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Applying Molecular and Phenotypic Tools to Characterize Flesh Texture and Acidity Traits in the Arkansas Peach Breeding Program and Understanding the Crispy Texture in the Arkansas Blackberry Breeding Program

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Applying Molecular and Phenotypic Tools to Characterize Flesh Texture and Acidity Traits in the Arkansas Peach Breeding Program and Understanding the Crispy Texture in the Arkansas Blackberry Breeding Program

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by

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ABSTRACT

Quantitative trait loci (QTL) utilizing a pedigree-based analysis (PBA) approach was performed in the peach breeding program for the first time. The pedigree consisted of seven-F_1 populations, their parents, ancestors, and cultivars. Flesh firmness and titratable acidity (TA) were examined in 2011, 2012, and 2013. For TA, a consistent and strong QTL was identified on the proximal end of linkage group (LG) 5 of the peach genome. For flesh firmness, two QTLs were located on LG 4. The first QTL was located on the chromosomal region where the slow-melting flesh (SMF) DNA test was identified, and the second QTL was identified in the region of the endopolygalacturonase (endoPG) gene. However, the QTLs on LG 4 were not always consistent. These results indicate that PBA approach for QTL analysis can be applied with success in this mature and ongoing peach breeding program with the aim to find molecular markers associated with relevant quality traits, which is the first step to apply marker-assisted breeding (MAB). Also, DNA tests associated with TA and flesh firmness were analyzed with data taken in 2013 and 2014 on this pedigree and other seedlings, selections, and cultivars to predict acidity levels and flesh texture with the final goal to validate these DNA tests and apply MAB. These tests were able to predict correctly the expected acidity levels and flesh textures of the tested individuals.

Firmness and texture are critical traits in blackberry for breeders, growers, and consumers. Crispy and extremely firm fruits were characterized for two blackberry selections which had been observed to have low color reversion (a postharvest disorder). Firmness of these selections and its seedlings were tested in 2013 and 2014. Results indicated that crispy selections had superior firmness and a higher postharvest storage potential compared to cultivars and other selections in the program. These results are important, because firmness will be increased and

color reversion will be reduced by use of this germplasm. This research also contributed to better understanding of the physical aspects of crispy and non-crispy genotypes, providing more information about this aspect of blackberries.

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TABLE OF CONTENTS

INTRODUCTION

The University of Arkansas (UA) has a long history of developing new fruit cultivars for fresh consumption and shipping markets, in particular blackberries (*Rubus* subgenus *Rubus* Watson), peaches [*Prunus persica* (L.) Batsch], table grapes (*Vitis vinifera* L. and V. *labrusca* L.), muscadine grapes (V. *rotundifolia* Michx.), blueberries (*Vaccinium* sp. L.), and strawberries (*Fragaria* x *ananassa* Duch.). In total, the UA breeding program has released 39 cultivars of grape, blackberry, blueberry, peach, and nectarine.

The fruit industry is dynamic, challenging, and is continuously imposing new and higher standards to all involved sectors, from new cultivar development to marketing technology. Consumers require or desire year-round supplies of flavorful, high-quality fruit and are very interested in the health benefits associated with their consumption (Sansavini et al., 2006). Growers demand high-yielding cultivars that produce quality fruit produced on disease-resistant plants. Also, they require cultivars that produce fruits of desirable size and flavors that have balanced content of sugars and acids, along with extended postharvest shelf-life potential (Crisosto, 2002). This last requirement is also important for shippers that normally ship fruit to distant markets. Fruits need to maintain high quality often for more than 14 d after harvest (Infante et al., 2006). Postharvest quality is closely related to fruit firmness and texture, and breeding programs developing new cultivars for the fresh and shipping industry must select new breeding material carrying “good texture and high firmness alleles”.

For peach, one of the industry problems is that consumers are not always satisfied with the fruit purchased (Crisosto, 2002). Often the peaches are dry, have a leathery texture, exhibit flesh browning, and lack flavor. Reasons for these problems include immature fruit harvest,

extended shipment distances coupled with long-term storage, and lack of cultivar suitability for this type of marketing (Crisosto and Valero, 2008).

Improving fruit quality is one of the most important objectives within a breeding program, and peach breeders focus their work in developing new cultivars with increased sweetness and other flavor enhancements, attractive color, high flesh firmness, large fruits, new shapes, and other characteristics (Sansavini et al., 2006). Breeders must select for these favorable traits to obtain new and better cultivars. The peach has been a model crop in *Prunus* for molecular research, and there are now several markers available for breeders to use for identifying quality attributes in seedling selection [termed marker-assisted breeding (MAB)]. National projects are working with genetic and phenotypic data to construct additional molecular markers that will be available to breeders in the future (Iezonni et al., 2010). One those projects is RosBREED, a Specialty Crop Research Initiative grant-funded initiative, which began in 2009 with the aim to incorporate MAB in *Rosaceae* breeding programs (www.rosbreed.org). This project was renewed with additional funding in 2014 with the expanded objectives to apply MAB for disease resistanceand postharvest storage potential.

The Arkansas peach breeding program maintains a diverse range of flesh types and textures [melting flesh (MF), non-melting flesh (NMF), slow-melting flesh (SMF), and non-softening flesh (NSF)] within its germplasm (Clark, 2011). Postharvest evaluations made on this germplasm show good storage and shipment potential (Clark and Sandefur, 2013a, 2013b). A previous study utilized flesh-type molecular markers related to endopolygalacturonase (endoPG) on some Arkansas genotypes, and these markers correctly identified flesh phenotypes approximately 89% of the time. This was a promising result for enabling MAB in this breeding program (Sandefur, 2011).

Peaches/nectarines having SMF texture remain firm for a longer period on the tree compared to the common MF fruits, but at full maturity SMF peaches melt reaching a juicy texture (Clark, 2011). This particular trait has not been mapped or identified as far as location within the peach genome, therefore the location of a DNA marker associated with this trait would be very important to select for this trait. This flesh type meets the goal of growers/shippers and consumers, since it holds firmness for a longer period (compared to melting peaches/nectarines) and when ripe develops a desirable texture that consumers prefer (J. Clark, personal communication).

The fresh-market blackberry industry has been growing during the last two decades due to several major reasons (Clark and Finn, 2014). The first reason is that consumers are interested in healthy foods, and berries are a leading food in this category. Consumers also favor year-round fresh products and blackberry fruits are now available almost 365 d a year in commercial markets, with this availability increasing the profile and consumption of blackberries (Clark and Finn, 2014). Further, there are more cultivars available that are adapted to a wide range of environments than in previous decades due to the incorporation of new traits such as primocane fruiting (Clark and Finn, 2014; Clark et al., 2007; Finn and Clark, 2012). This trait is beginning to impact this crop, extending production season and therefore increasing profitability to growers (Clark and Finn, 2014). Also, recently developed cultivars have incorporated improved flavors such ‘Osage’ (Clark, 2013).

Of these various characteristics in improved cultivars, postharvest handling has provided among the greatest advances for growers in market expansion. This has increased the need for breeding programs to incorporate evaluation protocols for traits related to postharvest handling

such as decay, leakiness of juice, weight loss, color and glossiness retention, and fruit firmness (Clark and Perkins-Veazie, 2011).

Flesh firmness of blackberry fruits is a critical factor for successful postharvest handling (Clark, 2005); therefore developing cultivars with increased flesh firmness is a high priority for different breeding programs across the U.S. and world. The UA blackberry breeding program has released several cultivars that maintain high quality during storage resulting in quality berries to the consumer. Further, the program has a wide range of genotypes with exceptional firmness characteristics, such as 'Navaho', 'Ouachita', 'Prime-Ark® 45', 'Natchez', 'Osage', and 'Prime-Ark® Traveler' (Clark, 2013; Clark and Moore, 2005, 2008; Clark and Perkins-Veazie, 2011; Moore and Clark, 1989). In the program, fruit with a crisp texture has been identified. It has further been observed that this crispy trait is inherited in seedlings (Clark, 2005). Currently, two thornless selections in the program have this trait, A-2453 and A-2454, are being used in crosses to transfer this trait into primocane- and floricane-fruiting plants to result in an enhancement of fruit quality, particularly firmness. After storage, it has been observed that these selections maintain this exceptional firmness and crisp texture (J. Clark, personal communication). A focus of my research was to evaluate further the morphological components that contribute to this trait and investigate its genetic potential for further enhancement in firmness in new cultivar development.

The main objectives of these studies were to understand the texture and firmness traits and their complexity in peaches and blackberries with the aim to implement the new knowledge in each breeding program.

Peach Objectives

- To enable MAB for different peach fruit quality traits within the Arkansas peach breeding program, and use it to complement parent and seedling selection.
- To develop a DNA test predictive of the SMF trait and validate endoPG-6 DNA test in the Arkansas breeding program.
- To quantify the flesh firmness of the different peach genotypes within the Arkansas peach breeding program (MF, NMF, SMF, and NMF).

Blackberry Objectives

- To determine the fruit morphological components associated with the firm ("crispy") trait found in genotypes within the Arkansas blackberry breeding program.
- To quantify the flesh firmness and color reversion of the firm and crispy trait.
- To determine the inheritance of the firm and crispy trait.

LITERATURE REVIEW

PEACH

Origin and Botany of Peach [*Prunus persica* (L.) Batsch]

Peach is a diploid species (2*n*=2*x*=16) having a self-compatible mating system and a juvenile period of usually 2 to 3 years. This crop belongs to the *Rosaceae* family, subfamily *Prunoideae*, genus *Prunus* (L.), subgenus *Amygdalus* (Byrne et al., 2012). Its origin is Asia and it was first domesticated in China, but its wild ancestor is not yet well known (Zheng et al., 2014). It is suggested that the speciation of *P. persica* occurred from allogamous species such as *P. scoparia* Schneider and *P. dulcis* (Mill.) D.A. Webb, and that peach and P. *dulcis, P. kansuensis* Rehder, *P. ferganensis* (Kost. & Riab) Kov. & Kost, *P. scoparia, P. mira* Koehne ex Sargent [syn. *Persica mira* (Koehne) Kov. et Kostina], and *P. davidiana* (Carrière) Franch. evolved from a common ancestor (Byrne et al., 2012).

Peach endocarp (pit/stone) stones collected from archeological sites in the Zhejiang Province in China indicated that peach use began 8000 BP (Zheng et al., 2014). Within this province in the Yangzi River valley is where early selection for favorable peach traits took place (Zheng et al., 2014). The oldest peach endocarp stones were found from 8000-7000 BP to 7000-6500 BP in two sites in China (Zheng et al., 2014). Peach stones from modern cultivars resemble peach stones in China from the Liangzhu culture (Zheng et al., 2014). From Asia, the peach spread to Persia and then to Europe (2,000 years ago) (Byrne et al., 2012). During the sixteenth century peach was brought to the Americas by Spaniards and Portuguese and was spread to several environments, such as South and Central America, Florida, Southern Brazil, Northern U.S., and Southern Canada; another introduction of peach to the U.S. was directly from China in the mid-1850s (Arus et al., 2012; Byrne et al., 2012; Layne and Bassi, 2008). The first genetic

drift of peach it is thought to have taken place in China 4,000-5,000 years ago due to the first domestication of this species followed by a second genetic-related drift due to the introduction of peach to the U.S. (The International Peach Genome Initiative, 2013). Dispersion of peach to China through Persia to Europe and recently to the U.S. represented the second bottleneck, since only a few cultivars were brought to the U.S. and this can be observed in the decrease of nucleotide diversity in the western material (The International Peach Genome Initiative, 2013).

Peach trees are medium in height, usually up to 8 m high at maturity (Bassi and Monet, 2008). Leaves are lanceolate, glabrous, and serrated with glandular petioles (Bassi and Monet, 2008). Flowers and fruit of peach develop from buds on one-year-old shoots (Bassi and Monet, 2008). Each node on the shoot usually has one to two flower buds and one vegetative bud at the same location as the flower buds (Bassi and Monet, 2008). Flower bud development is a character influenced or determined by genotype or cultivar, and is further affected by environmental or other conditions, including age and health of the tree, density and distribution of buds on the shoots, maturity of the shoot tissues, and rootstock (Németh and Szalay, 2012). By studying quantitative parameters of flower bud development (pistil growth, bud weight, and water content), Németh and Szalay (2012) identified significant differences in the floral bud development located on short (up to 20 cm) or long shoots (20 to 40 cm). In short shoots, endodormancy period was 5 to 30 d shorter compared to buds on the long shoots depending on the genotypes and this difference decreased by the time of blooming (Németh and Szalay, 2012).

The principal growth habits in peach are arching, columnar, compact, open, spreading, spur (in which the canopy size could be semi-dwarf or dwarf), standard, upright, and weeping (Bassi and Monet, 2008). Internode length can vary greatly among growth habits with dwarf

peach trees having internode lengths as short as 10 mm along with larger and thicker leaves than other growth habits, resulting in a dense canopy (Bassi and Monet, 2008).

Peach flowers are hermaphroditic and perigynous, the calyx is gamosepalous and detaches after the initial swelling of the fruitlet. Flowers vary in color from white to dark red, with pink being the most common color (Bassi and Monet, 2008). Petals can be large, termed "showy" or small and "non-showy" (Bassi and Monet, 2008). Usually the flower has five petals, although petals can increase in number from 12 to 24 in semi-double to double-flowered genotypes (Bassi and Monet, 2008). The period between pollination and fertilization can vary from 24 h to 12 d, depending on temperature. The first division of the zygote occurs about two weeks after fertilization (Bassi and Monet, 2008).

Fruit can be pubescent (peach) or glabrous (nectarine), and has a fleshy mesocarp and a stony endocarp that is pitted, furrowed, and very hard (Bassi and Monet, 2008). Peach fruit undergoes four growth development stages following a double sigmoid curve (Bassi and Monet, 2008; DeJong, 2006). The first stage is one of rapid growth marked by cell division, followed by a slower stage (stage 2) in which dry matter is used for pit hardening, seed, and embryo growth. The third stage exhibits rapid growth of the fruit due to cell enlargement and elongation. Lastly, the last (fourth) stage is the ripening phase in which ethylene production in fruit increases (Bassi and Monet, 2008; Tonutti et al., 1991).

Peach Production and its Economic Importance

Peach and nectarine are 10th in total fruit crop production in the world. The peach is the third-most important temperate tree fruit species after apples (*Malus* x *domestica* Borkh.) and grapes, with total production estimated at over 21.6 Mt in 2013 (FAO, 2015a). China produces

55% of the total world production (12 Mt) followed by Italy (1.4 Mt), Spain (1.3 Mt), U.S. (0.9 Mt), and Greece (0.7 Mt) (Byrne et al., 2012; Layne and Bassi, 2008). The average worldwide yield in 2013 was 14.1 $t \cdot ha^{-1}$compared to the average yield of 19.3 $t \cdot ha^{-1}$ in the U.S. and China averaging 15.4 $t \cdot ha^{-1}$ (FAO, 2015a). In 2013, the total harvested area in the world was 1,538,174 ha with 50.5% of this in China, 5.5% in Spain, 3.2% in the U.S., and 1.2% in Chile (FAO, 2015a).

In 2011, the top five exporting countries were Spain, Italy, U.S., Greece, and Chile (in decreasing order) (FAO, 2015b) and the top five importers were Germany, Russia, France, Poland, and Italy (FAO, 2015b). China in 2011 exported 0.038 Mt which was 0.4% of total production of that year (FAO, 2015b), indicating that Chinese production is primarily for internal consumption.

Peaches and nectarines destined for the fresh market must be large, usually round, have red skin and a yellow ground color, freestone, have short pubescence or be glabrous (nectarines), firm enough to be transported, and have good eating quality. In recent years, the market trends have broadened and now include different flesh colors, acidities, textures, and shapes (Layne and Bassi, 2008).

Peach production for the processing industry represents nearly 10% of the total world production (Byrne et al., 2012). Australia, Chile, China, European Union, Japan, Turkey, and the U.S. are the primary countries producing peaches for these markets with China as the major producer (Byrne et al., 2012; Perez and Plattner, 2012). Processing peaches are handled as a bulk commodity, requiring uniformity, durability for handling, and greater yields to compensate for lower market prices. The final product of a processed peach can be canned fruit, jellies, jams, juice, and pulp used for yogurt (Byrne et al., 2012; Layne and Bassi, 2008).

In 2014, Arkansas peach production totaled 650 tons (USDA, 2014). Total peach production area in Arkansas reported in 2014 was 263 ha with an average yield of 2.5 $t \cdot ha^{-1}$ (USDA, 2014). The amount of production in 2014 was the lowest compared to prior recent years (USDA, 2012). In 2010, Arkansas produced a total of 3,000 tons, in 2011 produced 1,800 tons, and in 2012 production was 3,500 tons (USDA, 2012).

Peach and Nectarine Breeding

A. University of Arkansas Peach and Nectarine Breeding Program

The UA peach and nectarine breeding program, currently directed by Dr. John R. Clark, was initiated in 1964 by Dr. Jim Moore and Dr. Roy Rom. The program's activities have been based at the Fruit Research Station, Clarksville. Initially, the program focused primarily on developing yellow, NMF peaches for baby food. This type of flesh is very firm and has a rubbery texture at maturity which makes it suitable for processing. It also maintains firmness in storage. There was a small effort in the program to develop primarily NMF nectarines and white-flesh peaches for fresh-market also. The objectives of the program shifted in the mid to late 1990s to working only on fresh market cultivars and that same focus area remains today. For the fresh-market effort, firm MF and NMF peach and nectarines were hybridized to develop white- and yellow-flesh breeding material.

In the late 1980s and into the 1990s, the program incorporated additional germplasm including SMF and NSF. Dr. Fred Hough, a peach breeder at Rutgers University, sent seeds from crosses made at Rutgers in 1982 to Arkansas. These seeds were planted and selections made from them in the mid-1980s. The populations segregated for acidity level and flesh type, and it is believed that within those seeds the SMF and NSF traits were introduced into the UA program

(Clark, 2011; J. Clark, personal communication). Selections from these populations were used in additional crosses of NMF, SMF, NSF, and MF in the 1990s, and 2000s to further blend the various flesh genetics.

Fruit having SMF remains very firm until full maturity, then softens, usually a few days after harvest (Clark, 2011). This can be a useful trait for the shipping industry since growers and shippers need firm fruit for handling but a softer, melting texture is usually preferred by consumers. Non-softening and NM peaches and nectarines maintain high firmness throughout the ripening process. Conversely, MF softens at early maturity and must be harvested less mature for handling than other texture types. Textures other than MF can express a crispy texture, particularly at their early maturity stages (Clark, 2011).

As of 2015, the UA program has released 11 peach and five nectarine cultivars. The first five cultivars released were NM yellow peaches for processing: 'Goldnine', 'Goldilocks', 'Allgold', 'GoldJim', and 'Roygold' (Clark, 2011; Clark and Moore, 2001). Between 2002 and 2009, the first white-flesh peaches were released: 'White River' (MF) (Clark and Moore, 2003), 'White County' (SMF), 'White Rock' (NMF) (Clark et al., 2005b), 'White Diamond' (SMF) (Clark and Moore, 2011), and 'White Cloud' (NMF) (Clark and Moore, 2011).

In 2001, several nectarines with different flesh types were released including 'Arrington', 'Bradley' (both yellow-flesh, NMF), and 'Westbrook' (yellow-flesh, MF) (Clark et al., 2001). In 2012, 'Amoore Sweet' (yellow-flesh, NSF) and 'Bowden' (white-flesh NSF) were released (Clark and Sandefur, 2013a). 'Souvenirs', the first SMF and yellow-flesh peach for the fresh market from the UA program was released in 2012 (Clark and Sandefur, 2013b).

In 2009-2013, the UA program was a demonstration peach breeding participant of the Specialty Crop Research Initiative RosBREED project. The aim of this project was to

incorporate MAB into *Rosaceae* crops, including peach, sweet and tart cherry [*Prunus avium* (L.) L.], apple, and strawberry utilizing phenotypic and genotypic information collected on various traits. In the UA breeding program, more accurate flesh characterization was emphasized including the application of the endoPG DNA test which is related to flesh texture of peach and nectarines and is capable to differentiate between MF, NMF, and NSF. This enzyme is involved in the flesh softening process and the DNA test can identify if a peach or nectarine is MF, NMF, or NSF. However, this test is not capable of identifying SMF.

Another important item of progress in the UA breeding program was the development of a postharvest protocol to evaluate the storage and shelf-life potential of new selections and cultivars of the program. The protocol was developed by Paul J. Sandefur as part of his master's degree thesis (Sandefur, 2011). This protocol includes subjective measurements of skin color, skin quality, flesh color, flesh quality, browning of flesh, juiciness, and taste.

B. Peach Breeding Programs Worldwide

The first peach breeding program in North America was established in Geneva, NY in 1895, the year that the cultivar Chinese Cling was imported from China to North America (Byrne et al., 2012). Progeny of 'Chinese Cling', such as 'Elberta', 'Belle of Georgia', and 'J.H. Hale', and progeny of these foundation parents became important cultivars in the U.S. (Byrne et al., 2012). Other programs were started in Iowa (1905), Illinois (1907), Ontario (1911), New Jersey (1914), Virginia (1914), Massachusetts (1918), and New Hampshire (1918) (Byrne et al., 2012). The breeding program of the University of California at Riverside began in 1907 to develop low-chill peaches adapted to southern California (Okie et al., 2008). The cultivar Babcock was the

most important release from this low-chill program, which is low-acid and has white flesh (Okie et al., 2008).

In the southern states, Texas A&M University breeding program started in 1935 to develop low- to moderate-chill cultivars (Okie et al., 2008). In 1937, the USDA-ARS stone fruit breeding program in Georgia was initiated (Okie et al., 2008). A peach breeding program was begun in 1955 at North Carolina State University (Okie et al., 2008).

The USDA breeding efforts in the western U.S. started in 1920 at Palo Alto and later in Davis, CA to develop canning and drying peaches (Okie et al., 2008). Later, the processing cling peach breeding program was established in the University of California Davis and the fresh-market breeding program was relocated to Fresno, CA in 1954 (Okie et al., 2008).

In Latin America, breeding programs were initiated in Brazil (mid 1950s), Mexico (1980s), Chile (2004), Uruguay, and Argentina for the fresh and processing market industries (Byrne et al., 2012). The germplasm utilized in the peach breeding program in Mexico based at the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias introduced genotypes from Bolivia, Peru, Brazil, U.S. (Northeast, Southwest, Central, and Southeast), Italy, Spain, China, Korea, and South Africa (Pérez et al., 2012).

In Europe, peach breeding programs were initiated in Italy and France in the 1920s and 1960s, respectively, and additional breeding efforts were started in Spain (several ongoing breeding programs), Romania (1950s), Serbia (1950s), Greece, Bulgaria (initiated in 1974-1975), Ukraine, and Poland (the mid-1960s) (Byrne et al., 2012; Okie et al., 2008).

In Australia, the peach breeding program currently located in Queensland focuses on the development of low-chilling cultivars for subtropical areas (Topp et al., 2012). Initial populations used in the program were mostly originated from open-pollinated seeds from

University of Florida cultivars and selections and other subtropical selections (Topp et al., 2012). Additional parental germplasm used was from Australia, Brazil, Mexico, California, Georgia, Florida, and China (Topp et al., 2012).

Private companies working with *Prunus* also operate breeding programs to develop cultivars for industry use. One of the largest efforts is that of Zaiger Genetics Inc., a family owned breeding organization founded in Modesto, CA in 1958 (Okie et al., 2008). This company initially conducted breeding for white-flesh peaches for the Asian market along with low-acid, yellow-flesh, and low-chilling cultivars (Okie et al., 2008). Some cultivars released from this company include 'Super Rich', 'Brittney Lane', 'Spring Snow', 'Country Sweet', 'Honey Blaze', 'Artic Snow', 'Artic Mist', and 'Red Roy' (Okie et al., 2008). Bradford Genetics Inc. is another private company developing peach and nectarine cultivars (along with other *Prunus*) for different markets (Okie et al., 2008). This company has been conducting breeding since the 1940s and is based at Le Grand, CA (Okie et al., 2008). Recent developments and areas of emphasis include white-flesh nectarines and peaches, very high soluble solid content peaches and nectarines, along with apricots, cherries, and plums (Bradford Genetics, 2015). Some cultivars developed by the Bradford program are 'Diamond Bright', 'Kay Sweet', 'August Pearl', 'Ruby Sweet', 'Ivory Princes', and 'Crimson Lady' (Okie et al., 2008).

Important Traits of Improvement in Breeding Programs

Peach breeding programs around the world work to accomplish their objectives, such as specific environmental adaptation, disease resistance, extended harvest periods to expand marketing season, enhanced fruit quality (shape, color, flavor, aroma, texture, and firmness), and

postharvest potential. However, all programs have a common and unique focus, to produce new and improved cultivars (Infante et al., 2006).

Breeders should take into consideration the entire market chain (growers, shippers, target market, and consumers) to make breeding decisions and to develop new cultivars. For example, as a part of the RosBREED project, a survey was conducted of 66 fresh peach producers and 26 processing peach growers at the Mid-Atlantic Fruit and Vegetable meeting, PA, the Southeast Regional Fruit and Vegetable Conference, GA, and California Stone Fruit meeting (Yue et al., 2014) to determine the most important traits of interest of these varied grower groups. Growers were included in the survey from Alabama, California, Georgia, South Carolina, North Carolina, New Jersey, Pennsylvania, Maryland, and Virginia (Yue et al., 2014). For southeastern fresh peach producers, the most important quality traits were fruit flavor and size (Yue et al., 2014). Also, they reported that fresh peach producers make cultivar decisions based on their perception of consumer's preferences but also consider plant adaptation to their location, cultivar availability, and market type (Yue et al., 2014). Southeastern producers considered fruit skin color more important and fruit flavor less important than mid-Atlantic producers (Yue et al., 2014).

Major Trait Inheritance

A. Qualitative Characters:

1. Tree Architecture: Due to the relatively low productivity and high pruning costs of peach compared to apple for example, innovative fruit production methods are needed in a peach orchard (Byrne et al., 2012). To help address needed innovations, breeders can develop dwarfing rootstocks, or select scion cultivars with modified tree architecture to allow high

density orchards (Byrne, 2005; Sansavini et al., 2006). Architecture phenotypes can vary from dwarf to weeping and columnar, with interactions between these major architectural classifications (Byrne et al., 2012). Tree architecture is a qualitative trait and has Mendelian inheritance (Monet and Bassi, 2008). Examples of tree architecture are columnar (*br/br*) which has an incomplete dominance (Fan et al., 2010). Upright architecture is expressed when the *Br* allele is heterozygous with the alleles for the standard, dwarf, compact, or weeping growth habits (Fan et al., 2010).

2. Leaves: Peach leaves vary in shape, leaf margins, and color (Byrne et al., 2012). For example, narrow shape (*Nl*) is dominant over wide (*nl*) and smooth margin is dominant over wavy margins (*wa*) (Byrne et al., 2012).

3. Flower Type: There are several flower characteristics that combined give rise to the flower produced from a particular genotype. One of these traits is that peach flowers can be non-showy or non-showy with non-showy being the dominant allele (*Sh*) (Byrne et al., 2012). Also, peach flowers can have single (*Dl*) or double petals (*dl*), in which case single flower is the dominant character. Color of petals is another trait that varies from white to dark pink (Byrne et al., 2012). For example, red petal color is dominant over pink and dark pink is dominant over light pink (Byrne et al., 2012; Monet and Bassi, 2008).

4. Fruit Shape: Peach fruit can be saucer (also called flat) or round. Flat fruit is dominant (*S*), but the homozygous dominant genotype is lethal (Monet and Bassi, 2008).

5. Skin Pubescence: Fruits with glabrous skin are termed nectarines and lack of pubescence (*g*) is recessive to the pubescence or fuzz allele (*G*) (Monet and Bassi, 2008).

6. Flesh Color: Flesh and skin color are two traits important within many breeding programs. Flesh color varies from white to yellow to dark red, with white being the dominant phenotype (*Y*)

(Byrne et al., 2012; Monet and Bassi, 2008). In recent years, there has been an expansion in the development of white-flesh peaches, which until the 1960s, were primarily consumed in Asia and in some European countries. Now it is possible to find this type of peach in markets outside Asia and several breeding programs are currently developing white-flesh peaches (Byrne et al., 2012).

6. Stone Adhesion: Peach fruits can be freestone or clingstone (Monet and Bassi, 2008). In the case of freestone peaches/nectarines, the stone can be separated from the flesh, a situation that does not occur with clingstone fruit. Freestone fruit is more utilized for the fresh market industry and fruit from clingstone genotypes is utilized for processing (Monnet and Bassi, 2008). This trait is controlled by a single gene located on chromosome 4, very close to the gene that controls flesh type (Peace et al., 2005a). Freestone is dominant over clingstone (Peace et al., 2005a).

7. Fruit Acidity: Fruit acidity is controlled primarily by a major locus located on chromosome 5 with low acidity dominant (Monet and Bassi, 2008). This inheritance has not always been found to be completely qualitative, as a study of inheritance of fruit acidity was estimated to be 19% (Monet and Bassi, 2008) to 31% by de Souza et al. (1998). In a study done by Boudehri et al. (2009), it was concluded, by using a molecular marker linked to this locus, that the low acidity trait (controlled by the D locus) is partially dominant.

B. Quantitative Characters:

1. Disease and Insect Resistance: Efforts are being made to integrate pest and disease management to reduce chemicals used in the peach industry (Byrne, 2002, 2005). In recent years, restrictions in the use and availability of chemicals in agriculture have become major concerns including safety of agricultural workers, environmental contamination, production costs, and

food safety. For this reason, breeding for pest and disease resistance is one step in this process and breeding programs across the world are trying to incorporate resistance genes (Sansavini et al., 2006). Examples of these are resistance to Sharka disease (plum pox virus), powdery mildew [*Podosphaera pannosa* (Wallr.) de Bary], brown rot [*Monilinia fructicola* (G. Winter) Honey], and leaf curl [*Taphrina deformans* (Berk.) Tul.], and resistance to bacterial diseases such as bacterial spot [*Xanthomonas arboricola* pv. *pruni* (Smith) Dye] (Sansavini et al., 2006). Resistance to nematodes is also a concern and work has been done specifically in *Meloidogyne* species in China and the U.S. (Byrne et al., 2012; Sansavini et al., 2006).

2. Environmental Adaptation: Breeding for this objective has led to the expansion of production zones and the extension of the harvest season (Byrne, 2005). To address the adaptation objectives, breeders must select for chilling, heat requirement, heat adaptation, cold tolerance, and resistance to biotic and abiotic stresses (Byrne et al., 2012). An example is that a significant number of breeders are developing low-chill cultivars with short fruit development periods as a priority to create early season fruit production adapted to reduced-chill environments. This innovation, in combination with the complementary production from the northern and southern hemispheres, allows year-round production and marketing of peaches in many countries (Byrne, 2005; Byrne et al., 2012). Chilling and heat requirement were reported to have broad-sense heritability of 80% and 54%, respectively, indicating progress in genetic advancement of these traits should be achieved (Fan et al., 2010).

3. Bloom Date: Chilling requirement is the major factor determining bloom date (Fan et al., 2010). Since genotypes with low chilling requirement usually bloom early and in cold regions/years, they can be susceptible to late frost damage (Fan et al., 2010). Also, genotypes with high chilling requirement can suffer inadequate chilling in warm regions resulting in

irregular floral and leaf bud break (Fan et al., 2010). Broad sense heritability for this trait was reported to be 85% in the study done by Fan et al. (2010), and full bloom heritability was reported to be 39%and 78% by Monet and Bassi (2008) and de Souza et al. (1998), respectively.

4. Harvest Season: Extension of the harvest season has been one of the goals of several breeding programs, resulting in an expansion of production by 1 to 2 months (Byrne et al., 2012). This has resulted in a fruiting season that can be as long as 8 months in a single location or region (Byrne et al., 2012). This trait has quantitative inheritance with an estimated heritability of 84% reported by Monet and Bassi (2008) and 94% estimated by de Souza et al. (1998).

5. Fruit Size and Weight: These traits are important in all breeding programs, because they are tied to consumer preferences and yield. Fruit size and weight are related and exhibit quantitative inheritance. Fruit length inheritance was estimated to be 31% and 38% by Monet and Bassi (2008) and de Souza et al. (1998), respectively. Also, inheritance of fruit weight was reported to be 32% (de Souza et al., 1998).

7. Fruit Firmness: Fruit firmness is essential for postharvest handling and marketing (Byrne, 2002) and its inheritance has been reported to be about 13% (Monet and Bassi, 2008). This trait is closely related to flesh texture, characteristics that are explained in detail in the "The Diversity of Flesh of Peach" section.

8. Skin Color: Skin color is very important for fresh-market cultivars and less important for the non-melting genotypes used most often for processing (Byrne et al., 2012). For example, fresh market consumers in Europe and U.S. prefer a red skin covering over 80% of the surface (Byrne et al., 2012). Fruit skin color inheritance was estimated to be 68% by de Souza et al. (1998).

9. Fruit Sweetness: This trait is critical for consumer acceptance, and because of previous breeding, it is now possible it have mid- and late-season peaches/nectarines with more than 20%

soluble solid concentration (SSC) and a range of fruit acidity levels (Byrne, 2005; Byrne et al., 2012). Fruit sweetness inheritance was estimated to be 33% by de Souza et al. (1998).

Molecular Genetics of Peach

Peach is one of the most widely grown and genetically characterized species in the *Rosaceae* family (Zhebentyayeva et al., 2008). Diploid peach nuclear DNA content is estimated to be 0.60 pg (Baird et al., 1994).

The genome of the doubled haploid cultivar Lovell was studied by The International Peach Genome Initiative (2013) and it was estimated that the genome size of 'Lovell' was be 265 Mb. A total of 27,852 protein-coding genes and 28,689 protein-coding transcripts were predicted (The International Peach Genome Initiative, 2013). When comparing the gene content of peach to apple and grape, this study found that the gene number in peach was lower than apple and similar to grape (57,386 genes in apple and 30,434 genes in grape); peach gene density was found to be higher than apple (1.22 and 0.78 genes per 10 kb, respectively) (The International Peach Genome Initiative, 2013).

Peach nucleotide diversity was studied by sequencing *P. persica* accessions and wild relatives such as *P. ferganensis*, *P. kansuensis*, *P. davidiana*, and *P. mira* (The International Peach Genome Initiative, 2013). Nucleotide diversity of peach was $1.5x10^{-3}$, lower than other species, such as wild and cultivated soybean [*Glycine max* (L.) Merr.], and marked differences in diversity were observed among chromosomal regions. These differences may have resulted from breeding activities, such as selection and crossing for specific traits (The International Peach Genome Initiative, 2013).

The first genetic map of *P. persica* was constructed by Chaparro et al. (1994). A year later Rajapakse et al. (1995) used restricted fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) techniques to construct an improved map. Since then, different linkage maps of peach and interspecific hybrids between peach and other members of *Prunus* have been constructed (Byrne et al., 2012). One of the interspecific maps, produced using an almond ('Texas') x peach ('Earlygold') F_2 progeny (T x E map), is now used in the *Prunus* scientific community as a reference map for peach and other *Prunus* species (Arus et al., 2012). Using this interspecific map, Dirlewanger et al. (2004) identified the position on the chromosomes of 28 major genes affecting morphological or horticultural traits in different *Prunus* crops. For example, for peach, linkage group 1 (LG 1) has genes that segregate for fruit flesh color and evergreen, as well quantitative trait loci (QTL) for chilling requirement (Dirlewanger et al., 2004). On LG 2 was located the gene for double flower (Dirlewanger et al., 2004). On LG 3 were located genes for flesh color around the stone and polycarpel and flower color (Dirlewanger et al., 2004). On LG 4 was located the gene that segregated for flesh adhesion, and a gene for ecodormancy release (Dirlewanger et al., 2004). On LG 5, genes for acid/non-acid fruit and surface pubescence were identified (Dirlewanger et al., 2004). Further, on LG 6, genes for leaf shape, plant height, male sterility, and fruit shape were present (Dirlewanger et al., 2004). On LG 7, genes for resistance to powdery mildew were found along with and leaf gland type (Dirlewanger et al., 2004). Later, Dirlewanger et al. (2006) constructed a new linkage map using 208 individuals of an F_2 peach population ('Ferjalou Jalousia' x 'Fantasia'). They reported that on the upper part of LG 5 are QTL for pH and titratable acidity (TA). Also Ettiene et al. (2002) detected QTL for malic and citric acid in this region using the same population. Quantitative trait loci for sugar concentration were located on LG 4, LG 5, LG 6, and LG 7 and

QTL related to phenolic concentration were detected on LG 1, LG 2, LG 4, and LG 6 by Dirlewanger et al. (2006). Ettiene et al. (2002) detected on LG 4 QTL for ripening date and fruit development period, and on the same genomic region were QTL for glucose, sucrose, and SSC. The study reported by Dirlewanger et al. (2006) was an update of previous work made by Dirlewanger et al. (1999), where they used 63 individuals of an F_2 progeny of 'Ferjalou Jalousia' x 'Fantasia' (Dirlewanger et al., 1999). A fruit quality genetic map was developed by Ogundiwin et al. (2009) using a peach intraspecific cross called "Pop-DG" of 'Dr. Davis' (canning peach) x 'Georgia Belle' (fresh-market peach), and also DNA samples from 'Texas' x 'Earlygold'. In this map they determined the genomic location of 133 fruit quality candidate genes related to fruit ripening, softening, flavor, pigmentation, and chilling injury resistance. Also, the "Pop-DG" map was almost entirely collinear with the *Prunus* reference T x E map, meaning that the locations of markers and QTL located on "Pop-DG" could be cross-referenced to T x E and other *Prunus* maps.

Synteny between peach, sweet cherry, and 11 other species of *Rosaceae* and others families was studied by Dirlewanger et al. (2002) by testing 41 simple sequence repeat (SSR) molecular markers generated from a genomic library of the peach cultivar Merrill O´Henry. All SSR markers amplified in peach and 80.5% (33 markers) on sweet cherry, indicating that these 33 SSR-markers amplified in both species. Among these 33 markers, 13 were polymorphic in peach and sweet cherry, 19 were polymorphic only in peach, and one was polymorphic only in cherry (Dirlewanger et al., 2002). Also, 29.3% amplified in all analyzed *Rosaceae* species, 75.6% amplified in the six *Prunus* species tested, 80.5% amplified on chestnut (*Castanea sp*.), 43.9% on grape, and 31.7% on walnut (*Juglans regia* L.) (Dirlewanger et al., 2002). High amplification was also obtained when the transferability of SSR markers was analyzed across

Rosaceous crops (Mnejja et al., 2010). In this study, eight cultivars from almond, peach, apricot (*Prunus armerniaca* L.), Japanese plum (*Prunus salicina* Lindl.), European plum (*Prunus domestica* L.), sweet cherry, apple, pear (*Pyrus communis* L.), and strawberry were tested with molecular markers of almond, peach, Japanese plum, and apricot. Most of the primer pairs (83.6%) amplified bands of the expected size range in other *Prunus.* Thirty-one SSR amplified and were polymorphic in all *Prunus* studied (Mnejja et al., 2010). Twelve of these were proposed as the universal *Prunus* set and while 16.3% were transferable in species of other *Rosaceae* genera (Mnejja et al., 2010).

Another study was conducted to discover the QTL affecting reproductive phenology in peach (Romeu et al., 2014). They studied the segregation of different traits of the population 'V6' x 'Granada'. They discovered a QTL related to chilling requirement, endodormancy and ecodormancy release, bloom date, heat requirement for ecodormancy release, and harvest date on LG 1. On LG 3, QTL for chilling requirement, bloom date, and heat accumulation were also found by Romeu et al. (2014). On LG 4, QTL for ecodormancy release, heat requirement for bloom, and harvest date were also found (Romeu et al., 2014). On LG 5, Romeu et al. (2014) also found a QTL for ecodormancy release. More QTL for chilling requirement, eco- and endodormancy release, bloom and harvest date, and heat requirement for bloom were also found on LG 6 and LG 7 (Romeu et al., 2014).

Polygenic resistance may contribute to the development of cultivars with effective and durable resistance to biotic stress (Dirlewanger et al., 2004). Three *Prunus* species (*P. davidiana*, *P. kansuensis*, and *P. mira*) which are closely related to peach are possible sources of peach pest and disease resistance such as peach aphid (*Aphis gossypii* Glover), peach leaf curl [*Taphrina*

deformans (Berk.) Tul.], and powdery mildew [*Sphaeroteca pannosa* var. *persicae* (Wallr.) de Bary] (Dirlewanger et al., 2004).

The Diversity of Flesh Types of Peach

Peach fruits have a fruit development period exhibiting a double sigmoid growth curve, and the development stages have been well established in prior research. Stage I, which begins just after fertilization, is characterized by cell division and initial growth (the length of this period is the same for all cultivars). Stage II is the period of embryo development and hardening of the endocarp. During Stage III, a rapid increase in fruit size is achieved by cell enlargement and elongation. Lastly, Stage IV is the onset of fruit ripening, in this stage ethylene production increases, water and sugar accumulates, and the final fruit size is achieved (Hayama et al., 2001; Layne and Bassi, 2008).

Fruit ripening is a complex process that when completed, the different flesh textures are expressed and differentiated. There is high diversity in peach flesh types, which have been described and characterized in recent years with advances in phenotypic and genotypic characterization. Different textures or flesh types are due to the great diversity of this species coming from different locations and breeding programs, such as MF, NMF, slow-ripening flesh (SRF), SMF, and stony-hard flesh (SHF).

Traditionally, peaches and nectarines have been classified as MF and NMF. Melting flesh has a rapid final stage of ripening where there is substantial loss of firmness due to the loss or reduction of cell wall adhesion in the fruit mesocarp (Byrne et al., 2012). In general, fruit ripening is a coordinated series of modifications to the polysaccharide components of the primary cell wall and middle lamella, resulting in a weakening of the structure (Brummell,

2006). When MF peaches or nectarines ripen, there is a major solubilization of flesh polymers and an increased water-soluble fraction (Ortiz et al., 2012). According to Brummell (2006), MF peaches show two phases of rapid softening (the first at the beginning of ripening and the second at the beginning of melting). The beginning of softening was in the pre-climacteric stage of fruit ripening, when the fruit still has green background color and coincides with the beginning of depolymerization of hemicelluloses (Brummell, 2006). Ghiani et al. (2011b) compared endoPG behavior in MF and NMF peaches. Their results implied that the loss of cell turgidity of pericarp tissue during softening is a process common to MF and NMF (Ghiani et al., 2011a). However, the loss of cell adhesion was exclusively observed in the pericarp tissue of MF peaches and no loss or reduction of cell adhesion was observed in NMF or unripe MF peaches (Ghiani et al., 2011a).

Non-melting-flesh cultivars have historically been developed for processing purposes and were usually avoided by breeders in the U.S. for fresh-market objectives (Sherman et al., 1990). However, in recent years this flesh type has become popular in the fresh market. Non-melting cultivars lack this final melting phase, maintaining most of their firmness even when fully ripe, and have a rubbery texture (Ghiani et al., 2011a, 2011b; Lester, 1994). This type of texture is usually associated with the clingstone trait. One of the first attempts to utilize NMF cultivars in the fresh market was led by the University of Florida breeding program, by introducing characteristics of the MF selections/cultivars in the NMF type, such as high acidity and red overcolor (Peace and Norelli, 2009; Sherman et al., 1990). In NMF genotypes, it has been demonstrated that during softening the number of exocarp cells per mm^2 were reduced, while in the mesocarp the number of cells per mm^2 increased indicating an increase and a decrease in cell size in both tissues, respectively (Ghiani et al., 2011a). Morphological analysis of MF and NMF

revealed that mesocarp cells of NMF not only lost turgidity, but also cells put pressure on each other (Ghiani et al., 2011a). This pressure seemed to come from the exocarp layer in which cells increased their volume during softening, behavior not observed in MF fruits (Ghiani et al., 2011a). 'White Rock' is a white-flesh peach, released in 2005 by the UA peach breeding program (Clark et al., 2005b), which it was thought to belong to the NSF texture due to its impressive firmness (Clark et al., 2005b; J. Clark, personal communication). However, after genotypic characterization it was revealed that this cultivar is NMF (J. Clark, personal communication). It maintains its high firmness during full maturity, and when overripe the fruit remains firm even after dropping from the tree (Clark et al., 2005b). This particular flesh texture and extraordinary firmness could be due to other sources of NM flesh or another type of mutation in the endoPG enzyme.

The SRF genotypes show a very slow rate of flesh softening, reduced CO_2 and ethylene production, and remain firm in the field even during the beginning of autumn (Ramming, 1991). With this flesh type, fruit development appears to cease before the end of cell expansion phase (Tataranni et al., 2010). The flesh is crisp and firm, never softens (or softens very slightly), but does not have the texture of NMF (Ramming, 1991). It is assumed that this character is monogenic and recessive due to the segregation ratios of fruit obtained of a selfed, normal-ripening tree having one slow-ripening allele (3 normal : 1 slow-ripening) and of the segregation obtained from a controlled slow-ripening, selfed population (0 normal :1 slow-ripening) (Ramming, 1991; Tataranni et al., 2010). Also, with this type flesh the ground skin color and the flesh color maintain a green color (Tataranni et al., 2010).

The SHF trait, briefly described before, is another flesh type that has sugar and acid content and appearance similar to common peaches, but it has a crispy texture and maintains its

firmness after harvest (Hayama et al., 2008). This flesh type can be either melting or non-melting because the SH locus (*Sh*), which is recessive and monogenic, is independent and epistatic from the melting locus (Goffreda, 1992; Haji et al., 2005; Liverani et al., 2002).The softening process of SHF genotypes is blocked due to the lack of ethylene production and SHF fruits produce little or no ethylene, have low respiration rate, tend to ripen later than non-stony hard fruit, and also have a lower percentage of blush (red over color). They also maintained their firmness after 5 d of storage at 5 °C (Bassi et al., 1998; Goffreda, 1992; Hayama et al., 2006). However, firmness of SHF fruits sprayed with ethephon prior to storage decreased significantly (Goffreda, 1992). In a study done by Bassi et al. (1998) it was found that calcium bound to insoluble pectins ratio in this type of flesh was 35% higher compared to MF and NMF genotypes, so it could be possible that SHF peaches/nectarines are more able to allocate calcium ions in bridges among galacturonic acid units of pectins that strengthen the cell wall structure. Similar results were also found by Mignani et al. (2006).

Another flesh type, SMF, maintains firmness for a longer period on the tree than MF (similar to SHF genotypes), but at maturity melts completely, reaching firmness values similar to conventional MF genotypes (Sandefur, 2011). Ghiani et al. (2011b) studied the postharvest performance of MF, NMF, and the peach cultivar Big Top, which was classified as SHF (this cultivar has the firmness and crispness of a SHF at harvest, but it melts at a slow pace and develops ethylene during softening). They concluded that ‘Big Top’ belongs to the SMF category, because the postharvest behavior of ‘Big Top’ was very different from that observed for SHF by other researchers. This cultivar evolved ethylene 5 d after harvest, when it reached the melting phase, and this was contrary to what happened with the SHF ‘Ghiaccio’, which did not evolve ethylene during postharvest and maintained its firmness 5 d after harvest (Ghiani et

al., 2011b). ‘White County’ and ‘White Diamond’ are white-flesh peaches released in 2005 from the UA peach breeding program and are thought to be SMF because the flesh is very firm until full maturity when it softens substantially. ‘Souvenirs’, the latest yellow-flesh peach cultivar released from this breeding program, also is a SMF genotype (Clark and Moore, 2011; Clark and Sandefur, 2013b; Clark et al., 2005b). Further, MF individuals could be separated in two distinct groups of texture based on their softening (also called melting) rate and/or their fruit ethylene production after harvest (RosBREED, 2015). The first group contains individuals that melt in a quick rate and their ethylene production increases at the second or third day after harvest, which in previous literature are referred to just as MF. This type of texture in this Dissertation is referred to as quick-melting flesh (QMF). The second group of the melting texture contains individuals that melt at a slow rate and their ethylene production rate after harvest is slow during the first days of postharvest, increasing later, such as ‘Big Top’ (Ghiani et al., 2011b). This type of texture in this Dissertation is called SMF.

Non-softening flesh peaches are another genotype having a distinct flesh that could be confused with NMF given its high firmness after ripening. This genotype loses very little firmness and maintains a crispy texture during the ripening process (Peace and Norelli, 2009). This texture type is associated with the clingstone trait and has a complete deletion of the endoPG gene in the Freestone-Melting (F-M) locus (Peace and Norelli, 2009). This flesh type has been used in the UA breeding program since the mid-80s. Within the Arkansas program this texture was thought to be NMF, but after investigation utilizing the endoPG DNA molecular marker, it was discovered that NSF texture was present in the program. It was also found that the Arkansas cultivars Roygold, Amoore Sweet, and Bowden are NSF and not NMF (unpublished data).

Flesh adherence to the endocarp is a quality trait closely related to the flesh type trait in peaches, and cultivars can be classified as freestone or clingstone. The freestone trait is dominant over clingstone, and although semi-clingstone individuals can be found among populations, they are usually classified as freestone or clingstone because the semi-cling characteristic can vary among years and degree of fruit maturity for the same individual (Bailey and French, 1932). Early evidence reported by Bailey and French (1932) showed that these two traits are genetically related, since freestone, non-melting flesh genotypes were not found in their investigations, and they concluded that these two traits were linked. Later research indicated that these two traits are controlled by a single locus (F-M locus) with two separate genes (Martinez-Garcia et al., 2012; Peace et al., 2005a, 2005b). Freestone NMF types still have not been found (Peace et al., 2005a). An extensive and complete literature review of peach texture was wrote by Sandefur et al. (2013) in which the biochemical, genetic, and physiological bases of this character are explained in detail.

Freestone – Melting Locus and the Endopolygalacturonase Enzyme

Polygalacturonase enzymes were first identified over 45 years ago and are involved in the disassembly of pectins, particularly in tissues that require cell separation such as fruit (Hadfield and Bennett, 1998). Endopolygalacturonase (endoPG, EC 3.2.1.15) function is to soften fruits during the ripening process by hydrolysis of the pectate chain randomly in peach cell walls (Pressey and Avants, 1973, 1976). In a study done on fruits of 'Flavorcrest' (midseason FMF peach), low endoPG activity level was detected in fruits that were substantially softer (7 to 10 Kgf) compared with unripe fruit (>10 Kgf) in which endoPG activity was barely detectable (Orr

and Brady, 1993). Below 2 Kgf the activity of the enzyme increased several-fold compared to fruit with a level of force superior to 7 Kgf (Orr and Brady, 1993).

Endopolygalacturonase is found in several fruits, such as tomato (*Solanum lycopersicum* L.), melon (*Cucumis melo* L.), and apple (DellaPena et al., 1986; Giovannoni et al., 1989; Hadfield and Bennett, 1998; Wu et al., 1993). In peach, this enzyme was first characterized by Pressey and Avants (1973) and according to these authors has the ability to solubilize pectins, as mentioned before. Pectin disassembly is associated with the later stage of ripening and with fruit deterioration to overripe. During disassembly, pectins are solubilized, the middle lamella swells and disappears, and the microfibrillar network becomes disorganized (Hadfield and Bennett, 1998).

Recent studies have determined that freestone and melting traits are controlled by a single locus, now referred to as the F-M locus, which has two copies of the same gene located near the end of peach chromosome LG 4 (Peace et al., 2005a, 2005b, 2007). The second copy of this gene is located less than 50 kilobase pairs (kbp) upstream of the first texture gene (Peace and Norelli, 2009). One copy controls the Melting locus and the other the Freestone locus (Peace et al., 2007). Thus the F-M locus has four alleles controlling both traits; cultivars can be freestone melting flesh (FMF), clingstone melting-flesh (CMF), clingstone non-melting flesh (CNMF), and clingstone non-softening flesh (CNSF) (Peace et al. 2005a, 2007). It has been proposed that in MF cultivars, that during softening the activity of the endoPG enzyme increases along with the endoPG gene expression during the melting phase, and that in CNMF the expression of the endoPG gene is reduced and there is almost no endoPG activity (Peace et al., 2005a).

The findings of Peace et al. (2005a) are similar to those obtained by Callahan et al. (2004), in which the amount of mRNA PRF5 transcripts (a transcript associated with the endoPG

gene) was much lower in NMF compared to MF cultivars. Previously, Lester et al. (1994), while studying endoPG differences between MF and NMF genotypes, detected no significant endoPG activity in the NMF types. In a more recent study, endoPG expression was observed in all MF and NMF fruit except for unripe NMF and SRF (Ghiani et al., 2011a). Lester et al. (1996) found two genetic sources of NMF, one having a deletion in the gene, and in the other source the related genes of endoPG were present but in a different form (at least one of the alleles was found in MF genotypes). Callahan et al. (2004) utilized five MF cultivars and eight NMF cultivars and also found more than one NMF source. One of the sources resulted in a complete deletion of the genes segregating for endoPG, and the other two sources had deletions of a subset of those genes (Callahan et al., 2004). Freestone melting-flesh genotypes can be obtained from four different allele combinations, FF, Ff, Ff1, and Fn (n corresponds to the null allele). Clingstone MF genotypes result from the allelic combinations ff, ff1, and fn. Cultivars having the CNMF genotype have the combination f1f1, and f1n, while CNSF arises only with the nn combination. Further, the f allele is recessive to the F allele, the f1 allele is recessive to the f and to F alleles, and the null allele is recessive to all (Peace and Norelli, 2009; Peace et al., 2005a).

As a part of the RosBREED project, a genotyping analysis was done for the first time in the UA breeding program in 2011 and 2012 with the aim to validate endoPG DNA markers and hopefully to find DNA markers for SMF (Sandefur, 2011). Endopolygalacturonase markers (endoPG-1 and endoPG-6) matched correctly to the phenotype 89% of the time with cultivars classified as MF and NMF, but no differentiation when the genotyping was found between for MF and SMF (Sandefur, 2011). These results indicated that the endoPG marker, specifically endoPG-6, could be used to differentiate between NMF and MF types, but that one or more loci, different from the M-F locus, are likely responsible for SMF (Sandefur, 2011).

Other Enzymes and Proteins Involved in the Peach Softening and Ripening Processes

Endopolygalacturonase is only one of several enzymes involved in the ripening and softening process in peaches. Pectin methylesterase (PME, EC 3.1.1.15) is another enzyme which has been associated with a massive solubilization and depolymerization of pectins in MF cultivars (Nilo et al., 2010). The principal function of PME is demethylation of polyuronides so they can be degraded by endoPG (Nilo et al., 2010). Pectin methylesterase activity increased at an early stage of fruit ripening and remained constant or decreased throughout the melting phase in MF genotypes (Nilo et al., 2010), and according to Kao et al. (2012) its activity in NMF genotypes is lower than MF cultivars, before and after ripening. The results of Nilo et al. (2010) were similar to those found previously by Glover and Brady (1995) and Lurie et al. (2002) in which the activity of PME was higher in green fruit and during harvest than in ripe fruit. Kao et el. (2012) concluded that PME activity may be more directly related to softening than endoPG and that endoPG activity of NMF cultivars was not different from that of MF genotypes. Further, they found firmness of NMF cultivars were almost five times greater at maturity compared to MF genotypes. In their study it was concluded that endoPG activity may not completely explain the delayed softening of NMF cultivars. Differing from the results of Lester et al. (1994), Kao et al. (2012) suggested that lower PME activity observed in NMF cultivars is a limitation for the generation of the substrate for endoPG. In another study, PME activity was not affected by exogenous ethylene applications in MF and SHF genotypes and no correlation between PME level and flesh firmness was observed (Kao et al., 2012). Similar results were not found with exopolygalacturonase (exoPG) and endoPG levels, since correlations were observed for these enzymes and flesh firmness in the SHF cultivar Manani (Hayama et al., 2006). The function of exoPG is probably to complete the hydrolysis of pectate initiated by endoPG (Pressey and

Avants, 1976). Also, it is proposed that the role exoPG could be the cleavage of linkages between the pectic polysaccharides and the protein rather than in degradation of pectin (Pressey and Avants, 1973, 1976).

The enzyme β-(1,4)-Glucanase (also referred as Egase) increased its activity during abscission along with the EGase genes that are expressed during this period (Hadfield and Bennett, 1998). The activity of this enzyme was suppressed by auxin and increased by ethylene (Hadfield and Bennett, 1998). It was found by Lurie et al. (2002) that activity of Egase and its transcript were barely detectable at harvest and no increase was observed during softening after harvest. In peach, the gene ppEG1 is a member of a multigene family coding for EGase (Trainottti et al., 1997). This gene is up-regulated by ethylene and its expression is high during leaf and fruit abscission and during a very late stage of fruit ripening cooperating with PG during the softening process (Trainotti et al., 1997).

Expansins (Exp) are cell wall proteins with the ability to extend cell walls that are under tensile stress, and their intervention can facilitate the action of other enzymes such as endoPG (Hayama et al., 2003; Nilo et al., 2012). These proteins can be detected in high amounts in stages I and III, but not in Stage II of peach ripening, suggesting a role in fruit softening (Hayama et al., 2001; Obenland et al., 2003). Studies have identified that the mRNA levels of PpExp1 were abundant in ripe peach fruit, but no differences between MF and SHF peaches were found for PpExp1. To elucidate the above, Hayama et al. (2003) studied two new expansin proteins (PpExp2 and PpExp3) in ripe peach fruit of different genotypes as well PpExp1. Their results indicated that all three expansins showed different accumulation patterns and were only detected in fruit tissue (not in the flower bud, stem, or leaf). In the SHF ‘Manami’ and in the MF ‘Akatsuky’ cultivars, the proteins PpExp1 and PpExp2 were detected during cold storage, but

PpExp3 was detected at high levels in ‘Akatsuky’ and barely detected in ‘Manami’. When ‘Manami’ fruits were treated with ethylene during cold storage, the firmness and the transcript abundance of mRNA PpExp3 were almost the same as ‘Akatsuky’. In a later study, Hayama et al. (2006) concluded that the rapid softening at late stages of ripening requires significant amounts of ethylene and that this ethylene-dependent pathway is at least in-part correlated with endoPG, exoPG, and PpExp3 activities. Nilo et al. (2012) also found increasing accumulations of Exp proteins in soft fruits but these differed by cultivar.

Also, it was found that Exp proteins were not detected in mealy tissue (a postharvest disorder) and that the suppression of Exp proteins begins to occur very early in the development of the disorder, prior to any detectable symptom (Obenland et al., 2003). So, it is possible that the lack of expansin protein is related to the inhibition of ethylene production observed during development of mealiness as expansin expression is ethylene-regulated (Obenland et al., 2003).

Ethylene Cycle and its Role in Ripening and Softening

Ethylene (C_2H_4) is a gaseous hormone biosynthesized from S-adenosyl-L-methionine (SAM) (Yang and Hoffman, 1984). The amino acid methionine is the first component of this cycle which is converted to SAM, and later SAM is converted to 1-amino-cyclopropane-carboxylate (ACC). This is then converted to ethylene (Yang and Hoffman, 1984). Ethylene has a simple hydrocarbon structure, which has effects on plant growth, senescence and abscission of leaves and fruits, flowering, apical dominance, and flower induction (Binder, 2008; Hartmann et al., 2011). Its internal concentration can vary over a wide range. In Arabidopsis [*Arabidopsis thaliana* (L.) Heynh.] for example, responses to ethylene between 0.2 $nL \cdot L^{-1}$ to 100 $\mu L \cdot L^{-1}$ have been reported (Binder, 2008). In peach, ethylene concentration can be less than 1.0 $\mu L \cdot L^{-1}$ during

fruit growth and development (Layne and Bassi, 2008). The regulation of ripening by ethylene includes different factors such as ethylene biosynthesis, its reception by target cells such as ethylene receptors (ETRs), signal transduction cascade involving positive and negative regulators, and regulation of the target gene expression by transcription factors such as ethylene response factors (ERFs) (Bapat et al., 2010). Two ethylene biosynthesis pathways have been identified and described. The first one (System 1) corresponds to low ethylene production in the pre-climacteric period of climacteric fruits and is present during the development and ripening of non-climacteric fruit (Bapat et al., 2010). The other pathway (System 2) refers to an auto-stimulated (usually referred as autocatalytic synthesis), massive ethylene production that is specific to climacteric fruit (Bapat et al., 2010). In climacteric fruit, increases in ethylene production during fruit ripening correlate with a burst of respiration (Tatsuki et al., 2006).

Responses to ethylene are mediated by a family of receptors, for example in *A. thaliana* there are five receptor isoforms all of which can bind ethylene. In tomato, there are six receptor isoforms, five of which have been found to bind ethylene with high affinity (Binder, 2008). Despite the different number of receptor isoforms in different species, they share many structural features in common (Binder, 2008).

In peach, differences in fruit ethylene production have been observed in different breeding programs and studies. For example, 'Flordagold', a cultivar of the University of Florida breeding program released in the 1970s, has a remarkable firmness and a delayed softening and it was thought that its firmness was related to low ethylene production (Biggs, 1976). When the ethylene production of 'Flordagold' was measured and compared with 'Early Amber' (a medium-firm peach), 'Flordagold' produced 100-fold less ethylene than 'Early Amber' and when external ethylene was applied to both cultivars it was observed that 'Flordagold' softening

was accelerated which did not occur with ‘Early Amber’ (Biggs, 1976). Similar results were observed when the ethylene production rate was studied on selections and cultivars of SHF, MF, NMF, and very soft melting flesh (MVF) (Mignani et al., 2006). Stony hard fruit showed no or a very low production of ethylene, always less than 10 $\mu L \cdot Kg^{-1} \cdot h^{-1}$, MF fruits produced between 5-25 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ of ethylene, NMF always showed a high level of the hormone, more than 10 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ and often higher than 25 $\mu L \cdot Kg^{-1} \cdot h^{-1}$, and MVF produced the highest rate of ethylene usually more than 60 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ (Mignani et al., 2006).

According to Kao et al. (2012), ethylene in peaches measured at harvest indicated that MF cultivars needed only low levels of this hormone to initiate ripening. However, NMF flesh cultivars generally had higher peak rates of ethylene production than MF cultivars during ripening, and it is likely that the NMF trait is not related to low ethylene production (Kao et al., 2012). The same results were found by Brovelli et al. (1999), who showed that the climacteric peak of ethylene production was higher in NMF cultivars compared to MF genotypes.

It is thought that SHF fruits cannot produce ethylene due to the inability to convert SAM to ACC, by the 1-amino-cyclopropane-carboxylate synthase (ACS, EC 4. 4. 1. 14). When ACC was applied to SHF fruit, ethylene was detected in all treated fruits and it was correlated with ACC concentrations applied (Hayama et al., 2008), confirming that the limiting step in the ethylene production in SHF peaches was ACC. Also, as was expected, the degree of flesh firmness was inversely correlated with ACC concentration (Hayama et al., 2008). The findings of Tatsuki et al. (2006), who studied the ethylene production and the activity of enzymes and genes of the ethylene cycle, indicated that during ripening the expression of Pp-ACS1 was suppressed in SHF peaches, resulting in low levels of ethylene production and the inhibition of

softening. Also, they indicated that the stony hard locus could be related to the regulation of expression of Pp-ACS1 (Tatsuki et al., 2006).

The final step of ethylene synthesis is catalyzed by the enzyme 1-aminocyclopropane-1-carboxilic acid oxydase (ACO, EC 1. 14. 17. 4), which utilizes ACC as a substrate (Yang and Hoffman, 1984). Lombardo et al. (2011) reported that in the melting freestone peach cultivar Dixiland the levels of PpACO1 (an encoding peach ACO) were relatively low and constant during almost all peach fruit development. However, at ripening, a 10-fold increase was observed (Lombardo et al., 2011). The same increase was observed on fruits measured after harvest. Increased levels of ACO during the last stage of peach fruit development and ripening were also reported by Nilo et al. (2010) and Prinsi et al. (2011). Nilo et al. (2010) found that high levels of ACO were highly correlated with high levels of ethylene, and these results were confirmed two years later by Nilo et al. (2012). In the study by Nilo et al. (2012), using a proteomic approach to assess fruit softening of different peach genotypes, they observed that ethylene synthesis reached a peak that was concomitant with the higher levels of ACS and ACO transcripts. Another interesting result was that almost 90% of the change in protein accumulation between firm and soft fruit were not common to all cultivars analyzed, and that only a low number of proteins displayed qualitative changes indicating that the transition from firm to ripe fruit was due to quantitative variations in the fruit proteome.

Another hormone, auxin, can stimulate the synthesis of climacteric ethylene by inducing the expression of the enzyme ACS (Tatsuki et al., 2013; Trianoti et al., 2007). The indole-3-acetic acid (IAA, most common auxin) concentration increased just before harvest in MF peaches coinciding with System 2 of ethylene production, which did not occur in SHF peaches (Tatsuki et al., 2013). It has been found that at least nine different auxin/indoleacetic butyric acid

genes appear to increase their expression at the onset of ripening and that the increment of IAA in the mesocarp seems to have been produced by an endogenous biosynthesis of the hormone (Trianoti et al., 2007). The ACS gene is up-regulated by the action of auxin (Trianoti et al., 2007).

Destructive and Non-Destructive Texture Measurements

Texture of fresh peaches is considered to be as important as flavor and aroma for consumer preference (Sistrunk, 1985). Harvest maturity is the stage when fruits should be picked, so that high quality remains for extended storage period (Vanolli and Buccheri, 2012). Fruit firmness is an important quality characteristic in peaches and it is related to ripeness. It can be used as an indicator of postharvest treatments, storage conditions, and shelf life (Yurtlu, 2012). Texture can be a difficult parameter to evaluate with a high degree of precision. This problem is caused by personal preferences, temperature, juiciness, sweetness, and acidity (Sistrunk, 1985). A trained panel was trained to denote firmness as the force to compress between molars, elasticity as the tendency to regain the original shape after compression, and mouthfeel as a moist or dry feeling in the mouth during chewing (Sistrunk, 1985).

Firmness has been measured traditionally by a Magness-Taylor (MT) device (Sistrunk, 1985), using the maximum force required to penetrate the fruit with a rounded plunger. This device measures the resistance of fruit flesh to penetration. This test is simple, easy to use, and correlates well with human perception, but it has high variability and low repeatability (Vanolli and Buccheri, 2012; Yurtlu, 2012). Firmness of transportable nectarines must be between 22 and 43 Newton (N), ripe nectarines between 9 N and 17 N, and when nectarines are overripe firmness values are less than 5 N (Vanolli and Buccheri, 2012). In a panel evaluation, values of

consumer acceptance ranged from 31 to 52 N, whereas firmness values over 54 N resulted in unacceptable fruit quality (Miceli et al., 2010).

Penetration measurements are considered a destructive evaluation, as well as compression measurements that use a flat plate over a 30-mm of diameter and a loading deformation force; both penetration and compression measure the elastic portions of firmness (Stommel et al., 2005). Zerbini et al. (2006) studied the intra- and inter-fruit variability to predict softening, and they concluded that it is better to make firmness measurements from both fruit cheeks and record the average value.

An interesting value is the viscoelastic portion of firmness, this measurement obtained by holding the deformation force for a determined period of time and then follow the force relaxation. This type of evaluation was done in tomato to study the inheritance of elastic and viscoelastic components of firmness. The results indicated that compression was related among parental lines and their seedlings, but the viscoelastic portion was independent thus not genetically controlled (Stommel et al., 2005).

In peaches, non-destructive methods of measuring firmness have been studied (Delwiche et al., 1987; Scorza et al., 2004). Non-destructive firmness testing may be used to evaluate new germplasm for potential use in breeding programs, for genetic mapping to marker development, and other types of molecular research (Scorza et al., 2004). One of these methods is impact force (Scorza et al., 2004), which has shown to correlate to traditional (destructive) firmness measurements (Zhang et al., 1994). Also, in the study done by Zhang et al. (1994), sonic impulse measurements showed correlations with destructive force measurement in peaches. Studies done in this area showed that the suture around the fruit softens earlier than the top portion of the fruit (Delwiche et a., 1987). A later study done on ‘Redhaven’ showed that the shoulder portion

bruised at a much lower height of dropping than a suture of equivalent firmness, therefore the authors of this study concluded that to avoid bruising, firmness measurements must be based at the shoulder, not suture or cheek (Schulte et al., 1994). Another non-destructive measurement of firmness used commonly is the acoustic method, and this procedure is carried out by applying a small amount of force sufficient to deform the fruit with a metallic plunger but without causing damage. The device used for this measurement is known as Durofel (Vanolli and Biccheri, 2012).

Molecular Advances in Peach Breeding

The application of MAB in horticultural crops needs a statically robust procedure for validating QTL in germplasm relevant to breeding programs. For investigations in this area, germplasm needs to be chosen to represent important members of larger pedigree-connected genepools (Peace et al., 2014). Commonly, to validate if the alleles of QTL-linked markers initially detected in an experimental population are useful, they are examined in a set of cultivars; however, those cultivars might not be related to breeding germplasm (Peace et al., 2014). Due to the cost and time required to phenotype important traits, breeders usually conduct QTL studies on a pre-selected subset of germplasm limiting or truncating the phenotypic variation (Peace et al., 2014). To resolve this problem, a protocol was designed by Peace et al. (2014) to strategically select important breeding parents (IBPs), their numerous unselected progenies and close relatives, and all available and intermediate ancestors in apple, peach, and sweet cherry. This protocol, based on pedigree selections plus the use of pedigree-based analysis (PBA) using Bayesian approach, is a useful approach to identify and/or validate QTL in breeding germplasm (Bink et al., 2014; Peace et al., 2014).

In conventional QTL linkage mapping, independence among parental alleles is assumed, but Bayesian QTL linkage mapping offers the flexibility to study multiple full-sib families with known pedigrees simultaneously (Bink et al., 2012, 2014). This increases the probability of detecting QTL and their magnitudes across different genetic backgrounds as well increasing the improvement of mapping accuracy and power of genetic relationships among parents (Bink et al., 2012, 2014). This type of analysis alleviates the issues that usually are observed in QTL analysis such as: using a small portion of the germplasm (one biparental population), limited fraction of the total genetic variance present in the breeding program, useful alleles can be missed due to not present or they don't segregate into specific single mapping families, no or low information about the QTL mode of action (Bink et al., 2014).

Breeders and molecular scientists have reasons to study complex populations derived from multiple founders or from germplasm taken from ongoing breeding programs (Bink et al., 2008). Some of the reasons are (1) improved exploration of QTL variation (if a population arises from many founders where multiple alleles are present it will increase the probability to detect the most valuable QTL allele); (2) higher relevance of identified QTL alleles is found (experimental crosses, such as bi-parental populations, usually do not represent commercial breeding populations); (3) an improved cost-effectiveness of QTL mapping is possible (Bink et al., 2008).

Important breeding parents, mentioned before, are individuals in a breeding program designated by breeders and by pedigree examination of current families, elite or advanced selections, or if the breeder has knowledge about their value in future crosses. These IBPs are used in combination to create populations within breeding programs from which new parents or potential cultivars will arise (Peace et al., 2014). An IBP has two alternatives alleles (A and B,

for example) at a locus and the probabilities that an IBP's relatives carry a specific allele can be calculated using principles of identity by descent (IBD). Average allelic representation (AAR) is a measure of the representation of the alleles of IBPs provided by relatives in a germplasm set. It measures the probability that a given allele at a random locus of an individual is identical by descent to an allele at that locus in another individual (Peace et al., 2014). So, if one IBP is considered, each F_1 offspring of an IBP represents the IBP and the other parent by 0.5 ARR units, then AAR is reduced by half for every subsequent generation (Peace et al., 2014). According to Peace et al. (2014), 12.5 ARR units is the minimum for statistical power in representing the alleles of IBPs, which is equivalent to 25 F_1 seedlings (12.5 individuals carrying allele A and 12.5 individuals carrying allele B, numbers that are subject to laws of inheritance). Further, this procedure provides for choosing a germplasm set for detailed phenotypic and genotypic characterization to maximize the allele representation and validate QTL. This is of particular importance for perennial crops that have a long juvenile period (Peace et al., 2014).

The advantages of using a population of multiple families are increased chances of having good representation of available relevant QTL and QTL alleles, and power of QTL detection by searching multiple QTL simultaneously. And, after the larger QTL are detected, the remaining residual variance is reduced so smaller QTL can be detected (Bink et al., 2014). Lastly, the use of multiple families provides insight into the QTL contribution across different genetic backgrounds (Bink et al., 2014).

A major difference between the Bayesian approach and other genomics methods is that it considers QTL as a factor in the model while the other genomic selection methods utilize markers as explanatory variables (Bink et al., 2014). Also, in this method the number of alleles can be modeled as a random variable (Bink et al., 2014).

Molecular Markers and Application of MAB in Peach

Molecular markers can be used for important functions in a breeding program such as (1) identifying outstanding parents; (2) enhancing selection of elite alleles at loci controlling important traits; (3) pyramiding favorable alleles at multiple loci affecting either a single or several traits, and (4) cultivar fingerprinting for identification, intellectual property, and patent rights (Bliss, 2010; Testolin and Cipriani, 2010).

Molecular marker technology has rapidly evolved in recent years. The first type of molecular maker was the restriction fragment length polymorphism (RFLP) marker, but due to several issues, including the high cost of the technique and the large amount of DNA needed, they were replaced by other types of markers (Testolin and Cipriani, 2010). With the development of polymerase chain reaction (PCR) and the use of arbitrary designed primers (random amplified polymorphic DNA-RAPD), markers were easier to use and had reduced cost, resulting in more popularity among scientists (Testolin and Cipriani, 2010). Later, RAPD markers were replaced by amplified fragment length polymorphism (AFLP) markers, since they were easier to produce and attainable in larger numbers compared to RAPD markers. Microsatellites (also called SSR) markers are PCR-amplified markers, they are codominant, highly polymorphic, have conserved flanking regions that enable their amplification in entire taxa, and allow differentiation even in taxa that are distantly related (Testolin, 2004; Testolin and Cipriani, 2010). Lastly, single nucleotide polymorphism (SNP) is another class of marker that can be identified by comparing DNA sequences (Testolin and Cipriani, 2010).

Fruit crops have long juvenile periods, so some traits cannot be evaluated until their first fruiting season which can be several years after planting in the field. As pointed out before, *Prunus* species have a high level of synteny, and several genes studied in different populations of

various species have been integrated in a single map (Dirlewanger et al., 2004). Some of these genes have been used for selection of resistance to root-knot nematodes in *Prunus* rootstocks, due to markers tightly linked to a resistant gene (*Ma/ma*) from Myrobalan plum located on LG 7 (Dirlewanger et al., 2004). This gene, plus another from peach found in the peach rootstock 'Nemared' on LG 2 have been screened with markers searching for rootstocks that pyramid both resistance genes (Dirlewanger et al., 2004).

Molecular markers allow genomic localization of genes involved in the expression of horticultural traits, and the use of these markers linked to these genes can be used for marker-assisted selection (MAS) (Arús et al., 2012). Breeding programs can easily generate large populations of seedlings, but large plant numbers have high management costs and low selection efficiency. Use of MAS can allow breeders to improve selection efficiency especially in traits difficult to select early in seedling life or when the desired trait is recessive (Byrne et al., 2012). Mapping of traits that have a great impact in the peach industry (especially those affecting fruit quality and disease resistance) plus map-based cloning that is underway, is providing the opportunity to apply MAS in peach (Arús et al., 2012). A good example is internal breakdown (IB) or chilling injury that occurs in peaches, a term used to describe various disorders occurring in long-stored fruits, such as mealiness, flesh browning, loss of flavor, and bleeding or red pigmentation (Crisosto et al., 1999). Peace et al. (2005b) postulated that IB has considerable genetic control, because the expression of these traits (those involved in IB) were quite consistent within each progeny studied, and also mealiness was expressed only in melting-flesh types. Peace et al. (2005b) found a major QTL for this disorder at the Freestone-Melting flesh-endoPG locus. Moreover, two unlinked simple sequence repeat (SSR) markers were associated with mealiness. These three markers accounted for approximately 90% of the variation found

after two weeks of fruit storage, and the authors expressed this as a promising finding for the future development of MAS for IB or chilling injury (Peace et al., 2005b).

Before applying MAB in a new or ongoing breeding program, some considerations should be considered to determine if MAB is feasible and /or how to utilize this tool. It is inefficient, unwarranted, and costly to attempt to select for too many traits using MAB, so the value and contribution of the trait to cultivar performance is more important than availability of markers (Bliss, 2010). The use of molecular markers for a trait controlled by one or a few loci accounting for most of the genetic variability is straight forward and effective compared to traits where the number of loci controlling is numerous. In the last case, the value of MAS for each individual locus becomes less (Bliss, 2010). The use of robust makers for selection across different populations and breeding programs is desirable, since the use of specific markers for each population is less efficient and more costly (Bliss, 2010). The use of molecular markers intended for MAS must be reliable (i.e. producing markers/alleles that can be used confidently to distinguish desirable and undesirable alleles) (Bliss, 2010).

An example of MAB in peach is fruit acidity, which is an important quality trait for breeders and consumers and is a major selection criterion (Boudehri et al., 2009). Low fruit acidity is controlled by the D locus and is located on LG 5 of the peach genome and is co-localized with major QTL for pH, titratable acidity (TA), and other organic acid concentrations (Boudehri et al., 2009). Several markers in the vicinity of D locus were genotyped in a segregating population for acidity, and the marker CPPCT040 was tightly linked with this major locus indicating that this marker could be used for MAB (Boudehri et al., 2009).

RosBREED Project

The RosBREED project had as a major objective to incorporate MAB in five *Rosaceae* crops: apple, sweet cherry, tart cherry, peach, and strawberry. This project was funded for four years and had the objectives: (1) enhance the likelihood of new cultivar adoption, enlarge market potential, and increase consumption of *Rosaceae* fruits with socio-economic knowledge objectively used in breeding decisions, (2) establish a sustainable technical infrastructure for an efficient MAB Pipeline in *Rosaceae*, (3) integrate breeding and genomics resources with a standardized breeding information management system incorporating PBA, (4) implement MAB in the associated RosBREED breeding programs with focus on fruit quality traits, and (5) enhance sustainability of cultivar development with MAB technology transfer to current and future U.S. *Rosaceae* breeders (Iezzoni et al., 2010). This project had five components, corresponding to each objective.

- Trait and market segment breeding target establishment: economic weights of traits were determined from information on trait values obtained from breeders and key supply chain members.
- Genome scans and comparative genomics: SNP markers for genome scans were developed. These SNP markers were generated from SNP markers from existing projects and new SNPs detected in whole genome sequences using synteny and orthology information.
- Pedigree-based breeding information management system: a common breeding information management system (BIMS) aligned with the PBA approach was established for streamlined collection, archiving, analysis, and interpretation of integrated breeding and genomic data. Pedigree breeding analysis is a statistical framework to identify,

validate, and use QTL information from pedigree-linked individuals to inform breeding decisions-making.

- Marked-assisted breeding pipeline implementation: a stepwise process introduced breeders to BIMS functions and MAB capability.
- Extension: meetings and workshops were conducted to engage, educate and train *Rosaceae* breeders, scientist, producers/processors, marketing groups, and trade organizations.

In the case of peach, four universities participated in this project: Clemson University, UC Davis, the University of Arkansas, and Texas A&M University. These universities worked as a team in different aspects. They utilized the standardized phenotyping protocol for fruit quality traits in peach (Frett et al., 2012). Characteristics such as 50% bloom date, bloom type, leaf gland type, fruit set, flower type, fruit ripening date, and fruit characters including size, firmness, internal and external color, acidity, sugar content, and pH (Frett et al., 2012).

The ongoing breeding programs of these universities chose representative populations, important breeding parents, selections, and ancestors to be included in the project. They also identified genotypes that were connected within each breeding program and among these four breeding programs. Also, all four universities genotyped their pedigrees by utilizing the 9K-SNP peach array developed by the International peach SNP consortium (IPSC) (Verde et al., 2012). This array was done by re-sequencing the genome of 56 peach breeding accessions using the Illumina and Roche/454 sequencing technologies. These 56 peaches were relevant accessions assembled with the goal of achieving an efficient coverage of the genetic background of cultivated peach (Verde et al., 2012). These accessions were founders, intermediate ancestors, and important breeding parents used in international peach breeding programs (Verde et al.,

2012). The SNPs detected were validated and filtered to get a final number of 9,000 SNPs to be included in the array (Verde et al., 2012). The array was evaluated using 709 accessions containing peach cultivars and three wild related *Prunus* species or their hybrids with peach (Verde et al., 2012).

Sharing knowledge and data is of importance for the scientific community to reach certain goals and objectives. The Genome Database for *Rosaceae* (GDR) is an example of this. It is a long-standing repository of data mining and a resource for *Rosaceae* research, and in the last 10 years it has been enhanced and updated with genetic and breeding data (Sook et al., 2013). This repository was established in 2003 with the aim of integrating publicly available genetic and genomic data and to provide genome analysis tools. The new type of data found in GDR includes identified QTL and Mendelian trait loci (MTL), genes, and markers for important agricultural traits (Sook et al., 2013). About two million genetic markers used in genetic maps or genetic diversity studies, including SNPs, are available in this repository. Information and data of the whole genome assemblies of *P. persica* genome v.1.0, *Malus* x *domestica* genome v1.0. *Malus* x *domestica* genome v.1.0p, *Fragaria vesca* L. genome v1.0, and *F. vesca* v1.0p are publically available in GDR (Sook et al., 2013). Also, genetic markers anchored to the whole genome sequences include SNP markers from array development such us IRSC apple 9k, cherry 6k, UC Davis 6k, and IRSC peach 9k (Sook et al., 2013). Lastly, breeding data such as phenotypic data, genotypic data, and germplasm and pedigree data from the RosBREED project is available in GDR (Sook et al., 2013).

BLACKBERRY

Economic Importance and Use of Blackberries

In 2005, a total of 20,035 ha of blackberries were estimated to be produced in the world, but in the last 10 years blackberry production area has increased to more than 5,000 ha making a total of more than 25,000 ha worldwide (Clark and Finn, 2014; Strik et al., 2007). The production within the U.S. has shown the greatest expansion compared with the rest of the world, especially in California along with new commercial shipping plantings in Georgia, North Carolina, Arkansas, and Texas (Clark and Finn, 2014). The expansion is due to several factors, the result is increased consumption due to the increased in plantings and production.

In 2005, Europe had 7,692 ha, and Serbia was the leading country with 69% of the area in Europe (Strik et al., 2007). Serbia continues as one of the leading blackberry producers in the world with more than 5,000 ha planted (Clark and Finn, 2014).

Mexican production increased from 2,300 ha in 2005 to 6,500-8,000 ha in 2013, principally in the states of Michoacán and Jalisco (Central Mexico) with 'Tupy' the most widely planted cultivar. For Mexican production to be successful, cultural manipulations were developed to allow floricane-fruiting blackberries, such us 'Brazos' and 'Tupy' to produce fruit without a dormancy period (Clark and Finn, 2014). Most of the Mexican production is destined for export to the fresh market in the U.S. and Europe and it spans from October to June (Clark and Finn, 2014; Strik et al., 2007).

Central and South America accounted for 1,640 ha (1,590 t) and 1,597 ha (6,380 t), respectively in 2005 (Strik et al., 2007). Guatemala has increased its production in the last 10 years (Clark and Finn, 2014). Chile shifted from a blackberry producer for the fresh market industry in the 1990s and early 2000s to a producer for the processing industry more recently due

to the higher cost of shipping fruit to the U.S. and the expansion of the Mexican production (Clark and Finn, 2014). For processed fruit, the primary products are individual quick frozen (IQF), bulk frozen, canned or dried, or use in other minor products (Finn and Clark, 2012).

Currently, the blackberry industry is in expansion around the world and some reasons for this are as follows (Clark and Finn, 2014; Clark et al., 2007):

1. Blackberry is a new crop in many areas of the world.
2. Red raspberry (*Rubus ideaeus* L.) and blackberry share many similarities and where raspberry production develops blackberries often follow.
3. Blackberry plants usually do not have many diseases to control and do not have to be replanted as often as raspberries.
4. New blackberry cultivars ship better, allowing for extension of the harvest and maturity season as well as having better fruit quality.
5. Blackberry fruits have high levels of anthocyanins and antioxidants, with increasing appeal to consumers.
6. Year-round production allows to growers, packers, and processors continue profitability and to be part of the expansion of the market.

Fall-fruiting or primocane-fruiting blackberry cultivars developed by the UA breeding program have also contributed to the expansion of the market, since this type of plant has several advantages such us later-season fruit production, potential of two crops on the same plant in the same year (i.e. floricane followed by primocane), reduction in pruning costs by mowing of canes, avoidance of winter injury, and production of fruit in locations with low- or no-chilling accumulation (Clark and Finn, 2014; Clark et al., 2007). Primocane-fruiting has the potential to supply fruit in the U.S. from September to November, which is the window when almost all

summer production in the U.S. is complete and before Mexican production/imports begin (Clark and Finn, 2014). One example of the success of fall-fruiting cultivars is 'Prime-Ark® 45' which has begun to make an impact in the U.S. market for August to October production and marketing (Clark and Finn, 2014; Clark and Perkins-Veazie, 2011).

Another factor having a great impact in the expansion of the market is the introduction of new production techniques such as production under high-tunnels to avoid the damage produced by detrimental environmental conditions such as precipitation during harvest (Clark and Finn, 2014). Another environmental problem is the occurrence of extremely low winter temperatures (-20 °C or less) or spring freezes (-4 °C or less) that can damage canes or kill the plants, and also high temperatures and high solar radiation levels that cause white drupelets in blackberries causing reduction in fruit sales (Takeda et al., 2013). To reduce the negative impacts of temperature, the novel trellis referred to as the rotating cross-arm (RCA) has been utilized, and plants subjected to -18 °C produced more than 5.5 kg per plant (Takeda et al., 2013).

Blackberry yields, whether floricane- or primocane-fruiting, usually range from 8,000 to 20,000 kg· ha^{-1}. Prices paid to the grower vary with season depending of the amount of fruit available in the market (Clark and Finn, 2014).

Origin of Blackberry

Blackberries belong to *Rosaceae* family, genus *Rubus* subgenus *Rubus* (formerly *Eubatus*). Blackberries, red raspberries (R. *idaeus* L.; *Idaeobatus*), and black raspberries (R. *occidentalis* L.; *Idaeobatus*) are the most widely grown commercial *Rubus* (Finn and Clark, 2012). These species are designated as caneberries or brambles (Clark and Finn, 2011). It is estimated that between 900 to 1,000 species belong to the genus *Rubus*, distributed across the

world (Thompson, 1997). Species of *Rubus* are found on all continents with the exception of Antarctica and the highest number of species is found in Eurasia and North America (Swanson et al., 2011).

Blackberries have been used by man for centuries. Theiphrastus, the Greek writer, mentioned them in 370 B.C. (Jennings, 1988). Aeschylus and Hippocrates from 500 to 400 B.C. discussed caneberries. Also, at Newberry Crater near Bend, OR artifacts of food remnants containing *Rubus* date to 8,000 B.C. (Finn and Clark, 2012). The first image of a *Rubus* that survived antiquity is from the Juliana Anicia Codex, an illustrated manuscript based on Dioscorides work from around 512 B.C. (Finn and Clark, 2012).

Blackberry cultivation in the U.S. began in the early 1900s, using wild selections and chance discoveries. These and other wild plants of various blackberry species in the eastern and western U.S. along with some use of red raspberry species have provided breeders a tremendous and diverse germplasm pool (Clark and Finn, 2011). The primary groups of blackberry species are:

1. European Blackberries: This group of blackberries is derived from diploid and polyploid species. This species has a center of origin in Armenia and is well distributed throughout Europe and have been introduced in Asia, Oceania, and America (Clark et al., 2007; Hummer and Janick, 2007). Most species are facultative apomictics, so they can freely hybridize with distantly related species (Thompson, 1997). In this group, there was an equilibrium in succeeding generations alternating apomictic and sexual reproduction providing segregation and a rapid dispersal of a single genotype (Thompson, 1997).

2. Erect, Semi-erect Blackberries, and Trailing Dewberries: This group was domesticated from diploid and tetraploid species from eastern North America (Clark et al., 2007). Eastern North

American species have some similarities and differences with European species (Thompson, 1997). One similarity is the facultative apomict character and interspecific hybridization, so boundaries between species are difficult to determine (Thompson, 1997). A difference between these two groups is that among the eastern North American species are several diploid species compared to only a few diploids among European species (Thompson, 1997).

3. Trailing Blackberries: These generated from polyploid species from western North America (Clark et al., 2007). Predominated by *R. ursinus,* this group is present from southern California to southern British Columbia, with the greatest presence in the Oregon in the Pacific Northwest (Thompson, 1997).

Taxonomy and Morphology

Blackberry is derived from the sections *Moriferi* and *Ursini* of the subgenus *Rubus* (formerly *Eubatus*), and almost all cultivated blackberries are derived from at least two or more species. This subgenus inhabits the temperate zones of northwestern Asia, Europe, northern Africa, North America, and the mountains of South America (Clark and Finn, 2011; Clark et al., 2007; Moore and Skirvin, 1990). The ploidy levels vary from 2*n*=14 to 2*n*=84 and with the chromosome number in multiples of seven (Shoemaker and Sturrock, 1959; Swanson et al., 2011). The UA blackberry breeding program has focused since 1964 in developing erect blackberries that are tetraploid and share similar genetic background with the semi-erect blackberries (Clark and Finn, 2011).

Trailing blackberry species are crown-forming and grow at or near ground level. Trailing canes must be bundled and tied to a trellis and their production is concentrated primarily in the Pacific Northwest state of Oregon. Major trailing cultivars include ´Marion', 'Thornless

Evergreen', 'Obsidian', and more recently 'Black Diamond' (Finn and Strik, 2014; Finn et al., 2005a). Erect-type blackberries have canes that grow in a more upright direction and often sucker beneath the soil line from the crown or roots. Although the erect types can be grown without trellis support, commercial growers use a trellis with supporting wires to maintain canes in an upright orientation. The cultivars Navaho, Ouachita, Natchez, and Osage are examples of erect-cane blackberries (Clark, 2013; Clark and Moore, 2005, 2008; Moore and Clark, 1989). Lastly, semi-erect blackberries are also crown-forming and require a trellis. Mature canes of semi-erect cultivars reach 1.0 m in height before arching over to a horizontal orientation. The cultivars Chester Thornless, Triple Crown, Black Satin, Hull Thornless, Dirksen Thornless, and Loch Ness are examples of this type of blackberry (Clark, 2013; Clark and Moore, 2008; Clark et al., 2007; Strik et al., 2012).

Blackberry plants are perennial and have a biennial growth cycle which starts when a bud below the soil develops into a primocane. The first-year canes are called primocanes, and after a dormant period they are called floricanes (Clark and Finn, 2011). The floricanes flower, fruit, and die while new vegetative primocanes are growing (Finn and Clark, 2012). In the spring of the second year, after chilling-hour accumulation is completed during winter, the vegetative canes of the first year's growth become fruiting canes and axillary buds develop into fruiting laterals (Jennings, 1988). However, a new type of plant, the primocane-fruiting blackberry, which is able to fruit on first-year canes, was first developed in Arkansas. The first cultivars to be released having this particular trait were 'Prime-Jan®' and 'Prime-Jim®' in 2004, both thorny (Clark et al., 2007). The UA has continued working with this trait and released 'Prime-Ark® 45' (Clark and Perkins-Veazie, 2011), 'Prime-Ark® Freedom' (Clark, 2014), and lastly 'Prime-Ark® Traveler' (Clark, 2015) in recent years.

The blackberry flower receptacle has multiple ovaries, styles, and stigmas and it is surrounded by white or pink petals (Finn and Clark, 2012). The fruit type is an aggregate fruit (Fig. 1), because each pistil develops into a single and miniature drupe or drupelet containing a single hard pyrene, which contains the true seed (Moore and Skirvin, 1990). This aggregate fruit has a central torus or receptacle and it is surrounded by drupelets that cling to each other and to the receptacle (Clark and Finn, 2011; Moore and Skirvin, 1990). The number of drupelets per fruit can vary from 50 to over 100 with a range of set percentage from 40 to near 90% (Strik et al., 1996). The cohesion of these drupelets depends of the entanglement of epidermal hairs (unicellular, linear trichomes arising from surface cells). In blackberry, these hairs are less profuse and the drupelets less compressed compared to red raspberry. Drupelets are arranged on a fleshy and elongated receptacle and the drupelets and receptacle are removed from the plant at harvest (Crandall, 1995; Jennings, 1988).

The period of development from bloom to ripening usually varies from 40 to 60 d, depending on the cultivar, and fruit weight can vary from less than 5 g up to 20 g or more (fruits of ‘Natchez’ weighing 24 g have been observed at the field) (J. Clark, personal communication; Moore and Skirvin, 1990). The largest berries are produced by the primary flower in a cluster, and the secondary flowers of the inflorescence usually produce slightly smaller fruit (Moore and Skirvin, 1990). Blackberries follow a development pattern similar to other drupe fruits (e.g. peach, apricot),with rapid growth after pollination due to cell division, followed by slower fruit growth in which the embryo develops and the endocarp becomes hardened. The final stage of development has rapid growth due to cell enlargement due to the accumulation of water and sugars (Jennings, 1988).

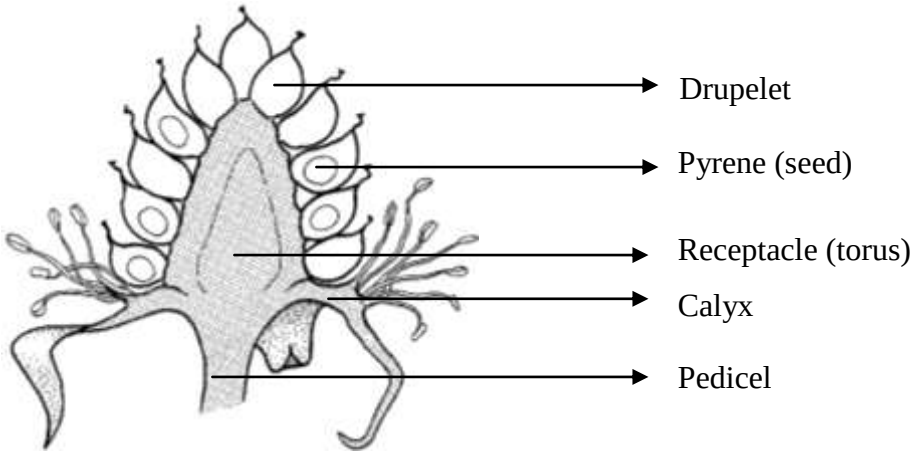

Fig.1. Scheme of a longitudinal section of a blackberry fruit (Copyright D. G. Mackean).

University of Arkansas Blackberry Breeding Program

The UA blackberry breeding program, currently led by Dr. John R. Clark, began in 1964 under the direction of Dr. James N. Moore, and since then has been developing erect blackberry cultivars originating mainly from eastern North American species. The aim is to provide high quality cultivars destined for the fresh market and shipping industry (Clark and Finn, 2011; Finn and Clark, 2012). This program has developed tetraploid cultivars with improved fruit size, high quality fruit produced by plants adapted to the mid to upper south of U.S. by merging germplasm from different sources (Finn and Clark, 2012). One good example of this combining of blackberries species is the incorporation of the thornless trait by hybridizing 'Merton Thornless' offspring with erect blackberry cultivars (Finn and Clark, 2012). The first result of this in the UA program was the thornless cultivar Navaho released in 1989 (Moore and Clark, 1989). Other thornless blackberry cultivars from this breeding program are 'Apache' (Clark and Moore, 1999), 'Arapaho' (Moore and Clark, 1993), 'Ouachita' (Clark and Moore, 2005), 'Natchez' (Clark and Moore, 2008), 'Osage' (Clark, 2013), 'Prime-Ark® Freedom' (Clark, 2014), 'Prime-Ark® Traveler' (Clark, 2015).

A notable highlight of the UA breeding program is the development of the primocane-fruiting character of blackberries. This trait was introduced in 1967 when Dr. Moore crossed "Hillquist" x 'Brazos' at the Fruit Research station located in Clarksville, AR. "Hillquist", although not a released cultivar, was a wild plant found in Ashland, VA and is known as the primocane source due to its rudimentary level of primocane fruiting (Clark and Finn, 2011). During the last two decades this trait has been improved and transferred to thorny and thornless blackberry plants bearing high-quality fruits with shipping potential. So far, three primocane-fruiting, thorny cultivars have been released from the UA breeding program including 'Prime Jim®', 'Prime-Jan®', and 'Prime-Ark® 45' (Clark et al., 2005a; Clark and Perkins-Veazie, 2011). Also, two primocane-fruiting, thornless cultivars Prime-Ark® Freedom and Prime-Ark® Traveler have been released in 2014 and 2015, respectively (Clark, 2014, 2015).

Blackberry Breeding Programs Worldwide

Located in Oregon, the USDA-ARS blackberry breeding program started in 1928 is the oldest active program (Finn and Clark, 2012). The focus of this program is to develop trailing blackberry cultivars primarily for processing. It has released 'Black Diamond' (Finn et al., 2005a), 'Onyx' (Finn et al., 2011), 'Wild Treasure' (Finn et al., 2010a), 'Newberry' (Finn et al., 2010b), 'Obsidian' (Finn et al., 2005c), 'Nightfall' (Finn et al., 2005b), 'Black Pearl' (Finn et al., 2005d), 'Marion' (Finn et al., 1997), and others. The principal source of germplasm was *R. ursinus* individuals or populations with winter hardiness along with *R. lacianiatus* Willd. and *R. armeniacus* (Finn, 2001). Also, this material was merged with seed populations obtained from the National Clonal Germplasm Repository (NCGR) located in Oregon and from H. Daubeny in British Columbia (Finn, 2001). The ploidy level of the *Rubus* germplasm utilized in this breeding

program ranged from 2x to 12x and cultivars with ploidy levels of 6x, 7x, 8x+2, and 9x have been released (Finn, 2001).

North Carolina State University has a public caneberry breeding program which released raspberry and blackberry cultivars adapted to North Carolina conditions. This program is led by Dr. Gina Fernandez and has released the cultivar Von (Fernandez et al., 2013). 'Von' was obtained by crossing 'Navaho' x NC 194 (derived from the Arkansas selection A-593) (Fernandez et al., 2013).

Driscoll's Strawberry Associates in California has conducted a private breeding program since 1991 (Finn and Clark, 2012). This is one of the largest fresh-market breeding programs in the world, and develops proprietary cultivars. This effort has utilized primarily eastern-US germplasm.

Many programs were important in the past, but have been discontinued including the USDA-ARS, Beltsville, MD and Plant and Food Research Institute in New Zealand (Swanson et al., 2011).

Fruit quality improvement is always a major goal across on breeding programs and breeders invest considerable time improving traits related to quality, as well other traits such as yield, plant health, etc (Clark and Finn, 2008, 2011). This is true whether the effort is for the fresh or processing markets. As blackberry production has diversified geographically, more interest in adaptation to specific climates, such as low chill, has become or greater interest (Clark and Finn, 2011).

Blackberry Postharvest and its Importance for the Fresh-Market Industry

Fresh-market and shipping industries base their success primarily on the quality of blackberry fruits after a period of cold storage. The main goal here is that the fruit must maintain its high quality characteristics during the entire commercialization process. The quality of fruits for the fresh-market is determined by how the genotype responds to storage and handling from the day of harvest until purchased and eaten by the consumer (Finn and Clark, 2012). Blackberries have been considered one of the most difficult fruits to ship due to softening and leakage during postharvest (Clark, 2005). Increased firmness that has been maintained during storage has been achieved in new blackberry cultivars, but advances have been challenging in breeding, and firmness is considered an intractable trait, indicating difficulty in improvement (Clark, 2005). Perkins-Veazie et al. (1996), studied important postharvest quality characteristics, such as weight loss, decay, leakiness, firmness, reddening, ethylene production, pH, SSC, and TA of 'Cheyenne', 'Choctaw', 'Navaho', and 'Shawnee' after 7 d of cold storage at 2 °C and at different ripeness stages (mottled, shiny black, and dull black). Important results were that weight loss (which is an indirect indicator of dehydration of fruits) was influenced by cultivar and color stage, varying from 0.8% to 3.3%. Fruit skin firmness was affected by ripeness stage, cultivar, and storage duration, and 'Choctaw' was the softest cultivar and fruits harvested at dull-black stage expressed the softest values of fruit and skin firmness. Subjective firmness ratings were positively correlated with objective measurements. Dull-black fruits had higher SSC values than mottled fruits in all cultivars although no differences in SSC between shiny and dull-black fruits were found among cultivars except for 'Navaho', which did not have differences between these last two stages. Titratable acidity decreased 50% between mottled and shiny black fruit and

from 10% to 30% between shiny and dull-black fruit, depending on cultivar. Decay was also affected by ripeness stage and cultivar.

Storage temperature is another important factor affecting shelf-life of blackberries. Berries stored at 5 °C had almost twice the amount of decay compared to those stored at 2 °C (Perkins-Veazie et al., 1999). They suggested that when fruit is held at 5 °C the storage period is reduced by 50% compared to fruit stored at 2 °C (Perkins-Veazie et al., 1999).

An important postharvest disorder affecting blackberry fruit destined for the fresh market industry is color reversion (also called reddening) (Clark and Finn, 2011). Drupelets of blackberry showing this disorder turn red when exposed to room temperature after being in cold storage, and also can develop red color while they remain in cold storage (Clark and Finn, 2011; Finn and Clark, 2012). Retention of black color can be selected for in breeding, but it cannot be determined in the field; therefore, postharvest evaluations must be done to verify if resistance to reversion is present or not (Clark and Finn, 2011). When color reversion was measured in the study of Perkins-Veazie et al. (1996), shiny-black fruits showed higher reversion than dull-black berries, with ‘Navaho’ having the lowest values for this postharvest disorder.

Ethylene in fruits increased with ripeness and was significantly different among cultivars with ‘Navaho’ expressing the lowest values and ‘Choctaw’ the highest. Further studies done on ‘Navaho’ showed that its fruit quality is suitable for shipment, but temperature, transport, and handling conditions have a great effect on the final quality (Perkins-Veazie et al., 1997).

The UA blackberry breeding program, concerned with the importance of postharvest quality of fruits for a successful commercialization process, started in 2008 to characterize important postharvest traits on different advanced breeding material (J. Clark, personal communication). Traits such as weight loss, decay, leakiness, firmness, color reversion, and

shininess were included in a postharvest protocol, which were measured after 7 d in cold storage at 5 °C (Clark and Perkins-Veazie, 2011). Every year around 40-45 genotypes of advanced breeding material, including known cultivars, are evaluated using this protocol generating valuable information for postharvest potential (J. Clark, personal communication). As breeding continues and potential new cultivars are being improved every year, storage quality is also increasing since it has been observed that new breeding material within UA breeding program has the potential to be stored 14 d (J. Clark, personal communication).

Blackberry Texture and Fruit Firmness

One of the challenges for blackberry breeders have been to combine different traits such as high sweetness with flavor components in shiny-black and firm berries (Clark and Finn, 2011). High fruit firmness was critical to develop a commercial shipping industry for this crop (Clark and Finn, 2011). Fruit firmness is considered a quantitative trait in other fruits such as tomato (Stommel et al., 2005) and apple (Marondedze and Thomas, 2013). Inheritance values of flesh firmness and fruit softening vary with the species. For example apple heritability values of fruit softening were intermediate (h^2=0.55) (Iwanami et al., 2008). Inheritance of fruit firmness in blackberry has not been reported.

In general, modifications of the polysaccharide components of the primary cell wall and middle lamella of fruits during ripening result in a weaker structure at the end of the process due to the increase of cell separation, softening and swelling of the cell wall creating fruit softening (Brummell, 2006). Alteration in the bonding between polymers along with degradation of polysaccharide can cause an increase in cell separation and softening and swelling of the cell wall; this, combined with alterations in cell turgor, causes fruit softening and textural changes

(Brummell, 2006). In particular, fruit such as blackberries increase their pectin solubility activity during ripening (Brummell, 2006).

Fruit skin firmness of blackberries depends on cultivar, ripeness stage, and storage duration (Perkins–Veazie et al., 1996) and is a critical characteristic in postharvest evaluation. Fruits with high values of firmness are difficult to consistently achieve in progeny (Clark, 2005). Also, it was learned that if a blackberry genotype is firm in the field it does not always retain firmness during postharvest storage and that is why it is important to measure firmness during postharvest storage (Clark, 2005; Finn and Clark, 2012). Perkins-Veazie et al. (1996) found that 'Choctaw' fruit were softer compared to 'Navaho' or 'Cheyenne' at all maturity stages (mottled, shiny- and dull-black). Dull-black fruit had the lowest skin firmness and mottled fruit the highest. Skin firmness was occasionally higher after storage, which could be related to dehydration of fruits during storage (Perkins-Veazie et al., 1996). Compression and resistance to penetration in drupelet and receptacle tissues decreased as blackberries ripened, and the receptacle and drupelet tissue of dull-black fruits had only 4% and 25%, respectively, of the resistance to penetration measured at the green stage. Compression decreased more rapidly than resistance to penetration from the red to black stages of maturity (Perkins-Veazie et al., 2000).

Several years ago, it was found within the UA breeding program a floricane-fruiting, thorny plant bearing fruit with a crispy texture, the selection was coded as A-1790 (Clark, 2005). Fruits of this selection not only had this crispy texture, they also showed an increased firmness. This selection was utilized in crosses and years later another floricane-fruiting thorny plant with improved fruit size was selected (A-2218). The use of these selections in different crosses in the UA program led to transfer this trait into two floricane-fruiting thornless selections having improved fruit size, higher yield, and better plant health compared to the original A-1790 (J.

Clark, personal communication). This trait is of interest because its higher firmness has showed an increased postharvest potential after cold storage, which can be an advantage for growers and shippers.

Flesh firmness can be measured using different objective and subjective methodologies, although firmness measurements are not widely used in the industry. Breeding programs primarily apply these measurements in postharvest evaluations, because fresh-market potential of a genotype is determined by how a genotype responds to storage and handling practices (Finn and Clark, 2012).

Objective measurements provide certain advantages compared to subjective scales, such as reproducibility, reduction in sampling error, and more precise measurements using instruments. Below is a brief description of the firmness methodology that has been applied in blackberry fruits.

1. Penetration: The objective of this procedure is to measure epidermal firmness of drupelets. Typically a penetrometer is utilized with a pin adapted for drupelet epidermal penetration. Previous work used a pin of 0.3 mm diameter (Perkins-Veazie et al., 1996, 2000).

Receptacle firmness is measured by cutting each fruit longitudinally. Then, one half is utilized to measure firmness in the basal, center, and distal location of the fruit receptacle (Perkins-Veazie et al., 2000).

2. Compression: Fruit compression can be assessed by placing individual fruits, shoulder-side down, on a flat surface and firmness measured on the opposite shoulder with a texture analyzer (using a cylinder 3 cm in diameter) (Perkins-Veazie et al., 2000). Another method to measure compression is to place the fruit vertically upside down and measure the firmness of the distal part of the fruit with a texture analyzer (P. Perkins-Veazie, personal communication).

3. Subjective Firmness: The UA blackberry breeding program utilizes a subjective scale to classify fruit firmness after cold storage. Firmness is classified using a scale from 1 to 5, with 1 being firm fruit and 5 being mushy fruit. These measurements are done after 7 d of cold storage (Clark and Perkins-Veazie, 2011).

Other Traits Improved in Blackberry Breeding Programs

1. Fruit Quality: This is a major area of improvement for breeders regardless if the final product will be for processing or fresh market (Clark and Finn, 2008, 2011). Advances in fruit quality from the wild selections and the first cultivars have been substantial (Swanson et al., 2011). An example of this is flavor which is critical for both the fresh-market and processing industry. Flavor is composed of sweetness, acidity, bitterness, astringency, and aromatic components. The flavor of the section *Ursini* is desired by many consumers, and 'Marion' is a leading cultivar having this type of flavor (Clark and Finn, 2008; Clark et al., 2007). Another important aspect is enhancing the sweetness of the berries, and a SSC of 10 to12% or more is desired to provide a "sweet" eating experience; SSC levels can be further be increased up to 15% by crossing sweet parents (Clark and Finn, 2011; Clark et al., 2007). Very sweet fruits that have reduced seediness (for some consumers, noticeable "seediness" is unacceptable) and are firm enough to ship when ripe are essential for the fresh market (Clark and Finn, 2011). Acidity is another flavor component important for breeders and consumers and by reducing the acidity level, berries could be perceived as sweeter, but the risk of this is reduced acidity can result in berries with a flat flavor (Clark and Finn, 2011). Color, firmness, and ease of removal of the fruit at harvest, are other fruit quality traits important for breeders (Clark and Finn, 2011).

2. Productivity: High yields are important for the economic viability of commercial production, and depends on the cultivar, cultural management, and location (Clark and Finn, 2011). Breeding for this quantitative trait is usually a challenge, because of the complex nature of components that contribute to yield (Clark and Finn, 2011; Clark et al., 2007).

Fruit weight, number of canes, cane length, and cane diameter are components of yield (Clark and Finn, 2011). The crossing of two parents that are high yielding can produce high-yield seedlings, and then selections can be made for yield expression on fruit size, fruits per lateral, number of fruiting laterals per cane, and numbers of plants per meter of row (Clark et al., 2007).

3. Thornlessness: Blackberry plants can vary from being totally thornless to having very dense thorns on their canes (Clark and Finn, 2011). This trait has been important for many years and the number of thornless blackberry cultivars is increasing (Clark and Finn, 2008). There are several sources of thornlessness, one is the recessive tetraploid source from R. *ulmifolius* Schott. that gave rise to 'Merton Thornless'. This cultivar was used in several breeding programs in the U.S., and a number of cultivars have been released such as 'Arapaho', 'Navaho', 'Apache', 'Triple Crown', 'Ouachita', 'Pecos', 'Osage', 'Natchez', and others (Clark, 2013; Clark and Moore 2008; Clark et al., 2007). Other sources of thornlessness are the dominant gene S_f from the cultivar Austin Thornless, the non-chimeric derivatives of 'Thornless Evergreen', and the 'Lincoln Logan' thornless gene S_{fL} (Clark et al., 2007).

4. Primocane Fruiting: The main source of the primocane fruiting trait in blackberries was the wild blackberry diploid selection "Hillquist", which was found in Ashland, VA by L.G. Hillquist. Years after discovery, his wife provided plants of this genotype to the New York State Agricultural Experiment Station in 1949 (Clark and Finn, 2011). In 1967, J. N. Moore at UA

made a cross using ‘Brazos’ as a female parent and “Hillquist” as a male parent, and the tetraploid selection Ark-593 was obtained from this cross. This selection did not express the primocane-fruiting trait (Clark and Finn, 2011). Years later this selection was selfed at North Carolina State University by Dr. James Ballington, from which primocane seedlings were recovered and the breeding selection NC-194 was released (Ballington and Moore, 1995; Clark and Finn, 2011). As pointed out before, the UA breeding program has been a world leader in the development of a wide range of primocane-fruiting breeding material combined with other advanced traits such as thornlessness, sweet and firm fruit, disease resistance, high yields along with having expanded shelf-life. From the UA effort, five cultivars carrying this trait include ‘Prime-Jan®’, ‘Prime-Jim®’, ‘Prime-Ark® 45’, Prime-Ark® Freedom’, and ‘Prime-Ark® Traveler’ (Clark, 2014, 2015; Clark and Finn, 2008, 2011; Clark et al., 2005a, 2007). This trait is influenced by environmental conditions and a good example of this is that primocane fruits of ‘Prime-Jan®’ and ‘Prime-Jim®’ produced in Aurora, OR (where only mild temperatures are present during the summer) were larger than fruit produced in Clarksville, AR in 2001 and 2002 and plants also had higher yields at this location compared to Arkansas (Clark et al., 2005a). Cultivars carrying this trait have several advantages over floricane-fruiting as previously described.

5. Plant Habit: Habit of blackberry canes varies from procumbent to very erect. Most trailing blackberries are used for processing while erect and semi-erect types are usually used for fresh market. The Arkansas breeding program has focused on erect types since its beginning. Dr. Moore utilized the cultivars Darrow and Brazos as foundation parents early in the program to accomplish this objective (Clark, 2005). Breeding for this erect type of cane was challenging. One of the problems was that the thornlessness source (another trait important when the program

was initiated) was a semi-erect genotype that when used in crossing did not produce fully erect offspring. Another difficulty was the quantitative inheritance nature of this trait along with associated negative traits such as tart flavor and poor seed germination. The first erect thornless blackberry was released in 1989 and named ‘Navaho’ (Clark and Finn, 2011; Clark and More, 1993; Clark et al., 2007).

Trailing blackberries offer advantages for mechanical harvesting and production is concentrated primarily in Oregon and to a much lesser extent in California. Releases from the USDA-ARS breeding program in Corvallis include ‘Marion’, ‘Thornless Evergreen’, ‘Black Pearl’, ‘Obsidian’, and others (Clark et al., 2007).

6. Plant Disease Resistance: Blackberries are mostly free of serious disease and insect pest problems in much of their range (Clark and Finn, 2008). Within a region and depending on the type of blackberry cultivated, different diseases can be a problem. No blackberry breeding program actively screens for diseases or pests, but rather screening is done in an indirect way by not selecting genotypes that have a serious disease or by discarding genotypes that develop diseases symptoms during evaluation.

Important diseases for blackberries are cane spot (*Mycosphaerella rubi* Roark), spur blight [*Didymella applanata* (Niessl) Sacc.], cane botrytis [*Botrytis cinerea* (De Bary) Whetzel], purple blotch [*Septocyta ruborum* (Lib.) Petr.], botrytis fruit rot [*Botrytis cinerea* (De Bary) Whetzel], downy mildew (*Peronospora sparsa* Berk.), anthracnose [*Elsinoë veneta* (Burkh.) Jenkins,], botryosphaeria cane canker [*Botryosphaeria dothidea* (Moug.: Fr.) Cesati & De Notaris], orange rust [*Arthuriomyces peckianus* (Howe) Cummins & Y.Hirats], double blossom/rosette [*Cercosporella rubi* (G.Winter) Plakidas], and crown gall (*Agrobacterium tumefaciens* Smith & Townsend), etc. An example of research on disease resistance was a study

done to examine the resistance to the bacterium *Erwinia amylovora* (Burr.) Winsl. et al. that causes fire blight disease (Stewart et al., 2005).

Double blossom or rosette was considered a limiting factor for blackberry production in the southern region of the U.S. and thorny blackberries are very susceptible (Clark, 2005). When the thornless trait was transferred to the erect individuals it was noted that this disease were not expressed in the thornless cultivar 'Navaho' (Clark, 2005). This was important because this disease was the most limiting factor in southern U.S. production. Breeding for resistance was undertaken in Mississippi, and resistance was found to be quantitative and heritability of resistance was estimated to be 0.48 (Gupton and Smith, 1997).

Virus diseases are also important for blackberry, raspberry bushy dwarf virus (RBDV), tomato ringspot virus (ToRSV), tobacco ringspot virus (TRSV) are mostly identified in blackberry production regions (Finn and Clark, 2012). Recently, impatiens necrotic spot virus (INSV) and blackberry yellow-vein associated virus (BYVaV) have been reported in the southeastern US (Finn and Clark, 2012).

Important insect pests for blackberry are red-necked caneborer (*Agrilus ruficollis* F.), strawberry weevil (*Otiorhynchus ovatus* L.), brown stink bug (*Euschistus servus* Say), green stink bug (*Acrosternum hilare* Say), Japanese beetle (*Popillia japonica* Newman), and thrips (Clark et al., 2007).

Literature Cited

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

Bailey, J. and P. French. 1932. The inheritance of certain characters in the peach. Proc. Amer. Soc. Hort. Sci. 29: 127-130.

Baird, W., A. Estager, and J. Wells. 1994. Estimating nuclear DNA content in peach and related diploid species using laser flow cytometry and DNA hybridization. J. Amer. Soc. Hort. Sci. 119:1312-1316.

Ballington, J. and J. Moore. 1995. NC 194 primocane-fruiting thorny erect tetraploid blackberry germplasm. Fruit. Var. 49:101-102.

Bapat, V., P. Trivedi, A. Ghosh, V. Sane, T. Ganapathi, and P. Nath. 2010. Ripening of fleshy fruit: Molecular insight and the role of ethylene. Biotechnology Advances 28:94-107.

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

Bassi, D., I. Mignani, and M. Rizzo. 1998. Calcium and pectin influence in peach texture. Acta Hort. 465: 433-438.

Biggs, R.H. 1976. Biological basis for firmness in the 'Flordagold' peach. Proc. Fla. State Hort. Soc. 89:213-214.

Binder, B. 2008.The ethylene receptors: Complex perception for a simple gas. Plant Sci. 175:8–17.

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

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

Bink, M., M. Boer, C. ter Braak, J. Jansen, R. Voorrips, and W. van de Web. 2008. Bayesian analyses of complex traits in pedigreed plant populations. Euphytica 161:85-96.

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

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

Bradford Genetics. 2015. 2 March 2015. Main Menu. <http://www.bradfordgenetics.com/mainmenu.htm>

Brovelli, E., J. Brecht, W. Sherman, and C. Sims. 1999. Non-melting flesh trait in peaches is not related to low ethylene production rates. HortScience 34:313–315.

Brummel, D. 2006. Cell wall disassembly in ripening fruit. Functional Plant Biol. 33:103-119.

Byrne, D. 2002. Peach breeding trends: A worldwide perspective. Acta Hort. 592:49-59.

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

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

Callahan, A., R. Scorza, C. Bassett, M. Nickerson, and F. Abeles. 2004. Deletions in an endopolygalacturonase gene cluster correlate with non-melting texture in peach. Functional Plant Biol. 31:159-168.

Carvalho, T., J. Clark, and M. Thomsen. 2009. History of development and characterization of the U.S. blackberry market. Discovery, the student journal of the Dale Bumpers College of Agricultural, Food and Life Sciences 10:10-16.

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

Clark, J. 2005. Intractable traits in eastern U.S. blackberries. HortScience 40:1954-1955.

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

Clark, J. 2013. 'Osage' thornless blackberry. HortScience 48:909-912.

Clark, J. 2014. 'Prime-Ark® Freedom' Primocane-fruiting thornless blackberry. HortScience 49:1097-1101.

Clark, J. 2015. A new blackberry: 'Prime-Ark® Traveler' thornless, primocane-fruiting blackberry. Small Fruit News, the Newsletter of the Southern Region Small Fruit Consortium 15(2):16-18.

Clark, J. and C. Finn. 2008. New trends in blackberry breeding. Acta Hort. 777:41-47.

Clark, J. and C. Finn. 2011. Blackberry breeding and genetics. Fruit, Veg. and Cereal Sci. and Biotechnol 5:27-43.

Clark, J. and C. Finn. 2014. Blackberry cultivation in the world. Rev. Bras. Frutic. 36:46-57.

Clark J. and J. Moore. 1993. 'Arapaho' erect, thornless blackberry. HortScience 28:861-862.

Clark, J. and J. Moore. 1999. 'Apache' thornless blackberry. HortScience 34:1291-1293.

Clark, J. and J. Moore. 2001. 'Roygold' peach. HortScience 36:1344-1346.

Clark, J. and J. Moore. 2003. 'White River' peach. HortScience 38:1257-1259.

Clark, J. and J. Moore. 2005. 'Ouachita' thornless blackberry. HortScience 40:258-260.

Clark, J. and J. Moore. 2008. ‘Natchez’ thornless blackberry. HortScience 43:1897-1899.

Clark, J. and J. Moore. 2011. ‘White Diamond’ and ‘White Cloud’ peaches. HortScience 46:665-667.

Clark, J. and P. Perkins-Veazie. 2011. ‘APF-45’ Primocane-fruiting blackberry. HortScience 46:670-673.

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

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

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

Clark, J., E. Stafne, H. Hall, and C. Finn. 2007. Blackberry breeding and genetics. Plant Breeding Rev., Timber Press, Portland, Ore. 29:19-144.

Clark, J., J. Moore, J. Lopez-Medina, C. Finn, and P. Perkins-Veazie. 2005a. ‘Prime-Jan’ (‘APF-8’) and ‘Prime-Jim’ (‘APF-12’) primocane-fruiting blackberries. HortScience 40:852-855.

Clark, J., J. N. Moore, and P. Perkins-Veazie. 2005b. ‘White Rock’ and ‘White County’ peaches. HortScience 40:1561-1565.

Clark, J., J. N. Moore, and R. Rom. 2001. ‘Westbrook’, ‘Bradley’, and ‘Arrington’ nectarines. HortScience 36:1164-1167.

Crandall, C. 1995. Bramble production: the management and marketing of raspberries and blackberries. 1st ed. The Haworth Press, Inc. Binghamton, N.Y.

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

Crisosto C. and D. Valero. 2008. Harvesting and postharvest handling of peaches for the fresh market, p. 575-596. In: D.R. Layne and D. Bassi (eds.), The peach: botany, production, and uses. CAB Intl., Oxfordshire, UK.

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

DeJong, T. 2006. Physiological and developmental principles of peach tree and fruit growth related to management practices. Acta Hort. 713:161-167.

Dellapena, D., D. Alexander, and A. Bennett.1986. Molecular cloning of tomato fruit polygalacturonase: Analysis of polygalacturonase mRNA levels during ripening. Proc. Natl. Acad. Sci. 83:6420-6424.

Delwiche, M., T. McDonald, and S. Bowers. 1987. Determination of peach firmness by analysis of impact forces. Amer. Soc. Agricultural Eng. 30:249-254.

De Souza, V., D. Byrne, and J. Taylor. 1998. Heritability, genetic, and phenotypic correlations, and predicted selection response of quantitative traits in peach: II. An analysis of several fruit traits. J. Amer. Soc. Sci. 123:604-611.

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

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

Dirlewanger, E., P. Cosson, C. Renaud, R. Monet, J. Poessel, and A. Moing. 2006. New detection of QTLs controlling major fruit quality components in peach. Acta Hort. 713:65-72.
Dirlewanger, E., P. Cosson, M. Tavaus, M. Aranzana, C. Poizat, A. Zanetto, P. Arus, and F. Laigret. 2002. Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in generic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor. Appl. Genet. 105:127-138.

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

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

Fernandez, G., J. Ballington, and P. Perkins-Veazie. 2013. 'Von' thornless blackberry. HortScience 48:654-656.

Finn, C. 2001. Trailing blackberries: from clear-cuts to your table. HortScience 36:236-238.

Finn, C., and B. Strik. 2014. Blackberry cultivars for Oregon. EC 1617.

Finn, C., and J. Clark. 2012. Blackberry, p. 151-190. In: M. Badenes and D. Byrne (eds). Fruit breeding. Springer Science + Business Media, New York.

Finn, C, B. Strik, B. Yorgey, M. Qian, R. Martin, and M. Peterson. 2010a. 'Wild Treasure' thornless trailing blackberry. HortScience 45:434-436.

Finn, C, B. Strik, B. Yorgey, and R. Martin. 2011. 'Onyx' trailing blackberry. HortScience 46:657-659.

Finn, C, B. Strik, B. Yorgey, R. Martin, and M. Stahler. 2010b. 'Newberry' trailing blackberry. HortScience 45:437-440.

Finn, C., B. Strik, and F.J. Lawrence. 1997. Marion trailing blackberry. Fruit Var. J. 51: 130-132.

Finn, C., B. Yorgey, B. Strik, H. Hall, R. Martin, and M. Qian. 2005a. 'Black Diamond' thornless trailing blackberry. HortScience 40:2175-2178.

Finn, C., B. Yorgey, B. Strik, and R. Martin. 2005b. 'Nightfall' thornless trailing blackberry. HortScience 40:2182-2184.

Finn, C., B. Yorgey, B. Strik, R. Martin, and C. Kempler. 2005c. ‘Obsidian’ thornless trailing blackberry. HortScience 40:2185-2188.

Finn, C., B. Yorgey, B. Strik, R. Martin, and M. Qian. 2005d. ‘Black Pearl’ thornless trailing blackberry. HortScience 40:2179-2181.

Food and Agricultural Organization of the United Nations (FAO). 2015a. Peaches and Nectarines world production. 21 January 2015. < http://faostat3.fao.org/browse/Q/QC/E>

Food and Agricultural Organization of the United Nations (FAO). 2015b. Peaches and Nectarines world production. 21 January 2015. < http://faostat3.fao.org/browse/T/TP/E >

Fournier, D., E. Costes, and Y. Guedon. 1998. A comparison of different fruiting shoots of peach tree. Acta Hort. 465:557-565.

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

Giovannoni, J., D. DellaPenna, A. Bennett, and R. Fischer. 1989. Expression of a chimeric polygalacturonase gene in transgenic *rin* (ripening inhibitor) tomato fruit results in polyuronide degradation but not fruit softening. The Plant Cell 1:53-63.

Ghiani, A., E. Onelli, R. Aina, M. Cocucci, and S. Citterio. 2011a. A comparative study of melting and non-melting flesh peach cultivars reveals that during fruit ripening endopolygalacturonase (endo-PG) is mainly involved in pericarp textural changes, not in firmness reduction. J. Experimental Bot. 62:4043–4054.

Ghiani, A., N. Negrini, S. Morgutti, F. Baldin, F. Nocito, A. Spinardi, I. Mignani, D. Bassi, and M. Cocucci. 2011b. Melting of Big Top nectarine fruit: Some physiological, biochemical, and molecular aspects. J. Amer. Soc. Hort. Sci. 136:61-68.

Glover, H., and C.J. Brady. 1995. Pectinesterases from mature, unripe peach fruit bind strongly to pectic polysaccharides. Austral. J. Plant Physiol. 22:977-985.

Goffreda, J. 1992. Stony hard gene of peach alters ethylene biosynthesis, respiration, and other ripening related characteristics. HortScience 27:610 (Abstr.).

Gupton, C. and B. Smith. 1997. Heritability of rosette resistance in blackberry. HortScience 32:940.

Hadfield, K. and A. Bennett. 1998. Polygalacturonases: Many genes in search of a function. Plant Physiol. 117:337–343.

Haji, T., H. Yaegaki, and M. Yamaguchi. 2005. Inheritance and expression of fruit texture melting, non-melting and stony hard in peach. Scientia Hort. 105:241–248.

Hartmann, H., D. Kester, F. Davies, and R. Geneve. 2011. Plant propagation. Principles and practice. (8^{th} ed.). Prentice Hall. New York.

Hayama, H., A. Ito, T. Moriguchi, and Y. Kashimura. 2003. Identification of a new expansin gene closely associated with peach fruit softening. Postharvest Biol. Technol. 29:1-10.

Hayama, H., M. Tatsuki, A. Ito, and Y. Kashimura. 2006. Ethylene and fruit softening in the stony hard mutation in peach. Postharvest Biol. Technol. 41:16-21.

Hayama, H., M. Tatsuki, H. Yoshioka, and Y. Nakamura. 2008. Regulation of stony hard peach softening with ACC treatment. Postharvest Biol. Technol. 50:231-232.

Hayama, H., T. Shimada, A. Ito, H. Yoshioka, and Y. Kashimura. 2001. Changes in the levels of mRNA for putative cell wall-related genes during peach fruit development. Scientia Hort. 91:239-250.

Hummer, K. and J. Janick. 2007. *Rubus* iconography: antiquity to the renaissance. Acta Hort. 759:89-105.

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

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

Iwanami, H., S. Moriya, N. Kotoda, S. Takahashi, and K. Abe. 2008. Estimations of heritability and breeding value for postharvest fruit softening in apple. J. Amer. Soc. Hort. Sci. 133:92-99.

Jennings, D. 1988. Raspberries and blackberries: their breeding, diseases, and growth. 1st ed. Academic Press Inc. San Diego, CA.

Kao, M., J. Brecht, J. Williamson, and D. Huber. 2012. Ripening development and quality of melting and non-melting flesh peach cultivars. HortScience 47:879–885.

Layne D. and D. Bassi. 2008. The peach. Botany, production and uses. CABI. Cambridge, MA.

Lester, D., J. Speirs, C. Orr, and C. Brady. 1994. Peach (*Prunus persica)* endopolygalacturonase cDNA isolation and mRNA analysis in melting and non-melting peach cultivars. Plant Physiol. 105:225-231.

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

Liverani, A., D. Giovannini, and F. Brandi. 2002. Increasing fruit quality of peaches and nectarines: the main goals of ISF-FO (Italy). Acta Hort. 592:507-518.

Lombardo, V., S. Osorio, J. Borsani, M. Lauxmann, C. Bustamante, C. Budde, C. Andreo, M. Lara, A. Fernie, and M. Drincovich. 2011. Metabolic profiling during peach fruit development and ripening reveals the metabolic networks that underpin each developmental stage. Plant Physiol. 157:1696–1710.

Lurie, S., A. Lers, H.W. Zhou, and L. Dong. 2002. The role of ethylene in nectarine ripening following storage. Acta Hort. 592:607-613.

Marondedze, C. and L. Thomas. 2013. Genes and quality trait loci (QTLs) associated with firmness in *Malus x domestica*. African J. Biotechnol. 12:996-1003.

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

Miceli C., R. Infante, and P. Inglese. 2010. Instrumental and sensory evaluation of eating quality of peaches and nectarines. Europ. J. Hort. Sci. 75:97–102.

Mignani, I., C. Ortugno, and D. Bassi. 2006. Biochemical parameters for evaluation of different peach flesh types. Acta Hort. 713:441-448.

Mnejja, M., J. Garcias-Mas, J. Audergon, and P. Arus. 2010. *Prunus* microsatellite marker transferability across rosaceous crops. Tree Genet. & Genomes 6:689-700.

Monet, R. and D. Bassi. 2008. Classical genetics and breeding, p. 61-84. In: D.R. Layne and D. Bassi (eds.), The peach: botany, production, and uses. CAB Intl, Oxfordshire, UK.

Moore, J. N. and J. R. Clark. 1989. 'Navaho' thornless blackberry. HortScience 24:863-865.

Moore J., and R. Skirvin. 1990. Blackberry management, p. 214-244. In: G. Galletta and D. Himelrick (eds). Small fruit crop management. Prentice-Hall, Inc, Englewood Cliffs, N.J.

Németh, S. and L. Szalay. 2012. Quantitative parameters of peach and apricot flower bud development. Acta Hort. 962:253-259.

Nilo, R., C. Saffie, K. Lilley, R. Baeza-Yates, V. Cambiazo, R. Campos-Vargas, M. González, L. Meisel, J. Retamales, A. Orellana, and H. Silva. 2010. Proteomic analysis of peach fruit mesocarp softening and chilling injury using difference gel electrophoresis (DIGE). BMC Genomics 11:43.

Nilo, R., P. Campos-Vargas, and A. Orellana. 2012. Assessment of *Prunus persica* fruit softening using a proteomics approach. J. Proteomics 75:1618–1638.

Obenland, D.M., C.H. Crisosto, and J.K.C. Rose. 2003. Expansin protein levels decline with the development of mealiness in peaches. Postharvest Biol. Technol. 29:11-18.

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

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

Orr, G., and C. Brady. 1993. Relationship of endopolygalacturonase activity to fruit softening in a freestone peach. Postharvest Biol. Technol. 3:121-130.

Ortiz, A., G. Echeverría, J. Graell, and I. Lara. 2012. Cell wall disassembly during on-tree maturation, ripening and senescence of 'Snow Queen' nectarines. Acta Hort. 962:523-530.

Peace, C. and J. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae, p. 19-53. In K. Folta and S. Gardiner (eds). Genetics and genomics of Rosaceae. Springer Science + Business Media, New York.

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

Peace, C., C. Crisosto, and T. Gradziel. 2005a. Endopolygalacturonase: a candidate gene for freestone and melting flesh in peach. Mol. Breeding 16:21–31.

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

Peace, C., R. Ahmad, T. Gradziel, A. Dandekar, and C. Crisosto. 2005b. The use of molecular genetics to improve peach and nectarine post-storage quality. Acta Hort. 682:403-409.

Perez, A. and K. Plattner. 2012. Fruit and tree nuts outlook. A report from the economic research service. FTS-352. Economic research service, U.S. Dept. Agr.

Perez, S., M. Fernandez, R. Parra, A. Rumayur, C. Mondragon, and R. Roa.2012. Peach Genetic Resources and Breeding Strategies in Mexico. Acta Hort. 962:69-73.

Perkins-Veazie, P., J. Clark, D. Huber, and E. Baldwin. 2000. Ripening physiology in 'Navaho' thornless blackberries: Color, respiration, ethylene production, softening, and compositional changes. J. Amer. Soc. Hort. Sci. 125:357–363.

Perkins-Veazie, P., J. Collins, and J. Clark. 1993. Fruit characteristics of some erect blackberry cultivars. HortScience 28:853.

Perkins-Veazie, P., J. Collins, and J. Clark. 1996. Cultivar and maturity affect postharvest quality of fruit from erect blackberries. HortScience 31: 258-261.

Perkins-Veazie, P., J. Collins, J. Clark, and L. Risse. 1997. Air shipment of 'Navaho' blackberry fruit to Europe is feasible. HortScience 32:132.

Perkins-Veazie, P., J. Collins, and J. Clark. 1999. Cultivar and storage temperature effects on the shelf life of blackberry fruit. Fruit Var. J. 53:201-208.

Pressey, R. and J.Avants.1973. Separation and characterization of endopolygalacturonase and exopolygalacturonase from peaches. Plant Physiol. 52:252-256.

Pressey, R., and J. Avants. 1976. Pear polygalacturonases. Phytochem. 15:1349–1351.

Prinsi, B., A. Simone, C. Fedeli, S. Morgutti, N. Negrini, M. Cocucci, and L. Espen. 2011. Peach fruit ripening: A proteomic comparative analysis of the mesocarp of two cultivars with different flesh firmness at two ripening stages. Phytochemistry 72:1251–1262.

Rajapakse, S., L. Belthoff, G. He, A. Estager, R. Scorza, I. Verde, R. Ballard, W. Baird, A. Callahan, R. Monet, and A. Gabott. 1995. Genetic linkage mapping in peach using morphological, RFLP and RAPD markers. Theor. Appl. Genet. 90:503-510.

Ramming, D. 1991. Genetic control of a slow-ripening fruit trait in nectarine. Canadian J. Plant Sci. 71:601-603.

Romeu, J., A. Monforte, G. Sanchez, A. Granell, J. Garcia-Brunton, M. Bedenes, and G. Rios. 2014. Quantitative trait loci affecting reproductive phenology in peach. BMC Plant Biology 14:52-67.

RosBREED. 2015. Ppe-Texture: DNA Tests for Peach Texture. 21 October 2015. < https://www.rosbreed.org/breeding/dna-tests/peach/texture>

Rubio-Cabetas, M. 2012. Present and future trends in peach rootstock breeding worldwide. Acta Hort. 962:81-89.

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

Sandefur, P., J. Clark, and C. Peace. 2013. Peach texture, p. 241-302. In: J. Janick (ed.). Horticultural reviews. John Wiley & Sons, Inc., Hoboken, New Jersey.

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

Schulte, N., E. Timm, and G. Brown. 1994. 'Redhaven' peach impact damage thresholds. HortScience 29:1052–1055.

Scorza, R., W. Anger, D. Peterson, K. Bett, E. Champagne, J. Beaulieu, and D.A. Ingram. 2004. Non-destructive evaluation of post-harvest peach fruit softening. Acta Hort. 663:269-273.

Sherman, W., B. Topp, and P. Lyrene. 1990. Non-melting flesh for fresh market peaches. Proc. Fla. State Hort. Soc. 103:293-294.

Shoemaker, J. and T. Sturrock. 1959. Chromosome relations in blackberries. Proc. Florida State Hort. Soc. 72:327-330.

Sistrunk, W. 1985. Peach quality assessment: fresh and processed, p. 1-46. In: H.E. Patted (ed.), Evaluation of quality of fruits and vegetables. AVI, Westport, CN.

Sook, J., S. Ficklin, T. Lee, C. Cheng, P. Zheng, J. Yu, A. Bombarely, I. Cho, A. Blenda, S. Ru, C. Peace, A. Abbott, L. Mueller, M. Olmstead, D. Main, and K. Evans. 2013. The genome database for Rosaceae (GDR): year 10 update. Nucl. Acids Res. doi: 10.1093/nar/gkt1012.

Stewart, P., J. Clark, and P. Fenn. 2005. Sources and inheritance of resistance to fire blight (*Erwinia amylovora*) in eastern U.S. blackberry genotypes. HortScience 40:39-42.

Stommel, J., J. Abbot, T. Campbell, and D. Francis. 2005. Inheritance of elastic and viscoelastic components of tomato firmness derived from intra- and interspecific genetic backgrounds. J. Amer. Soc. Hort. Sci. 130:598-604.

Strik, B., J. Clark, C. Finn, and M. Bañados. 2007. Worldwide production of blackberries. Acta Hort. 777:209-217.

Strik, B., J. Mann, and C. Finn. 1996. Percent drupelet set varies among blackberry genotypes. J. Amer. Soc. Hort. Sci. 121:371–373.

Strik, B., J. Clark, C. Finn, and G. Buller. 2012. Management of primocane-fruiting blackberry: impacts on yield, fruiting season, and cane architecture. HortScience 47:593-598.

Swanson J., J. Carlson, F. Fernandez-Fernandez, C. Finn, J. Graham, C. Weber, and D. Sargent. 2011. Blackberries and raspberries, p. 64-113. In: K. Folta and C. Kole (eds). Genetics, genomics and, breeding of berries. Science Publishers, Enfield, NH.

Takeda, F., D. Glenn, and T. Tworkoski. 2013. Rotating cross-arm trellis technology for blackberry production. J. Berry Res. 3:25-40.

Tataranni, G., A. Spada, C. Pozzi, and D. Bassi. 2010. AFLP-based bulk segregant analysis for tagging the slow-ripening trait in peach [*Prunus persica* (L.) Batsch]. J. Hort. Sci. & Biotechnol. 85:78–82.

Tatsuki, M., N. Nakajima, H. Fujii, T.Shimada, M. Nakano, K. Hayashi, H. Hayama, H. Yoshioka, and Y. Nakamura. 2013. Increased levels of IAA are required for system 2 ethylene synthesis causing fruit softening in peach (*Prunus persica* L. Batsch). J. Experimental Bot. 64:1049–1059.

Tatsuki, M., T. Haji, and M. Yamaguchi. 2006. The involvement of 1-aminocyclopropane-1-carboxylic acid synthase isogene, Pp-ACS1, in peach fruit softening. J. Expt. Bot. 57:1281-1289.

Testolin, R. 2004. Marker-assisted selection in stone fruits. Acta Hort. 622:163-176.

Testolin, R. and G. Cipriani. 2010. Molecular markers for germplasm identification and characterization. Acta Hort. 859:59-72.

The International Peach Genome Initiative. 2013. The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genet. 45:487-497.

Thompson, M. 1997. Survey of chromosome number in *Rubus* (Rosaceae: Rosoideae). Ann. Missouri Botanical Garden 84:128-164.

Tonnuti P., P Casson, and A. Ramina. 1991. Ethylene biosynthesis during peach fruit development. J. Amer. Soc. Hort. Sci. 116:274-279.

Topp, B., G. Bignel, P. Wilk, and D. Russel. 2012. Breeding low-chill peaches in subtropical Queensland. Acta Hort. 962:109-115.

Trainotti T., A. Tadiello, and G. Casadoro. 2007. The involvement of auxin in the ripening of climacteric fruits comes of age: the hormone plays a role of its own and has an intense interplay with ethylene in ripening peaches. J. Exp. Bot. 58:3299–3308.

Trainotti, L., S. Spolaore, L. Ferrarese, G. Casadoro. 1997. Characterization of ppEG1, a member of a multigene family which encodes endo-ß-1,4-glucanase in peach. Plant Mol. Biol. 34:791-802.

United States Department of Agriculture. 2012. Arkansas peach production report. 7 July 2015. < http://www.nass.usda.gov/Statistics_by_State/Arkansas/Publications/Crop_Releases/Fruit_Nut_Vegetable/2012/peachprod12.pdf>

United States Department of Agriculture. 2014. State agriculture overview. Arkansas. 2 June 2015. <http://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=ARKANSAS>

Vanolli, M. and M. Buccheri. 2012. Overview of the methods for assessing harvest maturity. Stewart Postharvest Rev.1(4): 1-11.

Verde, I., N. Bassil, S. Scalabrin, B. Gilmore, C. Lawley, K. Gasic, D. Micheleti, U. Rosyara, F. Cattonaro, E. Vendramin, D. Main, V. Aramini, A. Blas, T. Mockler, D. Bryant, L. Wilhelm, M. Troggio, B. Sosinski, M. Aranzana, P. Arus, A. Iezzoni, M. Morgante, and C. Peace. 2012. Development and Evaluation of a 9K SNP Array for Peach by Internationally Coordinated SNP Detection and Validation in Breeding Germplasm. PLoS ONE 7:1-13.

Wu, Q., M. Szakács-Dobozi, M. Hemmat, and C. Hrazdina. 1993. Endopolygalacturonase in apples *(Malus domestica)* and its expression during fruit ripening. Plant Physiol.102:219-225.

Yang, S. and N. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. 35:155-189.

Yue, C., R. Gallardo, J. Luby, A. Rihn, J. McFerson, V. McCracken, T. Gradziel, K. Gasic, G. Reighard, J. Clark, and A. Iezzoni. 2014. An evaluation of U.S peach producers' trait prioritization: evidence from audience surveys. HortScience 49:1309-1314.

Yurtlu, Y. 2012. Comparison of nondestructive impact and acoustic techniques for measuring firmness in peaches. J. Food and Agr. Environ. 10:180-185.

Zerbini, P., M. Vanoli, M. Grassi, A. Rizzolo, R. Cubeddu, A.Pifferi, L. Spinelli, and A. Torricelli. 2006. Effect of inter and intra-variability in predicting softening of nectarines. J. Fruit and Ornamental Plant Res. 14:161-166.

Zhang, X., M.L. Stone, D. Chen, N.O. Maness, and G.H. Brusewitz. 1994. Peach firmness determination by puncture resistance, drop impact, and sonic impulse. Trans. Am. Soc. Agr. Biol. Eng. 37:495-500.

Zhebentyayeva, T., G. Swire-Clark, L. Georgi, L. Garay, S. Jung, S. Forrest, A. Blenda, B. Blackmon, J. Mook, R. Horn, W. Howad, P. Arús, D. Main, J. Tomkins, B. Sosinski, W. Baird, G. Reighard, and A. Abbott. 2008. A framework physical map for peach, a model *Rosaceae* species. Tree Genetics & Genomes 4:745–756.

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

Chapter 1

QUANTITATIVE TRAIT LOCI ANALYSIS OF PEACH TO ENABLE MARKER-ASSISTED BREEDING

Abstract

Acidity is one of the most important components of flavor of peach [*Prunus persica* L. (Batsch)] and can determine if the fruit of a certain cultivar will be acceptable to consumers. At the same time, fruit firmness is an important trait for growers and shippers who sell and ship their fruit to distant markets. Fruit having the desirable balance between acidity and sweetness along with the ability to maintain firmness until reaching the grocery store or consumer's house will be successful in the fresh-market industry. Selecting for these traits using traditional breeding methods could take several years, due to the long juvenile period of peach trees and also due the complexity of the genetic control of the traits. To improve breeding efficiency, DNA tests linked with quantitative trait loci (QTL) of the traits of interest should be incorporated as marker–assisted breeding (MAB). Titratable acidity (TA) and flesh firmness were analyzed following a pedigree based analysis (PBA) using a Bayesian approach with the objective to confirm and find new QTLs associated with both traits in the Arkansas breeding program for the first time. Quantitative trait loci on the proximal end of linkage group (LG) 5 were statistically related with TA and were at the same chromosomal location of a major locus for low-acid fruit (D-locus). In the case of fruit firmness, a QTL was located on the distal end of LG 4, where the gene determining three types of peach flesh texture is located (endoPG gene). Also, a QTL downstream to the previous QTL was also located and related to fruit firmness. However, these

last QTLs on LG 4 showed low posterior probabilities, indicating low probability for association with this choromosmal region.

Introduction

Improving fruit quality is one of the most important objectives within a fruit breeding program, and peach breeders focus their work in developing new cultivars with increased sweetness and other flavor enhancements, attractive color, higher flesh firmness, larger fruits, new shapes, and other characteristics (Byrne et al., 2012). The peach has been a model crop within the *Prunus* genus and now the first draft of the peach genome sequence is available (Arus et al., 2012).

National projects are working with genetic and phenotypic data to construct molecular markers that will be available to breeders in the future. One of them was the RosBREED project (Iezzoni et al., 2010) which had as an objective to apply MAB in five *Rosaceae* crops, apple (*Malus* x *domestic*a Borkh.), sweet cherry [*Prunus avium* (L.) L.], tart cherry (*Prunus cerasus* L.), strawberry (*Fragaria* x *ananassa* Duchesne), and peach, with primary focus on fruit quality traits important for breeders, growers, shippers, and consumers (Iezzoni et al., 2010).

The application of MAB in horticultural and fruit crops needs a statically robust procedure for validating QTLs in germplasm relevant to breeding programs (Peace et al., 2014). The protocol designed by Peace et al. (2014) proposes a step-by-step procedure to select the most efficient and informative germplasm to pursue a QTL analysis in ongoing breeding programs. In conventional QTL linkage mapping, independence among parental alleles is assumed, but Bayesian QTL linkage mapping offers the flexibility to study multiple full-sib families with known pedigrees simultaneously. These results in an increased probability of detecting QTLs

and their magnitude across different genetic backgrounds as well increasing the improvement of mapping accuracy and power (Bink et al., 2012; 2014). This type of analysis alleviates the issues that usually are observed in QTL analysis such as a small portion of the germplasm (one bi-parental population), limited fraction of the total genetic variance present in the breeding program, useful alleles can be missed due to not present or they don't segregate into specific single mapping families, and no or little information about the QTL mode of action (Bink et al., 2014).

Fruit acidity and flesh firmness are important quality traits that affect acceptance of peach by consumers and are major selection criterion for breeders (Boudehri et al., 2009; Crisosto, 2002). Also, firmness is an important attribute for shippers and growers that want to ship fruit to distant markets, so they need fruit that maintains high quality for more than 14 d (Infante et al., 2006). There are several reports of bi-parental studies, including a report of a QTL associating low fruit acidity with a major locus, named D-locus, located on the proximal end of LG 5 (Bliss et al., 2002; Boudehri et al., 2009; Dirlewanger et al., 1999, 2006; Etienne et al., 2002). In the study of Dirlewanger et al. (1999), they found two other QTLs (in addition to the one on LG 5) on LG 1 and 6. This major locus was co-localized with major QTLs for pH, TA, and several organic acid concentrations (Boudehri et al., 2009). Several markers in the vicinity of D-locus were genotyped in a segregating population for acidity, and the marker CPPCT040 was tightly linked with this major locus indicating that this marker could be used for MAB (Boudehri et al., 2009).

Endopolygalacturonase (endoPG) is associated with three flesh textures of peaches which are melting flesh (MF), non-melting flesh (NMF), and non-softening flesh (NSF) (Peace et al., 2005a). The candidate gene for this enzyme is located in the Freestone-melting (F-M) locus on

the distal end of LG 4 (Peace et al., 2005a, 2005b, 2007). This gene has two copies separated by less than 50,000 bp, one copy controls the melting trait and the second copy controls the adhesion to the pit character (freestone trait) (Peace and Norelli, 2009). Also, another study on peach fruit texture identified 26 candidate genes which spanned most of the linkage groups (Illa et al., 2011). These genes were associated with expansin proteins, glucanase, pectin methylesterases, and other enzymes and proteins (Illa et al., 2011).

After a QTL is detected, the next step is to design DNA tests that will validate the QTL within a breeding program. The use of DNA tests linked to these major QTLs or genes can be used for MAB (Arús et al., 2012). In this context they can be used for important functions in a breeding program such as (1) identifying outstanding parents; (2) enhancing selection of elite alleles at loci controlling important traits; (3) pyramiding favorable alleles at multiple loci affecting either a single trait or several traits and (4) cultivar fingerprinting for intellectual property and patent rights (Bliss, 2010; Testolin and Cipriani, 2010). Before applying MAB in a new or ongoing breeding program, some considerations should be taken into account in reference to how to utilize this tool. It is inefficient, unwarranted, and costly to attempt to select for too many traits using MAB, so the value and contribution of the trait to cultivar performance is more important than availability of markers (Bliss, 2010).

The University of Arkansas (UA) peach breeding program began in 1964 and has released several cultivars (Clark, 2011). This is a traditional program, so no molecular DNA methods have been used to select parents and advanced breeding material. The UA breeding program was part of the RosBREED project and phenotyped and genotyped populations, parents, selections, cultivars, and ancestors that were related to each other within the breeding program (and related at the same time with other three ongoing peach breeding programs, University of

California, Davis, Texas A&M University and Clemson University). This project followed the PBA approach to confirm previously found QTLs and find new QTLs for different quantitative traits related to fruit quality with the aim to incorporate molecular tools and technologies as a routine activity in the program.

The objective of this study was to apply for the first time in the Arkansas peach breeding program the PBA methodology to discover and confirm QTLs related to important fruit quality traits, such as fruit titratable acidity, pH, and flesh firmness.

Material and Methods

Plant Material

All phenotypic work was conducted at the University of Arkansas Fruit Research Station, Clarksville [west-central Arkansas (west-central Arkansas, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]. In all testing, trees were either open-center trained and spaced 5.5 m between trees and rows, or trained to a perpendicular-V system with trees spaced 1.9 m in rows spaced 5.5 m apart. All trees were dormant pruned and fertilized annually with a single application of 640 Kg $\cdot ha^{-1}$ of complete fertilizer (19:19:19 of N:P:K) and were sprinkler or drip irrigated as needed. Pests were managed using a program typical for commercial orchards in the area (Smith, 2015; Studebaker et al., 2015). Fruits were thinned to a distance of 12 to 15 cm between fruit after shuck split, but before pit hardening.

Pedigree identification

The germplasm used in this study was part of the RosBREED project and it was chosen to effectively represent alleles currently found within the Arkansas breeding program (Peace et al., 2014). Ancestors, important breeding parents, cultivars, selections, and populations were identified and integrated in a comprehensive pedigree. A pedigree chart was constructed using PediMap 1.2 (Fig. 1) (Voorrips et al., 2012).

Analyzed cultivars were 'Amoore Sweet', 'Arrington', 'Bradley', 'Souevenirs', 'White County', and 'Winblo', as well Arkansas selections A-665, A-672, A-699, A-708, A-716, A-760, A-772, A-773 A-776, A-778, A-783, and A-789. Seedlings from 7-F_1 segregating populations that were interrelated and related with common ancestors were utilized: 49 seedlings from population ArPop_1 ('White County' x A-672), 16 seedlings from ArPop_0801 (A-776 x A-783), 15 seedlings from ArPop_0803 ('Amoore Sweet' x A-778), 12 seedlings from ArPop_0813 (A-772 x A-672), nine seedlings from ArPop_0817 (A-789 x A-699), 23 seedlings from ArPop_0819 (A-708 x A-773), and 17 seedlings from ArPop_0825 ('Souvenirs' x A-760). In 2011, harvest period of this pedigree ranged from 7 June to 25 August. In 2012, harvest period ranged from May 30 to 8 August. In 2013, harvest period ranged from 27 June to 25 August.

Phenotypic Evaluation

Phenoptypic data was taken in 2011, 2012, and 2013. For phenotyping measurements, 20-25 fruits were selected from mid-canopy of only healthy trees. According to the RosBREED phenotyping protocol for peach, for fruit sample collection the tree was checked to have a few edible fruits and then the fruit collected for measurement was early ripe, a stage called "tree-ripe" (Frett et al., 2012; Gasic et al., 2010). Only fruit exhibiting uniform shape and background color, and lacking any insect or disease damage were included in samples. All fruit were hand-

harvested directly into 0.24 L corrugated trays (FormTex Plastics Corp., Houston, TX). Also, transportation from the field to the laboratory was done carefully (avoiding sudden movements to decrease the probability that fruits hit with each other), since any damage on the fruit could have a negative effect on the final results. For the phenotyping evaluation, at least five fruit from each individual tree were selected and subjected to the evaluation procedure as follows utilizing the peach phenotyping protocol (Frett et al., 2012).

In 2011 and 2012, flesh firmness of fruits was measured using a hand-held penetrometer with an 8-mm tip (model FT 327; Effegi, Torino, Italy). In 2013, firmness was measured using automated texturometer with an 8-mm tip (iCon Texture Analyzer, Texture Technologies Corp. Hamilton, MA). Titratable acidity and pH values of fruit juice were measured in 2011, 2012, and 2013 using a Methrohm 877 Titrino Plus automatic titrator with a LL Unitrode combination pH (Matrohm AG, Herisau, Switzerland).

The following equation (Gasic et al., 2010) was used to calculate titratable acidity (the milliequivalent factor used corresponded to malic acid, 0.067):

$$\text{Titratable acidity (\%)} = \frac{[\text{NaOH titrated (ml)} * 0.1\text{ N (NaOH)} * \text{milliequivalent factor} * 100]}{6\text{ g of juice}}$$

The milliequivalent factor corresponds to the equivalent amount of material that will react with 1 g of N.

SNP Data Set

All individuals were previously genotyped using the IPSC 9K SNP Array for Peach (Verde et al., 2012) as part of the RosBREED project (Iezzoni et al., 2010). The polymorphic SNPs were filtered to eliminate those with a high proportion of inheritance errors and heterozygosity excess, resulting in 1,947 informative SNPs spread over the eight LGs [this process was completed by Dr. Cameron Peace, Washington State University (WSU)]. The

number of markers on each LG varied based on the length. For example, LG 4 was the longest with 424 SNPs, and LG 5 was the shortest with 137 SNPs. The average density of markers across the whole genome was estimated to be one SNP per 2.5 centiMorgan (cM). In addition, the MapChart software (Voorrips et al., 2002) was used to visualize the LGs. Scoring of SNP was determined using the Illumina® Genome Studio software. This was conducted by Dr. Ksenija Gasic and Dr. Peace at Clemson University, Clemson, SC and WSU, respectively.

QTL Analysis

Genotypic and phenotypic data were analyzed by the genetic software FlexQTL™ version 099128 (www.flexQTL.nl). Titratable acidity, pH, and flesh firmness data were analyzed with this software with data collected over seasons 2011, 2012, and 2013, and the across-year averages (average of 2011, 2012, and 2013 data). FlexQTL™ software estimated the number and position of QTLs given a pedigree and marker linkage map. FlexQTL™ utilizes a Bayesian approach to infer the number of QTLs by comparison of models using posterior estimates through Markov Chain Monte Carlo (MCMC) simulations. In all analyses, a 5000,000 simulation chain length was enough to store 1000 samples (QTL models) with a thinning of 500.

Locations of QTLs were identified based on the values of Bayes Factors (BFs) primarily. Bayes Factors are the evidence favoring the presence of a number of QTLs and the genetic model proposed (Bink et al., 2012, 2014). Based on this, when BF values are between zero and two the evidence of a significant QTL is considered low, when values are between two and five the evidence is positive, when BFs are between five and 10 the evidence is strong, and when the values are greater than 10 the evidence is decisive. The main criteria to determine major QTLs per trait was the exhibition of the QTL with at least positive evidence (BF>2), minimum effective chain size (ECS) of 100, and a 0.1 threshold of posterior intensity to determine the

stability of the QTL. Also, trace plots for convergence and stability of the evaluated genetic model per trait and year were performed to determine QTLs were reliable. Traceability of MCMC was calculated by FlexQTL™ and was visualized by MapChart software (Voorrips et al., 2001). Trace plots are shown in Appendix A.

Initially, a genome-wide analysis was performed, in which all the LGs were included in the analysis to discover QTL(s) associated with the traits. After genome-wide QTL analyses results were analyzed and the location of major QTL were identified, specific analyses were performed to better locate the major QTL(s) and to identify other QTL(s) located on other LG by removing the effects of the specific LG where the major QTL is located. The two types of analyses were:

1. QTL analysis utilizing all the LGs, except the LG where the major QTL is located. The reason to perform this type of analysis is because some QTL are so strong that they could "hide" the effect of other QTL(s) that explain a part of the phenotypic variation of the trait. So, by removing the effects of the major QTL, other QTLs can arise as explanatory variables.
2. QTL analysis utilizing only the LG where the major QTL is located by removing the effects of all the other LGs.

Broad sense heritability (H^2) was calculated using values of phenotypic variance(σ_P^2) and error (s_e^2) for each trait. Narrow sense heritability (h^2) was calculated using the weighted additive variance of the QTL [probability*additive variance a the QTL, (σ_A^2)]. The values of σ_P^2, σ_e^2, and σ_A^2 were obtained from the FlexQTL™ outputs and H^2 and h^2 were calculated using the following formulas:

$$H^2 = \frac{\sigma_P^2 - \sigma_e^2}{\sigma_P^2} = \frac{\sigma_G^2}{\sigma_P^2}$$

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

Estimated Breeding Values

Estimated breeding values (EBVs) were obtained utilizing FlexQTL™ outputs. Individual EBVs were obtained for each chromosome segment with at least positive evidence (BF between 2 and 5).

Statistical Analysis

Mean, maximum, minimum, and standard deviation values per year per trait of Arkansas RosBREED germplasm were obtained utilizing FlexQTL™ outputs.

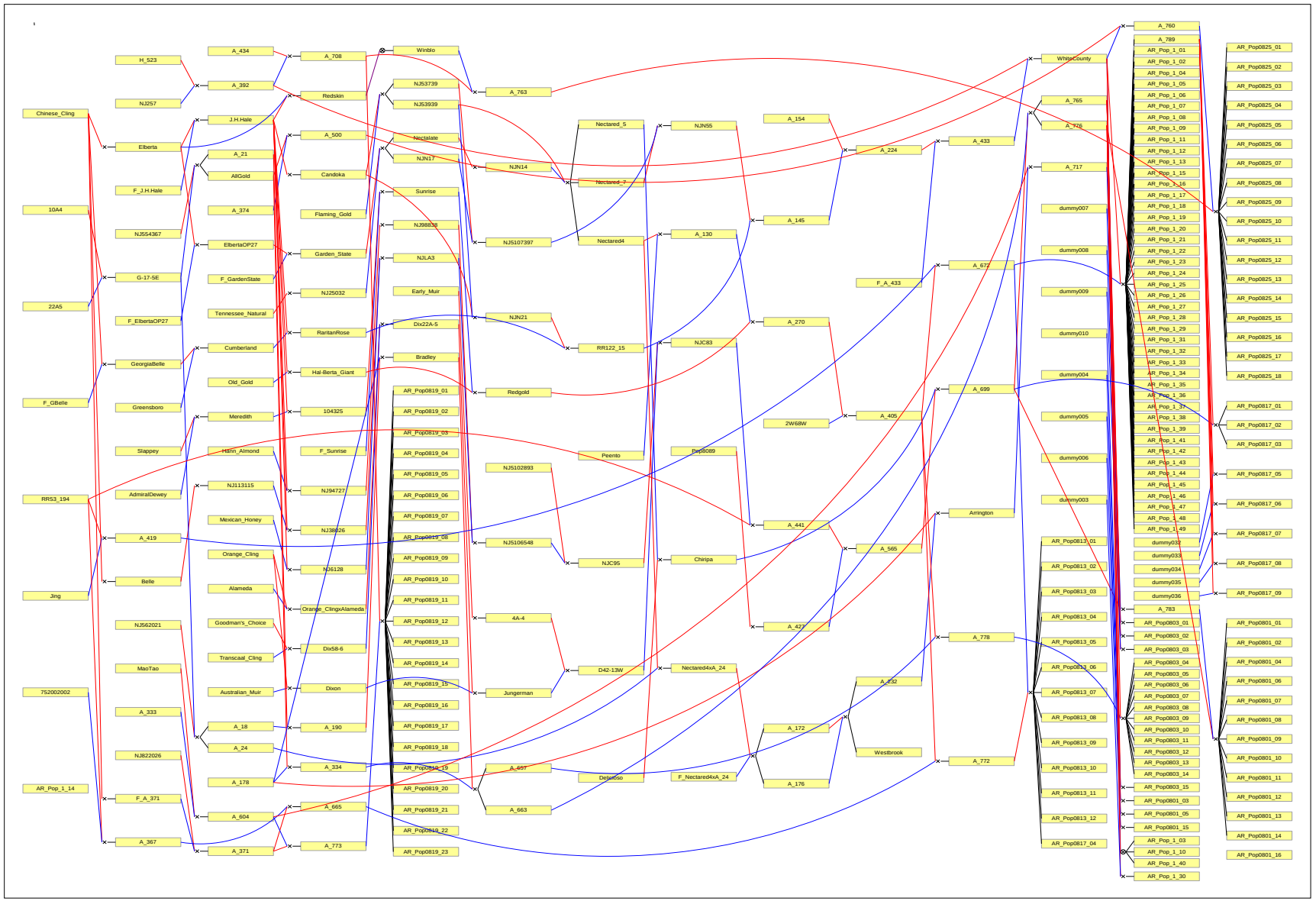

Fig. 1. Arkansas RosBREED germplasm. Maternal parents indicated with red lines and paternal parent indicated with blue lines.

Results and Discussion

Phenotypic Data Analysis

Acidity, measured as TA, averaged across all samples was 0.54% in 2011, 0.49% in 2012, and 0.65% in 2013, with an average across the years of 0.56% (Table 1). Values among all years ranged from a high of 1.40 % to a low 0.10% (Table 1). Previous studies done in segregating populations found values ranging from 0.36 to 1.10% (Boudehri et al., 2009), and my phenotypic values exceeded this range. Within the Arkansas program, low acid cultivars such as 'Souvenirs', 'White County', and 'White Rock' averaged in 2011 and 2012 a TA value of ~0.24 to 0.26% (Clark and Sandefur, 2013). The standard-acid 'Redhaven' averaged in Arkansas, during seasons 2011 and 2012, a TA value of 0.64% (Clark and Sandefur, 2013). My means indicated that samples from the genotypes sampled ranged from low to high acidity, indicating wide diversity for acidity in the parents and populations of the Arkansas RosBREED germplasm (Table 2).

There is a major locus, called "D-locus" that segregates for acidity, in which the low-acid character is dominant or partially dominant (Boudehri et al., 2009). This segregation can be seen in the titratable acidity histograms (Figs. 2 to 5), in that there is a higher frequency of individuals with low acidity (mostly within 0.30 to 0.50%) which is characteristic behavior of a dominant character.

Table 1. Mean, maximum value, minimum value, standard deviation, and number of observations of titratable acidity (%) for 2011, 2012, 2013, and across-year average. RosBREED Arkansas seedlings.

Year	TA (%)				Number of observations
	Mean	Max.	Min.	Std. dev.	
2011	0.54	1.3	0.2	0.30	120
2012	0.49	1.2	0.1	0.23	125
2013	0.65	1.4	0.3	0.26	112
Across-year average	0.56	1.2	0.2	0.23	137

Table 2. Titratable acidity (%) values of parental individuals of Arkansas RosBREED germplasm of years 2011, 2012, and 2013.

Progeny	Parentage		TA (%)		
			2011	2012	2013
ArPop_1	Female	White County	0.3	0.2	0.3
	Male	A-672	0.6	0.5	0.7
ArPop_0801	Female	A-776	0.5	0.4	-
	Male	A-783	1.2	0.7	0.9
ArPop_0803	Female	Amoore Sweet	0.5	0.7	-
	Male	A-778	0.9	0.5	0.9
ArPop_0813	Female	A-772	0.4	0.2	0.4
	Male	A-672	0.6	0.5	0.7
ArPop_0817	Female	A-789	0.4	0.4	0.4
	Male	A-699	0.6	0.5	0.5
ArPop_0819	Female	A-708	0.3	0.9	0.4
	Male	A-773	0.5	-	-
ArPop_0825	Female	Souvenirs	0.3	0.3	-
	Male	A-760	0.2	0.2	0.3

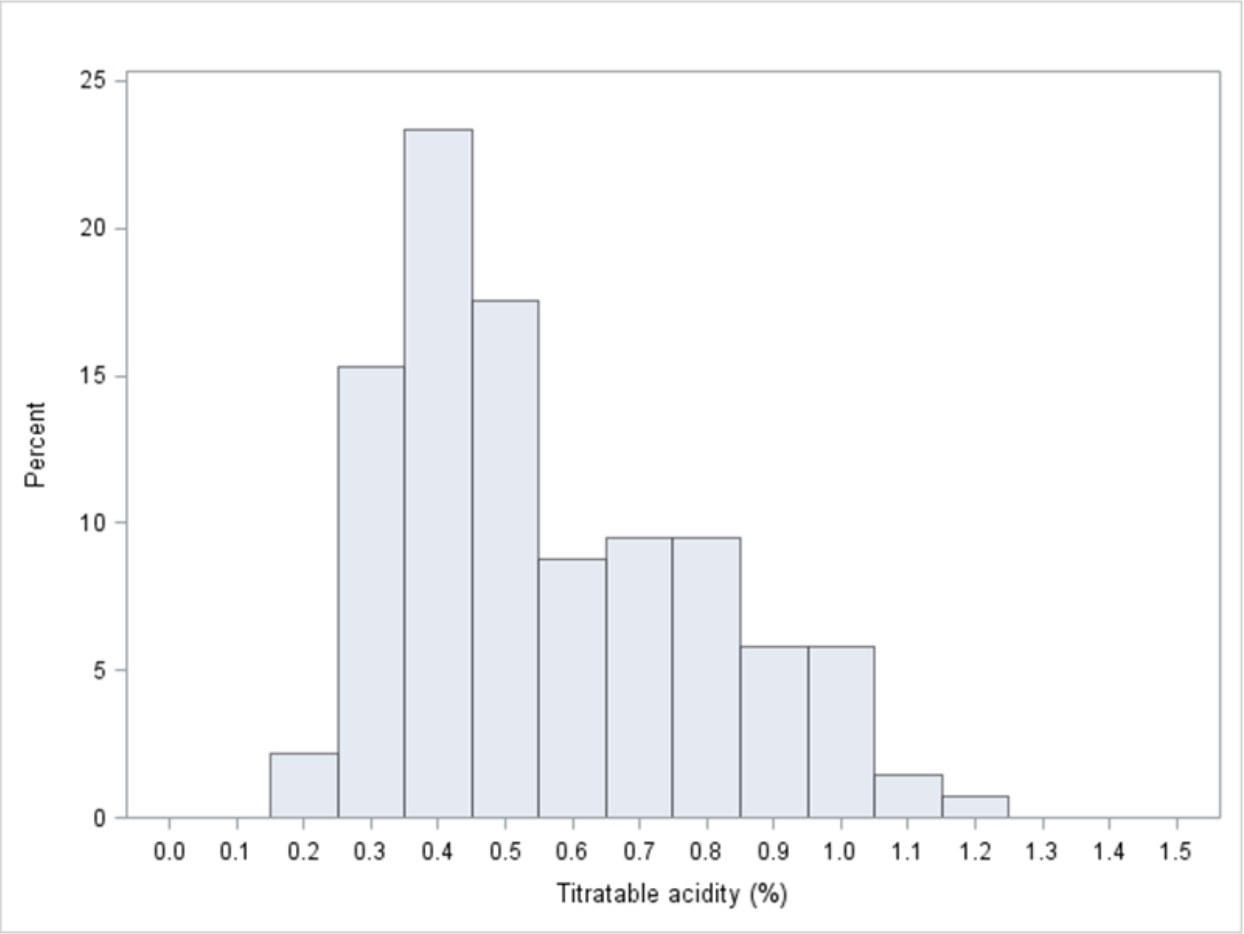

Fig. 2. Distribution (%) of the seedlings within titratable acidity (%) values, across-year average. Arkansas RosBREED seedlings. N=137.

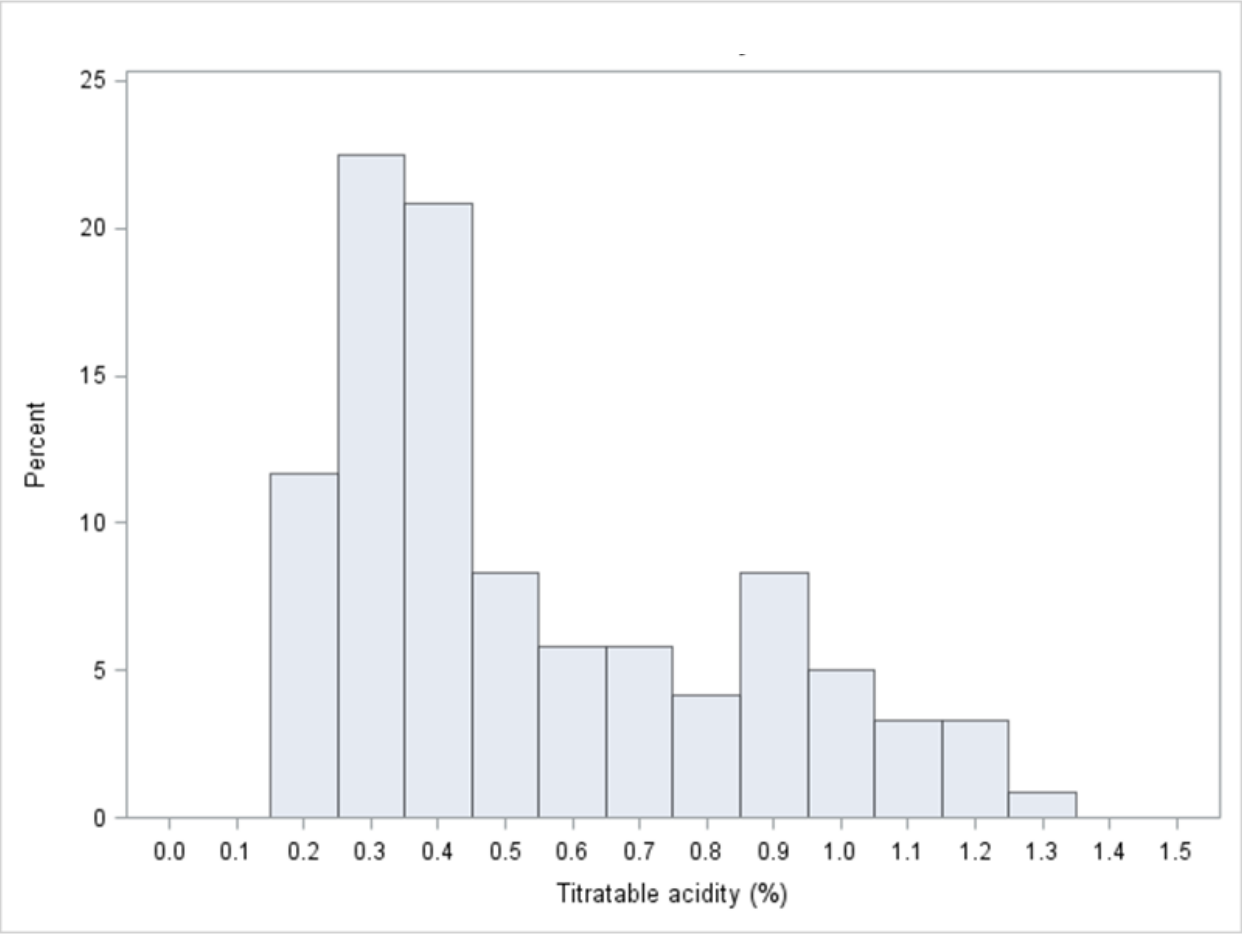

Fig. 3. Distribution (%) of the seedlings within titratable acidity (%) values for 2011. Arkansas RosBREED seedlings. N=120.

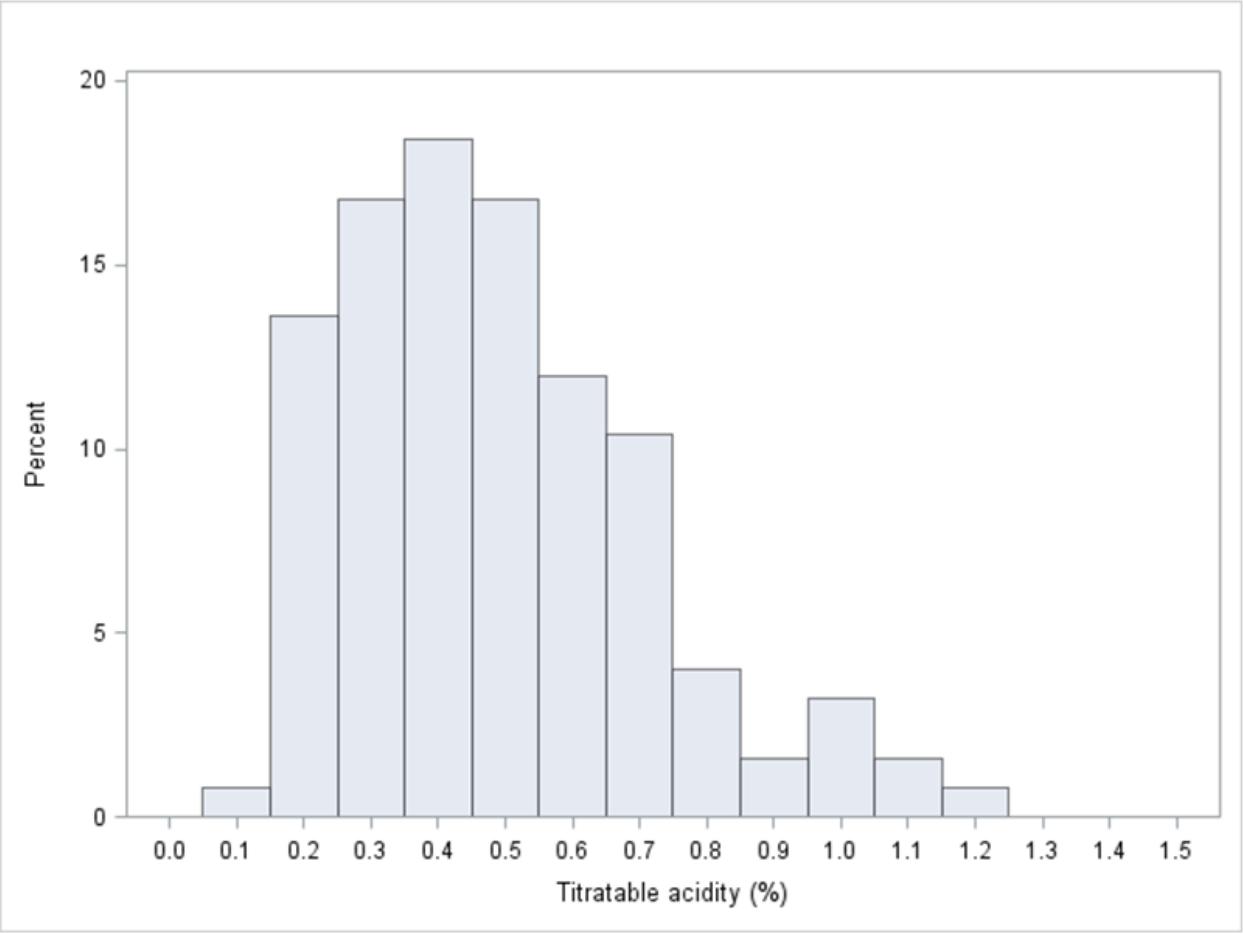

Fig. 4. Distribution (%) of the seedlings within titratable acidity (%) values for 2012. Arkansas RosBREED seedlings. N=125.

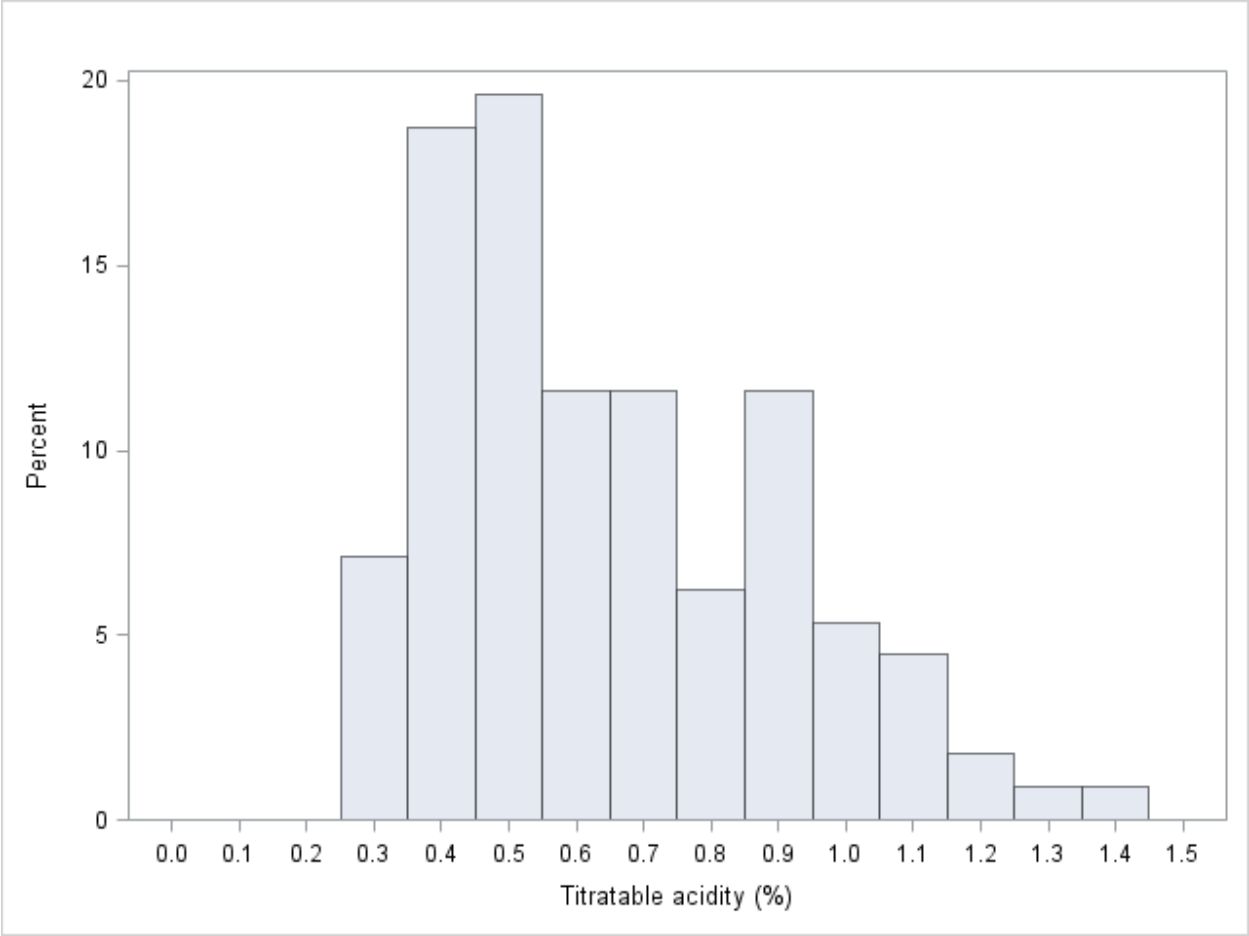

Fig. 5. Distribution (%) of the seedlings within titratable acidity (%) values for 2013. Arkansas RosBREED seedlings. N=112.

Fruit pH is another trait related to fruit acidity which was also measured during the period of the study. Mean values across all populations ranged from 3.8 in 2011 to 4.1 in 2012 with an average across all years of 4.0. Extreme values ranged from 2.8 to 5.5 (Table 3). In a study done in a segregating population, pH values ranged from 3.3 to 4.6 (Boudehri et al., 2009). Mean pH values of low-acid Arkansas cultivars were found to be 4.2 to 4.5 (Clark and Sandefur, 2013). Conversely, 'Redhaven', a standard-acid cultivar, had an average pH of 3.6 (Clark and Sandefur, 2013). Frequency distributions of pH indicated a higher frequency of individuals within the pH range of 3.7 to 4.2 and fewer individuals exceeding 5.0 pH (Figs. 6 to 9). The diversity in fruit pH was substantial in the genotypes evaluated in my study, which can be due to the parents utilized in the RosBREED populations (Table 4).

Table 3. Mean, maximum value, minimum value, standard deviation, and number of observations of fruit pH for 2011, 2012, 2013, and across-year average. RosBREED Arkansas seedlings.

Year	pH				Number of observations
	Mean	Max.	Min.	Std. dev.	
2011	3.8	4.5	2.9	0.36	121
2012	4.1	5.5	2.8	0.44	126
2013	3.9	5.0	3.1	0.32	116
Across-year average	4.0	4.8	3.0	0.33	136

Table 4. Values for pH of parental individuals of Arkansas RosBREED germplasm of years 2011, 2012, and 2013.

Progeny	Parentage		pH		
			2011	2012	2013
ArPop_1	Female	White County	4.1	4.4	4.1
	Male	A-672	3.8	3.8	3.7
ArPop_0801	Female	A-776	4.0	4.5	-
	Male	A-783	3.5	3.8	3.6
ArPop_0803	Female	Amoore Sweet	3.1	3.7	-
	Male	A-778	3.4	4.0	3.5
ArPop_0813	Female	A-772	3.9	4.5	4.3
	Male	A-672	3.8	3.8	3.7
ArPop_0817	Female	A-789	4.0	4.2	4.2
	Male	A-699	4.0	3.9	4.1
ArPop_0819	Female	A-708	4.4	4.5	4.2
	Male	A-773	4.1	-	-
ArPop_0825	Female	Souvenirs	4.2	4.1	-
	Male	A-760	4.4	4.8	4.5

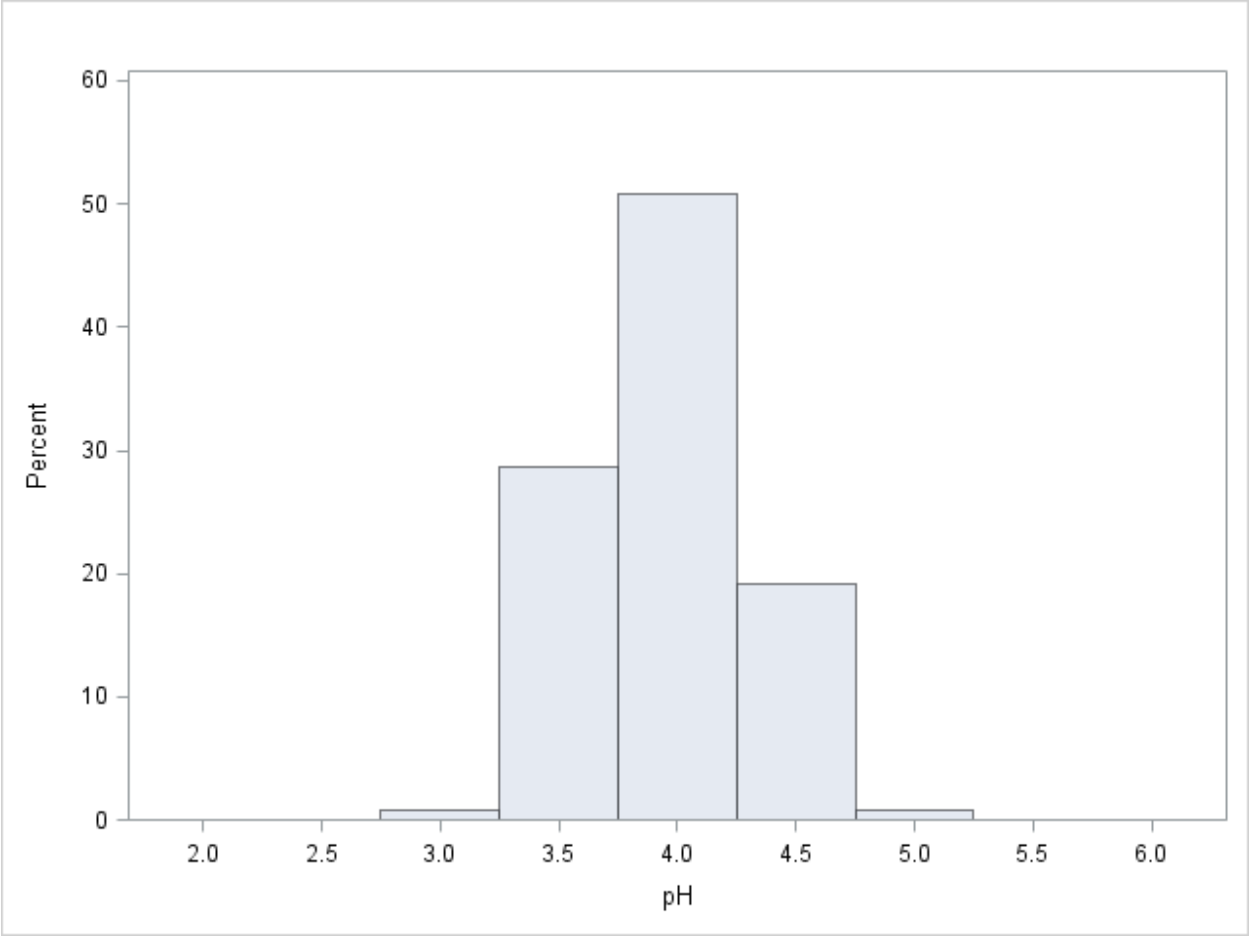

Fig. 6. Distribution (%) of the seedlings within pH values, across-year average. Arkansas RosBREED seedlings. N=136.

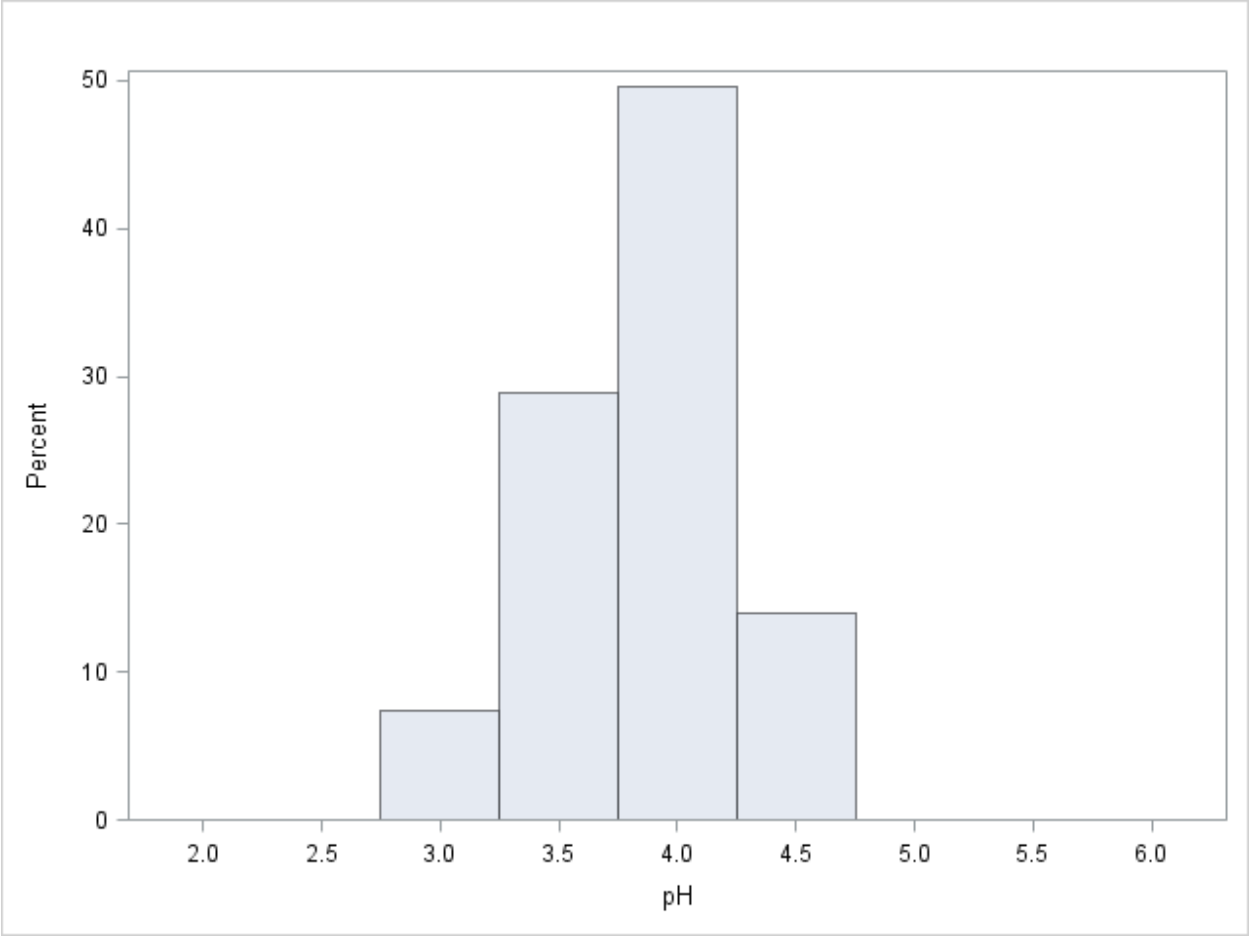

Fig. 7. Distribution (%) of the seedlings within pH values for 2011. Arkansas RosBREED seedlings. N=121.

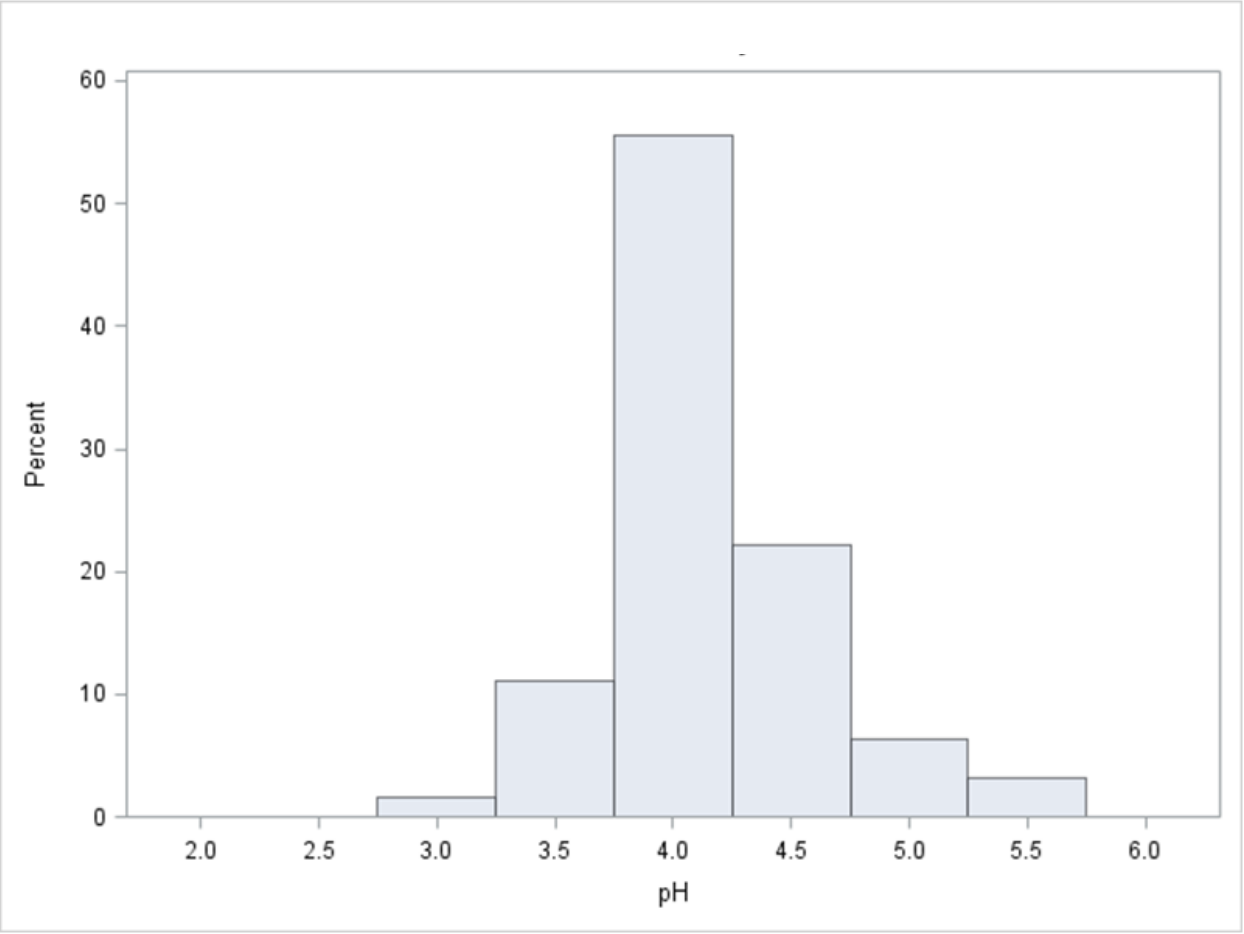

Fig. 8. Distribution (%) of the seedlings within pH values for 2012. Arkansas RosBREED seedlings. N=126.

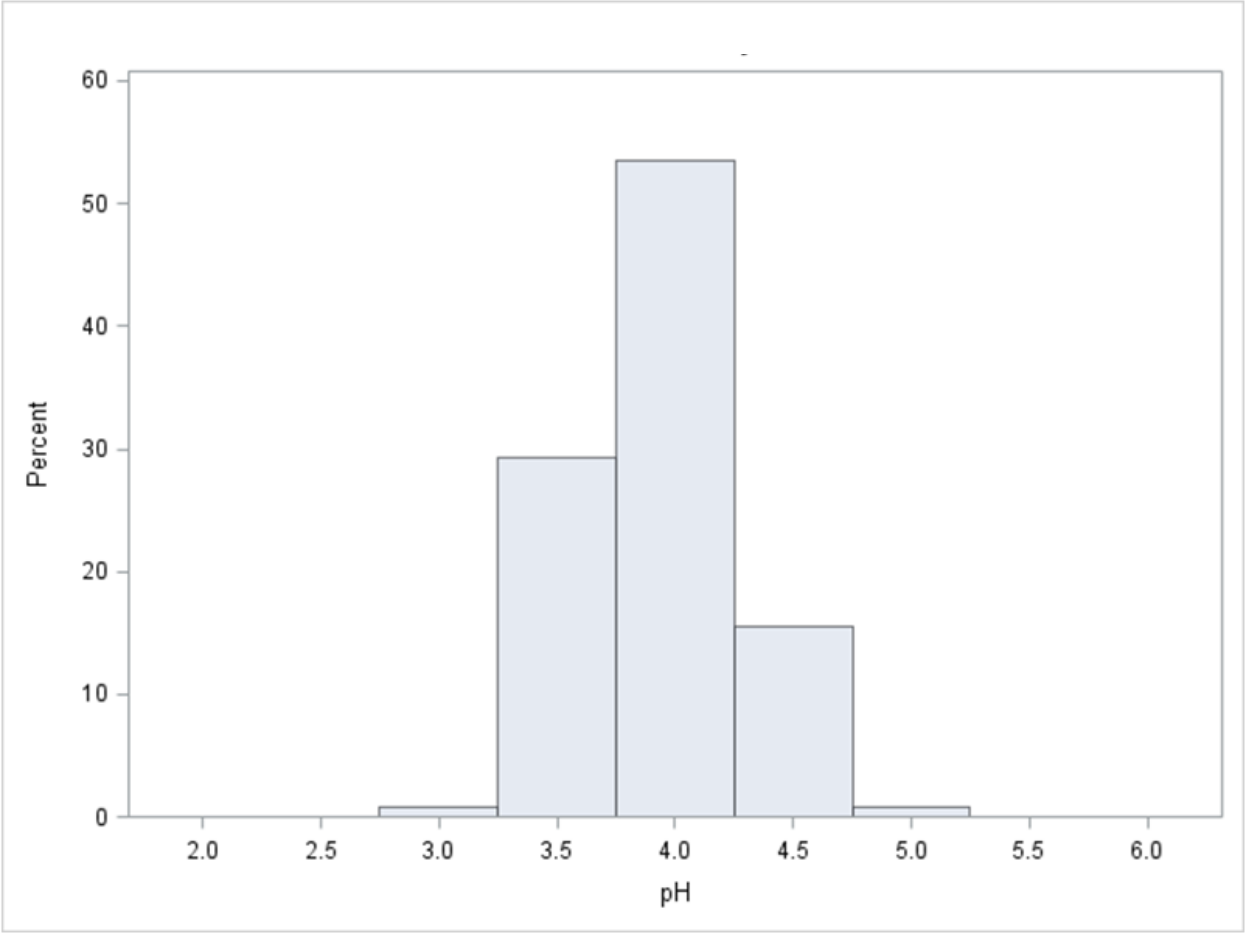

Fig. 9. Distribution (%) of the seedlings within pH values for 2013. Arkansas RosBREED seedlings. N=116.

Fruit firmness is an important trait for the peach fresh-market and processing industries (Crisosto, 2002). Breeders select new peaches and nectarines that are firm and can hold their flesh firmness after harvest (Infante et al., 2006). The Arkansas breeding program maintains a diversity range of flesh textures which were represented in the RosBREED germplasm (Tables 5 and 6). Means of fruit firmness ranged from 21.3 N in 2011 to 30.1 N in 2013, with an average across all years and genotypes of 27.3 N (Table 5), and these values are within the range of consumer acceptance (Crisosto and Kader, 2000).

As stated before, the analyzed germplasm contained MF, SMF, NMF, and NSF individuals which expressed a wide range of firmness values when they go through the ripening process. Melting flesh individuals can average ~15.0 to 18.0 N when ripe with a range of 8.0 to 25.0 N (Karakurt et al., 2000; Tonutti et al., 1997; Valero et al., 2007). Non-melting individuals can range from 22.0 to 32.0 N (Karakurt et al., 2000). Firmness values of SMF were similar to those of MF (Sandefur, 2011) and NSF individuals were similar or higher to those of NMF (Sandefur, 2011).

Firmness histograms (Figs. 10 to 13) show distribution of firmness values of RosBREED germplasm in 2011, 2012, 2013, and the across-year average. It can be observed that there was a high frequency of individuals between 20 to 40 N and fewer individuals over 40 N. This range of values was expected due to the range in texture types in the measured germplasm, and is very important to have this variation to confirm QTLs related to this important trait and to apply MAB as a tool in the breeding program.

Table 5**.** Mean, maximum value, minimum value, standard deviation, and number of observations of flesh firmness for 2011, 2012, 2013, and across-year average. RosBREED Arkansas seedlings.

Year	Flesh firmness (N)				Number of observations
	Mean	Max.	Min.	Std. dev.	
2011	21.3	57.9	2.3	12.7	122
2012	30.3	69.6	7.8	13.7	131
2013	30.1	94.0	1.2	18.3	117
Across-year average	27.3	55.3	5.5	11.4	136

Table 6. Flesh firmness values of parental individuals of Arkansas RosBREED germplasm of years 2011, 2012, and 2013.

Progeny	Parentage		Flesh firmness (N)		
			2011	2012	2013
ArPop_1	Female	White County	20.0	29.4	28.4
	Male	A-672	2.2	20.1	23.5
ArPop_0801	Female	A-776	18.9	22.1	-
	Male	A-783	27.8	28.4	-
ArPop_0803	Female	Amoore Sweet	26.7	25.5	42.1
	Male	A-778	4.5	26.5	34.3
ArPop_0813	Female	A-772	22.3	36.3	25.5
	Male	A-672	2.2	20.1	23.5
ArPop_0817	Female	A-789	11.1	37.2	24.4
	Male	A-699	11.1	29.4	31.4
ArPop_0819	Female	A-708	33.4	35.8	35.3
	Male	A-773	21.2	-	-
ArPop_0825	Female	Souvenirs	13.4	24.0	25.5
	Male	A-760	2.2	18.1	-

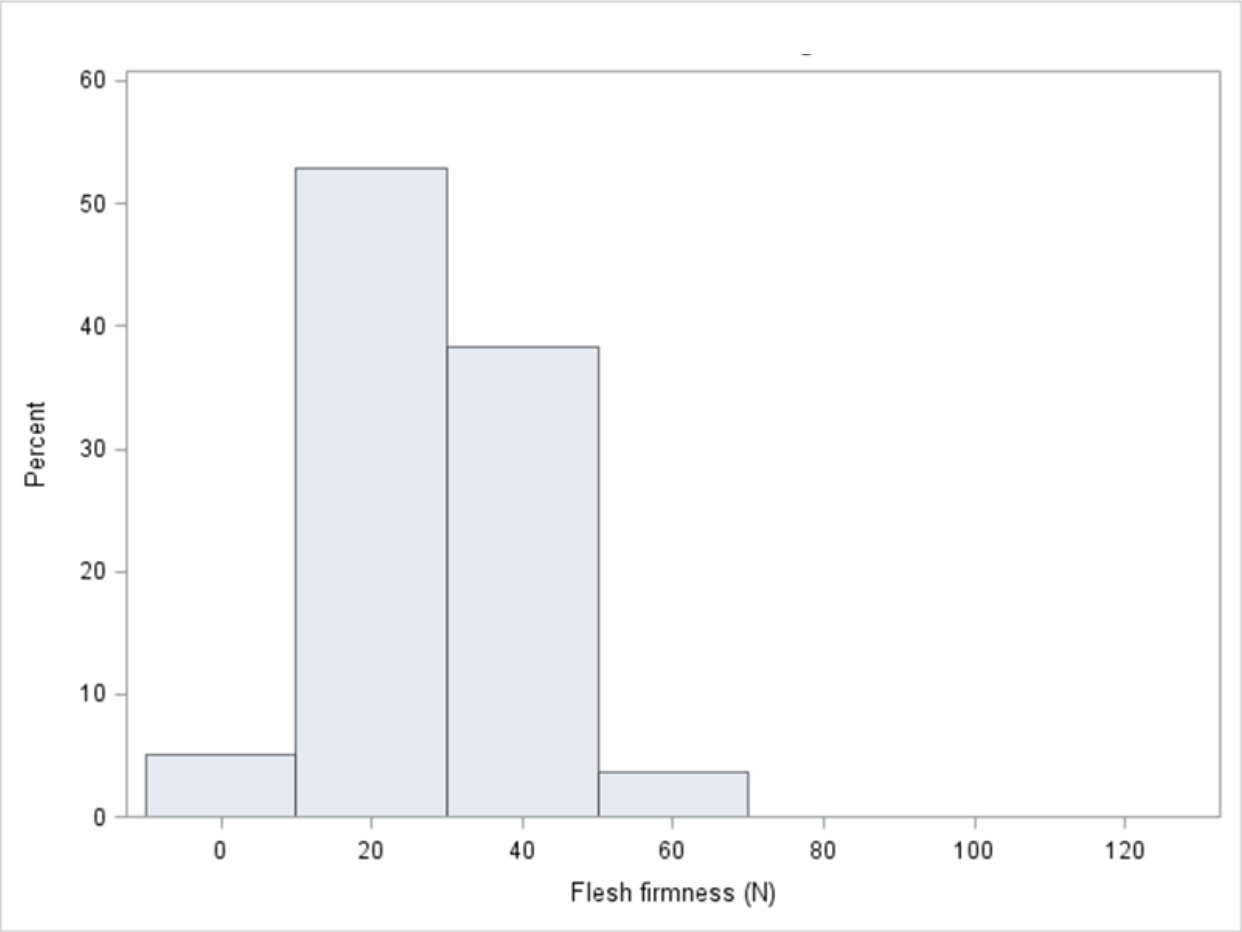

Fig. 10. Distribution (%) of the seedlings within firmness values of flesh firmness (N), across-year average. Arkansas RosBREED seedlings. N= 136.

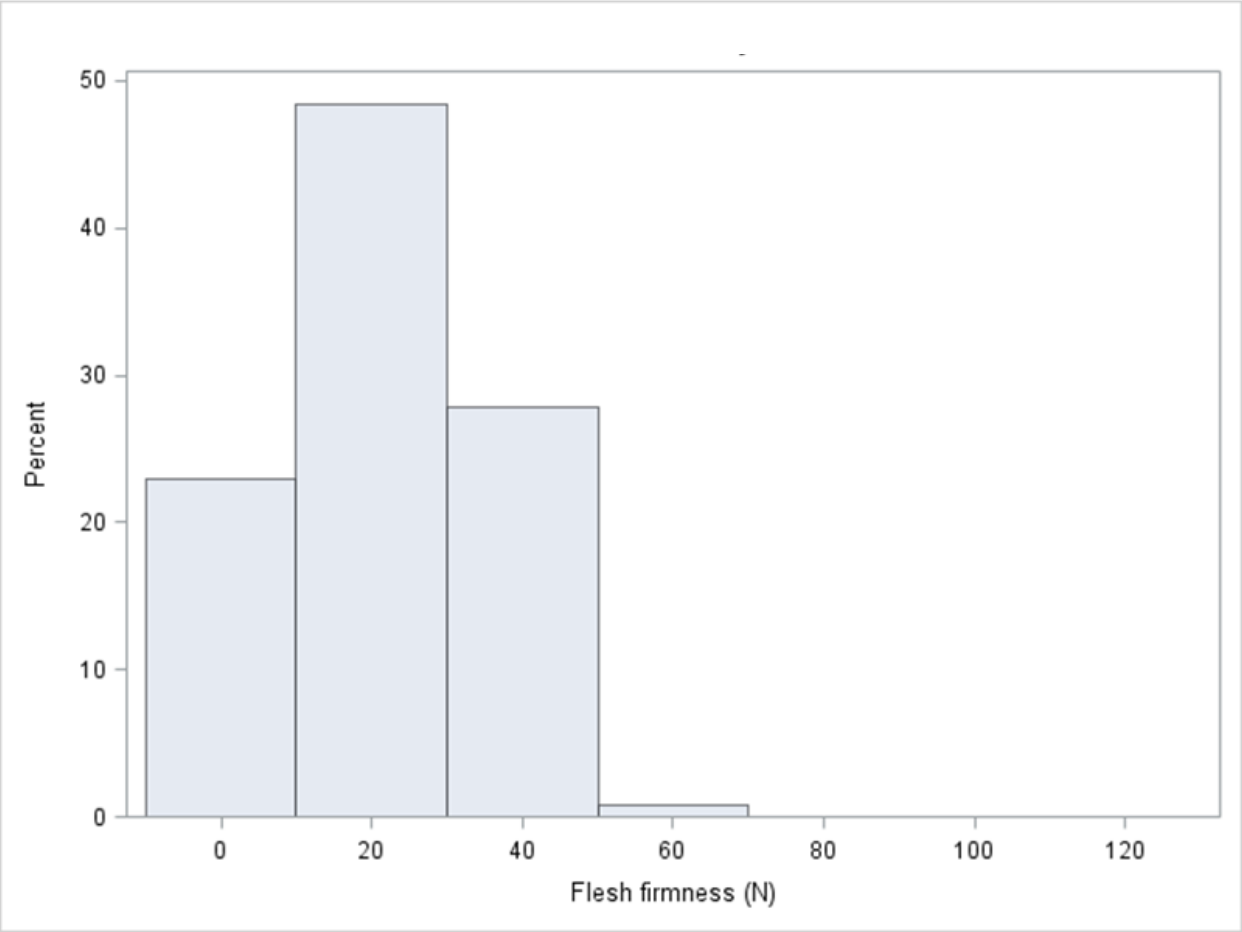

Fig. 11. Distribution (%) of the seedlings within firmness values of flesh firmness (N) for 2011. Arkansas RosBREED seedlings. N= 122.

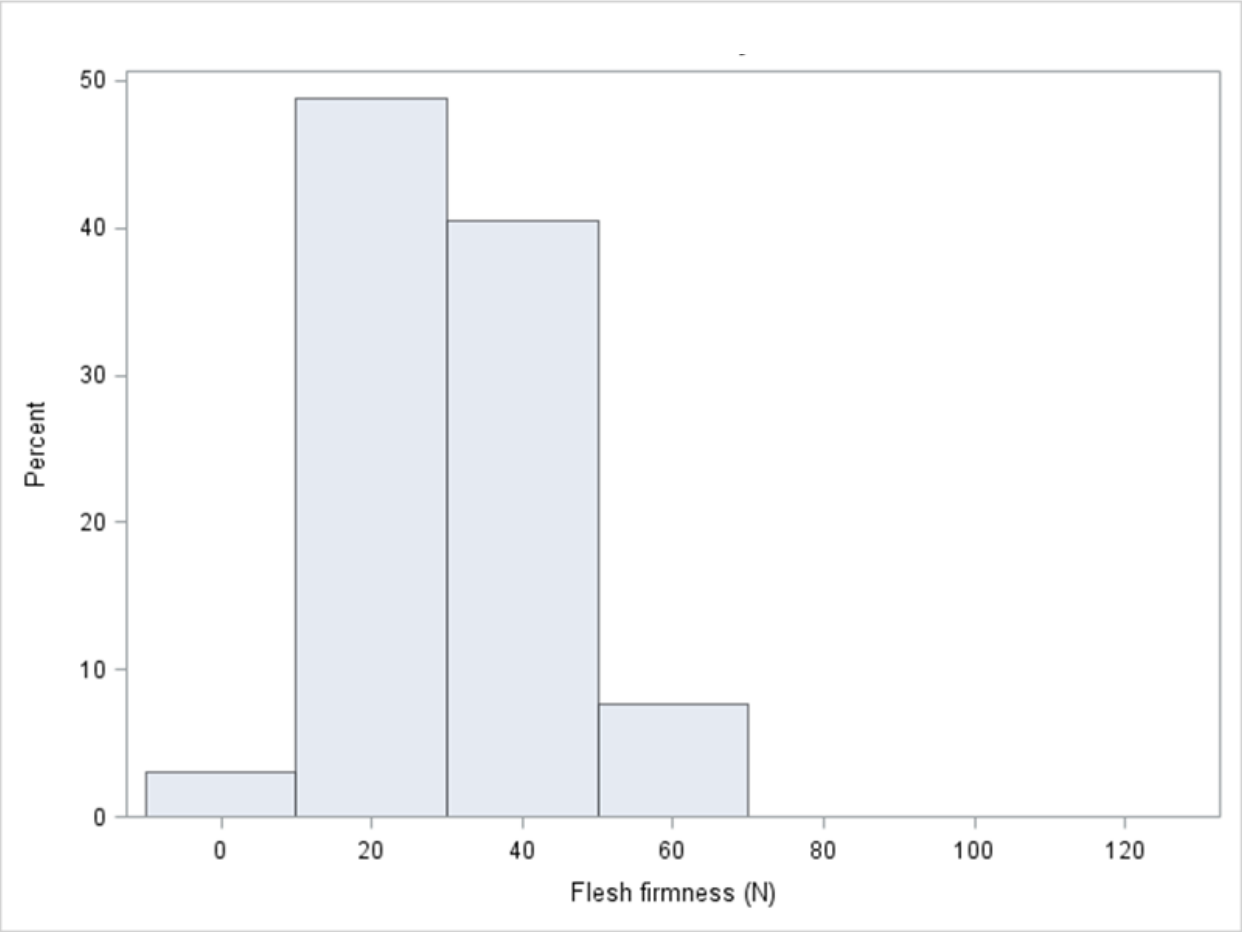

Fig. 12. Distribution (%) of the seedlings within firmness values of flesh firmness (N) for 2012. Arkansas RosBREED seedlings. N= 131.

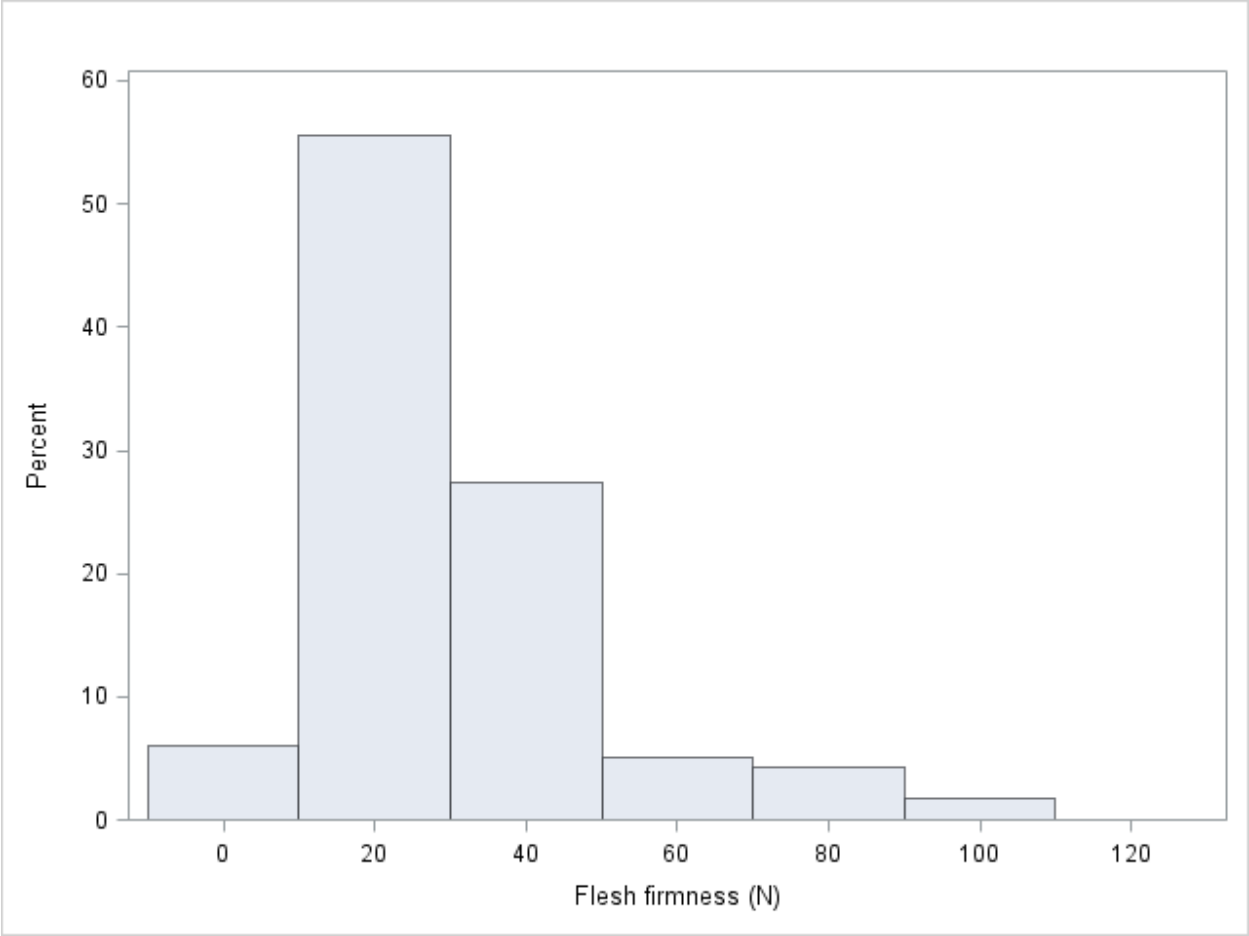

Fig. 13. Distribution (%) of the seedlings within firmness values of flesh firmness (N) for 2013. Arkansas RosBREED seedlings. N= 117.

Genome-Wide QTL Analysis

Titratable Acidity and pH

Chromosomal locations, flanking markers, BFs, and broad and narrow sense heritabilities of QTLs for TA evaluated in 2011, 2012, 2013, and across-year averages are shown in Table 7. The PBA approach found QTLs associated with TA consistently on LG 5 across the three years of the study and in the across-year average (Table 7). The BF showed strong (BF 5 ≥) and decisive evidence (BF) ≥ 10 (Table 7). The H^2 of TA ranged from 0.38 in 2011 to 0.64 in the across-year average.

The QTL located on the proximal end of LG 5, between snp_5_274324 and ss_547211 (0.7-3.0 cM) was consistent and showed decisive evidence across years. The variation explained by this QTL (narrow sense heritability, h^2) ranged from 0.35 in 2013 to 0.62 in 2011 (Table 7). This QTL location coincides with the location of a major gene controlling the low-acidity character in peaches (Boudehri et al., 2009; Etienne et al., 2002). Dirlewanger et al. (2006) and Ogundiwin et al. (2009) also discovered a QTL for TA on the same location found in my study. Dirlewanger et al. (2006) indicated that the year effect was small and that the QTL detected in one year was also detected in another year. I found a similar occurrence since the QTL detected on the proximal end of LG 5 was detected across all years of study at the same chromosomal location.

The QTL located between ss_582516 and ss_588676 (20.7-25.9 cM) showed only positive (2 <BF> 5) evidence in the across-year average, so this QTL was not consistent and reliable, since it did not show strong association with TA in the years of study (Table 7). This QTL has not been previously reported, indicating that this QTL might not be real.

The genomic region containing the D gene was associated with variation of 44% (h^2) of TA in the study reported by Etienne et al. (2002), a value similar to those found in 2012 and 2013 in this study (43 and 35%, respectively), and lower than the values found in 2011 and across-year average (62 and 59%, respectively). Also, QTLs for pH, malic, and citric acid content have been mapped close to the D locus (Etienne et al., 2002).

For pH, the PBA approach detected QTLs on LG 3, 4, and 5 in 2011, LG 4 and 5 in 2012, and LG 5 in 2013 (Table 8). The H^2 ranged from 0.48 in 2012 to 0.62 in across-year average. The QTL on LG 3 in 2011 was located at the same position as the across-year average, between 51.2 and 52.4 cM (BF = 2.2) and between 50.0 and 53.9 cM (BF = 3.2). On LG 4, the QTL was located between 67.1 to 67.5 cM in 2011 (BF = 2.6), and from 26.5 to 27.4 cM in 2012 (BF = 2.3). On LG 5, a QTL was located on the proximal end of this chromosome between 0.7 and 3.0 cM consistently across years, with BFs of 32.4 in 2011 and 32.5 in 2012 and 2013. When values were averaged (across-year average), the QTL on LG 5 was located between 1.2 and 3.0 cM with a Bayes factor of 32.4. These values of LG 5 show that this QTL was reliable, stable, and consistent across all years of study.

The pH QTL found on the proximal end of LG 5 was located on the same chromosomal position as the TA QTL discovered in this study (0.7-3.0 cM), corresponding with prior studies which found TA and pH QTLs were co-localized on LG 5 (Boudehri et al., 2009; Etienne et al., 2002, 2006). The phenotypic variation explained in my study by this QTL ranged from 47% in 2011 to 58% in the across-year average, lower than the 90% accounted for by Etienne et al. (2002). This difference could be due to the different structure of the germplasm utilized in my study (multifamily germplasm) compared with the bi-parental population utilized by Etienne et al. (2002).

Quantitative trait loci for quinic acid and total organic acids have been found previously in the middle section of LG 4 (Dirlewanger et al., 2006) which could be related to the QTL found in my study on this same LG. Molecular makers flanking the QTLs for quinic and total organic acid are the SSRs BPPCT023 and CPPCT24b which in some linkage maps are close to the chromosomal location of this QTL found on LG 4 (67.1 to 67.5 cM) (Dirlewanger et al., 2006). However, to confirm the finding of this minor QTL, measurements of quinic and other organic acid contents would need to be taken on this germplasm or utilize another germplasm structure to find minor QTLs. A similar situation could be occurring with the QTL found on LG 3 at 50.0 to ~54.0 cM in 2011 and across-year average.

These findings on fruit TA and pH validate the use of PBA and Bayesian approach to find QTLs related with these traits, since they were located on the same location as previous studies (Boudehri et al., 2009; Dirlewanger et al., 2006; Etienne et al., 2002; Ogundiwin et al., 2009). Major QTLs of TA and pH per year for LG 5 are shown in Fig. 14.

Visual inspections of the trace plots for convergence and stability of the evaluated genetic model per year were performed to determine if the TA and pH QTLs were reliable. In the case of TA, the QTL found on the upper end of LG 5 (0.7-3.0 cM) was stable. It showed a posterior probability higher than 0.1 and converged each year (traceability plots of TA per year of LG 5 are shown in Appendix A, Figs. A.1-A.3). For pH, the QTL on the proximal end of LG 5 (same location of the TA QTL) also was stable and converged every year with a posterior probability over 0.1 (traceability plots of pH per year of LG 5 are shown in Appendix A, Figs. A.4-A.6), a situation that did not occur with the QTLs on LG 3 and LG 4. This indicated that for TA and pH, the QTL on the proximal end of LG 5 is reliable and could be used within the Arkansas breeding

program and MAB can be applied for both of these traits using DNA tests that contain molecular markers flanking this QTL.

Table 7. Chromosomal location for putative titratable acidity (%) QTLs per year, average evidence per year (Bayes factors), broad sense heritability per year, and narrow sense (additive) heritability per year.

Year	Linkage group	Flanking markers[z]	Physical location[y]	Genetic position (cM)	BF[x]	H^{2w}	h^{2v}
2011	5	snp_5_274325	274,325	0.7	32.2	0.53	0.62
		ss_547211	1,190,216	3.0			
2012	5	snp_5_274325	274,325	0.7	32.4	0.43	0.43
		ss_547211	1,190,216	3.0			
2013	5	snp_5_274325	274,325	0.7	32.5	0.38	0.35
		ss_547211	1,190,216	3.0			
Across-year	5	snp_5_274325	274,325	0.7	31.8	0.64	0.59
		ss_547211	1,190,216	3.0			
average	5	ss_582516-	8,270,399	20.7	4.7		0.04
		ss_588676	10,341,037	25.9			

[z] Flanking SNP markers of QTL regions.
[y] Physical location of the QTL's chromosomal region.
[x] Bayes factors. Indicator for the evidence of a QTL. Range from 2.0 to 5.0 suggest positive evidence, 5.0-10.0 suggest strong evidence, and >10.0 suggest decisive evidence.
[w] Broad sense heritability.
[v] Narrow sense heritability (proportion of phenotypic variation explained by the QTL).

Table 8. Chromosomal location for putative pH QTLs per year, average evidence per year (Bayes factors), broad sense heritability per year, and narrow sense (additive) heritability per year.

Year	Linkage group	Flanking markers[z]	Physical location[y]	Genetic position (cM)	BF[x]	H^{2w}	h^{2z}
2011	3	ss_365455	20,475,126	51.2	2.2	0.50	0.019
		ss_366432	20,963,264	52.4			
	4	ss_521563	26,843,329	67.1	2.6		0.031
		ss_524981	27,077,999	67.5			
	5	snp_5_274325	274,325	0.7	32.4		0.47
		ss_547211	1,190,216	3.0			
2012	4	ss_410134	10,615,885	26.5	2.3	0.48	0.03
		ss_411637	10,971,100	27.4			
	5	snp_5_274325	274,325	0.7	32.5		0.49
		ss_547211	1,190,216	3.0			
2013	5	snp_5_274325	274,325	0.7	32.5	0.46	0.55
		ss_547211	1,190,216	3.0			
Across-year average	3	ss_364330	20,017,024	50.0	3.2	0.62	0.02
		ss_367359	21,555,813	53.9			
	5	ss_543942	481,014	1.2	32.4		0.58
		ss_547211	1,190,216	3.0			

[z] Flanking SNP markers of QTL regions.
[y] Physical location of the QTL's chromosomal region.
[x] Bayes factors. Indicator for the evidence of a QTL. Range from 2.0 to 5.0 suggest positive evidence, 5.0-10.0 suggest strong evidence, and >10.0 suggest decisive evidence.
[w] Broad sense heritability.
[v] Narrow sense heritability (proportion of phenotypic variation explained by the QTL.

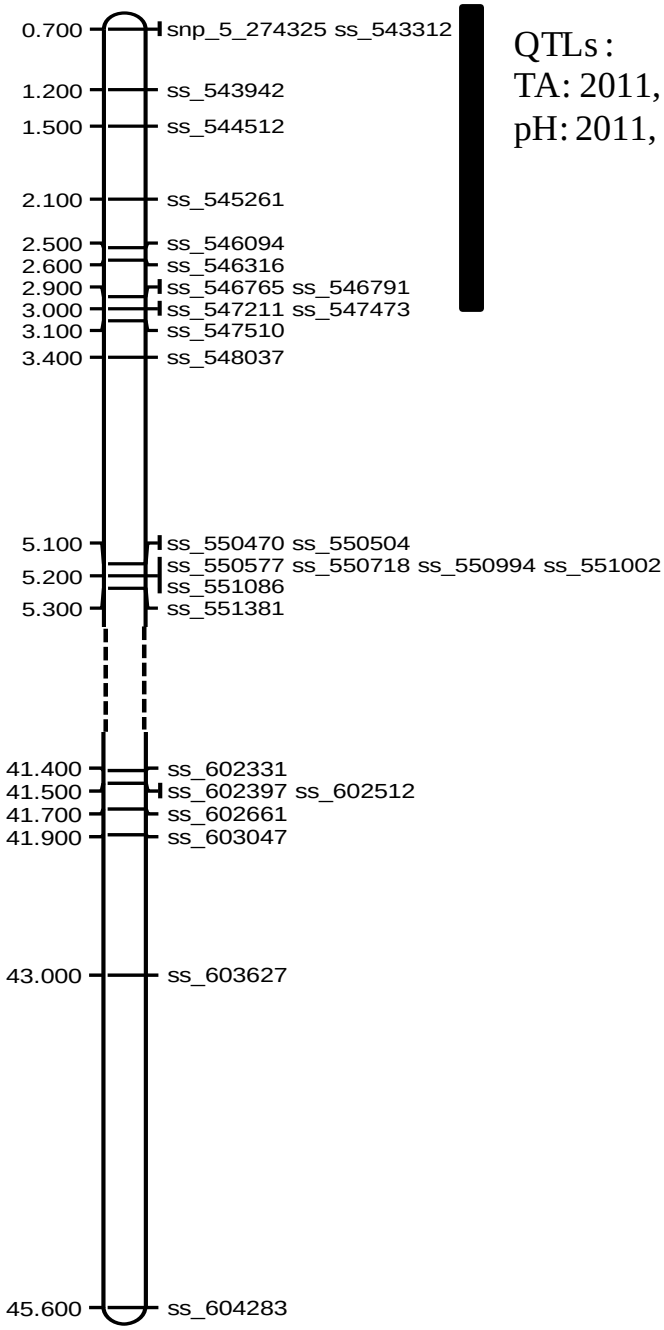

Fig. 14. QTLs of titratable acidity (%) and pH per year (2011, 2012, 2013, and across-year average). The black vertical line indicates the position of the QTLs on LG 5. QTLs were detected in the region between 0.7 to 3.0 cM.

Fruit Firmness

Quantitative trait loci were detected for flesh firmness on LG 4 across years, two QTLs in 2011 and in the across-year average and one QTL each in 2012 and 2013 (Table 9). In 2011 and in the across-year average the QTL on the distal end of LG 4 overlapped (55.8 to 58.2 cM in 2011 and 42.7 to 56.2 cM in year average). Also, there was an overlap between the locations of the QTL found in 2012, 2013, and the across-year average (26.5 to 40.5 cM in 2012, 38.4 to 45.0 cM in 2013, and 27.4 to 36.4 cM in across-year average). Bayes factors were above 5.0 in 2011, 2012, and 2013, which indicated strong evidence of a QTL, and decisive evidence [(BF) ≥ 10] on the across-year average for firmness (Table 9). Broad sense heritability (H^2) ranged from 0.20 to 0.68 for the trait, and additive heritability (h^2) of the QTL found at the distal end of LG 4 ranged from 0.2 to 0.5. The wide range of H^2 values for years is likely an indication of the environmental effect on this trait.

Firmness is a quantitative trait and can be affected by environmental conditions, maturity, and harvest handling of fruit (Crisosto and Valero, 2008; Crisosto et al., 1997). Harvest and postharvest handling procedures during the years of my study were uniform to minimize the impact of errors in the results. However, temperatures and rainfall were very different across years. Average high temperatures from 1 June to 31 Aug. were 34, 35, and 31 °C in 2011, 2012, and 2013, respectively. Average minimum temperatures from 1 June to 31 Aug. were 23, 22, and 20 °C in 2011, 2012, and 2013, respectively. Also, the amount of rain was variable during the years of my study with a total of 20 cm of rain in 2011, 12 cm in 2012, and 33 cm in 2013 from 1 June to 31 Aug. (data collected at the Fruit Research Station weather station). Lastly, days exceeding 32 °C were 96 in 2011, 92 in 2012, and only 54 in 2013 between 1 June and 31 Aug. These data show that the environment was quite different among years, especially when

comparing 2011 and 2012 to 2013, the first two years were hot and very dry compared to 2013; these differences in temperatures and total rain could explain in part the variation in the H^2 of flesh firmness (0.20 to 0.68).

Previous studies reported that endopolygalacturonase (endoPG) is closely related to peach flesh texture and its action determines if a peach or nectarine will be MF, NMF, or NSF (Peace et al., 2005). Peach flesh texture is also related to flesh firmness, since peaches or nectarines having NMF are firmer than MF at the end of the ripening process (Sandefur, 2011).

The QTL found on the distal end of LG 4 in this study is located on the same chromosomal region where Freestone-Melting (F-M) locus and endoPG candidate genes were reported to be (~52-56 cM) (C. Peace personal communication; Dirlewanger et al., 2004; Peace and Norelli, 2009). The F-M locus is related to pit adhesion and flesh texture characters (Peace and Norelli, 2009). The melting gene (ppa006839m) encodes for endoPG enzyme (a family member of the pectinase super family protein) which impacts fruit texture at the end of the ripening process differentiating peaches into MF, NMF, and NSF texture classifications. The function of endoPG is to soften fruit during the ripening process by hydrolysis of the pectate chain in peach cell walls (Pressey and Avants, 1973, 1976). The melting gene covers a total of 3,098 bp on LG 4 (22,649,519 to 22,652,617 bp) (Genome Data for Rosaceae, 2015). The freestone gene (ppa006857m) encodes for the polygalaturonase (PG) enzyme, another member of the pectinase super family protein, covering a total of 2,659 bp on LG 4 (22,684,500 to 22,687,169 bp) (Genome Data for Rosaceae, 2015). The distance between both genes is 31,883 bp (Genome Data for Rosaceae, 2015).

My findings validate the application of the PBA approach to find QTLs in ongoing breeding programs, since the QTL region on the distal end of LG 4 overlapped with the location

of the F-M locus reported in a bi-parental study (Peace et al., 2005, 2007). The F-M locus and the related DNA tests (that differentiate MF, NMF, and NSF textures) were discovered based on two progeny populations which segregated for flesh type and also for adhesion to the pit (Peace et al, 2005a). However, the bi-parental approach has certain disadvantages, particularly for an ongoing fruit breeding program. These include that they usually represent a small portion of the germplasm present in peach, display a limited fraction of the total genetic variance present in a breeding program, useful alleles can be missed due to not being present or they don't segregate into specific single mapping families, and there may be no or little information about the QTL mode of action (Bink et al., 2012, 2014; Peace et al., 2014). For these reasons, a PBA using a Bayesian approach was utilized to discover QTL(s) segregating for flesh firmness in the established Arkansas breeding program is a valuable new approach to discover and confirm QTLs for important traits.

Another interesting finding of this study is that the QTLs on LG 4 were related to flesh firmness, a quantitatively measured trait in my study. The research reported here did not use melting and non-melting categorical classifications as used in previous studies (Peace et al., 2005, 2007). This confirms that flesh firmness is closely related to the type of flesh texture in peaches, because the QTL located at the distal end of LG 4, is located where the endoPG gene was found using categorical scales (Peace et al., 2005a, 2007).

The other QTL on LG 4 (located downstream to the QTL related to F-M locus) found in 2012 covered a distance of 14 cM on LG 4 (26.5-40.5 cM). This is a distance that is considered large and reduces the chances of this being confirmed as a true QTL (Bernardo, 2010). However, when years were averaged (across-year average), the QTL located between 27.4 cM and 36.4 cM was within the QTL found on 2012. In my following Chapter, I will explain a new DNA test that

was developed to distinguish slow-melting flesh texture (SMF), which it is not possible to detect with the DNA tests developed for the F-M locus. The SNP marker utilized in the DNA test to detect SMF (SMF-SNP) is located on 30.1 cM of this LG. Major QTLs of flesh firmness per year of LG 4 are shown in Fig. 15.

Visual inspections of the trace plots for convergence and stability of the evaluated genetic models per year were also performed to determine if the QTLs were reliable. For flesh firmness, the QTLs were not stable, showing posterior intensities lower than 0.1. Traceability of flesh firmness was calculated, but trace plots of this trait are not shown since the QTLs for flesh firmness did not converge. Despite the lack of confidence, the QTLs found on LG 4 spanned locations that are closely related to peach flesh texture. This lack of confidence, due to the low posterior probabilities of the flesh firmness QTLs, could be due to the several candidate genes associated with texture (Illa et al., 2011). This finding likely indicates a quantitative nature of the trait as reported previously by Illa et al. (2011) (with several minor QTLs) and is probably strongly affected by environmental conditions.

Table 9. Chromosomal location for putative flesh firmness QTLs per year, average evidence per year (Bayes factors), broad sense heritability per year, and narrow sense (additive) heritability per year.

Year	Linkage group	Flanking markers[z]	Physical location[y]	Genetic position (cM)	BF[x]	H^{2w}	h^{2v}
2011	4	ss_441887	18,520,777	46.3	9.4	0.33	0.2
		ss_451930	20,505,479	51.3			
		ss_463805	22,150,620	55.8	9.3		
		ss_475922	23,307,725	58.3			
2012	4	ss_410134	10,372,524	26.5	8.0	0.20	0.17
		ss_434331	16,182,179	40.5			
2013	4	ss_429127	15,345,460	38.4	7.7	0.68	0.50
		ss_440116	17,996,235	45.0			
Across-year average	4	ss_411601	10,965,493	27.4	10.1	0.49	0.36
		ss_424415	14,624,215	36.4			
		ss_437516	17,094,116	42.7	9.8		
		ss_465820	22,485,848	56.2			

[z] Flanking SNP markers of QTL regions.
[y] Physical location of the QTL's chromosomal region.
[x] Bayes factors. Indicator for the evidence of a QTL. Range from 2.0 to 5.0 suggest positive evidence, 5.0-10.0 suggest strong evidence, and >10.0 suggest decisive evidence.
[w] Broad sense heritability.
[v] Narrow sense heritability (proportion of phenotypic variation explained by the QTL).

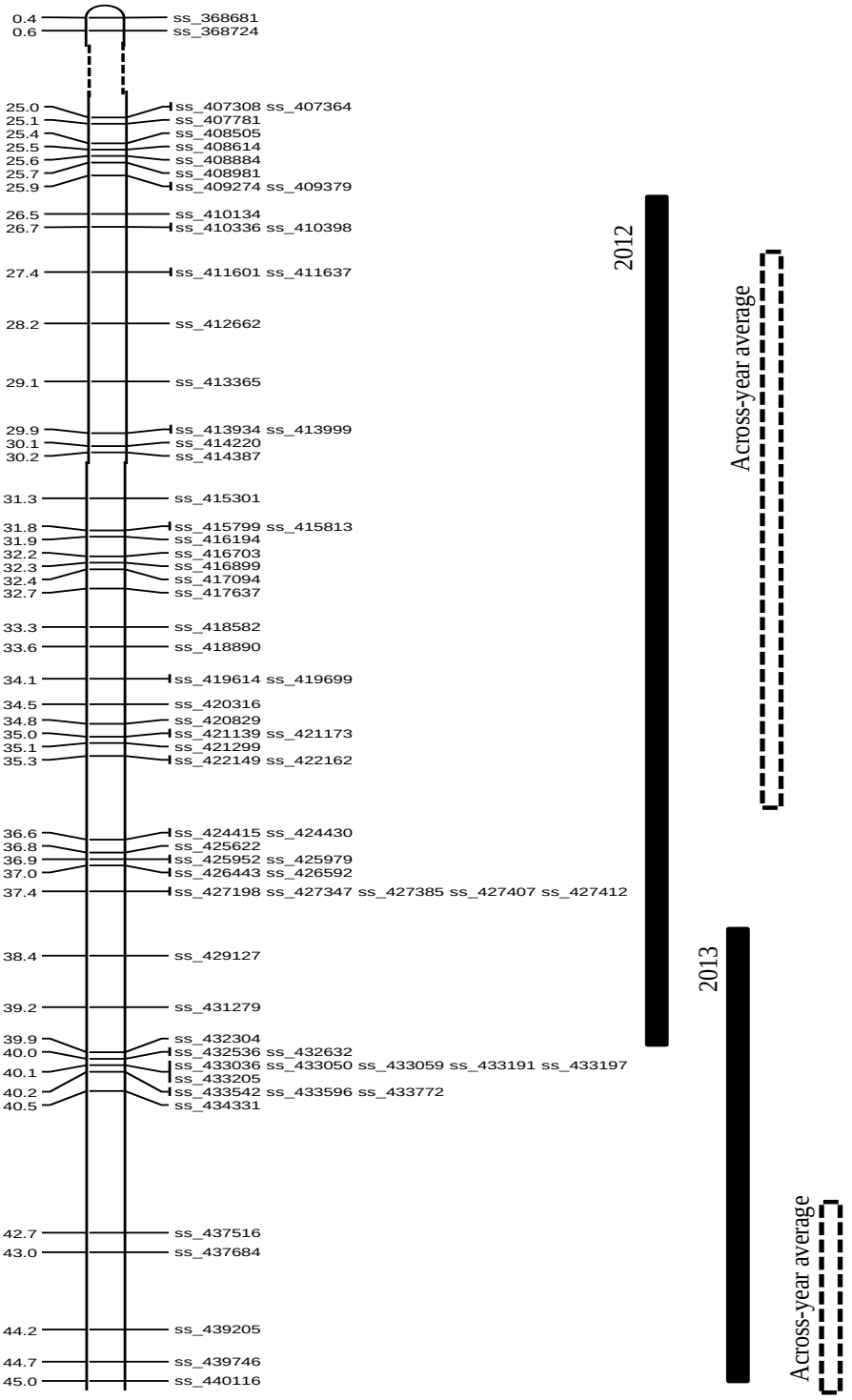

Fig. 15. QTLs for flesh firmness for year (2011, 2012, 2013, and across-year average) on LG 4. The black vertical line indicates the position of the QTL. The dotted vertical line indicates across-year average QTLs.

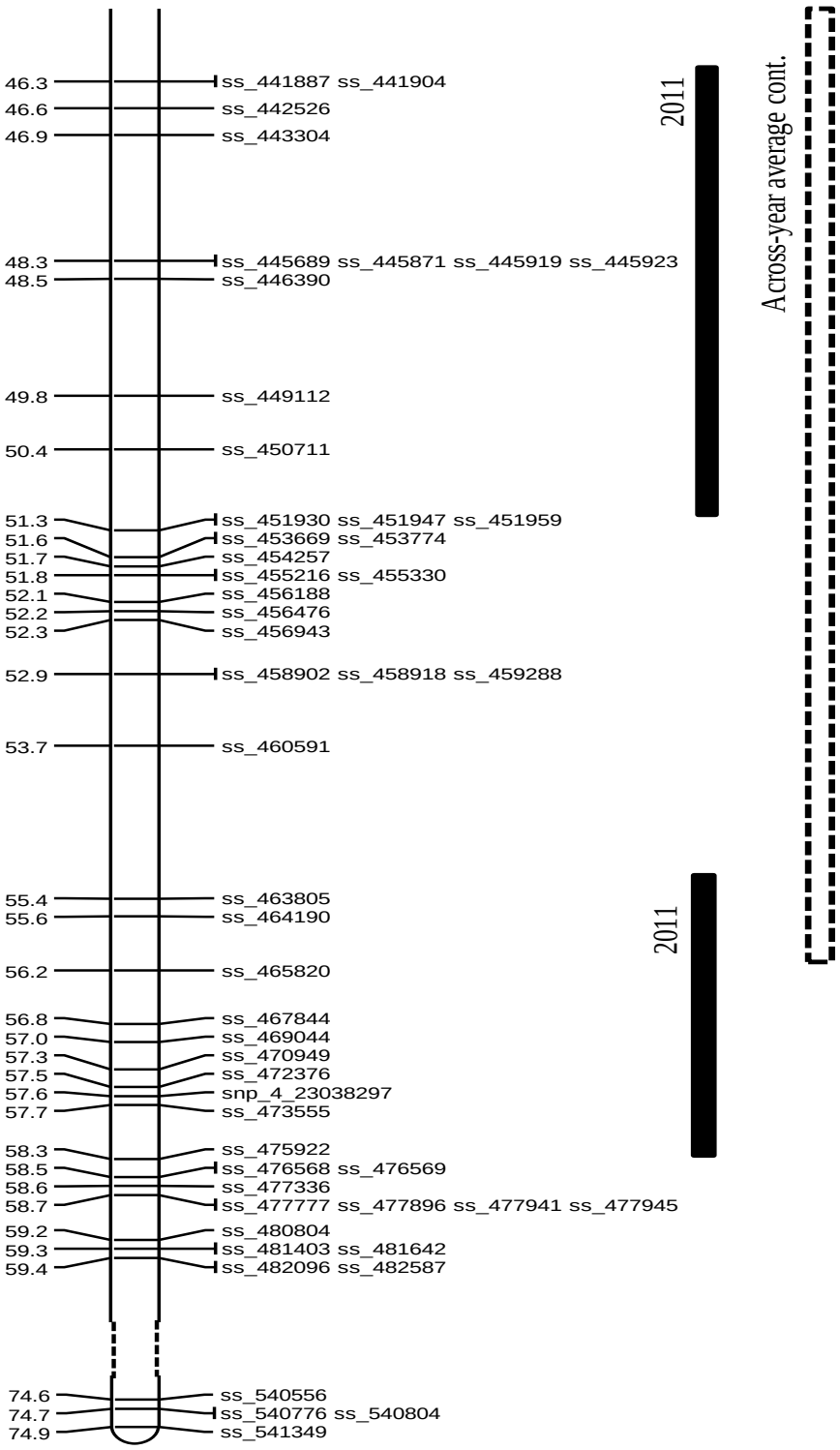

Fig. 15. QTLs of flesh firmness for year (2011, 2012, 2013, and across-year average) on LG 4. The black vertical line indicates the position of the QTL. The dotted vertical line indicates across-year average QTLs. (Cont.).

Specific QTL Analyses

In the case of TA and pH, a QTL analysis was performed by removing the effects of LG 5 and then a QTL analysis utilizing only LG 5 was performed. In the case of flesh firmness, a QTL analysis was performed by removing the effects of LG 4 and then a QTL analysis utilizing only LG 4 was performed. In the genome-wide QTL analyses of TA, there was one major QTL at the proximal end of LG 5 and no other stable QTL(s) associated with this trait. When the effects of LG 5 were removed, no reliable QTLs were associated with this trait in the years of study in other LGs. In 2011, a QTL with positive evidence (BF=3.4) was found on LG 2. In 2012, no QTLs with positive evidence were found. In 2013, a QTL in LG 8 with positive evidence (LG=3.5) was located. In across-year average, only one QTL was located on LG 4 with positive evidence (BF=4.5). None of these QTLs were located in the same chromosomal region across years. This could be an indication that to find another QTL related to this trait another type of analysis should be conducted. Since this germplasm contains individuals with high and low acidity, only the QTL that segregated for high and low TA was found. Another explanation could be that the effects of the QTLs on LG 2, LG 4, and LG 8 were too small to be identified by FlexQTL™. When LG 5 was run alone (by removing the effects of all the other LGs) the QTL on the proximal end was also found. In this case, this QTL was narrowed down in 2011, 2012, and the across-year average from 0.7-2.1 cM. In 2013, the QTL spanned the same region in the genome-wide analysis (0.7-3.0 cM).

For fruit pH, when the effects of LG 5 were removed, QTLs on LGs 2, 3, and 4 were found. The QTL on LG 2 was only found in 2011 and it was not found in the genome-wide analysis. The QTL found on LG 3 was found in the same location in 2011 and 2012, separated by less of 5.0 cM. The QTL on LG 3 found using across-year average spanned a large area of

this LG. The QTL on LG 4 found in 2011 was not consistent with the QTL on this same LG found in 2012 and across-year average. In 2013, there were no QTLs associated with pH in this type of analysis. The QTLs associated with pH found in these QTLs by this type of analysis were not consistent and had low posterior probabilities (less than 0.1).When LG 5 was analyzed alone (by silencing all the other LGs) the major QTL found initially in the genome-wide analysis was also detected. As in the case of TA, this QTL was also narrowed down. In 2011, 2012, and 2013 the QTL spanned a region from 0.7-2.1 cM. In across-year average this QTL spanned a chromosomal region from 0.7-2.3 cM on LG 5.

For flesh firmness, when LG 4 was silenced, QTLs were found on LGs 1, 2, 5, 7, and 8. However, these QTLs were not consistent across years and had low posterior probabilities (less than 0.1). Linkage group 4 was analyzed individually (by removing the effects of all the other LGs), in 2011, 2013, and across-year average, the QTL that spans the region of the F-M locus was found. However, the posterior probabilities were low, a similar result as the genome-wide analysis that was performed for this trait. However, in 2011, 2012, and across-year average, a QTL located downstream from the QTL of the F-M locus was associated with flesh firmness with positive evidence in 2012 and decisive evidence in 2011, 2013 and across-year average. Also, posterior probabilities in 2012 and across-year average were ~ 0.1 and visual inspection of trace plots showed increased stability and convergence of the genetic model. This QTL is on the same chromosomal region as the one found in 2012 and across-year average in the genome-wide analysis. This QTL, as mentioned before, is located in the region where the SMF-SNP was found (30.1 cM). These findings increase the confidence of the genome-wide analysis results regarding this QTL. The QTL in 2011 was between 21.1 and 35.0 cM, in 2012 between 27.4 and 33.3 cM, and in across-year average between 26.5 and 32.2 cM.

This type of analysis of removing the effects of a specific LG(s) and/or removing the effects of all the LGs with the exception of where the major QTL(s) are located is useful and contributed to better understanding the QTLs underlying the traits. For TA and pH, no other QTLs were associated with these traits when LG 5 was removed from the analysis, confirming that this QTL explains most of the phenotypic variation. If other QTL(s) are to be found for these traits, the analysis should be performed using another pedigree design, as stated before. When LG 5 was analyzed alone, the major QTL on the proximal end of this LG was confirmed and narrowed down. In the case of flesh firmness, this type of analysis helped to confirm the QTL that had low posterior probabilities in the genome-wide analysis. Thus, this type of analysis increased the confidence of the DNA tests flanking or within these chromosomal regions associated with the traits to apply MAB.

Estimated Breeding Values

Breeders select parents depending on desirable characteristics such as large size, high or low acidity, and high firmness for crossing. Likewise, they choose advanced selections for release as a new cultivar. However, at the moment of selection, breeders often do not know if that new selection will pass their positive genetic components to the next generation in crossing.

Titratable acidity and pH estimated genome-wide breeding values (EBVs) were obtained from FlexQTL^{TM} outputs using the Bayesian approach (Bink et al., 2014). Estimated breeding values from the major QTLs on LG 5 for both traits are shown in Fig. 16. There was a significant correlation between EBVs for both traits (Fig. 16). This means that by selecting an individual with negative TA EBV (which will decrease the acidity of the population if used in a cross), that individual will have a positive pH EBV. Further, this indicates that by using that individual the pH of the population will increase and will be less acid. On the contrary, by selecting an

individual with high TA EBV as a parent, the TA value of the population is expected to increase (making it more acidic) and at the same time the pH EBV of that individual will be negative meaning that the pH of that population is expected to decrease by using that individual as a parent. This relation is reflected also in the significant correlation between the phenotypic values of both traits, in which a higher TA was correlated with a lower pH (Fig. 17). Correlation between phenotypic and EBVs per trait and per year were positive and significant, and ranged from 0.46 to 0.63 for TA and from 0.55 to 0.57 for pH. The use and application of EBVs will optimize the information generated by the estimation of the QTLs underlying these traits and will allow more efficient application of MAB in an ongoing breeding program. The EBVs will reflect the capacity of an individual to pass their additive genetic effect to the next generation. Estimated breeding values of each individual of the Arkansas RosBREED pedigree of TA and pH and year are in Table B.1 in the Appendix B. Estimated breeding values of flesh firmness are not shown due to the low stability of the discovered QTLs.

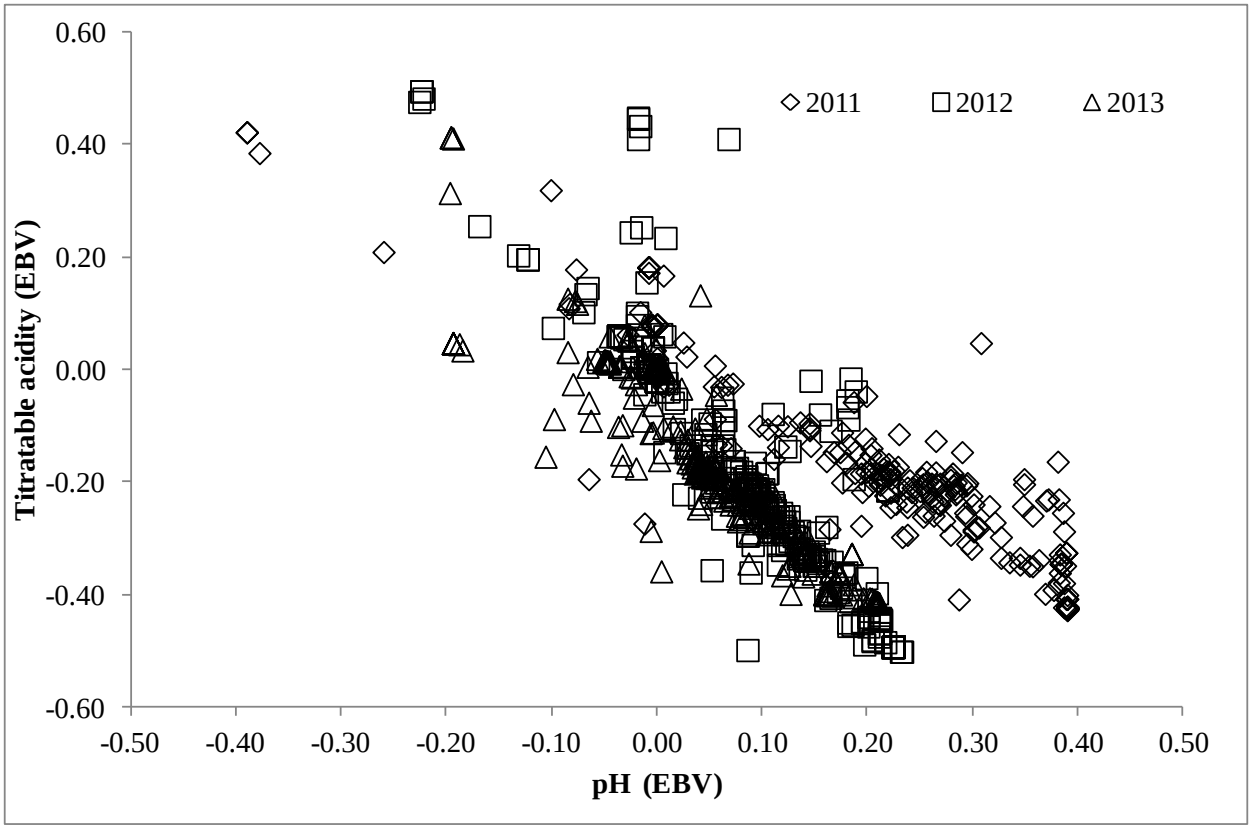

Fig 16. Correlation of titratable acidity and pH estimated breeding values (EBV) of Arkansas RosBREED germplasm for years 2011 (◊), 2012 (□), and 2013 (Δ). Coefficient of determination (R^2) of 2011, 2012, and 2013 were 0.87, 0.79, and 0.82, respectively.

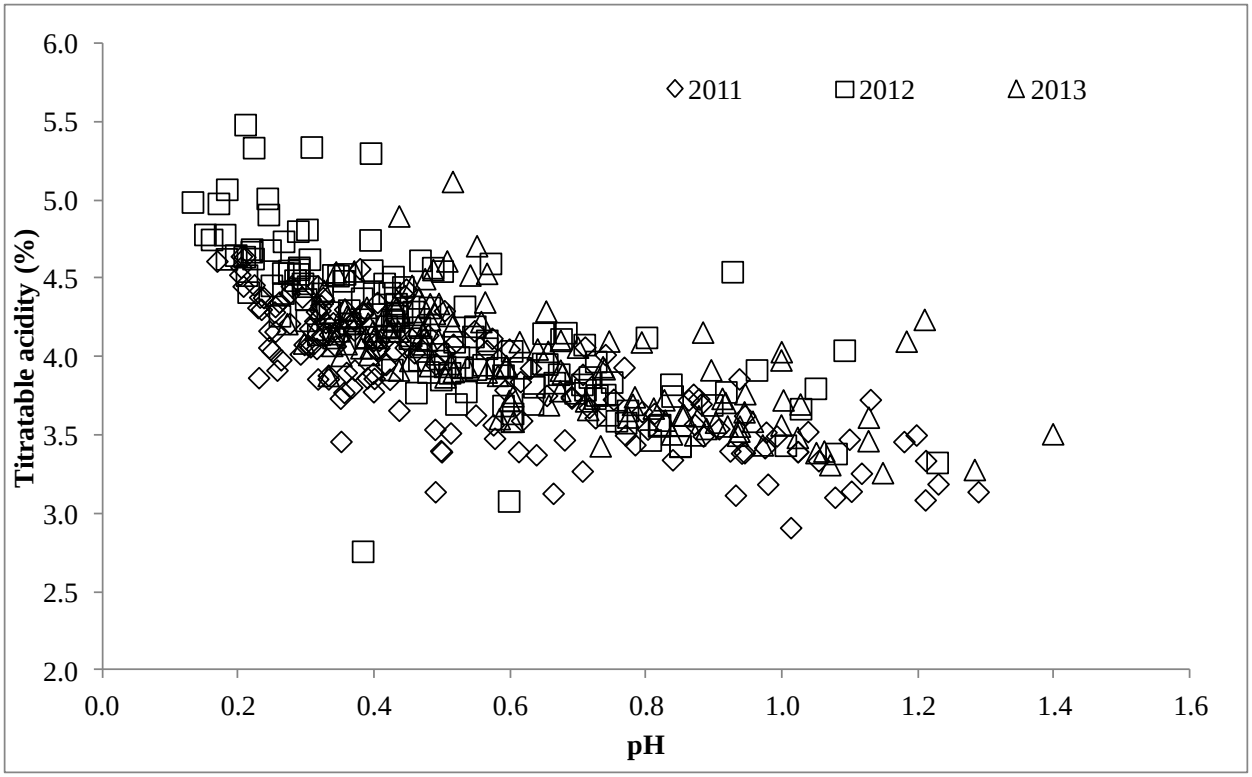

Fig 17. Correlation of titratable acidity and pH phenotypic values of Arkansas RosBREED germplasm for years 2011(◊), 2012 (□), and 2013 (Δ). Coefficient of determination (R^2) of 2011, 2012, and 2013 were 0.62, 0.54, and 0.50, respectively.

Conclusions

Quantitative trait loci identification is the first step to apply MAB for traits that are critical for breeding programs, such as fruit acidity and firmness in the case of the Arkansas peach breeding program. Frequently, the experimental design to discover QTL utilizes an F_2 segregating population for the trait of interest, and for fruit tree crops this design implies that the breeder will have to wait at least 7 to 8 (or even more) years for the first fruit phenotyping data. A better design needs to be applied to make QTL discoveries more efficient and easier for this type of breeding programs.

The objective of this study was to determine if the application of PBA using Bayesian approach which utilizes a procedure to carefully select common ancestors, important breeding parents, related cultivars, advanced selections, and seedlings can be used as an alternative experimental design to discover QTLs associated with fruit quality traits and others. All these individuals are connected and are representative of the breeding program. This procedure was applied for the first time in the Arkansas peach breeding program and results are promising.

Genome-wide QTL analysis for fruit TA and pH were consistent across years and were co-localized on the proximal end of LG 5 (0.7-3.0 cM), a location that coincides with QTLs previously found by other authors which used a bi-parental design. This means that the use of DNA tests, flanking that particular region of LG 5, will facilitate the application of MAB for TA and pH. Despite the fact that QTLs on LG 4 were not stable and had low posterior probabilities, indicating no reliable QTLs, they were located on the same region of which a major gene for flesh texture is located (endoPG) and on the same region where a new DNA test for distinguishing slow-melting flesh texture is located. The results of QTL analysis by removing the

effects of all the LGs, with the exception of LG 4 flesh firmness and LG 5 in for TA and pH, increased the confidence of the QTLs associated with these traits on LG 4 and 5.

These results are important and they provide interesting highlights about QTL analysis in the ongoing Arkansas peach breeding program. To complete a QTL analysis with success, the chosen statistical procedure should be adjusted to the design of the germplasm to be analyzed. In this case, the PBA approach was chosen and applied with successful results. The confirmation or discovery of new QTLs will facilitate the design of DNA tests flanking the genomic region of the QTL, which after validation will make possible the application of molecular tools like MAB in the form of marker-assisted parent selection (MAPS) and marker-assisted seedling selection (MASS) increasing the efficiency of the breeding process.

Literature Cited

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

Bernardo, R. 2010. Mapping quantitative trait loci, p. 85-112. In R. Bernardo (ed.). Breeding for quantitative traits in plants. Stemma Press, Woodbury, MN.

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

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

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

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

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

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

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

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

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

Crisosto, C. and A. Kader. 2000. Peach postharvest quality maintenance guidelines. Department of Pomology, University of California. Davis.

Crisosto, C., R. Johnson, T. Dejong, and K. Ray. 1997. Orchard factors affecting postharvest stone fruit quality. HortScience 32:820-823.

Crisosto, C. and D. Valero. 2008. Harvesting and postharvest handling if peaches for flesh market, p. 575-596. In: D.R. Layne and D. Bassi (eds.), The peach: botany, production, and uses. CAB Intl, Oxfordshire, UK.

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

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

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

Etienne, C., E. Dirlewanger, P. Cosson, L. Savanella-Dumas, C. Bodenes, C. Plomion, C. Rothan, and J. Kervella. 2002. QTLs and genes controlling peach fruit quality. Acta Hort. 592 253-258.

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

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

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

Gasic, K., T. Gradziel, C. Crisosto, D. Byrne, and J. Clark. 2010. Phenotyping in peach. 15 March 2013 < https://www.rosbreed.org/sites/default/files/files/RosBREED_2010-Phenotyping_protocol_P_persica.pdf>

Genome Data for Rosaceae. 2015. Prunus persica Whole Genome v1.0 Assembly & Annotation. 25 August 2015. < http://rosaceae.org/gb/gbrowse/prunus_persica/>

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

Illa, E., I. Eduardo, J. Audergon, F. Barale, E. Dirlewanger, X. Li, A. Moing, P. Lambert, L. Le Dantec, Z. Gao, J. Poessel, C. Pozzi, L. Rossini, A. Vecchietti, P. Arús, and W. Howard. 2011.

Saturating the *Prunus* (stone fruits) genome with candidate genes for fruit quality. Mol. Breeding 28:667-982.

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

Karakurt Y., D. Huber, and W. Sherman. 2000. Quality characteristics of melting and non-melting flesh peach genotypes. J. Sci. Food. Agri. 80:1848-1853.

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

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

Peace, C. and J. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae, p. 19-53. In K. Folta and S. Gardiner (eds). Genetics and genomics of Rosaceae. Springer Science + Business Media, New York.

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

Peace, C., C. Crisosto, and T. Gradziel. 2005a. Endopolygalacturonase: a candidate gene for freestone and melting flesh in peach. Mol. Breeding 16:21–31.

Peace, C., R. Ahmad, T. Gradziel, A. Dandekar, and C. Crisosto. 2005b. The use of molecular genetics to improve peach and nectarine post-storage quality. Acta Hort. 682:403-409.

Pressey, R. and J.Avants.1973. Separation and characterization of endopolygalacturonase and exopolygalacturonase from peaches. Plant Physiol. 52:252-256.

Pressey, R., and J. Avants. 1976. Pear polygalacturonases. Phytochem. 15:1349–1351.

Romeu, J., A. Monforte, G. Sanchez, A. Granell, J. Garcia-Brunton, M. Bedenes, and G. Rios. 2014. Quantitative trait loci affecting reproductive phenology in peach. BMC Plant Biology 14:52-67.

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

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

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

Testolin, R. and G. Cipriani. 2010. Molecular markers for germplasm identification and characterization. Acta Hort. 859:59-72.

Tonutti, P., C. Bonghi, B. Ruperti, G. Tornielli, and A. Ramina. 1997. Ethylene evolution and 1-aminocyclopropane-1carboxylate oxidase gene expression during early development and ripening of peach fruit. J. Amer. Hort. Sci. 122:642-647.

Valero, C., C. Crisosto, and D. Slaughter. 2007. Relationship between non-destructive firmness measurements and commercially important ripening fruit stages for peaches, nectarines, and plums. Postharvest Biol. Technol. 44:248-253.

Verde, I., N. Bassil, S. Scalabrin, B. Gilmore, C. Lawley, K. Gasic, D. Micheleti, U. Rosyara, F. Cattonaro, E. Vendramin, D. Main, V. Aramini, A. Blas, T. Mockler, D. Bryant, L. Wilhelm, M. Troggio, B. Sosinski, M. Aranzana, P. Arus, A. Iezzoni, M. Morgante, and C. Peace. 2012. Development and Evaluation of a 9K SNP Array for Peach by Internationally Coordinated SNP Detection and Validation in Breeding Germplasm. PLoS ONE 7(4):1-13.

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

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

Appendix A. Trace Plots of Titratable Acidity and pH per Year

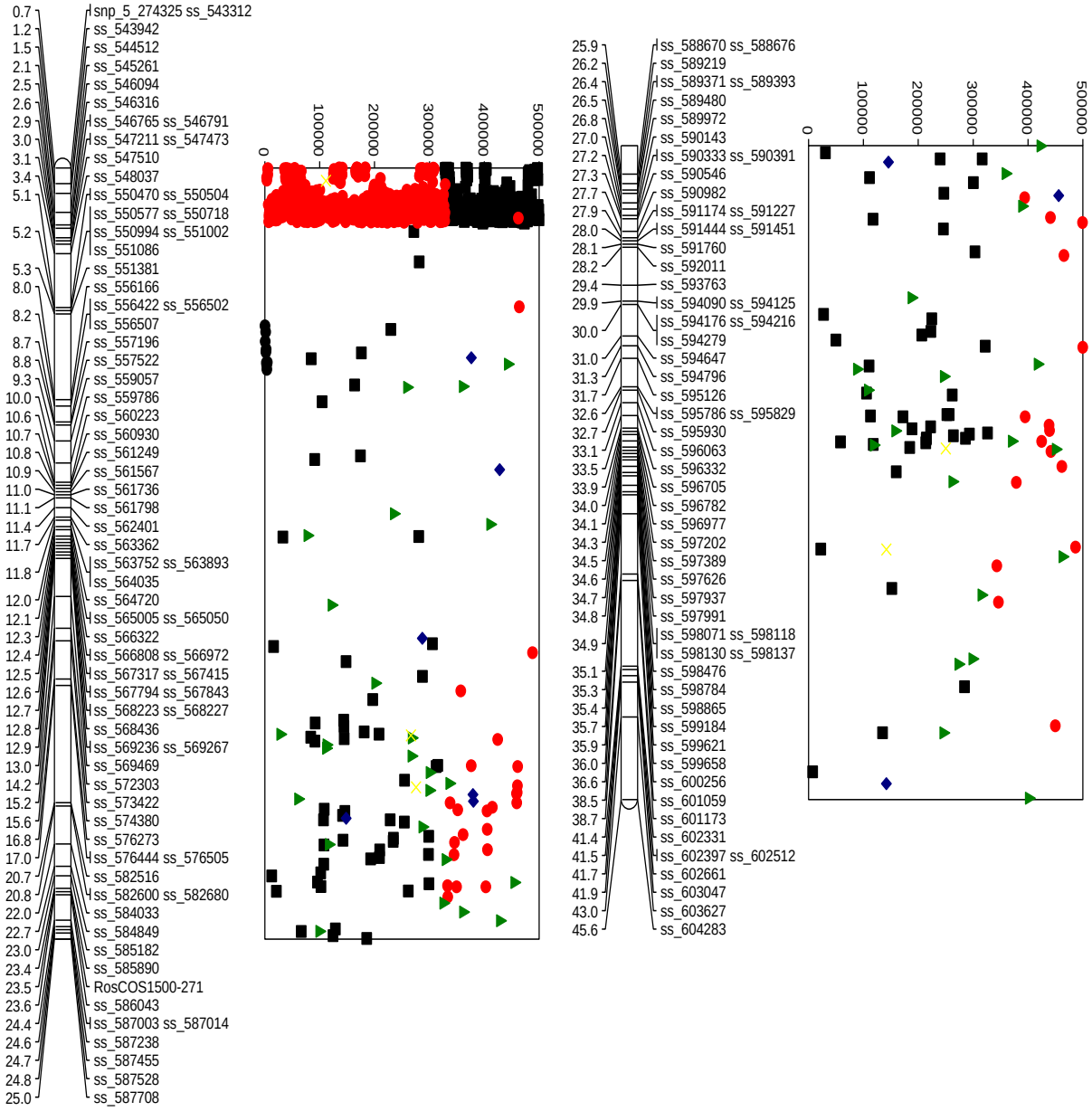

Fig. A.1. Trace plot of titratable acidity (%) of 2011 showing convergence of 500,000 Markov chain Monte Carlo (MCMC) of linkage group 5. Stable patterns indicate good mixing of Markov chain. Different colors and shapes do not have a biological interpretation. Linkage group 5 is divided in two sections to fit on one page.

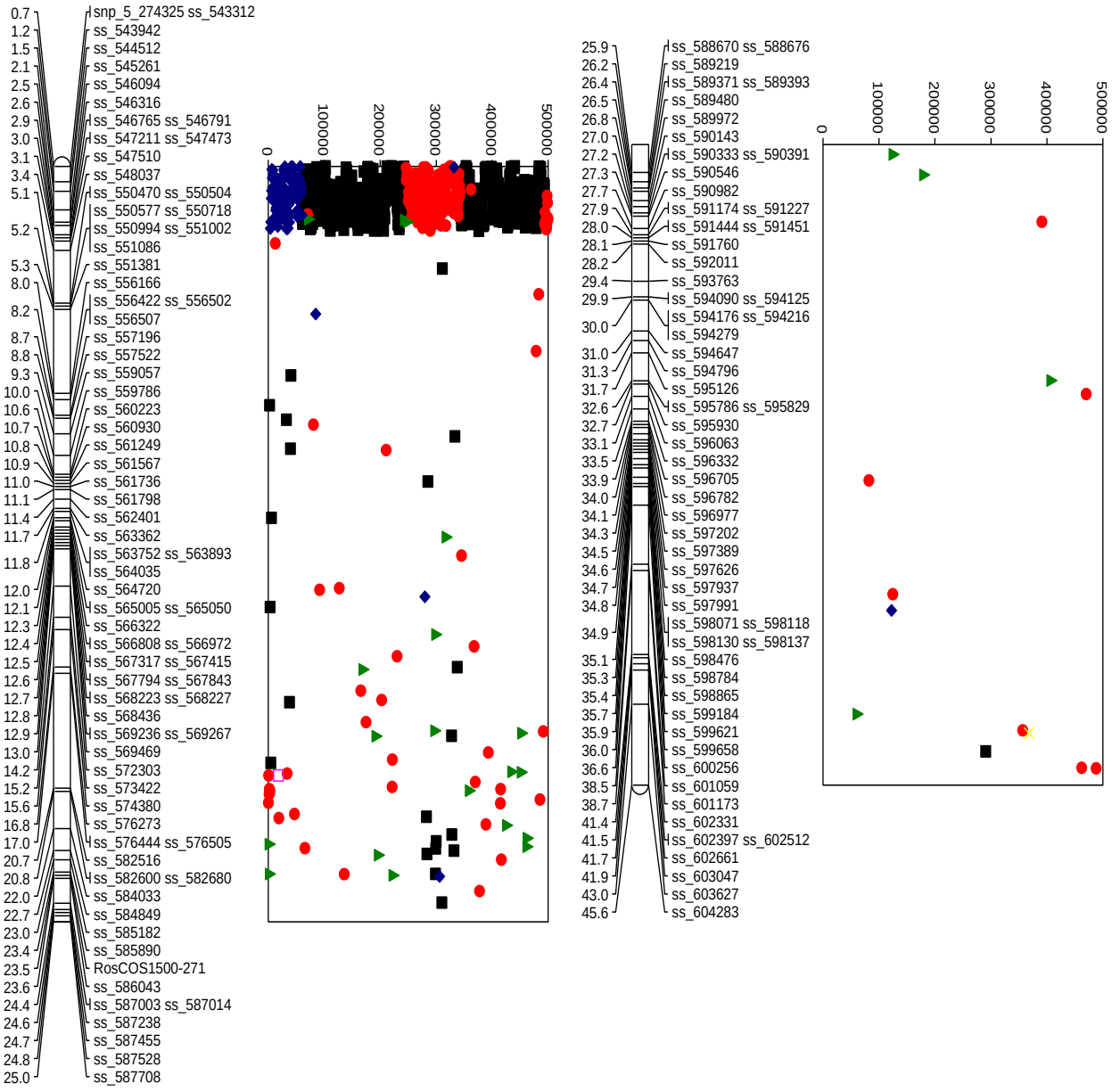

Fig. A.2. Trace plot of titratable acidity (%) of 2012 showing convergence of 500,000 Markov chain Monte Carlo (MCMC) of linkage group 5. Stable patterns indicate good mixing of Markov chain. Different colors and shapes do not have a biological interpretation. Linkage group 5 is divided in two sections to fit on one page.

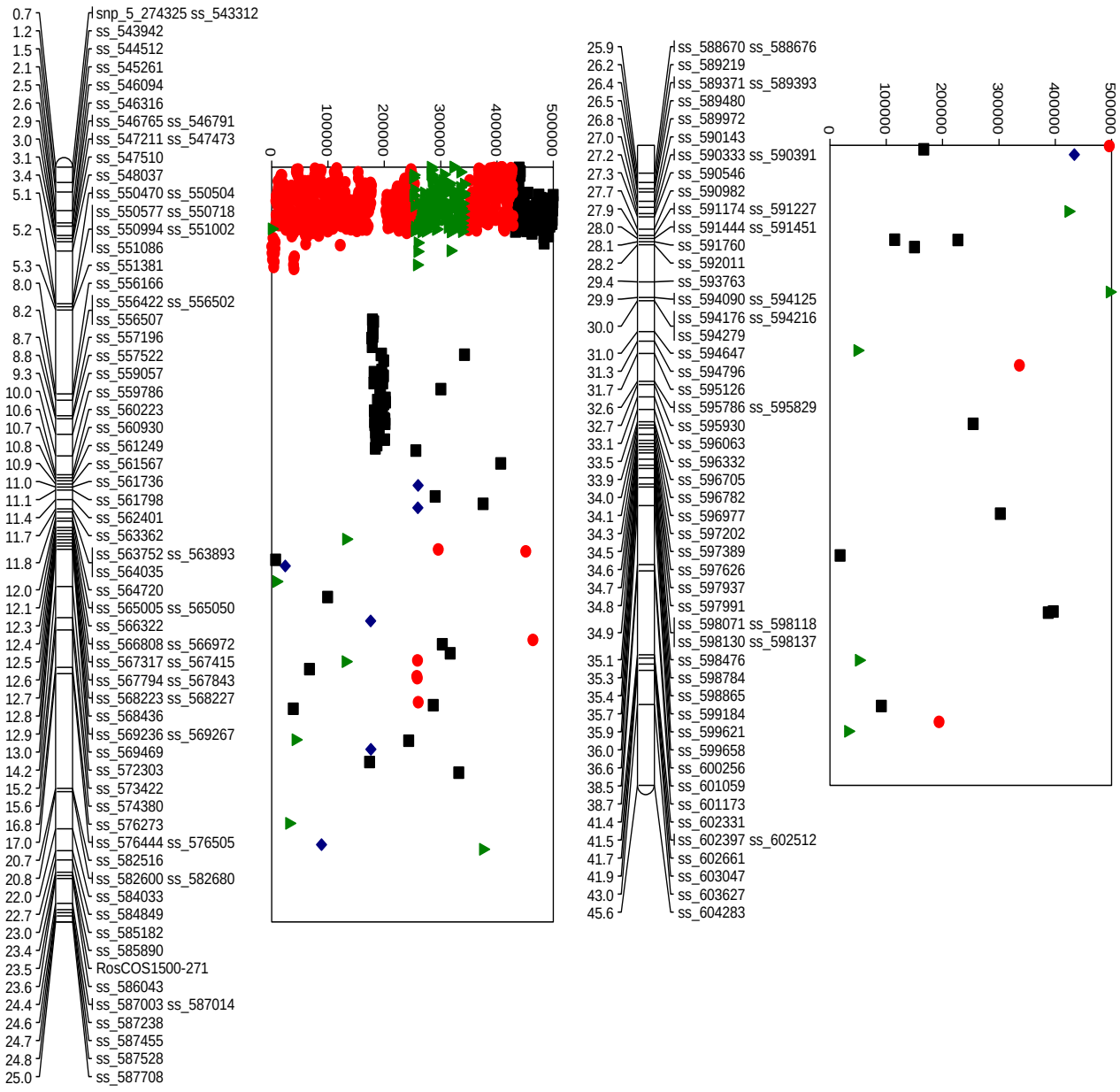

Fig. A.3. Trace plot of titratable acidity (%) of 2013 showing convergence of 500,000 Markov chain Monte Carlo (MCMC) of linkage group 5. Stable patterns indicate good mixing of Markov chain. Different color and shapes do not have a biological interpretation. Linkage group 5 is divided in two sections to fit on one page.

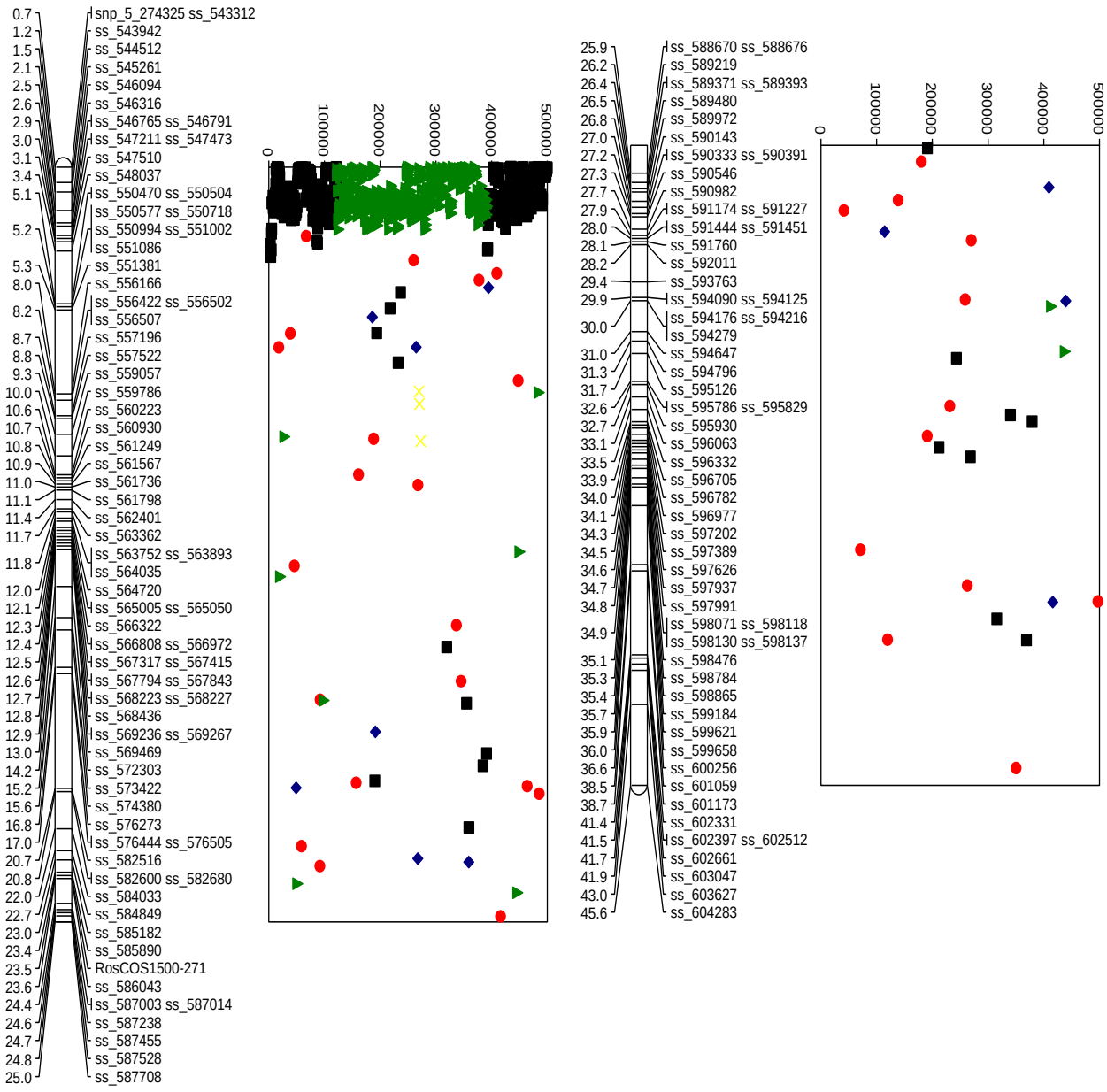

Fig. A.4. Trace plot of pH of 2011 (%) showing convergence of 500,000 Markov chain Monte Carlo (MCMC) of linkage group 5. Stable patterns indicate good mixing of Markov chain. Different color and shapes do not have a biological interpretation. Linkage group 5 is divided in two sections to fit on one page.

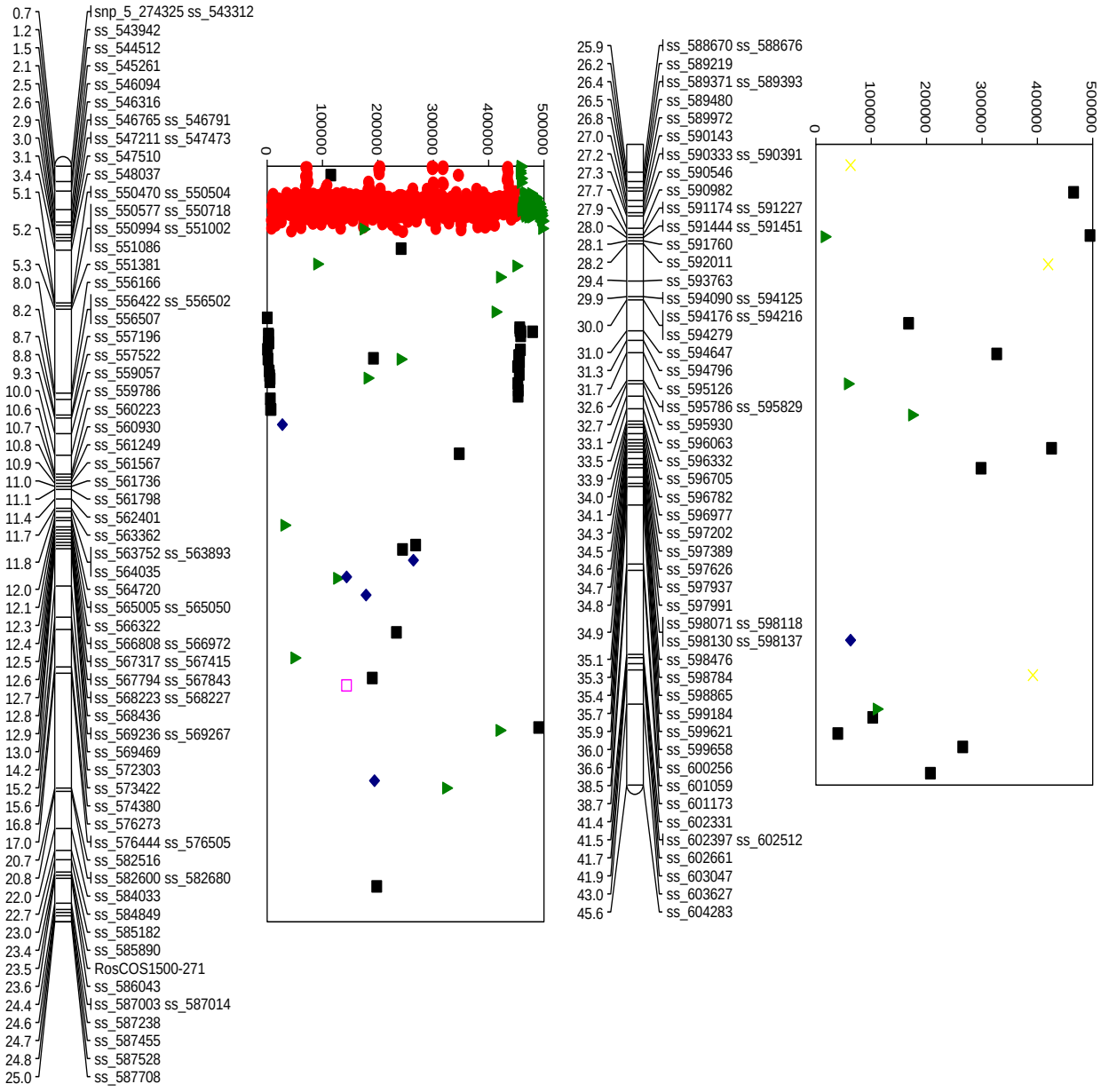

Fig. A.5. Trace plot of pH of 2012 (%) showing convergence of 500,000 Markov chain Monte Carlo (MCMC) of linkage group 5. Stable patterns indicate good mixing of Markov chain. Different color and shapes do not have a biological interpretation. Linkage group 5 is divided in two sections to fit on one page.

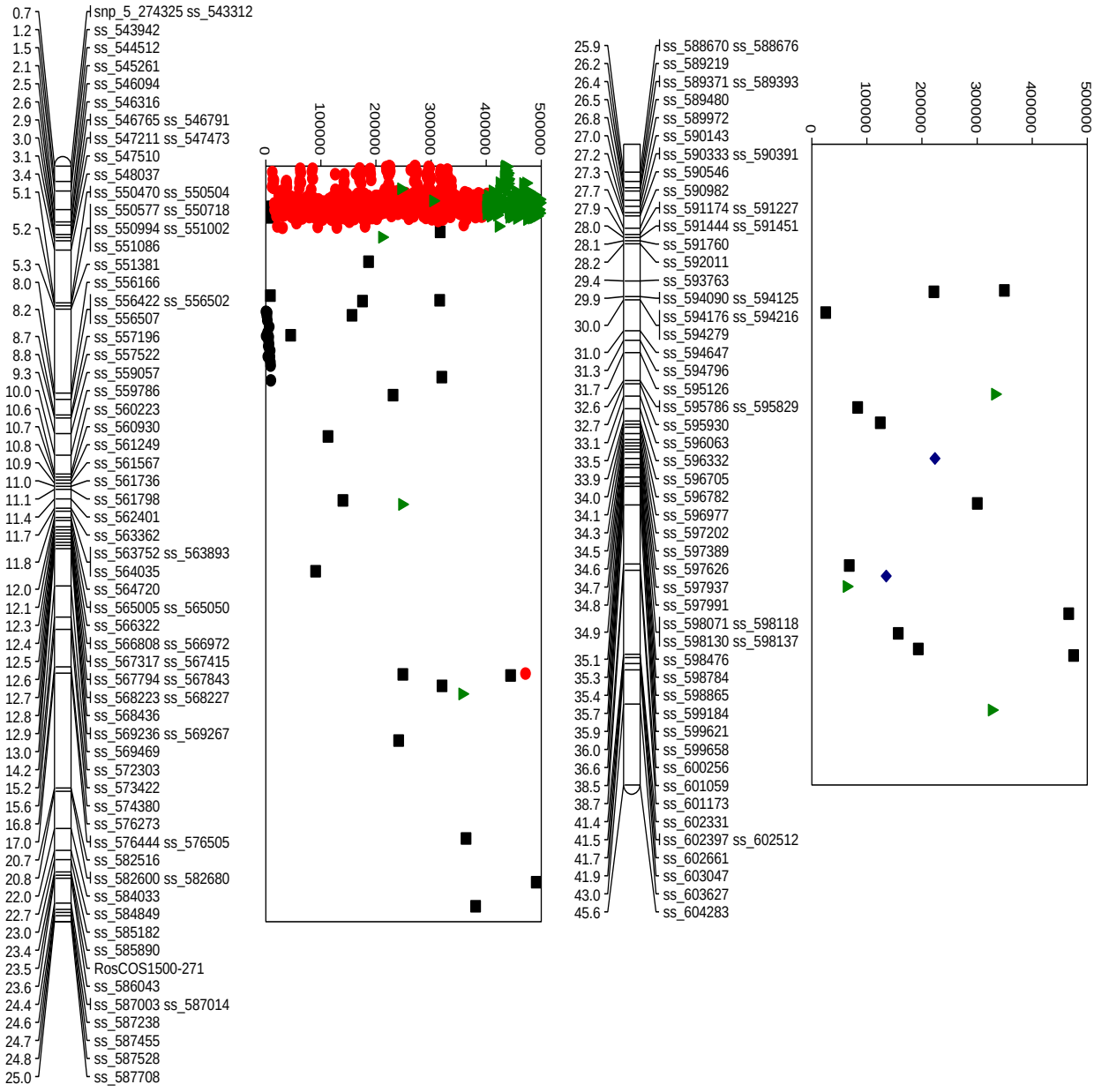

Fig. A.6. Trace plot of pH of 2013 (%) showing convergence of 500,000 Markov chain Monte Carlo (MCMC) of linkage group 5. Stable patterns indicate good mixing of Markov chain. Different color and shapes do not have a biological interpretation. Linkage group 5 is divided in two sections to fit on one page.

Appendix B. Detailed Information of Breeding Values of Titratable Acidity and pH per Year of the Arkansas RosBREED Pedigree

Table B.1. Breeding values per trait and year of the Arkansas RosBREED pedigree.

Accession	Titratable acidity (%)				pH			
	2011	2012	2013	Average	2011	2012	2013	Average
104325	0.30	0.15	0.08	0.22	-0.20	-0.34	-0.24	-0.36
752002002	0.10	0.03	0.04	0.07	-0.10	-0.15	-0.11	-0.18
10A4	0.23	0.11	0.09	0.16	-0.24	-0.26	-0.21	-0.36
22A5	0.22	0.10	0.09	0.15	-0.25	-0.27	-0.23	-0.34
2W68W	0.30	0.14	0.12	0.22	-0.29	-0.32	-0.29	-0.36
4A_4	0.20	0.10	0.04	0.15	-0.18	-0.23	-0.17	-0.29
A_130	0.29	0.10	0.08	0.22	-0.26	-0.24	-0.21	-0.37
A_145	0.18	0.06	0.02	0.13	-0.11	-0.19	-0.10	-0.22
A_154	0.12	0.05	0.01	0.08	-0.10	-0.12	-0.10	-0.18
A_172	0.27	0.13	0.13	0.24	-0.24	-0.26	-0.33	-0.42
A_176	0.27	0.12	0.14	0.24	-0.24	-0.26	-0.34	-0.42
A_178	0.36	0.17	0.14	0.27	-0.35	-0.34	-0.33	-0.45
A_18	0.26	0.13	0.11	0.20	-0.26	-0.30	-0.28	-0.38
A_190	0.30	0.15	0.15	0.22	-0.32	-0.35	-0.33	-0.42
A_21	0.24	0.12	0.13	0.16	-0.29	-0.31	-0.30	-0.40
A_224	0.07	0.02	-0.02	0.06	-0.03	-0.06	-0.03	-0.11
A_232	0.30	0.15	0.15	0.25	-0.28	-0.29	-0.36	-0.44
A_24	0.25	0.12	0.11	0.19	-0.26	-0.28	-0.27	-0.37
A_270	0.33	0.15	0.12	0.24	-0.30	-0.34	-0.27	-0.40
A_333	0.27	0.13	0.09	0.20	-0.21	-0.15	-0.24	-0.34
A_334	0.39	0.17	0.17	0.29	-0.41	-0.41	-0.36	-0.47
A_367	0.06	0.01	0.05	0.05	-0.09	-0.15	-0.09	-0.13
A_371	0.20	0.06	-0.11	0.11	-0.05	-0.14	-0.16	-0.23
A_374	0.26	0.05	0.05	0.16	-0.18	-0.14	-0.19	-0.27
A_392	0.00	0.01	0.01	0.00	0.00	-0.01	0.00	0.00
A_402	0.33	0.16	0.14	0.24	-0.34	-0.34	-0.33	-0.43
A_405	0.38	0.20	0.19	0.28	-0.39	-0.44	-0.38	-0.46
A_419	0.28	0.15	0.08	0.21	-0.29	-0.32	-0.27	-0.35
A_427	0.26	0.11	0.06	0.18	-0.23	-0.28	-0.23	-0.34
A_433	-0.08	-0.07	-0.09	-0.07	0.12	0.15	0.13	0.10
A_434	0.31	0.17	0.11	0.21	-0.28	-0.11	-0.23	-0.37

Table B.1. Breeding values per trait and year of the Arkansas RosBREED germplasm (Cont.).

Accession	Titratable acidity (%)				pH			
	2011	2012	2013	Average	2011	2012	2013	Average
A_441	0.24	0.12	0.07	0.18	-0.24	-0.31	-0.24	-0.32
A_500	0.27	0.05	0.11	0.15	-0.27	-0.20	-0.27	-0.36
A_565	0.30	0.14	0.09	0.22	-0.29	-0.36	-0.29	-0.37
A_604	0.32	0.16	0.14	0.24	-0.24	-0.08	-0.31	-0.39
A_657	0.39	0.18	0.17	0.29	-0.41	-0.36	-0.36	-0.47
A_663	0.39	0.18	0.21	0.29	-0.43	-0.45	-0.41	-0.48
A_665	-0.03	-0.06	-0.07	-0.03	0.06	0.01	0.01	0.00
A_672	0.39	0.22	0.16	0.29	-0.42	-0.49	-0.40	-0.48
A_699	0.00	-0.02	-0.04	0.00	0.00	0.02	0.01	0.00
A_708	0.00	0.01	-0.01	0.00	0.02	0.23	0.08	0.01
A_716	0.15	0.04	-0.01	0.08	-0.14	-0.23	-0.09	-0.19
A_717	0.36	0.18	0.17	0.27	-0.34	-0.02	-0.37	-0.44
A_760	-0.01	-0.13	-0.03	-0.06	0.08	0.20	0.05	0.07
A_763	0.00	-0.01	0.00	0.00	0.00	0.00	0.00	0.00
A_765	0.00	-0.02	0.00	0.00	-0.01	0.05	0.00	0.00
A_772	0.00	-0.01	0.00	0.00	0.00	-0.01	0.00	0.00
A_773	0.38	0.18	0.00	0.23	-0.16	-0.39	-0.36	-0.47
A_776	0.00	-0.03	0.00	0.00	-0.02	0.00	0.00	0.00
A_778	0.39	0.21	0.21	0.29	-0.43	-0.40	-0.41	-0.47
A_783	0.39	0.19	0.17	0.29	-0.41	-0.04	-0.40	-0.47
A_789	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
Admiral Dewey	0.22	0.09	0.05	0.14	-0.18	-0.21	-0.18	-0.27
Alameda	0.21	0.09	0.05	0.15	-0.19	-0.23	-0.16	-0.28
Allgold	0.17	0.12	0.15	0.10	-0.36	-0.35	-0.33	-0.46
AR_Pop_1_01	0.00	-0.01	-0.01	0.00	0.00	0.01	0.00	0.00
AR_Pop_1_02	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop_1_03	0.39	0.23	0.21	0.29	-0.43	-0.50	-0.42	-0.48
AR_Pop_1_04	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop_1_05	0.39	0.22	0.16	0.29	-0.43	-0.49	-0.40	-0.48
AR_Pop_1_06	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop_1_07	0.39	0.23	0.21	0.29	-0.43	-0.49	-0.42	-0.48

Table B.1. Breeding values per trait and year of the Arkansas RosBREED pedigree (Cont.).

Accession	Titratable acidity (%)				pH			
	2011	2012	2013	Average	2011	2012	2013	Average
AR_Pop_1_08	0.39	0.23	0.21	0.29	-0.43	-0.49	-0.41	-0.48
AR_Pop_1_09	0.39	0.23	0.21	0.29	-0.43	-0.49	-0.42	-0.48
AR_Pop_1_10	0.39	0.23	0.21	0.29	-0.43	-0.50	-0.42	-0.48
AR_Pop_1_11	0.39	0.23	0.16	0.29	-0.43	-0.49	-0.40	-0.48
AR_Pop_1_12	0.39	0.23	0.16	0.29	-0.43	-0.49	-0.40	-0.48
AR_Pop_1_13	0.39	0.23	0.21	0.29	-0.43	-0.49	-0.41	-0.48
AR_Pop_1_14	0.01	0.09	-0.09	0.02	-0.03	-0.31	0.03	-0.17
AR_Pop_1_15	0.39	0.22	0.16	0.29	-0.43	-0.49	-0.40	-0.48
AR_Pop_1_16	0.00	-0.01	0.00	0.00	0.00	0.01	0.00	0.00
AR_Pop_1_17	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop_1_18	0.29	0.18	0.09	0.26	-0.41	-0.46	-0.35	-0.46
AR_Pop_1_19	0.00	-0.01	0.00	0.00	0.00	0.01	0.00	0.00
AR_Pop_1_20	0.39	0.23	0.21	0.29	-0.43	-0.49	-0.42	-0.48
AR_Pop_1_21	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop_1_22	0.00	-0.01	-0.05	0.00	0.00	0.01	0.01	0.00
AR_Pop_1_23	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop_1_24	0.39	0.23	0.21	0.29	-0.43	-0.49	-0.41	-0.48
AR_Pop_1_25	0.39	0.23	0.16	0.29	-0.43	-0.49	-0.40	-0.48
AR_Pop_1_26	0.00	-0.01	0.00	0.00	0.00	0.01	0.00	0.00
AR_Pop_1_27	0.00	-0.01	0.00	0.00	0.00	0.01	0.00	0.00
AR_Pop_1_28	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop_1_29	0.39	0.22	0.16	0.29	-0.43	-0.49	-0.40	-0.48
AR_Pop_1_30	0.35	0.20	0.16	0.27	-0.34	-0.45	-0.39	-0.47
AR_Pop_1_31	0.00	-0.01	0.00	0.00	0.00	0.01	0.00	0.00
AR_Pop_1_32	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop_1_33	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop_1_34	0.00	-0.01	0.00	0.00	0.00	0.01	0.00	0.00
AR_Pop_1_35	0.00	-0.01	0.00	0.00	0.00	0.01	0.00	0.00
AR_Pop_1_36	0.00	-0.01	-0.01	0.00	0.00	0.01	0.00	0.00
AR_Pop_1_37	0.39	0.22	0.16	0.29	-0.43	-0.49	-0.40	-0.48
AR_Pop_1_38	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00

Table B.1. Breeding values per trait and year of the Arkansas RosBREED pedigree (Cont.).

Accession	Titratable acidity (%)				pH			
	2011	2012	2013	Average	2011	2012	2013	Average
AR_Pop_1_39	0.39	0.23	0.16	0.29	-0.43	-0.49	-0.40	-0.48
AR_Pop_1_40	-0.26	-0.23	-0.20	-0.18	0.21	0.48	0.31	0.22
AR_Pop_1_41	0.19	0.03	0.08	0.06	-0.06	-0.22	-0.20	-0.08
AR_Pop_1_42	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop_1_43	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop_1_44	0.00	-0.01	0.00	0.00	0.00	0.01	0.00	0.00
AR_Pop_1_45	0.39	0.23	0.21	0.29	-0.43	-0.49	-0.41	-0.48
AR_Pop_1_46	0.00	-0.01	0.00	0.00	0.00	0.01	0.00	0.00
AR_Pop_1_47	0.39	0.23	0.21	0.29	-0.43	-0.49	-0.42	-0.48
AR_Pop_1_48	0.39	0.22	0.16	0.29	-0.43	-0.49	-0.40	-0.48
AR_Pop_1_49	0.00	-0.01	-0.05	0.00	0.00	0.01	0.02	0.00
AR_Pop0801_01	0.39	0.18	0.20	0.29	-0.42	-0.06	-0.41	-0.47
AR_Pop0801_02	0.00	-0.02	-0.04	0.00	0.00	0.02	0.01	0.00
AR_Pop0801_03	0.39	0.09	0.19	0.28	-0.26	-0.50	-0.41	-0.48
AR_Pop0801_04	0.00	-0.02	0.00	0.00	-0.01	0.45	0.01	0.00
AR_Pop0801_05	0.38	0.09	0.16	0.26	-0.33	-0.36	-0.38	-0.46
AR_Pop0801_06	0.39	0.15	0.13	0.29	-0.40	-0.02	-0.40	-0.46
AR_Pop0801_07	0.10	0.07	0.06	0.12	-0.22	0.41	-0.05	0.00
AR_Pop0801_08	0.00	-0.03	-0.03	0.00	0.00	0.24	0.01	0.00
AR_Pop0801_09	0.00	-0.02	0.00	0.00	-0.01	0.43	0.01	0.00
AR_Pop0801_10	0.00	-0.02	-0.01	0.00	-0.01	0.41	0.01	0.00
AR_Pop0801_11	0.39	0.18	0.20	0.29	-0.43	-0.09	-0.41	-0.47
AR_Pop0801_12	0.00	-0.02	0.00	0.00	-0.01	0.45	0.01	0.00
AR_Pop0801_13	0.00	-0.02	-0.04	0.00	0.00	0.03	0.01	0.00
AR_Pop0801_14	0.39	0.18	0.20	0.29	-0.42	-0.07	-0.41	-0.47
AR_Pop0801_15	0.39	0.20	0.12	0.29	-0.42	-0.49	-0.37	-0.48
AR_Pop0801_16	0.00	-0.02	-0.04	0.00	0.00	0.02	0.01	0.00
AR_Pop0803_01	0.38	0.16	0.12	0.25	-0.38	-0.28	-0.35	-0.43
AR_Pop0803_02	0.03	-0.02	0.04	-0.01	0.05	0.25	0.13	0.23
AR_Pop0803_03	-0.02	-0.01	0.06	-0.01	0.10	0.16	-0.20	0.06
AR_Pop0803_04	0.39	0.21	0.21	0.29	-0.43	-0.45	-0.41	-0.48

Table B.1. Breeding values per trait and year of the Arkansas RosBREED pedigree (Cont.).

Accession	Titratable acidity (%)				pH			
	2011	2012	2013	Average	2011	2012	2013	Average
AR_Pop0803_05	0.39	0.21	0.21	0.29	-0.43	-0.45	-0.41	-0.48
AR_Pop0803_06	0.01	-0.02	0.01	0.00	-0.03	0.08	-0.01	-0.02
AR_Pop0803_07	0.39	0.21	0.21	0.29	-0.43	-0.45	-0.41	-0.48
AR_Pop0803_08	0.20	0.09	0.11	0.14	-0.22	-0.17	-0.22	-0.23
AR_Pop0803_09	0.39	0.21	0.21	0.29	-0.43	-0.44	-0.41	-0.48
AR_Pop0803_10	0.39	0.21	0.21	0.29	-0.43	-0.44	-0.41	-0.48
AR_Pop0803_11	0.00	-0.02	0.00	0.00	-0.01	0.10	0.01	0.00
AR_Pop0803_12	0.00	-0.02	0.00	0.00	-0.01	0.10	0.01	0.00
AR_Pop0803_13	0.39	0.21	0.21	0.29	-0.43	-0.44	-0.41	-0.48
AR_Pop0803_14	0.39	0.21	0.21	0.29	-0.43	-0.45	-0.41	-0.48
AR_Pop0803_15	0.24	0.12	0.11	0.18	-0.25	-0.26	-0.24	-0.32
AR_Pop0813_01	0.00	0.00	-0.05	0.00	0.00	-0.02	0.01	0.00
AR_Pop0813_02	0.00	0.00	-0.05	0.00	0.00	-0.02	0.01	0.00
AR_Pop0813_03	0.00	0.00	0.00	0.00	0.00	-0.02	0.01	0.00
AR_Pop0813_04	0.00	0.00	0.00	0.00	0.00	-0.02	0.00	0.00
AR_Pop0813_05	0.00	0.00	-0.05	0.00	0.00	-0.02	0.01	0.00
AR_Pop0813_06	0.39	0.21	0.21	0.29	-0.42	-0.47	-0.41	-0.48
AR_Pop0813_07	0.00	0.00	-0.05	0.00	0.00	-0.02	0.01	0.00
AR_Pop0813_08	0.39	0.21	0.16	0.29	-0.42	-0.47	-0.40	-0.48
AR_Pop0813_09	0.00	0.00	-0.05	0.00	0.00	-0.02	0.01	0.00
AR_Pop0813_10	0.00	0.00	0.00	0.00	0.00	-0.02	0.00	0.00
AR_Pop0813_11	0.39	0.21	0.21	0.29	-0.42	-0.47	-0.41	-0.48
AR_Pop0813_12	0.00	0.00	0.00	0.00	0.00	-0.02	0.00	0.00
AR_Pop0817_01	0.18	-0.01	-0.10	-0.05	-0.20	-0.04	-0.09	-0.05
AR_Pop0817_02	0.06	0.03	0.00	0.04	-0.14	-0.14	-0.11	-0.12
AR_Pop0817_03	0.12	0.07	-0.02	0.10	-0.10	-0.09	-0.05	-0.07
AR_Pop0817_04	0.16	0.06	-0.07	0.01	-0.28	-0.09	-0.06	-0.14
AR_Pop0817_05	0.07	0.02	-0.01	0.03	-0.14	-0.11	-0.11	-0.11
AR_Pop0817_06	0.11	0.06	-0.06	0.01	-0.11	-0.07	0.02	-0.05
AR_Pop0817_07	0.22	0.01	-0.08	-0.01	-0.22	-0.04	-0.03	-0.07
AR_Pop0817_08	0.32	0.11	-0.02	0.15	-0.27	-0.08	-0.18	-0.17

Table B.1. Breeding values per trait and year of the Arkansas RosBREED pedigree (Cont.).

Accession	Titratable acidity (%)				pH			
	2011	2012	2013	Average	2011	2012	2013	Average
AR_Pop0817_09	0.27	0.06	-0.06	0.06	-0.24	-0.05	-0.09	-0.13
AR_Pop0819_01	0.39	0.22	0.19	0.29	-0.33	-0.22	-0.33	-0.46
AR_Pop0819_02	-0.01	-0.04	-0.19	-0.06	0.18	0.06	0.05	0.01
AR_Pop0819_03	0.00	0.00	0.00	0.00	0.08	0.04	0.01	0.00
AR_Pop0819_04	-0.01	-0.04	-0.19	-0.06	0.18	0.06	0.05	0.01
AR_Pop0819_05	0.00	0.00	0.00	0.00	0.08	0.04	0.01	0.00
AR_Pop0819_06	0.00	0.00	0.00	0.00	0.08	0.04	0.01	0.00
AR_Pop0819_07	0.00	0.00	0.00	0.00	0.08	0.04	0.01	0.00
AR_Pop0819_08	0.38	0.19	-0.01	0.24	-0.23	-0.20	-0.29	-0.46
AR_Pop0819_09	0.39	0.22	0.19	0.29	-0.33	-0.21	-0.33	-0.46
AR_Pop0819_10	-0.01	-0.04	-0.19	-0.06	0.18	0.06	0.05	0.01
AR_Pop0819_11	-0.01	-0.04	-0.19	-0.06	0.18	0.06	0.05	0.01
AR_Pop0819_12	-0.01	-0.04	-0.19	-0.06	0.18	0.06	0.05	0.01
AR_Pop0819_13	0.01	-0.03	-0.19	-0.05	0.17	0.06	0.03	0.00
AR_Pop0819_14	0.39	0.22	0.19	0.29	-0.33	-0.22	-0.33	-0.46
AR_Pop0819_15	-0.01	-0.04	-0.19	-0.06	0.18	0.06	0.05	0.01
AR_Pop0819_16	-0.01	-0.04	-0.19	-0.06	0.18	0.06	0.05	0.01
AR_Pop0819_17	0.00	0.00	0.00	0.00	0.08	0.04	0.01	0.00
AR_Pop0819_18	-0.01	-0.04	-0.19	-0.06	0.18	0.06	0.05	0.01
AR_Pop0819_19	-0.01	-0.04	-0.19	-0.06	0.18	0.06	0.05	0.01
AR_Pop0819_20	-0.01	-0.03	-0.19	-0.06	0.17	0.05	0.05	0.00
AR_Pop0819_21	0.39	0.22	0.19	0.29	-0.33	-0.21	-0.33	-0.46
AR_Pop0819_22	0.00	0.00	0.00	0.00	0.08	0.04	0.01	0.00
AR_Pop0819_23	0.00	0.00	0.00	0.00	0.08	0.04	0.01	0.00
AR_Pop0825_01	-0.39	-0.22	-0.20	-0.29	0.42	0.50	0.41	0.48
AR_Pop0825_02	0.38	0.09	0.17	0.23	-0.35	-0.30	-0.36	-0.41
AR_Pop0825_03	-0.01	-0.12	-0.03	-0.06	0.08	0.20	0.05	0.07
AR_Pop0825_04	0.38	0.09	0.17	0.23	-0.35	-0.30	-0.36	-0.41
AR_Pop0825_05	0.38	0.09	0.17	0.23	-0.35	-0.30	-0.36	-0.41
AR_Pop0825_06	-0.01	-0.12	-0.03	-0.06	0.08	0.20	0.05	0.07
AR_Pop0825_07	0.38	0.06	0.14	0.23	-0.34	-0.27	-0.30	-0.40

Table B.1. Breeding values per trait and year of the Arkansas RosBREED pedigree (Cont.).

Accession	Titratable acidity (%)				pH			
	2011	2012	2013	Average	2011	2012	2013	Average
AR_Pop0825_08	-0.01	-0.12	-0.03	-0.06	0.08	0.20	0.05	0.07
AR_Pop0825_09	-0.10	-0.17	-0.08	-0.14	0.32	0.26	0.12	0.12
AR_Pop0825_10	-0.38	-0.22	-0.19	-0.28	0.39	0.48	0.41	0.43
AR_Pop0825_11	-0.39	-0.22	-0.20	-0.29	0.42	0.50	0.41	0.48
AR_Pop0825_12	-0.39	-0.22	-0.20	-0.29	0.42	0.50	0.41	0.48
AR_Pop0825_13	-0.08	-0.10	-0.05	-0.11	0.18	0.07	0.06	0.03
AR_Pop0825_14	0.00	-0.02	0.00	0.00	0.00	0.00	0.00	0.00
AR_Pop0825_15	0.38	0.09	0.17	0.23	-0.35	-0.30	-0.36	-0.41
AR_Pop0825_16	0.38	0.09	0.17	0.23	-0.35	-0.30	-0.36	-0.41
AR_Pop0825_17	0.00	-0.02	0.00	0.00	0.00	0.00	0.00	0.00
AR_Pop0825_18	0.00	-0.07	-0.01	-0.02	0.03	0.10	0.03	0.03
Arrington	0.38	0.20	0.18	0.29	-0.36	-0.37	-0.39	-0.47
Australian Muir	0.06	0.09	0.04	0.03	-0.13	-0.23	-0.18	-0.18
Belle	0.22	0.09	0.04	0.14	-0.18	-0.22	-0.18	-0.27
Bradley	0.39	0.20	0.20	0.29	-0.38	-0.43	-0.41	-0.48
Candoka	0.28	0.13	0.07	0.21	-0.20	-0.34	-0.21	-0.33
Chinese Cling	0.37	0.20	0.05	0.29	-0.23	-0.46	-0.18	-0.41
Chiripa	-0.08	-0.07	-0.08	-0.07	0.11	0.13	0.12	0.10
Cumberland	0.39	0.15	0.04	0.29	-0.29	-0.34	-0.17	-0.45
D42_13W	0.19	0.11	0.05	0.13	-0.19	-0.27	-0.19	-0.29
Delicioso	0.06	0.01	-0.02	0.02	-0.03	-0.04	-0.01	-0.07
Dix_16_3	0.28	0.14	0.05	0.18	-0.22	-0.29	-0.18	-0.33
Dix_22A_5	0.25	0.11	0.05	0.17	-0.21	-0.25	-0.19	-0.31
Dix_58_6	0.23	0.09	0.05	0.15	-0.18	-0.22	-0.17	-0.27
Dixon	0.12	0.14	0.04	0.04	-0.14	-0.30	-0.18	-0.23
dummy003	0.26	0.13	0.08	0.20	-0.20	-0.31	-0.26	-0.38
dummy004	0.29	0.05	0.10	0.22	-0.15	-0.36	-0.29	-0.38
dummy005	0.29	0.05	0.08	0.18	-0.21	-0.22	-0.27	-0.35
dummy006	0.30	0.16	0.04	0.22	-0.31	-0.34	-0.25	-0.38
dummy007	0.29	0.11	0.04	0.18	-0.26	-0.18	-0.24	-0.34
dummy008	0.06	0.00	0.00	0.01	0.01	0.06	0.04	0.02

Table B.1. Breeding values per trait and year of the Arkansas RosBREED pedigree (Cont).

Accession	Titratable acidity (%)				pH			
	2011	2012	2013	Average	2011	2012	2013	Average
dummy009	0.03	0.01	0.00	0.01	0.02	0.06	-0.16	-0.05
dummy010	0.20	0.09	0.05	0.14	-0.18	-0.21	-0.18	-0.27
Early Crawford	0.34	0.16	0.08	0.25	-0.34	-0.41	-0.26	-0.46
Elberta	0.36	0.20	0.08	0.27	-0.26	-0.45	-0.26	-0.41
Elberta OP27	0.28	0.14	0.05	0.21	-0.21	-0.32	-0.21	-0.34
F_A_371	0.20	0.08	-0.03	0.12	-0.12	-0.18	-0.15	-0.26
F_A_427	0.25	0.10	0.06	0.17	-0.20	-0.24	-0.19	-0.31
F_A_433	0.05	0.01	-0.03	0.03	-0.03	-0.02	-0.01	-0.08
F_A_699	0.22	0.04	-0.04	0.06	-0.22	-0.09	-0.10	-0.12
F_Candoka	0.20	0.08	0.04	0.14	-0.19	-0.22	-0.17	-0.29
F_Elberta OP27	0.21	0.08	0.03	0.14	-0.16	-0.20	-0.17	-0.27
F_Garden State	0.20	0.09	0.04	0.14	-0.15	-0.19	-0.16	-0.25
F_Ga. Belle	0.27	0.15	0.04	0.21	-0.13	-0.34	-0.18	-0.36
F_JH Hale	0.27	0.14	0.10	0.21	-0.23	-0.33	-0.27	-0.35
F_Nectared4xA_24	0.24	0.11	0.11	0.21	-0.21	-0.25	-0.29	-0.37
F_NJ113115	0.21	0.10	0.04	0.15	-0.20	-0.21	-0.17	-0.27
F_NJ98838	0.22	0.09	0.04	0.15	-0.18	-0.22	-0.16	-0.27
F_NJN14	0.17	0.07	0.03	0.13	-0.15	-0.16	-0.14	-0.24
F_Sunrise	0.22	0.09	0.04	0.14	-0.20	-0.22	-0.18	-0.30
G_17_5E	0.23	0.12	0.13	0.17	-0.30	-0.32	-0.29	-0.40
Garden State	0.23	0.10	0.04	0.17	-0.17	-0.25	-0.19	-0.29
Ga. Belle	0.35	0.21	0.04	0.28	-0.20	-0.48	-0.17	-0.45
Goodmans Choice	0.22	0.09	0.03	0.15	-0.19	-0.21	-0.17	-0.27
Greensboro	0.30	0.09	0.04	0.22	-0.23	-0.21	-0.18	-0.36
H_523	0.31	0.02	0.02	-0.05	0.05	-0.11	-0.03	-0.20
HalBerta Giant	0.29	0.15	0.09	0.21	-0.21	-0.35	-0.24	-0.35
Hann Almond	0.22	0.09	0.04	0.15	-0.17	-0.21	-0.18	-0.28
Honeydew Hale	-0.07	0.06	0.04	0.16	-0.20	-0.10	-0.17	-0.08
Jefferson	0.35	0.18	0.03	0.27	-0.35	-0.41	-0.15	-0.45
JH Hale	0.35	0.19	0.10	0.28	-0.20	-0.45	-0.27	-0.39
Jing	0.26	0.13	0.06	0.18	-0.25	-0.27	-0.23	-0.33

Table B.1. Breeding values per trait and year of the Arkansas RosBREED pedigree (Cont.).

Accession	Titratable acidity (%)				pH			
	2011	2012	2013	Average	2011	2012	2013	Average
Jungerman	0.19	0.12	0.05	0.10	-0.18	-0.28	-0.19	-0.27
M_A_789	0.11	0.05	-0.03	0.03	-0.16	-0.10	-0.10	-0.12
MaoTao	0.27	0.12	0.09	0.19	-0.22	-0.14	-0.25	-0.34
Meredith	0.24	0.10	0.05	0.15	-0.20	-0.21	-0.18	-0.33
Mexican Honey	0.21	0.09	0.04	0.14	-0.21	-0.20	-0.19	-0.28
Nectalate	0.17	0.08	0.03	0.15	-0.15	-0.20	-0.16	-0.25
Nectared4	0.18	0.08	0.06	0.14	-0.13	-0.18	-0.18	-0.26
Nectared4 x A_24	0.24	0.11	0.11	0.20	-0.22	-0.24	-0.28	-0.38
Nectared 5	0.07	0.02	0.00	0.06	-0.03	-0.05	-0.06	-0.11
Nectared 7	0.14	0.05	0.02	0.11	-0.09	-0.14	-0.12	-0.20
NJ113115	0.21	0.10	0.03	0.15	-0.19	-0.22	-0.17	-0.27
NJ25032	0.16	0.07	0.04	0.14	-0.16	-0.18	-0.16	-0.25
NJ257	-0.01	0.10	0.03	0.22	-0.27	-0.19	-0.15	-0.16
NJ38026	0.28	0.14	0.07	0.22	-0.19	-0.34	-0.23	-0.33
NJ5102893	0.22	0.10	0.05	0.15	-0.19	-0.21	-0.18	-0.28
NJ5106548	0.27	0.13	0.07	0.21	-0.20	-0.32	-0.23	-0.32
NJ5107397	0.19	0.09	0.04	0.12	-0.15	-0.22	-0.15	-0.25
NJ53739	0.20	0.09	0.04	0.13	-0.17	-0.20	-0.15	-0.27
NJ53939	0.15	0.05	0.03	0.10	-0.10	-0.15	-0.14	-0.20
NJ554367	0.19	0.11	0.10	0.11	-0.28	-0.29	-0.26	-0.38
NJ562021	0.26	0.11	0.07	0.17	-0.20	-0.24	-0.21	-0.32
NJ6128	0.28	0.14	0.07	0.21	-0.20	-0.33	-0.23	-0.33
NJ822026	0.23	0.07	-0.03	0.13	-0.12	-0.18	-0.17	-0.25
NJ94727	0.28	0.14	0.07	0.21	-0.19	-0.33	-0.22	-0.33
NJ98838	0.25	0.12	0.05	0.18	-0.19	-0.29	-0.19	-0.30
NJC83	0.23	0.12	0.06	0.17	-0.21	-0.29	-0.22	-0.32
NJC95	0.25	0.12	0.06	0.18	-0.22	-0.27	-0.22	-0.31
NJLA3	0.28	0.14	0.08	0.22	-0.21	-0.34	-0.24	-0.33
NJN14	0.15	0.06	0.03	0.13	-0.10	-0.14	-0.13	-0.21
NJN17	0.20	0.09	0.05	0.14	-0.14	-0.24	-0.18	-0.26
NJN21	0.18	0.08	0.04	0.15	-0.17	-0.21	-0.16	-0.25

Table B.1. Breeding values per trait and year of the Arkansas RosBREED pedigree (Cont.).

Accession	Titratable acidity (%)				pH			
	2011	2012	2013	Average	2011	2012	2013	Average
NJN55	0.15	0.05	0.02	0.10	-0.11	-0.17	-0.11	-0.19
OldGold	0.22	0.10	0.06	0.15	-0.19	-0.23	-0.20	-0.29
Orange Cling	0.35	0.18	0.04	0.20	-0.24	-0.36	-0.19	-0.39
Orange Cling x Alameda	0.29	0.13	0.05	0.17	-0.22	-0.31	-0.17	-0.34
Peento	0.39	0.11	0.06	0.29	-0.35	-0.25	-0.19	-0.46
Pop8089	0.24	0.10	0.05	0.16	-0.21	-0.24	-0.21	-0.30
Raritan Rose	0.37	0.17	0.06	0.29	-0.23	-0.40	-0.22	-0.41
Redgold	0.30	0.15	0.10	0.21	-0.24	-0.33	-0.23	-0.36
Redskin	0.35	0.21	0.13	0.27	-0.35	-0.48	-0.35	-0.46
RR122_15	0.27	0.12	0.05	0.21	-0.18	-0.29	-0.16	-0.31
RR53_194	0.20	0.09	0.06	0.14	-0.21	-0.26	-0.20	-0.27
Slappey	0.26	0.10	0.05	0.16	-0.21	-0.22	-0.17	-0.40
Sunrise	0.27	0.13	0.07	0.18	-0.20	-0.29	-0.22	-0.34
Transcaal Cling	0.21	0.09	0.06	0.15	-0.19	-0.21	-0.17	-0.28
Westbrook	0.26	0.12	0.18	0.29	-0.24	-0.26	-0.40	-0.47
White County	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Winblo	0.37	0.21	0.14	0.28	-0.40	-0.48	-0.37	-0.47

Chapter 2

CHARACTERIZATION OF THE SLOW-MELTING FLESH TRAIT OF PEACH TO FIND AN ASSOCIATED DNA TEST FOR THE TRAIT

Abstract

Marker-assisted breeding (MAB) is a molecular technology which will allow peach [*Prunus persica* Batsch (L.)] breeders to increase efficiency of the breeding cycle and decrease program costs. The peach breeding program of the University of Arkansas, as a part of the RosBREED project, recently began applying MAB and it will become a routine activity within the program. Flesh texture in peach is important for breeders. Melting flesh (MF) and non-melting flesh (NMF) are the most common textures in most breeding programs. However, other flesh types such as non-softening flesh (NSF) and slow-melting flesh (SMF) have been found within the Arkansas program, potentially having higher postharvest potential. The endopolygalacturonase (endoPG) enzyme is involved with pectin depolymerization, and depending on the allelic combination of the candidate gene, this enzyme will determine if a peach will become MF [also called quick melting flesh (QMF)], NMF, or NSF when ripe. Currently, a DNA test is available to apply MAB on these three flesh types. The SMF has a crispy texture when early ripe and a slow melting rate until fully ripe when it becomes completely melting. The endoPG test is not able to differentiate SMF from QMF, so this different melting rate was theorized to be due to a different ethylene fruit production rate and not due to differences in endoPG activity. During 2013 and 2014, ethylene fruit production and fruit softening rates were measured on populations segregating for QMF and SMF with the objective to find a molecular marker associated with these textures and develop a DNA test able to

distinguish SMF from QMF. Results indicated that a relevant SNP associated with this trait was located on chromosome 4 of the peach genome. Six simple sequence repeat (SSR) markers were designed and tested. One of these was polymorphic and able to differentiate between SMF and other the flesh types when screened along with the endoPG-6 DNA test. This will allow further implementation of flesh type MAB methods in the Arkansas peach breeding program.

Introduction

In peach, flesh texture varies among cultivars and selections, and it depends on the different enzymes and proteins participating in the complex process of ripening and softening. Endopolygalacturonase (endoPG), pectin methylesterase (PME), 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), expansins (Exp), and 1-aminocyclopropane-1-carboxylic acid synthase (ACS) are enzymes and proteins involved in peach flesh softening. Previous research identified the presence of these molecules in peach mesocarp during ripening and softening, as well differences in the action of these molecules depending on the type of flesh texture (Glover and Brady, 1995, Hayama et al., 2006; Kao et al., 2012, Lurie et al., 2002; Nilo et al., 2010; Peace and Norelli, 2009; Peace et al., 2005).

In peach, differences in fruit ethylene production have been observed in different breeding programs and studies. For example, ‘Flordagold’ (a cultivar from the University of Florida released in the 1970s) has remarkable firmness and delayed softening (Biggs, 1976). When its ethylene production was compared with ‘Early Amber’ (a mid-firm peach), ‘Flordagold’ produced 100-fold less ethylene than ‘Early Amber’. When external ethylene was applied to both cultivars it was observed that ‘Flordagold’ softening was accelerated, but was not for ‘Early Amber’ (Biggs, 1976). Similar results were observed when the ethylene production

rate was studied on selections and cultivars of SHF, MF, NMF, and very soft melting flesh (MVF) (Mignani et al., 2006).

Endopolygalacturonase enzyme and PME have been associated with solubilization and depolymerization of pectins in melting flesh (MF) cultivars. The principal function of PME is demethylation of polyuronides so they can be degraded by endoPG (Nilo et al., 2010). Pectin methylesterase activity increases at an early stage of fruit ripening and remains constant or decreases throughout the melting phase in MF genotypes (Nilo et al., 2010). According to Kao et al. (2012), its activity in non-melting flesh (NMF) genotypes is lower than MF cultivars, before and at maturity. The function of exopolygalacturonase (exoPG) is probably to complete the hydrolysis of pectates initiated by endoPG (Pressey and Avants, 1976). Also, it is proposed that the role of exoPG could be the cleavage of linkages between the pectic polysaccharides and the protein rather than in degradation of pectin (Pressey and Avants, 1973, 1976). Expansins (Exp) are cell wall proteins with the ability to extend cell walls that are under tensile stress, and their intervention can facilitate the action of other enzymes such as endoPG (Hayama et al., 2003; Nilo et al., 2012).

Softening at late stages of ripening requires significant amounts of ethylene and the ethylene-dependent pathway is at least in-part correlated with endoPG, exoPG, and PpExp3 activities (Hayama et al., 2006). Two ethylene biosynthesis pathways have been identified and described. The first one (System 1) corresponds to low ethylene production in the pre-climacteric period of climacteric fruits and is present during the development and ripening of non-climacteric fruits (Bapat et al., 2010). The other pathway (System 2) refers to an auto-stimulated (usually referred as autocatalytic synthesis), with significant ethylene production that is specific

to climacteric fruits (Bapat et al., 2010). In climacteric fruit, such as peach, increases in ethylene production during fruit ripening correlate with a burst of respiration (Tatsuki et al., 2006).

According to Kao et al. (2012), ethylene production in peach measured at harvest indicated that MF cultivars needed only low levels of this hormone to initiate ripening. However, NMF flesh cultivars generally had higher peak rates of ethylene production than MF cultivars during ripening, and it is likely that the NMF trait is not related to low ethylene production (Kao et al., 2012). These results were also found by Brovelli et al. (1999), who showed that the climacteric peak of ethylene production was higher in NMF cultivars compared to the MF genotypes in their study. The final step of ethylene synthesis is catalyzed by the enzyme 1-aminocyclopropane-1-carboxilic acid oxydase (ACO), which utilizes 1-aminocyclopropane-1-carboxilic (ACC) as a substrate (Yang and Hoffman, 1984). Lombardo et al. (2011) reported that in the MF freestone peach cultivar Dixiland the levels of PpACO1 (an encoding peach ACO gene) were relatively low and constant during almost all fruit development, however, at ripening a 10-fold increase was observed.

Peaches having a distinctive texture have been identified in the UA peach breeding program referred to as SMF (Clark, 2011). These peaches and nectarines are characterized by a crispy texture at early maturity and by maintaining firmness during ripening for a longer period than conventional MF genotypes. The Arkansas cultivars White County, White Diamond, and Souvenirs have been characterized to have this particular type of flesh. These genotypes achieve a melting and juicy flesh, similar to MF individuals, when softening is completed. This particular trait is thought to be different and independent of the F-M endo-PG locus which segregates for the MF, NMF, and NSF types, because previous studies indicated no differences between MF and SMF peaches when they were genotyped with the endoPG-6 DNA test (all SMF were

identified as MF by this DNA test) (Sandefur, 2011). For this reason, further research needed to be conducted to differentiate genetically the MF individuals from the SMF individuals and apply marker-assisted breeding (MAB) for the flesh texture trait with the ultimate goal to predict if a specific seedling will have MF, NMF, NSF, and SMF. One approach to elucidate the differences between MF and SMF is to measure ethylene fruit production after harvest for a certain period. This methodology was followed by Ghiani et al. (2011) in which the ethylene fruit production after harvest of MF cultivar Bolero, NMF cultivar Oro A, SHF cultivars Ghiaccio and Yumyeong, and the SMF cultivar Big Top were measured during 9 d after harvest. After measuring ethylene it was noted that 'Big Top' (previously considered SHF) did evolve low amounts of ethylene after harvest, but its values of ethylene production were comparable to the ones of the MF 'Bolero' at day 5 (Ghiani et al., 2011). These authors concluded that the different rate of softening of 'Big Top' is not due to a difference of the endoPG gene (Ghiani et al., 2011).

Further, melting flesh individuals could be separated in two distinct groups of texture based on their softening (also called melting) rate and/or their fruit ethylene production after harvest (RosBREED, 2015b). The first group contains individuals that melt at a quick rate and their ethylene production increases on the second or third day after harvest, which in previous literature are referred just as MF. This type of texture in this Chapter is called quick melting flesh (QMF). The second group of the melting texture contains individuals that melt in a slow rate and their ethylene production rate after harvest is slow during the first days of postharvest, increasing lately, such as 'Big Top' (Ghiani et al., 2011). This type of texture in this Chapter is called SMF.

The objective of this study was to phenotypically characterize populations segregating for QMF and SMF textures, associate this phenotypic data to SNP data, and design a DNA test able to differentiate QMF from SMF individuals.

Materials and Methods

Plant Material

All fruit phenotypic measurements were conducted at the University of Arkansas Fruit Research Station, Clarksville [west-central Arkansas (west-central Arkansas, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]. In all testing, trees were either open-center trained and spaced 5.5 m between trees and rows, or trained to a perpendicular-V system with trees spaced 1.9 m in rows spaced 5.5 m apart. All trees were dormant pruned and fertilized annually with a single application of 640 $Kg \cdot ha^{-1}$of complete fertilizer (19:19:19 of N:P:K) and sprinkler or drip irrigated as needed. Pests were managed using a program typical for commercial orchards in the area (Smith, 2015; Studebaker et al., 2015). Fruit were thinned to a distance of 12 to 15 cm between fruit after shuck split but before pit hardening.

Thirty six individuals (trees) of the 49-tree population ArPop_1 (‘White County’ x A-672) were used for texture characterization using compression and penetration measurements and fruit ethylene measurements in 2013. Harvest period of ArPop_1, in 2013, ranged from 15 July to 12 August, 2013. In 2014 the same measurement procedures were applied to the fruit harvested. However, during the spring of 2014 high infection of bacterial spot [*Xanthomonas arboricola* pv. *pruni* (Smith)] developed on trees in this population, damaging leaves and fruits. Trees of ArPop_1 were affected by this bacteria and most of the trees that produced fruit that did not have acceptable, representative fruit quality to perform the measurements. For this reason, only 2013 results of ArPop_1 are presented in this chapter. The same phenotypic data were collected in 2014 on six additional populations (five individuals of ArPop_0819, three individuals of ArkPop_0825, six individuals of population 0821, 14 individuals of population

1002, 11 individuals of 1004, and nine individuals of 1007). In 2014, for the 2010 populations, harvest period ranged from 17 July to 5 August, 2014.

Lastly, additional selections and cultivars included were A-672, A-699, A-708, A-760, 'Eastern Glo', 'Flavor Top', 'Loring', 'Sugar Lady', 'Westbrook', 'White County', 'White Diamond', 'Winblo', 'Yumm Yumm', and 14 Clemson University (CU) selections.

For phenotyping measurements, 20-25 fruits were selected from mid-canopy of only healthy trees. According to the RosBREED phenotyping protocol for peach, for fruit sample collection the tree was checked to have a few edible fruits and then the fruit collected for measurement was early ripe, a stage called "tree-ripe" (Frett et al., 2012; Gasic et al., 2010). Only fruit exhibiting uniform shape and background color, and lacking any insect or disease damage were included in samples. Also, transportation from the field to the laboratory was done carefully (avoiding sudden movements to decrease the probability that fruits hit with each other), since any damage on the fruit could have a negative effect on the final results.

Classification of melting individuals which were not SMF were designated QMF (the same as MF) for clarity of classification for my study.

Phenotypic Evaluation

For ethylene measurements, non-destructive analyses were performed. Four fruits were collected from each seedling tree. Each fruit was placed individually in a 900-mL jar with hermetic enclosure for 3 min every 24 h for 6 d after harvest and ethylene evolution was measured. After each measurement, fruits were left at room temperature (~20 °C) in an open room until the next day when a new measurement was performed. Since this was a non-destructive measurement, the same fruit was measured during the entire 6-d period, allowing the most accurate measurements of ethylene evolution of each seedling measured. The ethylene

concentration in the jar was measured in ppm by the ethylene analyzer model ETH-1010 (Fluid Analytics, Rio Rancho, NM).

After measuring the ethylene concentration, the rate of fruit ethylene production was calculated by the following formula (provided by the ethylene analyzer manufacturer Fluid Analytics):

Rate of ethylene

$$= \frac{\left[\frac{(Volume\ of\ container - volume\ of\ fruit) * (Ethylene\ concentration)}{\frac{measurement\ period}{60\ minutes}}\right]}{Fruit\ weight}$$

Volume of fruit was measured in mL, ethylene concentration in ppm, measurement period in minutes, and fruit weight in kilograms. Units of rate of ethylene evolution were recorded as $\mu L \cdot Kg^{-1} \cdot h^{-1}$. Weight and volume of fruits were measured at day of harvest.

Ethylene data was classified into five discrete categories based on ethylene evolution over the 6 d period. These categories were designated full rise, D4, D3, D3-peak, and single-peak. Ratings within category were assigned to each seedling tree. For each category, the ethylene evolution followed these patterns and assigned ratings:

Category full rise:

- rating 1 = seedling that maintained low ethylene (below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) until day 6 when it increased from 1.5 to 5.0 times the ethylene production of the previous day.
- rating 2 = seedling where ethylene was low (below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) until day 5, then it increased from 1.5 to 5.0 times the ethylene production of the previous day.
- rating 3 = seedling where ethylene was low (below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) until day 4, then it increased from 1.5 to 5.0 times the ethylene production of the previous day.

- rating 4 = seedling where ethylene was low (below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) until day 3 d, then it increased from 1.5 to 5.0 times the ethylene production of the previous day.

- rating 5 = seedling where ethylene was low (below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) until day 2 then it increased from 1.5 to 5.0 times the ethylene production of the previous day.

Category D4:

- rating 1 = seedling that maintained low ethylene (below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) for the entire 6-d period or at day 6 increased its ethylene production from 1.5 to 5.0 times the ethylene production of the previous day.

- rating 2 = seedling where ethylene was low (below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) for 4 d, then increased to approx. 100 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ on day 4, then decreased.

- rating 3 = seedling where ethylene was low (below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) for 4 d, then increased on day 4 (increased to about 100 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) followed by a continued increase in ethylene through day 6.

Category D3:

- rating 1 = seedling that maintained low ethylene (below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) through day 6 or at day 6 increased its ethylene production from 1.5 to 5.0 times the ethylene production of the previous day.

-rating 2 = seedling that increased to approx. 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ on day 3, then decreased below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ through day 6.

- rating 3 = seedling that increased to approx. 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ on day 3 then remained steady at this level for the remaining 3 d.

- rating 4 = seedling that increased on day 3 to approx. 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ then continued to increase to higher levels through day 6.

Category D3-peak:

- rating 1 = seedling that maintained low ethylene (below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) through day 6 or at day 6 increased its ethylene production from 1.5 to 5.0 times the ethylene production of the previous day.
- rating 2 = seedling where ethylene increased to approx. 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ on day 3 then decreased.
- rating 3 = seedling where ethylene increased to approx. 100 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ on day 3 then decreased.

Category single-peak:

- rating 1 = seedling that maintained low ethylene (below 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$) through day 6 or at day 6 increased its ethylene production from 1.5 to 5.0 times the ethylene production of the previous.
- rating 2 = seedling where ethylene increased on day 5 to about 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ then decreased.
- rating 3 = seedling where ethylene increased on day 4 to approx. 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ then decreased
- rating 4 = seedling where ethylene increased on day 3 to approx. 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ then decreased.
- rating 5 = seedling where ethylene increased on day 2 to about 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ then decreased
- rating 6 = seedling where ethylene increased on day 1 to about 50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ then decreased.

For fruit firmness, five fruits were hand-harvested directly into 0.24 L corrugated trays (FormTex Plastics Corp., Houston, TX), transported to the laboratory, and left at room temperature. Fruit firmness was measured with two different methodologies at harvest day (0 d), 3 d, and 6 d after harvest. The two methodologies were:

A. Compression: Fruit compression was performed by placing round pieces of flesh 1 cm diameter on a flat surface, and then using a cylindrical and plane probe of 7.6 cm diameter to compress the flesh 10 mm (iCon Texture Analyzer, Texture Technologies Corp. Hamilton, MA).

B. Penetration: From both cheeks of each peach, skin was removed using a fruit skin peeler and a probe of 8-mm tip was utilized to measure fruit firmness by penetrating the fruit 10 mm (iCon Texture Analyzer, Texture Technologies Corp. Hamilton, MA).

Analysis of Genetic Information and Statistical Analysis

The analyzed population was part of the Arkansas RosBREED germplasm. This is a comprehensive pedigree in which seven-F1 Arkansas populations, parents, ancestors, important selections, and cultivars are a part. All individuals in this pedigree were previously genotyped using the IPSC 9K SNP Array for Peach (Verde et al., 2012) as part of the RosBREED project (Iezzoni et al., 2010). The polymorphic SNPs were filtered to eliminate those with a high proportion of inheritance errors and heterozygosity, resulting in 1,947 informative SNPs spread over the eight LGs [this process was completed by Dr. Cameron Peace, Washington State University (WSU)]. The number of markers on each LG varied based on chromosome length. For example, LG 4 was the longest with 424 SNPs, and LG 5 was the shortest with 137 SNPs. Scoring of SNPs was determined using the Illumina® Genome Studio software. This was conducted by Dr. Ksenija Gasic and Dr. Cameron Peace at CU, Clemson, SC and WSU, Pullman, WA, respectively.

As a part of the RosBREED project, association files were developed by Dr. Cameron Peace (WSU). These association files contained the genetic information of the 1,947 SNPs of the IPSC 9K SNP Array for Peach of all the RosBREED germplasm. During the development of the project, the files were utilized to associate the genetic information to different traits. Association files could be managed to analyze the data of all RosBREED germplasm or individual populations. The last option was utilized in this study, in which only ArkPop_1 (member of the Arkansas RosBREED germplasm) was analyzed. Using the 1,947 SNPs of the IPSC 9K SNP, the coefficient of determination (R^2) was calculated from a linear regression for each SNP and those with the higher R^2 were selected as candidate SNPs.

Associations between flesh texture and allelic combinations with fruit ethylene production and flesh firmness values were performed utilizing the PROC GLIMIX procedure (SAS® 9.4, SAS Institute Inc. Cary, NC). Least square means were calculated for mean comparisons ($P \leq 0.05$).

Marker Conversion

Once a relevant(s) SNP(s) was identified by the association files for ArPop_1, they were localized within the *P. persica* Whole Genome v1.0 Assembly and Association (www.rosaceae.org). By using the "pseudomolecules in GBrowse" tool, 10,000 to 20,000 base pair (bp) sequences upstream and downstream of the SNP were downloaded. By using "BatchPrimer3" (Rozen and Skaletski, 2000) web tool, SSR markers were screened in the target region. Also, forward and reverse primers were designed flanking the SSR sequence using "BatchPrimer3". Primers were designed following criteria such as product size between 100 and 400 bp, optimum melting temperature of 60 °C, and primer sizes of a minimum of 18 bp, an optimum of 21 bp, and a maximum of 23 bp. Lastly, primers were designed to have a GC Clamp

of two at the 3'end. Selected SSRs were checked for specific amplification in only the target region using NCBI BLAST on the Peach Genome v1.0 database software (www.rosaceae.org/tools/ncbi_blast).

Leaf Sample Collection and DNA Extraction

Approximately 30-60 mg of young leaf tissue was collected during spring of 2013 and 2014 of all the analyzed material in this study (except for CU selections in which case extracted DNA was sent to the Fruit Breeding Genotyping Laboratory of the University of Arkansas from CU). In 2013, leaf tissue was placed in individual 1.5 mL tubes (Eppendorf, Hauppauge, NY) containing a 4 mm stainless steel bead (McGuire Bearing Company, Salem, OR). Samples were stored at -80 °C until DNA extraction. DNA was extracted following the protocol on Appendix A. In 2014, young leaf samples were collected using coin envelopes, then samples were lyophilized for 7 d utilizing a lyophylizer Freezone® 12 model 77540 (Labconco Corporation, Kansas City, MO). When leaf samples were dry they were loaded into 96-deep well plates containing approximately 2 g of silica-gel in each well, including three negative control (empty wells containing only silica-gel). Then, DNA was extracted following the Edge-Garza et al. (2014). This protocol was used because it is high-throughput and cost efficient for extracting DNA in peaches and other *Prunus* species.

Polymerase Chain Reaction (PCR)

Extracted DNA was amplified utilizing optimized PCR reaction at the Fruit Breeding Genotyping Laboratory of the University of Arkansas. To identify QMF individuals (from NMF and NSF), the endoPG-6 DNA test was utilized to amplify the F-M locus (Table 1). Polymerase chain reactions consisted of a denaturalization step at 95 °C for 5 min, then 35 cycles of 95 °C for 45 s, 50 °C for 45 s, and 72 °C for 1 min, and lastly a final extension at 72°C for 7 min,

utilizing a thermocyler (BIO RAD, model T100, Hercules CA). Each PCR-plate included three negative controls.

Polymerase chain reactions for SMF-SSR DNA consisted of a denaturalization step at 95 °C for 30 s, then 30 cycles of 95 °C for 5 min, then 35 cycles of 95 °C for 45 s, 52 °C for 45 s, and 72 °C for 1 min, and lastly a final extension at 72 °C for 7 min, using a thermocycler (BIO RAD, model T100). Each PCR-plate included three negative controls.

Individual 25.0 µL PCR reactions were utilized using 5.0 µL of PCR buffer (Promega Corp., Madison, WI), 1.5 µL of $MgCl_2$ (Promega), 1.5 µL of 10 µM dNTPs (Promega), 0.5 µL of forward and reverse primer each (Integrated DNA Technologies, Coralville, IA), 0.5 µL of 5X Taq polymerase (Gene and Cell Technologies, San Vallejo, CA), 15.5 µL of ultra pure water, and 1.5 µL of DNA template.

SSR Allele Analysis

Six primer pairs were designed for the SMF DNA test (Table 2), following the previous methodology. Designed primer sequences were sent to Dr. Cameron Peace's molecular laboratory at WSU to test for polymorphisms using polyacrylamide gel electrophoresis (PAGE) for initial visualization. This initial visualization was completed with fragment separation on denaturing gels containing 4% acrylamide and 7.5 M urea in 1× TBE buffer run on a 50-cm Sequi-Gen GT system (Bio Rad, Hercules, CA) for ~2 h at 85 W. Gels were stained with Promega Silver Sequence™ Staining System (Promega) as per manufacturer's instructions. Samples in this initial visualization were 'Bradley', 'Pentoo', 'Sunrise', A-665, A-672, A-699, ArPop_1-03, ArPop_1-17, SC_1A122, and SC_1A124.

Once initial visualization was performed and polymorphisms of SSRs were tested, PCR reactions of all the individuals including in this study were resolved utilizing a Fragment

Analyzer™, model AdvanCE FS96 (Advanced Analytical Technologies, Inc., Ames, IA), as per manufacturer instruction. The Fragment Analyzer™ was located at the wheat breeding laboratory, University of Arkansas. This technology is based on capillary gel electrophoresis for DNA separation. Allele scoring was conducted utilizing PROSize® v.1 software (Advanced Analytical Technologies, Inc., Ames, IA).

Table 1. Physical location on LG 4 of peach genome and nucleotide sequence of forward and reverse primers of endoPG-6 DNA test.

Physical location (bp)	Forward	Reverse
22,650,569 22,650,975	CGGGGTTACCATATCAGGTG	TTAGGGATGCCAATCCACTC

Table 2. SSR markers developed within proximity to SNP ss_414220, their physical location, motif, forward and reverse primers, and expected band size.

Marker	Physical location (bp)	Motif	Forward	Reverse	Expected size (bp)
SSR#1	12,040,501 12,040,839	TA x 11	AAGGAAAAGCGTGAGATAATCG	TTCGCATAGTTGAAGAGATTTCC	339
SSR#2	12,046,352 12,046,690	GTCAC x 2	AGAGGGCTTCTCAAAAAGTGG	ATAAGGAAGGGTGCAAGTGG	339
SSR#3	12,058,451 12,058,828	TA x 9	CCTCTTCGCAGTGCTTCC	TGCTCAATTTTGTATTGATTTGG	378
SSR#4	12,062,077 12,062,437	AT x 9	TTTTTAGTTGCCTTTTGATTAGG	AGCGGTATTGGAAAAGAAAGG	361
SSR#5	12,063,539 12,063,875	GA x 12	GTGTGCTGAGCAGTTTTTGG	CCTAGGATCGAGGGAAATCG	337
SSR#6	12,066,572 12,066,910	TTA x 7	TGGGCCTCTTTCTTGAATACC	TGTTGGAGCTCCTGTTTTAGC	339

Results and Discussion

Phenotypic Analysis

Total production of ethylene ranged from 64 to 697 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ among seedlings of ArPop_1, with a population average of 252 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ for the 6 days of analyses. Ethylene fruit production segregated within this population. Individuals classified as QMF averaged a total of and of 305.1 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ ranged from 132 to 697 $\mu L \cdot Kg^{-1} \cdot h^{-1}$. Individuals classified as SMF averaged a total of 161 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ and ranged from 64 to 330 $\mu L \cdot Kg^{-1} \cdot h^{-1}$. After 5 d of ethylene measurements, the average ethylene production for SMF fruit was 19 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ with a range of 11 to 32 $\mu L \cdot Kg^{-1} \cdot h^{-1}$, values that were similar to those of 'Big Top' after 5 d of storage (Ghiani et al., 2011). In the study with 'Big Top', ethylene measurement continued until day 9 and had mean values of ~50 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ with a highest value of ~70 $\mu L \cdot Kg^{-1} \cdot h^{-1}$, and these levels were at day 5 or 6 in my study.

The next major assessment of the data was assignment of seedlings to ethylene production categories (Table 3). Since SMF peaches were reported to soften several days after postharvest and to have lower ethylene production rates prior to softening, it was anticipated that ethylene production would be low until the end of the postharvest period of 6 d. My phenotypic analysis therefore included ethylene evolution measurements as an additional method of differentiating SMF individuals, because the difference between SMF and QMF is the rate of softening and not in the final texture (both are melting eventually). The data showed distinct ethylene evolution patterns. Slow-melting individuals, characterized by late ethylene evolution, were identified by having a score of 1 in all ethylene production categories or a 2 in the full-rise scale (Table 3). A total of 13 individuals of ArPop_1 were classified as SMF in 2013. All the

other seedlings assigned in the other categories were considered as QMF, because their ethylene production was higher (compared to SMF) from the second or third day of evaluation. For example, ArPop_1-02 and ArPop_1-17 received a rating of 5 in the “full rise” category, because they started to produce twice the amount of ethylene in the second day of measurement compared to the first day. These classification categories were then analyzed as traits with the association files.

Table 3. Fruit ethylene rate production categories (ethylene traits), assigned flesh type (quick melting flesh=QMF and slow melting flesh=SMF), total ethylene production, and daily average ethylene production for seedlings in Arpop_1, year 2013, Clarksville, AR.

Individual	Ethylene trends (traits) [z]					Flesh type	Ethylene production ($\mu L \cdot Kg^{-1} \cdot h^{-1}$)	
	Full rise	D4	D3	D3 peak	Single peak		Total[y]	Per day[x]
01	-	-	2	2	-	QMF	132	18.9
02	5	-	-	-	-	QMF	600	85.7
03	1	1	1	1	1	SMF	132	18.9
04	-	-	-	3	4	QMF	286	40.9
07	1	1	1	1	1	SMF	143	20.4
08	1	1	1	1	1	SMF	171	24.4
09	-	-	-	3	4	QMF	238	34.0
10	2	-	-	-	-	SMF	196	28.0
11	1	1	1	1	1	SMF	118	16.9
12	-	-	3	-	-	QMF	227	32.4
14	2	-	-	-	-	SMF	178	25.4
17	5	-	-	-	-	QMF	697	99.6
19	3	3	-	-	-	QMF	378	54.0
20	1	1	1	1	1	SMF	107	15.3
21	-	2	-	-	3	QMF	302	43.1
22	4	-	4	-	-	QMF	286	40.9
23	-	-	-	-	6	QMF	272	38.9
24	-	-	3	-	-	QMF	208	29.7
26	-	-	3	-	-	QMF	233	33.3
27	-	-	-	-	6	QMF	241	34.4
30	1	1	1	1	1	SMF	105	15.0
32	4	-	4	-	-	QMF	404	57.7
33	1	1	1	1	1	SMF	120	17.1

[z] For a complete description of each ethylene rate production categories see the Phenotypic Evaluation section in Material and Methods.

[y] Total ethylene production produced during the entire analysis period.

[x] Average daily ethylene production.

Table 3. Fruit ethylene rate production categories (ethylene traits), assigned flesh type (quick melting flesh=QMF and slow melting flesh=SMF), total ethylene production, and daily average ethylene production for seedlings in Arpop_1, year 2013, Clarksville, AR. (Cont.).

Individual	Ethylene trends (traits) [z]					Flesh type	Ethylene production ($\mu L \cdot Kg^{-1} \cdot h^{-1}$)	
	Full rise	D4	D3	D3 peak	Single peak		Total[y]	Per day[x]
34	-	-	2	2	-	QMF	155	22.1
36	-	-	-	3	4	QMF	373	53.3
37	3	3	-	-	-	QMF	271	38.7
38	2	-	-	-	-	SMF	229	32.7
39	1	1	1	1	1	SMF	64	9.1
40	2	-	-	-	-	SMF	330	47.1
41	2	-	-	-	-	SMF	200	28.6
42	4	-	4	-	-	QMF	352	50.3
43	3	3	-	-	-	QMF	269	38.4
46	3	3	-	-	-	QMF	406	58.0
47	-	-	2	2	-	QMF	190	27.1
49	-	-	2	2	-	QMF	193	27.6

[z] For a complete description of each ethylene rate production categories see the Phenotypic Evaluation section in Material and Methods.

[y] Total ethylene production produced during the entire analysis period.

[x] Average daily ethylene production.

In comparing average daily ethylene production, QMF individuals produced significantly higher average ethylene on days 2, 3, and 4 (Fig. 1) compared to SMF, while means for days 1, 5, and 6 for ethylene production were not significantly different between textures. This indicates that SMF genotypes reached the same levels of ethylene as QMF toward the end of the storage period as was anticipated (Fig. 1). It is unclear why there was not a significant difference for day 1, although the mean values were substantially different; this reflects greater variation in the data for day 1 measurements resulting in lack of significant differences.

This trend of ethylene evolution is unique to SMF texture and no other type of peach texture behaves in this way, since QMF and NMF individuals have been found to produce ethylene immediately after harvest and their ethylene rates increased every day after harvest (Haji et al., 2003, 2005; Lu et al., 2008). Conversely, SHF individuals (not present in the Arkansas program) do not produce ethylene when ripe or in a postharvest period unless they are stimulated by external factors such as ethylene application or cold treatments (Ghiani et al., 2011; Haji et al., 2003, 2005).

Further, when total ethylene produced by individuals of ArPop_1 was divided by the number of days of measurement (7 d, counting the harvest day), textures showed significant differences, QMF produced 43.6 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ ethylene per day, significantly higher than SMF that produced 22.9 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ per day.

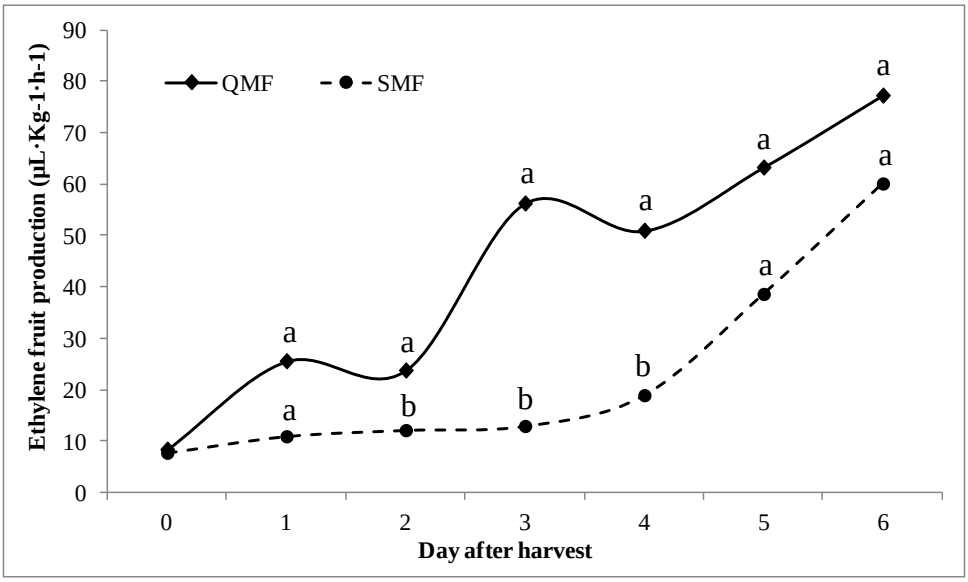

Fig. 1. Least square means of ethylene evolution per day after harvest of ArPop_1 individuals having either quick melting flesh (QMF) or slow melting flesh (SMF) textures. Means with the same letter on the same day are not significantly different ($P \leq 0.05$), year 2013. Sampling period of ArPop_1 seedlings ranged from 15 July to 12 August, 2013.

Penetration and compression firmness values of QMF and SMF individuals decreased during postharvest (Figs. 2 and 3). Quick-melting individuals ranged from 23.4 (harvest day) to 5.9 N at day 6 for penetration and from 69.3 (harvest day) to 14.1 N at day 6 for compression force. Slow-melting individuals ranged from 31.9 for harvest day to 7.5 N at day 6 and from 99.4 at harvest day to 25.5 N at day 6 for penetration and compression forces, respectively.

Slow-melting flesh individuals showed higher compression and penetration firmness at harvest day (day 0) compared to QMF individuals. At days 3 and 6, textures were statistically similar for penetration force, indicating that after three days at room temperature, both types of flesh had similar texture and a fruit of a SMF texture could not be differentiated from QMF. However, the rate of softening between days 0 and 3 was much more rapid for QMF as had been observed before (Sandefur, 2011). The melting texture was reported to be most desirable by consumers (Crisosto and Velero, 2008). These results are similar to those obtained by previous studies in which MF, NMF, SMF, and SHF were analyzed (Haji et al., 2003, 2005; Hayama et al. 2006, Kao et al. 2012; Rasori et al., 2002; Sandefur, 2011; Truque et al., 2012). For flesh compression, SMF values were significantly higher for all days of measurement compared to QMF, showing a difference between the two types of force measurements used. This difference in types of force measurements could be because penetration is more related to deformation of the flesh of fruits, and compression is more associated to resistance of the flesh breaking apart when compressed.

On average, SMF individuals had higher firmness and lower ethylene production compared to QMF individuals. In climacteric fruits, like peaches, ethylene is an activator of the "ripening machinery" and it is well reported that when these types of fruit reach the climacteric point (high ethylene production), enzymes like endoPG, exoPG, PME, and expansins are

activated and as a consequence the softening rate of fruits is also increased (Brummel et al., 2004; Ghiani et al., 2011; Hayama et al., 2006; Kao et al., 2012). Depending on the type of change or mutation in any of the alleles controlling these enzymes, the texture of a particular peach will be expressed, for example then NMF peach texture has a deletion in the endoPG gene, thus the last stage of softening does not occur (Peace et al., 2007).

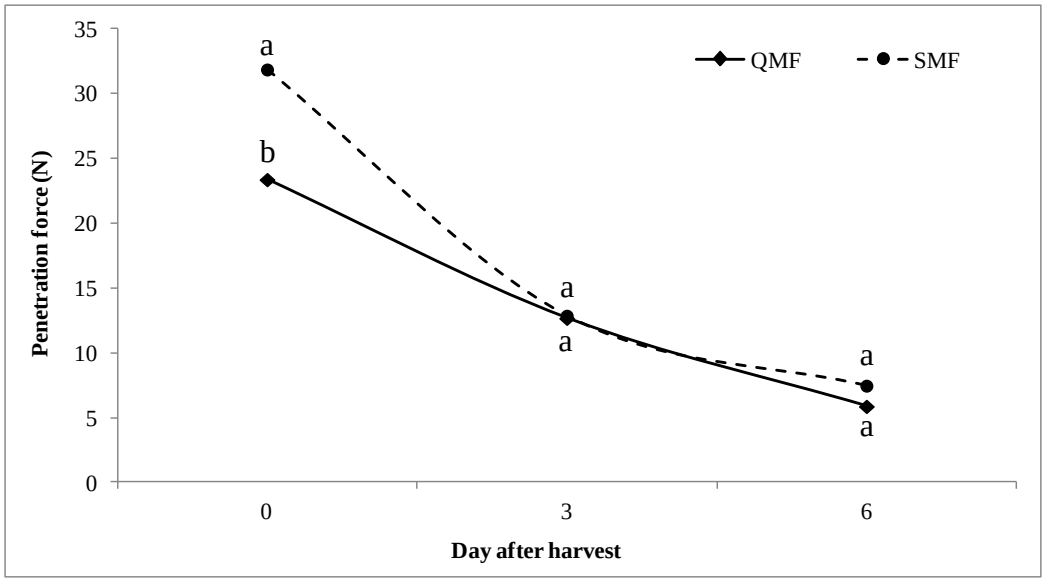

Fig. 2. Least square means of penetration force (N) of ArPop_1 individuals having either quick melting flesh (QMF) or slow melting flesh (SMF) textures. Means with the same letter on the same day are not significantly different ($P \leq 0.05$), year 2013. Sampling period of ArPop_1 seedlings ranged from 15 July to 12 August, 2013.

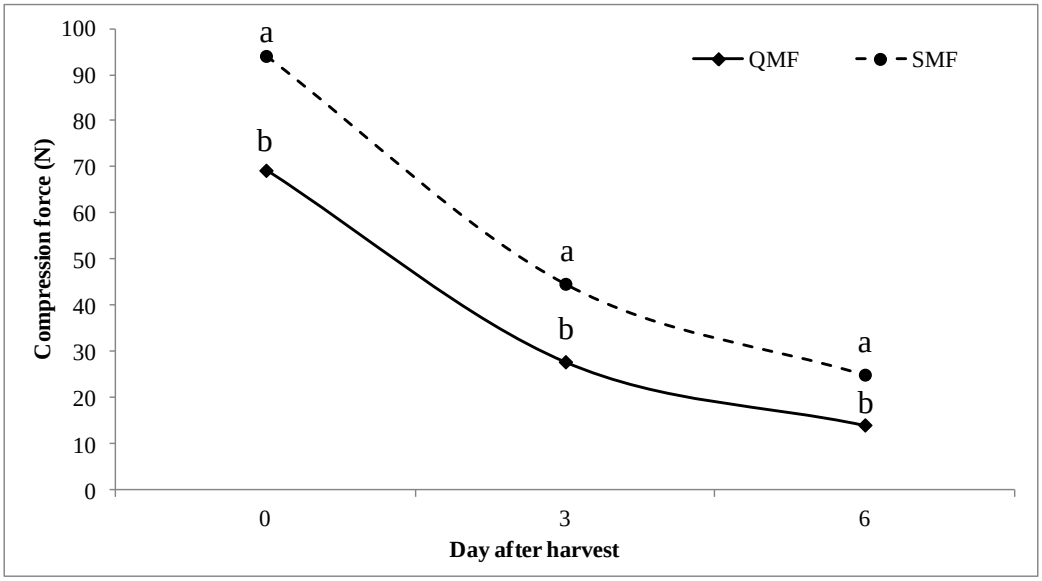

Fig. 3. Least square means of compression force (N) (N) of ArPop_1 individuals having either quick melting flesh (QMF) or slow melting flesh (SMF) textures. Means with the same letter on the same day are not significantly different ($P \leq 0.05$), year 2013. Sampling period of ArPop_1 seedlings ranged from 15 July to 12 August, 2013.

Association Analysis

The endoPG gene, located on the distal end of LG 4, controls MF, NMF, and NSF textures, but the DNA test associated with this gene is not able to distinguish between MF (QMF) and SMF (Peace et al., 2005, 2007; Sandefur, 2011), suggesting that this enzyme is not responsible for SMF texture. Thus, discovering a location in the genome able to differentiate between QMF and SMF will increase the knowledge about this trait and to create a more powerful DNA test for differentiating flesh types.

Results of endo-PG6 DNA test indicated that all individuals of this population and its parents are MF. So, no NMF and NSF individuals were present in this population.

All ethylene production classifications presented in Table 3 were run on the association files to discover relevant SNPs. Relevant SNPs were found on LG 2, LG 3, LG 4, LG 5, and LG 6. The SNP ss_414220 (designated SMF-SNP) located at 30.1 cM on LG 4 explained the highest proportion of the phenotypic variation of the ethylene production category full rise, D3-peak, D4, and single-peak (Table 4). The proportion of the variation explained for these traits varied from 14% for the single-peak pattern to 92% for D4.

Individuals of the population were heterozygous AB and homozygous BB for the ss_414220 SNP. Heterozygous individuals produced 42.6 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ per day on average, significantly higher than 29.5 $\mu L \cdot Kg^{-1} \cdot h^{-1}$ per day produced by "BB" individuals. This could indicate a possible partial dominant control of the trait (although ethylene was not measured on "AA" individuals, because they were not present in the population). Similar values were associated with QMF and SMF, indicating that QMF corresponded to heterozygous AB individuals and SMF corresponded to homozygous BB individuals. There were no homozygous

AA individuals in this population due to the allelic composition of its parents, which are ‘White County’ (BB) and A-672 (AB).

Table 4. Phenotypic variation explained (%) of SNP ss_414220 located on LG 4 in relation to fruit ethylene production rate categories.

Characteristic	Full rise	D4	D3-peak	Single peak
Prop Var Expl	0.33	0.92	0.79	0.14
Prob AA > Avg	-	-	-	-
Prob AB > Avg	0.74	0.84	0.79	0.69
Prob BB > Avg	0.31	0.20	0.18	0.40

SSR and DNA Test

Utilizing the peach genome sequence (*P. persica* whole genome v1.0), six SSRs were located around the SMF-SSR (Table 2). These six sequences were sent to Dr. Cameron Peace's laboratory at WSU to test if the observed SSR allelic combinations were polymorphic and matched the allelic combination of SMF-SNP.

Of the six SSRs, the second one (SSR#2) produced clear polymorphisms and was able to differentiate individuals that produced ethylene in a high concentration almost immediately after harvest from individuals that produced low amounts of ethylene the first days after harvest but at day 5 or 6 their ethylene production increased. This SSR amplified two alleles polymorphic for this trait. One of them was associated with individuals that produced high amounts of ethylene after harvest and softened at a quick rate. It was designated as the 'Q' allele and was associated with QMF. In the Arkansas program this allele was present only in the heterozygous form, similar to the SMF-SNP allele AB combination. The other allele was related to individuals that the first days after harvest produced low amounts of ethylene until day 5 or 6 when their ethylene production increased and also softened in a slow rate. It was designated as the "S" allele and associated with SMF texture; also it was associated with the B allele of the SMF-SNP. This allele was present as homozygous (SS) and heterozygous in combination with the Q allele (QS). The Q allele amplified a band of ~330 bp and the S allele a band of ~340 bp (Figs. 4-7). Significant differences in average ethylene production per day were observed between QS and SS individuals (42.7 and 29.6 $\mu L \cdot Kg^{-1} \cdot h^{-1}$, respectively), the same differences when individuals were grouped by AB and BB.

More evaluations after the initial test were performed to validate the SMF-DNA test. These evaluations included individuals of other RosBREED breeding programs along with

accessions outside the RosBREED germplasm to test if they matched with the alleles of the SMF-DNA test (Table 5). As stated before, within ArPop_1 population there were no homozygous AA individuals (of the SMF-SNP); however among the 14 genotypes from CU, all had this allele combination and were amplified successfully and matched the expected allelic combination of the SMF-SSR DNA test (QQ). Table 5 provides a subset of the individuals initially tested to validate the DNA test (from AR Pop_1). It can be observed with these individuals that AA alleles matched with the QQ alleles of the QMF individuals, the AB alleles matched with the QS alleles of QMF individuals, and BB alleles matched with the SS alleles of SMF individuals. These results indicate that this new DNA test along with endoPG-6 can be used to successfully distinguish QMF, SMF, NMF, and NSF. In apples (*Malus* x *domestica* Borkh.), there are two QTLs associated with ethylene fruit production at postharvest on LG 10 and 15, and two DNA tests flanking those QTLs are already being utilized in apple breeding programs to selected apples with postharvest potential (Costa et al., 2014; RosBREED, 2015a).

The location of the SMF-SSR DNA test is located near the maturity date locus, separated by ~2.0 cM (Dirlewanger et al., 2012; Eduardo et al., 2015). Two of the early ripening cultivars from the Arkansas program, 'Westbrook' and 'Yumm Yumm', have always been considered to have very melting flesh in field evaluations, but DNA analysis showed they were homozygous SS indicating that they are SMF (also 'Westbrook' was homozygous "BB" for the SMF-SNP, 'Yumm Yumm' is not part of the RosBREED germplasm, so no SMF-SNP allelic information was obtained from it). An explanation for this could be that the "early" trait is also associated with a high or fast ethylene production trait and it its epistatic and dominant to the SMF locus. Also, five individuals of ArPop_1 had the same issue (10% of the population). 'Westbrook' and these five individuals of ArPop_1 carry at least one early allele of the G4Mat locus (unpublished

data). The G4Mat locus is associated with peach maturity date; it was found by analyzing the ripening date of the peach RosBREED germplasm (unpublished data). Further tests and analyses need to be done to discover the genetic relationship between these two loci which are separated by approximately 760,000 bp (unpublished data). By studying slow-ripening flesh (SRF) individuals of two segregating populations and maturity day (MD) there was found a relationship between these two traits (Eduardo et al., 2015). Slow-ripening individuals produce no ethylene, never get ripe, have a poor flavor, and maintain their green background color, all characteristics than make them undesirable for breeding purposes (Eduardo et al., 2015; Tataranni et al., 2010). It is possible that the SRF allele is one of the series of the MD alleles, so when one SRF allele is present in combination with any other MD allele, individuals mature at an intermediate date (mid-season). If there are no SRF alleles, individuals mature early in the season, and if individuals are homozygous for the SRF locus they would not ripen at all (Eduardo et al., 2015).

In 2014, ethylene measurements were performed on six other populations that segregated for QMF and SMF in order to further validate the DNA test (Table 6). Ethylene data was taken following the same protocol as in 2013. Allelic combinations of the SMF DNA test coincided in 90% of the tested progenies in 2014, since only five "SS" (SMF) individuals (1002_010, 1004_003, 1004_026, 1004_059, and 1004_60) were phenotyped as QMF. This could be due to the same potential genetic relationship of this locus and maturity date (G4Mat) locus described earlier, in which if individuals carry an "early" allele the individual melts quickly despite if the SMF locus indicates that the individual has SMF texture (there was no G4Mat allele information for these individuals). Further analyses should be done to study this interesting relationship. A potential solution for this is when analyzing the results of early season individuals to apply MAB, one should take into consideration that some of the "SS" individuals could be QMF, so it

might be possible to only apply MAB on mid- and late-season individuals until more information can be obtained about the relationship between these two loci.

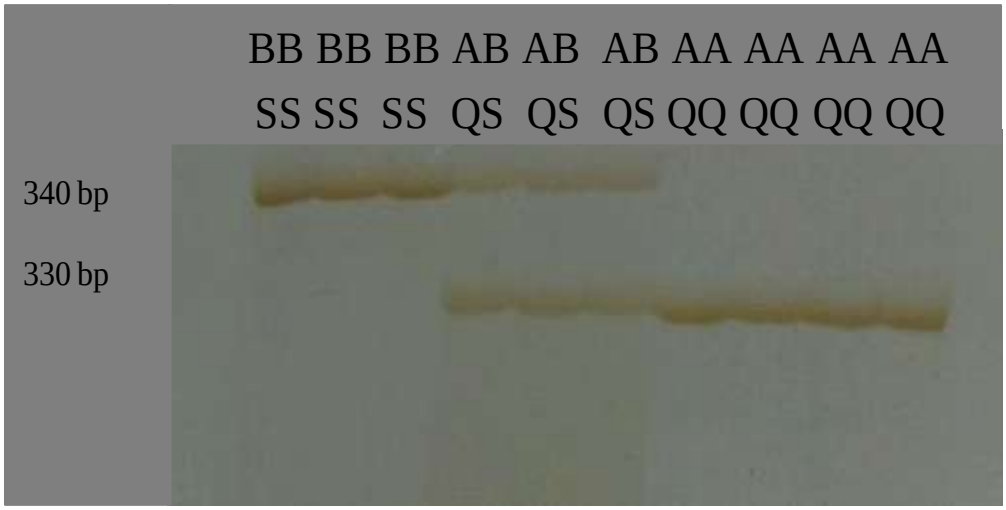

Fig. 4. PAGE results of SSR#2. AA (QQ) and AB (QS) alleles represent quick melting flesh (QMF) texture. BB (SS) individuals represent slow melting flesh (SMF) texture. Q band is ~330 bp and S band is ~340 bp.

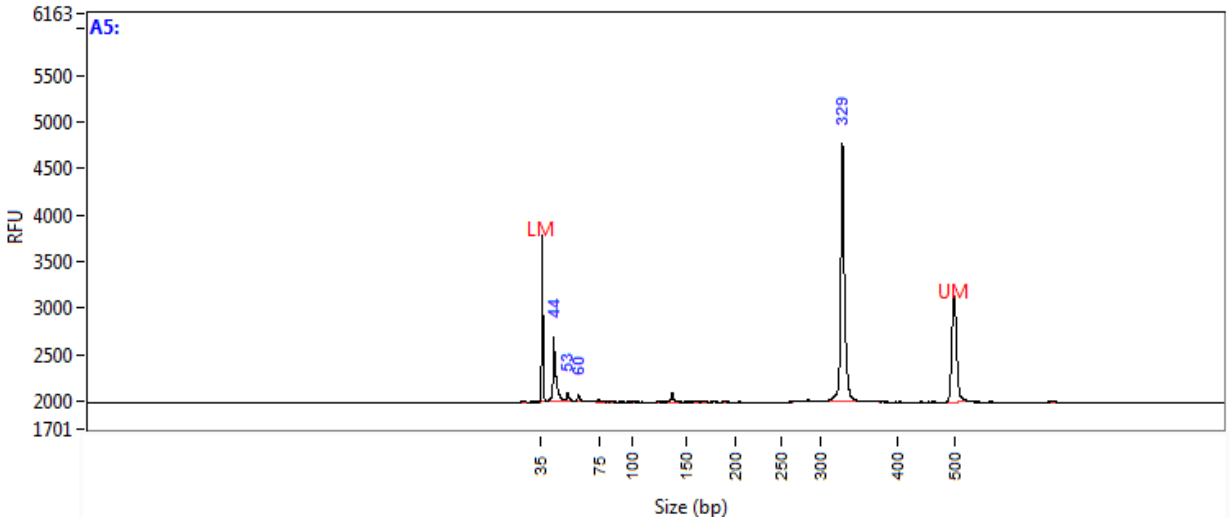

Fig. 5. Peak height (relative fluorescence units, RFU) of homozygous quick melting flesh (QQ) individual amplifying a band of 329 base pairs (bp). SMF DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. A5 corresponds to the row letter and column number of the location of sample on the plate.

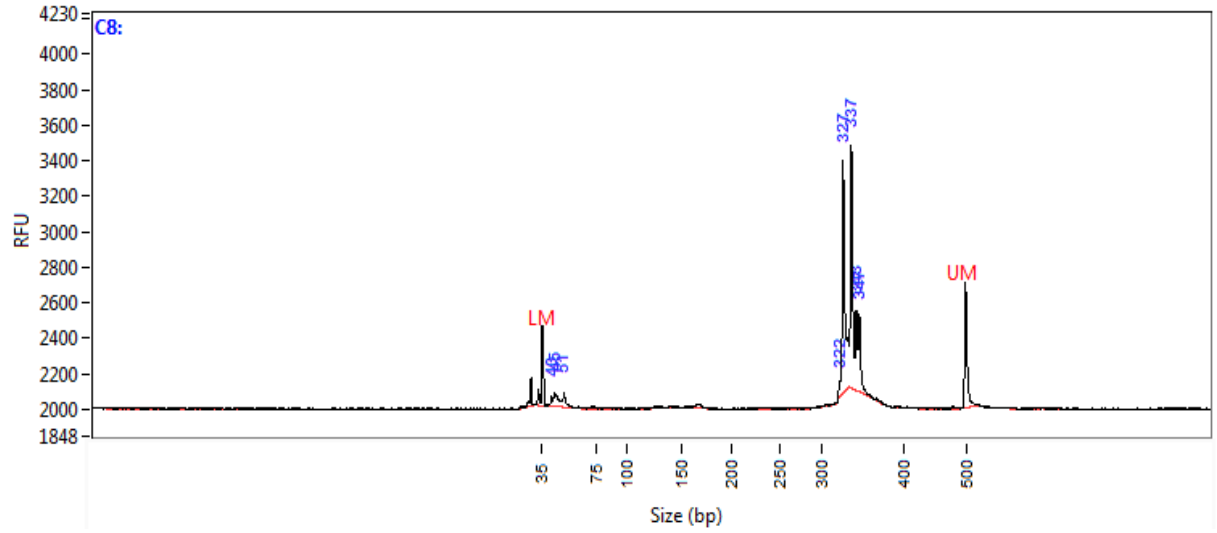

Fig. 6. Peak height (RFU) of heterozygous quick melting flesh (QS) individual amplifying bands of 327 and 337 base pairs (bp). SMF DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. C8 corresponds to the row letter and column number of the location of sample on the plate.

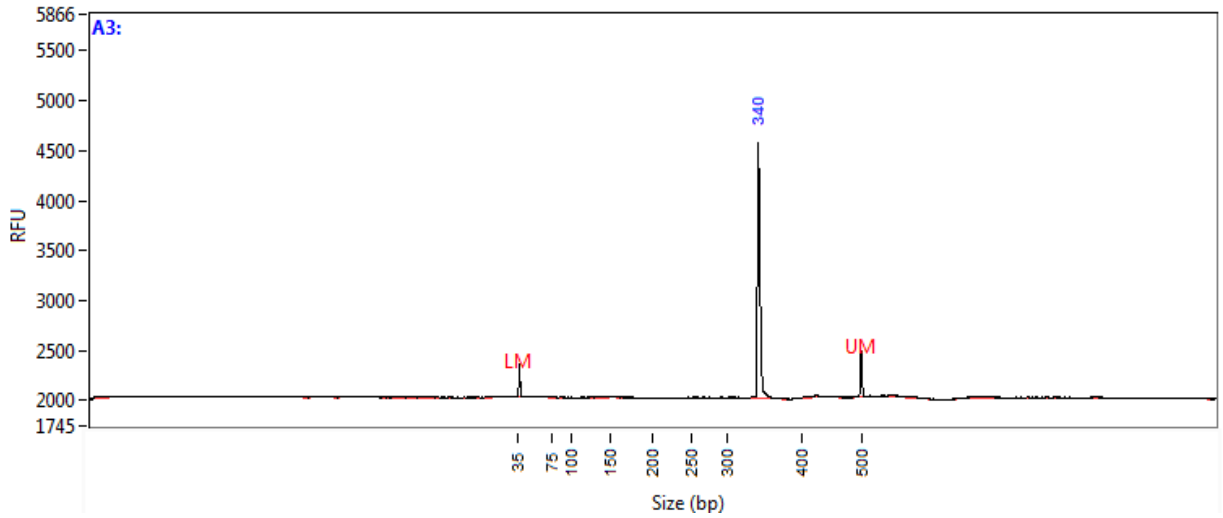

Fig. 7. Peak height (RFU) of homozygous slow melting flesh (SS) individual amplifying a band 340 base pairs (bp). SMF DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. A3 corresponds to the row letter and column number of the location of sample on the plate.

Table 5. Alleles of SMF-SNP and SMF-SSR of different accessions and their flesh texture (evaluated phenotypically). The origin of each cultivar/selection is also provided.

Accession	SSR allele	SNP allele	Texture[z]	Origin
A-672	QS	AB	QMF	U. of Arkansas
A-699	QS	AB	QMF	U. of Arkansas
A-708	SS	BB	SMF	U. of Arkansas
A-760	SS	BB	SMF	U. of Arkansas
ArPop_1-01	QS	AB	QMF	U. of Arkansas
ArPop_1-02	SS	BB	QMF	U. of Arkansas
ArPop_1-03	SS	BB	SMF	U. of Arkansas
ArPop_1-04	QS	AB	QMF	U. of Arkansas
ArPop_1-05	SS	BB	-	U. of Arkansas
ArPop_1-06	SS	BB	-	U. of Arkansas
ArPop_1-07	SS	BB	SMF	U. of Arkansas
ArPop_1-08	SS	BB	SMF	U. of Arkansas
ArPop_1-09	QS	AB	QMF	U. of Arkansas
ArPop_1-10	SS	BB	SMF	U. of Arkansas
ArPop_1-11	SS	BB	SMF	U. of Arkansas
ArPop_1-12	SS	BB	QMF	U. of Arkansas
ArPop_1-14	SS	BB	SMF	U. of Arkansas
ArPop_1-15	SS	BB	-	U. of Arkansas
ArPop_1-17	QS	AB	QMF	U. of Arkansas
ArPop_1-18	SS	BB	-	U. of Arkansas
ArPop_1-19	QS	AB	QMF	U. of Arkansas
ArPop_1-20	SS	BB	SMF	U. of Arkansas
ArPop_1-21	QS	AB	QMF	U. of Arkansas
ArPop_1-22	SS	BB	QMF	U. of Arkansas
ArPop_1-23	SS	BB	QMF	U. of Arkansas
ArPop_1-24	SS	BB	QMF	U. of Arkansas
ArPop_1-25	QS	AB	QMF	U. of Arkansas
ArPop_1-26	QS	AB	QMF	U. of Arkansas
ArPop_1-27	SS	BB	QMF	U. of Arkansas
ArPop_1-28	SS	BB	-	U. of Arkansas
ArPop_1-29	QS	AB	-	U. of Arkansas
ArPop_1-30	SS	BB	SMF	U. of Arkansas
ArPop_1-32	QS	AB	QMF	U. of Arkansas
ArPop_1-33	SS	BB	SMF	U. of Arkansas
ArPop_1-34	QS	AB	QMF	U. of Arkansas

[z] QMF and SMF refer to quick melting flesh and slow melting flesh, respectively.

Table 5. Alleles of SMF-SNP and SMF-SSR of different accessions and their flesh texture (evaluated phenotypically). The origin of each cultivar/selections also provided. (Cont.)

Accession	SSR allele	SNP allele	Texture[z]	Origin
ArPop_1-36	QS	AB	QMF	U. of Arkansas
ArPop_1-37	QS	AB	QMF	U. of Arkansas
ArPop_1-38	SS	BB	SMF	U. of Arkansas
ArPop_1-39	SS	BB	SMF	U. of Arkansas
ArPop_1-40	SS	BB	SMF	U. of Arkansas
ArPop_1-41	QS	AB	SMF	U. of Arkansas
ArPop_1-42	QS	AB	QMF	U. of Arkansas
ArPop_1-43	QS	AB	QMF	U. of Arkansas
ArPop_1-44	SS	BB	-	U. of Arkansas
ArPop_1-45	SS	BB	-	U. of Arkansas
ArPop_1-46	QS	AB	QMF	U. of Arkansas
ArPop_1-47	QS	AB	QMF	U. of Arkansas
ArPop_1-48	QS	AB	QMF	U. of Arkansas
ArPop_1-49	QS	AB	QMF	U. of Arkansas
Eastern Glo	SS	-	SMF	Zaiger genetics, CA
Flavor Top	SS	-	SMF	Agro Selections Fruits, France
Loring	QS	AB	QMF	State Fruit Exp. Station, MO.
SC_Pop1-A008	QQ	AA	QMF	Clemson University
SC_Pop1-A075	QQ	AA	QMF	Clemson University
SC_Pop1-A104	QQ	AA	QMF	Clemson University
SC_Pop1-A122	QQ	AA	QMF	Clemson University
SC_Pop1-A124	QQ	AA	QMF	Clemson University
SC_Pop1-A133	QQ	AA	QMF	Clemson University
SC_Pop1-A137	QQ	AA	QMF	Clemson University
SC_Pop1-A138	QQ	AA	QMF	Clemson University
SC_Pop1-A146	QQ	AA	QMF	Clemson University
SC_Pop1-A190	QQ	AA	QMF	Clemson University
SC_Pop1-B077	QQ	AA	QMF	Clemson University
SC_Pop1-B097	QQ	AA	QMF	Clemson University
SC_PopB-1602	QQ	AA	QMF	Clemson University
SC_PopB-1604	QQ	AA	QMF	Clemson University
Sugar Lady	QS	-	QMF	Zaiger genetics, CA
Westbrook	SS	BB	QMF	U. of Arkansas
White County	SS	BB	SMF	U. of Arkansas
White Diamond	SS	BB	SMF	U. of Arkansas
Winblo	QS	AB	QMF	U. of Arkansas
YummYumm	SS	BB	QMF	U. of Arkansas

[z] QMF and SMF refer to quick melting flesh and slow melting flesh, respectively.

Table 6. Alleles of SMF-SSR of different accessions and their flesh texture (evaluated phenotypically). The origin of each cultivar/selection is also provided, year 2014.

Accession	SSR allele	Texture[z]	Origin
0821-01	SS	SMF	U. of Arkansas
0821-32	SS	SMF	U. of Arkansas
0821-74	SS	SMF	U. of Arkansas
0821-75	SS	SMF	U. of Arkansas
0821-85	SS	SMF	U. of Arkansas
0821-96	SS	SMF	U. of Arkansas
0819-04	SS	SMF	U. of Arkansas
0819-13	SS	SMF	U. of Arkansas
0819-15	SS	SMF	U. of Arkansas
0819-19	SS	SMF	U. of Arkansas
0819-21	SS	SMF	U. of Arkansas
0825-01	SS	SMF	U. of Arkansas
0825-08	SS	SMF	U. of Arkansas
0825-11	SS	SMF	U. of Arkansas
1002-010	SS	QMF	U. of Arkansas
1002-011	SS	SMF	U. of Arkansas
1002-019	SS	SMF	U. of Arkansas
1002-024	SS	SMF	U. of Arkansas
1002-039	SS	SMF	U. of Arkansas
1002-048	SS	SMF	U. of Arkansas
1002-069	SS	SMF	U. of Arkansas
1002-072	SS	SMF	U. of Arkansas
1002-100	SS	SMF	U. of Arkansas
1002-109	-	SMF	U. of Arkansas
1002-113	-	SMF	U. of Arkansas
1002-117	-	SMF	U. of Arkansas
1002-118	-	SMF	U. of Arkansas
1002-121	-	SMF	U. of Arkansas

[z]QMF and SMF refer to quick melting flesh and slow melting flesh, respectively.

Table 6. Alleles of SMF-SSR of different accessions and their flesh texture (evaluated phenotypically). The origin of each cultivar/selection is also provided, year 2014. (Cont.)

Accession	SSR allele	Texture[z]	Origin
1004-001	SS	SMF	U. of Arkansas
1004-003	SS	QMF	U. of Arkansas
1004-006	SS	SMF	U. of Arkansas
1004-009	SS	SMF	U. of Arkansas
1004-011	SS	SMF	U. of Arkansas
1004-012	SS	SMF	U. of Arkansas
1004-013	SS	SMF	U. of Arkansas
1004-018	SS	SMF	U. of Arkansas
1004-026	SS	QMF	U. of Arkansas
1004-059	SS	QMF	U. of Arkansas
1004-060	SS	QMF	U. of Arkansas
1007-031	SS	SMF	U. of Arkansas
1007-039	SS	SMF	U. of Arkansas
1007-077	SS	SMF	U. of Arkansas
1007-088	SS	SMF	U. of Arkansas
1007-098	SS	SMF	U. of Arkansas
1007-104	SS	SMF	U. of Arkansas
1007-126	SS	SMF	U. of Arkansas
1007-155	SS	SMF	U. of Arkansas
1007-193	SS	SMF	U. of Arkansas

[z] QMF and SMF refer to quick melting flesh and slow melting flesh, respectively.

Conclusions

The objective of this study was to develop a DNA test able to distinguish SMF individuals from QMF by using phenotypic and genotypic information with the ultimate goal to complement the endoPG-6 DNA test and potentially apply MAB for peach flesh texture.

The ArPop_1 ethylene production and softening rate was measured for 6 d after harvest at room temperature. It was possible to group individuals with two different ethylene production rates, which coincided with the observed softening rates. Individuals with a slow ethylene production rate coincided with a slow flesh softening rate were classified as SMF. Individuals with a high rate of ethylene and a quick rate of flesh softening were classified as QMF. Average ethylene production per day of SMF individuals was significantly lower than QMF and initial flesh firmness was significantly higher in SMF compared to QMF during the days of measurement. Exceptions were the third and last days of postharvest in which both textures had the same firmness. This confirms that during postharvest, SMF genotypes maintain their firmness and have a slow rate of ethylene production, but when they are totally mature and ripe their flesh is comparable to QMF which is the texture that consumers prefer to eat.

Association genome-wide analyses (by using the association files) determined a relevant SNP located at 30.1 cM on LG 4 that was able to distinguish between QMF and SMF individuals. Further analyses led to the design of six SSR makers around this SNP in which only one was polymorphic for the trait and it was called SMF-SSR. This DNA test, as stated before, is able to identify SMF-individuals from QMF, when used along with endoPG-6 DNA marker. This last marker will differentiate which peaches/nectarines are going to melt at the end of the ripening process and will separate them from NMF and NSF. However, this marker is not able to differentiate if melting individuals are going to be QMF or SMF. Thus, running the endoPG-6

maker along with the SMF-SSR will provide more informative DNA test results by distinguish QMF, NMF, NSF, and SMF. However, special attention needs to be paid when utilizing the SMF DNA test on early season selections/populations, due to the possible incorrect scoring of SMF individuals which really are QMF. This is probably due to a potential interaction of SMF with G4Mat locus which are located close to each other on LG 4.

Literature Cited

Bapat, V., P. Trivedi, A. Ghosh, V. Sane, T. Ganapathi, and P. Nath. 2010. Ripening of fleshy fruit: Molecular insight and the role of ethylene. Biotechnology Advances 28:94-107.

Barry, C. and J. Giovannoni. 2007. Ethylene and fruit ripening. J. Plant Growth Regulat. 26:143-159.

Biggs, R.H. 1976. Biological basis for firmness in the 'Flordagold' peach. Proc. Fla. State Hort. Soc. 89:213-214.

Brovelli, E., J. Brecht, W. Sherman, and C. Sims. 1999. Non-melting flesh trait in peaches is not related to low ethylene production rates. HortScience 34:313–315.

Brummel, D., V. Cin, S. Lurie, C. Crisosto, and J. Labavitch. 2004. Cell wall metabolism during the development of chilling injury in cold-stored peach fruit: association of mealiness with arrested disassembly of cell wall pectins. J. Experimental Botany 55:2041-2052.

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

Crisosto C. and D. Valero. 2008. Harvesting and postharvest handling of peaches for the fresh market, p. 575-596. In: D.R. Layne and D. Bassi (eds.), The peach: botany, production, and uses. CAB Intl., Oxfordshire, UK.

Costa, F., L. Cappellin, B. Farneti, A. Tadiello, A. Romano, C. Soukoulis, S. Sansavini, R. Velasco, and F. Biasiolo. 2014. Advanced in ethylene production in apple (*Malus* x *domestica* Borkh.). Postharvest Biol. Technol. 87:126-132.

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

Edge-Garza, D., T. Rowland, S. Haendiges, and C. Peace. 2014. A high-throughput and cost-efficient DNA extraction protocol for the tree fruit crops of apple, sweet cherry, and peach relying on silica beads during tissue sampling. Mol. Breeding 34: 2225-2228.

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

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

NCBI Blast. 2015. Genome Data for *Rosaceae*. 25 February 2015. < https://www.rosaceae.org/tools/ncbi_blast>

Gasic, K., T. Gradziel, C. Crisosto, D. Byrne, and J. Clark. 2010. Phenotyping in peach. 15 March 2013 < https://www.rosbreed.org/sites/default/files/files/RosBREED_2010-Phenotyping_protocol_P_persica.pdf>

Ghiani, A., N. Negrini, S. Morgutti, F. Baldin, F. Nocito, A. Spinardi, I. Mignani, D. Bassi, and M. Cocucci. 2011. Melting of Big Top nectarine fruit: Some physiological, biochemical, and molecular aspects. J. Amer. Soc. Hort. Sci. 136:61-68.

Glover, H., and C.J. Brady. 1995. Pectinesterases from mature, unripe peach fruit bind strongly to pectic polysaccharides. Austral. J. Plant Physiol. 22:977-985.

Haji, T., H. Yaegaki, and M. Yamaguchi. 2003. Softening of stony hard peach by ethylene and the induction of endogenous ethylene by 1-aminocyclopropane-1-carboxylic acid (ACC). J. Japan Soc. Hort. Sci. 72:212-217.

Haji, T., H. Yaegaki, and M. Yamaguchi. 2005. Inheritance and expression of fruit texture melting, non-melting and stony hard in peach. Scientia Hort. 105:241–248.

Hayama, H., A. Ito, T. Moriguchi, and Y. Kashimura. 2003. Identification of a new expansin gene closely associated with peach fruit softening. Postharvest Biol. Technol. 29:1-10.

Hayama, H., M. Tatsuki, A. Ito, and Y. Kashimura. 2006. Ethylene and fruit softening in the stony hard mutation in peach. Postharvest Biol. Technol. 41:16-21.

Hayama, H., M. Tatsuki, H. Yoshioka, and Y. Nakamura. 2008. Regulation of stony hard peach softening with ACC treatment. Postharvest Biol. Technol. 50:231-232.

Hayama, H., T. Shimada, A. Ito, H. Yoshioka, and Y. Kashimura. 2001. Changes in the levels of mRNA for putative cell wall-related genes during peach fruit development. Scientia Hort. 91:239-250.

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

Kao, M., J. Brecht, J. Williamson, and D. Huber. 2012. Ripening development and quality of melting and non-melting flesh peach cultivars. HortScience 47:879–885.

Kieber, J. 1997. The ethylene response pathway in Arabidopsis. Plant Mol. Biol. 48:277-296.

Lester, D., J. Speirs, C. Orr, and C. Brady. 1994. Peach (*Prunus persica)* endopolygalacturonase cDNA isolation and mRNA analysis in melting and non-melting peach cultivars. Plant Physiol. 105:225-231.

Lombardo, V., S. Osorio, J. Borsani, M. Lauxmann, C. Bustamante, C. Budde, C. Andreo, M. Lara, A. Fernie, and M. Drincovich. 2011. Metabolic profiling during peach fruit development and ripening reveals the metabolic networks that underpin each developmental stage. Plant Physiol. 157:1696–1710.

Lu, M., C. Song, C. Huang, and S. Ou. 2008. Changes in flesh firmness and ethylene production of different peach types during fruit ripening. Acta Hort. 768:153-159.

Lurie, S., A. Lers, H.W. Zhou, and L. Dong. 2002. The role of ethylene in nectarine ripening following storage. Acta Hort. 592:607-613.

Mignani, I., C. Ortugno, and D. Bassi. 2006. Biochemical parameters for evaluation of different peach flesh types. Acta Hort. 713:441-448.

Nilo, R., C. Saffie, K. Lilley, R. Baeza-Yates, V. Cambiazo, R. Campos-Vargas, M. González, L. Meisel, J. Retamales, A. Orellana, and H. Silva. 2010. Proteomic analysis of peach fruit mesocarp softening and chilling injury using difference gel electrophoresis (DIGE). BMC Genomics 11:43.

Nilo, R., R. Campos-Vargas, and A. Orellana. 2012. Assessment of *Prunus persica* fruit softening using a proteomic approach. J. Proteomics 75:1618-1638.

Obenland, D., C. Crisosto, and J. Rose. 2003. Expansin protein levels decline with the development of mealiness in peaches. Postharvest Biol. Technol. 29:11-18.

Peace, C. and J. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae, p. 19-53. In K. Folta and S. Gardiner (eds). Genetics and genomics of Rosaceae. Springer Science + Business Media, New York.

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

Peace, C., C. Crisosto, and T. Gradziel. 2005. Endopolygalacturonase: a candidate gene for freestone and melting flesh in peach. Mol. Breeding 16:21–31.

Pressey, R. and J.Avants.1973. Separation and characterization of endopolygalacturonase and exopolygalacturonase from peaches. Plant Physiol. 52:252-256.

Pressey, R., and J. Avants. 1976. Pear polygalacturonases. Phytochem. 15:1349–1351.

Ramina, A., P. Tonutti, and W. McGlasson. 2008. Ripening, nutrition, and postharvest physiology, p. 550-574. In: D.R. Layne and D. Bassi (eds.), The peach: botany, production, and uses. CAB Intl., Oxfordshire, UK.

Rasori, A., B. Ruperti, C. Bonghi, P. Tonutti, and A. Ramina. 2002. Characterization of two putative ethylene receptor genes expressed during peach fruit development and abscission. J. Experimental Botany 379:2333-2339.

Rozen, S. and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers, p. 365-386. In: Krawetz S, Misener S, (ed.). Bioinformatics Methods and Protocols: Methods in Molecular Biology. Totowa, NJ.

RosBREED. 2015a. Apple fruit storability/ethylene biosynthesis. 9 September 2015. < https://www.rosbreed.org/breeding/dna-tests/apple/storability>

RosBREED. 2015b. Ppe-Texture: DNA Tests for Peach Texture. 21 October 2015. < https://www.rosbreed.org/breeding/dna-tests/peach/texture>

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

SAS institute Inc., 2013. SAS® 9.4. Cary, NC.

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

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

Tataranni, A., A. Spada, C. Pozzi, and D. Bassi. AFLP-based bulk segregant analysis for tagging the slow-ripening trait in peach [*Prunus persica* (L.) Batsch]. J. Hort. Sci. Biotechnol. 85:78-82.

Tatsuki, M., N. Nakajima, H. Fujii, T.Shimada, M. Nakano, K. Hayashi, H. Hayama, H. Yoshioka, and Y. Nakamura. 2013. Increased levels of IAA are required for system 2

ethylene synthesis causing fruit softening in peach (*Prunus persica* L. Batsch). J. Experimental Bot. 64:1049–1059.

Tatsuki, M., T. Haji, and M. Yamaguchi. 2006. The involvement of 1-aminocyclopropane-1-carboxylic acid synthase isogene, Pp-ACS1, in peach fruit softening. Experimental Bot. 57:1281-1289.

Truque, E., E. Aguayo, F. Artes-Hernandez, P. Gomez, and F. Artes. 2012. Controlled atmosphere for the export of 'Miraflores' peaches. Acta Hort. 962:585-590.

Verde, I., N. Bassil, S. Scalabrin, B. Gilmore, C. Lawley, K. Gasic, D. Micheleti, U. Rosyara, F. Cattonaro, E. Vendramin, D. Main, V. Aramini, A. Blas, T. Mockler, D. Bryant, L. Wilhelm, M. Troggio, B. Sosinski, M. Aranzana, P. Arus, A. Iezzoni, M. Morgante, and C. Peace. 2012. Development and Evaluation of a 9K SNP Array for Peach by Internationally Coordinated SNP Detection and Validation in Breeding Germplasm. PLoS ONE 7:1-13.

Yang, S. and N. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. 35:155-89.

Appendix A. DNA Extraction Procedure

- Place tubes in compartments for shaker and place in liquid nitrogen.
- Make sure tissue is still frozen.
- Attach compartments to shaker. Set machine to run for 3 mins at a frequency of 25/s.
- Turn tubes around in compartments and run shaker at same time and speed (may put back in liquid nitrogen to keep frozen if necessary).
- Ensure the tissue doesn't thaw out until it is in the extraction buffer.
- Add 1200 µl (remember 2-mercaptoethanol) of Delaporta and vortex to evenly mix and liquefy (if doesn't liquefy too much tissue).
- Add 80 µl of 20% SDS to break down membranes. Vortex again and be sure there is no clumping (make sure it liquefies) of tissue in tube.
- 65 ^{0}C water bath for 30 min.
- Add 400 µl of 5M Potassium acetate and mix by inversion.
- Put on ice for 20 min (can be up to 30 min for apple) (*potentially leave this for longer if you need to but not more than 2 h).*
- Spin at 4 ^{0}C at 12,000 rpm for 20min.
- Pre-add 400 µl isopropanol (cold -20 ^{0}C) to two 1.5 mL tubes *(potentially stop here and put in +4 C fridge in needed)*.
- Once samples are done spinning split the sample equally (~800µl each / total volume ~1.2 ml).
- 1.5ml tubes; invert several times mixing **gently** (you can see DNA strands at this point).
- Spin at 4^{0}C 12,000 rpm for 10 to 15min.
- Pour off supernatant (make sure pellet doesn't dislodge), dry tube on tissue.
- Wash with 800µl of 70% EtOH (use cold -20 ^{0}C, helps remove isopropanol); invert several times **gently** *(potentially stop here and put in +4 C fridge in needed)*.
- Spin at 4 ^{0}C 12,000 rpm for 10 to 15 min.
- Decant and dry on tissue (careful pellet may be loose). *Leave overnight in flow hood.*
    - Optional instead of drying overnight: Place in thermomixer at 45 ^{0}C for 15 min (be sure samples completely dry, but do not overdry them).
- Set water bath to 37 ^{0}C for the next day
- NEXT DAY
- Add 200 µl TE buffer mixed with RNAse to each sample (1ml AE/1µl RNAse). After adding flick to mix. Quick spin then check to make sure DNA is dislodged from tube wall. Make sure DNA completely re-suspends.

    [optional***Stop here……place samples in +4 ^{0}C fridge overnight]
- RNAse treatment at 37 ^{0}C bath for 30 min (Make sure DNA is all dissolved).

- Combine samples in one 1.5 ml tube, wash empty tube with 100 µl TE buffer to get all material transferred (set pipette greater than sample size to get all material).
- Add 50 µl 3M sodium acetate and 350 µl of cold isopropanol (cold, -20 ^{0}C), mix by inversion.
- Place samples in -20 ^{0}C for a minimum of 30 min (45 is good).

  [optional***Stop here……place samples in -20 ^{0}C overnight]
- Spin samples at +4 ^{0}C at 12,000 rpm for 20 min. Decant and dry on tissue.
- Wash pellets with 70% EtOH (cold -20 ^{0}C)(~800 µl) and spin as directed above.
- Decant tubes and dry *Leave overnight in flow hood.*
    - optional instead of drying overnight: in thermomixer at 45 ^{0}C for 10 min - be sure samples completely dry.
- NEXT DAY
- Re-suspend (flick tubes so DNA dislodges and mixes) DNA in 100 µl of TE buffer and place in +4 ^{0}C fridge.
- Next day put in -20 ^{0}C freezer….better to freeze and thaw out….than keep in +4 ^{0}C fridge.
    - Don't keep in +4 fridge for more than a few days
- Check concentration (Nanodrop machine, and then run on gel).
- PCR followed by fragment analyzer (Dr. Mason's machine).

Solutions for DNA extraction

Delaporta Extraction buffer:

Final concentrations	For 300ml
0.1M Tris-HCl pH 8.0	30 ml 1M Tris HCl pH 8.0
0.05M EDTA pH 8.0	30 ml 0.5M EDTA pH 8.0
0.5M NaCl	30 ml 5M NaCl
PVP 40 000	1% (optional, add 2% if tissue high in polyphenolics, tannins etc., for peach and cherry use **2%)**
2-mercaptoethanol	90µl per 100 ml buffer – add just before use

The buffer minus the 2-mercaptoethanol can be **autoclaved and stored at room temperature** (RT)

20% SDS for 250 ml

Dissolve 50 g of SDS in 250 ml water.

Heat to 65 ºC to dissolve.

Store at RT, warm to remove precipitates before use.

5M Potassium acetate for 100 ml

Potassium acetate	49.1g
Dissolve in	90 ml of water

When in solution make volume up to 100 ml with water **(+4 °C fridge).**

3M Sodium acetate for 200 ml

TE (Tris:EDTA)

Final concentrations:	For 1 L
10mM Tris-HCl pH 8.0	10 ml 1.0 M Tris-HCl pH 8.0
1mM EDTA	2 ml 0.5M EDTA pH 8.0

Make volume to 1 liter with water. **Autoclave**

70% Ethanol for 100 ml

Ethanol (absolute)	70 ml
Water	30 ml

Chapter 3

APPLYING MARKER-ASSISTED BREEDING FOR FLESH TEXTURE IN THE ARKANSAS PEACH BREEDING PROGRAM

Abstract

Marker-assisted breeding (MAB) is a molecular technology which allows breeders to increase efficiency of the breeding cycle and potentially to decrease breeding program costs. Fruit breeding programs especially could take advantage of this technology due to the long juvenile period required before fruit is produced. The peach [*Prunus persica* (Batsch)] breeding program of the University of Arkansas (UA), as a part of the SCRI RosBREED project, has begun to apply MAB and it should become a routine activity within the program. Molecular markers associated with several fruit quality traits are being validated. One of these traits is flesh texture, a trait that most often is classified as melting flesh (MF) [also referred as quick-melting flesh (QMF)] or non-melting (NMF). However, other flesh types have been found within the UA program, and contribute toward higher postharvest storage potential. These include non-softening (NSF) and slow-melting flesh (SMF). The endopolygalacturonase (endoPG) enzyme is involved with pectin depolymerization in peach flesh, and depending on the allelic combination of its candidate gene, this enzyme determines if a peach will be MF (either QMF or SMF), NMF, or NSF when ripe. Currently, the endoPG-6 DNA test is available to apply MAB on these three flesh types in the program. The SMF has a crispy texture and a slower melting rate compared to QMF. This different melting rate was theorized to be due to lower ethylene production and not due to differences in endoPG activity. During the 2013 and 2014 seasons, flesh softening rates were measured on QMF, NMF, NSF, and SMF accessions, with the objective to validate

endoPG-6 and SMF DNA tests on these four flesh textures. Results indicated that these four flesh textures had different softening rates, and that QMF and SMF reach the same firmness at the end of evaluation period and that both were softer than NMF and NSF (the last the firmeramong all four). Also, endoPG-6 and SMF DNA tests together were able to distinguish QMF, SMF, NMF, and SMF, which are important results to begin the application of MAB for this trait.

Introduction

Fruit ripening is a complex process in peaches, that when completed, different flesh textures are expressed or differentiated. There is a high diversity in peach flesh types, which have been described and characterized in the previous years with advances in phenotypic and genotypic characterization. Different textures or flesh types are due to the great diversity of this species coming from different locations and breeding programs, such as MF, NSF, NMF, slow-ripening flesh (SRF), SMF, and stony-hard flesh (SHF) (Byrne et al., 2012).

According to Brummell (2006), MF peaches show two phases of rapid softening (the first at the beginning of ripening and the second at the beginning of melting). The beginning of softening occurs in the pre-climacteric stage of fruit ripening, when the fruit still has a green background color and coincides with the beginning of depolymerization of glycans and the onset of ripening was marked by a continued depolymerization of glycans and an increase in the rate of pectin demethylesterification (Brummell, 2006). Ghiani et al. (2011b) compared the endoPG behavior in MF and NMF peaches. Their results imply that the loss of cell turgidity of pericarp tissue is a common process during softening in MF and NMF (Ghiani et al., 2011a). However, the loss of cell adhesion was exclusively observed in the pericarp tissue of MF peaches and no

loss of cell adhesion was observed in NMF or unripe MF peaches (Ghiani et al., 2011a). Morphological analysis of MF and NMF revealed that mesocarp cells of NMF not only lose turgidity, but also cells put pressure on each other (Ghiani et al., 2011a). This pressure was suggested to come from the exocarp layer in which cells increased their volume during softening, a behavior not observed in MF fruits (Ghiani et al., 2011a).

Non-melting flesh cultivars were mostly developed for processing uses and this trait or flesh type was avoided by breeders in the U.S. for fresh market objectives (Sherman et al., 1990), but now this flesh type is becoming popular for the fresh market industry. Non-melting flesh cultivars lack this final melting phase, maintaining most of their firmness even when fully ripe and have a rubbery texture (Ghiani et al., 2011a,b; Lester, 1994). The University of Florida breeding program was the first to attempt to introgress characteristics of the MF selections/cultivars into the NMF types, such as high acidity, red overcolor, and in some cases the lack of the rubbery texture common of the NMF (Peace and Norelli, 2009; Sherman et al., 1990).

The SRF genotypes show a very slow rate of flesh softening, reduced CO_2 and ethylene production, and can remain on the tree even during the beginning of autumn in the dry conditions of California (Ramming, 1991). In these genotypes, fruit development appears to cease before the end of the cell expansion phase (Tataranni et al., 2010). The flesh is crisp and firm, never softens (or softens very slightly), but does not have the same texture of NMF (Ramming, 1991). It is assumed that this character is monogenic and recessive due to the segregation ratios of fruit obtained from a selfed, MF tree having one slow-ripening allele (3 MF : 1 slow-ripening) and of the segregation obtained from a controlled slow-ripening selfed population (0 normal : 1 slow-ripening) (Ramming, 1991; Tataranni et al., 2010).

Stony-hard flesh texture can be either MF or NMF, because the SHF locus (*Sh)* is independent and epistatic from the melting locus (Goffreda, 1992; Haji et al., 2005; Liverani et al., 2002).The softening process of SHF genotypes is blocked due to the lack of ethylene production and SHF fruits produced little or no ethylene, had low respiration rate, and tended to ripen later than non-stony hard fruit. These genotypes also had a lower percentage of blush (red overcolor) and maintained their firmness after 5 d of storage at 5 °C (Bassi et al., 1998; Goffreda, 1992; Hayama et al., 2006). Also, in this flesh type the background skin color and the flesh color remained greenish (Tataranni et al., 2010).

Genotypes having SMF texture maintain firmness for a longer period on the tree (similar to SHF genotypes), but at maturity they melt completely, reaching firmness values similar to conventional MF genotypes (Ghiani et al., 2011b; Sandefur, 2011). Non-softening-flesh (NSF) is another texture having a distinct flesh that could be confused with NMF given its high firmness after ripening. This genotype loses very little firmness and maintains a crispy texture during the ripening process and after harvest (Peace and Norelli, 2009). This texture type is associated with the clingstone trait and has a complete deletion of the endoPG gene in the Freestone-Melting (F-M) locus (Peace and Norelli, 2009).

Polygalacturonase enzymes were first identified over 45 years ago and have been suggested to be involved in the disassembly of pectins, particularly in tissues that require cell separation (Hadfield and Bennett, 1998). Endopolygalacturonase function is to soften fruits during the ripening process by hydrolysis of the pectate chain randomly in peach cell walls (Pressey and Avants, 1973, 1976). Endopolygalacturonase is found in several species, such as tomato (*Solanum lycopersicum* L.), melon (*Cucumis melo* L.), and apple (*Malus* x *domestica* Borkh.) (DellaPena et al., 1986; Giovannoni et al., 1989; Hadfield and Bennet., 1998; Wu et al.,

1993). In peach, this enzyme was first characterized by Pressey and Avants (1973). Pectin disassembly is associated with the later stage of ripening and with fruit deterioration to overripe. During disassembly, pectins are solubilized, the middle lamella swells and disappears, and the microfibrillar network becomes disorganized (Hadfield and Bennet, 1998).

Recent studies have determined that freestone and melting traits are controlled by a single locus, now referred to as the F-M locus, which has two copies of the same gene located near the distal end of peach LG 4 (Peace et al., 2005a, 2005b; Peace et al., 2007). The second copy of this gene is located less than 50 kilobase pairs (kbp) upstream from the first (Peace and Norelli, 2009). One copy controls the Melting locus and the other the Freestone locus (Peace et al., 2007). Thus the F-M locus has four alleles controlling both traits; cultivars can be freestone melting flesh (FMF), clingstone melting-flesh (CMF), clingstone non-melting flesh (CNMF), and clingstone non-softening flesh (CNSF) (Peace et al., 2005a, 2005b; Peace et al., 2007). Several studies proposed that during softening of MF cultivars, the activity of the endoPG enzyme increases along with the endoPG gene expression during the melting phase, and that in CNMF the expression of the endoPG gene is reduced and there is almost no endoPG activity (Peace et al., 2005a; Callahan et al., 2004; Ghiani et al., 2011a; Lester et al., 1994). Also, studies have found more than one genetic source of NMF (Callahan et al., 2004; Lester et al., 1996). These variations were reported to be related to different types of deletions of the genes segregating for endoPG (Callahan et al., 2004; Lester et al., 1996)

Freestone melting flesh genotypes can be obtained from four different allele combinations, FF, Ff, Ff1, and Ff2 (f2 corresponds to the null allele). Clingstone melting flesh genotypes result from the allelic combinations ff, ff1, and ff2. Clingstone non-melting flesh genotypes have the combination f1f1, and f1f2, while CNSF results only with the f2f2

combination (Peace et al., 2005a; Peace and Norelli, 2009). Further, the f allele is recessive to the F allele, the f1 allele is recessive to f and to F alleles, and the null allele is recessive to all (Peace et al., 2005a; Peace and Norelli, 2009).

Further, MF individuals could be separated in two distinct groups of texture based on their softening (also called melting) rate and/or their fruit ethylene production after harvest (RosBREED, 2015). The first group contains individuals that melt at a quick rate and their ethylene production increases at the second or third day after harvest, which in previous literature are referred to just as MF. This type of texture in this Chapter is referred to as quick-melting flesh (QMF). The second group of the melting texture contains individuals that melt at a slow rate and their ethylene production rate after harvest is slow during the first days of postharvest, increasing later, such as 'Big Top' (Ghiani et al., 2011b). This type of texture in this Chapter is referred to as SMF. A DNA test was developed by the UA which is able to predict if a certain peach/nectarine will melt in a quick or slow rate (Chapter 2 of this Dissertation). This DNA test was named SMF DNA test.

The peach breeding program of the UA began in 1964, and several peach and nectarine cultivars have been developed. The first peaches released were CNMF with yellow-flesh color destined for baby food (Clark, 2011). In recent years the objectives of the program have changed to focus on fresh-market cultivars, having different textures, flavors, flesh and skin colors, shapes, and harvest dates. As a part of the RosBREED project (Iezonni et al., 2010), a genotyping analysis was done for the first time in this breeding program to validate endoPG DNA markers and hopefully to find DNA markers for SMF (Sandefur, 2011). Endopolygalacturonase markers (endoPG-1 and endoPG-6) matched correctly to the phenotype 89% of the time with cultivars classified as QMF and NMF, but no differentiation was found

when the genotyping was focused for QMF and SMF. These results indicated that the endoPG marker, specifically endoPG-6, could be used to differentiate between NMF, MF, and NSF textures, but that other loci, different from the F-M locus, were likely responsible for SMF (Sandefur, 2011). The slow-melting and non-softening traits are present in the breeding program (Clark, 2011) and cultivars such as 'White County', 'Souvenirs', 'Amoore Sweet', and 'Bowden' have been recently released and have these particular textures. Further research is necessary to fully confirm the phenotypes and genotypes in the breeding program.

The objective of this study was to analyze the softening rate during 6 d after harvest of different selections and cultivars having different textures to test if the endoPG-6 and SMF DNA tests are able to distinguish correctly the four different peach flesh textures within the UA breeding program and apply MAB on these four textures.

Materials and Methods

Plant Material

All fruit phenotypic measurements were conducted at the University of Arkansas Fruit Research Station, Clarksville [west-central Arkansas (west-central Arkansas, lat. 35°31'58''N and long. 93°24'12''W; U.S. Dept of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]. In all testing, trees were either open-center trained and spaced 5.5 m between trees and rows, or trained to a perpendicular-V system with trees spaced 1.9 m in rows spaced 5.5 m apart. All trees were dormant pruned and fertilized annually with a single application of 640 Kg $\cdot ha^{-1}$ of complete fertilizer (19:19:19 of N:P K) and were sprinkler or drip irrigated as needed. Pests were managed using a program typical for commercial orchards

in the area (Smith, 2015; Studebaker et al., 2015). Fruit were thinned to a distance of 12 to 15 cm between fruit after shuck split but before pit hardening.

In 2013 and 2014, analyzed selections were A-663, A-665, A-672, A-699, A-708, A-743, A-758, A-760, A-761, A-772, A-778, A-794, A-816, A-860, A-861, A-864, and A-888. Analyzed cultivars were 'Amoore Sweet', 'Arrington', 'Bowden', 'Bradley', 'Roygold', 'Souvenirs', 'White County', 'White Diamond', 'White River', 'White Rock', and 'Winblo'.

In 2014, a second group of individuals composed of 207 selections and cultivars plus 158 individuals of seven 2010-seedling populations were added to data collection (22 individuals of population 1002, 35 individuals of population 1003, 13 individuals of population 1004, 22 individuals of population 1006, nine individuals of population 1007, 40 individuals of population 1011, 17 individuals of population 1015). This second group of individuals were field evaluated only (the softening rate was not measured on these as the two-year measured group, but rather rated subjectively, as the breeder in the program does routinely based on maturity and feel of the fruit). This second group was added to increase the amount of data to contribute to test validation.

For phenotyping measurements, 20-25 fruits were selected from mid-canopy of only healthy trees. According to the RosBREED phenotyping protocol for peach, for fruit sample collection the tree was checked to have a few edible fruits and then the fruit collected for measurement was early ripe, a stage called "tree-ripe" (Frett et al., 2012; Gasic et al., 2010). Only fruit exhibiting uniform shape and background color, and lacking any insect or disease damage were included in samples. All fruit were hand-harvested directly into 0.24 L corrugated trays (FormTex Plastics Corp., Houston, TX). For the initial 2-year analyzed group transportation from the field to the laboratory was done carefully (avoiding sudden movements

to decrease the probability that fruits hit with each other), since any damage on the fruit could have a negative effect on the final results. Immediately after harvest, each fruit was labeled and left at room temperature for 6 d after harvest.

Classification of melting individuals which are not SMF were designated QMF (the same as MF) for clarity of classification for my study.

Phenotypic Evaluation

Fruit firmness was measured with two different methodologies at harvest day (0 d), 3 d, and 6 d after harvest. The two methodologies were:

A. Compression: Fruit compression was performed by placing round pieces of flesh of 1 cm diameter on a flat surface, and then using a cylindrical and plane probe of 7.6 cm diameter to compress the flesh 10 mm (iCon Texture Analyzer, Texture Technologies Corp. Hamilton, MA).

B. Penetration: From both cheeks of each peach, skin was removed using a skin peeler and a probe of 8-mm tip was utilized to measure fruit firmness by penetrating the fruit 10 mm (iCon Texture Analyzer, Texture Technologies Corp. Hamilton, MA).

Due to limited fruit availability, compression measurements were not able to be conducted on seven individuals of the initial group (Table 2).

Leaf Sample Collection and DNA Extraction

Approximately 30-60 mg of young leaf tissue was collected during spring of 2013 and 2014 of all the analyzed material in this study (except for CU selections in which case extracted DNA was sent to the Fruit Breeding Genotyping Laboratory of the University of Arkansas from CU). In 2013, leaf tissue was placed in individual 1.5 mL tubes (Eppendorf, Hauppauge, NY) containing a 4 mm stainless steel bead (McGuire Bearing Company, Salem, OR). Samples were stored at -80 °C until DNA extraction. DNA was extracted following the protocol on Appendix

A. In 2014, young leaf samples were collected using coin envelopes, then samples were lyophilized for 7 d utilizing a lyophylizer Freezone® 12 model 77540 (Labconco Corporation, Kansas City, MO). When leaf samples were dry they were loaded into 96-deep well plates containing approximately 2 g of silica-gel in each well, including three negative control (empty wells containing only silica-gel). Then, DNA was extracted following the Edge-Garza et al. (2014). This protocol was used because it is high-throughput and cost efficient for extracting DNA in peaches and other *Prunus* species.

Polymerase Chain Reactions (PCR)

Extracted DNA was amplified utilizing optimized PCR reaction at the Fruit Breeding Genotyping Laboratory of the UA. The endoPG-6 DNA test was utilized to amplify the F-M locus (Table 1). Reactions consisted of a denaturalization step at 95 °C for 5 min, then 35 cycles of 95 °C for 45 s, 50 °C for 45 s, and 72 °C for 1 min, and lastly a final extension at 72 °C for 7 min using a thermocycler (BIO RAD, model T100, Hercules, CA). Each PCR-plate included three negative controls.

For SMF-SSR DNA (Table 1) test reactions consisted of a denaturalization step at 95 °C for 30 s, then 30 cycles of 95 °C for 5 min, then 35 cycles of 95 °C for 45 s, 52 °C for 45 s, and 72 °C for 1 min, and lastly a final extension at 72 °C for 7 min, using a thermocycler (BIO RAD, model T100). Each PCR-plate included three negative controls.

Individual 25.0 µL PCR reactions were utilized using 5.0 µL of PCR buffer (Promega Corp., Madison, WI), 1.5 µL of $MgCl_2$ (Promega), 1.5 µL of 10 µM dNTPs (Promega), 0.5 µL of forward and reverse primer each (Integrated DNA Technologies, Coralville, IA), 0.5 µL of 5X Taq polymerase (Gene and Cell Technologies, San Vallejo, CA), 15.5 µL of ultra pure water, and 1.5 µL of DNA template.

SSR Allele Analysis

All PCR reactions of all individuals included in this study (the initial and second groups) were resolved utilizing a Fragment Analyzer™, model AdvanCE FS96 (Advanced Analytical Technologies, Inc., Ames, IA), as per manufacturer instruction. The Fragment Analyzer™ was located at the wheat breeding laboratory, UA. This technology is based on capillary gel electrophoresis for DNA separation. Allele scoring was conducted utilizing PROSize® v.1 software (Advanced Analytical Technologies, Inc., Ames, IA).

The PCR reaction of these DNA tests should be performed together or, if not possible, they should be resolved together after the PCR. This is because the NSF individuals do have a deletion in the endoPG gene and no band amplification is expected for the endoPG-6 DNA test. Thus, if this test is not used along with SMF DNA test, it will not be possible to determine if the null amplification of the endoPG-6 DNA test is due to a NSF individual or due to a failed amplification.

Statistical Analysis

For the initial group, data for penetration and compression were analyzed by analysis of variance (ANOVA) as a two-factor analysis, with sources of variation year (2013 and 2014) and texture (QMF, SMF, NSF and NMF) by the PROC GLIMIX procedure (SAS® 9.4. Cary, NC). Least square means were calculated for mean comparisons ($P \leq 0.05$).

Table 1. Nucleotide sequence of forward and reverse primers of endoPG-6 and SMF DNA molecular markers on LG 4.

DNA test	Forward primer	Reverse primer
EndoPG-6	CGGGGTTACCATATCAGGTG	TTAGGGATGCCAATCCACTC
SMF	AGAGGGCTTCTCAAAAAGTGG	ATAAGGAAGGGTGCAAGTGG

Results and Discussion

EndoPG-6 and SMF DNA tests

DNA tests were performed on 28 cultivars and Arkansas selections plus their penetration and compression softening rate was taken and analyzed during 2013 and 2014 to test if endoPG-6 and SMF DNA tests were able to identify and differentiate the four peach flesh textures present in the UA peach breeding program (the initial group). After analysis of the DNA tests, 10 NMF accessions, five NSF accessions, three QMF accessions, and 10 SMF accessions were identified (Table 2). This is the first time that this type of information is available for the UA breeding program, in which by using genetic information the four textures present in the program are confirmed. 'White County' in a previous study was classified as QMF by endoPG-6 DNA test, but by phenotypic characterization was described as SMF (Clark, 2011; Sandefur, 2011). However, by adding the data generated by the SMF DNA it was confirmed that 'White County' is SMF. With this set of data, the breeder will be able to make more informed decisions on textures when using this information to apply MAB and deciding which selections to use as a parent for a certain cross. Band sizes of SMF DNA test are shown in Figs. 1 to 3. Band sizes of the endoPG-6 DNA test are shown in Figs. 4 to 6.

Table 2. Texture type and results of endoPG-6 and slow melting flesh (SMF) DNA tests performed on different peach and nectarine accessions, years 2013 and 2014. Also, indicated is if penetration and compression force tests were performed on them.

Accession	Texture[z]	DNA test alleles		Firmness test	
		EndoPG-6[y]	SMF[x]	Penetration	Compression
A-663	NSF	f2f2	SS	Yes	Yes
A-665	NSF	f2f2	SS	Yes	Yes
A-672	QMF	ff	QS	Yes	No
A-699	QMF	Ff2	QS	Yes	Yes
A-708	SMF	FF	SS	Yes	Yes
A-743	NMF	f1f1	SS	Yes	No
A-758	NMF	f1f1	SS	Yes	Yes
A-760	SMF	FF	SS	Yes	No
A-761	SMF	f_*	SS	Yes	Yes
A-772	SMF	ff2	SS	Yes	Yes
A-778	SMF	ff2	SS	Yes	Yes
A-794	NMF	f1_*	SS	Yes	No
A-816	NMF	f1_*	SS	Yes	Yes
A-860	NMF	f1f1	SS	Yes	Yes
A-861	NMF	f1_*	SS	Yes	No
A-864	NMF	f1f	SS	Yes	Yes
A-888	SMF	Ff	SS	Yes	No
Amoore Sweet	NSF	f2f2	SS	Yes	Yes
Arrington	NMF	f1f2	SS	Yes	No
Bowden	NSF	f2f2	SS	Yes	Yes
Bradley	NMF	f1f2	SS	Yes	Yes
RoyGold	NSF	f2f2	SS	Yes	Yes
Souvenirs	SMF	FF	SS	Yes	Yes
White County	SMF	FF	SS	Yes	Yes
White Diamond	SMF	FF	SS	Yes	Yes
White River	SMF	Ff	SS	Yes	Yes
White Rock	NMF	f1f1	SS	Yes	Yes
Winblo	QMF	FF	QS	Yes	Yes

[z] QMF and SMF refer to quick melting flesh and slow melting flesh, respectively.

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

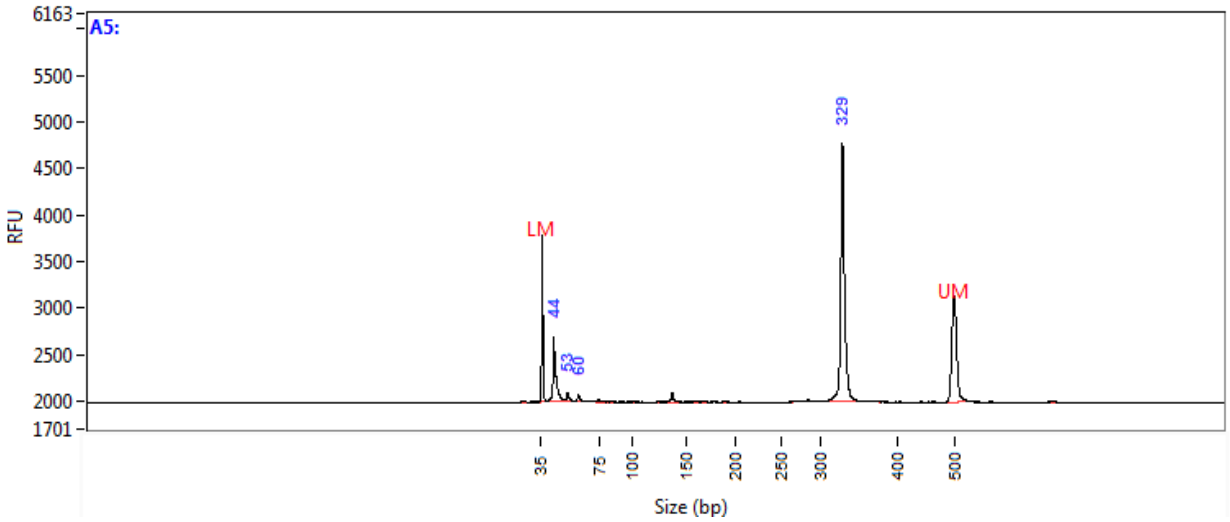

Fig. 1. Peak height (relative fluorescence units, RFU) of homozygous quick melting flesh (QQ) individual amplifying a band of 329 base pairs (bp) using the SMF DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. A5 corresponds to the row letter and column number of the location of sample on the plate.

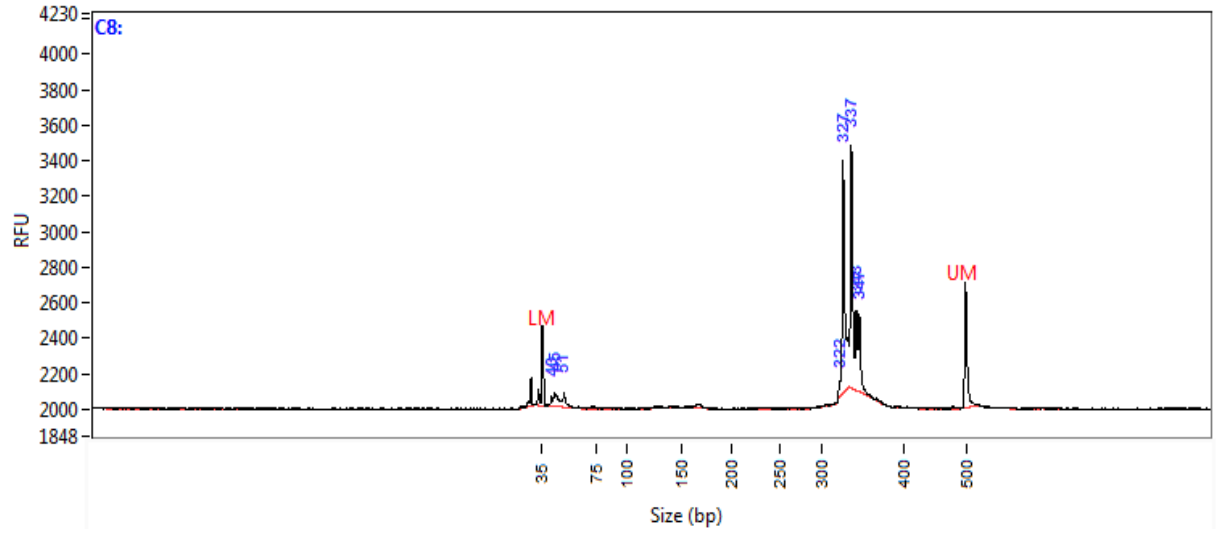

Fig. 2. Peak height (relative fluorescence units, RFU) of heterozygous quick melting flesh (QS) individual amplifying bands of 327 and 337 base pairs (bp) using the SMF DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. C8 corresponds to the row letter and column number of the location of sample on the plate.

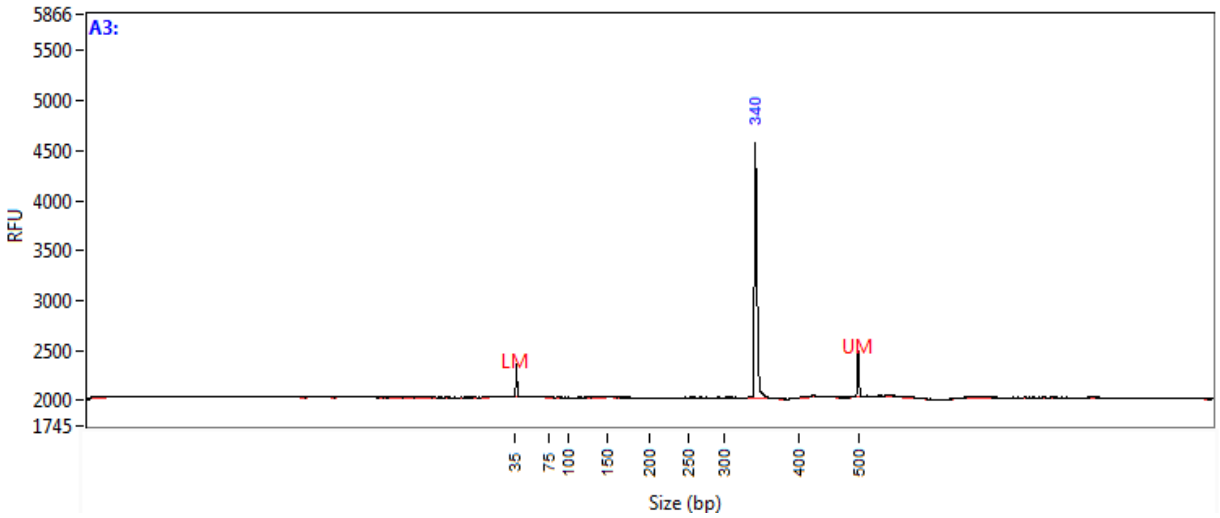

Fig. 3. Peak height (relative fluorescence units, RFU) of homozygous slow melting flesh (SS) individual amplifying a band 340 base pairs (bp) using the SMF DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. A3 corresponds to the row letter and column number of the location of sample on the plate.

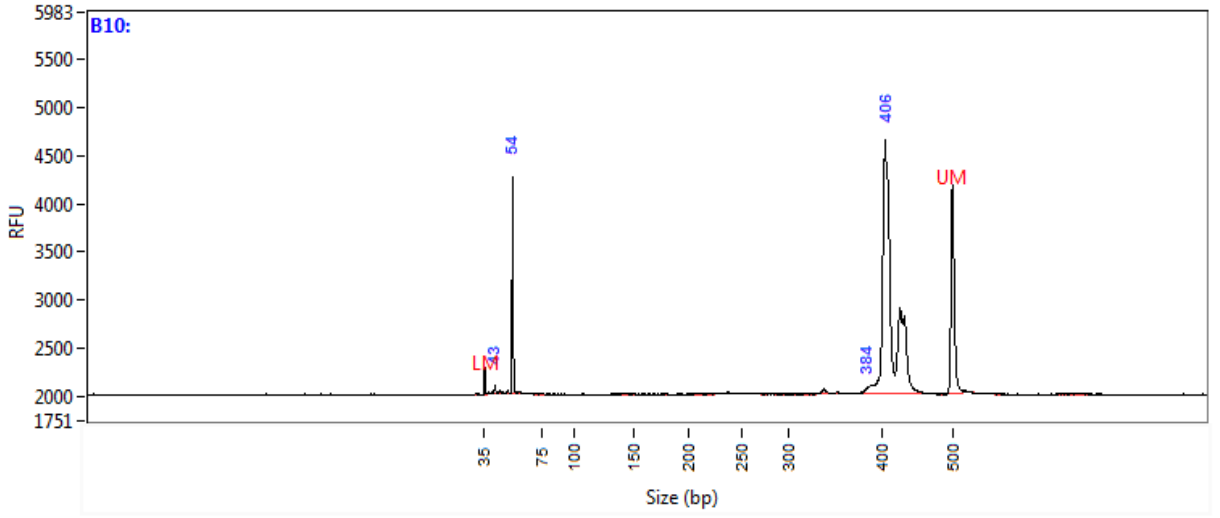

Fig. 4. Peak height (relative fluorescence units, RFU) of F allele, 406 base pairs (bp) band (FMF individual) using the EndoPG-6 DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. B10 corresponds to the row letter and column number of the location of sample on the plate.

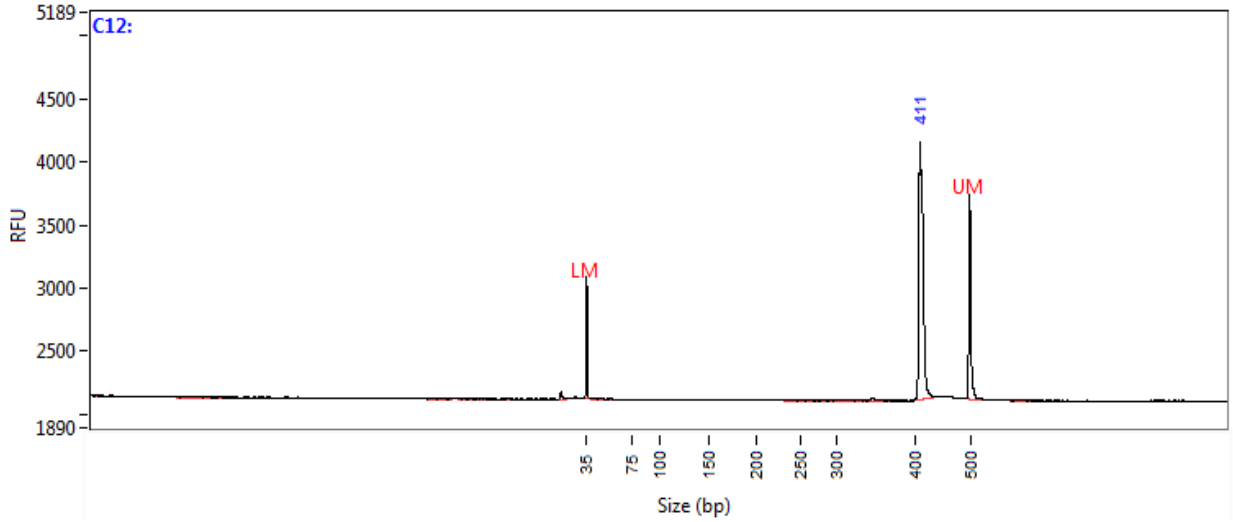

Fig. 5. Peak height (relative fluorescence units, RFU) of f allele, 411 base pairs (bp) band (CMF individual) using the EndoPG-6 DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. C12 corresponds to the row letter and column number of the location of sample on the plate.

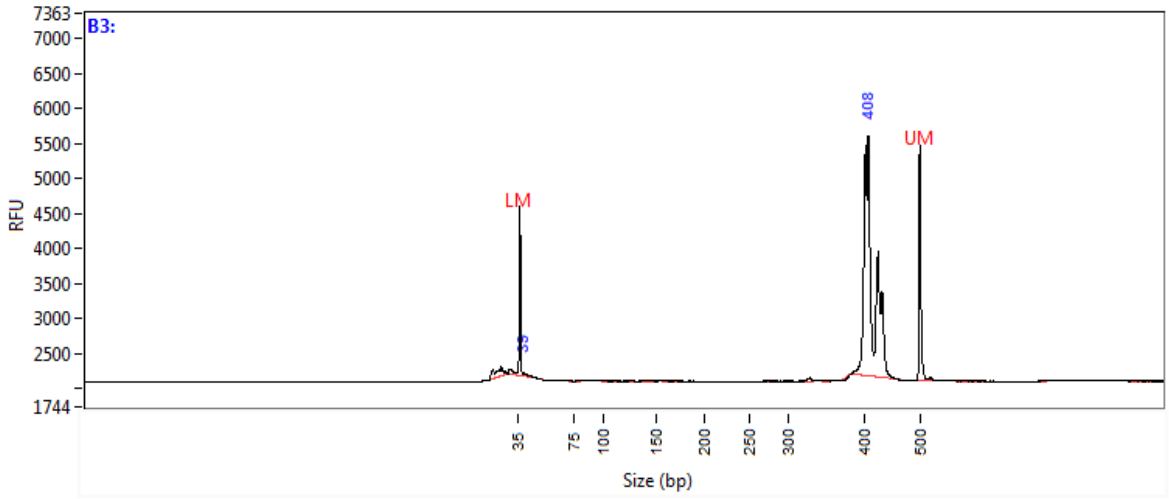

Fig. 6. Peak height (relative fluorescence units, RFU) of f1 allele, 408 base pairs (bp) band (CNMF individual) using the EndoPG-6 DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. B3 corresponds to the row letter and column number of the location of sample on the plate.

Softening Rate Analysis

During 2013 and 2014 seasons, fruit softening rates of different textures (QMF, SMF, NMF, and NSF) in the initial group were analyzed during 6 d after harvest. Softening rates were measured as penetration and compression forces at harvest day (day 0), 3 d, and 6 d after harvest. Analysis of variance of penetration measurements determined significance sources of variation for year and texture for days 0, 3, and 6, and year x texture interaction for days 3 and 6 (Table 3). Compression firmness had significant sources of variation for year for day 6, texture for days 3 and 6, and the interaction of year by texture was significant for days 3 and 6 (Table 4). This indicated that flesh texture varied for softening rate and that part of the variation could be explained by year, likely reflecting environmental influences (2013 season, on average, was warmer and rainy than 2014 season).

As stated before, year x texture interaction was not significant at day 0 for compression and penetration variables (Tables 3 and 4). Also, texture main effect was not significant at day 0 for compression, but it was significant for penetration. So, texture main effects are shown in Figs. 7 and 8 for penetration and compression, respectively. For penetration, at day 0 NMF and QMF were similar and were significantly softer than NSF texture; SMF was similar to NSF and NMF (Fig. 7). At day 3, NSF and NMF were not similar, but both were significantly higher than SMF and QMF (these last two textures are not significantly different). At day 6, NSF was significantly higher in firmness compared to the other three, while NMF texture was significantly firmer than SMF and QMF (these last two were not different). The trends for softening as measured by compression were very similar to that for penetration, but at day 0 all textures were similar (Fig. 8). This could be due to higher variation for this measurement at day of harvest. These results paralleled previous research which also found that for penetration force at day 6,

SMF and QMF were not significantly different and that NMF texture at day 6 was firmer than SMF and QMF (Sandefur, 2011). Values for SMF texture reported here are in the range to those of the SMF cultivar Big Top after 5 d of harvest (2.5±1.1 N) (Ghiani et al., 2011b) .

Table 3. Analysis of variance, and F-test p-value (P) of penetration firmness of different peach textures (NMF, NSF, QMF, and SMF) at harvest day (day 0), day 3, and day 6, years 2013 and 2014.

Source	*P*-value		
	Day 0	Day 3	Day 6
Year	<.0001	<.0001	<.0001
Texture	0.0112	<.0001	<.0001
Year*Texture	0.6889	0.0017	<.0001

Table 4. Analysis of variance, and F-test p-value (P) of compression firmness of different peach textures (NMF, NSF, QMF, and SMF) at harvest day (day 0), day 3, and day 6, years 2013 and 2014.

Source	*P*-value		
	Day 0	Day 3	Day 6
Year	0.6004	0.1060	0.0071
Texture	0.9790	<.0001	<.0001
Year*Texture	0.7837	<.0001	<.0001

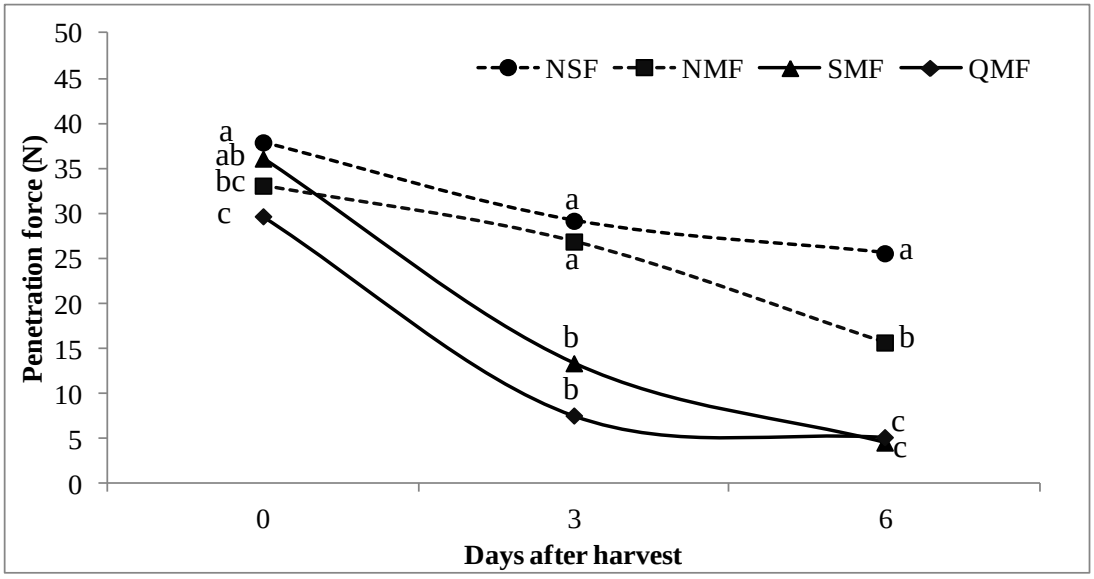

Fig. 7. Least square means of penetration softening rate from 0 to six days after harvest non-melting flesh (NMF), non-softening flesh (NSF), quick-melting flesh (QMF), slow-melting flesh (SMF), years 2013 and 2014. Means with the same letter on the same day are not significantly different ($P \leq 0.05$).

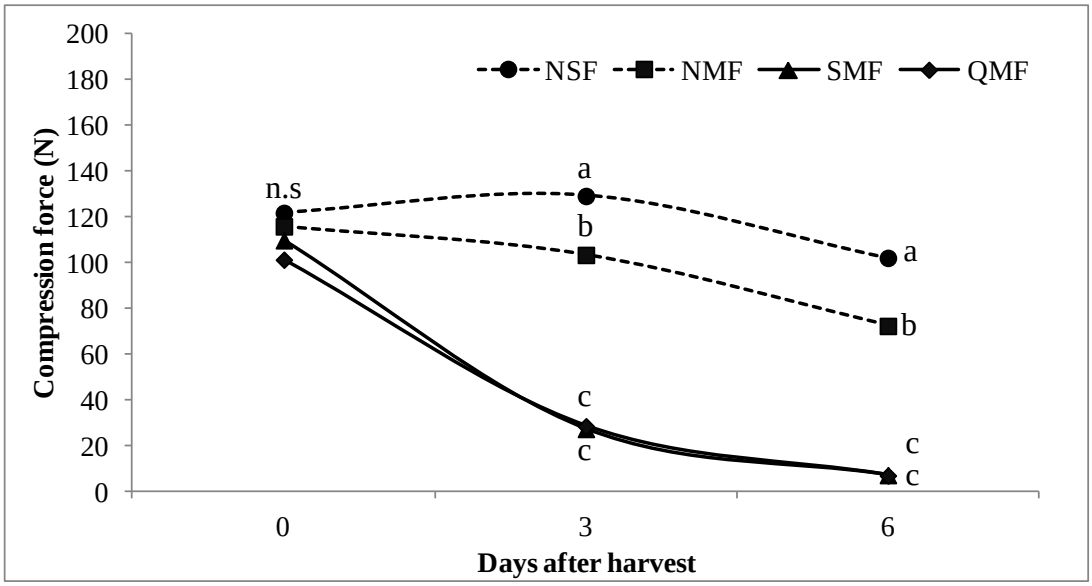

Fig. 8. Least square means of compression softening from 0 to six days after harvest non-melting flesh (NMF), non-softening flesh (NSF), quick-melting flesh (QMF), slow-melting flesh (SMF), years 2013 and 2014. Means with the same letter on the same day are not significantly different ($P \leq 0.05$).

Interaction means for year by texture for penetration at day 3 indicated that NSF, and NMF textures were significantly firmer in both years than QMF. Slow-melting flesh was less firm than NSF and NMF both years in within-year comparisons; SMF was similar to QMF both years (Table 5). The same interaction means for compression for day 6 showed similar results, with NSF and NMF the firmest although NSF was significantly firmer than NMF in all comparisons. Means for QMF and SMF were similar except for QMF for 2013 that was significantly higher (Table 5). The findings at the end of measurement period (day 6) indicated QMF and SMF usually reached similar levels of firmness measured by penetration confirmed that SMF individuals had a melting texture at the end of ripening and softening period, although SMF took longer to reach a similar level of firmness compared to NMF. This a promising result, because SMF individuals maintain their firmness for a longer period which is an advantage for growers and shippers, but once at the consumer's house the texture and firmness is the same as a melting peach (which consumers are used to purchasing for fresh consumption) (Crisosto and Velero, 2008).

For compression, interaction means for year by texture for days 3 and 6 showed similar results to those for penetration (Table 6). The major findings include that at days 3 and 6, NSF and NMF were the firmest textures compared to SMF and QMF, and SMF and QMF were usually similar for both days. Similar results have been previously reported (Sandefur, 2011). The similarity between SMF and QMF textures at the end of the softening period can explain in part why the endoPG-6 DNA test cannot distinguish between them, because both textures are scored as melting by this DNA test. The action of endoPG enzyme is likely not different in SMF individuals compared to QMF, but instead there is another enzyme or group of enzymes that

contribute to the delayed action of endoPG and the softening of the fruit (Ghiani et al., 2011b). The SMF DNA test helps to explain this difference (See Chapter 2).

The environmental conditions for each year likely explain much of the significant year x environment interactions. Harvest period in 2013 was from 15 July to 11 Aug. and in 2014 from 8 July to 8 Aug. According to the Fruit Research Station weather station, average maximum and minimum temperatures were ~2 °C lower during the harvest period in 2014 compared with the harvest period of 2013. Also, in 2014 there were 7 days over 32 °C while in 2013 11 days over 32 °C were recorded. Lastly, a total of 16.5 and 25.8 cm of rain were reported during harvest period in 2014 and 2013, respectively. This information shows that during the 2013 harvest period, environmental conditions were warmer with more days over 32 °C and with a total rainfall 1.6 times higher compared to the harvest period of 2014. The higher rainfall and warmer conditions likely influenced firmness in peaches during 2013.

Since penetration and compression analyses provided the same result to differentiate the four types of textures, it might be possible, for future analyses, to only use one of these measurements to reduce the amount of analysis and provide more efficient phenotyping within the peach breeding program. In general, penetration analysis is more commonly used in peach firmness and postharvest analysis (Crisosto and Labavitch, 2002; Crisosto et al., 1999; Ghiani et al., 2011b; Ortiz et al., 2012; Severa et el., 2012).

Table 5. Least square interaction means for year by texture for fruit penetration force (N) of four peach flesh textures at days 3 and day 6, years 2013 and 2014.

Year	Texture[z]	Penetration force (N)	
		Day 3	Day 6
2014	NMF	30.2 ab[y]	16.2 c
2013	NMF	23.5 cd	15.1 c
2014	NSF	34.1 a	29.0 a
2013	NSF	24.3 bc	22.1 b
2014	QMF	8.2 e	6.5 d
2013	QMF	6.9 e	3.8 e
2014	SMF	14.0 de	4.5 de
2013	SMF	12.9 e	4.8 de

[z] NMF: non-melting flesh, NSF: non-softening flesh, QMF: quick-melting flesh, SMF: slow-melting flesh.
[y] Means in the same column followed by the same letter are not significantly different, $P \leq 0.05$.

Table 6. Least square interaction means for year by texture for fruit compression force (N) of four peach flesh textures at days 3 and day 6, years 2013 and 2014.

Year	Texture[z]	Compression force (N)	
		Day 3	Day 6
2014	NMF	117.3 b[y]	59.2 c
2013	NMF	89.5 bc	85.4 b
2014	NSF	161.9 a	131.7 a
2013	NSF	96.4 b	72.5 b
2014	QMF	44.8 cd	8.4 d
2013	QMF	11.9 d	5.2 d
2014	SMF	33.0 d	7.7 d
2013	SMF	21.5 d	6.2 d

[z] NMF: non-melting flesh, NSF: non-softening flesh, QMF: quick-melting flesh, SMF: slow-melting flesh.
[y] Means in the same column followed by the same letter are not significantly different, $P \leq 0.05$.

Detailed information of SMF and endoPG-6 DNA tests results, prediction of flesh texture by these two DNA tests, and phenotypic information of cultivars, Arkansas selections, and Arkansas 2010-seedlings (the second group) are shown in Tables D.1 and D.2, respectively, in Appendix D. These analyses were performed to provide information for the Arkansas peach breeding program and to further verify if the markers were working consistently. For these individuals, phenotypic evaluation was only done subjectively in the field (softening rate was not measured), which is what most breeders do when they select a plant from seedlings when no genotypic analysis has been done on the seedlings prior. Most of the NSF individuals were classified phenotypically as NMF. This happened because NMF and NSF individuals share common characteristics of their flesh, but NMF individuals have a rubbery texture whereas NSF individuals have a texture that is very firm much like NMF but usually has a crispy aspect which can be difficult to differentiate in the field. Thus, applying a DNA test to differentiate both textures will be useful and will increase accuracy of identifying and confirming these textures. The same situation occurred with some individuals that have a melting texture. In the field, it can be difficult to differentiate QMF from SMF individuals (especially when SMF individuals are fully ripe and also on very hot days), but with the help of these DNA tests the necessary information will be generated to distinguish both textures.

Out of the 207 cultivars/selections, 10 did not match the DNA test predictions with the flesh phenotypic data, which is 4.8% of the individuals tested. A similar result occurred with the seedlings, where six out of 158 did not match, 3.8% of the total (Tables B.1 and B.2, respectively). This incorrect association of genotypic and phenotypic data could be due to the subjective character of the phenotypic taken of this second group, and also due to the single year of data, particularly in the 2010-seedlings. Thus, if one is not sure about the phenotypic data of a

particular individual, especially if that individual will be used as a parent, more than one year of phenotypic data should be used to confirm the texture or allele combination of that sample.

Cultivars and selection results of DNA tests are useful in marker-assisted parent selection (MAPS) to design crosses with more accurate information about parents and predict segregation ratios of the resulting populations. For example, if the breeder is planning a cross only to obtain CNSF individuals (for their high postharvest potential), only individuals with f2f2 allele combination should be chosen as parents, these being A-663, A-665, A-818, and A-833 (Table B.1). Seedlings results are useful to predict the flesh texture of very small plants (at the greenhouse stage) and apply marker-assisted seedling selection (MASS). This procedure will allow keeping only the individuals carrying the desired alleles and discarding others. For example, if only FSMF individuals were desired to be planted of family 1002, individuals 1002-10, 1002-11, 1002-22, and 1002-46 would be removed from that population since they are predicted to be CSMF (Table B.2).

Conclusions

Peach flesh texture is a major trait that breeders evaluate and select for depending on the objective of the breeding program or of the specific objective of a particular cross. The endoPG enzyme determines if a peach will be MF, NMF, or NSF with the MF allele being dominant over the others and the endoPG-6 DNA test is able to distinguish these three texture types. However, a fourth flesh texture is present in the UA program called SMF in which the endoPG-6 DNA test cannot differentiate this texture from MF individuals. To resolve this, a new DNA test associated with ethylene fruit production rate after harvest, was tested to differentiate QMF and SMF textures. The objective of this study was to verify if endoPG-6 and SMF DNA tests were able to

distinguish NMF, NSF, QMF, and SMF textures present in the UA Arkansas peach breeding program.

Both tests were analyzed on several selections, cultivars, and seedlings. Results indicated that when the dominant F allele (whether homozygosis or heterozygosis) of endoPG-6 is combined with the Q allele in homozygosis or heterozygosis of the SMF DNA test, the individual is predicted to be QMF. Conversely, when the F allele determined from the endoPG-6 DNA test (whether homozygosis or heterozygosis) is combined with the S allele (homozygosis) of the SMF DNA test, the individual is predicted to be a SMF texture. The same situation can happen with the f allele, with the difference that the peach in this case will be clingstone QMF or clingstone SMF. The f1 allele will determine a NMF peach and the f2f2 allele combination will determine NSF, independent of the allele combination of the SMF-DNA test. These tests are able to predict in juvenile trees the type of flesh texture the adult plant will produce. This information is useful to establish MAB in the program to select parents to be used in crossing that carry desired alleles for texture, and also to select or discard seedlings at the early stage of development. These tests should be used together in a PCR reaction or resolved together on a capillary electrophoresis system (or other system) to predict these four types of texture. Further, if there is no PCR amplicon of the endoPG-6 DNA test, this could be due to a NSF individual (null allele) being present or to a failed PCR amplification. So, to determine if a peach is a NSF (and not a failed sample) the SMF DNA test should amplify a band (Q or S). In Arkansas material, all of NSF individuals were homozygous SS for the SMF DNA test.

Lastly, this type of information, DNA tests results, of a particular individual (cultivar, selection, and seedlings) will not change over time, since the allelic profile will be the same from one season to the next or from one location to another). This is useful when making decisions for

crossing or selecting a potential new cultivar, because DNA information will tell the potential performance of an individual even if the trait of interest is quantitative and a high percentage of its variation is explained by the environment.

Literature Cited

Bassi, D., I. Mignani, and M. Rizzo. 1998. Calcium and pectin influence in peach texture. Acta Hort. 465: 433-438.

Brummel, D. 2006. Cell wall disassembly in ripening fruit. Functional Plant Biol. 33:103-119.

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

Callahan, A., R. Scorza, C. Bassett, M. Nickerson, and F. Abeles. 2004. Deletions in an endopolygalacturonase gene cluster correlate with non-melting texture in peach. Functional Plant Biol. 31:159-168.

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

Crisosto, C., R. Johnson, T. Dejong, and K. Ray. 1997. Orchard factors affecting postharvest stone fruit quality. HortScience 32:820-823.

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

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

Crisosto, C. and D. Valero. 2008. Harvesting and postharvest handling if peaches for flesh market, p. 575-596. In: D.R. Layne and D. Bassi (eds.), The peach: botany, production, and uses. CAB Intl, Oxfordshire, UK.

Dellapena, D., D. Alexander, and A. Bennett.1986. Molecular cloning of tomato fruit polygalacturonase: Analysis of polygalacturonase mRNA levels during ripening. Proc. Natl. Acad. Sci. 83:6420-6424.

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

Gasic, K., T. Gradziel, C. Crisosto, D. Byrne, and J. Clark. 2010. Phenotyping in peach. 15 March 2013 < https://www.rosbreed.org/sites/default/files/files/RosBREED_2010-Phenotyping_protocol_P_persica.pdf>

Ghiani, A., E. Onelli, R. Aina, M. Cocucci, and S. Citterio. 2011a. A comparative study of melting and non-melting flesh peach cultivars reveals that during fruit ripening endopolygalacturonase (endo-PG) is mainly involved in pericarp textural changes, not in firmness reduction. J. Experimental Bot. 62:4043–4054.

Ghiani, A., N. Negrini, S. Morgutti, F. Baldin, F. Nocito, A. Spinardi, I. Mignani, D. Bassi, and M. Cocucci. 2011b. Melting of Big Top nectarine fruit: Some physiological, biochemical, and molecular aspects. J. Amer. Soc. Hort. Sci. 136:61-68.

Giovannoni, J., D. DellaPenna, A. Bennett, and R. Fischer. 1989. Expression of a chimeric polygalacturonase gene in transgenic *rin* (ripening inhibitor) tomato fruit results in polyuronide degradation but not fruit softening. The Plant Cell 1:53-63.

Goffreda, J. 1992. Stony hard gene of peach alters ethylene biosynthesis, respiration, and other ripening related characteristics. HortScience 27:610 (Abstr.).

Iezzoni, A., C. Weebadde, J. Luby, C. Yue, E. van de Weg, G. Fazio, D. Main, C. Peace, N. Bassil, and J. McFerson. RosBREED: enabling marker-assisted breeding in Rosaceae. Acta Hort. 859:389-394.

Hadfield, K. and A. Bennett. 1998. Polygalacturonases: Many genes in search of a function. Plant Physiol. 117:337–343.

Hayama, H., M. Tatsuki, H. Yoshioka, and Y. Nakamura. 2008. Regulation of stony hard peach softening with ACC treatment. Postharvest Biol. Technol. 50:231-232.

Hayama, H., M. Tatsuki, A. Ito, and Y. Kashimura. 2006. Ethylene and fruit softening in the stony hard mutation in peach. Postharvest Biol. Technol. 41:16-21.

Haji, T., H. Yaegaki, and M. Yamaguchi. 2005. Inheritance and expression of fruit texture melting, non-melting and stony hard in peach. Scientia Hort. 105:241–248.

Lester, D., J. Speirs, C. Orr, and C. Brady. 1994. Peach (*Prunus persica)* endopolygalacturonase cDNA isolation and mRNA analysis in melting and non-melting peach cultivars. Plant Physiol. 105:225-231.

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

Liverani, A., D. Giovannini, and F. Brandi. 2002. Increasing fruit quality of peaches and nectarines: the main goals of ISF-FO (Italy). Acta Hort. 592:507-518.

Ortiz, A., G. Echeverría, J. Graell, and I. Lara. 2012. Cell wall disassembly during on-tree maturation, ripening and senescence of 'Snow Queen' nectarines. Acta Hort. 962:523-530.

Peace, C. and J. Norelli. 2009. Genomics approaches to crop improvement in the Rosaceae, pp. 19-53. In K. Folta and S. Gardiner (eds). Genetics and genomics of Rosaceae. Springer Science + Business Media, New York.

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

Peace, C., C. Crisosto, and T. Gradziel. 2005a. Endopolygalacturonase: a candidate gene for freestone and melting flesh in peach. Mol. Breeding 16:21–31.

Peace, C., R. Ahmad, T. Gradziel, A. Dandekar, and C. Crisosto. 2005b. The use of molecular genetics to improve peach and nectarine post-storage quality. Acta Hort. 682:403-409.

Pressey, R. and J.Avants.1973. Separation and characterization of endopolygalacturonase and exopolygalacturonase from peaches. Plant Physiol. 52:252-256.

Pressey, R., and J. Avants. 1976. Pear polygalacturonases. Phytochem. 15:1349–1351.

Ramming, D. 1991. Genetic control of a slow-ripening fruit trait in nectarine. Canadian J. Plant Sci. 71:601-603.

RosBREED. 2015. Ppe-Texture: DNA Tests for Peach Texture. 21 October 2015. < https://www.rosbreed.org/breeding/dna-tests/peach/texture>

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

SAS institute Inc., 2013. SAS® 9.4. Cary, NC.

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

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

Severa, L., S. Nedomova, J. Buchar, J. Trnka, and P. Dvorakova. 2012. Impact test as a tool for peach firmness. Acta Hort. 962:577-585.

Sherman, W., B. Topp, and P. Lyrene. 1990. Non-melting flesh for fresh market peaches. Proc. Fla. State Hort. Soc. 103:293-294.

Tataranni, G., A. Spada, C. Pozzi, and D. Bassi. 2010. AFLP-based bulk segregant analysis for tagging the slow-ripening trait in peach [*Prunus persica* (L.) Batsch]. J. Hort. Sci. Biotechnol. 85:78–82.

Wu, Q., M. Szakács-Dobozi, M. Hemmat, and C. Hrazdina. 1993. Endopolygalacturonase in apples *(Malus domestica)* and its expression during fruit ripening. Plant Physiol.102:219-225.

Appendix A. DNA Extraction Procedure

- Place tubes in compartments for shaker and place in liquid nitrogen.
- Make sure tissue is still frozen.
- Attach compartments to shaker. Set machine to run for 3 mins at a frequency of 25/s.
- Turn tubes around in compartments and run shaker at same time and speed (may put back in liquid nitrogen to keep frozen if necessary).
- Ensure the tissue doesn't thaw out until it is in the extraction buffer.
- Add 1200 µl (remember 2-mercaptoethanol) of Delaporta and vortex to evenly mix and liquefy (if doesn't liquefy too much tissue).
- Add 80 µl of 20% SDS to break down membranes. Vortex again and be sure there is no clumping (make sure it liquefies) of tissue in tube.
- 65 ^{0}C water bath for 30 min.
- Add 400 µl of 5M Potassium acetate and mix by inversion.
- Put on ice for 20 min (can be up to 30 min for apple) (*potentially leave this for longer if you need to but not more than 2 h).*
- Spin at 4 ^{0}C at 12,000 rpm for 20min.
- Pre-add 400 µl isopropanol (cold -20 ^{0}C) to two 1.5 mL tubes *(potentially stop here and put in +4 C fridge in needed)*.
- Once samples are done spinning split the sample equally (~800µl each / total volume ~1.2 ml).
- 1.5ml tubes; invert several times mixing **gently** (you can see DNA strands at this point).
- Spin at 4^{0}C 12,000 rpm for 10 to 15min.
- Pour off supernatant (make sure pellet doesn't dislodge), dry tube on tissue.
- Wash with 800µl of 70% EtOH (use cold -20 ^{0}C, helps remove isopropanol); invert several times **gently** *(potentially stop here and put in +4 C fridge in needed)*.
- Spin at 4 ^{0}C 12,000 rpm for 10 to 15 min.
- Decant and dry on tissue (careful pellet may be loose). *Leave overnight in flow hood.*
    - Optional instead of drying overnight: Place in thermomixer at 45 ^{0}C for 15 min (be sure samples completely dry, but do not overdry them).
- Set water bath to 37 ^{0}C for the next day
- NEXT DAY
- Add 200 µl TE buffer mixed with RNAse to each sample (1ml AE/1µl RNAse). After adding flick to mix. Quick spin then check to make sure DNA is dislodged from tube wall. Make sure DNA completely re-suspends.

  [optional***Stop here……place samples in +4 ^{0}C fridge overnight]
- RNAse treatment at 37 ^{0}C bath for 30 min (Make sure DNA is all dissolved).

- Combine samples in one 1.5 ml tube, wash empty tube with 100 µl TE buffer to get all material transferred (set pipette greater than sample size to get all material).
- Add 50 µl 3M sodium acetate and 350 µl of cold isopropanol (cold, -20 ^{0}C), mix by inversion.
- Place samples in -20 ^{0}C for a minimum of 30 min (45 is good).

  [optional***Stop here……place samples in -20 ^{0}C overnight]
- Spin samples at +4 ^{0}C at 12,000 rpm for 20 min. Decant and dry on tissue.
- Wash pellets with 70% EtOH (cold -20 ^{0}C)(~800 µl) and spin as directed above.
- Decant tubes and dry *Leave overnight in flow hood.*
    - optional instead of drying overnight: in thermomixer at 45 ^{0}C for 10 min - be sure samples completely dry.
- NEXT DAY
- Re-suspend (flick tubes so DNA dislodges and mixes) DNA in 100 µl of TE buffer and place in +4 ^{0}C fridge.
- Next day put in -20 ^{0}C freezer….better to freeze and thaw out….than keep in +4 ^{0}C fridge.
    - Don't keep in +4 fridge for more than a few days
- Check concentration (Nanodrop machine, and then run on gel).
- PCR followed by fragment analyzer (Dr. Mason's machine).

Solutions for DNA extraction

Delaporta Extraction buffer:

Final concentrations	For 300ml
0.1M Tris-HCl pH 8.0	30 ml 1M Tris HCl pH 8.0
0.05M EDTA pH 8.0	30 ml 0.5M EDTA pH 8.0
0.5M NaCl	30 ml 5M NaCl
PVP 40 000	1% (optional, add 2% if tissue high in polyphenolics, tannins etc., for peach and cherry use **2%)**
2-mercaptoethanol	90µl per 100 ml buffer – add just before use

The buffer minus the 2-mercaptoethanol can be **autoclaved and stored at room temperature** (RT)

20% SDS for 250 ml

Dissolve 50 g of SDS in 250 ml water.

Heat to 65 ºC to dissolve.

Store at RT, warm to remove precipitates before use.

5M Potassium acetate for 100 ml

Potassium acetate	49.1g
Dissolve in	90 ml of water

When in solution make volume up to 100 ml with water **(+4 °C fridge).**

3M Sodium acetate for 200 ml

TE (Tris:EDTA)

Final concentrations:	For 1 L
10mM Tris-HCl pH 8.0	10 ml 1.0 M Tris-HCl pH 8.0
1mM EDTA	2 ml 0.5M EDTA pH 8.0

Make volume to 1 liter with water. **Autoclave**

70% Ethanol for 100 ml

Ethanol (absolute)	70 ml
Water	30 ml

Appendix B. Detailed Allelic Information of SMF and EndoPG-6 DNA Tests for Different Cultivars, Selections, and Seedlings in the UA Breeding Program (Second Group)

Table B.1. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different accessions.

	DNA test results		DNA test prediction[x]	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]		Pit adhesion[v]	Texture[u]
A-554	SS	f1_*	CNMF	C	NMF
A-641	SS	F_*	FSMF	F	MF
A-647	SS	f2f2	CNSF	C	NMF
A-662	SS	-	-	C	NMF
A-663	SS	f2f2	CNSF	C	NMF
A-665	SS	f2f2	CNSF	C	NMF
A-668	SS	f2f2	CNSF	C	NMF
A-672	QS	ff	CQMF	C	MF
A-699	QS	Ff2	FQMF	F	MF
A-708	SS	FF	FSMF	F	SMF
A-716	SS	FF	FSMF	F	MF
A-743	SS	f1f1	CNMF	C	NMF
A-758	SS	f1f1	CNMF	C	NMF
A-760	SS	FF	FSMF	F	SMF
A-761	SS	f_*	CSMF	C	MF
A-765	SS	f2f2	CNSF	C	NSF
A-766	QS	FF	FQMF	F	MF
A-768	SS	FF	FSMF	F	MF
A-770	SS	f2f2	CNSF	C	NMF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.1. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different accessions (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
A-772	SS	ff2	CSMF	C	MF
A-778	SS	ff2	CSMF	C	MF
A-783	SS	f2f2	CNSF	C	NSF
A-786	SS	F_*	FSMF	F	MF
A-790	SS	F_*	FSMF	F	MF
A-792	SS	f2f2	CNSF	C	NMF
A-794	SS	f1_*	CNMF	C	NMF
A-797	SS	f2f2	CNSF	C	NMF
A-798	SS	f1_*	CNMF	C	NMF
A-799	SS	f2f2	CNSF	C	NMF
A-801	SS	f1_*	CNMF	C	NMF
A-803	SS	f1_*	CNMF	C	NMF
A-804	SS	f1_*	CNMF	C	NMF
A-805	SS	f1_*	CNMF	C	NMF
A-806	SS	f1_*	CNMF	C	NMF
A-808	SS	F_*	FSMF	F	MF
A-809	SS	F_*	FSMF	F	MF
A-810	SS	f2f2	CNSF	C	NMF
A-811	SS	f1_*	CNMF	C	NMF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).
[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).
[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.
[w] Subjective data from field observations.
[v] C: clingstone, F: freestone.
[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.1. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different accessions (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
A-813	SS	f2f2	CNSF	C	NMF
A-814	SS	f1_*	CNMF	C	NMF
A-815	SS	f2f2	CNSF	C	NMF
A-816	SS	f1_*	CNMF	C	NMF
A-818	SS	f2f2	CNSF	C	NMF
A-819	SS	f1_*	CNMF	C	NMF
A-820	SS	F_*	FSMF	F	MF
A-821	SS	F_*	FSMF	F	MF
A-822	SS	F_*	FSMF	C	NM
A-824	SS	f_*	CSMF	C	MF
A-825	SS	f1_*	CNMF	C	MF
A-826	SS	F_*	FSMF	F	MF
A-827	SS	F_*	FSMF	F	MF
A-828	SS	f1_*	CNMF	C	MF
A-829	SS	F_*	FSMF	F	MF
A-830	-	-	-	C	MF
A-832	SS	f1_*	CNMF	C	NMF
A-833	SS	f2f2	CNSF	C	NMF
A-836	SS	f1_*	CNMF	C	NMF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.1. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different accessions (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
A-837	SS	FF	FSMF	F	SMF
A-839	SS	f_*	CSMF	C	MF
A-840	SS	-	-	F	NMF
A-841	QS	f1_*	CNMF	C	NMF
A-842	SS	f1_*	CNMF	C	NMF
A-843	SS	f_*	FSMF	F	MF
A-844	SS	f1_*	CNMF	C	NMF
A-845	SS	F	FSMF	F	MF
A-846	SS	F_*	FSMF	C	NMF
A-847	QS	f1_*	CNMF	C	MF
A-848	SS	F_*	FSMF	F	MF
A-849	SS	F_*	FSMF	F	MF
A-850	SS	F_*	FSMF	F	SMF
A-851	SS	F_*	FSMF	F	MF
A-852	QS	f2f2	CNSF	C	NMF
A-853	SS	F_*	FSMF	F	MF
A-854	SS	F_*	FSMF	F	MF
A-855	QS	f1_*	CNMF	C	MF
A-856	QS	FF	FQMF	F	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.1. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different accessions (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
A-857	SS	F_*	FSMF	F	MF
A-858	QS	F_*	FQMF	F	MF
A-859	SS	F_*	FSMF	F	MF
A-860	SS	f1f1	CNMF	C	NMF
A-861	QS	f1_*	CNMF	C	NMF
A-862	SS	-	-	F	MF
A-864	SS	f1f	CNMF	C	NMF
A-865	QS	-	-	F	MF
A-866	SS	F_*	FSMF	F	MF
A-867	QS	f1_*	CNMF	C	NMF
A-868	QS	f1_*	CNMF	C	NMF
A-869	SS	-	-	C	NMF
A-870	QS	F_*	FQMF	F	MF
A-871	SS	f_*	CSMF	C	MF
A-872	SS	F_*	FSMF	F	MF
A-873	SS	f1_*	CNMF	C	NMF
A-874	SS	F_*	FSMF	F	MF
A-875	SS	f2f2	CNSF	C	NMF
A-876	QS	f1_*	CNMF	C	NMF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).
[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).
[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.
[w] Subjective data from field observations.
[v] C: clingstone, F: freestone.
[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.1. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different accessions (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
A-877	SS	F_*	FSMF	F	MF
A-878	SS	F_*	FSMF	F	MF
A-879	SS	F_*	FSMF	F	MF
A-880	SS	F_*	FSMF	F	MF
A-881	SS	-	-	F	SMF
A-882	SS	F_*	FSMF	F	MF
A-883	SS	F_*	FSMF	F	MF
A-884	SS	F_*	FSMF	F	MF
A-885	SS	F_*	FSMF	F	MF
A-886	SS	f1_*	CNMF	C	NMF
A-887	SS	-	-	F	MF
A-888	SS	F_*	FSMF	F	MF
A-889	SS	-	-	C	NMF
A-890	SS	f1_*	CNMF	C	NMF
A-891	SS	F_*	FSMF	F	MF
A-892	QS	f1_*	CNMF	C	NMF
A-893	-	-	-	F	MF
A-894	SS	F_*	FSMF	F	MF
A-895	-	-	-	C	NMF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).
[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).
[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.
[w] Subjective data from field observations.
[v] C: clingstone, F: freestone.
[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.1. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different accessions (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
A-896	SS	f2f2	CNSF	C	NMF
A-897	SS	F_*	FSMF	C	NMF
A-898	SS	f1_*	CNMF	C	NMF
A-899	SS	F_*	FSMF	C	MF
A-900	SS	F_*	FSMF	-	-
A-901	SS	F_*	FSMF	F	-
A-902	SS	f1_*	CNMF	-	MF
A-903	SS	f1_*	CNMF	C	NMF
A-904	SS	F_*	FSMF	F	NMF
A-905	-	-	-	-	NMF
A-906	SS	F_*	FSMF	-	NMF
A-907	SS	-	-	C	NMF
A-908	SS	f2f2	CNSF	C	NMF
A-909	SS	-	-	C	NMF
A-910	SS	F_*	FSMF	F	MF
A-911	QS	-	-	C	NMF
A-912	SS	F_*	FSMF	F	-
A-913	-	F_*	FSMF	-	-
A-914	SS	f_*	CSMF	-	-

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).
[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).
[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.
[w] Subjective data from field observations.
[v] C: clingstone, F: freestone.
[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.1. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different accessions (Cont.).

Accession	DNA test results		DNA test prediction[x]	Phenotypic data[w]	
	SMF[z]	EndoPG-6[y]		Pit adhesion[v]	Texture[u]
A-915	-	f_*	-	-	-
A-916	SS	f_*	CSMF	-	-
A-917	-	-	-	-	-
A-918	QS	f_*	FQMF	-	-
Allgold	SS	f1_*	CNMF	C	NMF
Amoore Sweet	SS	f2f2	CNSF	C	NMF
Arrington	SS	f1f2	CNMF	C	NMF
Autumn Prince	-	F_*	-	F	MF
Autumn Star	-	-	-	F	MF
Bounty	QS	F_*	FQMF	F	MF
Bowden	SS	f2f2	CNSF	C	NSF
Bradley	SS	f1f2	CNMF	C	NMF
Bright Star	QS	F_*	FQMF	F	MF
Challenger	SS	F_*	FSMF	F	MF
China Pearl	SS	F_*	FSMF	F	MF
Contender	SS	F_*	FSMF	F	MF
Cresthaven	SS	F_*	FSMF	F	MF
Crimson Lady	-	-	-	C	NMF
Crimson Snow	SS	F_*	FSMF	F	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).
[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).
[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.
[w] Subjective data from field observations.
[v] C: clingstone, F: freestone.
[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.1. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different accessions (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
CVN-13w	-	F_*	FSMF	F	MF
Denman	SS	F_*	FSMF	F	MF
Early Star	QQ	-	-	F	MF
Emeraude	SS	F_*	FSMF	F	MF
Eastern Glo	SS	-	-	C	MF
Flavor Top	SS	F_*	FSMF	F	MF
Gladiator	SS	F_*	FSMF	-	-
Gloria	QS	F_*	FQMF	F	MF
Goldilocks	SS	f2f2	CNSF	C	NMF
Goldjim	SS	f2f2	CNSF	C	NMF
Jade	SS	F_*	FSMF	-	-
KV175	-	F_*	-	-	-
KV357	SS	F_*	FSMF	-	-
KV398	QS	F_*	FQMF	-	-
KV401	SS	F_*	FSMF	-	-
KV501	SS	F_*	FSMF	F	MF
KV601	SS	F_*	FSMF	-	-
KV606	SS	F_*	FSMF	-	-
KV701	SS	F_*	FSMF	-	-

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.1. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different accessions (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
KV801	-	f1_*	-	-	-
Loring	-	F_*	-	F	MF
Manon	SS	F_*	FSMF	F	MF
Messina	QS	F_*	FQMF	F	MF
PF 1	SS	F_*	FSMF	-	-
PF 11	-	-	-	F	MF
PF 24-007	QS	F_*	FQMF	F	MF
Lovell	SS	F_*	FSMF	F	MF
PF 5B	SS	-	-	C	-
PF 5D Big	-	-	-	C	-
PF 8 Ball	-	-	-	C	MF
PF 9A-007	SS	F_*	FSMF	F	MF
PF Lucky 13/L	-	F_*	-	F	MF
PF-19-007	QS	F_*	FQMF	F	MF
Redhaven	SS	F_*	FSMF	F	MF
Rising Star	SS	F_*	FSMF	F	MF
Roygold	SS	f2f2	CNSF	C	NMF
Ruby Prince	SS	-	-	F	MF
Saturn	QS	F_*	FQMF	F	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.1. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different accessions (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
Scarlet Prince	SS	F_*	FSMF	F	MF
Souvenirs	SS	FF	FSMF	F	SMF
Spring Snow	-	F_*	-	-	-
Sugar Giant	-	F_*	-	F	MF
Sugar Lady	QS	F_*	FQMF	F	MF
Sweet Star	QS	F_*	FQMF	-	-
Tango	SS	-	-	C	MF
Tango-II	QS	-	-	-	-
Westbrook	SS	F_*	FSMF	C	MF
White County	SS	F_*	FSMF	F	SMF
White Diamond	SS	F_*	FSMF	F	SMF
White Lady	SS	F_*	FSMF	F	MF
White River	SS	Ff	FSMF	F	MF
White Rock	SS	f1f1	CNMF	C	NMF
White Cloud	SS	f1_*	CNMF	C	NMF
Winblo	QS	FF	FQMF	F	MF
Yumm Yumm	SS	F_*	FSMF	C	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

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Table B.2. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different Arkansas seedlings.

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
1002-002	SS	FF	FSMF	F	MF
1002-004	-	Ff1	-	C	MF
1002-007	SS	Ff1	FSMF	F	MF
1002-008	SS	Ff1	FSMF	F	SMF
1002-010	SS	ff	CSMF	F	SMF
1002-011	SS	ff1	CSMF	F	SMF
1002-013	-	ff1	-	F	-
1002-016	-	Ff1	-	F	MF
1002-019	SS	FF	FSMF	F	MF
1002-022	SS	ff1	CSMF	F	MF
1002-025	-	Ff1	-	F	SMF
1002-026	SS	Ff1	FSMF	F	SMF
1002-046	SS	ff1	CSMF	F	MF
1002-048	SS	FF	FSMF	F	MF
1002-050	-	-	-	F	MF
1002-053	SS	F_*	FSMF	F	MF
1002-056	SS	F_*	FSMF	F	MF
1002-057	SS	F_*	FSMF	F	MF
1002-059	SS	FF	FSMF	F	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.2. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different Arkansas seedlings (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
1002-072	SS	F_*	FSMF	F	MF
1002-091	SS	F_*	FSMF	F	MF
1002-094	SS	F_*	FSMF	F	MF
1003-010	SS	Ff	FSMF	F	MF
1003-014	SS	Ff	FSMF	F	MF
1003-018	SS	FF	FSMF	F	MF
1003-026	SS	FF	FSMF	F	MF
1003-027	SS	FF	FSMF	F	MF
1003-032	SS	FF	FSMF	F	MF
1003-035	SS	FF	FSMF	F	MF
1003-044	SS	FF	FSMF	F	MF
1003-045	SS	FF	FSMF	F	MF
1003-048	SS	FF	FSMF	F	MF
1003-049	SS	FF	FSMF	F	MF
1003-050	SS	FF	FSMF	F	MF
1003-054	SS	FF	FSMF	F	MF
1003-057	SS	FF	FSMF	F	MF
1003-063	SS	FF	FSMF	F	MF
1003-067	SS	Ff	FSMF	F	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.2. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different Arkansas seedlings (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
1003-068	SS	Ff	FSMF	F	MF
1003-069	SS	Ff	FSMF	F	MF
1003-071	SS	Ff	FSMF	F	MF
1003-075	SS	-	-	F	MF
1003-076	SS	Ff	FSMF	F	MF
1003-089	SS	FF	FSMF	F	MF
1003-090	SS	FF	FSMF	F	MF
1003-094	SS	FF	FSMF	F	MF
1003-096	SS	FF	FSMF	F	MF
1003-105	SS	FF	FSMF	F	MF
1003-106	SS	FF	FSMF	F	MF
1003-116	SS	F_*	FSMF	F	MF
1003-120	SS	Ff	FSMF	F	MF
1003-123	SS	Ff	FSMF	F	MF
1003-125	SS	FF	FSMF	F	MF
1003-167	SS	FF	FSMF	F	MF
1003-168	SS	-	-	F	MF
1003-206	SS	FF	FSMF	F	MF
1003-207	SS	FF	FSMF	F	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.2. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different Arkansas seedlings (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
1004-003	SS	F_*	FSMF	F	MF
1004-009	SS	Ff1	FSMF	F	MF
1004-012	SS	Ff1	FSMF	F	MF
1004-015	SS	FF	FSMF	F	MF
1004-020	SS	FF	FSMF	F	MF
1004-033	SS	Ff	FSMF	F	MF
1004-042	SS	FF	FSMF	F	MF
1004-051	SS	Ff1	FSMF	F	MF
1004-053	QS	FF	FQMF	C	NSF
1004-055	SS	FF	FSMF	F	MF
1004-057	QS	FF	FQMF	F	MF
1004-059	SS	FF	FSMF	F	MF
1004-060	SS	FF	FSMF	C	NSF
1006-003	SS	Ff	FSMF	F	MF
1006-004	SS	Ff	FSMF	F	MF
1006-006	SS	Ff	FSMF	F	MF
1006-011	SS	FF	FSMF	F	MF
1006-012	SS	FF	FSMF	F	MF
1006-013	SS	FF	FSMF	F	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.2. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different Arkansas seedlings (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
1006-024	SS	FF	FSMF	F	MF
1006-025	SS	Ff	FSMF	F	MF
1006-031	SS	Ff	FSMF	F	MF
1006-034	SS	Ff1	FSMF	F	MF
1006-035	SS	Ff1	FSMF	F	MF
1006-041	SS	FF	FSMF	F	MF
1006-043	SS	FF	FSMF	F	MF
1006-048	SS	FF	FSMF	F	MF
1006-049	SS	FF	FSMF	F	MF
1006-051	SS	FF	FSMF	F	MF
1006-054	SS	Ff	FSMF	F	MF
1006-055	SS	Ff	FSMF	F	MF
1006-074	SS	FF	FSMF	F	MF
1006-094	SS	FF	FSMF	F	MF
1006-106	SS	FF	FSMF	F	MF
1006-152	SS	FF	FSMF	F	MF
1007-024	SS	FF	FSMF	F	MF
1007-031	SS	FF	FSMF	F	MF
1007-036	SS	FF	FSMF	F	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.2. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different Arkansas seedlings (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
1007-063	SS	FF	FSMF	F	MF
1007-097	SS	Ff	FSMF	F	MF
1007-116	SS	Ff	FSMF	F	MF
1007-155	SS	FF	FSMF	F	MF
1007-192	SS	FF	FSMF	F	MF
1007-193	SS	FF	FSMF	F	MF
1011-001	SS	FF	FSMF	F	MF
1011-005	SS	FF	FSMF	F	MF
1011-014	SS	FF	FSMF	F	MF
1011-020	SS	FF	FSMF	F	MF
1011-023	SS	Ff2	FSMF	F	MF
1011-025	SS	Ff2	FSMF	F	MF
1011-026	SS	F_*	FSMF	F	MF
1011-027	QS	Ff2	FQMF	F	MF
1011-029	SS	Ff2	FSMF	F	MF
1011-034	SS	Ff2	FSMF	F	MF
1011-035	SS	Ff2	FSMF	F	MF
1011-040	SS	Ff	FSMF	F	MF
1011-046	SS	Ff	FSMF	F	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.2. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different Arkansas seedlings (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
1011-050	SS	Ff	FSMF	F	MF
1011-051	SS	Ff2	FSMF	F	MF
1011-055	SS	Ff2	FSMF	F	MF
1011-057	SS	Ff	FSMF	F	MF
1011-058	SS	Ff	FSMF	F	MF
1011-060	SS	Ff2	FSMF	F	MF
1011-062	QS	Ff2	FQMF	F	MF
1011-070	SS	Ff2	FSMF	F	MF
1011-071	SS	Ff2	FSMF	F	MF
1011-078	SS	Ff2	FSMF	F	MF
1011-081	SS	Ff2	FSMF	F	MF
1011-082	SS	Ff2	FSMF	F	MF
1011-083	SS	Ff2	FSMF	F	MF
1011-089	SS	Ff2	FSMF	F	MF
1011-093	SS	Ff2	FSMF	F	MF
1011-094	QS	Ff2	FQMF	F	MF
1011-099	QS	Ff2	FQMF	F	MF
1011-108	SS	Ff2	FSMF	F	MF
1011-114	SS	Ff2	FSMF	F	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.2. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different Arkansas seedlings (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
1011-117	SS	Ff2	FSMF	F	MF
1011-129	SS	Ff2	FSMF	F	MF
1011-131	SS	Ff2	FSMF	F	MF
1011-135	SS	F_*	FSMF	F	MF
1011-136	SS	Ff2	FSMF	F	MF
1011-139	QS	Ff2	FQMF	F	MF
1011-140	SS	Ff2	FSMF	F	MF
1011-154	QS	Ff2	FQMF	F	MF
1015-003	SS	FF	FSMF	F	MF
1015-004	SS	FF	FSMF	F	MF
1015-008	SS	FF	FSMF	F	MF
1015-012	QS	FF	FQMF	F	MF
1015-014	SS	FF	FSMF	C	MF
1015-015	SS	FF	FSMF	F	MF
1015-016	SS	FF	FSMF	C	MF
1015-017	QS	FF	FQMF	C	MF
1015-021	QS	FF	FQMF	F	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Table B.2. Allele combination of SMF and endoPG-6 DNA tests, texture prediction, and phenotypic data of different Arkansas seedlings (Cont.).

	DNA test results		DNA test	Phenotypic data[w]	
Accession	SMF[z]	EndoPG-6[y]	prediction[x]	Pit adhesion[v]	Texture[u]
1015-022	QS	FF	FQMF	F	MF
1015-029	SS	FF	FSMF	F	MF
1015-031	SS	-	-	C	MF
1015-040	QS	FF	FQMF	F	MF
1015-041	SS	-	-	C	MF
1015-044	SS	-	-	C	NMF
1015-050	QS	ff	CQMF	C	MF
1015-056	SS	FF	FSMF	C	MF

[z] Allele combination of SMF DNA test. SS refers to slow-melting flesh (SMF), QS refers to quick-melting flesh (QMF).

[y] Allele combination of endoPG-6 DNA test. F: freestone melting flesh allele, f: clingstone melting flesh allele, f1: clingstone non-melting flesh allele, f2: non-softening flesh allele. Accessions with an * at the side indicate that the accession could be homozygous or the second allele could be null (f2).

[x] CNMF: clingstone non-melting flesh, CNSF: clingstone non-softening flesh, CQMF: clingstone quick-melting flesh, CSMF: clingstone slow-melting flesh, FQMF: freestone quick-melting flesh, and FSMF: freestone slow-melting flesh.

[w] Subjective data from field observations.

[v] C: clingstone, F: freestone.

[u] MF: melting flesh, NMF: non-melting flesh, NSF: non-softening flesh, and SMF: slow-melting flesh.

Chapter 4

APPLICATION OF MARKER-ASSISTED BREEDING IN THE UNIVERSITY OF ARKANSAS PEACH BREEDING PROGRAM TARGETING THE MAJOR LOCUS FOR FRUIT ACIDITY

Abstract

Peach [*Prunus persica* (L.) Batsch] is the third-most important temperate tree fruit crop produced in the world. It is a diploid species that belongs to the *Rosaceae* family. Fruit quality characteristics such as flavor, acidity, color, flesh texture, size, shape, and shelf life are important attributes on which breeding programs focus to produce new and improved peach cultivars. The Arkansas peach and nectarine breeding program began in 1964 and was initially focused on clingstone, non-melting, yellow-flesh peach cultivars destined for the baby food industry. In recent years, the objectives of the program have changed to breeding fresh-market cultivars with different textures, flavors, flesh and skin colors, and harvest dates. Fruit acidity evaluated in the field and measured as titratable acidity (TA) is an important component of flavor and within the Arkansas peach breeding program a wide range of TA levels is present. As part of the RosBREED project, phenotypic and genotypic data were collected on numerous seedlings, selections, and cultivars in 2011, 2012, 2013, and 2014 with the objective of implementing marker-assisted breeding (MAB) for this trait to complement the traditional breeding process. The simple sequence repeat (SSR) marker CPPCT040, located on the chromosomal region in which the D-locus/gene (which segregates for low and high acidity peaches) is found was screened. This DNA test was able to distinguish homozygous (DD) and heterozygous (Dd) low-

acid individuals and homozygous (dd) high-acid individuals. These results are promising and provide the basis for the application of MAB for TA in this traditional breeding program.

Introduction

Improving fruit quality is one of the most important objectives within a breeding program, and peach breeders focus on developing new cultivars with increased sweetness and other flavor enhancements, attractive color, higher flesh firmness, larger fruits, new shapes, and other characteristics. Breeders must select for these favorable traits in order to obtain new and better cultivars. The peach has been a model crop within the *Prunus* genus and has recently been used in molecular research, and there are now markers available for breeders to use for selection in young seedlings and apply MAB.

Peach is one of the most widely grown and genetically characterized species in the *Rosaceae* family (Zhebentyayeva et al., 2008). Diploid peach nuclear DNA content is estimated to be 0.60 pg (Baird et al., 1994).

Molecular markers can be used for important functions in a breeding program such us (1) identifying outstanding parents, (2) enhancing selection of elite alleles at loci controlling important traits, (3) pyramiding favorable alleles at multiple loci affecting either a single trait or several traits, and, (4) cultivar fingerprinting and intellectual property and patent rights (Bliss, 2010; Testolin and Cipriani, 2010).

The application of MAB in horticultural and more specifically fruit crops needs a statically robust procedure for validating quantitative trait loci (QTL) in germplasm relevant to breeding programs. For this approach, the germplasm chosen should represent important members of larger pedigree-connected gene pools (Peace et al., 2014). Commonly, to validate if

the alleles of QTL-linked markers initially detected in an experimental population they are examined in a set of cultivars. However, these cultivars might not be related to the breeding germplasm of the program (Peace et al., 2014). Due to the cost and time required to phenotype important traits, breeders usually conduct QTL studies on a preselected subset of germplasm, thus limiting or truncating the phenotypic variation (Peace et al., 2014).

Fruit acidity is an important quality trait for breeders and consumers and is a major selection criterion (Boudehri et al., 2009). Low fruit acidity is controlled by the D-locus and is located on the proximal end of LG 5 of the peach genome. It is co-localized with major QTLs for pH, TA, and other organic acid concentrations such as malic and citric acid (Boudehri et al., 2009). It was first described by Monet in 1979, who determined that the D allele was dominant (Monet and Bassi, 2008). Several markers in the vicinity of the D-locus were genotyped in a segregating population for acidity, and the marker CPPCT040 was found to be tightly linked with this major locus (Boudehri et al., 2009). This indicates that this marker could be used for MAB (Boudehri et al., 2009). On LG 5, genes for acid/non-acid fruit and skin pubescence were identified (Dirlewanger et al., 2004). Later, Dirlewanger et al. (2006) constructed a new linkage map using 208 individuals of an F_2 peach population (‘Ferjalou Jalousia’ x ‘Fantasia’), and QTLs were detected. They reported that on the upper part of LG 5 are QTLs for pH and TA. The CPPCT040 SSR marker amplifies a chromosomal region from 993,688 to 994,003 base pairs (bp), at ~ 1.0 centiMorgan (cM) on LG 5, which is within the region where the D-locus/gene is located (990,843 to 994,898 bp) (NCBI Blast, 2015.)

The objective of this study was to apply for the first time the CPPCT040 SSR DNA molecular marker (D-locus DNA test) in the Arkansas breeding program to distinguish low- and

high-acid peach individuals, and validate the effectiveness of this marker to apply MAB as a new tool of selection in this breeding program.

Material and Methods

Plant Material

All fruit phenotypic measurements were conducted at the University of Arkansas Fruit Research Station, Clarksville [west-central Arkansas (west-central Arkansas, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]. In all testing, trees were either open-center trained and spaced 5.5 m between trees and rows, or trained to a perpendicular-V system with trees spaced 1.9 m in rows spaced 5.5 m apart. All trees were dormant pruned and fertilized annually with a single application of 640 $Kg{\cdot}ha^{-1}$ of complete fertilizer (19:19:19 of N:P:K) and were sprinkler or drip irrigated as needed. Pests were managed using a program typical for commercial orchards in the area (Smith, 2015; Studebaker et al., 2015). Fruit were thinned to a distance of 12 to 15 cm between fruit after shuck split but before pit hardening.

For phenotyping measurements, 20-25 fruits were selected from mid-canopy of only healthy trees. According to the RosBREED phenotyping protocol for peach, for fruit sample collection the tree was checked to have a few edible fruits and then the fruit collected for measurement was early ripe, a stage called “tree-ripe” (Frett et al., 2012; Gasic et al., 2010). Only fruit exhibiting uniform shape and background color, and lacking any insect or disease damage were included in samples. Also, transportation from the field to the laboratory was done carefully (avoiding sudden movements to decrease the probability that fruits hit with each other), since any damage on the fruit could have a negative effect on the final results.

Pedigree Identification

In 2011, 2012, and 2013, the germplasm used in this study was part of the RosBREED project and it was chosen to effectively represent alleles currently found within the Arkansas breeding program (Peace et al., 2014). Ancestors, important breeding parents, cultivars, selections, and populations were identified and integrated in a comprehensive pedigree.

Analyzed cultivars were 'Amoore Sweet', 'Arrington', 'Bradley', ' Souvenirs', 'White County', and 'Winblo', as well Arkansas selections A-665, A-672, A-699, A-708, A-716, A-760, A-772, A-773 A-776, A-778, A-783, and A-789. Seedlings from seven-F_1 segregating populations that were interrelated and related with common ancestors were utilized: 49 seedlings from population ArPop_1 ('White County' x A-672), 16 seedlings from ArPop_0801 (A-776 x A-783), 15 seedlings from ArPop_0803 ('Amoore Sweet' x A-778), 12 seedlings from ArPop_0813 (A-772 x A-672), nine seedlings from ArPop_0817 (A-789 x A-699), 23 seedlings from ArPop_0819 (A-708 x A-773), and 17 seedlings from ArPop_0825 ('Souvenirs' x A-760). In 2014, Arkansas populations of crosses made in 2010 (individuals measured in these populations ranged from four to 30 individuals per population) were analyzed to validate the fruit acidity DNA test. This last-measured group of seedlings was not part of the RosBREED project.

Also in 2013 and 2014, a set of additional selections and cultivars (not part of RosBREED project) included, A-544, A-743, A-758, A-761, A-766, A-768, A-770, A-786, A-790, A-792, A-794, A-797, A-798, A-799, A-801, A-803, A-804, A-805, A-806, A-808, A-809, A-811, A-813, A-814, A-815, A-816, A-818, A-819, A-820, A-821, A-822,A-834, A-825, A-836, A-827, A-828, A-829, A-833, A-840, A-841, A-842, A-844, A-846, A-848, A-849, A-850, A-850, A-851, A-852, A-853, A-854, A-856, A-857, A-858, A-859, A-860, A-862, A-864, A-866, A-868, A-869, A-872, A-873, A-874, A-875, A-876, A-877, A-878, A-881, A-882, A-883,

A-884, A-885, A-886, A-887, A-888, A-891, ‘Bowden’, ‘Challenger’, ‘Cresthaven’, CVN-13w, ‘EasternGlo’, ‘Goldilocks’, ‘Goldjim’, ‘Goldnine’, KV501, ‘Loring’, ‘Manon’, ‘Messina’, ‘Lovell’, ‘Redhaven’, ‘Roygold’, ‘Tango’, ‘White Diamond’, ‘White Lady’, ‘White River’, ‘White Rock’, and ‘Yumm Yumm’.

Phenotypic Evaluation

For phenotyping measurements, 20-25 fruits were selected from mid-canopy of only healthy trees. According to the RosBREED phenotyping protocol for peach, for fruit sample collection the tree was checked to have a few edible fruits and then the fruit collected for measurement was early ripe, a stage called “tree-ripe” (Frett et al., 2012; Gasic et al., 2010). Only fruit exhibiting uniform shape and background color, and lacking any insect or disease damage were included in samples. All fruit were hand-harvested directly into 0.24 L corrugated trays (FormTex Plastics Corp., Houston, TX). Also, transportation from the field to the laboratory was done carefully (avoiding sudden movements to decrease the probability that fruits hit with each other), since any damage on the fruit could have a negative effect on the final results. For the phenotyping evaluation, at least five fruit from each individual tree were selected and subjected to the evaluation procedure as follows utilizing the peach phenotyping protocol (Frett et al., 2012).

In 2011, 2012, and 2013 TA was measured using a Methrohm 877 Titrino Plus automatic titrator with a LL Unitrode combination pH (Matrohm AG, Herisau, Switzerland). In 2014, TA was measured using a Methrohm 862 Compact Titrisampler (Matrohm AG, Herisau, Switzerland).

The following equation (Gasic et al., 2010) was used to calculate TA (the milliequivalent factor used corresponded to malic acid, 0.067):

$$\text{Titratable acidity (\%)} = \frac{[\text{NaOH titrated (ml)} * 0.1\text{ N (NaOH)} * \text{milliequivalent factor} * 100]}{6\text{ g of juice}}$$

The milliequivalent factor corresponds to the equivalent amount of material that will react with 1 g of N.

Leaf Sample Collection and DNA Extraction

Approximately 30-60 mg of young leaf tissue was collected during spring of 2013 and 2014 of all the analyzed material in this study (except for CU selections in which case extracted DNA was sent to the Fruit Breeding Genotyping Laboratory of the University of Arkansas from CU). In 2013, leaf tissue was placed in individual 1.5 mL tubes (Eppendorf, Hauppauge, NY) containing a 4 mm stainless steel bead (McGuire Bearing Company, Salem, OR). Samples were stored at -80 °C until DNA extraction. DNA was extracted following the protocol on Appendix A. In 2014, young leaf samples were collected using coin envelopes, then samples were lyophilized for 7 d utilizing a lyophylizer Freezone® 12 model 77540 (Labconco Corporation, Kansas City, MO). When leaf samples were dry, they were loaded into 96-deep well plates containing approximately 2 g of silica-gel in each well, including three negative control (empty wells containing only silica-gel). Then, DNA was extracted following the Edge-Garza et al. (2014). This protocol was used because it is high-throughput and cost efficient for extracting DNA in peaches and other *Prunus* species.

Polymerase Chain Reactions (PCR)

Extracted DNA of all samples/individuals was amplified utilizing optimized PCR reaction for the CPPCT040 DNA marker at the Fruit Breeding Genotyping Laboratory of the University of Arkansas. Forward and reverse sequences of this primer are shown in Table 1. Reactions consisted in a denaturalization step at 95 °C for 5 min, then 35 cycles of 95 °C for 45 s, 52 °C for 45 s, and 72 °C for 1 min, and lastly a final extension at 72°C for 7 min, utilizing a

thermocyler (BIO RAD, model T100, Hercules CA). Each PCR-plate included three negative controls.

Individual 25.0 µL PCR reactions were utilized using 5.0 µL of PCR buffer (Promega), 1.5 µL of $MgCl_2$ (Promega), 1.5 µL of 10 µM dNTPs (Promega), 0.5 µL of forward and reverse primer each, 0.5 µL of 5X Taq polymerase, and 15.5 µL of ultra pure water, and 1.5 µL of DNA template.

SSR Allele Analysis

PCR reactions of all individuals included in this study were resolved utilizing a Fragment Analyzer™, model AdvanCE FS96 (Advanced Analytical Technologies, Inc., Ames, IA), as per manufacture instruction. The Fragment Analyzer™ is located at the wheat breeding laboratory, University of Arkansas. This technology is based on capillary gel electrophoresis for DNA separation. Allele scoring was conducted utilizing PROSize® v.1 software (Advanced Analytical Technologies, Inc., Ames, IA).

Statistical Analysis

Titratable acidity values of Arkansas RosBREED populations and Arkansas 2010-seedlings were tested for normality and later transformed using the base 10 logarithm of each value to approach normality. Associations between TA and allele combinations were performed by least square means comparisons ($P \leq 0.05$) by utilizing the PROC GLIMIX procedure (SAS® 9.4. Cary, NC). In the case of Arkansas RosBREED populations the ANOVA test was performed by using data of years 2011, 2012, and 2013. In the case of Arkansas 2010-seedlings, no year effect was analyzed since only one year of data was analyzed (year 2014). Chi-square test was performed in each Arkansas RosBREED population to test if the allelic segregation fit the

expected segregation ratios. This test was not performed on the 2010-seedlings, because not all of the individuals amplified a band and/or did not have phenotypic data to use in the analysis.

Table 1. Nucleotide sequence of forward and reverse primers of *D*-locus DNA test (CPPCT040 SSR marker) on LG 5.

Physical location (bp)	Forward	Reverse
993,688 994,003	TGAAATAAAATTACGCCAACAGG	GTTTGAAGATGGGATTGGAAATGG

Results and Discussion

Titratable Acidity

Seven-F_1 populations were tested for association of TA and their allele combination of the *D*-locus DNA test. Analysis of variance indicated significant year, allele, and year x allele interaction effects (Table 2). Even though the interaction was significant, the main effect means for TA for years are provided to elucidate the year-to-year variation. Interaction means are shown later in my discussion. Means for TA for 2013 were significantly higher than for 2012 and 2011; 2012 and 2011 TA values were not significantly different (Table 3). These results indicate that TA was likely affected by environmental conditions. This trait in peaches is controlled by a major locus located on the proximal end of LG 5 and was reported to be qualitative with the low-acid character expression to be dominant (Monet and Bassi, 2008). However, this trait has not always been found to be qualitative, and was reported to have incomplete dominance in molecular studies (Boudeheri et al., 2009, de Souza et al., 1998). My findings (reported in Chapter 1 of this dissertation) of the QTL analysis of this trait by FlexQTL™ indicated that the major locus on LG 5 explained 35% to 62% of the phenotypic variation. Also, weather data showed that the environmental factors of rainfall and temperature varied among years, especially when comparing 2011 and 2012 to 2013; the first two years were hot and very dry compared to 2013. Average high temperatures from 1 June to 31 Aug. were 34, 35, and 31 °C in 2011, 2012, and 2013, respectively. Average minimum temperatures from 1 June to 31 Aug. were 23, 22, and 20 °C in 2011, 2012, and 2013, respectively. Days exceeding 32 °C were 96 in 2011, 92 in 2012, and only 54 in 2013 between 1 June and 31 Aug. Also, the amount of rain was variable during the years of my study with a total of 20 cm of rain in 2011,

12 cm in 2012, and 33 cm in 2013 from 1 June to 31 Aug. (data collected at the Fruit Research Station weather station).

Trait distribution is shown in Fig. 1, in which the mean was centered in the lower values of TA of the curve. Titratable acidity values ranged from 1.25% (high acidity) to 0.15% (low acidity). This range shows high variation from low to high acidity represented in the Arkansas RosBREED germplasm, with high diversity as needed for this study. Parents of each population and their corresponding TA values per year are presented in Table 4. Table 5 shows mean, max., min., and standard deviation values of across-year average of TA values per family per trait.

Table 2. Analysis of variance, degrees of freedom (DF), and F-test p-value (*P*) for titratable acidity (%), Arkansas RosBREED populations, years 2011, 2012, and 2013.

Source	DF	P
Year	2	0.0001
Allele	2	<0.0001
Year*Allele	4	0.02170

Table 3. Least square main effect means of titratable acidity (TA) (%) for years 2011, 2012, and 2013, Arkansas RosBREED populations.

Year	TA (%)
2013	0.69 a[z]
2011	0.58 b
2012	0.52 b

[z] Means in the same column followed by the same letter not significantly different as determined by least square means $P \leq 0.05$.

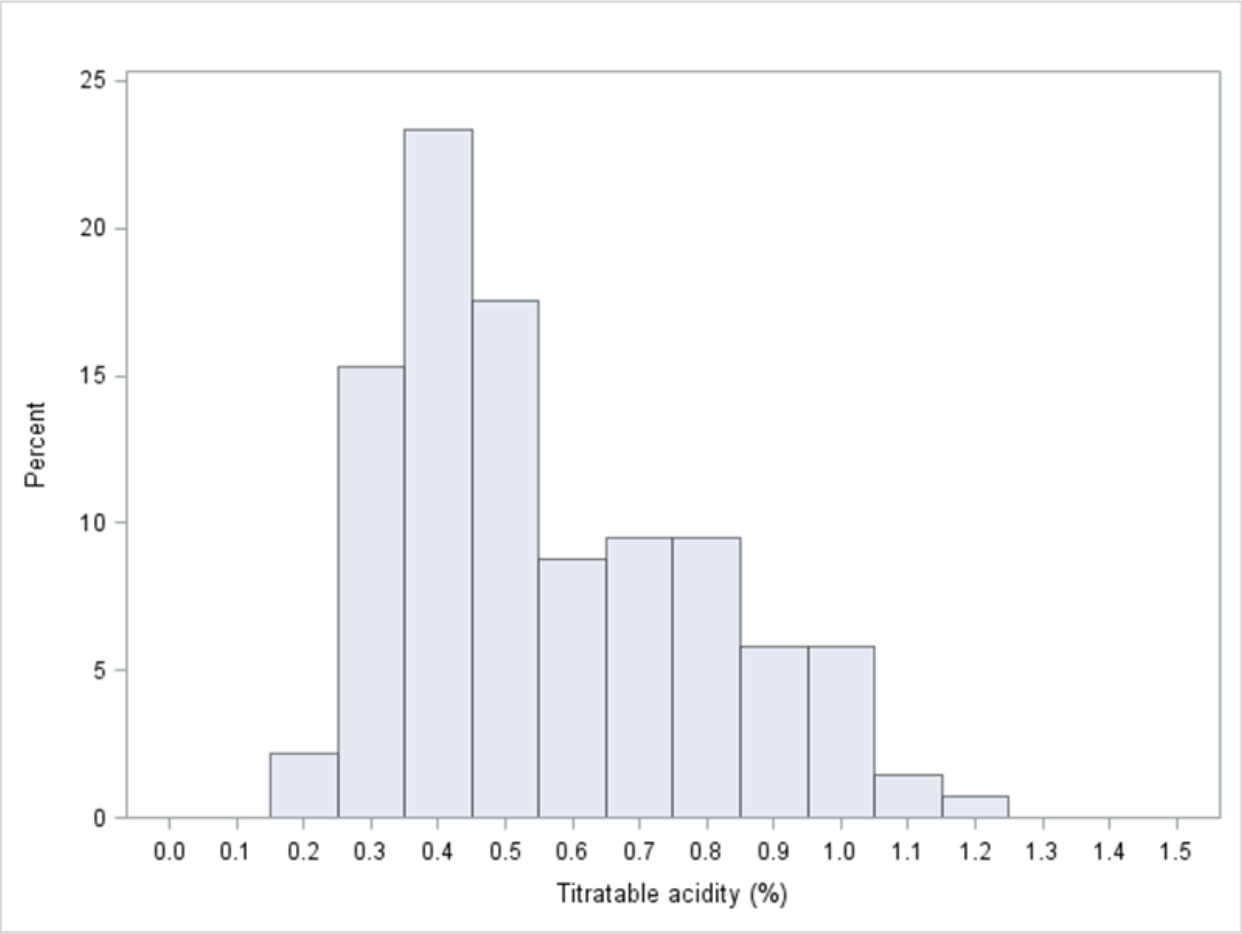

Fig 1. Distribution (%) of the seedlings within titratable acidity (%) values of across-year average, Arkansas RosBREED seedlings.

Table 4. Titratable acidity (TA) (%) values of parental individuals of Arkansas RosBREED germplasm for years 2011, 2012, 2013, and across-year average.

Progeny	Parentage		TA (%)			Across-year average
			2011	2012	2013	
ArPop_1	Female	White County	0.30	0.20	0.30	0.27
	Male	A-672	0.60	0.50	0.70	0.60
ArPop_0801	Female	A-776	0.50	0.40	-	0.45
	Male	A-783	1.20	0.70	0.90	0.93
ArPop_0803	Female	Amoore Sweet	0.50	0.70	0.25	0.48
	Male	A-778	0.90	0.50	0.90	0.77
ArPop_0813	Female	A-772	0.40	0.20	0.40	0.33
	Male	A-672	0.60	0.50	0.70	0.60
ArPop_0817	Female	A-789	0.40	0.40	0.40	0.40
	Male	A-699	0.60	0.50	0.50	0.53
ArPop_0819	Female	A-708	0.30	0.90	0.40	0.53
	Male	A-773	0.5	-	-	0.5
ArPop_0825	Female	Souvenirs	0.30	0.30	-	0.30
	Male	A-760	0.20	0.20	0.30	0.23

Table 5. Mean, maximum, minimum, and standard deviation value of titratable acidity (TA) (%) values of Arkansas RosBREED seedlings, across-year average (2011-2013).

Progeny	TA (%)				Number of observations
	Mean	Max.	Min.	Std. dev.	
ArPop_1	0.60	1.20	0.20	0.24	48
ArPop_0801	0.59	1.00	0.30	0.22	16
ArPop_0803	0.65	1.00	0.20	0.27	12
ArPop_0813	0.58	0.90	0.30	0.19	12
ArPop_0817	0.51	0.80	0.30	0.15	9
ArPop_0819	0.49	1.10	0.30	0.22	23
ArPop_0825	0.47	0.90	0.20	0.23	17

Association of *D*-locus DNA Test with TA levels

Population Analyses

The D-locus DNA test was evaluated for the first time in the Arkansas peach breeding program in this study, with the objective to reliably predict acidity levels of peach fruit. Initially the material utilized was individuals included in the Arkansas RosBREED project to confirm the functionality of the DNA test in the UA peach breeding program. Then in 2014, a second group of individuals, composed of 2010-seedlings, were used to validate the utility of the DNA test in this same breeding program.

This DNA test evaluates nucleotide sequences located within the region where the D-locus is located and it is able to distinguish between high and low fruit acidity in peaches (Boudehri et al., 2009). Also, the location of this marker coincides with the location where FlexQTL^{TM} identified the major locus controlling this trait in Chapter 1. Malic and citric acid are the principal organic acids that contribute to peach acidity and in fleshy fruits in general (Boudehri et al., 2009, Dirlewanger et al., 2013). Malic acid synthesis occurs mainly in the cytosol by phosphoenolpyruvate carboxylase (PEPC) and NAD-dependent malate dehydrogenase (MDH) enzymes, while citric acid synthesis takes place in the mitochondria through the tricarboxilic acid cycle (Etienne et al., 2002; 2013). On LG 5, the D-gene has been associated and co-localized with pH, malic acid, citric acid, and sucrose content (Etienne et al., 2002, Boudehri et al., 2009).

In 2011, 2012, and 2013, a total of 130 individuals were analyzed including only Arkansas RosBREED seedlings. Individuals were grouped in three allelic combinations: homozygous low acidity (DD), heterozygous low acidity (Dd), and homozygous high acidity (dd). These genotypes were clearly differentiated during the genotyping and allele scoring

processes (Figs. 2, 3, and 4). Band sizes were ~306 to ~312 bp for the D allele and ~313 to 318 bp for the d allele.

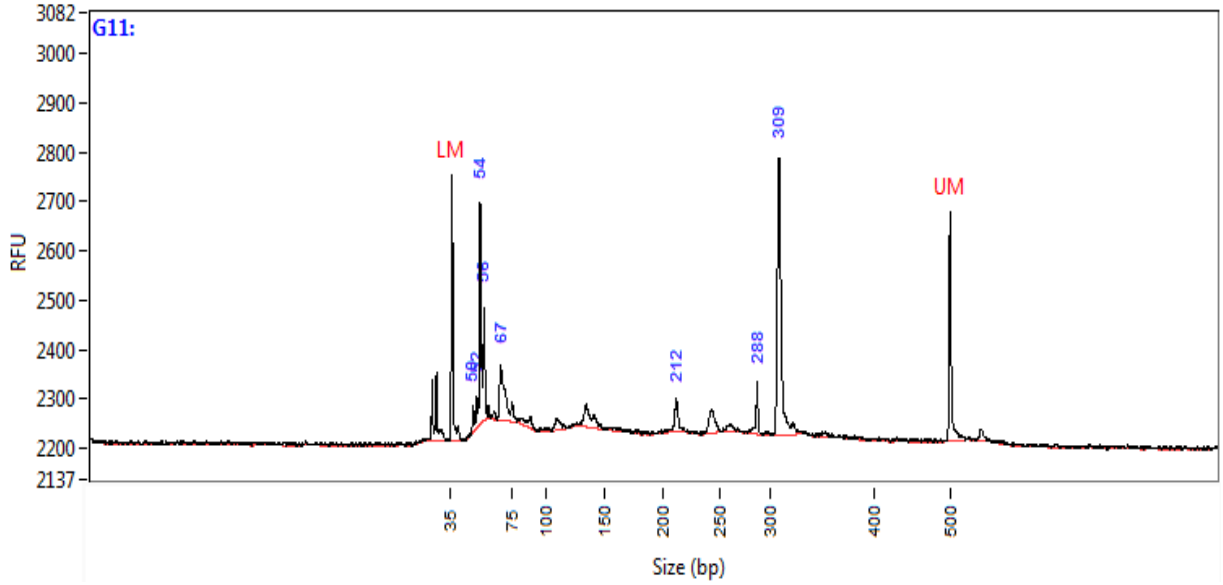

Fig. 2. Peak height (relative fluorescence units, RFU) of homozygous low-acid (DD) individual amplifying a band of 309 base pairs (bp). D-locus DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. G11 corresponds to the row letter and column number of the location of the sample on the plate.

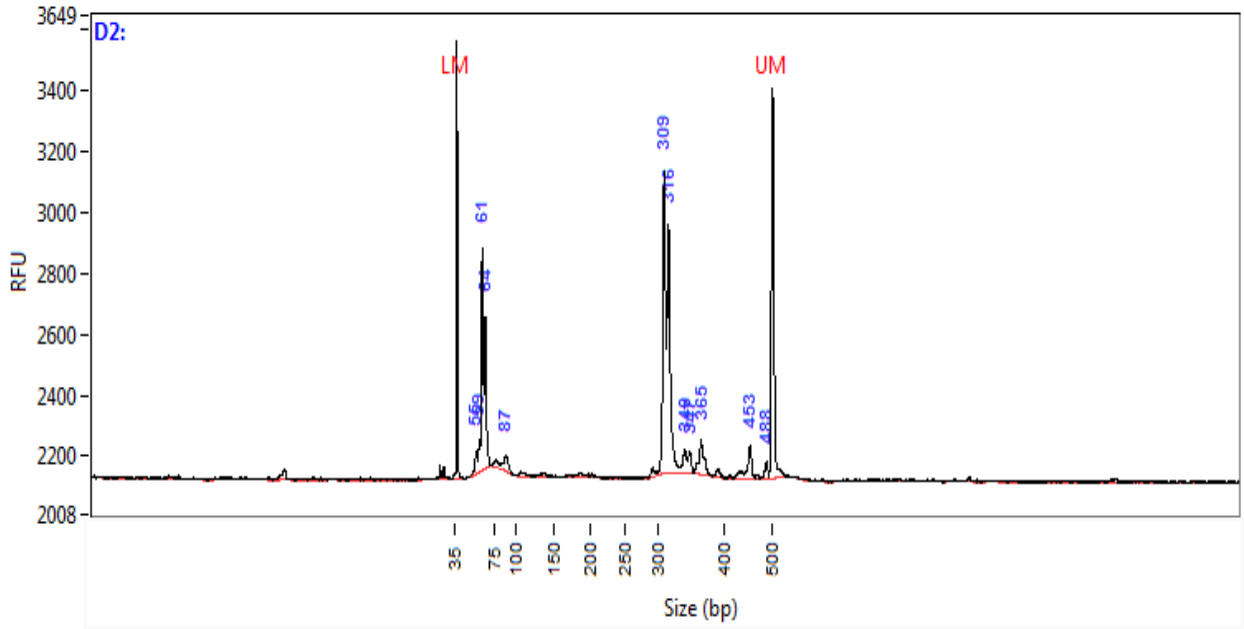

Fig. 3. Peak height (relative fluorescence units, RFU) of heterozygous low-acid (Dd) individual amplifying two bands of 309 and 316 base pairs (bp). D-locus DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. D2 corresponds to the row letter and column number of the location of the sample on the plate.

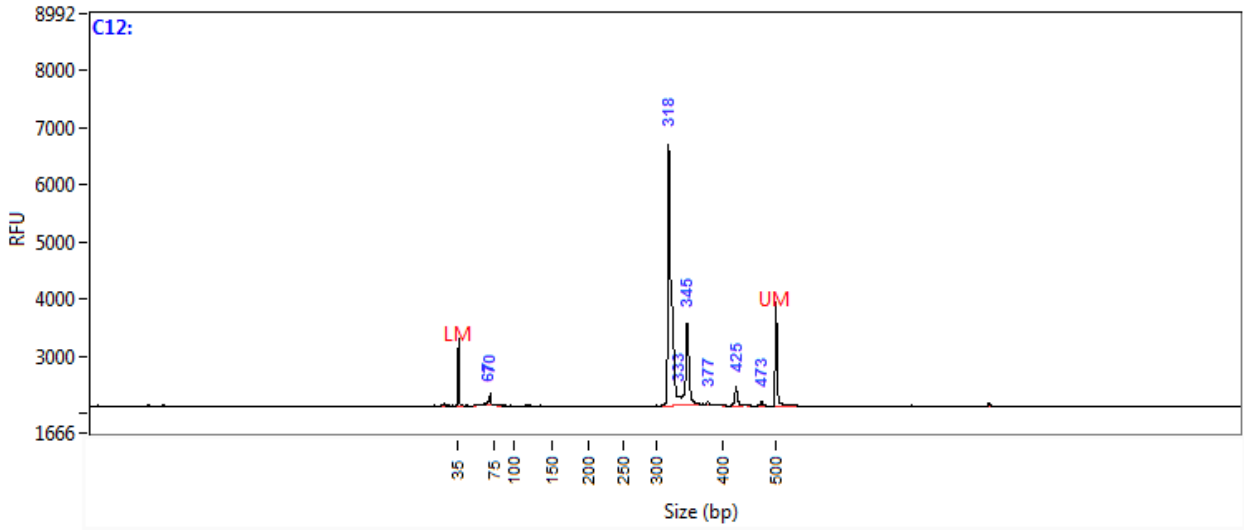

Fig. 4. Peak height (relative fluorescence units, RFU) of homozygous high-acid (dd) individuals amplifying a band of 318 base pairs (bp). D-locus DNA test. Lower marker (LM) is at 35 bp and upper marker (UM) is at 500 bp. C12 corresponds to the row letter and column number of the location of the sample on the plate.

As stated before, significant allele ($P < 0.001$) and year x allele interaction ($P=0.0001$) effects were observed for TA in this study (Table 2) for the Arkansas RosBREED populations. Main effect of allele mean separation of the allele combinations of this DNA test indicated incomplete dominance control of the low-acid character (Table 6). These values are similar to those ones found by (Boudehri et al., 2009) in which homozygous DD individuals averaged a TA value of 0.37%, heterozygous Dd individuals 0.48%, and homozygous dd individuals 1.10%.

For the interaction means for allele and year, homozygous dd allele individuals in 2011, 2012, and in 2013 were not significantly different from each other, as expected, but were different from heterozygous Dd and homozygous DD individuals (Table 7). Titratable acidity values of homozygous dd individuals ranged from 0.90 to 0.72%. Homozygous DD and heterozygous Dd individuals had some significantly different values among them, but the allelic combinations were not consistent in differentiation based on the homozygous and heterozygous values. This year x allele effect for allele combinations and their overlapping values suggests dominance or possibly incomplete dominance of the low-acid character (Table 7). The overlapping between DD and Dd individuals could be due other loci or alleles affecting this trait but providing minor effects. Eduardo et al. (2014) analyzed this molecular marker in a collection of cultivars and seedlings. They found an allele associated with the low-acid character either in homozygosis or heterozygosis allelic combinations (Eduardo et al., 2014). Incomplete dominance of the major locus controlling apple (*Malus* x *domestica* Borkh.) TA, the Ma locus, has been reported (Bai et al., 2012, Xu et al., 2012). The Ma locus on LG 16 of apple genome explained 17.0 to 42.3% of the variation in TA and pH, but there were two other QTLs on LG 1 and LG 6 that explained part of the portion not explained by the *Ma*-locus also, and these likely explained the overlapping between MaMa and Mama individuals (Liebhard et al., 2003, Xu et

al., 2012). These other two QTLs were found to be associated with modification of TA values in the high/medium acid range in apples (Xu et al., 2012). A similar situation could be occurring in the data here, and in phenotypic evaluations of selections and seedlings in the Arkansas peach breeding program, mid-acid genotypes are often found, not corresponding to the distinct classes of dominance and recessive as inferred by a simple qualitative trait (J. Clark, personal communication).

Of the evaluated Arkansas RosBREED seedlings, 3.1% were classified as DD, 67.0% were Dd, and 30.0% were dd, meaning that 70.1% of the tested individuals included in the Arkansas germplasm corresponded to low fruit acid individuals supporting low-acidity dominance of this trait. Chi-square test results indicated that five populations (ArPop_1, ArPop_0801, ArPop_0803, ArPop_0813, ArPop_0817, and ArPop_0825) fit the expected genotypic segregation ratios. ArPop_0819 did not fit the expected segregation ratio, which could be due to the low number of individuals in this population that could cause a deviation from the expected ratio, which in this case is 1:2:1. Also, due to the characteristic of the cross, ArPop_0819 was expected to have homozygous DD individuals of which were not found. Allelic and phenotypic values of the parental individuals of Arkansas RosBREED populations are shown in Table 9. Detailed information between the D-locus DNA test and association with TA values of Arkansas RosBREED seedlings is provided in Appendix B, Table B.1.

To validate the utility and accuracy of this DNA test, in 2014 TA was measured on 300 seedlings from 20 crosses made in 2010 and the acidity DNA test was performed on these individuals. The ANOVA indicated a significant allele effect ($P < 0.0001$). The three expected allelic combinations were observed (homozygous DD, heterozygous Dd, and homozygous dd). Allele combination means had significant differences (Table 8). Homozygous dd individuals, as

expected, reported the highest TA values among all allele combinations. Heterozygous Dd individuals presented significantly higher TA values compared to homozygous DD individuals, indicating a possible incomplete dominance genetic control of this trait, this behavior was previously reported by Boudehri et al., (2009). Of the 300 2010-seedlings measured, 33% corresponded to homozygous DD, 56% to heterozygous Dd, and 11% to homozygous dd; this indicates that 89% of the tested individuals were in the low-acid category. Detailed information for the D-locus DNA test and its association with TA values of 2010-seedlings is provided in Appendix B, Table B.2.

Titratable acidity values of DD, Dd, and dd individuals of Arkansas RosBREED seedlings were higher compared to the 2010 seedlings evaluated in 2014. This difference could be explained because parents of RosBREED seedlings had a high range of variation in several traits including TA resulting in individuals with high, mid, and low acidity. Conversely, 2010-seedlings measured were not selected with any special procedure, instead four to 30 seedlings per population of each 2010-population were chosen for analysis. However, not all seedlings of each population were possible to associate the alleles with TA value because some samples did not amplify a band or the TA value was not measured (because the tree did not produce enough fruit with acceptable quality to take measurements). Titratable acidity values of 2010-seedlings are shown in Table 10 and parental values of 2010-seedlings plus their corresponding allele combination are shown in Table 11 (not all parents of these populations were possible to evaluate due to some being discarded from the program). Despite the difference of TA values between the Arkansas RosBREED seedlings and 2010-seedlings, the acidity DNA test was able to distinguish in both cases low-acid individuals from high-acid individuals.

Information of allele combination of important traits of selection is very useful for decreasing cost, labor, and the use of land and at the same time increasing the efficiency of a breeding program. For example, if the breeding objectives of population1020 are to select low-acid individuals, by running this DNA when the plants had one-year old the breeder would have known that individuals 1021-21, 1020-30, 1020-33, and 1020-41 (31% of the analyzed plants of this population in this study) carried the dd allelic combination (high acid) and could take the decision of discard those plants early in the breeding process, saving 31% of the space, labor, and field cost of destined for that population.

Table 6. Least square means of titratable acidity (TA) (%) of D-locus DNA test allele combinations of Arkansas RosBREED seedlings, across-year average.

Alleles	TA (%)
dd	0.84 a[z]
DD	0.52 b
Dd	0.44 c

[z] Means in the same column followed by the same letter not significantly different as determined by least square means $P \leq 0.05$.

Table 7. Least square interaction means for year and alleles for titratable acidity (TA) (%) of D-locus DNA test allele combinations of Arkansas RosBREED seedlings, years 2011, 2012, and 2013.

Year	Alleles	TA (%)
2013	dd	0.90 a[z]
2011	dd	0.89 a
2012	dd	0.72 a
2013	DD	0.67 b
2013	Dd	0.53 bc
2011	DD	0.47 bcd
2012	DD	0.43 cd
2012	Dd	0.40 d
2011	Dd	0.39 d

[z] Means in the same column followed by the same letter not significantly different as determined by least square means $P \leq 0.05$.

Table 8. Least square main effect means for titratable acidity (TA) (%) of D-locus DNA test allele combinations of 2010-seedlings, year 2014.

Alleles	TA (%)
dd	0.56 a[z]
Dd	0.39 b
DD	0.33 c

[z] Means in the same column followed by the same letter not significantly different as determined by least square means $P \leq 0.05$.

Table 9. Allele combination of D-locus DNA test and titratable acidity (TA) (%) values of parental individuals of Arkansas RosBREED germplasm, across-year average.

Progeny	Parentage		Alleles	TA (%)
ArPop_1	Female	White County	Dd	0.27
	Male	A-672	dd	0.60
ArPop_0801	Female	A-776	Dd	0.45
	Male	A-783	dd	0.93
ArPop_0803	Female	Amoore Sweet	Dd	0.60
	Male	A-778	dd	0.77
ArPop_0813	Female	A-772	Dd	0.33
	Male	A-672	dd	0.60
ArPop_0817	Female	A-789	Dd	0.40
	Male	A-699	Dd	0.53
ArPop_0819	Female	A-708	Dd	0.53
	Male	A-773	Dd	0.50
ArPop_0825	Female	Souvenirs	Dd	0.30
	Male	A-760	Dd	0.23

Table 10. Mean, maximum, minimum, and standard deviation of titratable acidity (TA) (%) values of Arkansas 2010-seedlings, year 2014.

Progeny	TA (%)				Number of observations
	Mean	Max.	Min.	Std. dev.	
1001	0.29	0.76	0.15	0.20	13
1002	0.24	0.59	0.10	0.14	15
1003	0.26	0.63	0.11	0.18	23
1004	0.43	1.03	0.13	0.25	12
1006	0.24	0.53	0.03	0.14	19
1007	0.46	1.18	0.13	0.30	28
1008	0.25	0.57	0.09	0.15	12
1009	0.25	0.60	0.13	0.15	13
1011	0.36	1.00	0.09	0.24	30
1012	0.35	1.02	0.15	0.26	15
1013	0.22	0.60	0.09	0.19	6
1018	0.70	0.91	0.54	0.14	10
1019	0.30	0.84	0.13	0.20	13
1020	0.53	0.71	0.34	0.09	13
1021	0.41	1.04	0.18	0.23	26
1022	0.29	0.67	0.14	0.15	16
1023	0.75	0.93	0.61	0.15	4
1024	0.76	1.05	0.45	0.19	10
1025	0.73	1.16	0.39	0.26	6
1026	0.55	1.73	0.20	0.42	13

Table 11. Allele combination of D-locus DNA test and titratable acidity (TA) (%) values of parental individuals of Arkansas 2010-seedlings, across-year average.

Progeny	Parentage		Alleles	TA (%)
1001	Female	A-665	dd	0.40
	Male	A-800	-	-
1002	Female	A-760	Dd	0.23
	Male	A-708	Dd	0.53
1003	Female	White Diamond	Dd	0.25
	Male	A-760	Dd	0.23
1004	Female	A-753	-	-
	Male	Souvenirs	Dd	0.30
1006	Female	White County	Dd	0.27
	Male	Souvenirs	Dd	0.30
1007	Female	A-775	-	-
	Male	Souvenirs	Dd	0.30
1008	Female	A-746	-	-
	Male	A-785	-	-
1009	Female	A-746	-	-
	Male	A-823	-	-
1011	Female	A-786	Dd	0.40*
	Male	A-773	Dd	0.50*
1012	Female	A-773	Dd	0.50*
	Male	A-774	-	-
1013	Female	A-772	Dd	0.33
	Male	A-774	-	-
1018	Female	Bowden	dd	0.38*
	Male	A-761	dd	-
1019	Female	A-779	-	-
	Male	A-776	-	0.50*
1020	Female	Bowden	dd	0.38*
	Male	A-758	Dd	0.82*
1021	Female	A-778	Dd	0.77
	Male	A-777	-	-
1022	Female	Amoore Sweet	Dd	0.60*
	Male	A-779	-	-
1023	Female	A-761	dd	-
	Male	A-768	dd	1.00*
1024	Female	A-757	-	-
	Male	A-807	-	-
1025	Female	A-770	-	0.62*
	Male	A-768	dd	1.00*
1026	Female	A-816	Dd	0.50*
	Male	A-772	Dd	0.33

*Indicates that the value of TA is only from 2014 measurement.

Cultivars and Selections Analyses

Arkansas selections and cultivars along with cultivars from other breeding programs were evaluated in 2011, 2012, 2013, and 2014 (Tables 12 and 13). The D-locus DNA test was run on 116 selections and cultivars and the results were compared with their corresponding TA values.

Cultivars and selections of the Arkansas RosBREED germplasm (a total of 19 individuals) carried heterozygous Dd and homozygous dd individuals and they were associated with low (0.40%) and high (0.77%) average TA values, respectively, in 2011, 2012, and 2013 (Table 12). There were no dominant DD individuals in this group. The values corresponded to my previous findings on the seedlings in which dd individuals were associated with high TA values and Dd individuals were associated with low TA values. The second set of selections and cultivars, which included selections from the Arkansas program as well as cultivars from the Arkansas and other breeding programs were evaluated in 2013 and 2014, adding a total of 97 genotypes (Table 13). In this set, DD, Dd, and dd individuals were present. Homozygous DD individuals had average TA value of 0.30%, Dd individuals averaged a TA value of 0.37%, and homozygous dd individuals averaged a value of 0.79%. Of this set, three individuals, A-770, A-794, and A-803, (3.1% of the total samples evaluated) were classified as Dd, but their TA values were above 0.74% (values that correspond to homozygous dd individuals). This could be due to error in the phenotyping process and the possible environmental influences on this trait, since two of these three samples had only data for one year.

This is the first time that genetic and phenotypic information for this trait has been obtained and analyzed in the Arkansas breeding program. Selections and cultivars having phenotypic data paired with allelic information could be of great utility in a breeding program to implement as a tool for marker-assisted parent selection (MAPS) for choosing parents to create

progenies with the desired levels of fruit acidity. For example, if the breeder desires to create a population composed of only high-acid individuals, by crossing the selections A-768 and A-818 (both carry the dd allelic combination) one will attain the desired high-acid population. This tool could then later be applied as marker-assisted seedling selection (MASS) to test the progeny to remove plants that do not carry the desired allelic combination. In tart cherry (*Prunus cerasus* L.), a DNA test was developed for cherry leaf spot resistance [*Blumeriella jaapii* (Rehm)] and self-compatibility/incompatibility. In strawberry (*Fragaria* x *ananassa* Duchesne), red stele (*Phytophthora fragariae* Hickman var. *fragariae* Wilcox & Duncan) resistance has been tested by utilizing a DNA test (www.rosbreed.org).

Another advantage of these types of tools is that they will work independent of environmental conditions. In Arkansas, summer rainfall occurs during peach season, which can affect quality, including acidity, thereby impacting effectiveness of summer evaluations and phenotyping. Thus, DNA tests analysis could complement summer evaluations since they will work under any environmental conditions if the leaf tissue was collected during the spring time and all the genotyping process was followed correctly. Further, variations in maturity can affect the perception of acidity of fruit in evaluations in the field particularly when fruit is at early maturity, and having data from a marker for acidity genotype ensures greater accuracy during evaluations for various potential uses of a genotype.

Table 12. Allele combination of D-locus DNA test and titratable acidity (TA) (%) values of selections and cultivars of Arkansas RosBREED germplasm, years 2013, 2012, 2011, and across-year average.

Accession	Alleles	TA (%)			
		2011	2012	2013	Across-year average
A-663	dd	1.00	0.30	1.00	0.77
A-665	Dd	0.30	0.30	0.60	0.40
A-672	dd	0.60	0.50	0.70	0.60
A-699	Dd	0.60	0.50	0.50	0.53
A-708	Dd	0.30	0.90	0.40	0.53
A-716	Dd	0.50	0.40	0.50	0.47
A-760	Dd	0.20	0.20	0.30	0.23
Amoore Sweet	Dd	0.50	0.70	0.25	0.48
A-772	Dd	0.40	0.20	0.40	0.33
A-776	Dd	0.50	0.40	-	0.45
A-778	dd	0.90	0.50	0.90	0.77
A-783	dd	1.20	0.70	0.90	0.93
Allgold	dd	0.40	-	-	0.40
Arrington	dd	0.90	0.80	0.90	0.87
Bradley	dd	0.80	0.80	1.10	0.90
Souvenirs	Dd	0.30	0.30	-	0.30
Westbrook	dd	-	-	1.00	1.00
White County	Dd	0.30	0.20	0.30	0.27
Winblo	dd	0.84	0.50	0.80	0.71

Table 13. Allele combination of D-locus DNA test and titratable acidity (TA) (%) values of selections and cultivars, years 2013 and 2014.

Accession	Alleles	TA (%)	
		2013	2014
A-554	dd	0.74	0.63
A-743	DD	-	0.39
A-758	dd	0.61	0.82
A-761	dd	1.13	-
A-766	Dd	-	0.29
A-768	dd	-	1.04
A-770	Dd	0.86	0.62
A-786	Dd	-	0.40
A-790	Dd	-	0.20
A-792	Dd	-	0.55
A-794	Dd	-	1.03
A-797	Dd	0.70	0.49
A-798	Dd	0.26	0.13
A-799	dd	0.71	0.76
A-801	Dd	0.39	0.27
A-803	Dd	-	0.88
A-804	Dd	0.35	0.39
A-805	Dd	-	0.48
A-806	Dd	0.36	0.28
A-808	Dd	-	0.18
A-809	Dd	-	0.19
A-811	Dd	-	0.71
A-813	Dd	-	0.42
A-814	Dd	0.28	-
A-815	Dd	0.24	0.37
A-816	Dd	0.39	0.50
A-818	dd	0.88	0.83
A-819	Dd	-	0.29
A-820	DD	-	0.25
A-821	Dd	-	0.24
A-822	Dd	-	0.29
A-824	Dd	-	0.34
A-825	Dd	0.35	0.37
A-826	DD	0.40	0.19
A-827	Dd	0.28	0.26

Table 13. Allele combination of D-locus DNA test and titratable acidity (TA) (%) values of selections and cultivars, years 2013 and 2014 (Cont.).

Accession	Alleles	TA (%)	
		2013	2014
A-828	dd	-	0.67
A-829	Dd	-	0.41
A-833	Dd	0.36	-
A-840	Dd	0.47	-
A-841	dd	0.85	0.66
A-842	Dd	0.38	0.62
A-844	Dd	0.41	0.57
A-846	Dd	0.34	0.27
A-848	Dd	-	0.22
A-849	DD	0.32	0.13
A-850	Dd	0.32	0.26
A-851	Dd	-	0.45
A-852	Dd	-	0.35
A-854	Dd	0.17	-
A-853	DD	-	0.33
A-856	Dd	0.36	0.35
A-857	Dd	0.46	0.39
A-858	Dd	0.38	0.46
A-859	Dd	0.62	-
A-860	Dd	-	0.19
A-862	Dd	0.37	0.24
A-864	Dd	-	0.23
A-866	Dd	-	0.32
A-868	Dd	-	0.22
A-869	Dd	-	0.33
A-872	Dd	0.22	-
A-873	dd	-	0.97
A-874	Dd	-	0.46
A-875	Dd	-	0.35
A-876	Dd	0.52	0.23
A-877	Dd	-	0.19
A-878	Dd	0.30	0.24
A-881	Dd	-	0.34
A-882	Dd	-	0.51
A-883	Dd	-	0.15

Table 13. Allele combination of D-locus DNA test and titratable acidity (TA) (%) values of selections and cultivars, years 2013 and 2014 (Cont.).

Accession	Alleles	TA (%)	
		2013	2014
A-884	Dd	-	0.21
A-885	Dd	-	0.14
A-886	dd	-	0.58
A-887	Dd	-	0.19
A-888	Dd	-	0.22
A-891	dd	-	0.78
Bowden	dd	0.77	0.38
Challenger	Dd	-	0.68
Cresthaven	dd	-	0.85
CVN-13w	dd	-	0.51
EasternGlo	dd	-	0.98
Goldilocks	dd	0.47	0.69
Goldjim	dd	0.61	0.45
Goldnine	dd	-	0.82
KV501	dd	-	0.98
Loring	dd	-	0.48
Manon	Dd	0.25	0.36
Messina	dd	-	0.66
Lovell	dd	-	0.83
Redhaven	dd	-	0.65
Roygold	dd	0.58	0.63
Tango	dd	-	0.71
White Diamond	Dd	0.41	0.25
White Lady	dd	-	0.59
White River	dd	0.62	0.66
White Rock	Dd	0.23	0.39
Yumm Yumm	dd	1.04	-

Conclusions

Peach fruit acidity is one of the main traits that breeders evaluate by taste and/or by measuring the TA to characterize a particular plant from a breeding program. If a particular individual has the desired level of acidity, along with other quality traits, the breeder will select this plant to consider it as a potential new cultivar or use it as parent for future crosses.

Several QTLs have been identified and related to fruit quality traits in peach fruit, one of them is TA. A major locus located at the proximal end of LG 5 has been identified for TA which is co-localized with fruit pH and concentration of other organic acid such as malic and citric acids, the main acids found in peaches. This locus, called D-locus, has been studied and several markers have been mapped, one of them is CPPCT040 SSR marker that in previous studies has been associated with low and high TA levels.

This DNA test was applied for the first time in the UA peach breeding program with the objective to associate its allelic combinations with different values of TA. Numerous seedlings, selections, and cultivars were analyzed. Homozygous DD, heterozygous Dd, and homozygous dd individuals were found in the evaluated material. The two first types of allelic combinations were more abundant and were associated with low levels of acidity. Conversely, homozygous dd individuals were found at a lower frequency within the analyzed material and associated with the higher levels of TA. Tested populations, selections, and cultivars showed similar results.

The results obtained with this study will complement the information that the breeder requires to make informative decisions in the different steps of a breeding program. Specifically, this DNA test could be used to predict the fruit acidity levels of peach and nectarine trees. Efficiency of breeding programs could be increased by the application of MAB to select seedling plants a few days or weeks after germination, allowing determination of high or low acidity

(retention of seedlings depending of the objective of each cross/program objectives). Also, by using the DNA information from advanced selections and cultivars, crosses can be designed to obtain offspring that carry only the desired alleles. This will reduce land use, program cost, and work hours necessary to grow the plants that will be discarded later in the breeding process.

Literature Cited

Bai, Y., L. Dougherty, M. Li, G. Fazio, L. Cheng, and K. Xu. 2012. A natural mutation-led truncation in one of the two aluminum-activated malate transporter –like genes at the *Ma* locus is associated with low fruit acidity in apple. Mol. Genet. Genomics 287:663-678.

Baird, W., A. Estager, and J. Wells. 1994. Estimating nuclear DNA content in peach and related diploid species using laser flow cytometry and DNA hybridization. J. Amer. Soc. Hort. Sci. 119:1312-1316.

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

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

De Souza, V., D. Byrne, and J. Taylor. 1998. Heritability, genetic, and phenotypic correlations, and predicted selection response of quantitative traits in peach: II. An analysis of several fruit traits. J. Amer. Soc. Sci. 123:604-611.

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

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

Edge-Garza, D., T. Rowland, S. Haendiges, and C. Peace. 2014. A high-throughput and cost-efficient DNA extraction protocol for the tree fruit crops of apple, sweet cherry, and peach relying on silica beads during tissue sampling. Mol. Breeding 34: 2225-2228.

Eduardo, I., E. Lopez-Girona, I. Batlle, G. Reig, I. Iglesias, W. Howad, P. Arus, M. Aranzana. 2014. Development of diagnostic markers for selection of the subacid trait in peach. Tree Genet. & Genomes 10:1695-1709.

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

Etienne A., M. Genard, P. Lobit, D. Mbeguie-A-Mbeguie, and C. Bugaud. 2013. What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. J. Experimental Bot. 64:1451-1469.

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

Gasic, K., T. Gradziel, C. Crisosto, D. Byrne, and J. Clark. 2010. Phenotyping in peach. 15 March 2013 < https://www.rosbreed.org/sites/default/files/files/RosBREED_2010-Phenotyping_protocol_P_persica.pdf>

Liebhard, R., M. Kellerhals, W. Pfammatter, M. Jertmini, and G. Gessler. 2003. Mapping quantitative physiological traits in apple (*Malus* x *domestica* Borkh). Plant Mol. Biol. 52:511-526.

Monet, R. and D. Bassi. 2008. Classical genetics and breeding, p. 61-84. In: D.R. Layne and D. Bassi (eds.), The peach: botany, production, and uses. CAB Intl, Oxfordshire, UK.

NCBI Blast. 2015. Genome Data for *Rosaceae*. 25 February 2015. < https://www.rosaceae.org/tools/ncbi_blast>

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

RosBREED. 2015. RosBREED 1 deliverables. 22 September 2015. <https://www.rosbreed.org/breeding/deliverables>

SAS institute Inc., 2013. SAS® 9.4. Cary, NC.

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

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

Testolin, R. and G. Cipriani. 2010. Molecular markers for germplasm identification and characterization. Acta Hort. 859:59-72.

Xu, K., A. Wang, and S. Brown. 2012. Genetic characterization of the *Ma* locus with pH and titratable acidity in apple. Mol. Breeding 30:899-912.

Zhebentyayeva, T., G. Swire-Clark, L. Georgi, L. Garay, S. Jung, S. Forrest, A. Blenda, B. Blackmon, J. Mook, R. Horn, W. Howad, P. Arús, D. Main, J. Tomkins, B. Sosinski, W. Baird, G. Reighard, and A. Abbott. 2008. A framework physical map for peach, a model Rosaceae species. Tree Genetics & Genomes 4:745–756.

Appendix A. DNA Extraction Procedure

- Place tubes in compartments for shaker and place in liquid nitrogen.
- Make sure tissue is still frozen.
- Attach compartments to shaker. Set machine to run for 3 mins at a frequency of 25/s.
- Turn tubes around in compartments and run shaker at same time and speed (may put back in liquid nitrogen to keep frozen if necessary).
- Ensure the tissue doesn't thaw out until it is in the extraction buffer.
- Add 1200 µl (remember 2-mercaptoethanol) of Delaporta and vortex to evenly mix and liquefy (if doesn't liquefy too much tissue).
- Add 80 µl of 20% SDS to break down membranes. Vortex again and be sure there is no clumping (make sure it liquefies) of tissue in tube.
- 65 ^{0}C water bath for 30 min.
- Add 400 µl of 5M Potassium acetate and mix by inversion.
- Put on ice for 20 min (can be up to 30 min for apple) (*potentially leave this for longer if you need to but not more than 2 h).*
- Spin at 4 ^{0}C at 12,000 rpm for 20min.
- Pre-add 400 µl isopropanol (cold -20 ^{0}C) to two 1.5 mL tubes *(potentially stop here and put in +4 C fridge in needed)*.
- Once samples are done spinning split the sample equally (~800µl each / total volume ~1.2 ml).
- 1.5ml tubes; invert several times mixing **gently** (you can see DNA strands at this point).
- Spin at 4^{0}C 12,000 rpm for 10 to 15min.
- Pour off supernatant (make sure pellet doesn't dislodge), dry tube on tissue.
- Wash with 800µl of 70% EtOH (use cold -20 ^{0}C, helps remove isopropanol); invert several times **gently** *(potentially stop here and put in +4 C fridge in needed)*.
- Spin at 4 ^{0}C 12,000 rpm for 10 to 15 min.
- Decant and dry on tissue (careful pellet may be loose). *Leave overnight in flow hood.*
    - Optional instead of drying overnight: Place in thermomixer at 45 ^{0}C for 15 min (be sure samples completely dry, but do not overdry them).
- Set water bath to 37 ^{0}C for the next day
- NEXT DAY
- Add 200 µl TE buffer mixed with RNAse to each sample (1ml AE/1µl RNAse). After adding flick to mix. Quick spin then check to make sure DNA is dislodged from tube wall. Make sure DNA completely re-suspends.

  [optional***Stop here……place samples in +4 ^{0}C fridge overnight]
- RNAse treatment at 37 ^{0}C bath for 30 min (Make sure DNA is all dissolved).

- Combine samples in one 1.5 ml tube, wash empty tube with 100 µl TE buffer to get all material transferred (set pipette greater than sample size to get all material).
- Add 50 µl 3M sodium acetate and 350 µl of cold isopropanol (cold, -20 ^{0}C), mix by inversion.
- Place samples in -20 ^{0}C for a minimum of 30 min (45 is good).

  [optional***Stop here……place samples in -20 ^{0}C overnight]
- Spin samples at +4 ^{0}C at 12,000 rpm for 20 min. Decant and dry on tissue.
- Wash pellets with 70% EtOH (cold -20 ^{0}C)(~800 µl) and spin as directed above.
- Decant tubes and dry *Leave overnight in flow hood.*
    - optional instead of drying overnight: in thermomixer at 45 ^{0}C for 10 min - be sure samples completely dry.
- NEXT DAY
- Re-suspend (flick tubes so DNA dislodges and mixes) DNA in 100 µl of TE buffer and place in +4 ^{0}C fridge.
- Next day put in -20 ^{0}C freezer….better to freeze and thaw out….than keep in +4 ^{0}C fridge.
    - Don't keep in +4 fridge for more than a few days
- Check concentration (Nanodrop machine, and then run on gel).
- PCR followed by fragment analyzer (Dr. Mason's machine).

Solutions for DNA extraction

Delaporta Extraction buffer:

Final concentrations	For 300ml
0.1M Tris-HCl pH 8.0	30 ml 1M Tris HCl pH 8.0
0.05M EDTA pH 8.0	30 ml 0.5M EDTA pH 8.0
0.5M NaCl	30 ml 5M NaCl
PVP 40 000	1% (optional, add 2% if tissue high in polyphenolics, tannins etc., for peach and cherry use **2%)**
2-mercaptoethanol	90µl per 100 ml buffer – add just before use

The buffer minus the 2-mercaptoethanol can be **autoclaved and stored at room temperature** (RT)

20% SDS for 250 ml

Dissolve 50 g of SDS in 250 ml water.

Heat to 65 ºC to dissolve.

Store at RT, warm to remove precipitates before use.

5M Potassium acetate for 100 ml

Potassium acetate	49.1g
Dissolve in	90 ml of water

When in solution make volume up to 100 ml with water **(+4 °C fridge).**

3M Sodium acetate for 200 ml

TE (Tris:EDTA)

Final concentrations:	For 1 L
10mM Tris-HCl pH 8.0	10 ml 1.0 M Tris-HCl pH 8.0
1mM EDTA	2 ml 0.5M EDTA pH 8.0

Make volume to 1 liter with water. **Autoclave**

70% Ethanol for 100 ml

Ethanol (absolute)	70 ml
Water	30 ml

Appendix B. Detailed Information Allelic Combination of D-locus DNA Test of Arkansas Populations

Table B.1. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of Arkansas RosBREED populations, years 2011, 2012, 2013, and across year average.

Accession	Alleles	TA (%)			
		2011	2012	2013	Average
ArPop_1-01	Dd	0.40	0.60	0.30	0.43
ArPop_1-02	Dd	0.40	0.70	0.70	0.60
ArPop_1-03	dd	0.90	-	0.80	0.85
ArPop_1-04	Dd	0.40	0.70	0.40	0.50
ArPop_1-05	dd	0.90	0.60	0.60	0.70
ArPop_1-06	Dd	0.60	0.30	0.50	0.47
ArPop_1-07	dd	0.70	1.00	0.70	0.80
ArPop_1-08	dd	0.80	0.80	-	0.80
ArPop_1-09	dd	1.10	0.60	1.30	1.00
ArPop_1-10	dd	0.80	-	0.90	0.85
ArPop_1-11	dd	1.30	0.90	-	1.10
ArPop_1-12	dd	0.80	0.50	0.80	0.70
ArPop_1-14	Dd	0.30	0.50	0.30	0.37
ArPop_1-15	dd	0.60	0.50	0.70	0.60
ArPop_1-17	Dd	0.40	0.50	0.50	0.47
ArPop_1-18	Dd	0.50	0.60	0.70	0.60
ArPop_1-19	Dd	0.20	0.50	0.50	0.40
ArPop_1-20	dd	1.20	0.70	1.10	1.00
ArPop_1-21	Dd	0.40	0.40	0.40	0.40
ArPop_1-22	Dd	0.30	0.30	0.50	0.37
ArPop_1-23	Dd	0.40	0.40	0.70	0.50
ArPop_1-24	dd	0.70	0.60	0.90	0.73
ArPop_1-25	dd	0.90	1.00	-	0.95
ArPop_1-26	Dd	0.40	0.50	-	0.45
ArPop_1-27	Dd	0.20	0.30	0.60	0.37
ArPop_1-28	Dd	0.30	0.20	0.30	0.27
ArPop_1-29	dd	0.60	0.70	0.80	0.70
ArPop_1-30	dd	0.70	0.70	0.90	0.77
ArPop_1-31	Dd	0.30	0.30	-	0.30
ArPop_1-32	Dd	0.40	0.50	0.40	0.43
ArPop_1-33	Dd	0.30	0.40	-	0.35
ArPop_1-34	Dd	0.30	0.30	0.50	0.37

Table B.1. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of Arkansas RosBREED populations, years 2011, 2012, 2013, and across year average (Cont.).

Accession	Alleles	TA (%)			
		2011	2012	2013	Average
ArPop_1-35	Dd	0.50	0.60	-	0.55
ArPop_1-36	Dd	0.30	0.40	0.40	0.37
ArPop_1-37	dd	1.20	0.70	1.00	0.97
ArPop_1-38	Dd	0.30	0.30	0.50	0.37
ArPop_1-39	dd	1.00	0.80	0.70	0.83
ArPop_1-40	Dd	0.50	0.40	0.70	0.53
ArPop_1-41	Dd	0.60	0.30	-	0.45
ArPop_1-42	Dd	0.20	0.20	-	0.20
ArPop_1-43	Dd	0.30	-	-	0.30
ArPop_1-44	Dd	0.30	0.20	1.20	0.57
ArPop_1-45	Dd	0.50	0.70	0.60	0.60
ArPop_1-46	Dd	0.30	0.50	0.50	0.43
ArPop_1-47	dd	0.90	1.20	1.40	1.17
ArPop_1-48	dd	0.60	0.80	-	0.70
ArPop_1-49	Dd	0.40	0.40	0.90	0.57
ArPop_0801-01	dd	1.00	0.50	0.70	0.73
ArPop_0801-02	Dd	0.60	0.40	0.40	0.47
ArPop_0801-03	dd	0.90	0.40	1.10	0.80
ArPop_0801-04	Dd	0.40	0.20	0.80	0.47
ArPop_0801-05	dd	0.90	0.40	0.90	0.73
ArPop_0801-06	Dd	1.20	0.60	-	0.90
ArPop_0801-07	Dd	0.50	0.40	0.60	0.50
ArPop_0801-08	Dd	0.30	0.20	0.70	0.40
ArPop_0801-09	Dd	0.40	0.50	-	0.45
ArPop_0801-10	Dd	0.30	0.40	0.30	0.33
ArPop_0801-11	dd	1.20	0.70	1.00	0.97
ArPop_0801-12	Dd	0.20	0.20	0.60	0.33
ArPop_0801-15	dd	1.00	1.10	0.70	0.93
ArPop_0801-16	Dd	0.20	0.40	0.50	0.37
ArPop_0803-01	dd	0.90	0.60	-	0.75
ArPop_0803-02	Dd	0.30	0.20	0.50	0.33
ArPop_0803-03	Dd	0.20	0.20	0.50	0.30
ArPop_0803-04	dd	0.60	0.60	0.80	0.67
ArPop_0803-05	DD	-	0.50	1.10	0.80
ArPop_0803-06	Dd	-	0.20	-	0.20

Table B.1. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of Arkansas RosBREED populations, years 2011, 2012, 2013, and across year average (Cont.).

Accession	Alleles	TA (%)			
		2011	2012	2013	Average
ArPop_0803-07	Dd	0.70	1.00	0.60	0.77
ArPop_0803-11	Dd	-	0.40	0.50	0.45
ArPop_0803-12	dd	-	-	0.70	0.70
ArPop_0803-14	dd	1.10	0.90	0.90	0.97
ArPop_0813-01	Dd	-	0.40	0.70	0.55
ArPop_0813-02	Dd	0.50	0.50	0.60	0.53
ArPop_0813-03	Dd	0.30	0.40	0.40	0.37
ArPop_0813-04	dd	0.40	0.50	0.60	0.50
ArPop_0813-05	dd	0.40	0.40	-	0.40
ArPop_0813-06	dd	0.90	0.80	1.10	0.93
ArPop_0813-07	Dd	0.20	0.30	0.40	0.30
ArPop_0813-08	dd	0.70	0.50	0.90	0.70
ArPop_0813-09	Dd	0.40	0.70	-	0.55
ArPop_0813-10	Dd	1.00	0.60	-	0.80
ArPop_0813-11	dd	1.00	0.70	0.80	0.83
ArPop_0813-12	Dd	0.40	0.50	-	0.45
ArPop_0817-01	Dd	-	0.30	0.30	0.30
ArPop_0817-02	Dd	0.40	0.60	0.70	0.57
ArPop_0817-03	Dd	0.50	0.50	-	0.50
ArPop_0817-04	DD	0.50	0.50	0.40	0.47
ArPop_0817-05	Dd	-	0.30	0.60	0.45
ArPop_0817-06	Dd	0.40	-	0.50	0.45
ArPop_0817-07	Dd	-	0.20	0.30	0.25
ArPop_0817-08	dd	0.90	0.80	0.80	0.83
ArPop_0817-09	Dd	-	-	0.60	0.60
ArPop_0819-01	Dd	0.30	0.30	0.50	0.37
ArPop_0819-02	Dd	0.40	0.60	0.40	0.47
ArPop_0819-03	Dd	0.30	0.30	0.40	0.33
ArPop_0819-04	Dd	0.40	0.40	0.40	0.40
ArPop_0819-05	Dd	0.30	0.40	-	0.35
ArPop_0819-06	Dd	0.40	0.70	0.50	0.53
ArPop_0819-07	Dd	0.30	0.40	-	0.35
ArPop_0819-08	Dd	0.50	0.30	0.40	0.40
ArPop_0819-09	dd	1.00	1.10	1.20	1.10
ArPop_0819-11	Dd	0.40	0.50	0.40	0.43

Table B.1. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of Arkansas RosBREED populations, years 2011, 2012, 2013, and across year average (Cont.).

Accession	Alleles	TA (%)			
		2011	2012	2013	Average
ArPop_0819-12	Dd	-	0.40	0.40	0.40
ArPop_0819-13	Dd	-	0.40	0.60	0.50
ArPop_0819-14	dd	1.10	1.00	1.00	1.03
ArPop_0819-15	Dd	0.40	0.40	0.40	0.40
ArPop_0819-16	Dd	0.30	0.30	0.40	0.33
ArPop_0819-17	Dd	0.30	0.30	0.40	0.33
ArPop_0819-18	Dd	0.30	0.30	0.50	0.37
ArPop_0819-19	Dd	0.30	0.50	0.30	0.37
ArPop_0819-20	Dd	0.20	-	0.50	0.35
ArPop_0819-21	dd	0.80	-	0.90	0.85
ArPop_0819-22	Dd	0.40	0.40	0.60	0.47
ArPop_0819-23	Dd	0.40	0.70	1.00	0.70
ArPop_0825-01	Dd	0.30	0.30	1.00	0.53
ArPop_0825-02	DD	0.50	0.50	0.90	0.63
ArPop_0825-03	Dd	0.40	0.20	0.50	0.37
ArPop_0825-04	Dd	0.70	0.60	0.90	0.73
ArPop_0825-05	DD	-	-	0.40	0.40
ArPop_0825-06	Dd	0.20	0.20	0.50	0.30
ArPop_0825-07	dd	1.10	0.60	0.90	0.87
ArPop_0825-08	Dd	0.20	0.20	-	0.20
ArPop_0825-10	Dd	0.30	0.30	0.50	0.37
ArPop_0825-11	Dd	0.20	0.30	0.30	0.27
ArPop_0825-12	Dd	0.20	0.20	0.50	0.30
ArPop_0825-13	Dd	-	0.20	0.50	0.35
ArPop_0825-14	Dd	-	0.20	0.40	0.30
ArPop_0825-15	dd	-	0.70	1.00	0.85
ArPop_0825-16	dd	-	0.60	0.90	0.75
ArPop_0825-17	Dd	-	0.10	0.50	0.30

Table B.2. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of 2010-Arkansas populations, year 2014.

Accession	Alleles	TA (%)
1001-06	Dd	0.20
1001-09	Dd	0.67
1001-14	Dd	0.21
1001-18	Dd	0.15
1001-23	Dd	0.76
1001-24	Dd	0.25
1001-25	Dd	0.25
1001-32	Dd	0.26
1001-43	Dd	0.22
1001-45	Dd	0.15
1001-50	Dd	0.18
1001-55	Dd	0.24
1001-61	Dd	0.26
1002-008	Dd	0.26
1002-010	Dd	0.20
1002-011	Dd	0.16
1002-024	Dd	0.22
1002-039	Dd	0.59
1002-046	DD	0.17
1002-047	Dd	0.19
1002-048	Dd	0.22
1002-050	Dd	0.17
1002-053	DD	0.12
1002-069	Dd	0.28
1002-072	DD	0.19
1002-091	Dd	0.10
1002-094	DD	0.51
1002-100	Dd	0.15
1003-013	Dd	0.15
1003-014	Dd	0.16
1003-015	Dd	0.17
1003-018	Dd	0.17
1003-023	DD	0.20
1003-027	Dd	0.63
1003-036	DD	0.54

Table B.2. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of 2010-Arkansas populations, year 2014 (Cont.).

Accession	Alleles	TA (%)
1003-037	DD	0.15
1003-044	Dd	0.49
1003-049	DD	0.15
1003-054	dd	0.61
1003-067	DD	0.24
1003-068	DD	0.20
1003-069	DD	0.16
1003-071	DD	0.59
1003-075	Dd	0.49
1003-090	Dd	0.13
1003-106	DD	0.12
1003-116	DD	0.15
1003-120	DD	0.11
1003-123	DD	0.17
1003-125	DD	0.11
1003-207	DD	0.13
1004-001	dd	0.48
1004-009	DD	0.13
1004-010	DD	0.75
1004-011	DD	0.31
1004-012	Dd	0.35
1004-015	Dd	0.37
1004-026	DD	0.45
1004-033	DD	0.52
1004-042	dd	0.36
1004-051	DD	1.03
1004-055	DD	0.27
1004-062	DD	0.18
1006-003	DD	0.42
1006-004	DD	0.21
1006-006	DD	0.26
1006-007	DD	0.03
1006-012	DD	0.11
1006-025	DD	0.53
1006-031	DD	0.35

Table B.2. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of 2010-Arkansas populations, year 2014 (Cont.).

Accession	Alleles	TA (%)
1006-034	DD	0.52
1006-043	DD	0.14
1006-055	DD	0.21
1006-060	DD	0.12
1006-094	DD	0.17
1006-102	Dd	0.27
1006-106	DD	0.30
1006-111	DD	0.14
1006-112	Dd	0.38
1006-139	Dd	0.12
1006-140	DD	0.18
1006-152	Dd	0.14
1007-012	Dd	0.61
1007-014	Dd	1.07
1007-022	Dd	0.53
1007-024	Dd	0.55
1007-031	DD	0.47
1007-034	DD	0.25
1007-036	DD	0.18
1007-039	DD	0.21
1007-041	DD	0.38
1007-051	DD	0.54
1007-063	DD	0.42
1007-073	DD	0.82
1007-077	DD	0.23
1007-083	DD	0.23
1007-088	DD	1.12
1007-097	DD	0.57
1007-116	DD	0.28
1007-126	Dd	0.21
1007-131	DD	1.18
1007-132	DD	0.43
1007-141	DD	0.57
1007-143	DD	0.19
1007-155	DD	0.22

Table B.2. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of 2010-Arkansas populations, year 2014 (Cont.).

Accession	Alleles	TA (%)
1007-174	DD	0.17
1007-178	Dd	0.28
1007-187	DD	0.85
1007-192	DD	0.21
1007-193	Dd	0.13
1008-011	DD	0.09
1008-012	Dd	0.18
1008-019	DD	0.20
1008-027	Dd	0.11
1008-028	DD	0.57
1008-033	DD	0.24
1008-043	DD	0.22
1008-046	Dd	0.28
1008-051	Dd	0.18
1008-054	Dd	0.31
1008-059	DD	0.15
1008-061	DD	0.51
1009-018	Dd	0.21
1009-021	DD	0.44
1009-022	Dd	0.17
1009-024	DD	0.14
1009-027	DD	0.60
1009-029	DD	0.17
1009-040	DD	0.20
1009-045	DD	0.22
1009-054	DD	0.25
1009-061	DD	0.16
1009-062	DD	0.13
1009-063	DD	0.44
1009-066	DD	0.17
1011-001	DD	0.43
1011-003	DD	0.32
1011-005	DD	0.42
1011-006	DD	0.15
1011-008	DD	0.71

Table B.2. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of 2010-Arkansas populations, year 2014 (Cont.).

Accession	Alleles	TA (%)
1011-014	DD	0.33
1011-016	Dd	0.09
1011-020	Dd	0.19
1011-023	Dd	0.17
1011-025	Dd	0.78
1011-026	Dd	0.36
1011-027	Dd	0.20
1011-029	DD	0.24
1011-031	Dd	0.16
1011-034	Dd	0.30
1011-035	Dd	1.00
1011-038	Dd	0.92
1011-040	Dd	0.15
1011-050	Dd	0.29
1011-051	Dd	0.26
1011-052	Dd	0.63
1011-057	Dd	0.43
1011-060	Dd	0.22
1011-061	Dd	0.21
1011-062	Dd	0.33
1011-064	Dd	0.14
1011-065	Dd	0.71
1011-071	DD	0.36
1011-081	dd	0.23
1011-083	Dd	0.11
1012-011	DD	0.26
1012-019	Dd	0.35
1012-027	Dd	0.32
1012-028	dd	0.86
1012-029	Dd	0.21
1012-038	Dd	0.19
1012-045	Dd	0.22
1012-056	Dd	0.29
1012-060	DD	0.21
1012-063	dd	1.02

Table B.2. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of 2010-Arkansas populations, year 2014 (Cont.).

Accession	Alleles	TA (%)
1012-066	Dd	0.19
1012-069	Dd	0.17
1012-071	Dd	0.52
1012-074	Dd	0.22
1012-081	Dd	0.15
1013-004	Dd	0.20
1013-005	Dd	0.19
1013-009	Dd	0.09
1013-016	Dd	0.60
1013-029	Dd	0.15
1013-072	dd	0.11
1018-026	DD	0.65
1018-030	dd	0.89
1018-053	dd	0.77
1018-054	dd	0.60
1018-062	dd	0.54
1018-063	DD	0.78
1018-070	Dd	0.54
1018-077	dd	0.60
1018-089	DD	0.72
1018-090	Dd	0.91
1019-005	Dd	0.30
1019-028	Dd	0.28
1019-033	Dd	0.84
1019-037	Dd	0.18
1019-039	Dd	0.20
1019-043	Dd	0.19
1019-046	Dd	0.30
1019-048	DD	0.13
1019-054	Dd	0.20
1019-056	DD	0.24
1019-057	DD	0.26

Table B.2. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of 2010-Arkansas populations, year 2014 (Cont.).

Accession	Alleles	TA (%)
1019-059	Dd	0.17
1019-100	Dd	0.59
1020-005	Dd	0.56
1020-007	Dd	0.49
1020-021	dd	0.54
1020-025	Dd	0.56
1020-027	Dd	0.56
1020-028	Dd	0.34
1020-029	Dd	0.38
1020-030	dd	0.44
1020-033	dd	0.56
1020-035	Dd	0.71
1020-036	Dd	0.50
1020-040	Dd	0.53
1020-041	dd	0.59
1021-008	dd	0.48
1021-013	Dd	0.32
1021-019	Dd	0.18
1021-045	Dd	0.27
1021-062	Dd	0.27
1021-086	Dd	0.22
1021-088	Dd	0.22
1021-089	dd	0.26
1021-092	Dd	0.27
1021-095	dd	0.47
1021-104	Dd	0.48
1021-105	dd	0.80
1021-107	dd	0.33
1021-124	Dd	0.23
1021-134	Dd	0.19
1021-136	dd	0.55
1021-140	dd	0.49
1021-141	dd	0.72
1021-142	Dd	0.23
1021-143	Dd	0.28

Table B.2. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of 2010-Arkansas populations, year 2014 (Cont.).

Accession	Alleles	TA (%)
1021-144	dd	0.67
1021-145	Dd	0.23
1021-146	dd	1.04
1021-148	dd	0.37
1021-149	Dd	0.34
1021-150	Dd	0.84
1022-001	DD	0.32
1022-004	DD	0.23
1022-005	Dd	0.19
1022-006	Dd	0.14
1022-009	dd	0.67
1022-010	dd	0.16
1022-011	Dd	0.24
1022-012	Dd	0.49
1022-018	DD	0.26
1022-019	Dd	0.38
1022-020	Dd	0.23
1022-021	Dd	0.21
1022-022	Dd	0.23
1022-023	Dd	0.29
1022-024	Dd	0.17
1022-025	Dd	0.50
1023-001	Dd	0.61
1023-002	Dd	0.81
1023-005	Dd	0.93
1023-006	Dd	0.65
1024-002	Dd	0.86
1024-003	Dd	0.69
1024-007	Dd	0.96
1024-029	Dd	0.70
1024-041	Dd	0.45
1024-042	Dd	0.65
1024-043	Dd	1.05
1024-044	Dd	0.55
1024-048	Dd	0.83

Table B.2. Allele combination of *D*-locus DNA test and titratable acidity (TA) (%) values of 2010-Arkansas populations, year 2014 (Cont.).

Accession	Alleles	TA (%)
1024-085	Dd	0.90
1025-009	Dd	0.79
1025-017	Dd	0.67
1025-018	Dd	0.39
1025-019	Dd	0.79
1025-020	Dd	0.58
1025-048	Dd	1.16
1026-004	Dd	0.36
1026-005	Dd	0.38
1026-011	Dd	0.23
1026-012	Dd	0.35
1026-016	Dd	0.49
1026-025	Dd	0.94
1026-026	Dd	0.76
1026-027	Dd	0.68
1026-028	Dd	0.20
1026-030	Dd	0.36
1026-047	dd	0.30
1026-052	Dd	1.73
1026-053	Dd	0.35

Chapter 5

EVALUATION AND CHARACTERIZATION OF FRUIT FIRMNESS IN "CRISPY" BLACKBERRY GENOTYPES IN THE BLACKBERRY BREEDING PROGRAM OF THE UNIVERSITY OF ARKANSAS

Abstract

The University of Arkansas blackberry breeding program was begun in 1964, and since then the program has released cultivars with the aim to provide high-quality fruit to the fresh-market industry. One of the critical traits for successful blackberry (*Rubus* subgenus *Rubus* Watson) postharvest handling is flesh firmness, so developing cultivars with high firmness is a top priority for the majority of breeding programs across the world. In particular, the Arkansas blackberry program has a wide range of genotypes with exceptional firmness characteristics, including fruit with a unique crispy texture and firmness. During 2013 and 2014, fruit firmness measurements were done on 15 crispy and non-crispy Arkansas genotypes. Firmness measurements consisted of fruit compression, skin drupelet penetration, and receptacle penetration. Also, color reversion was evaluated among these genotypes after storage. Finally, in 2014 confocal photos were taken on sections of berries of a subset of crispy and non-crispy genotypes. Compression force values differentiated crispy and non-crispy genotypes, with average values of 11.8 N and 8.0 N, respectively. Drupelet penetration force was also higher for crispy genotypes averaging 0.23 N and non-crispy 0.15 N; similarly, receptacle penetration force averaged 0.20 N for crispy and 0.18 N for non-crispy genotypes. However, penetration values were more variable than compression values. Visual inspection of fruit mesocarp revealed that drupelet and receptacle cells and cell walls of crispy genotypes maintained their structure during ripening and did not break apart, while non-crispy genotypes did not maintain their structure and

cellular integrity. Color reversion is a postharvest disorder in which drupelets of blackberry fruits turn red after being black at harvest. Therefore, this trait, it has a negative impact for growers, shippers, and consumers. After storage at 5 ^{o}C for 7 d, crispy genotypes expressed low levels of reversion compared to non-crispy genotypes. For crispy genotypes, 13.2% of drupelets developed color reversion, whereas a 41.0% developed this disorder in non-crispy genotypes.

Introduction

Blackberry fruit production in the U.S. has shown great expansion compared to most of the world, especially in California along with new commercial shipping in Georgia, North Carolina, Arkansas, and Texas (Clark and Finn, 2014). This expansion is due to several factors, one of them an increase in demand and resulting consumption in the U.S. and Europe (Clark and Finn, 2014). In 2005, Europe had 7,692 ha of blackberries in commercial production, and Serbia was the leading country with 69% of the area in Europe (Strik et al., 2007). Serbia continues as one of the top blackberry producers in the world with more than 5,000 ha planted (Clark and Finn, 2014) although this production is primarily for processing. Mexico leads the world with over 6,500 ha in production, almost all production for the fresh market and exported (Clark and Finn, 2014).

The blackberry industry is in expansion and reasons for this phenomenon are several, but include that blackberry is a new crop in many areas of the world, new blackberry cultivars ship better allowing for extension of the harvest and maturity season, fruit quality is improved in newer cultivars, blackberry fruits have high levels of anthocyanins and antioxidants providing for increasing appeal to consumers, and longer-season production allows for year-round

marketing enhancing grower, packer, and processor profitability (Clark and Finn, 2014; Clark et al., 2007).

The quality of fruits for the fresh-market is determined largely by how the genotype responds to storage and handling from the day of harvest until purchased and eaten by the consumer (Finn and Clark, 2012). Blackberries have been considered one of the more difficult fruits to ship due to softening and leakage during postharvest (Clark, 2005). Blackberry is one crop that firmness is crucial, especially during postharvest handling. Fruit firmness is suggested to be an intractable trait, meaning that it is a difficult character to improve in breeding. Firmness varies with cultivar, ripeness stage, and storage duration (Clark, 2005; Perkins–Veazie et al., 1996). If a blackberry genotype is firm in the field it does not always retain firmness during postharvest storage, so measuring firmness after cold storage is crucial to determine fresh-market potential (Clark, 2005; Finn and Clark, 2012).

Generally in fruits, firmness is related to modifications of the polysaccharide components of the primary cell wall and middle lamella during fruit ripening, resulting in a weaker fruit structure at the end of the ripening process (Brummell, 2006; Winkler et al., 2015). Alterations in the bonding between polymers along with degradation of polysaccharides can cause an increase in cell separation, softening, and swelling of the cell wall (Brummell, 2006). These alterations, combined with changes in cell turgor, cause fruit softening and textural changes in fruits (Brummell, 2006). Blackberries increase their pectin solubility activity during ripening (Brummell, 2006). In apples (*Malus* x *domestica* Borkh), ripening is accompanied by decreasing strength of the middle lamella, resulting in reduced intercellular adhesion and cell separation under stress (Atkinson et al., 2012). A suppression of the gene that promotes polygalacturonase (PG) activity resulted in firmer ‘Royal Gala’ apples due to different distribution of pectins along

with increased integrity of the middle lamella (Atkinson et al., 2012). Also, in the PG-suppressed genotypes, cells in the hypodermal layers of the fruit below the cuticle remained densely packed (Atkinson et al., 2012).

An important postharvest disorder affecting blackberry fruit destined for the fresh market is color reversion (also called reddening or red drupelet) (Clark and Finn, 2011). Affected drupelets of blackberry fruits turn red, often during cold storage or when exposed at room temperature after being in cold storage (Clark and Finn, 2011; Finn and Clark, 2012). In a mature fruit cell, approximately 90% of the volume is occupied by the vacuole, a cell organelle that is dynamic and multifunctional and provides the primary site of macromolecule storage and metabolism (Fontes et al., 2011). The vacuole accumulates sugars, aromas, flavors, ions, and water; all these compounds are transported across the tonoplast (vacuole membrane) by a specific transporter protein (Fontes et al., 2011).

Retention of black color can be selected for in breeding, but it cannot be determined in the field. Therefore, postharvest evaluations must be done to verify if resistance to reversion is present (Clark and Finn, 2011). The UA blackberry breeding program, with a focus on postharvest quality of fruits for successful postharvest storage for the fresh market, began postharvest evaluations in 2008 to characterize postharvest traits of advanced breeding selections (Clark and Perkins-Veazie, 2011).

Crispy fruit with high firmness was first observed in the UA blackberry breeding program a number several years ago on a floricane-fruiting, thorny selection. Since that time, this texture trait has been advanced to improved selections, and thornless selections that express the crispy texture consistently and have been used in crosses with the aim to transfer the crispiness into improved seedlings and resulting selections with increased yield, fruit size, fruit flavor, and

primocane-fruiting. Two of these, A-2453 and A-2454, are believed to hold the most promise for use in breeding for this trait. These crispy genotypes show improved postharvest performance compared to previously released cultivars from the breeding program. They maintain the high firmness observed in the field and after storage, and also show reduced color reversion (drupelets developing red color) after 7 d of cold storage (J. Clark, unpublished data).

The objectives of this study focused on characterization of crispy and non-crispy genotypes including compression and penetration forces in different tissues of the fruit. Also, analysis of cell structure of drupelet and receptacle tissue of crispy and non-crispy genotypes was conducted to reveal physical aspects contributing to the unique texture.

Materials and Methods

Plant Material

All fruit collection and firmness measurements were conducted at the University of Arkansas Fruit Research Station, Clarksville [west-central Arkansas (west-central Arkansas, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]. Confocal image analysis for cell structure analysis was done at the University of Arkansas, Fayetteville campus. Blackberry plants were grown with cultural components including annual routine plant management practices such us fertilization, weed control, and irrigation. All plantings received a single application of liquid lime sulfur (94 L≅ha^{-1}) at budbreak for control of anthracnose [*Elsinoë veneta* (Burkh.) Jenkins] (Smith, 2015). This was the only fungicide applied to any plantings in any year. Raspberry crown borer (*Pennisetia marginata* Harris) was controlled by a single application of a labelled insecticide in October of each year (Smith, 2015). Insecticides labelled for commercial use in

Arkansas were used for spotted-wing drosophila (*Drosophila suzukii* Matsumura) control. All plots were irrigated as needed using overhead sprinkler irrigation.

Genotypes used in this study were crispy and non-crispy cultivars/selections. Within the crispy group the selections used were A-1790, A-2218, A-2453, and A-2454. Within the non-crispy group the cultivars/selections were 'Prime-Ark® 45', 'Natchez', 'Osage', 'Ouachita', A-1960, A-2252, A-2297, A-2416, A-2417, A-2418, and A-2428. Harvest period was between 12 June and 30 June in 2013, and between 9 June and 3 July in 2014.

During the floricane season, each cultivar/selection (genotype) was harvested into 260-g clamshells (FormTex Plastics Corp., Houston, TX) at the shiny-black maturity stage. Genotypes were harvested between two and six times per season, depending on fruit availability (some selections were only available in one single plot at the research station and sometimes was difficult to obtain high quality fruit to evaluate more than two times per season. However, some selections were present in multiple plots and they had a higher amount of high quality fruit available to harvest more than two times per season). In 2013, two clamshells and an additional 10 berries were harvested at each harvest date for each genotype. The fruits of one clamshell (randomly selected) were used to measure fruit compression (15-20 fruits) and the other clamshell was used to measure drupelet penetration (10 fruits) and receptacle penetration (10 fruits). The additional 10 berries were used for reversion measurements after storage. In 2014, four clamshells were harvested at each harvest date. Two clamshells were randomly selected and used to measure firmness at harvest day (day 0) and the other two were used to measure firmness and reversion after one week (day 7) of cold storage at ~5 °C (Clamshells were stored in a closed plastic tub within the refrigerator to avoid dehydration caused by the excessive air circulation). At each day of measurement (day 0 and 7), the fruits of one clamshell were used to measure

compression (15-20 fruits) and the other clamshell was used to measure drupelet penetration (10 fruits) and receptacle penetration (10 fruits). In the case of cold storage, only two harvests per season were analyzed.

Phenotypic Evaluation

Fruit Firmness was measured using an iCon Texture Analyzer (Texture Technologies Corp. Hamilton, MA) utilizing two different methodologies:

1. Compression: Fruit compression was performed by placing individual fruits horizontally on a flat surface using a cylindrical and plane probe of 7.6 cm diameter (Fig. 1).

2. Penetration: Each fruit was cut in half longitudinally. One half was used for drupelet penetration and the other half to measure the receptacle firmness.

a. Drupelet penetration: Drupelet skin firmness was assessed using a probe of 1 mm diameter. For this, three drupelets of similar shape and size were used per berry (Fig. 2).

b. Receptacle penetration: Measured using a probe of 1 mm diameter in the middle of the receptacle (Fig. 3).

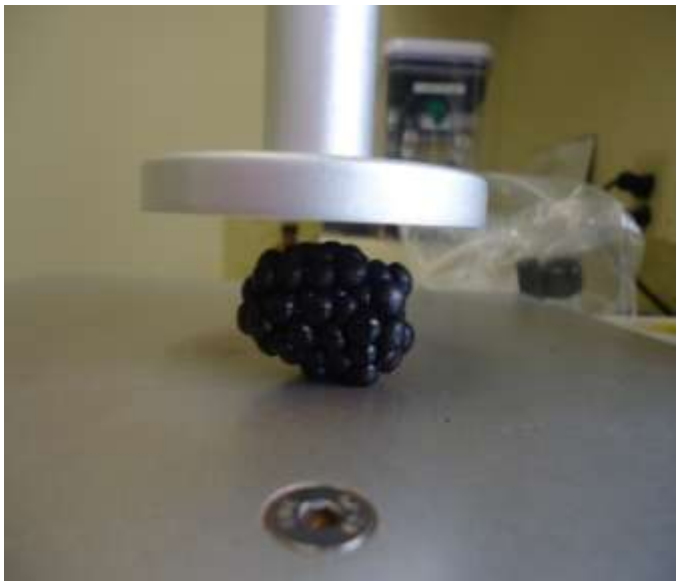

Fig. 1. Fruit compression measurement procedure utilizing a flat surface and a cylindrical and plane probe of 7.6 cm diameter.

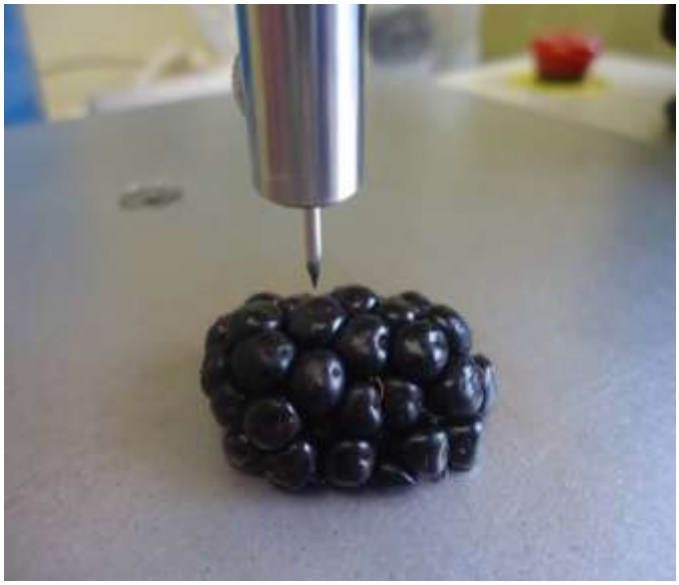

Fig. 2. Skin drupelet penetration measurement procedure utilizing a probe of 1 mm diameter.

Fig. 3. Receptacle penetration measurement procedure utilizing a probe of 1 mm diameter.

In 2014, fresh tissue of individual toruses and drupelets of two crispy genotypes (A-2453 and A-2454) and two non-crispy genotypes ('Natchez' and 'Shawnee') were analyzed for fruit structural differences. Of the two non-crispy cultivars, 'Natchez', an industry standard with good firmness (Clark and Moore, 2008) is considered substantially firmer than 'Shawnee' (Perkins-Veazie et al., 2000a) ('Shawnee' was added to the fruit structure analysis, because it is an older, very soft-fruited Arkansas cultivar and useful to compare it with very firm cultivar/selection). Berries were hand-sectioned with a razor blade in thin layers and mounted on a microscope slide with a cover slip. Slides were examined with 20X magnification with a confocal microscope (Nikon Eclipse 90i, Nikon Instrument Inc. Melville, NY) to examine cellular consistency. Photos were taken immediately after placement to allow evaluation of cellular differences among the genotypes.

Color reversion after one week of cold storage at ~5 °C was evaluated in 2013 and 2014. All genotypes indicated in the Plant Material section were included in this evaluation by exception of non-crispy genotypes A-2416 and A-2418 (due to low fruit availability). For color reversion evaluation, each fruit was categorized using the following multinomial color reversion scale:

- Zero: No red drupelets.
- Low to medium levels: Percentage of berries showing one to three red drupelets after cold storage.
- High level: Percentage of berries having more than four red drupelets after cold storage.

Data Analysis

Fruit compression force, skin drupelet penetration, and receptacle penetration were subjected to a split-plot ANOVA in which sources of variation were year (2013 and 2014),

texture (crispy and non-crispy), and genotype (genotype is nested in texture). For the storage analysis, data was analyzed as a split-plot ANOVA in which sources of variation were storage (storage and non-storage), texture (crispy and non-crispy), and genotype (genotype is nested within texture). The PROC GLIMIX procedure was utilized (SAS® 9.4. Cary, NC). Least square means test was performed for mean comparisons ($P \leq 0.05$). For color reversion, data was analyzed as a split-plot ANOVA in which sources of variation were year (2013 and 2014), texture (crispy and non-crispy), and genotype (genotype is nested in texture). The ANOVA test was performed utilizing a multinomial distribution and the cumulative probability of a genotype to be in one of the three levels of color reversion was estimated (zero, low to medium, and high). The PROC GLIMIX procedure was utilized (SAS® 9.4. Cary, NC). Least square means test was performed for mean comparisons ($P \leq 0.05$).

Results and Discussion

Fruit Firmness

A significant year (2013 and 2014) x genotype (texture) (crispy and non-crispy) interaction was found for all force measurements for the study (Table 1). This means that genotypes, depending on their texture, expressed different firmness values in 2013 and 2014. This also indicates that different environmental conditions for both years affected firmness and this was reflected in all three types of firmness measurements. As in other fruits, such as apple and red raspberry (*Rubus ideaeus* L.), firmness is a quantitative trait (Iwanami et al., 2008; Stephens et al., 2012), and is difficult to improve in blackberry (Clark, 2005). Also, its quantitative behavior was supported in this study since there was a significant year x texture interaction in all force measurements (Table 1).

In examining year x texture effects, compression values ranged from a high of 13.3 N for the crispy category in 2014 to 8.2 N for non-crispy in 2013, and 10.2 N for crispy in 2013 to 7.7 N for non-crispy in 2014 (Table 2). When comparing within-texture means among years, crispy values were different, with 2013 value of 13.3 N and in 2014 10.2 N (Table 2), while non-crispy means among years were also different (8.2 N and 7.7 N). In 2013 and 2014, values of skin drupelet penetration force of crispy texture was higher than non-crispy (Table 3). Skin drupelet penetration force value for within crispy texture in 2103 was significantly higher than in 2014 (0.29 N and 0.18 N, respectively); also value of skin drupelet penetration force within non-crispy texture was higher in 2013 compared to 2014 (0.17 and 0.12 N, respectively) (Table 3). In 2013, mean of receptacle penetration force of crispy texture was higher than non-crispy texture, but in 2014 they were not significantly different (Table 4). Receptacle penetration firmness value of within crispy texture among years was different, in 2013 was significantly higher compared to 2014 (0.27 and 0.12 N, respectively), and within non-crispy texture in 2013 was significantly higher than in 2014 (0.22 and 0.13 N, respectively) (Table 4). It is well known that high temperatures in the field, rainfall before harvest, and an incorrect temperature in handling of the harvested fruits in storage have negative impacts on blackberry fruit firmness (and other traits) and subsequent shelf-life (Clark and Finn, 2011; Perkins-Veazie et al., 1999). According to the data obtained from the weather station of FRS during the harvest period in 2014, the amount of rain was 3.3 times higher compared to 2013 (1.7 cm in 2013 and 5.6 cm in 2014). Also, during the harvest period the amount of rainy days in 213 was two and in 2014 was nine. These data indicate that the environment was different between these two seasons and the higher amount of rain in 2014 likely had a significant negative impact on fruit firmness.

As stated before, a significant year x genotype (texture) source of variation effect was found for all types of force measurements (Table 1). In 2013, compression force of crispy genotypes ranged from 12.2 to 14.1 N and in 2014 ranged from 7.7 to 13.4 N (Table 5). For non-crispy genotypes, there was a substantial range in compression values from 6.0 to 11.4 N in 2013 and from 5.9 to 9.6 N in 2014 (Table 6). In 2013, all crispy selections expressed higher firmness compared to the non-crispy ones, with the lowest values for a crispy selection, A-2454, averaged a compression force of 12.2 N and the firmest non-crispy selection, A-2418, averaged 11.4 N. However, in 2014 this situation was not the same since the non-crispy genotype with higher firmness, A-2416, averaged 9.6 N, a value that was within the range of the crispy genotypes. Again, environmental effects likely contribute to these results.

Skin drupelet penetration values of crispy genotypes ranged from 0.19 to 0.42 N in 2013 and from 0.16 to 0.19 N in 2014 (Table 7). Non-crispy genotypes ranged from 0.12 to 0.27 N in 2013 and from 0.10 to 0.14 N in 2014 (Table 8). These values are comparable to the 0.20 N skin penetration force value reported by Perkins-Veazie et al. (2000b) for shiny-black fruits of 'Navaho' (non-crispy but firm cultivar). The mean value of crispy genotypes during both years of the study was also higher than the firmness value of 'Navaho' reported by Perkins-Veazie et al. (2000b).

Receptacle penetration values of crispy genotypes ranged from 0.24 to 0.31 N in 2013 and from 0.11 to 0.14 N in 2014 (Table 9). Non-crispy genotype values for receptacle penetration ranged from 0.13 to 0.33 N in 2013 and from 0.10 to 0.17 N in 2014 (Table 10). Mean value of 'Navaho' reported by Perkins-Veazie et al. (2000b) was 0.10 N, a value that was more comparable to the firmness observed of non-crispy genotypes in the present study.

Table 1. Split-plot ANOVA, degrees of freedom (DF), and F-test p-value (*P*) for compression firmness, drupelet skin penetration firmness, and receptacle penetration firmness. Data are from two years, two textures, and 15 genotypes.

Source	DF	Compression *P*	Drupelet *P*	Receptacle *P*
Year	1	<.0001	<.0001	<.0001
Texture	1	0.0006	0.0391	<.0001
Year*Texture	1	<.0001	<.0001	<.0001
Genotype (Texture)	13	<.0001	<.0001	<.0001
Year*Genotype (Texture)	13	<.0001	<.0001	<.0001

Table 2. Least square interaction means of compression force (N) of year (2013 and 2014) by texture (crispy and non-crispy) on the day of harvest.

Year	Texture			
	Crispy		Non-crispy	
2013	13.3[z]	A[y]a[x]	8.2	Bc
2014	10.2	Ab	7.7	Bd

[z] Values correspond to compression force measured in Newton (N).

[y] Means in the same row followed by the same upper-case letter are not significantly different by least square means, $P \leq 0.05$.

[x] Means in columns and rows followed by the same lower-case letter are not significantly different by least square means, $P \leq 0.05$.

Table 3. Least square interaction means of skin drupelet penetration force (N) of texture (crispy and non-crispy) by year (2013 and 2014) on the day of harvest.

Year	Texture			
	Crispy		Non-crispy	
2013	0.29[z]	A [y] a[x]	0.17	Bb
2014	0.18	Ab	0.12	Bc

[z] Values correspond to skin drupelet penetration force measured in Newton (N).
[y] Means in the same row followed by the same upper-case letter are not significantly different by least square means, $P \leq 0.05$.
[x] Means in columns and rows followed by the same lower-case letter are not significantly different by least square means, $P \leq 0.05$.

Table 4. Least square interaction means of receptacle penetration force (N) of texture (crispy and non-crispy) by year (2013 and 2014) on the day of harvest.

Year	Texture			
	Crispy		Non-crispy	
2013	0.27[z]	A [y] a[x]	0.22	Bc
2014	0.12	Ab	0.13	Ad

[z] Values correspond to receptacle penetration force measured in Newton (N).
[y] Means in the same row followed by the same upper-case letter are not significantly different by least square means, $P \leq 0.05$.
[x] Means in columns and rows followed by the same lower-case letter are not significantly different by least square means, $P \leq 0.05$.

Table 5. Least square means of fruit compression force (N) of four crispy selections at harvest day, years 2013 and 2014.

Genotype	Fruit compression (N)			
	2013		2014	
A-2218	14.1	a[z]	7.7	c
A-1790	13.9	a	9.4	b
A-2453	13.0	ab	13.4	a
A-2454	12.2	bc	10.4	b

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Table 6. Least square means of fruit compression force (N) of 11 non-crispy selections at harvest day, years 2013 and 2014.

Genotype	Fruit compression (N)			
	2013		2014	
A-2418	11.4	a[z]	8.8	a
Natchez	10.1	ab	9.3	a
A-2416	9.0	ab	9.6	a
Prime-Ark®45	9.8	b	8.8	a
A-2417	8.4	bc	7.3	bc
A-2252	7.7	cd	7.3	bc
A-2428	7.3	cd	6.2	bc
Osage	7.0	cd	8.6	ab
Ouachita	6.8	cd	6.3	bc
A-2297	6.1	d	5.9	d
A-1960	6.0	d	6.3	bc

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Table 7. Least square means of skin drupelet penetration (N) of four crispy selections at harvest day, years 2013 and 2014.

Genotype	Skin drupelet penetration(N)			
	2013		2014	
A-1790	0.42	a[z]	0.18	ab
A-2218	0.33	b	0.19	a
A-2454	0.24	c	0.16	b
A-2453	0.19	d	0.19	a

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Table 8. Least square means of skin drupelet penetration (N) of 11 non- crispy selections at harvest day, years 2013 and 2014.

Genotype	Skin drupelet penetration(N)			
	2013		2014	
A-2297	0.27	a[z]	0.11	c
Prime-Ark®45	0.19	b	0.11	c
Natchez	0.17	b	0.10	c
A-2428	0.17	bc	0.11	c
Ouachita	0.16	bc	0.14	a
A-2417	0.16	bc	0.14	ab
Osage	0.16	bc	0.12	c
A-2252	0.15	bc	0.13	ab
A-2416	0.14	bc	0.12	bc
A-2418	0.14	bc	0.13	ab
A-1960	0.12	bc	0.11	c

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Table 9. Least square means of fruit receptacle penetration (N) of four crispy selections at harvest day, years 2013 and 2014.

Genotype	Receptacle penetration (N)	
	2013	2014
A-2454	0.31 a[z]	0.11 a
A-2218	0.30 a	0.11 a
A-1790	0.25 b	0.13 a
A-2453	0.24 b	0.14 a

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Table 10. Least square means of fruit receptacle penetration (N) of 11 non-crispy selections at harvest day, years 2013 and 2014.

Genotype	Receptacle penetration(N)	
	2013	2014
A-2418	0.33 a[z]	0.17 a
A-2416	0.28 b	0.14 bc
A-2297	0.24 c	0.10 d
A-2417	0.24 c	0.14 bc
Prime-Ark®45	0.24 c	0.12 cd
A-2428	0.23 c	0.15 ab
Natchez	0.22 c	0.11 cd
A-2252	0.21 cd	0.14 bc
Ouachita	0.20 cd	0.12 cd
A-1960	0.16 de	0.11 cd
Osage	0.13 e	0.10 d

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Cold Storage

Blackberry is one of the most difficult fruits to ship due to high leakiness and softening (Clark, 2005). However, breeding improvement is one tool to get better genotypes, and crispy texture genotypes represent a good alternative for use as parents to generate progeny with better firmness and postharvest shelf-life, since there is a positive relationship between fruit firmness and postharvest shelf-life (Perkins-Veazie et al., 1996). In 2014, crispy and non-crispy genotypes were stored for 7 d with the objective to compare their firmness after cold storage at 5 °C. The ANOVA indicated a significant storage by genotype (texture) for all firmness variables measured (Table 11). Significant storage x texture interaction was observed for compression (P= 0.0485) but not for drupelet skin and receptacle penetration (Table 11). Genotype (texture) was significant for all variables (Table 11). The main effects of storage and texture were not significant for any variables, except storage for receptacle penetration (Table 11).

After 7 d of cold storage for crispy texture, a significant loss of 1.2 N of compression force was found (initially 10.0 N on harvest day to 8.8 N after storage) (Table 12). Non-crispy values were the same before and after storage, 7.7 N (Table 12). In comparing crispy to non-crispy, there were differences in non-storage means but not for storage means. Decrease in firmness after a period of cold treatment it is expected as previous studies have indicated (Perkins-Veazie et al., 1996), but the idea is to retain a high percentage of the initial firmness combined with high soluble solids and flavor components (Clark and Finn, 2011). It was expected that after storage, crispy texture would maintain a higher firmness than non-crispy texture, because crispy texture was higher in firmness before storage compared to non-crispy texture. However, this was not the finding. This could be due to that only one year of data was taken in this particular storage study and repeating the study another year could improve the

conclusions of this study. Another reason for this could be that the non-crispy genotypes included in this study were advanced selections carrying high quality attributes, including high firmness, and that could be influencing the similarity between crispy vs non-crispy textures after storage (because firmness was likely fixed in the non-crispy genotypes). Further, the high rainfall in 2014 could have further influenced results.

Also, it was expected that skin drupelet and receptacle penetration forces of crispy and non-crispy texture would be significantly higher before storage than after storage, but there were no significant interactions between these variables (P=0.6765 for skin drupelet penetration and P=0.7128 for receptacle penetration). One more year of data could help to elucidate this to better understand the different effects of cold storage on crispy and non-crispy genotypes.

Table 11. Split-plot ANOVA, degrees of freedom (DF), and F-test p-value (*P*), values for compression firmness, drupelet skin penetration firmness, and receptacle penetration firmness. Two cold storage treatments, one year, two textures, two replications, 15 genotypes.

Source	DF	Compression *P*	Drupelet *P*	Receptacle *P*
Storage	1	0.0514	0.1430	0.0149
Texture	1	0.1276	0.2929	0.7940
Storage*Texture	1	0.0485	0.6765	0.7128
Genotype (Texture)	13	<.0001	<.0001	<.0001
Storage*Genotype (Texture)	13	<.0001	<.0001	<.0001

Table 12. Least square interaction means of compression force (N) of texture (crispy and non-crispy) by storage (non-storage and storage).

Storage	Texture			
	Crispy		Non-crispy	
Non-storage	10.0[z]	A[y] a[x]	7.7	Bb
Storage	8.8	Ab	7.7	Ab

[z] Values correspond to compression force measured in Newton (N).
[y] Means in the same row followed by the same upper-case letter are not significantly different by least square means, $P \leq 0.05$.
[x] Means in columns and rows followed by the same lower case-letter are not significantly different by least square means, $P \leq 0.05$.

As indicated in Table 11, storage treatments had significant effects on compression values of genotypes depending on their texture. Compression firmness of crispy selection A-2453 was significantly higher than all other crispy selections before storage (Table 13). After 7 d of cold storage, A-2453 was still in the higher range of firmness and was similar to A-2454 and A-1790 (Table 13). For non-crispy genotypes, before storage 'Natchez' and A-2416 were in the higher range of compression firmness, but after 7 d in cold storage the selection A-2252 was the firmest (Table 14). For skin drupelet penetration force before storage, A-2454 had a lower value than all of the other crispy genotypes and similar values were found among most genotypes after storage (Table 15). For non-crispy genotypes, 'Ouachita', A-2417, and A-2252 were in the higher range of skin drupelet firmness before storage, but after storage 'Ouachita' was in the lower range of firmness and A-2252 maintained its high firmness compared to all the other genotypes (Table 16). For receptacle penetration firmness, genotypes A-1790 and A-2453 had the highest firmness compared to the other crispy genotypes, but after 7 d of cold storage these genotypes were similar in receptacle firmness (Table 17). Lastly, A-2418 was the genotype that presented the highest receptacle penetration firmness before storage, but its higher firmness was not maintained after storage, instead genotype A-2428 was the genotype with highest firmness (Table 18). The differences among genotypes of each type of texture within storage treatment was expected due to the significant interaction of storage x genotype (texture), which all had a p-value of <0.0001. In the case of non-crispy genotypes, the effect of storage was very diverse among genotypes for all measured variables, meaning that the effect on firmness of the cold storage treatments was different depending on the particular genotype.

Compression and penetration forces (skin drupelet and receptacle), after storage, of crispy genotypes, did not express high variation among them (in most of the cases there were no

significant differences among them). This could be due to genetic relatedness of these genotypes. Selection A-1790 is a parent of A-2218, and A-2218 is a parent of A-2453 and A-2454 (these last two are siblings) (J. Clark, personal communication). So, it might be expected that the effect of cold storage would be very similar on these selections. This situation that did not occur in the non-crispy genotypes, since there was variation among them for all forces measured. These genotypes were not that closely related as in the case of crispy genotypes. This indicates that there is a large amount of variation within those genotypes that still can be exploited, and if crossed with the crispy genotypes, the improvement in firmness and postharvest potential could be even greater. Also, in some cases the firmness after storage was higher compared to before storage, which can be explained by some level of weight loss and epidermal desiccation during cold storage (although clamshells were within a plastic tub during all storage treatment). A similar situation occurred in ‘Navaho’ at the shiny-black stage (Arkansas cultivar not included in this study) when Perkins-Veazie et al. (1996) did early studies on the effects of postharvest storage on fruit quality for this cultivar and others.

Table 13. Least square means of fruit compression (N) of four non-crispy selections. Non-storage and storage treatments.

Genotype	Fruit compression (N)			
	Non-storage		Storage	
A-2453	12.6	a[z]	9.3	a
A-2454	9.7	b	9.9	a
A-1790	8.6	b	9.9	a
A-2218	8.3	b	6.4	b

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Table 14. Least square means of fruit compression (N) of 11 non-crispy selections, non-storage and storage treatments.

Genotype	Fruit compression (N)			
	Non-storage		Storage	
A-2416	9.8	a[z]	8.1	c
Natchez	9.7	a	7.3	cd
Prime-Ark®45	9.0	ab	6.9	cd
A-2418	9.0	ab	8.2	bc
Osage	8.6	ab	6.2	d
A-2417	7.6	bc	6.6	d
A-2252	7.5	bc	11.3	a
Ouachita	6.6	cd	6.2	d
A-1960	6.5	cd	9.8	b
A-2428	6.5	cd	9.2	b
A-2297	5.9	d	6.6	d

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Table 15. Least square means of skin drupelet penetration (N) of four non-crispy selections. Non-storage and storage treatments.

Genotype	Skin drupelet penetration (N)			
	Non-storage		Storage	
A-2218	0.16	a[z]	0.15	a
A-1790	0.16	a	0.16	a
A-2453	0.15	a	0.14	ab
A-2454	0.13	b	0.14	b

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Table 16. Least square means of skin drupelet penetration (N) of 11 non-crispy selections. Non-storage and storage treatments.

Genotype	Skin drupelet penetration (N)			
	Non-storage		Storage	
Ouachita	0.16	a[z]	0.11	e
A-2417	0.15	a	0.13	cd
A-2252	0.14	ab	0.17	a
A-2418	0.14	bc	0.12	de
A-2416	0.13	bc	0.13	cd
A-1960	0.13	cd	0.15	b
A-2428	0.13	cd	0.13	cd
Prime-Ark®45	0.13	cd	0.12	de
Osage	0.13	cde	0.11	e
Natchez	0.11	de	0.11	e
A-2297	0.11	e	0.14	bc

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Table 17. Least square means of receptacle penetration (N) of four non-crispy selections. Non-storage and storage treatments.

Genotype	Receptacle penetration (N)			
	Non-storage		Storage	
A-1790	0.14	a[z]	0.13	a
A-2453	0.14	a	0.12	a
A-2218	0.12	b	0.12	a
A-2454	0.11	c	0.12	a

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Table 18. Least square means of receptacle penetration (N) of 11 non-crispy selections. Non-storage and storage treatments.

Genotype	Receptacle penetration (N)			
	Non-storage		Storage	
A-2418	0.17	a[z]	0.12	bc
A-2428	0.16	b	0.17	a
A-2416	0.14	bc	0.13	b
A-2252	0.14	bc	0.16	a
A-2417	0.13	cd	0.10	cd
Prime-Ark®45	0.12	de	0.11	cd
Ouachita	0.12	cde	0.10	cd
A-1960	0.12	ef	0.11	bcd
Natchez	0.11	ef	0.11	cd
Osage	0.10	fg	0.10	d
A-2297	0.010	g	0.11	cd

[z] Means in the same column followed by the same letter are not significantly different by least square means, $P \leq 0.05$.

Fruit Morphology

Fruit morphology examination for cellular structure, using the confocal microscope, showed visual differences between crispy and non-crispy genotypes (Figs. 4 to 11). Drupelet mesocarp cells and cell walls of the crispy selections A-2454 and A-2453 were visually differentiated (Figs. 4 and 5, respectively), while 'Natchez' cells and cell walls were not differentiated and appeared to break apart corresponding with its non-crispy texture (Fig. 6). For 'Shawnee' (Fig. 7), cellular structures (cell and cell walls) were observed in the drupelet mesocarp, but cells appeared to have more intercellular space compared to crispy genotypes, suggesting loss of cell-cell adhesion and potentially soft berries. Similar results were found by Atkinson et al. (2012) in apples when fruits of 'Roya Gala' from normal and transgenic plants (which had the endo-polygalacturonase gene, a gene associated with fruit softening in apples suppressed) were compared. This loss of cell-cell adhesion could be due to disintegration of the middle lamella. The middle lamella is a pectin layer that acts as "glue" holding neighboring cells together, which contributes to the overall textural change during fruit ripening (Toivonen and Brummel, 2008). In a study done in red raspberry, the differences in cell wall composition between a soft and a firm cultivar were analyzed (Stewart et al., 2001). It was determined that pectin is one of the key factors determining firmness and it was observed that a reduced level of pectin backbone and methyl ester hydrolysis contributed to firm cultivars being more able to maintain cell wall cohesion (Brummell, 20006; Stewart et al., 2001). Since red raspberry and blackberry are in the same genus, these same processes may be involved in both. In my study, drupelet mesocarps of crispy genotypes maintained their cell walls and cell to cell adhesion during ripening due to the complete integrity of their middle lamella (Figs. 4 and 5). Receptacle cells of the crispy genotypes (Figs. 8 and 9) had more clearly defined tissue structure of cells and

maintained their structure compared to non-crispy genotypes (Figs. 10 and 11), especially for ‘Natchez’ in which it was very difficult to distinguish individual cells.

Softening is primarily associated with changes in the cell wall of parenchyma cells resulting in tissue failure. This failure normally occurs first by cell fracture, then by cell rupture, and finally by cell-to cell de-bonding (Iwanami et al., 2008). Also, it was found by Atkinson et al. (2012) that transgenic apples with suppressed polygalaturonase (PG) enzyme activity had reduced expansion of cells in the hypodermis resulting in a more densely packed cell layer.

Cell turgor also plays a critical role in fruit softening and a reduction in turgor during ripening can also reduce firmness (Toivonen and Brummel, 2008; Winkler et al., 2015). Firmness is determined by the anatomy of the tissue, in particular by cell size, shape, and packing, cell wall thickness and strength, cell-to-cell adhesion, and also by turgor status (Toivonen and Brummel, 2008). Water loss from the tissue can lead to a reduction in turgor, which at the same time produces a reduction in crispiness (Toivonen and Brummel, 2008). Therefore, including turgor measurements to compare crispy and non-crispy genotypes would open another area to explore and could lead to a better understanding of what is happening at the cellular level of crispy blackberry genotypes.

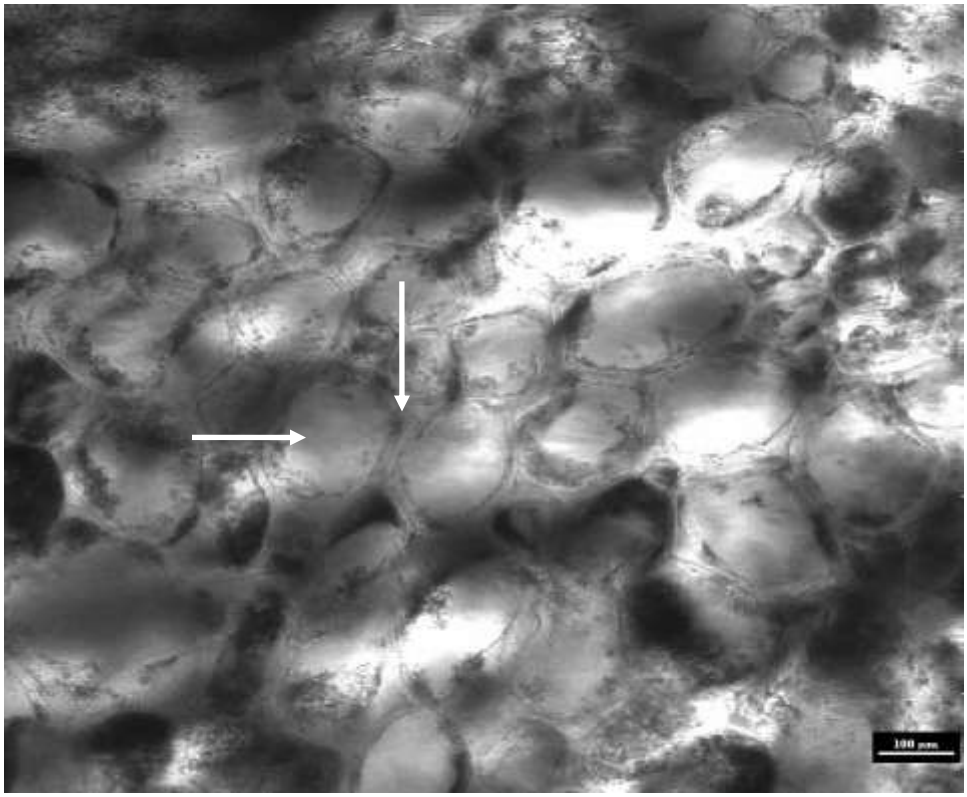

Fig. 4. Ripe drupelet mesocarp image taken by confocal microscope of crispy selection A-2454. Bars = 100 µm. Horizontal arrow indicates individual cell. Vertical arrow indicates cell wall/middle lamella.

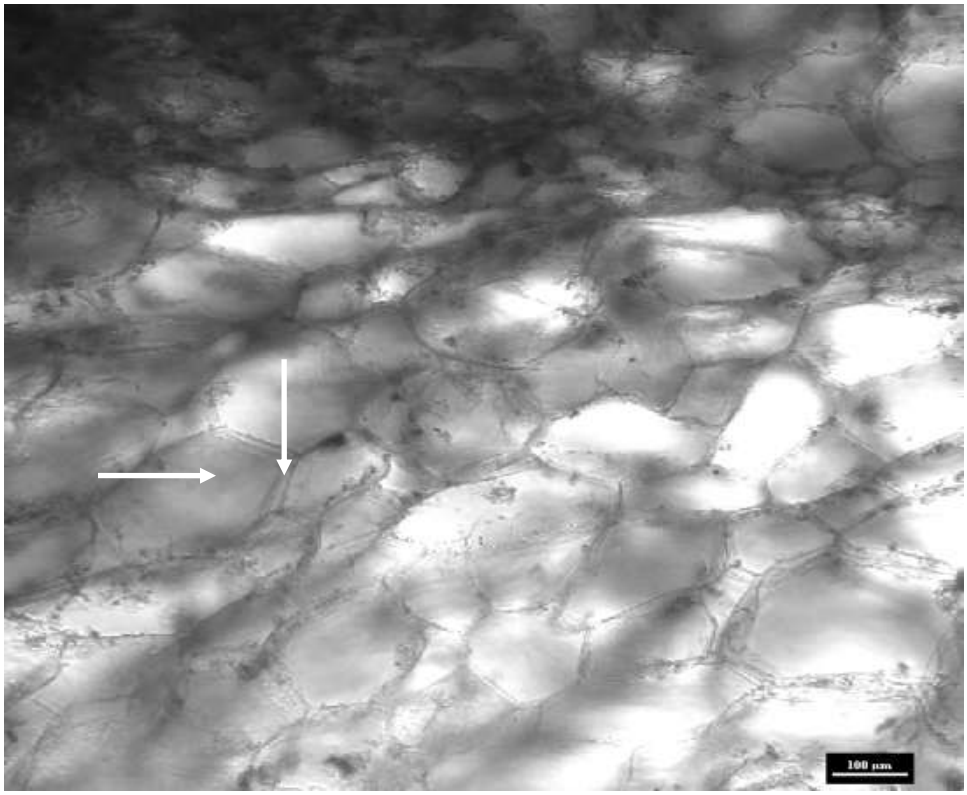

Fig. 5. Ripe drupelet mesocarp image taken by confocal microscope of crispy selection A-2453. Bars = 100 µm. Horizontal arrow indicates individual cell. Vertical arrow indicates cell wall/middle lamella.

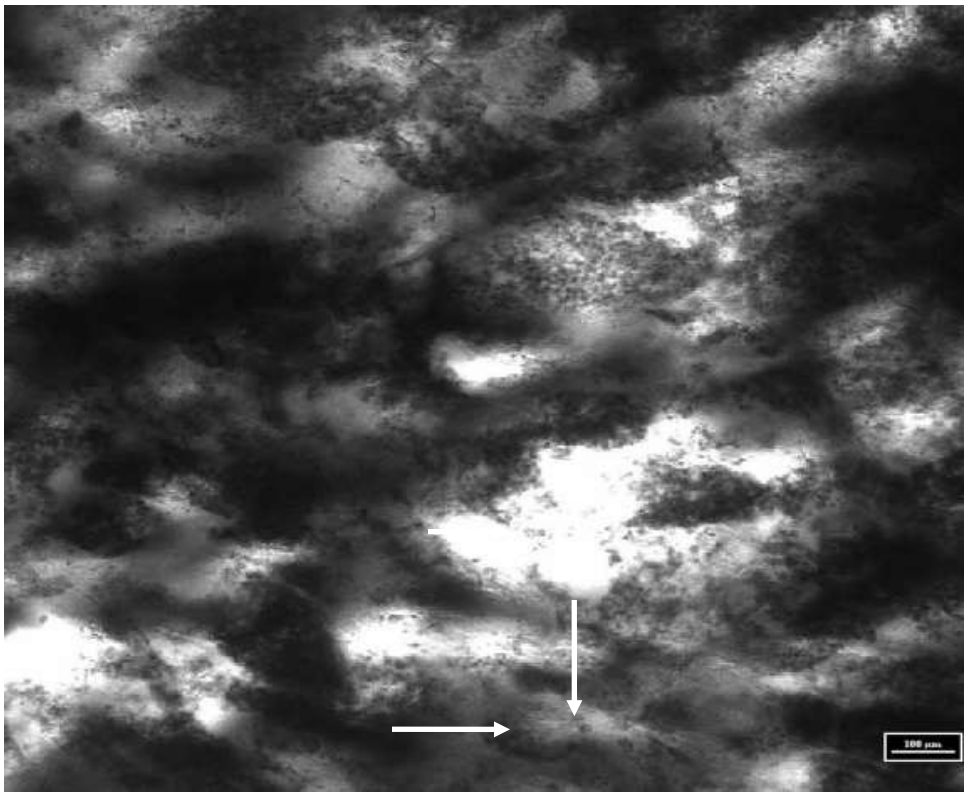

Fig. 6. Ripe drupelet mesocarp image taken by confocal microscope of non-crispy cultivar Natchez. Bars = 100 µm. Horizontal arrow indicates individual cell. Vertical arrow indicates cell wall. Vertical arrow indicates cell wall/middle lamella.

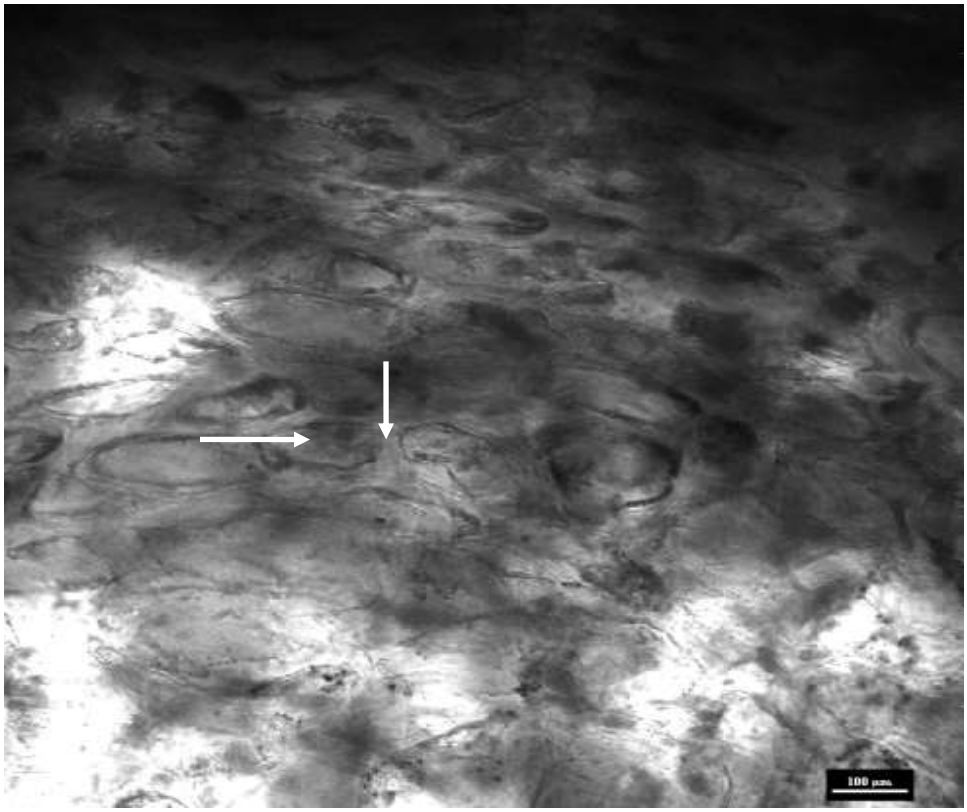

Fig. 7. Ripe drupelet mesocarp image taken by confocal microscope of non-crispy cultivar Shawnee. Bars = 100 µm. Horizontal arrow indicates individual cell. Vertical arrow indicates cell wall/middle lamella.

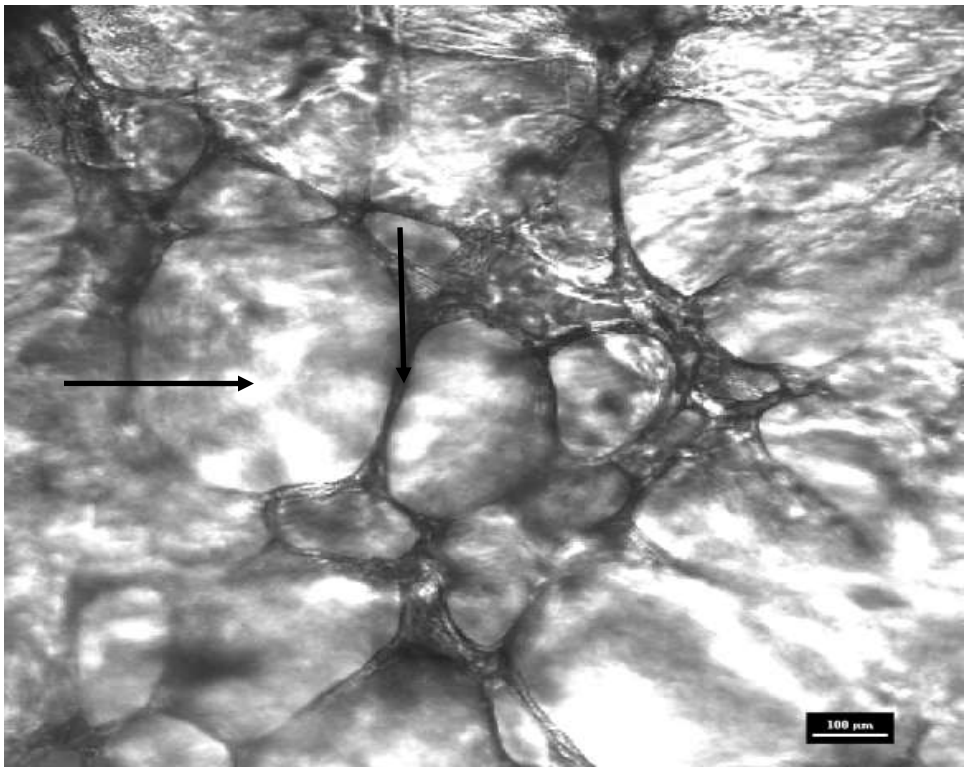

Fig. 8. Ripe receptacle image taken by confocal microscope of crispy selection A-2454. Bars = 100 µm. Horizontal arrow indicates individual cell. Vertical arrow indicates cell wall/middle lamella.

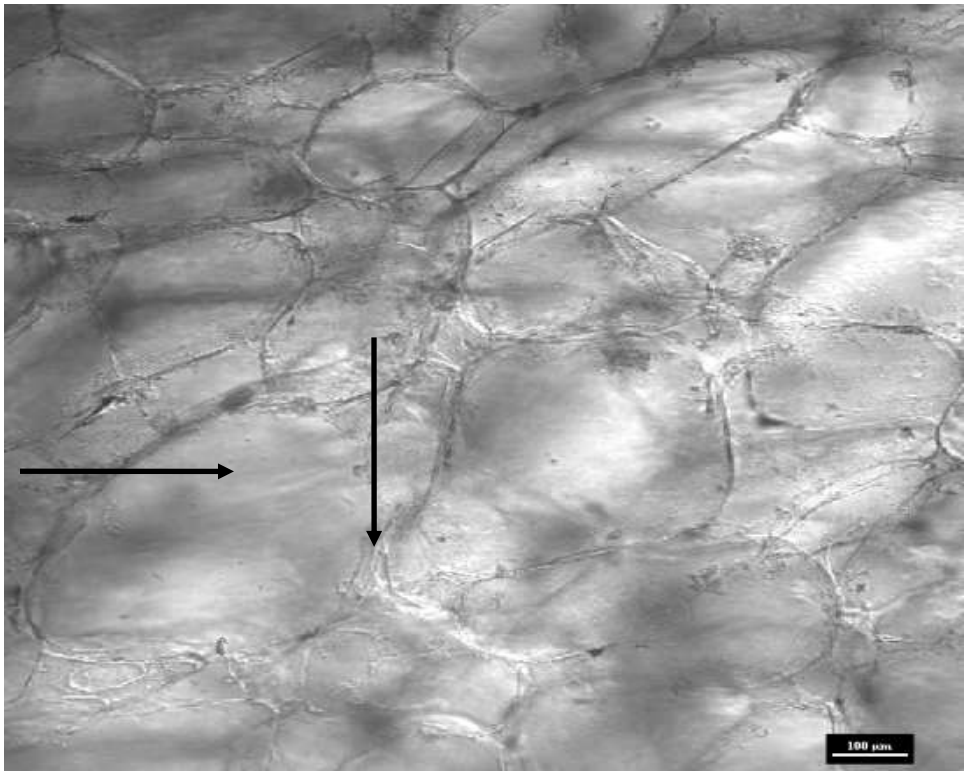

Fig. 9. Ripe receptacle image taken by confocal microscope of crispy selection A-2453. Bars = 100 µm. Horizontal arrow indicates individual cell. Vertical arrow indicates cell wall/middle lamella.

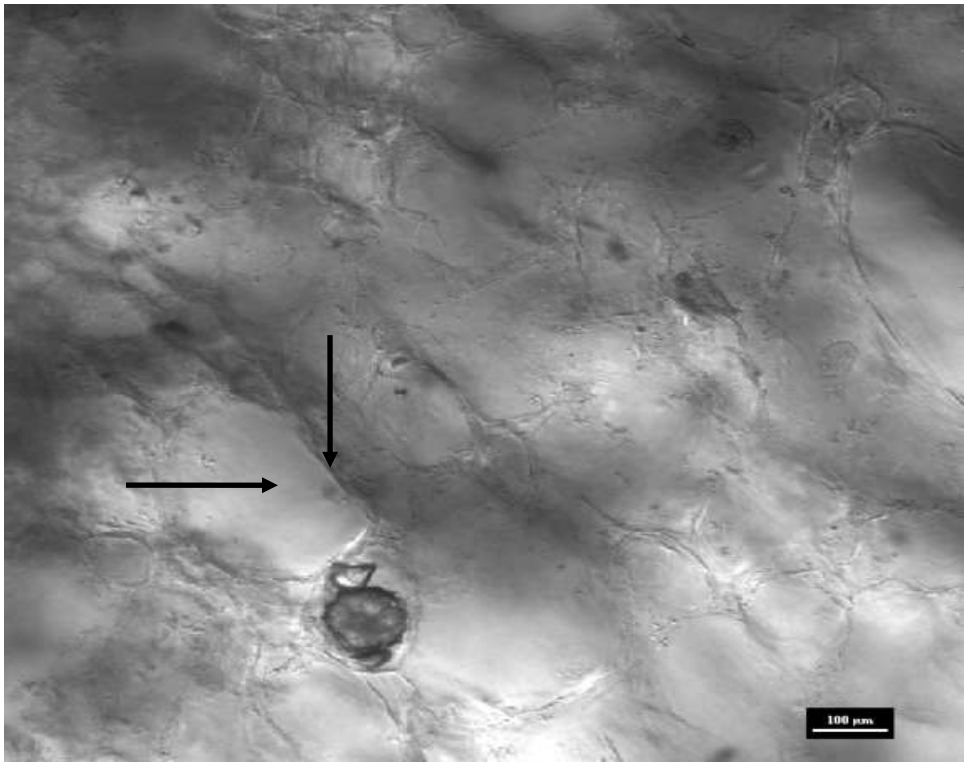

Fig. 10. Ripe receptacle image taken by confocal microscope of non-crispy cultivar Natchez. Bars = 100 µm. Horizontal arrow indicates individual cell. Vertical arrow indicates cell wall/middle lamella.

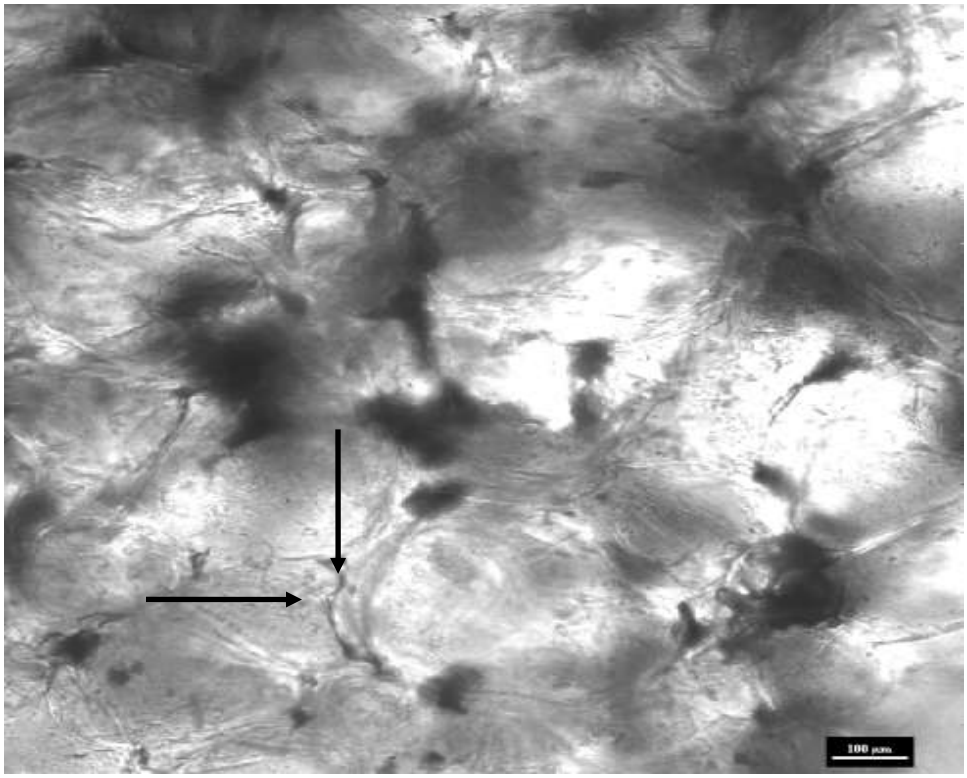

Fig. 11. Ripe receptacle image taken by confocal microscope of non-crispy cultivar Shawnee. Bars = 100 µm. Horizontal arrow indicates individual cell. Vertical arrow indicates cell wall/middle lamella.

Color Reversion

Color reversion is a postharvest disorder with genetic and environmental influences (Clark, 2005; Clark and Finn, 2011) and drupelets of berries expressing this disorder turn red during cold storage or after being in cold storage and exposed to room temperature (Clark and Finn, 2011; Finn and Clark, 2012). Color reversion was evaluated in 2013 and 2014 after one week of cold storage utilizing a multinomial scale. The ANOVA indicated the only significant source of variation was texture (P= 0.0112) and non-significant sources were year, year by texture, genotype (texture), or year by genotype (texture) (Table 19).

For non-crispy genotypes, 28.0% of berries developed a low-to-mid level of color reversion and 13.0% developed a high level of this disorder (Fig. 12). Color reversion value of crispy genotypes was significantly lower than non-crispy genotypes, with 10.1% of the berries in the low-mid level and 3.2 in the high level of development of this disorder. Percentage of fruits with no development or expression of this disorder after storage was 59.0% of non-crispy genotypes and 86.8% for crispy genotypes (data not shown). These results indicate that crispy genotypes have superior postharvest potential compared to non-crispy genotypes due to their low levels of color reversion development.

In a mature fruit cell, approximately 90% of the volume is occupied by the vacuole, a cell organelle that is dynamic and multifunctional and provides the primary site of macromolecule storage and turnover (Fontes et al., 2011). The vacuole accumulates sugars, aromas, flavors, ions, and water; these compounds are transported across the tonoplast (vacuole membrane) by a specific transporter protein (Fontes et al., 2011). When the vacuole membrane is interrupted, its contents can leak into the cytoplasm changing the cytoplasm pH. It is possible that color reversion could be due to a breaking apart of cell membranes and cell wall, but crispy genotypes,

due to their improved structure of cells, maintain the stability of their cell membranes. These genotypes likely have a reduced incidence of acid leakage into the cytoplasm. Previous studies indicated a relationship between high firmness and a decreased development of color reversion in blackberries (Perkins-Veazie et al., 1996).

Table 19. Split-plot ANOVA, degrees of freedom (DF), and F-test p-value (*P*), values for color reversion. Data are from two years, two textures, and 13 genotypes.

Source	DF	*P*
Year	1	0.3354
Texture	1	0.0112
Year*Texture	1	0.2228
Genotype (Texture)	11	0.7620
Year*Genotype (Texture)	7	0.4938

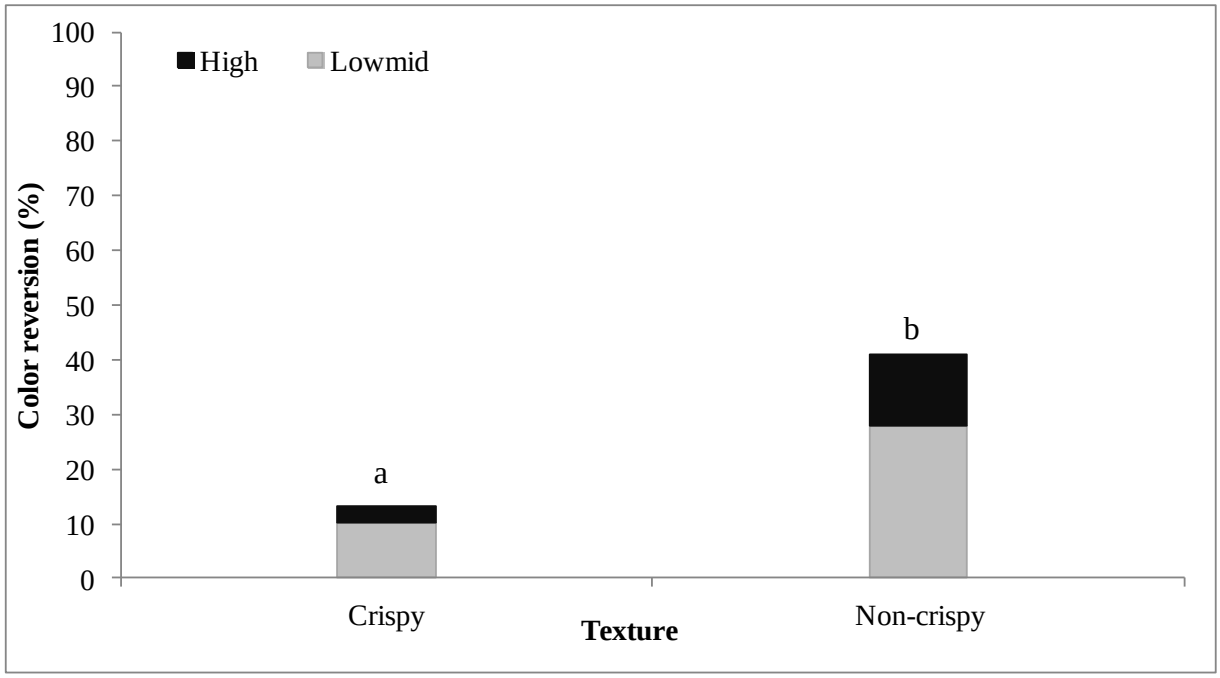

Fig. 12. Percentage of color reversion after one week of cold storage at 5 °C of four crispy and nine non-crispy genotypes, means of years 2013 and 2014. Low to mid: Percentage of berries showing one to three red drupelets after cold storage. High: Percentage of berries having more than four red drupelets after cold storage. Significant difference between textures is indicated by different letters ($P \leq 0.05$).

Conclusions

The objective of this study was to analyze the differences in firmness components, cell structure, and color reversion between crispy and non-crispy genotypes. Crispy genotypes had superior firmness compared to non-crispy genotypes during both years of study. Compression firmness and drupelet skin penetration were the measurements that better represented blackberry firmness in general and also the measurements that better differentiated crispy and non-crispy genotypes. This also indicates these measurements are most appropriate in differentiating firm berries versus the non-firm berries in phenotypic evaluations of for berry firmness. Receptacle penetration was less effective in firmness differentiation, as differences were found only in one year (2013) between crispy and non-crispy genotypes.

After cold storage, no significant differences were observed between stored and non-stored berries within crispy and non-crispy genotype groups with the exception of fruit compression in which case non-stored crispy berries were significantly higher in firmness compared to all the other combinations. To further substantiate these results and gain additional understanding of the effect of storage on crispy genotypes, this experiment should be repeated.

Confocal image analysis of crispy and non-crispy selections/cultivars showed clear differences between both types of textures in cell characteristics. Drupelet mesocarp cells of crispy genotypes maintained their structure during the ripening process and cell walls did not break apart, compared to non-crispy genotypes where cells did not remain intact. Visual evidence indicated that non-crispy genotypes did have loss of integrity of cell walls, likely allowing the sugars and acids to be leaked. This was particularly observed for ‘Natchez’. For ‘Shawnee’, cells appeared to maintain most of their structure during ripening, but cells were observed to separate, likely contributing to loss of firmness.

Color reversion is considered one of the most frequent and not yet understood postharvest disorders of blackberry fruit. An exciting finding was that crispy genotypes after one week of cold storage showed lower levels of color reversion compared to non-crispy genotypes indicating higher postharvest potential.

Literature Cited

Atkinson, R., P. Sutherland, S. Johnston, K. Gunaseelan, D. Mitra, D. Brummell, R. Schröder, J. Johnston, I. Hallett, and R. Schaffer. 2012. Down-regulation of Polygalacturonase1alters firmness, tensile strength and water loss in apple (*Malus x domestica*) fruit. BMC Plant Biol.12:129-141.

Brummel, D. 2006. Cell wall disassembly in ripening fruit. Functional Plant Biol. 33:103-119.

Clark, J. R. 2005. Intractable traits in eastern U.S. blackberries. HortScience 40:1954-1955.

Clark, J. R. and C. E. Finn. 2011. Blackberry breeding and genetics. Fruit, Vegetable and Cereal Science and Biotechnology 5 (Special Issue 1) Global Science Books:27-43.

Clark, J. R. and C. E. Finn. 2014. Blackberry cultivation in the world. Rev. Bras. Frutic. 36:46-57.

Clark, J.R. and J. N. Moore. 2008. 'Natchez' thornless blackberry. HortScience 43:1897-1899.

Clark, J. R. and P. Perkins-Veazie. 2011. 'APF-45' Primocane-fruiting blackberry. HortScience 46:670-673.

Clark, J R., E. Stafne, H. Hall, and C. E. Finn. 2007. Blackberry breeding and genetics. Plant Breeding Rev., Timber Press, Portland, Ore. 29:19-144.

Finn, C.E., and J. R. Clark. 2012. Blackberry, p. 151-190. In: M. Badenes and D. Byrne (eds). Fruit breeding. Springer Science + Business Media, New York.

Fontes N., H. Geron, and S. Delrot. 2011. Grape berry vacuole: a complex and heterogeneous membrane system specialized in the accumulation of solutes. Amer. J. Enol. Viticul. 62:270-279.

Iwanami, H., S. Moriya, N. Kotoda, S. Takahashi, and K. Abe. 2008. Estimations of heritability and breeding value for postharvest fruit softening in apple. J. Amer. Soc. Hort. Sci. 133:92-99.

Marondedze, C. and L. Thomas. 2013. Genes and quality trait loci (QTLs) associated with firmness in *Malus x domestica*. African J. Biotechnol. 12:996-1003.

Perkins-Veazie, P., J.K. Collins, and J. R. Clark. 2000a. Shelf-life and quality of 'Navaho' and 'Shawnee' blackberry fruit stored under retail storage conditions. J. Food Qual. 22:535-544.

Perkins-Veazie, P., J. R. Clark, D. Huber, and E. Baldwin. 2000b. Ripening physiology in 'Navaho' thornless blackberries: Color, respiration, ethylene production, softening, and compositional changes. J. Amer. Soc. Hort. Sci. 125:357–363.

Perkins-Veazie, P., J. Collins, and J. R. Clark. 1996. Cultivar and maturity affect postharvest quality of fruit from erect blackberries. HortScience 31:258-261.

Perkins-Veazie, P., J. Collins, and J. R. Clark. 1999. Cultivar and storage temperature effects on the shelflife of blackberry fruit. Fruit Var. J. 53:201-208.

SAS institute Inc., 2013. SAS® 9.4. Cary, NC.

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

Stewart, D., P. Ianneta, and H. Davies. 2001. Ripening-related changes in raspberry cell wall composition and structure. Phytochemistry 56:423-428.

Stephens, M., P. Alspach, R. Beaton, C. Winefield, and E. Buck. 2012. Genetic parameters and development of a selection index for breeding red raspberries for processing. J. Amer. Soc. Hort. Sci. 137:236-242.

Strik, B., J. R. Clark, C. E. Finn, and M. Bañados. 2007. Worldwide production of blackberries. Acta Hort. 777:209-217.

Stommel, J., J. Abbot, T. Campbell, and D. Francis. 2005. Inheritance of elastic and viscoelastic components of tomato firmness derived from intra- and interspecific genetic backgrounds. J. Amer. Soc. Hort. Sci. 130:598-604.

Toivonen, P. and D. Brummel. 2008. Biochemical bases of appearance and texture in fresh-cut fruit and vegetables. Postharvest Biol. Tech. 48:1-14.

Winkler A., M. Ossenbrink, and M. Knoche. Malic acid promotes cracking of sweet cherry fruit. J. Amer. Soc. Hort. Sci. 140:280-287.

Chapter 6

INHERITANCE OF THE FIRMNESS AND CRISPY TRAIT IN BLACKBERRIES IN THE BLACKBERRY BREEDING PROGRAM OF THE UNIVERSITY OF ARKANSAS

Abstract

Flesh firmness of blackberry (*Rubus* subgenus *Rubus* Watson) fruits is critical for successful postharvest handling. Therefore, this trait is a priority in the development of new cultivars in breeding programs. The University of Arkansas (UA) blackberry breeding program has released several cultivars with excellent postharvest quality for the fresh and shipping industries. Fruits having a crispy texture and exceptional firmness have been identified in the program. Two Arkansas selections carry this trait, A-2453 and A-2454. They were used in 2011 in crosses with other Arkansas selections with the intention of increasing firmness in progeny. Another objective for these crosses was to evaluate the inheritance of firmness. Fruit firmness of five populations (ArPop_1145, ArPop_1146, ArPop_1147, ArPop_1148, and ArPop_1151) was measured. All populations were crosses of firm x crispy parents. In 2013, four to 27 plants per population were evaluated, and in 2014, 30 to 37 plants were evaluated per population. Fruit compression, skin penetration, and drupelet penetration forces were measured. For penetration and compression, 1.0 mm and 7.2 cm cylinder probes were utilized, respectively. Broad sense inheritance values were lower than expected ranging from 0.018 for fruit compression and receptacle penetration to 0.034 for skin drupelet penetration. Fruit compression values indicated that in 2013 there was no population that averaged higher values than the mid-parent value, with the exception of ArPop_1147; and in 2014, only ArPop_1145 exceeded the mid-parent value. Skin drupelet penetration mean value of populations ArPop_1145 and ArPop_1151 exceeded

their mid-parent values in 2013; no populations exceeded the mid-parent values in 2014. Mean value receptacle penetration of populations ArPop_1145 and ArPop_1147 exceeded their mid-parent values in 2013. No progeny were measured to be in the "soft" category due to all parents being firm and/or crispy. Skin and receptacle penetration firmness measurement values were less consistent and values did not parallel the phenotype of the parents. Compression force values better explained the firmness of a genotype compared to penetration values.

Introduction

Fresh-market and shipping industries base their success primarily on the quality of blackberry fruits after a period of cold storage. The main goal is that fruit must maintain its high quality characteristics during the entire commercialization process. The quality of fruits for the fresh market is determined by how the genotype responds to storage and handling from the day of harvest until purchased and eaten by the consumer (Finn and Clark, 2012). Blackberries have been considered one of the more difficult fruits to ship due to softening and leakage during postharvest storage (Clark, 2005). Increased firmness during shelf-life has been achieved in new blackberry cultivars, but advances have been challenging and firmness is considered an intractable trait (Clark, 2005).

One of the challenges for blackberry breeders have been to combine different traits such as high sweetness with flavor components in shiny-black and firm berries (Clark and Finn, 2011). High fruit firmness was critical to develop a commercial shipping industry for this crop (Clark and Finn, 2011). Fruit firmness is considered a quantitative trait in other fruits such as tomato (*Solanum lycopersicum* L.) (Stommel et al., 2005) and apple (*Malus x domestica* Borkh.) (Marondedze and Thomas, 2013). Inheritance values of flesh firmness and fruit softening vary

with species. For example apple narrow sense heritability value for fruit softening was moderate (h^2=0.55) in the study done by Iwanami et al. (2008) and high in tomato (h^2=0.94) in the study done by Stommel et al. (2005).

Modifications of the polysaccharide components of the primary cell wall and middle lamella during fruit ripening can result in a weaker structure at the end of the process (Brummell, 2006). Alteration in the bonding between polymers along with degradation of polysaccharide could cause an increase in cell separation and softening and swelling of the cell wall; this combined with alterations in cell turgor causes fruit softening and textural changes (Brummell, 2006). In particular, some fruits such as blackberries increase their pectin solubility activity during ripening (Brummell, 2006).

Fruit skin firmness depends on cultivar, ripeness stage, and storage duration (Perkins–Veazie et al., 1996) and is a critical characteristic in postharvest evaluation. Several years ago it was found within the UA breeding program a floricane-fruiting, thorny plant bearing fruit with a crispy texture, the selection was coded as A-1790 (Clark, 2005). Fruits of this selection not only had this crispy texture, they also showed increased firmness compared to all other selections in the program. This selection was utilized in crosses and years later another floricane-fruiting thorny plant with improved fruit size was selected (A-2218). The use of these selections in different crosses in the UA program led to the transfer of this trait into two floricane-fruiting thornless selections having an improved fruit size and slightly higher yield compared to the previous crispy selections (Clark, personal communication). This trait is of interest because its higher firmness has showed an increased postharvest life after cold storage, which can be an advantage for growers and shippers. It has been observed that this trait can be inherited, but more research is necessary to confirm and quantify this inheritance.

The objective of this study was to determine the heritability of this firm and crispy trait.

Materials and Methods

Plant Material

All fruit collection and firmness measurements were conducted at the University of Arkansas Fruit Research Station, Clarksville [west-central Arkansas (west-central Arkansas, lat. 35°31’58’’N and long. 93°24’12’’W; U.S. Dept of Agriculture (USDA) hardiness zone 7a; soil type Linker fine sandy loam (Typic Hapludult)]. Blackberry plants were grown with cultural components including annual routine plant management practices such us fertilization, weed control, and irrigation. All plantings received a single application of liquid lime sulfur (94 L≅ha^{-1}) at budbreak for control of anthracnose [*Elsinoë veneta* (Burkh.) Jenkins] (Smith, 2015). This was the only fungicide applied to any plantings in any year. Raspberry crown borer (*Pennisetia marginata* Harris) was controlled by a single application of a labelled insecticide in October of each year (Smith, 2015). Insecticides labelled for commercial use in Arkansas were used for spotted-wing drosophila (*Drosophila suzukii* Matsumura) control. Individuals analyzed were the parental genotypes A-2218, A-2453, A-2454, A-2417, A-2416, A-2297, and A-2428. Seedlings from the Arkansas breeding program available to be used (it was not possible to measure all seedlings in some populations due to limited or no crop, thus seedling number was determined prior to berry harvest and the beginning of measurement initiation) included 30-37 seedlings from populations ArPop_1145 (A-2297 x A-2454), ArPop_1146 (A-2416 x A-2453), ArPop_1147 (A-2416 x A-2454), ArPop_1148 (A-2417 x A-2454), and ArPop_1151 (A-2428 x A-2453). Data was taken during the floricane-fruiting seasons in 2013 and 2014.

Phenotypic Evaluation

In 2013 and 2014, on harvest days, fruits were picked before noon to avoid high temperatures at the shiny-black maturity stage. A minimum of 10 fruits per seedling were evaluated for fruit compression firmness and a minimum of 10 fruits were evaluated for skin drupelet and receptacle penetration firmness. Fruits were transported immediately to the laboratory for firmness measurement. In 2013, evaluations were done between 20 June and 17 July and in 2014 between 17 June and 12 July. Fruit Firmness was measured utilizing two different methodologies:

1. Compression: Fruit compression was performed by placing individual fruits horizontally on a flat surface using a cylindrical and plane probe of 7.6 cm diameter (iCon Texture Analyzer, Texture Technologies Corp. Hamilton, MA) (Fig. 1).

2. Penetration: Each fruit was cut in half longitudinally. One half was used for drupelet penetration and the other half to measure the receptacle firmness (iCon Texture Analyzer, Texture Technologies Corp. Hamilton, MA).

a. Drupelet penetration: Drupelet skin firmness was assessed using a probe of 1 mm diameter. For this, three drupelets of similar shape and size were measured per berry (Fig 2).

b. Receptacle penetration: Measured using a probe of 1 mm diameter in the middle of the receptacle (Fig. 3).

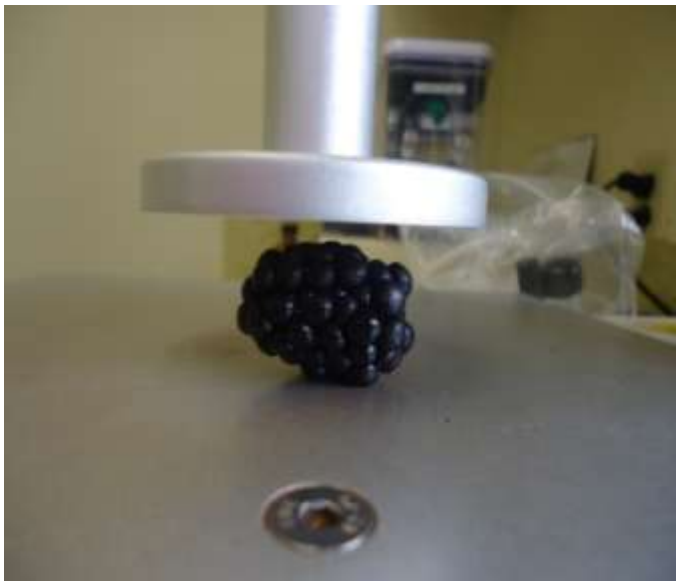

Fig. 1. Fruit compression measurement procedure utilizing a flat surface using a cylindrical and plane probe of 7.6 cm diameter.

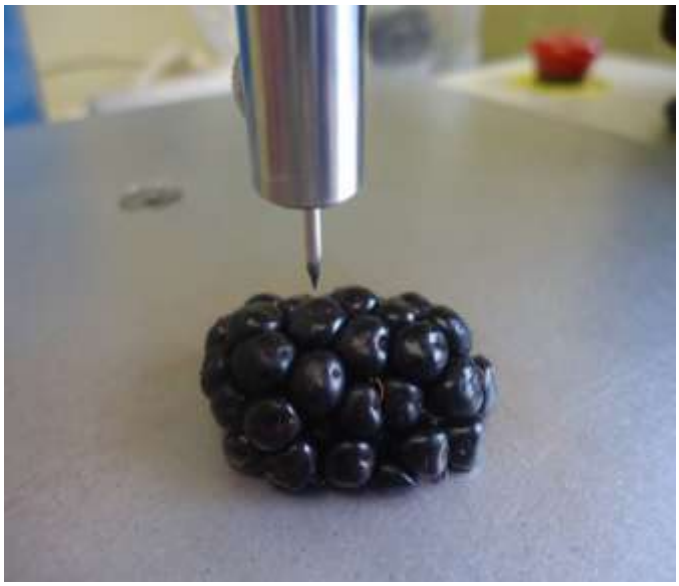

Fig. 2. Skin drupelet penetration measurement procedure utilizing a probe of 1 mm diameter.

Fig. 3. Receptacle penetration measurement procedure utilizing a probe of 1 mm diameter.

Data Analysis

Genetic (σ^2_G), environment (year) variance(σ^2_Y), genetic x environment(σ^2_{GY}), and residual variance ($\sigma^2_{residual}$) variances were estimated using a restricted maximum likelihood (REML) with all random effects. To calculate the variance components PROC VARCOMP was utilized (SAS® 9.4. Cary, NC). The PROC GLIMIX procedure was utilized (SAS® 9.4. Cary, NC) to calculate least square means test for mean comparisons ($P \leq 0.05$).

Broad sense heritability estimates of fruit compression and penetration firmness were calculated as:

$$H^2 = \frac{\sigma^2_G}{\sigma^2_G + \frac{\sigma^2_{GxY}}{e} + \sigma^2_{residual}}$$

Where,

σ^2_G = estimated genetic variance

σ^2_Y = estimated year variance

σ^2_{GxY} = estimated genetic x enviroment variance

$\sigma^2_{residual}$ = estimated residual variance

e = number of environments (year). In this case, Y = 2

Mid-parent values were calculated by averaging the mean firmness value (compression, skin drupelet and receptacle penetration) of the corresponding parents of each cross.

Results and Discussion

Analysis of variance was calculated for fruit compression and skin drupelet and receptacle penetration firmness (Table 1). Calculated broad sense heritability (H^2) using the estimated variance for fruit compression was 0.018, for drupelet skin penetration was 0.034, and for receptacle penetration was 0.018. These values are lower than expected since it has been reported in the literature heritability values for firmness of 0.54 in red raspberry (*Rubus idaeus* L.) and 0.55 in apple (Iwanami et al., 2008; Stephens et al., 2012). Apple is an inter-specific hybrid that behaves as a diploid (Brown, 2012) and raspberry is a diploid species (Kempler et al., 2012) while blackberry is tetraploid (Finn and Clark, 2012), and this difference in ploidy level could explain in part the lower values of inheritance. A population of tetraploids contains a potential double number of copies of each allele compared to a population of similar size of a diploid species (Meirmans and Tienderen, 2013). The previous statement means, that in tetraploid species, it will take a higher number of "firm" alleles to fix the locus compared to a diploid species, that normally take one allele (if the trait is dominant for the locus) or two alleles (if the trait is recessive for the locus). If one considers that firmness is a quantitative trait, multiple genes can influence the trait. Further, the populations used in this study were crosses of firm by crispy parents, so it is possible that the firmness locus was already largely fixed in these crosses, thus segregation of this trait was low. This behavior can be experienced in quantitative traits when no new variation for a trait is introduced in the studied germplasm. After a certain number of generations, the loci and alleles at each locus for that particular trait can become fixed and there is limited to no segregation (Bernardo, 2010). Finally, population sizes were rather small for this study, and larger numbers of individuals measured could have provided higher heritability values.

Year effect was significant ($P \leq 0.05$) for skin drupelet and receptacle penetration, but not for fruit compression (Table 1). Population (genetic effect) was significant only for fruit compression and year x population interaction was significant for the three forces measured (Table 1). Additive inheritance was not possible to calculate due to the experimental design of this study in which no particular mating design was utilized (such as a partial or complete diallel). The crosses were simply those of firm x crispy parents.

In 2014, skin drupelet and receptacle penetration means were significantly lower compared to 2013 when averaged across all individuals measured; and in the case of fruit compression, 2013 values were not significantly different to 2014 (Table 2). Fruit firmness is a quantitative trait in which the environment has a great impact (Clark, 2005). In the case of blackberry, rain has a negative impact on firmness, making fruits softer than normal which reduces berry shelf-life and shipping-quality (Clark, 2005). Rain during summer is a common phenomenon in Arkansas which can occur during blackberry fruiting season. In 2013, only one rainy day was registered during the sampling period (20 June to 17 July) with a total of 0.2 cm of rain. In 2014, the number of rainy days during the sampling period (17 June to 12 July) was 7 d with a total of 7.8 cm of rain (data from Fruit Research Station weather station). Therefore, during 2014 the number of days with rain was seven times higher compared to 2013 and the amount of rainfall was 39 times higher than in 2013, which is likely related to the lower firmness measured in populations during 2014.

Other models were tried to test if inheritance values would differ from those presented here. However, results were similar to those described preciously. The other models tested were:
1. Calculation of inheritance by utilizing only 2014 firmness data, so no year x population interaction.

2. Calculation of inheritance by utilizing only data of plants that fruited both years. So, in this model a reduced number of plants were utilized, but year x population interaction was possible to calculate.

Table 1. Analyses of variance of fruit compression, drupelet skin penetration, and receptacle penetration, years 2013 and 2014.

Source of variation	Analysis of variance		
	Fruit compression	Drupelet penetration	Receptacle penetration
Year	0.15	0.0026*	0.013*
Population	0.27*	0.0002	0.0001
Year x population	0.11*	0.0012*	0.0009*
Residual	14.9	0.005	0.005
Total	15.5	0.009	0.018

* Significant at $P \leq 0.05$.

Table 2. Least square means of fruit compression, skin drupelet penetration, and receptacle penetration, years 2013 and 2014.

Year	Fruit compression (N)	Skin drupelet penetration (N)	Receptacle (N)
2013	9.5 a[z]	0.20 a	0.29 a
2014	8.9 a	0.12 b	0.13 b

[z] Means in the same column followed by the same letter not significantly different, $P \leq 0.05$.

Population and parental means per year and by type of force were calculated (Tables 3 to 5). Population ArPop_1147 was significantly higher in compression firmness (10.8 N) compared to ArPop_1145, ArPop_1146, and ArPop_1151 in 2013. This population was significantly higher, with a value of 9.6 N, compared to ArPop_1146, ArPop_1146, and ArPop_1148 among the two years (Table 3). Fruit compression force ranged from 10.8 N (ArPop_1147, 2013) to 8.2 N (ArPop_1146, 2013). Population ArPop_1145 for 2013 averaged higher skin force and was significantly higher than all other populations in both years (Table 4). However, it was not different from all other 2014 means when compared within this year only. This force ranged from 0.29 N (ArPop_1145, 2013) to 0.11 N (ArPop_1151, 2014), with 2013 averaging overall higher values than 2014. Receptacle penetration ranged from 0.35 N (ArPop_1147, 2013) to 0.11 N (ArPop_1145, 2014) (Table 5). Population ArPop_1147 in both years was significantly higher than all other populations, including this population's value in 2014.

Fruit compression values of populations averaged almost similar or lower than their respective mid-parent values, with the exception of ArPop_1145 in 2014 and ArPop_1147 in 2013 (Table 3). Similar findings for skin drupelet penetration were observed as there were only two populations exceeding their respective mid-parent (ArPop_1145 and ArPop_1151 in 2013) values in both years of study (Table 4). In the case of receptacle penetration, populations ArPop_1147 and ArPop_1145 in 2013 exceeded their respective mid-parent values (in 2014 these populations averaged receptacle penetration values that were similar to their mid-parent values). The other populations averaged similar values compared to their mid-parent values (Table 5). These results parallel the low inheritance values obtained for the firmness measurements in this study.

Table 3. Least square means of fruit compression of populations ArPop_1145, ArPop_1146, ArPop_1147, ArPop_1148, and ArPop_1151 and their mid-parent values, years 2013 and 2014.

Population	Year	Fruit compression (N)	
		Population	Mid-parent
ArPop_1147	2013	10.8 a[z]	10.2
ArPop_1148	2013	10.1 ab	10.3
ArPop_1147	2014	9.6 bc	10.0
ArPop_1151	2013	9.3 cd	10.2
ArPop_1151	2014	9.1 cd	9.8
ArPop_1145	2013	8.8 cd	9.2
ArPop_1148	2014	8.8 d	8.9
ArPop_1145	2014	8.7 d	8.2
ArPop_1146	2014	8.5 d	11.0
ArPop_1146	2013	8.2 d	11.4

[z] Means in the same column followed by the same letter not significantly different, $P \leq 0.05$.

Table 4. Least square means of skin drupelet penetration of populations ArPop_1145, ArPop_1146, ArPop_1147, ArPop_1148, and ArPop_1151 and their mid-parent values, years 2013 and 2014.

Population	Year	Skin drupelet penetration force (N)	
		Population	Mid-parent
ArPop_1145	2013	0.29 a[z]	0.26
ArPop_1148	2013	0.19 b	0.20
ArPop_1151	2013	0.19 b	0.18
ArPop_1147	2013	0.18 b	0.19
ArPop_1146	2013	0.15 bc	0.17
ArPop_1148	2014	0.14 c	0.16
ArPop_1145	2014	0.13 c	0.14
ArPop_1147	2014	0.13 c	0.14
ArPop_1146	2014	0.11 c	0.16
ArPop_1151	2014	0.11 c	0.16

[z] Means in the same column followed by the same letter not significantly different, $P \leq 0.05$.

Table 5. Least square means of receptacle penetration of populations ArPop_1145, ArPop_1146, ArPop_1147, ArPop_1148, and ArPop_1151 and their mid-parent values, years 2013 and 2014.

Population	Year	Receptacle penetration force (N)	
		Population	Mid-parent
ArPop_1147	2013	0.35 a[z]	0.30
ArPop_1145	2013	0.32 a	0.28
ArPop_1148	2013	0.28 b	0.28
ArPop_1151	2013	0.25 b	0.24
ArPop_1146	2013	0.23 b	0.26
ArPop_1147	2014	0.14 c	0.13
ArPop_1146	2014	0.13 d	0.14
ArPop_1148	2014	0.13 d	0.13
ArPop_1151	2014	0.12 d	0.15
ArPop_1145	2014	0.11 d	0.11

[z] Means in the same column followed by the same letter not significantly different, $P \leq 0.05$.

Conclusions

Blackberry firmness is a trait of importance for breeders, growers, shippers, and consumers. Genotypes carrying a unique crispy and firm texture were selected within the UA blackberry breeding program several years ago with the aim to incorporate this trait into new generations of seedlings to improve the fruit and shipping quality of the future cultivar releases. Calculations of fruit blackberry firmness inheritance were done on five Arkansas populations for two years. These populations were crosses of a firm, crispy parent by a firm, non-crispy, parent. Firmness measurements were fruit compression, skin drupelet penetration, and receptacle penetration. Inheritance was low for all firmness measurements, indicating that obtaining individuals of superior firmness could take several generations and large populations would likely be needed to allow for segregation of substantially firmer progeny. Despite the low values of inheritance, firmness can be improved in blackberry, because some populations showed mean values higher than their mid-parent values in all types of forces measured. Also, in the field it has been observed that seedlings of a cross of one or two high firmness parents can have higher firmness than their parents (J. Clark, personal observation). Rain has a negative impact on blackberry quality traits and fruit firmness is highly impacted. Firmness was lower in 2014, compared with 2013 results, and that can be explained in part by the higher amount of rain during the 2014 harvest period.

To obtain better results of blackberry firmness inheritance, this study should be repeated using an improved mating design and/or repeat it in a location where rain during the harvest period will not affect the trait under study. Also, population(s) of a firm parent by a soft parent should be utilized to increase the trait segregation. Finally, larger population sizes should be utilized.

Literature Cited

Bernardo, R. 2010. Mapping quantitative trait loci, p. 85-112. In R. Bernardo (ed.). Breeding for quantitative traits in plants. Stemma Press, Woodbury, MN.

Brown, S. 2012. Apple, p. 329-367. In: M. Badenes and D. Byrne (eds). Fruit breeding. Springer Science + Business Media, New York.

Brummel, D. 2006. Cell wall disassembly in ripening fruit. Functional Plant Biol. 33:103-119.

Clark, J. R. 2005. Intractable traits in eastern U.S. blackberries. HortScience 40:1954-1955.

Clark, J. R. and C. E. Finn. 2011. Blackberry breeding and genetics. Fruit, Vegetable and Cereal Science and Biotechnology 5 (Special Issue 1) Global Science Books:27-43.

Finn, C.E., and J. R. Clark. 2012. Blackberry, p. 151-190. In: M. Badenes and D. Byrne (eds). Fruit breeding. Springer Science + Business Media, New York.

Iwanami, H., S. Moriya, N. Kotoda, S. Takahashi, and K. Abe. 2008. Estimations of heritability and breeding value for postharvest fruit softening in apple. J. Amer. Soc. Hort. Sci. 133:92-99.

Kempler, C., H. Hall, and C. E. Finn. 2012. Raspberry, p. 263-304. In: M. Badenes and D. Byrne (eds). Fruit breeding. Springer Science + Business Media, New York.

Marondedze, C. and L. Thomas. 2013. Genes and quality trait loci (QTLs) associated with firmness in *Malus x domestica*. African J. Biotechnol. 12:996-1003.

Meirmans P. and P. Tienderen. 2013. The effects of inheritance in tetraploids on genetic diversity and population divergence. Hered. 110:131-137.

Perkins-Veazie, P., J. Collins, and J. R. Clark. 1996. Cultivar and maturity affect postharvest quality of fruit from erect blackberries. HortScience 31:258-261.

SAS institute Inc., 2013. SAS® 9.4. Cary, NC.

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

Stephens, M, P. Alspach, R. Beaton, C. Winefield, and E. Buck. 2012. Genetic parameters and development of a selection index for breeding red raspberries for processing. J. Amer. Soc. Hort. Sci. 137:236-242.

Stommel, J., J. Abbot, T. Campbell, and D. Francis. 2005. Inheritance of elastic and viscoelastic components of tomato firmness derived from intra- and interspecific genetic backgrounds. J. Amer. Soc. Hort. Sci. 130:598-604.

CONCLUSION

These studies focused on understanding the genetics underlying the traits of flesh texture and flesh acidity in the Arkansas peach breeding program and in understanding the trait of flesh texture and flesh firmness in the Arkansas blackberry breeding program. The objectives included the implementation of this new knowledge in both breeding programs.

Quantitative trait loci identification is the first step to apply marker-assisted breeding (MAB) for traits that are critical for breeding programs, such as fruit acidity and firmness in the case of the Arkansas peach breeding program. Genome-wide QTL analysis for fruit TA and pH were consistent across years and were co-localized on the proximal end of LG 5 (0.7-3.0 cM), a location that coincides with QTLs previously found by other authors who used a bi-parental design. This means that the use of DNA tests, flanking that particular region of LG 5, will facilitate the application of MAB for TA and pH. This locus, called D-locus, has been studied and several markers have been mapped, one of them is the CPPCT040 SSR marker that in previous studies has been associated with low and high TA levels. This DNA test was applied in the Arkansas peach breeding program with the objective to associate its allelic combinations with different values of TA. The allelic combinations DD and Dd were more abundant and were associated with low levels of acidity. Conversely, homozygous dd individuals were found at a lower frequency within the analyzed material and were associated with the higher levels of TA.

Also, QTLs for flesh firmness were located on the same region of which a major gene for flesh texture is located (endoPG) and on the same region where the new DNA test for distinguishing slow-melting flesh (SMF) texture is located. When this new DNA test was utilized together with the endoPG-6 DNA test, the four flesh textures present in the Arkansas peach breeding program were differentiated (QMF, SMF, non-melting flesh, non-softening flesh) on a

molecular level, indicating that these tests are able to predict in juvenile trees the type of flesh texture of adult plant.

These results are important and provide interesting highlights about QTL analysis in the ongoing Arkansas peach breeding program. The confirmation or discovery of new QTLs will facilitate the design of DNA tests flanking the genomic region of the QTL, which after validation will make possible the application of molecular tools like MAB in the form of marker-assisted parent selection (MAPS) and marker-assisted seedling selection (MASS) increasing the efficiency of the breeding process. This type of data will complement the information that the breeder requires to make informative decisions in the different steps of a breeding program. Efficiency of this and other peach breeding programs will be increased by the application of MAB to select seedling plants for superior traits a few days or weeks after germination, allowing determination of high or low acidity and different textures. Also, by using the DNA information from advanced selections and cultivars, the optimum parents can be chosen for crosses and designed to obtain offspring that carry only the desired alleles. This will reduce land use, program cost, and work hours necessary to grow the plants that will be discarded later in the breeding process.

In blackberries, crispy genotypes expressed superior flesh firmness compared to non-crispy genotypes during both years of study. Compression firmness and drupelet skin penetration were the measurements that better represented blackberry firmness in general and also the measurements that better differentiated crispy and non-crispy genotypes. After cold storage, no significant differences were observed between stored and non-stored berries within crispy and non-crispy genotype groups with the exception of fruit compression in which case non-stored crispy berries were significantly higher in firmness compared to all the other combinations. An

exciting finding was that crispy genotypes after one week of cold storage showed lower levels of color reversion compared to non-crispy genotypes indicating higher postharvest potential.

Drupelet mesocarp cells of crispy genotypes maintained their structure during the ripening process and cell walls did not break apart, compared to non-crispy genotypes where cells did not remain intact. Visual evidence indicated that non-crispy genotypes had a loss of integrity of cell walls, likely allowing the sugars and acids to be leaked. This was particularly observed for 'Natchez'. For 'Shawnee', cells appeared to maintain most of their structure during ripening, but cells were observed to separate, likely contributing to loss of firmness.

Inheritance was low for all firmness measurements (fruit compression and skin and drupelet penetration), indicating that obtaining individuals of superior firmness could take several generations and large populations would likely be needed to allow for segregation of substantially firmer progeny. Despite the low values of inheritance, firmness can be improved in blackberry, because some populations showed mean values higher than their mid-parent values in all types of forces measured.