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Enabling Marker Assisted Breeding (MAB) for Phytochemical Compounds in Peach [*Prunus persica* (L.) Batsch]

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ENABLING MARKER ASSISTED BREEDING (MAB) FOR PHYTOCHEMICAL
COMPOUNDS IN PEACH [*PRUNUS PERSICA* (L.) BATSCH]

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Plant and Environmental Sciences

by
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December 2016

Accepted by:
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Dr. Gregory Reighard
Dr. Douglas Bielenberg
Dr. Feng Chen

ABSTRACT

Genetic control and location of QTLs associated with phytochemical compounds in peach were evaluated using bi-parental mapping and genome wide association (GWAS). The bi-parental mapping was conducted in an F₂ population (ZC²) derived from cross between ‘Zin Dai’ x ‘Crimson Lady’. GWAS was performed on an association panel representing modern peach cultivars available and/or produced for the U.S. market. Antioxidant capacity and phenolic compound accumulation (total phenolics, flavonoids and anthocyanins) were evaluated for two years on all materials. The ZC² progeny were genotyped using IPSC 9K peach SNP array v1., and the association panel was genotyped using genotyping-by-sequencing. The genetic linkage map, constructed with 908 SNP markers distributed among eight linkage groups, covers a genetic distance of ~ 336 cM, with an average marker density of 1.07 cM/marker. Total of 6 QTLs associated with phytochemical traits were identified on 5 linkage groups (LGs). Two major QTLs were observed on LG 6 and 8. *qPC.ZC-6.1* was associated with all phytochemical compounds, while *qPC.ZC-8.1* exhibited association only with total phenolics and anthocyanin content. GWAS, performed on a dataset of 18,085 SNPs and all phytochemical compounds, revealed a significant association ($P < 0.05$) for 129 SNPs covering the entire genome. A Majority of SNPs (121) were associated with anthocyanin accumulation and spread across all LGs, while 8 SNPs on LG7 were associated with both antioxidant capacity and flavonoid content. Overlap was observed between SNPs associated with anthocyanin accumulation, detected via GWAS, and major QTL for phytochemical compounds on LG6, identified using the bi-parental population.

Validation of functional alleles associated with accumulation of phytochemical compounds will enable development of a simple DNA test(s) to predict phenotype response to accumulation of these compounds in young seedlings without the need for fruit evaluation.

DEDICATION

I dedicate this dissertation to my wonderful husband, Faisal El-gandouz, and my lovely daughter, Jana El-gandouze. Special dedication to my dear parents. My father, Abdelghafar Mohammed, and my mother, Fujriah Ibrahim, with my love and respect.

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CHAPTER ONE

LITERATURE REVIEW

Introduction to peach

Peach [*Prunus persica* (L.) Batsch] belongs to Prunoideae subfamily, and Rosaceae family. Rosaceae family is one of the major angiosperm families and covers many economically significant fruits and ornamental plants worldwide, such as apples (*Malus domestica*), strawberries (*Fragaria × ananassa*), European pears (*Pyrus communis* L.), blackberries (*Rubus fruticosus*), raspberries (*Rubus idaeus*), and *Rosa* species. In addition, *Prunus* species include many stone fruits, such as European plum (*P. domestica* L.), Japanese plum (*P. salicina*), apricot (*P. armeniaca* L.), sweet cherry (*P. avium* L.), sour cherry (*P. cerasus* L.), and almond (*P. amygdalus* L.).

Peach is native to China where it was domesticated near 2000 B.C. and is considered a symbol of long life (Huang et al., 2008). This fruit crop spread to the Mediterranean through Persia and then was brought to America by Spanish explorers (Hedrick, 1917). Peach is one of the most economically important fruit crops consumed worldwide (Rice-Evans et al., 1996), and is ranked the third most important fruit after apple and pear (Font i Forcada et al., 2014). In the United States, peach culture and production is highly successful on the western and eastern coasts (Hancock et al., 2008; Scorza and Sherman, 1996). China, the center of origin for peach, constitutes the first major production country of peaches, accounting for about 44% of the total world supply followed by Italy, Spain, and the USA (Huang et al., 2008). South Carolina and Georgia rank second and third, respectively, in US fresh market peach production (NASS, 2013).

Peach cultivation in South Carolina dates back to early 1700s and has great health, economical, historical, and landscape benefits. The production of South Carolina peaches is steady in the last 5 years, with an annual average of 47,574 tons (SCDA; data update Sep. 13, 2015). Detailed description for many of peach cultivars used in the United States is provided by Okie (1998). Most cultivars in the U.S. market are yellow-fleshed, freestone, melting peaches and nectarines, while those from Asian countries, such as China, Japan, and South Korea; are predominantly white-fleshed (Della Strada and Fideghelli, 2003).

Peach is regarded as a summer fruit with special taste and health benefits in the human diet. In addition, it is rich in many minerals, such as potassium for regular heart rate and blood pressure, iron required for red blood cell formation, and fluoride for component of bones and teeth. Moreover, peaches do provide a source of dietary fiber that is essential for the suitable function of the digestive systems.

Phytochemical compounds

Phytochemical compounds are naturally found in fruits and vegetables.

Consumption of a variety of fruits and vegetables provide a wide range of nutrients which are easily absorbed and together with vitamins, minerals, and fibers promote good health in humans. For example, a highly significant negative correlation has been found between total intake of fruits and vegetables and different chronic diseases (e.g. cancer, cardiovascular diseases, diabetes, and stroke) and mortality, through improving antioxidant defenses in the human body (Acheson and Williams, 1983; Arts and Hollman, 2005; Cantín et al., 2009; Giampieri et al., 2012; Prior and Cao, 2000; Verlangieri et al., 1985; Vizzotto et al., 2007). Therefore, to prevent free radical activities

that cause chronic and degenerative diseases, human diet should contain fruit and vegetables that have high rates of free radical scavengers.

Antioxidants are substances that when present at low concentrations prevent or delay oxidation of cell contents (Lúcio, 2009). Antioxidative properties of phenolic compounds arise from their high reactivity as electron or hydrogen donors against the damaging effects of reactive oxygen species (ROS) and/ or reactive nitrogen species (RNS) (Fig. 1.1), otherwise called free radicals, such as singlet oxygen, super oxide, peroxy radicals, hydroxyl radicals and peroxynitrite which result in oxidative stress triggering cellular damage (Blokhina et al., 2003; Gill and Tuteja, 2010; Kaur and Kapoor, 2001; Mattson and Cheng, 2006). DNA damage, lipid peroxidation, protein oxidation, and enzyme inhibition are some of the injuries caused by overproduction ROS under numerous abiotic stresses leading to disease conditions (Uddin et al., 2008; Gill and Tuteja, 2010). Antioxidants scavenging the harmful free radicals by enzymatic [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)] and non-enzymatic [vitamin E (tocopherol), vitamin C (ascorbic acid), glutathione GSH tripeptide (glutamyl,-cysteinyl-glycine), carotenoids, and flavonoids] antioxidants (Ahmad et al., 2010; Blokhina et al., 2003; Gill and Tuteja, 2010). Antioxidants, such as phenolic compounds, including total phenolics, flavonoids and their subclass anthocyanin, not only improve fruit quality but also increase shelf life of fruit, inhibit growth of pathogenic microorganisms due to their natural antimicrobial properties, and reduce occurrence of post-harvest diseases (Cevallos-Casals et al, 2006; Tajkarimi and Ibrahim, 2012; Khanizadeh et al., 2012).

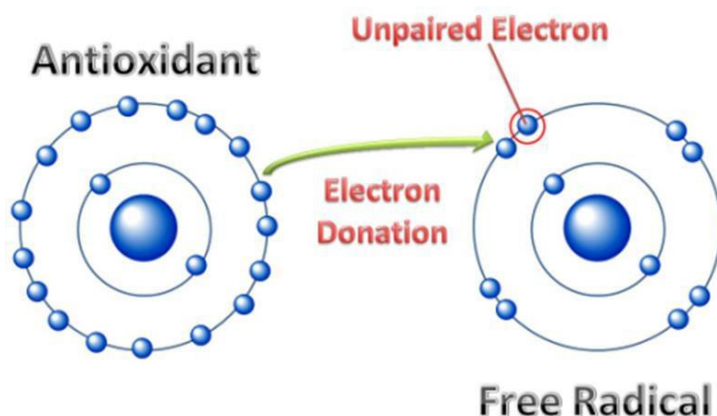


Figure 1. 1. Antioxidants fight the damage of free radicals (adapted from <http://normsfarms.com/what-are-antioxidants/>).

Phenolic compounds are a large and varied group of molecules, which construct many different families of aromatic secondary metabolites in plants and play an important role in peach fruit color, flavor, and health attributes (Ravaglia, 2010). Phenolics are divided into two groups; non-soluble compounds, such as lignins, condensed tannins, and cell- wall bound hydroxycinnamic acids, and soluble compounds, such as phenolic acids, flavonoids, phenylpropanoids, and quinines (Rispaill et al., 2005). Polyphenols include a wide range of compounds with antioxidant activities, therefore, they are considered the main sources of antioxidants (Gil et al., 2002). The main phenolic compounds reported in peach are hydroxycinnamates (chlorogenic and neochlorogenic acid); flavan 3-ols (catechin, epicatechin, and procyanidin B1); flavonols (quercetin 3-glucoside and quercetin 3-rutinoside); and anthocyanins (cyanidin 3- glucoside and cyanidin 3-rutinoside) (Kim et al., 2003; Tomas-Barberan et al., 2001). Phenolic compounds are widely distributed within the fruit tissues, with higher concentration in the exocarp of the fruit (Cevallos-Casals et al., 2006).

Flavonoids are a diverse group of polyphenolic compounds that are widely distributed in the plant kingdom, and are categorized into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones (Buhler and Miranda, 2000; Peluso, 2006; Risipail et al., 2005). Flavonoids are a large family of compounds found in fruits, vegetables, leaves, flowering tissues, pollens, and certain beverages having strong antioxidant effects. They contribute to the flavor and pigmentation in flowers and seed, taste, metabolic activity, and health-promoting properties. In addition, flavonoids play an important role in plant reproduction and fertility and in protection during stressful conditions (Buhler and Miranda, 2000; Forkmann and Martens, 2001; Peluso, 2006; Risipail et al., 2005; Weisshaar and Jenkins, 1998; Winkel-Shirley, 2001). The consumption of flavonoids is inversely correlated with mortality caused by cardiovascular disease and cancer (Buhler and Miranda, 2000; Peluso, 2006). These secondary metabolites are strong antioxidants, in which they inhibit hydrolytic and oxidative enzymes (phospholipase A2, cyclooxygenase, lipoxygenase), and have anti-inflammatory action (Frankel, 1995).

Anthocyanins (water-soluble vacuolar pigments) are one of the most important bioactive compounds that belong to the flavonoid family. They are classified as many different compounds based on their structure and molecular weights. Anthocyanins linked to monosaccharides (glucose, galactose, rhamnose, and arabinose), di, or tri- saccharides (Bureau et al., 2009) are described. The main anthocyanins reported in peach are cyanidin-3-glycoside and cyanidin-3-rutinoside (Kim et al., 2003; Tomas-Barberan et al., 2001; Wu and Prior, 2005). These pigments are important for food quality because of

their contribution to color and appearance. Anthocyanins are good sources of antioxidants that benefit human health (Edenharder et al., 2003; Moline et al., 2000; Sun et al., 2002; Wang et al., 1997, 1999; Wrolstad, 2001; Zhou et al., 2004).

All these phytochemical compounds are excellent scavengers of ROS/ RNS, which can be significantly affected by numerous abiotic environmental stresses, such as chilling, salinity, water deficiency, or UV-B irradiation (Agati et al., 2012).

In addition to environment, genotype, horticultural practices, harvest time, and post-harvest conditions also influence the accumulation of phytochemical compounds in fruit (Brown et al., 2014; Cevallos-Casals et al., 2006; Gil et al., 2002; Lee and Kader, 2000; Martínez-Espla et al., 2014; Romandini et al., 2008; Zhang et al., 2012).

Phenolic compounds, including flavonoids and their subclass anthocyanins, are synthesized by the shikimic acid metabolic pathway that consists of a number of enzymatic steps (Figures 1. 2 and 1. 3). Each of these enzymes catalyzes a progressive reaction for flavonoid synthesis. The first step in this pathway is to produce aromatic amino acid phenylalanine, which allows/ catalyzes the production of phenolic compounds via phenylalanine-ammonia lyase (PAL) (the key enzyme in bioactive compounds synthesis). Enzymes required for flavonoid synthesis are chalcone synthase (CHS), which catalyzes the first step by using malonyl CoA and 4-coumaroyl CoA as substrates to produce chalcones; chalcone isomerase (CHI) produces flavonones; flavanone 3-hydroxylase (F3H) produces dihydroflavonols; dihydroflavonol-4-reductase (DFR) synthesizes leucoanthocyanidins; and leucoantho-cyanidin dioxygenase (LDOX) synthesizes anthocyanidins. Specific enzymes for various flavonoid compounds include:

UDP-glucose: flavonoid 3-O-glucosyltransferase (UFGT) for anthocyanin synthesis; leucoanthocyanidin reductase (LAR) and ANR, in which anthocyanidin reductase synthesizes flavan-3-ols (proanthocyanidins); and flavonol synthase (FLS) for flavonols synthesis (Davies and Schwinn, 2003; Patra et al., 2013; Ravaglia et al., 2013; Winkel-Shirley, 2001) (Figures 1. 2 and 1. 3).

Since these enzymes catalyze the biosynthesis of flavonoids, any change in DNA coding sequence, such as deletion, insertion, inversion, duplication, affect these enzymes and the biosynthesis pathway by increasing or decreasing metabolic efficiency. Furthermore, some transcription factors (TFs) regulate gene expression either as activators or as repressors. Ravaglia et al. (2013) reported three TFs (MYB10, MYBPA1, and bHLH3) that activate flavonoid biosynthesis and two (MYB111 and MYB16) that repress the transcription of the biosynthetic genes.

Total antioxidant capacity and phenolic compound accumulation have been determined in different Rosaceae crops; plum germplasm (Vizzotto et al., 2007), strawberry and berries (Häkkinen and Törrönen, 2000), apple (Jelodarian et al., 2015; Muresan et al., 2014), blueberry (Prior et al., 1998), and wines of different fruit sources (Rupasinghe and Clegg, 2007).

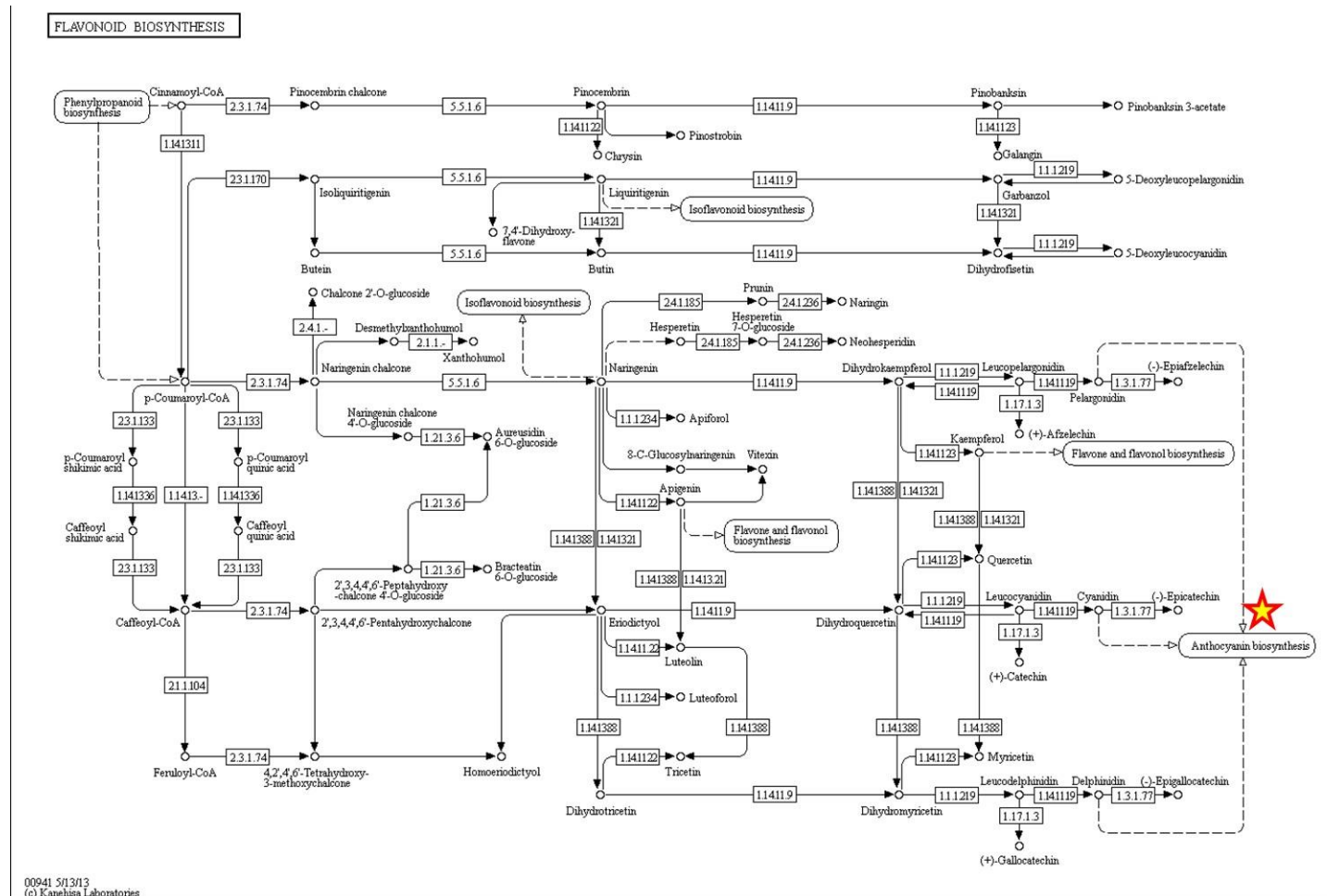


Figure 1. 2. Flavonoid biosynthesis pathway. Flavonoids are a main class of plant secondary metabolites that assists numerous functions including pigments and antioxidant activity (KEGG database, Kanehisa Labs, 1995-2016 http://www.kegg.jp/kegg-bin/highlight_pathway?scale=1.0&map=map00941&keyword=Flavonoids).

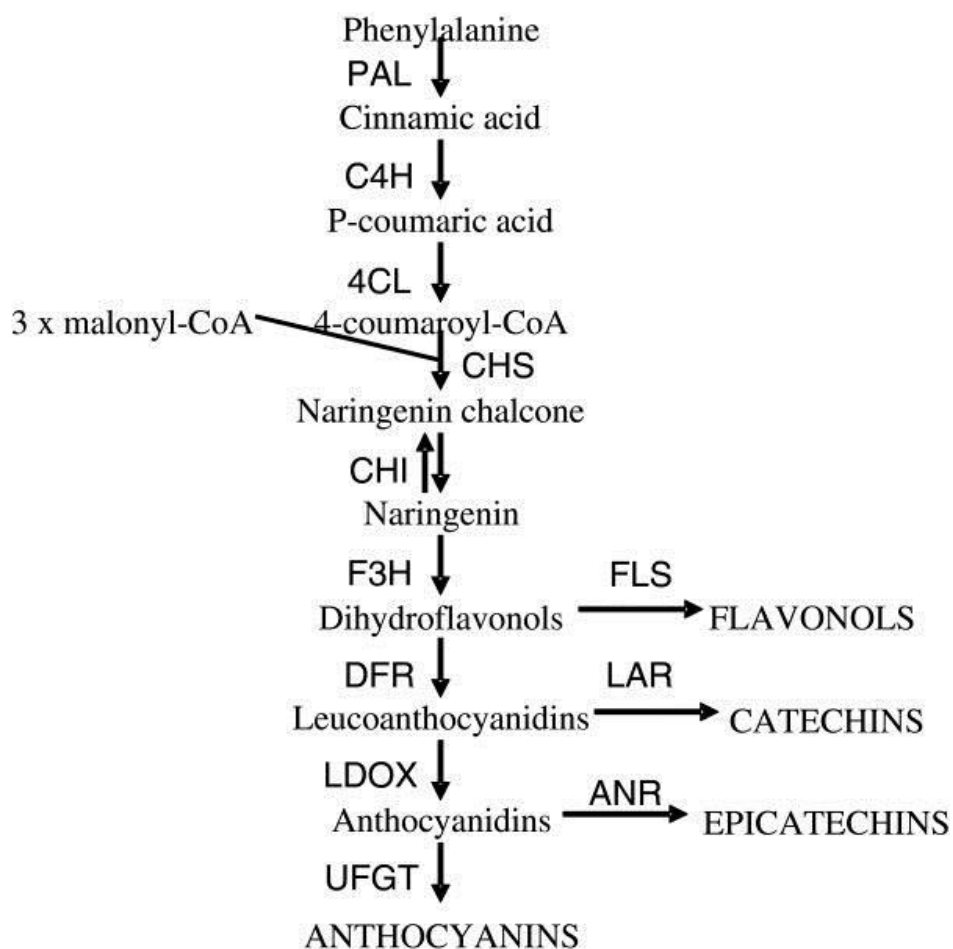


Figure 1. 3. Simplified pathway of flavonoids biosynthesis (adapted from Ravaglia et al., 2013).

Peach, like many other fruits, is high in bioactive compounds (Brown et al., 2014; Cantín et al., 2009; Cevallos-Casals et al., 2006; Dalla Valle et al., 2007; Gil et al., 2002; Kim et al., 2003; Tomas-Barberan et al., 2001; Vizzotto et al., 2007). Analyzing different flesh colored peaches revealed the influence of flesh color on phytochemical content in different locations around the world (Cantín et al., 2009; Dalla Valle et al., 2007; Gil et al., 2002; Vizzotto et al., 2007). Red-flesh peaches exhibited higher antioxidant activity,

phenolic content, and anthocyanin than light-colored flesh peaches and plums (Vizzotto et al., 2007). In general, the yellow-flesh peach cultivars showed lower antioxidant capacity than the white flesh ones (Cantín et al., 2009; Gil et al., 2002). Furthermore, the distribution of the phenolic compounds differs within the tissue of fruits. Although the fruit skin (exocarp) has the higher concentration of phytochemical compounds (Brown et al., 2014; Cevallos-Casals et al., 2006; Jiao et al., 2014; Zhang et al., 2015), it only represents ~8% of the total fresh flesh weight, accumulating ~30% of total phenolic compounds per fruit (Cevallos-Casals et al., 2006). Furthermore, fleshy part of the fruit is mostly consumed without the peel due to a consumer perception of skin being contaminated by sprays or human disease agents (Remorini et al., 2008). A positive correlation was found between antioxidant activity and total phenolics rather than some other phytochemicals in peach (Abidi et al., 2011; Cantín et al., 2009; Cevallos-Casals et al., 2006; Gil et al., 2002; Vizzotto et al., 2007). The wide variation of phenolic compounds and antioxidant capacity observed in peach provides genetic opportunities for breeding programs.

Although peaches have been found to exhibit lower antioxidant capacity than blueberry and plum, peach phenolic compounds showed higher antioxidant kinetics indicating their potential for faster removal of radical species (Cevallos-Casals et al., 2006). Further, it has been reported that phytochemical compounds from peach improved plasma total radical-trapping potential in humans (Dalla Valle et al., 2007) and selectively killed breast cancer cells (Noratto et al., 2009). Consequently, this has encouraged the interest of breeders in developing new cultivars with higher levels of antioxidants (Dalla

Valle et al., 2007; Vizzotto et al., 2007). Molecular techniques have been recently available for peach crop breeding and could be used to improve accumulation of the phytochemical compounds in newly developed cultivars.

Breeding and genetics

Traditional and molecular breeding

Since last century, the traditional genetic improvement tools, such as crossing, selection, statistical design, and evaluation of superior lines and *in vitro* propagation of new cultivars (Hancock et al., 2008; Iezzoni, 2008; Okie and Hancock, 2008) have successfully increased productivity and development of new cultivars with desirable qualities to satisfy commercial requirements by assuring more than acceptable quality levels. Early breeding programs focused on the improvement of physiological and quality characteristics of peach, including fruit color, size, firmness, attractiveness, taste, ripening time, cold hardiness, and adaptation to various environmental conditions. Recent increased awareness of health benefits of bioactive compounds found in fruits and vegetables has intensified breeder's efforts to incorporate enhancement of these compounds in newly developed varieties.

Consequently, there has been a growing interest in breeding programs to obtain information on phenolic content and antioxidant capacity of existing germplasm and its potential to provide enhanced health benefits to consumers (Brown et al 2014; Cantín et al., 2009; Orazem et al., 2011). However, one of the obstacles in development of new cultivars with desired traits is narrow genetic diversity of peach germplasm due to selfing and limited number of parents used in breeding programs (Byrne, 1999; Scorza et al., 1985; Scorza and Okie, 1990). This leads to reduce diversity in peach through

using modern cultivars in breeding that share a few common ancestors (Aranzana et al., 2003). A full characterization of germplasm is essential to discover new genetic backgrounds and introduce them to peach breeding programs, which could expand the genetic base of modern peach.

Traditional fruit tree breeding is a time consuming, expensive, and laborious process. For peach trees, it takes at least 3 years to mature and to produce fruit before the breeder can take any fruit quality data on the progeny (Dirlewanger et al., 1998, 2004a). Once the trees bear fruit, it takes about 15 years or more to do phenotypic analysis, selection, and regional testing to release a new cultivar. In addition, peach farms require a significant amount of space and maintenance practices, such as planting, pruning, thinning, irrigating, fertilizing, pesticide, fungicide, and herbicide spraying.

Molecular breeding [marker-assisted breeding (MAB)] would increase the efficiency of traditional breeding techniques and accelerate development of new cultivars with improved fruit quality traits. Understanding the genetics of different traits is very important for the development of new cultivars through implementation of MAB.

The application of MAB requires well developed genetic resources. Within the Rosaceae family, peach is highly genetically characterized and considered a model species for the Rosaceae family because of the vast molecular resources available (Dirlewanger et al., 2004a; Eduardo et al., 2013; Martínez-García et al., 2013; Verde et al., 2012, 2013), having a short juvenile phase (2 - 3 years) compared to other fruit trees, and being a self-fertile diploid species ($2n=16$) with a relatively small genome (~ 220-230 Mbp) twice that of *Arabidopsis thaliana* (157 Mb) (Baird et al., 1994;

Dirlewanger et al., 2004a; Verde et al., 2013). In addition to peach, strawberry, raspberry, and rose have diploid and small genomes (200 – 300 Mb); however, they show a wide diversity in growth habit. Therefore, availability of whole-genome sequences of diploid species is crucial to identify unique patterns of genetic diversity, domestication, and genome evolution (Verde et al., 2013). Availability of high-quality peach genome assembly enables the rapid translation of genetic knowledge to actual genes in specific gene families because of the resolution of mapped trait-containing intervals in peach for different important traits. The improved version of the peach genome sequence (v2.0.a1) (www.rosaceae.org) was recently released by the International Peach Genome Initiative (Verde et al., 2013), which is the basis for comparative analysis with other *Prunus* species and other trees. The available *Prunus* reference map (Dirlewanger et al., 2004a) along with release of a high-quality peach genome v2 (Verde et al., 2013) and developed SNP genotyping resources (Verde et al., 2012) offer vast resources for marker detection and MAB application.

Molecular markers

Molecular marker (genetic marker) is a fragment of DNA that is associated with certain location within the genome. The principle of using these markers is to identify a particular sequence of DNA that tightly linked to genes controlling the traits of interest. These markers have been used in analysis of various aspects of the *Prunus* genome, such as genetic variability, genome finger printing, genome mapping, gene location, and plant breeding.

Molecular markers (i.e., DNA-based markers) offer important advantages over use of biochemical (isozymes). A unique DNA sequence can be identified by a range of

molecular techniques, such as Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPD), DNA Amplification Fingerprinting (DAF), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) (microsatellite), and Single Nucleotide Polymorphisms (SNPs). DNA-based markers are highly polymorphic, exhibit simple inheritance, abundant throughout the genome, and easy and fast to detect (Arús et al., 2003; Ball et al., 2010; Martínez-García et al., 2013; Wünsch and Hormaza, 2002).

Both RFLPs and RAPD markers have been used to saturate the linkage map in *Prunus* (Chaparro et al., 1994; Dettori et al., 2001; Joobeur et al., 2000; Quarta et al., 1998). AFLP fragments were widely used in *Prunus* to construct linkage maps (Blenda et al., 2007; Dirlewanger et al., 1998, 1999; Olukolu et al., 2009). The co-dominant markers (SSRs) have been used in development of a new linkage map in peach (Illa et al., 2009; Yamamoto et al., 2005), apricot (Dondini et al., 2007; Illa et al., 2009), sweet cherry (Guajardo et al., 2015), plum and almond-peach hybrid (Dirlewanger et al., 2004b), and almond (Joobeur et al., 2000).

SNP markers represent a single nucleotide difference between DNA sequences in different individuals. These markers are the most abundant and are currently used to highly saturate the *Prunus* and other fruit crops linkage maps. SNPs have been used to construct linkage maps in peach (Bielenberg et al., 2015; da Silva Linge et al., 2015; Frett et al., 2014; Yang et al., 2013; Zeballos et al., 2016), apple rootstock (Antanaviciute et al., 2012), strawberry (Mahoney et al., 2016), sweet cherry (Guajardo et al., 2015), and peanut (Zhou et al., 2014). Next generation sequencing technology provides a way to

obtain low-cost and high density genotype information of numerous individuals in genetic mapping and population studies (Elshire et al., 2011). SNP markers have several advantages as they (1) having fewer evaluation errors than SSRs (Ball et al., 2010; Hamblin et al., 2007), (2) stable from generation to generation thru the genome with low mutation rate (Batley and Edwards, 2009), (3) applicable for quantitative trait loci (QTL) mapping with greater precision than is possible with SSRs or RFLPs (Yu et al., 2011), and (4) their frequency is high, compared with other molecular markers (Infante et al., 2008). Recently, progress in genome sequencing and SNP genotyping have allowed association analysis for QTL mapping and for genome-wide association scan mapping in different crops (Morgante and Salamini, 2003; Rafalski, 2010).

Genotyping -By-Sequencing (GBS)

Genotyping -By-Sequencing (GBS) using next generation sequencing technique is applicable approach for germplasm characterization, breeding, and trait mapping in different organisms. In addition, it is a powerful method that reduces the complexity of a genome using restriction enzymes (REs) and generates a high density of genetic markers, such as SNPs at low cost (Elshire et al., 2011). Even though this approach could generate considerable amount of missing data and a varying distribution of sequence reads (Beissinger et al., 2013), it has been proven as a rapid and cost- competitive genotyping method for peach (Bielenberg et al., 2015). Recent release of high quality peach genome sequence and generated diverse pools of SNP markers (Aranzana et al., 2012; Martínez-García et al., 2013; Verde et al., 2012, 2013) are providing necessary resources for enabling marker assisted selection (MAS) in peach breeding programs.

Linkage maps in *Prunus* and peach

Linkage maps (genetic maps) show the position and genetic distances between markers along chromosomes (Paterson, 1996a; Collard et al., 2005). Linkage map development requires a segregating population. Genetic map construction is based on recombination events that occur during meiosis to achieve a high resolution (Collard et al., 2005; Paterson, 1996a). The first genetic map for peach was developed by Chaparro et al., (1994). After that, interspecific almond peach ('T × E') genetic map was generated and used as the *Prunus* reference map (Dirlewanger et al., 2004a; Joobeur et al., 1998; Illa et al., 2011). The initial 'T × E' linkage map had 8 linkage groups (LGs) with 235 RFLP and 11 isozyme markers and spanning a total distance of 491 centimorgans (cM) (Aranzana et al., 2003; Dirlewanger et al., 2004a; Joobeur et al., 1998). Anchor markers (i.e. transferable markers throughout *Prunus*) with known map locations mapped in 'T × E' *Prunus* reference map, enabled comparative analysis of peach and *Prunus* species and facilitated construction of different *Prunus* linkage maps (Dirlewanger et al., 2004a; Howad et al., 2005; Pozzi and Vecchiatti, 2009). More than twenty genetic maps have been constructed with peach and other *Prunus* species (Abbott et al., 2008). Many of these maps were created for specific breeding objectives using specific mapping populations with different lengths, degree of saturation, and type of markers. Other *Prunus* interspecific maps include: *Prunus dulcis* (almond) × *Prunus persica* (peach) (Jáuregui et al., 2001); *Prunus persica* (peach selection) × *Prunus dulcis* almond (Foolad et al., 1995); *Prunus persica* × *P. ferganensis* (Dettori et al., 2001; Quarta et al. 1998); *Prunus persica* × *P. davidiana* (Dirlewanger et al. 1996); and *Prunus cerasifera* (plum) × [*Prunus dulcis* × *Prunus persica* hybrid] (Dirlewanger et al. 2004b). Published

intraspecific *Prunus* linkage maps, using different molecular markers, include those of almond (Joobeur et al., 2000; Viruel et al., 1995), apricot (Dondini et al., 2007; Hurtado et al., 2002; Lambert et al., 2004), sweet cherry (Olmstead et al., 2008), and peach: ‘New Jersey Pillar’ × ‘KV77119’ F2 (Abbott et al., 1998); ‘Ferjalou Jalousia’ × ‘Fantasia’ F2 (‘J x F’; Dirlewanger et al., 1998, 2006); ‘Lovell’ × ‘Nemared’ F2 (Lu et al., 1998); ‘New Jersey Pillar’ and ‘KV 77119’ (Rajapakse et al., 1995); ‘Akame’ × ‘Juseito’ F2 (Yamamoto et al., 2001); ‘Zin Dai’ × ‘Crimson Lady’ F2 (Frett et al., 2014). Additional peach linkage maps, including inter and intraspecific maps, are available on the Genomic Database for Rosaceae (GDR; <http://www.rosaceae.org/>). The construction of *Prunus* genetic linkage maps facilitates QTL analysis and development of DNA tests for marker-assisted breeding (MAB).

Quantitative trait loci (QTL) mapping

Quantitative trait loci (QTLs) are defined as the regions within genomes that contain genes associated with a particular quantitative trait (Collard et al., 2005; McCouch and Doerge, 1995; Mohan et al., 1997; Paterson, 1996a, b). Segregating populations are required for QTL linkage mapping, in which progeny derived from a cross between parents with contrasting phenotypes segregate for the trait of interest (Takagi et al., 2013). The majority of agronomically important traits are quantitative in nature (complex traits), controlled by multiple genes which presents significant challenges for genetic manipulation in trait improvement. Therefore, understanding the genetic control of quantitative traits facilitates development of new cultivars through different approaches (Peace and Norelli, 2009). Releasing the new version of peach genome with the availability of T × E *Prunus* reference map and the development of different

markers provide the opportunity to determine the inheritance of many quantitative traits at the molecular level. In addition, high conservation between the genomes of different *Prunus* species (Dirlewanger et al., 2004a; Dondini et al., 2007; Lambert et al., 2004) allows positioning of many markers/genes on each chromosome of one species to one or two chromosomes of the other, and also allows transferring knowledge obtained in other species to species of interest (Dirlewanger et al., 2004a; Jung et al., 2012; Vilanova et al., 2008). Several QTLs that associated with important traits in peach, such as flesh color, flesh adherence to the stone, soluble solids content, bloom and harvest dates, sugars, skin color, fruit shape, and titratable acidity have been mapped on the *Prunus* reference map (Arús et al. 2012; Dirlewanger and Arús, 2004; Dirlewanger et al. 2004a; Frett et al., 2014; Illa et al. 2011; Monet et al., 1996; Ogundiwin et al. 2009). In addition, QTLs associated with disease resistance, chilling and heat requirement, and bloom time (Fan et al., 2010; Yang et al, 2013) and with phytochemical compounds (Zeballos et al., 2016) in peach also have been detected. Limited studies exist about availability of QTLs associated with phytochemical compounds across rosaceae family. QTLs linked to polyphenolic composition (Chagné et al., 2012; Verdu et al., 2014) and vitamin C (Davey et al., 2006) in apple using single nucleotide polymorphism (SNP) markers, and QTLs linked to phytochemical compounds in peach (Zeballos et al., 2016) were detected. Zeballos et al. (2016) detected several QTLs for different phytochemical compounds using SNP and SSR markers in an F1 nectarine population derived from cross between ‘Venus’ and ‘Big Top’. Two relative antioxidant capacity QTLs were detected, one on linkage group (LG) 4 of the ‘Venus’ map and one on LG8 of the ‘Big Top’ map. One

QTL for total phenolics was detected on LG2 for both parental maps. Furthermore, QTLs associated with flavonoid accumulation were mapped on LGs 2, 3, 4, and 7 for the ‘Venus’ map and on LG2 on the ‘Big Top’ map, while a single QTL for anthocyanin was detected on LG 5 of the ‘Big Top’ map. Many important agronomic characters of *Prunus* species have not yet been mapped (Dirlewanger et al. 2004a), and most of these traits can be affected by environmental conditions.

Genome-wide association (GWA)

In addition to QTL mapping, genome-wide association (GWA) is a powerful approach that is used to examine genetic variation among different individuals to determine regions in the genome associated with the traits of interest by identifying genes with small effects via markers covering the entire genome (Meuwissen, 2007; Riedelsheimer et al., 2012; Zhao et al., 2011; Zhu et al. 2008). Association mapping uses natural populations or panels of diverse cultivars with the purpose of detecting recombination events that contribute to a higher resolution power for the association and greater ability for detecting more alleles (Meuwissen, 2007; Riedelsheimer et al., 2012; Sonah et al., 2015; Zhao et al., 2011; Zhu et al., 2008). Recent advances in next generation sequencing technology are providing low-cost, high throughput options for genome-wide SNP genotyping of numerous individuals (Elshire et al., 2011; Bielenberg et al., 2015). Availability of high throughput genotyping technique together with improvements in DNA sequencing in the presence of population structure are required for a successful association mapping (Atwell et al., 2010; Chan et al., 2011; Rafalski, 2010; Yu et al., 2006). Genes / markers controlling chilling injury in peach (Dhanapal and Crisosto, 2013), pomological traits in peach and nectarine (Font i Forcada et al, 2013),

fruit quality in peach germplasm (Micheletti et al., 2015), chemical and physical traits in almond (Font i Forcada et al., 2015), and fruit quality traits in Japanese pear (Iwata et al., 2013) have been detected using GWAS.

Marker assisted breeding (MAB)

Once tightly linked markers to the traits of interest are identified, a marker assisted breeding (MAB) approach can be used to simplify the evaluation of valuable fruit traits (Xu and Crouch, 2008). Selection using molecular markers is a useful and simpler method, compared to phenotypic screening as it (1) speeds up selection by saving time and resources especially for tree crops that have very long juvenile period, (2) reduces population sizes and the cost until individuals mature in the field, and (3) selects for traits that are difficult or expensive to measure, exhibit low heritability, or are expressed late in development (Dirlewanger et al., 2004a; Font i Forcada et al., 2013; Xu and Crouch, 2008). In addition, MAB for fruit breeding programs (4) estimates haplotype frequencies and haplotype–phenotype associations (Bielenberg et al., 2009; Pozzi and Vecchiatti, 2009).

Enabling MAB for phytochemical compounds in peach

There is a need to develop MAB for accumulation of phytochemical compounds in peach. The investigation of definitive QTL associated with polyphenolic composition (Chagné et al., 2012; Verdu et al., 2014) and vitamin C (Davey et al., 2006) has been performed in apple and for nutritional and antioxidant contents in tomato (Rousseaux et al., 2005). However, there have only been few reports on genetics behind accumulation of these compounds in peach fruit using genome-wide-association (GWA) (Font i Forcada et al., 2013) and bi-parental mapping for an F1 nectarine population derived from cross

between ‘Venus’ and ‘Big Top’ cultivars (Zeballos et al., 2016). Understanding the genetic control of quantitative traits will facilitate developing of new cultivars through breeding programs (Peace and Norelli, 2009).

Project objectives

The aim of this research is to enable MAB for improved phytochemical compounds in peach fruit through the following objectives:

1. Phenotyping of peach germplasm and breeding material for fruit quality traits and phytochemical compounds
2. QTL mapping using bi-parental approach and developing haplotypes for regions in the genome associated with phytochemical traits, and associating them with phenotype
3. Genome wide association (GWAS) using the next generation sequencing approach: genotyping- by- sequencing (GBS).

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CHAPTER TWO

ANTIOXIDANT CAPACITY AND PHYTOCHEMICAL COMPOUNDS OF MODERN PEACH [*Prunus persica* (L.) Batsch] GERMPLASM

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INTRODUCTION

Many fruits and vegetables are considered as functional foods and have always been considered important in human diet. Numerous studies showed that increased consumption of fruits and vegetables has a protective effect against chronic-degenerative diseases and improves antioxidant defenses of the human body (Arts and Hollman, 2005; Cantín et al., 2009b; Giampieri et al., 2012; Prior and Cao, 2000; Vizzotto et al., 2007). In addition, phytochemical compounds, such as phenolics, including total phenolics, flavonoids and their subclass anthocyanins, increase the shelf life of food, reduce the occurrence of post-harvest diseases, and inhibit growth of pathogenic microorganisms due to their natural antimicrobial properties (Cevallos-Casals et al., 2006; Khanizadeh et al., 2012; Tajkarimi and Ibrahim, 2012). Antioxidant enzyme systems that protect plants against free radicals can be grouped as enzymatic (superoxide dismutase SOD, catalase CAT, and glutathione peroxidase GPx) and non-enzymatic [vitamin E (tocopherol), vitamin C (ascorbic acid), glutathione GSH tripeptide (glutamyl,-cysteinyl-glycine), carotenoids, and flavonoids] antioxidants systems (Ahmad

et al., 2010; Blokhina et al., 2003; Gill and Tuteja, 2010). Accumulation of phytochemical compounds has been determined in many important fruit such as peach (Cantín et al., 2009b; Cevallos-Casals et al., 2006; Dalla Valle et al., 2007), peach and plum (Vizzotto et al., 2007), strawberry and berries (Häkkinen and Törrönen, 2000), apple (Jelodarian et al., 2015; Muresan et al., 2014), and in wines of different fruit sources (grape, apple, black currant, blueberry, cherry, cranberry, elderberry, peach, pear, plum and raspberry) (Rupasinghe and Clegg, 2007). Peaches have been found to exhibit lower antioxidant capacity than for example, blueberry and plum, but their phenolic compounds showed higher antioxidant kinetics indicating their potential for faster removal of free radicals (Cevallos-Casals et al., 2006). Furthermore, phytochemical compounds from peach improved total radical- trapping potential of plasma in humans (Dalla Valle et al., 2007) and selectively killed breast cancer cells (Noratto et al., 2009).

The main phenolic compounds reported in peach [*Prunus persica* (L.) Batsch] are hydroxycinnamates (chlorogenic and neochlorogenic acid), flavan 3-ols (catechin, epicatechin, and procyanidin B1), flavonols (quercetin 3-glucoside and quercetin 3-rutinoside), and anthocyanins (cyanidin 3-glucoside and cyanidin 3-rutinoside) (Kim et al., 2003; Tomás-Barberán et al., 2001). All these bioactive compounds are excellent scavengers of free radicals and could be affected/ elicited by numerous abiotic environmental stresses, such as chilling, salinity, heavy metals, water deficiency, or UV-B irradiation (Agati et al., 2012). It has been reported that UV light induces anthocyanin accumulation and biosynthesis in different peach and nectarine tissues

(Ravaglia et al., 2013).

In addition to environment, the genotype, horticultural practices, maturity date, pre- harvest applications, and post-harvest conditions also influence the accumulation of phytochemicals and fruit quality traits (Brown et al., 2014; Cevallos-Casals et al., 2006; Eduardo et al., 2011; Gil et al., 2002; Lee and Kader, 2000; Martínez-Espla et al., 2014; Romandini et al., 2008; Zhang et al., 2012). Phenolic compounds are widely distributed within the peach fruit, with higher concentration in the exocarp (Cevallos-Casals et al., 2006; Zhang et al., 2015). Even though the fruit skin (exocarp) has the higher concentration of phytochemical compounds, it only represents ~8% of the total fresh flesh weight, accumulating ~30% of total phenolic compounds per fruit and is usually removed prior to consumption (Cevallos-Casals et al., 2006; Remorini et al., 2008).

Recent increased awareness of health benefits of bioactive compounds found in fruits and vegetables has intensified breeder's efforts to enhance these compounds in newly developed varieties. Consequently, there has been a growing interest in breeding programs to obtain information on phenolic compounds and antioxidant capacity of existing germplasm and its potential to provide enhanced health benefits to consumers (Brown et al 2014; Cantín et al., 2009b; Orazem et al., 2011; Vizzotto et al., 2007). Therefore, the goal of this study was to evaluate modern peach breeding germplasm for its potential to provide sources for improvement of accumulation of phenolic compounds and relative antioxidant capacity in peach breeding program. Improved knowledge of nutritional quality of modern peach germplasm will facilitate

development of new peach cultivars with increased bioactive compounds.

MATERIALS AND METHODS

Plant material

One hundred and thirty-two peach and nectarine cultivars and one advanced selection (Table 2. 1), maintained within the *Prunus* collection at Clemson University, were included in this study. Fruit quality and phytochemical compounds evaluation was performed over two years (2013 and 2014). Analyzed material was comprised of yellow and white flesh peach and nectarine cultivars available and / or grown in the U.S. market with addition of the red-flesh advanced selection. The red- fleshed advanced selection was included to provide the variability in flesh colors and to allow assessment of health benefits that red flesh might bring to the market. The collection is maintained at the Musser Fruit Research Center, Seneca, SC (34.605202 latitude and - 82.877995 longitudes) under a warm, humid temperate climate and standard commercial practices for irrigation, fertilization, and pest and disease control. The trees were at least 8 years old, grafted on Guardian®™ rootstock, grown in triplicate, with 2 x 6 m spacing and perpendicular V training system. The maturity groups were based on average Julian ripening date recorded at the Musser Fruit Research Center over 6 years. Out of 133 accessions, a subset of 21 peach and nectarine cultivars and the red-fleshed advanced selection were analyzed over three seasons (2012-2014) to evaluate seasonal effects on phytochemical profiles.

Table 2. 1. Accessions used in this study. Individuals are listed by the ripening time.

Name	Fruit type	Flesh color	Ripening time ^y	Ripening Seaso	Origin
Rich May	Peach	Yellow	142	Early	USA, CA
Crimson Lady^z	Peach	Yellow	149	Early	USA, CA
Spring Snow	Peach	White	149	Early	USA, CA
Carored	Peach	Yellow	153	Early	USA, SC
Desiree	Peach	Yellow	153	Early	USA, NJ
Springold	Peach	Yellow	153	Early	USA, GA
Westbrook	Nectarine	Yellow	153	Early	USA, AR
Arctic Star	Nectarine	White	156	Early	USA, CA
Earlstar	Peach	Yellow	156	Early	USA, CA
Manon	Peach	White	156	Early	France
PF 1	Peach	Yellow	156	Early	USA, MI
Arctic Glo	Nectarine	White	160	Early	USA, CA
Candor	Peach	Yellow	160	Early	USA, NC
Honey Blaze	Nectarine	Yellow	160	Early	USA, CA
PF 5D Big	Peach	Yellow	160	Early	USA, MI
Sugar May	Peach	White	160	Early	USA, CA
Sweet Scarlet	Peach	Yellow	160	Early	USA, CA
Country Sweet	Peach	Yellow	163	Early	USA, CA
Early Red Free	Peach	Yellow	163	Early	USA, CA
Garnet Beauty	Peach	Yellow	163	Early	Canada
Glenglo	Peach	Yellow	163	Early	USA, WV
Sentry	Peach	Yellow	163	Early	USA, WV
7 Ball	Peach	Yellow	167	Early	USA, MI
Arctic Sweet	Nectarine	White	167	Early	USA, CA
D88-147	Peach	White	167	Early	USA, NJ
Easternglo	Nectarine	Yellow	167	Early	USA, CA
Gala	Peach	Yellow	167	Early	USA, LA
Jade	Nectarine	White	167	Early	France
PF-7A	Peach	Yellow	167	Early	USA, MI
Risingstar	Peach	Yellow	167	Early	USA, MI
Snowbrite	Peach	White	167	Early	USA, CA
Vulcan	Peach	Yellow	167	Early	Canada
Galaxy	Peach	White	174	Early	USA, CA
Karla Rose	Nectarine	White	174	Early	USA, CA
PF 8 Ball	Peach	Yellow	174	Early	USA, MI

Table 2.1 continued

Name	Fruit type	Flesh color	Ripening time ^y	Ripening season	Origin
Blazingstar	Peach	Yellow	177	Early	USA, MI
Caroking	Peach	Yellow	177	Early	USA, SC
PF 11 Peach	Peach	Yellow	177	Early	USA, MI
Redhaven	Peach	Yellow	177	Early	USA, MI
Starfire	Peach	Yellow	177	Early	USA, MI
Coronet	Peach	Yellow	181	Mid	USA, GA
Klondike White	Peach	White	181	Mid	USA, CA
PF 15A	Peach	Yellow	181	Mid	USA, MI
Redstar	Peach	Yellow	181	Mid	USA, MI
Reliance	Peach	Yellow	181	Mid	USA, NH
Summer Beaut	Nectarine	Yellow	181	Mid	USA, CA
White lady	Peach	White	181	Mid	USA, CA
Raritan Rose	Peach	White	184	Mid	USA, NJ
ZinDai	Peach	White	184	Mid	China
10 Ball	Peach	Yellow	188	Mid	USA, MI
11 Ball	Peach	Yellow	188	Mid	USA, MI
Burpeachfive	Peach	Yellow	188	Mid	USA, CA
Carolina Belle	Peach	White	188	Mid	USA, NC
Coralstar	Peach	Yellow	188	Mid	USA, MI
Crimson Rocket	Peach	Yellow	188	Mid	USA, WV
Ernies Choice	Nectarine	Yellow	188	Mid	USA, NJ
Glohaven	Peach	Yellow	188	Mid	USA, MI
Harrow Beauty	Peach	Yellow	188	Mid	Canada
John Boy II	Peach	Yellow	188	Mid	USA, PA
PF 17	Peach	Yellow	188	Mid	USA, MI
Sunhigh	Peach	Yellow	188	Mid	USA, NJ
White County	Peach	Yellow	188	Mid	USA, AR
Winblo	Peach	Yellow	188	Mid	USA, NC
9 Ball	Peach	Yellow	191	Mid	USA, MI
Allstar	Peach	Yellow	191	Mid	USA, MI
Arctic Belle	Nectarine	White	191	Mid	USA, CA
Arctic Jay	Nectarine	White	191	Mid	USA, CA
Bounty	Peach	Yellow	191	Mid	USA, WV
Flavortop	Nectarin	Yellow	191	Mid	USA, CA
Flavrburst	Peach	Yellow	191	Mid	USA, WV

Table 2.1 continued

Name	Fruit type	Flesh color	Ripening time ^y	Ripening season	Origin
Intrepid	Peach	Yellow	191	Mid	USA, NC
Sweet Dream	Peach	Yellow	191	Mid	USA, CA
Beaumont	Peach	Yellow	195	Mid	USA, MI
Beekman	Peach	Yellow	195	Mid	USA, NJ
Canadian Harmony	Peach	Yellow	195	Mid	Canada
Contender	Peach	Yellow	195	Mid	USA, NC
Early Loring	Peach	Yellow	195	Mid	USA, PA
Julyprince	Peach	Yellow	195	Mid	USA, GA
Late Large 23	Peach	Yellow	195	Mid	USA, MI
Loring	Peach	Yellow	195	Mid	USA, MO
Majestic	Peach	Yellow	195	Mid	USA, LA
PF-24-007	Peach	Yellow	195	Mid	USA, MI
Redglobe	Peach	Yellow	195	Mid	USA, MD
Summer Gold	Peach	Yellow	195	Mid	USA, GA
Suncrest	Peach	Yellow	195	Mid	USA, OR
99p4388	Peach	Red	198	Mid	USA, GA
Diamond Princess	Peach	Yellow	198	Mid	USA, CA
Late 24-007	Peach	Yellow	198	Mid	USA, MI
PF Lucky 24B	Peach	Yellow	198	Mid	USA, MI
Redrose	Peach	White	198	Mid	USA, NJ
Sweet Breeze	Peach	Yellow	198	Mid	USA, PA
Arctic Gold	Nectarine	White	202	Late	USA, CA
Gloria	Peach	Yellow	202	Late	USA, NJ
Glowingstar	Peach	Yellow	202	Late	USA, MI
PF 2050	Peach	Yellow	202	Late	USA, MI
Sweet N Up	Peach	Yellow	202	Late	USA, WV
Belle of Georgia	Peach	White	205	Late	USA, GA
Blushingstar	Peach	Yellow	205	Late	USA, MI
China Pearl	Peach	White	205	Late	USA, NC
Cresthaven	Peach	Yellow	205	Late	USA, MI
Fantasia	Nectarine	Yellow	205	Late	USA, CA
Messina	Peach	Yellow	205	Late	USA, NJ
Blake	Peach	Yellow	209	Late	USA, NJ
Carolina Gold	Peach	Yellow	209	Late	USA, NC
Summer Fire	Nectari	Yellow	209	Late	USA, CA

Table 2.1 continued

Name	Fruit type	Flesh color	Ripening time ^y	Ripening season	Origin
Summerfest	Peach	Yellow	209	Late	USA, WV
Zephyr	Nectarine	White	209	Late	France
Glacier	Peach	White	211	Late	USA, CA
Madison	Peach	Yellow	211	Late	USA, VA
PF 25	Peach	Yellow	211	Late	USA, MI
PF 28-007	Peach	Yellow	211	Late	USA, MI
Ambre	Nectarine	Yellow	216	Late	France
Elberta	Peach	Yellow	216	Late	USA, GA
Jerseyqueen	Peach	Yellow	216	Late	USA, NJ
Lady Nancy	Peach	White	216	Late	USA, NJ
PF 27A	Peach	Yellow	216	Late	USA, MI
Redskin	Peach	Yellow	216	Late	USA, MD
Augustprince	Peach	Yellow	219	Late	USA, GA
Autumnstar	Peach	Yellow	219	Late	USA, MI
Burpeachfour	Peach	Yellow	219	Late	USA, CA
Flameprince	Peach	Yellow	219	Late	USA, GA
PF 30-007	Peach	Yellow	219	Late	USA, MI
Snow Giant	Peach	White	219	Late	USA, CA
Encore	Peach	Yellow	223	Late	USA, NJ
Snow King	Peach	White	226	Late	USA, CA
Laurol	Peach	Yellow	230	Late	USA, NJ
Victoria	Peach	Yellow	230	Late	USA, NJ
Autumn Red	Peach	Yellow	233	Late	USA, CA
Caro Tiger	Peach	Yellow	237	Late	USA, SC
September Snow	Peach	White	237	Late	USA, CA
Snow Gem	Peach	White	237	Late	USA, CA
Tra-Zee	Peach	Yellow	244	Late	USA, CA
Autumn Flame	Peach	Yellow	251	Late	USA, CA

^z Bold - accessions analyzed in three years (2012- 2014).

^y Ripening time expressed in Julian date.

Fruit sampling

Fruits were harvested at commercial maturity and the Index of Absorbance Difference (I_{AD}) calculated as the difference between fruit absorbance at the wavelengths of 670 and 720 nm (chlorophyll-a absorbance peak and background of the spectrum) (Ziosi et al., 2008) to ensure uniform fruit maturity for all cultivars included in the study. Five fruits in the ripening stage equivalent to $I_{AD} = 0.6$ were selected and analyzed for fruit quality and phytochemical content.

Fruit quality measurement

Fruit quality traits were measured according to Frett et al. (2012) phenotyping protocol. Fruit texture analyzer (FTA) was used to measure fruit size (diameter) (mm), fruit weight (g), and fruit firmness (kg) on both cheeks. Soluble solids concentration (SSC) (Brix %) of juice was determined using digital refractometer (Atago 3810 PAL-1). Titratable acidity (TA) (malic acid %) was measured by titration with NaOH 0.1 N to pH 8.2. Additionally, ripening index (RI) was calculated as sugar/ acid ratio (SSC/ TA).

Phytochemical Analyses

After fruit quality parameters were measured, two slices of mesocarp per individual fruit were excised from opposing cheeks for phytochemical analyses. After removal of fruit exocarp and red tissue around the pit, tissue was sliced, individually packed for each fruit, frozen in liquid nitrogen (LN2), and stored at -20 °C until needed. A composite sample per accession was prepared by combining equivalent amount of tissue from each of five fruits, and grinding in liquid nitrogen using Freezer/Mill (SPEX® SamplePrep, Metuchen, NJ, USA). Ground tissue was stored at -

80 °C until needed. One milliliter of 80% methanol was used to extract 500 mg of frozen composite powder in five replicates. The extract was incubated at +4 °C overnight and then centrifuged for 10 min at 12,000 g at +4 °C to collect the supernatant. This hydroalcoholic extract was stored at -80°C and used in subsequent analyses. The total phenolics, flavonoid content, anthocyanin, and antioxidant capacity were measured in 5 replicates following the protocols of Cantín et al. (2009b).

The phenolic compounds, expressed in milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (FW), were determined according to the Folin–Ciocalteu method (Singleton and Rossi, 1965). The method consisted of mixing 10 µL of the extract with 75 µL of Folin–Ciocalteu’s reagent diluted in water and allowed to react for 1-8 min before the addition of 15 µL of 20% sodium carbonate (Na_2CO_3). The reaction mixture was incubated for 2 hours at room temperature and measurements of absorbance were taken at 765 nm using a spectrophotometer (Bio-Tek HT Synergy Multidetector microplate reader, Winooski, VT).

The flavonoid content was measured using the protocol of Zhishen et al. (1999) and the results were expressed as milligrams of catechin equivalents (CE) per 100g of FW. 10 µL of methanolic extract was diluted with 40 µL distilled water and 3 µL of 5% NaNO_2 , was added and incubated for 5 minutes. Then, 3 µL of 10% AlCl_3 was added to the solution, mixed and incubated for 6 minutes. Finally, 20 µL of 1N NaOH was added and absorbance was measured at 510 nm against catechin as standard.

Total anthocyanin content of the hydroalcoholic extracts was determined using the method of Fuleki and Francis (1968) adapted to peach tissue, and quantified as

milligrams of cyanidin-3-glucoside per kilogram of FW. Aliquots of the clear methanol extract were used for spectrophotometric readings at 535 nm by subtracting the absorbance at 700 nm (due to turbidity). The spectrophotometer was zeroed with the anthocyanins extraction solvent as the blank. Anthocyanins were quantified as milligrams of cyanidin-3-glucoside per kilogram of FW using a molar extinction coefficient of $25965 \text{ cm}^{-1} \text{ M}^{-1}$ and a molecular weight of 494 using following formula:

$$C (\text{mg/ kg}) = A/\epsilon L * MW * \text{Dilution factor} * (\text{Weight}+1)/ \text{Weight}$$

Where, A= $A_{535} - A_{700}$; ϵ = molar extinction coefficient of $25965 \text{ cm}^{-1} \text{ M}^{-1}$; L= the pathlength in cm (1cm); MW= molecular weight of 494.

The antioxidant capacity was measured using the DPPH method adapted from Brand-Williams et al. (1995) and modified by Crisosto lab (personal communication). The antioxidant capacity was expressed as Trolox (6- hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid) equivalent (TE) per gram of flesh weight. Briefly, 5 μl of the methanolic extract was added to 95 ml of fresh DPPH radical solution (0.1 mM in methanol) mixed and incubated overnight at room temperature. The absorbance of the samples was measured at 515 nm. The readings were used for calculation of the relative antiradical capacity (RAC), which indicates the antiradical capacity of the sample compared to Trolox for a specific reaction time.

Statistical Analysis

All data was expressed as means \pm SD of five replicates. Data for each accession were averaged over the years of study and mean values were used to estimate phenotypic values. The fresh peach market in the U.S. is almost exclusively yellow and

white flesh peach and nectarines; thus the red flesh accession was included only in the flesh color analysis. For the purpose of statistical analysis, cultivars ripening from May to the end of June (< 180 Julian date) were considered early, from July 1 – 20 (181-201) mid-season, and after July 21st (>202) late maturing. Analysis of variance was performed and means were compared with the Student-Newman-Keuls's test and t test ($P < 0.05$). Correlation analysis using Pearson's correlation coefficients at $P < 0.01$, was applied to assess the relationship between analyzed traits. Principal Components Analysis (PCA) was performed to study associations among the interested traits. All statistical analyses were performed in SPSS v. 23 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp).

RESULTS AND DISCUSSION

Variation in accumulation of phytochemical compounds was observed within the analyzed material and between experimental years. A large deviation from the mean, observed in our results, is due to the different environments affecting the accumulation of phytochemical compounds. Accumulation of bioactive compounds was highly influenced by genotype, flesh color, ripening season, and environmental conditions during years of study.

Nutritional composition and content in peach fruit is often measured using flesh tissue, based on the assumption that consumers usually remove skin (peel) prior to consuming the flesh, and that the flesh tissue accounts for most of the peach fruit (Cevallos-Casals et al., 2006; Remorini et al., 2008). To determine if there are differences in the total phenolic content between different peach tissues, peaches were evaluated with different tissues (peel and flesh, separately and together) for the total phenolic content and antioxidant capacity in a separate study. Results showed that total phenolics were not significantly different between fruit tissues (data not shown). Thus, regardless of how peach fruit is consumed, with or without peel, approximately the same amount of total phenolics will be consumed.

Generally, no significant difference in the average of observed accumulation of bioactive compounds was detected between yellow and white flesh color nor between peaches and nectarines, except for total phenolics, which were higher in peaches (53.2 mg GAE/100 g FW) (Table 2. 2). This difference could be due to the lower representation of nectarine cultivars included in this study. Red flesh

Table 2. 2. Accumulation of phytochemical compounds in 114 peaches and 18 nectarines, different flesh colors and ripening groups.

	Total phenolics (mg GAE/100g) ^y	Flavonoids (mg CE/100g) ^y	Anthocyanin (mg C3GE/kg) ^y	Antioxidants (μg TE/g) ^y
Fruit type				
Peach	53.2 ± 34.7 b ^z	14.4 ± 12.8 a	5.6 ± 5.5 a	646 ± 418.3 a
Nectarine	45.3 ± 32.6 a	15.8 ± 13.2 a	6.5 ± 6.3 a	612.9 ± 330.7 a
Flesh color				
White	48.6 ± 32.2 a	16.3 ± 13.7 a	6.4 ± 5.6 a	672.5 ± 389 a
Yellow	53.2 ± 35.1 a	14.1 ± 12.6 a	5.6 ± 5.7 a	632.7 ± 412.5 a
Red ^x	73.4 ± 21.7 b	33.1 ± 3.2 b	29.9 ± 14.1 b	1254.7 ± 480 b
Ripening season				
Early	37.6 ± 19.7 a	10.8 ± 9.5 a	4.0 ± 3.3 a	454.7 ± 230.4 a
Mid	52.2 ± 33.6 b	12.4 ± 10.1 a	6.0 ± 6.2 b	578.2 ± 337.7 b
Late	65.8 ± 40.5 c	21.0 ± 15.9 b	7.2 ± 6.3 c	894.9 ± 483.6 c

^xData obtained for red flesh accession used only in flesh color comparisons.

^z Different letters indicate significant differences at $P < 0.05$ according to Student-Newman-Keuls's and t test.

^y Data indicates mean ± standard deviation for each character.

Abbreviations: GAE, Gallic acid equivalents; CE, Catechin equivalents; C3GE, Cyaniding-3-glucoside equivalents; TE, Trolox equivalents.

accession '99p4388' exhibited 2-5 fold difference in the antioxidant capacity and the accumulation of total phenolics, flavonoids, and anthocyanins when compared to white and yellow flesh cultivars (Table 2. 2). On the other hand, significant differences were observed between different ripening seasons, with increasing antioxidant capacity and phytochemical content as the season progressed (Table 2. 2; Figure 2. 1). Significantly higher accumulation of bioactive compounds in red-fleshed peaches and plums has been observed by Vizzotto et al. (2007). Tomás-Barberán et al. (2001) reported no clear differences in phenolic content of nectarines and peaches or between white and yellow flesh, which is in agreement with our results. In contrast,

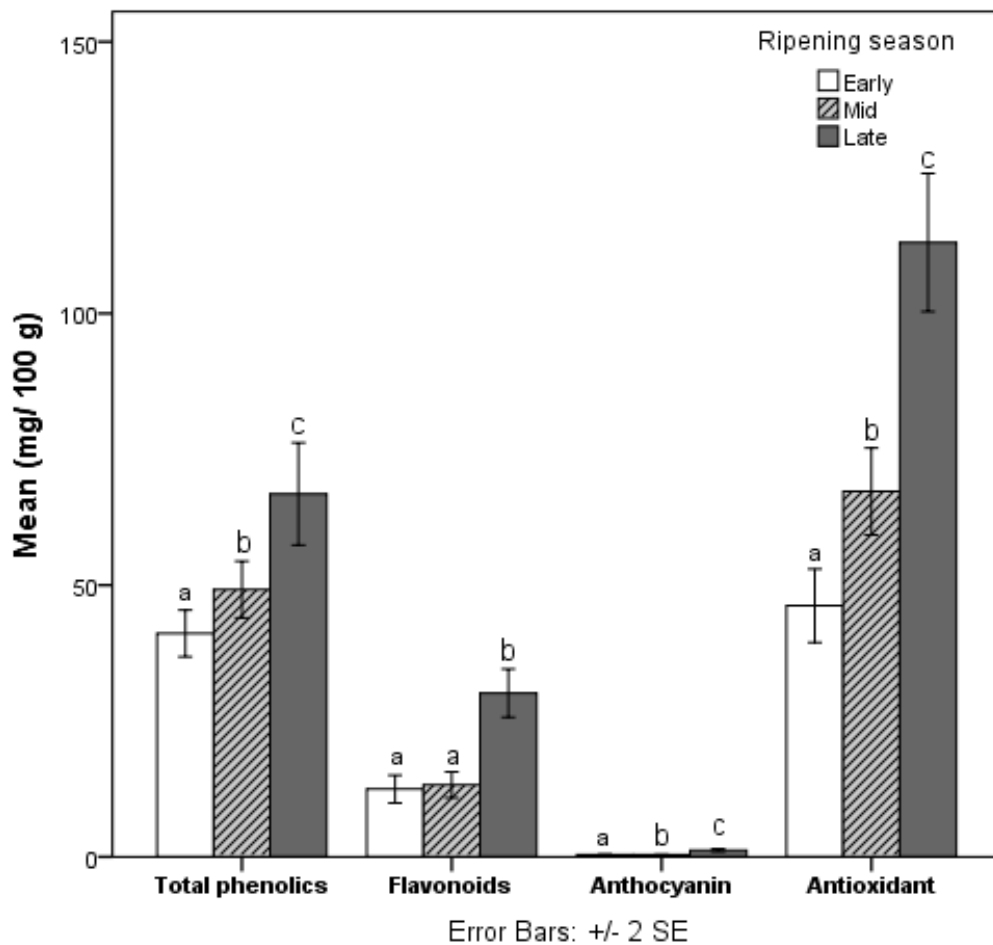


Figure 2. 1. Phytochemicals accumulation in different flesh color and ripening groups across two seasons.

Font i Forcada et al. (2014) study showed that peaches significantly accumulated more phytochemicals than nectarines. The highest accumulation of the bioactive compounds detected in the cultivars with later maturity could be due to increased amount of soluble solids concentration (SSC). It has been previously reported that maturity date correlated positively with SSC in peach (Eduardo et al., 2011). In addition, a significant and positive correlation between SSC and RAC, phenolics, and flavonoids has been observed in

several studies on peaches and nectarines (Abidi et al., 2011; Cantín et al., 2009b; Font i Forcada et al., 2014), supporting the essential role of sugars in the regulation of synthesis of phenolic compounds (DeJong, 1999; Font i Forcada et al., 2014).

Phenolic compounds

Accumulation of total phenolics, measured as mg of Gallic Acid Equivalent (GAE) per 100 g of FW, ranged from 22.9 to 110.7 mg GAE/100 g FW (Tables 2.3 and 2.4) in yellow-fleshed ‘Blazingstar’ and white-fleshed ‘Belle of Georgia’, respectively. The influence of flesh color on the accumulation of total phenolics has been observed. Among white-fleshed cultivars, the lowest accumulation of total phenolics (29.3) was observed in ‘Sugar May’, while the highest (110.7) was observed in ‘Belle of Georgia’ (Table 2. 3). Yellow-fleshed cultivars also exhibited differences in the accumulation of the total phenolics, with ‘Loring’ exhibiting the highest accumulation (98.3) (Table 2. 4). When different ripening seasons are considered, accumulation of total phenolics ranged from 37.6 to 65.8 (in early to late ripened genotypes, respectively) (Table 2. 2; Figure 2. 1). Early ripening cultivar Blazingstar exhibited the lowest phenolics (22.9), while mid ripening peaches, such as ‘Loring’ (98.3), ‘Beaumont’ (95.6), and Harrow Beauty’ (93.8) accumulated the most. Among all evaluated accessions, the majority of analyzed individuals accumulated < 70 mg GAE/100 g FW of total phenolics (Table 2. 3 and Table 2. 4).

Table 2. 3. Phytochemical accumulation in white flesh accessions in different ripening seasons.

Genotype	Total phenolics (mg GAE/100g) ^y	Flavonoids (mg CE/100g) ^y	Anthocyanin (mg C3GE/kg) ^y	Antioxidants (μg TE/g) ^y
Early season				
Arctic Glo	32.5 ± 15.7 a ^z	23.4 ± 21.7 d-g	13.7 ± 5.5 e	452.4 ± 251.3 b-f
Arctic Star	37.8 ± 25 ab	5.5 ± 0.8 ab	1.9 ± 0.9 a	267.2 ± 247.6 a-c
Arctic Sweet	38.4 ± 23.3 ab	11.8 ± 7.7 a-e	6.2 ± 4.3 a-d	431.3 ± 155.4 b-f
D88-147	45.1 ± 6.2 a-c	10.2 ± 2 a-d	8.1 ± 5 a-e	595 ± 121 d-i
Early Red	48.8 ± 32.5 a-c	23.5 ± 13.7 d-g	4.9 ± 4.4 a-c	843.7 ± 241.6 h-l
Galaxy	52.3 ± 27.2 a-c	15.6 ± 11.2 a-f	2.7 ± 1.1 a-c	569.4 ± 127.9 d-h
Jade	32 ± 4.4 a	31.3 ± 9.9 g	6.4 ± 1.7 a-d	775.4 ± 39.6 g-k
Karla Rose	36.8 ± 6.8 ab	5.8 ± 1.6 ab	3.7 ± 1.3 a-c	339.9 ± 104.9 a-e
Manon	30.3 ± 16.3 a	12.4 ± 4.4 a-e	3.3 ± 0.8 a-c	603.3 ± 28.9 e-i
Snowbrite	30.3 ± 4.3 a	4.4 ± 2.6 a-d	3.7 ± 1.2 a-c	203.7 ± 128.1 ab
Spring Snow	30.9 ± 9.6 a	20.1 ± 5 b-g	7.3 ± 3.9 a-e	698.3 ± 10.9 f-j
Sugar May	29.3 ± 3.2 a	7.1 ± 3.4 a-c	4.4 ± 1.7 a-c	435.4 ± 37.6 b-f
Mid-season				
Arctic Belle	42.1 ± 15.1 a-c	11.4 ± 4.3 a-e	3.8 ± 1.5 a-c	627.6 ± 185 f-i
Arctic Jay	52 ± 13.5 a-c	14.4 ± 1.8 a-e	5.9 ± 2.6 a-d	757.5 ± 223.3 g-k
Carolina	69.9 ± 24 a-c	16.3 ± 7.3 a-f	8.2 ± 7.7 a-e	828.7 ± 88.3 h-k
Klondike	32.4 ± 19.1 a	14.3 ± 1.8 a-e	3.7 ± 1.1 a-c	691.9 ± 26.6 f-j
Raritan Rose	34 ± 11.3 ab	14.1 ± 8.3 a-e	3.6 ± 2 a-c	655.6 ± 243.8 f-j
Redrose	67.1 ± 46.5 a-c	16 ± 12.7 a-f	12.1 ± 9.4 de	319.3 ± 119.6 a-d
White County	55.4 ± 35.7 a-d	26.3 ± 2.8 i	8.2 ± 7 a-c	1066.3 ± 492.2 i
White Lady	41.7 ± 9.1 a-c	4.9 ± 2 ab	3.7 ± 0.8 a-c	516.7 ± 19.9 c-g
Zin Dai	45.2 ± 31 a-c	10.7 ± 11.1 a-e	6.7 ± 1.3 a-d	318.8 ± 315.7 a-d
Late season				
Arctic Gold	82.5 ± 71.5 c	32.5 ± 22.8 g	9.1 ± 7.8 a-e	919.1 ± 351.2 j-l
Belle of	110.7 ± 36.5 d	50 ± 7 h	13.4 ± 6.3 e	1679.3 ± 50.1 n
China Pearl	77.7 ± 51.4 bc	22.4 ± 9 c-g	5.8 ± 2.6 a-d	1013.4 ± 158.2 kl
Glacier	45.6 ± 16.2 a-c	2.1 ± 0.9 a	2.2 ± 0.7 a	136.9 ± 78.7 a
Lady Nancy	67.2 ± 49.5 a-c	25.9 ± 15.2 e-g	9.9 ± 8.2 b-e	1088.7 ± 440.3 l
September	52.6 ± 18.4 a-c	13.3 ± 2.1 a-e	5.4 ± 1.9 a-d	863 ± 222.9 i-l
Snow Gem	31.5 ± 14.1 a	7.6 ± 4.2 a-c	2.5 ± 0.5 ab	611 ± 155.7 e-I

Table 2. 3 continued

Genotype	Total phenolics (mg GAE/100g) ^y	Flavonoids (mg CE/100g) ^y	Anthocyanin (mg C3GE/kg) ^y	Antioxidants (μg TE/g) ^y
Snow Giant	71 ± 28.8 a-c	28.9 ± 10.8 fg	13.5 ± 9.4 e	1470.6 ± 252.7 m
Snow King	50.2 ± 31.7 a-c	7.6 ± 3.4 a-c	10.2 ± 9.3 c-e	464 ± 34 b-f
Zephyr	39.4 ± 5.8 ab	21.6 ± 7.6 c-g	6.3 ± 0.8 a-d	999.2 ± 325.1 kl

^zDifferent letters indicate significant differences at $P < 0.05$ according to Student-Newman-Keuls's test for differences between cultivars.

^y Means ± standard deviation of five replicates for 2 or 3 years (each replicate came from 5 fruit). Abbreviations: GAE, Gallic acid equivalents; CE, Catechin equivalents; C3GE, Cyaniding-3-glucoside equivalents; TE, Trolox equivalents.

Flavonoid accumulation, expressed as milligrams of Catechin Equivalents (CE) per 100g of FW, also showed variability among different accessions and different flesh colors. Cultivars with white flesh showed large variation in the accumulation of flavonoids ranging from 2.1 in Glacier to 50 in Belle of Georgia (Table 2. 3). Yellow flesh cultivars also showed significant differences in the accumulation of flavonoids with highest accumulation detected in Jerseyqueen (56) and lowest (2.5) in Country Sweet (Table 2. 4). Even though, Cantín et al. (2009b) evaluated phytochemical compounds in 218 genotypes derived from 15 controlled bi-parental crosses with different genetic origins and with a large phenotypic variability, the average of flavonoid accumulations observed in our material were higher (8.8 vs. 53 mg CE/ 100 g of FW). In addition, flavonoid accumulations observed in our study were higher than those previously reported in the F1 population ‘Venus’ × ‘Big Top’ nectarines (one of crosses included in Cantín et al., 2009b study) (Abidi et al., 2011) (12.5 mg CE/ 100 g of FW). This result could be due to differences in analyzed materials. Our material includes a diverse collection with different fruit characterizations and origins (Table 2.1), while Abidi et al. (2011) included only F1 yellow nectarine progeny from Spanish material. In addition, accumulation of flavonoids was influenced by ripening season. In the early ripening season, flavonoid accumulation was low (10.8) compared to mid and late ripening cultivars (Table 2. 2; Figure 2. 1). Early ripening ‘Country Sweet’ exhibited low accumulation of flavonoids (2.5), whereas late ripening ‘Jerseyqueen’ had the highest (55.9).

Table 2. 4. Phytochemical accumulation in yellow flesh accessions in different ripening seasons.

Genotype	Total phenolics (mg GAE/100g) ^y	Flavonoids (mg CE/100g) ^y	Anthocyanin (mg C3GE/kg) ^y	Antioxidants (µg TE/g) ^y
Early season				
7 Ball	39.3 ± 17.9 a-c ^z	10.4 ± 5.1 a-g	7.3 ± 2.3 f	509 ± 72.5 e-i
Blazingstar	22.9 ± 14.5 a	3.6 ± 2.1 ab	1.9 ± 0.6 a-c	245 ± 69 a-c
Candor	32.1 ± 6.9 a-c	6.9 ± 1.7 a-d	1.6 ± 0.8 a	371.4 ± 55.4 b-g
Caroking	48.8 ± 21.4 a-d	8 ± 1.5 a-e	2.1 ± 0.8 a-c	328.3 ± 127.7 b-f
Carored	42.5 ± 14.5 a-c	4 ± 1.4 a-c	2.9 ± 1 a-c	312.8 ± 40.1 b-e
Country Sweet	29.6 ± 15.5 ab	2.5 ± 1.1 a	3.1 ± 1.4 a-c	156.4 ± 27.2 ab
Crimson Lady	37.2 ± 22.9 a-c	3.8 ± 2 ab	1.6 ± 1.3 a	93.3 ± 84.5 a
Desiree	28 ± 5.5 ab	4.9 ± 2.7 a-c	2.1 ± 0.9 a-c	267.3 ± 36.1 a-d
Earlystar	44 ± 9.9 a-c	15.6 ± 5.6 e-h	2.2 ± 0.8 a-c	601.4 ± 19.8 h-j
Easternglo	42 ± 36.4 a-c	4.2 ± 1.8 a-c	6.6 ± 1.7 d-f	308.5 ± 36.9 b-e
GaLa	58.7 ± 26.1 cd	11.9 ± 7.2 b-g	3.3 ± 1.2 a-c	677 ± 236.9 i-k
Garent Beauty	31.5 ± 10.2 a-c	10.2 ± 4.1 a-g	2.2 ± 1 a-c	487.5 ± 16.2 d-i
Glenglo	44.8 ± 19.3 a-d	17.5 ± 6.1 gh	4.8 ± 1.9 b-e	793.9 ± 101.8 k
Honey Blaze	34.5 ± 9.4 a-c	16.1 ± 9.4 f-h	2.7 ± 1.1 a-c	589.5 ± 135.2 h-j
PF 1	33.2 ± 17.9 a-c	9.5 ± 5.3 a-f	2.2 ± 1.2 a-c	411.1 ± 40.8 c-h
PF 11 Peach	38 ± 19.1 a-c	9 ± 2.2 a-f	2.7 ± 0.5 a-c	566.5 ± 173.9 g-j
PF 5D Big	24.2 ± 11.8 a	3.6 ± 1.7 ab	1.7 ± 0.4 ab	266.9 ± 50.3 a-d
PF 7A	27.9 ± 6 ab	6.3 ± 2.5 a-d	4.3 ± 2.8 a-d	331.2 ± 86.6 b-f
PF 8 Ball	27.4 ± 16.9 ab	10.4 ± 10.1 a-g	6.9 ± 2.9 ef	340 ± 273.3 b-f
Redhaven	67.9 ± 36.7 d	20.9 ± 17.5 h	3.3 ± 2.1 a-c	561.6 ± 197.1 g-j
Rich May	28.4 ± 13.8 ab	14.4 ± 1.9 d-h	4.6 ± 3 a-e	548.6 ± 211.3 f-j
Risingstar	41.2 ± 8.7 a-c	3.7 ± 2.2 ab	5 ± 6 c-e	342.5 ± 72.9 b-f
Sentry	35.4 ± 7.9 a-c	17.6 ± 3.3 gh	3.5 ± 1 a-c	731.8 ± 188.9 j-k
Springold	41.3 ± 6.7 a-c	12.4 ± 7.1 c-g	4.4 ± 2.6 a-d	620.7 ± 464.7 h-k
Starfire	52.1 ± 16.9 a-d	8.3 ± 1.5 a-f	3.4 ± 2 a-c	307.8 ± 211.8 b-e
Sweet Scarlet	28 ± 12.5 ab	7.3 ± 2.4 a-d	2.3 ± 0.4 a-c	475 ± 26.6 c-i
Vulcan	54.1 ± 29.7 b-d	5.9 ± 1.1 a-c	1.6 ± 0.4 a	361.4 ± 21.8 b-g
Westbrook	23.6 ± 13.1 a	6.5 ± 4.6 a-d	2.2 ± 1 a-c	368.4 ± 59.6 b-g
Mid-season				
10 Ball	45.5 ± 17.3 a-d	4.8 ± 1.2 a-c	6.3 ± 4.4 a-c	341.5 ± 86.6 ab
11 Ball	23.3 ± 9.7 a	3.7 ± 2.3 ab	2.1 ± 0.6 ab	281.6 ± 33.1 ab

Table 2. 4 continued

Genotype	Total phenolics (mg GAE/100g) ^y	Flavonoids (mg CE/100g) ^y	Anthocyanin (mg C3GE/kg) ^y	Antioxidants (µg TE/g) ^y
9 Ball	33.6 ± 19.6 ab	2.7 ± 1.8 a	2.3 ± 1 ab	315.8 ± 87.7 ab
Allstar	36.1 ± 19.9 ab	7.1 ± 1.8 a-e	3.4 ± 1.2 ab	492.7 ± 58.4 a-e
Beaumont	95.6 ± 77.6 d	9.6 ± 5.1 a-h	8.7 ± 7.9 a-c	341.2 ± 125.3 ab
Beekman	77.1 ± 42.5 a-d	22.7 ± 6.3 hi	10.5 ± 7.4 a-c	797.3 ± 96.3 e-i
Bounty	47.5 ± 12 a-d	14.5 ± 3.5 a-i	1.9 ± 0.9 ab	706.7 ± 58.2 c-g
Burpeachfive	49 ± 9.3 a-d	6.3 ± 2.1 a-e	6.3 ± 1.4 a-c	280.5 ± 93.2 ab
Canadian Harmony	41.8 ± 14 a-c	7.9 ± 3.2 a-f	7.4 ± 3.6 a-c	388.7 ± 29.4 a-c
Contender	49.4 ± 23.1 a-d	11.8 ± 8.3 a-h	3.3 ± 1.9 ab	636.1 ± 376.1 b-g
Coralstar	40.2 ± 23.6 ab	17.3 ± 12.9 b-i	4.5 ± 1.7 ab	489.4 ± 111.9 a-e
Coronet	55.4 ± 18 a-d	11.5 ± 5.5 a-h	3.4 ± 1.1 ab	622.4 ± 273.4 b-g
Crimson Rocket	38.5 ± 24.6 ab	12.1 ± 7 a-h	2.3 ± 1 ab	548.4 ± 107.7 a-f
Diamond Princess	56.2 ± 21.4 a-d	4.4 ± 1.4 a-c	6.1 ± 4 a-c	279.4 ± 28.6 ab
Early Loring	63.2 ± 35 a-d	10.2 ± 8.6 a-h	8.2 ± 8 a-c	559.1 ± 163.1 a-f
Ernies Choice	67.3 ± 51.2 a-d	19.1 ± 13.9 e-i	19 ± 15.9 e	836.3 ± 304.8 f-i
Flavortop	37.4 ± 23.6 ab	21.8 ± 14.5 g-i	5.7 ± 1.2 a-c	848.6 ± 446.3 f-i
Flavrburst	34.4 ± 16.8 ab	5.3 ± 1.1 a-d	4.4 ± 0.8 ab	521.1 ± 58.7 a-f
Glohaven	52.9 ± 29.5 a-d	17.6 ± 8.6 c-i	3.7 ± 1.8 ab	807.9 ± 273.4 e-i
Harrow Beauty	93.8 ± 60.2 cd	6.3 ± 4.3 a-e	9.8 ± 8.7 a-c	211.5 ± 87.5 a
Intrepid	68.5 ± 32.8 a-d	21.8 ± 3.5 g-i	4.9 ± 1.6 a-c	1111 ± 115.6 i
John Boy II	45.7 ± 11.7 a-d	6.1 ± 1.3 a-e	5.9 ± 2.7 a-c	303.6 ± 88.8 ab
Julyprince	60.4 ± 20.7 a-d	6.4 ± 1.3 a-e	4.9 ± 3 a-c	515.7 ± 268.4 a-f
Late 24-007	38.3 ± 27.5 ab	10 ± 9.9 a-h	5.8 ± 1.4 a-c	341.2 ± 238.5 ab
Late Large 23	49.7 ± 7.4 a-d	6.5 ± 2.9 a-e	2.1 ± 1 ab	299.5 ± 51.7 ab
Loring	98.3 ± 74.2 d	18.5 ± 11.1 d-i	6.3 ± 3.7 a-c	894.8 ± 451.9 g-i
Majestic	29.2 ± 8.2 a	5.2 ± 3.7 a-d	5.2 ± 6 a-c	419.7 ± 101.7 a-d
PF 15A	46.2 ± 20.8 a-d	5.6 ± 2.1 a-e	6.5 ± 1.9 a-c	246.7 ± 86.1 a
PF 17	62.1 ± 47.7 a-d	12.9 ± 7.2 a-h	8.6 ± 7.9 a-c	462.2 ± 85.4 a-d
PF 24-007	62.1 ± 32.7 a-d	17.5 ± 12.3 c-i	11.1 ± 9.2 bc	745.9 ± 292.4 d-h
PF Lucky 24B	68.9 ± 29.2 a-d	11.1 ± 8 a-h	3.5 ± 2 ab	335.2 ± 45.5 ab
Redglobe	42.7 ± 30.7 a-c	20.6 ± 6.8 f-i	5.5 ± 2.1 a-c	1039.4 ± 31.1 hi
Redstar	33.3 ± 9.7 ab	5.8 ± 2.8 a-e	2 ± 0.3 ab	369.7 ± 110.6 a-c
Reliance	54.1 ± 15.4 a-d	10 ± 5.8 a-h	1.7 ± 0.8 a	293 ± 16.3 ab
Summerbeaut	46 ± 11.5 a-d	10 ± 4.6 a-h	5.4 ± 1.5 a-c	396.4 ± 72 a-c
Summergold	48.3 ± 23.1 a-d	21.1 ± 5.5 g-i	13.3 ± 5.6 cd	923.6 ± 336.6 g-i
Suncrest	87.2 ± 52.6 b-d	33.8 ± 17.7 j	17.3 ± 15.8 de	1374.5 ± 255.2 j
Sunhigh	41.1 ± 23.4 a-c	7.2 ± 4 a-e	2.6 ± 1.4 ab	420.2 ± 94.5 a-d

Table 2. 4 continued

Genotype	Total phenolics (mg GAE/100g) ^y	Flavonoids (mg CE/100g) ^y	Anthocyanin (mg C3GE/kg) ^y	Antioxidants (μ g TE/g) ^y
Late season				
Sweet Breeze	55.4 \pm 31.8 a-d	11.8 \pm 6.6 a-h	6.8 \pm 7.1 a-c	546.8 \pm 173.4 a-f
Sweet Dream	36.4 \pm 9.8 ab	9.1 \pm 1.2 a-g	2.9 \pm 0.3 ab	736.1 \pm 81.4 d-h
Winblo	58.5 \pm 16.3 a-d	21.6 \pm 14.1 g-i	4.1 \pm 3.1 ab	1046.2 \pm 616 hi
Ambre	59.5 \pm 44.7	28.3 \pm 12.7 b-d	5.4 \pm 3.2 ab	1060.2 \pm 387.1 e-g
Augustprince	84.1 \pm 54.5	19.3 \pm 10.9 a-c	13.8 \pm 11.6 cd	437.1 \pm 340.8 ab
Autumn Flame	50.6 \pm 29.6	13.2 \pm 7.4 ab	5 \pm 1 ab	594.5 \pm 175.4 a-d
Autumn Red	56.3 \pm 12.1	44.6 \pm 6.5 ef	4.8 \pm 0.5 ab	1255.2 \pm 50.2 gh
Autumnstar	81.8 \pm 51.6	30.9 \pm 17.8 cd	6.3 \pm 3.7 a-c	1216.6 \pm 314.1 f-h
Blake	39.2 \pm 19	19.6 \pm 12.5 a-c	4.9 \pm 2.4 ab	875.8 \pm 342.6 c-f
Blushingstar	52.4 \pm 32.2	12.8 \pm 7.9 ab	11.9 \pm 11 b-d	586.2 \pm 150.7 a-d
Burpeachfour	79.5 \pm 42.2	17.2 \pm 10.6 a-c	7.8 \pm 1.4 a-c	982 \pm 287.9 d-g
Carolina Gold	78.4 \pm 18.4	17.5 \pm 4.1 a-c	10.4 \pm 8.4 a-c	944.6 \pm 338.2 d-g
CaroTiger	67.9 \pm 11.9	22 \pm 9 a-c	9.4 \pm 2.3 a-c	1379.5 \pm 80.6 h
Cresthaven	35.1 \pm 7	20.9 \pm 15.5 a-c	7.9 \pm 5.2 a-c	816.7 \pm 372.7 b-e
Elberta	68.1 \pm 25.9	51.9 \pm 14.6 fg	13.5 \pm 7.9 cd	1868.9 \pm 93.2 i
Encore	89.9 \pm 54.8	40.3 \pm 14.3 de	8.7 \pm 6.2 a-c	1427.4 \pm 188.7 h
Fantasia	50.3 \pm 19.7	13.1 \pm 3.1 ab	5.4 \pm 3.2 ab	640 \pm 30.2 a-e
Flameprince	51.4 \pm 24.1	12.3 \pm 3.6 ab	4.1 \pm 2.3 ab	714.7 \pm 74.9 a-e
Gloria	72.7 \pm 55.1	12.5 \pm 9.4 ab	2.7 \pm 1.5 a	655.3 \pm 265.4 a-e
Glowingstar	47.1 \pm 6.2	7.4 \pm 1.2 a	2.7 \pm 1.8 a	314.6 \pm 97.2 a
Jerseyqueen	82.8 \pm 33.1	56 \pm 21.7 g	9.5 \pm 3.8 a-c	2115.3 \pm 352.6 j
Lauroi	87.2 \pm 42.7	31.5 \pm 14.5 cd	9.6 \pm 3.8 a-c	1401.8 \pm 280.9 h
Madison	61.8 \pm 21	15.3 \pm 6 a-c	3 \pm 0.9 a	673 \pm 78.2 a-e
Messina	52.2 \pm 18.3	9.5 \pm 3.3 a	2.6 \pm 1.1 a	466.3 \pm 53.8 a-c
PF 2050	87.7 \pm 55.6	12 \pm 6.2 ab	4.1 \pm 2 ab	674.6 \pm 205.2 a-e
PF 25	53.7 \pm 33.5	20.2 \pm 16.4 a-c	4.2 \pm 2 ab	954.9 \pm 535.5 d-g
PF 27A	63.2 \pm 35.2	18.3 \pm 9.4 a-c	5.1 \pm 3 ab	777.9 \pm 264.4 b-e
PF 28-007	65.6 \pm 32.9	13.8 \pm 10.3 ab	4.6 \pm 2.1 ab	679.8 \pm 394.6 a-e
PF 30-007	75.8 \pm 69	11.1 \pm 5.1 ab	7.7 \pm 6.5 a-c	402.6 \pm 99 ab
Redskin	73.9 \pm 19.7	19.1 \pm 6.1 a-c	8.8 \pm 8.1 a-c	866.1 \pm 74.1 c-f
Summer Fire	60.5 \pm 52.6	7.8 \pm 6.3 a	8.6 \pm 6.8 a-c	415.6 \pm 141.9 ab
Summerfest	83.6 \pm 71.1	17.1 \pm 13.1 a-c	4.6 \pm 3.8 ab	781.2 \pm 408.5 b-e

Table 2. 4 continued

Genotype	Total phenolics (mg GAE/100g) ^y	Flavonoids (mg CE/100g) ^y	Anthocyanin (mg C3GE/kg) ^y	Antioxidants (μg TE/g) ^y
Sweet N Up	67.6 ± 58.2	19.4 ± 7.7 a-c	3.4 ± 1.3 ab	804.4 ± 236.3 b-e
Tra-Zee	73.2 ± 24.2	16.7 ± 10.4 a-c	17.1 ± 10.6 d	576.4 ± 529.8 a-d
Victoria	84.1 ± 36.9	17.1 ± 5.6 a-c	5.4 ± 3 ab	982.3 ± 47.4 d-g

^z Different letters indicate significant differences at $P < 0.05$ according to Student-Newman-Keuls's test for differences between cultivars.

^y Means ± standard deviation of five replicates for 2 or 3 years (each replicate came from 5 fruit). Abbreviations: GAE, Gallic acid equivalents; CE, Catechin equivalents; C3GE, Cyaniding-3-glucoside equivalents; TE, Trolox equivalents.

Anthocyanin accumulation, expressed as milligrams of cyanidin-3-glucoside per kilogram of FW (mgC3GE/kg), was higher in red-fleshed advanced selection 99p4388 (29.8) than in yellow and white cultivars (1.6 – 19). This result was in agreement with the report of Vizzotto et al. (2007), who reported that red-fleshed peaches accumulated more anthocyanins than those with light-colored flesh. Cyanidin is the main pigment responsible for red coloration in peach and nectarine, with cyanidin 3- glucoside as the main anthocyanin reported in peach (Tomás-Barberán et al., 2001; Wu and Prior, 2005). Anthocyanin accumulation exhibited a similar range (1.6 to 17-19 mg C3GE/kg FW) regardless of flesh color, excluding red flesh, and ripening season. Among white-fleshed cultivars, the highest (13.7) and lowest (1.9) accumulation of cyanidin-3-glucoside was observed in ‘Arctic Glo’ and ‘Arctic Star’, respectively (Table 2. 3). Yellow-fleshed cultivars also exhibited huge variability in the accumulation of anthocyanins with mean values ranging from 1.6 mg C3GE/kg FW observed in early ripening ‘Candor’, ‘Crimson Lady’ and ‘Vulcan’ all the way to 17 in ‘Tra-Zee’ and ‘Suncrest’ and 19 mg C3GE/kg FW in ‘Ernies Choice’ (Table 2. 4). Comparing the three different ripening seasons, anthocyanin accumulation was the lowest in early ripening (4.0) and the highest (7.2) in late ripening season cultivars (Table 2. 2; Figure 2. 1).

Antioxidant capacity

The antioxidant capacity, measured as micrograms of Trolox Equivalent (TE) per gram of fresh weight (FW), ranged from 93.3 – 2115.3 in yellow fleshed ‘Crimson Lady’ and ‘Jerseyqueen’, respectively (Table 2. 4; Figure 2. 2).

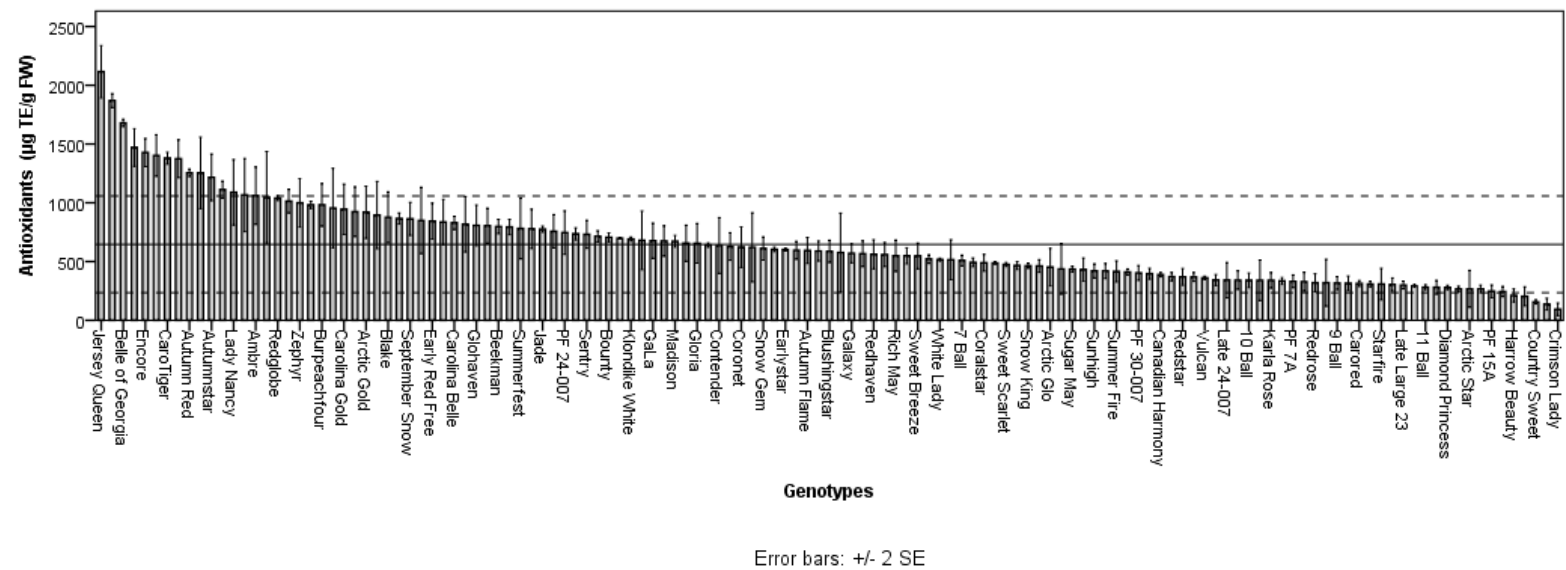


Figure 2. 2. Mean of antioxidant capacity in 133 individuals. Solid line represents overall mean, while dotted lines represent standard deviations.

Heirloom cultivars, such as Elberta and Belle of Georgia, represent the ancestors of modern peach germplasm and are some of the cultivars with significant capacity of antioxidants (1868.9 and 1679.3, respectively). The antioxidant capacity, in the two years of study, exceeded values reported in other studies evaluating Spanish material (Abidi et al., 2011; Cantín et al., 2009b; Reig, et al., 2013) (464.2, 405.0, and 338.3, respectively). This could be due to the differences in the evaluated material as well as to the environmental conditions during the study. Antioxidant capacity in peach germplasm with white flesh color ranged from 136.9 in ‘Glacier’ to 1679.3 $\mu\text{g Trolox/g}$ FW in ‘Belle of Georgia’ (Table 2. 3). In general, our results did not reveal significant differences in the average of antioxidant capacity between white and yellow peaches (Table 2.2), which is in agreement with Tomás-Barberán et al. (2001). Observed antioxidant capacity was significantly different between different ripening seasons, with the lowest (454.7) observed in the early ripening individuals and the highest (894.9) observed in late ripening ones (Table 2. 2; Figure 2. 1). Early ripening peaches, such as ‘Crimson Lady’, ‘Glacier’, and ‘Country Sweet’ exhibited the lowest capacity of antioxidants (93.3, 137 and 156.4, respectively), while late ripening cultivars Jerseyqueen (2115.3), Elberta (1869), and Belle of Georgia (1679.3) had the highest (Table 2. 3; Table 2. 4). The highest antioxidant capacity detected in the cultivars that ripen later could be due to increased amount of soluble solids concentration (SSC) (data not shown). It has been previously reported that maturity date correlated positively with SSC in peach (Eduardo et al., 2011). Further, it has been found that sugar positively correlated with accumulation of phytochemical compounds in peach (Font i Forcada et

al., 2014; Abidi et al., 2011; Cantín et al., 2009 a, b), revealing the essential role of sugars in the biosynthesis of bioactive compounds. The significant accumulation of non-enzymatic antioxidants (total, phenolics, flavonoids and their subclass anthocyanins) (Ahmad et al., 2010; Blokhina et al., 2003; Gill and Tuteja, 2010) in peach and nectarine revealed/ confirmed the importance of these fruits for human health. In addition, high antioxidant capacity observed in heirloom cultivars indicates that breeders should consider using early heirloom cultivars when breeding for improving fruit quality traits in newly released cultivars.

Correlations between different phenolic compounds in two years, measured by Pearson's correlation coefficient, were significant and positive between antioxidant capacity (AC) and the accumulation of bioactive compounds, as well as within specific bioactive compounds in different flesh colors (Table 2. 5). Among all fruit quality parameters, SSC (Brix %) showed the highest positive correlation with all phytochemical compounds (Table 2. 6). The positive correlation between SSC and phytochemical compounds in peach has been reported before by other authors (Font i Forcada et al., 2014; Abidi et al., 2011; Cantín et al., 2009 a, b). A positive correlation was also observed between flavonoids and total phenolics, with the highest correlation observed in the white fleshed tissues ($r = 0.686$, $P < 0.01$). The positive correlation of AC with total phenolics has been previously reported in peach and nectarine (Abidi et al., 2011; Cantín et al., 2009b) (0.738 and 0.606, $P < 0.01$, respectively), with accumulation of total phenolics being one of the most important compounds in stone fruits that correlated with antioxidant capacity. The highest correlation in this study was

Table 2. 5. Pearson's correlation coefficients between antioxidant capacity (AC) and different phytochemical compounds in different flesh colors.

Flesh color	Total phenolics (mg GAE/100g FW)	Flavonoids (mg CE/100g FW)	Anthocyanin (mg C3GE/kg FW)
White	0.534 ^{** z}	0.764 ^{**}	0.410 ^{**}
Yellow	0.415 ^{**}	0.844 ^{**}	0.348 ^{**}

^z ^{**} = Significant correlation at $P < 0.01$.

^y Abbreviations: GAE, Gallic acid equivalents; CE, Catechin equivalents; C3GE, Cyaniding-3-glucoside equivalents.

Table 2. 6. Pearson's correlation coefficients between phytochemicals and fruit quality traits observed over 2 years in the *P. persica* accessions.

Trait	FS ^x	FW ^x	FF ^x	SSC ^x	TA ^x	Ri ^x
antioxidant capacity (μ g TE/g FW) ^y	0.100 ^{*z}	0.121 ^{**}	ns	0.326 ^{**}	ns	Ns
Total phenolics (mg GAE/100g FW)	-0.0125 ^{**}	-0.342 ^{**}	0.358 ^{**}	0.384 ^{**}	ns	Ns
Flavonoids (mg CE/100g FW)	ns	-0.110 [*]	0.232 ^{**}	0.376 ^{**}	ns	Ns
Anthocyanin (mg C3GE/kg FW)	-0.192 ^{**}	-0.272 ^{**}	0.132 ^{**}	0.303 ^{**}	0.254 [*]	Ns

^z ^{*} = Significant correlation at $P < 0.05$; ^{**} = Significant correlation at $P < 0.01$; ns = not significant

^y Phytochemical compounds abbreviations: TE, Trolox equivalents; GAE, Gallic acid equivalents; CE, Catechin equivalents; C3GE, Cyaniding-3-glucoside equivalents.

^x Fruit quality abbreviations: FS, Fruit size; FW, Fruit weight; FF, Fruit firmness; SSC, Soluble solids concentration; TA, Titratable acidity; RI, Ripening index.

observed between AC and flavonoid contents in yellow-fleshed genotypes ($r = 0.844$).

However, the lowest correlation was observed between AC and anthocyanins in both white and yellow flesh individuals, which could be attributed to low accumulation of anthocyanin observed in peaches and nectarines in this study. Furthermore, AC exhibited a linear positive relationship with both flavonoids and total phenolics

regardless of the flesh color. The highest linear relationship ($r = 0.822$) was observed between antioxidant capacity and flavonoids of different genotypes (Figure 2. 3), supporting the fact that these phenolic compounds play an important role as non-enzymatic antioxidants.

Principal components analysis (PCA) of 133 individuals showed that 52 % of the observed variance could be explained by the first two components (PC1 and 2). PC1 includes phytochemical compounds and some fruit quality parameters, such as SSC, fruit firmness (FF), and index of absorbance difference (I_{AD}) (DA), while PC2 represents fruit size (FS), fruit weight (FW) and ripening index (RI) (Figure 2. 4). The PC1 and PC2 axes explained 31.5 and 20.4 % of total variability, respectively. Results showed a close relationship between bioactive compounds and SSC as they strongly correlated with each other, representing the essential role of sugars in regulation of phenolic compounds synthesis (Font i Forcada et al., 2014; DeJong, 1999). In addition, both FF and I_{AD} , the index for fruit maturity, separated from other variables and grouped together as they significantly and positively associate with each other (Figure 2. 4). High correlation between I_{AD} and flesh firmness has been previously observed (Infante 2012), indicating more mature fruit being less firm. However, I_{AD} is highly genotype dependent (Ziosi et al., 2008; Gasic et al., 2014) so while in melting, freestone peach and nectarine cultivars this observation is true, it does not hold true in slow-softening type cultivars, such as Sweet Dream. Flesh firmness in slow-softening cultivars at the time of harvest is high although I_{AD} value is close or equal to 0.

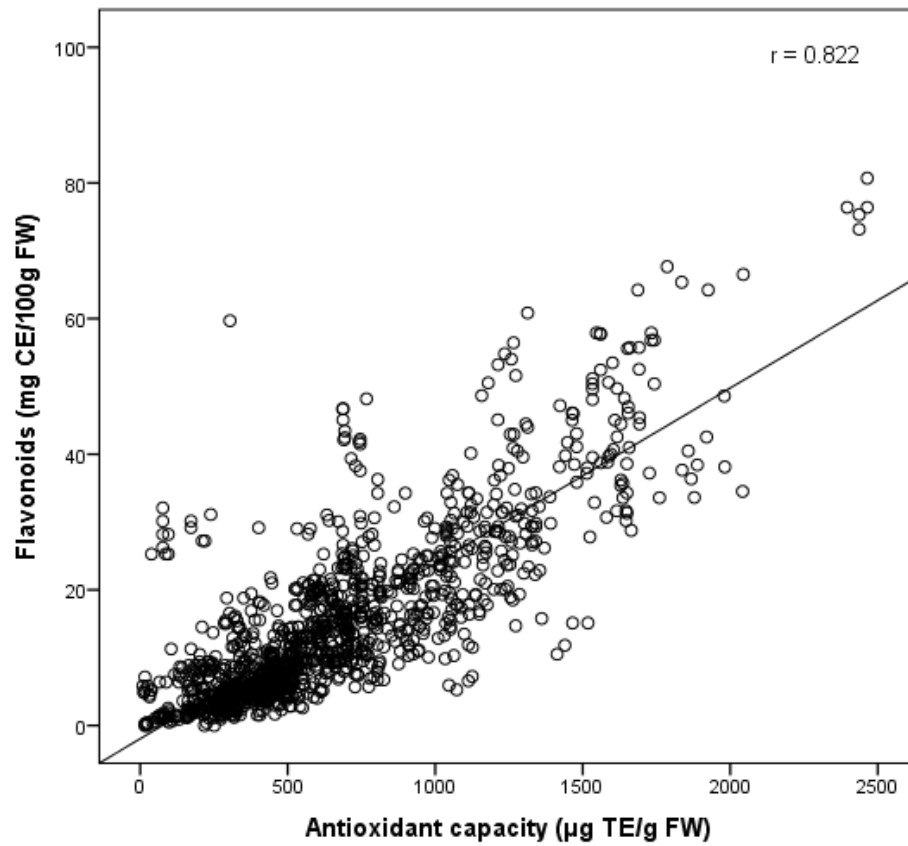


Figure 2. 3. Linear regression ($P < 0.01$) between antioxidant capacity and flavonoid accumulation in the *P. persica* accessions.

The distribution of traits in PC 2 is obviously due to a strong correlation between them, such as high positive correlation between FW and FS, as observed in previous studies in peach (Yamaguchi et al., 2002) and other fruits (Bohner and Bangerth, 1988; Ho, 1996), revealing that final fruit size and weight are determined by cell division and expansion beside carbohydrate dilution within cells.

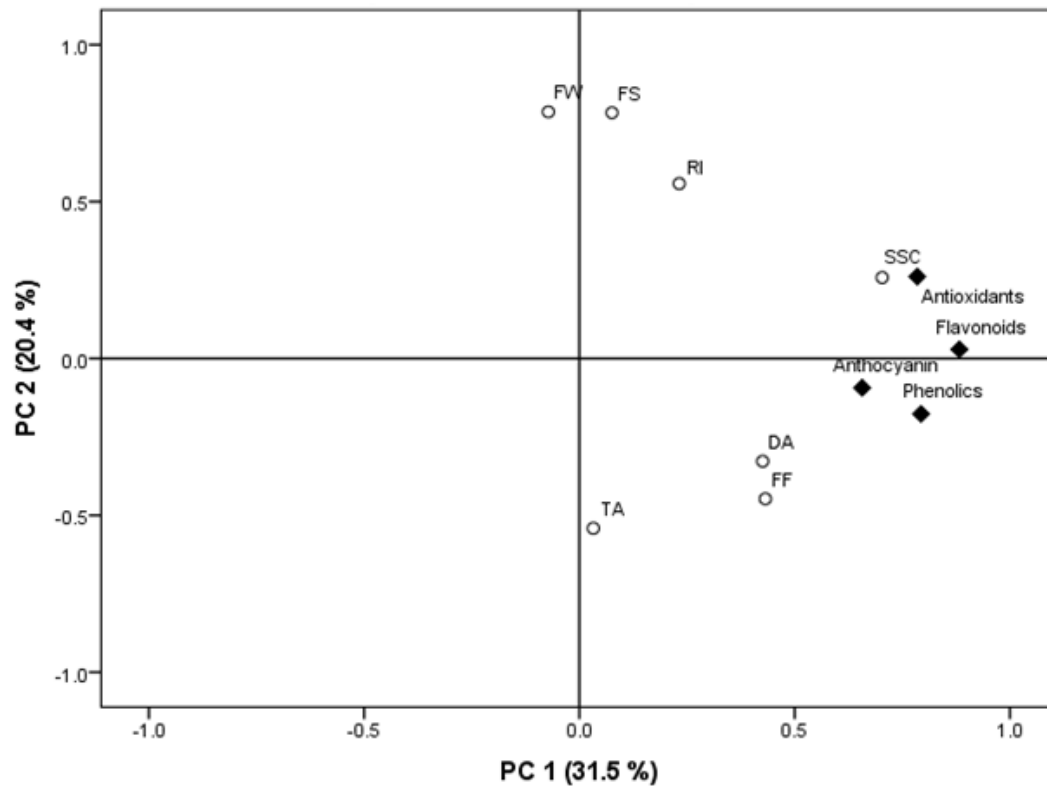


Figure 2. 4. Principal component analysis of the phytochemical compounds (black diamonds) and fruit quality (white circles) evaluated on 133 peach and nectarine genotypes. Fruit quality abbreviations: FS, Fruit size; FW, Fruit weight; FF, Fruit firmness; SSC, Soluble solids concentration; TA, Titratable acidity; SSC/TA, Ripening index (RI); DA, Index of Absorbance Difference (I_{AD}).

In addition, grouping TA and RI separately in PC2 (positive side and negative side, respectively) could be interpreted by the negative correlation between these two traits, where the RI was determined as SSC/ TA ratio (data not shown), giving that more ripe fruit has less sourness and better taste for some consumers.

Different environmental conditions over the three experimental years in the subset of 22 individuals (14 yellow and 7 white flesh peach and nectarines, plus one red-

fleshed advanced selection) affected the accumulation of bioactive compounds and their relative antioxidant capacity. Antioxidant capacity among all materials was significantly higher in 2013 (819.11) than in 2012 (222.79) and 2014 (708.87), while accumulation of total phenolics (69.12) and flavonoids (22.21) were significantly higher in 2014. On the other hand, the highest accumulation of anthocyanins was observed in 2012 (24.60) (Table 2. 7). Environmental conditions between experimental years were different. During ripening season (May to August), 25 days exceeding a maximum daily temperature of 35°C in 2012 compared to one day in 2013 and zero days in 2014 were recorded (Figure 2. 5). Total rainfall from May to August of 51.3 cm in 2012, 102.3 cm in 2013, and 35.7 cm in 2014 was recorded. Furthermore, daily rainfall at the experimental location exceeded 3 cm 14 times during 2013 season, while only 5 times during each of the 2012 and 2014 seasons. Average per year accumulation of total phenolics and flavonoids increased in 2014. The highest antioxidant capacity observed during the wet summer of 2013, might be attributed to the up-regulated biosynthesis of antioxidants under the stress conditions caused by the overproduction of reactive oxygen species (ROS) (Agati et al., 2012; Gill and Tuteja, 2010; Kassim et al., 2009). The toxic effects of ROS are counteracted by enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) as well as non-enzymatic (tocopherol, ascorbic acid, glutathione, carotenoids, and phenolic compounds) antioxidative systems (Ahmad et al., 2010; Blokhina et al., 2003; Gill and Tuteja, 2010) therefore depleting phenolic compounds in the tissue. Phenolics are diverse secondary metabolites that include simple phenols (total phenolics) and polyphenols (flavonoids and their subgroup anthocyanins) (Goleniowski

Table 2. 7. Accumulation of total phenolics, flavonoids, anthocyanin, and antioxidants in a subset of 22 *P. persica* accessions across three seasons.

Year	Total phenolics (mg GAE/100g) ^y	Flavonoids (mg CE/100g) ^y	Anthocyanin (mg C3GE/kg) ^y	Antioxidants (μg TE/g) ^y
2012	6.0 ± 3.4 a ^z	8.2 ± 8 a	24.6 ± 19.9 b	222.8 ± 161.7 a
2013	34.9 ± 14.2 b	14.6 ± 12.4 b	5.2 ± 3.7 a	819.1 ± 504 c
2014	69.1 ± 29.5 c	22.2 ± 17.1 c	8.1 ± 6.7 a	708.87 ± 472.8

^z Different letters indicate significant differences at $P < 0.05$ according to Student-Newman-Keuls's test.

^y Data indicates means ± standard deviation. Abbreviations: GAE, Gallic acid equivalents; CE, Catechin equivalents; C3GE, Cyaniding-3-glucoside equivalents; TE, Trolox equivalents.

et al., 2013). Phenolic compounds possess an ideal structured chemistry for free radical scavenging activity; therefore, their depletion is an effect of increased antioxidant productivity in protecting plants against oxidative stress damages (Table 2. 7). High anthocyanin accumulation observed during 2012 could be explained by the daily average temperatures of 25 °C, which is 10 °C warmer than 2013. Furthermore, solar radiation (837.6 W/m²) recorded in 2012 was very high compared to 2013 and 2014 (data not showed). It has been shown that solar radiation has a huge influence on expression of genes, such as bHLH3, WD40, and MYBPA1 that are influenced by UV-B radiation and control anthocyanin biosynthesis in peach peel and flesh (Ravaglia et al., 2013). Environmental impact on the accumulation of bioactive compounds has been reported for other fruit species as well. For example, in grape berries, anthocyanin accumulation and biosynthesis increased under low night temperature (15°C) and decreased during high night temperatures (30°C). The decrease has been attributed to the degradation of

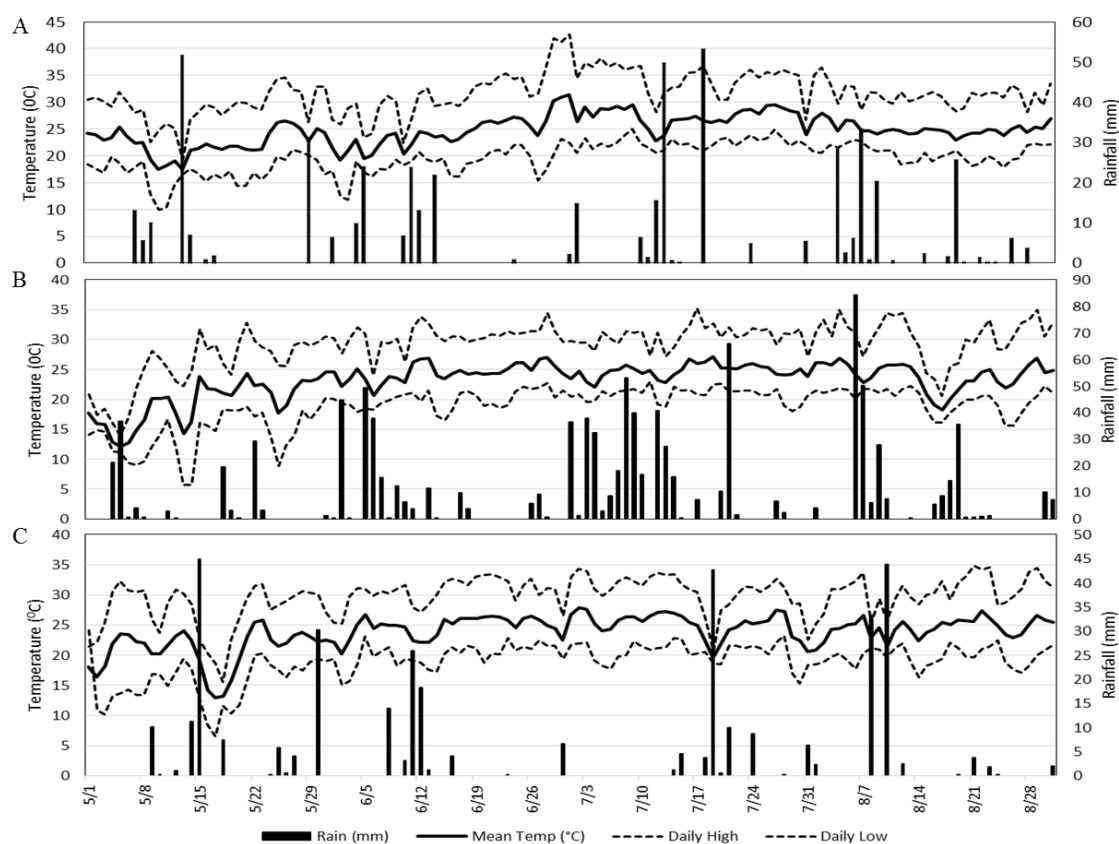


Figure 2. 5. Weather conditions during the ripening season. Daily air temperature (°C) and rainfall (mm), at the Musser Fruit Research Center, Seneca, SC, over three years [2012 (A), 2013 (B), and 2014 (C)].

anthocyanin and the inhibition of mRNA transcription of the anthocyanin biosynthetic genes (Mori et al., 2005, 2007). Moreover, postharvest application of UV-B visible irradiation in apple and red Chinese sand pears (*Pyrus pyrifolia* Nakai) improved anthocyanin accumulation and consequently coloration of the fruit skin (Arakawa, 1991; Zhang et al., 2012). Average per year accumulation of total phenolics and flavonoids increased in 2014, which exhibited average temperatures and rainfall for the experimental location, and was considered a normal season.

CONCLUSION

Wide variation in the accumulation of bioactive compounds observed in modern peach breeding germplasm provides genetic opportunities for breeding programs to continue enhancement of these healthy traits in newly developed varieties with keeping the other fruit qualities constant. It also portrays peach as a valuable source of health promoting compounds in human consumption, and provides valuable marketing tools to growers and retailers for delivering healthier food choices for consumers. Significant variation in the antioxidant capacity and bioactive compounds was observed in peach germplasm with different flesh color, ripening season, and across different years. In general, results indicated that fruits that ripen late, such as white-fleshed ‘Belle of Georgia’, yellow-fleshed ‘Jerseyqueen’ and ‘Elberta’, and red-fleshed advanced selection 99p4388 have the highest accumulation of phenolic compounds and their antioxidant capacity. Both Belle of Georgia and Elberta are heirloom cultivars and ancestors of the modern U.S. peach germplasm. Further, they play an essential role in the development of the U.S. peach industry. Improvement of phytochemical compounds in newly developed cultivars is one of the objectives in many breeding programs besides improving fruit quality traits. Further study regarding analyzing individual phenolic compounds is needed to account for different health effects that individual phenolic compounds have on human body.

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CHAPTER THREE

MAPPING QTLs FOR PHYTOCHEMICAL COMPOUNDS IN PEACH [*Prunus persica* (L.) Batsch]

INTRODUCTION

Numerous epidemiological studies suggest that increased fruit and vegetable consumption reduces mortality rate and risks of chronic diseases and improve antioxidant defenses of human body (Arts and Hollman, 2005; Dillard & German, 2000; Garcia- Closas et al., 1999; Joseph et al., 1999; Prior & Cao, 2000; Steinmetz & Potter, 1991; Wargovich, 2000). Fruits and vegetables are beneficial for human health due to being rich in different phytochemical compounds. Phenolic compounds are excellent scavengers of reactive oxygen species (ROS) and/ or reactive nitrogen species (RNS) under numerous abiotic environmental stresses, such as chilling, salinity, heavy metals, water deficiency, or UV- B irradiation (Agati et al., 2012). The toxic effects of ROS and RNO are counteracted by enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) as well as non-enzymatic (tocopherol, ascorbic acid, glutathione, carotenoids, and phenolic compounds) antioxidative systems (Ahmad et al., 2010; Blokhina et al., 2003; Gill and Tuteja, 2010).

Several Rosaceae fruit trees contribute significant levels of antioxidants to human diet and health. Total antioxidant capacity and phenolic compounds have been determined in peach and plum germplasm (Cantín et al., 2009; Cevallos-Casals et al., 2006; Vizzotto et al., 2007), in strawberry and berries (Häkkinen and Törrönen, 2000), in wines of different fruit sources (grape, apple, black currant, blueberry, cherry,

cranberry, elderberry, peach, pear, plum and raspberry) (Rupasinghe and Clegg, 2007), and in apple (Jelodarian et al., 2015; Muresan et al., 2014). Further, it has been found that phytochemical compounds from peach improved total radical-trapping potential of plasma in humans (Dalla Valle et al., 2007) and showed capability to selectively kill breast cancer cells (Noratto et al., 2009). Consequently, this has encouraged the interest of breeders in developing new peach cultivars with higher levels of antioxidants (Dalla Valle et al., 2007; Vizzotto et al., 2007).

Peach (*Prunus persica*) is a diploid species that belongs to genus *Prunus* and *Rosaceae* family. It is considered the third most important consumed fruit in the world after apple and pear (Font i Forcada et al., 2014; Rice-Evans et al., 1996). Peach fruit is high in bioactive compounds (Cantín et al., 2009; Cevallos-Casals et al., 2006; Vizzotto et al., 2007). Variation in the accumulation of phytochemical compounds has been observed in peach germplasm (Cantín et al., 2009; Cevallos-Casals et al., 2006; Gil et al., 2002; Vizzotto et al., 2007). For example, accumulation of phenolic compounds in peach ranged from 12.7 to 71.3 mg / 100g FW (Cantín et al., 2009). Those levels are higher than those reported in blueberry 4.4 - 9.2 mg/100g FW (Hakkinen and Torronen 2000). Analysis of different flesh colored peaches revealed influence of flesh color on phytochemical content (Cantín et al., 2009; Dalla Valle et al., 2007; Gil et al., 2002; Vizzotto et al., 2007). In general, the yellow-flesh peach cultivars showed lower antioxidant capacity than the white flesh ones (Gil et al., 2002; Cantín et al., 2009). The wide range of phytochemical content and antioxidant capacity observed in peach germplasm provides genetic opportunities for breeding programs to

continue enhancing health benefits in newly developed peach cultivars.

The main phenolic compounds reported in peach are hydroxycinnamates (chlorogenic and neochlorogenic acid), flavan 3-ols (catechin, epicatechin, and procyanidin B1), flavonols (quercetin 3-glucoside and quercetin 3-rutinoside), and anthocyanins (cyanidin 3-glucoside and cyanidin 3-rutinoside) (Kim et al., 2003; Tomas-Barberan et al., 2001). Phenolic compounds (total phenolics, flavonoids and their subclass anthocyanins) are synthesized by shikimic acid metabolic pathway that consists of a number of enzymatic steps. Each of these enzymes catalyzes a progressive reaction for flavonoid synthesis in different organelles of plant cells (Davies and Schwinn, 2003; Ravaglia et al., 2013; Winkel-Shirley, 2001). These secondary metabolites are upregulated when plants are exposed to stressful conditions in order to protect them against free radicals (Agati et al., 2012; Gill and Tuteja, 2010; Patra et al., 2013).

Peach is one of the most genetically characterized species in the Rosaceae family. A short juvenile phase (2-3 years) compared to other fruit trees, a diploid state ($n=8$), relatively small genome (~ 220-230 Mbp) twice the size of *Arabidopsis* (Baird et al., 1994), and vast molecular resources available (Dirlewanger et al., 2004; Verde et al., 2013; Martínez-García et al., 2013; Scorza and Sherman, 1996) make peach an ideal model for understanding biology and genetics of key traits. Therefore, the peach genome serves as the model genome for the Rosaceae family and other tree species (Verde et al., 2013). Many genetic maps have been constructed with peach and other *Prunus* species (Abbott et al., 2008). A consensus

map from an interspecific F2 cross between almond cv. ‘Texas’ and peach cv. ‘Earlygold’ is also considered the reference map for the *Prunus* genus (Dirlewanger et al., 2004; Illa et al., 2011; Joobeur et al., 1998). Conservation of synteny is greatly increased when comparing species of the same family such as Rosaceae and genera such as *Prunus*. This allows mapping of many markers on each chromosome of one species to one or two chromosomes of the other, and transferring knowledge obtained in other species to species of interest (Dirlewanger et al., 2004; Jung et al., 2012; Vilanova et al., 2008), as demonstrated for red coloration development in apple (Ban et al., 2007), cherry (Sooriyapathirana et al., 2010) and peach (Frett et al., 2014) fruit.

The high quality of the peach genome, the availability of the T × E *Prunus* reference map, and the availability of high throughput genotyping platforms such as the 9K peach SNP array (Verde et al., 2012) provide the opportunity to determine the inheritance of many quantitative traits. Many genes / quantitative trait loci (QTLs) for various traits, such as fruit quality traits, disease resistance, chilling and heat requirement, and bloom time in peach have been genetically mapped (Arús et al., 2012; Bielenberg et al., 2009,2015; Dirlewanger et al., 1999, 2004; Dirlewanger and Arús, 2004; Fan et al., 2010; Fresnedo- Ramírez et al., 2015, 2016; Frett et al., 2014; Illa et al., 2011; Monet et al., 1996; Ogundiwin et al., 2009; Okie et al., 2008; Yang et al., 2013). Most QTL identifications in peach have been done using bi-parental mapping approach (Frett et al., 2014; Fan et al., 2010; Yang et al., 2013; Zeballos et al., 2016). Recently, QTL mapping using pedigree based analysis (Fresnedo- Ramírez et al., 2015, 2016) and genome-wide association (GWAS) (Micheletti et al., 2015) have been

reported.

Although many QTLs associated with fruit quality in peach germplasm have been detected, there are only few reports regarding genetics of nutritional quality in peach (Font i Forcada et al, 2013; Ravaglia et al., 2013; Zeballos et al., 2016). Limited information exists in Rosaceae on QTLs associated with phytochemical compounds. QTLs linked to polyphenolic composition (Chagné et al., 2012; Verdu et al., 2014) and vitamin C (Davey et al., 2006) were detected in apple using single nucleotide polymorphism (SNP) markers. Recently, in an F1 nectarine population derived from cross between ‘Venus’ and ‘Big Top’, Zeballos et al. (2016) detected several QTLs for different phytochemical compounds using both single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers. Although the linkage map was not well saturated, indication of 2 QTLs responsible for relative antioxidant capacity were shown, one on linkage group (LG) 4 of the ‘Venus’ map and one on LG8 of the ‘Big Top’ map, and one QTL for total phenolics on LG2 for both parental maps. Furthermore, QTLs associated with flavonoid accumulation were mapped on LGs 2, 3, 4, and 7 for the ‘Venus’ map and on LG2 on the ‘Big Top’ map, and a single QTL for anthocyanins was detected on LG5 of the ‘Big Top’ map. The quantitative nature of these traits and environmental effects on their expression (Font i Forcada et al, 2013; Kassim et al., 2009; Zeballos et al., 2016), make it challenging to detect the candidate genes associated with them. Understanding the genetic control of quantitative traits will facilitate developing of new cultivars through breeding programs (Peace and Norelli, 2009). Therefore, the main objective of this study was to use a bi-parental mapping

approach to understand the inheritance of the accumulation of phytochemical compounds in 'Zin Dai' x 'Crimson Lady' F2 population.

MATERIALS AND METHODS

Plant material

Ninety individuals from an F2 population, designated ZC² (Frett et al., 2014), segregating for accumulation of phytochemical compounds were used in this study. It originated from self-pollination of an F1 (yellow flesh) hybrid created by crossing two peach cultivars ‘Zin Dai’ (white flesh) × ‘Crimson Lady’ (yellow flesh) (Figure 3. 1). The seed parent, ‘Zin Dai’, is a non-melting, low-acid peach, originating in China. The pollen donor ‘Crimson Lady’ is a non-melting peach with high – acid flesh, originated in CA. The F1 individual ‘BY02p4019’ has melting and semi-low acid flesh. The plant material is maintained at the Clemson University Musser Fruit Research Center, in Seneca, SC, under a warm, humid, temperate climate and standard commercial practices for irrigation, fertilization, and pest and disease control. The trees were at least 5 years old, grafted on Guardian®™ rootstock, planted in duplicate at 1.5 m x 4 m spacing and trained to a perpendicular V system. Out of a total of 93 genotypes, 73 in 2013 and 81 in 2014 were available for phytochemical analysis.

Phenotyping

Five fruits from each individual were selected at commercial ripe stage by measuring the Index of Absorbance Difference (I_{AD}) (Ziosi et al., 2008). To ensure uniformity of maturity for all individuals, five fruits in the ripe stage equivalent to $I_{AD} = 0.6$ were selected and analyzed for fruit quality and phytochemical contents in 2013 and 2014.

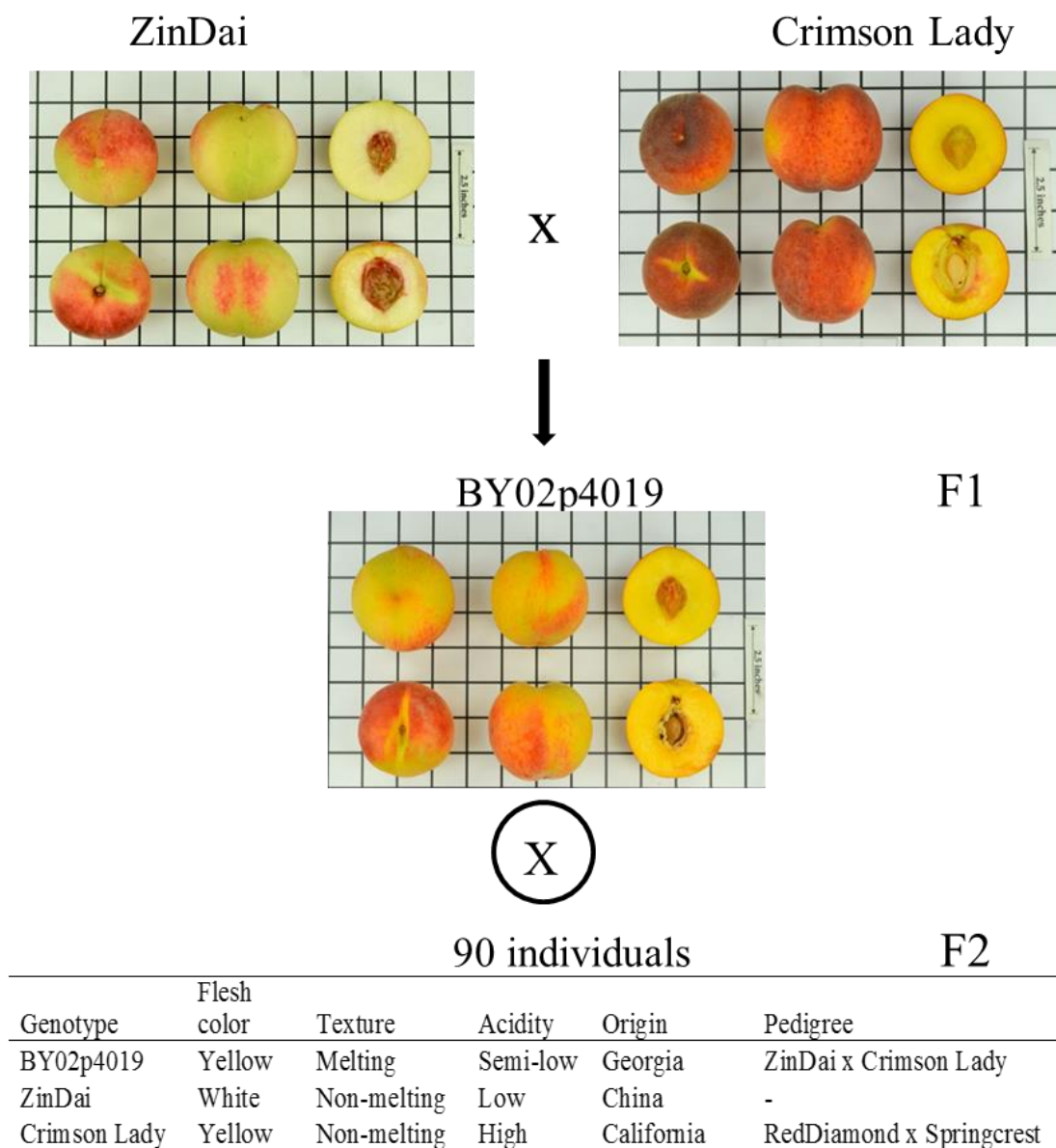


Figure 3. 1. Development and characteristics of ZC² mapping population and parental genotypes, ‘Zin Dai’, ‘Crimson Lady’ and BY02p4019.

Fruit quality traits, such as fruit size (diameter) (mm), fruit weight (g), fruit firmness (kg), soluble solids concentration (SSC) (Brix %), titratable acidity (TA) (malic acid %), and ripening index (RI) were evaluated according to Frett et al. (2012) phenotyping protocol (for detail protocol see chapter II; page 50).

Phytochemical analyses were performed on two slices of mesocarp excised from opposing cheeks of each of the five fruits previously subjected to fruit quality phenotyping. Fruit exocarp and red tissue around the pit, if present, were removed. Flesh was cut into small pieces, individually packed for each fruit, and frozen in LN₂. The frozen tissues were stored at -20 °C until analyzed. Composite samples, comprised of equal tissue from each of the 5 fruits, was prepared per individual and ground in liquid nitrogen to a fine powder and kept at -80 °C until needed. Methanol extraction was performed in 1 ml of 80% methanol using 500 mg of frozen composite powder in five replicates. The extract was incubated at +4 °C overnight and then centrifuged for 10 min at 12,000 g at +4 °C to collect the supernatant. This hydroalcoholic extract was stored at -80 °C and used in subsequent analysis. Relative antioxidant capacity (RAC) (µg Trolox Equivalent/ g FW), total phenolics (mg GAE / 100g FW), flavonoids (mg CE/ 100g FW) and their subclass anthocyanin (mgC3GE/kg FW) assays were performed in accordance with protocols published in Cantín et al., (2009) (described in chapter II in details; pages 50 - 52).

DNA extraction and genotyping

A subset of 25 individuals was previously used in mapping QTLs for blush in peach (Frett et al., 2014). For an additional 65 siblings from the ZC² population, DNA

extraction was performed according to Dellaporta et al. (1983). The ZC² population, F1, 'Zin Dai' and 'Crimson Lady' were genotyped using the IPSC 9K peach SNP array v1 (Verde et al., 2012; Frett et al., 2014). Concentrations of DNAs were adjusted to a minimum of 50 ng/ µl and submitted to the Research Technology Support Facility at Michigan State University (East Lansing, MI, USA) where the Infinium assay was performed following the manufacturer's protocol (Illumina Inc.) as previously described in Frett et al. (2014). SNP genotypes were scored with the Genotyping Module of Genome Studio Data Analysis software (Illumina Inc.). A GenTrain score of > 0.4 and a GenCall 10% of > 0.2 were applied to remove SNPs that did not cluster (homozygous) or had ambiguous clustering.

Genetic map Construction

The existing ZC² map (Frett et al., 2014) was updated with genotyping data from an additional 65 individuals, and a new revised version of ZC² linkage map was developed using JoinMap 4.1 software (Van Ooijen, 2006). SNPs homozygous for alternate alleles in two parents as well as SNPs homozygous in one and heterozygous in the other parent were considered for mapping. F2 population type codes were applied according to Van Ooijen (2006). Names of the SNP markers were edited by adding the chromosome number in front of the name to simplify the mapping process. Chi-square-goodness-of-fit test ($P < 0.05$) was used to test the deviations from Mendelian ratio. Linkage groups (LG's) were determined using a minimum 3.0 logarithm of odds (LOD) and maximum recombination frequency of 0.40. Marker distances were calculated using the Kosambi (1944) mapping functions. Map figures were generated using MapChart 2.3 software (Voorrips, 2002).

Linkage map comparison to peach physical map

The revised ZC² map was compared to the peach physical map v.2 to confirm LG names and orientations. The set of SNPs mapped in each linkage group were aligned with their position on the peach genome using MapChart2.3 (Voorrips 2002), and collinearity between the linkage and physical maps was evaluated.

QTL mapping

Phenotypic data were combined with the improved ZC² linkage map in MapQTL6.0 software (Van Ooijen, 2009) for QTL analysis. Blush phenotypic data were collected in 2007 and 2008 (Frett et al., 2014) and in 2014. All phenotypic data sets were tested for the normality of distribution using the Shapiro-Wilk normality test ($P < 0.05$) in R software. Data that failed normality test were transformed using either log or square root transformations in R. Detection of putative QTLs was performed separately for each dataset applying Kruskal-Wallis test (P values ranged from < 0.01 to < 0.0001) in MapQTL 6.0. Graphical presentation of QTLs on the ZC² linkage map was generated with MapChart 2.3 (Voorrips, 2002). To simplify map presentation, SNPs with identical segregation pattern (mapping at the same genetic position) were grouped into blocks (bins). Blocks were named as following: BL_1_11, where B= block; L= LG; 1 = block number in ascending order within the LG; 11 = number of SNPs in the block. QTLs were named as qTT_L.1 where q = QTL; TT = trait acronym; L = linkage group number; 1= numbers to identify different QTL for the same trait.

Haplotyping and candidate gene analysis

SNP-based haplotypes and candidate gene analysis were performed for major QTL on chromosome 6. The complete coding sequences were obtained from peach genome v2.0 assembly (GDR: www.rosaceae.org). The coding sequences and the translated proteins were blasted using both blastn and tblastn against NCBI nr.

Statistical Analysis

All phenotypic data were expressed as means of five fruits. Differences were compared by one-way ANOVA and Student-Newman-Keuls's test at $P < 0.05$. Correlation analysis between evaluated traits was performed using Pearson's correlation coefficients at $P < 0.01$. An association test between phytochemical compounds and haplotypes was performed by Student-Newman-Keuls's at $p < 0.05$. All statistical analyses were performed in SPSS v. 23 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). Test of normality, performed by Sharpiro-Wilk normality test ($P < 0.05$), and variance compounds for broad-sense heritability (H^2) were calculated in R. Histograms with normality curves for each trait were created for each year separately. H^2 was estimated using the following equation:

$$H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2/n)$$

Where: σ_g^2 = genotypic variance; σ_e^2 = environmental variance; n = sample size).

RESULTS

Phenotypic data

Phytochemical compounds and fruit quality parameters were evaluated in the ZC² progeny and grandparent cultivars Zin Dai and Crimson Lady as well as in F1 accession in two years (2013 and 2014), and 33 datasets were obtained. Seventy- three individuals were analyzed for phytochemical compounds in 2013 and 81 individuals in 2014, due to either tree death or lack of fruit, while 62 individuals were phenotyped in both years. The ZC² progeny segregated for all phenotypic traits. Shapiro- Wilk normality test revealed few traits normally distributed [fruit diameter (FS; 2013 and 2014), soluble solids concentration (SSC; 2013 and 2014), and titratable acidity (TA; 2014)] ($P < 0.05$). Traits that failed the normality test required either log transformation, such as antioxidant capacity (RAC; 2013), total phenolics (GAE; 2013 and 2014), flavonoids (CE; 2013), anthocyanins (C3GE; 2013 and 2014), fruit firmness (FF; 2013), ripening index (RI; 2013 and 2014), and index of absorbance difference (I_{AD}; 2013), or square root transformation, such as antioxidant capacity (RAC in 2014), flavonoids (CE; 2014), fruit weight (FW; 2013 and 2014), and index of absorbance difference (I_{AD}; 2014). However, even after transformation, traits were still not normally distributed except for FW (appendix I, and Figure 3. 2 and 3. 3).

Statistically significant differences ($P < 0.05$) were observed for average phenotypic data sets, with highest accumulation of all fruit quality traits and phytochemical compounds observed in 2014 except for anthocyanin level, which was higher in 2013 (6.1 mg C3GE /kg) (data not shown).

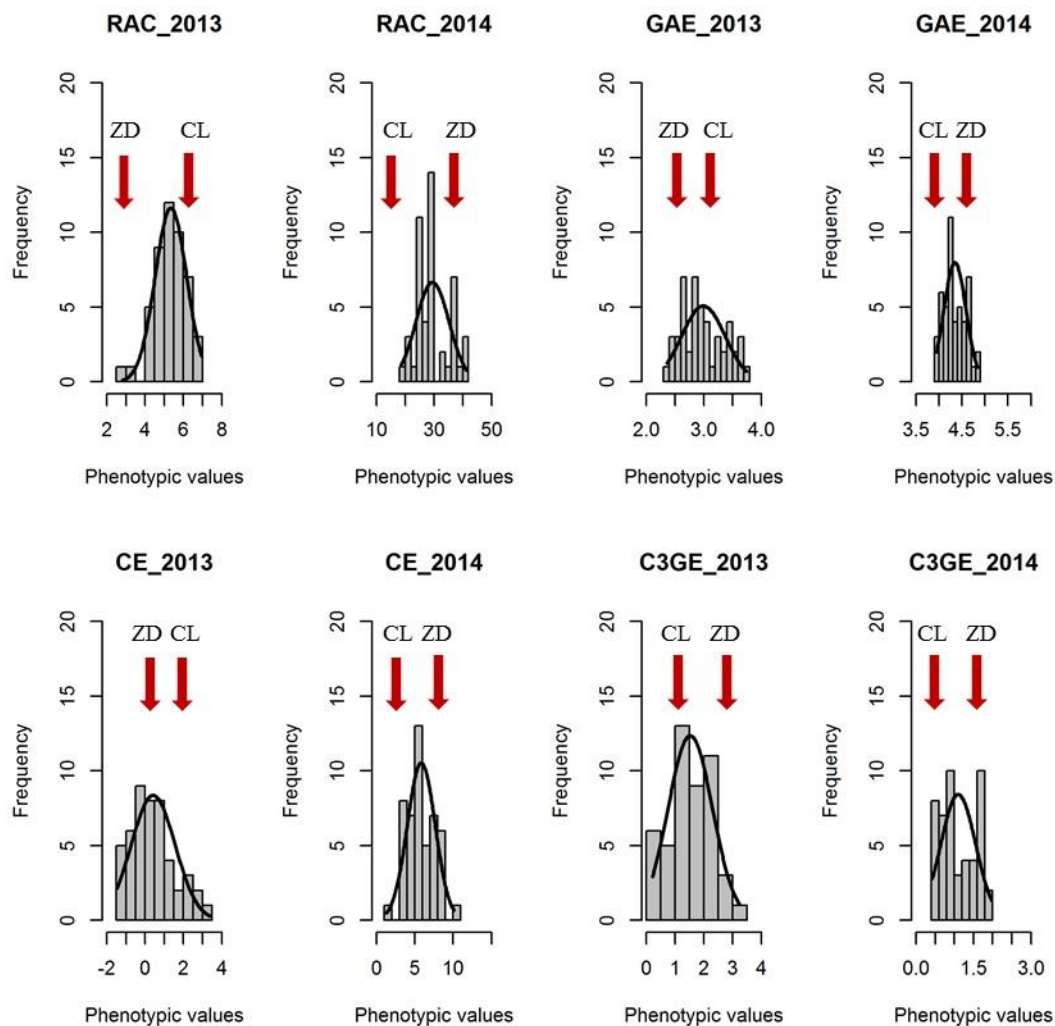


Figure 3. 2. Distribution of phytochemical compounds in ZC² progeny organized in 8 datasets with normality curves. Red arrows indicate the values measured for the grand parental individuals, Zin Dai (ZD) and Crimson Lady (CL).

Accumulation of phytochemical compounds between grandparent cultivars Zin Dai and Crimson Lady, F1 accession (BY02p4019) as well as in F2 progeny exhibited variability during years of study. Grandparent cultivars Zin Dai and Crimson Lady were significantly different in their antioxidant capacity, 318.8 and 93.3 µg TE/g FW, respectively (Tables 2.3 and 2.4; chapter II), while

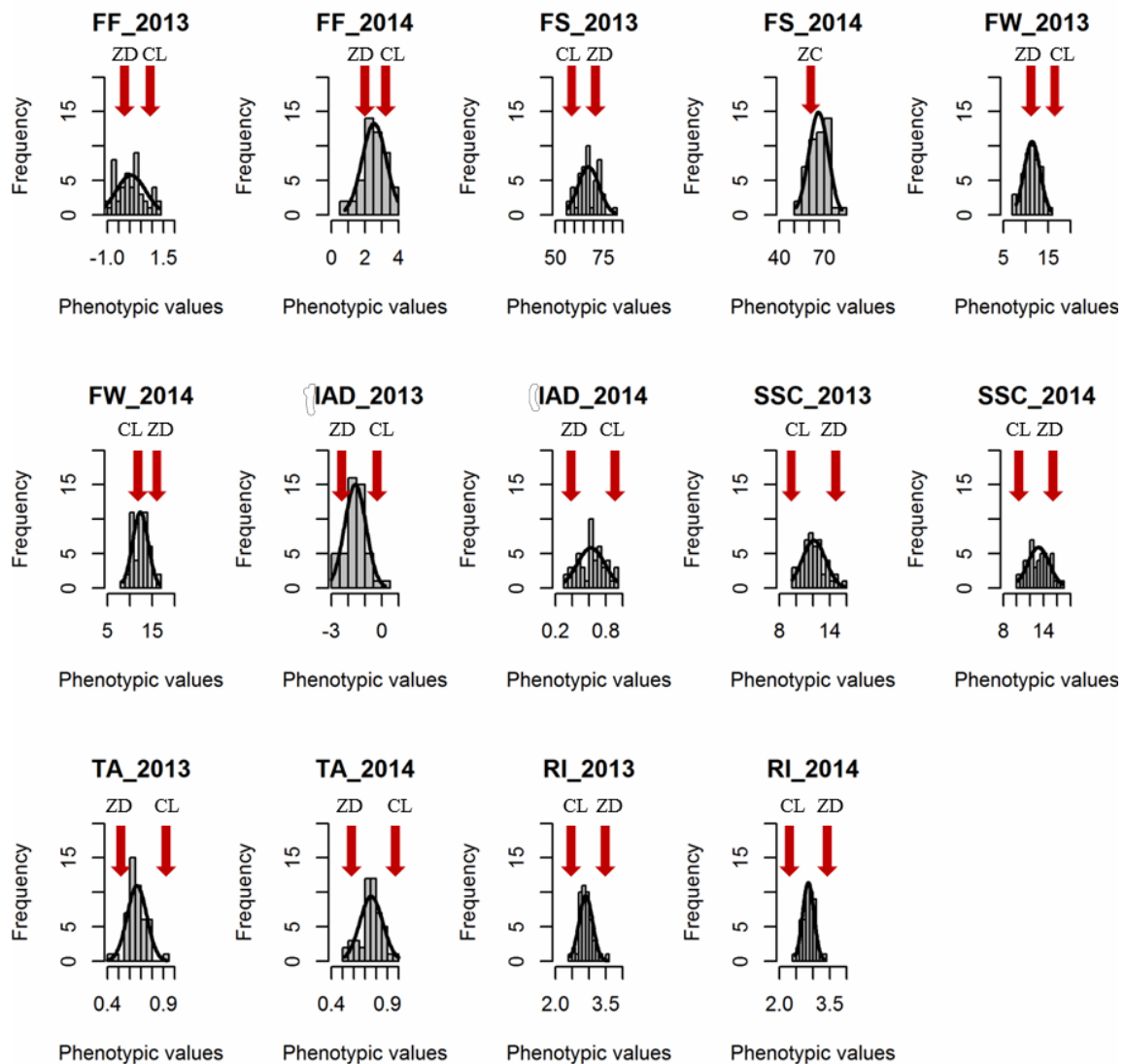


Figure 3. 3. Distribution of fruit quality traits in ZC² progeny organized in 14 datasets with normality curves. Red arrows indicate the values measured for the grand parental individuals, Zin Dai (ZD) and Crimson Lady (CL).

‘BY02p4019’, parent of the ZC² population, exhibited positive transgressive segregation with 498.07 µg TE/g FW. Majority of ZC² progeny exhibited a higher accumulation of antioxidants (> 500 µg TE/g FW) than ‘Zin Dai’, ‘Crimson Lady’, and ‘BY02p4019’.

Variability was also observed for accumulation of total phenolics between grandparent cultivars and F1 accession (BY02p4019), with average accumulation of 37.2 in ‘Crimson Lady’, 45.2 in ‘Zin Dai’ (Tables 2.3 and 2.4; chapter II), and 41.8 mg GAE/100g FW in ‘BY02p4019’. Transgressive segregation was also evident in accumulation of flavonoids between grandparent cultivars and F1 parent, with an average accumulation from 3.8 in ‘Crimson Lady’ (Table 2.4; chapter II) to 21.2 mg CA/100g FW in ‘BY02p4019’. In addition, variability was observed for average accumulation of anthocyanin as well, with ‘Crimson Lady’ accumulating 1.6, ‘Zin Dai’ 6.7 (Tables 2.3 and 2.4; chapter II), and ‘BY02p4019’ 4.7 mg C3GE /kg. ‘Crimson Lady’ exhibited low accumulation of flavonoids (3.8) and their subgroup anthocyanin (1.6).

ZC² progeny segregated for antioxidant capacity (RAC), with minimum 15 and maximum 1,723 µg TE/g FW, with mean 575 µg TE/g FW, and average accumulation of total phenolics, 50.3 mg GAE/100g FW, with minimum 8 and maximum 145, flavonoids, 19.4 mg CE/100g FW, with minimum 2 and maximum 115, and anthocyanin, 4.6 mgC3GE/kg FW, with minimum 0 and maximum 30 (Table 3.1).

Phenotypic variation for evaluated fruit quality traits was also observed in grandparent cultivars, F1 parent and ZC² progeny. Both ‘Zin Dai’ and ‘Crimson Lady’ exhibited the lowest percentage of titratable acidity (TA) (0.4%) and soluble solids concentration (SSC) (9.3 %), respectively (data not shown).

Table 3. 1. Phytochemical traits and fruit quality parameters observed over 2 years in ZC² population. TE, Trolox equivalent; GAE, gallic acid equivalents; CE, catechin equivalents; C3GE, cyaniding-3-glucoside equivalents; SSC, Soluble solids concentration; TA, Titratable acidity; RI, Ripening index; I_{AD}, Index of Absorbance Difference.

Trait	Unit	Min	Max	Mean	MSE	SD	H ²
Antioxidant	µg TE/g FW	15	1723	575.1	17.4	423.8	0
Phenolics	Mg GAE/100g FW	8	145	50.3	1.3	32.4	0.02
Flavonoids	Mg CA/100g FW	2	115	19.4	0.9	22.6	0.03
Anthocyanin	mg C3GE /kg	0	30	4.6	0.2	3.7	0.99
Fruit firmness	Kg	.2	5.4	2.0	0.0	1.2	0.12
Diameter	Mm	49.5	89.0	66.9	0.3	6.7	1
Fruit Weight	G	35.0	360.0	146.5	2.0	48.6	1
SSC	% Brix	9.5	16.8	12.6	0.2	1.6	1
TA	% Malic acid	0.4	1.0	0.7	0.0	0.1	0
RI	SSC/TA	8.4	35.6	18.0	0.4	3.8	1
I _{AD}		0	1	0.3	0.0	0.3	0.04

MSE = mean standard error, SD = standard deviation, H² = heritability.

Grandparent cultivars and F1 accession exhibited variability for majority of fruit quality traits. For example, ‘Zin Dai’, ‘Crimson Lady’, and ‘BY02p4019’ showed variation in fruit firmness (FF) (1.3, 2.8, and 1.6, respectively), fruit weight (FW) (151.7, 181.4, and 177.6, respectively), malic acid percentage (0.37, 0.88, and 0.64, respectively), and in ripening index (RI) (35.8, 10.6, and 28.6, respectively).

Transgressive segregation for all fruit quality data sets was evident in ZC² progeny for majority of traits. Average FF observed was 2 kg, with minimum and maximum values of 0.2 and 5.4, respectively (Table 3.1). Fruit size (FS) and FW ranged from 50 to 83.4 mm, and 67.2 to 285.6 g, respectively. Soluble solids concentration (%) was also variable, with the 9.5 – 16.8 % Brix observed in progeny. Average malic acid percentage observed in ZC² progeny ranged from 0.37 to 0.99 and RI ratio ranged from (8.4) to (35.6) in the progeny.

High broad-sense heritability ($H^2 \geq 0.99$) was estimated for accumulation of anthocyanin, FS, FW, SSC, and RI. Most phytochemical compounds exhibited very low H^2 (Table 3. 1).

Pearson’s correlation coefficients between different phenolic compounds and fruit quality traits (Table 3. 2) revealed significant correlations only for antioxidant capacity (AC) and the total phenolics, flavonoids, FF, FW, SSC, TA, and I_{AD}. Negative correlation was observed between FS with AC ($r = -0.095$, $P < 0.05$), while no correlation was observed between anthocyanin accumulation and AC. The AC was significantly and positively correlated with accumulation of flavonoids and total phenolics ($r = 0.849$ and $r = 0.763$, $P < 0.01$, respectively). The highest correlation was observed in 2013

Table 3. 2. Pearson's correlation coefficients between antioxidant capacity (AC) and different pomological traits in two years.

Trait \ Year	2013	2014	2 years
Total phenolics	0.440**	0.600**	0.763**
Flavonoids	0.877**	0.772**	0.849**
Anthocyanin	0.109*	0.493**	ns
FF	0.309**	N	0.408**
Diameter	ns	-0.128*	-0.095*
FW	ns	-0.141**	0.088*
SSC	0.579**	0.557**	0.577**
TA	ns	N	0.363**
RI	ns	0.228*	ns
I _{AD}	0.168**	0.137*	0.249**

** = Significant correlation at $P < 0.01$; * = Significant correlation at $P < 0.05$; ns= not significant.

between AC and flavonoid content ($r = 0.877$) and between AC and SSC ($r = 0.579$). A positive correlation was also observed between flavonoids and total phenolics with average data set ($r = 0.755$, $P < 0.01$). Results revealed no correlation between antioxidant capacity and fruit quality traits except for FF in 2013, SSC in 2013 and 2014, RI in 2014, and I_{AD} in both years. The highest correlation among fruit quality traits was between FS and FW ($r = 0.983$, $P < 0.01$) and between SSC and RI ($r = 0.569$, $P < 0.01$) in 2014. However, RI and TA were negatively correlated ($r = -0.693$, $P < 0.01$) (data not shown).

Linkage map construction

The improved ZC^2 genetic linkage map was constructed using 1,710 SNPs obtained from genotyping data from all 90 F2 individuals. Out of 8,144 SNPs available on the peach SNP array only those homozygous for alternate alleles in two grandparents as well as homozygous in one and heterozygous in the other grandparent were selected. Nine hundred and eight markers were successfully grouped in eight groups creating the ZC^2 linkage map, with 347 SNPs mapped at a unique position. Eight linkage groups, LG, corresponded to 8 peach chromosomes (Table 3. 3; Fig. 3. 4). Appendix II defines the SNPs included in each block. The revised map covers a genetic distance of ~ 336 centiMorgans (cM) with an average marker density of 1.10 cM/ marker. The number of unique map positions mapped on each linkage group, ranged from 21 on LG5 to 63 on LG8, with an average of 43 markers per LG. The length of LGs was variable, with LG1 being the largest, 58.9 cM, and LG4 covering the shortest distance, 30 cM (Table 3. 3). The largest gap was observed on LG1 (19.7 cM) between SNP_IGA_103771 and SNP_IGA_135137.

Linkage map comparison to peach physical map

Revised ZC^2 genetic map exhibited high homology with the physical map with all SNPs in agreement with their positions on the scaffolds of the peach genome v2. The ZC^2 map covered about 58.9 % of the peach genome v 2.0 physical length. LG3 exhibited the largest coverage (89.35 %), while the lowest coverage (24.09 %) was observed in LG4. In addition, the estimated average coverage per marker on the pseudomolecules ranged from 17.95 kb/cM on LG4 to 782.80 kb/cM on LG3 (Table 3. 4).

Table 3. 3. ZC² linkage map description.

LG#	Length (cM)	Mapped markers	Uniquely mapped	SNPs mapped to the same position	Smallest gap (cM)	Largest gap (cM)
LG1	58.9	67	33	34	0.2	19.7
LG2	38.2	208	61	147	0.1	11.2
LG3	31.1	101	36	65	0.4	0.6
LG4	30	105	29	76	3.5	3.9
LG5	43.9	38	21	17	0.7	10.4
LG6	53.1	115	53	62	0.1	15.1
LG7	40.7	164	51	113	0.1	9.4
LG8	40.1	110	63	47	0.1	4.7
ZC ² map	336	908	347	561	0.1	19.7

OTL analysis

QTL analysis was performed for each trait separately for each year. Using Kruskal-Wallis test in MapQTL 6.0, a total of 1,419 markers were significantly mapped for all traits in different years, including SNPs that associated with more than one trait on different linkage groups (Appendix III). A total of 28 QTLs were identified with at least one QTL on each chromosome, except for chromosome 5, with different sources and years. Seven QTLs were detected with datasets from 2013, 17 with datasets from 2014, 4 blush QTLs were also detected using blush phenotypic data collected in 2007 and 2008 (Frett et al., 2014), and 12 QTLs with average datasets. The highest number of QTLs was detected on LG6 followed by LG3 and LG4 (Fig. 3. 5).

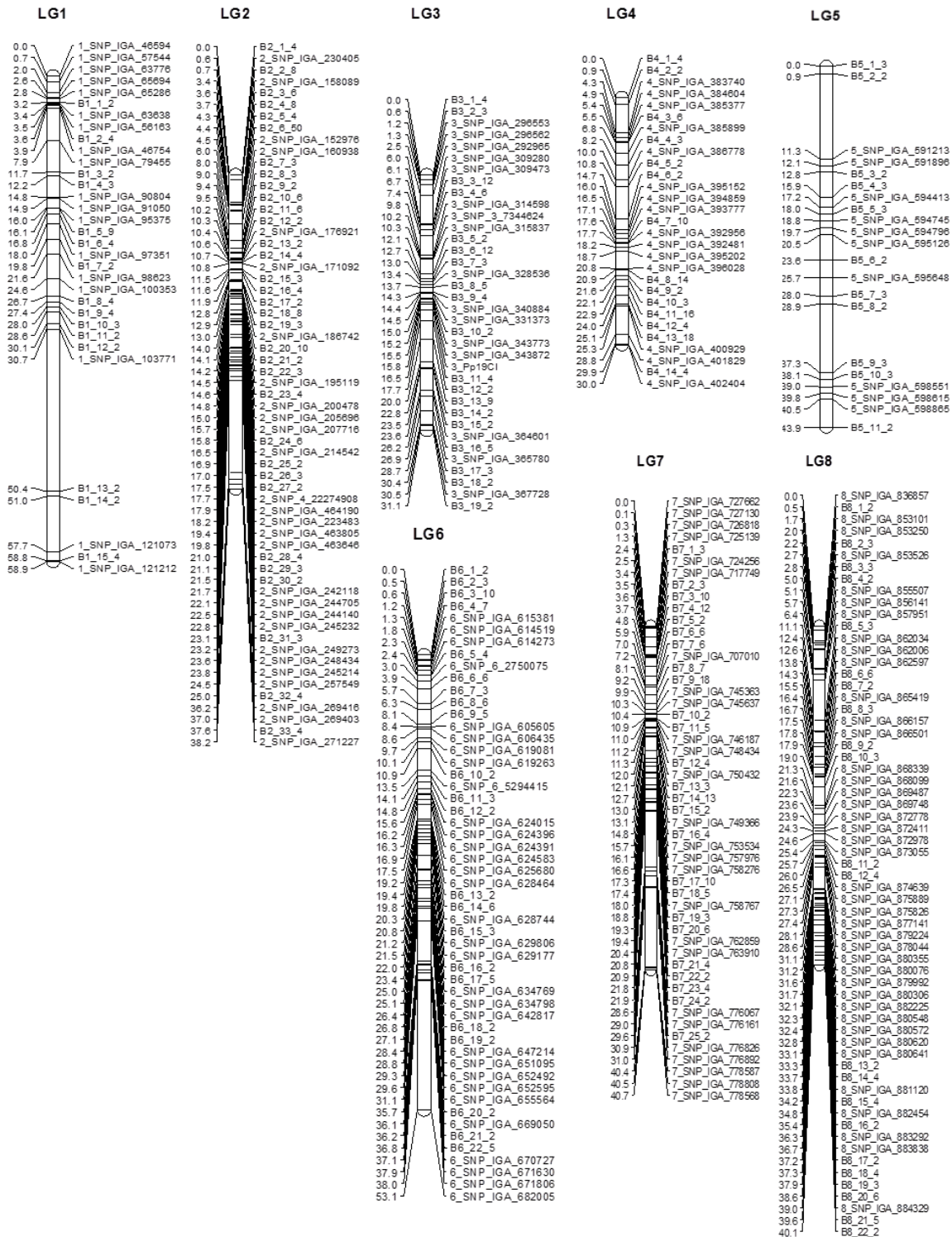


Figure 3. 4. ZC² genetic linkage map. Names on the right represent SNP markers (one SNP per loci), while numbers on the left side represent the distance between markers in cM.

Table 3. 4. Comparison of the ZC² linkage map with the peach physical map v 2.0.

LGs	ZC ² linkage map				Marker density		Average coverage (kb/cM)
	SNP markers (inverted)	Genetic distance (cM)	Physical length (Mb)	Physical coverage (%)	cM	Kb	
LG1	33 (-)	58.9	25.68	53.82	1.78	778.3	436.04
LG2	61 (-)	38.2	19.85	65.67	0.63	325.4	519.65
LG3	36 (-)	31.1	24.35	89.35	0.86	676.3	782.80
LG4	29 (-)	30	6.11	24.09	1.03	18.6	17.95
LG5	21 (-)	43.9	4.82	26.47	2.09	229.5	109.76
LG6	53 (-)	53.1	24.16	79.55	1.00	455.9	455.03
LG7	51 (-)	40.7	15.47	69.43	0.80	303.4	380.19
LG8	63 (-)	40.1	11.39	50.88	0.64	180.8	284.04

Phytochemical compounds QTLs

Two QTLs for antioxidant capacity (RAC) were detected with the 2014 data set; one on each LG3 ($P < 0.005$) spanned 7 cM (*qRAC.ZC_3.1*) and peaked at 28.7 cM [SNP_IGA_366194 ($K = 13.1$)] and LG6 ($P < 0.01$) (*qRAC.ZC_6.1*) from 8 – 11 cM peaked at 10.1 cM at SNP_IGA_619263 ($K = 12.2$). The most significantly linked SNP within RAC QTL on LG3 was inherited from the seed parent ‘Zin Dai’, while the most linked SNP on LG6 was inherited from both ‘Zin Dai’ and ‘Crimson Lady’ (Fig. and Table 3.5). Three QTLs were detected for accumulation of total phenolics (GAE). QTL on LG1 (*qGAE.ZC_1.1*) ($K = 10.1$) was detected with the average data set, spanned 28 - 30 cM and peaked at 30.1 cM. Two QTLs on LG6 and 8 were detected with the 2014 data set, *qGAE.ZC_6.1* ($K = 12.2$; $P < 0.005$) spanned 5 - 16 cM and peaked at 5.7 cM, and *qGAE.ZC_8.1* ($K = 12.2$; $P < 0.01$) spanned 1 - 3 cM and peaked at 1.7 cM (Fig. and Table 3.5). Three QTLs were detected for accumulation of flavonoids, two with the 2013 dataset ($P < 0.01$); one on LG2 (*qCE.ZC_2.1*) spanned 23 – 25 cM, peaked at 23.1 cM and one on LG3 (*qCE.ZC_3.1*) from 6 – 8 cM, peaked at 6.7 cM (SNP_IGA_309881), and one with the 2014 dataset, on LG6 (*qCE.ZC_6.1*) ($P < 0.005$) spanned 21 cM, peaked at 10.1 cM at SNP_IGA_619263 with $K = 14.0$ (Fig. and Table 3.5). QTLs for accumulation of anthocyanins were observed with data sets for yearly phenotypic observations. QTL with the 2013 dataset, was mapped on LG8 (*qC3GE.ZC_8.1*) spanned 3 cM and peaked at SNP_IGA_836857 ($K = 11.6$), while QTL with the 2014 dataset was mapped on LG6 (*qC3GE.ZC_6.1*) from 0 – 17 cM, peaked at 13.5 cM at SNP_6_5294415 ($K = 13.1$) (Fig. and Table 3.5).

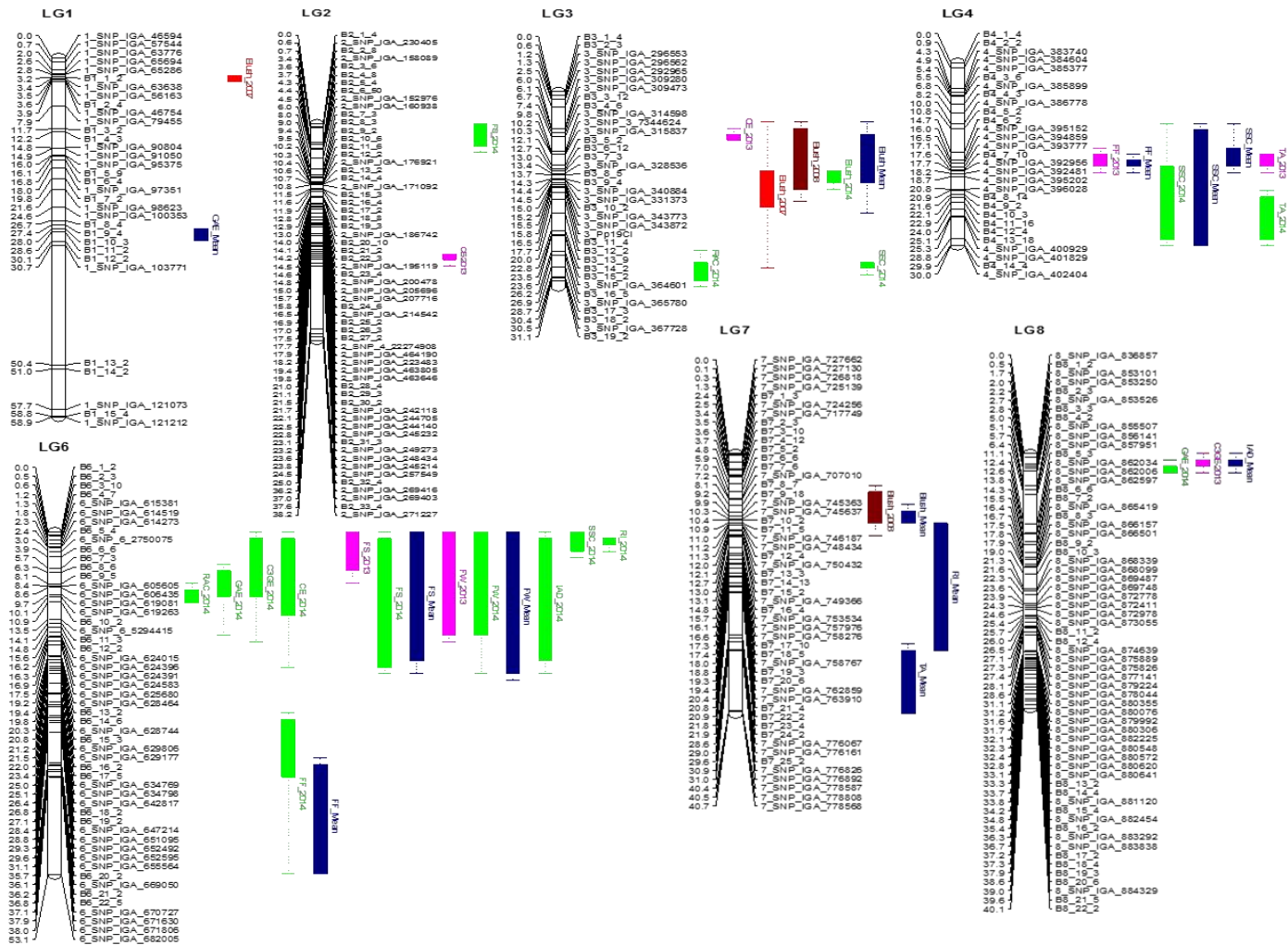


Figure 3. 5. QTLs mapped on the ZC² linkage map. Bars in red indicate QTLs detected with 2007 datasets, in brown indicate QTLs detected with 2008 datasets, in pink indicate QTLs detected with 2013 datasets, green bars indicate QTLs detected with 2014 datasets, while blue bars indicate QTLs detected with average datasets.

A major QTL for phytochemical compounds was detected on LG6 *qPC.ZC-6.1*. The closest and most significantly associated marker to this QTL was SNP_IGA_619263 (K= 12.2) at 10.1 cM, and it was inherited from both grandparents.

Fruit quality QTLs

Three QTLs for blush, two minor on LG1 and 7, and one major on LG3, were detected (Fig. 3.5). A minor QTL on LG1 (*qBlush.ZC_1.1*), detected with the 2007 data set, spanned 3 – 4 cM and peaked at 3.6 cM and QTL on LG7 (*qBlush.ZC_7.1*) spanned 5 – 13 cM and peaked at 10.3 cM for 2008 and average data sets. A major QTL on LG3 (*qBlush.ZC_3.1*), detected with all data sets (2007, 2008, 2014, and average), spanned 5 – 29 cM, peaked at 15 cM at SNP_IGA_12878608 (K= 15.3). The most significantly linked SNP within blush QTLs on LG7 was SNP_IGA_745637 (K= 11.2). SNPs in the region of *qBlush.ZC_3.1* QTL were inherited from ‘Zin Dai’, while the SNPs in QTL region on LGs1 and 7 were inherited from ‘Crimson Lady’ (Fig. and Table 3.5). Two QTLs for fruit firmness were detected; one on LG4 *qFF.ZC_4.1* with the 2013 and average datasets spanned 14 – 18 cM and peaked at ~ 16 cM, and one QTL on LG6 *qFF.ZC_6.1* with the 2014 and average data sets spanned 53 cM and peaked at 37 cM. The most linked markers within FF QTLs, SNP_IGA_395152 on LG4 (K = 18.8) and SNP_IGA_670727 (K= 16.1) on LG6 were inherited from both grandparents (‘Zin Dai’ and ‘Crimson Lady’) (Fig. and Table 3.5). Two fruit size (FS, diameter) QTLs were mapped, one on each LG2 (*qFS.ZC_2.1*) and LG6 (*qFS.ZC_6.1*). *qFS.ZC_2.1* spanned 4 cM, peaked at 0.6 cM at SNP_IGA_230405 (K= 6.8), while *qFS.ZC_6.1* spanned 22 cM, and peaked at SNP_IGA_614519 (K = 18.7) at 1.2 cM

Table 3. 5. Summary of QTLs mapped in ZC² F2 progeny by Kruskal-Wallis (non-parametric mapping) and their closest SNPs for 12 traits evaluated in different years. RAC, relative antioxidant capacity; GAE, gallic acid equivalents; CE, catechin equivalents; C3GE, cyaniding-3-glucoside equivalents; FF, fruit firmness; FS, fruit size(diameter); FW, fruit weight; I_{AD}, Index of Absorbance Difference; SSC, soluble solids concentration; TA, titratable acidity; RI, ripening index.

Trait	Year	QTL	Group	Closest SNP	Position (cM)	K* ^a	Source ^b	Signif. ^c
RAC	2014	<i>qRAC.ZC_3.1</i>	LG3	B3_17_3	28.68	13.1	ZD	*****
RAC	2014	<i>qRAC.ZC_6.1</i>	LG6	SNP_IGA_619263	10.09	12.23	ZC	****
GAE	Average data set	<i>qGAE.ZC_1.1</i>	LG1	SNP_IGA_103507	30.12	10.14	ZC	***
GAE	2014	<i>qGAE.ZC_6.1</i>	LG6	B6_7_3	5.7	12.21	ZC	****
GAE	2014	<i>qGAE.ZC_8.1</i>	LG8	SNP_IGA_853101	1.71	10.26	ZC	***
CE	2013	<i>qCE.ZC_2.1</i>	LG2	SNP_IGA_245182	23.12	9.21	ZC	***
CE	2013	<i>qCE.ZC_3.1</i>	LG3	SNP_IGA_309881	6.72	6.95	ZD	***
CE	2014	<i>qCE.ZC_6.1</i>	LG6	SNP_IGA_619263	10.09	14.01	ZC	*****
C3GE	2013	<i>qC3GE.ZC_8.1</i>	LG8	SNP_IGA_836857	0	11.58	ZC	****
C3GE	2014	<i>qC3GE.ZC_6.1</i>	LG6	SNP_IGA_5294415	13.49	13.14	ZC	****
Blush	2007	<i>qBlush.ZC_1.1</i>	LG1	SNP_IGA_15750387	3.58	7.38	CL	***
Blush	All data sets	<i>qBlush.ZC_3.1</i>	LG3	SNP_IGA_12878608	15	15.31	ZD	*****
Blush	2008 & average	<i>qBlush.ZC_7.1</i>	LG7	SNP_IGA_745637	10.34	11.16	CL	*****
FF	2013 & average	<i>qFF.ZC_4.1</i>	LG4	SNP_IGA_395152	15.98	18.8	ZC	*****
FF	2014 & average	<i>qFF.ZC_6.1</i>	LG6	SNP_IGA_670727	37.1	16.12	ZC	*****
FS	2014	<i>qFS.ZC_2.1</i>	LG2	SNP_IGA_230405	0.58	6.8	CL	***
FS	All data sets	<i>qFS.ZC_6.1</i>	LG6	SNP_IGA_614935	1.16	24.11	ZC	*****
FW	All data sets	<i>qFW.ZC_6.1</i>	LG6	B6_1_2	0	28.1	ZC	*****

Table 3. 5 continued

Trait	Year	QTL	Group	Closes SNP	Position (cM)	K* ^a	Source ^b	Signif. ^c
I _{AD}	2014	<i>qI_{AD}.ZC_6.1</i>	LG6	SNP_IGA_5294415	13.49	20.84	ZC	*****
I _{AD}	Average data set	<i>qI_{AD}.ZC_8.1</i>	LG8	SNP_IGA_853101	1.71	11.47	ZC	****
SSC	2014	<i>qSSC.ZC_3.1</i>	LG3	SNP_IGA_366194	28.68	8.21	ZD	****
SSC	2014 & average	<i>qSSC.ZC_4.1</i>	LG4	SNP_IGA_401829	28.84	20.85	CL	*****
SSC	2014	<i>qSSC.ZC_6.1</i>	LG6	SNP_IGA_607528	0	11.3	ZC	****
TA	2013	<i>qTA.ZC_4.1</i>	LG4	SNP_IGA_393777	17.11	10.99	ZC	****
TA	2014	<i>qTA.ZC_4.2</i>	LG4	B4_11_16	22.88	8.67	CL	****
TA	Average data set	<i>qTA.ZC_7.1</i>	LG7	SNP_IGA_778587	40.44	15.54	CL	*****
RI	2014	<i>qRI.ZC_6.1</i>	LG6	SNP_IGA_2750075	2.97	10.29	ZC	**
RI	Average data set	<i>qRI.ZC_7.1</i>	LG7	SNP_IGA_748434	11.22	7.3	CL	***

^a K* = Kruskal-Wallis test.

^b ZD = 'Zin Dai'; CL = 'Crimson Lady'; ZC = 'Zin Dai' and 'Crimson Lady'.

^c *** = $P < 0.01$; **** = $P < 0.005$; ***** = $P < 0.001$; ***** = $P < 0.0005$; ***** = $P < 0.0001$.

(Fig. and Table 3.5). A fruit weight QTL detected on LG6 *qFW.ZC_6.1* ($P < 0.005$) spanned 23 cM, was stable in both years of study. Two I_{AD} QTLs were detected on LG6 and 8. QTL on LG6 (*qI_{AD}.ZC_6.1*), detected with the 2014 data set, spanned 22 cM, peaked at 13.5 cM (SNP_6_5294415, $K = 20.8$; $P < 0.005$), while QTL on LG8 (*qI_{AD}.ZC_8.1*), detected with the average data set, spanned 3 cM, and peaked at 1.7 cM at SNP_IGA_853101 ($K = 11.5$) (Fig. and Table 3.5). Three QTLs for SSC were mapped, one on each LGs 3, 4 and 6; *qSSC.ZC_3.1* (spanned 28 – 30 cM), *qSSC.ZC_4.1* (spanned 10 – 30 cM), and *qSSC.ZC_6.1* (spanned 4 cM). The most linked marker among the 3 SSC QTLs was SNP_IGA_401829 ($K = 20.8$) at 28.8 cM on LG4, and it was inherited from the pollen donor ‘Crimson Lady’ (Fig. and Table 3.5). Three QTLs were detected for titratable acidity (TA, malic acid). Two QTLs on LG4 were detected with yearly data sets ($P < 0.005$); *qTA.ZC_4.1* in 2013, spanned 15 – 18 cM and *qTA.ZC_4.2* in 2014, spanned 21 – 30 cM. QTL on LG7 *qTA.ZC_7.1*, detected with the average data set, spanned 30 – 41 cM. The most significantly linked marker within TA QTLs was SNP_IGA_778587 on LG7 at 40.4 cM ($K = 15.5$), and it was inherited from the pollen donor (Fig. and Table 3.5). Lastly, two QTLs for RI were detected on LGs 6 and 7. *qRI.ZC_6.1* ($P < 0.01$) on LG6 detected with the 2014 data set spanned 1 – 3 cM, peaked at ~ 3 cM at SNP_IGA_2750075 ($K = 10.3$), while QTL on LG7, *qRI.ZC_7.1*, spanned 11 – 31 cM, peaked at 11.2 cM at SNP_IGA_748434 ($K = 7.3$) and was detected with the average data set (Fig. and Table 3.5).

Some QTLs were mapped in the same region creating clusters. For instance, on LG3 cluster of 2 QTLs spanning 24 cM included *qBlush.ZC_3.1* and *qCE.ZC_3.1*, and

cluster of 2 QTLs spanning 6 cM included *qSSC.ZC_3.1* and RAC_2014 QTL. In addition, QTLs for TA (*qTA.ZC_4.1* and *qTA.ZC_4.2*), SSC (*qSSC.ZC_4.1*), and FF (*qFF.ZC_4.1*) overlapped on LG4 from 10 – 30 cM. Furthermore, QTLs for FS, FW, SSC, RI, I_{AD}, RAC, CE, GAE, and C3GE were all overlapped on LG6 creating a cluster spanning 23 cM. A small cluster of GAE *qGAE.ZC_8.1*, C3GE *qC3GE.ZC_8.1*, and I_{AD} *qIAD.ZC_8.1* QTLs were mapped on LG8 spanning 3 cM (Fig. 3. 5).

Haplotyping and candidate gene analysis

Functional alleles were determined for the major QTL on LG6, *qPC.ZC-6.1*, associated with accumulation of all phytochemical compounds. A major QTL encompassed a region from 8 – 11 cM (4305044 – 4777153 bp) with 9 SNPs (SNP_IGA_605605, SNP_IGA_605863, SNP_IGA_605980, SNP_IGA_605986, SNP_IGA_606059, SNP_IGA_606086, SNP_IGA_606435, SNP_IGA_619263, and SNP_IGA_619845). Haplotype analysis revealed two haplotypes, *a* and *b*. ‘Zin Dai’ was homozygous for haplotype *a* and ‘Crimson Lady’ was homozygous for haplotype *b*. Therefore, two grandparental, *aa* and *bb*, and one heterozygote, *ab*, diplotypes were observed in the ZC² progeny (Table 3. 6). Progeny that received the *a* haplotype (*a/a*) from the white fleshed ‘Zin Dai’ showed significantly higher antioxidant capacity ($P < 0.05$) than those that received *b* haplotype (*a/b* and *b/b*) from yellow fleshed ‘Crimson Lady’ (Table 3. 6).

Candidate genes analysis was performed on the major QTL *qPC.ZC-6.1* region on LG6 for each of the 4 compounds separately. Coding sequences were extracted from

Table 3. 6. Phenotypic effect calculated for functional alleles detected in ZC² progeny at the major QTL region on LG6 *qPC.ZC-6.1* associated with relative antioxidant capacity (RAC).

Trait	QTL	Haplotype combination	# of progeny	Mean	Min	Max
RAC	<i>qPC.ZC-6.1</i>	aa	27	753.1 b	355.23	1103.2
		ab	46	518.4 a	187.74	1147
		bb	16	509.1 a	283.3	861.6

Different letters indicate significant differences at $P < 0.05$ according to Student-Newman-Keuls's.

QTL regions of the peach genome v2 (www.rosaceae.org) and blasted using both blastn and tblastn against NCBI nr. to identify potential candidate genes associated with accumulation of phytochemical compounds in peach. Blasting QTL coding sequences of all compounds revealed 20 homologous sequences for 20 candidate genes belonging to 4 genes in 8 species (Table 3. 7). Three genes with functional enzyme acyl-coenzyme A thioesterase and putative esterase from *qRAC.ZC_6.1* region were associated with antioxidant capacity. Homologous sequences were present in different fruit crops; Japanese apricot, apple, and Chinese white pear, and they were highly identical to the peach genome (97, 86, and 86 %, respectively). In *qGAE.ZC_6.1* region, 4 predicted genes belonging to enzymes that could be responsible for total phenolic accumulation. Both *qCE.ZC_6.1* and *qC3GE.ZC_6.1* were detected in the same region. Thirteen candidate genes belonging to nuclear protein (Importin-5) were defined in this region and were involved in the flavonoid pathway. The homologous sequences (> 90%) were observed with Japanese apricot, apple, and Chinese white pear (Table 3. 7).

Table 3. 7. Prediction of candidate genes for phytochemical compounds in major QTL on LG6.

QTL	Predicted gene	Source	Functional annotation	e-value
<i>qRAC.ZC_6.1</i>	LOC103320424	<i>Prunus mume</i>	Acyl-coenzyme A thioesterase 13-like	97% (2e-66)
	LOC103427159	<i>Malus × domestica</i>	Putative esterase F42H10.6	86% (7e-36)
	LOC103963229	<i>Pyrus × bretschneideri</i>	Acyl-coenzyme A thioesterase 13-like	86% (3e-34)
<i>qGAE.ZC_6.1</i>	LOC103341567	<i>Prunus mume</i>	Phytoene dehydrogenase, chloroplastic/chromoplastic	96% (0)
	LOC103418736	<i>Malus × domestica</i>	Phytoene dehydrogenase, chloroplastic/chromoplastic-like	85% (0)
	LOC105637484	<i>Jatropha curcas</i>	Phytoene dehydrogenase, chloroplastic/chromoplastic-like	71% (6e-62)
	LOC102616469	<i>Citrus sinensis</i>	Phytoene dehydrogenase, chloroplastic/chromoplastic-like	74% (2e-36)
<i>qCE.ZC_6.1</i> and <i>qC3GE.ZC_6.1</i>	LOC103327964	<i>Prunus mume</i>	Importin-5	98% (0)
	LOC103407527	<i>Malus × domestica</i>	Importin-5-like	98% (0)
	LOC103964618	<i>Pyrus × bretschneideri</i>	Importin-5-like	98% (0)
	LOC103446988	<i>Malus × domestica</i>	Importin-5-like	88% (0)
	LOC103414914	<i>Malus × domestica</i>	Importin-5-like	87% (0)
	LOC103954091	<i>Pyrus × bretschneideri</i>	Importin-5-like	87% (0)
	LOC101293385	<i>Fragaria vesca</i> subsp. <i>vesca</i>	Importin-5	86% (0)
	LOC103964628	<i>Pyrus × bretschneideri</i>	Importin-5-like	88% (0)
	LOC100265730	<i>Vitis vinifera</i>	Importin-5	79% (0)
	LOC102619446	<i>Citrus sinensis</i>	Importin-5-like	79% (0)
	LOC105630016	<i>Jatropha curcas</i>	Importin-5	78% (0)
	LOC100805849	<i>Glycine max</i>	Importin-5	78% (0)
	LOC103338967	<i>Prunus mume</i>	Importin-5-like	76% (9e-179)

DISCUSSION

Phenotypic analysis

Phenolic compounds are diverse secondary metabolites that include total phenolics, flavonoids and their subgroup anthocyanins. These bioactive compounds are classified under non- enzymatic antioxidants that play significant roles against toxic effects of reactive oxygen species (ROS) (Ahmad et al., 2010; Gill and Tuteja, 2010). The wide variation for all phytochemical data sets observed in this population support the quantitative nature of these traits.

Phenotypic variation was caused either by genotype or environment variation. Grandparental cultivars had contrasting values for phytochemical compounds and fruit quality traits, and transgressive segregation was observed in F1 parent and F2 progeny for most traits, making the ZC² population suitable for mapping these traits. Accumulation of phytochemical compounds, such as total phenolics, flavonoids, and their antioxidant capacity was significantly influenced by different experimental seasons. Environmental conditions between experimental years were very variable (Fig. 2. 5; chapter II; page 75). Variable environmental conditions during experimental years influenced accumulation of phytochemical compounds and their antioxidant capacity. The high accumulation of total phenolics, flavonoids, and their antioxidant capacity during summer 2014, compared to 2013, might be attributed to the daily average temperature of 24 °C and solar radiation (data not shown). It has been shown that solar radiation has a huge influence on expression of genes, such as bHLH3, WD40, and MYBPA1 that are influenced by UV-B radiation and control flavonoid biosynthesis in peach peel and flesh (Ravaglia et al.,

2013). However, the anthocyanin accumulation in our material increased under a wet summer in 2013. This could be due to the production of various anthocyanins with unique chemical structure (Andersen and Markham, 2006), and each anthocyanin profile possesses different biological functions against different free radicals (Garcia-Alonso et al., 2005; Kovinichet al., 2014). Therefore, increased anthocyanin accumulation during a wet, rainy summer might be explained as the reaction of the trees to stress caused by root suffocation resulting in an increase/ up-regulation of cyanidin 3-glucoside activity against superoxide and hydroxyl anion radicals. Observed depletion of anthocyanins under high temperature is in agreement with what has been reported in grape berries, where anthocyanin accumulation and biosynthesis increased under low night temperature (15°C) and decreased during high night temperatures (30°C). The decrease has been attributed to the degradation of anthocyanin and the inhibition of mRNA transcription of the anthocyanin biosynthetic genes (Mori et al., 2005, 2007). Fruit quality traits also showed variability between the two contrasting seasons, with majority of traits at optimum during a normal, sunny summer in 2014.

High heritability (H^2) estimated for fruit quality traits suggested that most of observed phenotypic variance was due to genetic factors. In contrast, H^2 for phytochemical compounds except for anthocyanin (0.99) was very low, suggesting that development of these traits in the ZC^2 population was highly influenced by the environment.

The observed positive correlation of RAC with accumulation of total phenolics and flavonoids is in agreement with previous reports for peach and nectarine (Abidi et

al., 2011; Cantín et al., 2009; Font i Forcada et al., 2014; Vizzotto et al., 2007). This supports the fact that these phenolic compounds play an important role as non-enzymatic antioxidants. The positive correlation between soluble solid concentration (SSC) and phytochemical compounds observed in our material has been previously reported in peach (Abidi et al., 2011; Cantín et al., 2009; Font i Forcada et al., 2014), revealing the essential role of sugars in the biosynthesis of bioactive compounds. Significant positive correlation between FF and I_{AD} (the index for fruit maturity) in peach and nectarine has been previously reported by Infante (2012). Even though I_{AD} is highly genotype dependent, its high correlation with firmness agreed with more mature fruit being less firm in melting freestone peach and nectarine cultivars (Ziosi et al., 2008; Gasic et al., 2014). In addition, a significant positive correlation observed for FW and FS was found in previous studies in peach (Yamaguchi et al., 2002) and tomato (Bohner and Bangerth, 1988; Ho, 1996), suggesting that final fruit size and weight is determined by cell division, expansion, and carbohydrate dilution within cells. The negative correlation between TA and RI has been previously reported in peach by Byrne et al. (1991). This negative correlation is expected since RI is calculated as the SSC/TA ratio. SSC/TA ratio in mature fruit is higher due to higher SSC and results in better taste.

Linkage map construction

Genotyping the full ZC^2 progeny with the 9K SNP array for peach (Verde et al., 2012) provided sufficient data for construction of highly saturated ZC^2 linkage map. The ZC^2 population was previously used for linkage and QTL mapping (Frett et al., 2014). The higher number (90) of genotyped progeny, compared to 25 from Frett et

al. (2014), from the ZC² population resulted in an increased number of total (908) and uniquely mapped markers (347), supporting use of a larger population size to obtain higher resolution mapping (Collard et al., 2005).

The 9K peach SNP array v. 1 provides a large number of high quality SNPs for map construction. It was recently used in development of other peach linkage maps (da Silva Linge et al., 2015; Nuñez-Lillo et al., 2015; Yang et al., 2013; Zeballos et al., 2016). The resolution and number of SNPs in common among published peach maps and the ZC² map were variable, which is probably due to different cultivars/accessions used to create the segregating progeny and/or different size of the mapping populations. The revised ZC² linkage map was comparable to the F2 peach maps from the cross between ‘NJ Weeping’ and ‘Bounty’ (da Silva Linge et al. 2015), and the cross between ‘O’Henry’ and ‘Clayton’ (Yang et al., 2013) with coverage of 58.9 % of the peach genome v 2.0 physical length was obtained in the ZC² linkage map vs. 93.6% and 63 % of the peach genome v.1 in the NB and OC maps, respectively. In addition, a linkage map obtained from the self-pollination of the nectarine ‘Venus’ published by Nunez-Lillo et al. (2015) covered a genetic distance of 389.2 cM, which was similar or close to our genetic coverage (~ 336 cM). The number of mapped SNPs in the revised ZC² map (908) was higher than that published by Zeballos et al. (2016) in F1 population (104 in ‘Venus’ map and 122 in ‘Big Top’ map). The number of uniquely mapped markers in the ZC² linkage map (347) was also higher than in a recently published ‘Venus’ x ‘Venus’ genetic map (332) (Nuñez-Lillo et al., 2015) and OC linkage map (258) (Yang et al., 2013). Even though a high resolution map we obtained, there are still some large

gaps that might be related to either the population size or the number of markers still not large enough to fill these gaps. The largest gaps in our genetic map were observed on LGs 1 and 6 (19.7 and 15.1 cM, respectively). These values are similar to the ones reported by Yang et al. (2013) and Frett et al. (2014) who used the same genotyping strategy.

QTL analysis for pomological traits

The accuracy of phenotypic evaluation is very important for the accuracy and the reliability of QTL mapping. Evaluation of traits in different years provides accuracy of QTLs through reducing experimental error (Salazar et al., 2013). In the current study, majority of traits were not normally distributed even after transformation, which could be due to presence of some extreme values or that some values were close to zero. Therefore, the non- parametric Kruskal-Wallis test was best suited for QTL analysis.

Numerous candidate genes/ quantitative trait loci (QTLs) for different traits have been mapped in peach (Arús et al., 2012; Bielenberg et al., 2009,2015; da Silva Linge et al., 2015; Dirlewanger et al., 2004; Font i Forcada et al, 2013; Fresnedo- Ramírez et al., 2015, 2016; Frett et al., 2014; Illa et al., 2011; Nuñez-Lillo et al., 2015; Ogundiwin et al., 2009; Yang et al., 2013; Zeballos et al., 2016). Few studies reported QTLs associated with phytochemical compounds in Rosaceae and *Prunus* species. In apple, many polyphenolic compositions and vitamin C QTLs have been identified using SNPs (Chagné et al., 2012; Verdu et al., 2014; Davey et al., 2006), while in F1 nectarine population, Zeballos et al. (2016) have identified 7 QTLs linked to phytochemical compounds using SNP and SSR markers.

QTLs associated with accumulation of all phytochemical compounds and fruit quality in peach were successfully detected using the revised ZC² map.

Flavonoid QTL detected on LGs 2 and 3 in ZC² progeny is in agreement with a recently reported QTL by Zeballos et al. (2016) using a F1 nectarine population derived from ‘Venus’ and ‘Big Top’ with the pomological traits evaluated over 4 years. The rest of phytochemical compound QTLs that detected in this study; on LG1 for total phenolics *qGAE.ZC_1.1*, LG3 for antioxidant capacity and flavonoid accumulation *qRAC.ZC_3.1* and *qCE.ZC_3.1*, LG6 for all phytochemical compounds *qRAC.ZC_6.1*, *qGAE.ZC_6.1*, *qCE.ZC_6.1*, and *qC3GE.ZC_6.1*, and on LG8 for the accumulation of total phenolics and anthocyanin *qGAE.ZC_8.1* and *qC3GE.ZC_8.1* are reported for the first time in peach. Abidi et al. (2010) reported that none of the QTLs associated with biochemical traits and antioxidant capacity in peach were mapped on LG6 of a F1 population derived from the cross ‘Venus’ × ‘Big Top’ nectarines using SSRs. Our results revealed a major QTL *qPC.ZC-6.1* for all phenolic compounds and antioxidant capacity on LG6, indicating the presence of genes involved in phytochemical accumulation in this region. This linkage group also includes QTLs for fruit quality traits, confirming the correlation between bioactive compounds and fruit quality, in which they play an important role in the visual appearance (pigmentation and browning) and taste (astringency) of fruit, in addition to their health-promoting properties (Cantín et al., 2009; Rice-Evans et al., 1996). Moreover, numerous QTLs associated with soluble solids and compounds involved in peach aroma were reported on LG6 (Dirlewanger et al., 1999; Eduardo et al., 2013).

Blush QTLs were detected on three linkage groups (1, 3, and 7) in the revised ZC² map, which corroborated blush QTLs on LG3 and LG7 previously reported by Frett et al. (2014) using the same population. A major blush QTL on LG3, *qBlush.ZC_3.1*, was mapped with all datasets. SNP_IGA_341962 was one of the closest markers to the *qBlush.ZC_3.1* and is positioned 5.49 kbp upstream of *PprMYB10* (Frett et al., 2014). Fruit size QTL, *qFS.ZC_2.1*, mapped on LG2 supports the QTL for FS detected using pedigree based analysis (Fresnedo- Ramírez et al., 2015). A fruit weight QTL, *qFW.ZC_6.1*, was stable in the two years and detected with all FW datasets on LG6. QTLs for FW on LG6 have been previously reported in peach (da Silva Linge et al., 2015; Dirlewanger et al., 1999; Eduardo et al., 2011). QTLs for FF, *qFF.ZC_4.1*, SSC, *qSSC.ZC_4.1*, and TA, *qTA.ZC_4.1* detected on LG4 are in agreement with those reported by Cantín et al. (2010), Dirlewanger et al. (1999), and Eduardo et al. (2011). A QTL for fruit firmness, *qFF.ZC_4.1*, mapped on LG4, collocates with the previously reported QTL for maturity date (Eduardo et al., 2011; Etienne et al., 2002; Nuñez-Lillo et al., 2015), which supports the suggestion that the maturity process includes and/or regulates several fruit quality traits, such as softening rate in peach (Dirlewanger et al., 2012). Mapping SSC QTLs on LGs 3 and 4 is in agreement with other studies in peach (Eduardo et al., 2011) and apricot (Socquet-Juglard et al., 2012). A QTL for TA, *qTA.ZC_4.1*, detected on LG4 has also been reported in apricot (Salazar et al., 2013).

In this study, some QTLs for different traits were co-localized in the same region on linkage groups 3, 4, 6, and 8 creating clusters. Clustering of QTLs could be explained by correlations between those traits. For instance, overlapping between QTLs

for phytochemical compounds and SSC could be interpreted by the high correlation between these traits, supporting the essential role of sugars in the regulation of synthesis of phenolic compounds (DeJong, 1999; Font i Forcada et al., 2014).

Furthermore, overlapping of the QTLs for FW and FS on LG6 are in agreement with the positive correlation between these two traits as it has been previously reported in other fruit crops (Bohner and Bangerth, 1988; Ho, 1996). Mapping QTLs for TA and RI on separate linkage groups (LG 4 and 6, respectively), and SSC and RI on the same LG (6) supports the observed negative correlation between TA and RI, and positive correlation between RI and SSC. RI is determined as SSC / TA ratio, therefore its higher value suggests that more mature fruit is less sour and has better taste in standard peach cultivars. However, in sub acid peach cultivars RI is usually very high due to a low TA and does not always support higher SSC or better taste.

No overlapping of QTLs for anthocyanin and blush was detected. This could be because only anthocyanins in flesh tissue were analyzed (the skin and the red tissue around the stone were removed) and the likely regulators (MYB10.1 and MYB10.3, with bHLH3) of anthocyanin biosynthesis in peach fruit are highly expressed in the skin and mesocarp around the pit, but show low expression in the mesocarp (Abdur Rahim et al., 2014).

QTLs associated with phytochemical compounds were mapped on different linkage groups with different datasets, indicating the influence of the environment in the year of the study on the accumulation of these compounds. Therefore, evaluation of accumulation of phenolic compounds and their antioxidant capacity in additional years

are needed to increase the accuracy of the detected QTLs. It has been found that different environmental and seasonal conditions influence both the concentration of anthocyanin in red apricot (Bureau et al., 2009) and the accumulation and identification of QTLs for anthocyanin's antioxidant contents in red raspberry (Kassim et al., 2009). QTLs for total phenolics and flavonoids accumulation were distributed on different LGs, and none of them were repeated over the years of study, which agrees with Zeballos et al. (2016) observations based on 4 years of phenotypic data. Anthocyanin accumulation and the composition of the accumulated anthocyanins have been reported to be variable in different tissue. For example, three anthocyanin compounds were found only in skin for one apricot cultivar, while same compounds were presented in both skin and flesh in another apricot cultivar (Bureau et al., 2009). It has been suggested that different individual compounds that constitute phenolic compounds have different effects and potential for improving human health (Brown et al., 2014; Pandey and Rizvi, 2009). Therefore, further analysis of specific compounds is needed to understand the genetics behind their accumulation. In addition, detection of QTLs for phytochemical compounds on different linkage groups support the quantitative nature of these traits as suggested previously (Font i Forcada et al, 2013; Kassim et al., 2009; Zeballos et al., 2016). QTLs for fruit quality traits, such as *qFW.ZC_6.1* and *qFS.ZC_6.1* on LG6 and *qBlush.ZC_3.1* on LG3 were highly stable in both years of evaluation, suggesting that they were not affected by climatic variations which was also supported by H^2 . FW QTLs at the same position have been reported recently by Zeballos et al. (2016). Identifying QTLs at different locations over different years is commonly found in QTL analysis for peach and

other *Prunus* species (Dirlewanger et al. 1999, 2012; Etienne et al. 2002; Eduardo et al. 2011; Salazar et al. 2013).

Haplotyping and candidate genes for phytochemical compounds in peach

Phenolic compounds are excellent scavengers of free radicals due to their antioxidant capacity. These compounds play an important role in the visual appearance and taste of fruit. The major QTL for phytochemical compounds, *qPC.ZC_6.1*, was mapped on LG6 and haplotyping and candidate gene study were performed on this region. This region includes clusters of QTLs for all measured phenolic compounds and their antioxidant capacity, confirming that phytochemical compounds (phenolics, flavonoids and their subgroup anthocyanin) serve as non-enzymatic antioxidants and have the antioxidant capacity against free radicals (Ahmad et al., 2010; Blokhina et al., 2003; Gill and Tuteja, 2010). Moreover, this region overlapped with several fruit quality QTLs, confirming the strong correlation between accumulation of bioactive compounds and fruit quality traits suggested previously (Cantín et al., 2009; Rice-Evans et al., 1996).

Two haplotypes detected in the F2 progeny were designated *a* and *b*. The highest antioxidant capacity for all phenolic compounds was observed in progeny homozygous for *a* haplotype. This haplotype was observed in the white-fleshed grandparent ‘Zin Dai’, supporting previous observations that more phenolic compounds are accumulated in white-fleshed peaches than yellow-fleshed ones (Brown et al., 2014; Cantín et al., 2009; Gil et al., 2002). Low accumulation of phytochemical compounds has been observed in yellow-fleshed ‘Crimson Lady’ that exhibited *bb* diplotype.

A candidate gene study was performed to uncover genes within the major QTL on LG6 associated with accumulation of phytochemical compounds. Four types of candidate genes involved in the biosynthesis pathway of phytochemical compounds were identified in this study. Two genes were annotated as acetyl CoA type in the *qRAC.ZC_6.1* region. In plant peroxisomes, acetyl CoA reacts with glyoxylate to yield malate, which is converted to oxaloacetate and used for glucose synthesis (Cooper, 2000). Glucose is involved in the initial steps of the flavonoid pathway (Davies and Schwinn, 2003; Patra et al., 2013). In addition, 4 candidate genes detected in *qGAE.ZC_6.1* region were annotated as phytoene dehydrogenase, which involves in one of the non- enzymatic antioxidant biosynthesis pathway, (Hirschberg et al., 1997; Ruiz-Sola and Rodríguez-Concepción, 2012). Another interesting candidate gene, detected in flavonoids and their subclass anthocyanin QTL region, is importin-5 protein. Importin-5 (IPO-5) protein is one of the nuclear proteins that plays an important role in the eukaryotic cell by transporting molecules between the cytoplasm and the nucleus (OMIM:<http://www.omim.org/>). This protein could be involved in transporting phenolic compounds from the endoplasmic reticulum, their biosynthesis location, to different cellular organelles. In addition, only one candidate gene belongs to putative esterase F42H10.6 found in apple and involved in fatty acid synthesis (KEGG:<http://www.genome.jp>). Nine SNPs mapped in the major QTL, *qPC.ZC-6.1*, on LG6 are good candidates for MAB for accumulation of phytochemical compounds in peach.

CONCLUSION

Quantitative traits are influenced by the complex action of many genes and environments. Phenotypic and genetic diversity was observed in the ZC² population. Significant correlations were observed between different phenolic compounds and fruit quality traits. A highly saturated linkage map consisting of 8 linkage groups and spanning a total length of ~ 336 cM with an average marker density of 1.10 cM/marker was created and used in QTL mapping. Twenty-eight QTLs were successfully mapped with different sources and years. Three QTLs, *qFW.ZC_6.1* and *qFS.ZC_6.1* on LG6 and *qBlush.ZC_3.1* on LG3 were highly stable with all data sets, suggesting that more years of evaluation are needed to confirm some of the QTLs. Five QTL clusters were identified on four linkage groups, with largest cluster including all phytochemical compounds and majority of fruit quality traits on LG6, showing the significant correlation between the traits and suggesting tight linkage between genes controlling these traits. These discovered markers/ QTLs provide the first step in development of DNA test for implementation of MAB for phytochemical compounds.

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CHAPTER FOUR
WHOLE-GENOME ANALYSIS OF DIVERSITY AND SNP-MAJOR
GENE ASSOCIATION IN MODERN PEACH BREEDING
GERMPLASM

INTRODUCTION

Peach (*Prunus persica* L.) belongs to the *Rosaceae* family, *Prunoideae* subfamily, and *Prunus* genus, which includes many stone fruits, such as plum, apricot, cherry, and almond. Peach is native to China where it was domesticated 2000 years B.C. (Huang et al., 2008), spread to the Mediterranean through Persia and then brought to America by Spanish explorers (Hedrick, 1917). Peach has great economic and social importance in the U.S.A. and worldwide. Peach breeding germplasm has a narrow genetic diversity due to limited number of parents used in breeding programs (Byrne, 1999; Scorza et al., 1985; Scorza and Okie, 1990). This leads to reduce diversity through using modern cultivars that share a few common ancestors (Aranzana et al., 2003). A full characterization of available germplasm is essential to provide breeders with valuable information on parental selection, which could expand the genetic base of modern peach germplasm. Genetic improvement of peach has resulted in the development of many cultivars worldwide, and many of these cultivars are used in the United States commercially or for breeding (Okie, 1998). Most cultivars in the U.S. market are yellow-fleshed, freestone, melting peaches and nectarines, while those from Asian countries, such as, China, Japan, and South Korea are predominantly white-fleshed (Della Strada and Fideghelli, 2003). Understanding genetic diversity and

population structure of the breeding germplasm will provide helpful genetic information for breeding and genetic studies to explore the markers/ QTLs associated with important traits in peach. Genetic diversity has been extensively studied in *Prunus* species, such as peach (Cao et al., 2012; Font i Forcada et al., 2013; Li et al., 2013; Micheletti et al., 2015), apricot (Maghuly et al., 2005; Wang et al., 2014), cherry (Barac et al., 2014), and almond (Font i Forcada et al., 2015) using different molecular markers.

Population structure has been studied in different crops using different markers such as on peach and nectarine cultivars (Aranzana et al., 2010; Cao et al., 2012; Font i Forcada et al., 2013; Li et al., 2013; Micheletti et al., 2015), sweet cherry (Mariette et al., 2010), apricot (Wang et al., 2014), rice germplasm (Jin et al., 2010), and wheat (Maccaferri et al., 2005). Several crops have been reported to have complex population structures resulting from their domestication and breeding history (Flint-Garcia et al., 2003). It is very important to understand population structure to avoid nonfunctional, spurious associations in association mapping.

Release of the peach genome sequence (Verde et al., 2013) and 9K peach SNP array (Verde et al., 2012) has facilitated an exponential increase in genetic studies and their application in breeding (Aranzana et al., 2012; Martínez-García et al., 2013; Verde et al., 2012). In addition, recent advances in next generation sequencing technology are providing low-cost, high throughput options for genome-wide single nucleotide polymorphism (SNP) genotyping of numerous individuals facilitating genetic mapping and population studies (Elshire et al., 2011; Bielenberg et al., 2015).

Genotyping -By-Sequencing (GBS) is a cost-effective, high throughput genotyping method that reduces genome complexity by using restriction enzymes and in process generates high number of genetic markers (Elshire et al., 2011). The GBS has been proven as rapid and cost-competitive genotyping method for peach (Bielenberg et al., 2015) even though it could generate considerable amount of missing data and a varying distribution of sequence reads (Beissinger et al., 2013).

Most genetic studies in peach have been done using bi-parental mapping approach (Abbott et al., 2008). Recently, with the availability of high throughput genotyping platforms, such as Illumina 9K peach SNP array (Verde et al., 2012), attempts of mapping quantitative trait loci (QTL) using pedigree based analysis (Fresnedo- Ramírez et al., 2015, 2016) and genome- wide association (GWAS) (Micheletti et al., 2015) have been reported.

GWAS examines many genetic variants among different individuals to determine regions in genome associated with the traits of interest by identifying high number of markers covering the entire genome (Meuwissen, 2007; Riedelsheimer et al., 2012; Zhao et al., 2011; Zhu et al., 2008). Many association genetic (association mapping) studies for different pomological traits in peach and nectarine cultivars (Cao et al., 2012; Dhanapal and Crisosto, 2013; Font i Forcada et al., 2013; Micheletti et al., 2015), in almond (Font i Forcada et al., 2015), and in Japanese pear (Iwata et al., 2013) have been recently published. Mapped markers associated with different pomological traits in peach could be applied to other *Prunus* and *Rosaceae* species due to the high synteny within the Rosaceae family (Dirlewanger et al., 2004).

One of the goals of breeding programs, besides improving fruit quality, productivity and disease resistance, is to increase the accumulation of the nutritionally beneficial compounds (phytochemical compounds). Understanding the level of diversity of these compounds in the modern peach breeding germplasm will advise breeding programs on the feasibility of improving accumulation of phytochemicals in newly developed cultivars. Therefore, there is a need to identify and select genes that control accumulation of these metabolic products through marker-assisted breeding (MAB) while keeping the other fruit qualities constant. The objective of this study was to evaluate diversity and population structure in modern U.S. peach breeding germplasm and use GWAS for detecting genes with genome wide effects on accumulation of phytonutritional compounds and fruit quality. The results would inform the growers, consumers and breeders of the level of diversity and nutritional benefits in the peach and nectarine cultivars available on the U.S. market and further enable development of a DNA test for early selection of individuals with improved accumulation of phytochemical compounds.

MATERIALS AND METHODS

Plant material

A collection of ninety-six peach and nectarine [*Prunus persica* (L.) Batsch] cultivars and fifteen advanced selections, maintained at Clemson University Musser Fruit Research Center near Seneca, SC, were used in this study (Table 4. 1). This germplasm covers a wide range of geographic origins (North America, Europe, and Asia). The collection is grown under a warm, humid, temperate climate and standard commercial practices for irrigation, fertilization, pest and disease control. The trees were at least 8 years old, grafted on Guardian®TM rootstock, grown in triplicate, with 2 x 6 m spacing and perpendicular V training system.

Phenotyping

Phenotypic variation for fruit quality and phytochemical compounds was evaluated over two years (2013 and 2014). Five fruits from each accession were selected at commercial ripe by measuring Index of Absorbance Difference (I_{AD}) (Ziosi et al., 2008). Five fruits in the ripening stage equivalent to $I_{AD} = 0.6$ were selected to ensure uniformity of maturity for all accessions.

Phenotyping protocols for fruit quality and phytochemical compounds were provided in detail in chapter II (pages 50 - 52). The following fruit quality traits were measured: fruit size (mm), fruit weight (g), fruit firmness (kg), soluble solids concentration (SSC) (Brix %), titratable acidity (TA) (malic acid %), and ripening index (RI), using the standardized peach phenotyping protocol (Frett et al., 2012).

Table 4. 1. Main fruit characteristics, origin, and pedigree of the accessions included in the study.

Genotype	Fruit type	Flesh color	Origin	Breedig program	Release date	Pedigree
7 Ball	P	Y	Michigan	Private		-
Arctic Belle	N	W	California	Private	1998	141LB505 (Arctic Queen OP) OP
Arctic Blaze	N	W	California	Private	1998	23R236 x 63EC404
Arctic Gold	N	W	California	Private	1995	Ruby Gold x Redwing Peach
Arctic Pride	N	W	California	Private	1993	[Ruby GoldxRedwing] x [O’HenryxGiant Babcock]
Arctic Star	N	W	California	Private	1995	White Nectarine x May Glo
Arctic Sweet	N	W	California	Private	1996	97ED497 x June Glo
Arrington	N	Y	Arkansas	Public	2002	A_178 x A_232
Augustprince	P	Y	Georgia	Public	2006	Sunprince x BY92P2710
Autumn Flame	P	Y	California	Private	1996	A48-70 x 1-14S-28
Autumnred	P	Y	California	Public	1996	O’Henry x Fairtime)
Belle of Georgia	P	W	Georgia	Public	1875	Chinese Cling OP
Blazeprince	P	Y	Georgia	Public	1997	BY81P2840 OP
Blazingstar	P	Y	Michigan	Private	1995	Fayette x Newhaven
Bradley	N	Y	Arkansas	Public	2002	A_190 x A_178
BY00p4945	P	R	Georgia	Public	1893	-
BY01p9239	P	W	Georgia	Public	1893	-
BY02p4019	P	Y	Georgia	Public	1893	ZinDai x Crimson Lady
BY02P3997	P	Y	Georgia	Public	1893	ZinDai x Crimson Lady
BY02p4826	P	W	Georgia	Public	1893	-
BY02p4840	P	Y	Georgia	Public	1893	-
BY92P2710	P	Y	Georgia	Public	1893	Flameprince x BY87P943
BY95p5518	P	Y	Georgia	Public	1893	-
BY99p4388	P	R	Georgia	Public	1893	-

Table 4. 1 continued

Genotype	Fruit type	Flesh color	Origin	Breedig program	Release date	Pedigree
BY99p4570	P	R	Georgia	Public	1893	BY95p6384 OP
Carogem	P	Y	South Carolina	Public	1989	Redglobe x Ranger
Caroking	P	Y	South Carolina	Public	1987	Fantasia x Loring
Carored	P	Y	South Carolina	Public	2005	Springbrite x pollen mix
China Pearl	P	W	North Carolina	Public	2001	Contender x PI134401
Chinese Cling	P	W	China	Public	1850	-
Clayton	P	Y	North Carolina	Public	1976	Pekin x Candor
Contender	P	Y	North Carolina	Public	1987	Winblo x NC64
Coronet	P	Y	Georgia	Public	1953	[Halehaven x Halehaven] x Dixigem
Crimson Lady	P	Y	California	Private	1992	RedDiamond x Springcrest
CVN-1	P	Y	Tennessee	Private	-	-
Dixired	P	Y	Georgia	Public	1945	Halehaven x Halehaven
Early Elegant Lady	P	Y	California	Private	1990	Elegant Lady MUT
Elberta	P	Y	Georgia	Public	1889	Chinese Cling x EarlyCrawford
Empress	P	Y	California	Private	1964	[(Flory Dwarf x Red Grand) F2]
Encore	P	Y	New Jersey	Public	1980	NJ585414 x Autumnglo
Fireprince	P	Y	Georgia	Public	1985	FV6_1130 x FV324_25
Flameprince	P	Y	Georgia	Public	1993	BY68_3877 OP
Flavorich	P	Y	California	Private	-	[MayGrand opxSamHouston] x [TastyGoldxMaycrest]
Flavortop	N	Y	California	Public	1969	Fairtime OP
Gala	P	Y	Louisiana	Public	1992	Harvester O.P.
Galaxy	P	W	California	Public	1995	P34_106 x D33_1
Glacier	P	W	California	Private	2001	(36RB243 x 103ED581) OP
Glenglo	P	Y	West Virginia	Private	1996	-

Table 4. 1 continued

Genotype	Fruit type	Flesh color	Origin	Breedig program	Release date	Pedigree
Goldcrest	P	Y	California	Public	1984	FV9_164 OP
Hakuho	P	W	Japan	-	?	Hakuto x Tachibana Wase
Harcrest	P	Y	Canada	Public	1983	Redskin x H_4219
Harrow Diamond	P	Y	Canada	Public	1984	Redskin x Harbinger
Harvester	P	Y	Louisiana	Public	1973	Redskin x SouthernGlow
Honey Blaze	N	Y	California	Private	1998	(36EB86 x 9GC175) F2
Honey Kist	N	Y	California	Private	1995	36ER86 x 9GC175
Intrepid	P	Y	North Carolina	Public	2002	[Redhaven x (Reliance x Biscoe)]
Jade	N	W	France		1993	Alpes x RR53272?
Jayhaven	P	Y	Michigan	Public	1976	SH333 x SH348
Joanna Sweet	P	Y	California	Private	1999	55GA106 x 33EB323
Julyprince	P	Y	Georgia	Public	-	L75_A50_20 x BY89P2787
Juneprince	P	Y	Georgia	Public	1985	FV325-58 x Junegold
Karla Rose	N	W	California	Privae	1975	24GA795 x 24R215
KV021693	P	Y	West Virginia	Public	-	KV981056 OP
KV980330601	P	Y	West Virginia	Public	-	-
KV980677801	P	Y	West Virginia	Public	-	-
Lauroi	P	Y	New Jersey	Private	1994	Jerseyqueen MUT
Loring	P	Y	Missouri	Public	1946	Frank x Halehaven
May Lady	P	Y	California	Private	1968	Gemfree x Blazing Gold
NC97-45	P	Y	North Carolina	Public	-	-
O'Henry	P	Y	California	Private	1970	Merrill Bonanza OP
Parade	P	Y	California	Private	1960	?
PF12B	P	Y	Michigan	Private	2002	-

Table 4. 1 continued

Genotype	Fruit type	Flesh color	Origin	Breedig program	Release date	Pedigree
PF23	P	Y	Michigan	Private	1993	-
PF Lucky 13	P	Y	Michigan	Private	2003	-
Raritan Rose	P	W	New Jersey	Public	1936	J.H. Hale x Cumberland
Redglobe	P	Y	Maryland	Public	1954	[(Admiral Dewey x St. John) x Fireglow]
Redhaven	P	Y	Michigan	Public	1940	Halehaven x Kalhaven
Redrose	P	W	New Jersey	Public	1940	J.H. Hale x Delicious
Rubira	P	W	France		1980	-
Rubyprince	P	Y	Georgia	Public	1997	Fireprince x BY78ANG55
Scarletpearl	P	W	Georgia	Public	1989	[Biscoe x Redgold] OP
Scarletprince	P	Y	Georgia	Public	-	Blazeprince OP
Snowbrite	P	W	California	Private	1993	[(O'Henry x Giant Babcock) x (May GrandxSam Houston)]
Snow Prince	P	W	California	Private	1997	(24GA795 x 24R215) F2
Spring Lady	P	Y	California	Private	1981	-
Springold	P	Y	Georgia	Public	1966	FV89_14 x Springtime
Springprince	P	Y	Georgia	Public	1998	Springcrest OP
Starfire	P	Y	Michigan	Private	1995	Fayette x Newhaven
Stark Saturn	P	W	New Jersey	Public	1985	NJ602903 x Pallas
Sugarlady	P	W	California	Private	1991	[(O'Henry xGiant Babcock) x MayGrand OP)]
Sugar May	P	W	California	Private	1991	29G560 x 54G262
Summergold	P	Y	Georgia	Public	1970	[(J.H. HalexValiant)OP] x Redglobe
Summerprince	P	Y	Georgia	Public	1992	[(Summerset x BY4_7364)OP]
Summer Sweet	P	W	California	Private	1992	23R236 x 44EB108
Sunbrite	P	Y	Georgia	Public	1976	FV7_873 x FV9_327
Sunprince	P	Y	Georgia	Public	1982	Redglobe x FV9_6288

Table 4. 1continued

Genotype	Fruit type	Flesh color	Origin	Breedig program	Release date	Pedigree
Suzi Q	P	Y	South Carolina	Private	1982	-
Sweet Blaze	P	Y	California	Private	2000	39EB706 x 34GA1155
Sweet Dream	P	Y	California	Private	1998	Unknown.
Sweet N Up	P	Y	West Virginia	Public	2004	[(Bounty x pillar) x (Firered x pillar)]
Sweet Scarlet	P	Y	California	Private	1996	39EB706 x Sweet Gem
Topaz	P	Y	Missouri	Public	1976	Loring x Loring
UFGold	P	Y	Florida	Public	1998	Fla84_18C x Fla9_20C
Vulcan	P	Y	Canada	Public	1994	Veecling x NJC95
Westbrook	N	Y	Arkansas	Public	2002	A_172 x A_176
White Lady	P	W	California	Private	1986	[(O'Henry x Giant Babcock) x (May Grand x Sam Houston)]
Wildrose	P	W	New Jersey	Public	1947	J.H. Hale x Delicious
Winblo	P	Y	North Carolina	Public	1972	Redskin x Redskin
Yukon King	P	W	California	Private	1997	07ED101 x 97GF518
Zephyr	N	W	France		1992	-
Zin Dai	P	W	China		?	-

Fruit type: peach (P), nectarine (N); flesh color: yellow (Y), white (W), and red (R); parent(s) unknown (-); open pollinated (OP); mutation (MUT); Arkansas (A); New Jersey (NJ); Fort Valley (FV); plant introduction (PI).

Phenolic compounds and their antioxidant capacity were measured using methanol extracts from 500 mg of frozen composite powder. The extracts were incubated at +4 °C overnight, centrifuged for 10 min at 12,000 g at +4 °C to collect the supernatant, and stored at -80°C until needed. The following compounds were measured: total phenolics, expressed as mg GAE / 100g FW; flavonoids, expressed as mg CE/ 100g FW, and their subclass anthocyanin expressed as mgC3GE/kg FW; and relative antioxidant capacity (RAC) expressed as µg Trolox Equivalent/ g FW, using protocols published in Cantín et al., (2009b) (details in chapter II; pages 50 - 52).

Statistical Analysis

All statistical analyses were performed in SPSS v. 23 (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). All data are expressed as means \pm SE of five replicates. Differences were compared by one-way ANOVA and Student-Newman-Keuls's test at $P < 0.05$.

Genotyping

Fresh young leaves from each of the 111 individuals (Table 4. 1) were harvested, refrigerated during transportation, and then frozen in liquid nitrogen. Two grams of frozen tissue was ground in liquid nitrogen using pre-chilled mortar and pestle, and stored at -80°C until needed. Genomic DNA was extracted following Dellaporta et al. (1983) method with specific adaptations for peach. DNA quantity and quality were measured using a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and Bioteck (Bio-tek HT Synergy Multidetector microplate reader, Winooski, VT). Final concentration of 100 ng of DNA was used for

downstream application. Genotyping-by-sequencing (GBS) was performed following the original protocol described in Elshire et al (2011) with specific modifications for peach (Bielenberg et al 2015). Ninety-six double- stranded forward adaptors each with a unique barcode and a single common double- stranded reverse adaptor were created from a set of 194 single-stranded oligonucleotides (IDT, Coralville, IA, U.S.A.). Each adaptor contained a three base overhang for ligation with *Ape*KI digested DNA. The ApeKI compatible barcode set was that published in Elshire et al. (2011). Adaptors were ligated to restriction-digested DNA following protocol described in Elshire et al (2011). Each sample was pooled into 96 multiplex and cleaned up with Qiagen PCR cleanup kit (QIAquick PCR Purification Kit). PCR primers were added and libraries amplified to increase the fragment pool. After, another clean up with Qiagen PCR cleanup kit, the library pool was sent to the David H. Murdock Research Institute (Kannapolis, NC) for sequencing on the Illumina HiSeq® 2000 platform.

Processing of sequence reads

Sequence reads were processed using default parameters of the TASSEL 4.0 GBS pipeline (Glaubitz et al., 2014) as described in Bielenberg et al. (2015). Assembled scaffolds of the peach genome v2.0 were downloaded from the Genome Database of Rosaceae (www.rosaceae.org; Verde et al., 2013) and used as the reference sequence for alignment of sequenced reads with Bowtie v2.1 (Langmead and Salzberg, 2012). SNP calls from duplicate taxa and duplicate SNPs from opposite strands were merged and exported as hapmap and vcf files. Hapmap files were imported to the TASSEL GUI 5.0 for visualization of results and initial filtering of samples with fewer reads and SNPs

with missing data (Bradbury et al. 2007).

SNPs were named according to scaffold and base pair position within the peach genome v2.0 build, as explained in Bielenberg et al (2015). In short, SNP names contain the scaffold number, and three to eight characters denoting the base position.

Filtering and minor allele frequency (MAF)

Site and taxa filter tools in TASSEL software (version 5.0) were used (Bradbury et al., 2007). SNPs and accessions showing more than 25% missing data were excluded from further analyses. In addition, the minor allele frequency was calculated for each SNP, and SNPs with $MAF \geq 0.05$ were discarded from further analyses.

Phylogenetic tree

The distance matrix was calculated using TASSEL and visualized using Archaeopteryx Tree in TASSEL 5.0. TASSEL 5.0 was also used to create files for subsequent analysis in *fastSTRUCTURE* (Raj et al., 2014).

Population structure

Principle component analysis (PCA) using SNP data was performed in TASSEL 5.0 (Bradbury et al., 2007). Population structure was studied using the *fastSTRUCTURE* v.1.0 (Raj et al., 2014) software. *fastSTRUCTURE* performs inference for the simplest, independent-loci, admixture model, clustering individuals into multiple subpopulations with a different coefficient, with the sum of all being equal to 1 based on k subgroups. Choosing the appropriate number of model components that explain structure in the dataset, was performed using the algorithm for multiple choices of K, provided in the *fastSTRUCTURE*. A utility tool is used to parse through the output of these runs and

provide a reasonable range of values for the model complexity appropriate for the dataset. Visualization of the expected admixture proportions inferred by *fastSTRUCTURE*, have been performed using a simple tool to generate DISTRUCT (Rosenberg, 2004) plots using the mean of the variable posterior distribution over admixture proportions. The samples in the plot were grouped according to population labels inferred by *fastSTRUCTURE*.

Association analysis

The association analysis was performed using general linear model (GLM) in TASSEL v 5.0 (Bradbury et al., 2007). This function performs association analysis using a least squares fixed effects linear model accounting to test for association between segregating sites and phenotypes and accounts for population structure using covariates (Q matrix) that indicate degree of membership in underlying populations. A main effects only model was automatically built using all variables in the input data. A separate model was built and solved for each trait and marker combination. Any factors, covariates, reps or locations are included in every model as main effects. Bonferroni multiple test corrections ($P < 0.05$) were used to detected significant association between SNP markers and traits.

RESULTS

Sequencing and detection of SNPs

Genotyping -By-Sequencing (GBS) generated about 134.2 Mb barcode reads in total, with an average of 1.19 million sequence reads per genotype. Barcode count of more than 500K has been observed in 64% of accessions and more than 200K barcode count has been detected in 79.8% of accessions. TASSEL GBS pipeline using peach genome v 2.0 as a reference genome identified 115,361 SNP markers in 111 accessions, evenly distributed across all scaffolds of the peach genome. After filtering for SNPs with $MAF < 0.05$, and accessions with more than 25% data missing, 97,276 SNPs and 25 accessions were excluded. The final dataset comprised 86 individuals and 18,085 SNPs that were used in further analysis. The highest number of SNPs was observed on chromosome 1(3,470), while chromosome 5 exhibited the lowest number of SNPs (1,793) (Appendix IV). The 18,085 SNPs provided an average coverage of 78.6 SNPs/Mb of total peach genome size (230 Mbp).

Breeding Germplasm characterization

Majority of the breeding germplasm originated from North America (80), while a few cultivars originated from Asia and Europe. Among the North American accessions, 54 are from public breeding programs, and 26 from private breeding programs. Only 3 cultivars originated in Asia (Chinese Cling, Hakuho, and Zin Dai), and 3 in Europe (Jade, Zephyr, and rootstock Rubira). A phylogenetic tree constructed from the SNP data divided the 86 germplasm accessions based on their genetic diversity and relatedness into three subclusters (Figure 4. 1). Heirloom cultivars, such as Elberta and Chinese Cling were clustered close to each other. Cultivars from Asia were also clustered close to each

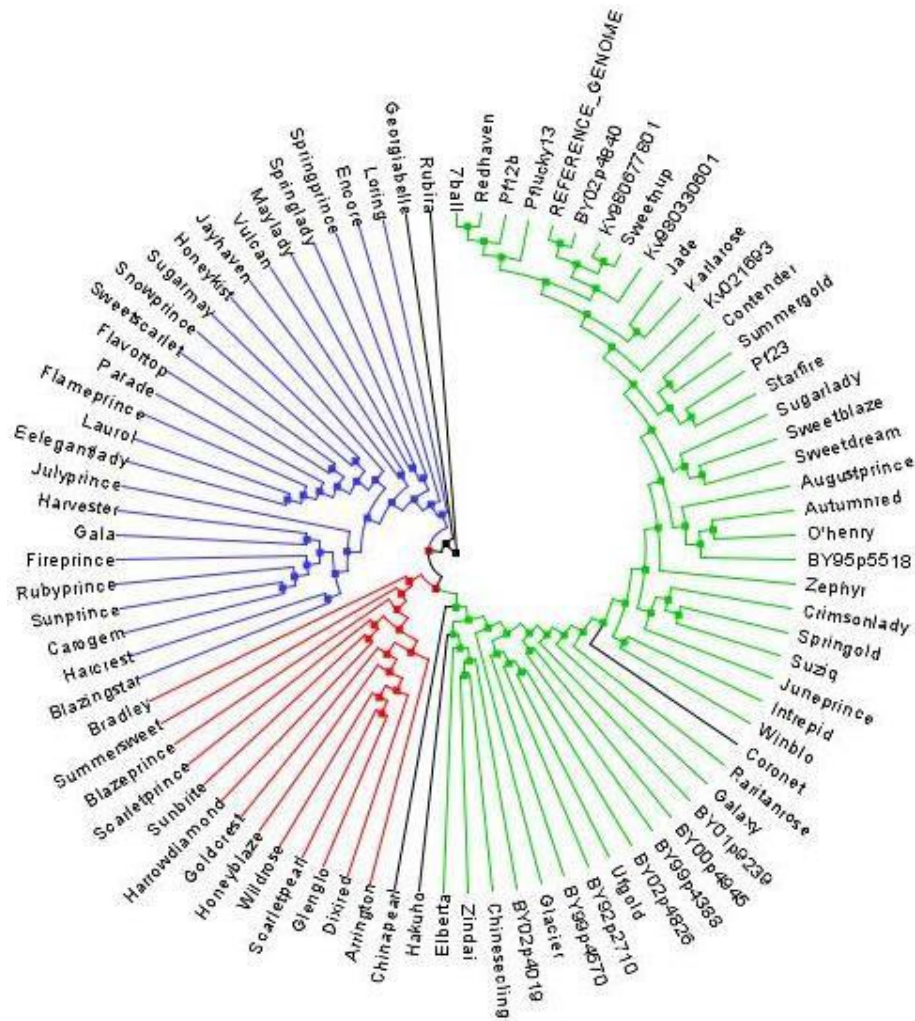


Figure 4. 1. UPGMA dendrogram showing structure of the modern peach breeding germplasm. Colors indicate the assignment into populations based on *fastSTRUCTURE* analysis at $k=3$; black lines, unstructured accessions (admixture).

other according to the similarity in their origin and genetic background. Further, these cultivars were highly homozygous (99% of their SNP genotypes identical). Advanced selections, included in the panel, also exhibited high homozygosity and were clustered together, as shown in the phylogenetic tree (Figure 4. 1).

Cultivar variability and population structure

A total of 86 accessions were genotyped with 18,085 SNPs. The observed mean heterozygosity (H_o) per SNP was 0.27, ranging from 0.011 in S2_20249597 to 0.98 in S5_10085932. The lowest heterozygosity was observed in chromosome 6 ($H_o = 0.193$), while the highest heterozygosity was in chromosome 7 ($H_o = 0.205$). The H_o between individuals ranged from 0.16 in ‘Scarletpearl’ to 0.45 in ‘Belle of Georgia’, with an average of 0.27. The proportion of missing alleles ranged from 0.2 % in ‘Sweet Blaze’ to 22 % in ‘Scarletpearl’.

The first approximation of population structure, obtained by principle component analysis (PCA) using 18,085 SNP dataset, separated the germplasm into three groups (subpopulations) (Figure 4. 2), confirming the phylogenetic results.

The *fastSTRUCTURE* and DISTRUCT software were used to investigate in more detail stratification in the germplasm panel. The 86 peach and nectarine accessions, from different continents (North America, Europe, and Asia), grouped into three subpopulations ($k = 3$) (Figure 4. 3 a) based on membership coefficient ($Q \geq 80$ %), congruently with the results from the phylogenetic tree (Figure 4. 1) and PCA analysis (Figure 4. 2). Five cultivars (China Pearl, Coronet, Belle of Georgia, Hakuho, and Rubira) did not cluster with any group, considering them as unstructured individuals (admixed) (Figure 4. 3 b). *fastSTRUCTURE* divided the panel into 3 subpopulations corresponding with cultivar origin and some with breeding program (public and private). For example, when looking at accessions that clustered together, those from similar origin, such as Chinese Cling and Zin Dai (from Asia) were grouped together as well as those from the same breeding program, such as ‘Elberta’ and

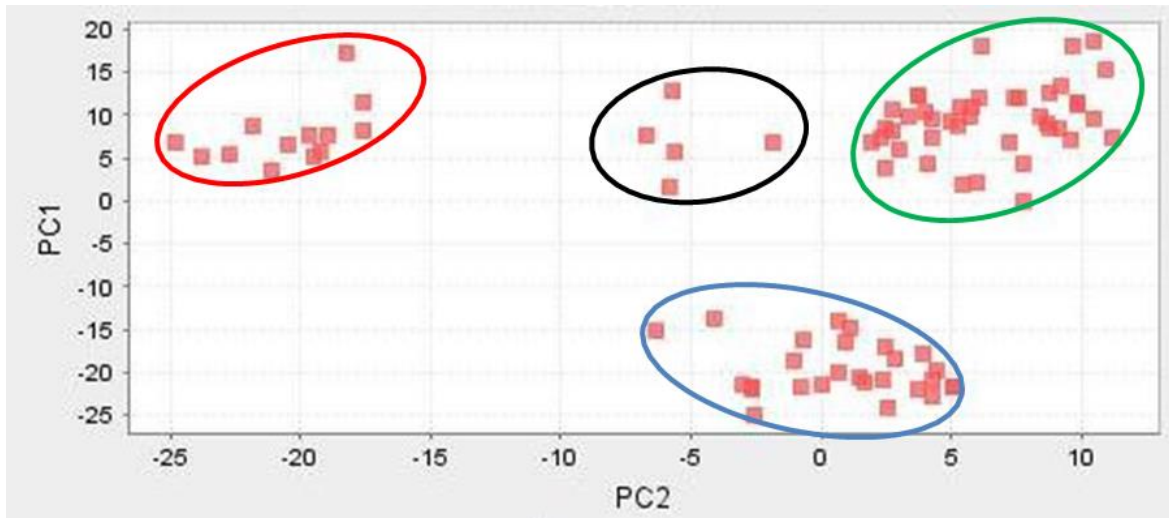


Figure 4. 2. Principle component analysis of the 18,085 SNPs in 86 accessions. Three subpopulations (in blue, red, and green) were separated. Admixtures are presented in black.

‘Chinese Cling’. In addition, all advanced selections that belong to a public breeding program (USDA Byron, GA) were clustered in the same subpopulation. Furthermore, some of the peach cultivars from early public breeding programs were grouped together sharing the same seed parent (Table 4. 1). Thus, ‘Carogem’ and ‘Sunprince’ are clustered together as they are half siblings sharing the same mother ‘Redglobe’. Similarly, this was observed for ‘Harcrest’ and ‘Harvester’, half siblings that share ‘Redskin’ as a seed parent.

No separation was observed between white and yellow -fleshed accessions. However, all 3 red-fleshed advanced selections included in this panel were clustered together. When the North American accessions were grouped in two populations based on breeding programs they originated from, public or private, also no clear separation was observed. Accessions from all 3 subpopulations were integrated/

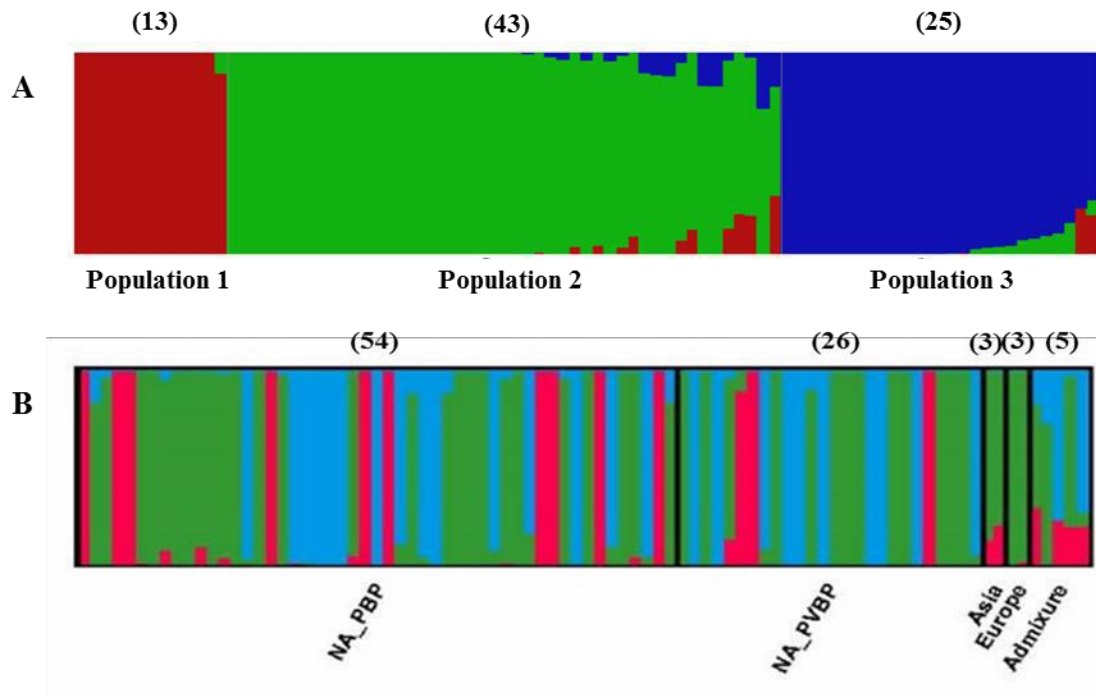


Figure 4. 3. Population structure in 86 *P. persica* genotypes ($k = 3$). Red, green, and blue bars represent accessions within three subpopulations, while individuals with $Q < 80\%$ considered as unstructured (admixture) are presented as unfilled bars (A). Five groups were separated by a black line and each individual is shown as a thin vertical line using DISTRUCT based on *fastSTRUCTURE*. (B). Numbers at the top of each column give the number of individuals included. NA_PBP= North American public breeding program; NA_PVBP= North American private breeding program.

distributed through both public and private breeding populations as shown in Figure 4. 3 b.

Genome-wide association

The association mapping was performed using SNPs present in 75% of individuals with $MAF \geq 0.05$ (18,085 SNPs), phenotypic data for all evaluated traits (10), and accounting for structure of the population applying general linear model (GLM) in TASSEL software.

Markers tightly associated with the traits of interest were widely distributed across the eight scaffolds of the peach genome. Significant associations were identified for 181 SNPs and six traits; three fruit quality traits (FF, RI, and TA) and three phenolic compounds (RAC, CE, and C3GE). Results of GLM analysis for all significantly associated SNPs with the six pomological traits are presented in Appendix V.

Fifty-two SNPs were associated with fruit quality (FF= 1, RI= 44, and TA= 7 SNPs) and 129 SNPs were associated with phytochemical compounds. Majority of SNPs (121) were associated with anthocyanin accumulation. For fruit quality traits, a single marker on chromosome 1 (S1_29416421) was tightly associated with fruit firmness ($P = 10^{-8}$) (Figure 4. 4 a). Seven markers were associated with TA (malic acid %) and 44 with RI. Three SNPs, S5_930892, S5_985948 and S5_987397, located on chromosome 5, were the most significantly associated with both TA and RI (Figure 4. 4 b).

Four SNPs located on chromosome 7 were significantly associated with both antioxidant capacity and flavonoids accumulation ($P = 10^{-7}$), with S7_12434842 being the most significantly associated marker to both compounds. Several markers were strongly linked to accumulation of anthocyanins; with the majority of SNPs (30) mapped on chromosome 1. The most significantly associated SNP, S4_5693485; $P = 10^{-9}$, was mapped on chromosome 4 (Figure 4. 5).

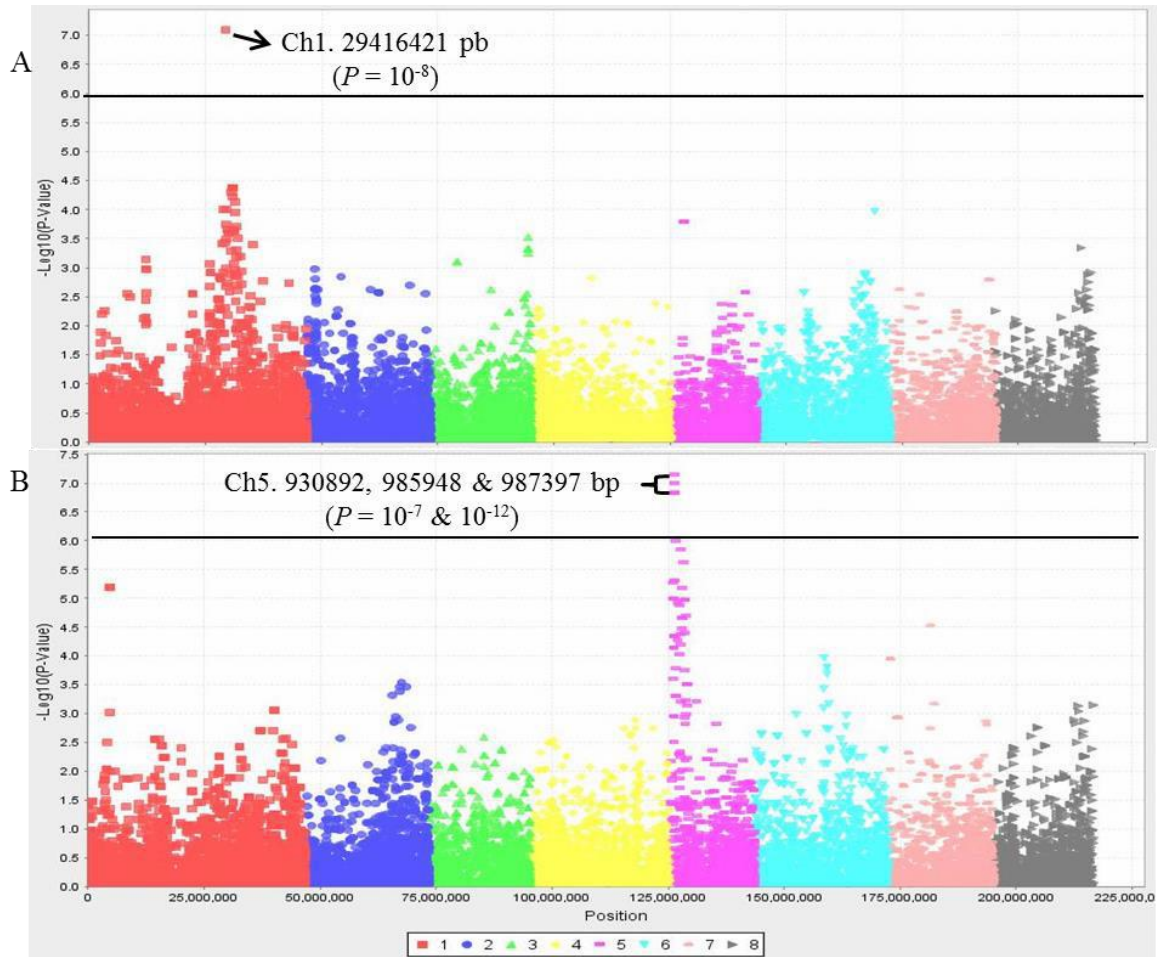


Figure 4. 4. Manhattan plot showing genome-wide association using general linear model (GLM) for (A) fruit firmness, and (B) titratable acidity and ripening index. Each color represents different chromosome. The horizontal solid line represents the significance threshold 2.7×10^{-6} at a significance level of $P < 0.05$ after Bonferroni multiple test correction.

For all significant markers associated with traits of interest except for titratable acidity, genotypes with major alleles showed a negative effect on the trait. Presence of major alleles (C, A, and G) for the most significantly linked markers to TA (S5_930892, S5_985948, and S5_987397, respectively) on LG 5 increased the trait (Table 4. 2). The

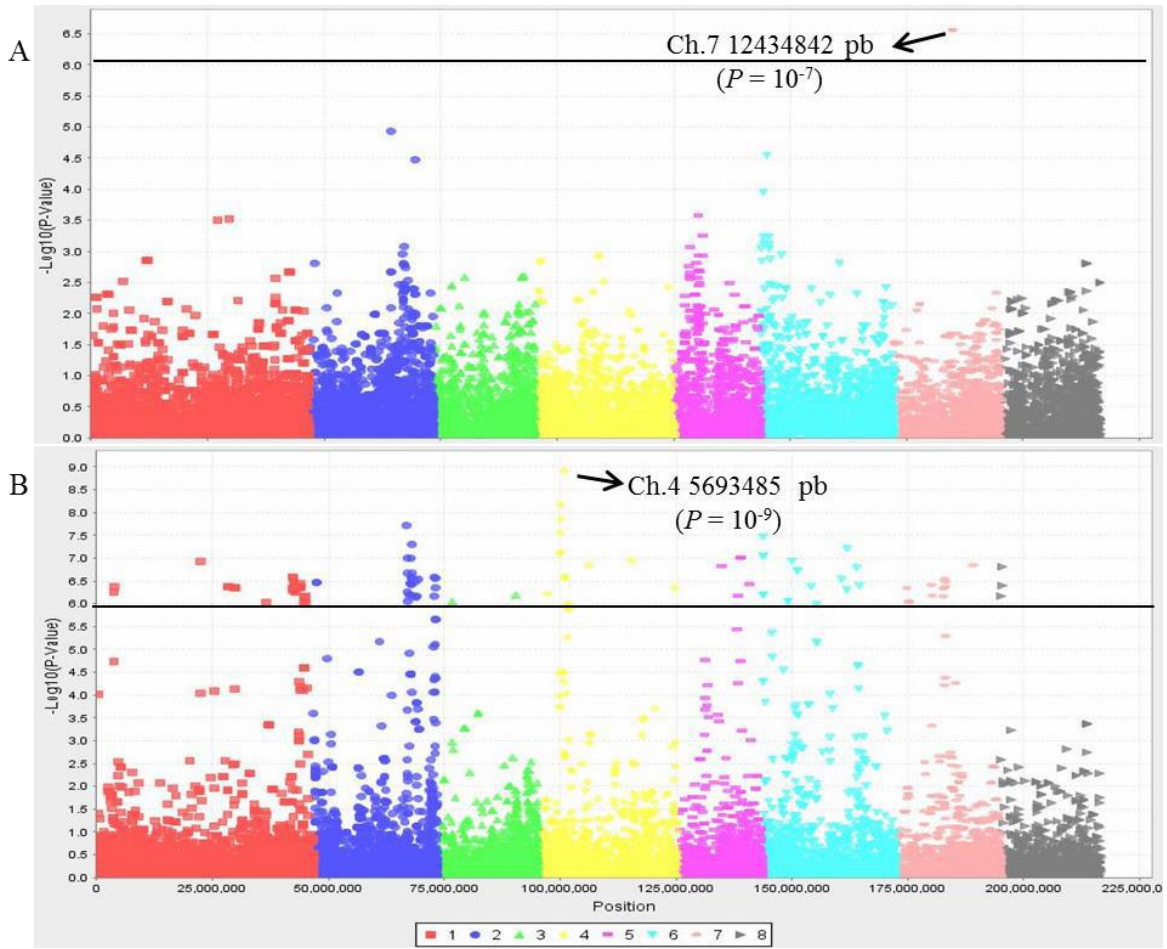


Figure 4. 5. Manhattan plot showing genome-wide association using general linear model (GLM) for (A) antioxidants and flavonoids, and (B) anthocyanin. Single nucleotide polymorphisms (SNPs) detected across the whole peach genome. Each color represents different chromosome. The horizontal solid line represents the significance threshold (2.7×10^{-6}) for the association of each trait.

same major alleles for these markers conversely affected the ripening index trait. Allele effects on phenotype expression and number of observed individuals in each genotype category for each significantly associated SNP were presented in Appendix VI.

Table 4. 2. GLM analysis for the most significantly associated markers with six traits based on Bonferroni multiple tests using GLM.

Trait	SNP name	P-value	Allele effect					
			Major Allele	Obs	Effect	Minor Allele	Obs	Effect
FF	S1_29416421	8.18E-08	G	16	-0.25	T	5	0
TA	S5_930892	9.99E-08	C	32	0.42	G	2	0.05
TA	S5_985948	7.07E-08	A	33	0.40	G	3	0
TA	S5_987397	1.47E-07	G	30	0.54	A	3	0
RI	S5_930892	4.21E-12	C	32	-23.72	G	2	-5.12
RI	S5_985948	7.86E-12	A	33	-23.21	G	3	0
RI	S5_987397	2.14E-11	G	30	-29.99	A	3	0
RAC	S7_12434842	2.75E-07	A	38	-1008.0	G	4	0
CE	S7_12434842	1.77E-07	A	38	-31.51	G	4	0
C3GE	S4_5693485	1.23E-09	G	38	-24.99	C	1	0

Effects of major and minor alleles on phenotypic traits (Effect) and number of genotypes observed (Obs) for each maker. FF, Fruit firmness; TA, Titratable acidity; RI, Ripening index; RAC, relative antioxidant capacity; CE, catechin equivalents; C3GE, cyanidin-3-glucoside equivalents.

Significant differences in phenotypic effect of the observed genotypes (GG, TT, and GT) for SNP S1_29416421 were only observed in two out of six traits (anthocyanin content and fruit firmness). There were 16 individuals with genotype GG, 5 with TT, and 21 with genotype GT. Individuals exhibiting genotype TT accumulated on average 9.1 mg/kg of anthocyanin and had 2.8 kg fruit firmness (Table 4. 3). Three genotypes were observed for the most significant marker on chromosome 4 S4_5693485 (GG, CC, and GC) associated with anthocyanin. Significant differences among these genotypes were observed for 5 out of 6 traits, with homozygous genotype CC having positive effect on the traits. There were 38 individuals with genotype GG, one individual, red-fleshed advanced selection BY99p4388, with CC genotype, and 2 individuals with genotype GC;

‘Augustprince’ and ‘Summergold’. Significant differences in phenotypic effects of different genotypes for SNP S5_930892 (CC, CG, and CG) were observed for TA and RI; where individuals with CC genotype (32) exhibited higher TA and lower RI.

Homozygous genotypes for major alleles A and G of SNPs S5_985948 and S5_987397, respectively, exhibited a positive effect on the expression of all traits except RI. There were 33 individuals with AA genotype and 30 with GG genotype. For the most significant SNP associated with antioxidant capacity and flavonoids accumulation on chromosome 7, S7_12434842, there were only two genotypes, AA and AG, with higher accumulation of phytochemical compounds observed in accessions with heterozygous genotype AG, such as ‘Elberta’, ‘Encore’, ‘Belle of Georgia’, and ‘Laurol’.

Table 4. 3. The phenotypic effect of the most significantly associated markers on six traits, observed genotype and number of individuals in each genotype class.

Marker name	Allele	Genotype	# of indivs	Antioxidant ($\mu\text{g TE/ gFW}$)	Flavonoids (mg CE/100gFW)	Anthocyanin (mg C3GE/kg)	FF (kg)	TA (%)	RI (SSC/TA)
S1_29416421	G/T	GG	16	717.9 a	17.3 a	5.4 a	1.7 a	0.6 a	21.7 a
		TT	5	853.5 a	21.1 a	9.1 b	2.8 b	0.7 a	16.5 a
		GT	21	714.1 a	17.1 a	5.7 a	1.8 a	0.8 a	18.3 a
S4_5693485	G/C	GG	38	719.5 a	17.1 a	5.0 a	2.2 a	0.7 a	19.8 a
		CC	1	1254.7 b	33.1 b	29.9 c	3.3 b	1.1 b	10.3 a
		GC	2	680.3 a	17.1 a	13.5 b	2.8 ab	0.7 a	16.9 a
S5_930892	C/G	CC	32	742.8 a	18.3 a	6.3 a	2.3 a	0.7 b	15.7 a
		GG	2	575.1 a	12.2 a	4.0 a	2.1 a	0.4 a	36.4 b
		CG	5	619.7 a	12.9 a	4.2 a	2.2 a	0.3 a	39.5 b
S5_985948	A/G	AA	33	761.2 b	18.8 b	6.5 b	2.2 a	0.8 c	15.1 a
		GG	3	506.8 a	11.9 a	3.3 a	2.6 a	0.5 b	27.5 b
		AG	6	685.3 b	14.4 ab	4.5 ab	2.2 a	0.3 a	38.4 c
S5_987397	G/A	GG	30	762.2 b	18.7 b	6.6 b	2.2 a	0.8 b	15.1 a
		AA	3	468.6 a	8.9 a	3.1 a	2.2 a	0.4 a	33.6 b
		GA	5	860.5 b	21.4 b	6.4 b	2.2 a	0.4 a	34.2 b
S7_12434842	A/G	AA	39	648.5 a	15.4 a	5.5 a	2.3 a	0.7 a	20.2 a
		AG	4	1594.4 b	43.4 b	11.3 b	2.0 a	0.8 a	16.5 a

Different letters indicate significant differences at $P < 0.05$ according to Student-Newman-Keuls's and t test.

TE, trolox equivalents; CE, catechin equivalents; C3GE, cyanidin-3-glucoside equivalents; FS, fruit size; FF, fruit firmness; TA, titratable acidity; RI, ripening index.

DISCUSSION

Association genetics could be a valuable tool in assessing diversity of peach germplasm as well as uncovering genetics behind the traits governed by many genes of small effects. One of the challenges peach breeders face is the narrow genetic base of peach germplasm (Aranzana et al., 2003). To overcome the narrow genetic base of modern peach cultivars, breeders need to widen genetic diversity and incorporate new genetic backgrounds by utilizing germplasm collections (Aranzana et al., 2010; Cao et al., 2012).

Material included in this study represents modern peach breeding germplasm grown or available in the U.S market. It contains important breeding parents, such as, ‘Elberta’ and ‘O’Henry’, recent releases from private and public breeding programs and few advanced selections. Majority of the accessions (80) originated in North American breeding programs, while a small number is from Europe and Asia. The mean observed heterozygosity per individual (0.21) observed in our material was lower than that reported by Micheletti et al. (2015) (0.28), which was expected because they analyzed material from 4 different European peach germplasm collections while our efforts concentrated on highly selected material with limited diversity.

Phylogenetic analysis, PCA, and population structure of the breeding germplasm all revealed 3 major clusters consistent with genetic background and origin, but not breeding programs. Phylogenetic analysis agreed with pedigree, clustering half siblings ‘Carogem’ and ‘Sunprince’ close to each other, as they share the same seed parent ‘Redglobe’. In addition, ‘Elberta’ and ‘Hakuho’ clustered close to ‘Chinese

Cling’, confirming the former two being the descendants of the ‘Chinese Cling’ (Okie, 1998; Li et al., 2013). Cultivars of Asian origin, such as Chinese Cling and Zin Dai were clustered close to each other supporting the similarity in their origin and fruit characteristics. The majority of advanced selections, included in the study clustered together revealing shared genetic background and similar lineage utilized in the USDA-ARS, Byron, GA breeding program, from where they originated.

Numerous peach cultivars are grown all over the world, and new ones are released every year that exhibit variability in their color, size and growing characteristics based on their origin (Della Strada and Fideghelli, 2003). Population structure revealed separate clusters based on origin with cultivars from Asia, Chinese Cling and Zin Dai, and from Europe, Jade and Zephyr, clustering together. Some cultivars originating from public and private breeding programs clustered separately revealing separation in the origin and diversity in the newly released cultivars, possibly due to the decrease in number of releases by public breeding programs and increase in cultivars released by private programs over last 20 years (Byrne, 2005).

Genome wide association (GWA) is a powerful approach used to examine genetic variation between different individuals by identifying large number of markers covering the entire genome in order to determine regions in the genome associated with the traits of interest. Only few reports on markers / QTLs for nutritional quality in peach and nectarine are available (Font i Forcada et al., 2013; Zeballos et al., 2016). Font i Forcada et al. (2013) have identified simple sequence repeat (SSR) markers that are significantly associated with reactive antioxidant capacity, total phenolics, and

flavonoids. QTLs for different phytochemical compounds using both SSRs and SNPs also have been recently detected in an F1 nectarine population derived from ‘Venus’ and ‘Big Top’ by Zeballos et al. (2016).

In our study, GWA revealed 181 markers with significant associations with six traits: fruit firmness (FF), titratable acidity (TA), ripening index (RI), antioxidant capacity (RAC), and accumulation of flavonoids (CE) and anthocyanin (C3GE). A significantly linked marker to TA detected on chromosome 5 was previously reported in peach germplasm using the GWAS approach (Micheletti et al., 2015), and using the bi-parental QTL mapping approach (Dirlewanger et al., 1999; Etienne et al., 2002; Quilot et al., 2005). Similarly, marker associated with ripening index on chromosome 5 detected in our work, supports ripening QTLs mapped in peach on the same LG (Zeballos et al., 2016). Markers associated with malic acid and ripening index, identified on chromosome 5, are in the same region where QTLs for FF and SSC in nectarine were recently reported (Zeballos et al., 2016) using the bi-parental QTL mapping approach, supporting observed correlation between these traits, in which more mature fruit usually is less firm, less sour, and more sweet in standard cultivars. SNPs associated with anthocyanin accumulation on chromosomes 4 and 6 were collocated with QTLs for SSC previously reported in peach (Dirlewanger et al., 2005; Etienne et al., 2002; Font i Forcada et al., 2013; Quilot et al., 2004). This observation is supported by reported significant correlation between sugars and anthocyanin biosynthesis (Abidi et al., 2011; Cantín et al., 2009a b; Font i Forcada et al., 2014). SNPs linked to anthocyanin accumulation detected on chromosome 6 collocate with QTLs associated

with different compounds in peach, involved in the flavonoid pathway and function, such as sugars and volatile organic compounds (Dirlewanger et al., 1999; Eduardo et al., 2013). SNPs associated with anthocyanin accumulation on chromosome 5 have recently been reported by Zeballos et al. (2016) in bi-parental nectarine progeny between ‘Venus’ and ‘Big Top’ using 4 years of phenotypic data.

The wide distribution of significantly associated markers with evaluated traits across the peach genome observed in this study, especially those associated with anthocyanin accumulation, could be interpreted by the differences in the environmental conditions observed between years of study (Fig. 2.5 Chapter II). It has been observed previously that environmental conditions can affect accumulation of phenolic compounds. For example, UV light increases accumulation of phenolic compounds like anthocyanin in the peach skin and flesh (Andreotti et al., 2009; Ravaglia et al., 2013).

The effects of major and minor alleles on some traits are supported with observed correlations in this study (data not shown). For instance, the contrasting effect of S5_930892, S5_985948 and S5_987397 major alleles (C, A, and G, respectively) on the TA and RI traits confirms the negative correlation between these two traits found in this study ($r = -0.882$, $P < 0.01$), in which the majority of ripe fruit had less sourness and better taste.

Associations were detected between many SNPs and traits, in which major alleles showed negative effect on the accumulation of phytochemical compounds, suggesting the decrease of positive alleles in modern breeding germplasm. Potential explanation might be that breeding for traits such as size and appearance eliminated

alleles with positive effects on accumulation of phytonutritional compounds. It is known that under stress plants tend to increase accumulation of phenolic compounds to support antioxidant scavenging of free radicals. Given that peach breeding is mostly based on phenotypic observation it would be expected that accessions under stress would suffer from poor fruit size and/or appearance and therefore would be eliminated from breeding program. On the other hand, minor alleles exhibited positive effects on the traits. For instance, the red-fleshed advanced selection with homozygous genotype (CC) of minor allele for the most significantly SNP associated with anthocyanon accumulation, exhibited the highest accumulation of anthocyanin, confirming what have reported about accumulating significantly higher anthocyanin in red-fleshed peaches and plums (Vizzotto et al., 2007).

CONCLUSION

Preservation and utilization of genetic diversity is necessary for food security.

Breeding programs depend on genetic diversity being preserved so when the need is presented, the source of the beneficial allele(s) can readily be identified and is available. Unfortunately, breeding activities tend to involuntarily decrease diversity of the germplasm by focusing on few, at the time deemed important, traits.

Understanding the diversity of the breeding germplasm and its structure is the first step in its preservation. Molecular tools, if and when available, allow for fast and efficient screening of the available germplasm. Identifying alleles/genes that control favorite traits (simple and/or complex) in peach helps the development of DNA tools and increases efficiency of improving and releasing new cultivars through marker-assisted breeding (MAB). This phenotypic study revealed huge variability in the pomological traits in the modern peach breeding germplasm that could not always be explained on a molecular level. Population structure grouped breeding germplasm into 3 subpopulations supporting what is known about its background and geographic origin. GWAS successfully identified markers associated with six out of ten pomological traits. Close evaluation of marker effects on phenotypic performance provided useful information for further understanding the combination of alleles needed to maximize trait expression. Analyses also revealed the importance and necessity of repetitive phenotypic data to account for environmental effects on evaluated traits, especially accumulation of phenolic compounds. Marker-trait associations based on either a single marker or haplotype have identified genetic variants of quantitative traits providing useful resources for enabling marker-assisted breeding in peach.

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CHAPTER FIVE

DISCUSSION

Peach is one of the most economically important fruit tree crops worldwide along with other fruit species that belong to the genus *Prunus* and *Rosaceae* family. It is regarded as a summer fruit with a special taste and healthy benefits in the human diet. Phenolic compounds that accumulate in peach fruit provide good sources of antioxidants and protect against free radicals, thereby decreasing the risk for different chronic diseases in humans (Arts and Hollman, 2005; Cantín et al., 2009b; Giampieri et al., 2012; Prior and Cao, 2000; Vizzotto et al., 2007). Medical studies have shown that phytochemical compounds from peach fruit improve total radical-trapping potential of plasma in humans (Dalla Valle et al., 2007) and selectively kill breast cancer cells (Noratto et al., 2009). Consequently, there has been a growing interest in breeding programs to obtain information on phenolic compounds and antioxidant capacity of existing germplasm and its potential to provide enhanced health benefits to consumers (Brown et al 2014; Cantín et al., 2009b; Orazem et al., 2011; Vizzotto et al., 2007). Improvement of the fruit quality characteristics of peach have been the focus of many breeding programs (Byrne et al., 1991; Cantín et al., 2010b) driving research to understand the genetics behind these traits. Consumer interest in functional foods resulted in nutritional improvement becoming an important goal in many breeding programs. Numerous studies on composition and accumulation of the phytochemical

compounds in peach fruit revealed the potential of wild and cultivated germplasm for improvement of these compounds (Brown et al 2014; Cantín et al., 2009b; Vizzotto et al., 2007). However, only a few reports on the genetics behind accumulation of phytochemical compounds in peach fruit are available (Font i Forcada et al, 2013; Zeballos et al., 2016). Phenotypic evaluation of accumulation of these compounds is time consuming and requires fruit. For breeding programs to efficiently and effectively incorporate health promoting compounds in newly developed cultivars, DNA tests are necessary. For this reason, more studies are needed to identify QTLs and genes associated with phytochemical compounds in peach. Moreover, synteny and collinearity among *Prunus* species and within the *Rosaceae* family allow using a comparative marker framework for map-based predictions of gene locations that determine traits of economic importance within different species of the family (Dirlewanger et al., 2004).

A narrow genetic base and low diversity observed in peach germplasm is due to its self-compatibility and a limited number of parents used in breeding programs (Byrne, 1999; Scorza et al., 1985; Scorza and Okie, 1990). Reduced diversity is further promoted by using modern cultivars that share a few common ancestors as parents in breeding programs (Aranzana et al., 2003). A full characterization of available germplasm is essential to provide breeders with valuable information on its potential for parental selection, which could expand the genetic base of modern peach.

In this study, peach germplasm evaluation for phytochemical compounds revealed two heirloom cultivars, white-fleshed Belle of Georgia and yellow-fleshed

Elberta, with the highest potential for accumulation of bioactive compounds and antioxidant capacity. Both ‘Belle of Georgia’ and ‘Elberta’ are direct descendants of ‘Chinese Cling’, which played a pivotal role in the development of the U.S. peach industry, and most of the U.S. peach industry is based on a very narrow genetic base that traces back to ‘Elberta’ as a progenitor. Modern peach breeding germplasm although variable in its potential to accumulate phytochemical compounds showed lower levels in these compounds than its ancestors. Breeding for fruit quality, size, appearance and productivity inadvertently decreased the phytochemical value of modern peach cultivars. Considering that improvement of the level of phytochemical compounds in newly developed cultivars is becoming one of the main objectives in many breeding programs, and that the modern peach germplasm presents a valuable resource for improvement of these traits, breeders are poised to make significant improvements in health promoting properties of this delicious fruit.

Variation in phytochemical compounds and fruit quality observed within analyzed materials and between experimental years could be due to genetic complexity (the quantitative nature) of the traits and/or to the effect of different environments. In addition to environment, accumulation of bioactive compounds was highly influenced by genotype, flesh color, and ripening season. Pearson’s correlation coefficient showed significant correlations between studied traits. Among all fruit quality parameters, soluble solid contents (SSC; Brix %) correlated positively with all phytochemical compounds, which is in agreement with observations made by Abidi et al. (2011), Cantín et al. (2009 a, b), and Font i Forcada et al. (2014), supporting the essential role

of sugars in the biosynthesis of bioactive compounds (Font i Forcada et al., 2014; DeJong, 1999). A positive correlation observed between antioxidants, flavonoids, and total phenolics has also been previously reported in prunes (Abidi et al., 2011; Cantín et al., 2009b; Vizzotto et al., 2007), supporting the supposition that these phenolic compounds play an important role as non-enzymatic antioxidants.

Phytochemical compounds have been found to be under polygenic inheritance and influenced by different environmental seasons (Font i Forcada et al., 2013; Kassim et al., 2009; Zeballos et al., 2016), making it challenging for breeders to work on improvement of these traits. Understanding the genetic control of quantitative traits facilitates development of new cultivars through different approaches of breeding programs (Peace and Norelli, 2009). Release of the peach genome (Verde et al., 2013), availability of the *Prunus* reference map (Howad et al., 2005), and SNP genotyping platforms (Elshire et al., 2011; Verde et al., 2012) provide the opportunity to determine the inheritance of many quantitative traits at the molecular level. In addition, association mapping provides an alternative approach for discovery of new markers/QTLs to enable marker-assisted selection and breeding. Genetic mapping is based on recombination and requires genetic diversity to achieve high resolution mapping (Collard et al., 2005; Paterson, 1996).

An improved, highly saturated linkage map using additional progeny from the ZC² population (Frett et al., 2014) was developed. Genotyping additional progeny with the same SNP array (Verde et al., 2012) provided necessary recombination events for higher resolution mapping (Collard et al., 2005). The number of uniquely mapped

markers (347) in an improved ZC^2 map was higher than in recently published peach maps of 190 (Frett et al., 2014), 332 (Nuñez-Lillo et al., 2015) and 258 (Yang et al., 2013) markers, which were developed using the same genotyping platform.

Two mapping approaches have been used to increase the possibility and precision of identifying locations of QTLs associated with phytochemical compounds and fruit quality traits; bi-parental mapping and genome wide association (GWAS). Bi-parental mapping although powerful is constricted with the variability existing in the mapping population. In contrast, GWAS provides a genome wide perspective on markers and genes associated with traits of interest. Together, they could be used as a complimentary way to map and confirm the regions associated with traits of interest.

Bi-parental mapping is one of the most frequently used approaches to determine QTLs associated with traits of interest. This approach requires segregating populations derived from crosses between contrasting genotypes and it has been extensively used in peach (da Silva Linge et al., 2015; Frett et al., 2014; Yang et al., 2013). QTLs associated with all pomological traits in peach were successfully detected in this study using the revised ZC^2 map. QTLs associated with soluble solids concentration, *qSSC.ZC_4.1*, titratable acidity, *qTA.ZC_4.1*, and fruit firmness, *qFF.ZC_4.1*, all detected on linkage group 4 have previously been reported by Cantín et al. (2010a), Dirlewanger et al. (1999), and Eduardo et al. (2011). QTLs for maturity date were also mapped on the same linkage group (4) (Eduardo et al., 2011; Etienne et al., 2002; Nuñez-Lillo et al., 2015), supporting involvement of the maturity process in regulation of several fruit quality traits, such as softening rate and ripening index (SSC/TA ratio) in peach. Major QTL, *qPC.ZC-6.1*, for

phytochemical compounds was mapped on LG6. This region encompasses some of fruit quality traits as well. Overlapping QTLs for phytochemicals and fruit quality traits support the strong correlation between these traits (Cantín et al., 2009b; Rice-Evans et al., 1996), and suggest pleiotropic effect of genes in this region.

Association mapping seeks to identify specific functional variants (i.e., loci, alleles) linked to phenotypic differences in a trait, to facilitate detection of trait-causing DNA sequence polymorphisms and/or selection of genotypes that closely resemble the phenotype (Oraguzie and Wilcox, 2007). It is a multidisciplinary field, involving components of genomics, statistical genetics, molecular biology, and bioinformatics, which together form the basis for selecting, evaluating, and associating genomic regions for correlation with trait variation. Genome wide association involves the use of unstructured or loosely structured populations – usually intraspecific – that are both phenotypically and genotypically characterized to detect statistical associations between genetic polymorphisms and heritable trait variation (Meuwissen, 2007; Riedelsheimer et al., 2012; Sonah et al., 2015; Zhao et al., 2011; Zhu et al., 2008). Markers associated with malic acid, ripening index and anthocyanin accumulation identified on chromosome 5 agree with QTLs for fruit firmness and soluble solids concentration mapped on the same chromosome in different *Prunus* species (Salazar et al., 2013; Zeballos et al., 2016); revealing the correlation between these traits, where more mature fruit usually has less firmness and less sourness, but more sweetness and higher level of anthocyanins in standard cultivars.

Association mapping has much in common with QTL mapping. Both methods

apply statistical inference to detect co-segregation of polymorphic genetic markers with genes underpinning trait variation. However, conventional QTL mapping is largely limited to those loci that have large effects on quantitative trait variation. These loci have large effects compared with the environmental effect. Furthermore, individuals in segregating populations can usually be assigned to discrete groups corresponding directly to their genotypes. Unlike these Mendelian traits, for which (usually) alleles at single loci determine the phenotype in a predictable manner, complex trait phenotypes are determined by alleles at many loci. Not only is the number of loci unknown, the phenotypic effects of alleles at each locus may also be influenced by the environment (Oraguzie and Wilcox, 2007). In this study, overlap between the QTLs and markers detected by association mapping was observed, as well as association between the numerous markers on linkage groups with no QTLs detected by bi-parental mapping for the same trait. These differences are mostly due to the complex nature of the inheritance of nutritional traits and the effect that environment has on their accumulation. In addition, different genotyping platforms contributed to the different results of QTL and association mapping. Modern peach germplasm used in association mapping was genotyped using genotyping-by-sequencing (GBS), which reduces genome complexity with restriction enzymes (Elshire et al., 2011). TASSEL pipeline software uses a reference genome to detect SNPs that are different in the genotyped material, thereby masking those that are the same as the reference used. On the other hand, the ZC² population was genotyped using the IPSC 9K peach SNP array v1 (Verde et al., 2012), where nine thousand SNPs

are spread across the whole peach genome with at least one SNP at every 5cM. SNPs associated with accumulation of phytochemical compounds and fruit quality traits were either overlapped with the QTLs or found in flanking regions. In addition, association mapping revealed SNPs associated with one trait that were collocated or overlapped with QTLs detected for different traits. For instance, majority of SNPs associated with anthocyanin on chromosome 4 overlapped with QTLs for fruit firmness, soluble solids concentration, and titratable acidity. Overlapping was also observed between SNPs associated with anthocyanin accumulation and ripening index on chromosome 6 with QTL cluster of phytochemical compounds, fruit size, fruit weight, and index of absorbance difference, suggesting pleiotropic effects and/or shared biosynthesis pathways. SNPs associated with ripening index on LG7 flanked *qBlush.ZC_7.1* QTL, while SNP associated with anthocyanin accumulation on chromosome 7, *S7_7758465*, overlapped with *qRI.ZC_7.1* QTL. Furthermore, *S7_7547042* associated with anthocyanin on chromosome 7 collocated with SNP mapped within blush QTL (at 7514430 bp). Collocation of SNPs detected via association and QTL mapping is supported with observed positive correlation between those traits as well. Higher ripening index usually means higher sugar content, which is the main component in anthocyanin pigmentation (Byrne et al., 1991; Delwiche and Baumgardner, 1983).

Haplotyping of the major QTL, *qPC.ZC-6.1*, on LG6 revealed functional alleles, *a* and *b*, and their effects on accumulation of phytochemical compounds. Progeny homozygous for haplotype *a* showed highest antioxidant capacity. This haplotype originated from the white-fleshed grandparent ‘Zin Dai’. Yellow-fleshed ‘Crimson

Lady’, homozygous for haplotype *b*, exhibited low accumulation of phytochemical compounds, confirming previous observations about white-fleshed peaches accumulating more phenolic compounds than yellow-fleshed ones (Cantín et al., 2009b; Gil et al., 2002).

Association mapping provides marker effects on expression of the evaluated traits by revealing positive and negative effects of major and minor alleles for each SNP. Higher accumulation of phytochemical compounds was observed for heterozygous genotype (AG) of S7_12434842, as observed in ‘Elberta’, ‘Encore’, ‘Belle of Georgia’, and ‘Lauro’. On the other hand, homozygous genotype (AA) exhibited lower accumulation of phytochemical compounds as observed in modern peach germplasm. Connecting phenotypic data with the allele effects allowed detailed understanding of each marker effect on the accumulation of phytochemical compounds. Further analysis is needed to combine effects of all markers and elucidate which combination gives the highest accumulation. Given that environment highly influences association of complex traits such as phytochemicals, additional phenotyping data covering more seasons and/or environments is needed to elucidate inheritance of these traits and use this data in support of development of DNA tools for breeding.

Overlap of SNPs associated with anthocyanin accumulation with *qPC.ZC-6.1*, a major QTL for accumulation of phytochemical compounds on LG6, supports further investigation into the candidate genes residing in this region. Candidate genes identified within *qPC.ZC-6.1*, are included in the phytochemical biosynthesis pathway

(Cooper, 2000; Davies and Schwinn, 2003; Patra et al., 2013; Hirschberg et al., 1997; Ruiz-Sola and Rodríguez-Concepción, 2012).

This is the first comprehensive report on using both QTL and association mapping in peach to determine the genetics of complex traits under polygenic inheritance, such as nutritional compounds. As such, it provides insight in what modern peach germplasm has to offer in breeding for these important compounds and enables further work in development of DNA tools for MAB. The complex nature of these traits and environmental effect on their accumulation requires validation of presented results before the DNA test can be developed and utilized. Validation can be achieved either with additional bi-parental populations, pedigree base analysis and/or addition of new phenotypic data from more seasons/environments. Nevertheless, this work provides a base for enabling marker-assisted breeding for the phytonutritional traits in peach.

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CHAPTER SIX

OVERALL CONCLUSION AND FUTURE WORK

The principal goal of this study was to test the feasibility of marker-assisted selection for phytochemical compounds in peach. Modern peach germplasm showed considerable variability in the capacity to accumulate phytochemical compounds despite its narrow genetic base. Two SNP genotyping platforms, Illumina SNP array and next generation sequencing proved to be valuable tools for genotyping closely related material. The wide variation in the accumulation of bioactive compounds in our material provided genetic opportunities to identify candidate gene regions associated with the traits of interest. Identified regions in the genome associated with the phytochemical accumulation and fruit quality traits will be valuable in development of the DNA tools to complement traditional breeding methods.

Accumulation of phytochemical compounds is under quantitative inheritance and highly influenced by the genotype and environment. A major QTL, *qPC.ZC-6.1*, detected on LG6 reveals a hot spot in the peach genome for phytochemical compound accumulation. It also encompasses some fruit quality traits (FS, FW, and IAD), and therefore presents a good starting point in deciphering the genetics behind accumulation and inheritance of these compounds in peach. Several SNPs associated with anthocyanin accumulation and ripening index, detected by GWAS overlapped with *qPC.ZC-6.1* supporting the hypothesis of the cluster of genes associated with accumulation of nutritional compounds. Candidate gene analysis, using complete coding sequence of *qPC.ZC-6.1* revealed several genes homologous among Rosaceae

species that might play an important role in the biosynthetic pathway of phenolic compounds.

Development of a DNA test to enable MAB for phytochemical compounds requires additional phenotypic data on existing material and related populations to stabilize detected QTLs and provide functional alleles for phytochemical accumulation. In addition, genotypic data from peach germplasm, available through another Rosaceae genotyping project could be used to determine complexity of the *qPC.ZC-6.1* region in close and distant material. This will increase the accuracy of the prediction for the alleles detected and enable development of a DNA test for further validation. In addition, pedigree based analysis could be used on the Clemson University peach breeding program material to confirm the QTL and association mapping results and potentially uncover additional alleles through pedigree. DNA test validation will be performed on the advanced selections from other U.S. public breeding programs that are evaluated in a Clemson University peach evaluation trial to determine their prediction power. Different genotyping platforms will be explored for DNA test development and cost effectiveness and the most accurate and efficient platform will be chosen for final validation.

Phenolic compounds are known to increase the shelf life of the fruit and provide attractive attributes. Therefore, postharvest disorders in cultivars with increased phenolic content should be evaluated as well as consumer acceptance of the fruit with increased phenolic content. Development and application of DNA tests will boost breeding efficiency and efficacy allowing breeders to focus on fewer hybrids and

additional traits. Consumer acceptance of the peach fruit with increased phenolic compounds will ensure profitability and prosperity of the peach industry while providing tastier and healthier summer fruit to the consumers.

APPENDICES

Appendix I:

Shapiro-wilk normality test performed for 12 evaluated pomological traits ($P < 0.05$). Highlighted tests were used for QTL mapping

Trait	Shapiro-Wilk normality test		
	Raw data (original data)	Log transformation	Square root transformation
RAC_2013	W = 0.81923, p-value < 2.2e-16	W = 0.97863, p-value = 3.1e-05	W = 0.95666, p-value = 6.738e-09
RAC_2014	W = 0.93702, p-value = 4.591e-12	W = 0.75697, p-value < 2.2e-16	W = 0.95423, p-value = 6.853e-10
GAE_2013	W = 0.86102, p-value < 2.2e-16	W = 0.94297, p-value = 1.223e-10	W = 0.90624, p-value = 2.995e-14
GAE_2014	W = 0.92748, p-value = 4.197e-13	W = 0.98716, p-value = 0.001216	W = 0.96473, p-value = 2.691e-08
CE_2013	W = 0.65155, p-value < 2.2e-16	W = 0.97653, p-value = 4.468e-05	W = 0.84473, p-value < 2.2e-16
CE_2014	W = 0.92988, p-value = 7.503e-13	W = 0.94389, p-value = 2.998e-11	W = 0.98754, p-value = 0.00153
C3GE_2013	W = 0.85176, p-value < 2.2e-16	W = 0.98241, p-value = 0.0001978	W = 0.95182, p-value = 1.509e-09
C3GE_2014	W = 0.82276, p-value < 2.2e-16	W = 0.9393, p-value = 9.149e-12	W = 0.89906, p-value = 1.099e-15
FF_2013	W = 0.86275, p-value < 2.2e-16	W = 0.97185, p-value = 2.725e-06	W = 0.94357, p-value = 2.997e-10
FF_2014	W = 0.98908, p-value = 0.004057	W = 0.91956, p-value = 6.737e-14	W = 0.97294, p-value = 7.646e-07
FS_2013	W = 0.99225, p-value = 0.06808	W = 0.99455, p-value = 0.2553	W = 0.99454, p-value = 0.253
FS_2014	W = 0.9942, p-value = 0.1266	W = 0.98665, p-value = 0.0008875	W = 0.99167, p-value = 0.02256
FW_2013	W = 0.97332, p-value = 4.941e-06	W = 0.9861, p-value = 0.002003	W = 0.99448, p-value = 0.2441
FW_2014	W = 0.98395, p-value = 0.0001831	W = 0.98615, p-value = 0.0006571	W = 0.99515, p-value = 0.2375
SSC_2013	W = 0.97469, p-value = 0.1741	W = 0.98816, p-value = 0.7622	W = 0.9828, p-value = 0.4606
SSC_2014	W = 0.98937, p-value = 0.7484	W = 0.98361, p-value = 0.3897	W = 0.98789, p-value = 0.6489
TA_2013	W = 0.9603, p-value = 0.02602	W = 0.9091, p-value = 9.042e-05	W = 0.94053, p-value = 0.002387
TA_2014	W = 0.98122, p-value = 0.2812	W = 0.93704, p-value = 0.0006154	W = 0.96433, p-value = 0.02354
RI_2013	W = 0.89146, p-value = 2.053e-05	W = 0.96577, p-value = 0.05547	W = 0.93442, p-value = 0.001321
RI_2014	W = 0.86883, p-value = 6.463e-07	W = 0.96611, p-value = 0.0305	W = 0.93147, p-value = 0.000316

I _{AD} _2013	W = 0.84825, p-value < 2.2e-16	W = 0.97843, p-value = 0.0001626	W = 0.95153, p-value = 1.944e-08
I _{AD} _2014	W = 0.93403, p-value = 8.22e-12	W = 0.97218, p-value = 1.39e-06	W = 0.98415, p-value = 0.0004013
Blush_2007	W = 0.88684, p-value < 2.2e-16	W = 0.8284, p-value < 2.2e-16	W = 0.87174, p-value < 2.2e-16
Blush_2008	W = 0.89249, p-value < 2.2e-16	W = 0.85182, p-value < 2.2e-16	W = 0.88305, p-value < 2.2e-16
Blush_2014	W = 0.88728, p-value = 1.694e-15	W = 0.83535, p-value < 2.2e-16	W = 0.87177, p-value < 2.2e-16

Appendix II:

All SNPs represented in number of blocks

Block	SNP marker	Position
B1_1_2	1_SNP_IGA_63746	3.229
	1_SNP_IGA_63603	3.239
B1_2_4	1_SNP_1_15750387	3.582
	1_SNP_IGA_56198	3.582
	1_SNP_IGA_53531	3.582
	1_SNP_IGA_55903	3.582
B1_3_2	1_SNP_IGA_86925	11.681
	1_SNP_IGA_86968	11.681
B1_4_3	1_SNP_IGA_87433	12.187
	1_SNP_IGA_87572	12.233
	1_SNP_IGA_87577	12.233
B1_5_9	1_SNP_IGA_94254	16.058
	1_SNP_IGA_95412	16.058
	1_SNP_IGA_95417	16.058
	1_SNP_IGA_95715	16.058
	1_SNP_IGA_95095	16.058
	1_SNP_IGA_95426	16.058
	1_SNP_IGA_95389	16.058
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	1_SNP_IGA_102464	28
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B5_8_2	5_SNP_IGA_596359	28.864
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	5_SNP_IGA_597636	37.301
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	6_SNP_IGA_606086	8.074
	6_SNP_IGA_605986	8.074
	6_SNP_IGA_606059	8.074
	6_SNP_IGA_605980	8.108
B6_10_2	6_SNP_IGA_620099	10.921
	6_SNP_IGA_619845	10.937
B6_11_3	6_SNP_IGA_621593	14.093
	6_SNP_IGA_621562	14.093
	6_SNP_IGA_621556	14.094
B6_12_2	6_SNP_IGA_621914	14.809
	6_SNP_IGA_621925	14.837
B6_13_2	6_SNP_IGA_626080	19.364
	6_SNP_IGA_627574	19.364
B6_14_6	6_SNP_IGA_627934	19.767
	6_SNP_IGA_627396	19.794

	6_SNP_IGA_627350	19.794
	6_SNP_IGA_627328	19.794
	6_SNP_IGA_627415	19.794
	6_SNP_IGA_627535	19.814
B6_15_3	6_SNP_IGA_628841	20.779
	6_SNP_IGA_628993	20.779
	6_SNP_6_7910869	20.779
B6_16_2	6_SNP_IGA_631014	22.002
	6_SNP_IGA_631212	22.002
B6_17_5	6_SNP_IGA_632123	23.383
	6_SNP_IGA_631845	23.383
	6_SNP_IGA_632110	23.383
	6_SNP_IGA_631948	23.383
	6_SNP_IGA_632049	23.383
B6_18_2	6_SNP_IGA_641542	26.774
	6_SNP_IGA_641339	26.795
B6_19_2	6_SNP_IGA_640430	27.108
	6_SNP_IGA_640928	27.128
B6_20_2	6_SNP_IGA_667746	35.655
	6_SNP_IGA_668442	35.715
B6_21_2	6_SNP_IGA_669740	36.216
	6_SNP_IGA_669440	36.244
B6_22_5	6_SNP_IGA_670781	36.785
	6_SNP_IGA_670280	36.785
	6_SNP_IGA_670509	36.785
	6_SNP_IGA_670348	36.802
	6_SNP_IGA_670494	36.802
<hr/>		
B7_1_3	7_SNP_IGA_724309	2.413
	7_SNP_IGA_724271	2.443
	7_SNP_IGA_723701	2.448
B7_2_3	7_SNP_IGA_722889	3.484
	7_SNP_IGA_719131	3.516
	7_SNP_IGA_715579	3.516
B7_3_10	7_SNP_IGA_722956	3.558
	7_SNP_IGA_722921	3.574
	7_SNP_IGA_722928	3.574
	7_SNP_IGA_715837	3.574
	7_SNP_IGA_720963	3.579

	7_SNP_IGA_719923	3.608
	7_SNP_IGA_718919	3.624
	7_SNP_IGA_718436	3.624
	7_SNP_IGA_722952	3.637
	7_SNP_IGA_719120	3.637
B7_4_12	7_SNP_IGA_718094	3.653
	7_SNP_IGA_722771	3.653
	7_SNP_IGA_716297	3.653
	7_SNP_IGA_717776	3.653
	7_SNP_IGA_718633	3.653
	7_SNP_IGA_716322	3.653
	7_SNP_IGA_721564	3.653
	7_SNP_IGA_717591	3.653
	7_SNP_IGA_722801	3.656
	7_SNP_IGA_721444	3.656
	7_SNP_IGA_719178	3.656
	7_SNP_IGA_722899	3.667
B7_5_2	7_SNP_IGA_714572	4.778
	7_SNP_IGA_714207	4.782
B7_6_6	7_SNP_IGA_710792	5.873
	7_SNP_IGA_713301	5.873
	7_SNP_IGA_710802	5.873
	7_SNP_IGA_711056	5.873
	7_SNP_IGA_710377	5.873
	7_SNP_IGA_710787	5.879
B7_7_6	7_SNP_IGA_706926	6.97
	7_SNP_IGA_704173	6.97
	7_SNP_IGA_707691	6.97
	7_SNP_IGA_705226	6.999
	7_SNP_IGA_704234	6.999
	7_SNP_IGA_704075	6.999
B7_8_7	7_SNP_IGA_732397	8.069
	7_SNP_IGA_730950	8.087
	7_SNP_IGA_730927	8.087
	7_SNP_IGA_732284	8.087
	7_SNP_IGA_732383	8.087
	7_SNP_IGA_732377	8.087
	7_SNP_IGA_703549	8.104

B7_9_18	7_SNP_IGA_740845	9.176
	7_SNP_IGA_740785	9.176
	7_SNP_IGA_740837	9.176
	7_SNP_IGA_740791	9.176
	7_SNP_IGA_741704	9.202
	7_SNP_IGA_740801	9.202
	7_SNP_IGA_741769	9.208
	7_SNP_IGA_738856	9.222
	7_SNP_IGA_740927	9.222
	7_SNP_IGA_742067	9.222
	7_SNP_IGA_740796	9.222
	7_SNP_IGA_741985	9.222
	7_SNP_IGA_741063	9.222
	7_SNP_IGA_742048	9.222
	7_SNP_IGA_742392	9.222
	7_SNP_IGA_741178	9.222
	7_SNP_IGA_743057	9.222
	7_SNP_IGA_738499	9.222
B7_10_2	7_SNP_IGA_743069	10.353
	7_SNP_IGA_744253	10.359
B7_11_5	7_SNP_IGA_746147	10.923
	7_SNP_IGA_747413	10.938
	7_SNP_IGA_746204	10.939
	7_SNP_IGA_746989	10.944
	7_SNP_IGA_745712	10.944
B7_12_4	7_snp_7_7722966	11.275
	7_SNP_IGA_748210	11.296
	7_SNP_IGA_748306	11.296
	7_SNP_IGA_748637	11.325
B7_13_3	7_SNP_IGA_749383	12.078
	7_SNP_IGA_749396	12.078
	7_SNP_IGA_750219	12.088
B7_14_13	7_SNP_IGA_750944	12.654
	7_SNP_IGA_752549	12.654
	7_SNP_IGA_751463	12.654
	7_SNP_IGA_750914	12.667
	7_SNP_IGA_751250	12.667
	7_SNP_IGA_750721	12.667

	7_SNP_IGA_750960	12.667
	7_SNP_IGA_751547	12.667
	7_SNP_IGA_752104	12.667
	7_SNP_IGA_751667	12.667
	7_SNP_IGA_751049	12.667
	7_SNP_IGA_751123	12.667
	7_SNP_IGA_750921	12.674
B7_15_2	7_SNP_IGA_749816	12.992
	7_SNP_IGA_749812	13.018
B7_16_4	7_SNP_IGA_753360	14.795
	7_SNP_IGA_753122	14.795
	7_SNP_IGA_753541	14.795
	7_SNP_IGA_753146	14.836
B7_17_10	7_SNP_IGA_759002	17.328
	7_SNP_IGA_758556	17.328
	7_SNP_IGA_758722	17.328
	7_SNP_IGA_759178	17.328
	7_SNP_IGA_759247	17.328
	7_SNP_IGA_759122	17.328
	7_SNP_IGA_759386	17.328
	7_SNP_IGA_759146	17.328
	7_SNP_IGA_759382	17.328
	7_SNP_IGA_759111	17.335
B7_18_5	7_SNP_IGA_758772	17.356
	7_SNP_IGA_759519	17.356
	7_SNP_IGA_759117	17.36
	7_SNP_IGA_759141	17.36
	7_SNP_IGA_759024	17.36
B7_19_3	7_SNP_IGA_760572	18.812
	7_SNP_IGA_760615	18.816
	7_SNP_IGA_760608	18.828
B7_20_6	7_SNP_IGA_762942	19.262
	7_SNP_IGA_762895	19.262
	7_SNP_IGA_763311	19.281
	7_SNP_IGA_762538	19.317
	7_SNP_IGA_763224	19.317
	7_SNP_IGA_763204	19.338
B7_21_4	7_SNP_IGA_768215	20.839

	7_SNP_IGA_769084	20.839
	7_SNP_IGA_768368	20.839
	7_SNP_IGA_768843	20.839
B7_22_2	7_SNP_IGA_769471	20.867
	7_SNP_IGA_768226	20.886
B7_23_4	7_SNP_IGA_769682	21.778
	7_SNP_IGA_769843	21.821
	7_SNP_IGA_770009	21.821
	7_SNP_IGA_769927	21.821
B7_24_2	7_SNP_IGA_769687	21.852
	7_SNP_IGA_769675	21.852
B7_25_2	7_SNP_IGA_776214	29.647
	7_SNP_IGA_776348	29.647
B8_1_2	8_SNP_IGA_834505	0.504
	8_SNP_IGA_834321	0.539
B8_2_3	8_SNP_IGA_853233	2.211
	8_SNP_IGA_853257	2.211
	8_SNP_IGA_853239	2.216
B8_3_3	8_SNP_IGA_853473	2.756
	8_SNP_IGA_853986	2.756
	8_SNP_IGA_853728	2.798
B8_4_2	8_SNP_IGA_855459	5.047
	8_SNP_IGA_855356	5.047
B8_5_3	8_SNP_IGA_860069	11.118
	8_SNP_IGA_859441	11.134
	8_SNP_IGA_859602	11.134
B8_6_6	8_SNP_IGA_862801	14.282
	8_SNP_IGA_862489	14.313
	8_SNP_IGA_862825	14.313
	8_SNP_IGA_862368	14.313
	8_SNP_IGA_863252	14.313
	8_SNP_IGA_863130	14.313
B8_7_2	8_SNP_IGA_863869	15.464
	8_SNP_IGA_864110	15.464
B8_8_3	8_SNP_IGA_865041	16.667
	8_SNP_IGA_865003	16.667
	8_SNP_IGA_865412	16.667
B8_9_2	8_SNP_IGA_866213	17.852

	8_SNP_IGA_865709	17.852
B8_10_3	8_SNP_IGA_866829	19
	8_SNP_IGA_866785	19.019
	8_SNP_IGA_866691	19.019
B8_11_2	8_SNP_IGA_873491	25.706
	8_SNP_IGA_874263	25.706
B8_12_4	8_SNP_IGA_873768	26.006
	8_SNP_IGA_873803	26.006
	8_SNP_IGA_873743	26.006
	8_SNP_IGA_873750	26.021
B8_13_2	8_SNP_IGA_881509	33.304
	8_SNP_IGA_881453	33.335
B8_14_4	8_SNP_IGA_880789	33.672
	8_SNP_IGA_881154	33.677
	8_SNP_IGA_881135	33.677
	8_SNP_IGA_881173	33.677
B8_15_4	8_SNP_IGA_882091	34.154
	8_SNP_IGA_881804	34.183
	8_SNP_IGA_881810	34.183
	8_SNP_IGA_881815	34.192
B8_16_2	8_SNP_IGA_882788	35.372
	8_SNP_IGA_882680	35.377
B8_17_2	8_SNP_IGA_883524	37.159
	8_SNP_IGA_884078	37.159
B8_18_4	8_SNP_IGA_884050	37.327
	8_SNP_IGA_883429	37.338
	8_SNP_IGA_883625	37.338
	8_SNP_IGA_883381	37.342
B8_19_3	8_SNP_IGA_884135	37.854
	8_SNP_IGA_884153	37.87
	8_SNP_IGA_884218	37.938
B8_20_6	8_SNP_IGA_884755	38.64
	8_SNP_IGA_884527	38.64
	8_SNP_IGA_884721	38.64
	8_SNP_IGA_884498	38.64
	8_SNP_IGA_884455	38.64
	8_SNP_IGA_884657	38.64
B8_21_5	8_SNP_IGA_884847	39.579

	8_SNP_IGA_885070	39.585
	8_SNP_IGA_885177	39.585
	8_SNP_IGA_884899	39.585
	8_SNP_IGA_885121	39.585
B8_22_2	8_SNP_IGA_885335	40.138
	8_SNP_IGA_885430	40.138

Appendix III:

QTLs mapped in ZC² linkage map by Kruskal-Wallis.

QTL	Group	Position	Locus	K*	Df	Signif.
RAC_2014	LG3	28.68	SNP_IGA_366194	13.1	1	*****
	LG3	28.696	SNP_IGA_366233	12.658	1	*****
	LG6	10.086	SNP_IGA_619263	12.228	2	****
	LG3	28.674	SNP_IGA_366432	11.615	1	*****
	LG6	10.921	SNP_IGA_620099	11.111	2	****
	LG6	10.937	SNP_IGA_619845	11.111	2	****
	LG3	30.443	SNP_IGA_367684	10.896	1	*****
	LG3	30.465	SNP_IGA_367728	10.568	1	****
	LG3	30.416	SNP_IGA_367668	9.955	1	****
	LG6	9.724	SNP_IGA_619081	9.605	2	***
	LG6	8.422	SNP_IGA_605605	9.306	2	***
	LG6	6.292	SNP_IGA_609630	8.788	2	**
	LG6	6.292	SNP_IGA_609485	8.788	2	**
	LG6	6.292	SNP_IGA_609463	8.788	2	**
	LG6	6.292	SNP_IGA_609531	8.788	2	**
	LG6	6.292	SNP_IGA_609501	8.788	2	**
	LG6	6.292	SNP_IGA_609723	8.788	2	**
	LG6	2.97	snp_6_2750075	8.736	2	**
	LG3	31.097	SNP_IGA_368077	8.406	1	****
	LG3	31.13	snp_3_21905073	8.406	1	****
	LG3	0	SNP_IGA_295644	8.398	1	****
	LG3	0	SNP_IGA_295658	8.398	1	****
	LG3	0	SNP_IGA_295649	8.398	1	****
	LG3	0	SNP_IGA_295664	8.398	1	****
GAE_2014	LG6	5.701	SNP_IGA_610531	12.212	2	****
	LG6	5.701	SNP_IGA_609984	12.212	2	****
	LG6	5.701	SNP_IGA_610487	12.212	2	****
	LG6	6.292	SNP_IGA_609630	11.958	2	****
	LG6	6.292	SNP_IGA_609485	11.958	2	****
	LG6	6.292	SNP_IGA_609463	11.958	2	****
	LG6	6.292	SNP_IGA_609531	11.958	2	****
	LG6	6.292	SNP_IGA_609501	11.958	2	****
	LG6	6.292	SNP_IGA_609723	11.958	2	****

	LG6	8.056	SNP_IGA_605863	10.39	2	***
	LG6	8.074	SNP_IGA_606086	10.39	2	***
	LG6	8.074	SNP_IGA_605986	10.39	2	***
	LG6	8.074	SNP_IGA_606059	10.39	2	***
	LG6	8.108	SNP_IGA_605980	10.39	2	***
	LG6	8.422	SNP_IGA_605605	10.309	2	***
	LG8	1.706	SNP_IGA_853101	10.261	2	***
	LG8	2.04	SNP_IGA_853250	10.261	2	***
	LG8	2.211	SNP_IGA_853233	10.261	2	***
	LG8	2.211	SNP_IGA_853257	10.261	2	***
	LG8	2.216	SNP_IGA_853239	10.261	2	***
	LG6	14.093	SNP_IGA_621593	10.029	2	***
	LG6	14.093	SNP_IGA_621562	10.029	2	***
	LG6	14.094	SNP_IGA_621556	10.029	2	***
	LG6	10.086	SNP_IGA_619263	9.803	2	***
	LG8	2.74	SNP_IGA_853526	9.699	2	***
	LG8	2.756	SNP_IGA_853473	9.699	2	***
	LG8	2.756	SNP_IGA_853986	9.699	2	***
	LG8	2.798	SNP_IGA_853728	9.699	2	***
	LG6	14.809	SNP_IGA_621914	9.534	2	***
	LG6	14.837	SNP_IGA_621925	9.534	2	***
	LG6	15.571	SNP_IGA_624015	9.513	2	***
	LG6	8.616	SNP_IGA_606435	9.312	2	***
GAE_Mean	LG1	28.564	SNP_IGA_102819	9.648	2	***
	LG1	28.585	SNP_IGA_102807	9.648	2	***
	LG1	30.121	SNP_IGA_103507	10.143	2	***
	LG1	30.138	SNP_IGA_103422	10.044	2	***
C3GE_2013	LG8	0	SNP_IGA_836857	11.577	2	*****
	LG8	1.706	SNP_IGA_853101	10.621	2	*****
	LG1	24.627	SNP_IGA_100353	10.46	2	***
	LG4	20.882	SNP_IGA_396340	7.187	1	***
	LG8	0.504	SNP_IGA_834505	10.484	2	***
	LG8	0.539	SNP_IGA_834321	10.377	2	***
	LG8	2.04	SNP_IGA_853250	9.456	2	***
	LG8	2.211	SNP_IGA_853233	9.456	2	***
	LG8	2.211	SNP_IGA_853257	9.456	2	***
	LG8	2.216	SNP_IGA_853239	9.456	2	***
	LG8	2.74	SNP_IGA_853526	9.589	2	***

	LG8	2.756	SNP_IGA_853473	9.456	2	***
	LG8	2.756	SNP_IGA_853986	9.456	2	***
	LG8	2.798	SNP_IGA_853728	9.589	2	***
C3GE_2014	LG6	0	SNP_IGA_607528	10.805	2	****
	LG6	0	SNP_IGA_607711	10.805	2	****
	LG6	0.501	SNP_IGA_616295	10.805	2	****
	LG6	0.501	SNP_IGA_607013	10.805	2	****
	LG6	0.501	SNP_IGA_616534	10.805	2	****
	LG6	0.591	SNP_IGA_607240	10.805	2	****
	LG6	0.591	SNP_IGA_607179	10.805	2	****
	LG6	0.591	SNP_IGA_616508	10.805	2	****
	LG6	0.591	SNP_IGA_617922	10.805	2	****
	LG6	0.591	SNP_IGA_618417	10.805	2	****
	LG6	0.591	SNP_IGA_607343	10.805	2	****
	LG6	0.591	SNP_IGA_616286	10.805	2	****
	LG6	0.591	SNP_IGA_618849	10.805	2	****
	LG6	0.591	SNP_IGA_618824	10.805	2	****
	LG6	0.591	SNP_IGA_618376	10.805	2	****
	LG6	1.158	SNP_IGA_614935	11.582	2	****
	LG6	1.186	SNP_IGA_616119	11.582	2	****
	LG6	1.186	SNP_IGA_616005	11.582	2	****
	LG6	1.186	SNP_IGA_614635	11.582	2	****
	LG6	1.186	SNP_IGA_615979	11.582	2	****
	LG6	1.186	SNP_IGA_615377	11.582	2	****
	LG6	1.201	SNP_IGA_616074	11.582	2	****
	LG6	1.277	SNP_IGA_615381	11.582	2	****
	LG6	1.761	SNP_IGA_614519	11.021	2	****
	LG6	2.338	SNP_IGA_614273	12.487	2	****
	LG6	2.38	SNP_IGA_614082	12.487	2	****
	LG6	2.38	SNP_IGA_614045	12.487	2	****
	LG6	2.38	SNP_IGA_614054	12.487	2	****
	LG6	2.38	SNP_IGA_614236	12.487	2	****
	LG6	2.97	snp_6_2750075	11.513	2	****
	LG6	3.936	SNP_IGA_611544	11.304	2	****
	LG6	3.936	SNP_IGA_611064	11.304	2	****
	LG6	3.936	SNP_IGA_611149	11.304	2	****
	LG6	3.936	SNP_IGA_611891	11.304	2	****
	LG6	3.936	SNP_IGA_611511	11.304	2	****

	LG6	3.936	SNP_IGA_611748	11.304	2	****
	LG6	5.701	SNP_IGA_610531	11.715	2	****
	LG6	5.701	SNP_IGA_609984	11.715	2	****
	LG6	5.701	SNP_IGA_610487	11.715	2	****
	LG6	6.292	SNP_IGA_609630	12.528	2	****
	LG6	6.292	SNP_IGA_609485	12.528	2	****
	LG6	6.292	SNP_IGA_609463	12.528	2	****
	LG6	6.292	SNP_IGA_609531	12.528	2	****
	LG6	6.292	SNP_IGA_609501	12.528	2	****
	LG6	6.292	SNP_IGA_609723	12.528	2	****
	LG6	8.056	SNP_IGA_605863	10.799	2	****
	LG6	8.074	SNP_IGA_606086	10.799	2	****
	LG6	8.074	SNP_IGA_605986	10.799	2	****
	LG6	8.074	SNP_IGA_606059	10.799	2	****
	LG6	8.108	SNP_IGA_605980	10.799	2	****
	LG6	8.422	SNP_IGA_605605	11.047	2	****
	LG6	8.616	SNP_IGA_606435	11.087	2	****
	LG6	9.724	SNP_IGA_619081	11.294	2	****
	LG6	10.086	SNP_IGA_619263	12.747	2	****
	LG6	10.921	SNP_IGA_620099	11.824	2	****
	LG6	10.937	SNP_IGA_619845	11.824	2	****
	LG6	13.493	snp_6_5294415	13.141	2	****
	LG6	14.093	SNP_IGA_621593	12.593	2	****
	LG6	14.093	SNP_IGA_621562	12.593	2	****
	LG6	14.094	SNP_IGA_621556	12.593	2	****
	LG6	14.809	SNP_IGA_621914	12.215	2	****
	LG6	14.837	SNP_IGA_621925	12.215	2	****
	LG6	15.571	SNP_IGA_624015	10.953	2	****
	LG6	16.24	SNP_IGA_624396	10.998	2	****
	LG6	16.25	SNP_IGA_624391	10.998	2	****
	LG6	16.949	SNP_IGA_624583	10.484	2	***
	LG6	17.458	SNP_IGA_625680	10.484	2	***
CE_2013	LG2	23.123	SNP_IGA_245182	9.211	2	***
	LG2	23.123	SNP_IGA_246838	9.211	2	***
	LG2	23.123	SNP_IGA_245204	9.211	2	***
	LG2	23.226	SNP_IGA_249273	9.211	2	***
	LG2	23.608	SNP_IGA_248434	9.211	2	***
	LG2	23.764	SNP_IGA_245214	9.211	2	***

	LG2	24.463	SNP_IGA_257549	9.355	2	***
	LG2	24.963	SNP_IGA_260053	7.264	1	***
	LG2	24.996	SNP_IGA_259667	7.264	1	***
	LG2	24.996	SNP_IGA_259741	7.264	1	***
	LG2	25.006	SNP_IGA_260373	7.176	1	***
	LG3	6.717	SNP_IGA_309881	6.954	1	***
	LG3	6.727	SNP_IGA_310057	6.954	1	***
	LG3	6.727	SNP_IGA_310566	6.954	1	***
	LG3	6.727	SNP_IGA_310760	6.954	1	***
	LG3	6.727	SNP_IGA_309635	6.954	1	***
	LG3	6.727	SNP_IGA_309872	6.954	1	***
	LG3	6.727	SNP_IGA_310338	6.954	1	***
	LG3	6.727	SNP_IGA_309554	6.954	1	***
	LG3	6.727	SNP_IGA_310548	6.954	1	***
	LG3	6.727	SNP_IGA_310467	6.954	1	***
	LG3	6.727	SNP_IGA_309338	6.954	1	***
	LG3	7.383	SNP_IGA_311300	7.744	1	***
	LG3	7.398	SNP_IGA_311192	7.744	1	***
	LG3	7.398	SNP_IGA_311011	7.744	1	***
	LG3	7.414	SNP_IGA_311941	7.231	1	***
	LG3	7.414	SNP_IGA_312052	7.231	1	***
	LG3	7.414	SNP_IGA_311196	7.231	1	***
	LG5	27.993	SNP_IGA_595930	6.934	1	***
	LG5	27.993	SNP_IGA_595786	6.934	1	***
	LG5	27.993	SNP_IGA_595829	6.934	1	***
CE_2014	LG6	10.086	SNP_IGA_619263	14.01	2	*****
	LG6	10.921	SNP_IGA_620099	13.82	2	*****
	LG6	10.937	SNP_IGA_619845	13.82	2	*****
	LG6	0	SNP_IGA_607528	11.237	2	****
	LG6	0	SNP_IGA_607711	11.237	2	****
	LG6	0.501	SNP_IGA_616295	11.237	2	****
	LG6	0.501	SNP_IGA_607013	11.237	2	****
	LG6	0.501	SNP_IGA_616534	11.237	2	****
	LG6	0.591	SNP_IGA_607240	11.237	2	****
	LG6	0.591	SNP_IGA_607179	11.237	2	****
	LG6	0.591	SNP_IGA_616508	11.237	2	****
	LG6	0.591	SNP_IGA_617922	11.237	2	****
	LG6	0.591	SNP_IGA_618417	11.237	2	****

LG6	0.591	SNP_IGA_607343	11.237	2	****
LG6	0.591	SNP_IGA_616286	11.237	2	****
LG6	0.591	SNP_IGA_618849	11.237	2	****
LG6	0.591	SNP_IGA_618824	11.237	2	****
LG6	0.591	SNP_IGA_618376	11.237	2	****
LG6	1.158	SNP_IGA_614935	12.118	2	****
LG6	1.186	SNP_IGA_616119	12.118	2	****
LG6	1.186	SNP_IGA_616005	12.118	2	****
LG6	1.186	SNP_IGA_614635	12.118	2	****
LG6	1.186	SNP_IGA_615979	12.118	2	****
LG6	1.186	SNP_IGA_615377	12.118	2	****
LG6	1.201	SNP_IGA_616074	12.118	2	****
LG6	1.277	SNP_IGA_615381	12.118	2	****
LG6	1.761	SNP_IGA_614519	12.4	2	****
LG6	2.338	SNP_IGA_614273	13.517	2	****
LG6	2.38	SNP_IGA_614082	13.517	2	****
LG6	2.38	SNP_IGA_614045	13.517	2	****
LG6	2.38	SNP_IGA_614054	13.517	2	****
LG6	2.38	SNP_IGA_614236	13.517	2	****
LG6	2.97	snp_6_2750075	13.519	2	****
LG6	8.422	SNP_IGA_605605	11.764	2	****
LG6	9.724	SNP_IGA_619081	12.114	2	****
LG3	0	SNP_IGA_295644	7.197	1	***
LG3	0	SNP_IGA_295658	7.197	1	***
LG3	0	SNP_IGA_295649	7.197	1	***
LG3	0	SNP_IGA_295664	7.197	1	***
LG6	3.936	SNP_IGA_611544	10.146	2	***
LG6	3.936	SNP_IGA_611064	10.146	2	***
LG6	3.936	SNP_IGA_611149	10.146	2	***
LG6	3.936	SNP_IGA_611891	10.146	2	***
LG6	3.936	SNP_IGA_611511	10.146	2	***
LG6	3.936	SNP_IGA_611748	10.146	2	***
LG6	5.701	SNP_IGA_610531	9.397	2	***
LG6	5.701	SNP_IGA_609984	9.397	2	***
LG6	5.701	SNP_IGA_610487	9.397	2	***
LG6	6.292	SNP_IGA_609630	10.26	2	***
LG6	6.292	SNP_IGA_609485	10.26	2	***
LG6	6.292	SNP_IGA_609463	10.26	2	***

	LG6	6.292	SNP_IGA_609531	10.26	2	***
	LG6	6.292	SNP_IGA_609501	10.26	2	***
	LG6	6.292	SNP_IGA_609723	10.26	2	***
	LG6	13.493	snp_6_5294415	10.035	2	***
	LG6	15.571	SNP_IGA_624015	9.973	2	***
	LG6	16.24	SNP_IGA_624396	9.375	2	***
	LG6	16.25	SNP_IGA_624391	9.375	2	***
	LG6	20.309	SNP_IGA_628744	10.077	2	***
	LG6	20.779	SNP_IGA_628841	10.396	2	***
	LG6	20.779	SNP_IGA_628993	10.396	2	***
	LG6	20.779	snp_6_7910869	10.396	2	***
	LG6	21.509	SNP_IGA_629177	9.825	2	***
Blush_2007	LG3	13.698	SNP_IGA_327093	11.698	1	*****
	LG3	13.698	SNP_IGA_328607	11.698	1	*****
	LG3	13.7	SNP_IGA_327678	11.44	1	*****
	LG3	13.703	SNP_IGA_328528	11.834	1	*****
	LG3	13.707	SNP_IGA_329177	11.44	1	*****
	LG3	14.327	SNP_IGA_330725	11.956	1	*****
	LG3	14.329	SNP_IGA_330917	11.698	1	*****
	LG3	14.335	SNP_IGA_330713	12.099	1	*****
	LG3	14.337	SNP_IGA_340919	11.834	1	*****
	LG3	14.411	SNP_IGA_340884	12.099	1	*****
	LG3	14.997	snp_3_12878608	11.698	1	*****
	LG3	14.997	SNP_IGA_341962	11.698	1	*****
	LG3	15.532	SNP_IGA_343872	11.318	1	*****
	LG3	9.838	SNP_IGA_314598	9.017	1	****
	LG3	13.027	SNP_IGA_325166	9.551	1	****
	LG3	13.029	SNP_IGA_339586	9.306	1	****
	LG3	13.034	SNP_IGA_325305	9.427	1	****
	LG3	13.401	SNP_IGA_328536	10.112	1	****
	LG3	14.465	SNP_IGA_331373	9.93	1	****
	LG3	15.197	SNP_IGA_343773	9.551	1	****
	LG3	15.804	Pp19Cl	8.949	1	****
	LG3	16.466	SNP_IGA_344712	8.842	1	****
	LG3	16.466	SNP_IGA_344628	8.842	1	****
	LG3	16.473	SNP_IGA_344612	8.609	1	****
	LG3	16.473	SNP_IGA_344789	8.609	1	****
	LG3	19.988	SNP_IGA_349682	7.962	1	****

	LG3	19.988	SNP_IGA_351038	7.962	1	****
	LG3	20.021	SNP_IGA_349789	7.962	1	****
	LG1	3.456	SNP_IGA_56163	7.102	1	***
	LG1	3.582	snp_1_15750387	7.381	1	***
	LG1	3.582	SNP_IGA_56198	7.381	1	***
	LG1	3.582	SNP_IGA_53531	7.381	1	***
	LG1	3.582	SNP_IGA_55903	7.381	1	***
	LG3	5.969	SNP_IGA_309280	6.73	1	***
	LG3	6.062	SNP_IGA_309473	6.973	1	***
	LG3	7.383	SNP_IGA_311300	7.679	1	***
	LG3	7.398	SNP_IGA_311192	7.801	1	***
	LG3	7.398	SNP_IGA_311011	7.801	1	***
	LG3	7.414	SNP_IGA_311941	6.973	1	***
	LG3	7.414	SNP_IGA_312052	6.973	1	***
	LG3	7.414	SNP_IGA_311196	6.973	1	***
	LG3	12.076	SNP_IGA_338469	7.001	1	***
	LG3	12.091	SNP_IGA_336711	7.23	1	***
	LG3	12.7	SNP_IGA_339178	7.23	1	***
	LG3	12.704	SNP_IGA_338615	7.575	1	***
	LG3	12.722	SNP_IGA_325855	7.342	1	***
	LG3	12.722	SNP_IGA_339170	7.342	1	***
	LG3	12.722	SNP_IGA_339568	7.342	1	***
	LG3	12.722	SNP_IGA_325718	7.342	1	***
	LG3	12.722	SNP_IGA_340016	7.342	1	***
	LG3	12.722	SNP_IGA_326457	7.342	1	***
	LG3	12.722	SNP_IGA_338533	7.342	1	***
	LG3	12.722	SNP_IGA_339037	7.342	1	***
	LG3	12.722	SNP_IGA_339719	7.342	1	***
	LG3	12.733	SNP_IGA_325850	7.575	1	***
	LG3	19.99	SNP_IGA_349831	7.711	1	***
	LG3	19.99	SNP_IGA_349233	7.711	1	***
	LG3	19.99	SNP_IGA_349707	7.711	1	***
	LG3	19.99	SNP_IGA_349097	7.711	1	***
	LG3	19.99	SNP_IGA_349757	7.711	1	***
	LG3	20.027	SNP_IGA_348931	7.526	1	***
	LG4	28.84	SNP_IGA_401829	7.208	1	***
Blush_2008	LG3	13.401	SNP_IGA_328536	12.183	1	*****
	LG3	13.698	SNP_IGA_327093	13.219	1	*****

LG3	13.698	SNP_IGA_328607	13.219	1	*****
LG3	13.7	SNP_IGA_327678	12.991	1	*****
LG3	13.703	SNP_IGA_328528	13.338	1	*****
LG3	14.327	SNP_IGA_330725	13.105	1	*****
LG3	14.329	SNP_IGA_330917	12.885	1	*****
LG3	14.335	SNP_IGA_330713	13.219	1	*****
LG3	14.337	SNP_IGA_340919	12.991	1	*****
LG3	14.411	SNP_IGA_340884	13.219	1	*****
LG3	14.997	snp_3_12878608	14.595	1	*****
LG3	14.997	SNP_IGA_341962	14.595	1	*****
LG3	15.197	SNP_IGA_343773	12.612	1	*****
LG3	15.532	SNP_IGA_343872	14.297	1	*****
LG3	15.804	Pp19Cl	12.126	1	*****
LG3	13.707	SNP_IGA_329177	11.75	1	*****
LG3	14.465	SNP_IGA_331373	11.318	1	*****
LG7	10.342	SNP_IGA_745637	11.16	1	*****
LG3	5.969	SNP_IGA_309280	8.895	1	*****
LG3	6.062	SNP_IGA_309473	9.125	1	*****
LG3	7.383	SNP_IGA_311300	9.628	1	*****
LG3	7.398	SNP_IGA_311192	9.746	1	*****
LG3	7.398	SNP_IGA_311011	9.746	1	*****
LG3	7.414	SNP_IGA_311941	9.125	1	*****
LG3	7.414	SNP_IGA_312052	9.125	1	*****
LG3	7.414	SNP_IGA_311196	9.125	1	*****
LG3	9.838	SNP_IGA_314598	9.095	1	*****
LG3	12.076	SNP_IGA_338469	9.753	1	*****
LG3	12.091	SNP_IGA_336711	9.974	1	*****
LG3	12.7	SNP_IGA_339178	8.514	1	*****
LG3	12.704	SNP_IGA_338615	8.828	1	*****
LG3	12.722	SNP_IGA_325855	8.602	1	*****
LG3	12.722	SNP_IGA_339170	8.602	1	*****
LG3	12.722	SNP_IGA_339568	8.602	1	*****
LG3	12.722	SNP_IGA_325718	8.602	1	*****
LG3	12.722	SNP_IGA_340016	8.602	1	*****
LG3	12.722	SNP_IGA_326457	8.602	1	*****
LG3	12.722	SNP_IGA_338533	8.602	1	*****
LG3	12.722	SNP_IGA_339037	8.602	1	*****
LG3	12.722	SNP_IGA_339719	8.602	1	*****

LG3	12.733	SNP_IGA_325850	8.828	1	****
LG3	13.027	SNP_IGA_325166	10.573	1	****
LG3	13.029	SNP_IGA_339586	10.338	1	****
LG3	13.034	SNP_IGA_325305	10.475	1	****
LG3	16.466	SNP_IGA_344712	10.446	1	****
LG3	16.466	SNP_IGA_344628	10.446	1	****
LG3	16.473	SNP_IGA_344612	10.254	1	****
LG3	16.473	SNP_IGA_344789	10.254	1	****
LG7	5.873	SNP_IGA_710792	8.503	1	****
LG7	5.873	SNP_IGA_713301	8.503	1	****
LG7	5.873	SNP_IGA_710802	8.503	1	****
LG7	5.873	SNP_IGA_711056	7.903	1	****
LG7	5.873	SNP_IGA_710377	7.903	1	****
LG7	6.97	SNP_IGA_706926	9.821	1	****
LG7	6.97	SNP_IGA_704173	9.821	1	****
LG7	6.97	SNP_IGA_707691	9.821	1	****
LG7	6.999	SNP_IGA_705226	9.967	1	****
LG7	6.999	SNP_IGA_704234	9.967	1	****
LG7	6.999	SNP_IGA_704075	9.967	1	****
LG7	7.218	SNP_IGA_707010	10.23	1	****
LG7	8.069	SNP_IGA_732397	10.642	1	****
LG7	8.087	SNP_IGA_730950	9.967	1	****
LG7	8.087	SNP_IGA_730927	9.967	1	****
LG7	8.087	SNP_IGA_732284	9.967	1	****
LG7	8.087	SNP_IGA_732383	9.967	1	****
LG7	8.087	SNP_IGA_732377	9.967	1	****
LG7	8.104	SNP_IGA_703549	10.23	1	****
LG7	9.176	SNP_IGA_740845	8.199	1	****
LG7	9.176	SNP_IGA_740785	8.199	1	****
LG7	9.176	SNP_IGA_740837	8.199	1	****
LG7	9.176	SNP_IGA_740791	8.199	1	****
LG7	9.202	SNP_IGA_741704	8.481	1	****
LG7	9.202	SNP_IGA_740801	8.481	1	****
LG7	9.208	SNP_IGA_741769	10.184	1	****
LG7	9.222	SNP_IGA_738856	9.56	1	****
LG7	9.222	SNP_IGA_740927	9.56	1	****
LG7	9.222	SNP_IGA_742067	9.56	1	****
LG7	9.222	SNP_IGA_740796	9.56	1	****

LG7	9.222	SNP_IGA_741985	9.56	1	****
LG7	9.222	SNP_IGA_741063	9.56	1	****
LG7	9.222	SNP_IGA_742048	9.56	1	****
LG7	9.222	SNP_IGA_742392	9.56	1	****
LG7	9.222	SNP_IGA_741178	9.56	1	****
LG7	9.222	SNP_IGA_743057	9.56	1	****
LG7	9.222	SNP_IGA_738499	9.56	1	****
LG7	10.353	SNP_IGA_743069	10.522	1	****
LG7	10.359	SNP_IGA_744253	10.645	1	****
LG7	10.944	SNP_IGA_746989	10.741	2	****
LG7	10.944	SNP_IGA_745712	10.741	2	****
LG7	11.22	SNP_IGA_748434	10.246	1	****
LG7	11.296	SNP_IGA_748210	10.723	1	****
LG7	11.296	SNP_IGA_748306	10.723	1	****
LG7	12.078	SNP_IGA_749383	7.932	1	****
LG7	12.078	SNP_IGA_749396	7.932	1	****
LG7	12.088	SNP_IGA_750219	8.034	1	****
LG7	12.992	SNP_IGA_749816	7.932	1	****
LG7	13.132	SNP_IGA_749366	8.205	1	****
LG3	6.654	SNP_IGA_310553	7.04	1	***
LG3	6.717	SNP_IGA_309881	7.385	1	***
LG3	6.727	SNP_IGA_310057	7.486	1	***
LG3	6.727	SNP_IGA_310566	7.486	1	***
LG3	6.727	SNP_IGA_310760	7.486	1	***
LG3	6.727	SNP_IGA_309635	7.486	1	***
LG3	6.727	SNP_IGA_309872	7.486	1	***
LG3	6.727	SNP_IGA_310338	7.486	1	***
LG3	6.727	SNP_IGA_309554	7.486	1	***
LG3	6.727	SNP_IGA_310548	7.486	1	***
LG3	6.727	SNP_IGA_310467	7.486	1	***
LG3	6.727	SNP_IGA_309338	7.486	1	***
LG3	10.243	snp_3_7344624	6.913	1	***
LG3	10.25	SNP_IGA_315837	6.791	1	***
LG3	17.692	SNP_IGA_346164	7.007	1	***
LG3	17.695	SNP_IGA_346196	6.93	1	***
LG7	3.516	SNP_IGA_719131	7.641	1	***
LG7	3.516	SNP_IGA_715579	7.641	1	***
LG7	3.608	SNP_IGA_719923	7.47	1	***

	LG7	3.624	SNP_IGA_718919	7.341	1	***
	LG7	3.624	SNP_IGA_718436	7.341	1	***
	LG7	3.637	SNP_IGA_722952	6.915	1	***
	LG7	3.637	SNP_IGA_719120	6.915	1	***
	LG7	3.653	SNP_IGA_718094	6.8	1	***
	LG7	3.653	SNP_IGA_722771	6.8	1	***
	LG7	3.653	SNP_IGA_716297	6.8	1	***
	LG7	3.653	SNP_IGA_717776	6.8	1	***
	LG7	3.653	SNP_IGA_718633	6.8	1	***
	LG7	3.653	SNP_IGA_716322	6.8	1	***
	LG7	3.653	SNP_IGA_721564	6.8	1	***
	LG7	3.653	SNP_IGA_717591	6.8	1	***
	LG7	3.656	SNP_IGA_722801	6.69	1	***
	LG7	3.656	SNP_IGA_721444	6.69	1	***
	LG7	3.656	SNP_IGA_719178	6.69	1	***
	LG7	3.667	SNP_IGA_722899	6.8	1	***
	LG7	5.879	SNP_IGA_710787	7.769	1	***
	LG7	9.939	SNP_IGA_745363	9.336	2	***
	LG7	10.923	SNP_IGA_746147	9.699	2	***
	LG7	10.938	SNP_IGA_747413	10.397	2	***
	LG7	10.939	SNP_IGA_746204	9.533	2	***
	LG7	11.275	snp_7_7722966	10.405	2	***
	LG7	11.325	SNP_IGA_748637	10.135	2	***
	LG7	13.018	SNP_IGA_749812	6.879	1	***
Blush_2014	LG3	13.027	SNP_IGA_325166	7.546	1	***
	LG3	13.034	SNP_IGA_325305	7.546	1	***
	LG3	13.698	SNP_IGA_327093	7.642	1	***
	LG3	13.698	SNP_IGA_328607	7.642	1	***
	LG3	13.703	SNP_IGA_328528	7.704	1	***
	LG3	13.707	SNP_IGA_329177	7.589	1	***
	LG3	14.327	SNP_IGA_330725	7.768	1	***
	LG3	14.329	SNP_IGA_330917	6.774	1	***
	LG3	14.335	SNP_IGA_330713	7.768	1	***
	LG3	14.337	SNP_IGA_340919	6.774	1	***
	LG3	14.411	SNP_IGA_340884	6.774	1	***
	LG3	14.997	snp_3_12878608	7.768	1	***
	LG3	14.997	SNP_IGA_341962	7.768	1	***
	LG3	15.532	SNP_IGA_343872	7.768	1	***

Blush_Mean	LG3	14.997	snp_3_12878608	15.31	1	*****
	LG3	14.997	SNP_IGA_341962	15.31	1	*****
	LG3	13.698	SNP_IGA_327093	13.739	1	*****
	LG3	13.698	SNP_IGA_328607	13.739	1	*****
	LG3	13.7	SNP_IGA_327678	12.879	1	*****
	LG3	13.703	SNP_IGA_328528	13.819	1	*****
	LG3	13.707	SNP_IGA_329177	12.666	1	*****
	LG3	14.327	SNP_IGA_330725	13.938	1	*****
	LG3	14.329	SNP_IGA_330917	13.094	1	*****
	LG3	14.335	SNP_IGA_330713	14.025	1	*****
	LG3	14.337	SNP_IGA_340919	13.197	1	*****
	LG3	14.411	SNP_IGA_340884	13.419	1	*****
	LG3	15.197	SNP_IGA_343773	13.037	1	*****
	LG3	15.532	SNP_IGA_343872	15.058	1	*****
	LG3	13.027	SNP_IGA_325166	11.737	1	*****
	LG3	13.029	SNP_IGA_339586	10.921	1	*****
	LG3	13.034	SNP_IGA_325305	11.663	1	*****
	LG3	13.401	SNP_IGA_328536	11.922	1	*****
	LG3	14.465	SNP_IGA_331373	11.844	1	*****
	LG3	15.804	Pp19Cl	11.937	1	*****
	LG3	16.466	SNP_IGA_344712	11.379	1	*****
	LG3	16.466	SNP_IGA_344628	11.379	1	*****
	LG3	6.062	SNP_IGA_309473	8.332	1	*****
	LG3	7.383	SNP_IGA_311300	9.064	1	*****
	LG3	7.398	SNP_IGA_311192	9.131	1	*****
	LG3	7.398	SNP_IGA_311011	9.131	1	*****
	LG3	7.414	SNP_IGA_311941	8.332	1	*****
	LG3	7.414	SNP_IGA_312052	8.332	1	*****
	LG3	7.414	SNP_IGA_311196	8.332	1	*****
	LG3	9.838	SNP_IGA_314598	9.111	1	*****
	LG3	12.076	SNP_IGA_338469	9.611	1	*****
	LG3	12.091	SNP_IGA_336711	10.419	1	*****
	LG3	12.7	SNP_IGA_339178	8.837	1	*****
	LG3	12.704	SNP_IGA_338615	9.126	1	*****
	LG3	12.722	SNP_IGA_325855	8.922	1	*****
	LG3	12.722	SNP_IGA_339170	8.922	1	*****
	LG3	12.722	SNP_IGA_339568	8.922	1	*****
	LG3	12.722	SNP_IGA_325718	8.922	1	*****

LG3	12.722	SNP_IGA_340016	8.922	1	****
LG3	12.722	SNP_IGA_326457	8.922	1	****
LG3	12.722	SNP_IGA_338533	8.922	1	****
LG3	12.722	SNP_IGA_339037	8.922	1	****
LG3	12.722	SNP_IGA_339719	8.922	1	****
LG3	12.733	SNP_IGA_325850	9.693	1	****
LG3	16.473	SNP_IGA_344612	10.567	1	****
LG3	16.473	SNP_IGA_344789	10.567	1	****
LG7	11.296	SNP_IGA_748210	7.977	1	****
LG7	11.296	SNP_IGA_748306	7.977	1	****
LG3	5.969	SNP_IGA_309280	7.551	1	***
LG3	6.717	SNP_IGA_309881	6.835	1	***
LG3	6.727	SNP_IGA_310057	6.89	1	***
LG3	6.727	SNP_IGA_310566	6.89	1	***
LG3	6.727	SNP_IGA_310760	6.89	1	***
LG3	6.727	SNP_IGA_309635	6.89	1	***
LG3	6.727	SNP_IGA_309872	6.89	1	***
LG3	6.727	SNP_IGA_310338	6.89	1	***
LG3	6.727	SNP_IGA_309554	6.89	1	***
LG3	6.727	SNP_IGA_310548	6.89	1	***
LG3	6.727	SNP_IGA_310467	6.89	1	***
LG3	6.727	SNP_IGA_309338	6.89	1	***
LG3	10.243	snp_3_7344624	6.669	1	***
LG3	17.692	SNP_IGA_346164	6.875	1	***
LG3	17.695	SNP_IGA_346196	6.83	1	***
LG3	19.988	SNP_IGA_349682	7.367	1	***
LG3	19.988	SNP_IGA_351038	7.367	1	***
LG3	19.99	SNP_IGA_349831	6.707	1	***
LG3	19.99	SNP_IGA_349233	6.707	1	***
LG3	19.99	SNP_IGA_349707	6.707	1	***
LG3	19.99	SNP_IGA_349097	6.707	1	***
LG3	19.99	SNP_IGA_349757	6.707	1	***
LG3	20.021	SNP_IGA_349789	6.915	1	***
LG7	8.069	SNP_IGA_732397	7.215	1	***
LG7	8.104	SNP_IGA_703549	6.653	1	***
LG7	9.208	SNP_IGA_741769	6.851	1	***
LG7	10.342	SNP_IGA_745637	7.238	1	***
LG7	10.359	SNP_IGA_744253	6.65	1	***

	LG7	11.22	SNP_IGA_748434	7.35	1	***
FF_2013	LG4	15.979	SNP_IGA_395152	18.804	2	*****
	LG4	16.541	SNP_IGA_394859	17.539	2	*****
	LG4	17.106	SNP_IGA_393777	18.103	2	*****
	LG4	17.568	SNP_IGA_394233	18.103	2	*****
	LG4	17.589	SNP_IGA_394015	18.103	2	*****
	LG4	17.589	SNP_IGA_393752	18.103	2	*****
	LG4	17.589	SNP_IGA_394034	18.103	2	*****
	LG4	17.589	SNP_IGA_393740	18.103	2	*****
	LG4	17.589	SNP_IGA_394026	18.103	2	*****
	LG4	14.698	SNP_IGA_390430	12.831	2	****
	LG4	14.698	SNP_IGA_390507	12.831	2	****
FF_2014	LG6	36.785	SNP_IGA_670781	16.124	2	*****
	LG6	36.785	SNP_IGA_670280	16.124	2	*****
	LG6	36.785	SNP_IGA_670509	16.124	2	*****
	LG6	36.802	SNP_IGA_670348	16.124	2	*****
	LG6	36.802	SNP_IGA_670494	16.124	2	*****
	LG6	37.096	SNP_IGA_670727	16.124	2	*****
	LG6	35.655	SNP_IGA_667746	13.522	2	****
	LG6	35.715	SNP_IGA_668442	13.522	2	****
	LG6	36.059	SNP_IGA_669050	13.784	2	****
	LG6	36.216	SNP_IGA_669740	13.784	2	****
	LG6	36.244	SNP_IGA_669440	12.365	2	****
	LG6	37.947	SNP_IGA_671630	12.854	2	****
	LG6	38.025	SNP_IGA_671806	11.402	2	****
	LG6	28.427	SNP_IGA_647214	9.659	2	***
	LG6	28.797	SNP_IGA_651095	9.659	2	***
	LG6	29.317	SNP_IGA_652492	9.982	2	***
	LG6	29.604	SNP_IGA_652595	9.982	2	***
	LG6	31.116	SNP_IGA_655564	10.161	2	***
	LG6	53.086	SNP_IGA_682005	9.262	2	***
FF_Mean	LG6	53.086	SNP_IGA_682005	15.29	2	*****
	LG4	15.979	SNP_IGA_395152	12.124	2	****
	LG4	17.568	SNP_IGA_394233	11.965	2	****
	LG4	17.589	SNP_IGA_394015	11.965	2	****
	LG4	17.589	SNP_IGA_393752	11.965	2	****
	LG4	17.589	SNP_IGA_394034	11.965	2	****
	LG4	17.589	SNP_IGA_393740	11.965	2	****

	LG4	17.589	SNP_IGA_394026	11.965	2	****
	LG6	36.785	SNP_IGA_670781	10.778	2	****
	LG6	36.785	SNP_IGA_670280	10.778	2	****
	LG6	36.785	SNP_IGA_670509	10.778	2	****
	LG6	36.802	SNP_IGA_670348	10.778	2	****
	LG6	36.802	SNP_IGA_670494	10.778	2	****
	LG6	37.096	SNP_IGA_670727	10.778	2	****
	LG4	16.541	SNP_IGA_394859	9.454	2	***
	LG4	17.106	SNP_IGA_393777	9.514	2	***
	LG5	40.54	SNP_IGA_598865	6.762	1	***
	LG6	35.655	SNP_IGA_667746	9.486	2	***
	LG6	35.715	SNP_IGA_668442	9.486	2	***
	LG6	36.059	SNP_IGA_669050	9.864	2	***
	LG6	36.216	SNP_IGA_669740	9.864	2	***
	LG6	36.244	SNP_IGA_669440	9.771	2	***
FS_2013	LG6	0	SNP_IGA_607528	18.231	2	*****
	LG6	0	SNP_IGA_607711	18.231	2	*****
	LG6	0.501	SNP_IGA_616295	17.707	2	*****
	LG6	0.501	SNP_IGA_607013	17.707	2	*****
	LG6	0.501	SNP_IGA_616534	17.707	2	*****
	LG6	0.591	SNP_IGA_607240	17.707	2	*****
	LG6	0.591	SNP_IGA_607179	17.707	2	*****
	LG6	0.591	SNP_IGA_616508	17.707	2	*****
	LG6	0.591	SNP_IGA_617922	17.707	2	*****
	LG6	0.591	SNP_IGA_618417	17.707	2	*****
	LG6	0.591	SNP_IGA_607343	17.707	2	*****
	LG6	0.591	SNP_IGA_616286	17.707	2	*****
	LG6	0.591	SNP_IGA_618849	17.707	2	*****
	LG6	0.591	SNP_IGA_618824	17.707	2	*****
	LG6	0.591	SNP_IGA_618376	17.707	2	*****
	LG6	1.158	SNP_IGA_614935	17.707	2	*****
	LG6	1.186	SNP_IGA_616119	17.707	2	*****
	LG6	1.186	SNP_IGA_616005	17.707	2	*****
	LG6	1.186	SNP_IGA_614635	17.707	2	*****
	LG6	1.186	SNP_IGA_615979	17.707	2	*****
	LG6	1.186	SNP_IGA_615377	17.707	2	*****
	LG6	1.201	SNP_IGA_616074	17.707	2	*****
	LG6	1.277	SNP_IGA_615381	17.707	2	*****

	LG6	1.761	SNP_IGA_614519	15.718	2	*****
	LG6	2.338	SNP_IGA_614273	14.861	2	*****
	LG6	2.38	SNP_IGA_614082	14.861	2	*****
	LG6	2.38	SNP_IGA_614045	14.861	2	*****
	LG6	2.38	SNP_IGA_614054	14.861	2	*****
	LG6	2.38	SNP_IGA_614236	14.861	2	*****
	LG6	2.97	snp_6_2750075	14.188	2	*****
	LG6	3.936	SNP_IGA_611544	14.188	2	*****
	LG6	3.936	SNP_IGA_611064	14.188	2	*****
	LG6	3.936	SNP_IGA_611149	14.188	2	*****
	LG6	3.936	SNP_IGA_611891	14.188	2	*****
	LG6	3.936	SNP_IGA_611511	14.188	2	*****
	LG6	3.936	SNP_IGA_611748	14.188	2	*****
	LG6	5.701	SNP_IGA_610531	11.824	2	*****
	LG6	5.701	SNP_IGA_609984	11.824	2	*****
	LG6	5.701	SNP_IGA_610487	11.824	2	*****
	LG6	6.292	SNP_IGA_609630	11.824	2	*****
	LG6	6.292	SNP_IGA_609485	11.824	2	*****
	LG6	6.292	SNP_IGA_609463	11.824	2	*****
	LG6	6.292	SNP_IGA_609531	11.824	2	*****
	LG6	6.292	SNP_IGA_609501	11.824	2	*****
	LG6	6.292	SNP_IGA_609723	11.824	2	*****
	LG6	8.056	SNP_IGA_605863	9.44	2	***
	LG6	8.074	SNP_IGA_606086	9.44	2	***
	LG6	8.074	SNP_IGA_605986	9.44	2	***
	LG6	8.074	SNP_IGA_606059	9.44	2	***
	LG6	8.108	SNP_IGA_605980	9.44	2	***
	LG6	8.422	SNP_IGA_605605	9.44	2	***
FS_2014	LG6	1.761	SNP_IGA_614519	18.712	2	*****
	LG6	2.338	SNP_IGA_614273	18.547	2	*****
	LG6	2.38	SNP_IGA_614082	18.547	2	*****
	LG6	2.38	SNP_IGA_614045	18.547	2	*****
	LG6	2.38	SNP_IGA_614054	18.547	2	*****
	LG6	2.38	SNP_IGA_614236	18.547	2	*****
	LG6	0	SNP_IGA_607528	16.531	2	*****
	LG6	0	SNP_IGA_607711	16.531	2	*****
	LG6	0.501	SNP_IGA_616295	16.531	2	*****
	LG6	0.501	SNP_IGA_607013	16.531	2	*****

LG6	0.501	SNP_IGA_616534	16.531	2	*****
LG6	0.591	SNP_IGA_607240	16.531	2	*****
LG6	0.591	SNP_IGA_607179	16.531	2	*****
LG6	0.591	SNP_IGA_616508	16.531	2	*****
LG6	0.591	SNP_IGA_617922	16.531	2	*****
LG6	0.591	SNP_IGA_618417	16.531	2	*****
LG6	0.591	SNP_IGA_607343	16.531	2	*****
LG6	0.591	SNP_IGA_616286	16.531	2	*****
LG6	0.591	SNP_IGA_618849	16.531	2	*****
LG6	0.591	SNP_IGA_618824	16.531	2	*****
LG6	0.591	SNP_IGA_618376	16.531	2	*****
LG6	1.158	SNP_IGA_614935	17.809	2	*****
LG6	1.186	SNP_IGA_616119	17.809	2	*****
LG6	1.186	SNP_IGA_616005	17.809	2	*****
LG6	1.186	SNP_IGA_614635	17.809	2	*****
LG6	1.186	SNP_IGA_615979	17.809	2	*****
LG6	1.186	SNP_IGA_615377	17.809	2	*****
LG6	1.201	SNP_IGA_616074	17.809	2	*****
LG6	1.277	SNP_IGA_615381	17.809	2	*****
LG6	2.97	snp_6_2750075	18.32	2	*****
LG6	8.422	SNP_IGA_605605	15.213	2	*****
LG6	9.724	SNP_IGA_619081	15.87	2	*****
LG6	10.086	SNP_IGA_619263	15.672	2	*****
LG6	10.921	SNP_IGA_620099	15.756	2	*****
LG6	10.937	SNP_IGA_619845	15.756	2	*****
LG6	3.936	SNP_IGA_611544	14.91	2	*****
LG6	3.936	SNP_IGA_611064	14.91	2	*****
LG6	3.936	SNP_IGA_611149	14.91	2	*****
LG6	3.936	SNP_IGA_611891	14.91	2	*****
LG6	3.936	SNP_IGA_611511	14.91	2	*****
LG6	3.936	SNP_IGA_611748	14.91	2	*****
LG6	5.701	SNP_IGA_610531	14.876	2	*****
LG6	5.701	SNP_IGA_609984	14.876	2	*****
LG6	5.701	SNP_IGA_610487	14.876	2	*****
LG6	6.292	SNP_IGA_609630	14.954	2	*****
LG6	6.292	SNP_IGA_609485	14.954	2	*****
LG6	6.292	SNP_IGA_609463	14.954	2	*****
LG6	6.292	SNP_IGA_609531	14.954	2	*****

	LG6	6.292	SNP_IGA_609501	14.954	2	*****
	LG6	6.292	SNP_IGA_609723	14.954	2	*****
	LG6	8.056	SNP_IGA_605863	12.141	2	****
	LG6	8.074	SNP_IGA_606086	12.141	2	****
	LG6	8.074	SNP_IGA_605986	12.141	2	****
	LG6	8.074	SNP_IGA_606059	12.141	2	****
	LG6	8.108	SNP_IGA_605980	12.141	2	****
	LG6	8.616	SNP_IGA_606435	12.919	2	****
	LG6	13.493	snp_6_5294415	11.56	2	****
	LG6	14.093	SNP_IGA_621593	11.802	2	****
	LG6	14.093	SNP_IGA_621562	11.802	2	****
	LG6	14.094	SNP_IGA_621556	11.802	2	****
	LG6	14.809	SNP_IGA_621914	12.079	2	****
	LG6	14.837	SNP_IGA_621925	12.079	2	****
	LG6	15.571	SNP_IGA_624015	11.401	2	****
	LG6	16.24	SNP_IGA_624396	11.253	2	****
	LG6	16.25	SNP_IGA_624391	11.253	2	****
	LG6	21.509	SNP_IGA_629177	10.699	2	****
	LG2	0.579	SNP_IGA_230405	6.802	1	***
	LG2	4.366	SNP_IGA_144919	6.691	1	***
	LG2	4.366	SNP_IGA_144913	6.691	1	***
	LG2	4.366	SNP_IGA_139783	6.691	1	***
	LG2	4.366	SNP_IGA_152301	6.691	1	***
	LG2	4.366	SNP_IGA_154600	6.691	1	***
	LG2	4.366	SNP_IGA_152330	6.691	1	***
	LG2	4.366	SNP_IGA_152809	6.691	1	***
	LG2	4.366	SNP_IGA_143346	6.691	1	***
	LG2	4.366	SNP_IGA_156070	6.691	1	***
	LG2	4.376	SNP_IGA_139433	6.691	1	***
	LG2	4.376	SNP_IGA_137625	6.691	1	***
	LG6	16.949	SNP_IGA_624583	9.825	2	***
	LG6	17.458	SNP_IGA_625680	9.825	2	***
	LG6	20.309	SNP_IGA_628744	10.176	2	***
	LG6	20.779	SNP_IGA_628841	10.395	2	***
	LG6	20.779	SNP_IGA_628993	10.395	2	***
	LG6	20.779	snp_6_7910869	10.395	2	***
FS_Mean	LG6	0	SNP_IGA_607528	22.732	2	*****
	LG6	0	SNP_IGA_607711	22.732	2	*****

LG6	0.501	SNP_IGA_616295	22.341	2	*****
LG6	0.501	SNP_IGA_607013	22.341	2	*****
LG6	0.501	SNP_IGA_616534	22.341	2	*****
LG6	0.591	SNP_IGA_607240	22.341	2	*****
LG6	0.591	SNP_IGA_607179	22.341	2	*****
LG6	0.591	SNP_IGA_616508	22.341	2	*****
LG6	0.591	SNP_IGA_617922	22.341	2	*****
LG6	0.591	SNP_IGA_618417	22.341	2	*****
LG6	0.591	SNP_IGA_607343	22.341	2	*****
LG6	0.591	SNP_IGA_616286	22.341	2	*****
LG6	0.591	SNP_IGA_618849	22.341	2	*****
LG6	0.591	SNP_IGA_618824	22.341	2	*****
LG6	0.591	SNP_IGA_618376	22.341	2	*****
LG6	1.158	SNP_IGA_614935	24.105	2	*****
LG6	1.186	SNP_IGA_616119	24.105	2	*****
LG6	1.186	SNP_IGA_616005	24.105	2	*****
LG6	1.186	SNP_IGA_614635	24.105	2	*****
LG6	1.186	SNP_IGA_615979	24.105	2	*****
LG6	1.186	SNP_IGA_615377	24.105	2	*****
LG6	1.201	SNP_IGA_616074	24.105	2	*****
LG6	1.277	SNP_IGA_615381	24.105	2	*****
LG6	1.761	SNP_IGA_614519	22.918	2	*****
LG6	2.338	SNP_IGA_614273	22.404	2	*****
LG6	2.38	SNP_IGA_614082	22.404	2	*****
LG6	2.38	SNP_IGA_614045	22.404	2	*****
LG6	2.38	SNP_IGA_614054	22.404	2	*****
LG6	2.38	SNP_IGA_614236	22.404	2	*****
LG6	2.97	snp_6_2750075	21.942	2	*****
LG6	3.936	SNP_IGA_611544	18.077	2	*****
LG6	3.936	SNP_IGA_611064	18.077	2	*****
LG6	3.936	SNP_IGA_611149	18.077	2	*****
LG6	3.936	SNP_IGA_611891	18.077	2	*****
LG6	3.936	SNP_IGA_611511	18.077	2	*****
LG6	3.936	SNP_IGA_611748	18.077	2	*****
LG6	5.701	SNP_IGA_610531	17.492	2	*****
LG6	5.701	SNP_IGA_609984	17.492	2	*****
LG6	5.701	SNP_IGA_610487	17.492	2	*****
LG6	6.292	SNP_IGA_609630	17.328	2	*****

LG6	6.292	SNP_IGA_609485	17.328	2	*****
LG6	6.292	SNP_IGA_609463	17.328	2	*****
LG6	6.292	SNP_IGA_609531	17.328	2	*****
LG6	6.292	SNP_IGA_609501	17.328	2	*****
LG6	6.292	SNP_IGA_609723	17.328	2	*****
LG6	8.422	SNP_IGA_605605	18.001	2	*****
LG6	9.724	SNP_IGA_619081	17.452	2	*****
LG6	10.086	SNP_IGA_619263	16.99	2	*****
LG6	10.921	SNP_IGA_620099	17.336	2	*****
LG6	10.937	SNP_IGA_619845	17.336	2	*****
LG6	8.056	SNP_IGA_605863	14.619	2	*****
LG6	8.074	SNP_IGA_606086	14.619	2	*****
LG6	8.074	SNP_IGA_605986	14.619	2	*****
LG6	8.074	SNP_IGA_606059	14.619	2	*****
LG6	8.108	SNP_IGA_605980	14.619	2	*****
LG6	8.616	SNP_IGA_606435	13.98	2	*****
LG6	13.493	snp_6_5294415	12.075	2	*****
LG6	14.093	SNP_IGA_621593	12.101	2	*****
LG6	14.093	SNP_IGA_621562	12.101	2	*****
LG6	14.094	SNP_IGA_621556	12.101	2	*****
LG6	14.809	SNP_IGA_621914	12.259	2	*****
LG6	14.837	SNP_IGA_621925	12.259	2	*****
LG6	15.571	SNP_IGA_624015	12.992	2	*****
LG6	16.24	SNP_IGA_624396	12.887	2	*****
LG6	16.25	SNP_IGA_624391	12.887	2	*****
LG6	16.949	SNP_IGA_624583	11.712	2	*****
LG6	17.458	SNP_IGA_625680	11.703	2	*****
LG6	20.309	SNP_IGA_628744	11.762	2	*****
LG6	20.779	SNP_IGA_628841	11.916	2	*****
LG6	20.779	SNP_IGA_628993	11.916	2	*****
LG6	20.779	snp_6_7910869	11.916	2	*****
LG2	0.579	SNP_IGA_230405	7.801	1	***
LG2	0.671	SNP_IGA_229248	7.52	1	***
LG2	0.681	SNP_IGA_230394	6.808	1	***
LG2	0.681	SNP_IGA_230715	6.808	1	***
LG2	0.681	SNP_IGA_229066	6.808	1	***
LG2	0.681	SNP_IGA_231766	6.808	1	***
LG2	0.742	SNP_IGA_230389	6.808	1	***

	LG2	0.742	SNP_IGA_232140	6.808	1	***
	LG2	0.742	SNP_IGA_230835	6.808	1	***
	LG2	4.376	SNP_IGA_139433	7.123	1	***
	LG2	4.376	SNP_IGA_137625	7.123	1	***
	LG6	19.364	SNP_IGA_626080	9.489	2	***
	LG6	19.364	SNP_IGA_627574	9.489	2	***
	LG6	21.509	SNP_IGA_629177	10.589	2	***
	LG6	22.002	SNP_IGA_631014	9.312	2	***
	LG6	22.002	SNP_IGA_631212	9.312	2	***
	LG8	5.688	SNP_IGA_856141	9.574	2	***
	LG8	6.35	SNP_IGA_857951	9.47	2	***
FW_2013	LG6	0	SNP_IGA_607528	26.642	2	*****
	LG6	0	SNP_IGA_607711	26.642	2	*****
	LG6	0.501	SNP_IGA_616295	26.532	2	*****
	LG6	0.501	SNP_IGA_607013	26.532	2	*****
	LG6	0.501	SNP_IGA_616534	26.532	2	*****
	LG6	0.591	SNP_IGA_607240	26.532	2	*****
	LG6	0.591	SNP_IGA_607179	26.532	2	*****
	LG6	0.591	SNP_IGA_616508	26.532	2	*****
	LG6	0.591	SNP_IGA_617922	26.532	2	*****
	LG6	0.591	SNP_IGA_618417	26.532	2	*****
	LG6	0.591	SNP_IGA_607343	26.532	2	*****
	LG6	0.591	SNP_IGA_616286	26.532	2	*****
	LG6	0.591	SNP_IGA_618849	26.532	2	*****
	LG6	0.591	SNP_IGA_618824	26.532	2	*****
	LG6	0.591	SNP_IGA_618376	26.532	2	*****
	LG6	1.158	SNP_IGA_614935	26.532	2	*****
	LG6	1.186	SNP_IGA_616119	26.532	2	*****
	LG6	1.186	SNP_IGA_616005	26.532	2	*****
	LG6	1.186	SNP_IGA_614635	26.532	2	*****
	LG6	1.186	SNP_IGA_615979	26.532	2	*****
	LG6	1.186	SNP_IGA_615377	26.532	2	*****
	LG6	1.201	SNP_IGA_616074	26.532	2	*****
	LG6	1.277	SNP_IGA_615381	26.532	2	*****
	LG6	1.761	SNP_IGA_614519	24.264	2	*****
	LG6	2.338	SNP_IGA_614273	23.604	2	*****
	LG6	2.38	SNP_IGA_614082	23.604	2	*****
	LG6	2.38	SNP_IGA_614045	23.604	2	*****

LG6	2.38	SNP_IGA_614054	23.604	2	*****
LG6	2.38	SNP_IGA_614236	23.604	2	*****
LG6	2.97	snp_6_2750075	22.159	2	*****
LG6	3.936	SNP_IGA_611544	22.159	2	*****
LG6	3.936	SNP_IGA_611064	22.159	2	*****
LG6	3.936	SNP_IGA_611149	22.159	2	*****
LG6	3.936	SNP_IGA_611891	22.159	2	*****
LG6	3.936	SNP_IGA_611511	22.159	2	*****
LG6	3.936	SNP_IGA_611748	22.159	2	*****
LG6	5.701	SNP_IGA_610531	19.077	2	*****
LG6	5.701	SNP_IGA_609984	19.077	2	*****
LG6	5.701	SNP_IGA_610487	19.077	2	*****
LG6	6.292	SNP_IGA_609630	19.077	2	*****
LG6	6.292	SNP_IGA_609485	19.077	2	*****
LG6	6.292	SNP_IGA_609463	19.077	2	*****
LG6	6.292	SNP_IGA_609531	19.077	2	*****
LG6	6.292	SNP_IGA_609501	19.077	2	*****
LG6	6.292	SNP_IGA_609723	19.077	2	*****
LG6	8.056	SNP_IGA_605863	16.98	2	*****
LG6	8.074	SNP_IGA_606086	16.98	2	*****
LG6	8.074	SNP_IGA_605986	16.98	2	*****
LG6	8.074	SNP_IGA_606059	16.98	2	*****
LG6	8.108	SNP_IGA_605980	16.98	2	*****
LG6	8.422	SNP_IGA_605605	16.98	2	*****
LG6	9.724	SNP_IGA_619081	15.325	2	*****
LG6	10.086	SNP_IGA_619263	15.325	2	*****
LG6	10.921	SNP_IGA_620099	15.63	2	*****
LG6	10.937	SNP_IGA_619845	15.63	2	*****
LG6	8.616	SNP_IGA_606435	13.968	2	*****
LG6	13.493	snp_6_5294415	11.95	2	****
LG6	14.093	SNP_IGA_621593	11.562	2	****
LG6	14.093	SNP_IGA_621562	11.562	2	****
LG6	14.094	SNP_IGA_621556	11.562	2	****
LG6	14.809	SNP_IGA_621914	10.841	2	****
LG6	14.837	SNP_IGA_621925	10.841	2	****
LG6	15.571	SNP_IGA_624015	13.105	2	****
LG6	16.24	SNP_IGA_624396	13.105	2	****
LG6	16.25	SNP_IGA_624391	13.105	2	****

	LG6	16.949	SNP_IGA_624583	12.466	2	****
	LG6	17.458	SNP_IGA_625680	11.779	2	****
FW_2014	LG6	1.158	SNP_IGA_614935	18.753	2	*****
	LG6	1.186	SNP_IGA_616119	18.753	2	*****
	LG6	1.186	SNP_IGA_616005	18.753	2	*****
	LG6	1.186	SNP_IGA_614635	18.753	2	*****
	LG6	1.186	SNP_IGA_615979	18.753	2	*****
	LG6	1.186	SNP_IGA_615377	18.753	2	*****
	LG6	1.201	SNP_IGA_616074	18.753	2	*****
	LG6	1.277	SNP_IGA_615381	18.753	2	*****
	LG6	1.761	SNP_IGA_614519	19.541	2	*****
	LG6	2.338	SNP_IGA_614273	19.372	2	*****
	LG6	2.38	SNP_IGA_614082	19.372	2	*****
	LG6	2.38	SNP_IGA_614045	19.372	2	*****
	LG6	2.38	SNP_IGA_614054	19.372	2	*****
	LG6	2.38	SNP_IGA_614236	19.372	2	*****
	LG6	2.97	snp_6_2750075	19.184	2	*****
	LG6	0	SNP_IGA_607528	17.71	2	*****
	LG6	0	SNP_IGA_607711	17.71	2	*****
	LG6	0.501	SNP_IGA_616295	17.71	2	*****
	LG6	0.501	SNP_IGA_607013	17.71	2	*****
	LG6	0.501	SNP_IGA_616534	17.71	2	*****
	LG6	0.591	SNP_IGA_607240	17.71	2	*****
	LG6	0.591	SNP_IGA_607179	17.71	2	*****
	LG6	0.591	SNP_IGA_616508	17.71	2	*****
	LG6	0.591	SNP_IGA_617922	17.71	2	*****
	LG6	0.591	SNP_IGA_618417	17.71	2	*****
	LG6	0.591	SNP_IGA_607343	17.71	2	*****
	LG6	0.591	SNP_IGA_616286	17.71	2	*****
	LG6	0.591	SNP_IGA_618849	17.71	2	*****
	LG6	0.591	SNP_IGA_618824	17.71	2	*****
	LG6	0.591	SNP_IGA_618376	17.71	2	*****
	LG6	3.936	SNP_IGA_611544	15.724	2	*****
	LG6	3.936	SNP_IGA_611064	15.724	2	*****
	LG6	3.936	SNP_IGA_611149	15.724	2	*****
	LG6	3.936	SNP_IGA_611891	15.724	2	*****
	LG6	3.936	SNP_IGA_611511	15.724	2	*****
	LG6	3.936	SNP_IGA_611748	15.724	2	*****

LG6	5.701	SNP_IGA_610531	15.7	2	*****
LG6	5.701	SNP_IGA_609984	15.7	2	*****
LG6	5.701	SNP_IGA_610487	15.7	2	*****
LG6	6.292	SNP_IGA_609630	15.738	2	*****
LG6	6.292	SNP_IGA_609485	15.738	2	*****
LG6	6.292	SNP_IGA_609463	15.738	2	*****
LG6	6.292	SNP_IGA_609531	15.738	2	*****
LG6	6.292	SNP_IGA_609501	15.738	2	*****
LG6	6.292	SNP_IGA_609723	15.738	2	*****
LG6	8.422	SNP_IGA_605605	15.998	2	*****
LG6	9.724	SNP_IGA_619081	16.43	2	*****
LG6	10.086	SNP_IGA_619263	16.225	2	*****
LG6	10.921	SNP_IGA_620099	16.289	2	*****
LG6	10.937	SNP_IGA_619845	16.289	2	*****
LG6	8.056	SNP_IGA_605863	12.811	2	****
LG6	8.074	SNP_IGA_606086	12.811	2	****
LG6	8.074	SNP_IGA_605986	12.811	2	****
LG6	8.074	SNP_IGA_606059	12.811	2	****
LG6	8.108	SNP_IGA_605980	12.811	2	****
LG6	8.616	SNP_IGA_606435	13.365	2	****
LG6	13.493	snp_6_5294415	12.206	2	****
LG6	14.093	SNP_IGA_621593	12.387	2	****
LG6	14.093	SNP_IGA_621562	12.387	2	****
LG6	14.094	SNP_IGA_621556	12.387	2	****
LG6	14.809	SNP_IGA_621914	12.719	2	****
LG6	14.837	SNP_IGA_621925	12.719	2	****
LG6	15.571	SNP_IGA_624015	12.191	2	****
LG6	16.24	SNP_IGA_624396	11.921	2	****
LG6	16.25	SNP_IGA_624391	11.921	2	****
LG6	20.309	SNP_IGA_628744	11.249	2	****
LG6	20.779	SNP_IGA_628841	11.459	2	****
LG6	20.779	SNP_IGA_628993	11.459	2	****
LG6	20.779	snp_6_7910869	11.459	2	****
LG6	21.509	SNP_IGA_629177	11.569	2	****
LG6	16.949	SNP_IGA_624583	10.406	2	***
LG6	17.458	SNP_IGA_625680	10.406	2	***
LG6	21.235	SNP_IGA_629806	9.58	2	***
LG6	22.002	SNP_IGA_631014	9.75	2	***

	LG6	22.002	SNP_IGA_631212	9.75	2	***
	LG8	24.636	SNP_IGA_872978	9.31	2	***
FW_Mean	LG6	0	SNP_IGA_607528	28.099	2	*****
	LG6	0	SNP_IGA_607711	28.099	2	*****
	LG6	0.501	SNP_IGA_616295	27.544	2	*****
	LG6	0.501	SNP_IGA_607013	27.544	2	*****
	LG6	0.501	SNP_IGA_616534	27.544	2	*****
	LG6	0.591	SNP_IGA_607240	27.544	2	*****
	LG6	0.591	SNP_IGA_607179	27.544	2	*****
	LG6	0.591	SNP_IGA_616508	27.544	2	*****
	LG6	0.591	SNP_IGA_617922	27.544	2	*****
	LG6	0.591	SNP_IGA_618417	27.544	2	*****
	LG6	0.591	SNP_IGA_607343	27.544	2	*****
	LG6	0.591	SNP_IGA_616286	27.544	2	*****
	LG6	0.591	SNP_IGA_618849	27.544	2	*****
	LG6	0.591	SNP_IGA_618824	27.544	2	*****
	LG6	0.591	SNP_IGA_618376	27.544	2	*****
	LG6	1.158	SNP_IGA_614935	28.271	2	*****
	LG6	1.186	SNP_IGA_616119	28.271	2	*****
	LG6	1.186	SNP_IGA_616005	28.271	2	*****
	LG6	1.186	SNP_IGA_614635	28.271	2	*****
	LG6	1.186	SNP_IGA_615979	28.271	2	*****
	LG6	1.186	SNP_IGA_615377	28.271	2	*****
	LG6	1.201	SNP_IGA_616074	28.271	2	*****
	LG6	1.277	SNP_IGA_615381	28.271	2	*****
	LG6	1.761	SNP_IGA_614519	26.515	2	*****
	LG6	2.338	SNP_IGA_614273	26.11	2	*****
	LG6	2.38	SNP_IGA_614082	26.11	2	*****
	LG6	2.38	SNP_IGA_614045	26.11	2	*****
	LG6	2.38	SNP_IGA_614054	26.11	2	*****
	LG6	2.38	SNP_IGA_614236	26.11	2	*****
	LG6	2.97	snp_6_2750075	25.501	2	*****
	LG6	3.936	SNP_IGA_611544	21.273	2	*****
	LG6	3.936	SNP_IGA_611064	21.273	2	*****
	LG6	3.936	SNP_IGA_611149	21.273	2	*****
	LG6	3.936	SNP_IGA_611891	21.273	2	*****
	LG6	3.936	SNP_IGA_611511	21.273	2	*****
	LG6	3.936	SNP_IGA_611748	21.273	2	*****

LG6	5.701	SNP_IGA_610531	20.867	2	*****
LG6	5.701	SNP_IGA_609984	20.867	2	*****
LG6	5.701	SNP_IGA_610487	20.867	2	*****
LG6	6.292	SNP_IGA_609630	20.308	2	*****
LG6	6.292	SNP_IGA_609485	20.308	2	*****
LG6	6.292	SNP_IGA_609463	20.308	2	*****
LG6	6.292	SNP_IGA_609531	20.308	2	*****
LG6	6.292	SNP_IGA_609501	20.308	2	*****
LG6	6.292	SNP_IGA_609723	20.308	2	*****
LG6	8.422	SNP_IGA_605605	22.362	2	*****
LG6	9.724	SNP_IGA_619081	21.268	2	*****
LG6	10.086	SNP_IGA_619263	20.418	2	*****
LG6	10.921	SNP_IGA_620099	20.679	2	*****
LG6	10.937	SNP_IGA_619845	20.679	2	*****
LG6	8.056	SNP_IGA_605863	18.35	2	*****
LG6	8.074	SNP_IGA_606086	18.35	2	*****
LG6	8.074	SNP_IGA_605986	18.35	2	*****
LG6	8.074	SNP_IGA_606059	18.35	2	*****
LG6	8.108	SNP_IGA_605980	18.35	2	*****
LG6	8.616	SNP_IGA_606435	17.068	2	*****
LG6	15.571	SNP_IGA_624015	15.31	2	*****
LG6	16.24	SNP_IGA_624396	15.419	2	*****
LG6	16.25	SNP_IGA_624391	15.419	2	*****
LG6	13.493	snp_6_5294415	14.355	2	*****
LG6	14.093	SNP_IGA_621593	14.161	2	*****
LG6	14.093	SNP_IGA_621562	14.161	2	*****
LG6	14.094	SNP_IGA_621556	14.161	2	*****
LG6	14.809	SNP_IGA_621914	13.928	2	*****
LG6	14.837	SNP_IGA_621925	13.928	2	*****
LG6	16.949	SNP_IGA_624583	14.603	2	*****
LG6	17.458	SNP_IGA_625680	14.479	2	*****
LG6	20.309	SNP_IGA_628744	14.195	2	*****
LG6	20.779	SNP_IGA_628841	13.982	2	*****
LG6	20.779	SNP_IGA_628993	13.982	2	*****
LG6	20.779	snp_6_7910869	13.982	2	*****
LG6	19.364	SNP_IGA_626080	11.232	2	****
LG6	19.364	SNP_IGA_627574	11.232	2	****
LG6	19.767	SNP_IGA_627934	11.62	2	****

	LG6	19.794	SNP_IGA_627396	11.62	2	****
	LG6	19.794	SNP_IGA_627350	11.62	2	****
	LG6	19.794	SNP_IGA_627328	11.62	2	****
	LG6	19.794	SNP_IGA_627415	11.62	2	****
	LG6	19.814	SNP_IGA_627535	11.62	2	****
	LG6	21.509	SNP_IGA_629177	13.127	2	****
	LG6	22.002	SNP_IGA_631014	11.642	2	****
	LG6	22.002	SNP_IGA_631212	11.642	2	****
	LG6	19.164	SNP_IGA_628464	9.929	2	***
	LG6	21.235	SNP_IGA_629806	10.492	2	***
	LG6	23.383	SNP_IGA_632123	9.252	2	***
	LG6	23.383	SNP_IGA_631845	9.252	2	***
	LG6	23.383	SNP_IGA_632110	9.252	2	***
	LG6	23.383	SNP_IGA_631948	9.252	2	***
	LG6	23.383	SNP_IGA_632049	9.252	2	***
	LG8	5.047	SNP_IGA_855459	9.481	2	***
	LG8	5.047	SNP_IGA_855356	9.481	2	***
	LG8	5.08	SNP_IGA_855507	9.481	2	***
	LG8	5.688	SNP_IGA_856141	10.509	2	***
	LG8	6.35	SNP_IGA_857951	10.046	2	***
IAD_2014	LG6	3.936	SNP_IGA_611544	19.655	2	*****
	LG6	3.936	SNP_IGA_611064	19.655	2	*****
	LG6	3.936	SNP_IGA_611149	19.655	2	*****
	LG6	3.936	SNP_IGA_611891	19.655	2	*****
	LG6	3.936	SNP_IGA_611511	19.655	2	*****
	LG6	3.936	SNP_IGA_611748	19.655	2	*****
	LG6	5.701	SNP_IGA_610531	19.994	2	*****
	LG6	5.701	SNP_IGA_609984	19.994	2	*****
	LG6	5.701	SNP_IGA_610487	19.994	2	*****
	LG6	8.616	SNP_IGA_606435	19.631	2	*****
	LG6	13.493	snp_6_5294415	20.839	2	*****
	LG6	14.093	SNP_IGA_621593	20.363	2	*****
	LG6	14.093	SNP_IGA_621562	20.363	2	*****
	LG6	14.094	SNP_IGA_621556	20.363	2	*****
	LG6	14.809	SNP_IGA_621914	19.45	2	*****
	LG6	14.837	SNP_IGA_621925	19.45	2	*****
	LG6	0	SNP_IGA_607528	15.704	2	*****
	LG6	0	SNP_IGA_607711	15.704	2	*****

LG6	0.501	SNP_IGA_616295	15.704	2	*****
LG6	0.501	SNP_IGA_607013	15.704	2	*****
LG6	0.501	SNP_IGA_616534	15.704	2	*****
LG6	0.591	SNP_IGA_607240	15.704	2	*****
LG6	0.591	SNP_IGA_607179	15.704	2	*****
LG6	0.591	SNP_IGA_616508	15.704	2	*****
LG6	0.591	SNP_IGA_617922	15.704	2	*****
LG6	0.591	SNP_IGA_618417	15.704	2	*****
LG6	0.591	SNP_IGA_607343	15.704	2	*****
LG6	0.591	SNP_IGA_616286	15.704	2	*****
LG6	0.591	SNP_IGA_618849	15.704	2	*****
LG6	0.591	SNP_IGA_618824	15.704	2	*****
LG6	0.591	SNP_IGA_618376	15.704	2	*****
LG6	1.158	SNP_IGA_614935	16.076	2	*****
LG6	1.186	SNP_IGA_616119	16.076	2	*****
LG6	1.186	SNP_IGA_616005	16.076	2	*****
LG6	1.186	SNP_IGA_614635	16.076	2	*****
LG6	1.186	SNP_IGA_615979	16.076	2	*****
LG6	1.186	SNP_IGA_615377	16.076	2	*****
LG6	1.201	SNP_IGA_616074	16.076	2	*****
LG6	1.277	SNP_IGA_615381	16.076	2	*****
LG6	1.761	SNP_IGA_614519	16.715	2	*****
LG6	2.338	SNP_IGA_614273	16.612	2	*****
LG6	2.38	SNP_IGA_614082	16.612	2	*****
LG6	2.38	SNP_IGA_614045	16.612	2	*****
LG6	2.38	SNP_IGA_614054	16.612	2	*****
LG6	2.38	SNP_IGA_614236	16.612	2	*****
LG6	2.97	snp_6_2750075	15.271	2	*****
LG6	6.292	SNP_IGA_609630	18.247	2	*****
LG6	6.292	SNP_IGA_609485	18.247	2	*****
LG6	6.292	SNP_IGA_609463	18.247	2	*****
LG6	6.292	SNP_IGA_609531	18.247	2	*****
LG6	6.292	SNP_IGA_609501	18.247	2	*****
LG6	6.292	SNP_IGA_609723	18.247	2	*****
LG6	8.056	SNP_IGA_605863	17.442	2	*****
LG6	8.074	SNP_IGA_606086	17.442	2	*****
LG6	8.074	SNP_IGA_605986	17.442	2	*****
LG6	8.074	SNP_IGA_606059	17.442	2	*****

	LG6	8.108	SNP_IGA_605980	17.442	2	*****
	LG6	9.724	SNP_IGA_619081	17.784	2	*****
	LG6	10.086	SNP_IGA_619263	16.528	2	*****
	LG6	10.921	SNP_IGA_620099	16.076	2	*****
	LG6	10.937	SNP_IGA_619845	16.076	2	*****
	LG6	15.571	SNP_IGA_624015	16.897	2	*****
	LG6	16.24	SNP_IGA_624396	17.586	2	*****
	LG6	16.25	SNP_IGA_624391	17.586	2	*****
	LG6	16.949	SNP_IGA_624583	16.006	2	*****
	LG6	17.458	SNP_IGA_625680	16.006	2	*****
	LG6	19.164	SNP_IGA_628464	15.689	2	*****
	LG6	19.364	SNP_IGA_626080	17.081	2	*****
	LG6	19.364	SNP_IGA_627574	17.081	2	*****
	LG6	19.767	SNP_IGA_627934	16.131	2	*****
	LG6	19.794	SNP_IGA_627396	16.131	2	*****
	LG6	19.794	SNP_IGA_627350	16.131	2	*****
	LG6	19.794	SNP_IGA_627328	16.131	2	*****
	LG6	19.794	SNP_IGA_627415	16.131	2	*****
	LG6	19.814	SNP_IGA_627535	16.131	2	*****
	LG6	21.235	SNP_IGA_629806	15.828	2	*****
	LG6	8.422	SNP_IGA_605605	13.654	2	*****
	LG6	20.309	SNP_IGA_628744	13.069	2	*****
	LG6	20.779	SNP_IGA_628841	12.406	2	*****
	LG6	20.779	SNP_IGA_628993	12.406	2	*****
	LG6	20.779	snp_6_7910869	12.406	2	*****
	LG6	21.509	SNP_IGA_629177	12.097	2	*****
	LG6	22.002	SNP_IGA_631014	10.082	2	***
	LG6	22.002	SNP_IGA_631212	10.082	2	***
IAD_Mean	LG6	53.086	SNP_IGA_682005	13.343	2	****
	LG8	1.706	SNP_IGA_853101	11.472	2	****
	LG8	2.04	SNP_IGA_853250	10.957	2	****
	LG8	2.211	SNP_IGA_853233	10.957	2	****
	LG8	2.211	SNP_IGA_853257	10.957	2	****
	LG8	2.216	SNP_IGA_853239	10.957	2	****
	LG8	0.504	SNP_IGA_834505	9.349	2	***
	LG8	0.539	SNP_IGA_834321	9.277	2	***
SSC_2014	LG4	20.813	SNP_IGA_396028	17.609	1	*****
	LG4	20.876	SNP_IGA_396308	17.322	1	*****

LG4	20.877	SNP_IGA_396958	17.609	1	*****
LG4	20.877	SNP_IGA_397165	17.609	1	*****
LG4	20.877	SNP_IGA_395529	17.609	1	*****
LG4	20.882	SNP_IGA_396340	16.214	1	*****
LG4	20.949	SNP_IGA_396034	17.609	1	*****
LG4	20.949	SNP_IGA_397015	17.609	1	*****
LG4	20.949	SNP_IGA_396171	17.609	1	*****
LG4	20.949	SNP_IGA_395621	17.609	1	*****
LG4	20.949	SNP_IGA_396443	17.609	1	*****
LG4	20.949	SNP_IGA_396351	17.609	1	*****
LG4	20.949	SNP_IGA_396345	17.609	1	*****
LG4	20.949	SNP_IGA_396006	17.609	1	*****
LG4	20.949	SNP_IGA_396439	17.609	1	*****
LG4	22.108	SNP_IGA_398213	18.461	1	*****
LG4	22.108	SNP_IGA_398075	18.461	1	*****
LG4	22.108	SNP_IGA_398373	18.461	1	*****
LG4	22.875	SNP_IGA_397710	15.445	1	*****
LG4	22.875	SNP_IGA_397732	15.445	1	*****
LG4	22.881	SNP_IGA_398915	15.445	1	*****
LG4	22.881	SNP_IGA_399569	15.445	1	*****
LG4	22.881	SNP_IGA_398228	15.445	1	*****
LG4	22.881	SNP_IGA_399565	15.445	1	*****
LG4	22.881	SNP_IGA_398368	15.445	1	*****
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LG4	22.881	SNP_IGA_399330	15.445	1	*****
LG4	22.881	SNP_IGA_399292	15.445	1	*****
LG4	22.881	SNP_IGA_399280	15.445	1	*****
LG4	22.881	SNP_IGA_399599	15.445	1	*****
LG4	22.881	SNP_IGA_398634	15.445	1	*****
LG4	22.881	SNP_IGA_397696	15.445	1	*****
LG4	22.881	SNP_IGA_398997	15.445	1	*****
LG4	24.018	SNP_IGA_399812	15.445	1	*****
LG4	24.018	SNP_IGA_399756	15.445	1	*****
LG4	24.018	SNP_IGA_399658	15.445	1	*****
LG4	24.034	SNP_IGA_399849	15.445	1	*****
LG4	25.14	SNP_IGA_401072	15.881	1	*****
LG4	25.14	SNP_IGA_400359	15.881	1	*****

LG4	25.14	Pp10Cl	15.881	1	*****
LG4	25.14	SNP_IGA_400613	15.881	1	*****
LG4	25.14	SNP_IGA_400090	15.881	1	*****
LG4	25.14	SNP_IGA_400774	15.881	1	*****
LG4	25.14	SNP_IGA_401100	15.881	1	*****
LG4	25.14	SNP_IGA_400811	15.881	1	*****
LG4	25.14	SNP_IGA_400907	15.881	1	*****
LG4	25.14	SNP_IGA_400954	15.881	1	*****
LG4	25.14	SNP_IGA_401089	15.881	1	*****
LG4	25.14	SNP_IGA_400963	15.881	1	*****
LG4	25.14	SNP_IGA_400506	15.881	1	*****
LG4	25.14	SNP_IGA_400661	15.881	1	*****
LG4	25.14	SNP_IGA_400126	15.881	1	*****
LG4	25.142	SNP_IGA_400572	15.881	1	*****
LG4	25.142	SNP_IGA_400521	15.881	1	*****
LG4	25.142	SNP_IGA_400858	15.881	1	*****
LG4	25.329	SNP_IGA_400929	15.881	1	*****
LG4	28.84	SNP_IGA_401829	20.851	1	*****
LG4	29.923	SNP_IGA_402192	20.2	1	*****
LG4	29.923	SNP_IGA_402416	20.2	1	*****
LG4	29.923	SNP_IGA_402569	20.2	1	*****
LG4	29.923	SNP_IGA_402456	20.2	1	*****
LG4	30.015	SNP_IGA_402404	20.2	1	*****
LG4	18.743	SNP_IGA_395202	12.291	1	*****
LG4	21.576	SNP_IGA_397228	14.663	1	*****
LG4	21.635	SNP_IGA_396770	14.663	1	*****
LG4	18.179	SNP_IGA_392481	10.853	1	*****
LG3	28.68	SNP_IGA_366194	8.211	1	****
LG4	17.106	SNP_IGA_393777	11.149	2	****
LG4	17.635	SNP_IGA_392560	8.501	1	****
LG4	17.635	SNP_IGA_392549	8.501	1	****
LG4	17.635	SNP_IGA_393684	8.501	1	****
LG4	17.635	SNP_IGA_393060	8.501	1	****
LG4	17.714	SNP_IGA_392956	9.465	1	****
LG6	0	SNP_IGA_607528	11.295	2	****
LG6	0	SNP_IGA_607711	11.295	2	****
LG6	0.501	SNP_IGA_616295	11.295	2	****
LG6	0.501	SNP_IGA_607013	11.295	2	****

LG6	0.501	SNP_IGA_616534	11.295	2	****
LG6	0.591	SNP_IGA_607240	11.295	2	****
LG6	0.591	SNP_IGA_607179	11.295	2	****
LG6	0.591	SNP_IGA_616508	11.295	2	****
LG6	0.591	SNP_IGA_617922	11.295	2	****
LG6	0.591	SNP_IGA_618417	11.295	2	****
LG6	0.591	SNP_IGA_607343	11.295	2	****
LG6	0.591	SNP_IGA_616286	11.295	2	****
LG6	0.591	SNP_IGA_618849	11.295	2	****
LG6	0.591	SNP_IGA_618824	11.295	2	****
LG6	0.591	SNP_IGA_618376	11.295	2	****
LG6	1.158	SNP_IGA_614935	11.43	2	****
LG6	1.186	SNP_IGA_616119	11.43	2	****
LG6	1.186	SNP_IGA_616005	11.43	2	****
LG6	1.186	SNP_IGA_614635	11.43	2	****
LG6	1.186	SNP_IGA_615979	11.43	2	****
LG6	1.186	SNP_IGA_615377	11.43	2	****
LG6	1.201	SNP_IGA_616074	11.43	2	****
LG6	1.277	SNP_IGA_615381	11.43	2	****
LG6	1.761	SNP_IGA_614519	11.365	2	****
LG6	2.338	SNP_IGA_614273	12.35	2	****
LG6	2.38	SNP_IGA_614082	12.35	2	****
LG6	2.38	SNP_IGA_614045	12.35	2	****
LG6	2.38	SNP_IGA_614054	12.35	2	****
LG6	2.38	SNP_IGA_614236	12.35	2	****
LG6	2.97	snp_6_2750075	13.197	2	****
LG3	28.696	SNP_IGA_366233	7.866	1	***
LG3	30.443	SNP_IGA_367684	7.758	1	***
LG3	30.465	SNP_IGA_367728	7.367	1	***
LG4	10.813	SNP_IGA_387198	7.388	1	***
LG4	10.813	SNP_IGA_387415	7.388	1	***
LG4	16.541	SNP_IGA_394859	9.333	2	***
LG4	17.568	SNP_IGA_394233	9.309	2	***
LG4	17.589	SNP_IGA_394015	9.309	2	***
LG4	17.589	SNP_IGA_393752	9.309	2	***
LG4	17.589	SNP_IGA_394034	9.309	2	***
LG4	17.589	SNP_IGA_393740	9.309	2	***
LG4	17.589	SNP_IGA_394026	9.309	2	***

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	LG6	3.936	SNP_IGA_611064	9.922	2	***
	LG6	3.936	SNP_IGA_611149	9.922	2	***
	LG6	3.936	SNP_IGA_611891	9.922	2	***
	LG6	3.936	SNP_IGA_611511	9.922	2	***
	LG6	3.936	SNP_IGA_611748	9.922	2	***
SSC_Mean	LG4	18.743	SNP_IGA_395202	16.249	1	*****
	LG4	20.813	SNP_IGA_396028	20.576	1	*****
	LG4	20.876	SNP_IGA_396308	20.17	1	*****
	LG4	20.877	SNP_IGA_396958	20.576	1	*****
	LG4	20.877	SNP_IGA_397165	20.576	1	*****
	LG4	20.877	SNP_IGA_395529	20.576	1	*****
	LG4	20.882	SNP_IGA_396340	19.132	1	*****
	LG4	20.949	SNP_IGA_396034	20.576	1	*****
	LG4	20.949	SNP_IGA_397015	20.576	1	*****
	LG4	20.949	SNP_IGA_396171	20.576	1	*****
	LG4	20.949	SNP_IGA_395621	20.576	1	*****
	LG4	20.949	SNP_IGA_396443	20.576	1	*****
	LG4	20.949	SNP_IGA_396351	20.576	1	*****
	LG4	20.949	SNP_IGA_396345	20.576	1	*****
	LG4	20.949	SNP_IGA_396006	20.576	1	*****
	LG4	20.949	SNP_IGA_396439	20.576	1	*****
	LG4	21.576	SNP_IGA_397228	17.549	1	*****
	LG4	21.635	SNP_IGA_396770	17.549	1	*****
	LG4	22.108	SNP_IGA_398213	20.524	1	*****
	LG4	22.108	SNP_IGA_398075	20.524	1	*****
	LG4	22.108	SNP_IGA_398373	20.524	1	*****
	LG4	22.875	SNP_IGA_397710	17.493	1	*****
	LG4	22.875	SNP_IGA_397732	17.493	1	*****
	LG4	22.881	SNP_IGA_398915	17.493	1	*****
	LG4	22.881	SNP_IGA_399569	17.493	1	*****
	LG4	22.881	SNP_IGA_398228	17.493	1	*****
	LG4	22.881	SNP_IGA_399565	17.493	1	*****
	LG4	22.881	SNP_IGA_398368	17.493	1	*****
	LG4	22.881	Pp17Cl	17.493	1	*****
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LG4	22.881	SNP_IGA_399280	17.493	1	*****
LG4	22.881	SNP_IGA_399599	17.493	1	*****
LG4	22.881	SNP_IGA_398634	17.493	1	*****
LG4	22.881	SNP_IGA_397696	17.493	1	*****
LG4	22.881	SNP_IGA_398997	17.493	1	*****
LG4	24.018	SNP_IGA_399812	17.549	1	*****
LG4	24.018	SNP_IGA_399756	17.549	1	*****
LG4	24.018	SNP_IGA_399658	17.549	1	*****
LG4	24.034	SNP_IGA_399849	17.549	1	*****
LG4	25.14	SNP_IGA_401072	17.221	1	*****
LG4	25.14	SNP_IGA_400359	17.221	1	*****
LG4	25.14	Pp10Cl	17.221	1	*****
LG4	25.14	SNP_IGA_400613	17.221	1	*****
LG4	25.14	SNP_IGA_400090	17.221	1	*****
LG4	25.14	SNP_IGA_400774	17.221	1	*****
LG4	25.14	SNP_IGA_401100	17.221	1	*****
LG4	25.14	SNP_IGA_400811	17.221	1	*****
LG4	25.14	SNP_IGA_400907	17.221	1	*****
LG4	25.14	SNP_IGA_400954	17.221	1	*****
LG4	25.14	SNP_IGA_401089	17.221	1	*****
LG4	25.14	SNP_IGA_400963	17.221	1	*****
LG4	25.14	SNP_IGA_400506	17.221	1	*****
LG4	25.14	SNP_IGA_400661	17.221	1	*****
LG4	25.14	SNP_IGA_400126	17.221	1	*****
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LG4	25.142	SNP_IGA_400521	17.221	1	*****
LG4	25.142	SNP_IGA_400858	17.221	1	*****
LG4	25.329	SNP_IGA_400929	17.221	1	*****
LG4	28.84	SNP_IGA_401829	22.319	1	*****
LG4	29.923	SNP_IGA_402192	21.577	1	*****
LG4	29.923	SNP_IGA_402416	21.577	1	*****
LG4	29.923	SNP_IGA_402569	21.577	1	*****
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LG4	30.015	SNP_IGA_402404	21.577	1	*****
LG4	17.106	SNP_IGA_393777	15.569	2	*****
LG4	17.635	SNP_IGA_392560	12.258	1	*****
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	LG4	17.635	SNP_IGA_393060	12.258	1	*****
	LG4	17.714	SNP_IGA_392956	13.412	1	*****
	LG4	18.179	SNP_IGA_392481	14.866	1	*****
	LG4	14.698	SNP_IGA_390430	14.139	2	*****
	LG4	14.698	SNP_IGA_390507	14.139	2	*****
	LG4	16.541	SNP_IGA_394859	14.117	2	*****
	LG4	10.813	SNP_IGA_387198	9.065	1	****
	LG4	10.813	SNP_IGA_387415	9.065	1	****
	LG4	15.979	SNP_IGA_395152	12.004	2	****
	LG4	17.568	SNP_IGA_394233	12.466	2	****
	LG4	17.589	SNP_IGA_394015	12.466	2	****
	LG4	17.589	SNP_IGA_393752	12.466	2	****
	LG4	17.589	SNP_IGA_394034	12.466	2	****
	LG4	17.589	SNP_IGA_393740	12.466	2	****
	LG4	17.589	SNP_IGA_394026	12.466	2	****
	LG4	10.039	SNP_IGA_386778	9.366	2	***
	LG6	2.97	snp_6_2750075	9.323	2	***
TA_2013	LG4	16.541	SNP_IGA_394859	10.815	2	****
	LG4	17.106	SNP_IGA_393777	10.986	2	****
	LG4	17.568	SNP_IGA_394233	10.986	2	****
	LG4	17.589	SNP_IGA_394015	10.986	2	****
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	LG4	17.589	SNP_IGA_394026	10.986	2	****
	LG7	28.616	SNP_IGA_776067	10.885	2	****
	LG7	30.939	SNP_IGA_776826	9.687	1	****
	LG7	30.976	SNP_IGA_776892	9.687	1	****
	LG7	40.437	SNP_IGA_778587	8.949	1	****
	LG7	40.473	SNP_IGA_778808	8.663	1	****
	LG7	40.713	SNP_IGA_778568	8.211	1	****
	LG4	15.979	SNP_IGA_395152	9.689	2	***
	LG7	29.01	SNP_IGA_776161	10.325	2	***
	LG7	29.647	SNP_IGA_776214	10.501	2	***
	LG7	29.647	SNP_IGA_776348	10.501	2	***
	LG8	36.706	SNP_IGA_883838	7.328	1	***
	LG8	37.938	SNP_IGA_884218	7.185	1	***
	LG8	39.044	SNP_IGA_884329	7.185	1	***

TA_2014	LG4	22.875	SNP_IGA_397710	8.671	1	****
	LG4	22.875	SNP_IGA_397732	8.671	1	****
	LG4	22.881	SNP_IGA_398915	8.671	1	****
	LG4	22.881	SNP_IGA_399569	8.671	1	****
	LG4	22.881	SNP_IGA_398228	8.671	1	****
	LG4	22.881	SNP_IGA_399565	8.671	1	****
	LG4	22.881	SNP_IGA_398368	8.671	1	****
	LG4	22.881	Pp17CI	8.671	1	****
	LG4	22.881	SNP_IGA_399337	8.671	1	****
	LG4	22.881	SNP_IGA_399330	8.671	1	****
	LG4	22.881	SNP_IGA_399292	8.671	1	****
	LG4	22.881	SNP_IGA_399280	8.671	1	****
	LG4	22.881	SNP_IGA_399599	8.671	1	****
	LG4	22.881	SNP_IGA_398634	8.671	1	****
	LG4	22.881	SNP_IGA_397696	8.671	1	****
	LG4	22.881	SNP_IGA_398997	8.671	1	****
	LG4	24.018	SNP_IGA_399812	8.671	1	****
	LG4	24.018	SNP_IGA_399756	8.671	1	****
	LG4	24.018	SNP_IGA_399658	8.671	1	****
	LG4	24.034	SNP_IGA_399849	8.671	1	****
	LG7	40.437	SNP_IGA_778587	8.673	1	****
	LG7	40.473	SNP_IGA_778808	8.475	1	****
	LG4	21.576	SNP_IGA_397228	7.841	1	***
	LG4	21.635	SNP_IGA_396770	7.841	1	***
	LG4	29.923	SNP_IGA_402192	7.41	1	***
	LG4	29.923	SNP_IGA_402416	7.41	1	***
	LG4	29.923	SNP_IGA_402569	7.41	1	***
	LG4	29.923	SNP_IGA_402456	7.41	1	***
	LG4	30.015	SNP_IGA_402404	7.41	1	***
	<u>LG7</u>	<u>40.713</u>	<u>SNP_IGA_778568</u>	<u>7.173</u>	<u>1</u>	<u>***</u>
TA_Mean	LG7	40.437	SNP_IGA_778587	15.544	1	*****
	LG7	40.473	SNP_IGA_778808	15.272	1	*****
	LG7	40.713	SNP_IGA_778568	13.837	1	*****
	LG7	30.939	SNP_IGA_776826	11.913	1	*****
	LG7	30.976	SNP_IGA_776892	11.913	1	*****
	LG7	28.616	SNP_IGA_776067	11.362	2	****
	LG7	29.01	SNP_IGA_776161	10.945	2	****
	LG7	29.647	SNP_IGA_776214	10.922	2	****

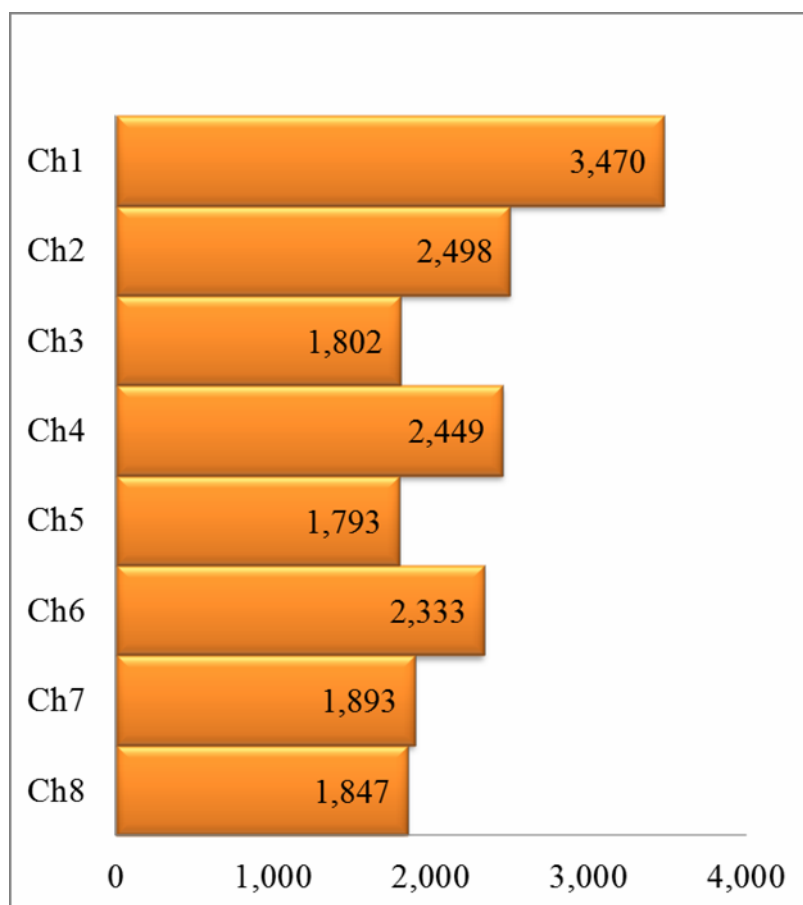
	LG7	29.647	SNP_IGA_776348	10.922	2	****
RI_2014	LG6	1.158	SNP_IGA_614935	9.242	2	***
	LG6	1.186	SNP_IGA_616119	9.242	2	***
	LG6	1.186	SNP_IGA_616005	9.242	2	***
	LG6	1.186	SNP_IGA_614635	9.242	2	***
	LG6	1.186	SNP_IGA_615979	9.242	2	***
	LG6	1.186	SNP_IGA_615377	9.242	2	***
	LG6	1.201	SNP_IGA_616074	9.242	2	***
	LG6	1.277	SNP_IGA_615381	9.242	2	***
	LG6	2.97	snp_6_2750075	10.286	2	***
	LG7	40.437	SNP_IGA_778587	6.961	1	***
	LG7	40.473	SNP_IGA_778808	6.897	1	***
RI_Mean	LG6	2.97	snp_6_2750075	9.858	2	***
	LG7	11.22	SNP_IGA_748434	7.303	1	***
	LG7	11.296	SNP_IGA_748210	6.913	1	***
	LG7	11.296	SNP_IGA_748306	6.913	1	***
	LG7	30.939	SNP_IGA_776826	7.06	1	***
	LG7	30.976	SNP_IGA_776892	7.06	1	***

^a K* = Kruskal-Wallis test.

^b*** = $P < 0.01$; **** = $P < 0.005$; ***** = $P < 0.001$; ***** = $P < 0.0005$; ***** = $P < 0.0001$.

Appendix IV:

Number of SNPs covering peach genome (v.2).



Appendix V:

Results of GLM analysis for significantly associated SNPs with the six pomological traits.

Trait	Marker	Chr	Position	Marker_f	marker_p	Marker_Rsq	marker_df
RAC	S7_12434835	7	12434835	39.25476	2.75E-07	0.50692	1
RAC	S7_12434837	7	12434837	39.25476	2.75E-07	0.50692	1
RAC	S7_12434839	7	12434839	39.25476	2.75E-07	0.50692	1
RAC	S7_12434842	7	12434842	39.25476	2.75E-07	0.50692	1
CE	S7_12434835	7	12434835	41.05419	1.77E-07	0.51944	1
CE	S7_12434837	7	12434837	41.05419	1.77E-07	0.51944	1
CE	S7_12434839	7	12434839	41.05419	1.77E-07	0.51944	1
CE	S7_12434842	7	12434842	41.05419	1.77E-07	0.51944	1
C3GE	S4_5693485	4	5693485	39.00819	1.23E-09	0.68051	2
C3GE	S4_4792501	4	4792501	33.78718	6.73E-09	0.65164	2
C3GE	S4_4772856	4	4772856	31.74666	1.37E-08	0.63572	2
C3GE	S2_20094779	2	20094779	30.81654	1.91E-08	0.62875	2
C3GE	S4_4766482	4	4766482	31.39701	2.84E-08	0.64897	2
C3GE	S6_144026	6	144026	30.1917	3.52E-08	0.63822	2
C3GE	S2_21251508	2	21251508	27.82245	4.96E-08	0.59769	2
C3GE	S6_18197408	6	18197408	46.17346	6.11E-08	0.55552	1
C3GE	S4_4745076	4	4745076	26.76764	7.54E-08	0.58858	2
C3GE	S4_4745104	4	4745104	26.76764	7.54E-08	0.58858	2
C3GE	S4_4745120	4	4745120	26.76764	7.54E-08	0.58858	2
C3GE	S4_4745122	4	4745122	26.76764	7.54E-08	0.58858	2
C3GE	S6_141593	6	141593	43.89168	9.00E-08	0.53412	1
C3GE	S6_171456	6	171456	43.89168	9.00E-08	0.53412	1
C3GE	S5_13649156	5	13649156	26.14748	9.70E-08	0.58302	2
C3GE	S5_13649187	5	13649187	26.14748	9.70E-08	0.58302	2
C3GE	S2_20268180	2	20268180	26.90166	9.90E-08	0.60924	2
C3GE	S2_21059153	2	21059153	44.54034	1.01E-07	0.55342	1
C3GE	S4_20140442	4	20140442	43.51886	1.12E-07	0.53994	1
C3GE	S6_6422408	6	6422408	25.74097	1.15E-07	0.57929	2
C3GE	S1_22463135	1	22463135	44.41512	1.20E-07	0.55695	1
C3GE	S7_16624661	7	16624661	25.22116	1.42E-07	0.57442	2
C3GE	S4_11007906	4	11007906	26.83185	1.44E-07	0.59588	2
C3GE	S5_9418012	5	9418012	25.48644	1.48E-07	0.58042	2
C3GE	S8_289969	8	289969	24.98604	1.57E-07	0.57217	2

C3GE	S6_20275784	6	20275784	24.89825	1.63E-07	0.57133	2
C3GE	S6_7541331	6	7541331	25.65339	1.90E-07	0.59816	2
C3GE	S6_7541341	6	7541341	25.65339	1.90E-07	0.59816	2
C3GE	S2_20249643	2	20249643	24.23402	2.15E-07	0.56484	2
C3GE	S2_21331276	2	21331276	40.22069	2.17E-07	0.51271	1
C3GE	S1_42381186	1	42381186	23.83563	2.55E-07	0.56084	2
C3GE	S2_21088292	2	21088292	25.74465	2.58E-07	0.61326	2
C3GE	S4_5693405	4	5693405	39.41484	2.64E-07	0.50774	1
C3GE	S4_5785756	4	5785756	39.41484	2.64E-07	0.50774	1
C3GE	S4_5828042	4	5828042	39.41484	2.64E-07	0.50774	1
C3GE	S4_5841655	4	5841655	39.41484	2.64E-07	0.50774	1
C3GE	S4_5841660	4	5841660	39.41484	2.64E-07	0.50774	1
C3GE	S2_26126457	2	26126457	23.75245	2.65E-07	0.56	2
C3GE	S1_42518320	1	42518320	23.65378	2.76E-07	0.55899	2
C3GE	S2_21083244	2	21083244	23.65378	2.76E-07	0.55899	2
C3GE	S2_26384297	2	26384297	23.65378	2.76E-07	0.55899	2
C3GE	S2_22606523	2	22606523	23.59254	2.84E-07	0.55836	2
C3GE	S7_10444885	7	10444885	23.58145	2.85E-07	0.55825	2
C3GE	S6_17046349	6	17046349	23.53557	2.91E-07	0.55778	2
C3GE	S2_22101908	2	22101908	23.32507	3.18E-07	0.55561	2
C3GE	S7_10651706	7	10651706	23.25577	3.28E-07	0.55489	2
C3GE	S1_42526732	1	42526732	23.87781	3.33E-07	0.57813	2
C3GE	S2_705719	2	705719	23.45949	3.44E-07	0.56282	2
C3GE	S2_705720	2	705720	23.45949	3.44E-07	0.56282	2
C3GE	S2_21051796	2	21051796	23.05152	3.59E-07	0.55275	2
C3GE	S2_20954251	2	20954251	23.00805	3.66E-07	0.55229	2
C3GE	S2_21028217	2	21028217	23.00805	3.66E-07	0.55229	2
C3GE	S1_44087286	1	44087286	23.30814	3.67E-07	0.56468	2
C3GE	S5_15404966	5	15404966	22.96912	3.72E-07	0.55188	2
C3GE	S2_21212899	2	21212899	23.96909	3.72E-07	0.58683	2
C3GE	S7_7547042	7	7547042	22.88743	3.86E-07	0.55102	2
C3GE	S8_373667	8	373667	22.83668	3.94E-07	0.55048	2
C3GE	S1_42888114	1	42888114	22.80308	4.00E-07	0.55012	2
C3GE	S1_42888115	1	42888115	22.80308	4.00E-07	0.55012	2
C3GE	S1_42888116	1	42888116	22.80308	4.00E-07	0.55012	2
C3GE	S6_20847811	6	20847811	22.75947	4.08E-07	0.54966	2
C3GE	S1_3855416	1	3855416	23.36621	4.12E-07	0.56718	2
C3GE	S1_28452145	1	28452145	23.34116	4.17E-07	0.56625	2
C3GE	S1_28452166	1	28452166	23.34116	4.17E-07	0.56625	2
C3GE	S6_10476825	6	10476825	22.68383	4.22E-07	0.54885	2

C3GE	S7_10559356	7	10559356	22.63141	4.32E-07	0.54829	2
C3GE	S1_44087072	1	44087072	22.92962	4.32E-07	0.55522	2
C3GE	S1_44087085	1	44087085	22.92962	4.32E-07	0.55522	2
C3GE	S1_29846347	1	29846347	22.92373	4.34E-07	0.55516	2
C3GE	S1_29846349	1	29846349	22.92373	4.34E-07	0.55516	2
C3GE	S1_29846350	1	29846350	22.92373	4.34E-07	0.55516	2
C3GE	S1_29846352	1	29846352	22.92373	4.34E-07	0.55516	2
C3GE	S1_29846353	1	29846353	22.92373	4.34E-07	0.55516	2
C3GE	S1_29846354	1	29846354	22.92373	4.34E-07	0.55516	2
C3GE	S1_43959689	1	43959689	22.61523	4.35E-07	0.54811	2
C3GE	S4_29436452	4	29436452	22.60033	4.38E-07	0.54795	2
C3GE	S2_26351033	2	26351033	22.54788	4.48E-07	0.54739	2
C3GE	S7_2335343	7	2335343	22.53382	4.51E-07	0.54724	2
C3GE	S1_42337680	1	42337680	23.11456	4.59E-07	0.56978	2
C3GE	S7_10153720	7	10153720	22.74759	4.68E-07	0.55868	2
C3GE	S1_42485853	1	42485853	23.25632	4.99E-07	0.5806	2
C3GE	S6_18197406	6	18197406	22.57392	5.05E-07	0.55691	2
C3GE	S1_42887977	1	42887977	22.74078	5.38E-07	0.5674	2
C3GE	S1_42888001	1	42888001	22.74078	5.38E-07	0.5674	2
C3GE	S2_20268354	2	20268354	23.8097	5.44E-07	0.58986	2
C3GE	S1_3826426	1	3826426	22.33212	5.61E-07	0.5527	2
C3GE	S4_1949827	4	1949827	22.42471	6.16E-07	0.55713	2
C3GE	S2_20656344	2	20656344	22.69978	6.30E-07	0.57787	2
C3GE	S6_149998	6	149998	22.35449	6.35E-07	0.56194	2
C3GE	S6_150024	6	150024	22.35449	6.35E-07	0.56194	2
C3GE	S2_21091430	2	21091430	23.01492	6.40E-07	0.58428	2
C3GE	S3_16995570	3	16995570	22.30645	6.48E-07	0.55539	2
C3GE	S7_7758465	7	7758465	22.26017	6.61E-07	0.55591	2
C3GE	S2_22289856	2	22289856	21.92632	6.71E-07	0.54751	2
C3GE	S5_12926264	5	12926264	21.90898	6.76E-07	0.54805	2
C3GE	S8_171597	8	171597	21.90678	6.77E-07	0.54802	2
C3GE	S1_45174720	1	45174720	22.87555	6.78E-07	0.58234	2
C3GE	S2_26054526	2	26054526	21.901	6.79E-07	0.54761	2
C3GE	S7_10194756	7	10194756	21.89331	6.81E-07	0.54787	2
C3GE	S2_22115305	2	22115305	22.04146	7.27E-07	0.55568	2
C3GE	S1_44808516	1	44808516	21.46641	7.29E-07	0.53542	2
C3GE	S2_20367277	2	20367277	22.28396	8.66E-07	0.56613	2
C3GE	S6_5473621	6	5473621	21.92026	8.77E-07	0.56042	2
C3GE	S6_5473638	6	5473638	21.92026	8.77E-07	0.56042	2
C3GE	S7_2817697	7	2817697	21.58981	8.86E-07	0.54785	2

C3GE	S3_3265127	3	3265127	21.54213	9.05E-07	0.55208	2
C3GE	S7_2817668	7	2817668	21.51736	9.15E-07	0.54704	2
C3GE	S1_36606934	1	36606934	21.4972	9.23E-07	0.55006	2
C3GE	S1_44698364	1	44698364	21.18864	9.34E-07	0.54032	2
C3GE	S1_45257407	1	45257407	22.36139	9.73E-07	0.58531	2
C3GE	S6_11654926	6	11654926	21.84876	1.04E-06	0.57081	2
C3GE	S4_6526337	4	6526337	34.42572	1.05E-06	0.48227	1
C3GE	S4_6526342	4	6526342	34.42572	1.05E-06	0.48227	1
C3GE	S4_6526358	4	6526358	34.42572	1.05E-06	0.48227	1
C3GE	S4_6526513	4	6526513	33.5881	1.30E-06	0.47582	1
C3GE	S4_6526524	4	6526524	33.5881	1.30E-06	0.47582	1
C3GE	S4_6526526	4	6526526	33.5881	1.30E-06	0.47582	1
C3GE	S4_6526530	4	6526530	33.5881	1.30E-06	0.47582	1
C3GE	S4_6526535	4	6526535	33.5881	1.30E-06	0.47582	1
C3GE	S2_26395209	2	26395209	19.32834	2.21E-06	0.51886	2
C3GE	S2_26395210	2	26395210	19.32834	2.21E-06	0.51886	2
FF	S1_29416421	1	29416421	26.56561	8.18E-08	0.55028	2
TA	S5_930892	5	930892	27.32921	9.99E-08	0.5395	2
TA	S5_985948	5	985948	26.92913	7.07E-08	0.53774	2
TA	S5_987389	5	987389	26.77284	1.47E-07	0.60136	2
TA	S5_987397	5	987397	26.77284	1.47E-07	0.60136	2
TA	S5_1212635	5	1212635	20.78295	9.99E-07	0.48078	2
TA	S5_2457236	5	2457236	20.07523	1.39E-06	0.47304	2
TA	S5_2973178	5	2973178	18.98066	2.35E-06	0.46049	2
RI	S5_930892	5	930892	64.20805	4.21E-12	0.69244	2
RI	S5_985948	5	985948	56.50473	7.86E-12	0.68898	2
RI	S5_987389	5	987389	58.29371	2.14E-11	0.74941	2
RI	S5_987397	5	987397	58.29371	2.14E-11	0.74941	2
RI	S5_2457236	5	2457236	43.13594	2.76E-10	0.64098	2
RI	S5_2973178	5	2973178	39.76534	7.67E-10	0.62538	2
RI	S1_4805216	1	4805216	38.14916	2.62E-09	0.63987	2
RI	S1_4805222	1	4805222	38.14916	2.62E-09	0.63987	2
RI	S5_669876	5	669876	36.1095	3.10E-09	0.6226	2
RI	S5_988373	5	988373	35.16149	4.24E-09	0.53407	2
RI	S5_1212635	5	1212635	32.89435	7.50E-09	0.58716	2
RI	S5_2979811	5	2979811	31.57956	1.20E-08	0.57864	2
RI	S5_3301033	5	3301033	31.57956	1.20E-08	0.57864	2
RI	S5_3301036	5	3301036	31.57956	1.20E-08	0.57864	2
RI	S5_3231053	5	3231053	30.59556	1.72E-08	0.57196	2
RI	S5_1708880	5	1708880	30.19707	2.40E-08	0.5852	2

RI	S6_14936842	6	14936842	28.48292	3.83E-08	0.55667	2
RI	S5_3458245	5	3458245	29.35406	3.93E-08	0.57329	2
RI	S7_403846	7	403846	28.40361	4.69E-08	0.5604	2
RI	S7_403851	7	403851	28.40361	4.69E-08	0.5604	2
RI	S7_403855	7	403855	28.40361	4.69E-08	0.5604	2
RI	S7_403868	7	403868	28.40361	4.69E-08	0.5604	2
RI	S7_403877	7	403877	28.40361	4.69E-08	0.5604	2
RI	S7_403879	7	403879	28.40361	4.69E-08	0.5604	2
RI	S5_2108950	5	2108950	27.77632	5.05E-08	0.55124	2
RI	S6_15628481	6	15628481	27.06877	7.85E-08	0.52344	2
RI	S5_2582148	5	2582148	26.04614	1.01E-07	0.5372	2
RI	S5_629595	5	629595	25.62369	1.20E-07	0.53361	2
RI	S5_2268935	5	2268935	25.4789	1.28E-07	0.53236	2
RI	S5_1991397	5	1991397	27.0367	1.33E-07	0.57834	2
RI	S5_894436	5	894436	25.12529	1.48E-07	0.52928	2
RI	S5_2731358	5	2731358	24.03732	3.62E-07	0.5491	2
RI	S5_2731396	5	2731396	24.03732	3.62E-07	0.5491	2
RI	S5_3724791	5	3724791	21.34344	8.71E-07	0.4988	2
RI	S5_3724837	5	3724837	21.34344	8.71E-07	0.4988	2
RI	S6_15591425	6	15591425	20.94125	9.28E-07	0.48854	2
RI	S6_15591430	6	15591430	20.94125	9.28E-07	0.48854	2
RI	S5_3167795	5	3167795	20.809	1.11E-06	0.50715	2
RI	S5_3717020	5	3717020	22.38625	1.12E-06	0.50744	2
RI	S5_886335	5	886335	20.7573	1.66E-06	0.50201	2
RI	S5_886346	5	886346	20.7573	1.66E-06	0.50201	2
RI	S5_2582150	5	2582150	19.44563	1.88E-06	0.47176	2
RI	S5_1694346	5	1694346	19.11033	2.21E-06	0.46782	2
<u>RI</u>	<u>S7_9061581</u>	<u>7</u>	<u>9061581</u>	<u>18.71335</u>	<u>2.68E-06</u>	<u>0.46305</u>	<u>2</u>

Appendix VI:

Results of GLM analysis for 181 significantly associated SNP markers with six traits with number of genotypes observed and effects of alleles on the phenotypic appearance.

Trait	Marker	Position	Obs	Allele	Effect
C3GE	S2_26054526	26054526	39	T	-3.71E-01
C3GE	S2_26054526	26054526	1	A	23.94536
C3GE	S2_26054526	26054526	1	W	0
C3GE	S2_26126457	26126457	38	T	-1.51E+01
C3GE	S2_26126457	26126457	2	C	5.36389
C3GE	S2_26126457	26126457	2	Y	0
C3GE	S2_26351033	26351033	39	G	-6.07E-01
C3GE	S2_26351033	26351033	1	C	23.73492
C3GE	S2_26351033	26351033	2	S	0
C3GE	S2_26384297	26384297	39	C	8.27528
C3GE	S2_26384297	26384297	1	T	32.56777
C3GE	S2_26384297	26384297	2	Y	0
C3GE	S2_26395209	26395209	36	T	-1.03E+01
C3GE	S2_26395209	26395209	2	G	9.27463
C3GE	S2_26395209	26395209	3	K	0
C3GE	S2_26395210	26395210	36	T	-1.03E+01
C3GE	S2_26395210	26395210	2	A	9.27463
C3GE	S2_26395210	26395210	3	W	0
C3GE	S2_705719	705719	21	T	-2.38E+01
C3GE	S2_705719	705719	19	Y	-2.51E+01
C3GE	S2_705719	705719	1	C	0
C3GE	S2_705720	705720	21	G	-2.38E+01
C3GE	S2_705720	705720	19	K	-2.51E+01
C3GE	S2_705720	705720	1	T	0
C3GE	S3_16995570	16995570	38	G	-2.17E-01
C3GE	S3_16995570	16995570	1	R	24.12757
C3GE	S3_16995570	16995570	1	A	0
C3GE	S3_3265127	3265127	37	T	0.68937
C3GE	S3_3265127	3265127	1	G	24.96658
C3GE	S3_3265127	3265127	2	K	0
C3GE	S4_11007906	11007906	36	C	-2.42E+01
C3GE	S4_11007906	11007906	1	T	-2.73E+01
C3GE	S4_11007906	11007906	1	Y	0
C3GE	S4_1949827	1949827	19	Y	-2.47E+01

C3GE	S4_1949827	1949827	20	T	-2.39E+01
C3GE	S4_1949827	1949827	1	C	0
C3GE	S4_20140442	20140442	37	T	-1.84E+01
C3GE	S4_20140442	20140442	4	K	0
C3GE	S4_29436452	29436452	37	T	0.54324
C3GE	S4_29436452	29436452	1	G	24.80154
C3GE	S4_29436452	29436452	4	K	0
C3GE	S4_4766482	4766482	33	A	-2.48E+01
C3GE	S4_4766482	4766482	4	W	-1.81E+01
C3GE	S4_4766482	4766482	1	T	0
C3GE	S4_4772856	4772856	34	T	-1.95E+01
C3GE	S4_4772856	4772856	5	Y	-1.01E+01
C3GE	S4_4772856	4772856	2	C	0
C3GE	S4_4792501	4792501	37	G	-2.49E+01
C3GE	S4_4792501	4792501	3	R	-1.80E+01
C3GE	S4_4792501	4792501	1	A	0
C3GE	S4_5693405	5693405	39	G	-1.41E+01
C3GE	S4_5693405	5693405	3	R	0
C3GE	S4_5693485	5693485	38	G	-2.50E+01
C3GE	S4_5693485	5693485	2	S	-1.64E+01
C3GE	S4_5693485	5693485	1	C	0
C3GE	S4_5785756	5785756	39	G	-1.41E+01
C3GE	S4_5785756	5785756	3	K	0
C3GE	S4_5828042	5828042	39	C	-1.41E+01
C3GE	S4_5828042	5828042	3	S	0
C3GE	S4_5841655	5841655	39	A	-1.41E+01
C3GE	S4_5841655	5841655	3	R	0
C3GE	S4_5841660	5841660	39	T	-1.41E+01
C3GE	S4_5841660	5841660	3	Y	0
C3GE	S4_6526337	6526337	39	G	-1.64E+01
C3GE	S4_6526337	6526337	2	R	0
C3GE	S4_6526342	6526342	39	A	-1.64E+01
C3GE	S4_6526342	6526342	2	M	0
C3GE	S4_6526358	6526358	39	C	-1.64E+01
C3GE	S4_6526358	6526358	2	M	0
C3GE	S4_6526513	6526513	39	C	-1.64E+01
C3GE	S4_6526513	6526513	2	Y	0
C3GE	S4_6526524	6526524	39	G	-1.64E+01
C3GE	S4_6526524	6526524	2	S	0

C3GE	S4_6526526	6526526	39	G	-1.64E+01
C3GE	S4_6526526	6526526	2	S	0
C3GE	S4_6526530	6526530	39	A	-1.64E+01
C3GE	S4_6526530	6526530	2	M	0
C3GE	S4_6526535	6526535	39	C	-1.64E+01
C3GE	S4_6526535	6526535	2	Y	0
C3GE	S5_12926264	12926264	33	A	-2.14E-01
C3GE	S5_12926264	12926264	1	G	24.13051
C3GE	S5_12926264	12926264	7	R	0
C3GE	S5_13649156	13649156	37	G	-2.41E+01
C3GE	S5_13649156	13649156	4	R	-2.76E+01
C3GE	S5_13649156	13649156	1	A	0
C3GE	S5_13649187	13649187	37	A	-2.41E+01
C3GE	S5_13649187	13649187	4	M	-2.76E+01
C3GE	S5_13649187	13649187	1	C	0
C3GE	S5_15404966	15404966	34	C	0.94405
C3GE	S5_15404966	15404966	1	T	25.09911
C3GE	S5_15404966	15404966	7	Y	0
C3GE	S5_9418012	9418012	21	K	-2.50E+01
C3GE	S5_9418012	9418012	19	T	-2.32E+01
C3GE	S5_9418012	9418012	1	G	0
C3GE	S6_10476825	10476825	40	M	1.48239
C3GE	S6_10476825	10476825	1	C	25.81264
C3GE	S6_10476825	10476825	1	A	0
C3GE	S6_11654926	11654926	11	Y	-2.42E+01
C3GE	S6_11654926	11654926	26	C	-2.43E+01
C3GE	S6_11654926	11654926	1	T	0
C3GE	S6_141593	141593	38	T	-1.84E+01
C3GE	S6_141593	141593	4	Y	0
C3GE	S6_144026	144026	37	G	-9.00E+00
C3GE	S6_144026	144026	1	R	15.29788
C3GE	S6_144026	144026	1	A	0
C3GE	S6_149998	149998	38	G	3.44855
C3GE	S6_149998	149998	1	K	27.78698
C3GE	S6_149998	149998	1	T	0
C3GE	S6_150024	150024	38	G	3.44855
C3GE	S6_150024	150024	1	K	27.78698
C3GE	S6_150024	150024	1	T	0
C3GE	S6_17046349	17046349	39	A	2.48632
C3GE	S6_17046349	17046349	1	G	26.63159

C3GE	S6_17046349	17046349	2	R	0
C3GE	S6_171456	171456	38	G	-1.84E+01
C3GE	S6_171456	171456	4	R	0
C3GE	S6_18197406	18197406	37	A	-2.43E+01
C3GE	S6_18197406	18197406	3	R	-2.50E+01
C3GE	S6_18197406	18197406	1	G	0
C3GE	S6_18197408	18197408	40	G	-2.43E+01
C3GE	S6_18197408	18197408	1	A	0
C3GE	S6_20275784	20275784	38	G	-2.41E+01
C3GE	S6_20275784	20275784	3	R	-2.74E+01
C3GE	S6_20275784	20275784	1	A	0
C3GE	S6_20847811	20847811	39	C	1.26136
C3GE	S6_20847811	20847811	1	T	25.482
C3GE	S6_20847811	20847811	2	Y	0
C3GE	S6_5473621	5473621	36	T	-2.06E+00
C3GE	S6_5473621	5473621	1	A	22.16087
C3GE	S6_5473621	5473621	2	W	0
C3GE	S6_5473638	5473638	36	T	-2.06E+00
C3GE	S6_5473638	5473638	1	C	22.16087
C3GE	S6_5473638	5473638	2	Y	0
C3GE	S6_6422408	6422408	34	T	-2.48E+01
C3GE	S6_6422408	6422408	7	W	-2.24E+01
C3GE	S6_6422408	6422408	1	A	0
C3GE	S6_7541331	7541331	34	G	-1.25E+00
C3GE	S6_7541331	7541331	1	T	23.4451
C3GE	S6_7541331	7541331	4	K	0
C3GE	S6_7541341	7541341	34	A	-1.25E+00
C3GE	S6_7541341	7541341	1	C	23.4451
C3GE	S6_7541341	7541341	4	M	0
C3GE	S7_10153720	10153720	34	G	-2.41E+01
C3GE	S7_10153720	10153720	6	R	-2.51E+01
C3GE	S7_10153720	10153720	1	A	0
C3GE	S7_10194756	10194756	35	C	-2.43E+01
C3GE	S7_10194756	10194756	5	Y	-2.44E+01
C3GE	S7_10194756	10194756	1	T	0
C3GE	S7_10444885	10444885	37	G	-2.45E+01
C3GE	S7_10444885	10444885	4	R	-2.18E+01
C3GE	S7_10444885	10444885	1	A	0
C3GE	S7_10559356	10559356	37	T	-2.44E+01
C3GE	S7_10559356	10559356	4	K	-2.36E+01

C3GE	S7_10559356	10559356	1	G	0
C3GE	S7_10651706	10651706	39	G	-2.44E+01
C3GE	S7_10651706	10651706	2	S	-2.21E+01
C3GE	S7_10651706	10651706	1	C	0
C3GE	S7_16624661	16624661	23	M	-2.49E+01
C3GE	S7_16624661	16624661	18	C	-2.30E+01
C3GE	S7_16624661	16624661	1	A	0
C3GE	S7_2335343	2335343	34	G	-2.36E-01
C3GE	S7_2335343	2335343	1	A	24.13119
C3GE	S7_2335343	2335343	7	R	0
C3GE	S7_2817668	2817668	37	G	-2.41E+01
C3GE	S7_2817668	2817668	2	R	-2.45E+01
C3GE	S7_2817668	2817668	1	A	0
C3GE	S7_2817697	2817697	35	T	-2.40E+01
C3GE	S7_2817697	2817697	4	Y	-2.46E+01
C3GE	S7_2817697	2817697	1	C	0
C3GE	S7_7547042	7547042	32	T	-2.40E+01
C3GE	S7_7547042	7547042	9	W	-2.49E+01
C3GE	S7_7547042	7547042	1	A	0
C3GE	S7_7758465	7758465	30	G	-2.41E+01
C3GE	S7_7758465	7758465	9	R	-2.49E+01
C3GE	S7_7758465	7758465	1	A	0
C3GE	S8_171597	171597	32	C	-2.43E+01
C3GE	S8_171597	171597	8	Y	-2.41E+01
C3GE	S8_171597	171597	1	T	0
C3GE	S8_289969	289969	32	G	-2.39E+01
C3GE	S8_289969	289969	9	S	-2.58E+01
C3GE	S8_289969	289969	1	C	0
C3GE	S8_373667	373667	31	T	-2.42E+01
C3GE	S8_373667	373667	10	K	-2.49E+01
C3GE	S8_373667	373667	1	G	0
C3GE	S1_3826426	3826426	26	G	-2.46E+01
C3GE	S1_3826426	3826426	14	R	-2.38E+01
C3GE	S1_3826426	3826426	1	A	0
C3GE	S1_3855416	3855416	33	G	-1.98E+00
C3GE	S1_3855416	3855416	1	A	22.38217
C3GE	S1_3855416	3855416	6	R	0
C3GE	S1_22463135	22463135	38	C	-2.43E+01
C3GE	S1_22463135	22463135	1	S	0
C3GE	S1_28452145	28452145	31	C	-2.46E+01

C3GE	S1_28452145	28452145	8	S	-2.32E+01
C3GE	S1_28452145	28452145	1	G	0
C3GE	S1_28452166	28452166	31	A	-2.46E+01
C3GE	S1_28452166	28452166	8	R	-2.32E+01
C3GE	S1_28452166	28452166	1	G	0
C3GE	S1_29846347	29846347	13	W	-2.44E+01
C3GE	S1_29846347	29846347	27	A	-2.43E+01
C3GE	S1_29846347	29846347	1	T	0
C3GE	S1_29846349	29846349	13	W	-2.44E+01
C3GE	S1_29846349	29846349	27	A	-2.43E+01
C3GE	S1_29846349	29846349	1	T	0
C3GE	S1_29846350	29846350	13	Y	-2.44E+01
C3GE	S1_29846350	29846350	27	T	-2.43E+01
C3GE	S1_29846350	29846350	1	C	0
C3GE	S1_29846352	29846352	13	M	-2.44E+01
C3GE	S1_29846352	29846352	27	A	-2.43E+01
C3GE	S1_29846352	29846352	1	C	0
C3GE	S1_29846353	29846353	13	Y	-2.44E+01
C3GE	S1_29846353	29846353	27	C	-2.43E+01
C3GE	S1_29846353	29846353	1	T	0
C3GE	S1_29846354	29846354	13	S	-2.44E+01
C3GE	S1_29846354	29846354	27	G	-2.43E+01
C3GE	S1_29846354	29846354	1	C	0
C3GE	S1_36606934	36606934	35	C	-2.43E+01
C3GE	S1_36606934	36606934	4	Y	-2.41E+01
C3GE	S1_36606934	36606934	1	T	0
C3GE	S1_42337680	42337680	36	A	-2.46E+01
C3GE	S1_42337680	42337680	3	R	-2.23E+01
C3GE	S1_42337680	42337680	1	G	0
C3GE	S1_42381186	42381186	23	T	-2.49E+01
C3GE	S1_42381186	42381186	18	K	-2.37E+01
C3GE	S1_42381186	42381186	1	G	0
C3GE	S1_42485853	42485853	35	A	3.36867
C3GE	S1_42485853	42485853	1	G	27.60364
C3GE	S1_42485853	42485853	3	R	0
C3GE	S1_42518320	42518320	39	G	8.27528
C3GE	S1_42518320	42518320	1	A	32.56777
C3GE	S1_42518320	42518320	2	R	0
C3GE	S1_42526732	42526732	36	C	3.34969
C3GE	S1_42526732	42526732	1	G	27.51587

C3GE	S1_42526732	42526732	3	S	0
C3GE	S1_42887977	42887977	38	T	2.07624
C3GE	S1_42887977	42887977	1	C	26.38859
C3GE	S1_42887977	42887977	1	Y	0
C3GE	S1_42888001	42888001	38	G	2.07624
C3GE	S1_42888001	42888001	1	A	26.38859
C3GE	S1_42888001	42888001	1	R	0
C3GE	S1_42888114	42888114	40	T	1.89449
C3GE	S1_42888114	42888114	1	C	26.15937
C3GE	S1_42888114	42888114	1	Y	0
C3GE	S1_42888115	42888115	40	G	1.89449
C3GE	S1_42888115	42888115	1	T	26.15937
C3GE	S1_42888115	42888115	1	K	0
C3GE	S1_42888116	42888116	40	T	1.89449
C3GE	S1_42888116	42888116	1	A	26.15937
C3GE	S1_42888116	42888116	1	W	0
C3GE	S1_43959689	43959689	39	K	1.03523
C3GE	S1_43959689	43959689	1	G	25.31707
C3GE	S1_43959689	43959689	2	T	0
C3GE	S1_44087072	44087072	35	A	-2.43E+01
C3GE	S1_44087072	44087072	5	W	-2.45E+01
C3GE	S1_44087072	44087072	1	T	0
C3GE	S1_44087085	44087085	35	C	-2.43E+01
C3GE	S1_44087085	44087085	5	Y	-2.45E+01
C3GE	S1_44087085	44087085	1	T	0
C3GE	S1_44087286	44087286	12	W	-2.37E+01
C3GE	S1_44087286	44087286	28	T	-2.48E+01
C3GE	S1_44087286	44087286	1	A	0
C3GE	S1_44698364	44698364	35	C	0.51423
C3GE	S1_44698364	44698364	4	G	18.82662
C3GE	S1_44698364	44698364	2	S	0
C3GE	S1_44808516	44808516	34	T	-6.50E-01
C3GE	S1_44808516	44808516	4	C	17.95418
C3GE	S1_44808516	44808516	4	Y	0
C3GE	S1_45174720	45174720	33	A	-3.28E+00
C3GE	S1_45174720	45174720	1	T	20.97002
C3GE	S1_45174720	45174720	4	W	0
C3GE	S1_45257407	45257407	33	T	3.14485
C3GE	S1_45257407	45257407	1	C	27.47421
C3GE	S1_45257407	45257407	3	Y	0

C3GE	S2_20094779	20094779	37	T	-5.79E+00
C3GE	S2_20094779	20094779	1	C	18.82385
C3GE	S2_20094779	20094779	3	Y	0
C3GE	S2_20249643	20249643	36	T	-2.14E+00
C3GE	S2_20249643	20249643	1	C	22.36633
C3GE	S2_20249643	20249643	5	Y	0
C3GE	S2_20268180	20268180	34	C	-3.36E+00
C3GE	S2_20268180	20268180	1	G	21.30931
C3GE	S2_20268180	20268180	5	S	0
C3GE	S2_20268354	20268354	34	A	-4.14E-01
C3GE	S2_20268354	20268354	1	C	23.78065
C3GE	S2_20268354	20268354	2	M	0
C3GE	S2_20367277	20367277	35	C	1.53969
C3GE	S2_20367277	20367277	1	A	25.86856
C3GE	S2_20367277	20367277	2	M	0
C3GE	S2_20656344	20656344	36	C	-1.34E+01
C3GE	S2_20656344	20656344	2	T	8.32737
C3GE	S2_20656344	20656344	1	Y	0
C3GE	S2_20954251	20954251	37	G	-1.78E+00
C3GE	S2_20954251	20954251	1	C	22.61323
C3GE	S2_20954251	20954251	4	S	0
C3GE	S2_21028217	21028217	37	A	-1.78E+00
C3GE	S2_21028217	21028217	1	T	22.61323
C3GE	S2_21028217	21028217	4	W	0
C3GE	S2_21051796	21051796	40	A	3.49904
C3GE	S2_21051796	21051796	1	T	27.81989
C3GE	S2_21051796	21051796	1	W	0
C3GE	S2_21059153	21059153	39	A	-2.43E+01
C3GE	S2_21059153	21059153	1	T	0
C3GE	S2_21083244	21083244	39	A	8.27528
C3GE	S2_21083244	21083244	1	G	32.56777
C3GE	S2_21083244	21083244	2	R	0
C3GE	S2_21088292	21088292	35	C	3.3875
C3GE	S2_21088292	21088292	1	G	27.83783
C3GE	S2_21088292	21088292	1	S	0
C3GE	S2_21091430	21091430	35	G	-1.87E+00
C3GE	S2_21091430	21091430	1	A	22.58219
C3GE	S2_21091430	21091430	2	R	0
C3GE	S2_21212899	21212899	36	G	-3.23E+00
C3GE	S2_21212899	21212899	1	A	21.17498

C3GE	S2_21212899	21212899	2	R	0
C3GE	S2_21251508	21251508	35	G	-3.30E+00
C3GE	S2_21251508	21251508	1	A	21.39266
C3GE	S2_21251508	21251508	6	R	0
C3GE	S2_21331276	21331276	40	A	-1.68E+01
C3GE	S2_21331276	21331276	2	R	0
C3GE	S2_22101908	22101908	34	G	-1.25E+00
C3GE	S2_22101908	22101908	1	A	23.29403
C3GE	S2_22101908	22101908	7	R	0
C3GE	S2_22115305	22115305	38	C	2.87526
C3GE	S2_22115305	22115305	1	T	26.99728
C3GE	S2_22115305	22115305	1	Y	0
C3GE	S2_22289856	22289856	38	G	-6.13E-01
C3GE	S2_22289856	22289856	1	A	23.7303
C3GE	S2_22289856	22289856	2	R	0
C3GE	S2_22606523	22606523	32	C	-1.35E+00
C3GE	S2_22606523	22606523	1	T	23.25774
C3GE	S2_22606523	22606523	9	Y	0
C3GE	S4_4745076	4745076	35	A	-2.49E+01
C3GE	S4_4745076	4745076	6	R	-2.15E+01
C3GE	S4_4745076	4745076	1	G	0
C3GE	S4_4745104	4745104	35	T	-2.49E+01
C3GE	S4_4745104	4745104	6	Y	-2.15E+01
C3GE	S4_4745104	4745104	1	C	0
C3GE	S4_4745120	4745120	35	C	-2.49E+01
C3GE	S4_4745120	4745120	6	S	-2.15E+01
C3GE	S4_4745120	4745120	1	G	0
C3GE	S4_4745122	4745122	35	C	-2.49E+01
C3GE	S4_4745122	4745122	6	Y	-2.15E+01
C3GE	S4_4745122	4745122	1	T	0
C3GE	S4_5693485	5693485	38	G	-2.50E+01
C3GE	S4_5693485	5693485	2	S	-1.64E+01
C3GE	S4_5693485	5693485	1	C	0
CE	S7_12434835	12434835	38	C	-3.15E+01
CE	S7_12434835	12434835	4	M	0
CE	S7_12434837	12434837	38	T	-3.15E+01
CE	S7_12434837	12434837	4	Y	0
CE	S7_12434839	12434839	38	T	-3.15E+01
CE	S7_12434839	12434839	4	K	0
CE	S7_12434842	12434842	38	A	-3.15E+01

CE	S7_12434842	12434842	4	R	0
FF	S1_29416421	29416421	21	K	-1.29E+00
FF	S1_29416421	29416421	16	G	-2.49E-01
FF	S1_29416421	29416421	5	T	0
RAC	S7_12434835	12434835	38	C	-1.01E+03
RAC	S7_12434835	12434835	4	M	0
RAC	S7_12434837	12434837	38	T	-1.01E+03
RAC	S7_12434837	12434837	4	Y	0
RAC	S7_12434839	12434839	38	T	-1.01E+03
RAC	S7_12434839	12434839	4	K	0
RAC	S7_12434842	12434842	38	A	-1.01E+03
RAC	S7_12434842	12434842	4	R	0
RI	S1_4805216	4805216	33	G	-2.32E+01
RI	S1_4805216	4805216	1	A	-8.75E+00
RI	S1_4805216	4805216	5	R	0
RI	S1_4805222	4805222	33	C	-2.32E+01
RI	S1_4805222	4805222	1	T	-8.75E+00
RI	S1_4805222	4805222	5	Y	0
RI	S5_1212635	1212635	31	T	-2.22E+01
RI	S5_1212635	1212635	4	C	-9.83E+00
RI	S5_1212635	1212635	7	Y	0
RI	S5_1694346	1694346	33	A	-2.50E+01
RI	S5_1694346	1694346	8	W	-6.69E+00
RI	S5_1694346	1694346	1	T	0
RI	S5_1708880	1708880	33	C	-2.33E+01
RI	S5_1708880	1708880	7	Y	-2.22E+00
RI	S5_1708880	1708880	1	T	0
RI	S5_1991397	1991397	32	A	-8.07E+00
RI	S5_1991397	1991397	3	R	20.40395
RI	S5_1991397	1991397	3	G	0
RI	S5_2108950	2108950	36	T	-2.14E+01
RI	S5_2108950	2108950	5	K	-2.45E-01
RI	S5_2108950	2108950	1	G	0
RI	S5_2268935	2268935	37	G	-1.91E+01
RI	S5_2268935	2268935	4	R	3.77106
RI	S5_2268935	2268935	1	A	0
RI	S5_2457236	2457236	37	A	-2.72E+01
RI	S5_2457236	2457236	3	R	-3.70E+00
RI	S5_2457236	2457236	2	G	0
RI	S5_2582148	2582148	36	C	-2.12E+01

RI	S5_2582148	2582148	5	Y	-4.60E-01
RI	S5_2582148	2582148	1	T	0
RI	S5_2582150	2582150	33	A	-1.50E+01
RI	S5_2582150	2582150	7	R	5.0102
RI	S5_2582150	2582150	2	G	0
RI	S5_2731358	2731358	32	A	-2.67E+01
RI	S5_2731358	2731358	4	G	-9.21E+00
RI	S5_2731358	2731358	3	R	0
RI	S5_2731396	2731396	32	C	-2.67E+01
RI	S5_2731396	2731396	4	T	-9.21E+00
RI	S5_2731396	2731396	3	Y	0
RI	S5_2973178	2973178	36	G	-2.34E+01
RI	S5_2973178	2973178	5	S	0.86183
RI	S5_2973178	2973178	1	C	0
RI	S5_2979811	2979811	35	G	-2.46E+01
RI	S5_2979811	2979811	6	S	0.44605
RI	S5_2979811	2979811	1	C	0
RI	S5_3167795	3167795	36	C	-2.68E+01
RI	S5_3167795	3167795	3	M	-9.26E+00
RI	S5_3167795	3167795	2	A	0
RI	S5_3231053	3231053	36	T	-8.14E+00
RI	S5_3231053	3231053	3	K	20.42695
RI	S5_3231053	3231053	3	G	0
RI	S5_3301033	3301033	35	A	-2.46E+01
RI	S5_3301033	3301033	6	W	0.44605
RI	S5_3301033	3301033	1	T	0
RI	S5_3301036	3301036	35	A	-2.46E+01
RI	S5_3301036	3301036	6	M	0.44605
RI	S5_3301036	3301036	1	C	0
RI	S5_3458245	3458245	33	A	-2.43E+01
RI	S5_3458245	3458245	6	R	0.58894
RI	S5_3458245	3458245	1	G	0
RI	S5_3717020	3717020	32	A	-2.17E+01
RI	S5_3717020	3717020	3	R	1.67668
RI	S5_3717020	3717020	1	G	0
RI	S5_3724791	3724791	35	T	-2.14E+01
RI	S5_3724791	3724791	5	W	2.65015
RI	S5_3724791	3724791	1	A	0
RI	S5_3724837	3724837	35	C	-2.14E+01
RI	S5_3724837	3724837	5	M	2.65015

RI	S5_3724837	3724837	1	A	0
RI	S5_629595	629595	33	C	-2.36E+01
RI	S5_629595	629595	8	Y	-5.46E+00
RI	S5_629595	629595	1	T	0
RI	S5_669876	669876	34	T	-1.41E+01
RI	S5_669876	669876	5	K	9.77341
RI	S5_669876	669876	2	G	0
RI	S5_886335	886335	26	T	-1.89E+01
RI	S5_886335	886335	3	C	-9.42E+00
RI	S5_886335	886335	9	Y	0
RI	S5_886346	886346	26	A	-1.89E+01
RI	S5_886346	886346	3	G	-9.42E+00
RI	S5_886346	886346	9	R	0
RI	S5_894436	894436	30	T	-1.94E+01
RI	S5_894436	894436	3	C	-9.47E+00
RI	S5_894436	894436	9	Y	0
RI	S5_930892	930892	32	C	-2.37E+01
RI	S5_930892	930892	2	G	-5.12E+00
RI	S5_930892	930892	5	S	0
RI	S5_985948	985948	33	A	-2.32E+01
RI	S5_985948	985948	6	R	1.26957
RI	S5_985948	985948	3	G	0
RI	S5_987389	987389	30	A	-3.00E+01
RI	S5_987389	987389	5	W	-8.20E+00
RI	S5_987389	987389	3	T	0
RI	S5_987397	987397	30	G	-3.00E+01
RI	S5_987397	987397	5	R	-8.20E+00
RI	S5_987397	987397	3	A	0
RI	S5_988373	988373	32	C	-1.80E+01
RI	S5_988373	988373	7	Y	2.84145
RI	S5_988373	988373	2	T	0
RI	S6_14936842	14936842	26	C	-3.38E+01
RI	S6_14936842	14936842	14	S	-2.73E+01
RI	S6_14936842	14936842	2	G	0
RI	S6_15591425	15591425	26	T	-2.67E+01
RI	S6_15591425	15591425	13	Y	-2.06E+01
RI	S6_15591425	15591425	3	C	0
RI	S6_15591430	15591430	26	T	-2.67E+01
RI	S6_15591430	15591430	13	Y	-2.06E+01
RI	S6_15591430	15591430	3	C	0

RI	S6_15628481	15628481	26	T	-3.32E+01
RI	S6_15628481	15628481	13	Y	-2.77E+01
RI	S6_15628481	15628481	2	C	0
RI	S7_403846	403846	28	T	-3.35E+01
RI	S7_403846	403846	11	Y	-2.64E+01
RI	S7_403846	403846	2	C	0
RI	S7_403851	403851	28	A	-3.35E+01
RI	S7_403851	403851	11	R	-2.64E+01
RI	S7_403851	403851	2	G	0
RI	S7_403855	403855	28	T	-3.35E+01
RI	S7_403855	403855	11	Y	-2.64E+01
RI	S7_403855	403855	2	C	0
RI	S7_403868	403868	28	C	-3.35E+01
RI	S7_403868	403868	11	M	-2.64E+01
RI	S7_403868	403868	2	A	0
RI	S7_403877	403877	28	T	-3.35E+01
RI	S7_403877	403877	11	Y	-2.64E+01
RI	S7_403877	403877	2	C	0
RI	S7_403879	403879	28	T	-3.35E+01
RI	S7_403879	403879	11	K	-2.64E+01
RI	S7_403879	403879	2	G	0
RI	S7_9061581	9061581	33	C	-3.40E+01
RI	S7_9061581	9061581	8	Y	-2.18E+01
RI	S7_9061581	9061581	1	T	0
TA	S5_1212635	1212635	31	T	0.40719
TA	S5_1212635	1212635	4	C	0.13595
TA	S5_1212635	1212635	7	Y	0
TA	S5_2457236	2457236	37	A	0.47471
TA	S5_2457236	2457236	3	R	0.05846
TA	S5_2457236	2457236	2	G	0
TA	S5_2973178	2973178	36	G	0.40376
TA	S5_2973178	2973178	5	S	-2.46E-02
TA	S5_2973178	2973178	1	C	0
TA	S5_930892	930892	32	C	0.4179
TA	S5_930892	930892	2	G	0.05389
TA	S5_930892	930892	5	S	0
TA	S5_985948	985948	33	A	0.39861
TA	S5_985948	985948	6	R	-4.59E-02
TA	S5_985948	985948	3	G	0
TA	S5_987389	987389	30	A	0.54325

TA	S5_987389	987389	5	W	0.14555
TA	S5_987389	987389	3	T	0
TA	S5_987397	987397	30	G	0.54325
TA	S5_987397	987397	5	R	0.14555
<u>TA</u>	<u>S5_987397</u>	<u>987397</u>	<u>3</u>	<u>A</u>	<u>0</u>