach harvest season. Our findings on predictive SNP haplotypes will help peach breeders in developing DNA assays such as simple sequence repeats (SSR), sequence-characterized amplified regions (SCARs), mini SNP arrays, and kompetitive allele-specific PCR (KASP) markers associated with important traits for MAB.

References

Alburquerque, N., F. Garcia-Montiel, A. Carrillo, and L. Burgos. 2008. Chilling and heat requirements of sweet cherry cultivars and the relationship between altitude and the probability of satisfying the chill requirements. *Environ. Exp. Bot.* 64:162-170.

Arús, P., I. Verde, B. Sosinski, T. Zhebentyayeva, and A.G. Abbott. 2012. The peach genome. *Tree Genet. & Genomes* 8:531-547.

Bailey, C.H. and L.F. Hough. 1959. A hypothesis for the inheritance of season of ripening in progenies from certain early ripening peach varieties and selections. *Proc. Amer. Soc. Hort. Sci.* 73:125-133.

Bink, M.C.A.M., M.P. Boer, C.J.F. ter Braak, J. Jansen, R.E. Voorrips, and W.E. van de Weg. 2008. Bayesian analysis of complex traits in pedigreed plant populations. *Euphytica* 161:85-96.

Bink, M.C.A.M., J. Jansen, M. Madduri, R.E. Voorrips, C.E. Durel, A.B. Kouassi, F. Laurens, F. Mathis, C. Gessler, D. Gobbin, F. Rezzonico, A. Patocchi, M. Kellerhals, A. Boudichevskaia, F. Dunemann, A. Peil, A. Nowicka, B. Lata, M. Stankiewicz-Kosyl, K. Jeziorek, E. Pitera, A. Soska, K. Tomala, K.M. Evans, F. Fernandez-Fernandez, W. Guerra, M. Korbin, S. Keller, M. Lewandowski, W. Plocharski, K. Rutkowski, E. Zurawicz, F. Costa, S. Sansavini, S. Tartarini, M. Komjanc, D. Mott, A. Antofie, M. Lateur, A. Rondia, L. Gianfranceschi, and W.E. van de Weg. 2014. Bayesian QTL analyses using pedigreed families of an outcrossing species, with application to fruit firmness in apple. *Theor. Appl. Genet.* 127:1073-1090.

Bink, M.C.A.M., L.R. Totir, C.J.F. Braak, C.R. Winkler, M.P. Boer, and O.S. Smith. 2012. QTL linkage analysis of connected populations using ancestral marker and pedigree information. *Theor. Appl. Genet.* 124:1097-1113.

Blake, M.A. 1932. The J.H. Hale as a Parent in Peach Crosses. *Proc. Amer. Soc. Hort. Sci.* 29:131-136.

Bliss, F.A. 2010. Marker-assisted breeding in horticultural crops. *Acta Hort.* 859:339-350.

Byrne, D.H. 2003. Founding clones of low chilling fresh market peach germplasm developed in the USA and Brazil. *Acta Hort.* 606:17-21.

Byrne, D.H. 2005. Trends in stone fruit cultivar development. *HortTechnol.* 15:494-500.

Byrne, D.H., W.B. Sherman, and T.A. Bacon. 2000. Stone Fruit Genetic Pool and Its Exploitation for Growing under Warm Winter Conditions, p. 157-230. In: A. Erez (ed.), Temperate Fruit Crops in Warm Climates. Springer Netherlands, Dordrecht.

Cantín, C.M., C.H. Crisosto, E.A. Ogundiwin, T. Gradziel, J. Torrents, M.A. Moreno, and Y. Gogorcena. 2010. Chilling injury susceptibility in an intra-specific peach [*Prunus persica* (L.) Batsch] progeny. *Postharvest Biol. Tec.* 58:79-87.

Citadin, I., M.C.B. Raseira, F.G. Herter, and J.B. da Silva. 2001. Heat requirement for blooming and leafing in peach. *HortScience* 36:305-307.

Citadin, I., M.d.C.B. Raseira, A.C. Quezada, and J.B.d. Silva. 2003. Herdabilidade da necessidade de calor para a antese e brotação em pessegueiro. *Revista Brasileira de Fruticultura* 25:119-123.

da Silva Linge, C., L. Antanaviciute, A. Abdelghafar, P. Arús, D. Bassi, L. Rossini, S. Ficklin, and K. Gasic. 2018. High-density multi-population consensus genetic linkage map for peach. *PLOS ONE* 13:e0207724.

de Pascual-Teresa, S., D.A. Moreno, and C. Garcia-Viguera. 2010. Flavanols and anthocyanins in cardiovascular health: A review of current evidence. *Intl. J. Mol. Sci.* 11:1679-1703.

de Souza, V.A.B., D.H. Byrne, and J.F. Taylor. 1998a. Heritability, genetic and phenotypic correlations, and predicted selection response of quantitative traits in peach: I. An analysis of several reproductive traits. *J. Amer. Soc. Hort. Sci.* 123:598-603.

de Souza, V.A.B., D.H. Byrne, and J.F. Taylor. 1998b. Heritability, genetic and phenotypic correlations, and predicted selection response of quantitative traits in peach: II. An analysis of several fruit traits. *J. Amer. Soc. Hort. Sci.* 123:604-601.

de Souza, V.A.B., D.H. Byrne, and J.F. Taylor. 2000. Predicted breeding values for nine plant and fruit characteristics of 28 peach genotypes. *J. Amer. Soc. Hort. Sci.* 125:460-465.

Dirlewanger, E., A. Moing, C. Rothan, L. Svanella, V. Pronier, A. Guye, C. Plomion, and R. Monet. 1999. Mapping QTLs controlling fruit quality in peach (*Prunus persica* (L.) Batsch). *Theor. Appl. Genet.* 98:18-31.

Dirlewanger, E., J. Quero-García, L. Le Dantec, P. Lambert, D. Ruiz, L. Dondini, E. Illa, B. Quilot-Turion, J.M. Audergon, S. Tartarini, P. Letourmy, and P. Arús. 2012. Comparison of the genetic determinism of two key phenological traits, flowering and maturity dates, in three *Prunus* species: peach, apricot and sweet cherry. *Hered.* 109:280-292.

Donoso, J.M., R. Picanol, O. Serra, W. Howad, S. Alegre, P. Arus, and I. Eduardo. 2016. Exploring almond genetic variability useful for peach improvement: mapping major genes and QTLs in two interspecific almond x peach populations. *Mol. Breeding* 36:1-17.

Eduardo, I., G. Chietera, R. Pirona, I. Pacheco, M. Troggio, E. Banchi, D. Bassi, L. Rossini, A. Vecchietti, and C. Pozzi. 2013. Genetic dissection of aroma volatile compounds from the

essential oil of peach fruit: QTL analysis and identification of candidate genes using dense SNP maps. *Tree Genet. & Genomes* 9:189-204.

Eduardo, I., I. Pacheco, G. Chietera, D. Bassi, C. Pozzi, A. Vecchietti, and L. Rossini. 2011. QTL analysis of fruit quality traits in two peach intraspecific populations and importance of maturity date pleiotropic effect. *Tree Genet. & Genomes* 7:323-335.

Egea, J., E. Ortega, P. Martinez-Gomez, and F. Dicenta. 2003. Chilling and heat requirements of almond cultivars for flowering. *Environ. Exp. Bot.* 50:79-85.

Etienne, C., C. Rothan, A. Moing, C. Plomion, C. Bodénès, L. Svanella-Dumas, P. Cosson, V. Pronier, R. Monet, and E. Dirlewanger. 2002. Candidate genes and QTLs for sugar and organic acid content in peach [*Prunus persica* (L.) Batsch]. *Theor. Appl. Genet.* 105:145-159.

Fan, S., D.G. Bielenberg, T.N. Zhebentyayeva, G.L. Reighard, W.R. Okie, D. Holland, and A.G. Abbott. 2010. Mapping quantitative trait loci associated with chilling requirement, heat requirement and bloom date in peach (*Prunus persica*). *New Phytologist* 185:917-930.

FAO. 2018. http://www.fao.org/faostat/en/#data/QC. Apr. 15, 2018

French, A.P. 1951. The peach, inheritance of time of ripening and other economic characters. Mass. Agric. Exp. Sta., Bull. 462.

Fresnedo-Ramírez, J., M. Bink, E. van de Weg, T.R. Famula, C.H. Crisosto, T.J. Frett, K. Gasic, C.P. Peace, and T.M. Gradziel. 2015. QTL mapping of pomological traits in peach and related species breeding germplasm. *Mol. Breeding* 35:166-166.

Frett, T.J. 2016. Genetic determinism of *Xanthomonas arboricola* pv. *pruni* (Xap) resistance, fruit quality, and phenological traits in peach and incorporation of marker-assisted

selection (MAS) in the University of Arkansas peach and nectarine breeding program. University of Arkansas, Fayetteville, AK, Ph.D Dissertation.

Frett, T.J., G.L. Reighard, W.R. Okie, and K. Gasic. 2014. Mapping quantitative trait loci associated with blush in peach [*Prunus persica* (L.) Batsch]. *Tree Genet. & Genomes* 10:367-381.

Hansche, P.E. 1986. Heritability of fruit quality traits in peach and nectarine breeding stocks dwarfed by the dw gene. *HortScience* 21:1193-1195.

Hansche, P.E. 1990. Heritability of Spring Bloom and Fall Leaf Abscission Dates in *Prunus persica*. *HortScience* 25:1639-1641.

Hansche, P.E., C.O. Hesse, and V. Beres. 1972. Estimates of genetic and environmental effects on several traits in peach. *J. Amer. Soc. Hort. Sci.* 97:76-79.

Hartmann, T.P. 2013. Heritability and Phenotypic Correlations in Peach *[Prunus persica* (L.) Batsch]. Texas A&M University, College Station, TX, Master of Science.

Hernández Mora, J.R., D. Micheletti, M. Bink, E. Van de Weg, C. Cantín, N. Nazzicari, A. Caprera, M.T. Dettori, S. Micali, E. Banchi, J.A. Campoy, E. Dirlewanger, P. Lambert, T. Pascal, M. Troggio, D. Bassi, L. Rossini, I. Verde, B. Quilot-Turion, F. Laurens, P. Arús, and M.J. Aranzana. 2017a. Integrated QTL detection for key breeding traits in multiple peach progenies. *BMC Genomics* 18:404.

Hernández Mora, J.R., D. Micheletti, M.C.A.M. Bink, W.E.v.d. Weg, D. Bassi, N. Nazzicari, A. Caprera, M.T. Dettori, S. Micali, E. Dirlewanger, P. Lambert, T. Pascal, E. Banchi, M. Troggio, L. Rossini, I. Verde, B. Quilot-Turion, F. Laurens, P. Arús, and M.J. Aranzana. 2017b. Discovering peach QTLs with multiple progeny analysis. *Acta Hort.* 1172:405-410.

Illumina Inc. 2010. GenomeStudio Genotyping module v1.0, User Guide. Illumina Inc., San Diego, CA, USA.

Joobeur, T., M.A. Viruel, M.C. de Vicente, B. Jáuregui, J. Ballester, M.T. Dettori, I. Verde, M.J. Truco, R. Messeguer, I. Batlle, R. Quarta, E. Dirlewanger, and P. Arús. 1998. Construction of a saturated linkage map for *Prunus* using an almond×peach F_2 progeny. *Theor. Appl. Genet.* 97:1034-1041.

Kass, R.E. and A.E. Raftery. 1995. Bayes Factors. *J. Amer. Stat. Assn.* 90:773-795.

Kenis, K., J. Keulemans, and M.W. Davey. 2008. Identification and stability of QTLs for fruit quality traits in apple. *Tree Genet. & Genomes* 4:647-661.

Monet, R. and Y. Bastard. 1982. Estimation du coefficient de régression enfant/parent de quelques caractères du pêcher dans le cas de familles issues d'autofécondations. *Agronomie* 2:347-358.

Mowrey, B.D. and W.B. Sherman. 1986. Flower bud set and relationship to vigor in 18-month peach seedlings. *Proc. Fla. State Hort. Soc.* 99:209-210.

Nuñez-Lillo, G., A. Cifuentes-Esquivel, M. Troggio, D. Micheletti, R. Infante, R. Campos-Vargas, A. Orellana, F. Blanco-Herrera, and C. Meneses. 2015. Identification of candidate genes associated with mealiness and maturity date in peach [*Prunus persica* (L.) Batsch] using QTL analysis and deep sequencing. *Tree Genet. & Genomes* 11:86.

Ogundiwin, E.A., C.P. Peace, T.M. Gradziel, D.E. Parfitt, F.A. Bliss, and C.H. Crisosto. 2009. A fruit quality gene map of *Prunus*. *BMC Genomics* 10:587.

Peace, C.P., C.H. Crisosto, and T.M. Gradziel. 2005. Endopolygalacturonase: A candidate gene for Freestone and Melting flesh in peach. *Mol. Breeding* 16:21-31.

Peace, C.P., J.J. Luby, W.E. van de Weg, M.C.A.M. Bink, and A.F. Iezzoni. 2014. A strategy for developing representative germplasm sets for systematic QTL validation, demonstrated for apple, peach, and sweet cherry. *Tree Genet. & Genomes* 10:1679-1694.

Pirona, R., I. Eduardo, I. Pacheco, C. Da Silva Linge, M. Miculan, I. Verde, S. Tartarini, L. Dondini, G. Pea, D. Bassi, and L. Rossini. 2013. Fine mapping and identification of a candidate gene for a major locus controlling maturity date in peach. *BMC Plant Biol.* 13:166.

Quilot, B., B.H. Wu, J. Kervella, M. Génard, M. Foulongne, and K. Moreau. 2004. QTL analysis of quality traits in an advanced backcross between *Prunus persica* cultivars and the wild relative species *P. davidiana*. *Theor. Appl. Genet.* 109:884-897.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Romeu, J.F., A.J. Monforte, G. Sánchez, A. Granell, J. García-Brunton, M.L. Badenes, and G. Ríos. 2014. Quantitative trait loci affecting reproductive phenology in peach. *BMC Plant Biol.* 14:52.

Ruiz, D., J.A. Campoy, and J. Egea. 2007. Chilling and heat requirements of apricot cultivars for flowering. *Environ. Exp. Bot.* 61:254-263.

Sánchez-Pérez, R., W. Howad, F. Dicenta, P. Arús, and P. Martínez-Gómez. 2007. Mapping major genes and quantitative trait loci controlling agronomic traits in almond. *Plant Breeding* 126:310-318.

Sansavini, S., A. Gamberini, and D. Bassi. 2006. Peach breeding, genetics and new cultivar trends. *Acta Hort.* 713:23-48.

Scorza, R. and W.B. Sherman. 1996. Peaches, p. 325-440. In: J. Janick and J.N. Moore (eds.). Wiley, New York, NY, USA.

Sorensen, D. and D. Gianola. 2002. Likelihood, Bayesian, and MCMC methods in quantitative genetics. Springer Science & Business Media.

Testolin, R. and G. Cipriani. 2010. Molecular Markers for Germplasm Identification and Characterization. *Acta Hort.* 859:59-72.

Vanderzande, S., J.L. Piaskowski, F. Luo, D.A. Edge Garza, J. Klipfel, A. Schaller, S. Martin, and C. Peace. 2018. Crossing the Finish Line: How to Develop Diagnostic DNA Tests as Breeding Tools after QTL Discovery. *J. Hort.* 05:1-6.

Verde, I., N. Bassil, S. Scalabrin, B. Gilmore, C.T. Lawley, K. Gasic, D. Micheletti, U.R. Rosyara, F. Cattonaro, E. Vendramin, D. Main, V. Aramini, A.L. Blas, T.C. Mockler, D.W. Bryant, L. Wilhelm, M. Troggio, B. Sosinski, M.J. Aranzana, P. Arús, A. Iezzoni, M. Morgante, and C. Peace. 2012. Development and Evaluation of a 9K SNP Array for Peach by Internationally Coordinated SNP Detection and Validation in Breeding Germplasm. *PLOS ONE* 7:e35668.

Verde, I., R. Quarta, C. Cedrola, and M.T. Dettori. 2002. QTL analysis of agronomic traits in a BC1 peach population. *Acta Hort.* 592:291-297.

Vileila-Morales, E.A., W.B. Sherman, C.J. Wilcox, and C.P. Andrews. 1981. Inheritance of short fruit development period in peach. *J. Amer. Soc. Hort. Sci.* 106:399-401.

Voorrips, R.E. 2002. MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. *J. Hered.* 93:77-78.

Voorrips, R.E., M.C.A.M. Bink, J.W. Kruisselbrink, H.J.J.K.-v. Putten, and W.E. van de Weg. 2016. PediHaplotyper: Software for consistent assignment of marker haplotypes in pedigrees. *Mol. Breeding* 36:119.

Weather Underground. 2013. http://www.wunderground.com/history/airport/KFAT/2012/8/10/MonthlyHistory.html. Sep. 10, 2013

Weinberger, J.H. 1948. Influence of temperature following bloom on fruit development period of Elberta peach. *Proc. Amer. Soc. Hort. Sci.* 51:175-178.

Yu, M., R. Ma, and X. Tang. 1997. Inheritance of ripening season in F_1 hybrids of peach. *Jiangsu J. Agr. Sci.* 13:176-181.

Zeballos, J. 2012. Identification of genomic region related to fruit quality traits in peach. Universidad de Lleida, Zaragoza, Spain, MS Thesis.

Zeballos, J.L., W. Abidi, R. Giménez, A.J. Monforte, M.Á. Moreno, and Y. Gogorcena. 2016. Mapping QTLs associated with fruit quality traits in peach [*Prunus persica* (L.) Batsch] using SNP maps. *Tree Genet. & Genomes* 12:37.

CHAPTER III

IDENTIFICATION OF QTLS FOR BLUSH, SOLUBLE SOLIDS CONTENT, AND TITRATABLE ACIDITY TRAITS IN PEACH

Synopsis

Fruit quality traits have a significant effect on consumer acceptance and subsequently on peach (*Prunus persica* L. Batsch) consumption. Pedigree-based analysis (PBA) using Visual FlexQTL software was conducted on seven low to medium chill F_1 families along with the founders and parents. Phenotypic data were collected over two years at a high chill (Fowler, CA) and medium chill (College Station, TX) locations and genotyped using the 9K SNP Illumina array. The objectives of this study were to 1) identify QTL(s) associated with fruit quality traits; 2) estimate QTL genotypes for important breeding parents; 3) identify predictive single-nucleotide polymorphism (SNP) or haplotype alleles for desired QTL alleles; and 4) determine source of the alleles for three important fruit quality traits, namely blush (BL), soluble solids content (SSC), and titratable acidity (TA) through pedigree-based analysis (PBA) on Texas peach/nectarine germplasm. Our analysis detected one major QTL on the central part of LG4 for blush at interval 42 – 44 cM that explained about 20 % of the total phenotypic variance (PVE). A major QTL for TA co-localized with the major locus for low-acid fruit (*D*-locus) at the proximal end of LG5 at 0 - 0 cM. This QTL was consistent across all data sets, explaining about 60 % of the phenotypic variance. There was a QTL at the distal end of LG5 at 52 - 62 cM that was associated with both TA and SSC, that explained about 15 % of the phenotypic variance. In addition, haplotype analyses for these QTLs revealed unique SNP haplotypes that are associated with the predictive SNP marker(s) of desired QTL alleles along with their original sources. Our

findings will help peach breeders develop new predictive DNA-based molecular marker tests by converting the trait linked SNP haplotypes to easy-to-use, (semi) high throughput markers such as simple sequence repeat (SSR), Kompetitive allele-specific PCR (KASP), or Sequence Characterized Amplified Region (SCAR) that can be used routinely in marker-assisted breeding (MAB) for enhancing peach quality traits.

Introduction

Peach [*Prunus persica* (L.) Batsch] is the third most important temperate fruit crop globally in terms of production (FAO, 2018). Peach fruit quality is significant for the industry as it has an impact on consumer preference. Traits of specific peach cultivars such as flesh texture, color, sweetness, acidity, and other organoleptic attributes may affect the consumption (Crisosto, 2002). Most of these quality traits are quantitatively inherited and their genetic control is still unclear (Eduardo et al., 2011). Thus, defining the genetic basis of these traits is essential to understand their genetic control and to develop specific methods to improve breeding efficiency (Peace and Norelli, 2009).

Compared to other tree fruit crops, peach has several advantages to facilitate genetic studies. It has a short juvenile period (Baird et al., 1992; Sosinski et al., 2009), a simple genome in terms of ploidy level and size (265 Mb), has a high-quality reference genome sequence (Verde et al., 2017), and has already been used as a model for genetic studies for Rosaceae crops (Carrasco et al., 2013).

In the last decade, the rate of fresh consumption has decreased from 2.3 to 1.3 kg per capita per year in the U.S. (USDA, 2018). Based on surveys, the lack of consistent quality is a main reason consumers do not purchase peaches (Byrne, 2005). In California, 50% of consumers were unsatisfied with the peaches available (Bruhn, 1995; Crisosto, 2006). The principle

complaints of peach consumers are poor firmness, lack of flavor, low sweetness, and non-ripening fruit (Crisosto and Kader, 2000; Scorza et al., 2004). In addition, consumers are willing to pay more for fruits of better quality (Opara et al., 2007) which is the reason for developing branded fruits that consistently provide high quality (Jaeger, 2006).

The primary reason for poor quality is harvesting at immature stages for storage and shipping reasons (Crisosto, 2002; Crisosto and Costa, 2008; Fideghelli et al., 1998; Sansavini et al., 2006). This negatively affects fruit quality since color, flavor, size, and other internal quality traits are developing while fruit is attached to the tree (Bielenberg et al., 2009). Other reasons for poor quality are a lack of good postharvest handling practices, the need for high yields but not necessarily high quality to make production profitable and the relative ease for selecting for external versus internal fruit traits. Recently, many breeders have become interested in increasing the internal quality of peach cultivars (Crisosto et al., 2006).

Peach fruits in the 1900s were improved in size, appearance, and firmness, but unfortunately, internal qualities traits such as sugar content, antioxidant content, tolerance to IB, and other traits lagged behind (Byrne et al., 2012). Recently, efforts concerning internal qualities have increased (Byrne, 2005; Cantín et al., 2010; 2009a; 2009b; Peace et al., 2006). A better understanding of the inheritance of these quality traits may improve breeding efficiency and thereby accelerate the development of new cultivars with improved fruit quality.

The genetic map construction with quantitative trait loci (QTL) analysis is vital for detecting candidate genes associated with quality traits. In the last two decades, abundant genetic maps of important crops have been established (Collard et al., 2005), including peach (Cantín et al., 2009a; Chaparro et al., 1994; Dirlewanger et al., 2006a; Sosinski et al., 2009; Yamamoto et al., 2005). QTLs of soluble solids content has been mapped to linkage groups (LGs) 2, 3, 4, 5,

and 6 (Abbott et al., 1998; Cantín et al., 2010; Eduardo et al., 2011; Etienne et al., 2002; Hernández Mora et al., 2017a; Quilot et al., 2004) and QTLs for organic acids have been mapped to LGs 1, 2, 4, 5, and 6 (Cantín et al., 2010; Dirlewanger et al., 1999; Etienne et al., 2002; Hernández Mora et al., 2017a). QTLs associated with chilling injury and maturity date were mapped on different LGs with diverse levels of reliability (Cantín et al., 2010; Hernández Mora et al., 2017a; Ogundiwin et al., 2009; Peace et al., 2005).

The blush on the skin surface is an important trait that enhances the aesthetic appeal to consumers. In addition, the anthocyanin compounds that create the skin color may have health benefits as a source of antioxidants (Balasundram et al., 2006; J and Paul, 2000; Sun et al., 2002). Therefore, improving the red blush in peach is an essential element for promoting the peach commercially (Crisosto, 2002; Crisosto and Costa, 2008). Several studies have reported QTLs associated with blush on peach fruits (Cantín et al., 2010; Eduardo et al., 2011; Frett et al., 2014; Quilot et al., 2004; Verde et al., 2002; Yamamoto et al., 2001; Yamamoto et al., 2005) on LG3, LG4, LG5, and LG6. The interval on LG3 where the major QTL for blush (Blush.Pp.ZC-3.1) is located contains the candidate genes for skin and flesh coloration of peach (PprMYB10), apple (MdMYB1/MdMYBA/MdMYB10), and cherry (PavMYB10).

Peach fruits are expected to have a sweet taste, and consumer acceptance is highly associated with ripe soluble solids concentration (RSSC) reaching a plateau at 10-12% for high acid and 15-16% for low acid cultivars (Crisosto and Crisosto, 2005). SSC has low to moderate heritability, which allows for enhancing sugar content even with the environmental, maturity, and production variations (Cantín et al., 2009a). A major QTL of soluble solids content (SSC) was consistently detected in the middle region of LG4 and close to the maturity date (MD) locus in intraspecific populations (Dirlewanger et al., 2006b; Eduardo et al., 2011; Quilot et al., 2004).

Other studies reported that SSC QTLs were found on LG5, LG6 (Dirlewanger et al., 2006b; Fresnedo-Ramírez et al., 2015; Hernández Mora et al., 2017b; Quilot et al., 2004), LG2 (Fresnedo-Ramírez et al., 2015; Hernández Mora et al., 2017b; Verde et al., 2002), and on LG1, 3,7, and 8 (Fresnedo-Ramírez et al. (2015).

Fruit acidity in peach is also one of the important quality traits for both consumers and breeders. Like SSC, acidity impacts consumer acceptance and is considered a major selection criterion for peach breeders (Boudehri et al., 2009; Crisosto, 2002). Many studies have reported that low fruit acidity is associated with the major locus (*D*-locus) which was identified on the proximal end of LG5 (Bliss et al., 2002; Boudehri et al., 2009; Dirlewanger et al., 2006b; 1999; Etienne et al., 2002; Fresnedo-Ramírez et al., 2015). This locus was co-localized with major QTLs for pH, titratable acidity (TA), and other organic acid concentrations (Boudehri et al., 2009). In a population segregating for acidity, the marker CPPCT040 was tightly linked to this locus and could be utilized for marker-assisted breeding, MAB (Boudehri et al., 2009). Several QTLs with minor effect have been mapped on LG1 and LG6 (Dirlewanger et al., 1999) and on LG2, 3, and 7 (Fresnedo-Ramírez et al., 2015).

Although numerous QTLs have been identified using linkage analysis methods for horticultural crops, only a few were translated into diagnostic DNA tests. For example, eight DNA tests were made available and used for several peach traits (Vanderzande et al., 2018). Fruit blush DNA test (Ppe-Rf-SSR) which predicts skin color accumulation is available and used for targeting a major Rf locus on LG3 which explained up to 70% of blush (Sandefur et al., 2017). A DNA test for acidity in peach (CPPCT040) is also available to target the *D*-locus at LG5 (Eduardo et al., 2015). Regarding SSC, no DNA test has been developed yet for this trait, even though several QTLs have been mapped (Abbott et al., 1998; Cantín et al., 2010;

Dirlewanger et al., 1999; Eduardo et al., 2011; Etienne et al., 2002; Hernández Mora et al., 2017a; Hernández Mora et al., 2017b; Quarta et al., 2000; Quilot et al., 2004; Sanchez et al., 2014; Zeballos et al., 2016).

The RosBREED project objective is to implement MAB in five Rosaceae crops, namely apple, strawberry, sweet cherry, tart cherry, and peach focusing on fruit quality traits (Iezzoni et al., 2010). A systematic, standardized, and statistically robust protocol and the FlexQTL program, Pedigree-Based Analysis (PBA) approach with multiple interrelated families allows the discovery of more QTL or QTL-alleles per locus across a range of genetic backgrounds. The output from this analysis increases mapping accuracy and the probability of identifying new and validating known QTLs across various genetic backgrounds (Bink et al., 2014; 2012).

The main goal is to identify QTL(s), to estimate QTL genotypes for important breeding parents, to identify predictive single SNP or haplotype alleles for desired QTL alleles and determine their original source for three important fruit quality traits: soluble solids content, titratable acidity, and blush through PBA on Texas peach/nectarine germplasm. Clarifying the genetic bases of these quality traits is essential for the peach industry because they can change consumer preferences and subsequently may increase fresh peach consumption. We hypothesized that quality-related markers can be developed and utilized by plant breeders in the development of excellent quality peach cultivars. QTLs were previously reported for these traits in germplasm of other breeding programs. Thus, results from this work will help peach breeding programs to design DNA assays linked to these QTLs or genes to be used for MAB

Materials and Methods

Plant materials

This study included 162 seedlings from seven related F_1 families derived from seven parents descending from 12 founders (Fig. III-1). Parents were medium to low chill selections from the Texas A&M University breeding program, and high chill selections from the USDA Stone Fruit Breeding Program in Parlier, CA. The number of seedlings in each family ranged from 8 to 36 with an average size of 20. These seedlings, along with parental genotypes, were budded onto 'Nemaguard' peach rootstocks and planted in College Station, TX, and Fowler, CA. Each site included one replicate of each seedling and three to four replicates of each parent.

Plot establishment and design

The College Station plot was randomized with one replicate of each seedling and four replicates of each parent, whereas planting at the Fowler site was organized by progeny with three replicates of each parent. Trees at College Station were planted in staggered double-rows, with 1.7 meters between rows, and 0.67 meter spacing within rows. Double rows were spaced five meters apart. All trees were trained as a central leader. Trees in the Fowler plot were trained as a two-scaffold ‘Y’ and spaced approximately one meter within rows spaced approximately 4 meters apart. At each location, irrigation, fertilization, pest and weed control, pruning, and fruit thinning were carried out according to typical commercial practice.

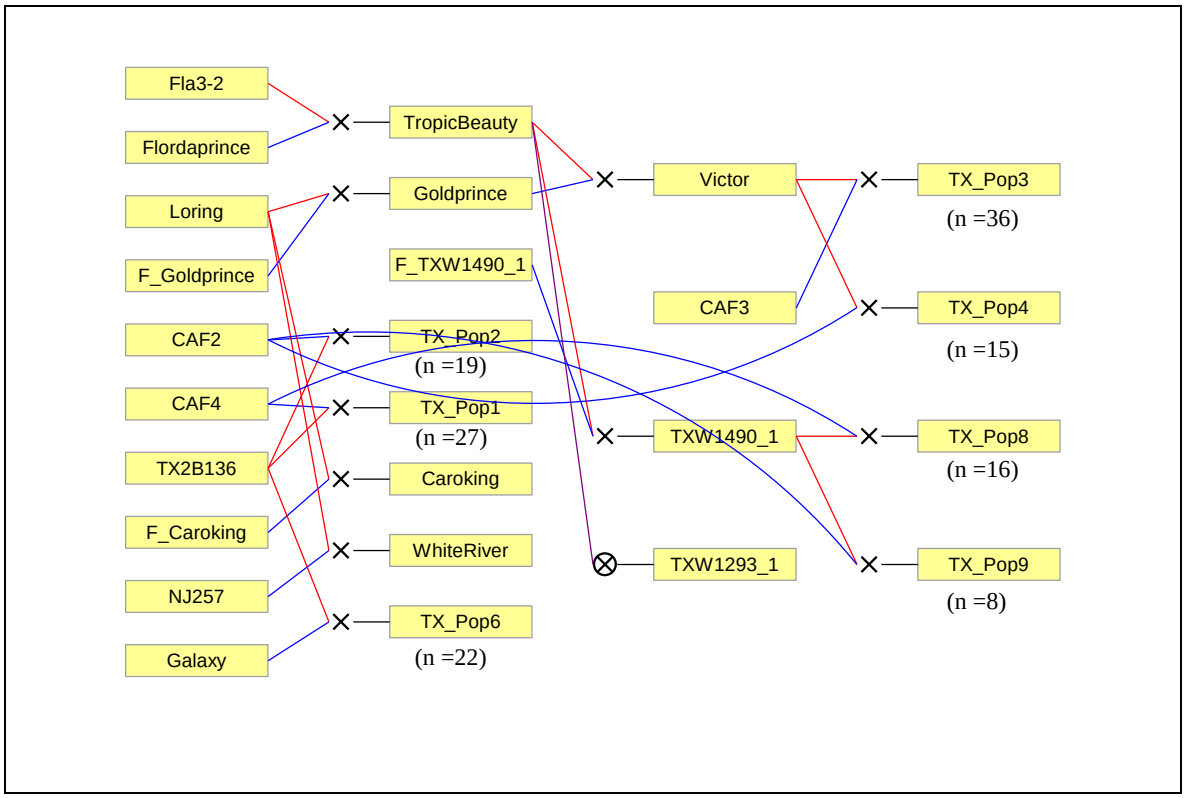

Fig. III-1. Pedigree of the seven peach populations and their progeny number. Red and blue lines link progeny to female and male parents, respectively.

Seedlings and parents were evaluated at the two locations over two years: Fowler, CA for 2011 and 2012, and College Station, TX for 2012 and 2013. College Station is located in east central Texas with a sub-humid and warm temperate climate with mild winters and warm to hot, humid summers. Fowler is located in the San Joaquin Valley in central California and is ideal for peach production with a semi-arid Mediterranean climate. The temperature in the two locations ranged between 4.0 to 36.5° C (Fowler) and between 6.8 – 35.0° C (College Station) for Min Ave. Jan. Temp. and Max Ave Jul. Temp., respectively. College Station has greater rainfall (1022 mm; 248 mm for Fowler), higher humidity (67.5% for College Station; 55.1% for Fowler), more cloudy days (College Station receives 27% less sunlight per year), and warmer night temperatures during fruit development (15.8° C for College Station and 12.4° C for

Fowler) (Weather Underground, 2013). In addition, College Station is more subject to late spring freezes, low chill accumulation and has a heavy textured soil. These environmental factors make College Station much less suitable for stone fruit production as compared to the California site.

Phenotypic evaluations

Data was recorded at both locations across two years (2011-2012 in CA, and 2012-2013 in TX) for all individual trees, on soluble solid content and blush. Titratable acidity was not taken in 2013 in Texas. Following harvest, samples of five fruits were placed in paper bags at College Station and plastic zip-lock bags at Fowler and placed in cold storage at 1 - 4° C for later evaluation. A five firm fruit sample was used for soluble solids content, titratable acidity, and blush evaluations.

Subjective scales were used to evaluate fruit blush (0 - 5 scale, 0 = <1%, 1 = 1% to 20%, 2 = 21% to 50%, 3 = 51% to 80%, 4 = 81% to 99%, and 5 = 100% red blush on fruit surface) as described by Frett (2016). For biochemical traits, a longitudinal slice of the fruit, approximately 2 cm wide, was taken to extract juice with a juicer for the measurement of soluble solids concentration (SSC) using a digital refractometer, and to measure titratable acidity (TA) using an automatic titrator (DL 22 Food and Beverage analyzer, Mettler Toledo, Columbus, OH, USA). TA was obtained by the titration of 2 mL peach juice to pH 8.2 with 0.1N NaOH and expressed as milliequivalents of malic acid. The following equation was used to calculate titratable acidity (the milliequivalent factor used corresponded to malic acid, 0.067) (Gasic et al., 2010):

$$TA = \frac{[NaOH\ titrated\ (ml) \times 0.1\ N\ (NaOH) \times milliequivalent\ factor \times 100}{6\ g\ of\ juice}$$

SNP genotyping and genetic linkage map

Individuals were previously genotyped as part of the US Peach Crop Reference Set and Breeding Pedigree Set established in the RosBREED project (Peace et al., 2014) using the IPSC 9K SNP array for peach (Verde et al., 2012). The raw iScan data from 9K SNP array was initially processed into the GenomeStudio software v2010.3 (Illumina Inc., 2010) using the Genotyping Module with a Gen Call threshold of 0.15. Parentage records and SNP data curation was performed following the procedures described in the pipeline recently developed for apple, sweet cherry and peach (Vanderzande et al., 2018). A subset of 4,005 informative SNPs that showed no null alleles and no parentage conflicts were retained after the pipeline steps. Genetic positions of the selected SNPs were obtained using the peach consensus map as a reference (da Silva Linge et al., 2018). In view of computing time, we decreased the number of markers by eliminating markers at identical map positions, which resulted in 1,487 informative SNP markers spread over the eight LGs.

QTL detection

Genotypic and phenotypic data for BL, SSC, and TA traits for all seedlings and seven parents were combined and analyzed by applying FlexQTL software (Bink et al., 2014). The software utilizes a Bayesian approach to estimate the number of QTLs. FlexQTL analyses were conducted three times, on data from each location and the overall (mean of both locations) with different chain length, prior and maximum QTL number to reach an effective chain size (ECS) (Sorensen and Gianola, 2002) of at least 100 for phenotypic mean, residual variance and number of QTLs as needed to make sound inferences and conclusions. The length of Markov Chain Monte Carlo (MCMC) simulations varied between 100,000 and 1,500,000 iterations, from which thousand simulations were sampled for statistical inference, thus storing one sample every

hundred to three thousand iterations for subsequent inference. The analysis was carried out with an additive genetic model and QTLs were detected as twice the natural logarithm of the obtained of Bayes Factors (BF) [*2ln(Bf)*] as described by Kass and Raftery (1995). The evidence of QTLs presence can be interpreted as hardly any (BF < 2), positive (2 ≤ BF < 5), strong (5 ≤ BF < 10), and decisive (BF ≥ 10). QTL intervals were defined as a series of successive 2-cM bins with intensities corresponding to 2lnBF > 2. For inferences on the number of QTLs, we considered loci that had a 2lnBF greater than 5 for at least one data set, or that had a 2lnBF greater than 2 for at least two independent data sets and being co-localized within ± 2 cM for identified regions, with effective chain size (ECS) at least 100, and explaining at least 15% of the phenotypic variation.

In addition, trace and intensity plots for each trait were evaluated to determine QTLs reliability. Additive variance (σ_A^2) for each trait was calculated by subtracting the residual variance (σ_e^2) from the phenotypic variance (σ_P^2) and the narrow sense heritability (h^2) was calculated as follows:

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2}$$

The proportion of phenotypic variance explained (PVE) by each QTL was estimated from FlexQTL output by dividing the additive variance explained by the QTL region ('AVt1') by the total phenotypic variance of the trait ('variance') as follows:

$$PVE = \frac{\sigma_A^2}{\sigma_P^2} \times 100 \quad \text{where } \sigma_A^2 = additive\ variance\ (AVT1)\ of\ QTL$$

Mapped QTLs were denoted with two or three letters for the trait, two letters and two-digit number representing location and evaluation year, and the letter ‘G’ followed by the linkage group number. The three parts of denotation separated by an underscore. If more than one QTL was present in the same genomic region, the letter a, b or c was added.

SNP haplotypes and QTL genotypes of important breeding parents

Considering the 1,487 informative SNP markers subset, SNPs within the interval of a significant QTL were chosen for haplotyping. Haplotypes were constructed across the germplasm using PediHaplotyper (Voorrips et al., 2016) as an R package (R Core Team, 2018) including, as input files, ‘mhaplotypes.csv’, ‘flexqtl.par’, ‘flexqtl.sort’, and ‘HaploBlocks.map’ files obtained from FlexQTL.

The non-parametric Kruskal-Wallis test with the Steele–Dwass nonparametric multiple comparison test ($P < 0.05$) using JMP Pro Version 13.2 (SAS Institute Inc., Cary, NC, 2016) was conducted to determine differences among haplotype and diplotype effects. Haplotype effects were deduced from combinations of diplotypes. For instance, the effects of haplotypes H1 and H2 can be obtained by comparing the effects of 3H|H1 and 3H|H2 diplotypes. Then, haplotypes were assigned to QTL allele categories (Q or q) based on the direction of their effects by increasing or decreasing of phenotypic values for each trait. Lastly, QTL genotypes were assigned to each individual based on its SNP haplotypes as *QQ*, *Qq*/*qQ*, and *qq*. Allele sequence of haplotypes and QTL genotypes along with pedigree records allowed the tracing back of favorable alleles to the original sources.

Results

Genome-wide QTL analysis

Blush (BL)

Narrow sense heritability (h^2) of blush ranged between low (0.31) for BL_CA11 to moderate (0.55) for BL_overall. The FlexQTL software has mapped several candidate QTLs associated with the blush on four different linkage groups (LG1, 4, 5, and 6) in different environments and the overall analysis. Among the candidate QTLs, the one located on LG4 passed our pre-defined inclusion threshold, showing strong to decisive evidence in each year and each location, except for 2011 when it did not give any signal (Table III-1; Fig. III-2).

The proportion of phenotypic variation explained (PVE) by the QTL detected on LG4 ranged between 20% and 32%, while the posterior QTL intensity was between 0.24 – 0.92, and the additive effect ranged between 0.53 and 0.63. (Table III-2). Peaks for the BL QTL co-localized across locations and years, having their mode at 42 and 44 cM on LG4, within the interval between 42 to 44 cM corresponding with the coordinates 10,582,092 to 11,208,347 bp on the peach genome sequence (Table III-2 and Table III-3; Fig. III-3).

Soluble solids content (SSC)

The narrow sense heritability (h^2) of SSC ranged between low (0.29) for SSC_CA11 to moderate (0.47) for SSC_CA12 (Table III-1). FlexQTL software detected several putative QTLs associated with SSC on three LGs across the environments and in the overall analysis, except for TX 2012. That could be attributed to a low number of records in this environment (n=53) (Fig. III-1). The QTL located at the distal end of LG5 showed consistency across the environments and in overall analysis with its reliability supported by trace plot patterns (Fig. III-4).

The proportion of phenotypic variation explained (PVE) by the QTL on LG5 ranged from 17 to 39% (Table III-2). The highest posterior QTL intensity (0.98) was for SSC_CA12 and the lowest (0.27) was for SSC_CA11. The highest additive effect (2.32° Brix) was associated with SSC_TX13. Interestingly, peaks of the SSC QTL co-localized across locations and years, having their mode at 54 and 62 cM at the distal end of LG5, within the interval from 52 to 62 cM and the physical position between 14,538,721 to 18,236,497 bp on the peach genome sequence v2.0 (Table III-2 and Table III-3; Fig. III-5).

Titratable acidity (TA)

The narrow sense heritability (h^2) of TA ranged between moderate (0.44) in TA_TX12 to high (0.88) in TA_CA12 (Table III-1). Three putative QTLs were detected for TA on LG5: one to three QTLs per environment. A QTL on the proximal end was common to all three environments (TA data was not taken for TX 2013) examined and the overall analysis (Table III-2; Fig. III-6). A second QTL on the distal end was common between TA_CA12 environment and TA_overall. The remaining QTL was environmental specific and was located on a more central part of LG5 and mapped only in CA11.

Table III-1. QTL mapped for the blush (BL), soluble solids content (SSC), and titratable acidity (TA) traits evaluated in four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean for 162 peach seedlings.

									2ln(BF)		
Trait	***MCMC***	***Records***	μ	σ^2_p	σ^2_e	σ^2_A	h^2	***LG***	***1/0***	***2/1***	***3/2***
BL_CA11	150,000	103	3.08	0.56	0.38	0.18	0.31	1	2.6	0.6	0.3
BL_CA12	150,000	138	2.79	0.60	0.29	0.31	0.52	4	13.2	1.1	0.8
								5	2.4	1.8	0.0
								6	3.9	1.0	-0.2
BL_TX12	150,000	62	3.18	0.62	0.41	0.20	0.33	4	5.7	0.9	0.8
BL_TX13	150,000	110	3.48	0.83	0.49	0.33	0.40	4	5.1	1.7	1.6
BL_overall	100,000	143	3.06	0.47	0.21	0.26	0.55	4	16.1	1.6	-0.5
								6	2.0	1.1	-0.9
SSC_CA11	100,000	105	11.87	4.94	3.52	1.42	0.29	5	2.6	0.9	na
SSC_CA12	100,000	137	11.61	3.35	1.79	1.56	0.47	5	13.8	4.0	1.3
SSC_TX13	100,000	111	12.84	6.63	4.59	2.04	0.31	4	2.3	0.4	0.8
								5	9.6	1.0	0.1
SSC_overall	100,000	137	11.90	2.46	1.43	1.03	0.42	4	6.1	0.3	-2.0
								5	11.8	0.9	-0.5
TA_CA11	150,000	95	0.78	0.14	0.03	0.11	0.80	5	na	30.4	4.7
TA_CA12	1,500,000	131	0.71	0.14	0.02	0.12	0.88	5	na	29.7	6.2
TA_TX12	150,000	43	0.55	0.06	0.04	0.03	0.44	5	5.9	0.1	-0.6
TA_overall	100,000	137	0.72	0.13	0.02	0.11	0.84	5	na	28.4	7.9

Markov chain Monte Carlo (MCMC) run length, phenotypic mean (μ), phenotypic variance (σ^2_P), residual variance(σ^2_e), additive variance(σ^2_A), narrow-sense heritability (h^2), the linkage groups (LG) that QTLs were mapped on, and the QTL evidence [*2ln(BF)*] which is hardly any (0-2); positive (2-5); strong (5-10); and decisive (>10).

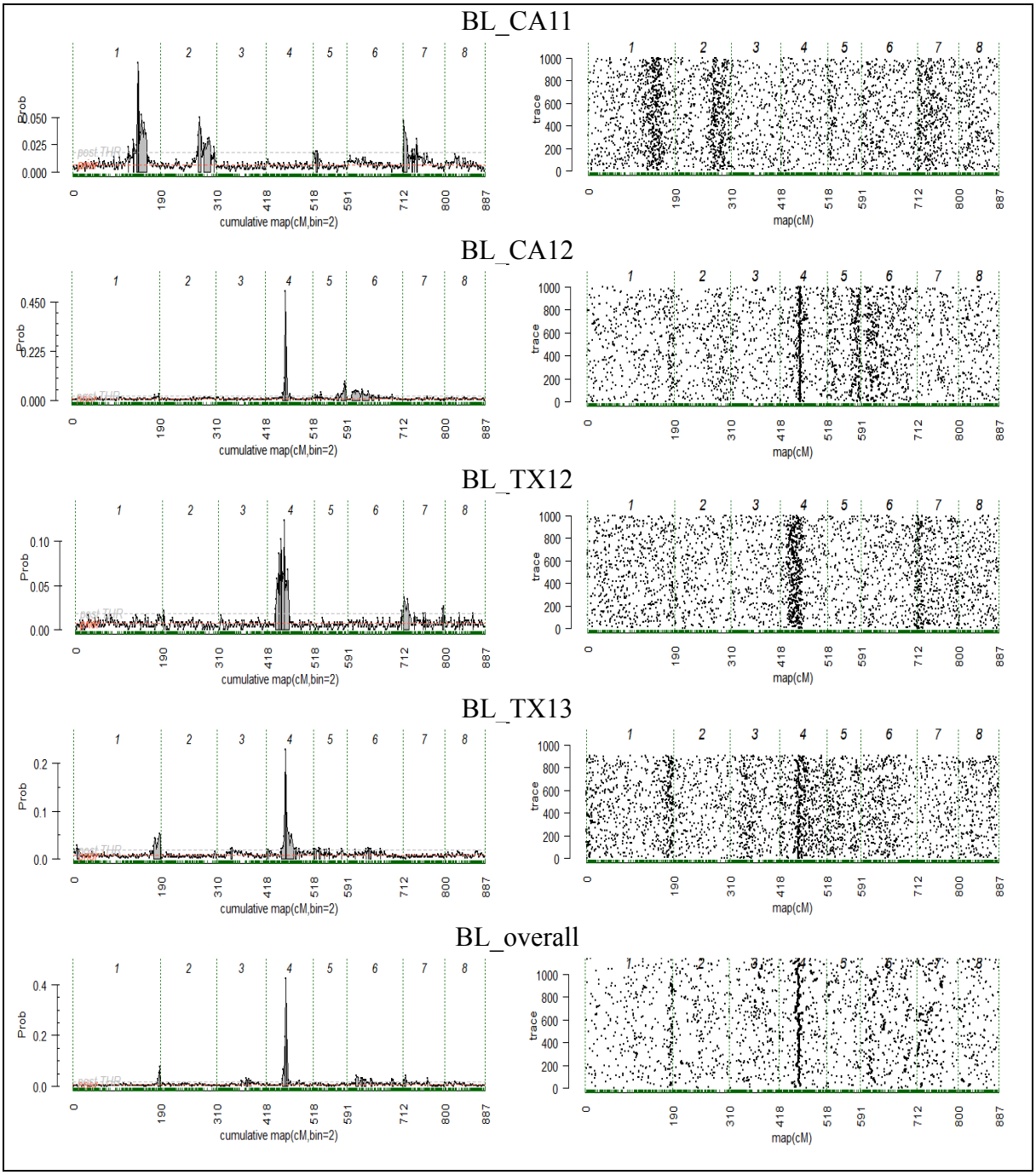

Fig. III-2. Posterior positions (left) and trace samples QTL positions (right) based on an additive model performed using Visual FlexQTL software (Bink et al., 2008) for the blush (BL) from four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean for 162 peach seedlings.

Table III-2. QTL name, linkage group, interval, mode peak, intensity, additive effect, and phenotypic variance explained (PVE) for the blush (BL), soluble solids content (SSC), and titratable acidity (TA) traits evaluated in four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean for 162 peach seedlings.

QTL name	***Linkage Group***	***Interval (cM)***	***Mode peak (cM)***	***Intensity***	***Additive Effect***	***PVE (%)***
BL_CA12_G4	4	[42, 44]	44	0.92	0.63	32
BL_TX12_G4	4	[42, 44]	44	0.24	0.62	31
BL_TX13_G4	4	[42, 44]	42	0.43	0.57	20
BL_overall_G4	4	[42, 44]	44	0.85	0.53	30
SSC_CA11_G5	5	[54, 62]	62	0.27	1.31	17
SSC_CA12_G5	5	[54, 62]	62	0.98	1.27	22
SSC_TX13_G5	5	[52, 62]	54	0.91	2.32	38
SSC_overall_G5	5	[54, 62]	62	0.91	1.42	39
TA_CA11_G5.a	5	[0, 0]	0	1.04	0.53	85
TA_CA11_G5.b	5	[18, 30]	24	0.78	0.22	11
TA_CA12_G5.a	5	[0, 0]	0	0.95	0.43	61
TA_CA12_G5.b	5	[56, 62]	62	0.96	0.26	21
TA_TX12_G5	5	[0, 0]	0	0.66	0.32	72
TA_overall_G5.a	5	[0, 0]	0	1.05	0.40	59
TA_overall_G5.b	5	[54, 62]	62	0.94	0.26	18

For each QTL reported, the evidence [*2ln(BF)*] is either positive (2-5), strong (5-10) or decisive (>10).

Table III-3. QTL name, linkage group, along with SNP name, genetic position, and physical location of flanking markers and nearest marker to the center of the mode for the blush (BL), soluble solids content (SSC), and titratable acidity (TA) traits evaluated in four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean for 162 peach seedlings.

		Flanking markers			***Nearest marker***		
QTL name	***Linkage group***	***Name***	***Genetic position (cM)***	***Physical location***	***Name***	***Genetic position (cM)***	***Physical location***
BL_CA12_G4	4	SNP_IGA_409901	41.98	10,582,092	SNP_IGA_410794	43.31	10,890,653
		SNP_IGA_412338	44.97	11,208,347			
BL_TX12_G4	4	SNP_IGA_409901	41.98	10,582,092	SNP_IGA_410794	43.31	10,890,653
		SNP_IGA_412338	44.97	11,208,347			
BL_TX13_G4	4	SNP_IGA_409901	41.98	10,582,092	SNP_IGA_410165	42.41	10,641,209
		SNP_IGA_412338	44.97	11,208,347			
BL_overall_G4	4	SNP_IGA_409901	41.98	10,582,092	SNP_IGA_410794	43.31	10,890,653
		SNP_IGA_412338	44.97	11,208,347			
SSC_CA11_G5	5	SNP_IGA_600072	53.16	14,538,721	SNP_IGA_604283	62.44	18,236,497
		SNP_IGA_604283	62.44	18,236,497			
SSC_CA12_G5	5	SNP_IGA_600509	54.45	14,888,402	SNP_IGA_603627	62.44	17,197,419
		SNP_IGA_604283	62.44	18,236,497			
SSC_TX13_G5	5	SNP_IGA_600072	53.16	14,538,721	SNP_5_15254637	55.49	15,249,344
		SNP_IGA_604283	62.44	18,236,497			
SSC_overall_G5	5	SNP_IGA_600072	53.16	14,538,721	SNP_IGA_602331	61.15	16,550,893
		SNP_IGA_604283	62.44	18,236,497			

Table III-3 Continued

QTL name	*Linkage group*	*Flanking markers*			*Nearest marker*		
		Name	*Genetic position (cM)*	*Physical location*	*Name*	*Genetic position (cM)*	*Physical location*
TA_CA11_G5.a	5	SNP_IGA_544428	0.00	557,504	SNP_IGA_548512	0.00	1,503,387
		SNP_IGA_550475	0.00	2,028,804			
TA_CA11_G5.b	5	SNP_IGA_589371	18.84	10,557,562	SNP_IGA_593629	24.34	11,738,391
		SNP_IGA_594279	30.94	12,884,759			
TA_CA12_G5.a	5	SNP_IGA_544428	0.00	557,504	SNP_IGA_546316	0.00	1,049,936
		SNP_IGA_550475	0.00	2,028,804			
TA_CA12_G5.b	5	SNP_5_15254637	55.49	15,249,344	SNP_IGA_603627	62.44	17,197,419
		SNP_IGA_604283	62.44	18,236,497			
TA_TX12_G5	5	SNP_IGA_544428	0.00	557,504	SNP_IGA_544961	0.00	698,215
		SNP_IGA_550475	0.00	2,028,804			
TA_overall_G5.a	5	SNP_IGA_544428	0.00	557,504	SNP_IGA_546316	0.00	1,049,936
		SNP_IGA_550475	0.00	2,028,804			
TA_overall_G5.b	5	SNP_IGA_600072	53.16	14,538,721	SNP_IGA_603627	62.44	17,197,419
		SNP_IGA_604283	62.44	18,236,497			

For each QTL reported, the evidence [*2ln(BF)*] is either positive (2-5), strong (5-10) or decisive (>10).

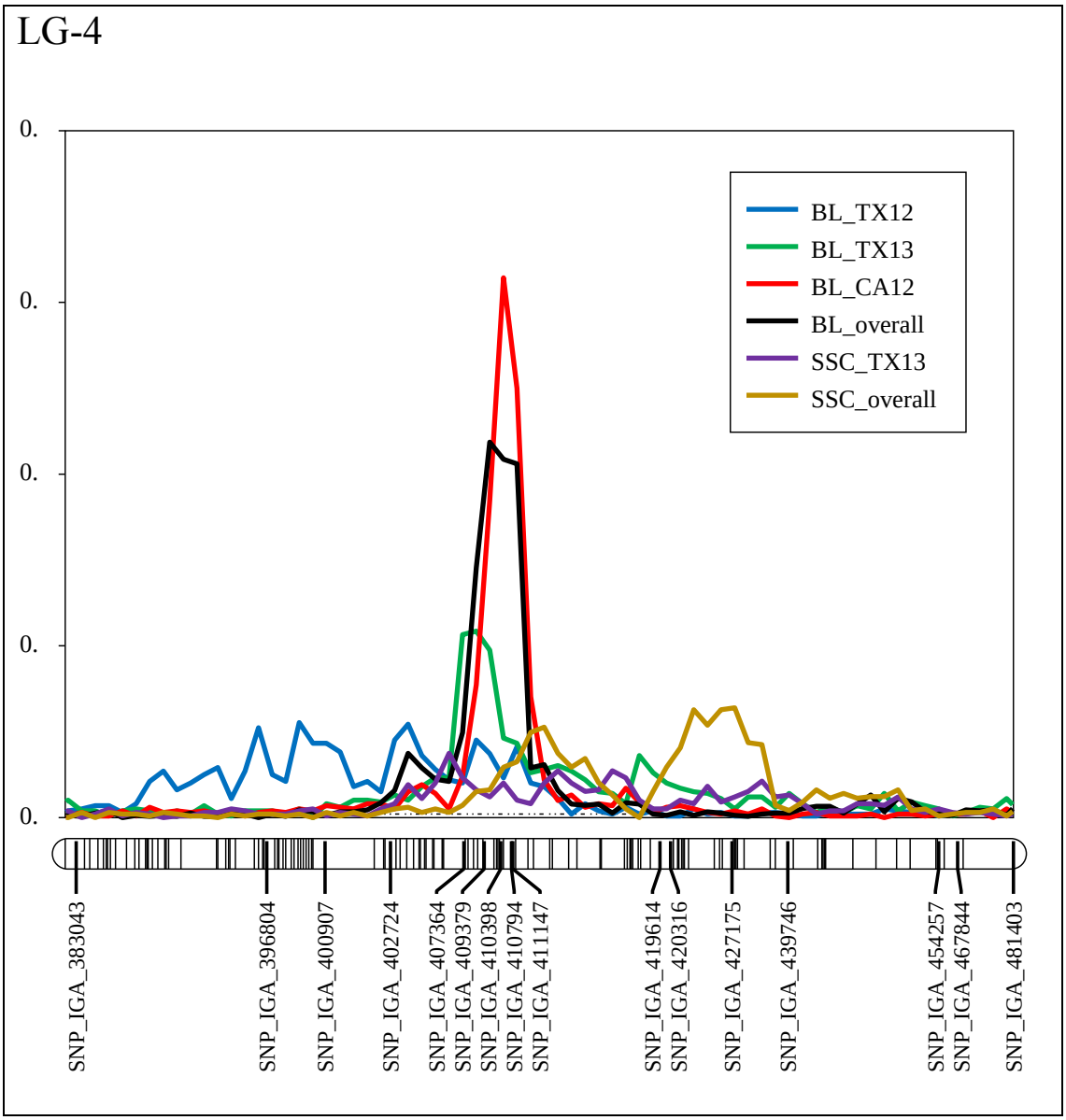

Fig. III-3. Position of putative QTLs controlling the blush (BL), soluble solids content (SSC), and titratable acidity (TA) traits at linkage group 4 (LG4) from four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean generated using MapChart software (Voorrips, 2002).

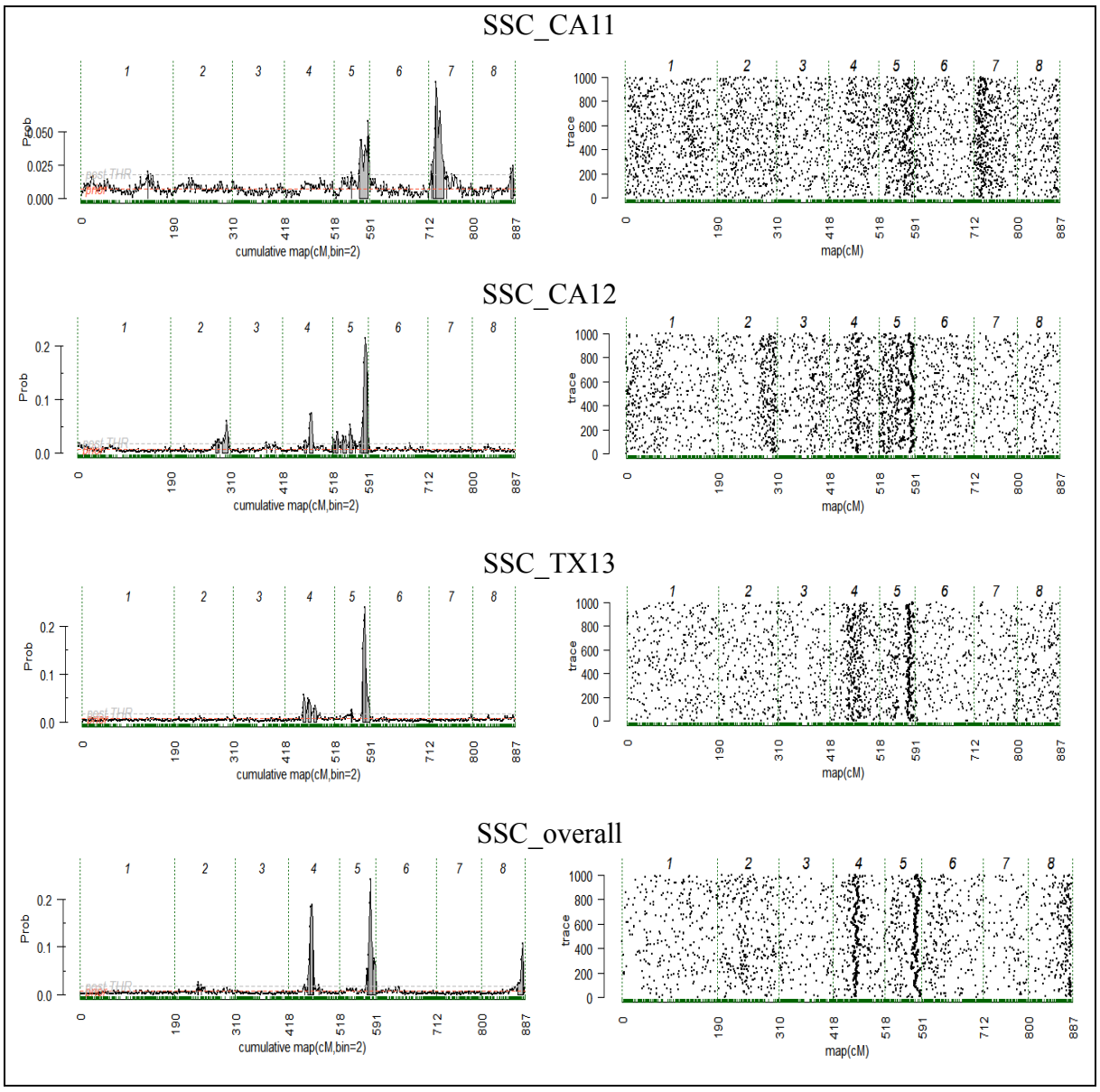

Fig. III-4. Posterior positions (left) and trace samples QTL positions (right) based on an additive model performed using Visual FlexQTL software (Bink et al., 2008) for the soluble solids content (SSC) from four environments (CA 2011, CA 2012, TX 2013), and the overall combined mean for 162 peach seedlings.

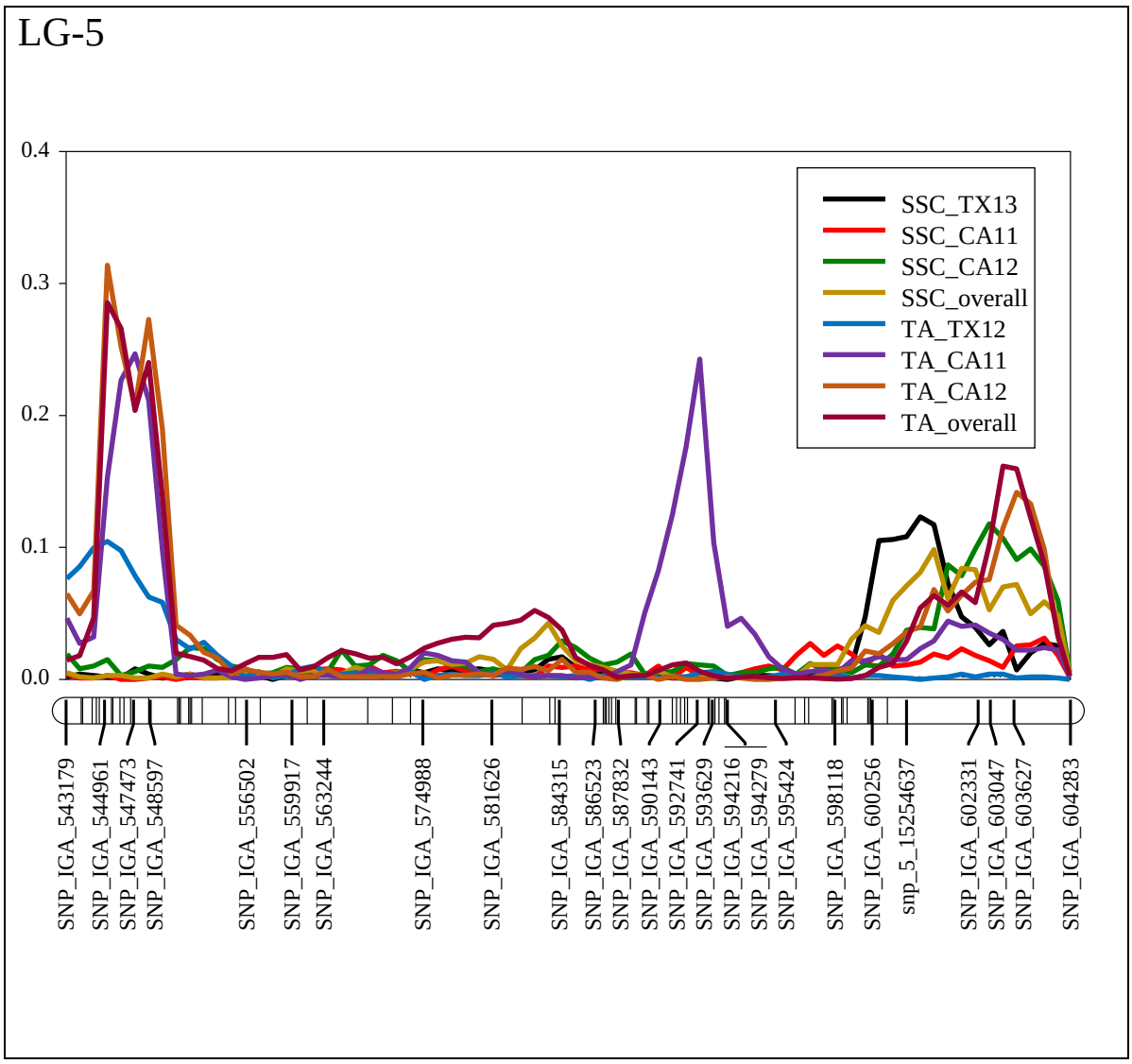

Fig. III-5. The position of putative QTLs controlling the soluble solids content (SSC), and titratable acidity (TA) traits at linkage group 5 (LG5) from four environments (CA 2011, CA 2012, TX 2012, TX 2013), and the overall combined mean generated using MapChart software (Voorrips, 2002).

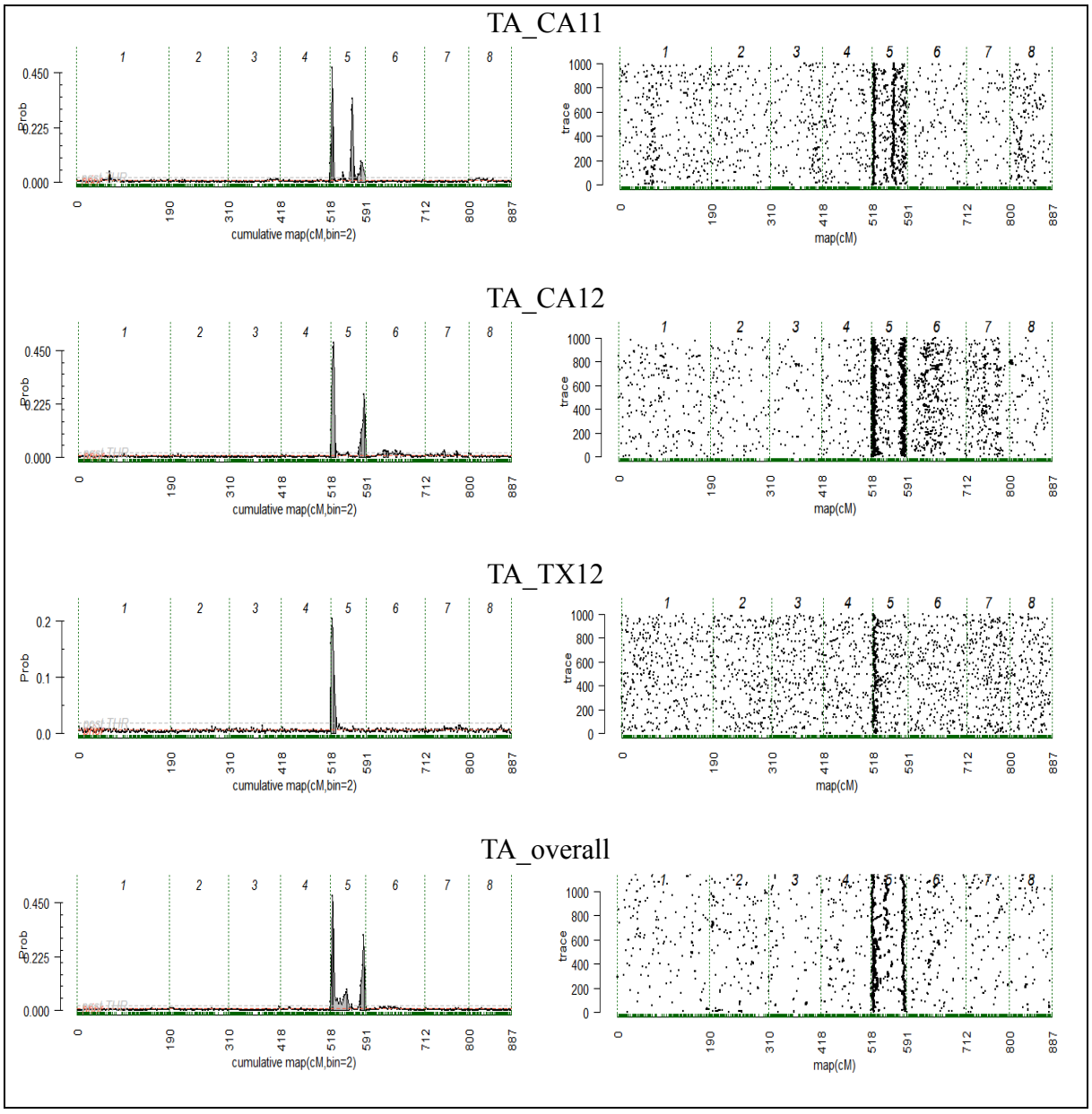

Fig. III-6. Posterior positions (left) and trace samples QTL positions (right) based on an additive model performed using Visual FlexQTL software (Bink et al., 2008) for the titratable acidity (TA) from three environments (CA 2011, CA 2012, TX 2012), and the overall combined mean for 162 peach seedlings.

The highest PVE value for TA was recorded for QTLs detected at the proximal end of LG5 (59 to 85%), while the QTLs at the distal end of LG5 had a PVE between 18 - 21% (Table III-2). The QTL at the proximal end of LG5 was within the interval from 0 - 0 cM and in the interval from 557,504 – 2,028,804 bp of the peach genome sequence v2.0, with a peak at 0 cM (Table III-3; Fig. III-5). The peak of the QTL on the distal end of LG5 was localized at 62 cM, within the interval between 52 - 62 cM and chromosomal position of 14,538,721 - 18,236,497 bp on the peach genome sequence v2.0 (Table III-2 and Table III-3). The highest posterior QTL intensity (1.05) was associated with TA_overall_G5.a, and the lowest intensity (0.66) was recorded for TA_TX12_G5, while the highest value of additive effect (0.53) was recorded for TA_CA11_G5.a (Table III-2).

Haplotype characterization, predictive markers, and validation of QTL effects

Blush (BL)

A total of 14 SNPs in the predicted QTL region of blush (41.98 - 44.97 cM) chosen for haplotyping, revealed four unique SNP haplotypes across the seven parents in which H1 and H3 were high prevalence haplotypes among these parents (Table III-4). The estimation of the diplotype effect showed that the lower blush mean values of individuals were for diplotypes containing H1, H3, and H4. The diplotypes for H4H1 and H1H1 were 2.6 and 3.1 (rating scale of 0 - 5), respectively Fig. III-7) suggesting that these haplotypes may be associated with *q*-alleles for decreasing fruit blush. These haplotypes were present in all important breeding parents as homozygous *qq* alleles except for ‘CAF3’ that was heterozygous *Qq*-alleles. The higher blush was seen in genotypes with the diplotypes of H1H2 and H4H2 (*Qq*-alleles) which had blush ratings of 3.8 and 3.5, respectively. Thus, H2 was the only haplotype associated with high blush

values in our germplasm and was assigned to *Q*-allele that was found only in 'CAF3'. We were unable to estimate the diplotype effect H2H2 because our germplasm did not have the *QQ*-allele combination. This H2 haplotype could be differentiated from all other haplotypes by the B-alleles of two adjacent SNP markers SNP_IGA_409901 (41.98 cM, ~10.6 Mb) and SNP_IGA_410134 (42.41 cM, ~10.6 Mb) (Table III-5). These favorable SNP alleles have been inherited from 'CAF3' that was considered as a founder in this study because we could not trace it back to its original source as earlier generations do not exist anymore. However, the A-allele at SNP marker SNP_IGA_409901 (41.98 cM, ~10.6 Mb) of H1 and H4, and the B-allele at SNP marker SNP_IGA_411188 (43.31 cM, 10.9 Mb) of H3, were associated with *q*-alleles for decreasing fruit blush.

We were unable to trace the source of the homozygous *q*-allele of 'CAF2', 'CAF4', 'Galaxy', and TX2B136 as they are considered as founders. On the other hand, the *q*-allele of both 'Victor' and TXW1490-1 was inherited from the maternal parent 'TropicBeauty' and derived from 'Fla3-2', while the *q*-allele of their paternal parents was traced back to the unknown paternal parents of 'Goldprince' and TXW1490-1, respectively.

Table III-4. QTL genotypes for blush (BL), soluble solids content (SSC), and titratable acidity (TA) traits of seven important breeding parents, with associated linkage groups, haplotype names, SNP haplotypes, and origin sources. QTL alleles for each parent cultivar are presented with ♀ and ♂ for maternal and paternal parent sources, respectively. Allele(s) for predictive SNP marker(s) associated with *Q* or *q*-alleles for increasing or decreasing a given trait, respectively, are shown in **underscored bold.**

Trait/LG	Parents	QTL allele	Hap.	SNP haplotype Allele sequence	Successive ancestors (founders in bold)
BL/LG4	CAF3	*Q* ♀	H2	**BB**BBBBBBBABBBB	**CAF3**
	CAF3	*q* ♂	H3	BAAAAAAAA**B**AAAA	**CAF3**
	CAF4	*q* ♂	H3	BAAAAAAAA**B**AAAA	**CAF4**
	Galaxy	*q* ♀	H3	BAAAAAAAA**B**AAAA	**Galaxy**
	CAF2	*q* ♀	H1	**A**BBBBBBBABBBB	**CAF2**
	CAF2	*q* ♂	H1	**A**BBBBBBBABBBB	**CAF2**
	CAF4	*q* ♀	H1	**A**BBBBBBBABBBB	**CAF4**
	Galaxy	*q* ♂	H1	**A**BBBBBBBABBBB	**Galaxy**
	Victor	*q* ♀	H4	**A**AABABAABAABBB	TropicBeauty > **Fla3-2**
	Victor	*q* ♂	H1	**A**BBBBBBBABBBB	Goldprince > **F_Goldprince**
	TX2B136	*q* ♀	H1	**A**BBBBBBBABBBB	**TX2B136**
	TX2B136	*q* ♂	H1	**A**BBBBBBBABBBB	**TX2B136**
	TXW1490_1	*q* ♀	H4	**A**AABABAABAABBB	TropicBeauty > **Fla3-2**
	TXW1490_1	*q* ♂	H1	**A**BBBBBBBABBBB	**F_TXW1490_1**
SSC/LG5	CAF2	*Q* ♀	H1	BBBA**AB**BB	**CAF2**
	CAF3	*Q* ♀	H1	BBBA**AB**BB	**CAF3**
	CAF3	*Q* ♂	H1	BBBA**AB**BB	**CAF3**
	CAF4	*Q* ♀	H1	BBBA**AB**BB	**CAF4**
	CAF4	*Q* ♂	H1	BBBA**AB**BB	**CAF4**
	Galaxy	*Q* ♂	H1	BBBA**AB**BB	**Galaxy**
	CAF2	*Q* ♂	H2	BBBA**AB**BA	**CAF2**
	TX2B136	*Q* ♂	H6	AAAB**AB**BB	**TX2B136**
	Victor	*q* ♀	H3	AAABBBAB	TropicBeauty > **Fla3-2**
	TX2B136	*q* ♀	H3	AAABBBAB	**TX2B136**
	TXW1490_1	*q* ♀	H3	AAABBBAB	TropicBeauty > **Fla3-2**
	TXW1490_1	*q* ♂	H3	AAABBBAB	**F_TXW1490_1**
	Galaxy	*q* ♀	H4	AAABBBBA	**Galaxy**
	Victor	*q* ♂	H5	BBBAAABA	Goldprince > **F_Goldprince**

Table III-4 Continued

Trait/LG	Parents	QTL allele	Hap.	SNP haplotype Allele sequence	Successive ancestors (founders in bold)
TA/LG5.a	CAF2	*Q* ♂	H2	**AB**BBBAABBBBB	**CAF2**
	CAF3	*Q* ♂	H2	**AB**BBBAABBBBB	**CAF3**
	CAF4	*Q* ♂	H2	**AB**BBBAABBBBB	**CAF4**
	Galaxy	*Q* ♂	H2	**AB**BBBAABBBBB	**Galaxy**
	Victor	*Q* ♂	H2	**AB**BBBAABBBBB	Goldprince > **F_Goldprince**
	Victor	*Q* ♀	H4	**AB**BBBAABBBBA	TropicBeauty > **Flordaprince**
	TX2B136	*Q* ♂	H5	**AB**ABBABBABAB	**TX2B136**
	TX2B136	*Q* ♀	H4	**AB**BBBAABBBBA	**TX2B136**
	TXW1490_1	*Q* ♀	H4	**AB**BBBAABBBBA	TropicBeauty > **Flordaprince**
	TXW1490_1	*Q* ♂	H4	**AB**BBBAABBBBA	**F_TXW1490_1**
	CAF2	*q* ♀	H1	**BA**AAABBAAAAB	**CAF2**
	CAF3	*q* ♀	H1	**BA**AAABBAAAAB	**CAF3**
	CAF4	*q* ♀	H3	**BA**ABBAABBBBB	**CAF4**
	Galaxy	*q* ♀	H1	**BA**AAABBAAAAB	**Galaxy**
TA/LG5.b	Victor	*Q* ♀	H3	**AAA**BBBAB	TropicBeauty > **Fla3-2**
	TX2B136	*Q* ♀	H3	**AAA**BBBAB	**TX2B136**
	TXW1490_1	*Q* ♀	H3	**AAA**BBBAB	TropicBeauty > **Fla3-2**
	TXW1490_1	*Q* ♂	H3	**AAA**BBBAB	**F_TXW1490_1**
	Galaxy	*Q* ♀	H4	**AAA**BBBBA	**Galaxy**
	TX2B136	*Q* ♂	H6	**AAA**BABBB	**TX2B136**
	Victor	*q* ♂	H5	**BBB**AAABA	Goldprince > **F_Goldprince**
	Galaxy	*q* ♂	H1	**BBB**AABBB	**Galaxy**
	CAF2	*q* ♀	H1	**BBB**AABBB	**CAF2**
	CAF2	*q* ♂	H2	**BBB**AABBA	**CAF2**
	CAF3	*q* ♀	H1	**BBB**AABBB	**CAF3**
	CAF3	*q* ♂	H1	**BBB**AABBB	**CAF3**
	CAF4	*q* ♀	H1	**BBB**AABBB	**CAF4**
	CAF4	*q* ♂	H1	**BBB**AABBB	**CAF4**

Soluble solids content (SSC)

Eight SNPs in the predictive QTL region (53.16 – 62.44 cM) were chosen for haplotyping for SSC (Table III-4and Table III-5). Haplotype analyses revealed six unique SNP haplotypes associated with SSC across the seven parents of the seven full-sib families. H1 and H3 were the common haplotypes among these parents. Estimation of the diplotype effect revealed that the higher SSC values were for individuals with diplotypes containing H1, H2, and H6 and with a mean value ~13° Brix in both H6H1 and H6H2 (Fig. III-7). Homozygous *Q*-alleles were present in 'CAF2', 'CAF3', and 'CAF4', while only one *Q*-allele was present in 'Galaxy' and TX2B136. In contrast, haplotypes H3, H4, and H5 were associated with lower SSC values and were assigned to *q*-alleles. We were unable to estimate their diplotype effects because of the limited number of individuals containing a combination of these haplotypes (only one individual of each H3H3 and H3H5). Both 'Victor' and TXW1490-1 breeding parents were homozygous *q*-allele. The mean values of diplotypes H5H2 and H6H4 (heterozygous *Q*-alleles) ranged between ~11 to 13° Brix.

Two specific SNP markers were associated with H1, H2, and H6 at two adjacent genetic positions (54.45 and 55.49 cM) of SSC QTL region. The two AB-alleles of the SNP markers SNP_IGA_600509 (~14.8 Mb) and SNP_5_15254637 (15.2 Mb) (Table III-5), were associated with *Q*-alleles for increasing SSC in peach. These favorable SNP alleles were inherited from 'CAF2', 'CAF3', 'CAF4', 'Galaxy', and TX2B136 parents.

The original sources of *Q*-alleles for high SSC of the breeding parents 'CAF2', 'CAF3', 'CAF4', 'Galaxy', and TX2B136 could not be traced back and they were considered as founders. However, the *q*-allele of both 'Victor' and TXW1490-1 was inherited from the maternal parent

‘TropicBeauty’ and derived from ‘Fla3-2’, while the *q*-allele of their paternal parents was traced back to the unknown paternal parents of ‘Goldprince’ and TXW1490-1, respectively.

Titratable acidity (TA)

The haplotyping analysis for TA was conducted on the two QTLs at the proximal and the distal ends of LG5 for TA. Twelve SNP markers in the first predictive QTL region (0 to 0 cM) were selected for haplotyping (Table III-4). Haplotype analyses discovered five unique SNP haplotypes linked to TA among the seven parents of this study, in which H2 and H4 were at high frequency. H2, H4, and H5 were associated with high TA and the mean value ranged between ~0.8 – 1.2% for H2H2 and H5H2, respectively (Fig. III-8), and were assigned to *Q*-allele. Three breeding parents (‘Victor’, TX2B136, and TXW1490-1) were homozygous for the *Q*-allele, while the remaining four parents have only one *Q*-allele. In contrast, the lower TA values were in individuals with diplotypes containing H1 and H3 and their effects could not be estimated because of the lack of individuals containing a combination of these two haplotypes. These haplotypes were present in ‘CAF2’, ‘CAF3’, ‘CAF4’, and ‘Galaxy’.

In addition, two distinct and adjacent SNP markers associated with H2, H4, and H5 were observed at this region. The two AB-alleles at SNP markers SNP_IGA_544428 (557,504 bp) and SNP_IGA_544495 (610,569 bp) were consistently linked to *Q*-alleles (Table III-4 and Table III-5). In the meanwhile, two predictive SNP markers (BA-alleles) were always associated with *q*-alleles at the same genetic positions of those linked to *Q*-allele and they were present only in ‘CAF2’, ‘CAF3’, ‘CAF4’, and ‘Galaxy’.

Table III-5. SNP name, genetic position (cM), and physical location for SNPs in each allele sequence of haplotypes identified for blush (BL), soluble solids content (SSC), and titratable acidity (TA) traits of seven important breeding parents.

SNP name	Genetic position (cM)	Physical location	Haplotype					
BL_G4			**H1**	**H2**	**H3**	**H4**		
SNP_IGA_409901	41.98	10,582,092	A	B	B	A		
SNP_IGA_410134	42.41	10,626,874	B	B	A	A		
SNP_IGA_410165	42.41	10,641,209	B	B	A	A		
SNP_IGA_410336	42.41	10,676,008	B	B	A	B		
SNP_IGA_410398	42.82	10,696,489	B	B	A	A		
SNP_IGA_410478	43.31	10,760,086	B	B	A	B		
SNP_IGA_410794	43.31	10,890,653	B	B	A	A		
SNP_IGA_410955	43.31	10,904,526	B	B	A	A		
SNP_IGA_411147	43.31	10,921,604	B	B	A	B		
SNP_IGA_411188	43.31	10,923,251	A	A	B	A		
SNP_IGA_411196	43.31	10,923,464	B	B	A	A		
SNP_IGA_411601	44.11	10,976,364	B	B	A	B		
SNP_IGA_411637	44.11	10,981,971	B	B	A	B		
SNP_IGA_412338	44.97	11,208,347	B	B	A	B		
SSC_G5			**H1**	**H2**	**H3**	**H4**	**H5**	**H6**
SNP_IGA_600072	53.16	14,538,721	B	B	A	A	B	A
SNP_IGA_600169	54.33	14,567,044	B	B	A	A	B	A
SNP_IGA_600230	54.33	14,610,097	B	B	A	A	B	A
SNP_IGA_600256	54.33	14,619,399	A	A	B	B	A	B
SNP_IGA_600509	54.45	14,888,402	A	A	B	B	A	A
SNP_5_15254637	55.49	15,249,344	B	B	B	B	A	B
SNP_IGA_603047	62.44	16,768,945	B	B	A	B	B	B
SNP_IGA_604283	62.44	18,236,497	B	A	B	A	A	B

Table III-5 Continued

SNP name	Genetic position (cM)	Physical location	Haplotype					
TA_G5.a			**H1**	**H2**	**H3**	**H4**	**H5**	
SNP_IGA_544428	0	557,504	B	A	B	A	A	
SNP_IGA_544495	0	610,569	A	B	A	B	B	
SNP_IGA_544961	0	698,215	A	B	A	B	A	
SNP_IGA_545261	0	821,356	A	B	B	B	B	
SNP_IGA_545448	0	850,261	A	B	B	B	B	
SNP_IGA_546094	0	987,686	B	A	A	A	A	
SNP_IGA_546316	0	1,049,936	B	A	A	A	B	
SNP_IGA_546987	0	1,166,290	A	B	B	B	B	
SNP_IGA_547473	0	1,216,762	A	B	B	B	A	
SNP_IGA_548512	0	1,503,387	A	B	B	B	B	
SNP_IGA_548597	0	1,518,366	A	B	B	B	A	
SNP_IGA_550475	0	2,028,804	B	B	B	A	B	
TA_G5.b			**H1**	**H2**	**H3**	**H4**	**H5**	**H6**
SNP_IGA_600072	53.16	14,538,721	B	B	A	A	B	A
SNP_IGA_600169	54.33	14,567,044	B	B	A	A	B	A
SNP_IGA_600230	54.33	14,610,097	B	B	A	A	B	A
SNP_IGA_600256	54.33	14,619,399	A	A	B	B	A	B
SNP_IGA_600509	54.45	14,888,402	A	A	B	B	A	A
snp_5_15254637	55.49	15,249,344	B	B	B	B	A	B
SNP_IGA_603047	62.44	16,768,945	B	B	A	B	B	B
SNP_IGA_604283	62.44	18,236,497	B	A	B	A	A	B

The original sources for Q and *q*-alleles could not be traced back for ‘CAF2’, ‘CAF3’, ‘CAF4’, TX2B136, and ‘Galaxy’ and they were treated as founders. The *Q*-allele of both ‘Victor’ and TXW1490-1 was inherited from the maternal parent ‘TropicBeauty’ that came from ‘Flordaprince’. Whereas the *Q*-allele of their paternal parents was traced back to the unknown paternal parents of ‘Goldprince’ and TXW1490-1, respectively.

Eight SNP markers in the second QTL region (53.16 to 62.44 cM) were selected for haplotyping (Table III-4). Six SNP haplotypes were associated with TA among the seven parents in which H1 and H3 were the most common haplotypes. H3, H4, and H6 were associated with high TA values, and they were assigned to *Q*-allele. However, as only a few of the evaluated individuals had combinations of these haplotypes, we could not estimate their values. The homozygous *Q*-allele was found in TX2B136 and TXW1490-1, while a single *Q*-allele was present in 'Victor' and 'Galaxy'. Low TA values were found in individuals with diplotypes containing H1, H2, and H5 and the mean value of H5H1 was ~0.6% (Fig. III-8). These haplotypes were present in 'CAF2', 'CAF3', and 'CAF4' as homozygous *q*-alleles, and as a single *q*-allele in 'Victor' and 'Galaxy' cultivars. The TA values ranged between ~0.7 to 1.0% in H6H1 and H3H1 (heterozygous *Q*-alleles), respectively.

Three Adjacent predictive SNP markers associated with TA were located at the distal end of LG5 (Table III-4). Their A-alleles (SNP_IGA_600072, SNP_IGA_600169, and SNP_IGA_600230) were always associated with a *Q*-allele for increasing TA level (Table III-4 and Table III-5). These predictive SNP marker alleles were homozygous in the parents TX2B136 and TXW1490-1, and heterozygous in 'Victor' and 'Galaxy'. The remaining three parents lacked these SNP marker alleles for high TA. The original source of both Q and *q*-alleles of 'CAF2', 'CAF3', 'CAF4', TX2B136, and 'Galaxy' were unknown as their ancestors were not available for genotyping. The original source of the *Q*-allele of both maternal parents 'Victor' and TXW1490-1 was traced back to 'TropicBeauty' that inherited it from the founder Fla3-2. The *Q*-allele for TXW1490-1 and *q*-allele for 'Victor' were traced back to the unknown paternal parent of TXW1490-1 and 'Goldprince', respectively.

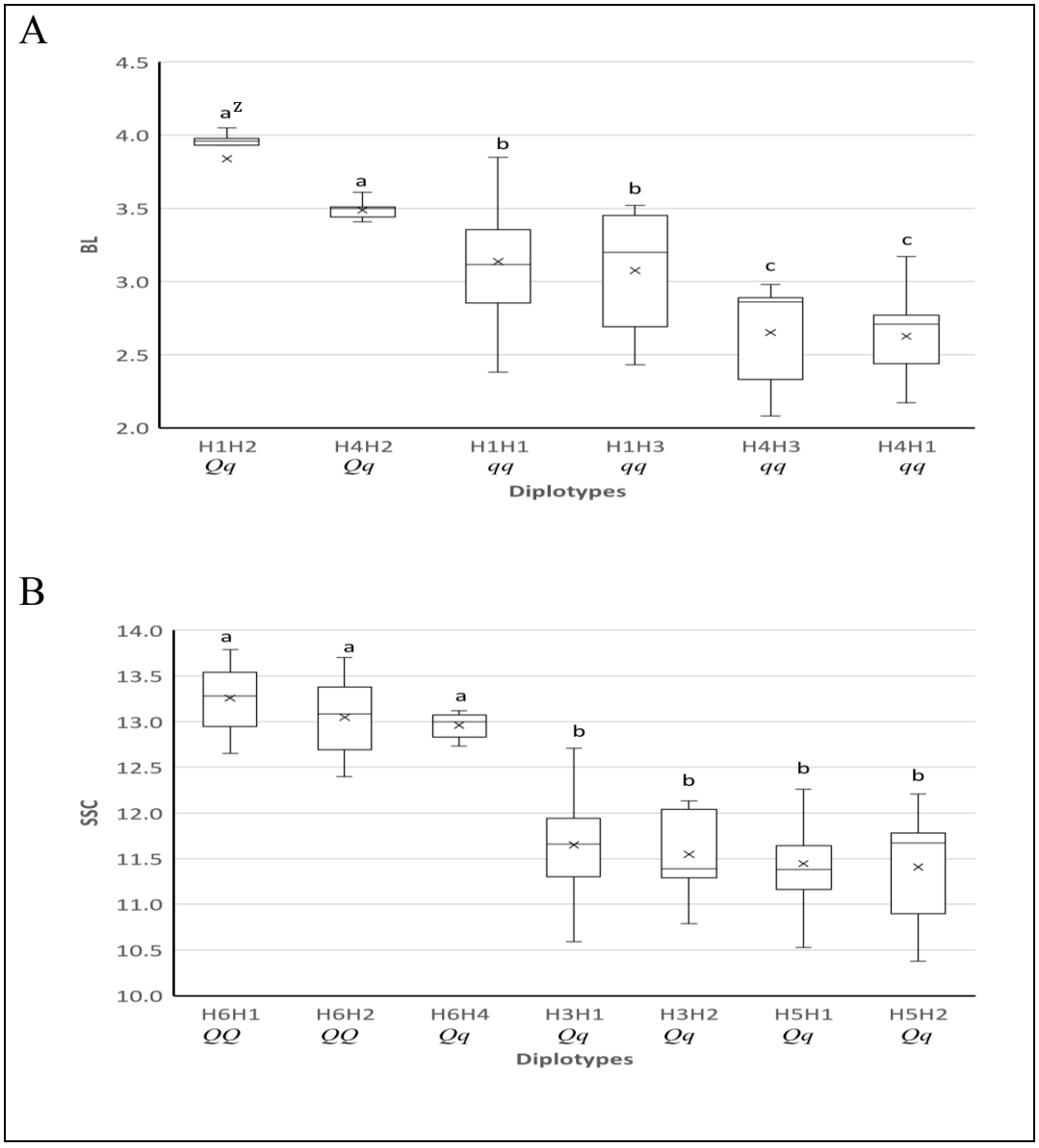

Fig. III-7. Diplotype effect of the most common haplotypes associated with fruit blush (BL) (A) and soluble solids content (SSC) (B) for the QTLs mapped on LG4 and LG5, respectively.

[z] Means not connected by the same letter are significantly different ($P<0.05$) within each linkage group using HSD test.

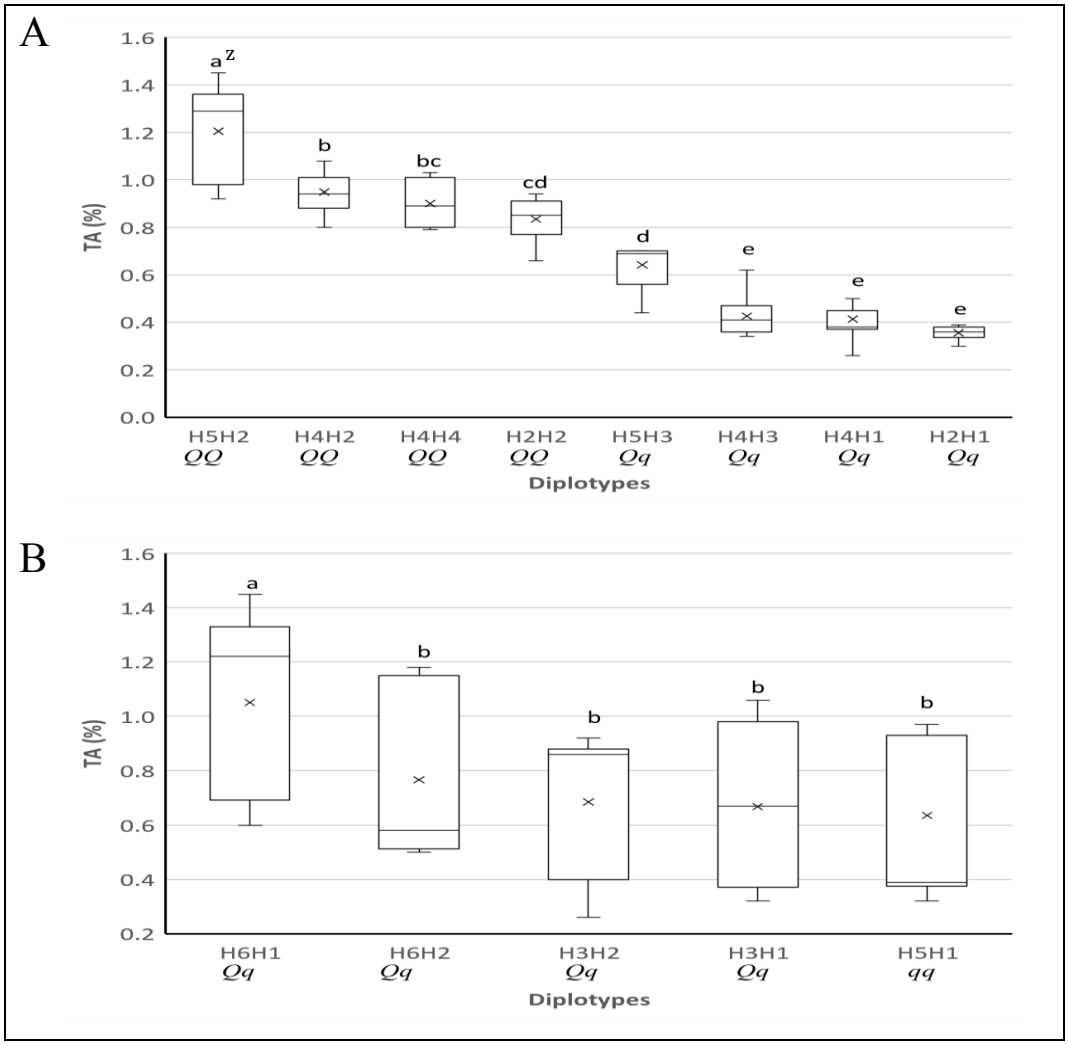

Fig. III-8. Diplotype effect of the most common haplotypes associated with titratable acidity (TA) for the QTLs mapped on LG4 (A) and LG5 (B).
[z] Means not connected by the same letter are significantly different ($P<0.05$) within each linkage group using HSD test.

To distinguish between effect of two QTLs at the upper (TA_overall_G5.a) and lower (TA_ overall_G5.b) parts of LG5 for TA, insight was needed on the effect (contribution) of each of these QTLs by studying the phenotypic means of their genotypes. Results showed that the average effect on TA increased with *Q*-allele dose at TA_ overall_G5.a from 0.40 to 1.00 % by increasing a single *Q*-dose from *Qq* to *QQ*-alleles (Table III-6). However, the effect of *Q*-doses

at TA_ overall_G5.b did not follow the same trend with different *Q*-doses at TA_ overall_G5.a. The population also had a non-uniform distribution of *Q/q* alleles with the ratio of *QQ*/*Qq*/*qq* individuals of 65/69/0 and 7/85/32 for the upper and the lower part QTLs, respectively. No individuals with *qq*-alleles for the QTL at the upper part were found. Although these results indicate that TA_G5.a has a greater effect on TA level than TA_G5.b in our peach population, the conclusion is limited by the small population and the poor representation of all allele combinations within the studied populations.

Interestingly in this study, the haplotype analysis showed an interplay between the two QTLs of SSC and TA on LG5.b at the genomic region between 53.16 to 62.44 cM (SNP_IGA_600072 to SNP_IGA_604283) which has not been previously reported. Haplotypes H1 and H2 that are associated with increased SSC levels were, at the same time, associated with decreased TA levels. These haplotypes were present as *QQ* alleles in SSC and as *qq* in TA in the parents 'CAF2', 'CAF3', and 'CAF4'. In addition, it was present as *Q*-allele and *q*-allele in SSC and TA, respectively, in the paternal parent 'Galaxy'. Likewise, H3 and H4 that are associated with decreased SSC were, at the same time, increased TA levels (Table III-4). These parents have common parentage and sometimes from both sides of the pedigree (Dr. David Byrne, personal communication).

Table III-6. Effect of *Q*-allele dose at TA_G5.a and TA_G5.b on TA contents in the average of TA content measured on 162 seedlings and seven parents.

	TA_G5.b											
	QQ			***Qq***			***qq***			**Total**		
TA_G5.a	**N**	**TA (%)**	**SD**	**N**	**TA (%)**	**SD**	**N**	**TA (%)**	**SD**	**N**	**TA (%)**	**SD**
QQ	4	1.08	0.150	45	1.01	0.311	16	0.93	0.148	65	1.00	0.273
Qq	3	0.33	0.058	40	0.45	0.155	16	0.41	0.082	59	0.40	0.137
qq	-	-	-	-	-	-	-	-	-	-	-	-
Total	7	0.70	0.412	85	0.73	0.376	32	0.67	0.288	124	0.70	0.356

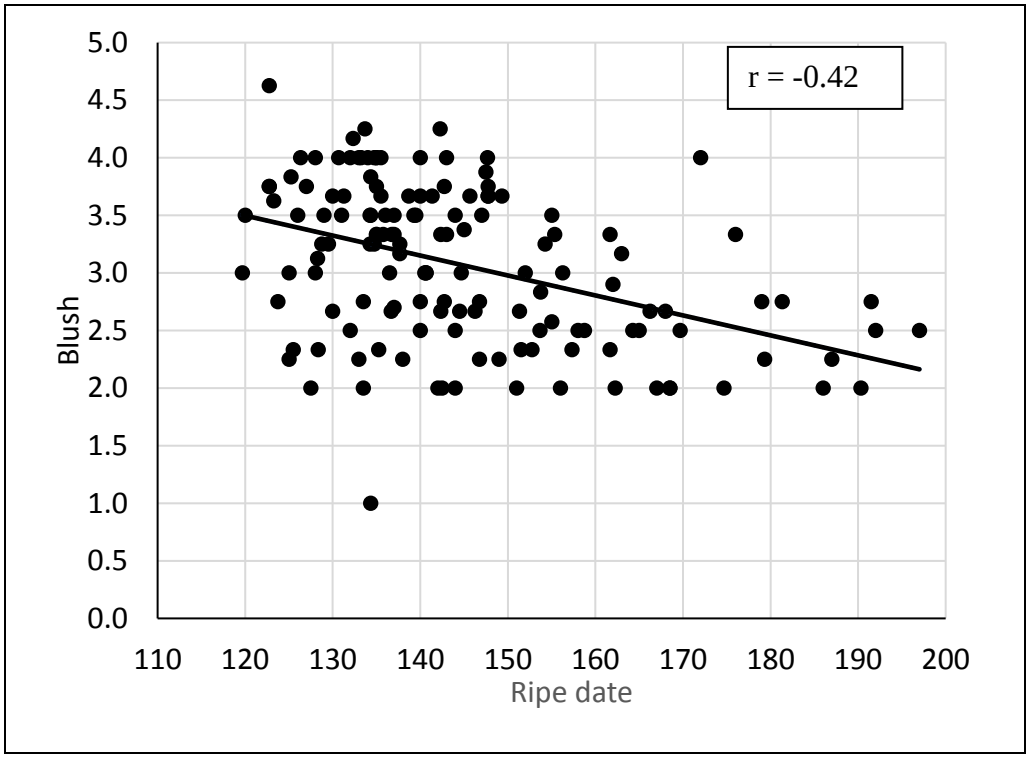

Fig. III-9. Correlation between fruit blush (BL) and ripe date (DR).

** Pearson correlation is significant at the 0.01 level (2-tailed).

Discussion

Blush (BL)

A high percentage of red blush on the fruit surface is desirable for the fresh market peaches and nectarines in the U.S. (Beckman et al., 2005; Hesse, 1975). Blush, which is a quantitative trait, is expressed during the final stage of fruit development and when the fruit is directly exposed to sunlight (Bassi and Monet, 2008; Frett et al., 2014). QTLs associated with blush on peach fruits have been reported (Cantín et al., 2010; Eduardo et al., 2011; Frett et al., 2014; Hernández Mora et al., 2017a; Quilot et al., 2004; Verde et al., 2002; Yamamoto et al., 2001; Yamamoto et al., 2005) on the linkage groups 2, 3, 4, 5, 6, and 7, indicating the polygenic nature of inheritance.

In this study, the narrow sense heritability of blush was between 0.31 (low) to 0.52 (moderate) (Table III-1). These values are in between those previously reported by Hansche (1986) (0.19 ± 0.04) and by de Souza et al. (1998) and Hernández Mora et al. (2017a) (0.70 and 0.71, respectively). Heritability is germplasm and environment specific; thus, different h^2 values may be expected among studies (Wert et al., 2009).

One QTL for blush which had a PVE between 20–32% was found on LG4 between the flanking markers SNP_IGA_409901 and SNP_IGA_412338 at the chromosomal location from ~10.5 to 11.2 Mb (Table III-2 and Table III-3). This genomic region is close to QTL positions for fruit blush previously reported on different peach germplasm. A QTL for blush on LG4 mapped near the marker BPPCT009 and the chromosomal position ~11.8 Mb using the peach linkage map (V x BT). This blush QTL explained ~69% of the phenotypic variance (Cantín et al., 2010). Likewise, Hernández Mora et al. (2017a), mapped a QTL for blush on LG4 between SNP_IGA_ 412338 and SNP_IGA_421930 markers with the interval between ~ 11.8 to 14.1 Mb.

Frett et al. (2014) also reported two minor QTLs for blush on LG4 with nearest markers SNP_IGA_384731 and SNP_IGA_386970 at the chromosomal position ~ 3.5 and 4.4 Mb, respectively. In addition, a minor QTL was found between flanking SNPs SNP_IGA_401100 and SNP_IGA_403004 on LG4 (~ 7.5 to 8.8 Mb) (Frett, 2016).

This specific genomic region on LG4 at the SNP_IGA_410794 marker (10.9 Mb) (Fig. III-3) co-localizes with the QTL of ripe date that mapped in this study (Chapter II) and other previous studies. Nuñez-Lillo et al. (2015) and Romeu et al. (2014) reported a major QTL for ripe date at the SNP_IGA_410398 (10.7 Mb) and at the SNP_IGA_411147 (10.9 Mb), respectively. A moderate correlation (r = -0.42) between ripe date and blush has been found in this study (Fig. III-9). This correlation was also reported by other studies with r=-0.57 (de Souza et al., 1998), -0.24, and -0.56 (Eduardo et al., 2011) on different datasets. This may be explained either by the presence of a single QTL with pleiotropic effects or by the linkage between the QTLs of these traits (Dirlewanger et al., 1999; Kenis et al., 2008).

A deviation in QTL detection at the environment level, particularly in CA11, was noticed in this study. We were unable to determine the reason for this behavior; however, it could be due to different orchard maintenance practices between locations like pruning, or fruits were picked from the inner parts instead of the outer parts of trees (Crisosto et al., 1997). In CA11, data taken from second leaf trees which were very vigorous and might increase shading and decrease blush development. The lower correlation coefficient between CA11 and TX12 (0.44) or TX13 (0.32) compared to CA12 with the same environments (0.62 and 0.53, respectively), may illustrate that blush in CA11 was different. All these factors could lead to less sunlight exposure of the fruit that would depress the activity of the light-inducible MYB gene regulating anthocyanin biosynthesis pathway (Kim et al., 2003) and subsequently skin color development.

Soluble solids content (SSC)

The narrow sense heritability (h^2) of SSC ranged from low (0.29) to moderate (0.47) which agrees with previous reports Cantín et al. (2009a); (de Souza et al., 1998). This range of heritability was expected since SSC is a quantitative trait with heritability strongly influenced by multiple environmental factors including temperature, canopy position, water availability, crop load, and agricultural practices during fruit development period (Crisosto et al., 1997; Westwood, 1993).

In this study, we mapped a QTL associated with SSC at the distal end of LG5 between SNP_IGA_600072 and SNP_IGA_ 604283 and at the interval from ~14.5 – ~18.2 Mb that exhibits a PVE from 17 to 39% (Table III-2 and Table III-3. This overlap with the QTL was reported by Hernández Mora et al. (2017a). In addition, Zeballos et al. (2016) reported two QTLs for SSC with a PVE between 13 to 17% at SNP_IGA_572589 and SNP_IGA_585182 at the positions ~5.8 and 9.2 Mb, respectively.

Titratable acidity (TA)

The narrow sense heritability (h^2) of TA was moderate (0.44) to high (0.88) (Table III-1) which was similar to that reported by Salgado Rojas (2015). This suggests the proportion of variation in this trait within our population is attributed more to the additive genetic component than the environment effects.

FlexQTL detected two QTLs associated with TA, the first QTL was at the upper part of LG5 between SNP IGA_ 544428 and SNP_IGA_550475 with a PVE between 59 – 85%, indicating this trait has a high genetic component (Table III-2; Fig. III-5). This QTL co-localizes with that reported by Zeballos et al. (2016) as well as with the *D*-locus that controls low fruit acidity in peaches (Boudehri et al., 2009; Etienne et al., 2002). In all the studies this QTL locus

controls the major part (60 to 87 %) of the phenotypic variance (Boudehri et al., 2009; Dirlewanger et al., 2006b; Etienne et al., 2002; Ogundiwin et al., 2009; Salgado Rojas, 2015; Zeballos et al., 2016).

The second QTL (TA_CA12_G5.b and TA_overall_G5.b) was mapped at the lower part of LG5 between SNP_IGA_600072 and SNP_IGA_604283 within the chromosomal positions between ~14.5 – 18.2 Mb (Table III-3). It was detected in two independent data sets with decisive evidence, was segregating in two parents (Galaxy and 'Victor') and explained 18 - 21% of the phenotypic variation for TA (Table III-1 and Table III-2). This QTL has not been previously reported, indicating this could be a novel QTL or environmentally specific.

These results indicate there is another locus governing TA besides the major QTL on the proximal part of LG5 that has been reported in several studies and across various breeding programs.

Conclusion

Pedigree-based analysis successfully detected the location of QTLs associated with BL, SSC, and TA traits among low-medium chill peach/nectarine germplasm. This technique allows the use of multiple segregating populations with increased genetic background to enhance the ability to identify both major and minor QTLs that are associated with these quality traits. Our analysis detected a QTL associated with BL at the central part of LG4 as did previous studies (Cantín et al., 2010; Frett et al., 2014; Hernández Mora et al., 2017a). This genomic region was associated with RD in this study and was supported by other studies (Frett, 2016; Nuñez-Lillo et al., 2015; Romeu et al., 2014). The proximal end of LG5 was related to TA and co-localized with the major locus for low-acid fruit (*D*-locus), while the distal end of LG5 was associated with both TA and SSC. These results agreed with previous studies and were consistent across

germplasm, geographical locations, and years despite some minor differences in genomic positions. Moreover, the results from haplotype analysis in this study revealed predominant SNP haplotypes associated with increasing or decreasing the levels of each trait. In addition, we were able to identify predictive SNPs and haplotype alleles for desired QTL alleles and their original source.

The knowledge about the genetic basis of these quality traits would help breeders in the crossing choice to pick combinations of parents that have desired haplotype alleles or in seedling selection to discard undesired seedlings in early stages. Our finding will improve these traits and, consequently, the industry as it can change with consumer preferences and impact peach consumption.

Future Studies

Our findings will help peach breeders develop new predictive, DNA-based molecular marker tests by converting the trait linked SNP haplotypes to easy-to-use, (semi) high throughput markers such as simple sequence repeat (SSR), Kompetitive allele-specific PCR (KASP), or Sequence Characterized Amplified Region (SCAR) markers that can be used routinely in MAB for enhancing peach quality traits. In addition, conducting additional PBA to discover molecular markers for other fruit quality traits of interest will be useful.

References

Abbott, A.G., S. Rajapakse, B. Sosinski, Z.X. Lu, K. Sossey-Alaoui, M. Gannavarapu, G. Reighard, R.E. Ballard, W.V. Baird, R. Scorza, and A. Callahan. 1998. Construction of saturated linkage maps of peach crosses segregating for characters controlling fruit quality, tree architecture and pest resistance. *Acta Hort.* 465:41-49.

Baird, V., L. Belthoff, R. Ballard, R. Scorza, A. Callahan, R. Monet, and A. Abbott. 1992. Analysis of the nuclear genome, and construction of a genetic map for peach [*Prunus persica* (L.) Batsch.]. *HortScience* 27:580 (abst.).

Balasundram, N., K. Sundram, and S. Samman. 2006. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chem.* 99:191-203.

Bassi, D. and R. Monet. 2008. Botany and taxonomy, p. 1-36. In: D. Layne and D. Bassi (eds.), The peach, botany, production and uses. CABI, Wallingford, UK.

Beckman, T.G., J. Rodriguez Alcazar, W.B. Sherman, and D.J. Werner. 2005. Evidence for qualitative suppression of red skin color in peach. *HortScience* 40:523-524.

Bielenberg, D., K. Gasic, and J.X. Chaparro. 2009. An Introduction to Peach (*Prunus persica*), p. 223-234. In: K.M. Folta and S.E. Gardiner (eds.), Genetics and Genomics of Rosaceae. Springer New York, New York, NY.

Bink, M.C.A.M., M.P. Boer, C.J.F. ter Braak, J. Jansen, R.E. Voorrips, and W.E. van de Weg. 2008. Bayesian analysis of complex traits in pedigreed plant populations. *Euphytica* 161:85-96.

Bink, M.C.A.M., J. Jansen, M. Madduri, R.E. Voorrips, C.E. Durel, A.B. Kouassi, F. Laurens, F. Mathis, C. Gessler, D. Gobbin, F. Rezzonico, A. Patocchi, M. Kellerhals, A.

Boudichevskaia, F. Dunemann, A. Peil, A. Nowicka, B. Lata, M. Stankiewicz-Kosyl, K. Jeziorek, E. Pitera, A. Soska, K. Tomala, K.M. Evans, F. Fernandez-Fernandez, W. Guerra, M. Korbin, S. Keller, M. Lewandowski, W. Plocharski, K. Rutkowski, E. Zurawicz, F. Costa, S. Sansavini, S. Tartarini, M. Komjanc, D. Mott, A. Antofie, M. Lateur, A. Rondia, L. Gianfranceschi, and W.E. van de Weg. 2014. Bayesian QTL analyses using pedigreed families of an outcrossing species, with application to fruit firmness in apple. *Theor. Appl. Genet.* 127:1073-1090.

Bink, M.C.A.M., L.R. Totir, C.J.F. Braak, C.R. Winkler, M.P. Boer, and O.S. Smith. 2012. QTL linkage analysis of connected populations using ancestral marker and pedigree information. *Theor. Appl. Genet.* 124:1097-1113.

Bliss, F.A., S. Arulsekar, M.R. Foolad, V. Becerra, A.M. Gillen, M.L. Warburton, A.M. Dandekar, G.M. Kocsisne, and K.K. Mydin. 2002. An expanded genetic linkage map of *Prunus* based on an interspecific cross between almond and peach. *Genome* 45:520-529.

Boudehri, K., M.A. Belka, G. Cardinet, G. Capdeville, C. Renaud, Y. Tauzin, E. Dirlewanger, A.M. Dirlewanger, C. Troadec, D. Jublot, M. Caboche, and A. Bendahmane. 2009. Toward the isolation of the d gene controlling the acidity of peach fruit by positional cloning. *Acta Hort.* 814:507-510.

Bruhn, C.M. 1995. Consumer and retailer satisfaction with the quality and size of California peaches and nectarines. *J. Food Qual.* 18:241-256.

Byrne, D.H. 2005. Trends in stone fruit cultivar development. *HortTechnol.* 15:494-500.

Byrne, D.H., M.B. Raseira, D. Bassi, M.C. Piagnani, K. Gasic, G.L. Reighard, M.A. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: L.M. Badenes and H.D. Byrne (eds.), Fruit Breeding. Springer US, Boston, MA.

Cantín, C.M., C.H. Crisosto, E.A. Ogundiwin, T. Gradziel, J. Torrents, M.A. Moreno, and Y. Gogorcena. 2010. Chilling injury susceptibility in an intra-specific peach [*Prunus persica* (L.) Batsch] progeny. *Postharvest Biol. Tec.* 58:79-87.

Cantín, C.M., Y. Gogorcena, and M.A. Moreno. 2009a. Analysis of phenotypic variation of sugar profile in different peach and nectarine *Prunus persica* (L.) Batsch breeding progenies. *J. Sci. Food Agr.* 89:1909-1917.

Cantín, C.M., M.A. Moreno, and Y. Gogorcena. 2009b. Evaluation of the antioxidant capacity, phenolic compounds, and vitamin C content of different peach and nectarine *Prunus persica* (L.) Batsch breeding progenies. *J. Agr. Food Chem.* 57:4586-4592.

Carrasco, B., L. Meisel, M. Gebauer, R. Garcia-Gonzales, and H. Silva. 2013. Breeding in peach, cherry and plum: from a tissue culture, genetic, transcriptomic and genomic perspective. *Biol. Res.* 46:219-230.

Chaparro, J.X., D.J. Werner, D. O'Malley, and R.R. Sederoff. 1994. Targeted mapping and linkage analysis of morphological isozyme, and RAPD markers in peach. *Theor. Appl. Genet.* 87:805-815.

Collard, B.C.Y., M.Z.Z. Jahufer, J.B. Brouwer, and E.C.K. Pang. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* 142:169-196.

Crisosto, C.H. 2002. How do we increase peach consumption? *Acta Hort.* 592:601-605.

Crisosto, C.H. 2006. Peach quality and postharvest technology. *Acta Hort.* 713:479-488.

Crisosto, C.H. and G. Costa. 2008. Preharvest factors affecting peach quality, p. 536-549. In: D.R. Layne and D. Bassi (eds.), The peach: botany, production and uses. CAB International.

Crisosto, C.H. and G.M. Crisosto. 2005. Relationship between ripe soluble solids concentration (RSSC) and consumer acceptance of high and low acid melting flesh peach and nectarine (*Prunus persica* (L.) Batsch) cultivars. *Postharvest Biol. Tec.* 38:239-246.

Crisosto, C.H., G.M. Crisosto, G. Echeverria, and J. Puy. 2006. Segregation of peach and nectarine (*Prunus persica* (L.) Batsch) cultivars according to their organoleptic characteristics. *Postharvest Biol. Tec.* 39:10-18.

Crisosto, C.H., R.S. Johnson, T. DeJong, and K.R. Day. 1997. Orchard factors affecting postharvest stone fruit quality. *HortScience* 32:820-823.

Crisosto, C.H. and A.A. Kader. 2000. Peach, Postharvest quality maintenance guidelines. *Department of Pomology. University of California. Davis*:1-10.

da Silva Linge, C., L. Antanaviciute, A. Abdelghafar, P. Arús, D. Bassi, L. Rossini, S. Ficklin, and K. Gasic. 2018. High-density multi-population consensus genetic linkage map for peach. *PLOS ONE* 13:e0207724.

de Souza, V.A.B., D.H. Byrne, and J.F. Taylor. 1998. Heritability, genetic and phenotypic correlations, and predicted selection response of quantitative traits in peach: I. An analysis of several reproductive traits. *J. Amer. Soc. Hort. Sci.* 123:598-603.

Dirlewanger, E., P. Cosson, K. Boudehri, C. Renaud, G. Capdeville, Y. Tauzin, F. Laigret, and A. Moing. 2006a. Development of a second-generation genetic linkage map for peach [*Prunus persica* (L.) Batsch] and characterization of morphological traits affecting flower and fruit. *Tree Genet. & Genomes* 3:1-13.

Dirlewanger, E., P. Cosson, C. Renaud, R. Monet, J.L. Poëssel, and A. Moing. 2006b. New detection of QTLs controlling major fruit quality components in peach. *Acta Hort.* 713:65-72.

Dirlewanger, E., A. Moing, C. Rothan, L. Svanella, V. Pronier, A. Guye, C. Plomion, and R. Monet. 1999. Mapping QTLs controlling fruit quality in peach (*Prunus persica* (L.) Batsch). *Theor. Appl. Genet.* 98:18-31.

Eduardo, I., I. Pacheco, G. Chietera, D. Bassi, C. Pozzi, A. Vecchietti, and L. Rossini. 2011. QTL analysis of fruit quality traits in two peach intraspecific populations and importance of maturity date pleiotropic effect. *Tree Genet. & Genomes* 7:323-335.

Eduardo, I., R. Picañol, E. Rojas, I. Batlle, W. Howad, M.J. Aranzana, and P. Arús. 2015. Mapping of a major gene for the slow ripening character in peach: co-location with the maturity date gene and development of a candidate gene-based diagnostic marker for its selection. *Euphytica* 205:627-636.

Etienne, C., C. Rothan, A. Moing, C. Plomion, C. Bodénès, L. Svanella-Dumas, P. Cosson, V. Pronier, R. Monet, and E. Dirlewanger. 2002. Candidate genes and QTLs for sugar and organic acid content in peach [*Prunus persica* (L.) Batsch]. *Theor. Appl. Genet.* 105:145-159.

FAO. 2018. http://www.fao.org/faostat/en/#data/QC. Apr. 15, 2018

Fideghelli, C., G. Della Strada, F. Grassi, and G. Morico. 1998. The peach industry in the world: Present situation and trend. *Acta Hort.* 465:29-40.

Fresnedo-Ramírez, J., M.C.A.M. Bink, E. van de Weg, T.R. Famula, C.H. Crisosto, T.J. Frett, K. Gasic, C.P. Peace, and T.M. Gradziel. 2015. QTL mapping of pomological traits in peach and related species breeding germplasm. *Mol. Breeding* 35:1-19.

Frett, T. 2012. Enabling marker-assisted breeding (MAB) for blush in peach [*Prunus persica* (L.) Batsch]. Clemson Univ., Clemson, SC., M.S. Thesis.

Frett, T.J. 2016. Genetic determinism of *Xanthomonas arboricola* pv. *pruni* (Xap) resistance, fruit quality, and phenological traits in peach and incorporation of marker-assisted selection (MAS) in the University of Arkansas peach and nectarine breeding program. University of Arkansas, Fayetteville, AK, Ph.D Dissertation.

Frett, T.J., G.L. Reighard, W.R. Okie, and K. Gasic. 2014. Mapping quantitative trait loci associated with blush in peach [*Prunus persica* (L.) Batsch]. *Tree Genet. & Genomes* 10:367-381.

Gasic, K., T. Gradziel, C. Crisosto, D. Byrne, and J. Clark. 2010. https://www.rosbreed.org/sites/default/files/files/RosBREED_2010-Phenotyping_protocol_P_persica.pdf. Jul. 10, 2017

Hansche, P.E. 1986. Heritability of fruit quality traits in peach and nectarine breeding stocks dwarfed by the dw gene. *HortScience* 21:1193-1195.

Hernández Mora, J.R., D. Micheletti, M. Bink, E. Van de Weg, C. Cantín, N. Nazzicari, A. Caprera, M.T. Dettori, S. Micali, E. Banchi, J.A. Campoy, E. Dirlewanger, P. Lambert, T. Pascal, M. Troggio, D. Bassi, L. Rossini, I. Verde, B. Quilot-Turion, F. Laurens, P. Arús, and M.J. Aranzana. 2017a. Integrated QTL detection for key breeding traits in multiple peach progenies. *BMC Genomics* 18:404.

Hernández Mora, J.R., D. Micheletti, M.A.M. Bink, W.E. Van de Weg, D. Bassi, N. Nazzicari, A. Caprera, M.T. Dettori, S. Micali, E. Dirlewanger, P. Lambert, T. Pascal, E. Banchi, M. Troggio, L. Rossini, I. Verde, B. Quilot-Turion, F. Laurens, P. Arús, and M.J. Aranzana. 2017b. Discovering peach QTLs with multiple progeny analysis. *Acta Hort.* 1172:405-410.

Hesse, C.O. 1975. Peaches, p. 285-347. In: J. Janick and J.N. Moore (eds.). Purdue Univ. Press, West Lafayette, IN.

Iezzoni, A., C. Weebadde, J. Luby, C.Y. Yue, E. van de Weg, G. Fazio, D. Main, C.P. Peace, N.V. Bassil, and J. McFerson. 2010. *Acta Hort.* 859:389-394.

Illumina Inc. 2010. GenomeStudio Genotyping module v1.0, User Guide. Illumina Inc., San Diego, CA, USA.

J, P.A. and B.G. Paul. 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agr.* 80:985-1012.

Jaeger, S.R. 2006. Non-sensory factors in sensory science research. *Food Quality and Preference* 17:132-144.

Kass, R.E. and A.E. Raftery. 1995. Bayes Factors. *J. Amer. Stat. Assn.* 90:773-795.

Kenis, K., J. Keulemans, and M.W. Davey. 2008. Identification and stability of QTLs for fruit quality traits in apple. *Tree Genet. & Genomes* 4:647-661.

Kim, S.-H., J.-R. Lee, S.-T. Hong, Y.-K. Yoo, G. An, and S.-R. Kim. 2003. Molecular cloning and analysis of anthocyanin biosynthesis genes preferentially expressed in apple skin. *Plant Sci.* 165:403-413.

Nuñez-Lillo, G., A. Cifuentes-Esquivel, M. Troggio, D. Micheletti, R. Infante, R. Campos-Vargas, A. Orellana, F. Blanco-Herrera, and C. Meneses. 2015. Identification of candidate genes associated with mealiness and maturity date in peach [*Prunus persica* (L.) Batsch] using QTL analysis and deep sequencing. *Tree Genet. & Genomes* 11:86.

Ogundiwin, E.A., C.P. Peace, T.M. Gradziel, D.E. Parfitt, F.A. Bliss, and C.H. Crisosto. 2009. A fruit quality gene map of *Prunus*. *BMC Genomics* 10:587.

Opara, L.U., F.A. Al-Said, and A. Al-Abri. 2007. Assessment of what the consumer values in fresh fruit quality: Case study of Oman. *N.Z. J. Crop Hort. Sci.* 35:235-243.

Peace, C. and J. Norelli. 2009. Genomics Approaches to Crop Improvement in the Rosaceae, p. 19-53. In: K.M. Folta and S.E. Gardiner (eds.), Genetics and Genomics of Rosaceae. Springer New York, New York, NY.

Peace, C.P., C.H. Crisosto, D.T. Garner, A.M. Dandekar, T.M. Gradziel, and F.A. Bliss. 2006. Genetic control of internal breakdown in peach. *Acta Hort.* 713:489-496.

Peace, C.P., C.H. Crisosto, and T.M. Gradziel. 2005. Endopolygalacturonase: A candidate gene for Freestone and Melting flesh in peach. *Mol. Breeding* 16:21-31.

Peace, C.P., J.J. Luby, W.E. van de Weg, M.C.A.M. Bink, and A.F. Iezzoni. 2014. A strategy for developing representative germplasm sets for systematic QTL validation, demonstrated for apple, peach, and sweet cherry. *Tree Genet. & Genomes* 10:1679-1694.

Quarta, R., M.T. Dettori, A. Sartori, and I. Verde. 2000. Genetic linkage map and QTL analysis in peach. *Acta Hort.* 521:233-241.

Quilot, B., B.H. Wu, J. Kervella, M. Génard, M. Foulongne, and K. Moreau. 2004. QTL analysis of quality traits in an advanced backcross between *Prunus persica* cultivars and the wild relative species *P. davidiana*. *Theor. Appl. Genet.* 109:884-897.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Romeu, J.F., A.J. Monforte, G. Sánchez, A. Granell, J. García-Brunton, M.L. Badenes, and G. Ríos. 2014. Quantitative trait loci affecting reproductive phenology in peach. *BMC Plant Biol.* 14:52.

Salgado Rojas, A.A. 2015. Applying Molecular and Phenotypic Tools to Characterize Flesh Texture and Acidity Traits in the Arkansas Peach Breeding Program and Understanding the Crispy Texture in the Arkansas Blackberry Breeding Program. University of Arkansas, Fayetteville, Ph.D. dissertation.

Sanchez, G., J. Martinez, J. Romeu, J. Garcia, A.J. Monforte, M.L. Badenes, and A. Granell. 2014. The peach volatilome modularity is reflected at the genetic and environmental response levels in a QTL mapping population. *BMC Plant Biol.* 14.

Sandefur, P., T. Frett, J. Clark, K. Gasic, and C. Peace. 2017. A DNA test for routine prediction in breeding of peach blush, Ppe-Rf-SSR. *Mol. Breeding* 37:11.

Sansavini, S., A. Gamberini, and D. Bassi. 2006. Peach breeding, genetics and new cultivar trends. *Acta Hort.* 713:23-48.

Scorza, R., W. Anger, D. Peterson, K. Bett, E. Champagne, J. Beaulieu, and D.A. Ingram. 2004. Non-destructive evaluation of post-harvest peach fruit softening. *Acta Hort.* 663:269-274.

Sorensen, D. and D. Gianola. 2002. Likelihood, Bayesian, and MCMC methods in quantitative genetics. Springer Science & Business Media.

Sosinski, B., V. Shulaev, A. Dhingra, A. Kalyanaraman, R. Bumgarner, D. Rokhsar, I. Verde, R. Velasco, and A.G. Abbott. 2009. Rosaceaous Genome Sequencing: Perspectives and Progress, p. 601-615. In: K.M. Folta and S.E. Gardiner (eds.), Genetics and Genomics of Rosaceae. Springer New York, New York, NY.

Sun, J., Y.-F. Chu, X. Wu, and R.H. Liu. 2002. Antioxidant and Antiproliferative Activities of Common Fruits. *J. Agr. Food Chem.* 50:7449-7454.

USDA. 2018. Fruit and Tree Nut Yearbook Tables. https://www.ers.usda.gov/data-products/fruit-and-tree-nut-data/fruit-and-tree-nut-yearbook-tables/,. Oct. 10, 2018.

Vanderzande, S., J.L. Piaskowski, F. Luo, D.A. Edge Garza, J. Klipfel, A. Schaller, S. Martin, and C. Peace. 2018. Crossing the Finish Line: How to Develop Diagnostic DNA Tests as Breeding Tools after QTL Discovery. *J. Hort.* 05:1-6.

Verde, I., N. Bassil, S. Scalabrin, B. Gilmore, C.T. Lawley, K. Gasic, D. Micheletti, U.R. Rosyara, F. Cattonaro, E. Vendramin, D. Main, V. Aramini, A.L. Blas, T.C. Mockler, D.W. Bryant, L. Wilhelm, M. Troggio, B. Sosinski, M.J. Aranzana, P. Arús, A. Iezzoni, M. Morgante, and C. Peace. 2012. Development and Evaluation of a 9K SNP Array for Peach by Internationally Coordinated SNP Detection and Validation in Breeding Germplasm. *PLOS ONE* 7:e35668.

Verde, I., J. Jenkins, L. Dondini, S. Micali, G. Pagliarani, E. Vendramin, R. Paris, V. Aramini, L. Gazza, L. Rossini, D. Bassi, M. Troggio, S. Shu, J. Grimwood, S. Tartarini, M.T. Dettori, and J. Schmutz. 2017. The Peach v2.0 release: high-resolution linkage mapping and deep resequencing improve chromosome-scale assembly and contiguity. *BMC Genomics* 18:225.

Verde, I., R. Quarta, C. Cedrola, and M.T. Dettori. 2002. QTL analysis of agronomic traits in a BC1 peach population. *Acta Hort.* 592:291-297.

Voorrips, R.E. 2002. MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. *J. Hered.* 93:77-78.

Voorrips, R.E., M. Bink, J.W. Kruisselbrink, H.J.J. Koehorst-van Putten, and W.E. van de Weg. 2016. PediHaplotyper: software for consistent assignment of marker haplotypes in pedigrees. *Mol. Breeding* 36:119.

Weather Underground. 2013. http://www.wunderground.com/history/airport/KFAT/2012/8/10/MonthlyHistory.html. Sep. 10, 2013

Wert, T.W., J.G. Williamson, J.X. Chaparro, E.P. Miller, and R.E. Rouse. 2009. The influence of climate on fruit development and quality of four low-chill peach cultivars. *HortScience* 44:666-670.

Westwood, M.N. 1993. Temperate-Zone Pomology. 3rd ed. Timber press, Portland, OR.

Yamamoto, T., T. Shimada, T. Imai, H. Yaegaki, T. Haji, N. Matsuta, M. Yamaguchi, and T. Hayashi. 2001. Characterization of morphological traits based on a genetic linkage map in peach. *Breeding Sci.* 51:271-278.

Yamamoto, T., M. Yamaguchi, and T. Hayashi. 2005. An integrated genetic linkage map of peach by SSR, STS, AFLP and RAPD. *J. Jpn. Soc. Hort. Sci.* 74:204-213.

Zeballos, J.L., W. Abidi, R. Giménez, A.J. Monforte, M.Á. Moreno, and Y. Gogorcena. 2016. Mapping QTLs associated with fruit quality traits in peach [*Prunus persica* (L.) Batsch] using SNP maps. *Tree Genet. & Genomes* 12:37.

CHAPTER IV

ASSESSMENT OF POSTHARVEST DURABILITY FOR IMPORTANT TEXAS PEACH AND NECTARINE GENOTYPES

Synopsis

The fruit quality is essential for selecting novel genotypes in breeding programs. The main reason for the decrease in peach consumption in the USA is problems with internal breakdown (also called chilling injury) disorders and inconsistent quality. These physiological disorders develop during cold storage and/or during ripening at ambient temperature and lead to reduced fruit quality. Thus, developing new genotypes with consistent high quality is an important objective for many breeders. The objective of this study is to implement a standardized phenotyping protocol for postharvest traits in important Texas peach and nectarine genotypes. The standardized postharvest phenotyping was conducted over three years (2016 - 2018) on 35 genotypes grown at two locations, Terrell and Fairfield, TX. Fruits from each genotype were harvested at a consistent ripening stage and evaluated for their quality after storage for two weeks at 5° C and 90% RH. Flesh firmness, fruit size, expressible juice, texture, mealiness, flavor, flesh browning, soluble solids content, pH and titratable acidity traits were evaluated for each genotype. The 35 genotypes differed in their performance in postharvest traits. We found that TX3C394N, ‘Royal Zest Two’, ‘Texstar’, ‘Royal Zest Three’, TX3B376LWP and ‘Harvester’ had poor postharvest quality, whereas ‘Flavorrich’, ‘White Delight One’, and TX2D357LW genotypes were outstanding. Early-season genotypes were less susceptible to chilling injury compared to the late-season genotypes. A similar trend was observed for yellow fleshed vs. white fleshed, peach vs. nectarine, and non-melting vs. melting genotypes. There was

a good correlation ($P<0.05$) for expressible juice with mealiness (r=-0.74) and texture (r=-0.73), and between mealiness and texture (r=0.63). The next step of this study is to combine phenotypic data of postharvest durability assessments with genotypic data to identify loci involved in post-storage fruit quality and, ultimately, enable marker-assisted breeding (MAB) for developing superior quality peach genotypes and increasing peach consumption.

Introduction

Peaches and nectarines [*P. persica* (L.) Batsch] are soft-fleshed fruits composed of about 87% water (Kader and Mitchell, 1989) and are highly perishable with a limited market life between 2-4 weeks (Kader, 2002). Fruits are commercially harvested at the firm mature stage and stored at cold temperatures until they go to market because they deteriorate quickly at ambient temperature. Maintaining low temperature near 0° C during storage and shipping can extend the peach shelf life (Crisosto et al., 1999b).

In the last decade, peach consumption has decreased due to inferior quality such as lack of aroma and flavor, flesh browning, mealiness and bleeding (Crisosto et al., 1999a; Lurie and Crisosto, 2005) as well as the lack of consistent quality (Byrne, 2005). This issue may be attributed to harvesting at immature stages for storage and shipping reasons (Crisosto, 2002; Crisosto and Valero, 2008; Fideghelli et al., 1998; Sansavini et al., 2006), the improper implementation of postharvest handling protocols, and the need for high yields to maintain a profitable operation and the breeders' emphasis on external quality for developing new genotypes rather than the internal quality of fruits (Crisosto et al., 2006).

Internal breakdown (IB), which is also known as chilling injury (CI) is one of the main reasons for the postharvest loss in peaches (Ceponis et al., 1987; Kader and Mitchell, 1989). IB develops more rapidly when fruits of susceptible genotypes are kept at temperatures between

2.2° C and 7.6° C (Kader and Mitchell, 1989). The symptoms of internal breakdown include mealiness (the lack of juice in the fruit), flesh or pit cavity browning, flesh translucency radiating from the pit, and red color pigment development in the flesh of some peach genotypes (Crisosto and Labavitch, 2002).

The development of mealiness may be related to a reduction in endo-polygalacturonase (endo-PG) activity or to changes in the degree of methylesterification of cell wall pectin (Lurie et al., 2003) or other changes in pectin metabolism (Brummell et al., 2004; King et al., 1989; Lurie and Crisosto, 2005). In a mealy fruit, pectins in the cell wall absorb the free water and form a gel in intercellular space that decreases intercellular adhesion, reduces cell rupturing, and prevents the release of the cellular contents (Brummell et al., 2004; King et al., 1989; Lurie and Crisosto, 2005).

The development of chilling injury (CI) varies with the storage temperature, length of the storage period, fruit maturity, and the genotype (Brovelli et al., 1998b; Ju et al., 2000).

In the short term, agronomic practices (fertilization, irrigation, tree thinning, canopy architecture, and genotype selection) can enhance postharvest qualities (Crisosto et al., 1997). Likewise, commercial practices to reduce postharvest deterioration such as calcium applications, controlled atmosphere (CA) storage (Anderson, 1979; Garner et al., 2001; Nanos and Mitchell, 1991), modified atmosphere packaging (MAP), and plant growth regulators (PGRs) have been used.

However, for long-term improvement, peach programs should focus on understanding the genetic basis of chilling injury, flavor, and antioxidant pathways (Crisosto, 2006). Selection for qualitative traits (firmness, fruit size, skin color, soluble solids content (SSC), and flavor) is complicated because they have polygenic control and are influenced by the environment. It is

hard to determine the number of genes involved in the expression of these characteristics (Della Strada et al., 1996).

Traditional fruit breeding is a long-term process. Peach trees have a 3-5 years breeding/evaluation cycle and require substantial land and resources for field evaluation. Thus, 12 to 20 years are needed to develop a novel genotype that satisfies consumer demands (Dirlewanger et al., 2006; Dirlewanger et al., 2004; Dirlewanger et al., 1998). Marker-assisted breeding (MAB) is an important objective in breeding programs because it could accelerate the breeding process by shortening the breeding cycle, reducing the number of seedlings that need to be evaluated in the field and improving parental selection (Bliss, 2010; Testolin and Cipriani, 2010).

During the 1900s, the peach was improved in size, appearance, and firmness, however, internal qualities such as sugar content, antioxidant content, tolerance to IB, and other traits lagged behind (Byrne et al., 2012). Recently, many efforts in these areas have increased (Byrne, 2005; Cantín et al., 2010a; Cantín et al., 2009a; Cantín et al., 2009b; Peace et al., 2006).

The USDA funded project RosBREED (http://www.rosbreed.org) involves the collaboration of numerous breeders and geneticists to develop DNA-tests for fruit quality traits and encourage the use of marker-assisted parental and seedling selection to accelerate the development of high-quality genotypes.

Phenotyping is an essential factor for QTL analysis to connect genetic variation with biological functions (Bassil and Volk, 2010). In RosBREED I a standardized phenotyping protocol was developed for peach productivity and fruit quality traits (Frett et al., 2012). This was used to phenotype peach germplasm including segregating families (513 accessions) for two seasons with four public breeding programs (Clemson University, the University of Arkansas,

the University of California at Davis, and Texas A&M University). These individuals were genotyped with the peach 9k SNP array. This project helped to identify QTLs for important fruit quality traits in these four breeding programs (Fresnedo-Ramírez et al., 2015; Frett, 2016) and established the Genome Database for Rosaceae (GDR: http://www.rosaceae.org).

In RosBREED II a standardized phenotyping protocol was developed to assess peach postharvest durability and mealiness (Rosbreed, 2016) and conducted across the four U. S. participating universities for two successive years. The germplasm phenotyped was genotyped with genome-wide SNP markers using the peach 16k SNP array. The FlexQTL software, a pedigree-based analytical approach was used to integrate the phenotypic, genotypic, and pedigree data to identify loci for postharvest storage fruit quality.

The objective of this study is to assess the postharvest traits in important peach genotypes in Texas for three years

Materials and Methods

Plant materials

During a three-year period (2016-2018), thirty-five peach and nectarine genotypes were assessed for their postharvest quality in this study (Table IV-1). However, the number of assessed genotypes was different in each year, in 2016 and 2018 the number was 34 and 32 genotypes, respectively, while in 2017 we were able to evaluate only 17 genotypes because of lack of chilling (warm winter temperatures) (Table IV-2). Most of the genotypes were grown at Cooper Farm in Fairfield, TX (31°44'28.61"N, 96°10'20.69"W) and few of them grown at Ham orchard in Terrell, TX (32°43'24.51"N, 96°12'21.65"W). Trees were budded onto 'Nemaguard' or ‘Nemared’ peach rootstocks and trained as an open center. General management (irrigation,

fertilization, pruning, fruit thinning, and pest and weed control) were carried out as required according to commercial practice at the two orchards.

During harvest (May to July), up to 20 fruits were harvested from each genotype at the same maturity stage, as determined by the nondestructive DA Meter (DA Meter 53500, T.R. Turoni Srl, Forli, Italy). Fruits with a DA-Meter reading from 0.5 to 1.5 were selected and used for evaluation. Fruits were transported to the lab and placed in the cold room. After labeling each fruit with a number, its DA-Meter reading, weight (g) and diameter (mm) were measured. Then, the fruits were placed in a labeled plastic tray and stored at 5°C and 90% RH for two weeks. Genotypes were categorized based on ripe date as Early season (up to May 20), Mid-season (May 21 to June 10), and Late season (later than June 10).

After two weeks, the fruits were removed from storage and checked for ripeness by touch or with the DA-Meter. If the fruit felt like it was at the 'Ready-to-Eat' stage (9 – 18 N flesh firmness) or the DA-Meter reading was below 0.5, it was considered ripe and subjected to the fruit quality evaluation. If the fruit was not ripe, it was placed at 20° C to ripen and was checked daily until the fruit was ripe and ready to evaluate.

Flesh firmness was measured using an electronic fruit texture analyzer (FTA) fitted with an 8-mm diameter tip (GÜSS Fruit Texture Analyzer; GÜSS Manufacturing (Pty) Ltd., Strand, South Africa). All readings were recorded as kilogram-force (kgf) and then converted to Newton (N) by multiplying the reading by 9.807. The fruit mealiness was estimated with both quantitative and sensory evaluations. For a quantitative measurement (expressible juice), 1.5 – 1.6 g sample of peach flesh was taken with a cork borer #7, placed in a 5 mL syringe and then gently forced into the labeled centrifuge tube. The homogenate was centrifuged for 20 min at

14,000 rpm to separate the juice (supernatant) and solid. Expressible juice (%) was calculated with the following formula:

$$Expressible\ juice\ (\%) = \frac{Supernatant\ wt.}{Initial\ sample\ wt.} \times 100$$

The sensory evaluation was conducted by trained screeners. Three wedge-shaped slices (0.75 - 1.0 cm wide longitudinal) without the skin attached were taken from each fruit to evaluate both flesh texture on a scale from 1 to 4 (1 = Juicy; 2 = Juicy and mealy; 3 = Mealy; 4 = Leathery), and the flavor using two categories, Flavor (1 = Yes, flavor perceived; 2 = No lack of flavor), and Off-Flavor (1 = Yes, fermented, bitter; 2 = No (no off-flavor).

Flesh browning and mealiness were qualitatively measured on visual scales from 1 to 6 (1= No browning; 6 = Extreme browning covering most of the flesh) for browning, and from 1 to 3 (1 = Juicy; 2 = Moderate; 3 = Severe mealiness) for mealiness (Table IV-3).

Table IV-1. Genotype name, seed- and pollen-parents, year of evaluation, flesh color, and acidity of plant materials.

Name	*Seed-Parent*	*Pollen-Parent*	*Year of evaluation*	*Flesh color* [x]	*Acidity* [y]	*Note* [z]
Fire Zest One	TX2B1	Springbaby	16, 17, 18	Y		NM
Flat Delight One	TexFirst	P1	16, 17, 18	W	L	Pantao
Flavorrich[w]			16, 17, 18	Y		
Galaxy	P34-106	D33-1	16, 17, 18	W	L	Pantao
Golden Zest	Crimsonlady	Agata	16, 17, 18	Y		NM
Goldprince	Loring	FV3-257	16,18	Y		
Harvester	Redskin	Southernglow	16,18	Y		
Junegold	Flamingo	Springtime	16, 17, 18	Y		
Royal Zest Four	Richlady	Victor	16, 17, 18	Y		
Royal Zest One	TX2293-1	Richlady	16, 17, 18	Y		
Royal Zest Three	Richlady	Victor	16, 17, 18	Y		
Royal Zest Two	Richlady	Victor	16, 17, 18	Y		
Scarletpearl	Biscoexredgold	Unknown	16,18	W		
Sentinel	FV556	Dixigem	16,18	Y		
Smooth Delight One	Sunmist	Arcticstar	16,18	W	L	
Smooth Delight Two	Sunmist	Arcticstar	16,18	Y	L	
Smooth Texan Three	Diamondray	Danmo	16,18	Y	L	
Texprince	P60-12	Flordaking	16,18	Y		
Texstar	Unknown	Unknown	16,18	Y		
TRH3A17	Victor	BRS-Kampai	16, 17, 18	Y	L	
TRH3C113LW	Smooth Delight One	TexFirst	16,18	W	L	
TRH3D38LWP	Victor	Galaxy	16	W	L	Pantao
TX2A297	TX2B1	Springbaby	16,18	Y		NM
TX2C465CW	White Delight Four	Y435-250PF	16,18	W	L	NM
TX2D357LW	Firepearl	TX1A129W	16,18	W	L	
TX3B298N	Crimsonbaby-NE	TX2C104N	17, 18	Y		
TX3B376LWP	TX4C198	Galaxy	16,18	W	L	Pantao
TX3C392LW	White Delight One	Unknown	16	W	L	
TX3C394N	TX2B271N	Unknown	16	Y		
White Delight Four	TXW1591-1	Zhaohongzhu	16, 17, 18	W	L	
White Delight One	TX2492-1	Chiyohime	16, 17, 18	W	L	
White Delight Three	TXW1591-1	Zhaohongzhu	16, 17, 18	W	L	
White Delight Two	TX4D46W	Summersweet	16, 17, 18	W	L	
White Zest One	TX3D45W	Tropicprince	16, 17, 18	W		
Y435-250PF			16,18	Y	L	Pantao

[w]. From Terrell, TX [x] Y=Yellow; W=White [y] L=Low acid [z] Pantao=flat shape; NM=non-melting

Table IV-2. Cumulative chill hours, based on the five common models, recorded for the three peach growing seasons 2016, 2017, and 2018 in Fairfield and Terrell, TX areas.

Period	Model	Chill hours*	
		Fairfield	Terrell
12/01/2015 – 1/31/2016	Below 45	460	567
	Between 45 and 32	395	518
	Utah	323	444
	Positive Utah	531	718
	Dynamic	21	29
12/01/2016 – 1/31/2017	Below 45	237	435
	Between 45 and 32	163	306
	Utah	119	288
	Positive Utah	355	587
	Dynamic	16	27
12/01/2017 – 1/31/2018	Below 45	713	666
	Between 45 and 32	469	469
	Utah	418	413
	Positive Utah	612	581
	Dynamic	30	28

*Chill hours data was obtained from Get Chill Hours! http://www.getchill.net

One or two drops of juice from each fruit was obtained to measure the soluble solids content (SSC) using a digital refractometer (Atago 3810 PAL-1, Atago Co., Ltd., Tokyo, Japan). For the measuring of pH and titratable acidity (TA), a composite juice sample obtained from the slices of each fruit. Sample slices were placed on a piece of cheesecloth and pressed by a manual (hand-press) juicer. About six grams of the juice was diluted with 50 mL of distilled water. Peach juice was titrated to an endpoint of pH 8.2 with 0.1N NaOH and expressed as milliequivalents of malic acid using an automatic titrator (DL 22 Food and Beverage analyzer, Mettler Toledo, Columbus, OH, USA) at Dr. Patil's Lab at the Vegetable and Fruit Improvement Center. The TA was calculated with the following the formula:

$$\%\ acid = \frac{[mls\ NaOH\ used] \times [0.1\ N\ NaOH] \times [\text{milliequivalent factor}^{*}]\ \times\ [100]}{grams\ of\ sample}$$

* milliequivalent factor for Malic acid = 0.067

Phenotyping protocol measurements are listed in Table IV-3.

Statistical analysis

Statistical analysis was performed using SPSS 21.0 (SPSS Inc., Chicago, USA). Data were subject to analysis of variance (ANOVA), followed by Tukey's test for mean separation. The effect size for genotype, year and their interaction were estimated by eta-squared statistics that describes the proportion of total variability attributable to a factor.

Table IV-3. Phenotyping protocol for fruit quality traits in peach

Fruit quality traits – Non-Destructive phenotyping		
DA-Meter		Chlorophyll contents (0.5 – 1.5)
Diameter		Widest part of the fruit (mm)
Weight		Grams
Fruit quality traits – Destructive phenotyping		
Flesh Firmness average		Newton (8mm tip size)
Brix		° Brix
pH		#
Malic Acid / Titratable Acidity		%
Expressible juice		%
Browning		1 – 6 visual rating scale: *1= None* *6 = Extreme browning covering most of the flesh*
Flesh mealiness		1 – 3 visual rating scale: *1 = Juicy* *2 = Moderate* *3 = Severe mealiness*
Sensory evaluation	Flavor	1 – 2 mouth rating scale: *1 = Yes (flavor perceived)* *2 = No (lack of flavor)*
	Off-flavor	1 – 2 mouth rating scale: *1 = Yes (fermented, bitter)* *2 = No (no off-flavor)*
	Texture	1 – 4 mouth rating scale: *1 = Juicy* *2 = Juicy and mealy* *3 = Mealy* *4 = Leathery*

Results and Discussion

Ripe date (RD)

The average ripe dates for 35 peach genotypes varied widely among genotypes in each year, although the changes were minor among years for the same genotype (Table IV-4). This is expected as this trait is dependent on the environmental conditions, especially temperature (Mounzer et al., 2008; Ruiz and Egea, 2008; Sánchez-Pérez et al., 2007).

The ripe date ranged from late-April to late-June. The earliest (TRH3A17 and TX2A297) were harvested at the end of April and the latest ripening genotypes ('White Delight Three' and 'White Delight Four') were harvested at the end of June. This variability was expected.

Table IV-4. Mean comparison for some phenological and fruit quality traits of 35 peach genotypes evaluated in three growing seasons 2016, 2017, and 2018.

Genotype	***Ripe date***	***Weight (g)***	***Diameter (cm)***	***Firmness (N)***	***pH***	***TA (%)***	***Brix (%)***
TRH3A17	Apr. 28	98.1 lmno*	5.61 lmn	11.01 l	4.3 abcdefg	0.5 abcdef	11.7 ijklm
TX2A297	Apr. 29	73.6 r	5.11 pq	29.95 defghi	3.5 eg	1.0 ab	12.1 fghijklm
Fire Zest One	May. 01	93.1 nop	5.54 mn	46.43 abc	3.9 abcdefg	0.6 abcdef	9.6 r
Flavorrich	May. 5	136.3 cde	6.15 ef	15.58 kl	3.6 e	0.8 abcdf	11.6 jklmn
TX3B298N	May. 07	96.3 lmnop	5.59 klmn	30.1 efgh	3.7 abcdefg	0.8 abcdef	10.4 pq
TX3B376LWP	May. 08	83.2 pqr	6.14 defg	26.81 efghij	4 abcdefg	0.4 bcdef	12.0 ghijkm
Royal Zest One	May. 11	109.5 j	5.78 ijk	15.87 jkl	4.2 abcdfg	0.4 def	11.4 klmn
Flat Delight One	May. 13	93.7 mnop	6.27 cde	35.78 de	4.5 a	0.3 e	13.3 cde
Smooth Delight One	May. 15	100.8 jklmno	5.71 ijklm	22.75 fghijk	4.1 abcdefg	0.4 def	13.1 cdef
Smooth Delight Two	May. 15	110.0 hijk	5.9 ghij	30.54 defgh	4.4 ab	0.3 e	13.0 cdef
TX2C465CW	May. 19	89.1 opq	5.42 no	48.95 ab	3.7 cdefg	0.6 abcdef	13.0 cdef
TX3C392LW	May. 20	120.8 fghi	5.94 ghi	18.3 ijkl	4.2 abdfg	0.4 def	12.3 fghi
TX3C394N	May. 20	107.3 ijkl	5.50 lmno	17.47 hijkl	3.7 cefg	0.6 abcdef	11.9 ghijklmn
White Delight One	May. 21	125.2 fg	6.04 fg	21.87 ghijk	3.9 abcdefg	0.8 abcdef	11.2 lmno
Y435-250PF	May. 21	53.4 s	5.32 op	31.34 ef	4 abcdefg	0.5 cdef	14.0 ab
Royal Zest Two	May. 23	100.0 klmn	5.80 ij	25.49 fghi	3.8 ce	0.8 abc	12.6 fg
Junegold	May. 24	124.4 fg	5.95 gh	26.07 fghi	3.9 bcdefg	0.5 cdef	11.2 lno
Goldprince	May. 26	107.9 jkl	5.72 ijklm	18.04 ijkl	3.8 bcdefg	0.5 cdef	12.0 ghijk
Texstar	May. 26	110.5 hijk	5.69 ijklm	30.72 defgh	3.7 cdefg	1.0 a	10.0 qr
TRH3C113LW	May. 26	100.4 jklmno	5.77 hijkl	17.62 ijkl	4 abcdefg	0.7 abcdef	12.5 efgh
TRH3D38LWP	May. 27	84.2 pqr	6.18 cdefg	34.04 cdefg	4.4 abcdf	0.3 ef	13.6 abcd
White Zest One	May. 29	139.4 cd	6.33 cd	20.29 ijk	4 abcdefg	0.6 bcdef	10.8 op
Smooth Texan Three	May. 30	103.9 jklm	5.69 jklm	26.73 efghi	4.2 abcdefg	0.3 cdef	11.7 hijklmn

Table IV-4 Continued

Genotype	*Ripe date*	*Weight (g)*	*Diameter (cm)*	*Firmness (N)*	*pH*	*TA (%)*	*Brix (%)*
Royal Zest Three	Jun. 01	110.5 j	5.83 hij	21.91 ghijk	4.1 abcdefg	0.4 cdef	12.9 def
TX2D357LW	Jun. 02	122.7 fgh	6.12 efg	12.81 kl	4.6 ab	0.3 cdef	11.0 nop
White Delight Two	Jun. 02	129.8 ef	6.33 cd	21.23 hijk	4 abcdefg	0.5 def	12.2 gh
Royal Zest Four	Jun. 04	107.7 j	5.84 hij	24.98 fghi	4 abcdefg	0.5 cdef	11.9 hijk
Texprince	Jun. 06	77.2 qr	4.97 q	28.91 efghi	3.7 bcdefg	0.8 abcd	12.9 cdefg
Galaxy	Jun. 08	141.4 c	7.22 a	47.42 a	4.2 abdfg	0.4 def	12.6 fg
Sentinel	Jun. 11	125.4 fg	6.00 fgh	21.56 fghijk	4.3 abd	0.4 ef	13.3 bcde
Golden Zest	Jun. 11	130.5 def	6.22 cdef	50.29 a	4.3 ab	0.3 e	13.7 abc
Scarletpearl	Jun. 14	170.5 a	6.68 b	49.09 ab	4.2 abcdefg	0.4 cdef	12.3 fghij
Harvester	Jun. 16	119.7 ghi	6.08 fg	28.67 efgh	3.8 abcdefg	0.5 bcdef	13.5 bcd
White Delight Three	Jun. 26	139.9 c	6.37 c	40.11 bcd	4 abcdefg	0.6 cdef	13.5 bc
White Delight Four	Jun. 30	158.8 b	6.55 b	51.36 a	4.1 abcdfg	0.4 e	14.2 a

* Within each column, means not connected by the same letter are significantly different ($P \leq 0.05$) using HSD test.

Table IV-5. Correlation coefficients among fruit quality and chilling injury traits.

	RD	***Expressible juice***	***Mealiness***	***Texture***	***Flavor***	***Weight***	***Diameter***	***Firm***	***pH***	***TA***
Expressible juice	-0.16									
Mealiness	0.17	-0.74**								
Texture	0.08	-0.73**	0.63**							
Flavor	-0.21	-0.56**	0.40*	0.56**						
Weight	0.62**	0.20	-0.06	-0.12	-0.25					
Diameter	0.52**	-0.05	0.13	0.02	-0.26	0.77**				
Firm	0.36*	-0.17	-0.02	0.24	0.11	0.19	0.31			
pH	0.19	0.03	0.14	-0.12	-0.45**	0.21	0.47**	0.03		
TA	-0.26	0.05	-0.15	-0.01	0.31	-0.21	-0.43**	-0.08	-0.84**	
Brix	0.49**	-0.23	0.17	0.03	-0.26	-0.01	0.19	0.32	0.28	-0.41*

* Significant at $P \leq 0.05$
** Significant at $P \leq 0.01$

Fruit size

Fruit size normally refers to both fruit weight (g) and diameter (cm). This trait is a major factor in determining fruit quality and consumer acceptability (Dirlewanger et al., 1999). The average fruit weight and diameter showed significant variability ($P<0.01$) among studied genotypes (Table IV-4). Fruit weight ranged from 53.4 g (Y435-250PF) to 170.5 g ('Scarletpearl'), while the diameter ranged from 5.0 cm ('Texprince') to 7.2 cm ('Galaxy'). These variations of fruit weight and diameter among genotypes were expected as both traits are genotype specific.

There is a tendency of having higher fruit weight and diameter in the peach genotypes that are harvested later in the season (r = 0.62 and 0.53, respectively) (Table IV-5). This agrees with previous reports of a positive correlation between harvest date and fruit weight (Dirlewanger et al., 1999; Lopez and Dejong, 2007; Ruiz and Egea, 2008).

Firmness

Firmness is a major fruit quality trait in peach fruit as it is directly related to mechanical damage susceptibility and bruising during postharvest (Crisosto et al., 2001b; Kunze et al., 1975).

The maximum level of fruit firmness for marketing fresh peaches and nectarines is set by the Commission Regulation (EC) (2004) of European Union (EU) at 63.7 N (6.9 kgf) with an 8 mm diameter tip. A wide range of fruit flesh firmness variability was noticed among studied genotypes. The range was between 11.0 N (TRH3A17) to 51.4 N ('White Delight Four') which was lower than the maximum levels of fruit firmness allowable for marketing fresh peaches and nectarines.

Among all genotypes, the highest fruit firmness was reported in non-melting fleshed genotypes (‘Golden Zest’, TX2C465CW, and ‘Fire Zest One’), and in a few late-season genotypes (‘Scarletpearl’, ‘White Delight Three’, and ‘White Delight Four’). The lowest firmness was found in the early-season, melting flesh selection TRH3A17 (Table IV-4).

Soluble solids content (SSC)

In general, consumer acceptance is attributed to high soluble solids concentration (SSC) in peaches (Crisosto et al., 2006). SSC values of the genotypes under investigation ranged from 9.6 to 14.2° Brix (Table IV-4). All of our genotypes (except ‘Fire Zest One’) had SSC levels greater than 10° Brix which is considered the minimum value for consumer acceptance (Kader, 1999). Thirty genotypes had SSC greater than 11° Brix that is usually preferred by consumers (Crisosto and Crisosto, 2005; Hilaire, 2003; Kader, 1994). The highest SSC values were observed in fruits of the late-season genotypes (r= 0.49), which was also reported by other researchers (Dirlewanger et al., 1999; Fallahi et al., 2009). Also, the results showed that among evaluated genotypes, flat shaped or white-fleshed fruits were a sweeter taste and higher SSC values compared to regular round or yellow-fleshed fruits. Similar findings were reported previously on different genotypes (Cantín et al., 2010a; Cantín et al., 2009a; 2010b; Crisosto et al., 2006; Crisosto et al., 2001a; Fallahi et al., 2009; Ma et al., 2003; Robertson et al., 1990; Wu et al., 2005).

Fruit acidity

Fruit acidity in peach is another major quality trait in terms of consumer acceptance (Boudehri et al., 2009; Crisosto, 2002). pH and titratable acidity (TA) values ($P<0.01$) varied among genotypes, which was expected (Table IV-4). The TA values ranged from 0.30 ('Smooth Delight Two') to 1.01 g malic acid per 100 g ('Texstar') while pH values ranged from 3.52 (TRH3A17) to 4.45 ('Flat Delight One'). Since the pH is a logarithmic scale and TA is a linear scale, a small change in pH may reflect a greater change in TA. In most cases, the higher pH and lower TA values were found in low acid nectarines, and low acid white-fleshed peaches, while lower pH and higher TA values were present in acid nectarines and acid yellow-fleshed peaches. Byrne et al. (1991) reported the low-acid peach genotypes had lower TA levels by three to five folds as compared to genotypes with traditional acidity.

In this study, we observed that the combination of TA values between 0.3 to 0.5 and SSC from 12 to 14° Brix were associated with low acid peach and nectarine genotypes. This balance between both acid and sugar content present in a fruit, aids in determining peach fruit quality (Colaric et al., 2005). However, optimal sugar and acid contents in peaches and nectarines are not universal criteria and they may change to match diverse consumer and ethnic preferences (Crisosto et al., 2006).

Overall assessment

Based on the overall assessments of these postharvest traits, most of the evaluated peach and nectarine genotypes grown in Texas were prone to develop moderately mealy flesh after storage and had satisfactory organoleptic properties in terms of texture, overall flavor, and other important quality traits.

Also, this work found that chilling injury (CI) symptoms varied among genotype groups in terms of harvest seasons (early, mid, late), flesh color (yellow, white), fruit type (peach, nectarine), and flesh type (melting, non-melting). Expressible juice was higher in early (E) season (50.0%) than in late (L) season (44.6%) genotypes (Fig 1 A), which corresponded to less mealiness and better texture (Fig. V-1 B and C). Crisosto et al. (1999b) reported early season peach and nectarine genotypes showed less susceptibility to CI than later season fruits. Regarding flesh color, yellow-fleshed (Y) fruits develop less CI symptoms and showed significantly ($P<0.05$) higher expressible juice (48.8%) (Fig. V-1 A) with less mealiness and better texture than white-fleshed (W) genotypes (Fig. IV-1 B and C). In this study, peach fruits (P) had slightly higher expressible juice (48.1%) as compared to nectarines (N) (46.3%), but they were similar in mealiness and fruit texture (Fig. IV-1 A, B, C). This is in contrast to several studies that have reported that nectarines are less susceptible to CI symptoms than peaches (Brovelli et al., 1998a; Crisosto et al., 1999b; Lester et al., 1996).

The non-melting flesh (NM) genotypes (TX2A297, TX2A296, TX2C465CW, and 'Golden Zest') had higher expressible juice (50.0%) than melting flesh (M) genotypes (Fig. IV-1 A) with less mealiness (Fig. IV-1 B). However, no difference was observed in fruit texture between the two flesh types (Fig. IV-1 C). Our result was in agreement with several previous studies that stated that NM genotypes are less susceptible to internal breakdown than MF

genotypes (Brovelli et al., 1998a; Crisosto et al., 1999b) due to the non-melting flesh endoPG gene (Peace and Norelli, 2009).

Moreover, ‘Flavorrich’, ‘White Delight One’, and TX2D357LW genotypes had good overall performance regarding fruit quality. Fruits of these genotypes were juicy (scored 1) based on both mealiness and texture scales and expressible juice (53 – 60%) and had outstanding flavor (SSC above 11° Brix and TA between 0.3 to 0.8 %), and large fruit weight (123 to 136 g).

Among all 12 quality traits, we observed that expressible juice, mealiness (visual assessment), and texture (mouth feel) were the most informative traits for assessing post storage traits in peaches and nectarines. These core traits underwent further analysis to assess their consistency over years. As only 16 of the cultivars yielded in 2017 when little winter chilling was received, the analysis was done first for the 16 genotypes that fruited all three years and then for the 31 genotypes that fruited in 2016 and 2018 seasons.

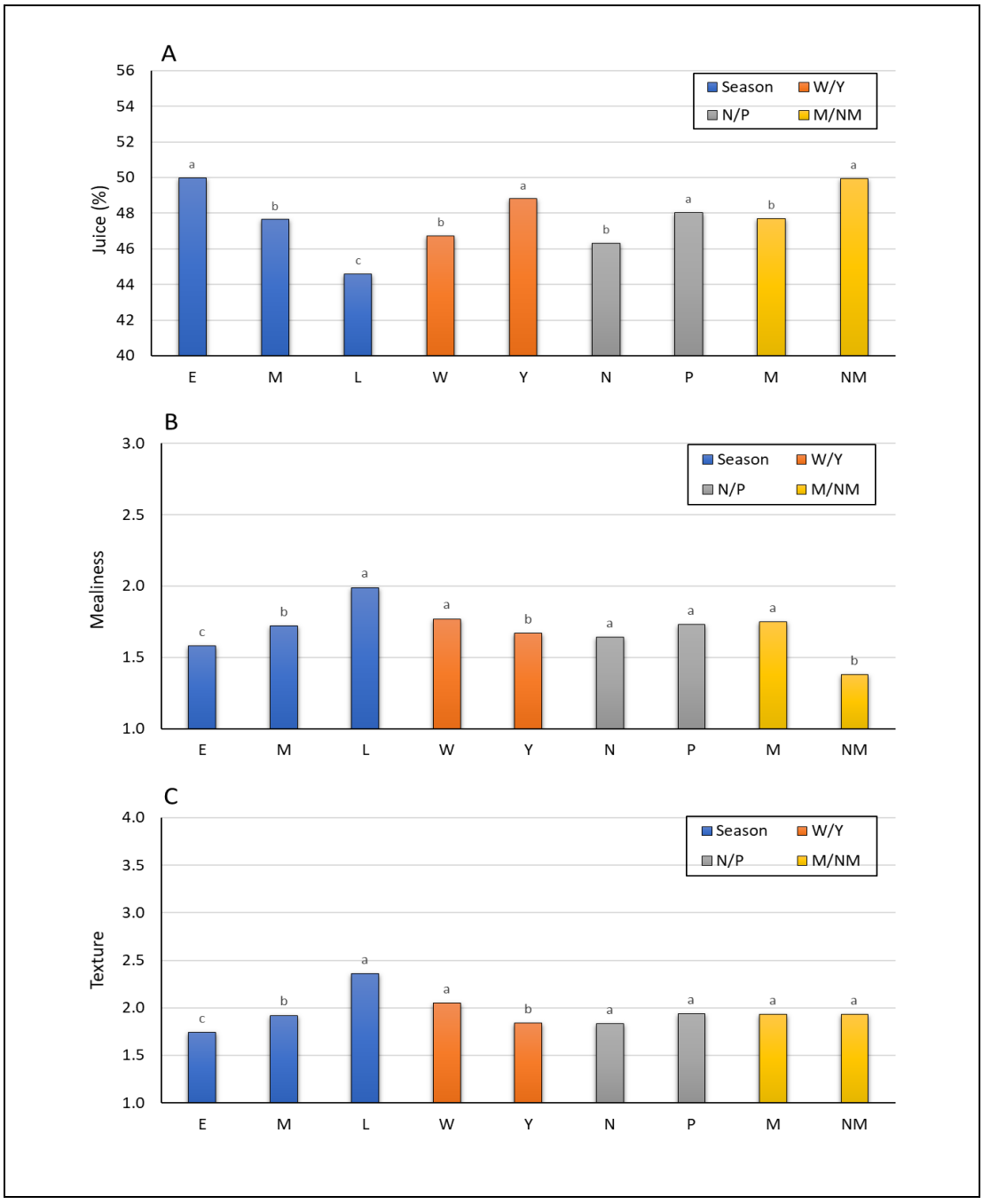

Fig. IV-1. Effect of ripe season (Early, Mid, Late), flesh color (White, Yellow), fruit type (Nectarine, Peach), and flesh type (Melting, Non-melting) on expressible juice (A), mealiness (B), and texture (C).

In each group, means not connected by the same letter are significantly different ($P \leq 0.05$) using Student's t-test.

Results from three years (2016, 2017, and 2018) CI evaluation for 16 genotypes showed significant (*P<0.05*) effects for the genotype (G), year (Y), and G × Y on expressible juice, mealiness, and texture. Although the effect of Y was statistically significant for expressible juice, the effect size (0.05) was small compared to the effects due to G (0.30) and GxY (0.23) (Table IV-6). The same was observed for mealiness and texture with Y effect 0.08 and 0.07, respectively. Over the three years of evaluation, 'Flavorrich', 'Junegold', and 'White Delight One' showed high and consistent performance with regard to expressible juice, mealiness, and texture. While 'Royal Zest One', 'Royal Zest Three', and TRH3A17 were sensitive to the year effect and showed fluctuating responses in all three traits (Fig. IV-2, 3, 4).

Results from two years (2016 and 2018) of CI evaluations for 31 genotypes showed significant effects for the genotype (G), year (Y), and G × Y on expressible juice, mealiness, and texture. As was seen in the previous analysis, the effect size for Y was small (0.03, 0.08, and 0.06 for expressible juice, mealiness, and texture, respectively) as compared to the G and GxY effects (Table IV-6). Among evaluated genotypes, 'Flavorrich', 'Junegold', 'White Delight One', TX2A297, were consistent and showed outstanding performance over the two years. In contrast, 'Harvester' showed consistent poor performance. (Fig. IV-5, 6, 7). The low acid selection TRH3A17 performed differently each year for the three evaluated traits, while the rest of the genotypes either did not show outstanding performance or were fluctuating over the years in one or more traits. The wide range of susceptibility to physiological disorders was expected because the type of chilling injury symptom is highly dependent on the genotype and year (Crisosto, 2002; Etienne et al., 2002; Martínez-García et al., 2012; Peace et al., 2005).

The role of genetics in controlling CI symptoms may make breeding for resistant or less susceptible cultivars achievable goal. Also, conducting consumer surveys would contribute in understanding the role of these quality attributes in consumer preference.

Table IV-6. Effect size[z] of genotype (G), year (Y), and genotype × year (G × Y) for expressible juice, mealiness, and fruit texture across two years (2016, 2018) and three years (2016, 2017, 2018) of evaluation.

Trait	**Effect**	**Two years**[y]	**Three years**[x]
Expressible juice	G	0.39	0.30
	Y	0.03	0.05
	G × Y	0.20	0.23
Mealiness	G	0.34	0.26
	Y	0.08	0.08
	G × Y	0.20	0.21
Texture	G	0.26	0.28
	Y	0.06	0.07
	G × Y	0.14	0.20

[z] Partial Eta-squared value
[y] The effect measured for 31 genotypes
[x] The effect measured for 16 genotypes

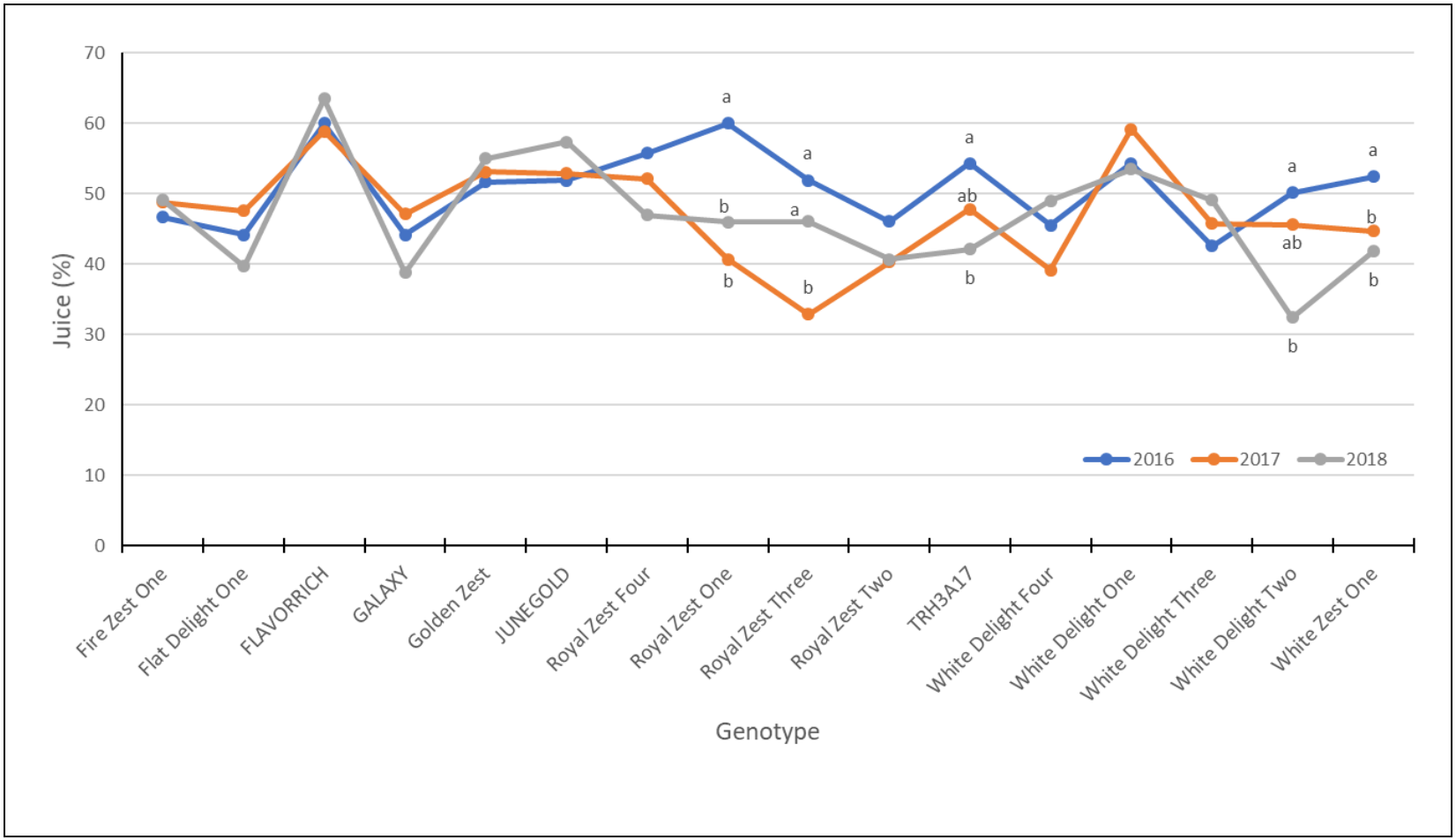

Fig. IV-2. Response of expressible juice in 16 genotypes evaluated over three years (2016, 2017, and 2018).

* For each genotype, years not connected by the same letter are significantly different ($P \leq 0.05$) using HSD test.

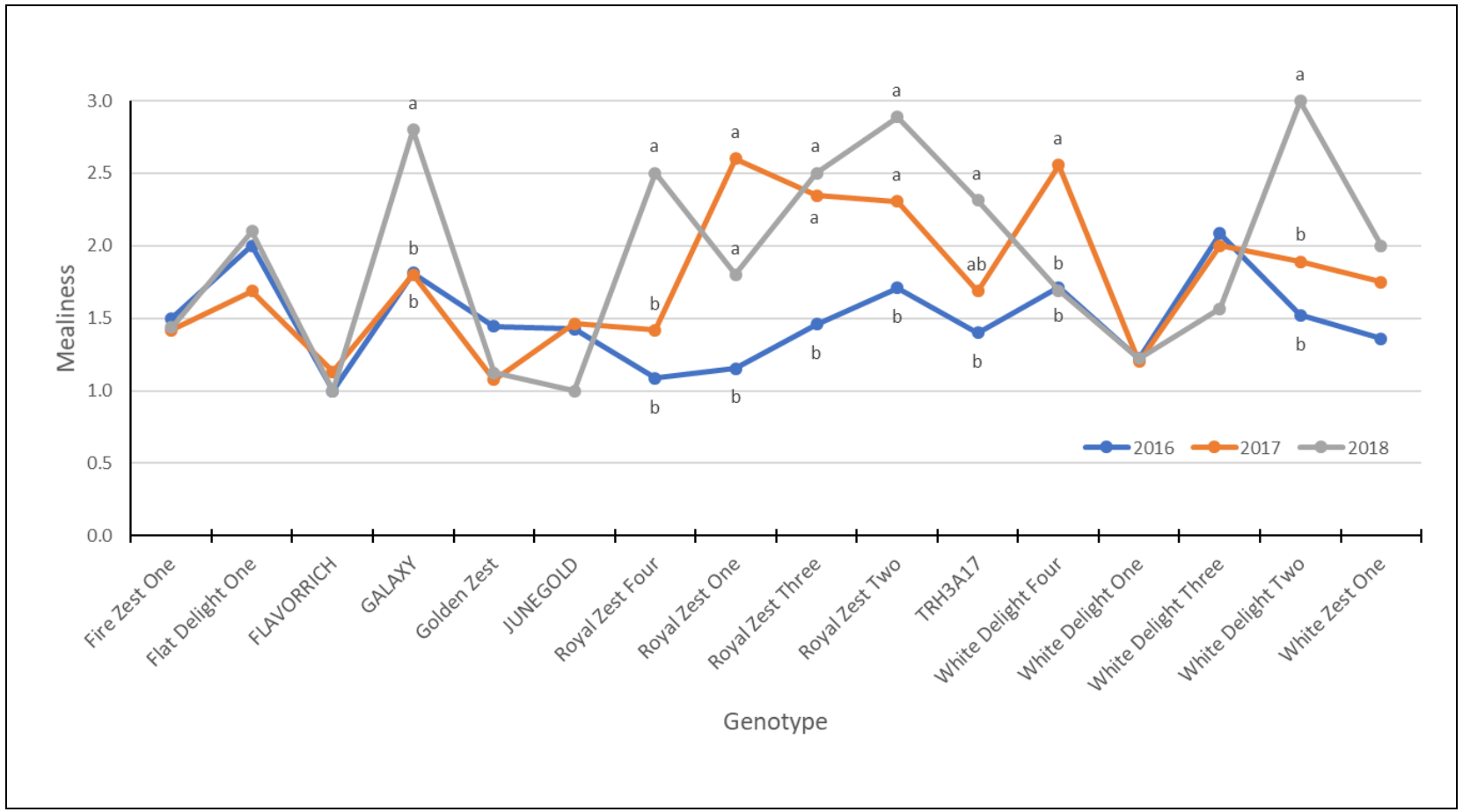

Fig. IV-3. Response of mealiness in 16 genotypes evaluated over three years (2016, 2017, and 2018).

* For each genotype, years not connected by the same letter are significantly different ($P \leq 0.05$) using HSD test.

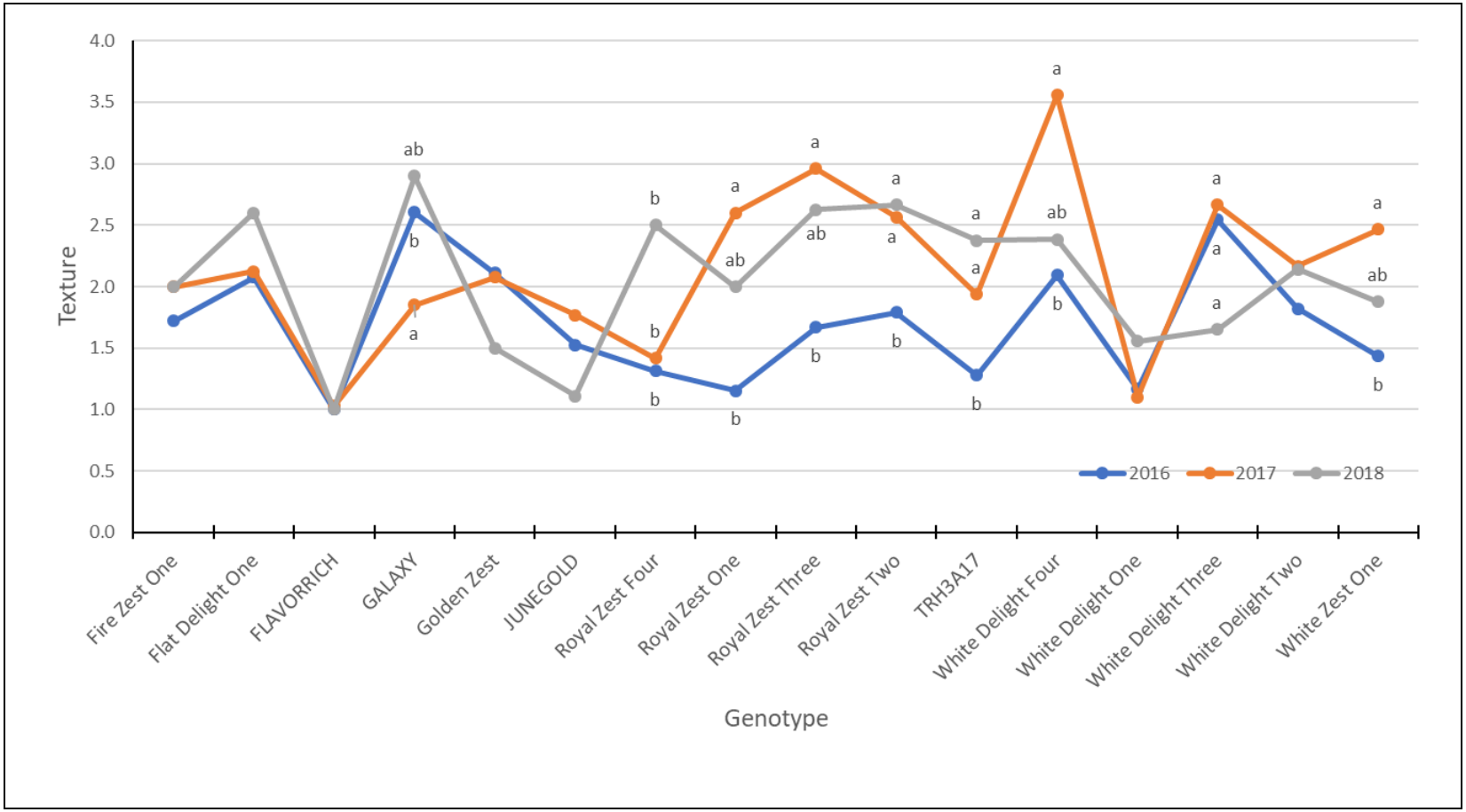

Fig. IV-4. Response of texture in 16 genotypes evaluated over three years (2016, 2017, and 2018).

* For each genotype, years not connected by the same letter are significantly different ($P \leq 0.05$) using HSD test.

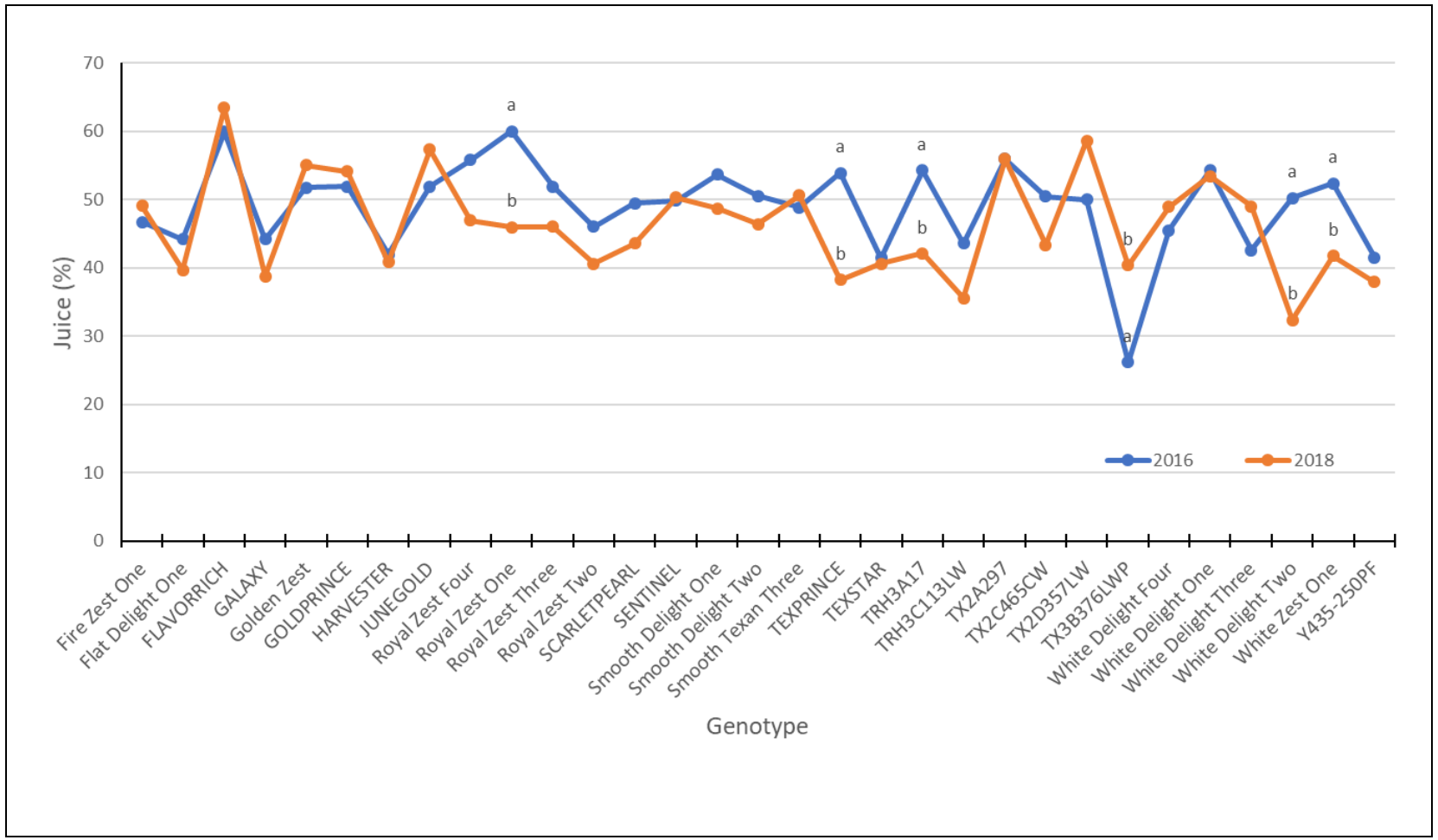

Fig. IV-5. Response of expressible juice in 31 genotypes evaluated over two years (2016 and 2018).

* For each genotype, years not connected by the same letter are significantly different ($P \leq 0.05$) using HSD test.

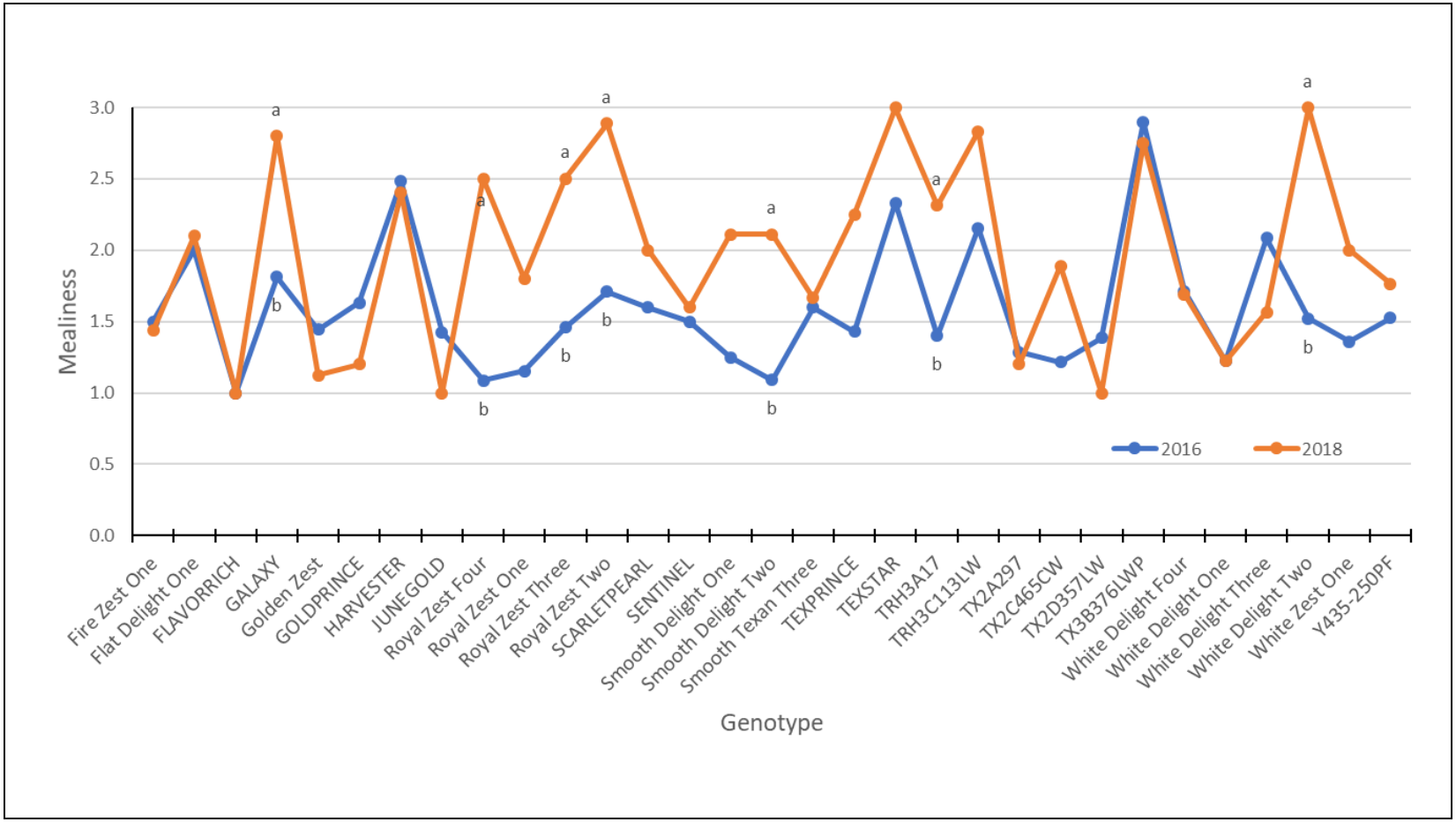

Fig. IV-6. Response of mealiness in 31 genotypes evaluated over two years (2016 and 2018).

* For each genotype, years not connected by the same letter are significantly different ($P \leq 0.05$) using HSD test.

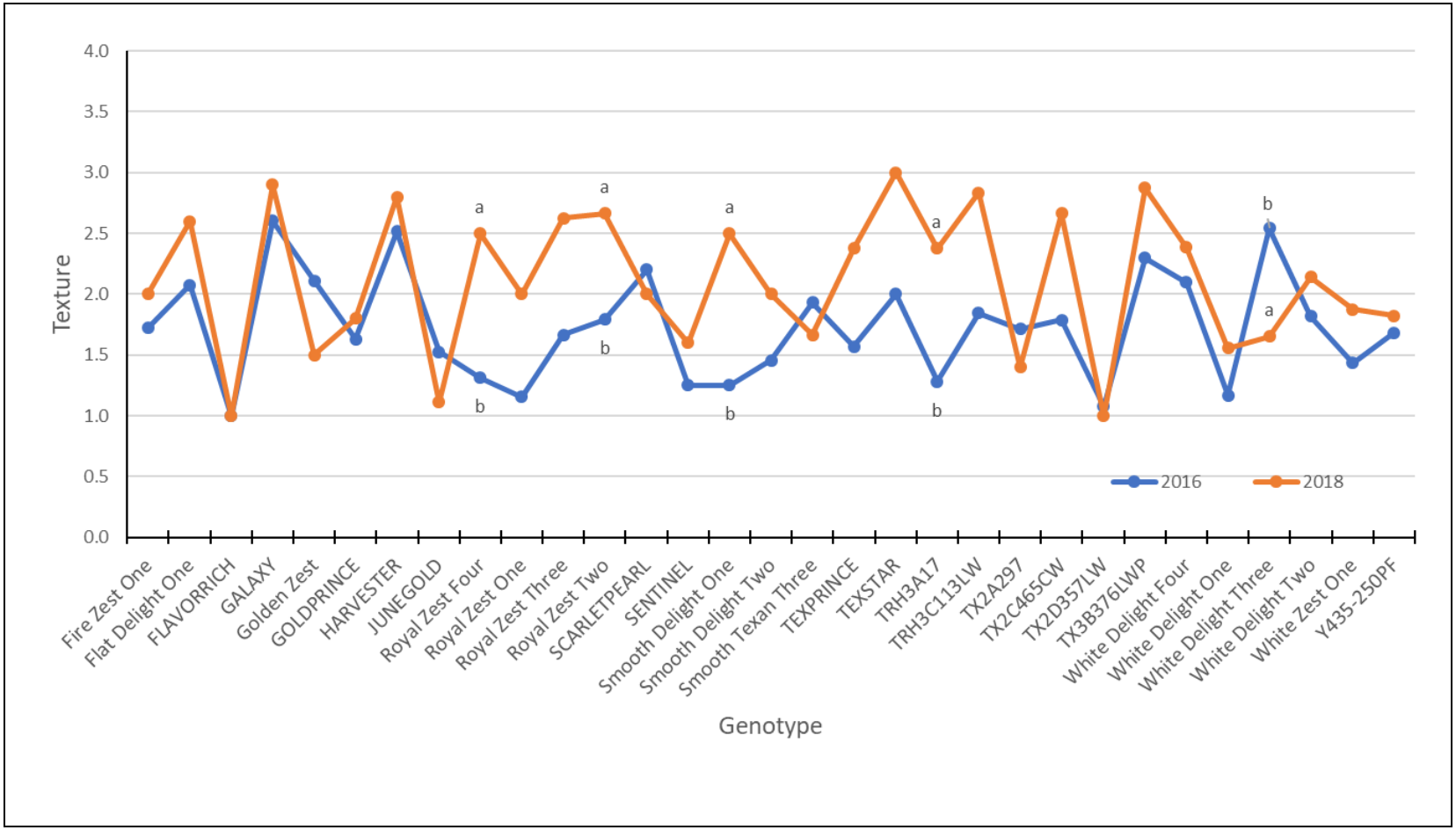

Fig. IV-7. Response of texture in 31 genotypes evaluated over two years (2016 and 2018).

* For each genotype, years not connected by the same letter are significantly different ($P \leq 0.05$) using HSD test.

Correlation among postharvest traits

Significant correlations (*P<0.05*) were found among some quality traits in this study, mainly among the chilling injury symptoms. A positive and significant correlation between mealiness and texture was observed (r=0.63) (Table IV-6), which was expected because the texture is the mouth feel of the visual mealiness. Moreover, a positive and significant correlation between flavor with both mealiness (r=0.40) and texture (r=0.56) was observed. On the other hand, we found negative correlations between expressible juice with mealiness (r=-0.74), texture (r=-0.79), and flavor (r=-0.52). These results indicate that those fruits that showed a dry appearance after squeezing and had a grainy texture in the mouth, had a little or no juice. Our results corroborated that these symptoms are associated with chilling injury disorders and negatively impact peach internal quality and correspond with results of previous works (Brummell et al., 2004; Crisosto et al., 1999b; Lurie and Crisosto, 2005) and with Cantín et al. (2010a) that indicated that off-flavor was positively correlated with mealiness and graininess (texture), which supports that chilling injury negatively affects fruit taste and flavor.

Additionally, a weak negative correlation was found between SSC and TA (r= - 0.41) although SSC was reported to have a weak positive correlation with TA (Cantín et al., 2010b; Dirlewanger et al., 1999; Hartmann, 2013; Wu et al., 2003) with other peach germplasm. As expected, TA was negatively correlated with pH (r= -0.84) and the two parameters for fruit size, fruit weight and diameter were positively correlated (r = 0.77) (de Souza et al., 1998).

Overall, the phenotypic correlations among all studied traits could be due to the selection of genotypes for or to linked genes. The response to breeding for a trait relies on the genotypic variation among individuals of the population as well as the genetic correlations among the traits.

Thus, information about the amount and direction of phenotypic correlation is important to take into consideration in breeding programs.

Conclusion

Peach and nectarine genotypes grown in Texas varied in their storage durability, as measured by expressible juice, mealiness, and texture traits over multiple years. Mealiness incidence developed in fruits after storing them for two weeks at 5° C and negatively impacted the internal quality traits of fruits. TX3C394N, 'Royal Zest Two', 'Texstar', 'Royal Zest Three', TX3B376LWP and 'Harvester' genotypes developed mealy flesh after storage, whereas 'Flavorrich', 'White Delight One', and TX2D357LW genotypes performed well in storage.

On the other hand, early-season genotypes, peach, yellow-fleshed, and non-melting genotypes were less susceptible to chilling injury as compared to other genotypes. A significant correlation found for expressible juice with mealiness (r=-0.74) and texture (r=-0.73), and between mealiness and texture (r=0.63). We noticed that expressible juice, mealiness, and texture are core and most informative traits to evaluate chilling injury symptoms and are complementary to each other by measuring the symptoms quantitatively (expressible juice), visually (mealiness), and organoleptically (texture).

Since these physiological disorders negatively affect internal fruit quality, they limit the marketing and consumption of the fruit. Thus, this study was an important step for developing new peach genotypes free from chilling injury symptoms for enhancing the peach industry.

References

Anderson, R.E. 1979. The influence of storage temperature and warming during storage on peach and nectarine fruit quality [chilling injury, internal breakdown]. *J. Amer. Soc. Hort. Sci.* 104:459-461.

Bassil, N.V. and G.M. Volk. 2010. Standardized phenotyping: Advantages to horticulture—Introduction to the workshop. *HortScience* 45:1306-1306.

Bliss, F.A. 2010. Marker-Assisted Breeding in Horticultural Crops. *Acta Hort.* 859:339-350.

Boudehri, K., M.A. Belka, G. Cardinet, G. Capdeville, C. Renaud, Y. Tauzin, E. Dirlewanger, A.M. Dirlewanger, C. Troadec, D. Jublot, M. Caboche, and A. Bendahmane. 2009. Toward the isolation of the d gene controlling the acidity of peach fruit by positional cloning. *Acta Hort.* 814:507-510.

Brovelli, E.A., J.K. Brecht, W.B. Sherman, and C.A. Sims. 1998a. Anatomical and physiological responses of melting- and nonmelting-flesh peaches to postharvest chilling. *J. Amer. Soc. Hort. Sci.* 123:668-674.

Brovelli, E.A., J.K. Brecht, W.B. Sherman, and C.A. Sims. 1998b. Quality of fresh-market melting- and nonmelting-flesh peach genotypes as affected by postharvest chilling. *J. Food Sci.* 63:730-733.

Brummell, D.A., V. Dal Cin, S. Lurie, C.H. Crisosto, and J.M. Labavitch. 2004. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. *J. Expt. Bot.* 55:2041-2052.

Byrne, D.H. 2005. Trends in stone fruit cultivar development. *HortTechnol.* 15:494-500.

Byrne, D.H., A.N. Nikolic, and E.E. Burns. 1991. Variability in sugars, acids, firmness, and color characteristics of 12 peach genotypes. *J. Amer. Soc. Hort. Sci.* 116:1004-1006.

Byrne, D.H., M.B. Raseira, D. Bassi, M.C. Piagnani, K. Gasic, G.L. Reighard, M.A. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: L.M. Badenes and H.D. Byrne (eds.), Fruit Breeding. Springer US, Boston, MA.

Cantín, C.M., C.H. Crisosto, E.A. Ogundiwin, T. Gradziel, J. Torrents, M.A. Moreno, and Y. Gogorcena. 2010a. Chilling injury susceptibility in an intra-specific peach [*Prunus persica* (L.) Batsch] progeny. *Postharvest Biol. Tec.* 58:79-87.

Cantín, C.M., Y. Gogorcena, and M.A. Moreno. 2009a. Analysis of phenotypic variation of sugar profile in different peach and nectarine *Prunus persica* (L.) Batsch breeding progenies. *J. Sci. Food Agr.* 89:1909-1917.

Cantín, C.M., Y. Gogorcena, and M.A. Moreno. 2010b. Phenotypic diversity and relationships of fruit quality traits in peach and nectarine *Prunus persica* (L.) Batsch breeding progenies. *Euphytica* 171:211-226.

Cantín, C.M., M.A. Moreno, and Y. Gogorcena. 2009b. Evaluation of the antioxidant capacity, phenolic compounds, and vitamin C content of different peach and nectarine *Prunus persica* (L.) Batsch breeding progenies. *J. Agr. Food Chem.* 57:4586-4592.

Ceponis, M.J., R.A. Cappellini, J.M. Wells, and G.W. Lightner. 1987. Disorders in plum, peach, and nectarine shipments to the new york market, 1972-1985. *Plant Dis.* 71:947-952.

Colaric, M., R. Veberic, F. Stampar, and M. Hudina. 2005. Evaluation of peach and nectarine fruit quality and correlations between sensory and chemical attributes. *J. Sci. Food Agr.* 85:2611-2616.

Commission Regulation (EC). 2004. Laying down the marketing standard applicable to peaches and nectarines. *Official Journal of the European Union* 47:L 325/310.

Crisosto, C.H. 2002. How do we increase peach consumption? *Acta Hort.* 592:601-605.

Crisosto, C.H. 2006. Peach quality and postharvest technology. *Acta Hort.* 713:479-488.

Crisosto, C.H. and G.M. Crisosto. 2005. Relationship between ripe soluble solids concentration (RSSC) and consumer acceptance of high and low acid melting flesh peach and nectarine (*Prunus persica* (L.) Batsch) cultivars. *Postharvest Biol. Tec.* 38:239-246.

Crisosto, C.H., G.M. Crisosto, G. Echeverria, and J. Puy. 2006. Segregation of peach and nectarine (*Prunus persica* (L.) Batsch) cultivars according to their organoleptic characteristics. *Postharvest Biol. Tec.* 39:10-18.

Crisosto, C.H., K.R. Day, G.M. Crisosto, and D. Garner. 2001a. Quality attributes of white flesh peaches and nectarines grown under California conditions. *J. Amer. Pomol. Soc.* 55:45-51.

Crisosto, C.H., D. Garner, L. Cid, and K. Day. 1999a. Peach size affects storage, market life. *Calif. Agr.* 53:33-36.

Crisosto, C.H., R.S. Johnson, T. DeJong, and K.R. Day. 1997. Orchard factors affecting postharvest stone fruit quality. *HortScience* 32:820-823.

Crisosto, C.H. and J.M. Labavitch. 2002. Developing a quantitative method to evaluate peach (*Prunus persica*) flesh mealiness. *Postharvest Biol. Tec.* 25:151-158.

Crisosto, C.H., F.G. Mitchell, and Z.G. Ju. 1999b. Susceptibility to chilling injury of peach, nectarine, and plum cultivars grown in California. *HortScience* 34:1116-1118.

Crisosto, C.H., D. Slaughter, D. Garner, and J. Boyd. 2001b. Stone fruit critical bruising thresholds. *J. Amer. Pomol. Soc.* 55:76-81.

Crisosto, C.H. and D. Valero. 2008. Harvesting and Postharvest Handling of Peaches for the Fresh Market, p. 575-596. In: D.R. Layne and D. Bassi (eds.), The Peach: Botany, Production and Uses. CABI, Wallingford, Oxfordshire, U.K.

de Souza, V.A.B., D.H. Byrne, and J.F. Taylor. 1998. Heritability, genetic and phenotypic correlations, and predicted selection response of quantitative traits in peach: II. An analysis of several fruit traits. *J. Amer. Soc. Hort. Sci.* 123:604-601.

Della Strada, G., C. Fideghelli, and F. Grassi. 1996. Peach and nectarine cultivars introduced in the world from 1980 to 1992. *Acta Hort.* 374:43-51.

Dirlewanger, E., P. Cosson, K. Boudehri, C. Renaud, G. Capdeville, Y. Tauzin, F. Laigret, and A. Moing. 2006. Development of a second-generation genetic linkage map for peach [*Prunus persica* (L.) Batsch] and characterization of morphological traits affecting flower and fruit. *Tree Genet. & Genomes* 3:1-13.

Dirlewanger, E., E. Graziano, T. Joobeur, F. Garriga-Calderé, P. Cosson, W. Howad, and P. Arús. 2004. Comparative mapping and marker-assisted selection in Rosaceae fruit crops. *Proc. Natl. Acad. Sci. United States Amer.* 101:9891-9896.

Dirlewanger, E., A. Moing, C. Rothan, L. Svanella, V. Pronier, A. Guye, C. Plomion, and R. Monet. 1999. Mapping QTLs controlling fruit quality in peach (*Prunus persica* (L.) Batsch). *Theor. Appl. Genet.* 98:18-31.

Dirlewanger, E., V. Pronier, C. Parvery, C. Rothan, A. Guye, and R. Monet. 1998. Genetic linkage map of peach [*Prunus persica* (L.) Batsch] using morphological and molecular markers. *Theor. Appl. Genet.* 97:888-895.

Etienne, C., C. Rothan, A. Moing, C. Plomion, C. Bodénès, L. Svanella-Dumas, P. Cosson, V. Pronier, R. Monet, and E. Dirlewanger. 2002. Candidate genes and QTLs for sugar and

organic acid content in peach [*Prunus persica* (L.) Batsch]. *Theor. Appl. Genet.* 105:145-159.

Fallahi, E., B. Fallahi, B. Shafii, and M.E. Amiri. 2009. Bloom and Harvest Dates, Fruit Quality Attributes, and Yield of Modern Peach Cultivars in the Intermountain Western United States. *HortTechnol.* 19:823-830.

Fideghelli, C., G. Della Strada, F. Grassi, and G. Morico. 1998. The peach industry in the world: Present situation and trend. *Acta Hort.* 465:29-40.

Fresnedo-Ramírez, J., M.C.A.M. Bink, E. van de Weg, T.R. Famula, C.H. Crisosto, T.J. Frett, K. Gasic, C.P. Peace, and T.M. Gradziel. 2015. QTL mapping of pomological traits in peach and related species breeding germplasm. *Mol. Breeding* 35:1-19.

Frett, T.J. 2016. Genetic determinism of *Xanthomonas arboricola* pv. *pruni* (Xap) resistance, fruit quality, and phenological traits in peach and incorporation of marker-assisted selection (MAS) in the University of Arkansas peach and nectarine breeding program. University of Arkansas, Fayetteville, AK, Ph.D Dissertation.

Frett, T.J., K. Gasic, J.R. Clark, D. Byrne, T. Gradziel, and C. Crisosto. 2012. Standardized phenotyping for fruit quality in peach [*Prunus persica* (L.) Batsch]. *J. Amer. Pomol. Soc.* 66:214-219.

Garner, D., C.H. Crisosto, and E. Otieza. 2001. Controlled atmosphere storage and aminoethoxyvinyl- glycine postharvest dip delay post cold storage softening of 'Snow King' peach. *HortTechnol.* 11:598-602.

Hartmann, T.P. 2013. Heritability and Phenotypic Correlations in Peach *[Prunus persica* (L.) Batsch]. Texas A&M University, College Station, TX, Master of Science.

Hilaire, C. 2003. The peach industry in France: state of art, research and development, p. 27-34. In: F. Marra and F. Sottile (eds.), Proc. First Mediterranean Peach Symp., Agrigento, Italy.

Ju, Z., Y. Duan, and Z. Ju. 2000. Leatheriness and mealiness of peaches in relation to fruit maturity and storage temperature. *J. Hort. Sci. Biotechnol.* 75:86-91.

Kader, A.A. 1994. Fruit maturity, ripening, and quality relationships. *Perishables Handling Newsletter* 80:2.

Kader, A.A. 1999. Fruit maturity, ripening, and quality relationships. *Acta Hort.* 485:203-208.

Kader, A.A. 2002. Postharvest Technology of Horticultural Crops. 3rd ed. UCANR Publications, UC-Davis.

Kader, A.A. and F.G. Mitchell. 1989. Postharvest physiology, p. 158-164. In: J.H. LaRue and R.S. Johnson (eds.), Peaches, Plums, and Nectarines: Growing and Handling for Fresh Market. Cooperative Extension, University of California, Division of Agriculture and Natural Resources, Oakland, CA.

King, G.A., K.G. Henderson, and R.E. Lill. 1989. Ultrastructural-changes in the nectarine cell-wall accompanying ripening and storage in a chilling-resistant and chilling-sensitive cultivar. *N.Z. J. Crop Hort. Sci.* 17:337-344.

Kunze, O.R., W.H. Aldred, and B.D. Reeder. 1975. Bruising characteristics of peaches related to mechanical harvesting. *Trans. ASAE (Amer. Soc. Agr. Engineers)* 18:939-941.

Lester, D.R., W.B. Sherman, and B.J. Atwell. 1996. Endopolygalacturonase and the Melting flesh (M) locus in peach. *J. Amer. Soc. Hort. Sci.* 121:231-235.

Lopez, G. and T.M. Dejong. 2007. Spring temperatures have a major effect on early stages of peach fruit growth. *J. Hort. Sci. Biotechnol.* 82:507-512.

Lurie, S. and C.H. Crisosto. 2005. Chilling injury in peach and nectarine. *Postharvest Biol. Tec.* 37:195-208.

Lurie, S., H.-W. Zhou, A. Lers, L. Sonego, S. Alexandrov, and I. Shomer. 2003. Study of pectin esterase and changes in pectin methylation during normal and abnormal peach ripening. *Physiol. Plant.* 119:287-294.

Ma, R.J., M.L. Yu, D. Ping, G. Hong, and H.F. Song. 2003. Evaluation of germplasm resources and breeding of flat peach. *Acta Hort.* 620:161-167.

Martínez-García, P.J., C.P. Peace, D.E. Parfitt, E.A. Ogundiwin, J. Fresnedo-Ramírez, A.M. Dandekar, T.M. Gradziel, and C.H. Crisosto. 2012. Influence of year and genetic factors on chilling injury susceptibility in peach (*Prunus persica* (L.) Batsch). *Euphytica* 185:267-280.

Mounzer, O.H., W. Conejero, E. Nicolás, I. Abrisqueta, Y.V. García-Orellana, L.M. Tapia, J. Vera, J.M. Abrisqueta, and M.d.C. Ruiz-Sánchez. 2008. Growth pattern and phenological stages of early-maturing peach trees under a mediterranean climate. *HortScience* 43:1813-1818.

Nanos, G.D. and F.G. Mitchell. 1991. High-temperature conditioning to delay internal breakdown development in peaches and nectarines. *HortScience* 26:882-885.

Peace, C. and J. Norelli. 2009. Genomics Approaches to Crop Improvement in the Rosaceae, p. 19-53. In: K.M. Folta and S.E. Gardiner (eds.), Genetics and Genomics of Rosaceae. Springer New York, New York, NY.

Peace, C.P., C.H. Crisosto, D.T. Garner, A.M. Dandekar, T.M. Gradziel, and F.A. Bliss. 2006. Genetic control of internal breakdown in peach. *Acta Hort.* 713:489-496.

Peace, C.P., C.H. Crisosto, and T.M. Gradziel. 2005. Endopolygalacturonase: A candidate gene for Freestone and Melting flesh in peach. *Mol. Breeding* 16:21-31.

Robertson, J.A., R.J. Horvat, B.G. Lyon, F.I. Meredith, S.D. Senter, and W.R. Okie. 1990. Comparison of Quality Characteristics of Selected Yellow-and White-fleshed Peach Cultivars. *J. Food Sci.* 55:1308-1311.

RosBREED. 2016. Publications and Presentations. https://www.rosbreed.org/publications. Oct. 3

Ruiz, D. and J. Egea. 2008. Phenotypic diversity and relationships of fruit quality traits in apricot (*Prunus armeniaca* L.) germplasm. *Euphytica* 163:143-158.

Sánchez-Pérez, R., E. Ortega, H. Duval, P. Martínez-Gómez, and F. Dicenta. 2007. Inheritance and relationships of important agronomic traits in almond. *Euphytica* 155:381-391.

Sansavini, S., A. Gamberini, and D. Bassi. 2006. Peach breeding, genetics and new cultivar trends. *Acta Hort.* 713:23-48.

Testolin, R. and G. Cipriani. 2010. Molecular Markers for Germplasm Identification and Characterization. *Acta Hort.* 859:59-72.

Wu, B., B. Quilot, J. Kervella, M. Génard, and S. Li. 2003. Analysis of genotypic variation of sugar and acid contents in peaches and nectarines through the principle component analysis. *Euphytica* 132:375-384.

Wu, B.H., B. Quilot, M. Génard, J. Kervella, and S.H. Li. 2005. Changes in sugar and organic acid concentrations during fruit maturation in peaches, *P. davidiana* and hybrids as analyzed by principal component analysis. *Scientia Hort.* 103:429-439.

CHAPTER V

SCREENING SENSITIVITY TO BACTERIAL LEAF SPOT IN PEACH TREES

Synopsis

Bacterial leaf spot (BLS), caused by *Xanthomonas arboricola* pv. *pruni* (Smith), is an economically important disease in stone fruit crops throughout the world. Disease infection and development are favored by warm and humid environments such as experienced by the eastern part of the U.S. Temperatures between 20 and 35° C along with frequent rains periods, and heavy dews from late bloom to pit hardening stages maximizes the disease severity. The disease causes necrotic lesions on leaves and fruits, and cankers on the twigs. The objective of this study was to screen the sensitivity to *X. arboricola* pv. *pruni* across seven F_1 peach populations using phenotypic data. Seven peach families grown in College Station, Texas were evaluated for their sensitivity to bacterial leaf spot across three years (2016 to 2018) using field and lab assessment methods. Our results revealed that all populations showed high susceptibility to bacterial leaf spot based on both assessment methods. The detached-leaf bioassay was an accurate approach for the assessment of bacterial leaf spot incidence even in a year in which the disease incidence in the field was low. The disease severity in the field varied with the environmental conditions with much less BLS incidence seen in 2018 when there was less rain during April to June in terms of quantity (~130 mm versus ~528-320 mm) and the number of events (11 versus 26-23) as compared to 2016 and 2017, respectively.

Introduction

Bacterial leaf spot (BLS) is caused by *Xanthomonas arboricola* pv. *pruni* (Smith) (Xap). It was first described in 1902 on plum trees in North America (Smith, 1903) and is an economically important disease in stone fruit crops throughout the world. This disease is found mainly on peach, nectarine, almond, plum, apricot, and sweet cherry (EPPO, 1997; 2006; Ritchie, 1995). The pathogen is found in almost all stone fruit production areas in North America, Europe, and Asia. Based on the European and Mediterranean Plant Protection Organization (EPPO), *X. arboricola* pv. *pruni* is a regulated quarantine pathogen, listed as an A2 pest (pests are locally present in the EPPO region) (EPPO, 2018).

Xap is classified as a mobile, gram-negative, and rod-shaped bacterium. This bacterium was first named *X. pruni* (Smith, 1903) and then classified as *X. campestris* pv. *pruni* in 1978 by D. W. Dye (Young et al., 1978). Most recently, it was reclassified as *X. arboricola* pv. *pruni* on the bases of DNA-DNA hybridization and the tree it infects (Vauterin et al., 1995). The disease can establish and develop in the field when the inoculum is present and favorable environmental conditions present (EPPO, 1997; Ritchie, 1995). Warm temperature between 20 – 35 ºC and humidity ≥ 75% are the most favorable conditions for BLS infection (Ritchie, 1995; Zehr et al., 1996), However, 28.9°C is the optimal temperature for infection (Morales et al., 2018). In addition, different studies mentioned the importance of warm temperatures and wetness periods (e.g. rainfall, dew) for increasing the disease severity (Battilani et al., 1999; Linvill, 2002; Stefani, 2010). Peach fruits are susceptible from late bloom to pit hardening stages, however, peach trees are prone to bacterial leaf spots infection when environmental conditions are optimal (Daines, 1961; EPPO, 1997; Randhawa and Civerolo, 1985; Ritchie, 1995; Zehr et al., 1996). Sandy soil sites generally experience more Xap than other soil types because wind-blown sand

may cause wounds on the plant surface that facilitate the entry of bacteria into the plant tissues (EPPO, 1997; Ritchie, 1995).

The disease causes necrotic lesions on leaves and fruits, and cankers on the twigs (EPPO, 1997; 2006; Ritchie, 1995). The spots initially appear on the lower surface of the leaf as small, angular, light-green to yellow, water-soaked lesions with a brownish-yellow center. As the disease progresses, these spots appear on the upper surface of the leaf and enlarge and darken in color to purplish-brown or black with the area surrounding the spots turning yellow. Symptoms appear and concentrate on the leaf tip, the midrib, and leaf margin, where water that carries the bacteria accumulates from rains or dews (EPPO, 2006; Ritchie, 1995).

Fruit symptoms are small, circular-brown spots on the fruit surface. These spots generally enlarge, become water-soaked with sunken spots and may develop into cracks and pits as the fruit grows (EPPO, 2006; Ritchie, 1995). Bacteria overwinter in the intercellular spaces of infected twigs. In the spring, bacteria develop a spring canker where the bacteria multiply to develop the primary inoculum (EPPO, 1997). The inoculum is spread to other tissues by rain, wind, insects, and wounding. Generally, this disease causes premature leaf drop and with time decreased tree vigor and an overall decline in fruit quality and production (Aarrouf et al., 2008; Ritchie, 1995).

Pesticide application such as copper compounds or antibiotics (oxytetracycline) is the primary method that is used to control Xap in the peach orchard (Ritchie, 1995; 1999). However, misuse of copper sprays may cause fruit and foliage damage in peach trees. In contrast, oxytetracycline can be applied during shuck split as it has a less detrimental effect on the fruit and foliage (Ritchie, 1995; 1999). The control of this pathogen becomes very difficult once it is established in the orchard, particularly if highly or moderately susceptible cultivars are present in

combination with favorable environmental conditions. Moreover, restrictions on using chemical pesticides in agriculture have developed in recent years due to environmental, health, economic, and food safety concerns (Ritchie, 1995). Planting resistant peach cultivars is the most effective and promising way to control the disease (Byrne et al., 2012; Sansavini et al., 2006; Werner et al., 1986). However, many of the available resistant cultivars are characterized by a lack of desirable fruit quality and marketing characteristics (Okie, 1998).

Traditional breeding for resistant cultivars is a difficult task because the response to BLS is polygenic in nature (Yang et al., 2010) and it is difficult to phenotype the resistance to this pathogen accurately. Several methods have been used for evaluating bacterial spot incidence. This includes field evaluation with natural infection (Hansche, 1983; Yang, 2012), or greenhouse evaluation using inoculation methods such as by immersing leaves in inoculum (Daines and Hough, 1951; Topp et al., 1993), or infiltrating by high pressure sprays (Civerolo and Keil, 1976; du Plessis, 1986), or in the lab by infiltrating with a needleless syringe (du Plessis, 1986; Frett, 2016; Hammerschlag, 1988; Randhawa and Civerolo, 1985; Topp et al., 1993) The visual evaluation of disease incidence or severity in the field is the primary method that has been used since the 19th century (Cobb, 1892). However, screening for resistance to BLS via the field evaluation is a challenging task as the accuracy is affected by environmental condition, distribution of the pathogen inoculum in the field, and the health of the host plant (Yang et al., 2013). To obtain more informative and precise phenotypic data for assessing Xap resistance, greenhouse inoculations and detached-leaf bioassays techniques have also been suggested (Frett, 2016).

Both continuous and discrete rating scales have been used to measure the disease severity in the crops (Sheskin, 2007). The ‘1-5’ visual rating scale was the first categorical scale

developed and used to measure the severity of disease (rust on wheat) (Cobb, 1892). Currently, '0-3' scale (0 = no symptoms and 3 = distinct, dark necrotic spot of > 2mm, with or without a chlorotic halo) has been applied for assessing bacterial leaf spot symptoms at each inoculation site in detached leaf or greenhouse evaluations (Civerolo and Keil, 1976; Hammerschlag, 1988; Hammerschlag et al., 1994; Randhawa and Civerolo, 1985) and a '0-5' scale (0 = no symptoms and 5 = highly susceptible) was used for field evaluation on peach trees inoculated with bacterial suspension by Yang et al. (2013).

The detached-leaf bioassay was developed to be a rapid, cheap, and reliable laboratory method for assessing peach trees susceptibility to BLS (Civerolo and Keil, 1976; Hammerschlag, 1988; Hammerschlag et al., 1994; Randhawa and Civerolo, 1985). In this method, leaves are collected from the host plant in the field or the greenhouse, infiltrated with the pathogen inoculum using a needle-less syringe, and then the inoculated leaves are incubated on Petri dishes (water agar) for two weeks. Later, a qualitative '0-3' rating scale is used to differentiate symptoms of infection at each inoculation site on the leaves (Civerolo and Keil, 1976; Hammerschlag, 1988; Hammerschlag et al., 1994; Randhawa and Civerolo, 1985).

The objective of this study was to screen *X. arboricola* pv. *pruni* sensitivity across seven F_1 peach populations using phenotypic data for three consecutive years (2016-2018) in field evaluations and for one year with a detached leaf bioassay.

Materials and Methods

Plant materials

Phenotyping evaluation for BLS resistance was performed on seedlings of seven F_1 peach families grown from seeds originating from crosses among important parents of low-medium chill peach genotypes (Table V-1).

In 2015, one-year-old seedlings were planted at the Texas A&M University Horticulture Research, Teaching, and Extension Center (HortTREC) in College Station, TX (30°31'30.43" N, 96°25'20.59" W). The orchard soil is calcareous and has a clay-loam texture (TAMU Soil Characterization Laboratory, 2018). The 192 individuals were planted in double rows with a spacing of 6 ft between the rows and 2 ft between seedlings. Sets of double rows were planted on 20 ft centers. Standard horticultural practices were applied. All rows were covered with a woven landscape fabric as a weed barrier for weed control.

After planting, seedlings were pruned and fertilized twice a year with water-soluble 20-20-20 (N-P-K) and iron chelate (Sprint 138). Seedlings received a single application of 640 $kg.ha^{-1}$ of 19-19-19 (N-P-K) in the spring and were drip irrigated as needed. Pests and diseases were managed with Malathion 5, and Ultra-fine Oil for stink bug, Sevin SL for leaf-footed bugs and Captec 4L for brown rot. The weeds were controlled with glyphosate and diquat around bed edges for general weed control, and Clethodim in beds for grass (mainly Bermuda grass) control.

Temperature, relative humidity, and rainfall data were obtained for College Station, TX for 2016, 2017, and 2018 for the period from Mar. 1 to Aug. 31 (Weather Underground, 2018).

Table V-1. The seven F_1 peach families and their female and pollen parents with the number of seedlings.

Population	Female parent (FP)	Pollen parent (PP)	Number of seedlings
FireZestOne×Y435-250PF	Fire Zest One	Y435-250PF	27
WhDelightOne×WhZestOne	White Delight One	White Zest One	46
WhDelightOne×TX4E311LWP	White Delight One	TX4E311LWP	9
TexFirst×FireZestOne	TexFirst	Fire Zest One	14
TX2B136×FireZestOne	TX2B136	Fire Zest One	33
TX2B136×TX2A297	TX2B136	TX2A297	51
TXW1A53×TX2A297	TXW1A53	TX2A297	12

Phenotypic evaluation

Field evaluation

Resistance to bacterial leaf spot is a trait of interest for peach breeding at Texas A&M University as many commercial cultivars are susceptible and the disease is common in Texas peach growing regions. However, the degree of disease severity varies across years and among peach cultivars. The warm temperatures and high humidity during spring and summer in the eastern part of Texas, including College Station where the peach orchard of this study is located, provided a conducive environment for the pathogen inoculation and spread.

Field evaluation for Xap infection was conducted for three consecutive years (2016, 2017, and 2018) at the HortTREC orchard of Texas A&M University under conditions of natural infection. During this period no antibiotic or copper-containing chemical sprays were applied to control the disease in the field. The incidence of the disease was assessed visually using a rating scale from 0 to 5 (Table V-2) that was developed by Yang (2012). The data was collected in June

of 2016 and 2017 from the seven seedling families but only from six families in June of 2018 because the individuals of one family were very weak.

Table V-2. Visual rating scale for *Xanthomonas arboricola* pv. *pruni* infection symptoms on peach leaves in the field (Yang et al., 2013).

Score	**Symptom**
0	no leaves with symptoms
1	1-5% diseased leaves or observed defoliation
2	6-10% diseased leaves or observed defoliation
3	11-25% diseased leaves or observed defoliation
4	25-50% diseased leaves or observed defoliation
5	> 50% diseased leaves or observed defoliation

Lab evaluation

Inoculum preparation

The strain of *X. arboricola pruni* used in the inoculations was obtained from several diseased leaves from the peach orchard at HortTREC at Texas A&M University in June of 2018 (Dr. D.N. Appel, personal communication). The Xap bacterium was isolated from peach leaves following a procedure developed by Civerolo et al. (1982). Infected peach leaves were collected from the orchard at HortTREC and placed inside sealable plastic bags that were then placed in a cooler to transport to the lab.

Tissue with lesions from the infected leaves was excised with a sterilized blade, placed in 2 mL tubes with 1 mL of phosphate buffered saline (PBS) and vortexed for 3 seconds. After removing the plant tissue, a loop of the bacterial solution was streaked onto nutrient agar (NA)

Petri plates (23 g commercial Difco™ NA in 1000 mL of distilled water amended with 2 ppm of the fungicide propiconazole). The cultures were incubated for 48 to 72 h at 30° C.

Xap identification

A single colony was taken from the incubated plate and re-streaked onto NA plates and allowed to grow for two days to obtain a pure culture. The culture identity was verified via morphological and molecular characterization and pathogenicity tests. The isolate of *X. arboricola pruni* developed colonies with a convex, smooth, mucoid and bright yellow appearance that darken to yellow-orange with age. The molecular confirmation of its identity was done with DNA extracted from the bacterial isolate using the Zymo Fungal/Bacterial DNA Kit and following the manufacturer's instructions (Zymo Research, Irvine, CA). The 16 S ribosomal RNA regions of the isolate were amplified by using Internal Transcribed Spacers (ITS 1 and ITS 4) then sequenced by Sangon sequencing. The sequenced nucleotide was assembled using Geneious software version 11.0.5 (Biomatters Inc., Newark, NJ) and the nucleotide sequences were deposited in GenBank with accession numbers MF351917 to MF351923. BLASTn analysis indicated 100% nucleotide sequence identity with *Xanthomonas arboricola* pv. *pruni* strains ZJ 195, SXD90, SD175, FZ51, DL131, CL189, and BGC239 present in the BLAST database.

Inoculum suspension

The bacterial cultures were washed from the NA Petri plates by adding 5 mL of PBS. The bacterial suspension was adjusted to an optical density of ~ 0.29 to 0.30 × 10^{-6} (600 nm) was prepared using Multiskan FC Microplate Photometer (Thermo Fisher Scientific, Waltham, MA). The inoculum suspension of medium concentration was prepared daily and used for the leaf assay.

Detached-leaf bioassay

A modified detached-leaf bioassay, as described by Randhawa and Civerolo (1985) was used to assess the disease severity. Ten fully expanded young, symptomless leaves from each seedling were collected and placed inside sealable plastic bags and then placed in a cooler to transport to the lab for inoculation. Based on leaf size, about $^{1}/_{5}$ to ⅓ of the leaf was removed from both proximal and distal ends to fit in the petri dish. The trimmed leaves were soaked in sterile distilled water for one minute followed by another min in 70% ethanol, and then rinsed for one min with sterile distilled water. Five leaves were assigned for each treatment (inoculated and the control). For inoculations, the bacterial suspension was loaded into a 3-mL needleless syringe, positioned firmly against the abaxial side and infiltrated carefully with pressure until a water-soaked area (2-3 mm) became visible. To minimize the pressure from the syringe on the leaf, several layers of sterile paper towels were placed underneath, and the excess inoculum around the inoculated sites was gently wiped off using a sterile paper towel. This was done on eight sites spaced about 1 cm apart per leaf.

Inoculated leaves were placed in plastic Petri dishes on water agar (1.5%) medium amended with 2 ppm propiconazole with the inoculated sides up. For incubation, all plates were placed inside clear plastic storage boxes at 25° C under fluorescent lights for 10 days. Data was

taken 10 days after inoculation using two approaches: a visual assessment and image analysis. The visual assessment scale of 0 to 3 grades (Table V-3) was used to differentiate symptoms of infections at each inoculation site (Frett, 2016; Hammerschlag, 1988; Hammerschlag et al., 1994). The populations were categorized into three groups based on the average visual rating score: resistant (0.00 - 1.50), intermediate (1.51 - 2.25), and susceptible (2.26 - 3.00) (Frett, 2016). For each population, the average rating score was obtained from each individual seedling.

Table V-3. Visual rating scale for *Xanthomonas arboricola* pv. *pruni* infection symptoms on peach leaves in the detached leaf bioassay (Frett, 2016).

Score	**Symptom**
0	no symptoms
1	distinct chlorotic spot (yellowing) and/or slight necrotic flecks
2	distinct but pale necrotic spot or grayish-white lesion, 2 mm in diameter
3	distinct, dark necrotic spot of > 2 mm in diameter, with or without a chlorotic halo

The lesion diameter from each of the eight inoculated sites on the leaf was measured by scanning the leaves with a flatbed scanner at 300 DPI resolution (MFC-J475DW, Brother Corporate, Bridgewater, NJ). The lesion diameter from scanned leaf images was estimated with the open source ImageJ software (version 1.52d). Visual rating score and lesion diameter for each individual seedling were calculated as the average of five leaves.

Statistical analysis

Descriptive statistics, mean, median, standard deviation, minimum and maximum values of phenotypic data of field and lab evaluations were calculated using JMP Pro Version 13.2 (SAS Institute Inc., Cary, NC, 2016). ANOVA and the non-parametric Wilcoxon rank sum

pairwise comparisons test ($P \leq 0.05$) was used to compare the mean scores among populations and years.

In addition, the Pearson's correlation coefficient (r) was calculated to measure the degree of association among the three assessment methods (average of the three-years field rating (0-5), lab visual rating (0 - 3), and lesion diameter).

Weather conditions, including temperature, relative humidity, precipitation, number of rainy days were obtained from Weather Underground (2018) for 2016, 2017, and 2018 for College Station, TX.

Results

Weather conditions analysis

Weather conditions in 2016, 2017, and 2018 during the period March to June (when disease symptoms appear on leaves) varied markedly from one year to another, especially for precipitation and rain frequency, however, no major differences were noticed on average temperature and relative humidity between years of evaluation. (Table V-4; Fig. V-1).

As expected, the temperature increased as the year progressed. The range of mean monthly temperatures was 18.4 to 19.5° C, 18.2 to 21.1° C, 23.2 to 25.9° C and 27.8 to 29.1° C for March, April, May, and June, respectively. The year 2016 and 2017 had the higher relative humidity (77.6% and 75.4% versus 72.6%), greater precipitation (632 mm and 360 mm versus 256 mm) and more rain days (32 and 29 versus 16 days) than did 2018 (Table V-4; Fig. V-1).

Table V-4. Temperature, relative humidity, precipitation, and number of rainy days for the period from March to June 2016, 2017, and 2018 in College Station, TX.

	Average temperature (°C)			Average relative humidity (%)			Accumulative precipitation (mm)			Total number of rainy days		
Month	**2016**	**2017**	**2018**	**2016**	**2017**	**2018**	**2016**	**2017**	**2018**	**2016**	**2017**	**2018**
March	18.4	19.5	18.4	73.6	74.1	69.5	104.2	40.6	126.9	6.0	6.0	5.0
April	20.3	21.1	18.2	78.1	75.6	72.1	157.5	86.3	38.1	12.0	6.0	4.0
May	23.2	23.4	25.9	81.3	74.5	74.7	307.3	119.3	38.1	9.0	9.0	3.0
June	28.0	27.8	29.2	77.5	77.3	74.2	63.4	114.1	53.3	5.0	8.0	4.0
Overall	22.5	22.9	22.9	77.6	75.4	72.6	632.4	360.4	256.4	32.0	29.0	16.0

Overall observations indicated that Xap infection levels were significantly greater ($P<0.05$) in 2016 (4.7) and 2017 (4.9) as compared to 2018 (3.7) (Table V-5; Fig. V-2). This is attributed to the more favorable weather conditions (higher % relative humidity, more precipitation and more rain days) for the spread and infection of the Xap inoculum.

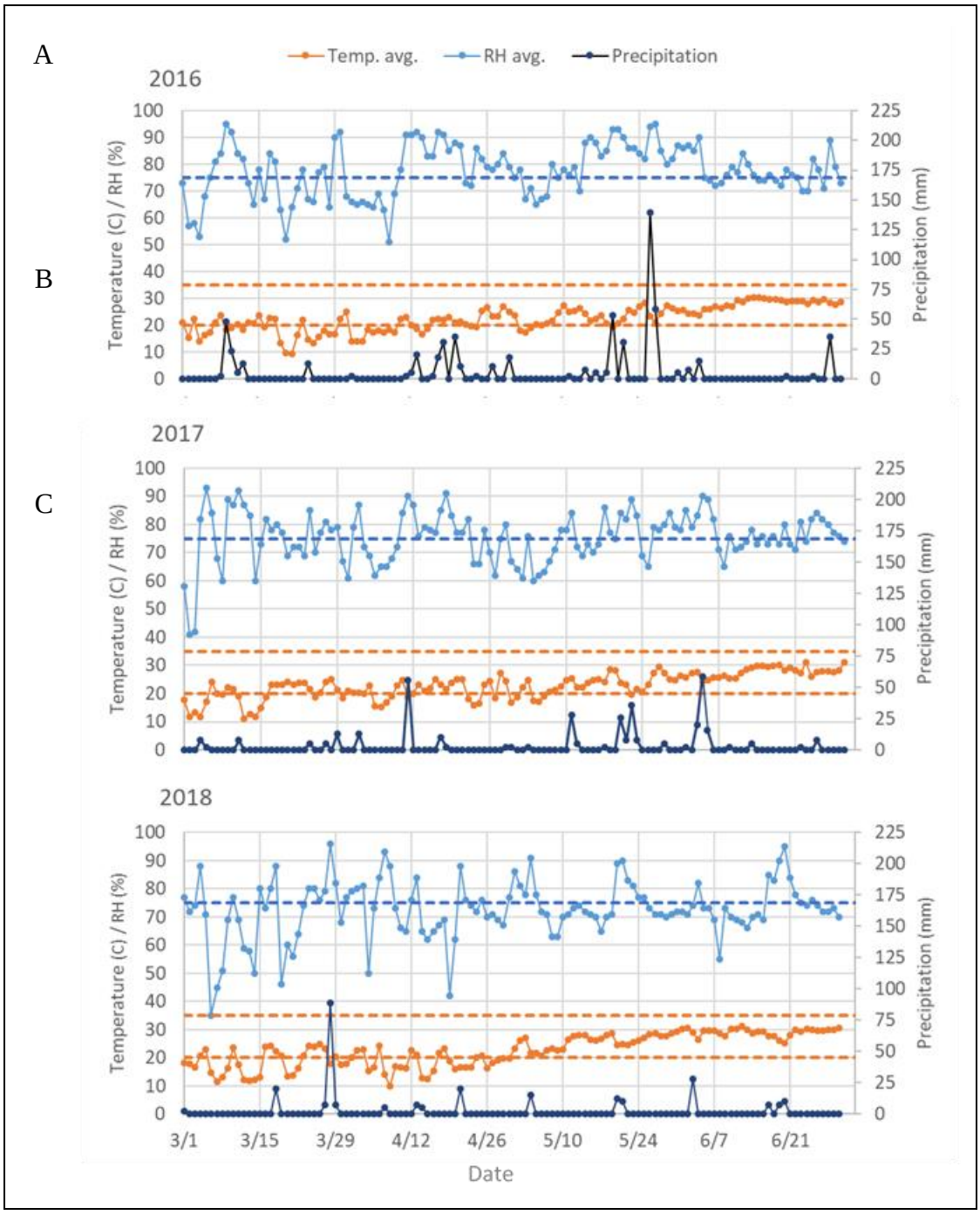

Fig. V-1. Temperature (°C), relative humidity (%), and precipitation (mm) for the period from March 1 to June 30 of the three years of evaluation 2016 (A), 2017 (B), and 2018 (C) in College Station, TX.

Table V-5. Field disease severity ratings for *Xanthomonas arboricola* pv. *pruni* in F_1 peach populations evaluated in College Station, TX for 2016, 2017, and 2018 using a 0 - 5 visual rating scale[z].

Population	N	2016	N	2017	N	2018
FireZestOne×Y435-250PF	27	4.8 a[y]	26	4.9 a	22	3.7 bcd
WhDelightOne×WhZestOne	46	4.1 b	46	4.8 a	41	3.9 bc
WhDelightOne×TX4E311LWP	9	4.4 ab	9	4.6 ab	-	-
TexFirst×FireZestOne	14	5.0 a	14	5.0 a	14	3.7 bcd
TX2B136×FireZestOne	33	4.9 a	33	4.8 a	33	3.5 cd
TX2B136×TX2A297	51	4.9 a	51	4.9 a	50	3.8 bc
TXW1A53×TX2A297	12	5.0 a	12	5.0 a	12	3.0 d

[z] Visual rating scale 0 = no leaves with symptoms, 1 = 1-5%, 2 = 6-10%, 3 = 11-25%, 4 = 25-50%, 5 = > 50% diseased leaves.
[y] Means not connected by same letter across populations and years are significantly different ($P \leq 0.05$) using HSD test.

Field evaluation (2016-2018)

The field rating with the 0 - 5 scale indicated that all of the seven F_1 populations in this study were susceptible to *X. arboricola* pv. *pruni* and that it was consistent over years. However, the level of disease incidence varied among the years (Table V-5; Fig. V-2). In 2016 and 2017, similar patterns for Xap infection were observed and all populations showed a mean rating greater than 4 (susceptible) (Table V-5). In 2016 and 2017, the ratings ranged from 4.1 to 5.0 and from 4.6 to 5.0, respectively, while in 2018 the range was 3.0 to 3.9 indicating that less infection was in 2018 (Table V-5). None of the populations exhibited resistance/tolerance (classes 0, 1, or 2) in the field evaluations (Fig. V-3). The minor effect size was observed for the population

(0.04) and the interaction between population and year (0.09) compared to the effect size of the year (0.37) (Table V-6), suggesting that all evaluated populations were highly susceptible to Xap, and the significant influence of year (weather) on the disease incidences.

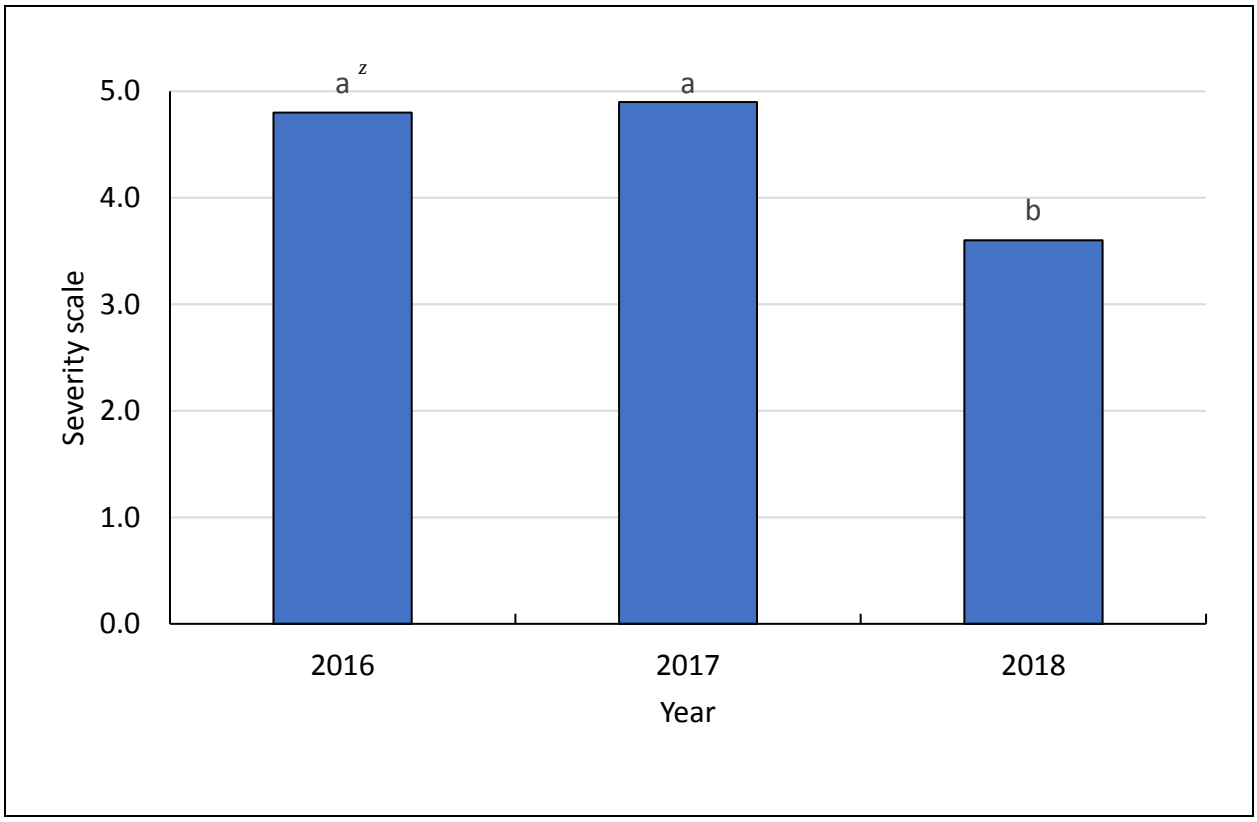

Fig. V-2. Bacterial leaf spot severity evaluated on seven peach families in the field over three years (2016, 2017, and 2018) using a 1-5 visual scale. Each value is an average of seven F_1 peach population.

[z] Means not connected by the same letter are significantly different ($P \leq 0.05$) using HSD test.

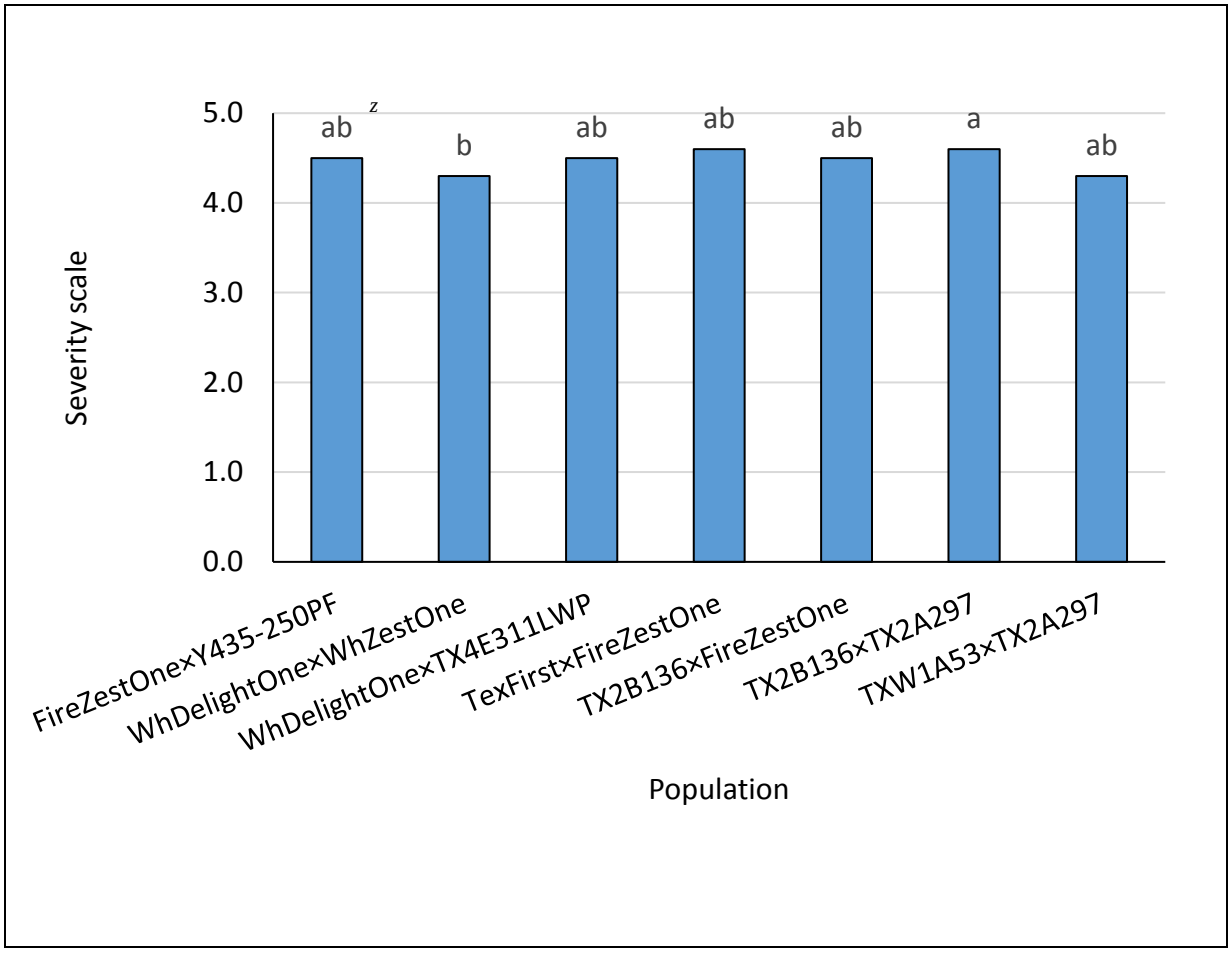

Fig. V-3. Bacterial leaf spot severity evaluated on seven F_1 peach populations in the field using a 1-5 visual scale. Each value is an average of three years of evaluation (2016, 2017, and 2018).

[z] Means not connected by the same letter are significantly different ($P \leq 0.05$) using HSD test.

Table V-6. Effect size for the population, year, and population × year effects on bacterial leaf spot severity evaluated in the field for seven F_1 peach populations across three years (2016, 2017, 2018) in College Station, TX.

Source	Effect size[z]
Population	0.04
Year	0.37
Population × Year	0.09

[z] Partial Eta-squared

Detached leaf bioassay

In June of 2018, a modified detached-leaf bioassay, as described by Randhawa and Civerolo (1985) was performed to assess Xap sensitivity across the F_1 peach populations. Results of visual assessment (0-3 ratings) and image analysis of the lesion area on leaf agreed with the field evaluation (0-5 ratings) and confirmed that the six F_1 peach populations in this study were sensitive to Xap infection (Table V-7). The visual ratings clustered between moderate (1.9 for TXW1A53×TX2A297) to highly susceptible (2.5 for FireZestOne×Y435-250PF) classes. Although the populations differed in their visual rating score (0 – 3), none of the populations scored as resistant (scores between 0.0 - 1.5) (Table V-7). No symptoms were detected on the eight inoculated sites of the five control leaves (inoculated with distilled sterile water). The lesion diameter on the leaf was consistent with visual rating scores and indicated that all the peach families were susceptible to bacterial leaf spot (Table V-7). The two lab-based assessments were highly correlated (r = 0.84) with each other and with the field assessments of disease incidence (r = 0.60-0.53) (Table V-8).

Discussion

The overall data sets from the field and lab evaluations revealed that all the peach populations are susceptible to *X. arboricola* pv. *pruni*, and that the disease severity varied across years. The variation in response to Xap infection between years can be attributed to the conducive environmental conditions in 2016 and 2017 versus 2018. Several previous studies have reported that the occurrence of Xap infection depends highly on environmental conditions with warm temperatures (20 – 30° C) and high moisture (high precipitation, frequent rain events and high humidity leading to prolonged leaf wetness) favoring bacterial leaf spot infection (Battilani et al., 1999; Frett, 2016; Medeiros et al., 2011; Yang, 2012).

All three years had favorable temperatures for bacterial leaf spot infections. The mean monthly temperature from April to June across the three years of evaluation, excluding April 2018, ranged from 20.3 to 29.2 °C (which was within the range of temperature of Xap multiplication (20 to 35º C), and was close to the optimal temperature for infection (28.9° C) during the evaluation period (June) (Morales et al., 2018).

Table V-7. Disease severity and total lesion diameter as a response to *Xanthomonas arboricola* pv. *pruni* on six F_1 peach populations using the detached leaf assay.

		Disease severity (0 -3)[z]					Lesion diameter (mm)				
Population	**N**	**Mean**	**Median**	**SD**	**Min**	**Max**	**Mean**	**Median**	**SD**	**Min**	**Max**
FireZestOne×Y435-250PF	21	2.5 a[y]	3	0.5	1	3	2.7 a	2.7	0.17	2.3	3.0
WhDelightOne×WhZestOne	40	2.3 ab	2	0.6	1	3	2.7 a	2.7	0.21	2.1	3.0
TexFirst×FireZestOne	10	2.2 ab	2	0.5	1	3	2.6 a	2.6	0.20	2.3	3.0
TX2B136×FireZestOne	32	2.0 b	2	0.5	1	30	2.6 a	2.6	0.15	2.1	3.0
TX2B136×TX2A297	50	2.1 ab	2	0.6	1	3	2.6 a	2.6	0.20	2.1	3.0
TXW1A53×TX2A297	12	1.9 b	2	0.3	1	3	2.6 a	2.6	0.19	2.2	2.8

[z] Visual rating scale 0 = no symptoms, 1 = distinct chlorotic spot (yellowing) and/or slight necrotic flecks, 2 = distinct but pale necrotic spot or grayish-white lesion, 2 mm in diameter, 3 = distinct, dark necrotic spot of > 2 mm in diameter, with or without a chlorotic halo

[y] Means not connected by the same letter within column are significantly different ($P \leq 0.05$) using HSD test.

Table V-8. Correlation coefficient among average bacterial leaf spot assessments in the field and in the lab on peach seedlings in 2018.

Correlations	Lab	Lesion diameter
Lesion diameter	0.84 **	
Field	0.60 **	0.53 **

** Significant at 0.01 level

The major difference between the high (2016 and 2017) versus the lower bacterial leaf spot year (2018) was the amount and distribution of precipitation. The more frequent rains especially with rain events occurring for 3 consecutive days, seen in May and June of 2016 and 2017 lead to prolonged leaf wetness a key factor for bacterial infection and consequently the higher disease severity seen in the first two years as compared to 2018 which experienced less precipitation in terms of both amount and frequency. This result is in agreement with Battilani et al. (1999) who reported that primary infection of BLS occurred when with a mean temperature between 14 and 19° C along with at least 3 successive rainy days and Morales et al. (2018) who reported temperatures close to 20° C along with wetness periods longer than 10 h or temperature between 25 and 35° C with wetness 5 h are necessary to cause high disease severity. Several other studies have reported on the importance of warm temperatures (20 – 30 °C) along with the duration of wetness period and high humidity for high infection rate of by *X. arboricola* pv. *pruni* (Battilani et al., 1999; EPPO, 1997; Linvill, 2002; Stefani, 2010).

Detached-leaf bioassays were shown to be an effective technique for screening for Xap sensitivity in breeding programs (Bock et al., 2010; Civerolo and Keil, 1976; Hammerschlag, 1988; Hammerschlag et al., 1994; Randhawa and Civerolo, 1985). Both data sets (lab visual rate

and area of the lesion) from the detached leaf assay differentiated peach populations' sensitivity to Xap and were consistent with field visual assessment. Scores of lab visual assessment (0 - 3) were clustered between moderate and high sensitivity classes (1.9 - 2.59) and the lesion diameter ranged between 2.6 – 2.7 mm. Likewise, the significant correlation among the three data sets indicated a good degree of association among these data sets.

Conclusion

All the peach families assessed for their sensitivity to bacterial leaf spot were susceptible by both field and lab-based assessments. The lab-based approach was an accurate approach for the assessment of bacterial leaf spot incidence even in a year in which the disease incidence in the field was low. The disease severity in the field varied with the environmental conditions with much less BLS incidence seen in 2018 when there was less rain during April to June in terms of quantity (~130 mm versus ~528-320 mm) and number of events (11 versus 26-23) as compared to 2016 and 2017.

Controlling Xap infection is a very challenging task particularly in Texas that is characterized by conducive climatic conditions for bacterial spot infection. Thus, the planting of resistant peach cultivars is highly recommended as an effective method for the control of the disease if accompanied by good orchard management practices and the application of chemicals in correct timing and rates. For future study, it is very important for the Texas peach program to focus on breeding peach cultivars resistant to Xap using molecular markers as tools improve the efficacy of traditional breeding methods.

References

Aarrouf, J., A. Garcin, Y. Lizzi, and M.E. Maataoui. 2008. Immunolocalization and histocytopathological effects of *Xanthomonas arboricola* pv. *pruni* on naturally infected leaf and fruit tissues of peach (*Prunus persica* L. Batsch). *J. Phytopathol.* 156:338-345.

Battilani, P., V. Rossi, and A. Saccardi. 1999. Development of *Xanthomonas arboricola* pv. *pruni* epidemics on peaches. *J. Plant Pathol.* 81:161-171.

Bock, C.H., G.H. Poole, P.E. Parker, and T.R. Gottwald. 2010. Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. *Critical Rev. Plant Sci.* 29:59-107.

Byrne, D.H., M.B. Raseira, D. Bassi, M.C. Piagnani, K. Gasic, G.L. Reighard, M.A. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: L.M. Badenes and H.D. Byrne (eds.), Fruit Breeding. Springer US, Boston, MA.

Civerolo, E.L. and H.L. Keil. 1976. Evaluation of *Prunus* spp resistance to *Xanthomonas pruni* by artificial inoculation. *Fruit Var. J.* 30:17-18.

Civerolo, E.L., M. Sasser, C. Helkie, and D. Burgage. 1982. Selective medium for *Xanthomonas-campestris* pv. *pruni*. *Plant Dis.* 66:39-43.

Cobb, N.A. 1892. Contributions to an economic knowledge of the Australian rusts (Uredineae). *Agricultural Gazette of New South Wales* 3:44-48.

Daines, R. 1961. What we know and don't know about bacterial spot of peach. *Hort. News* 42:49.

Daines, R.H. and L.F. Hough. 1951. Artificial inoculation of peach seedlings with *Xanthomonas pruni*. *Phytopathol.* 41:8-9.

du Plessis, H.J. 1986. Systemic migration and establishment of *Xanthomonas campestris* pv. *pruni* in plum leaves and twigs. *J. Phytopathol.* 116:221-227.

EPPO. 1997. *Xanthomonas arboricola* pv. *pruni*, p. 1096 - 1100. In: I.M. Smith, P.R. Scott, and M. Holderness (eds.), Quarantine Pests for Europe. CAB International, Wallingford, UK.

EPPO. 2006. *Xanthomonas arboricola* pv. *pruni*. *EPPO Bulletin (European and Mediterranean Plant Protection Organization)* 36:129-133.

EPPO. 2018. EPPO Standards, EPPO A1 and A2 lists of pests recommended for regulation as quarantine pests. EPPO, Paris.

Frett, T.J. 2016. Genetic determinism of *Xanthomonas arboricola* pv. *pruni* (Xap) resistance, fruit quality, and phenological traits in peach and incorporation of marker-assisted selection (MAS) in the University of Arkansas peach and nectarine breeding program. University of Arkansas, Fayetteville, AK, Ph.D Dissertation.

Hammerschlag, F.A. 1988. Selection of peach cells for insensitivity to culture filtrates of *Xanthomonas campestris* pv *pruni* and regeneration of resistant plants. *Theor. Appl. Genet.* 76:865-869.

Hammerschlag, F.A., D.J. Werner, and D.F. Ritchie. 1994. Stability of bacterial leaf spot resistance in peach regenerants under in vitro, greenhouse and field conditions. *Euphytica* 76:101-106.

Hansche, P.E. 1983. Response to selection, p. 154-171. In: J. J. and M.J. N. (eds.), Methods in Fruit Breeding. Purdue University Press, West Lafayette, USA.

Linvill, D.E. 2002. Use the proper environmental temperature to describe disease. *Acta Hort.* 592:689-694.

Medeiros, J.G.S., I. Citadin, I. dos Santos, and A.P. Assmann. 2011. Reaction of peach tree genotypes to bacterial leaf spot caused by *Xanthomonas arboricola* pv. *pruni*. *Sci. Agric.* 68:57-61.

Morales, G., C. Moragrega, E. Montesinos, and I. Llorente. 2018. Effects of leaf wetness duration and temperature on infection of *Prunus* by *Xanthomonas arboricola* pv. *pruni*. *PLOS ONE* 13:e0193813.

Okie, W.R. 1998. Handbook of peach and nectarine varieties: performance in the southeastern United States and index of names. USDA/ARS, Springfield, VA.

Randhawa, P.S. and E.L. Civerolo. 1985. A detached-leaf bioassay for *Xanthomonas campestris* pv *pruni*. *Phytopathol.* 75:1060-1063.

Ritchie, D.F. 1995. Bacterial spot, p. 50-52. In: J.M. Ogawa, E.I. Zehr, G.W. Bird, D.F. Ritchie, K. Uriu, and J.K. Uyemoto (eds.), Compendium of Stone Fruit Diseases. APS Press, St. Paul, MN.

Ritchie, D.F. 1999. Sprays for control of bacterial spot of peach cultivars having different levels of disease susceptibility, 1998. *Fungicide and Nematicide Tests* 54:63-64.

Sansavini, S., A. Gamberini, and D. Bassi. 2006. Peach breeding, genetics and new cultivar trends. *Acta Hort.* 713:23-48.

Sheskin, D.J. 2007. Handbook of parametric and nonparametric statistical procedures. 4th ed. Chapman & Hall/CRC.

Smith, E.F. 1903. Observations on a Hitherto Unreported Bacterial Disease the Cause of which Enters the Plant Through Ordinary Stomata. *Science* 17:456-457.

Stefani, E. 2010. Economic significance and control of bacterial spot/canker of stone fruits caused by *Xanthomonas arboricola* pv. *pruni*. *J. Plant Pathol.* 92:S99-S103.

TAMU Soil Characterization Laboratory. 2018. Texas Soil Database. http://soildata.tamu.edu/. Sep. 15, 2018

Topp, B.L., W.B. Sherman, R.E. Stall, G.V. Minsavage, and C.J. Wilcox. 1993. Comparison of Greenhouse Methods for Assessing Resistance to Bacterial Leaf Spot in Plum. *J. Amer. Soc. Hort. Sci.* 118:667-671.

Vauterin, L., B. Hoste, K. Kersters, and J. Swings. 1995. Reclassification of *Xanthomonas*. *Intl .J. Sys. Evol. Microbiol.* 45:472-489.

Weather Underground. 2018. Weather History for College Station, TX. https://www.wunderground.com/history/station/KCLL/2016/3/1/CustomHistory.html. Oct. 5, 2018

Werner, D.J., D.F. Ritchie, D.W. Cain, and E.I. Zehr. 1986. Susceptibility of peaches and nectarines, plant introductions, and other *Prunus* species to bacterial spot. *HortScience* 21:127-130.

Yang, N. 2012. Mapping quantitative trait loci associated with resistance to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in peach Clemson University, Clemson, SC, Ph.D. dissertation.

Yang, N., G. Reighard, K. Gasic, D. Ritchie, and W. Okie. 2013. Development of a genetic linkage map for identification of molecular markers associated with resistance to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in peach. *Acta Hort.* 976:561-566.

Yang, N., G. Righard, D. Ritchie, W. Okie, and K. Gasic. 2010. Construction of a genetic linkage map for identification of molecular markers associated with resistance to *Xanthomonas arboricola* pv. *pruni* in peach [*Prunus persica* (L.) Batsch]. *HortScience* 45:S304.

Young, J.M., D.W. Dye, J.F. Bradbury, C.G. Panagopoulos, and C.F. Robbs. 1978. A proposed nomenclature and classification for plant pathogenic bacteria. *N.Z. J. Agr. Res.* 21:153-177.

Zehr, E.I., D.P. Shepard, and W.C. Bridges. 1996. Bacterial spot of peach as influenced by water congestion, leaf wetness duration, and temperature. *Plant Dis.* 80:339-341.

CHAPTER VI

CONCLUSIONS

This research had three major parts. First was to identify QTLs and the predominant SNP haplotypes linked to the predictive SNP marker(s) for 3 phenological and 3 fruit quality traits. Second was to assess a collection of peaches for their postharvest durability and the third section was to screen peach seedlings for their susceptibility to bacterial leaf spot.

The first two research chapters (II and III) used Visual FlexQTL software, a pedigree-based analysis (PBA) approach and PediHaplotyper package of R software for haplotype analysis to identify QTLs and the predominant SNP haplotypes linked to the predictive SNP marker(s) for 3 phenological traits (bloom date (BD), ripe date (RD), and fruit development period (FDP)) and 3 fruit quality traits (blush (BL), soluble solids content (SSC), and titratable acidity (TA)). 162 individuals grown in four environments (CA 2011, CA 2012, TX 2012, and TX 2013) were phenotyped and genotyped (9K SNP Illumina array) for this research.

The QTL for RD and FDP was co-localized at the central part of LG4 (40 - 44 cM) and explained ~35 % of the phenotypic variance. Three QTLs were discovered for DB on LG1 (88 – 92 cM), LG4 (48 – 50 cM), and LG7 (40 – 44 cM), explaining between 17 - 94%, 11 - 55%, and 11 - 18% of the phenotypic variance respectively. Haplotype analyses for these QTLs revealed unique SNP haplotypes that are associated with the predictive SNP marker(s) of desired QTL alleles along with their original sources.

Our analysis detected one major QTL on the central part of LG4 for blush at interval 42 – 44 cM that explained ~20 % of the total phenotypic variance. A major QTL for TA co-localized with the major locus for low-acid fruit (*D*-locus) at the proximal end of LG5 at 0 - 0 cM. This

QTL was consistent across all data sets explaining ~60 % of the phenotypic variance. There was a QTL at the distal end of LG5 at 52 - 62 cM that was associated with both TA and SSC that explained ~15 % of the phenotypic variance. In addition, haplotype analyses for these QTLs revealed unique SNP haplotypes that are associated with the predictive SNP marker(s) of desired QTL alleles along with their original sources.

The haplotype results of these six traits would help breeders in choosing the best combinations of parents for generating breeding populations or in seedling selection to discard undesired seedlings at an early seedling stage. The next step is to convert these SNP haplotypes into easy-to-use, high throughput markers such as simple sequence repeat (SSR), Kompetitive allele-specific PCR (KASP), or Sequence Characterized Amplified Region (SCAR) markers that can be used routinely in MAB.

In Chapter IV, the standardized phenotyping protocol for the susceptibility to internal breakdown was implemented for three years (2016 - 2018) on 35 peach and nectarine genotypes grown in TX. Fruits from each genotype were harvested at a consistent ripening stage and evaluated for their quality after storage for two weeks at 5° C and 90% RH. Results showed that the 35 genotypes differed in their storage durability, as measured by expressible juice, mealiness, and texture traits. Mealiness incidence developed in fruits after storing them for two weeks at 5° C and negatively impacted the internal quality traits of fruits. TX3C394N, ‘Royal Zest Two’, ‘Texstar’, ‘Royal Zest Three’, TX3B376LWP and ‘Harvester’ genotypes developed mealy flesh after storage, whereas ‘Flavorrich’, ‘White Delight One’, and TX2D357LW genotypes performed well in storage.

On average, early-season genotypes, peach, yellow-fleshed, and non-melting genotypes were less susceptible to chilling injury as compared to late-season, nectarine, white-fleshed and

melting genotypes, respectively. A significant correlation found for expressible juice with mealiness (r=-0.74) and texture (r=-0.73), and between mealiness and texture (r=0.63). Expressible juice %, mealiness, and texture are the most informative traits for the evaluation of chilling injury symptoms and are complementary to each other by measuring the symptoms quantitatively (expressible juice), visually (mealiness), and organoleptically (texture). Since these physiological disorders negatively affect internal fruit quality and limit their marketing and consumption, this study was an important step for developing new peach genotypes free from chilling injury symptoms for enhancing the peach industry.

In Chapter V, seven peach families grown in College Station, TX were evaluated for their sensitivity to bacterial leaf spot across three years (2016 to 2018) using field evaluation and lab assessment methods. Our results revealed that all population were susceptible to bacterial leaf spot. The detached-leaf bioassay was an accurate approach for the assessment of bacterial leaf spot incidence even in a year in which the disease incidence in the field was low. The disease severity in the field varied with the environmental conditions with much less BLS incidence seen in 2018 when there was less rain during April to June in terms of quantity (~130 mm versus ~525-320 mm) and number of events (11 versus 23-26) as compared to 2016 and 2017.