5-2016

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

Terrence James Frett
University of Arkansas, Fayetteville

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

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Plant Science

by

Terrence J. Frett
Lawrence University
Bachelor of Arts in Biology, 2010
Clemson University
Master of Science in Plant and Environmental Sciences, 2012

May 2016
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

_____
Dr. John R. Clark
Dissertation Director

_____
Dr. Elena M. Garcia
Committee Member

_____
Dr. Richard E. Mason
Committee Member

_____
Dr. Burton H. Bluhm
Committee Member

_____
Dr. Ainong Shi
Committee Member

Abstract

For over two decades the incorporation of marker-assisted selection (MAS) has been discussed as an innovative approach to increase peach breeding efficiency. Although hundreds of quantitative trait loci (QTLs) have been identified, only a few have been converted into usable MAS tools. This highlights a disconnect between genomic discoveries and practical application in breeding programs, which has delayed use of MAS.

In this dissertation, three sequential steps were performed with the objective to bridge this gap and develop breeding-relevant DNA tests for MAS of fruit bacterial spot resistance (*Xap*1 and *Xap*6), fruit quality [blush (R_f), acidity (*D*), and acidity and soluble solid content (*G7Flav*)], and phenological traits [maturity date (*G4mat*), fruit type (*G*), and flesh color (*Y*)] across four RosBREED peach breeding programs. First, previously identified QTLs were further investigated and 32 SNPs were grouped into haplotypes and validated for association with breeding-relevant trait differences across RosBREED germplasm. The SNPs were divided into two mini-arrays and advanced breeding material from all programs were outsourced for design and testing. The validated SNP loci were used in marker-assisted parent selection (MAPS) in 2013-2015 at the University of Arkansas (UA) program to combine horizontal *Xap* resistance with high fruit quality spanning the season. Secondly, four of the SNP-based tests (*Xap*1, *Xap*6, *G*, and *Y* loci) were converted into sequence length polymorphism-based (SLP-based) tests (Ppe-*Xap*F1-SSR, Ppe-*Xap*F6-SSR, indelG, and PpCCD4b-SSR) and screened on the UA RosBREED germplasm and 22 additional F_1 populations. Results were compared across both DNA tests to identify the most efficient genotyping approach for each trait. In 2015, two of the SLP-based DNA tests, the indelG (pubescent vs. glabrous) and PpCCD4b-SSR (white vs. yellow flesh) were advanced to test in marker-assisted seedling selection (MASS).

In the final step, QTL analysis was conducted for *Xap* fruit, *Xap* leaf, and *Xap* leaf-assay resistance along with seven fruit quality and phenological traits using the Pedigree-Based Analysis (PBA) approach and the UA RosBREED pedigree. Overall 20 QTLs were identified, 10 for *Xap* resistance and 10 for quality and phenological traits. These 20 QTLs are optimal targets for future DNA test development, validation, and use in MAS.

Acknowledgments

First and foremost I would like to thank my advisor and mentor Dr. John R. Clark for his support, patience, guidance, and inspiration to grow substantially as a scientist, professional and young man throughout my time in his program. I will carry all his scientific, professional, and life lessons with me throughout my future career and life. Furthermore, I am certain I will experience multiple 'aha moments' in the field during my future career, when I realize the additional lessons he bestowed upon me for which at the time I did not fully grasp until I experienced them first hand.

Secondly, I want to express my gratitude to Drs. Shi, Bluhm, Garcia and Mason who served on my advisory committee. Their guidance and insightful feedback positively shaped my education here at The University of Arkansas and provided the foundation for my future life path. I am extremely grateful for all they and Dr. Clark have done for me, without their help this dissertation would not have been possible. They all went above and beyond what is asked of an advisory committee, by giving up their personal time to not only help me on my dissertation, but also share with me their professional advice as I transition into my future career as a fruit breeder at Sun World International. Through my work with them they have all become lifelong colleagues and more importantly friends.

I also want to thank both Dr. Clark and his predecessor Dr. James M. Moore, for their development of the peach material and populations I was fortunate to use in my studies. Furthermore, thanks to the entire Clarksville Fruit Research Station Farm staff: Dan Chapman, Katie Hanshaw, David Gilmore, Kay Buck, Dwain Ober II, Taunya Ernst, Jeff Inness, Alan Davis, Sara Griffith, and Jessie Clark, for all their assistance in peach tree maintenance and the setup of the bacterial spot leaf assay incubation room. Also thanks to Olivia Hines, Loren Anthony, Leslie Smith, and Jack McCoy for their help in completion of the bacterial spot leaf assay over the past two years, and to Dr. Clark for making this opportunity possible by forming this team and putting me in contact with his peach colleagues from 11 different states in the eastern U.S.

Special thanks goes out to all the encouragement and support through thick and thin from Andrew Jecmen (program associate), Alejandra Salgado (lab mate), Reet Gill (horticulture colleague), and Sandra Sleezer (former program technician). This dissertation would not have been possible without the support of these lifelong colleagues and friends.

I also want to express my gratitude to Brant Smith and Dr. Bluhm from the Department of Plant Pathology at The University of Arkansas. They generously supported and helped me to perform all methods on the pathogen side of this dissertation from isolate collection, culturing, and leaf inoculation to designing and screening of SSR markers to differentiate isolates from different states across the eastern U.S. During my time with them I focused on the pathogen side of the plant-pathogen interaction, which opened my eyes to a new way to look at disease resistance breeding. The techniques and knowledge I gained from them will be vital in my future career to differentiate myself and be successful in combining durable disease resistance with high fruit quality. They have also become lifelong colleagues and more importantly friends.

Special thanks to all the important colleagues that I have developed through my participation in RosBREED (http://www.rosbreed.org/). Thanks to Paul Sandefur and Cameron Peace, along with Daniel Edge-Garza and Terrence Rowland Jr., whom I was fortunate to visit and work with in their Washington State University (WSU) Tree Fruit Genetics Lab for two and a half weeks. My time with them enabled me to fine-tune the University of Arkansas Lab and begin our collaborative work on the development and validation of breeder-friendly peach DNA tests. Paul, Alejandra, and I worked together across our dissertations in the development and confirmation of several peach DNA tests, and Cameron served as our mentor. The collective dedication of all team members was instrumental to the success of this work.

My sincere thanks goes out to all those who helped me perform FlexQTL. This includes Eric van de Weg, and Marco Bink, the developers who first taught me about their software, Sujeet Verma and Jonathan Fresnedo, RosBREED colleagues who helped me through pedigree and SNP checking, along with data analysis, further QTL specific runs, and haplotyping. Moreover, thanks to the High Performance Computing (HPC) staff at Arkansas, especially Jeff Pummill and Pawel Wolinski for guidance on how to use the HPC system along with their help in enabling us to run FlexQTL over the system. The successful FlexQTL results in this dissertation would not have been achieved without the collective collaboration of all parties.

I would like to commend all the faculty, staff, and fellow graduate students in the Department of Horticulture for their assistance when I turned to them for help as well as their comradery. Lastly, thanks to all past professors/mentors, Dr. Clark, Terry Bacon, Dr. Mason, Dr. Garcia, Dr. Shi, Dr. Bluhm, Dr. Peace, Daniel Edge-Garza, Terry Rowland, Sujeet Verma, Jonathan Fresnedo, Dr. Evans, Dr. Reisch, Dr. Infante, Dr. Gasic, Dr. Reighard, Dr. Layne, Dr.

Byrne, Dr. Nicholas Maravolo, Dr. Walter Marin, and Mr. Cassini for sparking and expanding my interest in horticulture, genetics and fruit breeding. I can’t thank you all enough for helping me find and expand on my passion and path in life.

Dedication

I dedicate this dissertation to my family: my father Terry Frett, mother Lauree Frett, sisters Christina and Hannah Frett, brother in law Adam Swanson, and all extending family members. Their support and positive encouragement, especially during the stressful times, enabled me to complete this dissertation. My family will forever be the most important part of my life. To show my gratitude, I look forward to supplying them and my future family with endless amounts of fruit I work with in my future career.

Table of Contents

Abstract.....

Acknowledgments.....

Dedication.....

Table of Contents.....

Introduction..... 1

Peach Resistance *to Xanthomonas arboricola* pv. *pruni* (*Xap*) Objectives:..... 8

Xanthomonas arboricola pv. *pruni* (*Xap*) Objectives:..... 8

Peach Fruit Quality, and Phenological Trait Objectives:..... 8

Chapter One: Literature Review..... 9

Peach Introduction..... 9

Taxonomy..... 9

Botany..... 10

Origin..... 12

History of Peach Dispersal Across the World..... 14

Economic Importance..... 17

Peach Breeding History..... 18

Status of the Current Peach Industry in U.S.. 20

Breeding Objectives..... 20

Expanding the Diversity of Peaches to Develop High Quality, Disease-Resistant Cultivars 22

***Xanthomonas arboricola* pv. *pruni* (*Xap*)**..... 25

Taxonomy..... 25

Hosts and Damage..... 26

History and Spread..... 27

Optimal Conditions..... 28

Disease Life Cycle..... 29

Disease Symptoms..... 30

Disease Management..... 33

Development of Molecular Markers to Complement the Traditional Breeding Process. 34

The Traditional Steps Used to Enable MAS in Peach..... 39

Current Status of MAS in Peach 53
Current Status of MAS in Other Rosaceae Fruit Crops 54
Development, Validation and Deployment of DNA Tests for MAS of *Xap* Resistance, Fruit Quality, and Phenological Traits in Peach 56
Pedigree-Based QTL Analysis (PBA) 58
MASS Logistics for Rosaceae Fruit Breeding Programs 60
Importance of MAS for *Xap* Resistance, Fruit Quality, and Phenological Traits 61
Literature Cited 63
Chapter Two: Phenotypic Characterization of Bacterial Spot [*Xanthomonas arboricola* pv. *pruni* (*Xap*)] Resistance, and Pedigree-Based Quantitative Trait Loci Analysis (PBA), for *Xap* Resistance in the University of Arkansas Peach and Nectarine Breeding Program 81
Abstract 81
Introduction 82
Materials and Methods 94
Management Practices at FRS 94
Pedigree Construction 94
Field Phenotypic Evaluation 2013-2015 97
Detached-Leaf Bioassay 99
Descriptive Statistics 100
SNP Array Genotyping 100
PBA QTL Analysis 101
Genomic Estimated Breeding Values 102
Results 103
Weather Data 103
Xap Fruit Field Data Sets (2013-2015) 108
Xap Leaf Field Data Sets (2013-2015) 136
Xap Leaf Assay Data Sets 152
Genotypic Data 155
Pedigree-Based QTL Analysis (PBA) 155
Major and Minor QTLs 195
Genomic Estimated Breeding Values 199
Discussion 201

Future Work - SNP Data Set and Haplotype Construction 207
Literature Cited 208
Chapter Three: Pedigree-Based Quantitative Trait Loci Analysis (PBA) for Fruit Quality and Phenological Traits in the University of Arkansas Peach and Nectarine Breeding Program 218
Abstract 218
Introduction 219
Materials and Methods 225
Management Practices at FRS 225
Pedigree Construction 226
Phenotyping 228
Phenotypic Data Set Construction and Descriptive Statistics 229
SNP Array Genotyping 229
PBA QTL Analysis 230
Genomic Estimated Breeding Values 231
Results 231
Weather Data 231
Phenotypic Data 234
Genotypic Data 267
Pedigree-Based QTL Analysis (PBA) 267
Genomic Estimated Breeding Values 298
Discussion 299
Maturity Date (MD) 299
G and Y Loci (Pubescent vs. Glabrous and White vs. Yellow Flesh) 302
Blush Overcolor 304
Fruit Size (Diameter and Weight) 307
Soluble Solids Content (SSC) 310
Future Work - SNP Data Set and Haplotype Construction 313
Literature Cited 314
Chapter Four: SNP Haplotype DNA Test Development and Peach Mini-SNP Array v1.0 Analysis Across Four Pedigree-Connected U.S. RosBREED Peach Breeding Programs .. 322
Abstract 322

Introduction 323
Materials and Methods 326
Germplasm 326
Development and Validation of SNP-Haplotype DNA Tests 326
Outsourcing of SNP-haplotype DNA tests 329
Results 333
Development and Validation of SNP-Haplotype DNA Tests 333
Peach Mini-SNP Array v1.0 Analysis 378
Discussion 380
Fruit Bacterial Spot 380
Blush Overcolor 381
Maturity Date (MD) 382
Pubescent (Peach) vs. Glabrous (Nectarine) 383
White vs. Yellow Flesh Color 384
Titratable Acidity (TA) 384
Soluble Solids Content (SSC) and Titratable Acidity (TA) 385
Mini-SNP Array 386
Overall Conclusions and Future Work 386
Literature Cited 388
Chapter Five - Study One: G1*Xap*F and G6*Xap*F SNP Haplotype and Ppe-XapF1 and Ppe-XapF6 SSR DNA Tests for Routine Prediction in Breeding of Peach Fruit Bacterial Spot (*Xap*) Resistance 394
Abstract 394
Introduction 395
Materials and Methods 400
Management Practices at FRS 400
Germplasm Utilized 401
Xap Phenotyping 405
Leaf Collection and DNA Extraction 407
SNP Haplotype Validation and Conversion to SSR Marker 408
Statistical Analysis 417

Results 418
Phenotypic Data 418
G1XapF and *G6XapF* DNA Test Results 420
Non-Parametric Kruskal-Wallis test "Conversion Set." 423
Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR DNA Test Genotyping 425
SNP Haplotypes to Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR DNA Test Conversion 429
Ppe-*Xap*F6-SSR DNA Test (Additional Cultivars and Selections) 446
Ppe-*Xap*F6-SSR DNA Test "Confirmation Set" 451
Non-Parametric Kruskal-Wallis test "Confirmation Set" 454
Discussion 454
Literature Cited 460
Chapter Five - Study Two: IndelG, a DNA Test for Routine Prediction and Breeding of Peach and Nectarine 466
Abstract 466
Introduction 467
Materials and Methods 471
Management Practices at FRS 471
Germplasm Utilized 472
Pubescence Phenotyping 472
Leaf Collection and DNA Extraction 476
IndelG Genotyping 477
SNP Haplotypes to IndelG DNA Test Conversion 478
IndelG DNA Test Genotype Effects 480
Results 480
Phenotypic Data 480
IndelG DNA Test Genotyping 497
IndelG DNA Test "Conversion Set" 501
SNP Haplotype DNA Test Development 501
SNP Haplotypes to IndelG DNA Test Conversion 511
IndelG DNA Test (Additional Cultivars and Selections) 511
IndelG DNA Test "Confirmation Set" 513

Discussion 513
Literature Cited 516
Chapter Five - Study Three: PpCCD4b-SSR, a DNA Test for Routine Prediction and Breeding of Peach White and Yellow Flesh Colors 521
Abstract 521
Introduction 522
Materials and Methods 527
Management Practices at FRS 527
Germplasm Utilized 527
Flesh Color Phenotyping 528
Leaf Collection and DNA Extraction 532
PpCCD4b-SSR Design and Genotyping 533
SNP Haplotypes to PpCCD4b-SSR DNA Test Conversion 534
PpCCD4b-SSR DNA Test Genotype Effects 537
Results 537
Phenotypic Data 537
PpCCD4b-SSR DNA Test Genotyping 553
PpCCD4b-SSR DNA Test “Conversion Set” 556
SNP Haplotype DNA Test Development 556
SNP Haplotypes to PpCCD4b-SSR DNA Test Conversion 566
PpCCD4b-SSR DNA Test for Additional Cultivars and Selections 566
PpCCD4b-SSR DNA Test “Confirmation Set” 568
Discussion 568
Literature Cited 573
Chapter Six: Incorporation of MAPS and MASS in the University of Arkansas Peach and Nectarine Breeding Program 577
Abstract 577
Introduction 578
Materials and Methods 584
Management Practices at FRS 584
Marker Assisted Parent Selection (MAPS) and Marker Assisted Cross Selection (MACS) 584

Marker Assisted Seedling Selection (MASS) 587
Results 592
Marker Assisted Parent Selection (MAPS) and Marker Assisted Cross Selection (MACS) 592
Marker Assisted Seedling Selection (MASS) 616
Discussion 627
Marker Assisted Parent Selection (MAPS) and Marker Assisted Cross Selection (MACS) 627
Marker Assisted Seedling Selection (MASS) 635
Overall Conclusions 638
Literature Cited 639
Chapter Seven: Diversity and Virulence of XAP Isolates from 12 U.S. Locations 644
Abstract 644
Introduction 646
Materials and Methods 650
Management Practices at FRS 650
Xap Isolate Collection 651
Isolation and Culturing of *Xap* Isolates 654
Xap Identification 654
Diversity of *Xap* Isolates 655
Xap Inoculum 658
Detached-Leaf Bioassay 658
Results 661
Diversity of *Xap* Isolates 662
Virulence of *Xap* Isolates 667
Discussion 672
Xap Isolate Collection and Identification 672
Diversity of *Xap* Isolates 672
Virulence Assay 673
Literature Cited 675
Appendix A. Genomic estimated breeding values (GEBV) per trait and year for the AR RosBREED pedigree 678

Appendix B. High resolution melt (HRM) SNP-based genotyping for the G1*Xap*F 4-SNP haplotype DNA test 714
Overall Conclusions 718

Introduction

All fruit breeders share the same goal, to develop the "highest quality fruit possible," which to the general public might sound fairly simple. This is far from the case as fruit breeders must select for and combine what seems to be an endless array of traits to satisfy growers', distributers', marketers', and consumers' evolving demands. Each one of these entities desires different attributes: in general growers require high fruit productivity, extension of season and adaptation zone, large fruit size, adequate and consistent fruit quality and increased resistance to abiotic and biotic stress; distributors and marketers desire high firmness, low bruising potential, prolonged post-harvest, enhanced health benefits and new unique fruit types and flavors; consumers desire pristine appearance (color, shape, blemish free), the optimal eating experience (flavor, aroma, texture), adequate shelf life, and a growing trend towards organic, and or "non-GMO" fruit. The breeder's objective thus is to use his or hers' scientific and artistic skills to select parents with adequate genetic variation and design crosses to break linkages and combine all these characters into an array of total packages (cultivars) spanning the season, which can take ≥10-50 years of development depending on approach and resources of the breeding program.

Throughout the last century, multiple traditional peach breeding programs have developed and released hundreds of new cultivars throughout the world that have met some of these demands, yet today the majority of the peaches in the U.S. fresh market still lack optimal fruit quality and disease resistance. While peach [*Prunus persica* (L.) Batsch] is the third-most economically important temperate tree fruit species, with a total world production estimated at over 21.6 Mt (Byrne et al., 2012), unfortunately the U.S. peach industry has seen a slight decline in production, ~750,000 tonnes, from 1961 to 2013 (FAO, 2015b). This decline in production can be attributed to the need to harvest peaches at immature stages for storage and shipment

purposes, which negatively impacts fruit quality and increases the chances for postharvest problems such as mealiness, overall resulting in low fruit quality to consumers. Moreover, this fruit species and all other *Prunus* spp. are seriously threatened by bacterial spot [caused by *Xanthomonas arboricola* pv. *pruni* (*Xap* refers to the pathogen and disease)] thus, incorporation of resistant peach cultivars is pertinent to satisfy growers (EPPO/CABI, 1997; OEPP/EPPO, 2006; Ritchie, 1995).

High fruit quality and disease resistance can be combined through the development of new cultivars, however, more diversity must be sought, since all commercial peach cultivars trace back through their pedigree to similar founders with a very narrow genetic base and furthermore linkages must be broken. This is of high concern because a vast amount of diversity is necessary in order to breed for specific traits such as fruit quality and disease resistance. As said by Dr. John R. Clark, "Genetic variation (diversity) is the most important key to the chances for a breeder's success. All other things aside, without genetic variation, no progress in breeding is possible." The good news is that peach breeders still have an extensive amount of diversity to work with to reach these goals. There is much diversity in disease resistance and fruit quality found in the peach germplasm repositories as well as specific breeding programs.

However, access to other breeding programs germplasm has dramatically changed with the advent of plant intellectual property rights (IP rights) (Clark et al., 2012). These IP rights have re-shaped fruit breeding into an exceedingly private and competitive endeavor (in both public and private sectors). This shift has decreased germplasm exchange, and quite frequently exclusive contracts and breeding agreements are put into place to allow the exchange of genetic variation only between two programs. These agreements have commonly been associated with a transfer fee, along with plant royalties and have been and will continue to be critical for the

success of those 21st century fruit breeders who can successfully set up these agreements before their competitors (Byrne et al., 2012; Clark et al., 2012).

Nonetheless, enhancing genetic variation in a program will only give the breeder the ability to make progress. While genetic improvements can continue to be made with traditional seedling selection (TSS), unfortunately this approach is an expensive, laborious process, and has limitations in efficiency (Dirlewanger et al., 2004; Ru et al., 2015). It can take up to 10 years or more from the initial cross until a new peach cultivar can be released (Bliss, 2010; Dirlewanger et al., 2004b; Ru et al., 2015). The breeder must wait at least three years for peach trees to mature to fruit bearing capacity at most temperate locations before fruit quality can be assessed on the progeny (Bliss, 2010; Dirlewanger et al., 1998; Dirlewanger et al., 2004b; Dirlewanger et al., 2007). Once the trees bear fruit, it can then take an additional 10-15 years of phenotypic analysis, selection, and regional testing to release a new cultivar. Furthermore, after taking into account all maintenance costs from cross to initial selection or tree removal it was calculated to cost approximately $12 per peach seedling at Clemson University in 2015 (Ksenjia Gasic, and Cameron Peace, personal communication). This is comparable to the estimated $12 per apple seedling at the Washington State University (WSU) apple breeding program and $15 per cherry seedling at The Pacific Northwest (PNW) sweet cherry breeding program from 2001-2015 (Edge-Garza et al., 2010; Evans et al., 2012; Rowland et al., 2012). Considering $12 per peach seedling, if 2,000 new seedlings are planted, the overall cost to maintain these seedlings through initial selection phase (four years) totals $24,000. Crosses are typically made annually, thus TSS costs expand further as the breeding program matures.

Yet today innovative molecular tools (techniques) are nearing application, which can compliment the traditional breeding process by providing peach breeders with more informed

decision support to save resources and determine how to efficiently break linkages and combine traits such as disease and pest resistance with unique flavors and superior textures (Bliss, 2010; Byrne, 2005; Dirlewanger et al., 2004; Ru et al., 2015). One such tool, marker assisted selection (MAS), can enable breeders to decrease the costs of TSS and furthermore enhance their ability and efficiency to combine all desired traits into the next round of high quality peach cultivars (Bliss, 2010; Byrne, 2005; Collard et al., 2005; Edge-Garza et al., 2016; Ru et al., 2015). The MAS breeding strategy is based on a marker-locus-trait association (M-L-T), in which a predictive genetic marker is linked to a specific locus that contributes to the genetic variation for a specific phenotypic trait (Bliss, 2010; Ru et al., 2015). Therefore, the marker genotypes (through their association with the locus genotype) are used to select for the phenotype (Bliss, 2010; Collard et al., 2005; Collard et al., 2008; Ru et al., 2015).

When an M-L-T is screened on parental germplasm, MAS is called marker assisted parent selection (MAPS). In MAPS, allelic information of the parental pool can help direct the breeder to select parents with valuable alleles, and subsequently the genotypic information can help the breeder select favorable crosses with efficient combining abilities, through marker assisted cross selection (MACS). After the cross is made, the same M-L-T can be used to screen the seedlings to decide on which promising seedlings to grow in the field and which to discard based on their allelic makeup (Ru et al., 2015). This form of MAS is termed marker assisted seedling selection (MASS), and is useful in monitoring the incorporation of the desirable functional alleles at the locus from parent to progeny (Bliss 2010; Peace and Norelli 2009). The WSU Molecular Breeding Lab showed that TSS costs for the WSU apple and the PNW sweet cherry breeding programs can be substantially reduced by 50-60%, and 70-80% by using only one and two DNA tests for MASS, respectively (Edge-Garza et al., 2010; Edge-Garza et al.,

2016; Rowland et al., 2010; Rowland et al., 2012). Even more noteworthy, in general as more seedlings are screened, more DNA tests are used (in sequence rather than together), and culling rates are increased, the conventional breeding costs can be even further reduced (Cameron Peace, personal communication; Edge-Garza et al., 2016). These successful examples of MAS in apple (*Malus domestica* Borkh.) and sweet cherry (*Prunus avium* L.) substantiate the feasibility and value of conducting MAS in Rosaceae tree fruit breeding and provide insights into how to extend MAS adoption into more Rosaceae tree fruits (Ru et al., 2015).

An enormous array of manuscripts have been published in Rosaceae fruit crops on molecular marker development, genetic linkage map creation, quantitative trait loci (QTL) analysis, and candidate gene analysis, and the majority of these publications end with the same overall conclusion that the authors have enabled MAS for the traits they studied in their species of interest. With these equivalent conclusions documented by multiple researchers, why have only a few traits actually been documented for use in MAS of Rosaceae fruit crops? First and foremost, the lack of incentive to convert these M-L-Ts into DNA tests has created a valley of misconception, which has directly limited broad application of MAS in and across Rosaceae fruit breeding programs (Bliss, 2010; Iezzoni et al., 2010; Ru et al., 2015). In theory, traditional breeders and geneticists do not speak the same language, thus, to bridge this gap between them it is imperative that breeding relevant M-L-Ts are turned into DNA tests that will provide the traditional breeders with the information on which technique to use to screen the marker, as well as how to interpret and score the results.

Thus, the identified theoretical M-L-Ts should be investigated further, in order to make MAS practical in and across Rosaceae breeding programs, and ultimately to entice adoption. First, since almost all of these M-L-Ts were discovered using single bi-parental segregating

populations, the M-L-Ts should be tested across a wider array of germplasm, including the breeder's own material to validate the alleles from each marker are robust across germplasm as well as relevant in actual breeding material. Secondly, the reliability of the M-L-Ts to correctly predict the phenotypic variation must be determined; otherwise they will be misleading (Bliss, 2010; Collard et al., 2005; Collard et al., 2008; Ru et al., 2015).

In this dissertation, three sequential steps will be described with the overall objective to develop peach DNA tests for MAS of *Xap* resistance, fruit quality, and phenological traits across four peach breeding programs (The University of Arkansas (UA), Clemson University, Texas A&M University, and the University of California, Davis). First, previously identified breeding relevant M-L-Ts will be further investigated and developed into informative SNP-based DNA tests. Relevant single nucleotide polymorphisms (SNPs) associated with the traits of interest will be grouped into haplotypes and their robustness will be validated across the four RosBREED peach breeding program materials. Secondly, the SNP haplotypes will be converted into simple, straightforward breeder-friendly, SSR-based DNA tests and their robustness will be confirmed in and across the four breeding programs. Since SNP haplotype and SSR DNA tests each have their positives and negatives, and are both widely used, enabling the same DNA test to be screened across both platforms will give breeders more options, and thus further entice adoption of MAS.

Lastly, additional M-L-Ts for *Xap* resistance, fruit quality, and phenological traits will be identified through pedigree-based QTL analysis (PBA) using the UA pedigree. The PBA analysis uses multiple breeding populations connected in a pedigree to identify and/or validate QTL directly in the breeding germplasm (Bink et al., 2014; Peace et al., 2014). This method is a more powerful QTL statistical approach than bi-parental QTL analysis, and is used to simultaneously identify marker-trait associations, discover alleles for functional diversity, and

validate their robustness and applicability in individual breeding programs (Bink et al., 2014; van de Weg et al., 2004). The use of PBA analyses will increase the efficiency of the development of the next set of breeder-friendly DNA tests for the University of Arkansas peach breeding program.

Unfortunately, even after a DNA test has been validated for use in MASS, this tool can't be put into use until the logistics of organizing seedlings in the greenhouse, collecting leaf tissue, and identifying an economical platform for DNA extraction, PCR, allele sizing, and processing of data for subsequent culling of seedlings in the greenhouse have been developed. Interestingly, a questionnaire in 2013 to assess the level of MASS implementation in apple, sweet cherry, tart cherry (*P. cerasus* L.), strawberry (*Fragaria* × *ananassa* Duch.) and peach RosBREED demonstration tree fruit breeding programs revealed that the most prevalent challenge perceived by Rosaceae fruit breeders to perform MAS was in fact the difficulty in logistically enabling smooth integration of DNA testing into traditional breeding operations (Ru et al., 2015). The main reason for this perceived challenge could be due to the fact that successful DNA testing requires expertise in molecular data interpretation and management. Unfortunately this expertise is often lacking in breeding programs new to MASS (Ru et al., 2015).

Thus, a secondary objective of this dissertation was to build, implement and manage an entirely in-house MAS lab for the UA program in order to apply the developed DNA tests and transfer theoretical and practical knowledge to program associate, Andrew Jecmen, so that these tools can evolve over time in the peach program as well as extend to the blackberry (*Rubus* subgenus *Rubus* Watson), and muscadine grape (*Vitis rotundifolia* Michx.) breeding programs, upon development of DNA tests for these species. Enhancing diversity in one's fruit breeding program coupled with broad application of MAS for breeding relevant traits will enable the

"highest quality fruit possible," to be developed to meet the evolving growers', distributers', marketers', and consumers' demands, in a more timely and economical manner (Bliss, 2010).

Peach Resistance *to Xanthomonas arboricola* pv. *pruni* (*Xap*) Objectives:

1. To differentiate *Xap* resistance across 29 F_1 peach populations, selections, and cultivars from the University of Arkansas (UA) peach breeding program by taking high quality-controlled phenotypic data for three consecutive years (2013-2015).
2. To directly apply marker assisted parent selection (MAPS) and marker assisted cross selection (MACS) for *Xap* fruit resistance, in the UA program, by validating single nucleotide polymorphism (SNP) haplotypes at two QTL loci (G1*Xap*F and G6*Xap*F) using the collected phenotypic data.
3. To enable marker assisted seedling selection (MASS) for *Xap* fruit resistance in the UA program by converting these SNP haplotypes into SSR-based DNA tests.
4. To set the stage for the development of additional DNA tests for MAS of *Xap* by identifying additional QTL and SNP markers for *Xap* fruit and leaf resistance through pedigree-based quantitative trait loci (QTL) analysis (PBA), using the UA program. These DNA tests combined with G1*Xap*F and G6*Xap*F should allow peach breeders to combine horizontal *Xap* fruit and leaf resistance with high fruit quality.

***Xanthomonas arboricola* pv. *pruni* (*Xap*) Objectives:**

1. To investigate the diversity and virulence of *Xap* isolates across the eastern U.S. by genotyping and phenotyping different *Xap* isolates collected from 12 states in this region (including Arkansas).

Peach Fruit Quality, and Phenological Trait Objectives:

1. To document fruit quality and phenological traits across 29 F_1 peach populations and all selections and cultivars from the UA peach breeding program by taking phenotypic data for two consecutive years (2013-2014).
2. To enable MAPS and MACS across four U.S. RosBREED peach breeding programs (UA, Clemson University, Texas A&M University, and the University of California, Davis) by developing and validating informative SNP-based DNA tests for seven peach breeding-relevant fruit traits: Fruit resistance to bacterial spot (*Xap*1 and *Xap*6), maturity date (G4mat), fruit type (G), blush (R_f), flesh color (Y), acidity (D), and acidity and soluble solid content (G7Flav).
3. To enable MASS for fruit quality and phenological traits in the UA program by converting these SNP haplotypes into breeder-friendly DNA tests and confirming their accuracy.
4. To perform MAPS (2013-2015) and MASS (2015) and modify procedures and methods over the three years of this project to facilitate the UA fruit breeding program for continual use of MAS.

5. To set the stage for the development of additional DNA tests for MAS of fruit quality and phenological traits by identifying additional QTL and SNP markers through PBA of the UA fruit breeding program.

Chapter One: Literature Review

Peach Introduction

Taxonomy

Peach [*Prunus persica* (L.) Batsch] is a self-fertile diploid species (2n = 16), with a base chromosome number of x = 8, and belongs to the Rosaceae family, subfamily Prunoideae (Bassi and Monet, 2008; Byrne et al., 2012). The Rosaceae family encompasses several economically significant temperate fruits: *Malus domestica* Borkh. (apple), *Prunus avium* L. and *P. cerasus* L. (sweet and sour cherry), *P. domestica* L. and *P. salicina* Lindl. (European and Japanese plum), *P. armeniaca* L. (apricot), *P. dulcis* Mill. (almond), *Fragaria* × *ananassa* Duch. (garden strawberry), *Pyrus communis* L. (European pear), *Rubus* sp. (blackberry and raspberry) and ornamental plants such as *Rosa* sp. L. (rose). The subfamily Prunoideae comprises the largest genus of the Rosaceae family, *Prunus*.

Members of the *Prunus* genus are known as stone fruits (drupes), because they contain a fleshy/leathery mesocarp, enclosing a hard or stony endocarp and a seed. There are more than 400 *Prunus* species and the most economically important are fruit and nut species including: almond, peach and nectarine, sweet and sour cherry, European and Japanese plum, and apricot (Byrne et al., 2012). The peach and other *Prunus* species such as but not limited to *P. dulcis* (Mill.), *P. kansuensis* Rehd., *P. ferganensis* (Kost. and Rjab) Kov. and Kost., *P. scoparia* (Spach) C.K. Schneid, *P. mira* Koehne, and *P. davidiana* (Carr.) are all very closely related and believed to have evolved from a common ancestor, since several interspecific hybrids among them now exist, mainly for rootstock purposes (Byrne et al., 2012**;** Knight, 1969; Meader and

Blake, 1940). Several interspecific scion cultivars have additionally been developed in the last few decades, through different hybridizations between peach, cherry, plum and apricot (Fig. 1).

Fig. 1. Plum-cherry-apricot-peach hybrids; [A] photo by Terrence Frett in 2012 [B] photo compliments of David Karp (http://www.latimes.com/food/lat-karp1_l5gbb9nc20100719105721-photo.html).

Botany

Tree

Peach trees have a relatively short juvenility period; typically they begin fruit production in the second or third year and remain in commercial production for up to 15 years (Bassi and Monet, 2008). The trunk is typically straight and smooth. The roots reach up to 60 cm in depth. One-year-old shoots are red-green in color and turn dark grey as they age. Each node typically has three buds: one middle vegetative bud surrounded by two lateral reproductive buds. Lastly, there are six main growth habits documented in peach: standard, columnar, upright, compact, weeping, and open (Bassi, 2003; Bassi and Monet, 2008).

Leaves

The leaves of peach trees form following full bloom and are usually long and flat (Bassi and Monet, 2008) (Fig. 2A). A few recessive leaf traits have been discovered over time: wavy leaf form (wa/wa) (Scott and Cullinan, 1942); and narrow-width (wa2/wa2) (Chaparro et al., 1994; Okie and Scorza, 2001). Three different types of leaf glands are seen, located at the petiole and leaf blade base. The leaf glands show Mendelian inheritance with incomplete dominance:

reniform (kidney-shaped, homozygous dominant), globose (serrate margins, heterozygous) and eglandular (homozygous recessive) (Bassi and Monet, 2008; Connors, 1921).

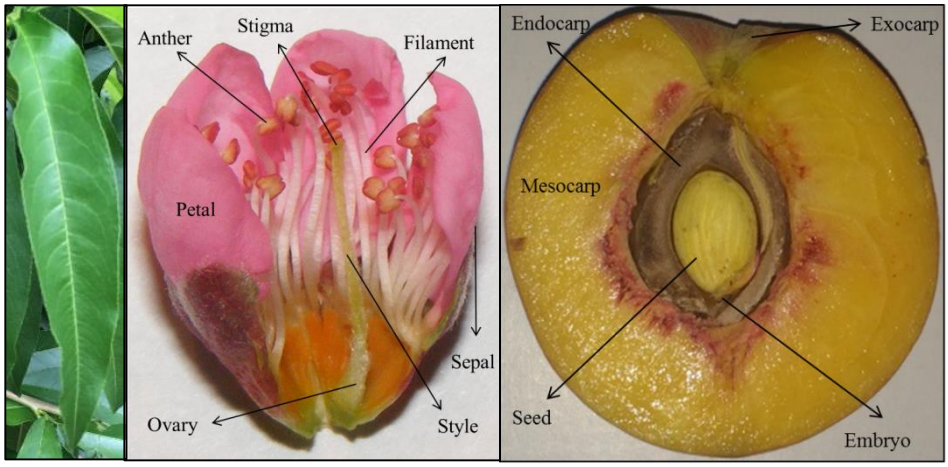

Fig. 2. Botanical pictures of peach: [A] leaf; [B] longitudinal section of a peach flower; [C] longitudinal section of a peach fruit (pictures by Terrence Frett, 2010-2015).

Flower

Peach has perfect, usually self-fertile, perigynous (half-inferior) flowers with separated petals (Fig. 2B). They have two corolla shapes which show Mendelian inheritance: showy (large petals, shaped like a rose, recessive sh/sh) and non-showy (small petals, bell-shaped; dominant Sh/Sh or Sh/sh) (Bassi and Monet, 2008) (Fig. 3). Typically, five petals are seen, ranging in color from white to pink, to dark red, to variegated (white and red) (Fig. 3). Additional mutations have occurred which have led to semi-double, and double flowers (12-24 petals) (Fig. 3). There is one pistil, and up to as many as 30 stamens with red anthers. Some peaches are male-sterile where the anthers look pale-yellow instead of red, since there is no pollen (these types are typically discarded from breeding programs).

Fig. 3. Flower variability seen across peach. (pictures by Terrence Frett, 2010-2012).

Fruit

The peach fruit is a drupe (stone fruit), which is composed of an exocarp (skin), fleshy mesocarp (flesh), and a hard endocarp (pit), which encloses the seed (Fig. 2C). The peach shape varies from round to elongated (elongated is dominant to round) (Bassi and Monet, 2008; Blake, 1932). Additionally the fruit can be flat ('pan-tao' is flat peach in Chinese) in appearance (flat is dominant to round or elongated) (Lesley, 1940). The fruit skin can be pubescent (fuzzy peach is dominant) or glabrous (no fuzz nectarine, recessive) (Blake, 1932; Rivers, 1906). The ground color (skin) and flesh color vary from white, yellow, orange, and red (Fig. 4). These varying colors are a result of different diversity and abundance of carotenoid and anthocyanin compounds. The flesh either adheres to the pit (clingstone) or does not (freestone). There are two main types of flesh textures, melting (flesh firmness softens), and non-melting (flesh maintains firmness with a rubbery texture), along with several others that have or still need to be characterized (stony hard, non-softening, and others) (Bassi and Monet, 2008).

Fig. 4. Fruit variability seen across peach (*****pictures from Lane and Bassi, 2008).

Origin

The peach originates from China where it is known as a symbol of long life/immortality, because it has always been important in Chinese culture (Byrne et al., 2012; Huang et al., 2008). The Chinese god called 'Shou' is a symbol of immortality (Fig. 5A) and as seen below he always carries a white peach. In one such Chinese custom, peach cakes are commonly served in birthday

celebrations for elders to express best wishes for a long prosperous life (Desmond Layne, personal communication). Drs. Desmond Layne, pomology specialist, and David Byrne, a peach breeder, are shown below looking at roadside peaches in China (Fig. 5B). Both spent extensive time traveling in China and learning about their history as well as current modified greenhouse production strategies for peach (Layne et al., 2013).

Fig. 5. [A] The Chinese god of immortality, 'Shou'; [B] Drs. Desmond Layne and David Byrne in China looking at peaches (*****pictures compliments of Dr. Desmond Layne – Clemson University, "Just Fruits" class fall 2010, peach lecture).

China has the longest history of peach cultivation in the world. In a recent study by Zheng et al. (2014), peach pits (stones) were discovered from an archeological site around the lower Yangzi River valley in the Zhejiang Province of China. These are the oldest peach stones found anywhere in the world, and the researchers were able to indicate that peach selection and domestication began ~8,000 years before present (BP). Other peach stones were also found in the lower Yangzi River valley, in Kuahuqiao (~7,000-8,000 BP) and Tianluoshan (~6,500-7,000 BP). These stones could be grouped into two sizes, thus indicating domestication of peach for preferred types (Zheng et al., 2014). Later, two peach stones were discovered in one of the ruin

sites of the Shang Dynasty (Goachen city, Hebei providence) which dated to around ~3,050-3,550 BP. These peach stones measured approximately the same size and had the same shape and surface grooves as current cultivars produced in China today (Chen, 1994; Huang et al., 2008).

History of Peach Dispersal Across the World

Following domestication in China, the species was moved to Persia along the silk trading routes. Later, around ~2,250 BP, the Greeks and Romans spread the peach throughout Europe. Western exploration of the Spaniards and Portuguese across the Atlantic Ocean led to the movement of the peach to Central America (and later North and South America) around the 1500s (Bassi and Monet, 2008; Byrne et al., 2012). This was the first introduction of peach into the Americas, which gave rise to several land races of the non-melting flesh texture (interestingly, today non-melting cultivars are more popular in the European fresh market, and melting flesh cultivars are the staple in the U.S.) (Bassi and Monet, 2008). By approximately the 1700s, peaches were being cultivated and commercially produced in Georgia and South Carolina. The Native Americans quickly adopted and spread the peach to a wide range of environments all the way from the tropical highlands of South and Central America, to the humid subtropics of Florida and Southern Brazil and to the coldest regions in northern U.S. and southern Canada (Bassi and Monet, 2008; Byrne et al., 2012; Faust and Timon, 1995; Hedrick, 1917; Hesse, 1975; Scorza and Okie, 1990).

Up until approximately the beginning of the 1900s, peaches in the U.S. and Europe were commonly propagated sexually by planting seeds instead of by vegetative means (Byrne et al., 2012). Seed propagation was used even longer in Central and South America, up until approximately the middle to later half of the 1900s. Because of sexual propagation, numerous

landraces of peaches were developed which went through centuries of extensive selection for adaptation and other characteristics throughout Asia, Europe, and the Americas (Bouhadida et al., 2011; Byrne et al., 2000; Byrne et al., 2012; Pérez, 1989; Pérez et al., 1993; Yamamoto et al., 2003).

Around the mid 1850s, a second wave of peach introduction occurred, directly from China to the U.S. The 'Chinese Cling' cultivar was grown for the first time in the U.S. at the Delaware Fruit Research Center (Bassi and Monet, 2008; Byrne et al., 2012). 'Chinese Cling' (a melting clingstone peach) was the mother of 'Elberta' (a melting freestone peach), which is the main ancestor of the majority of the cultivars grown today in the U.S. (majority are melting freestones; although there are non-melting, stony hard, non-softening, and other textures as well) as well as in other peach growing regions of the world (Bassi and Monet, 2008; Okie et al., 1985; Scorza et al., 1985).

Early commercial production of peaches in the U.S.

In 1895 the production of peaches in Georgia (as is seen in Figs. 6A-E) was a very intensive process involving extensive hand labor, and still is to this day (Desmond Layne, personal communication). Peaches were harvested by hand in the field (Fig. 6A) and then brought to packing sheds where they were inspected (Fig. 6B) and packaged into wooden boxes (Fig. 6C). The boxed up peaches were then loaded into 'refrigerated trains,' filled with big ice blocks to maintain a cool temperature, and distributed by trains to major cities in the Northeast for sale and consumption (Figs. 6D and 6E).

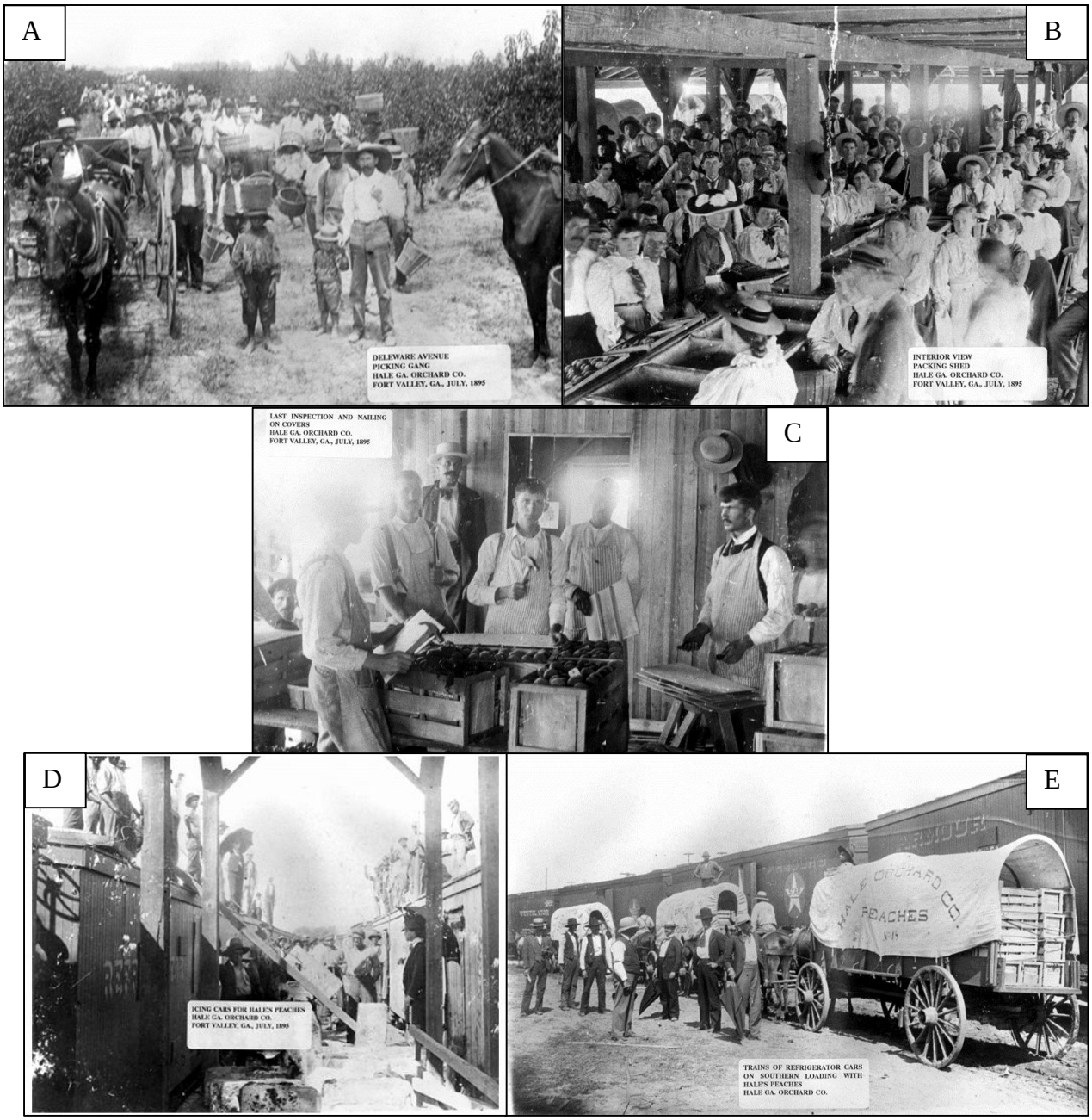

Fig. 6. Peach production in Georgia, U.S. around 1895: [**A**] peach harvest crew in the field; [B] the packing shed crew; [C] final inspections and packaging into wooden boxes; [D] loading of ice blocks into the train; [E] boxed up peaches ready to be loaded onto the 'refrigerated trains' (*pictures compliments of Dr. Desmond Layne – Clemson University, "Just Fruits" class fall 2010, peach lecture).

Economic Importance

The peach is a commercially important temperate fruit tree species, third-most important after apple and pear, with a total world production estimated at over 21.6 Mt (Byrne et al., 2012; FAO, 2015a). China was reported to be the main producer of peaches around 1993, and its production continues to grow (FAO, 2015a; Huang et al., 2008) (Fig. 7). In fact, in 2006 China was responsible for the production of 44% of the total global supply, while the other top producers of peaches, Italy, Spain, the U.S., and Greece, only produced 10%, 7%, 5%, and 5%, respectively (FAO, 2015a; Huang et al., 2008).

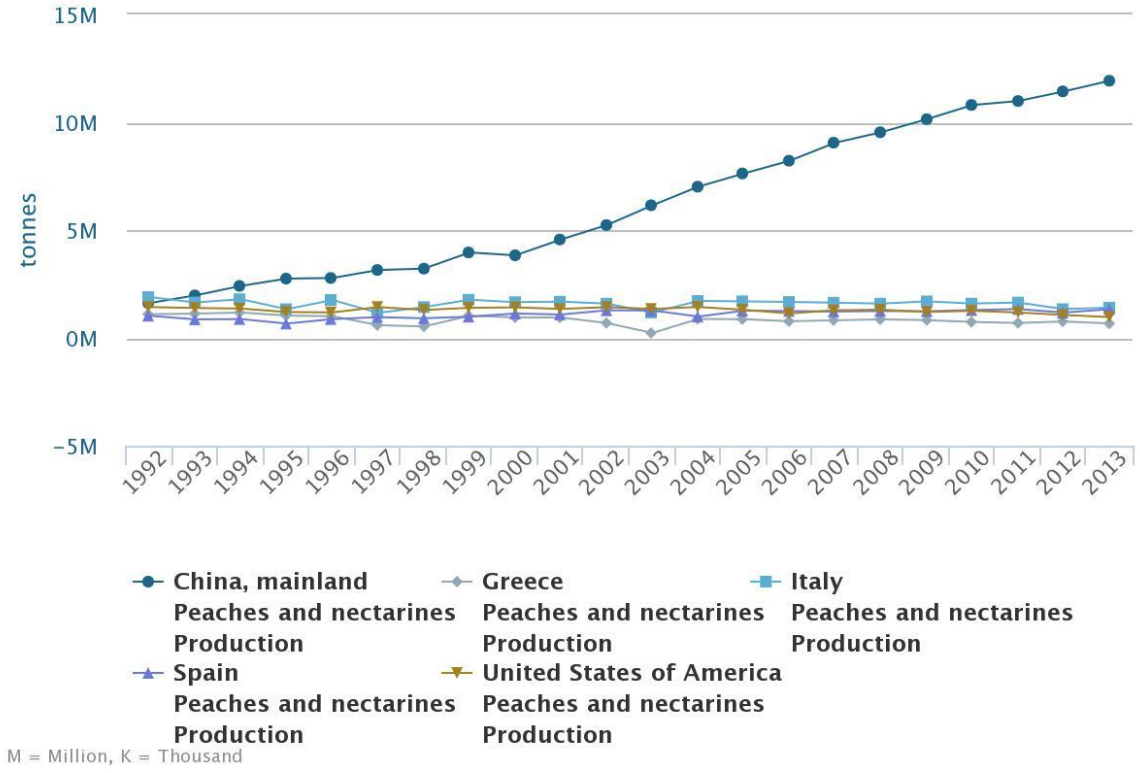

Fig. 7. Peach and nectarine production (tones = 1000 kg) of the top five producing countries during the period from 1992-2013 (FAO, 2015a).

In the U.S., peach is an economically important fruit. The peach industry in the U.S. has been dominated by California, followed by South Carolina, Georgia, and New Jersey. Across

2008, 2009, 2010, and 2011, California accounted for 74% of the peaches and nectarines produced in the U.S., while South Carolina, New Jersey, and Georgia accounted for 7%, 3% and 3% respectively (Pérez et al., 2011).

Peach Breeding History

As stated previously, around the mid-1850s a second wave of peach introduction occurred, directly from China to the U.S., during which the 'Chinese Cling' cultivar arrived (Bassi and Monet, 2008; Byrne et al., 2012). 'Chinese Cling' (a melting, clingstone peach) was the mother of 'Elberta', 'Belle of Georgia', and 'J.H. Hale.' These three peaches have served as key breeding parents in the development of important peach cultivars in the U.S. Today, nearly all fresh-market cultivars in the U.S. contain 'Chinese Cling,' 'Elberta,' 'Belle of Georgia,' and 'J.H. Hale' in their pedigrees (Byrne et al., 2012; Faust and Timon, 1995; Scorza et al., 1985).

Peach breeding in the U.S. officially started in 1895 with the establishment of a breeding program in Geneva, NY by Cornell's New York State Agricultural Experiment Station (NYSAES). After that, multiple peach breeding programs were established. From 1905-1920, breeding programs were established at public institutions in Iowa, Illinois, California, Ontario, New Jersey, Virginia, Massachusetts, New Hampshire, Maryland, and Michigan (Byrne et al., 2012). Later in the 1930s, Georgia and Texas commenced public breeding programs along with the first private breeding programs in California (Faust and Timon, 1995; Okie et al., 2008). Zaiger Genetics Inc., the company that expanded the idea of interspecific hybrids between peaches, plums, apricots, and cherry scions, was established in 1958. Other subsequent private California stone fruit breeding programs included Burchell Nursery, Bradford Genetics Inc., Sun World International Inc., and Gerawan Farming. In the 1950s, public breeding programs were developed in Louisiana, Florida, and North Carolina, and in the 1960s, Arkansas. The main goals

of all the programs were locally adapted, melting flesh (a few worked with non-melting flesh types as well) peach and nectarines destined for the fresh market. By 1970, 50% of the public breeding programs had been discontinued, and private companies in California began to flourish. This was due to several reasons including but not limited to consolidation of federal and state funding for fruit breeding research, and the expansion of intellectual property rights both in the U.S. and worldwide (Byrne et al., 2012; Clark et al., 2012).

Breeding programs were later established in Brazil around the 1950s (Byrne et al., 2012). The main focus of these programs were locally adapted non-melting and melting (emphasis in non-melting) flesh peaches and nectarines for the fresh and processing market. Later around the 1980s, Mexico began breeding non-melting peaches for the fresh market (Byrne et al., 2000; Byrne and Raseira, 2006). Additionally, around this time programs commenced in Chile, Uruguay, and Argentina.

Breeding programs were also established in Europe, primarily using cultivars developed in the U.S. The first European breeding program for peaches was established in Italy in the 1920s, followed by France in the 1960s. Later programs were started in Spain, Romania, Greece, Serbia, Ukraine, Bulgaria, and Poland (Llácer, 2009; Okie et al., 2008). Lastly, even though peaches had been cultivated in Asia for thousands of years, the first breeding program was established in Japan around the 1950-1960s, in China by the 1970s, and more recently in Thailand, Korea, and India (Byrne et al., 2000; Okie et al., 2008).

Status of the Current Peach Industry in U.S.

Throughout the past decade, the U.S. peach industry has seen a slight decline (Fig. 8), attributed to the necessity to harvest peaches at immature stages for storage and shipment purposes, negatively impacting fruit quality (Crisosto et al., 1995; Crisosto, 2002; Crisosto and Costa, 2008; Fideghelli et al., 1998; Sansavini et al., 2006). Several vital fruit quality traits including size, flavor (high sugar and moderate to low acidity), color, and blush (red skin pigmentation) develop as a peach ripens on the tree. Harvesting a peach at an immature stage limits the full development of these essential fruit quality traits, and furthermore increases the chances for postharvest problems such as mealiness, overall resulting in low fruit quality to consumers. Low peach consumption in the U.S. therefore can be overcome by finding a more precise balance with respect to fruit quality and the maturity stage at harvest (Bielenberg et al., 2009).

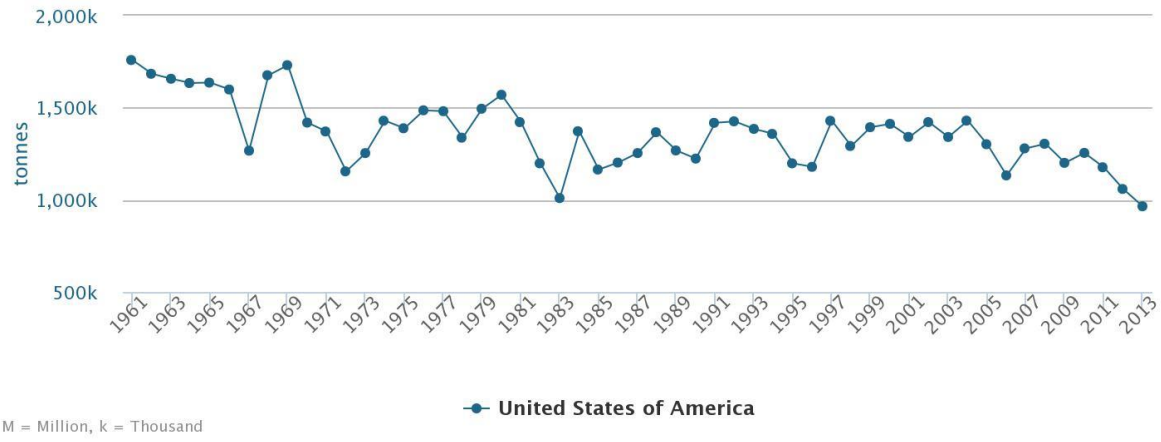

Fig. 8. Peach and nectarine production in the U.S. from 1961-2013 (FAO, 2015b).

Breeding Objectives

During the last century, peach breeding programs have worked diligently to develop and release hundreds of new cultivars throughout the world (Sansavini et al., 2006). The ultimate objective of all peach breeding programs worldwide is to develop cultivars that satisfy industry

and consumer demands. In general, peach breeding programs have aimed to produce productive cultivars spanning the season with environmental adaptation (abiotic and biotic), season extension, and improved fruit quality traits such as large size, unique shapes, low pubescence or glabrous (nectarines), appealing yellow or white ground color covered by extensive blush (red skin pigmentation), flavor with adequate eating quality, and increased firmness to resist damage associated with shipping (Bielenberg et al., 2009; Byrne et al., 2012; Howad et al., 2005; Okie et al., 2008; Sansavini et al., 2006).

Today these goals have been accomplished, yet the majority of the peaches in the U.S. fresh market still lack optimal fruit quality, postharvest life, and disease and pest resistance. Thus, programs are now focusing on fruit quality, enhanced flavors (high sugar with moderate to low acidity), pleasant aroma, improved texture, slow softening rates, expanded diversity of colors, enhanced postharvest life, and disease and pest resistance (Byrne, 2005; Okie et al., 2008).

Disease Resistance

Due to concerns of agricultural workers' safety, environmental contamination, economic concern, and food safety, restrictions on the use and availability of chemicals in agriculture have developed in recent years. Integrated pest management programs (IPM) are being incorporated to only use chemical sprays as the last resort. Disease resistant cultivars play a crucial role in these IPM practices (Byrne et al., 2012; Sansavini et al., 2006).

Because of these concerns, breeding programs across the world are currently trying to incorporate resistance genes for different pests and diseases into high-quality peach cultivars (Sansavini et al., 2006). The main pathogens targeted for resistance include ring nematodes [*Mesocriconema xenoplax* (Raski) Loof and de Grisse]; plum pox virus (Sharka disease), brown rot [*Monilinia fructicola* (G. Winter) Honey], armillaria root rot [*Armillaria mellea* (Vahl.: Fr.)

Kummer], peach leaf curl [*Taphrina deformans* (Berk.) Tul.], powdery mildew [*Podosphaera pannosa* (Wallr.) de Bary], and last but not least bacterial spot [caused by *Xanthomonas arboricola* pv. *pruni* (*Xap* refers to the pathogen and disease)] (Byrne et al., 2012; Sansavini et al., 2006).

The majority of cultivars used in the industry today have adequate fruit quality, but are very susceptible to *Xap*. Some cultivars have been developed with high *Xap* resistance, yet most still lack the desirable fruit quality characteristics required by the industry. Currently, there is a wide range of highly susceptible to resistant peach cultivars, however, no complete immunity has been reported (Ritchie, 1995). The majority of the cultivars produced in the western U.S. are highly susceptible to *Xap* because they lack *Xap* disease pressure during selection. On the other hand, several cultivars produced in the eastern U.S. show medium to high levels of resistance, since *Xap* pressure has enabled breeders to select for resistance (Okie, 1998). However, the majority of these breeding programs still apply copper, and oxytetracycline to keep the disease under control, thereby complicating selection of *Xap* resistance. Unfortunately, the majority of the best wholesale market peaches favored in the peach industry today are highly susceptible. This is because the varieties with *Xap* resistance commonly lack adequate fruit quality characteristics demanded by the industry.

Expanding the Diversity of Peaches to Develop High Quality, Disease-Resistant Cultivars

Fruit quality and disease and pest resistance can be improved through the development of new cultivars. However, more diversity must be sought (all commercial peach cultivars trace back through their pedigree to similar founders with a very narrow genetic base) and linkages must be broken. This is of high concern because a vast amount of diversity is necessary in order to breed for specific traits.

Simply put, without diversity (genetic variation) crop improvement through breeding is not possible. As said by Dr. John R. Clark, "Genetic variation is the most important key to the chances for a breeder's success. All other things aside, without genetic variation, no breeding progress is possible. If everything looks the same in the field, then no matter how many crosses you make, and how many seedlings per cross you look at, you will never be able to make improvements." Breeding is done to make improvements, thus if you can't make improvements there is no use in breeding. The good news, however, is that peach breeders today still have an extensive amount of diversity to work with to reach these goals. There is much diversity in disease and pest resistances found in the peach germplasm repositories, as well as certain breeding programs.

Peach Germplasm Repositories

There are several peach germplasm repositories worldwide. In the 1950s, the national peach germplasm survey began germplasm collections and establishment of three germplasm repositories in China (in Beijing, Zhengzhou, and Nanjing) (Byrne et al., 2012; Huang et al., 2008). During these explorations, more than 1,000 germplasm accessions were collected. This was an important feat to preserve this diversity, since the Chinese society and economy has greatly expanded since then. Germplasm explorations, screening, and evaluations within the repositories continued throughout the 1990s and greatly increased understanding of unique genotypes for effective use in breeding (Huang et al., 2008). The other peach germplasm repositories can be found in Japan, Korea, U.S., Brazil, Ukraine, France, Spain, and Italy (Byrne et al., 2012).

The *Prunus* National Clonal Germplasm Repository in Davis, CA (NCGR), serves as the repository in the U.S. This *Prunus* material has recently been genotyped by researchers at the

University of Arkansas (John R. Clark, Terrence J. Frett, and Alejandra A. Salgado) and Washington State University (Cameron P. Peace and Paul J. Sandefur) with multiple fruit quality and disease resistance DNA tests through the *Prunus* Crop Germplasm Committee (CGC) grant titled, "Genotypic evaluation of NCGR peach germplasm for high-value, breeding-relevant traits" (Project number 5306-21000-020-00D). The data generated will serve as an excellent resource for breeders and affiliated scientists to better understand trait genetics and the breeding value of the germplasm available at the Prunus NCGR.

Germplasm Exchange Between Breeding Programs

There is also much diversity in disease and pest resistances, as well as flavors and textures found in certain breeding programs. However, access to other breeding program's germplasm has dramatically changed with the advent of plant intellectual property rights (IP rights) (Clark et al., 2012). These IP rights have re-shaped fruit breeding into an exceedingly private and competitive endeavor (in both public and private sectors). This shift has decreased germplasm exchange, and quite frequently exclusive contracts and breeding agreements are put into place to allow the exchange of genetic variation only between two programs. These agreements have commonly been associated with a transfer fee, along with plant royalties and have been and will continue to be critical for the success of those 21st century fruit breeders who can successfully set up these agreements before their competitors (Byrne et al., 2012; Clark et al., 2012).

The University of Arkansas Breeding Program – High Fruit Quality Paired With Xap Resistance

Bacterial spot resistance has been a key trait of interest throughout the existence of peach breeding at the University of Arkansas (UA). This program has focused on breeding for resistance to *Xap* since 1964. Drs. Jim Moore and Roy Rom initiated the UA peach and nectarine breeding program, and their successor Dr. John R. Clark currently directs it. Unlike most other

peach breeding programs, antibiotic sprays to control the disease have never been applied, thus selection against *Xap* has been possible (John R. Clark, personal communication). The warm and humid spring and summers at the University's Fruit Research Station (FRS) near Clarksville, AR, create an ideal environment for the inoculation and spread of this pathogen. This environment has enabled breeding and selection of peaches with low *Xap* incidence.

The UA program also contains a diverse range of flesh textures and adherences including but not limited to: freestone melting flesh (FMF); clingstone melting flesh (CMF); clingstone non-melting flesh (CNMF); and clingstone non-softening flesh (CNSF). These unique textures maintain firmness for a longer period of time than traditional peaches in the market today, and thus can be retained on the tree longer and harvested at the correct maturity phase to develop full flavor. Furthermore, the textures have performed well in postharvest evaluations showing exceptional storage and shipment potential (Clark, 2011; Clark and Sandefur, 2013a; Clark and Sandefur, 2013b). This program serves as an example of a breeding program with which germplasm exchange could be possible to enhance diversity in other programs and pair high fruit quality with *Xap* resistance.

***Xanthomonas arboricola* pv. *pruni* (*Xap*)**

Taxonomy

Bacterial spot of peach [caused by *Xanthomonas arboricola* pv. *pruni* (*Xap* refers to the pathogen and disease)] is a mobile (one flagellum), gram-negative, rod bacterium (Fig. 9). This species was first named *X. pruni* (Smith, 1903) and then classified as *X. campestris* pv. *pruni* by Dye in 1978. Later the bacterium was reclassified as *X. arboricola* pv. *pruni* [Smith, 1903 (Vauterin et al., 1995)].

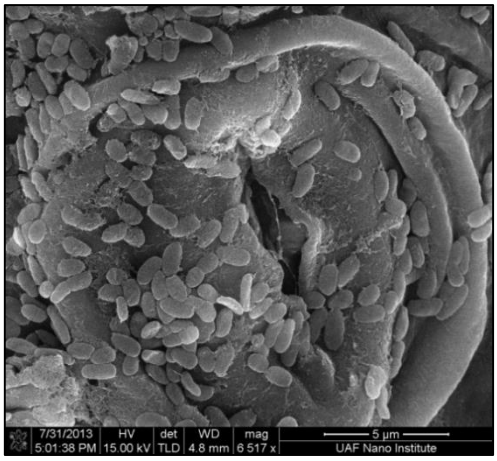

Fig. 9. Confocal microscope picture of *Xap* entering through a leaf stomate of peach (*picture compliments of Dr. Burton Bluhm and Brant Smith).

X. arboricola is a pathogenic bacterium comprised of seven pathovars that infect different hosts: *X. arboricola* pv. *pruni* [stone fruits (*Prunus spp.* L.)], *X. arboricola* pv. *corylina* [hazelnut (*Corylus americana* Marshall)], *X. arboricola* pv. *juglandis* [walnut (*Juglans regia* L.)], *X. arboricola* pv. *populi* [poplar (*Populus tremula* L.)], *X. arboricola* pv. *poinsettiicola* [poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch)], *X. arboricola* pv. *celebensis* [banana (*Musa* × *paradisiaca* L.)], and *X. arboricola* pv. *fragariae* [strawberry (*Fragaria* × *ananassa* Duch.)] (Hajri et al., 2012; Janse et al., 2001; Palleroni et al., 1993; Vauterin et al., 1995).

Hosts and Damage

Bacterial spot is a serious disease of *Prunus* spp. and their hybrids worldwide (EPPO/CABI, 1997; OEPP/EPPO, 2006; Ritchie, 1995). This disease has been reported on peach and nectarine, almond, cherry, plum, and apricot. The most severe infections have been seen on Japanese plum (*Prunus domestica* L.), Korean cherry (*P. japonica* Thunb.), plum hybrids, and on peach and nectarine (EPPO/CABI, 1997; OEPP/EPPO, 2006; Ritchie, 1995). Bacterial spot symptoms are generally characterized as various sized necrotic lesions on leaves and fruits and cankers on twigs (EPPO/CABI, 1997; OEPP/EPPO, 2006; Ritchie, 1995) (Fig. 10A-C). The

disease is commonly referred to as bacterial leaf spot, shot-hole, and black spot (Ritchie, 1995). In general, the disease leads to premature defoliation (Fig. 10D), weak vigor of the plant across years, unmarketable fruit with low fruit quality, and overall decline in production (Aarrouf et al., 2008; Ritchie, 1995).

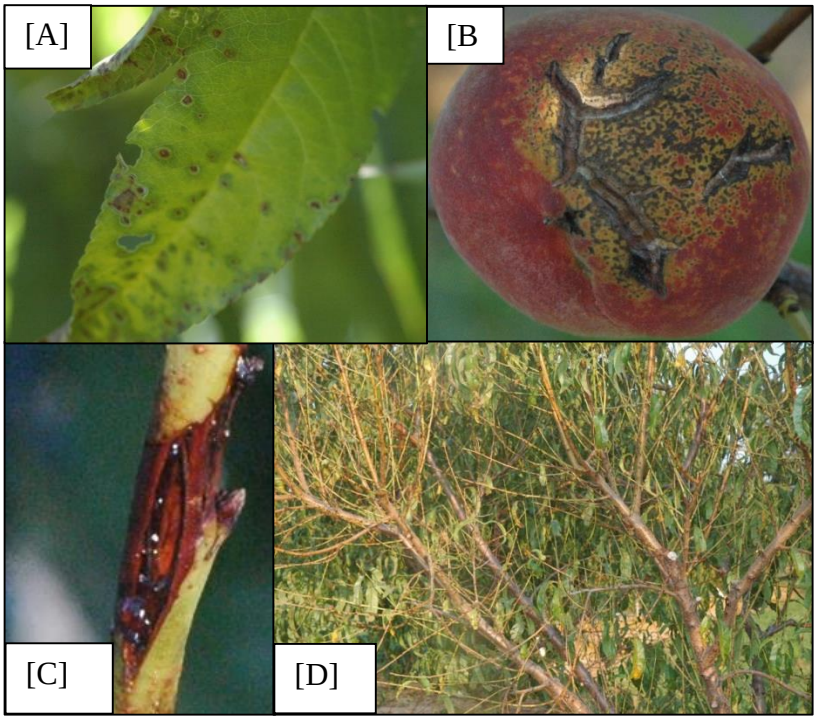

Fig. 10. Bacterial spot on [A] peach leaf, [B] peach fruit, [C] peach twig, and [D] entire peach tree (pictures by Terrence Frett in 2013-2015).

History and Spread

Bacterial spot was first defined on Japanese plums in Michigan in 1903 (Smith, 1903). Later, it was identified on peach and other stone fruits (Dunegan, 1932; Rolfs, 1915). The pathogen has rapidly spread across the world and today it is present in almost all continents where stone fruits are grown in North America, South America, Europe, Africa (South Africa), Asia, and Australia (OEPP/EPPO, 2006) (Fig. 11). The European Union currently subjects *Xap* to phytosanitary legislation through the EEC Directive no.92/103 (S.I. Nº 219, 2003) due to establishment and recent appearance in several countries of that region (EPPO, 2003).

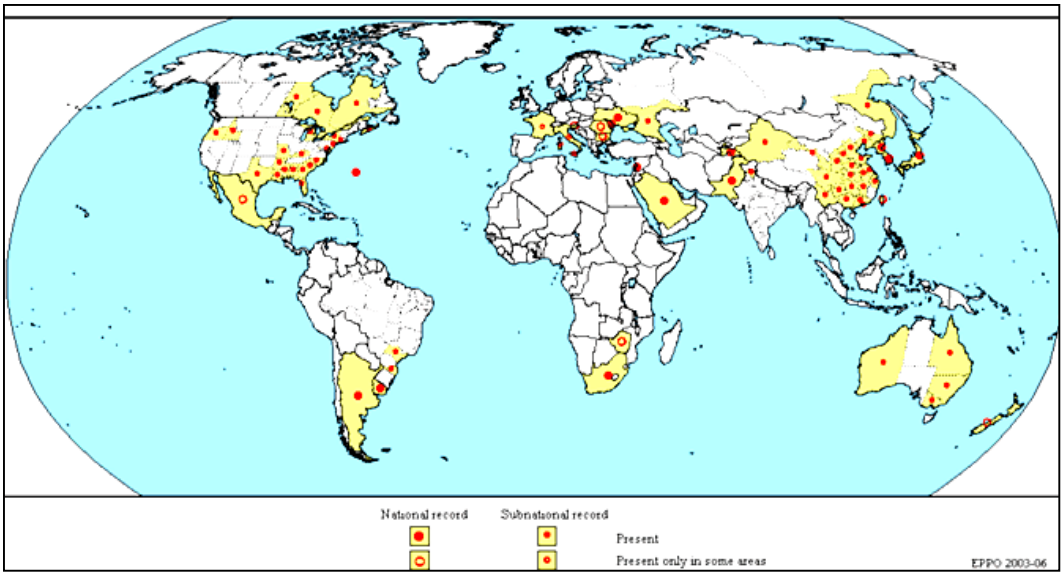

Fig. 11. Distribution of *Xanthomonas arboricola* pv. *pruni* around the world (*figure as in CABI/EPPO, 2006).

Optimal Conditions

Infection and development of *Xap* is highly dependent on environmental conditions. The disease is particularly devastating in warm, humid environments such as the eastern U.S. and other countries with similar climatic conditions. It rarely if ever is seen in dry arid climates such as the Central Valley of California. Locations with sandy soils are much more prone to *Xap* infection since wind-blown sand can create wounds for the bacterium to infect (EPPO/CABI, 1997; Ritchie, 1995). Periods with warm temperatures (20-35 °C), accompanied with light, frequent rains, extended heavy dews, very high humidity, along with the occurrence of substantial wind-blown rains during late bloom to pit hardening are most favorable for severe infection (Daines, 1961; EPPO/CABI, 1997; Randhawa and Civerolo, 1985; Ritchie, 1995; Zehr and Shepard, 1996). Conditions of at least 12 h of water saturation and high humidity (~90%) are needed for infection to occur, develop, and spread (Ritchie, 1995; Zehr and Shepard, 1996). Wind and water serve as vectors to move the bacterium around the orchard. Windy rains are needed for the bacterium to infiltrate the fruit and leaves (Daines, 1961; Randhawa and Civerolo,

1985). Infection of leaves and shoots can occur anytime environmental conditions are optimal. On the other hand, fruit infection is believed to be limited from petal fall to near or after pit-hardening (EPPO/CABI, 1997; Ritchie, 1995).

Disease Life Cycle

When the season culminates, the bacterium overwinters in the intercellular spaces of the cortex, phloem and xylem of cankers, and leaf scars on twigs (EPPO/CABI, 1997; Ritchie, 1995) (Fig. 12). During the following spring as temperatures warm, the bacterium begins to multiply in these intercellular locations, as leaf and flower buds swell and new tissue emerges. The primary inoculum originates from leaf scars and spring cankers and is then disseminated to the leaves by dripping dew and wind-blown rain. The bacterium is able to enter new leaf growth through stomata or wounds and spread through the vascular tissue (xylem and phloem) of the host (Aarrouf et al., 2008) (Figs. 9 and 12). Lesions developing on leaves exude the multiplied bacterium to allow secondary infections. Fruit is primarily infected between petal fall and near or after pit-hardening. Throughout the season the bacterium is able to continue to spread to other leaves and/or stems when environmental conditions are optimal (EPPO/CABI, 1997; Ritchie, 1995) (Fig. 12).

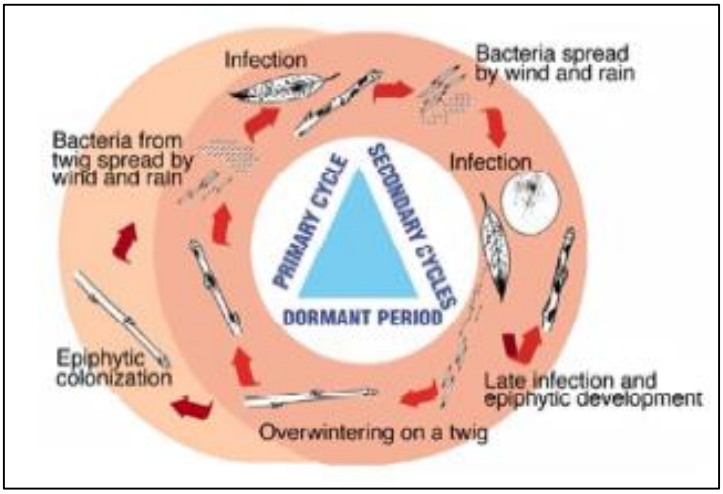

Fig. 12. *Xanthomonas arboricola* pv. *pruni* life cycle (*figure as in Jones and Sutton, 1996).

Disease Symptoms

On peach leaves, symptoms are generally concentrated on the leaf tip, and along the midrib and leaf margin, where rain or dew, which spread the bacterium, commonly accumulate (OEPP/EPPO, 2006; Ritchie, 1995) (Fig. 13). The symptoms are initially observed on the lower surface of peach leaves as small (~1-2 mm in size) light-green to yellow, angular-shaped, water-soaked lesions with a brownish-yellow center (Fig. 13A).

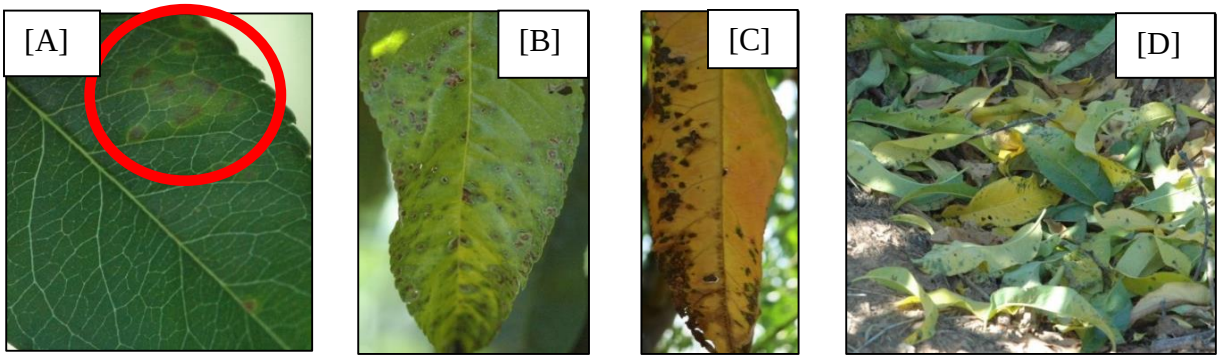

Fig. 13. Bacterial spot leaf symptoms: [A] initial water-soaked lesions; [B] coalesced shot holes; [C] yellowing; [D] premature leaf drop (pictures by Terrence Frett in 2013-2015).

As the disease progresses, spots can be seen on the upper surface of the leaf. They begin to enlarge to 2-3 mm (rarely exceeding 5 mm) and exhibit an angular shape since the pathogen is restricted by veins (OEPP/EPPO, 2006; Ritchie, 1995) (Fig. 13B). The angular spots have a

water-soaked appearance and eventually can darken to a dark-purple, brown, or black color. The surrounding area commonly becomes yellow (Fig. 13C). These diseased areas have been observed to detach after or prior to color change, leaving the leaf with a dark ring and a shot-hole appearance. Severely infected leaves turn yellow and frequently experience premature drop (Fig. 13D). The leaves are most susceptible to infection before they fully expand. When conditions are optimal, the first leaves to emerge can become infected and serve as secondary sources of inoculum for later emerging leaves and fruit. In general, leaf symptoms are observed 5-14 d after infection. Bacterial spot is differentiated from foliar lesions caused by pesticide sprays, insects, or other organisms, by the bacterium's characteristic smaller angular-shaped, water-soaked lesions. In comparison, pesticide sprays and insects usually create larger circular shaped lesions (OEPP/EPPO, 2006; Ritchie, 1995).

The symptoms of *Xap* on twigs are characterized as cankers (OEPP/EPPO, 2006; Ritchie, 1995) (Fig. 10C). These cankers are termed spring or summer cankers, depending on when they develop. Spring cankers occur around bloom from inoculum which has overwintered in leaf, flower, or terminal buds. The cankers extend down from the buds and are initially characterized as small (~1-2 cm), slightly dark, water-soaked blisters/lesions. As the season progresses, the cankers extend and the bark eventually darkens further and cracks. Cankers which develop from terminal buds eventually girdle the twig, commonly referred to as black tip. Summer cankers, on the other hand, form later in the season on current-season growth (OEPP/EPPO, 2006; Ritchie, 1995) (Fig. 10C).

On fruit, *Xap* infections are favored by warm, humid, moist conditions during petal fall to shuck split (OEPP/EPPO, 2006; Ritchie, 1995) (Fig. 14). Under these optimal environmental

conditions, symptoms can appear as early as 3 weeks after petal fall. Initial symptoms are small circular brown spots on the surface of the young fruitlet (Fig. 14A).

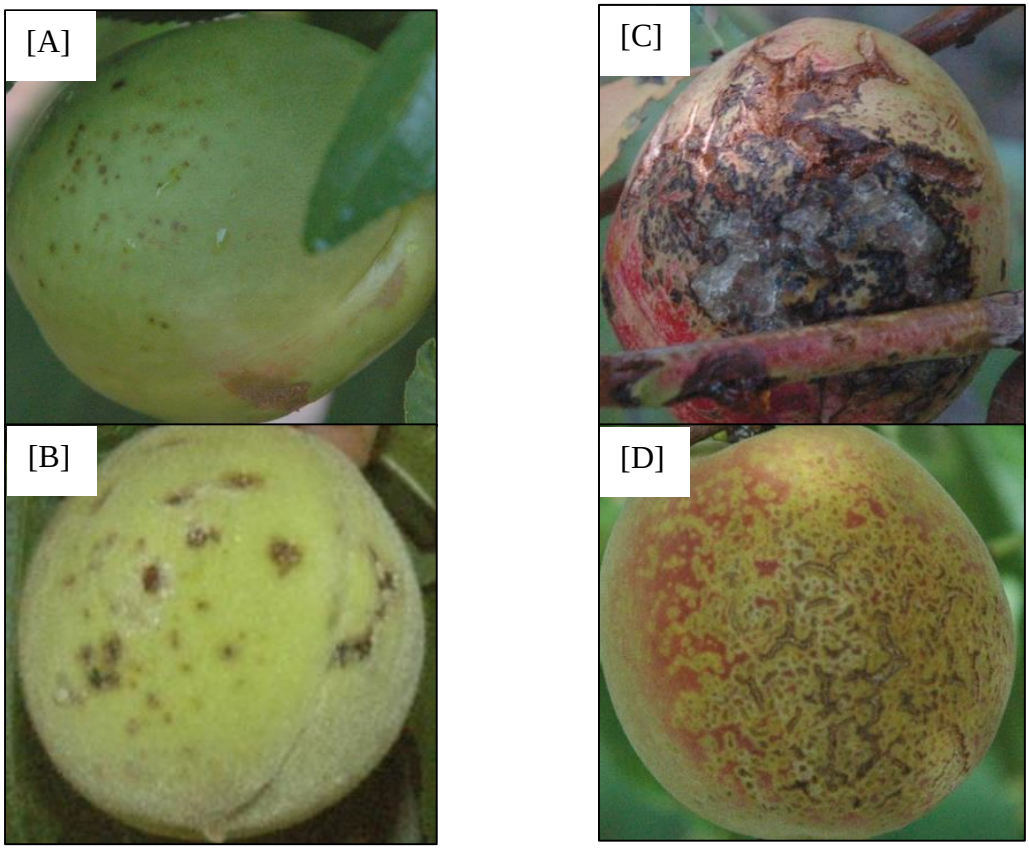

Fig. 14. Bacterial spot fruit symptoms: [A] small brown spot water soaked lesions; [B] mature lesions with sunken necrotic centers; [C] extended and coalesced lesions into flesh; [D] surface lesions (pictures by Terrence Frett in 2013-2015).

As the lesions develop, they appear water-soaked with a small sunken necrotic center (Fig. 14B). Later these lesions enlarge further, extend deep into the flesh, and develop a brown to black color (Fig. 14C). Infections that occur near or after pit-hardening characteristically are limited to the surface of the fruit skin (Fig. 14D). They may coalesce and cause the skin to crack, but do not extend into the flesh. Gum can exude from all *Xap* lesions, particularly after rainfall (OEPP/EPPO, 2006; Ritchie, 1995). This ‘xanthan gum’, is a polysaccharide used as food additive (soups, beverages, salad dressings, cake mixes, sauces, and frozen food), and a

thickening agent for toiletries (toothpaste), cosmetic creams, oil, and paper (Rosalam and England, 2006).

Disease Management

The first step in control of *Xap* is to grow and maintain healthy trees (Ritchie, 1995; Ritchie, 1999). Trees under stress are more severely affected by *Xap* than trees not experiencing stress. This includes nutrient stress, and stress related to other pathogenic organisms such as nematodes. Therefore, optimum soil fertility must always be maintained. High winds and blown sand are also known to spread the pathogen and create openings for entry. Ground covers can be used to minimize blowing of sand and windbreaks can be incorporated to withstand strong winds and only allow slight air movement through the orchard (Ritchie, 1995; Ritchie, 1999).

In the peach industry, the primary commercial control of *Xap* has been to rely heavily on pesticides, such as copper-containing compounds, and oxytetracycline (Ritchie, 1995; Ritchie, 1999). Correct timing of sprays and rates used are important. Relatively high rates of copper sprays are generally applied when only a limited amount of new growth is present early in the growing season, from dormancy through early shuck split. The idea of these applications is to cover the tree surface to create a barrier through which the bacterium must pass as it moves from overwintering sites. The majority of the inoculum is thought to die in this process of moving through the copper barrier. Copper sprays are continued as new growth emerges but rates are reduced because these compounds are known to cause fruit and foliage damage in peach, resulting in grayish discoloration, leaf shot holes, and premature leaf drop. The compound oxytetracycline is then incorporated when shuck split commences since it is less damaging to the fruit and foliage (Ritchie, 1995; Ritchie, 1999).

Unfortunately, if favorable environmental conditions occur, the disease can develop even when well-timed sprays have been applied (Ritchie, 1995; Ritchie, 1999). Generally, once the bacterium is established in the orchard, it is very difficult to control the disease, especially when highly and or moderately susceptible cultivars are used. Furthermore, due to concerns of agricultural worker safety, environmental contamination, economic concern, and food safety, restrictions on the use and availability of chemicals in agriculture have developed in recent years. Integrated pest management programs (IPM) are being incorporated to only use chemical sprays as the last resort and *Xap* resistant cultivars play a crucial role in these IPM practices (Byrne et al., 2012; Sansavini et al., 2006).

The genetic control of *Xap* resistance is quantitative in nature, however, dominant genes were suggested to be involved in peach (Sherman and Lyrene, 1981; Werner et al., 1986). Quantitative resistance is more stable than monogenic resistance since the pathogen must overcome the polygenic defense. Interestingly, the resistance of leaf and fruit in peach has been suggested to be controlled by alleles at different genetic loci due to inconsistent levels of leaf and fruit resistance (Keil and Fogle, 1974; Simeone, 1985; Werner et al., 1986; Yang et al., 2013).

Development of Molecular Markers to Complement the Traditional Breeding Process

Despite the considerable genetic improvements traditional peach breeders have made in the past century, traditional seedling selection (TSS) is a time consuming, expensive, and laborious process taking 10 years or more, from the initial cross until a new peach cultivar can be released (Bliss, 2010; Dirlewanger et al., 2004b; Ru et al., 2015). In temperate environments, the breeder must wait at least three years for peach trees to mature to fruit-bearing capacity before fruit quality data can be evaluated on progeny (Bliss, 2010; Dirlewanger et al., 1998; Dirlewanger et al., 2004b; Dirlewanger et al., 2007). Once the trees bear fruit, it can then take an

additional 10-15 years of phenotypic analysis, selection, and regional testing to develop a new cultivar. Moreover, peach breeding programs require a significant amount of space due to the large tree size along with continuous maintenance costs such as herbicide, pesticide and fungicide spraying, planting, pruning, thinning, and watering (Bliss, 2010; Dirlewanger et al., 2004b; Ru et al., 2015).

Taking into account all maintenance costs from cross to initial selection or tree removal, it was calculated to cost approximately $12 per peach seedling at Clemson University in 2015 (Ksenjia Gasic, and Cameron Peace, personal communication). This is comparable to the estimated $12 per apple seedling at the Washington State University (WSU) apple breeding program and $15 per cherry seedling at The Pacific Northwest (PNW) sweet cherry breeding program from 2001-2015 (Edge-Garza et al., 2010; Evans et al., 2012; Rowland et al., 2012). Considering $12 per peach seedling, if 2,000 new seedlings are planted, the overall cost to maintain these seedlings through initial selection phase (four years) totals $24,000. Crosses are typically made annually, thus TSS costs expand further as the breeding program matures.

Yet today innovative molecular tools (techniques) are nearing application, which can compliment the traditional breeding process. These can provide peach breeders with more informed decision support to save resources and determine how to efficiently break linkages and combine traits such as disease and pest resistance along with unique flavors and superior textures (Bliss, 2010; Byrne, 2005; Ru et al., 2015). One such tool, marker assisted selection (MAS), can enhance the ability and efficiency of breeders to combine all desired traits into the next round of high-quality peach cultivars (Bliss, 2010; Byrne, 2005; Collard et al., 2005; Ru et al., 2015) (Fig. 15). The MAS breeding strategy is based on a marker-locus-trait association (M-L-T), in which a predictive genetic marker is linked to a specific locus that contributes to the genetic variation for

a specific phenotypic trait (Bliss, 2010; Ru et al., 2015). Therefore, the marker genotype (through their association with the locus genotype) is used to select for the phenotype (Bliss, 2010; Collard et al., 2005; Collard et al., 2008; Ru et al., 2015).

When a M-L-T is screened on parental germplasm, MAS is called marker assisted parent selection (MAPS), and helps in decision making. In MAPS, allelic information of the parental pool can help direct the breeder to first select parents with valuable alleles, and subsequently the genotypic information can help the breeder select favorable crosses with efficient combining abilities, through marker assisted cross selection (MACS). After the cross is made, the same M-L-T can be used to screen the seedlings to decide on which promising seedlings to grow in the field and which to discard based on their allelic makeup (Ru et al., 2015). This form of MAS is termed marker assisted seedling selection (MASS), and is useful in monitoring the incorporation of the desirable functional alleles at the locus from parent to progeny (Bliss 2010; Peace and Norelli 2009).

The WSU Molecular Breeding Lab has been documenting MASS resource-use efficiency for the the WSU apple and the PNW sweet cherry breeding programs since ~2001 (Edge-Garza et al., 2016). They recently published an article on a new DNA-based diagnostic tool, the MASS Efficiency Calculator v1.0, which they developed to enable a more precise estimation of MASS resource-use efficiency (Edge-Garza et al., 2016). Using this tool, the WSU lab has shown that TSS costs for the WSU apple and the PNW sweet cherry breeding programs can be substantially reduced by 50-60%, and 70-80% by using only one and two DNA tests for MASS, respectively. Even more noteworthy, in general as more seedlings are screened, more DNA tests are used (in sequence rather than together) and culling rates are increased, the conventional breeding costs can be even further reduced (Cameron Peace, personal communication; Edge-Garza et al., 2016).

These successful examples of MASS in apple and sweet cherry substantiate the feasibility and value of conducting MASS in Rosaceae tree fruit breeding and provide insights into how to extend MAS adoption into more Rosaceae tree fruits (Ru et al., 2015; Edge-Garza et al., 2016).

The incorporation of MAS (MAPS, MACS, and MASS) can facilitate more informed breeding decisions, save resources, and complement, accelerate and enhance the traditional breeding process in order to enable more efficient cultivar development (Bliss, 2010; Byrne, 2005; Ru et al., 2015). The overall general breeding scheme that can be used by Rosaceous fruit tree breeders to incorporate MAS into the normal flow of the breeding program is shown in Fig. 15A (Ru et al., 2015). Additionally, a more animated version is shown in Fig. 15B. The Fig. 15C shows a visual timeline on how to incorporate MAS into the breeding cycle for peach.

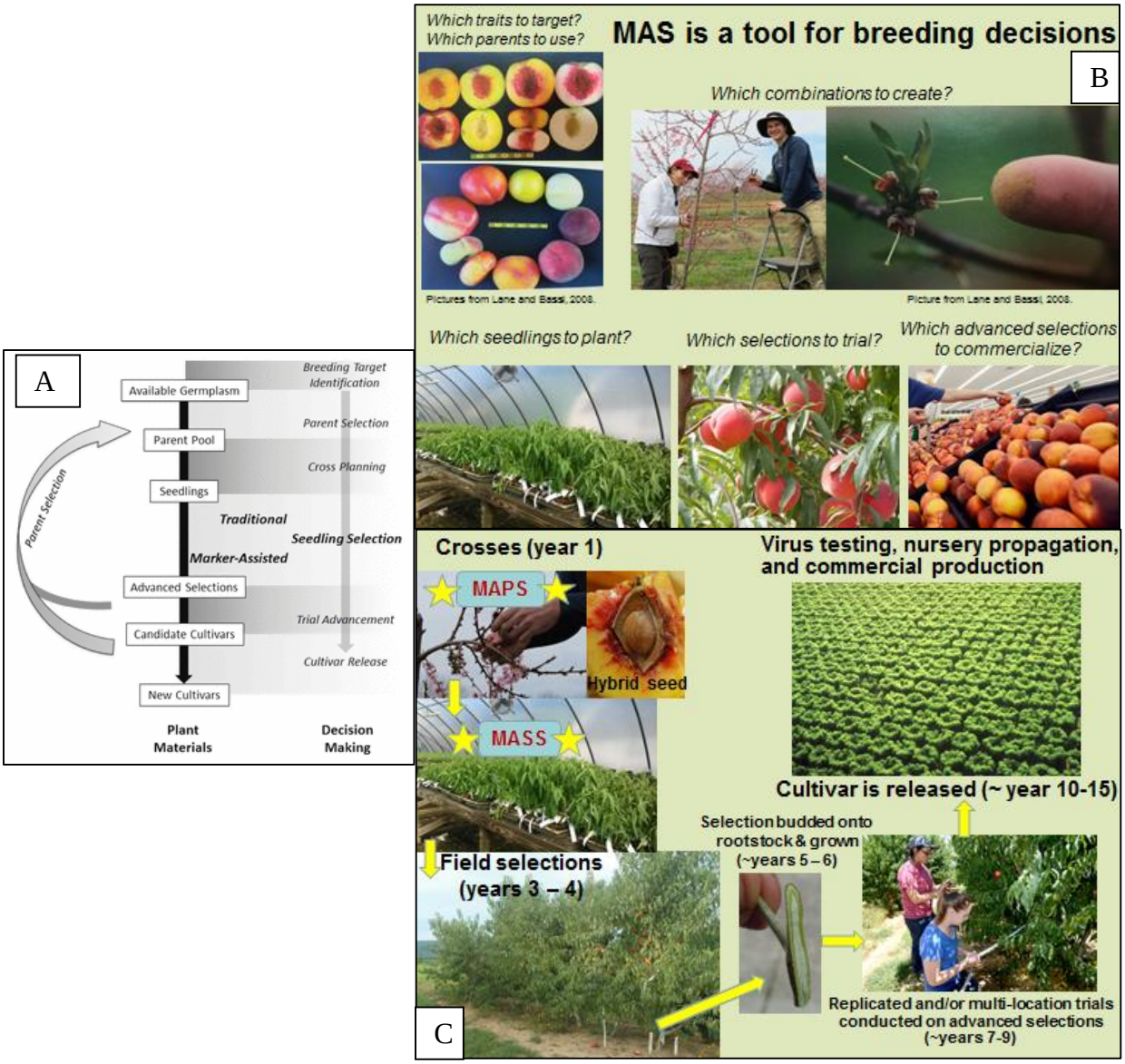

Fig. 15. Incorporation of MAPS, MACS, and MASS into the traditional Rosaceous tree fruit breeding scheme: [A] as in Ru et al., 2015; [B] MAS as a tool for breeding decisions (pictures by Terrence Frett in 2013-2015); [C] MAS visual timeline for peach (pictures by Terrence Frett in 2013-2015).

The Traditional Steps Used to Enable MAS in Peach

Several steps have been used in the past to try and reach deployment of MAS in peach. The typical M-L-T (QTL/MTL) analysis steps used in previous peach studies have included (Collard et al., 2005; Collard et al., 2008; Salazar et al., 2013):

(1) Creating a bi-parental population which segregates for the trait/s of interest

(2) Phenotyping the segregating population

(3) Genotyping the population

(4) Linkage map creation

(5) Marker-locus-trait (M-L-T) mapping

(6) Fine mapping

(7) The candidate gene (CG) approach

(1) Population Creation

A population which segregates for the traits of interest must be generated to perform linkage analysis and later M-L-T mapping. In general, two parents with contrasting phenotypes are selected for crossing, in order to develop seedlings which segregate for the traits of interest. M-L-T mapping can be performed using an F_1 segregating population derived from two heterozygous parents to discover M-L-T associations. However, to enhance recombination of the parental alleles, commonly one of the F_1 seedlings intermediate for the trait of interest is selected for selfing, and the F_2 population is used for linkage analysis and M-L-T mapping. More than 46 peach and other *Prunus* hybrid populations (F_1 and F_2 types combined) have been designed to study fruit quality, phenological, and biotic and abiotic resistance traits. Also, two populations have been designed to study the genetic control of *Xap* resistance in *Prunus* (Socquet-Juglard et al., 2013; Yang et al., 2010, 2011, 2012, and 2013).

(2) Phenotypic Data

Phenotypic data (measurements of observable plant traits) is a crucial component for M-L-T mapping, which connects genetic variation with biological activity, thus documenting gene function (Bassil and Volk, 2010). High quality phenotypic data is imperative in order for a M-L-T study to be successful; therefore, careful considerations must be taken on how to standardize and accurately phenotype the trait(s) of interest. The peach traits studied herein are grouped into fruit quality, phenological, and disease resistance traits.

Screening for Fruit Quality and Phenological Traits

Enhancing fruit quality and phenological traits is of high interest to fruit breeders, in order to satisfy growers', distributers', marketers', and consumers' evolving demands. Fruit quality traits include but are not limited to size, firmness, texture, internal and external color, sugar, acidity, other unique flavors, and postharvest potential. Phenological traits include but are not limited to bloom date, fruit set, and fruit ripening date. When harvesting fruit to phenotype fruit quality and phenological traits, determining a standardized maturity stage is critical, since these traits are known to vary depending on the environmental conditions, and the maturity state of the fruit. Additional details on the fruit quality and phenological traits studied herein can be found in Frett et. al. (2012).

Screening for Xanthomonas arboricola pv. *pruni (Xap) Resistance*

Screening for resistance to *Xap* in the field is not an easy task. In a host plant population the disease incidence or severity of each individual will be affected by the health of each individual and several environmental/pathogen factors (location, temperature, humidity, and distribution of pathogen inoculum). One way to control this is to inoculate plants in the field to ensure adequate distribution of the pathogen to each individual (Yang et al., 2013). Even further controlled methods to enhance the screening of *Xap* resistance and take more informative and

reliable phenotypic data have also been proposed: greenhouse inoculations and detached-leaf bioassays.

Greenhouse inoculations can utilize relatively controlled environmental factors, but the peach tree might still respond differently in the field. Socquet-Juglard et al., (2013) studied four different greenhouse inoculation techniques: (i) infiltration in the leaves using a needleless syringe; (ii) injuries caused by a needle; (iii) injuries caused by scissors; and (iv) dipping shoots of the plant into the inoculum. All techniques were found to be efficient except dipping shoots of the plant into the inoculum. However, they obtained the most quantitative data range (differentiation of susceptibility/resistance) through this dipping technique. Greenhouse inoculations were shown to be reliable; however, they are expensive to undertake and additionally are difficult to commence since peach seedlings take approximately three years to develop and bear fruit in temperate climates. Furthermore, resistance has been shown to be age-dependent. Socquet-Juglard et al., (2013) reported one- and two-year-old greenhouse seedlings were very susceptible to Xap. In fact, several of the one-year-old plants that were inoculated in the greenhouse died. This increase in resistance observed on the older plants was due to plant age effects instead of genetic susceptibility.

Detached-leaf bioassays offer a cheap and quick alternative to perform in vitro screening using peach leaves (Randhawa and Civerolo, 1985). A common detached-leaf bioassay has been performed in several previous studies (Civerolo and Keil, 1976; Hammerschlag, 1988; Hammerschlag et al., 1994; Randhawa and Civerolo, 1985). In short, leaves harvested from hosts in the field or in the greenhouse are infiltrated with the inoculum using a needle-less syringe and then are incubated on water agar for 2 weeks to allow symptoms to develop. Symptoms are later classified using a qualitative ‘0-3’ rating scale to differentiate symptoms of infections at each

inoculation site (Civerolo and Keil, 1976; Hammerschlag, 1988; Hammerschlag et al., 1994; Randhawa and Civerolo, 1985). This detached-leaf bioassay is a cheap and efficient way to screen for *Xap* resistance across the breadth of germplasm in a breeding program, as well as investigate the diversity and virulence of *Xap* isolates collected from multiple environments.

Scoring Method

For a study on disease screening to be successful, the correct method of scoring the phenotypic data must be determined. The main method to estimate disease incidence or severity has been through visual field estimation (Cobb, 1892). Several rating scales have been suggested to measure disease severity including nominal or descriptive scales, ordinal rating scales, and interval or categorical scales (Bardsley and Ngugi, 2012; Bock et al., 2010; Cobb, 1892). The disease rating is separated into three classes when using a nominal or descriptive scale: susceptible, tolerant, or resistant. This scale has very little value due to a high level of subjectivity and lack of quantitative definition (Bock et al., 2010). Ordinal rating scales are used to assign disease ratings into different classes that represent the increasing severity of symptoms. One example is a '0-4' scale: 0 = resistant, no symptoms; 1 =slightly resistant, 2 = moderately resistant, 3 = moderately susceptible; 4 = susceptible. Additionally, different qualitative words are used to classify the severity of the specific disease. This method is generally preferred for pathogens that are difficult to measure quantitatively (Madden et al., 2007). Interval or categorical scales are used to quantify and classify the percent area of the plant with symptoms (Bock et al., 2010). The Cobb scale (Cobb, 1892) was the first interval or categorical scale developed. It was a '1-5' scale used to assess severity of rust on wheat (*Triticum aestivum* L.) (1 = 1% disease; 2 = 5% disease; 3 = 10% disease; 4 = 20% disease; 5 = 50% disease).

Recent studies on *Xap* of peach used a combination of the ordinal and interval or categorical scale (Hammerschlag et al., 1994; Yang, 2012; Yang et al., 2013). A '0-5' scale was

used by Yang et al. (2013) to rate field-inoculated trees: 0 = resistant (no symptoms); 1 = slightly resistant (1-5% disease damage; water soaked lesions), 2 = moderately resistant (6-10% disease damage; tattered patterns on the leaf tip and leaf rib), 3 = moderately susceptible (11-25% disease damage; coalesced water-lesion and shot holes); 4 = susceptible (25-50% disease damage; yellow leaf); 5 = highly susceptible (> 50% disease damage; premature leaf drop). In Hammerschlag et al. (1994), a detached-leaf bioassay was rated using a 0-3 scale: 0 = no symptoms; 1 = distinct chlorotic spot and/or slight necrotic flecks; 2 = distinct but pale necrotic spot or grayish-white lesion, 2 mm in diameter; and 3 = distinct, dark necrotic spot of > 2 mm in diameter, with or without a chlorotic halo. These two combined ordinal and interval or categorical scales provided a rapid way for evaluators to accurately phenotype a large number of plants in a breeding program for resistance to *Xap* (Bock et al., 2010; Hammerschlag et al., 1994; Yang, 2012; Yang et al., 2013).

(3) Genotypic Data

Genetic markers reveal genetic differences between individuals. The two main types of genetic markers include morphological and molecular markers (Collard et al., 2005). Before screening genetic markers on the progeny, each must first be tested on the parents, and the F_1 that was selfed, if the population is an F_2, to determine if the markers will be polymorphic or monomorphic for the population. Polymorphic markers are informative co-dominant or dominant markers which discriminate between individuals by distinguishing between different genotypes, and therefore, can be used for constructing linkage maps (Collard et al., 2005). On the other hand, monomorphic markers are non-discriminatory, and therefore are not useful for constructing linkage maps. Morphological markers were the first genetic markers used to construct genetic linkage maps, however, their limited numbers and variability due to environmental effects hindered creation of extensive linkage maps (Winter and Kahl, 1995).

[a] Morphological Markers

Morphological markers are observable, qualitative phenotypic traits, associated with a major gene. These major genes were initially discovered by Gregor Mendel, the founder of modern genetics. Mendel made specific hybridizations of the model organism, *Pisum sativum* L. (the common pea plant), to generate pea populations segregating for different visible phenotypic traits. From these crosses, he observed that certain pea traits followed particular inheritance patterns, which were later termed the laws of Mendelian inheritance (Law of Segregation and the Law of Independent Assortment). In his work with pea he discovered the genetic control of seven discrete morphological traits: floral color (purple or white), floral position (axial or terminal), pod shape (inflated or constricted), pod color (green or yellow), seed shape (round or wrinkled), seed color (yellow or green), and stem length (long or short).

In *Prunus*, the position of 28 major genes controlling important agronomic traits (physiological, fruit quality, productivity, and disease resistance) have been located on the *Prunus* genetic reference map 'Texas' almond × 'Earlygold' ('T × E') (Dirlewanger et al., 2004b). Peach is the best genetically characterized *Prunus* species with 19 major genes mapped to their specific positions on the eight linkage groups (LG) of the 'T × E' reference map (Dirlewanger et al., 2004b; Etienne et al., 2002; Pozzi and Vecchietti, 2009; Sansavini et al., 2006).

Considering these 19 major genes, nine important fruit quality traits have been linked to molecular markers in the peach genome (Dirlewanger et al., 2004b; Dirlewanger et al., 2006; Mingliang et al., 2007) [**gene**; LG; *molecular marker*; distance from marker (cM)]: **[1]** fruit flesh color - white/yellow (**Y**; LG1; *UDP98-407*; 2.2) (Bliss et al., 2002; Mingliang et al., 2007); **[2]** red around the pit - red/no red (**Cs**; LG3; *OPO2/0.6*; 12.4) (Yamamoto et al., 2001); **[3]** flesh adhesion to pit - freestone/clingstone (**F**; LG4; *UDAp-431/b*; 1.2) (Dirlewanger et al., 2006)

(*BPPCT009/b*; 2.2; *AG12 and AG16b*; 2.0) (Dettori et al., 2001); **[4]** acidity - non-acid/acid fruit (**D**; LG5; *pTC-CTG/a* and *pGT-TTG/a*; 0) (Dirlewanger et al., 1998; Dirlewanger et al., 1999; Dirlewanger et al., 2006; Etienne et al., 2002); **[5]** pubescence - nectarine/peach (**G**; LG5; *eAC-CAA/a*; 0) (Dirlewanger et al., 2006) (*UDP96-018*; 4.5) (Mingliang et al., 2007); **[6]** fruit shape - flat/round (**S**; LG6; *MA040a*; 0) (Dirlewanger et al., 1998; Dirlewanger et al., 1999; Dirlewanger et al., 2006); **[7]** fruit skin color (**Sc**; LG6; *UDP96-015*; 3.7) (Yamamoto et al., 2001); **[8]** blood flesh (**bf**; LG4; *C41H*; 10.3) (Gillen and Bliss, 2005); and **[9]** aborting fruit (**Af**; LG6; *MA040a*; 0) (Dirlewanger et al., 2006). Since fruit flesh color, flesh adhesion to pit, acidity, pubescence, fruit shape, fruit skin color, and aborting fruit, have been linked tight enough (<5 cM) to their respective molecular marker, they have potential to be used in MAS (Collard et al., 2005; Dirlewanger et al., 2004b; Dirlewanger et al., 2006). However, the main limitation to morphological markers is their relatively low abundance in comparison to molecular markers.

[b] Molecular Markers

Molecular markers are specific sequences of DNA associated with a particular region in the genome (Jones et al., 1997; Winter and Kahl, 1995). They develop through several types of DNA mutations during meiosis (point, insertion or deletion, and replication error mutations, etc.) (Paterson, 1996). These mutations vary from individual to individual, thus screening molecular markers on genotypes of a population can be used to determine if the marker is polymorphic (different between genotypes) or monomorphic (all the same) for the population. These DNA molecular markers hold distinct advantages over morphological markers, in that they are highly abundant, can be analyzed at any time in the lab, and thus are not influenced by the stage of plant development and or the environment (Collard et al., 2005; Jones et al., 1997; Winter and Kahl, 1995). For these reasons, DNA-based markers have come to be the genetic markers most

commonly used for association analysis, linkage map construction, M-L-T analysis, fine mapping, CG analysis, and ultimately MAS (Bliss, 2010).

DNA molecular markers are divided into three classes; hybridization-based, PCR-based, and DNA sequence-based (Collard et al., 2005; Jones et al., 1997; Winter and Kahl, 1995). Important types of these DNA molecular markers include but are not limited to random amplified polymorphic DNA (RAPD; PCR-based), restriction fragment length polymorphisms (RFLP; hybridization-based), amplified fragment length polymorphic DNA (AFLP; PCR-based), simple sequence repeats (SSR; PCR-based), and single nucleotide polymorphisms (SNP; DNA sequence-based).

RAPDs are markers which are quick, simple and cost effective for use in genetic studies. These molecular markers, first documented by Williams et al., (1990), are arbitrary fragments of genomic DNA that contain single primers of random nucleotide sequence. A major advantage is that random RAPD can be amplified through PCR. The major disadvantages of RAPD markers include low reproducibility and non-transferability (Collard et al., 2005; Jones et al., 1997; Penner, 1996; Winter and Kahl, 1995).

The RFLPs are restriction enzyme sites which vary among individuals. They were the first molecular markers used, along with small numbers of morphological markers, to produce extensive genetic linkage maps of Rosaceae species (Peace and Norelli, 2009). They are co-dominant, highly reproducible, and transferable molecular markers. However, use of RFLPs is limited because they are time consuming and expensive to run (Beckmann and Soller, 1986; Collard et al., 2005; Jones et al., 1997; Kochert, 1994; Tanksley et al., 1989; Winter and Kahl, 1995).

The AFLPs are dominant, population-specific molecular markers which use a similar technique to RFLPs, only differing in that their PCR amplification technique is selective to specific restriction fragments. The DNA is first digested by restriction enzymes into restriction fragments. The sticky ends of the fragments are ligated to oligonucleotide adapters. Next, selected restriction fragments are amplified, and separated through gel electrophoresis to determine the AFLP banding pattern (Vos et al., 1995). Sometimes AFLPs can result in several bands, which only few are of significance. When this occurs, significant bands can be cut out from the gel sequence, and primers can be designed to only amplify those bands that are of significance to be implemented into genetic mapping. This process is known as cutting out sequence characterized amplified regions (SCARs). Downsides of AFLPs are that they require large amounts of DNA and are relatively complicated to screen (Collard et al., 2005; Jones et al., 1997; Vos et al., 1995; Winter and Kahl, 1995).

The SSRs are highly polymorphic, PCR-based markers, which arise from tandem repeat duplications of a specific string of two to six DNA nucleotides (Edwards et al., 1991). These markers are worthy for developing and comparing positions of genetic linkage maps, because of their co-dominant nature, frequent polymorphisms, and relatively high density in all plant genomes (McCouch et al., 1997; Powell et al., 1996; Taramino and Tingey, 1996). Specific forward and reverse primers can be generated in order to screen the SSR markers on plant DNA (Collard et al., 2005; Jones et al., 1997; McCouch et al., 1997; Paterson, 1996; Powell et al., 1996; Taramino and Tingey, 1996; Winter and Kahl, 1995). Downsides to SSRs are that sequence information is needed to design the primers, the majority are species specific, and they are low in abundance in plant genomes when compared to SNPs.

The SNPs are co-dominant, bi-allelic markers (present or absent) that develop over time through single nucleotide change mutations. The SNPs can be caused by insertions or deletions, transitions, or transversions (Vignal et al., 2002). These single nucleotide changes vary for different individuals of a species (Vignal et al., 2002). The SNPs have become very popular in the past decade and are currently the top marker of choice being used by genetic groups studying numerous plant species around the world. This popularity is due to their high density in plant genomes and relative ease in screening vast numbers (Vignal et al., 2002). They have enabled researchers to highly saturate linkage maps, such as what has been done for the *Prunus* reference map. In Rosaceae, SNPs hold an estimated frequency of 1/100 in intronic (non-coding) sequences and 1/225 and in exonic (coding) sequences, respectively (Illa et al., 2010; Sargent et al., 2009).

(4) Linkage Map Creation

A segregating population is required for linkage map development. Linkage maps approximate the genomic position and genetic distances between genetic markers through linkage analysis of the genotypic data (Collard et al., 2005; Jones et al., 1997; Paterson, 1996). The construction of a genetic linkage map is based on the events of meiosis. During meiosis, genetic recombination occurs between homologous chromosomes and leads to the development of recombinant genotypes. The recombination frequency (RF) between molecular markers in a segregating population is calculated based on the frequency of recombinant genotypes. The RF is used to determine the order and specific distance between the markers. The RF between the markers, and thus their positions on the linkage map, can be calculated by hand, but more frequently is done through high throughput computer software. The lower the RF between the molecular markers, the closer they are on the linkage group (Collard et al., 2005; Jones et al., 1997; Paterson, 1996). When markers show a RF >50%, they are termed unlinked, and thus

located on different linkage groups. The linkage group approximates all the alleles or markers which are linked on the same chromosome and excluding cross over events, remain together during meiosis.

History of Linkage Maps in Prunus and Peach

The first genetic linkage map for peach was developed by Chaparro et al. (1994). After this, an almond peach genetic map ('T × E') was generated, later used as the *Prunus* reference map (Foolad et al., 1995; Joobeur et al., 1998; Pozzi and Vecchietti, 2009). The 'T × E' *Prunus* reference map was developed through an interspecific F_2 cross between almond ('Texas') and peach ('Early Gold') (Foolad et al., 1995; Joobeur et al., 1998; Pozzi and Vecchietti, 2009). The 'T × E' map saturated the *Prunus* genome with 235 RFLP's and 11 isozymes (Aranzana et al., 2003; Joobeur et al., 1998). This 'T × E' linkage map showed all eight linkage groups and spanned a total distance of 491 cM. The reference map currently holds a total distance of 524 cM with 826 molecular markers leading to an average map density of 0.63 cM/marker (Dirlewanger et al., 2004b; Dirlewanger et al., 2007; Howad et al., 2005).

The 'T × E' *Prunus* reference map provided anchor markers (i.e. transferable markers throughout *Prunus*) with known map locations (Dirlewanger et al., 2004b; Dirlewanger et al., 2007; Howad et al., 2005; Pozzi and Vecchietti, 2009). These anchor markers enabled comparative genomics throughout peach, and *Prunus*, which facilitated the development of eight intraspecific peach linkage maps and several interspecific *Prunus* linkage maps (Dirlewanger et al., 2004b; Dirlewanger et al., 2007; Howad et al., 2005; Pozzi and Vecchietti, 2009).

The first eight linkage maps **[1-8]** generated for peach, include (F_2 = second generation population; *BC1*= backcross 1 population): **[1]** 'Ferjalou Jalousia' × 'Fantasia', F_2 ('J × F') (Dirlewanger et al., 1998; Pozzi and Vecchietti, 2009); **[2]** Peach *Prunus persica* × *P. ferganensis* (Kost. & Riab), *BC1* ('PxF') (Dettori et al., 2001; Pozzi and Vecchietti, 2009; Verde

et al., 2005); **[3]** 'Lovell' × 'Nemared', F_2 ('L × F') (Lu et al., 1998; Pozzi and Vecchietti, 2009; Sosinski et al., 1998); **[4]** 'Guardian' × 'Nemaguard', F_2 ('G × N') (Blenda et al., 2007; Pozzi and Vecchietti, 2009); **[5]** 'Akame' × 'Juseito', F_2 ('A × J') (Pozzi and Vecchietti, 2009; Yamamoto et al., 2001; Yamamoto et al., 2005); **[6]** 'Suncrest' × 'Bailey', F_2 ('Sc × B') (Pozzi and Vecchietti, 2009; Sosinski et al., 1998); **[7]** 'Harrow Blood' × 'Okinawa', F_2 - PMP2 ('HB × Oki') (Gillen and Bliss, 2005; Pozzi and Vecchietti, 2009); **[8]** 'New Jersey Pillar' × 'KV77119', F_2 ('NJ × KV') (Pozzi and Vecchietti, 2009; Sosinski et al., 1998).

There are currently 46 linkage maps generated for peach and related interspecific hybrids, which can be found on the Genomic Database for Rosaceae (GDR; http://www.rosaceae.org/) (Jung et al., 2008; Jung et al., 2014), and in Salazar et al. (2013). These genetic linkage maps continue to serve as powerful tools to compare to the 'T × E' Prunus reference map as well as the peach genome v2.0 sequence (Verde et al., 2015), for the localization and identification of M-L-T associations followed by candidate genes (CG) associated with the control of important qualitative and quantitative peach traits (Collard et al., 2005; Jones et al., 1997; Paterson, 1996; Tanksley et al., 1989; Winter and Kahl, 1995).

(5) Marker-Locus-Trait (M-L-T) Mapping

Marker-locus-trait (M-L-T) mapping associates the control of a known phenotype (quantitative or qualitative in nature) to a specific region on the linkage map. Computer software programs use the linkage map and phenotypic data to identify a M-L-T (QTL, MTL, or gene) association (Collard et al., 2005; Jones et al., 1997; Paterson, 1996; Winter and Kahl, 1995). Three main types of M-L-T analysis include: single-marker analysis, simple interval mapping (SIM), and composite interval mapping (CIM) (Liu, 1998; Tanksley, 1993). Single-marker analysis, the most basic M-L-T mapping tool, incorporates an analysis of variance (ANOVA) and linear regression to detect a M-L-T association (Collard et al., 2005). Unlike single-marker

analysis, the SIM M-L-T method is more powerful because it evaluates intervals in between adjacent linked markers along linkage maps simultaneously (Lander and Botstein, 1989; Liu, 1998). Considering all three M-L-T methods, the CIM is the most powerful and precise M-L-T mapping technique because it combines linear regression and interval mapping and also incorporates additional molecular markers (Jansen, 1993; Jansen and Stam, 1994; Zeng et al., 1993; Zeng et al., 1994).

Linkage between the marker/s and the QTL or MTL, is based on the frequency of recombination, as a result of meiosis events which occur through plant hybridizations (crosses). During meiosis, genetic recombination occurs between homologous chromosomes, which can break the linkage between the M-L-T, resulting in recombinant genotypes. The frequency of recombinant genotypes identified determines the recombination frequency (RF) between the M-L-T, and the RF ultimately determines the distance between the marker the QTL or MTL controlling the phenotype. The lower the RF, the closer the marker is to the locus responsible for the phenotypic variation observed (Bliss, 2010; Collard et al., 2005; Collard et al., 2008; Ru et al., 2015). The markers should only be used in MAS after they have been shown to be linked close enough to the locus [gene(s)] responsible for the phenotypic variation observed, otherwise they will be misleading (Bliss, 2010; Collard et al., 2005; Collard et al., 2008; Ru et al., 2015). Generally, a marker is determined reliable to be used in MAS when it is shown to be linked ideally <1 cM (centi-morgans), but not more than 5 cM from the locus (Bliss, 2010; Collard et al., 2005; Collard et al., 2008; Ru et al., 2015).

History of M-L-Ts in peach

A total of 568 different M-L-T (QTL or MTL) have been described in peach and or related interspecific hybrids and can be found on the Genomic Database for Rosaceae (GDR; http://www.rosaceae.org/) (Jung et al., 2008; Jung et al., 2014). Additionally, a partial list of

these QTL and MTL can be seen in Salazar et al. (2013). These QTL and MTL were linked to 75 different peach traits: 13 for tree development, 15 for flower and ripening, six for disease and pest resistance, and 41 for fruit quality (Salazar et al., 2013).

(6) Fine Mapping

Once the M-L-T has been discovered, subsequent steps are frequently required before the M-L-T can be used in MAS (Collard et al., 2005). Through fine mapping, more individuals and molecular markers which reside within the M-L-T of interest are incorporated (to increase the population size and marker density at the M-L-T) to identify a marker more tightly-linked to the locus of interest. There is no defined population size required for fine mapping, however, in previous studies, population sizes that have been used for fine mapping have consisted of >1000 individuals to identify a marker linked <1 cM to the locus which controls the trait variation (Blair et al., 2003; Chunwongse et al., 1997; Collard et al., 2005; Li et al., 2003). This strategy of fine mapping is very difficult for peach breeders to perform, since the perennial peach trees have very long juvenility periods and large populations are expensive to maintain in the field (Peace et al., 2014).

(7) The Candidate Gene (CG) Approach

The candidate gene (CG) approach is another option to develop a marker useful for MAS. The CG approach is used when assumptions are made in regards to the biological function of genes of interest (Byrne and McMullen, 1996; Pflieger et al., 2001). Previously sequenced structural or transcriptional regulating genes which co-locate within MTL or major QTL are useful in characterization of the major loci function. Primers can be designed for functional markers located within the CG, typically indels, SCARs, and SNPs. These markers can then be screened on different germplasm to validate their ability to predict the phenotypic variation of the trait of interest (Pflieger et al., 2001).

Current Status of MAS in Peach

Peach has been selected as the model species for Rosaceae genomics studies because it has a relatively short juvenility period (~2-3 years), simple genomic structure (227.4 Mb), and high amount of developed genetic resources (Abbott et al., 2002). Also, peach is the best genetically characterized *Prunus* species with 19 major genes mapped to specific loci on the highly saturated *Prunus* genetic reference map, 'T × E' (Aranzana et al., 2003; Dirlewanger et al., 2004b; Etienne et al., 2002; Joobeur et al., 1998; Pozzi and Vecchietti, 2009; Sansavini et al., 2006). A total of 46 linkage maps have been used for QTL analysis through traditional bi-parental mapping populations (Salazar et al., 2013). A total of 568 different M-L-T (QTL and MTL) have been described in peach and or related interspecific hybrids (Salazar et al., 2013). The Genomic Database for Rosaceae (GDR; http://www.rosaceae.org/) houses all of this molecular information to enabling further genetic studies of Rosaceae (Jung et al., 2008; Jung et al., 2014). Furthermore, the 'Lovell' di-haploid peach genome sequence v1.0 has been released (Verde et al., 2013), and later the chromosome assembly and genome annotation was improved and released as v2.0 sequence (Verde et al., 2015). In addition, a high-throughput Illumina Infinium® IPSC 9K SNP v1.0 genotyping array has been developed (Verde et al., 2012) and deployed across four peach breeding programs in the RosBREED 1 initiative (Iezzoni, 2013; Peace et al., 2010; Peace, 2011).

In peach, only three traits have been investigated enough to enable MASS across multiple peach breeding programs; flesh texture, flesh adherence to pit, and acidity. Texture and pit adherence traits were effectively mapped to a single locus (EndoPG), on linkage group four (LG 4) containing three endopolygalacturonase genes that code for proteins which break down the cell wall, leading to softening of the peach (Peace et al., 2005; Peace et al., 2006; Peace et al., 2007). Different alleles at the EndoPG locus have been associated with the development of the

different flesh and adherence phenotypes; FMF, CMF, CNMF, and CNSF. Each of these phenotypes are important for distinct markets; fresh market peach breeders typically develop FMF peach cultivars, while peach breeders in the canning market breed for CNMF varieties (Peace et al., 2005; Peace et al., 2006; Peace et al., 2007).

The major locus controlling peach fruit acidity, the D-locus [high vs. low titratable acidity (TA)], has also been identified and linked with a molecular marker on LG 5 of the peach genome. This is a key quality trait for breeders and consumers since it effects overall peach flavor (Boudehri et al., 2009; Dirlewanger et al., 1998). Several SSR markers flanking the D-locus were genotyped across a population which segregated for acidity, and the CPPCT040 marker was identified as the most tightly linked with the acidity locus (Boudehri et al., 2009; Dirlewanger et al., 1998).

Through the RosBREED project in 2010-2012, the EndoPG and CPPCT040 marker allele robustness to accurately predict acidity levels (high vs. low) and flesh texture and adherence, were validated across a diverse array of peach founders, cultivars, selections, and breeding populations from four pedigree-connected U.S. peach breeding programs (Iezzoni, 2013; Peace et al., 2010; Peace, 2011). Later, two studies on Arkansas individuals used this genetic information to show that the EndoPG markers correctly identified flesh phenotypes approximately 89% of the time and the CPPCT040b marker ~90% time (Salgado, 2015; Sandefur, 2011). These are the first three traits validated for use in MASS across the four RosBREED peach programs.

Current Status of MAS in Other Rosaceae Fruit Crops

In apple, MASS has been successfully incorporated in breeding for pyramiding of disease resistance alleles as well as for postharvest potential (Ru et al., 2015). The MAPS and MASS

strategies has been used to pyramid disease resistance alleles for apple scab [*Venturia inaequalis* Cooke (Wint.)] and apple fire blight [*Erwinia amylovora* (Burrill 1882)] resistance (Ru et al., 2015). Likewise, a postharvest potential marker, located within the Md-ACS1 gene (associated with ethylene production), has also been used for MAPS and MASS to enhance apple postharvest storability in the Washington State University (WSU) apple breeding program (Edge-Garza et al., 2010; Kumar et al., 2012; Ru et al., 2015). Culling of 89% of 3,000 individuals from one cross in 2006, using the Md-ACS1-indel, was shown to achieve an estimated net savings of ~$18,250 (~60%) over the traditional costs required without MASS (Edge-Garza et al., 2010; Ru et al., 2015). Later in 2008, culling of 54% of 5,400 individuals using the same DNA test achieved an estimated net savings of ~$44,000 (~70%) over the traditional breeding costs (Edge-Garza et al., 2010; Rowland et al., 2010). Furthermore, the University of Minnesota (UofM) and WSU apple breeding programs have performed MAPS (2011-present) and MASS (2013-present) for skin color, acidity, flesh texture, tartness, crispness, juiciness, bitter pit susceptibility, and apple scab resistance (Jim Luby, personal communication; Peace, 2013) and MAPS and MASS for storability, firmness, crispness, juiciness, and acidity (Kate Evans, personal communication; Ru et al., 2015; Sebolt, 2013). It is noteworthy that in general as more seedlings are screened, more DNA tests are used (in sequence rather than together), and culling rates are increased, conventional breeding costs are even further reduced (Cameron Peace, personal communication; Edge-Garza et al., 2016).

The Pacific Northwest (PNW) sweet cherry breeding program has been routinely using a DNA test for sweet cherry self-compatibility, as well as fruit size (Haldar et al., 2010; Iezzoni 2010; Rowland et al., 2012; Zhang et al., 2009) [self-compatibility tests have also been developed for apricot (Raz et al., 2009) and Japanese plum (Sapir et al., 2008)]. The S4' self-

compatibility allele is targeted in the self-compatibility DNA test (Haldar et al., 2010) and the fruit size DNA test targets alleles for large fruit size (Iezzoni, 2010; Zhang et al., 2009). Incorporation of MASS using these two DNA tests saved an estimated $25,000 (culling of ~60% of 837 seedlings) and $55,000 (culling of ~84% of 1439 seedlings) in the PNW sweet cherry breeding program in 2009 and 2010 respectively (Rowland et al., 2012).

These successful examples of MAS in apple and sweet cherry substantiate the feasibility and value of conducting MAS in Rosaceae tree fruit breeding and provide insights into how to extend MAS adoption into more Rosaceae tree fruits (Ru et al., 2015). This extension of MAS into other Rosaceae tree fruits is being completed as several new DNA tests are nearing application for strawberry, cherry, apple, and peach (https://www.rosbreed.org/node/482). The six new DNA tests for strawberry include remotancy, the y-decalactone gene, soluble solid content (SSC), red stele disease response (*Phytophthora fragariae* Hickman var. fragariae), angular leaf spot response (*Xanthamonas fragariae* (Kennedy & King)] and methyl anthranilate (https://www.rosbreed.org/node/501). The six new DNA tests for cherry include fruit firmness, fruit flesh and skin color (Sandefur et al., 2015; Stegmeir et al., 2015), powdery mildew resistance, bacterial canker response, and pedicel fruit retention force/abscission and soluble solids content (SSC; https://www.rosbreed.org/breeding/dna-tests/cherry). The three new DNA tests for apple are powdery mildew resistance, flesh color, and fructose content (sweetness; https://www.rosbreed.org/breeding/dna-tests/apple). The new peach DNA tests will be discussed in detail in the following sections.

Development, Validation and Deployment of DNA Tests for MAS of *Xap* Resistance, Fruit Quality, and Phenological Traits in Peach

While MAPS has been achievable in peach as well as apple, cherry, apricot, plum, strawberry, and other Rosaceae members, unlike most row crops where MASS is a common

practice, the use of markers for MASS in peach, and other Rosaceae members, is still in its infancy (Ru et al., 2015). Agronomic crop breeders have documented an increase in efficiency and saving of resources in their programs brought forth by incorporation of these new tools, however, fruit species have lagged behind due to several reasons including but not limited to substantially less funding, significantly longer juvenility periods, and a considerably higher investment cost per seedling.

In terms of peach, the majority of the 568 identified M-L-Ts (QTL or MTL) cannot currently be used in breeding programs for MASS for several reasons. First and foremost, the lack of incentive to convert these M-L-Ts into DNA tests has created a valley of misunderstanding, which has directly limited broad application of MASS in and across Rosaceae fruit breeding programs (Bliss, 2010; Iezzoni et al., 2010; Ru et al., 2015). Traditional breeders and geneticists do not speak the same language, thus to bridge this gap between them, it is imperative that breeding-relevant M-L-Ts are turned into DNA tests that will provide the traditional breeders with the information on which technique to use to screen the marker, as well as how to interpret and score the results.

Development of DNA tests for breeding relevant M-L-Ts would make MASS practical in and across Rosaceae breeding programs, and ultimately entice adoption. First, since almost all of these M-L-Ts were discovered using single bi-parental segregating populations, the M-L-Ts should be tested across a wider array of germplasm, including the breeder's own material to validate the alleles from each marker are robust across germplasm as well as relevant in actual breeding material. Secondly, the reliability of the M-L-Ts to correctly predict the phenotypic variation must be determined; otherwise they will be misleading (Bliss, 2010; Collard et al., 2005; Collard et al., 2008; Ru et al., 2015). If the marker is not found to be linked close enough

to the M-L-T, then fine mapping and or candidate gene analysis is pertinent to identify a marker linked close enough, or located within the gene(s) responsible for the phenotypic variation (Bliss, 2010; Collard et al., 2005; Collard et al., 2008).

In this dissertation, three sequential steps will be performed to develop peach DNA tests for MAS of *Xap* resistance, fruit quality and phenological traits across four peach breeding programs (UA, Clemson University, Texas A&M University, and the University of California, Davis). First, previously identified breeding relevant M-L-Ts will be further investigated and developed into informative SNP-based DNA tests. Relevant SNPs associated with the traits of interest will be grouped into haplotypes and their robustness will be validated in material across the four RosBREED peach breeding programs. Secondly, the SNP haplotypes will be converted into simple, straightforward breeder-friendly, SSR-based DNA tests and their robustness will be confirmed in and across the four breeding programs. Since SNP haplotype and SSR DNA tests each have their positives and negatives, and are both widely used, enabling the same DNA test to be screened across both platforms will give breeders more options, and thus further entice adoption of MAS. Lastly, additional M-L-Ts for *Xap* resistance, fruit quality, and phenological traits will be identified through pedigree-based QTL analysis (PBA) using the UA RosBREED peach pedigree.

Pedigree-Based QTL Analysis (PBA)

The PBA is a new approach that holds key advantages over single-population QTL analysis (Bink, 2004; Bink, 2005; Bink et al., 2008; Bink et al., 2012; van de Weg et al., 2004). The PBA strategy follows all the necessary steps of traditional bi-parental QTL analysis, except it simultaneously incorporates multiple breeding populations that segregate for the trait(s) of interest, which enhances the ability to detect all QTLs. This method is a more powerful QTL

statistical approach used to simultaneously identify marker-trait associations, validate their robustness and applicability in individual breeding programs, and discover alleles for functional diversity (Bink, 2004; Bink, 2005; Bink et al., 2008; Bink et al., 2012; van de Weg et al., 2004).

In order to perform PBA, germplasm first needs to be chosen to represent the most important parents of the breeding program (Peace et al., 2014). A protocol was developed by Peace et al. (2014) to strategically select important breeding parents (IBPs), by estimating the average allelic representation (AAR) in their relatives (unselected progenies, and all available direct and intermediate ancestors). The AAR is a measure of the representation of the alleles of IBPs provided by relatives in a germplasm set. An IBP has two alternative alleles, "A" and "B" at each locus, and the probability that an IBP's relatives carry the same allele can be calculated using the principles of "identity by descent" (IBD). Thus, the ARR measures the probability that a given allele at a random locus of an individual is IBD to an allele at that locus in another individual (Peace et al., 2014). Considering one IBP, each F_1 offspring represents the IBP and the other parent by 0.5 ARR units (Peace et al., 2014). The AAR units are further reduced in half for every subsequent generation. Peace et al. (2014) determined 12.5 ARR units as the minimum for statistical power in representing the alleles of IBPs. This is equivalent to 25 F_1 seedlings; 12.5 individuals carrying allele A and 12.5 individuals carrying allele B (the actual number of individuals carrying each allele is subject to the laws of inheritance) (Peace et al., 2014).

Once pedigrees have been selected and phenotypic and genotypic data have been collected, the PBA QTL analysis technique is used to integrate marker and phenotypic data over past, current, and future generations within and across breeding programs. The PBA approach is based on two complementary statistical approaches. The first identifies QTL regions based on Markov chain Monte Carlo (MCMC) simulations and Bayesian statistics (Bink, 2004; Bink,

2005; Bink et al., 2008). The second is based on "identity by descent" values of each allele of a genotype, taking the different alleles of founding cultivars as factors in statistical analysis (Bink, 2004; Bink, 2005; Bink et al., 2008). The PBA identifies networks of major genes and QTL associated with key breeding traits, and also elucidates their interaction and mines their functional allelic diversity (van de Weg et al., 2004). PBA QTL analysis effectively creates a flexible platform for marker identification, validation, and use in breeding material. This overall approach, based on selecting representative germplasm pedigrees (through estimation of ARR units for IBPs), followed by PBA, is an expanding platform approach to continuously identify, and validate QTL in breeding material for subsequent application of MAS (Bink, 2004; Bink, 2005; Bink et al., 2008; Bink et al., 2012; Peace et al., 2014; van de Weg et al., 2004; Yu and Buckler, 2006). This strategy is pertinent for fruit breeders since perennial woody species have very long juvenility periods and large populations are expensive to maintain in the field (Peace et al., 2014).

MASS Logistics for Rosaceae Fruit Breeding Programs

Even after a DNA test has been validated for use in MASS, this tool can't be put into use until the logistics of organizing seedlings in the greenhouse, collecting leaf tissue, and identifying an economical platform for DNA extraction, PCR, allele sizing, and processing of data for subsequent culling of seedlings in the greenhouse have been developed. Interestingly, a questionnaire in 2013 to assess the level of MASS implementation in apple, sweet cherry, tart cherry, and peach RosBREED demonstration tree fruit breeding programs revealed that the most prevalent challenge perceived by Rosaceae fruit breeders to perform MAS was in fact the difficulty in logistically enabling smooth integration of DNA testing into traditional breeding operations (Ru et al., 2015). The main reason for this perceived challenge could be due to the

fact that successful DNA testing requires expertise in molecular data interpretation and management. Unfortunately, this expertise is often lacking in breeding programs new to MASS (Ru et al., 2015).

The first step is to develop the logistics for greenhouse organization and leaf collection. Organization should not be overlooked, since sample organization throughout all subsequent steps is absolutely critical for success. Secondly, detail-oriented workers (or robots) need to be trained to collect the leaf tissue into organized 96-tube microracks (Edge-Garza et al., 2014). Next the 96-tube microracks are either outsourced for marker analysis or are processed in-house. Once the raw DNA test results are received, personnel with molecular expertise are required for allele calling and processing of data for subsequent culling of seedlings in the greenhouse (Ru et al., 2015).

While outsourcing is quick, performing MASS in-house is considerably more economical as price per sample can be reduced by >70% (Cameron Peace, personal communication). Furthermore, it is noteworthy that in general as more seedlings are screened, more DNA tests are used, and culling rates are increased, performing MASS in-house becomes even more economical than outsourcing (Cameron Peace, personal communication). However, it's important to keep in mind the initial investment required to set up the infrastructure to perform MASS, such as trained personnel with molecular expertise as well as equipment and supplies (Ru et al., 2015).

Importance of MAS for *Xap* Resistance, Fruit Quality, and Phenological Traits

The work herein is focused on the development and validation of SNP-based DNA tests for MAPS, MACS, and MASS for *Xap* resistance, fruit quality, and phenological traits in peach. The importance of these DNA tests will expand further through the conversion of the SNP-based

DNA tests into simple, straightforward, breeder-friendly, SSR-based DNA tests to enable the same DNA test to be screened on both SNP haplotype-based and SSR-based platforms. The DNA tests will then be confirmed and deployed in MASS for the UA peach and nectarine breeding program. Ultimately, through incorporation of MAS tools, traditional peach breeders can make more informed decisions, which will enable them to save time and resources, as well as increase the efficiency of combining all desired fruit traits into the next set of peach cultivars spanning the season, to meet growers', distributers', marketers', and consumers' evolving demands (Bliss, 2010; Byrne, 2005; Dirlewanger et al., 2004; Ru et al., 2015).

Literature Cited

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. of Phytopathol. 156:338-345.

Abbott, A.G., A.C. Lecouls, Y. Wang, L. Georgi, R. Scorza, and G. Reighard. 2002. Peach: The model genome for Rosaceae genomics. Acta Hort. 592:199-209.

Aranzana M.J., A. Pineda, P. Cosson, E. Dirlewanger, J. Ascasibar, G. Cipriani, C.D. Ryder, R. Testolin, A. Abbott, G.J. King, A.F. Iezzoni, and P. Arus. 2003 A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theor. Appl. Genet. 106:819-825.

Bardsley, S.J., and H.K. Ngugi. 2012. Reliability and accuracy of visual methods to quantify severity of foliar bacterial spot symptoms on peach and nectarine. Plant Pathol. 62:460-474.
Bassi, D. 2003. Growth habits in stone fruit trees. II Divulgatore, Bologna, Italy.

Bassi, D., and R. Monet. 2008. Botany and taxonomy, p. 1-30. In: D.R. Layne and D. Bassi (eds.). The peach, botany, production and uses. CAB International Press, Wallingford, Oxon, UK. 850.

Bassil, N.V., and G.M. Volk. 2010. Standardized phenotyping: advantages to horticulture introduction to the workshop. HortScience 45:1306-1306.

Beckmann J. and M. Soller. 1986. Restriction fragment length polymorphisms in plant genetic improvement. Oxford Surveys of Plant Mol. Biol. Cell Biol. 3:197-250.

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.

Bink, M. 2004. FlexQTL software. BIOMETRIS, Wageningen UR, The Netherlands <www.flexqtl.nl>.

Bink, M. 2005. FlexQTL software: efficient estimation of identity by descent probabilities and QTL mapping in pedigreed populations. In: Plant and Animal Genomes XII Conference, 15-19 January, San Diego, CA, U.S.

Bink, M., C. ter Braak, M. Boer, L. Totir, C. Winkler, and O. Smith. 2012. QTL linkage analysis of connected populations using ancestral marker and pedigree information. Theor. Appl. Genet. 124:1097–1113.

Bink, 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.

Blake, M.A. 1932. The J.H. Hale peach as a parent in peach crosses. Proc. Natl. Acad. Sci. U.S., 29:131-136.

Blair, M., A. Garris, A. Iyer, B. Chapman, S. Kresovich and S. Mc-Couch. 2003. High resolution genetic mapping and candidate gene identification at the xa5 locus for bacterial blight resistancein rice (*Oryza sativa* L.). Theor. Appl. Genet. 107: 62–73.

Blenda A.V., I. Verde, L.L Georgi, G.L. Reighard, S.D. Forrest, M. Munoz-Torres, W.V. Baird and A.G. Abbott. 2007. Construction of a genetic linkage map and identification of molecular markers in peach rootstocks for response to peach tree short life syndrome. Tree Genet. and Genomes 3:341-350.

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

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.

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. Crit. Rev. Plant Sci. 29:59-107.

Boudehri, K., A. Bendahmane, G. Cardinet, C. Troadec, A. Moing, E. Dirlewanger. 2009. Phenotypic and fine generic characterization of the D locus controlling fruit acidity in peach. Plant Biol. 9:59-72.

Bouhadida, M., M.A. Moreno, M.J. Gonzalo, J.M. Alonso, and Y. Gogorcena. 2011. Genetic variability of introduced and local Spanish peach cultivars determined by SSRs markers. Tree Genet. and Genomes 7:257–270.

Byrne, D.H. 2005. Trends in stone fruit cultivar development. HortTechnology 15:494–500.

Byrne, D.H. and M.C.B. Raseira. 2006. Inbreeding of the major commercial fresh market peach cultivars grown in southern Brazil. Acta Hort. 713:99–101.

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: Erez, A. (ed.). Temperate fruit crops in warm climates. Boston, Kluwer Academic Publishers.

Byrne, D.H., M. Bassols, D. Bassi, M. Piagnani, K. Gasic, G. Reighard, M. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: M. Badenes and D. Byrne (eds.). Fruit breeding. Springer Science, Business Media, New York.

Byrne, P.F., and M.D. McMullen. 1996. Defining genes for agricultural traits: QTL analysis and the candidate gene approach. Probe 7: 24-27.

CABI/EPPO. 2006. *Xanthomonas arboricola* pv. *pruni.* distribution maps of plant diseases. No. 340. CABI Head Office, Wallingford, UK.

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. and Appl. Genet. 87: 805-815.

Chen, W.H. 1994. Illustrated handbook of ancient relics of Chinese agriculture. Jiangxi Technology Press, Nanchang, China, p. 99-100.

Chunwongse, J., S. Doganlar, C. Crossman, J. Jiang and S.D. Tanksley. 1997. High-resolution genetic map the Lv resistance locus in tomato. Theor. Appl. Genet. 95:220–223.

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.

Clark, J. 2011. Peach breeding program of the University of Arkansas. Hort. News 91:1-5.

Clark, J. and P. Sandefur. 2013a. 'Bowden' and 'Amoore Sweet' Nectarines. HortScience 48:804-807.

Clark, J. and P. Sandefur. 2013b. 'Souvenirs' peach. HortScience 48:800-803.

Clark, J.R., A.B. Aust, and R. Jondle. 2012. Intellectual property protection and marketing of new fruit cultivars, p. 69-96. In: M. Badenes and D. Byrne (eds.). Fruit breeding. Springer Science, Business Media, New York.

Cobb, N.A. 1892. Contribution to an economic knowledge of the Australian rusts (Uredinae). Agrie. Gaz, NS Wales 3:181-212.

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.

Collard, B.C.Y., and D.J. Mackill. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B: Biol. Sci. 363: 557-572.

Connors, C.H. 1921. Inheritance of foliar glands of the peach. Proc. Amer. Soc. Hort. Sci. 18:20-26.

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

Crisosto C.H., and G. Costa. 2008. Preharvest factors affecting peach quality, Ch. 20:536-547In: Folta K.M., Gardiner S.E. (eds.) Genetics and Genomics of Rosaceae. Series Ed Jorgensen R. Springer, New York, Plant Genetics / Genomics, Vol 6.

Crisosto C.H., F.G. Mitchell, and R.S. Johnson. 1995. Factors in fresh market stone fruit quality. Postharvest News and Info. 6:17-21.

Daines, R. 1961. What we know and don't know about bacterial spot of peach. Hort. News 42:110-114.

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., 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., P. Cosson, W. Howad, G. Capdeville, N. Bosselut, M. Claverie, C. Voisin, R. Pozat, B. Lafargue, O. Baron, F. Laigret, M. Kleinhentz, P. Arús, and D. Esmenjaud. 2004a. Microsatellite genetic linkage maps of myrobalan plum and an almond-peach hybrid location of root-knot nematode resistance genes. Theor. Appl. Genet. 109:827-838.

Dirlewanger, E., E. Graziano., T. Joobeur., F. Garriga-Caldere., P. Cosson., W. Howad, and P. Arus. 2004b. Comparative mapping and marker-assisted selection in Rosaceae fruit crops. Proc. Natl. Acad. Sci. U.S. 101:9891-9896.

Dirlewanger E., P. Cosson, K. Boudrhri, C. Renaud, G. Capdeville., Y. Tauzin, F. Laihret, and A. Mong. 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. and Genomes. 3:1-13.

Dirlewanger, E., P. Cosson., K. Boudehri., C. Renaud., G. Capdeville., Y. Tauzin., F. Laigret, and A. Moing. 2007. 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. and Genomes 3:1-13.

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.

Dunegan J.C., 1932. The bacterial spot disease of the peach and other stone fruits. Tech. Bul. U.S. Dept. of Agr. No. 273:53.

Edwards A., A. Civitello, H.A. Hammond and C.T. Caskey. 1991. DNA typing and genetic-mapping with trimeric and tetrameric tandem repeats. Amer. J. of Human Genet. 49:746-756.

Etienne, C., C. Rothan., A. Moing., C. Plomion., C. Bodenes., 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.

Edge-Garza, D.A., C.P., Peace, C.P. and Y. Zhu. 2010. Enabling marker-assisted seedling selection in the Washington Apple Breeding Program. Acta Hort. 859:369-373.

Edge-Garza, D.A., J.J. Luby, and C.P. Peace. 2016. Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Acta Hort. In press.

EPPO. 2003. Data sheets on quarantine organisms. *Xanthomonas arborícola* pv. pruni. <www.eppo.org/QUARANTINE/BACTERIA/Xanthomonas_pruni/XANTPR_ds.pdf>.

EPPO/CABI 1997. *Xanthomonas arboricola* pv. *pruni*, p. 1096–1100. In: Quarantine pests for Europe, 2nd ed., CAB International, Wallingford (GB).

Food and Agricultural Organization of the United Nations (FAO). 2015a. Peach and nectarine world production of the top five producing countries, 1992-2014. August 25, 2015. <http://faostat3.fao.org/compare/E>.

Food and Agricultural Organization of the United Nations (FAO). 2015b. Peach and nectarine U.S. production, 1961-2014. August 25, 2015. <http://faostat3.fao.org/browse/Q/QC/E>.

Faust, M. and B. Timon. 1995. Origin and dissemination of the peach. Hort. Rev. 17:331–379.

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.

Foolad M.R., S. Arulsekar, V. Becerra, and F.A. Bliss. 1995. A Genetic Map of *Prunus* based on an interspecific cross between peach and almond. Theor. Appl. Genet. 91:262-269.

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

Gillen A.M. and F.A. Bliss. 2005. Identification and mapping of markers linked to the mi gene for root-knot nematode resistance in peach. J. Amer. Soc. for Hort. Sci. 130:24-33.

Hajri, A., J.F. Pothier, M. Fischer-Le Saux, S. Bonneau, S. Poussier, T. Boureau, B. Duffy, and C. Manceau. 2012. Type three effector gene distribution and sequence analysis provide new insights into the pathogenicity of plant-pathogenic *Xanthomonas arboricola*. Appl. Environ. Microbiol. 78:371-384.

Haldar, S., S. Haendiges, D.A. Edge-Garza, N.C. Oraguzie, J. Olmstead, and C.P. Peace. 2010. Applying genetic markers for self-compatibility in theWSU sweet cherry breeding program. Acta Hort. 859:375–380.

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.

Hedrick, H.P. 1917. The peaches of New York. NY Agr. Exp. Sta. NY, EUA.

Hesse, C.O. 1975. Peaches, p. 285–335. In: J. Janick and J.N. Moore (eds.), Advances in fruit breeding, Purdue University Press, W. Lafayette, IN.

Howad, W., T. Yamamoto, E. Dirlewanger, R. Testolin, P. Cosson, G. Cipriani, A.J. Monforte, L. Georgi, A.G. Abbott, and P. Arus. 2005. Mapping with a few plants: using selective mapping for microsatellite saturation of the *Prunus* reference map. Genet. 171:1305-1309.

Huang H., Z. Cheng, Z. Zhang, and Y. Wang. 2008. History of cultivation and trends in China, p. 37-57. In: D.R. Layne and D. Bassi (eds.). The peach, botany, production and uses. CAB International Press, Wallingford, Oxon, UK. 850.

Iezzoni, A. 2013. Jewels in the genome: peach flavor presented by RosBREED. <https://www.rosbreed.org/sites/default/files/RosBREED.Jewels.peach_.flavor.pdf>.

Iezzoni, A., C. Weebadde., J. Luby., Y. Chengyan., 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.

Illa E., I. Eduardo, J. Audergon, F. Barale, E. Dirlewanger, X. Li, A. Moing, P. Lambert, L. Le Dantec, Z. Gao, J.-L. Poëssel, C. Pozzi, L. Rossini, A. Vecchietti, P. Arús, and W. Howad. 2010. Saturating the *Prunus* (stone fruits) genome with candidate genes for fruit quality. Mol. Breeding 28:667-682.

Janse, J.D., M.P. Rossi, R.F.J. Gorkink, J.H. Derks, J. Swings, D. Janssens, and M. Scortichini. 2001. Bacterial leaf blight of strawberry (*Fragaria* (x) *ananassa*) caused by a pathovar of *Xanthomonas arboricola*, not similar to *Xanthomonas fragariae* Kennedy & King. Description of the causal organism as *Xanthomonas arboricola* pv. *fragariae* (pv. nov., comb. nov.). Plant Pathol. 50:653– 665.

Jansen R. 1993. Interval mapping of multiple quantitative trait loci. Genetics 135:205-211.

Jansen R. and P. Stam. 1994. High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447-1455.

Jones, A.L., and T.B. Sutton. 1996. Diseases of tree fruits in the East. Michigan State Univ. Ext., U.S.

Jones, N., H. Ougham, and H. Thomas. 1997. Markers and mapping: we are all geneticists now. New Phytol. 137:165–177.

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

Jung, S., M. Staton, T. Lee, A. Blenda, R. Svancara, A. Abbott, D. Main. 2008. GDR (Genome Database for Rosaceae): integrated webdatabase for Rosaceae genomics and genetics data. Nucl. Acids Res. 36:D1034–D1040.

Jung, S., S.P. Ficklin, T. Lee, C.-H. Cheng, A. Blenda, P. Zheng, J. Yu, A. Bombarely, I. Cho, S. Ru, K. Evans, C. Peace, A.G. Abbott, L.A. Mueller, M.A. Olmstead and D. Main. 2014. The genome database for rosaceae (GDR): year 10 update. Nucl. Acids Res. 42: D1237-D1244.

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

Knight, R.L. 1969. Abstract bibliography of fruit breeding and genetics. Easter Press, London.

Kochert G. 1994. RFLP technology, p. 8-38. In: R.L. Phillips and I.K. Vasil (eds.), DNA-based markers in plants. Kluwer Academic Publishers, Dordrecht, Netherlands.

Kumar, S., M.C. Bink, R.K. Volz, V.G. Bus, and D. Chagné. 2012. Towards genomic selection in apple (*Malus* × *domestica* Borkh.) breeding programmes: prospects, challenges and strategies. Tree Genet. and Genomes 8:1-14.

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

Layne, D.R., Z. Wang, and L. Niu. 2013. Protected cultivation of peach and nectarine in China–industry observations and assessments. J. Amer. Pomol. Soc. 67:18-28.

Lesley, J.W. 1940. A genetic study of saucer fruit shape and other characters in the peach. In Proc. Amer. Soc. Hort. Sci. 37:218-222.

Li, L., S. Lu, D. O'Halloran, D. Garvin and J. Vrebalo. 2003. High resolution genetic and physical mapping of the cauliflower high beta-carotene gene Or (Orange). Mol. Genet. Genomic 270:132–138.

Li, X. W., X.Q. Meng, H.J. Jia, M.L. Yu, R.J. Ma, L.R. Wang, K. Cao, Z.-j. Shen, L. Niu, J.-b. Tian, M.-j. Chen, M. Xie, P. Arus, Z.-s. Gao, and M.J. Aranzana. 2013. Peach genetic resources: diversity, population structure and linkage disequilibrium. BMC Genet. 14:84.

Liu B. 1998. Statistical genomics: linkage, mapping and QTL analysis. CRC Press, Boca Raton.

Llácer G. 2009. Fruit breeding in Spain. Acta Hort. 814:43–56.

Lu Z.X., B. Sosinski, G.L. Reighard, W.V. Baird and A.G. Abbott. 1998. Construction of a genetic linkage map and identification of AFLP markers for resistance to root-knot nematodes in peach rootstocks. Genome 41:199-207.

Luby J.J., and D.V. Shaw. 2001. Does marker-assisted selection make dollars and sense in a fruit breeding program? HortScience 36:872-879.

Madden, L.V., G. Hughes, and F. Van den Bosch. 2007. The study of plant disease epidemics. APS Press, St. Paul, MN.

McCouch S.R., X. Chen, O. Panaud, S. Temnykh, Y. Xu, Y. Cho, N. Huang, T. Ishii, and M. Blair. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. Plant Mol. Biol. 35:89-99.

Meader. E.M. and M.A. Blake. 1940. Some plant characteristics of second generation of *P. persica* x *P. kansuensis* crosses. Proc. Amer. Soc. Hort. Sci. 37:223–231.

Mingliang Y., M. Ruijuan, S. Zhijun, and Z. Zhen. 2007. Molecular markers linked to specific characteristics of *Prunus persica* (L.) Batsch. Acta Hort. 763:147-154.

OEPP/EPPO. 2006. *Xanthomonas arboricola* pv. *pruni*. OEPP/EPPO Bul. 36:129–133.

Okie, W.R. 1998. Handbook of peach and nectarine varieties. Performance in the Southeastern U.S. and index of names. Agriculture Handbook (Washington) 714.

Okie, W.R., and R. Scorza. 2001. Breeding peach for narrow leaf width. Acta Hort. 592:137-141.

Okie, W.R., D.W. Ramming, and R. Scorza. 1985. Peach, nectarine, and other stone fruit breeding by the USDA in the last two decades. HortScience 20:633-641.

Okie, W.R., T. Bacon, and D. Bassi. 2008. Fresh market cultivar development, p. 139–174. In: D.R. Layne and D. Bassi (eds.), The peach – botany, production and uses. CAB International.

Palleroni, N.J., D.C. Hildebrand, M.N. Schroth, and M. Hendson. 1993. Deoxyribonucleic acid relatedness of 21 strains of *Xanthomonas* species and pathovars. J. Appl. Bacteriol. 75:441–446.

Paterson, A.H. 1996. Making genetic maps, p. 23-39. In: A.H. Paterson (ed.). Genome mapping in plants. R.G. Landes Company, San Diego, CA.

Peace, C. 2011. Fast-tracked MAB pipelining in peach: the endoPG genetic test for fruit texture type. RosBREED's Community Breeders' Page: 1-3. <https://www.rosbreed.org/sites/default/files/RosBREED's.Fasttracked_endoPG.fruit_.texture.pdf >.

Peace C.P. 2013. RosBREED by the numbers. RosBREED Q Newsl 4:4.

Peace C.P., A. Callahan, E.A. Ogundiwin, D. Potter, T.M. Gradziel, F.A. Bliss, and C.H. Crisosto. 2007. Endopolygalacturonase genotypic variation in *Prunus*. Acta Hort. 738:639-646.

Peace, C.P., C.H. Crisosto., D.T. Garner., A.M. Dandekar., T. 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. and Genomes 10:1679-1694.

Peace C.P. and J.L. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae, Ch. 2:19-53. In: Folta K.M., Gardiner S.E. (eds.). Genetics and genomics of Rosaceae. Series Ed Jorgensen R. Springer, New York, Plant Genetics / Genomics, Vol 6.

Peace, C., N.V. Bassil, M.C.A.M. Bink, S.K. Brown, D.H. Byrne, J.R. Clark, T. Davis, K. Evans, G. Fazio, C. Finn, K. Gasic, T. Gradziel, J. Hancock, J. Luby, D. Main, N. Oraguzie, E. van de Weg, D. Wang, K. Xu, and A. Iezzoni. 2010. RosBREED's marker-assisted breeding pipeline. HortScience 45:S54-S54.

Penner G.A. 1996. RAPD analysis of plant genomes, p. 251-268. P.P. Jauhar (ed.), Methods of Genome Analysis in Plants, CRC Press, Boca Raton.

Pérez, A., K. Plattner and K. Baldwin. 2011. Fruit and tree nuts outlook. Economic Research Service, USDA, FTS-348 <http://www.ers.usda.gov/publications/fts/2011/07Jul/FTS348.pdf>.

Pérez, S. 1989. Characterization of Mexican peach population from tropical and subtropical regions. Acta Hort. 254:139–144.

Pérez, S., S. Montez. and C. Mejía. 1993. Analysis of peach germplasm in Mexico. J. Amer. Soc. Hort. Sci. 118:519–524.

Pflieger, S., V. Lefebvre, and M. Causse. 2001. The candidate gene approach in plant genetics: a review. Mol. Breeding 7:275-291.

Powell W., G. Machray, and J. Provan. 1996. Polymorphism revealed by simple sequence repeats. Trends Plant Sci. 1:215-222.

Pozzi, C. and A. Vecchietti. 2009. Peach structural genomics, p. 235-257. In: K.M. Folta. and S.E. Gardiner (eds.). Genetics and genomics of Rosaceae. Springer, New York.

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

Raz, A., R.A. Stern, D. Bercovich, and M. Goldway. 2009. SFB-based S-haplotyping of apricot (*Prunus armeniaca*) with DHPLC. Plant Breeding 128:707-711.

Ritchie, D.F. 1995. Bacterial spot, p. 50-52. In: J.M. Ogawa, E.I. Zehr and G.W. Bird (eds.). Compendium of stone fruit diseases. APS Press, St. Paul, MN, U.S.

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

Rivers, H.S. 1906. The cross-breeding of peaches and nectarines. In Rpt. 3d Int. Conf. Genet., London (Vol. 463467).

Rolfs F.M., 1915. A bacterial disease of stone fruits. New York Cornell Agricultural Experimental Station Memoir 8.

Rosalam, S., and R. England. 2006. Review of xanthan gum production from unmodified starches by *Xanthomonas comprestris* sp. Enzyme Microb. Technol. 39:197–207.

Rowland, T. Jr, D. Edge-Garza, K. Evans, and C.P. Peace. 2010. Routine marker-assisted seedling selection in the Washington apple breeding program provides resource savings. Abstracts of the 5th International Rosaceae Genomics Conference, Stellenbosch, November.

Rowland, T. Jr, D. Edge-Garza, N. Oraguzie, and C.P. Peace. 2012. Routine marker-assisted seedling selection in the Pacific Northwest sweet cherry breeding program provides resource savings. Poster presentation at 6th Rosaceous Genomics Conference, Mezzocorona, Italy.

Ru, S., D. Main, K. Evans, and C. Peace. 2015. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet. and Genomes 11:1-12.

Salazar, J.A., D. Ruiz, J.A. Campoy, R. Sánchez-Pérez, C.H. Crisosto, P.J. Martínez-García, A. Blenda, S. Jung, D. Main, P. Martínez-Gómez, and M. Rubio. 2013. Quantitative Trait Loci (QTL) and Mendelian Trait Loci (MTL) analysis in *Prunus*: a breeding perspective and beyond. Plant Mol. Biol. Rep. 1-18. DOI 10.1007/s11105-013-0643-7.

Salgado, 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. Ph.D dissertation University of Arkansas, Fayetteville, U.S.

Sandefur, P. 2011. Characterization and molecular analysis of University of Arkansas peach, *Prunus persica* (L.) Batsch, flesh types and development of a postharvest evaluation protocol for Arkansas peach and nectarine genotypes. MS thesis University of Arkansas, Fayetteville, U.S.

Sandefur, P., N. Oraguzie and C. Peace. 2015. PavRf-SSR, a DNA test for routine prediction in breeding of sweet cherry fruit color. Mol Breed In press.

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

Sapir, G., R.A. Stern, S. Shafir, and M. Goldway. 2008. S-RNase based S-genotyping of Japanese plum (*Prunus salicina* Lindl.) and its implication on the assortment of cultivar-couples in the orchard. Scientia Hort. 118:8-13.

Sargent D.J., A. Marchese, D.W. Simpson, W. Howad, F. Fernandez-Fernandez, A. Monfort, P. Arus, K.M. Evans and K.R. Tobutt. 2009. Development of "universal" gene-specific markers from *Malus* spp. cDNA sequences, their mapping and use in synteny studies within Rosaceae. Tree Genet. and Genomes 5:133-145.

Scorza, R. and W. Okie. 1990. Peaches, p. 175-232. In: J.N. Moore and J. R. Ballington Jr (eds.), Genetic resources of temperate fruit and nut crops. ISHS-Wageningen, The Netherlands.

Scorza, R., S.A. Mehlenbacher, and G.W. Lightner. 1985. Inbreeding and coancestry of freestone peach cultivars of the eastern United States and implications for peach germplasm improvement. J. Amer. Soc. for Hort. Sci. (U.S.).

Scott, D.H., and F.P. Cullinan. 1942. The inheritance of wavy-leaf character in the peach. J. Hered. 33:293-295.

Sherman, W.B., and P.M. Lyrene. 1981. Bacterial spot susceptibility in low-chilling peaches. Fruit Var. J. 35:74–77.

Simeone A.M. 1985. Study on peach and nectarine cultivars susceptibility to the main fungus and bacteria. Acta Hort. 173:541–551.

Smith, E. 1903. Observations on a hitherto unreported bacterial disease, the cause of which enters the plant through ordinary stomata. Sci. 17:456–457.

Socquet-Juglard D., B. Duffy, J.F. Pothier, D. Christen, C. Gessler, A. Patocchi. 2013. Identification of a major QTL for *Xanthomonas arboricola* pv. *pruni* resistance in apricot. Tree Genet. and Genomes 9:409-421.

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. Fourth Intl. Peach Symp. 1-2:61-68.

Stegmeir, T., L. Cai, F.R.A. Basundari, A.M. Sebolt, A.F. Iezzoni. 2015. A DNA test for fruit flesh color in tetraploid sour cherry (*Prunus cerasus* L.). Mol Breed. 35:149-159.

Tanksley S.D. 1993. Mapping polygenes. Annu. Rev. Genet. 27:205-233.

Tanksley, S.D., N.D. Young., A.H. Paterson, and M.W. Bonierbale. 1989. RFLP mapping in plant-breeding - new tools for an old science. Bio-Technol. 7:257-264.

Taramino G. and S. Tingey. 1996. Simple sequence repeats for germplasm analysis and mapping in maize. Genome 39:277-287.

van de Weg, W.E., R.E. Voorips., H.J. Finkers., L.P. Kodde., J. Jansen, and M. Bink. 2004. Pedigree genotyping: A new pedigree-based approach of QTL identification and allele mining. Acta Hort. 663:45-50.

Vauterin, L., B. Hoste., K. Kersters, and J. Swings. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45:472–489.

Verde, I., A.G. Abbott, S. Scalabrin, S. Jung, S. Shu, F. Marroni, T. Zhebentyayeva, M.T. Dettori, J. Grimwood, F. Cattonaro, A. Zuccolo, L. Rossini, J. Jenkins, E. Vendramin, L.A. Meisel, V. Decroocq, B. Sosinski, S. Prochnik, T. Mitros, A. Policriti, G. Cipriani, L. Dondini, S. Ficklin, D.M. Goodstein, P. Xuan, C. Del Fabbro, V. Aramini, D. Copetti, S. Gonzalez, D.S. Horner, R. Falchi, S. Lucas, E. Mica, J. Maldonado, B. Lazzari, D. Bielenberg, R. Pirona, M. Miculan, A. Barakat, R. Testolin, A. Stella, S. Tartarini, P. Tonutti, P. Arús, A. Orellana, C. Wells, D. Main, G. Vizzotto, H. Silva, F. Salamini, J. Schmutz, M. Morgante, and D.S. Rokhsar.

2013. The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genet. 45:487-494.

Verde, I., A.G. Abbott, S. Scalabrin, S. Jung, S. Shu, F. Marroni, T. Zhebentyayeva, M.T. Dettori, J. Grimwood, F. Cattonaro, A. Zuccolo, L. Rossini, J. Jenkins, E. Vendramin, L.A. Meisel, V. Decroocq, B. Sosinski, S. Prochnik, T. Mitros, A. Policriti, G. Cipriani, L. Dondini, S. Ficklin, D.M. Goodstein, P. Xuan, C. Del Fabbro, V. Aramini, D. Copetti, S. Gonzalez, D.S. Horner, R. Falchi, S. Lucas, E. Mica, J. Maldonado, B. Lazzari, D. Bielenberg, R. Pirona, M. Miculan, A. Barakat, R. Testolin, A. Stella, S. Tartarini, P. Tonutti, P. Arús, A. Orellana, C. Wells, D. Main, G. Vizzotto, H. Silva, F. Salamini, J. Schmutz, M. Morgante, and D.S. Rokhsar. 2015. The Peach v2.0 release: an improved genome sequence for bridging the gap between genomics and breeding in *Prunus*. Plant and Animal Genome XXIII Conference. Plant and Animal Genome.

Verde, I., M. Lauria, M. 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) x *P. ferganensis* BC_1 linkage map: saturation and coverage improvement. Theor. and Appl. Genet. 111:1013-1021.

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. DOI:10.1371/journal.pone.0035668.

Vignal A., D. Milan, M. SanCristobal and A. Eggen. 2002. A review on SNP and other types of molecular markers and their use in animal genetics. Genet. Selection Evolution 34:275-305.

Vos P., R. Hogers, M. Bleeker, M. Reijans, T. Vandelee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP - a new technique for DNA-fingerprinting. Nucl. Acids Res. 23:4407-4414.

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.

Williams J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic-markers. Nucl. Acids Res. 18:6531-6535.

Winter, P. and G. Kahl. 1995. Molecular marker technologies for plant improvement. World J. of Microbio. and Biotechnol. 11:438-448.

Yamamoto, T., K. Mochida and T. Hayashi. 2003. Shanhai Suimitsuto, one of the origins of Japanese peach cultivars. J. Japan. Soc. Hort. Sci. 72:116–121.

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

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.

Yang, N. 2012. Mapping quantitative trait loci associated with resistance to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in peach. Clemson University, Clemson, PhD Diss. UMI 3512202.

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

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

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

Zeng, Z.B. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc. Natl. Acad. Sci. U.S. 90:10972-10976.

Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. Genetics 136:1457-1468.

Zhang G., A. Sebolt, S. Sooriyapathirana, D. Wang, M. Bink, J. Olmstead, and A. Iezzoni. 2009. Fruit size QTL analysis of an F_1 population derived from a cross between a domesticated sweet cherry cultivar and a wild forest sweet cherry. Tree Genet. and Genomes 6:25–36.

Zheng, Y., G. Crawford, and X. Chen. 2014. Archaeological evidence for peach (*Prunus persica*) cultivation and domestication in China. PLoS ONE 9: 1-9.

Chapter Two: Phenotypic Characterization of Bacterial Spot [*Xanthomonas arboricola* pv. *pruni* (*Xap*)] Resistance, and Pedigree-Based Quantitative Trait Loci Analysis (PBA), for *Xap* Resistance in the University of Arkansas Peach and Nectarine Breeding Program

Abstract

The University of Arkansas (UA) peach breeding program has been breeding peach cultivars for resistance to *Xap* since the 1960s. Great progress has been made, however, the environmental impact on the disease can complicate selection for *Xap* resistance in the field, since *Xap* pressure is often not consistent from genotype to genotype and year to year. The objective of this study was to conduct genome-wide quantitative trait loci (QTL) analysis for *Xap* fruit (*Xap*F) and *Xap* leaf (*Xap*L) resistance using the Pedigree-Based Analysis (PBA) approach and the UA RosBREED peach breeding pedigree. The overall goal was to identify and validate QTLs and SNPs associated with *Xap*F and *Xap*L resistance in a large portion of this program's breeding material, for subsequent application of MAS. In total, 1,947 polymorphic SNP markers and three years of *Xap*F and *Xap*L field phenotypic data along with two *Xap* leaf assays using *Xap* isolates from AR (*Xap*L_{AR}), as well as one isolate from North Carolina (*Xap*L_{88}), from seven pedigree-connected F_1 families were analyzed using FlexQTL™ software. In total, PBA identified 19 reliable QTLs across seven linkage groups (LGs) (all except LG 7) using 35 *Xap* resistance data sets (21 *Xap*F, 12 *Xap*L, *Xap*L_{AR}, and *Xap*L_{88}). All 19 QTLs were compared across data sets to determine if any of the *Xap*F, *Xap*L, *Xap*L_{AR}, and *Xap*L_{88} QTLs co-localized and could be considered the same QTL. Interestingly, the 15 QTLs co-localized at six different loci (using both *Xap*F and *Xap*L data sets, as well as *Xap*L_{AR} in certain cases) (G1*Xap*F.2+L.2, G1*Xap*F.3+L_{AR}.1+L.1, G2*Xap*L.1+L_{AR}.1+F.1, G2*Xap*L.2+F.2+F.3, G5*Xap*L+F+ L_{AR}.1, and G6*Xap*F+L.1) and only four QTLs were identified to be associated with only *Xap*F data (G1*Xap*F.1, G2*Xap*F.3, G3*Xap*F.1, and G8*Xap*F.1). Out of all ten consensus QTLs, seven on

average were associated with ≥ 10% of the phenotypic variation for *Xap* resistance and were determined to be major QTLs (G1*Xap*F.1, G1*Xap*F.2+L.2, G1*Xap*F.3+L_{AR}.1+L.1, G2*Xap*L.1+L_{AR}.1+F.1, G2*Xap*L.2+F.2+F.3, G2*Xap*F.3, and G6*Xap*F+L.1). The other three QTLs explained < 10% of the phenotypic variation for *Xap* resistance, thus were considered minor QTLs (G3*Xap*F.1, G5*Xap*L+F+ L_{AR}.1, and G8*Xap*F.1). The genomic location of these seven major and three minor QTLs in the peach genome sequence v1.0 were compared to the locations noted in previous peach QTL studies in *Prunus*, and eight co-located within previously identified QTLs. Additionally, genomic estimated breeding values (GEBVs) for all traits were obtained through PostFlexQTL™ software analyses. The GEBV correlations for all 35 data sets ranged from 0.71-0.89 (R^2), and interestingly, the AR *Xap* isolate leaf assay (*Xap*L_{AR}) data set showed the highest positive correlation of all data sets (0.89). This PBA QTL analysis approach, followed by SNP data set and haplotype construction, effectively creates a flexible and continuously expanding platform for QTL and marker identification, validation, and use of MAS in the UA peach and nectarine breeding program. Additionally, the GEBVs generated through PostFlexQTL™ software analyses, can be used as a quantitative scale for decision support for choosing selections/cultivars to use as parents in crosses to combine horizontal *Xap* resistance with high fruit quality and advance in the UA breeding program.

Introduction

Bacterial spot [caused by *Xanthomonas arboricola* pv. *pruni* (*Xap* refers to the pathogen and disease)] is a serious disease of *Prunus* spp. and their hybrids worldwide (EPPO/CABI, 1997; OEPP/EPPO, 2006; Ritchie, 1995). This disease has been reported on peach and nectarine [*Prunus persica* (L.) Batch.], almond [*Prunus dulcis* (Mill.) D.A.Webb], sweet cherry (*Prunus avium* L.), plum (*Prunus salicina* Lindl.), and apricot [*Prunus armeniaca* (L.)]. The most severe

infections have been seen on Japanese plum (*Prunus domestica* L.), Korean cherry (*P. japonica* Thunb.), plum hybrids, and on peach and nectarine and their hybrids (EPPO/CABI, 1997; OEPP/EPPO, 2006; Ritchie, 1995). The pathogen has rapidly spread across the world and today it is present in almost all continents where stone fruits are grown in North America, South America, Europe, Africa (South Africa), Asia, and Australia (OEPP/EPPO, 2006). The European Union currently subjects *Xap* to phytosanitary legislation through the EEC Directive no.92/103 (S.I. Nº 219, 2003) due to establishment and recent appearance in several countries of that region (EPPO, 2003).

X. arboricola pv. *pruni* (*Xap*) is a mobile (one flagellum), gram-negative, rod bacterium. This species was first named *X. pruni* (Smith, 1903) and then classified as *X. campestris* pv. *pruni* by Dye in 1978. Later, the bacterium was reclassified as *X. arboricola* pv. *pruni* [Smith, 1903 (Vauterin et al., 1995)].

Infection and development of *Xap* is highly dependent on environmental conditions. The disease is particularly devastating in warm, humid environments such as the eastern U.S. and other countries with similar climatic conditions. It rarely, if ever, is seen in dry, arid climates such as the Central Valley of California. Locations with sandy soils are much more prone to *Xap* infection since wind-blown sand can create wounds for the bacterium to infect (EPPO/CABI, 1997; Ritchie, 1995). Periods with warm temperatures (20-35 °C), accompanied with light, frequent rains, extended heavy dews, very high humidity, along with the occurrence of substantial wind-blown rains during late bloom to pit hardening are most favorable for severe infection (Daines, 1961; EPPO/CABI, 1997; Randhawa and Civerolo, 1985; Ritchie, 1995; Zehr and Shepard, 1996). Conditions of at least 12 h of water saturation and high humidity (~75%) are needed for infection to occur, develop, and spread (Ritchie, 1995; Zehr and Shepard, 1996).

Wind and water serve as vectors to move the bacterium around the orchard. Bacterial spot symptoms are generally characterized as various-sized necrotic lesions on leaves and fruits and cankers on twigs (EPPO/CABI, 1997; OEPP/EPPO, 2006; Ritchie, 1995) (Fig. 1. A-D).

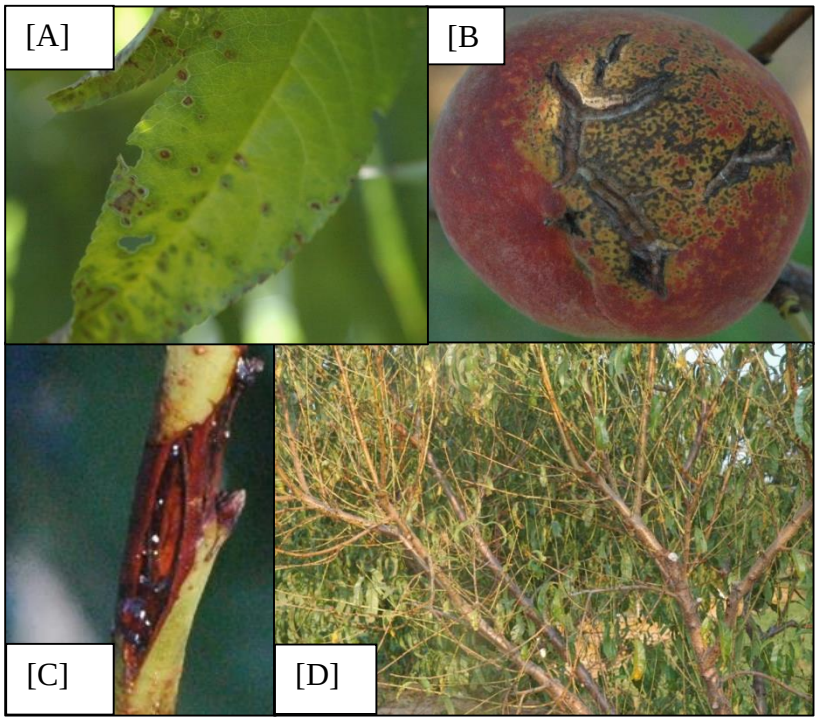

Fig. 1. Bacterial spot on [A] peach leaf, [B] peach fruit, [C] peach twigs, and [D] entire peach tree (pictures by Terrence Frett in 2013-2015, taken at the Fruit Research Station, Clarksville).

On peach leaves, symptoms are generally concentrated on the leaf tip, and along the midrib and leaf margin, where rain or dew, which spread the bacterium, commonly accumulate (OEPP/EPPO, 2006; Ritchie, 1995) (Fig. 1A). The symptoms are initially observed on the lower surface of peach leaves as small (~1-2 mm in size) light-green to yellow, angular-shaped, water-soaked lesions with a brownish-yellow center. As the disease progresses, spots can be seen on the upper surface of the leaf. They begin to enlarge to 2-3 mm (rarely exceeding 5 mm) and exhibit an angular shape since the pathogen is restricted by veins (OEPP/EPPO, 2006; Ritchie,

1995). The angular spots have a water-soaked appearance and eventually can darken to a dark-purple, brown, or black color. The surrounding area commonly becomes yellow. These diseased areas have been observed to detach after or prior to color change, leaving the leaf with a dark ring and a shot-hole appearance (Fig. 1A). Severely infected leaves turn yellow and frequently drop prematurely (Fig. 1D). The leaves are most susceptible to infection before they fully expand. In general, leaf symptoms are observed 5-14 d after infection (OEPP/EPPO, 2006; Ritchie, 1995).

On fruit, *Xap* infections can appear as early as 3 weeks after petal fall. Initial symptoms are small, circular-brown spots on the surface of the young fruitlet. As the lesions develop, they appear water-soaked with a small sunken necrotic center. Later, these lesions enlarge further, usually extend deep into the flesh, and develop a brown to black color (Fig. 1B). Infections that occur near or after pit-hardening characteristically are limited to the surface of the fruit skin. They may coalesce and cause the skin to crack, but do not extend into the flesh (OEPP/EPPO, 2006; Ritchie, 1995).

The symptoms of *Xap* on twigs are characterized as cankers (OEPP/EPPO, 2006; Ritchie, 1995) (Fig. 10C). When the season culminates, the bacterium overwinters in the intercellular spaces of the cortex, phloem, and xylem of these cankers, and/or leaf scars on twigs (EPPO/CABI, 1997; Ritchie, 1995).

During the following spring as temperatures warm, the bacterium begins to multiply in these intercellular locations as leaf and flower buds swell and new tissue emerges. The primary inoculum originates from leaf scars and spring cankers and is then disseminated to the leaves by dripping dew and wind-blown rain. The bacterium is able to enter new leaf growth through stomata or wounds and spread through the vascular tissue (xylem and phloem) of the host

(Aarrouf et al., 2008). Lesions developing on leaves exude the multiplied bacterium to allow secondary infections. Fruit is infected primarily between petal fall and near or after pit-hardening. Throughout the season, the bacterium is able to continue to spread to other leaves and/or stems when environmental conditions are optimal (EPPO/CABI, 1997; Ritchie, 1995). In general, the disease leads to premature defoliation (Fig. 10D), weak vigor of the plant across years, unmarketable fruit with low fruit quality, and overall decline in production (Aarrouf et al., 2008; Ritchie, 1995).

The first step in control of *Xap* is to grow and maintain healthy trees (Ritchie, 1995; Ritchie, 1999). Trees under stress are more severely affected by *Xap* than those not experiencing stress. This includes nutrient stress, and stress related to other pathogenic organisms such as nematodes. Therefore, optimum soil fertility must always be maintained (Ritchie, 1995; Ritchie, 1999). In the peach industry, the primary commercial control of *Xap* has been to rely heavily on pesticides, such as copper-containing compounds, and oxytetracycline (Ritchie, 1995; Ritchie, 1999). Correct timing of sprays and rates used are important. Relatively high rates of copper sprays are generally applied when only a limited amount of new growth is present early in the growing season, from dormancy through early shuck split. The idea of these applications is to cover the tree surface to create a barrier through which the bacterium must pass as it moves from overwintering sites. The majority of the inoculum is thought to die in this process of moving through the copper barrier. Copper sprays are continued as new growth emerges but rates are reduced because these compounds are known to cause fruit and foliage damage in peach, resulting in grayish discoloration, leaf shot holes, and premature leaf drop. The compound oxytetracycline is then incorporated when shuck split commences since it is less damaging to the fruit and foliage (Ritchie, 1995; Ritchie, 1999).

Unfortunately, if favorable environmental conditions occur, the disease can develop even when well-timed sprays have been applied (Ritchie, 1995; Ritchie, 1999). Generally, once the bacterium is established in the orchard, it is very difficult to control the disease, especially when highly and/or moderately susceptible cultivars are used. Furthermore, due to concerns of agricultural worker safety, environmental contamination, economic concern, and food safety, restrictions on the use and availability of chemicals in agriculture have developed in recent years. Integrated pest management programs (IPM) are being incorporated to only use chemical sprays as the last resort and *Xap*-resistant cultivars play a crucial role in these IPM practices (Byrne et al., 2012; Sansavini et al., 2006). Unfortunately, many good-quality cultivars favored in the peach industry are highly susceptible and cultivars with *Xap* resistance in general still lack desirable fruit quality characteristics required by the peach industry.

Bacterial spot resistance has been a key trait of interest throughout the existence of peach breeding at the University of Arkansas (UA). This program has focused on breeding for resistance to *Xap* since 1964. Drs. Jim Moore and Roy Rom initiated the UA peach and nectarine breeding program and their successor Dr. John R. Clark currently directs it. Unlike most other peach breeding programs, antibiotic sprays to control the disease have never been applied, thus, selection against *Xap* has been possible (J.R. Clark, personal communication). The warm and humid spring and summers at the University's Fruit Research Station (FRS) near Clarksville, AR, create an ideal environment for the inoculation and spread of this pathogen. This environment has enabled breeding and selection of peaches and nectarines with low *Xap* incidence.

For a study on disease screening to be successful, the correct method of scoring the phenotypic data must be determined. The main method to estimate disease incidence or severity

has been through visual field estimation (Cobb, 1892). The Cobb '1-5' scale (Cobb, 1892) was the first interval or categorical scale developed and was used to assess severity of rust on wheat (*Triticum aestivum* L.) (1 = 1% disease; 2 = 5% disease; 3 = 10% disease; 4 = 20% disease; 5 = 50% disease). Recent studies on *Xap* of peach used a combination of the ordinal and interval or categorical scale (Hammerschlag et al., 1994; Yang, 2012; Yang et al., 2013). A '0-5' scale was used by Yang et al. (2013) to rate field-inoculated trees: 0 = resistant (no symptoms); 1 = slightly resistant (1-5% disease damage; water soaked lesions), 2 = moderately resistant (6-10% disease damage; tattered patterns on the leaf tip and leaf rib), 3 = moderately susceptible (11-25% disease damage; coalesced water-lesion and shot holes); 4 = susceptible (25-50% disease damage; yellow leaf); 5 = highly susceptible (> 50% disease damage; premature leaf drop).

Yet, screening for resistance to *Xap* in the field is not an easy task. In a host-plant population, the disease incidence or severity of each individual will be affected by the health of each individual and several environmental/pathogen factors (location, temperature, humidity, and distribution of pathogen inoculum). One way to control this is to inoculate plants in the field to ensure adequate distribution of the pathogen to each individual (Yang et al., 2013). Even further, controlled methods to enhance the screening of *Xap* resistance and collect more informative and reliable phenotypic data have also been proposed such as a detached-leaf bioassay.

The detached-leaf bioassay offers a cheap and quick alternative to perform in vitro screening using peach leaves (Randhawa and Civerolo, 1985). A common detached-leaf bioassay has been performed in several previous studies (Civerolo and Keil, 1976; Hammerschlag, 1988; Hammerschlag et al., 1994; Randhawa and Civerolo, 1985). In short, leaves harvested from trees in the field or in the greenhouse are infiltrated with the inoculum using a needle-less syringe and then are incubated on water agar for 2 weeks to allow symptoms to develop. Symptoms are later

classified using a qualitative '0-3' rating scale to differentiate symptoms of infections at each inoculation site: 0 = no symptoms; 1 = distinct chlorotic spot and/or slight necrotic flecks; 2 = distinct but pale necrotic spot or grayish-white lesion, 2 mm in diameter; and 3 = distinct, dark necrotic spot of > 2 mm in diameter, with or without a chlorotic halo (Civerolo and Keil, 1976; Hammerschlag, 1988; Hammerschlag et al., 1994; Randhawa and Civerolo, 1985). This detached-leaf bioassay is an efficient way to screen for *Xap* resistance across the breadth of germplasm in a breeding program (Bock et al., 2010; Hammerschlag et al., 1994; Yang, 2012; Yang et al., 2013).

The genetic control of *Xap* resistance is quantitative in nature, however, dominant genes were suggested to be involved in peach (Sherman and Lyrene, 1981; Werner et al., 1986). Quantitative resistance is more stable than monogenic resistance since the pathogen must overcome the polygenic defense. Interestingly, the resistance of leaf and fruit in peach has been suggested to be controlled by different genetic locations due to inconsistent levels of leaf and fruit resistance (Keil and Fogle, 1974; Simeone, 1985; Werner et al., 1986; Yang et al., 2013).

Recently, studies were performed to determine the genetic control of *Xap* resistance in *Prunus* (Socquet-Juglard et al., 2013; Yang et al., 2010, 2011, 2012, and 2013). In these studies, two bi-parental populations were designed specifically to study the genetic control of *Xap* resistance in *Prunus*: an apricot population consisting of 101 F_1 individuals from a cross between 'Harostar' (resistant) (Ha) × 'Rogue de Mauves' (unknown) (RM); and an F_2 peach population consisting of 188 F_2 seedlings by crossing 'O'Henry' (susceptible) × 'Clayton' (resistant) (OC) (Socquet-Juglard et al., 2013; Yang et al., 2010, 2011, 2012, and 2013).

Using the HaRM F_1 population, Socquet-Juglard et al. (2013) constructed two low-density apricot parental linkage maps, 'Harostar' (81 AFLP fragments and 63 SSR markers) and

'Rouge de Mauves' (55 AFLP fragments and 53 SSR markers), both covering all eight apricot chromosomes. They located a major QTL for *Xap* leaf resistance [53% of the phenotypic variation explained (Vp %)] on linkage group 5 of 'Rouge de Mauves' apricot map, by inoculating actively growing peach shoots in a greenhouse setting. Microsatellite marker UDAp-452 was located at the peak, and BPCT037 and BPPCT038A flanked the QTL. Both BPPCT037 and BPPCT038A have high polymorphism in cherry and peach, and thus could be candidate markers for *Xap* resistance in peach (Dirlewanger et al., 2002). Socquet-Juglard et al. (2013) identified six candidate genes (CG) for resistance encoding for receptor-like protein kinases, leucin-rich repeat (LRR) proteins, or disease resistance proteins between BPPCT037 and BPPCT038A on chromosome 5 of the peach genome v1.0.

Later, using the OC F_2 population, Yang et al. (2013) developed a very dense OC linkage map using 63 of the 188 F_2 seedlings, consisting of 256 SNPs and two SSR markers, which covered all eight peach chromosomes. They identified 14 QTL with additive effects on *Xap* resistance using the OC population. The small population used in this study could have resulted in overestimation of QTL effect and detection of false positives. These QTL are spread throughout the OC LGs, some associated with leaf resistance, others with fruit resistance, and some with resistance to both leaf and fruit. The phenotypic variation explained (Vp %) by all QTL ranged from 15 to 56% in leaf data sets, and 33 to 60% in fruit data sets. Within these QTLs, they identified six different types of CG associated with *Xap* resistance in their study: NBS-LRR proteins; protein kinases; mildew resistance locus (MLO) gene family; glucanases, chitinases, and phytoalexins. Five of these QTL were denoted major QTL based on size and stability of additive effect and prior knowledge: *Xap.Pp.OC-4.1* and *Xap.Pp.OC-4.2* (LG4, leaf

resistance), *Xap.Pp.OC-5.1* (LG4, leaf and fruit resistance), *Xap.Pp.OC-1.2* (LG 1, fruit resistance), and *Xap.Pp.OC-6.1* (LG 2, fruit resistance).

These identified QTLs for *Xap* resistance are promising, however, these two studies (Socquet-Juglard et al., 2013; Yang et al., 2013) incorporated a bi-parental QTL analysis approach, and thus might not have detected all QTL loci responsible for *Xap* resistance. A new QTL analysis approach, pedigree-based QTL analysis (PBA), should be incorporated which uses a larger genetic background to enhance the ability to detect more QTL loci for *Xap* resistance. The small populations used in these two previous studies [HaRM F_1, 101 (Socquet-Juglard et al., 2013) and OC F_2, 63 (Yang et al., 2013)] could have resulted in overestimation of QTL effects and detection of false positives.

The PBA is a new approach that holds key advantages over single-population QTL analysis (Bink, 2004 and 2005; Bink et al., 2008 and 2012; van de Weg et al., 2004). The PBA strategy follows all the necessary steps of traditional bi-parental QTL analysis, except it simultaneously incorporates multiple breeding populations that segregate for the trait(s) of interest, which enhances the ability to detect all QTLs. This method is a more powerful QTL statistical approach used to simultaneously identify marker-trait associations, validate their robustness and applicability in individual breeding programs, and discover alleles for functional diversity (Bink, 2004 and 2005; Bink et al., 2008 and 2012; van de Weg et al., 2004). This strategy is pertinent for fruit breeders since perennial woody species have very long juvenility periods and large populations are expensive to maintain in the field (Peace et al., 2014).

In order to perform PBA, germplasm first needs to be chosen to represent the most important parents of the breeding program (Peace et al., 2014). A protocol was developed by Peace et al. (2014) to strategically select important breeding parents (IBPs), by estimating the

average allelic representation (AAR) in their relatives (unselected progenies and all available and intermediate ancestors). The AAR is a measure of the representation of the alleles of IBPs provided by relatives in a germplasm set. An IBP has two alternative alleles, "A" and "B" at each locus, and the probability that an IBP's relatives carry the same allele can be calculated using the principles of "identity by descent" (IBD). Thus, the AAR measures the probability that a given allele at a random locus of an individual is identical by descent (IBD) to an allele at that locus in another individual (Peace et al., 2014). Considering one IBP, each F_1 offspring represents the IBP and the other parent by 0.5 AAR units (Peace et al., 2014). The AAR units are further reduced in half for every subsequent generation. Peace et al. (2014) determined 12.5 AAR units as the minimum for statistical power in representing the alleles of IBPs. This is equivalent to 25 F_1 seedlings; 12.5 individuals carrying allele A and 12.5 individuals carrying allele B (the actual number of individuals carrying each allele is subject to the laws of inheritance) (Peace et al., 2014).

Once pedigrees have been selected and phenotypic and genotypic data have been collected, the PBA QTL analysis technique is used to integrate marker and phenotypic data over past, current, and future generations within and across breeding programs. The PBA approach is based on two complementary statistical approaches. The first identifies QTL regions based on Markov chain Monte Carlo (MCMC) simulations and Bayesian statistics (Bink, 2004 and 2005; Bink et al., 2008). The second is based on IBD values of each allele of a genotype, taking the different alleles of founding cultivars as factors in statistical analysis (Bink, 2004 and 2005; Bink et al., 2008). The PBA identifies networks of major genes and QTL associated with key breeding traits, and also elucidates their interaction and mines their functional allelic diversity (van de Weg et al., 2004). PBA QTL analysis effectively creates a flexible platform for marker

identification, validation, and use in breeding material. This overall approach, based on selecting representative germplasm pedigrees (through estimation of AAR units for IBPs), followed by PBA, is an expanding platform approach to continuously identify and validate QTLs in breeding material for subsequent application of marker-assisted selection (MAS) (Bink, 2004 and 2005; Bink et al., 2008 and 2012; Peace et al., 2014; van de Weg et al., 2004; Yu and Buckler, 2006).

The objective of this study was to use PBA to develop and validate QTL and SNPs for *Xap* fruit and leaf resistance in the UA RosBREED peach breeding pedigree for subsequent application of MAS. To do this, the majority of the founders, cultivars, selections, parents of the seven F_1 populations, and all seedlings in the UA RosBREED peach breeding pedigree were genotyped using the International Peach SNP Consortium (IPSC) 9 K peach SNP array v1.0 (Verde et al., 2012). Next, high-quality controlled phenotypic data was collected for three consecutive years on the majority of these individuals in the UA pedigree. The phenotypic and genotypic data were then used to perform PBA across the UA RosBREED peach breeding pedigree to identify and validate QTLs and SNPs associated with *Xap* fruit and leaf resistance directly in a large portion of this program's breeding material.

The SNPs which accurately depicted *Xap* resistance can then be used for marker assisted parent selection (MAPS) and marker assisted seedling selection (MASS). Incorporation of MAPS will enable quick genotypic screening of peach germplasm, and lead to more informed decisions on efficient cross combinations to introgress horizontal *Xap* resistance with high fruit quality throughout the entire season. The parents to use in a cross are identified through discovery of favorable alleles with efficient combining abilities. After the cross is made, MASS can be used to screen the seedlings, and decide on which seedlings to be grown in the field and which to discard (Bliss, 2010; Collard et al., 2005). Overall, incorporation of MAS for *Xap*

resistance and other fruit quality and phenological traits in the UA program can provide the UA peach breeders with more informed decision support to increase genetic gain per breeding cycle, improve selection efficiency, and significantly reduce breeding program operational costs (Bliss, 2010; Byrne, 2005; Edge-Garza et al., 2016; Ru et al., 2015). This study serves as a stepping stone for the ultimate goal realized in Chapter Six of this dissertation, to incorporate MAPS and MASS to enable the UA breeders continue to combine horizontal *Xap* resistance with high fruit quality throughout the peach season.

Materials and Methods

Management Practices at FRS

Phenotypic evaluation for *Xap* resistance was conducted on peach and nectarine material at FRS (west-central AR, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept. of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]). All trees were either open-center trained and spaced 5.5 m between trees and rows, or trained to a perpendicular-V system with trees spaced 1.9 m in rows spaced 5.5 m apart. All trees were dormant pruned and fertilized annually with a single application of 640 Kg ha^{-1} of complete fertilizer (19:19:19 of N:P:K) and were sprinkler or drip irrigated as needed. Pests were managed using a program typical for commercial orchards in the area (Smith, 2015; Studebaker et al., 2015). After shuck split but before pit hardening, fruitlets were thinned to a distance of 12 to 15 cm between each fruitlet. Temperature, humidity, and rainfall weather data from FRS were collected in 2013, 2014, and 2015. Figures were created to compare data across years.

Pedigree Construction

The germplasm used for pedigree-based QTL analysis (PBA) was selected to effectively represent a large portion of breeding-relevant alleles currently found within the UA breeding

program (Peace et al., 2014). Founders, important breeding parents, cultivars, selections, and seven F_1 populations populations ranging from nine to 48 seedlings (134 total) were identified and integrated in a comprehensive pedigree of 190 individuals (Table 1). Parentage records were confirmed or refuted first using four SSR markers, EndoPG1, EndoPG6, CPPCT040b, and BPPCT015, and then using SNPs from the International Peach SNP Consortium (IPSC) 9K peach SNP array v1.0 (C. Peace, personal communication; Verde et al., 2012). Pedimap software (Voorrips, 2007; Voorrips et al., 2012) was used to visualize the constructed pedigree (Fig. 1).

Table 1. Parental information and the number of F_1 seedlings for each of the seven RosBREED populations (N is number of individuals analyzed).

F_1 population	Female parent	Male parent	F_1 seedlings (N)
AR_Pop_1	White County (WC)	A-672	48
AR_Pop_0801	A-776	A-783	16
AR_Pop_0803	Amoore Sweet (AS)	A-778	9
AR_Pop_0813	A-772	A-672	12
AR_Pop_0817	A-789	A-699	9
AR_Pop_0819	A-708	A-773	23
AR_Pop_0825	Souvenirs (S)	A-760	17

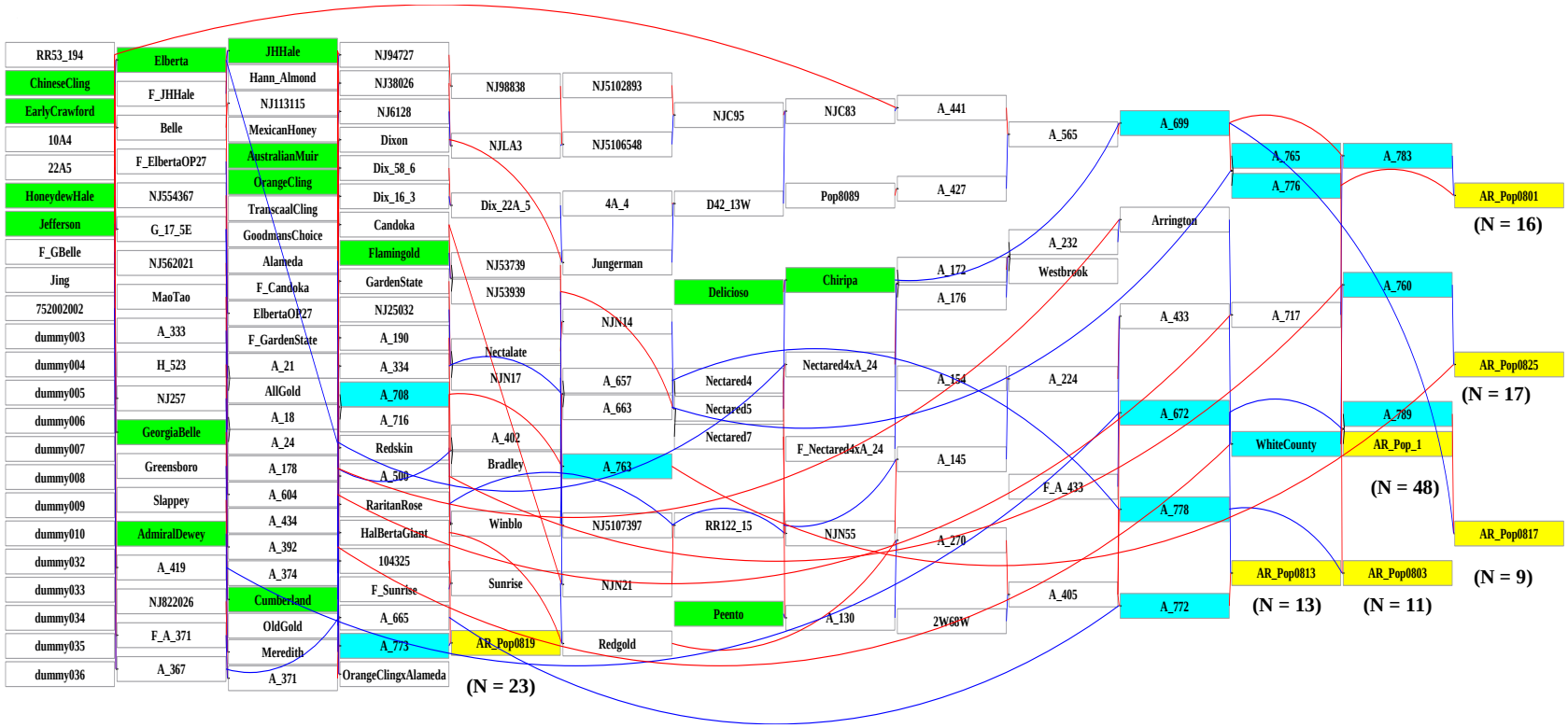

Fig. 2. Pedigree of the seven 2008 F_1 seedling populations used for FlexQTL analysis of *Xap* resistance; visualized through Pedimap software (Voorrips, 2007; Voorrips et al., 2012) (F_1 populations highlighted in yellow; Red line = *female parent*; Blue line = male parent; N = the number of progeny in each population).

Field Phenotypic Evaluation 2013-2015

All trees used in this study experienced routine exposure to *XAP* at FRS for infection, and were not field-inoculated. This approach was used because *XAP* is seen each year at FRS, although years vary to some degree in severity (J.R. Clark, personal communication). This dependability, in addition to multiple years of data collection, provided for adequate confidence in infection uniformity and phenotypic expression of degree of resistance or susceptibility.

In 2013-2015, phenotypic data was collected in the field across all material in the AR RosBREED pedigree (Table 1; Fig. 3). The combined ordinal and interval or categorical scale developed in the study by Yang (2012) was used to assess both leaf and fruit symptoms (Table 2; Fig. 3). Leaf symptoms were evaluated during a one-week period in May. Upon harvest of each selection, fruit symptoms were assessed and recorded. Additionally, a plus (+) or a minus (-) [± 0.5] was added to the rating when difficult to determine a whole-number rating. Upon collection of all data, these ± 0.5 were used to effectively create a second class of 12-point data sets for each year. Additionally, in 2014 and 2015, relative fruit symptoms, taking into account all fruit on the tree, were also scored following the leaf scale, termed fruit inoculation (designated, "inc") (Table 2; Fig. 3). This generated a third data set for 2014, 2015, and 2014-2015 avg. Lastly, since disease pressure was very high in 2014 and 2015, a fourth data set for each year was created, by subtracting one from all values, excluding those individuals which showed severe susceptibility. The generation of the multiple data sets for the same year, were used to comare to eachother in order to accurately identify QTLs. In total, 21 data sets were generated for *Xap* fruit resistance (*Xap*F) (2013, 2014, 2015, and 2013-2015 avg [(0-5); (0-5) ± 0.5; (0-5) – 1] and 2014, 2015, and 2014-2015 [(0-5) inc; (0-5) ± 0.5 inc; and (0-5) – 1 inc]), and 12 data sets for *Xap* leaf resistance (*Xap*L) (2013, 2014, 2015, and 2013-2015 avg [(0-5); (0-5) ± 0.5; (0-5) – 1]).

Table 2. Phenotypic scale used to assess bacterial spot (*Xap*) infection on peach leaves and fruits.

Class	Symptoms	
	Leaf	Fruit
0	No leaves with symptoms	No fruits with symptoms
1	1-5% diseased leaves or observed defoliation	1-5% fruit surface with spot lesions
2	6-10% diseased leaves or observed defoliation	6-10% fruit surface with spot lesions
3	11-25% diseased leaves or observed defoliation	11-25% fruit surface with spot lesions
4	25-50% diseased leaves or observed defoliation	25-50% fruit surface with spot lesions
5	> 50% diseased leaves or observed defoliation	> 50% fruit surface with spot lesions

*Note: For the purpose of genetic study of *Xap* resistance in peach, the phenotypic scoring for leaf was based on all leaves for symptoms on each individual tree. The phenotypic scoring for fruit was based on the most severe individual fruit symptom on each individual tree (Yang et al., 2012).

Fig. 3. Bacterial spot (*Xap*) symptom severity on peach leaf and fruit. A. Leaf symptoms are shown in six different severity categories based on 0-5 scale, with 0 – no symptoms, 1 – water soaked lesions, 2 – tattered patterns on the leaf tip and leaf rib, 3 – coalesced water-lesion and shot holes, 4 – yellow leaf, and 5 – premature leaf drop; B. 0-5 scale applied for fruit evaluation (as in Yang et al., 2013).

Detached-Leaf Bioassay

In May 2014, an emphasis was placed on collecting more quality-controlled phenotypic data, due to potential environmental differences in the field. A modified detached-leaf bioassay, as described by Randhawa and Civerolo (1985), was performed using two different inoculum mixtures: a group of six different *Xap* isolates from Arkansas (Xap_{AR}); and a highly virulent *Xap* isolate, *Xap*-88 (Xap_{88}) (originally from North Carolina State University, was used as a check to generate inoculum from outside Arkansas). Genotypes were condensed to only the seven 2008 F_1 seedling populations, and their parents and grandparents, which had been genotyped using the IPSC 9K peach SNP array v1.0 (Verde et al., 2012) or the mini-SNP array v1.0 (Table 1; Fig. 2). The assay was performed three times over three consecutive weeks during May, 2014, using each inoculum mixture. In each replication, four leaves were inoculated per *Xap* isolate group (Xap_{AR} and Xap_{88}).

In each replication, eight expanded young leaves (the third leaf from the tip of a shoot) were harvested from each genotype and brought into the lab (Randhawa and Civerolo, 1985). All leaves were first washed with water for 5 min to remove dirt. Next, leaves were surface-sterilized by soaking for 90 s in 70% ethanol, and then rinsed with sterile water for 90 s. Leaves were placed abaxial side up on four layers of sterile filter paper. A sterile 3 ml syringe (no needle) filled with inoculum (Xap_{AR} or Xap_{88}) was placed firmly against the leaf surface. Leaves were infiltrated by applying gentle pressure until a 2-4 mm diameter water-soaked spot appeared on the leaf. Excess inoculum spilled around the syringe tip at the infiltration site, so the leaves were lightly blotted to remove excess inoculum. Eight spots were applied, five per leaf half, ~1 cm apart. The infiltrated leaves were then placed inoculated side up in petri dishes on agar medium composed of 1.5% water amended with propiconazole to a concentration of 2.0 ppm. The leaves

were incubated at 25 °C under a 16 h photoperiod (fluorescent lights) for 2 weeks (Randhawa and Civerolo, 1985).

After two weeks of incubation, *Xap*$_{AR}$ and *Xap*$_{88}$ symptoms were assessed at each inoculation point, using a visual rating scale (0-3) as in Hammerschlag et al., (1994) and Hammerschlag (1988 and 1990) to differentiate symptoms of infections: 0 = no symptoms, 1 = distinct chlorotic spot and/or slight necrotic flecks, 2 = distinct but pale necrotic spot or grayish-white lesion, 2 mm in diameter, and 3 = distinct, dark necrotic spot of > 2 mm in diameter, with or without a chlorotic halo. Values for all eight inoculation points were averaged per leaf, and subsequently all four leaves were averaged per replication. Lastly, all three replications were averaged to generate two final data sets, *Xap* leaf assay AR (*Xap*$_{AR}$) and *Xap* leaf assay 88 (*Xap*L$_{88}$).

Descriptive Statistics

All data was organized into 35 datasets for QTL analysis: 21 data sets for *Xap* fruit resistance (*Xap*F); 12 data sets for *Xap* leaf resistance (*Xap*L); and two detached-leaf bioassay data sets (*Xap*L$_{AR}$ and *Xap*L$_{88}$). Descriptive statistics including the mean, minimum, maximum, and standard deviation for all 35 data sets were calculated for the entire AR RosBREED pedigree using a modified R script (R Core Team, 2013). Histograms for the entire UA RosBREED pedigree and each F_1 population were generated using a modified R script (R Core Team, 2013). Parental values for each F_1 population were noted in each histogram.

SNP Array Genotyping

All individuals in the UA RosBREED pedigree were previously genotyped using the International Peach SNP Consortium (IPSC) 9K peach SNP array v1.0 (Verde et al., 2012). Isolation of genomic DNA and subsequent Infinium assay were performed as explained in Verde

et al. (2012). SNP genotypes were scored with the Genotyping Module of GenomeStudio Data Analysis software (Illumina Inc., San Diego, CA). A GenTrain score of >0.4 and a GenCall 10% of >0.2 were applied to remove most SNPs that did not cluster (homozygous) or had ambiguous clustering (a high proportion of inheritance errors and/or heterozygosity excess). The average density of markers across the whole peach genome was estimated to be one SNP per 2.5 cM. The genetic locations for each SNP were positioned according to their relative physical locations (Verde et al., 2013). Map figures were generated using MapChart 2.2 software (Wageningen UR, Wageningen, Netherlands) (Voorrips 2002).

PBA QTL Analysis

All phenotypic and genotypic data sets were used for PBA QTL analysis performed through FlexQTL™ (Bink 2004 and 2005; Bink et al., 2008 and 2012; software version 099128; www.flexQTL.nl). The FlexQTL™ software estimated the number and position of QTLs given a pedigree and marker linkage map. FlexQTL™ utilizes a Bayesian approach to infer the number of QTLs by comparison of models using posterior estimates through Markov Chain Monte Carlo (MCMC) simulations. In all analyses, a 500,000 simulation chain length was enough to store 1000 samples (QTL models) with a thinning of 500 (Bink et al., 2013; Rosyara et al., 2013; Verma et al., 2016). The minimum requirements for detection of a QTL included a minimum effective chain size (ECS) value of 100, a posterior probability greater than 0.1 (threshold for a significant QTL), and a Bayes Factor (BF) greater than two (Bink et al. 2013; Rosyara et al. 2013; Verma et al., 2016). The BFs are the evidence favoring the presence of a number of QTLs and the genetic model proposed (Bink et al., 2012 and 2014). When BF values were between zero and two, the evidence of a significant QTL was considered low, when values were between two and five, the evidence was positive, when BFs were between five and 10, the evidence was

strong, and when the values were greater than 10, the evidence was decisive (Bink et al., 2012 and 2014). Additionally, PostQTL genome-wide trace and intensity plots were used to visualize and compare the convergence, stability, and probability of the evaluated genetic model per trait and year to further determine the reliability of the identified QTLs. The generation of the multiple data sets for the same year were used to comare to each other in order to accurately approximate QTLs. All reliable QTLs were subsequently named according to specifications of the Genome Database for Rosaceae: linkage group number and phenotypic trait symbol (Jung et al., 2008 and 2014).

QTL Effects Calculations

For all traits, the broad sense heritability (H^2) was calculated using the values of phenotypic variance $\left(\sigma_P^2\right)$ and error (σ_e^2) and the narrow sense (h^2) heritability was calculated using the weighted additive variance of the QTL [probability* weighted additive variance of QTL, $\left(\overline{\sigma_A^2}\right)$]. The values of $\left(\sigma_P^2\right)$, (σ_e^2), and $\left(\overline{\sigma_A^2}\right)$ were obtained from FlexQTL™ results and H^2 and h^2 were calculated using the following formulas: h^2: $H^2 = \frac{\sigma_P^2 - \sigma_e^2}{\sigma_P^2} = \frac{\sigma_G^2}{\sigma_P^2}$; and $h^2 = \frac{\sigma_A^2}{\sigma_P^2}$. The QTLs which explained ≥ 10% of phenotypic the variation for their respective trait (Vp %) were considered major QTLs and those which explained less were considered minor QTLs.

Genomic Estimated Breeding Values

The genomic estimated breeding values (GEBVs) based on all QTLs were calculated for all individuals in the UA RosBREED pedigree per trait per year through the *a posteriori* PBA results, using PostFlexQTL™ version 0.99110, (Bink et al., 2014; Fresnedo-Ramírez et al., 2015 and 2016). Prediction accuracy was calculated as the correlation between GEBVs and observed values per trait per year.

Results

Weather Data

Temperature and humidity data from FRS, collected in 2013, 2014, and 2015, showed that the temperature and humidity between 1 Mar. and 31 Aug. varied among years, especially when comparing 2014 and 2015 to 2013 (Figs. 4-6). Additionally, rainfall from 1 June to 31 Aug. was variable with a total of 19.7 cm of rain in 2013, 12.1 cm in 2014, and 33.4 cm in 2015 (Table 3). These varying conditions each year provided for a favorable environment of warm temperatures (20-35 °C), accompanied with light, frequent rains, extended heavy dews, and very high humidity (~75%), which enabled the occurrence for uniform *Xap* infection across the FRS. Overall observations indicated that *Xap* infection levels across the program were worse in 2014 and 2015 compared to 2013, as these last two years (2014 and 2015) in general were warmer and more humid.

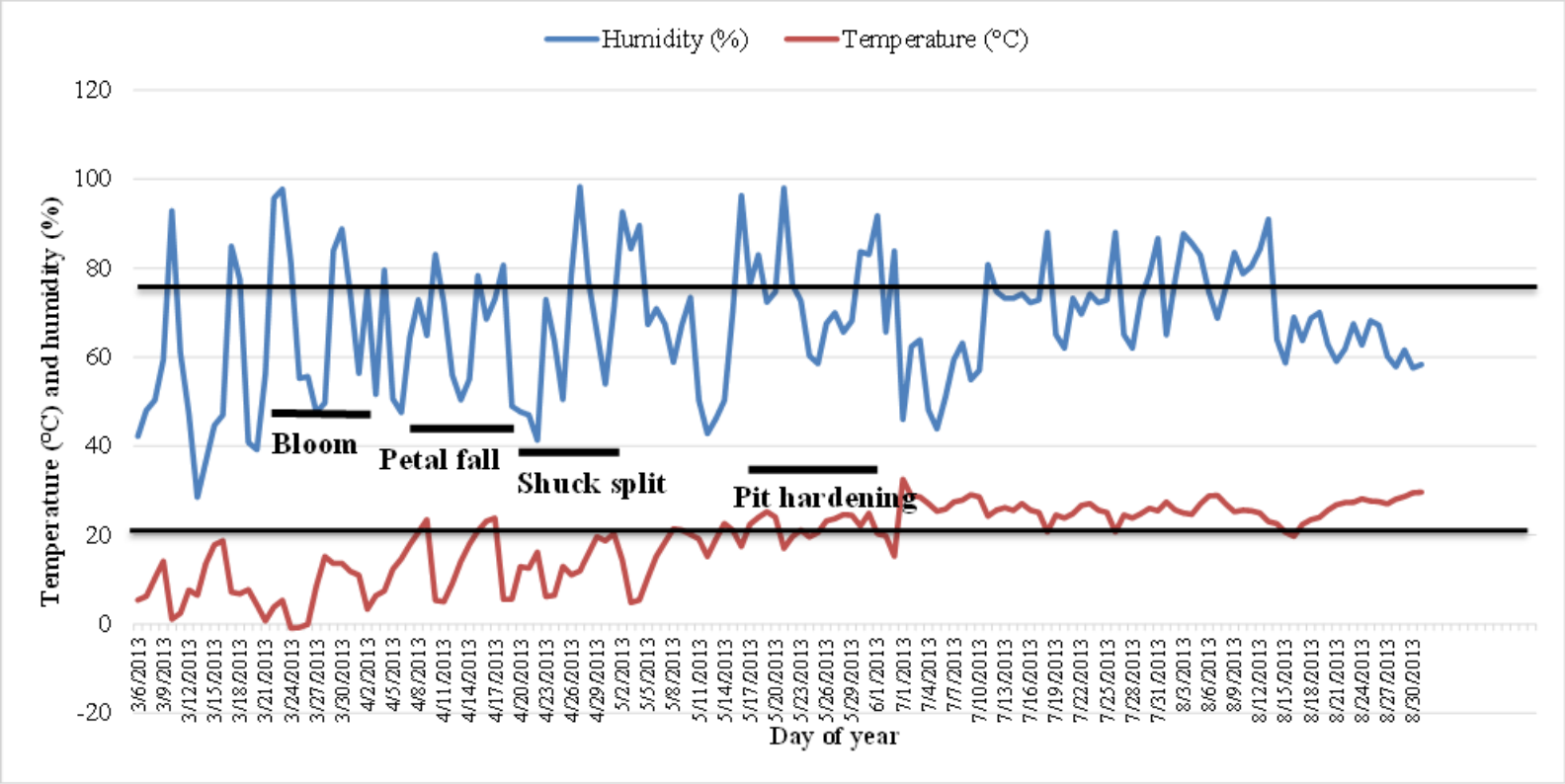

Fig. 4. Temperature and humidity weather condition data from FRS, collected in 2013. Relative humidity > 75% and temperatures between ~20-35 °C, are optimal for *Xap* infection (both are marked with a dark black line). Relative timing for bloom, petal fall, shuck split and pit hardening are also marked with dark black lines.

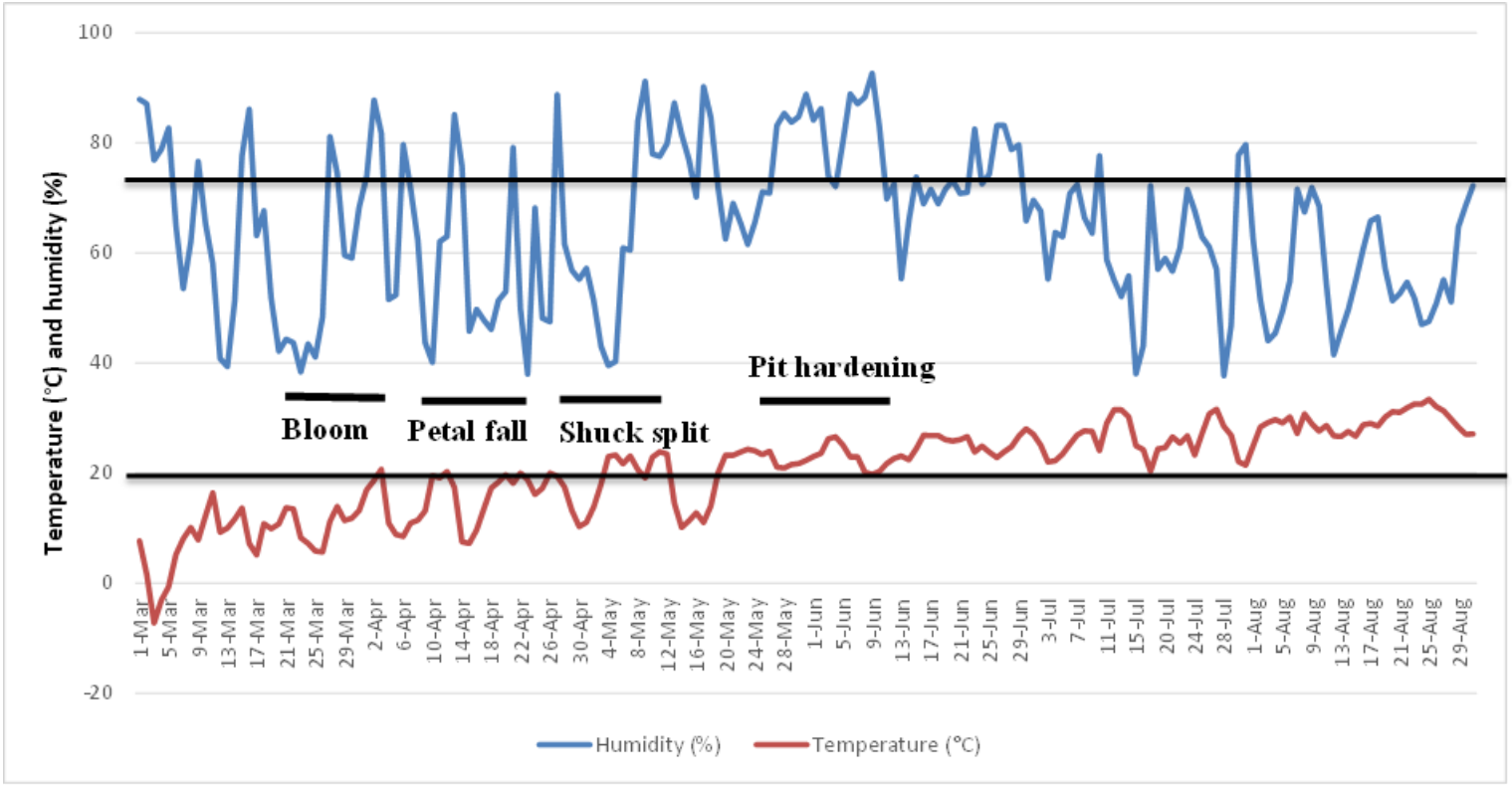

Fig. 5. Temperature and humidity weather condition data from the FRS collected in 2014. Relative humidity > 75% and temperatures between ~20-35 °C, are optimal for *Xap* infection (both are marked with a dark black line). Relative timing for bloom, petal fall, shuck split and pit hardening are also marked with dark black lines.

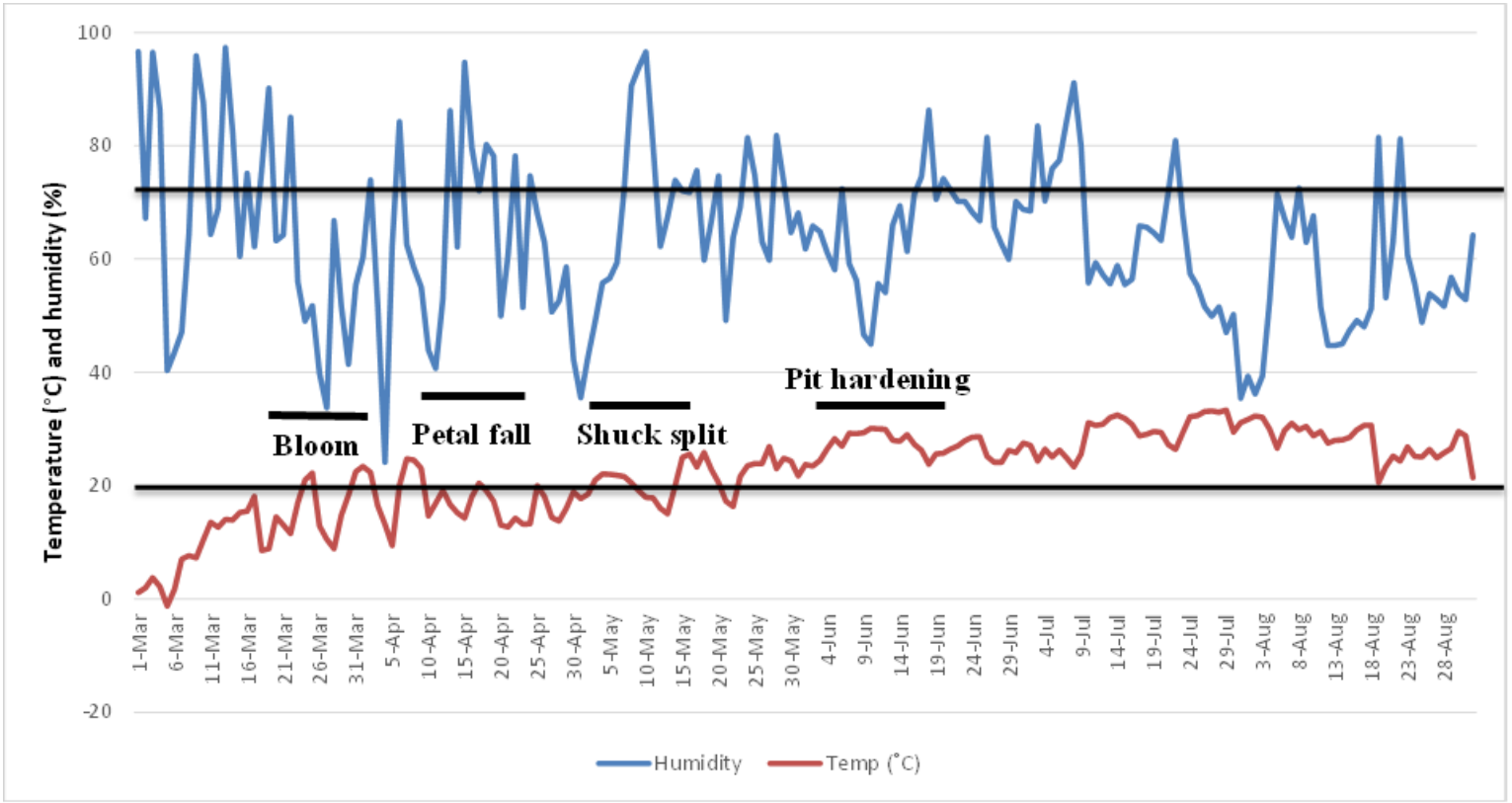

Fig. 6. Temperature and humidity weather condition data from the FRS, collected in 2015. Relative humidity > 75% and temperatures between ~20-35 °C, are optimal for *Xap* infection (both are marked with a dark black line). Relative timing for bloom, petal fall, shuck split and pit hardening are also marked with dark black lines.

Table 3. Total rainfall (cm) at the FRS, for each month (March-August) in 2013, 2014, and 2015.

Year	Month	Rainfall (cm)
2013	March	13.0
2013	April	12.1
2013	May	16.3
2013	March-May avg	13.8
2013	June	5.5
2013	July	10.0
2013	August	17.9
2014	March	2.8
2014	April	4.9
2014	May	7.3
2014	March-May avg	5.0
2014	June	5.8
2014	July	2.1
2014	August	0.1
2015	March	13.6
2015	April	8.6
2015	May	14.4
2015	March-May avg	12.2
2015	June	0.1
2015	July	1.2
2015	August	3.7

Xap Fruit Field Data Sets (2013-2015)

The *Xap* fruit (0-5) average ratings were 2.71 (2013), 3.90 (2014), 3.65 (2015), and 3.40 (2013-2015 avg) for all individuals in the AR RosBREED pedigree (Table 3). The year-to-year ratings paralleled the general observations of *Xap* occurrence at FRS, and reflect the weather pattern for the years particularly for 2014 and 2015 which were substantially more humid, and hot than 2013. The *Xap* fruit (0-5) ratings ranged from 0.00 (2013, 2015, and 2013-2015 avg) to 5.00 (all years). The standard deviation displayed across all years ranged from 1.03 (2014) to 1.55 (2013) (Table 3).

For the seven F_1 populations, different segregation patterns for *Xap* fruit (0-5) were observed across all years of the study (Figs. 7-10). In 2013, AR_Pop_1, AR_Pop_0801, AR_Pop_0803, AR_Pop_0813, and AR_Pop_0825 all segregated from 0.00-5.00 (Fig. 7). In contrast, AR_Pop_0817 and AR_Pop_0819 segregated from medium to high susceptibility. In 2014, only AR_Pop_0801 segregated from 0.00-5.00, and all other populations segregated from medium to high susceptibility (Fig. 8). In 2015, AR_Pop_1 and AR_Pop_0803 segregated from 0.00-5.00, and all other populations segregated from medium to high susceptibility, except for AR_Pop_0817, which were all scored as 5.00 (Fig. 9). For the 2013-2015 avg, AR_Pop_1 segregated from 0.00-5.00, and all other populations segregated from medium to high susceptibility, excluding AR_Pop_0817 where again the majority of the seedlings were scored as 5.00 (Fig. 10). Parental values from each year are also included in Figs. 7-10.

Table 3. Mean, minimum, maximum, and standard deviation of *Xap* fruit (0-5), *Xap* fruit [(0-5) ± 0.5], and *Xap* fruit [(0-5) – 1], for 2013, 2014, 2015, and 2013-2015 avg. Mean, minimum, maximum, and standard deviation of *Xap* fruit [(0-5) inc], *Xap* fruit [(0-5) ± 0.5 inc], and *Xap* fruit [(0-5) – 1 inc] for 2014, 2015, and 2014-2015 avg for the entire AR RosBREED pedigree (N is number of individuals).

Trait	Year	Mean	Min.	Max.	Std. dev.	N
Xap fruit (0-5)	2013	2.71	0.00	5.00	1.55	170
	2014	3.90	1.00	5.00	1.03	167
	2015	3.65	0.00	5.00	1.16	167
	2013-2015 avg	3.40	0.00	5.00	1.07	173
Xap fruit [(0-5) ± 0.5]	2013	2.73	0.00	5.50	1.59	170
	2014	3.91	1.00	5.00	1.02	167
	2015	3.62	0.00	5.00	1.15	167
	2013-2015 avg	3.39	0.00	5.00	1.09	173
Xap fruit [(0-5) – 1]	2013	1.90	0.00	5.00	1.40	170
	2014	3.13	0.00	5.00	1.21	167
	2015	2.85	0.00	5.00	1.31	167
	2013-2015 avg	2.61	0.00	5.00	1.13	173
Xap fruit [(0-5) inc]	2014	4.40	1.00	5.00	0.96	166
	2015	3.64	0.00	5.00	1.33	167
	2014-2015 avg	4.02	0.50	5.00	1.01	167
Xap fruit [(0-5) ± 0.5 inc]	2014	4.39	1.00	5.00	0.96	167
	2015	3.64	0.00	5.00	1.32	167
	2014-2015 avg	4.02	0.50	5.00	1.00	167
Xap fruit [(0-5) – 1 inc]	2014	3.58	0.00	5.00	1.01	167
	2015	2.83	0.00	5.00	1.46	167
	2014-2015 avg	3.20	0.00	5.00	1.11	167

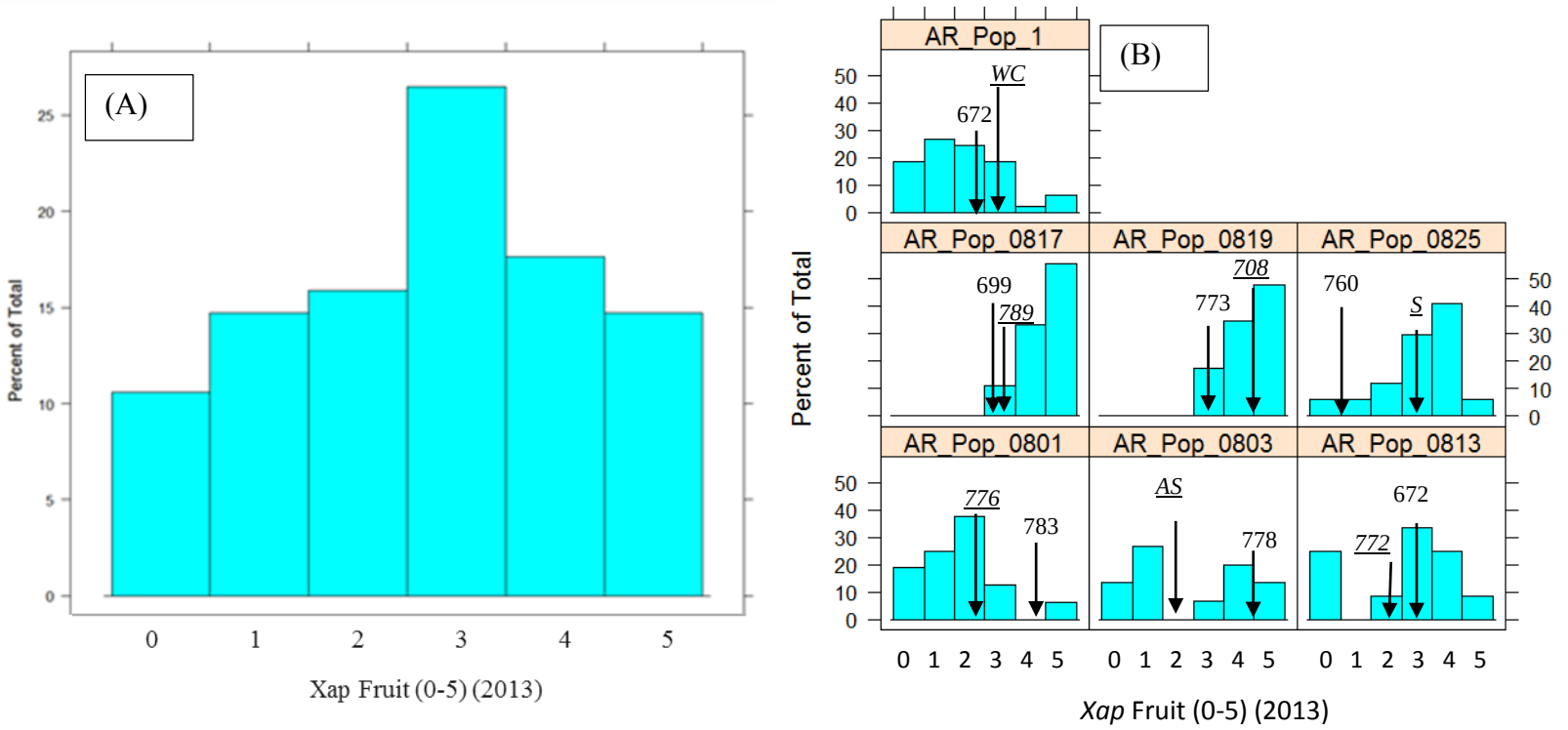

Fig. 7. Distribution (%) of *Xap* fruit (0-5) 2013 field ratings for the entire AR RosBREED pedigree (A) (N=170), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

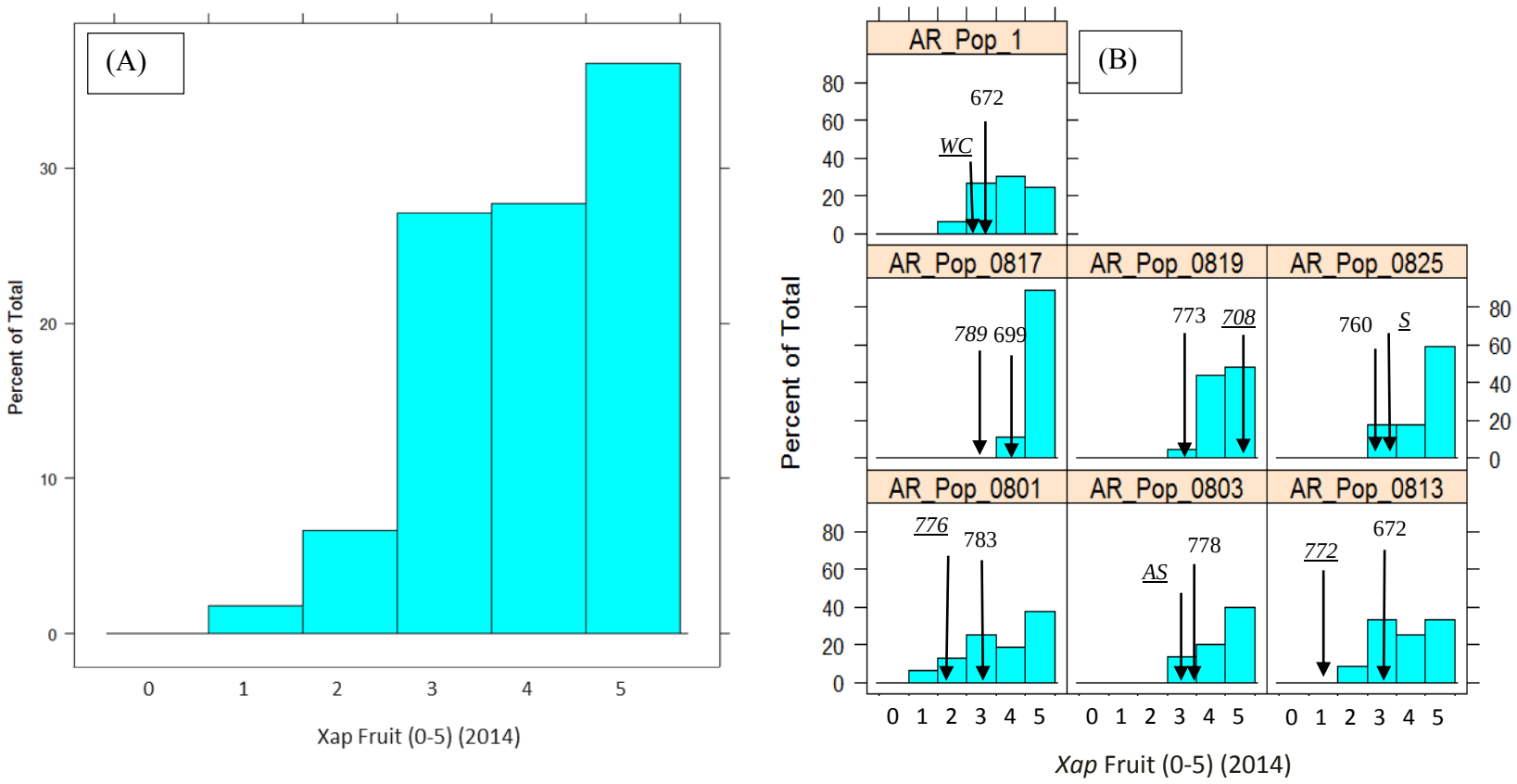

Fig. 8. Distribution (%) of *Xap* fruit (0-5) 2014 field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

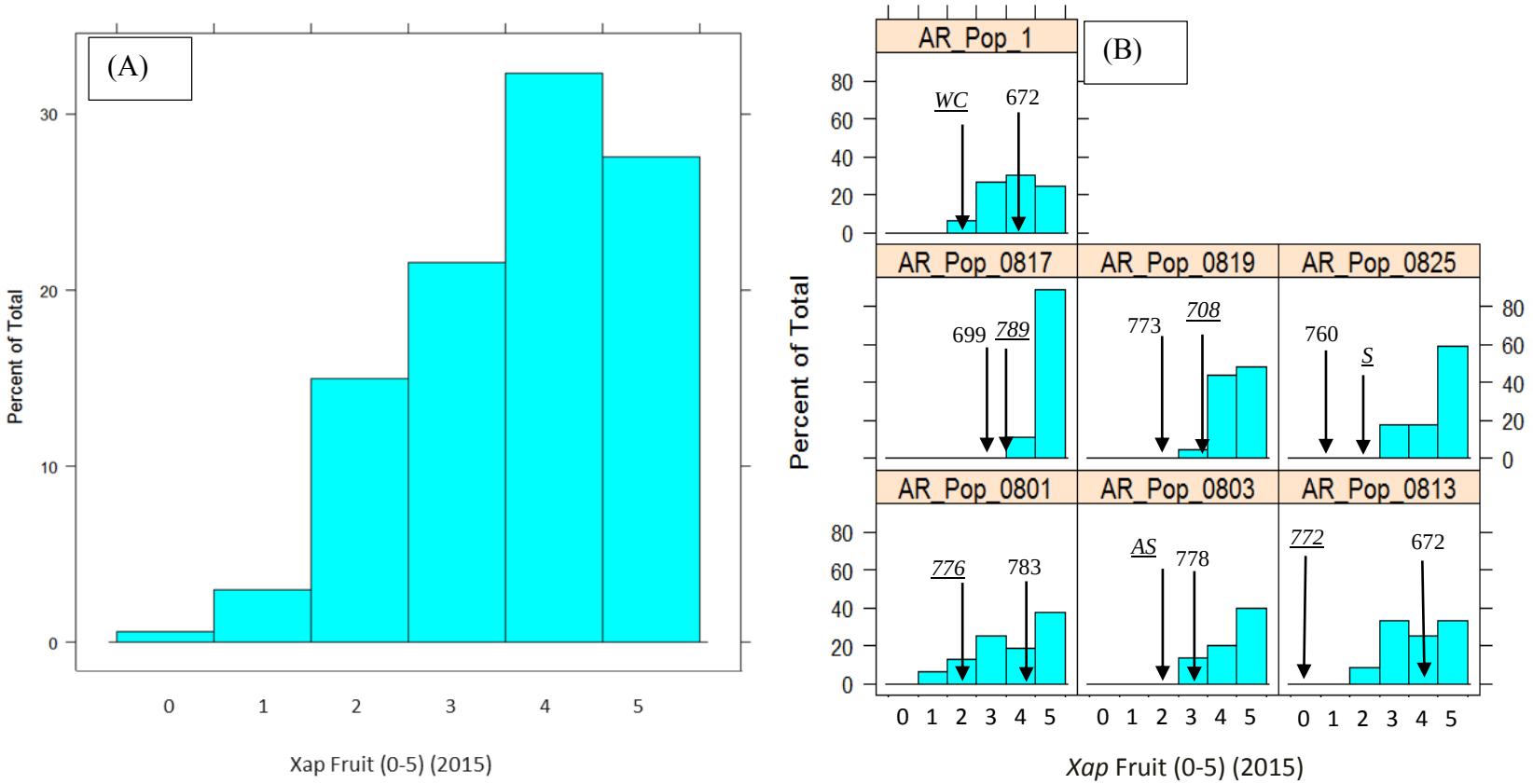

Fig. 9. Distribution (%) of *Xap* fruit (0-5) 2015 field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

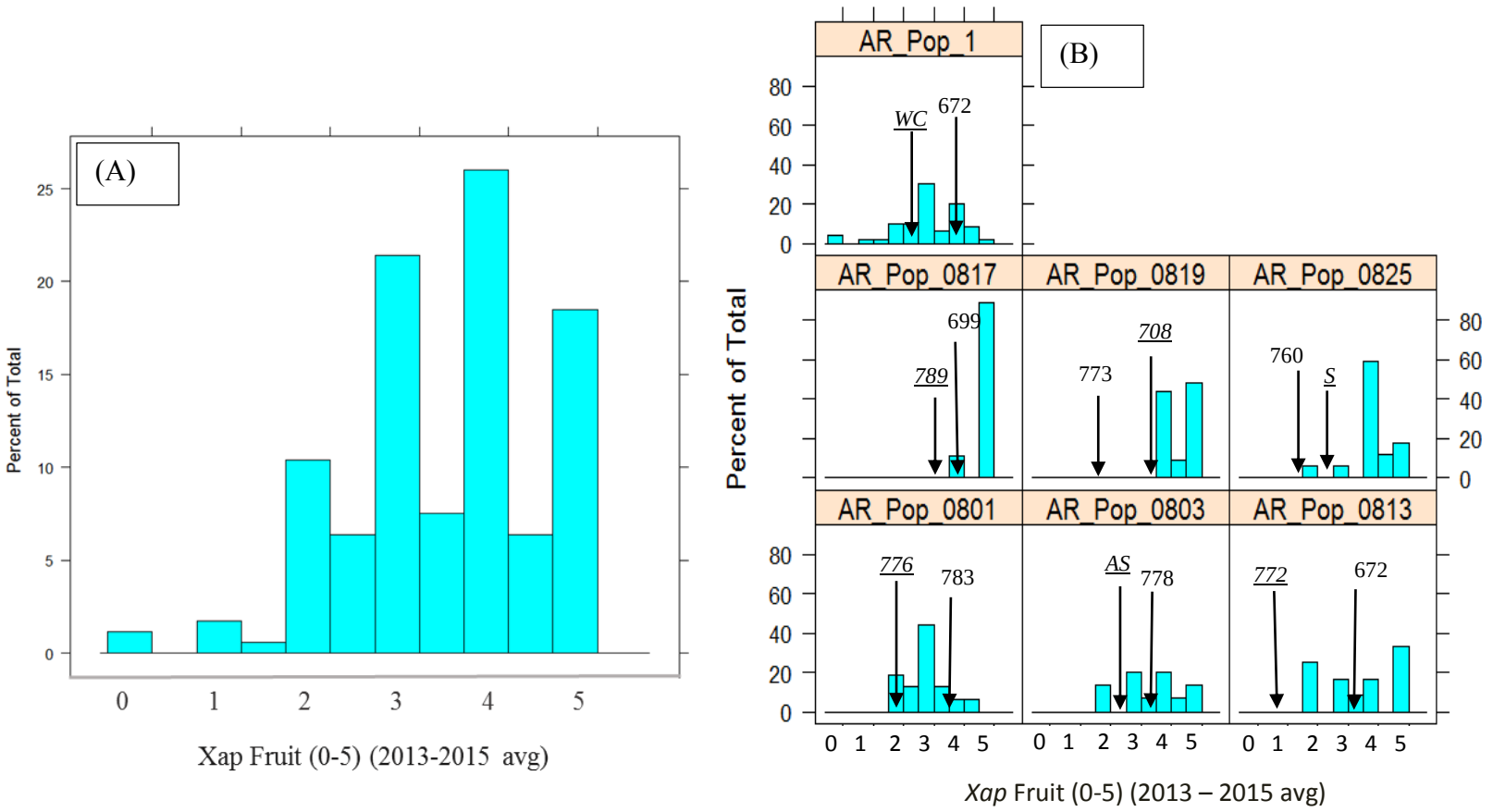

Fig. 10. Distribution (%) of *Xap* fruit (0-5) 2013-2015 avg field ratings for the entire AR RosBREED pedigree (A) (N=173), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

The *Xap* fruit [(0-5) ± 0.5] ratings exhibited an average of 2.73 (2013), 3.91 (2014), 3.62 (2015), and 3.39 (2013-2015 avg) for all individuals in the AR RosBREED pedigree (Table 3). The year-to-year ratings paralleled the general observations of *Xap* occurrence at FRS, and reflect the weather pattern for the years particularly for 2014 and 2015 which were substantially more humid, and hotter than 2013. The *Xap* fruit [(0-5) ± 0.5] ratings ranged from 0.00 (2013, 2015, and 2013-2015 avg) to 5.50 (2013). The standard deviation displayed across all years ranged from 1.02 (2014) to 1.59 (2013) (Table 3).

For the seven F_1 populations, different segregation patterns for *Xap* fruit [(0-5) ± 0.5] were observed across all years of the study (Figs. 11-14). In 2013, AR_Pop_1, AR_Pop_0801, AR_Pop_0803, AR_Pop_0813, and AR_Pop_0825 all segregated from 0.00-5.00 (Fig. 11). In contrast, AR_Pop_0817 and AR_Pop_0819 segregated from medium to high susceptibility. In 2014, only AR_Pop_0801 segregated from 0.00-5.00, and all other populations segregated from medium to high susceptibility, except for AR_Pop_0817, which were nearly all scored as 5.00 (Fig. 12). In 2015, AR_Pop_1, segregated from 0.00-5.00, and all other populations segregated from medium to high susceptibility, except for AR_Pop_0817, which were all scored as 5.00 (Fig. 13). In 2013-2015 avg, AR_Pop_1 segregated from 0.00-5.00, and all other populations segregated from medium to high susceptibility, excluding AR_Pop_0817, which again the majority of the seedlings were scored as 5.00, and AR_Pop_0801, which the seedlings segregated only within medium range values (Fig. 14). Parental values from each year are also included in Figs. 11-14.

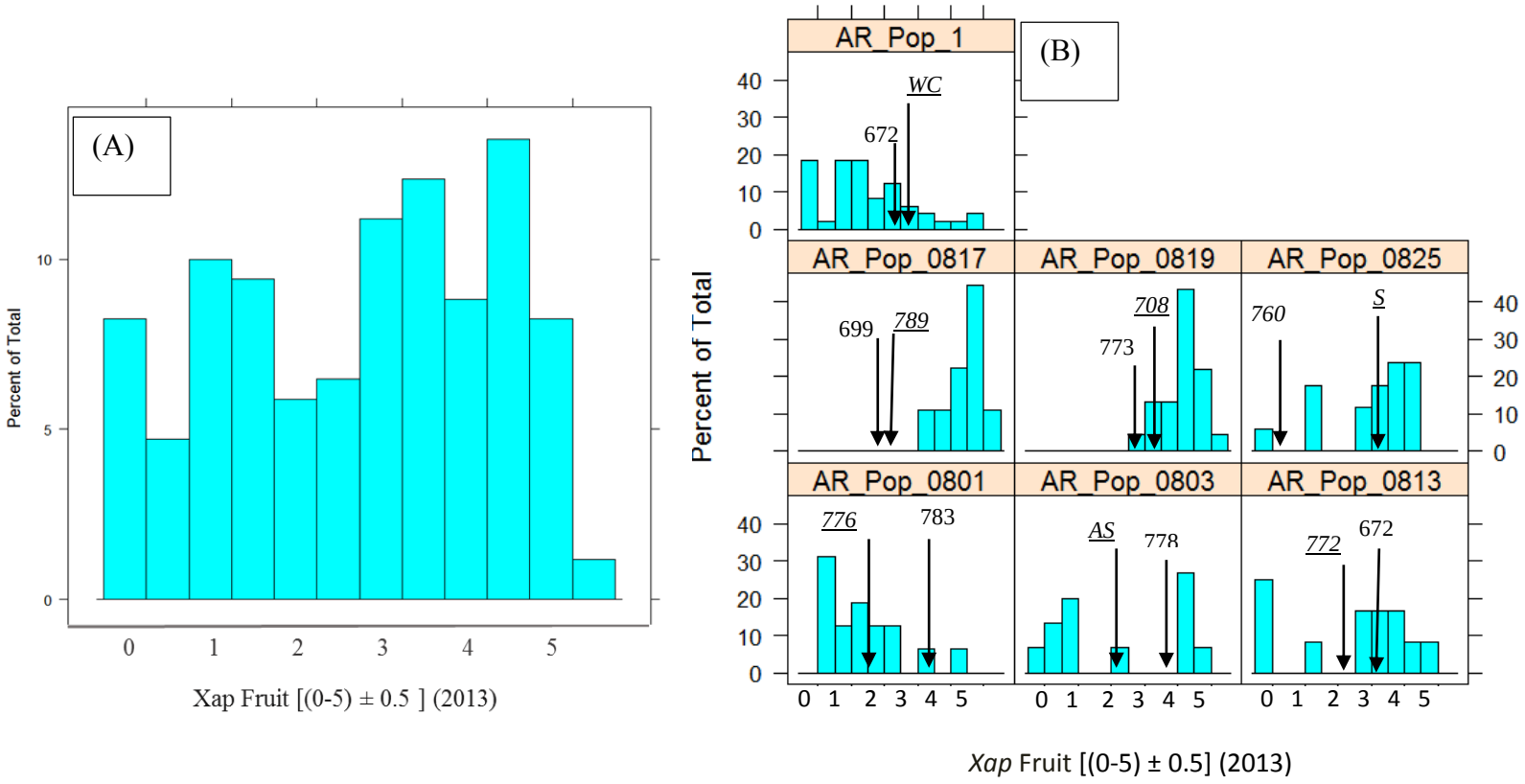

Fig. 11. Distribution (%) of *Xap* fruit [(0-5) ± 0.5] 2013 field ratings for the entire AR RosBREED pedigree (A) (N=170), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

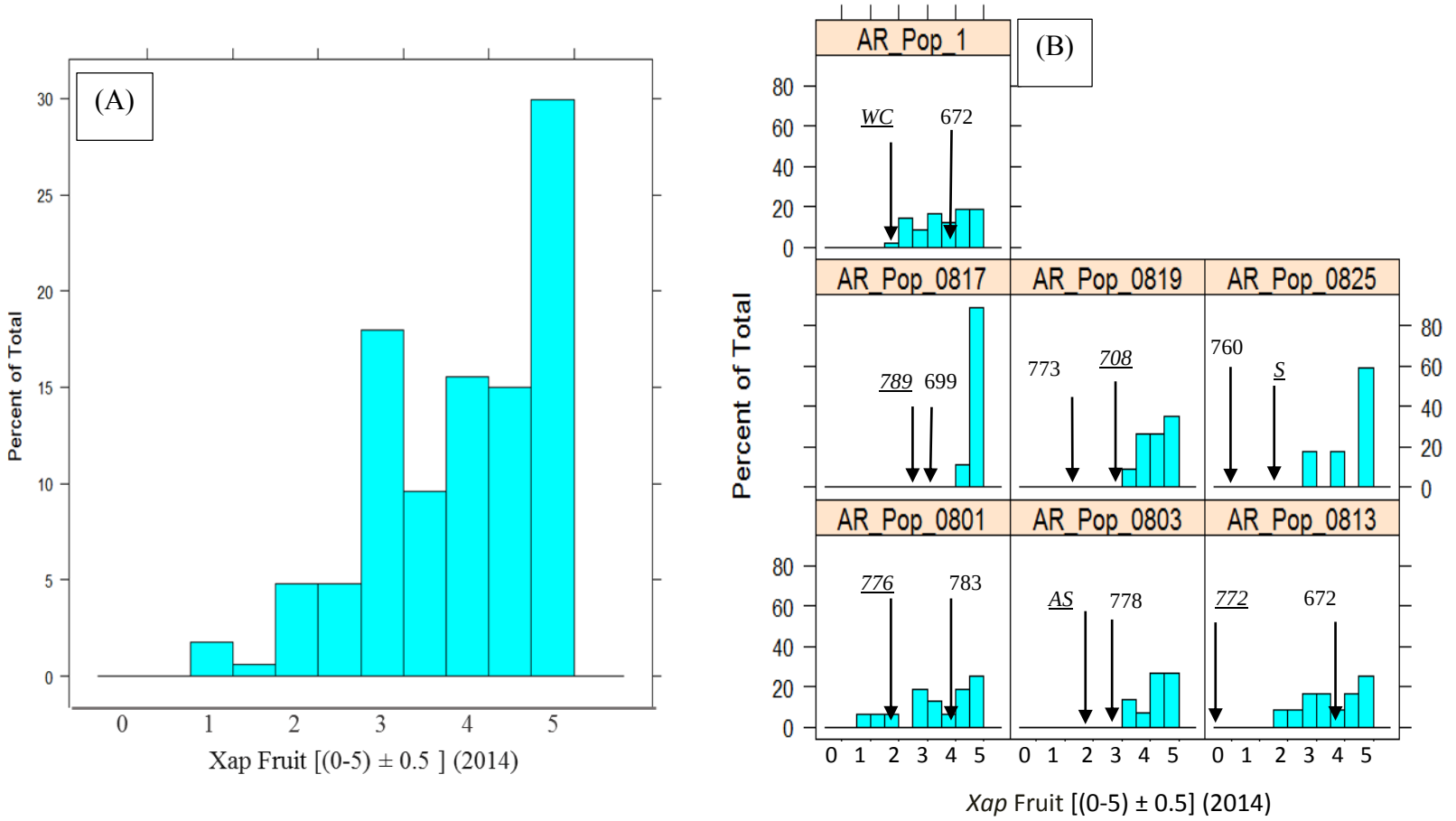

Fig. 12. Distribution (%) of *Xap* fruit [(0-5) ± 0.5] 2014 field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

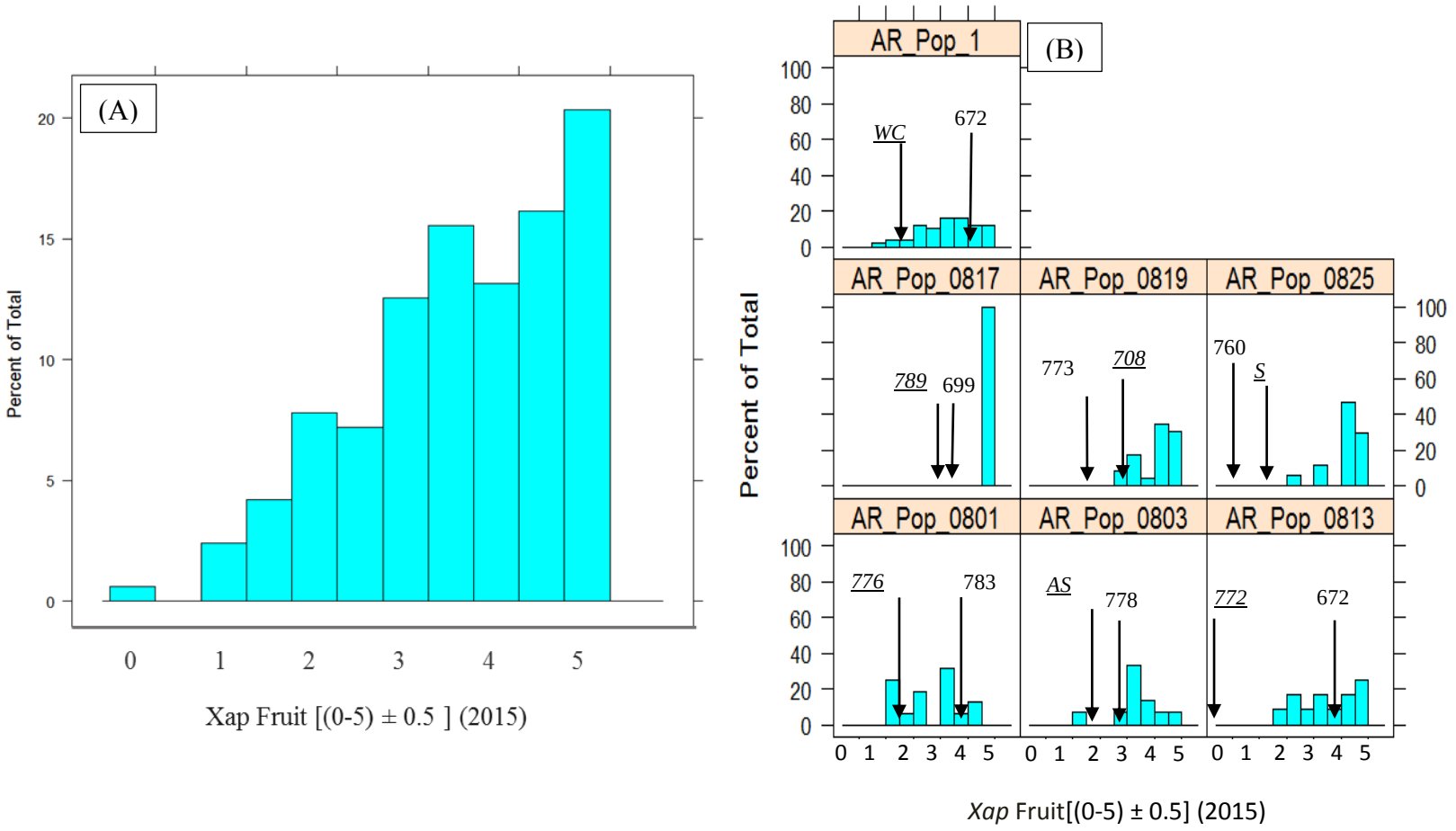

Fig. 13. Distribution (%) of *Xap* fruit [(0-5) ± 0.5] 2015 field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; *female parent* is italicized and underlined) (B).

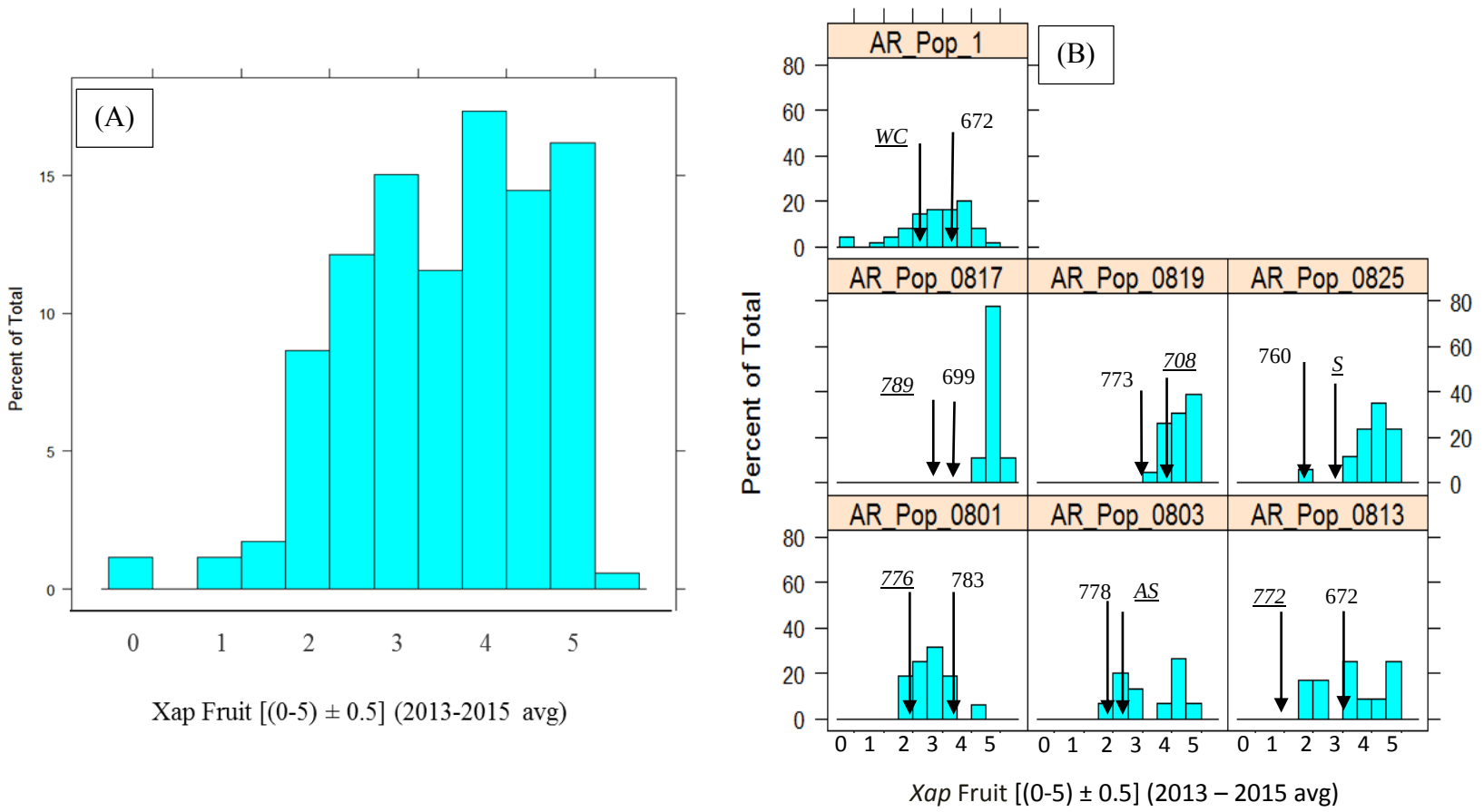

Fig. 14. Distribution (%) of *Xap* fruit [(0-5) ± 0.5] 2013-2015 avg field ratings for the entire AR RosBREED pedigree (A) (N=173), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

The *Xap* fruit [(0-5) – 1] ratings exhibited an average of 1.90 (2013), 3.13 (2014), 2.85 (2015), and 2.61 (2013-2015 avg) for all individuals in the AR RosBREED pedigree (Table 3). The year-to-year ratings paralleled the general observations of *Xap* occurrence at FRS, and reflect the weather pattern for the years particularly for 2014 and 2015 which were substantially more humid, and hotter than 2013. The *Xap* fruit [(0-5) – 1] ratings ranged from 0.00-5.00 across all years. The standard deviation displayed across all years ranged from 1.13 (2013-2015 avg) to 1.40 (2013) (Table 3).

In terms of the seven F_1 populations, different segregation patterns for *Xap* fruit [(0-5) – 1] were observed across all years of the study (Figs. 15-18). In 2013, AR_Pop_1, AR_Pop_0803, AR_Pop_0813, and AR_Pop_0825 all segregated from 0.00-4.00 (Fig. 15). In contrast, AR_Pop_0817 and AR_Pop_0819 segregated from medium to high susceptibility, and AR_Pop_0801 segregated from medium to low susceptibility. In 2014, only AR_Pop_0801 segregated from 0.00-5.00, and all other populations segregated from medium to high susceptibility, except for AR_Pop_0817, which were nearly all scored as 5.00 (Fig. 16). In 2015, AR_Pop_1 segregated from 0.00-5.00, and all other populations segregated from medium to high susceptibility, except for AR_Pop_0817, which were all scored as 5.00 (Fig. 17). In 2013-2015 avg, AR_Pop_1, and AR_Pop_0813 nearly segregated the entire range of ratings, while AR_Pop_0801 and AR_Pop_0803 segregated from low to medium susceptibility, and AR_Pop_0819 and AR_Pop_0825 segregated from medium to high susceptibility. As with other all other data sets, AR_Pop_0817 again contained seedlings which were nearly all scored as 5.00 (Fig. 18) Parental values from each year are also included in Figs. 15-18.

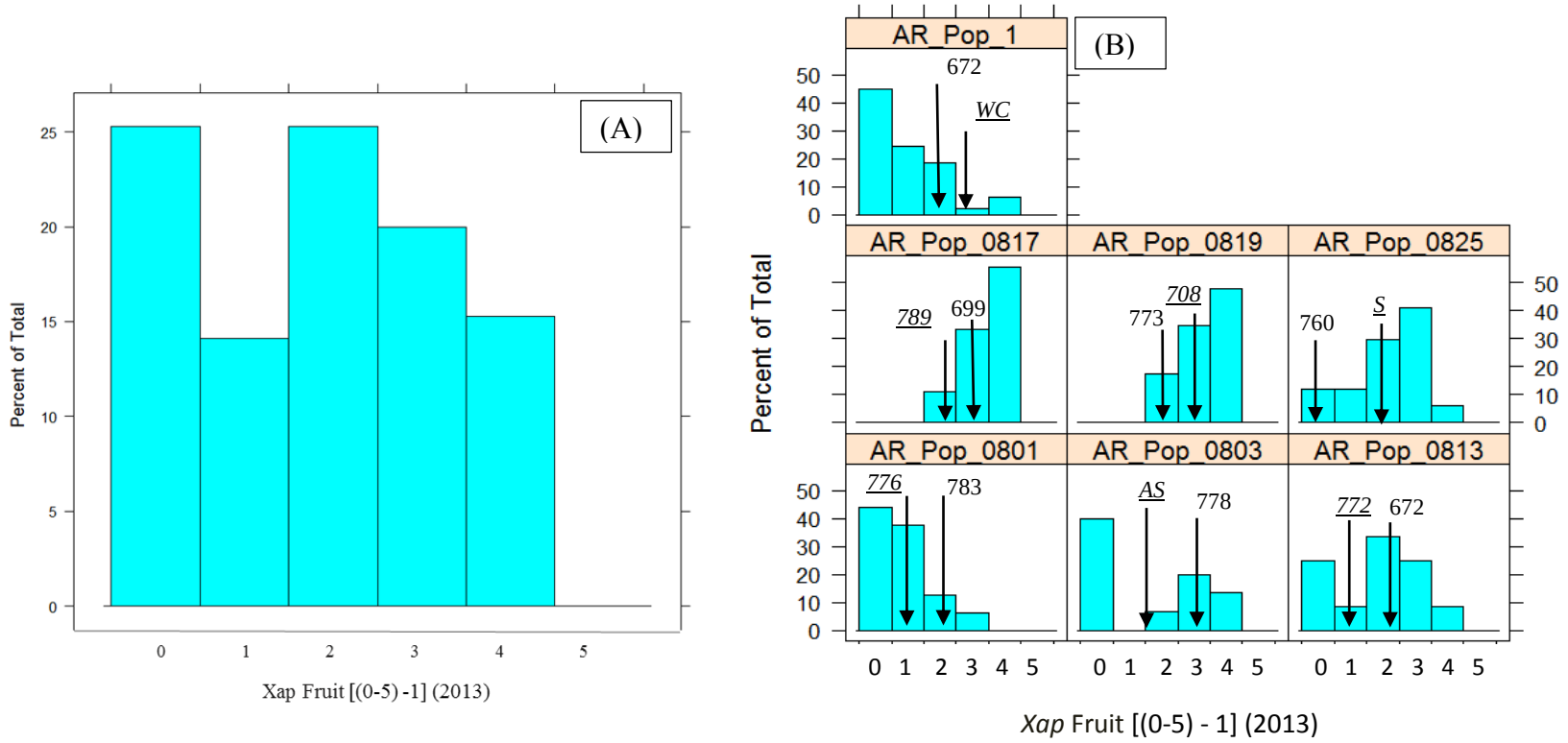

Fig. 15. Distribution (%) of *Xap* fruit [(0-5) – 1] 2013 field ratings for the entire AR RosBREED pedigree (A) (N=170), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

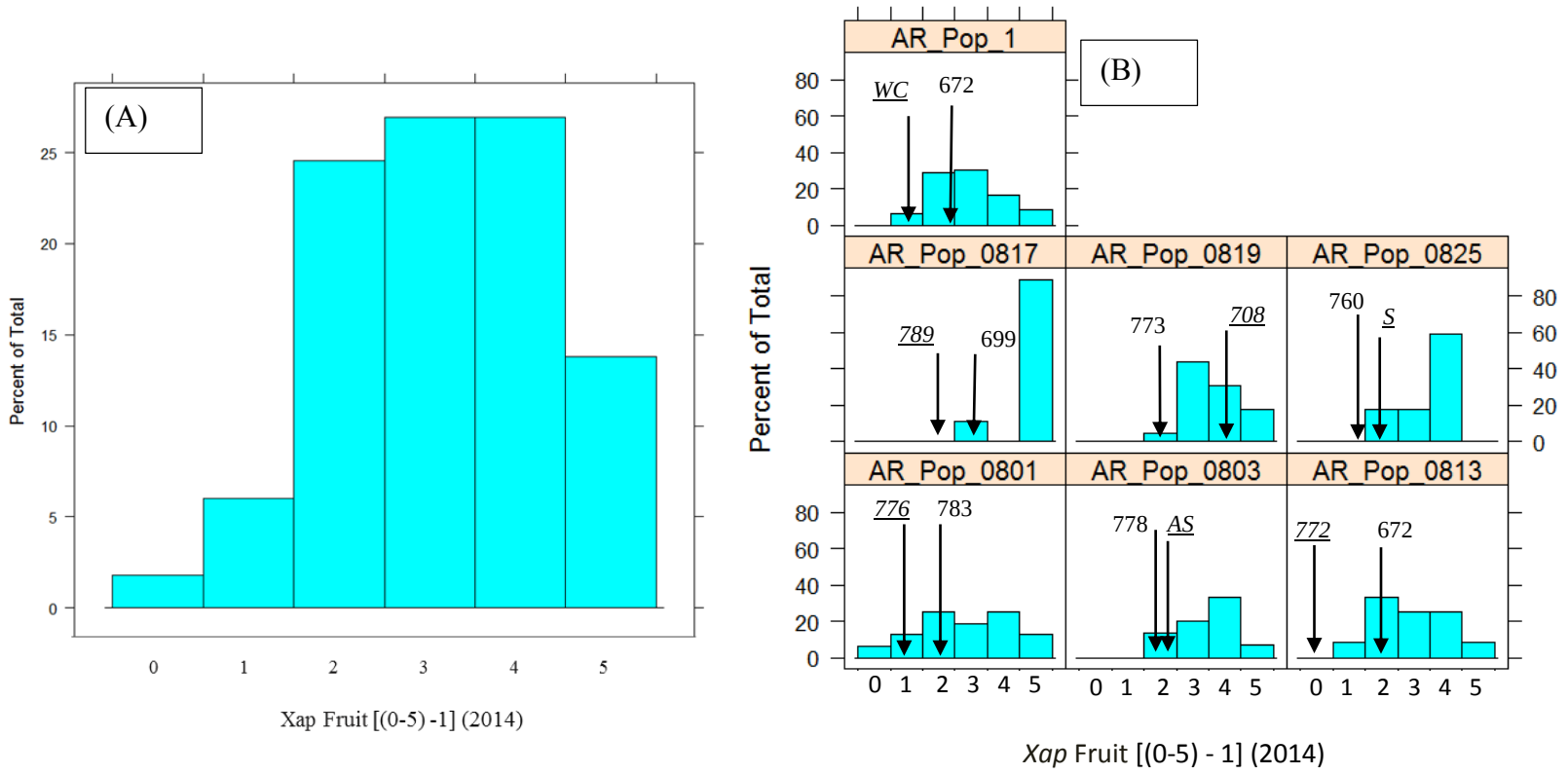

Fig. 16. Distribution (%) of *Xap* fruit [(0-5) – 1] 2014 field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

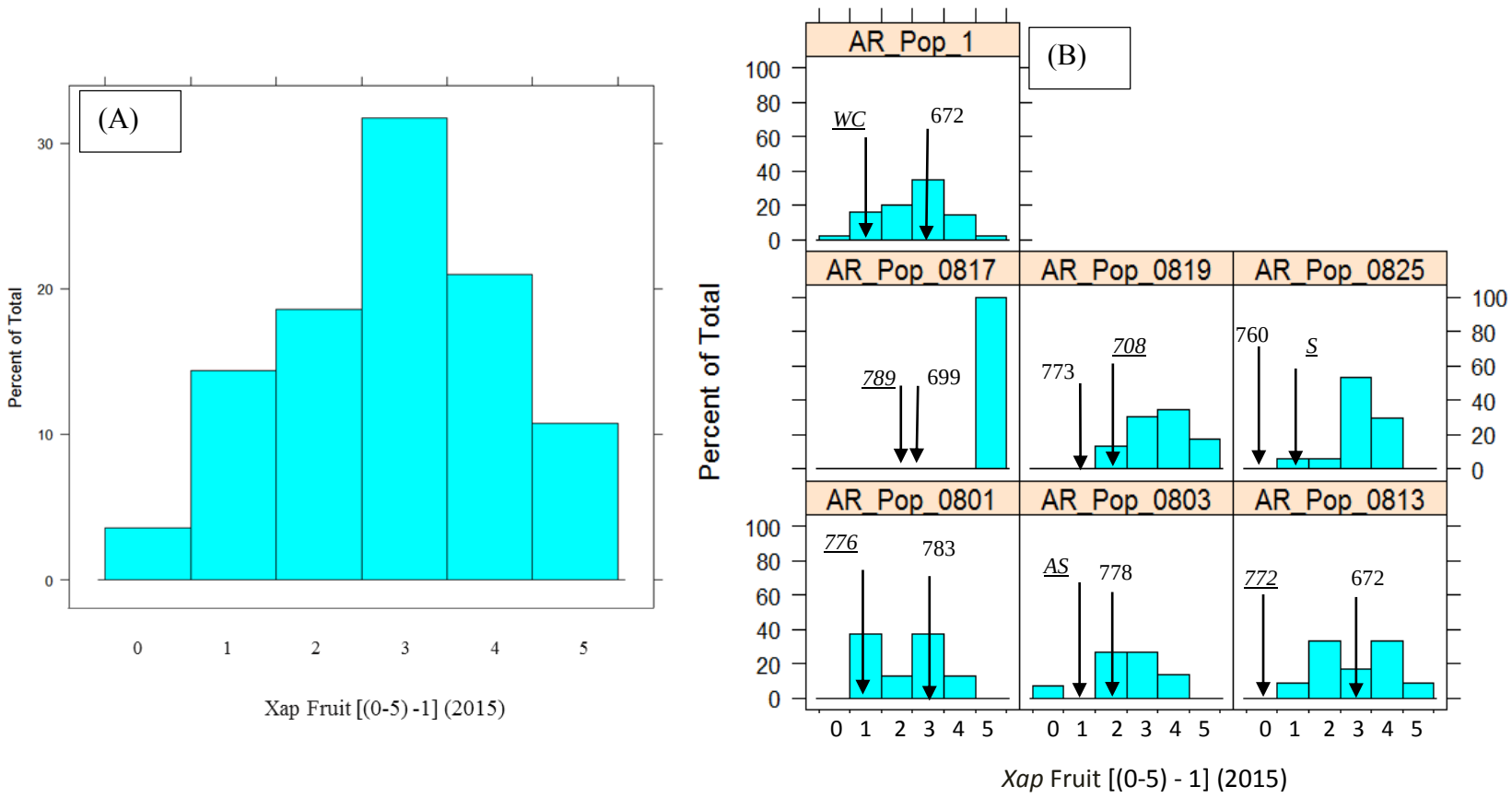

Fig. 17. Distribution (%) of *Xap* fruit [(0-5) – 1] 2015 field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

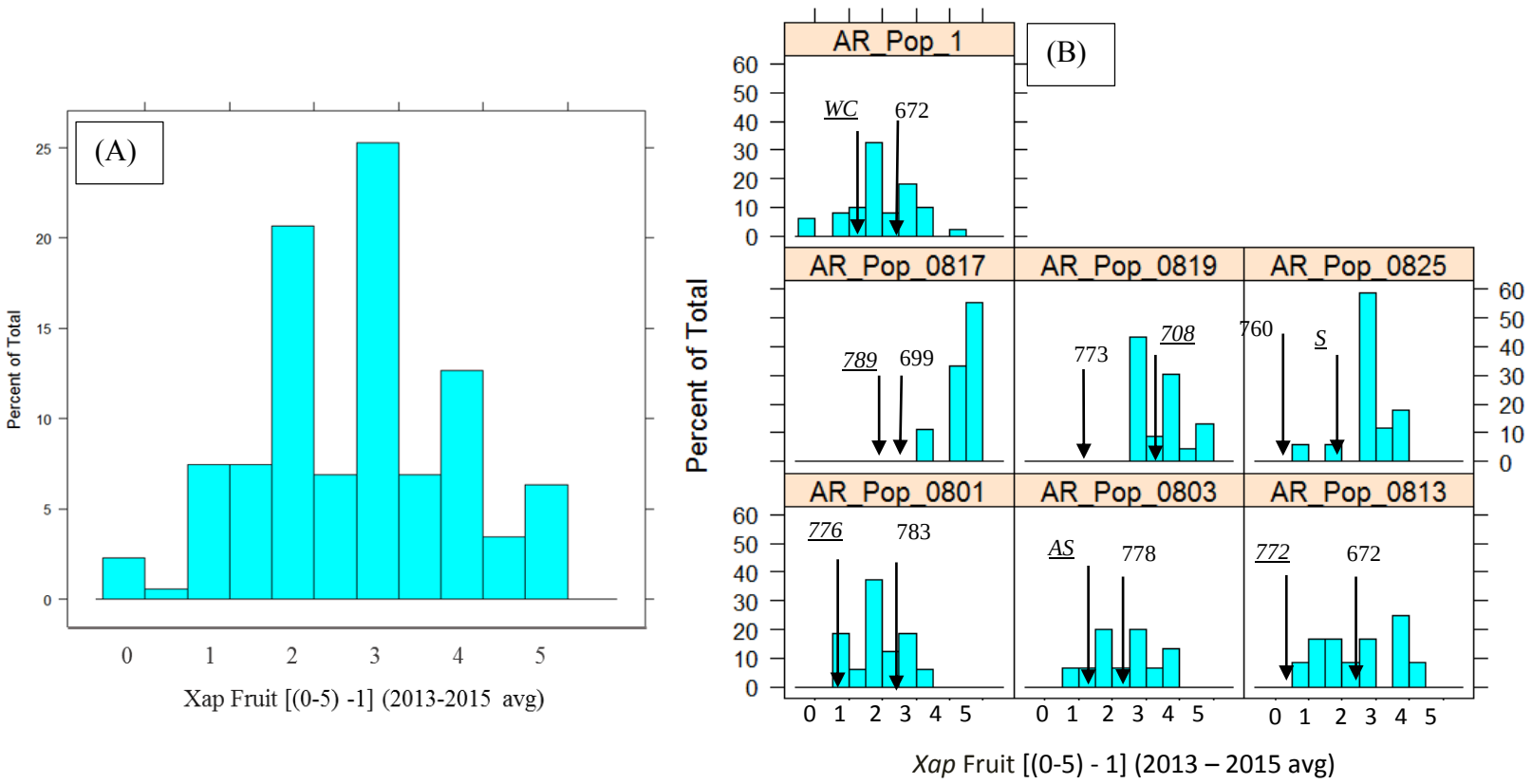

Fig. 18. Distribution (%) of *Xap* fruit [(0-5) – 1] 2013-2015 avg field ratings for the entire AR RosBREED pedigree (A) (N=173), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

The *Xap* fruit [(0-5) inc] ordinal ratings exhibited an average of 4.40 (2014), 3.64 (2015), and 4.02 (2014-2015 avg) for all individuals in the AR RosBREED pedigree (Table 3). The year-to-year ratings paralleled the general observations of *Xap* occurrence at FRS, as 2014 and 2015 were substantially humid and hot. The *Xap* fruit [(0-5) inc] ratings ranged from 0.00 (2015) to 5.00 (2014, 2015, and 2014-2015 avg). The standard deviation displayed across all years ranged from 0.96 (2014) to 1.33 (2015) (Table 3).

In terms of the seven F_1 populations, different segregation patterns for *Xap* fruit [(0-5) inc] were observed across all years of the study (Figs. 19-21). In 2014, all populations segregated from medium to high susceptibility, excluding AR_Pop_0817 and AR_Pop_0801, which were nearly all scored as 5.00 (Fig. 19). In 2015, AR_Pop_1, AR_Pop_0801, and AR_Pop_0803 segregated from 1.00-5.00, and all other populations segregated from medium to high susceptibility, except for AR_Pop_0817, which all were scored as 5.00 (Fig. 20). In 2014-2015 avg, only AR_Pop_1 segregated from 1.00-5.00, and all other populations segregated from medium to high susceptibility, excluding AR_Pop_0817, which were all scored as 5.00 (Fig. 21). Parental values from each year are also included in Figs. 19-21.

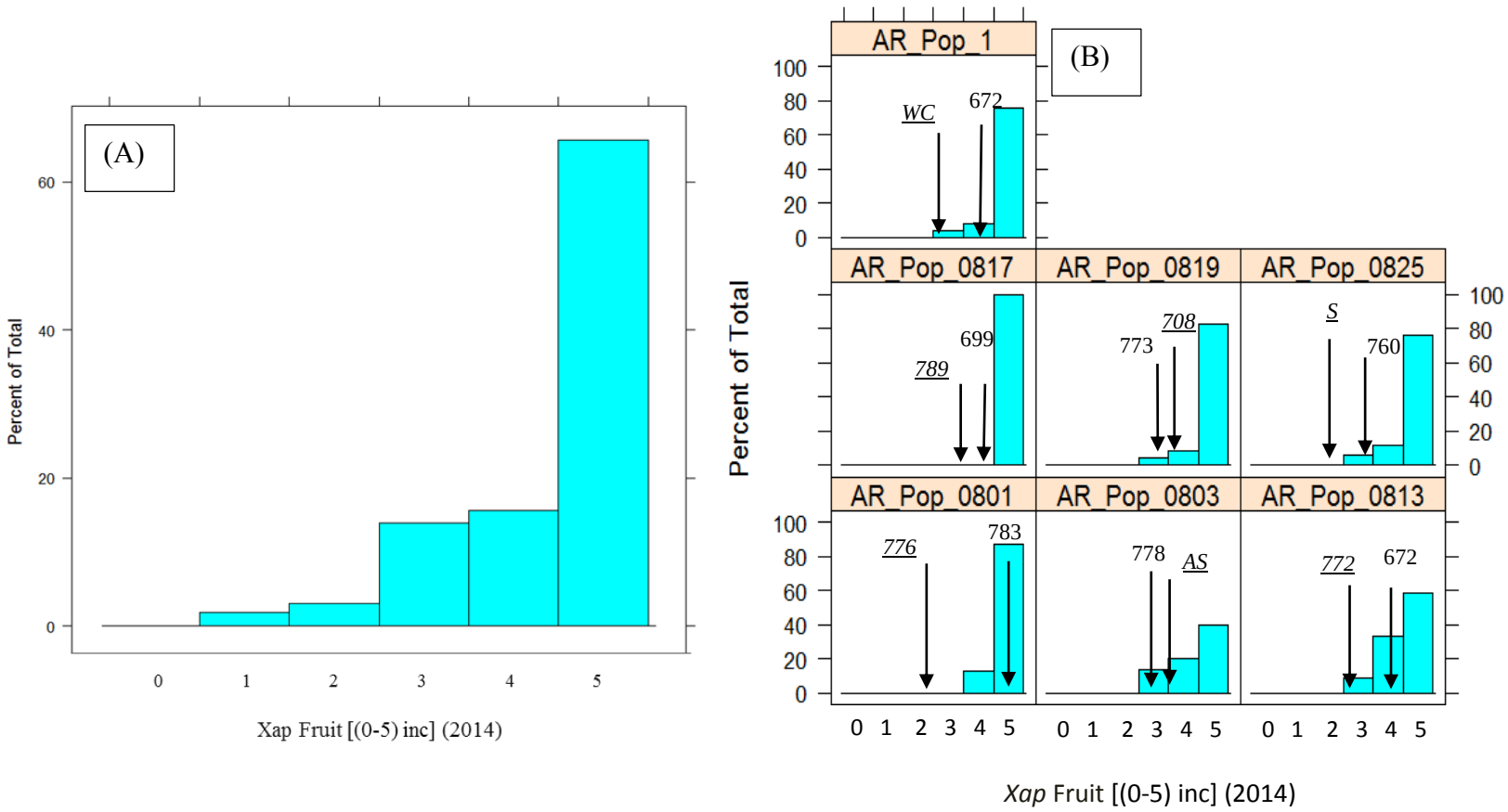

Fig. 19. Distribution (%) of *Xap* fruit [(0-5) inc] 2014 field ratings for the entire AR RosBREED pedigree (A) (N=166), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

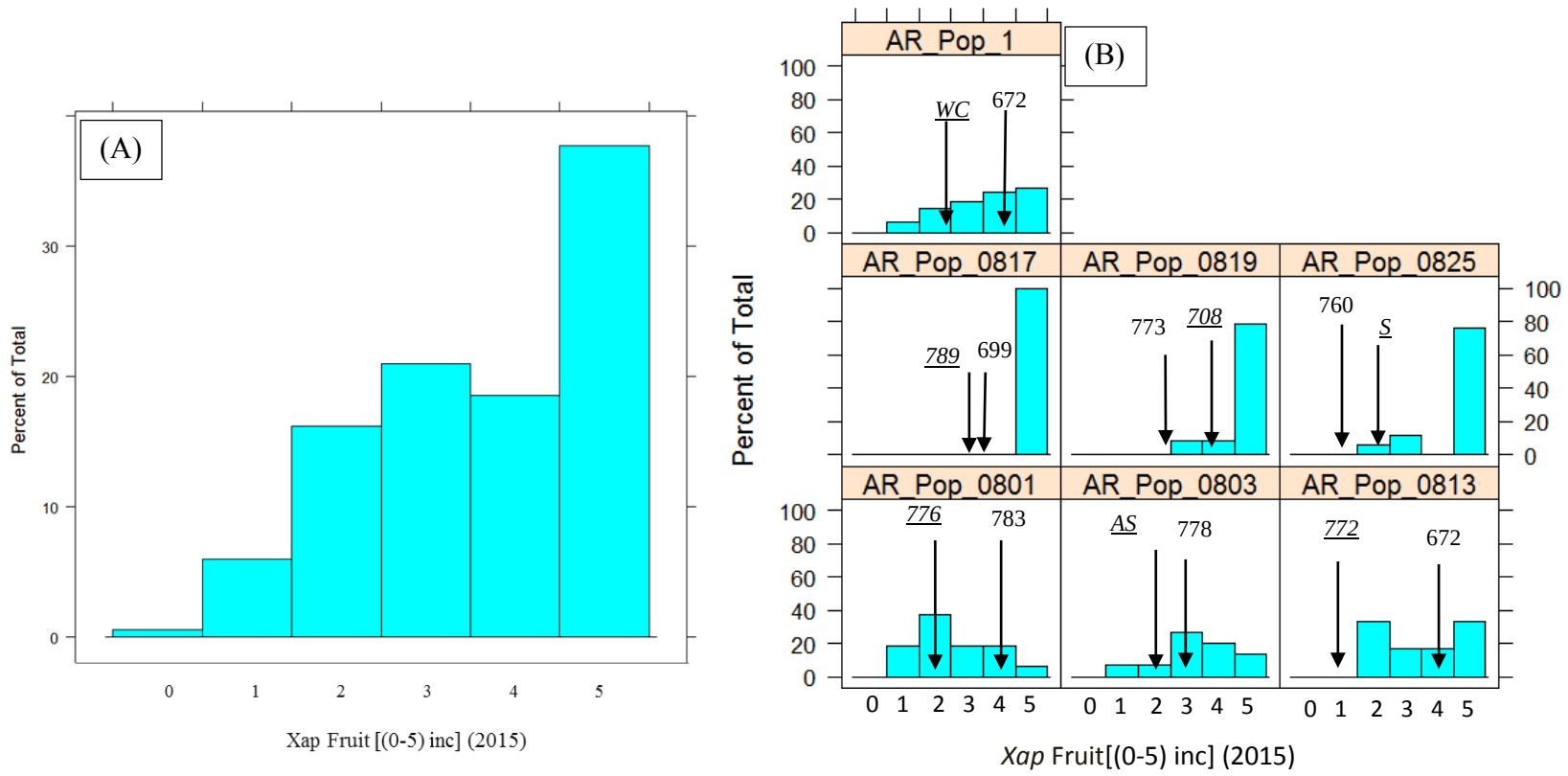

Fig. 20. Distribution (%) of *Xap* fruit [(0-5) inc] 2015 field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

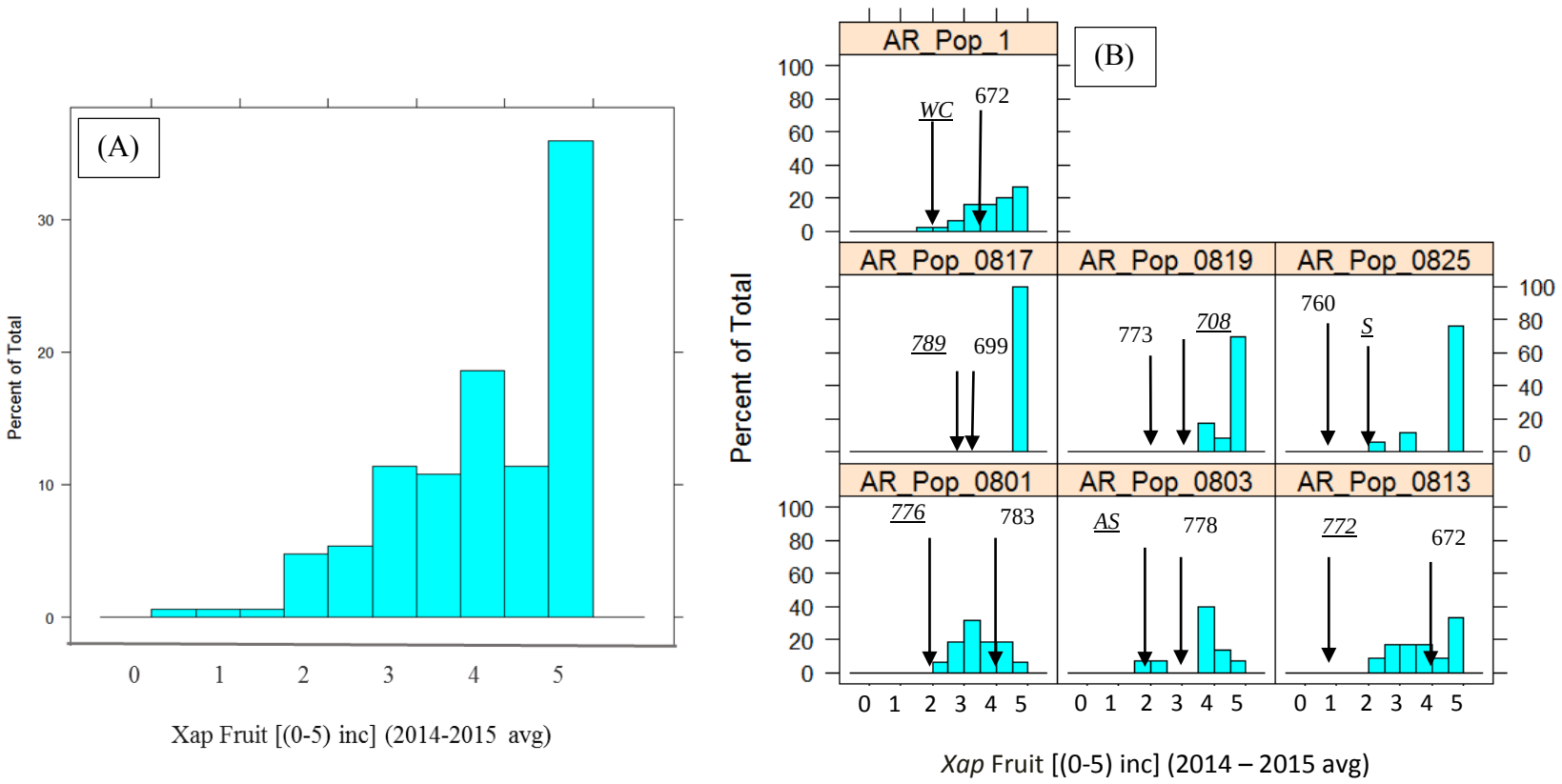

Fig. 21. Distribution (%) of *Xap* fruit [(0-5) inc] 2014-2015 avg field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

The *Xap* fruit [(0-5) ± 0.5 inc] ordinal ratings exhibited an average of 4.39 (2014), 3.64 (2015), and 4.02 (2014-2015 avg) for all individuals in the AR RosBREED pedigree (Table 3). The year-to-year ratings paralleled the general observations of *Xap* occurrence at FRS, as 2014 and 2015 were substantially humid and hot. The *Xap* fruit [(0-5) ± 0.5 inc] ratings ranged from 0.00 (2015) to 5.00 (2014, 2015, and 2014-2015 avg). The standard deviation displayed across all years ranged from 1.0 (2014-2015 avg) to 1.3 (2015) (Table 3).

For the seven F_1 populations, different segregation patterns for *Xap* fruit [(0-5) ± 0.5 inc] were observed across all years of the study (Figs. 22-24). In 2014, all populations segregated from medium to high susceptibility, excluding AR_Pop_0817 and AR_Pop_0801, which were nearly all scored as 5.00 (Fig. 22). In 2015, AR_Pop_1, AR_Pop_0801, and AR_Pop_0803 segregated from 1.00-5.00, and all other populations segregated from medium to high susceptibility, except for AR_Pop_0817, which all were scored as 5.00 (Fig. 23). In 2014-2015 avg, only AR_Pop_1, segregated from 2.00-5.00, and all other populations segregated from medium to high susceptibility, excluding AR_Pop_0817, which were all scored as 5.00 (Fig. 24). Parental values from each year are also included in Figs. 22-24.

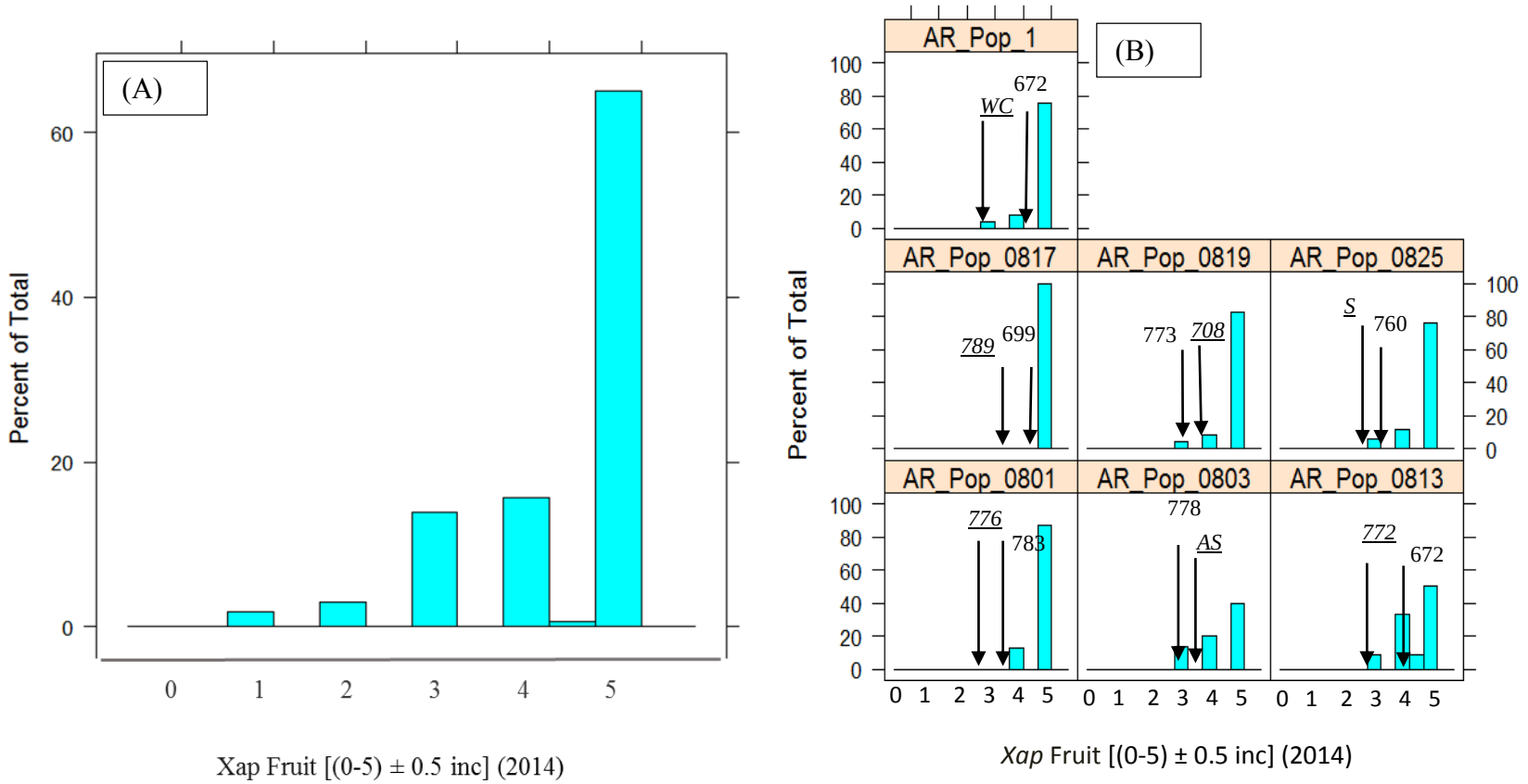

Fig. 22. Distribution (%) of *Xap* fruit [(0-5) ± 0.5 inc] 2014 field ratings for the entire AR RosBREED pedigree (A) (N=166), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

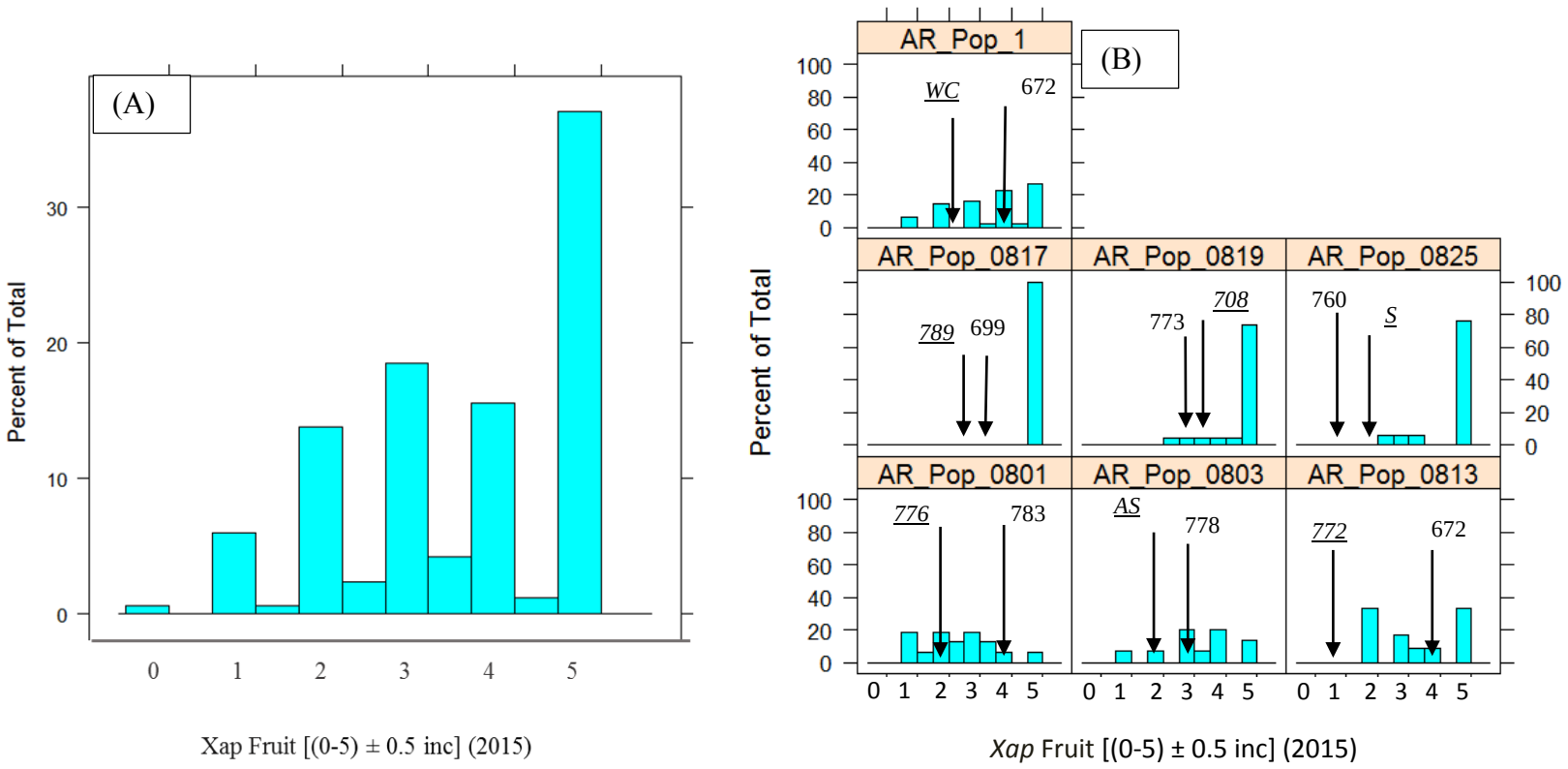

Fig. 23. Distribution (%) of *Xap* fruit [(0-5) ± 0.5 inc] 2015 field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

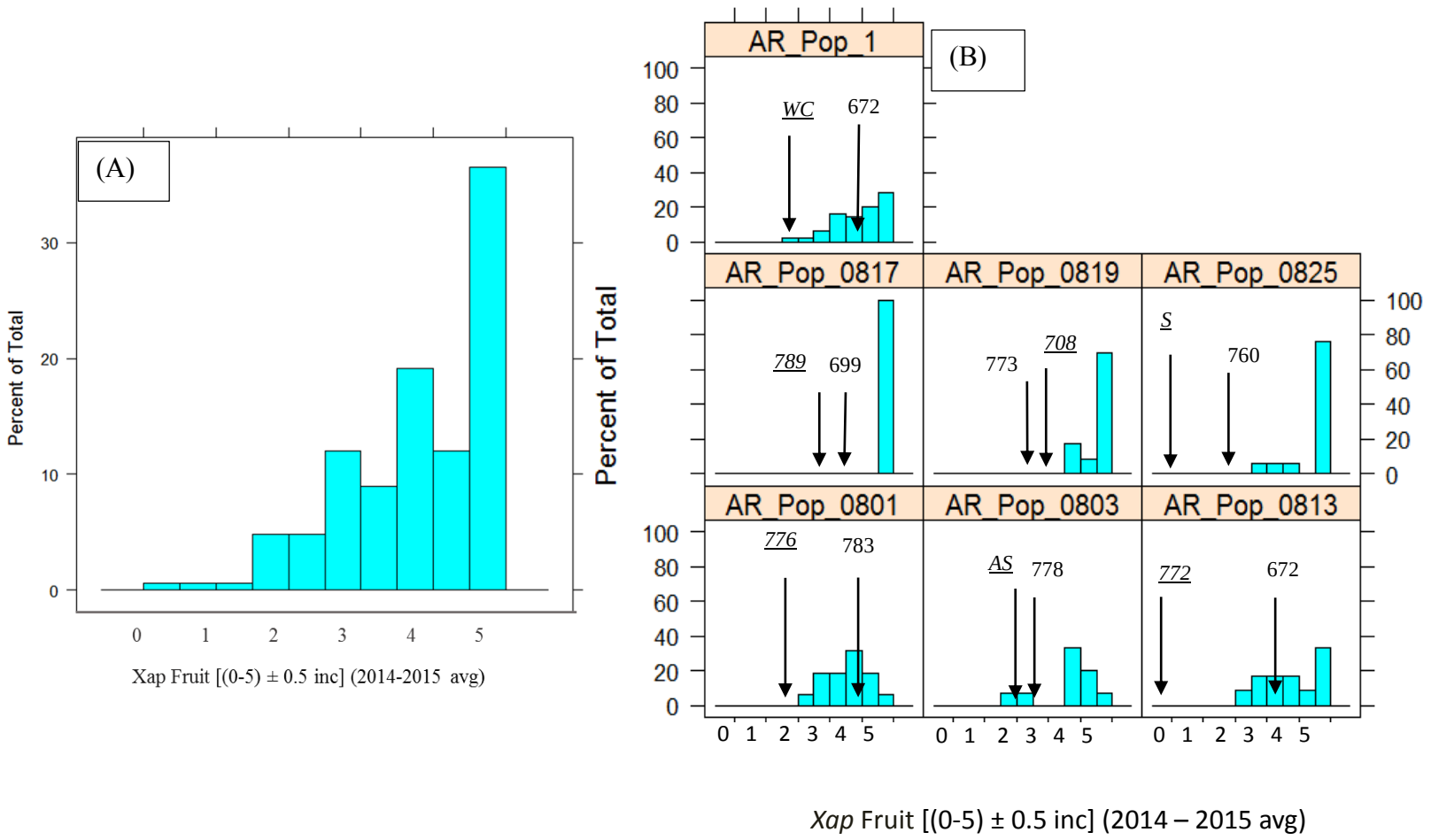

Fig. 24. Distribution (%) of *Xap* fruit [(0-5) ± 0.5 inc] 2014-2015 avg field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

The *Xap* fruit [(0-5) – 1 inc] ordinal ratings exhibited an average of 3.58 (2014), 2.83 (2015), and 3.20 (2014-2015 avg) for all individuals in the AR RosBREED pedigree (Table 3). The year-to-year ratings paralleled the general observations of *Xap* occurrence at FRS, as 2014 and 2015 were substantially humid and hot. The *Xap* fruit [(0-5) – 1 inc] ratings ranged from 0.00-5.00 (2014, 2015, and 2014-2015 avg). The standard deviation displayed across all years ranged from 1.1 (2014-2015 avg) to 1.4 (2015) (Table 3). In terms of the seven F_1 populations, different segregation patterns for *Xap* fruit [(0-5) – 1 inc] were observed across all years of the study (Figs. 25-27).

In terms of the seven F_1 populations, different segregation ratio patterns for *Xap* fruit [(0-5) – 1 inc] were observed across all years of the study (Figs. 25-27). In 2014, AR_Pop_1, AR_Pop_0813 and AR_Pop_0819 segregated from 2.00-5.00, while all other populations segregated from medium to high susceptibility, excluding AR_Pop_0817 which were all scored as 5.00 (Fig. 25). In 2015, AR_Pop_1, AR_Pop_0801, and AR_Pop_0803 segregated from 0.00-4.00. AR_Pop_0813 segregated from 1.00-5.00, and all other populations segregated from medium to high susceptibility, except for AR_Pop_0817, which were all scored as 5.00 (Fig. 26). In 2014-2015 avg, AR_Pop_1 and AR_Pop_0803 segregated from 0.00-4.00, and AR_Pop_0813 segregated from 1.00-5.00. All other populations segregated within medium susceptibility, excluding AR_Pop_0817, which were all scored as 5.00 (Fig. 27). Parental values from each year are also included in Figs. 25-27.

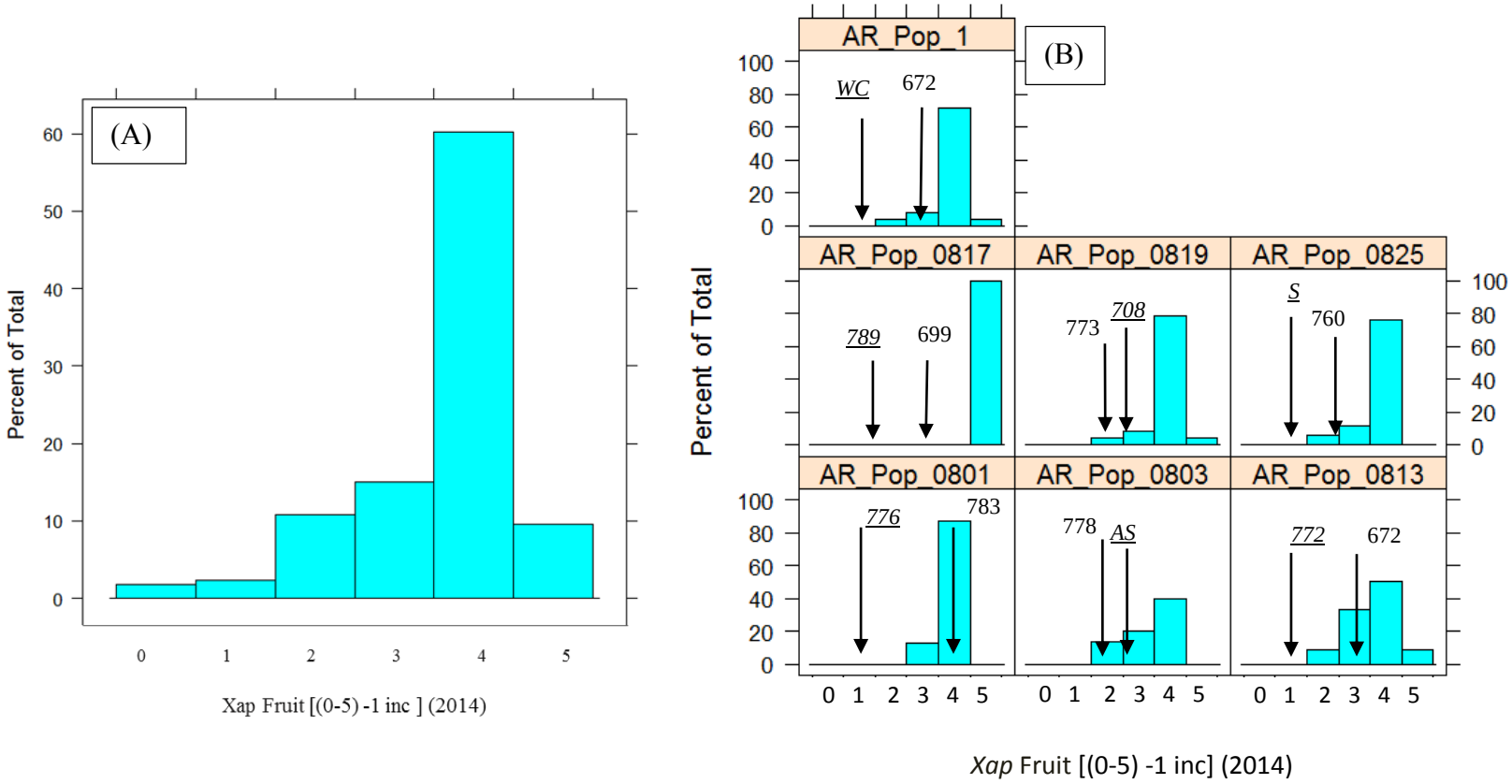

Fig. 25. Distribution (%) of *Xap* fruit [(0-5) – 1 inc] 2014 field ratings for the entire AR RosBREED pedigree (A) (N=166), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

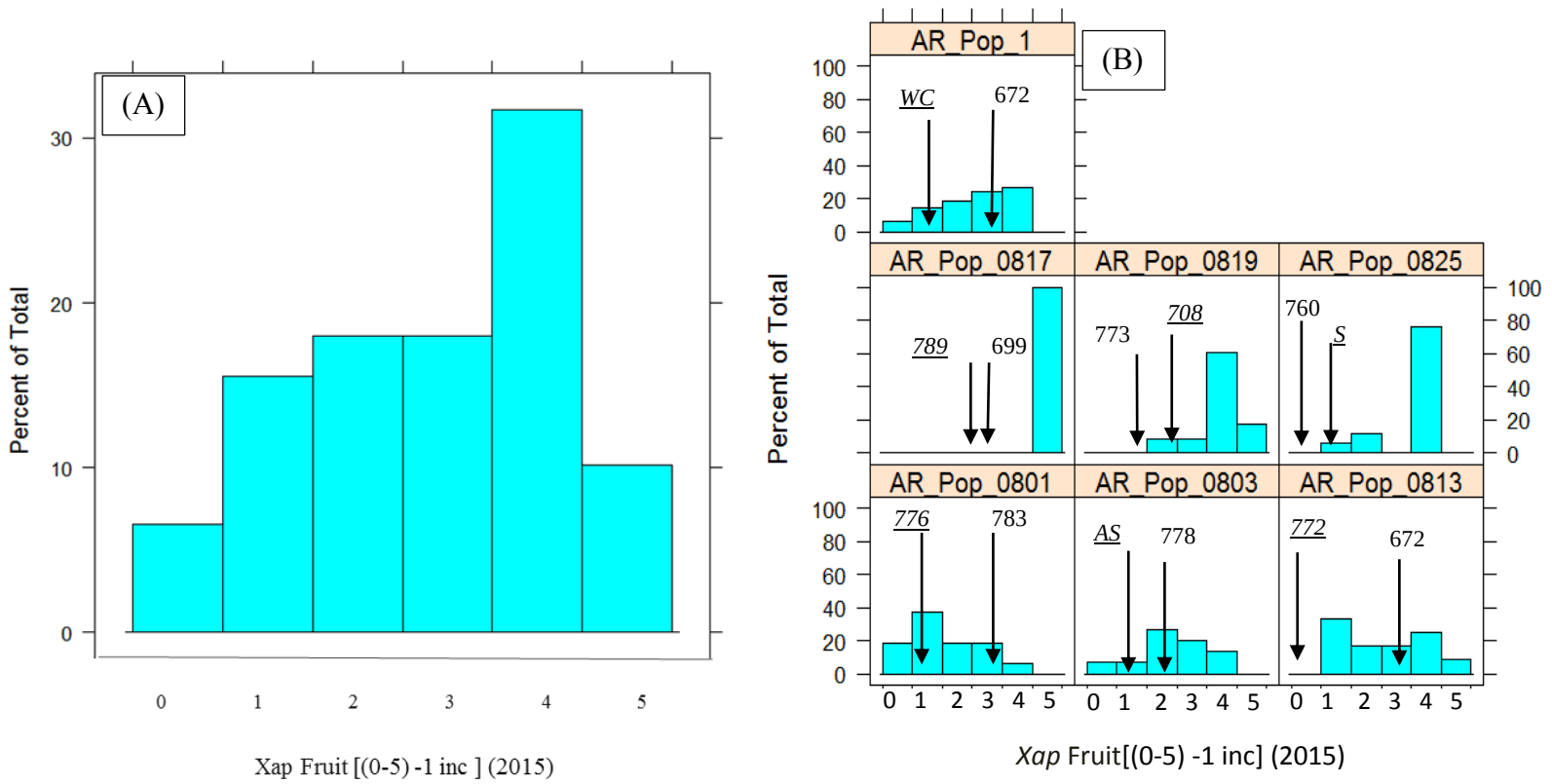

Fig. 26. Distribution (%) of *Xap* fruit [(0-5) – 1 inc] 2015 field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

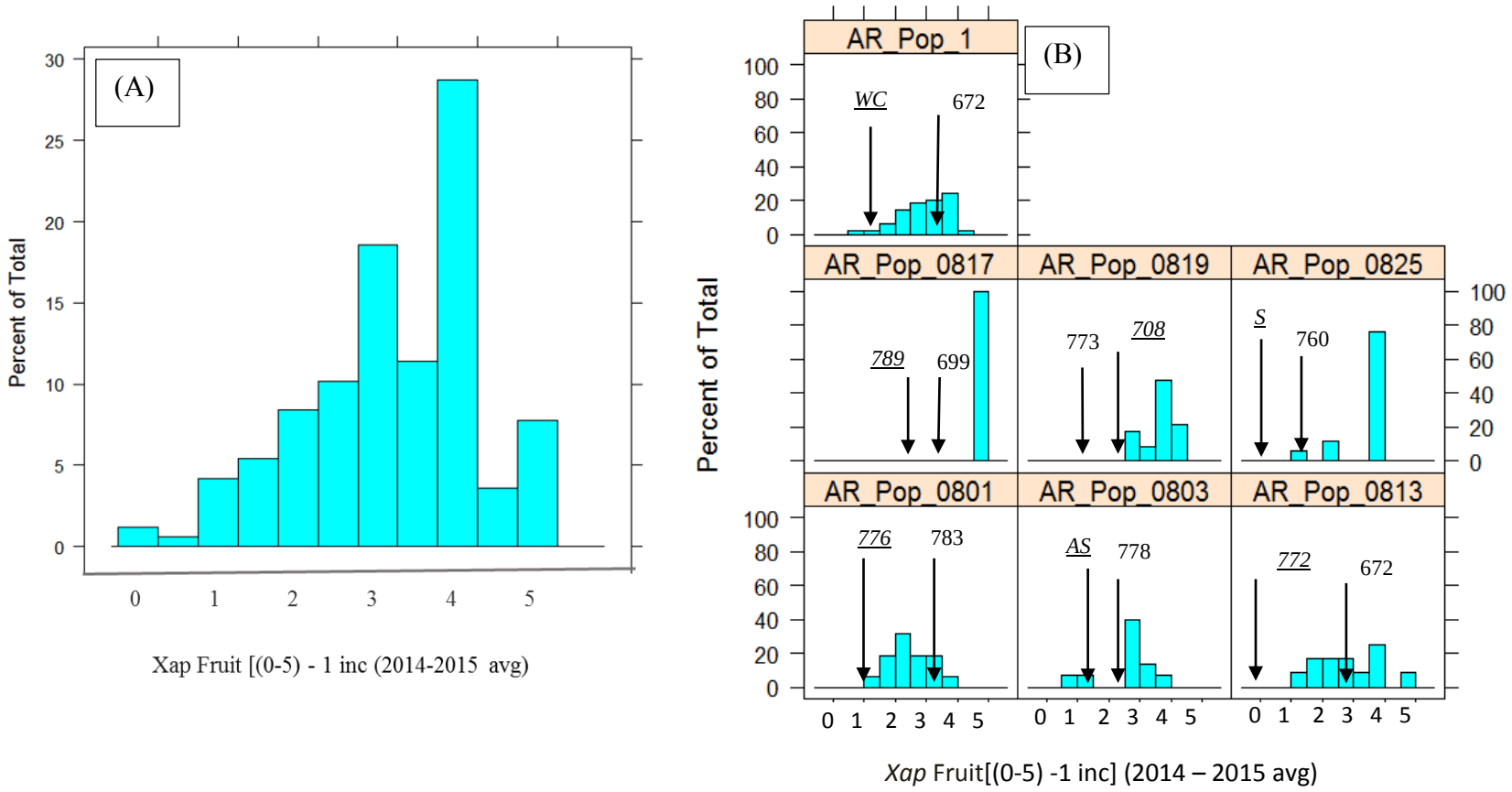

Fig. 27. Distribution (%) of *Xap* fruit [(0-5) – 1 inc] 2013-2015 avg field ratings for the entire AR RosBREED pedigree (A) (N=167), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

Xap Leaf Field Data Sets (2013-2015)

The *Xap* leaf (0-5) ratings exhibited an average of 2.65 (2013), 3.81 (2014), 3.47 (2015), and 3.33 (2013-2015 avg) for all individuals in the AR RosBREED pedigree (Table 4). As with Xap fruit ratings, the year-to-year ratings paralleled the general observations of *Xap* occurrence at FRS, and reflect the weather pattern for the years particularly for 2014 and 2015 which were substantially more humid, and hotter than 2013.The *Xap* leaf (0-5) ratings ranged from 0.00-5.00 (2013; all years). The standard deviation displayed across all years ranged from 1.03 (2013-2015 avg) to 1.48 (2013) (Table 4).

The seven F_1 populations showed different segregation patterns for *Xap* leaf (0-5) across all years of the study (Figs. 28-31). In 2013, AR_Pop_1, and AR_Pop_0803 segregated from 0.00-5.00 (Fig. 28). In contrast, AR_Pop_0801 segregated from medium to low susceptibility, while all the other populations segregated from medium to high susceptibility. In 2014, AR_Pop_1, AR_Pop_0801, AR_Pop_0813, and AR_Pop_0825 segregated from 2.00-5.00, and all other populations segregated from medium to high susceptibility, excluding AR_Pop_0817, which were all scored as 5.00 (Fig. 29). In 2015, AR_Pop_1, and AR_Pop_0803 segregated from 2.00-5.00. In contrast, AR_Pop_0801 segregated from medium to low susceptibility, while all the other populations segregated from medium to high susceptibility, except for AR_Pop_0817, which were all scored as 5.00 (Fig. 30). In 2013-2015 avg, AR_Pop_1 segregated from 1.00-5.00, and all other populations segregated from medium to high susceptibility, excluding AR_Pop_0801 which segregated from medium to low susceptibility (Fig. 31). Parental values from each year are also included in Figs. 28-31.

Table 4. Mean, minimum, maximum, and standard deviation of *Xap* leaf (0-5), [(0-5) ± 0.5], and [(0-5) – 1], for 2013, 2014, 2015, and 2013-2015 avg across the entire AR RosBREED pedigree (N is number of individuals).

Trait	Year	Mean	Min.	Max.	Std. dev.	N
Xap leaf (0-5)	2013	2.65	0.00	5.00	1.48	170
	2014	3.81	1.00	5.00	1.11	172
	2015	3.47	1.00	5.00	1.09	172
	2013-2015 avg	3.33	1.00	5.00	1.03	173
Xap leaf [(0-5) ± 0.5]	2013	2.71	0.00	5.50	1.52	170
	2014	3.81	1.00	5.00	1.11	172
	2015	3.46	1.00	5.00	1.11	172
	2013-2015 avg	3.34	1.00	5.20	1.05	172
Xap leaf [(0-5) – 1]	2013	1.79	0.00	5.00	1.40	170
	2014	2.99	0.00	5.00	1.24	172
	2015	2.62	0.00	5.00	1.24	172
	2013-2015 avg	2.50	0.00	5.00	1.12	173

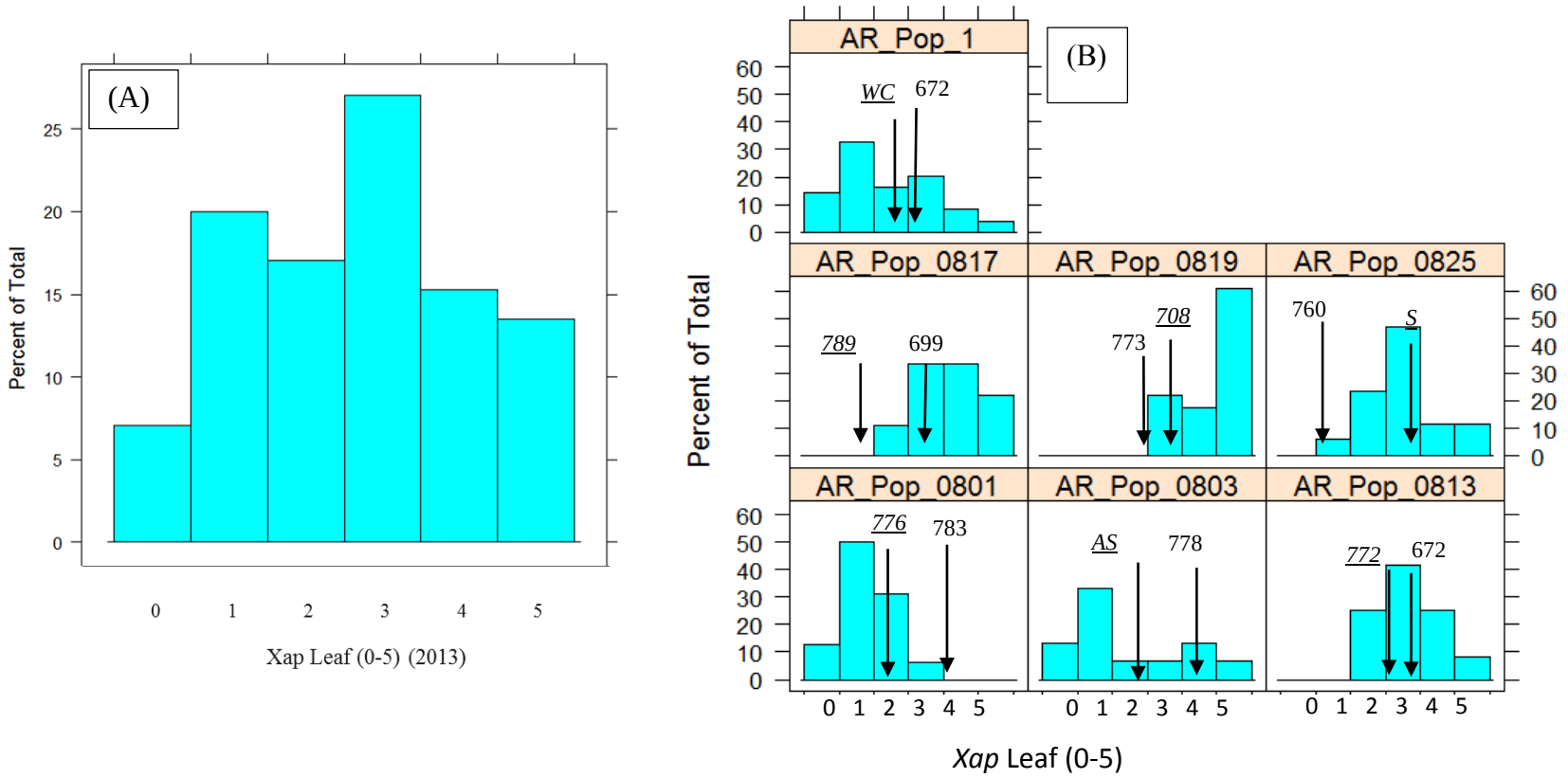

Fig. 28. Distribution (%) of *Xap* leaf (0-5) 2013 field ratings for the entire AR RosBREED pedigree (A) (N=170), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

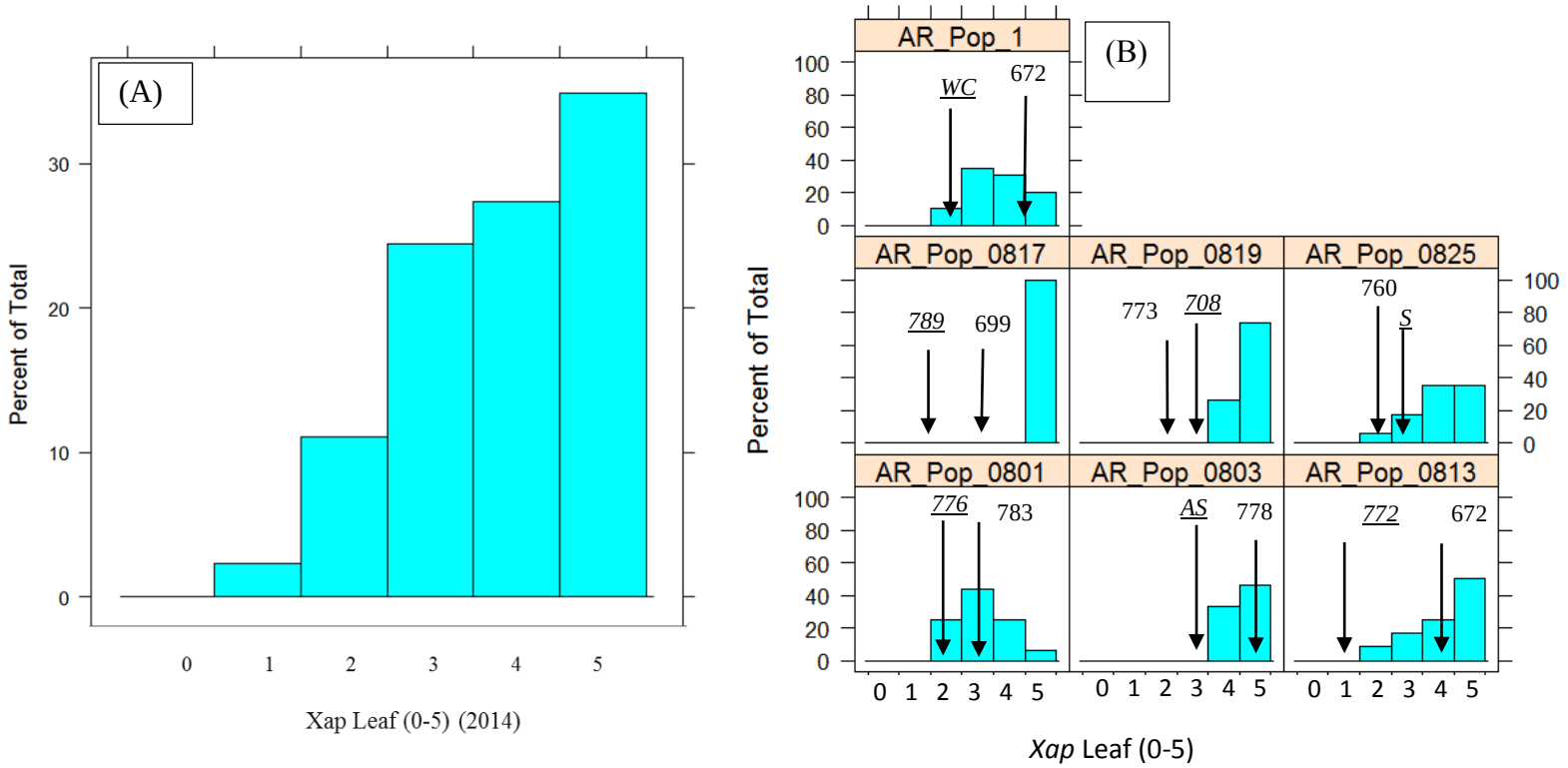

Fig. 29. Distribution (%) of *Xap* leaf (0-5) 2014 field ratings for the entire AR RosBREED pedigree (A) (N=172), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

.

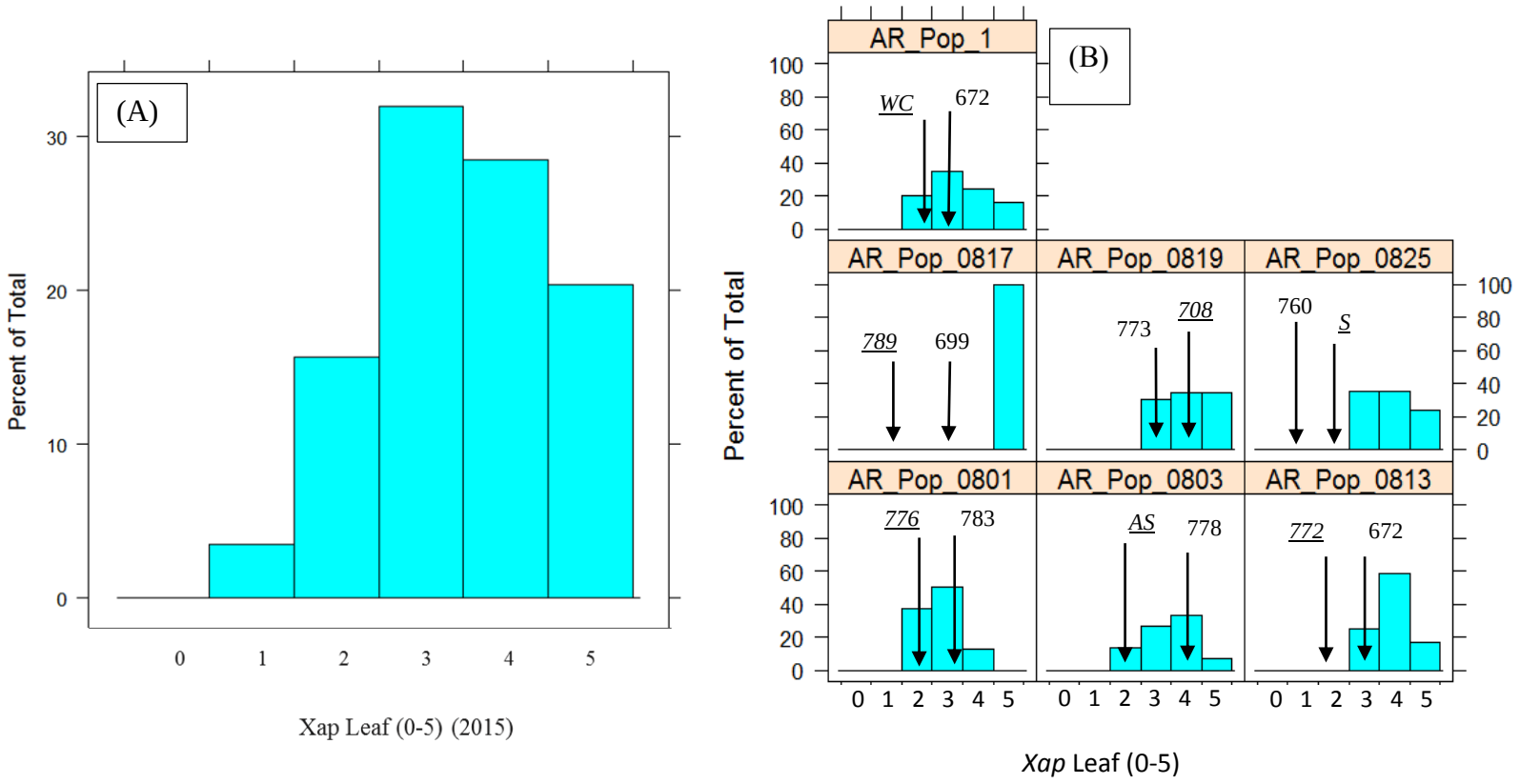

Fig. 30. Distribution (%) of *Xap* leaf (0-5) 2015 field ratings for the entire AR RosBREED pedigree (A) (N=172), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

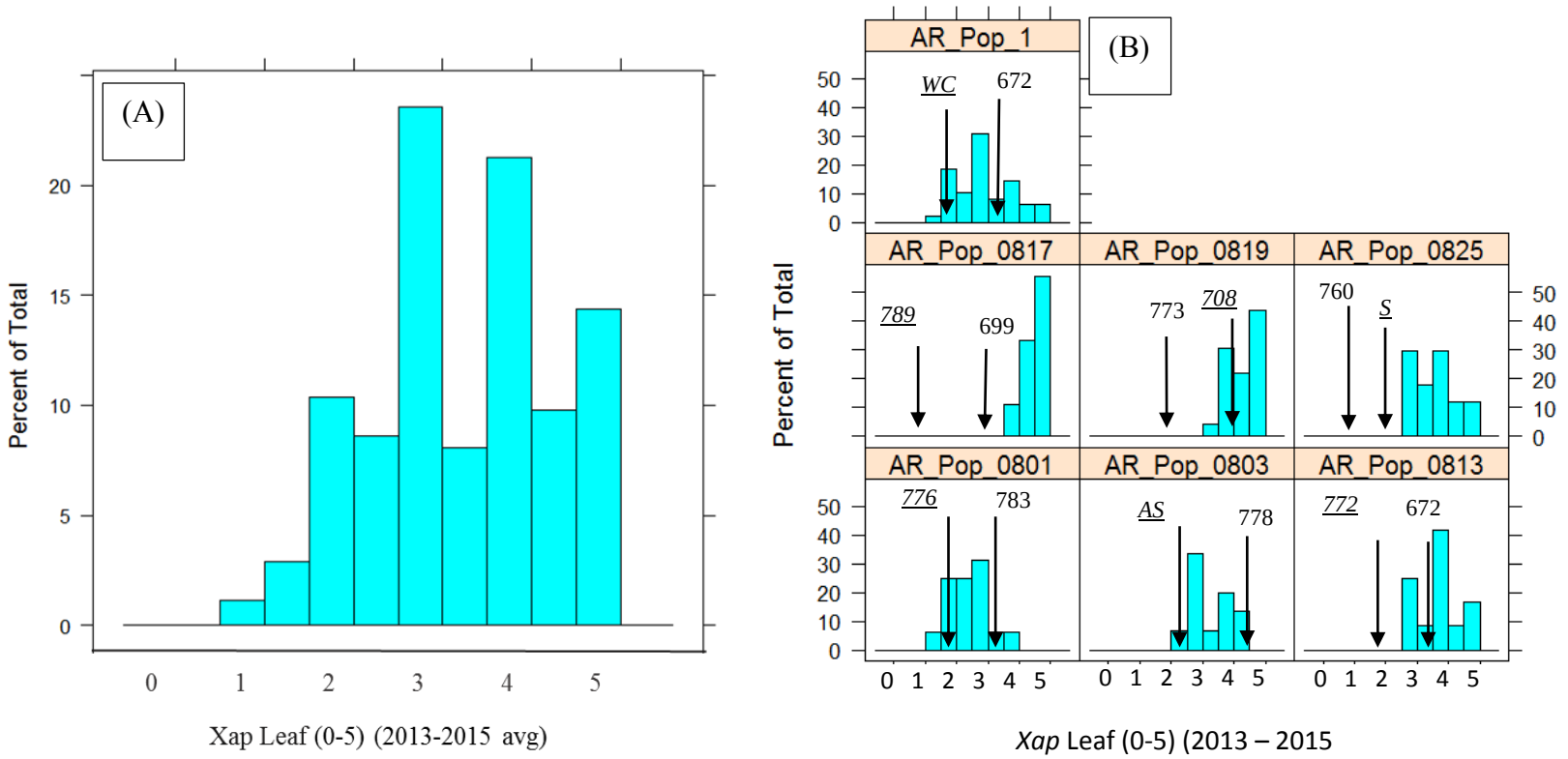

Fig. 31. Distribution (%) of *Xap* leaf (0-5) 2013-2015 avg field ratings for the entire AR RosBREED pedigree (A) (N=173), and the seven F_1 populations (parental values illustrated by arrows; *female parent* is italicized and underlined) (B).

The *Xap* leaf [(0-5) ± 0.5] ratings exhibited an average of 2.71 (2013), 3.81 (2014), 3.46 (2015), and 3.34 (2013-2015 avg) for all individuals in the AR RosBREED pedigree (Table 4). The year-to-year ratings paralleled the general observations of *Xap* occurrence at FRS, and reflect the weather pattern for the years particularly for 2014 and 2015 which were substantially more humid, and hotter than 2013. The *Xap* leaf [(0-5) ± 0.5] ratings ranged from 0.00-5.50 (2013). The standard deviation displayed across all years ranged from 1.04 (2013-2015 avg) to 1.51 (2013) (Table 4). For the seven F_1 populations, different segregation patterns for *Xap* leaf [(0-5) ± 0.5] were observed across all years of the study (Figs. 32-35).

In terms of the seven F_1 populations, different segregation patterns for *Xap* leaf [(0-5) ± 0.5] were observed across all years of the study (Figs. 32-35). In 2013, AR_Pop_1, AR_Pop_0803, and AR_Pop_0825 all segregated from 0.00-5.50 (Fig. 32). In contrast, and all other populations segregated from medium to high susceptibility, excluding AR_Pop_0801 which segregated from medium to low susceptibility. In 2014, AR_Pop_1, AR_Pop_0801, AR_Pop_0813, and AR_Pop_0825 segregated from 2.00-5.00, and all other populations segregated from medium to high susceptibility, excluding AR_Pop_0817, which were all scored as 5.00 (Fig. 33). In 2015, only AR_Pop_1, segregated from 1.00-5.00, and all other populations segregated from medium to high susceptibility, excluding for AR_Pop_0801 and AR_Pop_0803 which segregated within medium susceptibility and AR_Pop_0817 where again all seedlings were scored as 5.00 (Fig. 34). In 2013-2015 avg, AR_Pop_1 segregated from 1.00-5.00, and AR_Pop_1 segregated from medium to low susceptibility, while all other populations segregated from medium to high susceptibility (Fig. 35). Parental values from each year are also included in Figs. 32-35.

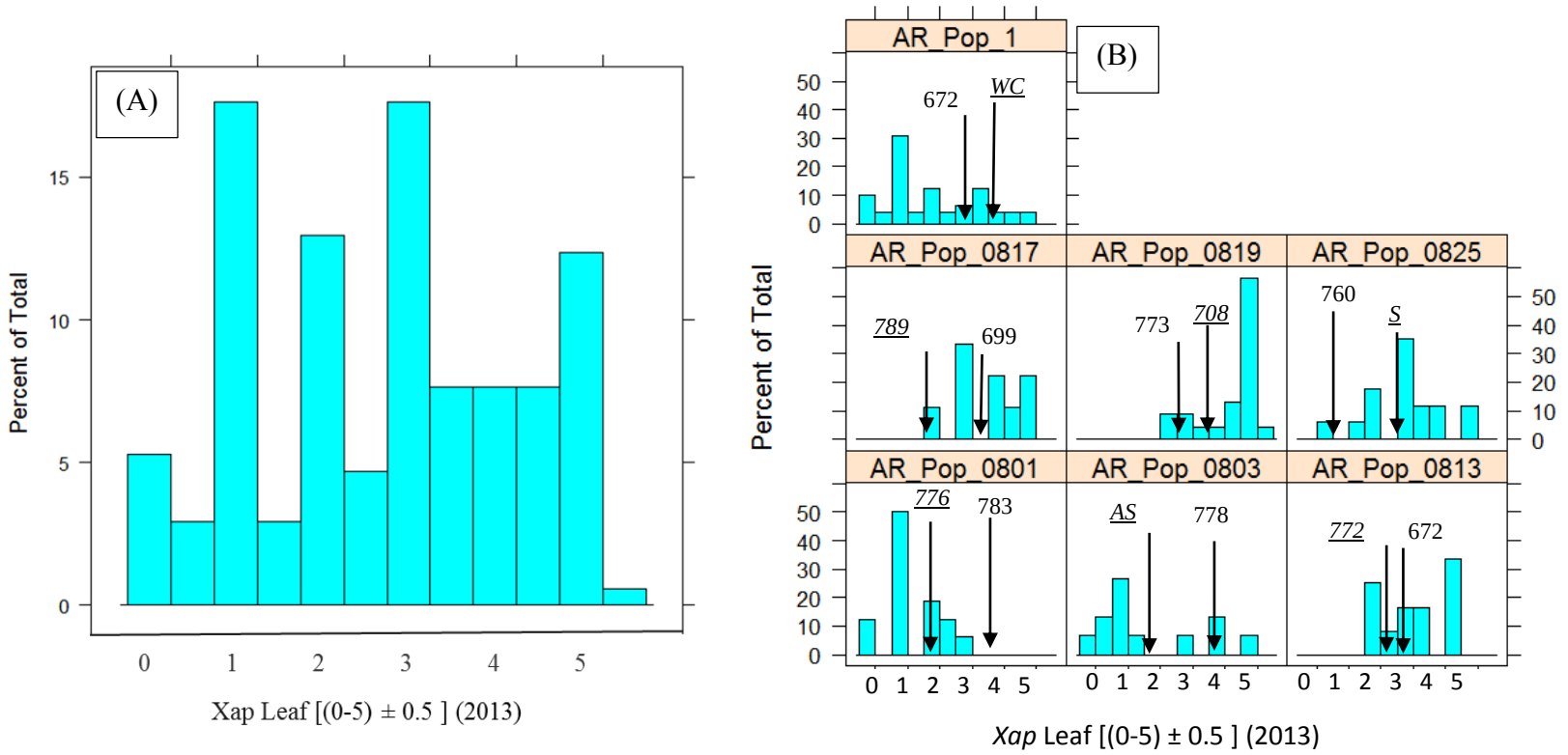

Fig. 32. Distribution (%) of *Xap* leaf [(0-5) ± 0.5] 2013 (field ratings for the entire AR RosBREED pedigree (A) (N=170), and the seven F_1 populations (parental values illustrated by arrows; *female parent* is italicized and underlined) (B).

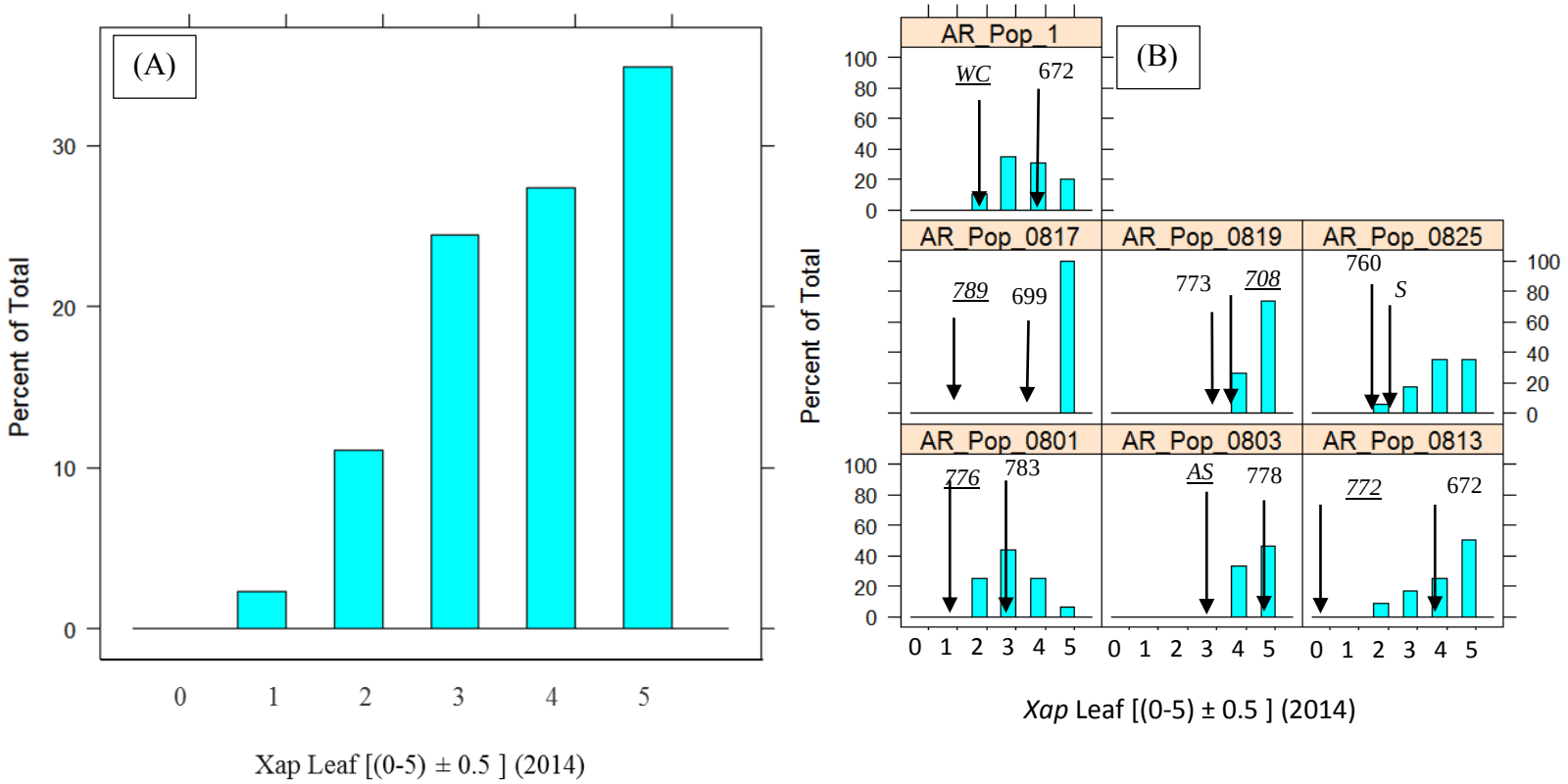

Fig. 33. Distribution (%) of *Xap* leaf [(0-5) ± 0.5] 2014 field ratings for the entire AR RosBREED pedigree (A) (N=172), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

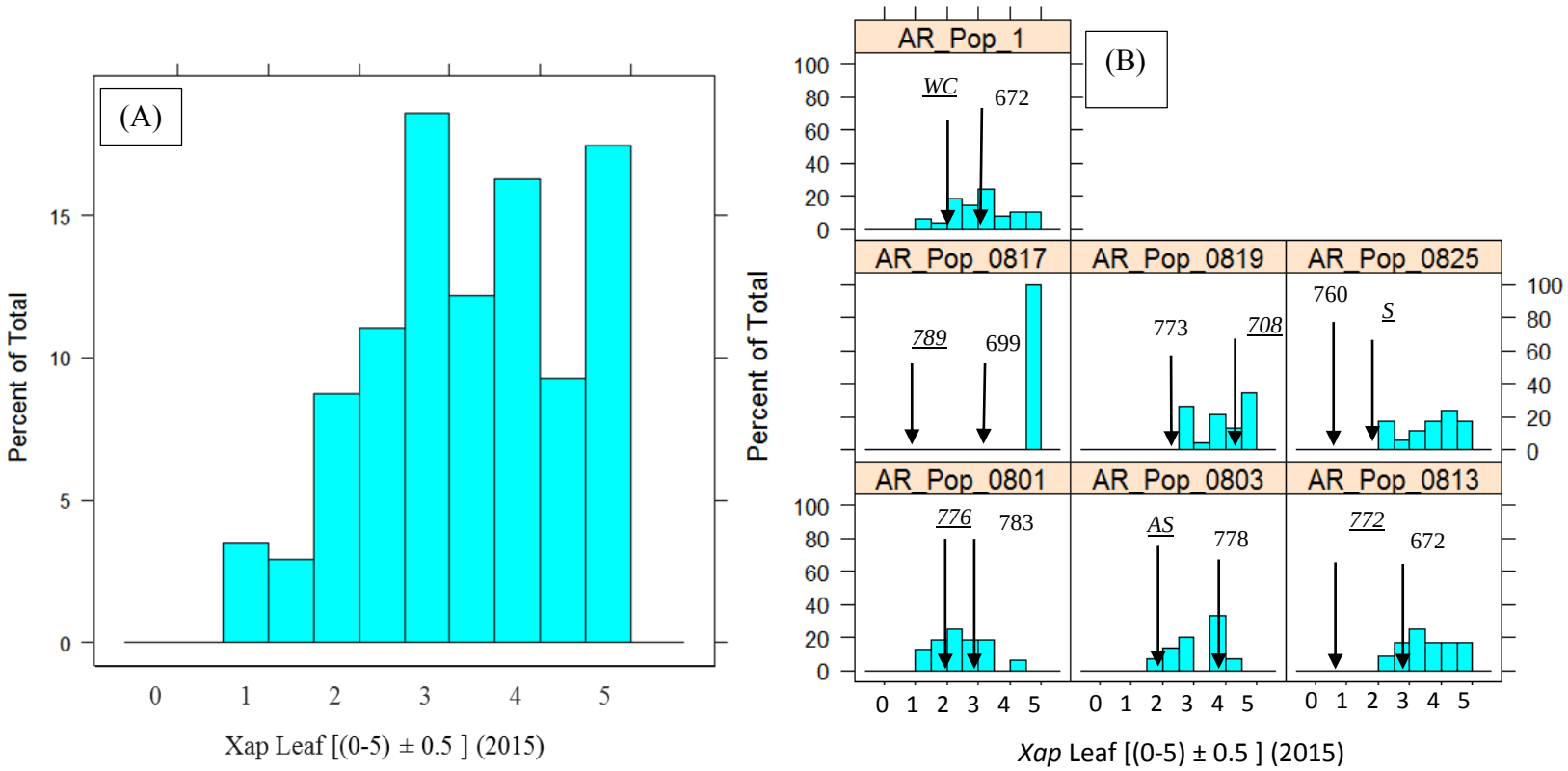

Fig. 34. Distribution (%) of *Xap* leaf [(0-5) ± 0.5] 2015 field ratings for the entire AR RosBREED pedigree (A) (N=172), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

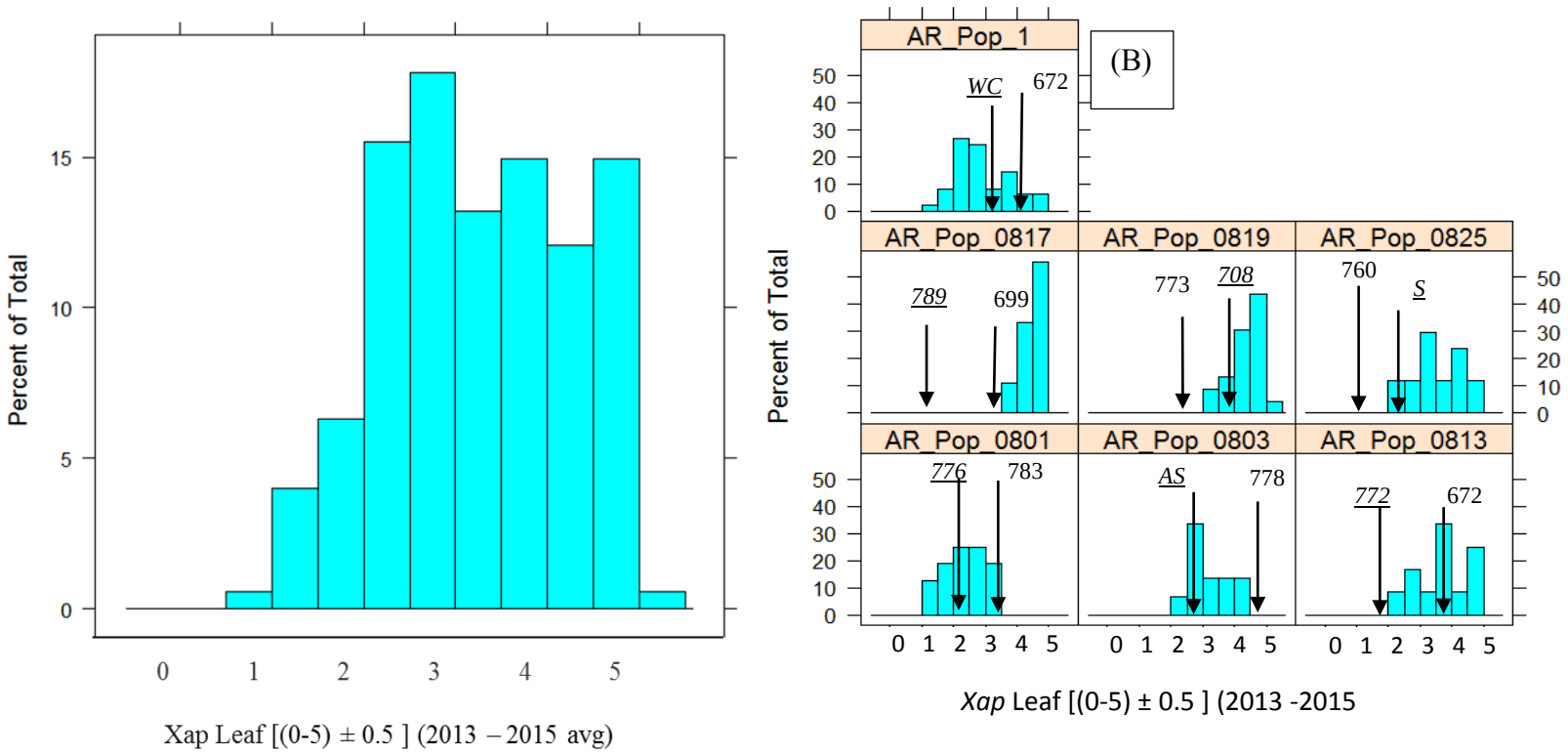

Fig. 35. Distribution (%) of *Xap* leaf [(0-5) ± 0.5] 2013-2015 avg field ratings for the entire AR RosBREED pedigree (A) (N=173), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

The *Xap* leaf [(0-5) – 1] ratings exhibited an average of 1.79 (2013), 2.99 (2014), 2.62 (2015), and 2.50 (2013-2015 avg) for all individuals in the AR RosBREED pedigree (Table 4). The year-to-year ratings paralleled the general observations of *Xap* occurrence at FRS, and reflect the weather pattern for the years particularly for 2014 and 2015 which were substantially more humid, and hotter than 2013. The *Xap* leaf [(0-5) – 1] ratings ranged from 0.00-5.00 (all years). The standard deviation displayed across all years ranged from 1.12 (2013-2015 avg) to 1.41 (2013) (Table 4). In terms of the seven F_1 populations, different segregation patterns for *Xap* leaf [(0-5) – 1] were observed across all years of the study (Figs. 36-39).

In terms of the seven F_1 populations, different segregation patterns for *Xap* leaf [(0-5) – 1] were observed across all years of the study (Figs. 36-39). In 2013, AR_Pop_1, AR_Pop_0803, and AR_Pop_0825 all segregated from 0.00-4.00 (Fig. 36). In contrast, all other populations segregated from medium to high susceptibility, excluding AR_Pop_0801 which segregated from medium to low susceptibility. In 2014, only AR_Pop_0813 segregated from 1.00-5.00, while AR_Pop_1, AR_Pop_0801, and AR_Pop_0825 segregated from medium to low susceptibility, and AR_Pop_0803 and AR_Pop_0819 segregated from medium to high susceptibility (Fig. 37). Once again, all seedlings in AR_Pop_0817 were scored as 5.00. In 2015, AR_Pop_1, AR_Pop_0801, and AR_Pop_0803 all segregated from medium to low susceptibility, and AR_Pop_0813, AR_Pop_0819, and AR_Pop_0825 all segregated within medium susceptibility (Fig. 38). As seen before, all seedlings in AR_Pop_0817 were scored as 5.00. In 2013-2015 avg, AR_Pop_1 segregated from 1.00-4.00, and AR_Pop_0801 segregated from medium to low susceptibility (Fig. 39). AR_Pop_0803 segregated within medium susceptibility, while all other populations segregated from medium to high susceptibility. Parental values from each year are also included in Figs. 36-39.

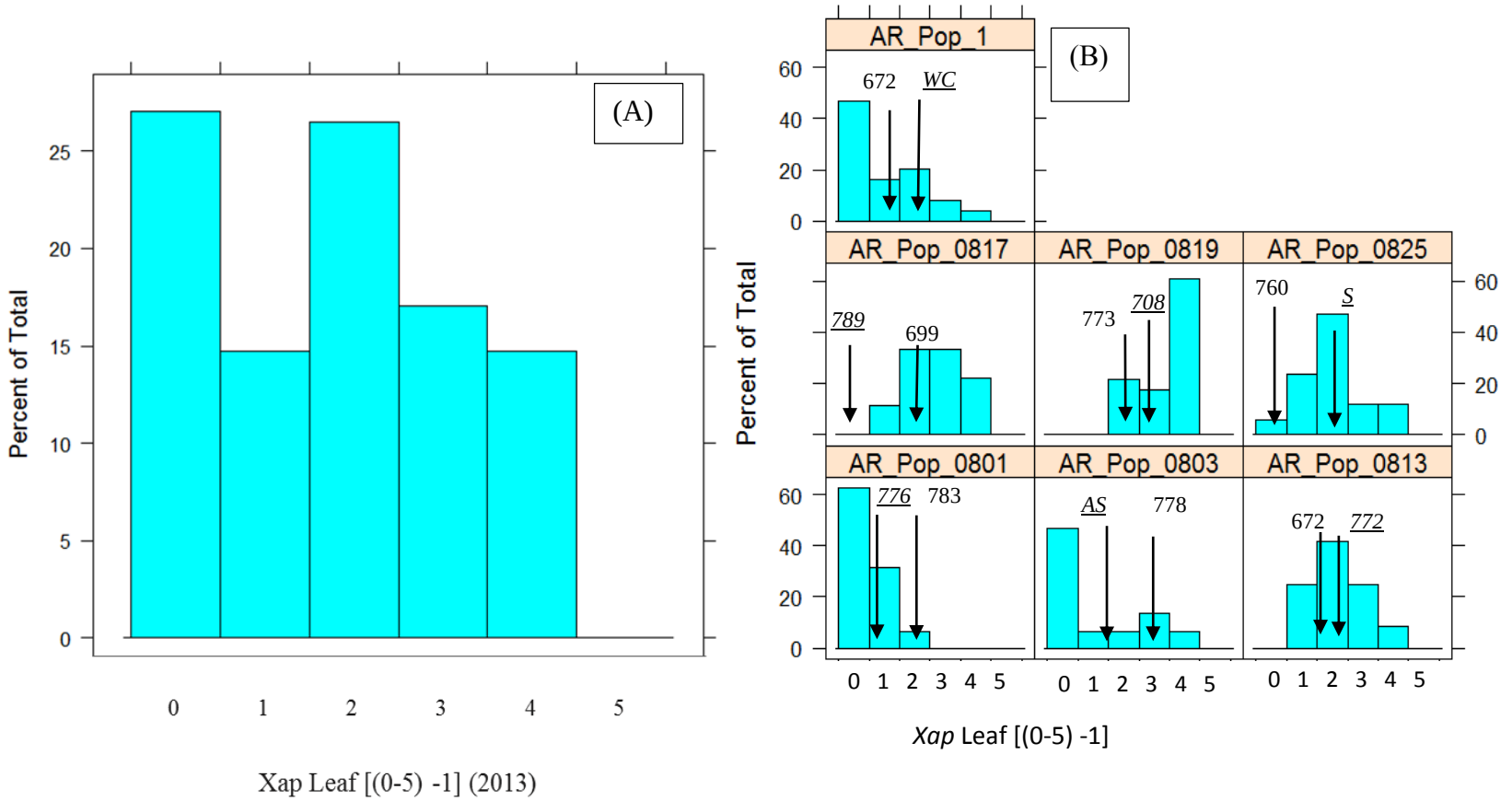

Fig. 36. Distribution (%) of *Xap* leaf [(0-5) – 1] 2013 field ratings for the entire AR RosBREED pedigree (A) (N=170), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

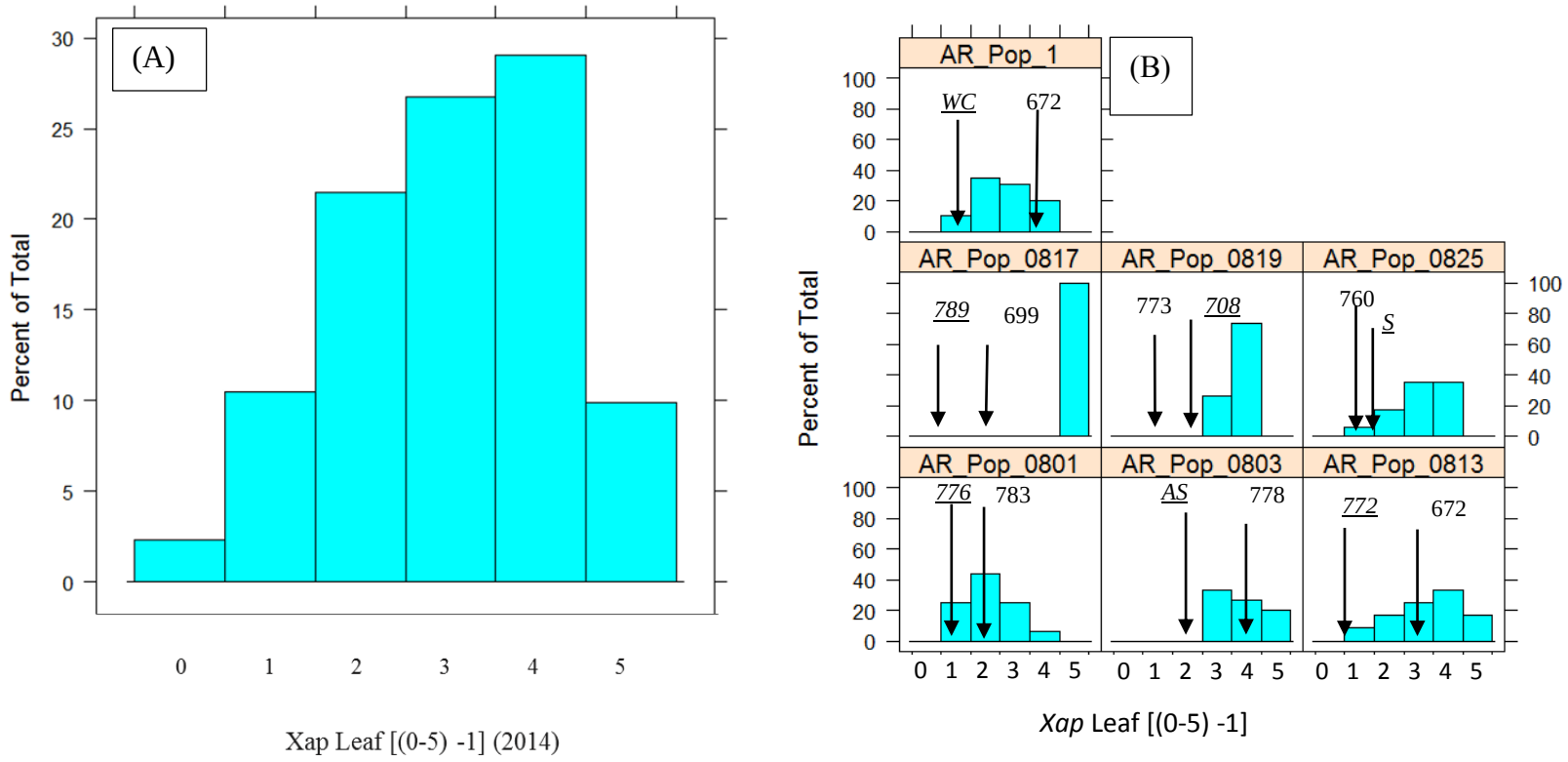

Fig. 37. Distribution (%) of *Xap* leaf [(0-5) – 1] 2014 field ratings for the entire AR RosBREED pedigree (A) (N=172), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

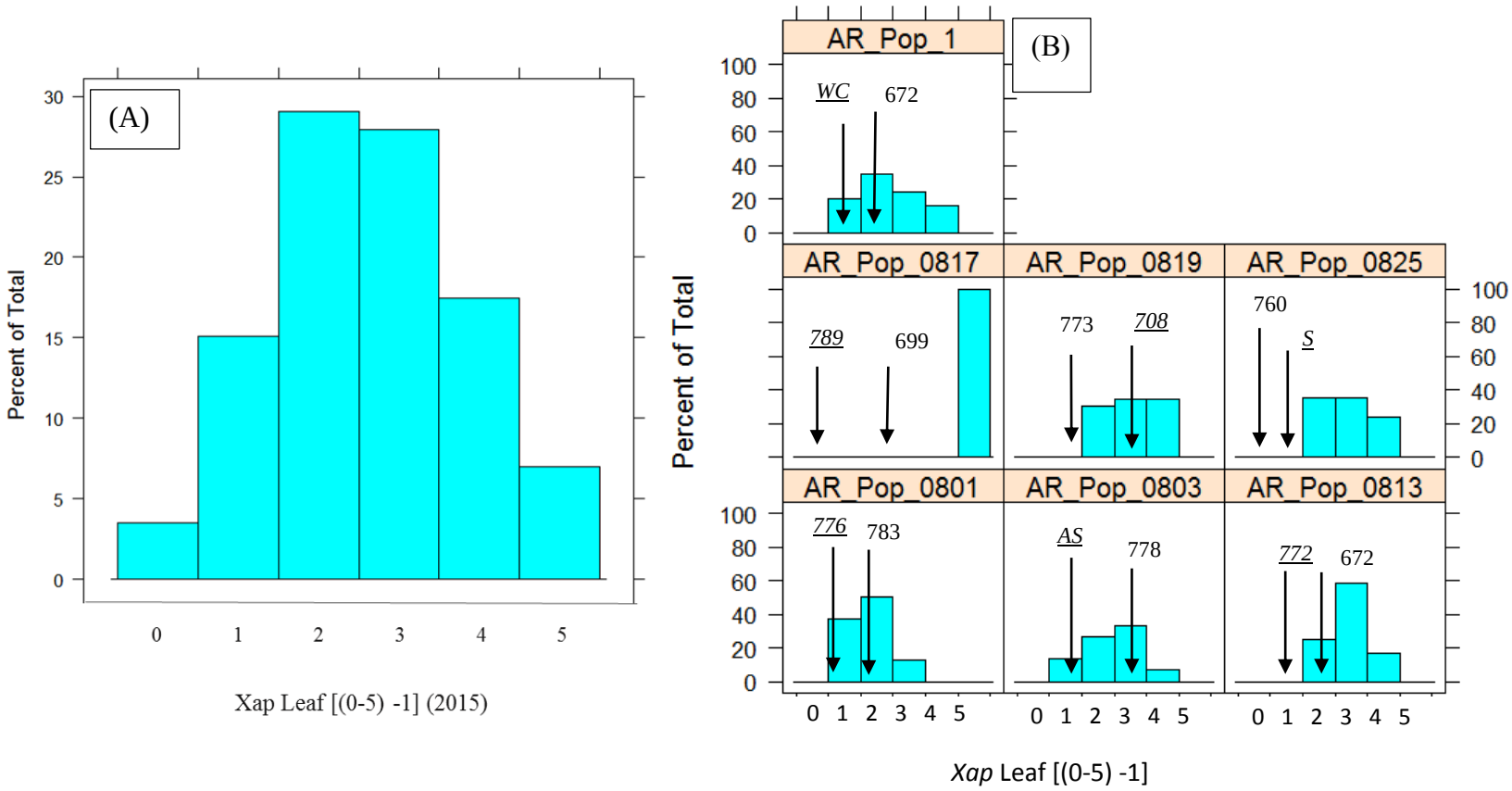

Fig. 38. Distribution (%) of *Xap* leaf [(0-5) – 1] 2015 field ratings for the entire AR RosBREED pedigree (A) (N=172), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

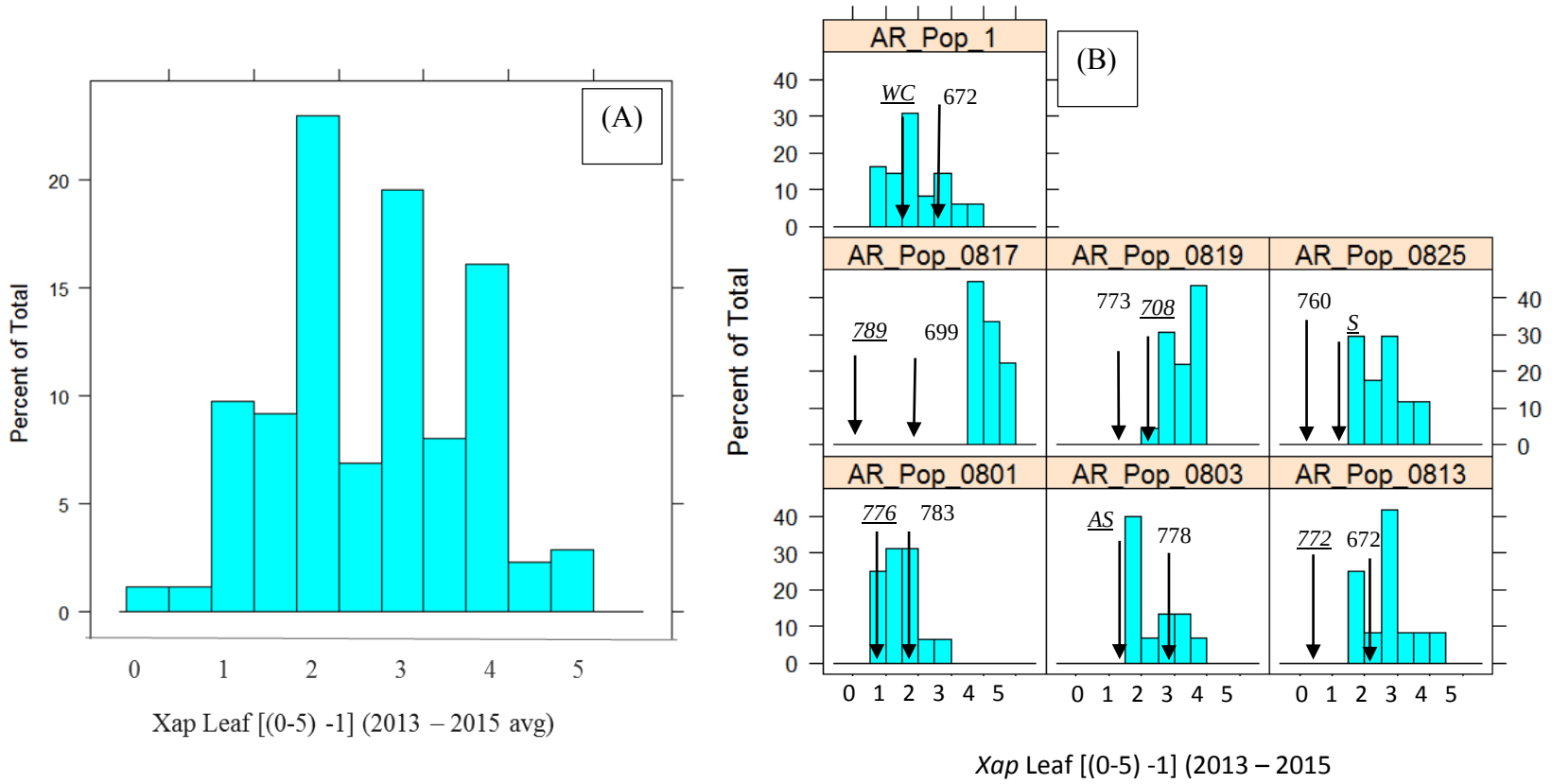

Fig. 39. Distribution (%) of *Xap* leaf [(0-5) – 1] 2013-2015 avg field ratings for the entire AR RosBREED pedigree (A) (N=173), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

Xap Leaf Assay Data Sets

The *Xap* leaf assay AR resistance (*Xap*L_{AR}) (0-3) ratings exhibited an average of 1.92 (2014), for all individuals in the AR RosBREED pedigree (Table 5). The ratings ranged from 0.80-2.90, and showed a standard deviation of 0.20 (Table 4).

In terms of the seven F_1 populations, different segregation patterns were observed for each population. AR_Pop_1, AR_Pop_0801, AR_Pop_0803, and AR_Pop_0825 each segregated from low to high susceptibility, ~1.00-2.50 (Fig. 40). In contrast, AR_Pop_0813, AR_Pop_0817, and AR_Pop_0819 each segregated from medium to high susceptibility, ~2.00-3.00. Parental values for *Xap*L_{AR} are also included in Fig. 40.

The *Xap* leaf assay 88 resistance (*Xap*L_{88}) (0-3) ratings exhibited an average of 2.50 (2014), for all individuals in the AR RosBREED pedigree (Table 4; Fig. 41). The ratings ranged from 2.10-3.00 (all years), and showed a standard deviation of 0.05 (Table 4; Fig. 41).

In terms of the seven F_1 populations, almost all populations segregated from medium to high susceptibility. AR_Pop_1, AR_Pop_0801, AR_Pop_0803, AR_Pop_0813, AR_Pop_0819 and AR_Pop_0825 each segregated from medium to high susceptibility, ~2.10-3.00 (Fig. 41). In contrast all AR_Pop_0817 seedlings were scored as highly susceptible, ~2.50-3.00. Parental values for *Xap*L_{88} are also included in Fig. 41.

Table 5. Mean, minimum, maximum, and standard deviation of *Xap* leaf assay 88 resistance (*Xap*L_{88}) and *Xap* leaf assay AR resistance (*Xap*L_{AR})**,** for the entire AR RosBREED pedigree, 2014 (N is number of individuals) (0-3 scale).

Trait	Mean	Min.	Max.	Std. dev.	N
XapL_{AR}	1.92	0.80	2.90	0.20	154
XapL_{88}	2.50	2.10	3.00	0.05	155

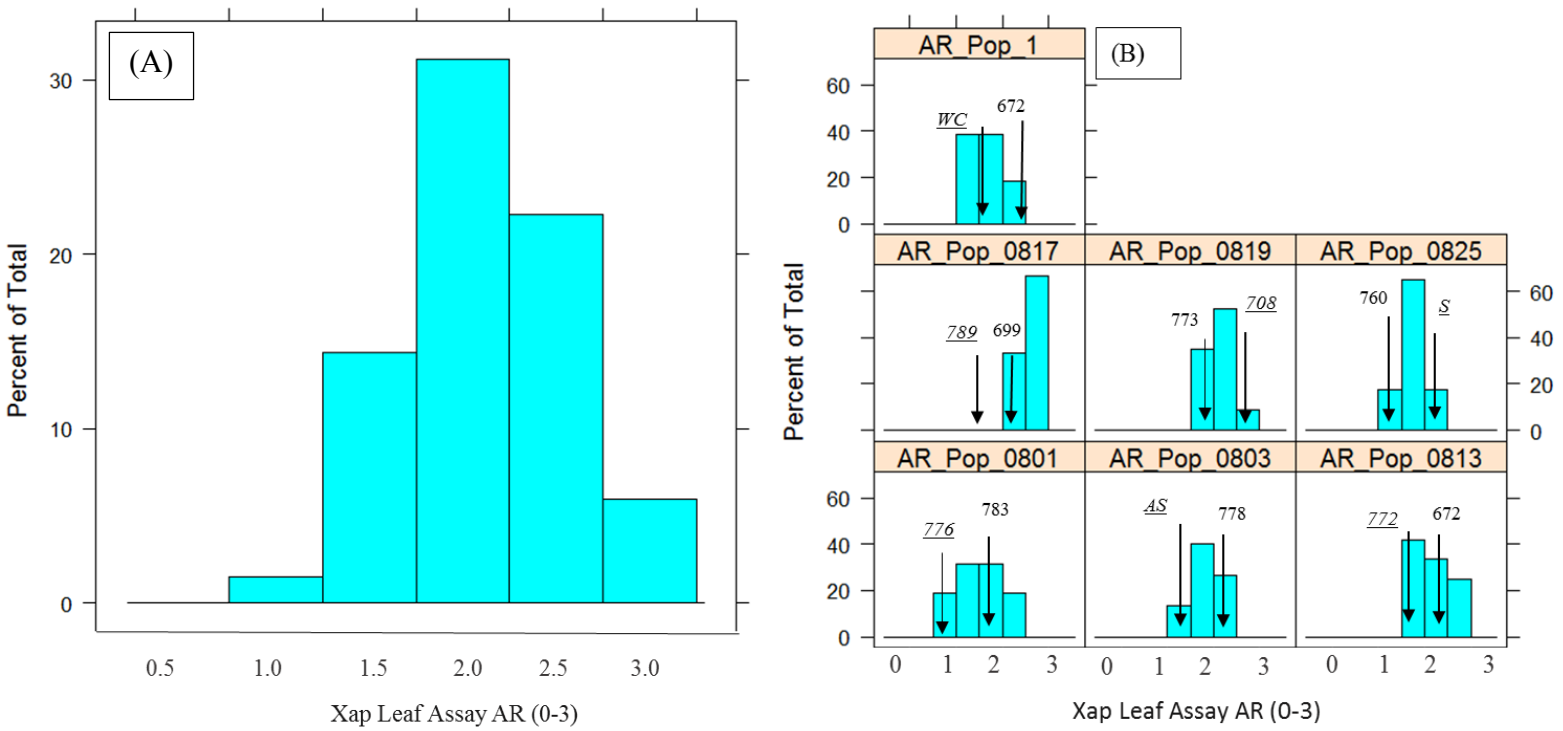

Fig. 40. Distribution (%) of *Xap* leaf assay (AR inoculum mix) 2014 ratings (0-3 scale) for the entire AR RosBREED pedigree (A) (N= 154), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

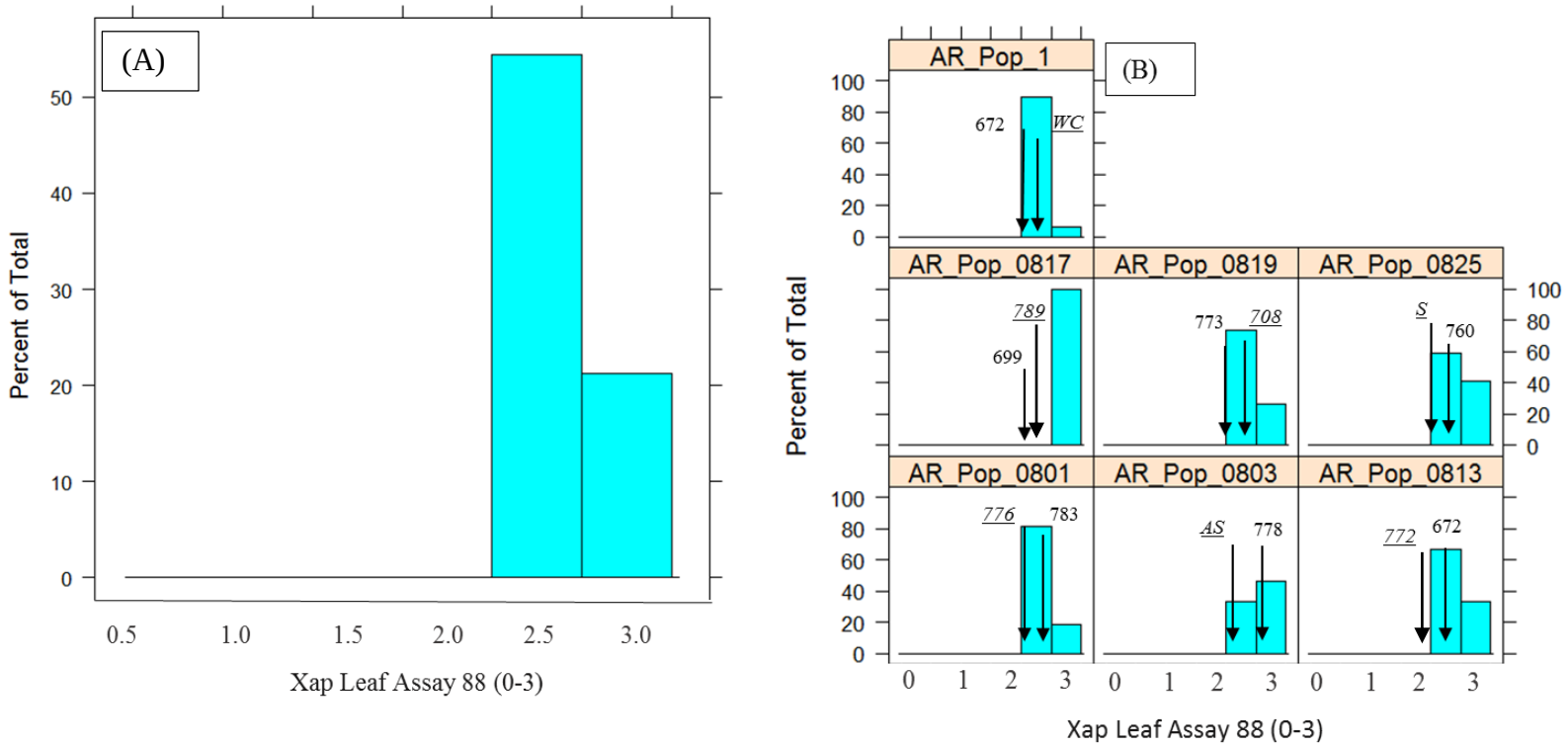

Fig. 41. Distribution (%) of *Xap* leaf assay (*Xap*-88 isolate) 2014 ratings (0-3 scale) for the entire AR RosBREED pedigree (A) (N=155), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

Genotypic Data

Of 8,144 SNPs on the IPSC peach 9 K SNP array v1.0, 1,947 (23.9%) were polymorphic and informative (GenTrain score of ≥ 0.4, and GenCall 10% of > 0.2) across the UA RosBREED pedigree. The 1,947 SNP marker locations were identified across the eight linkage groups (LGs), corresponding to the eight peach scaffolds. The number of SNPs and length of each LG ranged from 137 SNPs (0.69-45.61 cM) on LG5, to 424 SNPs (0.42-74.93 cM) on LG4.

Pedigree-Based QTL Analysis (PBA)

Xap Fruit (XapF) QTLs

Through PBA, a total of 10 QTLs across six LGs showed at least positive evidence (BF ≥ 2) to be associated with *Xap*F using 21 data sets (Tables 6-11; Figs. 42-53). The broad-sense heritability (H^2) ranged from 23-52% for *Xap*F_2015 and *Xap*F_2014-inc, respectively, and showed an average of 40% across all data sets. Moreover, the H^2 was always greater than the summation of the narrow sense heritability (h^2) for all QTLs identified using each data set (Tables 6-11). PostQTL intensity and trace plots were generated and organized in a way to visually compare QTLs identified across data sets, genome-wide (Figs. 42-53).

A total of three major QTLs associated with *Xap*F resistance were identified on LG1, termed, G1*Xap*F.1, G1*Xap*F.2, and G1*Xap*F.3. The G1*Xap*F.1 showed strong evidence (5.0-10.0 BF) to be associated with *Xap*F resistance across three 2013 data sets (*Xap*F_2013, *Xap*_F5_2013, and *Xap*F-1_2013) (Tables 6-8; Figs. 42-47). Using *Xap*F_2013 and *Xap*_F5_2013 data sets, G1*Xap*F.1 spanned 29.35-42.94 cM (BF 7.9 and 6.7) and was flanked by ss_31646 and ss_67620. The G1*Xap*F.1 was slightly broader using *Xap*F-1_2013, spanning 29.35-48.28 cM (BF 6.9). On average across all data sets, G1*Xap*F.1 explained 19% of the h^2 [phenotypic variation explained (Vp %)] for *Xap*F resistance (ranging from 16-22% V_P). Taking

into account all three data sets, 44 SNPs spanned G1*Xap*F.1 (Tables 6-8; Figs. 42-47). The second major QTL identified on LG1, G1*Xap*F.2, was located downstream of G1*Xap*F.1 on the bottom of the LG1, using eight data sets (*Xap*F-1_2013, *Xap*F-1_avg, *Xap*F-inc_avg, *Xap*F5_2014-inc_1, *Xap*F5-inc_avg, *Xap*F-1_2014-inc_2, and *Xap*F-1-inc_avg) (Tables 8-11; Figs. 46-53). The G1*Xap*F.2 showed decisive evidence (≥ 10.0, BF) to be associated with *Xap*F resistance using *Xap*F5_2014-inc (BF 30.5), strong evidence (5.0-10.0, BF) using *Xap*F-1_2014-inc (BF 6.1), and positive evidence (BF ≥ 2) using *Xap*F-1_2013, *Xap*F-1_avg, *Xap*F-inc_avg, *Xap*F5-inc_avg, and *Xap*F-1-inc_avg (BFs 2, 2, 2.5, 3.7, and 2.6, respectively). Taking into account all data sets, G1*Xap*F.2 spanned 102.57- 117.12 cM and was flanked by ss_122340 and ss_136096. On average across all data sets, G1*Xap*F.2 explained 20% of the h^2 (Vp %) for *Xap*F resistance (ranging from 3-50% V_P). A total of 27 SNPs spanned G1*Xap*F.2 (Tables 8-11; Figs. 46-53). The third major QTL identified on LG1, G1*Xap*F.3, was located upstream of G1*Xap*F.1 on the top of the LG1, using three 2014 data sets (*Xap*F_2014, *Xap*_F5_2014, and *Xap*F-1_2014) (Tables 6-8; Figs. 42-47). The G1*Xap*F.3 showed decisive evidence (≥ 10.0, BF) to be associated with *Xap*F resistance using *Xap*F_2014 (BF 10.2), and strong evidence (5.0-10.0, BF) using *Xap*_F5_2014 and *Xap*F-1_2014 (BFs 5.6 and 5.3). Using all three data sets, G1*Xap*F.3 spanned 11.96-14.71 cM and was flanked by ss_15268 and ss_18074. On average across all data sets, G1*Xap*F.3 explained 29% of the h^2 (Vp %) for *Xap*F resistance (ranging from 22-35% V_P). A total of five SNPs, spanned G1*Xap*F.3 (Tables 6-8; Figs. 42-47).

Two minor and one major QTLs associated with *Xap*F resistance were identified on LG2, termed G2*Xap*F.1, G2*Xap*F.2, and G2*Xap*F.3. The G2*Xap*F.1 showed positive evidence (BF ≥ 2) to be associated with *Xap*F resistance across three data sets (*Xap*F_2013, *Xap*F_avg, and *Xap*_F5_avg) (Tables 6-7; Figs. 42-45). Using *Xap*F_2013 and *Xap*F_avg data sets, G2*Xap*F.1

spanned 52.01-60.48 cM (BF 2.2 and 2.3) and was flanked by ss_280192 and ss_285807. The G2*Xap*F.1 was much broader using *Xap*_F5_avg, spanning 17.24-60.48 cM (BF 2.7). On average across all data sets, G2*Xap*F.1 explained 7% of the h^2 (Vp %) for *Xap*F resistance (ranging from 3-12% V_P). Taking into account only *Xap*F_2013, and *Xap*F_avg data sets, 18 SNPs spanned G2*Xap*F.1 (Tables 6-7; Figs. 42-45). The second minor QTL identified on LG2, G2*Xap*F.2, was located downstream of G2*Xap*F.1 (but overlapped with G2*Xap*F.1 using *Xap*_F5_avg) on the top of the LG2, using 11 data sets (*Xap*F_2015, *Xap*F_avg, *Xap*_F5_2015, *Xap*F-1_2015, *Xap*F-1_avg, *Xap*F_2015-inc, *Xap*F-inc_avg, *Xap*_F5_2015-inc, *Xap*F5-inc_avg, *Xap*F-1_2015-inc, and *Xap*F-1-inc_avg) (Tables 6-11; Figs. 42-53). The G2*Xap*F.2 showed positive evidence (BF ≥ 2) to be associated with *Xap*F resistance using all 11 data sets (BFs 2.3-4.6). Taking into account all data sets, G1*Xap*F.2 spanned 8.07-26.90 cM and was flanked by ss_172993 and ss_240333. On average across all data sets, G1*Xap*F.2 explained 8% of the h^2 (Vp %) for *Xap*F resistance (ranging from 3-13% V_P). A total of 76 SNPs, spanned G2*Xap*F.2 (Tables 6-11; Figs. 42-53). The third QTL identified on LG2, G2*Xap*F.3, slightly overlapped with G2*Xap*F.2, but spanned further upstream on the top of the LG2, using three data sets (*Xap*_F5_2013, *Xap*F_2014-inc, and *Xap*F-1_2014-inc) (Tables 7, 9 and 11; Figs. 44-45, 48-49, and 52-53). The G2*Xap*F.3 showed decisive evidence (≥ 10.0, BF) to be associated with *Xap*F resistance using *Xap*F_2014-inc (BF 10.2), and positive evidence (BF ≥ 2) using *Xap*_F5_2013 and *Xap*F-1_2014-inc (BFs 2.7 and 2.2). Using all three data sets, G1*Xap*F.3 spanned 0.64-12.00 cM and was flanked by ss_136625 and ss_195134. On average across all data sets, G1*Xap*F.3 explained 21% of the h^2 (Vp %) for *Xap*F resistance (ranging from 3-50% V_P). A total of 105 SNPs spanned G2*Xap*F.3 (Tables 7, 9, and 11; Figs. 44-45, 48-49, and 52-53).

One minor QTL associated with *Xap*F resistance was identified on LG3, termed G3*Xap*F.1. The G3*Xap*F.1 showed positive evidence (BF ≥ 2) to be associated with *Xap*F resistance across 12 data sets (*Xap*F_2013, *Xap*F_avg, *Xap*_F5_2013, *Xap*_F5_2014, *Xap*_F5_avg, *Xap*F-1_2014, *Xap*F-1_2015, *Xap*F-1_avg, *Xap*F-inc_avg, *Xap*F5-inc_avg, *Xap*F-1_2015-inc, and *Xap*F-1-inc_avg) (Tables 6-11; Figs. 42-53). Using all 12 data sets, G3*Xap*F.1 spanned 0.33-14.03 cM (BFs 2.0-4.8) and was flanked by snp_3_130507 and ss_309116. On average across all data sets, G3*Xap*F.1 explained 6% of the h^2 (Vp %) for *Xap*F resistance (ranging from 2-12% V_P). In total, 61 SNPs spanned G3*Xap*F.1 (Tables 6-11; Figs. 42-53).

Likewise, one minor QTL associated with *Xap*F resistance was identified on LG5, termed G5*Xap*F.1. The G5*Xap*F.1 showed strong evidence (5.0-10.0, BF) to be associated with *Xap*F resistance across two data sets (*Xap*F_2014 and *Xap*_F5_2014) and positive evidence (BF ≥ 2) across two data sets (*Xap*F-1_2014 and *Xap*F-1_avg) (Tables 6-8; Figs. 42-47). Using all four data sets, G5*Xap*F.1 spanned 29.45-41.94 cM (BFs 2.4-8.3) and was flanked by ss_593763 and ss_603047. On average across all data sets, G5*Xap*F.1 explained 5% of the h^2 (Vp %) for *Xap*F resistance (ranging from 2-7% V_P). In total, 40 SNPs spanned G5*Xap*F.1 (Tables 6-8; Figs. 42-47).

As with LG3 and LG5, one minor QTL associated with *Xap*F resistance was identified on LG6, termed G6*Xap*F.1. The G6*Xap*F.1 showed positive evidence (BF ≥ 2) to be associated with *Xap*F resistance across eight data sets (*Xap*F_2015, *Xap*F_avg, *Xap*_F5_2015, *Xap*F-1_2015, *Xap*F-1_avg, *Xap*_F5_2015-inc, and *Xap*F-1_2015-inc) (Tables 6-8 and 10-11; Figs. 42-47 and 50-53). Considering all eight data sets, G6*Xap*F.1 spanned 0.27-12.02 cM (BFs 2.0-4.2) and was flanked by ss_604834 and ss_620099. On average across all data sets, G6*Xap*F.1 explained 5%

of the h^2 (Vp %) for *Xap*F resistance (ranging from 1-11% V_P). In total, 41 SNPs, spanned G6*Xap*F.1 (Tables 6-8 and 10-11; Figs. 42-47 and 50-53).

Lastly, as with LG3, LG5, and LG6, one minor QTL associated with *Xap*F resistance was identified on LG8, termed G8*Xap*F.1. The G8*Xap*F.1 showed positive evidence (BF ≥ 2) to be associated with *Xap*F resistance across six data sets (*Xap*F_2015, *Xap*_F5_2015, *Xap*F-1_2015, *Xap*F_2015-inc, *Xap*_F5_2015-inc, and *Xap*F-1_2015-inc) (Tables 6-11; Figs. 42-53). Considering all six data sets, G8*Xap*F.1 spanned 16.50-42.14 cM (BFs 3.0-4.4) and was flanked by ss_832112 and ss_874263. On average across all data sets, G8*Xap*F.1 explained 7% of the h^2 (Vp %) for *Xap*F resistance (ranging from 6-10% V_P). In total, 99 SNPs spanned G8*Xap*F.1 (Tables 6-11; Figs. 42-53).

Table 6. *Xap* fruit (0-5) [2013, 2014, 2015, and 2013-2015 avg] QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL name	Data set	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G1*Xap*F.1	*Xap*F_2013	2013	170	1	ss_31646 ss_60312	29.35 42.94	11.74 17.20	7.9	0.46	0.22	0.29
G2*Xap*F.1				2	ss_280192 ss_285807	52.01 60.48	20.80 24.20	2.2		0.03	
G3*Xap*F.1				3	ss_306624 ss_307230	12.02 12.36	4.81 4.94	1.9		0.03	
G1*Xap*F.3	*Xap*F_2014	2014	167	1	ss_15268 ss_18074	11.96 14.71	4.78 5.89	10.2	0.43	0.35	0.42
G5*Xap*F.1				5	ss_593763 ss_603047	29.45 41.94	11.78 16.77	8.3		0.07	
G2*Xap*F.2	*Xap*F_2015	2015	167	2	ss_172993 ss_233804	8.07 24.31	3.23 9.72	3.8	0.23	0.12	0.19
G6*Xap*F.1				6	ss_604834 ss_618824	0.27 11.15	0.11 4.46	2		0.02	
G8*Xap*F.1				8	ss_834321 ss_866366	18.23 35.95	7.29 14.38	3		0.06	
G2*Xap*F.2	*Xap*F_avg	avg	173	2	ss_195134 ss_233804	12.00 24.31	4.80 9.72	2.3	0.40	0.04	0.21
G2*Xap*F.1				2	ss_280192 ss_285807	52.01 60.48	20.80 24.19	2.3		0.06	
G3*Xap*F.1				3	snp_3_130507 ss_306624	0.33 12.02	0.13 4.81	3.3		0.05	
G6*Xap*F.1				6	ss_613297 ss_620099	7.15 12.02	2.86 4.81	3		0.06	

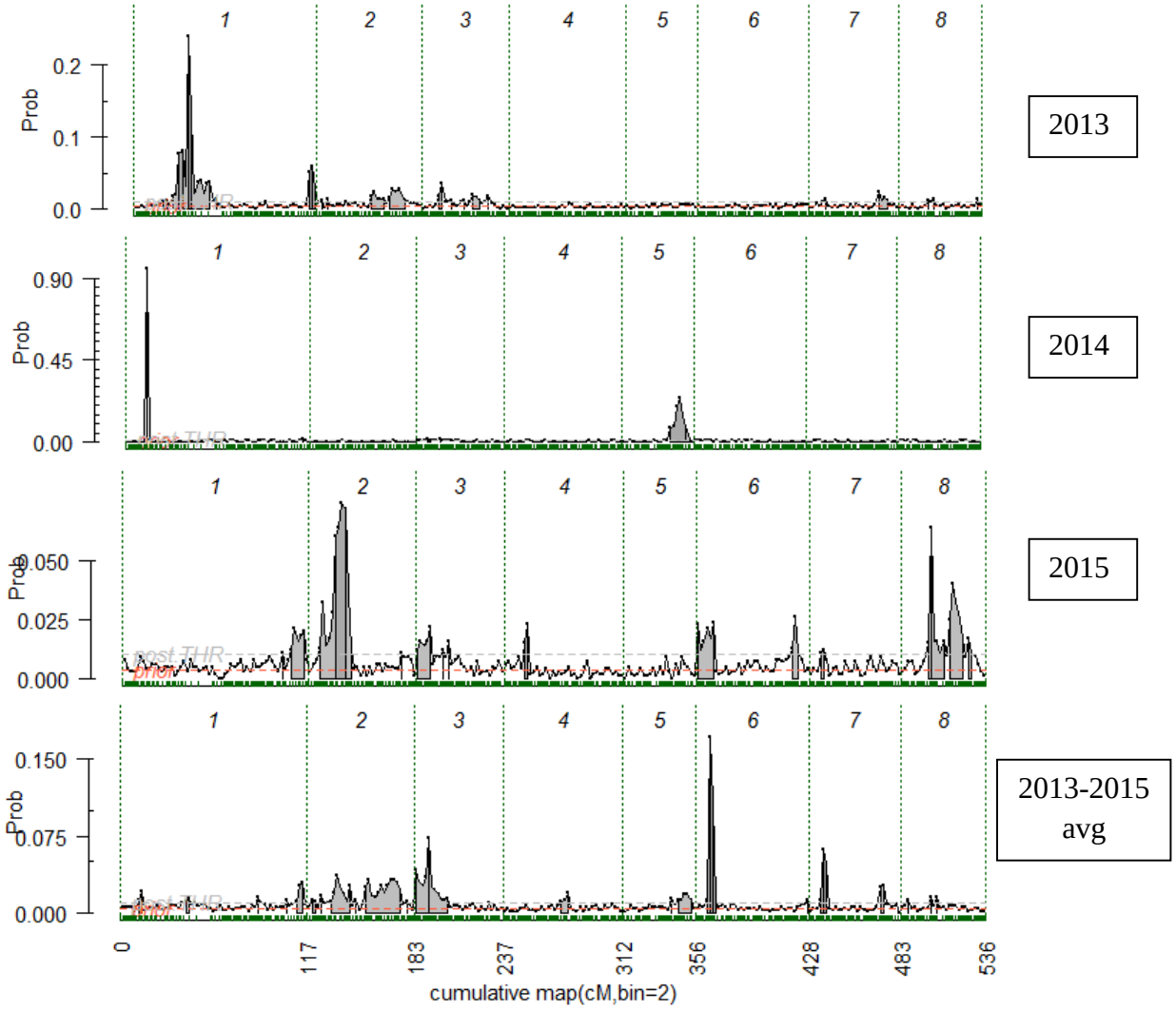

Fig. 42. PostQTL intensity plot positions for *Xap* fruit (0-5) [2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

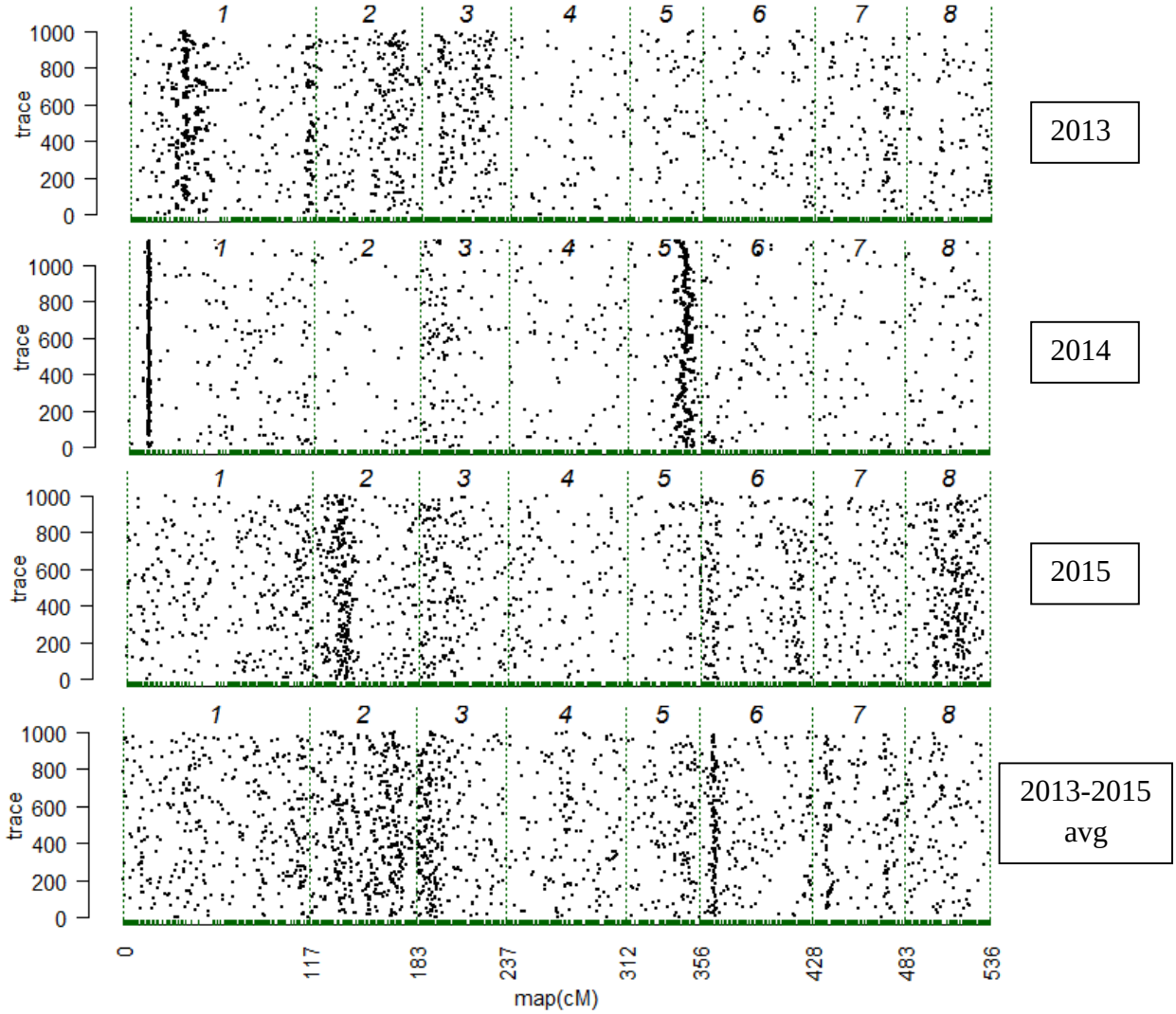

Fig. 43. PostQTL trace plots for *Xap* fruit (0-5) [(2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The colored dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Table 7. *Xap* fruit [(0-5) ± 0.5] [(2013, 2014, 2015, and 2013-2015 avg] QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL name	Data set	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G1*Xap*F.1	*Xap*_F5_2013	2013	170	1	ss_31646 ss_60312	29.35 42.94	11.74 17.18	6.7	0.50	0.20	0.26
G2*Xap*F.3				2	ss_136625 ss_178504	0.64 9.07	0.26 3.63	2.7		0.03	
G3*Xap*F.1				3	ss_306624 ss_307230	12.02 12.36	4.81 4.94	3.5		0.02	
G1*Xap*F.3	*Xap*_F5_2014	2014	167	1	ss_15268 ss_18074	11.96 14.71	4.78 5.89	5.6	0.44	0.30	0.42
G3*Xap*F.1				3	snp_3_130507 ss_307230	0.33 12.36	0.13 4.94	4.8		0.06	
G5*Xap*F.1				5	ss_593763 ss_603047	29.45 41.94	11.78 16.77	5		0.06	
G2*Xap*F.2	*Xap*_F5_2015	2015	167	2	ss_208760 ss_240333	15.04 26.90	6.02 10.76	3.7	0.27	0.07	0.15
G6*Xap*F.1				6	ss_604834 ss_618824	0.27 11.15	0.11 4.46	2.2		0.02	
G8*Xap*F.1				8	ss_832112 ss_867575	16.50 37.14	6.60 14.86	3.4		0.06	
G2*Xap*F.1	*Xap*_F5_avg	avg	173	2	ss_214703 ss_285807	17.24 60.48	6.89 24.19	2.7	0.47	0.12	0.32
G3*Xap*F.1				3	snp_3_130507 ss_306624	0.33 12.02	0.13 4.81	4.5		0.09	
G6*Xap*F.1				6	ss_614082 ss_620099	8.10 12.02	3.24 4.81	4.2		0.11	

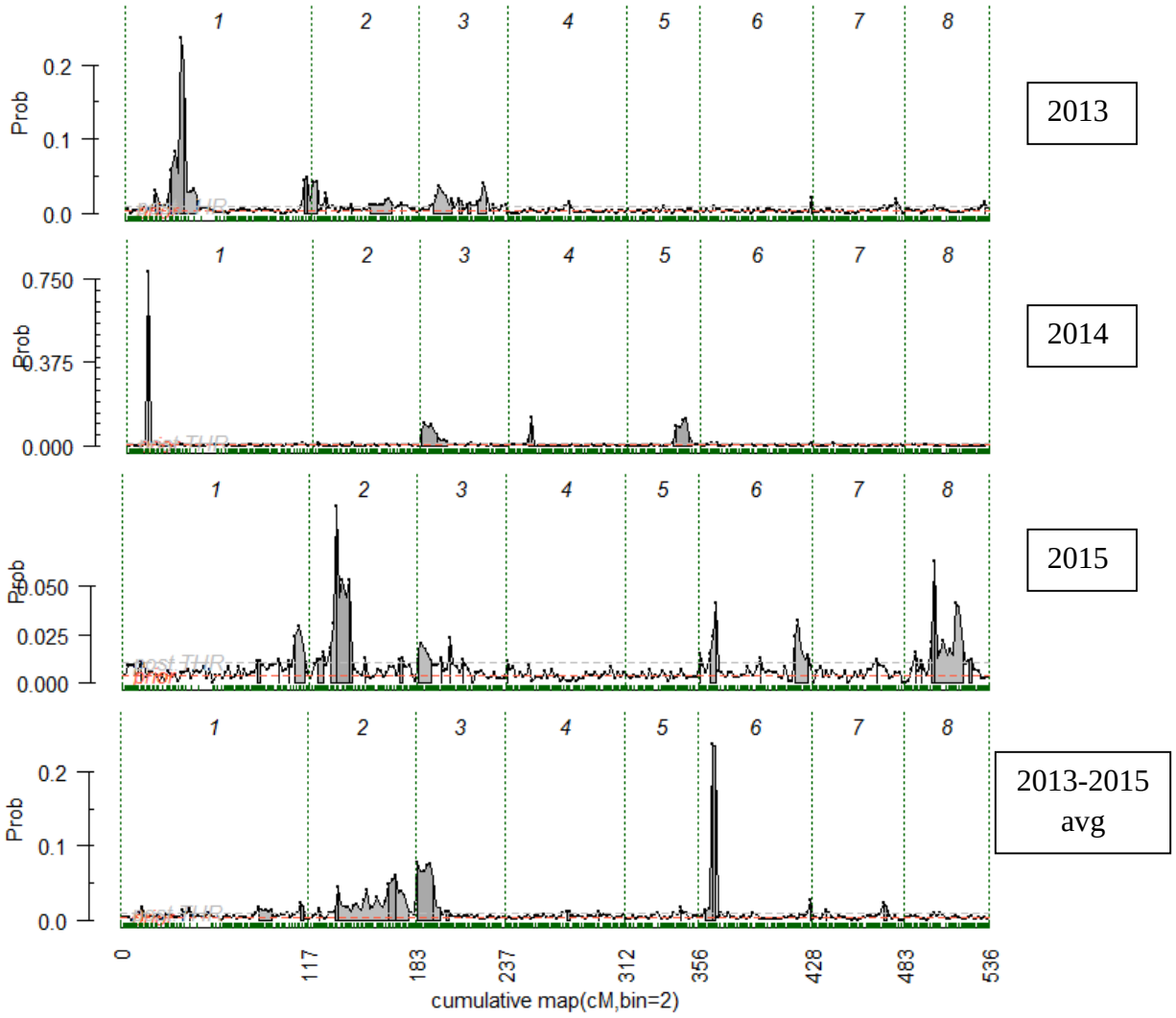

Fig. 44. PostQTL intensity plot positions for *Xap* fruit [(0-5) ± 0.5] [(2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

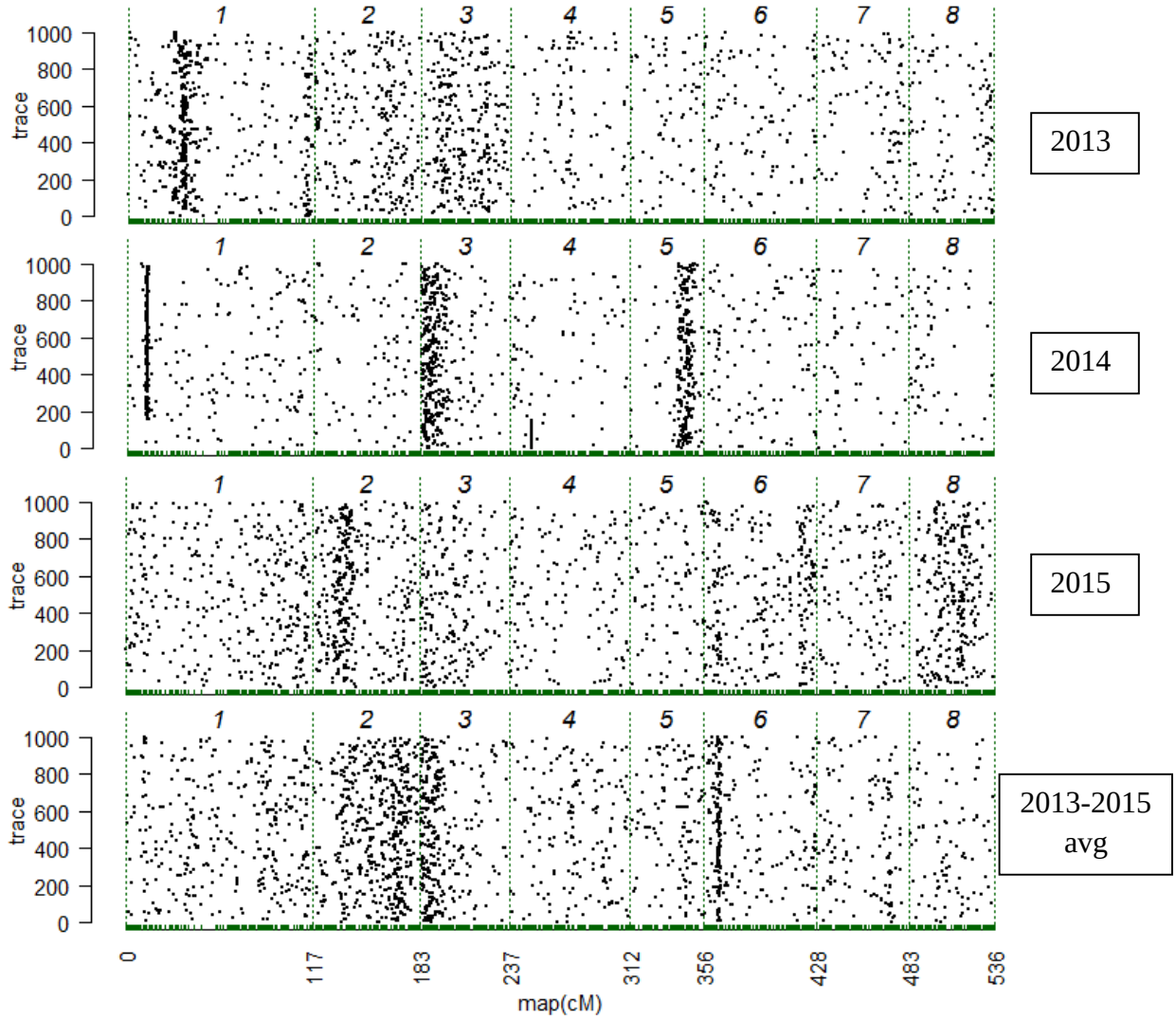

Fig. 45. PostQTL trace plots for *Xap* fruit [(0-5) ± 0.5] [(2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The colored dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Table 8. *Xap* fruit [(0-5) – 1] [(2013, 2014, 2015, and 2013-2015 avg] QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL name	Data set	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G1*Xap*F.1	*Xap*F-1_2013	2013	170	1	ss_31646 ss_67620	29.35 48.28	11.74 19.31	6.9	0.49	0.16	0.27
G1*Xap*F.2				1	ss_131801 ss_136096	112.03 117.12	44.81 46.85	2		0.11	
G1*Xap*F.3	*Xap*F-1_2014	2014	167	1	ss_15268 ss_18074	11.96 14.71	4.78 5.89	5.3	0.37	0.22	0.35
G3*Xap*F.1				3	snp_3_130507 ss_304082	0.33 10.12	0.13 4.05	3.7		0.07	
G5*Xap*F.1				5	ss_593763 ss_603047	29.45 41.94	11.78 16.77	4		0.06	
G2*Xap*F.2	*Xap*F-1_2015	2015	167	2	ss_195134 ss_240333	12.00 26.90	4.80 10.76	3.8	0.36	0.11	0.29
G3*Xap*F.1				3	snp_3_130507 ss_309116	0.33 14.03	0.13 5.61	3.7		0.08	
G6*Xap*F.1				6	ss_604834 ss_618824	0.27 11.15	0.11 4.46	2.2		0.03	
G8*Xap*F.1				8	ss_834321 ss_864805	18.23 34.78	7.29 13.91	3.3		0.08	

Table 8. *Xap* fruit [(0-5) – 1] [(2013, 2014, 2015, and 2013-2015 avg] QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font. (Cont.).

QTL name	Data set	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G1*Xap*F.2	*Xap*F-1_avg	avg	173	1	ss_131801 ss_135137	112.03 116.20	44.81 46.48	2	0.48	0.03	0.25
G2*Xap*F.2				2	ss_195134 ss_233804	12.00 24.31	4.80 9.72	2.6		0.03	
G3*Xap*F.1				3	snp_3_130507 ss_304082	0.33 10.12	0.13 4.05	4.1		0.10	
G5*Xap*F.1				5	ss_594647 ss_603047	31.04 41.94	12.41 16.77	2.4		0.02	
G6*Xap*F.1				6	ss_613297 ss_618824	7.15 11.15	2.86 4.46	3.2		0.07	

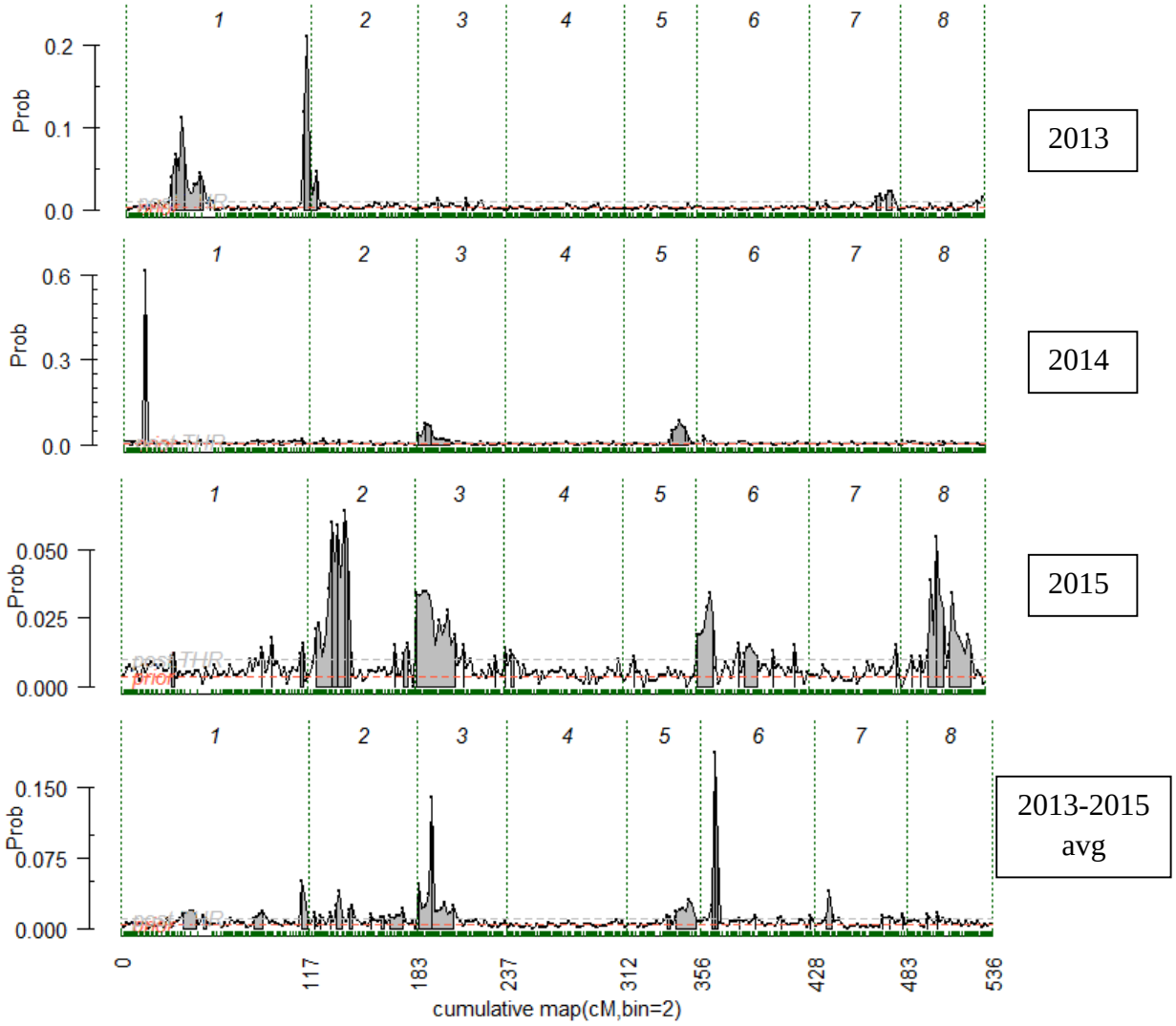

Fig. 46. PostQTL intensity plot positions for *Xap* fruit [(0-5) – 1] [(2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

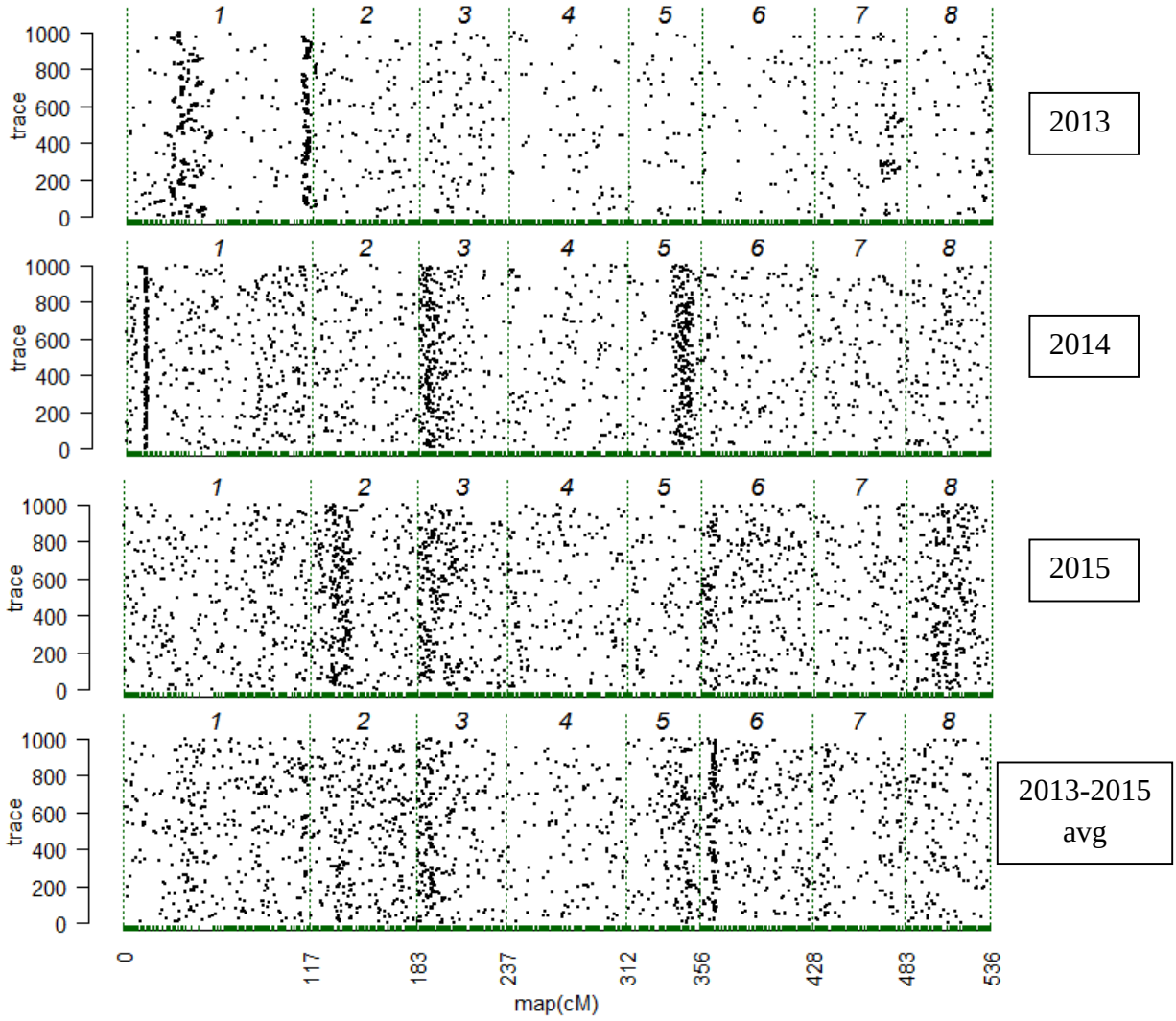

Fig. 47. PostQTL trace plots for *Xap* fruit [(0-5) – 1] [(2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The colored dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Table 9. *Xap* fruit [(0-5) inc] [(2013, 2014, 2015, and 2014-2015 avg] QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL name	Data set	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G2*Xap*F.3	*Xap*F_2014-inc	2014	166	1	ss_139036 ss_195134	1.66 12.00	0.66 4.80	10.1	0.52	0.50	0.50
G2*Xap*F.2	*Xap*F_2015-inc	2015	167	2	ss_195134 ss_224651	12.00 20.94	4.80 8.38	4.2	0.30	0.11	0.20
G8*Xap*F.1				8	ss_834321 ss_864805	18.23 34.78	7.29 13.91	4.4		0.10	
G1*Xap*F.2	*Xap*F-inc_avg	avg	167	1	ss_123719 ss_136096	105.71 117.12	42.28 46.85	2.5	0.30	0.10	0.24
G2*Xap*F.2				2	ss_195134 ss_228538	12.00 22.25	4.80 8.90	3.8		0.10	
G3*Xap*F.1				3	ss_293318 ss_306624	2.44 12.02	0.98 4.81	1.9		0.04	

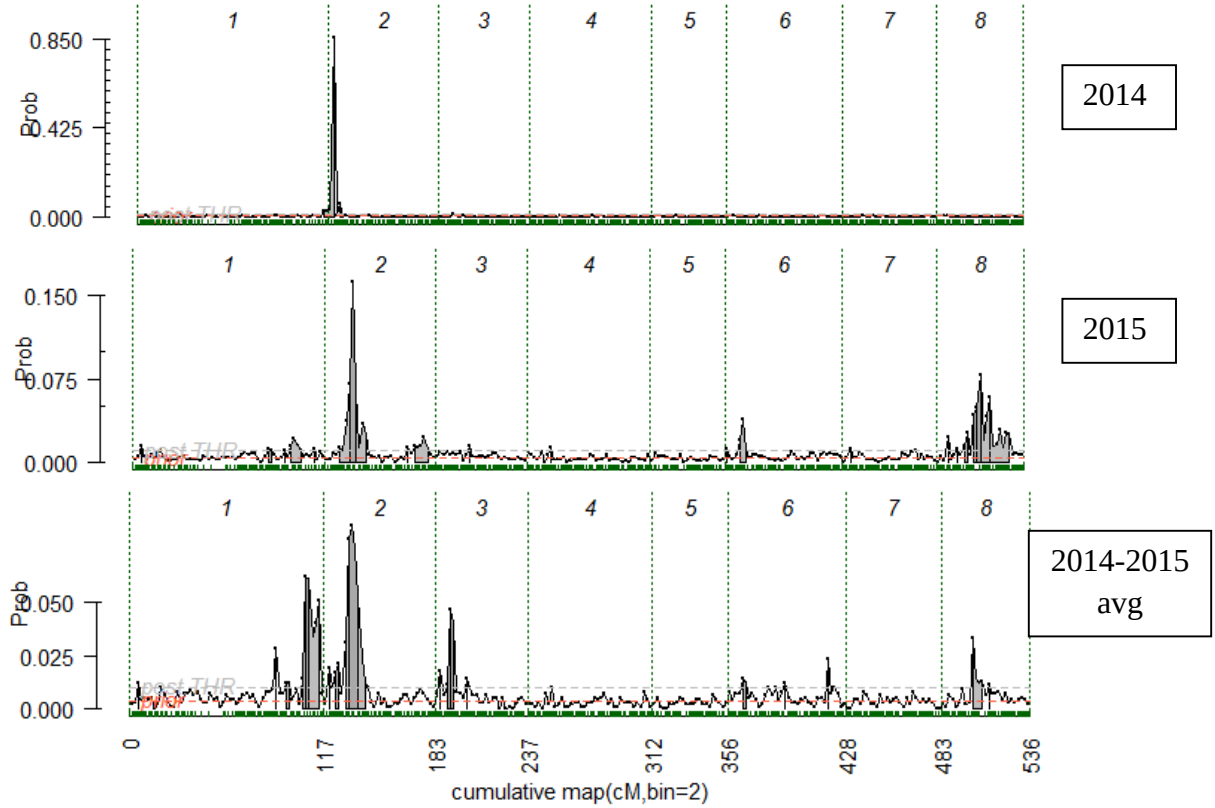

Fig. 48. PostQTL intensity plot positions for *Xap* fruit [(0-5) inc] [(2014, 2015, and 2014-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

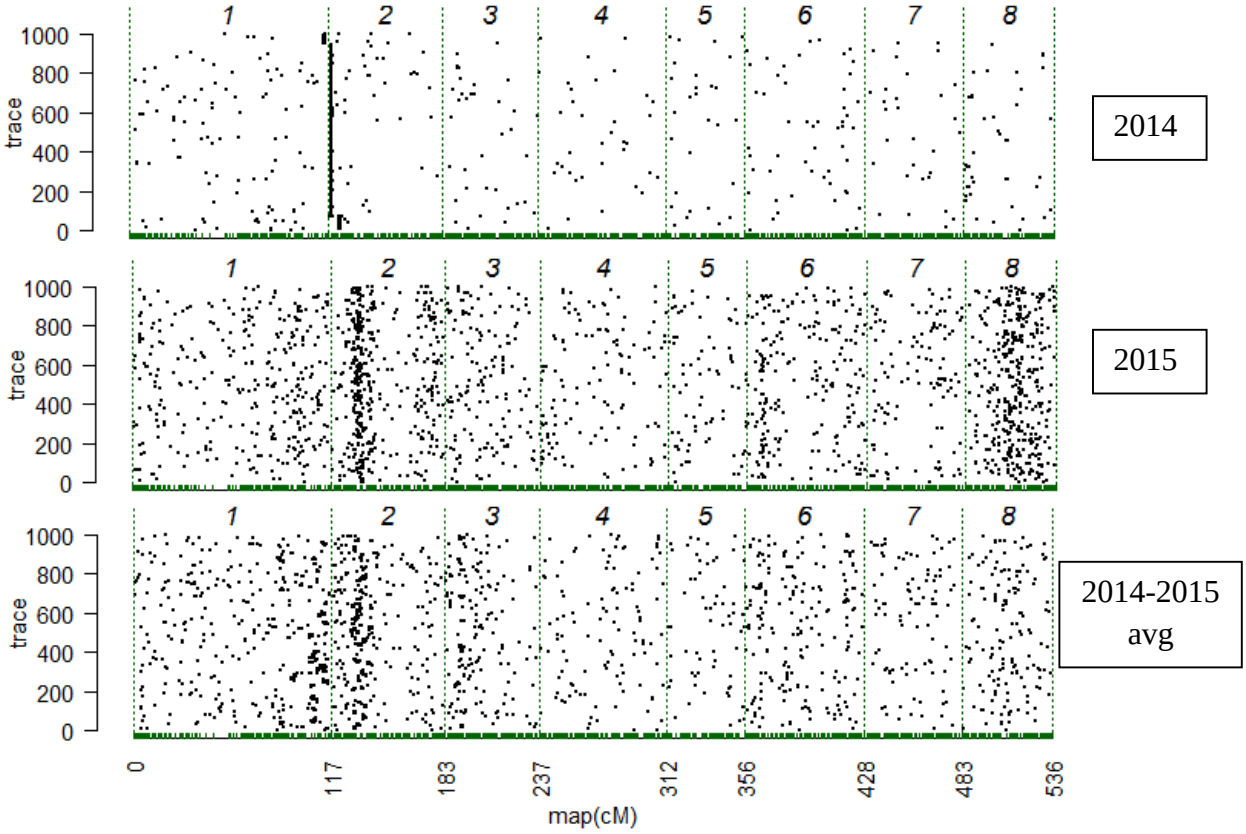

Fig. 49. PostQTL trace plots for *Xap* fruit [(0-5) inc] [(2014, 2015, and 2014-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The colored dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Table 10. *Xap* fruit [(0-5) ± 0.5 inc] [(2014, 2015, and 2014-2015 avg] QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL name	Data set	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G1*Xap*F.2	*Xap*F5_2014-inc	2014	166	1	ss_131801 ss_135137	112.03 116.20	44.81 46.48	30.5	0.52	0.50	0.50
G2*Xap*F.2	*Xap*F5_2015-inc	2015	167	2	ss_195134 ss_233804	12.00 24.31	4.80 9.72	3.8	0.30	0.10	0.19
G6*Xap*F.1				6	ss_613297 ss_618824	7.15 11.15	2.86 4.46	2		0.03	
G8*Xap*F.1				8	ss_851849 ss_874263	25.64 42.14	10.25 16.86	3.6		0.07	
G1*Xap*F.2	*Xap*F5-inc_avg	avg	167	1	ss_122340 ss_135137	102.57 116.20	41.03 46.48	3.7	0.31	0.19	0.29
G2*Xap*F.2				2	ss_195134 ss_230378	12.00 23.20	4.80 9.28	2.6		0.07	
G3*Xap*F.1				3	ss_293318 ss_306624	2.44 12.02	0.98 4.81	1.7		0.03	

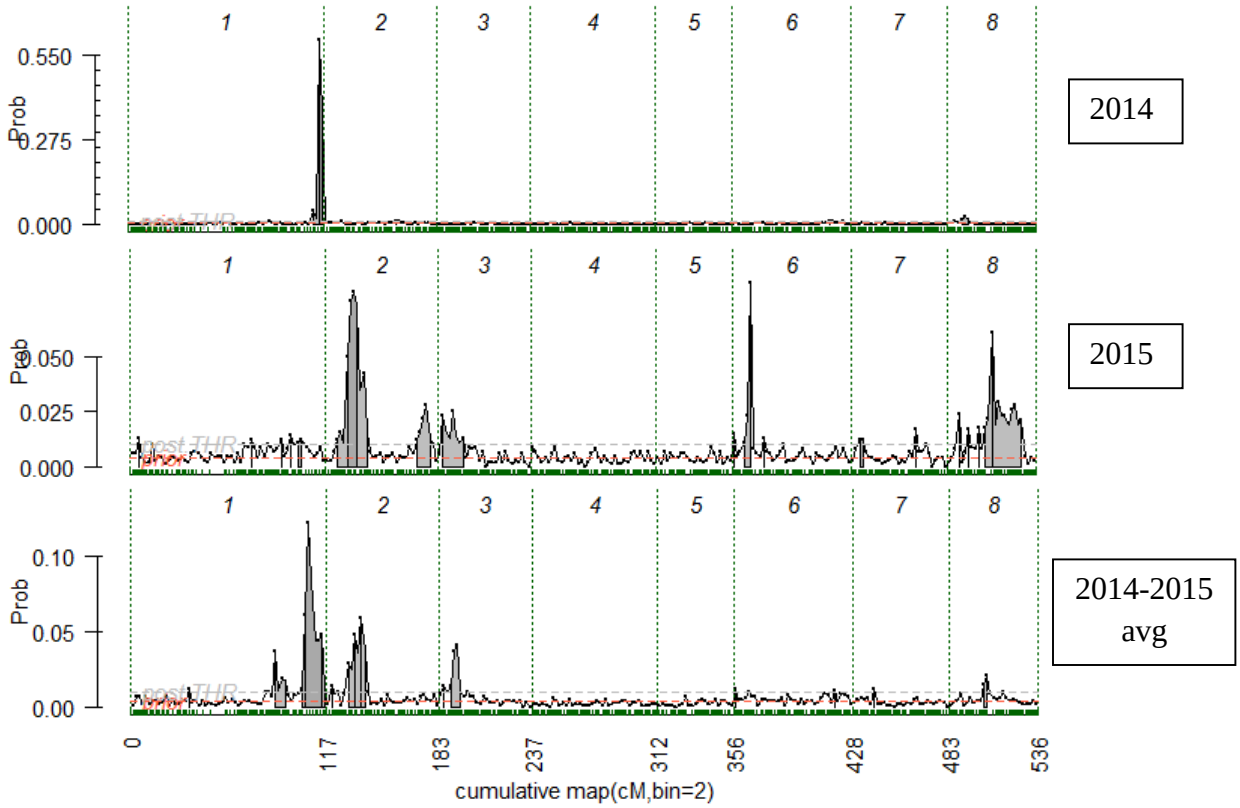

Fig. 50. PostQTL intensity plot positions for *Xap* fruit [(0-5) ± 0.5 inc] [(2014, 2015, and 2014-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

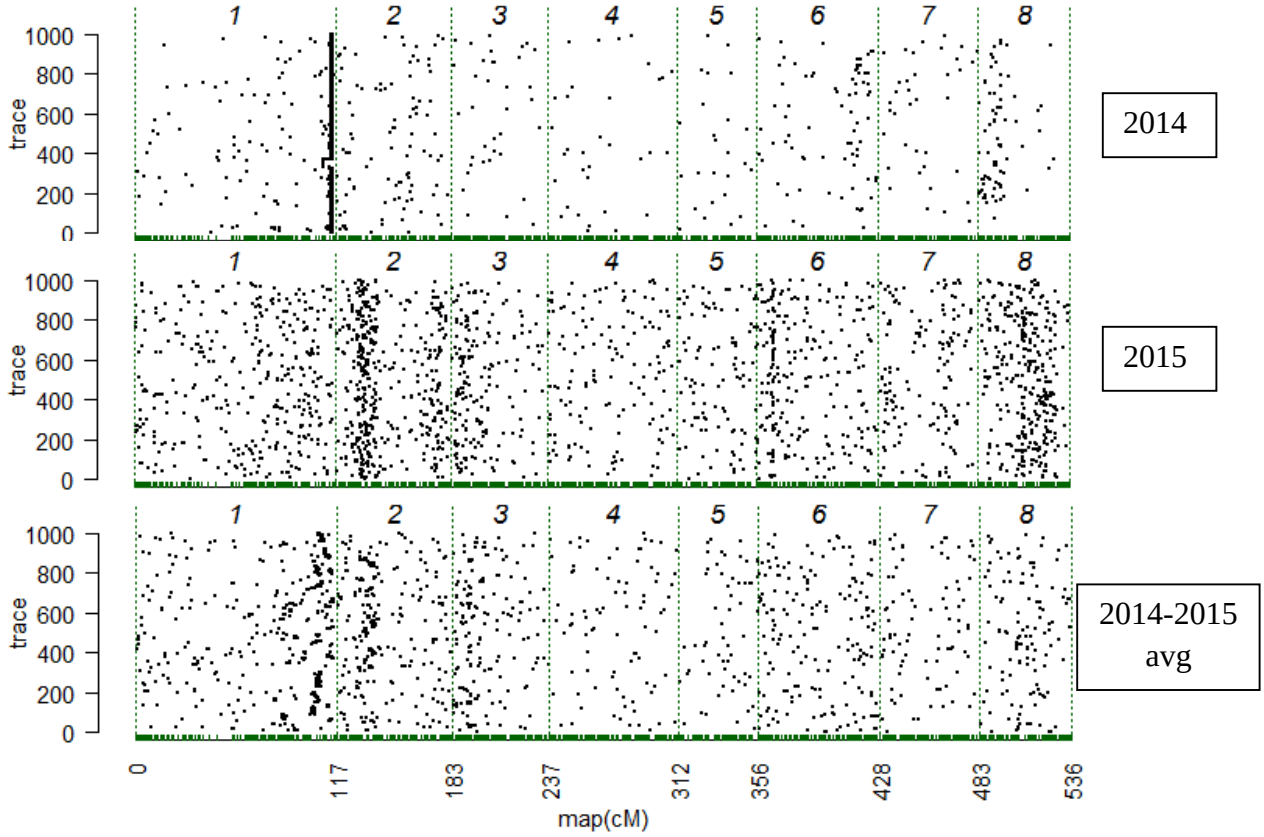

Fig. 51. PostQTL trace plots for *Xap* fruit [(0-5) ± 0.5 inc] [(2014, 2015, and 2014-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The colored dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Table 11. *Xap* fruit [(0-5) – 1 inc] [(2014, 2015, and 2014-2015 avg] QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL name	Data set	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G1*Xap*F.2	*Xap*F-1_2014-inc	2014	166	1	ss_131801 ss_135137	112.03 116.20	44.81 46.48	6.1	0.49	0.40	0.49
G2*Xap*F.3				2	ss_139036 ss_195134	1.66 12.00	0.66 4.80	2.2		0.09	
G2*Xap*F.2	*Xap*F-1_2015-inc	2015	167	2	ss_195134 ss_233804	12.00 24.31	4.80 9.72	4.6	0.39	0.13	0.25
G3*Xap*F.1				3	snp_3_130507 ss_307983	0.33 13.12	0.13 5.25	1.9		0.03	
G6*Xap*F.1				6	ss_613297 ss_618824	7.15 11.15	2.86 4.46	1.5		0.01	
G8*Xap*F.1				8	ss_832112 ss_862321	16.50 33.01	6.60 13.21	3.8		0.07	
G1*Xap*F.2	*Xap*F-1-inc_avg	avg	167	1	ss_124160 ss_135137	105.99 116.20	42.39 46.48	2.6	0.39	0.09	0.32
G2*Xap*F.2				2	ss_207716 ss_230378	14.69 23.20	5.88 9.28	3.8		0.10	
G3*Xap*F.1				3	ss_295913 ss_305895	5.39 11.17	2.16 4.47	3.8		0.12	

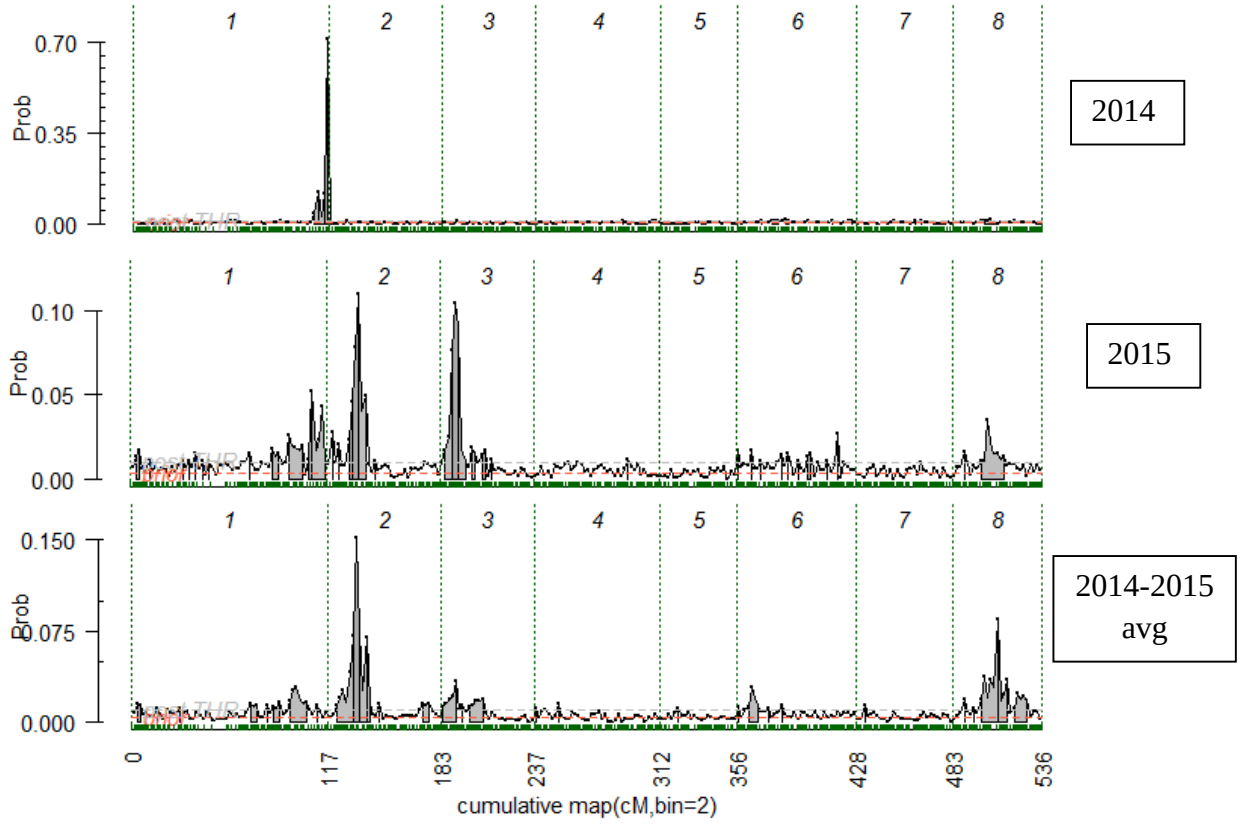

Fig. 52. PostQTL intensity plot positions for *Xap* fruit [(0-5) – 1 inc] [(2014, 2015, and 2014-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

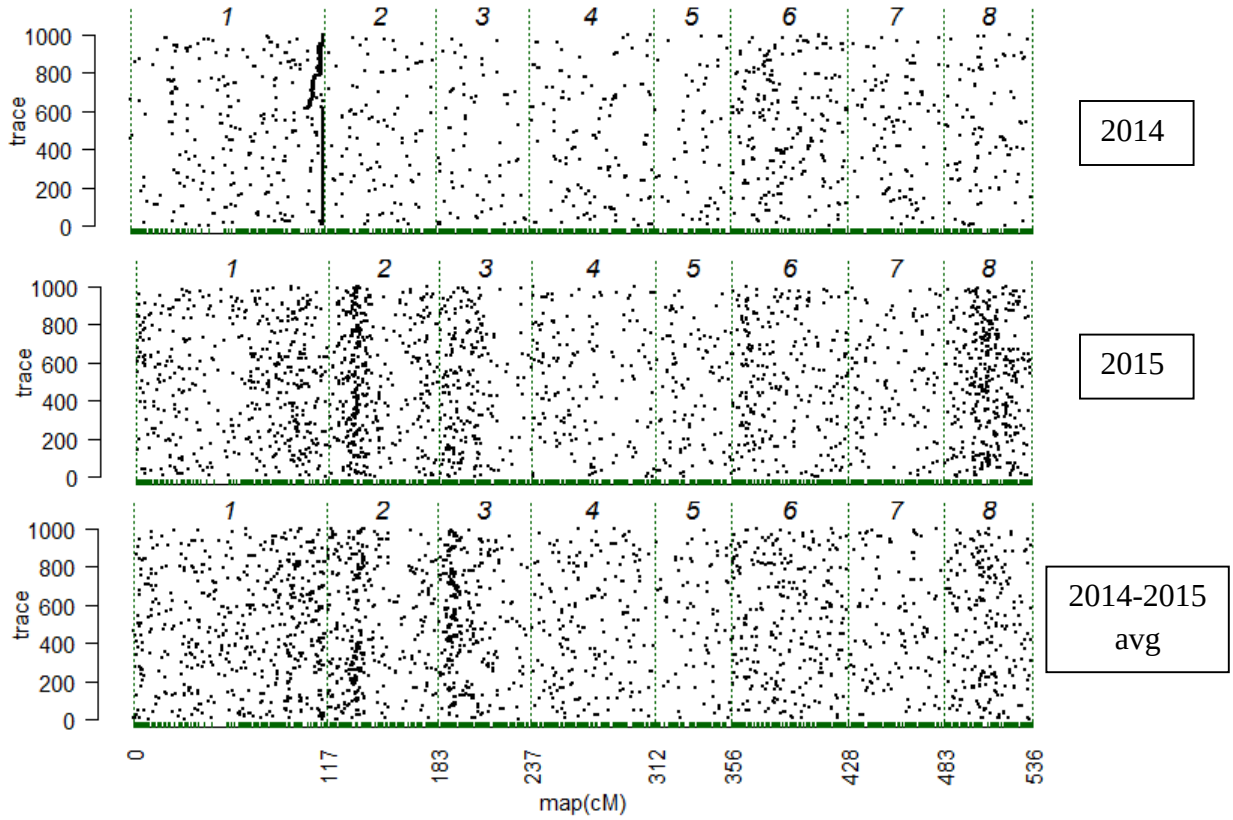

Fig. 53. PostQTL trace plots for *Xap* fruit [(0-5) – 1 inc] [(2014, 2015, and 2014-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The colored dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Xap Leaf (XapL) QTLs

Through PBA, a total of six QTLs across four LGs showed at least positive evidence (BF ≥ 2) to be associated with *Xap*L using 12 data sets (2013, 2014, 2015, and 2013-2015 avg [(0-5); (0-5) ± 0.5; and (0-5) – 1]) (Tables 12-14; Figs. 54-59). The broad-sense heritability (H^2) ranged from 37 to 57%, *Xap*L_2014 and *Xap*L-1_avg, and showed an average of 48% across all data sets. Moreover, the H^2 was always greater than the summation of the narrow sense heritability (h^2) for all QTLs identified using each data set (Tables 12-14). PostQTL intensity and trace plots were generated and organized in a way to visually compare QTLs identified across data sets, genome-wide (Figs. 54-59).

A total of two minor QTLs associated with *Xap*L resistance were identified on LG1, termed G1*Xap*L.1, and G1*Xap*L.2. The G1*Xap*L.1 showed positive evidence (BF ≥ 2) to be associated with *Xap*L resistance using data set *Xap*L-1_avg (Table 14; Figs. 58-59). This QTL spanned 12.48-20.90 cM (BF 2.1) and was flanked by ss_15977 and ss_24260. Using the *Xap*L-1_avg data set, G1*Xap*L.1 explained 5% of the h^2 (Vp %) for *Xap*L resistance. A total of 11 SNPs, spanned G1*Xap*L.1 (Table 14; Figs. 58-59). Likewise, G1*Xap*L.2 showed positive evidence (BF ≥ 2) to be associated with *Xap*L resistance using data set *Xap*L_2014 (Table 12; Figs. 54-55). This QTL spanned 86.15-116.90 cM (BF 2.8) and was flanked by ss_107432 and snp_1_46757382. Using the *Xap*L_2014 data set, G1*Xap*L.2 explained 11% of the h^2 (Vp %) for *Xap*L resistance. A total of 84 SNPs spanned G1*Xap*L.2 (Table 12; Figs. 54-55).

In contrast to LG1, two major QTLs associated with *Xap*L resistance were identified on LG2, termed G2*Xap*L.1, and G2*Xap*L.2. The G2*Xap*L.1 showed strong evidence (5.0-10.0, BF) to be associated with *Xap*L resistance across three data sets (*Xap*L_2013, *Xap*_L5_2013, and *Xap*L-1_2013) (Tables 12-14; Figs. 54-59) and showed positive evidence (BF ≥ 2) across two data sets (*Xap*_L_avg and *Xap*L-1_avg). Using all five data sets, G2*Xap*L.1 spanned 37.06-66.60

cM (BFs 2.9-9.4) and was flanked by ss_260838 and ss_290277. On average across all data sets, G2*Xap*L.1 explained 20% of the h^2 (Vp %) for *Xap*L resistance (ranging from 5-33% V_P). Taking into account all five data sets, 82 SNPs spanned G2*Xap*L.1 (Tables 12-14; Figs. 54-59). The second major QTL, associated with *Xap*L resistance, identified on LG2, G2*Xap*L.2 was located upstream of G2*Xap*L.1 on the top of LG2. The G2*Xap*L.2 showed strong evidence (5.0-10.0, BF) to be associated with *Xap*L resistance across two data sets (*Xap*L5_2014 and *Xap*L-1_2014) and showed positive evidence (BF ≥ 2) across two data sets (*Xap*_L_avg and *Xap*L-1_avg) (Tables 12-14; Figs. 54-59). Using all five data sets, G2*Xap*L.2 spanned 2.35-24.31 cM (BFs 2.9-7.1) and was flanked by ss_142214 and ss_233804. On average across all data sets, G2*Xap*L.2 explained 14% of the h^2 (Vp %) for *Xap*L resistance (ranging from 5-20% V_P). Taking into account all five data sets, 119 SNPs spanned G2*Xap*L.2 (Tables 12-14; Figs. 54-59).

In contrast to LG2, only one minor QTL associated with *Xap*L resistance was identified on LG5, termed G5*Xap*L.1. The G5*Xap*L.1 showed strong evidence (5.0-10.0, BF) to be associated with *Xap*L resistance across three data sets (*Xap*_L_avg, *Xap*L-1_2014, and *Xap*L-1_avg) (Tables 12 and 14; Figs. 54-55 and 58-59) and showed positive evidence (BF ≥ 2) across six data sets (*Xap*L_2013, *Xap*L_2014, *Xap*_L5_2013, *Xap*L5_2014, *Xap*_L5_avg, and *Xap*L-1_2015) (Tables 12-14; Figs. 54-59). Using all nine data sets, G5*Xap*L.1 spanned 10.00-41.39 cM (BFs 2.5-5.2) and was flanked by ss_559786 and ss_602331. On average across all data sets, G5*Xap*L.1 explained 9% of the h^2 (Vp %) for *Xap*L resistance (ranging from 3-14% V_P). Taking into account all five data sets, 103 SNPs spanned G5*Xap*L.1 (Tables 12-14; Figs. 54-59).

In contrast to LG5, one major QTL associated with *Xap*L resistance was identified on LG6, termed G6*Xap*L.1. The G6*Xap*L.1 showed decisive evidence (≥ 10.0, BF) to be associated with *Xap*L resistance across two data sets (*Xap*L_2015 and *Xap*L-1_2015) (Tables 12 and 14;

Figs. 54-55 and 58-59), strong evidence (5.0-10.0, BF) across two data sets (*Xap*L5_2014 and *Xap*L5_2015) (Table 13; Figs. 56-57), and positive evidence (BF ≥ 2) across five data sets (*Xap*L_2014, *Xap*L_avg, *Xap*L5_avg, *Xap*L-1_2014, and *Xap*L-1_avg) (Tables 12-14; Figs. 54-59). Using all nine data sets, G6*Xap*L.1 spanned 0.27-33.80 cM (BFs 2.4-12.8) and was flanked by ss_604834 and ss_652595. On average across all data sets, G6*Xap*L.1 explained 15% of the h^2 (Vp %) for *Xap*L resistance (ranging from 4-33% V_P). Taking into account all five data sets, 103 SNPs spanned G6*Xap*L.1 (Tables 12-14; Figs. 54-59).

Table 12. *Xap* leaf (0-5) [(2013, 2014, 2015, and 2013-2015 avg] QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL	Data set	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G2*Xap*L.1	*Xap*L_2013	2013	170	2	ss_264399 ss_290277	39.73 66.60	15.89 26.64	8.2	0.51	0.28	0.31
G5*Xap*L.1				5	ss_582516 ss_594279	20.68 30.02	8.27 12.01	3		0.04	
G1*Xap*L.2	*Xap*L_2014	2014	172	1	ss_107432 snp_1_46757382	86.15 116.90	34.46 46.76	2.8	0.37	0.11	0.37
G2*Xap*L.2				2	ss_157034 ss_214703	5.14 17.24	2.06 6.90	3.9		0.17	
G5*Xap*L.1				5	ss_590333 ss_602331	27.22 41.39	10.89 16.56	2.8		0.05	
G6*Xap*L.1				6	ss_614082 ss_622231	8.10 14.02	3.24 5.61	2.4		0.04	
G6*Xap*L.1	*Xap*L_2015	2015	172	6	ss_604834 ss_618417	0.27 10.80	0.11 4.32	12.8	0.39	0.33	0.33
G2*Xap*L.1	*Xap*L_avg	avg	173	2	ss_264399 ss_285807	39.73 60.48	15.90 24.19	3	0.49	0.05	0.32
G5*Xap*L.1				5	ss_589219 ss_601173	26.21 38.65	10.48 15.46	5.2		0.14	
G6*Xap*L.1				6	ss_604834 ss_621556	0.27 13.55	0.11 5.42	4.4		0.13	

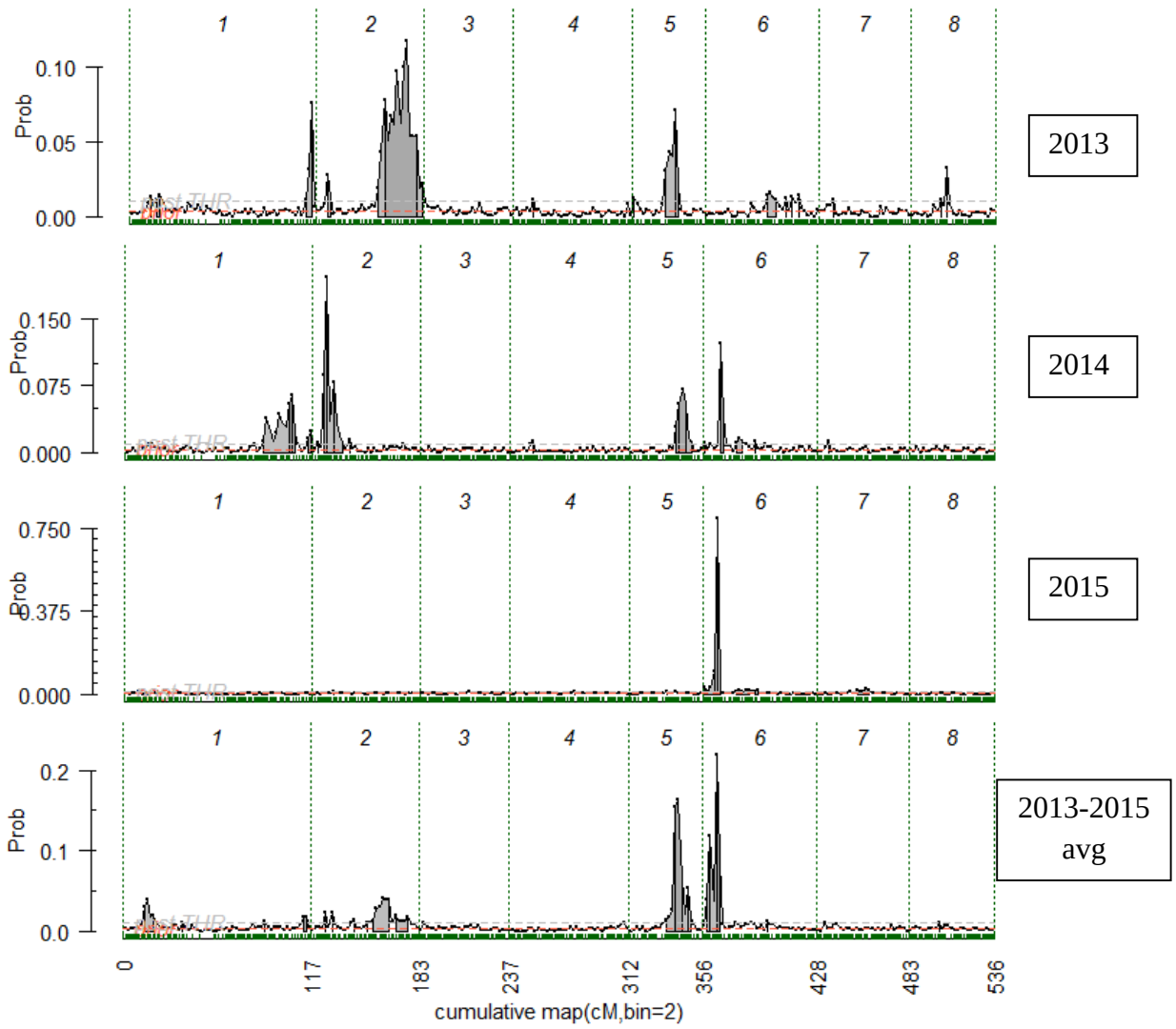

Fig. 54. PostQTL intensity plot positions for *Xap* leaf (0-5) [(2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

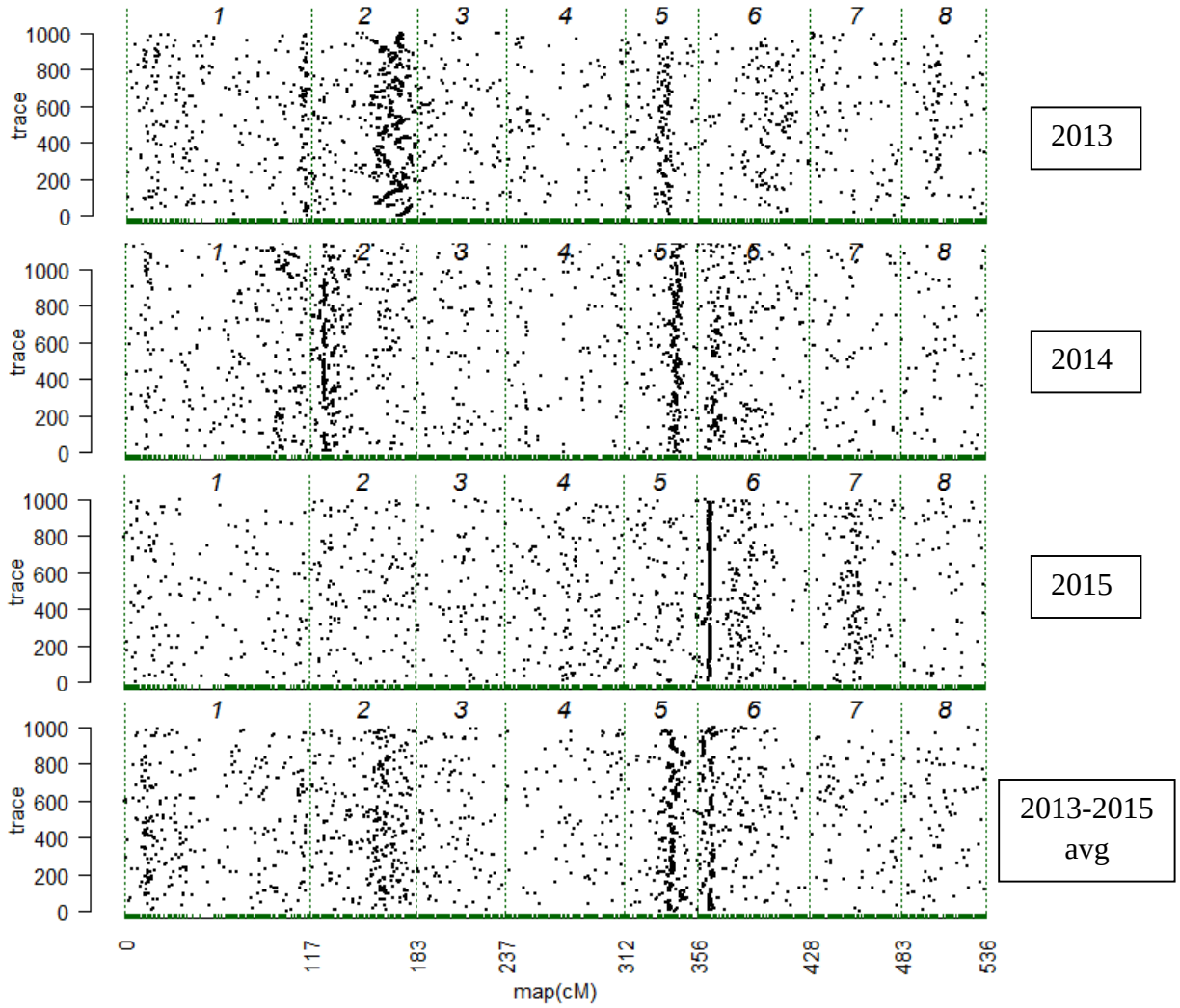

Fig. 55. PostQTL trace plots for *Xap* leaf (0-5) [(2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The colored dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Table 13. *Xap* leaf [(0-5) ± 0.5] [(2014, 2015, and 2014-2015 avg] QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL name	Data set	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G2*Xap*L.1	*Xap*L5_2013	2013	170	2	ss_260838 ss_289282	37.06 65.23	14.82 26.09	9.4	0.48	0.30	0.33
G5*Xap*L.1				5	ss_582516 ss_594216	20.68 30.02	8.27 12.00	2.6		0.03	
G2*Xap*L.2	*Xap*L5_2014	2014	172	2	ss_157034 ss_214703	5.14 17.24	2.06 6.90	7	0.45	0.17	0.31
G5*Xap*L.1				5	ss_590333 ss_601059	27.22 38.49	10.90 15.40	4.2		0.09	
G6*Xap*L.1				6	ss_615377 ss_627726	9.07 19.01	3.63 7.60	5		0.05	
G6*Xap*L.1	*Xap*L5_2015	2015	172	6	ss_604834 ss_652595	0.27 33.80	0.11 13.52	8.1	0.39	0.33	0.33
G2*Xap*L.2	*Xap*L5_avg	avg	173	2	ss_172993 ss_214703	8.07 17.24	3.23 6.90	3	0.47	0.05	0.26
G5*Xap*L.1				5	ss_588670 ss_600256	25.85 36.56	10.34 14.62	4.4		0.11	
G6*Xap*L.1				6	ss_604834 ss_620099	0.27 12.02	0.11 4.81	4.3		0.10	

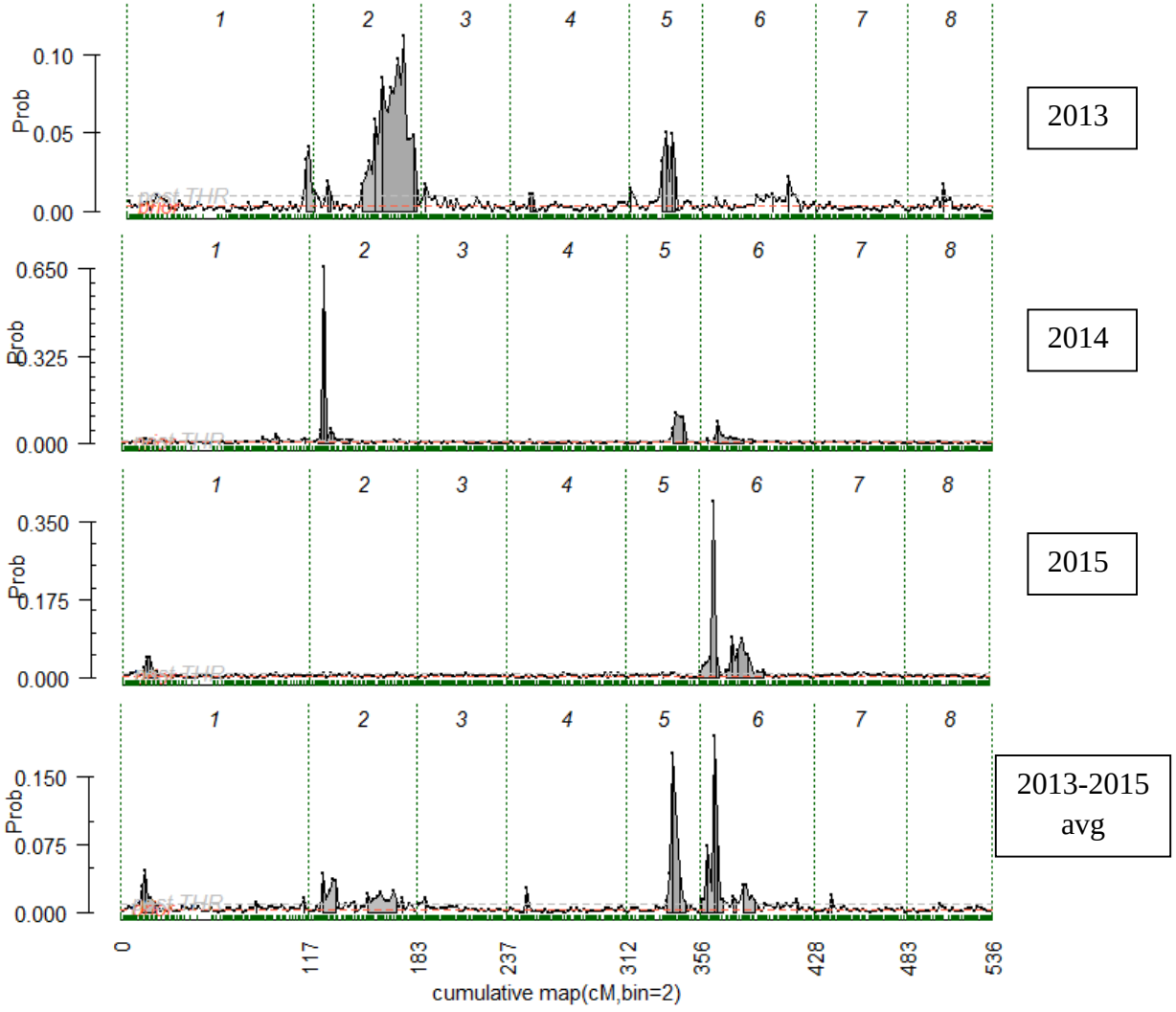

Fig. 56. PostQTL intensity plot positions for *Xap* leaf [(0-5) ± 0.5] [(2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

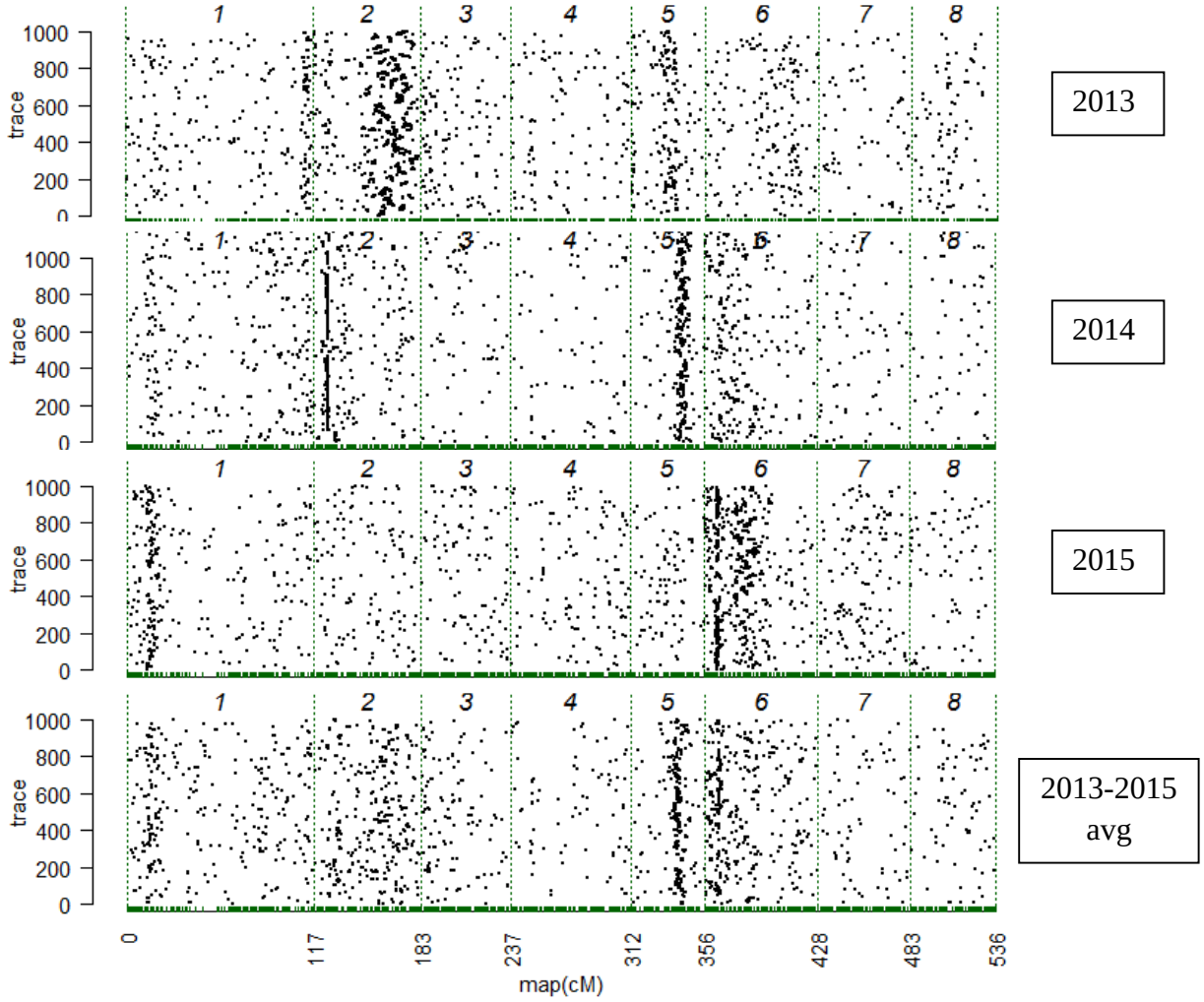

Fig. 57. PostQTL trace plots for *Xap* leaf [(0-5) ± 0.5] [(2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The colored dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Table 14. *Xap* leaf [(0-5) – 1] [(2014, 2015, and 2014-2015 avg] QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL name	Data set	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G2*Xap*L.1	*Xap*L-1_2013	2013	170	2	ss_269327 ss_286677	43.06 61.83	17.23 24.73	8.4	0.53	0.33	0.33
G2*Xap*L.2	*Xap*L-1_2014	2014	172	2	ss_142214 ss_233804	2.35 24.31	0.94 9.72	7.1	0.52	0.20	0.36
G5*Xap*L.1				5	ss_588670 ss_601173	25.85 38.65	10.34 15.46	5		0.11	
<u>G6*Xap*L.1</u>				6	ss_615377 ss_631014	9.07 21.16	3.63 8.46	2.6		0.06	
<u>G5*Xap*L.1</u>	*Xap*L-1_2015	2015	172	5	ss_559786 ss_594279	10.00 30.02	4.00 12.01	2.5	0.53	0.06	0.34
G6*Xap*L.1				6	ss_604834 ss_621556	0.27 13.55	0.11 5.42	10.5		0.28	
<u>G1*Xap*L.1</u>	*Xap*L-1_avg	avg	173	1	ss_15977 ss_24260	12.48 20.90	5.00 8.36	2.1	0.57	0.05	0.41
<u>G2*Xap*L.2</u>				2	ss_166779 ss_224651	7.24 20.94	2.90 8.38	2.9		0.10	
<u>G2*Xap*L.1</u>				2	ss_269327 ss_286677	43.06 61.83	17.23 24.73	2.9		0.05	
G5*Xap*L.1				5	ss_588670 ss_598476	25.85 35.10	10.34 14.04	5.2		0.14	
<u>G6*Xap*L.1</u>				6	ss_604834 ss_620099	0.27 12.02	0.11 4.81	2.9		0.07	

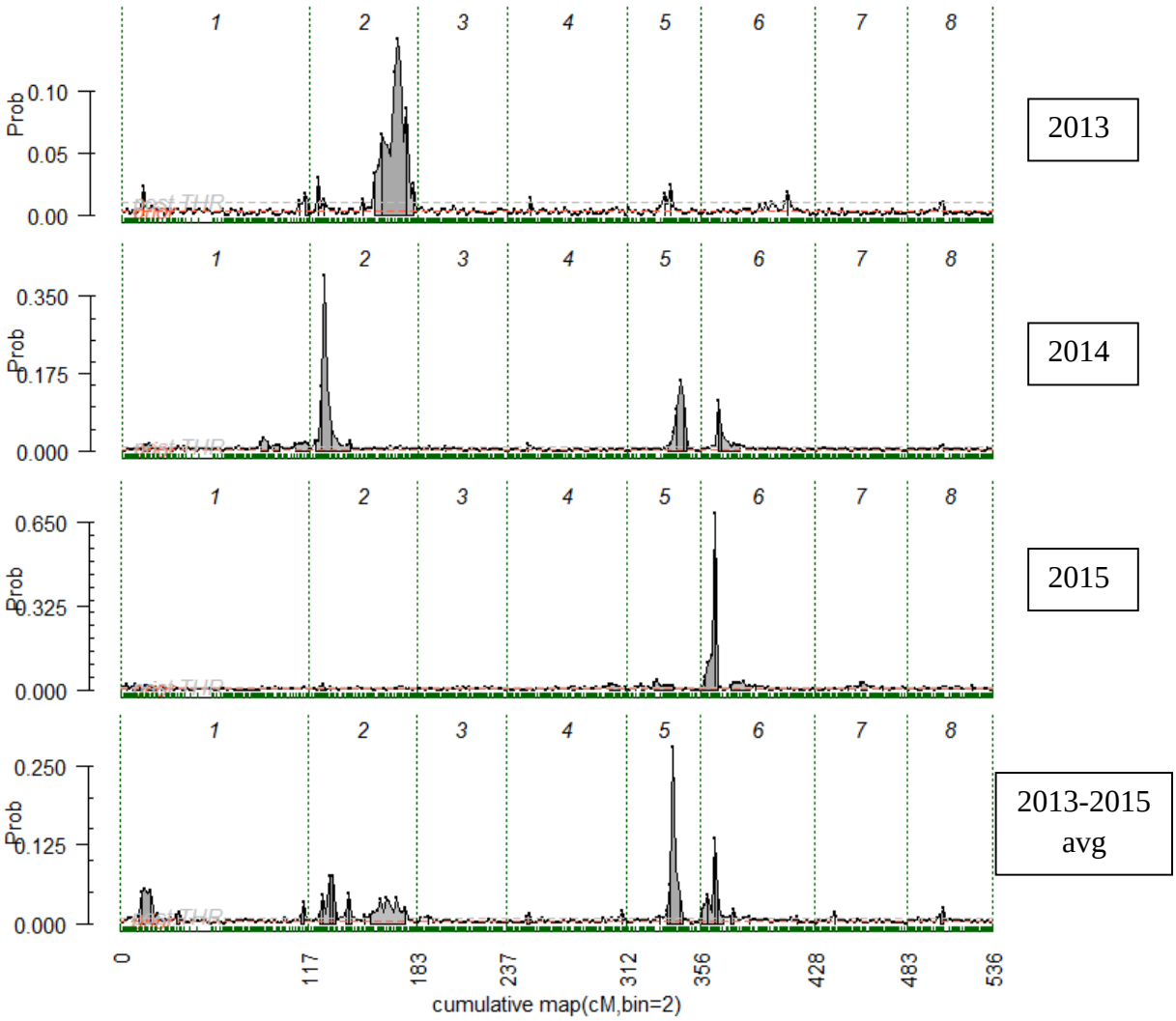

Fig. 58. PostQTL intensity plot positions for *Xap* leaf [(0-5) – 1] [(2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

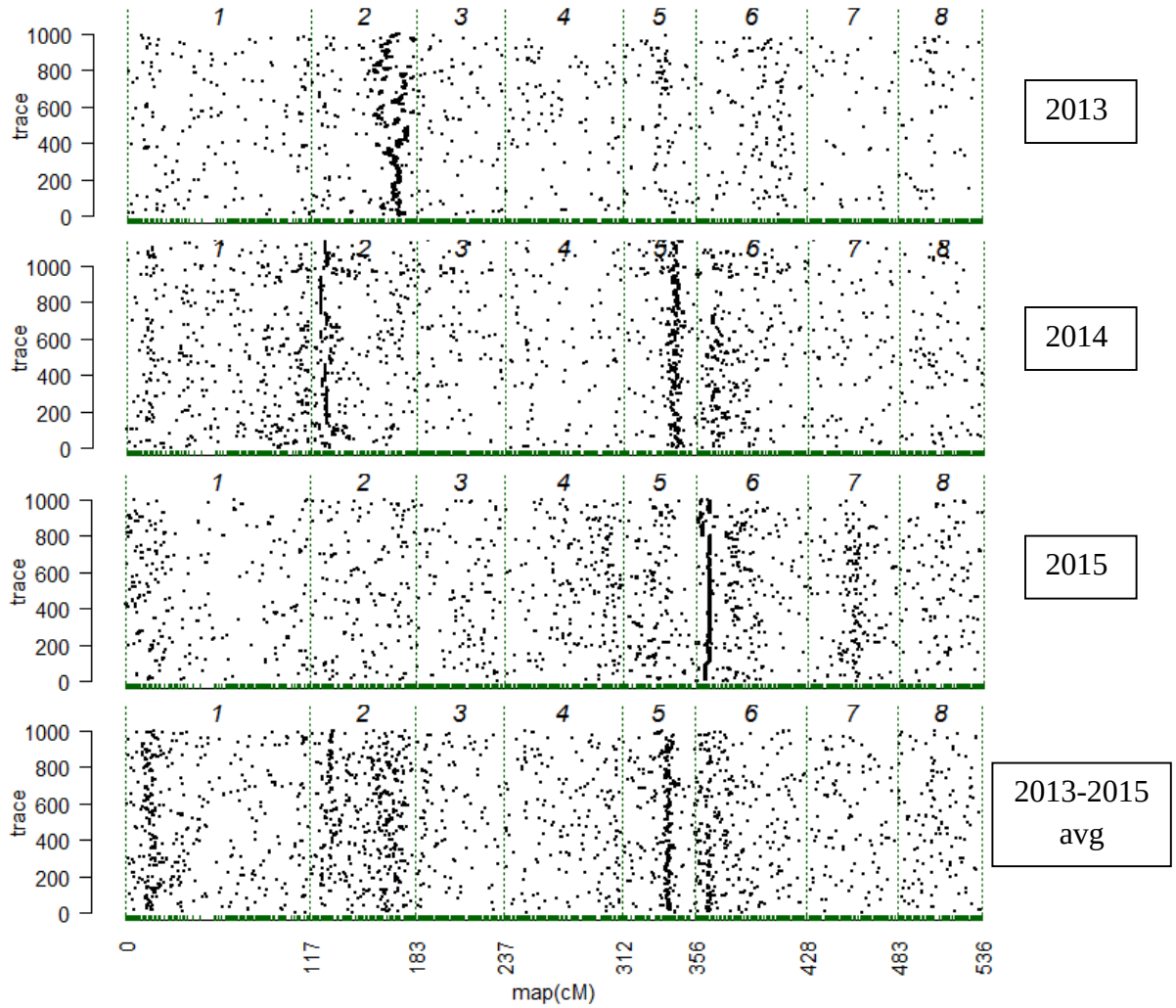

Fig. 59. PostQTL trace plots for *Xap* leaf [(0-5) – 1] [(2013, 2014, 2015, and 2013-2015 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The colored dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Xap Leaf Assay QTLs

Through PBA, a total of four QTLs across two LGs, and three QTLs across three LGs, showed at least positive evidence (BF ≥ 2) to be associated with *Xap* leaf assay 88 resistance (*Xap*L_{88}) and *Xap* leaf assay AR resistance (*Xap*L_{AR}), respectively (Table 15; Figs. 60-61). The broad-sense heritability (H^2) ranged from 42 (*Xap*L_{88}) to 62% (*Xap*L_{AR}), and showed an average of 52% across both data sets. Moreover, the H^2 was always greater than the summation of the narrow sense heritability (h^2) for all QTLs identified using each data set (Table 15). PostQTL intensity and trace plots were generated and organized in a way to visually compare QTLs identified across data sets, genome-wide (Figs. 60-61).

A total of four minor QTLs associated with *Xap* leaf assay 88 resistance (*Xap*L_{88}) were identified on LG1 (three) and LG3 (one), termed G1*Xap*L_{88}.1, G1*Xap*L_{88}.2, G1*Xap*L_{88}.3, and G3*Xap*L_{88}.1 (Table 15; Figs. 60-61). The G1*Xap*L_{88}.1 showed positive evidence (BF ≥ 2) to be associated with *Xap*L_{88} resistance using data set *Xap*L_{88}. This QTL spanned 11.96-14.71 cM (BF 2.0) and was flanked by ss_15268 and ss_18074. The G1*Xap*L_{88}.1 explained 2% of the h^2 (Vp %) for *Xap*L_{88} resistance, and a total of five SNPs spanned this QTL (Table 15; Figs. 60-61). Likewise, G1*Xap*L_{88}.2 showed positive evidence (BF ≥ 2) to be associated with *Xap*L_{88} resistance using data set *Xap*L_{88} (Table 15; Figs. 60-61). This QTL spanned 88.08-94.81 cM (BF 3.8) and was flanked by ss_109287 and ss_115560. The G1*Xap*L_{88}.2 explained 6% of the h^2 (Vp %) for *Xap*L_{88} resistance, and a total of 40 SNPs spanned this QTL (Table 15; Figs. 60-61). Yet, unfortunately after looking at PostQTL genome-wide trace and intensity plots to visualize and compare the convergence, stability, and probability of these QTLs, they were determined to not be reliable and instead could be false positives.

In contrast to *Xap*L_{88}, one major QTL and two minor QTLs associated with *Xap* leaf assay AR resistance (*Xap*L_{AR}) were identified on LG1, LG2, and LG5, termed G1*Xap*L_{AR}.1,

G2*Xap*L$_{AR}$.1, and G5*Xap*L$_{AR}$.1 (Table 15; Figs. 60-61). The G1*Xap*L$_{AR}$.1 showed strong evidence (5.0-10.0, BF) to be associated with *Xap*L$_{AR}$ resistance using data set *Xap*L$_{AR}$. This QTL spanned 12.48-29.35 cM (BF 5.7) and was flanked by ss_15977 and ss_31646. The G1*Xap*L$_{AR}$.1 explained 11% of the h^2 (Vp %) for *Xap*L$_{AR}$ resistance, and a total of 23 SNPs spanned this QTL (Table 15; Figs. 60-61). Likewise, G2*Xap*L$_{AR}$.1 showed strong evidence (5.0-10.0, BF) to be associated with *Xap*L$_{AR}$ resistance using data set *Xap*L$_{AR}$. This QTL spanned 31.10-54.84 cM (BF 9.3) and was flanked by ss_249781 and ss_282582. The G2*Xap*L$_{AR}$.1 explained 26% of the h^2 (Vp %) for *Xap*L$_{AR}$ resistance, and a total of 77 SNPs spanned this QTL (Table 15; Figs. 60-61).

Lastly, G5*Xap*L$_{AR}$.1 showed positive evidence (BF $\geq$ 2) to be associated with *Xap*L$_{AR}$ resistance using data set *Xap*L$_{AR}$. This QTL spanned 15.17-32.56 cM (BF 3.7) and was flanked by ss_573422 and ss_595786. The G5*Xap*L$_{AR}$.1 explained 5% of the h^2 (Vp %) for *Xap*L$_{AR}$ resistance, and a total of 48 SNPs spanned this QTL (Table 15; Figs. 60-61).

Table 15. *Xap* leaf-assay (0-3) (2014) (*Xap*L_{88} and *Xap*L_{AR}) QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL name	Data set	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G1*Xap*L_{88}.1	*Xap*L_{88}	2014	155	1	ss_15268 ss_18074	11.96 14.71	4.78 5.89	1.9	0.43	0.02	0.29
G1*Xap*L_{88}.2				1	ss_109287 ss_115560	88.08 94.81	35.23 37.92	1.9		0.06	
G1*Xap*L_{88}.3				1	ss_128148 ss_136096	110.13 117.12	44.05 46.85	1.9		0.08	
G3*Xap*L_{88}.1				3	ss_311192 ss_319883	15.07 20.34	6.03 8.14	3.5		0.12	
G1*Xap*L_{AR}.1	*Xap*L_{AR}	2014	154	1	ss_15977 ss_31646	12.48 29.35	5.00 11.74	5.7	0.62	0.11	0.41
G2*Xap*L_{AR}.1				2	ss_249781 ss_282582	31.10 54.84	12.44 21.93	9.3		0.26	
G5*Xap*L_{AR}.1				5	ss_573422 ss_595786	15.17 32.56	6.07 13.03	3.7		0.05	

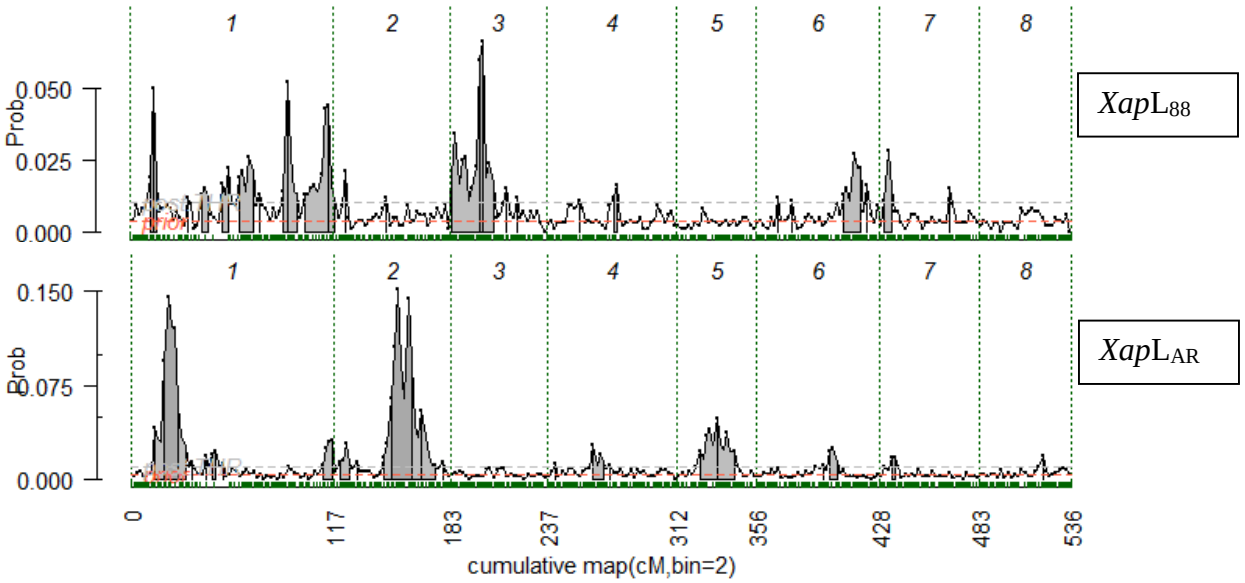

Fig. 60. PostQTL intensity plot positions for *Xap* leaf-assay (0-3) (2014) (*Xap*L_{88} and *Xap*L_{AR} data sets). Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

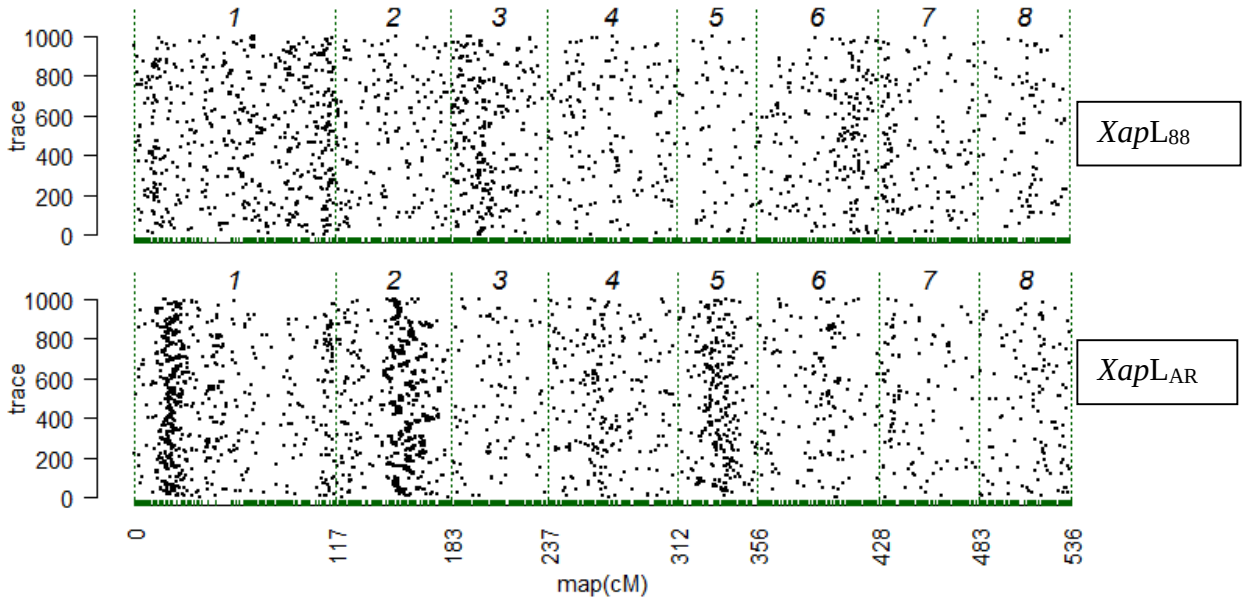

Fig. 61. PostQTL trace plots for positions for *Xap* leaf-assay (0-3) (2014) (*Xap*L_{88} and *Xap*L_{AR} data sets). Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The colored dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Major and Minor QTLs

In total, PBA identified 19 reliable QTLs for *Xap* resistance each showing at least positive evidence (BF ≥ 2): 10 QTLs for *Xap* fruit resistance (*Xap*F), six QTLs for *Xap* leaf resistance (*Xap*L), and three QTLs for *Xap* leaf assay AR resistance (*Xap*L_{AR}), using all 35 datasets (The four minor QTLs associated with *Xap* leaf assay 88 resistance (*Xap*L_{88}) were disregarded since they were determined to not be reliable). All 19 QTLs were thus compared across data sets to determine if the *Xap*F, *Xap*L, and *Xap*L_{AR} QTLs were in different locations [as previously reported for *Xap*F and *Xap*L QTLs in Yang et al. (2013)], or if they co-localized and could be considered the same QTL.

Interestingly, 15 of the QTLs in this study co-localized at six different loci using *Xap*F, *Xap*L, and *Xap*L_{AR} data sets, and only four QTLs were identified just using *Xap*F data sets. The combination of *Xap*F and *Xap*L data sets which co-located at these six loci were used to re-name the six consensus QTLs:

1. G1*Xap*F.2+L.2 (major QTL identified by seven *Xap*F and one *Xap*L data sets; on avg associated with 19% of the V_P for *Xap*F and *Xap*L resistance) (Table 16; Fig. 62).
2. G1*Xap*F.3+L_{AR}.1+L.1 (major QTL identified by the *Xap*L_{AR}, one *Xap*L, and three *Xap*F data sets; on avg associated with 21% of the V_P for *Xap*F, *Xap*L, and *Xap*L_{AR} resistance) (Table 16; Fig. 62).
3. G2*Xap*L.1+L_{AR}.1+F.1 (major QTL identified by five *Xap*L, the *Xap*L_{AR}, and three *Xap*F data sets; on avg associated with 16% of the V_P for *Xap*F, *Xap*L, and *Xap*L_{AR} resistance) (Table 16; Fig. 62).
4. G2*Xap*L.2+F.2+F.3 (major QTL identified by four *Xap*L and eight *Xap*F data sets; on avg associated with 14% of the V_P for *Xap*F and *Xap*L resistance) (Table 16; Fig. 62).

5. G5*Xap*L+F+ L_{AR}.1 (major QTL identified by nine *Xap*L, the *Xap*$L_{AR,}$ and four *Xap*F data sets; on avg associated with 7% of the V_P for *Xap*L, *Xap*L_{AR}, and *Xap*F resistance) (Table 16; Fig. 62).
6. G6*Xap*F+L.1 (major QTL identified by nine *Xap*L and eight *Xap*F data sets; on avg associated with 10% of the V_P for *Xap*L, and *Xap*F resistance) (Table 16; Fig. 62).

The four QTLs identified only using *Xap*F data sets were:

1. G1*Xap*F.1 (major QTL identified by three *Xap*F data sets; on avg associated with 20% of the V_P for *Xap*F resistance) (Tables 6-8; Figs. 42-47 and 62).
2. G2*Xap*F.3 (major QTL identified by three *Xap*F data sets, and associated with 21% of the V_P for *Xap*F) (Tables 7, 9, and 11; Figs. 44-45, 48-49, 52-53, and 62).
3. G3*Xap*F.1 (minor QTL identified by 12 *Xap*F data sets and on avg associated with 6% of the V_P for *Xap*F resistance) (Tables 6-11; Figs. 42-53, and 62).
4. G8*Xap*F.1 (minor QTL identified by six *Xap*F data sets and associated with 7% of the V_P for *Xap*F) (Tables 6-11; Figs. 42-53, and 62).

Table 16. The six concensus QTLs associated with both *Xap*F and *Xap*L resistance, identified within UA RosBREED pedigree. QTL name, data set (ds), linkage group (LG), SNPs flanking the consensus QTL, QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), average (avg) Bayes Factor (BF), and average (avg) narrow sense heritability (h^2), for all six consensus QTLs are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL name	Data set (ds)	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF avg	h^2 avg
G1*Xap*F.2+L.2	*Xap*F 2013, 2014, 2013-2015 avg, and 2014-2015 avg (7 ds); *Xap*L 2014 (1 ds);	1	ss_107432 ss_136096	86.15 117.12	34.46 46.85	6.5	0.19
G1*Xap*F.3+L_{AR}.1+L.1	*Xap*F 2014 (3 ds); *Xap*L_{AR} (1 ds); *Xap*L-1_avg (1 ds)	1	ss_15268 ss_24260	11.96 29.35	4.78 11.74	5.8	0.21
G2*Xap*L.1+L_{AR}.1+F.1	*Xap*L 2013 and 2013-2015 avg (5 ds); *Xap*L_{AR} (1 ds); *Xap*F 2013 and 2013-2015 avg (3 ds)	2	ss_249781 ss_290277	31.1 66.60	12.44 26.64	5.4	0.16
G2*Xap*L.2+F.2+F.3	*Xap*L 2014 and 2013-2015 avg (4 ds); *Xap*F 2013, 2014, 2015 and 2013-2015 avg (8 ds)	2	ss_139036 ss_233804	2.35 24.31	0.66 9.72	5.1	0.14
G5*Xap*L+F+ L_{AR}.1	*Xap*L 2013, 2014 and 2013-2015 avg (9 ds); *Xap*L_{AR} (1 ds); *Xap*F 2014 and 2013-2015 avg (4 ds)	5	ss_573422 ss_603047	15.17 41.94	6.07 16.77	5.0	0.07
G6*Xap*F+L.1	*Xap*L 2014, 2015, and 2013-2015 avg (9 ds); *Xap*F 2014 and 2013-2015 avg (8 ds);	6	ss_604834 ss_652595	0.27 33.80	0.11 13.52	5.2	0.10

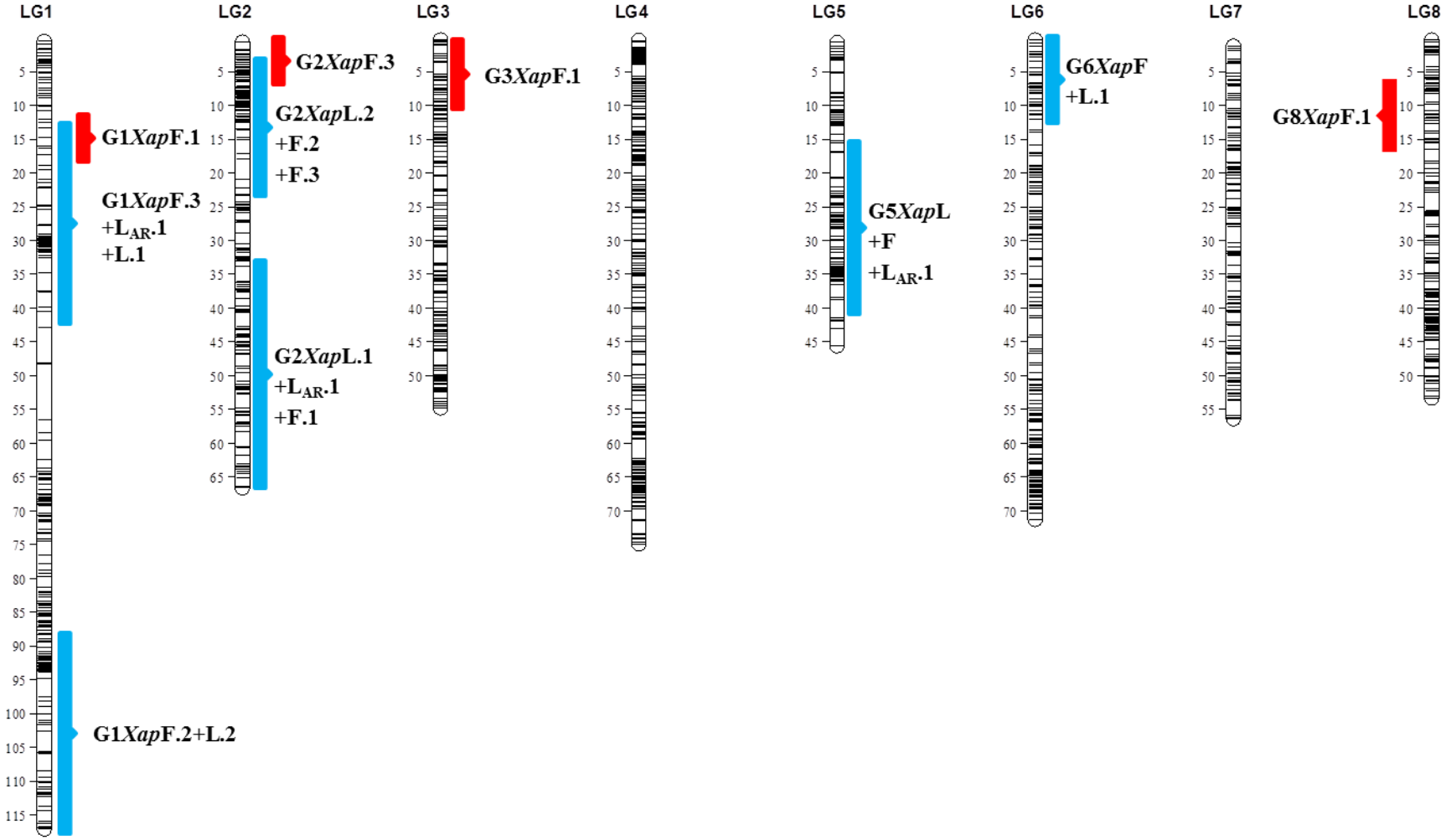

Fig. 62. The six concensus QTLs associated with both *Xap*F and *Xap*L resistance (blue), and four associated with only *Xap*F resistance (red), identified within UA RosBREED pedigree [linkage group (LG); cM noted on left of each LG; dashes on LGs represent SNPs].

Genomic Estimated Breeding Values

The genomic estimated breeding values (GEBVs) based on all QTLs for *Xap*F and *Xap*L (2013, 2014, 2015, and 2013-2015 avg) and *Xap*L_{88} and *Xap*L_{AR} (2014) were obtained from FlexQTL^{TM} (Bink et al., 2014). Overall, the GEBVs showed high positive correlations with the observed phenotypes for all traits per year (ranging from 0.71-0.89), signifying high accuracy (Table 17).

The *Xap*F data sets with highest positive GEBV correlations for each year were *Xap*_F5_2013 (0.80), *Xap*_F5_2014 (0.83), *Xap*F-1_2015 (0.79), *Xap*_F5_avg (0.83), *Xap*F5_2014-inc (0.78), *Xap*F-1_2015-inc (0.78), and *Xap*F-1_avg-inc (0.76) (Table 17). The *Xap*L data sets with highest positive correlations for each year were *Xap*L_2013 (0.82), *Xap*L-1_2014 (0.85), *Xap*L-1_2015 (0.81), and *Xap*L-1_avg (0.87). Interestingly, the *Xap*L_{AR} data set showed the highest positive correlation of all data sets (0.89), while the *Xap*L_{88} data set GEBV correlation was substantially lower (0.77) (Table 17). The GEBVs for each of the *Xap*F, *Xap*L, and *Xap*L assay data sets with highest positive correlations for each year are in Table A.1 and A.2 in Appendix A.

Table 17. Accuracy of genomic estimated breeding value (GEBV) expressed as its correlation (R^2) with the observed phenotype for each data set. The *Xap*F, *Xap*L and *Xap*L assay data sets with highest positive correlations for each year are represented in bold font.

*Xap*F data set	GEBV R^2		*Xap*L data set	GEBV R^2
*Xap*F_2013	0.77		***Xap*L_2013**	**0.82**
***Xap*_F5_2013**	**0.8**		*Xap*_L5_2013	0.8
*Xap*F-1_2013	0.78		*Xap*L-1_2013	0.8
*Xap*F_2014	0.77		*Xap*L_2014	0.77
***Xap*_F5_2014**	**0.83**		*Xap*_L5_2014	0.79
*Xap*F-1_2014	0.79		***Xap*L-1_2014**	**0.85**
*Xap*F_2015	0.72		*Xap*L_2015	0.72
*Xap*_F5_2015	0.73		*Xap*_L5_2015	0.74
***Xap*F-1_2015**	**0.79**		***Xap*L-1_2015**	**0.81**
*Xap*F_avg	0.79		*Xap*L_avg	0.83
***Xap*_F5_avg**	**0.83**		*Xap*_L5_avg	0.82
*Xap*F-1_avg	0.83		***Xap*L-1_avg**	**0.87**
*Xap*F_2014-inc	0.78		***Xap*L_{AR}**	**0.89**
***Xap*F5_2014-inc**	**0.78**		***Xap*L_{88}**	**0.77**
*Xap*F-1_2014-inc	0.71			
*Xap*F_2015-inc	0.73			
*Xap*F5_2015-inc	0.73			
***Xap*F-1_2015-inc**	**0.78**			
*Xap*F_avg-inc	0.71			
*Xap*F5_avg-inc	0.71			
***Xap*F-1_avg-inc**	**0.76**			

Discussion

In this study, pedigree-based analysis (PBA) under the Bayesian framework was used to identify and validate QTL and SNPs for *Xap* fruit (*Xap*F), *Xap* leaf (*Xap*L), and *Xap* leaf assay (*Xap*L_{AR} and *Xap*L_{88}) in the UA RosBREED peach breeding pedigree. The identified QTLs are supported by previous QTL studies in peach since all were subsequently compared with those reported in previous studies (Socquet-Juglard et al., 2013; Yang et al., 2013). The results for all major and minor QTLs identified for *Xap*F, *Xap*L, *Xap*L_{AR}, and *Xap*L_{88} will be discussed in the following sections.

Frequently, major and minor QTLs for *Xap*F and *Xap*L were only found in one or two years of the study and not across all three. Complex traits, such as *Xap* resistance, are greatly influenced by the environment, and these very contrasting environments (years) could have hampered the ability to identify the same QTL across all years. The temperature and rainfall weather condition data collected at the FRS in 2013, 2014, and 2015 showed that the temperature, humidity, and rainfall varied among all years, especially when comparing 2014 and 2015 to 2013. The second two years (2014 and 2015) were hotter and more humid in comparison to the milder 2013, which effectively created optimal conditions for *Xap* inoculum across more days in 2014 and 2015. This led to a higher selection pressure for *Xap* in 2014 and 2015, which is why *Xap*F and *Xap*L '0-5 scale' data sets were skewed towards the susceptible ratings, as compared to 2013 *Xap*F and *Xap*L data sets which showed a more even distribution from resistant to susceptible.

Interestingly, the conversion of *Xap*F and *Xap*L'0-5 scale' data sets to '0-5 ± 0.5', and '0-5 – 1' data sets in 2014, 2015, and 2013-2015 avg years resulted in higher GEBV R^2 values in comparison to the original '0-5 scale' (this same trend was not consistently observed across 2013

*Xap*F and *Xap*L data sets). Across all 2014, 2015, and 2013-2015 avg *Xap*L data sets, *Xap*L-1_2014 (0.85), *Xap*L-1_2015 (0.81), and *Xap*L-1_avg (0.87) showed the highest GEBV R^2. Similarly, across all 2014, 2015, and 2013-2015 avg *Xap*F data sets, *Xap*_F5_2014 (0.83), *Xap*F-1_2015 (0.79), and *Xap*_F5_avg (0.83) showed the highest GEBV R^2. Likewise, across all 2014, 2015, and 2013-2015 avg *Xap*F '(0-5) inc' data sets, *Xap*F5_2014-inc (0.78), *Xap*F-1_2015-inc (0.78), and *Xap*F-1_avg-inc (0.76) showed the highest GEBV R^2.

Previously, the resistance of leaf and fruit to *Xap* in peach has been suggested to be controlled by different genetic locations due to inconsistent levels of leaf and fruit resistance (Keil and Fogle, 1974; Simeone, 1985; Werner et al., 1986; Yang et al., 2013). The results in the study herein only support these previous findings for *Xap*F resistance, as four QTL were identified to only be associated with *Xap*F resistance (G1*Xap*F.1, G2*Xap*F.3, G3*Xap*F.1, and G8*Xap*F.1). On the other hand, the other six QTL were associated with both *Xap*F, *Xap*L and *Xap*L_{AR} (G1*Xap*F.2+L.2, G1*Xap*F.3+LAR.1+L.1, G2*Xap*L.1+LAR.1+F.1, G2*Xap*L.2+F.2+F.3, G5*Xap*L+F+ LAR.1, and G6*Xap*F+L.1). These ten QTLs identified in the study herein however were identified using the UA RosBREED pedigree. This program has focused on breeding for resistance to *Xap* since 1964, and unlike most other peach breeding programs, antibiotic sprays to control the disease have never been applied, thus, selection against *Xap* has been possible. These breeding efforts have generated individuals which frenquently show *Xap*L and *Xap*F resistance across seasons, such as seven of the twelve parents used to develop the seven F_1 populations in the UA RosBREED pedigree (A-760, A-772, A-773, A-776, 'Amoore Sweet', 'Souvenirs', and 'White County'). Because of this, the progeny of some populations segregated in a similar manner for both *Xap*F and *Xap*L resistance, and thus, consensus loci associated with both *Xap*F and *Xap*L resistance were identified.

The four QTLs identified in the study herein to only be associated with *Xap*F resistance, G1*Xap*F.1, G2*Xap*F.3, G3*Xap*F.1, and G8*Xap*F.1, all co-located with QTLs identified by Yang et al. (2013). The G1*Xap*F.1 flanked by ss_31646 and ss_67620 on chromosome 1 (11.74-19.31 Mbp), co-located with *Xap*.Pp.OC-1.2, in which two *Xap*F data sets identified SNP_IGA_34306 and SNP_IGA_63746 (12.01-18.60 Mbp). The *Xap.Pp.OC-1.2*, termed G1*Xap*F was previously haplotyped across a wide range of four pedigree-connected US RosBREED demonstration peach breeding programs (UA, Clemson University, Texas A&M University and University of California, Davis) (see Chapter Four for additional details). Interestingly, in this chapter the UA material was shown in general to contain a higher proportion of resistant alleles at G1*Xap*F than all three other RosBREED peach breeding programs. Likewise, G3*Xap*F.1 flanked by snp_3_130507 and ss_304082 on chromosome 3 (0.33-10.12 Mbp), co-located with *Xap*.Pp.OC-3.1, in which four *Xap*L, and one *Xap*F data set identified SNP_IGA_295433 and SNP_IGA_304307 (1.98-4.09 Mbp). It's interesting to note that in this case Yang et al. (2013) identified this region to be associated with both *Xap*F and *Xap*L resistance, while in our study the region was only associated with *Xap*F resistance. The G8*Xap*F.1, flanked by ss_832112 and ss_874263 on chromosome 8 (6.60-16.86 Mbp), spanned *Xap*.Pp.OC-8.1 and *Xap*.Pp.OC-8.2, which were instead identified using three *Xap*L data sets nearest to SNP_IGA_841298 and SNP_IGA_871727 (8.56-16.14 Mbp). The results for these three QTLs identified herein using *Xap*F data sets supports the previous QTLs identified by Yang et al. (2013), and furthermore, through comparison reveals that the co-located QTL on chromosome 1 is only associated with *Xap*F resistance, while the QTLs on chromosomes 3 and 8 are apparently associated with both *Xap*F and *Xap*L resistance.

Additionally, several of the six QTLs identified to be associated with both *Xap*F, *Xap*L, and *Xap*L_{AR} resistance, G1*Xap*F.2+L.2, G1*Xap*F.3+LAR.1+L.1, G2*Xap*L.1+LAR.1+F.1, G2*Xap*L.2+F.2+F.3, G5*Xap*L+F+ LAR.1, and G6*Xap*F+L.1, co-located with QTLs identified by Yang et al. (2013), while others did not. The G1*Xap*F.2+L.2, flanked by ss_107432 and ss_136096 on chromosome 1 (34.46-46.85 Mbp), co-located with *Xap*.Pp.OC-1.3, in which four *Xap*L data sets identified SNP_IGA_103422 and SNP_IGA_112042 (32.58-36.53 Mbp). The findings in the study herein add further evidence to this QTL, and additionally, reveal that the locus spans further upstream and is associated with both *Xap*F and *Xap*L resistance. The G1*Xap*F.3+L_{AR}.1+L.1, flanked by ss_15268 and ss_24260 on chromosome 1 (4.78-11.74 Mbp), co-located with *Xap*.Pp.OC-1.1, in which three *Xap*L data sets identified SNP_IGA_5891 and SNP_IGA_17833 (1.96-5.80 Mbp). Once again, the findings in this study add further evidence to this QTL, and additionally reveal that the locus spans further upstream and is associated with both *Xap*F and *Xap*L resistance. The G2*Xap*L.1+L_{AR}.1+F.1, flanked by ss_249781 and ss_290277 on chromosome 2 (12.44-26.64 Mbp), was only identified in the study herein. The bi-parental QTL analysis approach used in Yang et al. (2013) could have limited their ability to detect all QTL responsible for *Xap* resistance, such as G2*Xap*L.1+L_{AR}.1+F.1. On the other hand, the G2*Xap*L.2+F.2+F.3, flanked by ss_142214 and ss_233804 on chromosome 2 (0.94-9.72 Mbp), spanned *Xap*.Pp.OC-2.1 (associated with *Xap*L) and *Xap*.Pp.OC-2.2 (associated with *Xap*F) flanked by SNP_IGA_137253 and SNP_IGA_238077 (0.46-10.37 Mbp). Once again, the findings in the study herein add further evidence to these QTLs, and additionally, reveal that this locus is associated with both *Xap*F and *Xap*L resistance. The G5*Xap*L+F+ L_{AR}.1, flanked by ss_573422 and ss_603047 on chromosome 5 (6.07-16.77 Mbp), co-located with *Xap*.Pp.OC-5.1, in which one *Xap*L data set and one *Xap*F data set identified SNP_IGA_591439 and

SNP_IGA_594090 (11.20-12.05 Mbp). The findings in this study add further evidence to this QTL being associated with both *Xap*L and *Xap*F. On the other hand, the G6*Xap*F+L.1, flanked by ss_604834 and ss_652595 on chromosome 6 (0.11-13.52 Mbp), was not identified in Yang et al. (2013). Instead, they identified a QTL only associated with *Xap*F resistance by SNP_IGA_682531 (22.73 Mbp) which was termed G6*Xap*F and previously haplotyped across a wide range of four pedigree-connected US RosBREED demonstration peach breeding programs (UA, Clemson University, Texas A&M University and University of California, Davis) (see Chapter Four for additional details). Interestingly, in this chapter the UA material was shown in general to contain a higher proportion of resistant alleles at G6*Xap*F than all three other RosBREED peach breeding programs. Moreover, the UA material is almost fixed with *Xap*F resistant alleles (>80% of all alleles at this locus being the resistant alleles). These findings could be the major reason why G6*Xap*F was not identified using the UA PBA approach, simply due to the fact that there was no segregation for resistant and susceptible alleles at this locus in the UA RosBREED pedigree. Lastly, the five additional QTLs identified by Yang et al. (2013), *Xap*.Pp.OC-3.2, *Xap*.Pp.OC-4.1, *Xap*.Pp.OC-4.2, and *Xap*.Pp.OC-7.1 were not supported by the study herein. Either these QTLs are only associated with the lineages of their OC population, and not in the AR RosBREED pedigree, or instead they are false positive QTLs.

Interestingly, the G5*Xap*L+F+ L_{AR}.1 identified in the study herein as well as Yang et al. (2013) also co-located with a major QTL for *Xap* leaf resistance in apricot (Socquet-Juglard et al., 2013). Using the HaRM F_1 population (101 seedlings), this group identified a major QTL which explained 53% of XapL resistance. Of the V_P for *Xap* leaf resistance on LG 5 of the low-density 'Rouge de Mauves' apricot map (only 55 AFLP and 53 SSR markers, which covered all eight apricot chromosomes). The UDAp-452 marker was located at the peak, and BPPCT037

and BPPCT038A flanked the QTL. These markers are located at ~12.31 (BPPCT037), ~13.76 (UDAp-452), and ~ 14.66 (BPPCT038A) Mbp in the peach genome v1.0. The G5*Xap*L+F+ L_{AR}.1 identified in the study herein was located from 6.07-16.77 Mbp, considering 14 *Xap*L, *Xap*F, and *Xap*L_{AR} data sets. Furthermore, when only considering the six data sets which identified QTLs explaining the highest amount of V_P (7-14%), the overlapping location is narrowed down to 10.48-16.77 Mbp, which is closer to being as narrow as the 12.31-14.66 Mbp region identified in Socquet-Juglard et al. (2013). The main difference between both studies is that Socquet-Juglard et al. (2013) identified this region to be a major QTL (46-53% of V_P), while in the study herein this region was deemed a minor QTL (7-14% of V_P). As in Yang et al. (2013), Socquet-Juglard et al. (2013) only used a single bi-parental population, with only 101 seedlings, and a very low density linkage map (108 total markers, in comparison to the 1,947 SNP herein). The small F_1 population used in their study could have resulted in overestimation of QTL effect, as well as detection of false positives.

Lastly, it's also important to note that the four minor QTLs associated with *Xap* leaf assay 88 resistance (*Xap*L_{88}) were disregarded since they were determined to not be reliable, and thus, were regarded as false positives (after looking at PostQTL genome-wide trace and intensity plots to visualize and compare the convergence, stability, and probability of these QTLs). Unfortunately, it was apparent that the majority of all seedlings in the seven F_1 UA RosBREED populations, and their parents and grandparents, were highly susceptible to *Xap* isolate 88 (a mean of 2.50 across all individuals in comparison to a mean of 1.92 across all individuals screened with *Xap* isolates from AR, *Xap*L_{AR}), which was originally collected from peach orchards near North Carolina State University (K. Gasic, personal communication). The lack of segregation from resistant to susceptible, when using *Xap*L_{88} for the AR RosBREED pedigree (in

comparison to *Xap*L_{AR}), is why no reliable resistance QTLs were identified. Furthermore, in the field at FRS, there has been observed susceptibility of resistant cultivars bred in other states (found in the germplasm plots at FRS) to AR *Xap* isolates. These results support the findings of previous studies that *Xap* strain and population virulence levels from different locations were observed to differ remarkably among different peach and other stone fruit cultivars bred from different locations (Civerolo, 1975; Du Plessis, 1988; OEPP/EPPO, 2006; Scortichini et al., 1996).

Thus, it will be imperative to combine broad horizontal *Xap* resistance in order for cultivars that are planned to be grown in multiple different environments across the U.S. to maintain resistance to the *Xap* isolates from all regions. This indeed will be a daunting task for peach breeders to accomplish, yet through the incorporation of MAS for all nine QTL identified in the study herein, and those found in previous studies (Socquet-Juglard et al., 2013; Yang et al., 2013), breeders in all locations (even those in Central California, where no *Xap* is rarely seen) will be able to begin to combine resistance alleles at multiple loci, with high fruit quality, spanning the entire season.

Future Work - SNP Data Set and Haplotype Construction

Yet in order for this to be possible, these nine QTL must first be constructed into SNP haplotype DNA tests in order for breeders to use them routinely in their programs. The same steps used to haplotype G1*Xap*F and G6*Xap*F and across a wide range of four pedigree-connected U.S. RosBREED demonstration peach breeding programs (UA, Clemson University, Texas A&M University and University of California, Davis) (see Chapter Four for additional details) can be used. All individuals in the U.S. RosBREED pedigree were previously genotyped using the IPSC 9K peach SNP array v1.0 (Verde et al., 2012) with SNP genotypes determined

using the Illumina® Genome Studio software. A set of SNPs spanning a historically non-recombining QTL haploblock among all cultivars and ancestors included in the combined RosBREED demonstration program pedigree can be used for haplotype construction. These haplotypes can be initially constructed using FlexQTL™ software (Bink 2004 and 2005; Bink et al., 2008 and 2012), and subsequently confirmed based on inheritance and segregation in bi-parental populations. Functional haplotypes can then be determined for each unique SNP haplotype sequence after establishment of haplotype effects by comparing haplotypes with the phenotypic trait of interest. All RosBREED material genotyped with the IPSC 9K peach SNP array v1.0. can be used to validate the alleles associated with *Xap*F and *Xap*L resistance. Validated markers can then also be incorporated into MAS in the UA peach breeding program, as well as the other three RosBREED demonstration programs.

This PBA QTL analysis approach, followed by SNP data set and haplotype construction, effectively creates a flexible and continuously expanding platform for QTL and marker identification, validation, and use in the UA peach and nectarine breeding program. All future studies analyzing new populations for *Xap*F and *Xap*L resistance, as well as additional traits, can be added to the UA RosBREED pedigree for subsequent PBA. Additionally, the GEBVs generated through posteriori PBA results can be used as a quantitative scale for decision support on which selections/cultivars to use as parents in crossing (to pass on their additive genetic effects to progeny for *Xap*F and *Xap*L resistance), as well as which to advance in the UA breeding program.

Literature Cited

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. of Phytopathol. 156:338-345.

Abbott, A.G., A.C. Lecouls, Y. Wang, L. Georgi, R. Scorza, and G. Reighard. 2002. Peach: The model genome for Rosaceae genomics. Acta Hort. 592:199-209.

Ahmad, R., D. Potter, and S.M. Southwick. 2004. Identification and characterization of plum and pluot cultivars by microsatellite markers. J. Hort. Sci. Biotech. 79:164–169.

Bardsley, S.J., and H.K. Ngugi. 2012. Reliability and accuracy of visual methods to quantify severity of foliar bacterial spot symptoms on peach and nectarine. Plant Pathol. 62:460-474.

Bassi, D., and R. Monet. 2008. Botany and Taxonomy. p. 1-30. In: D.R. Layne and D. Bassi (eds.). The peach, botany, production and uses. CAB International Press, Wallingford, Oxon, UK. 850.

Bassil, N.V. and G.M. Volk. 2010. Standardized phenotyping: advantages to horticulture introduction to the workshop. HortScience 45:1306-1306.

Bink, M. 2004. FlexQTL software. BIOMETRIS, Wageningen UR, The Netherlands <www.flexqtl.nl>.

Bink, M. 2005. FlexQTL software: efficient estimation of identity by descent probabilities and QTL mapping in pedigreed populations. In: Plant and Animal Genomes XII Conference, 15-19 January, San Diego, CA, U.S.

Bink, M., C. ter Braak, M. Boer, L. Totir, C. Winkler, and O. Smith. 2012. QTL linkage analysis of connected populations using ancestral marker and pedigree information. Theor. Appl. Genet. 124:1097–1113.

Bink, 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.

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

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. Crit. Rev. Plant Sci. 29:59-107.

Breseghello, F., and M.E. Sorrells. 2006. Association analysis as a strategy for improvement of quantitative traits in plants. Crop Sci. 46:1323-1330.

Byrne, D.H. 2005. Trends in stone fruit cultivar development. HortTechnology 15:494–500.

Byrne, D., M. Bassols, D. Bassi, M. Piagnani, K. Gasic, G. Reighard, M. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: M. Badenes and D. Byrne (eds.). Fruit breeding. Springer Science, Business Media, New York.

Byrne, P.F., and M.D. McMullen. 1996. Defining genes for agricultural traits: QTL analysis and the candidate gene approach. Probe 7:24-27.

Civerolo, E.L. 1975. Quantitative aspects of pathogenesis of *Xanthomonas pruni* in peach leaves. Phytopathol. 65:258-264.

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. Burbage. 1982. Selective medium for *Xanthomonas campestris* pv. *pruni*. Plant Dis. 66:39-43.

Cobb, N.A. 1892. Contribution to an economic knowledge of the Australian rusts (Uredinae). Agrie. Gaz, NS Wales 3:181-212.

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.

Daines, R. 1961. What we know and don't know about bacterial spot of peach. Horticultural News 42:110-114.

Dirlewanger, E., P. Cosson, M. Tavaud, M.J. Aranzana, C. Poizat, A. Zanetto, P. Arús, and F. Laigret. 2002. Development of microsatellite markers in peach [*Prunus persica* (L) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L). Theor. Appl. Genet. 105:127–138.

Du Plessis, H.J. 1988. Differential virulence of *Xanthomonas campestris* pv. *pruni* to peach, plum, and apricot cultivars. Phytopathol. 78:1312-1315.

Edge-Garza, D.A., J.J. Luby, and C.P. Peace. 2016. Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Acta Hort. In press.

Etienne, C., C. Rothan., A. Moing., C. Plomion., C. Bodenes., 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.

EPPO/CABI 1997. *Xanthomonas arboricola* pv. *pruni*, p. 1096–1100. In: Quarantine Pests for Europe, 2nd ed., CAB International, Wallingford (GB).

EPPO. 2003. Data sheets on quarantine organisms. *Xanthomonas arborícola* pv. *pruni*. www.eppo.org/QUARANTINE/BACTERIA/Xanthomonas_pruni/XANTPR_ds.pdf

Fahy, P.C., and A.C. Hayward. 1983. Media and methods for isolation and diagnostic tests, p. 337-374. In: P.C. Fahy and G.J. Persley (eds.). Plant Bacterial Diseases, a Diagnostic Guide. Academic Press, Sydney, Australia.

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 Phytol. 185:917-930.

Flint-Garcia, S.A., J.M. Thornsberry, and E.S. Buckler. 2003. Structure of linkage disequilibrium in plants. Annu. Rev. Plant Biol. 54:357–374.

Fresnedo-Ramírez, J., M.C.A.M. Bink, W.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. Breed. 35:1-19.

Fresnedo-Ramírez, 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.C.A.M. Bink, W.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. Mol. Breed. In press.

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

Hajri, A., J.F. Pothier, M. Fischer-Le Saux, S. Bonneau, S. Poussier, T. Boureau, B. Duffy, and C. Manceau. 2012. Type three effector gene distribution and sequence analysis provide new insights into the pathogenicity of plant-pathogenic *Xanthomonas arboricola*. Appl. Environ. Microbiol. 78:371-384.

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. 1990. Resistance responses of plants regenerated from peach callus, cultures to *Xanthomonas campestris* pv. *pruni*. J. Amer. Soc. Hort. Sci. 115:1034-1037.

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.

Howad, W., T. Yamamoto, E. Dirlewanger, R. Testolin, P. Cosson, G. Cipriani, A.J. Monforte, L. Georgi, A.G. Abbott, and P. Arus. 2005. Mapping with a few plants: using selective mapping for microsatellite saturation of the *Prunus* reference map. Genet. 171:1305-1309.

Huang H., Z. Cheng, Z. Zhang, and Y. Wang. 2008. History of cultivation and trends in China, p. 37-57. In: D.R. Layne and D. Bassi (eds.). The peach, botany, production and uses. CAB International Press, Wallingford, Oxon, UK. 850.

Janse, J.D., M.P. Rossi, R.F.J. Gorkink, J.H. Derks, J. Swings, D. Janssens, and M. Scortichini. 2001. Bacterial leaf blight of strawberry (*Fragaria* (x) *ananassa*) caused by a pathovar of *Xanthomonas arboricola*, not similar to *Xanthomonas fragariae* Kennedy & King. Description of the causal organism as *Xanthomonas arboricola* pv. fragariae (pv. nov., comb. nov.). Plant Pathol. 50:653– 665.

Jones, A.L., and T.B. Sutton. 1996. Diseases of tree fruits in the East. Michigan State Univ. Ext., U.S.

Jones, N., H. Ougham, and H. Thomas. 1997. Markers and mapping: we are all geneticists now. New Phytol. 137:165–177.

Jung, S., M. Staton, T. Lee, A. Blenda, R. Svancara, A. Abbott, D. Main. 2008. GDR (Genome Database for Rosaceae): integrated webdatabase for Rosaceae genomics and genetics data. Nucleic Acids Res 36:D1034–D1040.

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

Madden, L.V., G. Hughes, and F. Van den Bosch. 2007. The study of plant disease epidemics. APS Press, St. Paul, MN.

OEPP/EPPO. 2006. *Xanthomonas arboricola* pv. *pruni*. OEPP/EPPO Bul. 36:129–133.

Okie, W.R., T. Bacon, and D. Bassi. 2008. Fresh market cultivar development, p. 140-169. In: D.R. Layne and D. Bassi (eds.). The peach, botany, production and uses. CAB International Press, Wallingford, Oxon, UK. 850.

Okie, W.R. 1998. Handbook of peach and nectarine varieties – performance in the Southeastern U.S. and index of names: USDA/ARS Agriculture.

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

Palleroni, N.J., D.C. Hildebrand, M.N. Schroth, and M. Hendson. 1993. Deoxyribonucleic acid relatedness of 21 strains of *Xanthomonas* species and pathovars. J. Appl. Bacteriol. 75:441–446.

Paterson, A.H. 1996. Making genetic maps, p. 23-39. In: A.H. Paterson (ed.). Genome mapping in plants. R.G. Landes Company, San Diego, CA.

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. and Genomes 10:1679-1694.

Perez, A., K. Plattner, and K. Baldwin. 2011. Fruit and tree nuts outlook. Economic Research Service, USDA, FTS-348 (http://www.ers.usda.gov/publications/fts/2011/07Jul/FTS348.pdf).

Pflieger, S., V. Lefebvre, and M. Causse. 2001. The candidate gene approach in plant genetics: a review. Mol. Breeding 7:275-291.

Pozzi, C. and A. Vecchietti. 2009. Peach structural genomics, p. 235-257. In: K.M. Folta. and S.E. Gardiner (eds.). Genetics and genomics of Rosaceae. Springer, New York.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org/. Accessed 12 November 2015.

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

Reich, D.E. 2001. Linkage disequilibrium in the human genome. Nature 411:199–204.

Ritchie, D.F. 1995. Bacterial spot, p. 50-52. In: J.M. Ogawa, E.I. Zehr and G.W. Bird (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 Nematicide Tests 54:63-64.

Rosalam, S., and R. England. 2006. Review of xanthan gum production from unmodified starches by *Xanthomonas comprestris* sp. Enzyme Microb. Technol. 39:197–207.

Ru, S., D. Main, K. Evans, and C. Peace. 2015. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet. and Genomes 11:1-12.

Salazar, J.A., D. Ruiz, J.A. Campoy, R. Sánchez-Pérez, C.H. Crisosto, P.J. Martínez-García, A. Blenda, S. Jung, D. Main, P. Martínez-Gómez, and M. Rubio. 2013. Quantitative trait loci (QTL) and mendelian trait loci (MTL) analysis in *Prunus*: a breeding perspective and beyond. Plant Mol. Biol. Rep. 1-18. DOI 10.1007/s11105-013-0643-7.

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

Scortichini, M., J. D.Janse., M.P. Rossi, and J.H.J. Derks. 1996. Characterization of *Xanthomonas campestris* pv. *pruni* strains from different hosts by pathogenicity tests and analysis of whole-cell fatty acids and whole-cell proteins. J. of Phytopathol. 144:69-74.

Sherman, W.B., and P.M. Lyrene. 1981. Bacterial spot susceptibility in low chilling peaches. Fruit Var. J. 35:74–77.

Simeone A.M. 1985. Study on peach and nectarine cultivars susceptibility to the main fungus and bacteria. Acta Hort. 173:541–551.

Socquet-Juglard, D., A. Patocchi., J.F. Pothier., D. Christen, and B. Duffy. 2012. Evaluation of *Xanthomonas arboricola* pv. *pruni* innoculation techniques to screen for bacterial spot. J. Plant Pathol. 94:1-91.

Socquet-Juglard D, B. Duffy, J.F. Pothier, D. Christen, C. Gessler, A. Patocchi. 2013. Identification of a major QTL for *Xanthomonas arboricola* pv. *pruni* resistance in apricot. Tree Genet. and Genomes 9:409-421.

Smith, E. 1903. Observations on a hitherto unreported bacterial disease, the cause of which enters the plant through ordinary stomata. Sci. 17:456–457.

Smith, S. 2015. Fruit tree diseases. 16 November 2015. <https://www.uaex.edu/publications/pdf/mp154/fruit-tree-diseases-commercial.pdf>.

Studebaker, G., J. Hopkins, and D. Johnson. 2015. Control peach tree borers on commercially grown peach and plum trees. 16 November 2015. <https://www.uaex.edu/publications/PDF/FSA-7504.pdf>.

Tanksley, S.D., N.D. Young., A.H. Paterson, and M.W. Bonierbale. 1989. RFLP mapping in plant-breeding - new tools for an old science. Bio-Technol. 7:257-264.

Vauterin, L., B. Hoste., K. Kersters, and J. Swings. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45:472–489.

van de Weg, W.E., R.E. Voorips., H.J. Finkers., L.P. Kodde., J. Jansen, and M. Bink. 2004. Pedigree genotyping: A new pedigree-based approach of QTL identification and allele mining. Acta Hort. 663:45-50.

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. DOI:10.1371/journal.pone.0035668.

Verma, S., J.J. Luby, K. Evans, U.R. Rosyara, Y. Guan, N. Bassil, M.C.A.M. Bink, W.E. van de Weg, and C.P. Peace. 2016. *Ma* locus functional alleles predict apple acidity within RosBREED Washington Apple Breeding Program Germplasm. Mol. Breed. In press.

Voorrips, R.E. 2001. MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. 93:77-78.

Voorrips, R.E. 2007. Pedimap: software for visualization of genetic and phenotypic data in pedigrees. Plant Research International, Wageningen, the Netherlands. <http://www.wageningenur.nl/en/show/Pedimap.htm>.

Voorrips, R., M. Bink, and W. van de Weg. 2012. Pedimap: software for the visualization of genetic and phenotypic data in pedigrees. J. Hered. 103:903-907.

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.

Winter, P. and G. Kahl. 1995. Molecular marker technologies for plant improvement. World J. of Microbiology & Biotechnol. 11:438-448.

Yang, N., G.L. Righard, D. Ritchie, W.R. 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 (abstr.).

Yang, N., G.L. Righard, D. Ritchie, W.R. Okie, and K. Gasic. 2011. Development of a genetic linkage map for identification of molecular markers associated with resistance to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in peach. XIII Eucarpia Symposium on Fruit Breeding and Genet., September 11–15, 2011 Warsaw, Poland.

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

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

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

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

Chapter Three: Pedigree-Based Quantitative Trait Loci Analysis (PBA) for Fruit Quality and Phenological Traits in the University of Arkansas Peach and Nectarine Breeding Program

Abstract

Despite the ever-growing availability of genomic resources in peach, the use of DNA tests for marker-assisted selection (MAS) is still in its infancy. To date, approximately 568 marker-locus-trait associations [M-L-T (QTL or MTL)] have been identified in peach, yet, the majority cannot currently be used in breeding programs for MAS for several reasons, including the fact that nearly all of these previous peach QTL studies used single bi-parental segregating populations, and therefore, the relevance of the majority of these findings may be limited only to specific lineages from the parents of those bi-parental populations. The objective of this study was to conduct genome-wide quantitative trait loci (QTL) analysis for seven peach fruit quality and phenological traits using the Pedigree-Based Analysis (PBA) approach and the University of Arkansas (UA) RosBREED peach breeding pedigree. The overall goal was to identify and validate QTLs and SNPs for fruit quality and phenological traits in a large portion of this program's breeding material, for subsequent application of MAS. In total, 1,947 polymorphic SNP markers and three years of phenotypic data for all traits from seven pedigree-connected families were analyzed using FlexQTL™ software. A total of 39 QTLs with at least positive evidence (BF $\geq$ 2) were identified using 2011, 2012, 2013, and 2011-2013 avg data sets for all seven phenotypic traits. Overall, nine major QTLs depicted $\geq$ 10% of the phenotypic variation for their respective trait: G4MD.1 (maturity date); G5Pub.1 (pubescent vs. glabrous); G1FC.2 (white vs. yellow flesh color), G3BL.1 (blush overcolor); G2FW.1, G4FW.1, and G5FW.1 (fruit weight) and G4SSC.1, G7SSC.1, and G7SSC.2 (soluble solids content). The location of all these

major QTLs in the peach genome sequence v1.0 were compared to the locations noted in previous peach QTL studies in *Prunus*. Several co-located within previous QTLs, while some did not. Additionally, genomic estimated breeding values (GEBVs) for all traits were obtained through PostFlexQTL^{TM} software analyses. The highest positive correlations were observed for maturity date (0.83-0.93), followed by fruit weight (0.74-0.92), soluble solids content (0.76-0.83) and blush overcolor (0.60-0.73). This PBA QTL analysis approach, followed by SNP data set and haplotype construction, effectively creates a flexible and continuously expanding platform for QTL and marker identification, validation, and use of MAS in the UA peach and nectarine breeding program. Additionally, the GEBVs generated through PostFlexQTL^{TM} software analyses can be used as a quantitative scale for decision support on which selections/cultivars to use as parents in cross, as well as which to advance in the UA breeding program.

Introduction

Peach [*Prunus persica* (L.) Batsch] is a self-fertile, diploid species (2n = 16), with a base chromosome number of x = 8, and belongs to the Rosaceae family, subfamily Prunoideae (Bassi and Monet, 2008; Byrne et al., 2012). Great advances in the understanding of the genetic control of several traits in peach have taken place in the past two decades, so much so that peach is now known as the model species for all Rosaceae fruit crops (Abbott et al., 2002; Shulaev et al., 2008). This model species has a relatively short juvenility period (~2-3 years), simple genomic structure (227.4 Mb), and high amount of developed genetic resources (Abbott et al., 2002; Salazar et al., 2013). Also, peach is the best genetically characterized *Prunus* species with 19 major genes mapped to specific loci on the highly saturated *Prunus* genetic reference map, 'T × E' (Aranzana et al., 2003; Dirlewanger et al., 2004; Etienne et al., 2002; Joobeur et al., 1998; Pozzi and Vecchietti, 2009; Sansavini et al., 2006). A total of 46 linkage maps have been used

for QTL analysis through traditional bi-parental mapping populations (Salazar et al., 2013). To date, numerous QTL studies have been performed in peach and a total of 568 different marker-trait-loci [M-L-T (QTL or MTL)] have been described in peach and/or related interspecific hybrids and can be found on the Genomic Database for Rosaceae (GDR; http://www.rosaceae.org/) (Jung et al., 2008; Jung et al., 2014). Additionally, a partial list of these QTL and MTL can be seen in Salazar et al. (2013). These QTL and MTL were linked to 75 different peach traits: 13 for tree development, 15 for flower and ripening, six for disease and pest resistance, and 41 for fruit quality (Salazar et al., 2013).

Unfortunately, despite the ever-growing availability of genomic resources in peach, the use of DNA tests for marker-assisted selection (MAS) is still in its infancy. Today, application of MAS using peach DNA-based tests is only a reality for a few breeding-relevant traits including but not limited to: texture (Peace et al., 2005; Peace and Norelli 2009), acidity (Eduardo et al. 2014), slow-melting flesh (Salgado, 2015), fruit bacterial spot resistance (see Chapter Five, Section One), white vs. yellow flesh (see Chapter Five, Section Three; Falchi et al., 2013), blush coverage (Sandefur et al., 2016a), and acidity and soluble solids content (Sandefur et al., 2016b). Furthermore, all previous peach QTL studies used single bi-parental segregating populations, excluding two recent publications by Fresnedo-Ramírez et al., (2015 and 2016), and therefore, the relevance of the majority of these findings may be limited only to specific lineages from the parents of the bi-parental populations (Peace et al., 2014).

The pedigree-based QTL analysis (PBA) (using the Bayesian Framework performed through FlexQTL^{TM}) has recently emerged as a superior strategy, with key advantages over bi-parental QTL analysis (Bink, 2004 and 2005; Bink et al., 2008 and 2012; Fresnedo-Ramírez et al., 2015; Fresnedo-Ramírez et al., 2016; Iezzoni 2010; van de Weg et al., 2004; Verma et al.,

2016). The PBA strategy follows all the necessary steps of traditional bi-parental QTL analysis, except it simultaneously incorporates multiple breeding populations that segregate for the trait(s) of interest, which enhances the ability to detect further QTL action. This method is a more powerful QTL statistical approach used to simultaneously identify marker-trait associations, validate their robustness and applicability in individual breeding programs, and discover alleles for functional diversity (Bink, 2004 and 2005; Bink et al., 2008 and 2012; van de Weg et al., 2004). The approach also allows estimation of genetic variances and genomic estimated breeding values (GEBVs) for all material in the pedigree (Bink et al., 2008).

The PBA approach for QTL mapping using linkage methods within pedigrees was first employed by the recent RosBREED I initiative (Fresnedo-Ramírez et al., 2016; Iezzoni 2010). Through the RosBREED I initiative, the PBA approach has been enabled across multiple fruit breeding programs including but are not limited to: apple (*Malus domestica* Borkh.) (Bink et al., 2014; Guan et al., 2015; Verma et al., 2016), sweet cherry (*Prunus avium* L.) (Rosyara et al., 2013), and peach (Fresnedo-Ramírez et al., 2015 and 2016). This framework will continue to aid the analysis for relevant complex traits across multiple Rosaceae breeding programs in RosBREED II, FruitBreedomics, and other collaborative projects (Fresnedo-Ramírez et al., 2016).

In terms of peach, Fresnedo-Ramírez et al. (2015 and 2016) performed two model PBA studies across two different sets of pedigree connected peach germplasm, to map QTLs (under the Bayesian Framework performed through FlexQTL^{TM}) associated with fruit quality and phenological traits directly in breeding material. In the first study they performed the first documented use of PBA in a peach breeding program, and identified well-supported QTLs for direct use in MAS for five out of nine traits investigated in the diverse processing peach breeding

program (introgressed with almond and other related species) at the Univerity of California (UC), Davis. Fresnedo-Ramírez et al. (2015) serves as a model study for PBA in a diverse peach breeding program, and the results highlight the ability of the PBA strategy (under the Bayesian Framework performed through FlexQTL^{TM}) to precisely identify genomic resources for direct incorporation in MAS. The adoption of the PBA strategy using the genomic resources developed in RosBREED facilitated the implementation of marker-assisted breeding in the processing peach breeding program at UC Davis (Fresnedo-Ramírez et al., 2015).

In the second study, Fresnedo-Ramírez et al. (2016) performed the first documented use of PBA across multiple peach breeding programs, to map QTLs for the complex traits associated with fruit size directly in a diverse breeding lineage. This is a model study for performing PBA across multiple diverse peach breeding program pedigrees, and the results support and highlight the ability of the PBA strategy to precisely identify genomic components of complex traits and for direct implementation of MAS within and between breeding programs. Such a strategy should facilitate the generation of more functional DNA tests, which are applicable across breeding programs, and so aid in the development of superior cultivars (Fresnedo-Ramírez et al., 2016).

In order to perform PBA, germplasm should be chosen to represent the most important parents of the breeding program (Peace et al., 2014). A protocol was developed by Peace et al. (2014) to strategically select important breeding parents (IBPs), by estimating the average allelic representation (AAR) in their relatives (unselected progenies, and all available and intermediate ancestors). The AAR is a measure of the representation of the alleles of IBPs provided by relatives in a germplasm set. An IBP has two alternative alleles, “A” and “B” at each locus, and the probability that an IBP’s relatives carry the same allele can be calculated using the principles

of "identity by descent" (IBD). Thus, the AAR measures the probability that a given allele at a random locus of an individual is IBD to an allele at that locus in another individual (Peace et al., 2014). Considering one IBP, each F_1 offspring represents the IBP and the other parent by 0.5 AAR units (Peace et al., 2014). The AAR units are further reduced in half for every subsequent generation. Peace et al. (2014) determined 12.5 AAR units as the minimum for statistical power in representing the alleles of IBPs. This is equivalent to 25 F_1 seedlings; 12.5 individuals carrying allele A and 12.5 individuals carrying allele B (the actual number of individuals carrying each allele is subject to the laws of inheritance) (Peace et al., 2014).

Once pedigrees have been selected and phenotypic and genotypic data have been collected, the PBA QTL analysis technique is used to integrate marker and phenotypic data over past, current, and future generations within and across breeding programs. The PBA approach is based on two complementary statistical approaches. The first identifies QTL regions based on Markov chain Monte Carlo (MCMC) simulations and Bayesian statistics (Bink, 2004 and 2005; Bink et al., 2008). The second is based on "identity by descent" values of each allele of a genotype, taking the different alleles of founding cultivars as factors in statistical analysis (Bink, 2004 and 2005; Bink et al., 2008). The PBA identifies networks of major genes and QTLs associated with key breeding traits, and also elucidates their interaction and mines their functional allelic diversity (van de Weg et al., 2004). PBA QTL analysis effectively creates a flexible platform for marker identification, validation, and use in breeding material. This overall approach, based on selecting representative germplasm pedigrees (through estimation of AAR units for IBPs), followed by PBA, is an expanding platform approach to continuously identify and validate QTLs in breeding material for subsequent application of MAS (Bink, 2004 and 2005; Bink et al., 2008 and 2012; Peace et al., 2014; van de Weg et al., 2004; Yu and Buckler,

2006). This strategy is pertinent for woody perennial fruit breeders since these species have very long juvenility periods and large populations are expensive to maintain in the field (Peace et al., 2014).

The objective of this study was to use PBA to identify and validate QTLs and SNPs for fruit quality and phenological traits in the University of Arkansas (UA) RosBREED peach breeding pedigree for subsequent application of MAS. To do this, the majority of the founders, cultivars, selections, and parents of seven F_1 populations, and all seedlings in the UA RosBREED peach breeding pedigree were genotyped using the International Peach SNP Consortium (IPSC) 9 K peach SNP array v1.0 (Verde et al., 2012). Next, high quality controlled phenotypic data was collected for three consecutive years on the majority of these individuals in the UA pedigree. The phenotypic and genotypic data were then used to perform PBA across the UA RosBREED peach breeding pedigree, to identify and validate QTLs and SNPs associated with fruit quality and phenological traits directly in a large portion of this program's breeding material.

The combination of SNPs which accurately depict each fruit quality or phenological trait can then be used for marker assisted parent selection (MAPS) and marker assisted seedling selection (MASS). Incorporation of MAPS will enable quick genotypic screening of peach germplasm, and lead to more informed decisions on efficient cross combinations to introgress high fruit quality throughout the entire season. The parents to use in a cross are identified through discovery of favorable alleles with efficient combining abilities. After the cross is made, MASS can be used to screen the seedlings, and decide on which seedlings to be grown in the field and which to discard (Bliss, 2010; Collard et al., 2005). Overall, incorporation of MAS for traits including fruit quality, phenological, and bacterial spot [caused by *Xanthomonas*

arboricola pv. *pruni* (*Xap* refers to the pathogen and disease)] resistance in the UA program, can provide the UA peach breeder with more informed decision support to increase genetic gain per breeding cycle, improve selection efficiency, and significantly reduce breeding program operational costs (Bliss, 2010; Byrne, 2005; Edge-Garza et al., 2016; Ru et al., 2015). This study also serves as a stepping stone for the ultimate goal realized in Chapter Six of this dissertation, to incorporate MAPS and MASS to enable the UA breeder to continue to combine high fruit quality with horizontal *Xap* resistance throughout the peach season.

Materials and Methods

Management Practices at FRS

Phenotypic evaluation for maturity date, pubescent vs. glabrous, white vs. yellow flesh color, blush overcolor, fruit weight diameter, and soluble solids content (SSC) was conducted on peach and nectarine material at the UA Fruit Research Station (FRS), Clarksville, AR (west-central AR, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept. of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]). All trees were either open-center trained and spaced 5.5 m between trees and rows, or trained to a perpendicular-V system with trees spaced 1.9 m in rows spaced 5.5 m apart. All trees were dormant pruned and fertilized annually with a single application of 640 Kg ha^{-1} of complete fertilizer (19:19:19 of N:P:K) and were sprinkler or drip irrigated as needed. Pests were managed using a program typical for commercial orchards in the area (Smith, 2015; Studebaker et al., 2015). After shuck split, but before pit hardening, fruitlets were thinned to a distance of 12 to 15 cm between each fruitlet. Temperature, humidity, and rainfall weather data from FRS were collected in 2011, 2012, and 2013. Figures were created to compare data across all three years.

Pedigree Construction

The germplasm used for PBA was selected to effectively represent a large portion of breeding relevant alleles currently found within the UA breeding program (Peace et al., 2014). Founders, important breeding parents, cultivars, selections, and seven F_1 populations ranging from nine to 48 seedlings (134 total) were identified and integrated in a comprehensive pedigree of 190 individuals (Table 1). Parentage records were confirmed or refuted first using four SSR markers, endoPG.1, endoPG.6, CPPCT040b, and BPPCT15 (Dirlewanger et al., 2002), followed by using SNPs on the International Peach SNP Consortium (IPSC) 9K peach SNP array v1.0 (C. Peace personal communication; Verde et al., 2012). Pedimap software (Voorrips, 2007; Voorrips et al., 2012) was used to visualize the constructed pedigree (Fig. 1).

Table 1. Parental information and the number of F_1 seedlings for each of the seven RosBREED populations (N is number of individuals analyzed).

F_1 population	Female parent	Male parent	F_1 seedlings (N)
AR_Pop_1	White County (WC)	A-672	48
AR_Pop_0801	A-776	A-783	16
AR_Pop_0803	Amoore Sweet (AS)	A-778	9
AR_Pop_0813	A-772	A-672	12
AR_Pop_0817	A-789	A-699	9
AR_Pop_0819	A-708	A-773	23
AR_Pop_0825	Souvenirs (S)	A-760	17

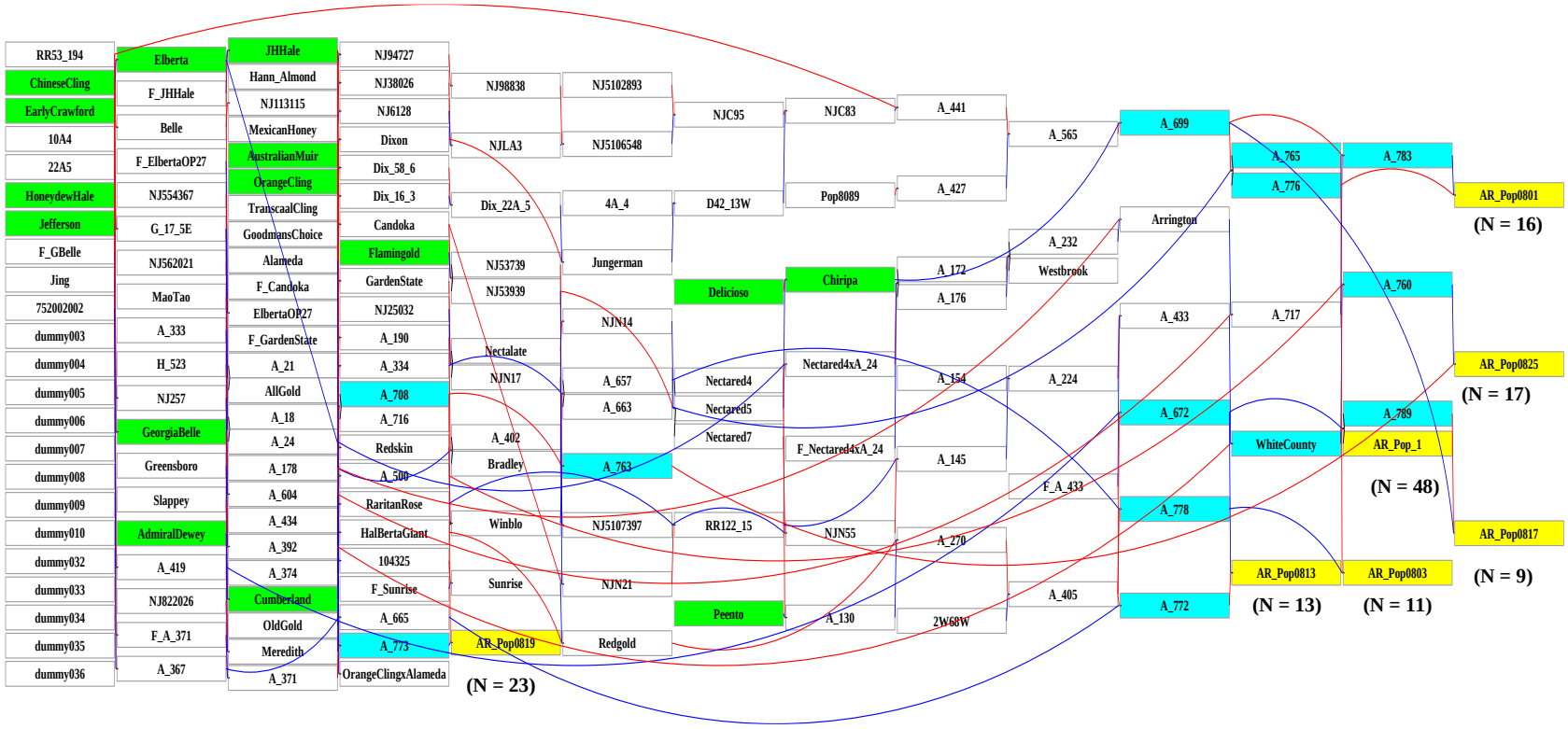

Fig. 1. Pedigree of the seven 2008 F_1 seedling populations used for PBA analysis of fruit quality and phenological traits; visualized through Pedimap software (Voorrips, 2007; Voorrips et al., 2012) (F_1 populations highlighted in yellow; Red line = female parent; Blue line = male parent; N = the number of progeny in each population).

Phenotyping

Phenotyping for maturity date, pubescent vs. glabrous, white vs. yellow flesh color, blush overcolor, fruit size (weight and diameter), and soluble solids content (SSC) was conducted in 2011-2013, following the RosBREED standardized peach phenotyping protocol (Frett et al., 2012). Fruit diameter measurements were additionally taken in 2013. In short, 10 fruit slightly firmer than tree ripe were harvested from the mid-canopy of each tree into 0.24 L corrugated trays (FormTex Plastics Corp., Houston, TX). Maturity date (day of year, 0-365) and visual estimation of the pubescence level was performed at harvest in the field, and then fruit were taken to the laboratory for additional measurements.

In the lab, fruit weight (g) and diameter (mm) measurements were recorded for the five largest fruit from each sample. Blush overcolor was estimated, using these same five fruit, on a scale from 0-5 (0 = no blush; 1 = 1-20%; 2 = 21-50%; 3 = 51-80%; 4 = 81-99%; 5 = 100%). Flesh color was determined using a 0-9 scale (1 = green; 2 = cream green; 3 = cream; 4 = cream yellow; 5 = yellow green; 6 = yellow; 7 = yellow orange; 8 = orange; 9 = red). Lastly, a longitudinal slice was taken from each of the five fruits, and juiced through a hand presser. Two to four drops of the juice were analyzed using a refractometer (3810 PAL-1 Digital Hand-Held Pocket Refractometer, Atago Inc., Bellevue,WA) to determine percent soluble solid content (SSC,%). Later, pubescence and flesh color ratings were converted to a 1 vs. 2 scale: for pubescence 3, 5 and 7 were all converted to 2 [pubescent (peach)], and 1 [glabrous (nectarine)]. For flesh color, 1, 2, and 3 ratings were converted to 1 (white) and 4, 5, 6, 7, 8 and 9 were all converted to 2 (yellow) (Frett et al., 2012).

Phenotypic Data Set Construction and Descriptive Statistics

All phenotypic data for the six traits taken in 2011-2013 (maturity date, pubescent vs. glabrous, white vs. yellow flesh, blush overcolor, fruit weight, and SSC) and one trait only in 2013 (fruit diameter) were organized into 19 data sets for PBA QTL analysis. Additionally, the 2011-2013 averages were calculated and included as a fourth data set for each trait, excluding fruit diameter, creating 25 final data sets. Descriptive statistics including the mean, minimum, maximum, and variance for all 25 data sets were calculated for the entire UA RosBREED pedigree using a modified R script (R Core Team, 2013). Histograms for the entire UA RosBREED pedigree and each F_1 population were additionally generated using a modified R script (R Core Team, 2013). Parental values for each F_1 population were noted in each histogram.

SNP Array Genotyping

All individuals in the UA RosBREED pedigree were previously genotyped using the International Peach SNP Consortium (IPSC) 9K peach SNP array v1.0 (Verde et al., 2012). Isolation of genomic DNA and subsequent Infinium assay were performed as explained in Verde et al. (2012). The SNP genotypes were scored with the Genotyping Module of GenomeStudio Data Analysis software (Illumina Inc., San Diego, CA). A GenTrain score of ≥0.4 and a GenCall 10% of ≥0.2 were applied to remove most SNPs that did not cluster (homozygous) or had ambiguous clustering (a high proportion of inheritance errors and/or heterozygosity excess). The average density of markers across the whole peach genome was estimated to be one SNP per 2.5 cM. The genetic locations for each SNP were positioned according to their relative physical locations (Verde et al., 2013). Map figures were generated using MapChart 2.2 software (Wageningen UR, Wageningen, Netherlands) (Voorrips 2001).

PBA QTL Analysis

All phenotypic and genotypic data sets were used for PBA QTL analysis performed through FlexQTL™ (Bink 2004 and 2005; Bink et al., 2008 and 2012; software version 099128; www.flexQTL.nl). The FlexQTL™ software estimated the number and position of QTLs given a pedigree and marker linkage map. FlexQTL™ utilizes a Bayesian approach to infer the number of QTLs by comparison of models using posterior estimates through Markov Chain Monte Carlo (MCMC) simulations. In all analyses, a 500,000 simulation chain length was enough to store 1000 samples (QTL models) with a thinning of 500 (Bink et al., 2014; Rosyara et al., 2013; Verma et al., 2016). The minimum requirements for detection of a QTL included a minimum effective chain size (ECS) value of 100, a posterior probability greater than 0.1 (threshold for a significant QTL), and a Bayes Factor (BF) greater than two (Bink et al. 2014; Rosyara et al. 2013; Verma et al., 2016). The BFs are the evidence favoring the presence of a number of QTLs under the genetic model proposed (Bink et al., 2012 and 2014). When BF values are between zero and two the evidence of a significant QTL is considered low, when values are between two and five the evidence is positive, when BFs are between five and 10 the evidence is strong, and when the values are greater than 10 the evidence is decisive (Bink et al., 2012 and 2014). Additionally, PostQTL genome wide trace and intensity plots were used to visualize and compare the convergence, stability, and probability of the evaluated genetic model per trait and year to further determine the reliability of the identified QTLs. All reliable QTLs were subsequently named according to specifications of the Genome Database for Rosaceae: linkage group number and phenotypic trait symbol (Jung et al., 2008 and 2014).

QTL Effects Calculation

For all traits, the broad sense heritability (H^2) was calculated using the values of phenotypic variance $\left(s_P^2\right)$ and error (s_e^2) and the narrow sense (h^2) heritability was calculated

using the weighted additive variance of the QTL [probability* weighted additive variance of QTL, $\left(\overline{\sigma^2_A}\right)$]. The values of $\left(\sigma^2_P\right)$, (σ^2_e), and $\left(\overline{\sigma^2_A}\right)$ were obtained from FlexQTL™ results and H^2 and h^2 were calculated using the following formulas: h^2: $H^2 = \frac{\sigma^2_P - \sigma^2_e}{\sigma^2_P} = \frac{\sigma^2_G}{\sigma^2_P}$; and $h^2 = \frac{\sigma^2_A}{\sigma^2_P}$. The QTLs which explained ≥ 10% of phenotypic the variation for their respective trait (Vp %) were considered major QTLs and those which explained less were considered minor QTLs.

Genomic Estimated Breeding Values

The genomic breeding values (GEBVs) based on all QTLs were calculated for all individuals in UA RosBREED pedigree per trait per year through the *a posteriori* PBA results, using PostFlexQTL™ version 0.99110, (Bink et al., 2014; Fresnedo-Ramírez et al., 2015 and 2016). Prediction accuracy was calculated as the correlation between GEBVs and observed values per trait per year.

Results

Weather Data

Temperature and rainfall weather condition data were collected at FRS in 2011, 2012, and 2013 using the FRS weather station (Table 2; Figs. 2-4). This data showed that the temperature and rainfall varied among years, especially when comparing 2011 and 2012 to 2013. The first two years (2011, and 2012) were very hot and dry compared to 2013. Average high temperatures from 1 June to 31 Aug. were 34, 35, and 31 °C in 2011, 2012, and 2013. Average minimum temperatures from 1 June to 31 Aug. were 23, 22, and 20 °C in 2011, 2012, and 2013. Days which exceeded 32 °F from 1 June to 31 Aug. were 96 in 2011, 92 in 2012, and only 54 in 2013. Lastly, rainfall from 1 June to 31 Aug. was variable with a total of 19.7 cm of rain in 2011, 12.1 cm in 2012, and 33.4 cm in 2013 (Table 2; Figs. 2-4).

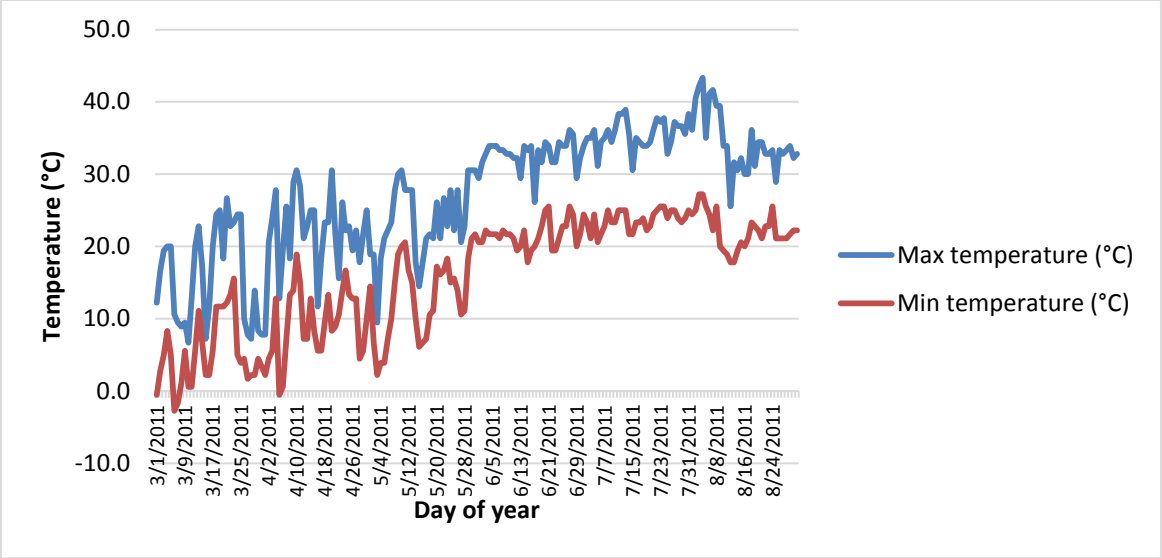

Fig. 2. Maximum (max) and minimum (min) temperature weather condition data from FRS, collected in 2011.

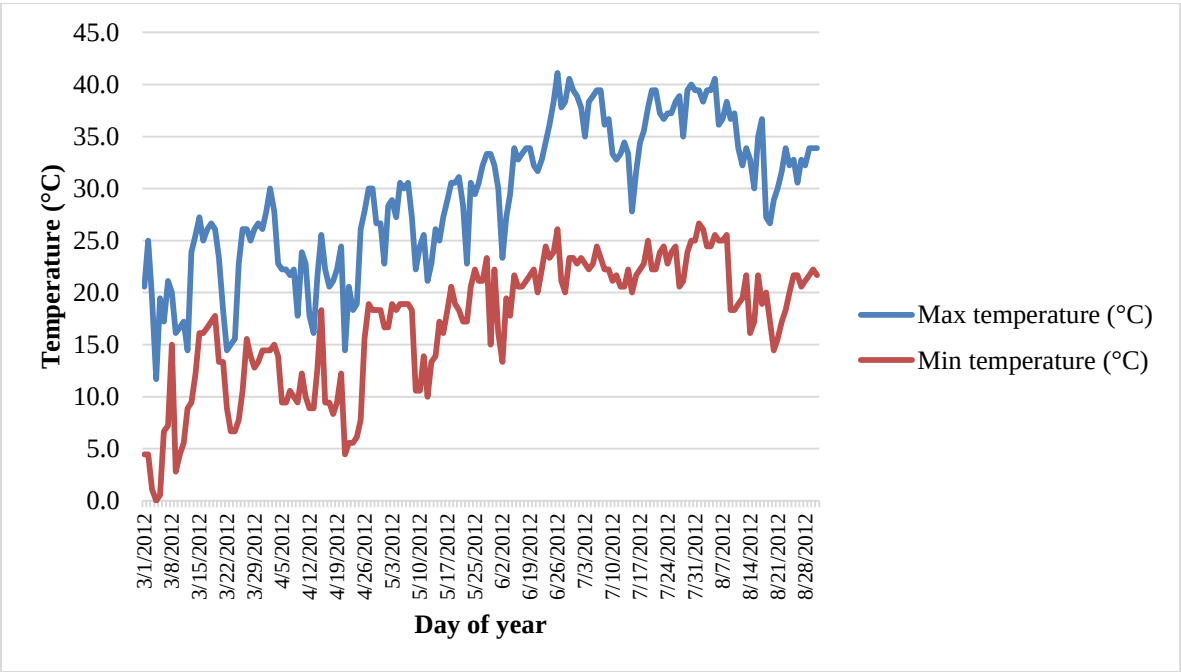

Fig. 3. Maximum (max) and minimum (min) temperature weather condition data from FRS, collected in 2012.

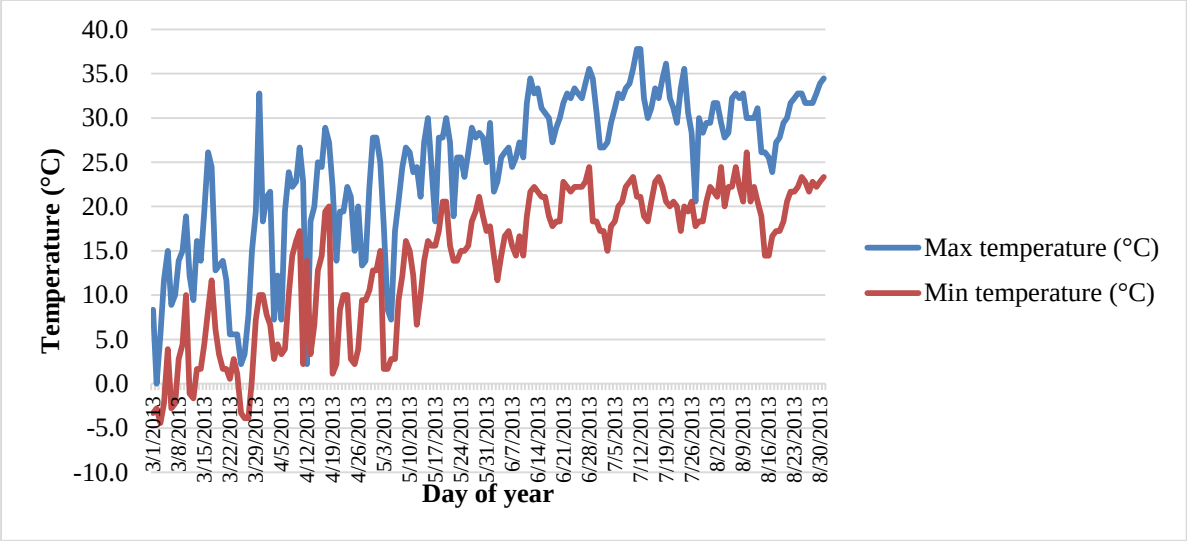

Fig. 4. Maximum (max) and minimum (min) temperature weather condition data from FRS, collected in 2013.

Table 2. Total rainfall (cm) at FRS, for each month (March-August) in 2011, 2012, and 2013.

Year	Month	Rainfall (cm)
2011	March	8.0
2011	April	29.0
2011	May	19.8
2011	June	5.5
2011	July	1.6
2011	August	12.5
2011	June-August avg	19.7
2012	March	20.0
2012	April	8.3
2012	May	1.9
2012	June	2.0
2012	July	3.8
2012	August	6.3
2012	June-August avg	12.1
2013	March	13.0
2013	April	12.1
2013	May	16.3
2013	June	5.5
2013	July	10.0
2013	August	17.9
2013	June-August avg	33.4

Phenotypic Data

Maturity Date (MD)

Maturity date (MD), measured as day of year (0-365), exhibited an average of 198.9 (2011), 180.5 (2012), 203.5 (2013), and 194.1 d (2011-2013 avg) for all individuals in the UA RosBREED pedigree (Table 3). This indicates differences in maturity date among all three years. The maturity date ranged within 151.0 (2012) to 237.0 d (2011 and 2012). The standard deviation displayed ranged from 12.9 (2013) to 17.5 d (2011) (Table 3).

For the seven F_1 populations, different segregation patterns for maturity date were observed across all years of the study (Figs. 5-8). In 2011, AR_Pop_0801 and AR_Pop_0803 contained all early season seedlings, and AR_Pop_1 and AR_Pop_0819 spanned the entire season (Fig. 5). In 2012 and 2013, AR_Pop_0801 and AR_Pop_0803 again contained all early season seedlings, and only AR_Pop_1 spanned the entire season (Figs. 6-7). In 2011-2013 avg, AR_Pop_0801 and AR_Pop_0803 once more contained all early season seedlings and AR_Pop_1 and AR_Pop_0819 nearly spanned the entire season (Fig. 8). Parental values from each year are also included in Figs. 5-8.

Table 3. Mean, minimum, maximum, and standard deviation of maturity date (day of year, 0-365) ratings for the entire AR RosBREED pedigree (N is number of individuals) in 2011, 2012, 2013, and 2011-2013 avg.

Year	Maturity date (day of year, 0-365)				N
	Mean	Min.	Max.	Std. dev.	
2011	198.9	158.0	237.0	17.5	154
2012	180.5	151.0	221.0	16.1	160
2013	203.5	178.0	237.0	12.9	143
2011-2013 avg	194.1	166.0	231.0	15.0	174

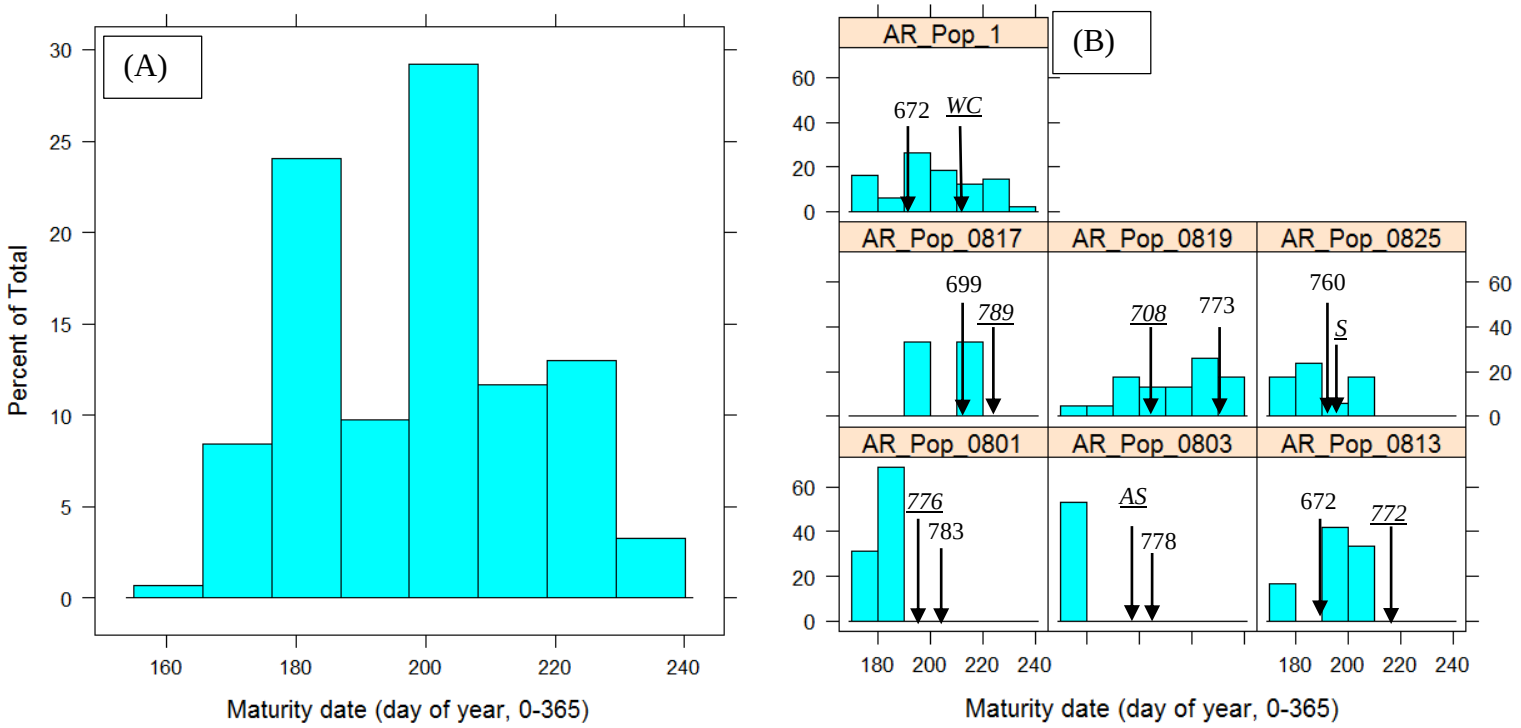

Fig. 5. Distribution (%) of maturity date 2011 field ratings for the entire UA RosBREED pedigree (A) (N=154), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

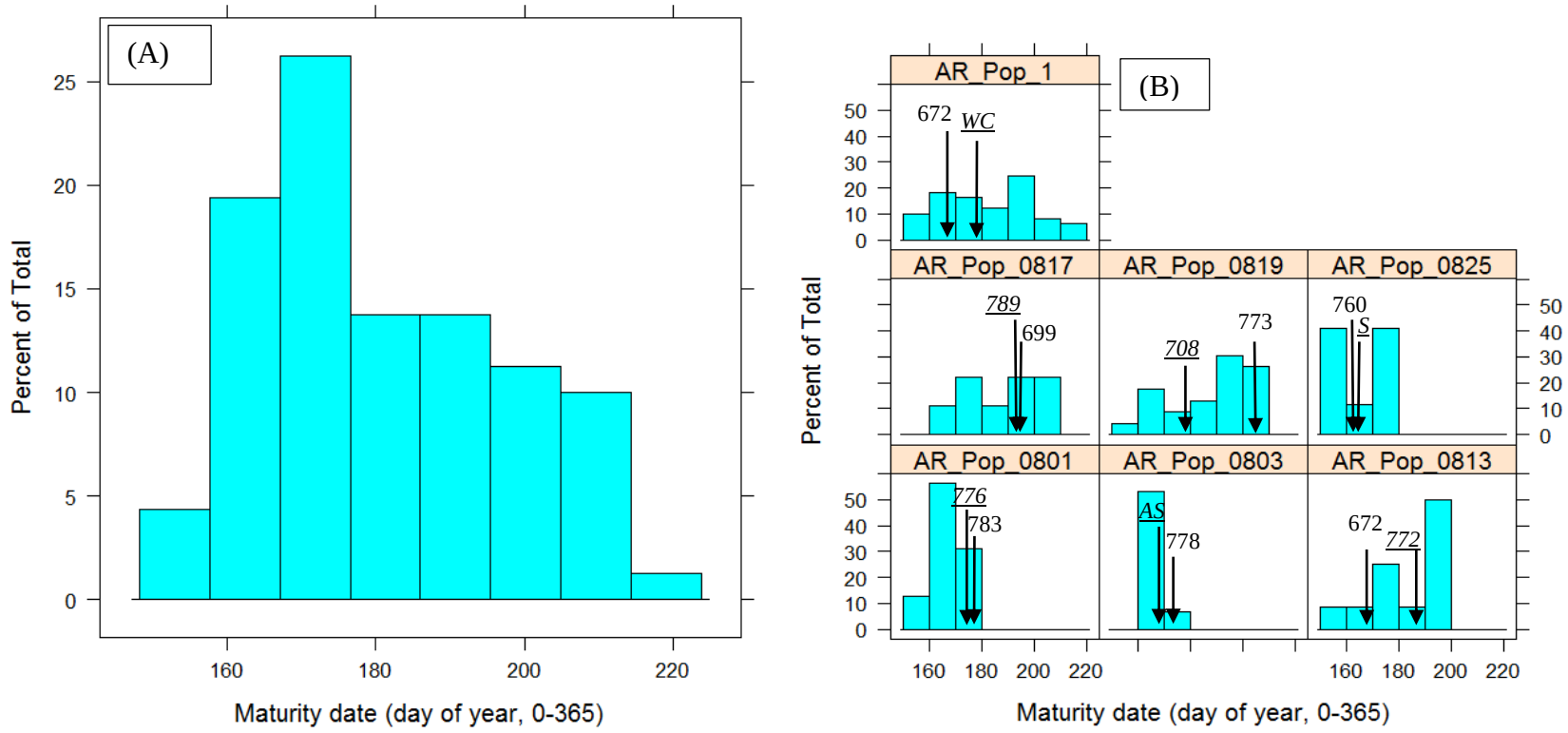

Fig. 6. Distribution (%) of maturity date 2012 field ratings for the entire UA RosBREED pedigree (A) (N=160), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

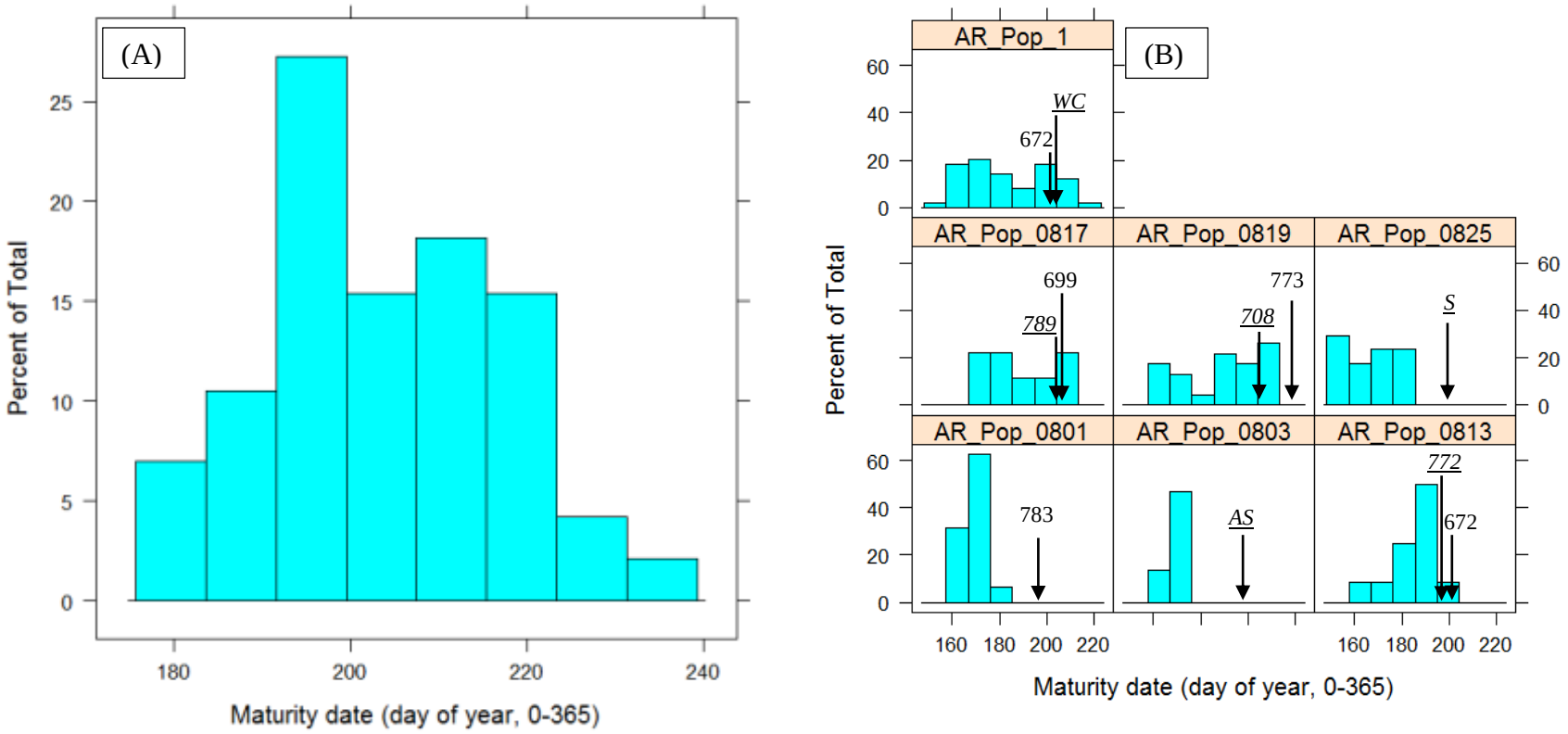

Fig. 7. Distribution (%) of maturity date 2013 field ratings for the entire UA RosBREED pedigree (A) (N=143), and the seven F_1 populations (parental values illustrated by arrows; parents not depicted were not phenotyped; ***female parent*** is italicized and underlined) (B).

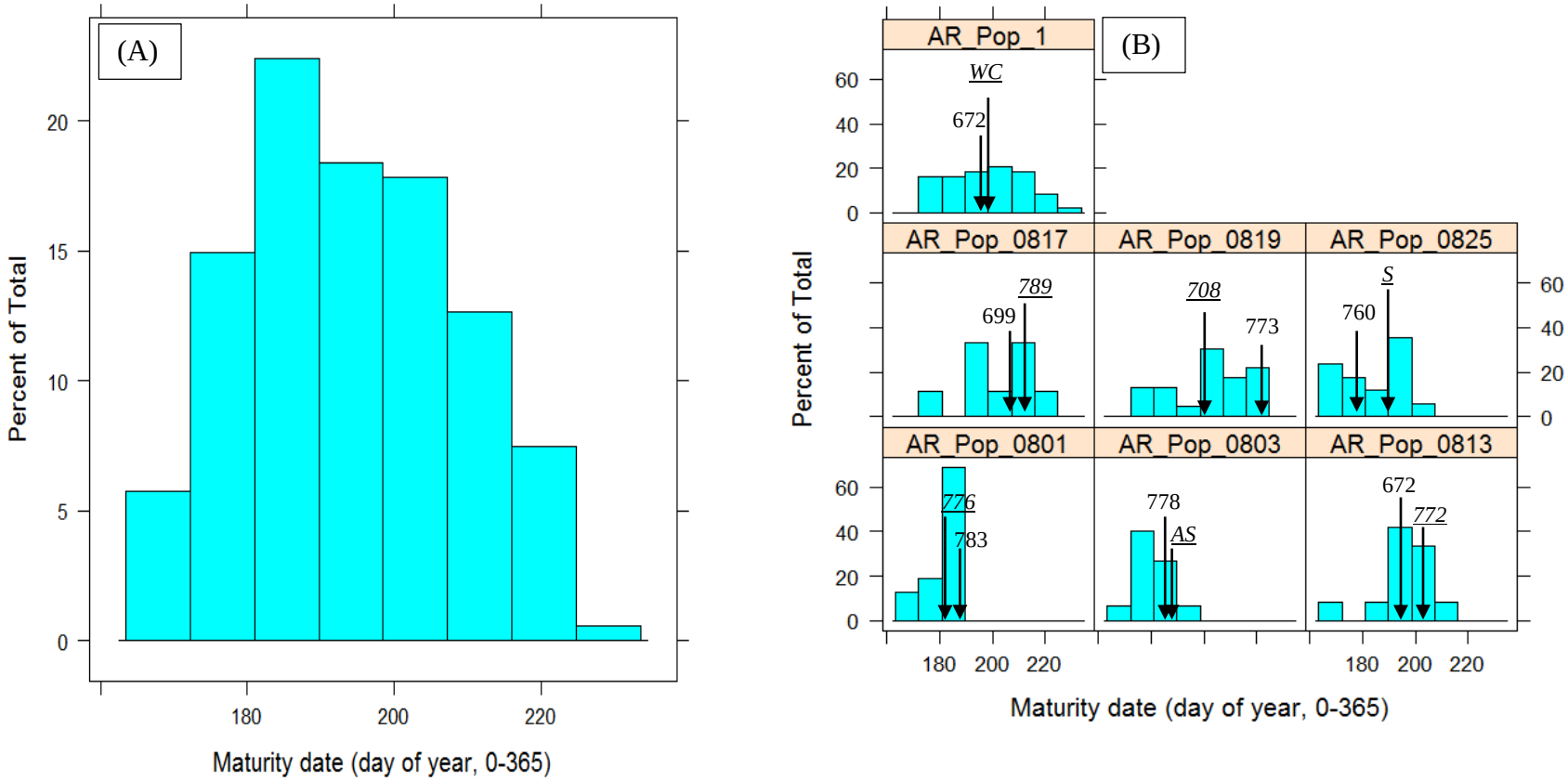

Fig. 8. Distribution (%) of maturity date 2011-2013 avg field ratings for the entire UA RosBREED pedigree (A) (N=174), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

G-locus (Pubescent vs. Glabrous)

Glabrous (1) vs. pubescent (2) exhibited an average of 1.7 for all individuals in the AR RosBREED pedigree, across all years of study (Table 4). The values ranged from 1.0-2.0 (all years), with a consistent standard deviation of 0.5 (all years) (Table 4).

For the seven F_1 populations, different segregation patterns for glabrous (1) vs. pubescent (2) were observed across all years of the study (Figs. 9-12). In total, seven parents were peaches and eight were nectarines. All progeny in AR_Pop_0801, AR_Pop_0803, and AR_Pop_0819 were nectarines across all years of the study. The progeny in AR_Pop_1 and AR_Pop_0813 showed ~3:1 peaches to nectarines across all years of the study. The progeny in AR_Pop_0817 and AR_Pop_0825 exhibited ~1:1 peaches to nectarines across all years, except for 2011, where AR_Pop_0817 only contained peaches (since less individuals were phenotyped that year for that population) (Figs. 9-12).

Table 4. Mean, minimum, maximum, and standard deviation of glabrous (1) vs. pubescent (2) ratings for the entire AR RosBREED pedigree (N is number of individuals) in 2011, 2012, 2013, and 2011-2013 avg.

Year	Pubescent (2) vs. glabrous (1)				N
	Mean	Min.	Max.	Std. dev.	
2011	1.7	1.0	2.0	0.5	154
2012	1.7	1.0	2.0	0.5	162
2013	1.7	1.0	2.0	0.5	153
2011-2013 avg	1.7	1.0	2.0	0.5	172

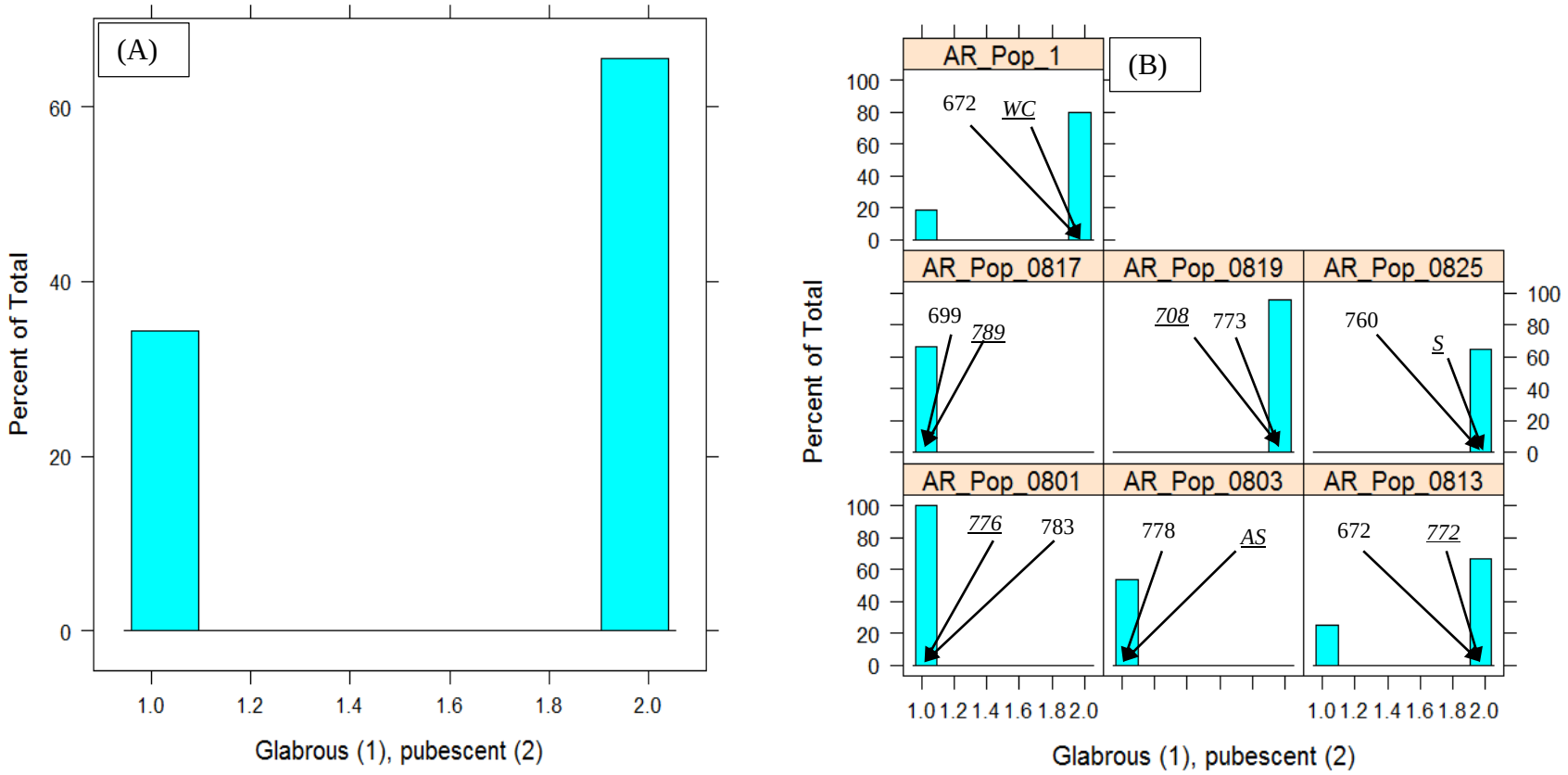

Fig 9. Distribution (%) of glabrous (1) vs. pubescent (2) 2011 field ratings for the entire UA RosBREED pedigree (A) (N=154), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

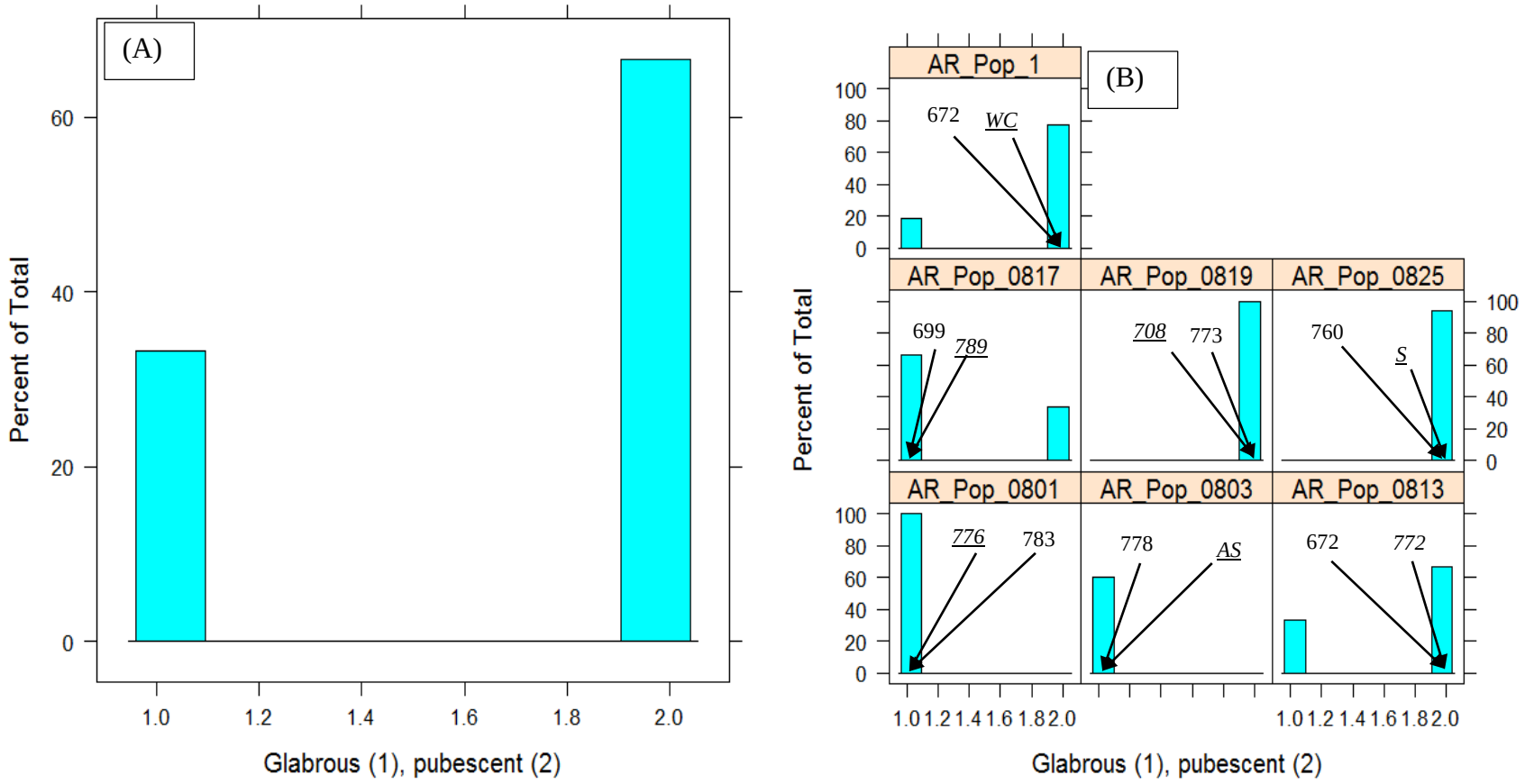

Fig 10. Distribution (%) of glabrous (1) vs. pubescent (2) 2012 field ratings for the entire UA RosBREED pedigree (A) (N=162), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

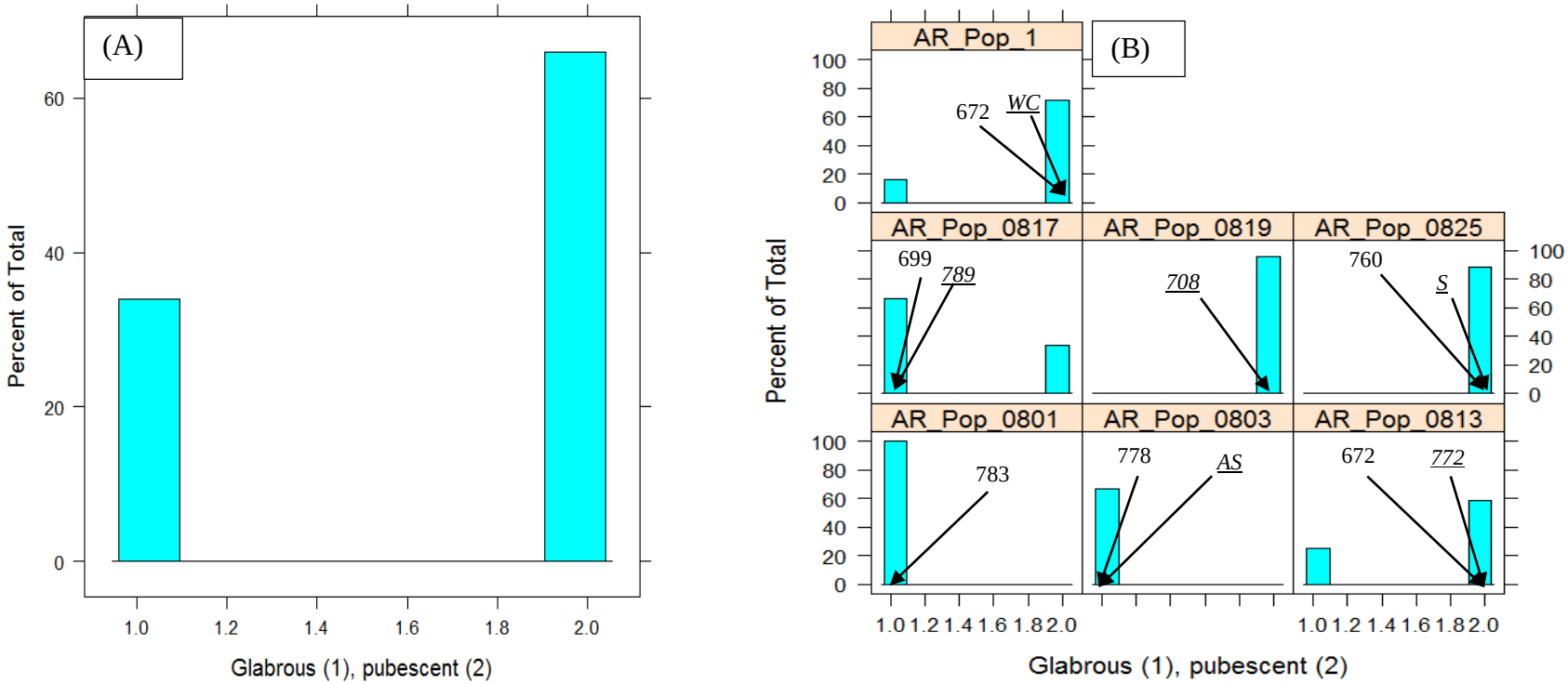

Fig 11. Distribution (%) of glabrous (1) vs. pubescent (2) 2013 field ratings for the entire UA RosBREED pedigree (A) (N=153), and the seven F_1 populations (parental values illustrated by arrows; parents not depicted were not phenotyped; *female parent* is italicized and underlined) (B).

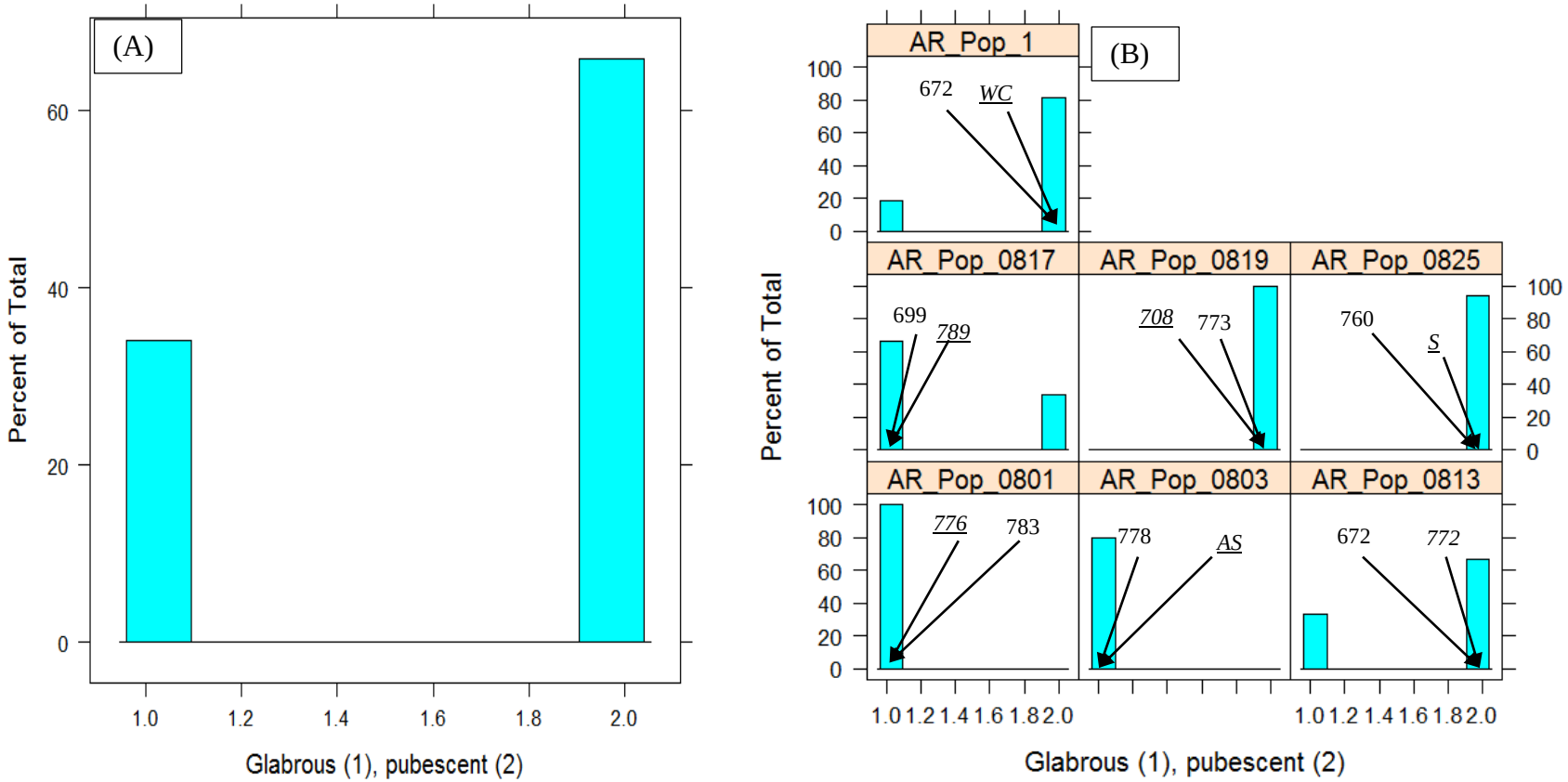

Fig 12. Distribution (%) of glabrous (1) vs. pubescent (2) 2011-2013 avg field ratings for the entire UA RosBREED pedigree (A) (N=170), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

Y-locus (White vs. Yellow Flesh)

White (1) vs. yellow (2) flesh exhibited an average of 1.5 for all individuals in the AR RosBREED pedigree, across all years of study (Table 5). The values ranged from 1.0-2.0 (all years), with a consistent standard deviation of 0.5 across all years (Table 5).

For the seven F_1 populations, different segregation patterns for white (1) vs. yellow (2) flesh were observed across all years of the study (Figs. 13-16). In total, nine parents were white flesh, and five were yellow flesh. All progeny in AR_Pop_0803 and AR_Pop_0825 were yellow flesh across all years of the study. Likewise, all progeny in AR_Pop_0817 were white flesh. The progeny in AR_Pop_1 and AR_Pop_0801 showed ~3:1 white to yellow flesh across all years of the study. The progeny in AR_Pop_0819 exhibited ~1:1 white to yellow flesh. Lastly, AR_Pop_0813 showed eight white flesh seedlings and one yellow flesh (Figs. 13-16).

Table 5. Mean, minimum, maximum, and standard deviation of white (1) vs. yellow (2) flesh ratings for the entire AR RosBREED pedigree (N is number of individuals) in 2011, 2012, 2013, and 2011-2013 avg.

Year	White (1) vs. yellow (2) flesh				N
	Mean	Min.	Max.	Std. dev.	
2011	1.5	1.0	2.0	0.5	164
2012	1.5	1.0	2.0	0.5	170
2013	1.5	1.0	2.0	0.5	173
2011-2013 avg	1.5	1.0	2.0	0.5	174

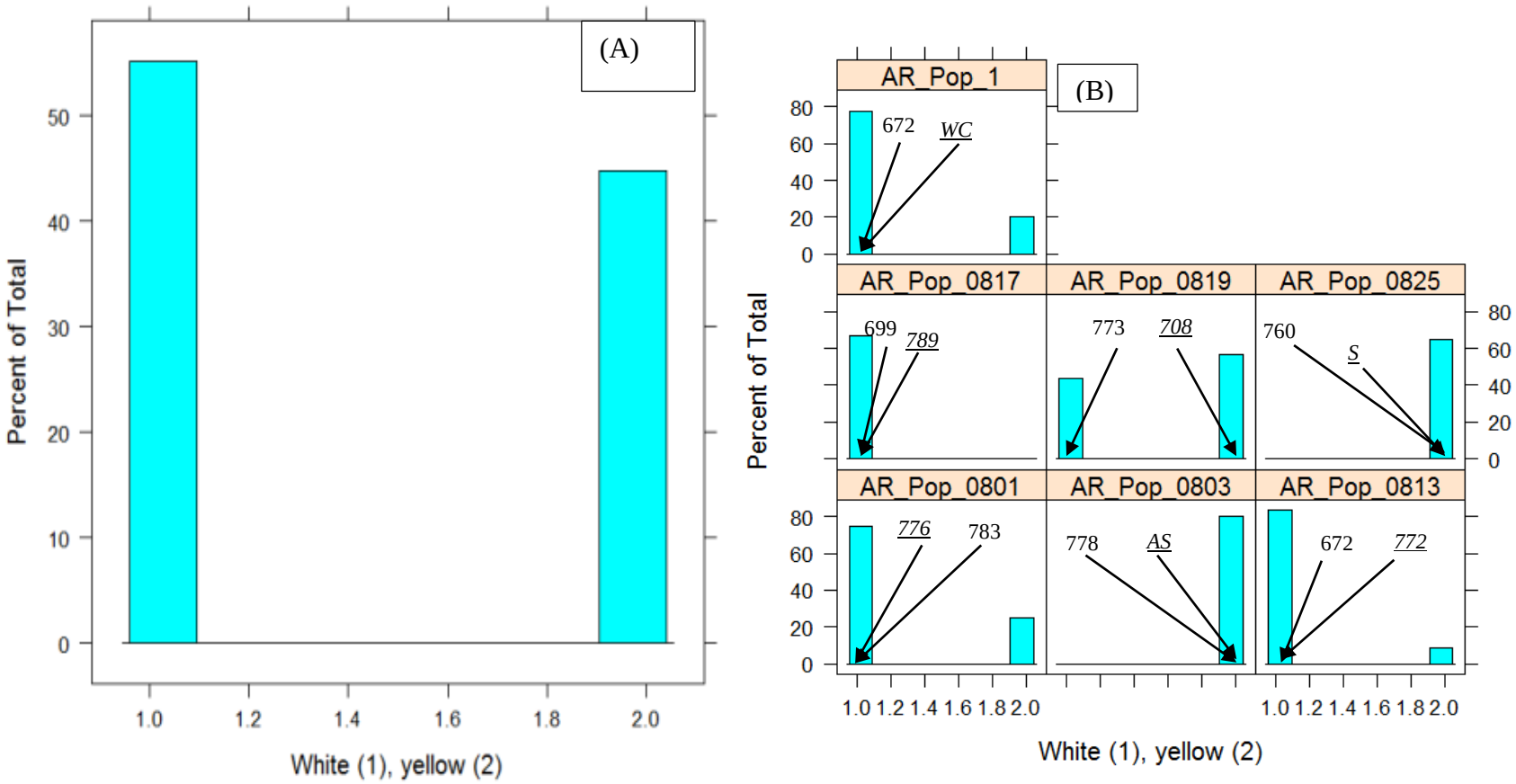

Fig 13. Distribution (%) of white (1) vs. yellow (2) flesh color 2011 field ratings for the entire UA RosBREED pedigree (A) (N=164), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

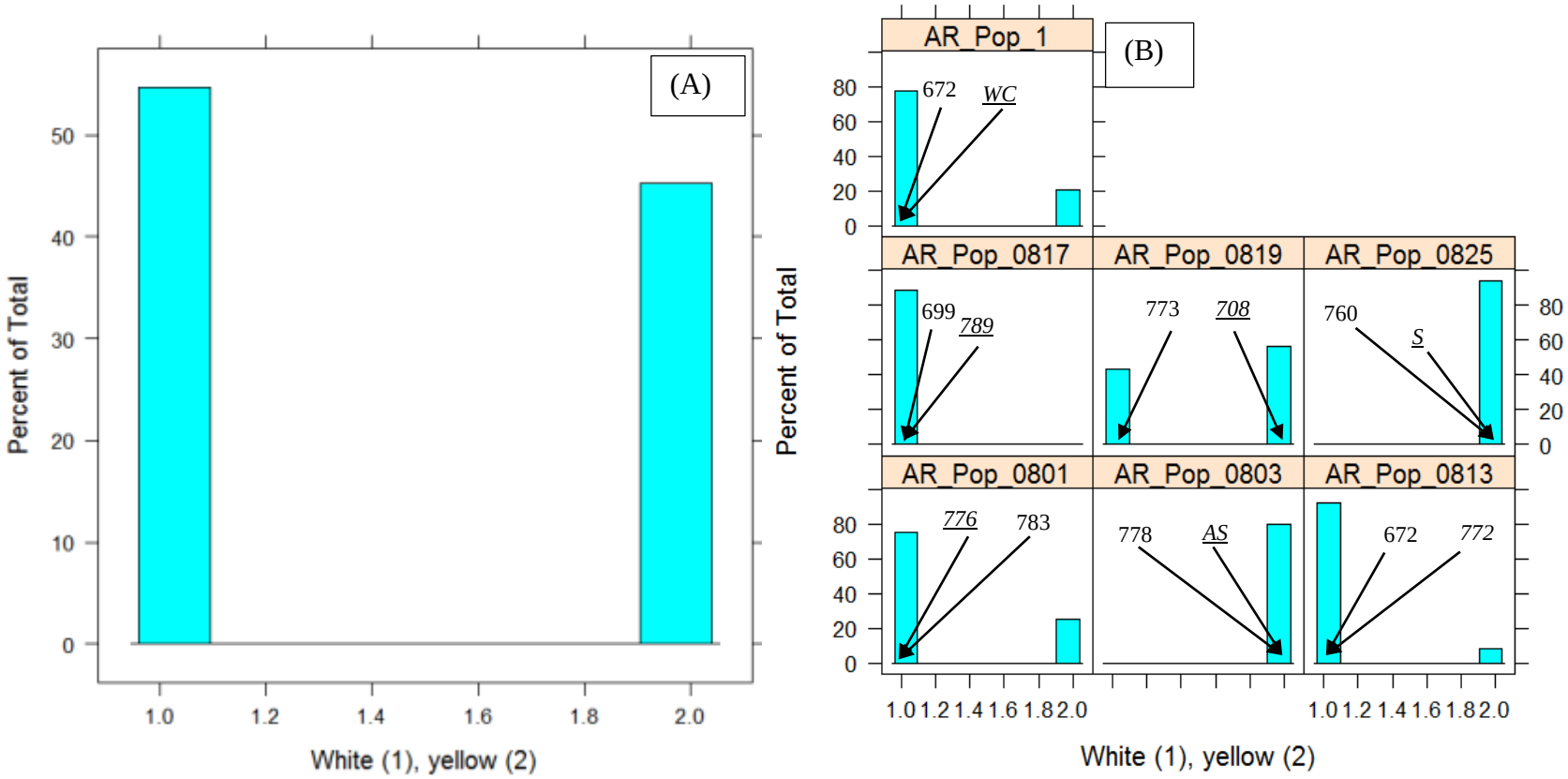

Fig 14. Distribution (%) of white (1) vs. yellow (2) flesh color 2012 field ratings for the entire UA RosBREED pedigree (A) (N= 170), and the seven F_1 populations (parental values illustrated by arrows; *female parent* is italicized and underlined) (B).

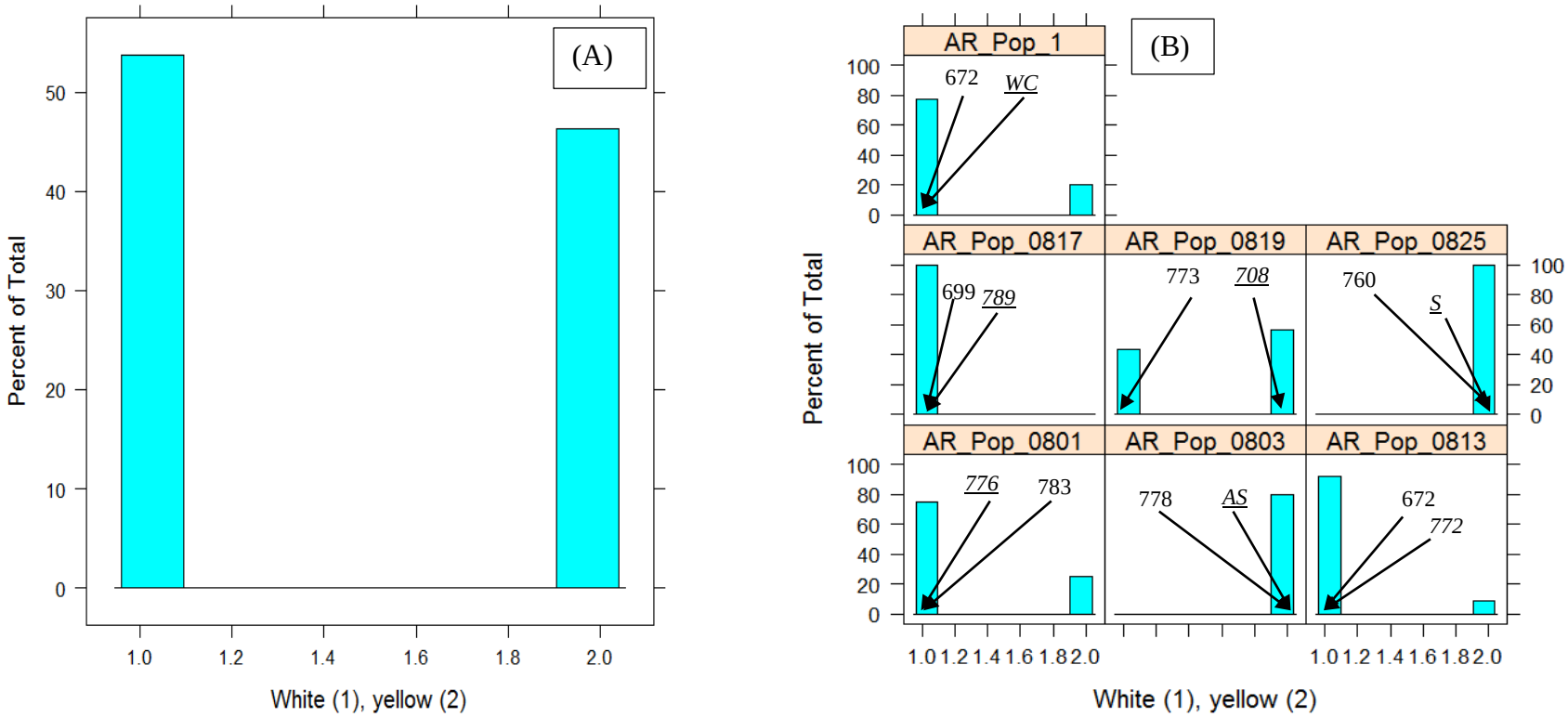

Fig 15. Distribution (%) of white (1) vs. yellow (2) flesh color 2013 field ratings for the entire UA RosBREED pedigree (A) (N=173), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

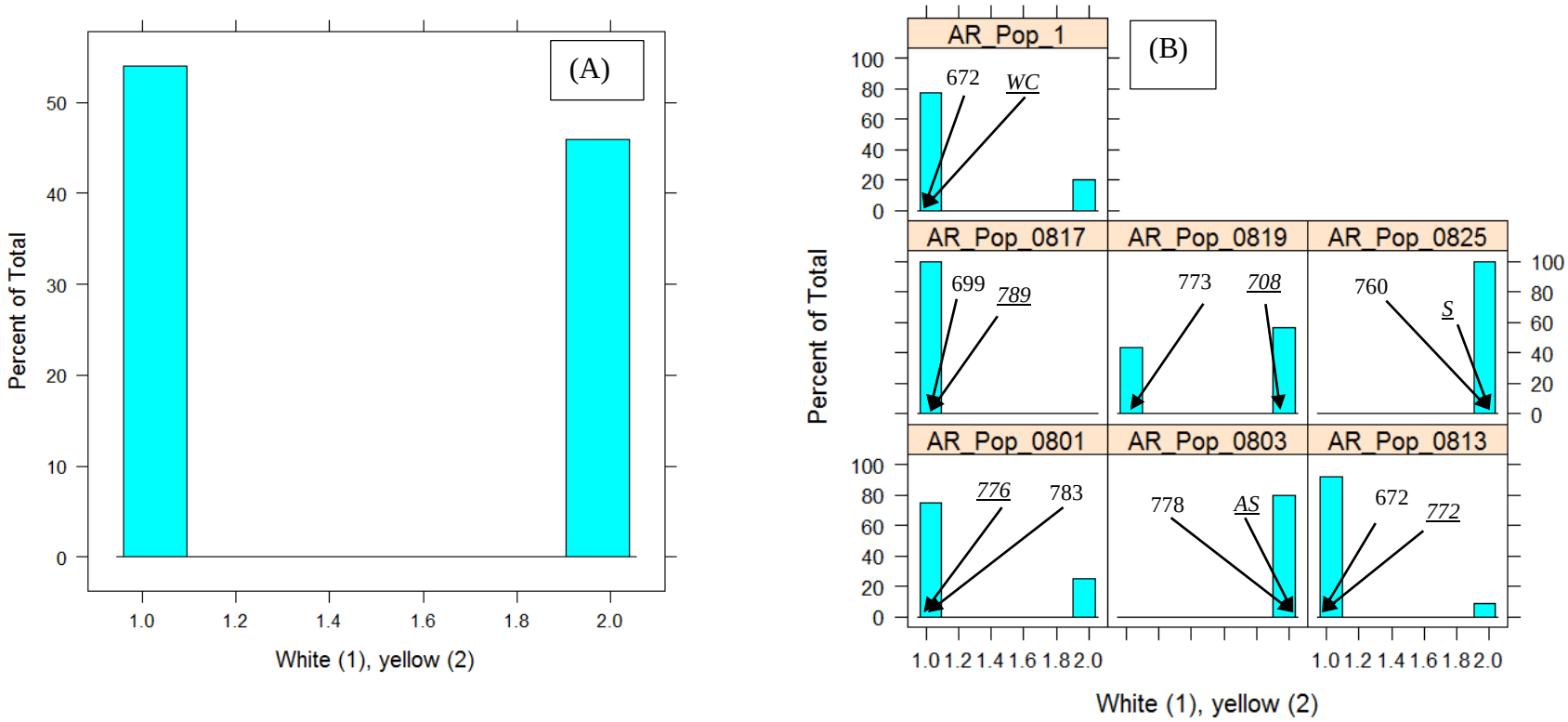

Fig 16. Distribution (%) of white (1) vs. yellow (2) flesh color 2011-2013 avg field ratings for the entire UA RosBREED pedigree (A) (N=174), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

Blush

Blush overcolor (0-5), exhibited an average of 3.5 (2011), 3.5 (2012), 3.4 (2013), and 3.5 (2011-2013 avg) for all individuals in the AR RosBREED pedigree (Table 6). The blush overcolor ranged from 2.0-5.0 in each respective year. The standard deviation displayed across all years ranged from 0.7 (2013) to 0.8 (2011, 2012, and 2011-2013 avg) (Table 6).

For the seven F_1 populations, different segregation patterns for blush overcolor were observed across all years of the study (Figs. 17-20). In 2011 and 2012, AR_Pop_0801, AR_Pop_0813, and AR_Pop_0819 all segregated from 2.0-5.0 (Figs. 17-18). In 2013, only AR_Pop_0813 segregated from 2.0-5.0 (Fig. 19). In 2011-2013 avg, AR_Pop_1, AR_Pop_0801, AR_Pop_0813, and AR_Pop_0819 all segregated from 2.0-5.0 (Fig. 20). Parental values from each year are also included in Figs. 17-20.

Table 6. Mean, minimum, maximum, and standard deviation of blush (0-5) ratings for the entire AR RosBREED pedigree (N is number of individuals) in 2011, 2012, 2013, and 2011-2013 avg.

Year	Blush (0-5)				N
	Mean	Min.	Max.	Std. dev.	
2011	3.5	2.0	5.0	0.8	164
2012	3.5	2.0	5.0	0.8	164
2013	3.4	2.0	5.0	0.7	162
2011-2013 avg	3.5	2.0	5.0	0.8	173

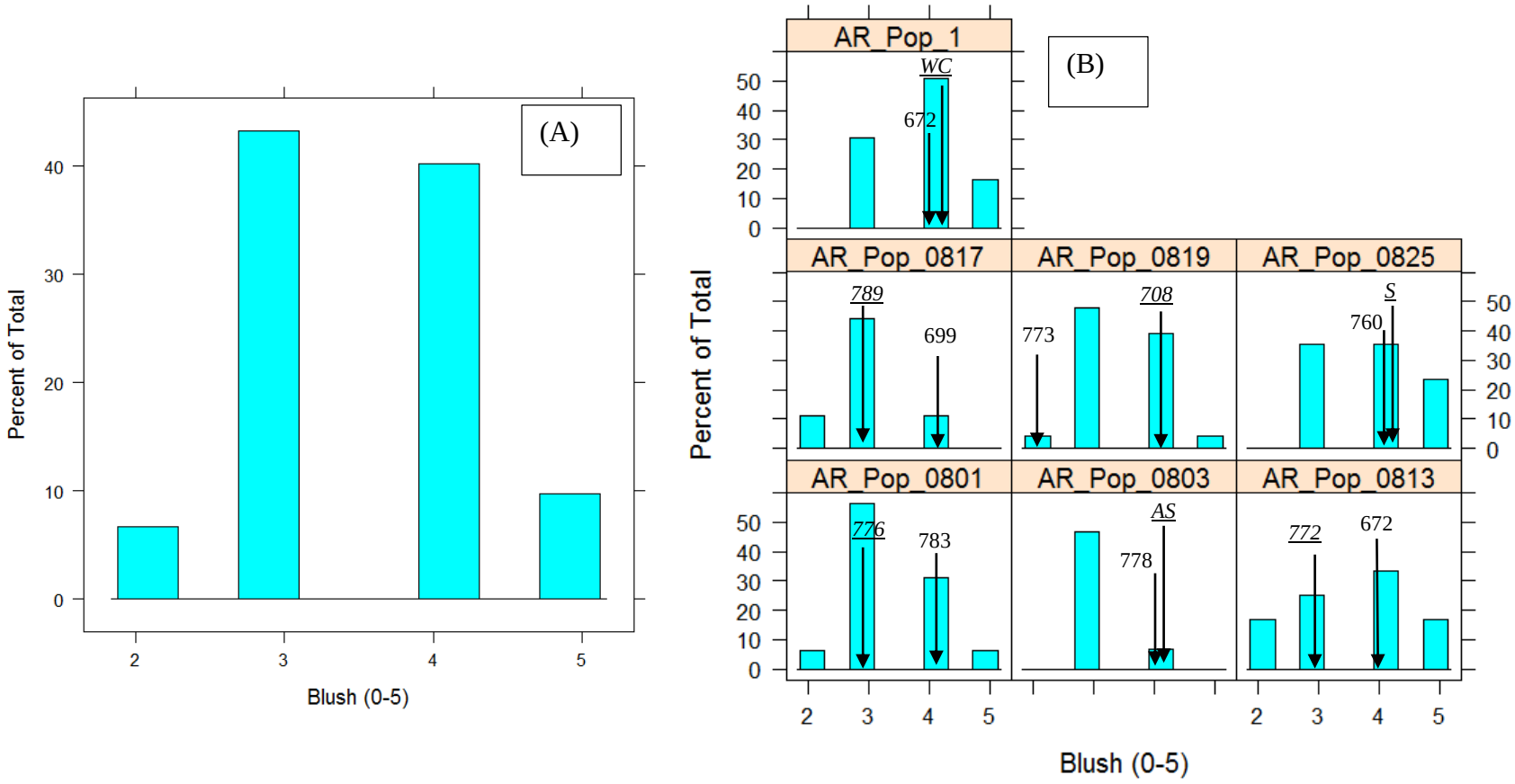

Fig 17. Distribution (%) of blush 2011 field ratings for the entire UA RosBREED pedigree (A) (N=164), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

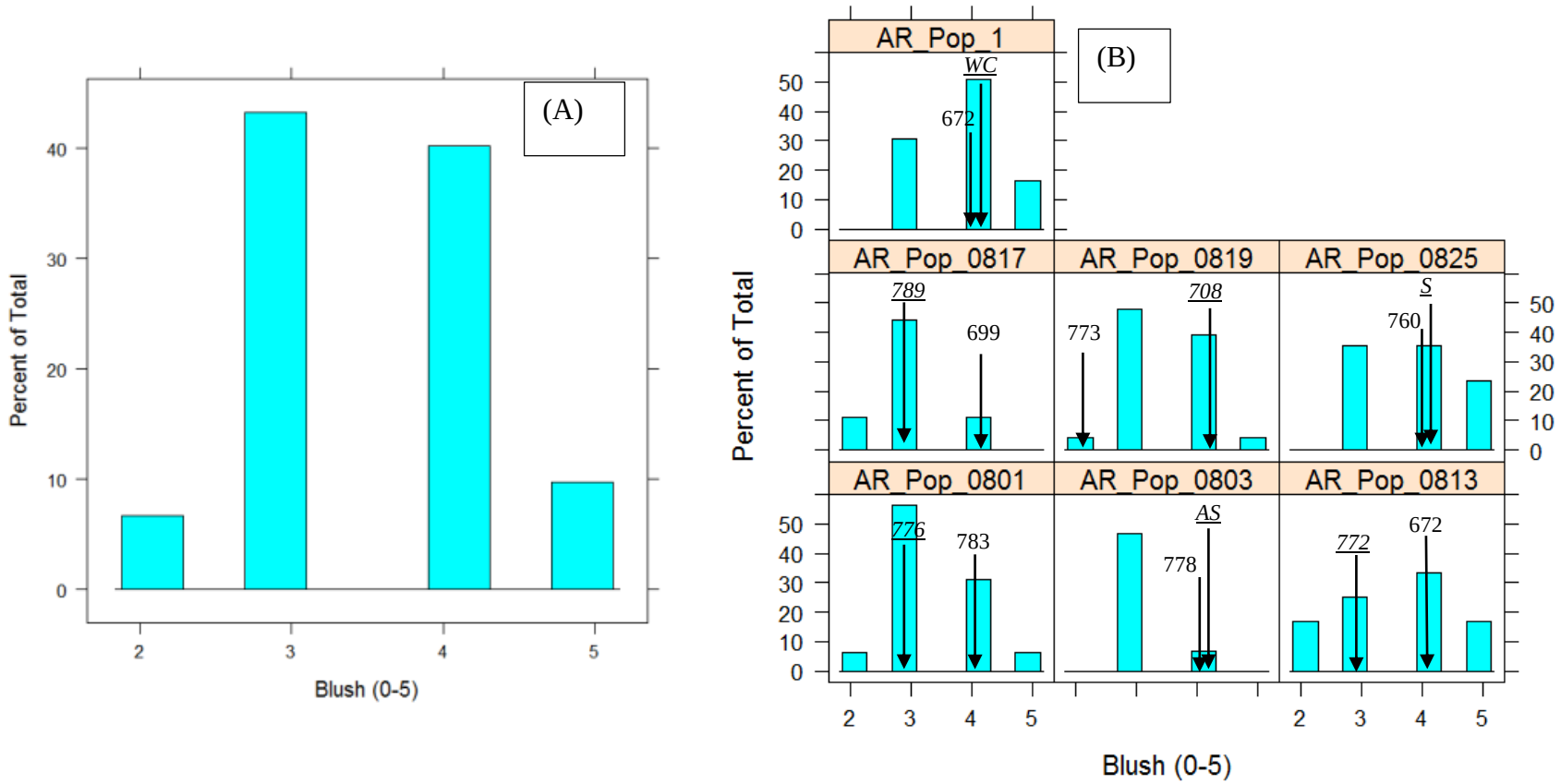

Fig 18. Distribution (%) of blush 2012 field ratings for the entire UA RosBREED pedigree (A) (N=164), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

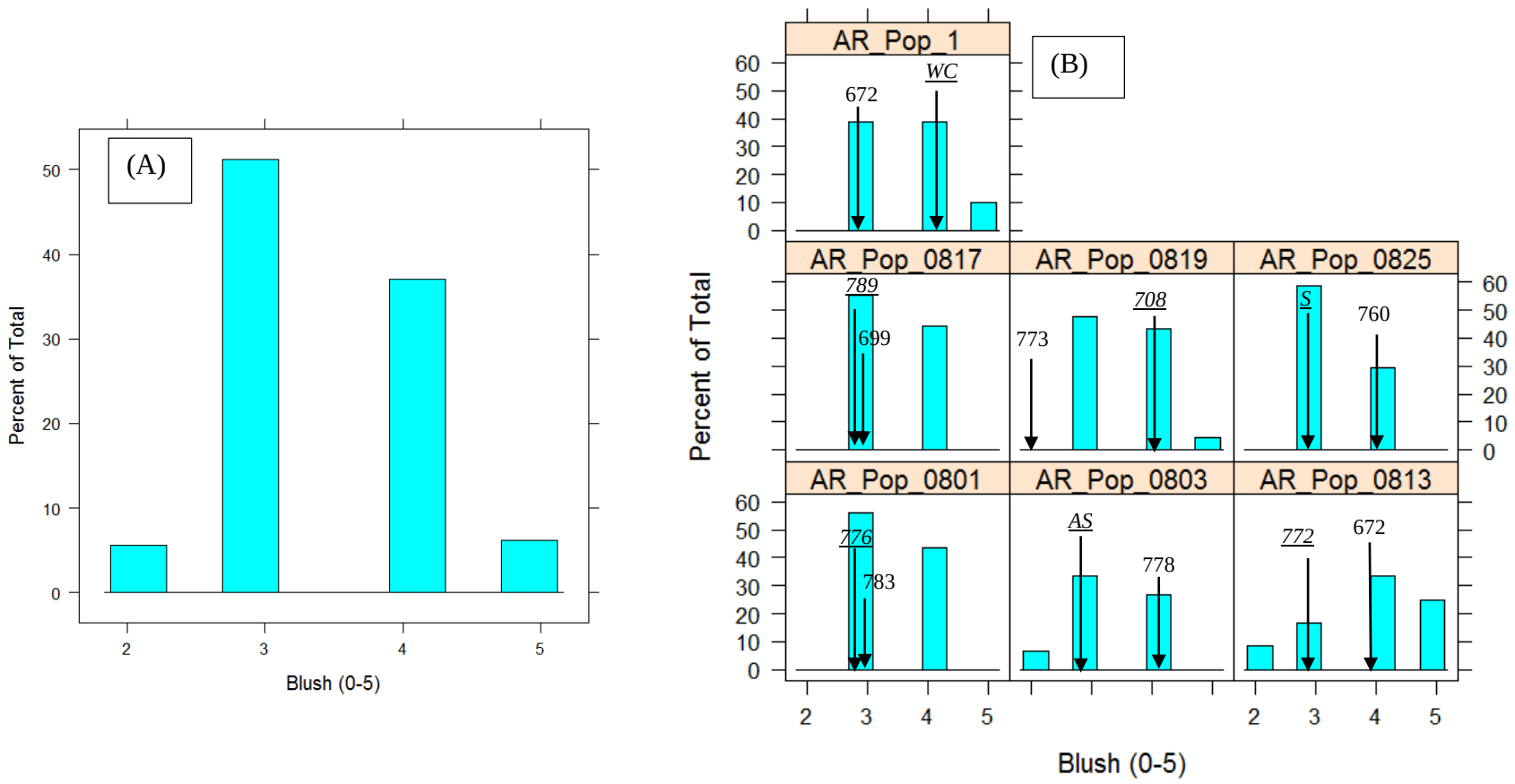

Fig 19. Distribution (%) of blush 2013 field ratings for the entire UA RosBREED pedigree (A) (N=162), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

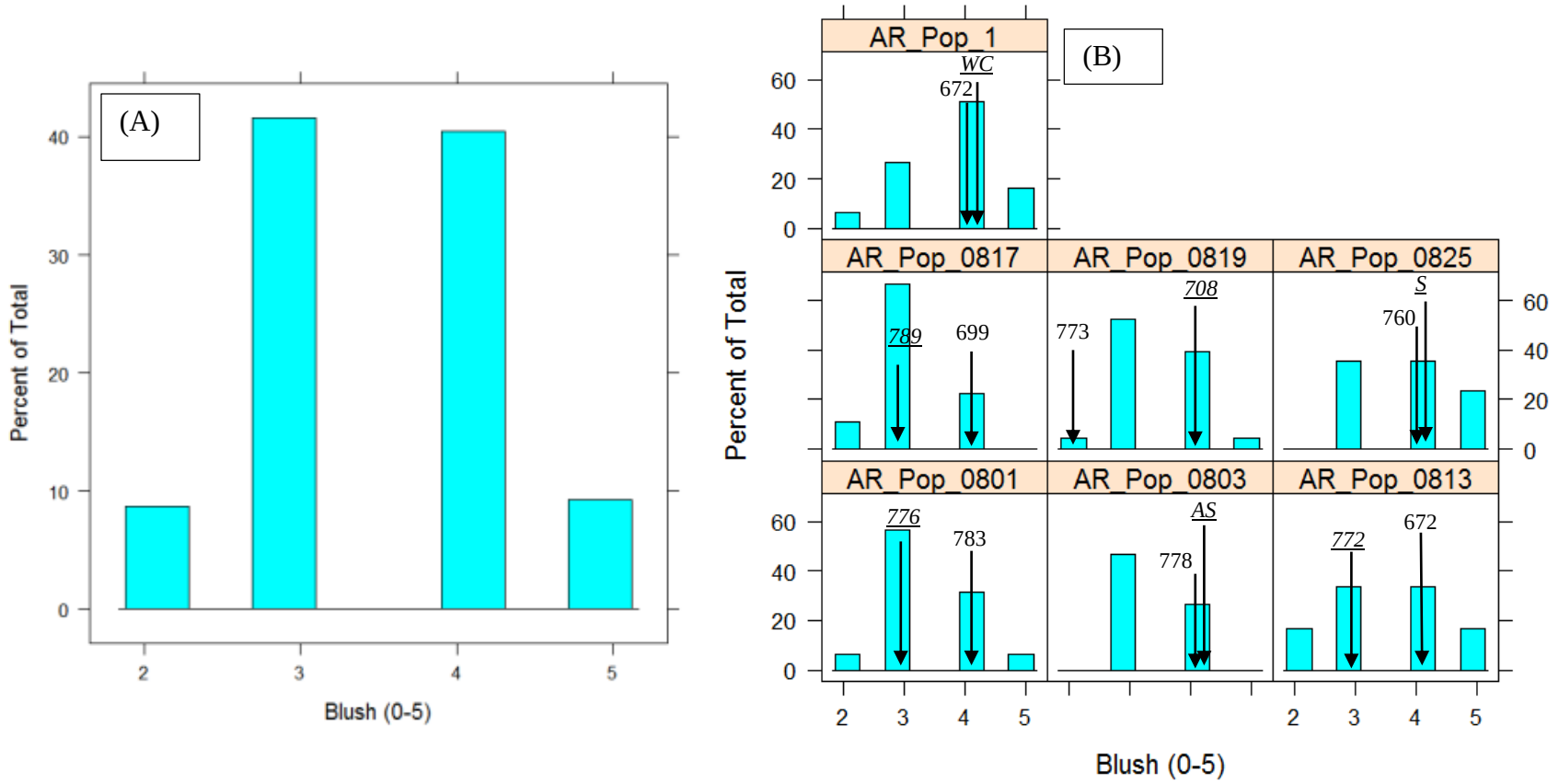

Fig 20. Distribution (%) of blush 2011-2013 avg field ratings for the entire UA RosBREED pedigree (A) (N=173), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

Size (Diameter and Weight)

Fruit weight (g), exhibited an average of 122.3 (2011), 102.6 (2012), 129.4 (2013), and 116.7 (2011-2013 avg) for all individuals in the AR RosBREED pedigree (Table 7). The fruit weight ranged from 30.2 (2012) to 253.8 g (2011) across all years. The standard deviation displayed across all years ranged from 37.5 (2011-2013 avg) to 45.4 (2011) (Table 7).

Fruit diameter (cm), displayed an average of 20.0 cm in 2013 (the only year measured) for all individuals in the AR RosBREED pedigree (Table 7). The fruit diameter ranged from 11.6-27.2 cm with a standard deviation of 2.9 in 2013 (Table 7).

In terms of the seven F_1 populations, different segregation patterns for fruit weight were observed across all years of the study (Figs. 21-24). In 2011, AR_Pop_0801, AR_Pop_0803 and AR_Pop_0817 contained seedlings that were the lowest in weight (Fig. 21). In contrast, AR_Pop_1 and AR_Pop_0819 consisted of seedlings which nearly spanned the entire weight spectrum in this study. In 2012, AR_Pop_0801 and AR_Pop_0813 contained seedlings that were again lowest in weight (Fig. 22). In contrast, only AR_Pop_1 consisted of seedlings which spanned the entire weight spectrum in this study. In 2013 and 2011-2013 avg, AR_Pop_0801 contained seedlings that were lowest in weight (Figs. 23-24). In contrast, as in 2012, only AR_Pop_1 consisted of seedlings which spanned the entire weight spectrum in this study. Parental values from each year are also included in Figs. 21-24.

In terms of fruit diameter (cm), in 2013 AR_Pop_0801 contained seedlings that were smallest in diameter (Fig. 25). In contrast only AR_Pop_1 consisted of seedlings which nearly spread across the entire diameter spectrum in this study. Parental values from 2013 are also included in Fig. 25.

Table 7. Mean, minimum, maximum, and standard deviation of fruit weight (g) ratings for the entire AR RosBREED pedigree (N is number of individuals) in 2011, 2012, 2013, and 2011-2013 avg. Mean, minimum, maximum, and standard deviation of fruit diameter (cm) ratings for the entire AR RosBREED pedigree (N is number of individuals) in 2013.

Year	Fruit weight (g)				N
	Mean	Min.	Max.	Std. dev.	
2011	122.3	40.5	253.8	45.4	153
2012	102.6	30.2	241.8	39.5	158
2013	129.4	51.2	233.9	42.2	125
2011-2013 avg	116.7	40.0	234.0	37.5	171
Year	Fruit diameter (cm)				N
	Mean	Min.	Max.	Std. dev.	
2013	20.0	11.6	27.2	2.9	135

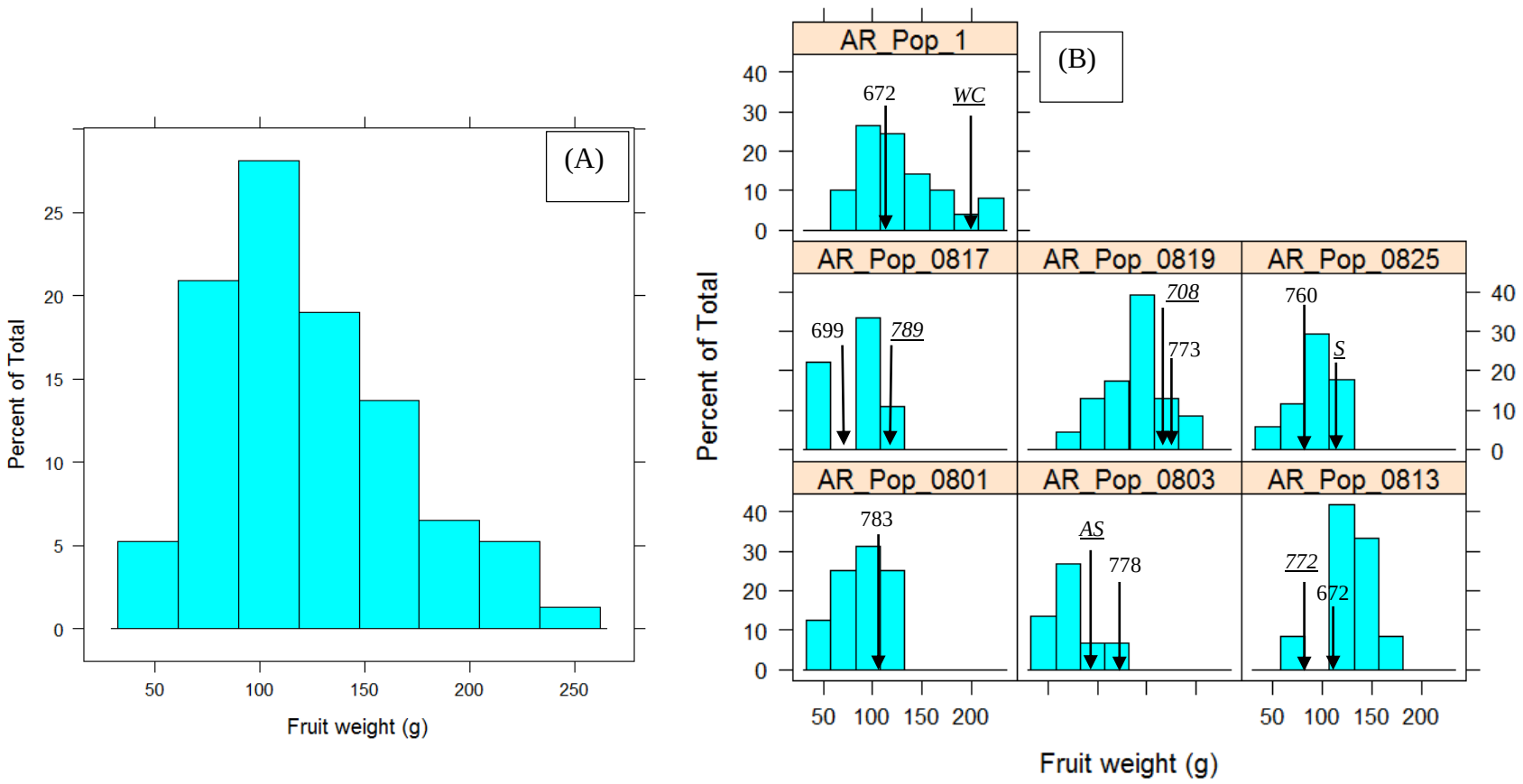

Fig 21. Distribution (%) of fruit weight 2011 field ratings for the entire UA RosBREED pedigree (A) (N=153), and the seven F_1 populations (parental values illustrated by arrows; parents not depicted were not phenotyped; *female parent* is italicized and underlined) (B).

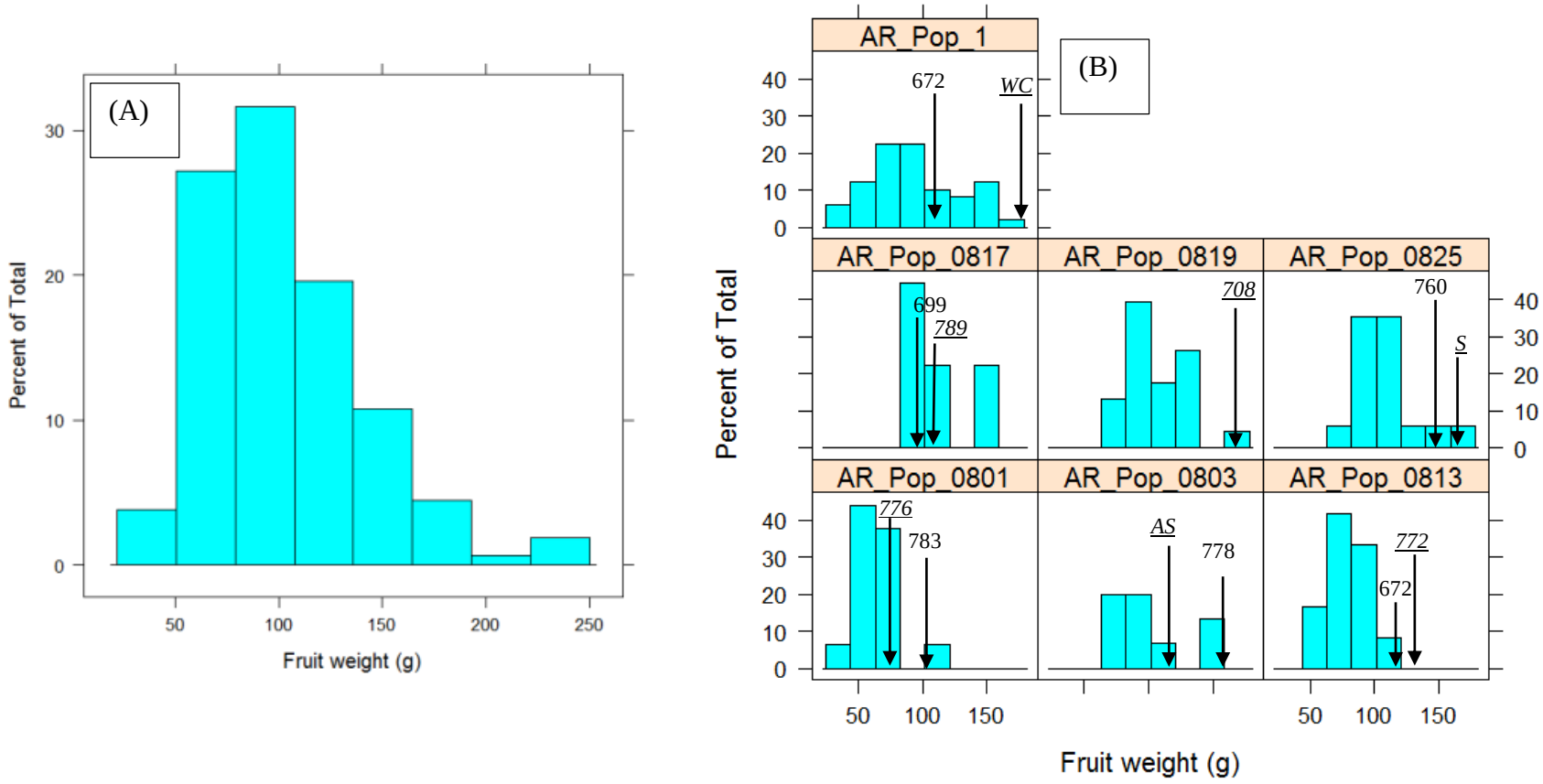

Fig 22. Distribution (%) of fruit weight 2012 field ratings for the entire UA RosBREED pedigree (A) (N=158), and the seven F_1 populations (parental values illustrated by arrows; parents not depicted were not phenotyped; *female parent* is italicized and underlined) (B).

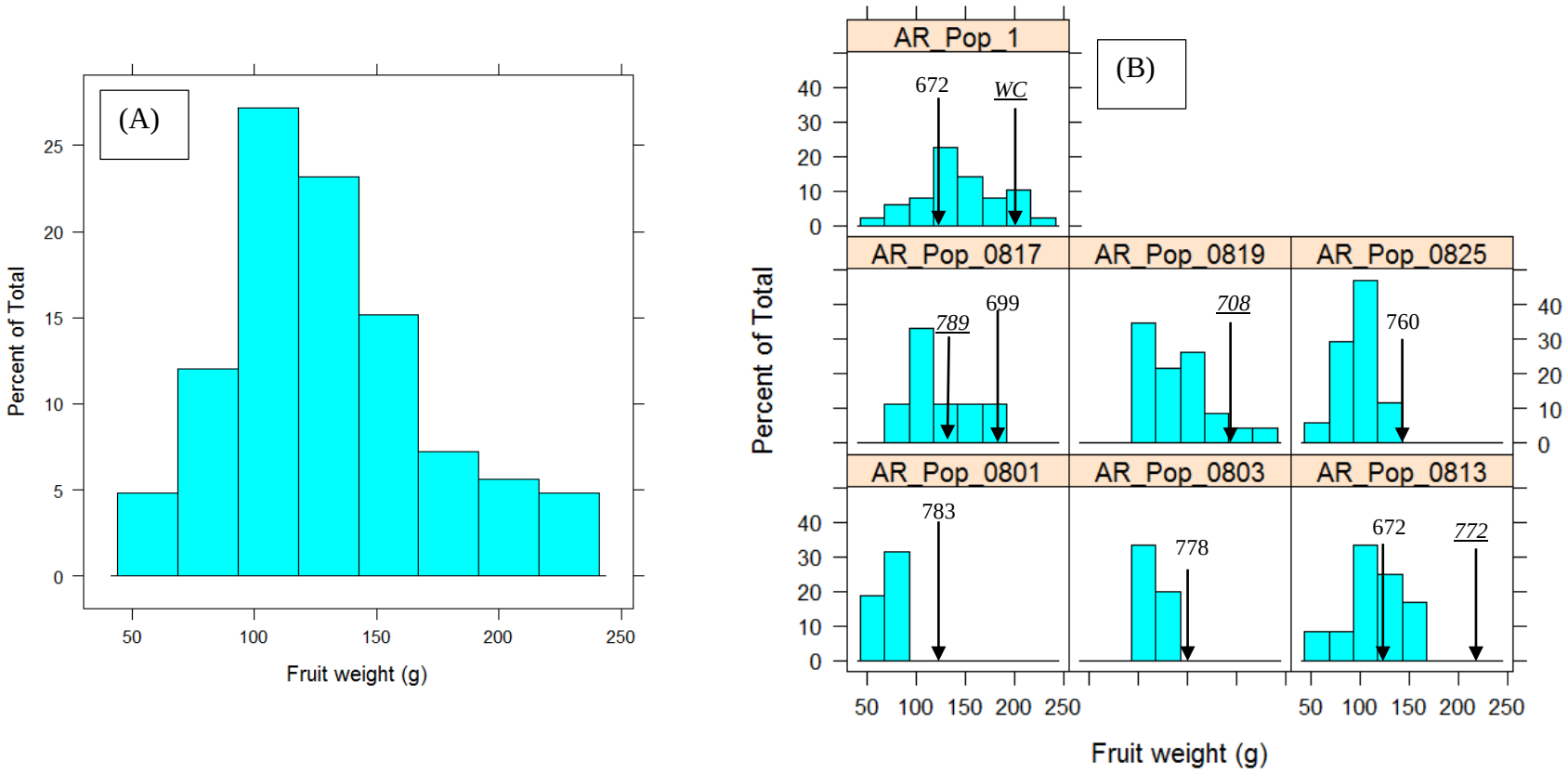

Fig 23. Distribution (%) of fruit weight 2013 field ratings for the entire UA RosBREED pedigree (A) (N=125), and the seven F_1 populations (parental values illustrated by arrows; parents not depicted were not phenotyped; *female parent* is italicized and underlined) (B).

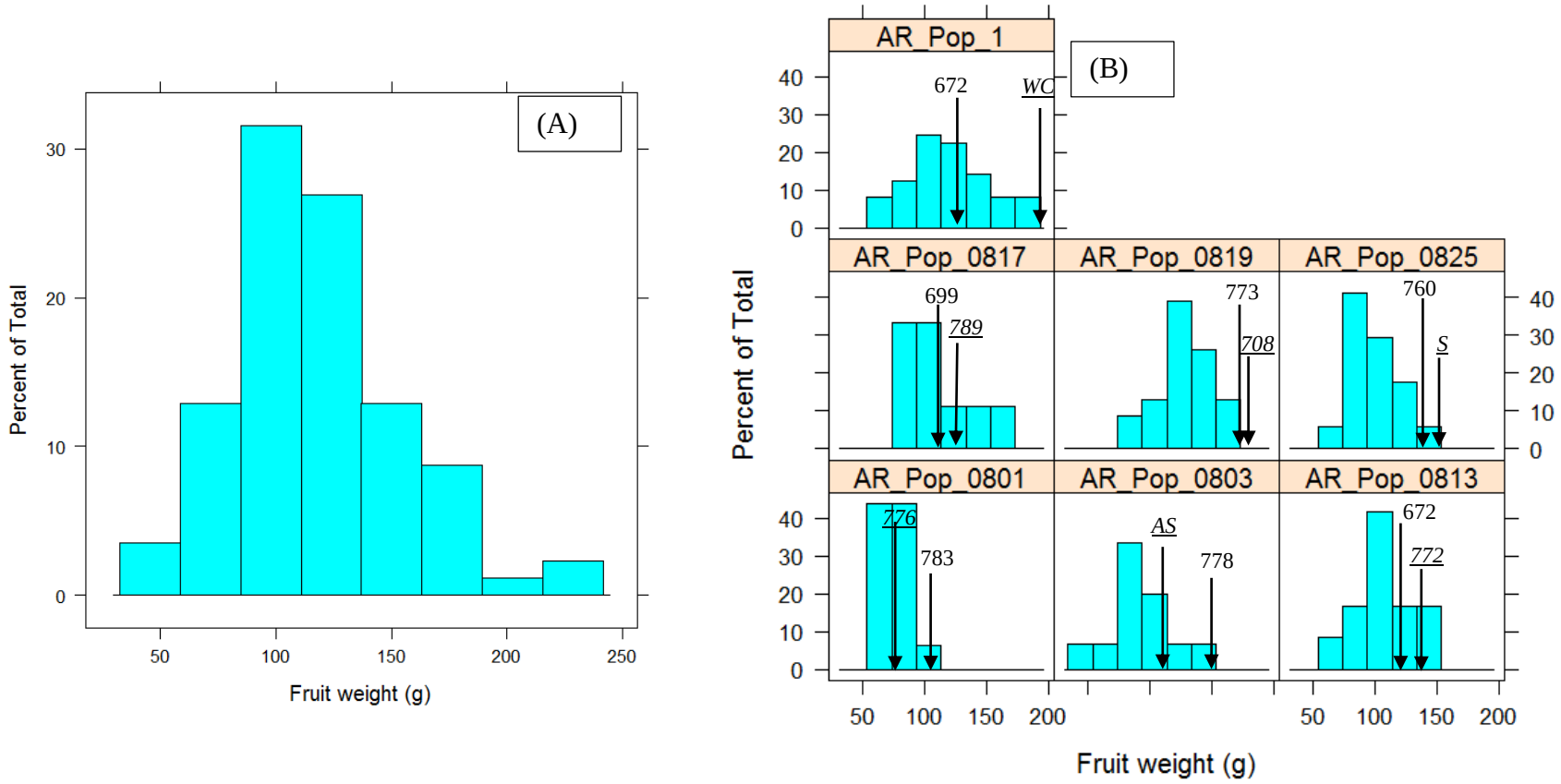

Fig 24. Distribution (%) of fruit weight 2011-2013 avg field ratings for the entire UA RosBREED pedigree (A) (N=171), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

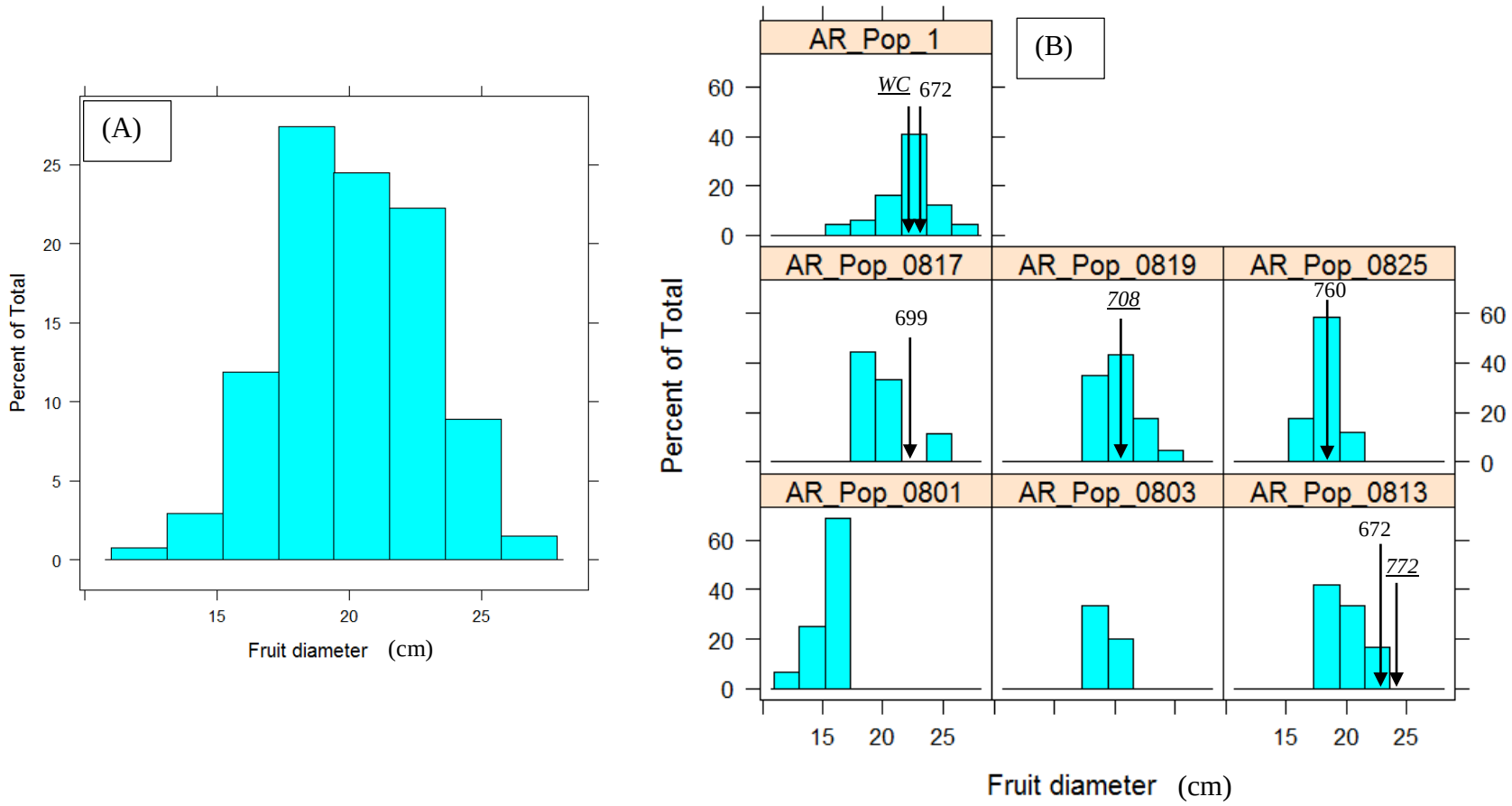

Fig 25. Distribution (%) of diameter 2013 field ratings for the entire UA RosBREED pedigree (A) (N=135), and the seven F_1 populations (parental values illustrated by arrows; parents not depicted were not phenotyped; *female parent* is italicized and underlined) (B).

Soluble Solids Content (SSC)

Soluble solids content (SSC) (%) exhibited an average of 15.7 (2011), 17.0 (2012), 13.3 (2013), and 15.5% (2011-2013 avg) for all individuals in the AR RosBREED pedigree (Table 8). The SSC ranged from 9.3 (2011) to 25.3% (2012) across all years. The standard deviation displayed across all years ranged from 2.0 (2013) to 3.2 (2012) (Table 8).

For the seven F_1 populations, different segregation patterns for SSC were observed across all years of the study (Figs. 26-29). In 2011, AR_Pop_0813 contained seedlings that spanned the entire SSC spectrum in this study (Fig. 26). In contrast, AR_Pop_1 and AR_Pop_0825 segregated from low to medium SSC, and AR_Pop_0801 and AR_Pop_0819 segregated from medium to high SSC. In 2012, AR_Pop_1 contained seedlings that spanned the entire SSC spectrum in this study (Fig. 27). Additionally, AR_Pop_0801, AR_Pop_0819, and AR_Pop_0813 nearly spanned the entire SSC spectrum, while AR_Pop_0825 and AR_Pop_0803 segregated from low to medium SSC. In contrast to 2011 and 2012, AR_Pop_0825 nearly spanned the entire SSC spectrum in 2013 (Fig. 28). During the same year, AR_Pop_1 and AR_Pop_0803 segregated from low to medium SSC, and AR_Pop_0801 and AR_Pop_0817 segregated from medium to high SSC. In 2011-2013 avg, AR_Pop_1, AR_Pop_0819, and AR_Pop_0825 segregated from low to medium SSC, while AR_Pop_0801, AR_Pop_0817, and AR_Pop_0803 segregated within medium SSC (Fig. 29). Parental values from each year are also included in Figs. 26-29.

Table 8. Mean, minimum, maximum, and standard deviation of soluble solids content (SSC) ratings for the entire AR RosBREED pedigree (N is number of individuals) in 2011, 2012, 2013, and 2011-2013 avg.

Year	Soluble solids content (SSC)%				N
	Mean	Min.	Max.	Std. dev.	
2011	15.7	9.3	21.0	2.5	153
2012	17.0	10.9	25.3	3.2	152
2013	13.3	9.8	17.9	2.0	125
2011-2013 avg	15.5	10.9	24.1	2.2	170

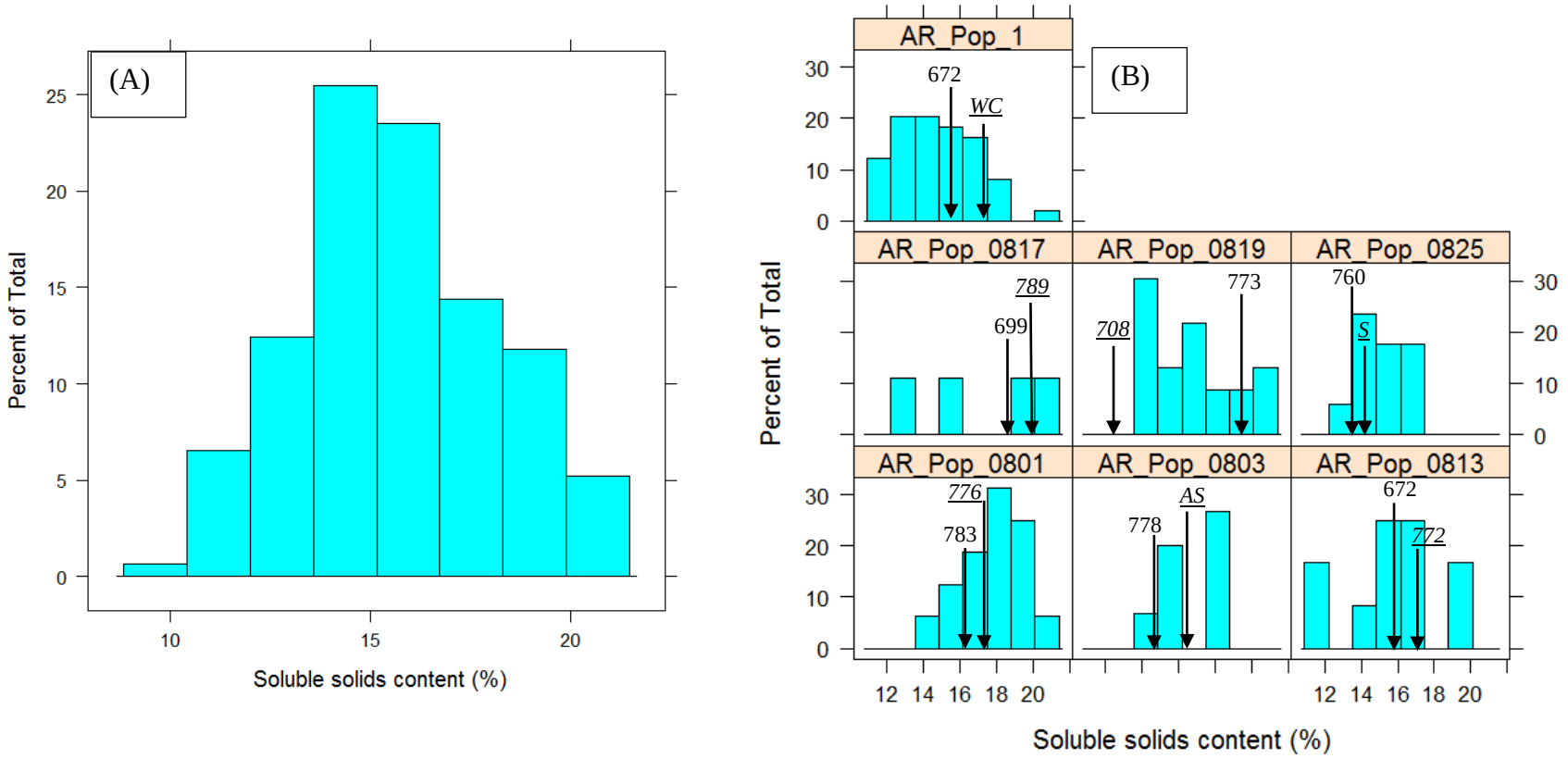

Fig 26. Distribution (%) of soluble solids content (%) 2011 field ratings for the entire UA RosBREED pedigree (A) (N=153), and the seven F_1 populations (parental values illustrated by arrows; *female parent* is italicized and underlined) (B).

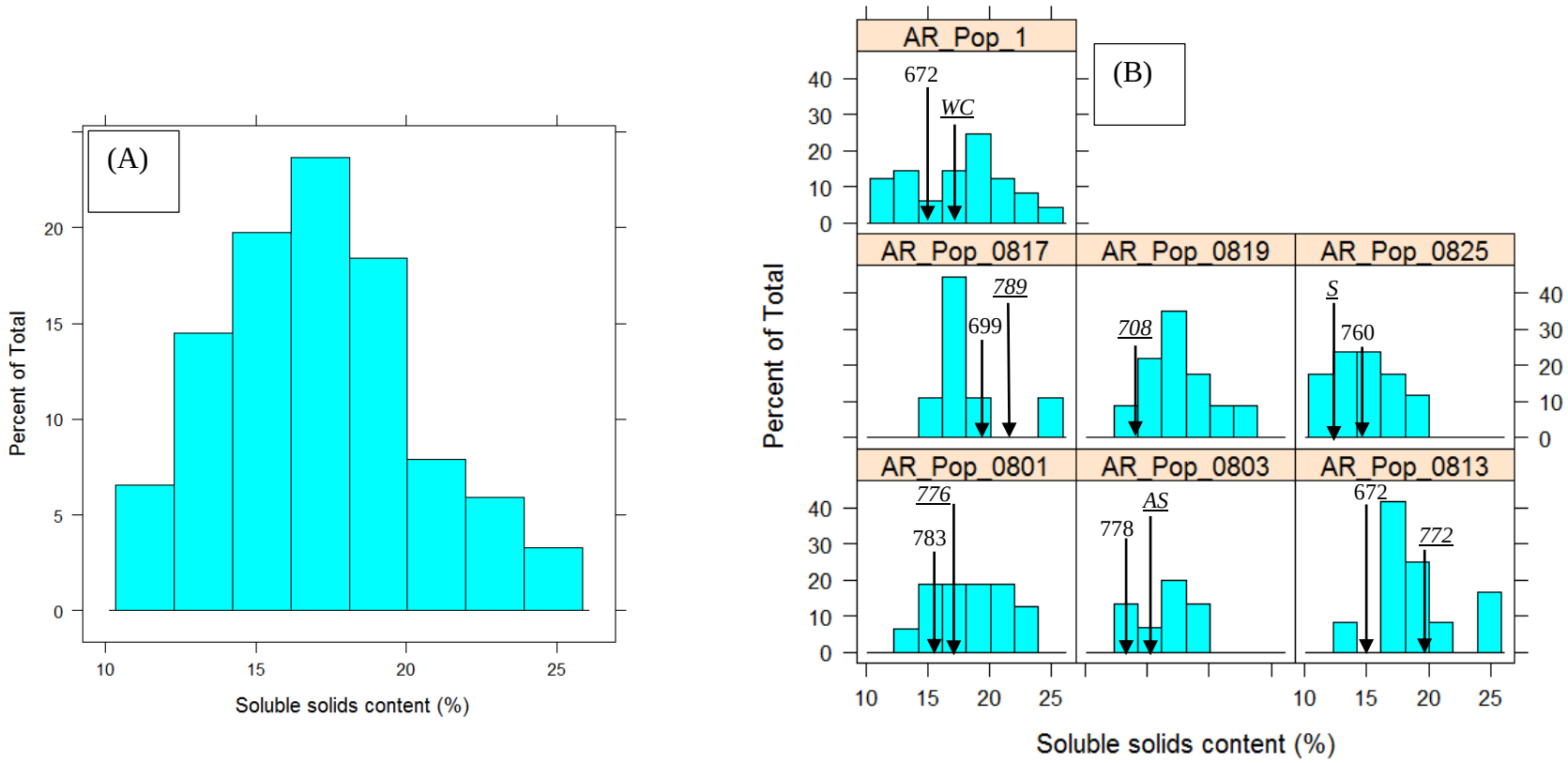

Fig 27. Distribution (%) of soluble solids content (%) 2012 field ratings for the entire UA RosBREED pedigree (A) (N=152), and the seven F_1 populations (parental values illustrated by arrows; parents not depicted were not phenotyped; *female parent* is italicized and underlined) (B).

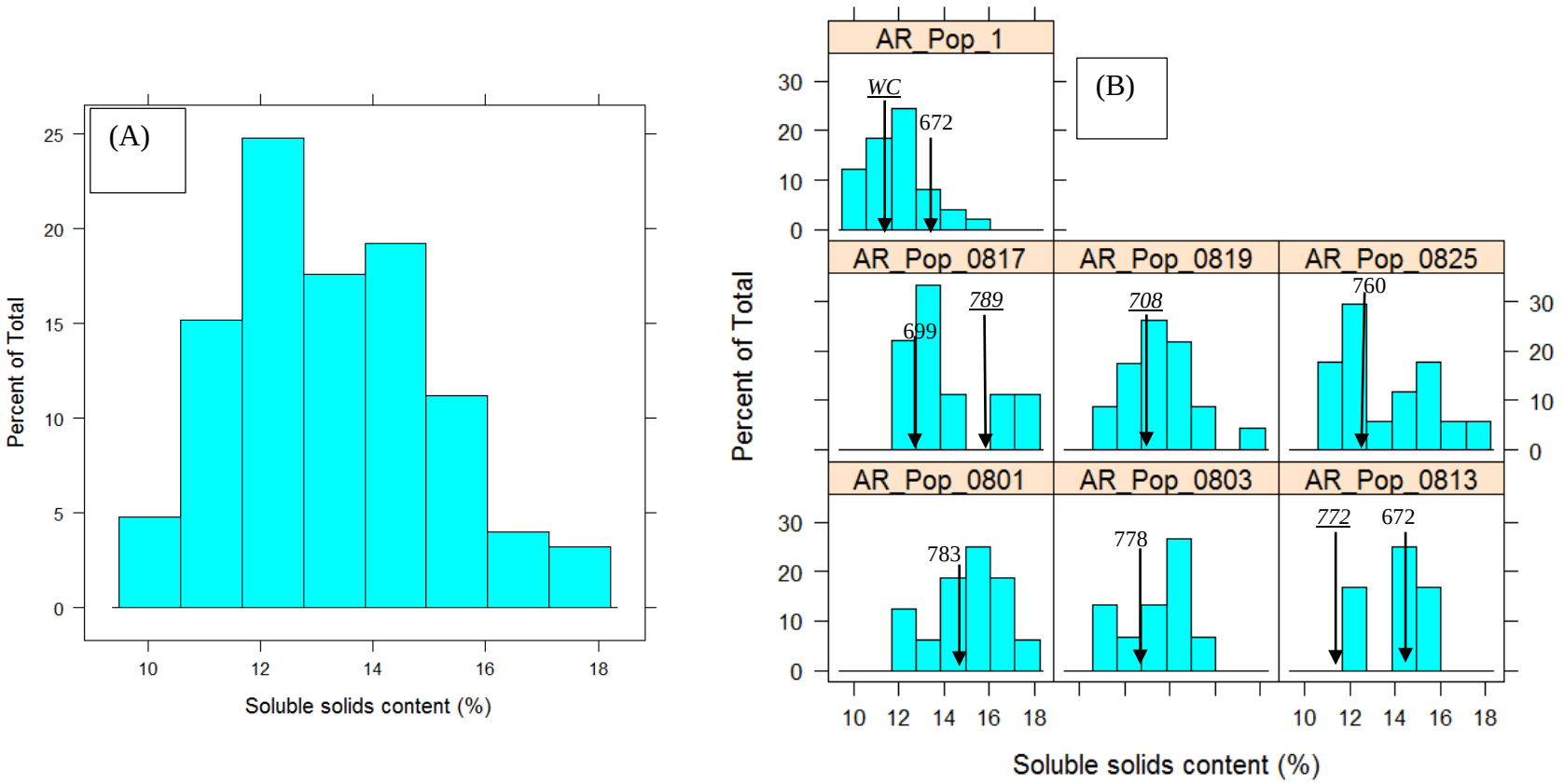

Fig 28. Distribution (%) of soluble solids content (%) 2013 field ratings for the entire UA RosBREED pedigree (A) (N=125), and the seven F_1 populations (parental values illustrated by arrows; parents not depicted were not phenotyped; ***female parent*** is italicized and underlined) (B).

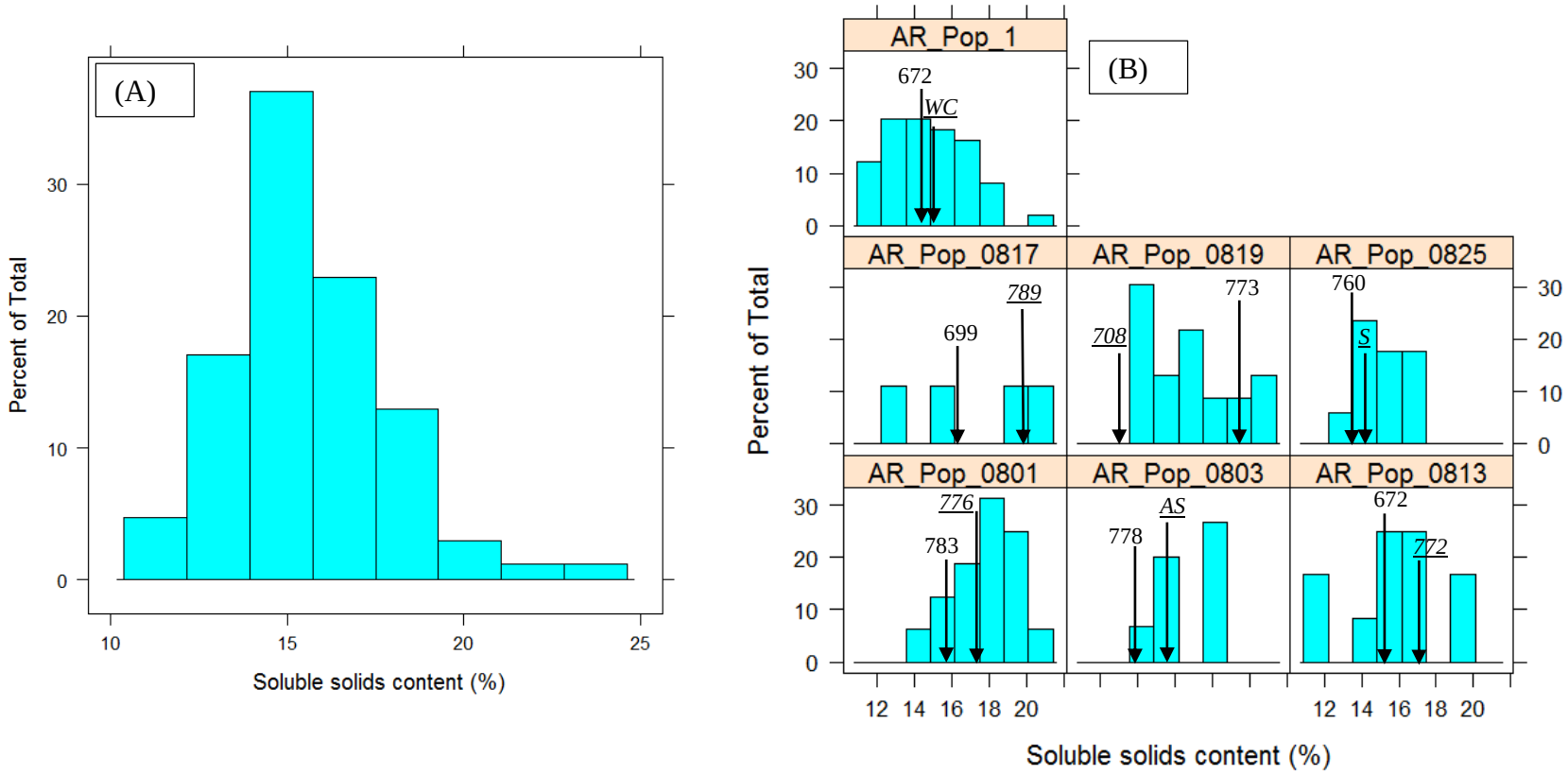

Fig 29. Distribution (%) of soluble solids content (%) 2011-2013 avg field ratings for the entire UA RosBREED pedigree (A) (N=170), and the seven F_1 populations (parental values illustrated by arrows; ***female parent*** is italicized and underlined) (B).

Genotypic Data

Of 8,144 SNPs on the IPSC peach 9 K SNP array v1.0, 1,947 (23.9%) were polymorphic and informative (GenTrain score of ≥ 0.4, and GenCall 10% of ≥ 0.2) across the UA pedigree. The 1,947 SNP marker locations were identified across the eight linkage groups (LGs), corresponding to the eight peach chromosomes. The number of SNPs and length of each LG ranged from 137 SNPs (0.69-45.61 cM) on LG5, to 424 SNPs (0.42-74.93 cM) on LG4.

Pedigree-Based QTL Analysis (PBA)

Pedigree-based QTL analysis (PBA) identified a total of 39 QTLs, each showing at least positive evidence (BF ≥ 2) using 2011, 2012, 2013, and 2011-2013 avg data sets for all seven phenotypic traits [maturity date (eight QTLs), pubescent vs. glabrous (four QTLs), white vs. yellow flesh (two QTLs), blush overcolor (three QTLs), fruit size (15 QTLs), and SSC (seven QTLs)]. All QTLs were named according to the guidelines of the Genome Database for Rosaceae: linkage group number and phenotypic trait symbol (Jung et al. 2008, 2014).

Maturity Date (MD)

Through PBA, a total of eight QTLs across seven LGs showed at least positive evidence (BF ≥ 2) to be associated with maturity date (MD) (day of year, 0-365) using 2011, 2012, 2013, and 2011-2013 avg data sets (Table 9; Figs. 30-32). The broad-sense heritability (H^2) ranged from 58-80%, years 2013 and 2011, and showed an average of 72% across all data sets. Moreover, the H^2 was always greater than the summation of the narrow sense heritability (h^2) for all QTLs identified using each data set (Table 9). PostQTL intensity and trace plots were generated and organized in a way to visually compare QTLs identified across all four data sets, genome-wide (Figs. 30-32).

One major QTL, which showed decisive evidence (BF ≥ 10) across all data sets, was identified on LG4, and termed G4MD.1 (Table 9; Figs. 30-32). Using 2011, 2012, and 2011-2013 avg data sets, G4MD.1 spanned 26.7-28.2 cM (BFs 31.1, 31.3) and was flanked by ss_410398 and ss_412662. The 2013 data set approximated G4MD.1 to span 26.7-29.1 (BF 31.3), flanked by ss_410398 and ss_413365. On average across all data sets, G4MD.1 explained 58% of the h^2 [phenotypic variation explained (Vp %)] for MD (ranging from 48-64% V_P). The PostQTL intensity plots for all four data sets from 23.0-32.7 cM on LG4 were lined up on top of each other to show the consistency of G4MD.1 across all data sets (Fig. 30). Taking into account all four data sets, four SNPs, ss_410398, ss_411601, ss_411637, and ss_412662 spanned G4MD.1 (Fig. 30).

Additionally, seven minor QTLs, which showed positive evidence (BF ≥ 2) to be associated with MD, were identified on LG1, LG2, LG3, LG5, LG6, and LG8. Using 2012 and 2011-2013 avg data sets, a minor QTL was identified on LG1 (termed G1MD.1), associated with an average of ~6% of the V_P for MD across both data sets. The G1MD.1 spanned 59.6-65.2 cM, and was flanked by ss_81682 and ss_88772. Next, using 2011 and 2012 data sets, a second minor QTL was identified on LG2 (termed G2MD.1), associated with ~5% of V_P for MD. Across both data sets, the G2MD.1 spanned 3.1-5.8 cM and was flanked by ss_146706 and ss_159881. A third minor QTL was identified on LG5 using 2012 and 2011-2013 avg data sets. This QTL, termed G5MD.1, localized to 2.05-36.56 cM flanked by ss_545261 and ss_600256 and on average was associated with ~2% of V_P for MD. Lastly, four additional minor QTLs were identified to be associated with 2-6% V_P for MD, using one data set each, on LG3 (G3MD.1), LG6 (G6MD.1, G6MD.2), and LG8 (G8MD.1).

Table 9. Maturity date (MD) QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in only one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected in two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in two or more data sets is represented in bold font.

QTL name	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G2MD.1	2011	154	2	ss_146706 ss_152111	3.05 4.26	1.22 1.70	2.8	0.80	0.09	0.75
G4MD.1			4	ss_410398 ss_412662	26.70 28.20	10.70 11.30	31.2		0.55	
G6MD.1			6	ss_608900 ss_611149	3.55 5.60	1.42 2.24	2.0		0.02	
G6MD.2			6	ss_631014 ss_638983	21.16 27.66	8.46 11.07	2.0		0.03	
G8MD.1			8	ss_842826 ss_851849	22.16 25.64	8.86 10.25	2.5		0.06	
G1MD.1	2012	160	1	ss_81682 ss_88772	59.60 65.20	23.40 26.10	3.6	0.75	0.07	0.74
G2MD.1			2	ss_152111 ss_159881	4.26 5.82	1.70 2.33	2.0		0.01	
G3MD.1			3	ss_350488 ss_354980	38.40 41.89	15.36 16.76	2.0		0.03	
G4MD.1			4	ss_410398 ss_412662	26.70 28.20	10.70 11.30	31.1		0.64	
G5MD.1			5	ss_545261 ss_600256	2.05 36.56	0.82 14.62	4.4		0.03	
G4MD.1	2013	143	4	ss_410398 ss_413365	26.7 29.1	10.7 13.1	31.3	0.58	0.48	0.48

Table 9. Maturity date (MD) QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in only one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected in two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in two or more data sets is represented in bold font. (Cont.).

QTL name	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G1MD.1	2011-2013 avg	174	1	ss_81682 ss_87685	59.6 64.7	23.4 31.2	3.5	0.74	0.05	0.72
G4MD.1			4	ss_410398 ss_412662	26.7 28.2	10.7 11.3	31.1		0.63	
G5MD.1			5	ss_545261 ss_594279	2.05 30.02	0.82 12.01	2.6		0.01	
G6MD.1			6	ss_608856 ss_617084	3.51 10.01	1.41 4.00	2.3		0.02	

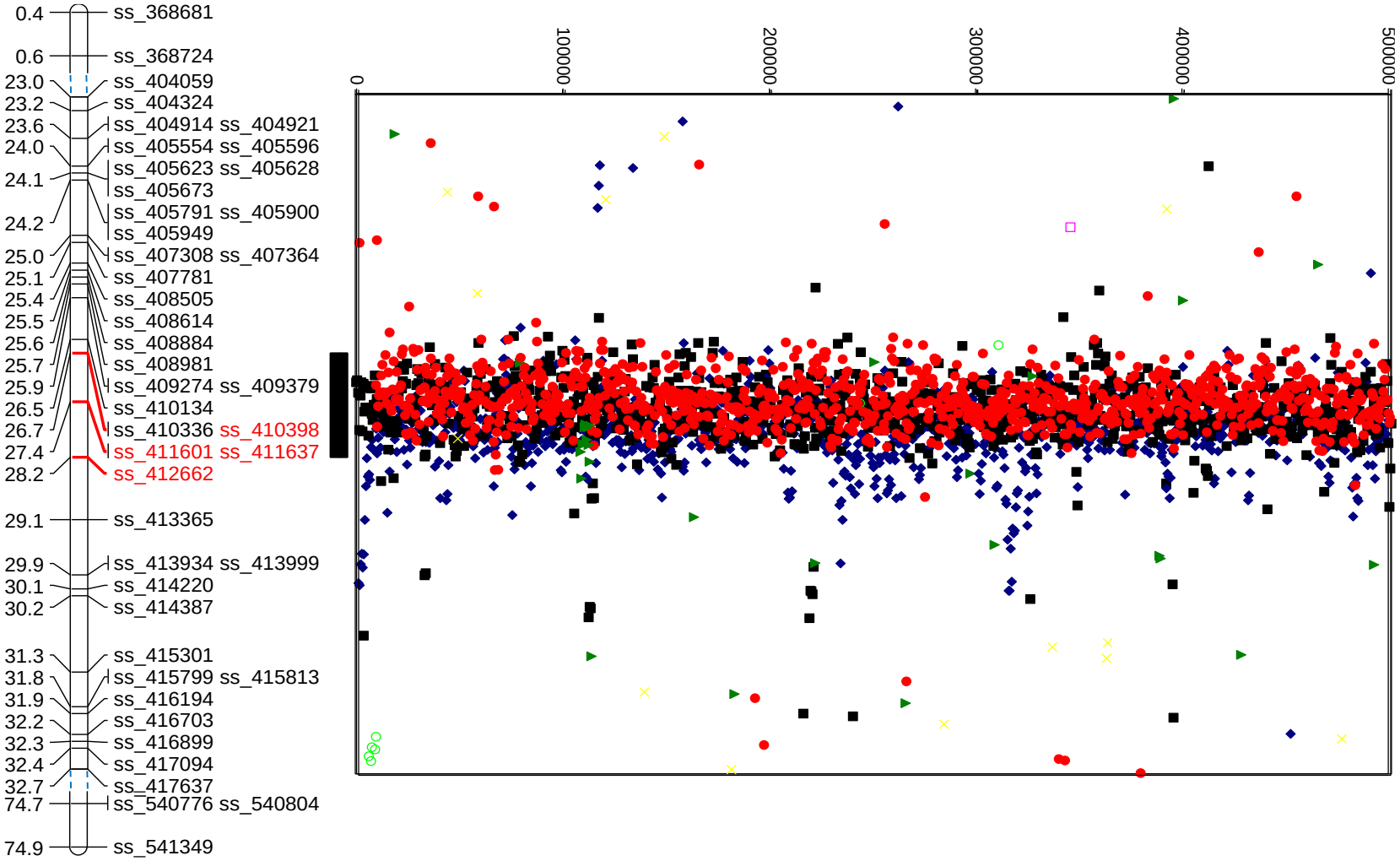

Fig. 30. Superimposed PostQTL trace plots of a subsection of LG4 (23.0-32.7 cM) for maturity date [2011, 2012, 2013, and 2011-2013 avg]. The black box indicates the position of the major QTL G4MD.1, and the four SNPs which span the QTL are highlighted in red. The y-axis represents the trace number iteration from 0-500,000. The colored dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide (Different colors and shapes do not have a biological interpretation). Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

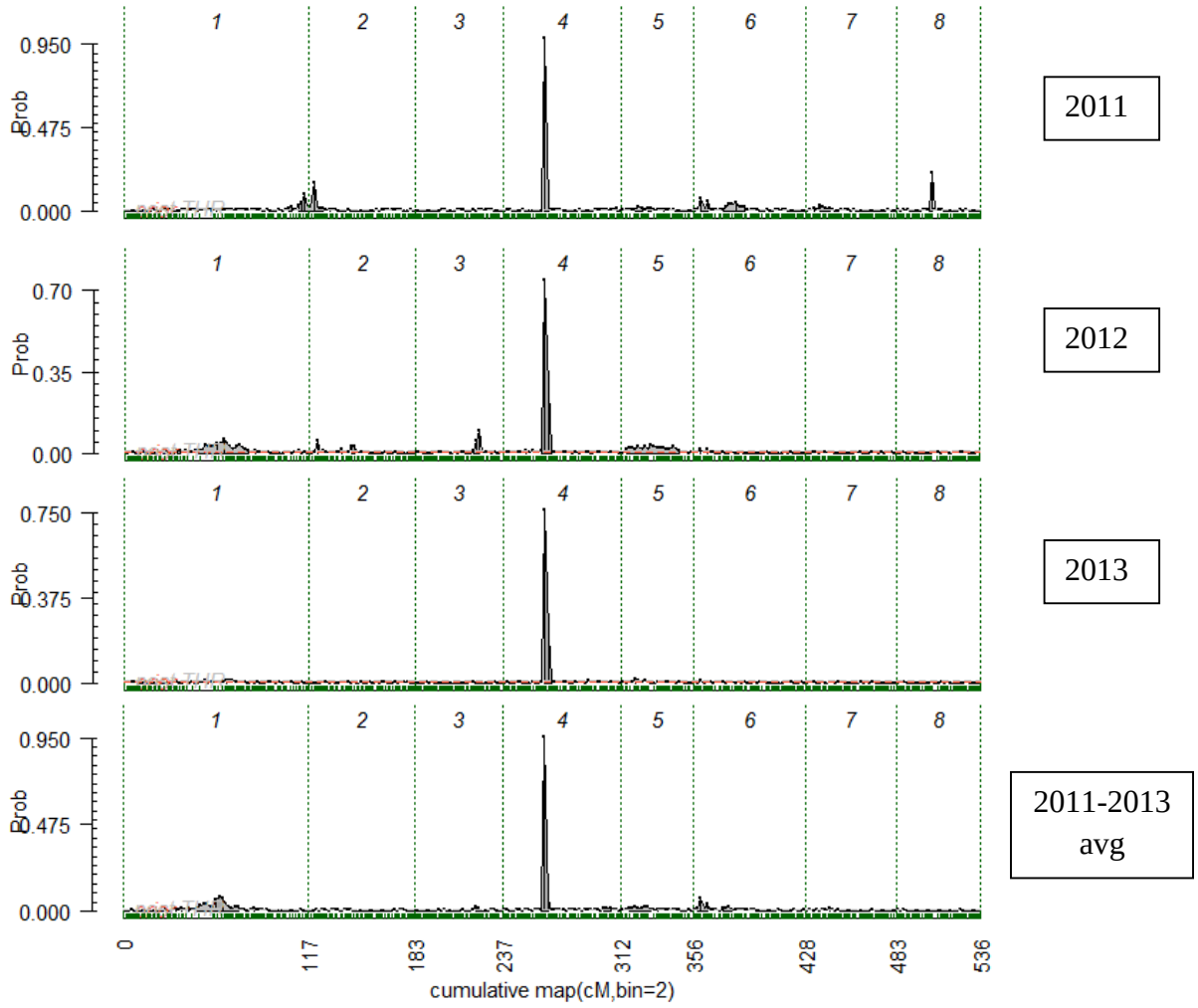

Fig. 31. PostQTL intensity plot positions for maturity date [2011, 2012, 2013, and 2011-2013 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

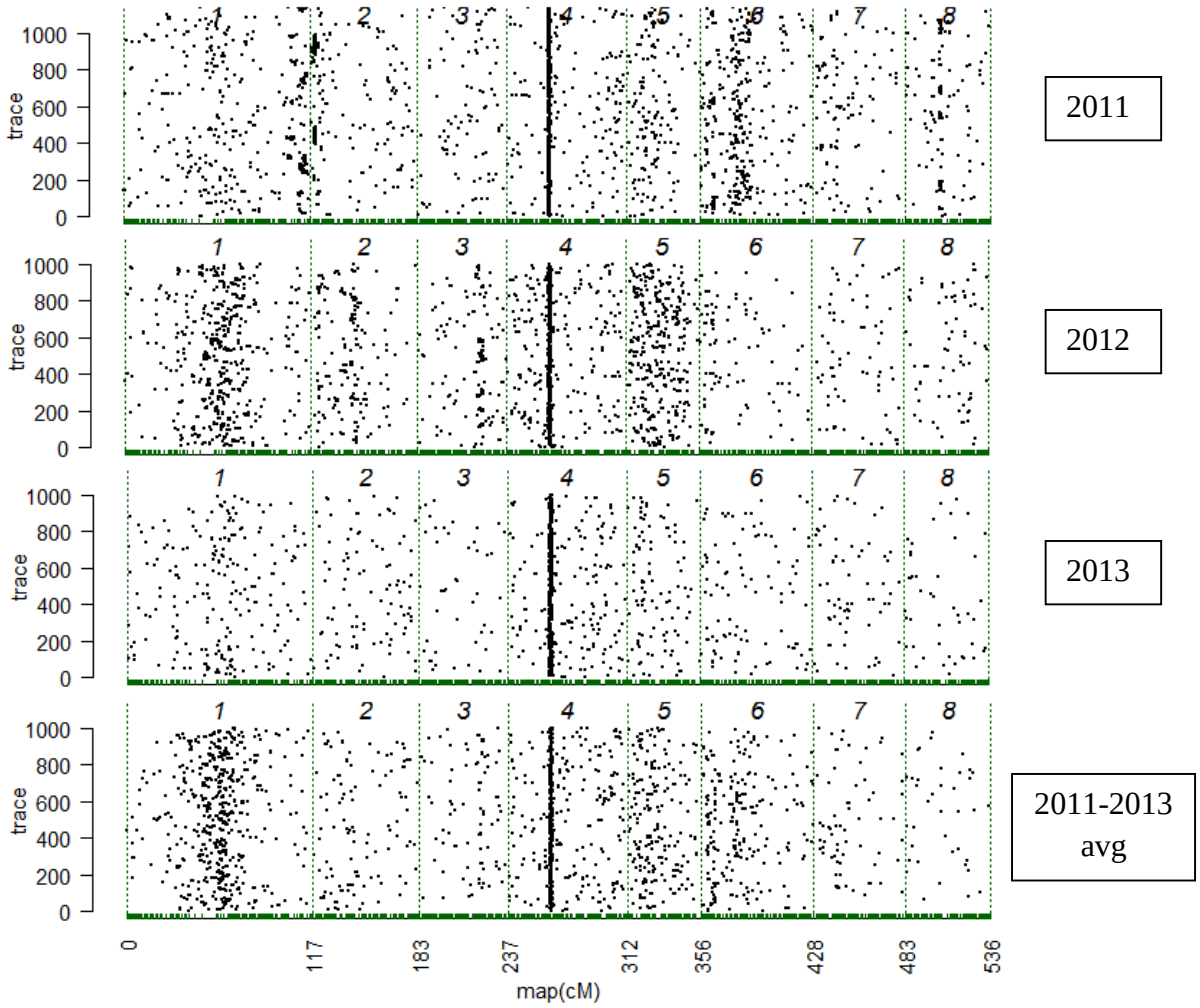

Fig. 32. PostQTL trace plots for maturity date [2011, 2012, 2013, and 2011-2013 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The black dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

G-locus (Pubescent vs. Glabrous)

Through PBA, only the pubescent vs. glabrous (Pub) 2011-2013 avg data set successfully converged. Four QTLs showed decisive evidence (BF ≥ 10) to be associated with Pub, using the Pub 2011-2013 avg data set (Table 10; Figs. 33-34). The first QTL, termed G1Pub.1 was located near the upper end of LG1, flanked by ss_21221 and ss_24260 (17.33-20.89 cM), and was associated with 9% of the V_P for Pub, using the Pub 2011-2013 avg data set. The second and third QTLs, termed G3Pub.1 and G3Pub.2, were located near the top of LG3. The G3Pub.1 QTL was flanked by ss_291987 and ss_294513 (1.00-3.46 cM), and was associated with 6% of the V_P for Pub, using the Pub 2011-2013 avg data set. The G3Pub.2 QTL was located just downstream of G3Pub.1, and was flanked by ss_300877 and ss_307230 (9.01-12.36 cM), and was associated with 16% of the V_P for Pub, using the Pub 2011-2013 avg data set. The fourth QTL, termed G5Pub.1, was identified near the bottom of LG5, flanked by ss_596063 and ss_602331 (33.09-41.39 cM), and was associated with 45% of the V_P for Pub, using the Pub 2011-2013 avg data set. The broad-sense heritability (H^2) for the Pub 2011-2013 avg data set was 99%, and the summation of the narrow sense heritability (h^2) for the four QTLs identified was less than the H^2 (Table 10). PostQTL intensity and trace plots were generated and organized in a way to visually compare QTLs identified using the Pub 2011-2013 avg data set, genome-wide (Figs. 33-34).

Table 10. Pubescent vs. glabrous QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented.

QTL	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G1Pub.1	2011-2013 avg	170	1	ss_21221 ss_24260	17.33 20.89	6.93 8.35	30.7	0.99	0.09	0.76
G3Pub.1			3	ss_291987 ss_294513	1.00 3.46	0.40 1.38	33.6		0.06	
G3Pub.2			3	ss_300877 ss_307230	9.01 12.36	3.60 4.94	33.6		0.16	
G5Pub.1			5	ss_596063 ss_602331	33.09 41.39	13.24 16.56	34.0		0.45	

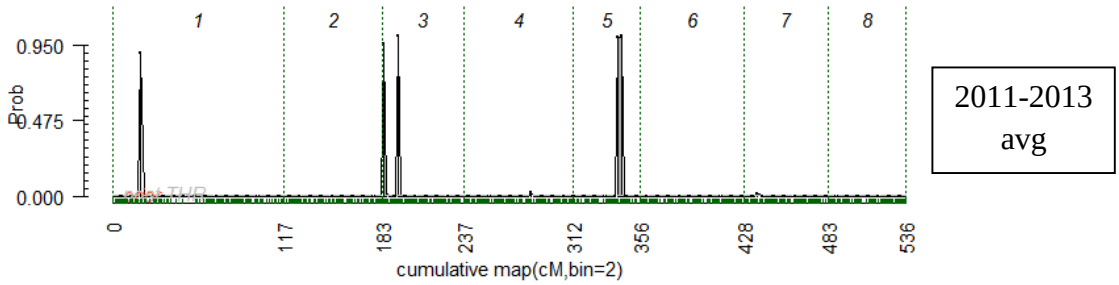

Fig. 33. PostQTL intensity plot positions for pubescent vs. glabrous [2011, 2012, 2013, and 2011-2013 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

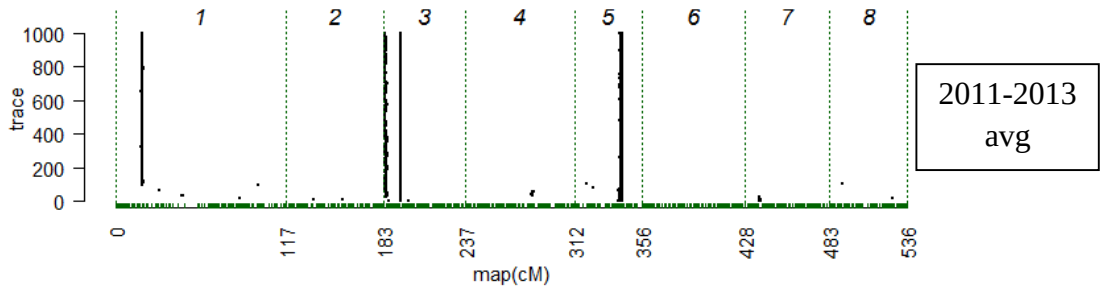

Fig. 34. PostQTL trace plots for pubescent vs. glabrous [2011, 2012, 2013, and 2011-2013 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The black dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Y-locus (White vs. Yellow Flesh)

Through PBA, only the flesh color (FC) 2011-2013 avg data set successfully converged. Two QTLs were identified on LG1, each showed decisive evidence (BF ≥ 10) to be associated with white vs. yellow flesh color (FC), using the FC 2011-2013 avg data set (Table 11; Figs 35-36). The first QTL was located near the upper end of LG1, flanked by ss_1103 (0.5 cM) and ss_7706 (5.9 cM), and was associated with 8% of the V_P for white vs. yellow FC. The second QTL was located near the middle of LG1 flanked by ss_67620 (42.9 cM) and ss_88772 (58.4 cM), and was associated with 83% of the V_P for white vs. yellow FC. The broad-sense heritability (H^2) for the FC 2011-2013 avg data was 94%, and the summation of the narrow sense heritability (h^2) for the two QTLs identified was less than the H^2 (Table 11). PostQTL intensity and trace plots were generated and organized in a way to visually compare QTLs identified using the FC 2011-2013 avg data set, genome-wide (Figs. 35-36).

Table 11. White vs. yellow flesh QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented.

QTL	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G1FC.1	2011-2013 avg	174	1	ss_1103 ss_7706	0.47 5.94	0.19 2.38	32.9	0.94	0.07	0.90
G1FC.2			1	ss_67620 ss_88772	48.28 65.22	19.31 26.10	32.9		0.83	

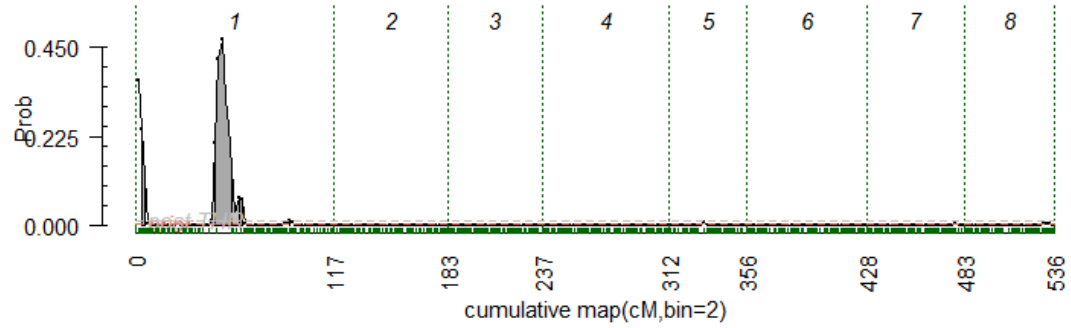

Fig. 35. PostQTL intensity plot positions for white vs. yellow flesh [2011-2013 avg]. Vertical dotted green lines separate linkage groups one-eight]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF $\geq$ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

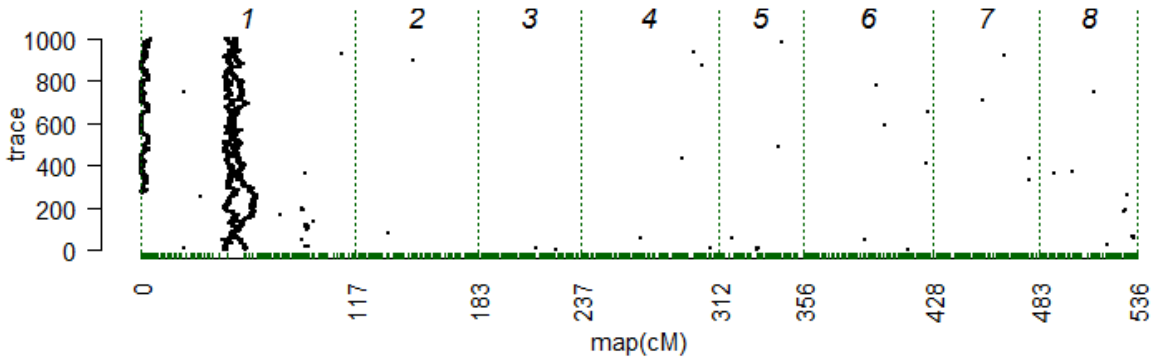

Fig. 36. PostQTL trace plots for white vs. yellow flesh [2011-2013 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The black dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Blush Overcolor

Through PBA, a total of three QTLs across three LGs showed at least positive evidence (BF ≥ 2) to be associated with blush overcolor using 2011, 2012, 2013, and 2011-2013 avg data sets (Table 12; Figs. 37-38). The broad-sense heritability (H^2) ranged from 13-36% for years 2013 and 2012, respectively, and showed an average of 28% across all data sets. Moreover, the H^2 was always greater than the summation of the narrow sense heritability (h^2) for all QTLs identified using each data set (Table 12). PostQTL intensity and trace plots were generated and organized in a way to visually compare QTLs identified across all four data sets, genome-wide (Figs. 37-38).

One major QTL, which showed strong (BF ≥ 5) or decisive evidence (BF ≥ 10) across three of the four data sets (2011, 2012, and 2011-2013 avg), was identified on LG3 and termed G3BL.1 (Table 12; Figs. 37-38). Using 2011, 2012, and 2011-2013 avg data sets, G3BL.1 spanned 30.8-35.1 cM (BFs 7.9, 10.8) and was flanked by ss_340465 and ss_345700. On average across all data sets, G3BL.1 explained 33% of the h^2 [phenotypic variation explained (V_P)] for maturity date (ranging from 30-36% V_P). Taking into account all three data sets, nine SNPs, ss_340465, ss_340919, ss_343311, ss_343432, ss_343773, ss_344064, ss_344612, ss_344755, and ss_345700 spanned G3BL.1 (Table 12; Figs. 37-38).

Additionally, two minor QTLs, which showed positive evidence (BF ≥ 2) to be associated with BL, were identified on LG2 and LG4 (Table 12; Figs. 37-38). Using 2011, 2012, and 2011-2013 avg data sets, a minor QTL was identified on LG2 (termed G2BL.1), associated with an average of ~4% of the V_P for blush across the three data sets. The G2BL.1 spanned 33.8-36.1 cM, and was flanked by ss_255903 and ss_258854. Next, using the 2013 data set, a second minor QTL was identified on LG4 (termed G4BL.1), associated with ~5% of V_P for BL. The

G4BL.1 spanned 18.9-22.1 cM and was flanked by ss_401100 and ss_403004 (Table 12; Figs. 37-38).

Table 12. Blush coverage (BL) QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in only one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected in two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in two or more data sets is represented in bold font.

QTL	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G2BL.1	2011	164	2	ss_255903 ss_258854	33.77 36.10	13.51 14.44	2.5	0.35	0.03	0.34
G3BL.1			3	ss_340465 ss_345700	30.81 35.06	12.32 14.02	10.1		0.31	
G2BL.1	2012	164	2	ss_255903 ss_258854	33.77 36.10	13.51 14.44	2.3	0.36	0.03	0.36
G3BL.1			3	ss_340465 ss_345700	30.81 35.06	12.32 14.02	10.8		0.33	
G4BL.1	2013	162	4	ss_401100 ss_403004	18.85 22.09	7.54 8.83	2.9	0.13	0.05	0.05
G2BL.1	2011-2013 avg	173	2	ss_255903 ss_258854	33.77 36.10	13.51 14.44	2.8	0.29	0.03	0.29
G3BL.1			3	ss_340465 ss_345700	30.81 35.06	12.32 14.02	7.9		0.26	

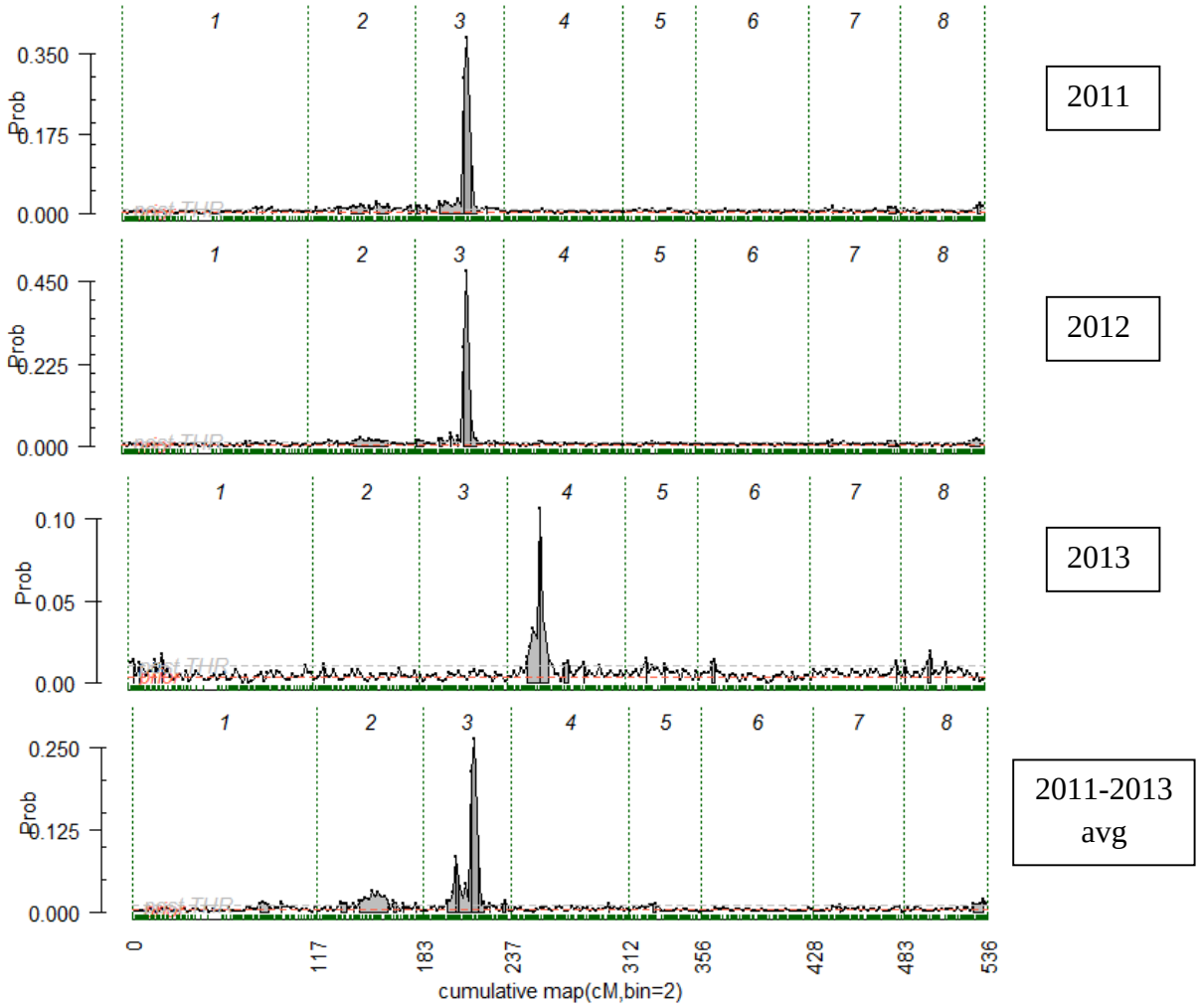

Fig. 37. PostQTL intensity plot positions for blush overcolor [2011, 2012, 2013, and 2011-2013 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

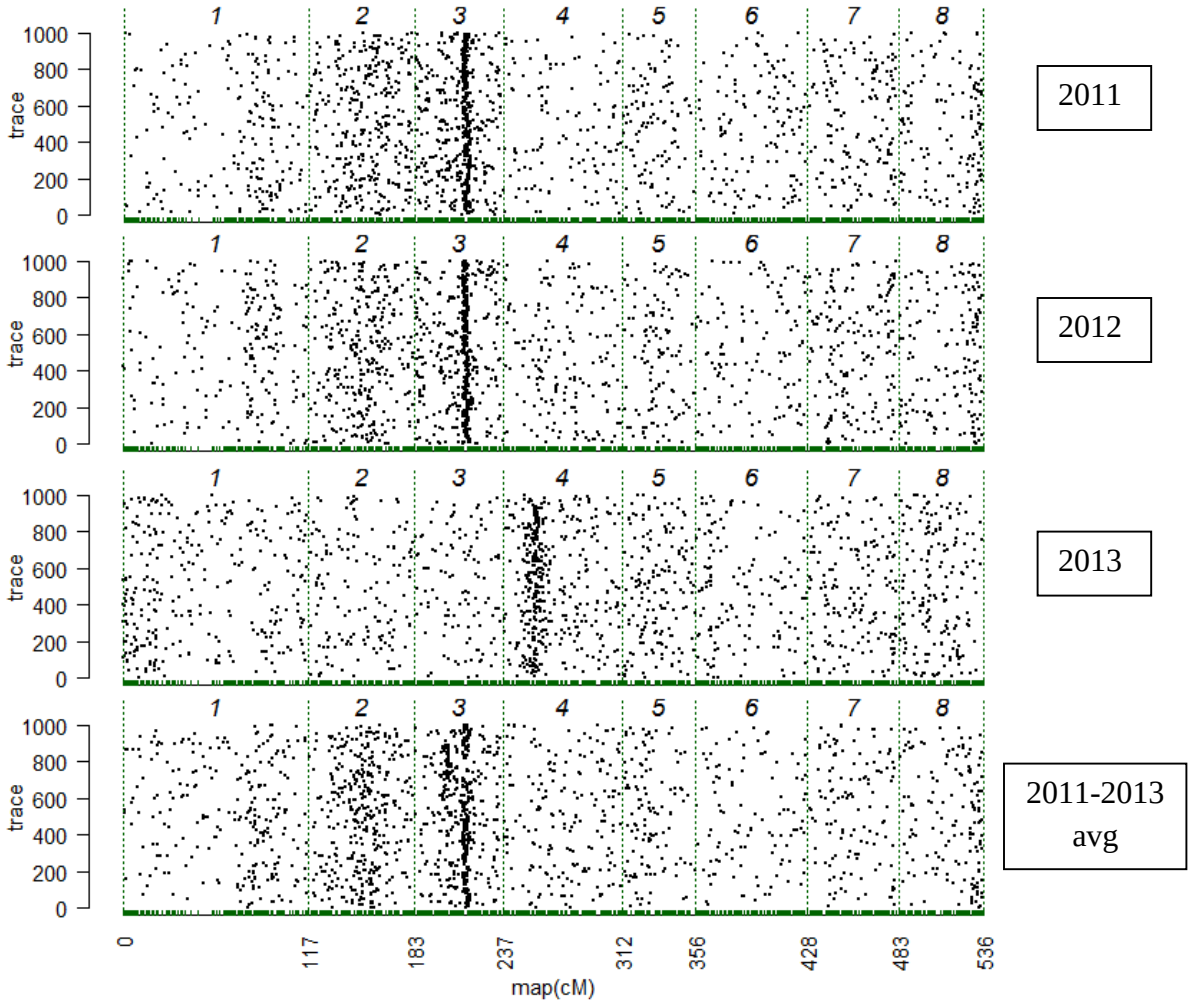

Fig. 38. PostQTL trace plots for blush overcolor [2011, 2012, 2013, and 2011-2013 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The black dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Fruit Size (Diameter and Weight)

Through PBA, a total of 15 QTLs across all eight LGs showed at least positive evidence (BF ≥ 2) to be associated with fruit size [fruit weight (FW) and or fruit diameter (FD)] using 2011, 2012, 2013, and 2011-2013 avg data sets (Tables 13-14; Figs. 39-40). The broad-sense heritability (H^2) for FW ranged from 19-73% for years 2013 and 2011, and showed an average of 47% across all data sets. Moreover, the H^2 for FW was always greater than the summation of the narrow sense heritability (h^2) for all QTLs identified using each data set (Table 13). The H^2 for FD in 2013 was 55%, which was greater than the summation of the narrow sense heritability (h^2) for all QTLs identified using the 2013 FD data set (Table 14). PostQTL intensity and trace plots were generated and organized in a way to visually compare QTLs identified across all five data sets, genome-wide (Figs. 39-40).

Three major QTLs were identified in this study, showing decisive evidence (BF ≥ 10) to be associated with FW on LG2, LG4, and LG5. The first showed decisive (BF ≥ 10) and positive evidence (BF ≥ 2) to be associated with FW using 2011 FW and 2011-2013 avg FW datasets, and was identified on the end of LG2, thus termed G2FW.1 (Table 13; Figs. 39-40). Using 2011 and 2011-2013 avg FW data sets, G2FW.1 spanned 52.01- 66.60 cM (BFs 31.4 and 2.7) and was flanked by ss_280192 and ss_290277. On average across the two data sets, G2FW.1 explained 36% of the h^2 (Vp %) for FW (ranging from 19-53% V_P). Taking into account both data sets, 31 SNPs (ss_280192 to ss_290277) spanned G2FW.1 (Table 13; Figs. 39-40). The second major QTL showed a BF of 31.2, decisive evidence (BF ≥ 10) to be associated with FW using the 2012 FW data set, and was identified near the middle of LG4, thus termed G4FW.1. Using the 2012 FW data set, G4FW.1 was spanned by 44 SNPs and flanked by ss_416703 and ss_437684 (32.22-43.01 cM). The G4FW.1 QTL explained 28% of the V_P for FW (Table 13; Figs. 39-40). The third major QTL, G5FW.1, showed a BF of 10.0, strong evidence (BF ≥ 10) to be associated

with FW using the 2011 FW data set, and was flanked by ss_588670 and ss_595126 (25.85-31.72 cM) on LG5. The G5FW.1 QTL was associated with 13% of the V_P for FW using the 2011 FW data set.

Additionally, 12 minor QTLs were identified across all eight LGs, which showed at least positive evidence (BF $\geq$ 2) to be associated with FW and or FD (Tables 13-14; Figs. 39-40). Only one minor QTL was identified towards the end of LG1 (termed G1FD.1), flanked by ss_126124 and ss_136096 (108.45-117.12 cM) using the 2013 FD data set. The G1FD.1 QTL was associated with 11% of the V_P for FD using this data set. On the contrary, two minor QTLs were identified and shown to flank each other at the top of LG2 (termed G2FW-FD.1 and G2FD-FW.2) using FD and FW data sets. The first QTL, G2FW-FD.1, was flanked by ss_214703 and ss_224651 (17.24-20.94 cM) when using both 2013 FW and 2013 FD data sets, and on average was associated with 4% of the V_P for FD and FW (ranging from 3-4% V_P). The second, G2FD-FW.2, was identified just upstream of G2FW-FD.1, flanked by ss_140938 and ss_177709 (1.95-8.92 cM) when using both 2012 FW and 2013 FD data sets, and on average was associated with 6% of the V_P for FD and FW (ranging from 4-7% V_P). A third minor QTL was identified on LG2 (termed G2FW.2), using the 2011 FW data set, between G2FW-FD.1 and G2FD-FW.2, flanked by ss_194408 and ss_214703 (11.84-13.68 cM). The G2FW.2 QTL was associated with 2% of the V_P for FW using the 2011 FW data set. Only one minor QTL was identified towards the end of LG3 (termed G3FW.1), flanked by ss_365455 and ss_367359 (51.19-53.89 cM) using the 2011-2013 avg FW data set. The G3FW.1 QTL was associated with 2% of the V_P for FW using this data set. On the contrary, two minor QTLs were identified to flank the major QTL for FW on LG4 (G4FW.1) using the 2011-2013 avg FW data set: the first was upstream of G4FW.1, flanked by ss_385004 and ss_387198 (9.08-11.31 cM) (termed G4FW.2), and the second was

downstream of G4FW.1, flanked by ss_469044 and ss_482587 (57.02-59.45 cM) (termed G4FW.3). The G4FW.2 and G4FW.3 QTLs were associated with 3 and 7% of the V_P for FW using the 2011-2013 avg FW data set. Likewise, two minor QTLs were also identified on LG5 using the 2011 FW and the 2013 FD data sets (termed G5FW.1 and G5FD.1). The G5FD.1 QTL was located just downstream of G5FW.1, flanked by ss_595930 and ss_601173 (32.72-38.65 cM) and was associated with 4% of the V_P for FD using the 2013 FD data set. Only one minor QTL was identified on LG6 (termed G6FW.1), using the 2011 FW data set. The G6FW.1 QTL was flanked by ss_637355 and ss_643414 (26.52-29.74 cM) and was associated with 4% of the V_P for FW, using the 2011 FW data set. On the contrary, two minor QTLs for FD were identified on LG7 (termed G7FD.1 and G7FD.2), using the 2013 FD data set. The G7FD.1 QTL was identified near the top of LG7, flanked by ss_725578 and ss_733833 (10.84-14.12 cM) and G7FD.2 was identified near the bottom of LG7, flanked by ss_778587 and ss_792745 (39.84-56.44 cM). The G7FD.1 QTL was associated with 5% of the V_P for FD and G7FD.2 was associated with 2% of the V_P for FD, using the 2013 FD data set. Lastly, two minor QTLs were identified on LG8 (termed G8FD-FW.1 and G8FD-FW.2), using FD and FW data sets. The first QTL, G8FD-FW.1, was flanked by ss_864805 and ss_885156 (34.78-52.23 cM), and on average was associated with 5% of the V_P for FD and FW using the 2013 FW, 2011-2013 avg FW, and 2013 FD data sets (ranging from 3-8% V_P). The second QTL, G8FD-FW.2, was flanked by ss_834321 and ss_844375 (18.23-22.85 cM), and on average was associated with 5% of the V_P for FD and FW using the 2011-2013 avg FW, and 2013 FD data sets (ranging from 3-7% V_P) (Table 13-14; Figs. 39-40).

Table 13. Fruit weight (FW) QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in only one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected in two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in two or more data sets is represented in bold font.

QTL	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G2FW.1	2011 (FW)	153	2	ss_284124 ss_290277	56.77 66.60	22.71 26.64	31.4	0.73	0.53	0.72
G2FW-FD.2			2	ss_194408 ss_214703	11.84 13.68	4.74 6.89	2.6		0.02	
G5FW.1			5	ss_588670 ss_595126	25.85 31.72	10.34 12.69	14.5		0.13	
G6FW.1			6	ss_637355 ss_643414	26.52 29.74	10.61 11.90	2.3		0.04	
G2FW-FD.2	2012 (FW)	158	2	ss_142214 ss_177709	2.35 8.92	0.94 3.57	3.3	0.49	0.07	0.35
G4FW.1			4	ss_416703 ss_437684	32.23 43.01	12.89 17.20	31.2		0.28	
G2FW-FD.3	2013 (FW)	125	2	ss_217794 ss_224651	18.01 20.94	7.21 8.38	2.0	0.19	0.04	0.08
G8FW-FD.1			8	ss_870337 ss_884078	39.03 50.95	15.61 20.38	2.3		0.04	

Table 13. Fruit weight (FW) QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in only one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected in two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in two or more data sets is represented in bold font. (Cont.).

QTL	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G2FW.1	2011-2013 avg (FW)	171	2	ss_280192 ss_284602	52.01 57.50	20.80 23.00	2.7	0.46	0.19	0.46
G3FW.1			3	ss_365455 ss_367359	51.19 53.89	20.48 21.56	2.8		0.02	
G4FW.2			4	ss_385004 ss_387198	9.08 11.31	3.63 4.52	5.5		0.03	
G4FW.3			4	ss_469044 ss_482587	57.02 59.45	22.81 23.78	3.2		0.07	
G8FW-FD.2			8	ss_834321 ss_844375	18.23 22.85	7.29 9.14	3.0		0.07	
G8FW-FD.1			8	ss_864805 ss_885156	34.78 52.23	13.91 20.89	3.0		0.08	

Table 14. Fruit diameter (FD) QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected across two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in one or more data sets is represented in bold font.

QTL	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G1FD.1	2013	135	1	ss_126124 ss_136096	108.45 117.12	43.38 46.85	2.5	0.55	0.11	0.36
G2FW-FD.2			2	ss_140938 ss_172993	1.95 8.07	0.78 3.23	2.0		0.04	
G2FW-FD.3			2	ss_214703 ss_224651	17.24 20.94	6.89 8.38	2.0		0.03	
G5FD.1			5	ss_595930 ss_601173	32.72 38.65	13.09 15.46	2.9		0.04	
G7FD.1			7	ss_725578 ss_733833	10.84 14.12	4.34 5.65	2.3		0.05	
G7FD.2			7	ss_778587 ss_792745	39.84 56.44	15.94 22.58	2.0		0.02	
G8FW-FD.1			8	ss_881815 ss_885156	47.90 52.23	19.16 20.89	3.4		0.03	
G8FW-FD.2			8	ss_834321 ss_844375	18.23 22.85	7.29 9.14	2.4		0.03	

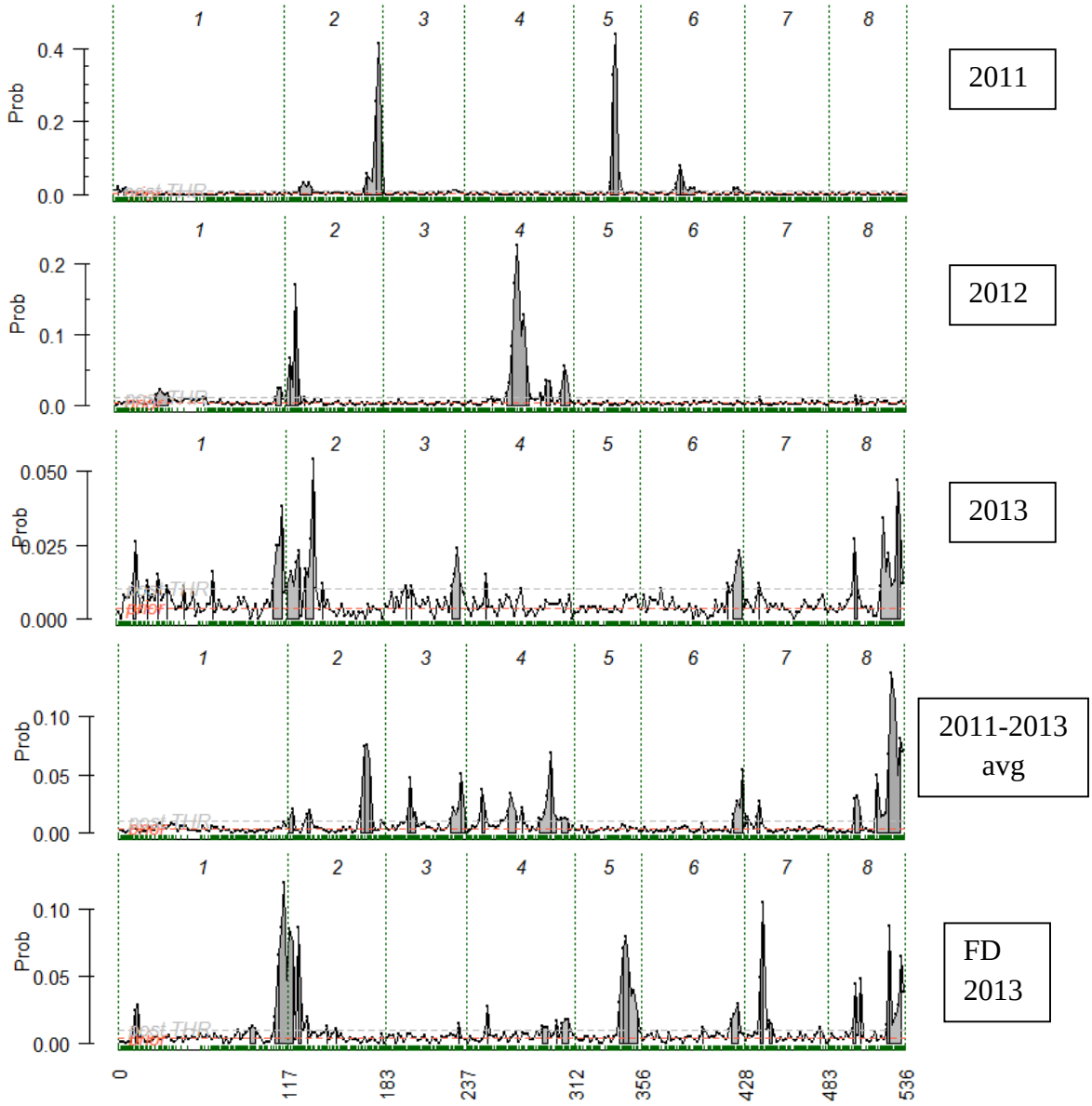

Fig. 39. PostQTL intensity plot positions for fruit weight (FW) [2011, 2012, 2013, and 2011-2013 avg], and fruit diameter (FD) (2013). Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

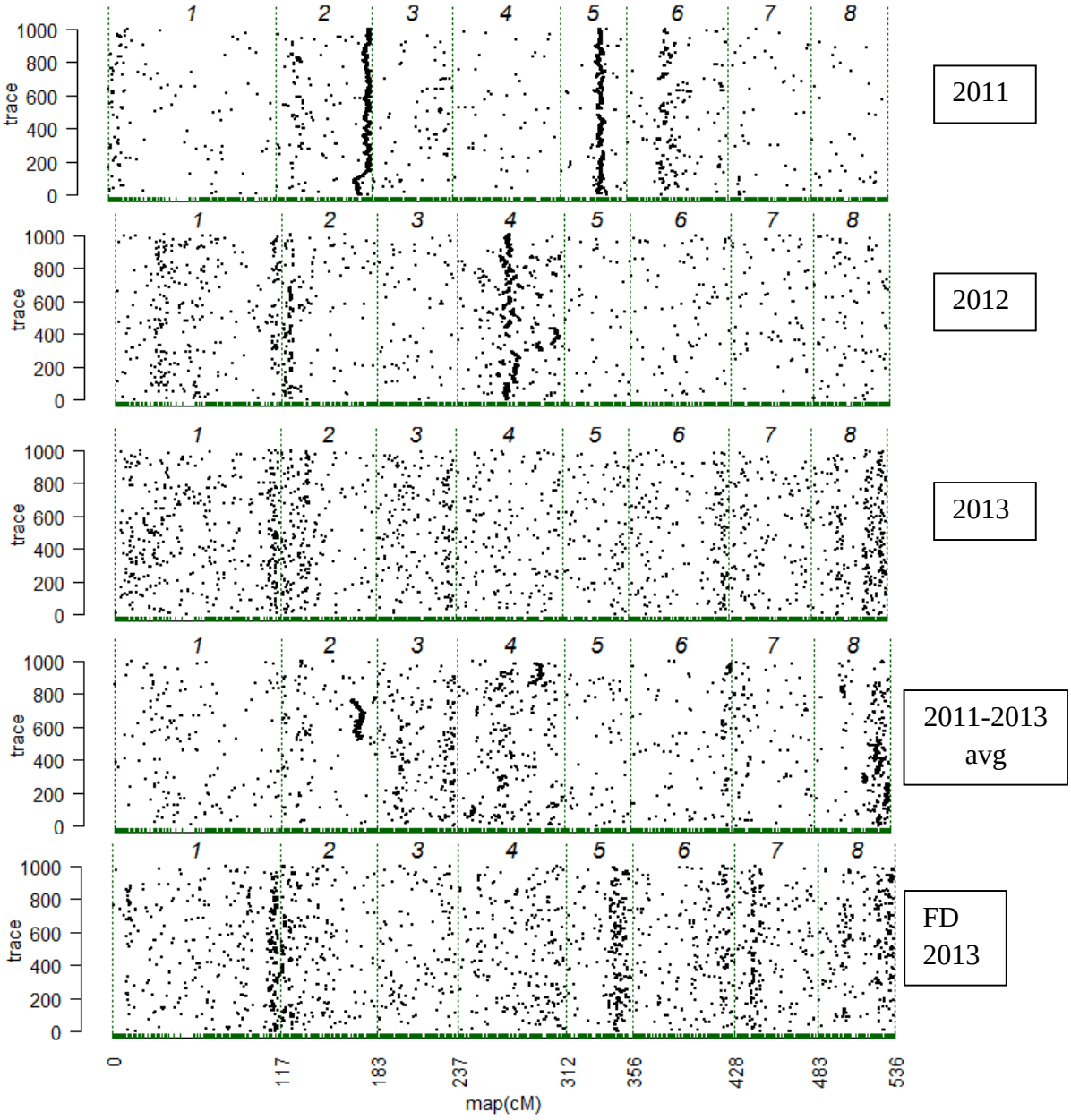

Fig. 40. PostQTL trace plots for fruit weight (FW) [2011, 2012, 2013, and 2011-2013 avg], and fruit diameter (FD) (2013). Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The black dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Soluble Solids Content (SSC)

Through PBA, a total of eight QTLs across five LGs showed at least positive evidence (BF ≥ 2) to be associated with SSC using 2011, 2012, 2013, and 2011-2013 avg data sets (Table 15; Figs. 41-42). The broad-sense heritability (H^2) for SSC ranged from 28-40%, years 2011-2013 avg and 2011, and showed an average of 36% across all data sets. Moreover, the H^2 was always greater than the summation of the narrow sense heritability (h^2) for all QTLs identified using each data set (Table 15). PostQTL intensity and trace plots were generated and organized in a way to visually compare QTLs identified across all five data sets, genome-wide (Figs. 41-42).

One major but broad QTL for SSC was identified on LG4 (25.01-63.89 cM) across two data sets, 2012 and 2011-2013 avg, flanked by ss_407364 and ss_502685. This QTL, termed G4SSC.1, showed a BF of 13.6, decisive evidence for a QTL (BF ≥ 10) in 2012 and explained 34% of the V_P for SSC (Table 15; Figs. 41-42). In 2011-2013 avg, G4SSC.1 showed a BF of 5.2, strong evidence (BF ≥ 5) for a QTL and explained 13% of the V_P for SSC. Overall 94 SNPs spanned G4SSC.1 (Table 15; Figs. 41-42).

Three QTLs were identified which showed at least positive evidence (BF ≥ 2) for a QTL and each explained ≥ 10% of the V_P for SSC in their respective data set (ranging from 11-23% V_P) (Table 15; Figs. 41-42). One QTL was identified on the lower end of LG7 (42.1-48.1 cM) across two data sets, 2011 and 2011-2013 avg. This QTL, termed G7SSC.1, explained 12% of the V_P for SSC in 2011, and 11% in 2011-2013 avg. A second QTL, termed G7SSC.2, was identified on the upper end of LG7, flanked by ss_711315 (3.84 cM) and ss_727662 (12.00 cM) and explained 23% of the V_P for SSC in 2011. A third QTL, termed G2SSC.1, was identified on the upper end of LG2, flanked by ss_136625 (0.6 cM) and ss_151067 (3.9 cM), and explained 19% of the V_P for SSC in 2013 (Table 15; Figs. 41-42).

Lastly, four minor QTLs were identified, which showed at least positive evidence (BF ≥ 2) for a QTL in their respective data set (Table 15; Figs. 41-42). One QTL, termed G2SSC.2, was identified just downstream of G2SSC.1, on the upper end of LG2, flanked by ss_136625 (0.6 cM) and ss_151067 (3.9 cM), using 2011-2013 avg, but only explained 2% of the V_P for SSC. A second minor QTL was identified near the bottom of LG5 flanked by ss_136625 (36.56 cM) and ss_151067 (45.61 cM), across two data sets, 2012 and 2011-2013 avg. This QTL, termed G5SSC.1, explained 3% of the V_P for SSC in 2012, and 5.0% in 2011-2013 avg. Lastly, two QTLs termed G8SSC.1 and G8SSC.2, were identified near the bottom of LG8, flanking each other, across two data sets, 2013 and 2011-2013 avg. The first, G8SSC.1, was flanked by ss_857742 (29.18 cM) and ss_865357 (34.93 cM) and explained 7% of the V_P for SSC in 2013 (Table 15; Figs. 41-42). The second, G8SSC.2, was located just downstream of G8SSC.1, flanked by ss_874263 (42.14 cM) and ss_875343 (42.94 cM) and explained 2% of the V_P for SSC in 2011-2013 avg (Table 15; Figs. 41-42).

Table 15. Soluble solids content (SSC) QTLs identified within UA RosBREED pedigree. QTL name, year of observation, number of individuals (N), linkage group (LG), SNPs flanking the QTL, Bayes Factor (BF), QTL confidence interval (CI) in cM (genetic location) and Mbp (physical location), broad (H^2) and narrow sense heritabilities (h^2), and sum of h^2 for all QTLs each year are presented. "Positive evidence" of QTL (2.0-5.0, BF), that is only detected in only one data set is represented by standard font. "Positive evidence" of a QTL (2.0-5.0, BF), that is detected in two or more data sets is underlined. "Strong evidence" (5.0-10.0, BF), or "decisive evidence" of a QTL (≥10.0, BF) that is detected in two or more data sets is represented in bold font.

QTL name	Year	N	LG	Flanking SNPs	CI (cM)	CI (Mbp)	BF	H^2	h^2	h^2 (sum)
G7SSC.1	2011	153	7	ss_783262 ss_785868	45.47 48.06	18.19 19.22	2	0.40	0.23	0.35
G7SSC.2			7	ss_711315 ss_727662	3.84 12.00	1.53 4.80	2		0.12	
G4SSC.1	2012	152	4	ss_424415 ss_502685	36.56 63.89	14.62 25.56	15.5	0.36	0.33	0.36
G5SSC.1			5	ss_600256 ss_601173	36.56 38.65	14.62 15.46	3.2		0.03	
G2SSC.1	2013	125	2	ss_136625 ss_151067	0.64 3.91	0.26 1.56	6	0.40	0.19	0.27
G8SSC.1			8	ss_857742 ss_865357	29.18 34.93	11.67 13.97	3.9		0.07	
G2SSC.2	Avg	170	2	ss_161939 ss_185608	6.20 10.07	2.48 4.03	2.2	0.28	0.02	0.28
G4SSC.1			4	ss_407364 ss_450711	25.01 50.41	10.01 20.17	5.2		0.12	
G5SSC.1			5	ss_600256 ss_604283	36.56 45.61	14.62 18.24	3.1		0.03	
G7SSC.1			7	ss_781082 ss_786682	42.12 48.73	16.85 19.49	3.6		0.10	
G8SSC.2			8	ss_874263 ss_875343	42.14 42.94	16.86 17.18	2.5		0.02	

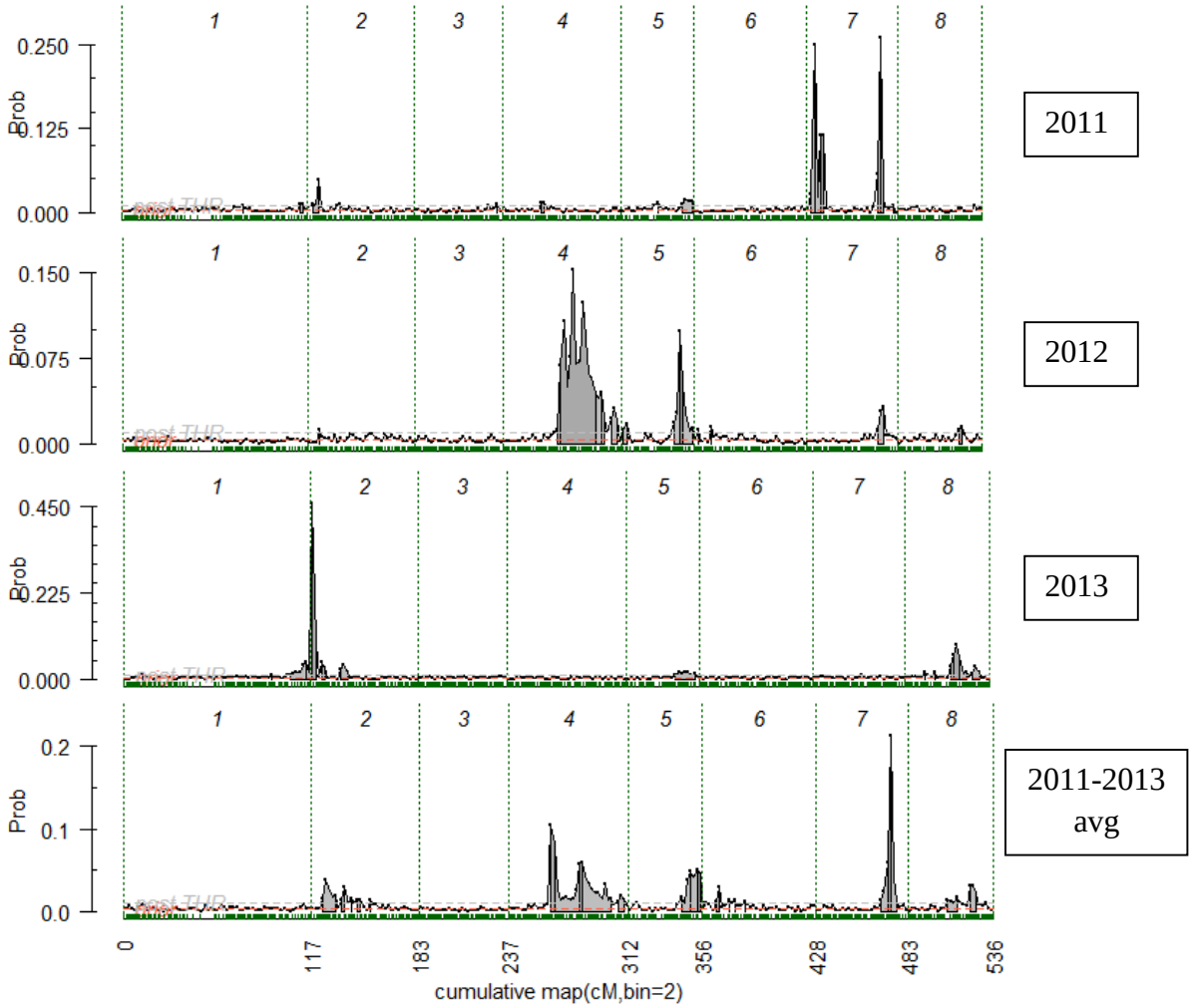

Fig. 41. PostQTL intensity plots positions for soluble solids content (SSC) [2011, 2012, 2013, and 2011-2013 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The filled in gray peaks, represent loci with at least positive evidence (BF ≥ 2) for QTL presence. The y-axis represents the posterior probability (prob) for the locations of each QTL.

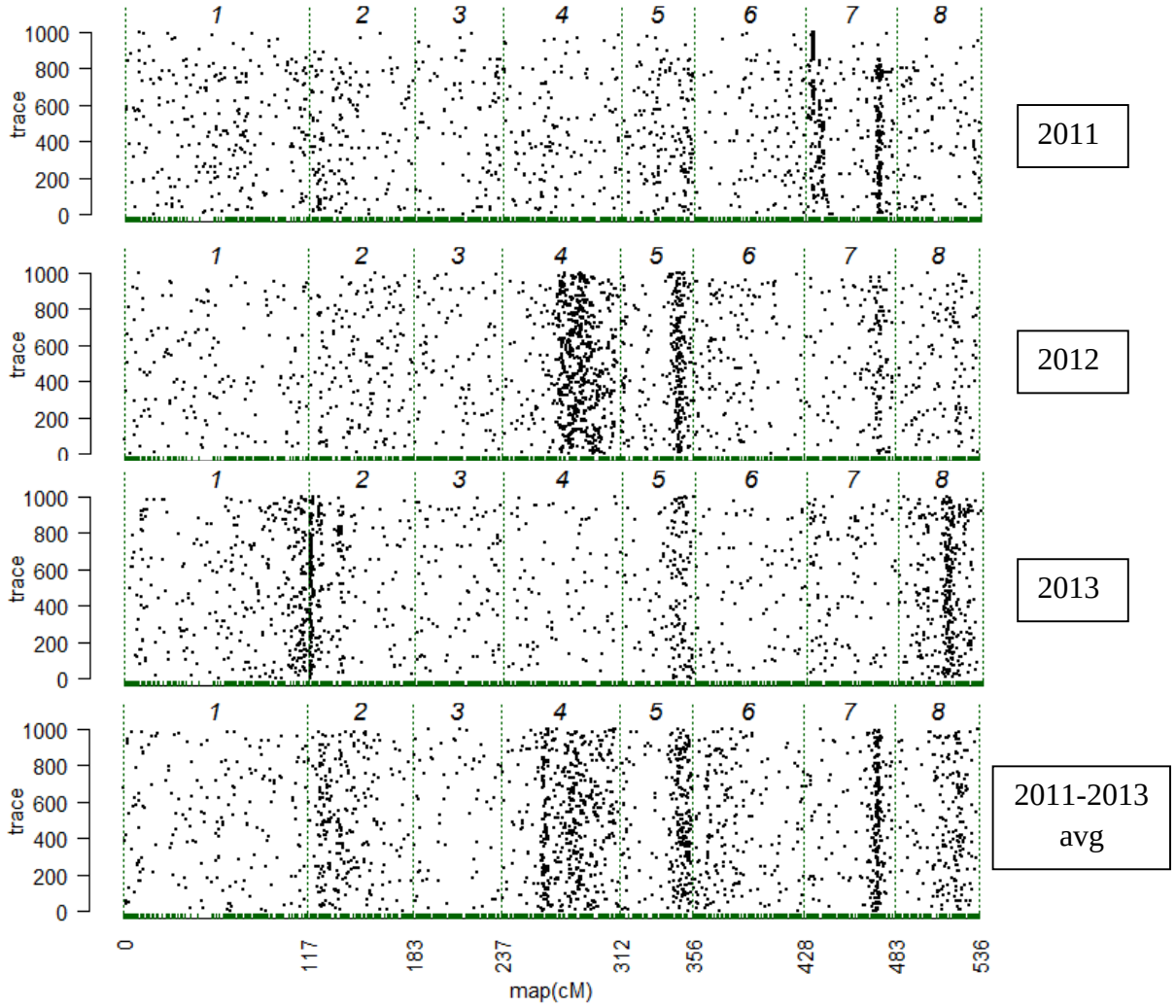

Fig. 42. PostQTL trace plots for soluble solids content (SSC) [2011, 2012, 2013, and 2011-2013 avg]. Vertical dotted green lines separate LG1-LG8 and green dashes on x-axis represent the SNPs which span each LG. The y-axis represents the trace number iteration from 0-1,000. The black dots represent the convergence of 500,000 Markov chain Monte Carlo (MCMC) positions, for the localization of QTLs genome-wide. Stable patterns indicate good mixing of Markov chain, and thus evidence for QTL presence.

Genomic Estimated Breeding Values

Maturity date, blush, fruit diameter, fruit weight, and soluble solids content genomic breeding values (GEBVs) based on all QTLs were obtained from FlexQTL™ (Bink et al., 2014). The GEBVs of each individual in the UA RosBREED pedigree for maturity date, blush, fruit diameter, fruit weight, and soluble solids content in all years (2011-2013) are in Table A.3 in Appendix A. Overall, the GEBVs showed high positive correlations with the observed phenotypes for all traits per year, signifying high accuracy (Table 16). The highest positive correlations were observed for maturity date (0.83-0.93) and the lowest for blush (0.60-0.73). Fruit weight and soluble solids content GEBVs positive correlations ranged from 0.74-0.92, and 0.76-0.83, respectively.

Table 16. Accuracy of genomic estimated breeding value (GEBV) expressed as its correlation (R^2) with the observed phenotype for each data set. The data set with highest positive correlations for each trait are represented in bold font.

Trait	Year	GEBV R^2
Maturity date	**2011**	**0.93**
Maturity date	2012	0.91
Maturity date	2013	0.83
Maturity date	avg	0.90
Blush	2011	0.73
Blush	**2012**	**0.73**
Blush	2013	0.60
Blush	avg	0.71
Fruit weight	**2011**	**0.92**
Fruit weight	2012	0.83
Fruit weight	2013	0.74
Fruit weight	avg	0.80
Fruit diameter	**2013**	**0.84**
Soluble solids content	**2011**	**0.83**
Soluble solids content	2012	0.77
Soluble solids content	2013	0.76
Soluble solids content	avg	0.79

Discussion

In this study, pedigree-based analysis (PBA) under the Bayesian framework was used to identify and validate QTL and SNPs for six peach breeding-relevant fruit quality and phenological traits in the UA RosBREED peach breeding pedigree. The identified QTLs are supported by previous QTL studies in peach since several were subsequently compared with those previously reported in *Prunus*.

Frequently, major and minor QTLs for the quantitative traits were only found in one or two years of the study and not across all three. The temperature and rainfall weather data collected at FRS in 2011, 2012, and 2013 showed that the temperature and rainfall varied among all years, especially when comparing 2011 and 2012 to 2013. The first two years (2011 and 2012) were very hot and dry compared to the mild and wet 2013. Complex traits such as fruit quality and phenological traits are greatly influenced by the environment, and these very contrasting environments (years) could have hampered the ability to identify the same QTL across all years. Additionally, the overall health of many of the F_1 populations was lower in 2013 (as well as total number of seedlings able to phenotype) in comparison to 2011 and 2012 (since these populations have been in the field on their own roots since 2008), which hampered precise QTL calculations using most 2013 data sets (FW, FD, SSC).

Maturity Date (MD)

One major QTL (G4MD.1) and seven minor QTLs (G1MD.1, G2MD.1, G3MD.1, G5MD.1, G6MD.1, G6MD.2, and G8MD.1) were detected for maturity date (MD) in this study, indicating polygenic nature of MD inheritance. The polygenic nature of MD inheritance is supported by previous reports which deemed MD in peach as a highly quantitative trait (Eduardo et al., 2011; Fresnedo-Ramírez et al., 2015; Pirona et al., 2013). Furthermore, the GEBVs

showed high positive correlations with the observed MD across all years (R^2 of 0.93 in 2011, 0.91 in 2012, 0.83 in 2013, and 0.90 in 2011-2013 avg) which indicates high accuracy and major contribution from additive genetic effects for MD. These GEBVs can now be used as a quantitative scale for the decision of selections/cultivars to use as parents in crossing (to pass on their additive genetic effects to progeny for MD), and/or to advance in the UA breeding program.

The major QTL, G4MD.1, showed decisive evidence (BF ≥ 10) and explained on avg 61% of the phenotypic variation (V_p) for MD across all four data sets (2011, 2012, 2013, and 2011-2013 avg). The G4MD.1 was located through PBA of the UA RosBREED pedigree to 10.7-11.3 Mbp on LG4. Pirona et al. (2013) used two F_2 populations to fine map a previously identified QTL for MD (qMD4.1) (Eduardo et al., 2011) to ~11.0-11.2 Mb on LG4. Moreover, they identified a candidate gene for the control of MD within this G4mat locus, ppa008301m. They concluded that this candidate gene could be of use in MAS if validated on more diverse material than just two segregating F_2 populations. In the study herein, PBA of the diverse UA RosBREED breeding pedigree identified G4MD.1 to nearly the same region on LG4, as Pirona et al. (2013) (10.7-11.3 vs. 11.0-11.2 Mbp). These results provide more evidence from more diverse material that this is the major locus controlling maturity date in peach, and furthermore, that ppa008301m is likely the candidate gene.

Additionally, seven minor QTLs were identified which showed positive evidence (BF ≥ 2) to be associated with MD on LG1 (G1MD.1), LG2 (G2MD.1), LG3 (G3MD.1), LG5 (G5MD.1), LG6 (G6MD.1 and G6MD.2), and LG8 (G8MD.1), further supporting the theory that MD is highly quantitative in nature (Eduardo et al., 2011; Fresnedo-Ramírez et al., 2015; Pirona et al., 2013). These findings are in agreement with several minor QTLs for MD identified in previous studies on: LG1, LG2, LG3, LG4, LG5, LG6, LG7, and LG8 (Eduardo et al. 2011;

Etienne et al., 2002; Fan et al., 2010; Fresnedo-Ramírez et al., 2015; Quarta et al., 2000 and 2001; Quilot et al., 2004; Yamamoto et al., 2001). While the locations of all these minor QTLs could be compared to the previously identified minor QTLs, to determine if they overlap, the main goal of this study was only to validate the major QTL on chromosome 4, G4MD.1, for incorporation of MAS for this major QTL. This said, it will be important for geneticists to continue to try and characterize additional minor QTLs and candidate genes to strive to understand the entire genetic pathway controlling MD for the future application of genomic selection for this and other traits.

During the last decade, one aim of the UA peach and nectarine breeding program has been to create peaches and nectarines pleasant to eat to satisfy a wide array of consumer palates across the entire season. Maturity date is an important trait for season extension, which enables Arkansas farmers to grow and sell peaches longer; therefore, MD is a breeding-relevant trait for extension of the UA program season, as well as other breeding programs. In this study, the four SNP markers which span G4MD.1 have been validated for use together as a 4-SNP haplotype DNA test for MAS across the four RosBREED demonstration peach breeding programs (see Chapter Four). In terms of just the UA program, the G4MD.1 SNP haplotype was vital to determining that the breeder can still extend their season by at least a few weeks, since very few late (L) and very late (VL) alleles are found within the program, and only one selection is homozygous for VL [A-789 (VL1 | VL2)]. Thus, in 2013-2015 MAPS and MACS was performed at the UA program using the results from the G4MD.1 SNP DNA test, coupled with the CPPCT040b-SSR, EndoPG1+6, indelG, PpCCD4b-SSR, Ppe-R_f-SSR, Ppe-XapF6-SSR, and 4-SNP *G1XapF* haplotype DNA tests with the goal of extending and diversifying a later part of the UA peach season in an efficient manner (late peach and nectarines with both melting, non-

melting, and non-softening flesh types, white and yellow flesh, high and low acid, high blush and resistance to fruit bacterial spot) (see Chapter Six for more details). While the G4MD.1 SNP DNA test works well, there still is the possibility of recombination occurring within the haplotype and leading to false positives. Thus, work should continue to be done to fully characterize the candidate gene for MD, ppa008301m (Pirona et al., 2013), to develop an even more precise intragenic DNA test.

G and Y Loci (Pubescent vs. Glabrous and White vs. Yellow Flesh)

Through PBA, only the pubescence (Pub) 2011-2013 avg and the flesh color (FC) 2011-2013 avg data sets successfully converged. These avg data sets contained more individuals phenotyped and genotyped (N=172 and 174, respectively), than the separate-year data sets (2011, 2012, and 2013), which could be why only the avg data sets converged. Another reason for the difficulty in convergence is due to the fact that FlexQTL PBA was designed to analyze quantitative traits (Bink, 2004 and 2005; Bink et al., 2008 and 2012). Since both Pub and FC are qualitative traits, it was more difficult for the program to accurately analyze them. This, in turn, lead to the identification of multiple false positive QTLs for both traits, yet nonetheless, previously identified major loci for Pub (G-locus) and FC (Y-locus) were identified using Pub 2011-2013 avg and FC 2011-2013 avg data sets.

In terms of Pub, four QTLs showed decisive evidence (BF $\geq$ 10) to be associated with Pub, using the Pub 2011-2013 avg data set. The first three QTLs, G1Pub.1, G3Pub.1, and G3Pub.2 were determined to be false positive QTLs since they did not localize to any previously identified loci. The fourth QTL, G5Pub.1, was associated with ~45% of the V_P for Pub (using the Pub 2011-2013 avg data set) and was identified on chromosome 5 from 13.24-16.56 Mbp, which

is in agreement with the fine mapped location of the G-locus in Vendramin et al. (2014) (15.85 - 16.49 Mbp).

These results are promising, however, since recently the R2R3-MYB gene *PpeMYB25*, at the G-locus, was proposed as the candidate gene for trichome formation on peach fruit skin (Vendramin et al., 2014). Later, a functional marker (indelG) on a long terminal repeat (LTR) retrotransposon inside exon 3 of *PpeMYB25* was identified as the putative cause of the loss-of-function mutation underlying the glabrous phenotype (Vendramin et al., 2014). This group screened the indelG on the F_2 C × A population, made up of 305 seedlings, as well as a panel of 95 cultivars, and determined the test was 100% predictive across all the material (Vendramin et al., 2014). Furthermore, in Chapter Five, Section Two of this dissertation, the indelG DNA test was 100% predictive across the UA RosBREED "conversion set," made up of 243 individuals [seven UA F_1 populations (138 total seedlings), and 105 UA cultivars and selections], as well as the "confirmation set," made up of 613 seedlings from 22 F_1 populations (ranging from 5-59 seedlings per population), and 69 additional UA selections and an array of 58 cultivars from other U.S breeding programs. This is a very diverse panel of individuals from the UA program and other breeding programs, and thus, provides additional evidence that *PpeMYB25* is indeed the candidate gene for Pub across a vast majority of peach material. The indelG DNA was subsequently incorporated into MAPS and MACS in 2013-2015 at the UA program, as well as for MASS in 2015 across 235 individuals from five F_1 populations ranging from 20-122 individuals (see Chapter Six).

In terms of white vs. yellow flesh, two QTLs showed decisive evidence (BF ≥ 10) to be associated with FC, using the FC 2011-2013 avg data set. The first QTL identified, G1FC.1, was determined to be a false positive QTL since it did not localize to any previously identified loci.

The second QTL, G1FC.2, was a major QTL (associated with 83% V_P for FC) identified near the bottom of LG5 (19.31-26.10 Mbp), which co-localizes with the recently fine-mapped region of the Y-locus (~23.35-27.37 Mbp) (Martínez-García et al., 2013; Verde et al., 2013).

As before with Pub, while these results are promising, recently, the candidate gene for FC at the Y-locus, carotenoid cleavage dioxygenase four (*PpCCD4*), has been identified using 37 peach cultivars (Brandi et al., 2011; Falchi et al., 2013). Furthermore, in Chapter Five, Section Three of this dissertation the intragenic PpCCD4b-SSR DNA test was 100% predictive across the seven UA RosBREED populations ("conversion set"), all 22 of the 2010 populations ("confirmation set"), and an array of cultivars from other U.S breeding programs. These results confirm PpCCD4b-SSR DNA test's predictiveness across a vast majority of the UA peach breeding program, thus subsequently this marker was incorporated into MAPS and MACS in 2013-2015 at the UA program, as well as for MASS in 2015 across 235 individuals from five populations ranging from 20-122 individuals (see Chapter Six).

Blush Overcolor

One major QTL (G3BL.1) and two minor QTLs (G2BL.1 and G4BL.1) for blush overcolor in peach were detected in this study indicating polygenic nature of blush inheritance, which supports previous reports suggesting blush in peach is a quantitative trait (Cantín et al. 2010; Frett et al., 2014; Quilot et al. 2004; Ogundiwin et al., 2007, 2008, 2009; Sandefur et al., 2016a). Unfortunately, the GEBVs only showed slightly positive correlations with the observed blush ratings across all years (R^2 of 0.73 in 2011, 0.73 in 2012, 0.60 in 2013, and 0.71 in 2011-2013 avg) which indicated environmental factors highly affected the development of blush over color. These results support previous findings that environmental factors (light throughout the canopy) influence the development of blush overcolor (Layne et al., 2001). The overall V_p of

blush is controlled by genetic factors, environmental factors (light throughout the canopy), as well as a genotype–environment interaction, and together, these three factors regulate highly conserved flavonoid and anthocyanin biochemical pathways (Frett et al., 2014; Schijlen et al., 2004). Since these GEBVs are low, it is not advised to incorporate them as a quantitative scale in the breeding decisions in the UA breeding program. Summer prunning was not performed at FRS, which did not allow adequate light to consistently penetrate through the canopy to the fruit. This ultimetly could have been the major reason why H^2 (avg 28% 2011-2013; low of 13% in 2013), h^2, and GEBVs R^2 were so low in this study in comparison to others such as Frett et al., (2014), where H^2 estimates were very high for all data sets, >0.99.

Nonetheless, the major QTL, G3BL.1, showed strong (BF ≥ 5) or decisive evidence (BF ≥ 10) and explained on avg 33% of the V_p for blush across three data sets (2011, 2012 and 2011-2013 avg) [The G3BL.1 identified in 2013 was not included, in this estimate, since the correlation between its GEBVs and the observed blush ratings in 2013 were considerably lower than all other years (R^2 of 0.60)]. Across 2011, 2012, and 2011-2013 avg, G3BL.1 was located to 12.32-14.02 Mbp on LG3. Recently, Frett et al. (2014) described a major QTL, termed *Blush.Pp.ZC-3.1*, located on LG3 (4.82-13.89 Mbp) which on average explained 64% of blush V_p in an F_2 population of 'Zin Dai' × 'Crimson Lady' (Z×CL). The G3BL.1 in the study herein overlaps with *Blush.Pp.ZC-3.1*, and additionally, narrowed the region of the blush locus to 12.32-14.02 Mbp. Furthermore, the candidate gene for red skin pigmentation (blush), *PprMYB10* (Lin-Wang et al. 2010), was located within both QTL regions (~12.841-12.842 Mbp), and more importantly near the peak of G3BL.1 from this study herein (Frett et al., 2014). These are promising results, which provide further evidence, across more diverse material, that *PprMYB10* is the candidate gene for blush. Additionally, recently three DNA tests were developed which

were tightly linked to the *PprMYB10*: a CAPS marker, CAPS_341962 (~5.5 kbp upstream) (Frett et al., 2014), a 5-SNP test (~700 kbp spanning and flanking) (Sandefur et al., 2016a), and a Ppe-Rf-SSR DNA test (located 76 kbp upstream) (Sandefur et al., 2016a). While all three DNA tests are now available for peach breeders to use, the predictiveness of the Ppe-Rf-SSR and the 5-SNP haplotype DNA tests were confirmed on 200 individuals representative of the North American peach breeding germplasm as well as more than 400 seedlings from 18 cross combinations of 28 important parents of the UA peach breeding program (Sandefur et al., 2016a). In comparison, the predictiveness of CAPS_341962 has only been confirmed on the Z×CL F_2 population and 69 peach cultivars. Additional limitations to running CAPS_341962 in comparison to the Ppe-Rf-SSR and the 5-SNP haplotype DNA tests is described in detail in Sandefur et al. (2016a).

Further, two minor QTLs showed positive evidence (BF ≥ 2) to be associated with BL, on LG2 (G2BL.1) and LG4 (G4BL.1). The G2BL.1 spanned 13.51-14.44 Mbp, and was associated with an average of ~4% of the V_P for blush across three data sets. No other studies up to this point have identified a QTL for blush on chromosome 2, indicating that the diverse pedigree background used for PBA was needed to uncover this minor locus. Next, using the 2013 data set, a second minor QTL was identified, G4BL.1, which spanned 7.54-8.83 Mbp on chromosome 4, and was associated with an average of ~4% of the V_P for BL. Previously, using the Z×CL F_2 population, Frett et al. (2014) also identified minor QTLs for blush on chromosome 4: Blush.Pp.ZC-4.1 (2.34-3.97 Mbp; 13% of V_P for blush) and Blush.Pp.ZC-4.2 (4.31-5.23 Mbp; 14% of V_P for blush). These three QTLs on chromosome 4 are located near each other. However, unfortunately none of them overlap. If they overlapped, they could potentially be associated with the same candidate structural gene(s) involved in the anthocyanin pathway. As of now the moment it appears each QTL is associated with a different loci, and thus different structural

gene(s) involved in the anthocyanin pathway. There are multiple structural genes in the anthocyanin pathway, thus supporting this hypothesis (Frett et al., 2014; Lin-Wang et al., 2010).

Fruit Size (Diameter and Weight)

Three major QTLs (G2FW.1, G4FW.1, and G5FW.1) and 12 minor QTLs (across all eight eight LGs) for FW and FD were identified in this study indicating the polygenic nature of FW and FD inheritance, which supports previous reports suggesting FW and FD in peach are very quantitative in nature (De Franceschi et al. 2013; Dirlewanger et al., 2006; Fresnedo-Ramírez et al., 2015 and 2016; Quilot et al., 2004). The GEBVs showed a high positive correlation with the observed FW or FD percentage across most years, the highest being in 2011 (FW R^2 of 0.92), 2013 (FD R^2 of 0.84), and 2012 (FW R^2 of 0.83), followed by 2011-2013 avg (FW R^2 of 0.80), and 2013 (FW R^2 of 0.74). These values indicate moderately high accuracy, and moderately high contribution from additive genetic effects for FW and FD. These GEBVs could potentially be used as a quantitative scale for breeding decision support of selections/cultivars to use as parents in crossing (to pass on their additive genetic effects to progeny for SSC), and/or to advance in the UA breeding program. Fruit thinning was not performed at the appropriate time period in 2013 (was done later than optimum), which could be a major reason why H^2 (19% in comparison to 73% in 2011), h^2, and GEBVs R^2 (0.74) were so low in this year, in comparison to 2011 and 2012.

The three major QTLs for FW identified in this study each showed decisive evidence (BF $\geq$ 10) to be associated with FW on LG2, LG4, and LG5. The first, G2FW.1, showed decisive (BF $\geq$ 10) and positive evidence (BF $\geq$ 2) to be associated with FW using 2011 FW, and 2011-2013 avg FW datasets, and explained on avg 36% of the h^2 (Vp %) for FW, and was identified on the lower end of chromosome 2 (20.80-26.64 Mbp). A major QTL for FW was also identified on

LG2 by Fresnedo-Ramírez et al., (2015 and 2016) for peach and Rosyara et al. (2013) for cherry. Neither publications QTLs co-located with G2FW.1 and instead were identified further upstream in the middle of chromosome 2. In Fresnedo-Ramírez et al. (2015 and 2016) and Rosyara et al. (2013), the major QTLs for FW on LG2 co-located near the middle, between markers CPSCT038 (15.06 Mbp) and BPPCT034 (16.49 Mbp) (Rosyara et al., 2013), and between markers ss_219973 (14.81 Mbp) and ss_244929 (16.92 Mbp) (Fresnedo-Ramírez et al., 2015 and 2016). A recent study by De Franceschi et al. (2013) identified 23 cell number regulator (*CNR*) candidate genes in the peach genome sequence, at least one CNR on each of the eight peach chromosomes. Four CNRs were identified on chromosome 2 (PpCNR12, PpCNR13, PpCNR14, and PpCNR15). The PpCNR12 candidate gene was located within the QTLs identified by Fresnedo-Ramírez et al. (2015 and 2016), and Rosyara et al. (2013) at ~15.65 Mbp. Conversely, PpCNR13, PpCNR14, and PpCNR15 were all co-located within G2FW.1 of this study, at ~ 23.21, 23.38, and 24.21 Mbp, respectively (De Franceschi et al. 2013). Therefore, although G2FW.1 did not co-locate with those QTLs identified in Fresnedo-Ramírez et al., (2015 and 2016) and Rosyara et al. (2013), these findings of CNR candidate genes adds support to both loci being major controllers of FW in peach.

The second and third major QTLs, G4FW.1, G5FW.1, also showed decisive evidence (BF $\geq$ 10) to be associated with FW. The G4FW.1 was identified using the 2012 FW data set, explained 28% of the V_P for FW, and was identified near the middle of chromosome 4 (12.89-17.20 Mbp). The G5FW.1 was identified using the 2011 FW data set, explained 13% of the V_P for FW, and was identified near the middle of chromosome 5 (10.34-12.69 Mbp). Two QTL for FW were previously identified on LG4 and LG5 in Fresnedo-Ramírez et al., (2016) [qFW.5 (3.66-8.70 Mbp)] and Fresnedo-Ramírez et al., (2015) [qFW.4 (5.40-9.00 Mbp)]. While G4FW.1

and G5FW.1 were located near these QTLs identified in Fresnedo-Ramírez et al., (2016) neither co-localized with them. The reason for this could be due to the fact that Fresnedo-Ramírez et al., (2015) performed PBA using the diverse processing peach breeding program at UC Davis, which uses directed introgression from different species such as almond [*Prunus dulcis* (Mill.) D.A.Webb], *P. argentea* (Lam.) Rehder, *P. davidiana* (Carrière) Franch, and *P. mira* Koehne (Fresnedo-Ramírez et al., 2015; Gradziel 2002 and 2003). Fresnedo-Ramírez et al., (2015) discussed that the information content of their marker set was limited due to germplasm introgression from related species. During the development and validation of the IPSC peach 9K SNP array (Verde et al. 2012), very few almond and interspecific hybrids were included, which ultimately resulted in missing data because several SNPs did not amplify in almond/interspecific hybrids. This resulted in less distinct identity by descent (IBD) probabilities, due to the difficulty in accurately tracing SNP alleles from founder to F_1/F_2 progeny (Fresnedo-Ramírez et al., 2015). Likewise, Fresnedo-Ramírez et al. (2016) performed PBA using a broad pedigree tracing back to the landmark cultivar 'Orange Cling' (syn. 'Orange Clingstone'), which spanned four public breeding programs: Clemson University, the University of Arkansas (UA), the University of California (UC) at Davis, and Texas A&M University. The introgression lines from UC Davis, as well as the fact that only one lineage was studied, could have led to this group identifying qFW.5 in a different location on LG5 than G5FW.1 identified in our study (Fresnedo-Ramírez et al., 2016).

Lastly, 13 minor QTLs which showed positive evidence (BF $\geq$ 2) to be associated with FW and FD were identified across all eight LGs, further supporting the theory that FW and FD are highly quantitative in nature (De Franceschi et al. 2013; Dirlewanger et al., 2006; Fresnedo-Ramírez et al., 2015 and 2016; Quilot et al., 2004). These findings are in agreement with several

minor QTLs for FW and FD identified in previous studies on all eight LGs (Eduardo et al. 2011; Etienne et al., 2002; Fresnedo-Ramírez et al., 2015 and 2016; Quilot et al., 2004). As with MD, while the locations of all these minor QTLs could be compared to the previously identified minor QTLs, to determine if they overlap, the main goal of this study was to identify and validate major QTLs for incorporation of MAS.

Soluble Solids Content (SSC)

Two major QTLs (G4SSC.1 and G7SSC.1) and six minor QTLs (G7SSC.2, G2SSC.1, G2SSC.2, G5SSC.1, G8SSC.1 and G8SSC.2) for SSC in peach were detected in this study indicating the polygenic nature of SSC inheritance, which supports previous reports suggesting SSC in peach is a quantitative trait (Dirlewanger et al., 2006; and Quilot et al., 2004). The GEBVs showed a moderately high positive correlation with the observed SSC across most years, the highest being in 2011 (R^2 of 0.83), and 2011-2013 avg (R^2 of 0.79), followed by 2012 (R^2 of 0.77) and 2013 (R^2 of 0.76), which indicates moderately high accuracy, and moderately high contribution from additive genetic effects for SSC. These GEBVs could potentially be used as a quantitative scale for breeding decision support of selections/cultivars to use as parents in crossing (to pass on their additive genetic effects to progeny for SSC), and/or to advance in the UA breeding program.

The major QTL on LG4, G4SSC.1, showed strong (BF ≥ 5) or decisive evidence (BF ≥ 10), explained on avg 23% of the V_p for maturity date across two data sets (2012 and 2011-2013 avg), and was located near the middle region of LG4 (25.01-63.89 cM). A previous bi-parental QTL study also identified a major QTL culter for SSC as well as three sugars (fructose, glucose, and sorbitol) on LG4 (Dirlewanger et al., 2006). Using an F_2 population [‘Ferjalou Jalousia’® × ‘Fantasia’ (J × F)], Dirlewanger et al. (2006) identified a region on LG4 of the J × F map which

showed a cluster of QLTs for both SSC as well as three sugars (fructose, glucose, and sorbitol). All QTLs were clustered within UDP97402-BPPCT035. Unfortunately, UDP97402 has not been located in the peach genome sequence v1.0. However, BPPCT035 and another marker, BPPCT023, which is ~ half way between UDP97402-BPPCT035 have been located in the peach genome sequence v1.0. The BPPCT023 marker is located at ~14.73 Mbp, and the BPPCT035 marker at ~19.15 Mbp on chromosome 4. Based on these results, it's apparent that the major QTL for SSC in this study, G4SSC.1, overlaps with this previous cluster of QTLs for SSC and three sugars identified in Dirlewanger et al. (2006). At the moment, the QTL cluster for SSC and sugars in Dirlewanger et al. (2006) appears to extend further upstream than G4SSC.1, and G4SSC.1 has been shown to extend further downstream. These differences could be due to the fact that Dirlewanger et al. (2006) only used a single bi-parental population, and a very low density linkage map, in comparison to the PBA approach and high density linkage map used herein. Nonetheless, both QTLs overlap, making this a major locus for SSC, which could next be fine mapped to narrow down the locus, followed by candidate gene mapping to characterize the actual gene(s) controlling this QTL region.

Additionally, two QTLs, which showed positive evidence (BF $\geq$ 2) to be associated with SSC, were identified on LG7 and associated with $\geq$ 10% of the V_P for SSC in their respective data set. The first, G7SSC.1, was identified on the lower end of LG7 (42.1-48.1 cM) across two data sets, 2011 and 2011-2013 avg, and explained on avg 12% of the V_P for SSC across both data sets. Using an advanced backcross progeny, derived from an interspecific cross between (*Prunus persica* × *P. davidiana*), Quilot et al. (2004) also identified a cluster of QTLs for sucrose, glucose, and fructose at the bottom of their LG7, between markers pchcms2 (43 cM) and CC132 (48 cM). Unfortunately, neither of these markers have been located in the peach

genome sequence v1.0, thus, direct comparisons on the chromosomal position of the QTLs identified in Quilot et al. (2004) cannot be made to G7SSC.1 in the study herein. The second QTL identified on LG7, G7SSC.2, was identified on the upper end of LG7, flanked by ss_711315 (3.84 cM) and ss_727662 (12.00 cM) and explained 23% of the V_P for SSC in 2011. In a previous PBA study, Fresnedo-Ramírez et al. (2015) also identified a QTL with strong evidence for SSC (qSSC.7), which co-locates with G7SSC.2, flanked by ss_708371 (1.13) and ss_752524 (8.34). While these overlapping QTLs on top of LG7 are promising, unfortunately, they were only able to be identified in one year, 2011 (Fresnedo-Ramírez et al., 2015). Nonetheless, the two QTLs on LG7 in the study herein, G7SSC.1 and G7SSC.2, are prime candidates to be investigated further through fine mapping to narrow down the locus, followed by candidate gene mapping to characterize the actual gene/s controlling this QTL region.

Lastly, four minor QTLs were identified which showed positive evidence (BF $\geq$ 2) to be associated with SSC on LG2 (G2SSC.1 and G2SSC.2), LG5 (G5SSC.1), and LG8 (G8SSC.1 and G8SSC.2), further supporting the theory that SSC is highly quantitative in nature (Dirlewanger et al., 2006; Quilot et al., 2004). These findings are in agreement with several minor QTLs for SSC identified in previous studies on all eight LGs (Eduardo et al. 2011; Etienne et al., 2002; Fresnedo-Ramírez et al., 2015; Quilot et al., 2004). As with MD and FW, while the locations of all these minor QTLs could be compared to the previously identified minor QTLs to determine if they overlap, the main goal of this study was to identify and validate major QTLs for incorporation of MAS for these major QTLs.

Lastly, while many of the QTLs identified herein co-located or were near regions previously reported, SSC using refractometry still is not as precise of a method to quantify all sugars as most researchers assume (K. Gasic, personal communication). In peach, SSC is known

to only be associated with ~80% of the total sugars in a sample. Fortunately a superior method, handheld nondestructive nearinfrared (NIR) spectroscopy, is available for future studies, to predict SSC and individual and combined concentrations of sucrose, glucose, and fructose.

Future Work - SNP Data Set and Haplotype Construction

For each major QTL identified for SSC (G4SSC.1, G7SSC.1, and G7SSC.2, G2SSC.1), and fruit size (G2FW.1, G4FW.1, G5FW.1), a set of SNPs spanning a historically non-recombining QTL haploblock among all cultivars and ancestors included in the combined RosBREED demonstration program pedigree (Clemson University, UA, the University of California at Davis, and Texas A&M University) can next be used for haplotype construction. These haplotypes can be initially constructed using FlexQTL™ software (Bink 2004 and 2005; Bink et al., 2008 and 2012), and subsequently confirmed based on inheritance and segregation in bi-parental populations. Functional haplotypes can then be determined for each unique SNP haplotype sequence after establishment of haplotype effects by comparing haplotypes with the phenotypic trait of interest. All RosBREED material genotyped with the IPSC 9K peach SNP array v1.0 can be used to validate the alleles associated with the trait of interest. Validated markers can then also be incorporated into MAS in the UA peach breeding program, as well as the other three RosBREED demonstration programs.

This PBA QTL analysis approach, followed by SNP data set and haplotype construction, effectively creates a flexible and continuously expanding platform for QTL and marker identification, validation, and use of MAS in the UA peach and nectarine breeding program. All future studies analyzing new populations for the same and/or additional traits can be added to the UA pedigree for subsequent PBA. Additionally, the GEBVs generated through posteriori PBA results can be used as a quantitative scale for decision support on which selections/cultivars to

use as parents in cross (to pass on their additive genetic effects to progeny for each trait of interest), as well as which to advance in the UA breeding program.

Literature Cited

Aranzana M.J., A. Pineda, P. Cosson, E. Dirlewanger, J. Ascasibar, G. Cipriani, C.D. Ryder, R. Testolin, A. Abbott, G.J. King, A.F. Iezzoni, and P. Arus. 2003 A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theor. Appl. Genet. 106:819-825.

Abbott, A.G., A.C. Lecouls, Y. Wang, L. Georgi, R. Scorza, and G. Reighard. 2002. Peach: The model genome for Rosaceae genomics. Acta Hort. 592:199-209.

Bassi, D., and R. Monet. 2008. Botany and Taxonomy. p. 1-30. In: D.R. Layne and D. Bassi (eds.). The peach, botany, production and uses. CAB International Press, Wallingford, Oxon, UK. 850.

Bink, M. 2004. FlexQTL software. BIOMETRIS, Wageningen UR, The Netherlands <www.flexqtl.nl≥.

Bink, M. 2005. FlexQTL software: efficient estimation of identity by descent probabilities and QTL mapping in pedigreed populations. In: Plant and Animal Genomes XII Conference, 15-19 January, San Diego, CA, U.S.

Bink, M.C.A.M., M. Boer, L. Totir, C. Winkler, and O. 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., 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. Fernández-Fernández, 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., 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.

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

Brandi, F., E. Bar, F. Mourgues, G. Horváth, E. Turcsi, G. Giuliano, A. Liverani, S. Tartarini, E. Lewinsohn, and C. Rosati. 2011. Study of 'Redhaven' peach and its white-fleshed mutant suggests a key role of CCD4 carotenoid dioxygenase in carotenoid and norisoprenoid volatile metabolism. BMC Plant Biol. 11: 24-38.

Byrne, D., M. Bassols, D. Bassi, M. Piagnani, K. Gasic, G. Reighard, M. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: M. Badenes and D. Byrne (eds.). Fruit breeding. Springer Science, Business Media, New York.

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

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 intraspecific peach [*Prunus persica* (L.) Bastch] progeny. Postharvest Biol. Technol. 58:79–87.

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.

De Franceschi, P., T. Stegmeir, A. Cabrera, E. van der Knaap, U.R. Rosyara, A.M. Sebolt, L. Dondini, E. Dirlewanger, J. Quero-Garcia, J.A. Campoy, and A.F. Iezzoni. 2013. Cell number regulator genes in *Prunus* provide candidate genes for the control of fruit size in sweet and sour cherry. Mol. Breed. 32:311-326.

Dirlewanger, E., P. Cosson, M. Tavaud, M.J. Aranzana, C. Poizat, A. Zanetto, P. Arús, F. Laigret. 2002. Development of microsatellite markers in peach [*Prunus persica* (L) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L). Theor. Appl. Genet. 105:127–138.

Dirlewanger, E. E. Graziano, T. Joobeur, F. Garriga-Caldere, P. Cosson, W. Howad, and P. Arus. 2004. Comparative mapping and marker-assisted selection in Rosaceae fruit crops. Proc. Natl. Acad. Sci. U.S. 101:9891-9896.

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

Etienne, C., C. Rothan., A. Moing., C. Plomion., C. Bodenes., 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.

Edge-Garza, D.A., J.J. Luby, and C.P. Peace. 2016. Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Mol. Breed. 35:1-15.

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. and Genomes 7:323-335.

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 Phytol. 185:917-930.

Falchi, R., E. Vendramin, L. Zanon, S. Scalabrin, G. Cipriani, I. Verde, G. Vizzotto, and M. Morgante. 2013. Three distinct mutational mechanisms acting on a single gene underpin the origin of yellow flesh in peach. Plant J. 76:175–187.

Fresnedo-Ramírez, J., M.C.A.M. Bink, W.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. Breed. 35:1-19.

Fresnedo-Ramírez, 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.C.A.M. Bink, W.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. Mol. Breed. In press.

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

Frett, T.J., G. Reighard, K. Gasic and W. Okie. 2014. Mapping quantitative trait loci associated with blush in peach. Tree Genet. and Genomes 10:367-381.

Gradziel, T.M. 2002. Almond species as sources of new genes for peach improvement. Acta Hort. 592:81-88.

Gradziel, T.M. 2003. Interspecific hybridizations and subsequent gene introgression within *Prunus subgenus Amygdalus*. Acta Hort. 622:249-255.

Guan, Y., C. Peace, D. Rudell, S. Verma, and K. Evans. 2015. QTLs detected for individual sugars and soluble solids content in apple. Mol. Breed. 35: 1-13.

Iezzoni, A., C. Weebadde., J. Luby., Y. Chengyan., 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.

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

Jung, S., M. Staton, T. Lee, A. Blenda, R. Svancara, A. Abbott, and D. Main. 2008. GDR (Genome Database for Rosaceae): integrated webdatabase for Rosaceae genomics and genetics data. Nucl. Acids Res. 36:D1034–D1040.

Jung, S., S.P. Ficklin, T. Lee, C.-H. Cheng, A. Blenda, P. Zheng, J. Yu, A. Bombarely, I. Cho, S. Ru, K. Evans, C. Peace, A.G. Abbott, L.A. Mueller, M.A. Olmstead, and D. Main. 2014. The genome database for rosaceae (GDR): year 10 update. Nucl. Acids Res. 42:D1237-D1244.

Layne, D.R., Z.W. Jiang, and J.W. Rushing. 2001. Tree fruit reflective film improves red skin coloration and advances maturity in peach. Hort Technol. 11:234–242.

Lin-Wang, K., K. Bolitho, K. Grafton, A. Kortstee, S. Karunairetnam, T.K. McGhie, R.V. Espley, R.P. Hellens, and A.C. Allan. 2010. An R2R3 MYB transcription factor associated with regulation of the anthocyanin biosynthetic pathway in Rosaceae. BMC Plant Biol 10:50. doi: 10.1186/1471-2229-10-50

Martinez-Garcia, P., D. Parfitt, E. Ogundiwin, J. Fass, H. Chan, R. Ahmad, S. Lurie, A. Dandekar, T. Gradziel, and C.H. Crisosto. 2012. High density SNP mapping and QTL analysis for fruit quality characteristics in peach (*Prunus persica* L.). Tree Genet Genomes 9:1–18. doi:10.1007/s11295-012-0522-7.

Ogundiwin, E.A., C.P. Peace, T.M. Gradziel, A.M. Dandekar, F.A. Bliss, and C.H. Crisosto. 2007. Molecular genetic dissection of chilling injury in peach fruit. Acta Hort 738:633–638.

Ogundiwin E.A., C.P. Peace, C.M. Nicolet, V.K. Rashbrook, T.M. Gradziel, F.A. Bliss, D. Parfitt, and C.H. Crisosto. 2008. Leucoanthocyanidin dioxygenase gene (PpLDOX): a potential functional marker for cold storage browning in peach. Tree Genet Genomes 4:543–554.

Ogundiwin, E.A., C.P. Peace, T.M. Gradziel, D.E. Parfit, 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. Breed. 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. and Genomes 10:1679-1694.

Peace C.P. and J.L. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae, Ch. 2:19-53. In: Folta K.M., Gardiner S.E. (eds.). Genetics and genomics of Rosaceae. Series Ed Jorgensen R. Springer, New York, Plant Genetics / Genomics, Vol 6.

Pirona, R., I. Eduardo, I. Pacheco, C.D. 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.

Pozzi, C. and A. Vecchietti. 2009. Peach structural genomics, p. 235-257. In: K.M. Folta. and S.E. Gardiner (eds.). Genetics and genomics of Rosaceae. Springer, New York.

Quarta, R., C. Cedrola, M.T. Dettori, and I. Verde. 2001. QTL Analysis of Agronomic Traits in a BC1 Peach Population. In V International Peach Symposium 592:291-297.

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. Genard, 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. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org/. Accessed 12 November 2015.

Rosyara, U.R., M.C.A.M. Bink, W.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. Breed. 32:875-887.

Ru, S., D. Main, K. Evans, and C. Peace. 2015. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet. and Genomes 11:1-12.

Salazar, J.A., D. Ruiz, J.A. Campoy, R. Sánchez-Pérez, C.H. Crisosto, P.J. Martínez-García, A. Blenda, S. Jung, D. Main, P. Martínez-Gómez, and M. Rubio. 2013. Quantitative Trait Loci (QTL) and Mendelian Trait Loci (MTL) analysis in Prunus: a breeding perspective and beyond. Plant Mol. Biol. Rep. 1-18. DOI 10.1007/s11105-013-0643-7.

Salgado, 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. Ph.D dissertation University of Arkansas, Fayetteville, U.S.

Sandefur, P., T. Frett, J. Clark, K. Gasic, and C. Peace. 2016a. PpeRf-SSR, a DNA test for routine prediction in breeding of peach blush. Mol. Breed. In press.

Sandefur, P., T. Frett, A. Salgado, J. Clark, K. Gasic, and C. Peace. 2016b. Ppe-Acidity, a combined DNA test for routine prediction in breeding of acidity and soluble solids content (SSC) in peach. Mol. Breed. In press.

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

Schijlen, E.G.W.M., C.H.R Ric de Vos, A.J. van Tunen, and A.G. Bovy. 2004. Modification of flavonoid biosynthesis in crop plants. Phytochem. 65:2631–2648.

Shulaev, V., S.S. Korban, B. Sosinski, A.G. Abbott, H.S. Aldwinckle, K.M. Folta, A. Iezzoni, D. Main, P. Arús, A.M. Dandekar, and K. Lewers. 2008. Multiple models for Rosaceae genomics. Plant Physiol. 147:985-1003.

Smith, S. 2015. Fruit tree diseases. 16 November 2015. <https://www.uaex.edu/publications/pdf/mp154/fruit-tree-diseases-commercial.pdf>.

Studebaker, G., J. Hopkins, and D. Johnson. 2015. Control peach tree borers on commercially grown peach and plum trees. 16 November 2015. <https://www.uaex.edu/publications/PDF/FSA-7504.pdf>.

van de Weg, W.E., R.E. Voorips., H.J. Finkers., L.P. Kodde., J. Jansen, and M. Bink. 2004. Pedigree genotyping: A new pedigree-based approach of QTL identification and allele mining. Acta Hort. 663:45-50.

Vendramin, E., G. Pea, L. Dondini, I. Pacheco, M. T. Dettori, L. Gazza, S. Scalabrin F. Strozzi, S. Tartarini, D. Bassi, I. Verde, and L. Rossini. 2014. A unique mutation in a MYB gene cosegregates with the nectarine phenotype in peach. PloS ONE 9:e90574. DOI: 10.1371/journal.pone.0090574.

Verde, I., A.G. Abbott, S. Scalabrin, S. Jung, S. Shu, F. Marroni, T. Zhebentyayeva, M.T. Dettori, J. Grimwood, F. Cattonaro, A. Zuccolo, L. Rossini, J. Jenkins, E. Vendramin, L.A. Meisel, V. Decroocq, B. Sosinski, S. Prochnik, T. Mitros, A. Policriti, G. Cipriani, L. Dondini, S. Ficklin, D.M. Goodstein, P. Xuan, C. Del Fabbro, V. Aramini, D. Copetti, S. Gonzalez, D.S. Horner, R. Falzchi, S. Lucas, E. Mica, J. Maldonado, B. Lazzari, D. Bielenberg, R. Pirona, M. Miculan, A. Barakat, R. Testolin, A. Stella, S. Tartarini, P. Tonutti, P. Arús, A. Orellana, C. Wells, D. Main, G. Vizzotto, H. Silva, F. Salamini, J. Schmutz, M. Morgante, and D.S. Rokhsar.

2013. The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genet. 45:487-494.

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. DOI:10.1371/journal.pone.0035668.

Verma, S., J.J. Luby, K. Evans, U.R. Rosyara, Y. Guan, N. Bassil, M.C.A.M. Bink, W.E. van de Weg, and C.P. Peace. 2016. *Ma* locus functional alleles predict apple acidity within RosBREED Washington Apple Breeding Program Germplasm. Mol. Breed. In press.

Voorrips, R.E. 2001. MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. 93:77-78.

Voorrips, R.E. 2007. Pedimap: software for visualization of genetic and phenotypic data in pedigrees. Plant Research International, Wageningen, the Netherlands. <http://www.wageningenur.nl/en/show/Pedimap.htm≥.

Voorrips, R., M. Bink, and W. van de Weg. 2012. Pedimap: software for the visualization of genetic and phenotypic data in pedigrees. J. Hered. 103:903-907.

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. Breed. Sci. 51:271-278.

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

Chapter Four: SNP Haplotype DNA Test Development and Peach Mini-SNP Array v1.0 Analysis Across Four Pedigree-Connected U.S. RosBREED Peach Breeding Programs

Abstract

Despite the ever-growing availability of genomic resources in peach, the use of DNA tests for marker-assisted selection (MAS) is still in its infancy. To date, approximately 568 marker-locus-trait associations [M-L-T (QTL or MTL)] have been identified, yet, the majority cannot currently be used in breeding programs for MAS for several reasons, including the lack of incentive to convert them into breeder-friendly DNA tests. This in turn has created a valley of misunderstanding, which has directly limited broad application of MAS in and across Rosaceae fruit breeding programs. To bridge this gap, it is imperative that breeding-relevant M-L-Ts be turned into DNA tests, and traditional breeders be provided with the information on which technique to use to screen the marker, as well as how to interpret and score the results. In this study, two sequential steps were followed with the overall objective to develop robust SNP-based DNA tests across four RosBREED peach demonstration breeding programs [the University of Arkansas (UA), Clemson University (CU), Texas A&M University (TX) and the University of California, Davis (CA)] for direct use of MAS for seven peach breeding-relevant fruit traits: Fruit resistance to bacterial spot (*Xap*1 and *Xap*6), maturity date (G4mat), fruit type (G), blush (R_f), flesh color (Y), acidity (D), and a combination flavor trait made up of acidity and soluble solid content (G7Flav). First, previously identified, breeding-relevant M-L-Ts were further investigated and developed into informative SNP-based DNA tests for each trait. To do this, relevant SNPs associated with the traits of interest were grouped into haplotypes and their robustness was validated in material across the four RosBREED programs (~1,000 pedigree connected individuals, termed “development set”). In total, 32 SNPs significantly associated with the seven traits and capable of distinguishing all functional alleles were selected. Second, these

32 SNPs were divided accordingly into one 16-SNP and one 20-SNP mini array. Leaf samples (240 total), representing advanced breeding material as well as several control samples (previously genotyped with the IPSC 9K peach SNP array v1.0) from all four programs, were outsourced to two DNA service providers for design and testing of the mini SNP arrays (termed "confirmation set"). Outsourcing was performed for two reasons: first to trial the DNA testing system to see if the service provider genotyping platforms could successfully translate the trait predictions from the 9K SNP array results; and secondly, for direct use in MAS as the raw results from the service providers were successfully translated into trait predictions and provided to each breeding program. Overall, depending on the material tested, genotypic data acquired accurately predicted phenotypic performance in ~75% of cases across material from all breeding programs. These results are due to the vast haplotypic diversity in U.S. peach breeding germplasm. The additional ~25% of material required specific attention to assigning the correct haplotypes to the correct phenotypes. Feasibility of using mini SNP arrays in breeding programs for cross planning and seedling selection as well as the level of service required from providers is discussed.

Introduction

Peach [*Prunus persica* (L.) Batsch] is a self-fertile, diploid species (2n = 16), with a base chromosome number of x = 8, and belongs to the Rosaceae family, subfamily Prunoideae (Bassi and Monet, 2008; Byrne et al., 2012). Great advances in the understanding of the genetic control of several traits in peach have taken place in the past two decades, so much so that peach is now known as the model species for all Rosaceae fruit crops (Abbott et al., 2002; Shulaev et al., 2008). This model species has a relatively short juvenility period (~2-3 years), simple genomic structure (227.4 Mb), and high amount of developed genetic resources (Abbott et al., 2002; Salazar et al., 2013). Peach is also the best genetically characterized *Prunus* species with 19

major genes mapped to specific loci on the highly saturated *Prunus* genetic reference map, 'T × E' (Aranzana et al., 2003; Dirlewanger et al., 2004; Etienne et al., 2002; Joobeur et al., 1998; Pozzi and Vecchietti 2009; Sansavini et al., 2006). A total of 46 linkage maps have been used for QTL analysis through traditional bi-parental mapping populations (Salazar et al., 2013). To date, numerous QTL studies have been performed in peach and a total of 568 different marker-locus-trait associations [M-L-T (QTL or MTL)] have been described in peach and/or related interspecific hybrids and can be found on the Genomic Database for Rosaceae (GDR; http://www.rosaceae.org/) (Jung et al., 2014). These QTL and MTL were linked to 75 different peach traits: 13 for tree development, 15 for flower and ripening, six for disease and pest resistance, and 41 for fruit quality (Salazar et al., 2013).

Unfortunately, despite the ever-growing availability of genomic resources in peach, the use of DNA tests for marker-assisted selection (MAS) is still in its infancy. In terms of peach, the majority of the 568 identified M-L-Ts (QTL or MTL) cannot currently be used in breeding programs for MAS for several reasons, one being that all previous peach QTL studies used single bi-parental segregating populations, excluding two recent publications by Fresnedo-Ramírez et al. (2015 and 2016). Therefore, the relevance of the majority of these findings may be limited only to specific lineages from the parents of the bi-parental genetic populations (Peace et al., 2014). Yet, an even more noteworthy obstacle is the lack of incentive to convert these M-L-Ts into breeder-friendly DNA tests, which breeders can understand and use in their programs (Bliss, 2010; Collard and Mackill 2008; Iezzoni et al., 2010; Ru et al., 2015). This lack of incentive has effectively created a valley of misunderstanding between geneticists and traditional breeders which has directly limited broad application of MAS in and across peach and other Rosaceae fruit breeding programs (Bliss, 2010; Iezzoni et al., 2010; Ru et al., 2015). To bridge this gap

between them, it is imperative that breeding relevant M-L-Ts are turned into DNA tests, and traditional breeders are provided with the information on which technique to use to screen the marker, as well as how to interpret and score the results

While agronomic crop breeders have documented an increase in efficiency and saving of resources in their programs by incorporation of molecular tools, peach and other perennial fruit tree breeders have unfortunately lagged behind due to several reasons including but not limited to substantially less funding, significantly longer juvenility periods, and a considerably higher investment cost per seedling (Ru et al., 2015). Perennial fruit breeders have much to gain from the incorporation of molecular techniques as these tools can provide them with more informed decision support to increase genetic gain per breeding cycle, improve selection efficiency, and significantly reduce breeding program operational costs (Bliss, 2010; Byrne, 2005; Edge-Garza et al., 2016; Ru et al., 2015). To date, application of MAS using peach DNA-based tests is only a reality for a few breeding-relevant traits including but not limited to: flesh texture and adherence (Peace et al., 2005; Peace and Norelli 2009), acidity (Eduardo et al., 2014), slow-melting flesh (Salgado, 2015), fruit bacterial spot resistance (see Chapter Five, Section One), pubescence (see Chapter Five, Section Two; Vendramin et al., 2014), white vs. yellow flesh (see Chapter Five, Section Three; Falchi et al., 2013), blush coverage (Sandefur et al., 2016a), acidity, and soluble solids content (Sandefur et al., 2016b).

The overall objective of this study was to develop and validate SNP-based DNA tests that are robust across four RosBREED peach demonstration breeding programs [University of Arkansas (UA), Clemson University (CU), Texas A&M University (TX) and University of California, Davis (CA)] for direct use in MAS of seven peach breeding-relevant fruit traits: fruit resistance to bacterial spot (*Xap*1 and *Xap*6), maturity date (G4mat), fruit type (G), blush (R_f),

flesh color (Y), acidity (D), and acidity and soluble solid content (G7Flav). A secondary objective was to outsource an array of advanced breeding material from all four programs for two primary reasons: first, to trial the DNA testing system to see if the service provider genotyping platforms can successfully translate the trait predictions from the 9K SNP array results; and secondly, upon successful confirmation, to provide each breeding program with the results for direct use in MAS in their program.

Materials and Methods

Germplasm

Through the RosBREED 1 initiative, material from four RosBREED pedigree-connected demonstration peach breeding programs: UA, CU, TX, and CA were included in the Crop Reference Set (CRS) and Breeding Pedigree Set (BPS) (~1,000 individuals in total) (termed "development set"). The CRS and BPS were originally chosen to contain a vast diversity of important cultivars, ancestors, founders, and progeny representative of North American breeding germplasm (Peace et al., 2014). Overall, 175 individuals were historically selected for commercial performance and the remaining individuals were un-selected seedlings in multiple small bi-parental families (Peace et al., 2014). Additionally, 240 advanced selections including several control samples (from the CRS/BPS) from all four programs were selected for outsourcing (72 individuals from UA; 48 individuals from CU; 72 individuals from TX; and 48 individuals from CA) (termed "confirmation set").

Development and Validation of SNP-Haplotype DNA Tests

Through the RosBREED 1 initiative, the CRS and BPS material were phenotyped for two years (2011 and 2012), using the RosBREED standardized phenotyping protocol (Frett et al., 2012), and genotyped with the IPSC 9K peach SNP array v1.0 (Verde et al., 2012). Using this

germplasm "development set," seven previously identified breeding-relevant M-L-T's associated with seven fruit quality, phenological, and disease resistance traits [fruit resistance to bacterial spot (*G1XapF and G6XapF; G1XapF*-locus and *G6XapF*-locus), maturity date (MD; *G4MD.1*-locus), fruit type [pubescent (peach) vs. glabrous (nectarine) (P vs. N); *G*-locus], blush overcolor (R_f-locus), flesh color [white vs. yellow (W vs. Y); *Y*-locus], titratable acidity (TA) (*D*-locus), and TA and soluble solids content (SSC) (*G7Flav*-locus)] were further investigated by selecting informative SNPs, which co-located within and or flanked the M-L-T's of interest, into SNP haplotype DNA tests (Table 1).

Two SNP-based Excel® (Microsoft, Redmond, WA) files developed by Dr. Cameron Peace (Co-Project Director and Marker-Assisted Breeding Pipeline leader of RosBREED) were used to select SNPs, and perform SNP haplotyping across the RosBREED CRS and BPS "development set". First, a SNP-trait-association file was utilized to select informative SNPs. The SNP-trait-association files contained a subset of 1,947 SNPs (from the IPSC 9K SNP array) which were polymorphic and robust across the RosBREED peach CRS and BPS, as well as phenotypic data for each individual in the file. Using the 1,947 SNPs of the IPSC 9K SNP, and the phenotypic data, the coefficient of determination (R^2) was calculated from a linear regression for each SNP and an array of SNPs with the highest R^2 were selected for each trait. Each DNA tests consisted of two or more SNPs, used in conjunction to additively explain the highest possible combined phenotypic variation for each respective trait. Secondly, the selected SNPs were added to a SNP-haplotyping file, which was used to develop functional haplotypes for each set of SNPs across the CRS and BPS.

Table 1. The 10 previously identified M-L-T's investigated in this study, including the publications/dissertation chapters they were identified in, as well as the locus they correspond to.

Trait	Previous M-L-T's/ candidate gene	Locus	Publication/dissertation chapter
Xap fruit resistance	*Xap.Pp.OC-1.2*	*Xap1*	Chapter Two; Chapter Five, Section One; Yang et al., 2013
Xap fruit resistance	*Xap.Pp.OC-6.1*	*Xap6*	
Titratable acidity	*D-locus*	*D*	Dirlewanger et al., 1998; Boudehri et al., 2009
Fruit type	*PpeMYB25*	*G*	Chapter Five, Section Two; Vendramin et al., 2014
Flesh color	*PpCCD4*	*Y*	Chapter Five, Section Three; Falch et al., 2013
Blush overcolor	*Blush.Pp.ZC-3.1*	R_f	Frett et al., 2014; Sandefur et al., 2016a
Maturity date	*G4MD.1*	*G4mat*	Chapter Three
Titratable acidity and soluble solids content	*qSSC.7*	*G7Flav*	Chapter Three; Fresnedo-Ramírez et al., 2015; Sandefur et al., 2016b

A graphical representation of the *P. persica* chromosome with the enlarged location of the locus and SNPs used for haplotyping were developed for each SNP haplotype. Physical locations were sourced from the *P. persica* whole genome v1.0 sequence (Verde et al., 2013) housed on the Genome Database for Rosaceae (Jung et al., 2014). Additional flanking markers and their estimated genetic positions based on the Prunus-TE-F_2 reference bin map were included in each figure to provide a general understanding of the genetic distance within each SNP used for haplotyping. Additionally, the unique haplotypes for each DNA test found within RosBREED peach breeding germplasm and their frequencies within total, selected (germplasm selected prior in seedling populations in routine breeding field selection activities), and un-selected (F_1 seedlings, not yet selected) material were calculated.

Histograms and box plots of TA, blush, MD, and SSC phenotypic data taken in 2011 and 2012, in and across all four RosBREED breeding programs were generated to visually check

distribution of data. Mosaic plot distributions of *G1XapF* and *G6XapF* (haplotypes) through RosBREED germplasm from UA, CU, TX, and CA were developed to make comparisons between programs. Mosaic plot distributions of 'P vs. N', 'W vs. Y' overall across all RosBREED material were developed to display the accuracy of the 'P vs. N' and 'W vs. Y' (diplotypes) to predict the correct phenotype.

Additionally, a three-way analysis of variance was performed for TA, MD, and SSC. The TA, MD, and SSC data sets were transformed using the base 10 logarithm of each value to approach normality. Associations between each trait, their corresponding haplotypes/diplotypes, and year were performed by least square means comparisons ($P \leq 0.05$, student's t-test, JMP ® 2012). The percent of phenotypic variation explained (Vp) by the SNP haplotypes for each respective trait was calculated using values from the ANOVA table for 2011-2012 avg data set by haplotypes/diplotypes and the formula: between group variance [sum of squares between groups (SS_B)] / total group variance [sum of squares total (SS_T)] = Vp %. Since blush was scored using a 0-5 scale, the non-parametric Kruskal-Wallis test with Wilcoxon rank sum pairwise comparisons test ($P \leq 0.05$) was used to evaluate differences among diplotypes and year for the proportion of blush they predict (JMP ® 2012).

Outsourcing of SNP-haplotype DNA tests

Leaf samples of the 240 advanced breeding selections, cultivars, and control samples from the four RosBREED demonstration peach breeding programs were harvested in the summer of 2013 ("confirmation set"). Positive control individuals, which were previously genotyped with the Infinium 9K SNP array, were included to determine successful translation of haplotypes (i.e. trait predictions) from the 9K array. In total, 32 SNPs associated with the traits and capable of distinguishing all functional alleles were chosen. The 32 SNPs were divided accordingly into one

16-SNP and one 20-SNP genotyping panel using two different outsourcing companies: The University of Arizona Genetics Core (AZ) and BioDiagnostics (BDI). The 72 UA and 72 TX individuals were screened on both SNP mini-arrays, while the 48 CA individuals were screened through the AZ 16-SNP panel, and the 48 Clemson individuals were screened through the BDI 20-SNP panel (Table 2). One of the service providers used sequenom technology for SNP analysis, while the other used Kompetitive Allele Specific PCR (KASP).

Data Analysis of Mini SNP Array Results

Genotypes for each trait were determined in different manners based off the number of heterozygous SNPs. Genotypes for "unambiguous individuals", those with one or zero heterozygous SNPs, were directly determined. "Unambiguous individuals (>2 SNP hetero)" were individuals that had two or more heterozygous SNPs. In this case, a pedigree approach in which the parents and grandparent alleles (from the 9K array), for each individual on the SNP mini-arrays, were used to trace the identity of each haplotype and decipher individuals with complicated alleles. Process of elimination was also used. Those individuals whose genotypes could not be determined, were labeled as "ambiguous".

Table 2. Set-up of two SNP mini-arrays submitted to service providers (1 and 2) (V_P = phenotypic variation explained; * signifies generalized phenotypic associations generated from SNP trait association file).

Trait	Locus	SNPs (N)	Service provider	Allele predictions	V_P
Bacterial fruit spot	*G1XapF*	4	1	AAAB (*very susceptible); BBAB (*intermediate); BBAA, BBBB (*resistant)	~7% (UA)
Bacterial fruit spot	*G6XapF*	4	1	BAAB (*very susceptible); AABB (*intermediate); ABAA, BBBB (*resistant)	~8% (UA)
Maturity date	*G4MD.1*	4	1 and 2	BBBA (*very early); ABBB (*early); ABBA (*mid); BBAA (*late); AAAA (*very late 1); AAAB (*very late 2); BAAB (*extra late); BBBB (*almond, also very late) (*later = higher SSC)	50%
Fruit type (peach vs. nectarine)	*G*	1	1	AA (*nectarine); AB, BB (*peach)	100%
		6	2,1,1, 1,1,1	BBBBBAA, BBABBBA, BBAAABB, BABABBA, BABABBB, BABAABB, ABBBBAA, ABBABAB, AABAABB (*peach); BBBBBBA, BBAABBA, ABAABBA, AABBBBA, AABABBA (*nectarine) (UA needs G2-4,6; CA needs G3-G8; SC needs all G2-8; and TX only needs G7)	
		1	1	BB (*Nectarine); AB, AA (*peach)	
		1	1	BB (*Nectarine); AB, AA (*peach)	

Table 2. Set-up of two SNP mini-arrays submitted to service providers (1 and 2) (V_P = phenotypic variation explained; * signifies generalized phenotypic associations generated from SNP trait association file) (Cont.).

Trait	Locus	SNPs (N)	Service provider	Allele predictions	V_P
Flesh color	*Y*	1	1	AA (yellow); AB, BB (white)	100%
		1	1	AA (yellow); AB, BB (white)	
		1	2	AA (yellow); AB, BB (white)	
		4	1,1,2,2	AAAAA, AAABB, ABBBA, ABBAA, ABBAB (yellow); BBBBB, BBBAB, BBBBA, BBBAA, BABAB, AABAA, AAABA (white)	
Blush	R_f	4	1,1,2,2	AAAA, ABAA, BBAA, BABB (high); BBBB, BBAB, BAAB, ABBB, ABBA, ABAB, AABB, AAAB (low)	55%
Titratable acidity (TA)	*D*	2	1	AA, AB (low); BB (high) (Primary SNP)	50%
				BB, AB (low); AA (high) (Secondary SNP)	
TA and soluble solids content (SSC)	*G7Flav*	2	2	AA (high); AB (med); BA (low)	5%

Results

Development and Validation of SNP-Haplotype DNA Tests

Fruit Bacterial Spot

The *G1XapF* spanned ~2 Mbp on *P. persica* chromosome 1, and the four SNP markers used for haplotype construction located within ~12.92 (SNP_IGA_39717) to 14.98 Mbp (SNP_IGA_46754) (Fig. 1). Using these four SNPs, functional *G1XapF* haplotypes were successfully developed for 971 individuals in the RosBREED pedigree "development set" (Table 3). Of these 971 individuals, four unique haplotypes were observed, and subsequently converted into functional haplotype groups based on haplotype effect on *Xap* fruit resistance/susceptibility [susceptible (SU), intermediate (I), resistant one and two (R1, R2)]. In total, the frequencies for each unique functional haplotype ranged from 3% (R2) to 48% (R1). In selected germplasm (germplasm selected prior in seedling populations in routine breeding field selection activities), the haplotype frequencies for 209 individuals ranged from 4% (R2) to 44% (R1). In un-selected germplasm (F_1 seedlings, not yet selected), the haplotype frequencies for 762 individuals ranged from 2% (R2) to 50% (R1) (Table 3).

In contrast, the *G6XapF* spanned a much smaller region, ~10 kbp, on *P. persica* chromosome 6 and the four SNP markers used for haplotype construction were located within 22.29 (SNP_IGT_680889) to 22.30 Mbp (SNP_IGA_681081) (Fig. 2). Using these four SNPs, functional *G1XapF* haplotypes were successfully developed for 964 individuals in the RosBREED pedigree "development set" (Table 4). Of these 964 individuals, four unique haplotypes were observed, and subsequently converted into functional haplotype groups based on haplotype effect on *Xap* fruit resistance/susceptibility [susceptible (SU), intermediate (I), resistant one and two (R1, R2)]. In total, the frequencies for each unique functional haplotype ranged from 2% (I) to 55% (SU). In selected germplasm, the haplotype frequencies for 203

individuals ranged from 7% (I) to 58% (SU). In un-selected germplasm, the haplotype frequencies for 761 individuals ranged from 1% (I) to 54% (SU) (Table 4).

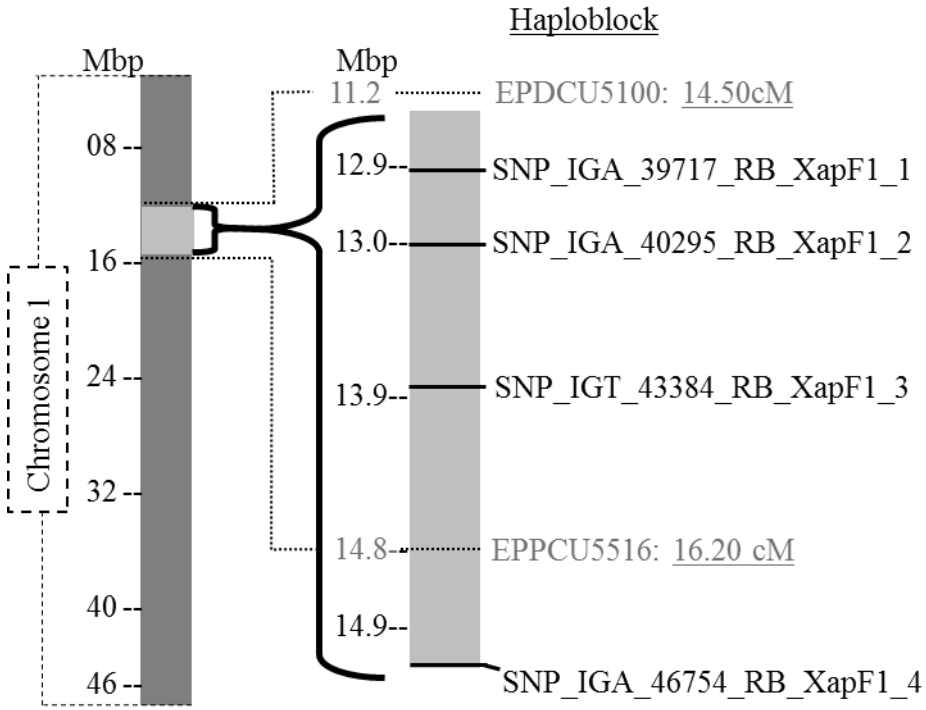

Fig. 1. Graphical representation of *P. persica* chromosome 1 with the enlarged location of the ~2 Mbp flanking the four SNP markers used for haplotyping at the *G1XapF* locus. Physical locations were sourced from the *P. persica* whole genome v1.0 sequence (Verde et al., 2013) housed on the Genome Database for Rosaceae (Jung et al., 2014). Two flanking markers and their estimated genetic positions based on the Prunus-TE-F_2 reference bin map are included in gray.

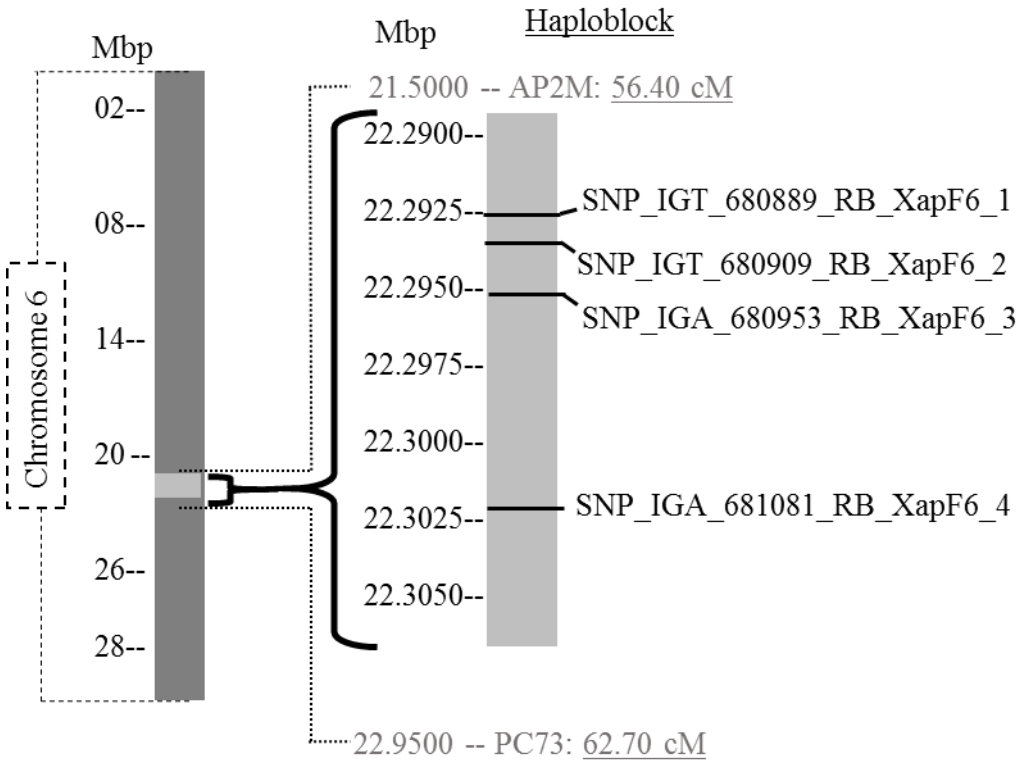

Fig. 2. Graphical representation of *P. persica* chromosome 6 with the enlarged location of the ~10 kbp flanking the four SNP markers used for haplotyping at the *G6XapF* locus. Physical locations were sourced from the *P. persica* whole genome v1.0 sequence (Verde et al., 2013) housed on the Genome Database for Rosaceae (Jung et al., 2014). Two flanking markers and their estimated genetic positions based on the Prunus-TE-F_2 reference bin map are included in gray.

Table 3. The four unique functional 4-SNP haplotypes (*G1XapF*) for distinguishing haplotype effect on *Xap* resistance among 971 total individuals (Selected = 209 cultivars and selections) (Un-selected = 762 F_1 seedlings) found within the RosBREED pedigree "development set" screened with the 9K array (N is number of haplotypes analyzed).

Locus	4-SNP haplotype		Total		Selected		Un-selected	
	Sequence	Function	N=1942	Frequency	N=418	Frequency[z]	N=1524	Frequency[y]
G1XapF	AAAB	SU	639	0.33	135	0.33	504	0.33
	BBAB	I	313	0.16	81	0.19	232	0.15
	BBAA	R1	938	0.48	185	0.44	753	0.50
	BBBB	R2	52	0.03	17	0.04	35	0.02

[z]Frequency of haplotypes in RosBREED cultivars or selections.
[y]Frequency of haplotypes in RosBREED un-selected germplasm (i.e. seedlings).

Table 4. The four unique functional 4-SNP haplotypes (*G6XapF*) for distinguishing haplotype effect on *Xap* resistance among 964 total individuals (Selected = 203 cultivars and selections) (Un-selected = 761 F_1 seedlings) found within the RosBREED pedigree "development set" screened with the 9K array (N is number of haplotypes analyzed).

Locus	4-SNP haplotype		Total		Selected		Un-selected	
	Sequence	Function	N=1928	Frequency	N=406	Frequency[z]	N=1522	Frequency[y]
G6XapF	BAAB	SU	1067	0.55	239	0.58	828	0.54
	AABB	I	31	0.02	26	0.07	5	0.01
	BBBB	R1	435	0.23	85	0.21	350	0.23
	ABAA	R2	395	0.2	56	0.14	339	0.22

[z]Frequency of haplotypes in RosBREED cultivars or selections.
[y]Frequency of haplotypes in RosBREED un-selected germplasm (i.e. seedlings).

The *G1XapF* haplotypes were successfully developed for 971 RosBREED pedigree individuals: 321 (CA), 330 (CU), 155 (TX), and 165 (UA). Analysis of distribution of *G1XapF* functional haplotypes in each individual breeding program revealed that in comparison to all other programs, UA contained the least proportion of SU haplotypes (20%) and the highest proportion of R1 and I alleles combined (80%) (Fig. 3).

Likewise, the *G6XapF* haplotypes were successfully developed for 964 RosBREED pedigree individuals: 318 (CA), 328 (CU), 154 (TX), and 164 (UA). Analysis of distribution of *G6XapF* functional haplotypes in each individual breeding program revealed again that in comparison to all other programs, UA contained the least proportion of SU haplotypes (14%) and the highest proportion of R1 and R2 alleles combined (87%) (Fig. 4).

Fig. 3. Mosaic plot distributions of G1*Xap*F haplotypes through germplasm of the RosBREED demonstration peach breeding programs [The University of Arkansas (AR), Clemson University (SC), Texas A&M University (TX), and The University of California, Davis (CA)] (JMP® 12).

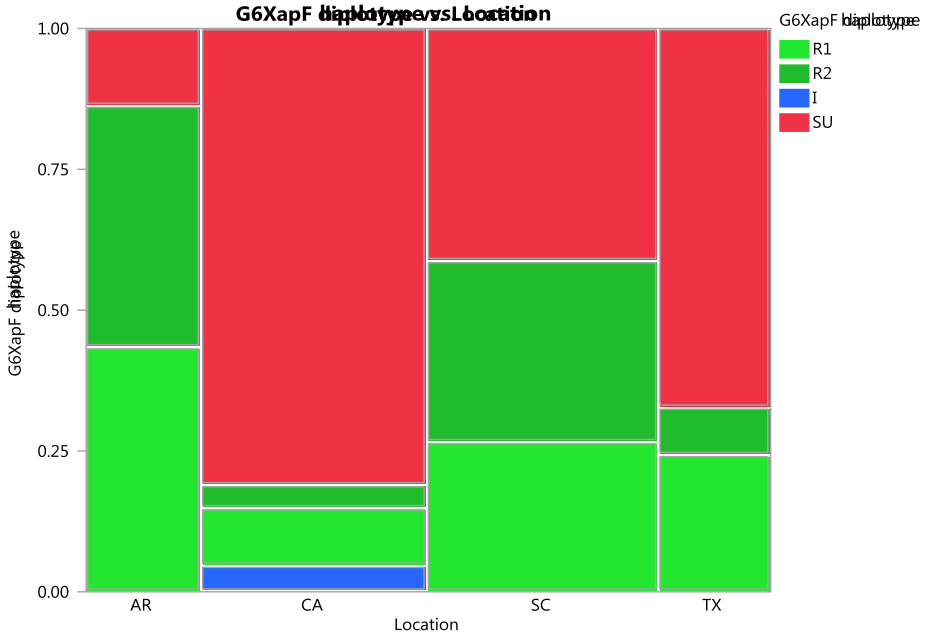

Fig. 4. Mosaic plot distributions of G6*Xap*F haplotypes through germplasm of the RosBREED demonstration peach breeding programs [The University of Arkansas (AR), Clemson University (SC), Texas A&M University (TX), and The University of California, Davis (CA)] (JMP® 12).

Blush Overcolor (Rf-locus)

Blush overcolor exhibited an average of 2.5 (2011), 2.3 (2012), and 2.4 (2011-2012 avg) and ranged from 0.0-5.0 (2011 and 2011-2012 avg) and 1.0-4.0 (2012) across all RosBREED material (Fig. 5; Table 5). The standard deviation displayed across all material was 1.3 (2011) and 1.2 (2012 and 2011-2012 avg). In UA material, blush overcolor exhibited an average of 3.5 (2011), 3.0 (2012), and 3.3 (2011-2012 avg), and ranged from 1.0-5.0 (2011 and 2011-2012 avg) and 1.0-4.0 (2012). The standard deviation displayed across all UA material was 1.2 (2012 and 2011-2012 avg) and 1.3 (2011), and all data sets were slightly skewed towards high blush ratings. In CA material, blush overcolor exhibited an average of 1.2 and ranged from 0.0-4.0 for all data sets. The standard deviation displayed across all CA material was 0.6 (2011), 1.3 (2012), and 1.0 (2011-2012 avg), and all data sets were heavily skewed towards low blush ratings. In TX material, blush overcolor exhibited an average of 3.1 (2011), 2.9 (2012), and 3.0 (2011-2012 avg), and ranged from 1.0-5.0 for all data sets. The standard deviation displayed across all data sets from TX material was 0.8, and all data sets were slightly skewed towards high blush ratings. In CU material, blush overcolor exhibited an average of 2.6 (2011), 2.5 (2012), and 2.6 (2011-2012 avg), and ranged from 0.0-4.0 (2011) and 0.0-5.0 (2012 and 2011-2012 avg). The standard deviation displayed across all CU material was 1.1 (2011 and 2011-2012 avg) and 1.0 for 2012, and all data sets were slightly skewed towards high blush ratings (Table 5; Fig. 5).

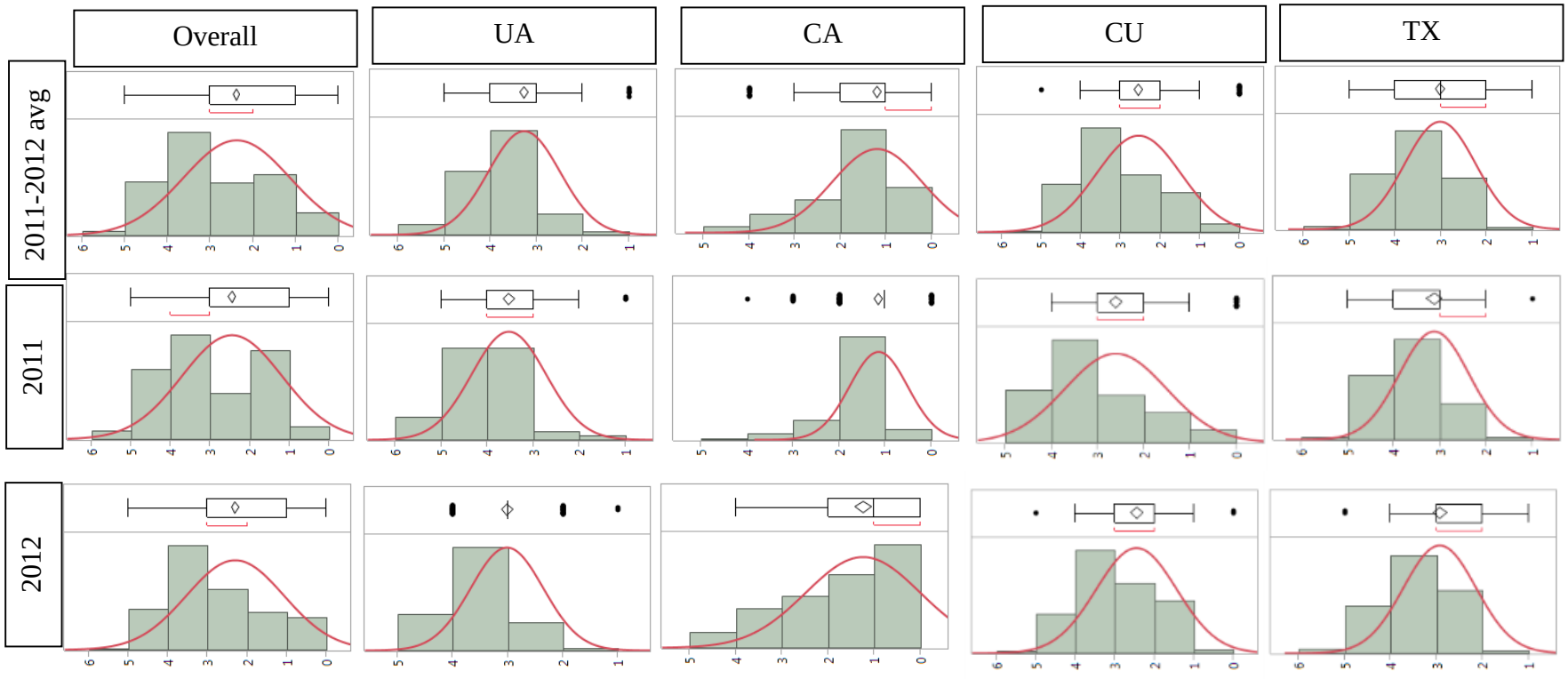

Fig. 5. Histograms and box plots of blush (0-5 scale; 0 = 0% blush, 5 = 100% blush) distribution in four RosBREED breeding programs [Arkansas (UA), UC Davis (CA), Texas A&M (TX), Clemson (CU)] across two years (2011, 2012) and 2011-2012 avg (*notice that UA, and TX scales start at 1, and CA scales stop at 4) (Normalized goodness of fit curves, are presented in red to highlight normality or skewness of data).

Table 5. Mean, median, minimum, maximum, and standard deviation of blush (0-5 scale; 0 = 0% blush, 5 = 100% blush) ratings for four RosBREED breeding programs [Arkansas (UA), UC Davis (CA), Texas A&M (TX), Clemson (CU)] in 2011, 2012, and 2011-2012 average (avg) (N is number of individuals).

Program	Year	Blush (0-5)					N
		Mean	Median	Min.	Max.	Std. dev.	
Overall	2011	2.5	3.0	0	5	1.3	664
Overall	2012	2.3	3.0	1	4	1.2	159
Overall	2011-2012 avg	2.4	3.0	0	5	1.2	1302
UA	2011	3.5	4.0	1	5	0.8	155
UA	2012	3.0	3.0	1	4	0.7	159
UA	2011-2012 avg	3.3	3.0	1	5	0.8	314
CA	2011	1.2	1.0	0	4	0.6	196
CA	2012	1.2	1.0	0	4	1.2	197
CA	2011-2012 avg	1.2	1.0	0	4	1.0	393
TX	2011	3.1	3.0	1	5	0.8	86
TX	2012	2.9	3.0	1	5	0.8	116
TX	2011-2012 avg	3.0	3.0	1	5	0.8	202
CU	2011	2.6	3.0	0	4	1.1	226
CU	2012	2.5	3.0	0	5	1.0	165
CU	2011-2012 avg	2.6	3.0	0	5	1.1	391

The *Rf-locus* spanned ~1 Mbp on *P. persica* chromosome 3, and the four SNP markers used for haplotype construction flanked the three peach *MYB10* homologs, *MYB10.1* (ppa026640m; *PprMYB10* candidate gene), *MYB10.2* (ppa016711m), and *MYB10.3* (ppa020385), within 12.32 (SNP_IGA_340465) to 13.42 Mbp (SNP_IGA_343773) (Zhou et al. 2014; Sandefur et al., 2016a) (Fig. 6). Using these four SNPs, functional blush haplotypes were successfully developed for 784 individuals in the RosBREED pedigree "development set" (Table 6). Of these 784 individuals, 11 unique haplotypes were observed, and subsequently converted into functional haplotype groups based on haplotype effect on blush development [high blush (Bl1, Bl2, Bl3, Bl4), and low blush (bl1, bl2, bl3, bl4, bl5, bl6, and bl7)]. All functional SNP haplotypes were additionally divided into two simple functional haplotype groups defined functional allele 'B' [high blush], and 'b' [low blush]. In total, the frequencies for each unique functional haplotype ranged from 1% (bl4) to 35% (Bl2). In selected germplasm, the haplotype frequencies for 105 individuals ranged from <1% (bl4) to 25% (Bl2). In un-selected germplasm, the haplotype frequencies for 679 individuals ranged from 1% (bl4) to 36% (Bl2) (Table 6).

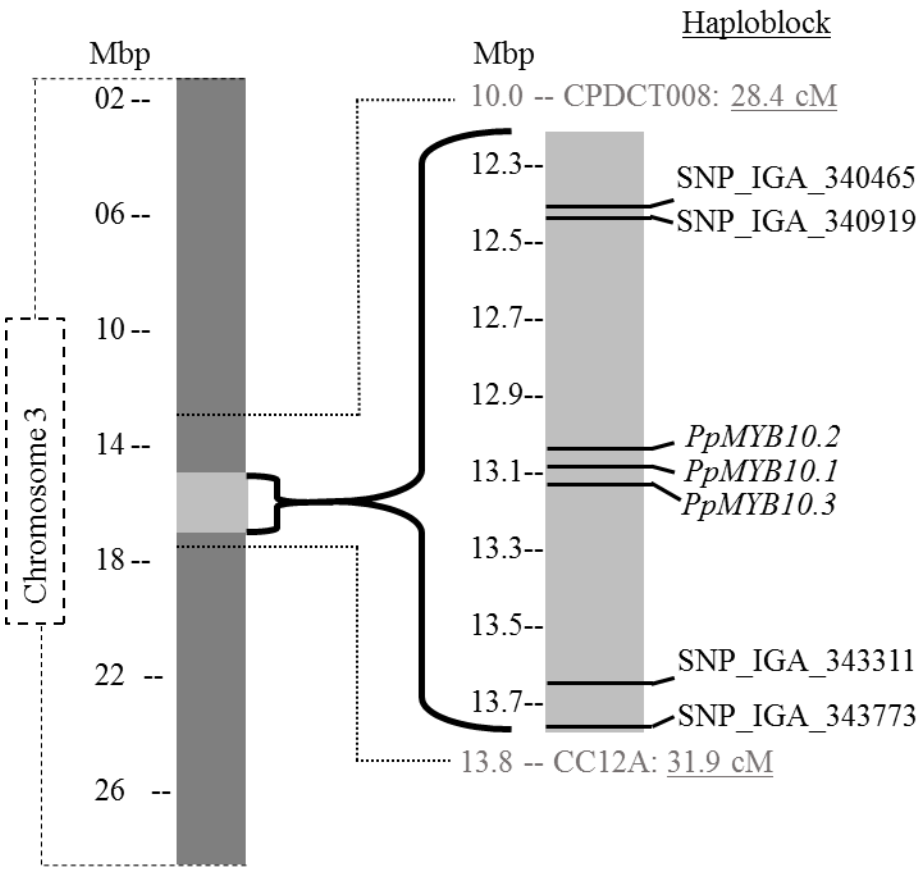

Fig. 6. Graphical representation of *P. persica* chromosome 3 with the enlarged location of the ~1 Mbp flanking the three *PpMYB10* transcription factors, including the four SNP markers used for haplotype construction at the *Rf-locus* (Sandefur et al., 2016a). Physical locations were sourced from the *P. persica* whole genome sequence v1.0 (Verde et al., 2013) found on the Genome Database for Rosaceae (Jung et al., 2014). Two flanking markers and their estimated genetic positions based on the Prunus-TE-F_2 reference map are included in gray.

Table 6. The 11 unique functional and simple functional 4-SNP haplotypes for distinguishing haplotype effect on blush development among 784 total individuals (Selected = 105 cultivars and selections) (Un-selected = 679 F_1 seedlings) found within the RosBREED pedigree "development set" screened with the 9K array (N is number of haplotypes analyzed).

Locus	4-SNP haplotype			Total		Selected		Un-selected	
	Sequence	Function	Simple function	N=1,568	Frequency	N=210	Frequency[z]	N=1,358	Frequency[y]
Rf	AAAA	B1	B	317	0.21	37	0.18	280	0.20
	BABB	B2	B	551	0.35	53	0.25	498	0.36
	BBAA	B3	B	83	0.05	11	0.05	72	0.05
	ABAA	B4	B	88	0.06	9	0.04	79	0.06
	BBBB	b1	b	100	0.06	23	0.11	77	0.06
	BAAB	b2	b	220	0.14	41	0.20	179	0.13
	ABBB	b3	b	82	0.05	17	0.08	65	0.05
	ABBA	b4	b	15	0.01	1	0.00	14	0.01
	ABAB	b5	b	36	0.02	2	0.01	34	0.03
	AABB	b6	b	28	0.02	4	0.02	24	0.02
	AAAB	b7	b	48	0.03	12	0.06	36	0.03

[z]Frequency of haplotypes in RosBREED cultivars or selections.

[y]Frequency of haplotypes in RosBREED un-selected germplasm (i.e. seedlings).

Individuals with simple functional diplotype B | B had significantly higher 2011-2012 avg blush medians (<.0001) than individuals with simple functional diplotype B | b or b | b (3.0 vs. 2.0 vs. 1.0) (Table 7). Likewise, individuals with simple functional diplotype B | b had significantly higher 2011-2012 avg blush medians (<.0001) than individuals with simple functional diplotype b | b (2.0 vs. 1.0).

Table 7. Medians of blush (0-5 scale; 0 = 0% blush, 5 = 100% blush) for blush simple functional diplotypes (B | B, B | b, b | b) across all RosBREED material, 2011-2012 avg.

Blush diplotype	Blush (0-5)
B | B	3.0 a[Z]
B | b	2.0 b
b | b	1.0 c

[Z] Medians followed by the same letter are not significantly different as determined by non-parametric Kruskal-Wallis test with Wilcoxon rank sum pairwise comparisons test ($P \leq 0.05$).

Maturity Date (MD)

Maturity date (MD), measured as day of the year (0-365), exhibited an average of 205.0 (2011), 191.4 (2012), and 198.3 (2011-2012 avg) and ranged from 111-264 (2011), 125-271 (2012), and 111-271 (2011-2012 avg) across all RosBREED material (Fig. 7; Table 8). The standard deviation displayed across all material was 29.8 (2011), 33.7 (2012), and 32.5 (2011-2012 avg). In UA material, maturity date exhibited an average of 198.9 (2011), 179.7 (2012), and 189.1 (2011-2012 avg), and ranged from 171-237 (2011), 151-220 (2012), and 151-237 (2011-2012 avg). The standard deviation displayed across all UA material was 17.2 (2011), 15.9 (2012), and 19.1 (2011-2012 avg). In CA material, maturity date exhibited an average of 231.4 (2011), 225.4 (2012), and 228.4 (2011-2012 avg), and ranged from 174-264 (2011), and 171-271 (2012 and 2011-2012 avg). The standard deviation displayed across all CA material was 19.3 (2011), 21.1 (2012), and 20.4 (2011-2012 avg), and all data sets were skewed towards the later season. In TX material, maturity date exhibited an average of 156.5 (2011), 146.8 (2012), and 150.6 (2011-2012 avg), and ranged from 111-233 (2011and 2011-2012 avg) and 125-187 (2012). The standard deviation displayed across all data sets from TX material was 20.1 (2011), 15.0 (2012), and 17.8 (2011-2012 avg), and all data sets were skewed towards the early season. In CU material, maturity date exhibited an average of 197.1 (2011), 184.3 (2012), and 191.5 (2011-2012 avg), and ranged from 151-249 (2011) and 144-249 (2012 and 2011-2012 avg). The standard deviation displayed across all CU material was 18.6 (2011), 16.7 (2012), and 18.9 (2011-2012 avg) (Table 8; Fig. 7).

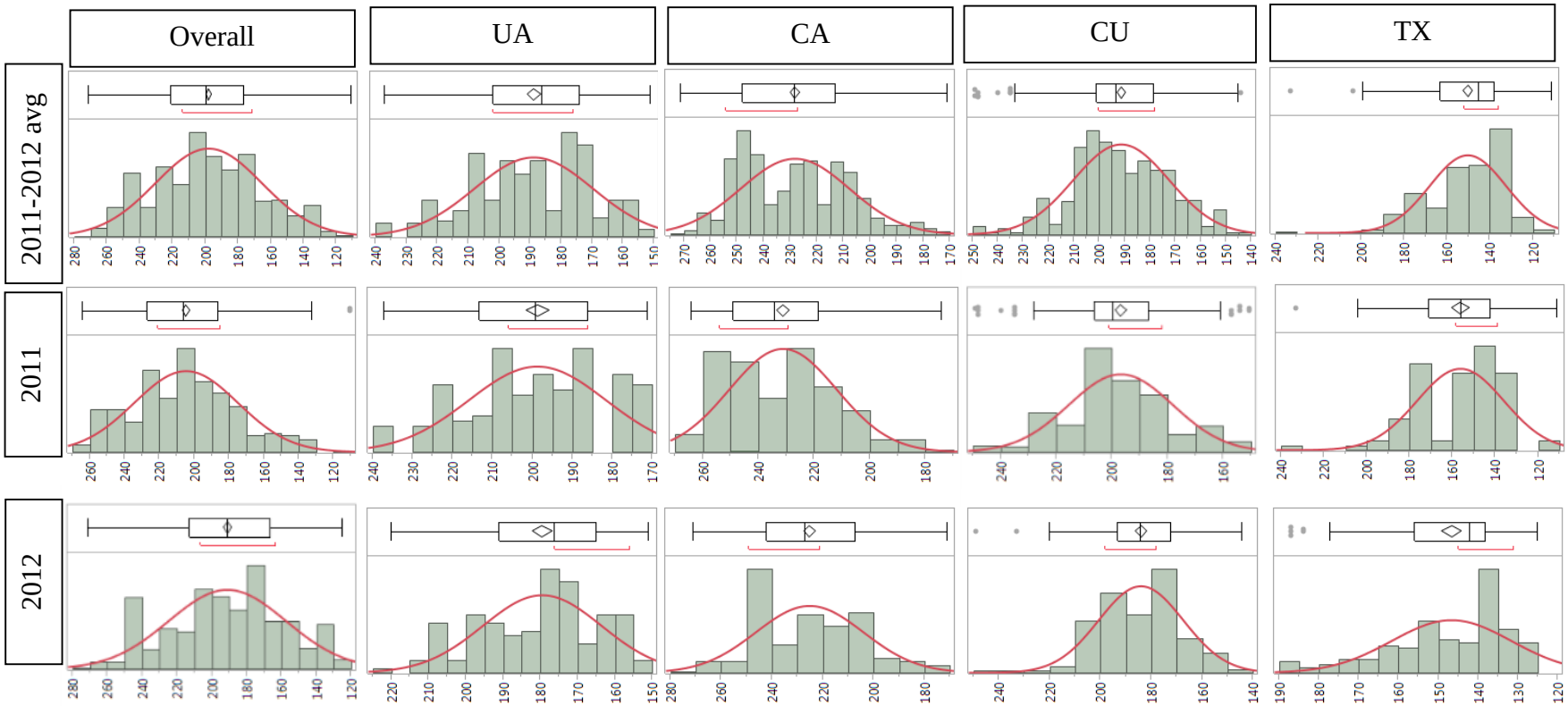

Fig. 7. Histograms and box plots of maturity date (day of year, 0-365) distribution in four RosBREED breeding programs [Arkansas (UA), UC Davis (CA), Texas A&M (TX), Clemson (CU)] across two years (2011, 2012) and 2011-2012 avg. (*note that the X-axis scales are different across the four programs) (Normalized goodness of fit curves, are presented in red to highlight normality or skewness of data).

Table 8. Mean, minimum, maximum, and standard deviation of maturity date (day of year, 0-365) ratings for four RosBREED breeding programs [Arkansas (UA), UC Davis (CA), Texas A&M (TX), Clemson (CU)] in 2011, 2012, and 2011-2012 average (avg) (N is number of individuals).

Program	Year	Maturity date (day of year, 0-365)				N
		Mean	Min.	Max.	Std. dev.	
Overall	2011	205.0	111	264	29.8	817
Overall	2012	191.4	125	271	33.7	806
Overall	2011-2012 avg	198.3	111	271	32.5	1623
UA	2011	198.9	171	237	17.2	142
UA	2012	179.7	151	220	15.9	149
UA	2011-2012 avg	189.1	151	237	19.1	291
CA	2011	231.4	174	264	19.3	288
CA	2012	225.4	171	271	21.1	287
CA	2011-2012 avg	228.4	171	271	20.4	575
TX	2011	156.5	111	233	20.2	91
TX	2012	146.8	125	187	15.0	145
TX	2011-2012 avg	150.6	111	233	17.8	236
CU	2011	197.1	151	249	18.6	294
CU	2012	184.3	144	249	16.7	223
CU	2011-2012 avg	191.5	144	249	18.9	517

A total of four informative SNP markers which spanned the previously identified QTL for MD at the *G4MD.1-locus* [10,685,500 (SNP_IGA_410398) to 11,288,514 bp (SNP_IGA_412662)] on scaffold 4 of the *P. persica* genome sequence v1.0 were selected for haplotyping (Chapter Three) (Fig. 8). Using these four SNPs, functional MD SNP haplotypes were successfully developed for 936 individuals in the RosBREED pedigree "development set" (Table 9). Of these 936 individuals, eight unique haplotypes were observed, and subsequently converted into functional haplotype groups based on haplotype effect on MD timing [very early season (VE), early season (E), mid-season (M), late season (L), very late season one and two (VL1, VL2), extra late season (XL), and almond (alm)]. In total, the frequencies for each unique functional haplotype ranged from 1% for XL to 42% for VE. In selected germplasm, the haplotype frequencies for 161 individuals ranged from 1% for XL to 39% for VE. In un-selected germplasm, the haplotype frequencies for 777 individuals ranged from 1% for XL to 42% for VE (Table 9).

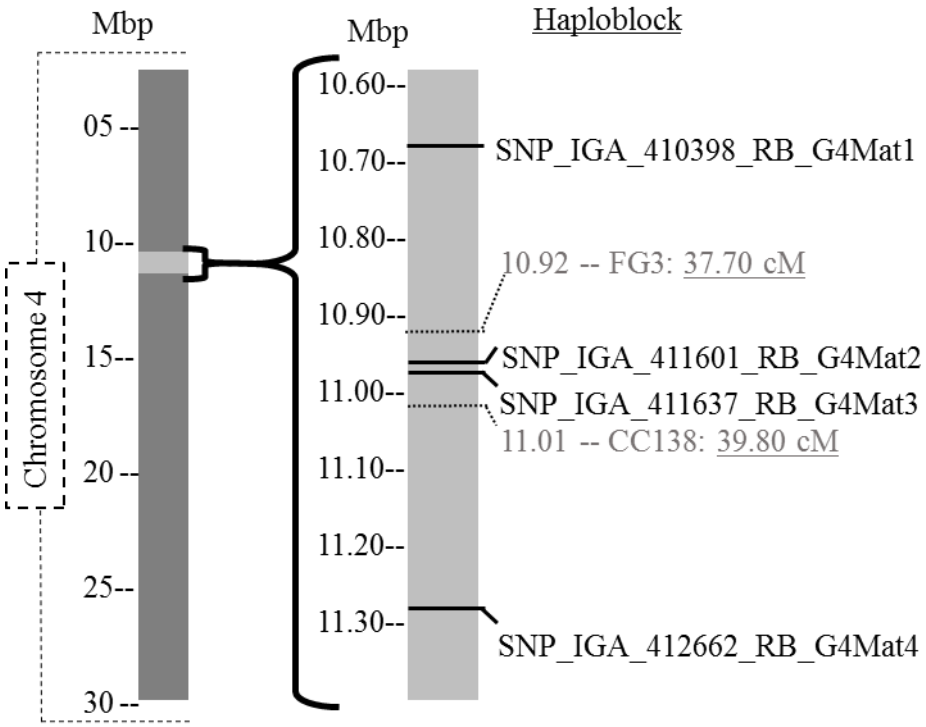

Fig. 8. Graphical representation of *P. persica* chromosome 4 with the enlarged location of the ~0.8 Mbp flanking the 4 SNP markers used for haplotyping at the *G4MD.1-locus*. Physical locations were sourced from the *P. persica* whole genome v1.0 sequence (Verde et al., 2013) on the Genome Database for Rosaceae (Jung et al., 2014). Two markers within the *G4MD.1* haplotype region and their estimated genetic positions based on the Prunus-TE-F_2 reference map are included in gray.

Table 9. The eight unique functional and simple functional *G4MD.1* 4-SNP haplotypes for distinguishing haplotype effect on MD timing among 936 total individuals (Selected = 161 cultivars and selections) (Un-selected = 777 F_1 seedlings) found within the RosBREED pedigree "development set" screened with the 9K array (N is number of haplotypes analyzed).

Locus	4-SNP haplotype		Total		Selected		Un-selected	
	Sequence	Function	N=1872	Frequency	N=322	Frequency[z]	N=1550	Frequency[y]
G4MD.1	BBBA	VE	793	0.42	127	0.39	666	0.42
	ABBB	E	59	0.03	8	0.03	51	0.03
	ABBA	M	509	0.27	99	0.31	410	0.27
	BBAA	L	80	0.04	7	0.02	73	0.05
	AAAA	VL1	60	0.03	4	0.01	56	0.03
	AAAB	VL2	297	0.17	35	0.11	262	0.17
	BAAB	XL	12	0.01	2	0.01	10	0.01
	BBBB	alm	62	0.03	40	0.12	22	0.02

[z]Frequency of haplotypes in RosBREED cultivars or selections.
[y]Frequency of haplotypes in RosBREED un-selected germplasm (i.e. seedlings).

The ANOVA for MD using G4mat functional haplotypes (VE, E, M, L, VL1, VL2, XL, and alm) and RosBREED breeding programs (CA, TX, SC, UA), across two years (2011, 2012) showed that breeding program, year, and G4mat haplotype, as well as the interactions between breeding program × G4mat haplotype, and breeding program × year, had significant effects on MD ($P \leq 0.05$) (Table 10). However, the interactions between year × G4mat haplotype and year × G4mat diplotype × breeding program did not have significant effects on MD (P = 0.3740 and P = 0.6482, respectively). Additionally, individuals with haplotypes alm, XL, M, L, VL2, and VL1 had significantly higher 2011-2012 avg MD least square means (alm = 235.3, XL = 212.6, M = 208.2, L =207.3, VL2 = 203.8, VL1 = 199.1) than individuals with haplotypes VE (185.3) and E (171.5) (Table 11). Individuals with alm haplotype had significantly higher 2011-2012 avg MD least square means than individuals with all other haplotypes, excluding XL.

Table 10. Analysis of variance, degrees of freedom (DF), and F-test p-value (*P*) for maturity date (day of year, 0-365) ratings, using G4mat functional haplotypes (VE, E, M, L, VL1, VL2, XL, and alm), and RosBREED material breeding programs [Arkansas (UA), UC Davis (CA), Texas A&M (TX), Clemson (CU)], across two years (2011, 2012).

Source	DF	P
Breeding program	3	<0.0001[Z]
Year	1	<0.0001
G4mat haplotype	22	<0.0001
Year*G4mat haplotype	24	0.3740
Breeding program*G4mat haplotype	26	<0.0001
Breeding program*Year	5	<0.0001
Year*G4mat diplotype*Breeding program	27	0.6482

Table 11. Least square means of maturity date (MD) (day of year, 0-365) for G4mat haplotypes (alm, XL, VL1, VL2, L, M, E, and VE) across all RosBREED material, 2011-2012 avg.

Haplotype	MD
alm	235.3 a[z]
XL	212.6 abc
M	208.2 b
L	207.3 bc
VL2	203.8 c
VL1	199.1 c
VE	185.3 d
E	171.5 d

[z] Means followed by the same letter are not significantly different as determined by least square means $P \leq 0.05$.

Pubescent (Peach) vs. Glabrous (Nectarine)

A total of 11 informative SNP markers which spanned the previously identified QTL for pubescence vs. glabrous at the *G-locus* [13,025,129 (SNP_IGA_595786) to 16,774,236 bp (SNP_IGA_603047)] on scaffold 5 of the *P. persica* genome sequence v1.0 were selected for haplotyping (Le Dantec et al., 2010) (Fig. 9). Using these 11 SNPs, functional *G-locus* SNP haplotypes were successfully developed for 663 individuals in the RosBREED pedigree "development set" (Table 12). Of these 663 individuals, 15 unique haplotype sequences were observed, and subsequently converted into functional haplotype groups based on haplotype effect on pubescence development [glabrous/nectarine (go, g1, g2, g4, g5) and pubescent/peach (G1, G2, G2b, G3, G3b, G4, G5, G6, G7, and Galm)]. The frequencies for each unique functional haplotype ranged from <1% (G5) to 25% (G3). In selected germplasm, the haplotype frequencies for 141 individuals ranged from ≤ 1% (go, g5, G5, and G7) to 36% (G3). In unselected germplasm, the haplotype frequencies for 492 individuals ranged from 0% (G5) to 26% (g1) (Table 12).

Additionally, all functional SNP haplotypes were divided into two simple functional haplotype groups defined functional allele ‘P’ [pubescent (peach)], and ‘n’ [glabrous (nectarine)]. Overall, all individuals with simple functional diplotype P | P were rated as peaches in the field (Fig. 10). Nearly all individuals with simple functional diplotype P | n were rated as peaches (99%), excluding two individuals (1%). On the other hand, only 91% of individuals with simple functional diplotype n | n were rated as nectarines, and the other 9% were rated as peaches.

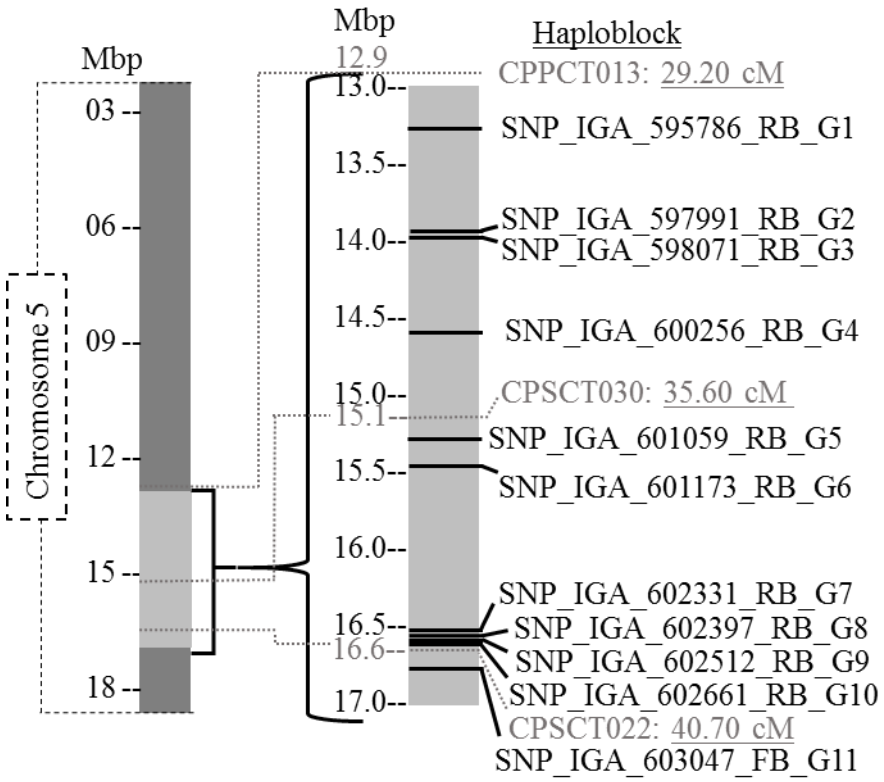

Fig. 9. Graphical representation of *P. persica* chromosome 5 with the enlarged location of ~4 Mbp including the 11 SNPs used for haplotyping at the *G-locus*. Physical locations were sourced from the *P. persica* whole genome v1.0 sequence (Verde et al., 2013) housed on the Genome Database for Rosaceae (Jung et al., 2014). Three markers within the *G-locus* SNP haploblock and their estimated genetic positions based on the Prunus-TE-F_2 reference map are included in gray.

Table 12. The 15 unique functional and simple functional *G*-locus 11-SNP haplotypes for distinguishing among 663 total pubescent/peach (P) or glabrous/nectarine (n) individuals (Selected = 141 cultivars and selections) (Un-selected = 492 F_1 seedlings) found within the RosBREED pedigree "development set" screened with the 9K array (N is number of haplotypes analyzed).

Locus	11-SNP haplotype			Total		Selected		Un-selected	
	Sequence	Function	Simple function	N=1266	Frequency	N=282	Frequency[z]	N=984	Frequency[y]
G-locus	ABAAABABAAA	go	n	58	0.05	1	0.00	57	0.06
	ABAAABBABBB	g1	n	294	0.24	31	0.11	263	0.26
	AAABBBBAAAA	g2	n	38	0.03	3	0.01	35	0.04
	AAABBBBBAAA	g4	n	32	0.02	6	0.02	26	0.02
	AAABBAABAAA	g5	n	34	0.02	1	0.00	33	0.03
	AABBBAABAAA	G1	P	40	0.03	11	0.04	29	0.03
	ABBBBAABAAA	G2	P	69	0.05	55	0.20	14	0.01
	AABBBAABBBB	G2b	P	80	0.06	3	0.01	77	0.08
	BBBBBAABAAB	G3	P	298	0.25	101	0.36	197	0.21
	BBBBBAABAAA	G3b	P	86	0.07	8	0.03	78	0.08
	BABBBAAABBB	G4	P	68	0.06	27	0.10	41	0.04
	BBBBBBBAABB	G5	P	1	0.00	1	0.00	0	0.00
	BBAABAABBBB	G6	P	86	0.07	3	0.01	83	0.09
	ABAABAABAAA	G7	P	36	0.02	1	0.00	35	0.04
	BBBBBABBBAB	Galm	P	46	0.03	30	0.11	16	0.01

[z]Frequency of haplotypes in RosBREED cultivars or selections.
[y]Frequency of haplotypes in RosBREED un-selected germplasm (i.e. seedlings).

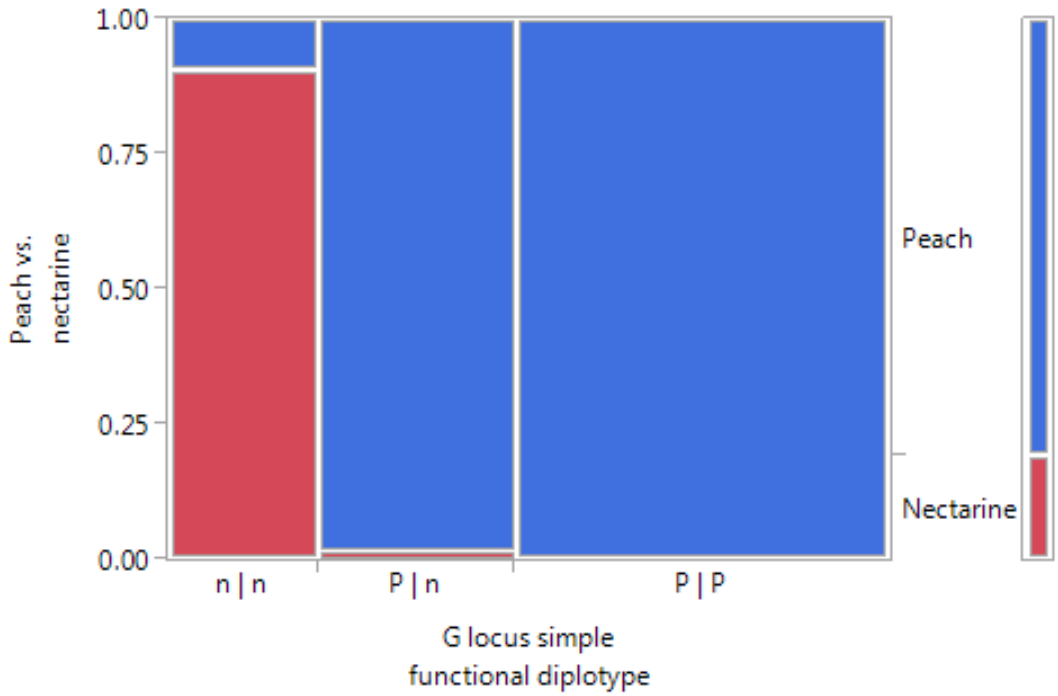

Fig. 10. Mosaic plot distributions of pubescent (peach) vs. glabrous (nectarine) simple functional diplotypes (P | P, P | n, and n | n) by actual phenotype across all germplasm in the RosBREED pedigree "development set" (JMP® 12).

White vs. Yellow Flesh Color

A total of 11 informative SNP markers which spanned the previously identified QTL for white vs. yellow flesh at the *Y-locus* [23,352,245 (SNP_IGA_81682) to 27,368,782 bp (SNP_IGA_89808)] on chromosome 1 of the *P. persica* genome sequence v1.0 were selected for haplotyping (Martínez-García et al., 2013) (Fig. 11). Using these 11 SNPs, functional *Y-locus* SNP haplotypes were successfully developed for 863 individuals in the RosBREED pedigree "development set" (Table 13). Of these 863 individuals, 19 unique haplotypes were observed, and subsequently converted into functional haplotype groups based on haplotype effect on flesh color development [yellow (y1, y2, y3, y4, y5, y6a, y6b, and y-St. John) and white (Y1a, Y1b, Y2, Y3, Y4, Y5, Yalm1, Yalm2, Hansen, Yumyeong, and Y-Galaxy)]. The frequencies for each unique functional haplotype ranged from ≤ 1% (y-St. John and Hansen) to 52% (y6a). In selected germplasm, the haplotype frequencies for 160 individuals ranged from 0% (Y5 and Y-Galaxy) to 50% (y6a). In un-selected germplasm, the haplotype frequencies for 703 individuals ranged from 0% (y-St. John, Yalm1, Yalm2, and Hansen) to 53% (y6a) (Table 13).

Additionally, all functional SNP haplotypes were divided into two simple functional haplotype groups defined functional allele 'W' (white flesh allele) and 'y' (yellow flesh allele). Overall, all three functional diplotypes, W | W, W | y, and y | y were ≥ 95% predictive. A total of 96% of individuals with simple functional diplotype W | W were scored as white flesh in the field, and the other 4% were rated as yellow flesh (Fig. 12). Likewise, 96% of individuals with simple functional diplotype y | y were scored as yellow flesh, and the other 4% were rated as white flesh. Lastly 95% of individuals with simple functional diplotype W | y were scored as white flesh in the field, and the other 5% were rated as yellow flesh.

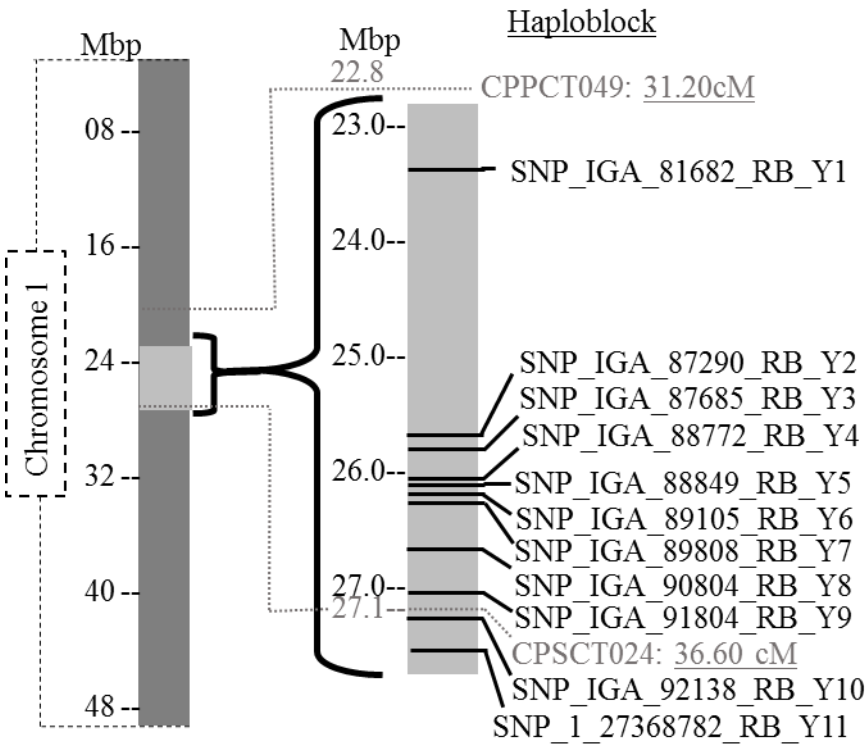

Fig. 11. Graphical representation of *P. persica* chromosome 1 with the enlarged location of the ~4 Mbp spanning the *Y*-locus including the 11 SNPs used for haplotyping. Physical locations were sourced from the *P. persica* whole genome v1.0 sequence (Verde et al., 2013) housed on the Genome Database for Rosaceae (Jung et al., 2014). Two markers within or just flanking the *Y*-locus SNP haploblock and their estimated genetic positions based on the Prunus-TE-F_2 reference map are included in gray.

Table 13. The 11 unique functional and simple functional 11-SNP haplotypes for distinguishing among 863 total white (W) or yellow (y) flesh individuals (Selected = 160 cultivars and selections) (Un-selected = 703 F_1 seedlings) found within the RosBREED pedigree "development set" screened with the 9K array (N is number of haplotypes analyzed).

Locus	11-SNP haplotype			Total		Selected		Un-selected	
	Sequence	Function	Simple function	N = 1726	Frequency	N = 320	Frequency[z]	N = 1406	Frequency[y]
Y-locus	ABAAABABAAA	y1	y	99	0.06	6	0.02	93	0.07
	ABAAABBABBB	y2	y	55	0.03	13	0.04	42	0.03
	AAABBBBAAAA	y3	y	107	0.06	17	0.05	90	0.06
	AAABBBBBAAA	y4	y	32	0.02	3	0.01	29	0.02
	AAABBAABAAA	y5	y	146	0.08	3	0.01	143	0.10
	AABBBAABAAA	y6a	y	910	0.52	160	0.50	750	0.53
	ABBBBAABAAA	y6b	y	189	0.11	58	0.18	131	0.09
	AABBBAABBBB	y-St. John	y	3	0.00	3	0.01	0	0.00
	BBBBBAABAAB	Y1a	W	33	0.02	5	0.02	28	0.02
	BBBBBAABAAA	Y1b	W	7	0.01	3	0.01	6	0.01
	BABBBAAABBB	Y2	W	34	0.02	5	0.02	29	0.02
	BBBBBBBAABB	Y3	W	21	0.01	1	0.00	20	0.01
	BBAABAABBBB	Y4	W	13	0.01	3	0.01	10	0.01
	ABAABAABAAA	Y5	W	17	0.01	0	0.00	17	0.01
	BBBBBABBBAB	Yalm1	W	23	0.01	22	0.07	0	0.00
	BBABBABBBAB	Yalm2	W	15	0.01	15	0.05	0	0.00
	BAABBAAABBB	Hansen	W	3	0.00	2	0.00	0	0.00
	BABBBBBAAAA	Yumyeong	W	7	0.01	1	0.00	6	0.01
	ABAAABBABBA	Y-Galaxy	W	12	0.01	0	0.00	12	0.01

[z]Frequency of haplotypes in RosBREED cultivars or selections.

[y]Frequency of haplotypes in RosBREED un-selected germplasm (i.e. seedlings).

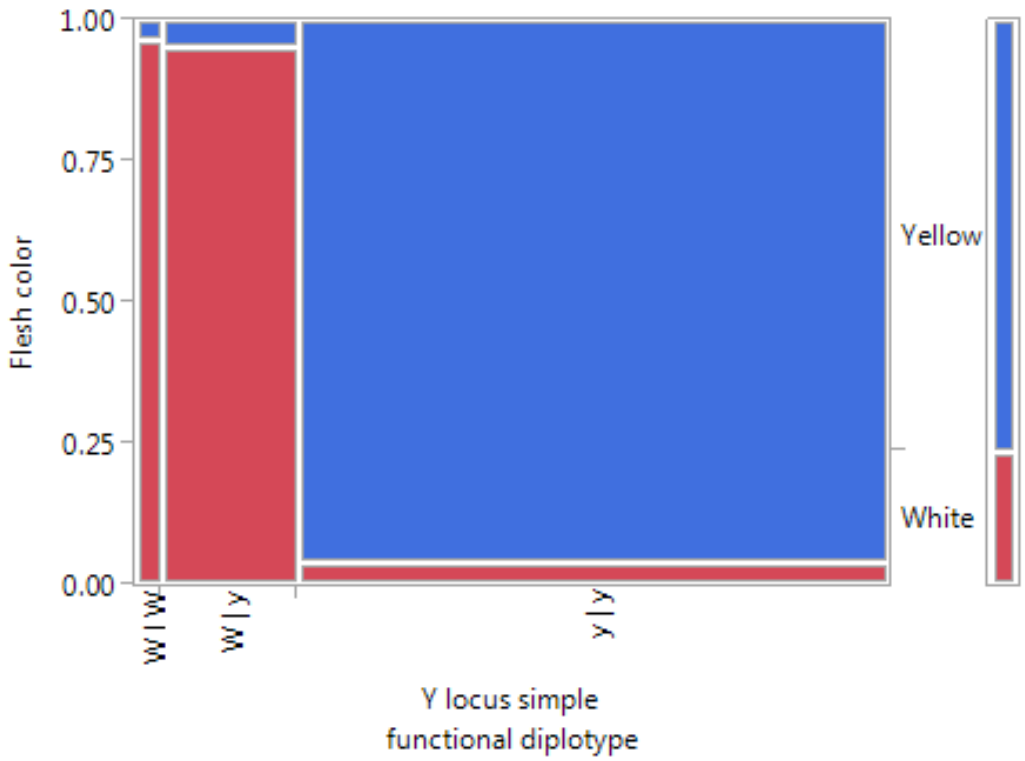

Fig. 12. Mosaic plot distributions of white vs. yellow flesh simple functional diplotypes (W | W, W | y, and y | y) by actual phenotype across all germplasm in the RosBREED pedigree "development set" (JMP® 12).

Titratable Acidity (TA)

Titratable acidity (TA) (%) exhibited an average of 0.65 (2011), 0.60 (2012), and 0.62 (2011-2012 avg) and ranged from 0.16-1.3 (2011) and 0.1-1.3 (2012 and 2011-2012 avg) across all RosBREED material (Fig. 13; Table 14). The standard deviation displayed across all material was 0.24 (2011 and 2011-2012 avg) and 0.25 (2012), and all data sets approached normality. In UA material, TA exhibited an average of 0.54 (2011), 0.49 (2012), and 0.52 (2011-2012 avg), and ranged from 0.2-1.29 (2011), 0.1-1.22 (2012), and 0.1-1.29 (2011-2012 avg). The standard deviation displayed across all UA material was 0.29 (2011), 0.23 (2012), and 0.27 (2011-2012 avg), and all data sets were skewed towards low TA. In CA material, TA exhibited an average of 0.61 (2011), 0.55 (2012), and 0.58 (2011-2012 avg), and ranged from 0.16-1.32 (2011) and 0.15-1.3 (2012 and 2011-2012 avg). The standard deviation displayed across all CA material was 0.20 (2011), 0.22 (2012), and 0.21 (2011-2012 avg), and all data sets were skewed towards low TA, with several high acid outliers. In TX material, TA exhibited an average of 0.71 (2011), 0.65 (2012), and 0.67 (2011-2012 avg), and ranged from 0.26-1.32 (2011), 0.21-1.25 (2012), and 0.21-1.32 (2011-2012 avg). The standard deviation displayed across all data sets from TX material was 0.29 (2011), 0.31 (2012), and 0.30 (2011-2012 avg), and all data sets showed a bimodal distribution, with a high acid and low acid group. In CU material, TA exhibited an average of 0.71 (2011), 0.69 (2012), and 0.70 (2011-2012 avg), and ranged from 0.28-1.34 (2011), 0.15-1.28 (2012), and 0.15-1.34 (2011-2012 avg). The standard deviation displayed across all CU material was 0.19 (2011), 0.22 (2012), and 0.20 (2011-2012 avg), and all data sets approached normality (Fig. 13; Table 14).

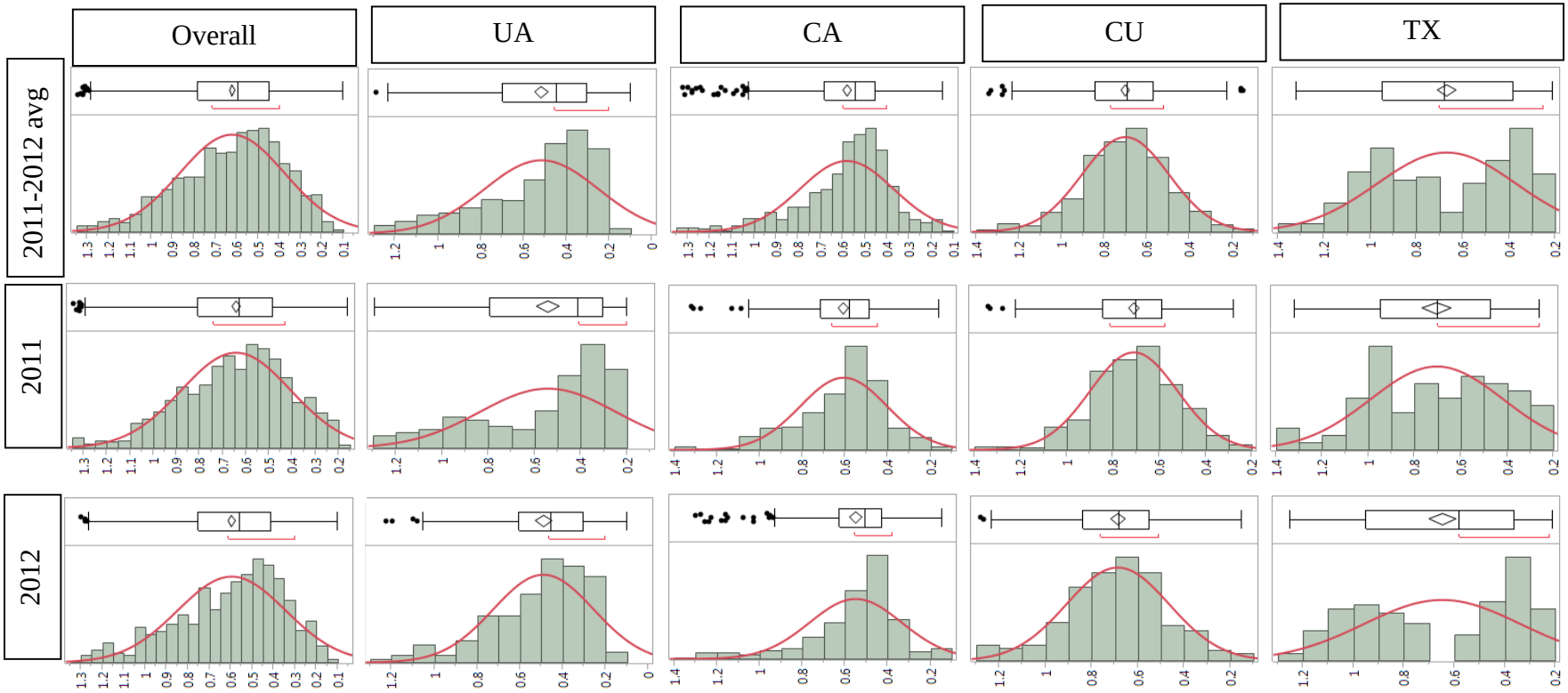

Fig. 13. Histograms and box plots of titratable acidity (TA) (%) distribution in four RosBREED breeding programs [Arkansas (UA), UC Davis (CA), Texas A&M (TX), Clemson (CU)] across two years (2011, 2012) across two years (2011, 2012) and 2011-2012 avg. (*note that the X-axis scales are slightly different across the four programs) (Normalized goodness of fit curves, are presented in red to highlight normality or skewness of data).

Table 14. Mean, minimum, maximum, and standard deviation of titratable acidity (TA) (%) for four RosBREED breeding programs [Arkansas (UA), UC Davis (CA), Texas A&M (TX), Clemson (CU)] in 2011, 2012, and 2011-2012 average (avg) (N is number of individuals).

Program	Year	TA%				N
		Mean	Min.	Max.	Std dev.	
Overall	2011	0.65	0.16	1.34	0.24	773
Overall	2012	0.60	0.10	1.30	0.25	701
Overall	2011-2012 avg	0.62	0.10	1.34	0.24	1474
UA	2011	0.55	0.20	1.29	0.30	140
UA	2012	0.49	0.10	1.23	0.23	144
UA	2011-2012 avg	0.52	0.10	1.29	0.27	284
CA	2011	0.61	0.16	1.32	0.20	269
CA	2012	0.55	0.15	1.30	0.22	222
CA	2011-2012 avg	0.58	0.15	1.32	0.21	491
TX	2011	0.71	0.26	1.32	0.29	81
TX	2012	0.65	0.21	1.25	0.31	127
TX	2011-2012 avg	0.67	0.21	1.32	0.30	208
CU	2011	0.71	0.28	1.34	0.19	280
CU	2012	0.69	0.15	1.28	0.22	205
CU	2011-2012 avg	0.70	0.15	1.34	0.20	485

The *D-locus* spanned ~200 kbp, on *P. persica* chromosome 5 and SNP_IGA_545261 (821,372 bp) was more informative than SNP_IGA_546094 (987,702 bp) (Fig. 14). Using SNP_IGA_545261, three unique functional genotypes (SNP_IGA_545261) were successfully developed for 989 individuals in the RosBREED pedigree "development set" (Table 15). In total, the frequencies for each unique functional genotype ranged 2% (D | D) to 82% (d | d). In selected germplasm, the haplotype frequencies for 175 individuals ranged from 1% (D | D), to 87% (d | d). In un-selected germplasm, the haplotype frequencies for 814 individuals ranged from 2% (D | D) to 81% (d | d).

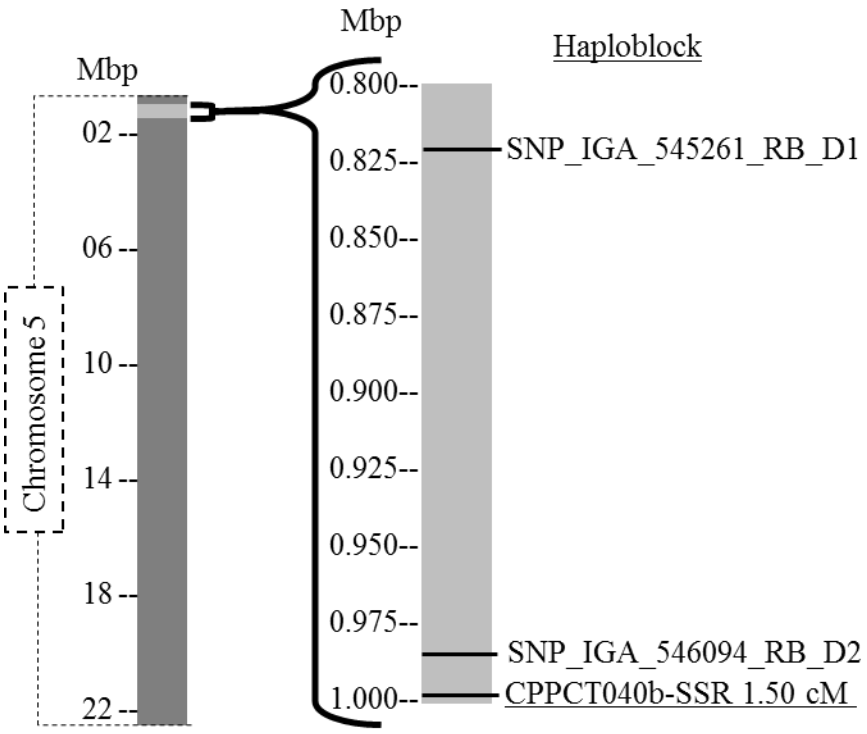

Fig. 14. Graphical representation of *P. persica* chromosome 5 including the 2 SNP markers and the CPPCT040b-SSR used for genotyping at the *D-locus* (~200 kbp region). Physical locations were sourced from the *P. persica* Whole Genome v1.0 (Verde et al., 2013) on the Genome Database for Rosaceae (Jung et al., 2014). Two flanking markers and their estimated genetic positions based on the Prunus-TE-F_2 reference map are included in gray.

Table 15. The three unique functional genotypes (SNP_IGA_545261) for distinguishing genotype effect on titratable acidity (TA) among 989 total individuals (Selected = 175 cultivars and selections) (Un-selected = 814 F_1 seedlings) found within the RosBREED pedigree "development set" screened with the 9K array (N is number of haplotypes analyzed).

Locus	Genotype		Total		Selected		Un-selected	
	Sequence	Function	N=989	Frequency	N=175	Frequency[z]	N=814	Frequency[y]
D	A | A	D | D	16	0.02	1	0.01	15	0.02
	A | B	D | d	163	0.16	21	0.12	142	0.17
	B | B	d | d	810	0.82	153	0.87	657	0.81

[z]Frequency of haplotypes in RosBREED cultivars or selections.

[y]Frequency of haplotypes in RosBREED un-selected germplasm (i.e. seedlings).

The ANOVA for TA (%) using SNP_IGA_545261 genotypes and RosBREED breeding programs (CA, TX, SC, UA), across two years (2011, 2012) showed that breeding program, year and genotype, as well as the interaction between year × genotype × breeding program were significant sources of variation for TA ($P \leq 0.05$) (Table 16). However, the interactions between year × genotype, breeding program × genotype, and breeding program × year did not have significant effect on TA (P = 0.2688, 0.06, and 0.64, respectively). Additionally, individuals homozygous d | d had a significantly higher 2011-2012 avg TA least square mean (0.68) than individuals which were D | d (0.38), and D | D (0.35) (Table 17). No significant differences were reported between individuals which were D | d or D | D.

Table 16. Analysis of variance, degrees of freedom (DF), and F-test p-value (*P*) for titratable acidity (TA) (%) using SNP_IGA_545261 genotypes (d | d, D | d, D | D), and RosBREED material breeding programs [Arkansas (UA), UC Davis (CA), Texas A&M (TX), Clemson (CU)], across two years (2011, 2012).

Source	DF	P
Breeding program	3	<0.0001[Z]
Year	1	<0.0001
Genotype	2	<0.0001
Year*Genotype	4	0.2688
Breeding program*Genotype	6	0.06
Breeding program*Year	5	0.64
Year*Genotype* Breeding program	8	<0.0001

Table 17. Least square means of titratable acidity (TA) (%) for SNP_IGA_545261 genotypes of all RosBREED material, 2011-2012 avg.

Genotype	TA (%)
d | d	0.68 a[Z]
D | d	0.38 b
D | D	0.35 b

[Z] Means followed by the same letter are not significantly different as determined by least square means $P \leq 0.05$.

Soluble Solids Content (SSC) and Titratable Acidity (TA)

Soluble solids content (SSC) (%) exhibited an average of 13.5 (2011), 13.8 (2012), and 13.6 (2011-2012 avg) and ranged from 7.2-25.7 (2011 and 2011-2012 avg) and 7.2-24.3 (2012) across all RosBREED material (Fig. 15; Table 18). The standard deviation displayed across all material was 2.8 (2011), 3.1 (2012), and 2.9 (2011-2012 avg), and all data sets were slightly skewed towards low SSC. In UA material, SSC (%) exhibited an average of 15.9 (2011), 17.1 (2012), and 16.5 (2011-2012 avg), and ranged from 11.3-24.3 (2011) and 10.9-24.3 (2012 and 2011-2012 avg). The standard deviation displayed across all UA material was 1.2 (2012 and 2011-2012 avg) and 1.3 (2011), and all data sets were slightly skewed towards low SSC%. In CA material, SSC (%) exhibited an average of 13.9 (2011), 14.8 (2012), and 14.3 (2011-2012 avg) and ranged from 7.2-25.7 (2012 and 2011-2012 avg) and 10.1-23.1 (2012). The standard deviation displayed across all CA material was 2.5 (2011), 1.9 (2012), and 2.3 (2011-2012 avg), and all data sets were slightly skewed towards low SSC. In TX material, SSC (%) exhibited an average of 11.7 (2011) and 11.8 (2012 and 2011-2012 avg), and ranged from 8.2-20.8 (2011), 8.8-21.1 (2012), and 8.2-21.1 (2011-2012 avg). The standard deviation displayed across all data sets from TX material was 2.2 (2011), 1.9 (2012), and 2.0 (2011-2012 avg) and all data sets were skewed towards low SSC. In CU material, SSC (%) exhibited an average of 12.2 (2011), 11.7 (2012), and 12.0 (2011-2012 avg), and ranged from 7.4-19.8 (2011), 7.2-16.3 (2012), and 7.2-19.8 (2011-2012 avg). The standard deviation displayed across all CU material was 2.3 (2011), 1.8 (2012), and 2.1 (2011-2012 avg), and all data sets approached normality (Fig. 15; Table 18).

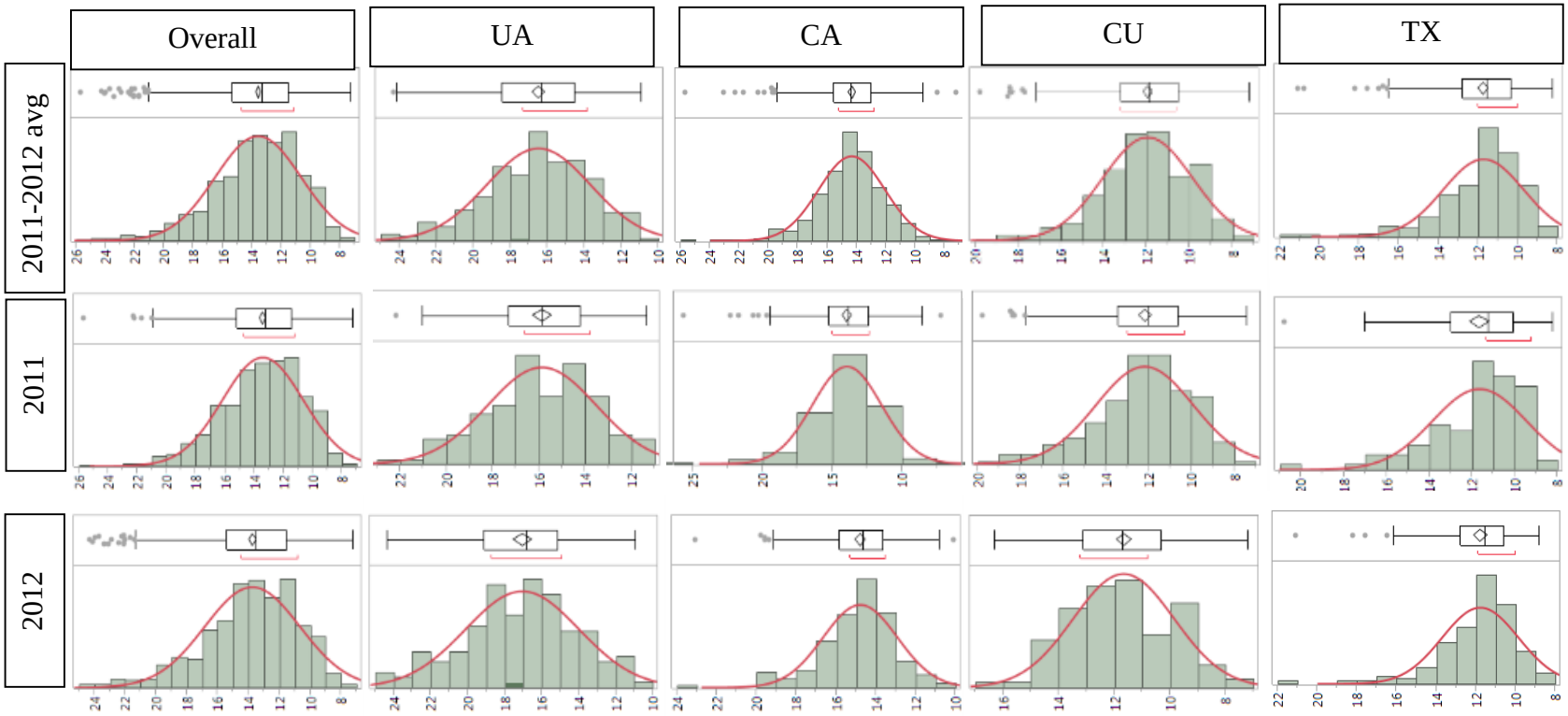

Fig. 15. Histograms and box plots of soluble solid content (SSC) (%) distribution in four RosBREED breeding programs [Arkansas (UA), UC Davis (CA), Texas A&M (TX), Clemson (CU)] across two years (2011, 2012) and 2011-2012 avg (*note that the X-axis scales are slightly different across the four programs) (Normalized goodness of fit curves, are presented in red to highlight normality or skewness of data).

Table 18. Mean, minimum, maximum, and standard deviation of soluble solid content (SSC) (%) ratings for four RosBREED breeding programs [Arkansas (UA), UC Davis (CA), Texas A&M (TX), Clemson (CU)] in 2011, 2012, and 2011-2012 average (avg) (N is number of individuals).

Program	Year	SSC%				N
		Mean	Min.	Max.	Std dev.	
Overall	2011	13.5	7.2	25.7	2.8	675
Overall	2012	13.8	7.2	24.3	3.1	645
Overall	2011-2012 avg	13.6	7.2	25.7	2.9	1320
UA	2011	15.9	11.3	24.3	2.4	136
UA	2012	17.1	10.9	24.3	3.1	143
UA	2011-2012 avg	16.5	10.9	24.3	2.8	279
CA	2011	14.0	7.2	25.7	2.5	218
CA	2012	14.8	10.1	23.1	1.9	182
CA	2011-2012 avg	14.4	7.2	25.7	2.3	400
TX	2011	11.7	8.2	20.8	2.2	90
TX	2012	11.8	8.8	21.1	1.9	135
TX	2011-2012 avg	11.8	8.2	21.1	2.0	225
CU	2011	12.2	7.4	19.8	2.3	229
CU	2012	11.7	7.2	16.3	1.8	183
CU	2011-2012 avg	12.0	7.2	19.8	2.1	412

The *G7Flav* spanned ~1 Mbp on *P. persica* chromosome 1 and the two SNP markers used for haplotype construction were located within ~14.07 (SNP_IGA_773659) to 14.95 Mbp (SNP_IGA_776348) (Fig. 16). Using these two SNPs, functional SNP haplotypes were successfully developed for 819 individuals in the RosBREED pedigree "development set" (Table 19). Of these 819 individuals, four unique haplotypes were observed, and subsequently converted into functional haplotype groups based on haplotype effect on TA and SSC [high TA and SSC (H), medium TA and SSC (M), low TA and SSC (L), and M/L recombinant]. In total, the frequencies for each unique functional haplotype ranged from 1% (M/L recombinant) to 40% (M). In selected germplasm, the haplotype frequencies for 103 individuals ranged from 1% (M/L recombinant) to 54% (M). In un-selected germplasm, the haplotype frequencies for 716 individuals ranged from 1% (M/L recombinant) to 54% (M) (Table 19).

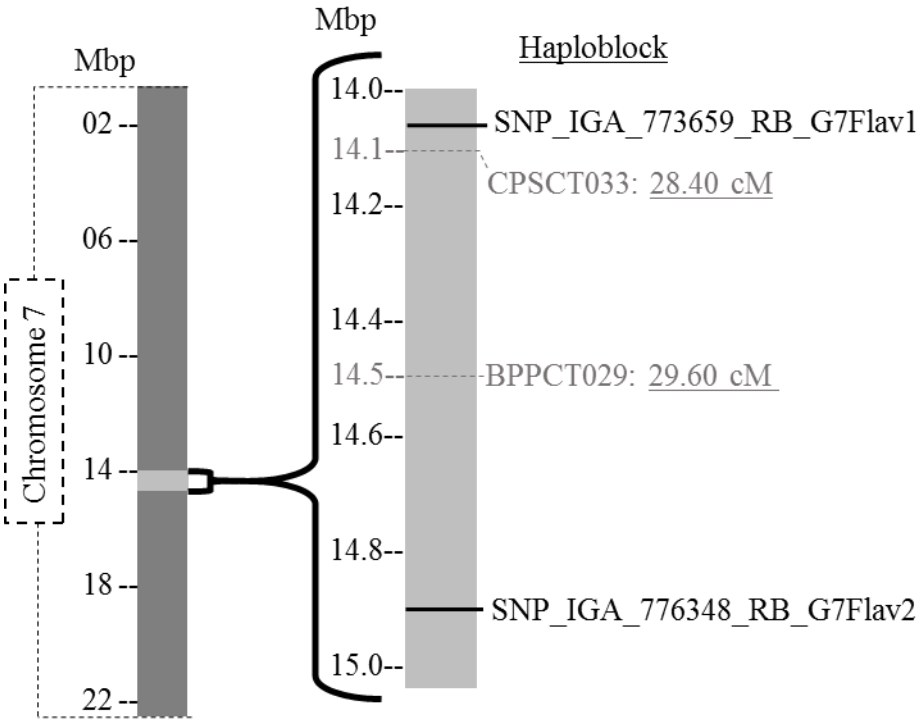

Fig. 16. Graphical representation of *P. persica* chromosome 7 with the enlarged location of the ~1 Mbp flanking the two SNPs used for haplotyping at the *G7Flav-locus*. Physical locations were sourced from the *P. persica* whole genome v1.0 sequence (Verde et al., 2013) housed on the Genome Database for Rosaceae (Jung et al., 2014). Two markers within the G4Mat haplotype region and their estimated genetic positions based on the Prunus-TE-F_2 reference map are included in gray.

Table 19. The four unique functional *G7Flav* 2-SNP haplotypes for distinguishing haplotype effect on titratable acidity (TA) (%) and soluble solids content (SSC) (%), among 819 total individuals (Selected = 206 cultivars and selections) (Un-selected = 716 F_1 seedlings) found within the RosBREED pedigree "development set" screened with the 9K array (N is number of haplotypes analyzed).

Locus	2-SNP haplotype		Total		Selected		Un-selected	
	Sequence	Function	N=1638	Frequency	N=206	Frequency[z]	N=1432	Frequency[y]
G7Flav	AA	H	522	0.32	53	0.26	469	0.33
	AB	M	651	0.40	112	0.54	539	0.38
	BA	L	454	0.27	40	0.19	414	0.28
	BB	M/L recombinant	11	0.01	1	0.01	10	0.01

[z]Frequency of haplotypes in RosBREED cultivars or selections.
[y]Frequency of haplotypes in RosBREED un-selected germplasm (i.e. seedlings).

The ANOVA for TA (%), using G7Flav diplotypes and RosBREED breeding programs (CA, TX, SC, UA), across two years (2011, 2012) showed that breeding program, year and G7Flav diplotype, as well as the interactions between breeding program × G7Flav diplotype and breeding program × year were significant sources of variation for TA ($P \leq 0.05$) (Table 20). However, the interactions between year × G7Flav diplotype and year × breeding program × G7Flav diplotype were not significant for TA ($P > 0.05$). Additionally, individuals with diplotypes that only contained H and M alleles had significantly higher 2011-2012 avg TA least square means (H | M = 0.696, M | M = 0.663, H | H = 0.660) than diplotypes which contained at least one L allele, L | L (0.583), H | L (0.578), and L | M (0.516) (Table 21).

The ANOVA for SSC, using G7Flav diplotypes and RosBREED breeding programs (CA, TX, SC, UA) across two years (2011, 2012), showed that breeding program, year, and G7Flav diplotype, as well as the interaction between year × G7Flav diplotype were significant sources of variation for SSC ($P \leq 0.05$) (Table 22). However, the interactions between breeding program × G7Flav diplotype, breeding program × year, and year × breeding program × G7Flav diplotype were not significant for SSC ($P > 0.05$). Additionally, individuals with the L | M diplotype had a significantly higher 2011-2012 avg SSC least square mean (14.6) than all other diplotypes, excluding H | L (13.8) (Table 23). Contrarily, individuals with homozygous L | L diplotype had a significantly lower 2011-2012 avg SSC least square mean (12.8) than all other diplotypes, excluding H | H (13.4).

Table 20. Analysis of variance, degrees of freedom (DF), and F-test p-value (*P*) for soluble solid content (SSC) (%), using G7Flav diplotypes (H | H, H | M, H | L, M | M, M | L, L | L), and RosBREED material breeding programs (CA, TX, SC, UA), across two years (2011, 2012).

Source	DF	P
Breeding program	3	<0.0001[Z]
Year	1	0.0019
G7Flav diplotype	5	<0.0001
Year*G7Flav diplotype	7	0.1629
Breeding program*G7Flav diplotype	9	0.0090
Breeding program*Year	5	<0.0001
Year*G7Flav diplotype*Breeding program	11	0.1190

Table 21. Least square means of titratable acidity (TA) (%) for G7flav diplotypes (H | H, H | M, H | L, M | M, M | L, L | L) of all RosBREED material, 2011-2012 avg.

Diplotype	TA (%)
H | M	0.696 a[Z]
M | M	0.663 a
H | H	0.660 a
L | L	0.583 b
H | L	0.578 b
L | M	0.516 b

[Z] Means followed by the same letter are not significantly different as determined by least square means $P \leq 0.05$.

Table 22. Analysis of variance, degrees of freedom (DF), and F-test p-value (*P*) for titratable acidity (TA) (%), using G7Flav diplotypes (H | H, H | M, H | L, M | M, M | L, L | L), and RosBREED material breeding programs (CA, TX, SC, UA), across two years (2011, 2012).

Source	DF	P
Breeding program	3	<0.0001
Year	1	<0.0001
G7Flav diplotype	5	<0.0001
Year*G7Flav diplotype	7	0.0228
Breeding program*G7Flav diplotype	9	0.0642
Breeding program*Year	5	0.3596
Year*G7Flav diplotype*Breeding program	11	0.1280

Table 23. Least square means of soluble solid content (SSC) (%) for G7flav diplotypes (H | H, H | M, H | L, M | M, M | L, L | L) of all RosBREED material, 2011-2012 avg.

Diplotype	SSC (%)
L | M	14.6 a[z]
H | L	13.8 ab
M | M	13.6 b
H | M	13.6 b
H | H	13.4 bc
L | L	12.8 c

[z] Means followed by the same letter are not significantly different as determined by least square means $P \leq 0.05$.

Peach Mini-SNP Array v1.0 Analysis

Once raw results were received from the two service providers, SNPs were grouped together into haplotypes for each trait. Control individuals, which were previously genotyped with the Infinium 9K array, contained matching haplotypes on the mini-SNP array for most traits. These control individuals were critical for determining successful translation of haplotypes (i.e. trait predictions) from the 9K array to the mini-SNP arrays.

Overall, genotypic data acquired accurately predicted phenotypic performance in ~75% of cases across material from all breeding programs (Table 24). This included all "Unambiguous individuals" representing those with one or zero heterozygous SNPs, which enabled each unambiguous individual's genotype to be directly determined. The additional ~25% of material required specific attention to assigning the correct haplotypes to the correct phenotypes. These results are due to the vast haplotypic diversity in U.S. peach breeding germplasm, where some germplasm carried unique haplotypes or had common haplotypes associated with an unusual trait level. "Unambiguous individuals (≥two SNP het)" represents those individuals that had two or more heterozygous SNP, and thus a pedigree-tracking approach in which the parents and grandparent haplotypes (from the 9K array), for each individual on the SNP mini-arrays, were tracked through identity by descent (IBD) and used to decipher individuals with complicated alleles. Process of elimination was also used in certain cases. If an individual's genotype could still not be determined, usually due to several heterozygous SNPs, they were labeled as "ambiguous individuals" and results were not reported.

Table 24. Results of the two mini-SNP arrays, received from service providers (1 and 2). "Unambiguous individuals" represent those with one or zero heterozygous SNPs; "unambiguous individuals (≥two SNP het)" represents those individuals that had two or more heterozygous SNP; "ambiguous individuals" represents individuals whose genotype could still not be determined, due to too many heterozygous SNPs.

Trait	Locus name	SNPs (N)	Service provider	Unambiguous	Unambiguous (≥two SNP het)	Ambiguous
Fruit bacterial spot	*G1XapF*	4	1	35	64	1
Fruit bacterial spot	*G6XapF*	4	1	37	62	1
Maturity date	*G4Mat*	4	1 and 2	65	20	15
Fruit type peach vs. nectarine	*G*	1	1	99	0	1
		2-8	-,2,-,1,1,1,1,1	75	15	10
		9	1	129	0	63
		10	1	190	0	2
Flesh color white vs. yellow	*Y*	1	1	99	0	1
		1	1	99	0	1
		1	2	87	0	13
		4	1,1,2,2	70	28	2
Blush	*Rf*	4	1,1,2,2	51	45	4
Titratable acidity (TA)	*D*	1-2	1	99	0	1
				99	0	1
TA, soluble solid content (SSC)	*G7Flav*	2	2	71	28	1

Discussion

Fruit Bacterial Spot

In comparison to all other programs, at both *Xap* fruit loci (*G1XapF* and *G6XapF*), UA contained the least proportion of SU haplotypes (20 and 14%) and the highest proportion of R1 and I haplotypes combined (80%) and R1 and R2 haplotypes (87%). These results have merit since UA has been breeding for *Xap* resistance since 1964 and unlike the other programs, antibiotic sprays to control the disease have never been applied, thus selection against *Xap* has been possible (J.R. Clark, personal communication). Additionally, the CA program contained two haplotypes unique only to their program, one for *G1XapF* (R2 haplotype) and one for *G6XapF* (I haplotype). Unlike the other three fresh-market peach breeding programs, CA focuses on processing peach breeding, and is a very diverse program which uses directed introgression from different species such as almond [*Prunus dulcis* (Mill.) D.A.Webb], *P. argentea* (Lam.) Rehder, *P. davidiana* (Carrière) Franch, and *P. mira* Koehne (Fresnedo-Ramírez et al., 2015; Gradziel 2002 and 2003). The R2 haplotype (*G1XapF*) and the I haplotype (*G6XapF*) originate from these other *Prunus.* species, which supports why only CA contains them.

However, both loci were only associated with a very small amount of Vp for *Xap* fruit resistance (~8% each). The results in Chapter Two of this dissertation supported the findings of previous studies that *Xap* strain and population virulence levels from different locations were observed to differ remarkably among different peach and other stone fruit cultivars bred from different locations (Civerolo, 1975; Du Plessis, 1988; OEPP/EPPO, 2006; Scortichini et al., 1996). Thus, it will be important to combine broad horizontal *Xap* resistance in order for cultivars that are planned to be grown in multiple different environments across the U.S. to maintain resistance to the *Xap* isolates from all regions. Although, this will be a difficult task for

peach breeders to accomplish, it can be attained by first developing and validating SNP haplotypes for the nine consensus major QTLs (G1*Xap*F.1, G1*Xap*F.2+L.2, G1*Xap*F.3+L_{AR}.1+L.1, G2*Xap*L.1+L_{AR}.1+F.1, G2*Xap*L.2+F.2+F.3, and G6*Xap*F+L.1) (each associated with > 10% Vp for *Xap* fruit resistance) identified in Chapter Two of this dissertation. Upon development, breeders from other programs can then screen on their own material to confirm that the DNA test is robust across their material. After confirmation, the breeders can then incorporate a wide array of DNA tests for *Xap* resistance for use in MAPS and MASS to efficiently combine resistance alleles at multiple loci, with high fruit quality, spanning the entire season.

Blush Overcolor

In general, across both years the UA and TX programs each were slightly skewed towards high blush ratings, and neither showed individuals with a 0 blush rating. Likewise, the CU program was slightly skewed towards high blush ratings, and spanned the 0-5 scale in 2012 and 2011-2012 avg. In contrast, the CA blush distributions were skewed towards lower blush ratings, and did not have an individual rated as 5 blush. The different blush distributions observed across the four programs follow suit with their different breeding objectives. Three of these programs breed for fresh-market peaches with high blush (UA, CU, TX), while CA traditionally has bred for low blush processing peaches, explaining why UA, CU, and TX distributions were skewed towards high blush ratings, and CA was skewed towards low blush ratings (J.R. Clark, K. Gasic, D. Byrne, and T. Gradziel, personal communication).

Using the RosBREED CRS and BPS "development set", the major blush QTL identified in Frett et al., (2014), *Blush.Pp.ZC-3.1*, was developed into an informative 4-SNP based DNA test, which was subsequently validated to accurately predict blush development. Individuals with

simple functional blush diplotype B | B had significantly higher 2011-2012 avg blush medians than individuals with B | b or b | b (3.0 vs. 2.0 vs. 1.0). Likewise, individuals with simple functional diplotype B | b had significantly higher 2011-2012 avg blush medians than individuals with b | b (2.0 vs. 1.0). Lastly, the 240 individuals screened with the peach mini-SNP array v1.0. ("confirmation set"), confirmed that the 4-SNP haplotype was successful using the service provider's platform. Additionally, genotypes of 96 out of 100 individuals screened with the blush 4-SNP DNA test were able to be determined due to a low amount of heterozygous SNPs.

Maturity Date (MD)

In general, across both years CA MD distributions were skewed towards later season, while TX distributions were skewed towards early season. In contrast, UA and CU in general showed a more normal distribution from early to late season across both years. The different MD distributions observed across the four programs follow suit with their different breeding objectives. The CA program has been breeding for mid-late season processing peaches and as stated before has used directed introgression from different species such as almond to extend the season later (Fresnedo-Ramírez et al., 2015; Gradziel 2002 and 2003). In contrast, the TX program has been breeding for low-chill, early to mid-season peaches (D. Byrne, personal communication). Lastly the UA and CU programs have been breeding for peaches which span the entire season (J.R. Clark and K. Gasic, personal communication).

Using the RosBREED CRS and BPS "development set", the major MD QTL identified in Chapter Three of this dissertation, *G4MD.1*, was developed into an informative 4-SNP based DNA test, which was subsequently validated to accurately predict MD timing. Individuals with functional MD haplotypes alm, XL, M, L, VL2, and VL1 had significantly higher 2011-2012 avg MD least square means than individuals with haplotypes VE and E. Lastly, the 240 individuals

screened with the peach mini-SNP array v1.0. ("confirmation set"), confirmed that the 4-SNP haplotype was successful using the service provider's platform. Yet, only genotypes of 85 out of 100 individuals screened with the MD 4-SNP DNA test were able to be determined due to several heterozygous SNPs.

Pubescent (Peach) vs. Glabrous (Nectarine)

Using the RosBREED CRS and BPS "development set", the previously identified QTL for pubescence vs. glabrous at the *G-locus* (Le Dantec et al., 2010) on chromosome 5 of the *P. persica* genome sequence v1.0. was developed into an informative 11-SNP based DNA test, which was subsequently validated to accurately predict peach vs. nectarine 90% of the time. The 10% error rate is most likely due to recombination events within the 11-SNP haplotype. In Chapter Five Section Two of this dissertation, the predictiveness of this 11-SNP based DNA test was compared to the intragenic indelG DNA test, previously developed by Vendramin et al. (2014). The comparison between the two DNA tests highlighted that the indelG DNA test is more predictive than the SNP haplotype DNA test, since it is located within the *PpeMYB25* pubescent vs. glabrous candidate gene, thus, the recombination events that affected the 11-SNP haplotypes did not affect the intragenic DNA test. Since the indelG DNA test was 100% predictive across the Arkansas RosBREED "conversion set" material, as well as all previous material in Vendramin et al. (2014), it was concluded to be more efficient to only proceed with that test. Furthermore, although the 240 individuals screened with the peach mini-SNP array v1.0. ("confirmation set"), confirmed that the 4-SNP haplotype was successful using the service provider's platform, several individuals diplotypes were not able to be determined due to several heterozygous SNPs.

White vs. Yellow Flesh Color

Using the RosBREED CRS and BPS "development set", the previously identified QTL for white vs. yellow flesh at the *Y-locus* (Martínez-García et al., 2013) on chromosome 1 of the *P. persica* genome sequence v1.0. was developed into an informative 11-SNP based DNA test, which was subsequently validated to accurately predict white vs. yellow flesh color ~ 95% of the time. The 5% error rate is most likely due to recombination events within the 11-SNP haplotype. In Chapter Five Section Three of this dissertation the predictiveness of this 11-SNP based DNA test was compared to the intragenic PpCCD4b-SSR DNA test, previously developed by Falchi et al., (2013). The comparisons between the two DNA tests highlighted that the PpCCD4b-SSR DNA test is more predictive than the 11-SNP haplotype DNA test. The PpCCD4b-SSR DNA test is located within the *PpCCD4* flesh color candidate gene, thus, the recombination events that affected the 11-SNP haplotypes did not affect the intragenic DNA test. Since the PpCCD4b-SSR DNA test was 100% predictive across the Arkansas RosBREED "conversion set" material, it was concluded to be more efficient to only proceed with that test. Furthermore, although the 240 individuals screened with the peach mini-SNP array v1.0 ("confirmation set") confirmed that the 11-SNP haplotype was successful using the service provider's platform, some individuals diplotypes were not able to be determined due to multiple heterozygous SNPs.

Titratable Acidity (TA)

In general, across both years AR and CA TA distributions were skewed towards low TA. In contrast, CU in general showed a more normal distribution from low to high acid. Additionally, TX data sets showed a bimodal distribution, with a high acid and low acid group. The different TA distributions observed across the four programs follow suit with their different breeding objectives. The AR program has focused on both high and low acid, with more of an emphasis on low-acid cultivars (J.R. Clark, personal communication). Likewise the CA program

has also focused on breeding for low-acid peaches (T. Gradziel, personal communication). In contrast the TX and CA programs have been breeding for both high- and low-acid cultivars, with more of an emphasis on high-acid cultivars (D. Byrne and K. Gasic, personal communication).

Using the RosBREED CRS and BPS "development set", one SNP at the major TA D-locus, SNP_IGA_545261, was developed into an informative 1-SNP based DNA test, which was subsequently validated to accurately predict TA. Individuals homozygous d | d had a significantly higher 2011-2012 avg TA least square mean (0.68) than individuals which were D | d (0.38), and D | D (0.35). Also, no significant differences were reported between individuals which were D | d or D | D. Additionally 99 out of 100 "confirmation set" individuals genotypes were able to be determined, since the test only has one SNP.

Soluble Solids Content (SSC) and Titratable Acidity (TA)

Across all RosBREED material "development set", all SSC data sets were slightly skewed towards low SSC. In general, across both years AR, CA and TX SSC distributions were slightly skewed towards low SSC. In contrast, and all data sets for CU SSC distributions approached normality. The AR, TX, and CU programs have focused on fresh-market peaches with at least 12% SSC, while the CA program has focused on processing peaches with adequate SSC (D. Byrne, J.R. Clark, K. Gasic, and T. Gradziel, personal communication).

Using the RosBREED CRS and BPS "development set", two SNPs at the minor TA and SSC *G7flav*-locus, were developed into an informative 2-SNP based DNA test, which was subsequently validated to accurately predict TA and SSC. Individuals with diplotypes that only contained H and M alleles had a significantly higher 2011-2012 avg TA least square mean than diplotypes which contained at least one L allele (L | L, H | L and L | M). Additionally, individuals homozygous L | L had a significantly lower 2011-2012 avg SSC least square mean

than all other diplotypes, excluding H | H. This DNA test was developed to screen with the *D*-locus DNA test, to help differentiate high and low acid in the high acid *D*-locus group, as well as high and low acid in the low acid *D*-locus group

Mini-SNP Array

The genotypic data successfully translated into trait predictions from the peach mini-SNP array v1.0. were provided in a breeder-friendly format to each breeding program for direct use in parentage verification, genetic potential description, and parental germplasm choice, as well as for facilitating crossing decisions [i.e. marker-assisted parent selection (MAPS) and marker-assisted cross selection (MACS)]. At UA, a MAPS template incorporating all 72 selections included on the mini-array and the 21 cultivars and selections on the 9K peach array v1.0. was developed that best suited the UA breeder for use of MAPS as a tool to design crosses in 2013-2015 (See Chapter Six for further details). Crosses were designed using this DNA information in 2013-2015 to extend and diversify the later part of the Arkansas peach season: both melting, non-melting, and non-softening flesh types, peach and nectarine, white and yellow flesh, high and low acid, high blush, and resistance to fruit bacterial spot.

Overall Conclusions and Future Work

Design and use of SNP mini-arrays is feasible for Rosaceae breeders, however molecular genetic expertise is required to decide the right SNPs to use, correctly interpret results (using pedigree tracking of alleles IBD to determine haplotypes for individuals with multiple heterozygous SNPs), and troubleshoot to develop more robust SNPs when needed. Furthermore, the importance of these SNP-based DNA tests expands further through the conversion and confirmation of the SNP-based DNA tests into simple, straightforward, breeder-friendly, sequence length polymorphism-based (SLP-based) DNA tests (such as SSRs, indels, or SCARs),

to enable the same DNA test to be screened on one or both SNP haplotype-based and SLP-based platforms, upon comparison and selection for each specific trait (see Chapter Five, Sections One-Three; Salgado, 2015; Sandefur et al., 2016a; and Sandefur et al., 2016b). At the moment, SLP-based DNA tests remain a more affordable and versatile option for multi-trait MAS, as they can be multiplexed and run separately on seedlings rather than being locked in all at once to all traits on a mini-SNP array. Yet high resolution melt (HRM) SNP-based genotyping (one SNP at a time) is proving to be comparable in resource and time savings to that of the SLP-based tests using fragment analysis. Primers of comparable price to the SLP-based tests can be bought, and HRM can be performed for each SNP on a Real-Time PCR System (qPCR). The main advantage is that fragment analysis is not required as the SNPs are called during qPCR, which reduces price per sample significantly. As to which method saves more resources and time, this is dependent on the viewpoint of the researcher, yet it is advantageous for a DNA test to be screened on both SNP haplotype-based and SLP-based platforms for more flexibility and options.

The future is bright for Rosaceae fruit breeders as the material from the four RosBREED pedigree connected demonstration peach breeding programs can continue to be used to develop and validate SNP haplotypes for additional previously identified M-L-T's, as well as M-L-T's identified in the future through genome-wide quantitative trait loci (QTL) analysis using the Pedigree-Based Analysis (PBA) approach. Upon validation, the same steps as described herein can be used to group the SNP haplotypes into mini-SNP arrays for conversion and confirmation using additional material not screened with the 9K SNP array. However, it is critical to remember to use positive control individuals, which were previously genotyped with the Infinium 9K SNP array, to determine successful translation of haplotypes (i.e. trait predictions) from the 9K array to the mini-SNP arrays. Lastly, successfully developed and validated SNP-

based DNA tests can continue to be converted into simple, straightforward, breeder-friendly, SLP-based DNA tests to enable the same DNA test to be screened on one or both SNP haplotype-based and SLP-based platforms, to increase options for breeders and further entice adoption of MAS in their programs.

Literature Cited

Abbott, A.G., A.C. Lecouls, Y. Wang, L. Georgi, R. Scorza, and G. Reighard. 2002. Peach: The model genome for Rosaceae genomics. Acta Hort. 592:199-209.

Aranzana M.J., A. Pineda, P. Cosson, E. Dirlewanger, J. Ascasibar, G. Cipriani, C.D. Ryder, R. Testolin, A. Abbott, G.J. King, A.F. Iezzoni, and P. Arus. 2003 A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theor. Appl. Genet. 106:819-825.

Bassi, D., and R. Monet. 2008. Botany and Taxonomy. p. 1-30. In: D.R. Layne and D. Bassi (eds.). The peach, botany, production and uses. CAB International Press, Wallingford, Oxon, UK. 850.

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

Boudehri, K., A. Bendahmane, G. Cardinet, C. Troadec, A. Moing, E. Dirlewanger. 2009. Phenotypic and fine generic characterization of the D locus controlling fruit acidity in peach. Plant Biol. 9:59-72.

Brandi, F., E. Bar, F. Mourgues, G. Horváth, E. Turcsi, G. Giuliano, A. Liverani, S. Tartarini, E. Lewinsohn, and C. Rosati. 2011. Study of 'Redhaven' peach and its white-fleshed mutant suggests a key role of CCD4 carotenoid dioxygenase in carotenoid and norisoprenoid volatile metabolism. BMC plant biology 11: 24-38.

Byrne, D.H. 2005. Trends in stone fruit cultivar development. HortTechnology 15:494–500.

Byrne, D., M. Bassols, D. Bassi, M. Piagnani, K. Gasic, G. Reighard, M. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: M. Badenes and D. Byrne (eds.). Fruit breeding. Springer Science, Business Media, New York.

Civerolo, E.L. 1975. Quantitative aspects of pathogenesis of *Xanthomonas pruni* in peach leaves. Phytopathol. 65:258-264.

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.

Collard, B.C.Y., and D.J. Mackill. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B: Biol. Sci. 363: 557-572.

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.

Dirlewanger, E., E. Graziano, T. Joobeur, F. Garriga-Caldere, P. Cosson, W. Howad, and P. Arus. 2004. Comparative mapping and marker-assisted selection in Rosaceae fruit crops. Proc. Natl. Acad. Sci. U.S. 101:9891-9896.

Du Plessis, H.J. 1988. Differential virulence of *Xanthomonas campestris* pv. *pruni* to peach, plum, and apricot cultivars. Phytopathol. 78:1312-1315.

Edge-Garza, D.A., J.J. Luby, and C.P. Peace. 2016. Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Mol. Breed. 35:1-15.

Eduardo, I., E. López-Girona, I. Batlle, F. Reig, I. Iglesias, W. Howad, P. Arús, and M.J. Aranzana. 2014. Development of diagnostic markers for selection of the subacid trait in peach. Tree Genet. and Genomes 10:1695-1709.

Etienne, C., C. Rothan., A. Moing., C. Plomion., C. Bodenes., 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.

Falchi, R., E. Vendramin, L. Zanon, S. Scalabrin, G. Cipriani, I. Verde, G. Vizzotto, and M. Morgante. 2013. Three distinct mutational mechanisms acting on a single gene underpin the origin of yellow flesh in peach. Plant J. 76:175–187.

Fresnedo-Ramírez, J., M. CAM 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. Molecular Breeding. 35:1-19.

Fresnedo-Ramírez, 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.C.A.M. Bink, W.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. Mol. Breed. In press.

Frett, T.J., K. Gasic., J.R. Clark., D. Byrne., T. Gradziel, and C.H. 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 association with blush in peach [*Prunus persica* (L.) Batsch]. Tree Genet. and Genomes 10:367-381.

Gradziel, T.M. 2002. Almond species as sources of new genes for peach improvement. Acta Hort. 592:81-88.

Gradziel, T.M. 2003. Interspecific hybridizations and subsequent gene introgression within *Prunus* subgenus *Amygdalus*. Acta Hort. 622:249-255.

Iezzoni, A., C. Weebadde., J. Luby., Y. Chengyan., 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.

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

Jung, S., S.P. Ficklin, T. Lee, C.-H. Cheng, A. Blenda, P. Zheng, J. Yu, A. Bombarely, I. Cho, S. Ru, K. Evans, C. Peace, A.G. Abbott, L.A. Mueller, M.A. Olmstead, and D. Main. 2014. The genome database for rosaceae (GDR): year 10 update. Nucl. Acids Res. 42:D1237-D1244.

Le Dantec, L., G. Cardinet, J. Bonet, M. Fouché, K. Boudehri, A. Monfort, J.-L. Poëssel, A. Moing, and E. Dirlewanger. 2010. Development and mapping of peach candidate genes involved in fruit quality and their transferability and potential use in other Rosaceae species. Tree Genet. and Genomes 6:995–1012.

Martínez-García P.J., D.E. Parfitt, E.A. Ogundiwin, J. Fass, H.M. Chan, R. Ahmad, S. Lurie, A. Dandekar, T.M. Gradziel, and C.H. Crisosto. 2013. High density SNP mapping and QTL analysis for fruit quality characteristics in peach (*Prunus persica* L.). Tree Genet Genomes 9:19-36. doi:10.1007/S11295-012-0522-7.

OEPP/EPPO. 2006. *Xanthomonas arboricola* pv. *pruni*. OEPP/EPPO Bul. 36:129–133.

Peace C.P., A. Callahan, E.A. Ogundiwin, D. Potter, T.M. Gradziel, F.A. Bliss, and C.H. Crisosto. 2007. Endopolygalacturonase genotypic variation in *Prunus*. Acta Hort. 738:639-646.

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. and J.L. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae, Ch. 2:19-53. In: Folta K.M., Gardiner S.E. (eds.). Genetics and genomics of Rosaceae. Series Ed Jorgensen R. Springer, New York, Plant Genetics / Genomics, Vol 6.

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. and Genomes 10:1679-1694.

Pozzi, C. and A. Vecchietti. 2009. Peach structural genomics, p. 235-257. In: K.M. Folta. and S.E. Gardiner (eds.). Genetics and genomics of Rosaceae. Springer, New York.

Ru, S., D. Main, K. Evans, and C. Peace. 2015. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet. and Genomes 11:1-12.

Salazar, J.A., D. Ruiz, J.A. Campoy, R. Sánchez-Pérez, C.H. Crisosto, P.J. Martínez-García, A. Blenda, S. Jung, D. Main, P. Martínez-Gómez, and M. Rubio. 2013. Quantitative trait loci (QTL) and mendelian trait loci (MTL) analysis in *Prunus*: a breeding perspective and beyond. Plant Mol. Biol. Rep. 1-18. DOI 10.1007/s11105-013-0643-7.

Salgado, 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. Ph.D dissertation University of Arkansas, Fayetteville, U.S.

Sandefur, P., T. Frett, J. Clark, K. Gasic, and C. Peace. 2016a. PpeRf-SSR, a DNA test for routine prediction in breeding of peach blush. Mol. Breed. In press.

Sandefur, P., T. Frett, A. Salgado, J. Clark, K. Gasic, and C. Peace. 2016b. Ppe-Acidity, a combined DNA test for routine prediction in breeding of acidity and soluble solids content (SSC) in peach. Mol. Breed. In press.

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

Scortichini, M., J. D.Janse., M.P. Rossi, and J.H.J. Derks. 1996. Characterization of *Xanthomonas campestris* pv. *pruni* strains from different hosts by pathogenicity tests and analysis of whole-cell fatty acids and whole-cell proteins. J. of Phytopathol. 144:69-74.

Shulaev, V., S.S. Korban, B. Sosinski, A.G. Abbott, H.S. Aldwinckle, K.M. Folta, A. Iezzoni, D. Main, P. Arús, A.M. Dandekar, and K. Lewers. 2008. Multiple models for Rosaceae genomics. Plant Physiol. 147:985-1003.

Vendramin, E., G. Pea, L. Dondini, I. Pacheco, M. T. Dettori, L. Gazza, S. Scalabrin F. Strozzi, S. Tartarini, D. Bassi, I. Verde, and L. Rossini. 2014. A unique mutation in a MYB gene cosegregates with the nectarine phenotype in peach. PloS ONE 9:e90574. DOI: 10.1371/journal.pone.0090574.

Verde, I., A.G. Abbott, S. Scalabrin, S. Jung, S. Shu, F. Marroni, T. Zhebentyayeva, M.T. Dettori, J. Grimwood, F. Cattonaro, A. Zuccolo, L. Rossini, J. Jenkins, E. Vendramin, L.A. Meisel, V. Decroocq, B. Sosinski, S. Prochnik, T. Mitros, A. Policriti, G. Cipriani, L. Dondini, S. Ficklin, D.M. Goodstein, P. Xuan, C. Del Fabbro, V. Aramini, D. Copetti, S. Gonzalez, D.S. Horner, R. Falzchi, S. Lucas, E. Mica, J. Maldonado, B. Lazzari, D. Bielenberg, R. Pirona, M. Miculan, A. Barakat, R. Testolin, A. Stella, S. Tartarini, P. Tonutti, P. Arús, A. Orellana, C. Wells, D. Main, G. Vizzotto, H. Silva, F. Salamini, J. Schmutz, M. Morgante, and D.S. Rokhsar. 2013. The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genet. 45:487-494.

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. DOI:10.1371/journal.pone.0035668.

Vignal A., D. Milan, M. SanCristobal, and A. Eggen. 2002. A Review on SNP and other types of molecular markers and their use in animal genetics. Genetics Selection Evolution 34:275-305.

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

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

Chapter Five - Study One: G1*Xap*F and G6*Xap*F SNP Haplotype and Ppe-XapF1 and Ppe-XapF6 SSR DNA Tests for Routine Prediction in Breeding of Peach Fruit Bacterial Spot (*Xap*) Resistance

Abstract

Bacterial spot [*Xanthomonas arboricola* pv. pruni (*Xap*)] is a serious disease of *Prunus* spp. worldwide that causes premature defoliation, weak vigor of the plant, unmarketable fruit, and decline in production. Effective control methods are lacking; anti-bacterial sprays (copper-based compounds and oxytetracycline) are only partially effective. Incorporating bacterial spot resistance into newly developed peach cultivars is a more promising control measure and has been a key trait of interest in the University of Arkansas (UA) peach and nectarine breeding program. The work herein is focused on the development and validation of two SNP-based DNA tests for *Xap* resistance (G1*Xap*F and G6*Xap*F). Both DNA tests were successfully screened on 240 out of 243 individuals from the UA RosBREED pedigree (termed "conversion set"): 104 cultivars and selections and seven F1 populations consisting of 136 seedlings. The resulting alleles at each SNP loci were associated with the phenotypic data to differentiate individuals with susceptible (SU) and or resistant (R) alleles. The Vp % for the *G1XapF* and *G6XapF* 4-SNP DNA tests in *Xap* fruit 2013-2015 avg (0-5 scale) were ~7% and ~8% respectively, and combined represented ~15% of the Vp % for *Xap*F resistance. Next, the importance of these DNA tests expanded further through the conversion of the SNP-based tests into simple, straightforward, breeder-friendly, SSR-based DNA tests (Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR) to enable the same DNA test to be screened on both SNP haplotype-based and SSR-based platforms. At the *G6XapF locus* both DNA tests for each UA RosBREED population in the "conversion set" showed that the Ppe-*Xap*F6-SSR genotypes and matching diplotypes for the *G6XapF* 4-SNP fit their expected parental segregation ratio patterns, using the chi-square (X^2) goodness-of-fit test ($p > 0.05$). Furthermore, the G6*Xap*F SNP diplotypes and Ppe-*Xap*F6-SSR

genotypes matched 100% of the time for all individuals in the "conversion set." Contrarily, at the *G1XapF locus* the Ppe-*Xap*F1-SSR and *G1XapF* 4-SNP haplotype DNA test results for the F_1 seedlings in the UA RosBREED populations only matched in three out of seven populations. Furthermore, the Ppe-*Xap*F1-SSR and *G1XapF* 4-SNP DNA test results only matched 50% of the time for all individuals in the "conversion set." Thus, only Ppe-*Xap*F6-SSR was advanced and successfully screened on 580 seedlings from 21 F_1 populations, ranging from 5-56 seedlings per population (termed "confirmation set"), to confirm the DNA test prediction accuracy in the UA breeding program. All F_1 populations of the "confirmation set" fit their parental expected genotypic segregation ratios for the Ppe-*Xap*F6-SSR using the chi-square (X^2) goodness-of-fit test ($p > 0.05$). Additionally, the Vp was ~10% when considering all 2010 seedlings. These results confirmed the predictiveness of the Ppe-*Xap*F6-SSR across a vast majority of the UA breeding material. The G1*Xap*F and Ppe-*Xap*F6-SSR DNA tests were deployed for routine marker-assisted selection (MAS) use in this breeding program (2013-2015) to enable further pyramiding of *R* alleles into high-quality UA cultivars spanning the season.

Introduction

Bacterial spot [caused by *Xanthomonas arboricola* pv. *pruni* (*Xap* refers to the pathogen and disease)] is a serious disease threat to the stone fruit industry and related *Prunus* hybrids worldwide (EPPO/CABI, 1997; OEPP/EPPO, 2006; Ritchie, 1995). Bacterial spot symptoms are generally characterized as various sized necrotic lesions on leaves and fruits and cankers on twigs (EPPO/CABI, 1997; OEPP/EPPO, 2006; Ritchie, 1995). Infection and development of *Xap* is highly dependent on environmental conditions. Favorable environmental conditions in the spring (high humidity, warm temperatures, and extended periods of rainfall) enable overwintered *Xap* inoculum to arise and spread throughout the orchard. Once *Xap* is established in an orchard

it is very difficult to control, especially when highly susceptible cultivars are used (Ritchie, 1995). This disease leads to premature defoliation, weak vigor of the plant across years, unmarketable fruit with low fruit quality, and overall decline in production (Aarrouf et al., 2008; Ritchie, 1995).

The primary commercial control of *Xap* in the peach industry has been to rely heavily on pesticides, such as copper-containing compounds and oxytetracycline (Ritchie, 1995 and 1999). Correct timing of sprays and rates used are important. Relatively high rates of copper sprays are generally applied when only a limited amount of new growth is present early in the growing season, from dormancy through early shuck split. The idea of these applications is to cover the tree surface to create a barrier through which the bacterium must pass as it moves from overwintering sites. The majority of the inoculum is thought to die in this process of moving through the copper barrier. Copper sprays are continued as new growth emerges but rates are reduced because these compounds are known to cause fruit and foliage damage in peach. The compound oxytetracycline is then incorporated when shuck split commences since it is less damaging to fruit and foliage (Ritchie, 1995 and 1999).

However, if favorable environmental conditions occur, the disease can develop even when well-timed sprays have been applied (Ritchie, 1995 and 1999). Furthermore, once the bacterium is established in the orchard it is very difficult to control the disease, especially when highly and/or moderately susceptible cultivars are used. Moreover, due to concerns of agricultural worker safety, environmental contamination, economic concern, and food safety, restrictions on the use and availability of chemicals in agriculture have developed in recent years. Integrated pest management programs (IPM) are now being incorporated to only use chemical sprays as the last resort and *Xap* resistant cultivars play a crucial role in these IPM practices

(Byrne et al., 2012; Sansavini et al., 2006). Unfortunately, many good quality cultivars favored in the peach industry are highly susceptible and cultivars with *Xap* resistance in general often lack desirable fruit quality characteristics required by the peach industry.

Bacterial spot resistance has been a key trait of interest in peach breeding at the University of Arkansas (UA). This program has focused on breeding for resistance to *Xap* since 1964. Drs. Jim Moore and Roy Rom initiated the UA peach and nectarine breeding program, and their successor Dr. John R. Clark currently directs it. Unlike most other peach breeding programs, antibiotic sprays to control the disease have never been applied, thus, selection against *Xap* has been possible (J.R. Clark, personal communication). The warm and humid spring and summers at the University's Fruit Research Station (FRS) near Clarksville, AR, create an ideal environment for the inoculation and spread of this pathogen. This environment has enabled breeding and selection of peaches and nectarines with low *Xap* incidence.

The genetic control of *Xap* resistance is quantitative in nature, however, dominant genes were suggested to be involved in peach (Sherman and Lyrene, 1981; Werner et al., 1986). Quantitative resistance is more stable than monogenic resistance since the pathogen must overcome the polygenic defense. Interestingly, the resistance of leaf and fruit in peach has been suggested to be controlled by different genetic locations due to inconsistent levels of leaf and fruit resistance (Keil and Fogle, 1974; Simeone, 1985; Werner et al., 1986; Yang et al., 2013).

Recently, studies were performed to determine the genetic control of *Xap* resistance in *Prunus* (Socquet-Juglard et al., 2013; Yang et al., 2010, 2011, 2012, and 2013). In these studies, two bi-parental populations were designed specifically to study the genetic control of *Xap* resistance in *Prunus*: an apricot population consisting of 101 F_1 individuals from a cross between 'Harostar' (resistant) (Ha) × 'Rogue de Mauves' (unknown) (RM); and an F_2 peach population

consisting of 188 F_2 seedlings by crossing ‘O’Henry’ (susceptible) × ‘Clayton’ (resistant) (OC) (Socquet-Juglard et al., 2013; Yang et al., 2010, 2011, 2012, and 2013).

Using the HaRM F_1 population, Socquet-Juglard et al. (2013) constructed two low-density apricot parental linkage maps, ‘Harostar’ (81 AFLP fragments and 63 SSR markers) and ‘Rouge de Mauves’ (55 AFLP fragments and 53 SSR markers), both covering all eight apricot chromosomes. They located a major QTL for *Xap* leaf resistance [53% of the phenotypic variation explained (Vp %)] on linkage group 5 of ‘Rouge de Mauves’ apricot map. Microsatellite marker UDAp-452 was located at the peak, and BPCT037 and BPPCT038A flanked the QTL. Both BPPCT037 and BPPCT038A have high polymorphism in cherry and peach, and thus could be candidate markers for *Xap* resistance in peach (Dirlewanger et al., 2002). Socquet-Juglard et al. (2013) identified six candidate genes for resistance encoding for receptor-like protein kinases, leucin-rich repeat (LRR) proteins, or disease resistance proteins between BPPCT037 and BPPCT038A on chromosome 5 of the peach genome v1.0.

Later, using the OC F_2 population, Yang et al. (2013) developed a very dense OC linkage map using 63 of the 188 F_2 seedlings, consisting of 256 SNPs and two SSR markers, which covered all eight peach chromosomes. They identified 14 QTL with additive effects on *Xap* resistance using the OC population. The small population used in this study could have resulted in overestimation of QTL effect and detection of false positives. These QTL are spread throughout the OC LGs, some associated with leaf resistance, others with fruit resistance, and some with resistance to both leaf and fruit. The phenotypic variation explained (Vp %) by all QTL ranged from 15 to 56% in leaf data sets, and 33 to 61% in fruit data sets. Within these QTLs, they identified six different types of candidate genes (CGs) associated with *Xap* resistance in their study: NBS-LRR proteins; protein kinases; mildew resistance locus (MLO) gene family;

glucanases, chitinases, and phytoalexins. Five of these QTL were denoted major QTL based on size and stability of additive effect and prior knowledge: *Xap.Pp.OC-4.1* and *Xap.Pp.OC-4.2* (LG4, leaf resistance), *Xap.Pp.OC-5.1* (LG4, leaf and fruit resistance), *Xap.Pp.OC-1.2* (LG 1, fruit resistance), and *Xap.Pp.OC-6.1* (LG 2, fruit resistance).

Of these five major QTLs, *Xap.Pp.OC-1.2* and *Xap.Pp.OC-6.1* were selected to be haplotyped at these two loci, termed G1*Xap*F and G6*Xap*F, across a wide range of germplasm in four pedigree connected US RosBREED demonstration peach breeding programs [The University of Arkansas (UA), Clemson University (CU), Texas A&M University (TX) and University of California, Davis (CA)] (see Chapter Four for additional details). In Chapter Four, the UA material was shown in general to contain a higher proportion of resistant alleles at both G1*Xap*F and G6*Xap*F loci than all three other RosBREED peach breeding programs. While these results of a large proportion of *Xap* fruit resistance alleles found in the UA peach breeding program are promising, more work needs to be done to validate the robustness of all resistant, intermediate, and susceptible SNP haplotypes at these two loci before incorporation of MAS for the trait. The robustness of a DNA test refers to the reliability of the DNA test to correctly predict the phenotypic variation for the desired breeding germplasm. This is critical to determine, otherwise the DNA test could be misleading (Bliss, 2010; Collard et al., 2005; Collard et al., 2008; Ru et al., 2015).

The objective of this study was to validate the robustness of the previously developed SNP-based DNA tests associated with *Xap* fruit resistance, G1*Xap*F and G6*Xap*F, across a vast array of the UA peach breeding material, for subsequent incorporation of MAS for *Xap* resistance. The importance of these DNA tests will expand further through the conversion of the SNP-based DNA tests into simple, straightforward breeder-friendly, SSR-based DNA tests. The

SSR-based DNA tests robustness will subsequently be confirmed in a vast array of the UA peach breeding material phenotyped for *Xap* resistance and genotyped with both DNA tests. Furthermore, the predictiveness of the two different types of DNA tests at both loci to accurately differentiate resistant vs. susceptible alleles will be determined and compared in the UA peach breeding material. The four DNA tests included the two 4-SNP haplotype tests (G1*Xap*F and G6*Xap*F) developed in Chapter Four of this dissertation, and the two SSR-based DNA tests developed in this study (Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR). The most predictive DNA test will be further confirmed across the program to enable both routine and accurate MAS for this trait in the UA program.

Materials and Methods

Management Practices at FRS

Phenotypic evaluation for fruit bacterial spot (*Xap*F) resistance was conducted on peach and nectarine material at FRS (west-central AR, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept. of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]). All trees were either open-center trained and spaced 5.5 m between trees and rows, or trained to a perpendicular-V system with trees spaced 1.9 m in rows spaced 5.5 m apart. All trees were dormant pruned and fertilized annually with a single application of 640 Kg ha-1 of complete fertilizer (19:19:19 of N:P:K) and were sprinkler or drip irrigated as needed. Pests were managed using a program typical for commercial orchards in the area (Smith, 2015; Studebaker et al., 2015). After shuck split but before pit hardening, fruitlets were thinned to a distance of 12 to 15 cm between each fruitlet.

Germplasm Utilized

The UA RosBREED "conversion set" was evaluated for bacterial spot incidence in 2013-2015 and utilized to convert the *G1XapF* and *G6XapF* SNP haplotypes (see Chapter Four of dissertation) to the Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR DNA tests. The "conversion set" consisted of all 138 individuals in the seven UA RosBREED F_1 populations, 36 UA cultivars and selections screened with the International Peach SNP Consortium (IPSC) 9K peach SNP array v1.0 (Verde et al., 2012), and 68 cultivars and selections screened with the mini SNP array v1.0, at the University of Arizona Genetics Core (AZ) and BioDiagnostics (BDI) (in total 241 individuals) (Table 1; Fig. 1). In 2013-2014, the remaining UA cultivars, selections (69), and an array of germplasm from different breeding programs primarily in the U.S. (58), not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0, were assessed (in total 127 individuals) (Table 2). Additionally, in 2014 a total of 613 seedlings from 22 F_1 populations (2010 crosses), ranging from 5-59 seedlings per population, were evaluated to confirm Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR DNA test allele effects in the UA breeding program (i.e. "confirmation set") (Table 3).

Table 1. Parental information and the number of F_1 seedlings for each of the seven RosBREED populations (N is number of individuals analyzed).

F_1 population	Female parent	Male parent	F_1 seedlings (N)
AR_Pop_1	White County	A-672	48
AR_Pop_0801	A-776	A-783	16
AR_Pop_0803	Amoore Sweet (AS)	A-778	9
AR_Pop_0813	A-772	A-672	12
AR_Pop_0817	A-789	A-699	9
AR_Pop_0819	A-708	A-773	23
AR_Pop_0825	Souvenirs	A-760	17

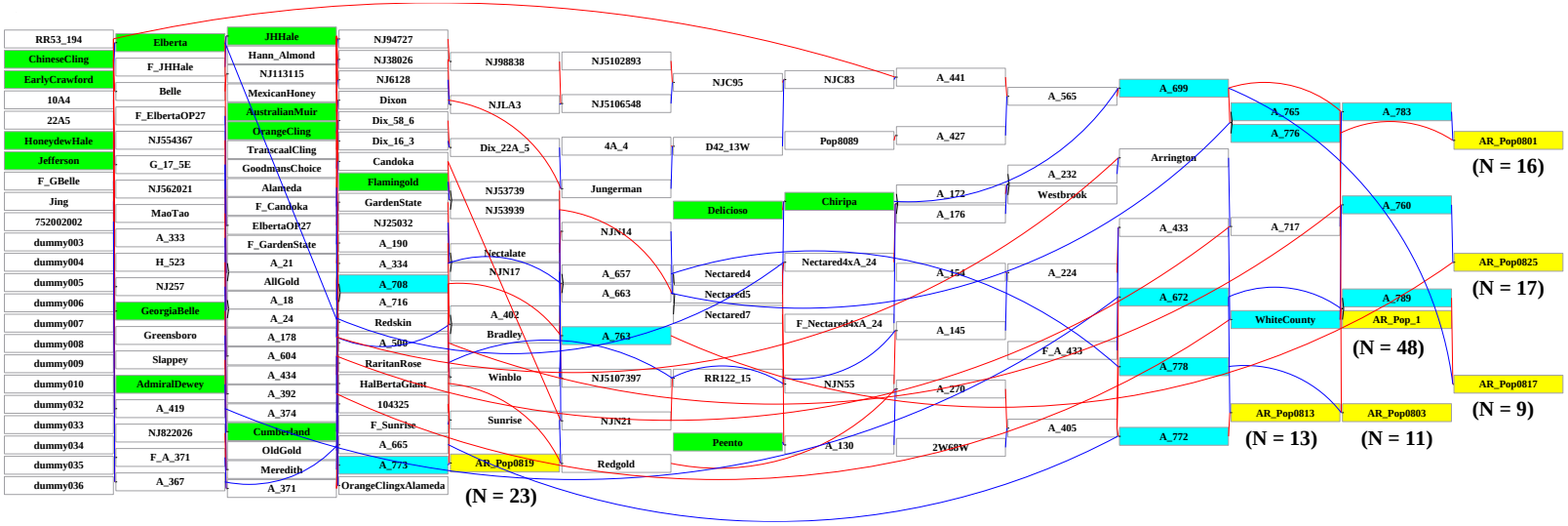

Fig. 1. Pedigree of the seven 2008 F_1 seedling populations in the UA RosBREED "conversion set" used to validate the predictiveness of the *G1XapF* and *G6XapF* SNP-based DNA tests, as well as confirm the successful conversion of these SNP-based DNA tests into the SSR-based tests, Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR. Visualized through Pedimap software (Voorrips, 2007; Voorrips et al., 2012) (F_1 populations highlighted in yellow; Red line = female parent; Blue line = male parent) (N is the number of progeny in each population).

Table 2. Arkansas selections and cultivars and an array of germplasm from different breeding programs phenotyped for fruit bacterial spot symptoms in 2013-2014 and genotyped in 2012-2015.

A-554	A-805[y]	A-845[y]	A-878[y]	A-910	Elberta[z]	PF 5D Big
A-662	A-806[y]	A-846	A-879[y]	A-911	Emeraude	PF Lucky 13
A-663[z]	A-808	A-847	A-880[y]	A-912	Flavortop	PF-19-007
A-665[zy]	A-809[y]	A-848[y]	A-881[y]	A-913	Georgia Belle[z]	Redhaven
A-668	A-810	A-849[y]	A-882[y]	A-914	Gladiator	Redskin[z]
A-672[zy]	A-811[y]	A-850[y]	A-883	A-915	Gloria[y]	Rising Star
A-699[z]	A-813[y]	A-851[y]	A-884	A-916	Goldilocks	Roygold
A-708[z]	A-814	A-852[y]	A-885	A-917	Goldjim	Ruby Prince
A-716[z]	A-815	A-853[y]	A-886	A-918	Goldnine	Saturn
A-743	A-816[y]	A-854[y]	A-887	Admiral Dewey[z]	Greensboro[z]	Slappey[z]
A-758	A-818[y]	A-855[y]	A-888	Allgold	Jade	Souvenirs[z]
A-760[z]	A-819[y]	A-856[y]	A-889	Amoore Sweet[z]	Jefferson[z]	Spring Snow
A-761	A-820[y]	A-857[y]	A-890	Arrington[z]	JH Hale[z]	Sugar Giant
A-766	A-821[y]	A-858	A-891	Autumn Prince	KV175	Sugar Lady
A-768	A-822[y]	A-859[y]	A-892	Autumn Star	KV175	Sweet Star
A-770	A-824	A-860[y]	A-893	Bounty	KV357	Tango
A-772[z]	A-825[y]	A-861[y]	A-894	Bowden[y]	KV398	Tango-II
A-773[z]	A-826[y]	A-862[y]	A-895	Bradley[zy]	KV401	Westbrook[z]
A-776[z]	A-827[y]	A-864[y]	A-896	Challenger	KV501	White Cloud
A-778[z]	A-828[y]	A-865[y]	A-897	China Pearl[z]	KV601	White County[zy]
A-783[z]	A-829[y]	A-866[y]	A-898	Chinese Cling[z]	KV606	White Diamond[y]
A-786[y]	A-830	A-867[y]	A-899	Contender	KV701	White Lady
A-789[z]	A-832[y]	A-868[y]	A-900	Cresthaven	KV801	White River[z]
A-790	A-833[y]	A-869[y]	A-901	Crimson Lady	Loring	White Rock[y]
A-792[y]	A-836[y]	A-870[y]	A-902	Crimson Snow	Manon	Winblo[z]
A-794	A-837[y]	A-871[y]	A-903	Cumberland[z]	Messina	Yumm Yumm
A-797[y]	A-839	A-872[y]	A-904	CVN13w	Orange Cling[z]	
A-798	A-840[y]	A-873[y]	A-905	Denman	Peento[z]	
A-799	A-841[y]	A-874[y]	A-906	Dixon[z]	PF 1	
A-801[y]	A-842[y]	A-875[y]	A-907	Early Crawford[z]	PF 24-007	
A-803	A-843[y]	A-876[y]	A-908	Early Star	PF 24C	
A-804[y]	A-844[y]	A-877[y]	A-909	Eastern Glo	PF 5B	

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 3. Additional 2010 F_1 seedling populations phenotyped for fruit bacterial spot symptoms in 2014, and genotyped in 2014-2015. (N is number of individuals analyzed).

F_1 population	Female parent	Male parent	F_1 seedlings (N)
AR_Pop_1001	A-665	A-800	38
AR_Pop_1002	A-760	A-708	37
AR_Pop_1003	White Diamond	A-760	43
AR_Pop_1004	A-753	Souvenirs	27
AR_Pop_1006	White County	Souvenirs	41
AR_Pop_1007	A-775	Souvenirs	34
AR_Pop_1008	A-746	A-785	13
AR_Pop_1009	A-746	A-823	17
AR_Pop_1011	A-786	A-773	66
AR_Pop_1012	A-773	A-774	33
AR_Pop_1013	A-772	A-774	28
AR_Pop_1014	A-685	A-773	10
AR_Pop_1015	A-789N	A-803CN	25
AR_Pop_1016	A-807CN	A-802CN	5
AR_Pop_1018	Bowden	A-761N	31
AR_Pop_1019	A-779CN	A-776CN	20
AR_Pop_1020	Bowden	A-758CN	33
AR_Pop_1021	A-778N	A-777CN	29
AR_Pop_1022	Amoore Sweet	A-779CN	26
AR_Pop_1024	A-757N	A-807CN	20
AR_Pop_1025	A-770CN	A-768N	6
AR_Pop_1026	A-816CN	A-772	31

Xap Phenotyping

All trees used in this study experienced routine exposure to *Xap* at FRS for infection, and were not field-inoculated. This approach was used because *Xap* is seen each year at FRS, although years vary to some degree in severity (J.R. Clark, personal communication). This dependability, in addition to multiple years of data collection, provided for adequate confidence in infection uniformity and phenotypic expression of degree of resistance or susceptibility.

In 2013-2015, phenotypic data was collected in the field for all individuals in the UA "conversion set" (Table 1; Fig. 1). The combined ordinal and interval or categorical scale developed in the study by Yang (2012) was used to assess both leaf and fruit symptoms (Table 4; Fig. 2). Leaf symptoms were evaluated during a one-week period in May. Upon harvest of each selection, fruit symptoms were assessed and recorded. Phenotypic data for the "confirmation set" was collected in the same manner, in 2014 (Table 3).

Table 4. Phenotypic scale used to assess bacterial spot (*Xap*) infection on peach leaves and fruits.

Class	Symptoms	
	Leaf	Fruit
0	No leaves with symptoms	No fruits with symptoms
1	1-5% diseased leaves or observed defoliation	1-5% fruit surface with spot lesions
2	6-10% diseased leaves or observed defoliation	6-10% fruit surface with spot lesions
3	11-25% diseased leaves or observed defoliation	11-25% fruit surface with spot lesions
4	25-50% diseased leaves or observed defoliation	25-50% fruit surface with spot lesions
5	> 50% diseased leaves or observed defoliation	> 50% fruit surface with spot lesions

*Note: For the purpose of genetic study of *Xap* resistance in peach, the phenotypic scoring for leaf was based on all leaves for symptoms on each individual tree. The phenotypic scoring for fruit was based on the most severe individual fruit symptom on each individual tree (Yang et al., 2012).

Fig. 2. Bacterial spot (*Xap*) symptom severity on peach leaf and fruit. A. Leaf symptoms are shown in six different severity categories based on 0-5 scale, with 0 – no symptoms, 1 – water soaked lesions, 2 – tattered patterns on the leaf tip and leaf rib, 3 – coalesced water-lesion and shot holes, 4 – yellow leaf, and 5 – premature leaf drop; B. 0-5 scale applied for fruit evaluation (as in Yang et al., 2013).

Leaf Collection and DNA Extraction

In the spring of 2013, approximately 50 mg of fresh, young leaf tissue was harvested in individual 1.5 mL tubes (Eppendorf, Hauppauge, NY) from all seedlings in the seven UA RosBREED F_1 populations as well as all UA cultivars and selections in the "conversion set" (243 individuals). Additionally, the remaining UA cultivars and selections, and an array of germplasm from different breeding programs, were collected (127 individuals). While collecting tissue, each F_1 seedling was labeled with a metal tag in order to correctly match phenotypic and genotypic data later. Tissue was refrigerated during transportation, and then stored at -80 °C until needed. Two 4 mm stainless steel beads (McGuire Bearing Company, Salem, OR) were placed into each 1.5 mL tube, and DNA was extracted following a modified Dellaporta (1983) protocol with specific adaptations for peach. DNA quantity and quality were measured using a spectrophotometer (NanoDrop 2000, NanoDrop Technologies, Wilmington, DE) and confirmed by electrophoresis on 1% TBE (1 M Tris, 0.9 M boric acid, and 0.01 M EDTA) agarose gel. Final dilutions of 25 ng/µl were created for genotyping.

In the spring of 2014, approximately 50 mg of fresh young leaf tissue was harvested into coin envelopes for all 613 seedlings from 2010 populations ("confirmation set"). While collecting tissue, each F_1 seedling was labeled with a metal tag in order to correctly match phenotypic and genotypic data later. Tissue was refrigerated during transportation, and then lyophilized (Freezone® 12 model 77540, Labconco Corporation, Kansas City, MO). Lyophilized plant tissue was then loaded into a 96 1.1 ml MicroTube Rack System (BioExpress, Kaysville, UT) containing approximately 0.25 g of technical grade 40, 6-12 mesh silica gel beads (Sigma Aldrich, St. Louis, MO). All DNA was extracted following a high-throughput and cost efficient extraction protocol developed for *Prunus* species by Edge-Garza et al. (2014).

SNP Haplotype Validation and Conversion to SSR Marker

In Chapter Four of this dissertation, two different 4-SNP haplotype DNA tests associated with *Xap* fruit resistance, G1*Xap*F and G6*Xap*F, were developed across 971 individuals in the combined RosBREED peach breeding pedigree [four universities: UA, Clemson University (CU), Texas A&M University (TX), and the University of California, Davis (CA)] (Figs. 3-4). All individuals in the UA "conversion set" (243 individuals) were used to validate the robustness of these previously developed SNP-based DNA tests, for subsequent incorporation of MAS in the UA program. Since *Xap*F was scored using a 0-5 scale, the non-parametric Kruskal-Wallis test with Wilcoxon rank sum pairwise comparisons test ($P \leq 0.05$) was used to evaluate differences among G1*Xap*F and G6*Xap*F diplotypes for the proportion of *Xap*F resistance they predict using all 243 UA "conversion set" individuals (JMP ® 2012).

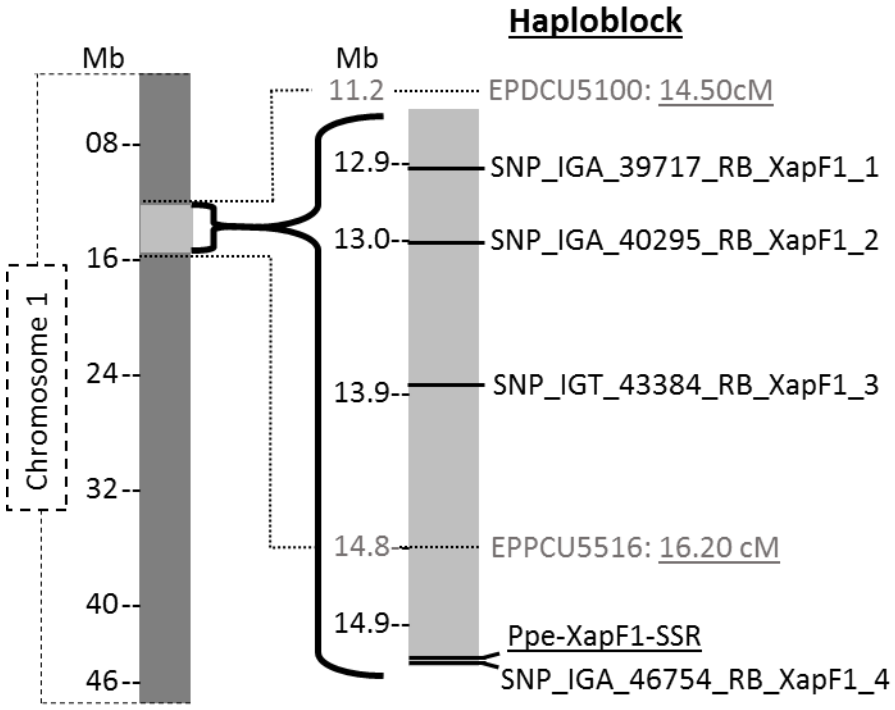

Fig. 3. Graphical representation of *P. persica* chromosome 1 with the enlarged location of the ~2 Mbp flanking the four SNP markers used for haplotyping at the *G1XapF* locus. The 4-SNP *G1XapF* DNA test was subsequently converted into Ppe-*Xap*F1-SSR (underlined in figure). Physical locations were sourced from the *P. persica* whole genome v1.0 sequence (Verde et al., 2013) housed on the Genome Database for Rosaceae (Jung et al. 2014). Two flanking markers and their estimated genetic positions based on the *Prunus*-TE-F2 reference bin map are included in gray. It's also important to note that there are 18 nucleotide-binding site leucine-rich repeat (NBS-LRR) resistance gene family candidate genes within the haploblock.

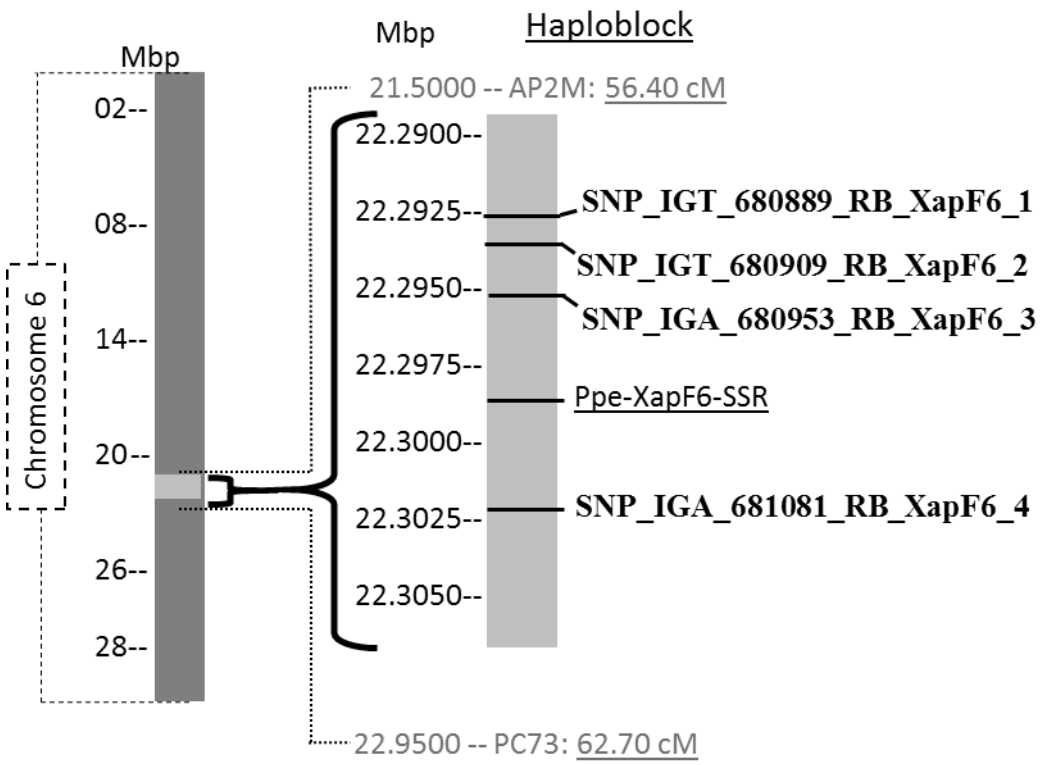

Fig. 4. Graphical representation of *P. persica* chromosome 6 with the enlarged location of the ~10 kbp flanking the four SNP markers used for haplotyping at the *G6XapF* locus. The 4-SNP *G6XapF* DNA test was subsequently converted into Ppe-*Xap*F6-SSR (underlined in figure). Physical locations were sourced from the *P. persica* whole genome v1.0 sequence (Verde et al., 2013) housed on the Genome Database for Rosaceae (Jung et al. 2014). Two flanking markers and their estimated genetic positions based on the *Prunus*-TE-F2 reference bin map are included in gray. It's also important to note that there are six nucleotide-binding site leucine-rich repeat (NBS-LRR) resistance gene family candidate genes within the haploblock.

To enable the same DNA test to be screened on both SNP-haplotype based and SSR-based platforms, the validated *G1XapF* and *G6XapF* SNP haplotypes were subsequently converted into simple, straightforward, breeder-friendly SSR-based DNA tests, using the UA RosBREED "conversion set" (243 individuals). To accomplish this, 27 and six SSR markers were designed targeting di-nucleotide repeats within the *G1XapF* and *G6XapF* loci using the *P. persica* genome sequence v1.0 (Verde et al., 2013) and the programs BatchPrimer3 (You et al., 2008; http://probes.pw.usda.gov/batchprimer3/) and Primer3Plus (Untergasser et al., 2012; www.primer3plus.com) (Tables 5-6). The criteria for SSR selection were the same as reported by Stegmeir et al. (2014) and Sandefur et al. (2016a). As numerous SSRs were found within both loci, only the largest SSRs (those with the largest number of tandem repeats) located within or flanking the *Xap* loci were selected for initial testing. All primer pairs selected contained a GC clamp of two (guanine and cytosine nucleotides) and were verified for specific amplification in only the *G1XapF* or *G6XapF* loci, using the NCBI BLAST function on the *P. persica* whole genome sequence v1.0 website (www.rosaceae.org/tools/ncbi_blast) (Verde et al., 2013). All suitable primer pairs were considered candidate assays (Tables 5-6).

Table 5. SSR markers developed upstream, downstream and within, *G1XapF* SNP locus, their physical locations, motif, and primers.

Name	Physical location[Z]	Motif	Forward primer	Reverse primer
G1-1_SSR1F	12906229-12906245	AT×8	ATCTAATGAGGTGGTTTAGGG	TGTAGGTTCAAACTTCATTCC
G1-1_SSR2F	12909195-12909217	TA×11	AACGTGGTCGATCAAGTATGC	CTGCTTGCTCACATGATACCC
G1-1_SSR3F	12920604-12920642	TC×19	CTGTCAATGGCTTCAGATTGG	TTCTTGAGCTCCAAAAACAGC
G1-1_SSR4F	12921627-12921657	CT×15	GATGAGGAGGAACCAAGTGC	AATTTAGGCTTCGGCTACTGC
G1-1_SSR9F	12961448-12961492	AT×10	TTGTTCAAGCTCATTGGTTAGG	ACCTTGCTTTAGGTTGATTGC
G1-1_SSR13	13006596-13006626	AG×15	GCAAAGAGAAAGCAAAGAATCG	TGAGAAAAGGGAAACATGTGC
G1-2_SSR14	13013155-13013185	AT×15	ACCTTTCGTCTTTCAAAATCC	AAGGACCATTTGCGATAGG
G1-2_SSR16	13013805-13013871	CT×33	GCAAACTAACAGAGTTTTTGACG	ATCAGCTTCGGAATGTCTAGG
G1-2_SSR18	13050049-13050069	TA×10	ATGGTGGTTTGCGTTTATACC	TTTCGATATCCAATTGTGTCC
G1-2_SSR19	13052199-13052221	AT×11	CTTTTTGACCACTTACCATGC	TGGTAAAGCACAAAACAAAGG
G1-2_SSR20	13054236-13054268	AG×16	CCCGTTTAATTGTCATTTCG	ATAAAGGCCCGTTTGATAACC
G1-2_SSR21	13075747-13075791	AG×22	CCTGACCTCCTCTCTCACG	TGATGTGTTTGATGTGAATGG
G1-2_SSR22	13075956-13076000	GA×22	TGCTTTCTACATTTATGCTTTGC	CTGTTCTACCATAGGGGAAGG

[Z]Marker locations were sourced from the Genome Database for Rosaceae (Jung et al., 2014).

Table 5. SSR markers developed upstream, downstream and within, *G1XapF* SNP locus, their physical locations, motif, and primers. (Cont.).

Name	Physical location[Z]	Motif	Forward primer	Reverse primer
G1-3_SSR24	13839572-13839887	GA×12	AAGATTTGGTGGGAAGAGTGC	CGTGTTTCCCAAACTAGAAGG
G1-3_SSR25	13875283-13875583	AG×17	CATTTAGGTTCCATCCCATGC	CTCCTCCATTGAAACACATGC
G1-3_SSR26	13920540-13920833	TC×12	TTCTCCTTCGTCTTTCATCTCC	GGATTCATGAACTGGAACACC
G1-3_SSR27	13923264-13923551	TC×12	GGTCATCCAGATGTAGCTTGC	TTGTTGTCGTTGAGCTTCTCC
G1-3_SSR28	13927369-13927645	GA×25	ATAACTGGAGGTGCCTCTTCG	ACGTGTTAGAGAAAGCGTTGC
G1-3_SSR30	13876363-13876653	ACC×9	GCATTCATTCAACGTGACTCC	AAAACCTGTGCCATTGTTGC
G1-3_SSR31	13923264-13923551	CTT×12	ATAACTGGAGGTGCCTCTTCG	ACGTGTTAGAGAAAGCGTTGC
G1-4_SSR25	14944775-14945129	TC×20	CTAGGCCCTCTTTGAAACTCC	AGCACCAACAGGATTCTAACG
G1-4_SSR26	14956295-15956607	CT×10	CCCTGTAGTCCGAGAGATCG	TTAGAGATTGCCTCACAAAACC
G1-4_SSR28	14965667-14965990	AG×18	TCCAAAAGAACCTGACTGTGG	TCAAATACTCGCCAAACATGC
G1-4_SSR30	15025410-15025732	AG×29	TAGGCCAGTTCCAACTCTTCC	GAGCAGTGTCTTGATCTTGAGG
G1-4_SSR31	14969274-14969653	TATAAT ×12	CGACATATGGATGGTGACACG	AATCAAACAAATTGTCATAGAGC
G1-4_SSR34[Y]	**14979998-14980318**	**ACAGAT ×12**	**CAACCAGGGAAACGTTATTGC**	**TTTCTGTTGAAGATTTGGATGG**
G1-4_SSR35	15016094-15016397	GTGGCA ×12	GGTTTGTGAGGGAGGTAGTGG	CCAGCACTATCACCATAATTGC

[Z]Marker locations were sourced from the Genome Database for Rosaceae (Jung et al., 2014).
[Y]The SSR marker in bold was chosen as *G1XapF* -SSR DNA test.

Table 6. SSR markers developed upstream, downstream and within, *G6XapF* SNP locus, their physical locations, motif, and primers.

Name	Physical location[Z]	Motif	Forward primer	Reverse primer
G6_SSR12	22246355-22246376	TCT×7	TTGAAAGCCAGGTAACATTGG	AACGTACCGTTGGAGTCACC
G6_SSR13	22270586-22270646	TC×30	TGACCGATTTTTAACTTTTTGG	TGAGAGAGGTAGACGCAGAGG
G6_SSR14	22271624-22271678	TC×27	AAGCCCAGTGGCATAATCG	GAAACCATACCTTGCTCATCG
G6_SSR15	22297527-22297553	AT×13	GTCCAAATTCCCAACAAAACC	TTGTTATCTCTTTCATCCTTTGC
G6_SSR16[Y]	**22298155-22298175**	**TC×10**	**TCGCCGACTGTTTATTCTAGC**	**TCACTTTGACACAAATCTCATGG**
G6_SSR17	22333236-22333258	AT×11	TCACAAACTTCACACCCATCC	CCCATGAATCTTGAATGTGG

[Z]Marker locations were sourced from the Genome Database for Rosaceae (Jung et al., 2014).
[Y]The SSR marker in bold was chosen as *G1XapF* -SSR DNA test.

A subset of individuals (n = 20) representing all SNP diplotype combinations possible were screened with all candidate assays, in a final total volume of 10.0 µl containing 1 µl DNA (~25 ng/µl), 4.0 µl of ultrapure molecular grade water [AccuGENE™ (Lonza Inc., Allendale, NJ)], 2.0 µl of Taq PCR buffer [×5 GoTaq® buffer (Promega Corp., Madison, WI)], 0.6 µl of $MgCl_2$ [25mM (Promega)], 0.2 µl deoxyribonucleotide triphosphates (dNTPs) [10 mM (Promega)], 0.5 µl of each primer (forward and reverse) [10mM (Integrated DNA Technologies, Coralville, IA)], 0.2 µl of Taq DNA polymerase (5U/µl) [GoTaq® (Promega Corp.)]. The PCR amplifications were performed in a BIORAD T100 thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA) under the following conditions: 5 min of initial denaturation at 95 °C followed by 35 cycles of 95 °C for 45 s, 60 °C for 45 s, 72 °C for 1.5 min, and then a final extension step at 72 °C for 7 min.

Visualization of PCR results for all candidate assays was completed with fragment separation on denaturing gels containing 4% acrylamide and 7.5 M urea in 1 × TBE buffer run on a 50-cm Sequi-Gen GT system (BioRad, Hercules, CA) for ~2 h at 85 W (as in Sandefur et al., 2016a). Gels were stained with Promega Silver Sequence™ Staining System (Promega Corp., Madison, WI) as per manufacturer's instructions. Amplified PCR fragments from each primer pair for the subset of individuals (n = 20) representing all SNP diplotype combinations possible were compared, and primer pairs producing fragments matching SNP haplotype patterns were selected for subsequent testing on a high-throughput platform to check suitability for routine breeding use. Visualization of the selected assay outcomes was completed with fragment separation on an ABI Prism 3730xl DNA Analyzer (Applied Biosystems by Life Technologies, Grand Island, NY), and size calling with GeneMarker® software (SoftGenetics, LLC, State

College, PA) (as in Sandefur et al., 2016a). The G1-4_SSR34 and G6_SSR16 candidate assays were selected and re-termed Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR, respectively (Tables 5-6).

Next, the extracted DNA from the UA RosBREED "conversion set" (243 individuals), and UA "confirmation set" (613 individuals) were amplified with the Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR DNA tests at the UA Horticulture Molecular Breeding Laboratory. Polymerase chain reaction (PCR) was performed for all samples as previously described. Amplified PCR fragments were resolved utilizing two different capillary gel electrophoresis machines: a Fragment Analyzer™, model AdvanCE FS96 (Advanced Analytical Technologies, Inc., Ames, IA) from the wheat breeding laboratory at UA, and an ABI Prism 3730xl DNA Analyzer (Applied Biosystems by Life Technologies, Grand Island, NY) at the fruit breeding laboratory at Washington State University. Results from the Fragment Analyzer™ were analyzed through PROSize® v.1 software (Advanced Analytical Technologies, Inc., Ames, IA), while results from the ABI Prism 3730xl were scored through GeneMarker® software (SoftGenetics, LLC, State College, PA). Lastly, direct comparisons between the UA RosBREED "conversion set" (243 individuals) *G1XapF* and *G6XapF* SNP diplotypes and Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR genotypes were made to confirm successful conversion from SNP to SSR DNA tests.

Statistical Analysis

The chi-square (X^2) goodness-of-fit test was performed for all F_1 populations of the "conversion set" and "confirmation set" to determine if the seedlings observed *G1XapF* and *G6XapF* SNP diplotypes and their Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR genotypes fit the expected segregation ratio patterns of their parents ($P > 0.05$). A pedigree allele tracking approach was used to determine parental genotypes for populations whose parents had previously been discarded from the program, and thus were not screened with the four *Xap* DNA tests. In this case, the parent alleles were determined based on their grandparent alleles to trace the identity of each allele. Lastly, process of elimination was used for parents whose alleles still could not be determined. In this scenario, the F_1 progeny segregation ratio and the other parent's genotype were used to determine what the unknown parent's genotype should be.

Since *Xap*F was scored using a 0-5 scale, the non-parametric Kruskal-Wallis test with Wilcoxon rank sum pairwise comparisons test ($P \leq 0.05$) was used to evaluate differences among diplotypes and their 2013-2015 avg *Xap*F medians (JMP ® 2012). Additionally, the percent of phenotypic variation explained (Vp %) by the SNP haplotypes (*G1XapF* and *G6XapF*) and SSR genotypes (Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR) for *Xap*F resistance was calculated using the coefficient of determination (R^2) calculated from a linear regression.

Results

Phenotypic Data

In the UA RosBREED pedigree "conversion set" material, *Xap*F (0-5) ratings exhibited an average of 2.5 (2013), 3.7 (2014), and 3.4 (2013-2015 avg), and a median of 2.0 (2013), 4.0 (2014), 4.0 (2015) and 3.0 (2013-2015 avg) (Table 7). The *Xap*F ratings ranged from 0.0-5.0 (2013, 2015, and 2013-2015 avg) and 1.0-5.0 (2014). The standard deviation displayed across all UA material was 1.5 (2013), 1.1 (2014 and 2013-2015 avg), and 1.3 (2015). The 2013 and 2013-2015 avg data sets were slightly skewed towards high *Xap*F ratings, and the 2014 and 2015 data sets were heavily skewed towards high *Xap*F ratings (Fig. 5).

Table 7. Mean, median, minimum, maximum, and standard deviation of *Xap*F (0-5 scale; 0 = resistant, 5 = susceptible) ratings for the UA RosBREED pedigree "conversion set" across 2013, 2014, 2015, and 2013-2015 avg data sets (N is number of individuals).

Year	*Xap* (0-5)					N
	Mean	Median	Min.	Max.	Std. dev.	
2013	2.5	2.0	0.0	5.0	1.5	232
2014	3.7	4.0	1.0	5.0	1.1	218
2015	3.4	4.0	0.0	5.0	1.3	222
2013-2015 avg	3.1	3.0	0.0	5.0	1.1	233

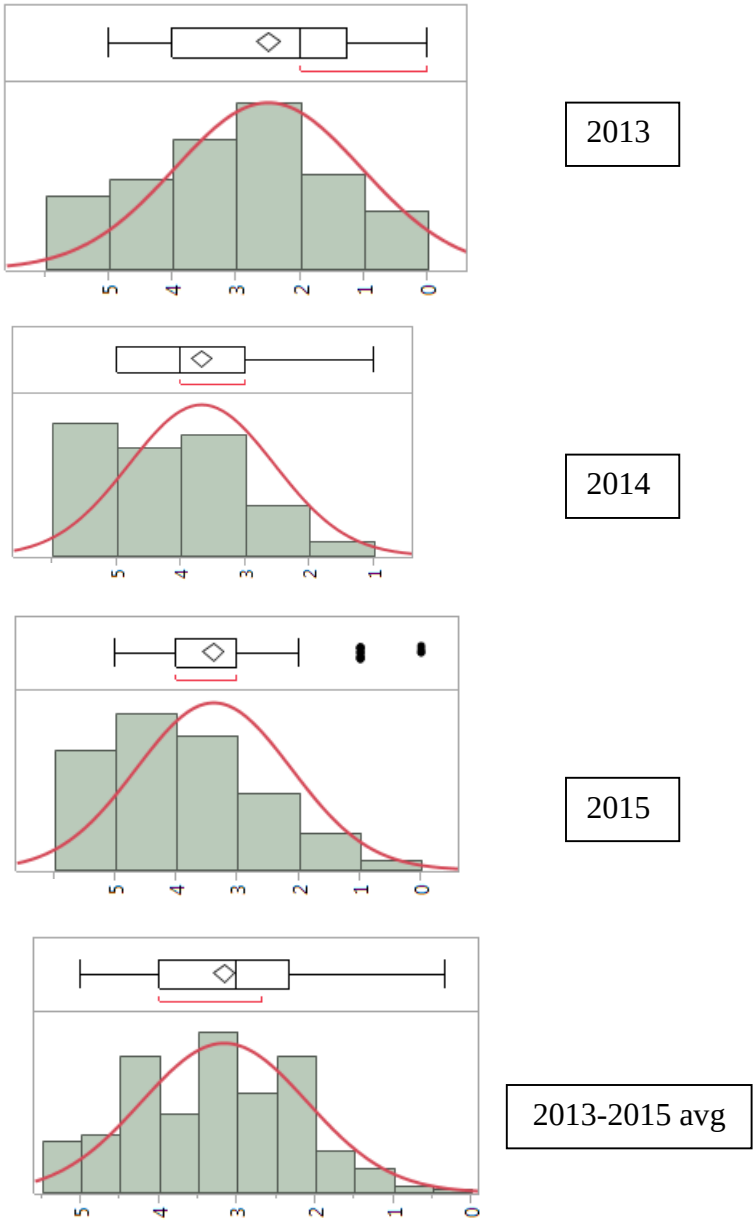

Fig. 5. Histograms and box plots of *Xap*F (0-5 scale; 0 = resistant, 5 = susceptible) distribution in the UA RosBREED pedigree "conversion set" across 2013, 2014, 2015 and 2013-2015 avg data sets (*notice that 2014 data set starts at 1) (Normalized goodness of fit curves, are presented in red to highlight normality or skewness of data).

G1XapF and *G6XapF* DNA Test Results

A total of four informative SNP markers which spanned (~2 Mbp) the previously identified QTL for *Xap*F resistance at the *G1XapF locus* [~12.92 (SNP_IGA_39717) to 14.98 Mbp (SNP_IGA_46754)] on *P. persica* chromosome 1 were used for haplotype construction (see Chapter Four for additional details) (Yang et al., 2013) (Fig. 3). Using these four SNPs and 240 out of 243 individuals in the UA RosBREED pedigree "conversion set," three unique *G1XapF* sequence haplotypes were observed and subsequently converted into functional haplotype groups based on haplotype effect on *Xap* fruit resistance/susceptibility [susceptible (SU), intermediate (I), and resistant (R1)] (Table 8). In total, the frequencies for each unique functional haplotype ranged from 24% (SU) to 39% (R1). In selected germplasm (germplasm selected prior in seedling populations in routine breeding field selection activities), the haplotype frequencies for 104 individuals ranged from 30% (I) to 38% (SU). In un-selected germplasm (F_1 seedlings, not yet selected), the haplotype frequencies for 136 individuals ranged from 13% (SU) to 44% (R1) (Table 8).

In contrast, the *G6XapF* spanned a much smaller region ~10 kbp, on *P. persica* chromosome 6 and the four SNP markers used for haplotype construction at the *G6XapF* locus were located within 22.29 (SNP_IGT_680889) to 22.30 Mbp (SNP_IGA_681081) (see Chapter Four for additional details) (Yang et al., 2013) (Fig. 4). Using these four SNPs and 240 out of 241 individuals in the UA RosBREED pedigree "conversion set," three unique *G1XapF* sequence haplotypes were observed and subsequently converted into functional haplotype groups based on haplotype effect on *Xap* fruit resistance/susceptibility [susceptible (SU), resistant one (R1), and resistant two (R2)] (Table 9). In total, the frequencies for each unique functional haplotype ranged from 15% (SU) to 54% (R1). In selected germplasm, the haplotype frequencies

for 104 individuals ranged from 18% (SU) to 50% (R1). In un-selected germplasm, the haplotype frequencies for 136 individuals ranged from 13% (SU) to 57% (R1) (Table 9).

Table 8. The three unique sequence and functional *G1XapF* 4-SNP haplotypes for distinguishing haplotype effect on *Xap*F ratings (0-5 scale; 0 = resistant, 5 = susceptible) found within the UA RosBREED pedigree "conversion set" screened with the 9K and/or mini-SNP array v1.0. The "conversion set" consisted of 240 total individuals (selected = 104 cultivars and selections and un-selected = 136 F1 seedlings) (N is number of haplotypes analyzed).

Locus	4-SNP haplotype		Total		Selected		Un-selected	
	Sequence	Function	N=480	Frequency	N=208	Frequency	N=272	Frequency
G1XapF	AAAB	SU	115	0.24	78	0.38	118	0.44
	BBAB	I	180	0.37	63	0.30	117	0.43
	BBAA	R1	185	0.39	67	0.32	37	0.13

Table 9. The three unique sequence and functional *G6XapF* 4-SNP haplotypes for distinguishing haplotype effect on *Xap*F ratings (0-5 scale; 0 = resistant, 5 = susceptible) found within the UA RosBREED pedigree "conversion set" screened with the 9K and/or mini-SNP array v1.0. The "conversion set" consisted of 240 total individuals (selected = 104 cultivars and selections and un-selected = 136 F1 seedlings) (N is number of haplotypes analyzed).

Locus	4-SNP haplotype		Total		Selected		Un-selected	
	Sequence	Function	N=480	Frequency	N=208	Frequency	N=272	Frequency
G6XapF	BAAB	SU	71	0.15	37	0.18	34	0.13
	ABAA	R1	258	0.54	103	0.50	155	0.57
	BBBB	R2	151	0.31	68	0.32	83	0.30

Non-Parametric Kruskal-Wallis test "Conversion Set."

In terms of *G1XapF,* all individuals in the UA RosBREED pedigree "conversion set" with *G1XapF* functional diplotype R1 | R1, R1 | I, and R1 | SU had a significantly lower 2013-2015 avg *Xap*F median (3.0) than individuals with diplotypes I | I, SU | I, and SU | SU (4.0) (< 0.0001) (Table 10). Additionally, the R^2 for the *G1XapF* functional diplotypes and *Xap*F resistance in 2013-2015 avg was ~7% for 240 individuals, thus the phenotypic variation explained (Vp %) was ~7%.

Table 10. Nonparametric medians of *Xap*F (0-5 scale; 0 = resistant, 5 = highly susceptible) for *G1XapF* diplotypes across 2013-2015 avg, in all individuals in the UA "conversion set".

G1XapF diplotype	*Xap*F median
R1 | R1	3.0 a[Z]
R1 | I	3.0 a
R1 | SU	3.0 a
I | I	4.0 b
SU | I	4.0 b
SU | SU	4.0 b

[Z] Medians followed by the same letter are not significantly different as determined by non-parametric Kruskal-Wallis test with Wilcoxon rank sum pairwise comparisons test ($P \leq 0.05$).

In terms of *G6XapF*, all individuals in the UA RosBREED pedigree "conversion set" with *G6XapF* functional diplotype R | R had a significantly lower 2013-2015 avg *Xap*F median (2.0) than individuals with diplotypes R | SU (3.0) or SU | SU (4.0) (< 0.0001) (Table 11). Furthermore, the 2013-2015 avg *Xap*F median for those with diplotype R | SU (3.0) was significantly lower than individuals which were SU | SU (4.0) (< 0.0001). Additionally the R^2 for the *G6XapF* functional diplotypes and *Xap*F resistance in 2013-2015 avg was ~8% for 240 individuals, thus the phenotypic variation explained (Vp %) was ~8%.

Table 11. Nonparametric medians of *Xap* (0-5 scale; 0 = resistant, 5 = highly susceptible) for *G6XapF* diplotypes across 2013-2015 avg, in all individuals in the UA "conversion set".

G6XapF diplotype	*Xap*F median
R | R	2.0 a[Z]
R | SU	3.0 b
SU | SU	4.0 c

[Z] Medians followed by the same letter are not significantly different as determined by non-parametric Kruskal-Wallis test with Wilcoxon rank sum pairwise comparisons test ($P \leq 0.05$).

Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR DNA Test Genotyping

Genetic screening for the Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR SSR DNA tests was successful across all individuals in the study except for a negligible sample failure rate of ~1-5%. Three alleles were identified in UA material for Ppe-*Xap*F1-SSR: 311 (I), 322 (R1), and 325 (SU) bp. Considering these three alleles, seven genotypes were observed across all material: 311 | 311 (I | I), 311 | 322 (I | R1), 311 | 325 (I | SU), 322 | 322 (R1 | R1), 322 | 325 (R1 | SU), and 325 | 325 (SU | SU). Representative alleles for the Ppe-*Xap*F1-SSR and DNA test using the ABI GeneMarker® software are depicted in Figs. (6-8). In contrast, only two alleles were identified in UA material for Ppe-*Xap*F6-SSR: 341 (SU) and 364 (R1) bp. Considering these alleles, three genotypes were observed across all material: 341 | 341 (SU | SU), 341 | 364 (SU | R1), and 364 | 364 (R1 | R1). Representative alleles for the Ppe-*Xap*F6-SSR and DNA test using the ABI GeneMarker® software and the Fragment Analyzer™ PROSize® v.1 software are depicted in Figs. (9-11) and (12-14).

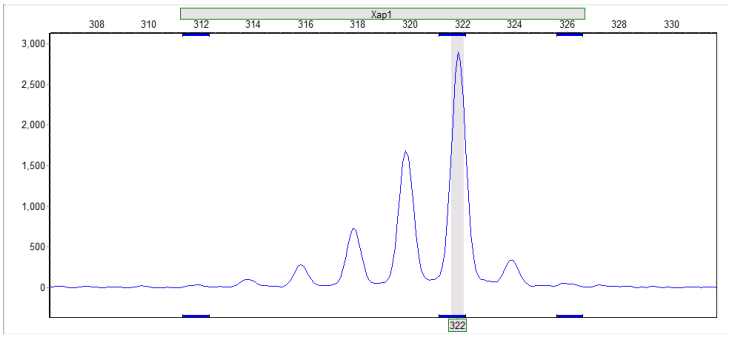

Fig. 6. Representative ABI output of an individual with genotype 322 | 322 (R1 | R1) bp using the Ppe-*Xap*F1-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

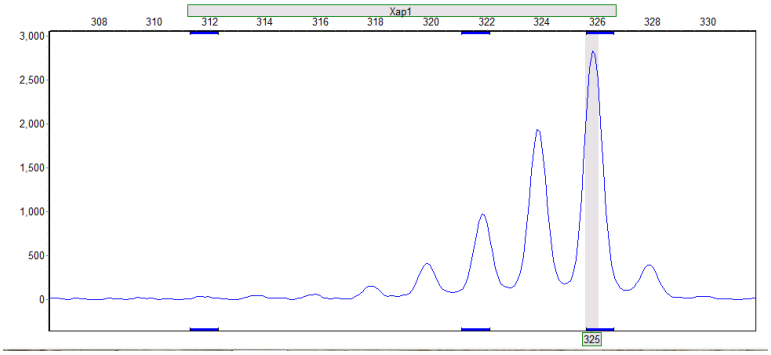

Fig. 7. Representative ABI output of an individual with genotype 325 | 325 (SU | SU) bp using the Ppe-*Xap*F1-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

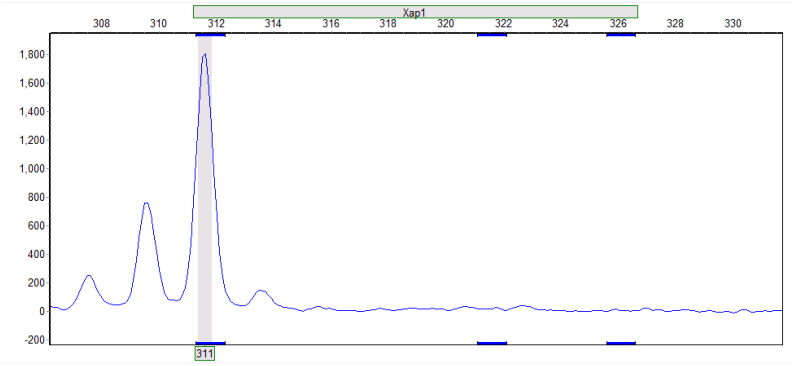

Fig. 8. Representative ABI output of an individual with genotype 311 | 311 (I | I) bp using the Ppe-*Xap*F1-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

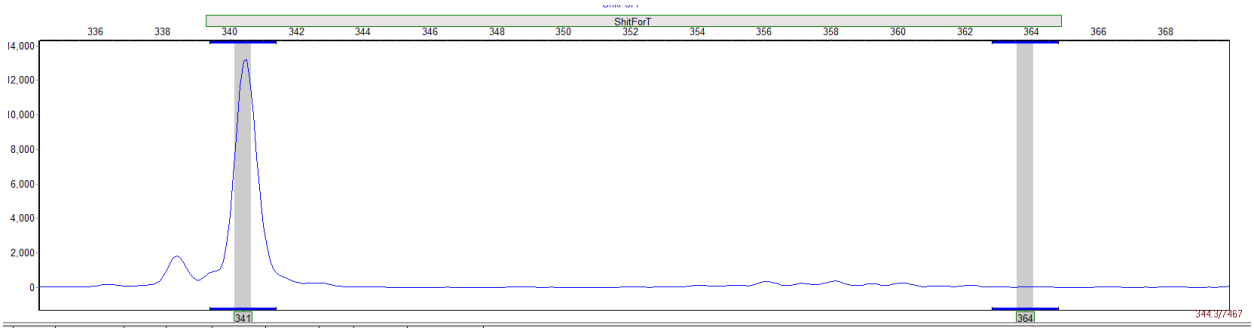

Fig. 9. Representative ABI output of an individual with genotype 341 | 341 (SU | SU) bp using the Ppe-*Xap*F6-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

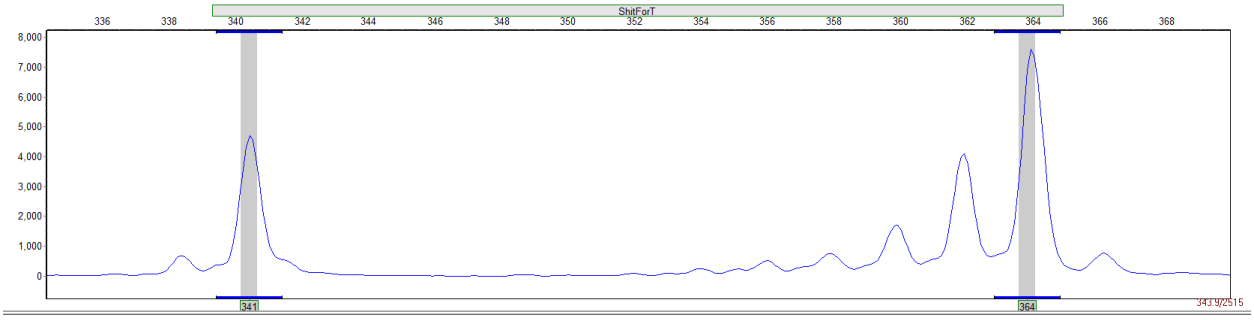

Fig. 10. Representative ABI output of an individual with genotype 341 | 364 (SU | R1) bp using the Ppe-*Xap*F6-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

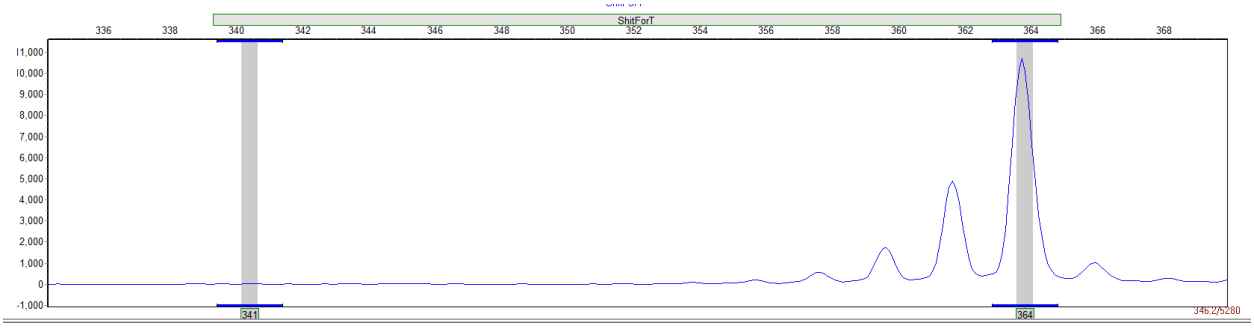

Fig. 11. Representative ABI output of an individual with genotype 364 | 364 (R1 | R1) bp using the Ppe-*Xap*F6-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

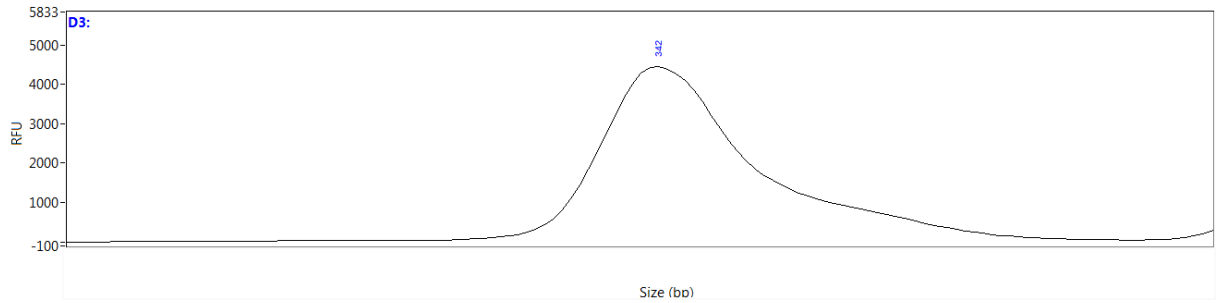

Fig. 12. Representative FA output of an individual with genotype 341 | 341 (SU | SU) bp using the Ppe-*Xap*F6-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

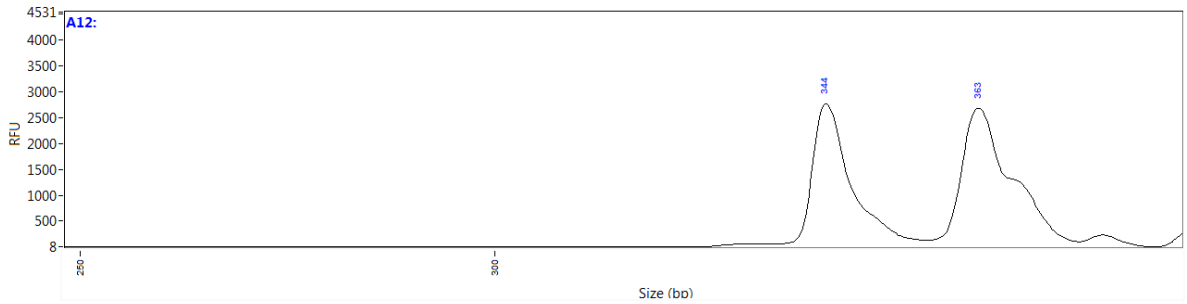

Fig. 13. Representative FA output of an individual with genotype 341 | 364 (SU | R1) bp using the Ppe-*Xap*F6-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

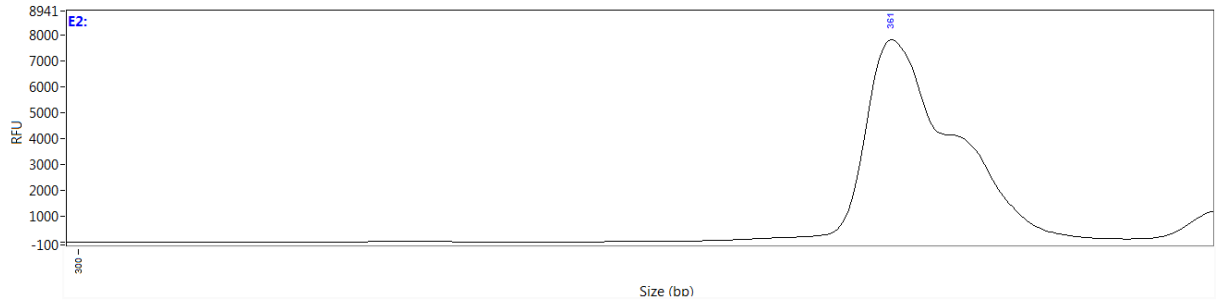

Fig. 14. Representative FA output of an individual with genotype 364 | 364 (R1 | R1) bp using the Ppe-*Xap*F6-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

SNP Haplotypes to Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR DNA Test Conversion

At the *G6XapF locus,* both DNA tests for each UA RosBREED population in the "conversion set" showed that the Ppe-*Xap*F6-SSR genotypes and matching *G6XapF* diplotypes fit their expected parental segregation ratio patterns (Table 12). It's important to note that only one resistant allele was identified using Ppe-*Xap*F6-SSR (364, R1) which corresponded to either of the two resistant alleles identified using the *G6XapF* DNA test (R1, R2). For AR_Pop_1, the female parent 'White County' and the male parent A-672 were both homozygous resistant for the Ppe-*Xap*F6-SSR (R | R, and R | R) and *G6XapF* (R1 | R1, and R1 | R2) DNA tests and their 47 F_1 progeny were R | R ($p = 1.0$). The remaining population chi-square p-values ranged from 0.32 (AR_Pop_0817) to 1.0 (AR_Pop_0801, AR_Pop_0803, AR_Pop_0813, and AR_Pop_0825) (Table 10). The genotypes and haplotypes for all seedlings are shown in Table 11.

Contrarily, at the *G1XapF locus*, the Ppe-*Xap*F1-SSR and *G1XapF* 4-SNP DNA test results for the F_1 seedlings in the seven UA RosBREED population in the "conversion set" only matched in the first three populations (AR_Pop_1, AR_Pop_0801, and AR_Pop_0803) (Table 14). Unfortunately, the Ppe-*Xap*F1-SSR and *G1XapF* 4-SNP DNA test results for the F_1 seedlings in the additional four populations contained inconsistencies (AR_Pop_0813, AR_Pop_0817, AR_Pop_0819, and AR_Pop_0825). However, the *G1XapF* diplotypes did fit their expected parental segregation ratio patterns in all seven populations. For AR_Pop_1, the female parent 'White County' and the male parent A-672 *G1XapF* diplotypes were homozygous R1 | R1 and heterozygous R1 | I. Their 45 F_1 progeny segregation ratio fit their parent's expected ratio as 25 were R1 | R1 and 20 were R | I ($p = 0.46$). The remaining populations chi-square p-values ranged from 0.22 (AR_Pop_0817) to 0.92 (AR_Pop_0801) (Table 12). The genotypes and haplotypes for all seedlings are shown in Table 13.

Table 12. The *G6XapF* functional SNP diplotypes and Ppe-*Xap*F6-SSR functional genotypes and fruit bacterial spot (*Xap*F) phenotypic ratings (0-5 scale) (avg 3 years) for parents of the seven F_1 UA RosBREED populations (2013-2015 avg) (N is number of samples analyzed) [*The chi-square p-value in brackets].

F_1 population	Progeny (N)	Parents		*Xap*F rating	*G6XapF*	Ppe-*Xap*F6-SSR	F_1 progeny allele segregation ratio
AR_Pop_1	46	Female	White County	1.7	R1 | R1	R | R	47 R | R [*1.00]
		Male	A-672	3.3	R1 | R2	R | R	
AR_Pop_0801	16	Female	A-776	2.0	R1 | R2	R | R	8 R | R : 8 R | SU [*1.00]
		Male	A-783	3.3	R1 | SU	R | SU	
AR_Pop_0803	11	Female	Amoore Sweet	1.7	R1 | R2	R | R	13 R | R [*1.00]
		Male	A-778	2.7	R1 | R2	R | R	
AR_Pop_0813	12	Female	A-772	0.7	R2 | R2	R | R	13 R | R [*1.00]
		Male	A-672	3.3	R1 | R2	R | R	
AR_Pop_0817	9	Female	A-789	2.7	R1 | R2	R | R	6 R | R : 3 R | SU [*0.32]
		Male	A-699	3.3	R1 | SU	R | SU	
AR_Pop_0819	22	Female	A-708	4.3	R1 | SU	R | SU	6 R | R : 12 R | SU : 5 SU | SU [*0.46]
		Male	A-773	2.7	R1 | SU	R | SU	
AR_Pop_0825	16	Female	Souvenirs	2.0	R1 | R1	R | R	17 R | R [*1.00]
		Male	A-760	1.7	R1 | R2	R | R	

Table 13. Relationships between SNP diplotypes (*G1XapF* and *G6XapF*), SSR genotypes (Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR), and fruit bacterial spot (*Xap*F) phenotypes (0-5 scale; 2013-2015 avg) for the seven F_1 populations in the AR RosBREED pedigree "conversion set". All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2013-2015). Individuals with inconsistencies between their SNP diplotypes and SSR genotypes are marked in **bold**, and the correct functional haplotype follows with ***( ).**

Individual	*G1XapF* functional diplotype	Ppe-*Xap*F1-SSR functional genotype	*G6XapF* functional diplotype	Ppe-*Xap*F6-SSR functional genotype	Phenotype
AR_Pop_1_01	R1 | R1	R1 | R1	R1 | R2	R | R	2.3
AR_Pop_1_02	R1 | R1	R1 | R1	R1 | R1	R | R	3.0
AR_Pop_1_03	R1 | R1	R1 | R1	R1 | R2	R | R	2.3
AR_Pop_1_04	R1 | R1	R1 | R1	R1 | R1	R | R	2.7
AR_Pop_1_05	R1 | I	R1 | I	R1 | R1	R | R	1.3
AR_Pop_1_06	R1 | I	R1 | I	R1 | R1	R | R	0.0
AR_Pop_1_07	R1 | R1	R1 | R1	R1 | R2	R | R	3.7
AR_Pop_1_08	R1 | R1	R1 | R1	R1 | R1	R | R	2.0
AR_Pop_1_09	R1 | R1	R1 | R1	R1 | R2	R | R	3.7
AR_Pop_1_10	R1 | R1	R1 | R1	R1 | R2	R | R	4.0
AR_Pop_1_11	R1 | R1	-	R1 | R1	R | R	3.0
AR_Pop_1_12	R1 | I	R1 | I	R1 | R1	R | R	2.0
AR_Pop_1_14	R1 | I	R1 | I	R1 | R2	R | R	3.7
AR_Pop_1_15	R1 | I	R1 | I	R1 | R1	R | R	1.0
AR_Pop_1_17	R1 | R1	R1 | R1	R1 | R1	R | R	3.0
AR_Pop_1_18	R1 | R1	R1 | R1	R1 | R2	R | R	0.0
AR_Pop_1_19	R1 | R1	R1 | R1	R1 | R1	R | R	2.7
AR_Pop_1_20	R1 | R1	R1 | R1	R1 | R1	R | R	2.7
AR_Pop_1_21	R1 | I	R1 | I	R1 | R2	R | R	4.3

Table 13. Relationships between SNP diplotypes (*G1XapF* and *G6XapF*), SSR genotypes (Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR), and fruit bacterial spot (*Xap*F) phenotypes (0-5 scale; 2013-2015 avg) for the seven F_1 populations in the AR RosBREED pedigree "conversion set". All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2013-2015). Individuals with inconsistencies between their SNP diplotypes and SSR genotypes are marked in **bold**, and the correct functional haplotype follows with ***( )**. (Cont.).

Individual	*G1XapF* functional diplotype	Ppe-*Xap*F1-SSR functional genotype	*G6XapF* functional diplotype	Ppe-*Xap*F6-SSR functional genotype	Phenotype
AR_Pop_1_22	R1 | R1	R1 | R1	R1 | R1	R | R	3.0
AR_Pop_1_23	R1 | R1	R1 | R1	R1 | R1	R | R	4.0
AR_Pop_1_24	R1 | R1	R1 | R1	R1 | R2	R | R	2.7
AR_Pop_1_25	R1 | R1	R1 | R1	R1 | R1	R | R	2.3
AR_Pop_1_26	R1 | R1	R1 | R1	R1 | R2	R | R	3.3
AR_Pop_1_27	R1 | I	R1 | I	R1 | R1	R | R	2.3
AR_Pop_1_28	R1 | R1	R1 | R1	R1 | R1	R | R	1.7
AR_Pop_1_29	R1 | R1	R1 | R1	R1 | R1	R | R	3.0
AR_Pop_1_30	R1 | I	R1 | I	R1 | R2	R | R	2.3
AR_Pop_1_31	R1 | I	R1 | I	R1 | R1	R | R	3.0
AR_Pop_1_32	R1 | I	R1 | I	R1 | R1	R | R	4.3
AR_Pop_1_33	R1 | I	R1 | I	R1 | R1	R | R	3.7
AR_Pop_1_34	R1 | I	R1 | I	R1 | R2	R | R	3.0
AR_Pop_1_35	R1 | I	R1 | I	R1 | R1	R | R	4.3
AR_Pop_1_36	R1 | R1	R1 | R1	R1 | R1	R | R	2.7
AR_Pop_1_37	R1 | I	R1 | I	R1 | R2	R | R	3.7
AR_Pop_1_38	-	-	R1 | R1	R | R	3.0
AR_Pop_1_39	R1 | I	R1 | I	R1 | R2	R | R	3.7
AR_Pop_1_40	R1 | R1	R1 | R1	R1 | R1	R | R	3.7
AR_Pop_1_41	-	R1 | I	-	R | R	5.0

Table 13. Relationships between SNP diplotypes (*G1XapF* and *G6XapF*), SSR genotypes (Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR), and fruit bacterial spot (*Xap*F) phenotypes (0-5 scale; 2013-2015 avg) for the seven F_1 populations in the AR RosBREED pedigree "conversion set". All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2013-2015). Individuals with inconsistencies between their SNP diplotypes and SSR genotypes are marked in **bold**, and the correct functional haplotype follows with ***( )**. (Cont.).

Individual	*G1XapF* functional diplotype	Ppe-*Xap*F1-SSR functional genotype	*G6XapF* functional diplotype	Ppe-*Xap*F6-SSR functional genotype	Phenotype
AR_Pop_1_42	R1 | I	R1 | I	R1 | R1	R | R	3.3
AR_Pop_1_43	R1 | R1	R1 | R1	R1 | R1	R | R	2.7
AR_Pop_1_44	R1 | I	R1 | I	R1 | R2	R | R	4.3
AR_Pop_1_45	R1 | I	R1 | I	R1 | R2	R | R	2.0
AR_Pop_1_46	R1 | I	R1 | I	R1 | R2	R | R	3.0
AR_Pop_1_47	R1 | I	R1 | I	R1 | R1	R | R	3.7
AR_Pop_1_48	R1 | R1	R1 | R1	R1 | R2	R | R	1.7
AR_Pop_1_49	R1 | R1	R1 | R1	R1 | R2	R | R	3.3
AR_Pop_0801_01	SU | I	SU | I	R2 | R2	R | R	3.0
AR_Pop_0801_02	SU | I	SU | I	R1 | SU	R | SU	2.7
AR_Pop_0801_03	**R1 | I**	**SU*(R1) | I**	R1 | R2	R | R	3.0
AR_Pop_0801_04	SU | R1	-	R2 | R2	R | R	3.7
AR_Pop_0801_05	**I | I**	**SU*(I) | SU*(I)**	R1 | R2	R | R	2.0
AR_Pop_0801_06	**R1 | I**	**R1 | R1*(I)**	R1 | SU	R | SU	3.3
AR_Pop_0801_07	SU | I	SU | I	R1 | R2	R | R	2.0
AR_Pop_0801_08	**R1 | I**	**R1 | R1*(I)**	R1 | R2	R | R	2.0
AR_Pop_0801_09	SU | I	SU | I	R2 | R2	R | R	3.0
AR_Pop_0801_10	**I | I**	**SU*(I) | SU*(I)**	R1 | SU	R | SU	3.0
AR_Pop_0801_11	R1 | I	R1 | I	R1 | SU	R | SU	2.3
AR_Pop_0801_12	SU | R1	SU | R1	R1 | SU	R | SU	2.3

Table 13. Relationships between SNP diplotypes (*G1XapF* and *G6XapF*), SSR genotypes (Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR), and fruit bacterial spot (*Xap*F) phenotypes (0-5 scale; 2013-2015 avg) for the seven F_1 populations in the AR RosBREED pedigree "conversion set". All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2013-2015). Individuals with inconsistencies between their SNP diplotypes and SSR genotypes are marked in **bold**, and the correct functional haplotype follows with ***( )**. (Cont.).

Individual	*G1XapF* functional diplotype	Ppe-*Xap*F1-SSR functional genotype	*G6XapF* functional diplotype	Ppe-*Xap*F6-SSR functional genotype	Phenotype
AR_Pop_0801_13	SU | R1	SU | R1	R1 | R2	R | R	3.0
AR_Pop_0801_14	I | I	-	R1 | SU		4.3
AR_Pop_0801_15	SU | R1	SU | R1	R1 | SU	R | SU	3.0
AR_Pop_0801_16	R1 | I	R1 | I	R1 | SU	R | SU	3.3
AR_Pop_0803_01	SU | SU	SU | SU	R1 | R2	R | R	1.7
AR_Pop_0803_02	SU | I	SU | I	R1 | R2	R | R	3.3
AR_Pop_0803_03	SU | SU	SU | SU	R1 | R1	R | R	2.7
AR_Pop_0803_04	SU | SU	SU | SU	R2 | R2	R | R	2.0
AR_Pop_0803_05	SU | I	SU | I	R2 | R2	R | R	2.7
AR_Pop_0803_06	SU | I	SU | I	R2 | R2	R | R	3.7
AR_Pop_0803_07	SU | SU	SU | SU	R1 | R2	R | R	3.0
AR_Pop_0803_09	SU | SU	SU | SU	R2 | R2		-
AR_Pop_0803_10	SU | SU	SU | SU	R1 | R2		-
AR_Pop_0803_11	SU | SU	SU | SU	R1 | R2	R | R	4.3
AR_Pop_0803_12	SU | I	SU | I	R2 | R2	R | R	4.7
AR_Pop_0803_13	SU | I	SU | I	R1 | R1		4.0
AR_Pop_0803_14	SU | I	SU | I	R1 | R1	R | R	4.7
AR_Pop_0803_15	-	-	R1 | R2		4.0
AR_Pop_0813_01	I | I	I | I	R1 | R2	R | R	5.0
AR_Pop_0813_02	**R1 | I**	**R1 | R1*(I)**	R1 | R2	R | R	3.7

Table 13. Relationships between SNP diplotypes (*G1XapF* and *G6XapF*), SSR genotypes (Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR), and fruit bacterial spot (*Xap*F) phenotypes (0-5 scale; 2013-2015 avg) for the seven F_1 populations in the AR RosBREED pedigree "conversion set". All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2013-2015). Individuals with inconsistencies between their SNP diplotypes and SSR genotypes are marked in **bold**, and the correct functional haplotype follows with ***( )**. (Cont.).

Individual	*G1XapF* functional diplotype	Ppe-*Xap*F1-SSR functional genotype	*G6XapF* functional diplotype	Ppe-*Xap*F6-SSR functional genotype	Phenotype
AR_Pop_0813_03	**R1 | I**	**R1 | R1*(I)**	R1 | R2	R | R	2.7
AR_Pop_0813_04	R1 | R1	R1 | R1	R1 | R2	R | R	4.0
AR_Pop_0813_05	I | I	I | I	R1 | R2	R | R	4.7
AR_Pop_0813_06	**R1 | I**	**R1 | R1*(I)**	R2 | R2	R | R	4.7
AR_Pop_0813_07	I | I	I | I	R2 | R2	R | R	2.0
AR_Pop_0813_08	**R1 | I**	**R1 | R1*(I)**	R2 | R2	R | R	3.3
AR_Pop_0813_09	**R1 | I**	**R1 | R1*(I)**	R1 | R2	R | R	4.7
AR_Pop_0813_10	I | I	I | I	R2 | R2	R | R	3.0
AR_Pop_0813_11	R1 | R1	R1 | R1	R2 | R2	R | R	1.7
AR_Pop_0813_12	R1 | R1	R1 | R1	R1 | R2	R | R	2.0
AR_Pop_0817_01	R1 | I	R1 | I	R1 | R2	R | R	5.0
AR_Pop_0817_02	**I | I**	**SU*(I) | I**	R1 | R2	R | R	5.0
AR_Pop_0817_03	R1 | R1	R1 | R1	R1 | R1	R | R	4.7
AR_Pop_0817_04	R1 | I	R1 | I	R1 | R2	R | R	5.0
AR_Pop_0817_05	**I | I**	**SU*(I) | I**	R1 | SU	R | SU	5.0
AR_Pop_0817_06	R1 | I	R1 | I	R1 | SU	R | SU	4.7
AR_Pop_0817_07	R1 | I	R1 | I	R1 | R2	R | R	4.0
AR_Pop_0817_08	**I | I**	**SU*(I) | I**	R1 | SU	R | SU	4.7
AR_Pop_0817_09	R1 | I	R1 | I	R1 | R2	R | R	5.0
AR_Pop_0819_01	**R1 | I**	**R1 | SU*(I)**	R1 | R2	R | SU	4.7

Table 13. Relationships between SNP diplotypes (*G1XapF* and *G6XapF*), SSR genotypes (Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR), and fruit bacterial spot (*Xap*F) phenotypes (0-5 scale; 2013-2015 avg) for the seven F_1 populations in the AR RosBREED pedigree "conversion set". All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2013-2015). Individuals with inconsistencies between their SNP diplotypes and SSR genotypes are marked in **bold**, and the correct functional haplotype follows with ***( )**. (Cont.).

Individual	*G1XapF* functional diplotype	Ppe-*Xap*F1-SSR functional genotype	*G6XapF* functional diplotype	Ppe-*Xap*F6-SSR functional genotype	Phenotype
AR_Pop_0819_02	**I | I**	**SU*(I) | SU*(I)**	R1 | SU	R | SU	4.3
AR_Pop_0819_03	**R1 | I**	**R1 | SU*(I)**	R1 | R2	R | R	5.0
AR_Pop_0819_04	**I | I**	**SU*(I) | SU*(I)**	R1 | SU	R | SU	4.0
AR_Pop_0819_05	**R1 | I**	**R1 | SU*(I)**	R1 | SU	R | SU	4.0
AR_Pop_0819_06	**R1 | I**	**R1 | SU*(I)**	R1 | R2	R | R	3.7
AR_Pop_0819_07	**I | I**	**SU*(I) | SU*(I)**	SU | SU	SU | SU	4.0
AR_Pop_0819_08	**R1 | I**	**R1 | SU*(I)**	R1 | R2	R | R	4.7
AR_Pop_0819_09	**R1 | I**	**R1 | SU*(I)**	R1 | R2	R | R	4.0
AR_Pop_0819_10	**I | I**	**SU*(I) | SU*(I)**	R1 | SU	R | SU	3.7
AR_Pop_0819_11	**I | I**	**SU*(I) | SU*(I)**	R1 | SU	R | SU	4.7
AR_Pop_0819_12	**I | I**	**SU*(I) | SU*(I)**	R1 | SU	R | SU	5.0
AR_Pop_0819_13	**R1 | I**	**R1 | SU*(I)**	SU | SU	SU | SU	4.3
AR_Pop_0819_14	**I | I**	**SU*(I) | SU*(I)**	R1 | SU	R | SU	4.0
AR_Pop_0819_15	**R1 | I**	**R1 | SU*(I)**	R1 | SU	R | SU	4.0
AR_Pop_0819_16	**I | I**	**SU*(I) | SU*(I)**	SU | SU	SU | SU	4.7
AR_Pop_0819_17	**R1 | I**	**R1 | SU*(I)**	R1 | SU	R | SU	5.0
AR_Pop_0819_18	**R1 | I**	**R1 | SU*(I)**	R1 | SU	R | SU	5.0
AR_Pop_0819_19	**I | I**	**SU*(I) | SU*(I)**	R1 | SU	R | SU	4.7
AR_Pop_0819_20	**R1 | I**	**R1 | SU*(I)**	R1 | SU	R | SU	4.0

Table 13. Relationships between SNP diplotypes (*G1XapF* and *G6XapF*), SSR genotypes (Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR), and fruit bacterial spot (*Xap*F) phenotypes (0-5 scale; 2013-2015 avg) for the seven F_1 populations in the AR RosBREED pedigree "conversion set". All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2013-2015). Individuals with inconsistencies between their SNP diplotypes and SSR genotypes are marked in **bold**, and the correct functional haplotype follows with ***( )**. (Cont.).

Individual	*G1XapF* functional diplotype	Ppe-*Xap*F1-SSR functional genotype	*G6XapF* functional diplotype	Ppe-*Xap*F6-SSR functional genotype	Phenotype
AR_Pop_0819_21	**R1 | I**	**R1 | SU*(I)**	SU | SU	SU | SU	4.7
AR_Pop_0819_22	**R1 | I**	**R1 | SU*(I)**	SU | SU	SU | SU	5.0
AR_Pop_0819_23	**I | I**	**SU*(I) | SU*(I)**	R1 | R2	R | R	3.7
AR_Pop_0825_01	SU | R1	SU | R1	R1 | R2	R | R	4.0
AR_Pop_0825_02	**SU | I**	**SU | SU*(I)**	R1 | R2	R | R	3.7
AR_Pop_0825_03	**R1 | I**	**R1 | SU*(I)**	R1 | R2	R | R	4.7
AR_Pop_0825_04	**SU | I**	**SU | SU*(I)**	R1 | R1	R | R	3.7
AR_Pop_0825_05	**R1 | I**	**R1 | SU*(I)**	R1 | R1	R | R	1.7
AR_Pop_0825_06	SU | R1	SU | R1	R1 | R2	R | R	4.3
AR_Pop_0825_07	**I | I**	**SU*(I) | SU*(I)**	R1 | R1	R | R	4.0
AR_Pop_0825_08	**R1 | I**	**R1 | SU*(I)**	R1 | R2	R | R	4.0
AR_Pop_0825_09	**R1 | I**	**R1 | SU*(I)**	R1 | R2	R | R	4.0
AR_Pop_0825_10	**I | I**	**SU*(I) | SU*(I)**	R1 | R1	R | R	4.3
AR_Pop_0825_11	**R1 | I**	**R1 | SU*(I)**	R1 | R2	R | R	4.7
AR_Pop_0825_12	**SU | I**	**SU | SU*(I)**	R1 | R2	R | R	4.7
AR_Pop_0825_13	**R1 | I**	**R1 | SU*(I)**	R1 | R1	R | R	4.0
AR_Pop_0825_14	**SU | I**	**SU | SU*(I)**	R1 | R2	R | R	3.7
AR_Pop_0825_15	**SU | I**	**SU | SU*(I)**	R1 | R1	R | R	3.0
AR_Pop_0825_16	**R1 | I**	**R1 | SU*(I)**	R1 | R2	R | R	3.7
AR_Pop_0825_17	**SU | I**	**SU | SU*(I)**	R1 | R1	R | R	4.0

Table 14. The *G1XapF* functional SNP diplotypes and Ppe-*Xap*F1-SSR functional genotypes and fruit bacterial spot (*Xap*F) phenotypic ratings (0-5 scale) for parents of the seven F_1 UA RosBREED populations (2013-2015 avg) (N is number of samples analyzed) [*The chi-square p-value in brackets]. Individuals with inconsistencies between their SNP diplotypes and SSR genotypes are marked in **bold**, and the correct functional haplotype follows with ***( )**.

F_1 population	Progeny (N)	Parents		*Xap*F rating	*G1XapF*	Ppe-*Xap*F1-SSR	F_1 progeny allele segregation ratio
AR_Pop_1	46	Female	White County	1.7	R1 | R1	R1 | R1	25 R | R : 20 R | I [*0.46]
		Male	A-672	3.3	R1 | I	R1 | I	
AR_Pop_0801	16	Female	A-776	2.0	SU | I	SU | I	4 SU | I : 5 R1 | I : 3 I | I : 4 SU | R1 [*0.92]
		Male	A-783	3.3	R1 | I	R1 | I	
AR_Pop_0803	11	Female	Amoore Sweet	1.7	SU | I	SU | I	7 S | S : 6 S | I [*0.78]
		Male	A-778	2.7	SU | SU	SU | SU	
AR_Pop_0813	12	Female	A-772	0.7	**R1 | I**	**R1 | R1*(I)**	3 R1 | R1 : 5 R1 | I : 4 I | I [*0.78]
		Male	A-672	3.3	R1 | I	R1 | I	
AR_Pop_0817	9	Female	A-789	2.7	R1 | I	R1 | I	1 R1 | R1 : 5 R1 | I : 3 R1 | I [*0.22]
		Male	A-699	3.3	**R1 | I**	**R1 | SU*(I)**	
AR_Pop_0819	22	Female	A-708	4.3	**R1 | I**	**R1 | SU*(I)**	10 I | I : 13 R1 | I [* 0.53]
		Male	A-773	2.7	**I | I**	**SU*(I) | SU*(I)**	
AR_Pop_0825	16	Female	Souvenirs	2.0	**I | I**	**SU*(I) | SU*(I)**	6 SU | I : 7 R1 | I [* 0.78]
		Male	A-760	1.7	**R1 | SU**	**R1 | I*(SU)**	

Next, the entire UA RosBREED "conversion set" of 243 individuals was used to make direct comparisons between each individual's SNP diplotype (*G1XapF* and *G6XapF*) and their SSR genotype (Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR). In terms of *G6XapF* and Ppe-*Xap*F6-SSR, at least one of the DNA tests failed for three individuals, thus, only 240 individuals were considered. In terms of *G1XapF* and Ppe-*Xap*F1-SSR, at least one of the DNA tests failed for 18 individuals, thus, only 225 individuals were considered.

For the *G6XapF* and Ppe-*Xap*F6-SSR DNA tests, in total, 181 individuals were homozygous resistant (R | R : R | R), 50 were heterozygous (R | SU : R | SU), and nine were homozygous susceptible (SU | SU : SU | SU) for both DNA tests (Table 13). In selected material, 73 individuals were homozygous resistant (R | R : R | R), 28 were heterozygous (R | SU : R | SU), and three were homozygous susceptible (SU | SU : SU | SU) for both DNA tests. In un-selected material, 108 individuals were homozygous resistant (R | R : R | R), 22 were heterozygous (R | SU : R | SU), and six were homozygous susceptible (SU | SU : SU | SU) for both DNA tests (Table 15). Overall the *G6XapF* SNP diplotypes and Ppe-*Xap*F6-SSR genotypes matched 100% of the time (Tables 12, 13, 15, 16, and 17).

Table 15. Relationships between the functional *G6XapF* SNP diplotypes and the Ppe-*Xap*F6-SSR DNA test genotypes in the UA RosBREED pedigree "conversion set" (N is number of samples analyzed). Inconsistencies between SNP diplotypes and Ppe-*Xap*F6-SSR genotypes are marked in **bold**, and the correct allele follows with ***( ).**

	DNA test				
Locus	*G6XapF* simple functional diplotype	Ppe-*Xap*F6-SSR functional genotype	Total (N)	Selected (N)	Un-selected (N)
G6XapF	R | R	R | R	181	73	108
	R | SU	R | SU	50	28	22
	SU | SU	SU | SU	9	3	6
	Total		240	103	136

Table 16. Relationships between the *G1XapF* and *G6XapF* functional diplotypes, the Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR functional genotypes, and the fruit bacterial spot (*Xap*F) phenotypic ratings (0-5 scale) (avg 3 years) for the cultivars and selections in the UA RosBREED pedigree "conversion set" (all individuals were genotyped with the IPSC 9K peach SNP array v1.0). Individuals with inconsistencies between their functional SNP diplotype and SSR functional genotype are marked in **bold**, and the correct SSR functional genotype follows with ***( ).**

Individual	*G1XapF*	Ppe-*Xap*F1-SSR	*G6XapF*	Ppe-*Xap*F6-SSR	*Xap*F rating
A-657[z]	SU | SU	SU | SU	R2 | R2	R | R	1.3
A-663[z]	**SU | SU**	**I*(SU) | I *(SU)**	R1 | R1	R | R	2.0
A-665[zy]	SU | I	SU | I	R1 | R2	R | R	2.0
A-672[zy]	R1 | I	R1 | I	R1 | R2	R | R	3.3
A-699[z]	**R1 | I**	**R1 | SU*(I)**	R1 | SU	R | SU	3.3
A-708[z]	**R1 | I**	**R1 | SU*(I)**	R1 | SU	R | SU	4.3
A-716[z]	**R1 | I**	**R1 | R1*(I)**	R1 | R2	R | R	2.0
A-760[z]	**R1 | SU**	**R1 | I*(SU)**	R1 | R2	R | R	1.7
A-772[z]	**R1 | I**	**R1 | R1*(I)**	R2 | R2	R | R	0.7
A-773[z]	**I | I**	**SU*(I) | SU*(I)**	R1 | SU	R | SU	2.7
A-776[z]	SU | I	-	R1 | R2	R | R	2.0
A-778[z]	SU | SU	-	R1 | R2	R | R	2.7
A-783[z]	R1 | I	R1 | I	R1 | SU	R | SU	3.3
A-789[z]	R1 | I	R1 | I	R1 | R2	R | R	2.7
Admiral Dewey[z]	**R1 | I**	**R1 | R1*(I)**	SU | SU	SU | SU	3.0
Amoore Sweet[z]	SU | I	SU | I	R1 | R2	R | R	1.7
Arrington[z]	**SU | SU**	**SU | I *(SU)**	R2 | R2	R | R	3.0
Bradley[zy]	**SU | SU**	**SU | I *(SU)**	R2 | R2	R | R	2.7
Chinapearl[z]	SU | I	**I*(SU) | I**	R1 | SU	R | SU	-
Chinese Cling[z]	**SU | I**	**R1*(SU) | R1*(I)**	R1 | SU	R | SU	4.0
Cumberland[z]	SU | I	-	R1 | SU	R | SU	3.0
Dixon[z]	R1 | SU	-	SU | SU	-	-
Early Crawford[z]	**SU | I**	**R1*(SU) | R1*(I)**	R1 | R1	R | R	-
Elberta[z]	**R1 | SU**	**R1 | R1*(SU)**	R2 | SU	R | SU	4.0
Georgia Belle[z]	SU | SU	-	R1 | R2	R | R	4.0

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 16. Relationships between the *G1XapF* and *G6XapF* functional diplotypes, the Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR functional genotypes, and the fruit bacterial spot (*Xap*F) phenotypic ratings (0-5 scale) (avg 3 years) for the cultivars and selections in the UA RosBREED pedigree, "conversion set" (all individuals were genotyped with the IPSC 9K peach SNP array v1.0). Individuals with inconsistencies between their functional SNP diplotype and SSR functional genotype are marked in **bold**, and the correct SSR functional genotype follows with ***( ).** (Cont.).

Individual	*G1XapF*	Ppe-*Xap*F1-SSR	*G6XapF*	Ppe-*Xap*F6-SSR	*Xap*F rating
Greensboro[z]	SU | I	-	SU | SU	-	2.0
Jefferson[z]	R1 | R1	R1 | R1	R2 | R2	R | R	3.0
JH Hale[z]	**R1 | SU**	**R1 | R1*(SU)**	R2 | SU	R | SU	5.0
Orange Cling[z]	R1 | R1	R1 | R1	R1 | SU	R | SU	-
Peento[z]	R1 | SU	-	SU | SU	SU | SU	5.0
Redskin[z]	R1 | SU	-	R1 | SU	-	-
Slappey[z]	SU | SU	-	R1 | R2	R | R	-
Souvenirs[z]	**I | I**	**SU*(I) | SU*(I)**	R1 | R1	R | R	2.0
Westbrook[z]	R1 | SU	R1 | SU	R2 | R2	R | R	3.0
White County[zy]	R1 | R1	R1 | R1	R1 | R1	R | R	1.7
White River[z]	R1 | R1	R1 | R1	R1 | R2	R | R	2.0
Winblo[z]	SU | SU	SU | SU	SU | SU	SU | SU	3.3

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 17. Relationships between the *G1XapF* and *G6XapF* functional diplotypes, the Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR functional genotypes, and the fruit bacterial spot (*Xap*F) phenotypic ratings (0-5 scale) (avg 3 years) for the 72 individuals screened with the Peach SNP mini array v1.0 (included in the "conversion set"). Individuals with inconsistencies between their functional SNP diplotype and SSR functional genotype are marked in **bold**, and the correct SSR functional genotype follows with ***( ).**

Individual	*G1XapF*	Ppe-*Xap*F1-SSR	*G6XapF*	Ppe-*Xap*F6-SSR	*Xap*F rating
A-665[zy]	SU | I	SU | I	R1 | R2	R | R	2.0
A-672[zy]	R1 | I	R1 | I	R1 | R2	R | R	3.3
A-786[y]	R1 | R1	R1 | R1	R1 | R2	R | R	3.3
A-792[y]	**R1 | I**	**R1 | R1*(I)**	R2 | R2	R | R	2.0
A-797[y]	SU | I	SU | I	R1 | R2	R | R	2.0
A-801[y]	**SU | I**	**I*(SU) | I**	R1 | R2	R | R	1.0
A-804[y]	**SU | I**	**SU | SU*(I)**	R1 | SU	R | SU	2.7
A-805[y]	**R1 | SU**	**R1 | I*(SU)**	R1 | SU	R | SU	1.7
A-806[y]	SU | I	SU | I	R2 | R2	-	1.3
A-809[y]	I | I	I | I	R1 | R2	R | R	2.0
A-811[y]	**SU | SU**	**SU | I *(SU)**	R1 | R2	R | R	2.0
A-813[y]	**R1 | I**	**R1 | R1*(I)**	R1 | SU	R | SU	3.3
A-816[y]	SU | I	-	R2 | R2	R | R	2.7
A-818[y]	SU | I	SU | I	R1 | SU	R | SU	4.3
A-819[y]	SU | I	SU | I	R1 | R2	R | R	1.3
A-820[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R2	R | R	0.7
A-821[y]	**R1 | SU**	**R1 | I*(SU)**	R1 | R2	R | R	0.3
A-822[y]	SU | I	SU | I	R1 | SU	R | SU	1.7
A-825[y]	R1 | R1	R1 | R1	R1 | SU	R | SU	4.3
A-826[y]	**R1 | SU**	**R1 | I*(SU)**	R1 | R1	R | R	2.0
A-827[y]	**R1 | SU**	**R1 | R1*(SU)**	R1 | R1	R | R	2.3
A-828[y]	SU | SU	SU | SU	R1 | R2	R | R	2.7
A-829[y]	**R1 | SU**	**R1 | R1*(SU)**	R1 | R2	R | R	1.7
A-832[y]	R1 | R1	R1 | R1	R1 | R2	R | R	1.7

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 17. Relationships between the *G1XapF* and *G6XapF* functional diplotypes, the Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR functional genotypes, and the fruit bacterial spot (*Xap*F) phenotypic ratings (0-5 scale) (avg 3 years) for the 72 individuals screened with the Peach SNP mini array v1.0 (included in the "conversion set"). Individuals with inconsistencies between their functional SNP diplotype and SSR functional genotype are marked in **bold**, and the correct SSR functional genotype follows with ***( ).** (Cont.).

Individual	*G1XapF*	Ppe-*Xap*F1-SSR	*G6XapF*	Ppe-*Xap*F6-SSR	*Xap*F rating
A-833[y]	SU | SU	-	R1 | R2	R | R	5.0
A-836[y]	**R1 | SU**	**R1 | R1*(SU)**	R1 | R2	R | R	2.7
A-837[y]	**I | I**	**SU*(I) | SU*(I)**	R1 | R1	R | R	2.0
A-840[y]	**SU | SU**	**I*(SU) | I *(SU)**	R1 | SU	R | SU	4.0
A-841[y]	**R1 | SU**	**R1 | R1*(SU)**	R2 | R2	R | R	2.7
A-842[y]	**R1 | SU**	**R1 | I*(SU)**	R1 | SU	R | SU	2.7
A-843[y]	**SU | SU**	**I*(SU) | I *(SU)**	R1 | SU	R | SU	4.3
A-844[y]	**R1 | SU**	**R1 | R1*(SU)**	R1 | SU	R | SU	4.3
A-845[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R1	R | R	1.0
A-848[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R1	R | R	2.0
A-849[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R1	R | R	2.3
A-850[y]	R1 | R1	R1 | R1	R1 | R1	R | R	3.3
A-851[y]	**SU | I**	**SU | SU*(I)**	R1 | R2	R | R	2.7
A-852[y]	**R1 | I**	**R1 | R1*(I)**	R1 | SU	R | SU	4.0
A-853[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R2	R | R	2.3
A-854[y]	**SU | I**	**SU | SU*(I)**	R1 | R2	R | R	2.7
A-855[y]	**I | I**	**SU*(I) | I**	R1 | R2	R | R	3.0
A-856[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R1	R | R	2.7
A-857[y]	**R1 | SU**	**R1 | R1*(SU)**	R1 | R2	R | R	2.3
A-859[y]	R1 | R1	R1 | R1	R1 | R1	R | R	2.5
A-860[y]	**SU | I**	**I*(SU) | I**	R2 | R2	R | R	3.0
A-861[y]	**SU | SU**	**I*(SU) | I *(SU)**	R1 | SU	R | SU	3.5
A-862[y]	**R1 | I**	**R1 | R1*(I)**	R1 | SU	R | SU	3.0
A-864[y]	**SU | SU**	**SU | I *(SU)**	R1 | R2	R | R	3.3

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 17. Relationships between the *G1XapF* and *G6XapF* functional diplotypes, the Ppe-*Xap*F1-SSR and Ppe-*Xap*F6-SSR functional genotypes, and the fruit bacterial spot (*Xap*F) phenotypic ratings (0-5 scale) (avg 3 years) for the 72 individuals screened with the Peach SNP mini array v1.0 (included in the "conversion set"). Individuals with inconsistencies between their functional SNP diplotype and SSR functional genotype are marked in **bold**, and the correct SSR functional genotype follows with ***( ).** (Cont.).

Individual	*G1XapF*	Ppe-*Xap*F1-SSR	*G6XapF*	Ppe-*Xap*F6-SSR	*Xap*F rating
A-865[y]	R1 | I	R1 | I	R1 | R2	R | R	3.5
A-866[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R1	R | R	2.0
A-867[y]	SU | I	SU | I	R1 | R2	R | R	2.0
A-868[y]	**SU | I**	**SU | SU*(I)**	R1 | R2	R | R	3.7
A-869[y]	R1 | R1	R1 | R1	R2 | R2	R | R	3.0
A-870[y]	SU | I	SU | I	R1 | SU	R | SU	2.5
A-871[y]	**I | I**	**SU*(I) | I**	R2 | R2	R | R	3.7
A-872[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R1	R | R	2.5
A-873[y]	**R1 | SU**	**R1 | I*(SU)**	R1 | SU	R | SU	3.7
A-874[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R1	R | R	3.3
A-875[y]	**SU | SU**	**SU | I *(SU)**	R2 | R2	R | R	4.0
A-876[y]	**SU | SU**	**I*(SU) | I *(SU)**	R2 | R2	R | R	3.7
A-877[y]	R1 | I	R1 | I	R1 | R1	R | R	3.0
A-878[y]	**SU | I**	**SU | SU*(I)**	R1 | R1	R | R	3.0
A-879[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R2	R | R	2.0
A-880[y]	R1 | R1	R1 | R1	R1 | R1	R | R	3.0
A-881[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R1	R | R	2.7
A-882[y]	**R1 | I**	**R1 | R1*(I)**	R1 | R1	R | R	2.7
Bowden[y]	SU | I	SU | I	R1 | R2	R | R	2.7
Bradley[zy]	**SU | SU**	**SU | I *(SU)**	R2 | R2	R | R	2.7
Gloria[y]	**R1 | SU**	**R1 | R1*(SU)**	R1 | SU	R | SU	-
White County[zy]	R1 | R1	R1 | R1	R1 | R1	R | R	1.7
White Diamond[y]	R1 | R1	R1 | R1	R1 | R2	R | R	1.3
White Rock[y]	SU | I	SU | I	R1 | SU	R | SU	3.3

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Contrarily, at the *G1XapF locus* the Ppe-*Xap*F1-SSR and *G1XapF* 4-SNP DNA test results for all individuals in the UA RosBREED "conversion set" only matched 50% of the time (Tables 13, 14, 16, 17, and 18). A total of 12 unique inconsistencies between *G1XapF* and the Ppe-*Xap*F1-SSR were observed. The inconsistency rate was much higher in selected material, in comparison to un-selected material (65% vs. 15%). Unfortunately, an overall 50% inconsistency rate between the *G1XapF* and the Ppe-*Xap*F1-SSR and 4-SNP DNA test results deems the Ppe-*Xap*F1-SSR to not be robust across material in the UA program, and thus it was not advanced to screen on the remaining 124 UA cultivars, selections and germplasm from other breeding programs, nor the 2010 populations "confirmation set".

Table 18. Relationships between the *G1XapF* functional diplotypes, and the Ppe-*Xap*F1-SSR genotypes in the UA RosBREED pedigree "conversion set" (N is number of samples analyzed). Inconsistencies between SNP diplotypes and Ppe-*Xap*F1-SSR genotypes are marked in **bold**, and the correct allele follows with ***( ).**

	DNA test				
Locus	*G1XapF* functional diplotype	Ppe-*Xap*F1-SSR functional genotype	Total (N)	Selected (N)	Un-selected (N)
G1XapF	R1 | R1	R1 | R1	40	12	28
	R1 | I	R1 | I	31	5	26
	I | I	I | I	5	1	4
	R1 | SU	R1 | SU	6	1	5
	SU | I	SU | I	21	11	10
	SU | SU	SU | SU	10	3	7
	R1 | I	**R1 | R1*(I)**	**26**	**19**	**7**
	R1 | SU	**R1 | R1*(SU)**	**9**	**9**	**0**
	R1 | I	**R1 | SU*(I)**	**22**	**2**	**20**
	R1 | SU	**R1 | I*(SU)**	**6**	**6**	**0**
	SU | I	**I*(SU) | I**	**3**	**3**	**0**
	SU | I	**R1*(SU) | R1*(I)**	**2**	**2**	**0**
	SU | SU	**I*(SU) | I *(SU)**	**5**	**5**	**0**
	SU | SU	**SU | I *(SU)**	**5**	**5**	**0**
	SU | I	**SU | SU*(I)**	**11**	**5**	**6**
	I | I	**SU*(I) | I**	**5**	**2**	**3**
	I | I	**SU*(I) | SU*(I)**	**17**	**3**	**14**
	R1 | I	**SU*(R1) | I**	**1**	**0**	**1**
	Total		225	94	131

Ppe-*Xap*F6-SSR DNA Test (Additional Cultivars and Selections)

Of the remaining 124 UA cultivars, selections and germplasm from other breeding programs not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0, 61 individuals were homozygous resistant, R **|** R, 57 were heterozygous R **|** SU, four were homozygous susceptible, SU **|** SU, and two individuals did not amplify using the Ppe-*Xap*F6-SSR (Table 19).

Table 19. Ppe-*Xap*F6-SSR DNA test genotypes and fruit bacterial spot (*Xap*F) phenotypic ratings (0-5 scale) (avg 3 years) of UA selections and cultivars (not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0) and germplasm from other breeding programs (2013-2015).

Individual	Ppe-*Xap*F6-SSR	*Xap*F rating
A-554	R | SU	3.7
A-662	R | R	1.0
A-668	R | R	3.0
A-743	R | R	1.7
A-758	R | R	2.3
A-761	R | R	2.3
A-766	R | SU	2.0
A-768	R | SU	4.0
A-770	R | R	1.7
A-790	R | R	2.0
A-794	R | R	2.3
A-798	R | R	2.3
A-799	R | R	2.0
A-803	R | R	1.3
A-808	R | R	1.7
A-810	R | SU	1.0
A-814	R | R	1.0
A-815	R | R	1.3
A-824	R | R	3.3
A-839	R | SU	4.7
A-846	R | R	3.3
A-847	R | R	4.0
A-858	R | R	3.3
A-883	R | R	2.5
A-884	R | SU	4.0
A-885	SU | SU	1.3
A-886	R | R	2.0
A-887	R | R	1.0
A-888	R | R	1.0
A-889	R | R	2.0
A-890	R | SU	3.0

Table 19. Ppe-*Xap*F1-SSR, and Ppe-*Xap*F6-SSR DNA test genotypes and fruit bacterial spot phenotypes (0-5 scale) (avg 3 years) of UA selections and cultivars (not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0) and germplasm from other breeding programs (2013-2015) (Cont.).

Individual	Ppe-*Xap*F6-SSR	*Xap*F rating
A-891	R | R	4.0
A-892	R | SU	4.0
A-893	R | R	3.0
A-894	R | R	4.0
A-895	R | R	-
A-896	R | R	4.0
A-897	R | R	3.0
A-898	R | SU	5.0
A-899	R | R	2.0
A-900	R | R	2.0
A-901	R | R	3.0
A-902	R | R	4.0
A-903	R | R	5.0
A-904	R | R	3.0
A-906	R | R	1.0
A-908	R | R	4.0
A-909	R | R	4.0
A-910	R | R	2.0
A-911	R | SU	-
A-912	R | R	4.0
A-913	R | R	-
A-914	R | R	-
A-915	R | R	-
A-916	R | R	-
A-917	R | SU	-
A-918	R | R	-
Admiral Dewey	SU | SU	3.0
Allgold	R | SU	1.5
Autumn Prince	R | SU	4.5
Autumn Star	-	4.0
Bounty	R | SU	3.0

Table 19. Ppe-*Xap*F1-SSR, and Ppe-*Xap*F6-SSR DNA test genotypes and fruit bacterial spot phenotypes (0-5 scale) (avg 3 years) of UA selections and cultivars (not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0) and germplasm from other breeding programs (2013-2015) (Cont.).

Individual	Ppe-*Xap*F6-SSR	*Xap*F rating
Bright Star	R | R	3.0
Challenger	R | R	2.0
China Pearl	R | SU	2.0
Chinese Cling	R | SU	4.0
Contender	R | SU	2.0
Cresthaven	R | SU	3.0
Crimson Lady	R | SU	5.0
Crimson Snow	R | SU	4.0
CVN13w	R | SU	3.0
Denman	SU | SU	-
Early Star	R | SU	3.0
Eastern Glo	R | SU	3.0
Elberta	R | SU	4.0
Emeraude	R | SU	5.0
Flavortop	R | SU	5.0
Georgia Belle	R | R	4.0
Gladiator	R | R	2.0
Goldilocks	R | SU	3.7
Goldjim	R | SU	3.5
Goldnine	R | SU	3.0
Greensboro	-	2.0
Jade	R | SU	
Jefferson	R | R	3.0
JH Hale	R | SU	5.0
KV175	R | SU	5.0
KV357	R | SU	4.5
KV398	R | SU	5.0
KV401	R | SU	3.5
KV501	R | SU	4.5
KV601	R | SU	5.0
KV606	R | SU	5.0
KV701	R | SU	4.5
KV801	R | SU	5.0

Table 19. Ppe-*Xap*F1-SSR, and Ppe-*Xap*F6-SSR DNA test genotypes and fruit bacterial spot phenotypes (0-5 scale) (avg 3 years) of UA selections and cultivars (not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0) and germplasm from other breeding programs (2013-2015). (Cont.).

Individual	Ppe-*Xap*F6-SSR	*Xap*F rating
Loring	R | R	3.0
Manon	R | SU	3.3
Messina	R | SU	3.5
Orange Cling	R | SU	-
Peento	SU | SU	5.0
PF 1	R | SU	3.5
PF 24-007	R | SU	3.0
PF 24C	R | R	3.0
PF 5B	R | SU	3.0
PF 5D Big	R | SU	3.0
PF Lucky 13	R | SU	3.0
PF-19-007	R | SU	3.8
Redhaven	R | SU	3.0
Rising Star	R | R	3.0
Roygold	R | R	2.5
Ruby Prince	R | SU	4.5
Saturn	R | R	4.0
Slappey	R | R	-
Spring Snow	R | R	4.5
Sugar Giant	R | SU	4.0
Sugar Lady	R | SU	4.5
Sweet Star	R | SU	3.8
Tango	R | R	3.0
Tango-II	R | R	3.5
Westbrook	R | R	3.0
White Cloud	R | SU	3.0
White Lady	R | SU	5.0
White River	R | R	2.5
Yumm Yumm	R | SU	3.8

Ppe-*Xap*F6-SSR DNA Test "Confirmation Set"

The F_1 seedlings alleles and matching phenotypes for each 2010 population ("confirmation set") fit the expected parental segregation ratio patterns for the Ppe-*Xap*F6-SSR DNA test (Table 20). For AR_Pop_1001, the female parent A-665 was a homozygous resistant, R | R, and the male parent A-800 was heterozygous, R | SU. Their F_1 progenies' genotypes fit their expected segregation ratio (22 R | R : 16 R | SU) ($p = 0.33$). The remaining populations chi-square p-values ranged from to 0.10 (AR_Pop_1025) to 1.00 (AR_Pop_1003, AR_Pop_1004, AR_Pop_1006, AR_Pop_1013, AR_Pop_1014, AR_Pop_1015, AR_Pop_1016, AR_Pop_1018, AR_Pop_1019, AR_Pop_1021, AR_Pop_1022, and AR_Pop_1026) (Table 20).

Table 20. Ppe-*Xap*F6-SSR DNA test functional genotypes and fruit bacterial spot phenotype ratings (*Xap*F) (0-5 scale) (2014) for parents of 21 F_1 UA 2010 populations (2014). (N is number of samples analyzed) [*The chi-square p-value in brackets]. Genotypes in **bold** were determined based of progeny and other parents' genotypes.

F_1 population	Progeny (N)	Parents		*Xap*F rating	Ppe-*Xap*F6-SSR	F_1 progeny allele segregation ratio
AR_Pop_1001	38	Female	A-665	2.0	R | R	22 R | R : 16 R | SU [*0.33]
		Male	**A-800**	-	**R | SU**	
AR_Pop_1002	37	Female	A-760	1.7	R | R	20 R | R : 11 R | SU [*0.11]
		Male	A-708	4.3	R | SU	
AR_Pop_1003	43	Female	White Diamond	1.3	R | R	43 R | R [*1.0]
		Male	A-760	1.7	R | R	
AR_Pop_1004	27	Female	**A-753**	-	**SU | SU**	25 R | SU [*1.0]
		Male	Souvenirs	2.0	R | R	
AR_Pop_1006	40	Female	White County	1.7	R | R	41 R | R [*1.0]
		Male	Souvenirs	2.0	R | R	
AR_Pop_1007	31	Female	**A-775**	-	**R | SU**	19 R | R : 12 R | SU [*0.21]
		Male	Souvenirs	2.0	R | R	
AR_Pop_1008	12	Female	**A-746**	-	**R | R**	4 R | R : 9 R | SU [*0.17]
		Male	**A-785**	-	**R | SU**	
AR_Pop_1009	12	Female	**A-746**	-	**R | R**	7 R | R : 8 R | SU [*0.80]
		Male	**A-823**	-	**R | SU**	
AR_Pop_1011	56	Female	A-786	3.3	R | R	24 R | R : 35 R | SU [*0.15]
		Male	A-773	2.7	R | SU	
AR_Pop_1012	32	Female	A-773	2.7	R | SU	13 R | R : 19 R | SU [*0.29]
		Male	**A-774**	-	**R | R**	
AR_Pop_1013	27	Female	A-772	0.7	R | R	28 R | R [*1.0]
		Male	**A-774**	-	**R | R**	

Table 20. Ppe-*Xap*F6-SSR DNA test functional genotypes and fruit bacterial spot phenotype ratings (*Xap*F) (0-5 scale) (2014) for parents of 21 F_1 UA 2010 populations (2014). (N is number of samples analyzed) [*The chi-square p-value in brackets]. Genotypes in **bold** were determined based of progeny and other parents' genotypes. (Cont.).

F_1 population	Progeny (N)	Parents		*Xap*F rating	Ppe-*Xap*F6-SSR	F_1 progeny allele segregation ratio
AR_Pop_1014	10	Female	**A-685**	-	**R | R**	5 R | R : 5 R | SU [*1.0]
		Male	A-773	2.7	R | SU	
AR_Pop_1015	21	Female	A-789N	2.7	R | R	22 R | R [*1.0]
		Male	**A-803CN**	-	**R | R**	
AR_Pop_1016	5	Female	**A-807CN**	-	**R | R**	5 R | R [*1.0]
		Male	**A-802CN**	-	**R | R**	
AR_Pop_1018	21	Female	Bowden	2.7	R | R	27 R | R [*1.0]
		Male	**A-761N**	-	**R | R**	
AR_Pop_1019	19	Female	**A-779CN**	-	**R | R**	19 R | R [*1.0]
		Male	A-776CN	2.0	R | R	
AR_Pop_1021	24	Female	A-778N	2.7	R | R	27 R | R [*1.0]
		Male	**A-777CN**	-	**R | R**	
AR_Pop_1022	17	Female	Amoore Sweet	1.7	R | R	24 R | R [*1.0]
		Male	**A-779CN**	-	**R | R**	
AR_Pop_1024	19	Female	**A-757N**	-	**R | SU**	9 R | R : 10 R | SU [*0.82]
		Female	**A-807CN**	-	**R | R**	
AR_Pop_1025	9	Male	**A-770CN**	-	**R | R**	7 R | R : 2 R | SU [*0.10]
		Female	**A-768N**	-	**R | SU**	
AR_Pop_1026	31	Male	A-816CN	2.7	R | R	31 R | R [*1.0]
		Female	A-772	0.7	R | R	

Non-Parametric Kruskal-Wallis test "Confirmation Set"

All 2010 seedlings "confirmation set" with Ppe-*Xap*F6-SSR functional genotype R | R had significantly lower 2014 *Xap*F medians (3.0) than individuals with Ppe-*Xap*F6-SSR functional genotype R | SU (4.0) (< 0.0001) (Table 21). Additionally the R^2 was ~10% for 551 2010 seedlings, thus the phenotypic variation explained (Vp %) was ~10%.

Table 21. Nonparametric medians of *Xap* (0-5 scale; 0 = resistant, 5 = highly susceptible) for Ppe-*Xap*F6-SSR genotypes in 2014 across all 2010 seedlings "confirmation set."

Ppe-*Xap*F6-SSR genotype	*Xap*F median
R | R	3.0 a[Z]
R | SU	4.0 b

[Z] Medians followed by the same letter are not significantly different as determined by non-parametric Kruskal-Wallis test with Wilcoxon rank sum pairwise comparisons test ($P \leq 0.05$).

Discussion

During the course of this study, the predictiveness of the previously developed SNP-based DNA tests associated with *Xap* fruit (*Xap*F) resistance, G1*Xap*F and G6*Xap*F (see Chapter Four), were tested across a vast array of 240 individuals the UA peach breeding program (the UA RosBREED "conversion set"). In terms of *G1XapF* all individuals in the UA RosBREED pedigree "conversion set," with *G1XapF* functional diplotype R1 | R1, R1 | I and R1 | SU had a significantly lower 2013-2015 avg *Xap*F median (3.0) than individuals with diplotypes I | I, SU | I, and SU | SU (4.0) (< 0.0001). Additionally the R^2 for the *G1XapF* 4-SNP haplotypes and *Xap*F resistance in 2013-2015 avg was ~7% for 240 individuals, the thus the phenotypic variation explained (Vp %) was ~7%. Likewise in terms of *G6XapF*, all individuals in the UA RosBREED pedigree "conversion set" with *G6XapF* functional diplotype R | R had a significantly lower 2013-2015 avg *Xap*F median (2.0) than individuals with diplotypes R | SU (3.0) or SU | SU (4.0)

(< 0.0001). Furthermore, the 2013-2015 avg *Xap*F median for those with diplotype R | SU (3.0) was significantly lower than individuals which were SU | SU (4.0) (< 0.0001). Additionally, the R^2 for the *G1XapF* 4-SNP haplotypes and *Xap*F resistance in 2013-2015 avg was ~8% for 240 individuals, thus the phenotypic variation explained (Vp %) was ~8%. The combined Vp % for the *G1XapF* and *G6XapF* 4-SNP DNA tests in *Xap*F 2013-2015 avg only represented ~15% of the Vp % for *Xap*F resistance. This is only a small portion of the Vp % for *Xap*F resistance. The genetic control of *Xap* resistance is quantitative in nature, however, dominant genes were suggested to be involved in peach (Sherman and Lyrene, 1981; Werner et al., 1986; Yang et al., 2013). The results of the study herein, and those in Chapter Two of this dissertation, add more support to the genetic control of *Xap*F resistance being very quantitative in nature.

The importance of these DNA tests expanded further through their conversion into simple, straightforward, breeder-friendly, SSR-based DNA tests. At the *G6XapF locus*, overall the *G6XapF* SNP diplotypes and Ppe-*Xap*F6-SSR genotypes matched 100% of the time for all individuals in the UA RosBREED "conversion set." Likewise results of each UA RosBREED population in the "conversion set" showed that the Ppe-*Xap*F6-SSR genotypes and matching diplotypes for the *G6XapF* 4-SNP, fit their expected parental segregation ratio patterns (X^2 p-value > 0.05). Furthermore, all 21, 2010 populations ("confirmation set") fit their parental expected segregation ratios (X^2 p-value > 0.05). Additionally, the Vp was ~10% when considering all 551 2010 seedlings. Moreover, all 2010 seedlings with Ppe-*Xap*F6-SSR functional genotype R | R had significantly lower 2014 *Xap*F medians (3.0) than individuals with Ppe-*Xap*F6-SSR functional genotype R | SU (4.0). These results confirmed the Ppe-*Xap*F6-SSR test's predictiveness across a vast majority of the UA peach breeding program; hence this marker can be incorporated into MAS in this program. A sample failure rate of ~5% was seen across

most populations, which is consistent with previous studies reported a negligible sample failure rate of ~1-5% (Edge-Garza et al., 2014).

Overall, the comparisons between the G6*Xap*F and Ppe-*Xap*F6-SSR highlights that both are equally predictive and robust, and thus either can be used for MAS. The Ppe-*Xap*F6-SSR DNA test is based on a single SSR marker that uses a standard PCR protocol, which was shown in this study to be effective even for low quality and quantity DNA. One PCR-based marker is more economical and simple to use than four SNPs. Using the one Ppe-*Xap*F6-SSR DNA test can reduce the PCR reactions needed to evaluate an individual's genotype, resulting in time and cost savings. Moreover, Ppe-*Xap*F6-SSR can be multiplexed with other DNA tests for fruit quality, phenological, or disease resistance traits, and pooled for analysis on a range of genotyping platforms, including but not limited to agarose gel, polyacrylamide gel, the Fragment Analyzer™, or ABI Prism 3730xl DNA Analyzer.

On the other hand, the comparisons between both DNA tests at the *G1XapF locus* (Ppe-*Xap*F1-SSR and *G1XapF* 4-SNP) revealed that while the 4-SNP *G1XapF* haplotype test was predictive across the UA RosBREED "conversion set", the Ppe-*Xap*F1-SSR was not robust across material in the UA program. At this locus the Ppe-*Xap*F1-SSR and *G1XapF* 4-SNP DNA test results for all individuals in UA RosBREED "conversion set" only matched 50% of the time, and in total 12 unique inconsistencies between *G1XapF* and the Ppe-*Xap*F1-SSR were observed. Additionally, the Ppe-*Xap*F1-SSR and *G1XapF* 4-SNP DNA test results for the F_1 seedlings in the seven UA RosBREED populations in the "conversion set" only matched in three of seven populations (AR_Pop_1, AR_Pop_0801, and AR_Pop_0803). These inconsistencies could be due to the Ppe-*Xap*F1-SSR being too far away from the candidate gene(s) responsible for the resistance. The 4-SNP *G1XapF* haplotype test spans the entire *Xap1* locus, ~12.9 – 15.0 Mbp on

P. persica chromosome 1, while the Ppe-*Xap*F1-SSR only flanks the *G1XapF* locus near the last SNP at ~15.0 Mbp on chromosome 1. This is a very large gap, spanning ~2.0 Mbp between the Ppe-*Xap*F1-SSR and the first SNP at ~12.9 Mbp, and moreover this locus is located relatively near the top of *P. persica* chromosome 1 (in general less recombination is known to occur the closer the marker is to the centromere), thus it became apparent that recombination occurred too frequently between the SSR alleles and the actual gene(s) responsible for the resistance, deeming the Ppe-*Xap*F1-SSR not robust enough to use in MAS at the UA program. Because of these inconsistencies the Ppe-*Xap*F1-SSR was not advanced to screen on the remaining 124 UA cultivars, selections and germplasm from other breeding programs, nor the 2010 populations "confirmation set." Overall these results indicate that the *G1XapF* and Ppe-*Xap*F1-SSR highlights are not equally predictive and robust, and thus only the *G1XapF* should be used for MAS. Nonetheless, the 4-SNP *G1XapF* haplotype test was predictive enough and thus can be used until an SSR test is developed which is located closer to the gene(s) responsible for the trait.

In this scenario, high resolution melt (HRM) SNP-based genotyping is comparable in resource and time savings to that of the SSR-based DNA tests using fragment analysis. Primers of comparable price to the SSR primers can be bought, and HRM can be performed for each SNP on a Real-Time PCR System (qPCR). The main advantage is that fragment analysis is not required as the SNPs are called during qPCR, which reduces price per sample significantly. As to which method saves more resources and time, this is dependent on the viewpoint of the researcher. Promisingly, preliminary testing of these four SNPs has been performed in collaboration with Dr. Seonghee Lee on a HRM machine at the University of Florida using a subset of individuals, which represented all haplotypes possible at this locus from the 9K SNP results (Appendix B). The results from HRM and the 9K matched for each individual tested, and

currently additional individuals are being tested for further confirmation to determine if this test can be used for MASS at the UA program.

Overall, the two DNA tests at the *G6XapF* locus (G6*Xap*F and Ppe-*Xap*F6-SSR) and the *G1XapF* 4-SNP haplotype at the *G1XapF* locus can provide the UA peach breeder with valuable DNA information to select parents (MAPS), accurately design crosses (MACS), and cull unwanted genotypes (MASS). Thus, the breeder can more efficiently design crosses to combine *Xap*F resistance (at two loci) with high fruit quality. After the crosses are made, unwanted seedlings with *Xap*F susceptible alleles can be discarded in the greenhouse, and only the most promising seedlings can be planted in the field. Both DNA tests were used for MAPS and MACS in 2013-2015 to combine *Xap* resistance with high fruit quality, spanning the season (Chapter Six). Deployment of MAPS, MACS, and next MASS for the *G1XapF G6XapF loci* will continue in 2016, and likely the Clemson University, Texas A&M University and University of California, Davis peach breeding programs will also begin routine deployment. Overall, implementation of MAS for DNA tests at the *G1XapF* and *G6XapF loci* in peach breeding programs substantiates the test's breeding utility and impact of predictive DNA tests in perennial fruit tree breeding. The work in this study additionally extends to other peach breeders, since the Ppe-*Xap*F6-SSR DNA test was 100% predictive across 59 cultivars from a range of U.S. breeding programs. While the Ppe-*Xap*F6-SSR and the *G1XapF* 4-SNP haplotype can be valuable DNA tests for all peach breeders, it's still advised to confirm their robustness and predictiveness in their own program's germplasm before widespread adoption.

Yet, together the *Xap1* and *Xap6* loci are only associated with ~15% of the V_P for *Xap* fruit resistance/susceptibility in UA material. Fortunately, through pedigree-based QTL analysis (PBA) using the UA pedigree, two additional major loci for fruit and leaf bacterial spot on LG1

(*G1XapF+L.1*, and *G1XapF+L.2*) and LG2 (*G2XapL+F.1*, and *G2XapL+F.2*), as well as one locus for fruit and leaf bacterial spot on LG5 (*G5XapL+F.1*) and LG6 (*G6XapF+L.1*) were identified and each represent > 10% of the Vp % for *Xap* fruit and leaf resistance/susceptibility (Chapter Two). These major QTLs are prime candidates for SNP and sequence length polymorphism-based (SLP-based) (i.e. SSR / indel) DNA test development to become the next set of DNA tests to use with the 4-SNP *G1XapF* haplotype and the Ppe-*Xap*F6-SSR to combine horizontal fruit and leaf bacterial spot resistance with high fruit quality.

Additionally, in Chapter Two, *Xap* leaf assay results using two sets of *Xap* isolates (one from Arkansas and the other from North Carolina) supported the findings of previous studies that *Xap* strain and population virulence levels from different locations were observed to differ remarkably among different peach and other stone fruit cultivars bred from different locations (Civerolo, 1975; Du Plessis, 1988; OEPP/EPPO, 2006; Scortichini et al., 1996). Thus, it will be important to combine broad horizontal *Xap* resistance in order for cultivars that are planned to be grown in multiple different environments across the U.S. to maintain resistance to the *Xap* isolates from all regions. This indeed will be a difficult task for peach breeders to accomplish, yet through the incorporation of MAS for the DNA tests at the *G1XapF* and *G6XapF loci* as well as the other nine QTL identified in Chapter Two, breeders in all locations (even those in Central California, where *Xap* is rarely seen) will be able to begin to combine resistance alleles at multiple loci, with high fruit quality, spanning the entire season.

Literature Cited

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. of Phytopathol. 156:338-345.

Blake, M.A. 1932. The J.H. Hale peach as a parent in peach crosses. Proc. Natl. Acad. Sci. U.S., 29:131-136.

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

Byrne, D.H. 2005. Trends in stone fruit cultivar development. HortTechnology 15:494–500.

Byrne, D.H., M. Bassols, D. Bassi, M. Piagnani, K. Gasic, G. Reighard, M. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: M. Badenes and D. Byrne (eds.). Fruit breeding. Springer Science, Business Media, New York.

Civerolo, E.L. 1975. Quantitative aspects of pathogenesis of *Xanthomonas pruni* in peach leaves. Phytopathol. 65:258-264.

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.

Collard, B.C.Y., and D.J. Mackill. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B: Biol. Sci. 363: 557-572.

Dellaporta, S., J. Wood, and J.B. Hicks. 1983. Plant Mol. Biol. Rept. 1:19-21.

Dirlewanger, E., P. Cosson, M. Tavaud, M.J. Aranzana, C. Poizat, A. Zanetto, P. Arús, and F. Laigret. 2002. Development of microsatellite markers in peach [*Prunus persica* (L) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L). Theor. Appl. Genet. 105:127–138.

Dirlewanger E., P. Cosson, K. Boudrhri, C. Renaud, G. Capdeville., Y. Tauzin, F. Laihret, and A. Mong. 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. and Genomes 3:1-13.

Du Plessis, H.J. 1988. Differential virulence of *Xanthomonas campestris* pv. *pruni* to peach, plum, and apricot cultivars. Phytopathol. 78:1312-1315.

Edge-Garza, D., T. Rowland, S. Haendiges, and C. Peace. 2014. A high-throughput and cost-efficient DNA extraction protocol for the tree fruit crops of apple, sweet cherry, and peach relying on silica beads during tissue sampling. Mol. Breeding 34:2225-2228.

Edge-Garza, D.A., J.J. Luby, and C.P. Peace. 2016. Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Mol. Breed. 35:1-15.

Eduardo, I., E. López-Girona, I. Batlle, F. Reig, I. Iglesias, W. Howad, P. Arús, and M.J. Aranzana. 2014. Development of diagnostic markers for selection of the subacid trait in peach. Tree Genet. and Genomes 10:1695-1709.

EPPO/CABI 1997. *Xanthomonas arboricola* pv. *pruni*, p. 1096–1100. In: Quarantine Pests for Europe, 2nd ed., CAB International, Wallingford (GB).

Falchi, R., E. Vendramin, L. Zanon, S. Scalabrin, G. Cipriani, I. Verde, G. Vizzotto, and M. Morgante. 2013. Three distinct mutational mechanisms acting on a single gene underpin the origin of yellow flesh in peach. Plant J. 76:175–187.

Food and Agricultural Organization of the United Nations (FAO). 2015. Peaches and nectarines world production. August 25, 2015. <http://faostat3.fao.org/compare/E>.

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

Jung, S., S.P. Ficklin, T. Lee, C.-H. Cheng, A. Blenda, P. Zheng, J. Yu, A. Bombarely, I. Cho, S. Ru, K. Evans, C. Peace, A.G. Abbott, L.A. Mueller, M.A. Olmstead and D. Main. 2014. The genome database for rosaceae (GDR): year 10 update. Nucl. Acids Res. 42:D1237-D1244.

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

OEPP/EPPO. 2006. *Xanthomonas arboricola* pv. *pruni*. OEPP/EPPO Bul. 36:129–133.

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. and J. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae, pp. 19-53. In K. Folta and S. Gardiner (eds.). Genetics and genomics of Rosaceae. Springer Science + Business Media, New York.

Ritchie, D.F. 1995. Bacterial spot, p. 50-52. In: J.M. Ogawa, E.I. Zehr and G.W. Bird (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 Nematicide Tests 54:63-64.

Ru, S., D. Main, K. Evans, and C. Peace. 2015. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet. and Genomes 11:1-12.

Salgado, 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. Ph.D dissertation University of Arkansas, Fayetteville, U.S.

Sandefur, P., T. Frett, J. Clark, K. Gasic, and C. Peace. 2016a. PpeRf-SSR, a DNA test for routine prediction in breeding of peach blush. Mol. Breeding (In press).

Sandefur, P., T. Frett, A. Salgado, J. Clark, K. Gasic, and C. Peace. 2016b. Ppe-Acidity, a combined DNA test for routine prediction in breeding of acidity and soluble solids content (SSC) in peach. Mol. Breeding (In press).

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

Scortichini, M., J. D.Janse., M.P. Rossi, and J.H.J. Derks. 1996. Characterization of *Xanthomonas campestris* pv. *pruni* strains from different hosts by pathogenicity tests and analysis of whole-cell fatty acids and whole-cell proteins. J. of Phytopathol. 144:69-74.

Sherman, W.B., and P.M. Lyrene. 1981. Bacterial spot susceptibility in low chilling peaches. Fruit Var. J. 35:74–77.

Simeone A.M. 1985. Study on peach and nectarine cultivars susceptibility to the main fungus and bacteria. Acta Hort. 173:541–551.

Socquet-Juglard D, B. Duffy, J.F. Pothier, D. Christen, C. Gessler, and A. Patocchi. 2013. Identification of a major QTL for *Xanthomonas arboricola* pv. *pruni* resistance in apricot. Tree Genet. and Genomes 9:409-421.

Smith, S. 2015. Fruit tree diseases. 16 November 2015. <https://www.uaex.edu/publications/pdf/mp154/fruit-tree-diseases-commercial.pdf>.

Stegmeir, T., L. Cai, F.R.A. Basundari, A.M. Sebolt, and A.F. Iezzoni. 2015. A DNA test for fruit flesh color in tetraploid sour cherry (*Prunus cerasus* L.). Mol Breed. 35:149-159.

Studebaker, G., J. Hopkins, and D. Johnson. 2015. Control peach tree borers on commercially grown peach and plum trees. 16 November 2015. <https://www.uaex.edu/publications/PDF/FSA-7504.pdf>.

Verde, I., A.G. Abbott, S. Scalabrin, S. Jung, S. Shu, F. Marroni, T. Zhebentyayeva, M.T. Dettori, J. Grimwood, F. Cattonaro, A. Zuccolo, L. Rossini, J. Jenkins, E. Vendramin, L.A. Meisel, V. Decroocq, B. Sosinski, S. Prochnik, T. Mitros, A. Policriti, G. Cipriani, L. Dondini, S. Ficklin, D.M. Goodstein, P. Xuan, C. Del Fabbro, V. Aramini, D. Copetti, S. Gonzalez, D.S. Horner, R. Falchi, S. Lucas, E. Mica, J. Maldonado, B. Lazzari, D. Bielenberg, R. Pirona, M. Miculan, A. Barakat, R. Testolin, A. Stella, S. Tartarini, P. Tonutti, P. Arús, A. Orellana, C. Wells, D. Main, G. Vizzotto, H. Silva, F. Salamini, J. Schmutz, M. Morgante, and D.S. Rokhsar. 2013. The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genet. 45:487-494.

Vendramin, E., G. Pea, L. Dondini, I. Pacheco, M. T. Dettori, L. Gazza, S. Scalabrin F. Strozzi, S. Tartarini, D. Bassi, I. Verde, and L. Rossini. 2014. A unique mutation in a MYB gene

cosegregates with the nectarine phenotype in peach. PloS ONE 9:e90574. DOI: 10.1371/journal.pone.0090574.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B.C. Faircloth, M. Remm, and S.G. Rozen. 2012. Primer3--new capabilities and interfaces. Nucl. Acids Res. 40:e115-127.

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. doi:10.1371/journal.pone.0035668.

Voorrips, R.E. 2007. Pedimap: software for visualization of genetic and phenotypic data in pedigrees. Plant Research International, Wageningen, the Netherlands. <http://www.wageningenur.nl/en/show/Pedimap.htm>.

Voorrips, R., M. Bink, and W. van de Weg. 2012. Pedimap: software for the visualization of genetic and phenotypic data in pedigrees. J. Hered. 103:903-907.

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., G.L. Righard, D. Ritchie, W.R. 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 (abstr.).

Yang, N., G.L. Righard, D. Ritchie, W.R. Okie, and K. Gasic. 2011. Development of a genetic linkage map for identification of molecular markers associated with resistance to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in peach. XIII Eucarpia Symp. on Fruit Breeding and Genet., September 11–15, 2011 Warsaw, Poland.

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

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

You, F.M., H. Huo, Y.Q. Gu, M.C. Luo, Y. Ma, D. Hane, G.R. Lazo, J. Dvorak, and O.D. Anderson. 2008. BatchPrimer3: a high throughput web application for PCR and sequencing primer design. BMC Bioinformatics 9:253-266.

Chapter Five - Study Two: IndelG, a DNA Test for Routine Prediction and Breeding of Peach and Nectarine

Abstract

Most consumers perceive peaches and nectarines as two different species, due to the presence or absence of skin pubescence. However, they are the same species, whose pubescence or lack thereof is known to be controlled by one gene at the *G-locus*, pubescent (peach) being dominant to glabrous (nectarine). In breeding programs, resources and attention are often differentially applied to each of these two market classes. A predictive DNA test for pubescent vs. glabrous (peach vs. nectarine) could aid in the differentiation of homozygous and heterozygous peaches. This would eliminate the need for breeders to do progeny testing, and allow them to accurately design crosses to introgress traits from peach into nectarine, and vice versa. Recently, a functional marker (indelG) on an LTR retrotransposon inside exon 3 of *PpeMYB25* was identified as the putative cause of the loss-of-function mutation underlying the glabrous phenotype. The objective of this study was to investigate two different types of DNA tests for pubescent vs. glabrous to determine and compare their predictiveness to differentiate peach and nectarine alleles in the University of Arkansas (UA) peach and nectarine breeding program. The two previously developed DNA tests include the 11-SNP haplotype that spans the *G-locus*, and the intragenic indelG. Both DNA tests were successfully screened on 214 out of 243 individuals from the UA breeding program RosBREED pedigree ("conversion set"): 88 cultivars and selections and seven F_1 populations consisting of 126 seedlings. The 11-SNP haplotype DNA test showed an inconsistency rate of 12%, while the indelG DNA test was 100% predictive. Thus, only the indelG DNA test was advanced and successfully screened on 558 seedlings from 22 F_1 populations, ranging from 5-59 seedlings per population ("confirmation set"), to confirm the DNA tests prediction accuracy in the UA breeding program. All F_1

populations of the “conversion set” and the “confirmation set” fit their parental expected phenotypic and genotypic segregation ratios for the indelG DNA test using the chi-square (X^2) goodness-of-fit test ($p > 0.05$). These results confirmed the 100% prediction rate of indelG across a vast majority of the UA breeding material. The indelG DNA test was deployed for routine marker-assisted selection (MAS) use in this breeding program to efficiently introgress traits from peach into nectarines, and vice versa.

Introduction

The peach, *Prunus persica* L. Batsch, and its smooth-skinned mutant, the nectarine, together constitute the third-most economically important temperate fruit tree species, behind apples (*Malus domestica* Borkh.) and pears (*Pyrus communis* L. subsp. *communis*), with a total world production estimated at over 21.6 Mt (Byrne et al., 2012; FAO, 2015a). The marketing of peach is dependent upon several class-defining traits such as blush overcolor, ground and flesh color, adhesion and texture, fruit shape, as well as the presence or absence of skin pubescence (trichomes) (Vendramin et al., 2014; Sandefur et al., 2016). Among these traits, skin pubescence has implications for consumer acceptance, since they are marketed separately, and some prefer the glabrous fruit (nectarine) over the pubescent (peach) due to its smooth skin (Vendramin et al., 2014).

Trichomes, which derive from epidermal cells, are hair-like appendages that are classified as unicellular or multicellular based on morphology, and glandular or non-glandular, based on secretory abilities (Vendramin et al., 2014). They are known to develop on different parts of the plant including but not limited to the leaf, fruit, and seed. The trichomes present on peach fruit are non-glandular and unicellular and develop on the ovary approximately four weeks before anthesis, and begin to die as the fruit ripens (Fig. 1). These trichomes serve as protective

barriers for peach, as with all plants, against biotic and abiotic stresses. Since nectarines lack this barrier, they commonly are more prone to environmental stresses (Vendramin et al., 2014).

Fig 1. An electron microscope image of peach fruit skin and trichomes.
(http://www.telegraph.co.uk/news/picturegalleries/earth/2646886/Fruits-under-the-electronmicroscope.html?image=5).

Pubescence is a qualitative trait and its presence is dominant to absence (glabrous) (Blake, 1932). This trait was later discovered to be controlled by the *G-locus* on linkage group 5 (Dirlewanger et al., 2006). Further Le Dantec et al., (2010) performed QTL analysis and determined that the *G-locus* spanned a 1.189 Mb interval from 15,126,681 to 16,315,341bp on scaffold 5 of the peach genome v1.0 (Verde et al., 2013).

Presence or absence of pubescence is a major consideration in peach breeding as resources and attention are often differentially applied to each market class. In general, nectarines are smaller than peaches and possess a distinct "nectarine flavor" both of which were originally thought to be due to pleiotropy at the *G-locus*. However, another theory is that size, and "nectarine flavor" may be due to other loci (J.R. Clark, personal communication). Traditional breeders have sometimes focused on peach and nectarine breeding as separate programs, with the exceptions that peaches have been crossed to nectarines to introgress peach size or other favorable traits into nectarines, and nectarines have been crossed to peaches to try and introgress the "nectarine flavor" into peaches (J.R. Clark, personal communication). To determine if a potential parent is homozygous or heterozygous for pubescence, traditional peach breeders have used conventional breeding techniques such as progeny testing, but unfortunately,

this technique is extremely time consuming and highly resource intensive due to peaches' long juvenility period and the need to perform extensive phenotyping (Byrne et al., 2012). Because of these limitations, most breeders have focused on peach and nectarine crossing without confirming the peaches' actual genotype.

While traditional peach and nectarine breeders have made considerable genetic improvements in the past century, unfortunately, traditional breeding is a time consuming, expensive, and laborious process taking 10 years or more, from the initial cross until a new peach cultivar can be released (Bliss, 2010; Byrne et al., 2012; Ru et al., 2015). Fortunately, today application of DNA-based information is a reality, and can provide peach breeders with more informed decision support to increase genetic gain per breeding cycle, improve selection efficiency, and significantly reduce breeding program operational costs (Bliss, 2010; Byrne, 2005; Edge-Garza et al., 2016; Ru et al., 2015). DNA tests for several breeding-relevant traits have been developed in peach including texture (Peace et al., 2005; Peace and Norelli, 2009), acidity (Eduardo et al., 2014), slow-melting flesh (Salgado, 2015), fruit bacterial spot resistance (see Chapter Five, Section One), white vs. yellow flesh (see Chapter Five, Section Three; Falchi et al., 2013), blush coverage (Sandefur et al., 2016a), and acidity and soluble solids content (Sandefur et al., 2016b). A predictive DNA test for pubescent vs. glabrous (peach vs. nectarine) could be important to accurately differentiate homozygous and heterozygous peaches across a breeding program. This would eliminate the need for breeders to do extensive progeny testing, and allow them to accurately select parents with desirable genotypes through marker-assisted parent selection (MAPS) as well as select favorable crosses with efficient combining abilities through marker-assisted cross selection (MACS), to introgress traits from peach into nectarines, and vice versa. After the cross, unwanted seedling types could be culled in the greenhouse

through marker-assisted seedling selection (MASS), or all peaches and nectarines could be planted into separate groups in the field, for more efficient field selection.

Promisingly, Vendramin et al., (2014) developed a DNA test for pubescent vs. glabrous. Through QTL analysis of an F_2 population of 'Contender' × 'Ambra' (C × A), they fine-mapped the *G-locus* to 15,853,006-16,488,104bp on scaffold 5 of the peach genome (Vendramin et al., 2014). Candidate genes within this region of the peach genome were studied, and coupled with variant discovery; this group successfully identified an R2R3-MYB gene *PpeMYB25* as the candidate gene for trichome formation on peach fruit skin (Vendramin et al., 2014). This group then performed genomic re-sequencing of the *PpeMYB25* for five peach/nectarines, aligned all sequences, and identified the insertion of a long terminal repeat (LTR) retrotransposon inside exon 3 of *PpeMYB25* as the putative cause of the loss-of-function mutation underlying the glabrous phenotype. Subsequently they developed a functional marker (indelG) on the LTR insertion which efficiently discriminated pubescent vs. glabrous plants across the C × A population as well as a panel of 95 cultivars, proposed to contain all known putative donors of the glabrous trait (Vendramin et al., 2014). The development of the functional marker, indelG, provides an efficient diagnostic tool for MAS of the pubescent/glabrous trait across the material studied. However, before incorporation of routine MAS in a breeding program for differentiating peach and nectarine individuals using indelG, it's advisable to confirm the tests' predictiveness across the target program's germplasm, since new mutations could have arisen in certain lineages.

Additionally, in Chapter Four of this dissertation, 11 informative SNP markers spanning the previously identified QTL at the *G-locus* (Le Dantec et al., 2010) were selected to develop a SNP haplotype DNA test for pubescent vs. glabrous. This 11-SNP haplotype DNA test was

shown to be ~95% predictive of peach vs. nectarine across 663 individuals from the four RosBREED peach demonstration breeding programs [the University of Arkansas (UA), Clemson University (CU), Texas A&M University (TX) and the University of California, Davis (CA)]. A total of 243 individuals from the UA breeding program were included in this effort.

The objective of this study was to investigate two different types of DNA tests for pubescent vs. glabrous to determine and compare their predictiveness to differentiate pubescent and glabrous alleles in the UA peach and nectarine breeding program. The two DNA tests included the 11-SNP haplotype that spans the *G-locus*, developed in Chapter Four of this dissertation, and the previously developed intragenic indelG. The most predictive DNA test was intended to be further confirmed across the UA program to enable routine MAS for this trait in the UA program.

Materials and Methods

Management Practices at FRS

Phenotypic evaluation for pubescent vs. glabrous was conducted on peach and nectarine material at the UA Fruit Research Station (FRS), Clarksville, AR (west-central AR, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept. of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]). All trees were either open-center trained and spaced 5.5 m between trees and rows, or trained to a perpendicular-V system with trees spaced 1.9 m in rows spaced 5.5 m apart. All trees were dormant pruned and fertilized annually with a single application of 640 Kg ha-1 of complete fertilizer (19:19:19 of N:P:K) and were sprinkler or drip irrigated as needed. Pests were managed using a program typical for commercial orchards in the area (Smith, 2015; Studebaker et al., 2015). After shuck split but before pit hardening fruitlets were thinned to a distance of 12 to 15 cm between each fruitlet.

Germplasm Utilized

The UA RosBREED "conversion set" was evaluated in 2011-2013 and utilized to convert the SNP haplotype (see Chapter Four of dissertation) to the indelG DNA test. The "conversion set" consisted of all 138 individuals in the seven UA RosBREED F_1 populations, 37 UA cultivars and selections screened with the International Peach SNP Consortium (IPSC) 9K peach SNP array v1.0 (Verde et al., 2012), and 68 cultivars and selections screened with the mini SNP array v1.0, at the University of Arizona Genetics Core (UA) and BioDiagnostics (BDI) (in total 243 individuals) (Table 1; Fig. 2). In 2013-2014, the remaining UA cultivars, selections (69), and an array of germplasm from different breeding programs primarily in the U.S. (58), not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0, were assessed (in total 127 individuals) (Table 2). Additionally, in 2014 a total of 613 seedlings from 22 F_1 populations (2010 crosses), ranging from 5-59 seedlings per population, were evaluated to confirm indelG DNA test allele effects in the UA breeding program (i.e. "confirmation set") (Table 3).

Pubescence Phenotyping

Phenotyping for pubescence was conducted in 2011-2014, as described in Frett et al., (2012). In short, five fruit slightly firmer than tree ripe, were harvested from the mid-canopy of each tree into 0.24 L corrugated trays (FormTex Plastics Corp., Houston, TX). Visual estimation of the pubescence level was performed at harvest, in the field (0 = glabrous or nectarine; 3 = slight ; 5 = medium ; 7 = heavy). Later 3, 5 and 7 were all converted to 1 or 0, so that pubescent (peach) (1) and glabrous (nectarine) (0) could be directly compared.

Table 1. Parental information and the number of F_1 seedlings for each of the seven RosBREED populations (N is number of individuals analyzed).

F_1 population	Female parent	Male parent	F_1 seedlings (N)
AR_Pop_1	White County	A-672	48
AR_Pop_0801	A-776	A-783	16
AR_Pop_0803	Amoore Sweet (AS)	A-778	9
AR_Pop_0813	A-772	A-672	12
AR_Pop_0817	A-789	A-699	9
AR_Pop_0819	A-708	A-773	23
AR_Pop_0825	Souvenirs	A-760	17

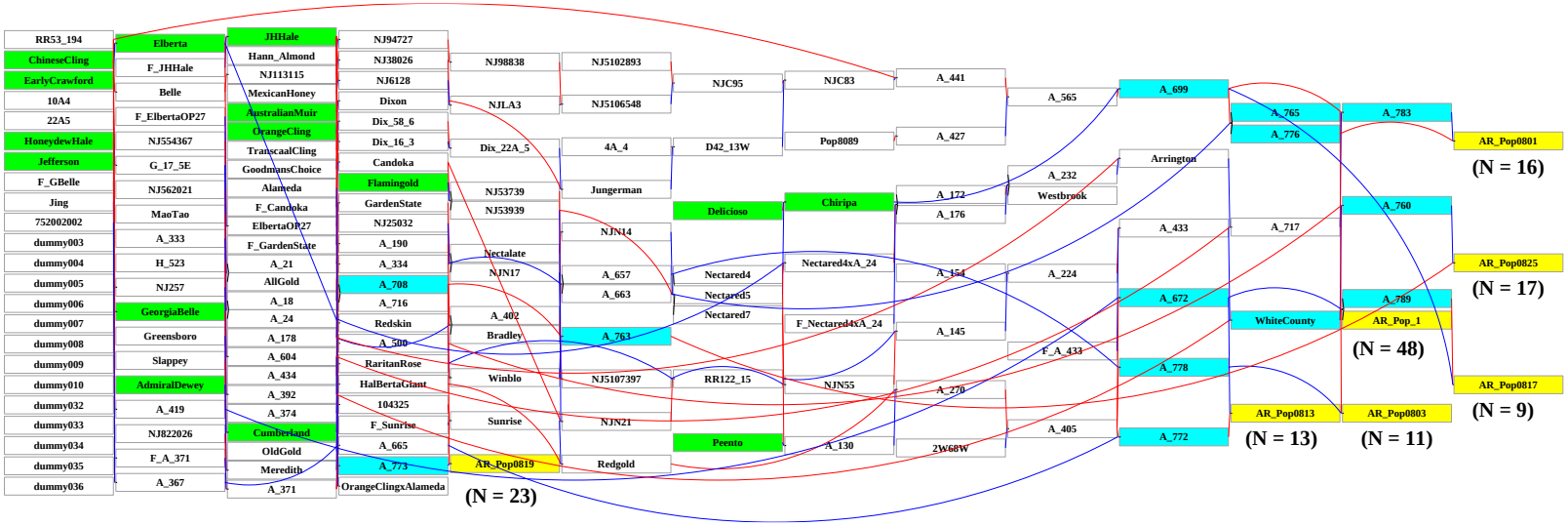

Fig. 2. Pedigree of the seven 2008 F_1 seedling populations included in the "conversion set" to validate the indelG DNA test. Visualized through Pedimap software (Voorrips, 2007; Voorrips et al., 2012) (F_1 populations highlighted in yellow; Red line = female parent; Blue line = male parent). N is the number of progeny in each population.

Table 2. Arkansas selections and cultivars and an array of germplasm from different breeding programs phenotyped for pubescence in 2013-2014 and genotyped in 2012-2015.

A-554	A-805[y]	A-845[y]	A-878[y]	A-910	Elberta[z]	PF 5D Big
A-662	A-806[y]	A-846	A-879[y]	A-911	Emeraude	PF Lucky 13
A-663[z]	A-808	A-847	A-880[y]	A-912	Flavortop	PF-19-007
A-665[zy]	A-809[y]	A-848[y]	A-881[y]	A-913	Georgia Belle[z]	Redhaven
A-668	A-810	A-849[y]	A-882[y]	A-914	Gladiator	Redskin[z]
A-672[zy]	A-811[y]	A-850[y]	A-883	A-915	Gloria[y]	Rising Star
A-699[z]	A-813[y]	A-851[y]	A-884	A-916	Goldilocks	Roygold
A-708[z]	A-814	A-852[y]	A-885	A-917	Goldjim	Ruby Prince
A-716[z]	A-815	A-853[y]	A-886	A-918	Goldnine	Saturn
A-743	A-816[y]	A-854[y]	A-887	Admiral Dewey[z]	Greensboro[z]	Slappey[z]
A-758	A-818[y]	A-855[y]	A-888	Allgold	Jade	Souvenirs[z]
A-760[z]	A-819[y]	A-856[y]	A-889	Amoore Sweet[z]	Jefferson[z]	Spring Snow
A-761	A-820[y]	A-857[y]	A-890	Arrington[z]	JHHale[z]	Sugar Giant
A-766	A-821[y]	A-858	A-891	Autumn Prince	KV175	Sugar Lady
A-768	A-822[y]	A-859[y]	A-892	Autumn Star	KV175	Sweet Star
A-770	A-824	A-860[y]	A-893	Bounty	KV357	Tango
A-772[z]	A-825[y]	A-861[y]	A-894	Bowden[y]	KV398	Tango-II
A-773[z]	A-826[y]	A-862[y]	A-895	Bradley[zy]	KV401	Westbrook[z]
A-776[z]	A-827[y]	A-864[y]	A-896	Challenger	KV501	White Cloud
A-778[z]	A-828[y]	A-865[y]	A-897	China Pearl[z]	KV601	White County[zy]
A-783[z]	A-829[y]	A-866[y]	A-898	Chinese Cling[z]	KV606	White Diamond[y]
A-786[y]	A-830	A-867[y]	A-899	Contender	KV701	White Lady
A-789[z]	A-832[y]	A-868[y]	A-900	Cresthaven	KV801	White River[z]
A-790	A-833[y]	A-869[y]	A-901	Crimson Lady	Loring	White Rock[y]
A-792[y]	A-836[y]	A-870[y]	A-902	Crimson Snow	Manon	Winblo[z]
A-794	A-837[y]	A-871[y]	A-903	Cumberland[z]	Messina	Yumm Yumm
A-797[y]	A-839	A-872[y]	A-904	CVN13w	Orange Cling[z]	
A-798	A-840[y]	A-873[y]	A-905	Denman	Peento[z]	
A-799	A-841[y]	A-874[y]	A-906	Dixon[z]	PF 1	
A-801[y]	A-842[y]	A-875[y]	A-907	Early Crawford[z]	PF 24-007	
A-803	A-843[y]	A-876[y]	A-908	Early Star	PF 24C	
A-804[y]	A-844[y]	A-877[y]	A-909	Eastern Glo	PF 5B	

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 3. Additional 2009 and 2010 F_1 seedling populations phenotyped in 2014, and genotyped in 2014-2015. (N is number of individuals analyzed).

F_1 population	Female parent	Male parent	F_1 seedlings (N)
AR_Pop_1001	A-665	A-800	38
AR_Pop_1002	A-760	A-708	37
AR_Pop_1003	White Diamond	A-760	43
AR_Pop_1004	A-753	Souvenirs	27
AR_Pop_1006	White County	Souvenirs	41
AR_Pop_1007	A-775	Souvenirs	34
AR_Pop_1008	A-746	A-785	13
AR_Pop_1009	A-746	A-823	17
AR_Pop_1011	A-786	A-773	66
AR_Pop_1012	A-773	A-774	33
AR_Pop_1013	A-772	A-774	28
AR_Pop_1014	A-685	A-773	10
AR_Pop_1015	A-789N	A-803CN	25
AR_Pop_1016	A-807CN	A-802CN	5
AR_Pop_1018	Bowden	A-761N	31
AR_Pop_1019	A-779CN	A-776CN	20
AR_Pop_1020	Bowden	A-758CN	33
AR_Pop_1021	A-778N	A-777CN	29
AR_Pop_1022	Amoore Sweet	A-779CN	26
AR_Pop_1024	A-757N	A-807CN	20
AR_Pop_1025	A-770CN	A-768N	6
AR_Pop_1026	A-816CN	A-772	31

Leaf Collection and DNA Extraction

In the spring of 2013, approximately 50 mg of fresh, young leaf tissue was harvested in individual 1.5 mL tubes (Eppendorf, Hauppauge, NY) from all seedlings in the seven UA RosBREED F_1 populations as well as all UA cultivars and selections in the "conversion set" (243 individuals). Additionally, the remaining UA cultivars and selections, and an array of germplasm from different breeding programs were collected (127 individuals). While collecting tissue, each F_1 seedling was labeled with a metal tag in order to correctly match phenotypic and genotypic data later. Tissue was refrigerated during transportation, and then stored at -80 °C until needed. Two 4 mm stainless steel beads (McGuire Bearing Company, Salem, OR) were placed into each 1.5 mL tube, and DNA was extracted following a modified Dellaporta (1983) protocol with specific adaptations for peach. DNA quantity and quality were measured using a spectrophotometer (NanoDrop 2000, NanoDrop Technologies, Wilmington, DE) and confirmed by electrophoresis on 1% TBE (1 M Tris, 0.9 M boric acid, and 0.01 M EDTA) agarose gel. Final dilutions of 25 ng/µl were created for genotyping.

In the spring of 2014, approximately 50 mg of fresh young leaf tissue was harvested into coin envelopes for all 613 seedlings from 2010 populations ("confirmation set"). While collecting tissue, each F_1 seedling was labeled with a metal tag in order to correctly match phenotypic and genotypic data later. Tissue was refrigerated during transportation, and then lyophilized (Freezone® 12 model 77540, Labconco Corporation, Kansas City, MO). Lyophilized plant tissue was then loaded into a 96 1.1 ml MicroTube Rack System (BioExpress, Kaysville, UT) containing approximately 0.25 g of technical grade 40, 6-12 mesh silica gel beads (Sigma Aldrich, St. Louis, MO). All DNA was extracted following a high-throughput and cost-efficient extraction protocol developed for *Prunus* species by Edge-Garza et al. (2014).

IndelG Genotyping

Extracted DNA was amplified with the indelG at the UA Horticulture Molecular Breeding Laboratory. Polymerase chain reaction (PCR) was performed for all samples in a final total volume of 10.0 µl containing 1.0 µl of DNA (~25 ng/µl), 4.0 µl of ultrapure molecular grade water [AccuGENE™ (Lonza Inc., Allendale, NJ)], 2.0 µl of Taq PCR buffer [×5 GoTaq® buffer (Promega Corp., Madison, WI)], 0.6 µl of $MgCl_2$ [25mM (Promega)], 0.2 µl deoxyribonucleotide triphosphates (dNTPs) [10 mM (Promega)], 0.5 µl of each primer (forward, reverse one and reverse two) [10mM (Integrated DNA Technologies, Coralville, IA)], 0.2 µl of Taq DNA polymerase (5U/µl) [GoTaq® (Promega Corp.)]. The PCR amplifications were performed in a BIORAD T100 thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA) under the following conditions: 5 min of initial denaturation at 95 °C followed by 35 cycles of 95 °C for 45 s, 60 °C for 45 s, 72 °C for 1.5 min, and then a final extension step at 72 °C for 7 min.

Table 4. The indelG DNA test forward and reverse primer sequences and physical locations on scaffold 5 [primer locations were sourced from the Genome Database for Rosaceae (Jung et al., 2014)].

Name	Physical location (bp)	Sequence
F	15,898,324	CTTGCACCTGAGTTCGATTCCG
R1	-	GGCTTCAATGGCAGAACAAGG
R2	15,899,264	GCAGGTGGTGGAGATTCATTCAT

The PCR reactions of all the individuals included in this study were resolved utilizing two different capillary gel electrophoresis machines: a Fragment Analyzer™, model AdvanCE FS96 (Advanced Analytical Technologies, Inc., Ames, IA) from the wheat breeding laboratory at UA, and an ABI Prism 3730xl DNA Analyzer (Applied Biosystems by Life Technologies, Grand Island, NY) in the fruit breeding laboratory at Washington State University. Results from the

Fragment Analyzer™ were analyzed through PROSize® v.1 software (Advanced Analytical Technologies, Inc., Ames, IA), while results from the ABI Prism 3730xl were scored through GeneMarker® software (SoftGenetics, LLC, State College, PA).

SNP Haplotypes to IndelG DNA Test Conversion

The UA RosBREED "conversion set" evaluated in 2011-2013 was utilized to convert the SNP haplotype (see Chapter Four of dissertation) to the indelG DNA test. Although the "conversion set" consisted of 243 individuals, only those that were screened with the IPSC 9K peach SNP array v1.0 were first considered for haplotype construction (175 individuals). A total of 11 informative SNP markers were used for haplotype construction (see Chapter Four of dissertation). The GBrowse tool on the Genome Database for Rosaceae (Jung et al., 2014; https://www.rosaceae.org/gb/gbrowse/prunus_persica/) was utilized to identify the precise location of the 11 informative SNP markers that spanned the previously identified QTL for pubescent vs. glabrous at the *G-locus* (13,025,129-16,774,236 bp) on scaffold 5 of the *Prunus persica* genome sequence v1.0 (Le Dantec et al., 2010; Verde et al. 2013) (Fig. 3). FlexQTL™ software was used to construct the initial functional SNP haplotypes that were subsequently manually confirmed based on inheritance analysis in bi-parental families (Bink, 2004; Bink, 2005; Bink et al., 2008; Bink et al., 2012).

Next, to make direct comparisons between each individuals SNP haplotypes and their indelG genotypes, all functional SNP haplotypes were divided into two simple functional haplotype groups defined functional allele 'P' (pubescent/peach allele) and 'n' (glabrous/nectarine allele). Only simple functional haplotype groups were developed for the 68 individuals screened with the peach mini SNP array v1.0, since less SNP markers were utilized,

which made it necessary to compare each individual's phenotypic data to accurately determine the correct simple functional haplotype group (see Chapter Four for more details).

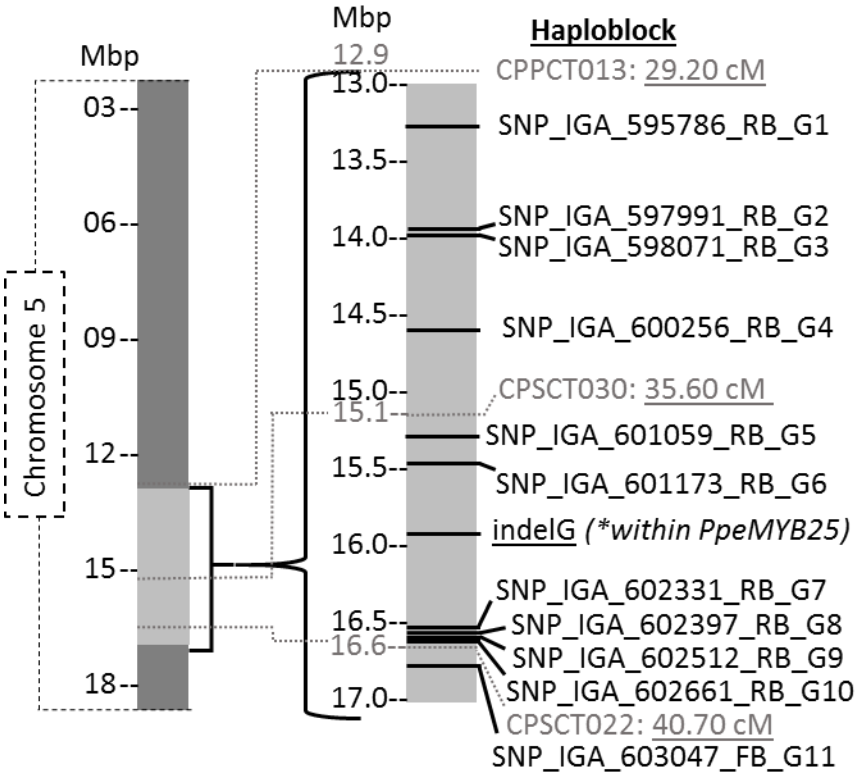

Fig. 3. Graphical representation of *P. persica* chromosome 5 with the enlarged location of the ~4 Mbp flanking the *PpeMYB25* transcription factor, including the 11 SNP markers used for haplotyping at the *G-locus* and the intragenic indelG DNA test. Physical locations were sourced from the *P. persica* whole genome v1.0 sequence (Verde et al., 2013) housed on the Genome Database for Rosaceae (Jung et al. 2014). Three markers within the *G-locus* SNP haploblock and their estimated genetic positions based on the Prunus-TE-F_2 reference map are included in gray.

IndelG DNA Test Genotype Effects

The chi-square (X^2) goodness-of-fit test was performed for all F_1 populations of the "conversion set" and the "confirmation set" to determine if the seedlings observed indelG genotype and pubescent vs. glabrous phenotype ratios fit the expected segregation ratio patterns of their parents ($p > 0.05$). A pedigree allele tracking approach was used to determine parental genotypes for populations whose parents had previously been discarded from the program and thus were not screened with the indelG DNA test. In this case, the parent alleles were determined based on their grandparent alleles to trace the identity of each allele. Lastly, process of elimination was used for parents whose alleles still could not be determined. In this scenario, the F_1 progeny segregation ratio and the other parent's genotype were used to determine what the unknown parent's genotype should be.

Results

Phenotypic Data

All individuals phenotyped were rated as pubescent (peach) (1) or glabrous (nectarine) (0). The UA RosBREED pedigree consisted of seven peach parents and eight nectarine parents (Table 5). All progeny in AR_Pop_0801, AR_Pop_0803, and AR_Pop_0819 were nectarines. Two populations showed ~3:1 peaches to nectarines: AR_Pop_1 (32:9) and AR_Pop_0813 (8:4). The remaining two populations exhibited ~1:1 peaches to nectarines: AR_Pop_0817 (3:6) and AR_Pop_0825 (8:4) (Table 5). The 105 UA cultivars and selections in the "conversion set" consisted of 59 peaches and 46 nectarines (Tables 6-7). Of the remaining UA cultivars, selections (69), and the array of germplasm from different breeding programs (58), not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0, 89 were peaches and 38 nectarines (127 total) (Table 8). The 22 F_1 populations from the 2010 "confirmation set"

consisted of 25 peach and 19 nectarine parents (Table 9). All progeny in AR_Pop_1001, AR_Pop_1002, AR_Pop_1003, AR_Pop_1004, AR_Pop_1006, AR_Pop_1007, AR_Pop_1008, AR_Pop_1011, AR_Pop_1012, AR_Pop_1013, and AR_Pop_1014 were peaches. All progeny in AR_Pop_1015, AR_Pop_1016, AR_Pop_1018, AR_Pop_1019, AR_Pop_1020, AR_Pop_1021, AR_Pop_1022, AR_Pop_1024, and AR_Pop_1025 were nectarines. The AR_Pop_1009 population showed ~3:1 peaches to nectarines (12:3) and the AR_Pop_1026 population exhibited ~1:1 peaches to nectarines (15:16) (Table 9).

Table 5. IndelG DNA test functional genotypes and pubescent [peach (P)] or glabrous [nectarine(n)] phenotypes for parents of the seven F_1 UA RosBREED populations (2011-2013) (N is number of samples analyzed) [*The chi-square p-value in brackets].

F_1 population	Progeny (N)	Parents		Phenotype	Functional genotype	F_1 progeny matching phenotype and allele segregation ratio
AR_Pop_1	41	Female	White County	Peach	P | n	10 P | P : 22 P | n : 9 n | n [*0.87]
		Male	A-672	Peach	P | n	
AR_Pop_0801	16	Female	A-776	Nectarine	n | n	16 n | n [*1.00]
		Male	A-783	Nectarine	n | n	
AR_Pop_0803	10	Female	Amoore Sweet	Nectarine	n | n	10 n | n [*1.00]
		Male	A-778	Nectarine	n | n	
AR_Pop_0813	12	Female	A-772	Nectarine	P | n	1 P | P : 7 P | n : 4 n | n [*0.40]
		Male	A-672	Nectarine	P | n	
AR_Pop_0817	9	Female	A-789	Nectarine	n | n	3 P | n : 6 n | n [*0.61]
		Male	A-699	Peach	P | n	
AR_Pop_0819	23	Female	A-708	Peach	P | P	23 n | n [*1.00]
		Male	A-773	Peach	P | P	
AR_Pop_0825	17	Female	Souvenirs	Peach	P | P	11 P | P : 6 P | n [*0.23]
		Male	A-760	Nectarine	P | n	

Table 6. Relationships between the functional 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] (2011-2014) for cultivars and selections in the UA RosBREED pedigree ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).**

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
A-657[z]	g1 | g1	n | n	197 | 197	n | n	Nectarine
A-663[z]	g1 | g1	n | n	197 | 197	n | n	Nectarine
A-665[zy]	G3a | G3b	P | P	941 | 941	P | P	Peach
A-672[zy]	**g1 | g2**	**n | n*(P)**	**941 | 197**	**P | n**	**Peach**
A-699[z]	g1 | g3	n | n	197 | 197	n | n	Nectarine
A-708[z]	G2a | G5	P | P	941 | 941	P | P	Peach
A-716[z]	G3b | G6	P | P	941 | 941	P | P	Peach
A-760[z]	G2c | g1	P | n	941 | 197	P | n	Peach
A-772[z]	g1 | G3b	P | n	941 | 197	P | n	Peach
A-773[z]	G6 | G3a	P | P	941 | 941	P | P	Peach
A-776[z]	g1 | g1	n | n	197 | 197	n | n	Nectarine
A-778[z]	g2 | g1	n | n	197 | 197	n | n	Nectarine
A-783[z]	g3 | go	n | n	197 | 197	n | n	Nectarine
A-789[z]	g1 | g2	n | n	197 | 197	n | n	Nectarine
Admiral Dewey[z]	G4 | G4	P | P	941 | 941	P | P	Peach
Amoore Sweet[z]	g1 | g1	n | n	197 | 197	n | n	Nectarine
Arrington[z]	g1 | g3	n | n	197 | 197	n | n	Nectarine

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 6. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] (2011-2014) for cultivars and selections in the UA RosBREED pedigree ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
Bradley[zy]	g1 | g1	n | n	197 | 197	n | n	Nectarine
Chinapearl[z]	G3b | G8	P | P	941 | 941	P | P	Peach
Chinese Cling[z]	G1 | G2a	P | P	941 | 941	P | P	Peach
Cumberland[z]	G4 | G3a	P | P	941 | 941	P | P	Peach
Dixon[z]	G3a | G4	P | P	941 | 941	P | P	Peach
Early Crawford[z]	G3a | G3a	P | P	941 | 941	P | P	Peach
Elberta[z]	G2a | G3a	P | P	941 | 941	P | P	Peach
Georgia Belle[z]	G1 | G4	P | P	941 | 941	P | P	Peach
Greensboro[z]	G3a | G3a	P | P	941 | 941	P | P	Peach
Jefferson[z]	-	-	941 | 941	P | P	Peach
JH Hale[z]	G2a | G2b	P | P	941 | 941	P | P	Peach
Orange Cling[z]	G3a | G3a	P | P	941 | 941	P | P	Peach
Peento[z]	-	-	941 | 941	P | P	Peach
Redskin[z]	G1 | G2a	P | P	941 | 941	P | P	Peach

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 6. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] (2011-2014) for cultivars and selections in the UA RosBREED pedigree ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
Slappey[z]	-	-	941 | 941	P | P	Peach
Souvenirs[z]	G2a | G2a	P | P	941 | 941	P | P	Peach
Westbrook[z]	g1 | g1	n | n	197 | 197	n | n	Nectarine
White County[zy]	g1 | G7	P | n	941 | 197	P | n	Peach
White River[z]	-	-	941 | 941	P | P	Peach
Winblo[z]	G2b | G1	P | P	941 | 941	P | P	Peach

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 7. Relationships between the simple functional 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] (2013-2014) for the 72 individuals screened with the Peach SNP mini array v1.0 ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).**

Individual	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
A-665[zy]	P | P	941 | 941	P | P	Peach
A-672[zy]	**n | n*(P)**	**941 | 197**	**P | n**	**Peach**
A-786[y]	P | n	941 | 197	P | n	Peach
A-792[y]	n | n	197 | 197	n | n	Nectarine
A-797[y]	n | n	197 | 197	n | n	Nectarine
A-801[y]	-	941 | 941	P | P	Peach
A-804[y]	n | n	197 | 197	n | n	Nectarine
A-805[y]	n | n	197 | 197	n | n	Nectarine
A-806[y]	n | n	197 | 197	n | n	Nectarine
A-809[y]	P | n	941 | 197	P | n	Peach
A-811[y]	n | n	197 | 197	n | n	Nectarine
A-813[y]	n | n	197 | 197	n | n	Nectarine
A-816[y]	n | n	197 | 197	n | n	Nectarine
A-818[y]	n | n	197 | 197	n | n	Nectarine
A-819[y]	-	941 | 197	P | n	Peach
A-820[y]	P | P	941 | 941	P | P	Peach

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 7. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] (2013-2014) for the 72 individuals screened with the Peach SNP mini array v1.0 ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
A-821[y]	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
A-822[y]	n | n	197 | 197	n | n	Nectarine
A-825[y]	-	941 | 941	P | P	Peach
A-826[y]	P | n	941 | 197	P | n	Peach
A-827[y]	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
A-828[y]	n | n	197 | 197	n | n	Nectarine
A-829[y]	n | n	197 | 197	n | n	Nectarine
A-832[y]	n | n	197 | 197	n | n	Nectarine
A-833[y]	n | n	197 | 197	n | n	Nectarine
A-836[y]	n | n	197 | 197	n | n	Nectarine
A-837[y]	-	941 | 941	P | P	Peach
A-840[y]	n | n	197 | 197	n | n	Nectarine
A-841[y]	n | n	197 | 197	n | n	Nectarine
A-842y	n | n	197 | 197	n | n	Nectarine
A-843y	n | n	197 | 197	n | n	Nectarine

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 7. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] (2013-2014) for the 72 individuals screened with the Peach SNP mini array v1.0 ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
A-844[y]	n | n	197 | 197	n | n	Nectarine
A-845[y]	P | n	941 | 197	P | n	Peach
A-848[y]	P | n	941 | 197	P | n	Peach
A-849[y]	P | n	941 | 197	P | n	Peach
A-850[y]	-	941 | 197	P | n	Peach
A-851[y]	-	941 | 941	P | P	Peach
A-852[y]	-	941 | 941	P | P	Peach
A-853[y]	-	941 | 941	P | P	Peach
A-854[y]	P | P	941 | 941	P | P	Peach
A-855[y]	P | n	941 | 197	P | n	Peach
A-856[y]	P | n	941 | 197	P | n	Peach
A-857[y]	P | P	941 | 941	P | P	Peach
A-859[y]	n | n	197 | 197	n | n	Nectarine
A-860[y]	-	941 | 197	P | n	Peach
A-861[y]	n | n	197 | 197	n | n	Nectarine

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 7. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] (2013-2014) for the 72 individuals screened with the Peach SNP mini array v1.0 ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
A-862[y]	-	941 | 941	P | P	Peach
A-864[y]	n | n	197 | 197	n | n	Nectarine
A-865[y]	n | n	197 | 197	n | n	Nectarine
A-866[y]	P | n	941 | 197	P | n	Peach
A-867[y]	n | n	197 | 197	n | n	Nectarine
A-868[y]	n | n	197 | 197	n | n	Nectarine
A-869[y]	n | n	197 | 197	n | n	Nectarine
A-870[y]	n | n	197 | 197	n | n	Nectarine
A-871[y]	n | n	197 | 197	n | n	Nectarine
A-872[y]	P | n	941 | 197	P | n	Peach
A-873[y]	n | n	197 | 197	n | n	Nectarine
A-874[y]	n | n	197 | 197	n | n	Nectarine
A-875[y]	n | n	197 | 197	n | n	Nectarine
A-876[y]	n | n	197 | 197	n | n	Nectarine
A-877[y]	-	941 | 197	P | n	Peach
A-878[y]	-	941 | 941	P | P	Peach

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 7. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] (2013-2014) for the 72 individuals screened with the Peach SNP mini array v1.0 ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
A-879[y]	P | n	941 | 197	P | n	Peach
A-880[y]	-	941 | 941	P | P	Peach
A-881[y]	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
A-882[y]	P | n	941 | 197	P | n	Peach
Bowden[y]	n | n	197 | 197	n | n	Nectarine
Bradley[zy]	n | n	197 | 197	n | n	Nectarine
Gloria[y]	-	-	-	Peach
White County[zy]	P | n	-	-	Peach
White Diamond[y]	P | P	941 | 941	P | P	Peach
White Rock[y]	P | n	941 | 197	P | n	Peach

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 8. IndelG DNA test genotypes and pubescent (peach) or glabrous (nectarine) phenotypes of UA selections and cultivars (not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0) and germplasm from other breeding programs, years 2013-2014.

Accession	IndelG genotype (bp)	IndelG functional genotype	Phenotype
A-554	941 | 197	P | n	Peach
A-662	197 | 197	n | n	Nectarine
A-668	197 | 197	n | n	Nectarine
A-743	941 | 197	P | n	Peach
A-758	197 | 197	n | n	Nectarine
A-761	197 | 197	n | n	Nectarine
A-766	941 | 941	P | P	Peach
A-768	197 | 197	n | n	Nectarine
A-770	197 | 197	n | n	Nectarine
A-790	941 | 941	P | P	Peach
A-794	197 | 197	n | n	Nectarine
A-798	941 | 197	P | n	Peach
A-799	197 | 197	n | n	Nectarine
A-803	197 | 197	n | n	Nectarine
A-808	941 | 941	P | P	Peach
A-810	197 | 197	n | n	Nectarine
A-814	941 | 197	P | n	Peach
A-815	197 | 197	n | n	Nectarine
A-824	941 | 941	P | P	Peach
A-830	197 | 197	n | n	Nectarine
A-839	941 | 197	P | n	Peach
A-846	941 | 197	P | n	Peach
A-847	941 | 197	P | n	Peach
A-858	197 | 197	n | n	Nectarine
A-883	941 | 197	P | n	Peach
A-884	941 | 197	P | n	Peach
A-885	941 | 197	P | n	Peach
A-886	941 | 197	P | n	Peach
A-887	-	-	Peach
A-888	941 | 197	P | n	Peach
A-889	197 | 197	n | n	Nectarine
A-890	941 | 197	P | n	Peach

Table 8. IndelG DNA test genotypes and pubescent (peach) or glabrous (nectarine) phenotypes of UA selections and cultivars (not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0) and germplasm from other breeding programs, years 2013-2014. (Cont.).

Accession	IndelG genotype (bp)	IndelG functional genotype	Phenotype
A-891	941 | 197	P | n	Peach
A-892	197 | 197	n | n	Nectarine
A-893	197 | 197	n | n	Nectarine
A-894	941 | 197	P | n	Peach
A-895	197 | 197	n | n	Nectarine
A-896	197 | 197	n | n	Nectarine
A-897	197 | 197	n | n	Nectarine
A-898	941 | 197	P | n	Peach
A-899	941 | 197	P | n	Peach
A-900	941 | 197	P | n	Peach
A-901	197 | 197	n | n	Nectarine
A-902	197 | 197	n | n	Nectarine
A-903	197 | 197	n | n	Nectarine
A-904	941 | 197	P | n	Peach
A-905	-	-	Peach
A-906	941 | 197	P | n	Peach
A-907	197 | 197	n | n	Nectarine
A-908	197 | 197	n | n	Nectarine
A-909	941 | 197	P | n	Peach
A-910	941 | 197	P | n	Peach
A-911	197 | 197	n | n	Nectarine
A-912	941 | 197	P | n	Peach
A-913	941 | 197	P | n	Peach
A-914	941 | 197	P | n	Peach
A-915	197 | 197	n | n	Nectarine
A-916	197 | 197	n | n	Nectarine
A-917	941 | 197	P | n	Peach
A-918	941 | 197	P | n	Peach
Admiral Dewey	941 | 941	P | P	Peach
Allgold	941 | 197	P | n	Peach
Autumn Prince	941 | 197	P | n	Peach
Autumn Star	-	-	Peach
Bounty	941 | 197	P | n	Peach

Table 8. IndelG DNA test genotypes and pubescent (peach) or glabrous (nectarine) phenotypes of UA selections and cultivars (not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0) and germplasm from other breeding programs, years 2013-2014. (Cont.).

Accession	IndelG genotype (bp)	IndelG functional genotype	Phenotype
Challenger	941 | 197	P | n	Peach
China Pearl	941 | 941	P | P	Peach
Chinese Cling	941 | 941	P | P	Peach
Contender	941 | 197	P | n	Peach
Cresthaven	941 | 941	P | P	Peach
Crimson Lady	941 | 941	P | P	Peach
Crimson Snow	197 | 197	n | n	Nectarine
CVN13w	941 | 197	P | n	Peach
Denman	941 | 197	P | n	Peach
Early Star	941 | 941	P | P	Peach
Eastern Glo	197 | 197	n | n	Nectarine
Elberta	941 | 941	P | P	Peach
Emeraude	197 | 197	n | n	Peach
Flavortop	197 | 197	n | n	Nectarine
Georgia Belle	941 | 941	P | P	Peach
Gladiator	941 | 197	P | n	Peach
Goldilocks	941 | 197	P | n	Peach
Goldjim	941 | 941	P | P	Peach
Goldnine	941 | 941	P | P	Peach
Greensboro	941 | 941	P | P	Peach
Jade	197 | 197	n | n	Peach
Jefferson	941 | 941	P | P	Peach
JH Hale	941 | 941	P | P	Peach
KV175	197 | 197	n | n	Nectarine
KV175	941 | 197	P | n	Nectarine
KV357	941 | 197	P | n	Peach
KV398	941 | 197	P | n	Peach
KV401	941 | 197	P | n	Peach
KV501	197 | 197	n | n	Nectarine
KV601	197 | 197	n | n	Nectarine
KV606	941 | 197	P | n	Peach
KV701	197 | 197	n | n	Nectarine
KV801	941 | 197	P | n	Peach

Table 8. IndelG DNA test genotypes and pubescent (peach) or glabrous (nectarine) phenotypes of UA selections and cultivars (not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0) and germplasm from other breeding programs, years 2013-2014. (Cont.).

Accession	IndelG genotype (bp)	IndelG functional genotype	Phenotype
Loring	941 | 941	P | P	Peach
Manon	941 | 941	P | P	Nectarine
Messina	941 | 197	P | n	Peach
Orange Cling	941 | 941	P | P	Peach
Peento	941 | 941	P | P	Peach
PF 1	941 | 941	P | P	Peach
PF 24-007	941 | 941	P | P	Peach
PF 24C	941 | 941	P | P	Peach
PF 5B	941 | 941	P | P	Peach
PF 5D Big	941 | 941	P | P	Peach
PF Lucky 13	941 | 941	P | P	Peach
PF-19-007	941 | 941	P | P	Peach
Redhaven	941 | 941	P | P	Peach
Rising Star	941 | 941	P | P	Peach
Roygold	941 | 941	P | P	Peach
Ruby Prince	941 | 197	P | n	Peach
Saturn	941 | 941	P | P	Peach
Slappey	941 | 941	P | P	Peach
Spring Snow	941 | 941	P | P	Peach
Sugar Giant	941 | 197	P | n	Peach
Sugar Lady	941 | 197	P | n	Peach
Sweet Star	941 | 941	P | P	Peach
Tango	941 | 941	P | P	Peach
Tango-II	941 | 197	P | n	Peach
Westbrook	197 | 197	n | n	Nectarine
White Cloud	941 | 941	P | P	Peach
White Lady	941 | 197	P | n	Peach
White River	941 | 941	P | P	Peach
Yumm Yumm	197 | 197	n | n	Nectarine

Table 9. IndelG DNA test functional genotypes and pubescent [peach (P)] or glabrous [nectarine(n)] phenotypes for parents of 22 F_1 UA 2010 populations (2013-2014). (N is number of samples analyzed) [*The chi-square p-value in brackets]. Genotypes in **bold** were determined based of progeny and other parents genotypes.

F_1 population	Progeny (N)	Parents		Phenotype	Functional genotype	F_1 progeny matching phenotype and allele segregation ratio
AR_Pop_1001	38	Female	A-665	Peach	P | P	16 P | P : 20 P | n [*0.50]
		Male	A-800	Peach	P | n	
AR_Pop_1002	31	Female	A-760	Peach	P | n	13 P | P : 18 P | n [*0.37]
		Male	A-708	Peach	P | P	
AR_Pop_1003	40	Female	White Diamond	Peach	P | P	19 P | P : 21 P | n [*0.75]
		Male	A-760	Peach	P | n	
AR_Pop_1004	19	Female	**A-753**	Peach	**P | P**	19 P | P [*1.00]
		Male	Souvenirs	Peach	P | P	
AR_Pop_1006	32	Female	White County	Peach	P | n	14 P | P : 18 P | n [*0.48]
		Male	Souvenirs	Peach	P | P	
AR_Pop_1007	28	Female	**A-775**	Peach	**P | n**	12 P | P : 16 P | n [*0.45]
		Male	Souvenirs	Peach	P | P	
AR_Pop_1008	13	Female	**A-746**	Peach	**P | n**	7 P | P : 6 P | n [*0.78]
		Male	**A-785**	Peach	**P | P**	
AR_Pop_1009	15	Female	**A-746**	Peach	**P | n**	5 P | P : 7 P | n : 3 n | n [*0.50]
		Male	**A-823**	Peach	**P | n**	
AR_Pop_1011	59	Female	A-786	Peach	P | P	59 P | P [*1.00]
		Male	**A-773**	Peach	**P | P**	
AR_Pop_1012	32	Female	**A-773**	Peach	**P | P**	32 P | P [*1.00]
		Male	A-774	Peach	P | P	
AR_Pop_1013	26	Female	A-772	Peach	P | n	12 P | P : 14 P | n [*0.69]
		Male	A-774	Peach	P | P	

Table 9. IndelG DNA test functional genotypes and pubescent [peach (P)] or glabrous [nectarine(n)] phenotypes for parents of 22 F_1 UA 2010 populations (2013-2014). (N is number of samples analyzed) [*The chi-square p-value in brackets]. Genotypes in **bold** were determined based of progeny and other parents genotypes. (Cont.).

F_1 population	Progeny (N)	Parents		Phenotype	Functional genotype	F_1 progeny matching phenotype and allele segregation ratio
AR_Pop_1014	9	Female	**A-685**	Peach	**P | P**	9 P | P [*1.00]
		Male	**A-773**	Peach	**P | P**	
AR_Pop_1015	23	Female	A-789N	Nectarine	n | n	23 n | n [*1.00]
		Male	A-803CN	Nectarine	n | n	
AR_Pop_1016	5	Female	A-807CN	Nectarine	n | n	5 n | n [*1.00]
		Male	**A-802CN**	Nectarine	**n | n**	
AR_Pop_1018	27	Female	Bowden	Nectarine	n | n	27 n | n [*1.00]
		Male	A-761N	Nectarine	n | n	
AR_Pop_1019	17	Female	A-779CN	Nectarine	n | n	17 n | n [*1.00]
		Male	A-776CN	Nectarine	n | n	
AR_Pop_1020	32	Female	Bowden	Nectarine	n | n	32 n | n [*1.00]
		Male	A-758CN	Nectarine	n | n	
AR_Pop_1021	28	Female	A-778N	Nectarine	n | n	28 n | n [*1.00]
		Male	**A-777CN**	Nectarine	**n | n**	
AR_Pop_1022	25	Female	Amoore Sweet	Nectarine	n | n	25 n | n [*1.00]
		Male	A-779CN	Nectarine	n | n	
AR_Pop_1024	20	Female	**A-757N**	Nectarine	**n | n**	20 n | n [*1.00]
		Female	**A-807CN**	Nectarine	**n | n**	
AR_Pop_1025	8	Male	A-770CN	Nectarine	n | n	8 n | n [*1.00]
		Female	A-768N	Nectarine	n | n	
AR_Pop_1026	31	Male	A-816CN	Nectarine	n | n	15 P | n : 16 n | n [*0.86]
		Female	A-772	Peach	P | n	

IndelG DNA Test Genotyping

Genetic screening for the indelG DNA test was successful across all individuals in the study except for a negligible sample failure rate of ~1-5%. Only three genotypes were observed across all material: homozygous dominant pubescent (P | P), heterozygous pubescent (P | n), and homozygous recessive glabrous (n | n). Representative genotypes for the indelG DNA test using gel electrophoresis are shown in Fig. 4. Using the gel electrophoresis platform, the glabrous allele (n) was consistently sized around ~200 bp and the pubescent allele (P) was consistently around ~920 bp. Representative alleles for the indelG DNA test using the Fragment Analyzer™ PROSize® v.1 software were consistently revealed (Figs. 5-7). Using this platform, the glabrous allele (n) was consistently between ~185-195 bp and the pubescent allele (P) was consistently between ~925-935 bp. Lastly, representative alleles for the indelG DNA test using the ABI GeneMarker® software were revealed (Figs. 8-10). Using this platform, the glabrous allele (n) was consistently ~192-198 bp and the pubescent allele (P) was consistently ~840-846 bp.

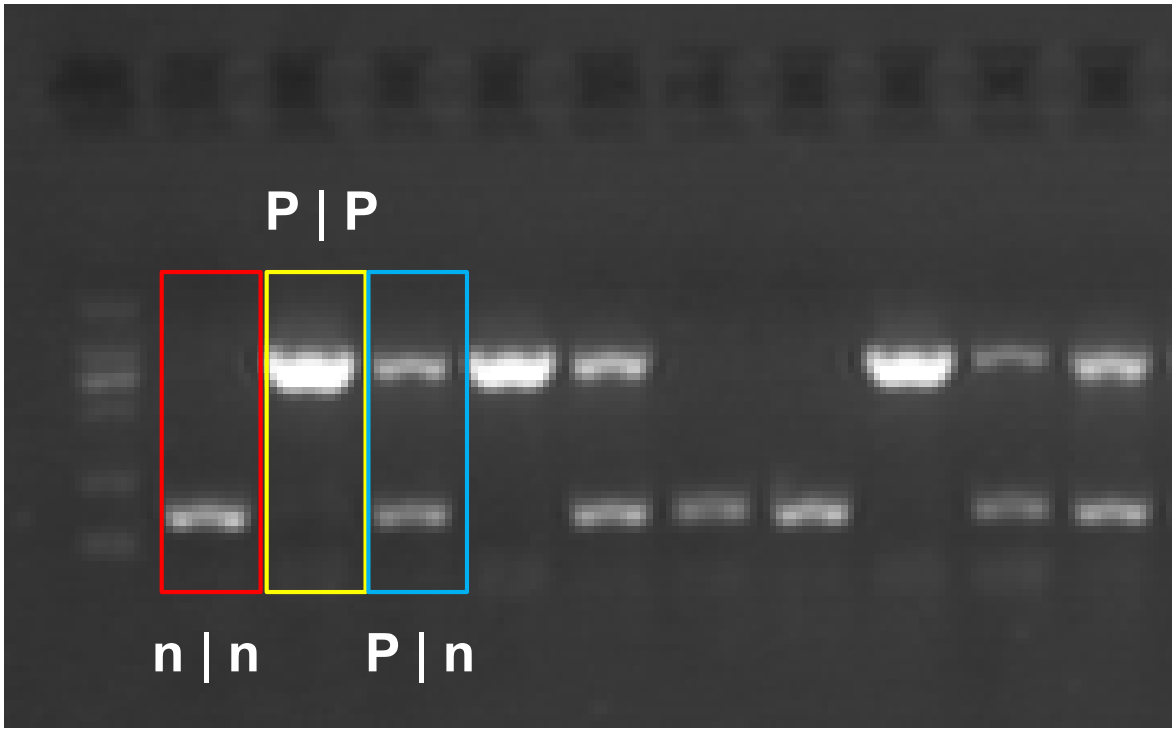

Fig. 4. Representative output of the indelG DNA test using gel electrophoresis. The bands represent the glabrous (nectarine) (n) and pubescent (peach) (P) alleles with approximate bp sizes of 200 bp for the n allele and 920 bp for the P allele. In red is glabrous individual, yellow homozygous pubescent, and blue heterozygous pubescent.

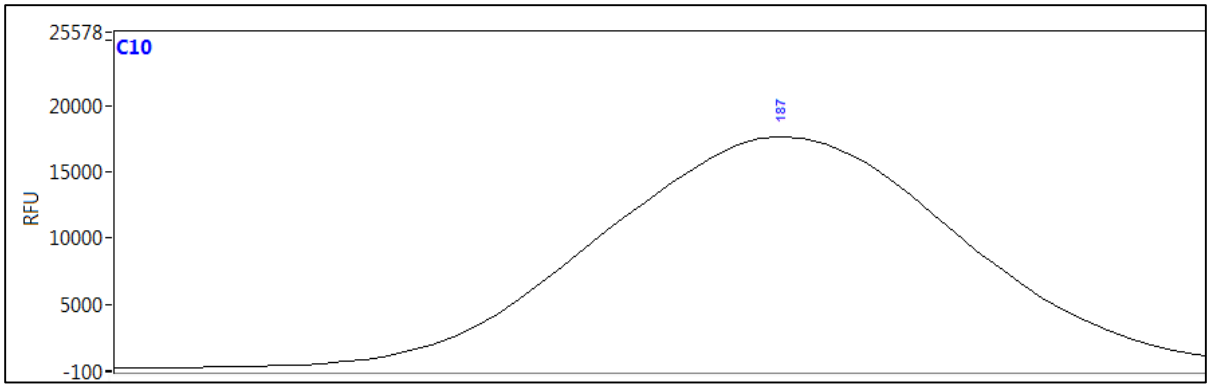

Fig. 5. Representative fragment analysis output of a homozygous glabrous (nectarine) (n | n) individual, amplifying a peak of 187 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

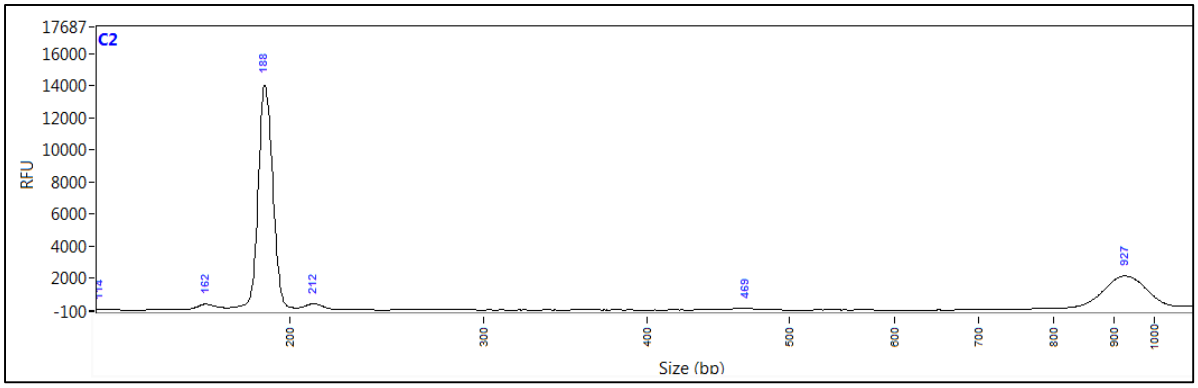

Fig. 6. Representative fragment analysis output of a heterozygous pubescent (peach) (P | n) individual, amplifying peaks of 188 and 927 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

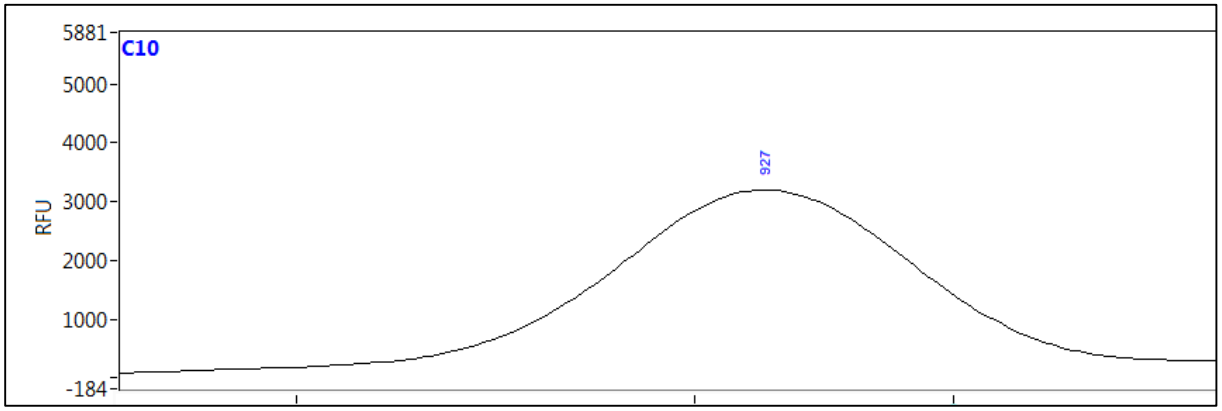

Fig. 7. Representative fragment analysis output of a homozygous pubescent (peach) individual (P | P), amplifying a peak of 927 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

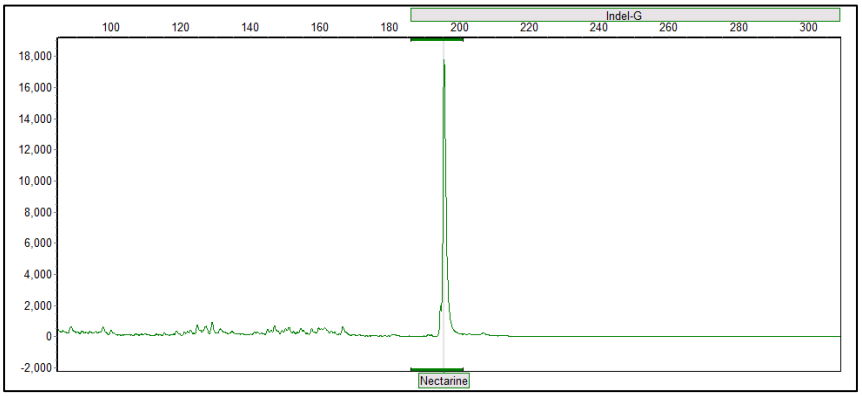

Fig. 8. Representative ABI output of a homozygous glabrous (nectarine) (n | n) individual, amplifying a peak of 197 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

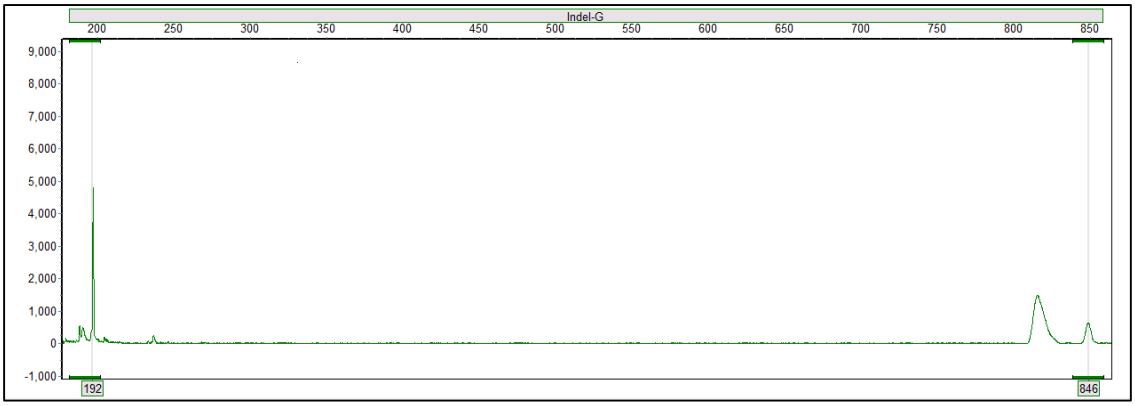

Fig. 9. Representative ABI output of a heterozygous pubescent (peach) individual (P | n), amplifying peaks of 192 and 846 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

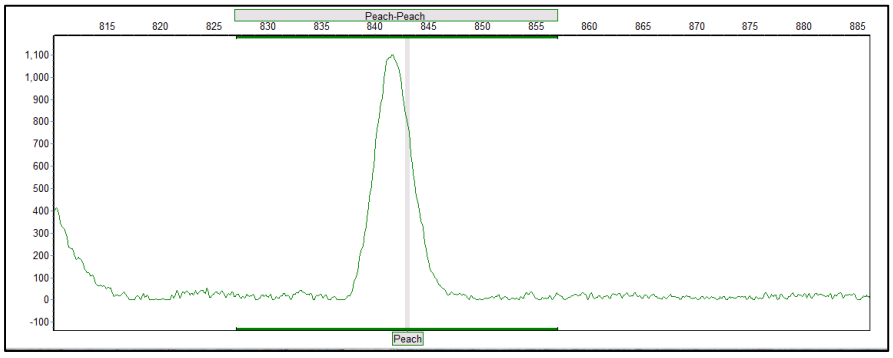

Fig. 10. Representative ABI output of a homozygous pubescent (peach) individual (P | P), amplifying a peak of 842 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

IndelG DNA Test "Conversion Set"

The indelG DNA test results of the F_1 seedlings alleles and matching phenotypes for each UA RosBREED population in the "conversion set" fit their expected parental segregation ratio patterns (Table 5). For AR_Pop_1, the female parent 'White County' and the male parent A-672 were both heterozygous pubescent (peach) (P | n), and their F_1 progeny alleles and matching phenotypes (10 P | P : 22 P | n : 9 n | n) fit the expected segregation ratio ($p = 0.87$). The remaining populations chi-square p-values ranged from 1.00 (AR_Pop_0801, AR_Pop_0803, AR_Pop_0819) to 0.40 (AR_Pop_0813) (Table 5). Additionally the 105 UA cultivars and selections in the "conversion set" consisted of 37 individuals homozygous pubescent (peach) (P | P), 22 heterozygous pubescent (peach) (P | n), 45 homozygous glabrous (nectarine) (n | n), and one individual did not amplify (Tables 6-7).

SNP Haplotype DNA Test Development

Functional SNP haplotypes were successfully developed for 162 out of the 175 individuals from the UA RosBREED "conversion set," that were screened with the IPSC 9K peach SNP array (Tables 6, 10, and 11). Of these 162 individuals, 15 unique haplotype sequences were observed, and subsequently converted into functional haplotypes groups as previously described in the Materials and Methods (Table 10). All functional SNP haplotypes were additionally divided into two simple functional haplotype groups defined functional allele 'P' [pubescent (peach)], and 'n' [glabrous (nectarine)]. The frequencies for each unique functional haplotype ranged from <1% for G1, G2b, G4, and G8, to 36% for haplotype g1. In selected germplasm (germplasm selected prior in seedling populations in routine breeding field selection activities), the haplotype frequencies for 33 individuals ranged from ≤ 3% for go, G2b, G2c, G5, G6, G7, and G8 to 30% for g1. In unselected germplasm (F_1 seedlings, not yet

selected) the haplotype frequencies for 129 individuals ranged from 0% for G1, G2b, G4, and G8 to 37% for g1 (Table 10).

Table 10. The functional and simple functional 11-SNP haplotypes for distinguishing among 162 total pubescent [peach (P)] or glabrous [nectarine (n)] individuals (Un-selected = 129 F1 seedlings) (Selected = 33 cultivars and selections) in the UA RosBREED pedigree ("conversion set") screened with the 9K array (N is number of haplotypes analyzed).

11-SNP haplotype			Total		Selected		Un-selected	
Sequence	Functional	Simple functional	N=324	Frequency	N=66	Frequency[z]	N=258	Frequency[y]
BBAAAABBABB	go	n	8	0.02	1	0.01	7	0.02
ABAAAABBABB	g1	n	115	0.36	20	0.30	95	0.37
AABAAABBABB	g2	n	36	0.11	3	0.05	33	0.13
BBBBAABBABB	g3	n	13	0.04	3	0.05	10	0.04
BBABAABBABB	G1	P	4	0.01	4	0.06	0	0.0
BBBBAABAAAB	G2a	P	37	0.11	7	0.11	30	0.12
BBBBAABAAAA	G2b	P	2	0.01	2	0.03	0	0.0
ABBBAABAAAB	G2c	P	13	0.04	1	0.01	12	0.05
AABAAAABBBA	G3a	P	21	0.06	11	0.17	10	0.04
BABAAAABBBA	G3b	P	12	0.04	4	0.06	8	0.03
AABABBABBBA	G4	P	5	0.02	5	0.09	0	0.0
BABAAABBBBA	G5	P	12	0.04	1	0.01	11	0.04
BBAAAAABBBA	G6	P	15	0.05	2	0.03	13	0.05
BABAAABBABB	G7	P	30	0.09	1	0.01	29	0.11
BBABBBBBABA	G8	P	1	0.00	1	0.01	0	0.0

[z]Frequency of haplotypes in UA ("conversion set") selected germplasm (i.e. cultivars or selections).
[y]Frequency of haplotypes in UA ("conversion set") un-selected germplasm (i.e. seedlings).

Table 11. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] for the 7 F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).**

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
AR_Pop_1_01	**G7 | g2*(G)**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_1_02	**g1 | g2*(G)**	**n | n*(P)**	**941 | 197**	**P | n**	**Peach**
AR_Pop_1_03	G2a| G7	P | P	941 | 941	P | P	Peach
AR_Pop_1_04	**G7 | g2*(G)**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_1_05	G7 | g2	P | n	-	-	Peach
AR_Pop_1_06	**g1 | g2*(G)**	**n | n*(P)**	**941 | 197**	**P | n**	**Peach**
AR_Pop_1_07	**g1 | g2*(G)**	**n | n*(P)**	**941 | 197**	**P | n**	**Peach**
AR_Pop_1_08	**g1 | g2*(G)**	**n | n*(P)**	**941 | 197**	**P | n**	**Peach**
AR_Pop_1_09	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_1_10	-	-	-	-	Peach
AR_Pop_1_11	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_1_12	**g1 | g2*(G)**	**n | n*(P)**	**941 | 197**	**P | n**	**Peach**
AR_Pop_1_14	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_1_15	**G7 | g2*(G)**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_1_17	**g1 | g2*(G)**	**n | n*(P)**	**941 | 197**	**P | n**	**Peach**
AR_Pop_1_18	**G7 | g2*(G)**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_1_19	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_1_20	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_1_21	g1 | g2	n | n	197 | 197	n | n	Nectarine
AR_Pop_1_22	G7 | g2	P | n	941 | 197	P | n	Peach

Table 11. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] for the 7 F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
AR_Pop_1_23	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_1_24	**-**	**-**	**-**	**-**	**Peach**
AR_Pop_1_25	**G7 | g1**	**P | n**	**941 | 197**	**P | n**	**Peach**
AR_Pop_1_26	**G7 | g2*(G)**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_1_27	**g1 | g2*(G)**	**n | n*(P)**	**941 | 197**	**P | n**	**Peach**
AR_Pop_1_28	**G7 | g2*(G)**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_1_29	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_1_30	g1 | G2	P | n	941 | 197	P | n	Peach
AR_Pop_1_31	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_1_32	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_1_33	G7 | g2	P | n	941 | 197	P | n	Peach
AR_Pop_1_34	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_1_35	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_1_36	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_1_37	**g1 | g2*(G)**	**n | n*(P)**	**941 | 197**	**P | n**	**Peach**
AR_Pop_1_38	**G7 | g2*(G)**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_1_39	**G7 | g2*(G)**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_1_40	g1 | go	n | n	197 | 197	n | n	Nectarine
AR_Pop_1_41	-	-	197 | 197	n | n	Nectarine
AR_Pop_1_42	**G7 | g2*(G)**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**

Table 11. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] for the 7 F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
AR_Pop_1_43	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_1_44	G7 | g2	P | n	941 | 197	P | n	Peach
AR_Pop_1_45	**G7 | g2*(G)**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_1_46	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_1_47	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_1_48	**G7 | g2*(G)**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_1_49	g1 | g2	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_01	g1 | go	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_02	g1 | g3	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_03	g1 | g3	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_04	g1 | g3	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_05	g1 | go	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_06	g1 | g3	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_07	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_08	g1 | g3	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_09	g1 | go	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_10	g1 | go	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_11	g1 | go	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_12	g1 | g3	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_13	g1 | g3	n | n	197 | 197	n | n	Nectarine

Table 11. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] for the 7 F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
AR_Pop_0801_14	g1 | go	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_15	g1 | g3	n | n	197 | 197	n | n	Nectarine
AR_Pop_0801_16	g1 | g3	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_01	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_02	g1 | g2	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_03	g1 | g2	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_04	g1 | g2	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_05	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_06	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_07	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_09	g1 | g2	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_10	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_11	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_12	g1 | g2	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_13	g1 | g2	n | n	-	-	Nectarine
AR_Pop_0803_14	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0803_15	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_0813_01	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0813_02	G3b | g1	P | n	941 | 197	P | n	Peach

Table 11. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] for the 7 F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
AR_Pop_0813_03	**G3b> | g2**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_0813_04	**G3b | g1**	**P | n*(P)**	**941 | 941**	**P | P**	**Peach**
AR_Pop_0813_05	G3b | g1	P | n	941 | 197	P | n	Peach
AR_Pop_0813_06	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0813_07	G3b | g1	P | n	941 | 197	P | n	Peach
AR_Pop_0813_08	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0813_09	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0813_10	G3b | g1	P | n	941 | 197	P | n	Peach
AR_Pop_0813_11	G3b | g1	P | n	941 | 197	P | n	Peach
AR_Pop_0813_12	G3b> | g1	P | n	941 | 197	P | n	Peach
AR_Pop_0817_01	G7 | g1	P | n	941 | 197	P | n	Peach
AR_Pop_0817_02	g1 | g3	n | n	197 | 197	n | n	Nectarine
AR_Pop_0817_03	g1 | g1	n | n	197 | 197	n | n	Nectarine
AR_Pop_0817_04	-	-	197 | 197	n | n	Nectarine
AR_Pop_0817_05	-	-	941 | 197	P | n	Peach
AR_Pop_0817_06	-	-	197 | 197	n | n	Nectarine
AR_Pop_0817_07	-	-	941 | 197	P | n	Peach
AR_Pop_0817_08	-	-	197 | 197	n | n	Nectarine
AR_Pop_0817_09	-	-	197 | 197	n | n	Nectarine
AR_Pop_0819_01	G6 | >G2a	P | P	941 | 941	P | P	Peach

> Marks recombination which was observed in an individuals SNP haplotype. The side of the haplotype which the > is on represents approximately where the recombination occurred.

Table 11. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] for the 7 F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
AR_Pop_0819_02	G3a | G5	P | P	941 | 941	P | P	Peach
AR_Pop_0819_03	G3a | G5	P | P	941 | 941	P | P	Peach
AR_Pop_0819_04	G6 | G5	P | P	941 | 941	P | P	Peach
AR_Pop_0819_05	G6 | G5	P | P	941 | 941	P | P	Peach
AR_Pop_0819_06	G2a | G6	P | P	941 | 941	P | P	Peach
AR_Pop_0819_07	G2a | G6	P | P	941 | 941	P | P	Peach
AR_Pop_0819_08	G2a | G3a	P | P	941 | 941	P | P	Peach
AR_Pop_0819_09	G6 | >G2a	P | P	941 | 941	P | P	Peach
AR_Pop_0819_10	G3a | G5	P | P	941 | 941	P | P	Peach
AR_Pop_0819_11	G2a | G3a	P | P	941 | 941	P | P	Peach
AR_Pop_0819_12	G6 | G5	P | P	941 | 941	P | P	Peach
AR_Pop_0819_13	G3a | >G2a	P | P	941 | 941	P | P	Peach
AR_Pop_0819_14	G2a | G6	P | P	941 | 941	P | P	Peach
AR_Pop_0819_15	G5 | G6	P | P	941 | 941	P | P	Peach
AR_Pop_0819_16	G6 | G5	P | P	941 | 941	P | P	Peach
AR_Pop_0819_17	G2a | G6	P | P	941 | 941	P | P	Peach
AR_Pop_0819_18	G2a | G3a	P | P	941 | 941	P | P	Peach
AR_Pop_0819_19	G2a | G3a	P | P	941 | 941	P | P	Peach
AR_Pop_0819_20	G2a | >G3a	P | P	941 | 941	P | P	Peach

> Marks recombination which was observed in an individuals SNP haplotype. The side of the haplotype which the > is on represents approximately where the recombination occurred.

Table 11. Relationships between 11-SNP diplotypes, indelG genotypes, and phenotypes [pubescent (peach) or glabrous (nectarine)] for the 7 F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and indelG genotypes are marked in **bold**, and the correct simple functional haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	IndelG genotype (bp)	IndelG functional genotype	Phenotype
AR_Pop_0819_21	G3a| G5	P | P	941 | 941	P | P	Peach
AR_Pop_0819_22	G6 | G5	P | P	941 | 941	P | P	Peach
AR_Pop_0819_23	G6 | G5	P | P	941 | 941	P | P	Peach
AR_Pop_0825_01	G2a | g1	P | n	941 | 197	P | n	Peach
AR_Pop_0825_02	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_03	G2a | g1	P | n	941 | 197	P | n	Peach
AR_Pop_0825_04	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_05	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_06	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_07	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_08	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_09	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_10	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_11	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_12	G2a | g1	P | n	941 | 197	P | n	Peach
AR_Pop_0825_13	G2a | g1	P | n	941 | 197	P | n	Peach
AR_Pop_0825_14	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_15	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_16	G2a | >G2c	P | P	941 | 941	P | P	Peach
AR_Pop_0825_17	G2a | g1	P | n	941 | 197	P | n	Peach

> Marks recombination which was observed in an individuals SNP haplotype. The side of the haplotype which the > is on represents approximately where the recombination occurred.

SNP Haplotypes to IndelG DNA Test Conversion

Next, the entire UA RosBREED "conversion set" of 243 individuals was used to make direct comparisons between each individuals SNP diplotypes and their indelG genotypes. At least one of the DNA tests failed for 29 individuals, thus only 214 individuals were considered. In total, 57 individuals were homozygous pubescent (peach) (P | P / P | P), 44 were heterozygous pubescent (P | n / P | n), and 88 were homozygous glabrous (nectarine) (n | n / n | n) for both DNA tests (Tables 6-7 and 10-12). In selected material, 22 individuals were homozygous pubescent (P | P / P | P), 16 were heterozygous pubescent (P | n / P | n), and 46 were homozygous glabrous (n | n / n | n) for both DNA tests. In un-selected material, 35 individuals were homozygous pubescent (P | P / P | P), 28 were heterozygous pubescent (P | n / P | n), and 42 were homozygous glabrous (n | n / n | n) for both DNA tests (Tables 6, 7, 10, 11, and 12). The total SNP diplotypes and indelG genotypes matched 88% of the time, yet 12% of the individuals contained inconsistencies (Table 12). In un-selected material, a higher inconsistency rate was observed, reaching 17%, and in selected material the inconsistency rate dropped to 4% (Table 12).

IndelG DNA Test (Additional Cultivars and Selections)

Of the remaining 127 UA cultivars, selections and germplasm from other breeding programs, not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0, 38 individuals were homozygous pubescent (peach) or (P | P), 48 were heterozygous pubescent (P | n), 38 were homozygous glabrous (nectarine) (n | n), and three individuals did not amplify using indelG (Table 8). The indelG genotype accurately predicted the phenotype in all cases.

Table 12. Relationships between simple 11-SNP diplotypes and indelG functional genotypes tested for distinguishing among pubescent (peach) and glabrous (nectarine) individuals in the UA RosBREED pedigree ("conversion set") (N is number of samples analyzed). Inconsistencies between SNP diplotypes and indelG genotypes are marked in **bold**, and the correct haplotype follows with ***( ).**

DNA test		Phenotype	Total		Selected		Un-selected	
Simple 11-SNP diplotype	IndelG functional genotype		N=214	Frequency	N=88	Frequency[z]	N=126	Frequency[y]
P | P	P | P	Peach	57	0.27	22	0.25	35	0.28
P | n	P | n	Peach	44	0.20	16	0.18	28	0.22
n | n	n | n	Nectarine	88	0.41	46	0.53	42	0.33
n | n*(P)	**P | n**	**Peach**	**9**	**0.04**	**1**	**0.01**	**8**	**0.07**
P | n*(P)	**P | P**	**Peach**	**16**	**0.08**	**3**	**0.03**	**13**	**0.10**

[z]Frequency of haplotypes in UA ("conversion set") selected germplasm (i.e. cultivars or selections).
[y]Frequency of haplotypes in UA ("conversion set") un-selected germplasm (i.e. seedlings).

IndelG DNA Test "Confirmation Set"

The F_1 seedlings alleles and matching phenotypes for each 2010 "confirmation set" population fit the expected parental segregation ratio patterns for the indelG DNA test (Table 9). For AR_Pop_1001, the female parent A-665 was homozygous pubescent (peach) (P | P), and the male parent A-800 was heterozygous pubescent (P | n). Their F_1 progenies' alleles and matching phenotypes (16 P | P : 20 P | n) fit their expected segregation ratio ($p = 0.50$). The remaining populations chi-square p-values ranged from 1.00 to 0.37 (AR_Pop_0813) (Table 9).

Discussion

During the course of this study two different types of DNA tests for pubescent vs. glabrous were investigated to determine and compare their predictiveness to differentiate pubescent (peach) and glabrous (nectarine) alleles. The two DNA tests included the 11-SNP haplotype, developed in Chapter Four of this dissertation, and the intragenic indelG, previously developed by Vendramin et al. (2014). At least one of the DNA tests failed for 29 individuals in the UA RosBREED "conversion set", thus, only 214 individuals were considered. This sample failure rate of ~6% per DNA test is near the sample failure rate that previous studies have observed, ~1-5% (Edge-Garza et al., 2014).

The SNP diplotypes and indelG genotypes for the UA RosBREED "conversion set" of 214 individuals matched 88% of the time, yet a 12% inconsistency rate was observed. In un-selected material a higher inconsistency rate of 17% was observed, and in selected material the inconsistency rate dropped to 5%. The selected material with inconsistencies included A-672, A-821, A-827, and A-881. These four selections were found to contain a pubescent (peach) SNP haplotype (AABAAABBABB = g2), disguised as a glabrous (nectarine) allele, most likely due to recombination events within the 11-SNP haplotype. The A-672 selection was a parent of

AR_Pop_1 and AR_Pop_0813, which contained seedlings that represented the 15% inconsistency rate in unselected material. A total of 19 seedlings from AR_Pop_1 and two from AR_Pop_0813 inherited the falsely associated glabrous SNP haplotype.

The comparison between the two DNA tests highlights that the indelG DNA test is more predictive than the SNP haplotype DNA test. The indelG DNA test is located within the *PpeMYB25* pubescent vs. glabrous candidate gene, thus, the recombination events that affected the SNP haplotypes did not affect the intragenic DNA test. Since the indelG DNA test was 100% predictive across the UA RosBREED "conversion set" material, as well as all previous material in Vendramin et al. (2014), it was more efficient to only proceed with that test. The indelG DNA test is based on a single indel marker that uses a standard PCR protocol, which was shown in the study herein to be effective even for low quality and quantity DNA. One PCR-based marker is more economical and simple to use than 11 SNPs. Using one indelG DNA test can reduce the amount of PCR reactions that are needed to evaluate an individuals' genotype, resulting in time and cost savings. Moreover, indelG can be multiplexed with other DNA tests for other fruit quality, phenological, or diseases resistance traits, and pooled for analysis on a range of genotyping platforms, including but not limited to agarose gel, polyacrylamide gel, the Fragment Analyzer™, or ABI Prism 3730xl DNA Analyzer.

The seven UA RosBREED populations ("conversion set") F_1 seedlings indelG alleles and matching phenotypes fit their parental expected segregation ratios (X^2 p-value > 0.05). Furthermore, all 25 of the 2010 populations ("confirmation set") fit their parental expected segregation ratios (X^2 p-value > 0.05). A sample failure rate of ~5% was observed across most populations, which is consistent with previous studies that reported a negligible sample failure rate of ~1-5% (Edge-Garza et al., 2014). These results add further evidence to the Vendramin et

al., (2014) finding that the transcription factor gene PpeMYB25 is the positive regulator of trichome formation in peach fruit, and that the Ty1-copia retrotransposon insertion in the third exon of PpeMYB25 caused a loss-of-function mutation, resulting in the glabrous phenotype. Furthermore, these results confirm the indelG test's predictiveness across a vast majority of the UA peach breeding program, hence, this marker can be incorporated into MAS in this program.

However as with any trait, there will always be the possibility of new mutations occurring in the PpeMYB25 candidate gene, as seen with the PpCCD4.1 candidate gene for white vs. yellow flesh color in Falchi et al., (2013). In this study, Falchi et al., (2013) showed that yellow flesh alleles across 37 peach and nectarine cultivars had arisen from three independent mutational events, and that the PpCCD4-SSR could only accurately differentiate yellow from white flesh for the first mutational event. Falchi et al., (2013) developed two additional DNA tests predictive of the other two mutational events, and thus breeders can now screen for one, or all three, depending on the mutational lineages in their program. Thus it's important to understand that a DNA test is only predictive for the mutational lineages it was developed for.

The indelG DNA test can provide the UA peach breeder with valuable DNA information to select parents (MAPS), accurately design crosses (MACS), and cull unwanted genotypes (MASS). The test eliminates the need to perform extensive (time consuming and highly resource intensive) progeny testing since it accurately determines the parental pool genotypes. As seen in this work, out of the 29 total F_1 crosses, only five were capable of introgressing traits from peach into nectarines (AR_Pop_1, AR_Pop_0813, AR_Pop_0817, AR_Pop_1009, and AR_Pop_1026) and vice versa. By using the indelG DNA test the breeder can now more efficiently design crosses between heterozygous peaches and homozygous nectarines to expand diversity in both

types. Furthermore, after the crosses are made, unwanted genotypes can be discarded in the greenhouse, or all the peaches and nectarines can be planted in different groups, to allow the breeder to be more efficient when walking seedlings.

The work in this study also extends to other peach breeders, since the indelG DNA test was 100% predictive across 58 cultivars from a range of U.S breeding programs as well as the 95 cultivars in Vendramin et al., (2014). While the indelG can be a valuable DNA test for all peach and nectarine breeders, it's still advised to confirm it's predictiveness in their own programs germplasm before widespread adoption, since as noted before independent mutational events could have, or still could occur in the PpeMYB25 candidate gene.

At the UA peach and nectarine breeding program, the indelG DNA test has been used for MAPS and MACS in 2013-2015 as well as for MASS in 2015 across 235 individuals from five populations ranging from 20-122 individuals (see Chapter Six in dissertation). This test was multiplexed and pooled with the PpCCD4b DNA test for resource savings. Deployment of MAS using the indelG DNA test will continue in 2016, and likely the Clemson University, Texas A&M University and UC Davis peach breeding programs will also begin routine deployment. Implementation of MAS for the indelG DNA test in peach breeding programs substantiates the tests breeding utility and impact of predictive DNA tests in perennial fruit tree breeding.

Literature Cited

Bink, M. 2004. FlexQTL software. BIOMETRIS, Wageningen UR, The Netherlands <www.flexqtl.nl>.

Bink, M. 2005. FlexQTL software: efficient estimation of identity by descent probabilities and QTL mapping in pedigreed populations. In: Plant and Animal Genomes XII Conference, 15-19 January, San Diego, CA, U.S.

Bink, M., C. ter Braak, M. Boer, L. Totir, C. Winkler, and O. Smith. 2012. QTL linkage analysis of connected populations using ancestral marker and pedigree information. Theor. Appl. Genet. 124:1097–1113.

Bink, 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.

Blake, M.A. 1932. The J.H. Hale peach as a parent in peach crosses. Proc. Natl. Acad. Sci. U.S., 29:131-136.

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

Byrne, D.H. 2005. Trends in stone fruit cultivar development. HortTechnology 15:494–500.

Byrne, D.H., M. Bassols, D. Bassi, M. Piagnani, K. Gasic, G. Reighard, M. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: M. Badenes and D. Byrne (eds.). Fruit breeding. Springer Science, Business Media, New York.

Dellaporta, S., J. Wood, and J.B. Hicks. 1983. Plant Mol. Biol. Rept. 1:19-21.

Dirlewanger E., P. Cosson, K. Boudrhri, C. Renaud, G. Capdeville., Y. Tauzin, F. Laihret, and A. Mong. 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. and Genomes 3:1-13.

Edge-Garza, D., T. Rowland, S. Haendiges, and C. Peace. 2014. A high-throughput and cost-efficient DNA extraction protocol for the tree fruit crops of apple, sweet cherry, and peach relying on silica beads during tissue sampling. Mol. Breeding 34:2225-2228.

Edge-Garza, D.A., J.J. Luby, and C.P. Peace. 2016. Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Mol. Breed. 35:1-15.

Eduardo, I., E. López-Girona, I. Batlle, F. Reig, I. Iglesias, W. Howad, P. Arús, and M.J. Aranzana. 2014. Development of diagnostic markers for selection of the subacid trait in peach. Tree Genet. and Genomes 10:1695-1709.

Falchi, R., E. Vendramin, L. Zanon, S. Scalabrin, G. Cipriani, I. Verde, G. Vizzotto, and M. Morgante. 2013. Three distinct mutational mechanisms acting on a single gene underpin the origin of yellow flesh in peach. Plant J. 76:175–187.

Food and Agricultural Organization of the United Nations (FAO). 2015. Peaches and nectarines world production. August 25, 2015. <http://faostat3.fao.org/compare/E>.

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

Jung, S., S.P. Ficklin, T. Lee, C.-H. Cheng, A. Blenda, P. Zheng, J. Yu, A. Bombarely, I. Cho, S. Ru, K. Evans, C. Peace, A.G. Abbott, L.A. Mueller, M.A. Olmstead and D. Main. 2014. The genome database for rosaceae (GDR): year 10 update. Nucl. Acids Res. 42:D1237-D1244.

Le Dantec, L., G. Cardinet, J. Bonet, M. Fouché, K. Boudehri, A. Monfort, J.-L. Poëssel, A. Moing, and E. Dirlewanger. 2010. Development and mapping of peach candidate genes involved in fruit quality and their transferability and potential use in other Rosaceae species. Tree Genet. and Genomes 6:995–1012.

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. and J. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae, pp. 19-53. In K. Folta and S. Gardiner (eds.). Genetics and genomics of Rosaceae. Springer Science + Business Media, New York.

Ru, S., D. Main, K. Evans, and C. Peace. 2015. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet. and Genomes 11:1-12.

Salgado, 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. Ph.D dissertation University of Arkansas, Fayetteville, U.S.

Sandefur, P., T. Frett, J. Clark, K. Gasic, and C. Peace. 2016a. PpeRf-SSR, a DNA test for routine prediction in breeding of peach blush. Mol. Breeding (In press).

Sandefur, P., T. Frett, A. Salgado, J. Clark, K. Gasic, and C. Peace. 2016b. Ppe-Acidity, a combined DNA test for routine prediction in breeding of acidity and soluble solids content (SSC) in peach. Mol. Breeding (In press).

Smith, S. 2015. Fruit tree diseases. 16 November 2015. <https://www.uaex.edu/publications/pdf/mp154/fruit-tree-diseases-commercial.pdf>.

Studebaker, G., J. Hopkins, and D. Johnson. 2015. Control peach tree borers on commercially grown peach and plum trees. 16 November 2015. <https://www.uaex.edu/publications/PDF/FSA-7504.pdf>.

Vendramin, E., G. Pea, L. Dondini, I. Pacheco, M. T. Dettori, L. Gazza, S. Scalabrin F. Strozzi, S. Tartarini, D. Bassi, I. Verde, and L. Rossini. 2014. A unique mutation in a MYB gene cosegregates with the nectarine phenotype in peach. PloS ONE 9:e90574. DOI: 10.1371/journal.pone.0090574.

Verde, I., A.G. Abbott, S. Scalabrin, S. Jung, S. Shu, F. Marroni, T. Zhebentyayeva, M.T. Dettori, J. Grimwood, F. Cattonaro, A. Zuccolo, L. Rossini, J. Jenkins, E. Vendramin, L.A. Meisel, V. Decroocq, B. Sosinski, S. Prochnik, T. Mitros, A. Policriti, G. Cipriani, L. Dondini, S. Ficklin, D.M. Goodstein, P. Xuan, C. Del Fabbro, V. Aramini, D. Copetti, S. Gonzalez, D.S. Horner, R. Falzchi, S. Lucas, E. Mica, J. Maldonado, B. Lazzari, D. Bielenberg, R. Pirona, M. Miculan, A. Barakat, R. Testolin, A. Stella, S. Tartarini, P. Tonutti, P. Arús, A. Orellana, C. Wells, D. Main, G. Vizzotto, H. Silva, F. Salamini, J. Schmutz, M. Morgante, and D.S. Rokhsar. 2013. The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genet. 45:487-494.

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. DOI:10.1371/journal.pone.0035668.

Voorrips, R.E. 2007. Pedimap: software for visualization of genetic and phenotypic data in pedigrees. Plant Research International, Wageningen, the Netherlands. <http://www.wageningenur.nl/en/show/Pedimap.htm>.

Voorrips, R., M. Bink, and W. van de Weg. 2012. Pedimap: software for the visualization of genetic and phenotypic data in pedigrees. J. Hered. 103:903-907.

Chapter Five - Study Three: PpCCD4b-SSR, a DNA Test for Routine Prediction and Breeding of Peach White and Yellow Flesh Colors

Abstract

Peach flesh color is a market-class defining trait, and has implications for consumer acceptance, since white and yellow flesh peaches are identified separately in the market. Both colors are associated with different carotenoid levels as well as different flavors and aroma. Traditional peach breeders have focused on crossing between white and yellow flesh to diversify flavors and aromas in both flesh colors, but usually don't confirm the parents' actual genotype, unless they perform progeny testing. A predictive DNA test for flesh color (white vs. yellow) could aid in the differentiation of homozygous and heterozygous individuals. This would eliminate the need for breeders to do progeny testing, and allow them to accurately design crosses to introgress traits from white flesh into yellow flesh peaches and nectarines, and vice versa. Peach flesh color has been associated with the *Y-locus* on linkage group 1 where white is dominant to yellow. At this locus, a candidate gene for flesh color, carotenoid cleavage dioxygenase four (PpCCD4), was proposed to be the major factor responsible for carotenoid degradation in white flesh peaches, which were shown to possess one or two copies of the functioning dominant allele for an intragenic SSR marker, PpCCD4, while yellow flesh peaches possess two copies of the non-functioning recessive allele. The objective of this study was to investigate two different types of DNA tests for white vs. yellow flesh to determine and compare their predictiveness to differentiate white and yellow flesh alleles in the University of Arkansas (UA) peach and nectarine breeding program. The two previously developed DNA tests included the 11-SNP haplotype test that spans the *Y-locus*, and the intragenic PpCCD4-SSR test. Both DNA tests were successfully screened on a total of 232 out 243 individuals from the UA

breeding program RosBREED pedigree "conversion set": 105 cultivars and selections and seven F_1 populations consisting of 127 seedlings. The 11-SNP haplotype showed an inconsistency rate of 32%, while the PpCCD4b-SSR DNA test was 100% predictive. Thus, only the PpCCD4b-SSR DNA test was advanced and successfully screened on 470 additional seedlings from 20 F_1 populations, ranging from 5-49 seedlings per population ("confirmation set"), to confirm the DNA test's prediction accuracy in the UA breeding program. All F_1 populations of the "conversion set" and the "confirmation set" fit their expected parental phenotypic and genotypic segregation ratios for the PpCCD4b-SSR DNA test, using the chi-square (X^2) goodness-of-fit test ($p > 0.05$). These results confirmed the 100% prediction rate of PpCCD4b-SSR across a wide diversity of the UA peach and nectarine breeding program. The PpCCD4b-SSR DNA test was deployed for routine marker-assisted selection (MAS) use in this breeding program to efficiently introgress traits from white flesh into yellow flesh peaches and nectarines, and vice versa.

Introduction

Peach flesh color is a market class-defining trait because white and yellow flesh peaches are identified separately in the market (Falchi et al., 2013). Flesh color has implications for consumer acceptance, since consumers have the option of yellow and white peaches (Falchi et al., 2013). Yet, the reason for this differentiation in the marketplace goes far beyond visual appearance, in that white and yellow flesh peaches are known to have strikingly different flavors and aromas. Yellow flesh peaches typically have a balanced sugar and acidic flavor, in comparison to the high sugar and low acid flavor along with intense aroma associated with most white flesh peaches (Brandi et al., 2011). These distinct flavors and aromas can be attributed to the different levels of carotenoid compounds that accumulate in the fruits mesocarp; yellow flesh

cultivars have been shown to possess higher quantities of β–cryptoxanthin and β–carotene at harvest than their white flesh counterparts (Brandi et al., 2011; Falchi et al., 2013).

Carotenoids are a class of pigments known to play several important functions in plants. These pigments are known to protect plants from UV light, attract insects to the flowers for pollination, as well as entice animals to eat the fruit and disperse seeds (Moise et al., 2005). Carotenoids are also known to have antioxidant properties which may reduce the risk of certain cancers (de la Rosa et al., 2009; Falchi et al., 2013). Additionally, carotenoids are known to be cleaved by dioxygenase enzymes to form volatile norisoprenoids, which possess strong aromatic properties exhibited by fruit, flower, and vegetative tissue of plants (Brandi et al., 2011).

In peach, flesh color is a qualitative trait with white dominant over yellow (Bailey and French, 1949; Connors, 1920). Peach flesh color was discovered to be controlled by the *Y-locus* on linkage group 1 (Bliss et al., 2002), and was recently fine-mapped to ~12,649,875-22,739,577 bp on chromosome 1 of the peach genome (Martínez-García et al., 2013; Verde et al., 2013).

Traditional breeders in some programs have focused on white and yellow flesh peach breeding as nearly separate programs due to the diversity of products and marketing of the two flesh colors. To determine if a parent is homozygous or heterozygous white flesh, traditional breeders have used conventional breeding techniques such as progeny testing, but unfortunately, this technique is extremely time consuming and highly resource intensive due to peaches' long juvenility period and the need to perform extensive phenotyping (Byrne et al., 2012). Because of these limitations most breeders have focused on white and yellow flesh crossing without always knowing the peaches' actual genotype.

While traditional peach breeders have made considerable genetic improvements in the past century, traditional breeding is a time consuming, expensive, and laborious process taking

10 years or more, from the initial cross until a new peach cultivar can be released (Bliss, 2010; Byrne et al., 2012; Ru et al., 2015). Fortunately, application of DNA-based information [marker-assisted selection (MAS)] is now a reality, and can provide peach breeders with more informed decision support to increase genetic gain per breeding cycle, improve selection efficiency, and significantly reduce breeding program operational costs (Bliss, 2010; Byrne, 2005; Edge-Garza et al., 2016; Ru et al., 2015). DNA tests for several breeding-relevant traits have been developed in peach including texture (Peace et al., 2005; Peace and Norelli, 2009), acidity (Eduardo et al., 2014), slow-melting flesh (Salgado, 2015), fruit bacterial spot resistance (see Chapter Five, Section One), pubescence (see Chapter Five, Section Two; Vendramin et al., 2014), blush coverage (Sandefur et al., 2016a), and acidity and soluble solids content (Sandefur et al., 2016b). A predictive DNA test for white vs. yellow flesh could be important to accurately differentiate homozygous and heterozygous white flesh individuals across the breeder's program. This would eliminate the need for breeders to do progeny testing, and allow them to accurately select parents with desirable genotypes through marker-assisted parent selection (MAPS) as well as select favorable crosses with efficient combining abilities through marker-assisted cross selection (MACS), to introgress traits from white into yellow flesh peach and nectarines, and vice versa. After the cross, unwanted seedling types could be culled in the greenhouse or all white and yellow flesh peaches could be planted into separate groups in the field if desired, for more efficient field selection.

Several additional molecular studies have been pursued to identify the candidate gene for flesh color. At the *Y-locus*, the carotenoid cleavage dioxygenase four (*PpCCD4*) was proposed as the candidate gene for flesh color and that yellow flesh fruits are homozygous for the recessive loss-of-function allele (Brandi et al., 2011) (Fig. 1). Differential expression of *PpCCD4* in

yellow flesh ‘Redhaven’ (RH) and its white flesh mutant ‘Redhaven Bianca’ (RHB) was observed, where *PpCCD4* was downregulated in RH and upregulated in RHB. The upregulation of *PpCCD4* generates *PpCCD4* enzymes which then cleave the carotenoid compounds, ultimately leading to the formation of volatile norisoprenoids in white peaches (Brandi et al., 2011). Falchi et al., (2013) later provided further evidence that *PpCCD4* is responsible for flesh color (Fig. 1), by performing comparative sequence analysis of this gene across 37 peach cultivars. Through this analysis, they confirmed that white flesh peaches possess one or two copies of the properly functioning dominant allele for the PpCCD4-SSR, and that yellow flesh fruits are homozygous recessive for the loss-of-function allele.

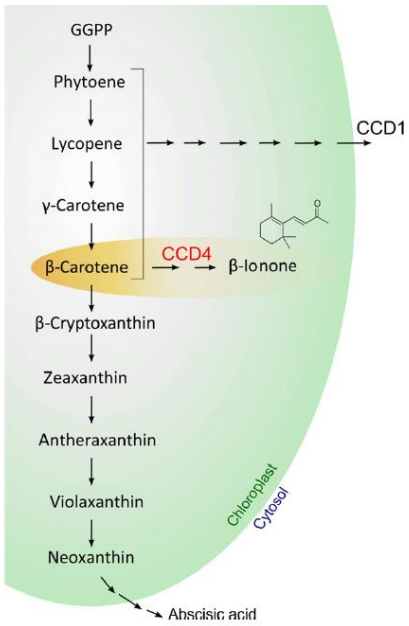

Fig. 1. Diagram of the carotenoid biosynthetic pathway in plants, highlighting where CCD4 cleaves carotenoids to form volatile norisoprenoids (such as β-ionone) which leads to the development of white flesh peaches (*figure as in Falchi et al., 2013).

Falchi et al., (2013), furthermore showed that yellow peach alleles across this material had arisen from two additional independent mutational events, and that the PpCCD4-SSR could

not accurately differentiate yellow from white flesh for these additional two mutational events. The second mutational event was a SNP, A to T transversion, occurring at position 1519, within the second exon of the *PpCCD4* gene, resulting in a premature stop codon. This mutation inactivated the properly functioning dominant allele (white flesh allele), but was only seen in five cultivars from Italy, Spain, and Brazil ('Oro A', 'Leonforte', 'Maruja', 'Leonforte1', and 'Bolinha'). The third mutational event was an intronic transposable element insertion, also leading to a truncated protien, thus inactivating the properly functioning dominant allele (white flesh allele). This mutation was identified in 'Babygold 8', 'Elberta', 'Redhaven', and *P. ferganensis*, all being yellow flesh individuals (Falchi et al., 2013).

Additionally, in Chapter Four of this dissertation, 11 informative SNP markers spanning the previously identified QTL at the *Y-locus* (Martínez-García et al., 2013; Verde et al., 2013) were selected to develop a SNP haplotype DNA test for white vs. yellow flesh. This 11-SNP haplotype DNA test was shown to be ~90% predictive of white vs. yellow flesh across 863 individuals from the four RosBREED peach demonstration breeding programs [the University of Arkansas (UA), Clemson University (CU), Texas A&M University (TX) and the University of California, Davis (CA)]. A total of 243 individuals from the UA breeding program were included in this effort.

The objective of this study was to investigate two types of DNA tests for white vs. yellow flesh to determine and compare their predictiveness to differentiate white vs. yellow flesh alleles in the UA peach and nectarine breeding program. The two DNA tests included the 11-SNP haplotype test that spans the *Y-locus*, developed in Chapter Four of this dissertation, and the previously developed intragenic PpCCD4b-SSR test. The most predictive DNA test was intended

to be further confirmed across the UA program to enable routine MAS for this trait in the UA program.

Materials and Methods

Management Practices at FRS

Phenotypic evaluation flesh color was conducted on peach and nectarine material at the UA Fruit Research Station (FRS), Clarksville, AR (west-central AR, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept. of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]). All trees were either open-center trained and spaced 5.5 m between trees and rows, or trained to a perpendicular-V system with trees spaced 1.9 m in rows spaced 5.5 m apart. All trees were dormant pruned and fertilized annually with a single application of 640 Kg ha-1 of complete fertilizer (19:19:19 of N:P:K) and were sprinkler or drip irrigated as needed. Pests were managed using a program typical for commercial orchards in the area (Smith, 2015; Studebaker et al., 2015). After shuck split but before pit hardening fruitlets were thinned to a distance of 12 to 15 cm between each fruitlet.

Germplasm Utilized

The UA RosBREED “conversion set” was evaluated in 2011-2013 and utilized to convert the 11-SNP haplotype (see Chapter Four of dissertation) to the PpCCD4b-SSR DNA test. The “conversion set” consisted of all 138 individuals in the seven UA RosBREED F1 populations, 37 UA cultivars and selections screened with the International Peach SNP Consortium (IPSC) 9K peach SNP array v1.0 (Verde et al., 2012), and 68 cultivars and selections screened with the mini SNP array v1.0, at the University of Arizona Genetics Core (AZ) and BioDiagnostics (BDI) (in total 243 individuals) (Table 1; Fig. 2). In 2013-2014, the remaining UA cultivars, selections

(69), and an array of germplasm from different breeding programs primarily in the U.S. (58), not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0, were assessed (in total 127 individuals) (Table 2). Additionally, in 2014 a total of 569 seedlings from 20 F_1 populations (2010 crosses), ranging from 5-49 seedlings per population, were evaluated to confirm PpCCD4b-SSR DNA test allele effects in the UA breeding program (i.e. "confirmation set") (Table 3).

Flesh Color Phenotyping

Phenoptyping for flesh color was conducted in 2011-2014, as described in Frett et al., (2012). In short, five fruit slightly firmer than tree ripe, were harvested from the mid-canopy of each tree into 0.24 L corrugated trays (FormTex Plastics Corp., Houston, TX). Visual estimation of the flesh color was performed at harvest, in the field (1 = green; 2 = cream green; 3 = cream; 4 = cream yellow; 5 = yellow green; 6 = yellow; 7 = yellow orange; 8 = orange; 9 = red). Later 1, 2, and 3 ratings were converted to 0 (white) and 4, 5, 6, 7, 8 and 9 were all converted to 1 (yellow), so that white flesh (0) and yellow flesh (1) could be directly compared.

Table 1. Parental information and the number of F_1 seedlings for each of the seven RosBREED populations (N is number of individuals analyzed).

F_1 population	Female parent	Male parent	F_1 seedlings (N)
AR_Pop_1	White County (WC)	A-672	48
AR_Pop_0801	A-776	A-783	16
AR_Pop_0803	Amoore Sweet (AS)	A-778	9
AR_Pop_0813	A-772	A-672	12
AR_Pop_0817	A-789	A-699	9
AR_Pop_0819	A-708	A-773	23
AR_Pop_0825	Souvenirs (S)	A-760	17

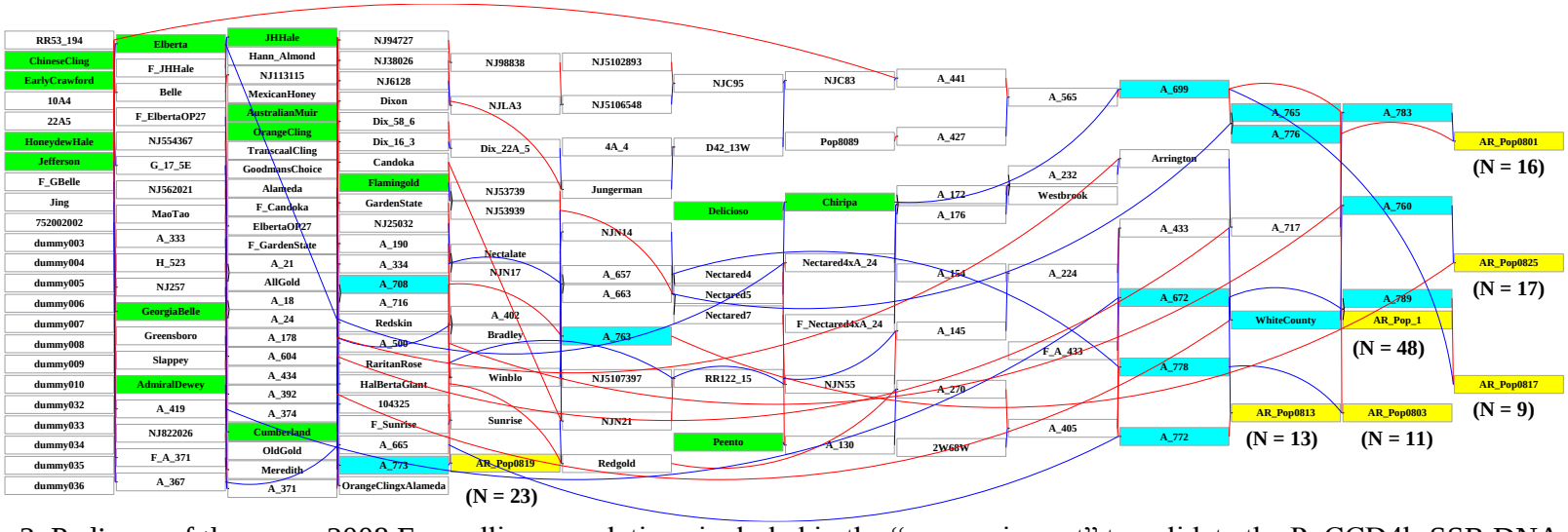

Fig. 2. Pedigree of the seven 2008 F_1 seedling populations included in the "conversion set" to validate the PpCCD4b-SSR DNA test; visualized through Pedimap software (Voorrips, 2007; Voorrips et al., 2012) (F_1 populations highlighted in yellow; Red line = female parent; Blue line = male parent). N is the number of progeny in each population.

Table 2. Arkansas selections and cultivars and an array of germplasm from different breeding programs phenotyped for flesh color in 2013-2014 and genotyped in 2012-2015.

A-554	A-805[y]	A-845[y]	A-878[y]	A-910	Elberta[z]	PF 5D Big
A-662	A-806[y]	A-846	A-879[y]	A-911	Emeraude	PF Lucky 13
A-663[z]	A-808	A-847	A-880[y]	A-912	Flavortop	PF-19-007
A-665[zy]	A-809[y]	A-848[y]	A-881[y]	A-913	Georgia Belle[z]	Redhaven
A-668	A-810	A-849[y]	A-882[y]	A-914	Gladiator	Redskin[z]
A-672[zy]	A-811[y]	A-850[y]	A-883	A-915	Gloria[y]	Rising Star
A-699[z]	A-813[y]	A-851[y]	A-884	A-916	Goldilocks	Roygold
A-708[z]	A-814	A-852[y]	A-885	A-917	Goldjim	Ruby Prince
A-716[z]	A-815	A-853[y]	A-886	A-918	Goldnine	Saturn
A-743	A-816[y]	A-854[y]	A-887	Admiral Dewey[z]	Greensboro[z]	Slappey[z]
A-758	A-818[y]	A-855[y]	A-888	Allgold	Jade	Souvenirs[z]
A-760[z]	A-819[y]	A-856[y]	A-889	Amoore Sweet[z]	Jefferson[z]	Spring Snow
A-761	A-820[y]	A-857[y]	A-890	Arrington[z]	JH Hale[z]	Sugar Giant
A-766	A-821[y]	A-858	A-891	Autumn Prince	KV175	Sugar Lady
A-768	A-822[y]	A-859[y]	A-892	Autumn Star	KV175	Sweet Star
A-770	A-824	A-860[y]	A-893	Bounty	KV357	Tango
A-772[z]	A-825[y]	A-861[y]	A-894	Bowden[y]	KV398	Tango-II
A-773[z]	A-826[y]	A-862[y]	A-895	Bradley[zy]	KV401	Westbrook[z]
A-776[z]	A-827[y]	A-864[y]	A-896	Challenger	KV501	White Cloud
A-778[z]	A-828[y]	A-865[y]	A-897	China Pearl[z]	KV601	White County[zy]
A-783[z]	A-829[y]	A-866[y]	A-898	Chinese Cling[z]	KV606	White Diamond[y]
A-786[y]	A-830	A-867[y]	A-899	Contender	KV701	White Lady
A-789[z]	A-832[y]	A-868[y]	A-900	Cresthaven	KV801	White River[z]
A-790	A-833[y]	A-869[y]	A-901	Crimson Lady	Loring	White Rock[y]
A-792[y]	A-836[y]	A-870[y]	A-902	Crimson Snow	Manon	Winblo[z]
A-794	A-837[y]	A-871[y]	A-903	Cumberland[z]	Messina	Yumm Yumm
A-797[y]	A-839	A-872[y]	A-904	CVN13w	Orange Cling[z]	
A-798	A-840[y]	A-873[y]	A-905	Denman	Peento[z]	
A-799	A-841[y]	A-874[y]	A-906	Dixon[z]	PF 1	
A-801[y]	A-842[y]	A-875[y]	A-907	Early Crawford[z]	PF 24-007	
A-803	A-843[y]	A-876[y]	A-908	Early Star	PF 24C	
A-804[y]	A-844[y]	A-877[y]	A-909	Eastern Glo	PF 5B	

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 3. Additional 2009 and 2010 F_1 seedling populations phenotyped in 2014, and genotyped in 2014-2015 (N is number of individuals analyzed).

F_1 population	Female parent	Male parent	F_1 seedlings (N)
AR_Pop_1001	A-665	A-800	38
AR_Pop_1002	A-760	A-708	37
AR_Pop_1003	White Diamond	A-760	43
AR_Pop_1004	A-753	Souvenirs	27
AR_Pop_1006	White County	Souvenirs	41
AR_Pop_1007	A-775	Souvenirs	34
AR_Pop_1008	A-746	A-785	13
AR_Pop_1009	A-746	A-823	17
AR_Pop_1011	A-786	A-773	66
AR_Pop_1012	A-773	A-774	33
AR_Pop_1013	A-772	A-774	28
AR_Pop_1014	A-685	A-773	10
AR_Pop_1015	A-789N	A-803CN	25
AR_Pop_1016	A-807CN	A-802CN	5
AR_Pop_1018	Bowden	A-761N	31
AR_Pop_1019	A-779CN	A-776CN	20
AR_Pop_1020	Bowden	A-758CN	33
AR_Pop_1021	A-778N	A-777CN	29
AR_Pop_1022	Amoore Sweet	A-779CN	26
AR_Pop_1024	A-757N	A-807CN	20
AR_Pop_1025	A-770CN	A-768N	6
AR_Pop_1026	A-816CN	A-772	31

Leaf Collection and DNA Extraction

In the spring of 2013, approximately 50 mg of fresh, young leaf tissue was harvested in individual 1.5 mL tubes (Eppendorf, Hauppauge, NY) from all seedlings in the seven UA RosBREED F_1 populations as well as all UA cultivars and selections in the "conversion set" (243 individuals). Additionally, the remaining UA cultivars and selections, and an array of germplasm from different breeding programs were collected (127 individuals). While collecting tissue, each F_1 seedling was labeled with a metal tag in order to correctly match phenotypic and genotypic data later. Tissue was refrigerated during transportation, and then stored at -80 °C until needed. Two 4 mm stainless steel beads (McGuire Bearing Company, Salem, OR) were placed into each 1.5 mL tube, and DNA was extracted following a modified Dellaporta (1983) protocol with specific adaptations for peach. DNA quantity and quality were measured using a spectrophotometer (NanoDrop 2000, NanoDrop Technologies, Wilmington, DE) and confirmed by electrophoresis on 1% TBE (1 M Tris, 0.9 M boric acid, and 0.01 M EDTA) agarose gel. Final dilutions of 25 ng/µl were created for genotyping.

In the spring of 2014, approximately 50 mg of fresh young leaf tissue was harvested into coin envelopes for all 569 seedlings from 20 F_1 populations (2010 crosses), ranging from 5-49 seedlings per populations ("confirmation set"). While collecting tissue, each F_1 seedling was labeled with a metal tag in order to correctly match phenotypic and genotypic data later. Tissue was refrigerated during transportation, and then lyophilized (Freezone® 12 model 77540, Labconco Corporation, Kansas City, MO). Lyophilized plant tissue was then loaded into a 96 1.1 ml MicroTube Rack System (BioExpress, Kaysville, UT) containing approximately 0.25 g of technical grade 40, 6-12 mesh silica gel beads (Sigma Aldrich, St. Louis, MO). All DNA was

extracted following a high-throughput and cost efficient extraction protocol developed for *Prunus* species by Edge-Garza et al., (2014).

PpCCD4b-SSR Design and Genotyping

The original primer sequences for the PpCCD4-SSR (Falchi et al., 2013) were re-designed to increase GC-content (guanine and cytosine nucleotides) to make the PCR reaction more stable, thus the marker was re-termed PpCCD4b-SSR (Table 4). The same primer design process as described in Stegmeir et al., (2014) was followed, utilizing Primer3Plus software (Untergasser et al., 2012; www.primer3plus.com). Subsequently, the NCBI BLAST on the *P. persica* genome sequence v1.0 website (www.rosaceae.org/tools/ncbi_blast) was used to confirm specific amplification of the re-designed primer pairs.

Table 4. The PpCCD4b-SSR DNA test forward and reverse primer sequences and physical locations on scaffold 1 (Tm = Melting temperature) [primer locations were sourced from the Genome Database for Rosaceae (Jung et al., 2014)].

Name	Physical location (bp)	GC content	Tm	Sequence
F	25,639,168	55%	56.1 °C	CAACCAACTGATCCCACACC
R	25,638,937	55%	56.9 °C	GTTTTGAAGCTGGTGGTGGG

Extracted DNA was amplified with the PpCCD4b-SSR at the UA Horticulture Molecular Breeding Laboratory. Polymerase chain reaction (PCR) was performed for all samples in a final total volume of 10.0 µl containing 1.0 µl of DNA (~25 ng/µl), 4.0 µl of ultrapure molecular grade water [AccuGENE™ (Lonza Inc., Allendale, NJ)], 2.0 µl of Taq PCR buffer [×5 GoTaq® buffer (Promega Corp., Madison, WI)], 0.6 µl of $MgCl_2$ [25mM (Promega)], 0.2 µl deoxyribonucleotide triphosphates (dNTPs) [10 mM (Promega)], 0.5 µl of each primer (forward, reverse one and reverse two) [10mM (Integrated DNA Technologies, Coralville, IA)], 0.2 µl of Taq DNA polymerase (5U/µl) [GoTaq® (Promega Corp.)]. The PCR amplifications were

performed in a BIORAD T100 thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA) under the following conditions: 5 min of initial denaturation at 95 °C followed by 35 cycles of 95 °C for 45 s, 60 °C for 45 s, 72 °C for 1.5 min, and then, a final extension step at 72 °C for 7 min.

The PCR reactions of all the individuals included in this study were resolved utilizing two different capillary gel electrophoresis machines: a Fragment Analyzer™, model AdvanCE FS96 (Advanced Analytical Technologies, Inc., Ames, IA) from the wheat breeding laboratory at UA, and an ABI Prism 3730xl DNA Analyzer (Applied Biosystems by Life Technologies, Grand Island, NY) at the fruit breeding laboratory at Washington State University. Results from the Fragment Analyzer™ were analyzed through PROSize® v.1 software (Advanced Analytical Technologies, Inc., Ames, IA), while results from the ABI Prism 3730xl were scored through GeneMarker® software (SoftGenetics, LLC, State College, PA).

SNP Haplotypes to PpCCD4b-SSR DNA Test Conversion

The UA RosBREED "conversion set" evaluated in 2011-2013 was utilized to convert the SNP haplotypes (see Chapter Four of dissertation) to the PpCCD4b-SSR DNA test. Although the "conversion set" consisted of 243 individuals, only those that were screened with the IPSC 9K peach SNP array v1.0 were first considered for haplotype construction (175 individuals). A total of 11 informative SNP markers were used for haplotype construction (see Chapter Four of dissertation). The GBrowse tool on the Genome Database for Rosaceae (Jung et al., 2014; https://www.rosaceae.org/gb/gbrowse/prunus_persica/) was utilized to identify the precise location of the 11 informative SNP markers that spanned the previously identified QTL for white vs. yellow flesh at the *Y-locus* (23,352,245-27,368,782 bp) on scaffold 1 of the *Prunus persica* genome sequence v1.0 (Martínez-García et al., 2013; Verde et al. 2013) (Fig. 3). FlexQTL™ software was used to construct the initial functional SNP haplotypes that were subsequently

manually confirmed based on inheritance analysis in bi-parental families (Bink, 2004; Bink, 2005; Bink et al., 2008; Bink et al., 2012).

Next, to make direct comparisons between each individual's SNP haplotypes and their PpCCD4b-SSR genotypes, all functional SNP haplotypes were divided into two simple functional haplotype groups defined as functional allele 'W' (white flesh allele) and 'y' (yellow flesh allele). Only simple functional haplotype groups were developed for the 68 individuals screened with the peach mini SNP array v1.0, since less SNP markers were utilized, which made it necessary to compare each individual's phenotypic data to accurately determine the correct simple functional haplotype group (see Chapter Four for more details).

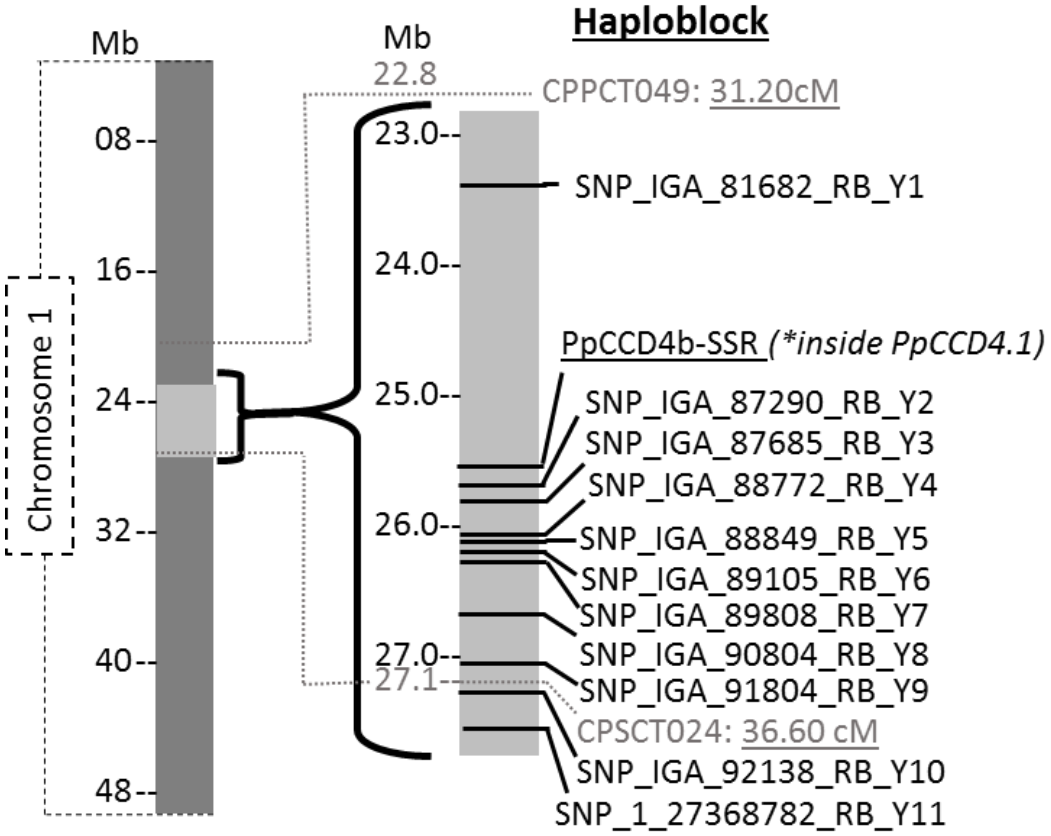

Fig. 3. Graphical representation of *P. persica* chromosome 1 with the enlarged location of the ~4 Mbp flanking the *PpCCD4.1* candidate gene, including the 11 SNP markers used for haplotyping and the intragenic PpCCD4b-SSR DNA test. Physical locations were sourced from the *P. persica* whole genome v1.0 sequence (Verde et al., 2013) housed on the Genome Database for Rosaceae (Jung et al., 2014). Two markers within or just flanking the *Y-locus* SNP haploblock and their estimated genetic positions based on the Prunus-TE-F_2 reference map are included in gray.

PpCCD4b-SSR DNA Test Genotype Effects

The chi-square (X^2) goodness-of-fit test was performed for all F_1 populations of the "conversion set" and the "confirmation set" to determine if the seedlings' observed PpCCD4b-SSR DNA test genotypes and white vs. yellow flesh phenotype ratios fit the expected segregation ratio patterns of their parents ($p > 0.05$). A pedigree allele tracking approach was used to determine parental genotypes for populations whose parents had previously been discarded from the program and thus, were not screened with the PpCCD4b-SSR. In this case, the parent alleles were determined based on their grandparent alleles to trace the identity of each allele. Process of elimination was used for parents whose alleles still could not be determined. In this scenario the F_1 progeny segregation ratio and the other parents' genotype were used to determine what the unknown parents' genotype should be. Lastly, process of elimination was used for parents whose alleles still could not be determined. In this case, the F1 progeny segregation ratio and the other parent's genotype were used to determine what the unknown parent's genotype should be.

Results

Phenotypic Data

All individuals phenotyped were rated as white (0) or yellow (1) flesh. The UA RosBREED pedigree consisted of nine white flesh parents and five yellow flesh parents (Table 5). All progeny in AR_Pop_0803, and AR_Pop_0825 were yellow flesh. Two populations showed ~3:1 white to yellow flesh: AR_Pop_1 (38:10) and AR_Pop_0801 (11:4). The AR_Pop_0819 exhibited ~1:1 white to yellow flesh (10:13) (Table 5). All nine seedlings in AR_Pop_0817 were white flesh, and AR_Pop_0813 showed eight white flesh and one yellow

flesh seedling (Table 5). The 105 UA cultivars and selections in the "conversion set" consisted of 55 white and 50 yellow flesh individuals (Tables 6-7). Of the remaining UA cultivars, selections (69), and the array of germplasm from different breeding programs (58), not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0, 60 were white flesh, and 67 were yellow flesh (Table 8). The 20 F_1 populations from the 2010 "confirmation set" consisted of 27 white, and 13 yellow flesh parents (Table 9). All progeny in AR_Pop_1001, AR_Pop_1015, and AR_Pop_1026 were white flesh. All progeny in AR_Pop_1002 were yellow flesh. Progeny in AR_Pop_1011, AR_Pop_1012, AR_Pop_1013, AR_Pop_1014, and AR_Pop_25 contained ~3:1 white to yellow flesh. Progeny in AR_Pop_1003, AR_Pop_1006, AR_Pop_1007, AR_Pop_1008, AR_Pop_1016, AR_Pop_1018, AR_Pop_1019, AR_Pop_1020, AR_Pop_1021, AR_Pop_1022, and AR_Pop_1024 contained ~1:1 white to yellow flesh (Table 9).

Table 5. The CCD4b-SSR DNA test functional genotypes and white (W) or yellow (y) flesh phenotypes and genotypes for parents of the seven F_1 UA RosBREED populations (2011-2013) (N is number of samples analyzed) [*The chi-square p-value in brackets].

F_1 population	Progeny (N)	Parents		Phenotype	Functional genotype	F_1 progeny matching phenotype and genotype segregation ratio
AR_Pop_1	48	Female	White County	White	W | y	12 W | W : 26 W | y : 10 y | y [*0.78]
		Male	A-672	White	W | y	
AR_Pop_0801	15	Female	A-776	White	W | y	4 W | W : 7 W | y : 4 y | y [*0.97]
		Male	A-783	White	W | y	
AR_Pop_0803	12	Female	Amoore Sweet	Yellow	y | y	12 y | y [*1.00]
		Male	A-778	Yellow	y | y	
AR_Pop_0813	9	Female	A-772	White	W | y	5 W | W : 3 W | y : 1 y | y [*0.10]
		Male	A-672	White	W | y	
AR_Pop_0817	9	Female	A-789	White	W | W	3 W | W : 6 W | y [*0.32]
		Male	A-699	White	W | y	
AR_Pop_0819	23	Female	A-708	Yellow	y | y	10 W | y : 13 y | y [*0.53]
		Male	A-773	White	W | y	
AR_Pop_0825	17	Female	Souvenirs	Yellow	y | y	17 y | y [*1.00]
		Male	A-760	Yellow	y | y	

Table 6. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes [white (W) vs. yellow (y) flesh] (2011-2014) for cultivars and selections in the UA RosBREED pedigree ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct SNP haplotype follows with ***( ).**

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
A-657[z]	y6a | y6a	y | y	231 | 231	y | y	Yellow
A-663[z]	y6a | y6a	y | y	231 | 231	y | y	Yellow
A-665[zy]	**Y1a | y2*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
A-672[zy]	y6a | Y2	W | y	229 | 231	W | y	White
A-699[z]	Y1a | y6a	W | y	229 | 231	W | y	White
A-708[z]	y6a | y6a	y | y	231 | 231	y | y	Yellow
A-716[z]	y6a | y6a	y | y	231 | 231	y | y	Yellow
A-760[z]	y6b | y6a	y | y	231 | 231	y | y	Yellow
A-772[z]	y6a | Y1a	W | y	229 | 231	W | y	White
A-773[z]	Y1b | y6a	W | y	-	-	White
A-776[z]	Y1a | y6a	W | y	-	-	White
A-778[z]	y6a | y6a	y | y	231 | 231	y | y	Yellow
A-783[z]	Y1a | y6a	W | y	229 | 231	W | y	White
A-789[z]	**y6a*(Y) | Y2**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
Admiral Dewey[z]	y6b | y6b	y | y	231 | 231	y | y	Yellow

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 6. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) (2011-2014) for cultivars and selections in the UA RosBREED pedigree ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
Amoore Sweet[z]	y6a | y6a	y | y	231 | 231	y | y	Yellow
Arrington[z]	y6a | y6a	y | y	231 | 231	y | y	Yellow
Bradley[zy]	y6a | y6a	y | y	231 | 231	y | y	Yellow
Chinapearl[z]	**y4*(Y) | Y3**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
Chinese Cling[z]	Y2 | y3	W | y	229 | 231	W | y	White
Cumberland[z]	**Y2 | y6b*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
Dixon[z]	y6a | y6b	y | y	231 | 231	y | y	Yellow
Early Crawford[z]	y6a | y6b	y | y	231 | 231	y | y	Yellow
Elberta[z]	y3 | y6a	y | y	229 | 231	W | y	Yellow
Georgia Belle[z]	**y3*(Y) | y6b*(Y)**	**y*(W) | y*(W)**	**229 | 229**	**W | W**	**White**
Greensboro[z]	Y2 | y6b	W | y	-	-	White
Jefferson[z]	y6a | y6a	y | y	231 | 231	y | y	Yellow
JH Hale[z]	y6a | y6a	y | y	231 | 231	y | y	Yellow
Orange Cling[z]	y6a | y6a	y | y	231 | 231	y | y	Yellow

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 6. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) (2011-2014) for cultivars and selections in the UA RosBREED pedigree ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
Peento[z]	**y6a | Y4**	**W | y(*W)**	**229 | 229**	**W | W**	**White**
Redskin[z]	y6b | y6a	y | y	231 | 231	y | y	Yellow
Slappey[z]	y3 | y3	y | y	231 | 231	y | y	Yellow
Souvenirs[z]	y6a | y6a	y | y	231 | 231	y | y	Yellow
Westbrook[z]	y6a | y6b	y | y	231 | 231	y | y	Yellow
White County[zy]	**y6a*(Y) | y6a**	**y*(W) | y**	**229 | 231**	**W | y**	**White**
White River[z]	**y6a*(Y) | y6a**	**W | y**	**229 | 231**	**W | y**	**White**
Winblo[z]	y4 | y6b	y | y	231 | 231	y | y	Yellow

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 7. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) (2013-2014) for the 72 individuals screened with the Peach SNP mini array v1.0 ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).**

Individual	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
A-665[zy]	**W | y*(W)**	**229 | 229**	**W | W**	**White**
A-672[zy]	W | y	229 | 231	W | y	White
A-786[y]	**y | y*(W)**	**229 | 231**	**W | y**	**White**
A-792[y]	W | y	229 | 231	W | y	White
A-797[y]	W | y	229 | 231	W | y	White
A-801[y]	W | y	229 | 231	W | y	White
A-804[y]	W | y	229 | 231	W | y	White
A-805[y]	W | y	229 | 231	W | y	White
A-806[y]	y | y	231 | 231	y | y	Yellow
A-809[y]	y | y	231 | 231	y | y	Yellow
A-811[y]	y | y	231 | 231	y | y	Yellow
A-813[y]	W | y	229 | 231	W | y	White
A-816[y]	W | W	229 | 229	W | W	White
A-818[y]	W | y	229 | 231	W | y	White
A-819[y]	y | y	231 | 231	y | y	Yellow
A-820[y]	y | y	231 | 231	y | y	White
A-821[y]	**y | y*(W)**	**229 | 231**	**W | y**	**White**
A-822[y]	W | y	229 | 231	W | y	White
A-825[y]	W | y	229 | 231	W | y	White
A-826[y]	y | y	231 | 231	y | y	Yellow
A-827[y]	y | y	231 | 231	y | y	Yellow
A-828[y]	y | y	231 | 231	y | y	Yellow
A-829[y]	W | y	229 | 231	W | y	White
A-832[y]	y | y	231 | 231	y | y	Yellow
A-833[y]	y | y	231 | 231	y | y	Yellow
A-836[y]	y | y	231 | 231	y | y	Yellow

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 7. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) (2013-2014) for the 72 individuals screened with the Peach SNP mini array v1.0 ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).** (Cont.).

Individual	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
A-837[y]	y | y	231 | 231	y | y	Yellow
A-840[y]	W | y	229 | 231	W | y	White
A-841[y]	W | y	229 | 231	W | y	White
A-842[y]	y | y	231 | 231	y | y	Yellow
A-843[y]	W | y	229 | 231	W | y	White
A-844[y]	**y | y*(W)**	**229 | 231**	**W | y**	**White**
A-845[y]	**y | y*(W)**	**229 | 231**	**W | y**	**White**
A-848[y]	y | y	231 | 231	y | y	Yellow
A-849[y]	y | y	231 | 231	y | y	Yellow
A-850[y]	**y | y*(W)**	**229 | 231**	**W | y**	**White**
A-851[y]	y | y	231 | 231	y | y	Yellow
A-852[y]	y | y	231 | 231	y | y	Yellow
A-853[y]	**y | y*(W)**	**229 | 231**	**W | y**	**White**
A-854[y]	y | y	231 | 231	y | y	Yellow
A-855[y]	**W | W*(y)**	**229 | 231**	**W | y**	**White**
A-856[y]	**y | y*(W)**	**229 | 231**	**W | y**	**White**
A-857[y]	y | y	231 | 231	y | y	Yellow
A-859[y]	**y | y*(W)**	**229 | 231**	**W | y**	**White**
A-860[y]	**y | y*(W)**	**229 | 231**	**W | y**	**White**
A-861[y]	-	229 | 231	W | y	White
A-862[y]	y | y	231 | 231	y | y	Yellow
A-864[y]	y | y	231 | 231	y | y	Yellow
A-865[y]	**y | y*(W)**	**229 | 231**	**W | y**	**White**
A-866[y]	y | y	231 | 231	y | y	Yellow

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 7. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) (2013-2014) for the 72 individuals screened with the Peach SNP mini array v1.0 ("conversion set"). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).** (Cont.).

Individual	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
A-867[y]	y | y	y | y	y | y	Yellow
A-868[y]	y | y	y | y	y | y	Yellow
A-869[y]	y | y	y | y	y | y	Yellow
A-870[y]	W | y	W | y	W | y	White
A-871[y]	W | y	W | y	W | y	White
A-872[y]	**y | y*(W)**	**W | y**	**W | y**	**White**
A-873[y]	y | y	y | y	y | y	Yellow
A-874[y]	W | y	W | W	W | W	White
A-875[y]	y | y	y | y	y | y	Yellow
A-876[y]	W | y	W | y	W | y	White
A-877[y]	-	W | W	W | W	White
A-878[y]	**y | y*(W)**	**W | y**	**W | y**	**White**
A-879[y]	y | y	y | y	y | y	Yellow
A-880[y]	**y | y*(W)**	**W | y**	**W | y**	**White**
A-881[y]	y | y	y | y	y | y	Yellow
A-882[y]	**y | y*(W)**	**W | y**	**W | y**	**White**
Bowden[y]	W | y	W | y	W | y	White
Bradley[zy]	y | y	y | y	y | y	Yellow
Gloria[y]	y | y	y | y	y | y	Yellow
White County[zy]	**y | y*(W)**	**W | y**	**W | y**	**White**
White Diamond[y]	**y | y*(W)**	**W | y**	**W | y**	**White**
White Rock[y]	W | W	W | W	W | W	White

[z] Genotyped with the IPSC 9K peach SNP array v1.0.
[y] Genotyped with the Peach mini SNP array v1.0.

Table 8. The PpCCD4b-SSR DNA test genotypes and white or yellow flesh phenotypes of UA selections and cultivars (not screened with the IPSC 9K peach SNP array v1.0, or Peach mini SNP array v1.0) and germplasm from other breeding programs, years 2013-2014.

Accession	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
A-554	229 | 231	W | y	White
A-662	231 | 231	y | y	Yellow
A-668	231 | 231	y | y	Yellow
A-743	231 | 231	y | y	Yellow
A-758	231 | 231	y | y	Yellow
A-761	231 | 231	y | y	Yellow
A-766	231 | 231	y | y	Yellow
A-768	229 | 231	W | y	White
A-770	229 | 231	W | y	White
A-790	231 | 231	y | y	Yellow
A-794	229 | 231	W | y	White
A-798	229 | 231	W | y	White
A-799	229 | 231	W | y	White
A-803	231 | 231	y | y	Yellow
A-808	-	-	White
A-810	231 | 231	y | y	Yellow
A-814	231 | 231	y | y	Yellow
A-815	229 | 231	W | y	White
A-824	231 | 231	y | y	Yellow
A-830	231 | 231	y | y	Yellow
A-839	229 | 231	W | y	White
A-846	231 | 231	y | y	Yellow
A-847	229 | 229	W | W	White
A-858	231 | 231	y | y	Yellow
A-883	231 | 231	y | y	Yellow
A-884	-	-	White
A-885	229 | 231	W | y	White
A-886	-	-	White
A-887	229 | 229	W | W	White
A-888	229 | 229	W | W	White
A-889	231 | 231	y | y	Yellow

Table 8. The CCD4b-SSR DNA test genotypes and white or yellow flesh phenotypes for UA selections and cultivars and germplasm from other breeding programs, years 2013-2014. (Cont.).

Accession	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
A-890	229 | 229	W | W	White
A-891	229 | 231	W | y	White
A-892	229 | 231	W | y	White
A-893	229 | 229	W | W	White
A-894	231 | 231	y | y	Yellow
A-895	-	-	White
A-896	-	-	White
A-897	231 | 231	y | y	Yellow
A-898	229 | 231	W | y	White
A-899	231 | 231	y | y	Yellow
A-900	229 | 231	W | y	White
A-901	229 | 231	W | y	White
A-902	231 | 231	y | y	Yellow
A-903	229 | 231	W | y	White
A-904	229 | 231	W | y	White
A-905	229 | 231	W | y	White
A-906	229 | 231	W | y	White
A-907	-	-	White
A-908	229 | 231	W | y	White
A-909	231 | 231	y | y	Yellow
A-910	229 | 231	W | y	White
A-911	229 | 231	W | y	White
A-912	229 | 231	W | y	White
A-913	229 | 231	W | y	White
A-914	229 | 231	W | y	White
A-915	229 | 231	W | y	White
A-916	229 | 231	W | y	White
A-917	229 | 231	W | y	White
A-918	229 | 231	W | y	White

Table 8. The CCD4b-SSR DNA test genotypes and white or yellow flesh phenotypes for UA selections and cultivars and germplasm from other breeding programs, years 2013-2014. (Cont.).

Accession	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
Admiral Dewey	231 | 231	y | y	Yellow
Allgold	231 | 231	y | y	Yellow
Autumn Prince	231 | 231	y | y	Yellow
Autumn Star	231 | 231	y | y	Yellow
Bounty	231 | 231	y | y	Yellow
Challenger	231 | 231	y | y	Yellow
China Pearl	229 | 229	W | W	White
Chinese Cling	229 | 229	W | W	White
Contender	231 | 231	y | y	Yellow
Cresthaven	231 | 231	y | y	Yellow
Crimson Lady	231 | 231	y | y	Yellow
Crimson Snow	229 | 231	W | y	White
Cumberland	229 | 229	W | W	White
CVN13w	229 | 229	W | W	White
Denman	231 | 231	y | y	Yellow
Early Star	231 | 231	y | y	Yellow
Eastern Glo	231 | 231	y | y	Yellow
Elberta	231 | 231	y | y	Yellow
Emeraude	-	-	White
Flavortop	231 | 231	y | y	Yellow
Georgia Belle	229 | 229	W | W	White
Gladiator	231 | 231	y | y	Yellow
Goldilocks	231 | 231	y | y	Yellow
Goldjim	231 | 231	y | y	Yellow
Goldnine	231 | 231	y | y	Yellow
Greensboro	229 | 231	W | y	White
Jade	-	-	White
Jefferson	231 | 231	y | y	Yellow
JH Hale	231 | 231	y | y	Yellow
KV175	231 | 231	y | y	Yellow

Table 8. The CCD4b-SSR DNA test genotypes and white or yellow flesh phenotypes for UA selections and cultivars and germplasm from other breeding programs, years 2013-2014. (Cont.).

Accession	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
KV357	231 | 231	y | y	Yellow
KV398	229 | 231	W | y	White
KV401	231 | 231	y | y	Yellow
KV501	231 | 231	y | y	Yellow
KV601	231 | 231	y | y	Yellow
KV606	231 | 231	y | y	Yellow
KV701	231 | 231	y | y	Yellow
KV801	231 | 231	y | y	Yellow
Loring	231 | 231	y | y	Yellow
Manon	-	-	White
Messina	231 | 231	y | y	Yellow
Orange Cling	231 | 231	y | y	Yellow
Peento	229 | 229	W | W	White
PF 1	229 | 231	W | y	White
PF 24-007	231 | 231	y | y	Yellow
PF 24C	231 | 231	y | y	Yellow
PF 5B	231 | 231	y | y	Yellow
PF 5D Big	231 | 231	y | y	Yellow
PF Lucky 13	231 | 231	y | y	Yellow
PF-19-007	231 | 231	y | y	Yellow
Redhaven	231 | 231	y | y	Yellow
Rising Star	-	-	Yellow
Roygold	231 | 231	y | y	Yellow
Ruby Prince	231 | 231	y | y	Yellow
Saturn	231 | 231	y | y	Yellow
Slappey	229 | 229	W | W	White
Spring Snow	229 | 231	W | y	White
Sugar Giant	229 | 231	W | y	White
Sugar Lady	229 | 231	W | y	White
Sweet Star	231 | 231	y | y	Yellow
Tango	231 | 231	y | y	Yellow

Table 8. The CCD4b-SSR DNA test genotypes and white or yellow flesh phenotypes for UA selections and cultivars and germplasm from other breeding programs, years 2013-2014. (Cont.).

Accession	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
Tango-II	231 | 231	y | y	Yellow
Westbrook	231 | 231	y | y	Yellow
White Cloud	229 | 231	W | y	White
White Lady	229 | 231	W | y	White
White River	229 | 231	W | y	White
Yumm Yumm	229 | 231	W | y	White

Table 9. The PpCCD4b-SSR DNA test functional genotypes and white or yellow flesh phenotypes for parents of 20 F_1 UA 2010 populations (across 2013-2014) (N is number of samples analyzed) [*The chi-square p-value in brackets]. Genotypes in **bold** were determined based on progeny and other parents genotypes.

F_1 population	Progeny (N)	Parents		Phenotype	Functional genotype	F_1 progeny matching phenotype and genotype segregation ratio
AR_Pop_1001	38	Female	A-665	White	W | W	18 W | W : 20 W | y [*0.75]
		Male	A-800	White	W | y	
AR_Pop_1002	30	Female	A-760	Yellow	y | y	30 y | y [*1.00]
		Male	A-708	Yellow	y | y	
AR_Pop_1003	41	Female	White Diamond	White	W | y	18 W | y : 23 y | y [*0.44]
		Male	A-760	Yellow	y | y	
AR_Pop_1006	30	Female	White County	White	W | y	19 W | y : 11 y | y [*0.14]
		Male	Souvenirs	Yellow	y | y	
AR_Pop_1007	33	Female	**A-775**	White	**W | y**	21 W | y : 12 y | y [*0.12]
		Male	Souvenirs	Yellow	y | y	
AR_Pop_1008	12	Female	**A-746**	White	**W | y**	4 W | y : 8 y | y [*0.25]
		Male	**A-785**	Yellow	**y | y**	
AR_Pop_1011	49	Female	A-786	White	W | y	17 W | W : 23 W | y : 9 y | y [*0.25]
		Male	**A-773**	White	**W | y**	
AR_Pop_1012	30	Female	**A-773**	White	**W | y**	7 W | W : 14 W | y : 9 y | y [*0.75]
		Male	A-774	White	W | y	
AR_Pop_1013	28	Female	A-772	White	W | y	6 W | W : 11 W | y : 11 y | y [*0.22]
		Male	A-774	White	W | y	

Table 9. The PpCCD4b-SSR DNA test functional genotypes and white or yellow flesh phenotypes for parents of 20 F_1 UA 2010 populations (across 2013-2014) (N is number of samples analyzed) [*The chi-square p-value in brackets]. Genotypes in **bold** were determined based on progeny and other parents genotypes. (Cont.).

F_1 population	Progeny (N)	Parents		Phenotype	Functional genotype	F_1 progeny matching phenotype and genotype segregation ratio
AR_Pop_1014	10	Female	**A-685**	White	**W | y**	3 W | W : 4 W | y : 3 y | y [*0.82]
		Male	**A-773**	White	**W | y**	
AR_Pop_1015	22	Female	A-789N	White	W | W	22 W | y [*1.00]
		Male	A-803CN	Yellow	y | y	
AR_Pop_1016	5	Female	A-807CN	White	y | y	4 W | y : 1 y | y [*0.18]
		Male	**A-802CN**	Yellow	**W | y**	
AR_Pop_1018	25	Female	Bowden	White	W | y	15 W | y : 10 y | y [*0.32]
		Male	A-761N	Yellow	y | y	
AR_Pop_1019	15	Female	A-779CN	White	W | y	9 W | y : 6 y | y [*0.44]
		Male	A-776CN	White	y | y	
AR_Pop_1020	30	Female	Bowden	White	W | y	13 W | y : 17 y | y [*0.47]
		Male	A-758CN	Yellow	y | y	
AR_Pop_1021	29	Female	A-778N	Yellow	y | y	11 W | y : 18 y | y [*0.19]
		Male	**A-777CN**	White	**W | y**	
AR_Pop_1022	20	Female	Amoore Sweet	Yellow	y | y	6 W | y : 14 y | y [*0.07]
		Male	A-779CN	White	W | y	
AR_Pop_1024	13	Female	**A-757N**	Yellow	**W | y**	4 W | y : 9 y | y [*0.17]
		Female	**A-807CN**	White	**y | y**	
AR_Pop_1025	6	Male	A-770CN	White	W | y	2 W | W : 3 W | y : 3 y | y [*0.69]
		Female	A-768N	White	W | y	
AR_Pop_1026	16	Male	A-816CN	White	W | W	7 W | W : 9 W | y [*0.62]
		Female	A-772	White	W | y	

PpCCD4b-SSR DNA Test Genotyping

Genetic screeing for the the PpCCD4b-SSR DNA test was successful across all individuals in the study, except for a negligible sample failure rate of ~5%. Only three genotypes were observed across all material: homozygous dominant white flesh (W | W), heterozygous white flesh (W | y), and homozygous recessive yellow flesh (y | y). Representative genotypes for the PpCCD4b-SSR DNA test using the Fragment Analyzer™ PROSize® v.1 software were consistently revealed (Figs. 4-6). Using this platform the white flesh allele (W) was consistently between ~225-235 bp and the yellow flesh allele (y) was consistently between ~240-250 bp. Representative genotypes for the PpCCD4b-SSR DNA test using the ABI GeneMarker® software were revealed (Figs. 7-9). Using this platform the white flesh allele (W) was consistently ~229 bp and the yellow flesh allele (y) was consistently ~231 bp.

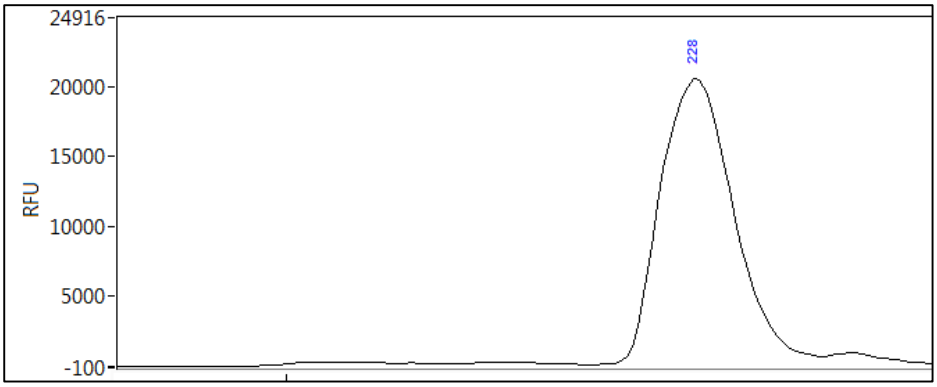

Fig. 4. Representative fragment analysis output of a homozygous white flesh individual (W | W), amplifying a peak of 228 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

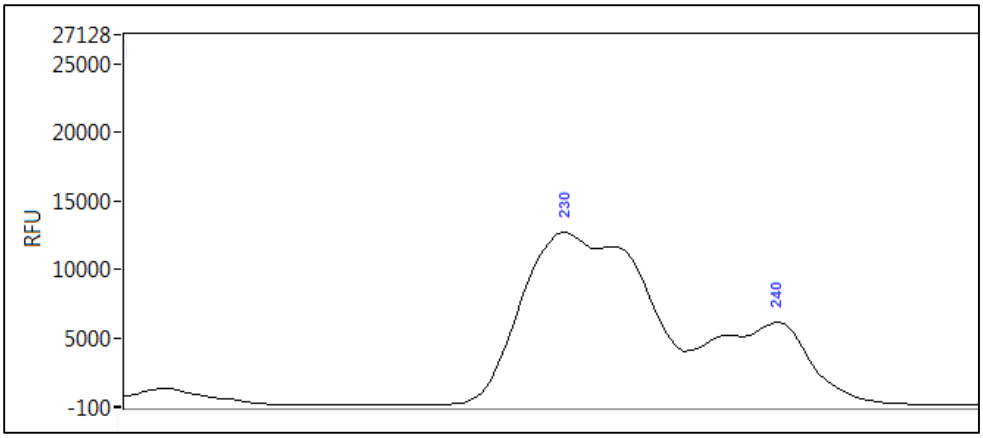

Fig. 5. Representative fragment analysis output of a heterozygous white flesh individual (W | y), individual, amplifying peaks of 230 and 240 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

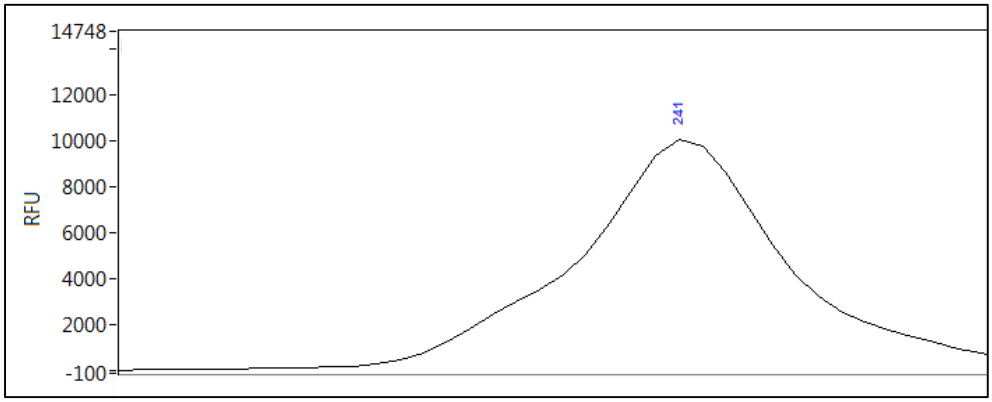

Fig. 6. Representative fragment analysis output of a yellow flesh individual (y | y), amplifying a peak of 241 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

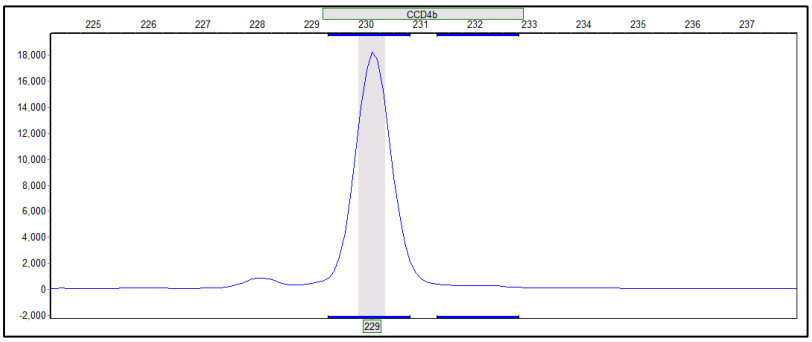

Fig. 7. Representative ABI output of a homozygous white flesh individual (W | W), amplifying a peak of 229 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU) (y-axis).

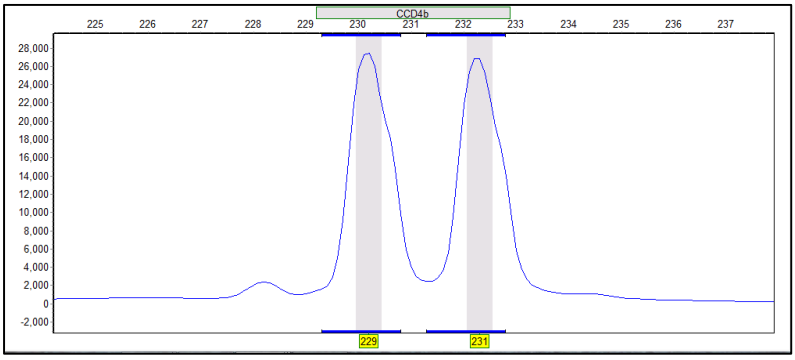

Fig. 8. Representative ABI output of a heterozygous white flesh individual (W | y), amplifying peaks of 229 and 231 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU) (y-axis).

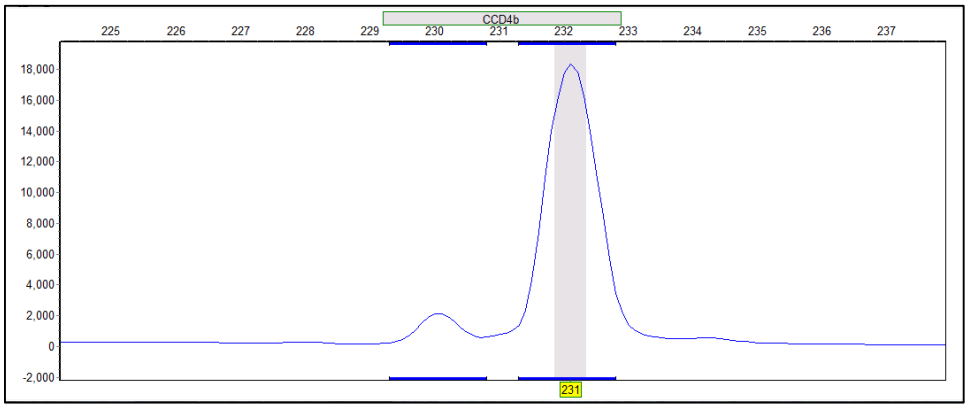

Fig. 9. Representative ABI output of a yellow flesh individual (y | y), amplifying a peak of 231 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU) (y-axis).

PpCCD4b-SSR DNA Test “Conversion Set”

The PpCCD4b-SSR DNA test reults of the F_1 seedlings alleles and matching phenotypes for each UA RosBREED population in the “conversion set” fit their expected parental segregation ratios (Table 5). For AR_Pop_1, the female parent ‘White County’ and the male parent A-672 were both heterozygous white flesh (W | y), and their F_1 progeny alleles and matching phenotypes (12 W | W : 26 W | y : 10 y | y) fit the expected segregation ratio ($p = 0.78$). The remaining populations’ chi-square p-values ranged from 1.00 (AR_Pop_0825, AR_Pop_0803) to 0.10 (AR_Pop_0813) (Table 5). Additionally, the 105 UA cultivars and selections in the “conversion set” consisted of 10 individuals homozygous white flesh (W | W), 41 heterozygous white flesh (W | y), 51 homozygous yellow flesh (y | y), and three individuals did not amplify (Tables 6-7).

SNP Haplotype DNA Test Development

Functional SNP haplotypes were successfully developed for 174 out of the 175 individuals from the UA RosBREED “conversion set,” that were screened with the IPSC 9K peach SNP array (Tables 6, 10, and 11). Of these 174 individuals, eight unique haplotypes were observed, and subseqently converted into functional haplotypes groups as previously described in the Materials and Methods (Table 10). All functional SNP haplotypes were additionally divided into two simple functional haplotype groups defined functional allele ‘W’ (white flesh allele) and ‘y’ (yellow flesh allele). In total, the frequencies for each unique functional haplotype ranged ≤ 1% for y2, y4, Y3, and Y4, to 67% for haplotype y6a. In selected germplasm (germplasm selected prior in seedling populations in routine breeding field selection activities) the haplotype frequencies for 37 individuals ranged from ≤ 1% for y2, Y1b, Y3 and Y4, to 55%

for y6a. In un-selected germplasm (F_1 seedlings, not yet selected) the haplotype frequencies for 137 individuals ranged from 0% for y2, y3, y4, y5, Y3 and Y4 to 70% for y6a (Table 10).

Table 10. The functional and simple functional 11-SNP haplotypes for distinguishing among 174 total white (W) or yellow (y) flesh individuals (Un-selected = 137 F1 seedlings) (Selected = 37 cultivars and selections) in the UA RosBREED pedigree ("conversion set") screened with the 9K array (N is number of haplotypes analyzed).

11-SNP haplotype			Total		Selected		Un-selected	
Sequence	Functional	Simple functional	N=348	Frequency	N=74	Frequency[z]	N=274	Frequency[y]
ABAAABABAAA	y1	y	0	0.00	0	0.00	0.00	0.00
ABAAABBABBB	y2	y	1	0.00	1	0.01	0.00	0.00
AAABBBBAAAA	y3	y	6	0.02	6	0.08	0.00	0.00
AAABBBBBAAA	y4	y	3	0.01	3	0.04	0.00	0.00
AAABBAABAAA	y5	y	0	0.00	0	0.00	0.00	0.00
AABBBAABAAA	y6a	y	233	0.67	40	0.55	193	0.7
ABBBBAABAAA	y6b	y	21	0.06	11	0.15	10	0.04
BBBBBAABAAB	Y1a	W	35	0.10	5	0.07	30	0.11
BBBBBAABAAA	Y1b	W	16	0.05	1	0.01	15	0.05
BABBBAAABBB	Y2	W	31	0.09	5	0.07	26	0.10
BBBBBBBAABB	Y3	W	1	0.00	1	0.01	0.00	0.00
BABBBAAAAAA	Y4	W	1	0.00	1	0.01	0.00	0.00

[z]Frequency of haplotypes in UA ("conversion set") selected germplasm (i.e. cultivars or selections).
[y]Frequency of haplotypes in UA ("conversion set") un-selected germplasm (i.e. seedlings).

Table 11. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) for the seven F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).**

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
AR_Pop_1_01	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_1_02	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_03	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_1_04	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_05	y6a | >y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_1_06	y6a | Y2	W | y	229 | 231	W | y	White
AR_Pop_1_07	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_08	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_1_09	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_10	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_11	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_12	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_14	**y6a*(Y) | y6a*(Y)**	**y*(W) | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_1_15	**y6a*(Y) | Y2**	**y*(W) | W**	**229 | 229**	**W | W**	**White**
AR_Pop_1_17	y6a | Y2	W | y	229 | 231	W | y	White
AR_Pop_1_18	y6a | Y2	W | y	229 | 231	W | y	White
AR_Pop_1_19	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_1_20	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_21	**y6a*(Y) | Y2**	**y*(W) | W**	**229 | 229**	**W | W**	**White**
AR_Pop_1_22	y6a | y6a>	y | y	231 | 231	y | y	Yellow
AR_Pop_1_23	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**

> Marks recombination which was observed in an individuals SNP haplotype. The side of the haplotype which the > is on represents approximately where the recombination occurred.

Table 11. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) for the seven F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
AR_Pop_1_24	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_25	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_26	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_27	**y6a*(Y) | Y2**	**y*(W) | W**	**229 | 229**	**W | W**	**White**
AR_Pop_1_28	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_1_29	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_30	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_31	**y6a*(Y) | Y2**	**y*(W) | W**	**229 | 229**	**W | W**	**White**
AR_Pop_1_32	**y6a*(Y) | Y2**	**y*(W) | W**	**229 | 229**	**W | W**	**White**
AR_Pop_1_33	y6a | Y2	W | y	229 | 231	W | y	White
AR_Pop_1_34	**y6a*(Y) | Y2**	**y*(W) | W**	**229 | 229**	**W | W**	**White**
AR_Pop_1_35	y6a | Y2	W | y	229 | 231	W | y	White
AR_Pop_1_36	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_1_37	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_1_38	**y6a*(Y) | Y2**	**y*(W) | W**	**229 | 229**	**W | W**	**White**
AR_Pop_1_39	y6a | Y2	W | y	229 | 231	W | y	White
AR_Pop_1_40	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_41	-	-	**229 | 231**	**W | y**	White
AR_Pop_1_42	**y6a*(Y) | Y2**	**y*(W) | W**	**229 | 229**	**W | W**	**White**
AR_Pop_1_43	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_44	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_1_45	y6a | Y2	W | y	229 | 231	W | y	White
AR_Pop_1_46	**y6a*(Y) | Y2**	**y*(W) | W**	**229 | 229**	**W | W**	**White**

Table 11. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) for the seven F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
AR_Pop_1_47	**y6a*(Y) | Y2**	**y*(W) | W**	**229 | 229**	**W | W**	**White**
AR_Pop_1_48	**y6a | y6a*(Y)**	**y | y*(W)**	**229 | 231**	**W | y**	**White**
AR_Pop_1_49	**y6a*(Y) | y6a*(Y)**	**y*(W) | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_0801_01	y6a | Y1a	W | y	229 | 231	W | y	White
AR_Pop_0801_02	y6a | Y1a	W | y	229 | 231	W | y	White
AR_Pop_0801_03	y6a | Y1a	W | y	229 | 231	W | y	White
AR_Pop_0801_04	Y1a | Y1a	W | W	229 | 229	W | W	White
AR_Pop_0801_05	Y1a | Y1a	W | W	229 | 229	W | W	White
AR_Pop_0801_06	y6a | Y1a	W | y	229 | 231	W | y	White
AR_Pop_0801_07	y6a | Y1a	W | y	229 | 231	W | y	White
AR_Pop_0801_08	y6a | Y1a	W | y	229 | 231	W | y	White
AR_Pop_0801_09	y6a | Y1a	W | y	229 | 231	W | y	White
AR_Pop_0801_10	Y1a | Y1a	W | W	229 | 229	W | W	White
AR_Pop_0801_11	y6a | >y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0801_12	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0801_13	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0801_14	Y1a | Y1a	W | W	229 | 229	W | W	White
AR_Pop_0801_15	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0801_16	Y1a | >Y1a	W | W	229 | 229	W | W	White
AR_Pop_0803_01	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0803_02	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0803_03	y6a | y6a	y | y	231 | 231	y | y	Yellow

> Marks recombination which was observed in an individuals SNP haplotype. The side of the haplotype which the > is on represents approximately where the recombination occurred.

Table 11. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) for the seven F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
AR_Pop_0803_04	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0803_05	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0803_06	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0803_07	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0803_09	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0803_10	y6a | y6a	y | y	-	-	-
AR_Pop_0803_11	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0803_12	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0803_13	y6a | y6a	y | y	-	-	Yellow
AR_Pop_0803_14	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0803_15	y6a | y6a	y | y	-	-	-
AR_Pop_0813_01	Y1a | Y2	W | W	229 | 229	W | W	White
AR_Pop_0813_02	Y1a | y6a	W | y	229 | 231	W | y	White
AR_Pop_0813_03	Y1a | Y2	W | W	229 | 229	W | W	White
AR_Pop_0813_04	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0813_05	Y1a | Y2	W | W	229 | 229	W | W	White
AR_Pop_0813_06	Y1a | Y1a	W | W	-	-	White
AR_Pop_0813_07	Y1a | Y2	W | W	-	-	White
AR_Pop_0813_08	Y1a> | Y2	W | W	-	-	White
AR_Pop_0813_09	Y1a | Y2	W | W	229 | 229	W | W	White
AR_Pop_0813_10	Y1a | Y1a	W | W	229 | 229	W | W	White

> Marks recombination which was observed in an individuals SNP haplotype. The side of the haplotype which the > is on represents approximately where the recombination occurred.

Table 11. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) for the seven F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
AR_Pop_0813_11	**Y1a | y6a**	**W | y**	**229 | 231**	**W | y**	**White**
AR_Pop_0813_12	**Y1a | y6a**	**W | y**	**229 | 231**	**W | y**	**White**
AR_Pop_0817_01	Y2 | y6a	W | y	229 | 231	W | y	White
AR_Pop_0817_02	Y2 | Y1b	**W | W**	**229 | 229**	**W | W**	White
AR_Pop_0817_03	Y2 | y6a	W | y	**229 | 231**	**W | y**	White
AR_Pop_0817_04	**y6a*(Y) | y6a**	**y*(W) | y**	**229 | 231**	**W | y**	**White**
AR_Pop_0817_05	**y6a*(Y) | y6a**	**y*(W) | y**	**229 | 231**	**W | y**	**White**
AR_Pop_0817_06	**y6a*(Y) | y6a**	**y*(W) | y**	**229 | 231**	**W | y**	**White**
AR_Pop_0817_07	**Y1b | y6a*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_0817_08	**y6a*(Y) | y6a**	**y*(W) | y**	**229 | 231**	**W | y**	**White**
AR_Pop_0817_09	**Y1b | y6a*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_0819_01	**y6a*(Y) | y6a*(Y)**	**y*(W) | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_0819_02	y6a | Y1b	**W | y**	**-**	**-**	White
AR_Pop_0819_03	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0819_04	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0819_05	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0819_06	**Y1b | y6a*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_0819_07	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0819_08	**Y1b | y6a*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_0819_09	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0819_10	**Y1b | y6a*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_0819_11	**Y1b | y6a*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**

Table 11. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) for the seven F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
AR_Pop_0819_12	**Y1b | y6a*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_0819_13	**Y1b | y6a*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_0819_14	**Y1b | y6a*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_0819_15	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0819_16	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0819_17	**Y1b | y6a*(Y)**	**W | y*(W)**	**229 | 229**	**W | W**	**White**
AR_Pop_0819_18	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0819_19	**y6a | >Y1b*(y)**	**y | W*(y)**	**231 | 231**	**y | y**	**Yellow**
AR_Pop_0819_20	**y6a | >Y1b*(y)**	**y | W*(y)**	**231 | 231**	**y | y**	**Yellow**
AR_Pop_0819_21	**y6a | >Y1b*(y)**	**y | W*(y)**	**231 | 231**	**y | y**	**Yellow**
AR_Pop_0819_22	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0819_23	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_01	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_02	y6a | y6b	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_03	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_04	y6a | y6b	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_05	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_06	y6a | y6b	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_07	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_08	y6a | y6b	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_09	y6a | y6b	y | y	231 | 231	y | y	Yellow

> Marks recombination which was observed in an individuals SNP haplotype. The side of the haplotype which the > is on represents approximately where the recombination occurred.

Table 11. Relationships between 11-SNP diplotypes, PpCCD4b-SSR genotypes, and phenotypes (white vs. yellow flesh) for the seven F_1 populations in the UA RosBREED pedigree ("conversion set"). All seedlings were genotyped with the IPSC 9K peach SNP array v1.0 and phenotyped for three years (2011-2013). Individuals with inconsistencies between their SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( ).** (Cont.).

Individual	Functional 11-SNP diplotype	Simple functional 11-SNP diplotype	PpCCD4b-SSR genotype (bp)	PpCCD4b-SSR functional genotype	Phenotype
AR_Pop_0825_10	y6a | y6b	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_11	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_12	y6a | y6b	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_13	y6a | y6a	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_14	y6a | y6b	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_15	y6a | y6b	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_16	y6a | y6b	y | y	231 | 231	y | y	Yellow
AR_Pop_0825_17	y6a | y6a	y | y	231 | 231	y | y	Yellow

SNP Haplotypes to PpCCD4b-SSR DNA Test Conversion

Next, the entire UA RosBREED "conversion set" of 243 individuals was used to make direct comparisons between each individual's SNP diplotypes and their PpCCD4b-SSR genotypes. At least one of the DNA tests failed for 13 individuals, thus, only 230 individuals were considered. In total, 13 individuals were homozygous white flesh (W | W : W | W), 41 were heterozygous white flesh (W | y : W | y), and 105 were homozygous yellow flesh (y | y : y | y) for both DNA tests (Tables 6, 7, 10, 11, and 12). In selected material, two individuals were homozygous white flesh (W | W : W | W), 22 were heterozygous white flesh (W | y : W | y), and 52 were homozygous yellow flesh (y | y : y | y) for both DNA tests. In un-selected material, 11 individuals were homozygous white flesh (W | W : W | W), 19 were heterozygous white flesh (W | y : W | y), and 53 were homozygous yellow flesh (y | y : y | y) for both DNA tests (Tables 6, 7, 10, 11, and 12). The total SNP diplotypes and PpCCD4b-SSR genotypes matched 69% of the time, yet 31% of the individuals contained inconsistencies (Table 12). In un-selected material, a higher inconsistency rate of 35% was observed, and in selected material the inconsistency rate dropped to 24% (Table 12).

PpCCD4b-SSR DNA Test for Additional Cultivars and Selections

Of the remaining 127 additional UA cultivars, selections, and germplasm from other breeding programs, 12 individuals were homozygous white flesh (W | W), 39 were heterozygous white flesh (W | y), 66 were homozygous yellow flesh (y | y), and 10 individuals did not amplify (Table 8). The PpCCD4b-SSR genotype accurately predicted the phenotype in all cases.

Table 12. Relationships between simple 11-SNP diplotypes, and PpCCD4b-SSR functional genotypes tested for distinguishing among white and yellow flesh peaches in the UA RosBREED pedigree ("conversion set") (N is number of genotypes analyzed). Inconsistencies between SNP diplotypes and PpCCD4b-SSR genotypes are marked in **bold**, and the correct haplotype follows with ***( )**.

DNA test		Phenotype	Total		Selected		Un-selected	
Simple 11-SNP diplotype	PpCCD4b functional genotype		N=230	Frequency	N=100	Frequency[z]	N=130	Frequency[y]
W | W	W | W	White	13	0.06	2	0.02	11	0.09
W | y	W | y	White	41	0.18	22	0.22	19	0.15
y | y	y | y	Yellow	105	0.46	52	0.52	53	0.41
y*(W) | y*(W)	**W | W**	**White**	**4**	**0.01**	**1**	**0.01**	**3**	**0.02**
W | y*(W)	**W | W**	**White**	**25**	**0.11**	**5**	**0.05**	**20**	**0.15**
y | y*(W)	**W | y**	**White**	**38**	**0.17**	**17**	**0.17**	**21**	**0.16**
y | W*(y)	**y | y**	**Yellow**	**4**	**0.01**	**1**	**0.01**	**3**	**0.02**

[z]Frequency of haplotypes in UA ("conversion set") selected germplasm (i.e. cultivars or selections).
[y]Frequency of haplotypes in UA ("conversion set") un-selected germplasm (i.e. seedlings).

PpCCD4b-SSR DNA Test "Confirmation Set"

The F_1 seedlings' alleles and matching phenotypes for each 2010 "confirmation set" population fit the parental alleles and matching phenotypes expected segregation ratio patterns for the PpCCD4b-SSR DNA test (Table 9). As in previous studies, a negligible sample failure rate of ~5% was seen for most populations (Edge-Garza et al., 2014). For population 1001, the female parent A-665 was homozygous white flesh (W | W), and the male parent A-800 was heterozygous white flesh (W | y). Their F_1 progenies alleles and matching phenotypes (18 W | W : 20 W | y) fit the expected segregation ratio ($p = 0.75$). The remaining populations chi-square p-values ranged from 1.00 (AR_Pop_1002, and AR_Pop_1015) to 0.07 (AR_Pop_1022) (Table 9).

Discussion

During the course of this study, two different types of DNA tests for white vs. yellow flesh were investigated to determine and compare their predictiveness to differentiate white and yellow flesh alleles. The two DNA tests included the 11-SNP haplotype test, developed in Chapter Four of this dissertation, and the intragenic PpCCD4b-SSR test, previously developed by Falchi et al., (2013). At least one of the DNA tests failed for 13 individuals in the UA RosBREED "conversion set", thus, only 230 individuals were considered. This sample failure rate of ~5% is consistent with previous studies that reported a negligible sample failure rate of ~1-5% (Edge-Garza et al., 2014).

The SNP diplotypes and PpCCD4b-SSR genotypes for the UA RosBREED "conversion set" of 230 individuals matched 69% of the time, however, a 31% inconsistency rate was still observed. In un-selected material a higher inconsistency rate was observed, reaching 36%, and in selected material the inconsistency rate dropped to 24%. The selected material with inconsistencies included selections A-665, A-786, A-798, A-821, A-844, A-845, A-850, A-853,

A-856, A-859, A-860, A-865, A-872, A-878, A-880, A-822, and cultivars Chinapearl, Cumberland, Georgia Belle, Peento, White County, White Diamond, and White River. These 24 selections and cultivars were found to contain five different white flesh SNP haplotypes, disguised as yellow flesh haplotypes [y2*(Y) = ABAAABBABBB; y3*(Y) = AAABBBBAAAA; y4*(Y) = AAABBBBBAAA; y6a*(Y) = AABBBAABAAA; and y6b*(Y) = ABBBBAABAAA]. The falsely associated yellow flesh SNP haplotype [y6a*(Y)] was the most abundant, present in 19 out of the 24 selections. These results are not surprising, after putting into perspective the distance of the 11 SNPs from the PpCCD4.1 candidate gene. As seen in Fig. 3, the first SNP in the 11-SNP haplotype, SNP_IGA_81682, is located ~2.3 Mbp away from the PpCCD4.1 candidate gene. All other SNPs are located downstream from the candidate gene, the furthest being ~1.7 Mbp away (snp_1_27368782). If the candidate gene's location would have been known before SNP haplotyping was performed, a narrower region could have been determined. However, since the SNP haplotype spanned ~ 4.0 Mbp, it's apparent that recombination events took place and generated these five falsely associated yellow flesh SNP haplotypes.

'White County' was a parent of AR_Pop_1, and passed on it's falsely associated yellow flesh SNP haplotype [y6a*(Y)] to 29 seedlings, representing nearly one half of the 39% inconsistency rate seen in un-selected material. Additionally, A-789, a selection from AR_Pop_1, passed on the falsely associated yellow flesh SNP haplotype [y6a*(Y)] it inherited from 'White County' to six seedlings in AR_Pop_0817. The remaining inconsistencies seen in un-selected material were nine seedlings in AR_Pop_0819 (A-708 × A-773) that inherited the falsely associated yellow flesh SNP haplotype [y6a*(Y)]. Yet, unlike the parents of AR_Pop_1 and AR_Pop_0817, the yellow flesh haplotype (y6a) was associated with yellow flesh for the

two parents of AR_Pop_0819, but later when passed onto the seedlings, this yellow flesh haplotype was falsely associated [y6a*(Y)] with white flesh. These nine discrepencies could likely be due to additional recombination events, outcrosses, or human error while working with the SNP data.

The comparisons between the two DNA tests highlight that the PpCCD4b-SSR DNA test is more predictive than the SNP haplotype DNA test. The PpCCD4b-SSR DNA test is located within the *PpCCD4* flesh color candidate gene, thus, the recombination events that affected the SNP haplotypes did not affect the intragenic DNA test. Since the PpCCD4b-SSR DNA test was 100% predictive across the UA RosBREED "conversion set" material, it was more efficient to only proceed with that test. The PpCCD4b-SSR DNA test is based on a single indel marker that uses a standard PCR protocol, which was shown in this study to be effective even for low quality and quantity DNA. One PCR-based marker is more economical and simple to use than 11 SNPs. Using the one PpCCD4b-SSR DNA test can reduce the PCR reactions needed to evaluate an individual's genotype, resulting in time and cost savings. Moreover, PpCCD4b-SSR can be multiplexed with other DNA tests for fruit quality, phenological, or disease resistance traits, and pooled for analysis on a range of genotyping platforms, including but not limited to agarose gel, polyacrylamide gel, the Fragment Analyzer™, or ABI Prism 3730xl DNA Analyzer.

The seven UA RosBREED populations ("conversion set") F_1 seedlings PpCCD4b-SSR alleles and matching phenotypes fit their parental expected segregation ratios (X^2 p-value > 0.05). Furthermore, all 20, 2010 populations ("confirmation set") fit their parental expected segregation ratios (X^2 p-value > 0.05). A sample failure rate of ~5% was seen across most populations, which is consistent with previous studies reported a negligible sample failure rate of ~1-5% (Edge-Garza et al., 2014). These results confirm the PpCCD4b-SSR test's predictiveness across a vast

majority of the UA peach breeding program, hence this marker can be incorporated into MAS in this program.

However, before crossing with new sources of germplasm it may be neccessary to screen potential parents with the PpCCD4b-SSR and for the long terminal repeat (LTR) retroelement insertion as described in Falchi et al., (2013). This group showed that yellow peach alleles across 37 cultivars had arisen from two additional independent mutational events, and that the PpCCD4-SSR could not accurately differentiate yellow from white flesh for these two additional mutations. The second mutational event was a SNP, A to T transversion, occurring at position 1519, within the second exon of the *PpCCD4* gene, resulting in a premature stop codon. This mutation inactivated the properly functioning dominant allele (white flesh allele) seen in five cultivars from Italy, Spain, and Brazil ('Oro A', 'Leonforte', 'Maruja', 'Leonforte1', and 'Bolinha'), but it appears the lineage of this mutation is not in UA germplasm. The third mutational event was an intronic transposable element insertion, also leading to a truncated protien, thus inactivating the properly functioning dominant allele (white flesh allele) (Falchi et al., 2013). This mutation was identified in 'Babygold 8', 'Elberta', 'Redhaven' and *P. ferganensis*, all with yellow flesh. 'Elberta' is in the background of the UA RosBREED pedigree, however it's apparent that the one non-functioning dominant white flesh allele 'Elberta' contains was not passed on through the generations in the UA pedigree. Nonetheless, if the UA breeder wants to be sure the mutant white flesh allele is not in their program, or new germplasm (from other programs) intended to cross with, one can also screen for the LTR retroelement insertion as described in Falchi et al., (2013).

The PpCCD4b-SSR DNA test can provide the UA peach breeder with valuable DNA information to select parents (MAPS), accurately design crosses (MACS), and cull unwanted

genotypes (MASS). The test eliminates the need to perform extensive (time consuming and highly resource intensive) progeny testing since it acccurately determines the parental pool's genotypes. Thus, the breeder can more efficiently design crosses between heterozygous white flesh and homozygous yellow flesh individuals to introgress traits from white flesh into yellow flesh peaches and nectarines, and vice versa. After the crosses are made, unwanted genotypes can be discarded in the greenhouse, and the white and yellow flesh peaches and nectarines can be planted in different groups, to allow the breeder to be more efficient when walking seedlings.

The work in this study also extends to other peach breeders, since the PpCCD4b-SSR DNA test was 100% predictive across 58 cultivars from a range of U.S. breeding programs. While the PpCCD4b-SSR can be a valuable DNA test for all peach and nectarine breeders, it's still advised to confirm it's predictiveness in their own program's germplasm before widespread adoption. The two additional mutations identified in Falchi et al., (2013) are a testament to this, yet both can be identified through additional DNA tests, thus breeders with these lineages can still perform MAS for the PpCCD4.1 candidate gene.

At the UA peach and nectarine breeding program, the PpCCD4b-SSR DNA test has been used for MAPS and MACS in 2013-2015 as well as for MASS in 2015 across 235 individuals from five populations ranging from 20-122 individuals (see Chapter Six). This test was multiplexed and pooled with the indelG DNA test for resource savings. Deployment of MAS using the PpCCD4b-SSR DNA test will continue in 2016, and likely the Clemson University, Texas A&M University and University of California, Davis peach breeding programs will also begin routine deployment. Implementation of MAS for the PpCCD4b-SSR DNA test in peach breeding programs substantiates the test's breeding utility and impact of predictive DNA tests in perennial fruit tree breeding.

Literature Cited

Bailey, J.S. and A.P. French. 1949. The inheritance of certain fruit and foliage characters in the peach. Amherst, MA: University of Massachusetts Press.

Bink, M. 2004. FlexQTL software. BIOMETRIS, Wageningen UR, The Netherlands <www.flexqtl.nl>.

Bink, M. 2005. FlexQTL software: efficient estimation of identity by descent probabilities and QTL mapping in pedigreed populations. In: Plant and Animal Genomes XII Conference, 15-19 January, San Diego, CA, U.S.

Bink, M., C. ter Braak, M. Boer, L. Totir, C. Winkler, and O. Smith. 2012. QTL linkage analysis of connected populations using ancestral marker and pedigree information. Theor. Appl. Genet. 124:1097–1113.

Bink, 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.

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

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.

Brandi, F., E. Bar, F. Mourgues, G. Horváth, E. Turcsi, G. Giuliano, A. Liverani, S. Tartarini, E. Lewinsohn, and C. Rosati. 2011. Study of 'Redhaven' peach and its white-fleshed mutant suggests a key role of CCD4 carotenoid dioxygenase in carotenoid and norisoprenoid volatile metabolism. BMC Plant Biol. 11: 24-38.

Byrne, D.H. 2005. Trends in stone fruit cultivar development. HortTechnology 15:494–500.

Byrne, D.H., M. Bassols, D. Bassi, M. Piagnani, K. Gasic, G. Reighard, M. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: M. Badenes and D. Byrne (eds.). Fruit breeding. Springer Science, Business Media, New York.

Connors, C.H. 1920. Some notes on the inheritance of unit characters in the peach. Proc. Amer. Soc. Hortic. Sci. 16:24–36.

de la Rosa, L.A., E. Alvarez-Parrilla, and G.A. Gonzalez-Aguilar. 2009. Fruit and vegetable phytochemicals: chemistry, nutritional value and stability. Ames, IA: John Wiley and Sons.

Dellaporta, S., J. Wood, and J.B. Hicks. 1983. Plant Mol. Biol. Rept. 1:19-21.

Dirlewanger E., P. Cosson, K. Boudrhri, C. Renaud, G. Capdeville., Y. Tauzin, F. Laihret, and A. Mong. 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. and Genomes 3:1-13.

Edge-Garza, D., T. Rowland, S. Haendiges, and C. Peace. 2014. A high-throughput and cost-efficient DNA extraction protocol for the tree fruit crops of apple, sweet cherry, and peach relying on silica beads during tissue sampling. Mol. Breeding 34: 2225-2228.

Edge-Garza, D.A., J.J. Luby, and C.P. Peace. 2016. Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Mol. Breed. 35:1-15.

Eduardo, I., E. López-Girona, I. Batlle, F. Reig, I. Iglesias, W. Howad, P. Arús, and M.J. Aranzana. 2014. Development of diagnostic markers for selection of the subacid trait in peach. Tree Genet. and Genomes 10:1695-1709.

Falchi, R., E. Vendramin, L. Zanon, S. Scalabrin, G. Cipriani, I. Verde, G. Vizzotto, and M. Morgante. 2013. Three distinct mutational mechanisms acting on a single gene underpin the origin of yellow flesh in peach. Plant J. 76:175–187.

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

Jung, S., S.P. Ficklin, T. Lee, C.-H. Cheng, A. Blenda, P. Zheng, J. Yu, A. Bombarely, I. Cho, S. Ru, K. Evans, C. Peace, A.G. Abbott, L.A. Mueller, M.A. Olmstead, and D. Main. 2014. The genome database for rosaceae (GDR): year 10 update. Nucl. Acids Res. 42:D1237-D1244.

Martinez-Garcia, P., D. Parfitt, E. Ogundiwin, J. Fass, H. Chan, R. Ahmad, S. Lurie, A. Dandekar, T. Gradziel, and C. Crisosto. 2013. High density SNP mapping and QTL analysis for fruit quality characteristics in peach (*Prunus persica* L.). Tree Genet. Genomes 9:19–36.

Moise, A.R., J. von Lintig and K. Palczewski. 2005. Related enzymes solve evolutionarily recurrent problems in the metabolism of carotenoids. Trends Plant Sci. 10, 178–186.

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. and J. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae, pp. 19-53. In K. Folta and S. Gardiner (Eds). Genetics and genomics of Rosaceae. Springer Science + Business Media, New York.

Ru, S., D. Main, K. Evans, and C. Peace. 2015. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet. and Genomes 11:1-12.

Salgado, 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. Ph.D dissertation University of Arkansas, Fayetteville, U.S.

Sandefur, P., T. Frett, J. Clark, K. Gasic, and C. Peace. 2016a. PpeRf-SSR, a DNA test for routine prediction in breeding of peach blush. Mol. Breeding (In press).

Sandefur, P., T. Frett, A. Salgado, J. Clark, K. Gasic, and C. Peace. 2016b. Ppe-Acidity, a combined DNA test for routine prediction in breeding of acidity and soluble solids content (SSC) in peach. Mol. Breeding (In press).

Stegmeir, T., M. Schuster, A. Sebolt, U. Rosyara, G. Sundin, and A. Iezzoni. 2014. Cherry leaf spot resistance in cherry (*Prunus*) is associated with a quantitative trait locus on linkage group 4 inherited from *P. canescens*. Mol. Breeding 34:927-935.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B.C. Faircloth, M. Remm, and S.G. Rozen. 2012. Primer3--new capabilities and interfaces. Nucl. Acids Res. 40:e115-e115.

Vendramin, E., G. Pea, L. Dondini, I. Pacheco, M. T. Dettori, L. Gazza, S. Scalabrin F. Strozzi, S. Tartarini, D. Bassi, I. Verde, and L. Rossini. 2014. A unique mutation in a MYB gene cosegregates with the nectarine phenotype in peach. PloS ONE 9:e90574. DOI: 10.1371/journal.pone.0090574.

Verde, I., A.G. Abbott, S. Scalabrin, S. Jung, S. Shu, F. Marroni, T. Zhebentyayeva, M.T. Dettori, J. Grimwood, F. Cattonaro, A. Zuccolo, L. Rossini, J. Jenkins, E. Vendramin, L.A. Meisel, V. Decroocq, B. Sosinski, S. Prochnik, T. Mitros, A. Policriti, G. Cipriani, L. Dondini, S. Ficklin, D.M. Goodstein, P. Xuan, C. Del Fabbro, V. Aramini, D. Copetti, S. Gonzalez, D.S. Horner, R. Falchi, S. Lucas, E. Mica, J. Maldonado, B. Lazzari, D. Bielenberg, R. Pirona, M. Miculan, A. Barakat, R. Testolin, A. Stella, S. Tartarini, P. Tonutti, P. Arús, A. Orellana, C. Wells, D. Main, G. Vizzotto, H. Silva, F. Salamini, J. Schmutz, M. Morgante, and D.S. Rokhsar. 2013. The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genet. 45:487-494.

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. DOI:10.1371/journal.pone.0035668.

Voorrips, R.E. 2007. Pedimap: software for visualization of genetic and phenotypic data in pedigrees. Plant Research International, Wageningen, the Netherlands. <http://www.wageningenur.nl/en/show/Pedimap.htm>.

Voorrips, R., M. Bink, and W. van de Weg. 2012. Pedimap: software for the visualization of genetic and phenotypic data in pedigrees. J. Hered. 103:903-907.

Chapter Six: Incorporation of MAPS and MASS in the University of Arkansas Peach and Nectarine Breeding Program

Abstract

Despite the considerable genetic improvements peach breeders have made over the last century, the majority of the peaches in the U.S. fresh market still lack consistent fruit quality, postharvest life, and disease and pest resistance. Moreover, traditional seedling selection (TSS) is a time consuming, expensive, and laborious process. Fortunately, application of DNA-based information [marker-assisted selection (MAS)] is now a reality, and can provide peach breeders with more informed decision support to increase genetic gain per breeding cycle, improve selection efficiency, and significantly reduce breeding program operational costs. Recently, several SNP and sequence length polymorphism-based (SLP-based) DNA tests for fruit bacterial spot [*Xanthomonas arboricola* pv. *pruni* (*Xap*)] resistance, fruit quality, and phenological traits have been validated for use in the UA program. The logistical incorporation of these DNA tests through MAS into an already successful peach breeding program at the University of Arkansas (UA) is discussed in this study. This work serves as a starting platform for the use of MAS in the UA program, which will continue to expand and evolve as additional DNA tests for the same or other breeding-relevant traits are developed and incorporated. This study furthermore serves as a case study for the feasibility and value of incorporating MAS into other Rosaceae tree fruit breeding programs.

Introduction

The peach [*Prunus persica* (L.) Batsch] is the third-most economically important temperate tree fruit species worldwide, with a total world production estimated at over 21.6 Mt (Byrne et al., 2012). However, in the U.S. the peach industry has seen a decline in production, ~750,000 tonnes, from 1961 to 2013 (FAO, 2015), which can be attributed to the necessity to harvest peaches at immature stages for storage and shipment purposes (Crisosto et al., 1995; Crisosto, 2002; Crisosto and Costa, 2008; Fideghelli et al., 1998; Sansavini et al., 2006) (Fig. 1). Unfortunately, several vital fruit quality traits including size, flavor (high sugar and moderate to low acidity), color, and blush (red skin pigmentation) develop as a peach ripens on the tree, thus harvesting a peach at an immature stage limits the full development of these essential fruit quality traits, and furthermore increases the chances for postharvest problems such as mealiness, overall resulting in low fruit quality to consumers. One solution to overcome the decline of peach production and consumption in the U.S. could be to develop peaches with unique textures that can maintain firmness for a longer period of time on the tree than traditional peaches in the market today. As a result, these peaches could be retained on the tree longer and harvested at the correct maturity.

Fig. 1. Peach and nectarine production in the U.S. from 1961-2013 (FAO, 2015).

Peach breeding in the U.S. started in 1895 with the establishment of a breeding program in Geneva, NY by Cornell's New York State Agricultural Experiment Station (NYSAES). After that, 16 public peach breeding programs were established from ~1905-1965, including the University of Arkansas (UA) peach and nectarine breeding program, initiated in 1964 by Dr. Jim Moore and Dr. Roy Rom, and currently directed by Dr. John R. Clark (Byrne et al., 2012; Faust and Timon, 1995; Okie et al., 2008). Later, by ~1970, > 50% of these public breeding programs were discontinued, and multiple private breeding companies in California began to flourish. This was due to several reasons including but not limited to the consolidation of federal and state funding for fruit breeding research, and the expansion of intellectual property rights both in the U.S. and worldwide (Byrne et al., 2012; Clark et al., 2012). In general, all these peach breeding programs aimed to produce productive cultivars spanning the season with environmental adaptation (abiotic and biotic), season extension, and improved fruit quality traits such as large size, unique shapes, low pubescence or glabrous (nectarines), appealing yellow or white ground color covered by extensive blush (red skin pigmentation), flavor with adequate eating quality, and increased firmness to resist damage associated with shipping (Bielenberg et al., 2009; Byrne et al., 2012; Howad et al., 2005; Okie et al., 2008; Sansavini et al., 2006).

Today, progress with most of these goals has been accomplished, yet despite these considerable genetic improvements, the majority of the peaches in the U.S. fresh market still lack consistent fruit quality, postharvest life, and disease and pest resistance. Moreover, traditional seedling selection (TSS) is a time consuming, expensive, and laborious process taking 10 years or more, from the initial cross until a new peach cultivar can be released (Bliss, 2010; Dirlewanger et al., 2004; Ru et al., 2015). In temperate environments, the breeder must wait at least three years for peach trees to mature to fruit-bearing capacity before fruit quality can be evaluated on progeny (Bliss, 2010; Dirlewanger et al., 1998; Dirlewanger et al., 2004; Dirlewanger et al., 2007). Once the trees bear fruit, it can then take an additional 10-15 years of phenotypic analysis, selection, and regional testing to develop and release a new cultivar. Furthermore, peach breeding programs require a significant amount of space due to the large tree size along with continuous maintenance costs such as herbicide, pesticide and fungicide spraying, planting, pruning, thinning, and irrigation (Bliss, 2010; Dirlewanger et al., 2004; Ru et al., 2015).

Taking into account all maintenance costs from cross to initial selection or tree removal, it was calculated to cost approximately $12 per peach seedling at Clemson University in 2015 (K. Gasic, and C. Peace, personal communication). This is comparable to the estimated $12 per apple (*Malus domestica* Borkh.) seedling at the Washington State University (WSU) apple breeding program and $15 per sweet cherry (*Prunus avium* L.) seedling at The Pacific Northwest (PNW) sweet cherry breeding program from 2001-2015 (Edge-Garza et al., 2010; Evans et al., 2012; Rowland et al., 2012). Considering $12 per peach seedling, if 2,000 new seedlings are planted, the overall cost to maintain these seedlings through initial selection phase (four years)

totals $24,000. Crosses are typically made annually, thus TSS costs expand further as the breeding program matures.

Fortunately, application of DNA-based information [marker-assisted selection (MAS)] is now a reality, and can provide peach breeders with more informed decision support to increase genetic gain per breeding cycle, improve selection efficiency, and significantly reduce breeding program operational costs (Bliss, 2010; Byrne, 2005; Edge-Garza et al., 2016; Ru et al., 2015). DNA tests for several breeding-relevant traits have been developed in peach including texture (Peace et al., 2005; Peace and Norelli, 2009), acidity (Eduardo et al., 2014), slow-melting flesh (Salgado, 2015), fruit bacterial spot resistance (see Chapter Five, Section One), pubescence (peach) vs. glabrous (nectarine) (see Chapter Five, Section Two; Vendramin et al., 2014), white vs. yellow flesh (see Chapter Five, Section Three; Falchi et al., 2013), amount of blush coverage (Sandefur et al., 2016a), and acidity and soluble solids content (Sandefur et al., 2016b). These DNA tests can provide peach breeders with more informed decision support to save resources and determine how to efficiently break linkages and combine all desired traits such as disease and pest resistance along with unique flavors, superior textures (that can maintain firmness for a longer period of time and thus be retained on the tree longer and harvested at the correct maturity), and enhanced postharvest life (Bliss, 2010; Byrne, 2005; Ru et al., 2015).

The MAS breeding strategy is based on a marker-locus-trait association (M-L-T), in which a predictive genetic marker is linked to a specific locus that contributes to the genetic variation for a specific phenotypic trait (Bliss, 2010; Ru et al., 2015). Therefore, the marker genotype (through its association with the locus genotype) is used to select for the phenotype (Bliss, 2010; Collard et al., 2005; Collard et al., 2008; Ru et al., 2015). When a M-L-T is screened on parental germplasm, MAS is called marker assisted parent selection (MAPS), and

helps in parent selection and cross combination decision making. In MAPS, allelic information of the parental pool can help direct the breeder to first select parents with valuable alleles, and subsequently select favorable crosses with efficient combining abilities, through marker assisted cross selection (MACS). After the cross is made, the same M-L-T can be used to screen the seedlings to decide on which promising seedlings to grow in the field and which to discard based on their allelic makeup (Ru et al., 2015). This form of MAS is termed marker assisted seedling selection (MASS), and is useful in monitoring the incorporation of the desirable functional alleles at the locus from parent to progeny (Bliss 2010; Peace and Norelli 2009).

The WSU Molecular Breeding Lab has been documenting MASS resource-use efficiency for the WSU apple and the PNW sweet cherry breeding programs since ~2001 (Edge-Garza et al., 2016). They recently published an article on a new DNA-based diagnostic tool, the MASS Efficiency Calculator v1.0, which they developed to enable a more precise estimation of MASS resource-use efficiency (Edge-Garza et al., 2016). Using this tool, the WSU lab has shown that TSS costs for the WSU apple and the PNW sweet cherry breeding programs can be substantially reduced by 50-60% and 70-80% by using only one and two DNA tests for MASS, respectively. Even more noteworthy, in general as more seedlings are screened, more DNA tests are used (in sequence rather than together) and culling rates are increased, the TSS costs can be even further reduced (C. Peace, personal communication; Edge-Garza et al., 2016). These successful examples of MASS in apple and sweet cherry substantiate the feasibility and value of conducting MASS in Rosaceae tree fruit breeding and provide insights into how to extend MAS adoption into more Rosaceae species (Edge-Garza et al., 2016; Ru et al., 2015).

Yet still, even after a DNA test has been validated for use in MASS, this tool can't be put into use until the logistics of organizing seedlings in the greenhouse, collecting leaf tissue, and

identifying an economical platform for DNA extraction, PCR, allele sizing, and processing of data for subsequent culling of seedlings in the greenhouse have been developed. Interestingly, a questionnaire in 2013 to assess the level of MASS implementation in apple, sweet cherry, tart cherry, and peach RosBREED demonstration tree fruit breeding programs revealed that the most prevalent challenge perceived by Rosaceae fruit breeders to perform MAS was in fact the difficulty in logistically enabling smooth integration of DNA testing into traditional breeding operations (Ru et al., 2015). The main reason for this perceived challenge could be due to the fact that successful DNA testing requires expertise in molecular data interpretation and management. Unfortunately this expertise is often lacking in breeding programs new to MASS (Ru et al., 2015).

The objective of this study was to document the incorporation of MAS into an already successful UA peach breeding program. In Chapter Four of this dissertation, multiple SNP-based DNA tests were developed, and validated for use in MAPS and MACS for fruit bacterial spot [caused by *Xanthomonas arboricola* pv. *pruni* (*Xap* refers to the pathogen and disease)] resistance, fruit quality, and phenological traits in the UA program as well as three other RosBREED demonstration peach breeding programs (Clemson University, Texas A&M University and University of California, Davis). The incorporation of these DNA tests through MAPS and MACS into the UA program in 2013 will first be discussed. The importance of these SNP-based DNA tests then expands further through the conversion and confirmation of the SNP-based DNA tests into simple, straightforward, breeder-friendly, sequence length polymorphism-based (SLP-based) DNA tests (such as SSRs, indel, or SCARs) to enable the same DNA test to be screened on one or both SNP haplotype-based and SLP-based DNA test platforms, upon comparison and selection for each specific trait (see Chapter Five, Sections One-Three; Salgado,

2015; Sandefur et al., 2016a; and Sandefur et al., 2016b). The most predictive and efficient method, SNP-based, or SLP-based, for each trait in the UA program will be decided on and displayed in a format useful for the UA breeder to accurately and efficiently perform MAPS, MACS, and MASS. Lastly, the deployment of MASS in 2015 for two of these SLP-based DNA tests, indelG and PpCCD4b, will be discussed. This work serves as a starting platform for the use of MAS in the UA program, which will continue to expand and evolve as additional DNA tests for the same or other breeding-relevant traits are developed, and incorporated.

Materials and Methods

Management Practices at FRS

Phenotypic evaluation for fruit bacterial spot [*Xanthomonas arboricola* pv. *pruni* (*Xap*)] resistance, fruit quality, and phenological traits were previously collected on peach and nectarine material at the UA Fruit Research Station (FRS), Clarksville, AR (west-central AR, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept. of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]). All trees were either open-center trained and spaced 5.5 m between trees and rows, or trained to a perpendicular-V system with trees spaced 1.9 m in rows spaced 5.5 m apart. All trees were dormant pruned and fertilized annually with a single application of 640 Kg ha-1 of complete fertilizer (19:19:19 of N:P:K) and were sprinkler or drip irrigated as needed. Pests were managed using a program typical for commercial orchards in the area (Smith, 2015; Studebaker et al., 2015). After shuck split but before pit hardening, fruitlets were thinned to a distance of 12 to 15 cm between each fruitlet.

Marker Assisted Parent Selection (MAPS) and Marker Assisted Cross Selection (MACS)

A MAPS and MACS template was created for the 72 individuals included on the mini-SNP array v1.0 (see Chapter Four), all cultivars and selections on the 9K Peach SNP array v1.0,

and all new selections genotyped with the SSR markers developed in Chapter Five of this dissertation (Salgado, 2015; Sandefur et al., 2016a; Sandefur et al., 2016b). This template includes genotypic and associated phenotypic data for all UA cultivars and selections. The 10 breeding-relevant traits documented for use in MAPS and MACS include: maturity date (day of year, 0-365); adherence (freestone vs. clingstone), flesh texture (melting, non-melting, non-softening), flesh melting rate (quick vs. slow); fruit bacterial spot resistance (resistant vs. intermediate vs. susceptible); pubescent vs. glabrous (peach vs. nectarine); white vs. yellow flesh; titratable acidity (TA; high vs. low); TA and soluble solids content (SSC; high vs. medium vs. low); and amount of blush overcolor (high vs. medium vs. low) (Table 1). Two DNA tests for each trait, SNP-based and SLP-based, were previously developed, converted, and confirmed across a vast array of the UA peach breeding program (see Chapters Four and Five). Of the two DNA tests, the most predictive one was selected for each trait and included in the MAPS and MACS template. The most predictive DNA test for each trait was also incorporated into the 2015 field selection data book, so the UA breeder could look at this data side by side with phenotypic data in the field. The amount of phenotypic variation explained (Vp %) by each DNA test was also included, to further inform the breeder on the utility of each specific DNA test (Table 1).

Table 1. Details on the respective SNP-based and SLP-based (SSR / indel) DNA tests for the 10 traits investigated in this study. The proportion of phenotypic variation each DNA tests explained for the respective trait is listed (Vp %) (TBD = yet to be determined genome-wide). Additionally, the dissertation chapter and or publication from which the two DNA tests for each trait were developed and compared are also listed.

	DNA test			
Trait	SNP-based	SLP-based	V_P	Dissertation chapter / publication
Xap fruit resistance	*G1XapF* locus (4-SNP)	Ppe-XapF1-SSR	~7%	Chapter Two; Chapter Four; Chapter Five, Section One
Xap fruit resistance	*G6XapF* locus (4-SNP)	Ppe-XapF6-SSR	~8%	
Acidity level	*D* locus (1-SNP)	CPPCT040b-SSR	~50%	Chapter Four; Salgado, 2015; Sandefur et al., 2016b
Acidity and sugar level	*G7Flav* locus (2-SNP)	G7-Flavor-7; G7-Flavor-16	~5% both traits	Chapter Three; Chapter Four; Sandefur et al., 2016b
Blush overcolor	*Rf* locus (4-SNP)	Ppe-R_f-SSR	~55%	Chapter Three; Chapter Four; Sandefur et al., 2016a
Texture and adherence	*EndoPG* locus (6-SNP)	EndoPG	100%	Chapter Four; Sandefur, 2011; Salgado, 2015
Slow-melting flesh	*SMF* locus (1-SNP)	SMF-SSR	TBD	Salgado, 2015
Fruit type	*G* locus (11-SNP)	indelG	100%	Chapter Three; Chapter Four; Chapter Five, Section Two
Flesh color	*Y* locus (11-SNP)	PpCCD4b-SSR	100%	Chapter Three; Chapter Four; Chapter Five, Section Three
Maturity date	*G4MD.1* (4-SNP)	G4Mat 3rd #6; PJS3; G4Mat Test#1	~50%	Chapter Three; Chapter Four

Marker Assisted Seedling Selection (MASS)

In the winter of 2015, five 2014 F_1 seedling populations were selected for testing MASS using two DNA tests confirmed across the UA breeding program (see Chapter Five, Sections Two-Three): indelG [pubescent (peach) (P) dominant to glabrous (nectarine) (n)]; and PpCCD4b-SSR (white (W) dominant to yellow (y) flesh] (Table 2). These five populations were produced from the following five crosses: AR_Pop_1403 (A-893N × A-820; 63 seedlings); AR_Pop_1405 (A-826 × A-880; 60 seedlings); AR_Pop_1406 (A-874N × A-872; 122 seedlings); AR_Pop_1414 (A-768N × A-805CN; 20 seedlings); and AR_Pop_1415 (A-868CN × A-861CN; 33 seedlings). Across the five populations a combined total of 295 F_1 seedlings, and their parents were investigated (Table 2).

Table 2. Crossing plan for five 2014 F_1 seedling populations, including parental phenotypes and functional genotypes for indelG and PpCCD4b-SSR DNA tests. Capitalized letters are dominant to lower-cased letters for both DNA tests.

				2013-15	IndelG	2013-15	PpCCD4b
F_1 population	Progeny (N)	Parents		Phenotype	Genotype[z]	Phenotype	Genotype[y]
AR_Pop_1403	63	Female	A-893N	Nectarine	n | n	White	W | W
		Male	A-820	Peach	P | P	Yellow	y | y
AR_Pop_1405	60	Female	A-826	Peach	P | n	Yellow	y | y
		Male	A-880	Peach	P | n	White	W | y
AR_Pop_1406	122	Female	A-874N	Nectarine	n | n	White	W | W
		Male	A-872	Peach	P | P	White	W | y
AR_Pop_1414	20	Female	A-768N	Nectarine	n | n	White	W | y
		Male	A-805CN	Nectarine	n | n	White	W | y
AR_Pop_1415	33	Female	A-868CN	Nectarine	n | n	Yellow	y | y
		Male	A-861CN	Nectarine	n | n	White	W | y

[z]P = peach allele, n = nectarine allele.
[y]W = white flesh allele, y = yellow flesh allele.

Germinated seeds from these five populations were planted in 28 well flats (7x4) and grown in the greenhouse at FRS. In January 2015, the seedling flats were organized into groups of three to mimic a 96-well DNA sampling plate, excluding row H, which served as positive and negative controls (Table 3). The organization of a subset of individuals in AR_Pop_1406 is depicted in Table 3. Each individual was labeled to its location in the 96 well DNA plate with a Weather Tough '27.9 × 2.5 cm' White Loop-Lock Laser Strip Tag. Leaf tissue was collected from the seedlings in coin envelopes, labeled, and lyophilized (Freezone® 12 model 77540, Labconco Corporation, Kansas City, MO). Lyophilized plant tissue was then organized into 96 1.1 ml MicroTube Rack Systems (BioExpress, Kaysville, UT) containing approximately 0.25 g of technical grade 40, 6-12 mesh silica gel beads (Sigma Aldrich, St. Louis, MO). DNA 96-well plate collection maps were developed to maintain organization from seedling in greenhouse to final data analysis (Table 3).

Table 3. Greenhouse organization of 28 well flats (different colors) to mimic 96 well DNA plate map, using a subset of AR_Pop_1406 as an example [Blank = negative controls; P1 (Parent 1) and P2 (Parent 2) of the population = positive controls].

	1	2	3	4	5	6	7	8	9	10	11	12
A	1406-1	1406-2	1406-3	1406-4	1406-5	1406-6	1406-7	1406-8	1406-9	1406-10	1406-11	1406-12
B	1406-12	1406-13	1406-14	1406-15	1406-16	1406-17	1406-18	1406-19	1406-20	1406-21	1406-22	1406-23
C	1406-24	1406-25	1406-26	1406-27	1406-28	BLANK	1406-29	1406_30	1406-31	1406-32	1406-33	1406-34
D	1406-35	1406-36	1406-37	1406-38	1406-39	1406-40	1406-41	1406-42	1406-43	1406-44	1406-45	1406-46
E	1406-47	1406-48	1406-49	1406-50	1406-51	BLANK	1406-52	1406-53	1406-54	1406-55	1406-56	1406-57
F	1406-58	1406-59	1406-60	1406-61	1406-62	1406-63	1406-64	1406-65	1406-66	1406-67	1406-68	1406-69
G	1406-70	1406-71	1406-72	1406-73	1406-74	1406-75	1406-76	1406-77	1406-78	1406-79	1406-80	1406-81
H	P1	P2	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	P1	P2

The DNA from each seedling was subsequently extracted using a high-throughput and cost efficient extraction protocol developed for *Prunus* species by Edge-Garza et al. (2014). This is a rapid DNA extraction protocol that has been shown to yield DNA extracts, with a quality of ~1.5-1.9 at 260nm/280nm and concentrations from ~4–180 ng/µl, which can be used immediately in PCR without any further dilutions (Edge-Garza et al., 2014). For confirmation purposes, DNA quantity and quality were measured for twelve samples from each 96 well DNA extraction plate in this study using a spectrophotometer (NanoDrop 2000, NanoDrop Technologies, Wilmington, DE) and confirmed by electrophoresis on 1% TBE (1 M Tris, 0.9 M Boric Acid, and 0.01 M EDTA) agarose gel. The expected success of PCR using DNA from this extraction protocol has been shown to be slightly greater than 95% across stone fruits and apple (Edge-Garza et al., 2014).

IndelG and PpCCD4b Genotyping

Extracted DNA was subsequently amplified with the indelG and PpCCD4b-SSR DNA tests at the University of Arkansas in the Horticulture Molecular Breeding Lab (Tables 4-5). Polymerase chain reaction (PCR) was performed for all samples in a final total volume of 10.0 µl containing 1.0 µl of DNA (~15-100 ng/µl), 4.0 µl of ultrapure molecular grade water [AccuGENE™ (Lonza Inc., Allendale, NJ)], 2.0 µl of Taq PCR buffer [×5 GoTaq® buffer (Promega Corp., Madison, WI)], 0.6 µl of $MgCl_2$ [25mM (Promega)], 0.2 µl deoxyribonucleotide triphosphates (dNTPs) [10 mM (Promega)], 0.5 µl of each primer (forward, reverse one and reverse two) [10mM (Integrated DNA Technologies, Coralville, IA)], 0.2 µl of Taq DNA polymerase (5U/µl) [GoTaq® (Promega Corp., Madison, WI)]. The PCR amplifications were performed in a BIORAD T100 thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA) under the following conditions: 5 min of initial denaturation at 95 °C followed by

35 cycles of 95 °C for 45 s, 60 °C for 45 s, 72 °C for 1.5 min, and then a final extension step at 72 °C for 7 min.

Table 4. The indelG DNA test forward and reverse primer sequences and physical locations on scaffold 5 [primer locations were sourced from the Genome Database for Rosaceae (Jung et al., 2014)].

Name	Physical location (bp)	Sequence
F	15,898,324	CTTGCACCTGAGTTCGATTCCG
R1	-	GGCTTCAATGGCAGAACAAGG
R2	15,899,264	GCAGGTGGTGGAGATTCATTCAT

Table 5. The PpCCD4b-SSR DNA test forward and reverse primer sequences and physical locations on scaffold 1 (Tm = Melting temperature) [primer locations were sourced from the Genome Database for Rosaceae (Jung et al., 2014)].

Name	Physical location (bp)	Sequence
F	25,639,168	CAACCAACTGATCCCACACC
R	25,638,937	GTTTTGAAGCTGGTGGTGGG

The indelG products were resolved using horizontal electrophoresis, and the PpCCD4b-SSR products were resolved utilizing two different capillary gel electrophoresis machines: a Fragment Analyzer™, model AdvanCE FS96 (Advanced Analytical Technologies, Inc., Ames, IA) from the wheat breeding laboratory at the University of Arkansas, and an ABI Prism 3730xl DNA Analyzer (Applied Biosystems by Life Technologies, Grand Island, NY) from the fruit breeding laboratory at WSU. The IndelG alleles were called based off distance they migrated in the gel. PpCCD4b-SSR alleles were called and quality checked using PROSize 1.0 software (Advanced Analytical Technologies, Ames, IA), for results from the Fragment Analyzer™ and GeneMarker® software (SoftGenetics, LLC, State College, PA), for results from the ABI Prism 3730xl.

Cost per Sample for MASS at UA Horticulture Molecular Breeding Lab

The cost per sample to perform DNA extraction, PCR, and fragment analysis at the UA Horticulture Molecular Breeding Lab was calculated using the MASS Efficiency Calculator v1.0 (https://www.rosaceae.org/breeders_toolbox/MASScalc), and additional manual calculations based on specific reagent costs used for MASS at the UA program (Edge-Garza et al., 2016).

Results

Marker Assisted Parent Selection (MAPS) and Marker Assisted Cross Selection (MACS)

The most efficient method, SNP-based, or SLP-based (SSR / indel), or both, were determined for each trait studied in the UA program (Table 6). These results were subsequently organized into the UA MAS breeder template v1.0, displayed in a format useful for the UA breeder to accurately and efficiently perform MAPS, MACS, and MASS (Table 7) (each respective DNA test is described in detail in each dissertation chapter, and/or publication listed in Table 7). The traits were furthermore organized in order of importance to the breeder to increase ease of use. Lastly, the most predictive DNA test for each trait was also incorporated into the 2015 peach field selection data book, so the UA breeder could look at this data side by side with phenotypic data in the field (Table 8).

Table 6. Details on the most predictive DNA test, SNP-based and or SLP-based (SSR / indel) are listed for all 10 traits investigated in this study. The proportion of phenotypic variation each DNA tests explains for their respective trait is listed (Vp %) (TBD = yet to be determined genome-wide). Additionally, the dissertation chapter and or publication from which the two DNA tests for each trait were developed and compared are also listed.

	DNA test			
Trait	SNP-based	SLP-based	V_P	Dissertation Chapter / Publication
Xap fruit resistance	*G1XapF* locus (4-SNPs)	-	~7%	Chapter Two; Chapter Four; Chapter Five, Section Two
Xap fruit resistance	*G6XapF* locus (4-SNPs)	Ppe-XapF6-SSR	~8%	
Acidity level	*D* locus (1-SNP)	CPPCT040b-SSR	~50%	Chapter Four; Salgado, 2015; Sandefur et al., 2016b
Acidity and sugar level	*G7Flav* locus (2-SNPs)	G7-Flavor-7 G7-Flavor-16	~5% Both traits	Chapter Three; Chapter Four; Sandefur et al., 2016b
Blush overcolor	-	Ppe-R_f-SSR	~55%	Chapter Three; Chapter Four; Sandefur et al., 2016a
Texture and adherence	-	EndoPG	100%	Chapter Four; Sandefur, 2011; Salgado, 2015
Slow-melting flesh	*SMF* locus (1-SNP)	SMF-SSR	TBD	Salgado, 2015
Fruit type	-	indelG	100%	Chapter Three; Chapter Four; Chapter Five, Section Two
Flesh color	-	PpCCD4b-SSR	100%	Chapter Three; Chapter Four; Chapter Five, Section Three
Maturity date	*G4MD.1* locus (4-SNP)	-	~50%	Chapter Three; Chapter Four

Table 7. The UA MAS breeder template v1.0.

	G4Mat[X]		EndoPG + SMF[W]		*G1XapF*[V]	XapF6-SSR[U]	XapF[T]
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]
A-554	-	190	CNMF	CNMF	-	SU | R	3.67
A-641	-	-	FMF	FMF	-	SU | R	-
A-657[z]	VE | VE	189	CNMF	CNMF	SU | SU	R2 | R2	1.33
A-662	-	194	-	CNMF	-	R | R	1.00
A-663[z]	VE | VE	-	CNSF	CNMF	SU | SU	R2 | R2	3.00
A-665[zy]	VE | M	199	CNSF	CNMF	SU | I	R1 | R2	2.00
A-668	-	194	CNSF	CNMF	-	R | R	3.00
A-672[zy]	VE | VL2	191	CQMF	CQMF	R1 | I	R1 | R2	3.33
A-699[z]	VE | E	210	FQMF	FQMF	R1 | I	SU | R1	3.33
A-708[z]	VE | M	196	FSMF	FSMF	R1 | I	SU | R1	4.33
A-716[z]	M | VL1	220	FMF	FMF	R1 | I	R1 | R2	2.00
A-743	-	190	CNMF	CNMF	-	R | R	1.67
A-758	-	190	CNMF	CNMF	-	R | R	2.33
A-760[z]	VE | M	191	FSMF	FSMF	SU | R1	R1 | R2	1.67
A-761	-	200	FMF	FMF	-	R | R	2.33
A-766	-	200	FMF	FMF	-	SU | R	2.00
A-768	-	203	FMF	FMF	-	SU | R	4.00
A-770	-	199	CNSF	CNMF	-	R | R	1.67
A-772[z]	VE | M	210	CSMF	CMF	R1 | I	R2 | R2	0.67
A-773[z]	VE | L	226	CNMF	CNMF	I | I	SU | R2	2.67
A-776[z]	VE | VE	182	CNSF	CNMF	SU | I	R1 | R2	2.00

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] *G4Mat* = Maturity date SNP haplotype: VE (very early); E (early); M (mid); L (late); VL (very late); XL (extra late) and P = [day of year 0-365].
[W] EndoPG + SMF = Adherence, flesh texture, and melting rate markers: FMF (freestone melting flesh); CMF (clingstone melting flesh); Q (quick melting); S (slow melting); CNMF (clingstone non-melting flesh); CNSF (clingstone non-softening flesh).
[V] *G1XapF* = Fruit bacterial spot SNP haplotype on LG1: R1 (resistant); I (intermediate); SU (susceptible) [0-5 scale].
[U] XapF6-SSR = Fruit bacterial spot SSR on LG6: R1 and R2 (resistant); SU (susceptible) [0-5 scale].
[T] XapF = Fruit bacterial spot phenotype [0-5 scale].
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.)

	G4Mat[X]		EndoPG + SMF[W]		*G1XapF*[V]	XapF6-SSR[U]	XapF[T]
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]
A-778[z]	VE | VE	191	CMF	CMF	SU | SU	R1 | R2	2.67
A-783[z]	VE | VE	191	CNSF	CNMF	R1 | I	SU | R2	3.33
A-786[y]	VE | VL1	203	FMF	FMF	R1 | R1	R1 | R2	3.33
A-789[z]	VL1 | VL2	214	FMF	FMF	R1 | I	R1 | R2	2.67
A-790	-	227	FMF	FMF	-	R | R	2.00
A-792[y]	VE | VE	187	CNSF	CNMF	R1 | R1	R2 | R2	2.00
A-794	-	190	CNMF	CNMF	-	R | R	2.33
A-797[y]	VE | VE	192	FMF	FMF	SU | I	R1 | R2	2.00
A-798	-	197	CNMF	CNMF	-	R | R	2.33
A-799	-	197	CNSF	CNMF	-	R | R	2.00
A-801[y]	VE | VE	200	CNMF	CNMF	SU | SU	R1 | R2	1.00
A-803	-	196	CNMF	CNMF	-	R | R	1.33
A-804[y]	VE | VE	206	CNMF	CNMF	SU | I	SU | SU	2.67
A-805[y]	VE | VE	203	CNMF	CNMF	SU | I	SU | SU	1.67
A-806[y]	VE | VE	206	CNMF	CNMF	SU | I	R2 | R2	1.33
A-808	-	217	FMF	FMF	-	R | R	1.67
A-809[y]	VE | VL1	215	FMF	FMF	I | I	R1 | R2	2.00
A-810	-	192	CNSF	CNMF	-	SU | R	-
A-811[y]	VE | VE	194	CNMF	CNMF	SU | SU	R1 | R2	2.00
A-813[y]	VE | VE	194	CNSF	CNMF	R1 | I	SU | SU	3.33
A-814	-	196	CNMF	CNMF	-	R | R	-

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] *G4Mat* = Maturity date SNP haplotype: VE (very early); E (early); M (mid); L (late); VL (very late); XL (extra late) and P = [day of year 0-365].
[W] EndoPG + SMF = Adherence, flesh texture, and melting rate markers: FMF (freestone melting flesh); CMF (clingstone melting flesh); Q (quick melting); S (slow melting); CNMF (clingstone non-melting flesh); CNSF (clingstone non-softening flesh).
[V] *G1XapF* = Fruit bacterial spot SNP haplotype on LG1: R1 (resistant); I (intermediate); SU (susceptible) [0-5 scale].
[U] XapF6-SSR = Fruit bacterial spot SSR on LG6: R1 and R2 (resistant); SU (susceptible) [0-5 scale].
[T] XapF = Fruit bacterial spot phenotype [0-5 scale].
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.)

	G4Mat[X]		EndoPG + SMF[W]		*G1XapF*[V]	XapF6-SSR[U]	XapF[T]
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]
A-815	-	196	CNSF	CNMF	-	R | R	1.33
A-816[y]	VE | VE	194	CNMF	CNMF	SU | I	R2 | R2	2.67
A-818[y]	VE | VE	197	CNSF	CNMF	SU | I	SU | SU	4.33
A-819[y]	VE | VE	200	FMF	FMF	SU | I	R1 | R2	1.33
A-820[y]	VE | VE	199	FMF	FMF	R1 | I	R2 | R2	0.67
A-821[y]	VE | VE	200	FMF	FMF	SU | R1	R1 | R2	0.33
A-822[y]	VE | VE	203	FMF	FMF	SU | I	SU | SU	1.67
A-824	-	203	CMF	CMF	-	R | R	3.33
A-825[y]	VE | VL1	204	FMF	FMF	R1 | R1	SU | SU	4.33
A-826[y]	VE | VL1	210	FMF	FMF	SU | R1	R1 | R1	2.00
A-827[y]	VE | M	215	FMF	FMF	SU | R1	R1 | R1	2.33
A-828[y]	VE | VE	186	CNMF	CNMF	SU | SU	R1 | R2	2.67
A-829[y]	VE | VE	187	CNMF	CNMF	SU | R1	R1 | R2	1.67
A-832[y]	VE | VE	192	CNMF	CNMF	R1 | R1	R1 | R2	1.67
A-833[y]	VE | VE	194	CNSF	CNMF	SU | R1	R1 | R2	5.00
A-836[y]	VE | VE	194	CNMF	CNMF	SU | R1	R1 | R2	2.67
A-837[y]	VE | VL1	199	FMF	FMF	I | I	R1 | R1	2.00
A-839	-	186	CMF	CMF	-	SU | R	4.67
A-840[y]	VE | VE	186	CNMF	CNMF	SU | SU	SU | SU	4.00
A-841[y]	VE | VE	187	CNMF	CNMF	SU | R1	R2 | R2	2.67
A-842[y]	VE | VE	191	CNMF	CNMF	SU | R1	SU | SU	2.67

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] *G4Mat* = Maturity date SNP haplotype: VE (very early); E (early); M (mid); L (late); VL (very late); XL (extra late) and P = [day of year 0-365].
[W] EndoPG + SMF = Adherence, flesh texture, and melting rate markers: FMF (freestone melting flesh); CMF (clingstone melting flesh); Q (quick melting); S (slow melting); CNMF (clingstone non-melting flesh); CNSF (clingstone non-softening flesh).
[V] *G1XapF* = Fruit bacterial spot SNP haplotype on LG1: R1 (resistant); I (intermediate); SU (susceptible) [0-5 scale].
[U] XapF6-SSR = Fruit bacterial spot SSR on LG6: R1 and R2 (resistant); SU (susceptible) [0-5 scale].
[T] XapF = Fruit bacterial spot phenotype [0-5 scale].
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.)

	G4Mat[X]		EndoPG + SMF[W]		*G1XapF*[V]	XapF6-SSR[U]	XapF[T]
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]
A-843[y]	VE | VE	192	CMF	CMF	SU | SU	SU | SU	4.33
A-844[y]	VE | VE	198	CNMF	CNMF	SU | SU	SU | SU	4.33
A-845[y]	VE | M	-	FMF	FMF	R1 | I	R1 | R1	1.00
A-846	-	192	CMF	CNMF	-	R | R	3.33
A-847	-	210	CNMF	CMF	-	R | R	4.00
A-848[y]	VE | M	201	FMF	FMF	R1 | I	R1 | R1	2.00
A-849[y]	VE | M	198	FMF	FMF	R1 | I	R1 | R1	2.33
A-850[y]	VE | VL1	207	FMF	FMF	R1 | R1	R1 | R1	3.33
A-851[y]	VE | VL1	200	FMF	FMF	SU | R1	R1 | R2	2.67
A-852[y]	VE | M	201	FMF	FMF	R1 | I	SU | SU	4.00
A-853[y]	VE | VL1	213	FMF	FMF	R1 | I	R1 | R2	2.33
A-854[y]	VE | VL1	220	FMF	FMF	SU | R1	R1 | R2	2.67
A-855[y]	VE | VE	182	CNMF	CNMF	I | I	R1 | R2	3.00
A-856[y]	VE | M	186	FMF	FMF	R1 | I	R1 | R1	2.67
A-857[y]	VE | VE	189	FMF	FMF	SU | R1	R1 | R2	2.33
A-858	-	191	FMF	FMF	-	R | R	3.33
A-859[y]	VE | M	191	FMF	FMF	R1 | R1	R1 | R1	2.50
A-860[y]	VE | VE	193	CNMF	CNMF	SU | SU	R2 | R2	3.00
A-861[y]	VE | VE	-	CNMF	CNMF	SU | SU	SU | SU	3.50
A-862[y]	VE | VE	191	FMF	FMF	R1 | I	SU | SU	3.00
A-864[y]	VE | VE	196	CNMF	CNMF	SU | SU	R1 | R2	3.33

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] *G4Mat* = Maturity date SNP haplotype: VE (very early); E (early); M (mid); L (late); VL (very late); XL (extra late) and P = [day of year 0-365].
[W] EndoPG + SMF = Adherence, flesh texture, and melting rate markers: FMF (freestone melting flesh); CMF (clingstone melting flesh); Q (quick melting); S (slow melting); CNMF (clingstone non-melting flesh); CNSF (clingstone non-softening flesh).
[V] *G1XapF* = Fruit bacterial spot SNP haplotype on LG1: R1 (resistant); I (intermediate); SU (susceptible) [0-5 scale].
[U] XapF6-SSR = Fruit bacterial spot SSR on LG6: R1 and R2 (resistant); SU (susceptible) [0-5 scale].
[T] XapF = Fruit bacterial spot phenotype [0-5 scale].
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.)

	G4Mat[X]		EndoPG + SMF[W]		*G1XapF*[V]	XapF6-SSR[U]	XapF[T]
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]
A-865[y]	VE | VL1	-	FMF	FMF	R1 | I	R1 | R2	3.50
A-866[y]	VE | M	200	FMF	FMF	R1 | I	R1 | R1	2.00
A-867[y]	VE | VE	196	CNMF	CNMF	SU | R1	R1 | R2	2.00
A-868[y]	VE | VE	192	CNMF	CNMF	SU | R1	R1 | R2	3.67
A-869[y]	VE | VE	194	CMF	CMF	R1 | R1	R2 | R2	3.00
A-870[y]	VE | VE	194	FMF	FMF	SU | R1	SU | SU	2.50
A-871[y]	VE | M	201	CMF	CMF	I | I	R2 | R2	3.67
A-872[y]	VE | M	198	FMF	FMF	R1 | I	R1 | R1	2.50
A-873[y]	VE | VE	198	CNMF	CNMF	SU | R1	SU | SU	3.67
A-874[y]	VE | VL1	206	FMF	FMF	R1 | I	R1 | R1	3.33
A-875[y]	VE | VE	200	CNMF	CNMF	SU | SU	R2 | R2	4.00
A-876[y]	VE | M	200	CNMF	CNMF	SU | SU	R2 | R2	3.67
A-877[y]	M | VL2	200	FMF	FMF	R1 | I	R1 | R1	3.00
A-878[y]	VE | VL1	203	FMF	FMF	SU | R1	R1 | R1	3.00
A-879[y]	VE | VL1	-	FMF	FMF	R1 | I	R1 | R2	2.00
A-880[y]	VE | VL1	232	FMF	FMF	R1 | R1	R1 | R1	3.00
A-881[y]	VE | VL1	217	FMF	FMF	R1 | I	R1 | R1	2.67
A-882[y]	VE | VL1	219	FMF	FMF	R1 | I	R1 | R1	2.67
A-883	-	177	FMF	FMF	-	R | R	2.50
A-884	-	192	FMF	FMF	-	SU | R	4.00
A-885	-	194	FMF	FMF	-	SU | SU	2.00

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] *G4Mat* = Maturity date SNP haplotype: VE (very early); E (early); M (mid); L (late); VL (very late); XL (extra late) and P = [day of year 0-365].
[W] EndoPG + SMF = Adherence, flesh texture, and melting rate markers: FMF (freestone melting flesh); CMF (clingstone melting flesh); Q (quick melting); S (slow melting); CNMF (clingstone non-melting flesh); CNSF (clingstone non-softening flesh).
[V] *G1XapF* = Fruit bacterial spot SNP haplotype on LG1: R1 (resistant); I (intermediate); SU (susceptible) [0-5 scale].
[U] XapF6-SSR = Fruit bacterial spot SSR on LG6: R1 and R2 (resistant); SU (susceptible) [0-5 scale].
[T] XapF = Fruit bacterial spot phenotype [0-5 scale].
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.)

	G4Mat[X]		EndoPG + SMF[W]		*G1XapF*[V]	XapF6-SSR[U]	XapF[T]
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]
A-886	-	196	CNMF	CNMF	-	R | R	2.50
A-887	-	196	-	FMF	-	R | R	-
A-888	-	201	FMF	FMF	-	R | R	2.50
A-889	-	192	-	CNMF	-	R | R	2.00
A-890	-	-	CNMF	CNMF	-	SU | R	2.50
A-891	-	192	FMF	FMF	-	R | R	4.00
A-892	-	192	CNMF	CNMF	-	SU | R	4.00
A-893	-	-	-	FMF	-	R | R	3.50
A-894	-	-	FMF	FMF	-	R | R	3.50
A-895	-	175	-	CNMF	-	-	2.00
A-896	-	175	CNSF	CNMF	-	R | R	4.00
A-897	-	183	FMF	CNMF	-	R | R	3.00
A-898	-	183	CNMF	CNMF	-	SU | R	5.00
A-899	-	183	FMF	CMF	-	R | R	1.00
A-900	-	183	FMF	FMF	-	R | R	2.00
A-901	-	190	FMF	FMF	-	R | R	3.00
A-902	-	190	CNMF	CMF	-	R | R	4.00
A-903	-	195	CNMF	CNMF	-	R | R	5.00
A-904	-	195	FMF	FNMF	-	R | R	3.00
A-905	-	195	-	CNMF	-	-	-
A-906	-	195	FMF	CNMF	-	R | R	1.00

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] *G4Mat* = Maturity date SNP haplotype: VE (very early); E (early); M (mid); L (late); VL (very late); XL (extra late) and P = [day of year 0-365].
[W] EndoPG + SMF = Adherence, flesh texture, and melting rate markers: FMF (freestone melting flesh); CMF (clingstone melting flesh); Q (quick melting); S (slow melting); CNMF (clingstone non-melting flesh); CNSF (clingstone non-softening flesh).
[V] *G1XapF* = Fruit bacterial spot SNP haplotype on LG1: R1 (resistant); I (intermediate); SU (susceptible) [0-5 scale].
[U] XapF6-SSR = Fruit bacterial spot SSR on LG6: R1 and R2 (resistant); SU (susceptible) [0-5 scale].
[T] XapF = Fruit bacterial spot phenotype [0-5 scale].
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.)

	G4Mat[X]		EndoPG + SMF[W]		*G1XapF*[V]	XapF6-SSR[U]	XapF[T]
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]
A-907	-	195	-	CNMF	-	-	3.00
A-908	-	196	CNSF	CNMF	-	R | R	4.00
A-909	-	203	-	CNMF	-	R | R	4.00
A-910	-	203	FMF	FMF	-	R | R	2.00
A-911	-	223	-	CNMF	-	SU | R	2.00
A-912	-	182	FMF	-	-	R | R	4.00
A-913	-	189	FMF	-	-	R | R	1.00
A-914	-	189	CMF	-	-	R | R	-
A-915	-	190	-	-	-	R | R	2.00
A-916	-	195	CMF	-	-	R | R	1.00
A-917	-	195	-	-	-	SU | R	3.00
A-918	-	195	FMF	-	-	R | R	3.00
Allgold	-	184	CNMF	CNMF	-	SU | R	1.67
Amoore Sweet[z]	VE | VE	188	CNSF	CNMF	SU | I	R1 | R2	1.67
Arrington[z]	VE | VE	175	CNMF	CNMF	SU | SU	R2 | R2	3.00
Bowden[y]	VE | VE	197	CNSF	CNMF	SU | I	R1 | R2	2.67
Bradley[zy]	VE | VE	183	CNMF	CNMF	SU | SU	R2 | R2	2.67
Goldilocks	-	-	CNSF	CNMF	-	SU | R	2.67
Goldjim	-	209	CNSF	CNMF	-	SU | R	2.67
Goldnine	-	200	-	CNMF	-	R | R	2.33
Roygold	-	185	CNSF	CNMF	-	R | R	1.67

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] *G4Mat* = Maturity date SNP haplotype: VE (very early); E (early); M (mid); L (late); VL (very late); XL (extra late) and P = [day of year 0-365].
[W] EndoPG + SMF = Adherence, flesh texture, and melting rate markers: FMF (freestone melting flesh); CMF (clingstone melting flesh); Q (quick melting); S (slow melting); CNMF (clingstone non-melting flesh); CNSF (clingstone non-softening flesh).
[V] *G1XapF* = Fruit bacterial spot SNP haplotype on LG1: R1 (resistant); I (intermediate); SU (susceptible) [0-5 scale].
[U] XapF6-SSR = Fruit bacterial spot SSR on LG6: R1 and R2 (resistant); SU (susceptible) [0-5 scale].
[T] XapF = Fruit bacterial spot phenotype [0-5 scale].
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.)

	G4Mat[X]		EndoPG + SMF[W]		*G1XapF*[V]	XapF6-SSR[U]	XapF[T]
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]
Souvenirs[z]	VE | M	190	FSMF	FSMF	I | I	R1 | R1	2.00
Westbrook[z]	VE | VE	168	FSMF	FQMF	SU | R1	R1 | R1	3.00
White Cloud	-	196	CNMF	CNMF	-	SU | R	2.00
White County[zy]	M | VL1	200	FSMF	FSMF	R1 | R1	R1 | R1	1.67
White Diamond[y]	VE | VL1	216	FSMF	FSMF	R1 | R1	R1 | R2	1.33
White Rock[y]	VE | VE	194	CNMF	CNMF	SU | I	SU | SU	2.00
WhiteRiver[z]	VE | M	212	FSMF	FSMF	R1 | R1	R1 | R2	3.33
Winblo[z]	M | XL	201	FQMF	FQMF	SU | SU	SU | SU	3.33
Yumm Yumm	-	169	FSMF	FQMF	-	SU | R	2.33

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] *G4Mat* = Maturity date SNP haplotype: VE (very early); E (early); M (mid); L (late); VL (very late); XL (extra late). P = day of year 0-365.
[W] EndoPG + SMF = Adherence, flesh texture, and melting rate markers: FMF (freestone melting flesh); CMF (clingstone melting flesh); Q (quick melting); S (slow melting); CNMF (clingstone non-melting flesh); CNSF (clingstone non-softening flesh).
[V] *G1XapF* = Fruit bacterial spot SNP haplotype on LG1: R1 (resistant); I (intermediate); SU (susceptible) [0-5 scale].
[U] XapF6-SSR = Fruit bacterial spot SSR on LG6: R1 and R2 (resistant); SU (susceptible) [0-5 scale].
[T] XapF = Fruit bacterial spot phenotype [0-5 scale].
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]
A-554	P | n	P	W | y	W	D | D	M | M	0.7	13.5	B_1 | b_1	4.0
A-641	-	-	y | y	Y	-	M | M	-	-	B_1 | B_1	-
A-657[z]	n | n	N	y | y	Y	d | d	M | M	0.9	16.6	B_1 | B_1	3.7
A-662	P | n	N	y | y	Y	d | d	M | M	0.9	18.3	B_2 | B_2	2.5
A-663[z]	n | n	N	y | y	Y	d | d	M | M	0.8	15.4	B_1 | B_1	3.0
A-665[zy]	P | P	P	W | W	W	D | d	L | M	0.4	15.0	B_1 | B_2	3.3
A-668	n | n	N	y | y	Y	d | d	M | M	0.7	14.8	B_2 | B_2	2.5
A-672[zy]	P | n	P	W | y	W	d | d	H | M	0.6	14.7	B_1 | B_1	3.8
A-699[z]	n | n	N	W | y	W	D | d	H | H	0.5	16.0	B_1 | b_1	3.3
A-708[z]	P | P	P	y | y	Y	D | d	L | L	0.4	14.5	B_1 | B_1	4.0
A-716[z]	P | P	P	y | y	Y	D | d	L | L	0.4	14.3	B_1 | B_1	3.0
A-743	P | n	P	y | y	Y	D | D	M | M	0.4	12.8	B_1 | B_1	5.0
A-758	n | n	N	y | y	Y	d | d	M | M	0.7	14.9	B_1 | B_2	3.5
A-760[z]	P | n	P	y | y	Y	D | d	L | M	0.2	13.9	B_1 | B_1	4.0
A-761	n | n	N	y | y	Y	-	M | M	1.0	16.2	B_1 | B_2	4.5

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]	G[S]
A-766	P | P	P	y | y	Y	D | d	L | L	0.4	14.4	B_1 | B_1	4.0
A-768	n | n	N	W | y	W	d | d	M | M	1.0	12.3	B_1 | B_2	4.0
A-770	n | n	N	W | y	W	D | d	M | M	0.7	16.5	B_1 | B_1	3.5
A-772[z]	P | n	P	W | y	W	D | d	L | M	0.3	16.7	B_1 | b_2	3.0
A-773[z]	P | P	P	-	W	D | d	L | M	0.5	19.4	B_1 | b_1	2.7
A-776[z]	n | n	N	-	W	D | d	M | H	0.4	17.2	B_1 | b_1	3.0
A-778[z]	n | n	N	y | y	Y	d | d	M | M	0.8	13.6	B_1 | B_2	4.0
A-783[z]	n | n	N	W | y	W	d | d	M | H	0.9	15.6	B_1 | b_1	3.5
A-786[y]	P | n	P	W | y	W	D | d	L | M	0.4	13.3	B_1 | B_1	4.0
A-789[z]	n | n	N	W | W	W	D | d	H | L	0.4	19.7	B_1 | B_1	4.0
A-790	P | P	P	y | y	Y	D | d	L | L	0.3	14.0	B_1 | B_1	-
A-792[y]	n | n	N	W | y	W	D | d	M | M	0.7	14.8	B_1 | B_1	3.5
A-794	n | n	N	W | y	W	D | d	M | M	1.0	15.6	B_1 | B_1	3.5
A-797[y]	n | n	N	W | y	W	D | d	H | M	0.6	19.2	B_1 | B_1	3.5
A-798	P | n	P	W | y	W	D | d	L | M	0.2	16.2	B_1 | B_2	4.0

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]	P
A-799	n | n	N	W | y	W	d | d	M | M	0.7	12.9	B_1 | B_1	3.0
A-801[y]	P | P	P	W | y	W	D | d	M | H	0.3	15.3	B_1 | B_2	3.0
A-803	n | n	N	y | y	Y	D | d	M | M	0.9	15.1	B_1 | B_2	4.0
A-804[y]	n | n	N	W | y	W	D | d	H | M	0.4	17.2	B_1 | B_2	3.0
A-805[y]	n | n	N	W | y	W	D | d	M | M	0.5	15.8	B_1 | B_2	4.0
A-806[y]	n | n	N	y | y	Y	D | d	L | M	0.3	16.1	B_1 | B_2	3.5
A-808	P | P	P	-	W	D | d	L | M	0.3	15.2	B_1 | B_1	3.0
A-809[y]	P | n	P	y | y	Y	D | d	L | M	0.3	13.3	B_1 | B_2	3.0
A-810	n | n	N	y | y	Y	-	M | M	-	-	B_1 | B_2	4.0
A-811[y]	n | n	N	y | y	Y	D | d	M | M	0.7	14.9	B_1 | B_1	2.5
A-813[y]	n | n	N	W | y	W	D | d	H | M	0.6	14.1	B_1 | B_2	3.5
A-814	P | n	P	y | y	Y	-	L | M	0.3	14.5	B_1 | B_1	3.0
A-815	n | n	N	W | y	W	D | d	M | M	0.3	16.9	B_1 | B_1	3.5
A-816[y]	n | n	N	W | W	W	D | d	H | M	0.4	16.7	B_1 | B_1	4.5

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]	G[S]
A-818[y]	n | n	N	W | y	W	d | d	H | M	0.9	14.2	B_1 | B_1	3.5
A-819[y]	P | n	P	y | y	Y	D | d	M | L2	0.3	13.5	B_1 | B_1	3.5
A-820[y]	P | P	P	y | y	W	D | D	L | M	0.3	14.2	B_1 | B_1	4.5
A-821[y]	P | P	P	W | y	W	D | d	M | M	0.3	15.5	B_1 | B_1	3.0
A-822[y]	n | n	N	W | y	W	D | d	H | M	0.3	16.2	B_1 | b_1	2.5
A-824	P | P	P	y | y	Y	D | d	L | M	0.3	14.2	B_1 | B_1	3.5
A-825[y]	P | P	P	W | y	W	D | d	L | L	0.4	13.6	B_1 | b_1	2.5
A-826[y]	P | n	P	y | y	Y	D | D	L | M	0.3	15.6	B_1 | B_1	3.0
A-827[y]	P | P	P	y | y	Y	D | d	L | L	0.3	13.3	B_1 | B_1	4.0
A-828[y]	n | n	N	y | y	Y	d | d	M | M	0.8	12.5	B_1 | B_1	4.0
A-829[y]	n | n	N	W | y	W	-	M | M	0.4	14.9	B_1 | B_1	4.0
A-832[y]	n | n	N	y | y	Y	-	M | M	0.4	18.2	B_1 | b_1	3.0
A-833[y]	n | n	N	y | y	Y	-	M | M	0.3	15.8	B_1 | b_1	3.0
A-836[y]	n | n	N	y | y	Y	-	M | M	0.4	15.4	B_1 | b_1	3.5

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]	G[S]
A-837[y]	P | P	P	y | y	Y	-	L | L	1.1	14.4	B_1 | B_1	3.0
A-839	P | n	P	W | y	W	-	L | M	0.5	14.7	B_1 | B_1	4.5
A-840[y]	n | n	N	W | y	W	-	L | M	0.5	19.1	B_1 | b_1	4.5
A-841[y]	n | n	N	W | y	W	d | d	M | M	0.8	15.1	B_1 | B_1	4.0
A-842[y]	n | n	N	y | y	Y	D | d	M | L2	0.5	15.5	B_1 | B_1	3.0
A-843[y]	n | n	N	W | y	W	-	M | M	0.4	16.8	B_1 | B_1	3.5
A-844[y]	n | n	N	W | y	W	D | d	M | M	0.6	17.7	B_1 | B_1	4.0
A-845[y]	P | n	P	W | y	W	-	M | H	-	-	B_1 | B_1	-
A-846	n | n	N	y | y	Y	D | d	M | M	0.3	15.2	B_1 | B_1	3.5
A-847	P | n	P	W | W	W	-	L | H	0.3	13.0	B_1 | B_1	4.0
A-848[y]	P | n	P	y | y	Y	D | d	M | H	0.2	-	B_1 | B_1	3.0
A-849[y]	P | n	P	y | y	Y	D | D	M | H	0.2	14.6	B_1 | B_1	3.5
A-850[y]	P | n	P	W | y	W	D | d	M | H	0.3	14.2	B_1 | B_1	2.5

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]	G[S]
A-851[y]	P | P	P	y | y	Y	D | d	L | M	0.4	14.6	B_1 | B_1	2.5
A-852[y]	P | P	P	y | y	Y	D | d	L | L	0.4	14.8	B_1 | b_1	2.5
A-853[y]	P | P	P	W | y	W	D | D	L | M	0.4	14.4	B_1 | B_1	3.0
A-854[y]	P | P	P	y | y	Y	-	L | L	0.2	14.0	B_1 | B_1	3.0
A-855[y]	P | n	P	W | y	W	-	M | H	0.4	12.9	B_1 | b_2	3.0
A-856[y]	P | n	P	W | y	W	D | d	L | L	0.4	12.5	B_1 | B_1	4.5
A-857[y]	P | P	P	y | y	Y	D | d	L | L	0.4	10.8	B_1 | B_1	3.5
A-858	P | n	P	y | y	Y	D | d	L | M	0.4	14.9	B_1 | B_1	4.0
A-859[y]	n | n	N	W | y	W	-	H | H	0.6	18.7	B_1 | B_1	3.5
A-860[y]	P | n	P	W | y	W	D | d	L | M	0.3	17.1	B_1 | B_1	3.5
A-861[y]	n | n	N	W | y	W	-	M | M	-	-	B_1 | b_1	-
A-862[y]	P | P	P	y | y	Y	D | d	L | L	0.3	14.6	B_1 | B_1	4.5
A-864[y]	n | n	N	y | y	Y	D | d	L | M	0.2	-	B_1 | B_2	4.0

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]	G[S]
A-865[y]	n | n	N	W | y	W	-	M | H	-	-	$B_1 \mid B_1$	-
A-866[y]	P | n	P	y | y	Y	D | d	L | L	0.3	14.1	$B_1 \mid B_1$	4.0
A-867[y]	n | n	N	y | y	Y	-	M | M	0.5	14.8	$B_1 \mid B_1$	3.0
A-868[y]	n | n	N	y | y	Y	D | d	M | M	0.3	14.4	$B_1 \mid B_2$	3.5
A-869[y]	n | n	N	y | y	Y	D | d	M | M	0.3	-	$B_1 \mid B_2$	3.0
A-870[y]	n | n	N	W | y	W	-	M | M	1.1	16.9	$B_1 \mid B_1$	4.0
A-871[y]	n | n	N	W | y	W	D | d	M | H	0.4	15.4	$B_1 \mid b_2$	2.5
A-872[y]	P | n	P	W | y	W	D | d	L | L	0.3	14.5	$B_1 \mid B_1$	4.0
A-873[y]	n | n	N	y | y	Y	d | d	M | M	1.0	17.7	$B_1 \mid B_1$	4.0
A-874[y]	n | n	N	W | W	W	d | d	M | H	0.5	15.9	$B_1 \mid B_1$	3.5
A-875[y]	n | n	N	y | y	Y	D | d	H | M	0.3	14.2	$B_1 \mid b_1$	4.0
A-876[y]	n | n	N	W | y	W	D | d	L | M	0.4	15.7	$B_1 \mid b_1$	4.5
A-877[y]	P | n	P	W | W	W	D | d	L | L	0.3	15.2	$B_1 \mid B_1$	4.5

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]	G[S]
A-878[y]	P | P	P	W | y	W	D | d	L | M	0.3	16.4	B_1 | B_1	4.0
A-879[y]	P | n	P	y | y	Y	D | d	L | L	-	-	B_1 | B_1	-
A-880[y]	P | P	P	W | y	W	D | d	H | H	0.4	11.5	B_1 | B_1	3.0
A-881[y]	P | P	P	y | y	Y	D | d	L | L	0.3	-	B_1 | B_1	4.0
A-882[y]	P | n	P	W | y	W	D | d	M | H	0.4	15.2	B_1 | B_1	3.0
A-883	P | n	P	y | y	Y	D | d	L | L	0.1	13.9	B_1 | B_1	5.0
A-884	P | n	P	-	W	D | d	L | L	0.2	15.4	B_1 | B_1	4.0
A-885	P | n	P	W | y	W	D | d	L | H	0.3	15.2	B_1 | B_1	5.0
A-886	P | n	P	-	W	d | d	L | M	0.5	12.3	B_1 | b_1	4.0
A-887	-	P	W | W	W	D | d	L | L	0.2	14.8	B_1 | B_2	4.5
A-888	P | n	P	W | W	W	D | d	L | M	0.2	13.5	B_1 | B_1	5.0
A-889	n | n	N	y | y	Y	D | d	H | M	-	16.3	B_1 | b_1	4.0
A-890	P | n	P	W | W	W	d | d	L | M	-	-	B_1 | B_1	-

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]	G[S]
A-891	P | n	P	W | y	W	d | d	L | L	0.8	11.8	B_1 | B_1	4.0
A-892	n | n	N	W | y	W	D | d	M | M	-	16.2	B_1 | B_2	4.0
A-893	n | n	N	W | W	W	D | d	L | M	-	-	B_1 | B_1	-
A-894	P | n	P	y | y	Y	-	L | M	-	-	B_1 | B_1	4.0
A-895	n | n	N	-	W	-	-	-	-	-	-
A-896	n | n	N	-	W	-	H | M	-	-	B_1 | B_1	3.0
A-897	n | n	N	y | y	Y	-	L | L	-	-	B_1 | B_1	5.0
A-898	P | n	P	W | y	W	-	L | M	-	-	B_1 | B_1	3.0
A-899	P | n	P	y | y	Y	-	L | M	-	-	B_1 | B_1	4.0
A-900	P | n	P	W | y	W	-	L | M	-	-	B_1 | B_1	4.0
A-901	n | n	N	W | y	W	-	L | M	-	-	B_1 | B_2	3.0
A-902	n | n	N	y | y	Y	-	H | H	-	-	B_1 | B_2	3.0
A-903	n | n	N	W | y	W	-	M | M	-	-	B_1 | B_1	4.0

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]	G[S]
A-904	P | n	P	W | y	W	-	M | M	-	-	B_1 | B_2	3.0
A-905	-	P	-	W	-	-	-	-	-	-
A-906	P | n	P	W | y	W	-	L | M	-	-	B_1 | B_1	4.0
A-907	n | n	N	-	W	-	-	-	-	-	-
A-908	n | n	N	W | y	W	-	L | M	-	-	B_1 | B_2	3.0
A-909	P | n	P	y | y	Y	-	L | L	-	-	B_1 | B_1	3.0
A-910	P | n	P	W | y	W	-	L | M	-	-	B_1 | B_1	5.0
A-911	n | n	N	W | y	W	-	L | M	-	-	B_1 | B_1	3.0
A-912	P | n	P	W | y	W	-	L | H	-	-	B_1 | B_1	4.0
A-913	P | n	P	W | y	W	-	L | M	-	-	B_1 | B_2	3.0
A-914	P | n	P	W | y	W	-	H | M	-	-	B_1 | B_2	3.0
A-915	n | n	N	W | y	W	-	L | M	-	-	B_1 | B_1	4.0
A-916	n | n	N	W | y	W	-	H | M	-	-	B_1 | B_1	3.0

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

Individual	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]	G[S]
A-917	P | n	P	W | y	W	-	L | H	-	-	B_1 | B_1	2.0
A-918	P | n	P	W | y	W	-	L | M	-	-	B_1 | B_2	3.0
Allgold	P | n	P	y | y	Y	-	M | M	0.4	14.2	B_2 | b_1	1.0
Amoore Sweet[z]	n | n	N	y | y	Y	D | d	M | H	0.5	16.6	B_1 | B_2	2.5
Arrington[z]	n | n	N	y | y	Y	d | d	M | M	0.9	15.1	B_1 | B_2	3.0
Bowden[y]	n | n	N	W | y	W	d | d	H | M	0.5	15.5	B_1 | b_1	2.5
Bradley[zy]	n | n	N	y | y	Y	d | d	M | M	0.8	14.3	B_1 | B_1	2.8
Goldilocks	P | n	P	y | y	Y	d | d	L | H	-	-	B_2 | b_1	-
Goldjim	P | P	P	y | y	Y	d | d	L | M	0.5	12.7	B_2 | B_2	2.0
Goldnine	P | P	P	y | y	Y	d | d	L | H	0.7	13.4	B_2 | b_1	2.0
Roygold	P | P	P	y | y	Y	d | d	L | M	0.6	11.1	B_2 | B_2	2.0
Souvenirs[z]	P | P	P	y | y	Y	D | d	L | L	0.4	13.8	B_1 | B_1	3.8
Westbrook[z]	n | n	N	y | y	Y	d | d	H | M	1.0	10.9	B_1 | B_1	4.3

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 7. The UA MAS breeder template v1.0. (Cont.).

	IndelG[X]		PpCCD4b[W]		CPPCT040b[V]	G7-Flav7+16[U]	TA (%)	SSC (%)	Ppe-Rf-SSR[T]	
Individual	G[S]	P[R]	G[S]	P[R]	G[S]	P[R]	G[S]	G[S]	P[R]	G[S]
White Cloud	P | P	P	W | y	W	d | d	-	0.3	13.4	B_1 | b_1	2.0
White County[zy]	-	P	W | y	W	D | d	H | L	0.3	15.1	B_1 | B_1	4.0
White Diamond[y]	P | P	P	W | y	W	D | d	L | L	0.3	11.2	B_1 | B_1	3.0
White Rock[y]	P | n	P	W | W	W	d | d	L | M	0.2	14.6	B_1 | b_1	1.5
White River[z]	P | P	P	W | y	W	D | d	L | M	0.7	12.5	B_1 | B_1	3.0
Winblo[z]	P | P	P	y | y	Y	d | d	L | L	0.8	15.3	B_1 | B_1	3.7
Yumm Yumm	n | n	N	W | y	W	d | d	H | M	-	-	B_1 | B_1	3.0

[Z] SNP genotyping by the IPSC 9K peach SNP array v1.0.
[Y] Peach mini SNP array v1.0 genotyping.
[X] IndelG = Peach vs. nectarine intragenic indel: n | n = nectarine (n); P | n / P | P = peach (P).
[W] PpCCD4b = Flesh color intragenic SSR: y | y = Yellow flesh (Y); W | y het / W | W = White flesh (W).
[V] CPPCT040b = High vs. low acidity SSR: d | d = High acid; D | d / D | D = Low acid [TA = titratable acidity].
[U] G7-Flav7+16 = TA and SSC (soluble solids content) SSRs: High (H) vs. mid (M) vs. low (L).
[T] Ppe-Rf-SSR = Amount of blush overcolor SSR: B_1, B_2 = high blush; b_1, b_2 = low blush. P = 0-5.
[S] G = Genotype.
[R] P = Phenotype [2013-2015 avg; except A-883 – A-918 (only 2015)].

Table 8. Incorporating functional alleles for 10 key breeding traits into the 2015 peach selection data book. 'White County' is used as an example. This is an example of the field book for rating of traits (1-10 scale) as traditionally used in the UA program, with the genotypic information added.

White County (A-392 × A-433)

Trait	Genotype	Rating
Maturity date[Z] (~50% V_P)	M | VL1	
Blush overcolor[Y] (~55% V_P)	B_1 | B_1	
Flesh type/stone [X] (intragenic)	F | F	
Flesh melting rate [W] (N/AV_P)	S | S	
Acidity[V] (~50% V_P)	D | d	
G7Flav[U] (~5% acidity and SSC V_P)	H | L	
Fuzz[T] (intragenic)	P | n	
Flesh color[S] (intragenic)	W | y	
Bact spot G1[R] (~7% fruit V_P)	R1 | R1	
Bact spot G6[Q] (~8% fruit V_P)	R1 | R1	

Trait	Rating
Size	
Shape	
Firm	
Finish	
Vigor	
Crop	
Health	

[Z] Maturity date = G4Mat SNP haplotype: VE (very early); E (early); M (mid); L (late); VL (very late); XL (extra late).
[Y] Blush overcolor = Ppe-Rf-SSR: B_1, B_2 = high blush; b_1, b_2 = low blush.
[X] Flesh type/stone = EndoPG1+6: FMF (freestone melting flesh); CMF (clingstone melting flesh); CNMF (clingstone non-melting flesh); CNSF (clingstone non-softening flesh).
[W] Flesh melting rate = SMF-SSR: Q (quick melting); S (slow melting).
[V] Acidity = CPPCT040b-SSR: d | d = high acid; D | d / D | D = low acid [TA = titratable acidity].
[U] G7Flav = G7-Flav7+16: L = low sugar / low acid, M = medium sugar / medium acid, H = high sugar / high acid. SSC = soluble solids content.
[T] Fuzz = indelG: n | n = nectarine (n); P | n / P | P = peach (P).
[S] Flesh color = PpCCD4b-SSR: y | y = yellow flesh (Y); W | y / W | W = white flesh.
[R] Bact spot G1 = *G1XapF* (fruit bacterial spot SNP haplotype on LG1): R1 (resistant); I (intermediate); SU (susceptible).
[Q] Bact spot G6 = XapF6-SSR (fruit bacterial spot SSR on LG6): R1 and R2 (resistant); SU (susceptible) (0-5 scale).

In terms of *Xap*, at the *Xap1* locus, the 4-SNP *G1XapF* haplotype test was predictive across the UA material studied as well as the entire RosBREED pedigree. However, the Ppe-XapF1-SSR was not (Table 6), thus only *G1XapF* alleles are depicted in Table 7 (see Chapter Four and Chapter Five Section One for more details). In contrast, at the Xap6 locus, both the 4-SNP *G6XapF* haplotype test and Ppe-XapF6-SSR DNA test genotypes matched for every individual (Table 6), and thus were equally predictive across the UA and RosBREED material studied (Table 7) (see Chapter Four and Chapter Five Section One for more details). However, more individuals were screened with the Ppe-XapF6-SSR DNA test, thus only genotypes from Ppe-XapF6-SSR are shown in Table 7. These *Xap* fruit resistance DNA tests explained a combined ~15% of the phenotypic variation percentage (V_P) for *Xap* fruit resistance/susceptibility (see Chapter Five, Section One).

In terms of the fruit quality traits studied, both the SNP based and SLP-based (SSR / indel) DNA tests were equally predictive across the UA material studied as well as the entire RosBREED pedigree, excluding the *D* (acidity level) and *EndoPG* loci (texture and adherence) tests. For acidity, while the CPPCT040b-SSR was predictive across ~90% of the material studied, the *D* locus SNP_IGA_545261 was ~ 98% predictive for all individuals screened with the 9K array (~50% V_P for acidity). Unfortunately, through the use of positive control individuals (those screened with 9K array), the alleles for the *D* locus SNP_IGA_545261 were not accurately determined for the majority of the 72 UA selections screened with the mini-SNP array, thus only the CPPCT040b-SSR genotypes for each individual are depicted in Table 7 (see Chapter Four). In contrast, the *G7Flav* 2-SNP haplotype and the G7-Flavor-7-SSR and G7-Flavor-16-SSR DNA tests were identified to be equally predictive across all material studied (~5% V_P for acidity and SSC). However, more individuals were screened with the G7-Flavor-7-

SSR and G7-Flavor-16-SSR DNA tests, thus only genotypes from these SSR tests are shown in Table 7 (see Chapter Four; Sandefur et al., 2016b). In regards to blush overcolor, both the *Rf* SNP-based and the Ppe-Rf-SSR DNA tests were equally predictive across all material studied (~55% V_P for red skin overcolor). However, more individuals were screened with the Ppe-Rf-SSR DNA test, thus only the Ppe-Rf-SSR DNA test genotypes are shown in Table 7 (see Chapter Four; Sandefur et al., 2016a). In contrast, only the SLP-based intragenic EndoPG markers for flesh type were predictive across all material studied, and thus only the genotypes from this DNA test are shown in Table 7 (see Chapter Four; Sandefur, 2011; Salgado, 2015). Lastly, the genotypes of the *SMF-locus SNP* ss_414220 and the SMF-SSR matched for all individuals (Vp % genome wide, to be determined). However, more individuals were screened with the SMF-SSR, thus only SMF-SSR genotypes of individuals that have been also phenotyped for ethylene production, are depicted in Table 7 (Salgado, 2015).

In terms of the phenological traits, the intragenic indelG marker for fruit type (pubescent vs. glabrous), and the intragenic PpCCD4b-SSR for flesh color (white vs. yellow) were more predictive than their respective 11-SNP haplotypes, while the *G4Mat* 4-SNP haplotype (~50% V_P for maturity date) was more predictive than the combined three SSR markers (G4Mat 3rd #6; PJS3; and G4Mat Test#1). Thus only the indelG, PpCCD4b-SSR, and *G4Mat* 4-SNP haplotype genotypes for each individual are depicted in Table 7 (Chapter Four; Chapter Five, Section Two; Chapter Five, Section Three).

Marker Assisted Seedling Selection (MASS)

IndelG and PpCCD4b Genotyping

The DNA quantity and quality measured for twelve samples from each 96-well DNA extraction plate ranged from ~20–200 ng/µl (quantity) with a quality of ~1.4-1.9 at 260 nm/280

nm. Since this is adequate DNA quantity and quality for PCR based markers, no further dilutions were necessary (Edge-Garza et al., 2014). Additionally, as in Edge-Garza et al., (2014), genetic screening with the indelG and PpCCD4b-SSR DNA tests were successful for ~95% of all individuals across the five F_1 populations. Representative alleles for the indelG test are shown in Figs. 3-9, and for the PpCCD4b-SSR in Figs. 10-15.

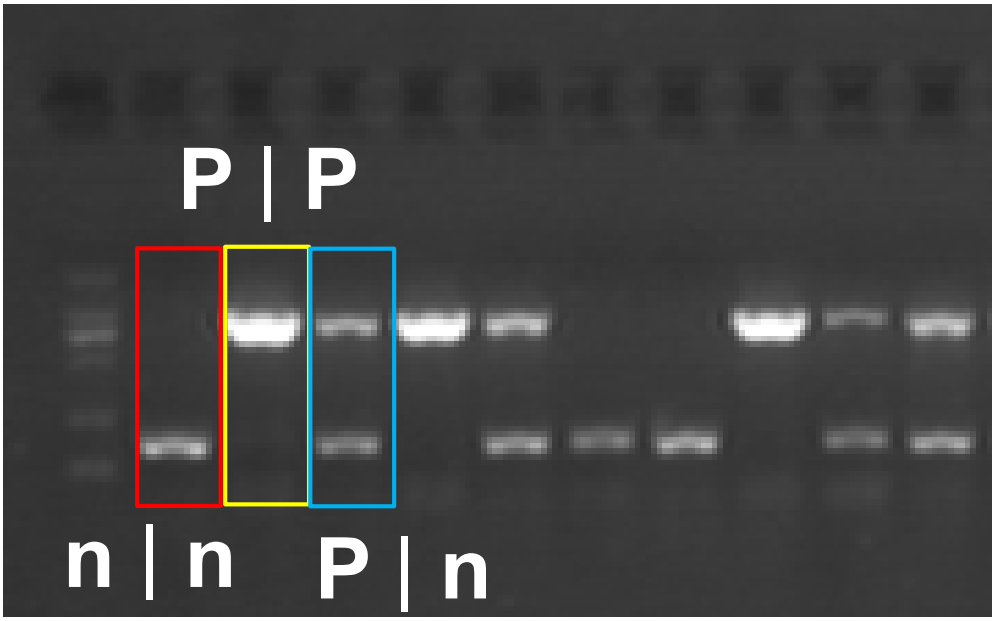

Fig. 3. Representative output from horizontal electrophoresis gel showing the resolved fragments of the indelG alleles. In red is nectarine individual (n | n), yellow homozygous peach (P | P), and blue heterozygous peach (P | n).

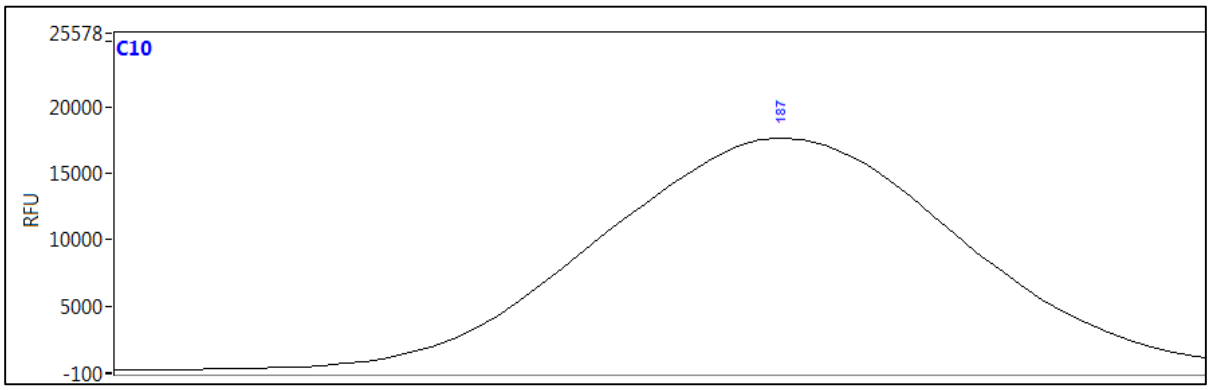

Fig. 4. Representative fragment analysis output of a homozygous glabrous (nectarine) (n | n) individual, amplifying a peak of 187 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

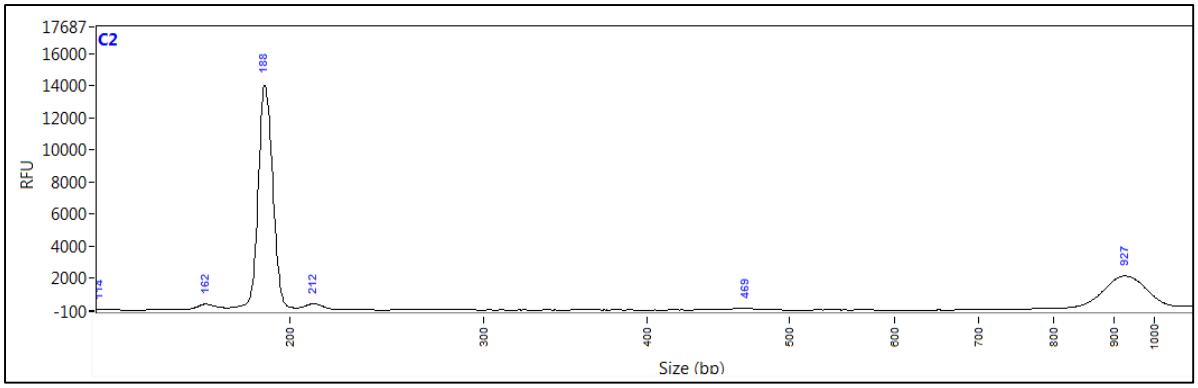

Fig. 5. Representative fragment analysis output of a heterozygous pubescent (peach) (P | n) individual, amplifying peaks of 188 and 927 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

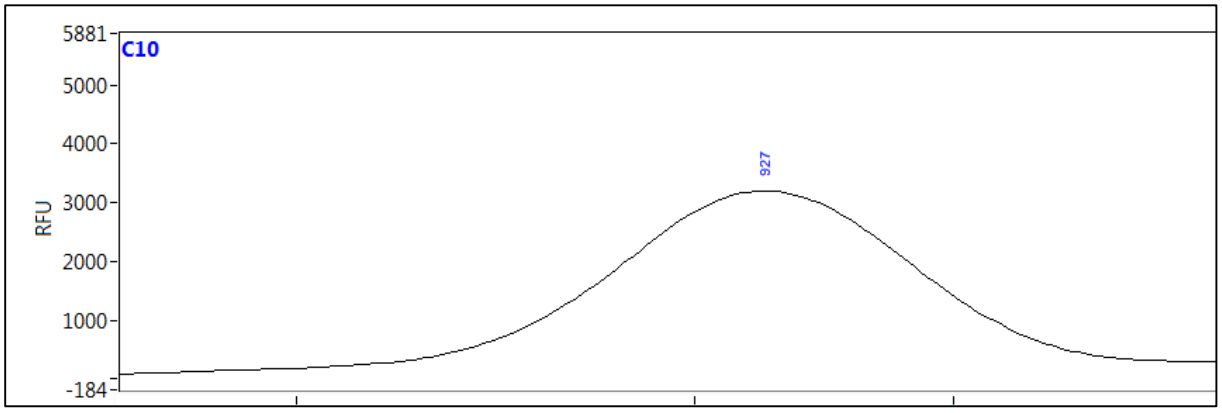

Fig. 6. Representative fragment analysis output of a homozygous pubescent (peach) individual (P | P), amplifying a peak of 927 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

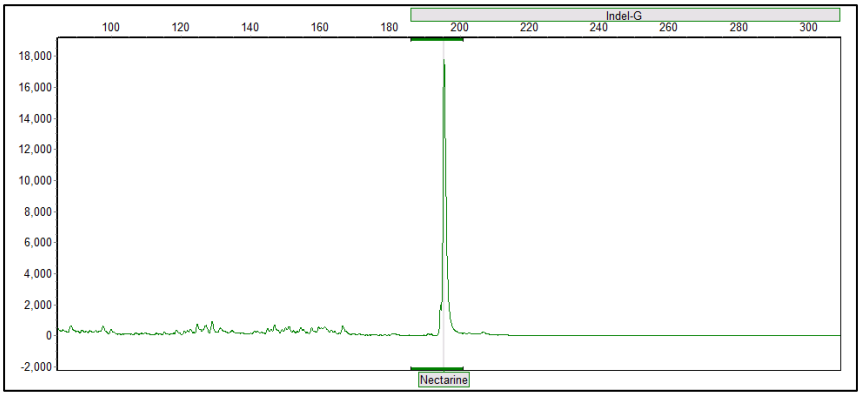

Fig. 7. Representative ABI output of a homozygous glabrous (nectarine) (n | n) individual, amplifying a peak of 197 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

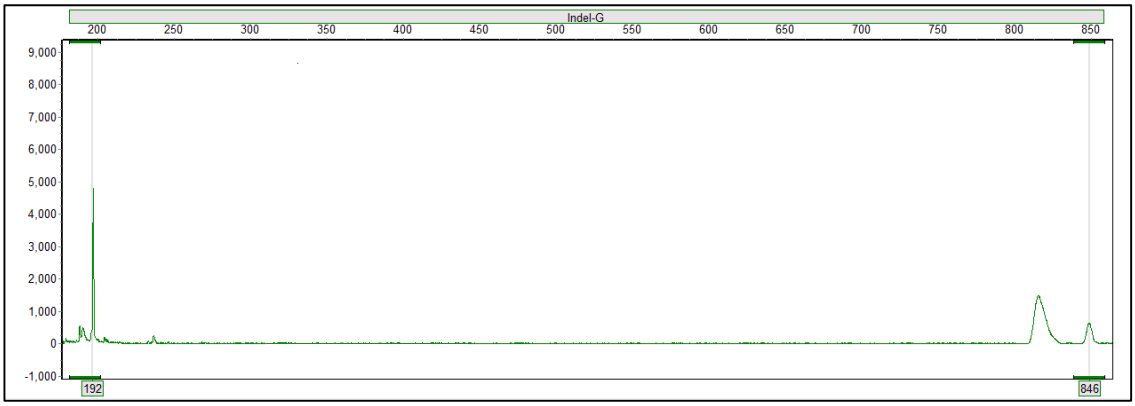

Fig. 8. Representative ABI output of a heterozygous pubescent (peach) individual (P | n), amplifying peaks of 192 and 846 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

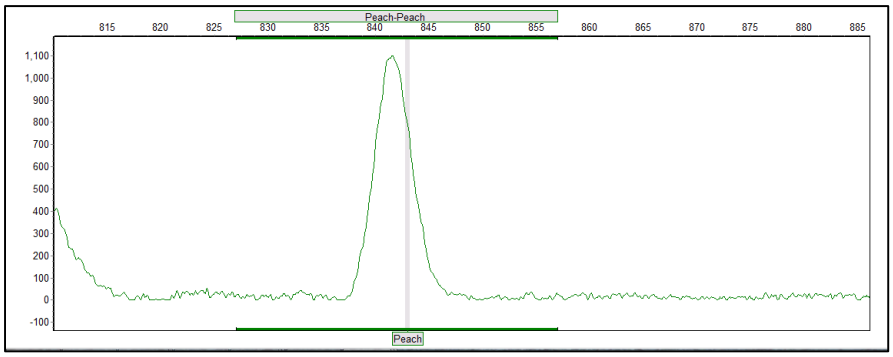

Fig. 9. Representative ABI output of a homozygous pubescent (peach) individual (P | P), amplifying a peak of 842 bp using the indelG DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

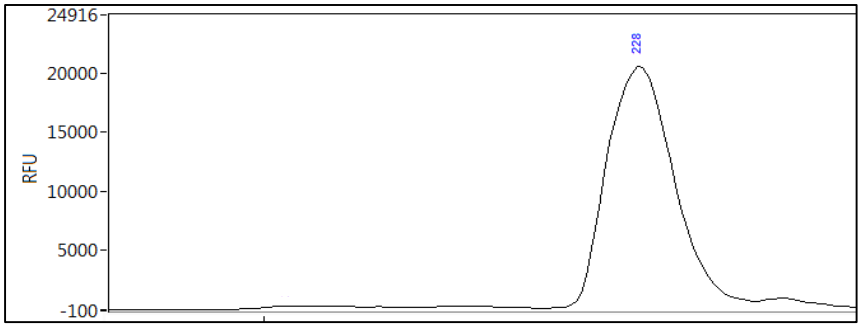

Fig. 10. Representative fragment analysis output of a homozygous white flesh individual (W | W), amplifying a peak of 228 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

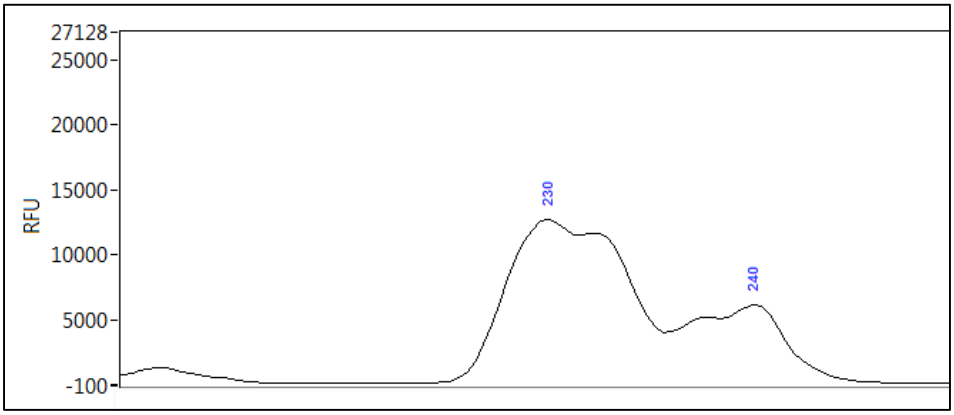

Fig. 11. Representative fragment analysis output of a heterozygous white flesh individual (W | y), individual, amplifying peaks of 230 and 240 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

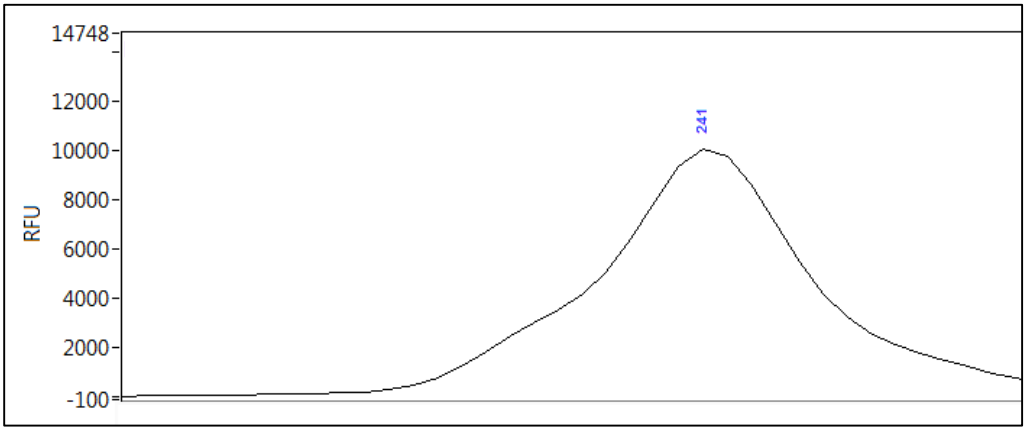

Fig. 12. Representative fragment analysis output of a yellow flesh individual (y | y), amplifying a peak of 241 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU).

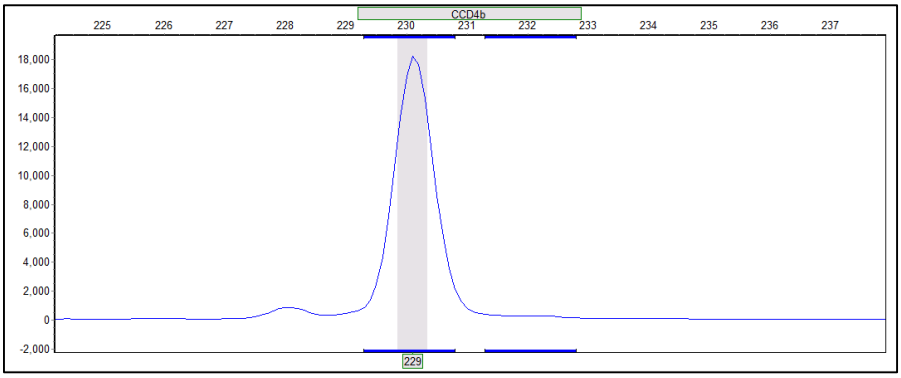

Fig. 13. Representative ABI output of a homozygous white flesh individual (W | W), amplifying a peak of 229 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU) (y-axis).

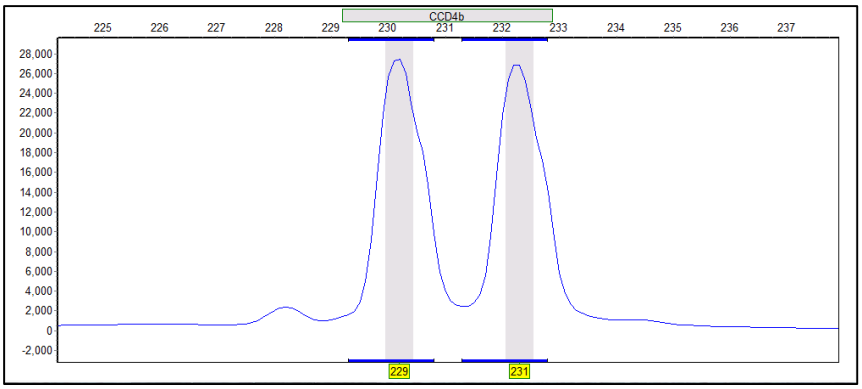

Fig. 14. Representative ABI output of a heterozygous white flesh individual (W | y), amplifying peaks of 229 and 231 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU) (y-axis).

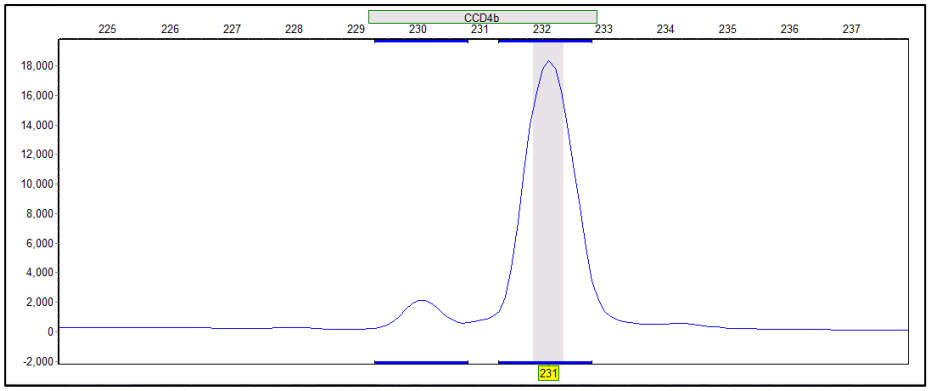

Fig. 15. Representative ABI output of a yellow flesh individual (y | y), amplifying a peak of 231 bp using the PpCCD4b-SSR DNA test. Peaks represent alleles with bp sizes indicated. Peak height indicates the relative fluorescence units (RFU) (y-axis).

The F_1 seedlings' genotypes for all five 2014 populations fit their expected parental segregation ratios for both the indelG and PpCCD4b-SSR DNA tests (Tables 9 and 10). For AR_Pop_1403, the female parent A-893N was a homozygous white flesh nectarine (W | W; n | n), and the male parent A-820 was homozygous yellow flesh peach (y | y; P | P). Their F_1 seedlings' genotypes (59 W | y; 61 P | n) fit the expected 1:1 segregation ratio (chi-square p = 1.0 and p = 1.0). The remaining population's indelG and PpCCD4b-SSR genotype chi-square p-values ranged from 1.0 (AR_Pop_1406, AR_Pop_1414, AR_Pop_1415) to 0.32 (AR_Pop_1405) for indelG (Table 9) and from 0.85 (AR_Pop_1406) to 0.16 (AR_Pop_1415) for PpCCD4b-SSR (Table 10). As in previous MASS fruit breeding studies, a negligible sample failure rate of ~1-5% was seen for both DNA tests (Edge-Garza et al., 2014); however, these individuals were simply re-screened in order to get results for all seedlings. This first round of MASS served as a trial run, since all seedlings were planted and later re-labeled with functional allele information in the field at the FRS for additional confirmation in 2017.

Table 9. The indelG DNA test genotypes (P=peach; n=nectarine) and pubescent (peach = P) or glabrous (nectarine = n) phenotypes for parents of the five 2014 F_1 populations, and the genotype segregation ratios for the five populations. [*The chi-square p-value in brackets]. Capitalized letters are dominant to lower-cased letters for indelG DNA test.

F_1 population	Progeny (N)	Parents		2013-15	IndelG	
				Phenotype	Genotype	F_1 genotype segregation ratios
AR_Pop_1403	63	Female	A-893N	Nectarine	n | n	61 P | n [*1.0]
		Male	A-820	Peach	P | P	
AR_Pop_1405	60	Female	A-826	Peach	P | n	18 P | P : 31 P | n : 10 n | n [*0.32]
		Male	A-880	Peach	P | n	
AR_Pop_1406	122	Female	A-874N	Nectarine	n | n	119 P | n [*1.0]
		Male	A-872	Peach	P | P	
AR_Pop_1414	20	Female	A-768N	Nectarine	n | n	20 n | n [*1.0]
		Male	A-805CN	Nectarine	n | n	
AR_Pop_1415	33	Female	A-868CN	Nectarine	n | n	33 n | n [*1.0]
		Male	A-861CN	Nectarine	n | n	

Table 10. The PpCCD4b-SSR DNA test genotypes and white or yellow flesh phenotypes for parents of the five 2014 F_1 populations, and the genotype segregation ratios for the five populations [*The chi-square p-value in brackets]. Capitalized letters are dominant to lower-cased letters for PpCCD4b-SSR DNA test.

F_1 population	Progeny (N)	Parents		2013-15	PpCCD4b-SSR	
				Phenotype	Genotype	F_1 genotype segregation ratios
AR_Pop_1403	63	Female	A-893N	White	W | W	59 W | y [*1.0]
		Male	A-820	Yellow	y | y	
AR_Pop_1405	60	Female	A-826	Yellow	y | y	32 W | y : 24 y | y [*0.29]
		Male	A-880	White	W | y	
AR_Pop_1406	122	Female	A-874N	White	W | W	55 W | y : 53 W | W [*0.85]
		Male	A-872	White	W | y	
AR_Pop_1414	20	Female	A-768N	White	W | y	4 W | W : 16 W | y : 4 y | y [*0.26]
		Male	A-805CN	White	W | y	
AR_Pop_1415	33	Female	A-868CN	Yellow	y | y	12 W | y : 20 y | y [*0.16]
		Male	A-861CN	White	W | y	

Cost per Sample for MASS at UA Horticulture Molecular Breeding Lab

The total cost per sample (295 individuals total) to perform DNA extraction, PCR, and fragment analysis for the two multiplexed DNA tests (IndelG and PpCCD4b-SSR) at the UA Horticulture Molecular Breeding Lab was calculated using the MASS Efficiency Calculator v1.0, and additional manual calculations based on costs of the specific reagents used for MASS at the UA program (Table 11) (Edge-Garza et al., 2016). After taking into account all maintenance costs from cross to initial selection or tree removal, it was calculated to cost approximately $12.00 per peach seedling to perform TSS at Clemson University in 2015 (K. Gasic, and C. Peace, personal communication).

This $12.00 cost per peach seedling was used as a reference for cost savings using MASS at the UA program (Fig. 16). The overall total cost to grow these 295 seedlings to maturity to perform TSS was calculated to cost ~$3,540. The total cost per seedling for all reagents necessary to perform DNA testing with both the IndelG and PpCCD4b-SSRs was calculated at $1.50 per seedling. The maintenance costs per initial seed, time spent to collect leaf tissue, screen the DNA tests, interpret the data, and perform culling was calculated at $1.50 per seedling. Taking into account both of these costs, the overall cost to perform MASS for each seedling was estimated at ~$3.00 per seedling, and when considering all 295 seedlings the total cost to perform MASS totals $885.00. Overall, for this example, the savings per seedling by using MASS was calculated to be $9.00. Overall, a substantial resource savings of ~$2,655 (75%) could be acquired through the incorporation of MASS for two DNA tests on only 295 seedlings (Fig. 16).

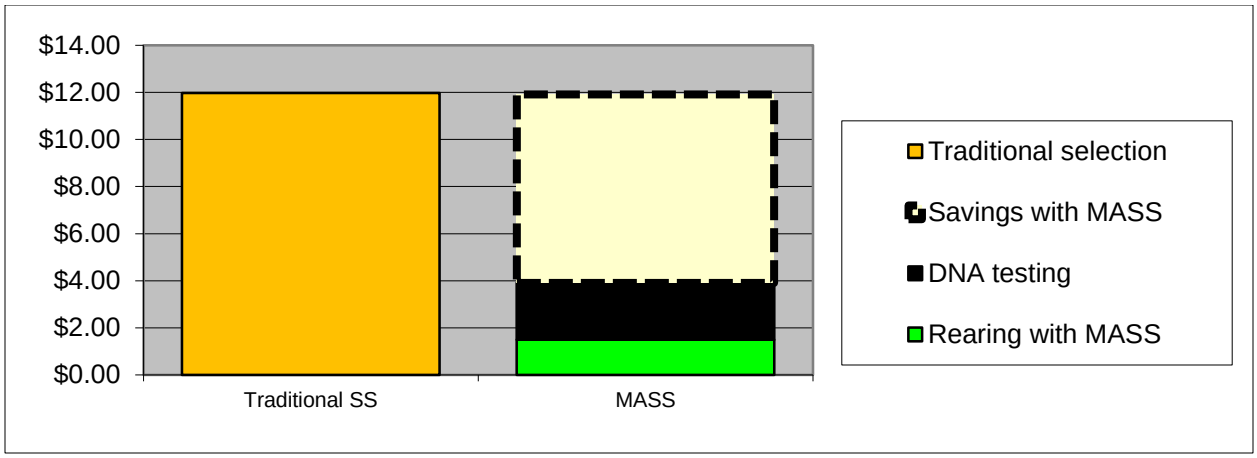

Fig. 16. Total cost per individual for traditional seedling selection (TSS), DNA testing, MASS rearing, and overall savings through MASS in the UA program 2015 [MASS Efficiency Calculator v1.0 (https://www.rosaceae.org/breeders_toolbox/MASScalc); Edge-Garza et al., 2016].

Table 11. Total cost per 96-well plate for DNA extraction, PCR, and fragment analysis at the UA. Total cost per sample for in-house MASS at the UA is listed at the bottom, at $1.50.

Items needed for DNA extraction			
Consumables / reagents	Units	Price ($)	Price per 96-well plate ($)
Latex gloves	100 / box	14.00	0.70
Deep 96-well extraction plates	50 / case	338.14	6.76
Sealing mats for 96-well plates	50 / box	212.77	4.25
Micro-tube 8-strip racked	50 / case	157.18	3.14
Paper towels	100 / case	1.73	0.34
Pipette tips 1 ml	1000 / bag	21.67	4.33
Pipette tips 200 ul	1000 / bag	19.70	0.20
SDS	100 mg / 1L	95.00	0.74
Tris HCL	1 L	30.00	0.15
EDTA	1 kg	97.16	0.60
PVPP	100 g	64.32	3.84
Ammonium acetate	2.5 kg / 5 L	60.92	0.15
Ethanol	3.78 L	20.00	0.16
Isopropanol	3.78 L	17.00	0.11
Total cost per 96-well plate for DNA extraction			25.47
Items needed for PCR			
Consumables / reagents	Units	Price ($)	Price per 96-well plate ($)
Latex gloves	100 / box	14.00	0.70
96-well PCR plates	100 / case	211.30	2.11
Sealing film	100 / box	66.54	0.66
Pipette tips 1 ml	1000 / bag	21.67	4.33
Pipette tips 200 ul	1000 / bag	19.70	0.20
1.5 ml microcentrifuge tubes	250 / bag	22.97	0.46
Taq polymerase (Promega)	2500 U	151.14	3.02
dNTP mix 10 MM (Omega)	2.5 ml	156.24	3.12
10X PCR MM (Promega)	2000 rxns	646.72	31.04
Total cost per 96-well plate for PCR			45.64
Items needed for fragment analysis			
Consumables / reagents	Units	Price ($)	Price per 96-well plate ($)
Latex gloves	100 / box	14.00	0.70
TE buffer (Tris HCL + EDTA)	0.1 M	1.20	0.20
FS96 kit 3000 resolutions	3000	2040.00	68.00
Total cost per 96-well plate for in-house fragment analysis			68.90
Total cost per 96-well plate for in-house MASS at the UA			140.01
Total cost per sample for in-house MASS at the UA (considering 93 samples per 96-well plate)			1.50

Discussion

The work herein serves two purposes: first, as a starting platform for the use of MAS in the UA program, which will continue to expand and evolve as additional DNA tests for the same or other breeding-relevant traits are developed and incorporated. Secondly, this reports serves as a case study for the incorporation of MAS into an already successful UA peach breeding program, and to highlight the feasibility and value of incorporating MAS into other Rosaceae tree fruit breeding programs (Edge-Garza et al., 2016; Ru et al., 2015). The most efficient method, SNP-based, or SLP-based (SSR / indel), or both, was determined for each trait studied in the UA program, for subsequent incorporation of MAPS, MACS, and MASS. Lastly, the deployment of MASS in 2015 for two of these DNA tests, indelG and PpCCD4b-SSR, was performed as a trial run.

Marker Assisted Parent Selection (MAPS) and Marker Assisted Cross Selection (MACS)

SNP haplotypes and SLP-based DNA tests each have their positives and negatives, and are both widely used. Enabling the same DNA test to be screened across both platforms gives breeders more options, and thus should further entice adoption of MAS. In this dissertation and other manuscripts, the robustness of the SNP-based and SLP-based DNA tests were determined by screening them across a wide array of germplasm in the UA program as well as three other RosBREED demonstration peach breeding programs (Clemson University, Texas A&M University and University of California, Davis) (Chapter Four; Chapter Five, Sections One-Three; Salgado, 2015; Sandefur et al., 2016a; Sandefur et al., 2016b). The most robust method, SNP-based, or SLP-based (SSR / indel), for each trait in the UA program was decided on and displayed in a format useful for the UA breeder to accurately and efficiently perform MAPS, MACS, and MASS, termed UA MAS breeder template v1.0. The robustness of a DNA test refers to the reliability of the DNA test to correctly predict the phenotypic variation for the desired

germplasm. This is critical to determine, otherwise the DNA test could be misleading (Bliss, 2010; Collard et al., 2005; Collard et al., 2008; Ru et al., 2015). It's important to understand that a DNA test is only robust for the mutational lineages it was developed from, and that it's always possible for interactions between the DNA test and other loci in the genome (epistatic, dominant, additive, etc.), which could impact it's robustness. Further, the distance the DNA test is from the candidate gene(s) can also impact it's robustness, as recombination during meiosis could lead to false positives if the DNA test is not linked close enough (< 5 cM from the candidate gene is adequate, but < 1 cM or inside the candidate gene is highly desired) (Bliss, 2010; Collard et al., 2005; Collard et al., 2008; Ru et al., 2015).

Intragenic Markers

In terms of the three intragenic markers; indelG for fruit type (pubescent vs. glabrous), PpCCD4b-SSR for flesh color (white vs. yellow), and EndoPG1+6 for fruit texture and adherence, all three markers were more predictive than their six or 11-SNP DNA tests, and were thus included in the UA MAS breeder template v1.0. In this case, one SLP-based (SSR / indel) DNA test is more economical and simple to use than six or 11-SNPs, since PCR reactions are reduced, resulting in time and cost savings. Moreover, further resource and time savings can be achieved through multiplexing these SLP-based (SSR / indel) DNA tests with other DNA tests for fruit quality, phenology, or disease resistance traits, and pooling for analysis on a range of genotyping platforms, including but not limited to agarose gel, polyacrylamide gel, the Fragment Analyzer™, or ABI Prism 3730xl DNA Analyzer. Even though these DNA tests are intragenic and highly predictive of their respective phenotype, it's important to take into account the initial mutational event that occurred which lead to the pubescent vs. glabrous alleles (at the PpeMYB25 candidate gene), the white vs. yellow flesh alleles (at the PpCCD4.1 candidate gene), and different flesh adherence and texture alleles (at the EndoPG locus).

Recent studies have determined that freestone and melting traits are controlled by a single locus, now referred to as the Freestone-Melting (*F-M*) locus, which has two copies of the same gene [endopolygalacturonase (endoPG)] flanking each other near the end of LG4 (Peace et al., 2005, 2007). The first copy controls the melting locus and the other controls the flesh adhesion to the pit (freestone trait) (Peace et al., 2007). Thus, the *F-M* locus has four alleles controlling both traits; individuals can be characterized as freestone melting flesh (FMF), clingstone melting-flesh (CMF), clingstone non-melting flesh (CNMF), and clingstone non-softening flesh (CNSF) (Peace et al. 2005, 2007). The NMF peach texture originated from a partial deletion in the endoPG gene, thus the last stage of softening does not occur (Peace et al., 2007). On the other hand, the NSF texture type arose from a complete deletion of the endoPG gene at the *F-M* locus (Peace and Norelli, 2009).

In contrast, Vendramin et al. (2014) proposed that the glabrous allele originated from only one loss-of-function mutational event, a Ty1-copia retrotransposon insertion in the third exon of the PpeMYB25 transcription factor gene which positively regulates trichome formation in peach fruit. The indelG DNA test was 100% predictive across a wide array of UA material (see Chapter Five, Section Two), as well as all previous material in Vendramin et al. (2014); thus it's apparent that the hypothesis by Vendramin et al. (2014) is valid.

However as with any trait, there will always be the possibility of new mutations occurring in the PpeMYB25 candidate gene, as seen with the PpCCD4.1 candidate gene for white vs. yellow flesh color in Falchi et al. (2013). In their study, Falchi et al. (2013) showed that yellow flesh alleles across 37 peach and nectarine cultivars had arisen from three independent mutational events, and that the PpCCD4-SSR could only accurately differentiate yellow from white flesh for the first mutational event. Falchi et al. (2013) developed two additional DNA

tests predictive of the other two mutational events, and thus breeders can now screen for one, or all three, depending on the mutational lineages in their program. Thus it's important to understand that a DNA test is only predictive for the mutational lineages it was developed for. At the UA program, it's apparent that only the first mutational event lineage, which can be screened for using the PpCCD4b-SSR, is found within the program's material (see Chapter Five, Section Two). Nonetheless, if the UA breeder wants to be sure the mutant white flesh allele is not in their program, or new germplasm is intended to cross with, one can also screen for the LTR retroelement insertion as described in Falchi et al. (2013).

Traits With Both DNA Tests Equally Predictive

The SNP-based and SLP-based (SSR) DNA tests for blush overcolor (*Rf* locus), *Xap* fruit resistance (*Xap6* locus), and acidity and sugar level (*G7Flavor* locus) were equally predictive across all material studied. However, more individuals were screened with the SLP-based (SSR) DNA tests, thus only the genotypes for these DNA tests were included in the UA MAS breeder template v1.0. As with the case above, currently it's still more economical to screen one SLP-based (SSR) DNA test to characterize two of these loci, *Rf* locus (Ppe-R_f-SSR) and *Xap6* locus (Ppe-XapF6-SSR), than four SNPs (*Rf* and *G6XapF*). Both tests can be multiplexed with other DNA tests, and pooled for analysis on a range of genotyping platforms.

In contrast, for the third locus, *G7Flav*, two SNPs and two SSRs were necessary to make the SNP-based and SLP-based (SSR) DNA tests predictive. In this scenario, high resolution melt (HRM) SNP-based genotyping is comparable in resource and time savings to that of the SLP-based (SSR / indel) tests using fragment analysis. Primers of comparable price to the SLP-based tests can be bought, and HRM can be performed for each SNP on a Real-Time PCR System (qPCR). The main advantage is that fragment analysis is not required as the SNPs are called during qPCR, which reduces price per sample significantly. As to which method saves more

resources and time, this is dependent on the viewpoint of the researcher. However, in any case, both SNP-based and SLP-based (SSR) DNA tests were equally predictive for all three traits, which gives breeders more options as to which DNA test platform they decide to proceed with.

Traits With Only One Predictive DNA Test

In terms of the *Xap1* locus, the 4-SNP *G1XapF* haplotype test was predictive across the UA material studied as well as the entire RosBREED pedigree. However, it was apparent that the Ppe-XapF1-SSR was too far away from the candidate gene(s) responsible for the resistance, thus only *G1XapF* alleles are depicted in Table 7. The 4-SNP *G1XapF* haplotype test spans the entire *Xap1* locus, ~12.9 – 15.0 Mbp on LG1, while the Ppe-XapF1-SSR only flanks the *Xap1* locus near the last SNP at ~15.0 Mbp on LG1 (see Chapter Four and Chapter Five, Section One for more details). This is a very large gap, spanning ~2.0 Mbp between the Ppe-XapF1-SSR and the first SNP at ~12.9 Mbp, and moreover this locus is located relatively near the top of LG1 (in general less recombination is known to occur the closer the marker is to the centromere), thus it became apparent that recombination occurred too frequently between the SSRs alleles and the actual gene/s responsible for the resistance, deeming the Ppe-XapF1-SSR not robust enough to use in MAS at the UA program. Nonetheless, the 4-SNP *G1XapF* haplotype test was predictive enough and thus can be used until an SSR test is developed which is located closer to the gene(s) responsible for the trait. In this scenario, HRM can be performed on a qPCR system to screen each SNP. As stated before, HRM SNP-based genotyping is comparable in resource and time savings to that of the SLP-based (SSR) tests. Promisingly, preliminary testing of these four SNPs has been performed in collaboration with Dr. Seonghee Lee on a HRM machine at the University of Florida using a subset of individuals, which represented all haplotypes possible at this locus from the 9K SNP results. The results from HRM and the 9K matched for each individual tested,

and currently additional individuals are being tested for further confirmation to determine if this test can be used for MASS at the UA program.

Yet, together the *Xap1* and *Xap6* loci are only associated with ~15% of the V_P for *Xap* fruit resistance/susceptibility. Fortunately, through pedigree-based QTL analysis (PBA) using the UA pedigree, two additional major loci for fruit and leaf bacterial spot on LG1 (*G1XapF+L.1*, and *G1XapF+L.2*) and LG2 (*G2XapL+F.1*, and *G2XapL+F.2*), as well as one locus for fruit and leaf bacterial spot on LG5 (*G5XapL+F.1*) and LG6 (*G6XapF+L.1*) were identified which represent more of the Vp % for *Xap* fruit and leaf resistance/susceptibility (Chapter Two). These major QTLs are prime candidates for SNP and SLP-based (SSR/indel) based DNA test development to become the next set of DNA tests to use with the 4-SNP *G1XapF* haplotype, and the Ppe-XapF6-SSR to combine horizontal fruit and leaf bacterial spot resistance with high fruit quality.

While the CPPCT040b-SSR was predictive across ~90% of the material studied (Salgado, 2015), the *D* locus SNP_IGA_545261 was ~ 98% predictive for all individuals screened with the 9K array (Chapter Four). Unfortunately, it's apparent that SNP genotyping errors occurred for SNP_IGA_545261 when the 72 UA selections and the other 168 selections/cultivars from the other three RosBREED breeding programs were screened with the Peach mini-SNP array v1.0. This was apparent since positive control individuals (those also screened with 9K SNP array) were also included, and their alleles for SNP_IGA_545261 did not match across both platforms (Chapter Four). The positive control individual SNP_IGA_545261 alleles were predictive of high vs. low acidity when using the SNP_IGA_545261 from the 9K SNP array, but were not predictive of high vs. low acidity when using the SNP_IGA_545261 from the Peach mini-SNP array v1.0. Thus for current application and use, only the CPPCT040b-

SSR genotypes for all UA individuals are depicted in Table 7. It's important to note however, that the CPPCT040b-SSR is only 90% predictive, thus it's not advisable to use in MASS. Instead, as with the 4-SNP *G1XapF*, primers for the SNP_IGA_545261 could be designed to test this SNP over HRM on a subset of individuals (again using positive controls) to confirm their correct genotypes for this SNP. Once promising primers have been identified, which correctly genotype individuals for the SNP_IGA_545261, then all UA selections can be screened over HRM, and the UA breeder can proceed with this marker (that has been shown to be ~ 98% predictive) for MAPS, MACS, and MASS. Even better yet, the candidate gene could one day be characterized, and an intragenic marker could be developed which would be 100% predictive.

The genotypes of the *SMF* locus SNP, ss_414220, and the SMF-SSR, matched for all individuals, but more individuals were screened with the SMF-SSR, thus only SMF-SSR genotypes of individuals that have been also phenotyped for ethylene production, are depicted in Table 7 (Salgado, 2015). Unfortunately, this single SNP was only able to be identified using 36 individuals from a single F_1 population (AR_Pop_1), and a SNP trait association file which only included SNPs from LG3 and LG4 (due to processing limitations of Excel® (Microsoft, Redmond, WA). This is a very low number of individuals, from only one population, and did not take into account the effects of all SNPs genome wide (LG1-LG8). Therefore, this could have greatly overestimated the effects of the initial *SMF* locus SNP ss_414220 identified. Furthermore, the same locus could not be identified using QTL analysis for the bi-parental population, nor PBA using the entire UA pedigree. Additionally, Salgado, (2015) noted that these DNA tests were not robust across all material genotyped and phenotyped (Salgado, 2015). She hypothesized that certain early season alleles at the G4Mat could be epistatic and dominant to the SMF locus, leading to false positives (Salgado, 2015). While this hypothesis could be

valid, another hypothesis is that a single mutation event has lead to this novel flesh texture in the UA program (A-392) (J.R. Clark, personal communication), and thus the ss_414220 and SMF-SSR SMF alleles are only robust from this lineage. 'White County' is the mother of AR_Pop_1, and the mother of this cultivar was A-392, which could explain how the *SMF* locus SNP ss_414220 was originally identified using the 36 seedlings from this population and the LG3 and LG4 SNP trait association file. Yet, to determine either of these hypotheses, additional phenotypic data (ethylene production) on more individuals, and pedigree tracking for all individuals desired to cross with will be imperative before using in MASS. Otherwise the alleles could be misleading. For the single mutational event hypothesis, pedigree tracking will be vital to determine if the SMF allele from the SNP-based and or SSR-based DNA tests is identical by descent (IBD) from the A-392 lineage, and thus robust, or only identical by state (IBS), and thus misleading. The UA breeder has already been keeping track of individuals with A-392 in their background, thus this pedigree knowledge coupled with either of the two SMF DNA tests, could be deemed robust enough to differentiate SMF alleles, and ultimately give the UA breeder enough confidence to proceed with MASS using either of these two DNA tests. Lastly, both SNP-based and SLP-based (SSR) DNA tests for SMF were equally predictive thus the breeder now has two comparable DNA test options (in cost and research time) to proceed with.

Last, but not least, in regards to the maturity date locus (*G4Mat*), the 4-SNP haplotype was more predictive than the combined three SSR markers. While the candidate gene(s) can continue to be studied within the *G4Mat* locus, the 4-SNP haplotype was shown to be very robust across all four RosBREED peach breeding programs and accounted for ~50% V_P for maturity date in the UA program (Chapter Three; Chapter Four). This 4-SNP haplotype was vital to determining that the UA program can still extend it's season by at least a few weeks using

current germplasm, since very few late (L) and very late (VL) alleles are found within the program, and only one selection is homozygous for VL [A-789 (VL1 | VL2)]. In 2013-2015, MAPS and MACS was performed using the results from the *G4Mat* 4-SNP DNA test, coupled with the CPPCT040b-SSR, EndoPG1+6, indelG, PpCCD4b-SSR, Ppe-R_f-SSR, Ppe-XapF6-SSR, and 4-SNP *G1XapF* haplotype DNA tests to diversify the later part of the UA peach season in a efficient manner (late peach and nectarines with both melting, non-melting, and non-softening flesh types, white and yellow flesh, high and low acid, high blush, and resistance to fruit bacterial spot). As with the 4-SNP *G1XapF* DNA test and the *D* locus SNP, SNP_IGA_545261, preliminary testing of these four SNPs has been performed by Dr. Seonghee Lee on his HRM machine at the University of Florida on a subset of individuals, which represented all haplotypes possible at this locus from the 9K SNP results. The results from HRM and the 9K matched for each individual tested, and thus additional individuals should be tested for further confirmation, before this test can be used for MASS at the UA program.

Marker Assisted Seedling Selection (MASS)

Yet, even after a DNA test has been validated for use in MASS, unfortunately, the tool can't be put into use until the logistics of organizing seedlings in the greenhouse, collecting leaf tissue, and identifying an economical platform for DNA extraction, PCR, allele sizing, and processing of data for subsequent culling of seedlings in the greenhouse have been developed. Interestingly, a questionnaire given out in 2013 to assess the level of MASS implementation in apple, sweet cherry, tart cherry (*P. cerasus* L.), strawberry (*Fragaria* × *ananassa* Duch.) and peach RosBREED demonstration tree fruit breeding programs revealed that the most prevalent challenge perceived by Rosaceae fruit breeders to perform MAS was in fact the difficulty in logistically enabling smooth integration of DNA testing into traditional breeding operations (Ru

et al., 2015). The main reason for this perceived challenge could be due to the fact that successful DNA testing requires expertise in molecular data interpretation and management, yet this expertise is often lacking in breeding programs new to MASS (Ru et al., 2015).

Thus, the first step to perform MASS is to develop the logistics for greenhouse organization and leaf collection. Organization should not be overlooked, since sample organization throughout all subsequent steps is absolutely critical for success. Secondly, detail-oriented workers (or robots) need to be trained to collect the leaf tissue into organized 96-tube microracks (Edge-Garza et al., 2014). The 96-well greenhouse and leaf collection organization method used for MASS in previous studies has been noted to greatly reduce potential errors that can occur on a large-scale MASS project (K. Evans and C. Peace, personal communication).

To test MASS in 2015, three 28-well flats were organized together to mimic the 96-well DNA plate map. The F_1 seedlings' genotypes for all five 2014 populations fit their expected parental segregation ratios for both the indelG and PpCCD4b-SSR DNA tests, indicating that this greenhouse organizational method was adequate to test MASS in the UA program on a small scale. However, it still leaves an entire blank row (H), and thus before performing large-scale MASS, it's highly advised to learn from the previous experiences at the WSU apple breeding program, and design or buy 96-well flats to plant seedlings. This complete 96-well flat method greatly enhances the likelihood that marker data points match seedlings, and thus the correct individuals to be culled can be identified (K. Evans and C. Peace, personal communication). The upfront costs to build or buy these 96-well flats will substantially outweigh the potential errors that could occur using the three, 28-well flat method.

Next, through incorporation of MASS for indelG and PpCCD4b-SSR DNA tests on the 295 seedlings, substantial resource savings of ~$2,655 (75% less than TSS) were noted, if

culling was performed. The $12.00 cost per peach seedling to perform TSS at Clemson University was used as a reference for cost savings using MASS at the UA program. These resource savings are comparable to those noted for the WSU apple and the PNW sweet cherry breeding programs (50-60% and 70-80% less than TSS using one and two DNA tests, respectively, for culling). Even more noteworthy, in general as more seedlings are screened, more DNA tests are used (in sequence rather than together) and culling rates are increased, the TSS costs can be even further reduced (C. Peace, personal communication; Edge-Garza et al., 2016). However, it's important to keep in mind the initial investment required to set up the infrastructure to perform MASS, such as trained personnel with molecular expertise as well as equipment and supplies (Ru et al., 2015).

This first round of MASS at the UA only served as a trial run, since all seedlings were planted and later re-labeled with functional allele information in the field at FRS for additional field confirmation in 2017. Now that the logistics have been put in place, the UA breeder will be able to significantly benefit from the increase in genetic gain per breeding cycle, improved selection efficiency, and significantly reduce breeding program operational costs brought forth through the use of MASS (Bliss, 2010; Byrne, 2005; Edge-Garza et al., 2016; Ru et al., 2015).

Furthermore, while outsourcing is quick and easy, performing MASS in-house is considerably more economical as price per sample can be reduced by >70% (C. Peace, personal communication). Through this study, the UA program in now set up to perform all in-house MAS, so resource savings can be maximized. Moreover, all the theoretical and practical knowledge required for molecular data interpretation and management has been successfully passed on to the breeding program associate, so that these tools can evolve over time in the peach program as well as extend to the blackberry (*Rubus* subgenus *Rubus* Watson), and muscadine

grape (*Vitis rotundifolia* Michx.) breeding programs, upon development of DNA tests for these species.

Overall Conclusions

This work serves as a starting platform for the use of MAS in the UA program, which will continue to expand and evolve as additional DNA tests for the same or other breeding-relevant traits are developed and incorporated. Most noteworthy is an array of significant QTLs for *Xap* resistance, fruit quality, and phenological traits that were identified through pedigree-based QTL analysis (PBA) using the UA pedigree in Chapters Two and Three of this dissertation. These major QTLs are prime candidates for SNP and SLP-based DNA test development to become the next set of tools added to the UA molecular peach breeding toolbox. These include: three major QTLs for fruit weight and fruit diameter (*G2FW.1*, and *G4FW.1*); a second major QTL for soluble solids content upstream of the *G7Flav* locus (*G7SSC.2*), as well as a third major locus for SSC on LG4 (*G4SSC.1*); two additional major loci for fruit and leaf bacterial spot on LG1 (*G1XapF+L.1*, and *G1XapF+L.2*) and LG2 (*G2XapL+F.1*, and *G2XapL+F.2*), as well as one locus for fruit and leaf bacterial spot on LG5 (*G5XapL+F.1*) and LG6 (*G6XapF+L.1*) (see Chapters Two and Three for more information).

The future is bright for the UA program, as the stage has been set for continued use of MAS, which will enable: increased genetic gain per generation cycle, resource savings, and extension of MAS into the blackberry and muscadine grape breeding programs upon successful development of DNA tests (Edge-Garza et al., 2010; Edge-Garza et al., 2016; Evans et al., 2012; Rowland et al., 2012; Ru et al., 2015). Moreover, this case study, of the incorporation of MAS into an already successful traditional peach breeding program, highlights the feasibility and value of incorporating MAS into other Rosaceae tree fruit breeding programs (Edge-Garza et al., 2016;

Ru et al., 2015). Ultimately through incorporation of MAS tools, other Rosaceae tree fruit breeders can also make more informed decisions, which will enable them to save time and resources, as well as increase the efficiency of combining all desired fruit traits into the next set of Rosaceae tree fruit cultivars spanning the season, to meet growers', distributers', marketers', and consumers' evolving demands (Bliss, 2010; Byrne, 2005; Dirlewanger et al., 2004; Ru et al., 2015).

Literature Cited

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.

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

Byrne, D.H. 2005. Trends in stone fruit cultivar development. HortTechnology 15:494–500.

Byrne, D.H., M. Bassols, D. Bassi, M. Piagnani, K. Gasic, G. Reighard, M. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: M. Badenes and D. Byrne (eds.). Fruit breeding. Springer Science, Business Media, New York.

Clark, J.R., A.B. Aust, and R. Jondle. 2012. Intellectual property protection and marketing of new fruit cultivars, p. 69-96. In: M. Badenes and D. Byrne (eds.). Fruit breeding. Springer Science, Business Media, New York.

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.

Collard, B.C.Y., and D.J. Mackill. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B: Biol. Sci. 363: 557-572.

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

Crisosto C.H., and G. Costa. 2008. Preharvest factors affecting peach quality, Ch. 20:536-547In: Folta K.M., Gardiner S.E. (eds.) Genetics and Genomics of Rosaceae. Series Ed Jorgensen R. Springer, New York, Plant Genetics / Genomics, Vol 6.

Crisosto C.H., F.G. Mitchell, and R.S. Johnson. 1995. Factors in fresh market stone fruit quality. Postharvest News and Info. 6:17-21.

Dirlewanger, E., E. Graziano., T. Joobeur., F. Garriga-Caldere., P. Cosson., W. Howad, and P. Arus. 2004. Comparative mapping and marker-assisted selection in Rosaceae fruit crops. Proc. Natl. Acad. Sci. U.S. 101:9891-9896.

Dirlewanger, E., P. Cosson., K. Boudehri., C. Renaud., G. Capdeville., Y. Tauzin., F. Laigret, and A. Moing. 2007. 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. and Genomes 3:1-13.

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.

Edge-Garza, D.A., C.P., Peace, C.P. and Y. Zhu. 2010. Enabling marker-assisted seedling selection in the Washington Apple Breeding Program. Acta Hort. 859:369-373.

Edge-Garza, D., T. Rowland, S. Haendiges, and C. Peace. 2014. A high-throughput and cost-efficient DNA extraction protocol for the tree fruit crops of apple, sweet cherry, and peach relying on silica beads during tissue sampling. Mol. Breeding 34:2225-2228.

Edge-Garza, D.A., J.J. Luby, and C.P. Peace. 2016. Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Mol. Breed. 35:1-15.

Eduardo, I., E. López-Girona, I. Batlle, F. Reig, I. Iglesias, W. Howad, P. Arús, and M.J. Aranzana. 2014. Development of diagnostic markers for selection of the subacid trait in peach. Tree Genet. and Genomes 10:1695-1709.

Evans, K.M., B. Konishi, L. Brutcher, D.A. Edge-Garza, T.J. Rowland, and C.P. Peace. 2012. The logistical challenges of marker-assisted seedling selection in an apple breeding program. Poster presentation at 6th Rosaceous Genomics Conference Mezzocorona, Italy.

Falchi, R., E. Vendramin, L. Zanon, S. Scalabrin, G. Cipriani, I. Verde, G. Vizzotto, and M. Morgante. 2013. Three distinct mutational mechanisms acting on a single gene underpin the origin of yellow flesh in peach. Plant J. 76:175–187.

Faust, M. and B. Timon. 1995. Origin and dissemination of the peach. Hort. Rev. 17:331–379.

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.

Food and Agricultural Organization of the United Nations (FAO). 2015. Peach and nectarine U.S. production, 1961-2014. August 25, 2015. <http://faostat3.fao.org/browse/Q/QC/E>.

Howad, W., T. Yamamoto, E. Dirlewanger, R. Testolin, P. Cosson, G. Cipriani, A.J. Monforte, L. Georgi, A.G. Abbott, and P. Arus. 2005. Mapping with a few plants: using selective mapping for microsatellite saturation of the *Prunus* reference map. Genet. 171:1305-1309.

Jung, S., S.P. Ficklin, T. Lee, C.-H. Cheng, A. Blenda, P. Zheng, J. Yu, A. Bombarely, I. Cho, S. Ru, K. Evans, C. Peace, A.G. Abbott, L.A. Mueller, M.A. Olmstead, and D. Main. 2014. The genome database for rosaceae (GDR): year 10 update. Nucl. Acids Res. 42:D1237-D1244.

Okie, W.R., T. Bacon, and D. Bassi. 2008. Fresh market cultivar development, p. 139–174. In: D.R. Layne and D. Bassi (eds.), The peach – botany, production and uses. CAB International.

Peace C.P., A. Callahan, E.A. Ogundiwin, D. Potter, T.M. Gradziel, F.A. Bliss, and C.H. Crisosto. 2007. Endopolygalacturonase genotypic variation in *Prunus*. Acta Hort. 738:639-646.

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. and J. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae, pp. 19-53. In K. Folta and S. Gardiner (Eds). Genetics and genomics of Rosaceae. Springer Science + Business Media, New York.

Rowland, T. Jr, D. Edge-Garza, N. Oraguzie, and C.P. Peace. 2012. Routine marker-assisted seedling selection in the Pacific Northwest sweet cherry breeding program provides resource savings. Poster presentation at 6th Rosaceous Genomics Conference, Mezzocorona, Italy.

Ru, S., D. Main, K. Evans, and C. Peace. 2015. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet. and Genomes 11:1-12.

Salgado, 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. Ph.D dissertation University of Arkansas, Fayetteville, U.S.

Sandefur, P. 2011. Characterization and molecular analysis of University of Arkansas peach, *Prunus persica* (L.) Batsch, flesh types and development of a postharvest evaluation protocol for Arkansas peach and nectarine genotypes. MS thesis University of Arkansas, Fayetteville, U.S.

Sandefur, P., T. Frett, J. Clark, K. Gasic, and C. Peace. 2016a. PpeRf-SSR, a DNA test for routine prediction in breeding of peach blush. Mol. Breeding (In press).

Sandefur, P., T. Frett, A. Salgado, J. Clark, K. Gasic, and C. Peace. 2016b. Ppe-Acidity, a combined DNA test for routine prediction in breeding of acidity and soluble solids content (SSC) in peach. Mol. Breeding (In press).

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

Smith, S. 2015. Fruit tree diseases. 16 November 2015. <https://www.uaex.edu/publications/pdf/mp154/fruit-tree-diseases-commercial.pdf>.

Studebaker, G., J. Hopkins, and D. Johnson. 2015. Control peach tree borers on commercially grown peach and plum trees. 16 November 2015. <https://www.uaex.edu/publications/PDF/FSA-7504.pdf>.

Vendramin, E., G. Pea, L. Dondini, I. Pacheco, M. T. Dettori, L. Gazza, S. Scalabrin F. Strozzi, S. Tartarini, D. Bassi, I. Verde, and L. Rossini. 2014. A unique mutation in a MYB gene cosegregates with the nectarine phenotype in peach. PloS ONE 9:e90574. DOI: 10.1371/journal.pone.0090574.

Chapter Seven: Diversity and Virulence of XAP Isolates from 12 U.S. Locations

Abstract

Xanthomonas arboricola pv. *pruni* (*Xap*) has been documented to have a low genetic diversity in comparison to other *X. arboricola* pathovars. However, even with potentially low genetic diversity, there are thought to be substantial differences in *Xap* populations and their degree of virulence on peach and other stone fruits. Furthermore, virulence of specific strains have been shown to vary among cultivars. Yet all these previous studies were limited by a low number of isolates, from a low number of locations, thus further studies should be undertaken to document differences in virulence of *Xap* strains in the U.S. In the study herein *Xap* isolates were collected from 12 U.S. states (including Arkansas), with the goal to study the diversity and virulence of strains from different locations. In total, 197 out of 471 single colony isolates screened positive for *Xap* using the Y17Co primers. Next eight polymorphic SSR markers were designed and screened on a representative set of 48 *Xap* isolates. A phylogenetic tree based on presence/absence of alleles for eight SSR markers for all 48 *Xap* isolates was generated. The phylogenetic tree showed two major groupings of the 48 *Xap* isolates: the monophyletic "group 1" contained the majority of isolates from northern and eastern states (New Jersey, Michigan, and Virginia), while the monophyletic "group 2" contained a major clade which included the majority of isolates from central states (Arkansas and Kentucky). These results provide evidence that there is a potential difference in *Xap* diversity in Central states, in comparison to the northern and eastern states. These results suggest that *Xap* indeed has a high genetic diversity, which differs from the low *Xap* diversity noted in previous studies. Next, 113 of the 197 *Xap* isolates were screened on detached leaves from 'Crimson Lady' (*Xap* susceptible) and A-772 (*Xap* resistant) genotypes to differentiate virulence levels. In total, 61 *Xap* isolates (~54% out of 113 isolates) were highly virulent on 'Crimson Lady' and A-772, and thus deemed highly

virulent strains. In terms of the additional 22 *Xap* isolates which were also highly virulent on 'Crimson Lady', 19 of them showed only medium virulence, and three showed low virulence on A-772. Likewise, more *Xap* isolates showed moderate and low virulence on the resistant cultivar, A-772, in comparison to the susceptible cultivar, Crimson Lady. In total, 52 *Xap* isolates (46%) showed moderate or low virulence on A-772, in comparison to only 30 *Xap* isolates (26%) with moderate or low virulence on 'Crimson Lady'. Lastly, there were 17 *Xap* isolates which showed low virulence on 'Crimson Lady' and A-772, and thus were deemed endophytic *Xap* strains (those which live in the host, yet apparently don't cause disease). While the *Xap* diversity and virulence findings in the study herein are exciting, these are only preliminary results from which future studies using more high throughput methods: sequencing, alignment, and SNP identification for diversity tree can be expanded upon.

Introduction

Bacterial spot, caused by *Xanthomonas arboricola* pv. *pruni* (*Xap*, referring to the pathogen and disease in this chapter), is a serious disease impacting the peach industry worldwide (EPPO/CABI, 1997; OEPP/EPPO, 2006; Ritchie, 1995). Bacterial spot symptoms are generally characterized as various-sized necrotic lesions on leaves and fruits and cankers on twigs (EPPO/CABI, 1997; OEPP/EPPO, 2006; Ritchie, 1995). Overall, this disease leads to premature defoliation, weak vigor of the plant across years, unmarketable fruit with low fruit quality, and overall reduced productivity (Aarrouf et al., 2008; Ritchie, 1995).

Infection and development of *Xap* is highly dependent on environmental conditions. The disease is particularly devastating in warm, humid environments such as the eastern U.S. and other countries with similar climatic conditions. Locations with sandy soils are much more prone to *Xap* infection since wind-blown sand can create wounds for the bacterium to infect (EPPO/CABI, 1997; Ritchie, 1995). Periods with warm temperatures (≥18 °C) accompanied with light, frequent rains, extended heavy dews, very high humidity, along with the occurrence of substantial wind-blown rains during late bloom to pit hardening are most favorable for severe infection (Daines, 1961; EPPO/CABI, 1997; Randhawa and Civerolo, 1985; Ritchie, 1995; Zehr and Shepard, 1996). Conditions of at least 12 h of water saturation and high humidity (≥75%) are needed for infection to occur, develop, and spread (Ritchie, 1995; Zehr and Shepard, 1996). Typically once *Xap* is established in an orchard it is very difficult to control and the bacterium can spread rapidly, especially with highly susceptible cultivars (Ritchie, 1995).

The first step in control of *Xap* is to grow and maintain healthy trees (Ritchie, 1995; Ritchie, 1999). Trees under stress are more severely affected by *Xap* than those not stressed. This includes nutrient stress, and stress related to other pathogenic organisms such as nematodes.

Therefore, optimum soil fertility must always be maintained. In the peach industry, the primary commercial control of *Xap* has been to rely heavily on pesticides, such as copper-containing compounds and oxytetracycline. Correct timing of sprays and rates used are important. Relatively high rates of copper sprays are generally applied when only a limited amount of new growth is present early in the growing season, from dormancy through early shuck split. The idea of these applications is to cover the tree surface to create a barrier through which the bacterium must pass as it moves from overwintering sites. The conventional thought is that the majority of the inoculum dies in this process of moving through the copper barrier. Copper sprays are continued as new growth emerges but rates are reduced because they are known to cause fruit and foliage damage in peach, resulting in grayish discoloration, shot holes, and premature leaf drop. The compound oxytetracycline is then incorporated when shuck split commences since it is less damaging to the fruit and foliage (Ritchie, 1995; Ritchie, 1999).

Unfortunately, if favorable environmental conditions occur, the disease can still develop even when well-timed spray schedules have been utilized (Ritchie, 1995; Ritchie, 1999). Moreover, due to a variety of concerns (agricultural worker safety, environmental contamination, economic, and food safety), restrictions have been placed on the use and availability of chemicals. Integrated pest management programs (IPM) are being incorporated to only use chemical sprays as the last resort, and *Xap*-resistant cultivars play a crucial role in these IPM practices (Byrne et al., 2012; Sansavini et al., 2006). Yet, many good quality cultivars favored in the peach industry tend to be highly susceptible while cultivars with *Xap* resistance often lack desirable fruit quality characteristics required by the peach industry.

Bacterial spot resistance has been a key trait of interest in peach breeding at the University of Arkansas (UA). This program has focused on breeding for resistance to *Xap* since

1964. Drs. Jim Moore and Roy Rom initiated the UA peach and nectarine breeding program, and their successor Dr. John R. Clark currently directs it. Unlike most other peach breeding programs, antibiotic sprays to control the disease have never been applied, thus selection against *Xap* has been possible (J.R. Clark, personal communication). The warm and humid springs and summers at the University's Fruit Research Station (FRS) near Clarksville, AR, create an ideal environment for the inoculation and spread of this pathogen. This environment has enabled breeding and selection of peaches with low *Xap* incidence.

Bacterial spot of stone fruit is caused by *Xap*, a mobile (one flagellum), gram-negative, rod bacterium (Fig. 1). This species was first named *X. pruni* (Smith, 1903) and then classified as *X. campestris* pv. *pruni* by Dye in 1978. Later, the bacterium was reclassified as *X. arboricola* pv. *pruni* [Smith, 1903 (Vauterin et al., 1995)]. *X. arboricola* encompasses seven pathovars with different hosts and of these pathovars *pruni, corylina,* and *juglandis* are the most closely related in phylogenetic analyses (Hajri et al., 2012).

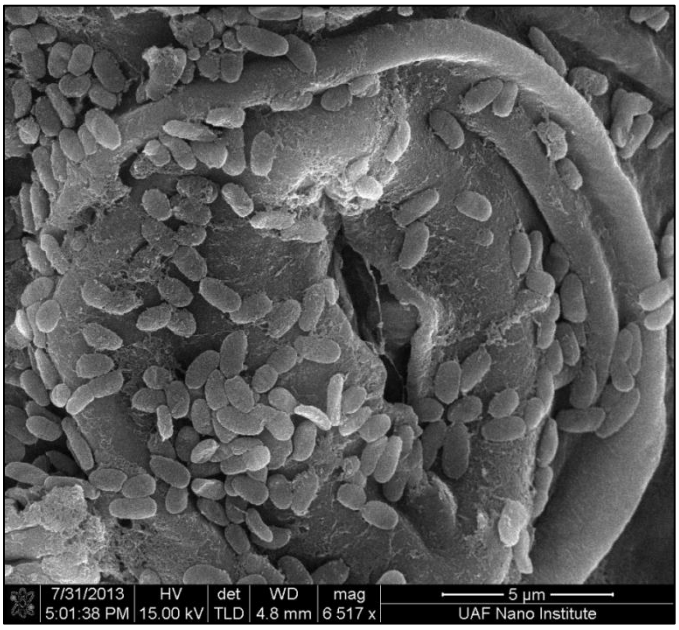

Fig. 1. A confocal microscope picture of *Xap* colonizing a peach leaf through its stomata (picture compliments of Dr. Burton Bluhm and Brant Smith, 2013, UA).

The *X. arboricola* species has been documented to have a low genetic diversity in comparison to other *X. arboricola* pathovars and partitioning at a geographical scale has not been observed (Hajri et al., 2012). However, *Xap* strain and population virulence levels are thought to differ substantially on peach and other stone fruits (Civerolo, 1975; OEPP/EPPO, 2006). Du Plessis (1988) and Scortichini et al. (1996) studied the difference in virulence of *Xap* isolates from different geographical areas and concluded that *Xap* varies in virulence among different cultivars. These combined studies were limited by a low number of isolates, contributing to a low amount of genetic diversity. Thus further studies should be undertaken to document differences in virulence of *Xap* strains in the U.S.

One method for assessing *Xap* resistance in peach is the detached-leaf bioassay which offers an inexpensive and rapid option for in vitro screening using peach leaves (Randhawa and Civerolo, 1985). A detached-leaf bioassay was performed in several previous studies (Civerolo and Keil, 1976; Hammerschlag, 1988; Hammerschlag et al., 1994; Randhawa and Civerolo, 1985). Leaves were inoculated and symptoms later classified using a qualitative '0-3' rating scale to differentiate symptoms of infections at each inoculation site (0 = no symptoms; 1 = distinct chlorotic spot and/or slight necrotic flecks; 2 = distinct but pale necrotic spot or grayish-white lesion, 2 mm in diameter; and 3 = distinct, dark necrotic spot of > 2 mm in diameter, with or without a chlorotic halo) (Civerolo and Keil, 1976; Hammerschlag, 1988; Hammerschlag et al., 1994; Randhawa and Civerolo, 1985). This detached-leaf bioassay is an effective method to screen for *Xap* resistance across a breeding program, as well as investigate the diversity and virulence of *Xap* isolates collected from multiple environments (Bock et al., 2010; Hammerschlag et al., 1994; Yang, 2012; Yang et al., 2013).

The objective of this study was to investigate the diversity and virulence of *Xap* isolates from Arkansas vs. other states, as there was observed susceptibility in *Xap* resistance in cultivars bred in other states. This study aims to classify isolates collected from 12 locations across the U.S.

Materials and Methods

Management Practices at FRS

Phenotypic evaluation for bacterial leaf spot resistance was conducted on peach and nectarine material at FRS (west-central AR, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept. of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]). All trees were either open-center trained and spaced 5.5 m between trees and rows, or trained to a perpendicular-V system with trees spaced 1.9 m in rows spaced 5.5 m apart. All trees were dormant pruned and fertilized annually with a single application of 640 Kg ha-1 of complete fertilizer (19:19:19 of N:P:K) and were sprinkler or drip irrigated as needed. Pests were managed using a program typical for commercial orchards in the area (Smith, 2015; Studebaker et al., 2015). After shuck split but before pit hardening, fruitlets were thinned to a distance of 12 to 15 cm between each fruitlet.

Xap Isolate Collection

To collect *Xap* isolates from Arkansas, leaves and fruit with bacterial spot symptoms were collected at FRS in April 2013 from the majority of peach seedlings of seven 2008 F_1 populations (Table 1). The leaves from these F_1 populations with high and low severity and an individual fruit with the highest severity were harvested from each genotype. Additionally, in April 2013, leaf samples showing *Xap* symptoms were collected from 11 additional locations throughout the U.S. by extension or research personnel at various universities, and shipped overnight to the Bluhm Lab (UA, Department of Plant Pathology) (Table 2; Figs. 2-4).

Table 1. The seven 2008 F_1 populations used for bacterial spot (*Xap*) leaf samples to collect the Arkansas *Xap* isolates.

F_1 population	Female parent	Male parent	F_1 seedlings (N)
AR_Pop_1	White County	A-672	48
AR_Pop_0801	A-776	A-783	16
AR_Pop_0803	A-765	A-778	15
AR_Pop_0813	A-772	A-672	12
AR_Pop_0817	A-789	A-699	9
AR_Pop_0819	A-708	A-773	23
AR_Pop_0825	Souvenirs	A-760	17

Table 2. The 12 different locations where bacterial spot (*Xap*) leaf samples were collected and provided to gather *Xap* isolates from locations across the U.S.

Institution	State	City	U.S. location
University of Arkansas	Arkansas	Clarksville	Central
Missouri State University	Missouri	Springfield	Central
University of Kentucky	Kentucky	Lexington	Central
Virginia Tech University	Virginia	Winchester	East
Michigan State University	Michigan	Benton Harbor	North
Penn State University	Pennsylvania	Biglerville	North East
Rutgers University	New Jersey	New Brunswick	North East
Auburn University	Alabama	Auburn	South
University of Florida	Florida	Gainesville	South
USDA-ARS Byron, GA	Georgia	Byron	South East
North Carolina State University	North Carolina	Raleigh	South East
Texas A&M University	Texas	College Station	South West

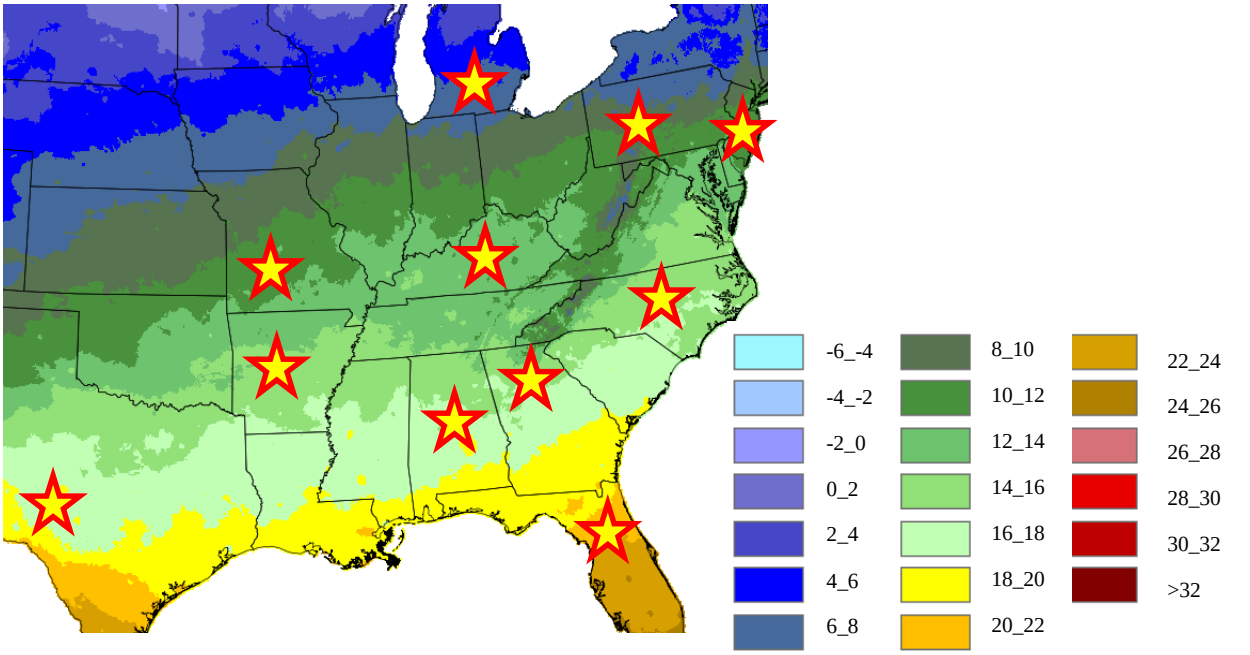

Fig. 2. April 2013 average daily temperature (°C) for 12 locations where peach leaves with *Xap* symptoms were collected (represented by stars) and provided to the University of Arkansas for the *Xap* isolate study (optimal temperature for *Xap* infection is ≥ 18°C) (Data from PRISM Climate Group©, Oregon State Univ., 2013) (National Climatic Data Center (NCDC) (http://cdo.ncdc.noaa.gov/climaps/rh2305.pdf).

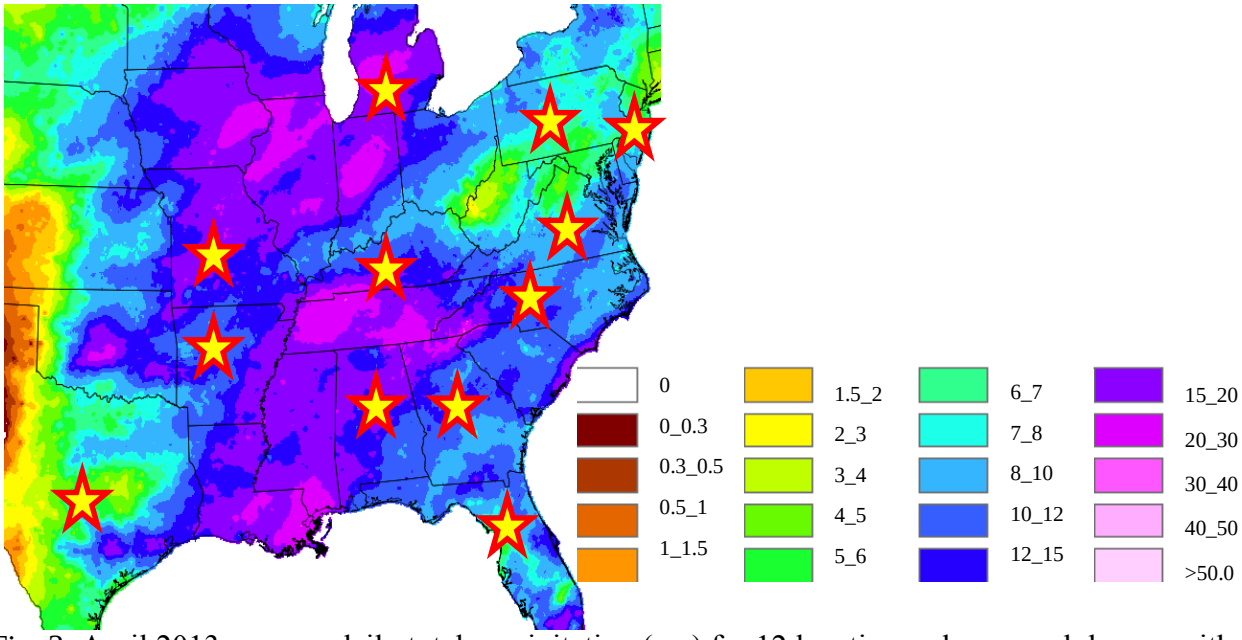

Fig. 3. April 2013 average daily total precipitation (cm) for 12 locations where peach leaves with *Xap* symptoms were collected (represented by stars) and provided to the University of Arkansas for the *Xap* isolate study (high rainfall is optimal for *Xap* infection) (Data from PRISM Climate Group©, Oregon State Univ., 2013) (National Climatic Data Center (NCDC) (http://cdo.ncdc.noaa.gov/climaps/rh2305.pdf).

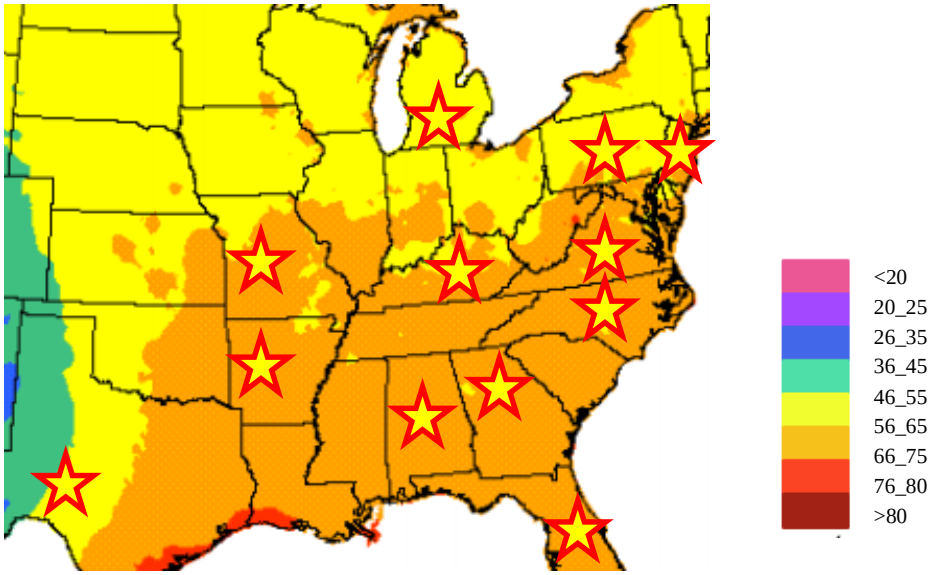

Fig. 4. April 2013 average daily humidity (%) for 12 locations *Xap* symptoms were collected from (represented by stars) and provided to the University of Arkansas for the *Xap* isolate study (optimal humidity% for *Xap* infection is ≥ 70%) (Data from PRISM Climate Group©, Oregon State Univ., 2013) (National Climatic Data Center (NCDC) (http://cdo.ncdc.noaa.gov/climaps/rh2305.pdf).

Isolation and Culturing of *Xap* Isolates

The *Xap* bacterium was isolated from leaf and fruit from FRS and leaves only from the 11 locations outside Arkansas (Tables 1 and 2) using a procedure adapted from Civerolo et al., (1982). A total of five single-lesion and five heavily diseased lesions were cut from the leaves. Likewise, a similar procedure was used for the Arkansas fruit samples of each genotype. Single-cut lesions and heavily diseased lesions were each placed in 2 mL Eppendorf tubes with 1 mL of phosphate buffered saline (PBS) and vortexed for 3 s. The tissue was then removed with forceps and glycerol stocks were made with solution at an end concentration of 12% glycerol. All *Xap* solutions were stored at -80 °C (Civerolo et al., 1982).

Bacterial spot populations were cultured on media plates as described in Civerolo et al., (1982). A loop of the bacterial glycerol solution was streaked for isolation onto NGA agar plates [23 g of nutrient agar (Difco Laboratories, Detroit, MI), 20 g of glucose, 80 μg/mL chlorothanonil, and distilled water added to provide total volume to 1 L (pH 6.6 to 7.0)]. Plates were incubated for 3 to 4 d at 27 °C.

Xap Identification

Identification of *Xap* isolates was determined by amplifying DNA from the single-colony isolates with the Y17Co primers, designed by Pagani (2004): Y17CoF (GACGTGGTGATCAGCGAGTCATTC) and Y17CoR (GACGTGGTGATGATGATCTGC), which yielded the 943-bp DNA fragment specific to *Xap*. Although Pagani (2004) suggested that the Y17Co primers may also amplify for endophytic *Xap* strains (those which live in the host, yet apparently don't cause disease), no appropriate alternative has been identified. Instead a pathogenicity screen was used in conjunction with the Y17Co primers to identify which strains were *Xap* positive.

ConcentrThermo Scientific MBS Satellite Thermal Cyclers (Thermo Fisher Scientific, Waltham, MA) were utilized for single-colony polymerase chain reaction (PCR). A total volume of 25µl of final concentrations of the following were produced: one colony on a tooth pick transferred from the respective isolation plate, 5µL 5x buffer, 1.25 mM MgCl2, 0.5 µL forward primer, 0.5 µL reverse primer, 0.5 µL dNTP, 0.1 µL Taq, and 18.9 µL H_20. Each colony was plated on NGA medium for culture maintenance.

The conditions used for all primer combinations were 3 min of denaturation at 94 °C, 30 s denaturation at 94 °C, 30 s annealing at 55-58 °C, and 1 min extension at 72 °C for 35 cycles, followed by a final extension step of 5 min at 72 °C. The annealing temp varied slightly based on primer design (melting temp) and size of the amplicon.

The PCR products were first separated by gel electrophoresis on 2% agarose gels (Invitrogen UltraPure Agarose, Carlsbad, CA), and visualized under UV light. The PCR products were next resolved using the AdvanCE FS96 dsDNA fragment analyzer (FA) (Advanced Analytical Technologies, Inc., Ames, IA). The program PROSize® v.1 software (Advanced Analytical Technologies, Inc., Ames, IA) was used for genotyping.

Diversity of *Xap* Isolates

After obtaining positively screened *Xap* isolates from 12 U.S. locations, all isolates were race typed using simple sequence repeat (SSR) markers to determine the diversity between *Xap* isolates in Arkansas versus other states (Table 2). To this end, 20 SSR primers were designed and screened by gel electrophoresis across a subset of *Xap* isolates (Microsatellite repeat finder, insilico.ehu.es) (Table 3). From these 20 SSR primers, eight were selected as putative markers for polymorphism among isolates. These eight polymorphic SSRs were screened next screened on 48 *Xap* isolates (preliminary set) using the same PCR methods and conditions stated before.

Likewise, the PCR products were analyzed using gel electrophoresis and the FA, and genotyped using PROSize® v.1 software. The program Bioedit® (Hall, 1999) was used to generate a phylogenetic tree (to visually determine genetic diversity of *Xap* isolates, within and across all locations) based on presence/absence of the alleles for the eight SSRs using the command "DNADist, neighbor phylogenetic tree."

Table 3. Details on the 20 SSR markers designed throughout the *X. arboricola* pv. *pruni* str. CFBP 5530 plasmid p*Xap*41, genome (Pothier et al., 2011) (http://www.ncbi.nlm.nih.gov/nuccore/351673878) The eight SSR markers in bold were chosen to screen across all isolates.

Name	Motif	Forward primer	Reverse primer
***Xap*1**	**GC × 4**	**TGCCGTGCTTTGCCAAGT**	**TATGCAGAGGGGCAGTAGAACT**
***Xap*2**	**CG × 3**	**GCTACCTGAGCAGAAGGAACTGA**	**GCCAGGGAATCCGTGCAT**
*Xap*3	GGT × 3	CACCAGCCAATGCGTTATCCA	CGTGTACGAGCAGAACCTACTGT
***Xap*4**	**CAG × 3**	**CGCAGGTAATGGCGCACT**	**GCAATGCTGCTCGACGAGAT**
*Xap*5	AGC × 3	GATGCGGTTGCAGGCAGAT	CCGCATCGACAGACGGATTC
*Xap*6	GGT × 3	CGATTGAACGACACCGTCTGAGT	CCGCCACTTCGGAGAAGT
*Xap*7	TGC × 3	CGGAGCCTCGTGCTTGA	GCAGATCGGCAACGGTGTA
*Xap*8	AC × 3	CGGCTACACCGTGATGGT	CGCCGATAGCGACAAGTAGGA
*Xap*9	AC × 3	TGACGCAGGCAGAGTTCATG	ACTTGCGGGCTACTCGTGAT
***Xap*10**	**CAG × 3**	**GCGTCATTGCCTACGAAGGT**	**GCTTGTGCTCTTCGGAGAACT**
***Xap*11**	**AC × 3**	**GCTCTCCGTGTTGGACCTAGT**	**ACCATCGGGAACGGTAGGA**
***Xap*12**	**AGC × 3**	**GCAGACGACGCAGCACAA**	**GCAAGTCCCACGTCGGTCTA**
*Xap*13	GA × 4	CCTGCCTTGGCGTTCAGT	GGCACTCGTCGGAATCCA
***Xap*14**	**CCG × 4**	**CCGCAGCGTCACCATG**	**CCCTGCTTGGTGGCATACAC**
*Xap*15	CCGA × 3	ACCGACTTCGTTGCACAACTTC	GCCTGTCGCCAAGACAGATTC
***Xap*16**	**GCGT × 3**	**CTCCGACACCGACCTATTGC**	**TTTGGCCCTGTTCGCTGT**
*Xap*17	GCC × 3	CGGAGTGGCGAACTCGAA	GCAAACCGACTTGGCAAAGCA
*Xap*18	AC × 3	TCAACGAGCTGCGGGATAG	GACACGGCAAACTGCACATCA
*Xap*19	CG × 3	ATAGCCGTCTGCCAGCAA	TCGGTGTCAGCGTCTGTGT
*Xap*20	CG × 3	CAACTGGTTGTAACGCACGATCT	CAAGCATGTGATGCCCAAAGC

Xap Inoculum

The *Xap* isolates from 12 locations across the US were prepared as inoculum for the detached-leaf bioassay. First, the *Xap* cultures were washed off their media with sterile water, suspended in sterile phosphate-buffered saline, and adjusted to an optical density of 0.2 at 600 nm (OD_{600} = 0.2) using a spectrophotometer (NanoDrop; Termo Fisher Scientific; Wilmington, DE). After concentration adjustments, bacterial suspensions were used for the leaf assay.

Detached-Leaf Bioassay

A modified detached-leaf bioassay, as described by Randhawa and Civerolo (1985), was performed using subset of 113 out of the total 197 *Xap* isolates which screened positive with the Y17Co primers (Pagani, 2004). The assay was performed three times over three consecutive weeks in May 2015. In each replication four leaves from A-772 (resistant) and 'Crimson Lady' (susceptible) were inoculated per *Xap* isolate, and placed in separate petri dishes (Fig. 5). Thus a total of 12 leaves per cultivar were inoculated per *Xap* isolate, and each leaf was placed inoculated side up in a separate petri dish on 1.5% water agar medium amended with propiconazole to a concentration of 2.0 ppm.

To perform the inoculations, expanded young leaves (the third leaf from the tip of a shoot) were harvested from A-772 and 'Crimson Lady' and brought into the lab at FRS (Randhawa and Civerolo, 1985). All leaves were washed with tap water for 5 min to remove dirt. Next, leaves were sterilized by soaking for 90 s in 70% ethanol, and then rinsed with sterile water for 90 s. Leaves were placed abaxial side up on four layers of sterile filter paper. A sterile, 3 ml syringe (no needle) filled with each inoculum was placed firmly against the leaf surface. Leaves were infiltrated by applying gentle pressure until a 2-4 mm diameter, water-soaked spot appeared. Eight spots were applied, four per leaf half, ~1 cm apart. There was a tendency for

excess inoculum to leak out around the syringe tip at the infiltration site, thus leaves were lightly blotted to remove excess inoculum. The infiltrated leaves were then be placed inoculated side up in petri dishes on 1.5% water agar medium amended with propiconazole to a concentration of 2.0 ppm. The leaves were incubated at 25 °C under a 16 h photoperiod [Bi-Pin cool white 15 watt fluorescent light bulbs (Bulbrite Industries, Moonachie, NJ)] for 2 weeks (Randhawa and Civerolo, 1985). After 2 weeks, leaves were assessed for *Xap* infection using a visual rating scale (0-3) as in Hammerschlag et al., (1994) and Hammerschlag (1988 and 1990) to differentiate symptoms of infections at each inoculation site. The ratings included: 0 = no symptoms, 1 = distinct chlorotic spot and/or slight necrotic flecks, 2 = distinct but pale necrotic spot or grayish-white lesion, 2 mm in diameter, and 3 = distinct, dark necrotic spot of > 2 mm in diameter, with or without a chlorotic halo.

Next, all eight inoculation point ratings were averaged together to attain a mean for each leaf. The leaves with a mean of 0.0-1.5, 1.5-2.25 and 2.25-3.0 were assigned into three groups resistant (0), intermediate (1) and susceptible (2), in terms of the host side, and three virulence groups low (0), medium (1) and high (2), in terms of the pathogen side, respectively (i.e. a categorical scale). Subsequently, the mode for each replication (i.e. four leaves) was calculated, and then the mode across the three replications was calculated which served as the final categorical rating for the isolate and cultivar (‘Crimson Lady’ or A-772) combination (JMP® 2012).

Fig. 5. Representative photos of ‘Crimson Lady’ (susceptible) and A-772 (resistant) genotypes at the FRS orchard (June, 2014).

Results

For isolating *Xap* from leaf tissue, we obtained 197/471 positive isolates, representing a 42% success rate (Table 4). *Xap* isolates were screened through PCR (Pagani, 2004) and then validated through a virulence assay. PCR screening of putative *Xap* colonies are represented in Fig 6. Representative bands underneath single-colony isolates 37, 40, and 41 amplified the Y17Co 943 bp fragment, thus were considered positive *Xap* isolates, while single-colony isolates 38 and 39 were not *Xap* isolates. The water well served as a negative control, illustrating that these bands were not amplified out of water (Fig. 6).

Table. 4. *Xap* isolates from 12 states screened with RAPD primers designed by Pagani (2004).

Institution	State	Positives (N)	Negatives (N)	Positive isolates (%)
University of Arkansas	Arkansas	35	49	41
Missouri State University	Missouri	13	0	100
Virginia Tech University	Virginia	26	23	53
Michigan State University	Michigan	19	30	38
Penn State University	Pennsylvania	8	6	57
Rutgers University	New Jersey	32	18	64
Auburn University	Alabama	10	2	83
University of Florida	Florida	24	65	27
USDA-ARS Byron, GA	Georgia	4	7	36
North Carolina State University	North Carolina	1	0	100
Texas A&M University	Texas	7	54	11
University of Kentucky	Kentucky	18	20	47
Total		197	274	42

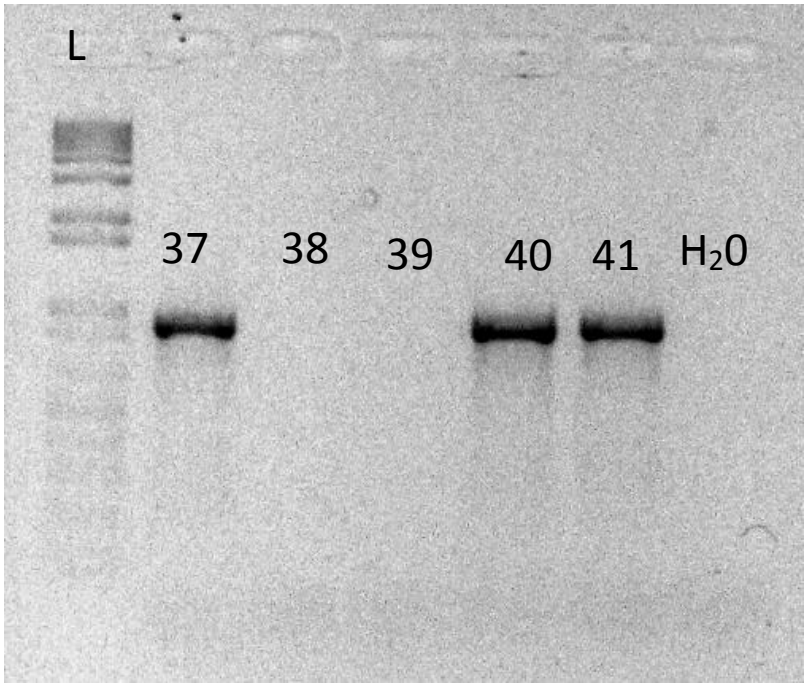

Fig. 6. Y17Co gel figure, showing single colony isolates 37, 40, and 41 as positive *Xap* isolates (L = ladder; H_20 = negative control).

Diversity of *Xap* Isolates

Out of twenty SSRs, eight were deemed polymorphic and screened on a representative set of 48 *Xap* isolates (Table 5). Even though this study obtained 197 positive *Xap* isolates, only 48 isolates were race typed. The successfully generated phylogenetic tree showed two major groupings of the 48 *Xap* isolates based on presence/absence of alleles for eight SSR markers (Fig. 7).

The first monophyletic group, highlighted with a blue bracket near the top of the figure, tends to include isolates from northern and eastern states, noteworthy are two New Jersey isolates, three Michigan isolates, and two Virginia isolates (Fig. 7). Additionally two Kentucky and one Arkansas isolate (central states) were also found in "group 1."

The second monophyletic grouping, highlighted with a red bracket, contained a major clade (indicated by the smaller red bracket) (Fig. 7). This major clade contained the majority of

isolates from central states, Arkansas (15) and Kentucky (five). Additionally, one Virginia isolate, one Florida isolate, two Michigan isolates, and one New Jersey isolate (northern and eastern states) were also found in the "group 2" clade 1. Lastly, two other smaller clades were found within "group 2" yet appear to be more random in terms of where the *Xap* isolates in these two clades are from.

Table 5. Presence (1) vs. absence (0) results for eight SSRs screened on the 48 *Xap* isolates from six different states [Arkansas (AR), Kentucky (KY), Michigan (MI), Virginia (VA), Florida (FL), and New Jersey (NJ)].

	SSR marker							
Isolate	1	2	4	10	11	12	14	16
AR_0817_4_L_HD-3	1	1	1	1	0	1	1	1
AR_0817_4_L_HD-5	1	1	1	1	0	1	1	1
AR_0817_4_L_HD-8	1	1	1	1	0	1	1	1
AR_0801_2L_HD-2	0	0	0	0	0	0	0	0
AR_0803_12L_SL-1	1	1	1	1	0	1	1	1
AR_0803_12L_SL-2	0	0	1	1	0	1	1	1
AR_0803_12L_SL-3	0	0	1	0	0	1	1	1
AR_0803_12L_SL-4	1	1	1	1	0	1	1	1
AR_0803_12L_SL-5	1	1	1	1	0	1	1	1
AR_0803_12L_SL-6	1	1	1	1	0	1	0	1
AR_0803_12L_SL-7	1	1	1	1	0	1	1	1
AR_0803_12L-SL-8	1	0	1	1	0	1	1	1
AR_0803_12L_SL-9	1	0	1	1	0	0	1	1
AR_0803_5L-HD-1	1	1	1	1	0	1	1	1
AR_0803_5L-HD-2	0	0	1	1	0	0	1	0
AR_0803_13L_HD-2	0	0	1	1	0	0	1	0
AR_0803_13L_HD-3	1	1	1	1	1	1	1	0
AR_0819_1L_HD-5	1	1	1	1	0	1	1	1
AR_0825_14L_HD-1	0	0	1	1	0	1	1	1
AR_0825_14L_HD-2	0	0	1	1	0	0	1	1
AR_0825_14L_HD-4	0	1	1	1	0	1	1	1
AR_0825_16F_SL-3	1	1	1	1	0	1	1	1
AR_0825_16F_SL-4	0	0	1	1	0	0	1	1
KY_1_L_HD-1	1	1	1	1	0	1	1	1
KY_1_L_HD-4	1	1	0	1	0	0	1	0
KY_1_L_HD-5	1	1	0	1	0	1	1	1
KY_1_L_HD-6	1	1	0	1	0	1	1	1
KY_2_L_HD-2	1	1	1	1	0	1	1	1
KY_4_L_HD-1	1	1	1	1	0	1	1	1
KY_4_L_HD-2	0	0	1	0	0	0	0	1
KY_4_L_HD-3	0	0	0	0	0	0	0	1
KY_4_L_HD-4	0	0	0	0	0	0	0	1
KY_4_L_HD-5	0	0	0	0	0	0	0	1

Table 5. Presence (1) vs. absence (0) results for eight SSRs screened on the 48 *Xap* isolates from six different states [Arkansas (AR), Kentucky (KY), Michigan (MI), Virginia (VA), Florida (FL), and New Jersey (NJ)]. (Cont.).

	SSR marker							
Isolate	1	2	4	10	11	12	14	16
MI_01_L_HD-1	1	1	1	1	0	1	1	0
MI_3_HD-2	1	1	1	1	1	1	1	1
MI_3_HD-4	0	0	0	0	0	0	0	1
MI_06_L_HD-1	1	1	1	1	0	1	1	1
MI_11_L_HD-3	1	1	1	1	0	1	1	1
VA_NJF-17_L_HD-3	1	1	1	1	0	1	1	1
VA_NJF-17_L_HD-5	1	1	1	1	0	1	1	1
VA_MPP_HD-9	1	1	1	1	1	1	1	1
VA_MPP_HD-11	1	1	1	1	0	1	1	1
VA_MPP_HD-12	1	1	1	1	1	1	1	1
VA_GP_L_HD	0	0	0	0	1	0	0	1
FL_UR_L_HD-5	0	0	0	1	0	0	0	0
NJ1_SD_L_HD-1	1	1	1	0	1	1	1	1
NJ1_SD_L_HD-2	1	1	0	1	1	1	1	1
NJ1_SD_L_HD-3	0	0	1	1	0	1	1	1

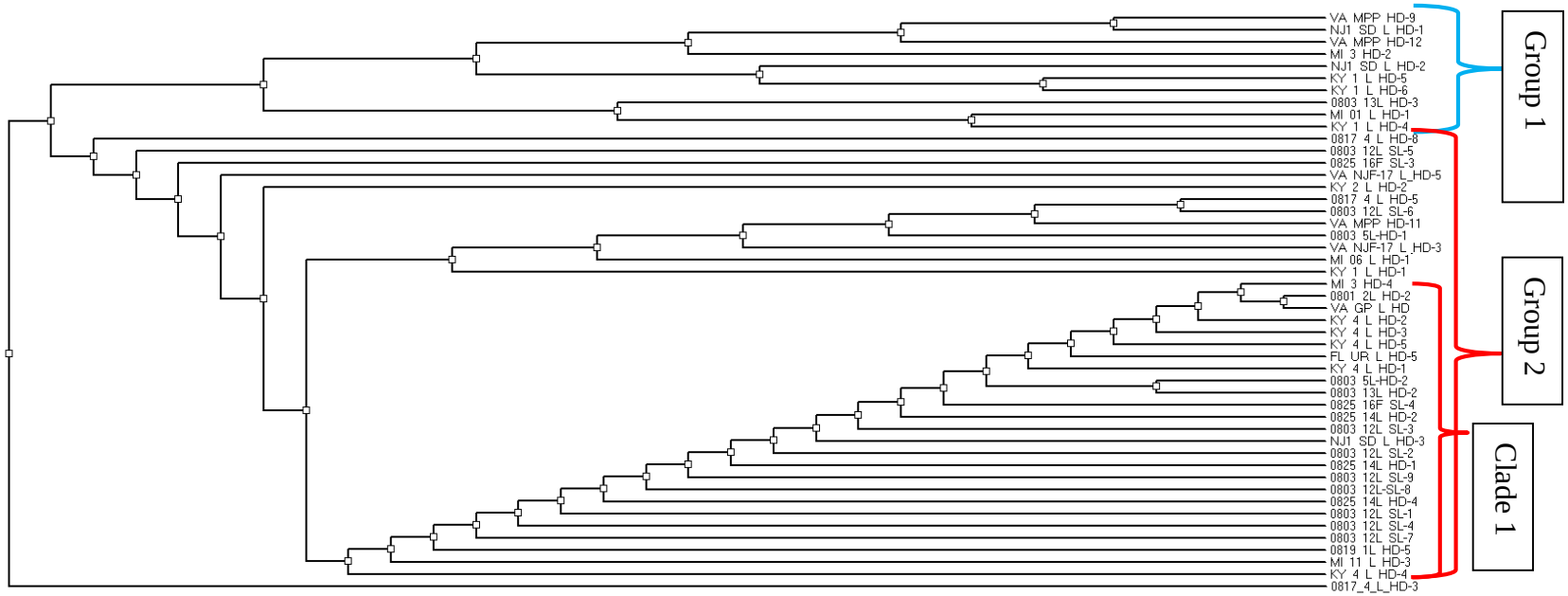

Fig. 7. The preliminary phylogenetic tree generated for 48 *Xap* isolates collected from six different locations in the U.S. based on genetic diversity (presence/absence of alleles for eight SSR markers) [Bioedit® (Hall, 1999)]. (Monophyletic "group 1" is highlighted with a blue bracket near the top of the figure. Monophyletic "group 2" is highlighted with a red bracket, and also contains a major "clade 1" indicated by the smaller red bracket).

Virulence of *Xap* Isolates

An important step to validating the *Xap* isolates obtained in this study was to screen for pathogenicity using a virulence assay. Results for the subset of 113 out of the total 197 *Xap* isolates which screened positive with the Y17Co primers (Pagani, 2004) are depicted in Table 6. In total, 83 *Xap* isolates (74% out of 113 isolates) were highly virulent on 'Crimson Lady', in comparison to only 61 (54%) highly virulent on A-772. The 61 *Xap* isolates that were highly virulent on A-772, were also highly virulent on 'Crimson Lady'. In terms of the 83 *Xap* isolates that were highly virulent on 'Crimson Lady', 19 of them showed only medium virulence, and three showed low virulence on A-772. A total of 13 and 17 *Xap* isolates showed medium and low virulence on 'Crimson Lady' (30 isolates combined; 26%), respectively. In comparison, 21 and 31 *Xap* isolates showed medium and low virulent on A-772 (52 isolates combined; 46%), respectively. The 17 *Xap* isolates which showed low virulence on 'Crimson Lady' also showed low virulence on A-772.

Table 6. Virulence [High(2) / Medium(1) / Low(0)] of 113 *Xap* isolates from 12 different locations in the U.S. on Crimson Lady (*Xap* susceptible) and A-772 (*Xap* resistant) peach cultivars. [Arkansas (AR), Florida (FL), Georgia (GA), Kentucky (KY), Michigan (MI), Missouri (MO), North Carolina (*Xap*-88), New Jersey one (NJ1), New Jersey two (NJ2), Pennsylvania (PA), and Virginia (VA).

Isolate		Virulence on	
Number	Name	Crimson Lady	A-772
2	AR_0803_5L_HD-2	High	High
8	AR_0817_4_L_HD-3	High	High
10	AR_0817_4_L_HD-5	High	High
13	AR_0817_4_L_HD-8	High	High
23	VA_MPP_HD-9	High	High
25	VA_MPP_HD-11	High	High
26	VA_MPP_HD-12	High	High
28	MI_3_HD-2	High	High
30	MI_3_HD-4	High	High
37	FL_UR_L_HD-5	High	High
40	NJ1_SD_L_HD-1	High	High
41	NJ1_SD_L_HD-2	High	High
42	NJ1_SD_L_HD-3	High	High
54	AR_0801_2L_HD-2	Low	Low
55	AR_0801_2L_HD-3	Low	Low
56	AR_0801_2L_HD-4	Low	Low
58	AR_0803_12L_SL-1	High	High
60	AR_0803_12L_SL-3	Low	Low
61	AR_0803_12L_SL-4	High	High
62	AR_0803_12L_SL-5	High	High
63	AR_0803_12L_SL-6	High	High
65	AR_0803_12L-SL-8	High	High
66	AR_0803_12L_SL-9	Low	Low
68	AR_0803_5L-HD-2	High	High
69	AR_0803_13L_HD-1	High	Medium
71	AR_0803_13L_HD-3	High	Medium
85	AR_0825_16F_SL-3	High	High
87	AR_0825_16F_SL-5	High	Medium
89	AR_0819_17L_HD-2	High	Medium
90	AR_0819_17L_HD-3	High	Medium
91	AR_0819_17L_HD-4	High	Medium
92	AR_0819_17L_HD-5	High	Medium
93	AR_0825_1L_HD-1	High	High

Table 6. Virulence [High(2) / Medium(1) / Low(0)] of 113 *Xap* isolates from 12 different locations in the U.S. on Crimson Lady (*Xap* susceptible) and A-772 (*Xap* resistant) peach cultivars. [Arkansas (AR), Florida (FL), Georgia (GA), Kentucky (KY), Michigan (MI), Missouri (MO), North Carolina (*Xap*-88), New Jersey one (NJ1), New Jersey two (NJ2), Pennsylvania (PA), and Virginia (VA). (Cont.).

Isolate		Virulence on	
Number	Name	Crimson Lady	A-772
98	AR_0813-3L-HD-4	Medium	Low
109	AR_0819_1L_HD-4	High	High
110	AR_0819_1L_HD-5	High	High
111	AR_0825_14L_HD-1	High	High
112	AR_0825_14L_HD-2	Medium	Low
125	VA_NJF-17_L_HD-3	High	High
127	VA_NJF-17_L_HD-5	High	High
132	MI_06_L_HD-1	High	Medium
133	KY_4_L_HD	High	High
135	KY_4_L_HD	High	Low
136	MI_01_L_HD-1	High	High
139	KY_2_L_HD-2	High	High
141	KY_4_L_HD	High	Medium
142	KY_4_L_HD	High	High
143	KY_4_L_HD-1	High	Medium
148	KY_1_L_HD-4	High	High
149	KY_1_L_HD-5	High	Medium
150	KY_1_L_HD-6	High	High
154	NJ1_R_L_HD-1	High	High
155	NJ1_R_L_HD-2	High	High
157	NJ1_R_L_HD-3	High	High
159	NJ1_R_L_HD-5	High	High
165	*Xap-88*	High	High
172	FL_SC_L_HD	Low	Low
275	*GA-06-LHD_2*	High	High
282	*PA(2)11-LHD_1*	High	Medium
284	*PA(9)13-LHD_3*	High	High
286	*PA(9)13-LSL_3*	High	Medium
288	*PA(9)9-LHD*	High	High
300	*MO-**-LSL_1*	High	High
301	*MO-**-LSL_2*	High	Medium
305	VA-NJF17-LSL_2	Medium	Medium
311	VA-SR-LSL_2	High	High

Table 6. Virulence [High(2) / Medium(1) / Low(0)] of 113 *Xap* isolates from 12 different locations in the U.S. on Crimson Lady (*Xap* susceptible) and A-772 (*Xap* resistant) peach cultivars. [Arkansas (AR), Florida (FL), Georgia (GA), Kentucky (KY), Michigan (MI), Missouri (MO), North Carolina (*Xap*-88), New Jersey one (NJ1), New Jersey two (NJ2), Pennsylvania (PA), and Virginia (VA). (Cont.).

Isolate		Virulence on	
Number	Name	Crimson Lady	A-772
319	VA-SR-LHD_3	Low	Low
325	VA-SR-LHD_3	Low	Low
326	VA-NJF17-LSL_1	High	High
328	VA-NJF17-LSL_2	High	High
330	VA-NJF17-LSL_3	High	High
332	NJ1-SS1-LSL_1	Medium	Low
336	NJ1-SS1-LSL_3	High	High
338	NJ1-SS1-LHD_1	Medium	Low
340	NJ1-SS1-LHD_2	High	High
342	NJ1-SS1-LHD_3	High	High
346	NJ1-SS1-LHD_2	Medium	Medium
358	KY-12-LHD_5	Medium	Low
360	KY-12-LHD_6	Low	Low
364	KY-12-LSL_2	Low	Low
369	MI-16_LHD_2	High	High
372	KY-12-LSL_3	High	High
373	MI-16_LHD_4	Medium	Low
374	MI-12-LHD_1	High	High
379	MI-14-LSL_3	High	Medium
380	MI-12-LHD_4	Medium	Low
381	MI-14-LSL_4	High	Low
384	MI-12-SL_2	High	High
386	MI-12-SL_3	High	High
388	MI-12-SL_4	High	Medium
399	NJ2-Gala1-LSL_2	Medium	Low
407	NJ2-SC6-LSL_1	High	High
411	NJ2-SC6-LSL_3	Medium	Low
413	FL-GK-LSL_1	High	Low
417	FL-GK-LSL_3	Medium	Low
419	FL-GK-LSL_4	High	Medium
428	FL-SC-LSL_4	Low	Low
433	FL-UFO-LSL_3	High	High

Table 6. Virulence [High(2) / Medium(1) / Low(0)] of 113 *Xap* isolates from 12 different locations in the U.S. on Crimson Lady (*Xap* susceptible) and A-772 (*Xap* resistant) peach cultivars. [Arkansas (AR), Florida (FL), Georgia (GA), Kentucky (KY), Michigan (MI), Missouri (MO), North Carolina (*Xap*-88), New Jersey one (NJ1), New Jersey two (NJ2), Pennsylvania (PA), and Virginia (VA). (Cont.).

Isolate		Virulence on	
Number	Name	Crimson Lady	A-772
439	FL-SR-LSL_1	High	Medium
440	FL-FGPK-LHD_1	Low	Low
442	FL-FGPK-LHD_2	High	High
449	MI-15-LSL_3	High	High
458	FL-GK-LSL_6	High	High
462	FL-GK-LSL_10	Low	Low
464	*MO-*-LHD_2*	High	Medium
465	*MO-*-LSL_1*	Low	Low
466	*MO-*-LSL_3*	Low	Low
467	*MO-BP2-LHD_1*	High	High
468	*MO-BP2-LSL_1*	High	High
469	*MO-SF2-LHD_1*	Low	Low
471	*MO-SF2-LSL_1*	High	High
472	*MO-SF2-LSL_2*	Medium	Low
474	*NJ2-OH2-LSL_1*	Low	Low

Table 7. Total number (N) and percentage (%) of *Xap* isolates which showed high/medium/low virulence on 'Crimson Lady' (*Xap* susceptible) and A-772 (*Xap* resistant) peach cultivars.

Virulence	Crimson Lady		A-772	
	N	Total%	N	Total%
High	83	74	61	54
Medium	13	11	21	19
Low	17	15	31	27
Total	113	100	113	100

Discussion

Xap Isolate Collection and Identification

This study began with observed segregation for *Xap* occurrence in the field at FRS. One observation that was noted suggested a difference in variation in occurence of *Xap* as New Jersey and Michigan *Xap*-resistant peach cultivars were in general susceptible at FRS, however they are known to be resistant in Michigan and New Jersey. Thus the idea was to expand and collect isolates from 12 U.S. states (including Arkansas), to then identify as *Xap* positive, and subsequently screen on two peach cultivars bred in two different locations: 'Crimson Lady' (from California, *Xap* susceptible) and A-772 (from UA, *Xap* resistant). In total, 197 out of 471 single colony isolates screened positive for *Xap* using the Y17Co primers developed in Pagani (2004). This is a relatively low success rate, which was due to screening by PCR, without using phenotypic cues (white vs. yellow color). The idea was to not exclude genetically different isolates, such as those with low xanthan production (the iconic yellow pigment produced by *Xanthomonas* species).

Diversity of *Xap* Isolates

The successfully generated phylogenetic tree showed two major groupings of the 48 *Xap* isolates based on presence/absence of alleles for eight SSR markers. In the developed phylogenetic tree, the monophyletic "group 1" contained the majority of isolates from northern and eastern states (New Jersey, Michigan, and Virginia) which suggests a potential difference in *Xap* diversity in these states, in comparison to the central states (Arkansas and Kentucky). Yet as expected, there were also a few Arkansas and Kentucky isolates in "group 1", which represents *Xap* diversity that is widespread. The monophyletic "group 2" contained a major clade which included the majority of isolates from central states (Arkansas and Kentucky), which add more

evidence that there is a potential difference in *Xap* diversity in central states, in comparison to the northern and eastern states (New Jersey, Michigan, and Virginia). Yet once again as expected, there were also a few New Jersey, Michigan, and Virginia isolates, and one Florida isolate in the "group 2" clade 1, which represents *Xap* diversity that is widespread. These differences in monophyletic groupings (one for central states and one for eastern states) indicate a difference in diversity in these locations. Additionally, as both monophyletic groupings share isolates from Kentucky, Arkansas, Michigan, Virginia, and New Jersey, there tends to be overlap.

Although these results are promising, sample number was low (48 isolates and eight SSRs), thus these are preliminary results and the data and phylogenetic tree could change as more individuals/markers are included. Nonetheless, these preliminary results support that the *Xap* isolates in the northern and eastern states (New Jersey, Michigan, and Virginia) are genetically different from those in the central states (Arkansas and Kentucky), as was previously expected. Moreover these preliminary results suggest that *Xap* indeed has a high genetic diversity, which opposes the low *Xap* diversity previously noted in Hajri et al., (2012).

Virulence Assay

In total, 61 *Xap* isolates (~54% out of all 113 isolates) were highly virulent on 'Crimson Lady' and A-772, and thus can be considered highly virulent strains. In terms of the additional 22 *Xap* isolates which were also highly virulent on 'Crimson Lady', 19 of them showed only medium virulence, and three showed low virulence on A-772, which makes sense since A-772 is known to be *Xap* resistant in the field, while 'Crimson Lady' is highly susceptible. Likewise more *Xap* isolates showed moderate and low virulence on the resistant cultivar, A-772, in comparison to the susceptible cultivar, Crimson Lady. In total 52 *Xap* isolates (46% out of 113)

showed moderate or low virulence on A-772, in comparison to only 30 *Xap* isolates (26% out of all 113) with moderate or low virulence on 'Crimson Lady'. Lastly, there were 17 *Xap* isolates which showed low virulence on 'Crimson Lady' and A-772, and thus these can be considered endophytic *Xap* strains (those which live in the host, yet apparently don't cause disease) [as Pagani (2004) suggested that the Y17Co primers may also amplify for endophytic *Xap* strains].

Although these results are promising, they are only preliminary results as the remaining 84 isolates which screened positive for *Xap* should also be tested. Nonetheless, these preliminary results support previous findings that *Xap* strain and population virulence levels are thought to differ substantially on peach and other stone fruits (Civerolo, 1975; OEPP/EPPO, 2006). Du Plessis (1988) and Scortichini et al. (1996) studied the difference in virulence of *Xap* isolates from different geographical areas and concluded that *Xap* varied in virulence among different cultivars. The results in the study herein add more evidence to these findings that *Xap* strain and population virulence levels differ substantially on peach. While the *Xap* diversity and virulence findings in the study herein are exciting, these are only preliminary results from which future studies using more high throughput methods: sequencing, alignment, and SNP identification for diversity tree can be expanded upon.

Literature Cited

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. of Phytopathol. 156:338-345.

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. Crit. Rev. Plant Sci. 29:59-107.

Byrne, D., M. Bassols, D. Bassi, M. Piagnani, K. Gasic, G. Reighard, M. Moreno, and S. Pérez. 2012. Peach, p. 505-569. In: M. Badenes and D. Byrne (eds.). Fruit breeding. Springer Science, Business Media, New York.

Civerolo, E.L., M. Sasser, C. Helkie, and D. Burbage. 1982. Selective medium for *Xanthomonas campestris* pv. *pruni* [Bacterial spot]. Plant Dis. 66:39–43.

Civerolo, E.L. 1975. Quantitative aspects of pathogenesis of *Xanthomonas pruni* in peach leaves. Phytopathol. 65:258-264.

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.

Daines, R. 1961. What we know and don't know about bacterial spot of peach. Horticultural News 42:110-114.

Du Plessis, H.J. 1988. Differential virulence of *Xanthomonas campestris* pv. *pruni* to peach, plum, and apricot cultivars. Phytopathol. 78:1312-1315.

EPPO/CABI 1997. *Xanthomonas arboricola* pv. *pruni*, p. 1096–1100. In: Quarantine Pests for Europe, 2nd ed., CAB International, Wallingford (GB).

Hajri, A., J.F. Pothier, M. Fischer-Le Saux, S. Bonneau, S. Poussier, T. Boureau, B. Duffy, and C. Manceau. 2012. Type three effector gene distribution and sequence analysis provide new insights into the pathogenicity of plant-pathogenic *Xanthomonas arboricola*. Appl. Environ. Microbiol. 78:371-384.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98.

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. 1990. Resistance responses of plants regenerated from peach callus, cultures to *Xanthomonas campestris* pv. *pruni*. J. Amer. Soc. Hort. Sci. 115:1034-1037.

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.

OEPP/EPPO. 2006. *Xanthomonas arboricola* pv. *pruni*. OEPP/EPPO Bul. 36:129–133.

Pagani, M.C. 2004. An ABC transporter protein and molecular diagnoses of *Xanthomonas arboricola* pv. *pruni* causing bacterial spot of stone fruits. Ph.D dissertation North Carolina State University, Raleigh, NC, U.S.

Pothier, J.F., F.J. Vorhölter, J. Blom, A. Goesmann, A. Pühler, T.H. Smits, and B. Duffy. 2011. The ubiquitous plasmid p*Xap*41 in the invasive phytopathogen *Xanthomonas arboricola* pv. *pruni*: complete sequence and comparative genomic analysis. FEMS microbiology letters 323:52-60.

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 and G.W. Bird (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 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.

Scortichini, M., J. D.Janse., M.P. Rossi, and J.H.J. Derks. 1996. Characterization of *Xanthomonas campestris* pv. *pruni* strains from different hosts by pathogenicity tests and analysis of whole-cell fatty acids and whole-cell proteins. J. of Phytopathol. 144:69-74.

Smith, E. 1903. Observations on a hitherto unreported bacterial disease, the cause of which enters the plant through ordinary stomata. Sci. 17:456–457.

Smith, S. 2015. Fruit tree diseases. 16 November 2015. <https://www.uaex.edu/publications/pdf/mp154/fruit-tree-diseases-commercial.pdf>.

Studebaker, G., J. Hopkins, and D. Johnson. 2015. Control peach tree borers on commercially grown peach and plum trees. 16 November 2015. <https://www.uaex.edu/publications/PDF/FSA-7504.pdf>.

Vauterin, L., B. Hoste., K. Kersters, and J. Swings. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45:472–489.

Yang, N. 2012. Mapping quantitative trait loci associated with resistance to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) in peach. Clemson University, Clemson, PhD Diss. UMI 3512202.

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

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

Appendix A. Genomic estimated breeding values (GEBV) per trait and year for the AR RosBREED pedigree

Table A.1. Genomic estimated breeding values (GEBV) per trait (*Xap* fruit (0-5) and *Xap* fruit (0-5) inc]) and year for the AR RosBREED pedigree.

Individual	*Xap* fruit (0-5)					*Xap* fruit (0-5) inc		
	2013 ± 0.5	2014 ± 0.5	2015 – 1	Avg ± 0.5		2014 inc	2015 – 1 inc	Avg – 1 inc
104325	0.75	0.78	0.49	0.47		0.75	0.71	0.50
752002002	0.09	0.52	-0.13	0.17		0.87	0.15	0.03
10A4	0.11	0.53	-0.11	0.11		0.86	0.12	0.07
22A5	-0.02	1.13	0.09	0.45		1.13	0.23	0.34
2W68W	0.31	0.69	0.08	0.25		0.78	0.30	0.22
4A_4	0.30	0.32	-0.11	0.07		0.32	0.07	-0.08
A_130	0.79	0.97	0.51	0.52		0.92	0.71	0.59
A_145	0.21	0.84	0.31	0.34		0.70	0.53	0.46
A_154	0.19	0.66	-0.02	0.15		0.53	0.20	0.20
A_172	0.50	0.39	-0.18	0.09		0.29	0.00	-0.19
A_176	0.46	0.37	-0.19	0.07		0.28	0.03	-0.14
A_178	0.04	0.10	-0.83	-0.48		-0.64	-0.65	-0.84
A_18	0.15	0.35	-0.30	0.11		0.96	-0.02	-0.14
A_190	0.00	0.14	-0.50	0.00		1.10	-0.15	-0.36
A_21	-0.08	0.32	-0.37	0.03		1.06	-0.04	-0.18
A_224	0.09	0.73	0.06	0.16		0.38	0.23	0.27
A_232	0.49	0.34	-0.27	0.05		0.41	-0.04	-0.24
A_24	0.20	0.42	-0.23	0.11		0.86	-0.01	-0.14
A_270	0.93	1.00	0.50	0.57		1.08	0.70	0.47
A_333	0.61	0.72	0.21	0.41		1.12	0.36	0.42
A_334	-0.76	-0.25	-1.00	-0.62		-0.03	-0.69	-0.96
A_367	0.15	-0.14	-0.49	-0.30		-0.06	-0.33	-0.38
A_371	0.87	0.35	-0.19	0.03		0.51	0.04	-0.20
A_374	0.18	0.73	0.02	0.33		0.91	0.30	0.25
A_392	0.17	1.14	-0.01	0.42		1.20	0.55	0.24
Bradley	0.21	0.10	-0.66	-0.23		0.23	-0.41	-0.60
A_405	0.48	1.37	0.28	0.68		1.47	0.46	0.43
A_419	-0.58	0.07	-0.26	-0.49		0.97	-0.11	0.16
A_427	0.35	0.90	0.04	0.29		0.37	0.18	0.24
A_433	-0.14	0.63	-0.28	-0.12		-0.11	-0.11	0.10
A_434	0.92	1.19	0.43	0.70		1.13	0.66	0.43
A_441	0.15	0.60	-0.06	0.05		0.32	-0.01	0.17

Table A.1. Genomic estimated breeding values (GEBV) per trait (*Xap* fruit (0-5) and *Xap* fruit (0-5) inc]) and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* fruit (0-5)					*Xap* fruit (0-5) inc		
	2013 ± 0.5	2014 ± 0.5	2015 – 1	Avg ± 0.5		2014 inc	2015 – 1 inc	Avg – 1 inc
A_500	-0.17	0.51	-0.30	0.17		1.21	0.07	0.10
A_565	0.32	0.99	-0.08	0.24		-0.01	-0.05	0.20
A_604	0.67	0.66	0.22	0.52		1.56	0.47	0.56
A_657	-0.79	-0.58	-1.33	-0.93		-1.54	-1.20	-1.58
A_663	-1.14	-0.59	-1.21	-0.71		-0.08	-0.95	-1.12
A_665	0.44	-0.63	-0.81	-0.47		-0.64	-0.68	-0.96
A_672	-0.21	0.67	-0.14	-0.12		1.55	-0.08	0.28
A_699	-0.13	1.04	-0.33	-0.05		0.18	-0.44	-0.11
A_708	1.45	1.58	0.52	1.12		1.53	1.04	0.55
A_716	0.54	0.38	-0.89	-0.15		0.09	-0.73	-0.71
A_717	0.23	0.21	-0.18	0.00		0.72	-0.04	-0.02
A_760	-0.44	0.93	-0.54	0.09		1.54	-0.02	0.12
Souvenirs	1.31	1.39	0.52	0.98		0.16	0.99	0.41
Amoore Sweet	-0.42	0.45	-0.89	-0.31		0.08	-0.90	-0.68
A_772	0.41	-0.03	-0.50	-0.13		-0.06	-0.52	-0.69
A_773	1.33	1.07	0.15	0.60		1.54	0.57	0.40
A_776	-0.69	0.06	-1.06	-0.83		0.08	-1.16	-0.97
A_778	-0.12	0.72	-0.41	-0.14		-0.06	-0.10	-0.51
A_783	-0.35	0.82	0.05	0.19		1.58	-0.17	0.41
A_789	0.31	0.85	-0.27	0.17		0.18	0.02	0.12
Admiral Dewey	0.33	0.31	0.10	0.05		0.06	0.34	0.16
Alameda	0.30	0.90	0.44	0.49		0.99	0.70	0.55
Allgold	-0.40	0.23	-0.46	-0.06		1.18	-0.13	-0.20
AR_Pop_1_01	-1.19	0.43	-0.32	-0.38		1.56	0.01	0.11
AR_Pop_1_02	-1.10	1.23	-0.23	-0.03		1.57	-0.02	0.17
AR_Pop_1_03	-0.90	0.63	-0.49	-0.57		1.54	-0.18	0.01
AR_Pop_1_04	-1.09	0.26	-0.42	-0.23		1.56	-0.05	0.15
AR_Pop_1_05	-0.53	0.44	-0.50	-0.36		1.54	-0.34	0.02
AR_Pop_1_06	-0.49	0.66	-0.45	-0.97		1.56	-0.19	0.14
AR_Pop_1_07	-1.23	0.70	-0.36	-0.61		1.57	-0.16	0.17
AR_Pop_1_08	-1.17	0.54	-0.16	0.01		1.56	0.16	0.23
AR_Pop_1_09	-0.82	1.42	-0.35	0.11		1.57	-0.22	0.13
AR_Pop_1_10	-0.84	0.95	-0.19	-0.17		1.57	0.13	0.09
AR_Pop_1_11	-1.04	0.97	-0.26	0.02		1.57	-0.15	0.17
AR_Pop_1_12	-0.47	0.97	-0.61	-0.44		1.56	-0.64	0.02

Table A.1. Genomic estimated breeding values (GEBV) per trait (*Xap* fruit (0-5) and *Xap* fruit (0-5) inc]) and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* fruit (0-5)					*Xap* fruit (0-5) inc		
	2013 ± 0.5	2014 ± 0.5	2015 – 1	Avg ± 0.5		2014 inc	2015 – 1 inc	Avg – 1 inc
AR_Pop_1_13	-1.22	0.47	-0.43	-0.60		1.57	-0.38	0.07
AR_Pop_1_14	-0.52	1.28	0.81	0.35		1.51	0.65	0.53
AR_Pop_1_15	-0.28	0.88	-0.43	-0.77		1.56	-0.20	0.11
AR_Pop_1_16	-1.01	0.71	-0.23	-0.11		1.57	0.01	0.15
AR_Pop_1_17	-1.13	0.53	-0.25	-0.04		1.56	0.16	0.23
AR_Pop_1_18	-1.19	0.62	-0.47	-1.06		1.56	-0.28	0.12
AR_Pop_1_19	-1.15	0.40	-0.56	-0.68		1.56	-0.34	0.04
AR_Pop_1_20	-1.39	0.71	-0.28	-0.42		1.56	-0.09	0.15
AR_Pop_1_21	-0.04	0.78	-0.13	0.23		1.56	0.21	0.21
AR_Pop_1_22	-0.93	0.42	-0.10	0.26		1.56	0.28	0.25
AR_Pop_1_23	-1.06	1.02	-0.11	0.28		1.57	0.23	0.22
AR_Pop_1_24	-0.96	0.91	-0.46	-0.42		1.54	-0.29	0.05
AR_Pop_1_25	-1.12	0.40	-0.28	-0.41		1.57	-0.19	0.18
AR_Pop_1_26	-1.16	0.46	-0.41	0.20		1.56	-0.27	0.07
AR_Pop_1_27	-1.09	0.69	-0.25	-0.68		1.57	-0.06	0.12
AR_Pop_1_28	-1.24	0.11	-0.57	-1.04		1.56	-0.40	0.07
AR_Pop_1_29	-0.86	1.13	-0.17	0.26		1.57	-0.08	0.16
AR_Pop_1_30	-1.00	1.02	-0.23	-0.13		1.55	0.28	0.27
AR_Pop_1_31	-0.38	0.34	-0.24	-0.08		1.56	0.14	0.20
AR_Pop_1_32	-0.04	1.29	-0.13	0.32		1.57	0.19	0.22
AR_Pop_1_33	-0.79	0.40	-0.42	-0.20		1.56	-0.29	0.05
AR_Pop_1_34	-0.15	0.76	-0.41	0.08		1.56	-0.26	0.14
AR_Pop_1_35	-0.08	1.20	-0.34	0.08		1.56	0.01	0.19
AR_Pop_1_36	-1.03	0.51	-0.35	-0.41		1.56	-0.05	0.17
AR_Pop_1_37	-0.44	0.92	-0.28	-0.24		1.56	-0.05	0.11
AR_Pop_1_38	-0.25	0.14	-0.45	-0.44		1.56	-0.23	0.15
AR_Pop_1_39	-0.29	0.56	-0.26	-0.31		1.56	0.05	0.17
AR_Pop_1_40	-0.94	1.13	-0.36	-0.07		1.56	-0.02	0.06
AR_Pop_1_41	0.28	1.24	-0.16	0.34		0.26	0.06	0.18
AR_Pop_1_42	-0.26	0.71	-0.37	0.16		1.56	-0.16	0.13
AR_Pop_1_43	-1.07	0.39	-0.15	-0.04		1.57	0.06	0.21
AR_Pop_1_44	-0.04	0.45	-0.44	0.08		1.57	-0.40	0.08
AR_Pop_1_45	-0.45	0.15	-0.42	-0.55		1.56	-0.04	0.12
AR_Pop_1_46	-0.42	0.91	-0.51	-0.53		1.56	-0.28	0.12
AR_Pop_1_47	-0.29	1.17	-0.18	-0.04		1.56	0.12	0.21

Table A.1. Genomic estimated breeding values (GEBV) per trait (*Xap* fruit (0-5) and *Xap* fruit (0-5) inc]) and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* fruit (0-5)					*Xap* fruit (0-5) inc		
	2013 ± 0.5	2014 ± 0.5	2015 – 1	Avg ± 0.5		2014 inc	2015 – 1 inc	Avg – 1 inc
AR_Pop_1_48	-1.19	0.22	-0.54	-0.70		1.57	-0.36	0.06
AR_Pop_1_49	-1.04	0.78	-0.50	-0.07		1.56	-0.48	0.09
AR_Pop0801_01	-0.84	1.39	-0.77	-0.39		1.56	-1.10	-0.46
AR_Pop0801_02	-0.92	1.24	-0.36	-0.35		1.56	-0.67	-0.14
AR_Pop0801_03	0.60	-0.67	-0.90	-0.43		1.20	-1.03	-0.73
AR_Pop0801_04	-1.30	1.28	-0.43	-0.29		1.57	-0.62	-0.32
AR_Pop0801_05	0.14	-0.88	-0.87	-0.67		0.19	-0.79	-0.59
AR_Pop0801_06	-0.30	0.48	-0.27	-0.24		1.57	-0.39	0.06
AR_Pop0801_07	-0.68	-0.36	-0.78	-0.47		1.57	-0.94	-0.40
AR_Pop0801_08	-0.55	0.30	-0.80	-0.48		1.58	-0.98	-0.31
AR_Pop0801_09	-0.82	0.64	-0.59	-0.33		1.57	-0.98	-0.34
AR_Pop0801_10	-0.03	0.39	-0.16	-0.25		1.57	-0.36	0.02
AR_Pop0801_11	-1.09	0.40	-0.37	-0.34		1.56	-0.75	-0.34
AR_Pop0801_12	-1.24	0.91	-0.87	-0.50		1.57	-1.14	-0.54
AR_Pop0801_13	-1.28	1.21	-0.27	-0.26		1.57	-0.47	-0.05
AR_Pop0801_14	0.08	1.22	-0.08	-0.12		1.57	-0.17	0.11
AR_Pop0801_15	-1.09	1.32	-0.18	-0.18		1.54	-0.09	0.06
AR_Pop0801_16	-0.58	1.34	-0.44	-0.21		1.58	-1.00	-0.32
AR_Pop0803_01	-0.85	0.43	-1.13	-0.45		0.20	-0.99	-0.93
AR_Pop0803_02	-0.37	1.22	-0.32	-0.11		1.52	-0.40	-0.13
AR_Pop0803_03	-1.13	1.30	-0.52	-0.16		1.54	-0.42	-0.01
AR_Pop0803_04	-0.23	0.53	-0.55	0.09		1.47	-0.37	-0.44
AR_Pop0803_05	-0.38	1.15	-0.43	-0.14		1.48	-0.12	-0.17
AR_Pop0803_06	-0.36	1.19	-0.01	0.26		1.45	0.08	0.02
AR_Pop0803_07	-0.15	1.10	-0.46	-0.19		1.45	-0.16	-0.54
AR_Pop0803_08	-0.60	0.30	-1.14	-0.63		-0.14	-1.08	-1.15
AR_Pop0803_09	-1.05	1.26	-0.30	0.09		1.44	-0.26	-0.20
AR_Pop0803_10	-1.03	1.36	-0.48	0.02		1.48	-0.45	-0.29
AR_Pop0803_11	-0.15	1.12	-0.64	0.04		1.45	-0.77	-0.98
AR_Pop0803_12	0.68	1.28	-0.18	0.34		1.49	-0.14	-0.02
AR_Pop0803_13	0.74	1.14	-0.22	0.29		1.50	0.22	0.00
AR_Pop0803_14	0.58	1.24	-0.10	0.34		1.51	-0.07	0.19
AR_Pop0803_15	0.69	1.36	-0.47	0.33		1.52	-0.30	-0.11
AR_Pop0813_01	1.01	1.36	0.10	0.32		1.50	0.17	0.21
AR_Pop0813_02	-0.08	0.32	-0.30	0.09		1.44	-0.35	-0.31

Table A.1. Genomic estimated breeding values (GEBV) per trait (*Xap* fruit (0-5) and *Xap* fruit (0-5) inc]) and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* fruit (0-5)					*Xap* fruit (0-5) inc		
	2013 ± 0.5	2014 ± 0.5	2015 – 1	Avg ± 0.5		2014 inc	2015 – 1 inc	Avg – 1 inc
AR_Pop0813_03	-0.24	-0.01	-0.19	-0.56		1.44	-0.10	-0.20
AR_Pop0813_04	-0.59	0.86	0.22	0.54		1.50	0.17	0.27
AR_Pop0813_05	0.85	0.77	0.11	0.70		1.51	0.15	0.20
AR_Pop0813_06	0.26	1.13	0.26	0.48		1.44	0.36	0.33
AR_Pop0813_07	-0.18	0.32	-0.25	-0.31		1.39	-0.14	-0.26
AR_Pop0813_08	0.09	0.82	-0.35	-0.02		1.44	-0.32	-0.19
AR_Pop0813_09	0.28	0.91	0.17	0.48		1.44	0.29	0.27
AR_Pop0813_10	0.77	0.71	0.07	0.36		1.45	0.12	0.15
AR_Pop0813_11	-0.99	0.34	-0.45	-0.50		0.87	-0.57	-0.52
AR_Pop0813_12	-0.98	0.63	-0.24	0.10		1.46	-0.46	-0.17
AR_Pop0817_01	1.41	1.52	1.28	1.17		1.56	1.39	1.30
AR_Pop0817_02	1.47	1.48	1.21	1.18		1.57	1.36	1.28
AR_Pop0817_03	1.28	1.53	1.32	1.13		1.57	1.49	1.41
AR_Pop0817_04	1.55	1.61	1.45	1.29		1.58	1.59	1.52
AR_Pop0817_05	1.55	1.61	1.45	1.27		1.58	1.58	1.52
AR_Pop0817_06	1.51	1.61	1.43	1.24		1.57	1.59	1.51
AR_Pop0817_07	1.49	1.60	1.45	1.27		1.58	1.57	1.52
AR_Pop0817_08	1.53	1.61	1.42	1.26		1.58	1.57	1.52
AR_Pop0817_09	1.55	1.61	1.45	1.28		1.57	1.60	1.53
AR_Pop0819_01	1.52	1.45	0.15	1.04		1.53	0.76	0.55
AR_Pop0819_02	1.37	1.18	0.35	0.84		1.55	0.93	0.63
AR_Pop0819_03	1.52	1.44	0.65	1.08		1.55	0.93	0.62
AR_Pop0819_04	1.46	1.27	0.41	0.77		1.55	0.78	0.47
AR_Pop0819_05	1.46	1.31	0.33	0.84		1.55	0.98	0.65
AR_Pop0819_06	1.37	1.24	0.26	0.81		1.55	0.73	0.51
AR_Pop0819_07	1.43	1.44	0.42	1.07		1.55	0.85	0.59
AR_Pop0819_08	1.46	1.53	0.68	1.07		1.55	0.99	0.73
AR_Pop0819_09	1.51	1.45	0.16	1.00		1.55	0.71	0.52
AR_Pop0819_10	1.32	1.15	0.38	0.63		1.55	0.90	0.60
AR_Pop0819_11	1.48	1.45	0.57	1.09		1.55	1.01	0.66
AR_Pop0819_12	1.37	1.37	0.61	0.98		1.55	1.03	0.64
AR_Pop0819_13	1.45	1.38	0.32	1.02		1.55	0.91	0.66
AR_Pop0819_14	1.42	1.43	0.30	1.02		1.55	0.92	0.63
AR_Pop0819_15	1.45	1.43	0.54	0.87		1.53	0.91	0.40
AR_Pop0819_16	1.52	1.42	0.57	1.13		1.55	0.98	0.65

Table A.1. Genomic estimated breeding values (GEBV) per trait (*Xap* fruit (0-5) and *Xap* fruit (0-5) inc]) and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* fruit (0-5)					*Xap* fruit (0-5) inc		
	2013 ± 0.5	2014 ± 0.5	2015 – 1	Avg ± 0.5		2014 inc	2015 – 1 inc	Avg – 1 inc
AR_Pop0819_17	1.53	1.52	0.75	1.15		1.55	1.07	0.77
AR_Pop0819_18	1.50	1.45	0.63	1.09		1.55	0.95	0.70
AR_Pop0819_19	1.40	1.34	0.39	0.87		1.55	0.97	0.60
AR_Pop0819_20	1.37	1.25	0.27	0.80		1.54	0.87	0.39
AR_Pop0819_21	1.51	1.50	0.59	1.14		1.55	1.00	0.67
AR_Pop0819_22	1.40	1.41	0.50	1.05		1.55	0.95	0.65
AR_Pop0819_23	1.32	1.16	0.22	0.78		1.54	0.78	0.45
AR_Pop0825_01	0.54	1.21	-0.02	0.51		1.55	0.53	0.31
AR_Pop0825_02	0.42	1.18	-0.01	0.58		1.49	0.59	0.33
AR_Pop0825_03	0.38	1.28	-0.05	0.65		1.49	0.57	0.34
AR_Pop0825_04	0.26	1.13	-0.05	0.39		1.54	0.50	0.28
AR_Pop0825_05	-0.08	1.08	-0.22	0.28		0.90	0.34	0.16
AR_Pop0825_06	0.62	1.24	0.00	0.68		0.15	0.28	0.13
AR_Pop0825_07	0.47	1.37	-0.04	0.54		1.55	0.57	0.34
AR_Pop0825_08	0.13	1.46	0.16	0.69		1.50	0.63	0.37
AR_Pop0825_09	0.48	1.27	0.10	0.65		1.49	0.60	0.37
AR_Pop0825_10	0.52	1.34	0.01	0.55		1.49	0.56	0.31
AR_Pop0825_11	0.34	1.46	0.17	0.78		1.55	0.58	0.34
AR_Pop0825_12	0.65	1.27	0.21	0.78		1.55	0.55	0.33
AR_Pop0825_13	0.48	1.45	0.01	0.58		1.50	0.49	0.28
AR_Pop0825_14	0.71	1.02	0.04	0.57		1.50	0.55	0.29
AR_Pop0825_15	0.52	0.89	-0.07	0.46		1.53	0.39	0.21
AR_Pop0825_16	0.13	1.45	0.05	0.61		1.50	0.56	0.31
AR_Pop0825_17	0.78	1.09	-0.07	0.54		0.19	0.37	0.18
AR_Pop0825_18	0.44	1.15	-0.02	0.54		0.90	0.49	0.28
Arrington	0.33	0.20	-0.57	-0.16		-0.12	-0.32	-0.54
Australian Muir	0.31	0.67	0.12	0.25		0.74	0.36	0.25
Belle	0.27	0.72	0.11	0.21		0.80	0.28	0.27
Bradley	-0.09	-0.12	-1.10	-0.46		-0.09	-0.86	-1.07
Candoka	0.56	0.95	0.38	0.49		1.08	0.66	0.50
Chinese Cling	0.38	1.00	0.66	0.51		0.17	0.82	0.65
Chiripa	-0.20	0.71	-0.09	-0.05		0.91	-0.09	0.00
Cumberland	-0.16	0.19	0.21	0.00		-0.38	0.40	0.14
D42_13W	0.28	0.73	0.08	0.29		0.71	0.31	0.25
Delicioso	0.02	0.68	-0.07	0.06		0.87	0.02	0.07

Table A.1. Genomic estimated breeding values (GEBV) per trait (*Xap* fruit (0-5) and *Xap* fruit (0-5) inc]) and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* fruit (0-5)					*Xap* fruit (0-5) inc		
	2013 ± 0.5	2014 ± 0.5	2015 – 1	Avg ± 0.5		2014 inc	2015 – 1 inc	Avg – 1 inc
Dix_16_3	0.26	0.83	0.24	0.37		0.88	0.51	0.40
Dix_22A_5	0.24	0.78	0.16	0.31		0.83	0.41	0.36
Dix_58_6	0.27	0.72	0.10	0.22		0.75	0.36	0.29
Dixon	0.31	0.71	0.09	0.24		0.75	0.34	0.20
dummy003	-0.29	0.77	-0.03	0.03		1.16	0.35	0.36
dummy004	0.68	0.20	-0.15	0.17		0.79	-0.10	0.01
dummy005	0.17	0.04	-0.15	-0.06		1.11	0.09	0.15
dummy006	-0.12	1.02	0.20	0.30		1.14	0.46	0.37
dummy007	-0.06	0.80	-0.35	-0.01		-0.16	-0.12	-0.14
dummy008	-0.38	0.87	0.27	0.19		1.15	0.27	0.29
dummy009	-0.28	0.82	-0.04	0.06		1.15	0.29	0.28
dummy010	0.64	1.03	0.37	0.55		1.21	0.61	0.54
Early Crawford	0.31	0.64	0.36	0.33		1.10	0.53	0.42
Elberta	0.42	0.88	0.74	0.53		0.14	0.88	0.70
Elberta OP27	0.34	0.77	0.46	0.39		0.43	0.61	0.50
F_A_371	0.53	0.28	-0.03	0.09		0.62	0.19	-0.01
F_A_427	0.29	0.77	0.04	0.29		0.59	0.26	0.21
F_A_433	0.06	0.65	-0.14	0.05		0.31	0.05	0.13
F_A_699	1.31	1.48	1.19	1.12		1.57	1.33	1.27
F_Candoka	0.23	0.69	0.06	0.23		0.71	0.32	0.27
F_Elberta OP27	0.26	0.63	0.12	0.24		0.75	0.34	0.27
F_Garden State	0.23	0.73	0.07	0.25		0.70	0.34	0.27
F_Ga. Belle	0.24	0.78	0.23	0.28		1.10	0.44	0.36
F_JH Hale	0.70	0.92	0.27	0.47		1.11	0.60	0.44
F_Nectared 4xA_24	0.46	0.52	-0.10	0.15		0.49	0.12	-0.01
F_NJ113115	0.26	0.68	0.11	0.23		0.79	0.36	0.24
F_NJ98838	0.28	0.67	0.10	0.24		0.78	0.29	0.26
F_NJN14	0.27	0.72	0.20	0.28		0.76	0.40	0.37
F_Sunrise	0.40	0.46	0.08	0.18		0.29	0.34	0.17
G_17_5E	-0.07	0.32	-0.32	0.05		1.00	-0.04	-0.17
Garden State	0.28	0.73	0.26	0.33		0.52	0.49	0.42
Ga. Belle	0.30	0.96	0.60	0.43		0.18	0.79	0.63
Goodmans Choice	0.27	0.66	0.07	0.24		0.70	0.28	0.27
Greensboro	-0.23	-0.28	-0.26	-0.37		-0.35	-0.07	-0.28
H_523	0.35	0.91	0.07	0.31		1.03	0.43	0.25

Table A.1. Genomic estimated breeding values (GEBV) per trait (*Xap* fruit (0-5) and *Xap* fruit (0-5) inc]) and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* fruit (0-5)					*Xap* fruit (0-5) inc		
	2013 ± 0.5	2014 ± 0.5	2015 – 1	Avg ± 0.5		2014 inc	2015 – 1 inc	Avg – 1 inc
HalBerta Giant	0.64	0.98	0.45	0.56		1.19	0.65	0.49
Hann Almond	0.28	0.71	0.11	0.23		0.79	0.35	0.20
Honeydew Hale	0.20	0.81	0.06	0.29		0.91	0.43	0.30
Jefferson	-0.10	0.44	0.07	0.05		0.12	0.37	0.21
JH Hale	0.87	1.18	0.75	0.74		1.49	0.99	0.73
Jing	-0.18	0.49	0.04	-0.11		0.95	0.18	0.23
Jungerman	0.29	0.74	0.14	0.30		0.77	0.36	0.29
M_A_789	1.36	1.54	1.26	1.16		1.57	1.41	1.33
MaoTao	0.34	0.65	0.13	0.37		1.14	0.42	0.37
Meredith	0.56	0.52	0.26	0.27		0.38	0.47	0.35
Mexican Honey	0.29	0.70	0.12	0.24		0.73	0.33	0.25
Nectalate	0.30	0.78	0.21	0.34		0.66	0.46	0.36
Nectared 4	0.36	0.54	0.23	0.12		0.16	0.43	0.27
Nectared 4 x A_24	0.32	0.38	-0.09	0.07		0.27	0.10	-0.08
Nectared 5	0.15	0.76	0.23	0.21		0.79	0.32	0.33
Nectared 7	0.20	1.17	0.68	0.67		1.44	0.84	0.78
NJ113115	0.23	0.71	0.11	0.25		0.80	0.33	0.28
NJ25032	0.29	0.75	0.14	0.29		0.79	0.35	0.25
NJ257	0.02	0.81	0.04	0.26		0.78	0.44	0.27
NJ38026	0.52	0.97	0.43	0.50		1.15	0.68	0.52
NJ5102893	0.29	0.72	0.09	0.26		0.74	0.28	0.22
NJ5106548	0.48	0.88	0.36	0.41		1.00	0.57	0.43
NJ5107397	0.28	0.71	0.13	0.23		0.65	0.35	0.27
NJ53739	0.24	0.67	0.10	0.20		0.70	0.28	0.24
NJ53939	0.24	0.78	0.32	0.26		0.71	0.45	0.40
NJ554367	-0.06	0.52	-0.21	0.09		1.02	0.10	0.03
NJ562021	0.36	0.57	-0.02	0.20		0.82	0.16	0.06
NJ6128	0.59	0.96	0.43	0.48		1.11	0.65	0.50
NJ822026	0.62	0.74	-0.09	0.14		0.65	0.20	0.05
NJ94727	0.60	0.96	0.46	0.48		1.13	0.64	0.48
NJ98838	0.43	0.82	0.26	0.37		0.92	0.47	0.35
NJC83	0.36	0.84	0.14	0.33		0.65	0.30	0.32
NJC95	0.41	0.81	0.22	0.35		0.82	0.40	0.34
NJLA3	0.56	0.94	0.47	0.48		1.14	0.67	0.50
NJN14	0.24	0.79	0.32	0.36		0.73	0.53	0.47

Table A.1. Genomic estimated breeding values (GEBV) per trait (*Xap* fruit (0-5) and *Xap* fruit (0-5) inc]) and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* fruit (0-5)					*Xap* fruit (0-5) inc		
	2013 ± 0.5	2014 ± 0.5	2015 – 1	Avg ± 0.5		2014 inc	2015 – 1 inc	Avg – 1 inc
NJN17	0.42	0.76	0.19	0.31		0.61	0.42	0.33
NJN21	0.30	0.87	0.35	0.33		0.87	0.52	0.46
NJN55	0.20	0.92	0.36	0.42		1.02	0.59	0.51
OldGold	0.31	0.68	0.11	0.29		0.80	0.31	0.25
Orange Cling	0.28	0.72	0.09	0.24		0.80	0.31	0.20
Orange Cling x Alameda	0.29	0.82	0.27	0.35		0.87	0.52	0.36
Peento	1.10	1.32	0.76	0.85		1.51	0.96	0.89
Pop8089	0.25	0.83	0.09	0.22		0.57	0.28	0.24
Raritan Rose	0.34	0.71	0.46	0.37		0.56	0.69	0.42
Redgold	0.72	0.77	0.41	0.43		0.72	0.60	0.38
Redskin	0.76	0.82	0.60	0.54		0.12	0.92	0.54
RR122_15	0.28	0.77	0.36	0.34		0.64	0.58	0.42
RR53_194	-0.09	0.16	-0.31	-0.26		0.42	-0.16	-0.03
Slappey	0.75	0.87	0.44	0.52		0.95	0.63	0.56
Sunrise	0.62	0.45	0.27	0.26		0.13	0.51	0.28
Transcaal Cling	0.27	0.74	0.12	0.21		0.81	0.35	0.26
Westbrook	0.60	0.26	-0.33	0.00		0.02	-0.11	-0.37
White County	-0.73	0.91	-0.52	-0.31		0.19	-0.17	-0.06
Winblo	0.93	0.79	0.52	0.53		0.12	0.88	0.47
Max-GBV	1.55	1.61	1.45	1.29		1.58	1.60	1.53
Avg-GBV	0.15	0.81	-0.01	0.21		1.06	0.21	0.19
Min-GBV	-1.39	-0.88	-1.33	-1.06		-1.54	-1.20	-1.58

Table A.2. Genomic estimated breeding values (GEBV) per trait [*Xap* leaf (0-5) and *Xap* leaf assay (*Xap*L_{AR} or *Xap*L_{88}) (0-3)] and year for the AR RosBREED pedigree.

Individual	*Xap* leaf (0-5)					*Xap*L_{AR} (0-3)	*Xap*L_{88} (0-3)
	2013	2014 – 1	2015 – 1	Avg – 1		2014	2014
104325	0.84	0.08	0.44	0.87		0.10	-0.02
752002002	0.15	-0.23	-0.69	-0.07		0.04	-0.06
10A4	0.15	-0.26	-0.61	-0.10		0.01	-0.06
22A5	0.46	-0.13	-0.23	0.33		-0.12	-0.07
2W68W	0.25	-0.16	-0.30	0.12		0.03	-0.08
4A_4	0.02	-0.48	-0.60	-0.12		0.06	-0.09
A_130	0.87	0.18	0.51	0.82		0.11	-0.08
A_145	0.16	-0.16	0.09	0.59		0.02	-0.07
A_154	0.09	-0.19	-0.53	0.04		-0.03	-0.10
A_172	0.70	-0.46	-0.90	-0.07		0.13	-0.09
A_176	0.69	-0.49	-0.91	-0.06		0.13	-0.09
A_178	-0.45	-0.98	-1.24	-0.63		0.08	-0.07
A_18	0.23	-0.31	-0.90	-0.20		0.05	-0.07
A_190	0.06	-0.36	-1.15	-0.42		0.05	-0.06
A_21	0.01	-0.35	-1.00	-0.35		0.03	-0.05
A_224	-0.04	-0.19	-0.40	0.21		-0.06	-0.11
A_232	0.77	-0.51	-0.93	-0.11		0.13	-0.10
A_24	0.29	-0.31	-0.88	-0.19		0.06	-0.07
A_270	0.92	0.74	0.51	0.84		0.18	-0.04
A_333	0.38	-0.17	-0.22	0.09		-0.16	-0.12
A_334	-0.63	-0.98	-1.74	-1.10		0.01	-0.01
A_367	-0.28	-0.71	-1.05	-0.54		0.12	-0.13
A_371	0.47	-0.53	-1.39	-0.28		0.11	-0.14
A_374	0.12	-0.25	-0.54	0.00		-0.01	-0.06
A_392	0.67	-0.04	0.13	-0.11		-0.03	-0.13
Bradley	-0.20	-0.67	-1.23	-0.54		0.13	-0.09
A_405	0.92	0.78	0.32	0.79		0.02	-0.03
A_419	-0.19	0.07	-0.99	-0.37		0.13	-0.13
A_427	0.25	-0.20	-0.57	0.06		0.04	-0.10
A_433	-0.42	-0.25	-0.96	-0.13		-0.18	-0.16
A_434	0.89	-0.16	-0.15	0.49		0.30	-0.03
A_441	0.05	-0.27	-0.75	-0.13		0.07	-0.11
A_500	-0.19	-0.39	-1.15	-0.43		-0.04	-0.03

Table A.2. Genomic estimated breeding values (GEBV) per trait [*Xap* leaf (0-5) and *Xap* leaf assay (*Xap*L_{AR} or *Xap*L_{88}) (0-3)] and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* leaf (0-5)					*Xap*L_{AR} (0-3)	*Xap*L_{88} (0-3)
	2013	2014 – 1	2015 – 1	Avg – 1		2014	2014
A_565	0.13	-0.28	-0.91	-0.14		0.03	-0.14
A_604	0.72	-0.18	-0.06	0.14		-0.16	-0.16
A_657	-0.83	-1.27	-1.82	-1.10		0.10	0.04
A_663	-1.02	-1.05	-1.91	-1.29		-0.18	0.00
A_665	-0.37	-1.25	-1.53	-1.01		0.21	-0.15
A_672	0.05	0.27	-0.57	0.00		0.06	-0.16
A_699	-0.21	-0.49	-1.12	-0.59		0.05	-0.19
A_708	1.52	0.62	0.19	0.74		0.33	-0.05
A_716	0.99	-1.51	-1.60	-0.64		0.05	-0.18
A_717	0.13	-0.51	-0.65	-0.27		-0.14	-0.15
A_760	-0.78	-0.88	-1.13	-0.93		-0.33	-0.11
Souvenirs	1.47	0.34	0.12	0.72		0.13	0.00
Amoore Sweet	-0.64	-0.48	-1.68	-0.90		-0.22	-0.08
A_772	0.82	-0.79	-0.96	-0.24		0.33	-0.13
A_773	1.43	-0.15	-0.77	0.27		0.07	-0.16
A_776	-1.20	-1.06	-1.76	-1.27		-0.34	-0.15
A_778	0.18	0.51	-0.37	0.17		0.17	0.10
A_783	-0.06	-0.16	-0.62	-0.40		-0.11	-0.18
A_789	-0.27	-1.23	-1.53	-0.94		0.10	-0.19
Admiral Dewey	0.40	-0.12	-0.18	0.32		0.04	-0.07
Alameda	0.24	-0.15	0.31	0.75		0.04	-0.07
Allgold	-0.21	-0.45	-1.28	-0.56		0.00	-0.01
AR_Pop_1_01	0.17	-0.40	-1.63	-0.62		-0.30	-0.21
AR_Pop_1_02	-0.03	-0.21	0.07	-0.43		0.07	-0.21
AR_Pop_1_03	-0.56	-0.89	-1.19	-0.83		-0.31	-0.24
AR_Pop_1_04	-0.07	-0.34	-0.85	-0.57		-0.22	-0.22
AR_Pop_1_05	-0.84	-0.18	-0.76	-0.55		-0.35	-0.21
AR_Pop_1_06	-0.93	-0.06	-0.78	-0.51		-0.45	-0.23
AR_Pop_1_07	-0.83	0.13	-0.68	-0.43		-0.31	-0.23
AR_Pop_1_08	-0.59	-0.26	0.08	-0.60		-0.12	-0.22
AR_Pop_1_09	-0.27	-0.01	-0.75	-0.26		-0.05	-0.21
AR_Pop_1_10	-0.83	-1.48	-1.07	-1.21		-0.26	-0.24
AR_Pop_1_11	-1.10	-0.02	-0.76	-0.51		-0.40	-0.22

Table A.2. Genomic estimated breeding values (GEBV) per trait [*Xap* leaf (0-5) and *Xap* leaf assay ($Xap\text{L}_{AR}$ or $Xap\text{L}_{88}$) (0-3)] and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* leaf (0-5)					$Xap\text{L}_{AR}$ (0-3)	$Xap\text{L}_{88}$ (0-3)
	2013	2014 – 1	2015 – 1	Avg – 1		2014	2014
AR_Pop_1_12	-0.90	-0.26	-0.85	-0.67		-0.23	-0.22
AR_Pop_1_13	-0.77	-0.13	-0.73	-0.27		-0.33	-0.22
AR_Pop_1_14	-0.48	-0.60	-1.22	-0.73		-0.28	-0.18
AR_Pop_1_15	-0.86	-0.30	-1.54	-0.62		-0.25	-0.22
AR_Pop_1_16	0.26	0.05	-0.70	-0.07		-0.06	-0.21
AR_Pop_1_17	0.10	-0.28	-0.06	-0.63		-0.36	-0.22
AR_Pop_1_18	-0.92	-0.37	-1.31	-0.67		-0.38	-0.22
AR_Pop_1_19	-0.62	0.04	-1.45	-0.29		-0.39	-0.22
AR_Pop_1_20	-0.89	0.11	-0.78	-0.50		-0.46	-0.23
AR_Pop_1_21	-0.35	-0.42	-0.08	-0.63		0.03	-0.22
AR_Pop_1_22	0.09	-0.12	0.10	-0.31		-0.03	-0.22
AR_Pop_1_23	-0.24	0.18	0.16	-0.16		-0.07	-0.22
AR_Pop_1_24	-0.08	-0.06	-0.74	-0.35		-0.10	-0.22
AR_Pop_1_25	-1.07	-0.38	-0.78	-0.69		-0.49	-0.22
AR_Pop_1_26	-0.50	-0.52	-0.77	-0.57		-0.45	-0.22
AR_Pop_1_27	-0.98	-0.47	-1.55	-1.01		-0.47	-0.21
AR_Pop_1_28	-0.92	-0.38	-1.49	-0.78		-0.49	-0.22
AR_Pop_1_29	-0.21	0.20	0.08	-0.06		0.12	-0.20
AR_Pop_1_30	-1.16	-1.24	-0.54	-0.86		-0.27	-0.23
AR_Pop_1_31	0.13	-0.12	-1.25	-0.47		-0.08	-0.22
AR_Pop_1_32	0.23	0.30	0.10	0.19		0.13	-0.21
AR_Pop_1_33	-0.60	-0.35	-0.84	-0.65		-0.32	-0.22
AR_Pop_1_34	-0.22	0.20	0.12	0.18		0.01	-0.21
AR_Pop_1_35	0.30	0.21	0.17	0.01		0.18	-0.21
AR_Pop_1_36	0.38	0.24	-0.70	0.07		-0.03	-0.22
AR_Pop_1_37	-0.85	-0.46	-0.75	-0.74		-0.19	-0.20
AR_Pop_1_38	-0.25	-0.14	-0.68	-0.42		0.02	-0.22
AR_Pop_1_39	-0.99	-0.13	-0.69	-0.47		-0.33	-0.21
AR_Pop_1_40	-1.28	-0.89	-0.93	-0.85		-0.38	-0.25
AR_Pop_1_41	0.48	0.10	-0.16	0.21		0.03	-0.17
AR_Pop_1_42	0.12	-0.11	0.04	-0.21		0.09	-0.21
AR_Pop_1_43	-0.85	-0.18	-0.03	-0.50		-0.36	-0.21
AR_Pop_1_44	-0.03	-0.35	-0.58	-0.05		-0.03	-0.21

Table A.2. Genomic estimated breeding values (GEBV) per trait [*Xap* leaf (0-5) and *Xap* leaf assay (*Xap*L_{AR} or *Xap*L_{88}) (0-3)] and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* leaf (0-5)					*Xap*L_{AR} (0-3)	*Xap*L_{88} (0-3)
	2013	2014 – 1	2015 – 1	Avg – 1		2014	2014
AR_Pop_1_45	-1.01	-0.16	-0.86	-0.72		-0.37	-0.22
AR_Pop_1_46	-0.60	0.26	-0.75	-0.01		-0.15	-0.22
AR_Pop_1_47	-0.59	0.28	-0.22	0.03		-0.13	-0.21
AR_Pop_1_48	0.18	-0.40	-1.57	-0.61		-0.04	-0.21
AR_Pop_1_49	-0.98	-0.73	-0.87	-0.97		-0.51	-0.22
AR_Pop0801_01	-0.84	-0.51	-1.31	-0.89		-0.52	-0.17
AR_Pop0801_02	-0.82	-0.65	-1.02	-0.91		-0.31	-0.16
AR_Pop0801_03	-0.85	-1.08	-1.43	-1.06		-0.57	-0.15
AR_Pop0801_04	-0.80	-0.72	-1.28	-0.91		-0.19	-0.20
AR_Pop0801_05	-0.81	-1.12	-1.44	-1.06		-0.41	-0.14
AR_Pop0801_06	-0.80	-0.35	-0.93	-0.72		-0.08	-0.16
AR_Pop0801_07	-0.59	-0.49	-0.92	-0.61		-0.09	-0.16
AR_Pop0801_08	-0.95	-0.67	-1.02	-0.93		-0.52	-0.17
AR_Pop0801_09	-0.66	-0.69	-1.11	-0.88		-0.35	-0.16
AR_Pop0801_10	-0.69	-0.40	-0.97	-0.70		-0.16	-0.17
AR_Pop0801_11	-0.62	-0.61	-1.04	-0.86		-0.17	-0.14
AR_Pop0801_12	-0.72	-0.68	-1.34	-0.96		-0.45	-0.16
AR_Pop0801_13	-0.65	-0.41	-1.22	-0.73		-0.01	-0.16
AR_Pop0801_14	-0.64	-0.55	-1.24	-0.81		-0.19	-0.21
AR_Pop0801_15	-1.10	-1.05	-1.42	-1.00		-0.39	-0.18
AR_Pop0801_16	-0.81	-0.80	-1.36	-1.05		-0.30	-0.20
AR_Pop0803_01	-1.05	-0.05	-1.09	-0.63		-0.43	-0.08
AR_Pop0803_02	-0.78	0.50	-1.45	-0.60		-0.34	-0.06
AR_Pop0803_03	-0.41	-0.01	-0.67	-0.35		-0.17	-0.03
AR_Pop0803_04	0.02	0.65	-0.64	-0.07		-0.04	0.02
AR_Pop0803_05	-0.73	0.67	-0.59	-0.19		-0.18	-0.03
AR_Pop0803_06	-0.75	0.86	-0.50	-0.13		0.07	-0.01
AR_Pop0803_07	-1.03	0.69	-0.61	-0.31		-0.19	0.09
AR_Pop0803_08	-0.65	-0.90	-1.52	-0.81		-0.10	0.02
AR_Pop0803_09	-0.76	0.71	-0.66	-0.21		-0.16	0.01
AR_Pop0803_10	0.36	0.57	-0.90	-0.23		0.11	-0.02
AR_Pop0803_11	0.42	0.80	-0.88	-0.19		0.09	0.08
AR_Pop0803_12	-0.98	1.02	-0.86	-0.32		-0.17	-0.04

Table A.2. Genomic estimated breeding values (GEBV) per trait [*Xap* leaf (0-5) and *Xap* leaf assay (*Xap*L_{AR} or *Xap*L_{88}) (0-3)] and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* leaf (0-5)					*Xap*L_{AR} (0-3)	*Xap*L_{88} (0-3)
	2013	2014 – 1	2015 – 1	Avg – 1		2014	2014
AR_Pop0803_13	0.41	1.03	-0.44	0.23		0.12	0.06
AR_Pop0803_14	-0.60	0.86	-0.86	-0.27		-0.04	-0.07
AR_Pop0803_15	0.52	0.47	-0.86	0.06		0.06	0.07
AR_Pop0813_01	0.72	0.82	-0.54	0.69		0.41	-0.11
AR_Pop0813_02	0.61	-0.15	0.09	0.03		0.11	-0.15
AR_Pop0813_03	0.15	-0.81	-0.77	-0.67		0.03	-0.17
AR_Pop0813_04	0.32	-0.17	0.05	0.00		0.14	-0.12
AR_Pop0813_05	0.74	0.74	0.21	0.52		0.43	-0.12
AR_Pop0813_06	0.44	0.81	-0.17	0.23		0.08	-0.11
AR_Pop0813_07	0.63	0.50	0.00	0.32		0.27	-0.16
AR_Pop0813_08	0.64	0.65	-0.58	0.32		0.27	-0.16
AR_Pop0813_09	0.52	0.84	0.07	0.35		0.16	-0.12
AR_Pop0813_10	0.51	0.52	-0.15	0.27		0.12	-0.11
AR_Pop0813_11	0.39	-0.56	-0.40	-0.22		0.08	-0.15
AR_Pop0813_12	0.17	-0.33	0.03	-0.16		0.12	-0.16
AR_Pop0817_01	0.43	1.62	1.32	1.09		0.44	0.15
AR_Pop0817_02	0.84	1.60	1.27	1.08		0.43	0.16
AR_Pop0817_03	0.33	1.75	1.32	1.06		0.50	0.19
AR_Pop0817_04	1.33	1.80	1.50	1.38		0.68	0.20
AR_Pop0817_05	1.31	1.79	1.46	1.37		0.66	0.20
AR_Pop0817_06	1.11	1.79	1.37	1.29		0.67	0.20
AR_Pop0817_07	1.22	1.81	1.49	1.35		0.68	0.20
AR_Pop0817_08	1.24	1.81	1.48	1.34		0.68	0.20
AR_Pop0817_09	1.20	1.80	1.50	1.31		0.67	0.21
AR_Pop0819_01	1.47	-0.14	-0.08	0.48		0.05	-0.12
AR_Pop0819_02	1.55	0.64	-0.08	0.56		0.30	-0.16
AR_Pop0819_03	1.36	0.70	0.08	0.54		0.22	-0.12
AR_Pop0819_04	1.51	0.64	-0.82	0.33		0.06	-0.12
AR_Pop0819_05	1.54	0.69	-0.51	0.56		0.30	-0.16
AR_Pop0819_06	1.44	0.65	-0.06	0.57		0.32	-0.10
AR_Pop0819_07	1.50	0.65	0.01	0.51		0.12	-0.05
AR_Pop0819_08	1.63	0.72	0.11	0.69		0.28	-0.08
AR_Pop0819_09	1.41	0.68	-0.16	0.46		0.08	-0.09

Table A.2. Genomic estimated breeding values (GEBV) per trait [*Xap* leaf (0-5) and *Xap* leaf assay (*Xap*L_{AR} or *Xap*L_{88}) (0-3)] and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* leaf (0-5)					*Xap*L_{AR} (0-3)	*Xap*L_{88} (0-3)
	2013	2014 – 1	2015 – 1	Avg – 1		2014	2014
AR_Pop0819_10	1.42	0.50	-0.74	0.37		0.23	-0.17
AR_Pop0819_11	1.52	0.71	-0.10	0.49		0.05	-0.09
AR_Pop0819_12	1.59	0.69	0.25	0.67		0.17	-0.15
AR_Pop0819_13	1.63	0.53	0.19	0.74		0.32	-0.05
AR_Pop0819_14	1.38	0.67	0.19	0.55		0.06	-0.17
AR_Pop0819_15	1.64	0.70	-0.26	0.63		0.32	-0.09
AR_Pop0819_16	1.48	0.69	0.33	0.65		0.29	-0.11
AR_Pop0819_17	1.58	0.72	0.38	0.81		0.31	-0.12
AR_Pop0819_18	1.57	0.69	0.05	0.63		0.33	-0.07
AR_Pop0819_19	1.67	0.69	-0.59	0.60		0.22	-0.16
AR_Pop0819_20	1.65	0.75	-0.26	0.74		0.26	-0.15
AR_Pop0819_21	1.66	0.71	0.35	0.81		0.30	-0.04
AR_Pop0819_22	1.53	0.65	0.20	0.70		0.34	-0.12
AR_Pop0819_23	1.28	0.61	-0.08	0.37		0.08	-0.11
AR_Pop0825_01	0.42	0.30	-0.13	0.11		-0.03	-0.02
AR_Pop0825_02	0.41	0.35	-0.13	0.05		-0.11	-0.07
AR_Pop0825_03	0.48	-0.55	-0.91	-0.26		-0.19	-0.10
AR_Pop0825_04	0.02	0.36	-0.09	-0.14		-0.22	-0.08
AR_Pop0825_05	0.07	-0.13	-0.23	-0.16		-0.13	-0.02
AR_Pop0825_06	0.36	-0.86	-0.17	-0.11		0.04	-0.02
AR_Pop0825_07	0.27	-0.16	-0.90	-0.30		-0.29	-0.07
AR_Pop0825_08	0.43	0.35	-0.06	0.07		-0.11	-0.05
AR_Pop0825_09	0.51	0.03	-0.85	0.01		0.04	-0.02
AR_Pop0825_10	0.41	-0.24	-0.84	-0.12		-0.10	-0.05
AR_Pop0825_11	0.59	0.33	-0.02	0.14		-0.02	0.00
AR_Pop0825_12	0.56	0.39	-0.16	0.03		-0.06	-0.02
AR_Pop0825_13	0.18	-0.17	-0.15	-0.14		-0.19	-0.12
AR_Pop0825_14	0.57	0.15	-0.69	0.03		-0.04	0.00
AR_Pop0825_15	0.18	0.16	-0.17	-0.08		-0.05	-0.07
AR_Pop0825_16	0.37	-0.07	-0.23	-0.18		-0.21	-0.07
AR_Pop0825_17	0.26	-0.83	-0.18	-0.04		-0.08	-0.06
AR_Pop0825_18	0.34	-0.27	-0.51	-0.09		-0.11	-0.06
Arrington	0.29	-0.79	-1.09	-0.39		0.08	-0.12

Table A.2. Genomic estimated breeding values (GEBV) per trait [*Xap* leaf (0-5) and *Xap* leaf assay (*Xap*L_{AR} or *Xap*L_{88}) (0-3)] and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* leaf (0-5)					*Xap*L_{AR} (0-3)	*Xap*L_{88} (0-3)
	2013	2014 – 1	2015 – 1	Avg – 1		2014	2014
Australian Muir	0.26	-0.13	-0.28	0.15		0.02	-0.07
Belle	0.26	-0.18	-0.31	0.09		0.04	-0.07
Bradley	-0.46	-0.84	-1.64	-0.76		0.06	-0.05
Candoka	0.61	-0.04	0.23	0.68		0.09	-0.02
Chinese Cling	0.35	-0.38	0.64	0.89		0.07	-0.03
Chiripa	-0.10	-0.41	-0.47	-0.21		0.07	-0.13
Cumberland	-0.16	-0.37	-0.16	0.30		0.05	-0.04
D42_13W	0.24	-0.16	-0.32	0.18		0.06	-0.09
Delicioso	0.03	-0.28	-0.51	-0.19		0.05	-0.10
Dix_16_3	0.24	-0.15	-0.01	0.44		0.04	-0.07
Dix_22A_5	0.20	-0.17	-0.17	0.29		0.04	-0.08
Dix_58_6	0.24	-0.16	-0.34	0.16		0.03	-0.08
Dixon	0.24	-0.16	-0.36	0.13		0.03	-0.07
dummy003	-0.24	-0.58	-0.63	-0.20		-0.06	-0.14
dummy004	0.00	-0.60	-0.67	-0.26		-0.23	-0.10
dummy005	0.05	-0.63	-0.65	-0.26		-0.09	-0.08
dummy006	-0.27	-0.63	-0.63	-0.25		-0.17	-0.13
dummy007	-0.22	-0.06	-0.34	0.03		-0.15	-0.11
dummy008	-0.01	0.36	-0.63	0.04		-0.08	-0.07
dummy009	0.25	-0.03	0.04	0.25		0.08	-0.01
dummy010	0.81	0.36	0.29	0.83		0.20	0.06
Early Crawford	0.60	0.02	0.01	0.39		0.07	-0.04
Elberta	0.57	-0.21	0.67	0.90		0.10	0.00
Elberta OP27	0.42	-0.19	0.20	0.54		0.07	-0.04
F_A_371	0.36	-0.38	-0.89	-0.12		0.05	-0.12
F_A_427	0.28	-0.22	-0.43	0.08		0.05	-0.09
F_A_433	-0.06	-0.24	-0.69	-0.11		-0.08	-0.11
F_A_699	0.62	1.59	1.23	1.09		0.45	0.15
F_Candoka	0.26	-0.19	-0.29	0.15		0.04	-0.07
F_Elberta OP27	0.26	-0.17	-0.29	0.15		0.04	-0.07
F_Garden State	0.25	-0.17	-0.32	0.24		0.04	-0.08
F_Ga. Belle	0.35	-0.13	-0.05	0.44		0.02	-0.08
F_JH Hale	0.63	-0.09	0.02	0.59		0.09	-0.01
F_Nectared 4xA_24	0.61	-0.42	-0.72	0.01		0.08	-0.08

Table A.2. Genomic estimated breeding values (GEBV) per trait [*Xap* leaf (0-5) and *Xap* leaf assay (*Xap*L_{AR} or *Xap*L_{88}) (0-3)] and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* leaf (0-5)					*Xap*L_{AR} (0-3)	*Xap*L_{88} (0-3)
	2013	2014 – 1	2015 – 1	Avg – 1		2014	2014
F_NJ113115	0.30	-0.14	-0.32	0.15		0.03	-0.07
F_NJ98838	0.22	-0.16	-0.32	0.12		0.04	-0.07
F_NJN14	0.26	-0.17	-0.10	0.28		0.05	-0.08
F_Sunrise	0.33	-0.09	-0.37	0.13		0.08	-0.07
G_17_5E	0.07	-0.35	-0.98	-0.31		0.03	-0.05
Garden State	0.37	-0.19	-0.03	0.43		0.06	-0.07
Ga. Belle	0.26	-0.37	0.48	0.79		0.06	-0.04
Goodmans Choice	0.27	-0.14	-0.32	0.14		0.02	-0.08
Greensboro	-0.18	-0.15	-0.70	-0.24		0.02	-0.07
H_523	0.55	-0.08	-0.10	0.04		-0.01	-0.08
HalBerta Giant	0.69	0.15	0.23	0.69		0.10	-0.02
Hann Almond	0.27	-0.17	-0.30	0.16		0.03	-0.07
Honeydew Hale	0.39	-0.15	-0.22	0.07		0.01	-0.08
Jefferson	-0.01	-0.14	-0.12	0.17		0.02	-0.10
JH Hale	1.06	0.13	0.76	1.16		0.14	0.03
Jing	0.08	0.07	-0.55	-0.02		0.08	-0.10
Jungerman	0.20	-0.16	-0.26	0.23		0.05	-0.08
M_A_789	0.84	1.67	1.21	1.12		0.51	0.16
MaoTao	0.61	-0.21	-0.16	0.19		0.03	-0.11
Meredith	0.61	-0.10	0.09	0.60		0.05	-0.06
Mexican Honey	0.24	-0.15	-0.35	0.13		0.03	-0.07
Nectalate	0.30	-0.19	-0.04	0.37		0.05	-0.08
Nectared 4	0.42	-0.16	-0.02	0.40		0.08	-0.08
Nectared 4 x A_24	0.51	-0.32	-0.67	0.01		0.12	-0.08
Nectared 5	0.15	-0.27	-0.03	0.32		0.06	-0.11
Nectared 7	0.23	-0.15	0.70	1.08		0.05	-0.09
NJ113115	0.28	-0.13	-0.32	0.15		0.04	-0.06
NJ25032	0.22	-0.19	-0.25	0.21		0.03	-0.08
NJ257	0.31	-0.12	-0.09	0.03		0.00	-0.12
NJ38026	0.65	-0.01	0.21	0.64		0.08	-0.01
NJ5102893	0.25	-0.17	-0.28	0.14		0.04	-0.08
NJ5106548	0.54	-0.04	0.05	0.50		0.07	-0.03
NJ5107397	0.31	-0.21	-0.29	0.24		0.04	-0.06
NJ53739	0.25	-0.18	-0.34	0.16		0.04	-0.07

Table A.2. Genomic estimated breeding values (GEBV) per trait [*Xap* leaf (0-5) and *Xap* leaf assay (*Xap*L_{AR} or *Xap*L_{88}) (0-3)] and year for the AR RosBREED pedigree (Cont.).

Individual	*Xap* leaf (0-5)					*Xap*L_{AR} (0-3)	*Xap*L_{88} (0-3)
	2013	2014 – 1	2015 – 1	Avg – 1		2014	2014
NJ53939	0.24	-0.19	0.09	0.50		0.05	-0.09
NJ554367	0.01	-0.31	-0.84	-0.23		0.02	-0.04
NJ562021	0.36	-0.23	-0.55	0.05		0.06	-0.07
NJ6128	0.66	-0.02	0.19	0.65		0.07	-0.02
NJ822026	0.39	-0.33	-0.80	-0.03		0.07	-0.09
NJ94727	0.67	-0.01	0.26	0.65		0.07	-0.02
NJ98838	0.45	-0.09	0.00	0.41		0.06	-0.04
NJC83	0.31	-0.14	-0.30	0.20		0.04	-0.08
NJC95	0.40	-0.10	-0.15	0.31		0.05	-0.06
NJLA3	0.66	-0.02	0.20	0.63		0.08	-0.02
NJN14	0.29	-0.19	0.11	0.48		0.06	-0.09
NJN17	0.42	-0.19	-0.15	0.30		0.06	-0.06
NJN21	0.29	-0.10	0.16	0.56		0.03	-0.08
NJN55	0.24	-0.19	0.16	0.65		0.04	-0.08
OldGold	0.26	-0.07	-0.28	0.15		0.04	-0.08
Orange Cling	0.25	-0.17	-0.38	0.13		0.02	-0.07
Orange Cling x Alameda	0.28	-0.18	-0.05	0.44		0.03	-0.07
Peento	1.21	0.05	0.90	1.18		0.06	-0.07
Pop8089	0.24	-0.18	-0.47	0.11		0.03	-0.08
Raritan Rose	0.41	-0.10	0.29	0.72		0.08	-0.01
Redgold	0.74	0.37	0.23	0.70		0.14	-0.02
Redskin	0.78	-0.17	0.40	0.84		0.15	0.06
RR122_15	0.31	-0.12	0.19	0.60		0.03	-0.04
RR53_194	-0.15	-0.34	-0.95	-0.39		0.11	-0.12
Slappey	0.80	-0.14	0.33	0.86		0.04	-0.07
Sunrise	0.61	0.11	0.04	0.54		0.10	-0.04
Transcaal Cling	0.23	-0.20	-0.36	0.16		0.05	-0.07
Westbrook	0.94	-0.63	-1.29	-0.19		0.21	-0.10
White County	-0.43	-0.91	-0.95	-0.79		-0.34	-0.22
Winblo	1.32	-0.20	0.22	0.78		0.18	0.08
Max-GEBV	1.67	1.81	1.50	1.38		**0.68**	**0.21**
Avg-GEBV	0.20	-0.05	-0.40	0.02		**0.00**	**-0.09**
Min-GEBV	-1.28	-1.51	-1.91	-1.29		**-0.57**	**-0.25**

Table A.3. Genomic estimated breeding values (GEBV) per trait [maturity date (day of year) and soluble solids content (SSC)] and year for the AR RosBREED pedigree.

Individual	Maturity date (day of year)					Soluble solids content (SSC)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
104325	-7.86	-8.14	-1.63	-3.98		-0.99	-0.73	0.39	-0.78
752002002	-13.43	-9.27	-7.18	-12.37		0.12	0.18	0.16	-0.06
10A4	-13.73	-9.38	-6.75	-12.08		-0.02	0.32	0.18	-0.11
22A5	-16.42	-13.37	-7.67	-16.00		0.24	0.73	0.67	0.18
2W68W	-8.46	-3.99	-4.61	-7.57		0.18	0.20	0.16	-0.02
4A_4	-10.61	-3.64	-4.23	-10.28		0.45	0.12	0.10	0.06
A_130	-16.85	-8.43	-7.68	-13.64		0.09	0.58	-0.08	0.10
A_145	-6.01	-3.83	-3.25	-6.73		0.27	0.13	0.09	-0.17
A_154	-5.85	-2.56	-2.81	-5.76		0.31	0.46	0.04	-0.10
A_172	-26.35	-13.70	-11.29	-20.06		-0.83	0.18	-0.53	-0.87
A_176	-26.82	-13.03	-11.38	-20.08		-0.80	0.11	-0.53	-0.84
A_178	-16.59	-19.80	-9.66	-16.89		1.08	-0.99	0.28	-0.02
A_18	-16.26	-12.15	-9.19	-14.53		0.15	0.17	-0.03	-0.15
A_190	-19.15	-15.57	-11.07	-17.68		0.50	0.10	-0.08	-0.13
A_21	-17.99	-12.97	-9.32	-15.66		0.18	0.21	0.21	-0.12
A_224	-2.34	-1.10	-0.57	-3.72		0.45	0.57	-0.03	-0.22
A_232	-29.74	-17.34	-11.85	-21.23		-0.59	0.07	-0.55	-0.84
A_24	-17.98	-11.83	-8.82	-15.06		-0.17	0.20	-0.05	-0.28
A_270	-17.30	-11.16	-8.33	-14.08		0.00	0.55	-0.04	0.21
A_333	-13.00	-9.36	-4.05	0.18		1.02	-0.02	0.95	0.73
A_334	-14.71	-19.47	-11.32	-12.97		0.70	-0.11	0.63	0.26
A_367	-7.48	0.96	-1.57	-7.78		0.75	0.52	0.07	0.30
A_371	14.47	4.91	3.37	7.66		2.02	1.00	0.17	1.11
A_374	-12.31	-9.96	-7.45	-12.66		0.00	-0.07	0.38	-0.23
A_392	-2.79	-2.14	0.31	-4.69		0.03	-0.67	-0.83	0.04
A_402	-17.63	-17.91	-10.48	-17.14		0.90	-0.38	0.05	-0.21
A_405	-24.29	-18.68	-11.29	-21.99		0.42	1.27	0.44	0.62
A_419	4.32	6.35	5.05	3.35		-0.55	1.38	-0.04	0.53
A_427	-2.12	0.17	-4.66	-3.05		0.71	0.33	0.08	0.26
A_433	4.58	5.52	4.32	1.82		0.79	1.16	-0.28	-0.36
A_434	-16.91	-14.80	-8.05	-15.55		0.22	-0.65	0.62	-0.31
A_441	1.78	5.35	-1.82	0.73		0.47	0.51	0.07	0.28
A_500	-18.72	-17.85	-10.65	-18.41		-0.22	-0.06	0.70	-0.26

Table A.3. Genomic estimated breeding values (GEBV) per trait [maturity date (day of year) and soluble solids content (SSC)] and year for the AR RosBREED pedigree (Cont.).

Individual	Maturity Date (day of year)					Soluble solids content (SSC)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
A_565	7.11	8.19	-2.42	4.30		1.18	0.57	-0.02	0.59
A_604	-14.10	-15.47	-6.84	-0.28		1.96	0.92	1.42	1.45
A_657	-11.81	-19.48	-13.05	-12.22		1.05	-1.59	-0.04	0.05
A_663	-13.75	-21.15	-12.58	-22.19		1.35	-0.22	0.76	0.49
A_665	3.95	-1.56	-0.87	-3.42		1.94	0.58	0.12	0.88
A_672	-5.41	-3.93	0.86	-4.28		-0.42	1.72	0.60	0.86
A_699	4.29	0.30	-7.32	-3.41		2.25	1.65	-0.10	1.26
A_708	-4.49	-3.86	1.32	-4.41		-0.21	-0.16	0.15	-0.17
A_716	14.52	17.37	-5.39	6.30		-0.32	-0.03	-0.21	-0.33
A_717	-25.51	-19.65	-2.97	0.73		1.21	-0.20	0.97	0.57
A_760	-21.87	-25.09	-14.19	-24.55		0.51	-0.10	0.11	-0.17
A_763	-5.23	-10.96	-0.12	-7.27		-0.66	-1.41	0.40	-0.45
A_765	-17.94	-19.23	-13.87	-21.72		2.37	0.40	0.50	1.07
A_772	1.13	0.73	0.02	-4.25		2.57	2.28	-0.19	1.67
A_773	13.72	2.50	1.41	4.53		2.56	1.99	0.61	1.93
A_776	-17.76	-19.87	-14.19	-23.15		2.42	1.97	0.76	1.82
A_778	-17.70	-19.71	-13.87	-21.70		-0.18	-1.13	-0.30	-0.39
A_783	-17.33	-16.78	-10.62	-15.73		2.10	1.50	1.19	1.73
A_789	17.44	12.30	13.60	11.49		2.00	3.09	0.64	1.61
Admiral Dewey	-9.52	-10.60	12.02	-2.07		0.07	0.23	0.14	-0.05
Alameda	-9.54	-4.53	-4.87	-8.91		0.08	0.25	0.17	-0.10
Allgold	-20.67	-16.27	-9.54	-16.40		-0.21	0.49	0.47	-0.03
AR_Pop_1_01	-2.01	-1.86	1.31	-2.50		-1.10	2.33	-1.11	0.03
AR_Pop_1_02	-5.18	-3.33	0.43	-4.13		-0.14	1.60	-0.70	0.42
AR_Pop_1_03	17.12	14.56	13.80	12.25		1.38	1.70	-2.04	0.13
AR_Pop_1_04	-2.57	-0.01	0.92	-3.22		0.38	2.58	-1.28	0.55
AR_Pop_1_05	-24.90	-23.79	-13.44	-23.92		-0.03	-1.32	-0.96	-0.51
AR_Pop_1_06	-24.87	-24.05	-13.54	-24.43		0.05	-1.06	-1.22	-0.60
AR_Pop_1_07	14.33	16.83	13.54	14.19		-0.54	1.47	-1.28	0.19
AR_Pop_1_08	-5.22	-3.79	0.48	-4.12		0.85	1.65	-1.05	0.85
AR_Pop_1_09	-2.57	0.83	1.28	-2.52		-0.39	2.71	-1.20	0.37
AR_Pop_1_10	17.14	15.92	13.52	12.42		1.57	-0.83	-1.99	-0.38
AR_Pop_1_11	-5.79	-2.74	0.33	-4.54		0.97	2.00	-0.70	1.21
AR_Pop_1_12	-25.05	-21.97	-13.24	-22.52		-0.03	-1.05	-0.75	-0.45
AR_Pop_1_13	16.94	18.22	14.58	15.17		-0.73	2.42	-1.12	0.52

Table A.3. Genomic estimated breeding values (GEBV) per trait [maturity date (day of year) and soluble solids content (SSC)] and year for the AR RosBREED pedigree (Cont.).

Individual	Maturity Date (day of year)					Soluble solids content (SSC)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
AR_Pop_1_14	-3.01	7.86	-13.18	-6.55		-0.98	0.99	0.14	0.21
AR_Pop_1_15	-24.76	-24.91	-13.57	-24.68		-1.09	-1.63	-1.00	-0.99
AR_Pop_1_16	-2.04	-0.46	1.25	-2.39		0.15	2.73	-1.15	0.51
AR_Pop_1_17	-4.95	-5.83	0.59	-6.14		-0.80	2.44	-1.15	0.38
AR_Pop_1_18	-22.82	-23.94	-13.49	-23.76		-0.78	-1.54	-1.31	-0.99
AR_Pop_1_19	-1.78	0.73	1.47	-2.17		0.22	2.53	-1.34	0.41
AR_Pop_1_20	-24.58	-19.31	-13.02	-21.21		0.50	0.13	-0.86	0.24
AR_Pop_1_21	16.56	12.45	14.00	11.67		1.20	3.09	-0.70	1.40
AR_Pop_1_22	-4.98	-4.58	0.83	-4.28		-0.70	2.21	-0.87	0.25
AR_Pop_1_23	-22.60	-19.07	-12.51	-20.56		0.56	0.97	-0.82	0.69
AR_Pop_1_24	-22.39	-19.74	-12.84	-20.66		-0.32	-0.96	-0.89	-0.59
AR_Pop_1_25	14.18	15.63	14.22	13.65		-0.32	2.26	-1.04	0.61
AR_Pop_1_26	17.36	17.58	14.78	15.36		-0.06	2.68	-0.78	1.14
AR_Pop_1_27	-22.73	-24.02	-13.42	-23.78		-0.68	-1.25	-1.08	-0.64
AR_Pop_1_28	-24.50	-22.10	-13.22	-21.90		0.16	-1.47	-1.29	-0.58
AR_Pop_1_29	-2.43	-1.56	1.54	-2.95		-0.58	2.12	-1.18	0.12
AR_Pop_1_30	-4.53	-1.07	1.27	-4.13		-0.04	1.06	-0.98	-0.23
AR_Pop_1_31	-4.65	-4.52	0.96	-5.98		-1.07	2.12	-0.89	0.21
AR_Pop_1_32	14.44	13.17	14.04	11.18		0.70	2.69	-1.24	0.99
AR_Pop_1_33	-3.40	-6.82	0.26	-6.71		-1.09	1.65	-0.94	0.02
AR_Pop_1_34	-4.24	-2.34	1.16	-5.28		-0.35	2.70	-0.83	0.67
AR_Pop_1_35	14.35	14.61	13.70	11.68		0.94	2.07	-1.24	1.09
AR_Pop_1_36	-4.34	-1.36	1.70	-3.48		0.40	2.10	-0.96	0.71
AR_Pop_1_37	-2.36	-0.31	4.23	-2.88		-0.58	2.39	-0.79	0.27
AR_Pop_1_38	-5.42	-6.39	0.18	-7.01		-0.81	0.92	-0.96	-0.20
AR_Pop_1_39	-25.21	-25.65	-13.55	-25.08		-0.68	-1.70	-0.98	-0.90
AR_Pop_1_40	-1.34	9.21	0.93	-2.23		1.62	2.65	-1.93	0.47
AR_Pop_1_41	-14.17	-15.46	-10.46	-15.65		1.51	2.18	0.01	1.04
AR_Pop_1_42	-4.98	-5.65	0.52	-6.45		-0.70	1.74	-0.95	0.07
AR_Pop_1_43	-4.87	-2.77	0.72	-3.84		-1.18	2.23	-1.14	0.18
AR_Pop_1_44	-21.98	-19.90	-12.85	-20.76		-0.08	-1.19	-1.19	-0.27
AR_Pop_1_45	-25.19	-24.58	-13.59	-24.67		-0.87	-1.63	-1.28	-0.91
AR_Pop_1_46	-4.26	-3.09	0.88	-5.44		-0.85	2.27	-1.33	-0.18
AR_Pop_1_47	14.01	14.34	13.71	11.39		-0.65	1.25	-1.15	0.30
AR_Pop_1_48	-2.80	-2.11	0.35	-3.37		-1.23	-1.45	-1.27	-0.80
AR_Pop_1_49	16.08	16.02	14.21	14.19		1.09	2.97	-1.11	1.27

Table A.3. Genomic estimated breeding values (GEBV) per trait [maturity date (day of year) and soluble solids content (SSC)] and year for the AR RosBREED pedigree (Cont.).

Individual	Maturity date (day of year)					Soluble solids content (SSC)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
AR_Pop0801_01	-22.89	-17.43	-11.18	-17.67		1.98	0.86	1.78	1.66
AR_Pop0801_02	-25.53	-18.05	-13.31	-20.13		2.73	2.02	1.51	1.91
AR_Pop0801_03	-28.00	-22.12	-14.24	-23.80		2.47	0.46	0.16	0.82
AR_Pop0801_04	-22.69	-16.73	-11.45	-18.30		2.44	1.93	1.70	1.77
AR_Pop0801_05	-27.93	-22.04	-14.18	-23.95		2.33	0.82	-0.24	0.81
AR_Pop0801_06	-25.71	-19.01	-13.79	-21.31		2.54	2.18	1.67	1.87
AR_Pop0801_07	-21.93	-18.22	-13.46	-19.33		2.29	2.57	1.66	2.04
AR_Pop0801_08	-21.99	-18.96	-12.23	-19.92		2.54	2.86	1.73	2.09
AR_Pop0801_09	-22.75	-16.82	-11.05	-17.54		2.41	1.17	1.75	1.70
AR_Pop0801_10	-22.71	-19.29	-13.46	-20.31		2.24	2.57	1.84	1.85
AR_Pop0801_11	-25.28	-19.64	-13.32	-21.31		2.23	0.86	1.78	1.68
AR_Pop0801_12	-22.13	-16.48	-11.27	-18.10		2.58	1.22	1.53	1.79
AR_Pop0801_13	-22.77	-16.68	-11.46	-18.63		2.28	2.31	1.71	1.86
AR_Pop0801_14	-23.54	-19.36	-13.37	-20.28		1.94	1.47	1.83	1.67
AR_Pop0801_15	-21.68	-17.49	-10.26	-19.25		0.42	0.74	0.87	0.77
AR_Pop0801_16	-23.10	-18.86	-13.38	-20.68		2.66	0.81	1.47	1.58
AR_Pop0803_01	-27.67	-17.58	-12.80	-22.38		0.89	0.55	0.62	0.45
AR_Pop0803_02	-26.26	-16.86	-11.45	-20.41		1.89	0.10	0.85	0.67
AR_Pop0803_03	-27.14	-17.65	-12.52	-22.27		1.71	1.11	0.29	0.94
AR_Pop0803_04	-27.39	-19.54	-13.72	-22.31		0.51	-1.70	0.46	-0.18
AR_Pop0803_05	-24.27	-19.59	-13.86	-22.46		1.66	0.80	0.24	0.59
AR_Pop0803_06	-26.08	-17.74	-13.71	-22.21		1.06	-0.19	0.17	0.56
AR_Pop0803_07	-27.33	-20.64	-14.20	-22.92		0.39	-1.58	-0.11	-0.29
AR_Pop0803_08	-9.46	-19.72	-13.92	-21.35		1.31	-0.89	0.41	0.12
AR_Pop0803_09	-27.45	-19.74	-13.97	-23.58		1.39	0.43	0.31	0.85
AR_Pop0803_10	-24.52	-19.94	-13.74	-23.31		1.30	-0.47	0.29	0.41
AR_Pop0803_11	-26.78	-18.71	-13.65	-21.92		0.76	-0.19	0.59	0.26
AR_Pop0803_12	-26.68	-19.44	-13.98	-20.60		0.86	-0.28	0.46	0.55
AR_Pop0803_13	-27.27	-19.51	-13.97	-21.78		1.39	-0.48	0.07	0.12
AR_Pop0803_14	-26.95	-19.38	-13.98	-21.27		1.24	-1.68	-0.07	-0.21
AR_Pop0803_15	-18.71	-11.89	-9.53	-15.05		1.09	-0.58	0.65	0.09
AR_Pop0813_01	-4.72	-0.96	-1.53	-5.77		1.15	2.12	-0.18	1.60
AR_Pop0813_02	-4.01	1.75	2.49	-2.41		1.90	2.00	0.81	1.83
AR_Pop0813_03	-3.33	-2.73	-1.47	-5.08		1.07	1.02	-0.22	1.33
AR_Pop0813_04	-4.28	0.05	-0.41	-2.92		0.13	2.46	-0.32	1.05
AR_Pop0813_05	-26.32	-21.41	-11.57	-24.12		1.27	1.45	-0.13	1.43

Table A.3. Genomic estimated breeding values (GEBV) per trait [maturity date (day of year) and soluble solids content (SSC)] and year for the AR RosBREED pedigree (Cont.).

Individual	Maturity Date (day of year)					Soluble solids content (SSC)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
AR_Pop0813_06	-5.38	0.74	-1.24	-4.74		0.41	1.86	0.87	1.23
AR_Pop0813_07	-23.83	-15.66	-7.40	-21.91		-0.04	2.46	-0.34	1.11
AR_Pop0813_08	-4.83	-3.75	-1.75	-6.50		1.73	1.55	0.76	1.33
AR_Pop0813_09	-4.65	-0.81	2.45	-5.27		0.66	3.04	0.79	1.53
AR_Pop0813_10	-22.84	-17.23	-11.13	-21.28		-0.06	1.88	0.00	1.09
AR_Pop0813_11	-22.77	-17.13	-10.98	-19.97		0.28	1.41	0.80	0.95
AR_Pop0813_12	-4.38	0.71	-0.81	-2.89		0.09	2.23	-0.36	0.95
AR_Pop0817_01	-3.22	4.14	4.88	0.58		1.00	0.71	0.10	0.36
AR_Pop0817_02	3.47	4.07	1.91	0.07		1.22	0.83	-0.11	-0.14
AR_Pop0817_03	-7.95	-2.65	2.13	-3.11		2.20	2.41	0.76	1.25
AR_Pop0817_04	-0.32	2.51	2.71	0.57		1.78	1.91	1.40	1.08
AR_Pop0817_05	-2.78	-11.50	-9.57	-17.56		0.97	1.61	0.89	0.61
AR_Pop0817_06	-5.30	-0.87	2.45	-1.49		0.95	1.93	1.04	0.83
AR_Pop0817_07	-3.06	2.97	2.65	0.77		0.82	1.91	0.67	0.73
AR_Pop0817_08	-6.00	-2.35	1.07	-3.53		0.65	1.75	0.91	0.75
AR_Pop0817_09	0.45	-3.13	2.73	1.39		1.50	1.73	1.34	0.98
AR_Pop0819_01	8.57	1.80	9.47	2.58		0.76	0.76	0.54	0.87
AR_Pop0819_02	-5.28	-9.98	-10.04	-11.38		1.77	0.84	0.49	0.86
AR_Pop0819_03	8.22	-1.60	1.36	-0.07		1.76	1.53	-0.01	1.08
AR_Pop0819_04	25.82	15.71	13.69	16.52		1.55	0.62	-0.13	0.93
AR_Pop0819_05	4.07	-1.69	1.26	-0.90		0.96	1.68	-0.16	1.22
AR_Pop0819_06	7.96	4.39	1.98	3.07		1.00	1.12	1.03	1.00
AR_Pop0819_07	1.97	-6.04	0.80	-2.83		1.02	0.28	-0.25	0.68
AR_Pop0819_08	7.70	3.83	1.90	2.64		1.98	0.47	-0.14	0.82
AR_Pop0819_09	23.35	12.68	13.31	14.97		0.72	0.89	0.63	0.91
AR_Pop0819_10	-12.33	-11.70	-10.56	-13.82		1.12	0.90	1.09	0.89
AR_Pop0819_11	8.53	6.48	6.38	5.30		1.91	1.07	-0.34	0.95
AR_Pop0819_12	5.86	1.16	1.82	0.90		0.82	1.61	-0.06	1.21
AR_Pop0819_13	4.88	3.18	2.98	1.06		1.88	1.58	0.96	1.09
AR_Pop0819_14	3.93	0.10	1.41	0.51		0.75	1.04	0.63	0.95
AR_Pop0819_15	10.76	8.47	3.94	4.54		0.88	1.36	-0.39	0.94
AR_Pop0819_16	-15.79	-20.53	-11.68	-19.28		0.85	0.53	-0.25	0.64
AR_Pop0819_17	-14.04	-20.58	-10.68	-16.68		0.61	0.34	0.46	0.68
AR_Pop0819_18	-11.82	-17.91	-11.39	-17.61		1.33	0.24	0.55	0.65
AR_Pop0819_19	24.31	14.91	13.91	15.58		1.68	1.45	0.30	1.12
AR_Pop0819_20	-16.61	-22.18	-11.80	-19.76		1.60	0.24	0.69	0.69

Table A.3. Genomic estimated breeding values (GEBV) per trait [maturity date (day of year) and soluble solids content (SSC)] and year for the AR RosBREED pedigree (Cont.).

Individual	Maturity Date (day of year)					Soluble solids content (SSC)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
AR_Pop0819_21	24.00	15.92	13.89	15.81		1.82	1.59	-0.07	1.11
AR_Pop0819_22	4.01	-0.35	1.56	-0.35		0.65	1.79	0.84	1.16
AR_Pop0819_23	5.41	2.48	1.34	0.52		1.04	1.39	0.36	1.09
AR_Pop0825_01	-3.98	-7.98	-0.31	-6.65		0.11	0.42	0.40	0.21
AR_Pop0825_02	-22.69	-28.36	-13.90	-25.24		-0.22	-0.78	-0.08	-0.62
AR_Pop0825_03	-24.45	-26.46	-13.95	-24.37		0.37	-1.30	-0.27	-0.57
AR_Pop0825_04	-24.48	-28.62	-14.28	-25.51		-0.74	-2.10	-0.61	-0.92
AR_Pop0825_05	-3.33	-8.92	-0.48	-6.86		-0.59	-1.97	0.79	-0.24
AR_Pop0825_06	-22.76	-28.76	-14.32	-25.65		-0.38	-1.83	0.07	-0.86
AR_Pop0825_07	-22.70	-26.02	-12.98	-23.77		-0.27	-0.41	0.19	-0.41
AR_Pop0825_08	-3.94	-9.27	-1.08	-6.76		0.24	-1.29	0.36	-0.13
AR_Pop0825_09	-24.97	-28.53	-14.37	-25.49		-0.09	-1.72	0.55	-0.65
AR_Pop0825_10	-22.76	-26.39	-13.74	-24.19		-0.29	-1.28	0.71	-0.53
AR_Pop0825_11	-3.70	-8.35	-0.17	-6.78		0.22	-0.19	0.39	0.00
AR_Pop0825_12	-4.58	-8.83	-0.45	-7.27		0.63	0.71	0.69	0.33
AR_Pop0825_13	-2.67	-7.74	0.02	-6.66		0.23	-0.44	-0.48	-0.22
AR_Pop0825_14	-23.71	-28.03	-14.44	-25.83		-0.12	-2.04	-0.40	-1.04
AR_Pop0825_15	-3.38	-10.00	-0.51	-7.67		-0.29	-0.38	0.30	-0.16
AR_Pop0825_16	-20.51	-16.30	-5.67	-15.33		-0.08	-0.56	-0.32	-0.54
AR_Pop0825_17	-5.78	-13.62	-3.89	-12.49		-0.10	-1.24	0.79	-0.35
AR_Pop0825_18	-13.46	-17.83	-7.16	-16.14		-0.09	-0.79	0.26	-0.31
Arrington	-28.50	-23.43	-9.52	-19.80		0.47	-0.47	-0.06	-0.32
Australian Muir	-11.71	0.35	-5.23	-9.96		-0.23	0.18	0.13	-0.35
Belle	-9.82	-5.00	-5.26	-7.96		0.16	0.21	0.15	-0.01
Bradley	-18.85	-20.88	-14.14	-21.37		1.12	-0.91	-0.15	-0.04
Candoka	-5.34	-6.80	-5.61	-3.75		-0.51	-0.73	0.45	-0.66
Chinese Cling	4.38	3.84	-3.30	-6.34		0.27	-1.19	0.30	-0.18
Chiripa	-11.44	-11.84	-9.87	-15.62		1.12	1.31	0.04	0.62
Cumberland	-17.67	-8.19	-4.18	-17.70		1.81	-0.30	0.27	1.22
D42_13W	-4.53	3.13	-4.55	-3.20		-0.38	0.01	0.10	-0.32
Delicioso	-8.96	-8.57	-7.06	-12.17		0.65	0.78	0.12	0.32
Dix_16_3	2.46	8.54	-4.86	3.20		-1.11	-0.27	0.13	-0.64
Dix_22A_5	-3.58	1.53	-4.40	-2.49		-0.49	0.00	0.12	-0.33
Dix_58_6	-8.72	-4.30	-4.06	-8.11		0.20	0.25	0.13	-0.06
Dixon	-0.09	14.32	-5.31	2.11		-1.63	-0.30	0.12	-1.07
dummy003	-16.94	-11.04	-7.91	-14.79		-1.29	-0.10	0.05	-0.84

Table A.3. Genomic estimated breeding values (GEBV) per trait [maturity date (day of year) and soluble solids content (SSC)] and year for the AR RosBREED pedigree (Cont.).

Individual	Maturity Date (day of year)					Soluble solids content (SSC)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
dummy004	-18.71	-14.18	-9.59	-16.09		1.00	-0.19	-0.28	-0.16
dummy005	-18.78	-13.21	-9.49	-16.19		0.92	-0.08	-0.14	-0.05
dummy006	-12.73	-10.28	-5.74	-12.35		-0.52	0.14	0.13	-0.15
dummy007	-17.15	-9.61	-8.28	-14.84		-0.12	0.16	0.24	-0.05
dummy008	-16.15	-9.55	-6.89	-14.54		0.60	0.83	0.40	0.37
dummy009	-19.40	-9.86	-7.52	-15.02		0.48	0.55	-0.02	0.10
dummy010	-8.98	-4.61	-4.74	-8.70		0.09	0.08	0.14	-0.05
Early Crawford	-2.65	5.36	-4.71	-2.93		-0.87	-1.12	0.43	-0.89
Elberta	10.27	9.04	-2.95	10.51		-0.90	-1.41	0.58	-0.84
Elberta OP27	0.04	1.51	-3.95	1.18		-0.35	-0.50	0.34	-0.44
F_A_371	3.01	-1.12	-1.16	-1.84		1.17	0.61	0.11	0.60
F_A_427	-6.13	-2.22	-4.86	-5.65		0.43	0.24	0.12	0.09
F_A_433	-2.36	1.14	-0.10	-3.22		0.36	0.62	-0.10	-0.25
F_A_699	-2.20	-3.12	-0.76	-5.79		1.24	1.17	0.45	0.40
F_Candoka	-9.86	-4.68	-5.02	-8.16		0.17	0.16	0.20	-0.11
F_Elberta OP27	-8.95	-5.24	-4.91	-8.36		0.17	0.32	0.11	-0.08
F_Garden State	-9.59	-4.19	-4.39	-8.92		0.05	0.29	0.14	-0.10
F_Ga. Belle	-6.15	-3.97	-4.79	-9.34		0.65	-0.05	0.15	0.36
F_JH Hale	-10.94	-12.46	-5.71	-11.11		-0.61	-1.06	0.41	-0.50
F_Nectared4xA_24	-20.53	-9.38	-8.85	-16.08		-0.57	0.17	-0.30	-0.60
F_NJ113115	-8.84	-4.25	-4.57	-8.56		0.19	0.25	0.14	-0.11
F_NJ98838	-8.75	-4.48	-5.22	-8.61		0.11	0.11	0.15	-0.02
F_NJN14	-11.29	-5.85	-6.33	-10.22		0.17	0.29	0.13	0.02
F_Sunrise	-10.15	-5.82	-4.72	-9.73		0.22	0.27	0.12	0.00
G_17_5E	-17.77	-13.24	-9.30	-15.75		0.00	0.26	0.15	-0.11
Garden State	-5.19	-1.50	-4.01	-4.14		-0.12	-0.05	0.16	-0.28
Ga. Belle	7.65	5.56	-3.15	-1.21		0.40	-0.91	0.28	0.18
Goodmans Choice	-8.75	-3.95	-4.36	-7.79		0.13	0.27	0.19	-0.03
Greensboro	-24.45	-14.63	-4.49	-22.39		1.04	0.19	0.19	0.66
H_523	3.65	5.46	-2.64	-15.29		-0.21	-0.15	-0.33	0.03
HalBerta Giant	-6.77	-8.24	-6.48	-5.90		-0.62	-0.71	0.35	-0.51
Hann Almond	-8.97	-4.46	-4.39	-8.49		0.01	0.20	0.14	-0.07
Honeydew Hale	-11.77	-9.51	-3.82	-3.70		0.48	0.03	-0.04	0.02
Jefferson	-12.73	0.85	-3.10	-7.87		-1.50	-0.54	-0.08	-0.65
JH Hale	-1.36	-9.59	-6.19	0.40		-1.38	-1.75	0.68	-1.12

Table A.3. Genomic estimated breeding values (GEBV) per trait [maturity date (day of year) and soluble solids content (SSC)] and year for the AR RosBREED pedigree (Cont.).

Individual	Maturity Date (day of year)					Soluble solids content (SSC)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
Jing	-3.14	-0.27	-0.10	-2.90		-0.39	0.79	0.07	0.20
Jungerman	-1.14	8.07	-5.06	-0.12		-1.00	-0.22	0.10	-0.63
M_A_789	-2.80	1.73	0.94	-1.33		0.92	1.24	0.40	0.42
MaoTao	-10.21	-10.82	-7.85	-9.67		1.04	0.98	0.68	0.72
Meredith	-13.79	-6.00	3.42	-8.38		-0.69	0.17	0.11	-0.48
Mexican Honey	-8.67	-3.87	-4.92	-8.99		0.01	0.18	0.18	-0.01
Nectalate	-8.37	-4.19	-4.92	-7.77		0.15	0.20	0.03	-0.17
Nectared4	-15.89	-8.65	-8.14	-13.62		0.16	0.52	-0.15	-0.02
Nectared4 x A_24	-22.29	-12.61	-10.27	-17.72		-0.33	0.27	-0.30	-0.50
Nectared 5	-12.68	-8.89	-8.19	-12.89		0.52	0.76	-0.05	0.19
Nectared 7	-11.66	-6.92	-6.64	-11.22		0.26	0.56	-0.04	0.06
NJ113115	-9.15	-4.97	-4.74	-8.52		0.13	0.24	0.15	-0.02
NJ25032	-9.50	-5.58	-4.82	-9.87		0.16	0.27	0.10	-0.11
NJ257	-15.79	-9.78	-1.73	0.89		-0.22	-0.45	-0.30	-0.23
NJ38026	-5.43	-7.13	-5.31	-4.10		-0.66	-0.80	0.42	-0.55
NJ5102893	-8.33	-3.56	-4.01	-8.56		0.24	0.17	0.17	-0.03
NJ5106548	-5.04	-5.92	-5.40	-4.55		-0.42	-0.50	0.32	-0.47
NJ5107397	-8.23	-5.65	-5.16	-8.34		0.11	0.07	0.19	-0.16
NJ53739	-7.95	-3.62	-4.39	-8.95		0.09	0.17	0.13	-0.03
NJ53939	-12.23	-8.34	-7.01	-12.05		0.30	0.52	-0.03	0.08
NJ554367	-15.17	-10.41	-7.03	-12.57		0.08	0.35	0.32	0.01
NJ562021	-12.76	-7.49	-6.53	-11.39		0.05	0.14	-0.01	-0.19
NJ6128	-4.59	-6.70	-5.48	-4.26		-0.62	-0.84	0.45	-0.58
NJ822026	2.59	1.67	-0.23	0.70		1.02	0.47	0.24	0.48
NJ94727	-4.42	-7.19	-5.42	-3.60		-0.66	-0.75	0.36	-0.56
NJ98838	-6.48	-6.28	-5.22	-5.94		-0.23	-0.31	0.21	-0.30
NJC83	-3.76	0.89	-4.55	-2.90		0.03	0.02	0.15	-0.14
NJC95	-6.10	-4.38	-4.89	-5.74		0.04	-0.12	0.24	-0.20
NJLA3	-4.15	-6.36	-5.56	-3.52		-0.65	-0.77	0.42	-0.58
NJN14	-12.40	-6.41	-6.71	-10.68		0.17	0.42	0.02	-0.02
NJN17	-8.59	-7.44	-6.43	-8.03		-0.11	-0.10	0.22	-0.30
NJN21	-6.64	-2.79	-3.73	-6.55		0.00	0.16	0.09	-0.21
NJN55	-8.89	-5.01	-5.09	-9.41		0.24	0.42	0.04	-0.06
OldGold	-10.49	-5.57	-5.54	-9.60		0.11	0.20	0.10	-0.05
Orange Cling	13.98	22.19	-5.18	14.37		-2.36	-0.72	0.12	-1.24

Table A.3. Genomic estimated breeding values (GEBV) per trait [maturity date (day of year) and soluble solids content (SSC)] and year for the AR RosBREED pedigree (Cont.).

Individual	Maturity date (day of year)					Soluble solids content (SSC)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
Orange Cling x Alameda	2.21	8.41	-4.60	2.67		-1.18	-0.25	0.12	-0.67
Peento	-13.40	-5.79	-5.33	-8.93		-0.21	0.37	0.09	-0.15
Pop8089	-5.29	-2.47	-5.14	-5.72		0.37	0.28	0.08	0.09
Raritan Rose	-8.23	-8.49	-4.85	-8.26		0.24	-1.00	0.44	0.05
Redgold	-10.91	-9.51	-5.80	-8.89		-0.37	-0.18	0.21	-0.27
Redskin	-0.78	-4.23	-4.19	-4.21		-1.09	-2.15	0.90	-0.83
RR122_15	-6.61	-4.80	-3.83	-6.49		0.16	-0.42	0.23	-0.10
RR53_194	-0.52	3.95	0.49	-1.73		0.31	0.82	0.02	0.41
Slappey	-17.61	-2.02	-4.66	-14.89		-1.36	0.10	0.10	-1.03
Sunrise	-10.27	-7.67	-3.51	-7.83		-0.32	-0.07	0.24	-0.28
Transcaal Cling	-8.96	-4.25	-4.50	-8.98		0.20	0.19	0.16	-0.07
Westbrook	-33.76	-13.33	-14.81	-24.95		-1.69	0.15	-0.97	-1.42
White County	-1.46	-3.30	0.18	-5.26		1.17	-0.16	-1.19	-0.31
Winblo	-2.08	-7.35	-4.24	-5.56		-1.06	-2.23	0.98	-0.80
Max-GEBV	**25.82**	**22.19**	**14.78**	**16.52**		**2.73**	**3.09**	**1.84**	**2.09**
Avg-GEBV	**-9.09**	**-7.15**	**-4.61**	**-9.03**		**0.41**	**0.46**	**0.07**	**0.24**
Min-GEBV	**-33.76**	**-28.76**	**-14.81**	**-25.83**		**-2.36**	**-2.23**	**-2.04**	**-1.42**

Table A.4. Genomic estimated breeding values (GEBV) per trait [fruit weight (g) and blush overcolor (0-5)] and year for the AR RosBREED pedigree.

Individual	Fruit weight (g)					Blush overcolor (0-5)			
	2011	2011	2011	Avg		2011	2011	2011	Avg
104325	-63.08	-35.99	-2.28	-52.25		-0.01	0.09	-0.02	0.01
752002002	-63.46	-28.45	-2.74	-45.27		-0.19	-0.19	-0.06	-0.13
10A4	-63.78	-28.11	-3.19	-43.98		-0.22	-0.17	-0.08	-0.14
22A5	-52.58	-38.24	-1.72	-42.21		0.14	0.17	0.14	0.20
2W68W	-53.68	-27.64	-1.86	-39.87		-0.08	-0.07	-0.03	-0.08
4A_4	-30.51	-29.41	4.45	-18.37		-0.04	-0.05	0.00	-0.01
A_130	-88.12	-31.73	-2.75	-59.59		-0.26	-0.24	0.04	-0.17
A_145	-46.11	-26.42	-2.05	-36.70		-0.12	-0.06	-0.01	-0.06
A_154	-53.22	-30.31	-0.35	-41.74		-0.14	-0.15	0.00	-0.12
A_172	-85.88	-36.69	-9.72	-59.89		0.02	0.05	0.11	0.04
A_176	-85.97	-36.31	-10.12	-59.54		0.02	0.06	0.11	0.05
A_178	-72.36	-24.13	-4.67	-54.82		-0.06	-0.06	-0.06	0.01
A_18	-71.69	-24.01	-0.96	-45.44		-0.20	-0.17	-0.06	-0.11
A_190	-80.93	-20.88	0.86	-44.07		-0.28	-0.25	-0.09	-0.16
A_21	-73.84	-27.99	-4.02	-47.98		-0.31	-0.29	-0.11	-0.21
A_224	-50.53	-30.07	-0.50	-41.85		-0.16	-0.13	0.02	-0.13
A_232	-90.31	-41.95	-11.55	-63.55		-0.02	0.02	0.10	0.01
A_24	-72.79	-29.20	-4.00	-49.87		-0.18	-0.12	-0.04	-0.08
A_270	-86.39	-33.16	-2.80	-53.16		-0.34	-0.32	0.12	-0.24
A_333	-55.95	-42.14	-5.62	-44.60		-0.02	-0.30	-0.03	-0.26
A_334	-86.35	-29.70	-11.45	-68.62		-0.22	-0.24	-0.12	-0.14
A_367	-18.63	-35.76	8.68	-6.70		0.10	0.11	0.04	0.10
A_371	-15.61	-45.12	12.63	-16.98		-0.18	-0.17	0.02	-0.18
A_374	-61.30	-27.10	-6.61	-48.34		0.19	0.23	-0.04	0.18
A_392	-6.82	-2.93	-3.59	-29.77		0.54	0.55	0.14	0.52
A_402	-76.67	-20.98	-2.22	-49.41		-0.17	-0.16	-0.07	-0.08
A_405	-79.65	-42.50	-3.24	-49.02		-0.14	-0.10	0.28	-0.03
A_419	-63.70	-47.48	0.26	-40.42		0.30	0.33	0.05	0.26
A_427	-59.27	-30.40	-2.33	-46.54		-0.01	0.00	-0.03	0.05
A_433	-54.40	-33.50	0.91	-45.45		-0.20	-0.18	0.05	-0.15
A_434	-45.52	13.15	-1.12	-36.75		0.21	0.25	0.11	0.23
A_441	-51.13	-32.24	-0.74	-36.08		0.11	0.14	-0.02	0.12
A_500	-76.33	-32.06	-14.20	-62.12		0.06	0.07	-0.11	0.10

Table A.4. Genomic estimated breeding values (GEBV) per trait [fruit weight (g) and blush overcolor (0-5)] and year for the AR RosBREED pedigree (Cont.).

Individual	Fruit weight (g)					Blush overcolor (0-5)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
A_565	-62.75	-35.70	-4.19	-50.54		0.13	0.16	-0.02	0.20
A_604	-63.10	-58.13	-2.63	-49.02		-0.14	-0.15	-0.01	-0.13
A_657	-76.31	1.17	-1.35	-56.78		0.02	0.03	-0.06	0.19
A_663	-97.24	-26.22	-10.80	-64.79		-0.21	-0.23	-0.13	-0.16
A_665	19.17	-43.16	19.62	18.26		0.02	0.04	0.07	0.01
A_672	-71.22	-56.80	-1.37	-47.92		0.53	0.57	0.22	0.50
A_699	-94.45	-49.60	-9.77	-72.22		0.37	0.42	0.01	0.38
A_708	-48.91	5.95	-6.35	-36.75		0.56	0.61	0.18	0.57
A_716	-13.63	-26.01	16.55	-8.84		-0.19	-0.16	-0.06	-0.14
A_717	-74.63	-54.52	-15.20	-65.82		0.02	0.03	-0.01	0.05
A_760	-102.08	-21.81	-17.57	-63.36		0.19	0.19	0.06	0.21
A_763	-73.64	-18.34	-14.65	-43.99		0.64	0.70	0.06	0.62
A_765	-110.25	-42.73	-15.19	-75.11		0.16	0.18	-0.05	0.24
A_772	-66.66	-48.03	7.76	-45.96		-0.14	-0.14	0.18	-0.10
A_773	-47.08	-59.26	12.42	-36.13		-0.42	-0.42	-0.02	-0.41
A_776	-104.59	-59.87	-24.08	-84.92		-0.03	-0.02	-0.03	-0.02
A_778	-84.79	-8.87	-2.26	-49.81		0.08	0.13	0.15	0.27
A_783	-73.03	-59.67	-17.19	-72.58		0.34	0.38	0.04	0.37
A_789	-48.75	-45.30	0.34	-49.29		0.07	0.10	0.11	0.16
Admiral Dewey	-49.77	-54.58	-0.80	-71.24		-0.09	-0.10	-0.01	-0.10
Alameda	-51.37	-28.21	-0.98	-39.21		-0.09	-0.09	-0.03	-0.08
Allgold	-77.81	-35.82	-10.85	-58.97		-0.48	-0.50	-0.19	-0.37
AR_Pop_1_01	0.14	-59.79	1.21	-48.66		0.78	0.80	0.27	0.73
AR_Pop_1_02	-94.20	-61.69	-2.59	-57.94		0.69	0.72	0.25	0.68
AR_Pop_1_03	-15.01	-60.06	6.85	-46.79		0.29	0.29	0.20	0.25
AR_Pop_1_04	-53.94	-60.90	4.72	-46.37		0.66	0.70	0.15	0.64
AR_Pop_1_05	-73.03	-11.34	5.86	-40.36		0.73	0.77	0.25	0.60
AR_Pop_1_06	-83.89	-11.01	4.19	-37.34		0.06	0.08	0.30	0.08
AR_Pop_1_07	-23.82	-59.83	7.73	-39.50		0.21	0.23	0.21	0.13
AR_Pop_1_08	-95.22	-60.13	-2.13	-53.84		0.67	0.72	0.27	0.61
AR_Pop_1_09	-77.23	-55.06	6.91	-42.82		0.19	0.21	0.22	0.23
AR_Pop_1_10	-102.58	-57.41	0.88	-49.81		0.31	0.31	0.16	0.29
AR_Pop_1_11	-86.66	-60.51	-0.10	-52.71		0.03	0.05	0.24	0.05
AR_Pop_1_12	-95.50	-20.32	4.32	-43.98		0.61	0.65	0.31	0.45
AR_Pop_1_13	-59.50	-59.84	2.93	-49.48		0.75	0.78	0.17	0.54

Table A.4. Genomic estimated breeding values (GEBV) per trait [fruit weight (g) and blush overcolor (0-5)] and year for the AR RosBREED pedigree (Cont.).

Individual	Fruit weight (g)					Blush overcolor (0-5)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
AR_Pop_1_14	-57.00	-51.23	8.42	-45.07		0.19	0.23	0.08	0.18
AR_Pop_1_15	-74.67	-15.56	3.70	-42.80		0.05	0.07	0.28	0.04
AR_Pop_1_16	-56.68	-60.31	-0.25	-51.51		0.75	0.77	0.15	0.71
AR_Pop_1_17	-29.94	-50.85	2.99	-43.83		0.19	0.21	0.19	0.22
AR_Pop_1_18	-72.61	-13.62	6.45	-37.40		0.07	0.09	0.28	0.09
AR_Pop_1_19	-57.72	-57.69	3.29	-45.54		0.21	0.22	0.17	0.20
AR_Pop_1_20	-83.72	-42.12	-5.88	-55.05		0.77	0.80	0.33	0.61
AR_Pop_1_21	-50.46	-58.86	3.90	-49.99		0.09	0.11	0.14	0.17
AR_Pop_1_22	-71.43	-61.75	0.46	-51.57		0.11	0.13	0.21	0.19
AR_Pop_1_23	-79.31	-30.70	0.05	-49.97		0.66	0.71	0.26	0.65
AR_Pop_1_24	-10.16	-23.10	2.82	-40.28		0.16	0.18	0.33	0.17
AR_Pop_1_25	-89.99	-62.19	0.69	-52.98		0.03	0.05	0.17	0.05
AR_Pop_1_26	-69.41	-62.21	3.56	-51.86		0.63	0.67	0.16	0.58
AR_Pop_1_27	-48.56	-13.55	-0.58	-43.68		0.79	0.82	0.32	0.71
AR_Pop_1_28	-73.86	-13.18	3.83	-37.60		0.14	0.16	0.21	0.14
AR_Pop_1_29	-8.27	-41.84	2.90	-44.15		0.80	0.83	0.28	0.71
AR_Pop_1_30	1.23	-43.62	11.55	-16.40		0.45	0.47	0.12	0.40
AR_Pop_1_31	2.92	-24.26	2.92	-39.25		0.77	0.81	0.17	0.67
AR_Pop_1_32	14.37	-59.21	6.86	-39.41		0.07	0.09	0.20	0.14
AR_Pop_1_33	-72.54	-58.24	4.41	-48.88		0.77	0.80	0.26	0.67
AR_Pop_1_34	-56.80	-40.42	13.61	-35.67		0.22	0.24	0.26	0.20
AR_Pop_1_35	-82.67	-57.72	7.14	-46.01		0.26	0.27	0.20	0.23
AR_Pop_1_36	-54.77	-60.51	1.53	-49.02		0.23	0.24	0.23	0.25
AR_Pop_1_37	-99.18	-59.84	0.51	-53.81		0.70	0.73	0.26	0.65
AR_Pop_1_38	0.87	-58.53	3.08	-47.46		0.29	0.30	0.24	0.30
AR_Pop_1_39	-87.15	-12.98	7.21	-39.57		0.07	0.10	0.31	0.08
AR_Pop_1_40	-86.29	-57.90	2.91	-51.29		0.31	0.32	0.23	0.26
AR_Pop_1_41	-75.14	-16.13	-4.05	-46.03		0.49	0.50	0.22	0.45
AR_Pop_1_42	-66.19	-53.96	5.25	-46.29		0.81	0.82	0.20	0.73
AR_Pop_1_43	-73.55	-55.72	-0.28	-50.70		0.17	0.18	0.16	0.21
AR_Pop_1_44	12.22	-12.17	10.96	-33.08		0.12	0.15	0.33	0.16
AR_Pop_1_45	-29.59	-11.19	10.01	-31.58		0.19	0.20	0.34	0.11
AR_Pop_1_46	-55.56	-54.81	7.58	-42.08		0.64	0.68	0.19	0.55
AR_Pop_1_47	-54.20	-56.54	4.78	-45.75		0.21	0.23	0.18	0.19
AR_Pop_1_48	2.46	-15.08	5.65	-33.37		0.79	0.82	0.20	0.74
AR_Pop_1_49	-98.52	-60.99	9.25	-44.24		0.54	0.59	0.20	0.42

Table A.4. Genomic estimated breeding values (GEBV) per trait [fruit weight (g) and blush overcolor (0-5)] and year for the AR RosBREED pedigree (Cont.).

Individual	Fruit weight (g)					Blush overcolor (0-5)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
AR_Pop0801_01	-80.63	-62.98	-25.49	-81.70		-0.17	-0.17	-0.05	-0.09
AR_Pop0801_02	-95.99	-63.87	-26.26	-83.04		0.00	0.02	0.03	0.06
AR_Pop0801_03	-106.23	-56.88	-22.00	-76.65		-0.28	-0.28	-0.02	-0.21
AR_Pop0801_04	-88.17	-63.94	-25.61	-81.71		0.44	0.49	0.03	0.44
AR_Pop0801_05	-107.01	-59.84	-21.09	-77.73		-0.20	-0.19	0.07	-0.14
AR_Pop0801_06	-94.07	-64.28	-24.51	-79.84		-0.02	-0.01	-0.01	0.04
AR_Pop0801_07	-84.62	-63.56	-22.75	-80.65		0.52	0.55	-0.02	0.48
AR_Pop0801_08	-89.89	-64.50	-24.74	-82.36		-0.06	-0.04	0.02	0.00
AR_Pop0801_09	-87.82	-64.18	-24.26	-82.66		-0.14	-0.13	0.01	-0.08
AR_Pop0801_10	-82.38	-64.69	-23.47	-83.05		-0.15	-0.15	0.05	-0.06
AR_Pop0801_11	-89.36	-63.77	-21.06	-80.54		-0.16	-0.13	0.05	-0.10
AR_Pop0801_12	-94.37	-63.58	-22.31	-81.60		0.35	0.39	0.04	0.34
AR_Pop0801_13	-83.11	-64.19	-22.08	-77.52		0.49	0.54	-0.02	0.46
AR_Pop0801_14	-81.43	-62.62	-21.24	-77.06		0.26	0.31	-0.02	0.25
AR_Pop0801_15	-96.99	-54.64	-19.48	-74.96		-0.33	-0.32	-0.08	-0.26
AR_Pop0801_16	-92.38	-63.95	-26.89	-82.82		-0.24	-0.23	0.00	-0.16
AR_Pop0803_01	-99.29	-51.64	-9.86	-68.36		-0.10	-0.10	-0.08	-0.03
AR_Pop0803_02	-91.07	-43.68	-14.26	-65.15		-0.10	-0.07	-0.08	-0.03
AR_Pop0803_03	-98.81	-56.25	-8.47	-71.90		-0.05	-0.05	-0.15	0.02
AR_Pop0803_04	-108.78	-14.02	-10.88	-57.47		0.18	0.22	0.06	0.25
AR_Pop0803_05	-107.01	-47.10	-16.06	-71.02		0.28	0.33	0.12	0.39
AR_Pop0803_06	-88.12	-32.97	-14.80	-70.24		0.18	0.18	0.04	0.33
AR_Pop0803_07	-85.64	-20.44	-12.45	-63.95		-0.05	-0.04	0.05	0.10
AR_Pop0803_08	-98.00	-12.93	-6.87	-61.43		0.16	0.20	0.00	0.25
AR_Pop0803_09	-110.83	-40.39	-12.53	-73.30		0.00	0.03	0.07	0.20
AR_Pop0803_10	-85.74	-33.00	-9.99	-61.72		0.22	0.26	0.07	0.29
AR_Pop0803_11	-88.99	-15.70	-11.23	-55.71		-0.06	-0.03	0.10	0.15
AR_Pop0803_12	-95.94	-26.08	-11.26	-57.15		0.21	0.24	0.07	0.29
AR_Pop0803_13	-107.44	-29.54	-12.54	-65.74		-0.10	-0.07	0.08	0.10
AR_Pop0803_14	-88.58	-23.84	-14.75	-65.13		0.32	0.35	-0.01	0.39
AR_Pop0803_15	-80.01	-34.87	-12.18	-58.18		0.06	0.09	-0.05	0.13
AR_Pop0813_01	-69.11	-56.86	-5.64	-56.72		-0.09	-0.09	0.21	-0.06
AR_Pop0813_02	-52.78	-57.67	-2.65	-49.44		0.61	0.66	0.18	0.56
AR_Pop0813_03	-71.73	-57.55	-5.99	-50.62		0.51	0.54	0.22	0.40
AR_Pop0813_04	-58.66	-59.19	-5.74	-55.40		-0.15	-0.12	0.25	-0.09
AR_Pop0813_05	-55.63	-54.84	-1.45	-48.33		0.43	0.49	0.33	0.44

Table A.4. Genomic estimated breeding values (GEBV) per trait [fruit weight (g) and blush overcolor (0-5)] and year for the AR RosBREED pedigree (Cont.).

Individual	Fruit weight (g)					Blush overcolor (0-5)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
AR_Pop0813_06	-57.23	-57.60	-8.40	-54.51		-0.15	-0.13	0.21	-0.12
AR_Pop0813_07	-53.12	-55.73	1.30	-46.13		0.60	0.63	0.32	0.51
AR_Pop0813_08	-78.94	-54.16	-2.51	-48.96		0.52	0.55	0.28	0.50
AR_Pop0813_09	-86.46	-57.81	-8.74	-55.75		-0.01	0.00	0.22	0.02
AR_Pop0813_10	-55.17	-55.88	-5.78	-52.72		-0.15	-0.13	0.11	-0.13
AR_Pop0813_11	-50.28	-55.76	-0.50	-48.52		0.58	0.63	0.34	0.51
AR_Pop0813_12	-57.70	-59.30	-3.83	-50.06		0.47	0.51	0.29	0.42
AR_Pop0817_01	-86.48	-13.47	-4.57	-45.00		-0.30	-0.28	0.07	-0.01
AR_Pop0817_02	-105.96	-39.19	-8.93	-61.52		-0.30	-0.29	0.01	-0.11
AR_Pop0817_03	-103.80	-33.91	-8.47	-56.63		-0.17	-0.15	0.06	-0.15
AR_Pop0817_04	-86.53	-30.69	-4.50	-53.88		-0.22	-0.20	0.00	-0.19
AR_Pop0817_05	-85.32	-31.19	-9.36	-57.89		-0.37	-0.36	0.02	-0.25
AR_Pop0817_06	-85.51	-33.05	-6.51	-53.53		-0.35	-0.32	0.02	-0.20
AR_Pop0817_07	-85.46	-28.94	-2.51	-46.91		-0.35	-0.33	0.01	-0.19
AR_Pop0817_08	-86.06	-35.08	-7.79	-57.37		-0.14	-0.13	0.06	-0.13
AR_Pop0817_09	-103.36	-33.60	-6.05	-55.57		-0.42	-0.42	0.01	-0.28
AR_Pop0819_01	-48.62	-40.68	2.17	-36.67		0.03	0.02	0.04	0.07
AR_Pop0819_02	-49.07	-12.11	5.13	-35.96		-0.06	-0.05	0.13	-0.01
AR_Pop0819_03	-46.80	-45.78	3.27	-35.98		0.02	0.06	0.03	0.07
AR_Pop0819_04	-47.48	-20.63	0.58	-37.19		0.02	0.04	0.05	0.06
AR_Pop0819_05	-44.62	-48.57	5.02	-35.84		0.07	0.11	0.00	0.08
AR_Pop0819_06	-53.38	-40.36	10.69	-34.83		-0.01	0.01	0.10	0.01
AR_Pop0819_07	-54.84	-11.39	-1.31	-37.87		0.20	0.24	0.13	0.18
AR_Pop0819_08	-48.65	-11.74	8.16	-35.36		-0.05	-0.01	0.15	0.00
AR_Pop0819_09	-47.44	-46.47	2.25	-36.57		0.04	0.06	0.03	0.07
AR_Pop0819_10	-43.45	-15.46	3.36	-35.95		0.01	0.05	0.11	0.03
AR_Pop0819_11	-51.77	-51.84	2.65	-38.21		-0.02	0.02	0.13	0.04
AR_Pop0819_12	-51.43	-51.90	2.03	-38.09		0.13	0.17	0.02	0.08
AR_Pop0819_13	-52.82	-49.86	4.47	-37.62		-0.02	0.01	0.13	0.00
AR_Pop0819_14	-53.12	-48.11	4.47	-36.98		0.11	0.14	0.06	0.12
AR_Pop0819_15	-59.79	-48.77	-1.46	-38.58		0.01	0.05	0.04	0.05
AR_Pop0819_16	-52.33	-12.53	0.13	-36.85		0.05	0.08	0.14	0.10
AR_Pop0819_17	-39.46	-7.43	4.09	-34.54		0.12	0.16	0.17	0.15
AR_Pop0819_18	-37.78	-9.23	1.57	-34.50		0.14	0.16	0.11	0.13
AR_Pop0819_19	-36.39	-49.70	7.01	-36.01		0.09	0.13	0.03	0.06
AR_Pop0819_20	-51.65	-11.61	-0.27	-37.00		0.18	0.22	0.17	0.15

Table A.4. Genomic estimated breeding values (GEBV) per trait [fruit weight (g) and blush overcolor (0-5)] and year for the AR RosBREED pedigree (Cont.).

Individual	Fruit weight (g)					Blush overcolor (0-5)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
AR_Pop0819_21	-42.58	-51.21	1.61	-36.81		-0.02	0.01	0.03	0.02
AR_Pop0819_22	-45.19	-48.55	6.58	-35.87		0.20	0.25	0.15	0.13
AR_Pop0819_23	-58.34	-50.26	4.83	-37.16		-0.01	0.03	0.00	0.03
AR_Pop0825_01	-87.72	-27.89	-15.74	-52.55		0.25	0.27	0.06	0.27
AR_Pop0825_02	-73.86	-29.30	-13.43	-52.28		0.19	0.19	0.08	0.20
AR_Pop0825_03	-103.47	-19.11	-16.65	-55.70		0.17	0.19	0.07	0.20
AR_Pop0825_04	-78.43	-24.27	-20.26	-62.97		0.68	0.73	0.04	0.66
AR_Pop0825_05	-75.89	-19.75	-20.13	-62.43		0.73	0.75	-0.01	0.68
AR_Pop0825_06	-75.40	-10.95	-16.30	-50.31		0.73	0.75	0.02	0.68
AR_Pop0825_07	-89.83	-30.07	-18.32	-56.20		0.79	0.84	0.18	0.71
AR_Pop0825_08	-74.45	-22.55	-16.73	-57.98		0.60	0.65	0.04	0.59
AR_Pop0825_09	-80.29	-20.93	-15.79	-51.85		0.07	0.10	0.11	0.12
AR_Pop0825_10	-88.26	-21.43	-17.17	-52.69		0.35	0.35	0.18	0.42
AR_Pop0825_11	-73.13	-38.03	-17.47	-53.58		0.65	0.69	-0.02	0.62
AR_Pop0825_12	-96.36	-29.75	-18.75	-49.76		0.69	0.74	0.05	0.67
AR_Pop0825_13	-94.53	-34.73	-14.33	-50.67		0.11	0.14	-0.01	0.16
AR_Pop0825_14	-95.26	-16.49	-19.64	-55.11		0.62	0.68	0.13	0.61
AR_Pop0825_15	-75.65	-36.52	-20.31	-60.25		0.15	0.17	-0.03	0.17
AR_Pop0825_16	-78.86	-35.98	-18.58	-55.84		0.56	0.62	0.03	0.56
AR_Pop0825_17	-86.00	-0.90	-15.29	-45.34		0.09	0.13	0.03	0.16
AR_Pop0825_18	-87.30	-19.24	-15.64	-53.73		0.41	0.44	0.07	0.42
Arrington	-90.21	-46.76	-13.42	-67.13		-0.16	-0.13	0.00	-0.10
Australian Muir	-26.42	6.25	-2.62	-12.99		-0.16	-0.07	-0.05	-0.06
Belle	-51.81	-27.89	-1.19	-39.88		-0.12	-0.08	-0.02	-0.08
Bradley	-78.49	-6.18	4.32	-42.77		-0.08	-0.08	-0.08	0.03
Candoka	-64.61	-27.31	-2.89	-43.72		0.12	0.16	0.00	0.12
Chinese Cling	7.77	21.54	0.68	16.42		-0.35	-0.34	-0.12	-0.29
Chiripa	-84.83	-42.17	-6.69	-59.92		0.17	0.19	0.01	0.14
Cumberland	53.39	-6.09	-0.15	14.39		-0.69	-0.69	-0.29	-0.60
D42_13W	-33.09	-9.30	-1.83	-21.12		-0.11	-0.13	-0.07	-0.13
Delicioso	-68.62	-34.30	-3.17	-49.00		0.05	0.08	-0.02	0.04
Dix_16_3	-26.30	-9.12	-0.57	-18.61		-0.16	-0.31	-0.12	-0.28
Dix_22A_5	-41.69	-18.63	-1.83	-28.95		-0.14	-0.20	-0.07	-0.18
Dix_58_6	-51.59	-28.86	-3.03	-38.95		-0.14	-0.09	-0.02	-0.08
Dixon	26.00	44.78	-1.35	33.49		-0.22	-0.31	-0.13	-0.25
dummy003	-24.16	-27.98	6.62	-17.57		-0.05	-0.01	0.01	-0.02

Table A.4. Genomic estimated breeding values (GEBV) per trait [fruit weight (g) and blush overcolor (0-5)] and year for the AR RosBREED pedigree (Cont.).

Individual	Fruit weight (g)					Blush overcolor (0-5)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
dummy004	-77.51	-37.43	-6.86	-51.16		-0.11	-0.10	-0.03	-0.11
dummy005	-77.26	-40.69	-8.28	-51.51		-0.16	-0.13	0.03	-0.12
dummy006	-73.17	-37.19	-5.66	-49.90		-0.10	-0.06	-0.05	-0.05
dummy007	-70.43	-36.49	-1.92	-50.44		-0.18	-0.18	-0.05	-0.13
dummy008	-61.48	-39.19	-5.38	-50.57		-0.20	-0.15	-0.06	-0.13
dummy009	-68.88	-39.97	-0.28	-49.73		-0.19	-0.20	-0.11	-0.16
dummy010	-51.94	-27.47	-2.38	-38.41		-0.10	-0.06	-0.01	-0.05
Early Crawford	-64.51	-53.16	2.98	-52.56		-0.06	-0.04	-0.04	-0.05
Elberta	18.82	3.09	4.81	21.78		-0.13	-0.10	-0.08	-0.12
Elberta OP27	-18.78	-14.78	1.50	-7.77		-0.17	-0.16	-0.07	-0.16
F_A_371	-31.60	-37.35	6.27	-28.15		-0.12	-0.12	-0.01	-0.13
F_A_427	-55.32	-28.81	-1.07	-43.55		-0.08	-0.04	-0.02	-0.01
F_A_433	-53.63	-32.74	0.03	-42.87		-0.15	-0.13	0.01	-0.11
F_A_699	-89.26	-27.99	-6.83	-52.72		-0.26	-0.25	0.03	-0.12
F_Candoka	-52.94	-28.11	-1.84	-40.04		-0.11	-0.08	-0.04	-0.08
F_Elberta OP27	-52.73	-28.01	-1.21	-38.27		-0.16	-0.15	-0.03	-0.13
F_Garden State	-56.17	-29.07	-1.71	-37.95		-0.10	-0.10	-0.02	-0.05
F_Ga. Belle	-66.59	-16.97	-2.52	-47.91		-0.31	-0.33	-0.13	-0.26
F_JH Hale	-72.82	-21.31	-4.27	-49.00		0.20	0.23	0.05	0.23
F_Nectared 4xA_24	-72.30	-33.02	-6.86	-51.18		0.02	0.03	0.07	0.03
F_NJ113115	-53.04	-26.58	-1.50	-38.84		-0.10	-0.09	-0.02	-0.04
F_NJ98838	-54.83	-26.98	-2.34	-39.98		-0.12	-0.09	-0.03	-0.07
F_NJN14	-59.72	-31.25	-2.44	-43.66		-0.09	-0.06	0.00	-0.04
F_Sunrise	-54.83	-28.47	-0.84	-38.13		-0.15	-0.15	-0.02	-0.11
G_17_5E	-74.46	-27.98	-4.98	-51.29		-0.30	-0.27	-0.10	-0.19
Garden State	-40.44	-24.14	-0.69	-24.22		-0.13	-0.12	-0.04	-0.09
Ga. Belle	-3.67	24.32	0.10	14.22		-0.57	-0.60	-0.23	-0.49
Goodmans Choice	-52.51	-28.71	-1.85	-36.81		-0.13	-0.08	-0.02	-0.07
Greensboro	-7.09	-28.07	-0.81	-48.56		-0.59	-0.62	-0.23	-0.52
H_523	-70.32	-23.06	-2.83	-33.94		0.22	0.26	0.06	0.22
HalBerta Giant	-67.65	-28.48	-2.78	-44.46		0.07	0.08	0.03	0.09
Hann Almond	-51.28	-27.92	-1.02	-39.54		-0.12	-0.08	-0.03	-0.06
Honeydew Hale	-42.43	-22.93	-1.12	-39.69		0.06	0.04	0.01	0.07
Jefferson	55.91	0.02	-2.43	13.92		-0.10	-0.07	-0.02	-0.04
JH Hale	-75.07	-27.13	-3.76	-47.84		0.34	0.39	0.05	0.33

Table A.4. Genomic estimated breeding values (GEBV) per trait [fruit weight (g) and blush overcolor (0-5)] and year for the AR RosBREED pedigree (Cont.).

Individual	Fruit weight (g)					Blush overcolor (0-5)			
	2011	2012	2013	Avg		2011	2012	2013	Avg
Jing	-61.33	-36.91	-2.57	-41.98		0.06	0.10	0.00	0.07
Jungerman	-8.84	12.05	-1.99	1.23		-0.17	-0.23	-0.10	-0.20
M_A_789	-99.44	-24.00	-5.46	-52.16		-0.19	-0.18	0.02	-0.09
MaoTao	-60.55	-42.79	1.41	-44.19		-0.25	0.09	0.01	0.07
Meredith	-46.95	-43.05	-0.45	-58.21		-0.32	-0.16	-0.10	-0.30
Mexican Honey	-53.06	-26.63	-2.68	-39.30		-0.11	-0.10	-0.03	-0.05
Nectalate	-51.93	-27.34	-2.58	-33.92		-0.11	-0.10	-0.02	-0.05
Nectared4	-72.31	-34.96	-5.30	-50.15		-0.09	-0.06	0.05	-0.03
Nectared4 x A_24	-81.42	-35.17	-7.47	-56.84		-0.08	-0.04	0.06	-0.02
Nectared 5	-72.26	-36.77	-5.08	-49.99		0.01	0.02	0.02	0.04
Nectared 7	-66.68	-33.40	-3.60	-45.86		-0.06	-0.04	0.01	-0.02
NJ113115	-53.18	-26.59	-1.73	-39.58		-0.10	-0.08	-0.02	-0.06
NJ25032	-56.46	-28.05	-2.10	-40.84		-0.10	-0.09	-0.03	-0.06
NJ257	22.06	-2.99	-2.17	-20.02		0.15	0.16	0.04	0.19
NJ38026	-63.71	-27.35	-3.09	-44.43		0.13	0.14	0.02	0.14
NJ5102893	-53.51	-30.50	-0.98	-39.55		-0.08	-0.08	-0.04	-0.04
NJ5106548	-60.93	-27.57	-3.66	-44.59		0.06	0.09	-0.01	0.08
NJ5107397	-57.44	-28.84	-1.96	-43.35		-0.12	-0.09	-0.03	-0.07
NJ53739	-51.98	-27.27	-0.45	-37.38		-0.12	-0.06	-0.03	-0.09
NJ53939	-66.49	-33.75	-4.01	-48.32		-0.05	-0.05	0.03	-0.01
NJ554367	-65.23	-31.96	-5.77	-47.55		-0.29	-0.32	-0.12	-0.25
NJ562021	-62.08	-25.76	0.13	-42.27		-0.08	-0.06	-0.01	-0.05
NJ6128	-64.49	-26.15	-3.01	-42.84		0.11	0.14	0.00	0.15
NJ822026	-35.90	-36.51	5.43	-29.16		-0.15	-0.14	0.01	-0.15
NJ94727	-61.22	-28.30	-2.90	-44.21		0.11	0.17	0.00	0.12
NJ98838	-57.75	-27.05	-3.08	-41.54		0.01	0.04	-0.02	0.01
NJC83	-48.07	-18.94	-3.46	-32.73		-0.03	-0.04	-0.05	-0.04
NJC95	-58.35	-30.14	-3.03	-42.14		-0.01	0.03	-0.03	0.00
NJLA3	-64.34	-27.35	-3.10	-43.85		0.13	0.14	0.01	0.16
NJN14	-64.17	-32.80	-3.78	-44.13		-0.07	-0.05	0.01	-0.04
NJN17	-63.63	-31.26	-3.22	-46.28		-0.11	-0.10	-0.03	-0.07
NJN21	-49.35	-25.95	-1.74	-32.75		0.05	0.05	0.02	0.05
NJN55	-63.14	-30.62	-3.06	-45.26		-0.11	-0.06	-0.01	-0.06
OldGold	-55.62	-28.98	-0.71	-41.39		-0.14	-0.13	-0.01	-0.10
Orange Cling	-5.59	10.67	-0.37	2.69		-0.21	-0.55	-0.20	-0.47

Table A.4. Genomic estimated breeding values (GEBV) per trait [fruit weight (g) and blush overcolor (0-5)] and year for the AR RosBREED pedigree (Cont.).

Individual	Fruit weight (g)					Blush overcolor (0-5)			
	2011	2012	2011	Avg		2011	2012	2011	Avg
Orange Cling x Alameda	-30.79	-8.57	-0.33	-17.45		-0.15	-0.31	-0.12	-0.27
Peento	-97.38	-28.05	-0.29	-66.29		-0.26	-0.24	-0.03	-0.18
Pop8089	-55.16	-29.77	-2.47	-43.47		-0.03	-0.05	-0.03	0.01
Raritan Rose	-9.45	-16.39	-2.41	-16.31		-0.16	-0.16	-0.11	-0.14
Redgold	-67.82	-31.27	-1.95	-46.01		-0.12	-0.08	0.05	-0.06
Redskin	-1.16	22.18	2.66	17.98		0.35	0.40	0.00	0.33
RR122_15	-28.29	-21.49	-2.29	-25.80		-0.08	-0.06	-0.05	-0.05
RR53_194	-47.08	-40.67	4.19	-31.52		0.16	0.20	0.02	0.17
Slappey	-43.16	-31.18	-0.49	-44.09		-0.54	-0.19	-0.19	-0.46
Sunrise	-61.48	-33.87	-1.71	-44.95		-0.12	-0.07	0.00	-0.06
Transcaal Cling	-54.79	-28.47	-2.74	-40.31		-0.12	-0.10	-0.03	-0.07
Westbrook	-96.41	-37.40	-13.98	-65.84		0.17	0.21	0.22	0.17
White County	-9.97	-1.98	2.55	-39.05		0.30	0.30	0.20	0.27
Winblo	-81.87	2.76	2.51	-42.46		0.42	0.46	0.00	0.39
Max-GEBV	**55.91**	**44.78**	**19.62**	**33.49**		**0.81**	**0.84**	**0.34**	**0.74**
Avg-GEBV	**-61.93**	**-33.89**	**-3.70**	**-45.84**		**0.07**	**0.09**	**0.05**	**0.10**
Min-GEBV	**-110.83**	**-64.69**	**-26.89**	**-84.92**		**-0.69**	**-0.69**	**-0.29**	**-0.60**

Appendix B. High resolution melt (HRM) SNP-based genotyping for the G1*Xap*F 4-SNP haplotype DNA test

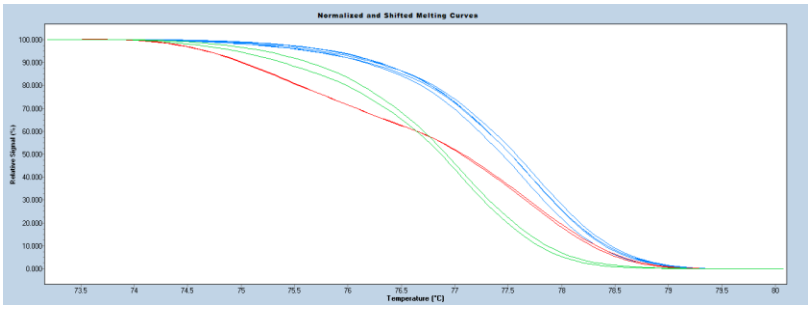

Fig. B.1. High resolution melt (HRM) SNP-based genotyping results for eight representative individuals using SNP 1.

Table B.1. High resolution melt (HRM) SNP-based genotyping results for eight representative individuals using SNP 1.

SNP 1			
Individuals tested	Expected	Haplotype 1	Haplotype 2
White County[zy]	R1 | R1	B	B
White Diamond[y]	R1 | R1	B	B
A-772[z]	R1 | I	B	B
A-672[zy]	R1 | I	B	B
A-665[zy]	I | SU	B	A
Westbrook	R1 | SU	B	A
Bradley	SU | SU	A	A
Arrington	SU | SU	A	A

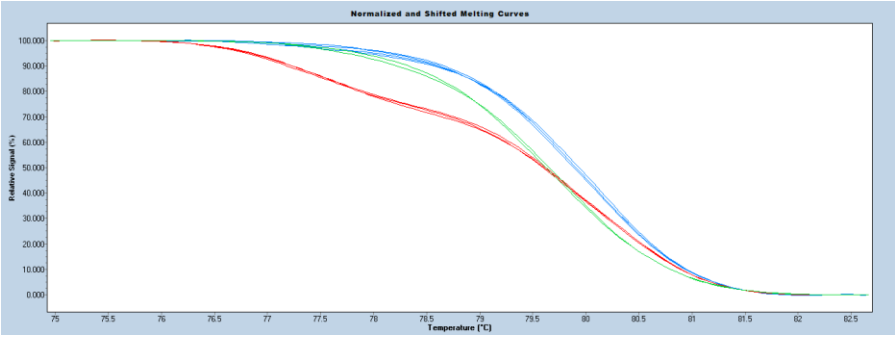

Fig. B.2. High resolution melt (HRM) SNP-based genotyping results for eight representative individuals using SNP 2.

Table B.2. High resolution melt (HRM) SNP-based genotyping results for eight representative individuals using SNP 2.

SNP 2			
Individuals tested	Expected	Haplotype 1 (SNP 2)	Haplotype 2 (SNP 2)
White County[zy]	**R1 | R1**	**B**	**B**
White Diamond[y]	**R1 | R1**	**B**	**B**
A-772[z]	**R1 | I**	**B**	**B**
A-672[zy]	**R1 | I**	**B**	**B**
A-665[zy]	**I | SU**	**B**	**A**
Westbrook	**R1 | SU**	**B**	**A**
Bradley	**SU | SU**	**A**	**A**
Arrington	**SU | SU**	**A**	**A**

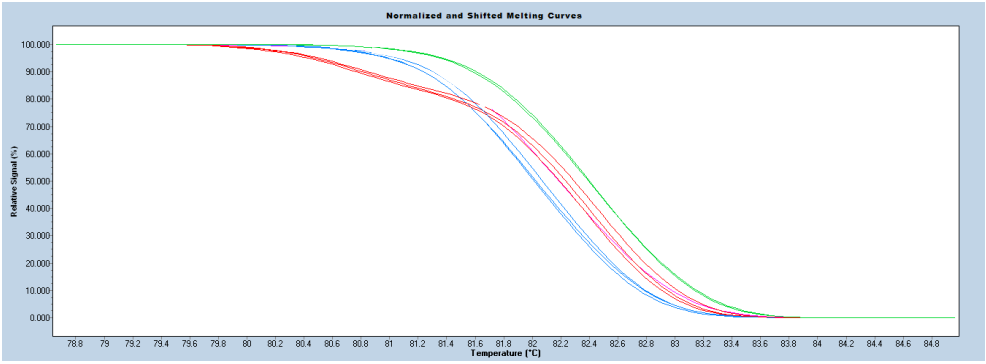

Fig. B.3. High resolution melt (HRM) SNP-based genotyping results for eight representative individuals using SNP 2.

Table B.3. High resolution melt (HRM) SNP-based genotyping results for eight representative individuals using SNP 2.

SNP 4			
Individuals tested	Expected	Haplotype 1	Haplotype 2
White County[zy]	R1 | R1	A	A
White Diamond[y]	R1 | R1	A	A
A-768	N/A	A	A
A-772[z]	R1 | I	A	B
A-672[zy]	R1 | I	A	B
Westbrook	R1 | SU	A	B
A-665[zy]	I | SU	B	B
Bradley	SU | SU	B	B
Arrington	SU | SU	B	B

Table B.4. High resolution melt (HRM) SNP-based observed genotyping results for eight representative individuals using the *G1XapF* 4-SNP haplotypes (SNP 3 was not tested since all individuals tested in this dissertation were shown to be monomorphic at this SNP). Expected functional haplotypes come from the IPSC 9K peach SNP array v1.0 (Verde et al., 2012), and or the peach mini-SNP array v1.0 (Chapter Four).

G1XapF 4-SNP haplotypes				
Individuals tested	Expected	Haplotype 1 (SNP 1-4)	Haplotype 2 (SNP 1-4)	Observed
White County[zy]	**R1 | R1**	**BB-A**	**BB-A**	**R1 | R1**
White Diamond[y]	**R1 | R1**	**BB-A**	**BB-A**	**R1 | R1**
A-772[z]	**R1 | I**	**BB-A**	**BB-A**	**R1 | I**
A-672[zy]	**R1 | I**	**BB-A**	**BB-A**	**R1 | I**
A-665[zy]	**I | SU**	**BB-B**	**AA-B**	**I | SU**
Westbrook	**R1 | SU**	**BB-A**	**AA-B**	**R1 | SU**
Bradley	**SU | SU**	**AA-B**	**AA-B**	**SU | SU**
Arrington	**SU | SU**	**AA-B**	**AA-B**	**SU | SU**

Overall Conclusions

In this dissertation, three sequential steps were performed to develop breeding-relevant DNA tests for MAS of fruit bacterial spot resistance (*Xap*1 and *Xap*6), fruit quality [blush (R_f), acidity (*D*), and acidity and soluble solid content (*G7Flav*)], and phenological traits [maturity date (*G4mat*), fruit type (*G*), and flesh color (*Y*)] across four RosBREED peach breeding programs. The validated DNA tests were used in marker-assisted parent selection (MAPS) in 2013-2015 at the University of Arkansas (UA) program to combine horizontal *Xap* resistance with high fruit quality spanning the season. Additionally in 2015, two of the SLP-based DNA tests, the indelG (pubescent vs. glabrous) and PpCCD4b-SSR (white vs. yellow flesh) were advanced to test in marker-assisted seedling selection (MASS). Lastly 20 QTLs were identified for *Xap* fruit, *Xap* leaf, and *Xap* leaf-assay resistance along with seven fruit quality and phenological traits using the Pedigree-Based Analysis (PBA) approach and the UA RosBREED pedigree. These 20 QTLs are optimal targets for future DNA test development, validation, and use in MAS. This work serves as a starting platform for the use of MAS in the UA program, which will continue to expand and evolve as additional DNA tests for the same or other breeding-relevant traits are developed and incorporated. The future is bright for the UA program, as the stage has been set for continued use of MAS, which will enable: increased genetic gain per generation cycle, resource savings, and extension of MAS into the blackberry and muscadine grape breeding programs upon successful development of DNA tests.